Residual dipolar couplings

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Background material


NMR structure determination

Bacteria → 13C,15N-Source → Sample → Assignment → Multi-D-NMR → Structure Calculation

Distance Restraints

Dihedral Restraints

J-Coupling (E.COSY, DQ/ZQ, FIDS, quant.J)

NOE/ROE-1/r (NOESY, ROESY)

Cross Correlated Relaxation

Projection Restraints

1) Dipole-Dipole
2) Dipole-CSA
3) CSA-CSA

1) Dipole-Susceptibility Tensor
2) Dipole-Mass Tensor

Chemical Shift Restraints

3D-Structure

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Why do we want to use dipolar couplings in solution NMR?

- all these parameters give essentially local information: distances <5Å; dihedral angles; intraresidual/sequential $\phi/\psi$ values, …
- long-range information is virtually non-existent

this leads to NMR-specific problems in structure determination:

- what is the exact angle between two non-parallel $\alpha$-helices in a protein?
- is a long helical structure (a-helical protein, DNA) straight or bent?
- what is the relative orientation of two protein domains?
Residual dipolar couplings (RDCs) can be observed in solution when a molecule is aligned with the magnetic field.

When alignment can be kept sufficiently weak:
- NMR spectra remain simple as in isotropic solution
- Quantitative measurement of a wide variety of RDCs

Several dilute liquid crystalline media are now available.

RDC measurements and analysis are highly efficient.

Residual dipolar couplings are a generally applicable tool for NMR structure determination.
The dipolar coupling
The classical dipolar interaction

Nuclear spin $\rightarrow$ magnetic dipole $\mu$ $\rightarrow$ magnetic field

$$ B_\mu (r) = \frac{\mu_0}{4\pi r^3} \left[ 3(\mu r)(r)-(r r)\mu \right] $$

2nd nuclear spin $\rightarrow$ magnetic moment in $B_\mu$ $\rightarrow$ interaction energy

$$ E_{\mu_1,\mu_2} = -B_\mu (r_{12}) \mu_2 = \frac{\mu_0}{4\pi} \frac{1}{r_{12}^3} \left[ \mu_1 \mu_2 - \frac{3}{r_{12}^2} (\mu_1 r_{12}) (\mu_2 r_{12}) \right] $$

Within strong external magnetic field $B_0$ $\rightarrow$ alignment

$$ \frac{\mu_1 r_{12}}{|r_{12}|} = \mu_1 \cos \theta $$

$$ E_{\mu_1,\mu_2} = \frac{\mu_0}{4\pi} \frac{1}{r_{12}^3} \mu_1 \mu_2 \left[ 1 - 3 \cos^2 \theta \right] $$

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The classical dipolar interaction

\[ E_{\mu_1,\mu_2} = \frac{\mu_0}{4\pi} \frac{1}{r_{12}^3} \mu_1 \mu_2 \left[ 1 - 3 \cos^2 \theta \right] \]

Isotropic reorientation

\[ \int_0^{\pi} (1 - 3 \cos^2 \theta) \sin \theta d\theta = 0 \]

Magic angle \( \sim 54^\circ \)
Quantum mechanical picture

\[ \text{spin } \mathbf{I} \rightarrow \mathbf{\mu} = \gamma_I \hbar \mathbf{I} \]

\[ H_D = -\frac{\mu_0}{4\pi} \frac{1}{r^3} \sum_{i,j=1}^{3} \mu_{ij} \left( 3 \frac{x_i x_j}{r^2} - \delta_{ij} \right) \mu_{2j} = -\frac{\gamma_I \gamma_s \hbar \mu_0}{4\pi r^3} \sum_{i,j=1}^{3} I_i \left( 3 \frac{x_i x_j}{r^2} - \delta_{ij} \right) S_j \]

\[ \begin{aligned}
I_z S_z : & 3 \frac{z^2}{r^2} - 1 \\
I_x S_z + I_z S_x : & 3 \frac{xz}{r^2} \\
I_y S_z + I_z S_y : & 3 \frac{yz}{r^2} \\
I_x S_y + I_y S_x : & 3 \frac{xy}{r^2} \\
I_x S_x : & 3 \frac{x^2}{r^2} - 1 \\
I_y S_y : & 3 \frac{y^2}{r^2} - 1 \\
I_x S_x + I_y S_y : & 3 \frac{3x^2 + 3y^2 - 2x^2 - 2y^2 - 2z^2}{2r^2} = -2z^2 + x^2 + y^2 - \left( \frac{3z^2}{r^2} - 1 \right) \frac{1}{2}
\end{aligned} \]
Quantum mechanical picture

