Chemical Shifts in Proteins and Nucleic Acids

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## Contents

1 Introduction: Physical origins of chemical shielding 5

2 The spin Hamiltonian account of NMR 7
   2.1 Chemical shielding ........................................... 8
   2.2 Chemical shifts and referencing .......................... 10

3 Molecular electronic structure and chemical shielding 13
   3.1 Classical mechanics ........................................ 13
   3.2 Electromagnetic fields and potentials .................... 14
   3.3 The transition to quantum mechanics ..................... 15
   3.4 Ramsey’s formula for chemical shielding ................. 16
   3.5 Density functional theory .................................. 17
      3.5.1 The total energy in density functional theory .... 18
      3.5.2 Exchange energy and the Fermi hole ................. 19
      3.5.3 Correlation for opposite spins and the Coulomb hole 20
      3.5.4 The Kohn-Sham approach ........................... 21
   3.6 Results for chemical shielding tensors ................... 22

4 Empirical approaches to biomolecular chemical shifts 25
   4.1 Models for induced currents ............................... 25
      4.1.1 Free electron precession and diamagnetism .......... 25
      4.1.2 Localized magnetic moments; the McConnell equation 26
      4.1.3 Higher-order moments ................................ 28
      4.1.4 Group susceptibilities ................................ 29
      4.1.5 Atom- and bond-based susceptibility models ...... 30
   4.2 Ring current models ......................................... 31
      4.2.1 The Johnson-Bovey model ............................ 31
      4.2.2 The Haigh-Mallion model ............................ 32
      4.2.3 Calibrating ring-current models .................... 34
   4.3 Electric field effects ...................................... 37
   4.4 Close contact interactions ................................ 41
   4.5 Hydrogen bond effects .................................... 44
      4.5.1 Natural bond orbitals and hydrogen bonds .......... 44
      4.5.2 Hydrogen-bond effects on chemical shifts ........ 46
   4.6 Paramagnetic chemical shifts ............................. 46
## Contents

### 5 Some sample applications
- 5.1 “Random-coil” shifts for peptides and nucleic acids ........................................ 47
- 5.2 Protein secondary structure identification .......................................................... 49
- 5.3 Chemical shifts and tertiary structure refinement ................................................ 50
  - 5.3.1 Diamagnetic proteins ...................................................................................... 50
  - 5.3.2 Paramagnetic proteins .................................................................................... 51
  - 5.3.3 Nucleic acids ................................................................................................. 52

### 6 Chemical shift anisotropies
- 6.1 Shielding anisotropies in proteins ......................................................................... 53
  - 6.1.1 The peptide group ........................................................................................ 53
  - 6.1.2 Cα and Hα anisotropies ................................................................................. 55
  - 6.1.3 Nucleic acid sugars ...................................................................................... 55
  - 6.1.4 Nucleic acid bases ....................................................................................... 56
  - 6.1.5 Shielding anisotropies in paramagnetic systems ............................................ 57
1 Introduction: Physical origins of chemical shielding

Chemical shifts (or shieldings) are the most direct parameters measured by NMR, since they determine the positions of the peaks in any spectrum. Once peaks are assigned to individual nuclei, they are often used just as a label to keep track of which spins are which. Such an approach throws away a lot of potentially useful information. These lecture notes describe current approaches to connecting chemical shifts to structure; they go way beyond what can be covered in a short course, but my hope is that you can use the information and citations here as a guide for a more careful study of this subject.

The general physical model for chemical shielding is illustrated in Fig. 1.1: the external (spectrometer) field will induce currents $J_{\text{ind}}$, 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. 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:

$$B_{\text{ind}} = -\frac{1}{c} \int \frac{r \times J_{\text{ind}}(r)}{r^3} \, dr$$  \hspace{1cm} (1.1)

Viewed in this way, the task of computing chemical shifts can be divided into two tasks: finding the current density $J_{\text{ind}}$, and computing fields through Eq. 1.1. There are two basic ways on can proceed. The first approach constructs models for $J_{\text{ind}}(r)$ (perhaps containing some adjustable parameters that may be fit to experiments), and uses these to compute the induced fields, and hence the shielding parameters; this is the basis of the empirical models outlined in Chap. 4. A second approach (chapter 3) uses methods of quantum chemistry to construct an approximate molecular wavefunction in the presence of an external magnetic field, and from this computes currents and shieldings. Both approaches are useful in developing connections between chemical shifts and molecular structure.

As a simple example, suppose the electrons undergo free precession about the applied field with the Larmor frequency $\omega_L = eB_0/2mc$, where $-e/2mc$ is the gyromagnetic ratio of the electron; here $e$ is the electron charge, $m$ is its mass, and $c$ is the speed of light. Then the electrons flow in circular paths around the field direction, and the current is:

$$J_{\text{free}} = e(\omega_L \times r) \rho(r)$$  \hspace{1cm} (1.2)

where $\rho$ is the electron density. The induced field becomes

$$B_{\text{ind}} = -\frac{e^2}{2mc^2} \int \frac{r \times (B_0 \times r)}{r^3} \rho(r) \, dr$$  \hspace{1cm} (1.3)

The triple vector product can be explicitly expanded to express this as a shielding tensor:
1 Introduction: Physical origins of chemical shielding

![Diagram of induced currents](image)

Figure 1.1: Schematic view of induced currents. The spectrometer field induces electronic currents ($j$), 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 moment $M$, which is generally opposed to the static field.

\[ \sigma = \frac{e^2}{2mc^2} \int \frac{r^2I - rr^T}{r^3} \rho(r)dr \]  

(1.4)

where $I$ is a unit matrix and $rr^T$ is the outer product of $r$ with itself, that is, a 3x3 matrix, with $kl$ element $r_k r_l$. For example, for the $zz$ component we would have:

\[ \sigma_{zz} = \frac{e^2}{2mc^2} \int \frac{x^2 + y^2}{r^3} \rho(r)dr \]  

(1.5)

This gives results of the correct order of magnitude, and can be used to understand some features of the anisotropy of shielding tensors, that is, of the reasons why $\sigma_{xx}$ and $\sigma_{yy}$ differ from $\sigma_{zz}$. Of course, for most useful predictions, we need a more complicated model than Eq. 1.2 to describe the response of the electron distribution to an external fields. Such models will be considered in Chap. 4.
The spin Hamiltonian account of NMR

One of the most fundamental and useful ways to describe NMR spectroscopy uses the concept of a spin Hamiltonian to represent the energetics of the system. Terms in such a Hamiltonian consist of operators that act on abstract spin variables, generally multiplied by parameters that describe the molecular system being studied. For example, the interaction energy between a nuclear magnetic moment and an external magnetic field is given by the so-called Zeeman interaction:

\[ E = -\mu \cdot B \]  

Here \( B \) is the magnetic field, and the magnetic moment \( \mu \) is related to the nuclear spin:

\[ \mu = \hbar \gamma I \]  

Here \( \hbar I \) is the angular momentum operator that mathematically represents the nuclear spin, and \( \gamma \) is the nuclear magnetogyric ratio, one of the parameters that describes the physical system. A (hypothetical) NMR experiment that determined the resonance frequency of a (bare) nucleus in a given field would enable one to estimate the value of \( \gamma \), and hence to learn something about the properties of an atomic nucleus. This distinction between abstract spin operators and “physical” parameters is key to practical discussions of NMR. As we mentioned in the preface, most books on NMR focus on the description of experiments that can be used to estimate the values of these physical parameters, whereas this book focuses more on the interpretation of the parameters in terms of the molecular properties of the sample. A few comments about our approach and notation are useful at this point:

1. It is not uncommon to use a more explicit notation to distinguish between the spin operators and other physical parameters, for example with a “hat” notation, so that \( I \) would be replaced with \( \hat{I} \). Since, for the most part, we are avoiding operator algebra here, we can use the somewhat simpler notation of Eq. 2.2. Nevertheless, it must be remembered that these operator quantities have to be manipulated according to the rules of quantum mechanics; in particular, products of operators in general do not commute.

2. Another very common convention, which we will adopt here, is to express spin Hamiltonians (and their associated energy levels) in units of \( \hbar \), so that they have the units of angular frequencies, or radians/sec. In this convention, we then have Zeeman spin Hamiltonian:

\[ \mathcal{H}_Z = -\gamma I \cdot B_0 \]  

Consistent with this convention, we can define the Larmor precession frequency, \( \omega_L = -\gamma B_0 \). Then, if the external field is along the z direction, we could write \( \mathcal{H}_Z = -\omega_L I_z \). In this convention, (which is followed by many but not all books on NMR), the so-called angular momentum or spin operator \( I \) is dimensionless; it is the combination \( \hbar I \) that has units of angular momentum.
3. At chemical energies, the value of $\gamma$ is a constant, independent of molecular conformation or motion. Most spin Hamiltonian parameters, on the other hand, are actually averages over an ensemble of conformations, and an appropriate average has to be taken. This is a non-trivial operation, since the nature of the averaging depends upon the time-scales and amplitudes of the motion. A good part of this book is devoted to describing how motional averaging affects spin Hamiltonian parameters.

For any given sample, testing a proposed spin Hamiltonian description is essentially an empirical procedure: is there a set of terms in the Hamiltonian (and parameters associated with them) that fit the observed experimental data? This turns out generally to be possible with spin Hamiltonian terms that involve only low powers of the spin operators and fields. For example, $H_Z$ is linear in the applied field and linear in the nuclear spin operator $I$. Other spin Hamiltonian terms have similar forms, and the ones of importance for this book are introduced in the next few sections.

### 2.1 Chemical shielding

The magnetic field that needs to be used in Eq. 2.1 is the (microscopic) effective field felt at each nucleus. This is different from the spectrometer field for two principal reasons. First, in a condensed phase the macroscopic polarization of the sample will affect the field seen by the nuclei. This effect is generally not of great importance, since in practice differences is shielding (called chemical shifts) are what are measured and interpreted. The macroscopic polarization (roughly the distinction between $B$ and $H$ in Maxwell’s equations) affects both the resonances and and reference compound in the same way; more careful considerations are needed in solid-state experiments. The second effect is much more interesting: the external field will induce currents in the electron distribution, that create secondary, or induced, fields at the nuclei. To a good approximation, these induced fields are proportional to the external field, and the term linear in field is given the symbol $\sigma$:

$$B_{\text{eff}} = (1 - \sigma) \cdot B_0 \quad (2.4)$$

Here $B_0$ is the effective external (spectrometer) field, and $\sigma$ is called the chemical shielding tensor. Hence the spin Hamiltonian for the nuclear Zeeman interaction becomes:

$$\mathcal{H}_Z = -\gamma I \cdot B_{\text{eff}} = -\gamma I \cdot (1 - \sigma) \cdot B_0 \quad (2.5)$$

The induced field (i.e. $B_{\text{eff}} - B_0$) is not necessarily in the same direction as the static field, as $\sigma$ is a tensorial quantity; this can be written out more explicitly:

$$\mathcal{H}_Z = -\gamma I \cdot B + \gamma \begin{bmatrix} I_x & I_y & I_z \end{bmatrix} \begin{bmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{bmatrix} \begin{bmatrix} B_x \\ B_y \\ B_z \end{bmatrix} \quad (2.6)$$

Since the shielding tensor is (by definition) the coefficient of the spin Hamiltonian term that is bi-linear in $B$ and $I$, it can also be written as a second derivative of the energy:

$$\sigma_{ij} = \left( \frac{\partial^2 E}{\partial \mu_i \partial B_j} \right) + \delta_{ij} \quad (2.7)$$
2.1 Chemical shielding

Here the derivative is taken in the limit that \( \mu, B \to 0 \), and the energy has has full units (i.e. is not divided by \( h \)). This expression is most useful in quantum chemistry calculations, to be discussed in Chapter 3, below.

The shielding tensor is small (on the order of \( 10^{-6} \) or parts per million) but can be measured to high accuracy and precision and encodes a great deal of information about molecular structure and dynamical averaging. In isotropic solutions, the relevant quantity for understanding the appearance of the spectrum is the orientational average of \( \sigma \), which is one-third its trace, or \( (\sigma_{xx} + \sigma_{yy} + \sigma_{zz})/3 \). This is called the isotropic shielding, or \( \sigma_{iso} \). Resonance positions are generally determined relative to a reference shift suitable for each type of nucleus. The proper referencing of shifts is an important practical topic,\[4, 5\] but is outside the scope of this book. By convention, the chemical shift is related to the difference in shieldings between the nucleus of interest and that of a reference compound. By this convention, which is nearly universal in liquid state NMR, the symbol \( \sigma \) is used for a shielding parameter, and \( \delta \equiv \sigma_{ref} - \sigma \). Note that the shielding decreases as the shift increases.

As a general second rank tensor, \( \sigma \) can be decomposed into a sum of tensors of rank 0, 1, and 2:

\[
\sigma = \sigma^{(0)} + \sigma^{(1)} + \sigma^{(2)}
\]

Here \( \sigma^{(0)} \) is the unit matrix (tensor) multiplied by the isotropic shielding, which is \( (\sigma_{xx} + \sigma_{yy} + \sigma_{zz})/3 \); this is a scalar quantity, independent of orientation. The rank 1 component \( \sigma^{(1)} \) is the antisymmetric component of the full tensor:

\[
\sigma^{(1)} \equiv (\sigma - \sigma^T)/2
\]  

(2.8)

and the rank 2 tensor is the (traceless) orientation-dependent part of the symmetric component:

\[
\sigma^{(2)} \equiv (\sigma + \sigma^T)/2 - \sigma^{(0)}
\]  

(2.9)

If we rotate the coordinate system to a frame where \( \sigma^{(2)} \) is diagonal (which can always be achieved for a real symmetric tensor), then we can denote the matrices as follows:

\[
\sigma^{(1)} = \begin{bmatrix}
0 & \sigma_{xy} & \sigma_{xz} \\
-\sigma_{xy} & 0 & \sigma_{yz} \\
-\sigma_{xz} & -\sigma_{yz} & 0
\end{bmatrix}
\]  

(2.10)

\[
\sigma^{(2)} = \begin{bmatrix}
\sigma_{xx} - \sigma_{iso} & 0 & 0 \\
0 & \sigma_{yy} - \sigma_{iso} & 0 \\
0 & 0 & \sigma_{zz} - \sigma_{iso}
\end{bmatrix}
\]  

\[
= \frac{1}{3} \sigma_x \begin{bmatrix}
2 & 0 & 0 \\
0 & -1 & 0 \\
0 & 0 & -1
\end{bmatrix} + \frac{1}{3} \sigma_y \begin{bmatrix}
-1 & 0 & 0 \\
0 & 2 & 0 \\
0 & 0 & -1
\end{bmatrix}
\]  

(2.11)

Here \( \sigma_x = \sigma_{xx} - \sigma_{zz} \), and \( \sigma_y = \sigma_{xy} - \sigma_{yz} \). The final line decomposes a general non-axial symmetric tensor into two axially symmetric parts, which can always be done. Note that the two tensors in the final line of Eq. 2.11 have values of \( \sigma_{\parallel} - \sigma_{\perp} \) of \( \sigma_x \) and \( \sigma_y \), respectively.
2 The spin Hamiltonian account of NMR

In order to facilitate comparisons, here we will discuss both calculated and experimental values in terms of absolute shieldings $\sigma$, using estimated values for the absolute shieldings of reference compounds to convert experimental chemical shifts into shieldings. Where tensor components are discussed, we shall take $\sigma_{11} < \sigma_{22} < \sigma_{33}$, i.e. that $\sigma_{11}$ is the least shielded principal component (eigenvalue) of the shielding tensor.

Generally, we will consider only the symmetric portion of the tensor, since this is expected to dominate relaxation in large systems,[6] but it is worth noting that the computed tensors often have significant antisymmetric parts. Ring current and magnetic dipole models that can be used to estimate the antisymmetric component in some situations are discussed below. Note that by convention, the first (row) index of $\sigma_{ij}$ refers to differentiation with respect to $\vec{\mu}$, and the second (column) index refers to differentiation with respect to $\vec{B}$.

Spin relaxation can be induced by molecular motions that lead to a time dependence in any of the above three components, $\sigma^{(0)}$, $\sigma^{(1)}$, or $\sigma^{(2)}$. Typically, motions that modulate the the isotropic shift need to be fairly slow (on the millisecond time scale or slower) in order to lead to significant relaxation; for historical reasons, such processes are called chemical exchange, since they often involve relatively slow “hopping” across a barrier from one conformer to a second one that has different isotropic shifts. Typical values of $\sigma_i$ or $\sigma_j$ (the “anistropies) are often much larger than changes in isotropic shifts caused by conformational change; for example, the dispersion (spread) of $^{15}$N isotropic shifts in proteins is about 20 ppm, whereas the change in the shift tensor as the molecule rotates is about 170 ppm. This larger variation means that much faster, nanosecond, rotational tumbling events can still be effective in relaxation; this is called chemical shift anisotropy (CSA) relaxation.

