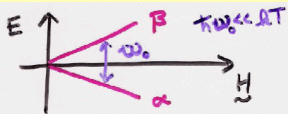


Connecting NMR data to biomolecular structure and dynamics

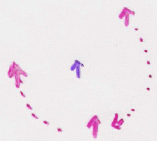
David A. Case

Methods in Molecular Biophysics, Spring, 2009



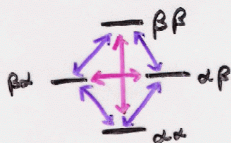
Chemical shift dispersion

$$H_{\text{eff}} = (1 - \sigma) H_0$$



red spin creates fluctuating field at purple spin

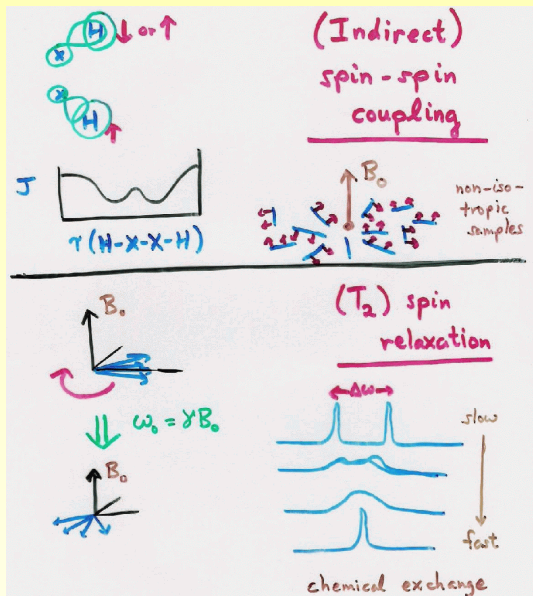
(T₁) Spin relaxation



Fourier components at $0, \omega_0, 2\omega_0$ will cause spin transitions

$$\sigma_{ij} = \left(\frac{\partial^2 E}{\partial \mu_i \partial B_j} \right)$$

$$\sigma \sim \frac{\tau_c S^2}{r^6}$$



$$J_{ij} = \left(\frac{\partial^2 E}{\partial \mu_i \partial \mu_j} \right)$$

$$R_{ex} = \rho_A \rho_B (\Delta\omega)^2 \tau_{ex}$$

$$\sigma_{\alpha\beta} = \left(\frac{\partial^2 E}{\partial B_{\alpha} \partial \mu_{\beta}} \right) \text{ chemical shielding}$$

$$J_{\alpha\beta} = \left(\frac{\partial^2 E}{\partial \mu_{\alpha} \partial \mu_{\beta}} \right) \text{ indirect spin - spin coupling}$$

$$\chi_{\alpha\beta} = \left(\frac{\partial^2 E}{\partial B_{\alpha} \partial B_{\beta}} \right) \text{ magnetic susceptibility}$$

Helgaker, Jaszunski, Ruud, *Chem. Rev.* **99**, 293 (1999)

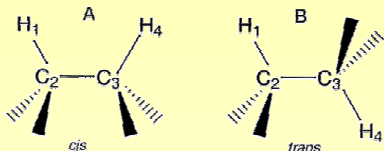
Can only “experts” do this?

```
#B3LYP/6-311G** NMR
```

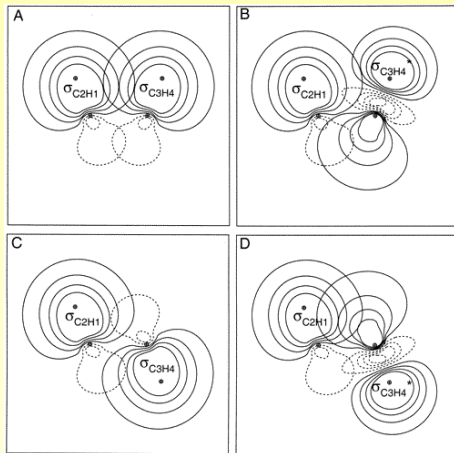
Example of Gaussian input to calculate shifts

```
0 1  
N x y z  
H x y z  
C1 x y z  
.....
```