\[ H_D = -\frac{\gamma_I \gamma_S \hat{h} \mu_0}{4\pi r^3} \left\{ \begin{array}{l}
I_z S_z - \frac{1}{2} (I_x S_x + I_y S_y) \left( 3 \frac{z^2}{r^2} - 1 \right) + \\
\left( I_x S_z + I_z S_x \right) 3 \frac{xz}{r^2} + \left( I_y S_z + I_z S_y \right) 3 \frac{yz}{r^2} \right. \\
+ \left. \left( I_x S_y + I_y S_x \right) 3 \frac{xy}{r^2} + \left( I_x S_y + I_y S_x \right) 3 \left( \frac{x^2 - y^2}{2r^2} \right) \right\} \]

spherical coordinates

\[ \frac{x}{r} = \sin \theta \cos \phi; \frac{y}{r} = \sin \theta \sin \phi; \frac{z}{r} = \cos \theta \]

\[ H_D = \sum_m (-1)^m F^m_2 (\theta, \phi) T_2^{-m} \]

where

\[ F^m_2 = -\frac{\gamma_I \gamma_S \hat{h} \mu_0}{4\pi r^3} \left( \frac{24\pi}{5} \right)^{\frac{1}{2}} \frac{1}{2} Y^m_2 \]

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Spherical harmonics

\[
Y^m_2(\theta, \phi) = \frac{1}{\sqrt{2}} \begin{cases} 
\frac{1}{2} & \text{for } m=0 \\
\pm \frac{1}{2} \sin \theta \cos \phi \exp(\pm i\phi) & \text{for } m=\pm1 \\
\pm \frac{1}{2} \sin^2 \theta \exp(\pm i2\phi) & \text{for } m=\pm2
\end{cases}
\]

\[
T_{2}^{-m} = \begin{cases} 
\frac{5}{4\pi} \frac{1}{2} (3\cos^2 \theta - 1) & \text{for } m=0 \\
\pm \frac{1}{2} [I_z S_z - \frac{1}{2} (I_x S_y + I_y S_x)] & \text{for } m=\pm1 \\
\pm \frac{1}{2} I_z S_z & \text{for } m=\pm2
\end{cases}
\]

\[
I_x = (I_x + iI_y) \quad I_y = (I_x - iI_y) \\
\exp(i\phi) = \cos \phi + i \sin \phi
\]
Secular approximation

\[ H_D = -\frac{\gamma_I \gamma_S \hbar \mu_0}{4\pi r^3} (3\cos^2 \theta - 1) \left[ I_z S_z - \frac{1}{4} (I_+ S_- + I_- S_+) \right] \]

\[ \frac{1}{2} (I_+ S_- + I_- S_+) = I_x S_x + I_y S_y \]

heteronuclear case

\[ H_D = -\frac{\gamma_I \gamma_S \hbar \mu_0}{4\pi r^3} (3\cos^2 \theta - 1) I_z S_z \]

\[ \frac{\gamma_I \gamma_S \hbar \mu_0}{4\pi r^3} \rightarrow r = 1.04 \rightarrow ^1\text{D(N,H)} = 21.7 \text{ kHz} \]
molecular tumbling/internal motion →

\[ D_{PQ} = -\gamma_p \gamma_q \hbar \mu_0 \frac{2}{4\pi r^3} \int \frac{P(\beta, \gamma) F_2^0(\theta, \phi)}{4\pi} (\cos \beta) d\gamma \]

sampled orientations

\[ D_{PQ} = -\gamma_p \gamma_q \hbar \mu_0 \frac{1}{2} \left( \frac{3\cos^2 \theta - 1}{2} \right) \]
Molecular alignment and RDCs
Molecular alignment and RDCs

