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Article

Interpreting dynamically-averaged scalar couplings in proteins

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Abstract

The experimental determination of scalar three-bond coupling constants represents a powerful method to probe both the structure and dynamics of proteins. The detailed structural interpretation of such coupling constants is usually based on Karplus relationships, which allow the measured couplings to be related to the torsion angles of the molecules. As the measured couplings are sensitive to thermal fluctuations, the parameters in the Karplus relationships are better derived from ensembles representing the distributions of dihedral angles present in solution, rather than from single conformations. We present a method to derive such parameters that uses ensembles of conformations determined through dynamic-ensemble refinement – a method that provides structural ensembles that simultaneously represent both the structure and the associated dynamics of a protein.

Abbreviations: DER – dynamic-ensemble refinement; DFT – density functional theory; TNfn3 – third fibronectin type III domain from human tenascin.

Introduction

Advances in NMR techniques have provided new quantitative insights into the relationship between structure and dynamics in proteins and other biological macromolecules (Case, 2002; Kay, 2005; Palmer, 2004). A powerful method for examining the local structure of a protein involves the measurement of three-bond coupling constants (^3J), which are sensitive probes of the intervening dihedral angles (θ) through a so called Karplus relationship (Bax et al., 1994; Bystrov, 1976; Karplus, 1959):

$$^3J(\theta) = A\cos^2(\theta + \delta) + B\cos(\theta + \delta) + C \qquad (1)$$

Since the measured couplings are averaged on a millisecond time scale or faster, they are also affected by the conformational averaging of dihedral angles. Therefore measurements of scalar couplings are particularly useful to examine at the same time not only the average structure of a protein but also the extent of conformational variability caused by thermal fluctuations (Best and Vendruscolo, 2004; Best et al., 2004; Brüschweiler and Case, 1994; Case et al., 2000; Chou et al., 2003; Hoch et al., 1984; Karimi-Nejad et al., 1994; Mierke et al., 1994).

In order to extract structural and dynamical information from measured coupling constants, an accurate estimate is required for the parameters A, B, C and δ. These parameters depend on the properties of the atoms and bonds that are involved in the couplings, and are usually determined

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through methods that combine the knowledge of both the experimental couplings and the geometry of the molecule. In these approaches, the parameters are determined to optimize the match between a set of experimental scalar couplings and the corresponding known values of θ. Thus, in order for these methods to be successful it is important to have accurate values of both the experimental couplings and the related dihedral angles.

The sensitivity of scalar couplings to native state dynamics has been considered in a series of studies (Brüschweiler and Case, 1994; Case et al., 2000; Chou et al., 2003; Hoch et al., 1984; Karimi-Nejad et al., 1994; Mierke et al., 1994; Schmidt et al., 1999) that have shown that not only the average dihedral angle ($\langle\theta\rangle$), but also the variation (σ_θ), around this angle can affect the measured value of ^3J and thus the parameters fitted for a Karplus relationship (Brüschweiler and Case, 1994). Therefore, for systems that display conformational heterogeneity, it is important to take conformational averaging into account when interpreting experimental couplings in terms of geometric properties.

Here we present a strategy to determine the parameters in a Karplus relationship through a method that explicitly includes experimental information about the structural fluctuations of proteins. Rather than using individual structures that represent the mean dihedral angles (as determined by traditional X-ray or NMR techniques), we use structural ensembles derived using experimental information about the native state heterogeneity provided by NMR relaxation experiments (Best and Vendruscolo, 2004). By using order parameters derived from these experiments as restraints in molecular dynamics simulations through the dynamic-ensemble refinement (DER) method (Lindorff-Larsen et al., 2005) we obtain ensembles of conformations that represent simultaneously both the native structure and the fluctuations from this structure. We first illustrate the method for a set of backbone scalar couplings in ubiquitin (Wang and Bax, 1996). Since the polypeptide backbone in ubiquitin displays only limited dihedral angle fluctuations (Lindorff-Larsen et al., 2005), the effects of dynamics on the coupling constants, although statistically significant, are rather small. In contrast, side chain dihedral angles may display larger variability, resulting both from local fluctuations and from the population of multiple rotameric states (Best and Vendruscolo, 2004; Best et al., 2004,2005; Chou et al., 2003; Lindorff-Larsen et al., 2005). In this case we show that if dynamically averaged scalar couplings reporting on side chain dihedral angles are used in combination with a single crystal structure to parameterize a Karplus relationship, the parameters that are obtained deviate significantly from those estimated either using independent measurements of the angles or from density functional theory (DFT) calculations. If structural ensembles representing the distributions of dihedral angles are instead used as the basis for the parameterization, we show that a set of parameters can be obtained that is consistent with these independent determinations.