Three-bond couplings in ethane

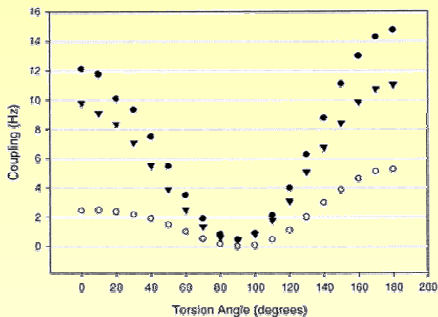


- construct localized bond and anti-bond orbitals
- look at their interactions in different geometries
- A&C: Pauli exchange repulsion
- B&D: hyperconjugation



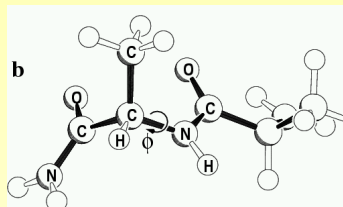
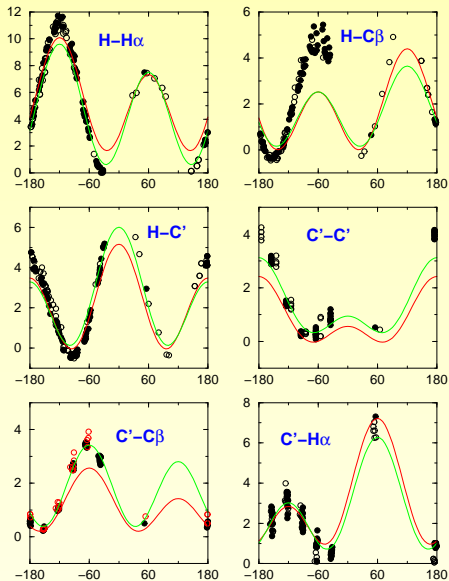
Wilkins, Westler, Markley, Weinhold, *JACS* 123: 12026, 2001

Three bond couplings in ethane (*cont.*)



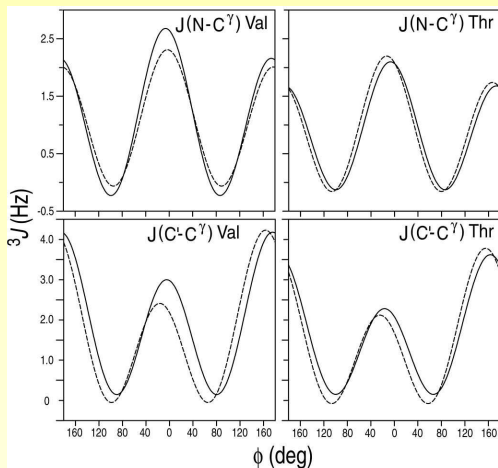
- open circles: hyperconjugation
- triangles: Pauli exchange repulsion
- closed circles: total
- (note that the orbitals representing the C-C bond don't enter at this level)

Three-bond backbone couplings in proteins



Case, Scheurer,
Brüschweiler, *JACS*
122:10390, 2000

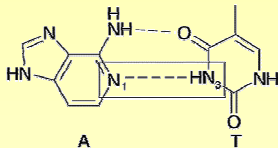
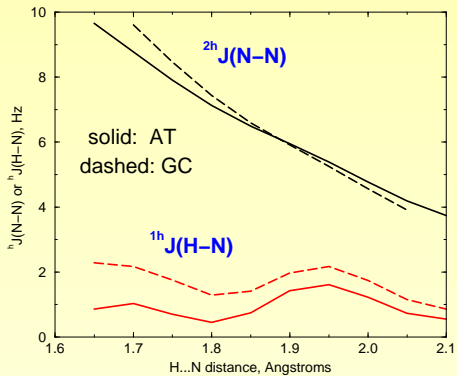
Three-bond side-chain couplings in proteins