\[ P_2(\cos \theta) = \frac{3}{2} \left\langle \left( \cos \beta_x(t) \cos \alpha_x + \cos \beta_y(t) \cos \alpha_y + \cos \beta_z(t) \cos \alpha_z \right)^2 \right\rangle - \frac{1}{2} \]

\[ C_i(t) = \cos \beta_i(t) \quad \text{and} \quad c_i = \cos \alpha_i \]

\[ \left\langle P_2(\cos \theta) \right\rangle = \frac{3}{2} \left[ \left\langle C_x(t) \right\rangle^2 c_x^2 + \left\langle C_y(t) \right\rangle^2 c_y^2 + \left\langle C_z(t) \right\rangle^2 c_z^2 + \frac{1}{2} \left\langle C_x(t) C_y(t) \right\rangle c_x c_y + 2 \left\langle C_x(t) C_z(t) \right\rangle c_x c_z + 2 \left\langle C_y(t) C_z(t) \right\rangle c_y c_z \right] - \frac{1}{2} \]

\[ \left\langle P_2(\cos \theta) \right\rangle = \frac{3}{2} \left( \frac{b_0^2 r_0^2}{2} - 1 \right) \]

\[ = \frac{3}{2} \left( r_x^0, r_y^0, r_z^0 \right) \left( \begin{array}{ccc}
\left\langle C_x(t) \right\rangle^2 & \left\langle C_x(t) C_y(t) \right\rangle & \left\langle C_x(t) C_z(t) \right\rangle \\
\left\langle C_y(t) C_x(t) \right\rangle & \left\langle C_y(t) \right\rangle^2 & \left\langle C_y(t) C_z(t) \right\rangle \\
\left\langle C_z(t) C_x(t) \right\rangle & \left\langle C_z(t) C_y(t) \right\rangle & \left\langle C_z(t) \right\rangle^2 
\end{array} \right) \left( \begin{array}{ccc}
r_x^0 \\
r_y^0 \\
r_z^0 
\end{array} \right) - \frac{1}{2} \]

orientational probability distribution

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Molecular alignment and RDCs

\[ S_{ij} = \frac{3}{2} \left\langle \cos \beta_i(t) \cos \beta_j(t) - \frac{1}{2} \delta_{ij} \right\rangle = \frac{3}{2} \left\langle C_i(t) C_j(t) \right\rangle - \frac{1}{2} \delta_{ij} \]

\[ \left\langle P_2(\cos \theta) \right\rangle = \sum_{i,j=x,y,z} S_{ij} \cos \alpha_i \cos \alpha_j \]

\[ \langle C_x \rangle^2 + \langle C_y \rangle^2 + \langle C_z \rangle^2 = 1 \quad \langle C_i C_j \rangle = \langle C_j C_i \rangle \]

S is real, traceless, symmetric \( \rightarrow 5 \) independent elements

principal alignment frame, i.e. diagonalization of \( S \rightarrow S^d \)

\[ D^{PQ}(\alpha_x, \alpha_y, \alpha_z) = \frac{3}{2} D^{PQ}_{\text{max}} \left[ \left( \langle C_x \rangle^2 c_x^2 + \langle C_y \rangle^2 c_y^2 + \langle C_z \rangle^2 c_z^2 \right) - 1 \right] \]

\[ \theta_{PQ} = \alpha_z; c_z = \cos \theta_{PQ}; c_x = \sin \theta_{PQ} \cos \phi_{PQ}; c_y = \sin \theta_{PQ} \sin \phi_{PQ} \]

\[ \langle C_i \rangle^2 = 1/3 + A_i \]

\[ D^{PQ}(\alpha_x, \alpha_y, \alpha_z) = \frac{3}{2} D^{PQ}_{\text{max}} \left[ \cos^2 \theta_{PQ} A_{zz} + \sin^2 \theta_{PQ} \cos^2 \phi_{PQ} A_{xx} + \sin^2 \theta_{PQ} \sin^2 \phi_{PQ} A_{yy} \right] \]

\[ D^{PQ}(\theta_{PQ}, \phi_{PQ}) = \frac{3}{2} D^{PQ}_{\text{max}} \left[ P_2(\cos \theta_{PQ}) A_{zz} \right. \left. + \frac{1}{2} \sin^2 \theta_{PQ} \cos 2\phi_{PQ} A_{xx} + \frac{1}{2} \sin^2 \theta_{PQ} \cos 2\phi_{PQ} A_{yy} \right] \]
Generalized degree of order (GDO):
Euclidean norm $\vartheta = \left( \frac{2}{3} \frac{4}{5} \pi \sum_{ij} S_{ij}^2 \right)^{1/2}$