2.2 Chemical shifts and referencing

By convention the chemical shift ($\delta$) relates the isotropic shielding to that of a reference compound:

$$\delta = \sigma_{iso}(ref) - \sigma_{iso}$$  \hspace{1cm} (2.12)

The proper referencing of chemical shifts is an important practical subject,[5, 7] but one we will not cover here. The convention of Eq. 2.12 is universally used in liquid-state NMR; solid-state studies often follow this convention, but may also use the symbol $\sigma$ for chemical shifts, or for the negative of the chemical shift, and reference standards are quite variable. Hence, care must be taken in extracting shifts from the literature or from on-line databases, and correction procedures may be needed to obtain consistent data sets for analysis.[8, 9]

It is important to remember that chemical shifts are relative frequency measurements, not absolute measurements. In organic chemistry and for compounds dissolved in organic solvents, TMS (tetramethylsilane) has been the de facto 1H and 13C chemical shift standard since the 1970’s. However, it has only been recently that a set of universal standards for chemical shift referencing in aqueous solutions has been set by the IUPAC and IUB.

The specific IUPAC recommendations for biological molecules are that internal DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid), a water soluble, pH insensitive form of TMS, should be the standard used for 1H and 13C referencing. In addition, external anhydrous liquid ammonia should be used for 15N referencing, external 100% trifluoroacetic acid should be used 19F referencing and internal
2.2 Chemical shifts and referencing

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Compound</th>
<th>Ξ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1\text{H})</td>
<td>DSS</td>
<td>1.000 000 000</td>
</tr>
<tr>
<td>(^{13}\text{C})</td>
<td>DSS</td>
<td>0.251 449 530</td>
</tr>
<tr>
<td>(^{15}\text{N})</td>
<td>liquid NH(_3)</td>
<td>0.101 329 118</td>
</tr>
<tr>
<td>(^{19}\text{F})</td>
<td>CF(_3)COOH</td>
<td>0.940 867 196</td>
</tr>
<tr>
<td>(^{31}\text{P})</td>
<td>(CH(_3))(_3)PO(_4)</td>
<td>0.404 808 636</td>
</tr>
</tbody>
</table>

Table 2.1: IUPAC/IUB recommended Ξ (Xi) ratios for indirect chemical shift referencing in biomolecular NMR (relative to DSS).

10% trimethylphosphate is recommended for 31P referencing. Because of the difficulties in working with some of these reference compounds, an alternative indirect referencing procedure has been strongly advocated. In particular, by using predetermined nucleus-specific frequency ratios (called Ξ or the Greek letter Xi) derived for DSS (13C), liquid ammonia (15N), trifluoroacetic acid (19F) and trimethylphosphate (31P), it is possible to determine the zero point reference for these compounds (and hence these nuclei) using the absolute 1H frequency of internal DSS. Some of the more commonly used Ξ values are presented in Table 1. A more extensive list is available at the BioMagResBank (www.bmrb.wisc.edu/bmrb).

As an example, let us assume you wished to reference the 15N dimension of a \(^{15}\text{N}-\text{H}\) HSQC experiment. First and foremost, your sample must contain a detectable amount of dissolved DSS (say 100 \(\mu\)M). Prior to collecting the spectrum, determine the spectrometer’s \(^1\text{H}\) carrier frequency (say 500,000,087.2 Hz). Second, determine the \(^1\text{H}\) DSS frequency relative to the carrier frequency (assume it is 2521.2 Hz upfield of the carrier). This implies the absolute DSS frequency is 500,000,087.2 - 2521.2 = 499,997,566.0 Hz. Third, multiply this DSS 1H frequency by the \(^{15}\text{N}\) Ξ ratio found in Table 1 (the result is 50,664,312.4 Hz). This value corresponds to the hypothetical \(^{15}\text{N}\) resonance frequency of external liquid ammonia, which by definition is 0 ppm. If the \(^{15}\text{N}\) carrier (decoupler) frequency is also known or measured (say it’s 50,670,450.8 Hz), then the \(^{15}\text{N}\) chemical shift scale, in ppm, can be fully determined. Because magnetic fields drift and spectrometer frequencies vary over time, this indirect referencing procedure must generally be repeated each time a new sample is placed in a spectrometer. For most spectrometers it is possible to write a simple computer program to routinely perform this referencing task. Regardless of whether one chooses to use the direct or indirect referencing procedures, properly referenced spectra are absolutely key to obtaining meaningful chemical shift information.
3 Molecular electronic structure and chemical shielding

The response of molecules to magnetic fields is clearly key to understanding NMR spectroscopy. The fields may be generated “externally” by a large magnet, or “internally” from the motion and spins of electrons and nuclei. In Chapter 2, we introduced the phenomenological approach, where the effect of magnetic fields is described in terms of a spin Hamiltonian. Here we take the complementary, microscopic view, in which magnetic behavior is written in terms of the response of molecular wavefunctions to magnetic fields. The theory of the behavior of electrons in a molecular environment is generally called “quantum chemistry.” There is space here for only a very brief overview of some parts of this broad subject that are needed in the rest of the text. Some excellent textbooks (at various levels of sophistication) are given at the end of the chapter. Since many introductory texts do not cover interactions with a magnetic field, we emphasize that topic here.

Our first task is to describe the way in which magnetic fields enter the Schrödinger equation, which we address by beginning with the corresponding classical ideas for fields and the forces they exert on charged particles. This leads directly to familiar formulas relating magnetic interactions to angular momenta. We follow this with an outline of how these formulas can be applied to compute chemical shieldings and indirect spin-spin couplings, which are the two most important NMR parameters that depend upon electronic structure.

3.1 Classical mechanics

Newton’s equations form the basis for classical mechanics. They are most easily written down in Cartesian coordinates:

\[ \mathbf{F} = \frac{dp}{dt} = m\mathbf{a} = m\frac{d^2\mathbf{r}}{dt^2} \]

Here \( \mathbf{F} \) is the force, \( \mathbf{p} \) the momentum, \( \mathbf{a} \) the acceleration, \( m \) the mass, and \( \mathbf{r} \) the position of the particle. The simple appearance of Eq. 3.1 only holds for Cartesian coordinates, and it is generally useful to consider generalized coordinates \( \{q\} \) and the corresponding generalized forces \( \{Q\} \). For conservative systems, the latter can be derived from a potential \( V(q,\dot{q}) \), where \( \dot{q} \equiv \frac{\partial q}{\partial t} \):

\[ Q_j = -\frac{\partial V}{\partial q_j} + \frac{d}{dt} \left( \frac{\partial V}{\partial \dot{q}} \right) \]

It is a characteristic feature of magnetism to have velocity dependent forces. If we define \( T \) as the kinetic energy, then the Lagrangian function \( L(q,\dot{q},t) \) is defined as

\[ L = T - V \]
This permits Newton’s equations of motion to be written in generalized coordinates

\[
\frac{d}{dt} \left( \frac{\partial L}{\partial \dot{q}_j} \right) - \frac{\partial L}{\partial q_j} = 0 \tag{3.4}
\]

The development of this generalized mechanics was a great achievement of eighteenth century mathematics; it is easy to show that Eq. 3.4 reduces to Eq. 3.1 when the coordinate system is Cartesian. A further generalization the momenta in Eq. 3.1 can be made by defining a generalized momentum as

\[
p_j = \frac{\partial L(q, \dot{q}, t)}{\partial \dot{q}} \tag{3.5}
\]

With this definition, the Hamiltonian function (which is most useful for making the transition to quantum mechanics) is defined as

\[
H(p, q, t) = \sum_j \dot{q}_j p_j - L(q, \dot{q}, t) \tag{3.6}
\]

The equations of motion in terms of the Hamilton function (i.e., the analogue of Eq. 3.4) can also be written down, but are not of direct interest here. Rather, we want to develop the Hamilton function for a charged particle in the presence of electric and magnetic fields, and use that to construct a quantum Hamiltonian operator.

### 3.2 Electromagnetic fields and potentials

We begin with a brief review of classical electromagnetic fields, and then consider how these are incorporated into the Schrödinger equation, and hence into quantum chemistry calculations. This will only be a very cursory overview: since magnetic phenomena are fundamentally a relativistic effect, a more proper treatment would have to begin with relativistic quantum theories (i.e., the Dirac equation). We will follow instead a simpler path, in which ...

The electric field \( \mathbf{E} \) and magnetic field \( \mathbf{B} \) are governed by Maxwell’s equations. In free space, and in Gaussian units, these have the form:

\[
\nabla \cdot \mathbf{B} = 0, \quad \nabla \cdot \mathbf{E} = 4\pi \rho \\
\nabla \times \mathbf{B} = \frac{1}{c} \frac{\partial \mathbf{E}}{\partial t} + \frac{4\pi}{c} \mathbf{j} \\
\nabla \times \mathbf{E} = -\frac{1}{c} \frac{\partial \mathbf{B}}{\partial t} \tag{3.7}
\]

Here \( \rho \) is the charge density and \( \mathbf{j} \) the current density. (See Appendix A for a discussion of units, and connections to the SI system.) It is generally more convenient to work with scalar and vector potentials \( \phi \) and \( \mathbf{A} \), which can be derived from the fields:

\[
\mathbf{E} = -\frac{1}{c} \frac{\partial \mathbf{A}}{\partial t} - \nabla \phi, \quad \mathbf{B} = \nabla \times \mathbf{A} \tag{3.8}
\]

Only the fields are physically meaningful; a gauge transformation using an arbitrary function \( f(\mathbf{r}, t) \):
3.3 The transition to quantum mechanics

\[ \mathbf{A} \rightarrow \mathbf{A}' + \nabla f(r,t), \quad \phi \rightarrow \phi' - \frac{1}{c} \frac{\partial f(r,t)}{\partial t} \]  

(3.9)

changes the potentials but leaves the fields unchanged, and hence can have no physical consequences.

A particle with charge \( q \) will act as if it was in a generalized potential:

\[ V = q \left[ \phi(r) - \frac{1}{c} \mathbf{A}(r) \cdot \mathbf{r} \right] \]

(3.10)

so that the momentum conjugate to \( r_i \) is

\[ p_j = \frac{\partial L}{\partial \dot{r}_i} = m \dot{r}_i + (q/c) A_i(r) \]

(3.11)

The Hamiltonian is then

\[ H = r \cdot p - L = \frac{1}{2m} \left[ p - (q/c) A(r) \right]^2 + q\phi(r) \]

(3.12)

3.3 The transition to quantum mechanics

A “prescription” for converting classical systems into corresponding quantum equations is well known: start with the classical Hamilton function given above, and replace the momentum with an operator

\[ \mathbf{p} \rightarrow -i\hbar \nabla. \]

Then we have a (time-independent) Schroedinger equation,

\[ \mathbf{H} \psi = E \psi \]

(3.13)

where \( \mathbf{H} \) is given by Eq. 3.12 with \( \mathbf{p} \) interpreted as an operator rather than a scalar, in the usual fashion. (Note that in this chapter, we are not dividing \( \mathbf{H} \) by \( \hbar \).) For a uniform external field \( \mathbf{B}_0 \), the vector potential can be given by \( A_0 = (1/2) \mathbf{B}_0 \times \mathbf{r} \). (Proof: substitute this directly into Eq. 3.7.) Then expanding out the square, and setting \( q = -e \), gives

\[ \mathbf{H} = \frac{p^2}{2m} - e\phi + \frac{e}{2mc} (\mathbf{p} \cdot \mathbf{A}_0 + \mathbf{A}_0 \cdot \mathbf{p}) + \frac{e^2}{2mc^2} A_0^2 \]

(3.14)

In the quantum operator algebra, the term \( \mathbf{p} \cdot \mathbf{A}_0 \) can be written as \( (\mathbf{p} \cdot \mathbf{A}_0) + \mathbf{A}_0 \cdot \mathbf{p} \), where \( (\mathbf{p} \cdot \mathbf{A}_0) \) means that \( \mathbf{p} \) operates only on the vector potential, and not on any wavefunction that follows it. It is conventional to work in the Coulomb gauge, \( \nabla \cdot \mathbf{A} = 0 \), so that the term linear in \( \mathbf{A}_0 \) becomes

\[ \mathbf{H}_Z = \frac{e}{mc} (\mathbf{A}_0 \cdot \mathbf{p}) = \frac{e}{2mc} (\mathbf{B}_0 \times \mathbf{r}) \cdot \mathbf{p} = \frac{e}{2mc} \mathbf{B}_0 \cdot (\mathbf{r} \times \mathbf{p}) \]

(3.15)

The subscript “Z” stands for Zeeman, a term conventionally applied for the Hamiltonian of a charged particle in a constant magnetic external magnetic field. Recognizing \( \mathbf{r} \times \mathbf{p} \) as the angular momentum \( \hbar \mathbf{L} \), we get:

\[ \mathbf{H}_Z = \frac{e\hbar}{2mc} \mathbf{B}_0 \cdot \mathbf{L} = \beta e \mathbf{B}_0 \cdot \mathbf{L} \]

(3.16)

Here \( \beta e \equiv e\hbar/2mc \) is called the Bohr magneton. If we were to make a gauge transformation, so that \( \mathbf{A}_0 = (1/2) \mathbf{B}_0 \times (\mathbf{r} - \mathbf{R}) \), where \( \mathbf{R} \) is some arbitrary location (say the position of some atomic
3 Molecular electronic structure and chemical shielding

nucleus), then it is straightforward to show that Eq. 3.16 still holds, with \( \mathbf{L} \) replaced by \( \mathbf{L}(\mathbf{R}) \), the angular momentum about the point \( \mathbf{R} \).

From perturbation theory[10] the wavefunction corrected to first order in \( \mathcal{H}_Z \) is

\[
\Psi = \Psi_0 + \sum_{n>0} \frac{\langle n | \mathcal{H}_Z | 0 \rangle}{E_0 - E_n} \Psi_n
\]

(3.17)

Here, \( \Psi_0 \) is the (ground-state) wavefunction in the absence of the field, with energy \( E_0 \), and \( \Psi_n \) and \( E_n \) are the wavefunction and energy of excited states. From this, one can compute the current density:

\[
\mathbf{J}^{\text{ind}}(\mathbf{r}) = -i \hbar \frac{q}{2m} (\Psi^\ast \nabla \Psi - \Psi \nabla \Psi^\ast) - \frac{q^2}{2m} \mathbf{A} \Psi \Psi^\ast
\]

(3.18)

This is a vector function of position, and it can be shown that it is gauge-invariant, and that it obeys the classical continuity equation expected of a current density. Here we are assuming a real wavefunction in the absence of the field, so that there are no zero-field currents; then Eq. 3.18 gives the induced currents generated by the external field.

Of course, moving charges or currents themselves generate magnetic fields, as Maxwell’s equations demand. If we know the current distribution as a function of position, the associated magnetic field can be determined from the Biot-Savart law:[1]

\[
\mathbf{B}^{\text{ind}} = -\frac{1}{c} \int \frac{\mathbf{r} \times \mathbf{J}^{\text{ind}}(\mathbf{r})}{r^3} d\mathbf{r}
\]

(3.19)

This induced (or local) field determines the shielding tensor, since (cf. Eq. ?)

\[
\mathbf{B}^{\text{ind}} = -\sigma \mathbf{B}_0
\]

As we pointed out in Chapter 2, this implies that the task of computing chemical shifts can be divided into two tasks: finding the current density \( \mathbf{J}^{\text{ind}} \), and computing fields through Eq. 4.1. This is a very general approach, and emphasizes the importance of currents generated by the external field. However, practical calculations often skip the intermediate step of computing the current, as we discuss in the following section.

