Studying Protein Backbone Conformational Dynamics using NMR Residual Dipolar Couplings

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Molecular motions, enabling changes in protein backbone or sidechain conformation, are thought to play a crucial role in both protein stability and function.[1-4] Despite the recognized importance of dynamics for biochemical activity, most approaches to protein structure determination, whether based on crystallographic or solution studies, propose three dimensional atomic representations of a single configuration, that takes little or no account of conformational fluctuation. Motional properties are routinely measured in solution, most commonly using Nuclear Magnetic Resonance (NMR) spin relaxation, [5-8] where rapid fluctuations, up to the range of the characteristic rotational correlation time of the molecule (around 10 ns for medium size proteins in aqueous solution at room temperature) can be characterised, [9-11] or using rotating frame relaxation dispersion experiments that can detect conformational exchange occurring on slower timescales. [12-13] However, the ability to elucidate both structural and dynamic aspects will provide direct access to the conformational space sampled by the native protein, as well leading to more accurate average conformations. NMR is uniquely suited to this purpose, with experimental techniques routinely probing time and ensemble-averaged conformation-dependent observables. These observables are generally used to extract a single conformation, but inherently encode, albeit in some potentially complex way, detailed information on conformational dynamics occurring on multiple timescales up to the millisecond range. These slower time scales are of particular interest, firstly because they are not probed routinely by spin relaxation, and secondly because functionally important biological processes, including enzyme catalysis, [14] signal transduction, [15] ligand binding or allosteric regulation, [16] requiring collective motions involving groups of atoms or amino acids, are expected to occur in this time range

Residual Dipolar Couplings

The dipolar coupling between two spins 1/2 (i,j) is described by the time and ensemble average of the dipolar Hamiltonian over all sampled orientations;

$$D_{ij} = -\frac{\gamma_i\gamma_j\mu_0 h}{8\pi^3}\left\langle \frac{P_2\left(cos\theta(t)\right)}{r_{ij}^3}\right\rangle \quad (1)$$

r_{ij} is the distance between the two nuclei, γ_i and γ_j are the gyromagnetic ratios of the two spins, h is Planck's constant and μ_0 the permittivity of free space. Note that the dipolar Hamiltonian depends on the orientation θ of the internuclear vector between the coupled spins, relative to the magnetic field, following a second order Legendre polynomial dependence ($P_2\cos\theta$). Time and ensemble averaging of this function, denoted by the angular brackets, reduces the measured coupling to zero under the conditions of orientational averaging found in isotropic solution. In order to measure a residual coupling (RDC) in solution it is necessary to induce partial alignment, or order, in the sample. It has been shown over ten years ago[31] that simple dissolution of a protein in a dilute liquid crystal solution of phospholipid bicelle, would allow the measurement of large (tens of Hertzs) couplings, while retaining the high quality spectra necessary for high resolution protein NMR. Very rapidly additional solvent systems were developed to provide partial alignment. [33-39]

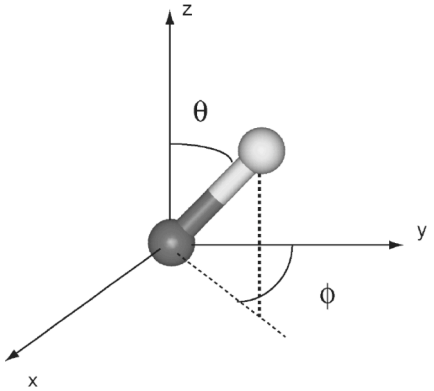

Figure 1. Orientation of the internuclear vector in the principle axis system of the molecular alignment tensor. The angles are those described in equation 3.

In the case of a macromolecule whose shape does not change significantly, the average in equation 1 can be described as a convolution of the restricted motion of the solute molecules, defined by the average over all orientations of the molecule relative to the magnetic field, and the orientation of the interspin vector relative to the molecule. The preferential orientational

averaging of the molecule is commonly described in terms of an alignment tensor **A** whose units are dimensionless, and whose trace is zero, reflecting its probabilistic nature. [40] It is convenient to describe the measured couplings in terms of their orientation relative to this alignment tensor or principal axis system (PAS) common to the whole molecule. The orientation of the PAS or alignment tensor with respect to the coordinate frame of the molecule can in return be defined simply via a three dimensional Euler rotation $R\{\alpha,\beta,\gamma\}$ One can describe the measured coupling in terms of $\{\theta,\phi\}$, the polar angles of the inter-spin vector in the eigenframe of the alignment tensor, with eigenvalues A_{xx}, A_{yy} and A_{zz} as:

$$D_{ij}(\theta,\phi) = -\frac{\gamma_i\gamma_j\mu_0 h}{8\pi^3 r_{ij}^3}\left[A_{zz}\cos^2\theta + A_{xx}\sin^2\theta\cos^2\phi + A_{yy}\sin^2\theta\sin^2\phi\right] \quad (2)$$

or

$$D_{ij}(\theta,\phi) = -\frac{\gamma_i\gamma_j\mu_0 h}{16\pi^3 r_{ij}^3}\left[A_a\left(3\cos^2\theta - 1\right) + \frac{3}{2}A_r\sin^2\theta\cos 2\phi\right] \quad (3)$$

where $A_a = A_{zz}/2$ is the axial component of the alignment tensor and $A_r = (1/3)(A_{xx} - A_{yy})$ is the rhombic component. The available orientations of an interaction vector for a single measured RDC in the presence of a known tensor are depicted in cartoon form in Figure 2 on the surface of a sphere.

