

[17] Hydrodynamic Models and Computational Methods for NMR Relaxation

By J. GARCÍA DE LA TORRE, P. BERNADÓ, and M. PONS

Abstract

Interpretation of NMR relaxation data of macromolecules is based on the analysis of their dynamic behavior in solution. For quasirigid molecules, in addition to a minor, separable contribution from local mobility, the main contribution corresponds to the overall rotational diffusion of the complete molecule. Therefore, theoretical descriptions and computational methodologies for hydrodynamic calculations, which yield the full, anisotropic rotational diffusion tensor of rigid molecules, are extremely helpful in the analysis of NMR relaxation. Recent approaches allow realistic predictions of the rotational diffusion tensor from structures at atomic detail. This enables measured relaxation rates and structural models to be compared. Such a comparison (1) provides an independent test of the structural model, (2) provides a framework for the interpretation of local motion, even for highly anisotropic systems, (3) provides a simple method for the detection of additional sources of relaxation, such as chemical exchange, and (4) provides a sensitive method for the detection of nonspecific aggregation or oligomer formation. Although hydrodynamic calculations usually assume a rigid structure, Brownian dynamics simulations extend their range of applications to flexible multidomain structures. Hydrodynamic applications are not restricted to globular proteins. Small DNA fragments, which could be otherwise considered cylindrical objects, can also be treated with atomic detail using the same methodology used for proteins.

Introduction

Biomolecular structures are intrinsically dynamic entities, and there is an increasing consensus that dynamics is often directly related to function. Together with a widespread accessibility of isotopically labeled samples, this fact has led to an increased interest in the study of biomolecular dynamics, especially through nuclear magnetic resonance (NMR) relaxation methods.

Backbone mobility is now routinely measured using ^{15}N NMR relaxation that probes the reorientation of N–H bonds. Additional information

can be obtained from ^{13}C relaxation, with a focus on carbonyl groups, although its use is limited by the higher costs of ^{13}C labeling and possible complications due to homonuclear coupling in uniformly labeled samples. Protein side chains have a much richer dynamics that is presently the object of great interest, but it is outside of the scope of this chapter. Heteronuclear NMR relaxation of backbone nuclei is usually interpreted in a model-free framework (Lipari and Szabo, 1982), whose central assumption is the absence of correlation between global and local motions, allowing their separation. Extensions of the model-free approach consider additional, uncorrelated, local fast motions or extra relaxation sources derived from chemical exchange. Experimental relaxation rates of individual nuclei are fitted to the model with the lowest number of statistically significant parameters, and the results are analyzed in terms of extracted parameters (Palmer *et al.*, 1996). Local motions are captured by order parameters, related to the amplitude of fluctuations, and characteristic time constants. Global motion, universally present in fluid phases, is accounted for by either a single correlation time, implying isotropic reorientation, or up to five correlation times for a fully anisotropic system. The assumption of axial symmetry may be remarkably misleading because, among other reasons, a careful description of global motion is required for the correct interpretation of local mobility.

Individual nuclei belonging to bonds with different orientations with respect to the principal axes of the rotational diffusion tensor relax at different rates. A structural model of the macromolecule is required to deconvolute anisotropic reorientation from other sources (e.g., local mobility) of variability in relaxation rates within a molecule. If the structure of the macromolecule is known and it can be effectively considered a rigid body, an alternative approach for interpreting NMR relaxation data is to compute the rotational diffusion tensor from the structure by applying hydrodynamic theory. A comparison of calculated and experimental relaxation rates will provide an independent confirmation of the model structure and the rigid body assumption and a framework for the interpretation of local variability in relaxation rates. In this chapter we shall discuss recent advances in the application of hydrodynamic theory to predict relaxation rates and some applications to the detection of local motion in rigid structures, characterization of protein oligomers, and detection of flexibility in multidomain structures. A recent review describes additional approaches to the interpretation and prediction of relaxation data (Brüschweiler, 2003). Background information on NMR relaxation has been extensively reviewed. (Fischer *et al.*, 1998; Korzhnev *et al.*, 2001).

