THERMOKINETIC MODELING AND MODEL REDUCTION OF REACTION NETWORKS

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Vorwort

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Stuttgart, im Juni 2009 Michael Ederer

Contents

List of Abbreviations

ATP	adenosine-triphosphate
ADP	adenosine-diphosphate
AMP	adenosine-monophosphate
Cor.	Corollary
Def.	Definition
GTP	guanosine-triphosphate
GDP	guanosine-diphosphate
mRNA	messenger ribonucleic acid
NADH	nicotinamide adenine dinucleotide reduced
NAD	nicotinamide adenine dinucleotide oxidized
ODE	ordinary differential equation
p.	page
P	inorganic phosphate
PTS	phosphotransferase system
Sec.	Section
TCA cycle	tricarboxylic acid cycle
TK	thermodynamic-kinetic, thermokinetic
TKM	thermodynamic-kinetic modeling, thermokinetc modeling

List of Symbols

Symbol	Unit	Meaning
Sub- and superscripts, modifiers etc.		
$i = 1 \dots i_0$	-	index for compounds; Sec. 2.2.1 (p. 28)
$j = 1 \dots j_0$	-	index for reactions; Sec. 2.2.1 (p. 28)
E, P	-	subscript for reactant- and product-related quantities
e	-	subscript for external, clamped quantities
eq	-	subscript for equilibrium quantities
0	-	subscript for initial conditions
$\circ$	-	superscript for standard state
$\hat{\cdot}$	-	transformed or translated quantities; Sec. 4.1 (p. 51)
$\tilde{\cdot}$	-	reduced quantities; Sec. 4.2 (p. 57)
$d\,\cdot$	-	differential change
$\delta\,\cdot$	-	finite change
$\Delta\,\cdot$	-	difference
Non-standard mathematical operations		
$\exp(\,\cdot\,)$	-	element-wise exponential function
$\log(\,\cdot\,)$	-	element-wise natural logarithm
$\circ$, $\#$	-	Hadamard and lin-log product; Def. 2.14 & 2.17 (p. 25)
$(\,\cdot\,)^{(-1)}$	-	Hadamard inverse; Def. 2.26 (p. 25)
$\mathrm{span}(\,\cdot\,)$	-	linear span of a matrix; Def. 2.2 (p. 23)
$\mathrm{null}(\,\cdot\,)$	-	null space of a matrix; Def. 2.3 (p. 23)
$\mathrm{inv}_{\cdot}(\,\cdot\,)$	-	pseudo-inverse; Def. 2.8 (p. 24)
$\Vert$	-	parallel resistances; Def. 6.6 (p. 113)
Thermodynamic quantities (Sec. 2.2, p. 28)		
U, G	J	internal energy and Gibbs energy
S	$\mathrm{J\,K^{-1}}$	entropy
V	L	volume
$u = U/V$, $g = G/V$	$\mathrm{J\,L^{-1}}$	volume-specific internal energy and Gibbs energy
$s = S/V$	$\mathrm{J\,K^{-1}\,L^{-1}}$	volume-specific entropy
T	K	temperature
p	Pa	pressure
pH	1	pH value
I	$\mathrm{mol\,L^{-1}}$	ionic strength
Constants		
$R^* = 8.314510\ \mathrm{J\,mol^{-1}\,K^{-1}}$		ideal gas constant
$F = 96485.309\ \mathrm{C\,mol^{-1}}$		Faraday constant
$c^\circ = 1\ \mathrm{mol\,L^{-1}}$		standard concentration

Symbol	Unit	Meaning
Models, transformations and translations		
M	-	thermodynamic or thermokinetic model; Def. 3.1 (p. 40) Def. 5.29 (p. 100)
T_c, T_J	-	transformation matrices; Cor. 4.2 (p. 52), Cor. 5.32 (p. 101)
$\delta\mu$, $\delta\xi$	-	translation vectors; Cor. 4.10 (p. 54), Cor. 5.34 (p. 102)
δN, δS	-	translation matrices; Cor. 5.37 (p. 104)
Compound X_i		
n_i	mol	amount; §2.29 (p. 28)
c_i	mol L^{-1}	concentration; §2.29 (p. 28)
μ_i	J mol^{-1}	chemical potential; §2.29 (p. 28)
$\Delta_f G_i$	J mol^{-1}	Gibbs formation energy; Def. 2.46 (p. 32)
ξ_i	1	thermokinetic potential; Def. 5.1 (p. 89)
C_i	mol L^{-1}	thermokinetic capacity; Def. 5.2 (p. 89)
z_i	1	charge number
Reaction j		
E_j, P_j	-	set of reactants and products; Sec. 2.2.1 (p. 28)
J_j	mol L^{-1} s^{-1}	flux; §2.29 (p. 28)
ΔG_j	J mol^{-1}	Gibbs reaction energy; § 2.36 (p. 30)
$\Delta\mu_j$	J mol^{-1}	thermodynamic force; §2.38 (p. 30)
$K_{eq,j}$	1	equilibrium constant; §2.51 (p. 33)
F_j	1	thermokinetic force; Def. 5.8 (p. 90)
$F_{E,j}$, $F_{P,j}$	1	thermokinetic reactant and product force; Def. 5.8 (p. 90)
k_{+j}, k_{-j}	mol L^{-1} s^{-1}	mass-action parameters; Def. 2.49 (p. 33)
$f_j(p, c)$	1	deviation from ideal mass-action: Def. 2.49 (p. 33)
R_j	J L s mol^{-2}	thermodynamic resistance; Def. 3.1 (p. 40), Sec. 3.3.2 (p. 46)
$\bar{R}_j$, R_j	mol^{-1} L s	thermokinetic resistance; Def. 5.13 (p. 92)
Stoichiometry		
ν_{ij}, $\nu_{E/P,ij}$	1	stoichiometric coefficients; Sec. 2.2.1 (p. 28)
N	$\mathbb{R}^{i_0\times j_0}$	stoichiometric matrix Sec. 2.2.1 (p. 28), Def. 3.1 (p. 40)
S	$\mathbb{R}^{i_{0,e}\times j_0}$	stoichiometric matrix Sec. 2.2.1 (p. 28), Def. 3.1 (p. 40)
N_e	$\mathbb{R}^{i_0\times j_{0,e}}$	stoichiometric matrix Sec. 2.2.1 (p. 28), Def. 3.1 (p. 40)
$N_{E/P}$	$\mathbb{R}^{i_0\times j_0}$	stoich. reactant and product matrices; Def. 5.29 (p. 100)
$S_{E/P}$	$\mathbb{R}^{i_{0,e}\times j_0}$	stoich. reactant and product matrices; Def. 5.29 (p. 100)
d_{j_0}	-	number of stoichiometric cycles; §2.54 (34)
d_{i_0}	-	number of conservation relations; §2.54 (34)

If the subscripts i or j are omitted, the vector of the respective quantities is meant. For example c is the vectors of concentrations, and J is the vector of fluxes: $c = (c_1 \dots c_{i_0})^T$ and $J = (J_1 \dots J_{j_0})^T$.

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Abstract

This work introduces a framework for thermodynamically consistent, kinetic modeling and model reduction of biochemical reaction networks.

Background. Kinetic models describe the dynamics of the concentrations and fluxes in a biochemical reaction network by means of the network stoichiometry and the kinetic rate equations. The laws of thermodynamics constrain the possible dynamics of reaction networks and thus constrain physically feasible kinetic models. The *second law of thermodynamics* demands that the entropy production of any process is non-negative at each point in time. Additionally, the *principle of detailed balance* states that all reaction fluxes vanish in thermodynamic equilibrium, i. e. that the forward rates equal the backward rates. From these laws the *generalized Wegscheider conditions* that constrain the set of possible kinetic parameters can be derived. The Wegscheider conditions express relations between the kinetic parameters of different reactions possibly belonging to different functional units. Especially for large networks, as they are considered in computational systems biology, finding thermodynamically consistent parameters can be difficult because the parameters may be constrained by many independent Wegscheider conditions.

Thermodynamic Modeling. A possibility for formulating thermodynamically consistent models is the use of the chemical potentials μ_i of the compounds and the Gibbs reaction energies ΔG_j. In the most simple form, a thermodynamic model of a reaction network is defined by the stoichiometric matrix N, a function $c(\mu)$ that describes the dependency of the vector of concentrations c on the vector of chemical potentials μ and a matrix of thermodynamic resistances $R(\mu)$. The matrix of thermodynamic resistances $R(\mu)$ is a positive semi-definite, symmetric matrix with $R(\mu) \cdot J = -\Delta G$ where J is the vector of reaction fluxes and ΔG the vector of Gibbs reaction energies. The symmetry and positive semi-definiteness of $R(\mu)$ guarantee the fulfillment of the second law of thermodynamics and the principle of detailed balance. If the stoichiometric matrix N, the derivative $\partial c(\mu)/\partial \mu$ or the matrix of thermodynamic resistances $R(\mu)$ satisfy certain rank conditions, algebraic relations between the model variables hold such that a reduced model with less compounds or reactions can be derived. Thus, this approach allows for thermodynamically consistent modeling and model reduction. However, neither are the concentrations proportional to the chemical potentials, nor are the Gibbs reaction energies proportional to the reaction fluxes. Even for simple systems the model equations are very complex, and it is impractical to use this approach for larger models.

Thermokinetic Modeling (TKM) Formalism. Based on the thermodynamic modeling and model reduction formalism, the thermokinetic modeling formalism (TKM) is derived. TKM is a convenient and user-friendly formalism to build thermodynamically consistent kinetic models. The TKM formalism is based on *thermokinetic potentials* ξ_i of compounds and *thermokinetic forces* F_j of reactions. These quantities are derived from chemical potentials and Gibbs reaction energies. In the case of ideal dilute solutions, thermokinetic potentials are proportional to the corresponding concentrations: $c_i = C_i \cdot \xi_i$. The constant proportionality factors C_i are the *thermokinetic capacities* of the compounds. In the case of mass-action kinetics, the thermokinetic forces and the reaction fluxes are proportional: $\bar{R}_j \cdot J_j = F_j$. The constant proportionality factors $\bar{R}_j$ are the *thermokinetic resistances* of the reactions. Non-ideal solutions or complex kinetics lead to non-constant, state-dependent capacities and resistances. Each model described by capacities and resistances is thermodynamically consistent and structurally fulfills the Wegscheider conditions. In addition, each thermodynamically consistent, kinetic model can be expressed by capacities and resistances. Thus, the use of these quantities provides a simple and comprehensive way for thermodynamically consistent modeling.

Transformation and Model Reduction. Thermokinetic models can be formally represented by a tuple of matrices and functions in a similar way as linear systems can be represented by a tuple of the matrices A, B, C and D. Analogously, equivalence transformations for thermokinetic models can be formulated. The concentrations and fluxes of the transformed model are linear combinations of the original concentrations and fluxes. If the matrices and the derivatives of the functions that describe a thermokinetic model fulfill certain rank conditions, the model size can be reduced by suited transformation and reduction steps. In particular, the model size can be reduced if the model contains conservation relations or stoichiometric cycles. Further, a reduction is possible if resistances or capacities have a value of zero. Capacities of zero correspond to quasi-stationary compounds and resistances of zero correspond to reactions in rapid equilibrium. Due to the formal structure of thermokinetic models, model reduction based on the rapid equilibrium assumption is particularly simple. It can be easily applied to reaction rules as they are used to describe protein-protein interaction networks with inherent combinatorial complexity.

Graphical Representation. The TKM formalism is similar to the modeling formalism for electrical networks and an analog graphical representation is possible. Thermokinetic models can be depicted in a diagram as a connection of basic network elements representing the compounds and reactions. Several model reduction methods can be formulated as graphical rules, which allow for a simple and intuitive reduction of the model size.

Oxygen Response of *Escherichia coli*. The TKM formalism is used to model the oxygen response of the bacterium *Escherichia coli*, which is strongly determined by thermodynamic constraints. In order to restrict the model to the relevant parameters and dynamics, model

reduction techniques are applied. The model is able to explain the measured metabolic fluxes and concentrations in the wild type and a regulatory mutant in dependence of the oxygen availability. This example also shows that TKM is useful for modeling large networks.

Conclusions. TKM unifies thermodynamic and kinetic approaches for the modeling of biochemical reaction networks in a natural and formally appealing way. In particular, it introduces thermodynamic flow-force relationships into kinetic modeling. In this way, TKM guarantees the thermodynamic consistency of the model equations. In the conventional kinetic modeling approach, the kinetic parameters are formally attributed to reactions but not compounds. However, the equilibrium constants that, in the conventional modeling approach, are ratios of kinetic parameters are solely determined by the thermodynamic properties of the compounds. This finally may lead to kinetic models violating thermodynamic constraints unless the Wegscheider conditions are explicitly considered. TKM clearly distinguishes between the thermodynamic parameters, i. e. the capacities, and the kinetic parameters, i. e. the resistances. Thus, TKM provides a thermodynamically consistent parameterization of kinetic models. TKM also provides thermodynamically consistent and conveniently usable model reduction methods. Altogether, TKM strongly simplifies the mathematical modeling of complex biochemical networks.

Deutsche Zusammenfassung

Diese Arbeit führt eine umfassende Gruppe von Methoden zur thermodynamisch konsistenten Modellierung und Modellreduktion biochemischer Reaktionsnetzwerke ein.

Hintergrund. Kinetische Modelle beschreiben die Dynamik von Konzentrationen und Flüssen in biochemischen Reaktionsnetzwerken auf Grundlage der Netzwerkstöchiometrie und der kinetischen Ratengleichungen. Die Gesetze der Thermodynamik beschränken die mögliche Dynamik von Reaktionsnetzwerken und damit auch physikalisch sinnvolle kinetische Modelle. Der *zweite Hauptsatz der Thermodynamik* fordert, dass die Entropieproduktion eines jeden physikalischen Prozesses zu jedem Zeitpunkt nicht negativ ist. Zusätzlich fordert das *Prinzip des detaillierten Gleichgewichts*, dass alle Reaktionsflüsse im thermodynamischen Gleichgewicht verschwinden, d. h. die Vorwärtsraten gleich den Rückwärtsraten sind. Aus diesen Gesetzen lassen sich die *verallgemeinerten Wegscheiderbedingungen*, die die Menge möglicher kinetischer Parameter beschränken, ableiten. Die Wegscheiderbedingungen sind Beziehungen zwischen den kinetischen Parametern verschiedener Reaktionen, die unter Umständen zu unterschiedlichen Funktionseinheiten des Reaktionsnetzwerks gehören. Insbesondere für große Netzwerke, wie sie in der Systembiologie untersucht werden, ist die thermodynamisch konsistente Parametrisierung schwierig, da die Parameterwerte durch viele unabhängige Wegscheiderbedingungen beschränkt sein können.

Thermodynamische Modellierung. Die Verwendung der chemischen Potenziale μ_i der Stoffe und der daraus abgeleiteten Gibbs-Reaktionsenergien ΔG_j bietet eine Möglichkeit zur Formulierung thermodynamisch konsistenter Modelle. In der einfachsten Form ist ein thermodynamisches Modell eines Reaktionsnetzwerks durch die stöchiometrische Matrix N, eine Funktion $c(\mu)$, die die Beziehung zwischen dem Vektor der Konzentrationen c und dem Vektor der chemischen Potenziale μ beschreibt, und durch die Matrix der thermodynamischen Widerstände $R(\mu)$ definiert. Die Matrix der thermodynamischen Widerstände $R(\mu)$ ist eine positiv semidefinite, symmetrische Matrix mit $R(\mu) \cdot J = -\Delta G$, wobei J der Vektor der Reaktionsflüsse und ΔG der Vektor der Gibbs-Reaktionsenergien ist. Die Symmetrie und positive Semidefinitheit von $R(\mu)$ garantieren die Einhaltung des zweiten Hauptsatzes der Thermodynamik und des Prinzips des detaillierten Gleichgewichts. Wenn die stöchiometrische Matrix N, die Ableitung $\partial c(\mu)/\partial\mu$ oder die Matrix der thermodynamischen Widerstände $R(\mu)$ gewisse Rangbedingungen erfüllen, gelten algebraische Beziehungen zwischen den Modellvariablen, die es erlauben ein reduziertes Modell mit weniger Stoffen oder Reaktionen herzuleiten. Dieser Ansatz bietet also die Möglichkeit der thermodynamisch konsistenten Modellierung und Modellreduktion. Jedoch sind weder die Konzentrationen proportional zu den chemischen Potentialen, noch sind die Gibbs-Reaktionsenergien proportional zu den Reaktionsraten. Schon für einfache Systeme werden die Modellgleichungen sehr komplex und es ist kaum möglich diesen Ansatz für größere Systeme zu benutzen.

Thermodynamisch-kinetische Modellierung (TKM). Aufbauend auf thermodynamischer Modellierung und Modellreduktion kann der Formalismus der thermodynamisch-kinetischen Modellierung (TKM) abgeleitet werden. TKM ist eine benutzerfreundliche Methode zur Erstellung thermodynamisch konsistenter Modelle. Der TKM-Formalismus basiert auf *thermokinetischen Potenzialen* ξ_i von Stoffen und *thermokinetischen Kräften* F_j von Reaktionen. Diese Größen sind von chemischen Potenzialen und Gibbs-Reaktionsenergien abgeleitet. Im Fall von verdünnten, idealen Mischungen sind die thermokinetischen Potenziale proportional zu den entsprechenden Konzentrationen: $c_i = C_i \cdot \xi_i$. Die konstanten Proportionalitätsfaktoren C_i sind die *thermokinetischen Kapazitäten* der Stoffe. Im Fall von Massenwirkungskinetiken sind die thermokinetischen Kräfte und die jeweiligen Reaktionsflüsse proportional: $\bar{R}_j \cdot J_j = F_j$. Die konstanten Proportionalitätsfaktoren $\bar{R}_j$ sind die *thermokinetischen Widerstände* der Reaktionen. Nicht-ideale Mischungen oder komplexe Kinetiken führen zu nicht-konstanten, zustandsabhängigen Kapazitäten und Widerständen. Jedes mit Kapazitäten und Widerständen formulierte Modell ist thermodynamisch konsistent und erfüllt strukturell die Wegscheiderbedingungen. Weiterhin kann jedes thermodynamisch konsistente, kinetische Modell in Abhängigkeit von Kapazitäten und Widerständen ausgedrückt werden. Die Verwendung dieser Größen stellt eine einfache und umfassende Möglichkeit zur thermodynamisch konsistenten Modellierung dar.

Transformation und Modellreduktion. Ähnlich wie lineare Systeme als ein Tupel von Matrizen A, B, C und D dargestellt werden können, können thermokinetische Modelle formal als ein Tupel von Matrizen und Funktionen dargestellt werden. Analog können auch Äquivalenztransformationen für thermokinetische Modelle definiert werden. Die Konzentrationen und Flüsse der transformierten Modelle sind Linearkombinationen der ursprünglichen Konzentrationen und Flüsse. Wenn die Matrizen und die Ableitungen der Funktionen, die ein thermokinetisches Modell beschreiben, bestimmte Rangbedingungen erfüllen, kann die Modellgröße durch entsprechende Transformations- und Reduktionsschritte verringert werden. Insbesondere kann die Modellgröße reduziert werden, wenn das Modell Erhaltungsbeziehungen oder stöchiometrische Zyklen enthält. Weiterhin ist eine Reduktion möglich, wenn Widerstände oder Kapazitäten den Wert Null annehmen. Kapazitäten vom Wert Null beschreiben quasistationäre Stoffe und Widerstände vom Wert Null beschreiben Reaktionen im schnellen Gleichgewicht. Aufgrund der formalen Struktur thermokinetischer Modelle ist die auf der Annahme eines schnellen Gleichgewichts basierende Modellreduktion besonders einfach anwendbar. Sie kann auch auf Reaktionsregeln angewandt werden, wie sie zur Beschreibung von Protein-Protein-Interaktionsnetzwerken mit inhärenter kombinatorischer Komplexität eingesetzt werden.

Grafische Darstellung. Der TKM-Formalismus ähnelt stark dem Modellierungsformalismus für elektrische Netzwerke und eine analoge grafische Darstellung ist möglich. Thermokinetische Modelle können als Verschaltung einfacher Netzwerkelemente, die Stoffe und Reaktionen beschreiben, dargestellt werden. Einige Modellreduktionsmethoden können als grafische Regeln, die eine einfache und intuitive Modellreduktion erlauben, formuliert werden.

Sauerstoffantwort von *Escherichia coli*. Der TKM-Formalismus wird zur Modellierung der Sauerstoffantwort des Bakteriums *Escherichia coli*, die stark durch thermodynamische Beschränkungen bestimmt wird, eingesetzt. Um das Modell auf die relevanten Parameter und die relevante Dynamik zu beschränken, werden Modellreduktionsmethoden eingesetzt. Das Modell beschreibt gemessene metabolische Flüsse und Konzentrationen im Wildtyp und in einer regulatorischen Mutante. Dieses Beispiel zeigt zudem die Nützlichkeit von TKM für die Modellierung großer Netzwerke.

Zusammenfassung. TKM verbindet thermodynamische und kinetische Ansätze zur Modellierung biochemischer Reaktionsnetzwerke in einer natürlichen und formal ansprechenden Art. Insbesondere führt TKM thermodynamische Fluss-Kraft-Beziehungen in die kinetische Modellierung ein. Auf dieser Weise garantiert TKM die thermodynamische Konsistenz der Modellgleichungen. Im konventionellen Modellierungsformalismus werden alle kinetischen Parameter als Eigenschaften der Reaktionen behandelt. Die Gleichgewichtskonstanten, die im konventionellen Modellierungsformalismus Verhältnisse von kinetischen Parametern darstellen, sind jedoch vollständig durch die thermodynamischen Eigenschaften der Reaktionspartner bestimmt. Wenn die Wegscheiderbedingungen nicht explizit beachtet werden, kann dies zu kinetischen Modellen führen, die thermodynamische Gesetze verletzen. TKM unterscheidet klar zwischen den thermodynamischen Parametern (den Kapazitäten) und den kinetischen Parametern (den Widerständen). Damit stellt TKM eine thermodynamisch konsistente Parametrisierung kinetischer Modelle zur Verfügung. Weiterhin bietet TKM Möglichkeiten zur einfachen und thermodynamisch konsistenten Modellreduktion. Damit vereinfacht TKM die thermodynamisch konsistente mathematische Modellierung und Modellreduktion komplexer biochemischer Netzwerke.

1. Introduction

This work introduces *Thermokinetic Modeling (TKM)*, a method for thermodynamically consistent, mathematical modeling and model reduction of reaction networks. Its goal is to simplify the mathematical modeling of the large and complex metabolic, signal transducing and regulatory networks that determine the behavior of cells. In order to introduce the main ideas of this work, the next paragraphs discuss the role of systems theory and mathematical modeling in engineering and biology. Major obstacles for kinetic modeling of biochemical networks are identified, and strategies to overcome them are suggested. This provides the motivation for the introduction of TKM.

Systems Theory and Engineering. Systems theory plays a pivotal role in modern engineering. Mathematical modeling and model analysis in conjunction with computational methods allow for solving complex analysis and design problems. Mathematical modeling allows one to gain a deep understanding of the dynamics of a given system and to systematically analyze and design complex systems. In particular, virtually all methods in advanced control engineering rely on mathematical models. The hierarchical structure of engineered systems often allows for the independent modeling of different levels of organization. For example, model-based methods are used for the planning of management strategies for container terminals with several cranes [109] and for the development of control strategies for single cranes [8]. These hierarchical levels are also associated with different time scales. For example, the duration for the loading of a single container is short compared to the time needed for the loading of several container ships in a container terminal. At each level of detail and each time scale interesting and challenging problems requiring tailored models arise.

Systems Theory and Biology. Similarly to classical engineering applications of systems theory, the mathematical modeling of biological and biotechnological systems provides important insight into their dynamics and allows for the systematic redesign of biological systems. For example, models of reaction networks proved to be helpful for the genetic engineering of microorganisms into production strains (see e. g. [59, 66, 72]) and are expected to be useful for the development of novel medical treatments [77, 91, 108].

However, the interest of systems engineering in biology is not only driven by biotechnological applications. Among the natural sciences, biology has a special role because we may assign functions to many biological systems. For example, the function of the central metabolism is the supply of the cell with precursor molecules for maintenance and growth. The complexity and robustness of the regulation of biological functions are astonishing and their understanding represents a challenge for systems theory and control engineering. Thus, biology can be partly understood as a *reverse engineering* effort with the goal to reveal the design principles of biological systems [29, 52, 101].

The application of systems theory to biological systems is often referred to as *systems biology*. However, the term systems biology is also used for approaches that seek to characterize the dynamics of biological systems by experimental high-throughput methods. Here, this term will be used only in the former sense.

Similarly to engineered systems, biological systems often exhibit a pronounced modularity and a hierarchical structure. Biological systems are organized for example hierarchically into populations, organisms, organs, cells, biochemical pathways and enzymatic reactions. This work focuses on the level of cellular reaction networks consisting of one or several biochemical pathways. Cellular reaction networks are also hierarchically organized in modules [44, 84].

Two main classes of biochemical reaction networks are metabolic networks and signal transduction networks. The function of metabolic networks is to transform chemical compounds into other chemical compounds, e. g. substrates found in the environment into precursors for cellular growth. Signal transduction networks are also based on the reaction of chemical compounds, but their function is the transmission and processing of cellular signals; e. g. to sense stress situations and to trigger adequate responses. Both kinds of networks closely interact in a similar way as plants and controllers interact in engineered systems. Signal transduction networks sense the state of metabolic networks and of the environment, and their output controls metabolic fluxes.

Modeling Biochemical Reaction Networks. The modeling of biochemical reaction networks currently suffers from a lack of quantitative, time resolved data. While the qualitative network structure is often known, the quantitative parameters needed for a detailed mathematical model are largely unknown. This problem can be partly solved by the advancement of measurement methods which is a major focus of the current experimental research. However, given the complexity and variability of biochemical networks, it is unlikely that the quantitative parameters of all biochemical reactions in medium or large sized biochemical networks can be measured completely and unambiguously in the near future.

Therefore, we also need systems theoretical answers for the problem of the missing quantitative data. It may not be necessary to experimentally determine all parameter values quantitatively to answer a given question or to solve a given design problem by means of a kinetic model. To address this problem two principal approaches are possible: (1) If some parameter values are not known, one may use a rough estimate of these values or one may study the model behavior in a certain parameter range. Here, it is important to explicitly acknowledge the basic physical constraints in order to avoid physically inconsistent model variants. (2) If the relevant system dynamics is not sensitive towards the unknown parameters, one may formulate a reduced-order model where these parameters are omitted.

Constraints on the Network Dynamics. The stoichiometry of biochemical networks determines the possible mass and energy flows in the system. It was shown that these structural and thermodynamic constraints determine key aspects of the network's functionality [34, 97]. This fact is exploited by the *constraint-based modeling* approach that mainly considers fluxes in quasi-stationary metabolic networks [85]. In fact, constraint-based modeling is one of the most successful systems biological approaches for biotechnological applications.

In the conventional kinetic modeling formalism that seeks to describe the dynamics of concentrations and fluxes in reaction networks, the thermodynamic constraints take the form of equality constraints relating the equilibrium constants of different reactions. These constraints are the so-called *generalized Wegscheider conditions* [45, 92]. Example 2.59 (p. 36) will show that in a genome-scale metabolic network the number of independent Wegscheider conditions is about 20% of the number of reactions. About 50% of the reactions participate in at least one Wegscheider condition. In many cases the Wegscheider conditions relate parameters of different functional units. The constraints on signal transduction networks that are based on the interaction of proteins are even stronger. In Example 2.60 (p. 37), every reaction participates in at least one Wegscheider condition. Already in moderate sized signal transduction networks, the number of independent Wegscheider conditions is about 70% of the number of reactions. This high number of Wegscheider conditions makes the correct parameterization of models in the traditional kinetic modeling formalism difficult. This task is complicated by the fact that the reaction parameters coupled by the Wegscheider conditions may lie in different modules of the network. For this reason, the development of a method for the explicit and systematic consideration of the basic thermodynamic constraints in kinetic, dynamic modeling is promising [26, 31, 32, 106].

Reduced-Order Modeling. Engineered systems can and have to be modeled on different levels of detail because their modular structure and the different associated time scales necessitate a certain degree of encapsulation. Modeling usually does not start at the most detailed, physically accessible level but is adapted to the problem under consideration. For example, it would be a very difficult problem to study the management of a container terminal by a model considering the oscillations of the loads of the single cranes. What matters for the management planning are not the parameters that characterize the details of the oscillations of a crane cable but the average time needed by a crane for the processing of a container. Similarly, by exploiting the hierarchical structure of biochemical networks it should be possible to develop models that are tailored for a specific research question or design task. These models contain a limited number of parameters that can be assessed experimentally. A common objection to this approach is that the parameters and variables in such models are composite quantities and not minimal in a physical sense. For this reason, it is believed that it is preferable to first build a detailed model and to perform *model reduction* rather than *reduced-order modeling.* However, it is important to see that the usage of the terms 'reduced' as opposed to 'detailed' makes only sense for the comparison of models. There is no ultimate detailed master model of a system. The fact that a model "is an approximation does not necessarily detract from its usefulness because all models are approximations. Essentially, all models are wrong, but some are useful" [21, p. 424].

The Focus of This Work. Above two major challenges for kinetic modeling were identified: (1) the explicit incorporation of basic thermodynamic constraints and (2) a reduced-order modeling approach that allows one to restrict the model to the important parameters. The TKM approach addresses both problems.

The theory of irreversible thermodynamics associates every storage variable of a system with an intensive variable [23, 41, 55]. For example, the electrical charge is associated with voltage, volume is associated with pressure, energy is associated with temperature and mass storages are associated with chemical potentials. Gradients of intensive variables define thermodynamic forces that drive fluxes. In a similar way, mechanical forces drive velocity changes. A flux and the associated force have the same direction. Because of their importance, the intensive variables are directly used in dynamic modeling. Models of electrical systems are formulated in terms of voltages, models of mechanical systems use mechanical forces and models of hydrodynamic systems use pressures. This is convenient because fluxes in such systems are often approximately proportional to the according forces. For example, in an Ohmic resistance the electrical current and the voltage difference is proportional and for constant mass mechanical force and acceleration are proportional. An exception are chemical reaction networks. The chemical potentials are rarely used directly for dynamic modeling. The reason for this is that the reaction fluxes usually are not proportional to the chemical potential gradients. Thus, kinetic models are usually formulated in terms of concentrations only. This would be analogous to a modeling formalism for electrical networks that uses charges, but does not refer to electrical voltages. The TKM formalism provides an alternative system of forces that is better suited for kinetic modeling than chemical potential differences. In this way, it avoids the problems that arise from the neglect of the chemical potentials in kinetic modeling and guarantees the thermodynamic feasibility of the resulting model equations.

The use of potential variables simplifies the application of the rapid-equilibrium assumption for reduced-order modeling. If a flux is very sensitive towards its force, a small perturbation from the equilibrium position leads to a large flux that counteracts the perturbation. Then one can often approximate the system by assuming a vanishing force. This reduces the order of the problem. For example, if the electrical resistance between two junctions is small, already a small voltage difference leads to a large flux. As a consequence, the voltages in both junctions are most of the time approximately equal and thus a single variable can be used to approximately describe both voltages. Another example is the rigid body assumption in mechanics. By neglecting the elasticity between two or more points of a body, one can simplify the model equations. The rapid equilibrium assumption is very important for the modeling of biochemical networks. It is used to derive approximate rate laws for complex reaction mechanisms, for example for enzymatic reactions [94]. It is also applied to simplify models of pathways [38], but its application is hindered by the somewhat complex calculations that are necessary for its application. Since TKM introduces potential variables, the application of the rapid equilibrium assumption is heavily simplified compared to conventional kinetic modeling. Besides the rapid equilibrium assumption, this work discusses further powerful methods for model reduction and reduced-order modeling of TK models.

The main results are presented in a formal way that allows for the implementation of the developed methods in computer programs. This is essential because the use of the thermokinetic modeling and model reduction methods is in particular interesting for large networks. The developed methods were implemented in the mathematical programming language *Mathematica* [104].

The Structure of This Work. This work develops a thermokinetic framework for mathematical modeling and model reduction of reaction networks. Whereas the first four chapters are concerned with the introduction of the general concepts, the following chapters develop practical and convenient methods for the application of these concepts. A reader who is mainly interested in the application of the methods can focus on the chapters five to nine.

At suited places, the development of the thermokinetic approach is interrupted by *excursuses*. They describe the application of the approach to problems where its applicability is not obvious but useful.

Chapter 2 *Preliminaries and Notation* introduces some basic concepts and the notation needed throughout the work. Chapter 3 *Thermodynamic Modeling* introduces a formal way for the formulation of kinetic models in terms of chemical potentials. Based on this formalism, Chapter 4 *Transformation and Reduction* discusses several possibilities for the transformation and reduction of thermodynamic models. The goal of the Chapters 3 and 4 is to systematically and rigorously introduce a general theory for the thermodynamically consistent modeling and model reduction. Chapter 5 *Thermokinetic Modeling* then derives a new, much more convenient modeling and reduction formalism by replacing chemical potentials by thermokinetic potentials. Chapter 6 *Model Reduction of Reaction Equations* shows that the relevant methods can be applied to a list of reaction equations. It also shows that for the reduction it is not necessary to write down the detailed model equations. This stresses the modular nature of the reduction methods and allows for reduced-order modeling as opposed to a pure model reduction. Chapter 7 *Graphical Representation of TK-Models* works out a graphical way to represent and manipulate thermokinetic models that is motivated by the graphical representation of electrical networks.

Chapter 8 *Modeling the Redox Regulation of Escherichia coli* introduces a thermokinetic model of the oxygen response of the bacterium *Escherichia coli* and compares its results to available experimental data. Thereby it demonstrates the use of TKM for the modeling of a large example network.

Finally, Chapter 9 *Comparison to Other Approaches* reviews several other methods for considering thermodynamic constraints in mathematical modeling of reaction networks and compares them with TKM.

Appendix A describes a prototypical modeling and model reduction computer tool for thermokinetic models.

2. Notations and Preliminaries

This chapter collects basics from different fields that are needed later on. Its task is to shortly introduce a few concepts and to fix the notation. It is by no means supposed to be a complete, self-contained introduction to the used concepts.

The first section is concerned with linear algebra. In particular, it introduces two mathematical operations that will simplify the notation of TKM models. The second section introduces the notation used for the description of reaction networks.

2.1. Linear Algebra and Matrix Operations

Definition 2.1 (Identity, one and zero matrix)**.** By I_n we denote the $n \times n$ identity matrix and by $1_{n_1 \times n_2}$ and $0_{n_1 \times n_2}$ the $n_1 \times n_2$ matrices where all elements are 1 and 0, respectively. The subscripts of the matrices $0_{n_1 \times n_2}$, $1_{n_1 \times n_2}$ and I_n will be suppressed if their dimensions are clear from the context.

2.1.1. Linear Equation Systems

A matrix $A \in \mathbb{R}^{n_1 \times n_2}$ defines two spaces: the linear span and the null space of A, which are introduced in the following two definitions.

Definition 2.2 (Linear span)**.** The linear span of a matrix $A \in \mathbb{R}^{n_1 \times n_2}$ is the space spanned by the columns of A:

$$\operatorname{span}(A) = \{x \in \mathbb{R}^{n_1} \ : \ x = A\,\tilde{x} \text{ with } \tilde{x} \in \mathbb{R}^{n_2}\}.$$

The linear span is also called the column space of A.

Definition 2.3 (Null space)**.** The null space of a matrix $A \in \mathbb{R}^{n_1 \times n_2}$ is the set of vectors mapped to 0 by this matrix:

$$\operatorname{null}(A) = \{x \in \mathbb{R}^{n_2} \ : \ A\,x = 0\}.$$

Corollary 2.4 (Orthogonal complement)**.** *The space* $\operatorname{null}(A)$ *is the orthogonal complement of* $\operatorname{span}(A^T)$*. This means, that if* $x \in \operatorname{span}(A^T)$ *and* $y \in \operatorname{null}(A)$*, then* $x^T y = 0$*. Further it holds that* $\mathbb{R}^{n_1}$ *is spanned by the union of the vectors of* $\operatorname{span}(A)$ *and* $\operatorname{null}(A^T)$*.*

The columns of A contain a basis of $\operatorname{span}(A)$. The basis of $\operatorname{null}(A)$ is given by the kernel matrix:

Definition 2.5 (Kernel matrix)**.** A kernel matrix $B \in \mathbb{R}^{n_2 \times (n_2 - \mathrm{rank}(A))}$ of a matrix $A \in \mathbb{R}^{n_1 \times n_2}$ is a matrix of full rank, i. e. $\mathrm{rank}(B) = n_2 - \mathrm{rank}(A)$, with

$$\mathrm{null}(A) = \mathrm{span}(B)$$

or equivalently

$$A\,B = 0.$$

§ 2.6 (Non-uniqueness of kernel matrix)**.** The kernel matrix B is not unique. In particular, every $B\,\Lambda$ with a quadratic matrix Λ of full rank is also a kernel matrix. A kernel matrix can be computed for example by Gauss elimination.

The columns of a kernel matrix span all homogeneous solutions of a linear equation system. It can be used to parameterize the solution set of a homogeneous linear equation system:

Corollary 2.7 (Homogeneous linear equation system)**.** *The set implicitly given by the homogeneous linear equation* $A\,x = 0$ *can be parameterized by* $x = B\,\tilde{x}$ *where* B *is a kernel matrix with* $A\,B = 0$ *and* $\tilde{x} \in \mathbb{R}^{n_2 - \mathrm{rank}(A)}$ *are the free parameters.*

The kernel matrix defines the homogeneous solutions. Similarly, we can define pseudoinverses that are matrices that yield particular solutions to inhomogeneous problems:

Definition 2.8 (Λ-inverse)**.** Let $A \in \mathbb{R}^{n_1 \times n_2}$ with $\mathrm{rank}(A) = n_1 \leq n_2$ and $\Lambda \in \mathbb{R}^{n_2 \times n_2}$ with $\mathrm{rank}(\Lambda) = n_2$. The matrix $\mathrm{inv}_\Lambda(A) \in \mathbb{R}^{n_2 \times n_1}$ is the Λ-inverse of A, respectively. It is defined by:

$$\mathrm{inv}_\Lambda(A) = \Lambda^{-1}\,A^T\,(A\,\Lambda^{-1}\,A^T)^{-1}.$$

For some applications of the Λ-inverse the choice of Λ does not play any role. In such cases, the subscript Λ is suppressed.

§ 2.9 (Possible generalizations)**.** The matrix $A\,\Lambda^{-1}\,A^T$ is invertible, because A has full row rank, i. e. $\mathrm{rank}(A) = n_1$. The above given formulas for the Λ-inverse rely on the existence of this inverse. The definitions can be extended to the case $rank(A) < n_1$ [83]. This, however, is not needed in this work.

Corollary 2.10 (Pseudoinverse)**.** *The matrix* $\mathrm{inv}_\Lambda(A)$ *is a right inverse of* A*. This means* $A\,\mathrm{inv}_\Lambda(A) = I$*. In particular, the matrix* $\mathrm{inv}_I(A)$ *is the Moore-Penrose inverse of* A*. If* $n_1 = n_2$*, then* $\mathrm{inv}_\Lambda(A) = A^{-1}$ *for any invertible matrix* Λ*.*

Corollary 2.11 (Inhomogeneous linear equation system)**.** *Let* $x = \mathrm{inv}_\Lambda(A)\,b$ *with* $x \in \mathbb{R}^{n_2}$*,* $b \in \mathbb{R}^{n_1}$*. Then* x *is a solution of* $A\,x = b$ *with* $x \in \mathrm{span}(\Lambda^{-1}\,A^T)$*.*

A parameterization of the set defined by $A\,x = b$ *is given by* $x = \mathrm{inv}_\Lambda(A)\,b + B\,\tilde{x}$*. Here,* $\Lambda \in \mathbb{R}^{n_2 \times n_2}$ *is an arbitrary, invertible matrix,* B *is a kernel matrix of* A *and* $\tilde{x} \in \mathbb{R}^{n_2 - \mathrm{rank}(A)}$ *is the vector of free parameters.*

2.1.2. Linear Operations in Logarithmic Scale

The thesis uses two non-standard binary operations on matrices, the Hadamard product and the lin-log product. Both operations are non-linear, but can be seen to be linear in a logarithmic scale. The following paragraphs introduce the notation and shortly discuss the properties of the products.

Here and throughout the thesis we will use $\log(A)$ and $\exp(A)$ to denote the natural element-wise logarithm and exponential function of a matrix A, respectively. Thus, $\log(A)$ and $\exp(A)$ do *not* denote matrix logarithm and exponential.

Definition 2.12 (Element-wise logarithm and exponential)**.** Let A be an $n_1 \times n_2$-matrix, then $C = \log(A)$ and $D = \exp(A)$ are $n_1 \times n_2$-matrices defined by

$$C_{i_1 i_2} = \log(A_{i_1 i_2}), \qquad D_{i_1 i_2} = \exp(A_{i_1 i_2}) \tag{2.1}$$

with $i_1 = 1 \dots n_1$ and $i_2 = 1 \dots n_2$.

Example 2.13. $\log\big((a_1, a_2)\big) = \big(\log(a_1), \log(a_2)\big)$.

Definition 2.14 (Hadamard product)**.** The Hadamard product of two matrices A and B with equal dimensions $n_1 \times n_2$ is the element-wise product of A and B:

$$C = A \circ B \;\text{ with }\; C_{i_1 i_2} = A_{i_1 i_2} \, B_{i_1 i_2}$$

with $i_1 = 1 \dots n_1$, $i_2 = 1 \dots n_2$ and $C \in \mathbb{R}^{n_1 \times n_2}$ [83].

Example 2.15. $(a_1, a_2) \circ (b_1, b_2) = (a_1 \, b_1, a_2 \, b_2)$.

Corollary 2.16 (Logarithmic scale)**.** *If the element-wise logarithms of the matrices A and B are defined, the Hadamard product is equivalent to matrix summation in logarithmic scale*

$$\log(A \circ B) = \log(A) + \log(B).$$

Definition 2.17 (Lin-log product)**.** The lin-log product $C \in \mathbb{R}^{n_1 \times n_3}$ of two matrices $A \in \mathbb{R}^{n_1 \times n_2}$ and $B \in \mathbb{R}^{n_2 \times n_3}$ is defined by

$$C = A \# B \;\text{ with }\; C_{i_1 i_3} = \prod_{i_2 = 1}^{n_2} B_{i_2 i_3}^{A_{i_1 i_2}}$$

for $i_1 = 1 \dots n_1$ and $i_3 = 1 \dots n_3$.

Example 2.18. $(a_1, a_2) \# (b_1, b_2)^T = b_1^{a_1} \, b_2^{a_2}$.

The term 'lin-log product' refers to the fact that it can be understood as a normal matrix product (inner product) in logarithmic scale of the second matrix.

Corollary 2.19 (Logarithmic scale)**.** *The lin-log product is equivalent to a matrix multiplication in logarithmic scale of the second matrix:*

$$\log(A\#B) = A\,\log(B).$$

§ 2.20. The lin-log product may be complex or undefined if $B_{i_2 i_3} \leq 0$. If we use the lin-log product, we implicitly assume that it exists and is real.

Definition 2.21 (Priorities of Hadamard and lin-log product)**.** To avoid the extensive use of brackets we define that the lin-log product has a higher priority than the Hadamard product and both have higher priority than addition:

$$A_1 \circ B_1\#C_1 + A_2 \circ B_2\#C_2 = (A_1 \circ (B_1\#C_1)) + (A_2 \circ (B_2\#C_2)).$$

Corollaries 2.16 and 2.19 often allow the application of tools from linear algebra for problems involving the Hadamard or the lin-log product. In the following corollaries we will list a few useful properties of these products. They can directly be derived with the help of the corollaries mentioned above.

Corollary 2.22 (Special matrices)**.** *As can be easily shown, the following holds for a matrix* $A \in \mathbb{R}^{n_1 \times n_2}$*:*

$$A \circ 1_{n_1 \times n_2} = A, \qquad A \circ 0_{n_1 \times n_2} = 0_{n_1 \times n_2}$$

and

$$A\#1_{n_2 \times n_3} = 1_{n_1 \times n_3}, \qquad A\#0_{n_2 \times n_3} = 0_{n_1 \times n_3}.$$

The last expression is only defined, if A has only positive entries. It further holds that

$$I_{n_1}\#A = A, \qquad 0_{n_3 \times n_1}\#A = 1_{n_3 \times n_2}$$

where the last expression is only defined, if the entries of A are different from zero.

Corollary 2.23 (Commutativity and Associativity)**.** *The Hadamard product inherits commutativity and associativity from the scalar multiplication:*

$$A \circ B = B \circ A, \qquad (A \circ B) \circ C = A \circ (B \circ C).$$

These properties do not hold for the lin-log product, but a law similar to associativity is valid:

$$(A\,B)\#C = A\#(B\#C).$$

Proof. Commutativity and associativity of the Hadamard product are straightforward. The above identity for the lin-log product can be proved by applying Corollary 2.19 to both sides of the equation. Left hand side: $\log((A\,B)\#C) = A\,B\,\log(C)$; right hand side: $\log(A\#(B\#C)) = A\,\log(B\#C) = A\,B\,\log(C)$. ☐

Corollary 2.24 (Distributivity)**.** *For matrices A, B and C of appropriate dimensions the following distributive and distributive-like laws hold:*

$$\begin{aligned} A \circ (B + C) &= A \circ B + A \circ C, \\ A\#(B \circ C) &= (A\#B) \circ (A\#C), \\ (A + B)\#C &= (A\#C) \circ (B\#C). \end{aligned}$$

Proof. The first identity follows directly from the according distributive laws for scalars. For proving the latter two identities we apply the element-wise logarithm to both sides and simplify the result: 1) left hand side: $\log(A\#(B \circ C)) = A\,\log(B \circ C) = A\,(\log(B) + \log(C)) = A\,\log(B) + A\,\log(C)$; right hand side: $\log((A\#B) \circ (A\#C)) = \log(A\#B) + \log(A\#C) = A\,\log(B) + A\,\log(C)$. 2) left hand side: $\log((A + B)\#C) = (A + B)\,\log(C)$; right hand side: $\log((A\#C) \circ (B\#C)) = A\,\log(C) + B\,\log(C) = (A + B)\,\log(C)$. ☐

Corollary 2.25 (Partitioned matrices)**.** *Let A, B, C and D be matrices of appropriate dimensions. It holds*

$$\begin{pmatrix} A & B \end{pmatrix} \# \begin{pmatrix} C \\ D \end{pmatrix} = (A\#C) \circ (B\#D).$$

Proof. Applying the logarithm to both sides of the equation and using Corollaries 2.16 and 2.19 proves the identity. ☐

For solving equation systems containing the Hadamard or the lin-log product suitable definitions of matrix inverses are useful.

Definition 2.26 (Hadamard inverse)**.** Let $A \in \mathbb{R}^{n_1 \times n_2}$ contain no zero entries. Then $A^{(-1)} \in \mathbb{R}^{n_1 \times n_2}$ is the Hadamard inverse of A defined by

$$\left(A^{(-1)}\right)_{i_1 i_2} = 1/A_{i_1 i_2}.$$

Corollary 2.27 (Hadamard equations)**.** *The matrix $A^{(-1)}$ is the right and left inverse of A under the Hadamard product:*

$$A^{(-1)} \circ A = A \circ A^{(-1)} = 1_{n_1 \times n_2}.$$

The solution of the matrix equation $A \circ X = B$ is $X = A^{(-1)} \circ B$.

Corollary 2.28 (Hadamard inverse of lin-log product)**.** *With the matrices $A \in \mathbb{R}^{n_1 \times n_2}$ and $B \in \mathbb{R}^{n_2 \times n_3}$ it holds that*

$$(A\#B)^{(-1)} = (-A)\#B = A\# \left(B^{(-1)}\right).$$

Proof. Applying the logarithm to both sides of the equation and using Corollary 2.16 and $-\log(B) = \log\left(B^{(-1)}\right)$ proves the identity. ☐

2.2. Reaction Networks

This section introduces some basics needed for the description of reaction networks. It is limited to the case of a homogeneous phase with clamped temperature and pressure. For the study of many cellular reaction networks this is a realistic assumption. Heterogeneous systems consisting of distinguishable homogeneous subsystems, e. g. cellular compartments, can be modeled by a connection of several homogeneous models.

§ 2.29 (Notation)**.** T [K], p [Pa] and V [L] denote temperature, pressure and volume, respectively. We assume that the phase contains i_0 distinguishable chemical species. Their amounts, concentrations and chemical potentials are denoted by $\mathrm{n_i}$ [mol], $\mathrm{c_i}$ [mol $\mathrm{L^{-1}}$] and μ_i [J $\mathrm{mol^{-1}}$], respectively. In the phase, j_0 reactions take place that interconvert the i_0 components. By $\mathrm{J_j}$ [mol $\mathrm{L^{-1}}$ $\mathrm{s^{-1}}$] we denote the rate of the jth reaction. The above defined symbols may appear without indices. Then they denote the respective vectors $n, c, \mu \in \mathbb{R}^{i_0}$ and $J \in \mathbb{R}^{j_0}$.

2.2.1. Stoichiometry

A reaction j interconverts the compounds according to the rule

$$\sum_{i \in E_j} \nu_{E,ij}\, X_i \overset{j}{\rightleftharpoons} \sum_{i \in P_j} \nu_{P,ij}\, X_i. \tag{2.2}$$

By E_j and P_j we denote the sets of the reactants and the products of reaction j, respectively. We distinguish the stoichiometric coefficients of reactants $\nu_{E,ij} > 0$ and products $\nu_{P,ij} > 0$. In this way we can directly model autocatalytic reactions as reactions where some of the components of P_j appear also in E_j. The overall stoichiometric coefficient is $\nu_{ij} = \nu_{P,ij} - \nu_{E,ij}$. It is positive for products and negative for reactants.

§ 2.30 (Stoichiometric Matrices)**.** The stoichiometric coefficients can be compiled into the reactant and product stoichiometric matrices N_E and N_P, respectively. The stoichiometric matrix N is the difference of the product and the reactant matrix:

$$(N_E)_{ij} = \nu_{E,ij}, \qquad (N_P)_{ij} = \nu_{P,ij}, \qquad N = N_P - N_E. \tag{2.3}$$

§ 2.31 (Mole Balances)**.** Using these definitions we may easily formulate the mole balances for a closed system. They can be transformed to the concentration balances:

$$\frac{1}{V}\dot{n} = N\,J, \qquad \dot{c} = N\,J - \frac{\dot{V}}{V}\,c. \tag{2.4}$$

For open systems additional exchange fluxes with the environment need to be considered in the balance equations.

2.2.2. Irreversible Thermodynamics

This section briefly introduces the aspects of irreversible thermodynamics that are needed for modeling homogeneous, isothermal and isobaric systems. Since only liquid phases are considered, one can safely assume that on a microscopic level dissipative collisions prevail such that the system is in *local equilibrium.* This means that it is possible to use quantities from classical equilibrium thermodynamics to describe non-equilibrium states. For a thorough analysis of this assumption see Glansdorf and Prigogine [41].

The goal of this section is to collect the relevant equations and to introduce the notation, but not to give a self-contained introduction. For a thorough introduction the reader may refer to Callen [23], Kaufman [55] and to Glansdorf and Prigogine [41].

2.2.2.1. Basic terms and relations

§ 2.32 (Entropy, Internal Energy and Gibbs Energy)**.** The entropy S $[\mathrm{J\,K^{-1}}]$, internal energy U [J] and Gibbs energy $\mathrm{G} = \mathrm{U} - \mathrm{T\,S} + \mathrm{p\,V}$ [J] are key quantities for the thermodynamics of reaction networks. The changes of the extensive variables entropy, energy, volume and mole numbers are related by the total differential

$$dU = T\,dS - p\,dV + \mu^T\,dn \qquad \text{or equivalently} \qquad dS = \frac{1}{T}\,dU + \frac{p}{T}\,dV - \frac{\mu^T}{T}\,dn.$$

In the following, we assume that the Gibbs energy is expressed as a fundamental equation $G(T, p, n)$. The total differential of $G(T, p, n)$ is

$$dG = -S\,dT + V\,dp + \mu^T\,dn.$$

Because G is homogeneous of degree one in n, i. e. $G(T, p, \lambda\, n) = \lambda\, G(T, p, n)$, we get the Gibbs-Duhem relation

$$0 = S\,dT - V\,dp + n^T\,d\mu$$

that is a restriction on the possible values of the intensive quantities T, p and μ. We further get that $G = \mu^T\,n$.

§ 2.33 (Specific Gibbs energy and entropy)**.** In homogeneous phases with constant volume it is of advantage to use the volume-specific quantities $s = S/V$ and $g = G/V$. The specific Gibbs energy can be expressed as a function $g(T, p, c)$. It holds that $g = \mu^T\,c$ and

$$dg = -s\,dT + dp + \mu^T\,dc.$$

§ 2.34 (Production and exchange flow)**.** The change of any extensive quantity X or specific quantity x can be divided into a production term ($P[X]$ and $\sigma[x]$) and an exchange term ($J[X]$ and $j[x]$) such that

$$\dot{X} = P[X] + J[X], \qquad\qquad \dot{x} = \sigma[x] + j[x].$$

The production term occurs due to processes in the considered system, whereas the exchange flow occurs due to an exchange of the system with the environment.

§ 2.35 (Entropy and Gibbs energy production)**.** In a closed, homogeneous system under isobaric and isothermal conditions with constant volume, the production rates of the entropy and Gibbs energy are given by

$$T\,P[S] = -P[G] = -\mu^T\,\dot{n} = -\mu^T\,N\,V\,J, \qquad T\,\sigma[s] = -\sigma[g] = -\mu^T\,\dot{c} = -\mu^T\,N\,J.$$

§ 2.36 (Gibbs reaction energies)**.** The vector of the Gibbs reaction energies is given by $\Delta G = N^T\,\mu$. With this we may write $P[S] = -T^{-1}\,\Delta G^T\,V\,J$ and $\sigma[s] = -T^{-1}\,\Delta G^T\,J$.

2.2.2.2. The basic principles of irreversible thermodynamics

Irreversible thermodynamics of reaction networks is mainly based on two postulates: (1) *The second law of thermodynamics* states that the entropy production of any process is non-negative. On this basis, thermodynamic forces can be defined that are directed towards a state of *thermodynamic equilibrium* where all forces and the entropy production vanish. (2) The *principle of detailed balance* or equivalently the *principle of microscopic reversibility* postulates that in thermodynamic equilibrium all fluxes vanish.

§ 2.37 (The Second Law of Thermodynamics)**.** The second law of thermodynamics states that the entropy production is non-negative. For a homogeneous phases one gets:

$$T\,P[S] = -\mu^T\,N\,V\,J \geq 0.$$

In a isobaric and isothermal homogeneous phase one has $P[G] = -T\,P[S] = V\,\mu^T\,N\,J \leq 0$.

§ 2.38 (Thermodynamic forces)**.** The entropy production is the product of thermodynamic forces and fluxes. Thus, $(-T^{-1}\,\Delta G) = -T^{-1}\,N^T\,\mu$ is the vector of thermodynamic forces for the respective chemical reactions. Because we consider systems with constant temperature T, we may alternatively use the vector $\Delta\mu = -\Delta G = -N^T\,\mu$ as the vector of thermodynamic forces. The thermodynamic force $\Delta\mu_j$ along a reaction with stoichiometric coefficients $\nu_{E,ij}$ and $\nu_{P,ij}$ consists of a term $\Delta\mu_{E,j}$ due to the reactants and a term $\Delta\mu_{P,j}$ due to the products:

$$\Delta\mu_j = -\sum_{i=1}^{i_0} \nu_{ij}\,\mu_i = \underbrace{\sum_{i\in E_j} \nu_{E,ij}\,\mu_i}_{\Delta\mu_{E,j}} - \underbrace{\sum_{i\in P_j} \nu_{P,ij}\,\mu_i}_{\Delta\mu_{P,j}}\,.$$

Definition 2.39 (Thermodynamic equilibrium)**.** A reaction is in thermodynamic equilibrium, if its force vanishes (i. e. $\Delta\mu_j = 0$). If all forces vanish simultaneously $\Delta\mu = 0$ the reaction system is in thermodynamic equilibrium.

§ 2.40 (The principle of detailed balance and microscopic reversibility)**.** The *principle of microscopic reversibility* states that in thermodynamic equilibrium any microscopic reaction event is as frequent as its reverse event. Let J_{+j} and J_{-j} denote the frequency of the forward and backward reaction events along a certain reaction coordinate. In thermodynamic equilibrium we have that $J_j = J_{+j} - J_{-j} = 0$ and thus the overall flux J_j vanishes. This is known as the *principle of detailed balance.*

Example 2.41 (Single reaction)**.** Consider a phase with a single reaction $A + B \rightleftharpoons C$. The thermodynamic force is $\Delta\mu = \mu_A + \mu_B - \mu_C$. The second law demands that $T\,V^{-1}\,P[S] = \Delta\mu\,J \geq 0$. Detailed balance demands further that $\Delta\mu = 0 \;\Rightarrow\; J = 0$. Thus, both laws together lead to the condition that $J = \gamma(T, p, n)\,\Delta\mu$, where $\gamma(T, p, n) \geq 0$ is a non-negative function of the state of the system. The function $\gamma(T, p, n)$ is the thermodynamic conductance of the reaction. The function $R(T, p, n) = 1/\gamma(T, p, n)$ is the thermodynamic resistance.

§ 2.42 (Reaction system in the linear region)**.** Near to the thermodynamic equilibrium the relation of the vector of fluxes J and the vector of forces $\Delta\mu$ in a homogeneous, isothermal, isobaric and closed phase of constant volume can be approximated by $J = \Gamma\,\Delta\mu$ where Γ is a symmetric, positive definite matrix. The positive definiteness of Γ follows from the non-negativity of the entropy production $T\,V^{-1}\,P[S] = \Delta\mu^T\,J = \Delta\mu^T\,\Gamma\,\Delta\mu \geq 0$. The symmetry of Γ is a consequence of Onsager's reciprocal relations [75, 76] that are based on the principle of detailed balance. The matrix Γ describes the conductance of the system. Thus, the matrix $R = \Gamma^{-1}$ is the thermodynamic resistance matrix. The matrix R is symmetric because the inverse of a symmetric matrix is symmetric.

2.2.2.3. Chemical potentials and Gibbs formation energies

Chemical potentials are central for the thermodynamic analysis of reaction systems. Here, we present standard approaches for their computation [55].

Definition 2.43 (Ideal mixtures)**.** A mixture is said to be ideal if the chemical potentials follow the law

$$\mu_i = \mu_i^\circ(T, p) + R^*\,T\,\log(n_i/n_\Sigma)$$

where $n_\Sigma = \sum_{i=1}^{i_0} n_i$ is the overall mole number of the phase and R^* is the ideal gas constant. The chemical standard potential μ_i° is independent of the concentrations, but may depend on T and p or any further intensive quantities.

In ideal mixtures, the chemical potentials depend on the overall mole number n_Σ. In highly diluted phases, where the overall mole number is mainly determined by the solvent, it can be assumed that n_Σ is constant and the relation of μ_i and c_i simplifies.

Definition 2.44 (Ideal dilute solutions)**.** A solution is an ideal dilute solution if the chemical potentials of the solutes follow the law

$$\mu_i = \mu_i^\circ(T, p) + R^*\,T\,\log(c_i/c^\circ)$$

where c° is the standard concentration, e. g. $c^\circ = 1$ M. The chemical standard potential μ_i° is independent of the concentrations, but may depend on T, p and further intensive quantities.

§ 2.45 (Assumption of an ideal dilute solution)**.** The assumption of an ideal dilute solution is a standard assumption for (bio)chemical reaction networks. In biochemical systems, the main source of non-ideality are ionic interactions that depend on the ionic strength I of the solution. Because many biochemical species are pools of chemical species with a different amount of protons, the chemical potential of biochemical species often depends on the pH-value. If the ionic strength I and the pH can assumed to be constant and no other non-ideal effects occur, the chemical potentials can be approximated by the quasi-ideal law $\mu_i = \mu_i^\circ(T, p, pH, I) + R^* T \log(c_i/c^\circ)$ (see Alberty [1]).

Since chemical potentials are not easily measurable, the value of the standard potentials μ_i° are usually not available. Instead, the Gibbs formation energies are tabulated for many chemical compounds.

Definition 2.46 (Gibbs energies of formation)**.** The standard Gibbs energy of formation $\Delta_f G_i^\circ$ of a compound X_i is the change in Gibbs energy if one mole of X_i in its standard state (ideal aqueous solution, $c_i = c^\circ = 1$ M) is formed from its elements in reference state (For the definition of the reference state see Alberty [1]). The Gibbs energy of formation in an ideal dilute solution is given by $\Delta_f G_i = \Delta_f G_i^\circ + R^* T \log(c_i/c^\circ)$.

§ 2.47 (Tables of Gibbs formation energies)**.** The standard Gibbs formation energies for many important metabolites are tabulated. Alberty [1] contains tables of Gibbs formation energies for different pH and ionic strength I for around 130 metabolites. Feist et al. [36] present a genome-scale constraint-based model of *Escherichia coli*. This model contains the standard Gibbs formation energies of around 870 metabolites that were estimated by the group contribution method from Jankowski et al. [53], Mavrovouniotis [73].

§ 2.48 (Gibbs formation energies and chemical potentials)**.** The chemical potentials enter the equations of thermodynamics mainly in the form of thermodynamic forces $\Delta\mu = -N^T \mu$. The thermodynamic forces are the differences of the chemical potentials of the reactants and the products. These differences can also be computed from the Gibbs energies of formation:

$$\Delta\mu = -N^T \mu = -N^T \Delta_f G.$$

Thus, for practical purposes chemical potentials μ_i and Gibbs formation energies $\Delta_f G_i$ are exchangeable. For the sake of simplicity we further write μ and $\Delta\mu$, even if in all practical examples Gibbs formation energies are used.

2.2.3. Kinetic Rate Laws

Kinetic rate laws can take various non-linear forms. However, mass-action kinetics are widely used as a basic kinetic law. It is the simplest rate law compatible with the equilibrium mass-action law. For dilute solutions it is mechanistically underpinned by statistical mechanics. Many complex kinetic rate laws are derived from it. Here, we consider generalized mass-action kinetics, that are a very flexible generalization of ideal mass-action kinetics.

Definition 2.49 (Generalized mass-action kinetics)**.** A reaction follows a *generalized mass-action rate law* [45, 89], if its reaction rate can be written as:

$$J_j = f_j(k, c) \left(k_{+j} \prod_{i \in E_j} \left(\frac{c_i}{c^\circ} \right)^{\nu_{E,ij}} - k_{-j} \prod_{i \in P_j} \left(\frac{c_i}{c^\circ} \right)^{\nu_{P,ij}} \right) \tag{2.5}$$

with a constant standard concentration c°, e. g. $c^\circ = 1$ M. The kinetic parameters $k_{\pm j}$ have the unit $[\mathrm{mol\ L^{-1}\ s^{-1}}]$ and are independent of the concentrations of the reactants but may depend on the the ionic strength I, temperature T, the pressure p, the electrical potential ϕ or other intensive variables. Because we assume that these quantities are constant, these dependencies are omitted in the notation. The unitless function $f_j(k, c) \geq 0$ may depend on parameters k, concentrations c and possibly further quantities that are omitted here. If J_j can be written with $f_j(k, c) = 1$, we say that J_j follows an *ideal mass-action law.*

Example 2.50 (Reversible Michaelis-Menten kinetics)**.** Consider the reaction $A \rightleftharpoons B$ with a reversible Michaelis-Menten kinetics: $J = (k_+ \, c_A - k_- \, c_B)/(k_1 + k_A \, c_A + k_B \, c_B)$. The flux J follows a generalized mass-action kinetics with k_+, k_- and $f(k, c) = (k_1 + k_A \, c_A + k_B \, c_B)^{-1}$.

§ 2.51 (Thermodynamic equilibrium)**.** According to the principle of detailed balance, the reaction flux vanishes in thermodynamic equilibrium and we get the equilibrium constant

$$K_{eq,j} = \frac{k_{+j}}{k_{-j}} = \frac{\prod_{i \in P_j} (c_{eq,i}/c^\circ)^{\nu_{P,ij}}}{\prod_{i \in E_j} (c_{eq,i}/c^\circ)^{\nu_{E,ij}}} = \exp\left(-\frac{\Delta G_j^\circ}{R^* \, T} \right) \tag{2.6}$$

where $\Delta G_j^\circ = -\Delta \mu^\circ = \sum_{i \in E_j \cup P_j} \nu_{ij} \, \mu_i^\circ$ is the standard Gibbs energy of the reaction in an ideal dilute solution.

The above equilibrium conditions can be written in the vectorial form

$$\log(K_{eq}) = N^T \log\left(\frac{c_{eq}}{c^\circ} \right), \qquad\qquad K_{eq} = N^T \# \left(\frac{c_{eq}}{c^\circ} \right) \tag{2.7}$$

where the logarithm has to be understood element-wise.

2.2.4. The Wegscheider Conditions

The second law of thermodynamics and the principle of detailed balance impose constraints on possible values of the equilibrium constants. These constraints are called the *Wegscheider conditions.*

§ 2.52 (Generalized Wegscheider Conditions)**.** From Equation 2.7 one sees that the vector $\log(K_{eq})$ is in the column space of N^T. Equivalently this means that

$$B^T \log(K_{eq}) = 0, \qquad\qquad B^T \# K_{eq} = 1 \tag{2.8}$$

where $B \in \mathbb{R}^{j_0 \times d_{j_0}}$ is a kernel matrix of $N \in \mathbb{R}^{i_0 \times j_0}$ with $N \, B = 0$. These are the so-called generalized *Wegscheider conditions* [45, 92]. The columns of the matrix B describe linear independent stoichiometric cycles in the network. Stoichiometric cycles correspond to circular flux distributions $J_{cycle} \in \operatorname{span}(B)$ that have no effect on the concentrations: $\dot{c} = N\, J_{cycle} = 0$. With the lin-log product the generalized Wegscheider conditions read $B^T \# K_{eq} = 1$. This means that the product of the equilibrium constants along any cycle is unity:

$$1 = \prod_{j=1}^{j_0} K_{eq,j}^{J_{cycle,j}}.$$

For example, in the simple cyclic reaction scheme $A \overset{1}{\rightleftharpoons} B \overset{2}{\rightleftharpoons} C \overset{3}{\rightleftharpoons} A$, the Wegscheider condition is $K_{eq,1}\, K_{eq,2}\, K_{eq,3} = 1$.

Kinetic parameters of networks that contain stoichiometric cycles are constrained by the Wegscheider conditions. A model that violates the Wegscheider conditions describes a physically impossible system.

§ 2.53 (Equivalence of the Wegscheider conditions and the usage of chemical potentials)**.** The derivation of the generalized Wegscheider conditions in §2.52 shows that the observance of the Wegscheider conditions in Eq. 2.8 and the usage of chemical potentials is equivalent. Whenever the equilibrium constants $K_{eq,j}$ in a kinetic model fulfill the Wegscheider conditions in Eq. 2.8, there exist chemical standard potentials μ_i° such that Eq. 2.6 is fulfilled and vice versa. When one is interested in a thermodynamically consistent parameterization of a mathematical model one has technically two possibilities: (1) choose standard potentials and compute the equilibrium constants by Eq. 2.6 or (2) choose the equilibrium constants of a subset of the reactions and compute the remaining equilibrium constants using Eq. 2.8.

§ 2.54 (Number of independent Wegscheider conditions)**.** The number of independent Wegscheider conditions can be calculated from the dimension and the rank of N. Let $N \in \mathbb{R}^{i_0 \times j_0}$ and $r = \operatorname{rank}(N)$. Then $d_{i_0} = i_0 - r$ is the number of conserved moieties and $d_{j_0} = j_0 - r = j_0 - i_0 + d_{i_0}$ is the number of independent cycles. This means that the network contains d_{j_0} independent Wegscheider conditions.

Example 2.55 (Simple stoichiometric cycle)**.** To illustrate the above, we study a reaction network describing the random-order complex formation of the three compounds A, B and C (example from Ederer and Gilles [32]):

$$\begin{aligned} A + B &\overset{1}{\rightleftharpoons} AB, \qquad & AB + C &\overset{2}{\rightleftharpoons} ABC, \\ B + C &\overset{3}{\rightleftharpoons} BC, \qquad & A + BC &\overset{4}{\rightleftharpoons} ABC. \end{aligned} \tag{2.9}$$

This system contains the cycle:

$$\begin{tikzcd} A + B + C \arrow[r, harpoon, shift left, "1"] \arrow[d, harpoon, shift left, "3"] & AB + C \arrow[l, harpoon, shift left] \arrow[d, harpoon, shift left, "2"] \\ A + BC \arrow[r, harpoon, shift left, "4"] \arrow[u, harpoon, shift left] & ABC. \arrow[l, harpoon, shift left] \arrow[u, harpoon, shift left] \end{tikzcd} \tag{2.10}$$

The kinetic model of the system is

$$\begin{aligned} \dot{c}_A &= -J_1 - J_4, & \dot{c}_{AB} &= +J_1 - J_2, \\ \dot{c}_B &= -J_1 - J_3, & \dot{c}_{BC} &= +J_3 - J_4, \\ \dot{c}_C &= -J_2 - J_3, & \dot{c}_{ABC} &= +J_2 + J_4 \end{aligned} \tag{2.11}$$

with ideal mass-action laws with parameters $k_{\pm j}$ for the fluxes J_j. From the second law of thermodynamics and the principle of detailed balance it follows that the thermodynamic equilibrium with vanishing fluxes exists: $J_1 = J_2 = J_3 = J_4 = 0$. This leads to the condition that the product of the equilibrium constants along the cycle has to be unity:

$$\begin{aligned} &\frac{c_{eq,AB}}{c_{eq,A}\,c_{eq,B}} \frac{c_{eq,ABC}}{c_{eq,AB}\,c_{eq,C}} \frac{c_{eq,BC}\,c_{eq,A}}{c_{eq,ABC}} \frac{c_{eq,B}\,c_{eq,C}}{c_{eq,BC}} = \\ &\frac{k_{+1}}{k_{-1}} \qquad \frac{k_{+2}}{k_{-2}} \qquad \frac{k_{-4}}{k_{+4}} \qquad \frac{k_{-3}}{k_{+3}} = 1. \end{aligned} \tag{2.12}$$

For all other parameter combinations the model would describe a physically impossible system because it does not contain a state of thermodynamic equilibrium with vanishing fluxes. Because the model would never reach thermodynamic equilibrium, it would describe a physically impossible *chemical perpetuum mobile*. If it would be real, the permanent deviation from equilibrium could be used to permanently perform work.

Example 2.56 (Formation of Shc-Grb2-Sos complex)**.** The formation of protein complexes at a scaffold as described in the example above is an ubiquitous motif in cellular signal transduction. Sos and phosphorylated Shc bind to the scaffold Grb2 during EGF signal transduction. Kholodenko et al. [57] presented a model of EGF signaling that explicitly acknowledges the Wegscheider conditions. Schöberl et al. [90] extended this model but varied some parameter values without observance of detailed balance. Later, Liu et al. [71] analyzed the model of Schöberl et al. [90] but again modified parameter values without observance of the detailed balance constraints. This means that the latter two models describe a thermodynamically impossible system where permanent cyclic fluxes occur.

Schöberl et al. [90] as well as Liu et al. [71] perform a sensitivity analysis. This means that the response of the system to small changes in each single parameter is computed. Because the parameters are not independent from each other but related by the Wegscheider conditions, such a sensitivity analysis considers physically impossible parameter variations. Thus, the results may be misleading.

§ 2.57 (Complete stoichiometry)**.** The formulation of the Wegscheider conditions is only possible if the complete stoichiometry of the reactions is considered. Often ubiquitous compounds, as for example adenosine-triphosphate ATP, adenosine-diphosphate ADP and inorganic phosphate P, are omitted. This is kinetically justified, if one assumes a constant concentration of these compounds. Then, their effect can be described by omitting them from the stoichiometric matrix N and considering their effect in the kinetic rate laws. However, the simplified stoichiometric matrix cannot be used for deriving the generalized Wegscheider conditions. Assume that reaction 1 in Example 2.55 would involve the hydrolysis of ATP. Then, the true stoichiometry of reaction 1 is $A+B+ATP \rightleftharpoons AB+ADP+P$. If the concentrations of ATP, ADP and P are constant, the rate law of this reaction can be written as a mass-action law with parameters $\tilde{k}_{\pm 1}$, but these parameters depend on the concentrations of ATP, ADP and P. By clamping these concentrations an external thermodynamic force is imposed to the system. This force prevents the system from reaching the thermodynamic equilibrium because it drives a cyclic flux through the network. The cycle is driven by a permanent inflow of ATP and outflow of ADP and P. For this reason such cycles are called *futile cycles*. In steady state, the free energy dissipation of the cycle is equal to the amount of chemical work needed to rephosphorylate the produced ADP. The modified network does not anymore contain a true cycle and thus does not contain a Wegscheider condition. The Wegscheider conditions hold only for *true cycles* but not for *futile cycles*. The distinction of true from futile cycles requires the knowledge of the complete stoichiometry of the reactions. However, it does not require the knowledge of all reactions in the network because a cycle in a subnetwork is also a cycle in a larger network.

§ 2.58 (Wegscheider conditions in large networks)**.** The generalized Wegscheider conditions impose constraints on the physically possible parameters of kinetic models. The small Example 2.56 showed that this is indeed an issue for the modeling of cellular reaction networks. Larger biological networks show a high degree of flexibility and robustness and contain a large number of partly redundant pathways [98]. In such networks, the number of reactions j_0 tends to be much greater than the number of species i_0. This leads to a high $d_{j_0} = j_0 - i_0 + d_{i_0}$ and thus to a high number of Wegscheider conditions. The next two examples demonstrate this for signal transduction and metabolic networks.

Example 2.59 (Metabolic networks)**.** Reed et al. [86] provide a constraint-based model of the metabolism of *E. coli* K-12 iJR904. By means of this network, one can assess the importance of the Wegscheider conditions for metabolic networks (example from Ederer and Gilles [32]). The model iJR904 consist of $i_0 = 762$ compounds, 931 metabolic reactions and 1 reaction describing cell growth ($j_0 = 932$). The stoichiometric matrix $N \in \mathbb{R}^{762\times 932}$ has $r = \operatorname{rank}(N) = 722$, i. e. the network contains $d_{j_0} = j_0 - r = 210$ independent Wegscheider conditions. This means that $210/932 = 23\%$ of the equilibrium constants are determined by Wegscheider conditions and cannot be freely adjusted. An example is the cycle

$$2\,ADP + GTP \rightleftharpoons AMP + ATP + GTP \rightleftharpoons ADP + ATP + GDP \rightleftharpoons 2\,ADP + GTP$$

with guanosine-diphosphate GDP and guanosine-triphosphate GTP. If the Wegscheider conditions in the cycle would be violated, a permanent deviation of the ATP/ADP ratio from its equilibrium value would occur. This cycle would provide ATP for free, i. e. without the consumption of energy-rich substrates. This is a physically impossible situation. Thus, detailed balance in such cycles is crucial for building correct and meaningful models.

Only 451 reaction, i. e. $451/932 = 48\%$, do not participate in any cycle and are not affected by the Wegscheider conditions. If one of the other 52% of the equilibrium constants is changed, it is also necessary to change the equilibrium constants of further reactions. The affected equilibrium constants are possibly distributed over several functional units. This makes it very difficult to adjust the parameters of the model or to assess the effect of parameter changes because changing certain equilibrium constants in the model may have effects on equilibrium constants of many other reactions.

