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Applications Note

Structural bioinformatics

Efficient identification and analysis of chemical exchange in biomolecules by $R_{1\rho}$ relaxation dispersion with *Amaterasu*

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Abstract

Summary: We introduce here a novel acquisition and processing methodology for cross-polarization based 1D rotating-frame relaxation dispersion NMR experiments. This easy-to-use protocol greatly facilitates the screening, acquisition, processing and model fitting of large on- and off-resonance $R_{1\rho}$ relaxation dispersion NMR datasets in an automated manner for the analysis of chemical exchange phenomena in biomolecules.

Availability and Implementation: The *Amaterasu* package including the spreadsheet, Bruker pulse programs and analysis software is available at www.moleng.kyoto-u.ac.jp/~moleng_01/amaterasu.

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1 Introduction

Protein and nucleic acid functions are central to all cellular processes. Whereas the function of many biomolecules seems to be explained by a single static structure, many macromolecules such as enzymes are highly dynamic and rely on sampling multiple conformations for their function. Accurate characterization of these dynamic conformations demands methods that can probe energetically excited minor states in solution. Relaxation dispersion NMR spectroscopy (RD NMR) is uniquely suited to probe motion on the biologically important timescale from microseconds to milliseconds, as highlighted by recent research on protein binding (Sugase *et al.*, 2007), enzymatic activity (Boehr *et al.*, 2006) and base mispairing (Kimsey *et al.*, 2015).

Relaxation Dispersion NMR facilitates the extraction of structural (chemical shift, $\Delta\omega$), kinetic (lifetime, $1/k_{ex}$) and thermodynamic (populations, p_i) information about the excited states by quantifying the broadening of resonance lines caused by the chemical exchange process. $R_{1\rho}$ RD resolves motion on the unique timescale of several tens of microseconds to milliseconds by screening the spin-lock power (ω_1) and/or offset (Ω) dependence of $R_{1\rho}$, the relaxation rate of a nucleus in the rotating frame. The state-of-the-art version of this experiment achieves superior temporal resolution by probing one resonance at a time using 1D NMR (Korzhnev *et al.*, 2005); however, it requires the time-consuming acquisition, processing and integration of $n * m$ free-induction decays (FIDs), where n is the number of resonances probed and m is the number of variable spin-lock power (ω_1) or offset (Ω) values. Accordingly, even for a biomolecule as small as an SH3 domain ($\sim$ 60 amino acids), very large 1D datasets must be acquired, processed and integrated before the $R_{1\rho}$ values can be fitted to a theoretical model. We considered that this labor-intensive procedure is limiting the broader applicability of this promising approach. Thus, our aim was to develop an efficient methodology to facilitate the investigation of chemical exchange in biomolecules by $R_{1\rho}$ RD.

2 Implementation

We addressed the challenges of $R_{1\rho}$ RD NMR by a developing a pipeline consisting of three modules. In the first, an $R_{1\rho}$ RD-specific spreadsheet automatically calculates acquisition parameters that

vary from one experiment to another. In the second, a novel pulse program acquires an entire set of 1D RD NMR data (*n* resonances * *m* spin-lock power and/or offset values) in a single acquisition run as a pseudo-2D experiment. This greatly simplifies data acquisition because only a single experiment has to be set up, instead of a large series of conventional 1D experiments. In the third, the newly written Unix software package *Amaterasu* (automated $R_{1\rho}$ analysis utility) processes all data from raw FIDs to the final model-fitted RD NMR curves.

The flow through our $R_{1\rho}$ RD NMR pipeline is illustrated in Figure 1. First, $^{1}H_N$ and ^{15}N chemical shifts are obtained from a standard heteronuclear single-quantum coherence (HSQC) experiment. Next, our spreadsheet calculates all experimental parameters such as spin-lock power values ω_1, the delay ζ, variable pulse lengths and offset Ω, which are passed to the pulse program as text files (for experimental details, see Korzhnev *et al.*, 2005). In most proteins, only a few residues report on chemical exchange; therefore, these residues are identified first by performing a quick screening experiment in which all resonances are probed by application of a weak and a strong spin-lock field, ω_1. In the absence of chemical exchange, the corresponding peak intensity is equal in both spectra, whereas a lower intensity in the spectra recorded at weak spin-lock fields indicates chemical exchange. The resulting pseudo-2D spectrum is split into its respective 1D components and passed to *Amaterasu* via a simple graphical user interface (Fig. 1).