Theory and Methods

Rotational Brownian motion (RBM) of a rigid particle is determined by the rotational diffusion tensor, \mathbf{D}_r , a symmetric 3×3 matrix whose six components depend on the size and shape of the particle and are proportional to the ratio of absolute temperature to solvent viscosity, T/η_0 . Diagonalization of \mathbf{D}_r provides three eigenvalues, D_i , where $i = 1, 2$, or 3 , and its principal axes are defined by the eigenvectors \mathbf{u}_i . For the simplest case of a spherical particle, \mathbf{D}_r is isotropic and all D_i s are identical; conversely, for an arbitrary particle, the D_i values are different and depend sensitively on the overall shape and therefore contain valuable structural information. The time course of observable properties related to RBM of a rigid body is determined by five relaxation time, τ_k , $k = 1, \dots, 5$, directly derived from D_i values. For an isotropic system, a single correlation time is enough to describe its motion. For an arbitrary system, it is sometimes useful to define the (harmonic) mean or correlation time, τ_c , whose reciprocal is given by

$$1/\tau_c = (1/5) \sum (1/\tau_k) = 2(D_1 + D_2 + D_3)$$

The Brownian reorientation of a particle-fixed vector \mathbf{v} , is usually represented by the time-dependent function $\langle P_2[\cos \beta(t)] \rangle$, where $\beta(t)$ is the angle subtended by an initial orientation of the vector and its orientation after time t has elapsed, $P_2[x] = (3x^2 - 1)/2$, and $\langle \dots \rangle$ means an average over the choices of the initial instant. RBM theory predicts that this function decays from 1 to 0 in the form of a sum of exponentials, $\sum a_k \exp(-t/\tau_k)$, where the time constants are the τ_k s and the amplitudes a_k depend on the vector \mathbf{v} being considered and are given by the coordinates of \mathbf{v} relative to the \mathbf{u}_i s.

In NMR relaxation, RBM is detected in the frequency domain, and the pertinent, frequency-dependent function is the spectral density, $J_0(\omega)$, which is the Fourier transform of $\langle P_2[\cos \beta(t)] \rangle$, given by

$$J_0(\omega) = \sum a_k \tau_k / (1 + \omega^2 \tau_k^2)$$

In heteronuclear NMR relaxation, the RBM of the macromolecular structure is measured mainly by the relaxation rates R1 and R2, and NOE values observed for a series of \mathbf{v} vectors, which are along the directions of X-H (X = N or C) bonds, usually one per each residue. These relaxation properties are determined by the values adopted by the $J_0(\omega)$ function for five particular frequencies that are combinations of the Larmor frequencies of H and X: $\omega = 0, \omega_X, \omega_H, \omega_X - \omega_H$, and $\omega_X + \omega_H$. Global motion is sensed differently by bond vectors with different orientations; therefore, relaxation measurements integrate details of the external, overall shape of the molecule with

information about its internal structure. Fast internal motions provide additional contributions to relaxation that are not accounted for in a rigid body approximation. Fortunately, R1 and R2 rates are affected similarly by internal motions, and the ratio R2/R1 provides a good approximation to the relaxation properties of the rigid body model (Kay *et al.*, 1989).

Even fairly rigid macromolecules, such as globular proteins, present some amount of internal mobility that contributes, along with overall tumbling, to the RBM of the residues. For most of them, the contribution of internal motion to R1 and R2 nearly cancels out in the ratio R2/R1 and is typically ignored in experimental results and theoretical calculations. For the sake of brevity, we have summarized the basic concepts of RBM and NMR relaxation of rotationally anisotropic molecules (Abragam, 1961; Woessner, 1962); the reader can learn more physical and mathematical details in the original references. The equations employed in the calculations are compiled in García de la Torre *et al.* (2000a).