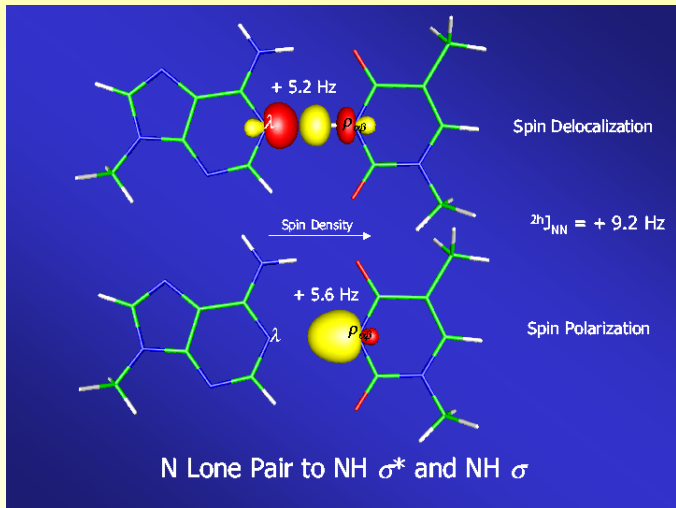
- solid: empirical fit
- dashed DFT results

Chou, Case, Bax,
JACS 125:8959, 2003

J-couplings across Watson-Crick hydrogen bonds

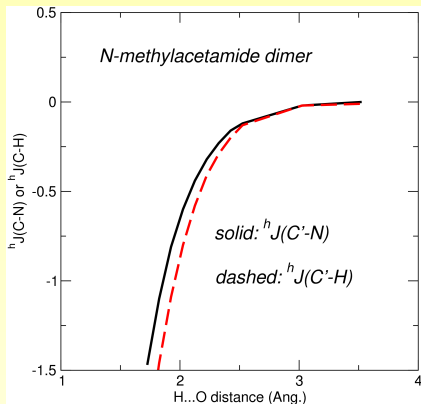


A localized orbital interpretation

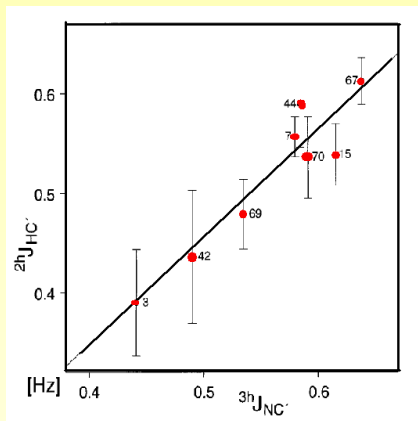


Wilkins, Westler, Weinhold, Markley, *JACS* 124: 1190, 2002

Couplings across peptide hydrogen bonds



D. Nguyen & D.A. Case, DFT calculations

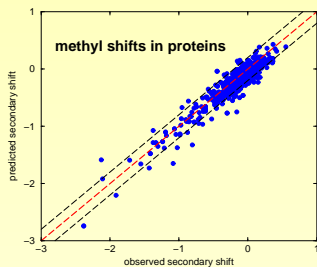


Cordier, Rogowski, Grzesiek, Bax, *JMR* **140**, 510 (1999)

Chemical shifts: Environmental effects for protons

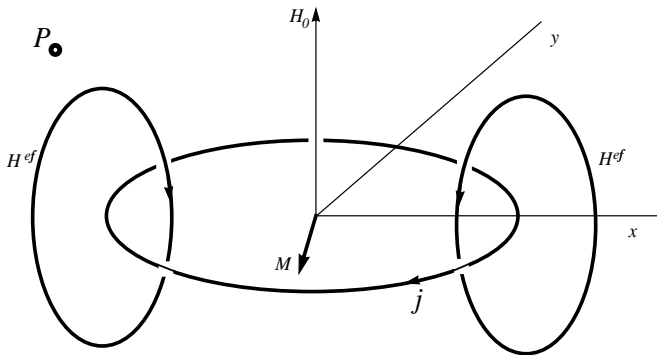
- Nearby groups with magnetic susceptibilities
 - ring currents
 - peptide group contributions
 - paramagnetic metal sites
- Electric fields
 - bond polarizability models
 - important in hydrogen bonds
- van der Waals (close contact) deshieldings
 - hydrogen bonds
 - general solvation effects
- local (“through-bond”) contributions