Amaterasu automatically reads all acquisition parameters and conducts apodization, zero-filling, Fourier-transform and phase correction on all FIDs (Chen *et al.*, 2002; Helmus and Jaroniec, 2013). Next, it performs peak-picking and intensity extraction of all spectra, while recognizing the correct peak on the basis of its a priori known chemical shift. If the intensity ratio indicates that the resonance exhibits chemical exchange, *Amaterasu* selects this resonance for a full experiment; unpromising resonances are discarded to save acquisition time. Optionally, the program can plot all spectra to check artifacts introduced due to the automated nature of the analysis. Next, a full RD experiment is recorded on the resonances identified in the screening step. This experiment is again recorded as a single pseudo-2D experiment, while the spin-lock power ω_1 and/or offset Ω values are varied between experiments. Raw data are then integrated by *Amaterasu*, and peak intensities as a function of ω_1 and/or Ω are directly passed to the fitting program GLOVE (Sugase *et al.*, 2013), which fits the experimental data to a theoretical model. For a standard dataset, a single run of *Amaterasu* from reading the raw FIDs to output of the model-fitted RD NMR curves finishes in <1 min on a standard desktop PC (e.g. Mac mini, 2015).

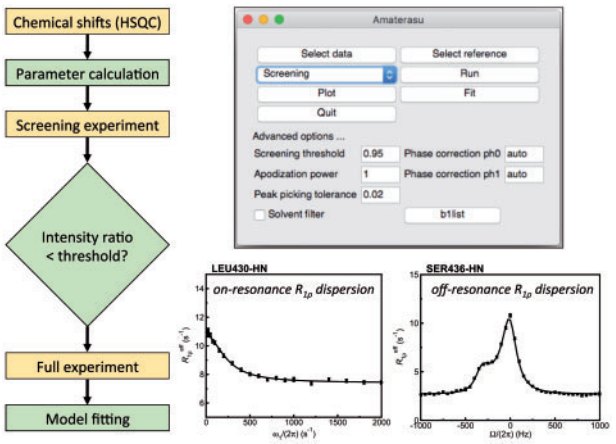

Fig. 1. Analysis of $R_{1\rho}$ RD NMR data with *Amaterasu*. After obtaining chemical shifts, variable experimental parameters are calculated. A screening experiment then probes all resonances for chemical exchange. Positively screened resonances are selected for a full experiment, in which the spin-lock power ω_1 and/or offset Ω are varied between experiments. The resulting data are automatically processed and fitted to a theoretical model by *Amaterasu*. Example RD curves for on- and off-resonance experiments on the p62 UBA domain are shown; yellow indicates experimental steps; green indicates steps automated by our pipeline

3 Results and conclusion

We tested the performance of *Amaterasu* on 3 NMR samples: the UBA domain of p62, superoxide dismutase 1 (SOD1) and ubiquitin. HSQC cross-peaks were picked and passed to our spreadsheet for calculation of the variable acquisition parameters (Fig. 1). Screening experiments identified 33 (p62), 37 (SOD1) and 6 (ubiquitin) resonances with a (weak/strong spin-lock) intensity ratio below a threshold of 0.95. As a specific example, analysis of p62 is discussed. Visual inspection of the spectral plots generated by *Amaterasu* revealed 2 resonances for which the analysis was deemed difficult due to resonance overlap. Accordingly, the remaining resonances were selected for a full $R_{1\rho}$ RD experiment in which 23 spin-lock power values ω_1 ranging from 25 to 2000 Hz on-resonance ($\Omega = 0$) were applied. The resulting FIDs were integrated by *Amaterasu* and fitted to a two-site exchange model (Miloushev and Palmer, 2005), thereby yielding residue-specific values of k_{ex}, $\Delta\omega$ and p_B. We also performed off-resonance $R_{1\rho}$ RD experiments with spin-lock offset Ω values ranging from −1000 to +1000 Hz and performed data fitting. Example RD curves are shown in Figure 1.

In conclusion, we have shown that our novel *Amaterasu* methodology greatly facilitates both the identification of those residues that report on chemical exchange phenomena in biomolecules and the thorough analysis of these site-specific probes by efficient quantification of on- and off-resonance $R_{1\rho}$ RD datasets. We anticipate that future $R_{1\rho}$ RD experiments in conjunction with *Amaterasu* will shed new light on the dynamic character of biomolecules beyond their ground-state structure.

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Conflict of Interest: none declared.

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