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REVIEW

An overview of recent developments in the interpretation and prediction of fast internal protein dynamics

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Abstract During the past decades, NMR spectroscopy has emerged as a unique tool for the study of protein dynamics. Indeed, relaxation studies on isotopically labeled proteins can provide information on the overall motions as well as the internal fast, sub-nanosecond, dynamics. Therefore, the interpretation and the prediction of spin relaxation rates in proteins are important issues that have motivated numerous theoretical and methodological developments, including the description of overall dynamics and its possible coupling to internal mobility, the introduction of models of internal dynamics, the determination of correlation functions from experimental data, and the relationship between relaxation and thermodynamical quantities. A brief account of recent developments that have proven useful in this domain are presented.

Keywords NMR · Relaxation · Protein dynamics · Order parameter

Introduction

Over the past decades, structures of proteins have been intensely studied, new folds and structures have been determined. However, the crucial role of internal dynamics for protein function has also emerged and has motivated a large amount of work. In this respect, nuclear magnetic resonance (NMR) has proved to be a unique tool, allowing a detailed, site-specific description of internal motions in proteins, and a variety of new NMR techniques have been developed to access internal mobility in proteins over time scales ranging from the picosecond to the millisecond or longer, mostly relying on spin relaxation measurements. Processes occurring in the sub-nanosecond time scale are due to fast motions involving chemical bonds in the molecule, such as rotations, vibrations and librations. Fast internal dynamics can be related to conformational entropy, and its alteration upon ligand binding, to changes of various thermodynamical quantities (Akke et al. 1993). Indeed, in the well-accepted view (Wand 2001a, b) (so-called ''induced fit''), binding with a ligand is associated with a reduction of conformational degrees of freedom. However, whilst binding is usually associated with a decrease of the local conformational entropy, it may also be associated with an increase of the latter in other parts of the protein, which could lead to a global favorable entropic contribution to the reaction (Zidek et al. 1999).

Alternatively, motions on a time scale slower (Palmer et al. 2001) than the microsecond are often associated with biological processes, such as catalytic activity or allostery, for instance. However, relationships between sub-nanosecond internal mobility and processes occurring at much slower time scales have been postulated, and reviewed recently (Jarymowicz and Stone 2006).

Methodological developments in liquid state high resolution NMR have opened the way to the measurement of relaxation rates associated with different nuclei and interactions, thus providing information on internal motions of proteins on a residue-per-residue basis. Thus, experimental measurement of ^{15}N longitudinal and transverse relaxation rates has developed into a valuable tool for studying protein-backbone dynamics (Palmer 2001; Korzhnev et al. 2001). In a protein, one commonly measures longitudinal and

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transverse relaxation rates R_1 and R_2 of ^{15}N, as well as heteronuclear ^{15}N{^1H} Overhauser enhancements for NH^N two-spin subsystems of each amide bond. In addition, the measurement of various spin relaxation rates due to interference effects, have allowed investigating fast (Pelupessy et al. 2003) and slow protein dynamics (Kloiber and Konrat 2000; Perazzolo et al. 2005; Wist et al. 2005; Kateb et al. 2006).

The interpretation of spin relaxation measurements in terms of molecular motions is a matter of primary importance. This difficult problem has benefited from the so-called model-free approach of Lipari and Szabo (a, b), where dynamics are described by an effective correlation time and an order parameter which is a measure of the restriction of internal motions. These model-free parameters must then be interpreted in terms of particular motional models to provide further insight. Many of the topics dealt with in the present account have been reviewed in great detail elsewhere (Daragan and Mayo 1997; Fischer et al. 1998; Korzhnev et al. 2001; Palmer 2001; Jarymowicz and Stone 2006; Igumenova et al. 2006). Here, we present an overview of some recent advances in the interpretation and prediction of NMR relaxation that have improved our understanding of protein dynamics.

NMR relaxation as a probe into internal motions

NMR relaxation experiments rely on the monitoring of spin magnetization or coherences to equilibrium following a perturbation by some radiofrequency excitation. NMR relaxation is caused by the fluctuations of magnetic interactions due to molecular motions, through the spatial part of the magnetic hamiltonians (Abragam 1961; Cavanagh et al. 1996; Levitt 2001). In the liquid state, these motions comprise the overall diffusion (''tumbling'') of the molecule and internal fluctuations caused by fast rotations, vibrations and librations of the chemical bonds.

The study of backbone dynamics commonly includes the measurement of longitudinal R_1 and transverse R_2 relaxation rates of amide ^{15}N, along with heteronuclear ^{15}N{^1H} nuclear overhauser effects. In the simple case of an amide ^{15}N spin, relaxation is mainly due the fluctuations of the dipolar interaction with its attached proton, and its chemical shift anisotropy. In the Abragam–Redfield theory of relaxation (Abragam 1961; Redfield 1965), spin relaxation rates are determined by the nature of the magnetic interactions (dipolar, CSA, etc.) and by the correlation functions $C(\tau)$ of the spatial functions comprised in the hamiltonians:

$$C(\tau) = \langle Y_{lm}(t) Y_{lm}(t-\tau) \rangle \tag{1}$$

where the brackets denote the ensemble average and Y_{lm} are the rank-l spherical haronics (Brink and Satchler 1968).