Methods

Determination of structural ensembles

Two different types of ensembles of conformations were used in the analysis presented here. In the first, experimentally determined order parameters (S^2) for both backbone amide groups and side chain methyl groups were used as restraints in the DER protocol to obtain ensembles of structures that display a variability compatible with the experimentally determined S^2 values. The determination of structural ensembles of TNfn3 and ubiquitin using this procedure has been described previously (Best and Vendruscolo, 2004; Lindorff-Larsen et al., 2005). These ensembles of conformations were used to parameterize the Karplus relationships as described below, i.e. no experimental scalar couplings were used as restraints in the structures used in the parameterization.

In addition to the ensembles determined using DER described above, we determined a second type of conformational ensemble for ubiquitin. This ensemble was determined to be compatible with the experimentally determined NOEs and scalar couplings. In short, the experimentally determined backbone and side chain scalar couplings (Chou et al., 2003; Wang and Bax, 1996) and NOEs (Cornilescu et al., 1998) (but no S^2 values) were applied as restraints on an ensemble of 16 copies of ubiquitin. The force constant (Best and Vendruscolo, 2004) used for the scalar coupling restraints was 10 000 kcal mol^{-1} Hz^{-4} and previously published Karplus parameters for Equation 1 were used (Chou et al., 2003; Wang and Bax, 1996); using this force constant we obtain a root

mean square deviation between experimental and calculated side chain scalar couplings of 0.2 Hz. Other details of the structure determination protocol were as described previously (Lindorff-Larsen et al., 2005) except that no S^2 restraints were used. Using a simulated annealing protocol (Lindorff-Larsen et al., 2005) we obtained 128 conformations that, as an ensemble, are compatible with the experimental scalar coupling and NOE restraints. We note that this ensemble of conformations was not used to parameterize the Karplus relationships, but to compare the S^2 order parameters obtained from relaxation data (S^2) with those obtained from ^3J restrained simulations ($S^2_{^3J}$).

For the parameterizations of Karplus relationships based on crystal structures, PDB entries (Berman et al., 2000) 1TEN (Leahy et al., 1992) and 1UBQ (Vijay-Kumar et al., 1987) were used for TNfn3 and ubiquitin, respectively.

Parameterization of Karplus relationships

Experimentally determined values of ^3J for the backbone atoms were obtained from data for ubiquitin (Wang and Bax, 1996): $^3J_{\mathrm{HNH}\alpha}$ (63 couplings), $^3J_{\mathrm{H}\alpha\mathrm{C}'}$ (65 couplings), $^3J_{\mathrm{HNC}\beta}$ (60 couplings) and $^3J_{\mathrm{HNC}'}$ (61 couplings). For the side chain atoms we used data for $^3J_{\mathrm{NC}_\gamma}$ couplings of Val or Ile residues from ubiquitin (Chou et al., 2003) (14 couplings) and the third fibronectin type III domain from human tenascin (TNfn3) (Best et al., 2004) (15 couplings).

