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Calculations of NMR dipolar coupling strengths in model peptides

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Abstract

Ab initio MP2 and density functional quantum chemistry calculations are used to explore geometries and vibrational properties of *N*-methylacetamide and of the alanine dipeptide with backbone angles characteristic of helix and sheet regions in proteins. The results are used to explore one-bond direct dipolar couplings for the N–H, Cα–Hα, C′–N, and Cα–C′ bonds, as well as for the two-bond C′–H interaction. Vibrational averaging affects these dipolar couplings, and these effects can be expressed as effective bond lengths that are 0.5–3% larger than the true bond lengths; bending and torsion vibrations have a bigger influence on the effective coupling than do stretching vibrations. Because of zero-point motion, these effects are important even at low temperature. Hydrogen bonding interactions at the amide group also increase the N-H effective bond length. Although vibrational contributions to effective bond lengths are small, they can have a significant influence on the extraction of order parameters from relaxation data, and a knowledge of relative bond lengths is needed when several types of dipolar couplings are to be simultaneously used for refinement. The present computational results are compared to both solid- and liquid-state NMR experiments. The analysis suggests that secondary structural elements in many proteins may be more rigid than is commonly thought.

Introduction

Spin-spin interactions between directly bonded nuclei can provide important information about protein structure and dynamics. Direct (through-space) dipolar couplings can be extracted from solid-state measurements (Roberts et al., 1987) or from studies on partially oriented liquid samples (Tolman et al., 1995; Tjandra et al., 1996; Tjandra and Bax, 1997; Prestegard, 1998). For proteins, the coupling strengths for a particular type of bond can show large variations from one residue to another that reflect both the mean bond orientation and angular fluctuations about this mean.

Information about dipolar couplings can also be extracted from liquid state relaxation studies (Abragam, 1961; Brüschweiler and Case, 1994a; Torchia, 1996). For certain nuclei, spin relaxation is dominated by dipolar interactions with directly bonded spins, and variations in relaxation times can be ascribed to variations in angular fluctuations, e.g. through order parameter analysis or spectral density mapping (Palmer, 1997; Fushman and Cowburn, 1998; Kay, 1998). The interpretation of these measurements is greatly facilitated by the fact that bond distances are nearly constant, and are approximately known for each type of bond, so that variations in dipolar splittings or relaxation parameters from one residue to another can be largely ascribed to changes in the mean angular orientation or to fluctuations about this mean. A typical analysis compares the observed results to those to be expected from a rigid molecule with fixed internal structure. For example, for short internal relaxation times, the 'Lipari–Szabo' order parameter S^2 is the ratio of the dipolar (cross-) relaxation rate in the real system to that of a hypothetical rigid system with the same mean structure (Lipari and Szabo, 1982; Yip and Case, 1991). Use of a rigid-molecular reference state is adequate for many purposes, but it must be recognized that, even at the lowest temperatures, zero-point vibrational motion ex-

ists that can affect dipolar interactions. I explore here a model in which the reference system, rather than being classically rigid, includes this zero-point motion, so that deviations from the reference model reflect only *thermally activated* motions. This change can be accommodated into standard analyses by replacing the actual mean bond length with an effective value that reflects the appropriate averaging over vibrational motion (Henry and Szabo, 1985; Ishii et al., 1997). Here I use quantum chemical calculations on peptide and dipeptide model compounds to obtain estimates for these effective bond lengths for the N–H, Cα–Hα, C′–N and Cα–C′ bonds that are typically studied in proteins. Models for hydrogen bonding and for backbone torsion angle variations are explored to obtain estimates of the extent to which both the actual and the effective bond lengths depend upon the environment.