3.4 Ramsey’s formula for chemical shielding

It is not always necessary, or even desirable, to separately compute the current densities in cases where the shielding itself is of direct interest. Following Ramsey,[11] one can consider simultaneously the joint effects of the vector potentials arising from from the external field and the nuclear moment. Rather than pursuing the complete expression, consider an external field and a magnetic dipole moment \( \mu \), both parallel to the \( z \) axis:

\[
\mathbf{A} = \frac{1}{2} (\mathbf{B}_0 \times \mathbf{r}) + r^{-3} (\mu \times \mathbf{r}) = (\frac{1}{2} B + \mu r^{-3})(-y, x, 0)
\]

(3.20)

Here the second contribution to \( \mathbf{A} \) is the vector potential arising from a nuclear dipole, \( \mu \), here taken to be at the origin. Terms in the energy that are proportional to \( \mu B \) will be just \( \sigma_{zz} \mu B \), giving us the
zz component of the shielding tensor. The perturbation Hamiltonian is just the final two terms of Eq. 3.14; making use of Eq. 3.15, we have

$$\mathcal{H}_1 + \mathcal{H}_2 = \frac{e}{mc} \sum_j \mathbf{A}_j \cdot \mathbf{p}_j + \frac{e^2}{2mc} \sum_j \mathbf{A}_j^2$$

(3.21)

Here the subscript $j$ indicates that one must sum over all electrons; our previous formulas have implicitly assumed a single electron. In order to obtain the terms proportional to $\mu_B$, we use first-order perturbation theory on $\mathcal{H}_2$ and second-order perturbation theory for $\mathcal{H}_1$:

$$E_{\text{pert}} = \langle 0 | \mathcal{H}_2 | 0 \rangle - \sum_n \frac{\langle 0 | \mathcal{H}_1 | n \rangle \langle n | \mathcal{H}_1 | 0 \rangle}{E_n - E_0}$$

(3.22)

The sum here is over all excited states of the system, with excitation energies $E_n - E_0$. Now, leaving out the $j$ subscript for the moment:

$$\mathbf{A}^2 = \left( \frac{1}{2} B + \mu r^{-3} \right) (x^2 + y^2)$$

$$\mathbf{A} \cdot \mathbf{p} = \left( \frac{1}{2} B + \mu r^{-3} \right) (xp_y - yp_x)$$

$$= -i \bar{h} \left( \frac{1}{2} B + \mu r^{-3} \right) \left( x \frac{\partial}{\partial y} - y \frac{\partial}{\partial x} \right)$$

$$= -i \bar{h} \left( \frac{1}{2} B + \mu r^{-3} \right) \frac{\partial}{\partial \phi}$$

(3.23)

where $\phi$ is the azimuthal angle of rotation about the $z$ axis. Picking out the cross terms in $\mu$ and $B$ yields:

$$\sigma_{zz} = \frac{e^2}{2mc^2} \left| \sum_j \frac{x_j^2 + y_j^2}{r_j^3} \right| 0 \rangle + \frac{e^2 \bar{h}^2}{m^2 c^2} \sum_n \left( \frac{0| \Sigma / \bar{\sigma} r^{-3} \frac{\partial}{\partial \bar{\sigma}} | n \rangle \langle n| \Sigma r_k^{-3} \frac{\partial}{\partial \bar{\sigma}} | 0 \rangle}{E_n - E_0} \right)$$

(3.24)

This is the so-called Ramsey formula;[11] other components can be calculated in a similar fashion. The first term is called the “diamagnetic” term, and the second term the “paramagnetic” one, but these designations are of mainly historical interest. Note that they do not correspond to the common distinction between diamagnetic (closed-shell) and paramagnetic (open-shell) molecular systems. Furthermore, a gauge transformation will change each of these values (but not their sum), so that no true physical interpretation can be attached to them.

### 3.5 Density functional theory

As it stands, Eq. 3.24 is of little direct use, since it assumes knowledge of the exact ground and excited states for the unperturbed problem (where $\mathbf{A} \equiv 0$), and such information is never available.
There is a large literature, which cannot really be surveyed here, dealing with the extensions of the above ideas to the realm of approximate wavefunctions.[12] Basically, the induced currents can be written in a form much like Eq. 3.18 (with approximate wavefunctions in place of the exact ones), but formulas like Eq. 3.17 can generally not be carried over.

There is one important practical case, however, where the above formalism is useful in a practical sense. This is in the so-called “sum over states” density functional theory (DFT) method. Density functional theory (as the term is used in quantum chemistry) derives from a theorem that the exact (non-relativistic) ground-state energy of an electronic charge distribution can be determined just from the electron density: there is no need in principle to know the molecular wavefunction. For any fixed nuclear configuration, the electronic energy can be determined as the value of $E[\rho(r)]$, where $\rho(r)$ is the electron density at point $r$. This functional is universal, and has a minimum for the exact density. The exact form of the functional is not known, but there are a number of approximate versions that generally give quite good results, especially for the closed-shell organic systems of principal interest in biomolecular NMR.

Density functional theory is now becoming widely recognized as a high-level method for carrying out quantum chemistry calculations, particularly for transition-metal clusters, which are difficult to handle by more conventional ab initio techniques. A distinctive feature of density-functional theory is that both the exchange and the correlation part of the electronic energy is approximated by terms that depend only on functionals of the electron density. In principle, there exists a universal and exact functional that yields the total ground state energy, given the electron density. Furthermore, the energy that arises from the true density is lower than that arising from any other density that integrates to the correct number of electrons, so that a variational procedure can be used to optimize the energy and corresponding electron density. A computational procedure developed by Kohn and Sham allows this optimization to be carried out (using approximate exchange-correlation functionals) in a manner similar to that of classical molecular orbital theory: given an orbital basis set, one constructs and diagonalizes a Fock-like matrix to determine the "Kohn-Sham" orbitals and energies. With this approach, one retains much of the conceptual simplicity and appeal of molecular orbital theory, obtaining numerical results that are generally better (and simpler to compute) than Hartree-Fock orbitals and energies. Because approximate density functional methods can scale as a lower power of basis set size than Hartree-Fock or more complex \textit{ab initio} methods, large basis sets and realistic ligand models are often feasible with a density functional approach.

### 3.5.1 The total energy in density functional theory.

In order to provide a framework for a qualitative understanding of recent trends in density functional theory, we begin with some basic concepts from the theory of reduced density matrices. Let a general coordinate $x = (r,s)$ represent both space and spin variables. The total energy is then:

$$E = \int_{x''=x} -\frac{\nabla^2}{2} \rho_1(x,x') dx + \int \rho(x) V_N(x) dx + \frac{1}{2} \int \rho_2(x_1,x_2) r_{12}^{-1} dx_1 dx_2$$  \hspace{1cm} (3.25)

Here the first term is the kinetic energy of the electrons, the second term is the nuclear-electron attraction energy, the third is the total electron-electron repulsion energy; $\rho_2(x_1,x_2)$ is a second order density matrix, giving the joint probability of finding electron 1 at $x_1$ and electron 2 at $x_2$. It is useful
3.5 Density functional theory

to separate this joint distribution into four terms, $\rho_2^{\alpha\alpha}$, $\rho_2^{\alpha\beta}$, $\rho_2^{\beta\alpha}$, and $\rho_2^{\beta\beta}$, involving only the two space variables $(r_1, r_2)$, indexed by the spin labels $\alpha$ and $\beta$.

For a system with $N$ electrons, we have the following conservation equations:

$$\int \rho_1(r) dr = N$$ (3.26)
$$\int \rho_2(r_1, r_2) dr_1 dr_2 = N(N-1)$$ (3.27)

Since the conservation equation applies equally to both spins, $N_\alpha + N_\beta = N$. Eq. 3.27 can be interpreted as saying that every electron in the system interacts with all other electrons (but not with itself). We can expand $N(N-1)$ in the following illuminating way

$$N(N-1) = (N_\alpha + N_\beta)(N_\alpha + N_\beta - 1)$$
$$= N_\alpha(N_\alpha - 1) + N_\beta(N_\beta - 1) + N_\alpha N_\beta + N_\beta N_\alpha$$ (3.28)

The individual terms on the second line are the integrals of $\rho_2^{\alpha\alpha}$, $\rho_2^{\beta\beta}$, $\rho_2^{\alpha\beta}$, and $\rho_2^{\beta\alpha}$, respectively. Hence, all $\alpha$ electrons interact with all $\beta$ electrons, and with the other $N_\alpha - 1$ electrons of the same spin.

3.5.2 Exchange energy and the Fermi hole

For $\rho_2^{\alpha\alpha}$, Eq. 3.27 implies that correct joint probability function deviates substantially from the classical uncorrelated form, since

$$\int \rho_2^{\alpha\alpha}_{\text{uncorr}}(r_1, r_2) dr_1 dr_2 = \int \rho^\alpha(r_1) \rho^\alpha(r_2) dr_1 dr_2 = N_\alpha^2$$ (3.29)

rather than $N_\alpha(N_\alpha - 1)$. The deviations from an uncorrelated pair distribution can be expressed in the following form:

$$\rho_2^{\alpha\alpha}(r_1, r_2) = \rho^\alpha(r_1) \rho^\alpha(r_2) [1 + f^{\alpha\alpha}(r_1, r_2)]$$ (3.30)

consisting of an uncorrelated part plus an additional term describing "Fermi correlation". Dividing both sides by $\rho^\alpha(r_2)$ yields the conditional probability for finding an electron of spin $\alpha$ at $r_1$ given that there is another spin $\alpha$ electron at $r_2$. The difference between this and the average density at $r_1$ is the "Fermi hole" density, i.e. the density deficit at $r_1$ due to the presence of an electron at $r_2$:

$$\left[ \frac{\rho_2^{\alpha\alpha}(r_1, r_2)}{\rho^\alpha(r_2)} - \rho^\alpha(r_1) \right] = \rho^\alpha(r_1) f^{\alpha\alpha}(r_1, r_2)$$ (3.31)

The integral of this density over $r_1$ is $-1$ for any $r_2$. Further, the Pauli exclusion principle requires that as $r_1 \to r_2$, $f^{\alpha\alpha}(r_1, r_2) \to -1$, so that no two $\alpha$ spin electrons can be at the same place. Thus, electrons of the same spin exhibit "Fermi correlation" in their interactions. Since this fundamentally arises from an antisymmetry requirement, it is present as well even in hypothetical "non-interacting"
systems where the electron-electron interaction is turned off. In such an "exchange-only" model, the electron-electron repulsion energy becomes

\[ U_{ee} = \frac{1}{2} \int \rho(r_1) \rho(r_2) r_{12}^{-1} dr_1 dr_2 \]

\[ + \frac{1}{2} \int \rho^\alpha(r_1) U^\alpha_x(r_1) dr_1 + \frac{1}{2} \int \rho^\beta(r_1) U^\beta_x(r_1) dr_1 \]

(3.32)

where

\[ U^\alpha_x(r_1) = \int \rho^\alpha(r_2) f^{\alpha\alpha}(r_1, r_2) r_{12}^{-1} dr_2 \]

(3.33)

with a corresponding equation for \( U^\beta_x \). The first term in Eq. 3.32 is the "classical" electron-electron repulsion, and the final two terms give the total exchange energy. A straightforward and often effective approximation is to replace these integrals by the corresponding integrals over the Fermi hole of a uniform electron gas having the same density as that of the real system at point \( r_1 \). This gives

\[ U^\alpha_x = -3 \left( \frac{3}{4\pi} \right)^{1/3} \rho^{1/3} \]

(3.34)

This is the "local spin density" (LSD) approximation, and (since detailed numerical properties of the uniform electron gas are known), it can be extended to include correlation effects as well. It turns out, however, that correlation between electrons of the same spin is much smaller in finite systems than in the uniform electron gas, so that ignoring or reducing the LSD correlation contribution for parallel spins is a common modification to the LSD model.

The study of the implications of the simple exchange model of Eq. 3.34 were for molecular and solid-state systems was pioneered by Slater, and a slight variant of this model called \( X\alpha \), where Eq. 3.34 is multiplied by \( 3\alpha/2 \) with \( \alpha \approx 0.7 \), was used for many early studies on transition metal complexes. As discussed below, models that are generally more accurate than LSD for total energies are now available, but it is not clear that these refined models predict better densities than those arising from LSD itself.

### 3.5.3 Correlation for opposite spins and the Coulomb hole.

Consider now the joint distribution function for electrons of opposite spin. We can write this in a form similar to Eq. 3.30:

\[ \rho^{\alpha\beta}_2(r_1, r_2) = \rho^\alpha(r_1) \rho^\beta(r_2) \left[ 1 + f^{\alpha\beta}(r_1, r_2) \right] \]

(3.35)

but now the "Coulomb hole density" integrates to zero for any \( r_2 \):

\[ \int \rho^\alpha(r_1) f^{\alpha\beta}(r_1, r_2) dr_1 = 0 \]

(3.36)
This follows directly from the fact that the integral of $\rho^2_{\alpha \beta}$ is $N_{\alpha}N_{\beta}$. The Coulomb hole is not necessarily small, despite the fact that it must integrate to zero over all space. The important physical principle is that as $r_1 \to r_2$, $\rho^2_{\alpha \beta}(r_1, r_2)$ becomes small, so that electrons of opposite spin avoid each other so as to reduce their Coulomb repulsion. The Coulomb hole density is thus negative (representing an electron deficit) at short distances and positive (electron surplus) at larger distances, to give a zero integral over all space.

As with parallel spins, these opposite-spin exchange-correlation effects could be approximated by using an $f^{\alpha \beta}$ function derived for a uniform electron gas. This gives generally reliable results for solid-state systems, but is often not sufficiently accurate to provide reliable bond energies or geometries in molecules. Over the past decade, considerable practical success has been achieved by extending the LSD model to write the exchange-correlation energy in terms of the densities and their local gradients:

$$E_{xc}^{GGA}[\rho^\alpha, \rho^\beta] = \int f(\rho^\alpha, \rho^\beta, \nabla \rho^\alpha, \nabla \rho^\beta) dr$$

(3.37)

Here "GGA" stands for "generalized gradient approximation"; the differences between the LSD and GGA models are commonly called "non-local" corrections. These functions can be constrained to comply with a variety of asymptotic limits, normalizations and scaling laws, and may include some amount of empirical adjustment as well. The largest adjustments to the LSD model involve the exchange energies, although non-local contributions to correlation can be important as well.

### 3.5.4 The Kohn-Sham approach

These non-local theories have had a dramatic effect in quantum organic chemistry, providing for the first time numerical results from density functional methods that systematically improve on Hartree-Fock theory for a variety of energies and properties. For many purposes, non-local DFT provides a useful level of approximation for considering geometries and chemical energetics. For small organic molecules, non-local density functional methods usually give bond energies accurate to 3 to 5 kcal/mol, bond lengths accurate to 0.02 Å, and bond angles accurate to a few degrees. Many GGA models show some cancelation of errors in their treatment of exchange and correlation, so that the sum is more accurate than the individual components. As with LSD, GGA accounts of correlation are expected to be more trustworthy for antiparallel spins than for parallel spins, although the practical consequences of this for open-shell systems are not yet clear.

Density functional methods are now available in many of quantum chemistry codes, and it is increasingly common to see systematic evaluations of results with different basis sets and energy functionals. The most extensive tests involve organic molecules, where comparisons can be made to a large body of well-calibrated gas-phase experimental data. Here the best overall results, particularly for energetics, currently appear to come from "hybrid" models in which a fraction of the Hartree-Fock exchange energy is combined with the density functional exchange terms described above. The "B3-LYP" functional is currently the most popular of these hybrid models.
3 Molecular electronic structure and chemical shielding

3.6 Results for chemical shielding tensors

Since Kohn-Sham DFT theory is based on one-electron orbitals, one can use the usual orbital-promotion ideas to construct a model for the excited states that are needed in second-order perturbation theory. In particular, the calculations required in Eq. 3.24 can be approached as follows: The ground state $|0\rangle$ can be taken as a single determinant constructed from Kohn-Sham orbitals, and the excited states as determinants in which electrons have been promoted from an occupied to an unoccupied orbital. Matrix elements of the form $<0|\hat{A}|n>$ can be reduced in the usual way to integrals over the one-electron orbitals that are involved in the promotion. If the operator $\hat{A}$ operates on only one electron at a time (as is the case in Eq. 3.24), the sum over excited states needs to consider only single-electron promotions. The energy denominator can be taken to be the difference in the orbital energies of the orbitals involved in the promotion. In this way, an intractable sum over exact excited states can be replaced (approximately, of course) by a fairly simple sum over single-electron excitations that involve only one-electron matrix elements.

This idea is called the "sum-over-states" approach, and can often give quite satisfactory results. It turns out that the energy denominators in Eq. 3.24 are systematically too small, so that the "paramagnetic" terms are overestimated. This deficiency can be largely rectified by modifying the energy denominators to partially account for the change in the exchange-correlation energy densities in the excited states. Some of these corrections have a plausible physical origin, and others are more explicitly empirical;[13–19] overall, however, modifications of the energy denominators appears to be a valuable correction, especially when correlation effects are large.

This approach of using second-order perturbation theory (through Eqs. 3.22 and 3.24) begins with an assumption that one has exact ground and excited-state wavefunctions for the zero-field problem, derives formulas based on this assumption, and then introduces approximations at the very end. An alternative route is to recognize from the beginning that only approximate wavefunctions are available, and to compute shielding tensors as the derivatives of these approximate functions, as in Eq. 2.7. The computational difficulties involved in this second approach depend strongly on what kind of approximate wavefunction is used, but for Hartee-Fock or DFT theories resulting computations are not especially onerous, and popular programs such as Gaussian adopt this model.