Thus, for two I = ^{15}N and S = ^1H, R_1, R_2 relaxation rates and NOE are given by:

$$\begin{aligned} R_1 &= \frac{1}{4} d^2 (J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)) + c^2 J(\omega_I) \\ R_2 &= \frac{1}{8} d^2 (4J(0) + J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_S) \\ &\quad + 6J(\omega_I + \omega_S)) + \frac{1}{6} c^2 (4J(0) + 3J(\omega_I)) \\ \mathrm{NOE} &= 1 + \frac{1}{4} d^2 \frac{\gamma_S}{\gamma_I} \frac{6J(\omega_I + \omega_S) - J(\omega_I - \omega_S)}{R_1} = 1 + \frac{\gamma_S}{\gamma_I} \frac{\sigma_{IS}}{R_1} \end{aligned} \tag{2}$$

where $c = \frac{\omega_I \Delta\sigma}{\sqrt{3}}$ and $d = \frac{\mu_0 h \gamma_I \gamma_S}{8\pi^2 \langle r^3 \rangle}$, $\Delta\sigma$ is the chemical shift anisotropy of spin I, γ_I and γ_S are the gyromagnetic ratios of spins I and S, and r is the distance between I and S nuclei. In Eq. 2, $J(\omega) = \int_0^\infty C(t) \cos \omega t dt$ defines the spectral density function, which contains the frequency distribution of the fluctuation processes.

Interference effects, or cross-correlated relaxation, between two different relaxation mechanisms also contribute to spin relaxation (Goldman 1984; Reif et al. 1997). Although experiments that measure these rates have been introduced to extract structural constraints on backbone conformation (Reif et al. 1997; Pelupessy et al. 1999a, b; Reif et al. 2000; Schwalbe et al. 1997), interference effects also provide valuable dynamical information, on both fast (Carlomagno et al. 2000) and slow (Kroenke et al. 1998; Millet et al. 2000; Kloiber and Konrat 2000; Wist et al. 2005; Perazzolo et al. 2005) time scales.

Recent developments in NMR methodology also focus on the measurement of fast side chain dynamics, including ^{13}C and ^{2}H relaxation of methylene (Yang et al. 1998; Idiyatullin et al. 2004) or methyl groups of protonated and partially deuterated methyl groups (Millet et al. 2002; Skrynnikov et al. 2002). These aspects have been reviewed recently (Igumenova et al. 2006).

As discussed above, the main goal of relaxation measurements is the determination of lattice correlation functions governing the fluctuations of the magnetic interactions. In isotropic liquids, the correlation functions decay to zero due to the absence of a preferential orientation of the molecules. However, the total correlation function results from the combination of different motional processes, commonly separated into internal and overall motions. Overall motion is usually accurately accounted for by a rotational diffusion process (''overall tumbling'') of the macromolecule (Favro 1960), whereas internal motions may be described in various ways. Folded proteins are dense objects, so that internal mobility is often thought to be restricted to relatively small amplitude fluctuations. This of course suffers many exceptions, especially for side chains, where the degree of mobility does not seem to be

correlated with local atomic packing or solvent accessibility (Igumenova et al. 2006).

To simplify matters and to allow for insightful analytical descriptions, most of the theoretical approaches to protein dynamics assume statistical independence of the internal and overall mobilities. Then, the further assumption of isotropic overall tumbling enables one to factorize the total correlation function $C(t)$ into the product of the correlation functions of overall and internal motions, $C_0(t)$ and $C_I(t)$ (Korzhnev et al. 2001):

$$C(t) = C_0(t)C_I(t) \tag{3}$$

Alternatively, in the case of anisotropic diffusion, relaxation data are analyzed using Woessner's equations (Woessner 1962), and the correlation function writes (Huntress 1968; Werbelow and Grant (1975; Daragan and Mayo 1997):

$$C(t) = \frac{4\pi}{5}\sum_{q=-2}^{2} \mathrm{e}^{-D_q t}\langle Y_{2q}[\Omega(t)]Y_{2q}^{*}[\Omega(0)]\rangle \tag{4}$$

where $\Omega = (\theta,\varphi)$ represents the polar angles defining the (say, NH) vector in the diffusing frame, and $Y_{2q}[\Omega(t)]$ are the spherical harmonics of rank 2 (Brink and Satchler 1968). The rotational diffusion coefficients D_q are defined as $D_q = 6D_\perp + q^2\,(D_\parallel - D_\perp)$.

Several approaches aiming at predicting diffusion tensors and based on the knowledge of the protein structure have been introduced. A bead model was developed some time ago, in which the atoms of the protein are modeled by small spheres. In this model, a shell representation of the protein is constructed from these elements to model the friction interaction between the molecular surface and the solvent (de la Torre and Bloomfield 1981; de la Torre et al. 2000a). This has been successfully applied to the modeling of ^{15}N and ^{13}C relaxation in proteins (Bernado et al. 2002; de la Torre et al. 2000b).

More recently, Fushman and co-workers (Ryabov et al. 2006) have developed a different method based on the work of Perrin (1934, 1936), who derived analytical expressions relating the diffusion properties of an ellipsoid to its geometry. Using the fact that the diffusional tensor of any object depends on six components, the authors propose to describe the protein by an ellipsoid, and thereby derive the components of the diffusion tensor for the equivalent ellipsoid.

In some cases, however, statistical correlation between internal and overall motions should be taken into account. This has been achieved through a mode-coupling approach (Tugarinov et al. 2001, 2002), based on the so-called slowly relaxing local structure (SRLS) theory (Polimeno and Freed 1993, 1995). This theory provides a framework for the description of amide ^{15}N relaxation when dynamical coupling between global and local diffusion is present, and has been successfully applied, and recently extended to side chain methyl relaxation (Meirovitch et al. 2006). Interestingly, the model-free approach (see below) can be seen as a limiting case of this more general theory (Tugarinov et al. 2003).