Example 2.60 (Protein-protein interaction networks)**.** Cellular signal transduction often relies on the interaction of proteins. The model-based analysis of signal transduction has a prominent role in systems biology because many diseases have their roots in defects of signal transduction. A typical motif is the binding of several proteins to a scaffold protein, e. g. the binding of extracellular hormones and intracellular signaling proteins to a transmembrane receptor. Example 2.55 discussed a simple system where two ligands A and C bind to the scaffold B. The present example generalizes considerations to scaffolds with more ligands. Stoichiometric cycles occur in such protein-protein interaction networks because a given complex can originate from a random order of association and dissociation events. We consider a scaffold protein with k binding sites for $\tilde{k}$ different ligand species (example adapted from [32]). We restrict the discussion to the cases where each binding site can only bind one specific ligand species, i. e. $\tilde{k} \leq k$. If $\tilde{k} < k$, one or more ligand species can bind to more than one binding site.

Since every binding site may be occupied or not occupied, the scaffold may form 2^k different complexes. Together with the free ligands we have $i_0 = 2^k + \tilde{k}$ species in the system. A ligand may bind to the scaffold if the corresponding binding site is free. Thus, we have 2^{k-1} distinguishable binding reactions per binding site. For k binding sites we have $j_0 = 2^k\, k/2$ reactions. The network contains $d_{i_0} = \tilde{k} + 1$ conserved moieties. Thus, the number of independent Wegscheider conditions is $d_{j_0} = j_0 - i_0 + d_{i_0} = (k/2 - 1)\, 2^k + 1$ (see §2.54, p. 34). Table 2.1 shows these numbers for different numbers of binding sites. In the table, it is assumed that the number of ligands $\tilde{k}$ is equal to the number of binding sites k. The number of reactions j_0 grows much faster than the number of species i_0 and the number of Wegscheider conditions grows exponentially with the number of binding sites.

Binding site numbers k as shown in Table 2.1 are realistic for large scaffolds. To illustrate this, we consider the protein Ste5p that plays a role in the mating response of the yeast [49]. The Ste5p dimer has at least $k = 7$ binding domains for $\tilde{k} = 4$ ligands. Thus, we have $d_{j_0} = 321$ Wegscheider conditions for $j_0 = 448$ different binding reactions. This means that $321/448 = 72\%$ of the equilibrium constants can be determined if the rest of the equilibrium constants is known. Any change of a reaction parameter in a model necessitates changes of parameters of other reactions because every reaction participates in a cycle. The Ste5P complex itself is only part of a larger complex. The relative number of cycles is presumably even higher for the larger complex.

§ 2.61 (Conclusions)**.** The Wegscheider conditions impose constraints on the physically feasible parameters of a kinetic model. For larger models it is getting increasingly difficult to determine consistent parameter values. Further, important model analysis tools that are based on the independent variation of parameter values may yield misleading results. A sensitivity analysis of the

$k = \tilde{k}$	1	2	3	4	5	6	7	8	9	10	...	$k, \tilde{k}$	...
i_0	3	6	11	20	37	70	135	264	521	1034	...	$2^k + \tilde{k}$	...
j_0	1	4	12	32	80	192	448	1024	2304	5120	...	$k/2 \cdot 2^k$	...
r	1	3	7	15	31	63	127	255	511	1023	...	$2^k - 1$	...
d_{i_0}	2	3	4	5	6	7	8	9	10	11	...	$\tilde{k} + 1$	...
d_{j_0}	0	1	5	17	49	129	321	769	1793	4097	...	$(k/2 - 1) \cdot 2^k$	...

Table 2.1.: Some numbers characterizing the binding of $\tilde{k}$ ligands to a scaffold with k binding sites (see Example 2.60): Numbers of species i_0, reactions j_0, conserved moieties d_{i_0} and stoichiometric cycles d_{j_0}; r is the rank of the stoichiometric matrix. For the numeric data, it is assumed that $k = \tilde{k}$.

model behavior towards independent perturbations of the parameter values is biased because the parameter values may not change independently. Similarly, parameter identification algorithms need to obey the Wegscheider conditions because otherwise they are likely to yield physically impossible parameter values. The explicit consideration of the Wegscheider conditions in the parameter identification is possible, but difficult. The Wegscheider conditions can be treated as equality constraints on the parameters. This turns the parameter identification problem in a constraint optimization problem that is considerably more difficult to solve. Another possibility is to distinguish between dependent and independent parameters (see e. g. Colquhoun et al. [26]). However, these calculations need to be redone for any structural modification of the model and lead to unintuitive dependencies of the parameters.

The Wegscheider conditions hinder the modeling of large metabolic networks in the conventional kinetic modeling formalism. In contrast to that, the TKM formalism that is developed in this work structurally guarantees the fulfillment of the Wegscheider conditions. It thus provides a possibility to avoid their explicit formulation and solution. For this reason, the TKM formalism is expected to be especially suited for the formulation of models of large networks.

In order to prepare the introduction of the TKM formalism in Chapter 5, the Chapters 3 and 4 develop the thermodynamic formalism for modeling and model reduction.

3. Thermodynamic Modeling

Kinetic models of reaction networks are usually described by kinetic rate equations $J_j(k, c)$ with concentrations c and kinetic parameters k. Such models disregard the existence of chemical potentials μ. Thus, it is not possible to directly model the thermodynamic condition that chemical potential differences $\Delta\mu$ determine the direction of reaction fluxes. To be nevertheless in accordance with the laws of thermodynamics, the kinetic parameters k have to obey the Wegscheider conditions (see Section 2.2.4, p. 33). Although the observance of the Wegscheider conditions and the usage of chemical potentials is equivalent (see §2.53), the Wegscheider conditions obstruct modeling and model analysis due to their 'non-local' nature: Wegscheider conditions are relations between kinetic parameters of different reactions possibly distributed over several functional modules. For this reason, parameters of a single reaction can often not be freely varied.

From a thermodynamic point of view, it is reasonable to formulate models that explicitly contain chemical potentials μ and chemical potential differences $\Delta\mu$. Fluxes J and driving forces $\Delta\mu$ are then related via the thermodynamic resistances R by the equation $R_j\, J_j = \Delta\mu_j$ (cf. Example 2.41 and §2.42, p. 31). However, such model formulations are usually only considered at or near equilibrium. The reason for this is the strong non-linear behavior of the thermodynamic resistances R_j far from equilibrium.

This chapter nevertheless introduces a formal approach to thermodynamic modeling of open reaction networks that are possibly far from equilibrium. Later, Chapter 4 shows that important model reduction techniques can be easily applied to such models using linear transformations. Due to its complexity this approach can be hardly used for larger networks. However, these considerations give us valuable insight of how to develop thermodynamically consistent, reduced-order models. In Chapter 5 the approach will be modified in order to arrive at the TKM formalism, a more handy modeling formalism that is suited for the modeling of large networks.

We describe the dynamics of an open reaction system by its stoichiometric matrices, the relation of concentrations c and chemical potentials μ, the state-dependent matrix of thermodynamic resistances R and initial conditions. This formal representation is the basis for the derivation of general mechanisms for manipulating these models by state space transformations in Chapter 4. In certain cases, we are able to transform the original system description in a form that directly allows applying model reduction techniques. Then a smaller system description with equivalent dynamics can be derived.

For the sake of simplicity, we consider only homogeneous phases with constant temperature T, pressure p and volume V. Consequently, we suppress the dependency of the occurring functions from these quantities. Later on, we show that the approach can be extended to heterogeneous systems consisting of homogeneous subsystems with varying volumes.

3.1. Formal Definition

This section introduces a formal definition of thermodynamic models by a tuple of matrices and functions. Transformation and reduction of thermodynamic models can then be formulated as manipulations of this tuple (Chapter 4).

Definition 3.1 (Thermodynamic model of a reaction system)**.** A *thermodynamic model* M of a reaction system with i_0 internal compounds, j_0 internal fluxes, $i_{0,e}$ compounds with clamped chemical potentials μ_e and $j_{0,e}$ clamped fluxes J_e is characterized by the stoichiometric matrices $N \in \mathbb{R}^{i_0 \times j_0}$, $N_e \in \mathbb{R}^{i_0 \times j_{0,e}}$, $S \in \mathbb{R}^{i_{0,e} \times j_0}$, the functions $c(\mu, \mu_e) \in \mathbb{R}^{i_0}$, $R(\mu, \mu_e) \in \mathbb{R}^{j_0 \times j_0}$ and the initial chemical potentials $\mu_0 \in \mathbb{R}^{i_0}$. For the functions $c(\mu, \mu_e)$ and $R(\mu, \mu_e)$ we demand:

$$\begin{aligned} R(\mu,\mu_e) &= R^T(\mu,\mu_e), & R(\mu,\mu_e) &\geq 0, \\ \left(\frac{\partial c}{\partial \mu}(\mu,\mu_e)\right) &= \left(\frac{\partial c}{\partial \mu}(\mu,\mu_e)\right)^T, & \frac{\partial c}{\partial \mu}(\mu,\mu_e) &\geq 0 \end{aligned}$$

for all $\mu \in \mathbb{R}^{i_0}$ and $\mu_e \in \mathbb{R}^{i_{0,e}}$. The environment is described by the clamped chemical potentials $\mu_e(t) \in \mathbb{R}^{i_{0,e}}$ and the clamped fluxes $J_e(t) \in \mathbb{R}^{j_{0,e}}$. The model equations read

$$\dot{c} = N\,J + N_e\,J_e, \qquad \Delta\mu = -N^T \mu - S^T \mu_e, \qquad c = c(\mu,\mu_e), \qquad R(\mu,\mu_e)\,J = \Delta\mu \tag{3.1}$$

with $\mu(0) = \mu_0$. We formally characterize a thermodynamic model of a reaction system by the tuple $M = [N, S, N_e, c(\mu,\mu_e), R(\mu,\mu_e), \mu_0]$.

§ 3.2 (Dimensions)**.** The quantities in the above definition have the following dimensions: c [mol L^{-1}], J, J_e [mol L^{-1} s^{-1}], $\mu, \mu_e, \Delta\mu$ [J mol^{-1}] and R [J L s mol^{-2}].

§ 3.3 (Symmetry and positive semi-definiteness of R and $\partial c/\partial\mu$)**.** The symmetry of the matrix $\partial c/\partial\mu$ follows from the symmetry of the Hessian of the Gibbs energy. Its positive semi-definiteness ($\partial c/\partial\mu \geq 0$) is related to the condition for diffusive stability. These conditions will be discussed in Section 3.2. The symmetry of the matrix R is related to the Onsager reciprocity relations. Its positive semi-definiteness ($R \geq 0$) follows from the non-negativity of the entropy production. These conditions are discussed in Section 3.3.

§ 3.4 (Three submodels)**.** The model equations presented in Definition 3.1 consist of three parts: The equations $\dot{c} = N\,J + N_e\,J_e$ and $\Delta\mu = -N^T\,\mu - S^T\,\mu_e$ describe the stoichiometry of the network. The function $c = c(\mu,\mu_e)$ describes the thermodynamic properties of the compounds. The equation $R(\mu,\mu_e)\,J = \Delta\mu$ describes the kinetics of the reactions.

§ 3.5 (ODE model)**.** The model in Definition 3.1 is formally given as a differential-algebraic equation system. If the matrices $R(\mu, \mu_e)$ and $\partial c/\partial\mu$ are invertible, the model equations can be easily expressed as an ordinary differential equation system:

$$\frac{\partial c}{\partial \mu}\,\dot{\mu} + \frac{\partial c}{\partial \mu_e}\,\dot{\mu}_e = -N\,R^{-1}\,N^T\,\mu - N\,R^{-1}\,S^T\,\mu_e + N_e\,J_e$$

or equivalently

$$\dot{\mu} = \left(\frac{\partial c}{\partial \mu}\right)^{-1}\left(-N\,R^{-1}\,N^T\,\mu - N\,R^{-1}\,S^T\,\mu_e + N_e\,J_e - \frac{\partial c}{\partial \mu_e}\,\dot{\mu}_e\right).$$

These representations of the model equations can be used as simulation equations.

Definition 3.6 (Closed and open system)**.** If $i_{0,e} = j_{0,e} = 0$ we say that M is *closed*, otherwise M describes an *open* system. In closed systems no mass fluxes cross the system boundary.

§ 3.7 (Systems theoretical interpretation of clamped variables)**.** The quantities $J_e(t)$ and $\mu_e(t)$ are clamped in the system. This means, they are assumed to be determined by an attached unmodeled system. This does not mean that they are necessarily constant. Physically, these quantities characterize the boundary conditions of the system. From a systems theoretical point of view, they are inputs of the system. If M is closed, the model equations are autonomous.

§ 3.8 (Block diagram)**.** Figure 3.1 shows a simplified block diagram of a thermodynamic model. The block diagram stresses the linear aspects of the model equations. The non-linear dynamics originate from the state dependency of the resistance matrix $R(\mu, \mu_e)$ and the function $c(\mu, \mu_e)$.

§ 3.9 (Clamped compounds and fluxes in reaction equations)**.** For the rest of this thesis, external compounds with clamped chemical potentials are indicated by brackets around the respective compounds in the reaction equation. For example, the reaction equation $A \rightleftharpoons B \rightleftharpoons (C)$ indicates that the chemical potential of C is clamped. Clamped fluxes are indicated by brackets around the flux variable in the reaction equations. The reactants or products of clamped reactions are often not part of the model. Unmodeled reactants or products are denoted by a zero in the reaction equation. For example, in the reaction equation $0 \overset{(J_e)}{\rightleftharpoons} A \rightleftharpoons B$ the flux J_e is clamped and has no internal reactant.

§ 3.10 (Differences to classical linear thermodynamics)**.** If the resistance matrix R is constant, the above definition is formally similar to a system description in linear irreversible thermodynamics (see Haase [42], Onsager [75, 76]). However, Definition 3.1 comprises a larger model class than is normally considered in linear irreversible thermodynamics. Linear thermodynamics is basically a black-box theory that works with the minimal number of fluxes that is necessary to describe the changes of the state variables. It does not require the knowledge of the underlying physical mechanism. Thus, a flux J_j does not necessarily describe a physical process that can be studied independently of other processes in the system. Consequently, it may be driven not

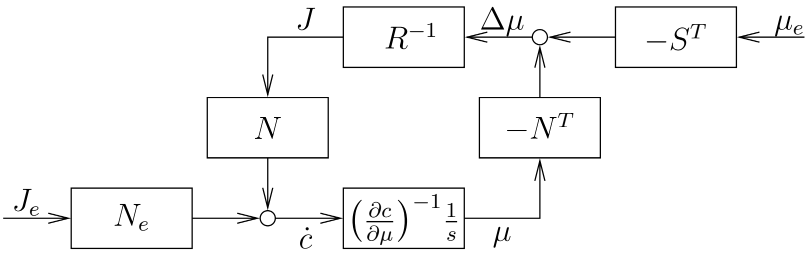


Figure 3.1.: Block diagram of the equations of a thermodynamic model. The blocks represent linear matrix multiplications. The term $1/s$ indicates integration over time. For the sake of simplicity, it is assumed that c depends only on μ but not on μ_e. The non-linear dependency of R and $\partial c/\partial \mu_e$ on μ and μ_e is not depicted.

only by its adjoint force $\Delta\mu_j$, but by all other forces of the same tensorial degree. Since we are interested in a setup allowing us to incorporate mechanistic knowledge, we will initially assume that the stoichiometric matrix N describes thermodynamically independent processes. This means that a flux is only driven by its adjoint forces and R is diagonal. If $\mathrm{null}(N) \neq \{\}$, cyclic flux distributions $J_{cycle} \in \mathrm{null}(N)$ that have no effect on the state c are stoichiometrically but not thermodynamically possible, and thus the flux vector J is not minimal.

Example 3.11 (Stoichiometric cycle)**.** The following example of a closed network will clarify the above remark:

$$A \overset{1}{\rightleftharpoons} B \overset{2}{\rightleftharpoons} C \overset{3}{\rightleftharpoons} A.$$

Sufficiently close to equilibrium, the resistance matrix R can assumed to be constant. The stoichiometric matrix can be compiled directly from the reaction network and the corresponding resistance matrix is diagonal:

$$N = \begin{pmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix}, \qquad R = \begin{pmatrix} R_1 & 0 & 0 \\ 0 & R_2 & 0 \\ 0 & 0 & R_3 \end{pmatrix}. \tag{3.2}$$

The system contains one cyclic flux distribution with $J_1 = J_2 = J_3$ that does not influence $\dot{c}$. Thus, the minimal number of fluxes that is necessary to describe $\dot{c}$ is two (and not three) and a model of classical linear thermodynamics would read for example:

$$\tilde{N} = \begin{pmatrix} -1 & 0 \\ 1 & -1 \\ 0 & 1 \end{pmatrix}, \qquad \tilde{R} = \begin{pmatrix} \tilde{R}_1 & \tilde{R}_{12} \\ \tilde{R}_{12} & \tilde{R}_2 \end{pmatrix}. \tag{3.3}$$

Here, $\tilde{J}_1$ and $\tilde{J}_2$ do not only represent the fluxes through the reactions 1 and 2, respectively, but also through reaction 3: $\tilde{J}_1 = J_1 - J_3$ and $\tilde{J}_2 = J_2 - J_3$. For this reason the matrix $\tilde{R}$ is not diagonal. However, it has to be symmetric, in order to meet the reciprocity conditions (see §2.42, p. 31). By a transformation and reduction step, the models in Equation 3.2 and Equation 3.3 can be transformed into each other. This will be discussed in Corollary 4.2 (p. 52).

3.2. Thermodynamic Submodel

The function $c(\mu, \mu_e)$ defines the thermodynamic properties of the system. When writing down Equation 3.1, we implicitly assumed that all chemical potentials μ are independent. In particular, we assumed that in principle arbitrary initial conditions for μ can be specified. This section discusses under what conditions this is true. Further, it is shown that for closed systems the Gibbs energy can be reconstructed from the function $c(\mu)$. Finally, we give expressions for $c(\mu, \mu_e)$ and the Gibbs energy for ideal dilute solutions.

§ 3.12 (Gibbs-Duhem equation – dependencies between intensive variables)**.** The Gibbs-Duhem relation ($0 = S\,dT - V\,dp + n^T\,d\mu$, §2.32, p. 29) is a differential form of an equation relating intensive state variables. That means that at given T and p a system cannot attend arbitrary chemical potentials μ. The feasible chemical potentials μ lie on an $(i_0 - 1)$-dimensional set. The same is true for the concentrations c. Thus, the Gibbs-Duhem equation would complicate the further considerations because one cannot describe the composition of a phase with i_0 compounds by i_0 independent state variables. However, in a highly diluted solution consisting of $i_0 - 1$ solutes and one solvent (e. g. water), concentrations and chemical potentials of solutes can vary freely because it can be assumed that the solvent's concentration and chemical potential balance the Gibbs-Duhem equation. If not stated otherwise, we will always assume that the modeled chemical potentials μ and thus also the concentrations c are independent because we consider a diluted solution.

§ 3.13 ($c(\mu, \mu_e)$ vs $\mu(c, c_e)$)**.** In Definition 3.1 the function $c(\mu, \mu_e)$ is used. Traditionally, the relation of concentrations and chemical potentials is stated in the form $\mu(c, c_e)$. Both representations are equivalent because c and μ both characterize the composition of the system uniquely. For our purposes, the use of functions $c(\mu, \mu_e)$ is advantageous over $\mu(c, c_e)$ because the former allows for the easy application of the rapid equilibrium assumption for model reduction. We will discuss this in detail later on.

§ 3.14 (Symmetry and positive semi-definiteness of $\partial c/\partial\mu$)**.** The matrix $\partial\mu/\partial n = \partial^2 G/\partial n^2$ is the Hessian of the Gibbs energy $G(n)$. For this reason it is symmetric. A sufficient condition for the local diffusive stability of an isothermal and isobaric, homogeneous phase is the positive definiteness of the Hessian of the molar Gibbs energy: $\partial^2 \bar{G}/\partial x^2 > 0$ with the molar Gibbs energy $\bar{G}(T, p, x) = G(T, p, n)/n_\Sigma$, the vector of mole fractions of the solutes $x = n/n_\Sigma$, the total mole number $n_\Sigma = \sum_{i=1}^{i_0} n_i + n_s$ and the mole number of the solvent n_s (see [43, §42, p. 161] and [41, Chapter IV, p. 47]). In a diluted solution with $n_\Sigma \approx n_s \approx$ const and constant volume, this yields $\partial\mu/\partial c > 0$ with $c = n/V$. Because the inverse of a symmetric and positive definite matrix is symmetric and positive definite, the matrix $\partial c/\partial\mu = (\partial\mu/\partial c)^{-1}$ is symmetric and positive definite in stable phases. As a limit case, this work also considers only semi-definite matrices $\partial c/\partial\mu \geq 0$.

§ 3.15 (Gibbs energy in a closed system)**.** In a diluted solution of constant volume, the chemical potentials μ_i are the partial derivatives $\partial g/\partial c_i = \partial G/\partial n_i$ of the volume-specific Gibbs energy

$g(c) = V^{-1}\, G(n)$ (see §2.33, p. 29). Thus, in a closed system, the function $c(\mu)$ defines a partial-differential equation for the Gibbs energy of the compounds $g(\mu)$ in dependence of their chemical potentials:

$$dg = \mu^T\, dc = \mu^T\, \frac{\partial c}{\partial \mu}\, d\mu$$

This equation is integrable because $\partial c/\partial \mu$ is symmetric.

§ 3.16 ($c(\mu, \mu_e)$ in ideal dilute solutions)**.** Ideal dilute solutions (Definition 2.44, p. 31) are characterized by

$$\mu_i = \mu_i^\circ + R^*\, T\, \log(c_i/c^\circ)$$

and thus we have

$$c_i(\mu_i) = c^\circ\, \exp\left(\frac{-\mu_i^\circ}{R^*\, T}\right)\, \exp\left(\frac{\mu_i}{R^*\, T}\right).$$

The matrix $\partial c/\partial \mu$ is diagonal with positive diagonal elements:

$$\frac{\partial c_i}{\partial \mu_i} = c^\circ \exp\left(\frac{-\mu_i^\circ}{R^*\, T}\right)\, \exp\left(\frac{\mu_i}{R^*\, T}\right)\, \frac{1}{R^*\, T} = \frac{c_i}{R^*\, T}.$$

§ 3.17 (Gibbs energy in ideal dilute solutions)**.** The Gibbs energy in ideal dilute solutions g is given by

$$dg = \sum_{i=1}^{i_0} \underbrace{\underbrace{(\mu_{i_0} + R^*\, T \log(c_i/c^\circ))}_{\mu_i}\, dc_i}_{dg_i},$$

where dg_i is the change of the Gibbs energy associated with compound i. Since dg_i depends only on c_i, we can integrate the summands individually. Thus, with every compound a part of the Gibbs energy is directly associated:

$$g_i(c_i) = c_i\left(\mu_i^\circ + R^*\, T\left(\log\left(\frac{c_i}{c^\circ}\right) - 1\right)\right), \qquad g_i(\mu_i) = c^\circ\, \exp\left(\frac{\mu_i - \mu_i^\circ}{R^*\, T}\right)\, (\mu_i - R^*\, T).$$

We get

$$g(c) = \sum_{i=1}^{i_0} g_i(c_i), \qquad g(\mu) = \sum_{i=1}^{i_0} g_i(\mu_i).$$

Note that these expressions are not consistent with the Euler relation $g = \sum_{i=1}^{i_0} \mu_i\, c_i$, which is a consequence of the homogeneity of $G(n)$. In the present setting $G(n)$ is not homogeneous because we neglect the solvent and assume a constant overall concentration. A doubling of the amount of only the solutes is not equivalent to the doubling of G.

3.3. Kinetic Submodel

The equation $R(\mu, \mu_e)\, J = \Delta\mu$ defines the kinetics of the reactions. The following subsections show how to calculate the entropy production of the reactions and discuss the properties of $R(\mu, \mu_e)$. Further, they derive an explicit expression for $R_j(\mu, \mu_e)$ of a generalized mass-action kinetics.

3.3.1. Entropy Production

§ 3.18 (Entropy production and dissipation of Gibbs energy)**.** The density of entropy production $\sigma[s]$ (see §2.35, p. 30) by the fluxes J is given by

$$T\,\sigma[s] = -\sigma[g] = \Delta\mu^T J = J^T R(\mu, \mu_e)\, J = \Delta\mu^T R(\mu, \mu_e)^{-1} \Delta\mu^T \geq 0. \tag{3.4}$$

The last expression is only defined if $R(\mu, \mu_e)$ is invertible. The function $\sigma[s]$ describes only the entropy production due to the fluxes J. The overall entropy production is larger because the clamped fluxes J_e and the clamping of the chemical potentials μ_e also produces entropy. However, from the information present in a thermodynamic model M, one cannot derive an expression for this external entropy production.

§ 3.19 (Positive semi-definiteness of $R(\mu, \mu_e)$)**.** The condition that the entropy production $\sigma[s]$ is always non-negative is a constraint on the resistance matrix $R(\mu, \mu_e)$ because we have that

$$T\,\sigma[s] = \Delta\mu^T R(\mu, \mu_e)^{-1} \Delta\mu^T \geq 0$$

for all μ and μ_e. Positive semi-definiteness as required by Definition 3.1 is sufficient, but not necessary for $\sigma[s] \geq 0$. Both, $\Delta\mu$ and $R(\mu, \mu_e)$, depend on μ and μ_e, and thus $\sigma[s]$ may be non-negative for all μ and μ_e even if $R(\mu, \mu_e)$ is not positive semi-definite for all μ and μ_e. However, positive definiteness of $R(\mu, \mu_e)$ is a reasonable assumption that is valid for all models we will study.

§ 3.20 (Symmetry of $R(\mu, \mu_e)$)**.** From Onsager's linear, near-equilibrium theory of thermodynamic systems we know that the resistance matrix at an equilibrium point $(\mu_{eq}, \mu_{e,eq})$ with $\Delta\mu_{eq} = 0$ is symmetric

$$R(\mu_{eq}, \mu_{e,eq}) = R^T(\mu_{eq}, \mu_{e,eq})$$

These conditions follows from the principle of microscopic reversibility [75, 76]. Symmetry of $R(\mu, \mu_e)$ for non-equilibrium states μ and μ_e is not necessary. However, we will study only examples with a symmetric $R(\mu, \mu_e)$.

§ 3.21 (Diagonal $R(\mu, \mu_e)$)**.** If the fluxes J are thermodynamically independent, the matrix $R(\mu, \mu_e)$ is diagonal. This can be assumed if the fluxes J describe sufficiently elementary processes. In particular, if the fluxes J describe elementary reactions or simple composed reactions, the matrix $R(\mu, \mu_e)$ is diagonal and thus symmetric. In this case, all diagonal elements $R_j(\mu, \mu_e)$ have to be non-negative, which leads to $R(\mu, \mu_e) \geq 0$. All models M that we will study originate from a description with diagonal $R(\mu, \mu_e)$. Later, we will study transformations and reductions of these models leading to non-diagonal resistance matrices $R(\mu, \mu_e)$. As will be shown, positive definiteness and symmetry of $R(\mu, \mu_e)$ is conserved under transformation and reduction. All models that we will consider, describe on some level of detail a system of thermodynamically independent processes. Such models have a symmetric and positive definite resistance matrix. For this reason, we restrict our considerations to this system class.

3.3.2. Resistances of Generalized Mass-Action Kinetics

Linear flow-force relationships are only valid in the vicinity of thermodynamic equilibrium. For systems far from equilibrium, the proportionality factor of thermodynamic force and flow – the thermodynamic resistance – is not constant. Chemical reactions often proceed in the non-linear region [23, Section 14.3]. In the following paragraphs we derive an expression for the thermodynamic resistance of a generalized mass-action kinetics that is also valid far from equilibrium. We give this resistance in dependence of the chemical potentials of reactants, products and possible effectors.

Consider a reaction

$$\sum_{i \in E} \nu_{E,i}\, X_i \rightleftharpoons \sum_{i \in P} \nu_{P,i}\, X_i.$$

Assume that its velocity can be described by a generalized mass-action law

$$J = f(k, c) \left(k_+ \prod_{i \in E} \left(\frac{c_i}{c^\circ} \right)^{\nu_{E,i}} - k_- \prod_{i \in P} \left(\frac{c_i}{c^\circ} \right)^{\nu_{P,i}} \right) \tag{3.5}$$

with $f(k, c) \geq 0$. The thermodynamic force

$$\Delta\mu = \underbrace{\sum_{i \in E} \nu_{E,i}\, \mu_i}_{\Delta\mu_E} - \underbrace{\sum_{i \in P} \nu_{P,i}\, \mu_i}_{\Delta\mu_P} \tag{3.6}$$

consists of forces exerted by the reactants $\Delta\mu_E$ and products $\Delta\mu_P$. Generalized mass-action kinetics allow the definition of equilibrium constants $K_{eq} = k_+/k_-$ that are independent of the concentrations. Thus, kinetic laws of this kind are consistent with ideal dilute solutions with concentration-independent standard Gibbs reaction energies ΔG° (see §2.51, p. 33). Assuming an ideal dilute solution with $c_i(\mu_i) = c^\circ\, \exp((\mu_i - \mu_i^\circ)/(R^*\, T))$ we get

$$J = \tilde{f}(k,\mu)\Bigg(k_+ \overbrace{\prod_{i\in E} \exp\left(-\frac{\nu_{E,i}\,\mu_i^\circ}{R^*\,T}\right)}^{\tilde{k}_+} \overbrace{\prod_{i\in E} \exp\left(\frac{\nu_{E,i}\,\mu_i}{R^*\,T}\right)}^{F_E} \\ - k_- \underbrace{\prod_{i\in P} \exp\left(-\frac{\nu_{P,i}\,\mu_i^\circ}{R^*\,T}\right)}_{\tilde{k}_-} \underbrace{\prod_{i\in P} \exp\left(\frac{\nu_{P,i}\,\mu_i}{R^*\,T}\right)}_{F_P} \Bigg)$$

where $\tilde{f}(k,\mu) = f(k, c(\mu))$. The equilibrium constant K_{eq} can be computed either from $J = 0$ or from $\Delta\mu = 0$. The former variant leads to $K_{eq} = k_+/k_-$, the latter leads with $\mu_i = \mu_i^\circ + R^*\,T\,\log(c_i/c^\circ)$ to $K_{eq} = \tilde{k}_-/\tilde{k}_+$. Since both variants are equivalent, we have that $\tilde{k} := k_+\,\tilde{k}_+ = k_-\,\tilde{k}_-$. Thus, we get for the thermodynamic resistance:

$$R = \frac{\Delta\mu}{J} = \underbrace{\frac{R^*\,T}{\tilde{f}(k,\mu)\,\tilde{k}}}_{\rho(k,\mu)} \underbrace{\frac{\sum_{i\in E}\frac{\nu_{E,i}\,\mu_i}{R^*\,T} - \sum_{i\in P}\frac{\nu_{P,i}\,\mu_i}{R^*\,T}}{\exp\left(\sum_{i\in E}\frac{\nu_{E,i}\,\mu_i}{R^*\,T}\right) - \exp\left(\sum_{i\in P}\frac{\nu_{P,i}\,\mu_i}{R^*\,T}\right)}}_{R_\circ(\Delta\mu_E/(R^*\,T),\,\Delta\mu_P/(R^*\,T))}$$

with $\Delta\mu_E$ and $\Delta\mu_P$ as in §2.38 (p. 30). This is a general expression for the resistance of a generalized mass-action kinetics. It consists of two factors: (1) $R_\circ(\Delta\mu_E/(R^*\,T), \Delta\mu_P/(R^*\,T))$ describes the ideal mass action behavior and (2) $\rho(k,\mu) > 0$ describes deviations from the ideal mass action behavior. To simplify the notation, we introduce the ideal resistance function $R_\circ(x_E, x_P)$ that describes an ideal mass action resistance.

Definition 3.22 (Ideal resistance function)**.** The *ideal resistance function* $R_\circ(x_E, x_P)$ is given by

$$R_\circ(x_E, x_P) = \frac{x_E - x_P}{\exp(x_E) - \exp(x_P)} \qquad \text{for } x_E \neq x_P.$$

For $x_E = x_P$ the ideal resistance is defined as the limit of the above expression:

$$R(x_E, x_P) = \lim_{x_E \to x_P} R_\circ(x_E, x_P) = \exp(-x_E) = \exp(-x_P) \qquad \text{for } x_E = x_P.$$

The arguments $x_E = \Delta\mu_E/(R^*\,T)$ and $x_P = \Delta\mu_P/(R^*\,T)$ are proportional to the thermodynamic forces exerted by products and reactants, respectively.

Example 3.23 (Mass-action kinetics)**.** Consider the reaction $A \rightleftharpoons B$ in an ideal dilute solution with an ideal mass-action kinetics: $J = k_+\, c_A/c^\circ - k_-\, c_B/c^\circ$. We have $\tilde{f}(k,\mu) = 1$ and $\tilde{k} = k_+\,\tilde{k}_+ = k_+\, \exp(-\mu_A^\circ/(R^*\,T))$. The thermodynamic resistance is given by $R = \rho\, R_\circ(\mu_A/(R^*\,T), \mu_B/(R^*\,T))$ where $\rho = R^*\,T/(\tilde{f}(k,\mu)\,\tilde{k}) = R^*\,T\,k_+^{-1}\, \exp(\mu_A^\circ/(R^*\,T))$ is positive and independent of μ_A and μ_B.

Example 3.24 (Reversible Michaelis-Menten kinetics)**.** Consider the reaction $A \rightleftharpoons B$ in an ideal dilute solution with a reversible Michaelis-Menten kinetics given by $J = (k_+ \, c_A/c^\circ - k_- \, c_B/c^\circ)/(k_0/c^\circ + k_A \, c_A/c^\circ + k_B \, c_B/c^\circ)$. Its thermodynamic resistance is

$$R = \left(\rho_0 + \rho_A \, \exp\left(\frac{\mu_A}{R^* \, T}\right) + \rho_B \, \exp\left(\frac{\mu_B}{R^* \, T}\right)\right) \cdot R_\circ \left(\frac{\mu_A}{R^* \, T}, \frac{\mu_B}{R^* \, T}\right)$$

where $\rho_0 = \hat{\rho} \, k_0$, $\rho_A = \hat{\rho} \, k_A$, $\rho_B = \hat{\rho} \, k_B$ and $\hat{\rho} = R^* \, T \, k_+^{-1} \, \exp(\mu_A^\circ/(R^* \, T))$.

§ 3.25 (Plots of $\Delta\mu$, $R_\circ$ and $J_\circ$)**.** Even an ideal mass-action resistance depends non-linearly on the chemical potentials of the reactants and products. To study this dependency, we consider the functions $\Delta x = \Delta\mu/(R^* \, T) = x_E - x_P$, $R_\circ(x_E, x_P)$ and an ideal mass action flux $J_\circ = \Delta x / R_\circ(x_E, x_P)$ with $\rho(k, \mu) = 1$ in dependency of $x_E = \Delta\mu_E/(R^* \, T)$ and $x_P = \Delta\mu_P/(R^* \, T)$ that are proportional to the forces exerted by reactants and products. The first row of Figure 3.2 shows the contour plots of these functions for small x_E and x_P, i. e. the near-equilibrium behavior. The second row shows the same functions for a larger interval of x_E and x_P. The ideal resistance $R_\circ$ is symmetric with respect to the line of equilibrium with $x_E = x_P$. Only very close to equilibrium the isolines of $J_\circ$ follow the parallel and straight isolines of Δx. Far from equilibrium, the form of the isolines of $J_\circ$ is dominated by the almost right-angled isolines of $R_\circ$. This means that for $x_E \gg x_P$ the resistance $R_\circ$ decreases and the flux $J_\circ$ increases with growing x_E, but is relatively insensitive to changes in x_P. The third row shows Δx, $R_\circ$ and $J_\circ$ over the extent of an example reaction. The linear behavior of $J_\circ$ of the example reaction in dependence of the concentrations is produced by the interaction of a nonlinear Δx with a nonlinear $R_\circ$. Observe that $R_\circ$ goes to infinity for low and high reaction extents, i. e. very low and high forces $\Delta\mu$. Thus, the finite reaction rate for the limit cases is due to a division of '∞/∞'. These considerations confirm that far from equilibrium the non-linearity of the thermodynamic resistance plays a major role and usually cannot be neglected.

3.4. Extension to Heterogeneous Systems

For the sake of simplicity, the above considerations were restricted to well-mixed homogeneous systems of constant volume. Making this simplification we were able to describe the system by the intensive variables μ [J mol^{-1}] and $\Delta\mu$ [J mol^{-1}] and the volume-specific variables c [mol L^{-1}] and J [mol L^{-1} s^{-1}]. The fluxes J are related via the resistances $R(\mu, \mu_e)$ with dimension [J mol^{-2} L s] to the forces $\Delta\mu$ and thus to the chemical potentials μ. The conductances $(R(\mu, \mu_e))^{-1}$ with the dimension [J^{-1} mol^2 L^{-1} s^{-1}] are volume-specific quantities.

The approach can be easily extended to heterogeneous systems consisting of several well-mixed homogeneous subsystems with varying volume. The state of such a system can be characterized by the vector (μ, V) where V is the vector of the volumes of the homogeneous subsystems. Here, chemical species occurring in more than one subsystem have a different chemical potential for each subsystem. Instead of the specific function $c(\mu, \mu_e)$ we need to use the extensive function $n(V, \mu, \mu_e)$. Instead of the specific fluxes J we need to use absolute fluxes $\breve{J}$ [mol s^{-1}]. We define

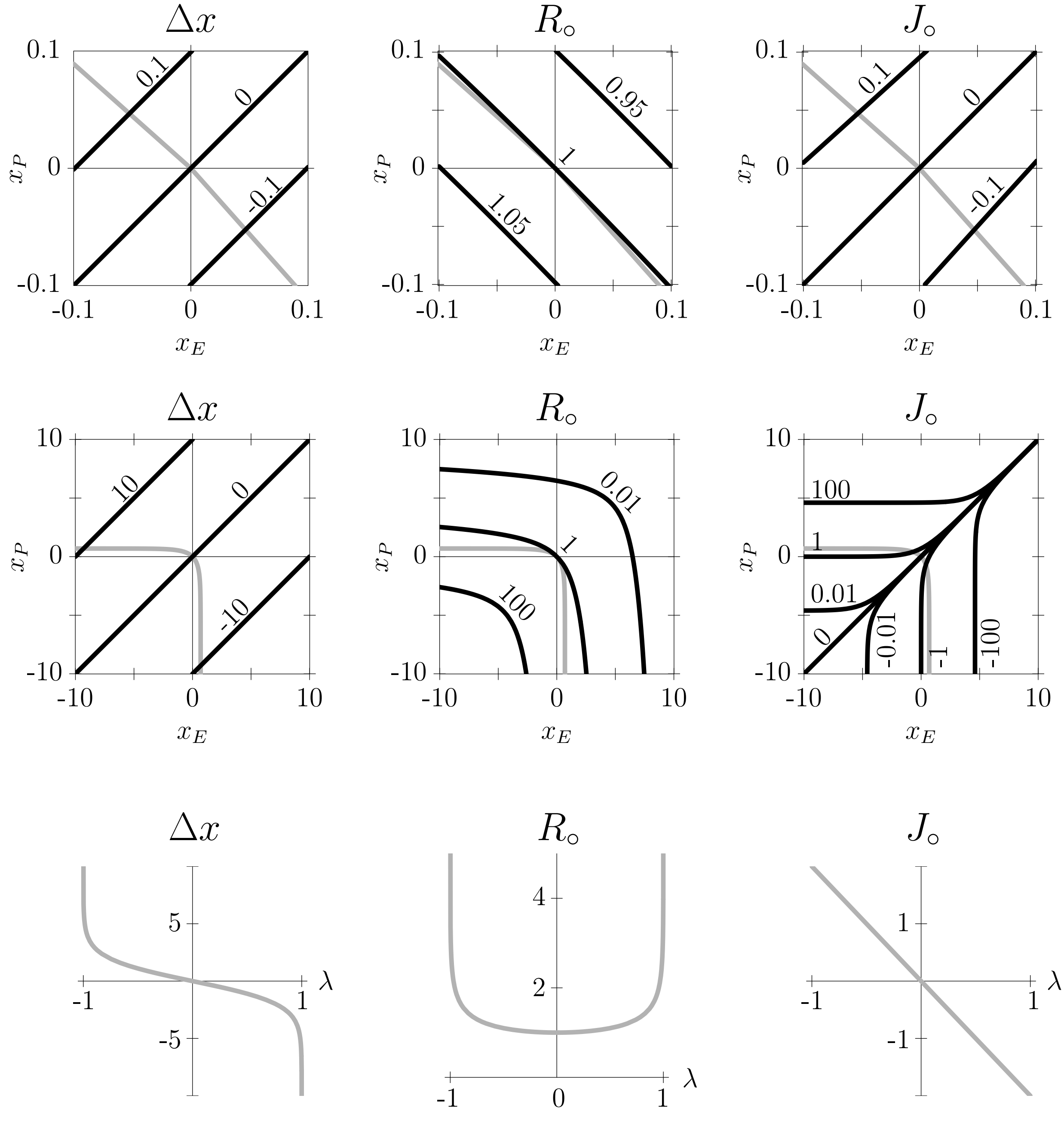


Figure 3.2.: Plots of $\Delta x = \Delta\mu/(R^* T)$, the ideal resistance functions $R_\circ(x_E, x_P)$ and an ideal mass action flux $J_\circ = \Delta x/R_\circ$. The black curves in the first and second row show isolines of the functions in the x_E-x_P-plane. While the second row shows a larger area of the x_E-x_P-plane and thus includes far-from-equilibrium situations, the first row focuses on small x_E and x_P and thus shows only the near-equilibrium behavior. The gray curve shows the trajectory of a reaction $A \rightleftharpoons B$ with $\mu_A^\circ = \mu_B^\circ = 0$. The parameter $-1 < \lambda < 1$ with $c_A = 1 - \lambda$, $c_B = 1 + \lambda$ describes the deviation of the reaction from equilibrium $c_{A,eq} = c_{B,eq} = 1$. The third row shows plots of Δx, $R_\circ$ and $J_\circ$ over λ.

the absolute resistances $\breve{R}(V, \mu, \mu_e)$ [J mol^{-2} s] such that $\breve{R}\,\breve{J} = \Delta\mu$. In order to formulate an equation for $\dot{V}$ we also need the functions $v(n)$ describing the partial molar volumes of the system such that $\dot{V} = v(n)\,\dot{n}$. The functions $v(n)$ can be computed from the function $V(n)$ by differentiation $v(n) = \partial V/\partial n$. If the volumes V also depend on further quantities such as e. g. the clamped potentials μ_e or if they are explicitly time dependent, this function has to be adapted.

The extended model equations read

$$\begin{aligned} \dot{n} &= N\,\breve{J} + N_e\,\breve{J}_e, & \dot{V} &= v(n)\,\dot{n}, \\ \Delta\mu &= -N^T\mu - S^T\mu_e, & n &= n(V, \mu, \mu_e), \\ \breve{R}(V, \mu, \mu_e)\,\breve{J} &= \Delta\mu. \end{aligned} \tag{3.7}$$

For the development of the transformation and reduction methods in the following sections, we will assume a homogeneous phase of constant volume. The consideration of heterogeneous systems with varying volumes only increases the complexity of notation and thus the length and number of equations but does not introduce new relevant aspects. We will come back to the above sketched generalized system description when discussing the implementation in the computer algebra system *Mathematica* (Appendix A, p. 185).

4. Transformation and Reduction

Transformations are an important tool in mathematical systems theory. They allow studying systems independent of the specific choice of coordinates made during the modeling process. In particular, transformations can be used to bring system equations to a form that allows for a simple analysis (e. g. stability, observability, controllability) or application of certain algorithms (e. g. model reduction, computation of minimal realization). We will see that a framework for transformation of thermodynamic models provides the basis for model reduction.

4.1. Transformations

From a mathematical point of view, arbitrary transformations of the model variables c, J, μ and $\Delta\mu$ are possible. However, this section introduces transformations that retain the thermodynamic interpretation of the model equations. In particular, this means that the transformed equations of a thermodynamic model M can be expressed as a transformed thermodynamic model $\hat{M}$. Model reduction methods defined on the basis of these transformations preserve the physical interpretability and validity of the models. The following Section 4.1.1 considers linear transformations of the model variables c, J, μ and $\Delta\mu$, i. e. the transformed model variables are linear combinations of the original model variables. Further, Section 4.1.2 discusses translations of the model variables, i. e. the vector of the transformed model variables is gained by adding a constant vector to the vector of original model variables.

4.1.1. Linear Transformations

Linear transformations of concentrations c, fluxes J, chemical potentials μ and thermodynamic forces $\Delta\mu$ correspond to the pooling of concentrations and fluxes. Because the transformed system $\hat{M}$ is required to have the form of a thermodynamic model, one cannot choose arbitrary transformation matrices. In particular, a transformation of concentrations c and fluxes J induces a transformation rule for the chemical potentials μ and the thermodynamic forces $\Delta\mu$, respectively because we require that the Gibbs energy and the entropy production are invariant under the transformation.

§ 4.1 (Conjugated variables)**.** Thermodynamic variables occur in conjugated pairs. An extensive or specific quantity is associated with a conjugated intensive quantity via the total differential of the Gibbs energy. Concentrations c or mole numbers n are conjugated to chemical potentials μ by $dg = \mu^T\, dc$ and $dG = \mu^T\, dn$. Reaction fluxes J are conjugated to thermodynamic forces $\Delta\mu$ by $\sigma[g] = \Delta\mu^T J$. Physically meaningful transformations conserve this structure and leave the Gibbs energy g and its production $\sigma[g]$ invariant. Thus, transformation rules for conjugated variables cannot be chosen independently. Let x and ϕ be vectors of conjugated variables with $dg = \phi^T dx$. A linear transformation $x = T\,\hat{x}$ leads to $dg = \phi^T T\, d\hat{x}$. Thus, the variables conjugated to $\hat{x}$ are $\hat{\phi} = T^T \phi$. This means that if the concentrations are transformed by $\hat{c} = T_c\, c$ the chemical potentials are transformed by $\mu = T_c^T \hat{\mu}$. If the fluxes are transformed by $J = T_J\, \hat{J}$, the thermodynamic forces are transformed by $\Delta\hat{\mu} = T_J^T \Delta\mu$.

Corollary 4.2 (Transformation of a thermodynamic model)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40), and let T_c, T_J, $T_{c,e}$ and $T_{J,e}$ be square matrices of full rank. Then, the solution of the system $\hat{M}$ with*

$$\begin{aligned} \hat{N} &= T_c\, N\, T_J, & \hat{S} &= T_{c,e}\, S\, T_J, & \hat{N}_e &= T_c\, N_e\, T_{J,e},\\ \hat{c}(\hat{\mu},\hat{\mu}_e) &= T_c\, c(T_c^T\hat{\mu}, T_{c,e}^T\hat{\mu}_e), & \hat{R}(\hat{\mu},\hat{\mu}_e) &= T_J^T\, R(T_c^T\,\hat{\mu}, T_{c,e}^T\,\hat{\mu}_e)\, T_J, & \hat{\mu}_0 &= T_c^{T,-1}\,\mu_0. \end{aligned}$$

is equivalent to that of M with

$$\begin{aligned} \hat{c} &= T_c\, c, & T_c^T\,\hat{\mu} &= \mu, & T_{c,e}^T\,\hat{\mu}_e &= \mu_e,\\ T_J\,\hat{J} &= J, & \Delta\hat{\mu} &= T_J^T\,\Delta\mu, & T_{J,e}\,\hat{J}_e &= J_e. \end{aligned}$$

Proof. The equivalence of M and $\hat{M}$ can be directly shown by writing down the model equations for M (see Equation 3.1, p. 40) and transforming them with $c = T_c^{-1}\,\hat{c}$, $\mu = T_c^T\,\hat{\mu}$, $\mu_e = T_{c,e}^T\,\hat{\mu}_e$, $J = T_J\,\hat{J}$, $\Delta\mu = T_J^{T,-1}\,\Delta\hat{\mu}$ and $J_e = T_{J,e}\,\hat{J}_e$. The transformed equations are equivalent to the model equations for $\hat{M}$. ☐

The transformation preserves the properties of a thermodynamic model demanded in Definition 3.1 (p. 40). Further, the Gibbs energy and the entropy production are invariant under transformation.

§ 4.3 (Symmetry and positive semi-definiteness of $\hat{R}$ and $\partial\hat{c}/\partial\hat{\mu}$)**.** The transformed matrices $\hat{R} = T_J^T\, R\, T_J$ and $\partial\hat{c}/\partial\hat{\mu} = T_c\,\partial c/\partial\mu\, T_c^T$ are symmetric and positive semi-definite because R and $\partial c/\partial\mu \geq 0$ are symmetric and positive semi-definite.

§ 4.4 (Entropy production)**.** The entropy produced by the fluxes J is $\sigma[s] = \Delta\mu^T J$. The entropy produced by the fluxes $\hat{J}$ is $\sigma[\hat{s}] = \Delta\hat{\mu}^T\,\hat{J}$. Applying the transformation rules this is $\sigma[\hat{s}] = (T_J^T\Delta\mu)^T\,(T_J^{-1}\,J) = \Delta\mu^T\,J = \sigma[s]$. Thus, the entropy production is invariant under transformation. This is not surprising because the transformation rules were designed such that conjugated variable pairs transform into conjugated variable pairs. The invariance of the entropy production is a design feature of the transformation.

§ 4.5 (Gibbs energy)**.** An analog argumentation can be used to show that the Gibbs energy of the original system $g(c)$ and of the transformed system $\hat{g}(\hat{c})$ are equivalent: We have that $d\hat{g} = \hat{\mu}^T\, d\hat{c} = (\mu^T\, T_c^{-1})\,(T_c\, dc) = \mu^T\, dc = dg$.

Example 4.6 (Transformation)**.** A transformation of the network model from Equation 3.2 in Example 3.11 (p. 42) with

$$T_c = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 1 & 1 & 1 \end{pmatrix}, \qquad T_J = \frac{1}{3} \begin{pmatrix} -1 & 1 & 1 \\ -1 & -2 & 1 \\ 2 & 1 & 1 \end{pmatrix}, \tag{4.1}$$

i. e. $\hat{c}_1 = c_A$, $\hat{c}_2 = c_B$, $\hat{c}_3 = c_A + c_B + c_c$. yields

$$\hat{N} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix}, \quad \hat{R} = \frac{1}{9} \begin{pmatrix} R_1 + R_2 + 4\,R_3 & -R_1 + 2\,R_2 + 2\,R_3 & -R_1 - R_2 + 2\,R_3 \\ -R_1 + 2\,R_2 + 2\,R_3 & R_1 + 4\,R_2 + R_3 & R_1 - 2\,R_2 + R_3 \\ -R_1 - R_2 + 2\,R_3 & R_1 - 2\,R_2 + R_3 & R_1 + R_2 + R_3 \end{pmatrix}.$$

Thus, the transformation diagonalizes the stoichiometric matrix N. The transformed matrix $\hat{N}$ consists of an identity matrix in the upper right corner and a zero row and column at the end; i. e. $\hat{J}_1 = \dot{\hat{c}}_1$, $\hat{J}_2 = \dot{\hat{c}}_2$ and $\dot{\hat{c}}_3 = 0$. The flux $\hat{J}_3$ causes no observable dynamics in this system. In the transformed system, $\hat{J}_3$ can be omitted and the differential equation for $\hat{c}_3$ can be replaced by $\hat{c}_3 = \hat{c}_{3,0}$. Thus, transformations provide a tool to bring the system equations to a form suited for model reduction. We will use this fact to develop model reduction techniques.

§ 4.7 (Partitioned transformation matrices – model equations)**.** The model reduction methods introduced in the following sections rely on the application of suited transformations of c and J that allow one to partition the system into two subsystems. From these two subsystems, the first forms the reduced-order system, whereas the second can be neglected. The model equations of a system transformed with $T_{c,e} = I$, $T_{J,e} = I$ and the partitioned matrices

$$T_c = \begin{pmatrix} T_{c,1} \\ T_{c,2} \end{pmatrix}, \qquad T_J = \begin{pmatrix} T_{J,1} & T_{J,2} \end{pmatrix}$$

read

$$\begin{aligned} \dot{\hat{c}}_1 &= (T_{c,1}\, N\, T_{J,1})\, \hat{J}_1 + (T_{c,1}\, N\, T_{J,2})\, \hat{J}_2 + (T_{c,1}\, N_e)\, J_e, \\ \dot{\hat{c}}_2 &= (T_{c,2}\, N\, T_{J,1})\, \hat{J}_1 + (T_{c,2}\, N\, T_{J,2})\, \hat{J}_2 + (T_{c,2}\, N_e)\, J_e, \\ \Delta\hat{\mu}_1 &= -(T_{c,1}\, N\, T_{J,1})^T\, \hat{\mu}_1 - (T_{c,2}\, N\, T_{J,1})^T\, \hat{\mu}_2 - (S\, T_{J,1})^T\, \mu_e, \\ \Delta\hat{\mu}_2 &= -(T_{c,1}\, N\, T_{J,2})^T\, \hat{\mu}_1 - (T_{c,2}\, N\, T_{J,2})^T\, \hat{\mu}_2 - (S\, T_{J,2})^T\, \mu_e, \\ (T_{J,1}^T\, R\, T_{J,1})\, \hat{J}_1 + (T_{J,1}^T\, R\, T_{J,2})\, \hat{J}_2 &= \Delta\hat{\mu}_1, \\ (T_{J,2}^T\, R\, T_{J,1})\, \hat{J}_1 + (T_{J,2}^T\, R\, T_{J,2})\, \hat{J}_2 &= \Delta\hat{\mu}_2, \\ \hat{c}_1(\hat{\mu}, \mu_e) &= T_{c,1}\, c(T_{c,1}^T\, \hat{\mu}_1 + T_{c,2}^T\, \hat{\mu}_2, \mu_e), \\ \hat{c}_2(\hat{\mu}, \mu_e) &= T_{c,2}\, c(T_{c,1}^T\, \hat{\mu}_1 + T_{c,2}^T\, \hat{\mu}_2, \mu_e). \end{aligned} \tag{4.2}$$

§ 4.8 (Partitioned transformation matrices – transformation rules)**.** The following equations explicitly give the forward and backward transformation rules for partitioned transformation matrices. The transformed variables can be computed from the original variables by

$$\begin{aligned} \hat{c}_1 &= T_{c,1}\, c, & \hat{c}_2 &= T_{c,2}\, c, & \hat{J}_1 &= \mathrm{inv}^T_{\Lambda_J^{-1}}(T^T_{J,1})\, J, & \hat{J}_2 &= \mathrm{inv}^T_{\Lambda_J^{-1}}(T^T_{J,2})\, J, \\ \Delta\hat{\mu}_1 &= T^T_{J,1}\, \Delta\mu, & \Delta\hat{\mu}_2 &= T^T_{J,2}\, \Delta\mu, & \hat{\mu}_1 &= \mathrm{inv}^T_{\Lambda_c^{-1}}(T_{c,1})\, \mu, & \hat{\mu}_2 &= \mathrm{inv}^T_{\Lambda_c^{-1}}(T_{c,2})\, \mu. \end{aligned}$$

The original variables can be computed from the transformed variables by

$$\begin{aligned} c &= \mathrm{inv}_{\Lambda_c^{-1}}(T_{c,1})\, \hat{c}_1 + \mathrm{inv}_{\Lambda_c^{-1}}(T_{c,2})\, \hat{c}_2, & J &= T_{J,1}\, \hat{J}_1 + T_{J,2}\, \hat{J}_2, \\ \Delta\mu &= \mathrm{inv}_{\Lambda_J^{-1}}(T^T_{J,1})\, \Delta\hat{\mu}_1 + \mathrm{inv}_{\Lambda_J^{-1}}(T^T_{J,2})\, \Delta\hat{\mu}_2, & \mu &= T^T_{c,1}\, \hat{\mu}_1 + T^T_{c,2}\, \hat{\mu}_2. \end{aligned}$$

In these equations the matrices Λ_c and Λ_J are symmetric, invertible matrices with $T_{c,1}\, \Lambda_c\, T^T_{c,2} = 0$ and $T^T_{J,1}\, \Lambda_J\, T_{J,2} = 0$. This means that $T_{c/J,1}$ and $T_{c/J,2}$ are orthogonal with respect to the scalar product $< x, y >= x^T\, \Lambda_{c/J}\, y$. Note that such matrices $\Lambda_{c/J}$ exist because the matrices T_c and T_J are invertible.

Proof. The above relations follow either directly from the transformation rules or can be directly proved by using the transformation rules and Definition 2.8 (p. 24). □

§ 4.9 (Computation of $T_{c/J,1/2}$ and $\Lambda_{c/J}$)**.** The above form of the transformation rules is especially suited for the use of partitioned transformations for model reduction. The original system is partitioned into a reduced system (subscript 1) and a negligible system (subscript 2). The transformation matrices can be determined in three steps: (1) define $T_{c/J,2}$ based on the knowledge of the negligible dynamics, (2) choose arbitrary invertible matrices Λ_c and Λ_J, e. g. $\Lambda_c = \Lambda_J = I$; (3) compute $T_{c,1}$ as a left kernel matrix of $\Lambda_c\, T^T_{c,2}$ with $T_{c,1}\, \Lambda_c\, T^T_{c,2} = 0$ and compute $T_{J,1}$ as a right kernel matrix of $T^T_{J,2}\, \Lambda_J$ with $T^T_{J,2}\, \Lambda_J\, T_{J,1} = 0$. Details of how to determine $T_{c/J,2}$ are given later, when discussing several model reduction methods.

4.1.2. Translations

Besides the linear transformation of the model variables, it is partly possibly to translate the vector of model variables by adding another vector. This is discussed in the following sections.

4.1.2.1. Translation of the Chemical Potentials

Chemical potentials can be translated by certain vectors. The details are given in the following corollary.

Corollary 4.10 (Translation of the chemical potentials)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40), and let $\delta\mu \in \mathbb{R}^{i_0}$ and $\delta\mu_e \in \mathbb{R}^{i_{0,e}}$ be vectors with $N^T\, \delta\mu + S^T\, \delta\mu_e = 0$. Then, the solution of the system $\hat{M}$ with*

$$\begin{aligned} \hat{N} &= N, & \hat{S} &= S, & \hat{N}_e &= N_e, \\ \hat{c}(\hat{\mu}, \hat{\mu}_e) &= c(\hat{\mu} - \delta\mu, \hat{\mu}_e - \delta\mu_e), & \hat{R}(\hat{\mu}, \hat{\mu}_e) &= R(\hat{\mu} - \delta\mu, \hat{\mu}_e - \delta\mu_e), & \hat{\mu}_0 &= \mu_0 - \delta\mu \end{aligned}$$

is equivalent to that of M with

$$\begin{aligned} \hat{c} &= c, & \hat{\mu} &= \mu + \delta\mu, & \hat{\mu}_e &= \mu_e + \delta\mu_e, \\ \hat{J} &= J, & \Delta\hat{\mu} &= \Delta\mu, & \hat{J}_e &= J_e. \end{aligned}$$

Proof. Write down the model equations of M. Now replace $\mu = \hat{\mu} - \delta\mu$ and $\mu_e = \hat{\mu}_e - \delta\mu_e$. This immediately leads to the functions $\hat{c}(\hat{\mu}, \hat{\mu}_e)$ and $\hat{R}(\hat{\mu}, \hat{\mu}_e)$ and the matrices $\hat{N}$, $\hat{S}$ and $\hat{N}_e$. To establish the identity of the thermodynamic forces we write: $\Delta\hat{\mu} = -N^T \mu - S^T \mu_e - N^T\delta\mu - S^T \delta\mu_e = -N^T \mu - S^T \mu_e = \Delta\mu$. □

§ 4.11 (Invariance of the entropy production)**.** The entropy production of the internal fluxes J is invariant under a translation of the chemical potentials because $\sigma[\hat{s}] = \Delta\hat{\mu}^T \hat{J} = \Delta\mu\, J = \sigma[s]$.

§ 4.12 (Change of the reference state)**.** Information on the absolute values of the chemical potentials is usually not available. Only differences of chemical potentials along reactions, i. e. thermodynamic forces, are measurable. Thus, the available tables give chemical potentials with respect to a chosen reference state (cf. Section 2.2.2.3, p. 31). The above described procedure for translation of the chemical potentials is equivalent to a change of this reference state.

§ 4.13 (Gibbs energy)**.** The Gibbs energy is *not* invariant under translation of the chemical potentials. We have $d\hat{g} = \hat{\mu}^T d\hat{c} = \mu^T dc + \delta\mu^T dc = dg + \delta\mu^T dc$. For constant $\delta\mu$ and suited initial conditions, an integration of this partial differential equation yields $\hat{g} = g + \delta\mu^T c$.

Example 4.14. Consider the closed system consisting of one reaction $A \rightleftharpoons B$ in an ideal dilute solution with

$$c_A(\mu_A) = c^\circ \exp\left(\frac{\mu_A - \mu_A^\circ}{R^* T}\right), \qquad c_B(\mu_B) = c^\circ \exp\left(\frac{\mu_B - \mu_B^\circ}{R^* T}\right).$$

We have $N^T = (-1, 1)$ and thus we can translate the chemical potentials by $\delta\mu^T = d \cdot (1, 1)$. We choose $d = -\mu_A^\circ$, i. e. $\hat{\mu}_A = \mu_A - \mu_A^\circ$ and $\hat{\mu}_B = \mu_B - \mu_A^\circ$, and get

$$\hat{c}_A(\hat{\mu}_A) = c^\circ \exp\left(\frac{\hat{\mu}_A}{R^* T}\right), \qquad \hat{c}_B(\hat{\mu}_B) = c^\circ \exp\left(\frac{\hat{\mu}_B - (\mu_B^\circ - \mu_A^\circ)}{R^* T}\right).$$

Thus, we transformed the system into an equivalent representation with $\hat{\mu}_A^\circ = 0$. The transformed Gibbs energy is given by

$$d\hat{g} = \underbrace{(\mu_A - \mu_A^\circ)}_{\hat{\mu}_A} dc_A + \underbrace{(\mu_B - \mu_A^\circ)}_{\hat{\mu}_B} dc_B = \underbrace{\mu_A\, dc_A + \mu_B\, dc_B}_{dg} - \mu_A^\circ\, (dc_A + dc_B).$$

4.1.2.2. Translation of the Fluxes

The translation of the chemical potentials is only constrained by the stoichiometric matrices N and S. However, possible translations of the fluxes depend on the stoichiometric matrices N and N_e and on the resistance matrix R.

Corollary 4.15 (Translation of the fluxes)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40) and let $\delta J \in \mathbb{R}^{j_0}$, $\delta J_e \in \mathbb{R}^{j_{0,e}}$ be vectors with $N\,\delta J + N_e\,\delta J_e = 0$ and $R(\mu, \mu_e)\,\delta J = 0$ for all μ and μ_e. The solution of the system $\hat{M}$ with*

$$\hat{N} = N, \qquad \hat{S} = S, \qquad \hat{N}_e = N_e,$$
$$\hat{c}(\hat{\mu}, \hat{\mu}_e) = c(\hat{\mu}, \hat{\mu}_e), \qquad \hat{R}(\hat{\mu}, \hat{\mu}_e) = R(\hat{\mu}, \hat{\mu}_e), \qquad \hat{\mu}_0 = \mu_0$$

is equivalent to that of M with

$$\hat{c} = c, \qquad \hat{\mu} = \mu, \qquad \hat{\mu}_e = \mu_e,$$
$$\hat{J} = J + \delta J, \qquad \Delta\hat{\mu} = \Delta\mu, \qquad \hat{J}_e = J_e + \delta J_e.$$

Proof. Write down the model equations of M. Now replace $J = \hat{J} - \delta J$ and $J_e = \hat{J}_e - \delta J_e$. It immediately follows that $\dot{c} = N\,\hat{J} + N_e\,\hat{J}_e - N\,\delta J - N_e\,\delta J_e = \dot{\hat{c}}$ and $\Delta\mu = R\,(\hat{J} - \delta J) = \hat{R}\,\hat{J} = \Delta\hat{\mu}$. Thus, the translation of the fluxes does not have effects on other model variables or functions. ☐

§ 4.16 (Invariance of the entropy production and the Gibbs energy)**.** The entropy production of the fluxes J is invariant under translation of the fluxes because $\sigma[\hat{s}] = \Delta\hat{\mu}^T\,\hat{R}\,\Delta\hat{\mu} = \Delta\mu\,R\,\Delta\mu = \sigma[s]$. The Gibbs energy is also invariant because $d\hat{g} = \hat{\mu}^T\,d\hat{c} = \mu\,dc = dg$.

§ 4.17 (Possible non-uniqueness of the fluxes)**.** If R is not singular, no translations of J but only of J_e are possible. If R and N share parts of their null space, J can be arbitrarily translated by a translation vector in the intersection of these null spaces ($N\,\delta J = 0$, $R\,\delta J = 0$ with $\delta J_e = 0$). This translation does not change any of the functions or matrices in the network description. This means that in such cases the fluxes J are not uniquely determined by the model equations. This will be discussed in detail in Section 4.2.2.2 that studies systems with singular resistance matrices R.

Example 4.18 (Translation of fluxes)**.** Consider a system with the reaction equation $0 \xrightleftharpoons{(J_e)} A \xrightleftharpoons{R=0} (B)$ and the function $c_A = c_A(\mu_A, \mu_B)$. We have that $N = -1$, $S = 1$, $N_e = 1$ and $R = 0$. According to the above corollary any translation with $\delta J = \delta J_e$ is valid. A resistance of $R = 0$ means that $0 = R\,J = \Delta\mu = \mu_A - \mu_{e,B}$ and thus $\mu_A = \mu_{e,B}$. Thus, we have that $c_A = c_A(\mu_{e,B}, \mu_{e,B})$. This means that μ_A, c_A and $\Delta\mu$ are determined solely by $R = 0$ and $\mu_{e,B}$. For a constant $\mu_{e,B}$ it follows from $c_A = c_A(\mu_{B,e}, \mu_{e,B})$ that $\dot{c}_A = 0$. Since $\dot{c}_A = J_e - J$, this means that $J = J_e$. However, since the fluxes J and J_e do no enter the equations for μ_A, c_A or $\Delta\mu$, their value can be arbitrarily translated with $\hat{J} = J + \delta J$ and $\hat{J}_e = J_e + \delta J_e$.

§ 4.19 (Time-variant translation)**.** The above introduced translation of fluxes does not require that δJ and δJ_e are constant. In fact, they may be arbitrary functions of time.

4.1.2.3. Translation of the Forces

The vector of the forces $\Delta\mu$ is uniquely determined by $\Delta\mu = R\,J = -N^T\,\mu - S^T\,\mu_e$. Thus, any translation of $\Delta\mu$ would lead to a change of the model variables J, μ and μ_e. Thus, it is not possible to formulate a transformed thermodynamic model where the transformed forces are the original forces plus a translation vector and the other model variables are unchanged. For this reason, a translation of the thermodynamic forces $\Delta\mu$ is not possible.

4.1.2.4. Translation of the Concentrations

Concentrations c can be translated by an arbitrary, constant translation vector $\delta c \in \mathbb{R}^{i_0}$. The transformed variables are $\hat{c} = c + \delta c$, $\hat{\mu} = \mu$, $\hat{J} = J$ and $\Delta\hat{\mu} = \Delta\mu$. The transformed thermodynamic system is characterized by $\hat{N} = N$, $\hat{S} = S$, $\hat{N}_e = N_e$, $\hat{c}(\hat{\mu}, \hat{\mu}_e) = c(\hat{\mu}, \hat{\mu}_e) + \delta c$, $\hat{R}(\hat{\mu}, \hat{\mu}_e) = R(\hat{\mu}, \hat{\mu}_e)$ and $\hat{\mu}_0 = \mu_0$. In a thermodynamic system according to Definition 3.1 (p. 40) the fluxes are determined by the equation $R(\mu, \mu_e)\,J = \Delta\mu$. This equation depends solely on the chemical potentials $\mu = \hat{\mu}$ but not on the concentrations c. For this reason the fluxes and the change of the concentrations are invariant ($J = \hat{J}$, $\dot{c} = \dot{\hat{c}}$) under a translation of the concentrations. The translation of the concentrations is not used in this thesis but is only included here for completeness.

4.2. Model Reduction

This section shows how transformations of thermodynamic models can be used to reduce a thermodynamic model to an equivalent lower dimensional model. The basic idea is to use partitioned transformation matrices as in §4.7 (p. 53) in order to bring the system to a form where the second subsystem can be neglected or directly solved. Cases where a lossless reduction is possible are characterized by rank deficiencies of certain matrices. We will also discuss how these methods can be used to gain approximately reduced models.

The first subsection *Reduction of the Stoichiometric Submodel* deals with a reduction of conservation relations and stoichiometric cycles that are characterized by the left and right null space of the stoichiometric matrices, respectively (cf. Example 4.6, p. 53). The subsections *Reduction of the Thermodynamic Submodel* and *Reduction of the Kinetic Submodel* discuss degenerated systems with singular $\partial c/\partial\mu$ and $R(\mu)$, respectively. The section *Reduction of the Boundary Conditions* explains a possibility to reduce the number of clamped chemical potentials and fluxes.

§ 4.20 (General procedure and notation)**.** The reduction methods introduced in the following sections are based on the transformation of an original thermodynamic model M with a partitioned transformation matrix into a model $\hat{M}$. The two parts of the transformed model $\hat{M}$ are indicated by subscripts. For example, the vector $\hat{\mu}$ is partitioned into two subvectors $\hat{\mu}^T = (\hat{\mu}_1^T, \hat{\mu}_2^T)$. The partitioned transformation matrices are chosen such that the subsystem 2 has a simple form and its generic solution can be computed. Substituting the solution of subsystem 2 in subsystem 1 yields the reduced system $\tilde{M}$. Thus, the variables of subsystem 1 and of $\tilde{M}$ are equal; for example $\tilde{\mu} = \mu_1$.

4.2.1. Reduction of the Stoichiometric Submodel

Conservation relations and stoichiometric cycles are related to the left and right null space of the stoichiometric matrices, respectively. Consider the mole balances of a thermodynamic model

$$\dot{c} = N\,J + N_e\,J_e.$$

Let $b \in \operatorname{null}([N, N_e]^T)$, then for all flux vectors J and J_e the conservation relation $b^T c = \text{const}$ holds. Conservation relations define an invariant manifold in the concentration space, and thus the system can be reduced to this manifold. The reduced system contains less components.

Let $b \in \operatorname{null}(N)$, then the effect of a certain flux distribution J on the change of concentrations $\dot{c}$ is indistinguishable from the effect of a flux distribution $J + b$. Thus, with respect to the concentrations c, the flux vector is redundant and the dynamics of the original system can be described by a reduced system with less fluxes.

4.2.1.1. Reduction of Conservation Relations

We transform the concentrations c by a partitioned transformation such that $\hat{c}_2$ is a constant quantity ($\dot{\hat{c}}_2 = 0$) and thus $\hat{c}_2(\hat{\mu}_1, \hat{\mu}_2, \hat{\mu}_e) = \hat{c}_{2,0}$. We solve this algebraic equation in order to gain a function $\hat{\mu}_2(\hat{\mu}_1, \hat{\mu}_e)$. Finally, after applying this function to all occurrences of $\hat{\mu}_2$ in the first subsystem, we consider the first subsystem independently from the second subsystem. Thus, we gain a reduced description of the original system. The following corollary discusses the details of this procedure.

Corollary 4.21 (Reduction of conservation relations)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40). Let $T_c = [T_{c,1}^T, T_{c,2}^T]^T$ be a square and invertible matrix with $T_{c,2}\,N = 0$ and $T_{c,2}\,N_e = 0$. Assume that the matrix $T_{c,2}\,\partial c/\partial\mu\,T_{c,2}^T$ is invertible for all $\mu \in \mathbb{R}^{i_0}$ and $\mu_e \in \mathbb{R}^{i_{0,e}}$. Let $\tilde{M}$ be a thermodynamic model with*

$$\begin{aligned} \tilde{N} &= T_{c,1}\,N, & \tilde{c}(\tilde{\mu}, \mu_e) &= T_{c,1}\,c(T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2(\tilde{\mu}, \mu_e), \mu_e),\\ \tilde{S} &= S, & \tilde{R}(\tilde{\mu}, \mu_e) &= R(T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2(\tilde{\mu}, \mu_e), \mu_e),\\ \tilde{N}_e &= T_{c,1}\,N_e, & \tilde{\mu}_0 &= \operatorname{inv}_{\Lambda_c^{-1}}^T(T_{c,1})\,\mu_0 \end{aligned}$$

where $\hat{\mu}_2(\tilde{\mu}, \mu_e)$ is a solution of

$$T_{c,2}\,c(T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2, \mu_e) = T_{c,2}\,c(\mu_0, \mu_{e,0})$$

and Λ_c is a symmetric, invertible matrix with $T_{c,1}\,\Lambda_c\,T_{c,2}^T = 0$. Then, a solution of M can be reconstructed from a solution of the reduced system $\tilde{M}$ by

$$\begin{aligned} c &= T_c^{-1}\begin{pmatrix} \tilde{c} \\ T_{c,2}\,c(\mu_0, \mu_{e,0}) \end{pmatrix}, & \mu &= T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2(\tilde{\mu}, \mu_e),\\ J &= \tilde{J}, & \Delta\mu &= \Delta\tilde{\mu}. \end{aligned}$$

Proof. Transform M with $\hat{c} = T_c\, c$ (cf. §4.7, p. 53). The first subsystem (subscript 1) corresponds to the reduced system $\tilde{M}$. Observe that $\dot{\hat{c}}_2 = 0$ and thus $\hat{c}_2 = T_{c,2}\, c(\mu_0, \mu_{e,0})$. From this we can compute a function $\hat{\mu}_2(\hat{\mu}_1, \mu_e)$ by solving the equation $T_{c,2}\, c(T_{c,1}^T\, \hat{\mu}_1 + T_{c,2}^T\, \hat{\mu}_2, \mu_e) = T_{c,2}\, c(\mu_0, \mu_{e,0})$. If the solution exists, it is locally unique because the matrix $T_{c,2}\, \partial c/\partial\mu\, T_{c,2}^T$ is invertible. This follows from the Inverse Function Theorem. If $T_{c,2}\, \partial c/\partial\mu\, T_{c,2}^T$ would be singular for all μ and μ_e, the model equations would not determine a locally unique trajectory for $\hat{\mu}_2$. If the function $\hat{\mu}_2(\hat{\mu}_1, \mu_e)$ does not exist or is not globally unique, the trajectory of M does not exist or is not unique, respectively. The possible non-uniqueness of the chemical potentials will be discussed in §4.44 (p. 66). With the function $\hat{\mu}_2(\hat{\mu}_1, \mu_e)$ we get $\mu = T_{c,1}^T\, \hat{\mu}_1 + T_{c,2}^T\, \hat{\mu}_2(\hat{\mu}_1, \mu_e)$. We use this relation to derive the functions $\hat{R}(\hat{\mu}_1, \mu_e)$ and $\hat{c}_1(\hat{\mu}_1, \mu_e)$. Observe that the first part of the equations ($\hat{c}_1$, $\hat{\mu}_1$) can now be considered independently of the second ($\hat{c}_2$, $\hat{\mu}_2$) and that $\hat{c}_2$ and $\hat{\mu}_2$ can be reconstructed from $\hat{c}_1$ and $\hat{\mu}_1$. Thus, the subsystem $\hat{M}_1$ is a reduced version of $\hat{M}$. Replacing the subscript 1 with a tilde ($\tilde{M} = \hat{M}_1$) yields the expressions given in the corollary. The initial value for the reduced chemical potentials $\hat{\mu}_{1,0} = \tilde{\mu}_{1,0}$ follows from the relations given in §4.8 (p. 53). The state variables of the original system can be reconstructed by using the inverse transformation matrix T_c^{-1}, $\hat{c}_2 = T_{c,2}\, c(\mu_0, \mu_{e,0})$ and the function $\hat{\mu}_2(\tilde{\mu}, \mu_e)$. ☐

§ 4.22 (Symmetry and positive semi-definiteness of $\tilde{R}$ and $\partial\tilde{c}/\partial\tilde{\mu}$)**.** The matrices $\tilde{R}$ and $\partial\tilde{c}/\partial\tilde{\mu}$ of the reduced model are symmetric and positive semi-definite.

