Supporting information

Co-translational Folding Trajectory of the HemK Helical Domain

Evan Mercier and Marina V. Rodnina*

Department of Physical Biochemistry, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Goettingen, Germany

*To whom correspondence should be addressed. E-mail: rodnina@mpibpc.mpg.de

Biochemical procedures

Experimental data are taken from (*1*). Translation was carried out in HiFi buffer (translation buffer J (HiFi, 50 mM Tris-HCl, pH 7.5, 70 mM NH4Cl, 30 mM KCl, 3.5 mM MgCl2, 0.5 mM spermidine, 8 mM putrescine, and 2 mM DTT) at 37°C. Translation products were separated on Tris-Tricine SDS-PAGE and visualized on a FLA-9000 fluorescent gel scanner. Stopped-flow experiments were carried out on a SX-20MV stopped-flow apparatus (Applied Photophysics).

Initial fits

As a first step in developing a kinetic model for co-translational HemK folding, a linear kinetic mechanism (Figure S1A) was developed, where each amino acid is added at the same rate (k_{trans}). This model was fitted to *in vitro* translation time courses of HemK 42, 56, 70, 84, 98, and 112 (Figure 1A) using KinTek Explore software (Version 4) (*2*). The resulting fits (Figure S1B) clearly indicate that a linear model with a constant rate of translation is insufficient for describing HemK translation.

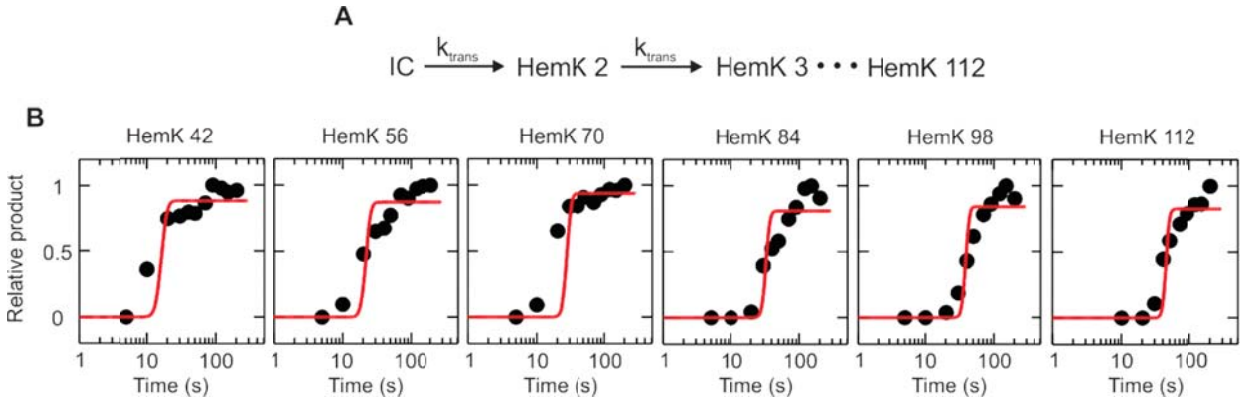

FIGURE S1. (A) Linear kinetic model wherein the HemK nascent chain is extended one amino acid at a time at a rate of k_{trans}. (B) Quantification of full-length product formation obtained from SDS-PAGE (black symbols, same as Fig. 1 C) plotted along with the fits obtained from global fitting to the linear model (red lines).

Global fitting

Global fitting was performed using KinTek Explorer software (Version 4) (*2*) to analyze co-translational folding of HemK based on FRET or PET time courses. The FRET dataset was obtained with BOP (donor) at the N-terminus and BOF (acceptor) at position 34 during donor excitation and acceptor emission detection. The PET data sets were obtained with BOP (donor)

at the N-terminus with (+Trp) or without (–Trp) tryptophan (quencher) at position 6 during donor excitation and donor emission detection. Independent global fits were performed for FRET, PET (+Trp), and PET (–Trp) data sets using the same kinetic model (Figure 1B). Each fit included one fluorescence (stopped-flow) time course (Figure 2A-C) and one translation time course (Fig. 1C) for each HemK construct (HemK 42, 56, 70, 84, 98, and 112) for a total of twelve experiments per fit. In order to fit multiple time courses ending at different positions on the same model, steps producing HemK 43, 57, 71, 85, and 99 were modeled as bimolecular reactions (HemK42 + B42 = HemK43, for example), with rate constants equal to (k_{trans}/1000 μM). Experiments with constructs longer than 42 amino acids, therefore, contained 1000 units of the 'phantom molecule' B42, while experiments with HemK42 did not. Since the total concentration of initiation complex in each experiment was 1 unit, these steps were pseudo first order with an apparent rate of k_{trans}, and did not slow down during the course of the simulated experiment. The same rate of translation (k_{trans}) was used for each step in the kinetic model, but the rate constants describing translational pausing ($k_{trap,38}$, $k_{rec,38}$, $k_{trap,78}$, and $k_{rec,78}$) were independent during the fitting procedure. Each fit, therefore, involved 5 independent rate constants. The fluorescence time courses simulated by the KinTek software (observables in the KinTek terminology) were defined by linear combinations of all 114 HemK constructs in the model, each multiplied by an intrinsic fluorescence intensity (IFI) (*3*). The IFIs for paused intermediates (HemK 38* and HemK 78* from Fig. 1. B) were assumed to be identical to the corresponding on-pathway intermediates (HemK 38 and HemK 78 respectively). For the PET data, it was necessary to apply scaling factors and fluorescence offsets to the observables for some experiments to account for day-to-day variation in signal amplitudes. Scaling factors were also applied to observables describing translation time courses in order to account for uncertainty in experimental endpoints.