Buckingham, Schaefer, Schneider, *JCP* 32: 1227, 1960



Ösapay & Case, *JACS*
113, 9436 (1991)

Chemical shielding and magnetic susceptibilities



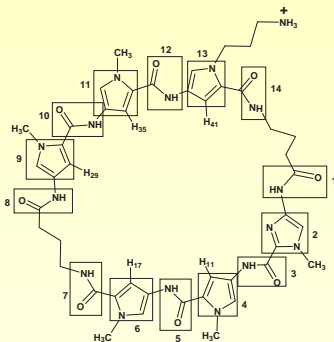
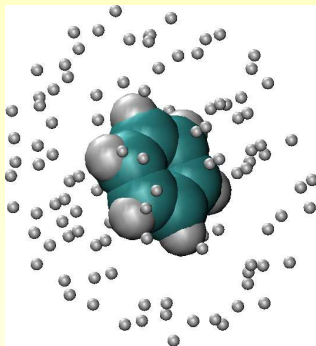
$$\sigma^G = \frac{\chi^G}{R^3} - \frac{3\chi^G \cdot \mathbf{RR}^T}{R^5}$$

$$\delta = (1/3)R^{-3}\Delta\chi(3\cos^2\theta - 1)$$

- Basis for ring-current and pseudocontact shifts
- Leads to automated fitting of χ to quantum mechanical calculations
 - can use atom, bond, or ring-based centers, with a “distributed” model
 - can be applied to arbitrary ligands

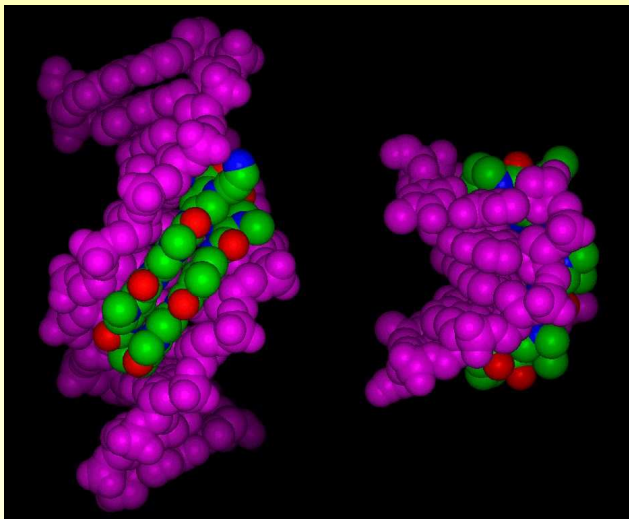
Calibrating ring-current and susceptibility models

- choose “dummy” points 2-3 Å from the molecular surface
- compute the DFT chemical shift tensor at each point
- fit susceptibility or ring-current intensities to these quantum data
- assemble larger molecules from fragments



Case, *JBNMR* 6: 341, 1995

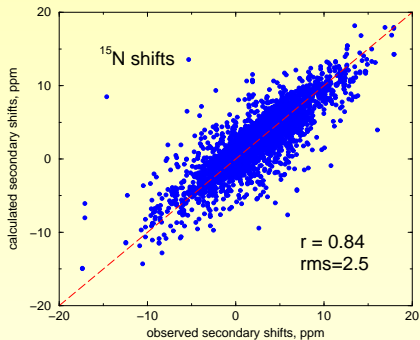
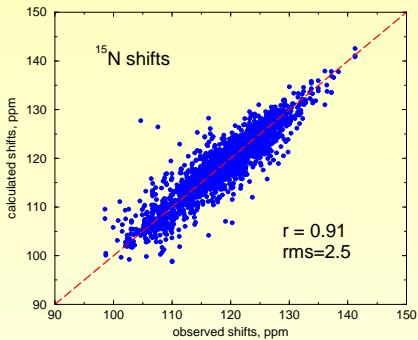
Chemical-shift driven structure refinement



