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Extracting Accurate Distances and Bounds from 2D NOE Exchangeable Proton Peaks

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Distance constraints derived from multidimensional NMR are used with distance geometry and molecular dynamics calculations to generate high-resolution structures of molecules in solution.[1,2] More accurate structures can be determined with more structural constraints,[1] with more accurate structural constraints, and with tighter but realistic constraint boundaries.[3] More numerous and more accurate distances between nonexchangeable protons can be determined from two-dimensional nuclear Overhauser effect (2D NOE) spectra using a complete relaxation matrix analysis, which accounts for all proton dipole–dipole interactions.[4–6] Determination of accurate distances to exchanging protons can present problems beyond that of the multispin effects noted already. Chemical exchange has been incorporated into the complete relaxation matrix formalism and demonstrated to influence 2D NOE peak intensities in the case of multiple conformations.[7–9] For the case of proton exchange from a dilute macromolecule to bulk solvent water, the total relaxation rate matrix can be modified so that chemical exchange is considered in addition to dipole–dipole relaxation:[10,11] $\mathbf{R}_\mathrm{T} = \mathbf{R}_\mathrm{DD} + \mathbf{K}$. For our case, the chemical exchange matrix **K** is diagonal; the diagonal terms correspond to off rates for exchangeable protons and are 0 for nonexchangeable protons. Another way of thinking about this is that exchange with bulk water contributes to direct relaxation, i.e., magnetization is lost via the physical "leakage" of the exchangeable proton into the pool of water. We have examined the influence of exchange on determination of distances and bounds for protons which can exchange with bulk water. The influence of solvent exchange rate and mixing time on proton cross-peak intensities for the simple case of two interacting protons is shown in Figure 1; it can be shown that the ordinate is independent of interproton distance and correlation time if the mixing time is short (<1 s). The mixing times and exchange rates shown suggest that, while exchange with water may be pertinent for some protons on proteins or other molecules, it is especially important for imino and amino protons on nucleic acids.

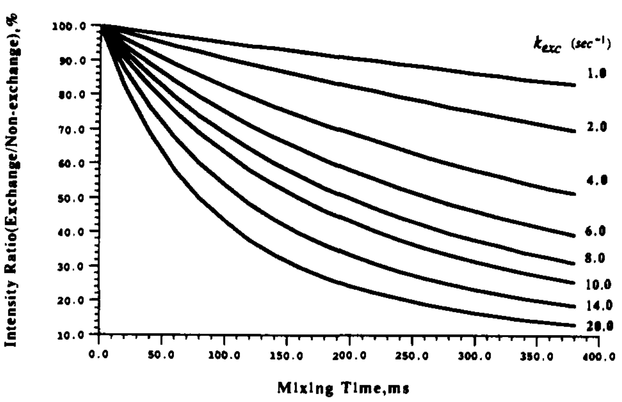

Figure 1. Diminution of 2D NOE cross-peak intensity for a system of two spins due to exchange of one proton of the pair with bulk water as a function of mixing time. The ordinate is the ratio of the cross-peak intensity in the presence to that in the absence of exchange. That ratio can be shown to be independent of interproton distance, number of exchangeable protons, and correlation time as long as the mixing time is short. The different curves are generated for protons at 500 MHz with a correlation time of 1 ns and exchange rates of (a), 1, (b) 2, (c) 4, (d) 6, (e) 8, (f) 10, (g) 14, and (h) 20 s^{-1}.

Table I. Comparison of Interproton Distances (Å) Calculated Using MARDIGRAS without and with Incorporation of Exchange from a Simulated 2D NOE Data Set for d(G1-T2-A3-T4-A5-A6-T7-G8)·d(C9-A10-T11-T12-A13-T14-A15-C16)[a]

proton interaction	calcd distance[b] without exchange	with exchange
T2-H3–T14-H3	4.69	4.01
T4-H3–T12-H3	4.59	3.92
T4-H3–T14-H3	4.36	3.71
T7-H3–T11-H3	4.24	3.61
T11-H3–T12-H3	3.63	3.07
T7-H3–G8-H1	4.31	3.99
T14-H3–A15-H2	4.99	4.68
T12-H3–A13-H2	4.77	4.47
T2-H3–A3-H2	4.61	4.32
T4-H3–A5-H2	4.54	4.25
G1-H1–T2-H3	3.58	3.30
A3-H2–T4-H3	4.03	3.76
A6-H2–T7-H3	4.05	3.78
A5-H2–T11-H3	4.96	4.70
A6-H2–T12-H3	3.89	3.63
A10-H2–T11-H3	3.77	3.52
A13-H2–T14-H3	3.62	3.37
A6-H2–T11-H3	2.98	2.77
T4-H3–A13-H2	2.97	2.76
A5-H2–T12-H3	2.97	2.77
T2-H3–A15-H2	2.96	2.76

[a] The simulated spectrum was generated for a mixing time of 100 ms using CORMA,[4,24] assuming (i) that the real structure is that previously determined for d(GTATAATG)·d(CATTATAC) at 15 °C,[18] (ii) an overall isotropic motion correlation time of 2.8 ns and fast three-state methyl jumps, and (iii) exchange rates of 10 s^{-1} for exchangeable AT protons and 1 s^{-1} for exchangeable GC protons (both amino and imino). [b] Using the simulated spectral intensities as "experimental" data, distances were calculated incorporating multispin effects via MARDIGRAS,[20,21] either incorporating or ignoring effects of exchange. Although several hundred distances are calculated, only those entailing imino or nonexchangeable protons which differ by more than 0.2 Å are listed. Of course, the distances calculated via MARDIGRAS agree within three significant figures to the model structure distance.