In some circumstances, the choice of a reference system is principally a matter of convenience: changing from a rigid reference system to one containing local bending and stretching vibrations will just scale order parameters of a particular type (such as the N–H group in a peptide) by a constant factor. This may have little effect on analyses of experimental relaxation data that concentrate on the differences among residues. The choice of reference system becomes important for quantitative considerations (such as for comparisons to molecular dynamics simulations), and can be crucial for comparisons of data from different pairs of nuclei (such as simultaneous analysis of ^{13}Cα–^{1}Hα and ^{15}N–^{1}H data). In this latter case, even small uncertainties in the absolute value of effective bond lengths can have a significant effect on geometric and fluctuation analyses. Furthermore, a quantitative analysis of the contributions of various types of motion to observed dipolar coupling strengths may help to clarify the conclusions that may be drawn from such measurements.

Methods

Two principal model systems were used here. The first is *N*-methylacetamide, which is a model for an isolated peptide group. The isolated molecule was geometry optimized with three levels of quantum theory. The first level employed MP2 theory and a 6-31G** basis set, and used the Gaussian 94 program (Frisch et al., 1995). Since correlation contributions increase with increasing bond lengths, the MP2 model gives bond lengths that are 0.01 to 0.02 Å longer than Hartree–Fock theory, and which should be more accurate. Approximate energy profiles for the N–H bond stretch were obtained by changing the N–H bond length in the MP2/6-31G** optimized structure, and constructing a curve from single point MP2/6-31G** energies. The second quantum level used the B3LYP density functional model (Kohn et al., 1996) with the 6-31G** basis set, using version 3.5 of the Jaguar program (Schödinger, Inc., Portland, OR, 1998). Normal modes were computed from a second derivative matrix constructed from finite difference calculations from first derivatives. A third quantum level added a continuum solvent term to the B3LYP/6-31G** calculations, with an external dielectric of 80.4, employing a boundary element method to carry out the self-consistent reaction field model, as described elsewhere (Cortis et al., 1996).

Since peptide groups in proteins are generally involved in hydrogen bonding interactions, two models for this were explored. The first used two water molecules, one hydrogen bonding to the N–H group and one to the C=O group. Hydrogen bond lengths were constrained to distances from 1.8 to 2.5 Å (in steps of 0.1 Å) for both groups, and the remaining degrees of freedom were geometry optimized at the MP2/6-31G** level. A second hydrogen bond model used a single acetate ion (to mimic the formation of a zwitterionic hydrogen bond); again, the N–H$\cdots$O hydrogen bond length was varied from 1.8 to 2.5 Å, and the remaining degrees of freedom were geometry optimized. Since the point of these studies was to estimate the effects of hydrogen bonds on NMR properties, I did not attempt to determine reliable energetics and ignored basis set superposition errors.

A second model was the ‘alanine dipeptide’ (*N*-acetyl, *N*′-methylalanineamide), which has been widely studied as a model for peptide conformations involving the ϕ and Ψ backbone angles. I looked mostly at a ‘sheet’ conformation, which is an unrestrained local minimum at the MP2/6-31G** level with $(\phi, \Psi) = (-156°, 165°)$. As with *N*-methylacetamide, approximate energy versus bond length profiles were computed by changing the Cα–Hα, Cα–C′or C′–N bond lengths and re-computing MP2/6-31G** single-point energies. Also in line with the *N*-methylacetamide calculations, normal modes were computed at the B3LYP/6-31G** level, both with and without a continuum solvent self-consistent reaction field term.

For comparison, I also looked at the alanine dipeptide with backbone angles characteristic of an α-helix.

Since there is no local minimum in this region on the gas-phase surface (Brooks and Case, 1993), I constrained the ϕ and Ψ angles to $-60°$, and optimized the remaining degrees of freedom at the MP2/6-31G** level.

The effects of normal mode motion on dipolar coupling strengths were computed using the Amber5 programs (Case et al., 1997), employing a formalism outlined below and described in detail previously (Brüschweiler and Case, 1994b). The Amber programs were modified to read in normal mode frequencies and eigenvectors as output from the Jaguar program. For comparison, normal modes for *N*-methylacetamide were also computed using the Amber 95 force field (Cornell et al., 1995), and used to compute motional effects on dipolar coupling strengths.