The model free approximation of $C(t)$

One of the most popular methods used in protein NMR is the so-called *model free* approach introduced by Lipari and Szabo (1982a, b). It is based on the assumption that the internal correlation equation in equation 3 can be approximated by a mono-exponentially decaying function toward the finite limit S^2:

$$C_I(t) = S^2 + (1 - S^2)\mathrm{e}^{-t/\tau_e} \tag{5}$$

The *generalized order parameter* S^2 can take values between 0 and 1, which define limiting cases of freely moving and totally rigid internal motions, respectively. The effective correlation time τ_e of the model-free correlation function sets the time scale of the internal processes. This simple model has been extended (Clore et al. (1990b) to account for more complex processes where internal motions are better described by two timescales, rather than one. Interestingly, a model-free analysis of experimental data does not rely on a particular motional model.

For the case of a protein with an axially symmetric diffusion tensor, the model-free spectral density function becomes (Halle and Wennerström 1981; Lipari and Szabo 1982a; Schurr et al. 1994):

$$J(\omega) = \sum_{q=0}^{2} A_q \left[\frac{S^2\tau_q}{1+\omega^2\tau_q^2} + \frac{(1-S^2)\tau_q'}{1+\omega^2\tau_q'^2}\right] \tag{6}$$

where θ is the angle between the direction of the vector $\mathbf{r}$ and the axis of the diffusion tensor, $A_0 = 0.25(3\cos^2\theta - 1)^2$, $A_1 = 3\sin^2\theta\cos^2\theta$, $A_2 = 0.75\sin^4\theta$, $\tau_q = D_q^{-1}$, and $\tau_q'^{-1} = \tau_q^{-1} + \tau_e^{-1}$.

In situations where the overall diffusion anisotropy is moderately small, an approximate diffusion tensor, leading to a ''local'' overall correlation time can be introduced using the quadric approximation (Brüschweiler et al. 1995; Lee et al. 1997; Fushman et al. 1999), so that the model-free spectral density function is:

$$J(\omega) \approx \left[\frac{S^2\tau_g}{1+\omega^2\tau_g^2} + \frac{(1-S^2)\tau_g'}{1+\omega^2\tau_g'^2}\right] \tag{7}$$

In Eq. 7, the overall correlation times τ_g are defined for each spin and depend on the orientation of the interaction

vectors with respect to the principal axis frame of the diffusion tensor. Strategies based on these model-free analyses of NMR relaxation data have been developed and implemented in dedicated softwares (Mandel et al. 1995; Blackledge et al. 1998; Dosset et al. 2000).

Model-based interpretation of NMR data

Order parameters and effective correlation times, as well as (partially) reconstructed spectral density functions can be given an interpretation in terms of a particular motional model. Indeed, models of internal motions include single-axis rotation, unrestricted (Woessner 1962) or restricted (London and Avitabile 1978; Wittebort and Szabo 1978) rotational diffusion, or rotational jumps (Torchia and Szabo 1982). The more general case of diffusion in an arbitrary potential has also been studied (Edholm and Blomberg 1979).

A specific model often used for the interpretation of internal motion of the bond vector is the diffusion-in-a-cone model, where the bond vector moves on the surface of a cone with semi-angle θ_0. In this case, the order parameter is related to θ_0 by: $S^2 = \left[\frac{1}{2}\cos\theta_0(1+\cos\theta_0)\right]^2$ (Lipari and Szabo 1982a, b). Alternatively, a two-site jump model, or a combination of diffusion-in-a-cone and two-site jump have been proposed (Clore et al. 1990a, b).

Proteins are very compact objects, in which motions are in many cases highly restricted. This is expected on the backbone, but may often be the case for sidechains. It is therefore reasonable to describe internal motions as fluctuations about an equilibrium position within a potential well. This idea has been developed by several authors and along different lines (Daragan and Mayo 1997).

A fruitful implementation of this is the 3D *G*aussian *A*xial *F*luctuation (3D GAF) model, which has been used for the description of anisotropic peptide-plane motions. Based on an analytical approach combined with molecular dynamics simulations, peptide plane motions are represented by independent harmonic angular fluctuations about three orthogonal axes (Bremi et al. 1997; Bremi and Brüschweiler 1997). Thus, experimental relaxation measurements can be analyzed in the framework of the 3D-GAF model, which relates observations to a physical picture of backbone motion where the peptide plane represents the structural unit.

Conformational entropy from S^2

As mentioned in the Introduction, one of the main motivations for the study of internal protein dynamics is the existence of possible relationships between fast (sub-nanosecond) time scale internal motions of the protein and its function (Jarymowicz and Stone 2006). In the context of NMR studies of proteins, it is possible to relate order parameters S^2 to vector conformational or statistical entropy, calculated from the distribution probability of orientations $p(\Omega)$ and defined as $S_p = -k\sum p(\Omega)\log p(\Omega)$. Observation of the dependence of S_p as a function of S^2 for various models allowed the derivation of empirical relationships. Thermodynamic quantities may then be estimated from NMR measurements. In particular, changes of conformational entropy between states a and b upon ligand binding can be calculated, with similar, albeit different expressions, depending on the model used. Thus, Akke et al. (1993) obtain:

$$\Delta S_p = k\sum_{j=1}^{N}\log\left(\frac{1-S_{jb}^2}{1-S_{ja}^2}\right) \tag{8}$$

whereas for Yang and Kay (1996):

$$\Delta S_p = k\sum_{j=1}^{N}\log\left(\frac{3-(1+8S_{jb})^2}{3-(1+8S_{ja})^2}\right) \tag{9}$$

where $S_{j\,a,b} = \sqrt{S_{j\,a,b}^2}$.

