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PERSPECTIVE

New opportunities for tensor-free calculations of residual dipolar couplings for the study of protein dynamics

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Abstract Residual dipolar couplings (RDCs) can provide exquisitely detailed information about the structure and dynamics of proteins. It is challenging, however, to extract such information from RDC measurements in conformationally heterogeneous states of proteins because of the complex relationship between RDCs and protein structures. To obtain new insights into this problem, we discuss methods of calculating the RDCs that do not require the definition of an alignment tensor. These methods can help in particular in the search of effective ways to use RDCs to characterise disordered or partially disordered states of proteins.

Keywords Residual dipolar couplings · Alignment tensor · Structural ensembles

Introduction

As the conformational fluctuations of proteins play decisive roles to enable their functions (Frauenfelder et al. 1991; Dobson et al. 1998; Boehr et al. 2006; Mittermaier and Kay 2006; Vendruscolo and Dobson 2006; Shaw et al. 2010; Kalodimos 2011; Sekhar and Kay 2013), an important goal is to pursue the development of approaches capable of providing accurate representations of these motions. Nuclear magnetic resonance (NMR) spectroscopy is particularly suitable for this purpose since this technique provides time- and ensemble-averaged measurements at atomic-level resolution (Palmer 2004; Boehr et al. 2006; Mittermaier and Kay 2006; Tolman and Ruan 2006; Vendruscolo and Dobson 2006; Kalodimos 2011; Salmon et al. 2011; Markwick and Nilges 2012; Jensen et al. 2013; Sekhar and Kay 2013). For this reason, NMR measurements on proteins can only be analysed approximately in terms of individual structures. This problem is not immediately evident in the case of highly structured native states, because when the conformational fluctuations are of small amplitude it is possible to identify an average structure that corresponds quite well to a given set of NMR measurements. By contrast, if the conformational fluctuations are of larger amplitude, it becomes necessary to represent the state of a protein by using an ensemble of structures, so that the average values of the NMR parameters over the ensemble reproduce closely the experimentally measured values (Kessler et al. 1988; Torda et al. 1989; Bonvin et al. 1994; Best and Vendruscolo 2004; Clore and Schwieters 2004; Lindorff-Larsen et al. 2005; Vendruscolo 2007; Krzeminski et al. 2009; Huang and Grzesiek 2010; Camilloni et al. 2012; Marsh and Forman-Kay 2012; Guerry et al. 2013; Jensen et al. 2013; Varadi et al. 2013). In this case, individual structures are not expected to exhibit values for the NMR parameters that match exactly the experimental ones. In this sense, the problem of calculating the values of NMR parameters from individual structures is not well defined, as such values are not measurable experimentally because of the averaging procedure that unavoidably takes place during the measurements. In the

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presence of conformational fluctuations, therefore, computational methods for characterising the behaviour of proteins should be based on the comparison between experimental parameters and average values estimated over ensembles of conformations (Kessler et al. 1988; Torda et al. 1989; Bonvin et al. 1994; Best and Vendruscolo 2004; Clore and Schwieters 2004; Lindorff-Larsen et al. 2005; Vendruscolo 2007; Krzeminski et al. 2009; Huang and Grzesiek 2010; Camilloni et al. 2012; Marsh and Forman-Kay 2012; Guerry et al. 2013; Jensen et al. 2013; Varadi et al. 2013).

The problem of extracting information about structure and dynamics from NMR parameters is particularly challenging in the case of residual dipolar couplings (RDCs) (Tjandra and Bax 1997; Tjandra et al. 1997; Tolman et al. 1997), since the values of these NMR parameters tend to have a strong structural dependence, and hence to experience large fluctuations as a protein explores its conformational space (Louhivuori et al. 2006; Salvatella et al. 2008). In practical terms, in the presence of conformational fluctuations of large amplitude even the most accurate methods for calculating the RDCs for a given structure (Zweckstetter and Bax 2000; Fernandes et al. 2001; Almond and Axelsen 2002; Azurmendi and Bush 2002; van Lune et al. 2002; Ferrarini 2003; Zweckstetter 2008; Berlin et al. 2009; Montalvao et al. 2011) may not provide values that can be expected to match the experimental ones. A close agreement between calculated and experimental RDCs can in these cases be obtained only when the calculated RDCs are averaged over an ensemble of structures representing the motions of the protein (Clore and Schwieters 2004; Best et al. 2006; Showalter and Bruschweiler 2007; Lange et al. 2008; De Simone et al. 2009; 2011; 2013a, b; Huang and Grzesiek 2010; Fenwick et al. 2011; Montalvao et al. 2011; Sgourakis et al. 2011; Lindorff-Larsen et al. 2012; Marsh and Forman-Kay 2012; Guerry et al. 2013; Jensen et al. 2013).

