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Chemical shifts in biomolecules

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Summary

NMR chemical shifts are sensitive probes of structure and dynamics in proteins. Empirical models, based on a large database of measured shifts, take an input structure and provide increasingly accurate estimates of the corresponding shifts. Quantum chemical calculations can provide the same information, with greater generality but (currently) with less accuracy. These methods are now providing new ways to approach NMR structure determination, and new insights into the conformational dynamics of proteins.

Introduction

High resolution NMR spectroscopy of biomolecules is made possible by the existence of chemical shift dispersion, so that nuclei with identical local chemical environments resonate over a range of frequencies. It is of considerable intellectual interest to understand the origins of this behavior, and is also of practical importance in obtaining information about biomolecular structure and dynamics from NMR. There has been impressive improvement since I surveyed the field for this journal fifteen years ago, [1] although the basic principles that drive chemical shift dispersion have been known for many years. [2–4] One force driving recent re-examinations of the origins of chemical shift dispersion has been the continued growth in the number and usefulness of assignments in the BioMagResBank database (<http://www.bmrb.wisc.edu>). [5, 6*]

The general physical model for chemical shielding is illustrated in Fig. 1: the external (spectrometer) field induces electron currents J^{ind} , which depend upon the orientation and chemical nature of the molecule. These currents in turn give rise to local fields that interact with nuclear magnetic moments. To a good approximation, these induced fields are proportional to the external field, and the term linear in field is given the symbol σ :

$$B^{ind} = -\sigma \cdot B_0 \quad (1)$$

Here B_0 is the effective external (spectrometer) field, and σ is called the chemical shielding tensor. Chemical shifts relate the absolute shielding to an agreed-upon reference shielding: $\delta \equiv \sigma^{ref} - \sigma$.

If we know the current distribution (which is a function of position r), the associated magnetic field can be determined from the Biot-Savart law:

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$$B^{ind} = \frac{-1}{c} \int \frac{\mathbf{r} \times \mathbf{J}^{ind}(\mathbf{r})}{r^3} d\mathbf{r} \quad (2)$$

Viewed in this way, the task of computing chemical shifts can be divided into two tasks: finding the current density $\mathbf{J}^{ind}$, and computing fields through Eq. 2.

For example, the induced current distribution within a chemical functional group (which might be an aromatic ring, a peptide bond, or a metal ion) can often be characterized by its magnetic moment (see Fig. 1). The connection between the induced magnetic moment and the applied field is just the magnetic susceptibility tensor:

$$\mathbf{M} = \chi \mathbf{B}_0 \quad (3)$$

Computing the corresponding currents and induced field yields the “McConnell equation”, [3, 7] giving the contribution to the shielding tensor arising from induced currents in a remote chemical group:

$$\sigma = \frac{\chi}{r^3} - \frac{3(\mathbf{r}\mathbf{r}^T)\chi}{r^5} \quad (4)$$

Here $\mathbf{r}$ is the vector from the center of the remote group to the probe nucleus, and $\mathbf{r}\mathbf{r}^T$ is the outer product of $\mathbf{r}$ with itself. It can easily be shown that the contribution to the isotropic shift at an arbitrary position P vanishes when the susceptibility is isotropic, so that observed shifts depend on the anisotropy of the susceptibility. All sorts of group susceptibilities can be used in Eq. 4, but aromatic and unsaturated groups tend to have the largest susceptibility anisotropies, and hence make the biggest contributions, so much so that these effects are often called “ring-current” contributions.

An especially attractive case for structure determination arises in paramagnetic proteins, where unpaired electrons at transition metal sites can make dominant contributions to susceptibility anisotropies. This contribution is completely analogous to that of aromatic groups, and is generally called the pseudo-contact term. Analysis of pseudo-contact shifts can provide both distance and angular information with respect to the metal ion, even at quite large distances. [8, 9]