Proof. The resistance matrices of the reduced system and of the original system are equal at corresponding state and input vectors: $\tilde{R}(\tilde{\mu}, \mu_e) = R(T_{c,1}^T\, \tilde{\mu} + T_{c,2}^T\, \hat{\mu}_2(\tilde{\mu}, \mu_e))$. Thus, $\tilde{R}$ is symmetric and positive semi-definite.

Differentiating the expression for $\tilde{c}(\tilde{\mu}, \mu_e)$ in Corollary 4.21 yields for the derivative of $\tilde{c}(\tilde{\mu}, \mu_e)$ that $\partial\tilde{c}/\partial\tilde{\mu} = T_{c,1}\, \partial c/\partial\mu\, T_{c,1}^T + T_{c,1}\, \partial c/\partial\mu\, T_{c,2}^T\, \partial\hat{\mu}_2/\partial\tilde{\mu}$. The total differential of the conservation relations in Corollary 4.21 is $T_{c,2}\, \partial c/\partial\mu\, T_{c,1}^T\, d\tilde{\mu} + T_{c,2}\, \partial c/\partial\mu\, T_{c,2}^T\, d\hat{\mu}_2 = 0$. This leads to $\partial\hat{\mu}_2/\partial\tilde{\mu} = -(T_{c,2}\, \partial c/\partial\mu\, T_{c,2}^T)^{-1}\, T_{c,2}\, \partial c/\partial\mu\, T_{c,1}^T$. Combining these results yields:

$$\partial\tilde{c}/\partial\tilde{\mu} = T_{c,1}\, \underbrace{\left(\partial c/\partial\mu - \partial c/\partial\mu\, T_{c,2}^T\, (T_{c,2}\, \partial c/\partial\mu\, T_{c,2}^T)^{-1}\, T_{c,2}\, \partial c/\partial\mu\right)}_{L}\, T_{c,1}^T.$$

Because the matrix L is symmetric, the derivative $\partial\tilde{c}/\partial\tilde{\mu}$ is symmetric.

If L is positive semi-definite, the derivative $\partial\tilde{c}/\partial\tilde{\mu}$ is positive semi-definite. However, from the expression for L given above it is not obvious if L is positive semi-definite. For this reason, an alternative expression for L that allows to prove its positive semi-definiteness is derived. Assume initially that $\partial c/\partial\mu$ is positive definite. Then, the matrices $W = T_{c,1}\, \Lambda_c\, (\partial c/\partial\mu)^{-1}$ and $Z = [W^T, T_{c,2}^T]$ can be defined. Let L' be the matrix $L' = \Lambda_c\, T_{c,1}^T\, (T_{c,1}\, \Lambda_c\, (\partial c/\partial\mu)^{-1}\, \Lambda_c\, T_{c,1}^T)^{-1}\, T_{c,1}\, \Lambda_c$. The matrices L' and L are equal because Z is a invertible matrix and $L\, Z = L'\, Z = (\Lambda_c\, T_{c,1}^T, 0)$. In this representation, it can be seen easily now that the matrix $L' = L$ is symmetric and positive definite. In the limit case where $\partial c/\partial\mu$ is only positive semi-definite, the limit of L may also be only positive semi-definite. ☐

§ 4.23 (Computation of $T_{c,1}$, $T_{c,2}$ and Λ_c)**.** Let Y be a matrix with full rank and with

$$\operatorname{span}(Y) = \bigcap_{\mu \in \mathbb{R}^{i_0}, \mu_e \in \mathbb{R}^{i_{0,e}}} \operatorname{span}\left(\frac{\partial c}{\partial \mu}(\mu, \mu_e)\right).$$

In all cases considered in this thesis, the matrix $\partial c/\partial \mu$ is diagonal (e. g. in an ideal dilute solution) or the result of a linear transformation of a system with a diagonal matrix $\partial c/\partial \mu$. Then, the column space $\operatorname{span}(\partial c/\partial \mu)$ is independent of μ and μ_e, and one may choose $Y = \partial c/\partial \mu(\mu^*, \mu_e^*)$ with an arbitrary state vector μ^* and input vector μ_e^*. If $\partial c/\partial \mu$ is invertible, then Y can be chosen to be the identity matrix ($Y = I$). If $\partial c/\partial \mu$ is diagonal, then Y can be chosen to be a diagonal matrix with diagonal elements $Y_{ii} = 1$ if $\partial c_i/\partial \mu_i \neq 0$ and $Y_{ii} = 0$ if $\partial c_i/\partial \mu_i = 0$ for $i = 1 \dots i_0$. With the matrix Y defined above, the matrix $Y^T \, \partial c/\partial \mu \, Y$ has always full rank. Using the matrix Y, the matrices $T_{c,1}$, $T_{c,2}$ and Λ_c with the properties demanded in Corollary 4.21 can be gained easily by computing kernel matrices: (1) Compute a kernel matrix X with $X\, Y^T N = 0$ and $X\, Y^T N_e = 0$. The choice $T_{c,2} = X\, Y^T$ guarantees that $T_{c,2}\, N = 0$, $T_{c,2}\, N_e = 0$ and that $T_{c,2}\, \partial c/\partial \mu \, T_{c,2}^T = X\, Y^T \, \partial c/\partial \mu \, Y\, X^T$ has full rank. (2) Choose an arbitrary symmetric and invertible Λ_c, e. g. $\Lambda_c = I$. (3) Compute $T_{c,1}$ as a right kernel matrix of $T_{c,2}^T\, \Lambda_c$ with $T_{c,2}^T\, \Lambda_c\, T_{c,1} = 0$.

§ 4.24 (Non-linear equation)**.** The reduction involves the solution of the non-linear equation $T_{c,2}\, c(\mu_0, \mu_{e,0}) = T_{c,2}\, c(T_{c,1}^T\, \hat{\mu}_1 + T_{c,2}^T\, \hat{\mu}_2, \mu_e)$. Thus, the computational complexity of the reduction of conservation relations may be large.

§ 4.25 (Invariance of the entropy production)**.** The entropy production of the internal fluxes J is invariant under the above described reduction method: $\sigma[\tilde{s}] = \Delta\tilde{\mu}^T\, \tilde{J} = \Delta\mu^T\, J = \sigma[s]$.

§ 4.26 (Invariance of the Gibbs energy)**.** For $d\hat{c}_2 = T_{c,2}\, dc = 0$, the differential of the Gibbs energy is invariant under the above described reduction method: $dg = \mu^T\, dc = (d\tilde{\mu}^T\, T_{c,1} + d\hat{\mu}_2\, T_{c,2})\, dc = d\tilde{\mu}^T\, d\tilde{c}$. The information on the dependency of the Gibbs energy on the change of the concentration of the conserved moiety $d\hat{c}_2 = T_{c,2}\, dc$ is lost during the reduction process.

Example 4.27 (Reduction of a conservation relation)**.** Consider a reaction $A \rightleftharpoons B$ with mass-action kinetics in an ideal dilute solution in a closed system:

$$N = \begin{pmatrix} -1 \\ 1 \end{pmatrix}, \quad c(\mu) = \begin{pmatrix} c^\circ \, \exp((\mu_A - \mu_A^\circ)/(R^*\, T)) \\ c^\circ \, \exp((\mu_B - \mu_B^\circ)/(R^*\, T)) \end{pmatrix}, \quad R(\mu) = \rho\, R_\circ(\mu_A/(R^*\, T), \mu_B/(R^*\, T)).$$

The system contains the conservation relation $c_A + c_B = \text{const}$. We choose the transformation matrices for the reduction as

$$T_{c,1} = \begin{pmatrix} -1 & 1 \end{pmatrix}, \qquad T_{c,2} = \begin{pmatrix} 1 & 1 \end{pmatrix}$$

with $\Lambda_c = I$. The variable $\hat{c}_2 = c_A + c_B$ is the concentration of the conserved moiety. The variable $\hat{c}_1 = c_B - c_A$ describes the difference of the concentrations of B and A. Although $\hat{c}_1$ may be negative, it is a concentration in the sense of Definition 3.1 (p. 40). We have $\mu_A = -\hat{\mu}_1 + \hat{\mu}_2$ and $\mu_B = \hat{\mu}_1 + \hat{\mu}_2$. From the conservation relation we get the following dependency of $\hat{\mu}_1$ and $\hat{\mu}_2$:

$$c_{A,0} + c_{B,0} = c^\circ \exp((-\hat{\mu}_1 + \hat{\mu}_2 - \mu_A^\circ)/(R^*\,T)) + c^\circ \exp((\hat{\mu}_1 + \hat{\mu}_2 - \mu_B^\circ)/(R^*\,T)).$$

Solving this equation for $\hat{\mu}_2$ and replacing $\tilde{\mu} = \hat{\mu}_1$ yields

$$\hat{\mu}_2(\tilde{\mu}) = R^*\,T \log\left(\frac{c_{A,0} + c_{B,0}}{c^\circ} \cdot \frac{1}{\exp((-\tilde{\mu} - \mu_A^\circ)/(R^*\,T)) + \exp((\tilde{\mu} - \mu_B^\circ)/(R^*\,T))}\right).$$

Substituting this expression into $\tilde{c} = \hat{c}_1 = -c_A + c_B$ with $c_i = c^\circ \exp((\mu_i - \mu_i^\circ)/(R^*\,T))$ and into $R = \rho\, R_\circ(\mu_A/(R^*\,T), \mu_B/(R^*\,T))$ yields the transformed functions

$$\tilde{c}(\tilde{\mu}) = (c_A^\circ + c_B^\circ) \cdot \frac{-\exp((-\tilde{\mu} - \mu_A^\circ)/(R^*\,T)) + \exp((+\tilde{\mu} - \mu_B^\circ)/(R^*\,T))}{+\exp((-\tilde{\mu} - \mu_A^\circ)/(R^*\,T)) + \exp((+\tilde{\mu} - \mu_B^\circ)/(R^*\,T))}$$

and

$$\hat{R}(\tilde{\mu}) = \rho\, R_\circ(-\tilde{\mu} + \hat{\mu}_2(\tilde{\mu}), \tilde{\mu} + \hat{\mu}_2(\tilde{\mu})).$$

Thus, the original system with two compounds and simple functions $c(\mu)$ and $R(\mu)$ can be reduced to one with one compound but more complex functions $\tilde{c}(\tilde{\mu})$ and $\tilde{R}(\tilde{\mu})$.

4.2.1.2. Reduction of Stoichiometric Cycles

The reduction of stoichiometric cycles reduces the number of fluxes. It proceeds analogously to the reduction of conservation relations. While the reduction of conservation relations is based on the left null space of the stoichiometric matrices, the reduction of stoichiometric cycles uses the right null space.

We transform the fluxes J by a partitioning transformation such that $\Delta\hat{\mu}_2 = 0$ and such that $\hat{J}_2$ vanishes from the mole balances.

Corollary 4.28 (Reduction of cycles)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40) and let $T_J = [T_{J,1}, T_{J,2}]$ be a square and invertible matrix with $N\,T_{J,2} = 0$ and $S\,T_{J,2} = 0$. Assume that the matrix $T_{J,2}^T\,R(\mu, \mu_e)\,T_{J,2}^T$ is invertible for all $\mu \in \mathbb{R}^{i_0}$ and $\mu_e \in \mathbb{R}^{i_{0,e}}$. Let Λ_J be a symmetric, invertible matrix with $T_{J,2}^T\,\Lambda_J\,T_{J,1} = 0$. Then, a trajectory of M can be reconstructed from a trajectory of the reduced system $\tilde{M}$ with*

$$\tilde{N} = N\,T_{J,1}, \qquad \tilde{S} = S\,T_{J,1}, \qquad \tilde{N}_e = N_e, \qquad \tilde{c}(\tilde{\mu}, \mu_e) = c(\tilde{\mu}, \mu_e), \qquad \tilde{\mu}_0 = \mu_0,$$

$$\tilde{R}(\tilde{\mu}, \mu_e) = (T_{J,1}^T\,R(\tilde{\mu}, \mu_e)\,T_{J,1}) - (T_{J,1}^T\,R(\tilde{\mu}, \mu_e)\,T_{J,2})\,(T_{J,2}^T\,R(\tilde{\mu}, \mu_e)\,T_{J,2})^{-1}\,(T_{J,2}^T\,R(\tilde{\mu}, \mu_e)\,T_{J,1})$$

where

$$c = \tilde{c}, \qquad \mu = \tilde{\mu},$$

$$J = (T_{J,1} - T_{J,2}\,(T_{J,2}^T\,R\,T_{J,2})^{-1}\,(T_{J,2}^T\,R\,T_{J,1}))\,\tilde{J}, \qquad \Delta\mu = \operatorname{inv}_{\Lambda_J^{-1}}(T_{J,1}^T)\,\Delta\tilde{\mu}.$$

Proof. Transform M with $J = T_J\,\hat{J}$ (see §4.7, p. 53). Observe that $\Delta\hat{\mu}_2 = 0$. Because $T_{J,2}^T\,R\,T_{J,2}$ is invertible, we can solve the equation $T_{J,2}^T\,R\,T_{J,1}\,\hat{J}_1 + T_{J,2}^T\,R\,T_{J,2}\,\hat{J}_2 = \Delta\hat{\mu}_2 = 0$ for $\hat{J}_2$ and get $\hat{J}_2 = -(T_{J,2}^T\,R\,T_{J,2})^{-1}\,(T_{J,2}^T\,R\,T_{J,1})\,\hat{J}_1$. If $T_{J,2}^T\,R\,T_{J,2}$ would be singular, the model equation would not uniquely determine the fluxes $\hat{J}_2$. The possible non-uniqueness of the fluxes will be discussed in Corollary 4.71 (p. 76). Entering the $\hat{J}_2$ computed above into the equation $T_{J,1}^T\,R\,T_{J,1}\,\hat{J}_1 + T_{J,1}^T\,R\,T_{J,2}\,\hat{J}_2 = \Delta\hat{\mu}_1$ yields

$$\underbrace{(T_{J,1}^T\,R(\tilde{\mu},\mu_e)\,T_{J,1}) - (T_{J,1}^T\,R(\tilde{\mu},\mu_e)\,T_{J,2})\,(T_{J,2}^T\,R(\tilde{\mu},\mu_e)\,T_{J,2})^{-1}\,(T_{J,2}^T\,R(\tilde{\mu},\mu_e)\,T_{J,1})}_{\tilde{R}}\;\underbrace{\hat{J}_1}_{\tilde{J}} = \underbrace{\Delta\hat{\mu}_1}_{\Delta\tilde{\mu}}\,.$$

The formulas for the reconstruction of the original variables from the reduced ones follow from the expressions given in §4.8 (p. 53). ☐

§ 4.29 (Symmetry and positive semi-definiteness of $\tilde{R}$ and $\partial\tilde{c}/\partial\tilde{\mu}$)**.** The matrices $\tilde{R}$ and $\partial\tilde{c}/\partial\tilde{\mu}$ of the reduced model are symmetric and positive semi-definite.

Proof. The derivative $\partial\tilde{c}/\partial\tilde{\mu}$ is symmetric and positive semi-definite because $\tilde{c}(\tilde{\mu},\mu_e) = c(\tilde{\mu},\mu_e)$ and $\partial c/\partial\mu$ is symmetric and positive semi-definite.

The symmetry of $\tilde{R}$ is obvious from the expression given in Corollary 4.28.

The resistance matrix $\tilde{R}$ resulting from the reduction of stoichiometric cycles and the derivative $\partial\tilde{c}/\partial\tilde{\mu}$ resulting from the reduction of conservation relations (see §4.22, p. 59) have the same structure. For this reason, the proof of the positive semi-definiteness of $\tilde{R}$ is completely analog to the proof of the positive semi-definiteness of $\partial\tilde{c}/\partial\tilde{\mu}$ in §4.22 (p. 59). ☐

§ 4.30 (Computation of $T_{J,1}$, $T_{J,2}$ and Λ_J)**.** Let Y be a matrix with full rank and with

$$\operatorname{span}(Y) = \bigcap_{\mu\in\mathbb{R}^{i_0},\mu_e\in\mathbb{R}^{i_{0,e}}} \operatorname{span}\left(R(\mu,\mu_e)\right).$$

In all cases considered in this thesis, the matrix R is diagonal or the result of a linear transformation of a system with a diagonal matrix R. Then, the column space $\operatorname{span}(R)$ is independent of μ and μ_e, and one may choose $Y = R(\mu^*,\mu_e^*)$ with an arbitrary state vector μ^* and input vector μ_e^*. If R is invertible, then Y can be chosen to be the identity matrix ($Y = I$). If R is diagonal, then Y can be chosen to be a diagonal matrix with diagonal elements $Y_{ii} = 1$ if $R_{ii} \neq 0$ and $Y_{ii} = 0$ if $R_{ii} = 0$ for $i = 1 \ldots i_0$. With the matrix Y defined above, the matrix $Y^T\,R\,Y$ has always full rank. Using the matrix Y, the matrices $T_{J,1}$, $T_{J,2}$ and Λ_J with the properties demanded in Corollary 4.28 can be gained easily by computing kernel matrices: (1) Compute a kernel matrix X with $N\,Y\,X = 0$ and $S\,Y\,X = 0$. The choice $T_{J,2} = Y\,X$ guarantees that $N\,T_{J,2} = 0$, $S\,T_{J,2} = 0$ and that $T_{J,2}^T\,R\,T_{J,2} = X^T\,Y^T\,R\,Y\,X$ has full rank. (2) Choose an arbitrary invertible, symmetric Λ_J, e. g. $\Lambda_J = I$. (3) Compute $T_{J,1}$ as a right kernel matrix of $T_{J,2}^T\,\Lambda_J$ with $T_{J,2}^T\,\Lambda_J\,T_{J,1} = 0$.

§ 4.31 (Invariance of entropy production)**.** The entropy production is invariant under the above described reduction method: $\sigma[\tilde{s}] = \sigma[s]$.

Proof. Using the equations for the reconstruction of the original states given in Corollary 4.28 we get for $\sigma[s] = \Delta\mu^T J^T$:

$$\sigma[s] = \Delta\tilde{\mu}^T \underbrace{(T_{J,1}^T \Lambda_J T_{J,1})^{-1} T_{J,1}^T \Lambda_J (T_{J,1} - T_{J,2} (T_{J,2}^T R T_{J,2})^{-1} (T_{J,2}^T R T_{J,1}))}_{I} \tilde{J} = \sigma[\tilde{s}].$$

□

§ 4.32 (Invariance of the Gibbs energy)**.** The differential of the Gibbs energy is invariant under the above described reduction method: $dg = \mu^T dc = \tilde{\mu}^T d\tilde{c} = d\tilde{g}$.

Example 4.33. The network in Example 3.11 (Equation 3.2, p. 42) contains a stoichiometric cycle. The matrices

$$T_{J,1} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 0 \end{pmatrix}, \qquad T_{J,2} = \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}, \qquad \Lambda_J = \begin{pmatrix} +1 & -1 & 0 \\ -1 & 0 & 1 \\ 0 & 1 & 1 \end{pmatrix}$$

fulfill the conditions $T_{J,2}^T \Lambda_J T_{J,1} = 0$ and $N T_{J,2} = 0$. Here, $T_{J,1}$ is chosen such that $\tilde{J}_1 = J_1$ and $\tilde{J}_2 = J_2$. This leads to the given matrix Λ_J. A reduction with these matrices yields a system with

$$\tilde{N} = \begin{pmatrix} -1 & 0 \\ 1 & -1 \\ 0 & 1 \end{pmatrix}, \qquad \tilde{R} = \frac{1}{R_1 + R_2 + R_3} \begin{pmatrix} R_1 (R_2 + R_3) & -R_1 R_2 \\ -R_1 R_2 & R_2 (R_1 + R_3) \end{pmatrix}.$$

This system contains a minimal number of fluxes and corresponds to the system given in Equation 3.3 (p. 42).

4.2.2. Reduction of the Thermodynamic and the Kinetic Submodel

The stoichiometric reduction methods discussed in the previous section rely on rank deficiencies of the stoichiometric matrices. Further reduction methods are applicable for models with a singular derivative $\partial c/\partial\mu$ or a singular resistance matrix R. Then, the system contains algebraic relations that can be used to derive a reduced formulation of the system. These reduction methods are equivalent to the quasi-steady state and rapid equilibrium assumptions that are often used to derive kinetic laws for enzyme-catalyzed reactions.

4.2.2.1. Reduction of the Thermodynamic Submodel

For $\partial c/\partial\mu_e \, \dot{\mu}_e = 0$, the concentration change $\dot{c} = \partial c/\partial\mu \, \dot{\mu}$ is constrained to the column space of $\partial c/\partial\mu$. If the derivative $\partial c/\partial\mu$ of the function $c(\mu, \mu_e)$ gets singular, certain concentration changes $\dot{c}$ are impossible. Concentration changes $\dot{c}$ that are not in span$(\partial c/\partial\mu)$ would correspond to an infinite change of the chemical potentials $\dot{\mu}$. This means that infinite thermodynamic forces would counteract the concentration change. In consequence, such concentration changes cannot occur and the fluxes that produce and consume the respective compounds are always equal. This case is equivalent to the classical quasi-steady state assumption for concentrations that, for example, is applied when deriving the Michaelis-Menten kinetics (see [45, 94]). If $\partial c/\partial\mu$ is singular and $\partial c/\partial\mu_e\,\mu_e \neq 0$, the concentration change $\dot{c}$ is restricted to the linear set $\mathrm{span}(\partial c/\partial\mu) - \partial c/\partial\mu_e\,\dot{\mu}_e$ and similar considerations can be made to reduce the system.

§ 4.34 (Singular $\partial c/\partial\mu$)**.** In an ideal dilute solution we have $c_i = c^\circ \exp((\mu_i - \mu_i^\circ)/(R^*\,T))$. The matrix $\partial c/\partial\mu$ is diagonal with diagonal elements $\partial c_i/\partial\mu_i = c_i/(R^*\,T)$ (see §3.16, p. 44). For $c_i > 0$ the matrix $\partial c/\partial\mu$ is always invertible. If one or several concentrations c_i are very small compared to the fluxes, one can approximate $c_i(\mu_i) = 0$ for all occurring μ_i. Then $\partial c/\partial\mu$ can be approximated by a singular matrix. The corresponding chemical potentials μ_i are no longer in a one to one relationship to the concentrations c_i because the functions $c_i(\mu_i) = 0$ are not invertible. Although the concentrations have a constant value of zero, the corresponding chemical potentials may vary. The assumption of small concentrations is also the basis of the classical *quasi-steady state assumption* in the conventional kinetic modeling formalism. Thus, the assumption of a singular $\partial c/\partial\mu$ implies the quasi-steady state assumption in the classical approach to kinetic modeling.

Example 4.35 (Singular $\partial c/\partial\mu$)**.** Consider the network $X \overset{J_1}{\rightleftharpoons} A \overset{J_2}{\rightleftharpoons} Y$. The compound A is very unstable. This means that A is very reactive and μ_A° is very large, such that we can approximate $c_A(\mu) = 0$. This leads to $\partial c_A/\partial\mu_A = 0$ and thus to $\dot{c}_A = J_1 - J_2 = 0$. The singularity of $\partial c/\partial\mu$ leads to a linear, algebraic constraint on the fluxes that is equivalent to the assumption of a quasi-stationary c_A.

Before developing the main corollary of this section that allows the reduction of the thermodynamic submodel, we have to discuss the existence and uniqueness of solutions with singular $\partial c/\partial\mu$.

Corollary 4.36 (Existence of a solution)**.** *Let M be a thermodynamic model with a positive semi-definite $\partial c/\partial\mu$. A necessary condition on the inputs μ_e and J_e for the existence of a solution at μ, μ_e and J_e is*

$$N_e\,J_e - \frac{\partial c}{\partial\mu_e}\,\dot{\mu}_e \in \mathrm{span}\left(\frac{\partial c}{\partial\mu}, N\right).$$

Proof. Due to the equation $\dot{c} = N\,J + N_e\,J_e$ we have that $N_e\,J_e - \partial c/\partial\mu_e\,\dot{\mu}_e = \partial c/\partial\mu\,\dot{\mu} - N\,J$. Thus, for any inputs J_e and μ_e that do not fulfill the given condition, the system equation $\dot{c} = N\,J + N_e\,J_e$ cannot be fulfilled. □

Definition 4.37 (Thermodynamically conflict-free networks)**.** A thermodynamic system M with

$$\mathrm{span}\left(N_e, \frac{\partial c}{\partial\mu_e}\right) \subseteq \mathrm{span}\left(\frac{\partial c}{\partial\mu}, N\right).$$

for all μ and μ_e is called *thermodynamically conflict-free.*

§ 4.38 (Interpretation)**.** If a thermodynamic model is *thermodynamically conflict-free*, the condition for the existence of the solution given in Corollary 4.36 is always fulfilled. However, the condition in Corollary 4.36 is only *necessary* for the existence of a solution. For example, it guarantees that the clamped fluxes J_e can stoichiometrically be balanced by the internal fluxes J. It does not guarantee that these fluxes can be realized with the given functions $R(\mu, \mu_e)$ and $c(\mu, \mu_e)$.

Example 4.39 (Conflicts of clamped fluxes J_e and the quasi-steady state assumption)**.** Corollary 4.36 means that the quasi-steady state assumption may lead to conflicts with clamped fluxes. An example will clarify this. Consider the system $0 \overset{(J_e)}{\rightleftharpoons} A \overset{J_1}{\rightleftharpoons} B$ with the functions $c_A(\mu, \mu_e) = 0$ and $c_B(\mu, \mu_e) = 0$ and a clamped flux $J_e \neq 0$. We have $N_e = (1, 0)^T$, $N = (-1, 1)^T$ and $\partial c/\partial \mu = 0$. Thus, the necessary condition for the existence of a solution given in Corollary 4.36 is only fulfilled for $J_e = 0$. Due to $c_A = c_B = 0$, we have that $\dot{c}_A = \dot{c}_B = 0$ for all times t. Because we have also that $\dot{c}_A = J_e - J_1$ and $\dot{c}_B = J_1$, we get the constraints $J_e = J_1 = 0$. Thus, the system equations are not solvable for a $J_e \neq 0$.

§ 4.40 (Resolving conflicts)**.** Conflicts of the inputs J_e and μ_e with the quasi-steady state assumption occur if the system is not thermodynamically conflict-free. There are two possibilities for resolving possible conflicts: (1) Unclamping of a flux means to move a column of the matrix N_e to the matrix N. Thus, the space $\operatorname{span}(\partial c/\partial \mu, N)$ gets larger and the space $\operatorname{span}(N_e, \partial c/\partial \mu_e)$ gets smaller. (2) If $\partial c/\partial \mu$ is diagonal, one may set a state function $c_i(\mu, \mu_e)$ to a non-singular value, i. e. $\partial c_i/\partial \mu_i \neq 0$. Then, the space $\operatorname{span}(\partial c/\partial \mu, N)$ gets larger, but the space $\operatorname{span}(N_e, \partial c/\partial \mu_e)$ does not change. Thus, by correcting the model in these ways, one eventually arrives at a structurally conflict-free system description with $\operatorname{span}(N_e, \partial c/\partial \mu_e) \subseteq \operatorname{span}(\partial c/\partial \mu, N)$. Then, no conflicts are possible.

Example 4.41 (Resolving conflicts of J_e)**.** In Example 4.39, a solution exists only for $J_e = 0$. This means that the system description imposes constraints on possible inputs J_e. Such constraints are not consistent with the usual systems-theoretical paradigm that inputs can be chosen independently of each other and independently of the state of the system. Most likely such models occur only due to modeling errors since the assumption of an independent clamping of two rigidly coupled variables is contradictory. Such situations can be easily resolved by either (1) unclamping one of the affected fluxes or (2) choosing a function $c_i(\mu, \mu_e)$ with a non-zero $\partial c/\partial \mu$ for one of the affected compounds. Which of these possibilities is more appropriate depends on the observed system dynamics. We discuss both possibilities for the Example 4.39: (1) An unclamping of J_e can be performed by extending the system to $(X) \overset{J_2}{\rightleftharpoons} A \overset{J_1}{\rightleftharpoons} B$. The compound X has a clamped potential $\mu_{e,X}$. The reaction flux J_2 replaces the clamped reaction flux J_e of the original Example 4.39. The flux J_2 is no longer a input of the system but an internal flux. Since $c_A = c_B = 0$, $\dot{c}_A = J_2 - J_1 = 0$ and $\dot{c}_B = J_1 = 0$ we get $J_1 = J_2 = 0$ and with $R\,J = \Delta\mu$ we get $\Delta\mu = 0$ and $\mu_A = \mu_B = \mu_X$. Thus, the solution of the modified system exists. (2) Alternatively, we may also introduce a non-singular function for either c_A or c_B. For example, we may use the functions $c_A(\mu, \mu_e) = c^\circ \exp((\mu_A - \mu_A^\circ)/(R^*\,T))$ and $c_B(\mu, \mu_e) = 0$.

Because $\dot{c}_B = J_1 = 0$ we get the constraint $J_1 = 0$. For the compound A, we get the equation $\dot{c}_A = J_e - J_1 = J_e$, which can be solved to $c_A = c_{A,0} + \int_0^t J_e \, dt$. Thus, the solution of the modified system exists.

§ 4.42 (Consistent initial conditions)**.** As discussed above, we get algebraic constraints on the fluxes J, if $\partial c/\partial \mu$ does not have full rank. Since $R(\mu, \mu_e)\, J = \Delta\mu$, this directly leads to constraints for the chemical potentials μ and in particular also for the initial chemical potentials μ_0. Multiplying the equation $\dot{c} = \partial c/\partial \mu \, \dot{\mu} + \partial c/\partial \mu_e \, \dot{\mu}_e = N\, J + N_e\, J_e$ with a kernel matrix $T_{c,2}$ with $T_{c,2}\, \partial c/\partial \mu = 0$ we get

$$T_{c,2} \, \frac{\partial c}{\partial \mu_e} \, \dot{\mu}_e = T_{c,2}\, N \, \underbrace{(R(\mu, \mu_e))^{-1} \, (-N^T \mu - S^T \mu_e)}_{J} + T_{c,2}\, N_e\, J_e \tag{4.3}$$

Valid chemical potentials μ and initial conditions μ_0 have to lie in the set defined by this equation. Since this equation is non-linear, it can be rather hard for the modeler to manually find suited initial conditions. Usually consistent initial conditions have to be computed numerically.

Example 4.43. Consider the network $(A) \overset{1}{\rightleftharpoons} B \overset{2}{\rightleftharpoons} C \overset{3}{\rightleftharpoons} (D)$ where $c_B(\mu, \mu_e) = 0$ and $c_C(\mu, \mu_e) = c^\circ \exp((\mu_C - \mu_C^\circ)/(R^*\, T))$. We have that $J_1 = J_2$ because $c_B = 0$ and thus $\dot{c}_B = J_1 - J_2 = 0$. Assuming mass-action kinetics we get the equation

$$(\mu_{e,A} - \mu_B)/R_1(\mu, \mu_e) = (\mu_B - \mu_C)/R_2(\mu, \mu_e).$$

where

$$R_1 = \rho_1\, R_\circ(\mu_{e,A}/(R^*\, T), \mu_B/(R^*\, T)), \qquad R_2 = \rho_2\, R_\circ(\mu_B/(R^*\, T), \mu_C/(R^*\, T))$$

and $R_\circ$ is the ideal resistance function (see Definition 3.22, p. 47). We can solve this equation to

$$\mu_B = R^*\, T\, \log\left(\frac{\exp\left(\frac{\mu_C}{R^*\, T}\right)\, \rho_1 + \exp\left(\frac{\mu_{e,A}}{R^*\, T}\right)\, \rho_2}{\rho_1 + \rho_2}\right).$$

Thus, the chemical potential of B is determined by the chemical potentials of A and C. In particular, μ_B is between μ_A and μ_C. For $\rho_1 \to 0$, we have $\mu_B \to \mu_A$ and for $\rho_2 \to 0$, we have $\mu_B \to \mu_C$.

§ 4.44 (Existence and uniqueness of the chemical potentials)**.** Equation 4.3 is an equation for the chemical potentials. If this equation has no solution, no vector μ exists that is consistent with the thermodynamic model. If the network is not thermodynamically conflict-free such situations occur for almost all inputs μ_e and J_e (see Example 4.39). Nevertheless, in a thermodynamically conflict-free network the existence of a solution is not guaranteed either. The equation can also have more than one solution. Then, the trajectory of the model equations is not unique. The following examples illustrate such cases.

Example 4.45 (Non-existing solution in a thermodynamically conflict-free model)**.** Consider a system $0 \xrightleftharpoons{(J_e)} A \overset{J}{\rightleftharpoons} (B)$ with the clamped quantities J_e and $\mu_{e,B}$. Assume the function $c_A(\mu_A) = 0$ and the resistance

$$R = \left(\rho_0 + \rho_A \exp\left(\frac{\mu_A}{R^*\,T}\right) + \rho_B \exp\left(\frac{\mu_B}{R^*\,T}\right)\right) \cdot R_\circ\left(\frac{\mu_A}{R^*\,T}, \frac{\mu_B}{R^*\,T}\right)$$

The given resistance realizes a reversible Michaelis-Menten kinetics (see Example 3.24, p. 48). We have $N = -1$, $N_e = 1$, $S = 1$ and $\partial c/\partial\mu = 0$. This leads to the constraint $J = J_e$. The system is thermodynamically conflict-free because from a stoichiometric view point J can balance J_e. However, it cannot balance high fluxes J_e because the flux J saturates for high chemical potentials of A. Equation 4.3 for the example system reads

$$0 = -\underbrace{\frac{\exp\left(\frac{\mu_A}{R^*\,T}\right) - \exp\left(\frac{\mu_{e,B}}{R^*\,T}\right)}{\frac{\mu_A}{R^*\,T} - \frac{\mu_{e,B}}{R^*\,T}} \left(\rho_0 + \rho_A \exp\left(\frac{\mu_A}{R^*\,T}\right) + \rho_B \exp\left(\frac{\mu_{e,B}}{R^*\,T}\right)\right)^{-1}}_{R^{-1}} (\mu_A - \mu_{e,B}) + J_e.$$

This can be solved to

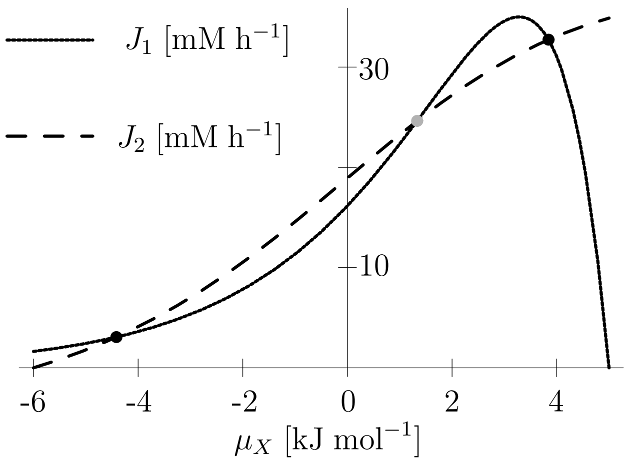
$$\mu_A = R^*\,T \log\left(\frac{\rho_0\,J_e + (\rho_B\,J_e + 1)\exp\left(\frac{\mu_{e,B}}{R^*\,T}\right)}{1 - J_e\,\rho_A}\right).$$

A real solution for μ_A only exists if $-\xi_B/(\rho_0 + \rho_B\,\xi_B) < J_e < \rho_A^{-1}$ where $\xi_B = \exp(\mu_{e,B}/(R^*\,T))$, i. e. if J_e is between the minimal and maximal value of J.

Example 4.46 (Infinitely many solutions)**.** Consider the system $A + (C) \rightleftharpoons B + (D)$ with $R > 0$. It contains a conservation relation $c_A + c_B = c_{A,0} + c_{B,0}$. Thus, its steady state depends on the initial concentrations. The steady state is characterized by $\dot{c}_A = -\dot{c}_B = -J = 0$ and thus $\Delta\mu = \mu_{e,C} - \mu_{e,D} + \mu_A - \mu_B = 0$. This equation only defines the difference $\mu_A - \mu_B$. By using the conservation relation $c_A(\mu, \mu_e) + c_B(\mu, \mu_e) = c_{A,0} + c_{B,0}$ we can uniquely compute the steady state potentials μ_A and μ_B. Now, assume a quasi-steady state for A and B with $c_A(\mu, \mu_e) = 0$ and $c_B(\mu, \mu_e) = 0$. We have again that $\dot{c}_A = -\dot{c}_B = -J = 0$ and thus $\Delta\mu = \mu_{e,C} - \mu_{e,D} + \mu_A - \mu_B = 0$. However, this equation cannot be solved uniquely since the conservation relation is trivially fulfilled. Thus, by using the quasi-steady state assumption with $c_i(\mu, \mu_e) = 0$ for all concentrations in a conservation relation, we lose the uniqueness of the corresponding chemical potentials. Since other parts of the model may depend on the value of these potentials, such a model cannot be easily simulated.

Example 4.47 (Finitely many solutions – Edelstein system)**.** Consider the system consisting of the reactions

$$(A) + X \overset{1}{\rightleftharpoons} 2X, \qquad\qquad X \overset{2}{\rightleftharpoons} (B)$$



$T = 298.15$ K
$\rho_1 = 1$ h mM^{-1} kJ mol^{-1}
$\rho_{2,0} = 0.08$ h mM^{-1} kJ mol^{-1}
$\rho_{2,X} = 0.08$ h mM^{-1} kJ mol^{-1}
$\rho_{2,B} = 0.08$ h mM^{-1} kJ mol^{-1}
$\mu_{e,A} = 5$ kJ mol^{-1}
$\mu_{e,B} = -3$ kJ mol^{-1}

Figure 4.1.: Multiple quasi-steady states of the Edelstein system (Example 4.47). The quasi-steady state condition $\dot{c}_X = J_1 - J_2 = 0$ is fulfilled for three values of μ_X. The two black dots correspond to statically stable points, whereas the gray dot corresponds to a statically unstable point.

(adapted from Edelstein [30]). Reaction 1 and 2 are modeled with mass-action and Michaelis-Menten kinetics, respectively:

$$R_1 = \rho_1 \, R_\circ \left(\frac{\mu_{e,A} + \mu_X}{R^* \, T}, \frac{2 \, \mu_X}{R^* \, T} \right),$$
$$R_2 = R_\circ \left(\frac{\mu_X}{R^* \, T}, \frac{\mu_{e,B}}{R^* \, T} \right) \left(\rho_{2,0} + \rho_{2,X} \, \exp\left(\frac{\mu_X}{R^* \, T} \right) + \rho_{2,B} \, \exp\left(\frac{\mu_{e,B}}{R^* \, T} \right) \right).$$

We assume a quasi-steady state with $c_X(\mu, \mu_e) = 0$. Then, for certain parameters and inputs, equation Equation 4.3 has three solutions. Figure 4.1 shows a plot of the fluxes J_1 and J_2 over μ_X for specific parameter values. The quasi-steady state condition $\dot{c}_X = J_1 - J_2 = 0$ is fulfilled at three distinct values of μ_X. Thus, the thermodynamic system has three distinct solutions. In this example, the stability of the three distinct solutions can be determined by a simple consideration. If a small fluctuation increases c_X and thus μ_X, the difference $J_1 - J_2$ is negative for the lower and upper steady state, but positive for the one between. Thus, the fluxes counteract a fluctuation at the lower and the upper state, but amplify a fluctuation of the middle state. Thus, the middle steady state is unstable, but the upper and the lower steady state are stable. This means that the model trajectory is not unique because two different stable quasi-steady states of c_X exist.

§ 4.48 (Assumption of a constant span$(\partial c / \partial \mu)$). For the formulation of the corollaries that describe the reduction method we assume that the span and thus also the null space of $\partial c / \partial \mu$ is constant. In ideal dilute solutions where $\partial c / \partial \mu$ is diagonal and all concentrations are strictly greater than zero, we have span$(\partial c / \partial \mu) = \mathbb{R}^{i_0}$ and the condition is obviously fulfilled. If a compound, say X_{i_0}, is reactive its diagonal entry is small and can be neglected. In this case, the space $\operatorname{span}(\partial c/\partial \mu) = \operatorname{span}(e_1 \dots e_{i_0-1})$ where e_i is the i^{th} unit vector is also constant. If a system with a constant $\operatorname{span}(\partial c/\partial \mu)$ is linearly transformed (see Corollary 4.2, p. 52) then the the transformed space $\operatorname{span}(\partial \hat{c}/\partial \hat{\mu}) = \operatorname{span}(T_c\, \partial \hat{c}/\partial \hat{\mu}\, T_c^T)$ is also constant. Thus, although $\partial c/\partial \mu$ is not constant, the space spanned by the columns of $\partial c/\partial \mu$ is usually constant and a kernel matrix $T_{c,2}$ exists with $T_{c,2}\, \partial c/\partial \mu = 0$ for all μ and μ_e.

Corollary 4.49 (Quasi-steady state assumption – simplified version)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40) and let $T_J = [T_{J,1}, T_{J,2}]$ and $T_c^T = [T_{c,1}^T, T_{c,2}^T]$ be invertible matrices with*

$$T_{c,2} \frac{\partial c}{\partial \mu} = 0, \qquad T_{c,2} \frac{\partial c}{\partial \mu_e} = 0, \qquad T_{c,2}\, N_e = 0, \qquad T_{c,2}\, N\, T_{J,1} = 0, \qquad T_{c,2}\, N\, T_{J,2} = I$$

for all μ and μ_e. Then, a trajectory of M can be reconstructed from a trajectory of the reduced system $\tilde{M}$ with

$$\tilde{N} = T_{c,1}\, N\, T_{J,1}, \qquad \tilde{S} = S\, T_{J,1}, \qquad \tilde{N}_e = T_{c,1}\, N_e, \qquad \tilde{\mu}_0 = \operatorname{inv}^T_{\Lambda_c^{-1}}(T_{c,1})\, \mu_0,$$

$$\tilde{R}(\tilde{\mu}, \mu_e) = T_{J,1}^T\, R(T_{c,1}^T\, \tilde{\mu} + T_{c,2}^T\, \hat{\mu}_2(\tilde{\mu}, \mu_e), \mu_e)\, T_{J,1}, \qquad \tilde{c}(\tilde{\mu}, \mu_e) = T_{c,1}\, c(T_{c,1}^T\, \tilde{\mu}, \mu_e)$$

where $\hat{\mu}_2(\tilde{\mu}, \mu_e)$ is a solution of

$$0 = T_{c,2}\, N\, (R(T_{c,1}^T\, \tilde{\mu} + T_{c,2}^T\, \hat{\mu}_2))^{-1}\, (-N^T\, (T_{c,1}^T\, \tilde{\mu} + T_{c,2}^T\, \hat{\mu}_2) - S^T \mu_e)$$

and Λ_c is a symmetric, invertible matrix with $T_{c,1}\, \Lambda_c\, T_{c,2}^T = 0$. The variables of the original system and the reduced system are related by

$$\begin{aligned} \mu &= T_{c,1}^T\, \tilde{\mu} + T_{c,2}^T\, \hat{\mu}_2(\tilde{\mu}, \mu_e), & c &= c(T_{c,1}^T\, \tilde{\mu}, \mu_e), \\ \Delta\mu &= -N^T\, \mu - S^T\, \mu_e, & J &= T_{J,1}\, \tilde{J}. \end{aligned}$$

Proof. Transform M with the given partitioned matrices T_c and T_J (see §4.7, p. 53). The system M_1 corresponds to the reduced system $\tilde{M}$. Premultiplying the equation $\dot{c} = \partial c/\partial \mu\, \dot{\mu} + \partial c/\partial \mu_e\, \dot{\mu}_e = N\, J + N_e\, J_e$ with $T_{c,2}$ yields $T_{c,2}\, N\, J = 0$. With $J = R^{-1} \cdot (-N^T\, \mu - S^T\, \mu_e)$ and $\mu = T_{c,1}^T\, \hat{\mu}_1 + T_{c,2}^T\, \hat{\mu}_2$ we get the expression defining the relation $\hat{\mu}_2(\tilde{\mu}, \mu_e)$. Substituting $J = T_{J,1}\, \hat{J}_1 + T_{J,2}\, \hat{J}_2$ in $T_{c,2}\, N\, J = 0$ yields $\hat{J}_2 = 0$. Now, the reduced model equations and the expressions for the reconstruction of the original from the reduced variables directly follow from §4.7 and §4.8 (p. 53). The result is simplified by using the relation $c(T_{c,1}^T\, \tilde{\mu} + T_{c,2}^T\, \hat{\mu}_2, \mu_e) = c(T_{c,1}^T\, \tilde{\mu}, \mu_e)$ that follows from $\partial c/\partial \mu\, T_{c,2}^T = 0$. □

§ 4.50 (Generalization)**.** The assumptions that $T_{c,2}\, \partial c/\partial \mu_e = 0$ and $T_{c,2}\, N_e = 0$ in the above corollary mean that the quasi-stationary compounds are not directly influenced by clamped fluxes or potentials. These assumptions simplify the formulation of the corollary because they lead to $\hat{J}_2 = 0$. If $T_{c,2}\, \partial c/\partial \mu_e \neq 0$ and $T_{c,2}\, N_e \neq 0$ premultiplying $\dot{c} = \partial c/\partial \mu\, \dot{\mu} + \partial c/\partial \mu_e\, \dot{\mu}_e = N\, J + N_e\, J_e$ with $T_{c,2}$ and substituting $J = T_{J,1}\, \hat{J}_1 + T_{J,2}\, \hat{J}_2$ yields $\hat{J}_2 = T_{c,2}\, \partial c/\partial \mu_e\, \dot{\mu}_e - T_{c,2}\, N_e\, J_e \neq$

0. The reduction method in the above corollary relies on the condition that $\hat{J}_2 = 0$ but it can be generalized. The generalized method that is introduced in the next corollary extends the vector of clamped potentials and clamped fluxes. The additional clamped fluxes are the fluxes associated with a change of the clamped chemical potentials $\dot{\mu}_e$ and the additional clamped potentials are the potentials that are associated with the clamping of the fluxes J_e.

Corollary 4.51 (Quasi-steady state assumption)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40) and let $T_J = [T_{J,1}, T_{J,2}]$ and $T_c^T = [T_{c,1}^T, T_{c,2}^T]$ be invertible matrices with*

$$T_{c,2}\,\frac{\partial c}{\partial \mu} = 0, \qquad\qquad T_{c,2}\,N\,T_{J,1} = 0, \qquad\qquad T_{c,2}\,N\,T_{J,2} = I$$

for all possible μ. Then, a trajectory of M can be reconstructed from a trajectory of the reduced system $\tilde{M}$ with

$$\tilde{N} = T_{c,1}\,N\,T_{J,1}, \qquad\qquad \tilde{S}^T = (T_{J,1}^T\,S^T, I), \qquad\qquad \tilde{\mu}_0 = \mathrm{inv}^T_{\Lambda_c^{-1}}(T_{c,1})\,\mu_0,$$

$$\tilde{N}_e = \big(T_{c,1}\,(I - N\,T_{J,2}\,T_{c,2})\,N_e, \quad T_{c,1}\,N\,T_{J,2}\,T_{c,2}\big)$$

$$\tilde{R}(\tilde{\mu}, \mu_e, \dot{\mu}_e, J_e) = T_{J,1}^T\,R(T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2(\tilde{\mu}, \mu_e, \dot{\mu}_e, J_e))\,T_{J,1}, \qquad \tilde{c}(\tilde{\mu}, \mu_e) = T_{c,1}\,c(T_{c,1}^T\,\tilde{\mu}, \mu_e)$$

where $\hat{\mu}_2(\tilde{\mu}, \mu_e, \dot{\mu}_e, J_e)$ is a solution of

$$T_{c,2}\,\frac{\partial c}{\partial \mu_e}\,\dot{\mu}_e = T_{c,2}\,N\,(R(T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2))^{-1}\,(-N^T\,(T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2) - S^T\,\mu_e) + T_{c,2}\,N_e\,J_e,$$

Λ_c is a symmetric, invertible matrix with $T_{c,1}\,\Lambda_c\,T_{c,2}^T = 0$ and

$$\tilde{\mu}_e = \begin{pmatrix} \mu_e \\ T_{J,1}^T\,R\,T_{J,2}\,T_{c,2}\,(N_e\,J_e - \frac{\partial c}{\partial \mu_e}\,\dot{\mu}_e) \end{pmatrix}, \qquad\qquad \tilde{J}_e = \begin{pmatrix} J_e \\ \frac{\partial c}{\partial \mu_e}\,\dot{\mu}_e \end{pmatrix}$$

The variables of the original system and the reduced system are related by

$$\begin{aligned} \mu &= T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2, & c &= c(T_{c,1}^T\,\tilde{\mu}, \mu_e), \\ \Delta\mu &= -N^T\,\mu - S^T\,\mu_e, & J &= T_{J,1}\,\tilde{J} + T_{J,2}\,T_{c,2}\,(\partial c/\partial \mu_e\,\dot{\mu}_e - N_e\,J_e). \end{aligned}$$

Proof. Transform M with the given partitioned matrices T_c and T_J (see §4.7, p. 53). As discussed in §4.50 we get $\hat{J}_2 = T_{c,2}\,(\partial c/\partial \mu_e\,\dot{\mu}_e - N_e\,J_e)$. With this one can verify that the model equations for the subsystem M_1 with subscript 1 are equivalent to the model equations of the reduced system $\tilde{M}$ with $\Delta\tilde{\mu} = \Delta\hat{\mu}_1 - (T_{J,1}^T\,R\,T_{J,2})\,\hat{J}_2$. The result is simplified by using the relation $c(T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2, \mu_e) = c(T_{c,1}^T\,\tilde{\mu}, \mu_e)$ that follows from $\partial c/\partial \mu\,T_{c,2}^T = 0$. □

§ 4.52 (Symmetry and positive semi-definiteness of $\tilde{R}$ and $\partial\tilde{c}/\partial\tilde{\mu}$)**.** The matrices $\tilde{R} = T_{J,1}^T\,R\,T_{J,2}$ and $\partial\tilde{c}/\partial\tilde{\mu} = T_{c,1}\,\partial c/\partial \mu\,T_{c,1}^T$ are symmetric and positive semi-definite if R and $\partial c/\partial \mu$ are symmetric and positive semi-definite.

§ 4.53 (Computation of $T_{c,1/2}$, $T_{J,1/2}$ and Λ_c)**.** Suited transformation matrices can be computed by the following steps: (1) compute $T_{c,2}$ as a left kernel matrix of $\partial c/\partial \mu$ with $T_{c,2}\, \partial c/\partial \mu = 0$; (2) choose an arbitrary invertible Λ_c, e. g. $\Lambda_c = I$; (3) compute $T_{c,1}^T$ as a right kernel matrix of $T_{c,2}\, \Lambda_c$ with $T_{c,2}\, \Lambda_c\, T_{c,1}^T = 0$; (4) compute $T_{J,1}$ as a right kernel matrix of $T_{c,2}\, N$ with $T_{c,2}\, N\, T_{J,1} = 0$; (5) compute $T_{J,2}$ such that $T_{c,2}\, N\, T_{J,2} = I$, e. g. $T_{J,2} = \mathrm{inv}(T_{c,2}\, N)$. Step (5) can only be conducted if $T_{c,2}\, N$ has full row rank.

§ 4.54 (The condition $T_{c,2}\, N\, T_{J,2} = I$)**.** If the matrix $T_{c,2}\, N$ does not have full row rank, the Corollaries 4.49 and 4.51 are not applicable because they require the existence of a matrix $T_{J,2}$ with $T_{c,2}\, N\, T_{J,2} = I$. If the matrix $T_{c,2}\, N$ does not have full row rank, a conserved moiety of N is completely in a quasi-steady state. In this case, either no trajectories exist (cf. Corollary 4.36, p. 64, and Example 4.39, p. 65) or the trajectories are not unique (cf. Example 4.46, p. 67). The formulation of a reduction method does not make sense for such degenerated cases.

Example 4.55 (Reduction of the thermodynamic submodel)**.** Consider the network $0 \overset{(J_e)}{\rightleftharpoons} X_2 \overset{J_2}{\rightleftharpoons} X_1 \overset{J_1}{\rightleftharpoons} (A)$ with $c_{X_2}(\mu, \mu_e) = 0$ and $c_{X_1} = c^\circ \exp((\mu_1 - \mu_1^\circ)/(R^*\, T))$. The matrices describing the system are

$$R = \begin{pmatrix} R_1 & R_{12} \\ R_{12} & R_2 \end{pmatrix}, \qquad N = \begin{pmatrix} -1 & 1 \\ 0 & -1 \end{pmatrix}, \qquad N_e = \begin{pmatrix} 0 \\ 1 \end{pmatrix}, \qquad S = \begin{pmatrix} 1 & 0 \end{pmatrix}.$$

For the sake of simplicity we assume that the resistance matrix is constant. With this non-diagonal resistance matrix the fluxes are defined by $J_1 = (-R_{12}\, \Delta\mu_2 + R_2\, \Delta\mu_1)/(R_1\, R_2 - R_{12}^2)$ and $J_2 = (-R_{12}\, \Delta\mu_1 + R_1\, \Delta\mu_2)/(R_1\, R_2 - R_{12}^2)$. We apply Corollary 4.49 (p. 69) with $T_{c,1} = (1, 0)$, $\Lambda_c = I$, $T_{c,2} = (0, 1)$, $T_{J,1}^T = (1, 0)$ and $T_{J,2}^T = (0, -1)$. We get $\tilde{\mu} = \hat{\mu}_1 = \mu_{X_1}$ and $\hat{\mu}_2 = \mu_{X_2}$. The chemical potential $\hat{\mu}_2 = \mu_{X_2}$ is given by the equation $0 = -J_2 + J_e = -(-R_{12}\, \Delta\mu_1 + R_1\, \Delta\mu_2)/(R_1\, R_2 - R_{12}^2) + J_e$ that can be solved to $\hat{\mu}_2 = \tilde{\mu} + R_2\, J_e - R_{12}\, R_1^{-1}\, (R_{12}\, J_e - \tilde{\mu} + \mu_{e,A})$. The reduced system matrices are $\tilde{N} = -1$, $\tilde{N}_e = (1, 0, -1)$, $\tilde{S} = 1$ and $\tilde{R} = R_1$. The new input vectors are $\tilde{J}_e^T = (J_e, 0, 0)$ and $\tilde{\mu}_e^T = (\mu_{e,A}, -R_{12}\, J_e)$. The new clamped fluxes introduced by the reduction are zero and can be omitted. The reduced system contains an additional clamped potential $-R_{12}\, J_e$ that depends on the clamped flux J_e. Due to the quasi-steady state of X_2 the flux via reaction 2 is effectively clamped: $J_2 = J_e$. Because the resistance matrix R contains off-diagonal elements, the clamped flux J_e has a direct influence on reaction 1. This influence can be modeled by introducing an additional clamped potential. Usually the resistance matrix is diagonal, i. e. $R_{12} = 0$. Then, an extension of the clamped vectors is not necessary since the additional inputs are zero. In this cases the reduced system corresponds to the reaction network $0 \overset{J_e}{\rightleftharpoons} \tilde{X}_1 \overset{\tilde{J}}{\rightleftharpoons} (A)$.

§ 4.56 (Transformation of the entropy production)**.** The entropy production of the internal fluxes is *not* invariant under the above described reduction method. In Example 4.55 with $R_{12} = 0$, the original entropy production is $\sigma[s] = (\mu_{X_2} - \mu_{X_1})\, J_2 + (\mu_{X_1} - \mu_{e,A})\, J_1$ whereas in the reduced system it is $\sigma[\tilde{s}] = (\tilde{\mu} - \mu_{e,A})\, \tilde{J} = (\mu_{X_1} - \mu_{e,A})\, J_1 \leq \sigma[s]$. The reason for this difference is that certain fluxes (in the example J_2) are directly determined by the boundary conditions and thus lose their status as internal fluxes.

§ 4.57 (Invariance of the Gibbs energy)**.** The Gibbs energy is invariant under the reduction in Corollaries 4.49 and 4.51: $dg = \mu^T\, dc = (\tilde{\mu}^T\, T_{c,1} + \hat{\mu}_2^T\, T_{c,2})\, dc = \tilde{\mu}^T\, T_{c,1}\, dc = \tilde{\mu}^T\, d\tilde{c} = d\tilde{g}$ because $T_{c,2}\, dc = 0$.

Example 4.58 (Michaelis-Menten kinetics)**.** Example 4.46 (p. 67) shows that the solution of a thermodynamic model is not unique if all compounds X_i in a conservation relation are in quasi-steady state. In this case no matrix $T_{J,2}$ with $T_{c,2}\, N\, T_{J,2} = I$ exists (see §4.54, p. 71). However, such a situation occurs when deriving enzyme kinetics where the free enzyme and the enzyme complexes are quasi-stationary. Such cases can be treated by first applying a reduction of conservation relations (see Corollary 4.21, p. 58) to the original system with a non-singular $\partial c/\partial \mu$. In the resulting, reduced system, one approximates $\partial c/\partial \mu$ by a singular matrix and applies Corollary 4.49 (p. 69) or Corollary 4.51 (p. 70) to reduce the system. We will demonstrate this procedure by means of an example.

Consider the reaction system $(A) + E \overset{J_1}{\rightleftharpoons} EA \overset{J_2}{\rightleftharpoons} (B) + E$ that describes the overall reaction $A \rightleftharpoons B$ catalyzed by the enzyme E. The compounds are in an ideal dilute solution $c_i = c^\circ \exp((\mu_i - \mu_i^\circ)/(R^*\, T))$ for $i \in \{E, EA\}$. The two reactions can be described by mass-action kinetics, i. e. the resistance matrix is diagonal with diagonal elements $R_1 = \rho_1\, R_\circ((\mu_A + \mu_E)/(R^*\, T), \mu_{EA}/(R^*\, T))$ and $R_2 = \rho_2\, R_\circ(\mu_{EA}/(R^*\, T), (\mu_B + \mu_E)/(R^*\, T))$. With $c^T = (c_E, c_{EA})^T$ and $\mu_e = (\mu_{e,A}, \mu_{e,B})$ the stoichiometric matrices are

$$N = \begin{pmatrix} -1 & +1 \\ +1 & -1 \end{pmatrix}, \qquad S = \begin{pmatrix} -1 & 0 \\ 0 & 1 \end{pmatrix}.$$

We apply Corollary 4.21 (p. 58) with $T_{c,2} = (1, 1)$ and $T_{c,1} = (0, 1)$. This yields $\mu_E = \hat{\mu}_2$ and $\mu_{EA} = \tilde{\mu} + \hat{\mu}_2$. The conservation relation

$$c_E + c_{EA} = c^\circ \exp((\hat{\mu}_2 - \mu_E^\circ)/(R^*\, T)) + c^\circ \exp((\tilde{\mu} + \hat{\mu}_2 - \mu_{EA}^\circ)/(R^*\, T)) = c_E^{tot} = \text{const}$$

can be solved to

$$\hat{\mu}_2(\tilde{\mu}, \mu_e) = R^*\, T \log\left(\frac{c_E^{tot}/c^\circ}{\exp(-\mu_E^\circ/(R^*\, T)) + \exp((\tilde{\mu} - \mu_{EA}^\circ)/(R^*\, T))}\right).$$

Substituting these results into the equations $\tilde{c} = c_{EA}$ and $\tilde{R}_1 = R_1$ and $\tilde{R}_2 = R_2$ yields

$$\tilde{c}(\tilde{\mu}, \mu_e) = c_E^{tot}\, \frac{\exp((\tilde{\mu} - \mu_{EA}^\circ)/(R^*\, T))}{\exp((-\mu_E^\circ)/(R^*\, T)) + \exp((\tilde{\mu} - \mu_{EA}^\circ)/(R^*\, T))}$$

and

$$\tilde{R}_1(\tilde{\mu}, \mu_e) = \rho_1 \cdot \frac{c^\circ}{c_E^{tot}} \cdot \left(\exp\left(\frac{-\mu_E^\circ}{R^*\, T}\right) + \exp\left(\frac{\tilde{\mu} - \mu_{EA}^\circ}{R^*\, T}\right)\right) \cdot R_\circ\left(\frac{\mu_{e,A}}{R^*\, T}, \frac{\tilde{\mu}}{R^*\, T}\right),$$

$$\tilde{R}_2(\tilde{\mu}, \mu_e) = \rho_2 \cdot \frac{c^\circ}{c_E^{tot}} \cdot \left(\exp\left(\frac{-\mu_E^\circ}{R^*\, T}\right) + \exp\left(\frac{\tilde{\mu} - \mu_{EA}^\circ}{R^*\, T}\right)\right) \cdot R_\circ\left(\frac{\tilde{\mu}}{R^*\, T}, \frac{\mu_{e,B}}{R^*\, T}\right).$$

The reduced stoichiometric matrices are $\tilde{N} = T_{c,1}\,N = (-1, 1)$ and $\tilde{S} = S$. The reduced system can be written as a reaction equation $(A) \overset{J_1}{\rightleftharpoons} \tilde{EA} \overset{J_2}{\rightleftharpoons} (B)$ where $\tilde{EA}$ symbolizes the compound with concentration $\tilde{c} = c_{EA}$ and chemical potential $\tilde{\mu}$.

Above we used the conservation relation $c_E + c_{EA} = c_E^{tot} = \text{const}$ to reduce the system. Now, we assume that the total enzyme concentration c_E^{tot} is very small such that we may approximate $\tilde{c}(\tilde{\mu}, \mu_e) = 0$ and thus $\partial\tilde{c}/\partial\tilde{\mu} = 0$. We apply Corollary 4.49 (p. 69) with $T_{c,1} = ()$, $T_{c,2} = 1$, $T_{J,1} = (1, 1)^T$ and $T_{J,2} = (0, 1)^T$. Thus, the reduced system does not contain an internal compound $\tilde{\tilde{N}} = ()$. The matrix $\tilde{\tilde{S}} = \tilde{S}\,T_{J,1} = (-1, 1)^T$ describes the reduced stoichiometry $(A) \rightleftharpoons (B)$. The quasi-steady state condition is

$$0 = \underbrace{(\mu_{e,A} - \tilde{\mu})/\tilde{R}_1(\tilde{\mu}, \mu_{e,A})}_{J_1} - \underbrace{(\tilde{\mu} - \mu_{e,B})/\tilde{R}_2(\tilde{\mu}, \mu_{e,A})}_{J_2}$$

and can be solved to

$$\tilde{\mu} = R^*\,T\,\log\left(\frac{\rho_1\,\exp(\mu_B/(R^*\,T)) + \rho_2\,\exp(\mu_A/(R^*\,T))}{\rho_1 + \rho_2}\right).$$

Computing the reduced resistance $\tilde{\tilde{R}}(\mu_e)$ yields

$$\begin{aligned}
\tilde{\tilde{R}}(\mu_e) &= T_{J,1}^T\,\tilde{R}\,T_{J,1} = \tilde{R}_1 + \tilde{R}_2 = \\
&= \Bigg(\overbrace{(\rho_1+\rho_2)\,\frac{c^\circ}{c_E^{tot}}\,\exp\left(\frac{-\mu_E^\circ}{R^*\,T}\right)}^{\rho_0} + \overbrace{\rho_2\,\frac{c^\circ}{c_E^{tot}}\,\exp\left(\frac{-\mu_{EA}^\circ}{R^*\,T}\right)}^{\rho_A}\,\exp\left(\frac{\mu_{e,A}}{R^*\,T}\right) \\
&\qquad + \underbrace{\rho_1\,\frac{c^\circ}{c_E^{tot}}\,\exp\left(\frac{-\mu_{EA}^\circ}{R^*\,T}\right)}_{\rho_B}\,\exp\left(\frac{\mu_{e,B}}{R^*\,T}\right)\Bigg)\cdot R_\circ\left(\frac{\mu_{e,A}}{R^*\,T}, \frac{\mu_{e,B}}{R^*\,T}\right)
\end{aligned}$$

This resistance is equivalent to the resistance of a reversible Michaelis-Menten kinetics that was derived in Example 3.24 (p. 48).

4.2.2.2. Reduction of the Kinetic Submodel

If the thermodynamic resistance matrix $R(\mu, \mu_e)$ gets singular, forces $\Delta\mu$ in the null space of $R(\mu, \mu_e)$ would lead to infinite fluxes J that counteract the forces. For this reason such forces $\Delta\mu$ do not occur. Thermodynamic forces $\Delta\mu$ lie in the column space of the resistance matrix R since $\Delta\mu = R\,J$. If the resistance matrix R is singular, the forces $\Delta\mu$ and thus also the potentials μ only can lie in a subspace of $\mathbb{R}^{j_0}$ and $\mathbb{R}^{i_0}$, respectively. These constraints can be used to reduce the model. A singular resistance matrix is equivalent to the rapid-equilibrium assumption of the reaction fluxes in the null space of $R(\mu, \mu_e)$.

§ 4.59 (Singular resistance matrix)**.** For generalized mass-action kinetics, the resistance matrix R is diagonal and the diagonal elements R_j are strictly positive. Thus, R cannot be singular.

However, if some reactions proceed rapidly compared to others, i. e. their resistance R_j is very low, the matrix R will be nearly singular and can be approximated by a singular resistance matrix where certain diagonal elements are zero. If a diagonal element of the resistance matrix is zero, the corresponding force vanishes since $\Delta\mu_j = R_j\, J_j = 0$. This means that the reactants and products of reaction j are in thermodynamic equilibrium. However, the flux J_j is not zero. The assumption of a singular resistance matrix is equivalent to the rapid-equilibrium assumption in the traditional kinetic modeling approach.

We first discuss the existence and uniqueness of the solution of a system with singular resistance matrix. Then, we give a method to reduce models with singular resistance matrices R.

§ 4.60 (Column and row space of $R(\mu, \mu_e)$)**.** In an ideal dilute solution with mass-action kinetics, the resistance matrix R is diagonal and all diagonal elements are positive and thus $\operatorname{span}(R) = \mathbb{R}^{j_0}$. Since the resistance matrix R is diagonal, its row space and column space are equal: $\operatorname{span}(R) = \operatorname{span}(R^T)$. If the kinetic constants of a reaction, say reaction j_0, are very large ($k_{\pm j_0} \to \infty$), the resistance is very low ($R_{j_0} \to 0$). In the limit the column space loses one dimension and we have $\operatorname{span}(R) = \operatorname{span}(e_1, \ldots, e_{j_0-1})$, where e_j are the coordinate vectors of $\mathbb{R}^{j_0}$. Although in general R is a function of μ and μ_e, the column space of R is usually independent of μ and μ_e. This means that $\operatorname{span}(R(\mu, \mu_e)) = \operatorname{span}(R(\mu', \mu_e'))$ for all μ, μ_e, μ' and μ_e'. If a system with a constant $\operatorname{span}(R(\mu, \mu_e))$ is linearly transformed (see Corollary 4.2, p. 52) then the transformed space $\operatorname{span}(\hat{R}) = \operatorname{span}(T_J^T\, R\, T_J)$ is also constant. A constant column space of R is a common property and strongly simplifies the computations because we can use methods from linear algebra to study such problems. In particular, we can determine a constant kernel matrix $T_{J,2}$ of $R(\mu, \mu_e)$ with $R(\mu, \mu_e)\, T_{J,2} = 0$ and use this kernel matrix to compute the reduced systems. In the following paragraphs we will only consider matrices $R(\mu, \mu_e)$ with a constant column space.

Corollary 4.61 (Existence of solution)**.** *Let M be a thermodynamic system with a singular $R(\mu, \mu_e)$. A necessary condition for the existence of a solution at μ and μ_e is that*

$$S^T\, \mu_e \in \operatorname{span}(R(\mu, \mu_e), N^T).$$

Proof. Due to the equation $R\, J = \Delta\mu = -N^T\, \mu - S^T\, \mu_e$ we have that $S^T\, \mu_e = -R\, J - N^T\, \mu$. This means that the vector $S^T\, \mu_e$ lies in $\operatorname{span}(R(\mu, \mu_e), N^T)$. Thus, for any pair μ and μ_e that does not fulfill the given condition the equation $R\, J = \Delta\mu$ cannot be fulfilled. □

Example 4.62 (Conflicts among clamped potentials μ_e)**.** Corollary 4.61 states that one cannot have rapid-equilibrium between clamped potentials that are not in equilibrium. An example will clarify this. Consider a system that contains the reactions $(A) \rightleftharpoons B \rightleftharpoons (C)$ with the resistances $R_1 = R_2 = 0$. Assume that the chemical potentials of A and C are clamped and not equal $\mu_{e,A} \neq \mu_{e,C}$. Then, the equations $R_j\, J_j = \Delta\mu_j$ read $0 = \mu_{e,A} - \mu_B$ and $0 = \mu_B - \mu_{e,C}$. Thus, the assumption $R_1 = R_2 = 0$ *physically* contradicts the assumption $\mu_{e,A} \neq \mu_{e,C}$. Consequently, the corresponding model equations do not have a solution.

Example 4.63 (Resolving conflicts of μ_e)**.** In the above example a solution only exists for $\mu_{e,A} = \mu_{e,C}$. However, the clamped chemical potentials are inputs of the system. A system description that imposes constraints on the inputs is not consistent with the usual systems-theoretical paradigm that inputs can be chosen in principle independently of each other. Most likely such models only occur due to modeling errors since the assumption of an independent clamping of two rigidly coupled variables is contradictory. Such situations can be easily resolved by either (1) unclamping one of the affected potentials or (2) setting one resistance $R_j(\mu, \mu_e)$ in the conflicting pathway to a strictly positive value. Which one is more appropriate depends on the observed system dynamics. In the example this means that (1) e. g. μ_C is unclamped or (2) e. g. reaction 1 is not assumed to be in rapid equilibrium ($R_1 > 0$). In the case (1) the equality $\mu_{e,A} = \mu_C$ is enforced by the rapid-equilibrium conditions. In case (2) no equality condition between $\mu_{e,A}$ and $\mu_{e,C}$ exists.

Definition 4.64 (Kinetically conflict-free networks)**.** A thermodynamic system M with

$$\operatorname{span}(S^T) \subseteq \operatorname{span}(R(\mu, \mu_e), N^T)$$

for all μ and μ_e is called *kinetically conflict-free* since then the necessary condition from Corollary 4.61 for the existence of a solution is structurally fulfilled.

§ 4.65 (Resolving conflicts)**.** Conflicts occur if $\operatorname{span}(S^T) \supset \operatorname{span}(R(\mu, \mu_e), N^T)$, i. e. if the system is not kinetically conflict-free. As is discussed in the example, there are two possibilities for resolving possible conflicts: (1) Unclamping of potentials means to move a row of the matrix S to the matrix N. Thus, the space $\operatorname{span}(R(\mu, \mu_e), N^T)$ gets larger and the space $\operatorname{span}(S^T)$ gets smaller. (2) If one sets resistances R_j of reactions in a conflicting pathway to strictly positive values, the $\operatorname{span}(R(\mu, \mu_e), N^T)$ gets larger, but the space $\operatorname{span}(S^T)$ does not change. Thus, by correcting the model in these ways, one eventually arrives at a structurally conflict-free system description with $\operatorname{span}(S^T) \subseteq \operatorname{span}(R(\mu, \mu_e), N^T)$.

§ 4.66 (Assumption of non-conflicting μ_e)**.** We discussed above that systems with conflicts between clamped chemical potentials coupled by rapid-equilibrium assumptions are physically meaningless. In a thermodynamic model M such conflicts can be easily resolved. Thus, for further analysis we may assume that no conflicts between clamped variables and the rapid-equilibrium assumption occur and that the system is kinetically conflict free. Because we assume a constant column space of $R(\mu, \mu_e)$, this assumption is equivalent to the assumption that for every value of the clamped potentials μ_e the equation

$$R(\mu, \mu_e)\, J + N^T \mu + S^T \mu_e = 0$$

has at least one solution (J, μ).

§ 4.67 (Consistent initial conditions μ_0 and $\mu_{e,0}$)**.** We discussed above that Corollary 4.61 restricts the combination of rapid-equilibrium assumptions and the clamping of chemical potentials. It also restricts possible initial conditions. If the relation given in Corollary 4.61 is violated by the initial conditions μ_0 and $\mu_{e,0}$, no solution starting at μ_0 and $\mu_{e,0}$ exists.

Example 4.68. Consider the network $A \overset{1}{\rightleftharpoons} B \overset{2}{\rightleftharpoons} C$ with $R_1 > 0$ and rapid equilibrium for reaction 2 ($R_2 = 0$). This means that $\mu_B = \mu_C$. Thus, we cannot have initial conditions $\mu_{B,0} \neq \mu_{C,0}$.

§ 4.69 (Choice of initial conditions)**.** The consistency conditions for initial chemical potentials μ_0 form a linear equation system. Thus, from a mathematical point of view, it is not difficult to choose consistent initial conditions. However, it may be rather tedious since the consistency conditions depend on the clamped potentials $\mu_{e,0}$ and on the null space of the resistance matrix R. Both properties typically change during the modeling process or even from simulation to simulation. Often a modeler does not want to spend too much effort in the choice of initial conditions. An implementation of the model reduction method should support the computation of consistent initial conditions from user-supplied, potentially inconsistent initial conditions. In the following paragraph, we develop an easily automatable method for the computation of consistent initial conditions.

§ 4.70 (Computing consistent initial conditions from inconsistent initial conditions)**.** Systems with initial conditions that are not near the thermodynamic equilibrium of the rapid reactions undergo rapid *relaxation dynamics*. In the short relaxation time, the fast fluxes go to equilibrium and the effect of the slow and the clamped fluxes is negligible. Let $T_{J,2}$ be a kernel matrix of R with $R\,T_{J,2} = 0$. The rows of $T_{J,2}$ span the space of rapid fluxes. During the relaxation time of the rapid fluxes we can neglect the slow fluxes and we have $J \in \operatorname{span}(T_{J,2})$. We further neglect the clamped fluxes and thus get $\dot{c} \in \operatorname{span}(N\,T_{J,2})$. During the relaxation time the conservation relation $T_{c,1}\,c = T_{c,1}\,c_0$ holds. Here, $T_{c,1}$ is a left kernel matrix of $N\,T_{J,2}$ with $T_{c,1}\,N\,T_{J,2} = 0$. Let μ'_0 and μ_0 be the user-specified, inconsistent initial conditions and the adapted, consistent initial conditions, respectively. From the invariance of the conservation relations, we have that $T_{c,1}\,c(\mu_0, \mu_{e,0}) = T_{c,1}\,c(\mu'_0, \mu_{e,0})$. From $R\,J = \Delta\mu$ we conclude further that consistent initial conditions fulfill the equilibrium condition $T_{J,2}^T\,\Delta\mu = -T_{J,2}^T\,S^T\,\mu_{e,0} - T_{J,2}^T\,N^T\,\mu_0 = 0$. These two equations allow the numerical computation of consistent initial conditions μ_0 from user-defined, inconsistent initial conditions μ'_0.

Corollary 4.71 (Possible non-uniqueness of the fluxes J)**.** *Let M be a thermodynamic system with a singular $R(\mu, \mu_e)$ and let*

$$X = \operatorname{null}(R) \cap \operatorname{null}(N) \neq \{0\}.$$

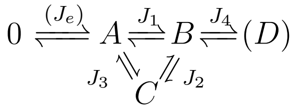
Let $\Delta J(t)$ be an arbitrary trajectory in X, i. e. $\Delta J(t) \in X$ for all t. Further, let $c(t)$, $\mu(t)$, $\Delta\mu(t)$ and $J(t)$ be a solution of M for given inputs $\mu_e(t)$ and $J_e(t)$. Then, the trajectory characterized by $c(t)$, $\mu(t)$, $\Delta\mu(t)$ and $J(t) + \Delta J(t)$ is also a solution of M for the inputs $\mu_e(t)$ and $J_e(t)$.