$A_a = \frac{3}{2} A_{zz} = S_{zz}^d$, $A_r = (A_{xx} - A_{yy}) = 2/3 \left( S_{xx}^d - S_{yy}^d \right)$

$D^{PO}(\theta_{PQ}, \phi_{PQ}) = D^{PO}_{\text{max}} \left[ P_2 \left( \cos \theta_{PQ} \right) A_a + \frac{3}{4} A_r \sin^2 \theta_{PQ} \cos 2\phi_{PQ} \right]$ 

$D^{PO}(\theta_{PQ}, \phi_{PQ}) = D^a_{PQ} \left[ (3 \cos^2 \theta_{PQ} - 1) + \frac{3}{2} R \sin^2 \theta_{PQ} \cos 2\phi_{PQ} \right]$ 

$D^a_{PQ} = \frac{1}{2} D^{PO}_{\text{max}} A_a$ : magnitude of alignment tensor ($A_a = 10^{-3} \rightarrow D_a^{\text{NH}} \sim 10$ Hz) 

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

use only a very slight orientational preference ("partial alignment"), i.e., only ca. 1 out of 1000 solute molecules
How to Get Alignment
Partial Alignment

- dipolar couplings are LARGE in solids (~22 kHz for $1^1D_{\text{HN}}$), but
- fortunately average out for isotropically fast tumbling (solution NMR)

⇒ full dipolar couplings are \textit{not} desirable in high-resolution NMR

Can we get orientational information WITHOUT messing up our nice NMR spectra?

\textbf{YES!}

- use only a very slight orientational preference ("\textit{partial alignment}"), i.e.,
  only ca. 1 out of 1000 solute molecules

→ result: dipolar coupling from the (0.1\%) non-isotropic fraction is scaled down to
  \textbf{residual dipolar couplings} (RDCs) of ± 20 Hz max.! (0.1\% of ±20 kHz)
Alignment – Anisotropic Tumbling

To extract dipolar coupling data, the molecule must behave anisotropically!

1) large magnetic susceptibility anisotropy
   • diamagnetic systems such as DNA (small anisotropy in each base)
   • metalloproteins with paramagnetic centers
   • lanthanide-binding tags

   field-dependent alignment of molecules

2) anisotropic environment
   • oriented liquid-crystalline phase
   • anisotropically compressed gel

   field-independent alignment
Alignment media

Requirements

• liquid crystalline at < 10% w/v  \( \Rightarrow \) order of biomolecules: \( \sim 0.002 \)

• aqueous

• uniform anisotropy over the whole sample volume,

• stable at different ionic strength, pH, temperature

• not too strongly charged < 0.5 e/nm\(^2\),

• solute should not bind (significantly) to medium
Bicelles

- Diskshaped particles made from DMPC and DHPC (q = 3:1)

- concentration usually 5% (w/v)

- degree of protein alignment can be “tuned” by adjusting the bicelle concentration

- alignment is temperature dependent (liquid crystal > 37°C)

- aligning with their normal perpendicular to the direction of the magnetic field

- degree of alignment can be determined by measuring the 2H quadrupolar splitting in the HDO resonance.
Bicelles (2)

- Isotropic bicelles ($q \sim 0.5$) for solubilization of integral membrane proteins → solution-state NMR

- Anisotropic bicelles for solid-state NMR and X-ray crystallography

Disadvantages:

- unstable in the presence of certain proteins

- offers only a limited temperature and pH range
Alignment media - Toolbox

- bicelles
- alkyl poly(ethylene glycol) based media
- filamentous phage (Pf1,fd; -0.47 e/nm²)
- polyacrylamide gel (charged, uncharged)
- cellulose crystallites
- purple membrane fragments
- cetylpyrimidinium-based media, ...
Alignment of Membrane Proteins

