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Rectifying system-specific errors in NMR relaxation measurements

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Abstract

^{15}N spin relaxation parameters provide a powerful tool for probing the internal dynamics and thermodynamics of proteins. The biological insight provided by these experiments often involves interpretation of small changes in relaxation parameters. This, in turn, requires careful data analysis, especially in the identification and treatment of systematic error. While progress continues on reduction of experiment-specific errors associated with pulse sequences, system-specific sources of error have received far less attention. The impact of these errors varies between facilities, spectrometers, and biological samples. We demonstrate that performing a series of control experiments along with relaxation measurements can help identify, quantify, and isolate sources of system-specific error, and, in some cases, correct for systematic changes. We further demonstrate that control experiments can be performed without significant loss of spectrometer time, and lead to more accurate relaxation parameter values.

Keywords: NMR; Relaxation dispersion; Protein dynamics; Systematic error

1. Introduction

^{15}N spin relaxation parameters provide a powerful tool for probing the internal dynamics and thermodynamics of proteins. They have been used to gain insight on protein folding [1], catalysis [2], and ligand binding [3], as well as conformational entropy and activation energies [4–6]. These biological insights often involve interpretation of small changes in relaxation parameters and require careful data analysis.

Careful data analysis requires cautious treatment of both random and systematic errors. A typical protocol for estimation of random errors includes measurement of duplicates or replicates [7,8], and setting of a minimum error (such as standard deviation of baseplane noise [9], or an ad hoc value, such as 2% [10,11]). Treatment of systematic errors is more complicated. Over the years, a number of sources of systematic error have been identified, and their impact on relaxation analysis mitigated. Examples of experiment-specific sources of error that have been addressed include scalar coupling [12], cross-relaxation and dipolar/CSA cross-correlation [12], off-resonance effects [13–16], sample heating [17], and decoupling effects [18]. Examples of system-specific sources of error that have been addressed include magnetic field inhomogeneity [19] and peak intensity analysis [20]. While progress continues on identification and elimination of experiment-specific sources of error, system-specific sources of error have received far less attention. The impact of these errors varies between facilities, spectrometers, and biological samples.

1.1. Historical treatments of systematic error were sufficient for historical measurements

Systematic changes in peak intensity over a series of relaxation measurements have long been observed and reported [8]. One of the most common ways to address these systematic changes is to fold them into the random error by sampling points along the decay curve in ''pseudo-random'' order, rather than sequentially in time. The impact of this ''random'' error is then reduced by sampling the intensity at a large number of time points along the decay curve with repeated measurements at individual time

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points [21,22]. This method has been sufficient for analysis of exponential decay functions, $I(t) = e^{-Rt}$. When measured over an appropriate range of time points, the amplitude of an exponential decay is much larger than the size of typical systematic and random errors. Small systematic errors have only a slight effect on the accurate extraction of R. For example, systematic errors in intensity of up to 5% in measurements of R_2 at 500 MHz cause deviations of only 0.4–1.3% in the extracted value of R_2, depending on the form of the underlying error function and the order in which data are acquired (vide infra). Hence, while various forms of systematic error can change the percent accuracy of extracted R_2 values by more than a factor of three, the least accurate value is still within 1.3% of the "true" value, suggesting that historical treatments of systematic error were sufficient for exponential decay measurements.

1.2. The effects of systematic error can be mitigated by transferring changes in intensity to changes in line shape

More recently, Orekhov et al. [23] have proposed that relaxation measurements be performed in an "interleaved manner." This approach transfers systematic error from the time domain into the frequency domain, and trades systematic changes in intensity, $I(t)$, for systematic changes in line-shape, $I(\omega_N)$. This method can be very effective in reducing the impact of systematic error. Employing this method requires implementation of four changes: (1) adapting the relaxation experiments to increment the relaxation delay for each ω_N before incrementing ω_N (trivial for Varian BioPack users); (2) separating the arrayed fids for data processing; (3) shifting from analysis of peak volumes to analysis of peak intensities; and (4) verifying that extracted values of chemical exchange are not correlated with ω_N (a phenomenon that may be observed if the relaxation rate changes as a function of time). Unfortunately, this method has not been widely adopted—a cited reference search reveals citations of this method by only four other groups.

1.3. New measurements require new treatments of systematic error

In recent years, relaxation dispersion measurements have been increasing in popularity, largely displacing traditional measurements of R_2 at a single ν_{CPMG} frequency. These dispersion curves can be difficult to characterize at low spectrometer frequencies (500–600 MHz), due to shallow curvature and large experimental error [24–26]. For example, at 500 MHz, systematic errors in intensity of up to 5% can cause deviations in the extracted value of $R_2(\nu_{CPMG} = \infty)$ that range from 1.3 to 36%, depending on the form of the underlying error function and the order in which data are acquired (vide infra). At higher spectrometer frequencies, dispersion curves are more easily characterized: at 800 MHz, systematic errors in intensity of up to 5% cause deviations of less than 1% in the extracted value of $R_2(\nu_{CPMG} = \infty)$ (vide infra). However, the extraction of meaningful exchange parameters from relaxation data requires knowledge of the exchange timescale [25], and hence, characterization of dispersion curves at multiple field strengths [24,27,28]. It is necessary, therefore, to reduce experimental errors sufficiently that dispersion curves can be characterized at low field strengths, as well.

We demonstrate that performing a series of control experiments along with relaxation measurements can help identify and isolate sources of systematic error, and, in some cases, correct for systematic changes in the state of the system. We further demonstrate that the benefits of control experiments can be gained without significant loss of spectrometer time and without alteration of pulse sequences or data processing protocols.

2. Results and discussion

2.1. Effect of systematic error and data acquisition order on extracted relaxation parameters

As noted in Section 1, three main factors determine the impact of systematic errors on the accuracy and precision of extracted relaxation rates: the shape of the curve, the underlying form of the error, and the order in which data points are collected. To evaluate the impact of systematic error on extracted relaxation rates we examined two types of curves, exponential decay and relaxation dispersion. For each curve we explored five different cases, sampling from two types of underlying systematic error (systematic increase or systematic decrease in intensity), and four possible collection orders (increasing increment, decreasing increment, alternating large and small increments [triangular], and "pseudo-random," see Supplemental Figs. S1 and S2). Models were constructed as described in Sections 4.6 and 4.7.

For the exponential decay curve, changes in the error function and collection order caused changes of more than a factor of three in the percent error of the fitted parameters; however, all extracted relaxation rates were within an acceptable range of accuracy (errors of 0.4–1.3%, see Supplemental Table S1A). In contrast, for the relaxation dispersion curve simulated at 500 MHz, changes in the error function and data collection order produced changes of more than an order of magnitude in the percent error of the fitted parameters, with errors in $R_2(\nu_{CPMG} = \infty)$ ranging from 1.3 to 36% and errors in τ_{ex} ranging from 13 to 94% (details in Supplemental Table S1B).

For the two curves and five cases we considered, the most accurate values of R_2 and $R_2(\nu_{CPMG} = \infty)$ were obtained for a systematic decrease in intensity with data points measured in order of decreasing increment (Supplemental Figs. S1a and S2a). The least accurate values of R_2 and $R_2(\nu_{CPMG} = \infty)$ were obtained for a systematic increase in intensity and points measured in order of alternating increment (triangular function, Supplemental Figs. S1b and S2b—notably, this function also provides insight into the