Proof. The fluxes J enter the model equations in the mole balances $\dot{c} = N\,J + N_e\,J_e$ and in the kinetic equations $R\,J = \Delta\mu$. Both equations are independent of $\Delta J(t)$:

$$\begin{aligned} \dot{c} &= N\,(J + \Delta J) + N_e^T\,J_e = N\,J + N_e^T\,J_e \\ \Delta\mu &= R\,(J + \Delta J) = R\,J. \end{aligned}$$

Thus, if the flux vector J fulfills these two equations, the flux vector $J + \Delta J$ also fulfills the equations. $\square$

Example 4.72 (Non-unique fluxes)**.** The case discussed in Corollary 4.71 occurs if all reactions in a stoichiometric cycle have resistance 0. Consider a system with the reactions



Assume that the resistance matrix is constant and diagonal with diagonal elements R_1, R_2, R_3 and R_4. For the sake of simplicity we only consider the *steady state*. In steady state, we have that $J_e = J_1 - J_3 = J_1 - J_2 = J_4$. Stoichiometrically possible flux distributions are linear combinations of the two flux distributions $J_a^T = J_e\,(1, 0, 0, 1)$ and $J_b^T = J_e\,(0, -1, -1, 1)$ with $J = x_a\,J_a + x_b\,J_b$ and $x_a + x_b = 1$. Their relative contributions x_a and x_b to the flux distribution depend on the resistances. We have that $R_1\,J_1 = R_1\,J_e\,x_a = \mu_A - \mu_B$, $R_2\,J_2 = -R_2\,J_e\,x_b = \mu_B - \mu_C$ and $R_3\,J_3 = -R_3\,J_e\,x_b = \mu_C - \mu_A$. Summing up the latter two equations and dividing the result by the first equation yields $(R_2 + R_3)\,J_e\,x_b = \mu_A - \mu_B$ and $x_a/x_b = (R_2 + R_3)/R_1$, respectively. The lower the resistance R_1, the more the flux distribution J_a via reaction 1 is favored. The lower resistances R_2 and R_3, the more the flux distribution J_b via reactions 2 and 3 is favored. If the resistances along the cycle are zero $R_1 = R_2 = R_3 = 0$, the ratio x_a/x_b is undetermined and the model equations do not uniquely determine the fluxes J_1, J_2 and J_3. However, the model equations uniquely determine $J_4 = J_e$. They also uniquely determine μ_A, μ_B and μ_C and thus c_A, c_B and c_C. From $R_4\,J_4 = \mu_B - \mu_{e,D}$ and $J_4 = J_e$ we get $\mu_B = \mu_{e,D} + R_4\,J_e$. With the equilibrium conditions this leads to $\mu_A = \mu_B = \mu_C = \mu_{e,D} + R_4\,J_e$. Thus, the non-uniqueness is limited to the fluxes J_1, J_2 and J_3.

§ 4.73 (Feasibility of non-unique fluxes)**.** In the above example, we could not determine the ratio x_a/x_b for $R_1 = R_2 = R_3 = 0$. Thus, from a mathematical point of view, all x_a and x_b with $x_a + x_b = 1$ define valid flux distributions. However, if we acknowledge the fact that zero resistances are a limit case with $R_j = \rho_j\,\epsilon$ with $\rho_j > 0$ and $\epsilon \to 0$, we get the additional condition that the ratio $x_a/x_b = (R_2 + R_3)/R_1 = (\rho_2 + \rho_3)/\rho_1$ is positive. Thus, x_a and x_b are restricted to $0 < x_a, x_b < 1$. This means that further physical constraints for non-unique fluxes exist that are not modeled by the equations with $R_i = 0$. This thesis does not further explore these constraints since for simulation one usually will either avoid situations with non-unique fluxes or ignore their values.

§ 4.74 (Model-reduction of non-unique J)**.** The reduction method that is presented below can deal with thermodynamic models with non-unique fluxes J. The reduced-order model has a unique solution. However, the reconstruction of the original fluxes J from the reduced fluxes $\tilde{J}$ is not unique.

§ 4.75 (Models with non-unique J)**.** Corollary 4.71 and Example 4.72 show that it is problematic to set all resistances along a cycle to zero. Then, the model equations do not contain information about the relative contributions of the different, stoichiometrically equivalent pathways through the network. However, the non-uniqueness is limited to the fluxes J. Since the different possible fluxes have the same stoichiometric effect, concentrations c, potentials μ and forces $\Delta\mu$ can be uniquely determined. Such a non-uniqueness of some model variables is unusual, but is not necessarily problematic. Consider a network function, e. g. the production of a precursor, that can be performed by two stoichiometrically equivalent pathways. Then, the network contains a cycle. If the reactions in both pathways are fast compared to the time scale of interest, the rapid-equilibrium assumption can be used. Then, the relative contributions of the two alternative pathways to the production of the precursor cannot be determined. However, the overall precursor production can be uniquely computed. This non-uniqueness is acceptable if the contributions of the respective pathways are not in the focus of interest.

§ 4.76 (Avoiding non-unique fluxes J)**.** A modeler can avoid situations with non-unique fluxes J by removing one reaction in the cycle, or equivalently setting the resistance of one reaction in the cycle to a strictly positive value. Then, only one branch of the cycle is used. If the relative contributions of the different branches of the cycle are of importance, the rapid-equilibrium assumption cannot be used for all reactions in the cycle, but one needs to assign positive values to at least one resistance in every branch of the cycle.

§ 4.77 (Model reduction)**.** For model reduction we transform the system with a partitioning transformation of concentrations and fluxes into a form where $\Delta\hat{\mu}_2 = 0$ and where $\hat{\mu}_2$ only depends on the clamped potentials μ_e. Then, the equations of the second subsystem can be easily solved and the subsystem with subscript 1 forms the reduced model. The details are discussed in the following corollary.

Corollary 4.78 (Rapid equilibrium assumption)**.** *Let M be a kinetically conflict-free thermodynamic model (see Definition 3.1, p. 40 and Definition 4.64, p. 75). Further, let $T_J = [T_{J,1}, T_{J,2}]$, $T_c^T = [T_{c,1}^T, T_{c,2}^T]$, Λ_c and Λ_J be square and invertible matrices with*

$$R\,T_{J,2} = 0, \quad T_{c,1}\,N\,T_{J,2} = 0, \quad \operatorname{rank}(T_{c,2}\,N\,T_{J,2}) = \operatorname{rank}(T_{c,2}), \quad T_{c,1}\,\Lambda_c\,T_{c,2}^T = 0, \quad T_{J,1}^T\,\Lambda_J\,T_{J,2} = 0.$$

Then, the dynamics of M can be reconstructed from the dynamics of the reduced system $\tilde{M}$ with

$$\begin{aligned} \tilde{N} &= T_{c,1}\,N\,T_{J,1}, & \tilde{S} &= S\,(T_{J,1} - T_{J,2}\,\operatorname{inv}(T_{c,2}\,N\,T_{J,2})\,T_{c,2}\,N\,T_{J,1}), \\ \tilde{N}_e &= T_{c,1}\,N_e, & \tilde{\mu}_0 &= \operatorname{inv}^T_{\Lambda_c^{-1}}(T_{c,1})\,\mu_0, \\ \tilde{c}(\tilde{\mu}, \mu_e) &= T_{c,1}\,c(\mu, \mu_e), & \tilde{R}(\tilde{\mu}, \mu_e) &= T_{J,1}^T\,R(\mu, \mu_e)\,T_{J,1} \end{aligned}$$

where

$$\begin{aligned} c &= c(\mu, \mu_e), & \mu &= T_{c,1}^T\,\tilde{\mu} - T_{c,2}^T\,\operatorname{inv}^T(T_{c,2}\,N\,T_{J,2})\,T_{J,2}^T\,S^T\,\mu_e \\ J &= T_{J,1}\,\tilde{J} + T_{J,2}\,\hat{J}_2, & \Delta\mu &= \operatorname{inv}_{\Lambda_J^{-1}}(T_{J,1}^T)\,\Delta\tilde{\mu} \end{aligned}$$

and

$$\hat{J}_2 = \operatorname{inv}(T_{c,2}\,N\,T_{J,2})\,T_{c,2}\,(\dot{c} - N\,T_{J,1}\tilde{J} - N_e\,J_e) + \Delta\hat{J}_2$$

with any $\Delta\hat{J}_2(t) \in \operatorname{null}(T_{c,2}\,N\,T_{J,2})$.

Proof. Transform M with the given partitioned matrices T_c and T_J (see §4.7, p. 53). The system M_1 with subscript 1 corresponds to the reduced system $\tilde{M}$. The corresponding model equations are

$$\begin{aligned}
\dot{\hat{c}}_1 &= (T_{c,1}\,N\,T_{J,1})\,\hat{J}_1 && && +(T_{c,1}\,N_e)\,J_e,\\
\dot{\hat{c}}_2 &= (T_{c,2}\,N\,T_{J,1})\,\hat{J}_1 && +(T_{c,2}\,N\,T_{J,2})\,\hat{J}_2 && +(T_{c,2}\,N_e)\,J_e,\\
\Delta\hat{\mu}_1 &= -(T_{c,1}\,N\,T_{J,1})^T\,\hat{\mu}_1 && -(T_{c,2}\,N\,T_{J,1})^T\,\hat{\mu}_2 && -(S\,T_{J,1})^T\,\mu_e,\\
\Delta\hat{\mu}_2 &= && -(T_{c,2}\,N\,T_{J,2})^T\,\hat{\mu}_2 && -(S\,T_{J,2})^T\,\mu_e,\\
\Delta\hat{\mu}_1 &= (T_{J,1}^T\,R\,T_{J,1})\,\hat{J}_1,\\
\Delta\hat{\mu}_2 &= 0,\\
\hat{c}_1(\hat{\mu},\mu_e) &= T_{c,1}\,c(T_{c,1}^T\,\hat{\mu}_1 + T_{c,2}^T\,\hat{\mu}_2,\mu_e),\\
\hat{c}_2(\hat{\mu},\mu_e) &= T_{c,2}\,c(T_{c,1}^T\,\hat{\mu}_1 + T_{c,2}^T\,\hat{\mu}_2,\mu_e).
\end{aligned} \tag{4.4}$$

Thus, we get a linear relation

$$0 = \Delta\hat{\mu}_2 = (T_{c,2}\,N\,T_{J,2})^T\,\hat{\mu}_2 + (S\,T_{J,2})^T\,\mu_e \tag{4.5}$$

of $\hat{\mu}_2$ and μ_e. Because the system is kinetically conflict-free the equation $R\,J = -N^T\,\mu - S^T\mu_e$ has at least one solution μ (§4.66, p. 75). Premultiplying this equation with $T_{J,2}^T$ and substituting $\mu = T_{c,1}^T\,\tilde{\mu} + T_{c,2}^T\,\hat{\mu}_2$ leads to Equation 4.5. This means that the solution vector $\hat{\mu}_2$ of Equation 4.5 exists for every μ_e. The solution $\hat{\mu}_2$ of this equation is unique because $T_{c,2}\,N\,T_{J,2}$ has full row-rank. Premultiplying the equation with $\mathrm{inv}_\Lambda^T(T_{c,2}\,N\,T_{J,2})$ with a positive definite and symmetric matrix Λ yields the solution

$$\hat{\mu}_2 = -\,\mathrm{inv}_\Lambda^T(T_{c,2}\,N\,T_{J,2})\,T_{J,2}^T\,S^T\,\mu_e.$$

Because the solution is unique, it is independent of the choice of Λ and the subscript Λ can suppressed. Using this result we get

$$\Delta\hat{\mu}_1 = -\underbrace{(T_{c,1}\,N\,T_{J,1})^T}_{\tilde{N}^T}\,\hat{\mu}_1 - \underbrace{\left(-(T_{c,2}\,N\,T_{J,1})^T\ \mathrm{inv}^T(T_{c,2}\,N\,T_{J,2})\,T_{J,2}^T\,S^T + T_{J,1}^T\,S^T\right)}_{\tilde{S}^T}\,\mu_e$$

With these equations and using §4.8 (p. 53), the derivation of the reduced model $\tilde{M}$ is straightforward. As we already discussed before, the reconstruction of the fluxes J from the reduced fluxes $\tilde{J}$ may not be unique. We can reconstruct the fluxes $\hat{J}_2$ from the balance equations $\dot{\hat{c}}_2 = \ldots$ in Equation 4.4. If the matrix $T_{c,2}\,N\,T_{J,2}$ is not quadratic, the solution is not unique. The expression for the reconstruction of $\hat{J}_2$ that is given above can be easily derived using Corollary 2.11 (p. 24). ☐

§ 4.79 (Computational complexity of the reduction)**.** The model reduction of systems with a singular resistance matrix requires only basic operations from linear algebra (matrix multiplication and inversion). The reduction does not involve the solution of non-linear equations. Thus, the application of the reduction method can be easily automated.

§ 4.80 (Computation of transformation matrices)**.** Suited matrices $T_{J,1}$, $T_{J,2}$, $T_{c,1}$, $T_{c,2}$, Λ_c and Λ_J can be gained easily by computing kernel matrices and inverses: (1) Compute $T_{J,2}$ as a kernel matrix of R with $R\,T_{J,2} = 0$. If R is diagonal $T_{J,2}$ consists of unit vectors e_j for every flux j with $R_{jj} = 0$. (2) Choose an arbitrary invertible, symmetric Λ_J, e. g. $\Lambda_J = I$; compute $T_{J,1}$ as a right kernel matrix of $T_{J,2}^T\,\Lambda_J$ with $T_{J,2}^T\,\Lambda_J\,T_{J,1} = 0$. (3) Compute $T_{c,1}$ as a left kernel matrix of $N\,T_{J,2}$ with $T_{c,1}\,N\,T_{J,2} = 0$. (4) Choose an arbitrary invertible, symmetric Λ_c, e. g. $\Lambda_c = I$; compute $T_{c,2}^T$ as a right kernel matrix of $T_{c,1}\,\Lambda_c$ with $T_{c,1}\,\Lambda_c\,T_{c,2}^T = 0$.

Since $T_{c,1}$ is constructed as a left kernel matrix of $N\,T_{J,2}$, the row space of $T_{c,1}$ is equal to the left null space of $N\,T_{J,2}$. Since the rows of $T_{c,1}$ and $T_{c,2}$ form a basis of $\mathbb{R}^{i_o}$, the row space of $T_{c,2}$ does not contain vectors in the left null space of $N\,T_{J,2}$. Thus, the matrices constructed above fulfill the rank condition: $\operatorname{rank}(T_{c,2}\,N\,T_{J,2}) = \operatorname{rank}(T_{c,2})$.

If $N\,T_{J,2}$ has full column rank, the fluxes in the system are unique. In this case it is most convenient to compute $T_{c,2}$ as a left inverse of $N\,T_{J,2}$ with $T_{c,2}\,N\,T_{J,2} = I$, i. e. $T_{c,2} = \operatorname{inv}^T_{\Lambda_c}(T_{J,2}^T\,N^T)$. Then, the reduced model equations and the relations for reconstruction of the original variables get much easier than in the general form given in the corollary.

§ 4.81 ($c(\mu, \mu_e)$ vs $\mu(c, c_e)$)**.** Definition 3.1 (p. 40) uses the function $c(\mu, \mu_e)$ to describe the relation of the chemical potentials μ and the concentrations c. Alternatively, one could express this relation by a function $\mu(c, c_e)$. The following considerations show that the use of the function $c(\mu, \mu_e)$ is advantageous with respect to the rapid-equilibrium assumption. For the sake of simplicity, the considerations are restricted to closed systems with $\mu_e = ()$.

The rapid-equilibrium conditions $T_{J,2}^T \Delta\mu = -T_{J,2}^T\,N^T\,\mu = 0$ are linear in the chemical potentials and thus their solution $\mu = T_{c,1}^T\,\tilde{\mu}$ is straightforward. Using this result, one can easily express the relation of the concentrations and chemical potentials in the reduced system by $\tilde{c}(\tilde{\mu}) = T_{c,1}\,c(T_{c,1}^T\,\tilde{\mu})$. In contrast, the rapid-equilibrium conditions are non-linear in the concentration $T_{J,2}^T\,\Delta\mu = -T_{J,2}^T\,N^T\,\mu(c) = 0$. For this reason, a parameterization $c(\tilde{c})$ of the solution set of this equation with independent parameters $\tilde{c}$ cannot be determined easily. This means that the rapid equilibrium assumption is more difficult to perform when using the function $\mu(c)$ instead of $c(\mu)$ to express the relation of c and μ. For this reason, Definition 3.1 (p. 40) uses the function $c(\mu, \mu_e)$.

§ 4.82 (Invariance of entropy production)**.** The entropy production is invariant under the above described reduction. With the expressions for the reconstruction of the original variables (see Corollary 4.78) we get

$$\sigma[s] = \Delta\mu^T\,J = \Delta\tilde{\mu}^T\,\operatorname{inv}^T_{\Lambda_J^{-1}}(T_{J,1}^T)\,(T_{J,1}\,\tilde{J} + T_{J,2}\,\hat{J}_2) = \Delta\tilde{\mu}^T\,\tilde{J} = \sigma[\tilde{s}].$$

§ 4.83 (Transformation of the Gibbs energy)**.** The differential of the Gibbs energy is not invariant under the above described reduction: $dg = \mu^T\,dc = (\tilde{\mu}^T\,T_{c,1} + d\mu_e^T\,M)\,dc = \tilde{\mu}^T\,T_{c,1}\,dc + d\mu_e^T\,M\,dc = \tilde{\mu}^T\,d\tilde{c} + d\mu_e^T\,M\,dc = d\tilde{g} + d\mu_e^T\,M\,dc$ with $M = S\,T_{J,2}\,\operatorname{inv}(T_{c,2}\,N\,T_{J,2})\,T_{c,2}$. The difference occurs because the reduction removes internal compounds that are in rapid-equilibrium with external compounds.

Example 4.84. Consider the network $X_1 \overset{J_1}{\rightleftharpoons} X_2 \overset{J_2}{\rightleftharpoons} X_3$ with $c_i(\mu_i) = c^\circ \exp((\mu_i - \mu_i^\circ)/(R^* T))$, $R_1(\mu) > 0$ and $R_2(\mu) = 0$. The network is characterized by

$$N = \begin{pmatrix} -1 & 0 \\ +1 & -1 \\ 0 & 1 \end{pmatrix}, \qquad R(\mu, \mu_e) = \begin{pmatrix} R_1(\mu, \mu_e) & 0 \\ 0 & 0 \end{pmatrix}.$$

The model can be reduced by the matrices

$$T_{J,1} = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \qquad T_{J,2} = \begin{pmatrix} 0 \\ 1 \end{pmatrix}, \qquad T_{c,1} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 1 \end{pmatrix}, \qquad T_{c,2} = \begin{pmatrix} 0 & 1 & -1 \end{pmatrix}$$

with $\Lambda_c = I$ and $\Lambda_J = I$. This leads to $\mu_1 = \tilde{\mu}_1$, $\mu_2 = \tilde{\mu}_2$ and $\mu_3 = \tilde{\mu}_2$. The reduced model is characterized by

$$\tilde{N} = \begin{pmatrix} -1 \\ +1 \end{pmatrix}, \qquad \tilde{R}(\tilde{\mu}, \mu_e) = R_1(\tilde{\mu}, \mu_e), \qquad \begin{pmatrix} \tilde{c}_1(\tilde{\mu}, \mu_e) \\ \tilde{c}_2(\tilde{\mu}, \mu_e) \end{pmatrix} = \begin{pmatrix} c_1(\tilde{\mu}_1) \\ c_2(\tilde{\mu}_2) + c_3(\tilde{\mu}_2) \end{pmatrix}.$$

This reduced model corresponds to a network $\tilde{X}_1 \overset{\tilde{J}}{\rightleftharpoons} \tilde{X}_2$ where $\tilde{c}_1 = c_1$ and $\tilde{c}_2 = c_2 + c_3$ is a pool of X_2 and X_3.

4.2.3. Reduction of the Boundary Conditions

The methods for the translation of the chemical potentials (Corollary 4.10, p. 54) and the fluxes (Corollary 4.15, p. 56) provide us with a possibility to reduce the size of the matrices S and N_e that describe the boundary conditions of the system.

4.2.3.1. Reduction of Clamped Potentials

In certain systems with constant clamped chemical potentials, we may reduce the number of columns of the matrix S and thus the number of clamped potentials μ_e by a suited translation of the chemical potentials. Clamped chemical potentials model the boundary conditions of open systems. One can distinguish between two kinds of clamped potentials. (1) The system $A + (ATP) \rightleftharpoons B + (ADP) + (P)$ can reach thermodynamic equilibrium ($\Delta\mu = \mu_A - \mu_B + \mu_{e,ATP} - \mu_{e,ADP} - \mu_{e,P} = 0$) for all values of $\mu_{e,ATP}$, $\mu_{e,ADP}$ and $\mu_{e,P}$. (2) In contrast the system consisting of the reactions $A + (ATP) \rightleftharpoons B + (ADP) + (P)$ and $A \rightleftharpoons B$ realizes a futile cycle that will not reach thermodynamic equilibrium for $\mu_{e,ATP} - \mu_{e,ADP} - \mu_{e,P} \neq 0$. The first system class behaves similar to a closed system and thus its model can be reduced to a closed system described by the reaction equation $\tilde{A} \rightleftharpoons \tilde{B}$. In a large system, clamped chemical potentials of both types may occur. The following corollary describes how such systems can be treated. This is helpful to remove ubiquitous compounds as for example water or protons from the system description.

Corollary 4.85 (Reduction of clamped potentials)**.** *Let M be a thermodynamic model (see Definition 3.1, p. 40) with $S^T = (S_1^T, S_2^T)$, $\mu_e^T = (\mu_{e,1}^T, \mu_{e,2}^T)$, and $\mu_{e,2} = \text{const}$. Let $\delta\mu$ and $\delta\mu_{e,1}$ be constant vectors with $N^T\,\delta\mu + S_1^T\,\delta\mu_{e,1} + S_2^T\,\mu_{e,2} = 0$. Then, the solution of M and of a system $\tilde{M}$ with*

$$\begin{aligned} \tilde{N} &= N, & \tilde{S} &= S_1, & \tilde{N}_e &= N_e, \\ \tilde{c}(\tilde{\mu}, \tilde{\mu}_e) &= c(\tilde{\mu} - \delta\mu, (\tilde{\mu}_e - \delta\mu_{e,1}, \mu_{e,2})), & \tilde{R}(\tilde{\mu}, \tilde{\mu}_e) &= R(\tilde{\mu} - \delta\mu, (\tilde{\mu}_e - \delta\mu_{e,1}, \mu_{e,2})), & \tilde{\mu}_0 &= \mu_0 + \delta\mu \end{aligned}$$

are equivalent. The solutions of M can be reconstructed from the solution of $\tilde{M}$ with the following equations:

$$c = \tilde{c} \qquad \mu = \tilde{\mu} - \delta\mu, \qquad J = \tilde{J}, \qquad \Delta\mu = \Delta\tilde{\mu} \qquad \mu_{e,1} = \tilde{\mu}_e - \delta\mu_{e,1}.$$

Proof. Apply Corollary 4.10 (p 54) with the translations $\delta\mu$ and $\delta\mu_e^T = (\delta\mu_{e,1}, -\mu_{e,2})$. Then, we have $\hat{\mu}_{e,2} = 0$, which can be omitted. □

Example 4.86. Consider a system $A + (X) \rightleftharpoons B$ with $c_i = c^\circ \exp((\mu_i - \mu_i^\circ/(R^*\,T)))$ and constant $\mu_{e,X}$. We have $N^T = (-1, 1)$ and $S = -1$. We set $S_1 = ()$ and $S_2 = -1$ and get the condition $-\delta\mu_A + \delta\mu_B - \mu_{e,X} = 0$. We choose $\delta\mu_B = 0$ and get $\delta\mu_A = -\mu_{e,X}$. The reduced system is $\tilde{A} \rightleftharpoons \tilde{B}$ with $\tilde{c}_i = c^\circ \exp((\tilde{\mu}_i - \tilde{\mu}_i^\circ)/(R^*\,T))$, $\tilde{\mu}_A^\circ = \mu_A^\circ - \mu_{e,X}$ and $\tilde{\mu}_B^\circ = \mu_B^\circ$. Observe that in the example the reduced system is closed, but the original system is open because the clamped potential of X is reduced from the system.

§ 4.87 (Entropy production, Gibbs energy)**.** Being a pure translation of chemical potentials, the above reduction method preserves the entropy production, but not the Gibbs energy of the internal compounds: $d\tilde{g} = \tilde{\mu}^T\,d\tilde{c} = \mu^T\,dc + \delta\mu^T\,dc = dg + \delta\mu^T\,dc$. With suited initial conditions this can be integrated to $\tilde{g} = g + \delta\mu^T\,c$.

§ 4.88 (Legendre Transformation)**.** Alberty [1, 2, 3] suggests the use of Legendre transforms of the Gibbs energy as a convenient way to study the thermodynamics of systems at constant pH ($\mu_{H^+} = \text{const}$), water activity ($\mu_{H_2O} = \text{const}$), ATP-ADP-energy gradient ($\mu_{ATP}, \mu_{ADP} = \text{const}$) or with a constant chemical potential of other compounds. Thus, the use of Legendre transforms and of Corollary 4.85 is equivalent because both provide a way to remove compounds with a constant clamped potential from the system description. In order to understand the relation of Corollary 4.85 to the use of Legendre transforms, it is instructive to consider the total Gibbs energy of the internal and external compounds g^{tot}. For the sake of simplicity, we assume that $\mu_{e,2} \in \mathbb{R}^1$ and $\mu_{e,1} = ()$. Then, g^{tot} is defined by $dg^{tot} = \mu^T\,dc + \mu_{e,2}\,dc_{e,2}$ where $c_{e,2}$ is the concentration of the clamped compound. The total Gibbs energy of the reduced system is given by $d\tilde{g}^{tot} = \tilde{\mu}^T\,d\tilde{c} = dg^{tot} + \delta\mu^T\,dc - \mu_{e,2}^T\,dc_{e,2}$. Let $a \in \mathbb{R}^{i_0}$ be a vector such that $N^T\,a + S_{e,2}^T = 0$. Then, a possible choice for the translation vector is $\delta\mu = -a\,\mu_{e,2}$ and one gets $d\tilde{g}^{tot} = dg^{tot} - \mu_{e,2}\,(a^T\,dc + dc_{e,2})$. Note that the expression $c_c = a^T\,c + c_{e,2}$ describes a conserved moiety of the system defined by the stoichiometric matrices N and $S_{e,2}$. For constant $\mu_{e,2}$, it follows from $d\tilde{g}^{tot} = dg^{tot} - \mu_{e,2}\,dc_c$ that $\tilde{g}^{tot} = g^{tot} - \mu_{e,2}\,c_c$. In this representation, one sees that $\tilde{g}^{tot}$ is a Legendre transform of g^{tot} (see [1–3, 23]). This means that the application of Corollary 4.85 is equivalent to the use of a Legendre transform of the Gibbs energy.

4.2.3.2. Reduction of Clamped Fluxes

Similarly to the reduction of the size of S by a translation of chemical potentials, we also may reduce the number of columns of N_e by a translation of fluxes. This is equivalent to the pooling of clamped fluxes.

Corollary 4.89. *Let M be a thermodynamic model (see Definition 3.1, p. 40) with a partitioned matrix $N_e = (N_{e,1}, N_{e,2})$ and vector $J_e^T = (J_{e,1}^T, J_{e,2}^T)$. Let δJ and $\delta J_{e,1}$ be vectors with $N\,\delta J + N_{e,1}\,\delta J_{e,1} + N_{e,2}\,J_{e,2} = 0$ and $R\,\delta J = 0$. Then, the solution of M and of a system $\tilde{M}$ with*

$$\begin{array}{lll} \tilde{N} = N, & \tilde{S} = S, & \tilde{N}_e = N_{e,1}, \\ \tilde{c}(\tilde{\mu}, \tilde{\mu}_e) = c(\tilde{\mu}, \tilde{\mu}_e), & \tilde{R}(\tilde{\mu}, \tilde{\mu}_e) = R(\tilde{\mu}, \tilde{\mu}_e), & \tilde{\mu}_0 = \mu_0 \end{array}$$

are equivalent. The solutions of M can be reconstructed from the solution of $\tilde{M}$ with the following equations:

$$c = \tilde{c} \qquad \mu = \tilde{\mu}, \qquad J = \tilde{J} - \delta J, \qquad \Delta\mu = \Delta\tilde{\mu}, \qquad J_{e,1} = \tilde{J}_e - \delta J_{e,1}.$$

Proof. Apply Corollary 4.15 (p. 56) with the translations δJ and $\delta J_e^T = (\delta J_{e,1}, -J_{e,2})$. Then, we have $\hat{J}_{e,2} = 0$, which can be omitted. □

Example 4.90 (Non-singular R)**.** Consider a system with $0 \xrightleftharpoons{(J_{e,1})} A$ and $0 \xrightleftharpoons{(J_{e,2})} A$. Then, we have $N_e = (1, 1)$. We set $N_{e,1} = 1$ and $N_{e,2} = 1$. Thus, we have $\delta J_{e,1} = -J_{e,2}$. The reduced system is $0 \xrightleftharpoons{\tilde{J}_{e,1}} A$ with $\tilde{J}_{e,1} = J_{e,1} + J_{e,2}$. Thus, in this case, the reduction method is equivalent to a simple pooling of clamped fluxes.

Example 4.91 (Singular R)**.** Consider a system with $0 \xrightleftharpoons{(J_e)} A \xrightleftharpoons{R=0} (B)$ (see Example 4.18, p. 56). We have that $N = -1$, $N_e = 1$, $S = 1$ and $R = 0$ and choose $N_{e,1} = ()$ and $N_{e,2} = 1$. Thus, we have $\delta J = J_e$. The reduced system is $A \xrightleftharpoons{R=0} (B)$. In this example, an open system could be reduced to a closed system. By applying Corollary 4.78 (p. 78) we could further reduce the system.

§ 4.92 (Entropy production, Gibbs energy)**.** Being a pure translation of fluxes, the above reduction method preserves the entropy production and the Gibbs energy (see §4.16, p. 56).

4.3. Conclusions and Discussion

This chapter introduced a series of model transformation and model reduction methods for thermodynamic models. According to Definition 3.1 (p. 40), a thermodynamic model M is characterized by a set of matrices and functions: The stoichiometric matrix N defines how the reaction fluxes act on the compounds. The stoichiometric matrices S and N_e define how the clamped potentials and fluxes act on the system. These matrices model the boundary conditions

compound based	reaction based
conservation relations (Section 4.2.1.1) $N^T v = 0$, $N_e^T v = 0$	stoichiometric cycles (Section 4.2.1.2) $N v = 0$, $S v = 0$
thermodynamic submodel (Section 4.2.2.1, quasi-steady state assumption) $(\partial c / \partial \mu)\, v = 0$	kinetic submodel (Section 4.2.2.2, rapid- equilibrium assumption) $R v = 0$
clamped potentials (Section 4.2.3.1) $N^T v_1 + S^T v_2 = 0$	clamped fluxes (Section 4.2.3.2) $N v_1 + N_e v_2 = 0$

Table 4.1.: Rank deficiencies of system matrices and the according reduction methods. The reduction methods are applicable if vectors v, v_1 and v_2 exist such that the conditions given in the table are fulfilled.

of the system. The resistance matrix $R(\mu, \mu_e)$ characterizes the relation of the thermodynamic forces and the fluxes. The thermodynamic state function $c(\mu, \mu_e)$ gives the relation of the chemical potentials and the concentrations. Thus, the matrix $\partial c/\partial \mu$ relates the changes of chemical potentials and concentrations.

The functions and matrices describing a thermodynamic model can be transformed into other coordinates such that the trajectories of the transformed and of the original model are equivalent. The transformation methods introduced in Section 4.1 (p. 51) are designed such that the transformed system can be written as a thermodynamic model. Based on the transformation methods, Section 4.2, (p. 57) derived model reduction methods. Model reduction is possible if the matrices describing a thermodynamic model have rank deficiencies. In this way, we can reduce the stoichiometric, kinetic and thermodynamic submodel, as well as the boundary conditions. We can derive an equivalent model with smaller, full-rank matrices and less variables. The following paragraphs compare the different model reduction methods and discuss their practical usability.

§ 4.93 (Duality of the reduction methods)**.** The methods for the reduction of singular network matrices appear in pairs (see Table 4.1). One method in a pair is associated with properties of compounds and one with properties of fluxes. The methods in a pair are partly dual to each other, in the sense that there are similarities in their application. However, the duality is not strong in the sense that the solution of a problem is equivalent to the solution of its dual problem.

§ 4.94 (Application and usability of the reduction methods)**.** Model reduction is performed for three main reasons: (1) simplification of the simulation equations; in particular, reduction of the numbers of state variables; (2) reduction of the number of parameters; in particular, reduction of the badly identifiable parameters; and (3) reduction of the stiffness of the equations. However,

reduction of the	reduction of the state variables	number of parameters	reduction of stiffness	computationally cheap
conservation relations	+	-	-	-
stoichiometric cycles	-	-	-	-
thermodynamic submodel	+	+	+	-
kinetic submodel	+	+	+	+
clamped potentials	-	-	-	+
clamped fluxes	-	-	-	+

Table 4.2.: Comparison of the reduction methods.

these advantages have to be seen in comparison to the computational costs of the reduction. Some of the developed reduction methods require the solution of a nonlinear equation which can be computationally expensive. The following paragraphs discuss these critical points for the reduction methods. The results of the discussion are collected in Table 4.2.

§ 4.95 (Reduction of the number of state variables)**.** The state variables of the simulation equations are the chemical potentials μ_i or the concentrations c_i (see §3.5, p. 41). The dimension of the simulation equations depends on the number of compounds i_0. The reduction of stoichiometric cycles lowers the number of reactions but not the number of compounds. The reduction of conservation relations lowers the number of compounds i_0 by the number of conservation relations. The number of compounds i_0 is also lowered by the reduction of the thermodynamic and the kinetic subsystems, i. e. the quasi-steady-state and the rapid-equilibrium assumption. The reduction of boundary conditions only lowers the number of inputs to the system.

§ 4.96 (Reduction of the number of thermodynamic and kinetic parameters)**.** The parameters of thermodynamic models are the stoichiometric coefficients and the parameters of the functions $c(\mu, \mu_e)$ and $R(\mu, \mu_e)$. Table 4.2 only refers to the number of parameters of the functions $c(\mu, \mu_e)$ and $R(\mu, \mu_e)$. The stoichiometric coefficients are usually much better known than these parameters and thus the knowledge of thermodynamic and kinetic parameters is the limiting factor for modeling. A reduction of the number of parameters is particularly advantageous if one does not need to assess the parameter values of the original model to formulate the reduced model, i. e. if one does not need a fully parameterized detailed model to derive the reduced model. Then, one can perform *reduced-order modeling* rather than *model reduction*. The reduction of boundary conditions and of the stoichiometric submodel lower the size of the stoichiometric matrices and thus only the number of stoichiometric coefficients. The reduction of the thermodynamic and the kinetic subsystems are always approximative because real systems do not have singular matrices R and $\partial c/\partial\mu$. In natural coordinates, these matrices are diagonal and non-singular. For the approximation of non-singular matrices R or $\partial c/\partial\mu$ by singular ones, one does not need to assess all elements of these matrices. In particular, the exact values of the small entries in the vector $c(\mu, \mu_e)$ and the matrix $R(\mu, \mu_e)$ need not to be known. It is sufficient to know that these entries are small enough to be approximated by zero. Thus, the methods for the reduction of the thermodynamic and the kinetic submodel provide a possibility for *reduced-order modeling* because they allow the formulation of the reduced model without quantitative knowledge of all parameters of the original model.

§ 4.97 (Reduction of the stiffness)**.** A differential equation system is stiff if it describes dynamics at a fast and at a slow time scale. Models of reaction networks tend to be stiff because kinetic constants can vary over a large range. Although the matrices R and $\partial c/\partial \mu$ are in real systems always positive definite, they are often nearly singular such that the system is stiff. If it is possible to approximate the system dynamics using singular matrices R and $\partial c/\partial \mu$, the stiffness can be reduced by the introduced model reduction methods. Thus, the reduction of thermodynamic and the kinetic submodel leads to a reduction of the stiffness of the equations. The other model reduction methods do not change the stiffness of the system.

§ 4.98 (Computational costs of reduction)**.** The methods for the reduction of the kinetic submodel and of the clamped potentials and fluxes only involve the solution of linear equations with constant coefficients. For this reason, the application of these methods is computationally cheap. To compute the reduced resistance matrix during a reduction of stoichiometric cycles, one needs to compute the inverse of $T_{J,2}^T\, R(\tilde{\mu}, \mu_e)\, T_{J,2}$. This computation should be performed symbolically because $R(\tilde{\mu}, \mu_e)$ is in general not constant. Thus, this is a problematic, computationally expensive step. The methods for the reduction of conservation relations and of the thermodynamic submodel involve the solution of nonlinear equation systems. In the case of a reduction of conservation relations, this system is usually uniquely solvable. For the reduction of the thermodynamic submodel, the solution neither always exists nor is always unique. An alternative to the symbolic solution of the non-linear equations before the simulation is the numerical solution during the simulation. Then, the model equations form a differential-algebraic equation system. This system has as many state variables as the original system, but the application of the reduction method may nevertheless be advantageous. The reduction of the kinetic submodel reduces the stiffness of the system and thus increases the minimal step size for simulation. A model with conservation relations contains limit-stable eigenmodes ("poles at zero") and thus the Jacobian of the system is singular. This leads to problems with some implementations of numerical analysis methods (e. g. integration with sensitivity analysis and continuation methods). The reduced model does not contain a limit-stable eigenmode, and thus has a non-singular Jacobian. This means that a reduction of the thermodynamic submodel and a reduction of conservation relations may be sensible, even if the computational costs are high.

§ 4.99 (Approximation quality)**.** The reduction of conservation relations and of the thermodynamic and kinetic submodel decrease the number of state variables of the system. For systems with rank-deficiencies of certain matrices (see Table 4.1) the reduced order model allows an exact reconstruction of the dynamics of the original model. In natural systems, the respective matrices usually do not have a rank deficiency but only are ill-conditioned. The original system needs to be approximated by a system with rank deficient matrices before applying these methods. This work only develops methods to derive the reduced models from the approximated, singular models, but does *not* intend to predict the approximation quality. The latter problem is in general very hard and for large, non-linear models it is impossible to develop a general and generic method to predict the approximation quality. This is explained in the following paragraphs.

§ 4.100 (Approximation quality – conservation relations)**.** If open systems are considered, a system does usually not contain real conservation relations. Substances with low production or consumption rates whose amounts do not change considerably in the relevant time scale are treated as conservation relations. An example for this are total enzyme concentrations in a model that neglects enzyme synthesis and degradation. If the production and consumption rates tend to zero, the behavior of the original model tends towards the behavior of the reduced model. However, without a thorough analysis of the system dynamics, it is not possible to assess the approximation quality. Depending on the sensitivity of the system, even a small production or consumption rate could lead to a largely different system behavior. For this reason, it is not possible to derive a generic measure for the anticipated approximation quality.

§ 4.101 (Approximation quality – thermodynamic and kinetic submodel)**.** In natural systems, the matrices $\partial c/\partial\mu$ and R are always non-singular. Thus, a reduction method based on the assumption of singularity is always approximative. In general, it is not guaranteed that the reduced and original models show a qualitatively similar behavior. Consider a non-singular system M_ϵ with a small parameter $\epsilon > 0$ that tends towards a singular system M_0 as ϵ tends to zero. For an ideal dilute solution with mass-action kinetics, the parameter ϵ could be a small resistance R_j or a small derivative $\partial c_i/\partial\mu_i$. Let $\xi(\epsilon, t)$ and $\xi(0, t)$ be the solutions of these models with equal initial conditions $\xi(\epsilon, 0) = \xi(0, 0)$. Tikhonov's Theorem (see e. g. Heinrich and Schuster [45], Wasow [103]) gives a sufficient condition for the convergence of the solutions, i. e. for $\lim_{\epsilon\to 0} \xi(\epsilon, t) = \xi(0, t)$. For non-linear systems the conditions of Tikhonov's Theorem are difficult to check since they involve the stability analysis of a non-linear system. Thus, for models of larger systems with partly unknown parameters it is virtually impossible to prove if the singular system behaves similar to the non-singular system as ϵ tends to zero.

If the conditions of Tikhonov's Theorem are fulfilled, it is guaranteed that a nearly singular system M_ϵ can be approximated by a singular system M_0 for $\epsilon \to 0$. However, the theorem does not make any assertions concerning the approximation quality for a finite ϵ. Additionally, the value of ϵ can usually not be experimentally assessed as small resistances R_j or small $\partial c_i/\partial\mu_i$ lead to fast dynamics. For this reason, these parameters can often not be determined quantitatively by a measurement on a slow time scale. Measurements on a slow time scale can only show that ϵ is small. Thus, even if it is possible to check the conditions of Tikhonov's Theorem, it is not clear if the approximation is justified. In the rare cases where the value of the small parameter ϵ is known, the approximation quality can be checked after the reduction by comparing the simulation results.

§ 4.102 (Reduced-order modeling – quantitative and qualitative parameters)**.** The above two paragraphs explained why it is not possible to develop generic and computationally feasible criteria that guarantee a good approximation quality for the reduction of the thermodynamic and kinetic submodel of non-singular models and for the reduction of conservation relations in open systems. Thus, the approximative model reduction techniques can normally only be applied "blindfold", i. e. without guarantees for the approximation quality. However, it is important to note that this is done anyhow in any modeling work. Most biochemical species are not elementary chemical species but pools of isomers or differentially protonated species. For modeling purposes it is assumed that these species are in equilibrium with each other (see Alberty [1, 2, 3]). Similarly, biochemical reactions are not elementary reaction steps but are composed from simpler reaction steps that are connected via quasi-stationary intermediate complexes. Thus, most if not all modeling efforts rely on an implicit, *a priori* reduction of the kinetic and thermodynamic submodel. The validity of the approach is usually implicitly assumed rather than formally checked.

From this perspective, the model parameters fall apart into qualitative and quantitative parameters. Qualitative parameters determine if a certain resistance R_j or a certain $\partial c_i/\mu_i$ can be approximated by zero. The quantitative parameters are the parameters of the remaining non-zero functions $c(\mu, \mu_e)$ and $R(\mu, \mu_e)$. In ideal dilute solutions with ideal mass action kinetics, the quantitative parameters are the chemical standard potentials μ_i° and the factor ρ that occurs in the resistance functions (see Section 3.3.2, p. 46). To describe the behavior of a system, quantitative and qualitative parameters need to be estimated from the measurement data. The proposed methods are not used for model reduction because there is no original model with quantitative values for all parameters, but for *reduced-order modeling.*

§ 4.103 (Conclusions)**.** Table 4.2 shows that the reduction of the kinetic submodel, i. e. the rapid-equilibrium assumption, is the most usable and powerful reduction method. The other methods either are intrinsically difficult to apply or do not lead to a significant simplification of the model equations. This does not mean that an application of these methods is not reasonable in special cases. However, we cannot expect to develop a general and scalable implementation for the methods that involve the solution of nonlinear equations. Methods that do not reduce the number of state variables or parameters have nevertheless their justification. For example, a reduction of clamped potentials often simplifies the following steps because it is possible to remove ubiquitous compounds as protons or ADP from the system. The approximative reduction methods do no require the knowledge of the parameter values that can be reduced. Thus, the proposed methods are reduced-order modeling methods rather than model reduction methods.

5. Thermokinetic Modeling

The previous chapter introduced a thermodynamic formalism for the mathematical modeling of chemical reaction networks. Methods for transformation and reduction of such models were presented. In particular, the reduction of the kinetic submodel by the rapid-equilibrium assumption is a promising tool to simplify models of large networks.

However, the application of the thermodynamic formalism to real systems is difficult because even for simple systems the mathematical expressions $c(\mu, \mu_e)$ and $R(\mu, \mu_e)$ are complex. The high complexity of the model equations makes it very cumbersome to treat example systems without the help of computers and even complicates the use of computer programs for symbolic computations. Numerical simulation suffers from the removable singularity in the expression of the ideal resistance function $R_\circ$ that is part of most resistances (see Definition 3.22, p. 47).

This chapter introduces the *thermokinetic modeling (TKM)* formalism, which is also called the *Thermodynamic-Kinetic Modeling (TKM)* formalism. It is directly derived from thermodynamic modeling and thus guarantees the thermodynamic feasibility of the model equations. However, the model equations are much simpler. Thus, the thermokinetic formalism is much better suited for modeling and model analysis. In the thermokinetic modeling formalism, we replace the chemical potentials μ_i and the thermodynamic forces $\Delta\mu_j$ by thermokinetic potentials ξ_i and forces F_j. In the simplest case of mass-action kinetics in ideal dilute solutions, thermokinetic potentials ξ_i and forces F_j are proportional to concentrations c_i and fluxes J_j, respectively. This leads to constant thermokinetic resistances and thermokinetic functions $c_i(\xi, \xi_e)$ that are linear in the thermokinetic potentials ξ_i.

A preliminary version of the TKM formalism was published in Ederer and Gilles [32].

5.1. Thermokinetic Potentials, Forces and Resistances

Definition 5.1 (Thermokinetic potential ξ_i)**.** The thermokinetic potential ξ_i of the compound X_i is defined by

$$\xi_i = \exp\left(\frac{\mu_i}{R^* T}\right).$$

Definition 5.2 (Thermokinetic capacity C_i)**.** The thermokinetic capacity C_i of the compound X_i is defined by $C_i = c_i/\xi_i$ or equivalently $c_i = C_i\, \xi_i$.

§ 5.3 (Relation of μ_i and ξ_i)**.** From the above definition we get directly:

$$\mu_i = R^* T \, \log(\xi_i).$$

In differential form we have that

$$d\xi_i = \frac{1}{R^*\,T}\,\exp\left(\frac{\mu_i}{R^*\,T}\right)\,d\mu_i, \qquad\qquad d\mu_i = \frac{R^*\,T}{\xi_i}\,d\xi_i.$$

§ 5.4 (Function $c(\xi, \xi_e)$ vs $c(\mu, \mu_e)$)**.** The information content of the functions $c(\mu, \mu_e)$ and $c(\xi, \xi_e)$ is equal, since ξ and μ are in a one-to-one correspondence. TKM uses the function $c(\xi, \xi_e)$ instead of $c(\mu, \mu_e)$, since this simplifies the treatment of ideal dilute solutions.

§ 5.5 ($c(\xi, \xi_e)$ in ideal dilute solutions)**.** In ideal dilute solutions with the relation $\mu_i = \mu_i^\circ + R^*\,T\,\log(c_i/c^\circ)$ we get the function

$$c_i(\xi, \xi_e) = \underbrace{c^\circ\,\exp\left(-\frac{\mu_i^\circ}{R^*\,T}\right)}_{C_i}\,\xi_i.$$

Here, the thermokinetic capacity C_i is a ξ-independent constant and thus a constant parameter of the system.

§ 5.6 (Equilibrium constants in ideal dilute solutions)**.** Using the relation in §5.5, the equilibrium constant of a reaction in an ideal dilute solution (see §2.51, p. 33) can be written in dependency on the capacities C_i:

$$K_{eq,j} = \frac{k_{+j}}{k_{-j}} = \exp\left(-\frac{\sum_{i=1}^{i_0}\nu_{ij}\,\mu_i^\circ}{R^*\,T}\right) = \prod_{i=1}^{i_0}\left(\frac{c_{eq,i}}{c^\circ}\right)^{\nu_{ij}} = \prod_{i=1}^{i_0}\left(\frac{C_i}{c^\circ}\right)^{\nu_{ij}}.$$

In vector notation we have

$$K_{eq} = \exp\left(-\frac{N^T\,\mu^\circ}{R^*\,T}\right) = N^T\#\left(\frac{c_{eq}}{c^\circ}\right) = N^T\#\left(\frac{C}{c^\circ}\right)$$

where N is the matrix of the stoichiometric coefficients ν_{ij} and # denotes the lin-log product (Definition 2.17, p. 25).

§ 5.7 (Relation of $\partial c/\partial\mu$ and $\partial c/\partial\xi$)**.** The Jacobians of the two functions $c(\mu, \mu_e)$ and $c(\xi, \xi_e)$ are related by

$$\frac{\partial c}{\partial \mu} = \frac{1}{R^*\,T}\,\frac{\partial c}{\partial \xi}\,\mathrm{diag}(\xi_i), \qquad\qquad \frac{\partial c}{\partial \xi} = R^*\,T\,\frac{\partial c}{\partial \mu}\,\mathrm{diag}(\xi_i^{-1}).$$

This follows directly from §5.3.

Definition 5.8 (Thermokinetic force F_j)**.** The thermokinetic force F_j along the reaction

$$\sum_{i\in E_j}\nu_{E,ij}\,X_i \rightleftharpoons \sum_{i\in P_j}\nu_{P,ij}\,X_i$$

is defined as

$$F_j = \underbrace{\prod_{i\in E_j}\xi_i^{\nu_{E,ij}}}_{F_{E,j}} - \underbrace{\prod_{i\in P_j}\xi_i^{\nu_{P,ij}}}_{F_{P,j}}$$

where F_{E_j} and F_{P_j} are the thermokinetic forces exerted by reactants and products, respectively.

§ 5.9 (Relation of $\Delta\mu_{E/P,j}$ and $F_{E/P,j}$)**.** The thermokinetic and thermodynamic forces exerted by reactants and products (see §2.38, p. 30) are related by

$$\begin{aligned} F_{E,j} &= \exp\left(\frac{\Delta\mu_{E,j}}{R^*\,T}\right), & \Delta\mu_{E,j} &= R^*\,T\,\log(F_{E,j}),\\ F_{P,j} &= \exp\left(\frac{\Delta\mu_{P,j}}{R^*\,T}\right), & \Delta\mu_{P,j} &= R^*\,T\,\log(F_{P,j}). \end{aligned}$$

In differential form we have:

$$\begin{aligned} dF_{E,j} &= \frac{1}{R^*\,T}\,\exp\left(\frac{\Delta\mu_{E,j}}{R^*\,T}\right)\,d\Delta\mu_{E,j}, & d\Delta\mu_{E,j} &= \frac{R^*\,T}{F_{E,j}}\,dF_{E,j},\\ dF_{P,j} &= \frac{1}{R^*\,T}\,\exp\left(\frac{\Delta\mu_{P,j}}{R^*\,T}\right)\,d\Delta\mu_{P,j}, & d\Delta\mu_{P,j} &= \frac{R^*\,T}{F_{P,j}}\,dF_{P,j}. \end{aligned}$$

These conversion formulas are equivalent to the formulas for the conversion between μ_i and ξ_i in §5.3.

§ 5.10 (Sign of F_j)**.** The thermokinetic force F_j has the same sign as the thermodynamic force $\Delta\mu_j$:

$$\operatorname{sign}(F_j) = \operatorname{sign}(\Delta\mu_j).$$

Proof. Observe that $R^*\,T\,\log(F_{E,j}) = \Delta\mu_{E,j}$ and $R^*\,T\,\log(F_{P,j}) = \Delta\mu_{P,j}$. Because the logarithm is a monotonic function, this proves the assertion. □

§ 5.11 (Relation of $\Delta\mu$ and F)**.** The thermodynamic force $\Delta\mu_j$ and the thermokinetic force F_j are related via the ideal resistance function. From Definition 3.22 (p.47) it follows directly that

$$R^*\,T\cdot R_\circ\left(\frac{\Delta\mu_{E,j}}{R^*\,T},\frac{\Delta\mu_{P,j}}{R^*\,T}\right) = \frac{\Delta\mu_j}{F_j}.$$

In matrix notation, we have that

$$\Delta\mu = R^*\,T\,\underline{R}_\circ\,F,$$

where $\underline{R}_\circ$ is the diagonal matrix of the ideal mass-action resistances for the reactions.

§ 5.12 (Relation of F and J)**.** With the above notation and with $R\,J = \Delta\mu$, we can directly derive that

$$\underbrace{(R^*\,T\,\underline{R}_\circ)^{-1}\,R}_{\bar{R}}\;J = F.$$

This allows defining the *thermokinetic resistance matrix* $\bar{R}$.

Definition 5.13 (Thermokinetic resistances)**.** The matrix of thermokinetic resistances $\bar{R}$ is defined by $\bar{R} = (R^* \, T \, \underline{R}_\circ)^{-1} \, R$ where R is the matrix of thermodynamic resistances and $\underline{R}_\circ$ is the diagonal matrix of the ideal mass-action resistances of the reactions. If the matrix of thermodynamic resistances R is diagonal, the matrix of thermokinetic resistances $\bar{R}$ is also diagonal. Then, in analogy to thermodynamic resistances (Section 3.3.2, p. 46), the thermokinetic resistance $\bar{R}_j$ of the reaction j is defined by $\bar{R}_j = F_j / J_j$ or equivalently $\bar{R}_j \, J_j = F_j$.

§ 5.14 (Thermokinetic resistance of generalized mass-action kinetics)**.** For generalized mass-action kinetics in ideal dilute solutions, the thermokinetic resistance matrix is diagonal. Section 3.3.2 (p. 46) derived an expression for the thermodynamic resistance of generalized mass-action kinetics: $R_j = \rho_j(k, \mu) \, R_\circ(\Delta\mu_{E,j}/(R^* \, T), \Delta\mu_{P,j}/(R^* \, T))$ where $\rho_j(k, \mu)$ is a function that defines the deviation from the ideal mass-action behavior. Thus, the thermokinetic resistance is $\bar{R}_j = R_j / (R^* \, T \, R_\circ(\Delta\mu_{E,j}/(R^* \, T), \Delta\mu_{P,j}/(R^* \, T))) = \rho_j(k, \mu)/(R^* \, T)$. With the expression for $\rho_j(k, \mu)$ given in Section 3.3.2 (p. 46) and the expression for C_i given in §5.5 (p. 90), this can be written in dependence of the capacities C_i, the kinetic parameters $k_{\pm j}$ and the function $f_j(k, c)$:

$$\begin{aligned} \bar{R}_j(\xi, \xi_e) &= k_{+j}^{-1} \prod_{i \in E_j} \left(\frac{C_i}{c^\circ} \right)^{-\nu_{E,ij}} (f_j(k, c(\xi, \xi_e)))^{-1} \\ &= k_{-j} \prod_{i \in P_j} \left(\frac{C_i}{c^\circ} \right)^{-\nu_{P,ij}} (f_j(k, c(\xi, \xi_e)))^{-1}. \end{aligned} \tag{5.1}$$

§ 5.15 (Thermokinetic resistance of ideal mass-action kinetics)**.** For ideal mass-action kinetics with $f(c, k) = 1$, the thermokinetic resistance matrix is diagonal and the diagonal elements $\bar{R}_j$ are constant. These constant diagonal elements are system parameters. Compared to the use of thermodynamic resistances, the use of thermokinetic resistances strongly simplifies the treatment of such rate laws. This is highly comfortable because the ideal mass-action law is an elementary kinetic rate law that is widely used for the description of biochemical networks.

§ 5.16 (Thermokinetic resistance of Michaelis-Menten type kinetics)**.** Simple enzyme-catalyzed reactions $A \rightleftharpoons B$ can often be described by the generalized mass-action law $J = c_E \, (k_+ \, (c_A/c^\circ) - k_- \, (c_B/c^\circ))/(k_0/c^\circ + k_A \, c_A/c^\circ + k_B \, c_B/c^\circ)$, where $k_\pm$, k_0, k_A and k_B are kinetic parameters and c_E is the enzyme concentration (cf. Example 3.24, p. 48). Here, the function $f(k, c)$ is $f(k, c) = c_E/(k_0/c^\circ + k_A \, c_A/c^\circ + k_B \, c_B/c^\circ)$. The thermokinetic resistance can be written as $\bar{R} = c^\circ/(k_+ \, f(k, c) \, C_A) = (\rho_0 + \rho_A \, \xi_A + \rho_B \, \xi_B)/c_E$ where $\rho_0 = k_+^{-1} \, C_A^{-1} \, k_0$, $\rho_A = k_+^{-1} \, k_A$ and $\rho_B = k_+^{-1} \, C_A^{-1} \, C_B \, k_B$ are constant, positive system parameters. Thus, the thermokinetic resistance consists of a part that is independent from the thermokinetic potentials of reactants and products plus a linear combination of the thermokinetic potentials of reactants and products. More complex reaction mechanisms typically lead to resistances that are polynomial in the thermokinetic potentials. The thermokinetic resistance is typically inversely proportional to the enzyme concentration.

Example 5.17 (Thermokinetic model)**.** Consider a system of two reactions with $A \overset{1}{\rightleftharpoons} B \overset{2}{\rightleftharpoons} C + D$. We assume an ideal dilute solution with the relation $c_i = \exp((\mu_i - \mu_i^\circ)/(R^* \, T))$

where the standard potentials μ_i° are constant system parameters. Ideal mass action kinetics are modeled by the thermodynamic resistances $R_1 = \rho_1 \, R_\circ(\mu_A/(R^*\,T), \mu_B/(R^*\,T))$ and $R_2 = \rho_2 \, R_\circ(\mu_B/(R^*\,T), (\mu_C + \mu_D)/(R^*\,T))$ where ρ_1 and ρ_2 are constant system parameters. The thermokinetic capacities C_i are constant and given by $C_i = c^\circ \exp(-\mu_i^\circ/(R^*\,T))$. The thermokinetic resistances $\bar{R}_1 = \rho_1/(R^*\,T)$ and $\bar{R}_2 = \rho_2/(R^*\,T)$ are constant as well. Thus, we get the thermokinetic model equations:

$$\dot{c}_A = -J_1, \qquad \dot{c}_B = J_1 - J_2, \qquad \dot{c}_C = \dot{c}_D = J_2$$

with

$$c_i = C_i\,\xi_i \text{ for } i \in \{A, B, C, D\}$$

and

$$\bar{R}_1\,J_1 = F_1 = \xi_A - \xi_B, \qquad \bar{R}_2\,J_2 = F_2 = \xi_B - \xi_C\,\xi_D.$$

These model equations can be simplified to the simulation equations:

$$\begin{aligned}
&C_A\,\dot{\xi}_A && = -(\xi_A - \xi_B)/\bar{R}_1, \\
&C_B\,\dot{\xi}_B && = +(\xi_A - \xi_B)/\bar{R}_1 - (\xi_B - \xi_C\,\xi_D)/\bar{R}_2, \\
&C_C\,\dot{\xi}_C = C_D\,\dot{\xi}_D && = \qquad\qquad\qquad\;\; +(\xi_B - \xi_C\,\xi_D)/\bar{R}_2
\end{aligned}$$

with the state vector $(\xi_A, \xi_B, \xi_C, \xi_D)$ and the system parameters C_A, C_B, C_C, C_D, $\bar{R}_1$ and $\bar{R}_2$. This thermokinetic representation is much more simple than the corresponding thermodynamic model.

Above we introduced thermokinetic capacities and resistances as an alternative set of parameters for kinetic models. The following two paragraphs discuss their relation to the traditional mass-action parameters.

§ 5.18 (Computation of the kinetic parameters from the thermokinetic resistances and capacities)**.** In an ideal dilute solution with generalized mass-action kinetics, the capacities C_i are constant and the thermokinetic resistance matrix is diagonal. The diagonal elements $\bar{R}_j(k, \xi_1, \dots \xi_{i_0})$ may depend on further system parameters k and the thermokinetic potentials ξ. If the thermokinetic capacities and resistances of a reaction j are known, its generalized mass-action parameters $k_{\pm j}$ and $f_j(p, c)$ can be computed by

$$k_{+j} = \frac{1}{\hat{k}} \prod_{i \in E_j} \left(\frac{C_i}{c^\circ}\right)^{-\nu_{E,ij}}, \quad k_{-j} = \frac{1}{\hat{k}} \prod_{i \in P_j} \left(\frac{C_i}{c^\circ}\right)^{-\nu_{P,ij}}, \quad f_j(k, c) = \frac{\hat{k}}{\bar{R}_j(k, C_1^{-1}\,c_1 \dots C_{i_0}^{-1}\,c_{i_0})}$$

where $\hat{k} > 0$ is an arbitrary positive constant. This expression results from substituting $\xi_i = c_i/C_i$ into $J_j = F_j/\bar{R}_j$ and comparing the result with a generalized mass-action kinetics (Definition 2.49, p. 33). The choice $\hat{k} = \bar{R}_j$ and $f_j(k, c) = 1$ is convenient if the resistance $\bar{R}_j$ is constant.

§ 5.19 (Computation of the thermokinetic resistances and capacities from the kinetic parameters)**.** The thermokinetic capacities can be computed from the chemical standard potentials $C_i = c^\circ \exp(-\mu_i^\circ/(R^* T))$. The chemical standard potentials, or more precisely the Gibbs energies of formation, are tabulated for many important metabolites [1, 36]. However, for many networks, e. g. for signal transduction networks, no such data is available. If a kinetic model with generalized mass-action laws is available, the thermokinetic capacities and resistances can be alternatively computed from the generalized mass-action parameters $k_{\pm j}$ and $f_j(k, c)$.

The thermokinetic capacities determine the equilibrium point and can be computed from the equations in §5.6 (p. 90). For given equilibrium constants $K_{eq,j}$, capacities C_i only exist if the generalized Wegscheider conditions (see §2.52, p. 33) are fulfilled. Otherwise, the given parameters $k_{\pm j}$ describe a physically infeasible system. If the generalized Wegscheider conditions are fulfilled, the equation system in §5.6 contains $\mathrm{rank}(N)$ independent relations for i_0 unknown capacities. This system is undetermined because $\mathrm{rank}(N) < i_0$. For each of the $d_{i_0} = i_0 - \mathrm{rank}(N)$ conservation relations of the system, the modeler has one degree of freedom to choose the capacities. Different choices lead to models that are related by a translation of chemical potentials (Section 4.1.2.1, p. 54). If the capacities are known, the thermokinetic resistances can be computed with Equation 5.1 (p. 92).

Example 5.20 (Model of the PTS dynamics)**.** In the following example we derive the capacities and resistances for a model of the *Escherichia coli* Phosphotransferase System (PTS) based on the model from Rohwer et al. [87]. We use the reference concentration $c^\circ = 1$ µM, since the concentrations of the PTS proteins are in this order of magnitude. The model contains the following reactions

$$
\begin{array}{rcccl}
(PEP) + EI & \xrightleftharpoons[k_{-1}=480000\ \mu\mathrm{M\ min}^{-1}]{k_{+1}=1960\ \mu\mathrm{M\ min}^{-1}} & Pyr{\cdot}P{\cdot}EI & \xrightleftharpoons[k_{-2}=294\ \mu\mathrm{M\ min}^{-1}]{k_{+2}=108000\ \mu\mathrm{M\ min}^{-1}} & (Pyr) + EI{\cdot}P \\
EI{\cdot}P + HPr & \xrightleftharpoons[k_{-3}=14000\ \mu\mathrm{M\ min}^{-1}]{k_{+3}=14000\ \mu\mathrm{M\ min}^{-1}} & EI{\cdot}P{\cdot}HPr & \xrightleftharpoons[k_{-4}=3360\ \mu\mathrm{M\ min}^{-1}]{k_{+4}=84000\ \mu\mathrm{M\ min}^{-1}} & EI + HPr{\cdot}P \\
HPr{\cdot}P + IIA & \xrightleftharpoons[k_{-5}=21960\ \mu\mathrm{M\ min}^{-1}]{k_{+5}=21960\ \mu\mathrm{M\ min}^{-1}} & HPr{\cdot}P{\cdot}IIA & \xrightleftharpoons[k_{-6}=3384\ \mu\mathrm{M\ min}^{-1}]{k_{+6}=4392\ \mu\mathrm{M\ min}^{-1}} & HPr + IIA{\cdot}P \\
IIA{\cdot}P + IICB & \xrightleftharpoons[k_{-7}=880\ \mu\mathrm{M\ min}^{-1}]{k_{+7}=880\ \mu\mathrm{M\ min}^{-1}} & IIA{\cdot}P{\cdot}IICB & \xrightleftharpoons[k_{-8}=960\ \mu\mathrm{M\ min}^{-1}]{k_{+8}=2640\ \mu\mathrm{M\ min}^{-1}} & IIA + IICB{\cdot}P \\
IICB{\cdot}P + (Glc) & \xrightleftharpoons[k_{-9}=389\ \mu\mathrm{M\ min}^{-1}]{k_{+9}=260\ \mu\mathrm{M\ min}^{-1}} & IICB{\cdot}P{\cdot}Glc & \xrightleftharpoons[k_{-10}=5.4^{-3}\ \mu\mathrm{M\ min}^{-1}]{k_{+10}=4800\ \mu\mathrm{M\ min}^{-1}} & IICB + (Glc{\cdot}P)
\end{array}
$$

where the dot "·" indicates a complex of the respective species. The reaction scheme describes how a phosphoryl group is transferred from phosphoenol-pyruvate PEP to glucose Glc via the proteins EI, HPr, IIA and $IICB$. The parameters above and below of the reaction arrows are mass-action parameters according to Definition 2.49 (p. 33) with $f_j(k, c) = 1$. For this reason the forward constant k_{+j} and the backward constant k_{-j} have the same unit. The overall concentrations of the four occurring proteins EI, HPr, IIA and $IICB$ are invariant. For this reasons, we have four degrees of freedom in choosing the capacities of the proteins. We choose

$$C_{EI} = C_{HPr} = C_{IIA} = C_{IICB} = 1\ \mu\text{M},$$

since the total concentrations of these proteins are in this order of magnitude. The number of phosphoryl groups $c_{PEP} + c_{Pyr\cdot P\cdot EI} + c_{EI\cdot P} + c_{EI\cdot P\cdot HPr} + c_{HPr\cdot P} + c_{HPr\cdot P\cdot IIA} + c_{IIA\cdot P} + c_{IIA\cdot P\cdot IICB} + c_{IICB\cdot P} + c_{IICB\cdot P\cdot Glc} + +c_{Glc\cdot P}$ is also invariant and we choose

$$C_{EI\cdot P} = 1\ \mu\text{M}.$$

Further, the concentrations $c_{PEP} + c_{Pyr}$ and $c_{Glc} + c_{Glc\cdot P}$ are invariant and we are free to choose in accordance with typical concentrations:

$$C_{Pyr} = C_{Glc} = 1000\ \mu\text{M}.$$

Alternatively, using the expression in §5.5 (p. 90), we could compute the capacities of the metabolites phosphoenol-pyruvate PEP, pyruvate Pyr, glucose Glc and glucose-6-phosphate $Glc{\cdot}P$ from suited tables of Gibbs formation energies, e. g. from Alberty [1] or Feist et al. [36]. The use of such tables is preferable because this guarantees a certain standardization of the capacities and resistances that makes them exchangeable between models (cf. Definition 2.46, p. 32). The degrees of freedom in choosing the capacities should normally only be used for species where no information on the Gibbs formation energies is available. For the sake of demonstration, we choose here a different approach and use the degrees of freedom to set the capacities of PEP and Glc.

We compute the remaining capacities from the given kinetic parameters. According to §5.6 (p. 90), we have that $C_{Pyr\cdot P\cdot EI} = K_{eq,2}^{-1}\, C_{Pyr}\, C_{EI\cdot P}/c^\circ$, where $K_{eq,2} = k_{+2}/k_{-2}$ is the equilibrium constant of reaction 2. Similarly, we have $C_{PEP} = K_{eq,1}^{-1}\, C_{Pyr\cdot P\cdot EI}\, C_{EI}^{-1}\, c^\circ$, $C_{EI\cdot P\cdot HPr} = K_{eq,3}\, C_{EI\cdot P}\, C_{HPr}/c^\circ$ and $C_{HPr\cdot P} = K_{eq,4}\, C_{EI\cdot P\cdot HPr}\, C_{EI}^{-1}\, c^\circ$. Performing the same calculations for the reactions 5 to 10 we can compute all capacities:

$$\begin{aligned}
C_{PEP} &= 666.7\ \mu\text{M}, & C_{Pyr\cdot P\cdot EI} &= 2.722\ \mu\text{M}, & C_{Pyr} &= 1000\ \mu\text{M},\\
C_{EI} &= 1\ \mu\text{M}, & C_{EI\cdot P\cdot HPr} &= 1\ \mu\text{M}, & C_{EI\cdot P} &= 1\ \mu\text{M},\\
C_{HPr} &= 1\ \mu\text{M}, & C_{HPr\cdot P\cdot IIA} &= 25\ \mu\text{M}, & C_{HPr\cdot P} &= 25\ \mu\text{M},\\
C_{IIA} &= 1\ \mu\text{M}, & C_{IIA\cdot P\cdot IICB} &= 32.45\ \mu\text{M}, & C_{IIA\cdot P} &= 32.45\ \mu\text{M},\\
C_{IICB} &= 1\ \mu\text{M}, & C_{IICB\cdot P\cdot Glc} &= 59638.7\ \mu\text{M}, & C_{IICB\cdot P} &= 89.23\ \mu\text{M},\\
C_{Glc} &= 1000\ \mu\text{M}, & & & C_{Glc\cdot P} &= 5.301 \times 10^{10}\ \mu\text{M}.
\end{aligned}$$

From Equation 5.1 (p. 92) we can compute the resistances, e. g. $\bar{R}_1 = k_{+1}^{-1}\,(c^\circ)^2\, C_{PEP}^{-1}\, C_{EI}^{-1} = k_{-1}^{-1}\, c^\circ\, C_{Pyr\cdot P\cdot EI}^{-1}$. Thus, we have two equivalent possibilities to compute $\bar{R}_1$. This equality can be used as a probe for the calculated capacities, since both expressions would yield different

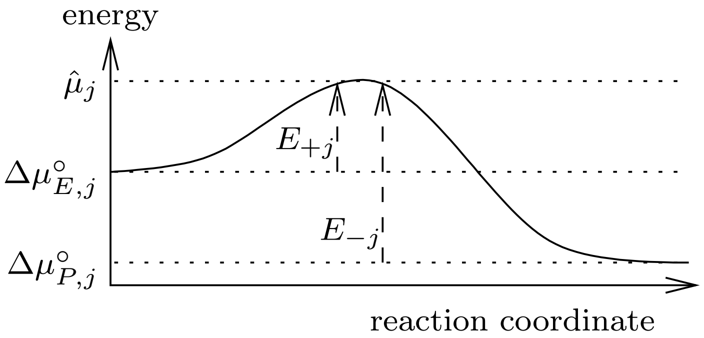


Figure 5.1.: Energy of the species along the reaction coordinate.

results if the capacities were wrong. We get

$$\bar{R}_1 = 0.7653 \times 10^{-6} \text{ min } \mu\text{M}^{-1}, \qquad \bar{R}_2 = 3.401 \times 10^{-6} \text{ min } \mu\text{M}^{-1},$$
$$\bar{R}_3 = 71.43 \times 10^{-6} \text{ min } \mu\text{M}^{-1}, \qquad \bar{R}_4 = 11.90 \times 10^{-6} \text{ min } \mu\text{M}^{-1},$$
$$\bar{R}_5 = 1.821 \times 10^{-6} \text{ min } \mu\text{M}^{-1}, \qquad \bar{R}_6 = 9.107 \times 10^{-6} \text{ min } \mu\text{M}^{-1},$$
$$\bar{R}_7 = 35.02 \times 10^{-6} \text{ min } \mu\text{M}^{-1}, \qquad \bar{R}_8 = 11.67 \times 10^{-6} \text{ min } \mu\text{M}^{-1},$$
$$\bar{R}_9 = 0.0431 \times 10^{-6} \text{ min } \mu\text{M}^{-1}, \qquad \bar{R}_{10} = 0.00349 \times 10^{-6} \text{ min } \mu\text{M}^{-1}.$$

The computed capacities and resistances spread over several orders of magnitude. Depending on the scaling of the system, this could be a hint to a potential for model reduction. We will discuss the reduction of this model in Example 7.10 (p. 148).

5.2. Excursus: The Theory of the Activated Complex

The theory of the activated complex provides a model for the functional dependency of the rate of simple reactions on several intensive variables as for example the temperature or the electrical potential [9]. It is based on the assumption that a molecular collision event needs a certain energy to trigger a reaction event. This means that the energy of the species along the reaction coordinate has a maximum as shown in Figure 5.1. The molecular conformation at the energy maximum is called the *activated complex.* The difference of the maximal energy and the energy of the reactants and products is called the *activation energy* of the forward and backward reaction, respectively.

This excursus demonstrates how the theory of the activated complex can be applied to a thermokinetic model. For this purpose, it first introduces the thermokinetic potential of the thermal energy, i. e. the energy of the collisions. Treating energy as a catalyst of a reaction directly leads to the corresponding rate laws.

§ 5.21. The total differential $dS = 1/T\,dU + p/T\,dV - \sum \mu_i/T\,dn_i$ (see §2.32, p. 29) shows that $1/T$ is the conjugated potential of the energy in the same way as $-\mu_i/T$ is the conjugated potential of compound X_i. This motivates the definition of a thermokinetic potential of the energy.

Definition 5.22 (Thermokinetic potential of the energy)**.** The thermokinetic potential of the energy ξ_U is defined by

$$\xi_U = \exp\left(-\frac{u^\circ}{R^* T}\right) \tag{5.2}$$

where $u^\circ = 1 \text{ J mol}^{-1}$ is the standard energy.

§ 5.23 (Extended reaction stoichiometry)**.** Reaction events with a collision energy lower than the activation energy do virtually not occur. Further, collisions with high energy are very improbable compared to collisions with low energy. For this reason, we can assume that most reaction events occur with a collision energy near the activation energy [9]. Thus, a certain amount of energy $\hat{\mu}_j$ is needed in a stoichiometric relation. We may write an extended reaction equation:

$$\sum_{i \in E_j} \nu_{E,ij}\, X_i + \underbrace{\nu_{\mathrm{U,j}} \text{ J mol}^{-1}}_{\hat{\mu}_j} \rightleftharpoons \sum_{i \in P_j} \nu_{P,ij}\, X_i + \underbrace{\nu_{\mathrm{U,j}} \text{ J mol}^{-1}}_{\hat{\mu}_j}. \tag{5.3}$$

It is crucial to note that the energy $\hat{\mu}_j$ is *not* the reaction heat. It is the collision energy that is needed to overcome the activation barrier. Thus, the energy $\hat{\mu}$ is only 'borrowed' from the environment and is released after the reaction event. The energy $\hat{\mu}_j$ enters the reaction equation as a catalyst.

§ 5.24 (Rate of a reaction with an activation barrier)**.** For the above reaction equation, we get the thermokinetic force $F = \xi_U^{\nu_{U,j}} \prod_{E_j} \xi_i^{\nu_{E,ij}} - \xi_U^{\nu_{U,j}} \prod_{P_j} \xi_i^{\nu_{P,ij}}$. In an ideal dilute solution with $C_i = c^\circ \exp(-\mu_i^\circ/(R^* T))$, i. e. $\xi_i = \exp(\mu_i^\circ/(R^* T))\, c_i/c^\circ$, and ideal mass-action kinetics with constant resistances $\bar{R}_j$, the reaction rates J_j read

$$\begin{aligned} J_j =& \bar{R}_j^{-1} \left(\xi_U^{\nu_{U,j}} \prod_{i \in E_j} \xi_i^{\nu_{E,ij}} - \xi_U^{\nu_{U,j}} \prod_{i \in P_j} \xi_i^{\nu_{P,ij}} \right) = \\ =& + \underbrace{\bar{R}_j^{-1} \exp\left(\frac{\Delta\mu_{E,j}^\circ - \hat{\mu}_j}{R^* T}\right)}_{k_{+j}} \prod_{i \in E_j} \left(\frac{c_i}{c^\circ}\right)^{\nu_{E,ij}} - \underbrace{\bar{R}_j^{-1} \exp\left(\frac{\Delta\mu_{P,j}^\circ - \hat{\mu}_j}{R^* T}\right)}_{k_{-j}} \prod_{i \in P_j} \left(\frac{c_i}{c^\circ}\right)^{\nu_{P,ij}}. \end{aligned} \tag{5.4}$$

§ 5.25 (Arrhenius Law)**.** The equations for the rate constants $k_{\pm j}$ given above are known as *Arrhenius Law*. The activation energies of the forward and the backward reaction are $E_{+j} = \hat{\mu}_j - \Delta\mu_{E,j}^\circ$ and $E_{-j} = \hat{\mu}_j - \Delta\mu_{P,j}^\circ$, respectively (see Figure 5.1). Thus, the assumption of a constant thermokinetic resistance for a reaction equation extended by the activation energy is equivalent to Arrhenius Law for the temperature dependency of the reaction rate.