Of course, there are some difficulties related to these approaches, which may lead to erroneous interpretations. Some are inherent to NMR relaxation experiments, such as the lack of sensitivity to slow mobility or certain types of motions. A more fundamental problem lies in the decoupling approximation which is explicit in all the approaches mentioned above, which may be overcome in the future by some of the approaches mentioned below.

Structure-based prediction of NMR relaxation parameters

In a folded protein, interactions between atoms determine their mobility, and the relationship between structure and internal dynamics has been questioned along different approaches. Although there does not seem to exist strong correlations between NMR order parameters and either the residue type or the structure elements (Goodman et al. 2000), several studies have demonstrated the intimate relationship between S^2 and local structure, which corroborates previous approaches relating the atomic mean square deviation (AMSD) with the local packing in proteins (Halle 2002).

Molecular dynamics

Over the past decades, molecular dynamics simulations have become extremely widespread and have demonstrated the ability of this approach to provide useful information on the sub-nanosecond time-scale evolution of proteins

(Palmer and Case 1992; Chandrashekar et al. 1992; Kördel and Teleman 1992; Schmidt et al. 1993; Fushman et al. 1994; Chatfield et al. 1998). Indeed, computer simulations can be used to predict NMR relaxation parameters, through the numerical computation of the correlation function (Bremi and Brüschweiler 1997; Lenin et al. 1998). However, the calculation of the global correlation function that includes the description of global and internal motions is possible only for relatively small molecules, for which simulations of the molecule dynamics over durations exceeding the value of the overall correlation time is achievable. In large molecules such as proteins, the overall tumbling time is too long to be adequately sampled by computer simulations. This is usually circumvented by eliminating overall degrees of freedom through alignment of the protein in successive snapshots with respect to a fixed frame. This procedure allows for the determination of internal correlation functions, provided that the decoupling approximation is valid (Eq. 3).

As a method derived from molecular dynamics simulations, the internal Reorientational Eigenmode Dynamics approach introduced by Brüschweiler et al. (Prompers and Brüschweiler 2002) is based on the computation of the covariance matrix of the spherical harmonics involved in the calculation of correlation functions. A principal component analysis of the covariance matrix enables one to obtain information such as mode collectivities and order parameters.

Approaches based on contact models

A contact model for the prediction of NMR order parameters has been proposed by (Zhang and Brüschweiler 2002). An empirical equation relating S_i^2 of the ith NH amide backbone vector to the three-dimensional structure was derived:

$$S_i^2 = \tan h\left(0.8C_{i,NH}\right) + b \tag{10}$$

where the contact sum $C_{i,NH} = \sum_k \mathrm{e}^{-d^O_{i-1,k}} + 0.8\mathrm{e}^{-d^H_{i,k}}$, $d^O{}_{i-1,k}$ is the the distance between the carbonyl oxygen of amino acid i–1 and heavy atom k and $d^H_{i,k}$ is the distance between the amide proton H^N and heavy atom k. The constant b of Eq. 10 was originally taken equal to 0.1 (Zhang and Brüschweiler 2002). This approach was subsequently extended and adapted to the prediction of methyl-group order parameters S^2 in protein side chains (Ming and Brüschweiler 2004). Thus, the authors propose a different expression:

$$S^2 = \tanh\left(aC_i/n_i^b\right) - c \tag{11}$$

where in the contact sum $C_i = \sum_k \mathrm{e}^{-r_{ij}/r_{\mathrm{eff}}}$, r_{ij} is the distance between the methyl carbon atom i and heavy atom k. The parameter n_i^b denotes the number of dihedral angles between the methyl carbon and the backbone C_α of the same residue. Parameters a, b, c, and r_{eff} were optimized on a group of proteins of known structure and for which relaxation data were available.

A recent extension of these ideas was the combination of a contact model with the elastic network model (ENM) (Ming and Brüschweiler 2006). An ENM model was originally introduced by Tirion for the efficient description of small motions in proteins via a potential which is the superposition of hookean pair potentials (Tirion 1997). This allowed a normal mode analysis that enables one to predict atomic mean square displacements and B-factors of C_α atoms in proteins of known 3D structure.

In the work of Ming and Brüschweiler (2006), pair potentials relate the angles between pairs of backbone NH vectors through their equilibrium angles defined by the three-dimensional structure. In addition, these pair potentials are weighted by the contact sums appearing in Eq. 10. This setup allows for an empiric determination of order parameters from the calculation of averaged squared displacements of the atoms involved (Wittebort and Szabo 1985; Brüschweiler and Case 1994).