In this Perspective, we review various approaches to calculate RDCs and discuss their possible advantages to characterise the conformational fluctuations of proteins from RDC measurements.

The RDC between two nuclear spins

The RDC between two nuclear spins of gyromagnetic ratios γ_1 and γ_2 at a given distance r can be expressed as (Bax 2003)

$$D = D_{\max}\left\langle \left(3\cos^2\vartheta - 1\right)/2 \right\rangle \quad (1)$$

where ϑ is the angle between the inter-nuclear vector and the external magnetic field, $D_{\max} = -\mu_0\gamma_1\gamma_2 h/8\pi^3 r^3$ is the maximal value of the dipolar coupling for the two nuclear spins, μ_0 is the magnetic constant and h is the Planck constant. The averaging specified by the angular brackets describes the variations in the orientation of the inter-nuclear vector with respect to the external magnetic field caused by thermal motions. In isotropic solutions RDCs average to zero because all directions are equivalent, but when the orientational symmetry is broken non-zero values of the RDCs may appear (Saupe and Englert 1963; Bothnerby et al. 1981; Tolman et al. 1995; 1997; Tjandra and Bax 1997; Tjandra et al. 1997; Bax 2003; Blackledge 2005; Thiele 2007).

Calculation of RDCs using alignment tensor methods

When a structural model of the protein is available, there are several ways to carry out the average in Eq. (1) to estimate the corresponding RDCs. The most common approaches involve the definition of an alignment tensor, either explicitly (Saupe and Englert 1963; Bothnerby et al. 1981; Tjandra and Bax 1997; Tjandra et al. 1997; Clore et al. 1998; Losonczi et al. 1999; Meiler et al. 2001; Bax 2003; Blackledge 2005; Thiele 2007; Habeck et al. 2008) or implicitly (Moltke and Grzesiek 1999; Sass et al. 2001), a procedure that is particularly convenient if a protein populates a rigid structure, so that the only important degrees of freedom in Eq. (1) concern the relative orientation of the molecule with respect to the alignment medium. In this case, one should consider just 5 degrees of freedom for the rotations and 3 further degrees of freedom for the translations of a protein molecule. More generally, if a protein undergoes conformational fluctuations, it is still possible to define an alignment tensor, although in this case the averaging has to be carried out not only over the rotations and translation of the molecule with respect to the alignment medium, but also with respect to its internal degrees of freedom.

The alignment tensor of a given protein conformation can be obtained through fitting procedures, such as the singular-value decomposition (SVD) method (Losonczi et al. 1999), in which the alignment tensor is chosen to optimise the agreement between calculated and experimental RDCs. Alternatively the alignment tensor can be determined by structure-based procedures in which this quantity is calculated on the basis of the shape and charge of the protein molecule and the alignment medium (Zweckstetter and Bax 2000; Fernandes et al. 2001; Almond and Axelsen 2002; Azurmendi and Bush 2002; van Lune et al. 2002; Ferrarini 2003; Zweckstetter 2008; Berlin et al. 2009; De Simone et al. 2011, 2013a, b; Montalvao et al. 2011), without reference to experimentally-measured RDCs.

These two approaches are differently suitable depending on the situation. This aspect can be understood in particular in the presence of conformational fluctuations of large

amplitude. In this case, the calculation of the average RDCs corresponding to an ensemble of conformations involves the definition of a different alignment tensor for each conformation in the ensemble. In approaches in which the RDCs are fitted to a structure, to simplify the calculations one can assume that all the conformations in the ensemble have the same alignment tensor, which, however, is often not an accurate approximation (De Simone 2013a, b). Alternatively, to achieve greater accuracy, one can obtain the alignment tensor of each individual conformation by a separate fitting to the experimental RDCs. In this case, however, an impractically large number of experimental RDCs is required in order to avoid overfitting. Therefore, fitting methods are at risk of failing to capture the full changes in the alignment tensor during the conformational fluctuations (De Simone et al. 2011, 2013a, b; Montalvao et al. 2011).