Results

The effects of vibrational motion on dipolar coupling strengths have been considered by Henry and Szabo (1985), whose notation I follow here. In this analysis, the vibrationally averaged dipolar coupling strength is related to an effective distance:

$$\omega_{eff} \equiv \hbar\gamma_i\gamma_S/r_{eff}^3 \tag{1}$$

where the γs are nuclear gyromagnetic ratios, and r_{eff} differs from the optimum bond length R (at the bottom of the potential well) in a way that reflects the effects of vibrational averaging on dipolar coupling. Using a Taylor series expansion of vibrational motion, one can write (Henry and Szabo, 1985):

$$\begin{aligned} r_{eff} &= R + \left[<\Delta_z> -2 <\Delta_z^2> /R \right] \\ &\quad + \left\{ (<\Delta_x^2> + <\Delta_y^2>)/2R \right\} \end{aligned} \tag{2}$$

Here we are in a coordinate frame where the *z* axis is along the bond vector, and Δ_i $(i = x, y, z)$ is the Cartesian deviation from the optimum bond vector. The $< >$ brackets indicate a vibrational averaging. The terms in square brackets arise from vibrational averaging of the internuclear distance (i.e. they correspond to $< r^{-3} >^{-1/3}$) whereas the terms in curly brackets arise from the averaging of the orientation of the internuclear vector (as might typically be represented by an S^2 order parameter). The vibrational averaging related to distances (the term in square brackets) can be further broken down into an anharmonic contribution ($<\Delta_z>$) that is generally positive, since the vibrationally averaged length is longer than the bottom-of-well distance, and a harmonic contribution ($-2 < \Delta_z^2 > /R$) that arises from the fact that averaging r^{-3} weights the small distances more than larger ones. It is worth emphasizing that r_{eff} is *not* simply $< r^{-3} >^{-1/3}$, but that it includes as well the orientational averaging terms given by the final terms in Equation 2, and that these latter terms typically have a bigger effect on r_{eff} than do the terms that involve distance averages alone.

Because of the large frequency mismatch between stretching and bending vibrations, the averages in Equation 2 can be approximately carried out in two steps, using an (anharmonic) one-dimensional potential for bond stretching and a conventional 3N-dimensional harmonic normal mode analysis for the bending contributions. It is possible to consider more complex models in which stretching and bending are coupled to one another (Ishii et al., 1997), but these will not be pursued here.

The averaging over the bond-stretching motion may be carried out by fitting quantum mechanical MP2/6-31G** energies to a cubic potential:

$$E = E_0 + \frac{1}{2}k(r-R)^2 + (1/6)\,f_{rrr}(r-R)^3 \tag{3}$$

where E_0, k, f_{rrr} and R are adjustable parameters. The parameter k is the conventional force constant and f_{rrr} is the cubic anharmonic force constant for the bond of interest. The mean-square fluctuation in bond length is then (McQuarrie, 1976):

$$< \Delta z^2 >= \frac{\hbar}{2\mu\omega} \coth\left(\frac{\hbar\omega}{2k_BT}\right) \tag{4}$$

where μ is the reduced mass, $\omega = (k/\mu)^{\frac{1}{2}}$ is the vibrational frequency, k_B is the Boltzmann constant and T the absolute temperature. The cubic term provides a coupling between normal modes. Using first order perturbation theory, the increase in average bond length can be written as (Henry and Szabo, 1985):

$$< \Delta z >= -\left(\frac{f_{rrr}}{2\mu\omega^2}\right) < \Delta z^2 > \tag{5}$$

Figure 1 shows MP2/6-31G** energies as a function of bond length for an isolated amide and for two models of hydrogen bonding. It is worth noting that the zero-point vibrational energy ($\hbar\omega/2$) is about 5 kcal/mol, so that a significant range of bond lengths is sampled by the ground-state vibrational wavefunction. Hydrogen bonding to water increases the optimal bond length (i.e. the value at the bottom