Acrylamide gels

DNA nanotubes

G-tetrad DNA

Douglas et al.
PNAS, 2007

Lorieau et al.
JACS, 2008
Modulation of alignment tensor
The problem: Orientational degeneracy

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

Ramirez & Bax
JACS, 1998
Strategies for Modulation of Alignment

- Alignment media with different properties (steric/electrostatic)
- Charged bicelles: doping with small charged amphiphiles to alter their charge
  Positive: CTAB / Negative: SDS
- Charged gels
- Variation of pH, ionic strength
- Mutation (introduction of charged residues)
- Paramagnetic alignment (different tags/lanthanides)
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

![Graph showing modulation of alignment tensor orientation by ionic strength changes](image)
Liquid crystal theory
Liquid crystal theory

Onsager (1949): concentration at which a solution undergoes a spontaneous first-order phase transition from an isotropic to a chiral nematic phase.

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

First-order transition \(\rightarrow\) coexistence region \(c_p \in [c_i, c_a]\)

For semi-flexible rods:

\[ c_i = 0.3588 \left[ (1-\sqrt{x})B_2 \right]^{-1} \]
\[ c_a = 0.3588 \left[ (x-\sqrt{x})B_2 \right]^{32} \]
Onsager theory

\[ \text{B}_2c_p > c_a \]

\[ \text{B}_2 = \pi \text{D}_{\text{eff}} L^2/4 \]

effective increase in rod diameter
\[ \text{D}_{\text{eff}} = \text{D} + \kappa^{-1} (\ln \omega + 0.7704) \]

contact potential \( \omega = 2\pi Z^2 [\beta \gamma x_0 K_1(x_0)]^{-2} Q \kappa^{-1} \exp(-2x_0) \)

contribution of the polyions to the ionic strength \( \kappa = 8\pi Q(c_s + \Gamma z_p c_p) \)
Liquid crystal theory

density of most nematogens is higher than for the solvent
⇒ average density of the nematic region is higher than for the isotropic region ⇒ gravity causes it to occupy the bottom region of the sample
Paranematic phase of Pf1 phage

For a fully nematic phase, the degree of alignment is independent of field strength above a typically very low threshold.
Measurement of RDCs
NOESY

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

required accuracy $< 5\% \cdot \text{Da}$

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

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

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

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

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

$^{1}H$-$^{1}H$: 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 (\(^1J_{\text{HN}}\))

IPAP-HSQC

Ottiger et al. JMR, 1998

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RDC measurement: Quantitative J correlation ($^{1}J_{CN}$)

Chou & Bax
JBNMR, 2001
Determination of a Molecular Alignment Tensor
Four Methods

1) RDC distribution analysis
2) Back-calculation of alignment tensor
3) Prediction of alignment from structure
4) Prediction of alignment from structure and charge distribution
1) Estimate for alignment tensor

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

with \( D_{ii}^{PQ} = D_{ii}^{PQ} \max S_{ii}^{d} \)

\[
\log( L( d_{1...n}^{PQ} \| D_a^{PQ}, R)) = \sum_{i=1,...,N} \log(P(d_i^{PQ}))
\]

no structure necessary!
2) Back-calculation of alignment tensor

if well-defined structure available

• singular value decomposition (SVD)
  ➔ very stable & with a minimum of five RDCs possible

• iterative least squares procedure (Levenberg-Marquardt minimization)
  \[ \chi^2 = \sum_{i=1,...,N} \frac{[d_{i}^{PQ}(\text{exp}) - d_{i}^{PQ}(\text{calc})]^2}{\sigma_{i}^{PQ}^2} \]
  ➔ fixing of alignment parameters (e.g. rhombic component zero due to three-fold or higher symmetry)
Can you trust a back-calculated alignment tensor?

Monte-Carlo type approach (RDC noise)

→ repeat SVD calculation many times (~1000 times)
→ each time add different Gaussian noise to experimental RDCs
→ accept only those solutions for which all back-calculated RDCs are within a given margin of the original experimental dipolar couplings

error in the data is dominated by the random measurement error in the dipolar couplings

*indirectly take into account uncertainties in the structure*

set the amplitude of the added noise two to three times higher than the measurement uncertainty
Evaluation of uncertainty in back-calculated alignment tensors (II)

Structural noise Monte-Carlo type approach

→ repeat SVD calculation many times (~1000 times)

→ each time add different Gaussian noise to the original structure (match the RMSD between the experimental and back-calculated RDCs)

→ spread in alignment parameters obtained for these noise-corrupted structures, when using the coupling constants calculated for the original structure (i.e., yielding a perfect fit if no structural noise were added)
3) Prediction of alignment from structure

no RDCs necessary!

