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The experimental accuracy of the uni-directional exact NOE

Dean Strotz, Julien Orts, Martina Minges, Beat Vögeli *

Laboratory of Physical Chemistry, Wolfgang-Pauli-Str. 10, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

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ABSTRACT

We have established protocols to calculate exact NOEs (eNOE) from NOE data. eNOEs lend unprecedented precision to the calculation of distance restraints used for structure calculation. Moreover, as eNOEs are averaged quantities over all conformations of a molecule, they may contain accessible information of the sampled conformational space. In practice, a prerequisite for an exact interpretation is the evaluation of both NOESY cross-peak buildups. For large molecular sizes, the fraction of NOEs which can only be obtained from one cross peak typically increases. Distance restraints derived from such NOEs must be used with a tolerance for errors associated with the broken symmetry of the individual magnetization transfer pathways. The correct choice of upper and lower limits is particularly important for multiple-state ensemble calculation, where too narrow tolerances may lead to incorrect spatial sampling. In order to dissect these pathways in heavy-atom resolved 3D NOESY experiments, we analyze 2D [^{1}H, ^{1}H]-NOESY experiments, which are the fundamental building blocks of the former. In combination with an analysis of excitation and inversion profiles of pulses on heavy atoms and relaxation effects during HXQC elements, we derive a rule for the correct choice of upper and lower distance limits derived from such uni-directional NOEs. We show that normalization of the cross- to the diagonal-peak intensities of the spins of magnetization destination rather than origin leads to similar errors of the distance restraints. This opens up the prospect of extended collection of unidirectional eNOEs.

1. Introduction

Conversion of the nuclear Overhauser enhancement (or effect, NOE) into approximate upper distance limits is the most valuable tool in biomolecular structure determination in the liquid state [1,2]. Building on previous work [3–10], we have established protocols to calculate exact NOEs (eNOE) from NOE data [11–13]. eNOEs lend unprecedented precision to the calculation of distance restraints used for structure calculation [14,15]. Moreover, when the eNOE's nature of an averaged quantity [16–22] is accounted for in structure determination, it also provides information of the sampled conformational space [11–13,23,24].

The exact determination of an eNOE requires the analysis of the buildups of both cross peaks [14]. In practice, many NOEs can only be determined from one cross peak due to asymmetry in the resolution of the ^{1}H-resolved dimensions or in the peak intensities [14,23]. As a consequence, the distance derived from a single cross-peak buildup has an additional error, which turns into an elevated upper limit and a reduced lower limit restraint in the structure determination [11,23]. It is not a trivial task to estimate the order of the error. In previous studies, the limits of the distances have been set to 15% or 20% higher and lower values. These limits were approximate estimates from comparisons with high-resolution structures [23]. Here, we rationalize the choice of these limits by an in-depth analysis of the asymmetry of the magnetization pathways leading to the two cross peaks.

NOE buildups that could not be normalized to the intensity of the diagonal peak of the spin of the origin magnetization have in the past been discarded in our protocol. Alternatively, we proposed to derive a more conservative upper distance restraint limit ('generic normalized eNOE') [25]. To define tighter distance limits for a subset of these distance restraints, we compare uni-directional eNOEs normalized to the origin of magnetization to such normalized to the destination of magnetization.

1.1. Dissecting multi-dimensional NOESY pulse sequences

In this work, the fates of the magnetization pathways leading to the cross and diagonal peaks, that ultimately determine the accuracy of the extracted cross-relaxation rate, are analyzed. Typical 2D and 3D NOESY pulse sequences and the spectral peaks are depicted in Fig. 1.

* Corresponding author at: Laboratory of Physical Chemistry, HCI F217, Vladimir-Prelog-Weg 2, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland.
E-mail address: beat.voegeli@phys.chem.ethz.ch (B. Vögeli).

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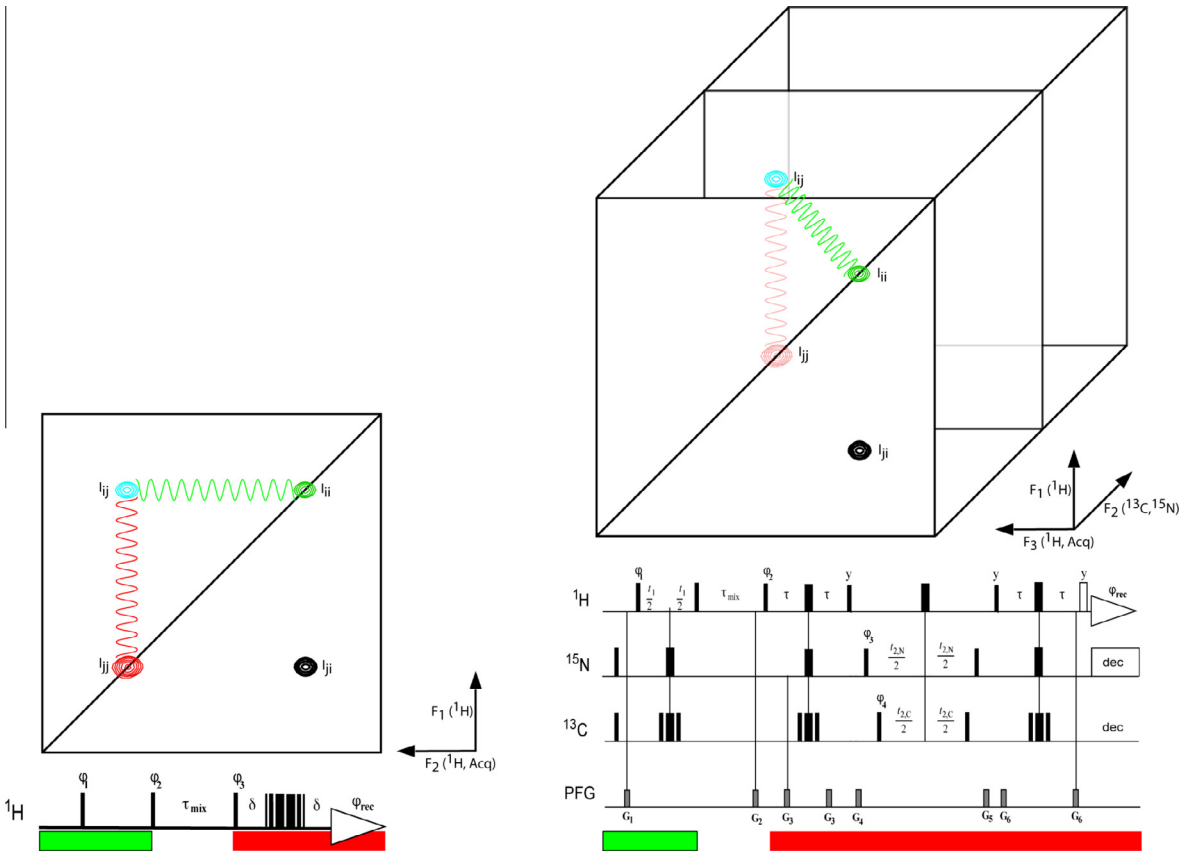