The core of the prediction of NMR relaxation consists of the calculation of D_r . In general, the calculation of hydrodynamic properties of rigid particles can be done employing bead models (García de la Torre and Bloomfield, 1981) as implemented in the HYDRO program (García de la Torre *et al.*, 1994). This possibility suggests a route applicable to macromolecules, described with chemical detail, at the level of residues or even individual atoms. Figure 1A displays an example in which all the nonhydrogen atoms of a globular protein are represented by identical

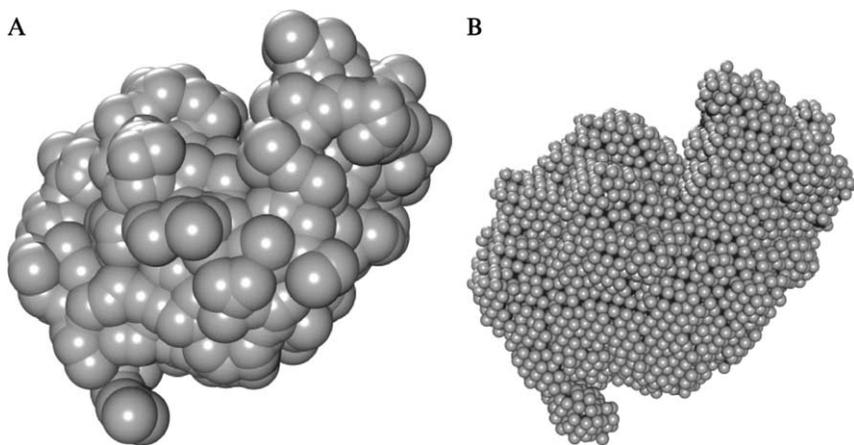


FIG. 1. (A) Primary hydrodynamic model for lysozyme. Each nonhydrogen atom is represented by a bead of radius $a = 3.2 \text{ \AA}$. (B) Shell model for lysozyme. The exposed surface of the primary hydrodynamic model is represented by a shell of minibeads with radius $\sigma = 0.7 \text{ \AA}$.

spherical beads of radius, a . To match the actual volume of the protein in solution, including immobilized hydration water, the radius of the beads must be appreciably larger than the atomic radius, and this produces an extensive overlap between beads. This is the primary hydrodynamic model (PHM) of the protein, whose properties are sought. The theory implemented in HYDRO was devised for nonoverlapping beads and is not applicable to the PHM. [García de la Torre *et al.* \(2000b\)](#) proposed a procedure, based on the so-called bead-shell modeling strategy ([Bloomfield *et al.*, 1967](#)), in which the exposed (but not the interior) surface of the overlapping spheres in the PHM is represented by a shell of touching but not overlapping “minibeads” of radius σ (see an example in [Fig. 1B](#)). Hydrodynamic properties of this shell model are calculated using the standard bead-model treatment for various values of σ , and the results are extrapolated to the limit of $\sigma = 0$, at which the shells represent exactly the hydrodynamic surface. This strategy was shown to yield quite accurate predictions of several hydrodynamic properties (including correlation times) for a number of globular proteins from their atomic structure, using a value of $a \approx 3.0\text{--}3.3 \text{ \AA}$ ([Bernadó *et al.*, 2002](#); [García de la Torre, 2001](#); [García de la Torre *et al.*, 2000b](#)). This radius is concordant with the van der Waals size of protein atoms increased by a contribution from hydration ([García de la Torre, 2001](#)) and can be regarded as a standard value for *ab initio* predictions.

The program HYDRONMR ([García de la Torre *et al.*, 2000b](#)) implements this methodology to calculate rigid body heteronuclear NMR relaxation rates from a known three-dimensional structure. From the atomic coordinates, \mathbf{D}_r is obtained by the shell-model procedure, and the X–H vectors are determined and referred to the eigenaxes of \mathbf{D}_r . Then, the procedure above described is employed to determine R1, R2, and NOE. The procedure is repeated for several values of a , treated as an adjustable parameter until the results fit the R2/R1 ratio (a more elaborated procedure for this fitting will be described below). However, it was found that the relative deviation of the series of $(R2/R1)_j$ ratios for the series of residues from its mean value, expressed as

$$\nabla_j = [(R2/R1)_j - \langle R2/R1 \rangle] / \langle R2/R1 \rangle$$

practically does not vary with a , and this serves as a direct, parameter-free test of agreement with experimental data.