§ 5.26 (Butler-Volmer Equation)**.** The transport of charged compounds between membrane-separated compartments with different electrical potential plays a pivotal role in the cellular metabolism. For example, many reactions in the electron transport chain are coupled to the transport of protons across a membrane.

The reaction coordinate of transport reactions can be associated with a spatial path through the interface between the compartments. Thus, the activated complex occurs at a specific electrical potential. As an example, consider the passive transport of a species A with charge number z_A from a phase ($'$) with electrical potential ϕ' to a phase ($''$) with electrical potential ϕ''. The activated complex $\hat{A}$ occurs at the electrical potential $\hat{\phi}$. The activation energy falls apart in a thermal and a electrical activation energy that both need to be considered in the extended reaction equation:

$$A' + \hat{\mu} + z_A\, F\, \hat{\phi} \rightleftharpoons A'' + \hat{\mu} + z_A\, F\, \hat{\phi}$$

where F is the Faraday constant. The electrochemical potential of A in an ideal dilute solution is $\mu_A = \mu_A^\circ + z_A\, F\, \phi + R^*\, T\, \log(c_i/c^\circ)$. Thus, its thermokinetic capacity is $C_A = c^\circ\, \exp(-(\mu_A^\circ + z_A\, F\, \phi)/(R^*\, T))$. A constant resistance $\bar{R}$ leads to a rate equation that can be written in the form

$$\begin{aligned} J =& \bar{R}^{-1} \underbrace{\left(\xi'_A\, \xi_U^{(\hat{\mu}+z_A\, F\, \hat{\phi})/u^\circ} - \xi''_A\, \xi_U^{(\hat{\mu}+z_A\, F\, \hat{\phi})/u^\circ}\right)}_{F} = \\ &+ \underbrace{\bar{R}^{-1} \exp\left(\frac{\mu_A^\circ - \hat{\mu}}{R^*\, T}\right) \exp\left((1-\alpha)\, \frac{z_A\, F\, \Delta\phi}{R^*\, T}\right)}_{k_{+j}} \frac{c'_A}{c^\circ} \\ &- \underbrace{\bar{R}^{-1} \exp\left(\frac{\mu_A^\circ - \hat{\mu}}{R^*\, T}\right) \exp\left(-\alpha\, \frac{z_A\, F\, \Delta\phi}{R^*\, T}\right)}_{k_{-j}} \frac{c''_A}{c^\circ} \end{aligned}$$

with the electrical potential difference $\Delta\phi = \phi' - \phi''$ and the transition factor α with $\hat{\phi} = \alpha\, \phi' + (1-\alpha)\, \phi''$ that determines the electrical potential of the activated complex relative to the electrical potentials of the phases (') and ("). This is the Butler-Volmer equation as it is used to model electrode processes and the transport of charged species [9, 93]. Thus, simple transport reactions can be modeled by extending the stoichiometric equations by the thermal and electrical activation energy and assuming a constant thermokinetic resistance.

§ 5.27 (Kinetic salt effect)**.** A major source of non-ideality in biochemical networks are ionic interactions. This means that the chemical potentials of ions depend on the ionic strength $I = (1/2)\, \sum_i z_i^2\, c_i$. According to the Debye-Hückel theory, the dependency can be approximated for small concentrations and a small ionic strength by $\mu_i = \mu_i^\circ - R^*\, T\, A\, z_i^2\, (I/c^\circ)^{1/2} + R^*\, T\, \log(c_i/c^\circ)$ with $A = 0.510651$ at 298.15 K [1]. The activated complex X of a reaction $B + C \rightleftharpoons D$ has the charge number $z_X = z_D = z_B + z_C$. Thus, the stoichiometric equation extended by the activation energy reads

$$B + C + \hat{\mu} - R^* \, T \, A \, (z_B + z_C)^2 \, (I/c^\circ)^{1/2} \rightleftharpoons D + \hat{\mu} - R^* \, T \, A \, (z_B + z_C)^2 \, (I/c^\circ)^{1/2}.$$

With a constant resistance $\bar{R}$ this leads to

$$\begin{aligned} J =& \bar{R}^{-1} \left(\xi_B \, \xi_C \, \xi_U^{(\hat{\mu} - R^* \, T \, A \, (z_B + z_C)^2 \, (I/c^\circ)^{1/2})/u^\circ} - \xi_D \, \xi_U^{(\hat{\mu} - R^* \, T \, A \, (z_B + z_C)^2 \, (I/c^\circ)^{1/2})/u^\circ} \right) = \\ &+ \bar{R}^{-1} \, \exp\left(\frac{\mu_B^\circ + \mu_C^\circ - \hat{\mu}}{R^* \, T} \right) \, \exp\left(-A \, (I/c^\circ)^{1/2} \, (z_B^2 + z_C^2 - (z_B + z_C)^2) \right) \, \frac{c_B}{c^\circ} \, \frac{c_C}{c^\circ} \\ &- \bar{R}^{-1} \, \exp\left(\frac{\mu_D^\circ - \hat{\mu}}{R^* \, T} \right) \, \frac{c_D}{c^\circ}. \end{aligned}$$

This rate equation describes the kinetic salt effect, i. e. the effect of the ionic strength on the reaction rates, in simple reactions as described in Atkins [9].

§ 5.28 (Conclusions)**.** The theory of the activated complex can be naturally integrated in the TKM formalism. For this purpose, one extends the stoichiometric reaction equation by the activation energy that acts as a catalyst. The amount of activation energy may depend on variables as the electrical potential or the ionic strength. Then, the thermokinetic force is formulated as if the activation energy was a further reactant with thermokinetic potential ξ_U. This means that activation energy is treated as if it were a chemical compound. In the simple situations that are considered above, a constant resistance is sufficient to describe the basic laws used to describe the dependency of the reaction rate on the temperature, the electrical potential and the ionic strength. Thus, TKM provides a natural and unifying way to model these dependencies.

This method can possibly be generalized to more complex situations. For example, one could model transport reactions between compartments of different temperature and ionic strength. Further, one could model a coupling of mass and heat flow, by assuming that the activation energy is acquired in one compartment but released in an other. It is beyond the scope of this work to study if the according TKM rate laws approximate the observed kinetics in such complex situations. In any case, the use of the TKM formalism guarantees the thermodynamic consistency of the model.

5.3. Thermokinetic Models

Section 5.1 introduced thermokinetic potentials ξ, thermokinetic forces F and the matrix of thermokinetic resistances $\bar{R}$. These quantities contain the same information as the chemical potentials μ, thermodynamic forces $\Delta\mu$ and thermodynamic resistances R, but their handling is much easier. This holds in particular for mass-action kinetics in ideal dilute solutions. Then, thermokinetic potentials ξ_i and forces F_j are proportional to concentrations c_i and fluxes J_j, respectively. Thus, model equations in these new thermokinetic variables are much simpler than in the original thermodynamic formulation.

The advantage of the thermokinetic formalism gets lost if non-diagonal resistance matrices occur, e. g. after certain transformation or reduction steps, since then the ideal resistance function $R_\circ$ that is described by a complex expression appears in the off-diagonal elements of the resistance matrix. For this reason, the further considerations are limited to diagonal resistance matrices. It will be discussed when and how non-diagonal matrices can be avoided during transformation and reduction.

We reformulate our original definition of thermodynamic models (Definition 3.1, p. 40) with thermokinetic variables. Since we consider only diagonal resistance matrices, it is sufficient to use the vector of the diagonal elements. For the sake of simplicity, we will suppress the bar $(\bar{\cdot})$ over the thermokinetic resistances from now on. Thus, we denote the vector of thermokinetic resistances simply by R.

The thermodynamic variables μ, $\Delta\mu_E$ and $\Delta\mu_P$ are proportional to the logarithm of the thermokinetic variables ξ, F_E and F_P, respectively. Thus, linear operations in μ, $\Delta\mu_E$ and $\Delta\mu_P$ are equivalent to lin-log operations as introduced in Section 2.1.2 (p. 25).

Definition 5.29 (Thermokinetic model (TK model) of a reaction system)**.** A *thermokinetic model* M of a reaction system with i_0 internal compounds, j_0 internal fluxes, $i_{0,e}$ compounds with clamped thermokinetic potentials and $j_{0,e}$ clamped fluxes is characterized by (1) stoichiometric matrices $N_E, N_P \in \mathbb{R}^{i_0 \times j_0}$, $N_e \in \mathbb{R}^{i_0 \times j_{0,e}}$, $S_E, S_P \in \mathbb{R}^{i_{0,e} \times j_0}$, (2) functions $c(\xi, \xi_e) \in \mathbb{R}^{i_0}$, $R(\xi, \xi_e) \in \mathbb{R}^{j_0}$ and (3) initial thermokinetic potentials $\xi_0 \in \mathbb{R}^{i_0}$. For the functions $c(\xi, \xi_e)$ and $R(\xi, \xi_e)$ we demand:

$$
\begin{aligned}
&R_j(\xi, \xi_e) \geq 0, \\
&\left(\frac{\partial c}{\partial \xi}\, \mathrm{diag}(\xi)\right) = \left(\frac{\partial c}{\partial \xi}\, \mathrm{diag}(\xi)\right)^T, \qquad \left(\mathrm{diag}(\xi)\, \frac{\partial c}{\partial \xi}\right) \geq 0
\end{aligned}
$$

for all $\xi \in \mathbb{R}_+^{i_0}$ and $\xi_e \in \mathbb{R}_+^{i_{e,0}}$. The environment is described by clamped thermokinetic potentials $\xi_e(t) \in \mathbb{R}^{i_{0,e}}$ and clamped fluxes $J_e(t) \in \mathbb{R}^{j_{0,e}}$. The model equations read

$$
\dot{c} = N\, J + N_e\, J_e, \quad F = N_E^T \# \xi \circ S_E^T \# \xi_e - N_P^T \# \xi \circ S_P^T \# \xi_e, \quad c = c(\xi, \xi_e), \quad R(\xi, \xi_e) \circ J = F
$$

with $N = N_P - N_E$, $S = S_P - S_E$ and $\xi(0) = \xi_0$. We formally characterize a thermokinetic model of a reaction system by the tuple $M = [N_E, N_P, S_E, S_P, N_e, c(\xi, \xi_e), R(\xi, \xi_e), \xi_0]$.

§ 5.30 (Matrices N_E, N_P, S_E and S_P)**.** The above definition distinguishes between the stoichiometric matrices of reactants N_E and S_E and the stoichiometric matrices of products N_P and S_P. The matrices N and S of the corresponding thermodynamic model are given by $N = N_P - N_E$ and $S = S_P - S_E$ (see §2.30, p. 28). This distinction is necessary to formulate the thermokinetic forces F.

The notation $N_{E/P}$ or $S_{E/P}$ will be used, Whenever an expression is valid for N_E and N_P or S_E and S_P, respectively.

Example 5.31. Consider the system of Example 5.17 (p. 92) with $A \overset{1}{\rightleftharpoons} B \overset{2}{\rightleftharpoons} C{+}D$ and constant thermokinetic capacities C_i and resistances R_j. The thermokinetic model of this system is given by $c(\xi, \xi_e) = (C_A\,\xi_A, C_B\,\xi_B, C_C\,\xi_C, C_D\,\xi_D)^T$, $R(\xi, \xi_e) = (R_1, R_2)^T$ and

$$N_E = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 0 \\ 0 & 0 \end{pmatrix}, \qquad N_P = \begin{pmatrix} 0 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{pmatrix}.$$

The matrices S_E, S_P and N_e are empty because the system is closed.

5.4. Transformation and Translation

The thermokinetic modeling formalism is a convenient reformulation of the thermodynamic modeling formalism. Both formalisms are basically equivalent and the transformation and translation methods for thermodynamic models (see Section 4.1.1, p. 51) can be applied.

Here we give the transformation and translation rules for thermokinetic models. Since we restrict ourselves to diagonal resistance matrices, we can only adapt methods where the occurrence of off-diagonal elements in the resistance matrix can be avoided.

Further, this section introduces an additional transformation method, namely the translation of stoichiometric coefficients that is specific for thermokinetic models.

5.4.1. Linear Transformation

Due to the restriction to diagonal resistance matrices, linear transformations of fluxes are not possible. Such transformations lead to non-diagonal resistance matrices. Thus, the following corollary considers only a transformation of concentrations.

Corollary 5.32 (Transformation of a thermokinetic model)**.** *Let M be a thermokinetic model (see Definition 5.29, p. 100), and let T_c, $T_{c,e}$ and $T_{J,e}$ be square matrices of full rank. Then, the solution of the system $\hat{M}$ with*

$$\begin{aligned} \hat{N}_E &= T_c\, N_E, & \hat{N}_P &= T_c\, N_P, \\ \hat{S}_E &= T_{c,e}\, S_E, & \hat{S}_P &= T_{c,e}\, S_P, \\ \hat{c}(\hat{\xi}, \hat{\xi}_e) &= T_c\, c(T_c^T \# \hat{\xi}, T_{c,e}^T \# \hat{\xi}_e), & \hat{R}(\hat{\xi}, \hat{\xi}_e) &= R(T_c^T \# \hat{\xi}, T_{c,e}^T \# \hat{\xi}_e), \\ \hat{N}_e &= T_c\, N_e\, T_{J,e}, & \hat{\xi}_0 &= T_c^{T,-1} \# \xi_0. \end{aligned}$$

is equivalent to that of M with

$$\begin{aligned} \hat{c} &= T_c\, c, & T_c^T \# \hat{\xi} &= \xi, & T_{c,e}^T \# \hat{\xi}_e &= \xi_e, \\ \hat{J} &= J, & \hat{F} &= F, & T_{J,e}\, \hat{J}_e &= J_e. \end{aligned}$$

Proof. This transformation rule follows from the Corollary 4.2 (p. 52). ☐

§ 5.33 (Negative stoichiometric coefficients)**.** In natural coordinates the entries of the matrices N_E, N_P, S_E and S_P are non-negative. After a transformation of concentrations, the entries of the corresponding transformed matrices may be negative.

5.4.2. Translation of the Variables

Section 4.1.2 (p. 54) introduced rules for the translation of the variables c, μ and J in thermodynamic models. Analog rules can be formulated for thermokinetic models. This section discusses only the translation of chemical potentials because this operation is needed for an appropriate scaling of the state variables of the simulation equations. It is equivalent to a change of the reference state of the Gibbs formation energies.

Corollary 5.34 (Scaling of a TK model)**.** *Let M be a thermokinetic model (see Definition 5.29, p. 100), and let $\delta\xi \in \mathbb{R}_+^{i_0}$, $\delta\xi_e \in \mathbb{R}_+^{i_{0,e}}$ be vectors with $N^T \log(\delta\xi) + S^T \log(\delta\xi_e) = 0$. Then, the solution of the system $\hat{M}$ with*

$$\hat{N}_{E/P} = N_{E/P}, \qquad \hat{S}_{E/P} = S_{E/P}, \qquad \hat{N}_e = N_e,$$
$$\hat{c}(\hat{\xi}, \hat{\xi}_e) = c\left(\hat{\xi} \circ \delta\xi^{(-1)}, \hat{\xi}_e \circ \delta\xi_e^{(-1)}\right), \qquad \hat{\xi}_0 = \xi_0 \circ \delta\xi^{(-1)}$$

and

$$\begin{aligned}\hat{R}(\hat{\xi}, \hat{\xi}_e) &= N_E^T\#\delta\xi \circ S_E^T\#\delta\xi \circ R\left(\hat{\xi} \circ \delta\xi^{(-1)}, \hat{\xi}_e \circ \delta\xi_e^{(-1)}\right)\\ &= N_P^T\#\delta\xi \circ S_P^T\#\delta\xi \circ R\left(\hat{\xi} \circ \delta\xi^{(-1)}, \hat{\xi}_e \circ \delta\xi_e^{(-1)}\right)\end{aligned}$$

is equivalent to that of M with

$$\hat{c} = c, \qquad \hat{\xi} = \xi \circ \delta\xi, \qquad \hat{\xi}_e = \xi_e \circ \delta\xi_e,$$
$$\hat{J} = J, \qquad \hat{F} = F, \qquad \hat{J}_e = J_e.$$

Proof. The proof follows the lines of the proof of Corollary 4.10 (p. 54). Write down the model equations of M. Now replace $J = \hat{J}$, $F = \hat{F}$, $\xi = \hat{\xi} \circ \delta\xi^{(-1)}$ and $\xi_e = \hat{\xi}_e \circ \delta\xi_e^{(-1)}$. This immediately leads to the functions $\hat{c}(\hat{\xi}, \hat{\xi}_e)$ and the matrices $\hat{N}_{E/P}$, $\hat{S}_{E/P}$ and $\hat{N}_e$. To prove the expression for $\hat{R}$ we start with the equation $\hat{R} \circ \hat{J} = \hat{F}$ and prove that it is equivalent to $R \circ J = F$. The equation $\hat{R} \circ \hat{J} = \hat{F}$ can be expanded to

$$\begin{aligned}&\overbrace{\underbrace{N_E^T\#\delta\xi \circ S_E^T\#\delta\xi}_{\delta F_E} \circ R}^{\hat{R}} \circ \hat{J} = \hat{F} = \hat{N}_E^T\#\hat{\xi} \circ \hat{S}_E^T\#\hat{\xi}_e - \hat{N}_P^T\#\hat{\xi} \circ \hat{S}_P^T\#\hat{\xi}_e\\ &\qquad = N_E^T\#\xi \circ S_E^T\#\xi_e \circ \underbrace{N_E^T\#\delta\xi \circ S_E^T\#\delta\xi_e}_{\delta F_E} - N_P^T\#\xi \circ S_P^T\#\xi_e \circ \underbrace{N_P^T\#\delta\xi \circ S_P^T\#\delta\xi_e}_{\delta F_P}. \qquad (5.5)\end{aligned}$$

Due to the specific choice of $\delta\xi$ and $\delta\xi_e$, we have $N_E^T \log(\delta\xi) + S_E^T \log(\delta\xi_e) = N_P^T \log(\delta\xi) + S_P^T \log(\delta\xi_e)$ and thus $\delta F_E = \delta F_P$. For this reason these terms cancel out and the equation $R \circ J = F$ remains. □

Example 5.35 (Scaling of capacities and resistances)**.** The above corollary is often necessary to numerically scale the simulation equations. Consider the single reaction of glucose-6-phosphate to fructose-6-phosphate ($g6p \rightleftharpoons f6p$) that is catalyzed by the phosphoglucose isomerase. Alberty [1] gives transformed Gibbs formation energies $\Delta_f G_i'^{\circ}$ at $pH = 7$, $T = 298.15$ K, $I = 0.25$ M and $c^{\circ} = 1$ M:

$$\Delta_f G_{g6p}'^{\circ} = -1318.92 \text{ kJ mol}^{-1}, \qquad \Delta_f G_{f6p}'^{\circ} = -1315.74 \text{ kJ mol}^{-1}.$$

These values may be used as chemical standard potentials μ_i° and lead to the capacities ($C_i = c^{\circ}\, m \exp(-\mu_i^{\circ}/(R^*\, T))$):

$$C_{g6p} = 1.160 \times 10^{231} \text{ M}, \qquad C_{f6p} = 3.217 \times 10^{230} \text{ M}.$$

Clearly, these values are not suited for direct use in numerical simulations. The tables from Alberty [1] are based on the convention that chemical elements in the standard state in their most stable form have $\Delta_f G_i'^{\circ} = 0$. Most relevant (bio)chemical species have a rather low, negative $\Delta_f G_i$ because the respective compounds are energetically more favorable than an analogous mixture of pure elements.

Using the above corollary, we can scale the capacities to values more suited for numerical computations. The stoichiometric matrix is $N^T = (-1, 1)$ and thus the condition for the scaling factors is $-\log(\delta\xi_{g6p}) + \log(\delta\xi_{f6p}) = 0$ or equivalently $\delta\xi_{g6p} = \delta\xi_{f6p}$.

Assume that $g6p$ has a typical concentration of 1 mM. From a numerical perspective, an optimal value of its capacity is 1 mM because then the thermokinetic potential ξ_{g6p} that is a state variable of the model varies around the typical value 1. We choose $\delta\xi_{g6p} = \delta\xi_{f6p} = 1.160 \times 10^{234}$ and get the new relation

$$c_{g6p} = \underbrace{C_{g6p}\, \delta\xi_{g6p}^{-1}}_{\hat{C}_{g6p}} \hat{\xi}_{g6p} \qquad c_{f6p} = \underbrace{C_{f6p}\, \delta\xi_{f6p}^{-1}}_{\hat{C}_{f6p}} \hat{\xi}_{f6p}$$

with $\hat{C}_{g6p} = 1$ mM and $\hat{C}_{f6p} = 0.2773$ mM. These values are moderate and thus can be used for numerical computations.

§ 5.36. The above example shows that tables of Gibbs formation energies can be used to determine the capacities. The standardized and widely accepted definition of the reference state that underlies the tables of Gibbs formation energies allows for the direct exchange of model parameters between different models. These tables should be used if they are available. This often leads to very large capacities because most Gibbs formation energies are rather low. To avoid numerical problems, the models can be scaled before simulation and analysis by applying the above corollary. This scaling of the model can be performed automatically by the modeling or simulation tool.

5.4.3. Translation of the Stoichiometric Coefficients

The thermokinetic modeling formalism explicitly distinguishes the stoichiometric matrices of reactants and products. In the thermodynamic modeling formalism, this distinction is not necessary because the information is coded in the expression for the thermodynamic resistances. For example, the ideal thermodynamic resistance function for mass-action kinetics depends on the thermodynamic forces exerted by reactants and products $\Delta\mu_{E/P,j} = \sum_{i\in E/P_j} \nu_{E/P,ij}\, \mu_{i,j}$ that depend on the stoichiometric coefficients of reactants and products (see Definition 3.22, p. 47). The explicit introduction of $N_{E/P}$ and $S_{E/P}$ in TKM allows for a further translation operation.

Two reactions with the same overall stoichiometry may differ in their reactant and product stoichiometry. Consider the reaction from A to B catalyzed by X. Its stoichiometry can be described by two equivalent variants: (1) The reaction equation $A + X \rightleftharpoons B + X$ shows that X is a catalyst of the reaction. (2) The formulation $A \rightleftharpoons B$ has the same overall stoichiometry but does not refer to the catalyst X. Assume mass-action kinetics and small concentrations of A and B such that saturation effects of the catalyst X can be neglected. Then, the kinetics of variant (1) can be described by a constant thermokinetic resistance $R_1 = \text{const}$, whereas the resistance of the variant (2) is ξ_X-dependent in order to model the catalyzing activity of X, i. e. R_2 is proportional to ξ_X^{-1}. From a formal point of view, both model variants are equivalent. Depending on the context, the modeler will prefer variant (1) or (2).

In the following corollary we establish the general rules that allow transforming such different representations into each other. These rules are based on a translation of the stoichiometric coefficients.

Corollary 5.37 (Translation of stoichiometric coefficients)**.** *Let M be a thermokinetic model (see Definition 5.29, p. 100) and let $\delta N \in \mathbb{R}^{i_0\times j_0}$ and $\delta S \in \mathbb{R}^{i_{0,e}\times j_0}$. Then, the solution of the system $\hat{M}$ with*

$$\begin{aligned} \hat{N}_{E/P} &= N_{E/P} + \delta N, & \hat{S}_{E/P} &= S_{E/P} + \delta S, & \hat{N}_e &= N_e, \\ \hat{c}(\hat{\xi}, \hat{\xi}_e) &= c(\hat{\xi}, \hat{\xi}_e), & \hat{R}(\hat{\xi}, \hat{\xi}_e) &= R(\hat{\xi}, \hat{\xi}_e) \circ \delta N\#\xi \circ \delta S\#\xi_e, & \hat{\xi}_0 &= \xi_0 \end{aligned}$$

is equivalent to that of M with

$$\begin{aligned} \hat{c} &= c, & \hat{\xi} &= \xi, & \hat{\xi}_e &= \xi_e, \\ \hat{J} &= J, & \hat{F} &= F \circ \delta N\#\xi \circ \delta S\#\xi_e, & \hat{J}_e &= J_e. \end{aligned}$$

Proof. We compare the model equations of M and $\hat{M}$ (see Definition 5.29, p. 100) under application of the above transformation rules.

Since $N = N_P - N_E = \hat{N}$ and $S = S_P - S_E = \hat{S}$, the mole balance equations are equal $\dot{\hat{c}} = \dot{c}$. Since $\hat{c} = c$, $\hat{\xi} = \xi$ and $\hat{\xi}_e = \xi_e$, the equations $\hat{c} = \hat{c}(\hat{\xi}, \hat{\xi}_e)$ and $c = c(\xi, \xi_e)$ are also equivalent. Further, we have that $\hat{F} = \hat{N}_E\#\hat{\xi} \circ \hat{S}_E\#\hat{\xi}_e - \hat{N}_P\#\hat{\xi} \circ \hat{S}_P\#\hat{\xi}_e = N_E\#\xi \circ \delta N\#\xi \circ S_E\#\xi_e \circ \delta S\#\xi_e - N_P\#\xi \circ \delta N\#\xi \circ S_P\#\xi_e \circ \delta S\#\xi_e = \delta N\#\xi \circ \delta S\#\xi \circ (N_E\#\xi \circ S_E\#\xi_E - N_P\#\xi \circ S_P\#\xi_P) = \delta N\#\xi \circ \delta S\#\xi \circ F$. Thus, the forces follow the given transformation rule. With this result it follows that $\hat{R}\,\hat{J} = \hat{F}$ is equivalent to $R\,J = F$. Thus, all four parts of the model equations given in Definition 5.29 are equivalent. □

Example 5.38 (Catalyzed reaction)**.** Let the reaction $A{+}X \rightleftharpoons B{+}X$ have a constant resistance R. The species X has an overall stoichiometric coefficient of zero ($\nu_X = \nu_{X,P} - \nu_{X,E} = 0$) and is therefore a catalyst of the reaction. With the ordering (A, B, X), we have $N_E^T = (1, 0, 1)$ and $N_P^T = (0, 1, 1)$. By using $\delta N^T = (0, 0, -1)$ we get the reaction $A \rightleftharpoons B$ with $\hat{N}_E^T = (1, 0, 0)$, $\hat{N}_P^T = (0, 1, 0)$ and a resistance $\hat{R} = R\,\xi_X^{-1}$. Thus, the effect of the catalyst could be moved from the stoichiometric submodel to the kinetic submodel. Observe that in the case of a constant C_X the model $\hat{M}$ can be reduced, since the concentration and the thermokinetic potential of the catalyst are constant: $\xi_X = C_x^{-1}\,c_x = \text{const}$. In the translated model ξ_X, can be treated as a model parameter and not as a state variable.

5.5. Model Reduction

5.5.1. Reduction of the Stoichiometric Submodel

The reduction of the stoichiometric submodel relies on rank deficiencies of the stoichiometric matrices. (see Section 4.2.1, p. 58). In the following sections, these reduction methods are discussed for thermokinetic models.

5.5.1.1. Reduction of Conservation Relations

The following corollary for thermokinetic models is analog to Corollary 4.21 (p. 58) for thermodynamic models. It shows how conservation relations can be reduced from thermokinetic models.

Corollary 5.39 (Reduction of conservation relations)**.** *Let M be a thermokinetic model (see Definition 5.29, p. 100). Let $T_c = [T_{c,1}^T, T_{c,2}^T]^T$ be a square and invertible matrix with $T_{c,2}\,N_E = 0$, $T_{c,2}\,N_P = 0$ and $T_{c,2}\,N_e = 0$. Assume that $T_{c,2}\,\partial c/\partial\xi\ \operatorname{diag}(\xi)\,T_{c,2}^T$ is invertible for all $\xi \in \mathbb{R}_+^{i_0}$ and $\xi_e \in \mathbb{R}_+^{i_{e,0}}$. Let $\tilde{M}$ be a thermokinetic model with*

$$\begin{gathered}
\tilde{N}_{E/P} = T_{c,1}\,N_{E/P}, \qquad \tilde{S}_{E/P} = S_{E/P}, \qquad \tilde{N}_e = T_{c,1}\,N_e, \qquad \tilde{\xi}_0 = \operatorname{inv}^T_{\Lambda_c^{-1}}(T_{c,1})\#\xi_0, \\
\tilde{c}(\tilde{\xi}, \xi_e) = T_{c,1}\,c(T_{c,1}^T\#\tilde{\xi} \circ T_{c,2}^T\#\hat{\xi}_2(\tilde{\xi}, \xi_e), \xi_e), \qquad \tilde{R}(\tilde{\xi}, \xi_e) = R(T_{c,1}^T\#\tilde{\xi} \circ T_{c,2}^T\#\hat{\xi}_2(\tilde{\xi}, \xi_e), \xi_e)
\end{gathered}$$

where $\hat{\xi}_2(\tilde{\xi}, \xi_e)$ is a solution of

$$T_{c,2}\,c(T_{c,1}^T\#\tilde{\xi} \circ T_{c,2}^T\#\hat{\xi}_2, \xi_{e,0}) = T_{c,2}\,c(\xi_0, \xi_{e,0})$$

and Λ_c is an invertible matrix with $T_{c,1}\,\Lambda_c\,T_{c,2}^T = 0$. Then, the dynamics of M can be reconstructed from the dynamics of the reduced system $\tilde{M}$ by

$$\begin{aligned}
c &= T_c^{-1}\begin{pmatrix} \tilde{c} \\ T_{c,2}\,c(\xi_0, \xi_{e,0}) \end{pmatrix}, & \xi &= T_{c,1}^T\#\tilde{\xi} \circ T_{c,2}^T\#\hat{\xi}_2(\tilde{\xi}, \xi_e), \\
J &= \tilde{J}, & F &= \tilde{F}.
\end{aligned}$$

Proof. The proof is analog to that of Corollary 4.21. In Corollary 4.21 the condition $T_{c,2}\,N = 0$ guarantees that $\Delta\hat{\mu} = \Delta\mu$. Here the stronger conditions $T_{c,2}\,N_E = 0$ and $T_{c,2}\,N_P = 0$ guarantee that $\Delta\hat{\mu}_{E/P} = \Delta\mu_{E/P}$ which is equivalent to $\hat{F}_{E/P} = F_{E/P}$ and thus $\hat{F} = F$. □

§ 5.40 (Role of the condition $T_{c,2}\,N_{E/P} = 0$)**.** Per se the conditions $T_{c,2}\,N_E = 0$ and $T_{c,2}\,N_P = 0$ are much stronger than the condition $T_{c,2}N = T_{c,2}(N_P - N_E) = 0$. They are hardly fulfilled in a system in natural coordinates and do not cover all conservation relations. By means of translation of stoichiometric coefficients (Corollary 5.37, p. 104), we can bring any system to a form where both conditions are equivalent. A trivial solution is to choose $\delta N = -N_E$. Then, the translated matrices are $\hat{N}_E = 0$ and $\hat{N}_P = N$ and the conditions $T_{c,2}\,\hat{N}_{E/P} = 0$ and $T_{c,2}\,N = 0$ are equivalent. Thus, the application of the above corollary usually requires a preprocessing of the system by a translation of stoichiometric coefficients.

Example 5.41 (Reduction of a conservation relation)**.** Consider a reaction $A \rightleftharpoons B$ with constant thermokinetic capacities C_A and C_B and resistance R. The system contains the conservation relation $c_A + c_B = c_{A,0} + c_{B,0} = c_{AB,0} = \text{const}$. Since $N_E^T = (1,0)$ and $N_P^T = (0,1)$, there is no matrix $T_{c,2} \neq 0$ with $T_{c,2}\,N_{E/P} = 0$. With Corollary 5.37 (p. 104) we translate this system to a system with a negative stoichiometric coefficient ($A - B \rightleftharpoons 0$) with resistance $\hat{R} = R\,\xi_B^{-1}$. Now we have that $\hat{N}_E^T = (1,-1)$, $\hat{N}_P^T = (0,0)$ and we can use the matrices $T_{c,2} = (1,1)$ and $T_{c,1} = (1,0)$ to reduce the conservation relation. This means that we apply the transformation $\tilde{c}_1 = \hat{c}_1 = c_A$ and $\hat{c}_2 = c_A + c_B$. According to the transformation rules we get $\xi_A = \hat{\xi}_1\,\hat{\xi}_2$ and $\xi_B = \hat{\xi}_2$. With $\tilde{\xi} = \hat{\xi}_1$ the conservation relation reads $C_A\,\tilde{\xi}\,\hat{\xi}_2 + C_B\,\hat{\xi}_2 = c_{AB,0}$ which can be solved to $\hat{\xi}_2 = c_{AB,0}/(C_A\,\tilde{\xi} + C_B)$. With this result we get the new capacity: $\tilde{C} = \tilde{c}/\hat{\xi}_1 = (C_A\,\xi_A)/\tilde{\xi} = C_A\,\hat{\xi}_2 = c_{AB,0}\,C_A/(C_A\,\tilde{\xi} + C_B)$. We get the reduced system $\tilde{X} \rightleftharpoons 0$ with the resistance $\tilde{R} = \hat{R} = R\cdot(C_A\,\tilde{\xi} + C_B)/c_{AB,0}$ and the capacity $\tilde{C}$. The zero at the right hand side of the reaction equation means that in the reduced system this reaction does not have a product. Its force is $\tilde{F} = \tilde{\xi} - 1$.

5.5.1.2. Reduction of Stoichiometric Cycles

In the general case, the reduction of stoichiometric cycles (Section 4.2.1.2, p. 61) leads to a non-diagonal resistance matrix. We will not further discuss the general reduction of stoichiometric cycles because we restrict ourselves to diagonal resistance matrices. However, the special case of reactions with the same stoichiometry can be treated easily in the TKM formalism. This special case will be discussed later when considering model reduction on the level of reaction equations (Section 6.2.2, p. 113).

5.5.2. Reduction of the Thermodynamic and the Kinetic Submodel

The reduction of the thermodynamic and the kinetic submodel as described in Section 4.2.2 (p. 63) relies on rank deficiencies of the matrices $\partial c/\partial\mu$ and the thermodynamic resistance matrix. This section discusses these cases for thermokinetic models.

5.5.2.1. Reduction of the Thermodynamic Submodel

According to Section 4.2.2.1 (p. 63) a reduction of the thermodynamic submodel is possible if $\partial c/\partial\mu$ is singular for all μ and μ_e. In an ideal dilute solution with $c_i = c^\circ \exp((\mu_i - \mu_i^\circ)/(R^*\, T))$, the matrix $\partial c/\partial\mu$ is diagonal and $\lim_{\mu_i^\circ\to\infty} \partial c_i/\partial\mu_i = 0$. Thus, $\partial c_i/\partial\mu_i$ goes to zero and $\partial c/\partial\mu$ gets singular if $C_i = c^\circ \exp(-\mu_i^\circ/(R^*\, T))$ goes to zero. In real systems, this condition can only approximately be fulfilled.

In the thermokinetic formalism, a capacity of zero (or close to zero) indicates a potential for an (approximative) model reduction. In the general case, the reduction of the thermodynamic submodel as described in Section 4.2.2.1 leads to a non-diagonal reduced thermodynamic resistance matrix. Thus, the reduced system cannot be expressed as a thermokinetic model with a diagonal resistance matrix. For this reason, we will not further discuss this reduction method here.

In Section 6.3.1 (p. 113) when studying the reduction on the reaction equation and reaction rule level, we will discuss special cases where the application of these reduction method is easily possible.

5.5.2.2. Reduction of the Kinetic Submodel

The kinetic submodel can be reduced if the thermodynamic resistance matrix is singular (Section 4.2.2.2, p. 73). For diagonal resistance matrices this is only the case if a diagonal element is zero. Thus, a thermokinetic model can be reduced if some of its thermokinetic resistances are (approximately) zero.

Corollary 4.78 (p. 78) describes the reduction of the kinetic submodel for thermodynamic models. For the reduction one needs to choose a matrix $T_J = [T_{J,1}, T_{J,2}]$ with certain properties. Then, the reduced thermodynamic resistance matrix can be computed from the original resistance matrix and the matrices $T_{J,1}$ and $T_{J,2}$. For any system with a diagonal resistance matrix where some of the diagonal elements are zero it is possible to choose a matrix $T_{J,1}$, such that the reduced system has a diagonal resistance matrix. Thus, it is possible to write the reduced system as a thermokinetic model. The following corollary gives the details of this reduction procedure.

Corollary 5.42 (Rapid equilibrium assumption)**.** *Let M be a kinetically conflict-free thermokinetic model. Let R, N_E, N_P, S_E and S_P be partitioned, such that*

$$R = \begin{pmatrix} R' \\ 0 \end{pmatrix}, \qquad N_{E/P} = \begin{pmatrix} N'_{E/P} & N''_{E/P} \end{pmatrix}, \qquad S_{E/P} = \begin{pmatrix} S'_{E/P} & S''_{E/P} \end{pmatrix}$$

with the vector $R' \in \mathbb{R}^{\tilde{j}_0}$ and the matrices $N'_E, N'_P \in \mathbb{R}^{i_0\times\tilde{j}_0}$, $N''_E, N''_P \in \mathbb{R}^{i_0\times(j_0-\tilde{j}_0)}$, $S'_E, S'_P \in \mathbb{R}^{i_{0,e}\times\tilde{j}_0}$ and $S''_E, S''_P \in \mathbb{R}^{i_{0,e}\times(j_0-\tilde{j}_0)}$. Further, let $T_c^T = [T_{c,1}^T, T_{c,2}^T]$ and Λ_c be square and invertible matrices with

$$T_{c,1}\, N'' = 0, \qquad \operatorname{rank}(T_{c,2}\, N'') = \operatorname{rank}(T_{c,2}), \qquad T_{c,1}\, \Lambda_c\, T_{c,2}^T = 0.$$

Then, the dynamics of M can be reconstructed from the dynamics of the reduced system $\tilde{M}$ with

$$\begin{aligned} \tilde{N}_E &= T_{c,1}\, N'_E, & \tilde{N}_P &= T_{c,1}\, N'_P, \\ \tilde{S}_E &= S'_E - S''\ \mathrm{inv}(T_{c,2}\, N'')\, T_{c,2}\, N'_E, & \tilde{S}_P &= S'_P - S''\ \mathrm{inv}(T_{c,2}\, N'')\, T_{c,2}\, N'_P, \\ \tilde{N}_e &= T_{c,1}\, N_e, & \tilde{\xi}_0 &= \mathrm{inv}^T_{\Lambda_c^{-1}}(T_{c,1})\#\xi_0, \\ \tilde{c}(\tilde{\xi},\xi_e) &= T_{c,1}\, c(\xi,\xi_e), & \tilde{R}(\tilde{\xi},\xi_e) &= R'(\xi,\xi_e) \end{aligned}$$

where

$$\begin{aligned} c &= c(\xi,\xi_e), & \xi &= T^T_{c,1}\#\tilde{\xi} \circ (-S''\ \mathrm{inv}(T_{c,2}\, N'')\, T_{c,2})^T\#\xi_e, \\ J^T &= (\tilde{J}^T, \hat{J}_2^T), & F^T &= \begin{pmatrix}\tilde{F}^T & 0^T\end{pmatrix} \end{aligned}$$

and

$$\hat{J}_2 = \mathrm{inv}(T_{c,2}\, N'')\, T_{c,2}\, (\dot{c} - N'\, \tilde{J} - N_e\, J_e) + \Delta\hat{J}_2$$

with any $\Delta\hat{J}_2(t) \in \mathrm{null}(T_{c,2}\, N'')$.

Proof. The proof follows the lines of the proof of Corollary 4.78 (p. 78). We choose the matrices $T_{J,1} \in \mathbb{R}^{j_0 \times \tilde{j}_0}$, $T_{J,2} \in \mathbb{R}^{j_0 \times (j_0 - \tilde{j}_0)}$ such that $T_J = [T_{J,1}, T_{J,2}] = I$ and $\Lambda_J = I$. Then, we have $N'_{E/P} = N_{E/P}\, T_{J,1}$, $N''_{E/P} = N_{E/P}\, T_{J,2}$, $S'_{E/P} = S_{E/P}\, T_{J,1}$ and $S''_{E/P} = S_{E/P}\, T_{J,2}$. We transform the system with Corollary 5.32 (p. 101).

The forces $\hat{F}_2 = F'' = (T_{c,1}\, N''_E)^T\#\xi' \circ (T_{c,2}\, N''_E)^T\#\xi'' \circ S''^T_E\#\xi_e - (T_{c,1}\, N''_P)^T\#\xi' \circ (T_{c,2}\, N''_P)^T\#\xi'' \circ S''^T_P\#\xi_e = 0$ vanish because $R'' = 0$. This equation simplifies to $(T_{c,2}\, N''_E)^T\#\xi'' \circ S''^T_E\#\xi_e - (T_{c,2}\, N''_P)^T\#\xi'' \circ S''^T_P\#\xi_e = 0$ because due to $T_{c,1}\, N'' = 0$ it holds $(T_{c,1}\, N''_E)^T\#\xi' = (T_{c,1}\, N''_P)^T\#\xi'$. The unique solution of the equation $F'' = 0$ is $\xi'' = (-\,\mathrm{inv}^T(T_{c,2}\, N'')\, S''^T)\#\xi_e$ (cf. proof of Corollary 4.78, p. 78). Substituting this result into the forces $F' = (T_{c,1}\, N'_E)^T\#\xi' \circ (T_{c,2}\, N'_E)^T\#\xi'' \circ S'^T_E\#\xi_e - (T_{c,1}\, N'_P)^T\#\xi' \circ (T_{c,2}\, N'_P)^T\#\xi'' \circ S'^T_P\#\xi_e = 0$ yields

$$\begin{aligned} F' = &(T_{c,1}\, N'_E)^T\#\xi' \circ (S'_E - S''\ \mathrm{inv}(T_{c,2}\, N'')\, T_{c,2}\, N'_E)^T\#\xi_e \\ &-(T_{c,1}\, N'_P)^T\#\xi' \circ (S'_P - S''\ \mathrm{inv}(T_{c,2}\, N'')\, T_{c,2}\, N'_P)^T\#\xi_e. \end{aligned}$$

From this expression, one can derive the matrices $\tilde{S}_{E/P}$. Observe that the result contains only the matrices S'' and N'' but not the matrices $S''_{E/P}$ and $N''_{E/P}$.

The other formulas in the corollary are equivalent the formulas in Corollary 4.78 (p. 78). □

5.5.3. Reduction of the Boundary Conditions

Section 4.2.3 (p. 81) introduced methods for the reduction of the number of the input vectors that define the boundary conditions in thermodynamic models. Methods to reduce the number of clamped potentials and clamped fluxes were discussed. These methods are based on suited translation of potentials and fluxes. Since these translation methods also apply to thermokinetic models (see Section 5.4.2, p. 102), the reduction of boundary conditions can be directly applied to thermokinetic models and needs not to be discussed here in detail.

5.6. Conclusions

This chapter introduced the Thermokinetic Modeling (TKM) formalism. The TKM formalism is derived from the thermodynamic formalism, but leads to much simpler model equations.

The complexity of the thermodynamic modeling formalism arises from the strongly nonlinear relation of thermodynamic forces and reaction rates. Whereas thermodynamic forces scale linearly with the logarithm of the concentrations, the rate laws of mass-action kinetics scale linearly with products of concentrations. Therefore, the thermodynamic resistances that are the ratio of thermodynamic forces and fluxes are described by complicated expressions.

This chapter introduced suited thermokinetic variables – namely the thermokinetic potentials ξ and the thermokinetic forces F – that are derived from thermodynamic potentials and forces. Further, it introduced thermokinetic capacities C_i that are the ratios of concentrations and thermokinetic potentials. Analogously, the thermokinetic resistances R_j are the ratios of thermokinetic forces and fluxes. In the standard case of mass-action kinetics in an ideal dilute solution, thermokinetic potentials and forces are proportional to concentrations and fluxes, respectively. This means that capacities C_i and resistances R_j are constant system parameters.

Many of the reduction methods that were derived in Chapter 4 for thermodynamic models apply with small modifications also to thermokinetic models. Only the reduction of stoichiometric cycles and the reduction of the thermodynamic submodels could not be extended to TK models in a general way. In general, these methods inherently lead to a non-diagonal resistance matrix. This is only a minor limitation of TKM because – as was discussed in Section 4.3 (p. 83) – the reduction of stoichiometric cycles does not lead to a considerable reduction of the model equations. In addition, the reduction of the thermodynamic submodel is often not possible due to the occurring non-linear equations. Important special cases of the reduction of stoichiometric cycles and of the thermodynamic submodel in thermokinetic models are discussed in Chapter 6. The reduction of the kinetic submodel, i. e. the application of the rapid equilibrium assumption, which is especially useful and simple, is applicable to TK models.

Although the thermodynamic modeling formalism is more general than the thermokinetic modeling formalism, the practical restrictions are minor, since non-diagonal resistance matrices usually do not occur in the kinetic modeling of reaction networks. The advantage of using TKM is its simple and elegant parameterization of the model equations. In contrast to the conventional kinetic modeling formalism, TKM structurally guarantees the thermodynamic consistency of the model equations. Compared to the thermodynamic modeling, the model equations of TKM and their handling are simple.

The methods developed in this chapter are formulated as operations on thermokinetic models. Thus, their application requires the complete formulation of the system matrices $N_{E/P}$, $S_{E/P}$, N_e and the functions $c(\xi, \xi_e)$ and $R(\xi, \xi_e)$ of the whole network. The derived methods are ideally suited for the development of a computer tool for modeling and model reduction. A prototypical implementation is available and described in Appendix A. The next chapter explores the possibilities of model reduction on the reaction equation level, which allows applying the reduction methods without explicitly building the system matrices. This simplifies modeling and shows the inherent modularity of the developed methods. The procedure developed in the next chapter is particularly useful for calculations with ‘paper and pencil’ and for systems defined by reaction rules.

6. Model Reduction of Reaction Equations

Chapters 3 and 4 developed a quite general thermodynamic modeling formalism for reaction networks. This allowed developing algorithms for model transformation and reduction. However, due to the complexity of the occurring mathematical expressions, the thermodynamic formalism is not suited for modeling larger networks. For this reason, Chapter 5 modified the thermodynamic formalism in order to arrive at the thermokinetic formalism that leads to much simpler model equations. However, the introduced model reduction methods are described for whole models of reaction networks in the form of matrix operations. For larger networks, model reduction as described in the previous chapters can only be done using computer programs.

This chapter introduces procedures that allow for the application of several model reduction techniques on the level of reaction equations. This means that rules are given that describe how to transform a set of reaction equations, with capacities and resistances to an equivalent reduced formulation. These methods can be easily applied without using a computer program but only with 'paper and pencil'. They can also be applied to reaction rules as they are often used for the description of combinatorial protein-protein interaction networks in signal transduction.

Definition 6.1 (Reaction Equations)**.** A reaction system is defined by a list of reaction equations

$$\sum_{i \in E_j} \nu_{E,ij}\, X_i \rightleftharpoons \sum_{i \in P_j} \nu_{P,ij}\, X_i$$

with stoichiometric coefficients $\nu_{E/P,ij}$ and indices $i = 1 \ldots i_0$, $j = 1 \ldots j_0$. Every reaction has a resistance $R_j(\xi) \geq 0$ and every compound X_i has a capacity $C_i(\xi)$.

§ 6.2 (Stoichiometric coefficients)**.** The overall stoichiometric coefficient of X_i in reaction j is $\nu_{ij} = -\nu_{E,ij} + \nu_{P,ij}$. Initially, $\nu_{E,ij}$ or $\nu_{P,ij}$ are positive, but models with negative $\nu_{E,ij}$ or $\nu_{P,ij}$ may occur during the model reduction. The overall stoichiometric coefficient ν_{ij} is negative if X_i is a reactant of reaction j and positive if it is a product.

§ 6.3 (Open vs. closed)**.** In the previous chapters, we distinguished between closed systems and open systems. In open systems, certain thermokinetic potentials ξ_i and fluxes J_j are clamped. Thus, the system does not necessarily reach thermodynamic equilibrium, but a continuous exchange of matter and energy with the environment may occur. In order to treat open systems, we introduced the external stoichiometric matrices N_e and S. This is a clear, formal way to include the boundary conditions. However, the goal in this chapter is to arrive at a methodology that is not only formally appealing and thermodynamically correct, but can also be easily applied with paper and pencil without using a computer program. A clear distinction of external and internal fluxes and compounds throughout the computations is tedious and increases notational complexity without adding to the comprehension. Thus, we always treat reaction equations as if they constituted a closed system. Clamping of potentials and fluxes can be done after the reduction steps. This is easily possible because, in the methods proposed below, fluxes and thermokinetic potentials of reduced and original system are equal (or of course removed by the reduction steps).

6.1. Transformations and Translations

The transformation and translation rules developed in the Sections 5.4.1 (p. 101) and 5.4.2 (p. 102) operate on whole networks rather than on single reaction equations or rules. The translation vector depends on properties of the whole network and not on properties of single reactions. Extensions or modifications of the network make it necessary to redo the calculations. This means that transformation and translation cannot be applied on the reaction equation level only.

An exception is the translation of stoichiometric coefficients (see Section 5.4.3, p. 104).

Corollary 6.4 (Translation of stoichiometric coefficients). *A reaction j with stoichiometry $\nu_{E,ij}$ and $\nu_{P,ij}$ and resistance R_j can be replaced by a reaction $\hat{j}$ with $\hat{\nu}_{E,ij} = \nu_{E,ij} + \delta\nu_i$, $\hat{\nu}_{P,ij} = \nu_{P,ij} + \delta\nu_i$ and $\hat{R}_j = R_j \prod_{i=1}^{i_0} \xi_i^{\delta\nu_i}$.*

Proof. This follows directly from Corollary 5.37 (p. 104). □

Example 6.5. Consider the reaction $A + E \rightleftharpoons B + E$ with resistance R. Here E has the role of a catalyst. The reaction is equivalent to the reaction $A \rightleftharpoons B$ with resistance $\hat{R} = R/\xi_E$.

6.2. Reduction of the Stoichiometric Submodel

6.2.1. Reduction of Conservation Relations

In the traditional formalism with concentration dependent kinetic rate laws, the reduction of conservation relations is straightforward. In TKM we seek for models with a guaranteed thermodynamic feasibility and thus use rate laws that depend on thermokinetic potentials in a defined way. As shown in Section 5.5.1.1 (p. 105) a reduction of conservation relations preserving the TKM formalism is possible but requires solving nonlinear equations. Thus, we cannot expect that this reduction technique is in general easily applicable within the framework of TKM. For this reason, we do not seek to formulate rules for this case.

6.2.2. Reduction of Stoichiometric Cycles

The reduction of stoichiometric cycles as discussed in Section 4.2.1.2 (p. 61) for thermodynamic models leads to a non-diagonal resistance matrix. This cannot be accounted for in the TKM formalism (see the discussion in Section 5.5.1.2, p. 106). However, we can give a rule for a simple but important case.

The simplest possible stoichiometric cycle is formed by two or more reactions with the same stoichiometry, e. g. isoenzymes. This is equivalent to a parallel connection of these reactions and analogous to the parallel connection of electrical resistances. To treat such cases, the following definition from electrical theory is helpful.

Definition 6.6 (Parallel resistances)**.** The operation $R_1 \parallel R_2 \parallel \ldots \parallel R_q$ is defined by

$$(R_1 \parallel R_2 \parallel \ldots \parallel R_q)^{-1} = R_1^{-1} + R_2^{-1} + \ldots + R_q^{-1}$$

Corollary 6.7 (Parallel reactions)**.** *Let $j = 1 \ldots q$ be the indices of reactions with equal stoichiometry ($\nu_{E,i1} = \ldots = \nu_{E,iq}$ and $\nu_{P,i1} = \ldots = \nu_{P,iq}$ for all $i = 1 \ldots i_0$). The q reactions can be replaced by one reaction with the resistance $R = R_1 \parallel R_2 \parallel \ldots \parallel R_q$.*

Proof. Since the stoichiometry is equal the forces are equal $F = F_1 = \ldots = F_q$. Thus, the overall flux is $J = J_1 + \ldots + J_q = R_1^{-1}\, F_1 + \ldots + R_q^{-1}\, F_q = (R_1^{-1} + \ldots + R_q^{-1})\, F = R^{-1}\, F$. □

Example 6.8 (Parallel reactions)**.** Consider a system that contains two reactions $A \overset{1}{\rightleftharpoons} B$ and $A \overset{2}{\rightleftharpoons} B$. Because both reactions have equal stoichiometry, they can be replaced by $A \rightleftharpoons B$ with a resistance of

$$R = R_1 \parallel R_2 = \frac{R_1\, R_2}{R_1 + R_2}.$$

6.3. Reduction of the Thermodynamic and Kinetic Submodel

If some capacities or resistances are zero, a reduction of the model size is possible. While for vanishing capacities we can only give rules for a special but important case, the reduction in the case of vanishing resistances, i. e. the rapid equilibrium assumption, is a very powerful tool.

6.3.1. Reduction of the Thermodynamic Submodel

The reduction of the thermodynamic submodel for a singular $\partial c / \partial \mu$ or vanishing capacities involves the solution of a nonlinear equation system (see Section 4.2.2.1, p. 63). The existence and uniqueness of the solution of this equation system is not guaranteed. Thus, a general and simple rule for this reduction method cannot be given. Further, as pointed out in Section 5.5.2.1 (p. 107) the reduction method in general leads to a non-diagonal resistance matrix and thus is not suited for TKM. However, for the special case of serial reactions that corresponds to a serial connection in electrical theory we may give a simple rule.

Corollary 6.9 (Serial reactions)**.** *Consider a network with two reactions* 1 *and* 2 *such that the products of reaction* 1 *are the reactants of reaction* 2 *(*$P_1 = E_2$ *and* $\nu_{P,i1} = \nu_{E,i2}$ *for all* i*). Let* $C_i = 0$ *for at least one* $i \in P_1 = E_2$*. Further, assume that the compounds in the set* $P_1 = E_2$ *with* $C_i = 0$ *do not appear anywhere else in the reaction network and that the resistances and capacities of the network do not depend on their thermokinetic potentials.*

These two reactions can be replaced by a single reaction with $E = E_1$*,* $P = P_2$*,* $\nu_{E,i} = \nu_{E,i1}$*,* $\nu_{P,i} = \nu_{P,i2}$ *and a resistance* $R = R_1 + R_2$*. The force* $F_{P,1} = F_{E,2}$ *can be reconstructed by*

$$F_{P,1} = F_{E,2} = \frac{R_2\, F_{E,1} + R_1\, F_{P,2}}{R_1 + R_2}.$$

Proof. We have that $F_{P,1} = F_{E,2}$ because the reactants of reaction 2 are the products of reaction 1. Since $C_i = 0$ for a compound $i \in P_1$ and since this compound does not occur anywhere else in the network, we get $J = J_1 = J_2$. Thus, it is $R_1\, J = F_{E,1} - F_{P,1}$ and $R_2\, J = F_{E,2} - F_{P,2}$. Since $F_{P,1} = F_{E,2}$, we get $(R_1 + R_2)\, J = F_{E,1} - F_{P,2}$ and thus $R\, J = F$. Further we get $F_{P,1} = F_{E,2} = F_{E,1} - R_1\, J$ which leads to the expression for $F_{P,1} = F_{E,2}$ given in the corollary. □

Example 6.10 (Serial reactions – simple case)**.** Let $A \overset{1}{\rightleftharpoons} B$ and $B \overset{2}{\rightleftharpoons} C$ be two reactions in a network with $C_B = 0$ and no further occurrences of B. Then, we can replace these reactions by $A \rightleftharpoons C$ with $R = R_1 + R_2$. The thermokinetic potential of the reduced compound B can be reconstructed by $\xi_B = F_{P,1} = F_{E,2} = (R_2\, \xi_A + R_1\, \xi_C)/(R_1 + R_2)$.

Example 6.11 (Serial reactions – loss of uniqueness)**.** Let $A \overset{1}{\rightleftharpoons} B_1 + B_2$ and $B_1 + B_2 \overset{2}{\rightleftharpoons} C$ be two reactions in a network with $C_{B_1} \geq 0$ and $C_{B_2} = 0$ and no further occurrences of B_1 and B_2. Then, we can replace these reactions by $A \rightleftharpoons C$ with $R = R_1 + R_2$. The force exerted by the compounds B_1 and B_2 can be reconstructed by $F_{E,1} = F_{P,2} = \xi_{B,1}\, \xi_{B,2} = (R_2\, \xi_A + R_1\, \xi_C)/(R_1 + R_2)$. If $C_{B,1} > 0$ then the thermokinetic potential $\xi_{B,1} = c_{B,1}/C_{B,1} = \xi_{B,1,0}$ is constant because the quasi-steady condition $J_1 = J_2$ leads to $\dot{c}_{B,1} = 0$. In this case, $\xi_{B,2}$ is given by $\xi_{B,2} = F_{E,1}/\xi_{B,1,0}$. If $C_{B,1} = 0$, we have no possibility to separately compute $\xi_{B,1}$ and $\xi_{B,2}$. This case is an example of the non-uniqueness of the solution as discussed in §4.44 (p. 66).

The above corollary can be generalized to a case where only some of the products of reaction 1 are reactants of reaction 2.

Corollary 6.12 (Partially serial reactions)**.** *Consider a system with two reactions* 1 *and* 2 *such that some of the products of reaction* 1 *are reactants of reaction* 2 *(*$X = P_1 \cap E_2 \neq \{\}$ *and* $\nu_{P,i1} = \nu_{E,i2}$ *for all* $i \in X$*). Let* $C_i = 0$ *for at least one* $i \in X$*. Further, assume that the compounds in the set* X *with* $C_i = 0$ *do not appear anywhere else in the reaction network and that the resistances and capacities of the network do not depend on their thermokinetic potentials.*

Then, these two reactions can be replaced by a single reaction with $E = (E_1 \cup E_2)/X$*,* $P = (P_1 \cup P_2)/X$*,* $\nu_{E,i} = \nu_{E,i1} + \nu_{E,i2}$*,* $\nu_{P,i} = \nu_{P,i1} + \nu_{P,i2}$ *and a resistance* $R = R_1 \prod_{i \in E_2/X} \xi_i^{\nu_{E,i2}} +$

$R_2 \prod_{i \in P_1/X} \xi_i^{\nu_{P,i1}}$. The force F_X exerted by the compounds in X can be reconstructed by

$$F_X = \prod_{i \in X} \xi_i^{\nu_{P,i1}} = \prod_{i \in X} \xi_i^{\nu_{E,i2}} = \frac{R_2\, F_{E,1} + R_1\, F_{P,2}}{R}.$$

Proof. Let $F_X = \prod_{i \in X} \xi_i^{\nu_{P,i1}} = \prod_{i \in X} \xi_i^{\nu_{E,i2}}$, $F_A = \prod_{i \in E_2/X} \xi_i^{\nu_{E,i2}}$ and $F_B = \prod_{i \in P_1/X} \xi_i^{\nu_{P,i1}}$. With these abbreviations we have that $F_{E,2} = F_X\, F_A$ and $F_{P,1} = F_X\, F_B$. Since $C_i = 0$ for a compound $i \in X$ and since this compound does not occur anywhere else in the network, we get $J = J_1 = J_2$. Thus, it is $R_1\, J = F_{E,1} - F_X\, F_B$ and $R_2\, J = F_X\, F_A - F_{P,2}$. Eliminating F_X yields $(R_1\, F_A + R_2\, F_B)\, J = F_{E,1}\, F_A - F_{P,2}\, F_B$ and thus $R\, J = F$. Eliminating J from the same equations yields the expression for F_X given in the corollary. The stoichiometry of the overall reactions follows from $J_1 = J_2$. □

Example 6.13 (Partially serial reactions)**.** Let $E \overset{1}{\rightleftharpoons} X + B$ and $X + A \overset{2}{\rightleftharpoons} P$ be two reactions in a network with $C_X = 0$ and no further occurrences of X. Then, we can replace these reactions by $E + A \rightleftharpoons P + B$ with $R = R_1\, \xi_A + R_2\, \xi_B$. The thermokinetic potential of X can be reconstructed by $\xi_X = F_X = (R_2\, \xi_E + R_1\, \xi_P)/(R_1\, \xi_A + R_2\, \xi_B)$.

6.3.2. Reduction of the Kinetic Submodel

If a network contains vanishing resistances, the corresponding thermokinetic forces are zero. The resulting algebraic equations allow for a reduction of the model (Section 5.5.2.2, p. 107). The approximative use of the reduction methods introduced below is possible if the resistances are small such that the respective reactions rapidly approach thermodynamic equilibrium.

This section presents simple but comprehensive precepts for the reduction of systems defined by reaction equations that contain fast reactions.

Corollary 6.14 (Reduction of reaction equations)**.** *Let M be a system defined by the reaction equations*

$$\sum_{i \in E_j} \nu_{E,ij}\, X_i \rightleftharpoons \sum_{i \in P_j} \nu_{P,ij}\, X_i$$

with resistances $R_j(\xi) \geq 0$ and capacities $C_i(\xi)$ for $i = 1 \dots i_0$ and $j = 1 \dots j_0$. Let at least one resistance be zero. Without loss of generality, we write $R_1 = 0$ and $\nu_{1,1} \neq 0$. Further, let $\tilde{M}$ be a system defined by

$$\sum_{i \in E_j} \tilde{\nu}_{E,ij}\, \tilde{X}_i \rightleftharpoons \sum_{i \in P_j} \tilde{\nu}_{P,ij}\, \tilde{X}_i$$

with stoichiometric coefficients

$$\tilde{\nu}_{E,ij} = \nu_{E,ij} - \nu_{E,1j}\, \frac{\nu_{i1}}{\nu_{11}}, \qquad \tilde{\nu}_{P,ij} = \nu_{P,ij} - \nu_{P,1j}\, \frac{\nu_{i1}}{\nu_{11}},$$

resistances $\tilde{R}_j(\tilde{\xi}) = R_j(\xi)$ *and capacities*

$$\tilde{C}_i(\tilde{\xi}) = C_i(\tilde{\xi}) - \frac{\nu_{i1}}{\nu_{11}}\,\frac{\xi_1}{\tilde{\xi}_i}\,C_1$$

for $i = 2 \dots i_0$ *and* $j = 2 \dots j_0$*. The solutions of* M *and* $\tilde{M}$ *are equivalent with*

$$\begin{aligned} &\xi_i = \tilde{\xi}_i, && c_i = \tilde{c}_i + \frac{\nu_{i1}}{\nu_{11}}\,c_1, && J_j = \tilde{J}_j, && F_j = \tilde{F}_j, \\ &\xi_1 = \prod_{k=2}^{i_0} \tilde{\xi}_k^{-\nu_{k1}/\nu_{11}}, && c_1 = C_1\,\xi_1, && J_1 = \frac{1}{\nu_{11}}\left(\dot{c}_1 - \sum_{j=2}^{j_0} \nu_{1j}\,\tilde{J}_j\right), && F_1 = 0 \end{aligned}$$

with $i = 2 \dots i_0$ *and* $j = 2 \dots j_0$*.*

Proof. The above follows from Corollary 5.42 (p. 107). There the fast subsystem is denoted by $('')$ and the slow subsystem by $(')$. Thus, when applying Corollary 5.42 we must take into account the different ordering of reactions and compounds. We have

$$R' = 1\begin{pmatrix} R_2 \\ \vdots \\ R_{j_0} \end{pmatrix}, \qquad N_{E/P} = \begin{pmatrix} \underbrace{\begin{matrix} \nu_{E/P,11} \\ a_{E/P} \end{matrix}}_{N''_{E/P}} & \underbrace{\begin{matrix} b_{E/P}^T \\ \bar{N}_{E/P} \end{matrix}}_{N'_{E/P}} \end{pmatrix}$$

with

$$a_{E/P} = \begin{pmatrix} \nu_{E/P,21} \\ \vdots \\ \nu_{E/P,i_0 1} \end{pmatrix}, \quad b_{E/P}^T = \begin{pmatrix} \nu_{E/P,12} & \dots & \nu_{E/P,1j_0} \end{pmatrix}, \quad \bar{N}_{E/P} = \begin{pmatrix} \nu_{E/P,22} & \cdots & \nu_{E/P,2j_0} \\ \vdots & \ddots & \vdots \\ \nu_{E/P,i_0 2} & \cdots & \nu_{E/P,i_0 j_0} \end{pmatrix}.$$

The transformation matrix T_c with $T_c^T = [T_{c,2}^T, T_{c,1}^T]$,

$$T_{c,1} = \begin{pmatrix} -\frac{1}{\nu_{11}}\,a & I_{i_0-1} \end{pmatrix}, \qquad T_{c,2} = \begin{pmatrix} 1 & 0 & \dots & 0 \end{pmatrix}$$

and with $a = a_P - a_E$ is square and of full rank. The matrices $T_{c,1}$ and $T_{c,2}$ fulfill the required conditions $T_{c,1}\,N'' = -a + a = 0$ and $\operatorname{rank}(T_{c,2}\,N'') = \operatorname{rank}(\nu_{11}) = 1 = \operatorname{rank}(T_{c,2})$. Thus, we can use these matrices to gain a reduced representation with

$$\underbrace{\begin{pmatrix} \xi_1 \\ \xi_2 \\ \vdots \\ \xi_{i_0} \end{pmatrix}}_{\xi} = \underbrace{\begin{pmatrix} -\frac{1}{\nu_{11}}\,a^T \\ I_{i_0-1} \end{pmatrix}}_{T_{c,1}^T} \# \underbrace{\begin{pmatrix} \tilde{\xi}_2 \\ \vdots \\ \tilde{\xi}_{i_0} \end{pmatrix}}_{\tilde{\xi}} = \begin{pmatrix} \prod_{k=2}^{i_0} \tilde{\xi}_k^{-\nu_{k1}/\nu_{11}} \\ \tilde{\xi}_2 \\ \vdots \\ \tilde{\xi}_{i_0} \end{pmatrix}$$

and

$$\underbrace{\begin{pmatrix} \tilde{c}_2 \\ \vdots \\ \tilde{c}_{i_0} \end{pmatrix}}_{\tilde{c}} = \underbrace{\begin{pmatrix} -\frac{1}{\nu_{11}}\, a & I_{i_0-1} \end{pmatrix}}_{T_{c,1}} \underbrace{\begin{pmatrix} c_1 \\ \vdots \\ c_{i_0} \end{pmatrix}}_{c} = \begin{pmatrix} c_2 - \frac{\nu_{21}}{\nu_{11}}\, c_1 \\ \vdots \\ c_{i_0} - \frac{\nu_{i_0 1}}{\nu_{11}}\, c_1 \end{pmatrix}.$$

The reduced system has stoichiometric matrices

$$\tilde{N}_{E/P} = \underbrace{\begin{pmatrix} -\frac{1}{\nu_{11}}\, a & I_{i_0-1} \end{pmatrix}}_{T_{c,1}} \underbrace{\begin{pmatrix} b^T_{E/P} \\ \bar{N}_{E/P} \end{pmatrix}}_{N'_{E/P}} = \bar{N}_{E/P} - \frac{1}{\nu_{11}}\, a\, b^T_{E/P}.$$

This is equivalent to the formula given for the new stoichiometric coefficients in the above corollary. According to Corollary 5.42, we have for the resistance $\tilde{R} = R'$. The function $\tilde{c}(\xi)$ is given by

$$\tilde{c}(\tilde{\xi}) = \underbrace{\begin{pmatrix} -\frac{1}{\nu_{11}}\, a & I_{i_0-1} \end{pmatrix}}_{T_{c,1}} \underbrace{(C \circ \xi)}_{c(\xi)} = \begin{pmatrix} C_2\, \xi_2 - \frac{\nu_{21}}{\nu_{11}}\, C_1\, \xi_1 \\ \vdots \\ C_{i_0}\, \xi_{i_0} - \frac{\nu_{i_0 1}}{\nu_{11}}\, C_1\, \xi_1 \end{pmatrix} = \underbrace{\begin{pmatrix} C_2 - \frac{\nu_{21}}{\nu_{11}}\, C_1\, \frac{\xi_1}{\tilde{\xi}_2} \\ \vdots \\ C_{i_0} - \frac{\nu_{i_0 1}}{\nu_{11}}\, C_1\, \frac{\xi_1}{\tilde{\xi}_{i_0}} \end{pmatrix}}_{\tilde{C}(\tilde{\xi})} \circ \tilde{\xi}.$$

Fluxes and forces of original and reduced system are related by

$$\underbrace{\begin{pmatrix} J_2 \\ \vdots \\ J_{j_0} \end{pmatrix}}_{J} = \underbrace{\begin{pmatrix} \tilde{J}_2 \\ \vdots \\ \tilde{J}_{j_0} \end{pmatrix}}_{\tilde{J}}, \qquad \underbrace{\begin{pmatrix} F_2 \\ \vdots \\ F_{j_0} \end{pmatrix}}_{J} = \underbrace{\begin{pmatrix} \tilde{F}_2 \\ \vdots \\ \tilde{F}_{j_0} \end{pmatrix}}_{\tilde{F}},$$

$F_1 = 0$ and

$$J_1 = \operatorname{inv} \left(\underbrace{\begin{pmatrix} 1 & 0 & \dots & 0 \end{pmatrix}}_{T_{c,2}} \underbrace{\begin{pmatrix} \nu_{11} \\ a \end{pmatrix}}_{N''} \right) \underbrace{\begin{pmatrix} 1 & 0 & \dots & 0 \end{pmatrix}}_{T_{c,2}} \left(\dot{c} - \underbrace{\begin{pmatrix} b^T \\ \bar{N} \end{pmatrix}}_{N'} \tilde{J} \right) = \frac{1}{\nu_{11}} \left(\dot{c}_1 - \sum_{j=2}^{j_0} \nu_{1j}\, \tilde{J}_j \right)$$

The above derived expressions are equivalent to the expressions given in the corollary. □

§ 6.15 (Step-by-step procedure)**.** The application of the above corollary to a list of reaction equations is straightforward. Here we give a step-by-step procedure for the reduction of networks with fast reactions:

1. Choice of the reaction equation and the compound that is to be reduced:

a) Choose a reaction assumed to be in rapid equilibrium ($R_1 = 0$):

$$\sum_{i=1}^{i_0} \nu_{E,i1}\, X_i \rightleftharpoons \sum_{i=1}^{i_0} \nu_{P,i1}\, X_i.$$

b) Choose a compound X_1 to eliminate. This compound must be a true reactant or product of the fast reaction ($\nu_{11} = \nu_{P,11} - \nu_{E,11} \neq 0$).

2. Computation of the reduced stoichiometry:

   a) Solve the rapid reaction equation for X_1 as if it is a linear equation:

   $$X_1 \rightleftharpoons \sum_{i=2}^{i_0} \underbrace{\frac{\nu_{E,i1} - \nu_{P,i1}}{\nu_{P,11} - \nu_{E,11}}}_{f_i = -\nu_{i1}/\nu_{11}} X_i$$

   (e. g. $A + B \rightleftharpoons C + D \quad \rightarrow \quad C \rightleftharpoons A + B - D$).

   b) Replace the compound X_1 in the remaining reaction equations by this solution. In order to distinguish the reduced model from the original model, add a tilde to all species in all reaction equations. In the example from the previous item, $C + E \rightleftharpoons F$ and $G + H \rightleftharpoons I$ become $\tilde{A} + \tilde{B} - \tilde{D} + \tilde{E} \rightleftharpoons \tilde{F}$ and $\tilde{G} + \tilde{H} \rightleftharpoons \tilde{I}$.

3. Reconstruction of original from reduced variables:

   a) We have $c_i = \tilde{c}_i - f_i\, c_1$ and $\xi_i = \tilde{\xi}_i$ for $i = 2 \ldots i_0$. Further it is $\xi_1 = \prod_{k=2}^{i_0} \xi^{f_k}$. With this we can reconstruct c_1 by $c_1 = C_1\, \xi_1$.

   b) The slow fluxes and forces of the original and reduced system are equal: $J_j = \tilde{J}_j$ and $F_j = \tilde{F}_j$ for $j = 2 \ldots j_0$. The fast force is $F_1 = 0$ and the fast flux can be reconstructed by $J_1 = (\dot{c}_1 - \sum_{j=2}^{j_0} \nu_{1j}\, J_j)/\nu_{11}$. In many cases, a reconstruction of the fast flux is not necessary.

4. Computation of the reduced parameters:

   a) The reduced resistances are equal to the original resistances $\tilde{R}_j = R_j$.

   b) The new capacities are given by

   $$\tilde{C}_i = C_i + f_i\, \frac{\xi_1}{\tilde{\xi}_i}\, C_1.$$

   c) If capacities $\tilde{C}$ and resistances $\tilde{R}$ depend on ξ, the results of step 3 can be used to express them in dependence on $\tilde{\xi}$.

5. If the network contains further reactions with resistance 0, apply the reduction method again until all remaining reactions have a strictly positive resistance.

§ 6.16 (Clamping of potentials and fluxes)**.** In §6.3 (p. 111) it was argued that it is sufficient to formally limit the development of the reduction method to closed systems because it is easily possible to clamp potentials and fluxes after the reduction step. The remaining potentials ξ_i and fluxes J_j of the reduced system are equal to the corresponding potentials and fluxes of the original system. Thus, the clamping of these compounds can be easily done after the reduction. During the reduction one has to take care that no clamped flux or potential is removed.

§ 6.17 (Conflicting potentials ξ_i)**.** During the application of the procedure in §6.15, it may not be possible to choose a compound that is to be removed because all compounds that participate in a fast reaction are going to be clamped. This situation corresponds to conflicts among clamped potentials as discussed in Corollary 4.61 (p. 74). Such situations need to be resolved as explained in §4.65 (p. 75) because they correspond to physically inconsistent model assumptions. Either a rapid-equilibrium assumption has to be relaxed, or the potential of a clamped compound has to be released.

§ 6.18 (Non-uniqueness of J_j)**.** During the application of the procedure in §6.15, it may happen that a network still contains fast reactions ($R_1 = 0$), but its overall stoichiometric coefficients ν_{i1} are all zero. In this case the reduction cannot be applied, since it requires at least one compound with $\nu_{i1} \neq 0$. In such cases, the rapid-equilibrium condition $F_1 = 0$ is trivially fulfilled and the flux J_1 is not uniquely determined. This situation corresponds to the non-uniqueness of fluxes that may occur under certain conditions (cf. Corollary 4.71, p. 76). A possibility to treat such situations is to completely remove the problematic reaction equation from the network (cf. §4.76, p. 78). This makes the fluxes unique, and only influences the values of the respective fluxes but no other quantities.

Example 6.19 (Linear reaction chain)**.** Consider the system $A \overset{R_1}{\rightleftharpoons} B \overset{R_2}{\rightleftharpoons} C$ with constant resistances $R_1 > 0$, $R_2 = 0$ and constant capacities C_A, C_B and C_C. Applying the procedure in §6.15 in order to remove species C and reaction 2 we get the reduced system: $\tilde{A} \overset{R_1}{\rightleftharpoons} \tilde{B}$ with $\tilde{C}_A = C_A$, $\tilde{C}_B = C_B + \xi_C/\tilde{\xi}_B \, C_C = C_B + C_C$, $c_A = \tilde{c}_A$, $c_B = \tilde{c}_B - c_C$, $c_C = C_C \, \xi_C$ and $\xi_C = \tilde{\xi}_B$. A more appropriate notation for $\tilde{B}$ would be BC, since the compound $\tilde{B}$ comprises the compounds B and C ($\tilde{c}_B = c_B + c_C$).