Network of coupled rotators

The description of internal dynamics of proteins by a network of coupled rotators (NCR) has been proposed. This approach is NMR-oriented in the sense that internal dynamics are described in the space of angles between bond vectors rather than in the cartesian space of atomic coordinates (Abergel and Bodenhausen 2005). The NCR model shares some common features with the contact-based approaches described above. Indeed, the overall simplified potential describing the force field is the superposition of pair potentials that couple pairs of vectors. Moreover, each of these pair potentials is weighted by the number of atomic neighbors that are located in the vicinity of the atoms that define the vectors involved. Each vector is assumed to behave as a rotating solid that obeys a diffusive motion in the above potential. The dynamics of the vectors are thus described by the rotational Langevin equations (Coffey et al. 1996). It is possible to show that from these stochastic differential equations, ordinary differential equations can be derived that define the evolution of averages of interest. The assumption of small amplitude motions of the vectors in the protein allows to derive analytical solutions from which order parameters and correlation functions are extracted (Abergel and Bodenhausen 2004, 2005). Whilst order parameters represent a measure of the degree of spatial restriction of the orientational motions, crystallographic B-factors account for dynamical disorder in proteins and are proportional to the atomic

mean square deviations (AMSD). A comparison of both shows a significant correlation (linear correlation coefficient ca 0.8) in the case of the protein 6-phosphogluconolactonase (Delarue et al. 2006) (Figs. 1, 2).

In a recent application, the NCR model was used to reproduce the variations of order parameters upon metal binding in the calcium-binding protein Calbindin (Dhulesia et al. 2007). This approach also gives access to the kinetics of the internal motions, such as effective correlation times, obtained from approximate solutions of the Langevin equations. An effective correlation time τ_e defined by the integral of the correlation function, as in the model-free approach, can thus be calculated. Alternatively, τ_e can be defined using the inverse of the initial slope of the correlation function: $\tau_e = \frac{S^2-1}{\dot{C}(0)}$. This definition is expected to yield better estimates in the case of fast processes, as it reflects the evolution of the correlation function at short times. Clearly, both definitions are equivalent in the case of an exponential correlation function.

A combination of these alternate definitions was used on the protein Calbindin to reproduce effective times which were obtained from a model-free or an extended model-free analysis of experimental data (Dhulesia et al. 2007). A more interesting prospect is the prediction of relaxation rates, which can also be calculated by the NCR approach (Nodet et al., in preparation).

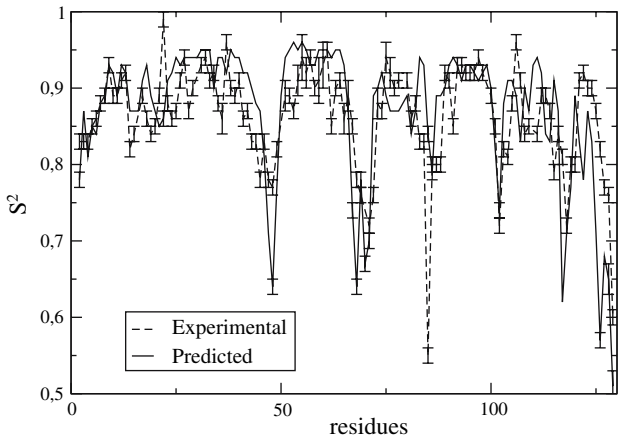

Fig. 1 Comparison of order parameters predicted by the NCR model and obtained experimentally on the lysozyme protein (Buck et al. 1995)

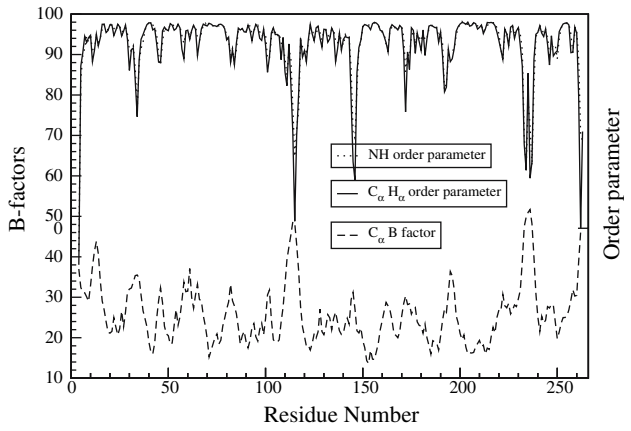

Fig. 2 Comparison of S^2 order parameters predicted by the NCR model for NH (*dotted line*) and $C_\alpha H_\alpha$ (*solid line*) vectors with experimental B-factors of C_α (*dashed line*) carbons on the protein 6PGL

Conclusion

NMR has proved to be a very powerful tool for the study of internal dynamics in proteins, and continues to develop and provide detailed, site-specific information on protein dynamics. Numerous dynamical models and theories for both overall and internal protein dynamics have been developed to provide an interpretation framework of experimental data. A selected number of those developments were briefly outlined in this review and some of their aspects highlighted. Important issues such as statistical separability of overall and internal motions, motion cooperativity, or side chain dynamics for instance, have been addressed by several authors. Although the validation of a model, a methodology or dynamics simulations should be eventually based on the comparison of predictions with relaxation experiments, this may sometimes be difficult due to a number of caveats (Philippopoulos et al. 1997; Case 2002). Among others, the possibility of fitting the same data to different models is one of the difficulties that should compel to apply these approaches to an ever larger number of proteins, in an effort to determine their ranges of applicability and to improve our understanding of protein dynamics.