Rigid Anisotropic Proteins

As an example of a straightforward application of HYDRONMR, we present a calculation for the outer surface protein (OspA, PDB file *losp*). The strongly anisotropic structure of OspA and the arrangement of its

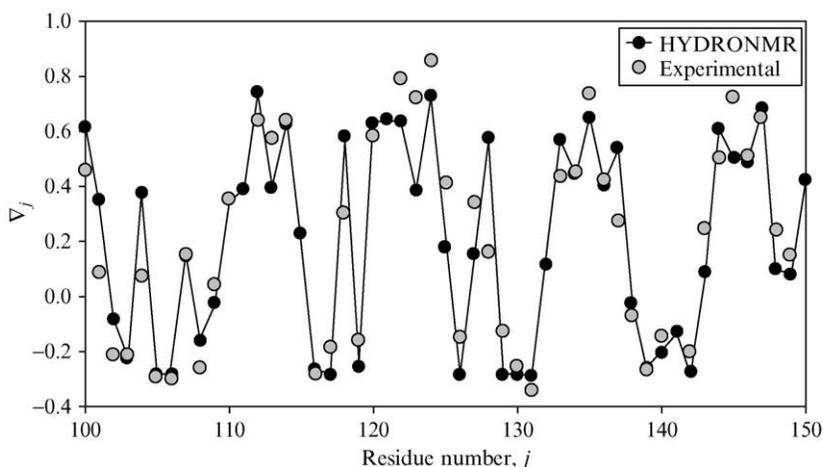


FIG. 2. Values of ∇_j for a series of consecutive residues (100–150) in Osp A.

secondary structure elements result in a remarkable periodicity of R2/R1 ratios along the sequence. This feature is accurately predicted by HYDRONMR, confirming that the residue variability of R2/R1 values is the result of anisotropic motion. Experimental relaxation data (Pawley *et al.*, 2002) are compared to the calculated results in Fig. 2, which plots ∇_j for a series of successive residues. We remark that the calculated values are practically independent, not only of radius a but also of other physical and instrumental conditions. We see how the calculated values correlate rather well with the experimental ones, and the trend of the ∇_j values along the polypeptide chain is predicted with notable accuracy. The experimental value of the correlation time of OSP at 318 K is 13.3 ns. Taking the “standard” value $a = 3.3$ Å, the calculated correlation time at 318 K is 14.3 ns, and the experimental value is fitted for $a = 2.9$ Å.

A detailed comparison of individual experimental and calculated R2/R1 values provides further insight into local deviations from the rigid body model. N-terminal residues are highly mobile and calculations overestimate R2/R1. The same situation is observed for a few internal residues (e.g., L98 and G163) suggesting local fast motion at these sites (see Fig. 3). Conversely, for residues G218, I224, and E239 the experimental R2/R1 value is higher than expected considering only dipole–dipole and chemical shift anisotropy (CSA) modulation due to molecular tumbling. Most likely, the additional relaxation mechanism is chemical exchange.