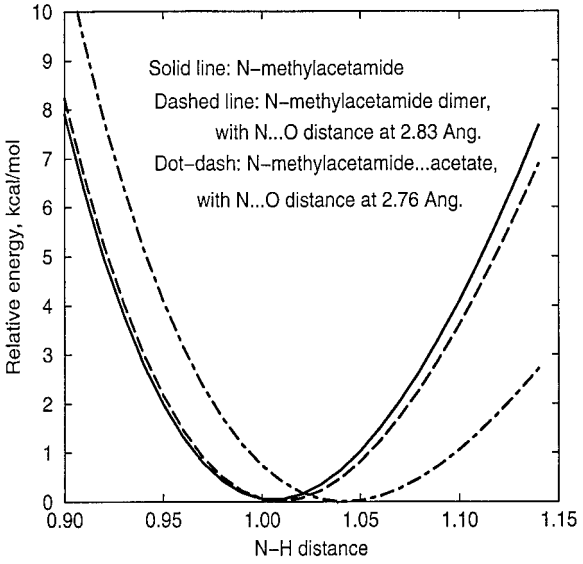

Figure 1. MP2/6-31G** energy versus bond length for *N*-methylacetamide and its complexes with two waters, or with an acetate anion.

of the well) by about 0.004 Å. This is in accord with earlier calculations by Guo and Karplus (1992), who studied a variety of hydrogen bonding environments and found N–H bond length increases upon formation of a hydrogen bond ranging from 0.001 to 0.009 Å. As Figure 1 shows, interactions with an anionic acceptor can lead to much larger bond length increases, although these sorts of interactions are rare in proteins. It is also worth noting that N–H bond lengths at the MP2 level are about 0.015 Å longer than those obtained from Hartree–Fock theory (Guo and Karplus, 1992). The vibrationally averaged N–H bond length (the sum of r_0 and Δz in Table 2, e.g. 1.025 Å for NMA/wat$_2$) is quite close to the values of 1.020 and 1.024 Å determined from neutron diffraction results on glycylglycine-H_2O (Kvick et al., 1977), suggesting that the present MP2 results are at a useful level of accuracy.

Table 1 gives the Taylor series expansions for various bonds in model compounds, and Table 2 shows the corresponding contributions to r_{eff}. The general pattern is the same for all bonds: anharmonic contributions increase r_{eff}, by roughly 0.015 for N–H or C–H bonds and by much smaller amounts (0.001–0.002 Å) for bonds not involving hydrogen. The harmonic correction, which reflects the fact that r^{-3} averaging weights smaller distances more heavily than larger ones, cancels much but not all of the anharmonic correction.

Table 1. Taylor series expansion for energy versus bond length

Molecule	Bond	R	k	f_{rrr}
NMA	H–N	1.0061	1152.0	−6798.6
NMA-wat	H–N	1.0096	1109.8	−6977.2
NMA-acetate	H–N	1.0419	773.7	−6541.7
Aladip(α)	Hα-Cα	1.0905	854.0	−5309.9
Aladip(β)	Hα-Cα	1.0922	842.4	−5309.7
Aladip(β)	C′–Cα	1.5256	1601.1	−2561.5
Aladip(β)	C′–N	1.3582	1772.5	−6348.0

NMA is *N*-methylacetamide; aladip is the alanine dipeptide (see text for details.) Parameters R, k and f_{rrr} are defined in Equation 3, and were fit to MP2/6-31G** energies. R is given in Å, k in kcal/mol Å^2 and f_{rrr} in kcal/mol Å^3.

The librational terms in Equation 1 can be estimated from conventional normal mode averages. The normal mode average of a scalar function $f(\mathbf{x})$ depending on the 3N-dimensional position vector $\mathbf{x}$, where N is the number of atoms in a system with an equilibrium conformation $\mathbf{x}_0$, is, to a second order Taylor expansion,

$$< f(\mathbf{x}) > = f(\mathbf{x}_0) + \tfrac{1}{2} \sum_{i}^{3N} \sum_{k,l} \frac{\partial^2 f(\mathbf{x}_0)}{\partial x_k \partial x_l} (m_k m_l)^{-1/2} Q_{ik} Q_{il} \sigma_i^2 \quad (6)$$