In the presence of conformational fluctuations it is more effective to use structure-based methods (Louhivuori et al. 2003; Bernado et al. 2005; Esteban-Martin et al. 2010; Huang and Grzesiek 2010; De Simone et al. 2011, 2013a, b; Montalvao et al. 2011). In this case, each member in a structural ensemble can be associated with its own alignment tensor without the need of using experimental data. In practice, the averaging in Eq. (1) is carried out both over the external degrees of freedom, which involve rotations and translations, and the internal ones, which involve conformational fluctuations of a protein.

Calculation of RDCs using tensor-free methods

As mentioned above, the alignment tensor was originally introduced as a convenient mathematical procedure to calculate RDCs in the cases when a protein structure could be considered as rigid (thus avoiding the explicit inclusion of the conformational fluctuations in the calculations) and the alignment tensor could be fitted from the RDC data (thus eliminating the need of considering explicitly the rotations and translations of the protein with respect to the alignment medium). However, starting from Eq. (1), one can also calculate an RDC numerically without the need of defining an alignment tensor as

$$D = D_{\max} \int P_B(R) \frac{1}{2} \left[3\cos^2 \vartheta(R) - 1\right] dR \tag{2}$$

In this expression, R is the vector of the positions of all the atoms of the protein and the alignment medium (including the solvent), and

$$P_B(R) = \frac{1}{Z} \exp\left[-\frac{E(R)}{k_B T}\right] \tag{3}$$

is the Boltzmann factor for the configuration R of the system, where T is the temperature, k_B is the Boltzmann constant, Z is the partition function and $E(R)$ is the energy of the system, which includes the internal energy of the protein and the interaction energy between the protein and the alignment medium.

With the availability of increasingly powerful computers it is becoming possible to calculate this statistical average without making strong assumptions to reduce the complexity of the system, in particular about the amplitude of its structural fluctuations, and to facilitate the use of RDC measurements in challenging cases such as those of conformationally heterogeneous states of proteins.

In this context, a major complication in calculating the integral in Eq. (2) comes from the need of obtaining an accurate estimate of the Boltzmann factors P_B. For the Boltzmann factors, the primary challenge is to account for the electrostatic interactions between the protein and the alignment medium, which represent the most complicated contributions to the total energy $E(R)$ of a given conformation R of the system. Effective available approaches are based on adaptations of the Gouy-Chapmman theory to describe the electrical double layer near the surface the alignment medium (Zweckstetter 2008; Montalvao et al. 2011).

Another major complication in calculating the integral in Eq. (2) comes from obtaining an accurate sampling of the conformational space. Standard approaches to this problem are based, as mentioned above, on the introduction of the alignment tensor, which involves a separation of the internal (i.e. of the protein) and external (i.e. of the alignment medium) degrees of freedom

$$D = D_{\max} \int P_B(R_i, R_e) \frac{1}{2} \left[3\cos^2 \vartheta(R_i, R_e) - 1\right] dR_i dR_e \tag{4}$$

where the integral over the internal degrees of freedom, R_i, can be calculated separately from that over the external ones, R_e. In this approach, as the angle ϑ depends on both the internal and external degrees of freedom, one performs first a change of variables by introducing an internal reference frame and its orientation with respect to the laboratory frame (Saupe and Englert 1963; Bothnerby et al. 1981; Tolman et al. 1995, 1997; Tjandra and Bax 1997; Tjandra et al. 1997; Bax 2003; Blackledge 2005; Thiele 2007). To separate the integral one also assumes that

$$P_B(R_i, R_e) = P_B(R_i) P(R_e) \tag{5}$$

which implies the assumption that the alignment medium does not affect the internal degrees of freedom of the protein molecules, but only their overall orientations with respect to it. This approximation holds if the interactions between the protein molecules and the alignment medium are weak, which may not be the case in particular in the presence of strong electrostatic interactions.