The effects of anisotropic motion and the possible complications derived from the presence of chemical exchange are well known (Tjandra

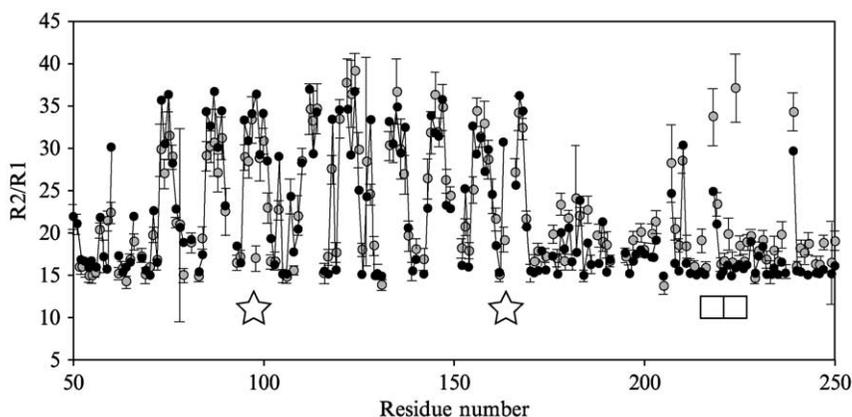


FIG. 3. Values of $R2/R1$ for a larger series of consecutive residues (50–250) in OspA, showing residues with high local mobility (stars) and exchange (squares).

et al., 1995), and there are experimental approaches to separate the two effects (Kroenke *et al.*, 1998), but hydrodynamic calculations offer a straightforward approach when a three-dimensional (3D) structure is available. Osborne and Wright (2001) used hydrodynamic calculations based on a PHM model to identify residues that are affected only by rotational diffusion.

A complete analysis of relaxation data requires the comparison of calculated and experimental values of $R2/R1$, in which the radius of the atomic elements can be treated as an adjustable parameter to optimize the agreement. An analysis of a number of proteins has shown that the optimized value of a can be used as a parameter to check the consistency of the 3D coordinates, the experimentally determined relaxation rates, and the assumption of a nonaggregating rigid-body molecule that is central to the HYDRONMR strategy. Additionally, relative deviations of individual $R2/R1$ values from the mean can be analyzed as a parameter-free test of the agreement with experimental data and can be used to identify sites of internal mobility or subjected to other sources of relaxation, such as chemical exchange. A detailed protocol, including filters to eliminate individual residues that are not representative of the global motion, has been described (Bernadó *et al.*, 2002) and is implemented in program HYDRORELAX.

Figure 4 shows the distribution of a values for a set of proteins in which three situations are observed. First, for rigid, nonaggregating proteins, HYDRONMR reproduces the experimental relaxation rates using a values

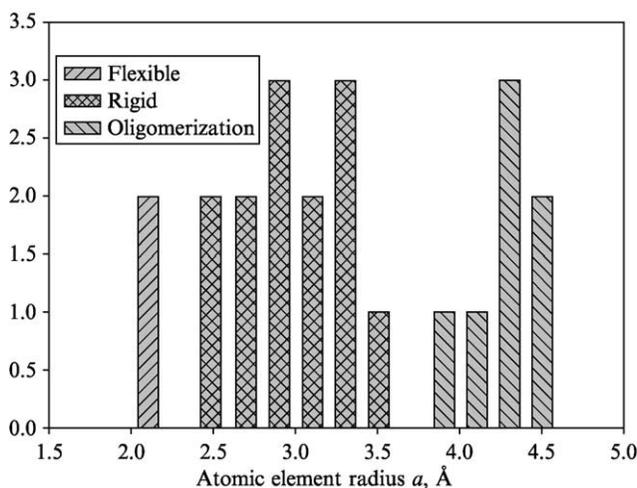


FIG. 4. Distribution of the a values for a large set of proteins. The central region corresponds to essentially rigid, nonaggregating structures. Cases with higher and lower a are attributed, respectively, to oligomerization and overall flexibility.

clustered around 3.3 \AA . The procedures indicated in this section provide an adequate data analysis in this case. Second, proteins known or suspected to be involved in oligomerization processes require substantially larger values of a . Finally, flexible proteins show an apparent smaller molecular volume that results in low values of a . In the following sections, we describe further applications of hydrodynamic modeling to proteins belonging to the two last cases.