In spite of the paucity of base protons to provide structural constraints, studies with double-stranded DNA generally have not utilized distances to imino protons because they exchange with

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Table II. Comparison of Interproton Distances (Å) Calculated Using MARDIGRAS without and with Incorporation of Upper Limits to Exchange Rates for an Experimental 2D NOE Data Set for d(C1-A2-T3-T4-T5-G6-C7-A8-T9-C10)·d(G11-A12-T13-G14-C15-A16-A17-A18-T19-G20)

	calculated distance[a]			calculated distance[a]	
proton interaction	lower bound[b]	upper bound[c]	proton interaction	lower bound[b]	upper bound[c]
T5-H3–G6-H1	2.98	3.88	T4-H3–A18-H2	2.97	3.60
A2-H2–T3-H3	3.18	3.82	C7-NHA–G14-H1	2.58	2.85
A8-H2–T13-H3	2.27	2.76	C7-NHB–G14-H1	2.84	3.13
T5-H3–A16-H2	2.29	2.77	G6-H1–C15-NHA	2.62	2.90
T4-H3–A17-H2	2.37	2.86	G6-H1–C15-NHB	2.80	3.09
T3-H3–A18-H2	2.34	2.84			

[a] Distances were analyzed using experimental 2D NOE spectral intensities (25 °C, 500 MHz, 140-ms mixing time, corrected for nonlinear radio frequency excitation)[22] via MARDIGRAS using an overall tumbling correlation time of 1.8 ns and fast three-state methyl jumps. Interactions listed are only those which differed by more than 0.2 Å when exchange effects were considered and are ordered by decreasing size of the difference. Hundreds of distances were affected negligibly by exchange. Two pairs of amino protons were resolved and included in the calculations. [b] Distance calculated from MARDIGRAS including exchange. For solution conditions employed, an upper limit to the exchange rate of 20 s^{-1} for exchangeable AT protons and 4 s^{-1} for exchangeable GC protons was estimated. [c] Distance calculated from MARDIGRAS ignoring exchange.

bulk water and thus yield observable resonances only in H_2O. Depending on the method of dealing with the potentially huge water proton signal, possible effects from nonlinear radio frequency excitation and accuracy of peak integrals for such spectra provoke concern, but the problems are tractable. The imino proton exchange rate with water can also affect the signal intensity; that rate depends on identity (T or G), temperature, pH, buffer type and concentration, inner or terminal base, and secondary structural features and slightly on sequence.[12-16] Generally, T imino protons exchange about 3–4 times faster than G imino protons. Unfortunately, amino proton exchange rates in nucleic acids are not well characterized. Relative pK_a values suggest that they should exchange more slowly than imino protons, but, to a first approximation, we will assume that they exchange at the same rate.[17]

Although the exchange rate with water has many variables, if ignored, typical rates can introduce significant error into distance determinations. This is shown by Table I. With exchange rates of 1 s^{-1} for exchangeable GC protons and 10 s^{-1} for exchangeable AT protons (on the high side of average) for a data set simulated assuming that the structure determined for d(GTATAATG)·d(CATTATAC) is the "true" structure,[18] overestimation of distances by nearly 20% occurs for some cases. It is not coincidental that the distances with greater error entail exchangeable protons with the largest exchange rate; indeed, the distances with greatest error entail two exchangeable protons. Distances involving nonexchangeable protons were affected negligibly by exchange. Although values are not shown, distance errors are even larger if multispin effects are ignored and the two-spin approximation is used to estimate distances.

As an adjutant to structural studies, it is possible to determine the imino proton exchange rate.[14,16,19] Our iterative complete relaxation rate matrix program, MARDIGRAS,[5,20,21] has been modified such that exchange rates can be accommodated in calculating interproton distances when exchange data is available. Alternatively, on the basis of exchange studies described in the literature, it is possible to estimate upper limits to exchange rates. Using these with MARDIGRAS will provide lower limits to distances involving exchangeable protons. Upper limits to distances can be obtained by ignoring the exchange rates. These limits can thus establish upper and lower distance bounds to be used for structure determination with subsequent distance geometry or restrained molecular dynamics calculations. Table II illustrates the approach using experimental 2D NOE data for d(CATTTGCATC)·d(GATGCAAATG).[22]

In general, imino protons can be included in practical calculations for DNA, but, if data are available, MARDIGRAS is set up to accommodate exchange rate information from other exchangeable protons, such as amino protons in nucleic acids or amide protons in proteins. We set distance bounds on the basis of (i) the minimum error dictated by the experimental noise level, (ii) the error calculated between an observed 2D NOE cross-peak intensity and the corresponding MARDIGRAS-converged matrix element intensity, (iii) distances calculated from spectra at different mixing times, and (iv) in the case of motional or overlap averaging, extreme distances calculated using worst-case geometries.[5,20,21,23] The majority of distances have upper and lower bounds differing by less than 10%. The results presented here, however, show that the range of bounds involving some exchangeable protons needs to be made somewhat larger and that a logical means of establishing those bounds exists.

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Synthesis and Structure of an Octasila[4.4]betweenallene

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To date there are very few examples of the bicyclic, doubly-bridged allenes[1] known as "betweenallenes".[2] Although examination of models suggests that the smallest all-carbon betweenallene capable of isolation should be a [5.5]betweenallene, the smallest yet reported[5] is [8.10]betweenallene, consisting of fused 11- and 13-membered rings. Highly strained, symmetrical betweenallenes would be of considerable interest owing to the fact that the theoretically predicted[6] relief of strain in small cyclic allenes by concomitant bending and twisting is rendered impossible. Thus strained symmetrical betweenallenes can gain no strain relief by bending, since what is gained in one ring is lost in the other, and therefore only twisting, as defined as a reduction in the normal

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