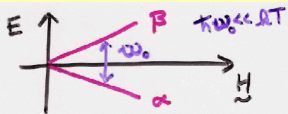
Zhang, Dwyer, Tsui, Case, Cho, Dervan, Wemmer, *JACS* 126: 7958, 2004

Current fits based on peptide DFT studies

^{15}N shifts for 20 proteins

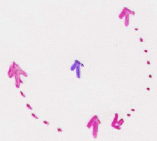


NMR 101, a second time



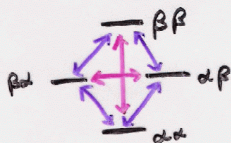
Chemical shift dispersion

$$H_{\text{eff}} = (1 - \sigma) H_0$$



red spin creates fluctuating field at purple spin

(T_1) Spin relaxation



Fourier components at $0, \omega_0, 2\omega_0$ will cause spin transitions

$$\sigma_{ij} = \left(\frac{\partial^2 E}{\partial \mu_i \partial B_j} \right)$$

$$\sigma \sim \frac{\tau_c S^2}{r^6}$$

More about dipolar relaxation

Simulating ^1H dipolar longitudinal relaxation

$$\dot{\underline{m}} = \underline{R} \underline{m} \Rightarrow \underline{m}(\tau) = \exp(\underline{R}\tau) \underline{m}(0)$$

$$R_{ij} \sim \frac{1}{r_{ij}^6} J(\omega), \quad J \sim J^{\text{rigid}} \omega^2$$

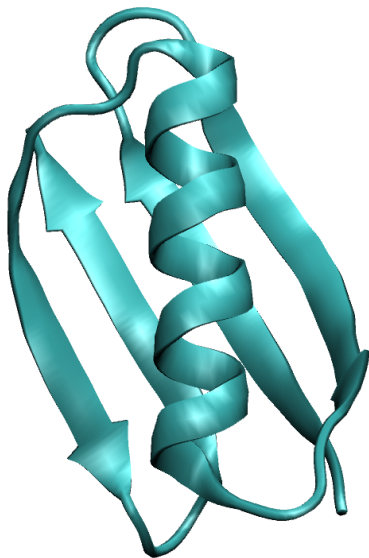
$$\omega^2 \sim \left\langle \frac{P_2(\mu(0) \cdot \mu(t))}{r^3(0) r^3(t)} \right\rangle \langle r \rangle^6$$

$$I^{\text{NOESY}} \sim \exp(\underline{R}\tau)$$

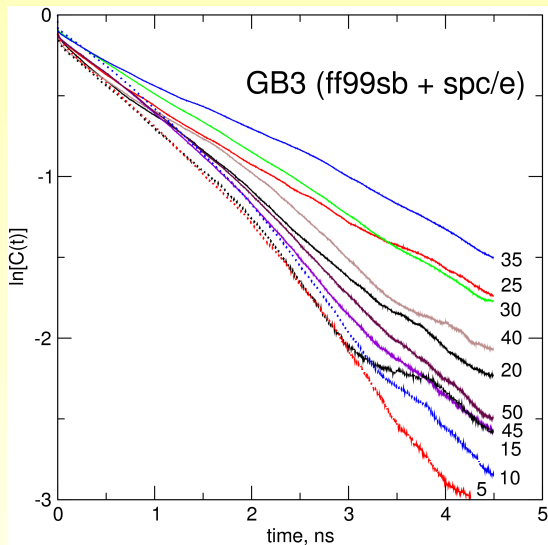
$$\frac{\partial I}{\partial x_i} \sim \begin{cases} \text{expand in } R_{ij} (i \neq j) & \tau < 0.1s \\ \text{Gaussian quad. on} & \tau > 0.1s \\ \text{integral expression} & \end{cases}$$