The parameters A, B, C and δ in Equation 1 were determined by least squares fitting to minimize:

$$\chi^2 = \frac{1}{N_J}\sum_{i=1}^{N_J}(^3J_{i,\mathrm{exp}} - {}^3J_{i,\mathrm{calc}})^2 \qquad (2)$$

In this equation N_J is the number of experimental couplings, $^3J_{i,\mathrm{exp}}$ is the i-th experimentally determined coupling and $^3J_{i,\mathrm{calc}}$ is the corresponding coupling constant calculated from protein structures using Equation 1. For the parameterization based on DER ensembles, $^3J_{i,\mathrm{calc}}$ was calculated as the average over the ensemble:

$$^3J_{i,\mathrm{calc}} = \frac{1}{N_e}\sum_{j=1}^{N_e} {}^3J^{(j)}_{i,\mathrm{calc}} \qquad (3)$$

where N_e is the size of the ensemble and $^3J^{(j)}_{i,\mathrm{calc}}$ is the coupling constant calculated using Equation 1 and the j-th conformation within the ensemble. The standard deviations of the parameters were estimated by a Monte Carlo procedure in which 500 synthetic datasets were generated by addition of Gaussian noise with a standard deviation of 0.1 Hz, corresponding approximately to the uncertainty in the experimental couplings (Best et al., 2004; Chou et al., 2003).

Results

Karplus relationships for backbone scalar couplings

We first analysed four different types of backbone couplings ($^3J_{\mathrm{HNH}\alpha}$, $^3J_{\mathrm{H}\alpha\mathrm{C}'}$, $^3J_{\mathrm{HNC}\beta}$ and $^3J_{\mathrm{HNC}'}$) that have been measured experimentally in ubiquitin (Wang and Bax, 1996) (Figure 1). The Karplus parameters A–C (we fixed $\delta = 0$, as is conventional for these couplings) that we obtain by fitting the experimental couplings to the dihedral angles in the X-ray structure of ubiquitin are very similar to those obtained previously using a similar approach (Wang and Bax, 1996).

To examine the effect of motional averaging in the parameterization of Karplus relationships we also used an ensemble of 128 ubiquitin conformations (DER structures) that we have determined to represent both the native structure and the associated dynamics of ubiquitin (Lindorff-Larsen et al., 2005) using experimental NOEs and S^2 values as restraints (i.e. no scalar couplings were used as restraints). When this ensemble is used as basis for the parameterization we obtain a different set of parameters (Table 1) giving rise to a Karplus relationship that displays slightly larger variations in ^3J as a function of θ. This observation is in good agreement with a model that describes the influence of harmonic fluctuations on the fitted parameters in Karplus relationships (Brüschweiler and Case, 1994), which predicts that if only the average dihedral angle is used to parameterize the relationship, the resulting Karplus curve is 'flatter' than that obtained using the correct distribution of dihedral angles. This effect is exactly the one that we observe when we compare the parameters obtained using the X-ray structure and the DER structures.

By assuming Gaussian fluctuations of uniform amplitude of the dihedral angle around the average value observed in the X-ray structure, we can