Protein Oligomers

Reversible formation of high-molecular-weight oligomers, even if present in small quantities, results in a substantial increase in the effective correlation time that is equivalent to an increased apparent molecular volume, and attempts to fit the observed relaxation rates require atomic elements with radii much larger than 3.3 \AA . If the structure of the oligomers is known, the ability to predict their relaxation rates quantitatively allows the use of relaxation measurements as a function of concentration as a method for the determination of small oligomerization constants. Conversely, a good agreement between measured and computed relaxation rates could be taken as sensitive proof for nonaggregation. Finally, in favorable cases, concentration-dependent relaxation rates can be fitted to

an oligomerization model to extract some characteristics of the high-molecular-weight species, in spite of the fact that the observed NMR spectrum corresponds to the monomeric form.

In a recent example, the structure of a tetramer of bovine protein tyrosine phosphatase (BTP) could be characterized by comparing the observed concentration dependence of relaxation rates and the known structures of a BTP monomer and its dimer. The ability to calculate the expected rates for the known species allowed the detection of a previously unknown tetramer that explained the observed deviations from the best fit. Interestingly, the same analysis identified a set of residues consistent with a tetramerization interface providing independent proof of the tetramer model. (Bernadó *et al.*, 2003).

Enhanced relaxation rates in high-molecular-weight species form the basis of widely used methods to detect weakly binding ligands to macromolecules. The ability to predict the expected relaxation rates of macromolecules of known structure opens the way to sensitive and structurally informative methods to characterize weak protein-protein complexes.

Flexible Multidomain Proteins

Large-scale interdomain motion may substantially modify the shape of a protein, thus having an appreciable influence in solution properties (García de la Torre *et al.*, 2003). In particular, rotational diffusion is not well described by a single time-independent tensor (Brüschweiler *et al.*, 1995). Baber *et al.* (2001) suggested the use of an extension of the model-free approach in which all residues belonging to the same domain would have a common order parameter and correlation time describing the amplitude and time scale of interdomain motion. This treatment of relaxation data is valid only when slow internal rearrangements and global tumbling are uncoupled. However, since the dimensions of individual domains are usually comparable, the time scales of interdomain and global motion will be similar and the two motions are likely to be strongly correlated. To separate them, additional information arising from molecular dynamic simulations is required.

Brüschweiler's group has developed a theoretical frame (RED) to combine NMR relaxation data and a molecular dynamic trajectory to study protein dynamics by uncoupling active motional modes (Prompers and Brüschweiler, 2002). Our group has recently applied this frame to a multidomain protein using a Brownian dynamics trajectory of a simplified model of protein PIN1 (Bernadó *et al.*, 2004). Brownian dynamics simulations of simple systems allow much longer simulations (μs) than molecular dynamics (ns). This is a requirement to correctly describe motions with

correlation times of several nanoseconds. Simplified models that realistically capture the rigid body motion of individual models can be produced by replacing each domain by a small cluster of spheres that collectively has the same hydrodynamic properties of the isolated domain as modeled using HYDRONMR.

Nucleic Acids

Although NMR relaxation measurements and interpretations based on rigid-body hydrodynamics are more frequently applied to globular proteins, it is noteworthy that such methodologies can also be applied to small nucleic acids. Thus, oligonucleotides can be hydrodynamically regarded as short rods, and indeed NMR relaxation of these molecules has sometimes been interpreted with the help of the hydrodynamic coefficients for cylindrical particles derived by García de la Torre and co-workers (Tirado and García de la Torre, 1980; Tirado *et al.*, 1984). A recent example has been provided by Boisbouvier *et al.* (2003). Actually, the hydrodynamic description of such pieces of nucleic acids can be done with atomic detail, using HYDRONMR, as described by Fernandes *et al.* (2002). This makes it possible to investigate fine details of DNA structure and large-scale dynamics by NMR relaxation.

Computer Programs

HYDRONMR and related computer programs can be downloaded from <http://leonardo.fcu.um.es/macromol>.