References

Abergel D, Bodenhausen G (2004) A simple model for NMR relaxation in the presence of internal motions with dynamical coupling. J Chem Phys 121:761

Abergel D, Bodenhausen G (2005) Predicting internal protein dynamics from structures using coupled networks of hindered rotators. J Chem Phys 123:204901

Abragam A (1961) Principles of nuclear magnetism. Clarendon Press, Oxford

Akke M, Brüschweiler R, Arthur G Palmer I (1993) NMR order parameters and free energy: an analytical approach and its application to cooperative ca^{2+} binding by calbindin d_{9k}. J Am Chem Soc 115:9832–9833

Bernado P, de la Torre JG, Pons M (2002) Interpretation of ^{15}N NMR relaxation of globular proteins using hydrodynamic calculations with hydroNMR. J Biomol NMR 23:139–150

Blackledge M, Cordier F, Dosset P, Marion D (1998) Precision and uncertainty in the characterization of anisotropic rotational diffusion by ^{15}N relaxation. J Am Chem Soc 120:4538–4539

Bremi T, Brüschweiler R (1997) Locally anisotropic internal polypeptide backbone dynamics by NMR relaxation. J Am Chem Soc 119:6672–6673

Bremi T, Brüschweiler R, Ernst R (1997) A protocol for the interpretation of side-chain dynamics based on NMR relaxation: Application to phenylalanines in antamanide. J Am Chem Soc 119:4272

Brink D, Satchler G (1968) Angular momentum. Clarendon Press, Oxford

Brüschweiler R, Liao X, Wright P (1995) Long-range motional restrictions in a multidomain zinc-finger protein from anisotropic tumbling. Science 268:886–889

Brüschweiler R, Case DA (1994) Collective NMR relaxation model applied to protein dynamics. Phys Rev Lett 72:940–943

Buck M, Boyd J, Redfield C, MacKenzie D, Jeenes D, Archer D, Dobson C (1995) Structural determinants of protein dynamics: analysis of ^{15}N NMR relaxation measurements for main-chain and side-chain nuclei of hen egg white lysozyme. Biochemistry 34:4041–4055

Carlomagno T, Maurer M, Hennig M, Griesinger C (2000) Ubiquitin backbone motion studied via NH^N-C′ C_α dipolar-dipolar and C′–C′C_α/ NH^N CSA-dipolar cross-correlation relaxation. J Am Chem Soc 122:5105–5113

Case D (2002) Molecular dynamics and NMR spin relaxation in proteins. Acc Chem Res 35:325–331

Cavanagh J, Fairbrother WJ, Palmer AG, Skelton NJ (1996) Protein NMR spectroscopy. Academic, New York

Chandrashekar I, Clore GM, Szabo A, Gronenborn A, Brooks B (1992) A 500 ps molecular dynamics simulation study of interleukin-1 beta in water. correlation with nuclear magnetic resonance spectroscopy and crystallography. J Mol Biol 226:239–250

Chatfield D, Szabo A, Brooks B (1998) Molecular dynamics of staphylococcal nuclease: comparison of simulation with ^{15}N and ^{13}C NMR relaxation data. J Am Chem Soc 120:5301–5311

Clore GM, Driscoll PC, Wingfield P, Gronenborn AM (1990a) Analysis of the backbone dynamics of interleukine-1 beta using two-dimensional inverse detected heteronuclear nitrogen-15-proton NMR spectroscopy. Biochemistry 29:7387–7401

Clore GM, Szabo A, Bax A, Kay LE, Driscoll PC, Gronenborn AM (1990b) Deviations from the simple two-parameter model-free approach of the interpretation of nitrogen-15 nuclear magnetic relaxation of proteins. J Am Chem Soc 112:4989–4991

Coffey WT, Kalmykov YP, Waldron JT (1996) The Langevin equation. World Scientific, Singapore

Daragan VA, Mayo KH (1997) Motional model analyses of protein and peptide dynamics using ^{13}C and ^{15}N NMR relaxation. Progr NMR Spectrosc 31:63–105

de la Torre JG, Bloomfield V (1981) Hydrodynamic properties of complex, rigid, biological macromolecules—theory and applications. Quart Rev Biophys 14:81–139

de la Torre JG, Huertas ML, Carrasco B (2000a) Calculation of hydrodynamic properties of globular proteins from their atomic-level structure. Biophys J 78:719–730

de la Torre JG, Huertas ML, Carrasco B (2000b) HydroNMR: prediction of NMR relaxation of globular proteins from atomic-level structures and hydrodynamic calculations. J Magn Reson 147:138–146

Delarue M, Duclert-Savatier N, Miclet E, Haouz A, Giganti D, Ouazzani J, Lopes P, Nilges M, Stoven V (2007) Three dimensional structure and implications for the catalytic mechanism of 6-phosphogluconolactonase from trypanosoma brucei. J Mol Biol 366:868–881

Dhulesia A, Abergel D, Bodenhausen G (2007) Networks of coupled rotators: Relationship between structures and internal dynamics in metal-binding proteins. application to apo- and holo-calbindin. J Am Chem Soc 129:4998–5006

Dosset P, Marion D, Blackledge M (2000) Tensor2. Copyright 1999, Institut de Biologie Structurale JP EBEL CEA-CNRS, Laboratoire de Resonance Magnetique nucleaire, Grenoble

Edholm O, Blomberg C (1979) Decay of angular-correlation functions by multiple rotational potential diffusion in polymer-chains, with applications to NMR relaxation in paraffin chains of lipid bilayers. Chem Phys 42:449

Favro LD (1960) Theory of the rotational brownian motion of a free rigid body. Phys Rev 119:53

Fischer M, Majumdar A, Zuiderweg E (1998) Protein NMR relaxation: theory, applications and outlook. Prog Nucl Magn Reson 33:207–272