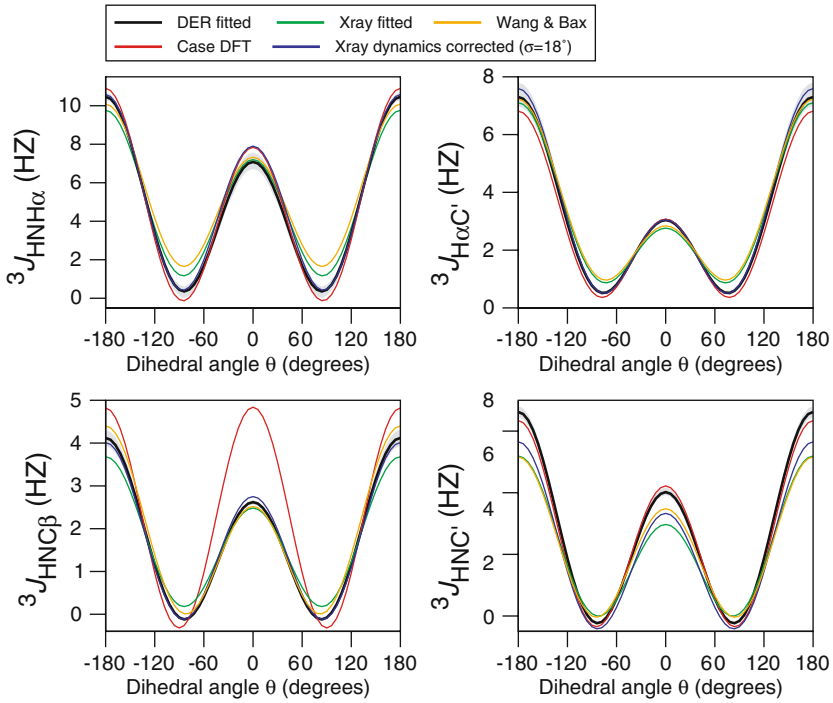

Figure 1. Comparison of different methods for deriving Karplus relationship parameters for four different types of scalar couplings that depend on the backbone dihedral angle ϕ. The relationships have been parameterized using (black) an ensemble of conformations representing the native state dynamics of ubiquitin (Lindorff-Larsen et al., 2005) (DER structures), (green) the crystal structure of ubiquitin, (red) density functional theory calculations (Case et al., 2000), (orange) using a combination of the X-ray structure and multiple scalar couplings (Wang and Bax, 1996) and (blue) using a harmonic correction (Brüschweiler and Case, 1994) to the X-ray-derived parameters. The value $\sigma = 18°$ in the Gaussian fluctuation model (Brüschweiler and Case, 1994) was determined to give the best agreement with the DER derived curves. Gray shaded areas indicates a 67% confidence interval for the curves derived using DER structures, but for the backbone couplings shown here this area is of the same size as the thickness of the black line and are therefore not visible.

remove the dynamical contributions to the parameters and estimate the Karplus relationship in the absence of motion (Brüschweiler and Case, 1994). The remarkably good agreement between the X-ray derived curve 'corrected' in this way and that derived using DER structures suggests that (i) the differences between the X-ray and DER derived parameters are principally due to fluctuations and (ii) that these fluctuations can be well described as harmonic motions. The value $\sigma = 18°$ was found to give the best agreement between the dynamics-corrected Karplus relationship and that derived using the DER structures, and is in good agreement with the average fluctuations of the ϕ dihedral ($\langle\sigma_\phi\rangle = 14°$) observed in the DER structures (Karplus curves obtained using standard deviations of 14° or 18° in the harmonic correction are very similar). It is also noteworthy that incorporating dynamics in the empirical parameterization, using either the DER structures or the dynamics-corrected X-ray parameters, results in a closer match to the Karplus relationship obtained using DFT calculations than that derived directly from fitting the data to a single structure. The only exception is the DFT estimate for the Karplus

Table 1. Parameters for Karplus relationships obtained using DER ensembles (*A*, *B* and *C* are in Hz and δ is in degrees)

Coupling constant	*A*	*B*	*C*	δ
$^3J_{HNH\alpha}$	8.33 ± 0.06	−1.69 ± 0.03	0.44 ± 0.05	0[a]
$^3J_{H\alpha C'}$	4.41 ± 0.06	−2.14 ± 0.03	0.77 ± 0.05	0[a]
$^3J_{HNC'}$	5.5 ± 0.1	−1.30 ± 0.04	−0.16 ± 0.02	0[a]
$^3J_{HNC\beta}$	3.4 ± 0.1	−0.75 ± 0.08	−0.08 ± 0.03	0[a]
$^3J_{NC_\gamma}$[b]	2.8 ± 0.2	0.4 ± 0.1	−0.4 ± 0.1	3 ± 1

[a] Fixed δ = 0.
[b] For Ile and Val residues.

relationship for $^3J_{HNC\beta}$, but this estimate also differs significantly from the other empirical estimates. Together, these results suggest that the backbone scalar couplings contain a small but significant contribution from the dynamical fluctuations described by the DER ensemble, and that the effects of these fluctuations can be reasonably well described as arising from harmonic motion. This is in good agreement with the results of molecular dynamics simulations that show that the fluctuations giving rise to backbone order parameters less than one can be well described by a harmonic model (Best et al., 2005; Buck and Karplus, 1999).