Acknowledgments

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References

- Abragam, A. (1961). "The Principles of Nuclear Magnetism." Clarendon Press, Oxford.
- Baber, J., Szabo, A., and Tjandra, N. (2001). Analysis of slow interdomain motion of macromolecules using NMR relaxation data. *J. Am. Chem. Soc.* **123**, 3953–3959.
- Bernadó, P., García de la Torre, J., and Pons, M. (2002). Interpretation of ¹⁵N NMR relaxation data of globular proteins using hydrodynamic calculations with HYDRONMR. *J. Biomol. NMR* **23**, 139–150.
- Bernadó, P., Akerud, T., García de la Torre, J., Akke, M., and Pons, M. (2003). NMR relaxation evidence for tetramers of low molecular weight protein. *J. Am. Chem. Soc.* **125**, 916–923.

- Bernadó, P., Fernandes, M. X., Jacobs, D., Fiebig, K., García de la Torre, J., and Pons, M. (2004). Interpretation of NMR relaxation properties of Pin1, a two-domain protein, based on Brownian dynamics simulation. *J. Biomol. NMR* **29**, 21–35.
- Bloomfield, V. A., Dalton, W. O., and van Holde, K. E. (1967). Frictional coefficient of multisubunit structures. I. Theory. *Biopolymers* **5**, 135–148.
- Boisbouvier, J., Wu, Z., Ono, A., Kainosho, M., and Bax, A. (2003). Rotational diffusion tensor of nucleic acids from ^{13}C NMR relaxation. *J. Biomol. NMR* **27**, 133–142.
- Brüschweiler, R. (2003). New approaches to the dynamic interpretation and prediction of NMR relaxation data from proteins. *Curr. Opin. Struct. Biol.* **13**, 175–183.
- Brüschweiler, R., Liao, X. B., and Wright, P. E. (1995). Long-range motional restrictions in a multidomain zinc-finger protein from anisotropic tumbling. *Science* **268**, 886–889.
- Fernandes, M. X., Ortega, A., López Martínez, M. C., and García de la Torre, J. (2002). Calculation of hydrodynamic properties of small nucleic acids from their atomic structure. *Nucleic Acids Res.* **29**, 3362–3376.
- Fischer, M. W. F., Majumdar, A., and Zuiderweg, E. R. P. (1998). Protein NMR relaxation: Theory, applications and outlook. *Prog. NMR Spectrosc.* **33**, 207–272.
- García de la Torre, J. (2001). Hydration from hydrodynamics: General considerations and applications of bead modelling to globular proteins. *Biophys. Chem.* **93**, 159–170.
- García de la Torre, J., and Bloomfield, V. A. (1981). Hydrodynamic properties of complex, rigid, biological macromolecules. Theory and applications. *Q. Rev. Biophys.* **14**, 81–139.
- García de la Torre, J., Navarro, S., López Martínez, M. C., Díaz, F. G., and Lopez Cascales, J. J. (1994). HYDRO: A computer software for the prediction of hydrodynamic properties of macromolecules. *Biophys. J.* **67**, 361–372.
- García de la Torre, J., Huertas, M. L., and Carrasco, B. (2000a). HYDRONMR: Prediction of NMR relaxation of globular proteins from atomic-level structures and hydrodynamic calculations. *J. Magn. Reson.* **147**, 138–146.
- García de la Torre, J., Huertas, M. L., and Carrasco, B. (2000b). Calculation of hydrodynamic properties of globular proteins from their atomic-level structure. *Biophys. J.* **78**, 719–730.
- García de la Torre, J., Pérez Sánchez, H. E., Ortega, A., Hernández Cifre, J. G., Fernández, M. X., and López Martínez, M. C. (2003). Calculation of solution properties of flexible macromolecules. Methods and applications. *Eur. Biophys. J.* **32**, 477–486.
- Kay, L., Torchia, D., and Bax, A. (1989). Backbone dynamics of proteins as studied by ^{15}N inverse detected heteronuclear NMR spectroscopy: Application to staphylococcal nuclease. *Biochemistry* **28**, 8972–8979.
- Korzhnev, D. M., Billeter, M. A., Arseniev, A. S., and Orekhov, V. Y. (2001). NMR studies of brownian tumbling and internal motions in proteins. *Prog. Nucl. Magn. Reson. Spectrosc.* **38**, 197–266.
- Kroenke, C. D., Loria, J. P., Lee, L. K., Rance, M., and Palmer, A. G., III (1998). Longitudinal and transverse ^1H - ^{15}N dipolar/ ^{15}N chemical shift anisotropy relaxation interference: Unambiguous determination of rotational diffusion tensors and chemical exchange effects in biological macromolecules. *J. Am. Chem. Soc.* **120**, 7905–7915.
- Lipari, G., and Szabo, A. (1982). Model-free approach to the interpretation of NMR relaxation in macromolecules. 1. Theory and range of validity. *J. Am. Chem. Soc.* **104**, 4546–4559.
- Osborne, M. J., and Wright, P. E. (2001). Anisotropic rotational diffusion in model-free analysis for a ternary DHFR complex. *J. Biomol. NMR* **19**, 209–230.
- Palmer, A. G., III, Williams, J., and McDermott, A. (1996). Nuclear magnetic resonance of biopolymer dynamics. *J. Phys. Chem.* **100**, 13293–13310.
- Pawley, N. H., Koide, S., and Nicholson, L. K. (2002). Backbone dynamics and thermodynamics of Borrelia outer surface protein A. *J. Mol. Biol.* **324**, 991–1002.