Fig. 1. Illustration of the NOESY pulse sequence segments and their relation to the recorded spectra. A 2D [^{1}H, ^{1}H]-NOESY sequence with water suppression [26] is shown in the left panel and a 3D simultaneous [^{15}N, ^{13}C]-resolved [^{1}H, ^{1}H]-NOESY-HMQC sequence in the right panel, respectively. Segments of the pulse sequences, during which the pathways giving rise to a specific cross and diagonal-peak pair are shared, are related by color code, illustrating the two possible ways to normalize cross-peak intensities thereby accounting for different experimental artifacts (see theory section). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Two-dimensional (2D) [^{1}H,^{1}H]-NOESY pulse sequences [2,9,10,27,28] are the fundamental building blocks of the 3D (or 4D) NOESY experiments typically recorded for macromolecules [29–33]. Such 2D NOESY experiments allow the analysis of NOESY effects isolated from other influences, not possible in multi-dimensional experiments. More specifically, one of the appeals of 2D NOESY experiments lies in their theoretical spectral symmetry [34]. In practice, however, even the symmetry of 2D NOESY is broken. This is illustrated with correlation plots of cross-relaxation rates obtained from cross peaks above the diagonal versus those below the diagonal (as demonstrated for the WW domain in Fig. 2).

The effect can be understood as different attenuation of the individual magnetization pathways throughout the entire pulse sequence. The cumulative attenuation without the relaxation processes during NOESY mixing can be visualized by the analysis of the dispersion of back-predicted diagonal-peak intensities at zero mixing time (Fig. 3a). The intensity dispersion over the entire domain is very broad ($s = 0.61$, see definition below). Including only H^{α} and H^{N} values in the β-sheet backbone, the back-predicted diagonal-peak intensities have a dispersion of 0.55 (Fig. 3b). For H^{N} spins, the dispersion is reduced to 0.40, since there is only one relevant J coupling, $^3J_{HN,H\alpha}$, that impacts the peak intensity during the evolution periods and the H^{N} frequencies are also far removed from the suppressed water frequency.

In the 3D ^{15}N- or ^{13}C-resolved [^{1}H, ^{1}H]-NOESY-HXQC, the different excitation profiles of ^{15}N and ^{13}C pulses and the different relaxation of ^{15}N and ^{13}C nuclei will enhance the non-symmetry of the experimental pathways.

In order to analyze the attenuation of the detected signal intensities, we introduce scaling factors α and magnetization transfer factors T of the individual pulse sequence segments. $M_i^{SS}(d_{int}, R_1)$ represents the magnetization of spin i prior to the first pulse of a scan and is a function of the interscan delay d_{int} and the longitudinal auto-relaxation rate constant R_1. The scaling factor associated with the xth evolution period (indirect or direct) resulting in the NOESY plane and the subsequent Fourier transforms, $\alpha_{x,i}^{EP}(proc, J, R_2)$, is determined by the user-set processing parameters *proc*, J-coupling induced FID modulation and the transverse relaxation rate constant R_2. $T_{ij}^{NOESY}(\tau_{mix})$ represents the transfer factor during NOESY mixing τ_{mix}. If a diagonal-peak is considered, i is equal to j, while i is different from j for cross-peaks. T_i^{HXQC} is the transfer factor (attenuation) of the magnetization of spin i during HXQC including processing effects. α_i^{WS} quantifies signal attenuation during water suppression. The complete attenuation of a signal throughout the entire pulse sequences shown in Fig. 1 originating from spin i and being detected on spin j can be expressed as:

$$I_{ij} = M_i^{SS}(d_{int}, R_1) \times \alpha_{1,i}^{EP}(proc, J, R_2) \times T_{ij}^{NOESY}(\tau) \times T_j^{HXQC} \times \alpha_j^{WS} \times \alpha_{2,j}^{EP}(proc, J, R_2) \quad (1)$$

For 2D [^{1}H, ^{1}H]-NOESY, the transfer factor $T^{HXQC} = 1$, which simplifies the analysis. Depending on the design, the element order of other NOESY pulse sequences may be rearranged, or some elements may be merged.

The evaluation protocol of eNOEs involves normalization of cross-peak intensities to diagonal-peak intensities (see theory