$$E_{\text{NoE}} = f(I^{\text{calc}} - I^{\text{exp}})$$

Fragment B3 of protein G



N-H correlation functions for GB3



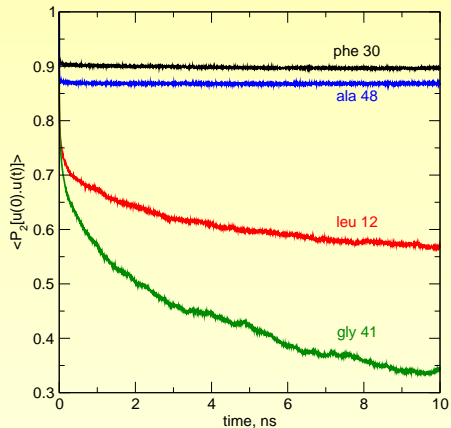
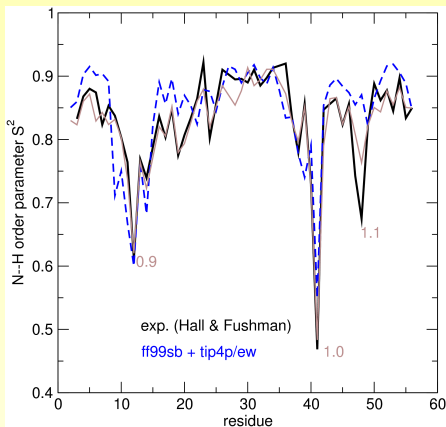
effective decay time

$$(\delta\tau)^{-1} = \mathbf{e}^T \cdot \mathbf{D} \cdot \mathbf{e}$$

But beware: statistical
uncertainty in $C(\tau)$ is
roughly

$$\left(\frac{2\tau}{T}\right)^{1/2} [1 - C(\tau)]$$

Now consider internal motions

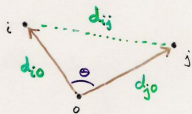


Hall & Fushman, *JBNMR*, 27, 261
(2003)

Converting distances to structures

Fundamentals of distance geometry

Define metric matrix $g_{ij} \equiv \tilde{x}_i \cdot \tilde{x}_j$



$$= d_{i0} d_{j0} \cos \theta$$

$$= \frac{1}{2}(d_{i0}^2 + d_{j0}^2 - d_{ij}^2)$$

Theorem: Distances correspond to 3-D object iff. \tilde{g} is a rank-3 matrix

$$\begin{bmatrix} \tilde{g} \\ \tilde{g} \end{bmatrix} = \begin{bmatrix} \tilde{w}^T \\ \tilde{w} \end{bmatrix} \begin{bmatrix} \lambda_1 & \lambda_2 & \lambda_3 & 0 \\ 0 & \dots & \dots & \dots \\ 0 & \dots & \dots & \dots \end{bmatrix} \begin{bmatrix} \tilde{w} \\ \tilde{w} \end{bmatrix}$$

$$g_{ij} = \sum_{k=1}^3 w_{ik} w_{jk} \lambda_k = \sum_{k=1}^3 x_{ik} x_{jk}$$

$$\therefore \boxed{x_{ik} = \lambda_k^{1/2} w_{ik}}$$

Molecular dynamics-based structure refinement

Fundamentals of MD refinement

$$E(\underline{x}) = E^{MM}(\underline{x}) + \sum_{\text{noe constraints}} K (d - d^u)^2$$

K for typical covalent bond 500 kcal/mol-Å^2

$K_{\text{noe}} = ??$ values from 1-40 are used.

$$-\frac{\partial E}{\partial \underline{x}} \equiv \underline{F} = m \underline{\ddot{x}}$$

integrate numerically, $\frac{3}{2} NkT = \text{K.E.}$