These induced currents affect chemical shifts directly, by contributing to the effective magnetic field at the probe nucleus. Nearby charges or dipoles can have an more indirect effect by polarizing chemical bonds containing the probe nucleus, which in turn affects the shielding. The isotropic proton shift due to polarization effects is generally written as an expansion in the field strength:

$$\sigma_{pol} = A(\mathbf{E} \cdot \mathbf{r}) + B E^2 \quad (5)$$

where $\mathbf{E}$ is the electric field, $\mathbf{r}$ is a unit vector along the bond direction, and A and B are proportionality constants specific to the X-H bond. Basically, fields that push electrons away from the H atom towards X will reduce the electron density near the H nucleus, tending to deshield it; fields in the opposite direction will increase the shielding. The quadratic term in Eq. 5 is generally thought to be considerably smaller than the linear term. Other environmental effects, such as close-contact deshielding, have been discussed elsewhere. [3]

Empirical models for biomolecular chemical shifts

Reasonably complete chemical shift assignments for proteins began to appear in the 1980's, and it was quickly realized that one way to estimate the unknown parameters in expressions like Eqs. 4 and 5 was to fit them to observed shifts, generally assuming that X-ray structures were good enough to provide the needed geometrical information. Early work, with "databases" consisting of a dozen or so proteins, led to correlations and parameters sets, especially for side-chain protons, that are still useful today. [10] But the addition of much more data, and the use of more flexible functional forms for fitting, has led to a variety of modern programs that do a much better job of predicting shifts from an input structure. Among programs that can compute such estimates (generally in seconds for an NMR-size protein) are **shifts** [11], **sparta+** [12, 13], **shiftx2** [14, 15**], **camshift** [16–18], **PPM** [19], and **4DSPOT** [20, 21], and **shAIC** [22]. These codes are roughly similar to each other, but emphasize different aspects of the problem. For example, **shiftx2** has an option to use observed data from homologous structures to improve the quality of the predictions; **camshift** is designed to have ready access to derivatives with respect to atomic coordinates, so that penalty functions based on its predictions can be readily added to molecular dynamics simulations; and **PPM** used snapshots from molecular dynamics simulations rather than X-ray structures as input for the calibration against experimental shifts. A limited view of current performance for backbone chemical shifts is given in Table 1, below.

Once routines are available to go in a "forward" direction (from assumed structure to predicted shifts), it is often feasible to construct search routines to do the "reverse", and find structures that are consistent with the observed data (and generally with other constraints as well). The ability to use chemical shift restraints in NMR structure refinement has been available for a long time [23, 24] but has experienced a recent renewal as better prediction algorithms and more robust search strategies have become available. [25–28**] In favorable cases, chemical shift information alone can be used to garner considerable structural information; this ability is especially important for the analysis of minor conformers whose existence is inferred from relaxation dispersion experiments, and where chemical shifts often are the only structural constraints available. [30] There is now a growing literature on the use of shifts to characterize such minor conformers in proteins. [30,31*,32*] Predicted chemical shift patterns are also being increasingly used to test the quality of molecular dynamics simulations and other procedures for generating dynamic ensembles. [21,33,34*, 35*] Such tests always face the problem that differences between computed and observed shifts arise both from errors in the conformational ensembles arising from imperfect force fields and from errors in the simplified models that are used to estimate the shifts for a given conformation. Nevertheless, some force fields seem to be consistently better than others, [34*], and the results can be used to improve existing ones. [36]

The ability to predict and understand chemical shifts in nucleic acids is much less well-developed than for proteins. Some older models work reasonably well for proton shifts, [37–39] where fairly well-understood ring-current contributions are often dominant. Empirical approaches for other nuclei have been hampered by a relative paucity (compared to proteins) of nucleic acids where both assignments and good-quality structural models are available. There is increased interest in this area, and it is likely that improved methods will become available soon.

Quantum chemical approaches to chemical shifts in macromolecules

Chemical shifts are an electronic structure property. The basic ideas of using quantum chemical methods to compute current distributions (and hence shielding tensors) are well established, [40] and have been greatly facilitated by efficient computer implementations.

[41, 42]. There is an enormous literature on calculations of shifts for small molecules that serve as fragments of proteins and nucleic acids, which cannot be covered here. [43] Of more direct interest here are attempts to compute shifts for intact biomolecules, either by adopting linear-scaling methods that can be applied to very large molecules, or by systematically breaking a large molecule into fragments. [44–49] A typical approach is the automated fragment-quantum mechanics/molecular mechanics (AF-QM/MM) model [50, 51*] illustrated in Fig. 2: each residue (and its immediate neighborhood) in turn is considered as a central fragment, treated with quantum mechanics, often at the density functional level. The rest of the biomolecule and the solvent environment are represented by point charges, either arising from the rest of the molecule, or fit on the molecular surface to reproduce the solvent reaction field as computed by Poisson-Boltzmann or integral equation methods.