Calculation of RDCs for highly-structured states

When the internal degrees of freedom can be considered as frozen, as the protein is assumed to undergo negligible conformational fluctuations, it is convenient to adopt tensor-based approaches, since in this way the external degrees of freedom, which are the only remaining ones in Eq. (2), are considered concisely through the definition of the alignment tensor. Thus, Eq. (2) can be recast as Eq. (4), which effectively becomes an algebraic expression. As discussed above, the alignment tensor can be obtained by a fitting procedure to experimental data (Losonczi et al. 1999), or by numerical methods based on the shape and charge of the protein (Zweckstetter and Bax 2000; Fernandes et al. 2001; Almond and Axelsen 2002; Azurmendi and Bush 2002; van Lune et al. 2002; Ferrarini 2003; Zweckstetter 2008; Berlin et al. 2009; De Simone et al. 2011, 2013a, b; Montalvao et al. 2011).

Calculation of RDCs for conformationally heterogeneous states

When the internal degrees of freedom of a protein molecule are considered as variable, they should be taken explicitly into account in Eq. (2) alongside the external ones. Thus, to calculate RDCs, one should devise efficient methods for carrying out the sampling of conformational space of the system composed by the protein and the alignment medium.

A powerful approach, which is based on rewriting Eq. (2) as Eq. (4), is to perform molecular simulations of the protein molecules in the absence of the alignment medium to obtain an ensemble of conformations with statistical weights corresponding to $P_B(R_i)$ in Eq. (5), and then, for obtaining the RDCs, to calculate for each conformation of the ensemble the integral over the external degrees of freedom (Mukrasch et al. 2007; Jensen et al. 2010; Terakawa and Takada 2011; Marsh and Forman-Kay 2012; Guerry et al. 2013).

An alternative strategy, if experimental measurements are available, is to use them to bias the sampling of conformational space (Kessler et al. 1988; Torda et al. 1989; Bonvin et al. 1994; Best and Vendruscolo 2004; Clore and Schwieters 2004; Lindorff-Larsen et al. 2005; Vendruscolo 2007; Huang and Grzesiek 2010). In this context, the use of the measurements as replica-averaged structural restraints in all-atom molecular dynamics simulations enables to modify a force field to match the experimental data according to the maximum entropy principle (Pitera and Chodera 2012; Cavalli et al. 2013; Roux and Weare 2013). If the experimental measurements used as restraints are the RDCs, it may still be convenient to separate the internal and external degrees of freedom in Eq. (4), as in this case the integral over the internal degrees of freedom can be considered effectively constant over a time interval (e.g. 0.1 ps) that is fairly long with respect to the integration time-step (e.g. 2 fs), thus avoiding its costly evaluation at every time-step. In practice, therefore, one reintroduces in this way an alignment tensor, with the difference that in this case the structure of the protein is approximated as rigid not in an absolute sense, but only on the sub-picosecond timescale, which can be fairly accurate even in highly dynamical states (De Simone et al. 2011, 2013a, b; Montalvao et al. 2011).

More generally, tensor-free approaches based on Eq. (2) can also be used to test the validity of the assumption that the introduction of the alignment medium does not alter the structure of a protein, which is required to separate the variables in Eq. (4) and thus define the alignment tensor. By comparing the RDCs calculated with Eq. (2) and Eq. (4) one may obtain an indication of the extent to which an alignment medium perturbs the conformational properties of a protein.

Correspondence between RDCs and protein structures

An aspect of RDC calculations that should be considered is whether one is interested in: (1) the problem of obtaining estimates of RDCs measured experimentally, or (2) the problem of estimating RDCs corresponding to individual structures. These two problems effectively coincide if a protein populates a highly-structured state with small conformational fluctuations, but are distinct in the case of conformationally heterogeneous states. This point can be understood with reference to Eq. (4) since the calculation of the RDCs corresponding to a given structure does not involve the integration over the internal degrees of freedom of the protein itself, but only over the external ones, which can indeed by achieved effectively by calculating an alignment tensor. By contrast, the prediction of RDCs measured experimentally should involve also the explicit integration over the internal degrees of freedom to obtain a conformational averaging.

Conclusions

We have discussed possible motivations for considering methods of calculating residual dipolar couplings that do not require the definition of an alignment tensor. Subject to the development of efficient computational strategies, these methods may offer novel opportunities in particular in the case of conformationally heterogeneous states of proteins.

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