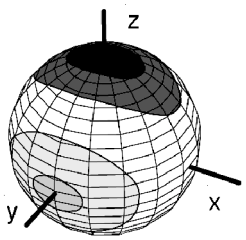

Figure 2. Cartoon representation of the dependence of measured dipolar couplings on the orientation of the internuclear vector. Dipolar coupling isocontours are shown as shaded bands – Black/ dark grey: positive coupling, White : intermediate and zero coupling, Light grey : negative coupling. The axes represent the axes of the alignment tensor.

Protein Dynamics from RDCs

Residual dipolar couplings are most commonly applied to the determination of static structures, but it is in terms of molecular dynamics that a second, equally powerful aspect of RDCs is revealed. RDCs are averaged over all orientations of the magnetic dipolar interaction vector sampled up to a timescale defined by the inverse of the alignment-induced coupling, thus reporting on averages up to the millisecond range under conditions of partial molecular alignment. [50]

Expressing the dependence of the dipolar coupling on the vector orientation with respect to the alignment tensor as in Equation 3, we implicitly assumed that the inter-spin vector was static with respect to the alignment tensor. In the presence of local internal motion the measured coupling is better represented by incorporating local conformational averaging over both time and ensemble :

$$\left\langle D_{jk}(\theta,\phi)\right\rangle = -\frac{\gamma_i\gamma_j\mu_0 h}{16\pi^3 r_{ij}^3}\left[A_a\left\langle 3\cos^2\theta - 1\right\rangle + \frac{3}{2}A_r\left\langle\sin^2\theta\cos 2\phi\right\rangle\right] \quad (4)$$

The angular brackets indicate conformational averaging. This provides access to information that is potentially highly complementary to the dynamic parameters routinely extracted from spin relaxation measurements. [51] Comparison of motional averaging on the two time-scales provides information on dynamics in the nano- to millisecond range. This relevance is particularly evident for first order averaging of dipolar interactions whose rapid reorientation also dominates experimental spin relaxation rates (for example ^{15}N-^{1}H couplings). The ability of RDCs to describe local conformational fluctuations over the nano to millisecond time range in proteins has been studied by a number of groups in recent years.

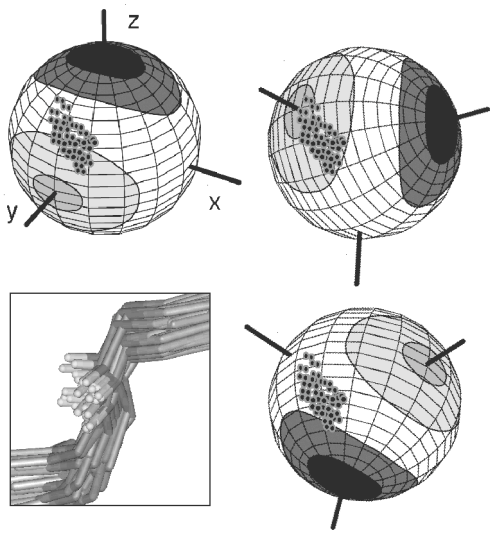

Figure 3. Representation of the effects of intramolecular motion on the dynamic averaging of residual dipolar couplings. In the presence of motion the effective measured coupling reports on all orientations sampled in the motional envelope (shown as an ensemble of vectors and as black circles). If the motional envelope is anisotropic, as shown here, the effective averaging depends on the orientation of the motional anisotropy with respect to the alignment tensor.

In the presence of different alignment tensors the averaging will sample dipolar couplings isocontours (shown as shaded bands) differently and give rise to differential averaging effects.