In addition to choices about the particular density functional to be used, DFT shielding calculations depend strongly on the basis set used. Figs. 3.1 and 3.2 illustrate some results for the amide group.

It is not straightforward to give general rules about the expected accuracy of chemical shielding calculations. It is important to distinguish to compute absolute or relative shieldings for atoms in widely different chemical environments from efforts to understand trends in a single type of shift as conformational parameters are varied. The latter are generally more reliable, and converge at a lower level of theory, than the former. We consider first test sets that span a wide range of chemical environments. Baldrige and Siegel[20] have shown that density functional calculations for proton shifts in simple hydrocarbons can be computed with an average error of about 0.1 ppm, but that extrapolations to more complicated molecules increases the expected error to about 0.3 ppm. Rablen et al.[21] report an RMS error versus experiment of 0.15 ppm for 80 organic molecules, using a slight linear scaling of density functional results. Errors for carbon and nitrogen shifts are significantly larger than for protons. Carbon shifts for a wide set environments are found to be within about 3-5 ppm of experiment in a number of studies.[14, 22–24] Computed nitrogen shifts are generally in
3.6 Results for chemical shielding tensors

Figure 3.1: Basis set dependence of $^{15}$N shieldings in amides.

Figure 3.2: Basis set dependence of $^{13}$C (carbonyl carbon) shieldings in amides.
somewhat worse agreement with experiment; this is probably because electron correlation effects can be both important and variable in a range of different chemical environments.

Comparisons that look at the same type of atom in different conformations (generally arising from rotations about single bonds) are expected to fare better. For example, Pearson et al.[25] looked at DFT simulations of the Cα and Cβ shifts in valine residues from three proteins. They found a mean deviation of less than 1 ppm for relative shifts (over a range of of 12 ppm for Cα and 10 ppm for Cβ). On a related note, it is common to find that conformation-dependent shifts are often insensitive to basis-set effects or to the method of geometry optimization. For example, studies of trends in peptides or sugars give nearly the same results for geometries optimized at the Hartree-Fock level versus those optimized using a molecular mechanics force field.[26, 27] Nevertheless, the general level of error cited here is large enough to suggest that empirical corrections or calibrations will continue to be used in combination with quantum chemical studies to extract the greatest amount of structural information from protein and nucleic acid chemical shifts.
4 Empirical approaches to biomolecular chemical shifts

4.1 Models for induced currents

As we discussed in Chap. 1, the general physical model for shieldings is illustrated in Fig. 1.1: the external (spectrometer) field induces a current distribution, $J^{\text{ind}}$, which depends upon the conformation and chemical nature of the molecule. These currents in turn give rise to local fields that interact with nuclear magnetic moments. If we know the current distribution as a function of position, the associated magnetic field can be determined from the Biot-Savart law:

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

(4.1)

Viewed in this way, the task of computing chemical shifts can be divided into two tasks: finding the current density $J^{\text{ind}}$, and computing fields through Eq. 4.1. The basic approach for most empirical schemes is to construct a model for $J^{\text{ind}}(\mathbf{r})$ (perhaps containing some adjustable parameters that may be fit to experiments), and to use this to compute the induced fields, and hence the shielding parameters. The $r^{-3}$ factor in Eq. 4.1 makes currents close to the nucleus most important in determining shifts, but differences between similar nuclei in a biomolecule often reflect longer-range behavior. In the next sections, we outline some ways in which this general approach is carried out.

4.1.1 Free electron precession and diamagnetism

Perhaps the simplest model for induced currents is one in which the electrons undergo free precession about the applied field with the Larmor frequency $\omega_L = eB_0/2mc$. Then the current is then

$$
\mathbf{J}^{\text{free}} = e(\omega_L \times \mathbf{r})\rho(\mathbf{r})
$$

where $\rho$ is the electron density. The induced field becomes

$$
\mathbf{B}^{\text{ind}} = -\frac{e^2}{2mc^2} \int \frac{\mathbf{r} \times (B_0 \times \mathbf{r})}{r^3} \rho(\mathbf{r})d\mathbf{r}
$$

(4.2)

The triple vector product can be explicitly expanded to express this as a shielding tensor:

$$
\mathbf{\sigma} = \frac{e^2}{2mc^2} \int \frac{\mathbf{r}^2 \mathbf{I} - \mathbf{r} \mathbf{r}^T}{r^3} \rho(\mathbf{r})d\mathbf{r}
$$

(4.3)

where $\mathbf{I}$ is a unit matrix and $\mathbf{r} \mathbf{r}^T$ is the outer product of $\mathbf{r}$ with itself. For example, for the $zz$ component,
Empirical approaches to biomolecular chemical shifts

\[ \sigma_{zz} = \frac{e^2}{2mc^2} \int \frac{x^2 + y^2}{r^3} \rho(r) dr \]  

(4.4)

Eq. 4.3 corresponds to the "diamagnetic" term in Ramsey's quantum mechanical derivation, given below in Chap. 3. Although this model for current is seriously incomplete, it helps explain some qualitative features of shielding anisotropies. In particular, external forces that tend to distort \( \rho \) in some direction (say along a bond in the \( z \) direction) are predicted from Eq. 4.4 to have a bigger effect on the shielding components perpendicular to the bond direction than they have on the shielding parallel to the bond. This continues to be true, as we discuss below, when the paramagnetic contributions are included as well.

4.1.2 Localized magnetic moments; the McConnell equation

A next step in parameterizing and understanding chemical shifts comes from treating the induced currents as being localized within functional groups. If we are concerned with effects far from such a functional group, the induced current distribution within that group can be characterized by its magnetic moment:

\[ M^{\text{ind}} = \frac{1}{2c} \int (r \times J^{\text{ind}}) dr \]  

(4.5)

This is the leading term in a multipole expansion,\[1, 28\] and should be most useful when the distance of the group to the probe nucleus is large compared to the dimensions of the group itself. The probe nucleus, whose shift we are interested in, has a magnetic moment \( \mu \) that interacts with the external field as in Eq. ??, and it interacts with \( M \) through the dipole-dipole interaction:

\[ E = -\mu \cdot B_0 + \frac{\mu \cdot M}{r^3} - \frac{3(\mu \cdot r)(r \cdot M)}{r^5} \]  

(4.6)

The connection between the induced magnetic moment and the applied field is just the magnetic susceptibility tensor:

\[ M = \chi B_0 \]  

(4.7)

(The susceptibility is conventionally measured on a molar basis, and is often denoted \( \chi_M \) to distinguish it from a volume susceptibility. Here \( \chi \) refers to an individual molecule, and is often called the magnetizability.) For isotropic diamagnetic molecules the components of \( \chi \) are negative and the magnetic moment is aligned opposite to \( B_0 \), as illustrated in Fig. 1.1. For anisotropic substances, \( M \) will not be colinear with \( B_0 \), but will still generally be opposed to it.

Combining Eqs. 2.7, 4.6 and 4.7 yields the "McConnell equation",\[29\] giving the contribution to the shielding tensor arising from induced currents in a remote chemical group:

\[ \sigma = \frac{\chi}{r^3} - \frac{3(rr^T)\chi}{r^5} \]  

(4.8)

Here \( r \) is the vector from the center of the remote group to the probe nucleus, and \( rr^T \) is the outer product of \( r \) with itself. We have written Eq. 4.7 in terms of molecular quantities; conversion factors
4.1 Models for induced currents

to molar susceptibilities and other units are given in Appendix A; in particular, one should note that susceptibilities expressed in SI units differ by a factor of \(4\pi\) from the Gaussian units used here. In particular, if susceptibilities are expressed in units like ppm-Å\(^3\), it is easy to use Eq. 4.8 when distances are expressed in Å.

Eq. 4.8 has most often been used to compute contributions to isotropic shifts, which are \(-1/3\) the trace of \(\sigma\). It is straightforward to show that the isotropic shift arises only from anisotropies in susceptibility tensors. If the susceptibility tensor is rotated to its principal coordinate frame (where off-diagonal elements of \(\chi\) vanish), the isotropic shift becomes:

\[
\delta = (1/3)r^{-3}\sum_{i} \chi_{ii}(3\cos^{2}\theta_{i} - 1) \tag{4.9}
\]

where \(\theta_{i}\) is the angle between the \(i\)th principal direction and \(B\). For the case of an axially symmetric magnetic anisotropy, this becomes

\[
\delta = (1/3)r^{-3}\Delta\chi(3\cos^{2}\theta - 1) \tag{4.10}
\]

where \(\theta\) is the angle between the vector \(r\) and the unique axis, and \(\Delta\chi\) is the difference between magnetic susceptibilities along the unique axis and perpendicular to it. Hence the contribution to the isotropic shift vanishes when the susceptibility is isotropic, i.e. when \(\Delta\chi = 0\). Less well-appreciated is the (tongue-twisting) counterpart that shielding anisotropies respond to the complete susceptibility tensors of neighboring groups, even when they are isotropic. This can be quite important for groups like water, for example, which has a significant (dia-)magnetic susceptibility that is nearly isotropic: neighboring water molecules have little contribution (by this mechanism) to isotropic shifts, but can significantly affect shielding anisotropies as well.

To illustrate these ideas, we consider the simple situation in which a peptide group (represented by NMA, N-methylacetamide) is brought up to argon, which has an isotropic susceptibility. Fig. 4.1 shows the shielding tensor of the proton as a rare-gas atom approaches the N-H group, along the N-H axis, as a hydrogen-bond acceptor might. The solid lines show the shielding tensor components parallel and perpendicular to the N-H bond computed from Eq. 4.8 using the experimental value for the magnetic susceptibility of argon. Since the argon susceptibility is isotropic, the computed isotropic shift is independent of the position of the argon atom. In contrast, the individual tensor components change markedly, with the component parallel to the bond increasing (toward higher shielding), and the components perpendicular to the bond decreasing. This effect is significant even at distances of 3 or 4 Å. In this simple model, the increased shielding of the parallel component is exactly twice the de-shielding of the perpendicular components, leading to no effect on the isotropic shift. Since all molecules have a significant isotropic diamagnetic susceptibility, we can expect the qualitative features seen here to be found in a variety of circumstances.

The squares in Fig. 4.1 give results from density-functional quantum mechanical calculations on the proton shielding tensor in the NMA-argon system. The quantum results map the solid lines of the McConnell equation quite closely, except at short distances, where there is a deshielding effect on the isotropic shift, that is of course also reflected in the shielding tensor components. This is a "close-contact" deshielding long identified in the theory of isotropic shifts, and will be discussed more fully in section 4.4, below. Calculations with helium or neon in the place of argon (not shown)
4 Empirical approaches to biomolecular chemical shifts

Figure 4.1: Interaction of NMA with argon. Red is $\sigma_{||}$, green is $\sigma_{\perp}$, black is the isotropic shift for the proton. See the text for a discussion of the geometries used. Solid lines are from Eq. 4.8; squares are results from density functional calculations. Adapted from Ref. [30].

follow very closely the predictions of Eq. 4.8 obtained by using the experimental susceptibilities of the lighter rare gases in place of argon; (again, there is a close-contact deviation that needs to dealt with separately.)

4.1.3 Higher-order moments

Since the McConnell equation comes from the leading term in a multipole expansion of the current distribution, it may break down for probe nuclei that come close to the group in question. Fig. 4.2 compares the predictions of Eq. 4.8 to quantum calculations of the shielding tensor at a variety of "dummy" points[31] around N-methyl-acetamide. The filled circles are for points evenly distributed on a surface 2.5 Å from the van der Waals surface of NMA. These fit the McConnell equation fairly well, with an r.m.s. error of 0.16 ppm. The open circles show results for a surface 1.5 Å from the van der Waals surface; since hydrogens have a van der Waals radius of about 1.2 Å, this is about as close as probe nuclei can get. Here the scatter is considerably greater, with a r.m.s. error of 0.52 ppm, although the general pattern of shifts is still well-reproduced. The basic ideas for extending the multipolar series to higher terms are known,[28] but practical applications have not yet been developed. Multi-center expansions may be a more efficient way to describe the spatial dependence
4.1 Models for induced currents

4.1.4 Group susceptibilities

Semiempirical theories have been used for many years to generate atom-based models for molecular magnetic susceptibilities.[32] More recently, renewed attention has been paid to the ability of ab initio calculations to compute accurate susceptibility tensors. The Hartree-Fock limit (at least for hydrocarbons) can be reached with relatively modest basis sets,[33, 34] and correlation effects appear to be modest, tending to reduce Hartree-Fock values by 2-10%.[35] Large basis-set density functional calculations also appear to give good results; some examples relevant to biomolecules are given in Table 4.1. discuss applications to DNA, etc.

Table 4.1 shows calculated results for a common peptide model, formamide, that are in fairly good agreement with gas-phase results from molecular Zeeman measurements. The susceptibility is roughly axial about an axis perpendicular to the peptide plane, with observed and calculated (HF) values of $\Delta \chi$ of -8.4 and -7.4 ppm-Å$^3$, respectively. However, a slightly larger peptide model, N-methyl-acetamide, has a computed tensor that is now roughly axial about an in-plane axis (rather than

Figure 4.2: Horizontal axis shows DFT results for the shift tensor at “dummy” points near N-methylacetamide; the vertical axis gives results from Eq. 4.8, using the NMA susceptibility tensor given in Table ?. Filled circles are for points 2.5 Å from the NMA van der Waals surface; open circles are at 1.5 Å.

of secondary fields. (discuss this more here).
4 Empirical approaches to biomolecular chemical shifts

<table>
<thead>
<tr>
<th>molecule</th>
<th>$\Delta \chi$</th>
<th>$R$</th>
<th>molecule (DFT)</th>
<th>$\Delta \chi$</th>
<th>$R$</th>
</tr>
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<tr>
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<td>imidazole</td>
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<tr>
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<td>indole</td>
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<tr>
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<td>cytosine</td>
<td>-38.7</td>
<td>0.06</td>
</tr>
<tr>
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<td></td>
<td>guanine</td>
<td>-0.915</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 4.1: Calculated susceptibility anisotropies, in ppm-Å$^3$/molecule. $\Delta \chi$ is $\chi_{33} - (\chi_{11} + \chi_{22})/2$, where $\chi_{33}$ is perpendicular to the molecular plane; the rhombicity $R = (\chi_{22} - \chi_{11})/\Delta \chi$. Experimental results are from Refs. [36] (benzene) and [37] (formamide). Hartree-Fock (HF) results are from Ref. [30] and use the cc-pVDZ basis. Density functional (DFT) results are from Ref. ?, and use the B3LYP functional and the 6-311++G(3df,3pd) basis.

Experimental results are from Refs. [36] (benzene) and [37] (formamide). Hartree-Fock (HF) results are from Ref. [30] and use the cc-pVDZ basis. Density functional (DFT) results are from Ref. ?, and use the B3LYP functional and the 6-311++G(3df,3pd) basis.

about an axis perpendicular to the peptide plane), and the out-of-plane vs. in-plane anisotropy of -11.6 ppm-Å$^3$ is larger than that of formamide. This suggests that any division into functional groups needs to be treated carefully and consistently to obtain good results.

4.1.5 Atom- and bond-based susceptibility models

Outside of aromatic ring systems which are known to be highly magnetically asymmetric, many chemical groups have relatively small magnetic anisotropies, as determined from susceptibility measurements on molecules in the gas phase. Due in part to its aromatic character, an exception is the peptide group. Data for formamide suggest that the susceptibility tensor for this group is roughly axially symmetric about the normal to the amide plane:

$$2\chi_{xx} - \chi_{yy} - \chi_{zz} = 2.2$$

$$2\chi_{yy} - \chi_{zz} - \chi_{xx} = 8.0$$

$$\delta \chi = -\frac{1}{2} (\chi_{xx} + \chi_{yy}) + \chi_{zz} = -5.1$$

where $z$ is a vector perpendicular to the peptide plane, $y$ bisects the peptide angle NCO, and the susceptibilities are in units of $10^{-6}$ cm$^3$/mol. For comparison, magnetic anisotropy values for water are an order of magnitude smaller.

