Residual dipolar couplings and orientational effects

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1) Why do we want to use dipolar couplings in solution NMR?
Nucleic acids – global structure
Protein-Protein complexes

IGF-II    mini-IGFBP-5
2) RDC theory

- Molecular reorientation
- Internal motion

$dipolar\ field$

$^{15}\text{N}$

$^{1}\text{H}$
\[ DPQ = DPQ_{\text{max}} <P_2(\cos \theta)> \quad \text{with} \quad DPQ_{\text{max}} = -\mu_0 \gamma_p \gamma_q h/(8\pi^3<r_{pq}^3>) \]

\[ P_2(x) = \frac{1}{2} (3 x^2 - 1) \]

\[ DPQ = DPQ_{\text{max}} \sum_{ij} S_{ij} \cos \phi_i^{PQ} \cos \phi_j^{PQ} \quad \text{with} \]

\[ S_{ij} = \frac{1}{2} <3 \cos \theta_i \cos \theta_j - \delta_{ij}> \]

\((i, j = x, y, z; \delta_{ij} = 1 \text{ for } i = j, \delta_{ij} = 0 \text{ for } i \neq j)\)

\[ \text{S: Saupe matrix, alignment tensor} \]
\[ \text{Real, symmetric, traceless 3x3 matrix} \]
\[ \Rightarrow \text{five independent elements} \]

principal alignment frame, i.e. diagonalization of \(S \rightarrow S^d\)
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eigenvectors of $S^d$ are axes of alignment tensor

\[ D^{PQ} = \frac{1}{2} D^{PQ}_{\text{max}} [A_a (3 \cos^2 \theta - 1) + 3/2 A_r \sin^2 \theta \cos(2\phi)] \quad \text{with} \]

$A_a$ and $A_r$ the axial, $S_{zz}^d$, and rhombic, $2/3 (S_{xx}^d - S_{yy}^d)$, components of the diagonalized alignment tensor $S^d (A_a \sim 10^{-3})$

\[ D^{PQ} = D_a^{PQ} [(3 \cos^2 \theta - 1) + 3/2 R \sin^2 \theta \cos(2\phi)] \]

$D_a^{PQ} = \frac{1}{2} D^{PQ}_{\text{max}} A_a$ : magnitude of alignment tensor ($D_a^{\text{NH}} \sim 10$ Hz)

$R = A_a / A_r$ : rhombicity of alignment tensor; $R \in [0; 2/3]$

$\theta, \phi$: polar coordinates of vector PQ relative to alignment tensor
3) How to get partial alignment of biomolecules
Dilute nematic liquid crystals

\[ B_2 c_p > c_a \]
\[ B_2 = \pi D_{\text{eff}} L^2/4 \]
Alignment media

requirement: liquid crystalline at < 10% w/v
➤ order of biomolecules: \(\sim 0.002\)
(aqueous, stable at different ionic strength,
not too strongly charged < 0.5 e/nm\(^2\))

- bicelles (steric \(!\); \(r < d/(2V_f)\))
- filamentous phage (Pf1,fd; -0.47 e/nm\(^2\); \(r < d/\sqrt(4V_f)\))
- alkyl poly(ethylene glycol) based media
- polyacrylamide gel
- cellulose crystallites, purple membrane fragments,
cetylpyrimidinium-based media, ...

Gaemers & Bax
JACS 2001
Pf1 bacteriophage

http://www.asla-biotech.com/asla-phage.htm
Pf1: magnetic field induced order

\[ Q_{cc}^{(2\text{H})} = 0.886 \, c_{\text{Pf1}} \]

Zweckstetter & Bax, JBNMR 2001
Attenuation of alignment strength by increasing the ionic strength

20 mg/ml Pf1
150 mM NaCl

450 mM NaCl

ubiquitin at 450 mM NaCl in 20 mg/ml Pf1
Modulation of alignment tensor orientation by ionic strength changes
Orientational degeneracy of RDC – use of multiple media

\[ D_{PQ}^{PQ} = D_a^{PQ} [(3 \cos^2 \theta - 1) + \frac{3}{2} R \sin^2 \theta \cos(2\phi)] \]

Ramirez & Bax
JACS, 1998
4) RDC measurement

NOESY

HSQC
Accuracy of measured splitting: $\Delta J = \frac{LW}{SN}$

required accuracy $< 5\% \times Da$

$^1J_{HN}$ [1]: IPAP-HSQC, DSSE-HSQC, 3D HNCO

$^1J_{C'C\alpha}$ [5]: 3D HNCO (CSA(C$'$) $\Rightarrow \sim 500$ MHz optimum)

$^1J_C$ & $^2J_{C'HN}$ [8.3]: 2D HSQC, 3D TROSY-HNCO

$^1J_{C\alpha H\alpha}$ [0.5]: 2D J$_{CH}$-modulated HSQC, (HA)CANH, HN(CO)CA

$^1J_{CH}$ (side-chain): 2D J$_{CH}$-mod. HSQC, CCH-COSY, SPITZE-HSQC

$^1H$-$^1H$: COSY, CT-COSY, HNHA, 3D SS-HMQC2 (long-range)

Chou & Bax JBNMR, 2001; Delaglio et al. JMR 2001; Wu & Bax, JACS, 2002;
RDC measurement: J splitting (\(^1J_{HN}\))