A number of methods have been developed that attempt to extract the extent and shape of the motional envelope of internuclear vectors from dipolar couplings measured in differently aligning media.Prestegard and co-workers interpreted local motions in terms of local alignment characteristics, and expressed these as a site-specific Generalized Degree of Order (GDO), [52] while Griesinger and co-workers used a very large number of datasets measured on Ubiquitin[53-56] to determine the

shape and size of the orientational averaging envelope for each N-H^N vector in the protein. Tolman has independently proposed and applied related approaches. [57,58] In an alternative approach, Bax and co-workers have attempted to define the limits of possible local dynamic amplitudes in protein GB3 by using refined static models, and comparing the ability of these models to describe the experimental data. [59] Finally, Clore et al have used ensemble averaging of RDCs to describe the conformational space available to both Ubiquitin and protein GB3. [60]

We have explored the possibility of using specific geometric models to describe local motional averaging of RDCs. Initial studies used a one-dimensional Gaussian Axial Fluctuation (GAF) model for peptide plane reorientation about the C^{α}_{i-1}-C^{α}_{i} axis, identifying a common anisotropic component of protein backbone dynamics from ^{15}N-^{1}H RDCs. [61-63] This simple approach demonstrated that statistically significant improvements could be made to the accuracy of the description of the overall molecular alignment tensor by taking into account local motions, even in the absence of site-specific detail. In the light of vigorous debate concerning the nature and extent of slow motions present in soluble proteins, and the ability of RDCs to describe these dynamics, we then undertook a detailed study of the presence of slow motions in protein GB3, interpreting an extensive set of RDCs[59,64] measured in multiple differently aligning media, in terms of the three dimensional Gaussian Axial Fluctuation model (3D-GAF). This general model of peptide plane dynamics allows for stochastic motions around three orthogonal axes attached to the peptide plane, [65] and our interpretation assumed a fixed time- and ensemble-averaged model of the average structure.

The study delivered a site-specific motional description of each peptide plane in the protein, and provided a quantitative estimate of the nature and extent of dynamics present on the protein backbone. [66] We identified a heterogeneous distribution of slower motions in the protein in comparison to ^{15}N spin relaxation data, [67] with local motions in some regions of the protein that are quantitatively the same as those detected using spin relaxation, for example in the α-helix and some surface loops and turns. We can therefore detect no additional (ns-ms) slow motions in these regions. In the β-sheet, and one of the surface loops however slower motions are observed, in particular in the region where the protein interacts with its physiological partner (β-strand II).

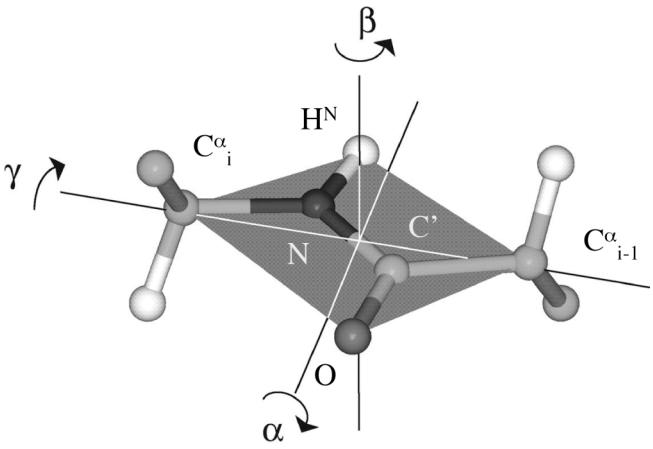

Figure 4. Three dimensional Gaussian axial fluctuation (GAF) model of peptide plane reorientation used for the modelling of dynamic reorientation as described in the text. One-dimensional GAF models imply rotations about any one of the three axes

The presence of these dynamic modes is verified using extensive cross validation of data that were not used in the analysis, and the dependence on the structural model was tested against two crystal conformations and an RDC refined structure, all of which gave similar motional distributions.

Analysis of trans-hydrogen bond scalar couplings in terms of these local dynamic amplitudes and directions also found strong evidence that the motion was correlated and that the collective motion transmitted across the β-sheet was propagated via the inter-strand hydrogen bonds. Although this kind of transmission of dynamics has been proposed, it has never been observed by other experimental methods, and is computationally challenging to simulate. The existence of these slow motional modes extending across the entire β-sheet carries clear implications for understanding the mechanisms of long-range signal propagation in proteins. In the case of protein G, these findings illustrate how the protein harnesses thermal motions via specific dynamic networks to enable molecular function at the interaction site.

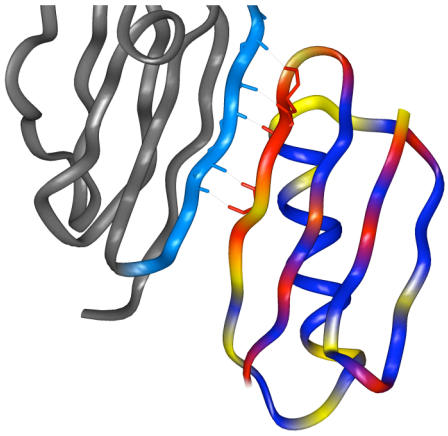

Figure 5. Representation of the collective motion traversing the b-sheet of protein GB3. The ribbon is coloured in this figure as a function of the amplitude of the component of the motion about the gamma axis shown in figure 4. Blue indicates little motion, yellow

regions have higher amplitude motion while red indicates the highest amplitude motions. The highest amplitude motions are clearly located in the interaction site of the protein that forms a complete b-sheet with Fab (shown in sky blue).

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