

ShereKhan—calculating exchange parameters in relaxation dispersion data from CPMG experiments

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ABSTRACT

Summary: Dynamics governing the function of biomolecule is usually described as exchange processes and can be monitored at atomic resolution with nuclear magnetic resonance (NMR) relaxation dispersion data. Here, we present a new tool for the analysis of CPMG relaxation dispersion profiles (ShereKhan). The web interface to ShereKhan provides a user-friendly environment for the analysis.

Availability: A stable version of ShereKhan, the web application and documentation are available at <http://sherekhan.bionmr.org>.

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1 INTRODUCTION

Functions of biomolecules are governed by their dynamics of conformational interconversions sometimes induced by binding of a second partner. The kinetics of these processes need to be explored. Normally, kinetics of the mentioned processes can be described with exchange processes. For example, proteins can be in conformational equilibrium, which may be characterized by two different conformations of the same molecule, or in chemical equilibrium, which may represent the bound and unbound state of molecules to a binding partner. The environments of the nuclei may differ in each state and thus their nuclear magnetic resonance (NMR) parameters (e.g. chemical shifts, scalar or dipolar couplings and relaxation) may also be different. We focus here on processes, in which at least one of the magnetically active nuclei exchanges between these states by either chemical exchange or conformational exchange.

The study of chemical exchange processes by NMR is already well established (Mittermaier and Kay, 2006; Wang *et al.*, 2001). Depending on the time scale at which the exchange process occurs, various NMR techniques such as line shape analysis, measurements of the spin-spin or spin-lattice relaxation rates, the off-resonance saturation method and pulse spin-echo techniques such as the Carr–Purcell–Meiboom–Gill (CPMG) experiment can be applied. In particular, the exchange processes occurring within the micro- to millisecond time window can be detected by the NMR relaxation dispersion experiments by using CPMG or off-resonance irradiation approaches. The CPMG

experiment can provide thermodynamic (relative populations of the species), kinetic (rates of exchange) and structural information (in form of chemical shifts) at atomic. Although CPMG relaxation dispersion experiments are in general well-established, their analysis is not a straightforward process. Currently existing programs are available on request from the authors (e.g. CPMGFit), demand a number of software libraries (Bieri and Gooley, 2011), which are often not commonly available on personal computers and result in compatibility problems or require proprietary software (Kleckner and Foster, 2012). Therefore, we developed ShereKhan, which is accessible through a web interface allowing a user-friendly selection of residues and suggesting models for the calculation of kinetic parameters like the relative populations of the species, the exchange rate and chemical shift information from the CPMG relaxation dispersion data. ShereKhan assumes a global two-state exchange process to fit the data with models for the slow or fast exchange.

2 FEATURES

The ShereKhan web application provides an easy way to calculate exchange parameters (rates, relative populations and their structural information) of molecules (Fig. 1). The workflow has been designed to guide the user through the process from uploading data to the calculation of the exchange rates and populations of the states. The input file for a dataset is a simple tab-delimited text file containing R_2 relaxation rate values including error estimates at various ν_{CPMG} values for each residue (Fig. 1A), which can also be calculated in the program with peak intensities from the two point sampling scheme (Mulder *et al.*, 2001). In addition, it must be specified with which resonance frequency and constant-time relaxation delay T_{ep} the data have been recorded. The residues must not be consecutive but should be numbered sequentially for calculating the chemical shift difference plot. ShereKhan accepts any number of datasets, e.g. data recorded at a pair or multiple field strengths. If the experimental datasets include relaxation dispersion data measured at two (or more) different magnetic fields, ShereKhan suggests the exchange regime (slow or fast exchange regime) for each given residue to facilitate the selection of an appropriate model (Millet *et al.*, 2000). Subsequently, specific sets of residues (e.g. with a certain exchange regime) or any combination of residues can be selected. In this way, residues containing ambiguous data can be deselected before starting the calculations. If high power $R_{1\rho}$ experiments that essentially represent the exchange free R_2

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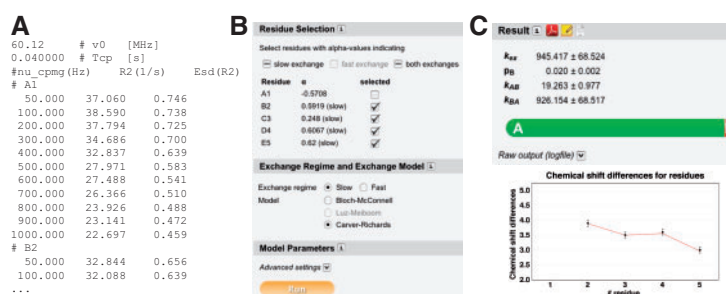


Fig. 1. (A) The input file for ShereKhan containing R_2 relaxation rate values including error estimates $Esd(R_2)$ at various ν_{CPMG} values for each residue. (B) Part of ShereKhan's user interface showing the residue selection and parameter options. (C) As output ShereKhan provides exchange regime-dependent kinetic parameters, a chemical shift difference plot and graphs with the fits (data not shown)

relaxation rate values are included (Ban *et al.*, 2012), the distinction between the fitting models is not necessary any more.

2.1 Applying different exchange regimes

In the current implementation, the user can choose between two exchange regimes (on the NMR time scale), slow or fast exchange (Fig. 1B). After assigning the regime, the user can select a fitting model, namely, the Carver–Richards model (Carver and Richards, 1972; Davis *et al.*, 1994) or the Luz–Meiboom model (Luz and Meiboom, 1963) for the slow or the fast exchange regimes, respectively. Alternatively, users can use the Bloch–McConnell equation (McConnell, 1958), which works for both regimes. Chemical shift variances and intrinsic R_2 relaxation rates [be they fit parameters or available from high-power $R_{1\rho}$ experiment (Ban *et al.*, 2012)] are residue specific, but kinetic parameters can be fitted globally. Starting values for the parameters of the model can either be suggested by ShereKhan or be specified by the user.

2.2 Displaying and exporting results

For residues in the fast exchange regime, only the exchange rates and their population-weighted chemical shift differences can be extracted from the fitting of NMR relaxation dispersion data, whereas for residues in the slow exchange regime, all parameters such as rate constants, populations and chemical shift differences can be obtained from the fitting. Fits of the model to the data can be browsed interactively or downloaded in various graphics formats.

3 IMPLEMENTATION

ShereKhan consists of a command-line program and a WWW-based tool, accessible through any modern web browser. The program was written in Python supplemented with the scipy (<http://www.scipy.org>) and matplotlib libraries (<http://matplotlib.sourceforge.net>) and the JSON parser. The *leastsq* fitting routine with the default convergence criteria was used. The web application framework is Ruby on Rails (<http://rubyonrails.org>). To present the user with a feature-rich interface, the site makes extensive use of modern Web 2.0 techniques like Ajax (Asynchronous JavaScript and XML) using jQuery

(<http://jquery.com>) and FancyBox (<http://fancybox.net>). Interactive graphs are drawn with the graphical toolkit Protovis [<http://protovis.org>; (Bostock and Heer, 2009)]. User-uploaded data are stored temporary on the server and deleted when leaving the application.

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