In addition to "group" susceptibility parameters, commonly used for aromatic rings or for the peptide group as discussed above, there is a strong tradition of analysis of susceptibility anisotropies in terms of "bond" contributions.[38–40] Bond-based models have long been used for saturated systems, where electron pair bonds dominate the electronic structure and there is little delocalization,[41, 42] These contributions are generally smaller than those from delocalized groups, but can nevertheless
4.2 Ring current models

lead to systematic shifts. A frequently cited example is the difference in chemical shift of 0.48 ppm for axial and equatorial protons in cyclohexane, which is thought to be due to anisotropy contributions from C-C and C-H bonds. Below, we study the ability of a model incorporating C-C and C-H bond anisotropies to model part of the torsional dependence of amide proton shifts in peptides. The calculations use the axially symmetric magnetic susceptibility values determined by Flygare[41]

\[ \Delta \chi_{C-C} = -7.7; \quad \Delta \chi_{C-H} = -2.5 \]

where \( z \) parallel to the C-X bond vector, and units are \( 10^{-6} \text{ cm}^3/\text{mol} \). The center of the C-C magnetic anisotropy is assumed to be midway between the two carbon atoms. For the C-H bond, the magnetic anisotropy center is taken as 0.77 Å away from the carbon along the C-H bond.

4.2 Ring current models

Rather than parameterizing currents in terms of magnetic dipoles, as in Eq. 4.5, one can try to guess a form for the current density directly, and compute shieldings from that. Considerable attention has been paid to unsaturated molecules, which tend to exhibit large susceptibility anisotropies, particularly for aromatic systems. The “ring-current” models postulate a simple physical picture for the induced currents of Eq. 4.1, generally with an adjustable parameter, the ring-current “intensity”, that is fit to experimental data. This approach has been extensively reviewed,[43, 44] and we only give highlights here.

4.2.1 The Johnson-Bovey model

For example, in the Johnson-Bovey model for aromatic systems like benzene,[45] the current is assumed to flow in circles above and below the plane of the benzene ring, in what would be the \( \pi \)-electron cloud. The field arising from this current can be computed from Eq. 4.1 and (in the simplest picture) the magnitude of the current in the rings can be adjusted so that the field at the ring protons matches the difference seen experimentally between benzene and a reference non-conjugated system. The currents can then be used to compute secondary fields at any position relative to the benzene ring.

Since aromatic rings are planar, it is convenient to use cylindrical coordinates to specify positions relative to the ring. The Johnson-Bovey model postulates two current loops of radius \( a \), 0.64 Å above and below the ring plane. If these loops have \( n \) electrons, then the isotropic shielding at position \((\rho, z, \phi)\) is:

\[
\sigma_R = -i \frac{ne^2}{6\pi m a c^2} \frac{1}{[(1 + \rho)^2 + z^2]^{1/2}} \left\{ K(k) + \frac{1 - \rho^2 - z^2}{(1 - \rho)^2 + z^2} E(k) \right\} \quad (4.11)
\]

Here \( K \) and \( E \) are complete elliptic integrals, \( \rho \) and \( z \) are cylindrical coordinates of the probe proton relative to the center of the current loop (measured in units of \( a \)), and \( k \) is:

\[
k = \left\{ \frac{4\rho}{(1 + \rho)^2 + z^2} \right\}^{1/2} \quad (4.12)
\]

If the ring radius \( a \) for benzene is taken to be that of a carbon-carbon bond, then the “intensity factor” \( i \) (obtained by an empirical fit) is very close to 1.0. For rings that are similar, but not identical
to benzene, adjusting $i$ slightly can provide an attractive alternative to fitting the radius and axial position of the electron loop. Note that for current rings above and below the plane, there would be two contributions like Eq. 4.11, one for each ring, each with $n=3$.

The model can be expanded to give the full shielding tensor.[46] Along the $x$ axis, the shielding tensor takes a simple form:

$$
\sigma_R = \begin{bmatrix}
0 & 0 & 3\sigma_1 \\
0 & 0 & 0 \\
0 & 0 & 3\sigma_R
\end{bmatrix}
$$

(4.13)

where

$$\sigma_1 = -i \frac{ne^2}{6\pi mac^2} \frac{z}{(1+\rho)^2 + z^2} \left\{ -K(k) + \frac{1+\rho^2 + z^2}{(1-\rho)^2 + z^2} E(k) \right\}
$$

(4.14)

Although this simple classical model for the currents can give good results when compared to quantum chemistry results on the same system,[46, 47] it should not be pushed too hard. For example, calculations of the current density for benzene[43] show that the current density induced by a field perpendicular to the molecular plane is not cylindrically symmetric, but rather has maxima in its modulus near the carbon atoms, with values only about half as great at the midpoints of the C-C bonds. Furthermore, the density is not localized in $\pi$ "rings" above and below the molecular plane, as in the Johnson-Bovey picture; rather, there are significant currents in all planes parallel to the molecular plane. (comment here on whether the JB model is really more accurate than a simple magnetic dipole model?)

4.2.2 The Haigh-Mallion model

Similar models can be derived from quantum-mechanical as well as classical pictures, and there is a rich history analyzing "ring-current" effects in isotropic shifts in organic chemistry and in proteins.[44, 48] One approach, which is very useful from both a practical and pedagogical point of view, derives originally from some assumptions made by London (and later Pople and others) about the molecular orbitals describing the $\pi$ electrons in an aromatic ring should respond to a magnetic field. The full explanation is more complex than can be considered here,[48] but an outline of the approach is useful to understand the flavor of the approximations that are involved.

Let us suppose we adopt the Huckel molecular orbital approach, and express the $\pi$ molecular orbitals of a planar system as a linear combination of $p_z$ orbitals on each ring atom:

$$
\Psi_J = \sum_i c_{ij} \phi_i^0
$$

(4.15)

Here $\phi_i$ is an atomic orbital on atom $i$, $J$ labels the various molecular orbitals, and the zero superscript denotes the fact that these are the orbitals one would have in the absence of an external field. One of the basic assumptions of the London model is that the orbitals in the presence of the field have the same form, but with modified atomic orbitals:

$$
\phi_i = \phi_i^0 \exp \left\{ -\frac{ie}{\hbar} A_i \cdot r \right\}
$$

(4.16)
4.2 Ring current models

Now, one can set up the usual secular equation to determine the coefficients:

$$|\mathbf{H} - E\mathbf{S}| = 0 \quad (4.17)$$

where \(\mathbf{H}\) and \(\mathbf{S}\) are Hamiltonian and overlap matrices in the space of the atomic orbitals:

$$H_{ij} = \langle \phi_i^* | \mathcal{H} | \phi_j \rangle$$

$$= \int \left\{ \exp \left[ \frac{-ie}{\hbar} \mathbf{A}_i \cdot \mathbf{r} \right] \phi_i^0 \left[ (\mathbf{p} + e\mathbf{A})^2 / 2m + V \right] \phi_j^0 \exp \left[ \frac{-ie}{\hbar} \mathbf{A}_j \cdot \mathbf{r} \right] \right\} d\tau$$

$$= \int \left\{ \exp \left[ \frac{ie}{\hbar} (\mathbf{A}_i - \mathbf{A}_j) \cdot \mathbf{r} \right] \phi_i^0 \left[ (\mathbf{p} + e\mathbf{A})^2 / 2m + V \right] \phi_j^0 \right\} d\tau \quad (4.18)$$

At this point, a further approximation is made, to take the vector \(\mathbf{r}\) in the exponential term to be just the midpoint of the bond between the two atoms:

$$\mathbf{r} = (\mathbf{R}_i + \mathbf{R}_j) / 2 \quad (4.19)$$

This can be very roughly rationalized by noting that this midpoint is roughly at the center of the overlap distribution of \(\phi_i \phi_j\). Given Eq. 4.19, we can take the exponential term outside of the integral in Eq. 4.18, leaving behind integrals (which will subsequently be parameterized) that involve the zero-field orbitals \(\phi_i^0\). Using \(\mathbf{A}_i = (\mathbf{B}_0 \times \mathbf{R}_i) / 2\), and taking \(\mathbf{B}_0\) to be perpendicular to the plane of the molecule, one can see that (use a figure here?) that

$$(\mathbf{A}_i - \mathbf{A}_j) \cdot (\mathbf{R}_i + \mathbf{R}_j) = 2S_{ij}B_0 \quad (4.20)$$

where \(S_{ij}\) is the signed area of the triangle formed by the origin and the bond \(i \rightarrow j\) of the conjugated network, counted positive if \(i \rightarrow j\) is right-handed about the \(\mathbf{B}_0\) axis, and negative otherwise. In solving the Hückel secular equation, one uses the usual approximations, setting diagonal elements \(H_{ii}\) to a parameter \(\alpha\), off-diagonal elements \(H_{ij}\) to \(\beta\) if the atoms are bonded to each other, and to zero otherwise. Solving the secular equations leads to expressions in which contains sums of terms like that in Eq. 4.20, with one term for each bond. The resulting calculation is too lengthy to present in full here, but the key ideas in that relate magnetic behavior to triangle areas (as in Eq. 4.20) follow these same approximations. In the end, the contribution to the isotropic chemical shift at a probe proton is given by:

$$\sigma_R = iB \sum_{bonds} s_{ij} \left\{ \frac{1}{r_i^3} + \frac{1}{r_j^3} \right\} \quad (4.21)$$

Here \(B\) is a constant, \(i\) is the ring-current intensity (taken to be 1.0 for benzene), \(r_i\) and \(r_j\) are the distances from the probe proton to two bonded atoms in the ring, and \(s_{ij}\) is the area of the projection into the plane of the aromatic ring of the triangle defined by the proton and ring atoms \(i\) and \(j\). In principle, the magnitude of \(B\) can be calculated from the Hückel parameters describing the ring, but in practice it is almost universally determined by fits to either experimental or quantum-mechanically calculated data. The separation of the overall constant into two terms, \(i\) and \(B\), is just for convenience,
4 Empirical approaches to biomolecular chemical shifts

so that \( i \) gives the magnitude of ring current shifts relative to that of a benzene ring. These ring current intensity factors were originally designed to be the same for the Johnson-Bovey and Haigh-Mallion models, but in practice fitted values (discussed below) are slightly larger for the latter.

In the end, predictions from Eqs. 4.11 and 4.21 are really quite similar for most geometries. It appears that the Johnson-Bovey model tends to overestimate ring-current effects in the deshielding region in the molecular plane, whereas the Haigh-Mallion model underestimates the shielding above and below the plane.[48] This is hard to establish experimentally, since both models predict a strong dependence of induced shift near the ring to the exact geometry. Fits to density-functional quantum mechanical data (where the geometries are exactly known) showed no significant difference between these models.[47] The functional form of Eq. 4.21 is simpler to deal with when derivatives with respect to atomic coordinates are required, which has prompted its use in some chemical shift refinement schemes.

In addition, for all but the closest interactions, the secondary shifts predicted by ring current models match closely those obtained from the magnetic-dipole approximation of Eq. 4.8. This is illustrated in Fig. ?, which compares the shifts predicted by the Haigh-Mallion model with those predicted by a susceptibility-anisotropy (magnetic dipole) model. As discussed below, the shifts were evaluated at a variety of points that are 1.2 to 1.8 Å from the molecular surface. For both benzene and 1-methyluracil, the predictions are essentially identical, with a root-mean-square difference of less than 0.01 ppm.

4.2.3 Calibrating ring-current models

As mentioned above, there are two main ways in which ring current intensity factors (or group susceptibility anisotropies) have been determined. The first uses an empirical scheme: ring current calculations are applied to proteins of known structure, adjusting values of \( i \) to give optimal agreement with measured chemical shifts. This of course requires that other contributions be estimated correctly; to the extent that these other contributions are ignored or mis-estimated, they may end up being assigned a ring-current origin. Furthermore, the actual (average) structure in solution almost certainly differs to some extent from models that are derived from X-ray or NMR structural studies. This adds an additional uncertainty to the fits, and may lead to underestimation of these contributions. Still, the results are useful as long as their limitations are understood. Table 4.2 shows intensity factors determined this way from surveys of both nucleic acids and proteins.

A second approach fits ring current intensities to quantum mechanical calculations, using density functional or other fairly accurate methods to provide the basic information. The basic approach works as follows: probe positions are placed at many points in the vicinity of a conjugated ring, and the chemical shifts at those positions are computed. The probes may either be just dummy points, or may be a small molecule like methane. Then the parameters of the ring-current models are adjusted to optimize the fit between the predictions of Eq. 4.11 or 4.21 and the corresponding quantum chemical results. This sort of “theory vs. theory” analysis has the advantage that one is sure that the geometries and chemical species exactly match. Using dummy points has the advantage of isolating the “pure” effect arising from magnetic-field induced currents in the ring, whereas using a real probe like methane includes effects that might arise from other sources, such as van der Waals or electrostatic interactions between the probe molecule and the ring.
4.2 Ring current models

<table>
<thead>
<tr>
<th>Ring</th>
<th>GP</th>
<th>C95</th>
<th>C04</th>
<th>emp</th>
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Table 4.2: Ring current intensity factors.

![Diagram of dummy points surrounding a benzene molecule.](image)

Figure 4.3: Dummy points surrounding a benzene molecule.
As an example, Figure 4.3 shows a set of dummy points surrounding the molecule, at which shieldings can be calculated. These points lie on two surfaces, one 1.2 Å from the molecular surface (i.e., where a hydrogen atom would be in van der Waals contact with the benzene,) and a second surface 1.8 Å from the surface. Table 4.2 gives some examples of fitting to a methane probe (labeled “C95”) and to dummy points (“C04”). In both cases, density functional calculations with large basis sets were used to construct the target function; details are give in Appendix ?. There are significant differences between the C95 and C04 fits, illustrating the difficulties in gaining secure estimates by this route. Since the C04 results use dummy points, those fits should be most representative of the “pure” ring-current contribution. The fits that used a methane probe had to separately estimate electrostatic and close-contact interactions (see sections ? and ??, below), in order to remove them from the total calculated shift. The uncertainties in this separation are the likely cause for these differences in ring-current intensities.

One can also then measure the extent to which ring-current models mimic the quantum results, providing an independent assessment of the assumptions that go into the ring current models. It turns out that simple ring current models actually do an outstanding job of reproducing quantum-chemical behavior. This is illustrated in Fig. 4.4, which compares secondary shifts of methane molecules near the side chains of aromatic amino acids with fits to Eq. 4.21.

Figure 4.4: Comparison of density functional results and Haigh-Mallion predictions for proton shifts in methane molecules placed near conjugated rings found in protein sidechains. Adapted from Ref. [47].
4.3 Electric field effects

Induced currents in neighboring groups affect chemical shifts by directly contributing to the effective magnetic field at the probe nucleus. Nearby charges or dipoles can have a 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:

$$\sigma_{pol} = A(E \cdot \hat{r} / r) + B E^2$$  \hspace{1cm} (4.22)

where $E$ is the electric field, $\hat{r}$ 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. 4.22 is generally thought to be considerably smaller than the linear term.

There have been a number of recent attempts to calibrate the magnitude of this effect, either through quantum chemistry calculations, or through empirical analyses of shielding changes when polar substituents are incorporated at various locations in organic molecules; an overview of these efforts is given in Chapter 4. In principle, this mechanism could provide a valuable probe for electric fields in biomolecules, but practical questions of disentangling the field effect from other contributions to shift dispersion have limited its use so far. Bond polarization models have also been used to good effect to model $^{13}$C shifts in organic molecules.[49, 50]

The susceptibility effects described above are "direct", in the sense that currents induced in neighboring groups give rise to secondary magnetic fields that contribute directly to the observed shielding at probe nuclei. A significant but more indirect contribution to chemical shifts also arises from distant polar groups, which polarize the electron cloud around the probe nucleus and thereby increase or decrease the local shielding by electrons. The most significant term for a proton is expected to be proportional to the projection of the local electric field onto the X-H bond vector, where X is the atom connected to H. The isotropic shift due to polarization effects is generally written as an expansion in the field strength:

$$\sigma_{pol} = A(E \cdot \hat{r}) + B E^2$$  \hspace{1cm} (4.23)

where $E$ is the electric field, $\hat{r}$ 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 (which have a positive projection on the X-H bond in the convention used here) will reduce the electron density near the H nucleus, tending to deshield it (cf. Eq. 4.4).

