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Looped-PROjected SpectroscopY (L-PROSY): A simple approach to enhance backbone/sidechain cross-peaks in ^{1}H NMR

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ABSTRACT

Cross-relaxation and isotropic mixing phenomena leading to the Nuclear Overhauser Effect (NOE) and to the TOCSY experiment, lie at the center of structural determinations by NMR. 2D TOCSY and NOESY exploit these polarization transfer effects to determine inter-site connectivities and molecular geometries under physiologically-relevant conditions. Among these sequences' drawback, particularly for the case of NOEs, are a lack of sensitivity arising from small structurally-relevant cross peaks. The present study explores the application of multiple Zeno-like projective measurements, to enhance the cross-peaks between spectrally distinct groups in proteins –in particular between amide and aliphatic protons. The enhancement is based on repeating the projection done by Ramsey or TOCSY blocks multiple times, in what we refer to as Looped, PROjected Spectroscopy (L-PROSY). This leads to a reset of the amide/aliphatic transfer processes; the initial slopes of the NOE- or J-transfer effects thus define the cross-peak growth, and a faster cross-peak buildup is achieved upon looping these transfers over the allotted time T_1. These projections also help to better preserve the magnetization originating in the amides, resulting in an overall improvement in sensitivity. L-PROSY's usefulness is demonstrated by incorporating it into two widely used protein NMR experiments: 2D ^{15}N-^{1}H HMQC-NOESY and ^{15}N-filtered 2D NOESY. Different parameters dictating the overall SNR improvement, particularly the protein correlation times and the amide-water chemical exchange rates, were examined, and L-PROSY's enhancements resulted for all tested proteins. The largest cross-peak enhancements were observed for unstructured proteins, where chemical exchanges with the solvent of the kind that tend to average out NOE cross-peaks in conventional NMR, boost L-PROSY's cross-peaks by replenishing the amide's magnetizations within each loop. Enhanced cross-peaks were also found in extensions involving TOCSY-based experiments when applied to proteins with unfolded segments.

1. Introduction

NMR provides unique vistas about molecular dynamics at atomic resolution, which are simply unobtainable by other forms of spectroscopy or imaging. The elucidation of dynamics by NMR has been going on for seventy years, ever since Bloembergen, Purcell and Pound (BPP) established the intimate connection between internuclear correlation times and spin relaxation [1–4]. Several distinct mechanisms further strengthen these bonds between NMR and dynamics, including the establishment of connectivities by NMR via chemical exchange and derived spectroscopies [5–11], and the realization that cross-relaxation between spins provides dynamic insight as well as new routes to enhance NMR sensitivity and to establish structural connectivities via the Overhauser effect [12–14]. Nuclear Overhauser Enhancement (NOE) involves transferring out-of-equilibrium nuclear polarization from one spin bath to another via dipolar interactions, providing insight about which atomic sites are in close proximity to one other [15–17]. NOE became a central tool in biophysical NMR determinations when incorporated into the two-dimensional NOE spectroscopy, 2D NOESY, where it can establish distances between spins that are proximate in space under native conditions in a general, broadband fashion [18–22]. Despite being one of the most important and widely performed 2D NMR experiments, NOESY suffers from a relatively low sensitivity, as the off-diagonal cross-peaks carrying the structurally relevant information only involve a small fraction of the total magnetization. The present study introduces a simple approach that we denominate Looped PROjected SpectroscopY (L-PROSY, as in leprosy, /leprəsē/), which provides a possibility to enhance cross-peaks between distinct sets of sites by several-fold.