In order to obtain reasonable estimates for the kinetic constants, a preliminary fit was performed where only the translation time courses were fitted to the kinetic model. Next, the fluorescence time courses were added to the fit in order determine the IFI values. The first fit provided very few defined IFIs since the uncertainty in many of the IFI values was larger than the IFI itself. To improve precision of the IFI values the number of IFIs was reduced by grouping intermediates of similar lengths and defining a single IFI for the group. Only neighboring IFIs were grouped: HemK50 and HemK51 might be grouped, for example, but HemK50 and HemK52 would not be grouped unless the group contained HemK50, 51, and 52. The six

constructs that were explicitly translated in the study (HemK42, 56, 70, 84, 98, and 112) were well defined at the outset and generally not included into groups. The one exception being a group of HemK41, 42, and 43 in the PET data set, which was necessary to define the intermediate lengths 41 and 43. Grouping was carried out in an iterative fitting procedure. Following a fit, intermediates represented by undefined IFIs would be grouped and another fit carried out. Intermediates were grouped first if neighboring groups had similar but undefined IFIs. If no such groups were present, neighboring groups with undefined IFIs were grouped, first in groups of two, then four, etc. A new round of fitting was carried out each time a group was changed, and the quality of the resulting fit was evaluated by χ^2 analysis as implemented in the KinTek software. Uncertainties in the maximum-likelihood parameters were determined based on the covariance matrix derived during non-linear regression in KinTek. For the PET data sets, PET(+Trp) was fitted first, and these groupings applied to the PET(–Trp) data sets. It was necessary, however, to split two groups (HemK 85-97 and 99-111) in order to obtain statistically significant fit to the PET(–Trp) data. Fitting and regrouping was then performed iteratively on PET(–Trp) and PET(+Trp) data sets until both data sets could be adequately fitted with the number of IFIs and the same groups of intermediates. The χ^2/DoF ratio calculated for each fit was 1.54, 1.66, and 0.42 for FRET, PET (+Trp), and PET (–Trp) respectively. Due to the complexity of the model, it was not possible to estimate the confidence contours by means of the FitSpace editor in KinTek Explorer. Thus, the standard error in the fit is reported for each fitting parameter.

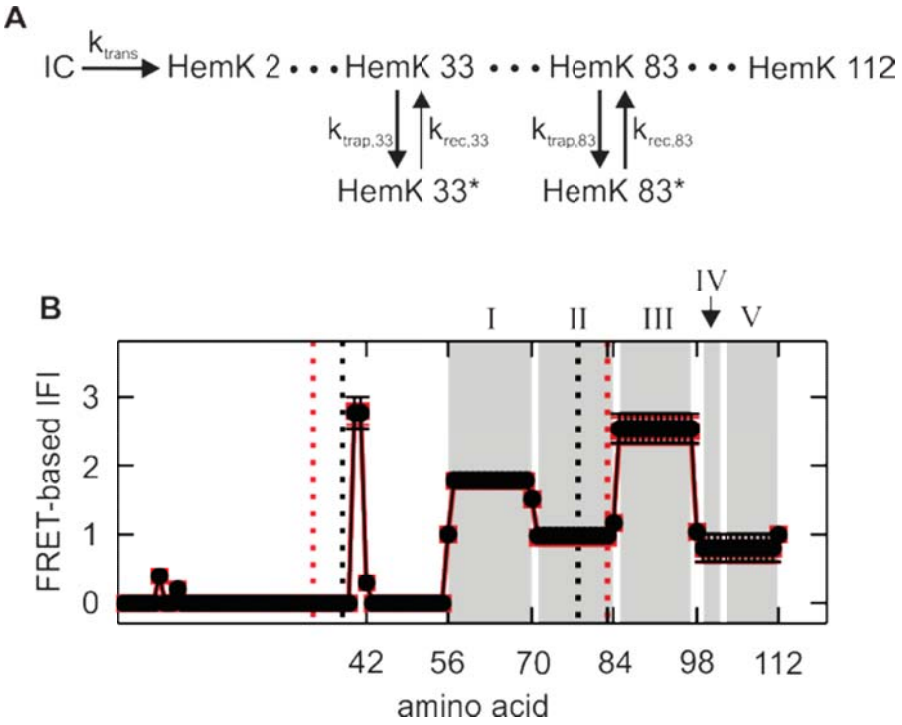

FIGURE S2. IFIs are insensitive to the exact position of translational pause sites. (A) Kinetic model of HemK translation with translational pause sites moved to positions 33 and 83. (B) IFIs calculated from global fitting of co-translational fluorescent changes in FRET-labeled HemK with translational pausing modeled at codons 33 and 83 (red) or 38 and 78 (black, as in Figure 3). Vertical dotted lines indicate positions where translational pausing was incorporated into the kinetic model (33/83: red, 38/78: black). Intermediates with distinct FRET IFIs are indicated by shaded areas and labeled I-V. The comparison shows that moving the translational pause sites by five codons (in opposite directions) has no effect on the calculated FRET-based IFIs, indicating the robustness of the model.

1. Holtkamp, W., Kokic, G., Jager, M., Mittelstaet, J., Komar, A. A., and Rodnina, M. V. (2015) Cotranslational protein folding on the ribosome monitored in real time. *Science 350*, 1104-1107.
2. Johnson, K. A. (2009) Fitting enzyme kinetic data with KinTek Global Kinetic Explorer. *Methods Enzymol 467*, 601-626.
3. Belardinelli, R., Sharma, H., Caliskan, N., Cunha, C. E., Peske, F., Wintermeyer, W., and Rodnina, M. V. (2016) Choreography of molecular movements during ribosome progression along mRNA. *Nat Struct Mol Biol 23*, 342-348.