Example 6.20 (Reaction chain with bimolecular reaction)**.** Consider the system $A + B \overset{R_1}{\rightleftharpoons} C \overset{R_2}{\rightleftharpoons} D$ with constant resistances $R_1 = 0$, $R_2 > 0$ and constant capacities C_A, C_B, C_C and C_D. We remove species C and reaction 1 from the system. The reduced system is $\tilde{A} + \tilde{B} \overset{R_2}{\rightleftharpoons} \tilde{D}$ with $c_A = \tilde{c}_A - c_C$, $c_B = \tilde{c}_B - c_C$, $c_C = C_C \, \xi_C$, $c_D = \tilde{c}_D$, $\xi_A = \tilde{\xi}_A$, $\xi_B = \tilde{\xi}_B$, $\xi_C = \tilde{\xi}_A \, \tilde{\xi}_B$, $\xi_D = \tilde{\xi}_D$, $\tilde{C}_A = C_A + C_C \, \xi_C/\tilde{\xi}_A = C_A + C_C \, \tilde{\xi}_B$ and $\tilde{C}_B = C_B + C_C \, \xi_C/\tilde{\xi}_B = C_B + C_C \, \tilde{\xi}_A$. Thus, the new compounds $\tilde{A}$ and $\tilde{B}$ are the pools of $\{A, C\}$ and $\{B, C\}$, respectively.

§ 6.21 (Simultaneous reduction of several fast reactions)**.** By successive application of the procedure in §6.15 we can completely reduce all reactions with a resistance zero from a reaction system. However, we can only remove one fast reaction at a time. To reduce several reactions simultaneously, one needs to fall back to Corollary 5.42 and compute the required null spaces (i. e. solve linear equations). However, for a simple but frequent case we can extend the procedure in §6.15 to reduce several reactions simultaneously: Assume that the first q reactions are fast ($R_j = 0$ for $j = 1 \dots q$). Further, assume that each fast reaction contains a compound X_j that occurs only in this fast reaction, but not in other fast reactions ($\nu_{ij} \neq 0$ for $i = j$, $\nu_{ij} = 0$ for $i \neq j$, $i, j = 1 \dots q$). Then the q reduction steps that are necessary to reduce the q fast reactions with procedure §6.15 can be done simultaneously. The only point where the q reduction steps interact is in the reconstruction of c_i and in the computation of the reduced capacities $\tilde{C}_i$. There one needs to add a corresponding summand for each reduced compound.

Example 6.22. Consider a network consisting of the four reactions: $A \xrightleftharpoons{R_1=0} B_1 \xrightleftharpoons{R_3>0} C_1$, $A \xrightleftharpoons{R_2=0} B_2 \xrightleftharpoons{R_4>0} C_2$. The reactions 1 and 2 are in rapid equilibrium and we will remove B_1 and B_2. Both reduction steps can be done simultaneously and lead to the reduced model $\tilde{A} \xrightleftharpoons{R_3>0} \tilde{C}_1$, $\tilde{A} \xrightleftharpoons{R_4>0} \tilde{C}_2$ with $\tilde{C}_A = C_A + C_{B_1} + C_{B_2}$, $\tilde{C}_{C_1} = C_{C_1}$, $\tilde{C}_{C_2} = C_{C_2}$, $\tilde{c}_A = c_A + c_{B_1} + c_{B_2}$, $\tilde{c}_{C_1} = c_{C_1}$ and $\tilde{c}_{C_2} = c_{C_2}$.

6.4. Reduction of the Boundary Conditions

The reduction of the number of clamped potentials and clamped fluxes requires the knowledge of the complete stoichiometry of the model. It is based on the null space of a combination of stoichiometric matrices (see Section 4.2.3, p. 81). By adding or removing reactions and compounds to the network, this null space changes. This means that the reduction method depends on properties of the whole network and not of single reactions or species only. For this reason, we do not give rules for the reduction of the boundary conditions on the reaction equation level.

6.5. Excursus: Combinatorial Protein Interaction Networks

In protein-protein interaction networks, the number of distinguishable protein-protein complexes and the number of distinguishable reactions is typically very large. This is due to the combinatorial explosion of these numbers for scaffold proteins with several binding sites. The number of species and reactions in such networks easily goes into millions even if only a few proteins are considered (see e. g. Hlavacek et al. [49]). The number of stoichiometric cycles and thus the number of Wegscheider conditions grows over-proportionally with the model size (Example 2.60, p. 37). Therefore, the thermodynamically consistent kinetic modeling of such networks gets increasingly involved.

Reaction rules are a possibility to describe models of these networks by a relatively short list of reaction equations [35]. We define a reaction rule as a reaction equation with an index variable, e. g. the reaction rule $A_{0,x_2,x_3} + B \rightleftharpoons A_{1,x_2,x_3}$ with the indices $x_2, x_3 \in \{0, 1\}$. This rule describes 4 single reaction equations. Several computer programs exist that build simulation equations from a set of indexed reaction rules; e. g. BioNetGen [18]. However, this approach gets easily intractable because the number of model equations grows exponentially with the number of involved proteins. Several model reduction techniques were developed for such systems. The method of Conzelmann et al. [28] relies on an observability analysis and on finding a minimal Kalman realization of the system. The approaches of Koschorreck [63], Koschorreck and Gilles [64], Koschorreck et al. [65] and Borisov et al. [20] use approximative assumptions for the reduction of the model size. The approximative approach of Koschorreck implicitly uses specific rapid equilibrium assumptions that allow avoiding a great deal of the combinatorial complexity. This approach is based on specific interaction motifs that occur frequently. The methods mentioned above are highly valuable for modeling protein-protein interaction networks, however, there is still no satisfying and general method for the reduced-order modeling of protein-protein interaction networks. The development of a more general method for the application of rapid equilibrium assumptions is a promising task. This excursus sketches a possible approach.

6.5.1. Reaction Rules and Interaction Factors

This section shows by means of an example how reaction rules can be formulated in TKM. This is a prerequisite for the consideration of complex protein interaction networks.

Example 6.23 (Complex formation at a scaffold)**.** Consider the binding of three ligands L_1, L_2 and L_3 to a scaffold protein S. The system can be described by 12 reactions that can be written by three reaction rules:

$$\begin{aligned} L_1 + S_{0,x_2,x_3} &\overset{1}{\rightleftharpoons} S_{1,x_2,x_3}, \\ L_2 + S_{x_1,0,x_3} &\overset{2}{\rightleftharpoons} S_{x_1,1,x_3}, \\ L_3 + S_{x_1,x_2,0} &\overset{3}{\rightleftharpoons} S_{x_1,x_2,1}. \end{aligned}$$

with $x_1, x_2, x_3 \in \{0, 1\}$. The three subscripts x_i indicate whether the corresponding binding site for ligand L_i is occupied ($x_i = 1$) or not ($x_i = 0$). The capacities and resistances of the reactions depend on the indices x_1, x_2 and x_3. We assume that we can describe the reaction kinetics by ideal mass-action laws. In contrast to the usual conventions, we allow that the rate constants $k_{\pm j}$ of the single reactions described by a reaction rule are different. Thus, we can describe the network by 11 constant capacities and 12 constant resistances. These numbers increase exponentially with the number of ligands (see Example 2.60, p. 37). The following two paragraphs introduce a convenient representation of the capacities and resistances by means of thermodynamic and kinetic interaction factors.

Thermodynamic interaction factors. The capacities $C_{S_{x_1,x_2,x_3}}$ of the scaffold complexes can be written as

$$C_{S_{x_1,x_2,x_3}} = C_S\, K_{C,1}^{x_1}\, K_{C,2}^{x_2}\, K_{C,3}^{x_3}\, K_{C,12}^{x_1\,x_2}\, K_{C,13}^{x_1\,x_3}\, K_{C,23}^{x_2\,x_3}\, K_{C,123}^{x_1\,x_2\,x_3}$$

with a constant base capacity C_S of the unoccupied scaffold and constant *thermodynamic interaction factors* $K_{C,\cdot}$. This way of denoting the dependency of the capacities on the binding state of S is especially appealing because it reflects the interaction structure of the scaffold. If we rewrite the above expression for $C_{S_{x_1,x_2,x_3}}$ in terms of chemical standard potentials (see §5.5, p. 90) we get:

$$\begin{aligned}\mu^\circ_{S_{x_1,x_2,x_3}} =& \underbrace{-R^*\,T\,\log\left(\frac{C_S}{c^\circ}\right)}_{\mu^\circ_{S_{0,0,0}}} - x_1\,\underbrace{R^*\,T\,\log K_{C,1}}_{\mu^\circ_1} - x_2\,\underbrace{R^*\,T\,\log K_{C,2}}_{\mu^\circ_2} - x_3\,\underbrace{R^*\,T\,\log K_{C,3}}_{\mu^\circ_3}\\ &- x_1\,x_2\,\underbrace{R^*\,T\,\log K_{C,12}}_{\mu^\circ_{12}} - x_1\,x_3\,\underbrace{R^*\,T\,\log K_{C,13}}_{\mu^\circ_{13}} - x_2\,x_3\,\underbrace{R^*\,T\,\log K_{C,23}}_{\mu^\circ_{23}}\\ &- x_1\,x_2\,x_3\,\underbrace{R^*\,T\,\log K_{C,123}}_{\mu^\circ_{123}}\end{aligned}$$

where $\mu^\circ_{S_{0,0,0}}$ is the standard potential of free S. The terms $\mu^\circ_{1/2/3}$ together with the standard potentials of the ligands $L_{1/2/3}$ determine the Gibbs binding energies of $L_{1/2/3}$ to free S. For example, the Gibbs binding energy of L_1 to free S is $\Delta G^\circ_{S_{0,0,0}+L_1 \rightleftharpoons S_{1,0,0}} = -\mu^\circ_{S_{0,0,0}} - \mu^\circ_{L_1} + \mu^\circ_{S_{1,0,0}} = -\mu^\circ_{L_1} - \mu^\circ_1$. The other terms describe interactions of the ligand bindings. If μ°_{12} is not equal zero, bound L_1 and bound L_2 influence each other with the Gibbs interaction energy μ°_{12}. Such an interaction occurs if L_1 and L_2 bind in direct neighborhood (i. e. with physical contact) at the scaffold protein and thus interact in this way. Another possibility is that the binding of L_1 stabilizes the scaffold in a certain conformation with an increased or decreased affinity for L_2. This kind of interaction occurs in signal transduction from extracellular ligands (e. g. hormones) to intracellular ligands. Observe that interactions are always reciprocal, i. e. the effect of bound L_1 on the Gibbs binding energy of L_2 is equal to the effect of bound L_2 on the Gibbs binding energy of L_1. If two bindings are independent, the respective $\mu^\circ_{i_1 i_2}$ equals zero, and the respective $K_{i_1 i_2}$ equals one . There is also the possibility of higher-order interactions ($K_{123} \neq 1$) that occur if the concerted effect of several ligand bindings influences the binding of a further ligand. For large scaffolds with only a few interactions, a simple list of all capacities would be very long, but a formulation in terms of interaction factors $K_{C,\cdot}$ is short because interaction factors equal to one can be omitted.

Kinetic interaction factors. The resistances of the reaction rules depend on the respective free index variables. We parameterize them in the following way:

$$\begin{aligned}R_{1,(x_2,x_3)} &= R_1\,\left(\frac{K_{R,1,2}}{K_{C,2}}\right)^{x_2}\,\left(\frac{K_{R,1,3}}{K_{C,3}}\right)^{x_3}\,\left(\frac{K_{R,1,23}}{K_{C,23}}\right)^{x_2\,x_3},\\ R_{2,(x_1,x_3)} &= R_2\,\left(\frac{K_{R,2,1}}{K_{C,1}}\right)^{x_1}\,\left(\frac{K_{R,2,3}}{K_{C,3}}\right)^{x_3}\,\left(\frac{K_{R,2,13}}{K_{C,13}}\right)^{x_1\,x_3},\\ R_{3,(x_1,x_2)} &= R_3\,\left(\frac{K_{R,3,1}}{K_{C,1}}\right)^{x_1}\,\left(\frac{K_{R,3,2}}{K_{C,2}}\right)^{x_2}\,\left(\frac{K_{R,3,12}}{K_{C,12}}\right)^{x_1\,x_2}.\end{aligned}$$

Here $R_{1/2/3}$ are the resistances of the binding of $L_{1/2/3}$ to the unoccupied scaffold $S_{0,0,0}$ and $K_{R,1/2/3,\cdot}$ are the kinetic interaction factors. They describe the effect of the binding state of the scaffold on the binding rate constant of $L_{1/2/3}$. For example, the rate constants of the binding of ligand 1 is $k_{+1,(x_2,x_3)} = R_1^{-1}\, C_S^{-1}\, C_{L_1}^{-1}\, K_{R,1,2}^{-x_2}\, K_{R,1,3}^{-x_3}\, K_{R,1,23}^{-x_2 x_3}$ (see §5.18, p. 93). The interaction factors $K_{R,1,2}$, $K_{R,1,3}$ and $K_{R,1,23}$ describe the influence of bound L_2, bound L_3 and the concerted effect of bound L_2 and L_3 on the binding of L_1. If for example $K_{R,1,2} < 1$, the association constant of L_1 to a S-L_2-complex is greater than to the empty scaffold S.

The definition of the kinetic interaction factors $K_{R,\cdot,\cdot}$ given above is advantageous because the forward rate constants k_{+j} are independent of the thermodynamic interaction factors $K_{C,\cdot\cdot}$. Thus, a change of the binding velocity is solely determined by the kinetic interaction factors. Often the diffusion of the ligand L_i is the rate-limiting step for the binding. In this case, the association constants k_{+i} are independent of the state of the scaffold S (see [15, 35]) and the kinetic interaction factors equal one; $K_{R,i,\cdot} = 1$. This means that the signal transmission in this diffusion limited case is solely determined by the thermodynamic interaction factors $K_{C,\cdot\cdot}$.

Independent processes. If the higher order thermodynamic interactions vanish ($K_{C,12} = K_{C,13} = K_{C,23} = 1$) and the kinetic interactions vanish ($K_{R,\cdot,\cdot} = 1$), the binding processes of L_1, L_2 and L_3 are mutually independent. Then, the rate constants for the reaction rules do not depend on the binding state of the scaffold.

§ 6.24 (Difference to other notations)**.** Other approaches for modeling by reaction rules usually assume that all reactions defined by a reaction rule have the same kinetic constants k_+ and k_- [19, 28, 65]. This means that the binding event described by a rule is independent of the state of the complexes that participate in the reaction rule. In the above example, this corresponds to the case of independent processes. To allow for interactions, the system needs to be written with more than three reaction rules. The parameterization of reaction rules as introduced in the above example is more general, since it is only assumed that resistances and capacities can be expressed in terms of the indices x_i that are used to write the reaction rules.

Example 6.25 (Effect of the interaction factors)**.** To illustrate the meaning of the interaction factors, we consider the signal transduction mediated by a scaffold S with two ligands L_1 and L_2. We start in thermodynamic equilibrium of the binding of L_1 and L_2. The ligands L_1 and L_2 are partly bound to the scaffold S. At a certain point in time, the total concentration of L_1 ($\hat{c}_{L_1} = c_{L_1} + c_{S_{1,0}} + c_{S_{1,1}}$) is increased by adding a certain amount of free L_1. In response to this pulse a part of the added free L_1 binds to the scaffold. Due to the interactions at the scaffold, this may have effects on the binding of L_2, i. e. the concentration of bound L_2 ($\hat{c}_{S_{\cdot,1}} = c_{S_{0,1}} + c_{S_{1,1}}$). If this is the case, the scaffold transmits a signal from ligand L_1 to ligand L_2. Figure 6.1 shows several cases with different interaction factors. In this example, the thermodynamic interaction factors determine the steady state response and the kinetic interaction factors characterize the transient behavior.

§ 6.26 (Conclusions)**.** Thermodynamic and kinetic interaction factors allow for the thermodynamically consistent modeling of information flow in protein-protein interaction networks. Such a description is the basis for the application of the rapid equilibrium assumption that is discussed in the following section.

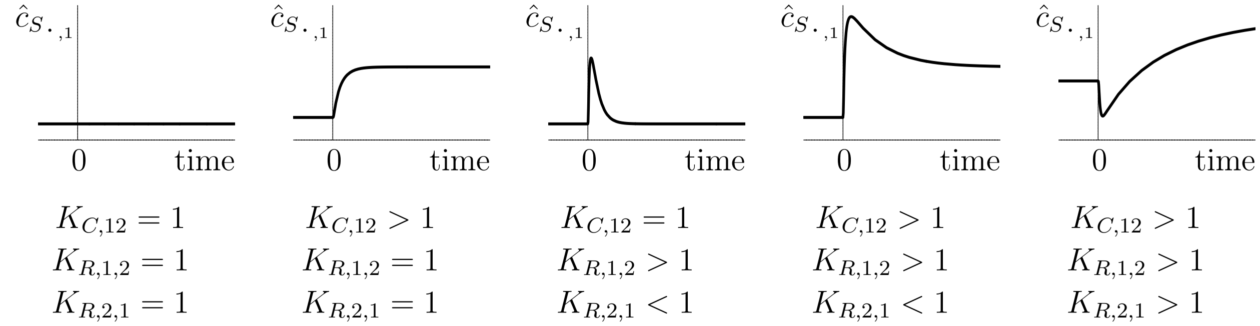


Figure 6.1.: Effect of the interaction factors on the signal transduction at a scaffold S. Free ligand L_1 is added at time $t = 0$. The plot show the concentration of bound ligand L_2 ($\hat{c}_{S_{\cdot,1}} = c_{S_{0,1}} + c_{S_{1,1}}$).

6.5.2. Model Reduction of Reaction Rules

The step-by-step procedure §6.15 (p. 117) for the reduction by rapid equilibrium assumptions can be applied to reaction rules. It is usually not necessary to completely expand the rules to a list of reaction equations, but it is possible to directly formulate the reduced reaction rules. This means that the formulation of the reduced-order model equations does not require the formulation of the detailed model equations. This is an essential advantage because the detailed model defined by the expanded reaction rules may contain an infeasibly high number of equations. We demonstrate this by means of a few examples.

Example 6.27 (Complex formation at a scaffold)**.** Consider a scaffold protein S with q binding sites for q ligands L_i ($i = 1 \dots q$). By $S_{x_1 \dots x_q}$ we denote the complexes of the scaffold, where $x_i = 0$ means that the i^{th} site is free and $x_i = 1$ means that L_i has bound to site i. The reaction rules

$$L_1 + S_{0,x_2 \dots x_q} \overset{1}{\rightleftharpoons} S_{1,x_2 \dots x_q}$$

$$\vdots$$

$$L_q + S_{x_1 \dots x_{q-1},0} \overset{q}{\rightleftharpoons} S_{x_1 \dots x_{q-1},1}$$

with $x_i \in \{0, 1\}$ describe all binding processes at the scaffold. The network contains $q \times 2^{q-1}$ reactions, 2^q scaffold complexes and q free ligands. Assume that the binding of L_1 is fast ($R_1 = 0$). We remove the respective reactions and the L_1-S-complexes $S_{1,x_2 \dots x_q}$ from the network. The new concentrations in the reduced network are $\tilde{c}_{L_1} = c_{L_1} + \sum_{x_2 \dots x_q} c_{S_{1,x_2 \dots x_q}}$ and $\tilde{c}_{S_{0,x_2 \dots x_q}} = c_{S_{0,x_2 \dots x_q}} + c_{S_{1,x_2 \dots x_q}}$. The quantity $\tilde{c}_{L_1}$ is the total concentrations of L_1. The concentrations $\tilde{c}_{S_{0,x_2 \dots x_q}}$ describe how much complexes with a binding state defined by $(x_2 \dots x_q)$ but arbitrary x_1 exist. The notation $\tilde{c}_{S_{0,x_2 \dots x_q}}$ is chosen systematically as described in §6.15, p. 117. An alternative and more intuitive notation for the same quantity is $\tilde{c}_{S_{\cdot,x_2 \dots x_q}}$. The original thermokinetic potential of the reduced complexes can be reconstructed by $\xi_{S_{1,x_2 \dots x_q}} = \tilde{\xi}_{L_1} \tilde{\xi}_{S_{0,x_2 \dots x_q}}$. The remaining concentrations are not changed by the reduction: $\tilde{c}_{L_i} = c_{L_i}$ for

$i = 2 \dots q$. The reduced capacities are $\tilde{C}_{L_1} = C_{L_1} + \sum_{x_2 \dots x_q} (\xi_{S_{1,x_2 \dots x_q}} / \tilde{\xi}_{L_1})\, C_{S_{1,x_2 \dots x_q}} = C_{L_1} + \sum_{x_2 \dots x_q} \tilde{\xi}_{S_{0,x_2 \dots x_q}}\, C_{S_{1,x_2 \dots x_q}}$, $\tilde{C}_{S_{0,x_2 \dots x_q}} = C_{S_{0,x_2 \dots x_q}} + (\xi_{S_{1,x_2 \dots x_q}} / \tilde{\xi}_{S_{0,x_2 \dots x_q}})\, C_{S_{1,x_2 \dots x_q}} = C_{S_{0,x_2 \dots x_q}} + \tilde{\xi}_{L_1}\, C_{S_{1,x_2 \dots x_q}}$ and $\tilde{C}_{L_i} = C_i$ for $i = 2 \dots q$.

We compute the reduced stoichiometry and the reduced resistances. For this purpose we use the binding of L_2 with resistance $R_{2,(x_1,x_3 \dots x_q)}$ as an example. The reaction rule for the binding of L_2 can be decomposed into two rules:

$$L_2 + S_{0,0,x_3 \dots x_q} \xrightleftharpoons{2a} S_{0,1,x_3 \dots x_q}, \qquad L_2 + S_{1,0,x_3 \dots x_q} \xrightleftharpoons{2b} S_{1,1,x_3 \dots x_q},$$

where only rule $2b$ contains the complexes $S_{1,x_2 \dots x_q}$. The resistances are $R_{2a,(x_3 \dots x_q)} = R_{2,(0,x_3 \dots x_q)}$ and $R_{2b,(x_3 \dots x_q)} = R_{2,(1,x_3 \dots x_q)}$. According to §6.15, we get the reduced stoichiometry:

$$\tilde{L}_2 + \tilde{S}_{0,0,x_3 \dots x_q} \xrightleftharpoons{\tilde{2}a} \tilde{S}_{0,1,x_3 \dots x_q}, \qquad \tilde{L}_2 + \tilde{L}_1 + \tilde{S}_{0,0,x_3 \dots x_q} \xrightleftharpoons{\tilde{2}b} \tilde{L}_1 + \tilde{S}_{0,1,x_3 \dots x_q}.$$

Reaction $\tilde{2}b$ can be simplified by a translation of stoichiometric coefficients (Corollary 6.4, p. 112) because L_1 does not enter its overall stoichiometry. Reaction $\tilde{2}b$ simplifies to $\tilde{L}_2 + \tilde{S}_{0,0,x_3 \dots x_q} \xrightleftharpoons{\hat{\tilde{2}}b} \tilde{S}_{0,1,x_3 \dots x_q}$ with a resistance $\hat{\tilde{R}}_{2b} = \tilde{R}_{2b,(1,x_3 \dots x_q)} / \tilde{\xi}_{L,1}$. Using Corollary 6.7 (p. 113), the rules $\tilde{2}a$ and $\hat{\tilde{2}}b$ can be combined to

$$\tilde{L}_2 + \tilde{S}_{0,0,x_3 \dots x_q} \xrightleftharpoons{\tilde{2}} \tilde{S}_{0,1,x_3 \dots x_q}$$

with resistance $\tilde{R}_{2,(x_3 \dots x_q)} = \tilde{R}_{2a,(0,x_3 \dots x_q)} \parallel (\tilde{R}_{2b,(1,x_3 \dots x_q)} / \tilde{\xi}_{L_1})$. Analog expressions can be given for the binding of the other ligands. The reduced system describes the binding of the remaining $q-1$ ligands $L_2, \dots L_q$. It contains $(q-1) \times 2^{q-2}$ reactions, 2^{q-1} scaffold proteins, $q-1$ free ligands ($\tilde{L}_2 \dots \tilde{L}_q$) and one ligand $\tilde{L}_1$ that represents the sum of free and bound ligand L_1. Thus, the reduced system contains $2^{q-1} = 50\%$ compounds less than the original system. The reaction rules, capacities and resistances of the reduced system can be derived without completely expanding the original reaction rules.

Example 6.28 (Interaction by stabilization of a conformation)**.** Let S be a scaffold with q ligands L_i. Assume that the ligands bind in sufficient spatial distance such that they do not interact directly. Such a situation occurs for example if a transmembrane receptor binds an extracellular ligand (e. g. a hormone) and an intracellular ligand (e. g. a kinase). This leads to the interaction factors $K_{C,i1} = \dots = K_{C,iq} = 1$ and $K_{R,i,\cdot} = 1$ because the ligands do not directly interact. Based on this interaction factors, the capacities have the form $C_{S_{x_1 \dots x_q}} = C_S\, K_{C,1}^{x_1} \dots K_{C,q}^{x_q}$. The following reaction rules and resistances describe the binding events:

$$\begin{aligned} L_1 + S_{0,x_2 \dots x_q} &\xrightleftharpoons{1} S_{1,x_2 \dots x_q}, & R_{1,(x_2 \dots x_q)} &= R_1\, K_{C,2}^{-x_2} \dots K_{C,q}^{-x_q} \\ &\vdots & &\vdots \\ L_q + S_{x_1 \dots x_{q-1},0} &\xrightleftharpoons{q} S_{x_1 \dots x_{q-1},1}, & R_{q,(x_1 \dots x_{q-1})} &= R_q\, K_{C,1}^{-x_1} \dots K_{C,q-1}^{-x_{q-1}}. \end{aligned}$$

	x_1	x_2	x_3	x_4
0	phosphatase form	dephosphorylated	no L_1 bound	no L_2 bound
1	kinase form	phosphorylated	L_1 bound	L_2 bound

Table 6.1.: The meaning of the subscripts of the scaffold protein S_{x_1,x_2,x_3,x_4} in Example 6.29.

We assume that the scaffold S can exist in an inactive conformation $\bar{S}$ that does not allow for the binding of any ligand. As a consequence, only free S can go to the inactive state. The capacity of the inactive state is $C_{\bar{S}} = C_S\,\bar{K}$ where $\bar{K} > 0$ is a constant. We further assume that there is a rapid equilibrium of the active and the inactive form:

$$S_{0\ldots0} \overset{0}{\rightleftharpoons} \bar{S}, \qquad\qquad R_0 = 0.$$

Applying the reduction method, we remove the inactive scaffold $\bar{S}$ from the system and get the new concentration $\tilde{c}_{S_{0\ldots0}} = c_{S_{0\ldots0}} + c_{\bar{S}}$. The other concentrations are not changed: $\tilde{c}_{L_i} = c_{L_i}$ and $\tilde{c}_{S_{x_1\ldots x_q}} = c_{S_{x_1\ldots x_q}}$ for at least one $x_i \neq 0$. The new capacities are $\tilde{C}_{S_{0\ldots0}} = C_{S_{0\ldots0}} + C_{\bar{S}} = C_S\,(\bar{K}+1)$ and $\tilde{C}_{L_i} = C_{L_i}$, $\tilde{C}_{S_{x_1\ldots x_q}} = C_{S_{x_1\ldots x_q}}$ for at least one $x_i \neq 0$. The capacities can be expressed in a more compact form as $\tilde{C}_{S_{x_1\ldots x_q}} = C_S\,K_{C,1}^{x_1}\ldots K_{C,q}^{x_q}\,(\bar{K}+1)^{\delta}$ with $\delta = \prod_{i=1}^{q}(1-x_i)$. If one does not experimentally distinguish between the active and the inactive forms of the free scaffold and thus measures only the concentration $\tilde{c}_{S_{0\ldots0}}$, one can only measure the apparent association $\tilde{k}_{+i} = R_i^{-1}\,C_S^{-1}\,C_{L_i}^{-1}\,(\bar{K}+1)^{-\delta}$ and dissociation constants $\tilde{k}_{-i} = R_i^{-1}\,C_S^{-1}\,K_{C,i}^{-1}$ (see §5.18, p. 93). Thus, the apparent dissociation constants $\tilde{k}_{-i}$ are independent of the binding state of S, but the apparent association constants $\tilde{k}_{+i}$ depend on the binding state.

In the examples above, the steady state signal flows at scaffold proteins are bidirectional. Whenever the binding of a ligand L_1 has influence on the binding of another ligand L_2, the binding of L_2 necessarily has an effect on the binding of L_1. This bidirectionality is an effect of the symmetry of the thermodynamic interaction factors and thus of the Wegscheider conditions. It can be broken if additional Gibbs energy (e. g. in the form of ATP) is supplied to the receptor. The next example derives the apparent association and dissociation constants for a model of such a mechanism.

Example 6.29 (Retroactivity-free signal transduction by a receptor kinase)**.** In the following example, we derive a reduced-order model of the signal transduction at a receptor kinase. Consider a receptor protein S_{x_1,x_2,x_3,x_4} (see Table 6.1). The receptor can spontaneously switch between two folding states ($x_1 \in \{0,1\}$) with auto-kinase ($x_1 = 1$) and phosphatase ($x_1 = 0$) activity, respectively. An extracellular ligand L_1 can bind to the kinase form ($x_3 = 1$ means that L_1 is bound). The resulting L_1-S complex cannot switch to phosphatase form. If the receptor is in the kinase form, it rapidly undergoes auto-phosphorylation. If it is in phosphatase form, it rapidly undergoes auto-dephosphorylation. The index $x_2 = 0$ indicates an unphosphorylated and $x_2 = 1$ a phosphorylated receptor. After phosphorylation, an intracellular ligand L_2 rapidly binds to the phosphorylation site ($x_4 = 1$ means that L_2 has bound). Bound L_2 protects the receptor from dephosphorylation.

Observe that not all combinations of x_1, x_2, x_3 and x_4 are possible. In particular, there are no L_1-S complexes with phosphatase activity (i. e. $x_1 = 0 \wedge x_3 = 1$ does not occur) and there are no L_2-S complexes that are dephosphorylated (i. e. $x_2 = 0 \wedge x_4 = 1$ does not occur).

We assume that binding, phosphorylation and conformation change are independent processes and get

$$C_{S_{x_1,x_2,x_3,x_4}} = C_S\, K_{C,1}^{x_1}\, K_{C,2}^{x_2}\, K_{C,3}^{x_3}\, K_{C,4}^{x_4}$$

and $K_{R,\cdot,\cdot} = 1$.

The above mechanism can be described by reaction rules for conformational change

$$S_{0,x_2,0,x_4} \xrightleftharpoons{1_{x_2,x_4}} S_{1,x_2,0,x_4}, \qquad R_{1,(x_2,x_4)} = R_1\, K_{C,2}^{-x_2}\, K_{C,4}^{-x_4},$$

phosphorylation and dephosphorylation

$$\begin{aligned} S_{1,0,x_3,0} + ATP &\xrightleftharpoons{2_{x_3}} S_{1,1,x_3,0} + ADP, & R_{2,x_3} &= 0,\\ S_{0,0,x_3,0} + P &\xrightleftharpoons{3_{x_3}} S_{0,1,x_3,0}, & R_{3,x_3} &= 0, \end{aligned}$$

and binding of L_1 and L_2

$$\begin{aligned} S_{1,x_2,0,x_4} + L_1 &\xrightleftharpoons{4_{x_2,x_4}} S_{1,x_2,1,x_4}, & R_{4,(x_2,x_4)} &= R_4\, K_{C,2}^{-x_2}\, K_{C,4}^{-x_4},\\ S_{x_1,1,x_3,0} + L_2 &\xrightleftharpoons{5_{x_1,x_3}} S_{x_1,1,x_3,1}, & R_{5,(x_1,x_3)} &= 0. \end{aligned}$$

Each of the states $S_{1,0,x_3,0}$, $S_{0,0,x_3,0}$ and $S_{x_1,1,x_3,1}$ occurs only in one of the three fast reaction rules 2, 3 and 5, respectively. Thus, we can reduce them simultaneously according to the step-by-step procedure §6.15 (p. 117) by applying the replacement rules:

$$S_{1,0,x_3,0} \rightleftharpoons S_{1,1,x_3,0} + ADP - ATP, \quad S_{0,0,x_3,0} \rightleftharpoons S_{0,1,x_3,0} - P, \quad S_{x_1,1,x_3,1} \rightleftharpoons S_{x_1,1,x_3,0} + L_2.$$

For the application of these replacement rules the reactions rules 1 and 4 need to be expanded. Since the situation $x_2 = 0 \wedge x_4 = 1$ cannot occur, rule 1 expands to three reactions. For two of those reactions the replacement rules are applicable:

$$\begin{aligned} S_{0,0,0,0} \xrightleftharpoons{1_{00}} S_{1,0,0,0} \quad &\to \quad \tilde{S}_{0,1,0,0} - P \xrightleftharpoons{\tilde{1}_{00}} \tilde{S}_{1,1,0,0} + \widetilde{ADP} - \widetilde{ATP}, & \tilde{R}_{1,(0,0)} &= R_1,\\ S_{0,1,0,0} \xrightleftharpoons{1_{10}} S_{1,1,0,0} \quad &\to \quad \tilde{S}_{0,1,0,0} \xrightleftharpoons{\tilde{1}_{10}} \tilde{S}_{1,1,0,0}, & \tilde{R}_{1,(1,0)} &= R_1\, K_{C,2}^{-1},\\ S_{0,1,0,1} \xrightleftharpoons{1_{11}} S_{1,1,0,1} \quad &\to \quad \tilde{S}_{0,1,0,0} + \tilde{L}_2 \xrightleftharpoons{\tilde{1}_{11}} \tilde{S}_{1,1,0,0} + \tilde{L}_2, & \tilde{R}_{1,(1,1)} &= R_1\, K_{C,2}^{-1}\, K_{C,4}^{-1}. \end{aligned}$$

Analogously, reaction rule 4 expands and reduces to

$$\begin{aligned} S_{1,0,0,0} + L_1 \xrightleftharpoons{4_{00}} S_{1,0,1,0} \quad &\to \quad \tilde{S}_{1,1,0,0} + \widetilde{ADP} - \widetilde{ATP} + \tilde{L}_1 \xrightleftharpoons{\tilde{4}_{00}} \tilde{S}_{1,1,1,0} + \widetilde{ADP} - \widetilde{ATP},\\ S_{1,1,0,0} + L_1 \xrightleftharpoons{4_{10}} S_{1,1,1,0} \quad &\to \quad \tilde{S}_{1,1,0,0} + \tilde{L}_1 \xrightleftharpoons{\tilde{4}_{10}} \tilde{S}_{1,1,1,0},\\ S_{1,1,0,1} + L_1 \xrightleftharpoons{4_{11}} S_{1,1,1,1} \quad &\to \quad \tilde{S}_{1,1,0,0} + \tilde{L}_1 + \tilde{L}_2 \xrightleftharpoons{\tilde{4}_{11}} \tilde{S}_{1,1,1,0} + \tilde{L}_2 \end{aligned}$$

with $\tilde{R}_{4,(0,0)} = R_4$, $\tilde{R}_{4,(1,0)} = R_4\, K_{C,2}^{-1}$ and $\tilde{R}_{4,(1,1)} = R_4\, K_{C,2}^{-1}\, K_{C,4}^{-1}$.

This list of reactions can be further simplified. By a translation of stoichiometric coefficients (Corollary 6.4, p. 112) the negative coefficients in $\tilde{1}_{00}$ can be removed and we get $\tilde{S}_{0,1,0,0} + \widetilde{ATP} \rightleftharpoons \tilde{S}_{1,1,0,0} + \widetilde{ADP} + \tilde{P}$ with resistance $\tilde{R}'_{1,(0,0)} = R_1\, \xi_{ATP}\, \xi_P$. Ligand $\tilde{L}_2$ enters the reaction $\tilde{1}_{11}$ only as a catalyst. By a translation of stoichiometric coefficients the reaction $\tilde{1}_{11}$ can be further simplified to $\tilde{S}_{0,1,0,0} \rightleftharpoons \tilde{S}_{1,1,0,0}$ with a resistance $\tilde{R}'_{1,(1,1)} = R_1\, K_{C,2}^{-1}\, K_{C,4}^{-1}\, \tilde{\xi}_{L_2}^{-1}$. Further, all three reactions $\tilde{4}_{00}$, $\tilde{4}_{10}$ and $\tilde{4}_{11}$ have the same overall stoichiometry $\tilde{S}_{1,1,0,0} + \tilde{L}_1 \rightleftharpoons \tilde{S}_{1,1,1,0}$ and thus the effect of $\tilde{\xi}_{ATP}$, $\tilde{\xi}_{ADP}$ and $\tilde{\xi}_{L_2}$ can be moved to the resistances: $\tilde{R}'_{4,(0,0)} = R_4\, \tilde{\xi}_{ATP}\, \tilde{\xi}_{ADP}^{-1}$, $\tilde{R}'_{4,(1,0)} = R_4\, K_{C,2}^{-1}$ and $\tilde{R}'_{4,(1,1)} = R_4\, K_{C,2}^{-1}\, K_{C,4}^{-1}\, \tilde{\xi}_{L_2}^{-1}$. After this simplification, several reactions have the same stoichiometry and can be treated as parallel reactions (see Section 6.2.2, p. 113). The stoichiometry of $\tilde{1}_{11}$ is equal to reaction $\tilde{1}_{10}$. We can also combine the three reactions $\tilde{4}_{00}$, $\tilde{4}_{10}$ and $\tilde{4}_{11}$.

Finally, the reduced system reads:

$$\tilde{S}_{0,1,0,0} + \widetilde{ATP} \xrightleftharpoons{\tilde{1}_{00}} \tilde{S}_{1,1,0,0} + \widetilde{ADP} + \tilde{P},$$

$$\tilde{S}_{0,1,0,0} \xrightleftharpoons{\tilde{1}_{1,x_4}} \tilde{S}_{1,1,0,0},$$

$$\tilde{S}_{1,1,0,0} + \tilde{L}_1 \overset{\tilde{4}}{\rightleftharpoons} \tilde{S}_{1,1,1,0}$$

with resistances

$$\begin{aligned}
\tilde{R}'_{1,(0,0)} &= R_1\, \tilde{\xi}_{ATP}\, \tilde{\xi}_P, \\
\tilde{R}_{1,(1,x_4)} &= \tilde{R}_{1,(1,0)} \parallel \tilde{R}'_{1,(1,1)} = R_1\, (\underbrace{K_{C,2} + K_{C,2}\, K_{C,4}\, \tilde{\xi}_{L_2}}_{\tilde{K}_A(\tilde{\xi})})^{-1}, \\
\tilde{R}_4 = \tilde{R}'_{4,(0,0)} \parallel \tilde{R}'_{4,(1,0)} \parallel \tilde{R}'_{4,(1,1)} &= R_4\, (\underbrace{K_{C,2} + K_{C,2}\, K_{C,4}\, \tilde{\xi}_{L_2} + \tilde{\xi}_{ADP}/\tilde{\xi}_{ATP}}_{\tilde{K}_B(\tilde{\xi})})^{-1}
\end{aligned}$$

with the state dependent factors $\tilde{K}_A(\tilde{\xi})$ and $\tilde{K}_B(\tilde{\xi})$. The new concentrations represent sums of the original concentrations:

$$\begin{array}{llllll}
\tilde{c}_{S_{0,1,0,0}} & = c_{S_{0,1,0,0}} & & + c_{S_{0,0,0,0}} & + c_{S_{0,1,0,1}} & = \sum_{x_2,x_4} c_{S_{0,x_2,0,x_4}}, \\
\tilde{c}_{S_{1,1,0,0}} & = c_{S_{1,1,0,0}} & + c_{S_{1,0,0,0}} & & + c_{S_{1,1,0,1}} & = \sum_{x_2,x_4} c_{S_{1,x_2,0,x_4}}, \\
\tilde{c}_{S_{1,1,1,0}} & = c_{S_{1,1,1,0}} & + c_{S_{1,0,1,0}} & & + c_{S_{1,1,1,1}} & = \sum_{x_2,x_4} c_{S_{1,x_2,1,x_4}}.
\end{array}$$

These are the overall concentration of the phosphatase form without L_1, the kinase form without L_1 and the kinase form with L_1, respectively. The concentration $\tilde{c}_{L_2}$ is the overall concentration of all L_2:

$$\tilde{c}_{L_2} = c_{L_2} + \sum_{x_1,x_3} c_{S_{x_1,1,x_3,1}} = c_{L_2} + \sum_{x_1,x_2,x_3} c_{S_{x_1,x_2,x_3,1}}.$$

The reduced capacities are

$$\begin{aligned}
\tilde{C}_{S_{0,1,0,0}} &= C_{S_{0,1,0,0}} & &+C_{S_{0,0,0,0}}\,\tilde{\xi}_P^{-1} &+C_{S_{0,1,0,1}}\,\xi_{L_2} &= C_S\,(\tilde{K}_A(\tilde{\xi}) + \tilde{\xi}_P^{-1}),\\
\tilde{C}_{S_{1,1,0,0}} &= C_{S_{1,1,0,0}} &+C_{S_{1,0,0,0}}\,\frac{\tilde{\xi}_{ADP}}{\tilde{\xi}_{ATP}} & &+C_{S_{1,1,0,1}}\,\tilde{\xi}_{L_2} &= C_S\,K_{C,1}\,\tilde{K}_B(\tilde{\xi}),\\
\tilde{C}_{S_{1,1,1,0}} &= C_{S_{1,1,1,0}} &+C_{S_{1,0,1,0}}\,\frac{\tilde{\xi}_{ADP}}{\tilde{\xi}_{ATP}} & &+C_{S_{1,1,1,1}}\,\tilde{\xi}_{L_2} &= C_S\,K_{C,1}\,K_{C,3}\,\tilde{K}_B(\tilde{\xi})
\end{aligned}$$

and

$$\begin{aligned}
\tilde{C}_{L_1} &= C_{L_1},\\
\tilde{C}_{L_2} &= C_{L_2} + \sum_{x_1,x_3} C_{S_{x_1,1,x_3,1}}\,\tilde{\xi}_{S_{x_1,1,x_3,0}}\\
&= C_{L_2} + C_S\,K_{C,2}\,K_{C,4}\left(\tilde{\xi}_{S_{0,1,0,0}} + K_{C,1}\,\tilde{\xi}_{S_{1,1,0,1}} + K_{C,1}\,K_{C,3}\,\tilde{\xi}_{S_{1,1,1,1}}\right).
\end{aligned}$$

The capacities of the energy carriers are unchanged: $\tilde{C}_{ATP} = C_{ATP}$, $\tilde{C}_{ADP} = C_{ADP}$ and $\tilde{C}_P = C_P$.

The binding of L_1 is independent of the other processes because $\tilde{K}_B(\tilde{\xi})$ cancels out in the rate parameters $k_{+\tilde{4}} = \tilde{R}_4^{-1}\,\tilde{C}_{S_{1,1,0,0}}^{-1}\,\tilde{C}_{L_1}^{-1} = R_4^{-1}\,C_S^{-1}\,K_{C,1}^{-1}\,C_{L_1}^{-1}$ and $k_{-\tilde{4}} = \tilde{R}_4^{-1}\,\tilde{C}_{S_{1,1,1,0}}^{-1} = R_4^{-1}\,C_S^{-1}\,K_{C,1}^{-1}\,K_{C,3}^{-1}$ are independent of the state of the system. Thus, the binding of L_1 is not influenced by the downstream processes. This shows that the above receptor model describes retroactivity-free signaling.

The original system consisting of 14 compounds (9 complexes, 2 ligands, ATP, ADP, P) and 13 reactions could be reduced to a system with 7 compounds (3 complexes, 2 ligands, ATP, ADP, P) and 3 reactions. The necessary calculations are lengthy but straightforward and can easily be automated.

6.5.3. Conclusions

This excursus developed an approach for the modeling of combinatorial complex protein-protein interaction networks in TKM. Thermodynamic and kinetic interaction factors provide a simple and intuitive way to characterize thermodynamically feasible signal flows. The reduction methods can often be applied without completely expanding the reaction rules. This means that the model reduction can be performed without formulating the detailed model equations based on a complete expansion of the reaction rules. Because in combinatorial networks the detailed model equations are very large, this is an essential advantage. It allows *reduced order-modeling* instead of *model reduction*. Thus, thermokinetic modeling and model reduction allows for the thermodynamically consistent modeling and thus the analysis of the interconnections of signal and energy flows in large signal transduction networks.

6.6. Excursus: Reduction of Uncontrollable and Unobservable Subsystems

The previous excursus demonstrated the application of the rapid-equilibrium assumption to combinatorial networks in the TKM formalism. Conzelmann et al. [27, 28] introduced a method for the reduction of such networks that is based on the reduction of unobservable modes in the system. The key step is the application of a state transformation that forms pool variables. Under certain conditions, the transformed system decomposes into uni-directionally coupled modules, the so called tiers. This means that the top-level modules can be analyzed without analyzing the downstream tiers.

The application of the rapid-equilibrium assumption leads to pool variables that are similar to the pool variables introduced by Conzelmann et al. [27, 28]. This section shows that the method that was developed for the reduction of fast reactions also is applicable for the reduction of unobservable modes.

This excursus shows by means of two examples, how the procedure for the reduction of rapid reactions in Corollary 6.14 and §6.15 (p. 115) can be modified for the reduction of uncontrollable and unobservable modes. This excursus provides not an exhaustive discussion but rather a starting point for including the approaches of Conzelmann et al. [27, 28] into TKM.

6.6.1. Reduction of Uncontrollable Subsystems

The clamped potentials and clamped fluxes describe how the system environment can provide the system with energy. If the energy cannot reach certain parts of the system, those parts inevitably go to thermodynamic equilibrium. If the system dynamics has followed the model equation for a long time before the experiment, these parts are in thermodynamic equilibrium. Then, we can reduce the system size. The following example demonstrates such a case.

Example 6.30 (Reduction of an uncontrollable subsystem)**.** We consider the independent binding of two ligands L_1 and L_2 to a scaffold protein S. We assume constant capacities and resistances. The interaction factors of the ligand bindings equal one ($K_{C,12} = K_{R,1,2} = K_{R,2,1} = 1$, see Section 6.5, p. 120) because the binding of L_1 and L_2 are independent. Thus, we have the capacities C_{L_1}, C_{L_2} and $C_{S_{i_1,i_2}} = C_S\, K_{C,1}^{i_1}\, K_{C,2}^{i_2}$. The following reaction rules describe the system:

$$
\begin{aligned}
(L_1) + S_{0,i_2} &\xrightleftharpoons{(1,i_2)} S_{1,i_2}, &\qquad R_{1,i_2} &= R_1\, K_{C,2}^{-i_2},\\
(L_2) + S_{i_1,0} &\xrightleftharpoons{(2,i_1)} S_{i_1,1}, &\qquad R_{2,i_1} &= R_2\, K_{C,1}^{-i_1}
\end{aligned}
$$

with $i_1, i_2 \in \{0, 1\}$. The clamped thermokinetic potentials of L_1 and L_2 are the inputs to the system. We get $k_{+1,0} = k_{+1,1} = R_1^{-1}\, C_S^{-1}\, C_{L_1}^{-1}$, $k_{-1,0} = k_{-1,1} = R_1^{-1}\, C_S^{-1}\, K_{C,1}^{-1}$, $k_{+2,0} = k_{+2,1} = R_2^{-1}\, C_S^{-1}\, C_{L_2}^{-1}$ and $k_{-2,0} = k_{-2,1} = R_2^{-1}\, C_S^{-1}\, K_{C,2}^{-1}$ (see §5.18, p. 93). This means that the association and dissociation constants of ligand L_1 do not depend on the binding state of L_2, and vice versa. Both binding reactions are independent.

Similar as Koschorreck [63], we introduce an additional, virtual reaction

$$S_{0,0} + S_{1,1} \overset{3}{\rightleftharpoons} S_{0,1} + S_{1,0}$$

with the thermokinetic force $F_3 = \xi_{S_{0,0}}\, \xi_{S_{1,1}} - \xi_{S_{0,1}}\, \xi_{S_{1,0}}$, resistance $R_3 = \infty$ and flux $J_3 = 0$. The time derivative of F_3 is

$$\dot{F}_3 = \dot{\xi}_{S_{0,0}}\, \xi_{S_{1,1}} + \xi_{S_{0,0}}\, \dot{\xi}_{S_{1,1}} - \dot{\xi}_{S_{0,1}}\, \xi_{S_{1,0}} - \xi_{S_{0,1}}\, \dot{\xi}_{S_{1,0}}.$$

With $\dot{\xi}_i = C_i^{-1}\, \dot{c}_i$ and $\dot{c}_i$ in dependence on the fluxes J_j we get

$$\dot{F}_3 = \frac{(J_{2,1} + J_{1,1})\, \xi_{S_{0,0}} + K_{C,2}\, (J_{2,1} - J_{1,0})\, \xi_{S_{0,1}} + K_{C,1}\, (J_{1,1} - J_{2,0})\, \xi_{S_{1,0}} - K_{C,1}\, K_{C,2} (J_{2,0} + J_{1,0})\, \xi_{S_{1,1}}}{C_S\, K_{C,1}\, K_{C,2}}.$$

Now, we apply $J_j = R_j^{-1}\, F_j$ and F_j in dependence on the thermokinetic potentials ξ_i and get

$$\dot{F}_3 = \underbrace{-\frac{K_{C,1}\, R_1\, (1 + K_{C,2}\, \xi_{L_2}) + K_{C,2}\, R_2 (1 + K_{C,1}\, \xi_{L_1})}{C_S\, K_{C,1}\, K_{C,2}\, R_1\, R_2}}_{\lambda}\, \underbrace{(\xi_{0,0}\, \xi_{1,1} - \xi_{0,1}\, \xi_{1,0})}_{F_3}.$$

The force F_3 asymptotically approaches zero because $\lambda < 0$ for all ξ_{L_1} and ξ_{L_2}. Although the rate of convergence depends on ξ_{L_1} and ξ_{L_2}, the set $F_3 = 0$ is invariant and globally, asymptotically stable for all ξ_{L_1} and ξ_{L_2}. If the system has followed the given model also before the initial time $t = 0$ for an infinitely long time, it is safe to assume that $F_3 = 0$, i. e. reaction 3 is in equilibrium. Then, we can proceed as if R_3 was zero because this assumption forces the system to an equilibrium of reaction 3.

We apply the step-by-step procedure in §6.15 (p. 117) to reduce S_{11} from the system (step 1). With $S_{11} \rightleftharpoons S_{0,1} + S_{1,0} - S_{0,0}$ we get in step 2 the new stoichiometry:

$$(\tilde{L}_1) + \tilde{S}_{0,0} \xrightleftharpoons{(1,0)} \tilde{S}_{1,0}, \qquad (\tilde{L}_1) + \tilde{S}_{0,1} \xrightleftharpoons{(1,1)} \tilde{S}_{0,1} + \tilde{S}_{1,0} - \tilde{S}_{0,0},$$

$$(\tilde{L}_2) + \tilde{S}_{0,0} \xrightleftharpoons{(2,0)} \tilde{S}_{0,1}, \qquad (\tilde{L}_2) + \tilde{S}_{1,0} \xrightleftharpoons{(2,1)} \tilde{S}_{0,1} + \tilde{S}_{1,0} - \tilde{S}_{0,0}.$$

Step 3 yields the relation of the original and the reduced variables:

$$c_{S_{0,0}} = \tilde{c}_{S_{0,0}} + c_{S_{11}}, \quad c_{S_{0,1}} = \tilde{c}_{S_{0,1}} - c_{S_{11}}, \quad c_{S_{1,0}} = \tilde{c}_{S_{1,0}} - c_{S_{11}}, \quad c_{S_{1,1}} = C_{S_{1,1}}\, \tilde{\xi}_{S_{0,1}}\, \tilde{\xi}_{S_{1,0}}\, \tilde{\xi}_{S_{0,0}}^{-1}$$

and $\tilde{c}_{L_1} = c_{L_1}$ and $\tilde{c}_{L_2} = c_{L_2}$. The reduced resistances are $\tilde{R}_j = R_j$ and the reduced capacities are

$$\tilde{C}_{S_{0,0}} = C_{S_{0,0}} - \frac{\tilde{\xi}_{S_{0,1}}\, \tilde{\xi}_{S_{1,0}}}{\tilde{\xi}_{S_{0,0}}^2}\, C_{S_{1,1}}, \quad \tilde{C}_{S_{0,1}} = C_{S_{0,1}} + \frac{\tilde{\xi}_{S_{1,0}}}{\tilde{\xi}_{S_{0,0}}}\, C_{S_{1,1}}, \quad \tilde{C}_{S_{1,0}} = C_{S_{1,0}} + \frac{\tilde{\xi}_{S_{0,1}}}{\tilde{\xi}_{S_{0,0}}}\, C_{S_{1,1}},$$

$\tilde{C}_{L_1} = C_{L_1}$ and $\tilde{C}_{L_2} = C_{L_2}$. The above equations define a reduced thermokinetic model that is equivalent to the original model for $F_3 = 0$. Since $F_3 = 0$ is a globally attractive set, the reduction error goes to zero with increasing time. If the system has followed the model dynamics for a long time, the reduced system and the original system are equivalent. Thus, this example shows that the method developed for model reduction by the rapid equilibrium assumption can be also used for the reduction of uncontrollable subsystems that approach thermodynamic equilibrium.

6.6.2. Reduction of Unobservable Subsystems

A typical system has a set of natural output variables. This may be signals that connect different modules of a signaling network, for example the concentrations of active transcription factors in a model of a signaling pathway. In particular, in the case of models of combinatorial protein-protein interaction networks one often is not interested in describing the whole combinatorial complexity, but only in the dynamics of the natural output variables and the experimental readouts. Conzelmann et al. [27, 28] showed that under certain conditions models of such systems can be reduced because they contain dynamics that are not observable from the output variables.

This section shows by means of an example how a non-observable subsystem can be identified and reduced in the TKM formalism. For this purpose, the unobservable subsystem is assumed to be in rapid equilibrium. To describe the error that is caused by this assumption virtual compounds are introduced. By a suited pooling of concentrations, a reduced system can be derived that does not contain the virtual compounds and thus is independent of the error introduced by the rapid equilibrium assumption. This means that the reduced system exactly describes the dynamics of the observable subsystem.

Example 6.31 (Reduction of an unobservable subsystem)**.** Consider the system from Example 6.30:

$$(L_1) + S_{0,i_2} \overset{(1,i_2)}{\rightleftharpoons} S_{1,i_2}, \qquad R_{1,i_2} = R_1\, K_{C,2}^{-i_2},$$

$$(L_2) + S_{i_1,0} \overset{(2,i_1)}{\rightleftharpoons} S_{i_1,1}, \qquad R_{2,i_1} = R_2\, K_{C,1}^{-i_1}$$

with $i_1, i_2 \in \{0, 1\}$, constant capacities C_{L_1}, C_{L_2} and $C_{S_{i_1,i_2}} = C_S\, K_{C,1}^{i_1}\, K_{C,2}^{i_2}$.

Since the bindings of L_1 and L_2 are independent, we can model L_1 binding independently from L_2 binding. The L_2 binding is unobservable if the binding state of L_1 is considered as the output of the system. Here, we discuss this in the framework of TKM and provide the reduced model equations.

The kinetics of L_2 binding are determined by the parameter R_2. We proceed as if R_2 was zero and thus as if the L_2 binding was in equilibrium. This assumption introduces an error that we can explicitly account for in the reaction network. For this purpose, we introduce two virtual species E_1 an E_2 that describe the error and replace the stoichiometric equation for reactions $(2, i_1)$ by the new equation:

$$(L_2) + S_{i_1,0} \xrightleftharpoons{(2,i_1)} S_{i_1,1} + (E_{i_1}), \qquad\qquad R_{2,i_1} = 0.$$

Since $R_2 = 0$ the equilibrium condition

$$\xi_{L_2}\, \xi_{S_{i_1,0}} = \xi_{S_{i_1,1}}\, \xi_{E_{i_1}} \tag{6.1}$$

holds. The newly introduced thermokinetic potentials ξ_{E_0} and ξ_{E_1} describe the deviation of the original reactions $(2, 0)$ and $(2, 1)$ from equilibrium. They describe the error that is introduced by the rapid equilibrium assumption. We treat the species E_1 and E_2 as species with a clamped thermokinetic potential and assume $C_{E_1} = C_{E_2} = 0$. This means that we may neglect their concentration $c_{E_1} = c_{E_2} = 0$ in the further considerations. From Example 6.30 we know that $\lim_{t\to\infty}(\xi_{S_{0,0}}\, \xi_{S_{1,1}} - \xi_{S_{0,1}}\, \xi_{S_{1,0}}) = 0$. With Equation 6.1 one can derive that $\xi_{S_{0,0}}\, \xi_{S_{1,1}} - \xi_{S_{0,1}}\, \xi_{S_{1,0}} = \xi_{S_{0,1}}\, \xi_{S_{1,1}}\, (\xi_{E,0} - \xi_{E,1})/\xi_{L,2}$. This means that $\lim_{t\to 0}(\xi_{E_0} - \xi_{E_1}) = 0$ for $\xi_{L_2}, \xi_{S_{i_1,i_2}} > 0$. Thus, if we restrict ourselves to the case when the uncontrollable subsystem is in equilibrium, we have $\xi_E = \xi_{E_0} = \xi_{E_1}$ and we can replace the virtual species E_1 and E_2 by the virtual species E. If we can show that its thermokinetic potential ξ_E does not interact with L_1 binding, we may safely use the reduced equations to model L_1 binding.

To reduce the system we follow the step-by-step procedure in §6.15 (p. 117). We reduce the species $S_{i_1,1}$ with $S_{i_1,1} \rightleftharpoons (L_2) + S_{i_1,0} - (E)$ (step 1) and get the stoichiometry (step 2):

$$(\tilde{L}_1) + \tilde{S}_{0,0} \xrightleftharpoons{(1,0)} \tilde{S}_{1,0}, \qquad\qquad (\tilde{L}_1) + \tilde{L}_2 + \tilde{S}_{0,0} - (\tilde{E}) \xrightleftharpoons{(1,1)} \tilde{L}_2 + \tilde{S}_{1,0} - (\tilde{E})$$

with resistances $\tilde{R}_{(1,i_2)} = R_{(1,i_2)}$. Reaction $(1, 1)$ can be simplified by a translation of stoichiometric coefficients (Corollary 6.4, p. 112). Then reactions $(1, 0)$ and $(1, 1)$ can be reduced to one equation:

$$(\tilde{L}_1) + \tilde{S}_{0,0} \xrightleftharpoons{1} \tilde{S}_{1,0}$$

with resistances $\tilde{R}_1 = R_{(1,0)} \parallel (R_{(1,1)}\, \xi_{L_2}^{-1}\, \xi_E) = R_1 \cdot (1 + \xi_{L_2}\, \xi_E^{-1}\, K_{C,2})^{-1}$. The relation of the original and reduced concentrations is given by (step 3):

$$c_{S_{i_1,0}} = \tilde{c}_{S_{i_1,0}} - c_{S_{i_1,1}}, \qquad\qquad c_{S_{i_1,1}} = C_{S_{i_1,1}}\, \xi_{L_2}\, \tilde{\xi}_{S_{i_1,0}}\, \tilde{\xi}_E^{-1}$$

and $c_{L_1} = \tilde{c}_{L_1}$ and $c_{L_2} = \tilde{c}_{L_2} - c_{S_{0,1}} - c_{S_{1,1}}$. Thus, the reduced concentrations $\tilde{c}_{L_2}$, $\tilde{c}_{S_{0,0}}$ and $\tilde{c}_{S_{1,0}}$ are the overall concentrations of L_2, scaffold with free L_1-binding site and scaffold-L_1 complex, respectively. The reduced resistance was already determined above. The reduced capacities are (step 4):

$$\tilde{C}_{S_{i_1,0}} = C_{S_{i_1,0}} + \xi_{L_2}\, \xi_E^{-1}\, C_{S_{i_1,1}} = C_S\, K_{C,1}^{-i_1}\, (1 + \xi_{L_2}\, \xi_E^{-1}\, K_{C,2})$$

and $\tilde{C}_{L_1} = C_{L_1}$. We see that the reduced capacities $\tilde{C}_{S_{i_1,0}}$ as well as the reduced resistance depend on ξ_{L_2} and the error ξ_E in the same manner. By a translation of the chemical potentials (Corollary 5.34, p. 102), we can rescale the system in order to remove this dependency. The scaling factors $\delta_{\tilde{\xi}_{S_{0,0}}} = 1 + \xi_{L_2}\, \xi_E^{-1}\, K_2$, $\delta_{\tilde{\xi}_{S_{0,1}}} = 1 + \xi_{L_2}\, \xi_E^{-1}\, K_2$ and $\delta_{\tilde{\xi}_{L_1}} = 1$ obey the condition $\tilde{N}^T \log(\delta\xi) + \tilde{S}^T \log(\delta\xi_e) = 0$. The scaling leads to the constant parameters

$$\hat{\tilde{C}}_{S_{i_1,0}} = C_S\, K_1^{-i_1}, \qquad \hat{\tilde{R}}_1 = R_1$$

for the reaction equation $\hat{\tilde{L}}_1 + \hat{\tilde{S}}_{0,0} \overset{1}{\rightleftharpoons} \hat{\tilde{S}}_{1,0}$. This model describes the binding of L_1 and does not depend on the second ligand ξ_{L_2} or on the approximation error ξ_E.

Thus, we could reduce the original system with 4 reactions and 6 species to a system with 1 reaction and 3 species. The reduced system does not describe the L_2 binding but only the L_1 binding. This is possible because the two binding reactions are independent.

§ 6.32 (Limitations of the approach). In the example above, we assumed that the uncontrollable subsystem is in equilibrium. Without this assumption, we cannot derive a reduced description for the L_1 binding independent of the L_2 binding using the methods discussed so far. Omitting the equilibrium assumption leads to $\xi_{E_1} \neq \xi_{E_2}$ and impedes to merge the two reactions for L_1 binding into one. Then we could not remove the dependency of L_1 binding system on L_2 binding. However, also in this case the reduced model relying on the pooling of all free and the pooling of all occupied L_1 binding sites is valid because the association and dissociation constants of L_1 are independent of L_2 binding. This is discussed by Conzelmann et al. [27, 28].

If the uncontrollable subsystem is not in equilibrium, i. e. $\xi_{E_1} \neq \xi_{E_2}$, we cannot prove the validity of the model reduction only by TKM operations that are introduced in this chapter. However, it is possible to prove the validity by comparing the model equations in terms of concentrations, as it is done by Conzelmann et al. [27, 28]. If a certain pooling scheme is known to lead to a valid reduction of the unobservable subsystem, an according reduced TKM system can be derived by neglecting the error variables ξ_{E_i} from the very beginning. Thus, the above proposed methodology is not optimal, when one intends to identify the unobservable subsystem, but it is well suited for doing the actual reduction step.

6.6.3. Conclusions

This excursus sketched a possibility to treat a reduction of uncontrollable and unobservable subsystems in TKM. By means of two examples it was shown that the step-by-step procedure §6.15 (p. 117) can be modified to reduce uncontrollable and unobservable subsystems. Thus, this approach provides a link to the work of Koschorreck et al. [63–65] and Conzelmann et al. [27, 28].

6.7. Excursus: Enzyme Kinetics in TKM

Enzymatic reactions consist of several elementary steps, but are often described by a single rate law that is derived on the basis of quasi-steady state or rapid equilibrium assumptions

[9, 45, 94]. This excursus demonstrates that the use of TKM greatly simplifies the use of rapid equilibrium assumptions for the derivation of enzyme kinetics. The following considerations have been published in Ederer and Gilles [31].

As explained in §5.14 (p. 92), any generalized mass-action law can be modeled in TKM. For example, the thermokinetic resistance of a Michaelis-Menten reaction $A \rightleftharpoons B$ is $R = \rho_0 + \rho_A\, c_A + \rho_B\, c_B$. This law is based on the assumption that the enzyme concentration is much smaller than the concentration of the substrate such that the concentrations of the enzyme complexes are in quasi-steady state.

However, situations with low metabolite but high enzyme concentrations may occur *in vivo*. Then, the enzyme concentrations cannot be assumed to be in a quasi-steady state. An alternative approach to derive a kinetic law for an enzymatic reaction is to assume that the binding of A and B to the enzyme E is in rapid equilibrium.

The reaction scheme

$$A + E \overset{A}{\rightleftharpoons} EA \overset{AB}{\rightleftharpoons} EB \overset{B}{\rightleftharpoons} B + E \tag{6.2}$$

describes a simple, reversible enzyme catalyzed reaction. We assume that $R_A = R_B = 0$ and $R_{AB} > 0$. This means that the conversion of A to B at the enzyme E is the rate-limiting step. Applying the reduction method, we get the reduced concentrations $\tilde{c}_A = c_A + c_{EA}$, $\tilde{c}_B = c_B + c_{EB}$ and $\tilde{c}_E = c_E + c_{EA} + c_{EB}$ that are the total concentrations of A, B and E, respectively. The reduced capacities are $\tilde{C}_A = C_A + \tilde{\xi}_E\, C_{EA}$, $\tilde{C}_B = C_B + \tilde{\xi}_E\, C_{EB}$ and $\tilde{C}_E = C_E + \tilde{\xi}_A\, C_{EA} + \tilde{\xi}_B\, C_{EB}$. With these pool variables the reaction scheme reduces to $\tilde{A} + \tilde{E} \overset{AB}{\rightleftharpoons} \tilde{B} + \tilde{E}$ with resistance $\tilde{R}_{AB} = R_{AB}$. This can be simplified by a translation of stoichiometric coefficients to

$$\tilde{A} \overset{AB}{\rightleftharpoons} \tilde{B}, \qquad\qquad \tilde{R}'_{AB} = R_{AB}/\tilde{\xi}_E. \tag{6.3}$$

The reduced model contains the pool compounds $\tilde{A}$, $\tilde{B}$ and $\tilde{E}$ but not the free species A, B, E, EA and EB. In fact, the consumption rate of free A is not equal to the production rate of free B ($-\dot{c}_A \neq \dot{c}_B \neq J_{AB}$) and thus there exists no rate law $J(c_A, c_B)$ for the reaction of the free species $A \to B$. The formulation of a kinetic law for the reaction of the pool compounds $\tilde{A} \to \tilde{B}$ is possible, since $-\tilde{c}_A = \tilde{c}_B = J_{AB}$. However, although the TKM expression $J_{AB}(\tilde{\xi}_A, \tilde{\xi}_B, \tilde{\xi}_E) = R_{AB}^{-1}\, \tilde{\xi}_E\, (\tilde{\xi}_A - \tilde{\xi}_B)$ is simple, the corresponding concentration dependent rate law $J_{AB}(\tilde{c}_A, \tilde{c}_B, \tilde{c}_E)$ is very complex. To derive an expression for $J_{AB}(\tilde{c}_A, \tilde{c}_B, \tilde{c}_E)$, the equations $\tilde{c}_i = \tilde{C}_i(\xi_A, \xi_B, \xi_E)\, \tilde{\xi}_i$ with $i \in \{A, B, C\}$ need to be solved for the thermokinetic potentials $\tilde{\xi}_i$. Because these are three coupled, nonlinear equations, the resulting expression is too complex to be shown here. Further, the resulting rate law $J_{AB}(\tilde{c}_A, \tilde{c}_B, \tilde{c}_E)$ is not a generalized mass-action law. The equilibrium constant for the pool compounds $K_{eq} = \tilde{c}_{B,eq}/\tilde{c}_{A,eq} = \tilde{C}_B/\tilde{C}_A$ depends on the total enzyme concentration $\tilde{c}_E$ and the overall amount $\tilde{c}_{AB} = \tilde{c}_A + \tilde{c}_B$. For $\tilde{c}_E \ll \tilde{c}_{AB}$, i. e. small $\tilde{\xi}_E$, the equilibrium constant is $K_{eq} = C_B/C_A$, whereas for large $\tilde{c}_E \gg \tilde{c}_{AB}$, i. e. large $\tilde{\xi}_E$, the equilibrium constant is $K_{eq} = C_{EB}/C_{EA}$. Thus, the rate J_{AB} can be described by a simple TKM model but the rate law $J_{AB}(\tilde{c}_A, \tilde{c}_B, \tilde{c}_E)$ is very complex.

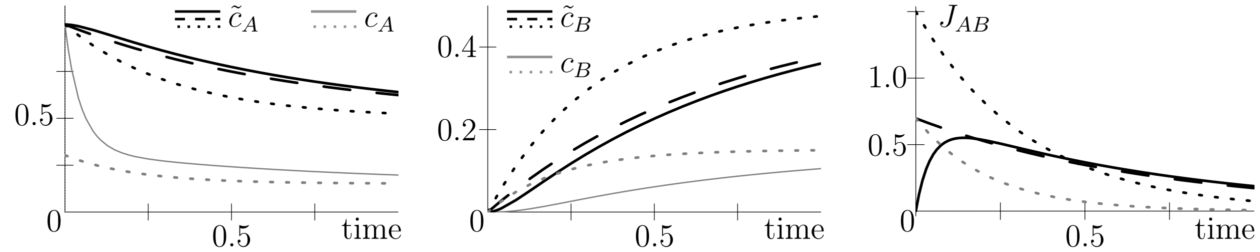


Figure 6.2.: Comparison of several models of an enzyme catalyzed reaction: (1) detailed model (solid); (2) reduced TKM (dashed); black lines for (1) and (2) show the total concentrations of A and B, whereas gray lines show the concentrations of free A and B); (3a) reversible Michaelis-Menten for total concentrations (dotted, black); (3b) reversible Michaelis-Menten for free concentrations (dotted, gray).

Under the additional assumption that the amount of enzyme-bound A and B is small compared to the total concentrations of A and B, we can further approximate $\tilde{c}_{A/B} \approx c_{A/B}$ and $\tilde{C}_{A/B} \approx C_{A/B}$. With this simplification, we can easily compute a concentration dependent rate law: $J_{AB} = R_{AB}^{-1}\, \tilde{c}_E \cdot (C_A^{-1}\, c_A - C_B^{-1}\, c_B)/(C_E + C_{EA}\, C_A^{-1}\, c_A + C_{EB}\, C_B^{-1}\, c_B)$. This is the classical reversible Michaelis-Menten rate law. However, in *in vivo* situations this last assumption is not always fulfilled.

Figure 6.2 compares simulation results of three different models: (1) the original, unreduced system in Equation 6.2, (2) the reduced TKM system in Equation 6.3, which is based on the rapid equilibrium assumption, and (3) the classical Michaelis-Menten approach, which is additionally based on the quasi-steady state of the enzyme complexes. We choose the capacities $C_i = 1$ for $i \in \{A, B, E, EA, EB\}$ and initial conditions $c_{EA,0} = c_{EB,0} = 0$ $\tilde{c}_{A,0} = 1$ and $\tilde{c}_{B,0} = 0$. The resistance of the actual conversion $R_{AB} = 1$ exceeds the resistances of the the binding processes $R_A = R_B = 0.2$ by a factor of five. The Michaelis-Menten approach (3) does not distinguish between the free and the total concentration of A. For the comparison, we assume that it either describes (3a) the concentrations of the pool compounds $c_{\mathrm{MM},A} \approx \tilde{c}_A$ or (3b) the concentration of free compounds $c_{\mathrm{MM},A} \approx c_A$ where $c_{\mathrm{MM},A}$ is the concentration of A in the Michaelis-Menten model. In the case (3a) the initial condition is $c_{\mathrm{MM},A,0} = \tilde{c}_{A,0}$. In the case (3b) the initial condition is computed to be consistent with the rapid equilibrium assumption: $c_{\mathrm{MM},A,0}\, c_{E,0}\, c_{EA,0}^{-1} = C_{A,0}\, C_{E,0}\, C_{EA,0}^{-1}$, $c_{\mathrm{MM},A,0} + c_{EA,0} = \tilde{c}_{A,0}$, $c_{E,0} + c_{EA,0} + c_{EB,0} = \tilde{c}_{E,0}$.

For low enzyme concentrations (e. g. $\tilde{c}_{E,0} = 0.1$) the approximative models (2), (3a) and (3b) are close to the detailed model (1) (data not shown), but for high enzyme concentrations ($c_{E,0} = 3$) the TKM model (2) given by Equation 6.3 is much closer to the detailed model (1) than the Michaelis-Menten model (3). While, the fluxes in the original model (1) and the reduced TKM model (2) are almost indistinguishable after a short induction period, both versions of the Michaelis-Menten model (3a) and (3b) show considerable deviations. These deviations of the Michaelis-Menten approach from the detailed model will be even higher if A, B and E participate in further reactions and exist in complexes with different enzymes (not shown). In this case, the deviations of free and total concentrations are greater than for a single reaction. In the specific simulation shown here, a better approximation of the original model (1) by the Michaelis-Menten model (3) can be achieved by fitting the parameters of the Michaelis-Menten rate law. However, because the Michaelis-Menten law gives the wrong functional dependencies of the reaction rate on the concentrations, it is not applicable if one aims for predictive models that need to describe a variety of cases.

This excursus showed that the TKM approach allows formulating a simple kinetic law for an enzymatic reaction assuming rapid equilibrium of the binding processes, but not an excess of substrate. The corresponding concentration dependent rate law is very complex and for this reason can hardly be used in kinetic models of larger reaction networks. The TKM model uses total concentrations of reactants and products instead of the free concentrations. This is more appropriate, since these are also the usual experimental readouts. The capacities of such pool concentrations are non-constant and consist of terms for the free and bound forms. For more complex reaction systems, e. g. if A, B and E participate in further reactions, these expressions are readily adapted. This demonstrates that the use of TKM does not only guarantee the thermodynamic feasibility of models but also extends the scope of kinetic modeling.

6.8. Conclusions

The thermodynamic modeling formalism for the modeling of reaction networks is directly based on concepts and quantities from irreversible thermodynamics (Chapter 3, p. 39). Chapter 4 (p. 51) systematically collected a variety of model reduction methods for the thermodynamic modeling formalism. However, the thermodynamic formalism leads to very complex equations and for this reason can hardly be used for realistic systems far from equilibrium. The Thermokinetic Modeling (TKM) formalism is based on the thermodynamic modeling formalism (Chapter 5, p. 89). Except for some minor restrictions, it is as powerful as the thermodynamic formalism but the thermokinetic model equations are much simpler than the thermodynamic model equations. Section 5.5 (p. 105) introduced several reduction methods for TK models that are based on matrix operations. However, one would also like to have modular procedures that can be easily applied to single reaction equations or reaction rules. In this chapter, we demonstrated the use of TKM to manipulate and reduce a list of reaction equations and their parameters, without explicitly formulating the system matrices or the model equations.

The reduction of the conservation relations and of the boundary conditions cannot be easily performed at the reaction equation level, since they rely on systemic rather than local properties of a model. The reduction of the stoichiometric cycles and of the kinetic submodel lead in general to a non-diagonal resistance matrix and thus can only be performed in special, but important, cases. The reduction of the kinetic submodel, which proved to be the most powerful method, is easily applicable at the reaction equation level. The proposed step-by-step procedure (§6.15, p. 117) leads to a natural pooling scheme where the reduced concentrations often correspond to meaningful and measurable sums of concentrations. The thermokinetic capacities of the pooled compounds are non-constant.

Using non-constant capacities, we can describe kinetic laws that cannot be described by generalized mass-action kinetics. Such kinetic laws appear under natural assumptions if the enzyme and substrate concentrations are in the same order of magnitude. These kinetic laws are very complex in a concentration-dependent notation but simple in the TKM notation. The use of the TKM formalism therefore extends the scope of kinetic modeling.

The step-by-step procedure (§6.15, p. 117) can be easily applied to combinatorial systems that are described by reaction rules without expanding the rules to a complete, detailed model. This is advantageous because it allows for the computationally cheap reduction of large combinatorial networks. The reduction method can also be modified for the reduction of uncontrollable and unobservable modes. Conzelmann et al. [27, 28] showed that the latter is a valuable tool for the reduction of combinatorial networks. Thus, this modification may provide a link between the TKM approach and the approach of Conzelmann.