Karplus relationships for side chain scalar couplings

The measurement of scalar couplings can also provide detailed information about the distribution of dihedral angles in side chains of proteins. As side chain atoms are known to be significantly more mobile than those in the polypeptide backbone, the dynamical effects on scalar couplings and Karplus relationships are expected to be much larger than in the case of backbone couplings. To demonstrate this effect we analysed in detail a Karplus relationship for $^3J_{NC_\gamma}$ couplings in Val and Ile residues using experimental data for ubiquitin (Chou et al., 2003) and TNfn3 (Best et al., 2004). The $^3J_{NC_\gamma}$ couplings of Val or Ile residues depend on the χ_1 dihedral angles of these residues, many of which are known to display a large conformational heterogeneity arising from both a variability within a given rotameric state and from rotameric transitions (Best and Vendruscolo, 2004; Best et al., 2005; Chou et al., 2003; Lindorff-Larsen et al., 2005). In Figure 2. we show the results of the parameterization of the Karplus relationship for these couplings using techniques similar to those described above. The figure reveals a good agreement between the Karplus relationship obtained using the DER structures and estimates based either on independent measurements of residual dipolar couplings (Chou et al., 2003) or DFT calculations (Chou et al., 2003). Notably, these Karplus relationships differ significantly from that obtained by fitting the experimental couplings to the dihedral angles present in the crystal structures of TNfn3 and ubiquitin. Further, in contrast to the backbone case, the DER-derived curve cannot be corrected by assuming an harmonic fluctuation model with the amplitude as a free parameter (Brüschweiler and Case, 1994); the best fit is shown in Figure 2. This result is caused by at least two effects: (i) the motion of side chain dihedrals is in many cases significantly anharmonic due to the population of multiple rotameric states and (ii) different Val and Ile residues vary in the amplitude of their fluctuations, making it impossible to fit the data using only a single value of σ. We note here that in cases for which a large number of experimental couplings can be measured for each dihedral it may be possible to extract a residue specific value of σ (Schmidt et al., 1999), overcoming the second problem but not the first.

Probing motion on different time scales

The structural ensembles determined using the DER method represent the dynamical heterogeneity probed by S^2 values obtained from heteronuclear relaxation experiments (S^2_{relax}). Such experiments probe the motion of side chain and backbone atoms on a time scale that is faster than that of overall rotation (about 4ns for ubiquitin and about 6ns for TNfn3). Scalar couplings, however, contain contributions from dynamical fluctuations up to the ms time scale, and may thus contain additional dynamical information from that contained in the S^2_{relax} values. Since the DER ensembles determined through the use of S^2 parameters may represent only a fraction of the heterogeneity that affects scalar couplings, the approach for the parameterization of Karplus relationships described above, although an improvement with respect to the use of individual X-ray structures, would still underestimate the dynamical contributions to the Karplus parameters. This effect could be particularly relevant for side chain atoms, which display larger variability than most atoms in the polypeptide backbone. To quantify the possible differences in the side chain dynamics probed by relaxation measurements and by scalar couplings we carried out an additional structure determination of ubiquitin to determine the dynamical contributions to the measured scalar couplings. In these calculations we enforced 289 scalar couplings (Wang and Bax, 1996; Chou et al., 2003) as well as distances from NOE experiments (Cornilescu et al., 1998) as restraints averaged on an ensemble of ubiquitin conformations; note here that no S^2 values were used as