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Spins in NOESY are excited and encoded by an evolution time t_1, which is followed by a storage pulse that projects their evolved magnetizations into the Bloch sphere longitudinal z-axis [5,6,23,24]. The ensuing Ramsey projections of the magnetizations [25,26] will in general be out of thermal equilibrium, leading to their cross-relaxation with other species. This in turn opens the possibility of establishing inter-site correlations upon interrogating their nature over the course of a second acquisition time t_2. To visualize how L-PROSY can magnify inter-site correlations we focus on the specific aim of establishing such NOE cross-peaks between distinct amide and aliphatic protons in a protein molecule –a common scenario, which is also analyzed experimentally below. The evolution of the ensuing cross-peaks can be analyzed by considering a model based on Solomon's equations [4,17,27] involving three distinct sites, where one represents the amide protons, the other represents the aliphatic protons, and for generality we also consider a potential water site which could also disturb the ^{1}H amide magnetization by mutual chemical exchanges. Furthermore, we consider that in the experiment in question it was the amide proton magnetization that was perturbed by the effects of the pulsing, and that it is its cross-relaxation to the aliphatic site that will generate the relevant NOESY peak. The buildup of this cross-peak will depend on the auto-relaxation rates $R_1^{H_N}$, $R_1^{H_C}$ and R_1^W of the amide, aliphatic and water protons, on the cross-relaxation rate σ between the aliphatic and amide protons, and on the chemical exchange rate $k_{W\to NH}$ between the amide protons and the water. For simplicity we assume the latter to represent the solution of the first-order kinetic reaction $k_{H_N\to W}[protein] = k_{W\to H_N}[water]$; the R's and σ rates will in turn depend on the internuclear amide/aliphatic distance r_{HH} (kept constant at 2.3 Å in the calculations below) and on the internuclear correlation tumbling time τ_c (varied as further described). The Solomon equations for such a system are then

$$\frac{d}{dt}\begin{pmatrix} M_{H_N}(t)-M_{H_N}^{eq} \\ M_{H_C}(t)-M_{H_C}^{eq} \\ M_W(t)-M_W^{eq}\end{pmatrix} = \begin{pmatrix} -R_1^{H_N}-k_{H_N\to W} & \sigma & k_{W\to NH} \\ \sigma & -R_1^{H_C} & 0 \\ k_{H_N\to W} & 0 & -R_1^W-k_{W\to NH}\end{pmatrix} \times \begin{pmatrix} M_{H_N}(t)-M_{H_N}^{eq} \\ M_{H_C}(t)-M_{H_C}^{eq} \\ M_W(t)-M_W^{eq}\end{pmatrix}$$

$$\frac{d}{dt}(M(t)-M^{eq}) = \hat{\hat{R}}(M(t)-M^{eq}) \qquad (1)$$

where the M's denote the normalized longitudinal magnetization of the different sites, and $\hat{\hat{R}}$ is their time evolution matrix. The thick purple lines in the various panels of Fig. 1 show the fate of the cross- peak predicted by Eq. (1) for a case where the amide peak has been taken entirely out of equilibrium, and all remaining reservoirs were left unchanged –a simulation done by propagating two experiments whose initial states were $M_1(0) = \begin{pmatrix}1\\1\\1\end{pmatrix}$ and $M_2(0) = \begin{pmatrix}-1\\1\\1\end{pmatrix}$, the equilibrium state $M^{eq} = \begin{pmatrix}1\\1\\1\end{pmatrix}$ was kept constant, and then the average amide and aliphatic magnetizations arising from each experiments were subtracted. This simple case can also be solved analytically (Appendix) leading to the well-known behavior whereby the aliphatic protons representing the NOE cross-peaks build up by receiving magnetization from the amides but then start decaying due to the auto-relaxation driving these spins irreversibly back towards zero. The buildup of these cross-peak intensities is initially dominated by cross-relaxation, and in the absence of auto-relaxation they should equilibrate with the amide intensities at 0.5 values; the R_1 and $k_{H_N\to W}$ processes, however, undermine this buildup, and eventually lead to the much smaller cross-peaks usually observed in NOESY experiments. Similarly, the amide peak decays multi-exponentially under the effects of auto- and cross-relaxation and of water exchanges over the course of the ensuing (mixing) time.