The methods introduced in this chapter can be used for the model reduction by ‘paper and pencil’ because the single steps are simple and can be applied to single reaction equations. The next chapter introduces a graphical representation of TK models and discusses graphical methods to perform the reduction. This further stresses the modular nature of the reduction methods.

7. Graphical Representation of TK Models

The TKM formalism is based on potentials and fluxes. The parameters are capacities and resistances. Thus, it is possible to use a graphical representation of reaction systems that is analog to the graphical representations of electrical systems. This chapter develops such a graphical representation. A preliminary version of the graphical representation was sketched in Ederer and Gilles [31, 33]. This chapter further shows how model reduction can be performed by manipulating the graphical representation. It also compares TKM to different aspects of the modeling of electrical networks.

7.1. Thermokinetic Components

§ 7.1 (Basic Components)**.** The basic components for the graphical representation of TK models are shown in Figure 7.1. Capacitors and resistors are known from electrical engineering and their behavior is described by analog equations. For TK models we need a further graphical element that describes the stoichiometry of reactions. We call this new element *stoichior*. Whereas ideal capacitors and resistors show linear behavior, the stoichior is an intrinsically non-linear element.

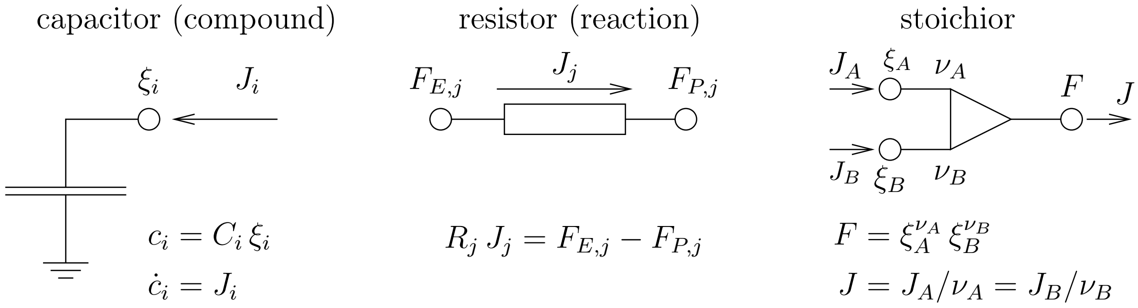


Figure 7.1.: Basic network elements of thermokinetic modeling. The following symbols are used: concentration c_i, thermokinetic potential ξ_i, capacity C_i, flux J_j, thermokinetic force F_j, thermokinetic force exerted by reactants/products $F_{E/P,j}$, resistance R_j, stoichiometric coefficients ν_i. For the sake of simplicity we will suppress stoichiometric coefficients of unity at stoichiors. Stoichiors can have more or less than three terminals. Open circles indicate terminals for the connection of different elements.

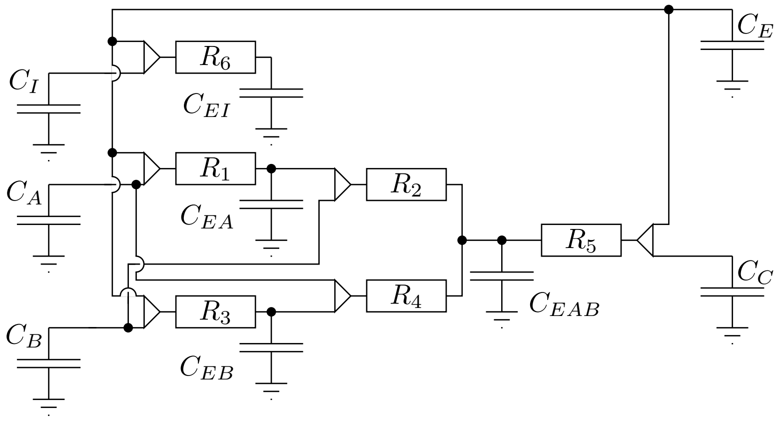


Figure 7.2.: Graphical representation of the example network in Equation 7.1 (enzyme catalyzed reaction with competitive inhibitor).

Example 7.2 (Enzymatic reaction)**.** We consider a simple example of a competitively inhibited enzymatic reaction:

$$\begin{aligned} A + E &\overset{1}{\rightleftharpoons} EA, & EA + B &\overset{2}{\rightleftharpoons} EAB, \\ B + E &\overset{3}{\rightleftharpoons} EB, & EB + A &\overset{4}{\rightleftharpoons} EAB, \\ EAB &\overset{5}{\rightleftharpoons} C + E, & I + E &\overset{6}{\rightleftharpoons} EI. \end{aligned} \tag{7.1}$$

The enzyme E catalyzes the reaction $A + B \rightleftharpoons C$ by a random-order mechanism. The inhibitor I may bind to free E, block it and thus inhibit catalysis. With the elements introduced above, we can give the graphical representation of this system shown in Figure 7.2.

Example 7.3 (PTS)**.** Figure 7.3 shows a graphical representation of the PTS model from Example 5.20 (p. 94). The thermokinetic potentials ξ_{PEP}, ξ_{Pyr}, ξ_{Glc} and $\xi_{Glc\cdot P}$ are clamped.

§ 7.4 (Kirchhoff's junction law)**.** Kirchhoff's junction law postulates that at each storage-free junction in a network, the sum of the incoming fluxes minus the sum of the outgoing fluxes is zero. This is a direct consequence of charge and mass conservation and thus also valid in TK models. In Figure 7.2 and Figure 7.3 the junctions are indicated by bold dots.

§ 7.5 (Kirchhoff's loop law.)**.** Kirchhoff's loop law in electrical systems states that the sum of potential differences along each cyclic path is zero. Analogously, the sum of $\Delta\mu_j$ around a stoichiometric cycle sums up to zero [81]. Let J_c be a vector describing a cyclic flux distribution and $\Delta\mu$ the vector of negative Gibbs reaction energies, then $\Delta\mu^T J_c = 0$. The example network in Equation 7.1 and Figure 7.2 contains the loop $E + A + B \overset{1}{\rightleftharpoons} EA + B \overset{2}{\rightleftharpoons} EAB \overset{4}{\leftrightharpoons} A + EB \overset{3}{\leftrightharpoons} E + A + B$. Thus, a cyclic flux vector is given by $J_c = (1, 1, -1, -1, 0, 0)^T$ and it is

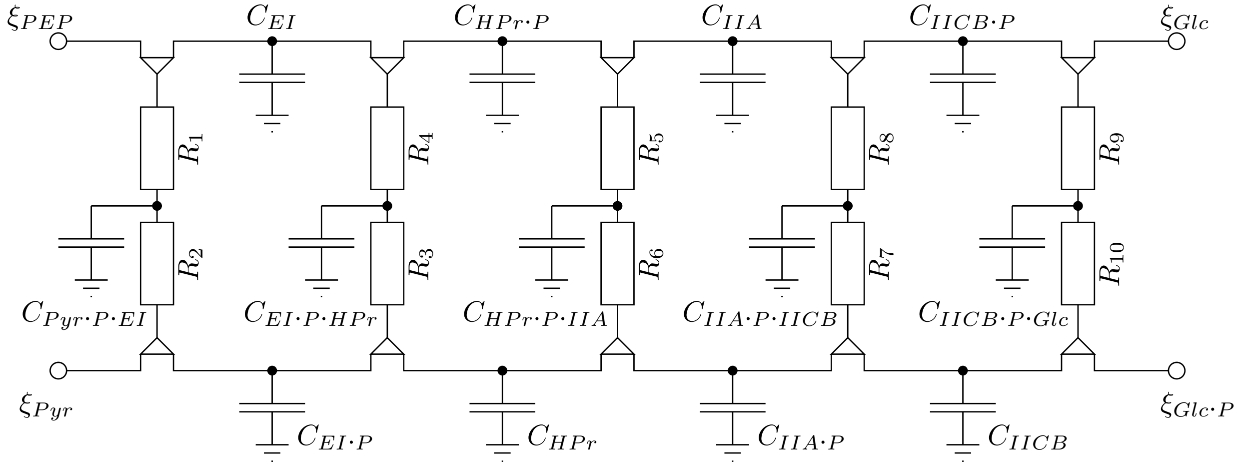


Figure 7.3.: Graphical representation of the PTS (see Example 7.3).

$\Delta\mu_1 + \Delta\mu_2 - \Delta\mu_4 - \Delta\mu_3 = 0$. Using Definition 5.8 and §5.9 (p. 90), Kirchhoff's loop law can be expressed by

$$\prod_j \left(\frac{F_{E,j}}{F_{P,j}}\right)^{J_{c,j}} = 1. \tag{7.2}$$

In the example network (Figure 7.2, Equation 7.1), we have that

$$F_{E,1}/F_{P,1} \cdot F_{E,2}/F_{P,2} \cdot F_{P,4}/F_{E,4} \cdot F_{P,3}/F_{E,3} = 1.$$

Due to the presence of stoichiors, the identification of loops in the graphical representation of a TK model is more difficult than in electrical networks. The identification of the loops is not necessary for modeling and simulation. because the use of the TKM formalism guarantees the fulfillment of Kirchhoff's loop law for all loops.

§ 7.6 (Degenerated components)**.** As for electrical networks, the graphical representation simplifies if the parameters take certain extreme values. The simplification rules in Figure 7.4 follow directly from the definition of the respective elements.

§ 7.7 (Operations with stoichiors)**.** Connections of stoichiors can be simplified to a single stoichior as shown in Figure 7.5. These rules follow directly from the equations of a stoichior.

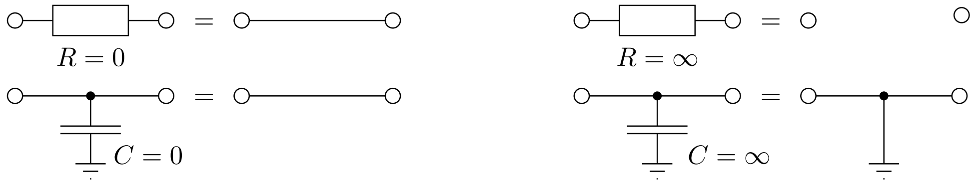


Figure 7.4.: Network simplifications for extreme values of capacities and resistances.

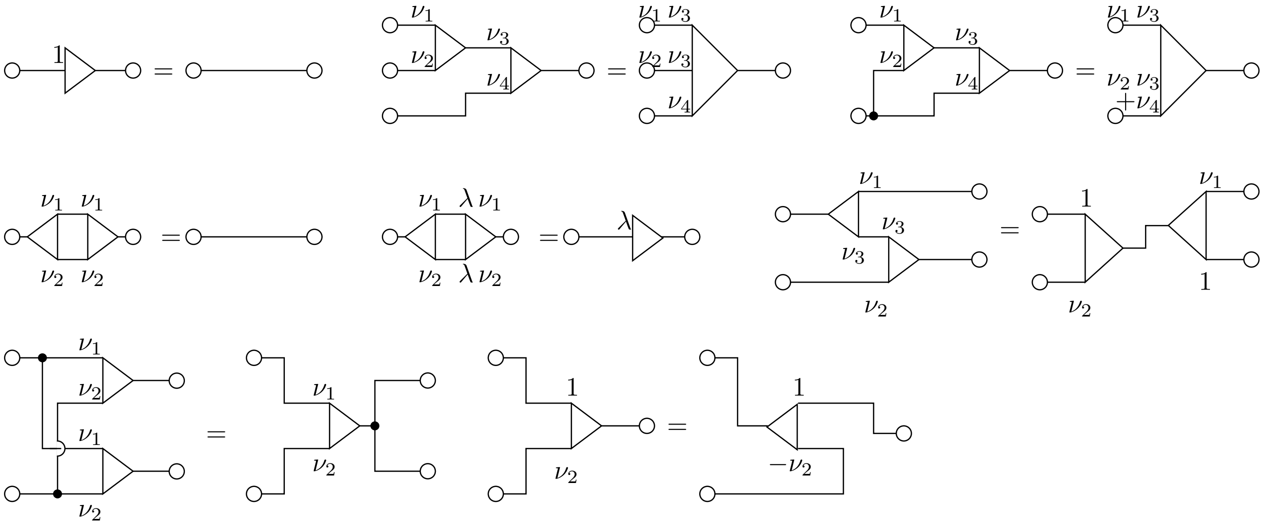


Figure 7.5.: Equivalence between connections of stoichiors. The rules are shown for stoichiors with two or three terminals but are readily extended to stoichiors with more or less terminals.

§ 7.8 (Translation of the stoichiometric coefficients)**.** A translation of the stoichiometric coefficients is sometimes useful to remove negative stoichiometric coefficients that may occur during model reduction. Figure 7.6 gives the graphical rule for a translation of the stoichiometric coefficients. More general cases can be brought to this form using the rules from Figure 7.5.

7.2. Model Reduction

Based on the results developed in Chapter 6, simple but powerful rules for the reduction of a graphical network representation can be formulated.

7.2.1. Parallel and Serial Reactions

The basic rules for the series and parallel connections of resistances in electrical networks are also valid in TKM. This was shown in Corollary 6.7 (p. 113) and Corollary 6.9 (p. 114), respectively. Partially serial reactions can be treated in a similar way (see Corollary 6.12, p. 114). The graphical simplification rules for parallel, serial and partially serial reactions are shown in Figure 7.7.

7.2.2. Vanishing Resistances

Resistors with a resistance of zero can be replaced by a simple connection (see Figure 7.4). The resulting model equations contain algebraic equations that can be used to reduce the

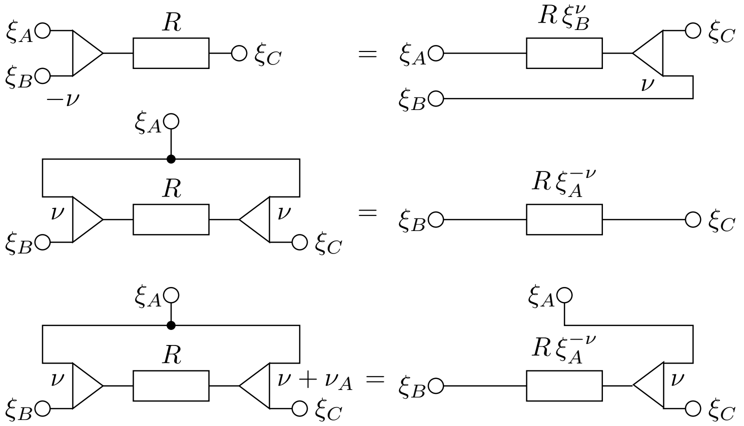


Figure 7.6.: Translation of the stoichiometric coefficients. The rules are shown for stoichiors with three terminals but are readily extended to stoichiors with more or less terminals.

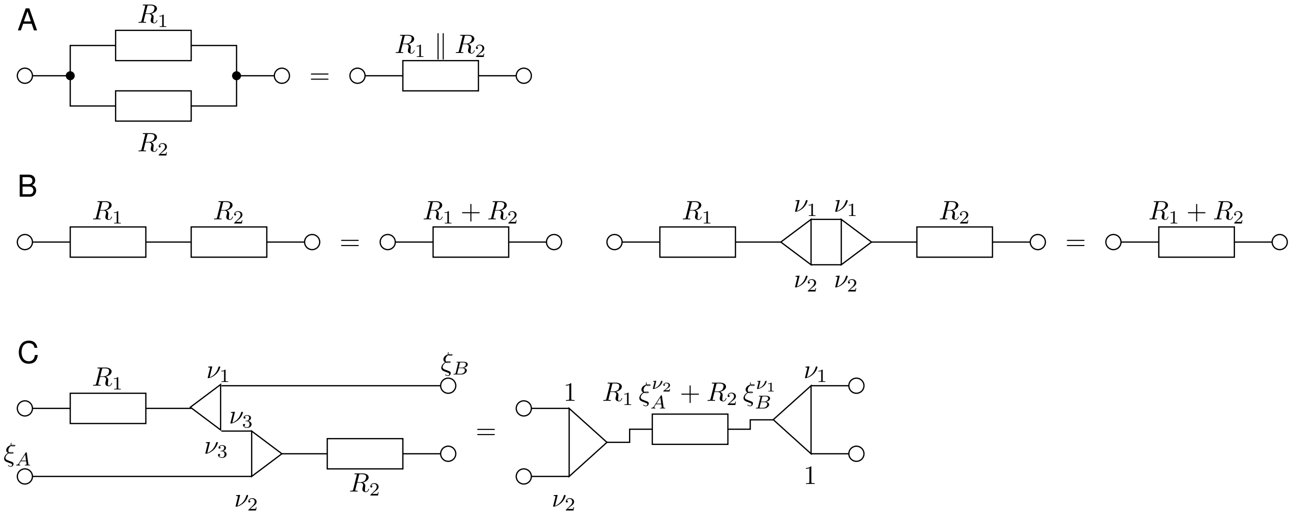
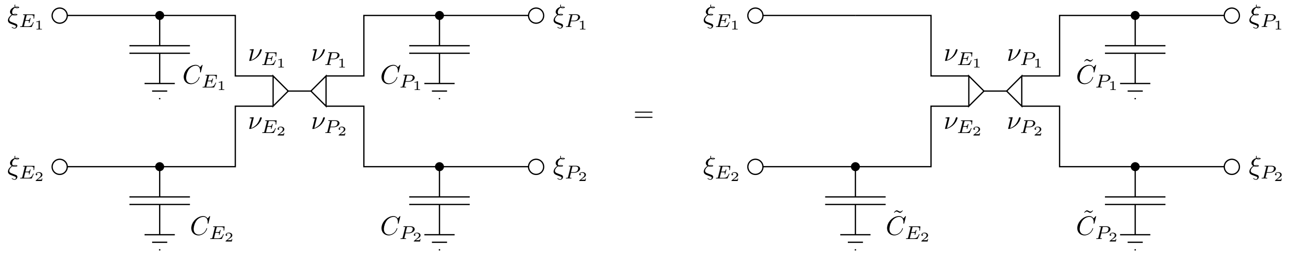


Figure 7.7.: Parallel (A), serial (B) and partially serial (C) reactions can be simplified into a single reaction.



$$\tilde{C}_{E_2} = C_{E_2} - \frac{\nu_{E_2}}{\nu_{E_1}} \frac{\xi_{E_1}}{\xi_{E_2}} C_{E_1}, \qquad \tilde{C}_{P_1} = C_{P_1} + \frac{\nu_{P_1}}{\nu_{E_1}} \frac{\xi_{E_1}}{\xi_{P_1}} C_{E_1}, \qquad \tilde{C}_{P_2} = C_{P_2} + \frac{\nu_{P_2}}{\nu_{E_1}} \frac{\xi_{E_1}}{\xi_{P_2}} C_{E_1},$$

$$\tilde{c}_{E_2} = c_{E_2} - \frac{\nu_{E_2}}{\nu_{E_1}} c_{E_1}, \qquad \tilde{c}_{P_1} = c_{P_1} + \frac{\nu_{P_1}}{\nu_{E_1}} c_{E_1}, \qquad \tilde{c}_{P_2} = c_{P_2} + \frac{\nu_{P_2}}{\nu_{E_1}} c_{E_1}$$

with $\xi_{E_1} = \xi_{P_1}^{\nu_{P_1}} \xi_{P_2}^{\nu_{P_2}} \xi_{E_2}^{-\nu_{E_2}}$

Figure 7.8.: Rapid equilibrium assumption: the network on the left with four capacities is equivalent to the network on the right with three capacities. Cases with more than two reactants and products can be treated analogously.

model. Section 6.3.2 (p. 115) introduced a method for the reduction of systems with vanishing resistances. The reduction method is based on a suited pooling of concentrations and removes implicit algebraic equations.

This method can be directly applied to a graphical representation. Figure 7.8 gives a generally applicable simplification rule for fast reactions. Additionally, Figure 7.9 shows two important special cases, namely a reduction of a fast reaction $E \rightleftharpoons P$ and of a reaction $E_1 + E_2 \rightleftharpoons P$. These rules follow directly from the application of the step-by-step procedure in §6.15 (p. 117).

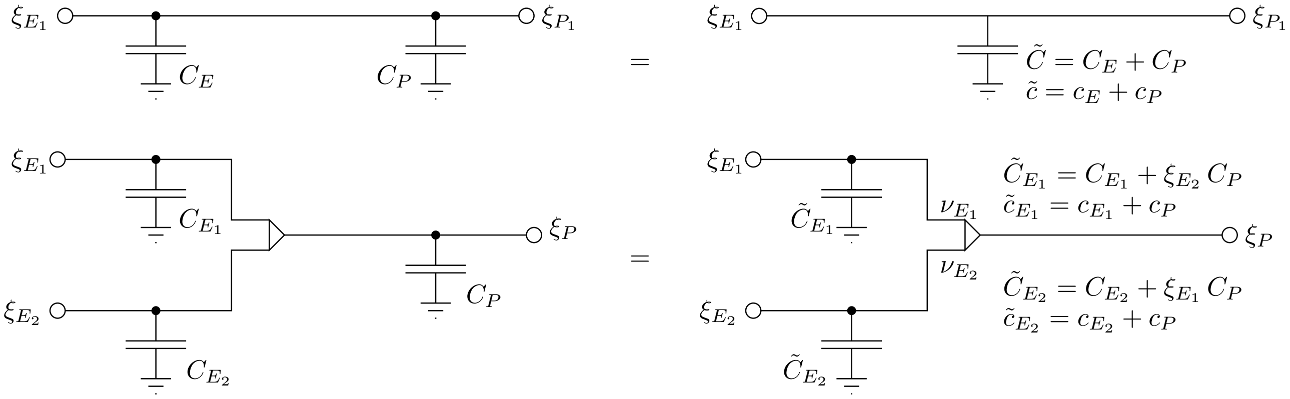
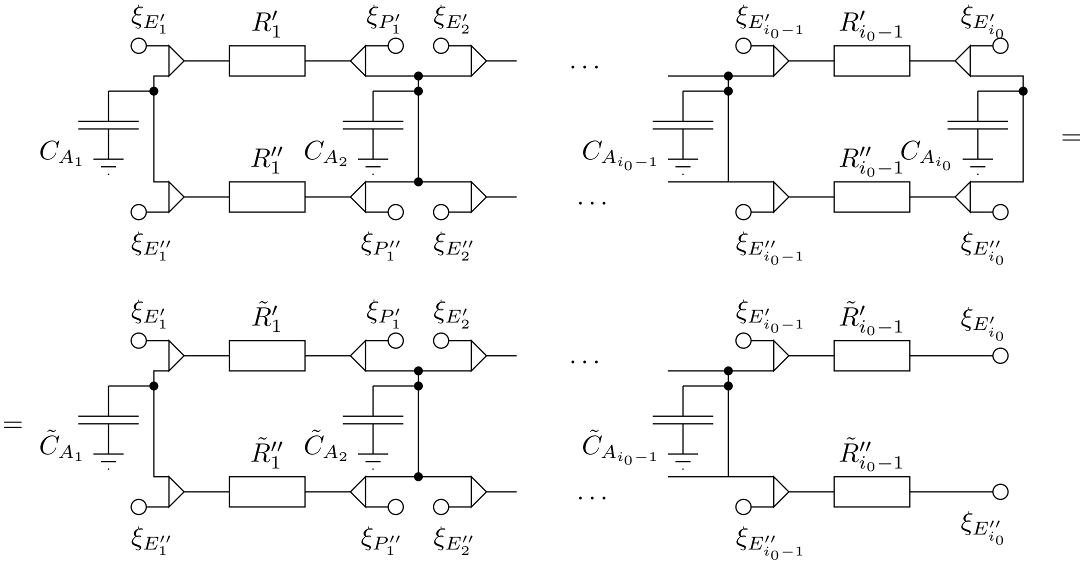


Figure 7.9.: Reduction of a fast reaction for two important special cases.



$$\begin{aligned}
\tilde C_{A_i} &= \frac{c_0}{C_0}\, C_{A_i}, & \tilde c_{A_i} &= c_{A_i}, & \tilde\xi_{A_i} &= \frac{C_0}{c_0}\, \xi_{A,i}, \\
\tilde R_i' &= \frac{C_0}{c_0}\, R_i', & \tilde J_i' &= J_i', & \tilde F_i' &= F_i'/\xi_{A_{i+1}}, \\
\tilde R_i'' &= \frac{C_0}{c_0}\, R_i'', & \tilde J_i'' &= J_i'', & \tilde F_i'' &= F_i''/\xi_{A_{i+1}}
\end{aligned}$$

with $i = 1 \ldots (i_0 - 1)$ and

$$c_0 = c_{A_1} + \ldots + c_{A_{i_0}} = \text{const}, \quad C_0 = C_{A_1}\,\tilde\xi_{A_1} + \ldots + C_{A_{i_0-1}}\,\tilde\xi_{A_{i_0-1}} + C_{A_{i_0}}, \quad \xi_{A_{i_0}} = c_0/C_0.$$

Figure 7.10.: Simplification rule for the reduction of conservation relations. The simplification rule is shown for a linear reaction chain. Rules for the reduction of branched or circular conservation relations are analog.

7.2.3. Conservation Relations

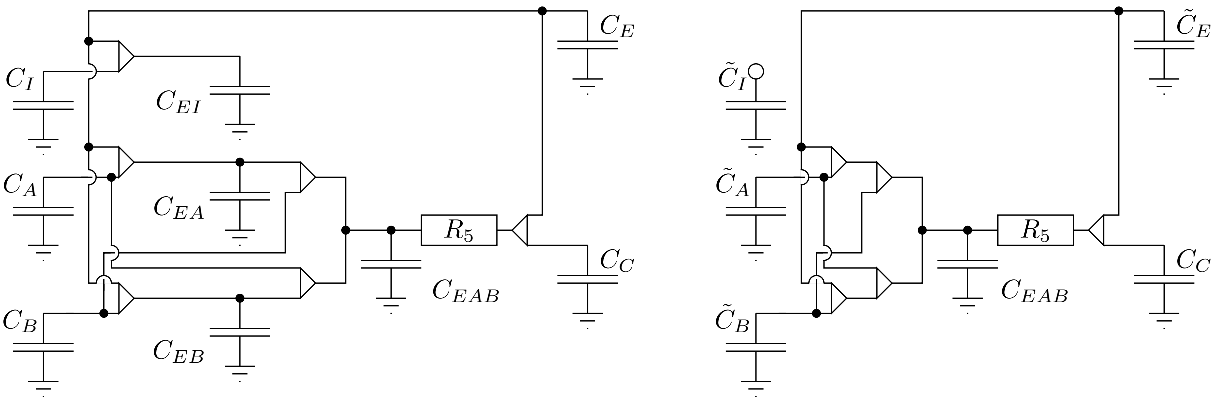
As discussed in Section 5.5.1.1 (p. 105), a reduction of conservation relations involves the solution of a non-linear equation. For this reason one cannot develop a simple and general rule for the reduction of conservation relations.

Figure 7.10 gives a rule for the important special case of a conservation relation where all coefficients are one, i. e. $c_{A_1} + \ldots + c_{A_2} = c_0 = \text{const}$. For example, the conservation relation $c_{ATP} + c_{ADP} + c_{AMP} = \text{const}$ could be reduced by this rule. This case is a generalization of Example 5.41 (p. 106) where only two compounds were considered. It can be derived analogously. If the capacities C_{A_i} depend on the potentials ξ_{A_i}, the application of the rule is not straightforward because then the function $\tilde C_{A_i}(\tilde\xi)$ is only implicitly given by the equations in Figure 7.10. In all other cases the rule can be used directly.

7.2.4. Examples

In order to demonstrate the application of the graphical simplification rules, we consider two examples. First, we reduce the model of the enzymatic reaction with competitive inhibition from Example 7.2 and Figure 7.2. Then, we consider the more complex PTS model from Figure 7.3.

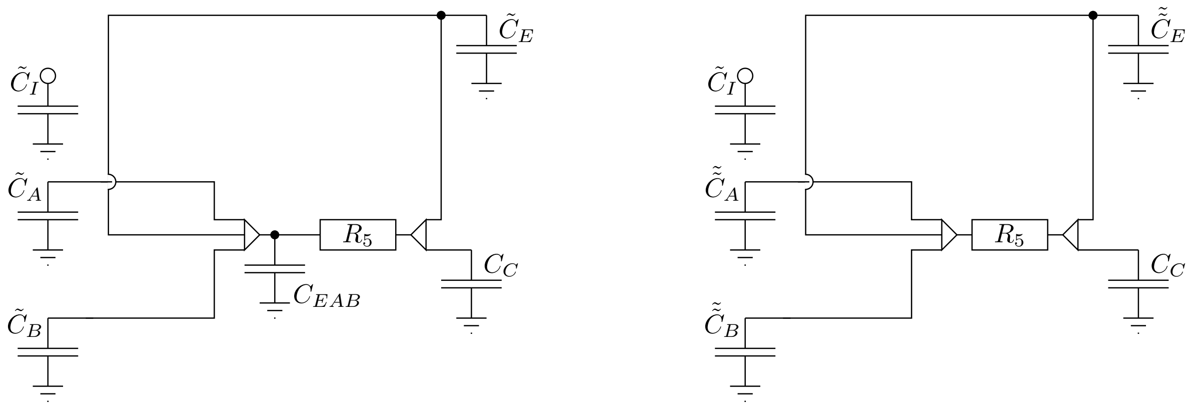
Example 7.9 (Enzymatic reaction)**.** In the network from Example 7.2 (p. 140) and Figure 7.2, we assume that the rate-limiting step is the actual conversion of the products A and B into the reactant C in reaction 5. Accordingly, as the first step towards a reduced model, we set $R_1 = R_2 = R_3 = R_4 = R_6 = 0$ (Figure 7.11 left). After applying the simplification rule from Figure 7.9 to reactions 1, 3 and 6 we get in step 2 the network in Figure 7.11 (right). By applying rules from Figure 7.5 this can be simplified in step 3 to Figure 7.12 (left). In step 4 we apply again the rule from Figure 7.9 and get Figure 7.12 (right). In step 5, a translation of the stoichiometric coefficients (Figure 7.6) finally leads to the reduced model in Figure 7.13. The figure shows that the inhibitor I and the enzyme E do not participate in the overall reaction, but they influence the reaction rate via 'side effects' of their thermokinetic potentials on the capacities $\tilde{C}_A$, $\tilde{C}_B$ and $\tilde{C}_C$. The reduced concentrations $\tilde{\tilde{c}}_A$, $\tilde{\tilde{c}}_B$, $\tilde{\tilde{c}}_E$ and $\tilde{c}_I$ represent the overall concentrations of A, B, E and I, respectively. This result is consistent with the discussion in Section 6.7 (p. 134).



$$\begin{aligned}\tilde{c}_E &= c_E + c_{EI} + c_{EA} + c_{EB},\\ \tilde{c}_I &= c_I + c_{EI},\\ \tilde{c}_A &= c_A + c_{EA},\\ \tilde{c}_B &= c_B + c_{EB},\end{aligned}$$

$$\begin{aligned}\tilde{C}_E &= C_E + \tilde{\xi}_I\, C_{EI} + \tilde{\xi}_A\, C_{EA} + \tilde{\xi}_B\, C_{EB},\\ \tilde{C}_I &= C_I + \tilde{\xi}_E\, C_{EI},\\ \tilde{C}_A &= C_A + \tilde{\xi}_E\, C_{EA},\\ \tilde{C}_B &= C_B + \tilde{\xi}_E\, C_{EB}.\end{aligned}$$

Figure 7.11.: Reduction of the enzymatic reaction model – step 1 and 2.



$$\begin{aligned}\tilde{\tilde{c}}_E &= \tilde{c}_E + \tilde{c}_{EAB}\\ &= c_E + c_{EI} + c_{EA} + c_{EB} + c_{EAB},\end{aligned}$$

$$\begin{aligned}\tilde{\tilde{c}}_A &= \tilde{c}_A + c_{EAB}\\ &= c_A + c_{EA} + c_{EAB},\end{aligned}$$

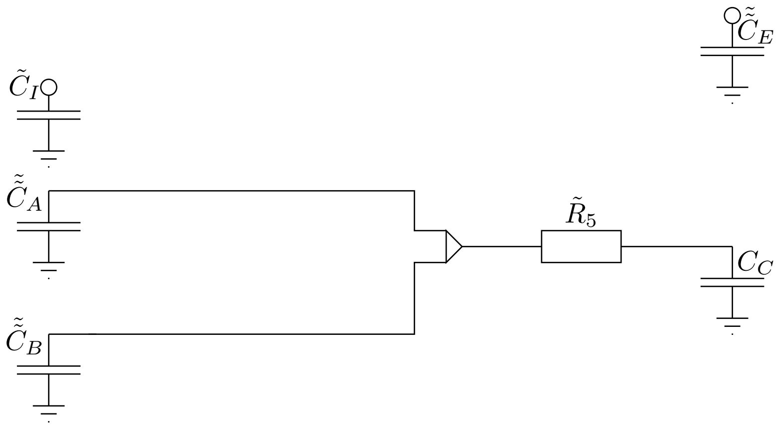
$$\begin{aligned}\tilde{\tilde{c}}_B &= \tilde{c}_B + c_{EAB}\\ &= c_B + c_{EB} + c_{EAB},\end{aligned}$$

$$\begin{aligned}\tilde{\tilde{C}}_E &= \tilde{C}_E + \tilde{\xi}_A\,\tilde{\xi}_B\,C_{EAB},\\ &= C_E + \tilde{\xi}_I\,C_{EI} + \tilde{\xi}_A\,C_{EA} + \tilde{\xi}_B\,C_{EB} + \tilde{\xi}_A\,\tilde{\xi}_B\,C_{EAB},\end{aligned}$$

$$\begin{aligned}\tilde{\tilde{C}}_A &= \tilde{C}_A + \tilde{\tilde{\xi}}_E\,\tilde{\xi}_B\,C_{EAB},\\ &= C_A + \tilde{\tilde{\xi}}_E\,C_{EA} + \tilde{\tilde{\xi}}_E\,\tilde{\xi}_B\,C_{EAB},\end{aligned}$$

$$\begin{aligned}\tilde{\tilde{C}}_B &= \tilde{C}_B + \tilde{\tilde{\xi}}_E\,\tilde{\xi}_A\,C_{EAB}\\ &= C_B + \tilde{\tilde{\xi}}_E\,C_{EB} + \tilde{\tilde{\xi}}_E\,\tilde{\xi}_A.\end{aligned}$$

Figure 7.12.: Reduction of the enzymatic reaction model – step 3 and 4.



$$\tilde{R}_5 = \tilde{\tilde{\xi}}_E^{-1}\,R_5$$

Figure 7.13.: Reduced model of the enzymatic reaction.

Example 7.10 (PTS)**.** In Example 5.20 (p. 94) the thermokinetic resistances and capacities for the PTS model from Rohwer et al. [87] were derived. The graphical representation of this model is given in Figure 7.3.

The model contains 10 resistances and 13 capacities. To reduce the model we approximate the 6 lowest resistances by zero: $R_1 = R_2 = R_5 = R_6 = R_9 = R_{10} = 0$. This leads to the approximated but unreduced model in Figure 7.14 with 4 resistances but still 13 capacities. By applying the rules for the reduction of the kinetic submodel (Figure 7.8), we get the reduced model in Figure 7.15. This model contains only 7 capacities but is not suited for a direct simulation because it contains stoichiors that are directly linked without intermediate capacities or resistances. This means that instead of the removed capacities C_{EI}, $C_{EI \cdot P}$, $C_{HPr \cdot P}$, $C_{IIA \cdot P}$, C_{IICB} and $C_{IICB \cdot P}$, the network in Figure 7.15 contains capacities of value zero. We can apply the rules for the reduction of partially serial reactions from Figure 7.7 to resolve this problem partially. In order to avoid crossing lines, the remaining elements need to be rearranged and one gets Figure 7.16.

By applying the rules for the equivalence of stoichior elements (Figure 7.5), we can completely resolve this problem in the two steps shown in Figure 7.17 and Figure 7.18. This network could be simulated as a TK model. However, the negative stoichiometric coefficients are uncommon and we can get rid of them by a translation of the stoichiometric coefficients (Figure 7.6) and get the reduced model in Figure 7.19. The model contains a conservation relation for each of the four proteins EI, HPr, IIA and $IICB$. Only the conservation relations for EI and $IICB$ in Figure 7.19 have the form required for using the simplification rule in Figure 7.10. After reduction of these two conservation relations we get the reduced model in Figure 7.20.

Figure 7.21 shows simulation results of the original and the reduced model. The respective curves are qualitatively similar but partly deviate quantitatively. The fluxes in the reduced model are overestimated in steady state because some of the resistances that add to the overall resistance of the PTS are set to zero. Thus, by increasing the remaining resistances we can get a much better fit of original and reduced model. In a real modeling work, the parameters of the detailed model are not known, but the reduced parameters are estimated using the measurement data. Then, the adapted resistance values can be directly identified from the measurement values.

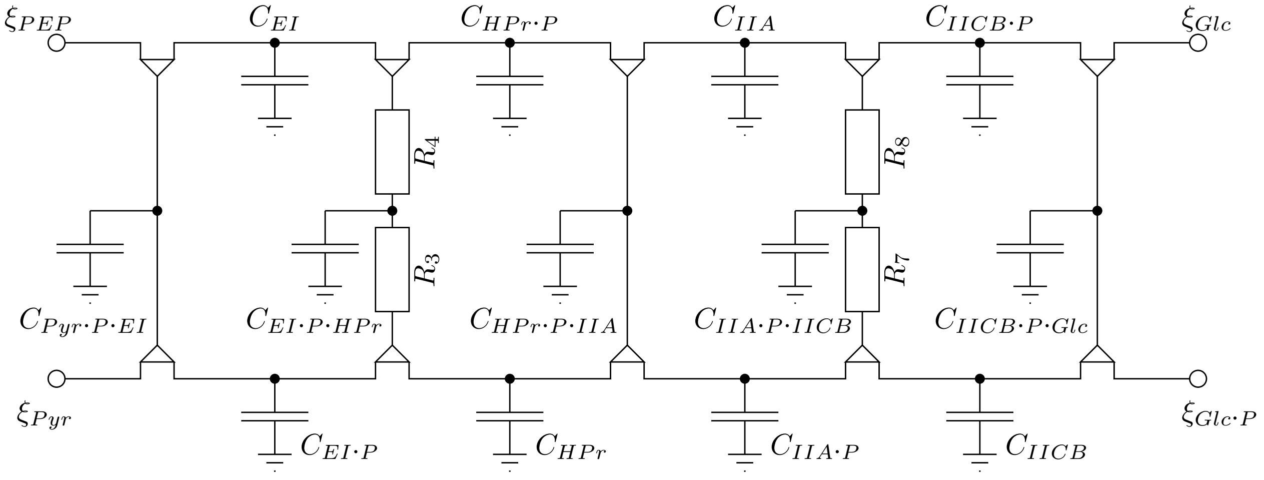
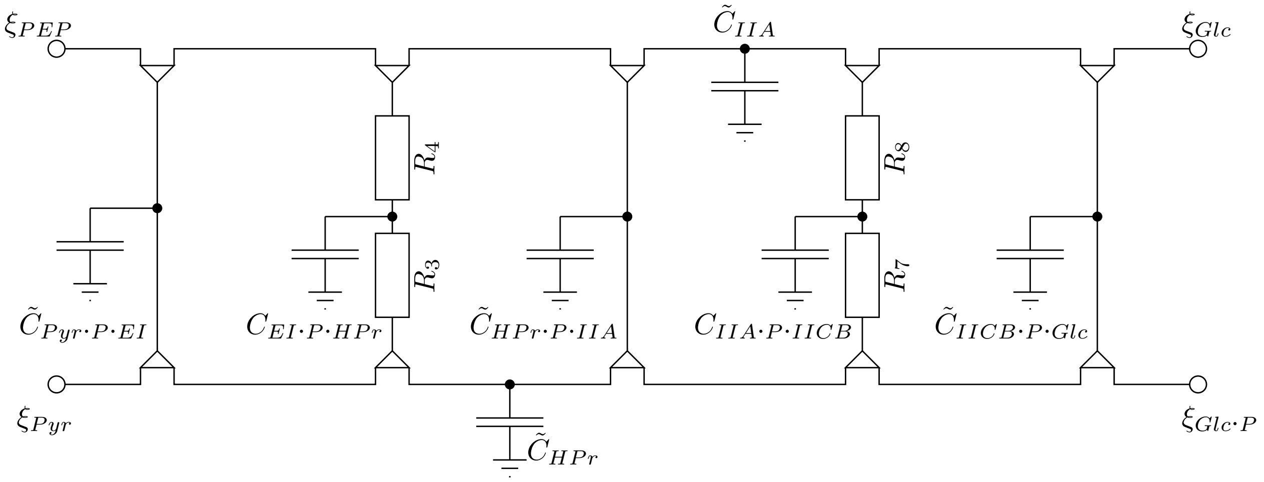
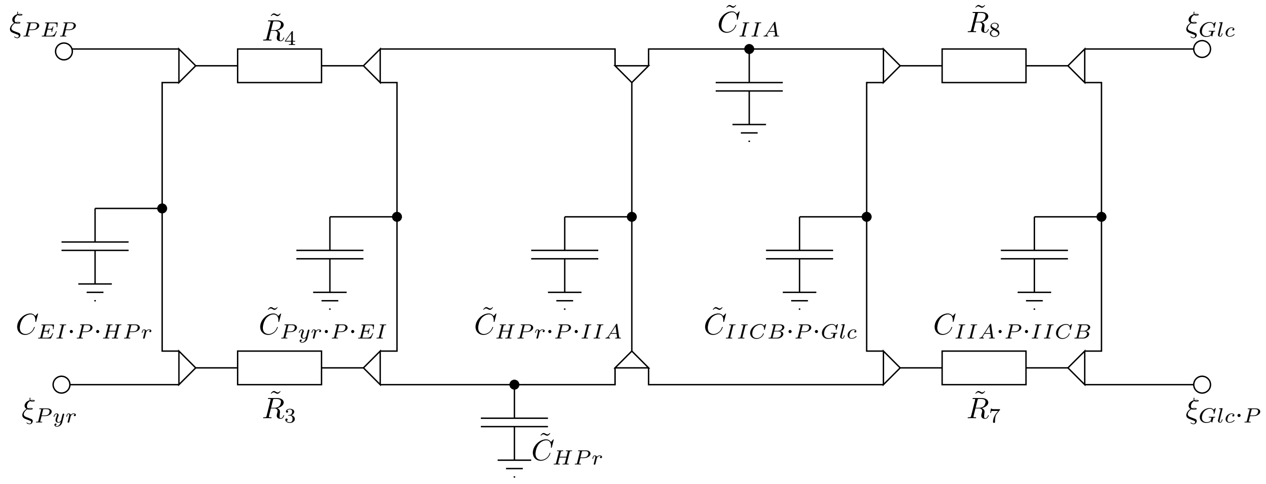


Figure 7.14.: Approximated but unreduced PTS model.



$$\begin{aligned}
\tilde{C}_{Pyr\cdot P\cdot EI} &= C_{Pyr\cdot P\cdot EI} + \xi_{PEP}^{-1}\, C_{EI} + \xi_{Pyr}^{-1}\, C_{EI\cdot P}, & \tilde{c}_{Pyr\cdot P\cdot EI} &= c_{Pyr\cdot P\cdot EI} + c_{EI} + c_{EI\cdot P},\\
\tilde{C}_{HPr\cdot P\cdot IIA} &= C_{HPr\cdot P\cdot IIA} + \tilde{\xi}_{IIA}^{-1}\, C_{HPr\cdot P} + \tilde{\xi}_{HPr}^{-1}\, C_{IIA\cdot P}, & \tilde{c}_{HPr\cdot P\cdot IIA} &= c_{HPr\cdot P\cdot IIA} + c_{HPr\cdot P} + c_{IIA\cdot P},\\
\tilde{C}_{IICB\cdot P\cdot Glc} &= C_{IICB\cdot P\cdot Glc} + \xi_{Glc\cdot P}^{-1}\, C_{IICB} + \xi_{Glc}^{-1}\, C_{IICB\cdot P}, & \tilde{c}_{IICB\cdot P\cdot Glc} &= c_{IICB\cdot P\cdot Glc} + c_{IICB} + c_{IICB\cdot P},\\
\tilde{C}_{IIA} &= C_{IIA} - \xi_{HPr\cdot P\cdot IIA}\, \tilde{\xi}_{IIA}^{-2}\, C_{HPr\cdot P}, & \tilde{c}_{IIA} &= c_{IIA} - c_{HPr\cdot P},\\
\tilde{C}_{HPr} &= C_{HPr} - \xi_{HPr\cdot P\cdot IIA}\, \tilde{\xi}_{HPr}^{-2}\, C_{IIA\cdot P}, & \tilde{c}_{HPr} &= c_{Hpr} - c_{IIA\cdot P}.
\end{aligned}$$

Figure 7.15.: Reduction of the PTS model – step 1.



$$\tilde{R}_3 = \xi_{Pyr}\, R_3, \qquad \tilde{R}_4 = \xi_{PEP}\, R_4, \qquad \tilde{R}_7 = \xi_{Glc\cdot P}\, R_7, \qquad \tilde{R}_8 = \xi_{Glc}\, R_8.$$

Figure 7.16.: Reduction of the PTS model – step 2.

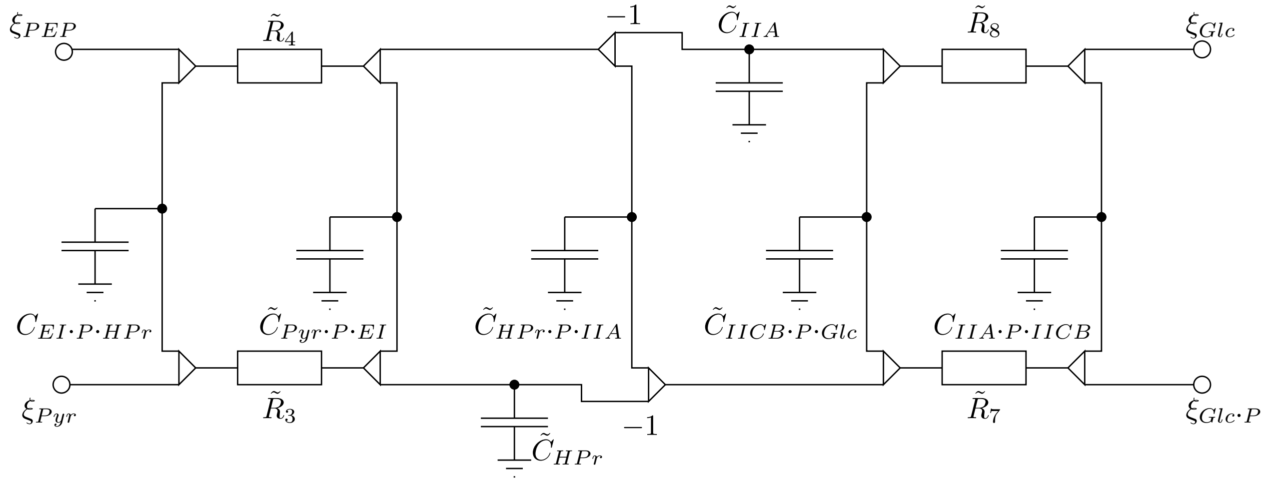


Figure 7.17.: Reduction of the PTS model – step 3.

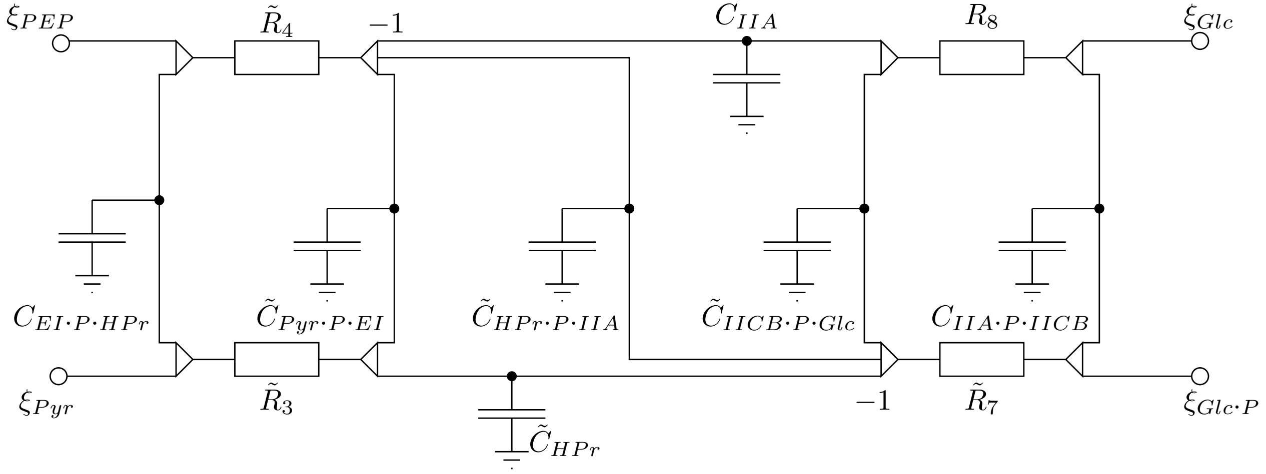
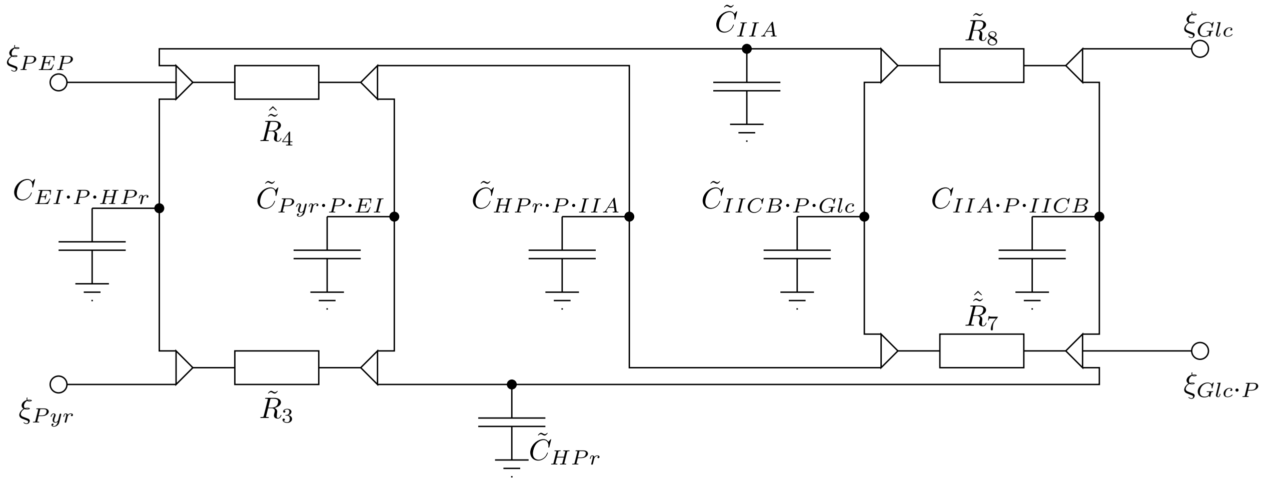
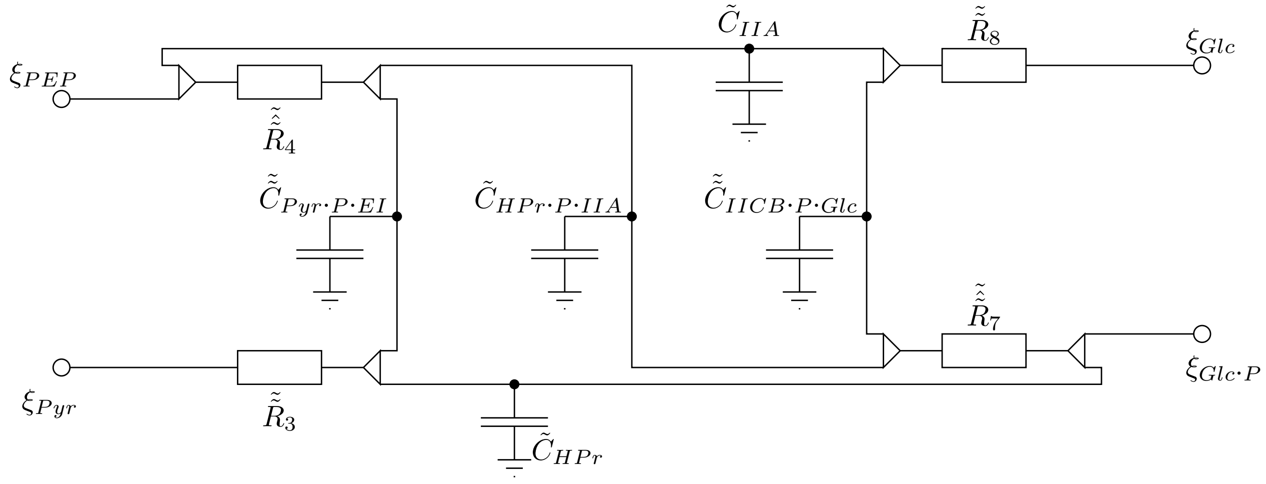


Figure 7.18.: Reduction of the PTS model – step 4.



$$\hat{\tilde{R}}_4 = \tilde{\xi}_{IIA}\,\tilde{R}_4 = \tilde{\xi}_{IIA}\,\xi_{PEP}\,R_4, \qquad \hat{\tilde{R}}_7 = \tilde{\xi}_{HPr}\,\tilde{R}_7 = \tilde{\xi}_{HPr}\,\xi_{Glc \cdot P}\,R_7.$$

Figure 7.19.: Reduction of the PTS model – step 6.



$$\tilde{\xi}_{Pyr\cdot P\cdot EI} = \frac{c_{EI,tot}}{C_{EI,tot}}\,\tilde{\tilde{\xi}}_{Pyr\cdot P\cdot EI}, \qquad \tilde{\xi}_{IICB\cdot P\cdot Glc} = \frac{c_{IICB,tot}}{C_{IICB,tot}}\,\tilde{\tilde{\xi}}_{IICB\cdot P\cdot Glc},$$

$$\begin{aligned}
\tilde{c}_{Pyr\cdot P\cdot EI} &= \tilde{\tilde{c}}_{Pyr\cdot P\cdot EI} = c_{Pyr\cdot P\cdot EI} + c_{EI} + c_{EI\cdot P},\\
\tilde{c}_{IICB\cdot P\cdot Glc} &= \tilde{\tilde{c}}_{IICB\cdot P\cdot Glc} = c_{IICB\cdot P\cdot Glc} + c_{IICB} + c_{IICB\cdot P},
\end{aligned}$$

$$\begin{aligned}
\tilde{\tilde{C}}_{Pyr\cdot P\cdot EI} &= \frac{c_{EI,tot}}{C_{EI,tot}}\,\tilde{C}_{Pyr\cdot P\cdot EI} = \frac{c_{EI,tot}}{C_{EI,tot}}\,(C_{Pyr\cdot P\cdot EI} + \xi_{PEP}^{-1}\,C_{EI} + \xi_{Pyr}^{-1}\,C_{EI\cdot P}),\\
\tilde{\tilde{C}}_{IICB\cdot P\cdot Glc} &= \frac{c_{IICB,tot}}{C_{IICB,tot}}\,\tilde{C}_{IICB\cdot P\cdot Glc} = \frac{c_{IICB,tot}}{C_{IICB,tot}}\,(C_{IICB\cdot P\cdot Glc} + \xi_{Glc\cdot P}^{-1}\,C_{IICB} + \xi_{Glc}^{-1}\,C_{IICB\cdot P}),
\end{aligned}$$

$$\begin{aligned}
\tilde{\tilde{R}}_3 &= \frac{C_{EI,tot}}{c_{EI,tot}}\,\tilde{R}_3 = \frac{C_{EI,tot}}{c_{EI,tot}}\,\xi_{Pyr}\,R_3, &\qquad \tilde{\hat{\tilde{R}}}_4 &= \frac{C_{EI,tot}}{c_{EI,tot}}\,\hat{\tilde{R}}_4 = \frac{C_{EI,tot}}{c_{EI,tot}}\,\tilde{\xi}_{IIA}\,\xi_{PEP}\,R_4,\\
\tilde{\hat{\tilde{R}}}_7 &= \frac{C_{IICB,tot}}{c_{IICB,tot}}\,\hat{\tilde{R}}_7 = \frac{C_{IICB,tot}}{c_{IICB,tot}}\,\tilde{\xi}_{HPr}\,\xi_{Glc\cdot P}\,R_7, &\qquad \tilde{\tilde{R}}_8 &= \frac{C_{IICB,tot}}{c_{IICB,tot}}\,\tilde{R}_8 = \frac{C_{IICB,tot}}{c_{IICB,tot}}\,\xi_{Glc}\,R_8,
\end{aligned}$$

with

$$\begin{aligned}
c_{EI,tot} &= \tilde{c}_{Pyr\cdot P\cdot EI} + c_{EI\cdot P\cdot HPr} = c_{EI} + c_{EI\cdot P} + c_{Pyr\cdot P\cdot EI} + c_{EI\cdot P\cdot HPr},\\
c_{IICB,tot} &= \tilde{c}_{IICB\cdot P\cdot Glc} + c_{IIA\cdot P\cdot IICB} = c_{IICB} + c_{IICB\cdot P} + c_{IICB\cdot P\cdot Glc} + c_{IIA\cdot P\cdot IICB},
\end{aligned}$$

$$\begin{aligned}
C_{EI,tot} &= \tilde{C}_{Pyr\cdot P\cdot EI}\,\tilde{\tilde{\xi}}_{Pyr\cdot P\cdot EI} + C_{EI\cdot P\cdot HPr}\\
&= (C_{Pyr\cdot P\cdot EI} + \xi_{PEP}^{-1}\,C_{EI} + \xi_{Pyr}^{-1}\,C_{EI\cdot P})\,\tilde{\tilde{\xi}}_{Pyr\cdot P\cdot EI} + C_{EI\cdot P\cdot HPr},\\
C_{IICB,tot} &= \tilde{C}_{IICB\cdot P\cdot Glc}\,\tilde{\tilde{\xi}}_{IICB\cdot P\cdot Glc} + C_{IIA\cdot P\cdot IICB}\\
&= (C_{IICB\cdot P\cdot Glc} + \xi_{Glc\cdot P}^{-1}\,C_{IICB} + \xi_{Glc}^{-1}\,C_{IICB\cdot P})\,\tilde{\tilde{\xi}}_{IICB\cdot P\cdot Glc} + C_{IIA\cdot P\cdot IICB}.
\end{aligned}$$

Figure 7.20.: Reduced PTS model.

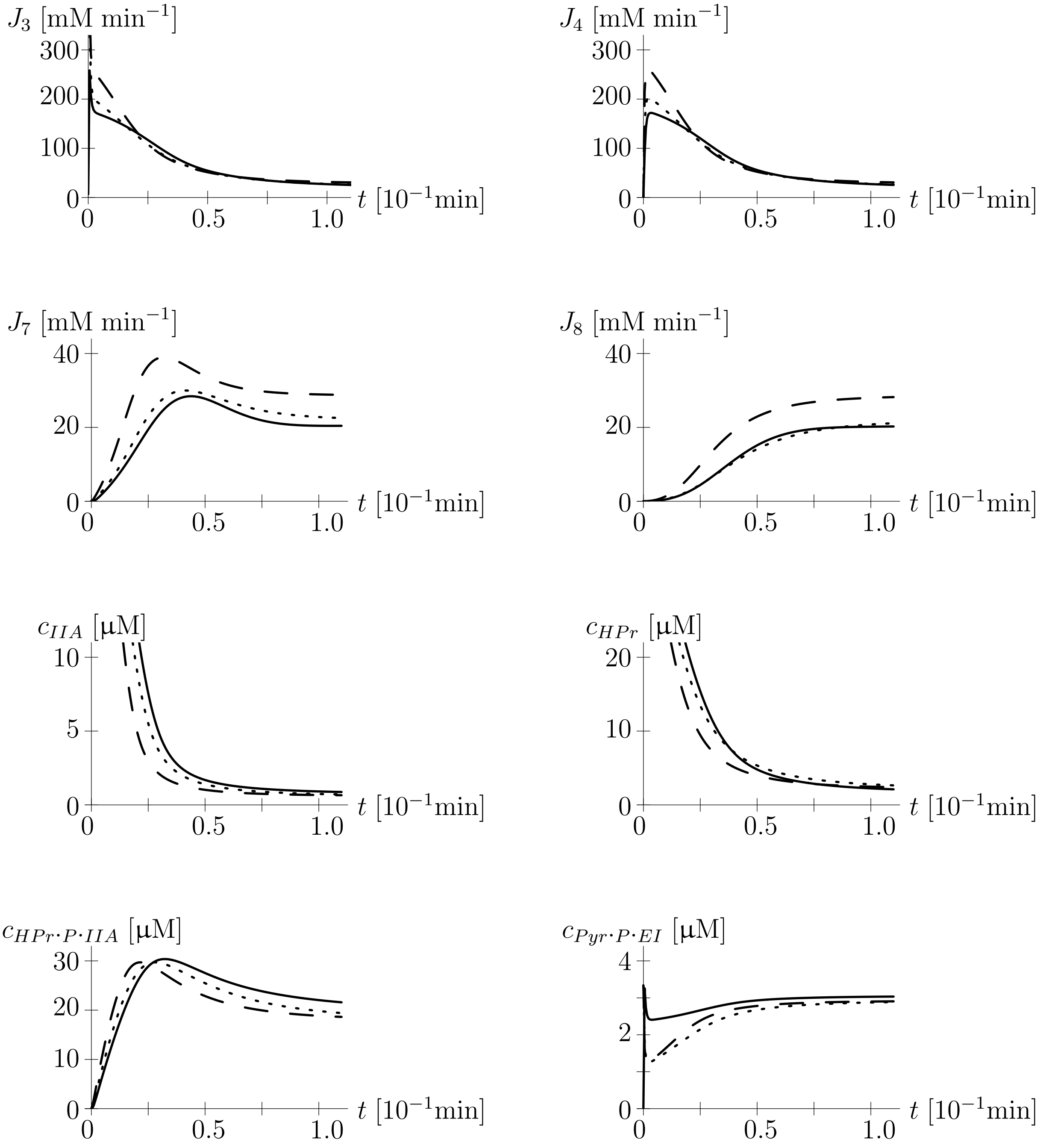


Figure 7.21.: Simulation results with the original (solid) and the reduced (dashed) PTS model. The dotted line shows the reduced model with corrected resistances: $R'_3 = 1.3\,R_3$, $R'_4 = 1.3\,R_4$, $R'_7 = 1.3\,R_7$ and $R'_8 = 1.3\,R_8$. The clamped concentrations are $c_{PEP} = 2800$ µM, $c_{Pyr} = 900$ µM, $c_{Glc} = 500$ µM, $c_{Glc\cdot P} = 50$ µM. The initial conditions of the detailed model are $c_{EI,0} = 5$ µM, $c_{HPr,0} = 50$ µM, $c_{IIA,0} = 40$ µM, $c_{IICB,0} = 15$ µM, $c_{EI\cdot P\cdot HPr,0} = c_{EI\cdot P,0} = c_{HPr\cdot P\cdot IIA,0} = c_{HPr\cdot P,0} = c_{IIA\cdot P\cdot IICB,0} = c_{IIA\cdot P,0} = c_{IICB\cdot P\cdot Glc,0} = c_{IICB\cdot P,0} = c_{Pyr\cdot P\cdot EI,0} = 0.01$ µM. The initial conditions of the reduced model were computed numerically from the initial conditions of the original model.

7.3. Comparison to Electrical Networks

The TK formalism works with potentials, capacities and resistances. In this respect, it is similar to the modeling formalism for electrical networks. The previous section introduced a graphical representation for thermodynamic models that resembles the graphical representation for electrical networks. The following subsections inspect this analogy more closely. They work out common features and differences. In particular, they show the chemical counterparts to some important electrical network elements.

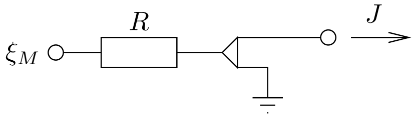
7.3.1. Conceptual Comparison

Thermokinetic potentials and forces are the key variables of the TKM formalism. This section discusses the relation of the thermokinetic and the electrical potentials, i. e the voltages.

§ 7.11 (Basic variables of electric theory)**.** The theory of electrical networks is based on four different quantities: The current I, the voltage U, the charge Q and the magnetic flux Φ. The charge is the integral of the current $dQ = I\,dt$ and the magnetic flux is the integral of the voltage $d\Phi = U\,dt$. Three of these four quantities have a direct analog in thermokinetic networks. Current I and charge Q correspond to flux J and concentration c, respectively. The analog of voltages U and voltage differences ΔU are thermokinetic potentials ξ_i and forces F_j, respectively. The magnetic flux does not have a chemical counterpart because neither the integral of the chemical potential nor of the thermokinetic potential nor of the thermokinetic force has an obvious physical interpretation.

§ 7.12 (Chemical and electrical potential)**.** The Gibbs energy G in a purely electrical network is equal to the electrical energy E. The energy E in an ideal electrical capacitor with capacitance C is given by $G = E = 1/2\, C^{-1}\, Q^2$. Thus, the chemical potential of the electrical charge is equal to the electrical voltage: $\mu_Q = \partial G/\partial Q = C^{-1}\, Q = U$. The chemical potential is a generalization of the electrical potential to electro-chemical systems.

§ 7.13 (Thermokinetic and electrical potential)**.** Many real electrical network elements are linear in dependence on the voltages, but most chemical networks are non-linear in dependence of the chemical potentials μ_i. For this reason, the kinetic modeling of reaction networks with chemical potential leads usually to complex model equations. The use of thermokinetic potentials $\xi_i = \exp(\mu_i/(R^*\, T))$ and forces F_j leads to a considerable simplification of the model equations. Ideal thermokinetic resistances and capacities are constant, whereas ideal thermodynamic resistances and capacities depend on the state of the system. Thus, although the electro-chemical potential μ_i and the thermodynamic force $\Delta\mu$ are the physical generalizations of the electrical potentials and the electrical potential differences, the thermokinetic potentials and the thermokinetic forces are in their formal handling more similar to the electrical counterparts. The most important difference between thermokinetic and electrical potential is that the electrical potentials can be negative, but thermokinetic potentials are always non-negative.



$$R = \rho_M \, \xi_M \, \xi_E^{-1}$$
$$J = R^{-1} \, (\xi_M - 0) = \rho_M^{-1} \, \xi_E$$

Figure 7.22.: TKM realization of a potential-controlled current source.

7.3.2. Sources and Amplifiers

Ideal voltage and current sources are equivalent to clamping of thermokinetic potentials and fluxes, respectively. As an example, the concentration of the energy carrier adenosine-triphosphate ATP is known to be tightly regulated by the cell and thus for many purposes may be modeled as an ideal thermokinetic potential source. Current sources may be realized by reactions with $F_{E,j} \gg F_{P,j}$ and clamped reactants. Then, the products do not significantly influence the reaction rate and the reaction rate is determined only by the reactant potentials.

§ 7.14 (Irreversible reactions and grounding)**.** The grounding of a terminal means that the respective potential is clamped to the value zero (see e. g. Figure 7.22). Because thermokinetic potentials are non-negative, this realizes an irreversible reaction towards the grounded terminal. In most biochemical systems, irreversible reactions are realized by a high clamped potential of a reactant; for example by coupling the reaction to the hydrolysis of ATP. Both possibilities, a grounded product and a clamped reactant with high potential, lead to a reaction that cannot be reversed under physiological conditions. The realization of an irreversible reaction by a grounded product is easier to depict. For this reason, grounded products are used to describe irreversible reactions in the following paragraphs. One has to keep in mind that the same behavior may be realized in a different way.

§ 7.15 (Controlled sources and amplifiers)**.** Potentials or fluxes of *controlled sources* are functions of other potentials or fluxes. Amplifiers are an example for controlled sources. In chemical networks, controlled sources can be realized in different ways. A particularly simple and ubiquitous solution is a irreversible, saturated, catalyzed reaction where the concentration of the enzyme determines the flux. One possibility to model a saturated enzymatic reaction $M \rightleftharpoons A + B$ is by ideal capacities and a resistance of $R = (\rho_0 + \rho_M \, \xi_M + \rho_A \, \xi_A + \rho_B \, \xi_B + \rho_{AB} \, \xi_A \, \xi_B) \, \xi_E^{-1}$ (cf. §5.16, p. 92). If ξ_B is kept on a low level and $\rho_M \, \xi_M$ is the dominant term in the resistance R, one may approximate $\xi_B = 0$ and $R = \rho_M \, \xi_M \, \xi_E^{-1}$. Then the flux $J = R^{-1} \, (\xi_M - \xi_A \, \xi_B) = \rho_M^{-1} \, \xi_E$ depends solely on the potential of the catalyst. Thus, such an enzymatic reaction realizes a potential-controlled current source. The necessary energy is supplied by the difference of the high potential ξ_M and the low potential ξ_B. Figure 7.22 shows a TKM diagram of this potential-controlled current source.

7.3.3. Passive Two-Terminal Components

Electrical network theory uses four basic, passive two-terminal elements: resistor, capacitor, inductor and memristor [25, 99]. Each element relates a pair of the four basic quantities current I, voltage U, charge Q and magnetic flux Φ. In particular, a resistor is characterized by $dU = R\,dI$, a capacitor by $dQ = C\,dU$, a inductor by $d\Phi = L\,dI$ and a memristor by $d\Phi = M\,dQ$. Together with the laws $dQ = I\,dt$ and $d\Phi = U\,dt$, they cover all six possible pairwise relations of the four basic quantities. The parameters R, C, L and M of the total differentials can depend on the independent variables I, U, I and Q, of the differentials above. In the following we will discuss the four elements and their thermokinetic analog in detail.

7.3.3.1. Resistor and Memristor

An electrical resistor is characterized by the law $dU = R\,dI$ with the differential resistance R. In the ideal case with $R = \text{const}$, we get $U = R\,I$. A memristor is characterized by $d\Phi = M\,dQ$ or equivalently $\dot{\Phi} = M\,\dot{Q}$ and thus $U = M\,I$. Thus, in the ideal case with $R, M = \text{const}$ resistors and memristors behave equally. In the non-ideal case, the resistance R depends on the current or the voltage, but the memristance M depends on the charge Q or in a generalized approach on an arbitrary storage variable. This means that the memristor is a resistor with a memory. Although the introduction of the memristor was suggested already in 1971 by Chua [25], implementations of electrical memristors without active elements were only recently experimentally demonstrated and afford nano-scale structures [99].

A thermokinetic resistor is characterized by $F = R\,J$. A thermokinetic resistor is able to show resistive and memristive behavior because R may depend on the potentials of reactants and products or arbitrary other storage variables of the system.

7.3.3.2. Capacitor

An electrical capacitor is characterized by the law $dQ = C\,dU$ where C is the differential capacitance and U is the voltage difference at the terminals of the capacitor. In the ideal case $C = \text{const}$, we get $Q = C\,U$. In the thermokinetic formalism, a compound is characterized by $c = C\,\xi$ and thus is analog to a grounded electrical capacitor. The behavior of ungrounded capacitors can be simulated by appropriate thermokinetic networks. Figure 7.23 (left) shows a circuit with capacitor-like dynamics. The underlying network consists of a complex formation $A + X \rightleftharpoons B$. Complex formation is a common motif in signal transduction. The compound X mimics the effect of the electric field in an electric capacitor. Upon excitation, it builds up a force that balances the original force and stops the flux. For $R \to 0$ and thus $\xi_A\,\xi_X = \xi_B$, the dynamics approaches $J = -C_X\,\dot{\xi}_X = -C_x\,d(\xi_B/\xi_A)/dt$. This is an ideal behavior because $J \neq 0$ only for non-constant ξ_B/ξ_A. It is however not the linear behavior of an ideal electrical capacity with $J = C \cdot (\dot{U}_A - \dot{U}_B)$.

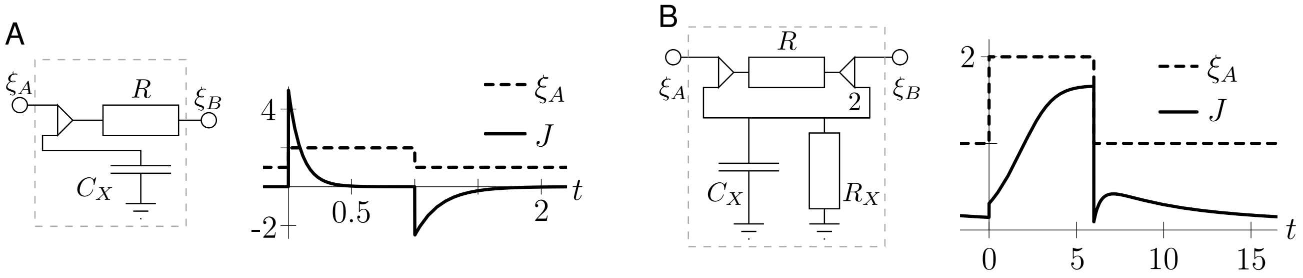


Figure 7.23.: TKM circuits with inductor and capacitor-like behavior. A: Capacitor-like behavior ($R = 0.2$, $C_X = 1$, $\xi_B = 1$). B: Inductor-like behavior ($R = 0.6$, $R_X = 0.6$, $C_X = 2$, $\xi_B = 1$).

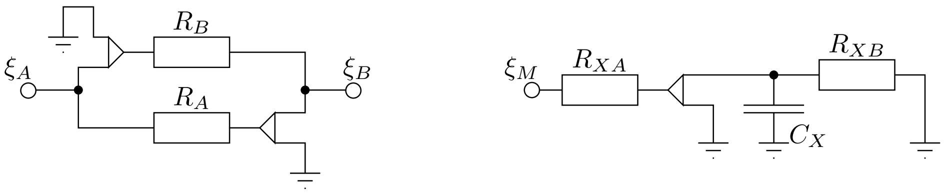
7.3.3.3. Inductor

The electrical inductor has the equation $d\Phi = L\, dI$ where L is the differential inductance. With $\dot{\Phi} = U$ this is equivalent to $U = L\, \dot{I}$. For a constant positive voltage U, the current I grows steadily. For a sinusoidal voltage $U = U^{\circ}\, sin(\omega t)$, the flux follows the voltage with a delay of $T = \omega^{-1}\, \pi/2$ because $I = L^{-1}\, U^{\circ}\, \omega^{-1}\, sin(\omega\, t - \pi/2)$. A ‘true’ thermokinetic inductor does not exist because thermokinetic networks do not contain a quantity comparable to the magnetic flux, but one may construct thermokinetic networks with an inductor-like behavior. Real electrical inductors are realized by electromagnetic coils and thus have a considerable resistive behavior. Further, they tend to be rather large in size. For this reason electrical engineers also often seek to replace inductors by networks of different elements with an inductor-like behavior [10].

Several chemical networks show inductive behavior. The average flux through an enzymatic reaction does not change immediately when the concentration of free reactant or product changes because the change is buffered by the intermediate complexes. The average flux reacts with a certain delay that is characteristic for inductive behavior. However, such a system contains more than one independent reaction flux and does not represent a simple network element in a strict sense.

The behavior of an autocatalytic reaction $A + X \rightleftharpoons B + 2\, X$ and $X \rightarrow 0$ as shown in Figure 7.23 (right) partly resembles the behavior of a real inductor. Upon a jump in the reactant concentration c_A the flux does not jump immediately to its steady-state value but increases slowly. Due to the asymmetry of the system with respect to A and B, we do not get this inductive behavior for all situations; for example a decrease of the reactant concentration does lead to a immediate decrease of the flux.