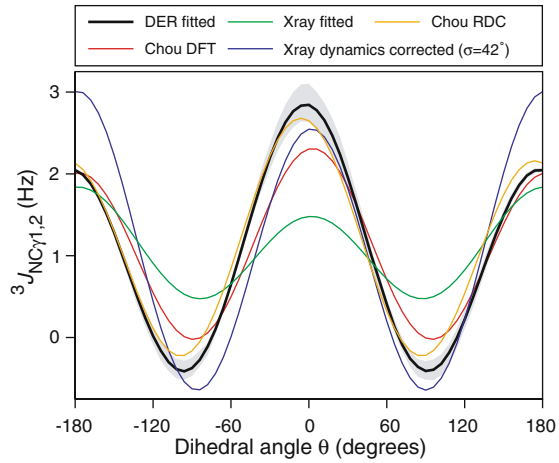

Figure 2. Karplus relationship for side chain $^3J_{NC_\gamma}$ couplings parameterized using different methods. A Karplus relationship for $^3J_{NC_{\gamma 1}}$ and $^3J_{NC_{\gamma 2}}$ in Ile and Val residues has been parameterized using (black) ensembles of conformations representing the native state dynamics of TNfn3 (Best and Vendruscolo, 2004) and ubiquitin (Lindorff-Larsen et al., 2005) (DER structures), (green) the native state crystal structure of TNfn3 and ubiquitin, (red) density functional theory calculations (Chou et al., 2003), (orange) using independent measurements of the χ_1 dihedral angle from residual dipolar couplings (Chou et al., 2003) and (blue) using a harmonic correction (Brüschweiler and Case, 1994) to the X-ray derived parameters. The value $\sigma = 42°$ in the Gaussian fluctuation model (Brüschweiler and Case, 1994) was determined to give the best agreement with the DER derived curve. The gray shaded area indicates a 67% confidence interval for the curve derived using DER structures.

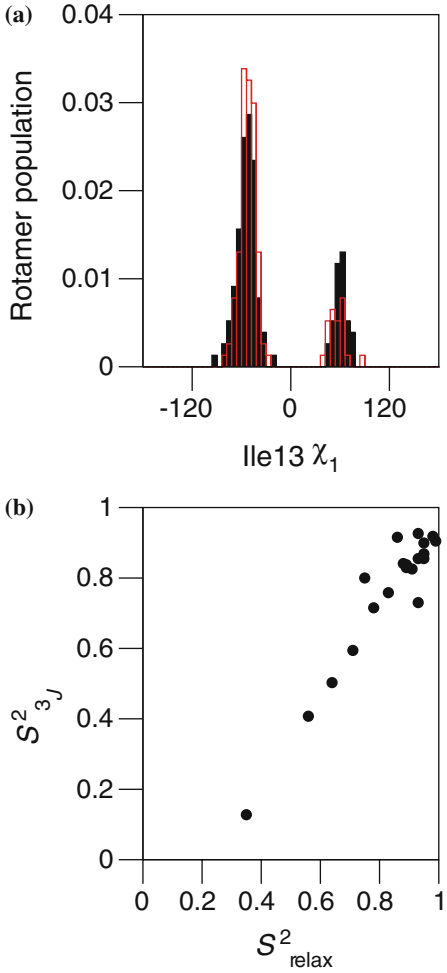

Figure 3. Comparison of the dynamics probed by relaxation experiments and by scalar couplings. (a) Distribution of the χ_1 dihedral angle for Ile13 in ubiquitin obtained using either (red) DER, which is based on relaxation experiments, or (black) ensemble simulations using scalar couplings as restraints. (b) Comparison between S^2 values determined from relaxation experiments, and those determined from scalar couplings as described in the text.

restraints in these calculations. We thereby obtained an ensemble of conformations fully compatible with the experimental restraints, without enforcing that the individual structures satisfy on their own the scalar coupling and NOE restraints. For example, while the ensemble as a whole is characterized by a high correlation ($r^2 = 0.96$) with the measured side chain scalar couplings, the individual conformations show a broad distribution of correlations ranging from $r^2 = 0.36$ to $r^2 = 0.89$ (mean 0.75).

As in the case of the DER-derived ensemble of ubiquitin, the ensemble determined using NOE and scalar couplings as restraints reveals broad distributions of side chain dihedral angles for many amino acid residues. As an example we show in Figure 3a the distribution of the χ_1 dihedral angle in Ile13 in both the DER and ^3J derived ensembles. The γ methyl group of Ile13 has $S^2_{relax} = 0.56$ (Lee et al., 1999) giving rise to the broad χ_1 distribution observed in the DER ensemble. Similarly, the measured values of $^3J_{NC_{\gamma 2}} = 1.4$ and $^3J_{CC_{\gamma 2}} = 1.7$ Hz (Chou et al., 2003) are not compatible with having only one rotameric state populated, and thus a broad distribution of χ_1 is obtained when these values are used as restraints in an ensemble simulation. Interestingly, the distribution obtained using scalar couplings is slightly broader than that obtained using the DER method, suggesting that for this residue the scalar couplings contain a slightly larger dynamical

contribution compared to the relaxation experiments.