Many years ago, Buckingham suggested that an appropriate value for $A$ for a C-H bond would be $-2 \times 10^{-12}$ e.s.u.$^{-1}$.[51] or -9.6 ppm-$\text{Å}^2$/e. Modern quantum mechanical methods can now be used to estimate the derivative of the proton shielding with respect to an external electric field. These calculations (see Table 4.3 for some examples) suggest larger values of $A$, close to 20 ppm-$\text{Å}^2$/e.[52, 53] Empirical estimates from fluorine-substituted hydrocarbons suggest a value of 18 ppm-$\text{Å}^2$/e.[54] A value of 14.9 $\pm$ 1.2 was obtained for the C-H bond in methane by performing density functional shift calculations on a methane probe near small ring molecules.[47] For protons in methane, $B$ has been estimated at -0.3 to -0.4 x10$^{-18}$ e.s.u.$^{-2}$ via shielding hyperpolarizability quantum chemistry.
4 Empirical approaches to biomolecular chemical shifts

\[
\sigma_{ab;c} = \left( \frac{\partial \sigma_{ab}}{\partial E_c} \right)
\]

(4.24)

where \(a,b,c\) can be any of \(x,y,z\). Hence, if the \(z\) direction is along the X-H bond, the conventional \(A\) coefficient gives the response of the isotropic shift to a field in the \(z\) direction:

\[
A_z = \frac{1}{3}(\sigma_{xx,z} + \sigma_{yy,z} + \sigma_{zz,z})
\]

(4.25)

For hydrogen and fluorine, which are involved in only one bond to a neighbor, we can expect the shift polarizabilities to display several qualitative features. First, since moving charge along the bond direction (i.e. changing the bond polarity) is easier than moving the electrons in a perpendicular direction, fields oriented along \(z\) should have more influence on shifts than those along \(x\) or \(y\); this should be especially true for hydrogen, where only the 1s orbital is occupied in any simple bonding picture. Second, fields along \(z\) should have the greatest effect on shielding components perpendicular to the bond: for \(\sigma_{zz}\), the numerator in the integral in Eq. 4.4 is \((x^2 + y^2)\), which should be insensitive to motions along \(z\), whereas the numerator for \(\sigma_{xx}\) would be \((y^2 + z^2)\), which will respond to charge motion along \(z\). Third, the polarizabilities should be negative, since (in our convention) a positive field along \(z\) will push electrons away from H and toward the atom it is bonded to, thereby deshielding the proton.

These qualitative expectations are borne out by quantum chemistry results, some of which are collected in Table 4.3. As expected, \(\sigma_{xx,z}\) is negative and about twice as big as \(\sigma_{zz,z}\), in accord with the simple arguments outlined above. The shielding polarizabilities for protons are only modestly dependent upon the hybridization of the carbon atom attached to it, and are even reasonably constant on going from a C-H bond to the N-H bond in N-methyl-acetamide. These general trends are also true for fluorine, but the response to external fields is much larger. This suggests that analysis of fluorine shifts in (chemically-modified) proteins may provide a useful probe of local electric fields at various positions.

An illustration of the effect of fields on shielding tensors is given in Fig. 4.5, which shows the shielding of the amide proton in N-methyl-acetamide as a water molecule approaches along the N-H...
bond to form a hydrogen bond with the amide group. As with argon, there is a direct effect on the proton shielding tensor arising from the susceptibility of the water, which is nearly isotropic and a little smaller than that of the argon example we considered above, as shown in Table insert table and cross-reference here! The shieldings parallel and perpendicular to the N-H bond computed from Eq. 4.8 are shown in the light solid lines. Unlike argon, the neighboring water molecule produces an electric field at the peptide group. The heavy solid lines are obtained by adding in electrostatic terms, using the shielding polarizabilities given in Table 4.3 and electric field at the amide proton arising from the water molecule. Both parallel and perpendicular components are deshielded by the electrostatic interaction, with the perpendicular components changing more than the parallel components, as indicated above. As in Fig. 4.1, the filled squares give results of density functional calculations for this system. Again, the empirical model fits the quantum chemistry results fairly well, expect at short distances where there is more deshielding in the quantum results than in our empirical models.

Electric field effects on $^{13}$C or $^{15}$N are undoubtedly more complex to unravel, not least since these atoms are involved in more than one bond, so that a model of bond polarization is more difficult to apply. Furthermore, multi-configuration SCF results indicate that correlation effects can sometimes

Figure 4.5: As in Fig. 4.1, but for water in place of the rare gas. The water molecule is in the plane of the NMA, with oxygen pointing toward the amide proton. Solid lines as in Fig 4.1; dashed lines include the electric field effect using polarizabilities given in Table 4.3; fields at the amide proton position due to water were computed from the TIP3P molecular mechanics charge model.
be quite important for $^{13}$C, changing the shielding polarizabilities in methane, for example by nearly a factor of two, compared to Hartree-Fock results.\cite{53} This is in contrast to proton results, which show only small effects of correlation, and may make it more difficult to calibrate empirical theories against quantum calculations for these heavier nuclei. Hartree-Fock SCF shielding polarizabilities have been reported for the N, C and H atoms in $N$-methylacetamide hydrogen bonded to either water (at the N–H bond) or formamide (at the C=O position).\cite{57} Fig. 4.6 shows the in-plane contributions to the $A$ values for these three nuclei, in ppm-$\text{Å}^2/e$; because the model molecule is planar, the out-of-plane values vanish by symmetry. These $A$ values, when multiplied by the local electric field in that direction, give the polarization contribution to the isotropic shielding. The value for $A$ for H along the N–H bond has been discussed above. A field perpendicular to the peptide plane has no first-order effect, and one perpendicular to the N–H bond, but in the peptide plane has an effect about one-fifth as big as a field along the bond direction. The results are all in qualitative accord with the notion that moving electrons back and forth along the bond direction has the greatest influence on the isotropic shielding at the proton.

As would be expected, shielding polarizabilities for C and N (as for F, discussed above) are much larger than for protons. Fields along the C–N bond are particularly important for nitrogen, whereas the polarizabilities are nearly equal for fields along the C=O and perpendicular to it in the plane of the molecule. If electric fields (or changes in electric fields) can be estimated from a structural model, then the predicted contributions to the isotropic shieldings are just $A \cdot E$. As an example, the shift changes in lysozyme upon ionization of residue Glu35 were estimated by computing the change in electric field at various backbone nuclei, due changing the carboxylate charge at this side chain. When a simple uniform dielectric model (with $\varepsilon = 3.5$) was used to estimate the fields, the resulting shifts were in reasonable agreement, in terms of both sign and magnitude, with those that were observed.\cite{57} Although systematic studies using this idea have not yet been carried out, this may be a promising way to connect shifts to electrostatics.

A bond polarization model\cite{49} appears to give a good account of many features of $^{13}$C tensors in a variety of environments. should expand on this here. The systems considered included sugars, amino
acids and some aromatic rings, and the derived empirical parameters reproduced observed tensor components with a mean error of under 10 ppm.

4.4 Close contact interactions

A third general environmental effect, especially important in solvation shifts, is a deshielding that occurs when the electron cloud of a non-bonded partner overlaps that of the probe nucleus. These close-contact de-shielding effects are thought to arise primarily from exchange-repulsion effects that result from the electron reorganization required when the electron clouds of neighboring atoms begin to overlap. It can be most clearly seen for rare gases (which have no polarity or susceptibility anisotropy), but is also an important aspect of the deshielding arising from hydrogen bond interactions, where contact distances can be quite short. It is a general rule that most nuclei are increasingly de-shielded as hydrogen bonds are formed, and these dependence can be a valuable indicator of both geometric and electronic structures. Because hydrogen bonds are key elements of non-covalent structure for both proteins and nucleic acids, we discuss in some detail in Section 4.5 general principles of electronic structures in hydrogen bonds. In this chapter we adopt a more empirical approach, surveying some of the observed and expected chemical shift patterns for H-bonds commonly found in macromolecules.

The study of close contact contributions to proton chemical shifts has a long and somewhat confusing history. At close proximity, London forces due to correlations of fluctuating dipoles can induce a buildup of electron density between molecules. The resultant loss of electron density near the nuclei is expected to decrease chemical shielding, by an amount that would be proportional to the mean square of the electric field, as in Eq. 4.23.[56, 58] In a simple Drude model for atoms, this would yield a "close contact" shift:

$$\delta_{cc} = B_{cc} \langle E^2 \rangle = B_{cc} \frac{3}{2} \frac{U_1 U_2}{U_1 + U_2} \frac{\alpha_2}{r^6}$$

(4.26)

Here $\langle E^2 \rangle$ is the average square fluctuating field at atom 1 induced by atom 2, $U_i$ is the ionization energy of atom 1, $\alpha_2$ is the polarizability of atom 2, $r$ is the distance between the atoms, and $B_{cc}$ is analogous to the parameter in Eq. 4.23.

Experimental measurements of changes in chemical shifts of nonpolar molecules as a function of density, temperature and solvent support the existence of a close contact or van der Waals contribution to chemical (de)shielding, that can be modeled with a $r^{-6}$ dependence.[59, 60] Although this has the same distance dependence as Eq. 4.26, two arguments suggest that dispersion effects are not the dominant interaction. First, Hartree-Fock or DFT calculations on rare gas dimers (and other non-polar interactions) show a deshielding in rough accord with observation,[30, 58] even though dispersion energetics are not correctly modeled at this level of theory. Second, mean square fields much larger than those arising from Eq. 4.26 would be required to explain the observed (and quantum-mechanically calculated) close-contact shifts.[54, 56, 60] It is more likely that close-contact de-shielding effects arise from exchange-repulsion effects that arise from the electron reorganization required when the electron clouds of neighboring atoms begin to overlap. This can have a steep distance dependence that may be difficult to distinguish from the $r^{-6}$ behavior predicted for dispersion.
An empirical value of $B$ in Eq. 4.26 that provides a good fit to isotropic shifts computed for rare gas interactions with methane and NMA, and for a variety of small peptide models is $2.9 \times 10^{-18}$ e.s.u.$^{-2}$ for interactions with oxygen and nitrogen, and $4.3 \times 10^{-18}$ e.s.u.$^{-2}$ for interactions with carbons.[26]

Realistic models for interactions in biomolecules involve a combination of susceptibility, bond polarization and close contact interactions. Figure 4.7 illustrates the application of all three terms to the amide proton shielding in an NMA dimer making a linear $C=O\ldots H-N$ hydrogen bond of given length. The lines show the prediction using Eqs. 4.8, 4.24 and 4.26, using parameters from Tables ? and ? and the $B_{cc}$ value given above, applied to the perpendicular tensor components. Circles show results from DFT calculations. Similar models work for a variety of model peptides.[26] Of course, reproducing quantum results is certainly easier than understanding all of the effects active in macromolecules, but we believe that this is a good first step toward such understanding.
Figure 4.8: Natural bond orbitals for the water dimer. The figure is drawn in the plane of a water molecule on the left, where the hydrogen and oxygen positions are marked by a cross, and perpendicular to the water molecule on the right, so that only its oxygen atom (also marked by a cross) is in the plane of the figure. An occupied “lone-pair” orbital is shown in both the top and bottom figure for the water molecule on the right. At the left are shown an occupied O–H bond orbital (bottom) and an unoccupied O–H antibond orbital (top).
4 Empirical approaches to biomolecular chemical shifts

4.5 Hydrogen bond effects

One useful definition of a hydrogen bond is: “a non-bonded interaction (involving hydrogen) in which interatomic distances are significantly less than the sum of their van der Waals radii.” This distinguishes these interactions from other non-bonded interactions, which are typically limited to longer distances. Since the van der Waals radius of hydrogen, nitrogen and oxygen are about 1.2, 1.55 and 1.5 Å, respectively, an N–H...O interaction in which the heavy atom (N–O) distance is less than about 3.2 Å, or an H...O distance of less than about 2.2 Å, would qualify. Identifying hydrogen bonds in this way naturally leads to the question of what sorts of (electronic structure) interactions are taking place that allow such short contacts, which would ordinarily be energetically quite unfavorable.

Quantum chemical calculations can provide accurate predictions of hydrogen bond behavior, although one must generally go beyond Hartree-Fock or density functional theory to obtain reliable energies. But these results by themselves do not necessarily provide any additional insight into which aspects of the molecular electronic structure are responsible for the hydrogen bond interaction. It has always been a goal of quantum chemistry calculations to provide not only numbers that can be compared to experiment (or which can be used where experimental data is not available), but also to provide an interpretive framework for understanding and correlating results. Such an interpretation should apply to NMR parameters as well as to energies.

4.5.1 Natural bond orbitals and hydrogen bonds

All schemes that decompose energies or NMR parameters into atomic or bond contributions, or which describe things in molecular orbital language, have a certain amount of arbitrariness in how the decomposition is carried out. Furthermore, in many cases a reasonable inventory of contributions yields a large of number of contributors (often with opposite signs!), which are of little aid to chemical intuition. Hence utility and transferability become hallmarks of a useful analysis: one must be able to abstract out a small number of key interactions that can be qualitatively transferred to different environments.

The scheme we use most here is called “natural bond orbital (NBO) analysis”, and derives primarily from the work of Frank Weinhold and his co-workers.[61–63] It is not feasible to give a full account of this model here, but the basic application to hydrogen bonds can be illustrated with reference to Fig. 4.8, which uses a water dimer as an example. The analysis begins by decomposing the complete wavefunction into localized orbitals that approximately represent the bonds and lone-pairs of a classical Lewis electron-pair (valence-bond) description. In addition to these sorts of orbitals, which will be (nearly) fully-occupied, there will be additional orbitals, with little or no occupation in the electronic ground state, that represent “higher-energy” potential configurations. The most important of these unoccupied orbitals are “anti-bonds”, which are paired with the traditional two-electron bonding orbitals, but which have nodes in between the two nuclei involved in the bonds.

Contour plots of three of these sorts of (non-orthogonal) localized orbitals are shown in Fig. 4.8. On the right are occupied lone-pair orbitals on an oxygen atom, and on the left are shown O–H bonding and antibonding orbitals, which are occupied and unoccupied, respectively. The overlap shown in the bottom figure, between two occupied orbitals, is unfavorable energetically. This repulsion arises in part because the negative charges of the two electron clouds repel each other, but even more so from the Pauli exchange principle, which requires the overall wavefunction to be antisymmetric upon
exchange of the position of two electrons. Imposing this antisymmetry requirement leads to an
orthogonalization of these orbitals, which increases the kinetic energy (and hence the total energy) of
the electrons. This Pauli exchange repulsion is the primary interaction that keeps non-bonded atoms
apart from each other.

In order to overcome the Pauli exchange repulsion, and hence to create a hydrogen bond with non-
bonded contacts that are shorter than typical van der Waals interactions, some additional, favorable
interactions must also be present. The most important of these (in the NBO analysis scheme) is illus-
trated at the top of Fig. 4.8. Here we have an interaction between an unoccupied antibonding, or $\sigma^*$
orbital (on the left) with the occupied lone-pair orbital (on the right). The energy of the system can
be lowered by a rearrangement of the electron density in a way that corresponds to a partial charge
transfer from the lone-pair orbital into the O–H $\sigma^*$ orbital. This is a very common sort of interaction,
known as hyperconjugation, and its efficiency is roughly proportional to the overlap between the two
orbitals involved. Many features of the torsional preferences of molecules can be explained qualita-
tively by an analysis of the way in which these sorts of overalps vary with conformation. It is worth
noting the the actual amount of charge transfer is quite small, (on the order of 0.01 to 0.02 electron
for this example,) but the energetic consequences can be significant.

When this sort of analysis is applied to the water dimer hydrogen bond, the details depend upon
what sort of wavefunction is being analyzed and what basis set is being used.[61] Nevertheless, the
basic ideas are fairly robust. For the water dimer at its equilibrium geometry, the exchange-repulsion
interaction is about +4.0 kcal/mol, whereas the charge-transfer stabilization contributes about −9.6
cal/mol, so that the net hydrogen bond enthalpy is about −5.6 kcal/mol. The exact numbers are not
of great importance here, and this analysis ignores other small contributions (which tend to nearly
cancel in this case); but the basic identification of the important interactions is one that will be useful
in a variety of situations.
4 Empirical approaches to biomolecular chemical shifts

4.5.2 Hydrogen-bond effects on chemical shifts

Section not yet written, but see Fig. 4.9.

4.6 Paramagnetic chemical shifts

Unpaired electrons, especially at transition metal sites, often make dominant contributions to magnetic susceptibilities. The contribution of this susceptibility to isotropic chemical shifts is usually called the pseudo-contact term, and it can lead to large shifts if the unpaired electron distribution is especially anisotropic. This effect has been extensively studied in a variety of metalloproteins, and structure refinement methods have been developed to fit calculated and observed pseudocontact shifts. Expand here on pseudocontact shifts.

Paramagnetic contributions to CSA should be even larger, since they will reflect the complete susceptibility, rather than just its anisotropy. For example, if there is only one thermally populated multiplet with spin $S$, the susceptibility is related to the $g$-tensor:[64]

$$\chi_{ii} = \frac{g_i^2 \beta_e^2 S(S+1)}{3kT} \quad (4.27)$$

where $\beta_e$ is the Bohr magneton. The mean value of the $g$-tensor is around 2 for $S = 1/2$ systems, whereas anisotropies are usually an order of magnitude smaller. For example, for $g=2$, $S=1/2$ and $T=300$, Eq. 4.27 gives a susceptibility of about 920 ppm-Å$^3$, a value about 5 times bigger than the susceptibility of benzene. This suggests that CSA measurements may see the effect of paramagnetic centers even at quite large distances, providing both distance and angular information with respect to the metal ion.