Here m_k is the mass of atom k and Q_{ik} is the k-th component of the i-th normal mode. The thermal averages of the second moments σ_i^2 of the amplitude distributions of the harmonic oscillators can be calculated for both classical and quantum statistics (McQuarrie, 1976):

$$\sigma_{i,class}^2 = kT/\omega_i^2 \quad (7)$$

$$\sigma_{i,qm}^2 = \frac{\hbar}{2\omega_i} \coth \frac{\hbar\omega_i}{2k_B T} \quad (8)$$

The two statistics coincide in the limits of low frequency or high temperature. For biomolecules, the most important difference is generally that higher frequency modes will have little amplitude in classical statistics but have non-negligible zero-point motion in quantum statistics. Henry and Szabo (1985) have shown how to remove the contribution of bond-length changes in computing the averages in Equation 6, and I have used that procedure here. The contributions to r_{eff} from angle bending can then be determined from the final terms of Equation 2. It is often useful to write these in terms of a corresponding order parameter, defined such that:

$$S^2/R^6 = 1/r_{eff}^6 \quad (9)$$

Table 2. Effective distances for peptide models

Model	Bond	r_0	Δz corr.	Δz^2 corr.	Zero-point libration	Total	Ottiger and Bax
NMA	N–H	1.006	+0.013	−0.009	+0.029	1.039	
NMA(s)	N–H	1.008	+0.013[a]	−0.009[a]	+0.025	1.037	
NMA/wat$_2$	N–H	1.010	+0.015	−0.009	+0.025[a]	1.041	1.041
NMA/acet.	N–H	1.042	+0.023	−0.011	+0.025[a]	1.079	
Aladip(β)	Cα–Hα	1.092	+0.017	−0.010	+0.014	1.113	1.117
Aladip(α)	Cα–Hα	1.090	+0.016	−0.010	+0.014[a]	1.110	
Aladip(β)	Cα–C′	1.526	+0.001	−0.002	+0.005	1.530	1.526
Aladip(β)	C′–N	1.358	+0.002	−0.002	+0.004	1.362	
Aladip(β)(s)	C′–N	1.337	+0.002[a]	−0.002[a]	+0.004[a]	1.341	1.329
NMA(s)	C′–H	2.024	–	–	+0.011	2.035	2.04–2.07

NMA is *N*-methylacetamide; aladip is the alanine dipeptide (see text for details.) Values marked '(s)' refer to calculations with a continuum solvent interaction, as described in the text. The final column is from Ottiger and Bax (1998).
[a]The value listed is assumed to be the same as for the row above it.

This is just the Lipari–Szabo 'model-free' order parameter when internal motion is fast relative to overall tumbling.

Table 3 shows librational averages (expressed as order parameters) for the N–H bond in *N*-methylacetamide using a vacuum quantum potential energy surface, a continuum solvent/quantum surface, and the Cornell et al. (1995) empirical potential energy function. For the solvated result, the table also gives the corresponding contributions r_{eff}. Shown are contributions from each mode (except for the C–H and N–H stretching modes, whose S^2 values are always with 10^{-6} of unity), as well as the overall value. These averages were calculated for quantum statistics at T = 0, i.e. including only zero-point vibrational motion. The out-of-plane motions and the 'amide III' N–H bending modes contribute the most to the reduction of the dipolar coupling strength. The continuum solvent model increases these frequencies compared to the gas-phase result, consistent with an increase in the double-bond character of the C′–N bond (discussed below), leading to slightly smaller librational corrections. The Amber empirical force field has frequencies generally slightly higher than the density functional results, and hence slightly smaller librational corrections (i.e. S^2 values that are closer to unity), but differences among the three models are minor.

Corresponding r_{eff} calculations have been carried out for other bonds of interest in peptides and proteins, and the resulting values are shown in Table 2. These corrections are significant (0.014 to 0.029 Å) for C–H and N–H bonds, and as expected are much smaller (about 0.005 Å) for bonds not involving hydrogens.