To quantify the similarities and differences between the heterogeneity probed by relaxation measurements and scalar couplings, we back-calculated (Henry and Szabo, 1985) order parameters ($S^2_{^3J}$) from the ensemble of structures that we determined using the ^3J values as restraints. The results for the 20 methyl groups in ubiquitin for which both experimental side chain scalar couplings and S^2_{relax} values are available are shown in Figure 3b. The correlation between S^2_{relax} and $S^2_{^3J}$ values is high ($r^2 = 0.9$), although the $S^2_{^3J}$ values are lower in general than the S^2_{relax} values. The difference can be related to the magnitude of the order parameter in an approximately linear way: $1 - S^2_{^3J} \approx 1.2(1 - S^2_{\mathrm{relax}})$. Lower order parameters, corresponding to larger amplitude motions, tend to show a larger contribution from slow motions.

Discussion

Experimentally determined scalar couplings provide important structural and dynamical information on proteins and other molecules. A crucial prerequisite for the conversion of scalar couplings to structural information is the availability of accurate parameters for the Karplus relationships. As is well known (Brüschweiler and Case, 1994), fitting experimental scalar couplings to a single structure (e.g. obtained from X-ray diffraction) that represents only the mean conformation, may result in rather inaccurate Karplus equation parameters, due to the effect of native state dynamics. Thus, if Karplus relationships derived in this way are used in combination with experimental couplings to restrain protein conformations in a structure determination protocol, the structures obtained may not represent faithfully the actual ensemble in solution. This effect may be particularly significant if the experimental scalar couplings are affected by anharmonic motions, or if ensemble simulations are used in an attempt to reconstruct the entire distribution of dihedral angles (Best and Vendruscolo, 2004; Mierke et al., 1994). We have shown that if structures representing dihedral angle distributions, not just the averages, are used to parameterize Karplus relationships, a set of parameters can be obtained that is in good agreement with independent estimates of these values. Such a distribution of structures can be obtained using DER in which NOEs and S^2 values are used as restraints to obtain an ensemble of conformations representing the heterogeneity of the native state of proteins. In addition, we find that while a Gaussian fluctuation model (Brüschweiler and Case, 1994) is sufficient to include dynamical effects for backbone couplings, it is necessary to consider the complete dihedral distribution for side chain couplings, for which larger and more anharmonic fluctuations may occur.

Because scalar couplings are averaged over a longer time scale than that probed by relaxation experiments, the approach described here, while still an improvement relative to using static structures, could potentially fail to include dynamical contributions resulting from time scales longer than that of overall rotational motion. To explore potential differences in motion on different time scales we have analysed two types ensembles of ubiquitin conformations; one ensemble was determined using NOEs and S^2 values as restraints and used to parameterize the Karplus relationship, and the other ensemble was determined using NOEs and scalar couplings as restraints and used to calculate the $S^2_{^3J}$ order parameters. As previously reported (Best and Vendruscolo, 2004; Lindorff-Larsen et al., 2005), the prediction of side chain scalar couplings is significantly improved by using DER ensembles obtained using S^2_{relax} values, suggesting that a large fraction of the motion probed by scalar couplings is also measured by relaxation experiments. In addition, we have shown here that the reverse also holds, namely that by using scalar couplings as restraints in ensemble simulations it is possible to predict S^2_{relax} values, again suggesting that the dynamical information provided by two different sets of measurements is similar, at least in the cases examined here. We have also discussed how the order parameters obtained from the scalar couplings are slightly lower than those from relaxation measurements, in agreement with previous findings from alternative models for side chain motion (Chou et al., 2003), and from the analysis of backbone residual dipolar couplings (Clore and Schwieters, 2004). Whilst there may be small differences between the motions probed by relaxation experiments and by scalar couplings, the results presented in this paper demonstrate that using ensembles calculated from

relaxation-derived data yields Karplus equation parameters which are in good agreement with *ab initio* results, and with independent experimental estimates.

In conclusion, we have discussed how conformational ensembles that represent simultaneously the native state structure and its dynamics may be used to parameterize Karplus relationships and, in a wider context, to study other relationships between protein structures and experimental data sensitive to the native dynamics.

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