Fushman D, Ohlenschläger O, Rüterjans H (1994) Determination of the backbone mobility of ribonuclease t1 and its 2′ GMP complex using molecular dynamics simulations and NMR relaxation data. J Biomol Struct Dyn 4:61–78

Fushman D, Xu R, Cowburn D (1999) Direct determination of changes of interdomain orientation on ligation: use of the orientational dependence of 15N NMR relaxation in abl sh(32). Biochemistry 38:10225–10230

Goldman M (1984) Interference effects in the relaxation of a pair of unlike spin-1/2 nuclei. J Magn Reson 60:437–452

Goodman JL, Pagel MD, Stone MJ (2000) Relationships between protein structure and dynamics from a database of NMR-derived backbone order parameters. J Mol Biol 295:963–978

Halle B (2002) Flexibility and packing in proteins. Proc Natl Acad Sci 99:1275–1279

Halle B, Wennerström H (1981) J Chem Phys 75:1928–1943

Huntress WT (1968) Effects of anisotropic molecular rotational diffusion on nuclear magnetic relaxation in liquids. J Chem Phys 48:3524–3533

Idiyatullin D, Daragan V, Mayo K (2004) A simple method to measure (ch2)-C-13 heteronuclear dipolar cross-correlation spectral densities. J Magn Reson 171:4–9

Igumenova TI, Frederick KK, Wand AJ (2006) Characterization of the fast dynamics of protein amino acid side chains using NMR relaxation in solution. Chem Rev 106:1672–1699

Jarymowicz VA, Stone M (2006) Fast time scale dynamics of protein backbones: NMR relaxation methods, applications, and functional consequences. Chem Rev 106:1624–1671

Kateb F, Abergel D, Blouquit Y, Duchambon P, Craescu C, Bodenhausen G (2006) Slow backbone dynamics of the C-terminal fragment of human centrin 2 in complex with a target peptide probed by cross-correlated relaxation in multiple-quantum NMR spectroscopy. Biochemistry 45:15011–15019

Kloiber K, Konrat R (2000) Differential multiple-quantum relaxation arising from cross-correlated time-modulation of isotropic chemical shifts. J Biomol NMR 18:33

Kördel J, Teleman O (1992) Backbone dynamics of calbindin d9k: comparison of molecular dynamics simulations and notrogen-15 NMR relaxation measurements. J Am Chem Soc 114:4934–4936

Korzhnev D, Billeter M, Arseniev A, Orekhov V (2001) NMR studies of brownian tumbling and internal motions in proteins. Prog NMR Spectrosc 38:197–266

Kroenke CD, Loria JP, Lee LK, Rance M, Palmer AG (1998) Longitudinal and transverse 1H–15N dipolar/15N 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

Lee LK, Rance M, Chazin WJ, Palmer AG (1997) Rotational diffusion anisotropy of proteins from simultaneous analysis of 15N and 13C^α nuclear spin relaxation. J Biomol NMR 9:287–298

Lenin F, Bremi T, Brutcher B, Ernst R (1997) Anisotropic intramolecular back-bone dynamics of ubiquitin characterized by NMR relaxation and md computer simulation. J Am Chem Soc 120:9870–9879

Levitt MH (2001) Spin dynamics: basics of nuclear magnetic resonance. Wiley, Chichester

Lipari G, Szabo A (1982a) Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 1. Theory and range of validity. J Am Chem Soc 104:4546

Lipari G, Szabo A (1982b) Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 2. Analysis of experimental results. J Am Chem Soc 104:4559

London R, Avitabile J (1978) Calculated carbon-13 NMR relaxation parameters for a restricted internal diffusionmodel. application to methionine relaxation in dihydrofolate reductase. J Am Chem Soc 100:7159–7165

Mandel AM, Akke M, Palmer III AG (1995) Backbone dynamics of escherichia coli ribonuclease hi: correlations with structure and function in an active enzyme. J Mol Biol 246:144–163

Meirovitch E, Polimeno A, Freed JH (2006) Methyl dynamics in proteins from NMR slowly relaxing local structure spin relaxation analysis: a new perspective. J Phys Chem B 110:20615–20628

Millet O, Loria JP, Kroenke CD, Pons M, Palmer AG (2000) The static magnetic field dependence of chemical exchange linebroadening defines the NMR chemical shift time scale. J Am Chem Soc 122:2867–2877

Millet O, Muhandiram D, Skrynnikov NR, Kay L (2002) Deuterium spin probes of side-chain dynamics in proteins. 1. Measurement of five relaxation rates per deuteron in ^{13}C-labelled and fractionally ^{2}H-enriched proteins in solution. J Am Chem Soc 124:6439–6448

Ming D, Brschweiler R (2004) Prediction of methyl side-chain dynamics in proteins. J Biomol NMR 29:363–368

Ming D, Brschweiler R (2006) Reorientational contact-weighted elastic network model for the prediction of protein dynamics: comparison with NMR relaxation. Biophys J 90:3382–3388

Palmer AG (2001) Annu Rev Biophys Biomol Struct 30:129

Palmer AG, Case D (1992) Molecular dynamics analysis of NMR relaxation in a zinc-finger peptide. J Am Chem Soc 114:9059–9067

Palmer AG, Kroenke CD, Loria JP (2001) Nuclear magnetic resonance methods for quantifying microsecond-to-millisecond motions in biological macromolecules. Meth Enzymol 339:204–238

Pelupessy P, Chiarparin E, Ghose R, Bodenhausen G (1999a) Efficient determination of angles subtended by C^{α} – H^{α} and $N–H^{N}$ vectors in proteins via dipole–dipole cross-correlation. J Biomol NMR 13:375–380