Discussion

Comparison to experiment

The results shown in Table 2 are most directly comparable to results reported by Ottiger and Bax (1998), who measured relative dipolar coupling strengths for H–N, Cα–N, Cα–Hα, Cα–C′ and C′–N bonds in a partially oriented sample of ubiquitin. Recognizing that bonds involving heavy atoms should have smaller vibrational corrections than those involving hydrogens, they converted the relative dipolar coupling strengths to effective bond distances by setting the C′–N distance to an average X-ray distance of 1.329 Å (Engh and Huber, 1991). The resulting values are shown in the final column of Table 2, and are in excellent agreement with the quantum chemistry values determined here. The biggest discrepancy is 0.01 Å for the C′–N bond, which is one of the most difficult to compute since it is sensitive to the environment. There are two main Lewis structures for a peptide bond, one with neutral atoms and a single bond between C′ and N, and a second with a negative oxygen and a C′–N double bond. The latter structure should be stabilized by a high-dielectric solvent that can stabilize the excess partial charges on the oxygen and nitrogen atoms. Stabilizing the C′–N double bond form should shorten its bond length, and this can be clearly seen in the distances reported in Table 2: the C′–N bond in a

Table 3. Normal modes and order parameters for *N*-methylacetamide

Mode	Vacuum		Solvated			Amber94	
	freq	S^2	freq	S^2	$10^3\delta r_{eff}$	freq	S^2
	60	0.9987	45	0.9998	0.03	51	0.9990
	88	0.9947	103	0.9990	0.16	72	0.9995
Peptide torsion	166	0.9944	180	0.9988	0.20	182	0.9987
	292	0.9990	297	0.9991	0.15	284	0.9988
	434	0.9999	438	0.9988	0.20	439	0.9998
N–H out-of-plane	431	**0.8993**	489	**0.9155**	14.89	587	**0.9724**
Amide IV	623	0.9997	622	0.9997	0.05	587	0.9999
C=O out-of-plane	629	0.9903	639	**0.9866**	2.25	687	**0.9501**
	874	0.9994	876	0.9992	0.13	800	0.9998
	999	0.9990	1010	0.9995	0.08	961	0.9959
	1055	1.0000	1054	1.0000	0.00	1025	0.9956
	1110	0.9984	1100	0.9985	0.25	1047	0.9995
	1157	0.9999	1149	0.9998	0.03	1079	0.9996
	1173	0.9967	1164	0.9971	0.48	1080	0.9991
Amide III	1274	**0.9894**	1294	**0.9879**	2.03	1204	**0.9772**
	1410	0.9999	1401	0.9998	0.03	1395	0.9994
	1446	0.9997	1439	0.9993	0.11	1399	0.9999
	1484	1.0000	1466	1.0000	0.00	1403	1.0000
	1499	0.9965	1470	0.9981	0.31	1411	1.0000
	1508	0.9983	1498	0.9981	0.31	1419	0.9946
	1516	0.9999	1510	0.9999	0.01	1509	0.9999
Amide II	1571	**0.9857**	1585	**0.9856**	2.42	1608	0.9969
Amide I	1789	0.9997	1708	0.9996	0.06	1693	0.9988
Total		0.8424		0.8630	24.99		0.8776

'Vacuum' and 'solvated' modes are from B3LYP/6-31G** density functional calculations; 'Amber94' uses an empirical force field (Cornell et al., 1995). Frequencies are in cm^{-1}. Computations assume quantum averaging and T = 0. Contributions from individual modes that are less than 0.99 are indicated in boldface.

dipeptide model is reduced by 0.021 Å (from 1.358 to 1.337) on moving from the gas phase to a continuum dielectric model. While this is clearly approximately the correct order of magnitude of bond shortening, the simple nature of the solvent correction makes it likely that this bond distance is the most uncertain, both in absolute and relative terms, of the quantum values reported in Table 2. For all other terms, the quantum values reported in Table 2 agree with those extracted from NMR measurements to within 0.005 Å.