This simplified description suggests a potential route to enhance the amide → aliphatic transfers: if instead of letting the cross-relaxation evolve until reaching its maximum amplitude as normally done in NOESY, one were to "freeze" it early and repeat it multiple times, the NOE cross-peaks could grow at the much more favorable rates characterizing their initial buildup. Indeed, repeating NOESY's Ramsey projection multiple times for short mixings, can suppress the dissipative effects in the amide and aliphatic evolution. This results in a Zeno-like effect [28,29] whereby evolution appears to proceed driven by NOE's fast initial buildup, enhancing the growth of the aliphatic cross-peak while protecting the amide's magnetization from auto-relaxation losses. Furthermore, the solvent exchange processes that were deleterious when considering single, long evolution mixings come now to the aid of the amide/aliphatic cross-peak buildups, by supplying fresh

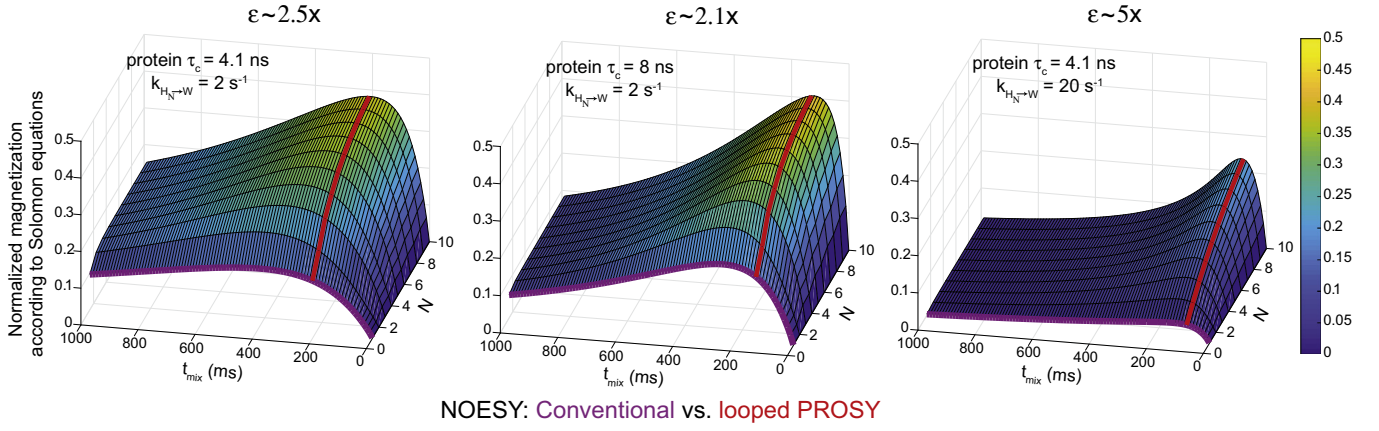

Fig. 1. Cross-peaks' evolution between idealized amide and aliphatic protons in the presence of a water reservoir, evaluated according to the Solomon Equations for three different sets of conditions: (a) Correlation time of 4.1 ns and amide-water exchange rate of 2 Hz, corresponding to average ubiquitin parameters ($M_W \approx 8.5$ KDa) at room temperature. (b) Slower correlation times characteristic of larger proteins. (c) Short correlation times but fast amide-water exchange rates characteristic of unstructured protein domains and of IDPs. The distance between dipolar-coupled amide and aliphatic protons was chosen as 2.3 Å. The purple lines show the expectation from conventional NOE transfer experiments, whereas the full surfaces represent the cross-peak intensities as a function of mixing time and of number of loops over which NOESY's Ramsey projection is executed – i.e., the number N of L-PROSY repetitions. Red lines emphasize the maximum L-PROSY enhancement for the various conditions. Maximal L-PROSY theoretical enhancements ε are indicated above each surface.