Pelupessy P, Chiarparin E, Ghose R, Bodenhausen G (1999b) Simultaneous detection of y and f angles in proteins from measurements of cross-correlated relaxation effects. J Biomol NMR 14:277–280

Pelupessy P, Ravindranathan S, Bodenhausen G (2003) Correlated motions of successive amide N–H bonds in proteins. J Biomol NMR 25:265–280

Perazzolo C, Wist J, Loth K, Poggi L, Homans S, Bodenhausen G (2005) Effects of protein-pheromone complexation on correlated chemical shift modulations. J Biomol NMR 33:233–242

Perrin F (1934) Journal de Physique du Radium 5:497–511

Perrin F (1936) Journal de Physique du Radium 7:1–11

Philippopoulos M, Mandel AM, Palmer AG III, Lim C (1997) Accuracy and precision of NMR relaxation experiments and md simulations for characterizing protein dynamics. Proteins Struct Funct Bioinformatics 28:481–493

Polimeno A, Freed J (1993) Adv Chem Phys 83:89–163

Polimeno A, Freed J (1995) Slow motional ESR in complex fluids—the slowly relaxing local-structure model of solvent cage effects. J Phys Chem 99:10995–11006

Prompers J, Brschweiler 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

Redfield AG (1965) Adv Magn Reson 1:1–32

Reif B, Diener A, Hennig M, Maurer M, Griesinger C (2000) Cross-correlated relaxation for the measurement of angles between tensorial interactions. J Magn Reson 143:45–68

Reif B, Hennig M, Griesinger C (1997) Direct measurement of angles between bond vectors in high-resolution NMR. Science 276:1230

Ryabov YE, Geraghty C, Varshney A, Fushman D (2006) An efficient computational method for predicting rotational diffusion tensors of globular proteins using an ellipsoid representation. Journal of the American Chemical Society 128:15432–15444

Schmidt JM, Brüschweiler R, Ernst RR, Dunbrack RL, Joseph D, Karplus M (1993) Molecular dynamics simulation of the proline conformational equilibrium and dynamics in antamanide using the charmm force field. J Am Chem Soc 115:8747–8756

Schurr JM, Babcock HP, Fujimoto BS (1994) A test of the model-free formulas—effects of anisotropic rotational diffusion and dimerization. J Magn Reson 105 B:211–224

Schwalbe H, Carlomagno T, Hennig M, Junker J, Reif B, Richter C, Griesinger C (1997) Methods Enzymol 338:35–81

Skrynnikov NR, Millet O, Kay LE (2002) Deuterium spin probes of side-chain dynamics in proteins 2 spectral density mapping and identification of nanosecond time-scale side-chain motions. J Am Chem Soc 124:6449–6460

Tirion M (1997) Large amplitude elastic motions in proteins from a single-parameter, atomic analysis. Phys Rev Lett 77:1905

Torchia D, Szabo A (1982) Spin-lattice relaxation in solids. J Magn Reson 49:107–121

Tugarinov V, Liang Z, Shapiro YE, Freed JH, Meirovitch E (2001) A structural mode-coupling approach to 15N NMR relaxation in proteins. J Am Chem Soc 123:3055–3063

Tugarinov V, Shapiro YE, Liang Z, Freed JH, Meirovitch E (2002) A novel view of domain flexibility in *E. coli* adenylate kinase based on structural mode-coupling 15N NMR relaxation. J Mol Biol 315:155

Tugarinov V, Shapiro YE, Liang Z, Freed JH, Meirovitch E (2003) Mode coupling srls versus mode-decoupled model-free N–H bond dynamics: mode-mixing and renormalization. J Phys Chem B 107:9898–9904

Wand J (2001a) Science 293:U1

Wand J (2001b) Dynamic activation of protein function: a view emerging from NMR spectroscopy. Nat Struct Biol 8:926–931

Werbelow L, Grant D (1975) The dipolar orientational correlation function. J Magn Reson 21:369

Wist J, Perazzolo C, Bodenhausen G (2005) Slow motions in nondeuterated proteins: concerted chemical shift modulations of backbone nuclei. Appl Magn Reson 29:251–259

Wittebort R, Szabo A (1978) Theory of NMR relaxation in macromolecules: restricted diffusion and jump models for multiple internal rotations in amino acid side chains. J Chem Phys 69:1722–1736

Wittebort R, Szabo A (1985) Influence of vibrational motion on solid state line shapes and NMR relaxation. J Chem Phys 82:4753–4761

Woessner DE (1962) Spin relaxation processes in a two-proton system undergoing anisotropic reorientation. J Chem Phys 36:1–4

Yang D, Kay LE (1996) Contributions to conformational entropy arising from bond vector fluctuations measured from NMR-

derived order parameters: application to protein folding. J Mol Biol 263:369–382

Yang D, Mittermaier A, Mok YK, Kay LE (1998) A study of protein side-chain dynamics from new 2H auto-correlation and 13C cross-correlation NMR experiments: application to the N-terminal SH3 domain from drk. J Mol Biol 276:939–954

Zhang L, Brüschweiler R (2002) Contact model for the prediction of NMR N–H order parameters in globular proteins. J Am Chem Soc 276:12654–12655

Zidek L, Novotny MV, Stone MJ (1999) Increased protein backbone conformational entropy upon hydrophobic ligand binding. Nat Struct Biol 6:1118–1121