There have also been several attempts to extract effective N–H bond distances from solid-state dipolar or quadrupolar coupling measurements (Roberts et al., 1987; Heaton et al., 1989). For crystalline amides, values between 1.04 and 1.06 Å have been reported, and it has long been recognized that vibrational averaging makes these effective distances greater than bond lengths extracted from X-ray or neutron diffraction studies. Table 2 makes it clear that most of this discrepancy arises from the influence of zero-point angular vibrations on the effective bond length, and that measurements and detailed quantum calculations are in close agreement with each other. There is a small dependence of the N–H bond length on hydrogen bonding, with the amide-water model having a bond length 0.004 Å longer than an isolated amide. For the less common situation of hydrogen bonding to an anionic acceptor, the hydrogen bond effect can be much greater, and in fact extremely strong hydrogen bonds are known where the proton is nearly equally shared between donor and acceptor. However, for most cases of interest in proteins, where the H-bond acceptor is either another amide group or a solvent molecule, the effect of hydrogen bonding on N–H bond length is expected to be small.

Implications for order parameters

It is by now quite a common practice to extract order parameters or spectral densities from heteronuclear NMR relaxation data (Palmer, 1997; Fushman and Cowburn, 1998; Kay, 1998), and to use these values to make conclusions about the extent of internal motion in proteins and its potential thermodynamic consequences (Akke et al., 1993; Yang and Kay, 1996; Yang et al., 1997). However, it is also well understood that the magnitudes of the extracted order parameters depend (among other things) upon assumptions about effective bond lengths. In this regard, it is useful to think of an order parameter as a ratio, relating the observed dipolar coupling strength to that which would be expected from some reference situation (Yip and Case, 1991). The most common reference is an internally rigid molecule with the same average structure as the actual molecule. In this case, it would be appropriate to use the R values listed in Table 1 for bond lengths, since in a truly rigid reference molecule there is no vibrational averaging. This is in a sense what is most commonly done, although for historical reasons, an amide N–H bond length of 1.02 Å is often employed. Using this bond length, values of S^2 for secondary structural elements in small proteins typically lie around 0.85, and such a value is often made more concrete by pointing out that it corresponds to diffusion in a cone of semi-angle 19°.

It is not obvious, however, that an internally rigid reference model is the most appropriate one, since it is based on classical ideas, and ignores the presence of zero-point averaging that must be present, even at absolute zero. If one instead adopts a 'quantum' reference for motions that includes such zero-point averaging (assuming that these intrinsic motions are applicable to peptides in all environments), it would be appropriate to use r_{eff} values like those given in Table 2. This will of course lead to derived order parameters that are much closer to unity.

When only relative order parameters are of importance, then the choice of reference state may be unimportant (with the understanding that the assumptions used should be made clear to promote communication). In particular, thermodynamic interpretations generally rely on changes in order parameters, which should be less sensitive to zero-point motion. However, the inevitable presence of local zero-point librations can be significant when absolute measures of mobility are concerned, as when comparisons are made to molecular dynamics simulations, or where simple physical pictures (such as diffusion in a cone) are used to illustrate the meaning of the extracted parameters. The key point here is that *many peptide groups in secondary structural elements of proteins have little more fast time-scale motion (as measured by NMR relaxation) than would an isolated peptide at 0 K.* This qualitative picture could be expressed in two ways: one could use (for N–H bonds) a value of r_{eff} near 1.041 and extract S^2 values near unity; or one could use an effective N–H bond length of 1.02 Å (as is current practice), but remember to compare the resulting order parameters to a value near 0.86 representative of model peptides even at low temperatures. The use of 1.04 Å rather than 1.02 Å would change a 'typical' N–H order parameter of 0.85 to 0.96; the corresponding semi-angle in the diffusion in a cone model would decrease from 19° to 9°. It is also worth restating the point, known for some time (Brüschweiler, 1992; Palmer and Case, 1992), that quantitative comparisons between molecular dynamics simulations and experiment can be significantly affected by zero-point vibrations that are not correctly represented in a classical dynamics simulation. The value of the analysis reported here lies not only in showing good agreement between quantum chemistry and experiment, but also in showing explicitly the connections between the actual bond length and the effective ones that are reflected in dipolar coupling strengths.

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