The above two examples for inductive behavior occur as side effects of enzymatic and autocatalytic reactions. Both reaction types are common motifs in metabolic networks. However, an inductive behavior may also be desired for filtering signals in signal transduction networks. Figure 7.24 shows a network that simulates the behavior of an ideal inductor. It consists of four irreversible, catalyzed reactions that realize controlled current sources (cf. Figure 7.22). The overall reaction rate J from A to B is given by $J = J_A - J_B = R_A^{-1}\, \xi_A - R_B^{-1}\, \xi_B = (\rho_A^{-1} - \rho_B^{-1})\, \xi_X$.



$$R_A = \rho_A\,\xi_X^{-1}\,\xi_A \qquad R_{XA} = \rho_X\,\xi_A^{-1}\,\xi_M$$
$$R_B = \rho_B\,\xi_X^{-1}\,\xi_B \qquad R_{XB} = \rho_X\,\xi_B^{-1}\,\xi_X$$

Figure 7.24.: TKM circuit with an ideal inductive behavior. Both parts of the circuit influence each other because the resistances in one part depend on the thermokinetic potentials of the other part.

We further have that $C_X\,\dot{\xi}_X = J_{XA} - J_{XB} = R_{XA}^{-1}\,\xi_M - R_{XB}^{-1}\,\xi_X = \rho_X^{-1}\,(\xi_A - \xi_B)$. In effect, we get an ideal inductive behavior:

$$\underbrace{((\rho_A^{-1} - \rho_B^{-1})^{-1}\,\rho_X\,C_X)}_{L}\,\dot{J} = \xi_A - \xi_B.$$

In this example, the ideal inductive behavior can only be realized by the introduction of controlled current sources, i. e. irreversible, saturated reactions. Thus, the passive inductive behavior can only be realized by using active elements. The example above is motivated by the so-called gyrator realization of electric inductive behavior without the use of electromagnetic coils but with active elements. General gyrators will be discussed in Section 7.3.4.2.

A possibility for the interpretation of the example above is to identify A and B with the dephosphorylated and phosphorylated form of a protein, respectively. Then the resistances R_A and R_B describe the phosphatase and kinase activity of the enzyme X, respectively. The synthesis or activation of X is controlled by A, whereas the degradation or inactivation is controlled by B.

7.3.4. Passive Two-Ports

The last section discussed basic network elements with two terminals. This section discusses the TK realization of two important two-port, four-terminal devices, namely the transformer and the gyrator.

7.3.4.1. Transformer

An ideal electrical transformer is a two-port device that is characterized by the equations shown in Figure 7.25 (right) [10]. The *turns ratio* n is the sole parameter of an transformer. Real transformers are realized by magnetically coupled coils. Due to the magnetic and electric

$$\begin{pmatrix}\mu_1\\ J_2\end{pmatrix}=\begin{pmatrix}0 & 1/\nu\\ -1/\nu & 0\end{pmatrix}\begin{pmatrix}J_1\\ \mu_2\end{pmatrix} \qquad \begin{pmatrix}U_1\\ I_2\end{pmatrix}=\begin{pmatrix}0 & n\\ -n & 0\end{pmatrix}\begin{pmatrix}I_1\\ U_2\end{pmatrix}$$

Figure 7.25.: The analog to an ideal transformer is a stoichior.

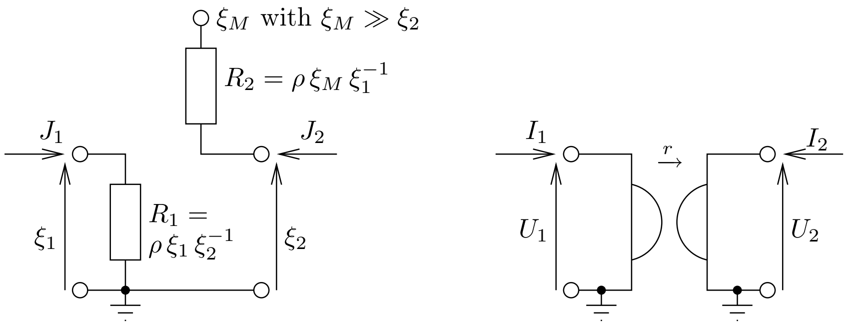
Figure 7.26.: Stoichiors with more than two terminals correspond to transformers with multiple primary coils.

losses, real transformers work only with alternating current but not with direct current. Ideal transformers without losses would work with direct current as well. A stoichior has analog equations (see Figure 7.25, left) if one uses chemical potentials $\mu_i = R^* T \log(\xi_i)$ as potential variables. For thermokinetic potentials ξ_i the behavior is accordingly non-linear. Figure 7.26 shows that the electrical analogy of a stoichior with more than two terminals is a transformer with multiple primary coils.

7.3.4.2. Gyrator

A gyrator is a two-port device with equations as shown in Figure 7.27 (right) [10]. Gyrators are non-reciprocal elements. This means, for example, that a capacitive behavior on terminal 2 leads to an inductive behavior at terminal 1. In electrical engineering, gyrators are often used to avoid the use of space-consuming coils to realize inductive behavior. Indeed, we already used a gyrator in our TK realization of an ideal inductive behavior in Figure 7.24. Figure 7.27 (left) shows a possible TK implementation of a grounded gyrator. It is based on saturated, enzymatic reactions, where the catalyst of reactions 1 and 2 are the compounds 2 and 1, respectively. This means that compound X_1 catalyzes the synthesis of compound X_2 and X_2 catalyzes the degradation of X_1.

The term *gyrator* has its origin from the fact that mechanical gyroscopes show such behavior. Although the equations of the gyrator are energy-conserving, there are no non-active electrical devices with gyrator behavior, but electrical gyrators are realized by circuits that contain amplifiers [10]. Irreversible, saturated enzyme kinetics are analog to amplifiers and thus electrical and TK gyrators are similar in their realization.



$$\begin{pmatrix}\xi_1\\ \xi_2\end{pmatrix} = \begin{pmatrix}0 & -\rho\\ \rho & 0\end{pmatrix}\begin{pmatrix}J_1\\ J_2\end{pmatrix} \qquad \begin{pmatrix}U_1\\ U_2\end{pmatrix} = \begin{pmatrix}0 & -r\\ r & 0\end{pmatrix}\begin{pmatrix}I_1\\ I_2\end{pmatrix}$$

Figure 7.27.: Implementation of a gyrator behavior by irreversible, catalyzed, reactant-saturated reactions.

7.4. Conclusions and Discussion

This chapter established a graphical representation of TK models that resembles the graphical representation of electrical networks.

We can give simple but powerful rules for the reduction of the graphical representations of TK models. As in the previous chapters, the reduction of reactions with vanishing resistances is the most powerful method and plays a crucial role. A graphical way for dealing with reaction network models is useful for the analysis of small models or model parts using paper and pencil. The graphical simplification procedures are intuitive but require multiple redrawing of the diagram. For larger networks, this is tedious and the reaction equation based methods from Chapter 6 are more efficient. For small models, the graphical procedure is advantageous because it provides a better feeling for the network structure than a list of reaction equations.

The TK circuit diagrams and the rules for their simplification stress the modular nature of the model reduction techniques. Several simplification rules can be applied locally to certain modules. This facilitates the building of model libraries containing modules on different level of detail. This modular nature of TKM provides a starting point for the integration of TKM into modular modeling tools as ProMoT [40] that is based on the network theory of chemical processes [39]. The network theory represents chemical processes as a connection of components and coupling elements. Components contain storages and coupling elements do not contain storages. In TKM, the capacities are the components and the stoichiors and resistances form the coupling elements.

Further, the analogy of notation may help to recognize important analogies in the design principles of electrical and chemical networks. For this purpose, the chapter listed the basic elements of electrical networks and discussed which chemical networks show an analog behavior.

8. Modeling the Redox Regulation of *Escherichia coli*

8.1. Introduction

The previous chapters introduced TKM, a method for the thermodynamically consistent modeling and model reduction of reaction networks. Here, this method is used to model the oxygen response of the bacterium *Escherichia coli*.

Upon a change of oxygen availability, the central metabolism of *Escherichia coli* undergoes major changes [69, Chapter 11]. If the oxygen availability is decreased, the metabolism switches in several steps from a purely respirative to a fermentative mode [4–7]. This oxygen response emerges from an interplay of metabolic pathways and their regulation. Several global and local regulatory systems, e. g. the ArcAB two-component system and the transcriptional regulator FNR, directly or indirectly respond to a change in the oxygen availability [88].

The oxygen response of *Escherichia coli* is an ideal example to test the thermokinetic approach developed throughout this thesis. The structure of the metabolic and regulatory network is largely known and accessible in databases, see e. g. [54, 86]. Further, the systemic behavior was experimentally studied and valuable data sets are available [4–7]. Since the oxygen response emerges from an interaction of several pathways, the model cannot be restricted to a single pathway or small subsystem, but one needs to model large parts of the central metabolism including the glycolysis, the tricarboxylic acid cycle (TCA), the fermentative pathways and the electron transport chain. The metabolic fluxes, in particular in the redox and energy metabolism, are strongly constrained by thermodynamic conditions [14]. Thermodynamic data, i. e. Gibbs formation energies, of the relevant compounds are available [1].

This chapter introduces a model that explains the emergence of the experimentally observed behavior from the interplay of different pathways and regulatory systems. The model describes steady-state concentrations and fluxes in the central metabolism of an *Escherichia coli* population growing in a chemostat.

Despite the large data basis, a strict bottom-up approach integrating only mechanistic details and relying only on measured kinetic parameters cannot be performed because quantitative data on enzyme kinetics and interaction strengths of regulatory interactions is missing or collected under *in vitro* conditions. For a thorough bottom-up approach, it would be necessary to determine all parameters under defined conditions in a defined strain, but the literature values are incomplete and refer to different strains under different conditions. Indeed, this is a typical situation, since for hardly any biochemical network sufficiently detailed and reliable kinetic data is available [48, 56]. For these reasons, the bottom-up approach is complemented by top-down elements. The available structural information is used in a bottom-up fashion. This means that the known stoichiometry and the known regulatory interactions determine the structure of the model. Simplified, generic expressions are used for enzyme kinetics and regulatory interactions. The corresponding parameter values are determined by adjusting them to fit the measurement data. This procedure has a top-down character because systemic data is used to identify the characteristic single interactions.

There is no guarantee that this procedure does initially lead to a unique model because there may be many combinations of parameter values where the simulation results fit the data. However, the model can be used to develop hypotheses concerning the functioning of the network. In particular, such a model is useful to hint for elements which are still unknown but important for the network behavior. Further, it can be used to suggest experiments that could help to identify such elements or clarify their function. In an iterative cycle of modeling, simulation and experiment the model converges to a correct and validated model.

8.2. Model Description

The model is structured into 7 compartments listed in Table 8.1. It contains variables describing the concentrations of 61 metabolic compounds, the concentration of biomass and 70 fluxes. The thermokinetic potentials of 12 of the compounds is assumed to be clamped and 31 of the fluxes are assumed to be in rapid equilibrium. Further, it contains the concentrations of 11 enzymes. Their synthesis rates are controlled by 5 transcription factors. The original model before the reduction contains 61 state variables (60 unclamped compounds plus biomass). After applying the rapid equilibrium assumption to 31 fluxes the reduced model is described by 30 state variables.

The model is implemented in Mathematica [104] using the package TKMOD (Appendix A, p. 185). The following section explains the structure of the model.

8.2.1. Metabolic Network

§ 8.1 (Network stoichiometry)**.** The metabolic network structure is based on a subset of the genome-scale flux balance model of *Escherichia coli* K-12 presented by Reed et al. [86]. This model contains the complete stoichiometry of the reactions which is necessary for the application of the TKM formalism. It further contains the charge of the dominant form of all species under physiological conditions. This number is used as an approximation of the average charge of the respective compounds. Table 8.2 lists the metabolites included in the model. Several thermokinetic potentials are clamped. The clamped values for the thermokinetic potentials of adp, coa, nad, $nadp$, pi and $q8h2$ are calculated from the list of typical concentrations given in Kümmel et al. [68]. By clamping adp, nad, $nadp$ and $q8h2$ one assumes that the ratios atp/adp, $nadh/nad$, $nadph/nadp$ and $q8h2/q8$ determine the behavior but not the single concentrations of adp, atp, nad, $nadh$, $nadp$, $nadph$, $q8$ and $q8h2$. Consequently, all kinetics in the model depend only on the ratios c_{atp}/c_{adp}, c_{nadh}/c_{nad}, c_{nadph}/c_{nadp} and c_{q8h2}/c_{q8} but not on the single concentrations. In this way, the detailed modeling of the de novo synthesis of adenosine, nicotineamide adenine dinucleotide and quinone is avoided and the model does only describe the ratios mentioned above but not the absolute concentrations. The clamped values for $h2o$ and h are determined by assuming a water concentration of 55.24 mol L and a $pH = 7.6$, which is the normal cytoplasmic pH of *Escherichia coli* [69, Section 28.3]. Table 8.3 lists the transport reactions between the compartments, Table 8.4 lists the reactions in the electron transport chain and Table 8.5 lists the remaining metabolic reactions in the model.

§ 8.2 (Gibbs formation energies and thermokinetic capacities)**.** Alberty [1] gives a list of Gibbs formation energies for many compounds of the central metabolism in dependence on pH and ionic strength I at a temperature $T = 298.15$ K. The table is available in Mathematica notebook format [104] at `http://library.wolfram.com/infocenter/MathSource/5704`. This data allows computing the thermokinetic capacities of most metabolites. Data for the remaining metabolites are taken from Kümmel et al. [67, Additional file 2] who extended the data set of Alberty [1]. The thermokinetic capacities used in the model are computed for the temperature $T = 310.15$ K from the Gibbs formation energies at $pH = 7.6$ and $I = 0.15$ M (see §5.5, p. 90). Because Alberty [1] does not list the temperature dependency of the Gibbs formation energies for many important metabolites, we follow the approach of Kümmel et al. [67] and neglect the temperature dependency of the Gibbs formation energies.

§ 8.3 (Reactions in rapid equilibrium)**.** Kümmel et al. [68] used the data set of Alberty [1] and measured metabolite concentrations to compile a list of reactions proceeding near thermodynamic equilibrium. For building the present model, this list was completed based on the assumption that only the reactions that are known to be tightly regulated proceed far from equilibrium. For the reactions proceeding near equilibrium, a thermokinetic resistance of zero is assumed that leads to a reduction of the model size and stiffness (see Section 5.5.2.2, p. 107). As an example, Figure 8.1 shows the TKM diagram for the tricarboxylic acid cycle. In the model, the behavior of the TCA cycle is determined by three reactions that are far from equilibrium, whereas the remaining reactions are assumed to be in rapid equilibrium.

§ 8.4 (Reactions far from equilibrium)**.** Irreversible, concentration-dependent kinetics are chosen for the reactions that are not in rapid equilibrium. This is possible because the steady state direction of the remaining reactions under physiological conditions is unique and the model is supposed to describe the steady state concentrations and fluxes. In TKM, irreversible reactions are modeled by clamped reaction fluxes. Technically, this means that the stoichiometry of these reactions is described by the stoichiometric matrix N_e, whereas the near-equilibrium reactions are described by the matrix N (see Definition 5.29, p. 100). Reactions that proceed far from equilibrium lead to differences of several orders of magnitude in the thermokinetic potentials. These differences may lead to difficulties in the numerical treatment of the reactions. Modeling the problematic reaction fluxes by irreversible reactions, reduces span(N) and the model can be scaled by a translation of chemical potentials (Corollary 5.34, p. 102). In this way the numerical difficulties can be overcome. Thus, the TKM formalism is used for the reactions in rapid equilibrium, but the remaining reactions are formulated in the conventional kinetic modeling formalism. This procedure shows that it is easily possible to combine the convenient model reduction possibilities of TKM with conventional kinetic rate laws.

§ 8.5 (Thermodynamic constraints)**.** The modeled network contains six independent stoichiometric cycles and thus six independent Wegscheider conditions. However, all of these cycles contain reactions that are assumed to proceed irreversibly in a way such that the cycle is broken and a cyclic flux is not possible. For example, *Escherichia coli* possesses several oxidases with different proton translocation efficiencies. A possible cycle consists of two oxidases, the ATP synthase and the hydrolysis of ATP. A proton translocating oxidase reduces oxygen to water and oxidizes NADH to NAD. The second oxidase oxidizes water to oxygen and reduces NAD to NADH without translocating protons. The ATP synthase uses protons translocated by the first oxidase to provide the energy for the phosphorylation of ADP to ATP. The cycle is completed by the spontaneous hydrolysis of ATP to ADP. However, because the oxidation of water to oxygen is thermodynamically highly unfavorable such that it does virtually not occur, the oxidases can be modeled by irreversible reactions and the cycle is broken. Because all occurring cycles are broken in this way, the thermodynamic constraints in the example model are not considered in the form of the Wegscheider conditions but in the form of the known, organism-independent Gibbs formation energies of the compounds. The organism-independent Gibbs formation energies (and thus the thermokinetic capacities) strongly restrict the values of the concentrations that are possible for the realization of a certain observed flux distribution. Henry et al. [46, 47] analyzed these conditions for a larger metabolism-wide flux balance model of *Escherichia coli* and showed that they strongly constrain concentrations of key metabolites.

§ 8.6 (Simplified kinetic laws)**.** For the sake of simplicity, Michaelis-Menten and Hill-type kinetics are used for the description of irreversible kinetics. For example, the reaction $A \rightleftharpoons B$ with enzyme E and inhibitor I is modeled by

$$J_j = \hat{J}_j \, c_E \, \frac{c_A^{k_{h,j,A}}}{c_A^{k_{h,j,A}} + k_{m,j,A}^{k_{h,j,A}}} \, \frac{c_I^{k_{h,j,I}}}{c_I^{k_{h,j,I}} + k_{m,j,I}^{k_{h,j,I}}} \tag{8.1}$$

where $\hat{J}_j$, $k_{m,j,\cdot}$ and $k_{h,j,\cdot}$ are constant parameters. Since A is a reactant and I an inhibitor, the parameters $k_{h,j,A}$ and $k_{h,j,I}$ are positive and negative, respectively. The basic hypothesis is that the dominating systemic behavior is not determined by the detailed form of the kinetic laws but by the network structure. For this reason often steep Hill kinetics with $|k_{h,j,\cdot}| \approx 5$ are assumed. The use of such kinetics allows a quasi-logical reasoning because reactions can be thought to be switched on and off dependent on the concentrations of reactants, products or effectors. However, a discrete, purely Boolean approach is not easily possible because the concentrations often stabilize near the threshold values $k_{m,j,\cdot}$, which would lead to continuous switching in a discrete model. The use of steep Hill kinetics provides an intuitive way to parameterize the model and analyze the simulation results. It proofed to be very helpful for the discussion with experimentalists because semi-quantitative statements (as for example: pyruvate formate lyase is inactive for oxygen concentrations above a certain threshold) can be easily implemented in the model.

The parameters were adjusted manually in an iterative way in order to get simulations that fit the data of Alexeeva et al. [5, 6, 7] and Alexeeva [4]. This data set will be discussed in Section 8.3.

§ 8.7 (Cellular growth and maintenance)**.** Cellular growth is modeled by an overall reaction from the precursor molecules to biomass. The stoichiometric coefficients are taken from Neidhardt et al. [74, Chapter 5]. A Hill-type kinetic law is assumed for the overall reaction. The behavior of the measured quantities proved to be robust against the parameters of this rate law. Basic data on the volume, surface and weight of a cell is taken from Sundararaj et al. [100]. The reaction ATPM represents the ATP consumption for maintenance needs, the spontaneous hydrolysis of ATP and the hydrolysis of ATP in unmodeled futile cycles. This flux is modeled by a Hill law depending on the ATP/ADP ratio with a k_m-value below physiological ATP/ADP ratios such that the ATPM flux is always saturated. The maximal ATPM flux is assumed to depend also on the activity of the transcription factor ArcA because measurement data of an $\Delta arcA$ mutant from Alexeeva et al. [7] show an increased electron transport activity that presumably originates from a higher ATP consumption caused by a futile cycle.

§ 8.8 (Chemostat equations)**.** The concentration of the medium constituents is determined by the chemostat equations [95]. Glucose is the sole carbon source and supplied in the inflow of the chemostat with a concentration of 45 mM. The dilution rate is 0.15 h^{-1}. The oxygen inflow into the chemostat is varied in order to adjust different levels of oxygen availability.

§ 8.9 (ATP and NADH in the model)**.** A hallmark of the model is the treatment of the ATP/ADP and the NADH/NAD ratio as state variables. These concentrations are often set constant because only a subset of the relevant reactions is modeled, see e. g. [16, 24, 78]. Because the present model contains the relevant parts of the central metabolism and overall reactions for the cellular growth and maintenance metabolism, it is possible to model this quantities as state variables.

8.2.2. Transcriptional Regulation

The transcriptional regulation network is described by phenomenological equations. The model describes the effect of each transcription factor on the gene expression by its activity a_i. The activity of a transcription factor is between 0 and 1 indicating minimal and maximal activity, respectively. The transcription factor activities a_i depend on their metabolic signals s_i that are

id	description	electrical potential
c	cytoplasm	$\phi_c = 0$
cm	cytoplasmatic membrane	$\phi_{cm} = (\phi_c + \phi_{p+})/2$
p+	charged boundary layer in periplasm	$\phi_{p+} \propto c_{h,p+} - c_{h,c}$
p	periplasm	$\phi_p = 0$
om	outer membrane	$\phi_{om} = 0$
e	cell exterior (medium)	$\phi_e = 0$

Table 8.1.: Compartments in the *Escherichia coli* model.

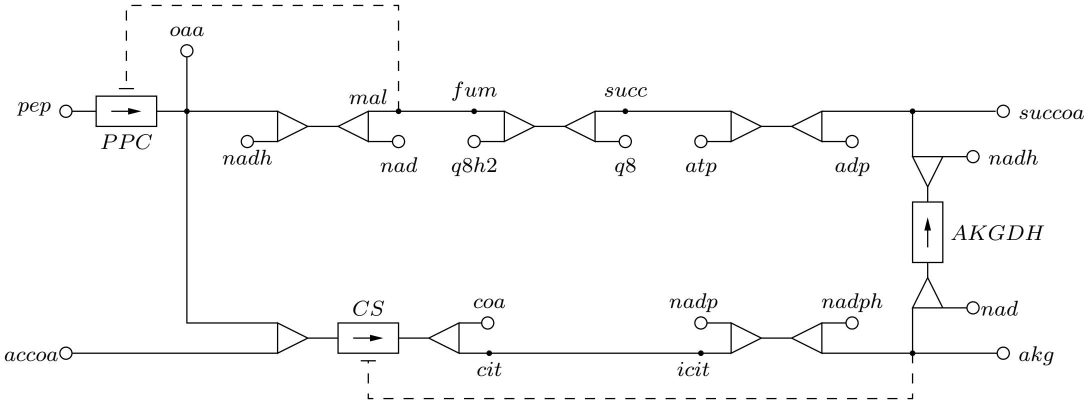


Figure 8.1.: TKM diagram of the citric acid cycle. The capacities are omitted because the model simulations are restricted to steady state conditions. The dashed lines indicate inhibitions. The arrow in the resistances indicates that the respective reactions are modeled by irreversible kinetics.

id	name	compartments (see Table 8.1)	clamped in compartments
13dpg	3-phospho-D-glyceroyl phosphate	c	-
2pg, 3pg	D-glycerate 2-phosphate, 3-phospho-D-glycerate	c	-
6pgc	6-phospho-D-gluconate	c	-
6pgl	6-phospho-D-glucono-1,5-lactone	c	-
ac	acetate	c, p, e	-
accoa, actp	acetyl-CoA, acetyl phosphate	c	-
akg	2-oxogluatate	c	-
amp	adenosine-monophosphate AMP	c	-
adp	adenosine-diphosphate ADP	c	c
atp	adenosine-triphosphate ATP	c	-
cit, icit	citrate, isocitrate	c	-
co2	carbon dioxide	c, p, e	e
coa	coenzyme A	c	c
dhap	dihydrixyacetone phosphate	c	-
e4p	D-erythrose 4-phosphate	c	-
etoh	ethanol	c, p, e	-
fdp	D-fructose 1.6-biphosphate	c	-
for	formate	c, p, e	-
fum	fumarate	c	-
g3p	glyceraldehyde 3-phosphate	c	-
g6p, f6p	D-glucose 6-phosphate, D-fructose 6-phosphate	c	-
glc-D	D-glucose	c, p, e	-
h	proton	c, p+	c
h2o	water	c	c
mal-L	L-malate	c	-
nad	nicotinamide adenine dinucleotide	c	c
nadh	reduced nad	c	-
nadp	nicotinamide adenine dinucleotide phosphate	c	c
nadph	reduced nadp	c	-
o2	oxygen	c, p, e	-
oaa	oxaloacetate	c	-
pep	phosphoenolpyruvate	c	-
pi	phosphate	c	c
pyr	pyruvate	c	-
q8	ubiquinone-8	cm	-
q8h2	ubiquinol-8	cm	c
r5p	α-D-ribose 5-phosphate	c	-
succ	succinate	c, e, p	-
succoa	succinyl-CoA	c	-

Table 8.2.: Metabolites in the *Escherichia coli* model.

Transport p → c (compartment cm):

			regulation	
id	name	R_j	genetic	enzymatic
ACt	acetate transport	0	-	-
CO2t	carbon dioxide transport	0	-	-
ETOHt	ethanol transport	0	-	-
FORt	formate transport	0	-	-
GLCpts	glucose PTS	irrev	-	-
O2t	oxygen transport	0	-	-
SUCCt	succinate transport	irrev	FNR: +	-

Transport e → p (compartment om):
compounds: ac, co2, etoh, for, glc, o2, succ; $R_j = 0$

Table 8.3.: Transport reactions from the extracellular medium (e) into the periplasm (p) and the cytoplasm (c). Reactions modeled by irreversible kinetics are marked with 'irrev' in the resistance column.

id	enzyme	R_j	genetic regulation
oxidases			
CYTBO3	Cyo (proton translocating)	irrev	ArcA: -, FNR: -
CYTBD	Cyd (proton translocating)	irrev	ArcA: +, FNR: -
CTYBD2	AppB	irrev	ArcA: +, FNR: -, AppY: +
NADH dehydrogenases			
NADHII	Ndh	irrev	FNR: -
WrbA	WrbA	irrev	ArcA: -, FNR: -
other			
SUCDH	succinate dehydrogenase fumarate reductase	0	-
ATPS4r	ATP synthase	0	-

Table 8.4.: Reactions in the electron transport chain model. Reactions modeled by irreversible kinetics are marked with 'irrev' in the resistance column. All reactions proceed at the cytoplasmatic membrane (compartment cm). The implemented genetic regulation is indicated by the transcription factors and the signs of the interactions (+: activation, -: inhibition).

				regulation	
id	enzyme	EC number	R_j	genetic	enzymatic
ACKRr	acetate kinase	2.7.2.1	0	-	-
ACONT	aconitase	4.2.1.3	0	-	-
ADHEr	acetaldehyde dehydrogenase	1.2.1.10	irrev	FNR: -	-
ADK1	adenylate kinase	2.7.4.3	0	-	-
AKGDH	2-oxoglutarate dehydrogenase		irrev	ArcA: -, FNR: - CRP: +, PdhR: -	-
ATPM	ATP maintenance drain		irrev	-	-
ATPS4r	ATP synthase	3.6.3.14	0	-	-
CS	citrate synthase	4.1.3.7	irrev	ArcA: -	akg: -
ENO	enolase	4.2.1.11	0	-	-
FBA	fdp aldolase	2.7.1.11	0	-	-
FUM	fumarase	4.2.1.2	0	-	-
G6PDH2r	g6p dehydrogenase	1.1.1.49	irrev	-	-
GAPD	g3p dehydrogenase	1.2.1.12	0	-	-
ICDHyr	isocitrate dehydrogenase	1.1.1.42	0	-	-
MDH	malate dehydrogenase	1.1.1.37	0	-	-
PDH	pyruvate dehydrogenase	1.2.1.-	irrev	FNR: -, PdhR: - ArcA: -, CRP:+	-
PFK	phosphofructokinase	2.7.1.11	irrev	-	pep: -
PFL	pyruvate formate lyase	2.3.1.54	irrev	FNR: +, ArcA: +	o2:-
PGI	g6p isomerase	5.3.1.9	0	-	-
PGK	phosphoglycerate kinase	2.7.2.3	0	-	-
PGL	6-phosphogluconolactonase	3.1.1.31	0	-	-
PGM	phosphoglycerate mutase	5.4.2.1	0	-	-
PPC	pep carboxylase	4.1.1.31	irrev	-	mal-L: -
PTAr	phosphotransacetylase	2.3.1.8	irrev	-	atp/adp: -
PYK	pyruvate kinase	2.7.1.40	irrev	-	fdp: +
SUCOAS	succinyl-CoA synthetase	6.2.1.5	0	-	-
TPI	triose-phosphate isomerase	5.3.1.1	0	-	-
GND, TKT1, TKT2	composite reactions describing reactions in the pentose phosphate pathway		0	-	-

Table 8.5.: Metabolic reactions in the cytoplasm of the *Escherichia coli* model. Reactions modeled by irreversible kinetics are marked with ‘irrev’ in the resistance column. The regulation that is implemented in the model is indicated by the transcription factors, the effectors and the signs of the interactions (+: activation, -: inhibition).

TF_i	s_i	$k_{h,i}$	reference
ArcA	c_{q8}/c_{q8h2}	-5	Georgellis et al. [37]
FNR	c_{o2}	-5	Unden et al. [102]
CRP	c_{pep}/c_{pyr}	+5	Bettenbrock et al. [17]
PdhR	c_{pyr}	-5	Quail and Guest [82]
AppY	c_{for}	+5	Brøndsted and Atlung [22]

Table 8.6.: Transcription factors TF_i and their metabolic signals s_i.

metabolite concentrations or ratios of metabolite concentrations in the form of a Hill equation:

$$a_i = \frac{s_i^{k_{h,i}}}{s_i^{k_{h,i}} + k_{m,i}^{k_{h,i}}} \tag{8.2}$$

where k_m and k_h are constant parameters. The modeled transcription factors together with their metabolic signals are listed in Table 8.6. The gene expression rates for the enzymes depend on the transcription factor activities. For example, the concentration of an enzyme E whose expression is activated by the transcription factor 1 but repressed by the transcription factor 2 can be modeled by

$$\dot{c}_E = \alpha + \beta \cdot a_1 \cdot (1 - a_2) - \mu \cdot c_E \tag{8.3}$$

where α and β are constants and μ is the specific growth rate. The mRNA concentration and the enzyme concentrations are assumed to be proportional. The database EcoCyc [54] lists the known transcriptional activators and repressors for many genes but does not contain information on the interaction strengths. The exact expression and the parameters of the transcriptional regulation model were adjusted to fit the measurement data from Alexeeva et al. [5, 6, 7] and Alexeeva [4]. It is not always necessary to include the full list of transcription factors listed in EcoCyc [54] of a gene to explain the observed behavior. In such cases, the influence of some transcription factors on the expression of certain genes was omitted and it is conjectured that this interaction does not play a significant role under the considered conditions. The genetic regulation of the enzymes that is implemented in the model is listed together with the metabolic reactions in the Tables 8.3, 8.5 and 8.4.

8.3. Comparison of the Simulations to Measurement Data

Alexeeva et al. [5, 6, 7] and Alexeeva [4] collected measurement data on the steady-state behavior of *Escherichia coli* in a glucose-limited chemostat at different levels of oxygen availability. They report on uptake and excretion fluxes, expression levels of key enzymes, the NADH/NAD ratio, the ArcA activity and the oxygen concentration. This data is given for the wildtype and a $\Delta arcA$ mutant.

§ 8.10 (Perceived aerobiosis)**.** The experimental adjustment and quantification of the oxygen availability is not straightforward. Significant metabolic changes occur at very low oxygen concentrations. These concentrations are difficult to measure and adjust with a sufficient accuracy. For this reason, Alexeeva et al. [5, 6] introduced "perceived aerobiosis as a quantifiable parameter which reflects the relative extent to which a cell uses oxidative catabolism." The oxygen supply in a chemostat is adjusted by varying the percentage of oxygen in the input gas. A fully anaerobic condition with no oxygen in the input gas ($J_{O_2,in} = 0$) is defined as 0% aerobiosis. The minimum oxygen input rate ($J_{O_2,in} = \hat{J}_{O_2,in}$) required for complete oxidation of glucose to carbon dioxide is defined as 100% aerobiosis. This means that 100% aerobiosis represents the minimal oxygen availability where no fermentation products are formed. Intermediate aerobiosis levels are defined via the oxygen input rate, e. g. 50% aerobiosis are defined as the metabolic state reached for $J_{O_2,in} = 0.5 \cdot \hat{J}_{O_2,in}$. Because $\hat{J}_{O_2,in}$ is different for every bioreactor, bioreactors need to be calibrated by identifying the respective $\hat{J}_{O_2,in}$. Perceived aerobiosis provides a quantitative and reproducible measure for the oxygen availability and is suited when comparing experimental results from different reactors. Thus, this parameter is an ideal basis for the comparison of simulation results with measurement data.

§ 8.11 (Comparison of simulation results with measurement data)**.** Figure 8.2 shows the comparison of the measurement data of Alexeeva et al. [5, 6, 7] and Alexeeva [4] with simulation results. All values are in steady state and are plotted over aerobiosis. The plots show the glucose-biomass yield Y_{Glc}, the transport rates J_{ACt}, J_{ETOHt}, J_{FORt}, J_{O2t} (see Table 8.3), the residual oxygen concentration in the medium $c_{o2(e)}$, the ratio of the concentrations of NADH and NAD c_{nadh}/c_{nad}, the cytochrome d content *cytd* that corresponds to the sum of the concentrations of oxidase *bd* (enzyme of reaction CYTBD) and *bd2* (enzyme of reaction CYTBD2), the mRNA level of the pyruvate formate lyase pfl (mRNA of reaction PFL) and the ArcA activity measured by a reporter construct. Further, Alexeeva et al. [5, 6, 7] and Alexeeva [4] present the values of fluxes that were not measured directly but are calculated from measured quantities by simple models. This data is not included in the comparison. Only directly measured quantities are shown in Figure 8.2. An exception is the flux through the pyruvate formate lyase J_{PFL} that produces formate. In the experiment, the formate is partly decomposed into carbon dioxide and hydrogen by the formate hydrogenlyase. Alexeeva et al. [7] compute the flux J_{PFL} from the measured formate excretion rate and other measured rates. Because the decomposition of formate into hydrogen and carbon dioxide is not included in the model, the formate excretion flux J_{FORt} is equal to J_{PFL} in the steady state of the model. Thus, the flux J_{FORt} of the model is not compared with the measured excretion rate of formate but with the calculated flux J_{PFL} given in Alexeeva et al. [7].

The simulation results fit the measured quantities well (Figure 8.2). The model reproduces the experimentally observed switching from fermentative metabolism with the formation of ethanol, formate and acetate under anaerobic conditions to purely respirative metabolism at and above 100% aerobiosis. It also reproduces the activity profile of ArcA, the cytochrome d content and the expression of *pfl* that show a complex, non-trivial behavior in dependence on aerobiosis. The model is also able to describe the $\Delta arcA$ mutant that (compared to the wildtype) shows a decreased yield Y_{Glc}, an increased oxygen uptake, an increased NADH/NAD ratio and a decreased residual oxygen concentration under microaerobic conditions (Figure 8.2). This shows that the structure as it is currently implemented in the model is coherent with the measurement values and that TKM is suited to describe large metabolic networks.

8.4. Conclusions

This chapter introduces a model of the steady state oxygen response of *Escherichia coli* in a chemostat culture. The model covers the central metabolism and the relevant regulation on the enzymatic and transcriptional level, both in the wildtype and a $\Delta arcA$ mutant.

The structure of the metabolic and regulatory network is largely known and was extracted from databases. An additionally available information allowing for model reduction is the knowledge which reactions proceed near thermodynamic equilibrium.

The Gibbs formation energies of the relevant metabolites are tabulated and thus the thermokinetic capacities can be computed. The reactions that proceed near thermodynamic equilibrium can be assumed to have a resistance of zero. However, reliable quantitative information on the remaining reaction kinetics and the strength of the regulatory interactions is scarce. For this reason, the kinetic rate laws and the regulatory interactions were modeled in a simplified manner. An interesting observation when building the model was that the model behavior is largely determined by the model structure and only to a lesser degree by the numerical values of the quantitative parameters. An important feature of the model is that it is able to quantitatively describe the data set of Alexeeva et al. [5, 6, 7] and Alexeeva [4]. To the knowledge of the author, it is currently the only kinetic model explaining the behavior of the central metabolism of *Escherichia coli* in dependence of the aerobiosis scale. The model is currently used to plan further experiments with the goal to extend and validate the model. This ongoing work is expected to further contribute to a deeper systemic understanding of the oxygen response[1].

Apart from the increased understanding of the oxygen response of *Escherichia coli*, the construction of the model and its performance show that the TKM formalism is highly useful as it allows the convenient reduced-order modeling of large reaction networks.

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[1] This is done in the framework of the SysMO-SUMO consortium.

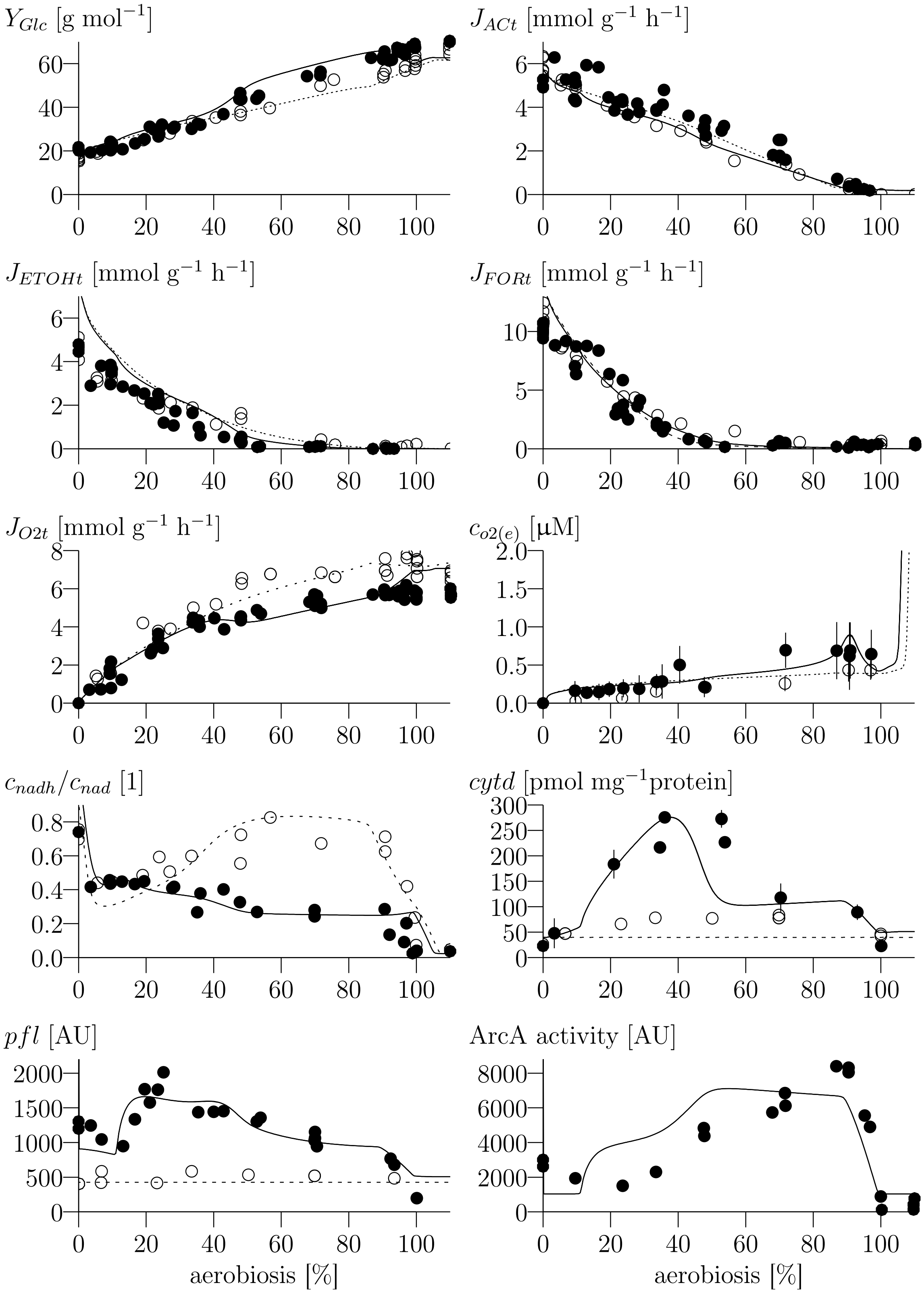

Figure 8.2.: Comparison of the simulation results (lines) and the experimental data (symbols) of the wildtype (filled symbols, solid lines) and a $\Delta arcA$ mutant (open symbols, dashed lines) over the aerobiosis scale. The plot labels are explained in §8.11.

9. Comparison to Other Approaches

The efforts of systems biology to arrive at large scale mathematical models require methods for the systematic analysis of thermodynamic constraints on reaction networks. For this reason, the consideration of thermodynamic constraints in the mathematical modeling of biochemical reaction networks received an increased attention in the last years. The focus of most current studies are the thermodynamics of metabolic networks. Such studies are possible due to the availability of tables of Gibbs formation energies for metabolites [1, 36] and of methods for their estimation [53, 73].

Several methods for the analysis of thermodynamic constraints in stoichiometric, constraint-based models of quasi-stationary metabolic networks are available. They help to reveal important principles of the functioning of large scale metabolic networks. For this reason Section 9.1 shortly reviews these methods. Section 9.2 is focusing on dynamic, kinetic modeling and compares TKM with several other approaches for the incorporation of thermodynamic constraints into dynamic, kinetic models. The discussion is restricted to approaches that are based on the structure of the biochemical network. Black-box approaches that are based on thermodynamic relationships but do not consider the detailed stoichiometry of the network are excluded from the discussion.

9.1. Thermodynamic Constraints in Constraint-Based Models

Constraint-based modeling seeks to formulate and analyze the basic laws that constrain the behavior of biochemical reaction networks. It is mainly used for modeling the fluxes in quasi-stationary metabolic networks characterized by stoichiometric matrices [85]. In this case, the main constraints are the quasi-steady state condition and the thermodynamic constraints.

Although constraint-based models do not provide a unique solution, they proved to be valuable for the analysis and redesign of metabolic networks. For example, Stelling et al. [97] showed that the quasi-steady state and the thermodynamic constraints on the metabolic network of *Escherichia coli* determines key aspects of its functionality and regulation.

Thermodynamic constraints on quasi-stationary flux distributions have the form of sign conditions; for example, in a given network under given boundary conditions, certain fluxes are irreversible, i. e. they can only proceed in one direction. These thermodynamic constraints are often determined heuristically [86]. Recently, formal methods were developed to computationally determine feasible flux directions. The following paragraphs review such approaches.

Beard and Qian [12], Beard et al. [13, 14], Price et al. [79], Qian and Beard [80], Qian et al. [81] and Yang et al. [105] introduce and apply energy balance analysis (EBA) that is a method for flux balance analysis under thermodynamic constraints. This method identifies and excludes thermodynamically infeasible cyclic flux distributions. It is applicable in situations where no thermodynamic data and no information on physiological concentration ranges is available because it only needs information on the stoichiometry of the network and on the boundary conditions.

If Gibbs energies of formation and bounds on physiological concentrations are known, stronger results can be achieved. Kümmel et al. [67, 68] present network-embedded thermodynamic (NET) analysis that uses the Gibbs energies of formation to determine the consistency of metabolomic data sets. Further, the method allows determining which reactions in the central metabolism proceed near thermodynamic equilibrium and which far from it. As expected, the far-from-equilibrium reactions are the rate limiting steps that are known to be strongly regulated and that determine the flux distributions. Zamboni et al. [107] present a computer tool for the application of NET analysis.

Henry et al. [46, 47] and Hoppe et al. [50] developed further methods to include thermodynamic constraints into constraint-based modeling. For this purpose, estimates for the maximal and minimal concentrations for the metabolites are used.

The methods sketched above analyze the thermodynamic constraints on the steady-state fluxes in metabolic networks. In contrast to that, TKM considers the thermodynamic constraints in dynamic, kinetic models. The next section discusses several alternative approaches to this problem and compares them to TKM.

9.2. Thermodynamic Constraints in Kinetic Models

The problem of how to consistently introduce thermodynamic constraints into kinetic modeling is discussed several times in the literature. This section shortly reviews several approaches. In particular, it works out their differences to the TKM approach. A preliminary version of this comparison was previously published in Ederer and Gilles [31].

9.2.1. Identification of a Cycle Base

Colquhoun et al. [26] discuss the problem of how to impose the detailed balance constraints in complex reaction mechanisms. Three methods have been suggested that rely on an explicit identification of independent stoichiometric cycles. In method 1, the cycles are subsequently identified and the according Wegscheider condition is used to eliminate a parameter, i. e. to express this parameter in dependency on the other parameters, whereby the order in which the cycles and parameters are considered is crucial. With a badly chosen ordering, this procedure leads to the elimination of all parameters in a cycle such that its Wegscheider condition is violated. Therefore, Colquhoun et al. [26] give rules to arrive at a valid ordering. Method

2 chooses a graph theoretical approach. Based on the spanning tree of the network, a set of independent cycles is computed that can be used to eliminate parameters. Here, the ordering is arbitrary. Method 3 uses the fact that the Wegscheider conditions form a linear equation system in the logarithms of the kinetic constants (see §2.52, p. 33). By basic linear algebra it is possible to divide the parameters into thermodynamically independent and dependent ones. Then, the dependent parameters can be computed as a function of the independent parameters. Method 1 and 2 are implemented in the parameter fitting tool HJCFIT.

The methods described above are developed for the purpose of automatic parameter fitting. For manual modeling and parameter adjustments prior to an automatic identification, there occur several problems. The identification of cycles and independent parameters has to be redone when extending or modifying the model. Hereby, different ordering or different spanning trees give different sets of independent parameters. Further, the independent parameters cannot be assigned to reactions or compounds, but changing a single parameter may have effects on the kinetic laws of several reactions. This makes parameter adjustment and interpretation difficult.

9.2.2. Reparameterization of Kinetic Rate Laws

From a mathematical point of view, TKM is equivalent to a transformation of the kinetic parameters into capacities and resistances. Yang et al. [106] and Liebermeister and Klipp [70] suggest similar parameters to avoid thermodynamic inconsistencies. First, both approaches are shortly discussed and then their relation to TKM is explored.

The approach of Yang et al. [106] is based on the formalism developed by Horn and Jackson [51], which uses the concept of reaction groups. A reaction group is a set of species participating in a reaction, either as reactants or products. In the example of an enzyme catalyzed reaction in Equation 7.1 (p. 140), one has the reaction groups $\{A, E\}$, $\{B, E\}$, $\{C, E\}$, $\{I, E\}$, $\{EA\}$, $\{EB\}$, $\{EI\}$, $\{EA, B\}$, $\{EB, A\}$ and $\{EAB\}$. A reaction transforms a reaction group into another reaction group. Now a matrix of kinetic parameters K can be built with $J = K^T g$ where g contains the mass action terms of the groups; in the example $g = (c_A\, c_E, c_B\, c_E, \ldots)$. Here J is a vector containing the rate of production for each reaction group. Let the matrix G be the diagonal matrix of the thermodynamic equilibrium values of g. Then, the elements of the matrix $G\, K$ are the forward and backward rates of the single reactions in thermodynamic equilibrium. The detailed balance relations, implying equality of forward and backward rates in equilibrium, take the form $G\, K = (G\, K)^T$. Thus, a valid parameter matrix K can be computed as $K = G^{-1} K_s$, where K_s is an arbitrary symmetric matrix. The independent parameters of the model are the equilibrium concentrations and the entries of the matrix K_s.

Detailed balance constrains the parameters only for true stoichiometric cycles. Thus, in a given scheme only a subset of the reaction parameters is constrained. For this reason, Yang et al. [106] further introduce a method to find the minimal subnetwork whose parameters are constrained by detailed balance. Any method imposing detailed balance on the parameters needs to work only on this subsystem.

Liebermeister and Klipp [70] suggest the use of so called *convenience kinetics* for the (semi)-automatic generation of kinetic models. Convenience kinetics are a special form of general mass-action kinetics that describe enzyme-catalyzed reactions with a random-order mechanism. Because these kinetics are quite flexible, they are suited as generic rate laws for (semi)-automatic model generation. In this context, also the question of how to find thermodynamically consistent parameters arises. This problem is solved by parameterizing the model by thermodynamically independent parameters: the energy constants for compounds $k_i^G = \exp(\Delta_f G_i^\circ/(R^* T))$, the velocity constant for reactions $k_j^V = (k_{+j}\, k_{-j})^{1/2}$ and the half saturation constants k_{ij}^M.

The parameterizations suggested by Yang et al. [106] and Liebermeister and Klipp [70] are similar to TKM. All three approaches divide the parameters into kinetic parameters related to reactions and thermodynamic parameters related to compounds. The thermodynamic parameters of the compounds are equal to or are the reciprocal value of the thermokinetic capacities: $C_i = c_{eq,i} = c^\circ/k_i^G$. The capacities C_i, the equilibrium concentrations $c_{eq,i}$ and the energy constants k_i^G are not unique and may be transformed to a different reference state (cf. Section 5.4.2, p. 102).

In the approach of Yang et al. [106], the entries of the matrix K_s are the forward and backward rates of the reactions in thermodynamic equilibrium defined by the concentrations $C_i = c_{eq,i}$. Thus, for mass-action kinetics the entries of the matrix K_S are equal to the reciprocal thermokinetic resistances. However, the approach by Yang et al. [106] is based on reaction groups. This concept is seldom used directly for kinetic modeling. In addition, there are usually much more reaction groups than species and thus complexity is added to the problem.

Although Liebermeister and Klipp [70] describe the approach for convenience kinetics, it is readily extended to generalized mass-action kinetics. TKM uses resistances R_j instead of velocity constants k_j^V to describe the kinetics. This is only a formal difference, but each variant has its own advantages. The standard Gibbs formation energies $\Delta_f G_i^\circ$ are tabulated with respect to a chosen, arbitrary reference state. If this reference state is changed, the capacities C_i and the parameter $k_j^G = c^\circ\, C_i^{-1}$ change accordingly (see Section 5.4.2, p. 102). A change of the reference state also affects the thermokinetic resistances R_j but not the velocity constants k_j^V. This means that the exchange of the velocity constant k_j^V between different models with different reference states is simpler than the exchange of thermokinetic resistances R_j. However, the use of thermokinetic resistances R_j is advantageous for parameter adjustments and sensitivity analysis: for the forward rate constant k_{+j} one gets in TKM $k_{+j} = R^{-1} \prod_{i \in E_j} C_i^{-|\nu_{E,ij}|}$ and in the convenience kinetics approach $k_{+j} = k_j^V \prod_i (k_i^G)^{-\nu_{ij}/2}$. In TKM, an adjustment of the thermodynamic parameter C_i of the products leaves the forward rate constant k_{+j} unchanged but has only influence on the backward constant k_{-j}. In the convenience kinetics approach, a change in the analog parameters k_i^G of the products changes both the forward and the backward rate constant. Thus, in the convenience kinetics approach, a variation of a thermodynamic parameter of a compound influences also the rate constant of mass-action terms that do not contain the respective concentration. Thus, for a clear distinction of thermodynamic and kinetic information, the use of thermodynamic resistances is advantageous.

Besides a parameterization of kinetic models by capacities and resistances, TKM introduces thermokinetic potentials and forces. In contrast to the reviewed approaches, TKM provides an intuitive, thermodynamic interpretation and stresses the relation to chemical potentials and thermodynamic flow-force relationships. For example, this allows using non-ideal capacities to model pools of compounds that are in equilibrium with each other. In this way, kinetic laws that would be very complex when expressed in dependence on the concentrations can be expressed in a simple manner (see Section 6.7, p. 134). None of the described, alternative approaches provides such a possibility.

9.2.3. Flow-Force Relationships

Kinetic rate laws that are derived from thermodynamic flow-force relationships are sometimes used to model biochemical reactions. TKM belongs to this class of approaches and thus one can directly compare the resulting rate equations.

For systems far from equilibrium, the complex dependency of the thermodynamic resistance on the chemical potentials μ_i (see Section 3.3.2, p. 46) renders the thermodynamic modeling formalism almost intractable for larger networks. Even first order reactions that would yield linear models in the kinetic modeling formalism have highly nonlinear thermodynamic resistances. Thus, thermodynamic flow-force relationships are usually used in an approximated form. We compare the TKM approach to two of these approximations by considering a model for the flux through the membrane-bound NADH dehydrogenase in the electron transport chain

$$NADH + Q \rightleftharpoons NAD^+ + QH_2 + 4\,\Delta H^+$$

where Q and QH_2 are ubiquinone and ubiquinol, respectively. Here ΔH^+ describes the translocation of protons over a membrane. Models of this reaction were recently presented by Beard [11] and Klamt et al. [58] as parts of their respective electron-transport chain models. In Alberty [1] one finds the following tables of transformed Gibbs energies of formation at $pH = 7$, $T = 298$ K and an ionic strength $I = 0.25$ mol L^{-1}:

$$\begin{aligned} \Delta_f G'^{\circ}_{Q} &= 3668.94\ \text{kJ mol}^{-1}, & \Delta_f G'^{\circ}_{QH_2} &= 3660.55\ \text{kJ mol}^{-1}, \\ \Delta_f G'^{\circ}_{NAD} &= 1059.11\ \text{kJ mol}^{-1}, & \Delta_f G'^{\circ}_{NADH} &= 1120.09\ \text{kJ mol}^{-1}. \end{aligned} \tag{9.1}$$

This yields a standard Gibbs reaction energy of $\Delta G^\circ = -\Delta_f G'^{\circ}_{NADH} - \Delta_f G'^{\circ}_{Q} + \Delta_f G'^{\circ}_{NAD} + \Delta_f G'^{\circ}_{QH_2} + 4\,F\,\Delta p$. Assuming a constant proton-motive force of $\Delta p = 180$ mV one gets $\Delta G^\circ = 0.11$ kJ mol^{-1}.

Klamt et al. [58] assumes proportionality of ΔG and J_K and use the law

$$J_K = -k_K \left(\Delta G^\circ + R^*\,T\,\log\left(\frac{c_{QH_2}\,c_{NAD}}{c_Q\,c_{NADH}} \right) \right).$$

Laws of this kind are also used for example by Korzeniewski [60] and Korzeniewski and Zoladz [62]. Beard [11] uses the law

$$J_B = k_B \left(\exp\left(\frac{-\Delta G^\circ}{R^*\,T} \right) \frac{c_Q}{c_{QH_2}}\,c_{NADH} - c_{NAD} \right).$$

The corresponding TKM law is

$$J_{TKM} = R^{-1}\left(\xi_{NADH}\,\xi_Q - \xi_{NAD}\,\xi_{QH_2}\,\xi_{\Delta H^+}^4\right)$$

where $\xi_{\Delta H^+} = \exp(F\,\Delta p/(R^*\,T)) = 1107.03$ and $\xi_i = c_i/C_i$ for $i \in \{Q, QH2, NAD, NADH\}$. Assuming a constant resistance R, the flux is proportional to the thermokinetic force. The capacities C_i can be computed from the data in Equation 9.1:

$$C_Q = 8.067 \times 10^{-644}\text{ M}, \qquad C_{QH_2} = 2.38 \times 10^{-642}\text{ M},$$
$$C_{NAD} = 2.28 \times 10^{-186}\text{ M}, \qquad C_{NADH} = 4.68 \times 10^{-197}\text{ M}.$$

A scaling of the system according to Section 5.4.2 (p. 102) with $\delta\xi_Q = \delta\xi_{QH2} = \sqrt{C_Q\,C_{QH_2}} = 4.39 \times 10^{-643}$ M and $\delta\xi_{NAD} = \delta\xi_{NADH} = \sqrt{C_{NAD}\,C_{NADH}} = 1.03 \times 10^{-191}$ M yields

$$C_Q = 0.18\text{ M}, \qquad C_{QH_2} = 5.44\text{ M},$$
$$C_{NAD} = 221. \times 10^{3}\text{ M}, \qquad C_{NADH} = 4.53 \times 10^{-6}\text{ M}.$$

We use $k_B = 0.54088\text{ s}^{-1}$ and adopt k_K and R such that in thermodynamic equilibrium the three approaches are equal up to the first order such that for small deviations from equilibrium $J_K \approx J_B \approx J_{TKM}$. Figure 9.1 compares the three models in dependence of c_{QH_2} for fixed $c_Q = c_{NAD} = c_{NADH} = 1$ mM. Deviations occur in particular for small c_{QH_2}. Then, the fluxes J_K and J_B are very large because their forward rate depends on the ratio c_Q/c_{QH_2}. However, one would not expect that the forward rate of a reaction is activated by the depletion of the product c_{QH_2}. In particular, there should be an upper bound for the forward flux independent of the concentration of the product c_{QH_2}. The flux J_{TKM} follows a kinetic mass-action law and thus the forward rate is independent of the product concentration c_{QH_2} and for small c_{QH_2} only the backward rate vanishes. Whereas the approach of Klamt et al. [58] shows problematic behavior for low and high concentrations of c_{NAD} and c_{QH2}, the approach by Beard [11] avoids this problem for c_{NAD} but not for c_{QH2}. The above laws are good approximations in a certain range of the concentrations, but they fail for low and high concentrations. Korzeniewski [61] observed a similar problem when comparing experimental flux data with the thermodynamic driving force of the cytochrome oxidase. In particular, Korzeniewski [61] showed that the flux through the oxidase even decreases with increasing thermodynamic force in a wide and physiological reasonable range.

Altogether, all three approaches for the NADH dehydrogenase yield similar results near thermodynamic equilibrium. Besides the known and tabulated Gibbs energies of formation they require only one additional parameter that has to be fitted to the measurement data. Far from equilibrium only the TKM approach shows a realistic behavior.

9.3. Discussion

In comparison to other approaches for the incorporation of thermodynamic constraints into kinetic models, the TKM approach has several advantages that are summarized in the following paragraphs.

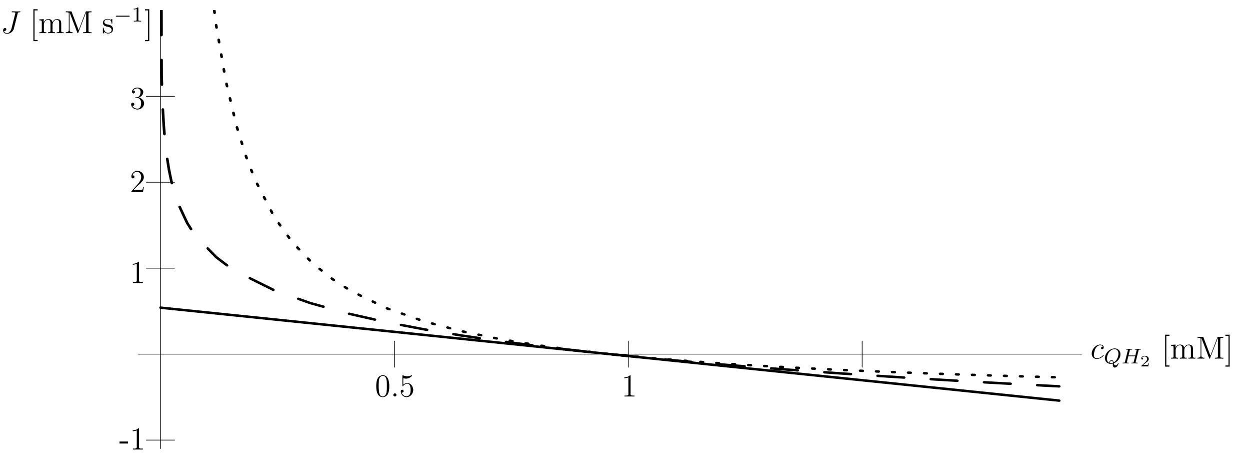

Figure 9.1.: Comparison of three different kinetic laws for the NADH dehydrogenase: TKM (solid); Klamt et al. [58] (dashed); Beard [11] (dotted).

Any model formulated using generalized mass-action kinetics can be translated into a TK model provided that the original model is thermodynamically consistent. Thus, TKM does not suffer from the scaling problems of linear thermodynamics for small and large concentrations.

TKM does not require the explicit enumeration of stoichiometric cycles, but provides an inherent thermodynamically consistent parameterization. The use of thermokinetic capacities and resistances distinguishes thermodynamic parameters (capacities) and kinetic parameters (resistances) in a clear way. For mass-action laws, TKM and the alternative parameterization schemes of Yang et al. [106] and Liebermeister and Klipp [70] are physically equivalent but differ in their formal properties. Yang et al. [106] apply the concept of reaction groups, which however is rarely directly used for modeling. Liebermeister and Klipp [70] describe their method for so called convenience kinetics, but it can be readily extended to generalized mass-action kinetics. This approach differs from TKM in the choice of the parameters of reactions. Changes in thermokinetic capacities influence only the mass-action terms that involve the respective concentrations, but not the rate constants of the reverse reaction. In the convenience kinetics approach, a change of the energy constants has effects on both directions of a reaction. Thus, TKM dissects thermodynamic and kinetic effects more clearly.

TKM unifies the approaches that are based on a thermodynamically consistent parameterization and those approaches that use flow-force relationships. A crucial point that is not addressed by any of the approaches discussed above is the introduction of thermokinetic potentials, forces, capacities and resistances. As shown in the Chapters 5 and 6, this simplifies the application of several model reduction techniques which lead to reduced models with non-constant capacities and resistances that depend on the system state. Non-constant resistances allow the modeler to describe all kinds of generalized mass-action kinetics, e. g. a reversible Michaelis-Menten reaction is described by a linear dependency of the resistance on the potentials (see Section 3.3.2, p. 46). Non-constant capacities can describe pools of species that are in rapid-equilibrium, e. g. the pool of free substrate and substrate-enzyme complex (see Section 6.7, p. 134). This is particularly interesting, since the respective concentration dependent rate laws do not follow a generalized mass-action law and are very complex, but the according TKM expressions are simple.

In conclusion, TKM unites the advantages of kinetic modeling and thermodynamic flow-force relationships. It makes the use of thermodynamic flow-force relationships feasible for networks far from equilibrium and extends the scope of kinetic modeling.

10. Conclusions and Discussion

Callen [23, p. 3] states that "thermodynamics sets limits (inequalities) on permissible physical processes, and it establishes relationships between apparently unrelated properties." In particular, thermodynamics constrains the physically possible dynamics of biochemical reaction networks. It determines the sign of reaction fluxes and establishes relationships between rate constant of different reactions. If no special care is taken, kinetic models may easily describe systems that are thermodynamically impossible.

It is the explicit goal of systems biology to derive large scale mathematical models of biochemical reaction networks [96]. To approach this goal, it is important to use methods that explicitly acknowledge the thermodynamic constraints [48]. The present thesis presents solutions for the thermodynamically consistent formulation and reduction of kinetic models. The key contribution is the introduction of the thermokinetic modeling (TKM) and model reduction formalism.

In general, thermodynamics distinguishes between extensive, specific and intensive variables. Extensive variables describe the amount of substance, charge, energy or other balanceable quantities. Specific variables are the ratio of extensive variables and a variable that describes the size of the system, e. g. its mass or volume. Examples for specific variables are concentrations, i. e. the ratios of the amounts of species and the volume of the system. Intensive variables describe the ability of an extensive variable to drive a flux. For example, temperatures, voltages and chemical potentials are intensive variables. Differences of intensive variables are the thermodynamic forces. For example, temperature, voltage and chemical potential differences drive heat fluxes, electrical currents and chemical reactions, respectively. The direction of the driving force determines the direction of the respective flux. For example, heat flows from hot to cool bodies. In contrast to extensive variables, intensive and specific variables are independent of the size of the system. Often, specific variables are called intensive variables because they share this property. Here, we follow Callen [23, Section 2.1] and reserve the term intensive variables for the variables whose differences are the thermodynamic forces, i. e. for the partial derivatives of the fundamental equation in the form $U(S, V, n, \ldots)$ or $S(U, V, n, \ldots)$. This distinction is necessary for the following discussion.

Dynamic models of thermodynamic systems generally consist of the balance equations and the rate equations. The balance equations describe the change of the extensive or specific variables in dependence on the fluxes. The rate equations describe the dependence of the fluxes on the state of the system. In most cases, the rate equations are formulated in terms of the driving forces, i. e. of intensive variables. For example, the heat flow and the electrical current can often assumed to be proportional to temperature and voltage differences, respectively. Such rate laws guarantee that the direction of the flux is thermodynamically feasible.

An exception is the conventional kinetic modeling formalism. Whereas electrical, thermal or diffusive systems often proceed sufficiently near to thermodynamic equilibrium such that the use of linear flow-force relationships is appropriate, reaction systems often proceed far from equilibrium such that the flow-force relationships are highly non-linear and the thermodynamic resistance in the flow-force relationships is not constant but depends strongly on the state of the system [23, Section 14.3]. For this reason, reaction rates are often more conveniently expressed in dependence on the concentrations, i. e. on specific but not intensive variables. An alternative is the use of approximated flow-force relationships that assume a functional, for example a linear, dependency of the fluxes from the forces but not from other state variables. Heinrich and Schuster [45, Chapter 2.2.3] discuss that approximated thermodynamic flow-force relationships are often inferior to concentration-dependent kinetic rate equations. The thermodynamic forces driving reaction fluxes do not contain the full information determining the reaction rates such that approximated flow-force relationships often fail to describe the occurring effects. In contrary, concentration-dependent kinetic rate equations allow one to incorporate detailed mechanistic knowledge and thus give deeper insight into the system.

Whereas flow-force relationships guarantee thermodynamic feasibility, concentration-dependent kinetic laws are more flexible and simple. To overcome this dilemma, TKM introduces flow-force relationships into kinetic modeling. TKM is based on the use of thermokinetic potentials and forces. Thermokinetic potentials and forces are derived from chemical potentials and thermodynamic forces. In the case of mass-action kinetics in an ideal dilute solution that is a standard assumption for many kinetic models, the thermokinetic flow-force relationships are linear. Compared to the conventional kinetic modeling formalism, TKM has two main features that render it attractive for the modeling of biochemical reaction networks. The use of TKM structurally guarantees the thermodynamic feasibility of the model equations, and any thermodynamically feasible kinetic model can be expressed in the TKM formalism. Additionally, TKM strongly simplifies the application of several model reduction techniques.

The advantages of TKM are related to its use of flow-force relationships. The use of flow-force relationships structurally guarantees the non-negativity of entropy production and the vanishing of the fluxes in thermodynamic equilibrium. Thus, it guarantees the thermodynamic feasibility of the model equations. If the flow-force relationships have certain properties, model reduction methods can be applied. A reaction is in rapid equilibrium if its force but not its flux is negligible, and a species is in quasi-steady state if its concentration change but not its contribution to the forces is negligible. In both cases, the size of the model can be reduced. TKM strongly simplifies the reduction of the model because it is able to directly incorporate the conditions on the flow-force relationships. In particular, it is possible to rigorously derive a reduced reaction scheme with less species and less reactions. Model reduction does not require the formulation of the full system equations but can be performed on a list of reaction equations or reaction rules, which additionally simplifies model reduction. The treatment of the rapid equilibrium assumption is particularly simple because it requires only linear operations.

Additionally, the use of flow-force relationships allows for a graphical representation of TK models analogously to the graphical representation of electrical networks. The model reduction methods can be formulated as graphical rules that allow their convenient application.

In conclusion, TKM unites the advantages of conventional kinetic modeling and of thermodynamic flow-force relationships. Further, it strongly simplifies model reduction. For this reasons, TKM is an ideal tool for building mathematical models of large biochemical reaction networks.

A. Mathematica Implementation of TKM

The use of Thermokinetic Modeling (TKM) and the thermokinetic model reduction methods is especially advantageous for large systems because it guarantees the thermodynamic feasibility of the model equations. The treatment of large systems requires a computer tool that assist the modeler in formulating and reducing the model equations. For this purpose the *Mathematica* package TKMOD (Thermodynamik-Kinetic MODeling tool) was built. Mathematica is a computer program that provides a highly developed programming language that is especially suited for mathematical applications [104]. It provides a large set of routines for symbolic and numerical computations. Due to its flexibility, it is an ideal tool to develop a thermokinetic modeling tool.

The Mathematica package TKMOD provides functions for modeling, model reduction and simulation of thermokinetic models. In particular, if provides functions to define compartments, compounds and reactions. TKMOD builds and simulates the model equations from this information. Additionally, it provides routines for the application of the rapid equilibrium assumption (see Section 5.5.2.2, p. 107). The model of the oxygen response of *Escherichia coli* that is presented in Chapter 8 (p. 161) was built with TKMOD. The following section shortly describes TKMOD.

A.1. Model Description in TKMOD

TK models are described by a data structure that is a textual list of model elements. This data structure may be defined in a model description file. The three most important types of model elements are compartments, compounds and fluxes. Further model elements allow the input of additional equations and the definition of export and visualization routines.

An example for the definition of a compartment is the definition of the cytoplasm in the model of *Escherichia coli*:

```
Compartment["c",
            "Name"                 -> "cytoplasm",
            "Size"                 -> n["X"][t] * Vc,
            "Scale"                -> Vc0,
            "Value"                -> Vc0,
            "ElectricalPotential" -> 0,
            "Temperature"          -> 310.15,
            "Flux"                 -> -DIL*V["c"][t]]
```

The definition consists of an expression with the head `Compartment`. The first element of the expression is the unique identifier `c`. The rest of the expression consists of tags that describe the compartment `c`. The tag `Name` has purely documentary function. `ElectricalPotential` and `Temperature` define the value of the respective intensive state variables for the compartment. `Size` describes a law for the computation of the volume of the compartment. In this case, the volume depends on the number of cells `n["X"][t]` and the volume of a cell `Vc`. The volume of the compartment is variable because the number of cells `n["X"][t]`is a function of time `t`. TKM uses the provided law for the volume of a compartment, e. g. $V(t) = V_c\, n_X(t)$, in its differentiated form, e. g. $\dot{V}(t) = V_c\, \dot{n}_X(t)$. For this reason, an initial value `Value` has to be supplied. The value `Scale` is used to internally scale the resulting equations for the compartment size in order to get more convenient numerical behavior. In a reactor cells grow and thus the intracellular compounds are accordingly diluted. This dilution of the compounds is determined by the growth rate of the compartment that is defined by the tag `Size`. In a chemostat, cells are continuously removed from the reactor. This means that although the overall size of the cytoplasm grows, the volume of cytoplasm in the reactor can be constant or even decrease. The tag `Flux` determines this dilution rate of the compartment, which results from a continuous flow through the reactor. Here, the term `-DIL*V["c"][t]` describes the dilution in a chemostat with dilution rate `DIL`.

Compounds are defined in a similar way as compartments. An example for a compound definition is that of glucose-6-phosphate `g6p`, which is given by:

```
Compound["g6p",
         "Compartment" -> "c",
         "Name"        -> "D-Glucose 6-phosphate",
         "Formula"     -> "C6H11O9P",
         "Charge"      -> -2,
         "Capacity"    -> Cap["g6p"],
         "Value"       -> 0.4 mM["g6p"],
         "Scale"       -> 0.4 mM["g6p"],
         "Clamped"     -> False]
```

`Capacity` defines the thermokinetic capacity at an electrical potential of zero. `Cap["g6p"]` is a function that computes the capacity of glucose-6-phosphate at an electrical potential of zero from a table of Gibbs formation energies. The true thermodynamic capacity is determined automatically by a multiplication by a correction term involving the `Charge` and the electrical potential of the phase:

$$C_i = C_i' \exp\left(\frac{-F\, z_i\, \phi}{R^*\, T}\right) \tag{A.1}$$

where C_i is the corrected capacity and C_i' is the user-supplied capacity (see §5.26, p. 98). The Boolean value `Clamped` determines if the thermokinetic potential is clamped to `Value` or if `Value` determines only the initial condition of the thermokinetic potential. The function `mM["g6p"]` defines the conversion factor of the unitless thermokinetic potential into the concentration of glucose-6-phosphate given in millimolar.

The definition of a reaction flux follows the same principle as the definition of compartments and compounds. An example is the reaction $g6p \rightleftharpoons f6p$ catalyzed by the glucose-6-phosphate isomerase:

```
Flux["PGI",
     "g6p" ->  "f6p",
     "Name"          -> "glucose-6-phosphate isomerase",
     "Subsystem"     -> "Glycolysis/Gluconeogenesis",
     "EC"            -> "EC-5.3.1.9",
     "Compartment"   -> "c",
     "Resistance"    -> 0,
     "Value"         -> kp*c["g6p"][t],
     "Clamped"       -> False]
```

Here the Boolean value `Clamped` decides if the thermokinetic resistance in the slot `Resistance` is used to compute the flux or if the expression given in `Value` is used instead. `Value` may depend on constants, e. g. `kp`, concentrations, e. g. `c["g6p"][t]` or other quantities. The tag `Value` is without effect, if the flux is not clamped. `Resistance` defines the thermokinetic resistance at an electrical potential of zero. This tag is without effect, if the flux is not clamped. The user supplied resistance value is multiplied with a voltage-dependent correction term that is unity at an electrical potential of zero:

$$R_j = R'_j \exp\left(\frac{F\, z_j\, \phi}{R^*\, T}\right) \tag{A.2}$$

where R_j is the corrected resistance, R'_j is the user supplied resistance and $z_j = \sum_{i\in E_j} \nu_{E,ij}\, z_i = \sum_{i\in P_j} \nu_{P,ij} z_i$. In the expression for the concentration-dependent reaction rate (§5.18, p. 93), the correction factors for the resistances and for the capacities (Equation A.1) cancel out if all reaction partners are on the same electrical potential. Thus, the reaction velocity depends on the electrical potential only if reactants and products are in different compartments with different electrical potentials.

Due the correction terms in Equations A.1 and A.2, the modeler needs to supply the resistances and capacities for an electrical potential of zero. TKMOD automatically adapts the expressions such that reaction fluxes in a compartment are independent of the electrical potential, and reaction fluxes between reactants and products of different compartments with different electrical potentials can be conveniently modeled. In the latter case, the reaction needs to be placed in an additional compartment that models the interface between the compartments of reactants and products. By adjusting the electrical potential $\hat{\phi}$ in the interface compartment, different transition factors α with $\hat{\phi} = \alpha\, \phi' + (1-\alpha)\, \phi''$ can be realized (see §5.26, p. 98).

Defining compartments, compounds and reactions as introduced above results in a complete TK model that can be reduced and simulated using the functions introduced in the next section.

A.2. Functions in TKMOD

TKMOD provides several functions for dealing with thermokinetic models. Here, the most important functions are shortly described.

`CheckNetworkDescription` tests the network description for common errors and prints warnings and errors. `NetworkDescription2TKM` reads in a network description and transforms it into an internal data structure for the description of thermodynamic kinetic models. `TKMScale` scales the model by a translation of chemical potentials (see Section 5.4.2, p. 102) in order to get numerically convenient model equations. `ComputeConsistentIC` computes consistent initial conditions for models with vanishing resistances (see §4.42, p.66). `TKMReduce` reduces the system by performing the reduction steps for vanishing resistances developed in Section 5.5.2.2 (p. 107). `TKMsim` finally allows to simulate the reduced model. `ShowView` and `MakeReport` produce plots and write csv files (numerical data in the comma-separated values format) of the simulation results, respectively.

A.3. Conclusions

This chapter gave a short overview over the TKMOD package for modeling and model reduction of thermokinetic models. The tool allows the convenient definition and simulation of thermokinetic models. A list of model elements that describe compartments, compounds and reactions constitutes the input of TKMOD.

As demonstrated in Chapter 8, the tool TKMOD can be used for the thermokinetic modeling of large reaction networks. Currently, TKMOD is able to treat vanishing resistances. In the future, it will be extended to the more difficult problem of vanishing capacities. Further, it is planned to include rule-based modeling and model reduction, which is a prerequisite for the modeling of complex signal transduction networks.

The Mathematica package TKMOD is a powerful tool for TKM that proved to be a valuable tool for method development and for the development of a large TK model of the oxygen response of *Escherichia coli*.

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