Because of the long-range effects of pseudo-contact shifts, precise information about atomic positions, global shape and domain orientation can be derived by including this information in the refinement process. The use of pseudocontact shifts in this regard is no different than the use of ring-current contributions; indeed, it is sometimes easier, since there is often only a single metal ion as a paramagnetic center, whereas there are often multiple rings that contribute to chemical shift dispersion. Paramagnetic shift refinement need not be limited to pseudo-contact shifts; a refinement strategy based on quantum mechanically calculated Fermi contact shifts can also be useful, but these analyses are almost always system-specific, so that general rules are not available. (Explain why) Given that 5 to 10% of all proteins either have or could accommodate a paramagnetic centre in a way that hardly distorts the structure, and than lanthanide spin-labels can be placed in many more locations, it is clear that paramagnetic shift refinement will play an increasingly important role in many future structural studies.
5 Some sample applications

5.1 “Random-coil” shifts for peptides and nucleic acids

One feature that we have not discussed very much so far is the local covalent chemical environment, which can often have a significant effect on the location resonances. Traditionally, this contribution has been assumed to be constant for a given residue type, and attempts have been made to extract empirical values from studies of short oligopeptides or oligonucleotides, under the assumption that these average over conformational space in a broad way, so that long-range, non-covalent contributions cancel. This is often a problematic assumption, as we discuss below. (An alternative approach would use quantum chemical calculations to estimate these local contributions for various residue types. To date, the empirical approach, using measured shifts from short peptides or oligonucleotides, has seemed to yield more useful results.)

Random coil shifts are defined as the characteristic chemical shifts of amino acid residues or nucleic acid bases in short, disordered polymers. They are often used in spin assignment and residue identification, but they can be equally useful in determining the so-called secondary chemical shift ($\Delta\delta$), which is the difference between the observed shift and the corresponding random coil value. Secondary chemical shifts primarily contain non-covalent structural or dynamic information, as opposed to simple covalent information. They can be valuable in identifying secondary structure, determining ring pucker, delineating flexible regions, locating hydrogen bonds, setting dihedral restraints and detecting aromatic stacking interactions.

For polypeptides, a considerable body of work on random coil shifts exists. A set of IUPAC referenced amino and phosphoamino acid backbone (including $^{13}$C$\beta$ and $^1$H$\beta$) random coil chemical shifts is listed in Table 5.1. These are primarily extracted from experiments on GGXGG peptides, which are probably reasonably flexible, although there is little direct evidence about their conformational preferences. In principle, such preferences could be estimated from force fields or from quantum calculations, and the resulting averaging of the long-range contributions to shifts could be calculated; in this case, these arise primarily from anisotropic susceptibility of the peptide groups. Since glycine and proline have unique backbone angle preferences, the measured “random-coil” values for these may reflect an amount of long-range contribution that is different from the other 18 amino acids.[65] But these sorts of estimates are sufficiently tentative that most uses of random-coil shifts treat all residues identically.

Defining random coil shifts for nucleic acids is a more difficult problem than for amino acids, primarily because shifts in polynucleotides (particularly $^1$H shifts) are much more sensitive to sequence effects than are amino acids in polypeptides. The use of chemical shifts from isolated nucleic acids or nucleic acid analogs instead of polynucleotides is also problematic because complications arising from intermolecular interactions make these measurements somewhat questionable.[66] Nevertheless, as has been previously shown with amino acids,[67] it is possible to generate a reasonably good
### Table 5.1: "Random coil" chemical shifts (in ppm) for the 20 common amino (and phosphoamino) acids measured at 25 °C, pH 5. Prefixes: r=reduced, o=oxidized, p=phosphorylated, c=cis. Data is taken from Ref. [5].

<table>
<thead>
<tr>
<th>AA</th>
<th>$^1$HN</th>
<th>$^{15}$N</th>
<th>$^1$H$\alpha$</th>
<th>$^{13}$C$\alpha$</th>
<th>$^1$H$\beta$</th>
<th>$^{13}$C$\beta$</th>
<th>$^{13}$CO</th>
</tr>
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<tr>
<td>Ala</td>
<td>8.24</td>
<td>123.8</td>
<td>4.32</td>
<td>52.5</td>
<td>1.39</td>
<td>19.1</td>
<td>177.8</td>
</tr>
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<td>rCys</td>
<td>8.32</td>
<td>118.8</td>
<td>4.55</td>
<td>58.2</td>
<td>2.93/2.93</td>
<td>28.0</td>
<td>174.6</td>
</tr>
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<td>oCys</td>
<td>8.43</td>
<td>118.6</td>
<td>4.71</td>
<td>55.4</td>
<td>3.25/2.99</td>
<td>41.1</td>
<td>174.6</td>
</tr>
<tr>
<td>Asp</td>
<td>8.34</td>
<td>120.4</td>
<td>4.64</td>
<td>54.2</td>
<td>2.72/2.65</td>
<td>41.1</td>
<td>176.3</td>
</tr>
<tr>
<td>Glu</td>
<td>8.42</td>
<td>120.2</td>
<td>4.35</td>
<td>56.6</td>
<td>2.06/1.96</td>
<td>29.9</td>
<td>176.6</td>
</tr>
<tr>
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<td>120.3</td>
<td>4.62</td>
<td>57.7</td>
<td>3.14/3.04</td>
<td>39.6</td>
<td>175.8</td>
</tr>
<tr>
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<td>108.8</td>
<td>3.96</td>
<td>45.1</td>
<td>——</td>
<td>——</td>
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<td>——</td>
<td>——</td>
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<tr>
<td>His</td>
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<td>——</td>
<td>56.3</td>
<td>30.8</td>
<td>38.8</td>
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<td>1.87</td>
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<td>4.32</td>
<td>56.2</td>
<td>1.84/1.75</td>
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<td>4.34</td>
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<td>42.4</td>
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<td>——</td>
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<td>119.8</td>
<td>4.34</td>
<td>55.7</td>
<td>2.12/1.99</td>
<td>29.4</td>
<td>176.0</td>
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<td>120.5</td>
<td>4.34</td>
<td>56.0</td>
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<td>30.9</td>
<td>176.3</td>
</tr>
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<td>Ser</td>
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<td>58.3</td>
<td>3.89/3.87</td>
<td>63.8</td>
<td>174.6</td>
</tr>
<tr>
<td>pSer</td>
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<td>115.5</td>
<td>4.60</td>
<td>57.4</td>
<td>4.22/4.13</td>
<td>66.8</td>
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<td>Thr</td>
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<td>Val</td>
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<td>8.25</td>
<td>121.3</td>
<td>4.66</td>
<td>57.5</td>
<td>3.29/3.27</td>
<td>29.6</td>
<td>176.1</td>
</tr>
<tr>
<td>Tyr</td>
<td>8.12</td>
<td>120.3</td>
<td>4.55</td>
<td>57.9</td>
<td>3.03/2.98</td>
<td>37.8</td>
<td>175.9</td>
</tr>
<tr>
<td>pTyr</td>
<td>8.21</td>
<td>120.1</td>
<td>4.61</td>
<td>57.0</td>
<td>3.15/3.02</td>
<td>38.7</td>
<td>176.7</td>
</tr>
</tbody>
</table>

5 Some sample applications
set of random coil nucleic acid chemical shifts by taking the average values for each $^1H$ type from previously assigned polynucleotides with known 3D structures.[68] Recently, it has been suggested that these values should be slightly modified so that sequence dependent effects and variations in sugar pucker could be more appropriately accommodated.[69] A proposed set of $^1H$, $^{13}C$ and $^{15}N$ random coil shifts for RNA and DNA are given in Table 5.2. update this with more recent/extensive values!!

### Table 5.2: “Random coil” chemical shifts (in ppm) relative to DSS for nucleic acid bases at 25$^\circ$ C.

<table>
<thead>
<tr>
<th>Position</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^1H$</td>
<td>$^{15}N$</td>
</tr>
<tr>
<td>A/C/G/T (sugar) 2’</td>
<td>2.24</td>
<td>40.5</td>
</tr>
<tr>
<td>A/C/G/T (sugar) 2”</td>
<td>2.17</td>
<td></td>
</tr>
<tr>
<td>A/C/G/T (sugar) 3’</td>
<td>4.36</td>
<td>78.4</td>
</tr>
<tr>
<td>A/C/G/T (sugar) 4’</td>
<td>4.00</td>
<td>86.7</td>
</tr>
<tr>
<td>A/C/G/T (sugar) 5’</td>
<td>3.72</td>
<td>67.6</td>
</tr>
<tr>
<td>A/C/G/T (sugar) 5”</td>
<td>3.60</td>
<td></td>
</tr>
<tr>
<td>A (sugar) 1’</td>
<td>5.23</td>
<td>85.1</td>
</tr>
<tr>
<td>A (base) 2</td>
<td>8.68</td>
<td>154.3</td>
</tr>
<tr>
<td>A (base) 8</td>
<td>8.60</td>
<td>141.2</td>
</tr>
<tr>
<td>A (base) 6</td>
<td>80.5</td>
<td></td>
</tr>
<tr>
<td>C (sugar) 1’</td>
<td>5.48</td>
<td>85.1</td>
</tr>
<tr>
<td>C (base) 4</td>
<td>97.8</td>
<td>158.7</td>
</tr>
<tr>
<td>C (base) 5</td>
<td>6.20</td>
<td>98.2</td>
</tr>
<tr>
<td>C (base) 6</td>
<td>7.80</td>
<td>142.2</td>
</tr>
<tr>
<td>G (sugar) 1’</td>
<td>5.25</td>
<td>85.1</td>
</tr>
<tr>
<td>G (base) 1</td>
<td>146.3</td>
<td></td>
</tr>
<tr>
<td>G (base) 2</td>
<td>73.8</td>
<td></td>
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<tr>
<td>G (base) 8</td>
<td>7.69</td>
<td>137.9</td>
</tr>
<tr>
<td>T (sugar) 1’</td>
<td>5.80</td>
<td>85.1</td>
</tr>
<tr>
<td>T (base) 3</td>
<td>158.8</td>
<td></td>
</tr>
<tr>
<td>T (base) 6</td>
<td>8.68</td>
<td>138.9</td>
</tr>
<tr>
<td>T (base) 7</td>
<td>8.60</td>
<td>14.4</td>
</tr>
<tr>
<td>U (sugar) 1’</td>
<td>5.60</td>
<td></td>
</tr>
<tr>
<td>U (base) 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U (base) 5</td>
<td>5.95</td>
<td></td>
</tr>
<tr>
<td>U (base) 6</td>
<td>7.85</td>
<td></td>
</tr>
</tbody>
</table>

5.2 Protein secondary structure identification

Perhaps the most widespread application of chemical shifts in protein structure generation has been in the area of secondary structure identification. See Ref. [5] for a more complete discussion.

As a general rule, $^{13}C\alpha$ shifts experience a downfield shift of about 2.5 ppm in helices and an
5 Some sample applications

upfield shift of 2.0 ppm in beta sheets. As with the Hα shifts, the origin of these trends is unclear. 13CO shifts are affected in a similar way with downfield shifts being characteristic of helices and upfield shifts being typical of beta sheets. In contrast, 13Cβ shifts are shifted downfield by about 2.5 ppm in beta sheets, but assume a near random coil value in helices. 13Cβ shifts are only strongly upfield shifted when a residue has a positive ϕ angle, making the 13Cβ shift a particularly good indicator of this unusual conformation.[70] Side chain orientation, particularly for beta-branched (Ile, Val, Thr) and aromatic (Phe, Trp, Tyr, His) amino acids, also likely plays a role in determining 13C shifts.[71]

The most popular approach has been the heteronuclear chemical shift index, or CSI plots.[72] (expand here on how these work) The heteronuclear CSI method, which makes simultaneous use of 1Hα, 13Cα, 13Cβ and 13CO chemical shift information, is quite accurate, with 93% agreement between X-ray secondary structure designations. Homonuclear methods (1Hα alone, 13Cα alone, etc.) typically have a lower level of agreement (80-85%). In general, the CSI results agree better with X-ray assignments for helices than beta-sheets, but this may be largely due to varying definitions of what constitutes a sheet. It has been argued that secondary structures derived from heteronuclear CSI methods are likely to be just as correct as those extracted from crystal structures.[72, 73]

5.3 Chemical shifts and tertiary structure refinement

5.3.1 Diamagnetic proteins

Given their sensitivity to backbone torsions, it is not surprising that chemical shifts are particularly useful in setting dihedral angle restraints. It now appears that if multiple backbone (1H, 13C and 15N) chemical shifts are appropriately combined, backbone dihedral angle constraints can be determined with surprisingly good precision. For example, Bax and co-workers have demonstrated very impressive results using the TALOS program. [74] This innovative approach to dihedral angle prediction is based on the simple observation that similar amino acid sequences with similar chemical shifts have similar backbone dihedral angles. In particular, TALOS breaks a query sequence into overlapping amino acid triplets. Each triplet and its corresponding heteronuclear chemical shifts (1Hα, 13Cα, 13CO, 13Cβ and 15N) is compared to a database of known sequences, shifts and dihedral angles. The dihedral angles from the closest sets of database triplets are used to "predict" the dihedral angle for the central residue of the query amino acid triplet. TALOS is able to predict about 70% of protein backbone dihedral angles to within 30o (summed difference of Δϕ + Δψ). A particular strength of the TALOS approach is that it accounts for nearest neighbor effects in a way that cannot easily be done with other approaches. Furthermore, as the database of known chemical shifts and dihedral angles grows, the accuracy of TALOS' predictions is expected to progressively grow as well.

"Direct" refinements against NMR data involve target functions that depend upon the difference between observed and calculated parameters, rather than using the more indirect information that is present in distance or dihedral angle restraints. Chemical shift refinement only became a possibility for peptides and proteins after reasonably accurate semi-empirical theories and sufficiently fast computer programs became widely available. Chemical shift refinement options are now available in Amber, XPLOR and CNS, with XPLOR/CNS offering both 1H shifts and 13C shift refinement. In general, 1H chemical shift refinement of non-exchangeable protons has been shown to improve the
quality of most structures and increase the definition of previously ill-defined segments. Typically the most significant improvements are seen in regions where there is close spatial proximity to aromatic groups. Despite the potential improvements available through 1H chemical shift refinement, this approach appears not to be widely used in the NMR community. As a simple example, Fig. 5.1 shows the degree to which methyl shifts in proteins can be predicted from crystal structures. Inverting this relation through the use of penalty functions based on ring current theories is expected (and observed) to improve local structures.

### 5.3.2 Paramagnetic proteins

Perhaps the most impressive application of direct chemical shift refinement can be found in recent work done on paramagnetic proteins.80,83,120 Electro-nuclear interactions involving paramagnetic metals such as iron, cobalt and various lanthanides can, under the right conditions, give rise to substantial (>10 ppm) chemical shifts.121 These shifts can be divided into two forms, a contact and a pseudo-contact form. The contact or Fermi contact shift is a scalar or through-bond phenomenon that fades rapidly after four or five bonds. The pseudo-contact shift is a dipolar or through-space phenomenon having a 1/r³ dependence that can extend up to 20 Å away from the paramagnetic centre.

In practical terms, paramagnetic shift refinement requires at least twice as much work as diamagnetic shift refinement, since it requires spectra for two forms of the protein, one with a diamagnetic metal and the other with a suitable paramagnetic metal. The observed chemical shift differences between the diamagnetic and paramagnetic form are assumed to arise entirely from the pseudo-contact shifts. Obviously if structural or solution conditions change, this is not a valid assumption. Care must be taken to reproduce the exact solution conditions of the first measurements and to ensure that one is looking at either a 100% diamagnetic or 100% paramagnetic form. It is possible to refine the g tensor.
in later steps or to alternately refine the g tensor and the protein coordinates during the minimization process. Computer programs and protocols to orient the g tensor have been written and are available (FANTASIAN, PSEUDOREM). AMBER and XPLOR have also been successfully modified to explicitly perform pseudo-contact 1H shift refinement.

Because of the long-range effects of pseudo-contact shifts, precise information about atomic positions, global shape and domain orientation can be derived by including this information in the refinement process. Indeed, in some cases it is possible to determine atomic positions with a precision of less than 0.1 Å. Being able to position 1H atoms with this kind of precision also aids in the assignment process. In particular, stereospecific 1H assignments can often be made because the shift differences between different diastereomers can be so precisely determined. In addition, this technique can provide independent evidence about the location of the paramagnetic metal atom. Paramagnetic shift refinement need not be limited to pseudo-contact shifts; a refinement strategy based on quantum mechanically calculated Fermi contact shifts has also been shown to yield detailed results about the geometry of the metal binding centre in rubredoxin (Wilkens et al., 1998). Given that between 5 and 10% of all proteins either have or could accommodate a paramagnetic centre, it is clear that paramagnetic shift refinement will play an increasingly important role in many future structural studies.