IPAP-HSQC

Ottiger et al. JMR, 1998
RDC measurement: Quantitative J correlation ($^1J_{C^\alpha N}$)

Chou & Bax
JBNMR, 2001
5) Determination of a molecular alignment tensor

1) RDC distribution analysis
2) Back-calculation of alignment tensor
3) Shape-prediction
4) Shape/Charge-prediction
Estimate for alignment tensor

\[ D_{zz}^{PQ} = 2 D_{a}^{PQ} \]
\[ D_{yy}^{PQ} = -D_{a}^{PQ} (1 + 1.5 R) \]
\[ D_{xx}^{PQ} = -D_{a}^{PQ} (1 - 1.5 R) \]

no structure necessary!

with \( D_{ii}^{PQ} = D_{ii}^{PQ_{\text{max}}} S_{ii}^d \)
Back-calculation of alignment tensor

- singular value decomposition (SVD)

→ very stable & with a minimum
  of five RDCs possible

- iterative least squares procedure (Levenberg-Marquardt minimization)

\[ \chi^2 = \sum_{i=1,\ldots,N} \frac{(d_i^{PQ}(\text{exp}) - d_i^{PQ}(\text{calc}))^2}{\sigma_i^{PQ}} \]

→ fixing of alignment parameters (e.g. rhombic component zero due to
  three-fold or higher symmetry)
Evaluation of uncertainty in back-calculated alignment tensors

Zweckstetter & Bax, JBNMR 2002
Steric model of alignment

no RDCs necessary!
Shape prediction of magnitude and orientation of alignment

Zweckstetter & Bax
JACS, 2000
Electrostatic model of alignment

\[ p = \exp\left[ -\frac{E_{\text{pot}}}{k_b T} \right] \]
Electrostatic potential

![Graph showing the electrostatic potential as a function of distance. The x-axis represents distance in nanometers (nm), and the y-axis represents the electrostatic potential (E_{pot}) in kT units. The graph shows a curve that increases as the distance increases, starting from zero potential at zero distance.](image-url)
Shape & charge prediction of alignment tensor

![Graph showing the comparison between predicted and measured 1DNH values for electrostatic and steric effects.]
PALES – software for analysis of RDC

http://spin.niddk.nih.gov/bax
6) RDC applications

- validation of structures
- analysis of inter-domain motion
- structure refinement (proteins, nucleic acids, oligosaccharides)
- identification of multimerization state
- determination of relative domain orientations
- structure determination of protein complexes
- analysis of slow dynamics
- improved assignment
- rapid structure determination
- ...
Validation of structures

\[ Q = \frac{\text{rms } (D^{\text{obs}} - D^{\text{calc}})}{\text{rms } (D^{\text{obs}})} \]

\[ Q \approx 0.2 \approx 1.5 \text{ Å X-ray} \]

- use only for RDC not included in structure determination!
- no translational validation
Conformational differences in solution: calmodulin

Q = 41%

Q = 25%

Flexibility of the inter-domain linker in solution

Baber et al. JACS, 2000

NH-dynamics from RDC: Peti et al. JACS 2002
Qualitative analysis of inter-domain motion
Quantitative analysis of interdomain motion
Structure refinement

\[ E_{\text{dip}} = k \left( D_{\text{calc}}^{\text{PQ}} - D_{\text{obs}}^{\text{PQ}} \right)^2 \]

k: $10^{-4} \Rightarrow 1 \text{ kcal/Hz}^2$

VEAN (intervector projection angles): Meiler et al. JBNMR, 2000

Chou, Li, Klee & Bax, Nature Struc Biol 2001

![Diagram of protein structure with annotations](image)
Determination of multi-module structures

in Lösung

im krystallinen Zustand

cyanovirin-N
Monomeric versus multimeric structures

$^{1}D_{NH}^{\text{predicted}}$ [Hz]

$^{1}D_{NH}^{\text{measured}}$ [Hz]
Translational information from shape-prediction

Bewley & Clore JACS, 2000
Rapid structure determination

assignment

MGSSHHHHHHSSGLVPRGSHMNNS
LDIKDVTTFYEEEDKHLIFGYTPTC
GTCKVSRMLDIANEILQLPLLKI
DLNFPQFCKDMQMSTPILLLMN
KDKEVKRIYAFKSVTDLLENLK
3D IPAP-(HA)CANH
Improved NMR assignment with RDC

Zweckstetter & Bax
JACS, 2001
Secondary structure

**backbone assignment**
- $\delta(C^\alpha)$
- $J+D(C^\alpha-H^\alpha)$
- CSA($C^\alpha$)

**3D structure**
- $\delta(C^\alpha)$
- $\delta(N)$
- $D(C^\alpha-H^\alpha)$
- $\phi, \psi$

**secondary structure**
- $J(C^\alpha-H^\alpha) > 145.5$ Hz
- $J(C^\alpha-H^\alpha) \sim 140.5$ Hz
- $J(C^\alpha-H^\alpha)$ & CSA($C^\alpha$) > 30 ppm
- $\phi > 0$
- $\phi < 0$
- helix
- sheet
Molecular fragment homology search
3D structure of molecular fragments

Zweckstetter & Bax
JACS, 2001
References:


Journal of American Chemical Society, Journal of Biomolecular NMR, Journal of Magnetic Resonance, ...