Table 1 gives a (very limited) idea of how these methods perform for backbone shifts of the small protein ubiquitin. I have rather arbitrarily chosen shift α as an example of the empirical models, since its results are among the best currently available. The AF-QM/MM approach is also represented, as are a “null-hypothesis” model in which each nucleus is assigned its average or “random-coil” value. (The latter is important, since for nuclei such as C α or C β , the overall dispersion is dominated by the side chain identity, and it is the deviations from the random-coil model that have structural information.)

These results lead to several conclusions. First, the empirical models uniformly out-perform the quantum fragment results, but sometimes by fairly small amounts. If the quantum results can be improved somewhat, they could be quite useful, since we know exactly what structures (or structural ensemble) are involved, avoiding the “structural noise” that arises in the empirical models from the fact that the structural ensemble leading to the observed shifts is not known. Second, there is an marked improvement in prediction when ensembles of structures are analyzed (compare the first and second entries in the Table). This is quite a common observation, at least for well-chosen ensembles, [21, 33*, 35*, 52] and the improvements in Table 1 are sometimes dramatic. Since quantum calculations are fairly expensive, more work is needed to determine how best to sample small yet representative ensembles. Third, the slopes of most of the fits are less than one, suggesting that even the best models are flattening the true extent of dispersion; this is presumably because some of the physical interactions leading to shift dispersion are not correctly represented. Finally, the quantum fragment results are especially poor for amide proton shifts, presumably due to a poor description of hydrogen bonding effects, but these shifts are also the most difficult for empirical models to predict.

Although quantum calculations of chemical shifts in proteins have not yet reached the level of accuracy obtained by empirical models, there are good reasons to push forward. Quantum models allow study of unusual conformations, including fibrils, partially disordered systems, or other unusual configurations that might not be represented in existing databases of shifts. They can take account of the effects of ligands or cofactors, and can be applied to carbohydrates, nucleic acids, and other biochemical entities.

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- Empirical models that predict chemical shifts in proteins have recently been improved
- This has led to an improved understanding of protein structure and dynamics
- Quantum chemistry methods are not yet as accurate, but show promise

Figure 1. Schematic view of induced currents. The spectrometer field induces electronic currents (J^{ind}), which in turn give rise to induced fields (B^{ind}) that are felt by nuclei at the probe positions P . The induced currents are often approximately equivalent to an induced magnetic moment M , which is generally opposed to the static field.

Figure 2. AF-QM/MM model for CSA Calculation. The central QM region, represented by ball-and-stick model, is calculated on using DFT methods. The rest of the protein, represented by sticks, are treated as AMBER charges. The entire protein is embedded in surface charges rendered as dots.

Table 1

Shift predictions for ubiquitin. For each nucleus, the Pearson correlation (R), slope of the best fit line, and the root-mean-squar (RMS) error are reported. The four values shown are are for shiftx+ [15**] (using Model1 of the 1D3Z pdb entry [53]); shiftx+ using a 64-member subset of the ERNST ensemble [54]; AF-QM/MM [49*, 50, 51*] using the “enhanced” erosII ensemble [33*]; and a random coil model (with values from sparta+ [13]).

nucleus	R	slope	RMS
N	.953/.959/.924/.739	.89/.87/.85/.40	1.98/1.90/2.65/4.40
C ^a	.992/.996/.980/.876	.98/1.00/.94/.84	0.55/0.40/0.89/2.17
C ^β	.998/.999/.992/.990	.99/.99/.98/.97	0.96/0.56/1.62/2.10
C	.926/.972/.725/.391	.85/.93/.65/.15	0.76/0.50/1.42/1.85
H ^a	.901/.994/.879/.201	.87/.93/.75/.06	0.21/0.06/0.25/0.50
H	.820/.922/.192/-.053	.67/.71/.19/.00	0.37/0.28/0.83/0.66