5.3.3 Nucleic acids

The use of chemical shifts in nucleic acid structure determination is much less advanced than for proteins. In large measure, this is due to concern that crystal structures for small nucleic acids may be more influenced by crystal packing forces than is the case for proteins. This means that the empirical methods used for proteins (which rely upon crystal structures during the parameter development stage) are less useful for nucleic acids. This situation may change as more reliable NMR structures become available, and as a larger database of assigned structures is used, which should allow errors arising from local crystal packing effects to be averaged out.

Two groups have considered the ability of ring-current, susceptibility and electrostatic models to predict proton shifts in nucleic acids.[68, 69] If the reference shift for each type of proton is taken as the average value found in a database of molecules for which X-ray or NMR structures are available, the root-mean-square error of the predictions is 0.17 ppm. Assigning reference shifts from fragments (as discussed above) increases the overall error to about 0.25, but does allow discrimination between protons at different chemical positions. The general trend that sugar protons in duplex DNA and RNA are downfield from their "random-coil" positions, and that base protons are upfield of their positions in isolated bases, are readily explained by ring-current effects. Base protons are generally more reliably predicted than are sugar protons. Nevertheless, the overall correlation of predicted to experimental results is significantly worse than for proteins. This may reflect inadequacies in the structures that were used, or it may reflect limitations of the computational models, particularly, in their treatment of the effects of the charges on the sugar-phosphate backbone.
6 Chemical shift anisotropies

6.1 Shielding anisotropies in proteins

6.1.1 The peptide group

A number of general features of CSA tensors in the peptide group are known from solid-state studies, and from liquid-state cross-correlation experiments. The general magnitudes and orientations of the tensors are determined by the local bonding environment, and are illustrated in Fig. 6.1. For N and H, the least shielded and most shielded components, respectively, lie roughly along the N–H bond, and for C, the middle tensor component is roughly along the C=O bond. Calculated values for N-methylacetamide hydrogen bonded to two water molecules are given in Table 6.1. These could be considered typical values, since peptide groups in proteins generally have some hydrogen-bonded partner; it should be clear from the discussion above that deviations from these general values should be expected, depending upon the environment. Analyses of dipole-CSA cross-correlation experiments in proteins suggests that the anisotropy of the N shielding tensor may be 10-15 ppm greater than shown in Table 6.1. This may be due to remaining deficiencies in the quantum calculations, or to considerable sensitivity of $^{15}$N shifts to environmental effects that are not considered here.

Table 6.1: Shielding tensors for NMA-(H$_2$O)$_2$

<table>
<thead>
<tr>
<th></th>
<th>$\sigma_{iso}$</th>
<th>$\sigma_{11}$</th>
<th>$\sigma_{22}$</th>
<th>$\sigma_{33}$</th>
<th>$\theta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>-65.1</td>
<td>11.5</td>
<td>88.2</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>-313.8</td>
<td>-156.2</td>
<td>327.5</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>128.7</td>
<td>27.1</td>
<td>155.2</td>
<td>203.9</td>
<td>18.7</td>
</tr>
<tr>
<td>H</td>
<td>14.1</td>
<td>22.4</td>
<td>33.9</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

A key question of interest is the extent to which these values respond to changes in hydrogen bonding to either the carbonyl or the N–H part of the peptide group. Hydrogen bonding effects on isotropic shieldings were discussed in Chap. ?, but much less is known about anisotropies. A hydrogen bond interaction typically involves all three of the environmental terms discussed above. Figure 4.7 shows the response of the H, N and C nuclei to the formation of a hydrogen bond between two NMA molecules. The deshielding effects discussed above are evident at short distances in the isotropic shieldings, although the shift for the carbonyl carbon is fairly insensitive to this change. Also plotted are the differences in shielding along and perpendicular to the N–H or C=O bonds. Here the N anisotropy changes only a little (except for distances below 2.0 Å), whereas both the H and C anisotropies are very sensitive to nearby groups, even when they are beyond conventional hydrogen-bonding distances.

Of course, hydrogen-bonding interactions in real systems do not consist of just a single group
making purely linear hydrogen bonds, as modeled in Fig. 4.7. There is often more than one hydrogen-bonding partner, and the interactions are often non-linear. As an example of real data, Figure 4.7(c) shows results for the H anisotropy in ubiquitin,[76] plotted against the distance to the closest hydrogen bond partner in the crystal structure. The isotropic shielding shows a small but systematic trend that is in good agreement with these simple dimer calculations. As predicted from the qualitative arguments above, the dependence of the anisotropy is much larger, and extends well beyond the distance range where hydrogen bonds have energetic significance. The measured values are smaller than those seen in the simple dimer model, but the overall trend is clearly the same. Analogous measurements have been made on the HU protein from *Bacillus stearothermophilus*.[77] These anisotropies show generally similar behavior, but with a significant number of larger anisotropies (>15 ppm) that were not found in the ubiquitin study. Results from a larger number of proteins should help establish the expected structural correlations with amide proton anisotropies.

Spectra with a fairly straightforward interpretation arise from cross-correlated relaxation between CSA and dipolar terms. Such experiments can provide the projection of the shielding tensor onto the dipolar frame, which is the same as the internuclear vector. The observed relaxation rates depend both on molecular motion and on the shielding tensor, but combinations of experiments can be used to isolate the dependence on the latter quantity. Several groups have extracted CSA-related information for amide groups in proteins in liquids, both for $^{15}$N[78–81] and for $^1$H.[76, 77, 82] A key point that is not yet fully resolved is the extent to which the $^{15}$N CSA in amides varies from one residue to another. The origins of this variability (as with the isotropic $^{15}$N shift itself) have so far eluded generally applicable structural interpretations.[83] Experiments analogous to those described above for the amide N-H spin pair can also be used to probe CSA-dipolar cross-correlated relaxation along the Cα-Hα bond,[84] or along Cα-C’ or H-C’, where C’ is the carbonyl carbon.[85–87] Since the orientation of the carbonyl carbon shielding tensor is closely related to the direction of the C=O bond vector, it can provide information about the structure and dynamics of the peptide plane which can be difficult to obtain by other means.[88, 89]

Shielding anisotropies in the liquid state can also be monitored by following the change in observed shift upon moving to a partially aligned medium. The need to know both the exact structure in solution

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Figure 6.1: Schematic view of typical tensor components and directions for a peptide group. $\sigma_{11}$ is the least shielded, and $\sigma_{33}$ the most shielded principal component.
6.1 Shielding anisotropies in proteins

and the contribution of dynamical effects, limits the precision that can be obtained by this method. Nevertheless, this method has been applied to both proteins and nucleic acids.[90–92]

6.1.2 Cα and Hα anisotropies

It is natural to expect that information about the φ and ψ backbone angles in proteins would be easiest to discern at the Cα and Hα positions, since these atoms are in the middle of the peptide backbone and since they should not be directly involved in hydrogen bonding interactions. The shielding anisotropy at the Cα position is also strongly correlated with secondary structure, with a variation that is considerably larger than that seen for the isotropic shift.[84, 93, 94] Fig. _#CAfld shows DFT results for the anisotropy in the alanine dipeptide as a function of φ and ψ. The φ and ψ dependencies are nearly independent, i.e. the shape of curves plotted as a function of φ does not depend upon ψ, and vice-versa. Values in the β-sheet region (broadly around φ ψ = −120, 120) are around 30 ppm, whereas those in the α-helix region (around φ ψ = −60, −60) are near zero or even negative. This variation with secondary structure is about five times as great as that seen for the isotropic shift, and the discriminatory power is quite good. For example, residues Thr44 and Glu45 in the calmodulin/M13 system have nearly isotropic Cα shifts that differ by only 0.5 ppm, yet in the structure, Thr44 is in a sheet region with (φ ψ) = (-81, 160) and Glu45 is helical at (-46, -48); an index based on isotropic shifts would not be able to predict this difference in backbone conformation, but the observed anisotropies show a clear distinction, with Thr44 at 26.8 ppm and Glu45 at 4.7 ppm.

Fig. _#CAfld also shows computed results for the anisotropy at the Hα position. As with isotropic shifts at Hα, variations in the φ angle have the greater effect. There is a clear difference of about 5 ppm between values typical of the sheet region and those typical of helix. While this variation is much smaller than that seen for the Cα anisotropy, it is about an order of magnitude larger than the difference in isotropic shift observed between sheet and helical regions.[67] There are only a few experimental measurements of HαCSA values, but these tentatively indicate that typical DFT calculations may be overestimating the actual values.

In measuring 13Cα chemical shift anisotropy one uses an (HA)Ca(C)NH experiment to detect intensity differences in 13Cα−1Hα doublets cause by relaxation interference between 13Cα dipolar and CSA effects. The anisotropy is defined as the difference between the shielding parallel to the C-H bond and the shielding orthogonal to this bond (σpar − σorth). In helices, this anisotropy is typically between 0 and 10 ppm, with an average value of 6 ppm. In beta sheets it is between 20 and 33 ppm, with an average value of 27 ppm. There is essentially no overlap between helical and beta-sheet values. This suggests that 13C CSA values could be used to both unambiguously determine secondary structure, and to define backbone dihedral angles.[30, 84]

6.1.3 Nucleic acid sugars

but much less is know about anisotropy trends. Fig. 6.2 summarizes some recent computational results on a deoxythymidine model system.[27] Here the base is in the anti position, and the anisotropy of three of the sugar carbons is plotted against the pseudorotation phase. It is noteworthy that the C1’ and C3’ values clearly discriminate between N and S sugars (with P near 0° and 180°, respectively). The difference between these conformers is about 30 ppm, which is about 3-4 times larger than the changes seen in the isotropic shifts. These large variations suggest that CSA-based measurements
could provide an important complement to coupling constant analysis in helping to determine sugar pucker conformations in different environments.

the relative CSA (projected along the C-H bond direction) for C1’ and C3’ appears to offer a sensitive way to determine sugar puckers.[27, 95]

6.1.4 Nucleic acid bases

It is often of considerable interest to understand the structure and dynamics of aromatic rings in proteins and nucleic acids. $^{13}$C and $^{15}$N atoms bonded to a single proton can be attractive probes, since dipolar interactions are dominated by the attached proton. This can permit the measurement of order parameters analogous to those more commonly measured along the polypeptide backbone in proteins. Furthermore, since aromatic groups often interact strongly with other rings (especially in helical regions of nucleic acids), an analysis of ring-current contributions to CSA may be useful in structural analysis as well.

Tables 6 and 7 collect some experimental and calculated shielding tensor information for models of aromatic groups in proteins and nucleic acids. As in the peptide group, $^{15}$N tensors are expected to be sensitive to environmental effects, but the comparisons to solid-state values for tryptophan, histidine and uracil shown in Table 6 suggest that calculations can be a useful complement to experimental studies. The $^{15}$N anisotropies shown are generally smaller than those found in the peptide group, with histidine Nδ1 and guanine N1 values in turn larger than those found for tryptophan Ne1 or uracil N3 sites.

The predicted effects of base pairing can be significant, as illustrated by the comparison between

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6.1 Shielding anisotropies in proteins

N3 positions in thymine and in an A-T based pair, or between N1 in guanine and a G-C base pair. The net $^{15}$N anisotropy is not greatly affected, but the adjacent base (or other hydrogen bonding partners) tends to increase the separation between $\sigma_{22,N}$ and $\sigma_{33,N}$; large effects are also evident in the proton tensor. These calculations are illustrative only: most nucleic acid base-pairs will also have stacking interactions that should be expected to have a significant influence on shieldings. Nevertheless, the strong sensitivity to environment (e.g. $\sigma_{11,H}$ changes by 15-20 ppm on forming a base pair) supports continued efforts to explore the sorts of structural information present in these quantities.

The $^{13}$C results collected in Table 7 are probably less sensitive to hydrogen bonding effects than the $^{15}$N tensors in Table 6, although their counterparts in biomolecules should still reflect susceptibility contributions from any nearby rings. The orientations of these tensors is much like the $^{15}$N tensors discussed above, with $\sigma_{33,C}$ always perpendicular to the ring, and $\sigma_{11,C}$ approximately along the C-H bond. The comparison to experiment for benzene suggests the general level of accuracy that may be expected. There is a range of in-plane vs. out-of-plane anisotropies, and it is worth noting that all of the $^{13}$C tensors are quite far from being axially symmetric. Both the nitrogen and carbon tensors shown provide useful approximations to the tensors in isolated systems, and may help in assigning error bounds for order parameters derived from dipolar relaxation experiments, and in assessing ring-current and other environmental effects in CSA-related measurements.

The DFT results in Tables 6 and 7 can be compared with coupled Hartree-Fock values reported some time ago by Schindler.[96] As with the NMA results shown in Table 2, the Hartree-Fock results for the bases tend to have somewhat higher anisotropies than the DFT results. In some cases, such as C6 in cytosine or C2 and C8 in purines, the differences can be as large as 40 ppm. For some molecules, Hartree-Fock results appear to overestimate anisotropies (e.g. for benzene, the HF result for $\Delta$ at the cc-pVTZ basis level is -181 ppm, versus a DFT result of -164 and an observed value of -157 ppm), but it is not clear whether this will always be the case. Trends in anisotropies for different types of carbons and nitrogens are mostly the same at the HF and DFT levels, and so may be more reliable. It is worth re-emphasizing the fact that the results shown here are for isolated molecules and do not incorporate environmental effects that may influence results in biomolecules.

6.1.5 Shielding anisotropies in paramagnetic systems

An enormous variety of interesting effects can be seen in biomolecular systems containing paramagnetic metal ions, and there is an important dependence on the time scale of electronic relaxation. One limit of considerable interest is the so-called "Curie limit",[97–99] where electron spin relaxation is fast enough that nuclear spins interact only with the thermal average of the electron spin (the "Curie spin"): hence the interaction between a remote nucleus and the metal center is modulated by molecular motion but not by the electronic spin relaxation itself. In this limit, the effects of the paramagnetic center on remote nuclei are the same as (but often larger than) that of other types of localized susceptibilities (such aromatic rings), and the fact that the system is "paramagnetic" (i.e. has unpaired electrons) becomes irrelevant.

Under these circumstances, the local susceptibility at the metal center influences chemical shielding tensors of remote nuclei through the pseudocontact shift mechanism discussed above. Metal centers can also have an important impact on chemical shift anisotropies. The simplest example occurs even for an isotropic metal spin: consider the projection of the shielding tensor along and perpendicular to some particular direction given by a unit vector $d$ (this might be the spectrometer field, or an N-H
Chemical shift anisotropies

bond direction, for example):

\[ \sigma_\parallel = d^T \cdot \sigma \cdot d; \quad \sigma_\perp = (tr\sigma - \sigma_\parallel)/2 \]

where \( tr \) is the trace operator. (Note that \( \sigma_\parallel \) and \( \sigma_\perp \) are not the principal components of the shielding tensor; these are rather the projections of the tensor along and perpendicular to the (arbitrary) direction \( d \).) If the susceptibility is isotropic, it is straightforward to show that:

\[ \sigma_\parallel - \sigma_\perp = -\frac{\chi}{r^3} P_2(\cos \theta) \]  

(6.1)

where \( \cos \theta \equiv d \cdot r \). This is true for any group susceptibility, but becomes readily measureable for paramagnetic metal ions. For example, for a \( \text{Fe}^{3+} \) ion with \( S = 5/2 \), the \( S(S+1) \) factor in Eq. 4.27 will be almost 12 times greater than for \( S = 1/2 \), leading to effects on CSA tensors (Eq. 6.1) of about 10 ppm at 10 Å, and 3 ppm at 15 Å. It is likely that these effects will be measureable in many circumstances, especially if one can compare two systems whose principal difference is in the spin at the metal ion site.

As mentioned above, shift anisotropies in solution can be approached through measurements of CSA relaxation and CSA/dipolar cross-correlated relaxation. The (potentially large) effect of metal ions on CSA makes this a potentially powerful technique for paramagnetic proteins. For example, Boisbouvier et al.\[100\] examined the effect of a metal ion on \( ^1\text{H}\) CSA/\(^{15}\text{N}-^1\text{H} \) dipolar cross correlation in cytochrome c’, seeing effects out to 25 Å, with enough to obtain useful geometric restraints out to 15 Å. A similar approach is likely to be useful for other metalloenzymes.
Bibliography


Bibliography


Bibliography
Bibliography