- Prompers, J. J., and Brüschweiler, R. (2002). General framework for studying the dynamics of folded and nonfolded proteins by NMR relaxation spectroscopy and MD simulation. *J. Am. Chem. Soc.* **124**, 4522–4534.
- Tirado, M. M., and García de la Torre, J. (1980). Rotational dynamics of rigid, symmetric top macromolecules. Application to circular cylinders. *J. Chem. Phys.* **73**, 1986–1993.
- Tirado, M. M., López Martínez, C., and García de la Torre, J. (1984). Comparison of theories for translational and rotational diffusion coefficients of rodlike macromolecules. Application to short DNA fragments. *J. Chem. Phys.* **81**, 2047–2052.
- Tjandra, N., Feller, S. E., Pastor, R. W., and Bax, A. (1995). Rotational diffusion anisotropy of human ubiquitin from ^{15}N NMR relaxation. *J. Am. Chem. Soc.* **117**, 12562–12566.
- Woessner, D. E. (1962). Nuclear spin relaxation in ellipsoids undergoing rotational Brownian motion. *J. Chem. Phys.* **37**, 647–654.

[18] Solution NMR Spin Relaxation Methods for Characterizing Chemical Exchange in High-Molecular-Weight Systems

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Abstract

Transverse relaxation optimized NMR spectroscopy (TROSY) techniques for ^1H – ^{15}N backbone amide moieties and for $^{13}\text{CH}_3$ methyl groups have permitted the development of Hahn spin echo and Carr–Purcell–Meiboom–Gill (CPMG) experiments for characterizing chemical exchange kinetic phenomena on microsecond–millisecond time scales in proteins with molecular masses >50 kDa. This chapter surveys the theoretical bases for TROSY in spin systems subject to chemical exchange linebroadening, the experimental methods that have been developed to quantitatively characterize chemical exchange in large proteins, and the emerging applications to triose phosphate isomerase, hemoglobin, and malate synthase G, with molecular masses ranging from 54 to 82 kDa.

Introduction

The ultimate goal of structural biology is to understand the relationship between protein structure and biological function. In this context, protein structure is usefully conceived not as a single conformational entity but rather as a dynamic ensemble of conformational states that is populated at equilibrium according to the Boltzmann distribution. With this in mind, under a set of “native” conditions, the lowest energy, or ground state, conformation is the most probable state. However, any given molecule in