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Computer experiment: PALES

\[ S_{ij} = \frac{1}{2} <3 \cos \Theta_i \cos \Theta_j - \delta_{ij}> \quad (i,j=x,y,z) \]

\[ \Rightarrow S^{\text{mol}} \text{ linear average over all non-excluded } S \text{ matrices} \]

Periodic boundary conditions

- \( r < d/(2V_f) \) (wall model), or \( r < d/(4V_f)^{1/2} \) (cylinder)
Shape prediction of magnitude and orientation of alignment

→ Protein alignment in bicelles is sterically induced
Weak alignment in Pf1 bacteriophage

http://www.asla-biotech.com/asla-phage.htm
4) Prediction of alignment from structure and charge distribution

\[ p_B = \exp \left[ -\frac{\Delta G_{el}(r, \Omega)}{k_B T} \right] \]

\[ \Delta G_{el}(r, \Omega) = \sum_i q_i \phi(r_i(r, \Omega)) \]

\[ S_{ij}^{\text{mol}} = \int S_{ij} p_B(r, \Omega) \, dr \, d\Omega / \int p_B(r, \Omega) \, dr \, d\Omega \]

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How to calculate the electrostatic energy?

- **Continuum electrostatic theory** (Debye and Hueckel 1923): protein embedded in a dielectric medium containing excess ions

- **non-linear Poisson-Boltzmann equation** (Chapman 1913; Gouy 1910)

- Further simplification: Protein = a particle in the external field of the liquid crystal → many approximations!

- protein = charges of their ionizable residues
- static dielectric constant of water $\varepsilon = 78.29$
- average surface charge of phages: $-0.47 \text{ e/nm}^2$
Electrostatic potential

$\phi$ [kT/e] vs distance from Pf1 [nm]

0 10 20

-4 -2 0
Experiment versus PALES prediction

- **steric**
- **electrostatic + steric**

Zweckstetter et al., Biophys. J. 2004

ubiquitin

DinI

GB3

GB1

DNA

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Ionic strength dependence

ubiquitin

Din1

GB3

GB1

DNA

magnitude

orientation

NMR workshop, Ro:
Weak alignment in surfactant liquid crystalline phases

3 nm

10-20 nm

PALES

B₀

Lα

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Residual dipolar couplings in cetylpyridinium bromide/hexanol/sodium bromide

steric and electrostatic interactions dominate weak alignment of biomolecules in polar liquid crystalline media

Zweckstetter, submitted

$^{1}D_{NH}$ pred. CPBR/hexanol/NaBr [Hz]

$^{1}D_{NH}$ exp. C

ubiquitin
Partial alignment at pH 3

Barrientos et al., JMR 2001

Zweckstetter, Eur. Biophys. J. 2005
PH dependence of alignment

\[ \text{GB3} \]

\[ \text{ubiquitin} \]
RDCs are a sensitive probe of protein electrostatics

DinI (PDB code: 1GHH)

Zweckstetter et al., Biophys. J. 2004
Charge/Shape prediction: applications

- differentiation of monomeric and homodimeric states
  (Zweckstetter and Bax 2000)

- conformational analysis of dynamic systems such as oligosaccharides
  (Azurmendi and Bush 2002)

- refinement of nucleic acid structures
  (Warren and Moore, 2001)

- determination of the relative orientation of protein domains
  (Bewley and Clore 2000)

- validation of structures of protein complexes
  (Bewley 2001)

- classify protein fold families on the basis of unassigned NMR data
  (Valafar and Prestegard 2003)

- Probing surface electrostatics of proteins and nucleic acids,
  refinement of side chain orientations in proteins, …
RDCs in Structure Calculation
RDC-based refinement of structures

**PROBLEM:**
Potential energy surface is very rough ➔ many local/false minima ➔ convergence problem

RDC-based refinement of starting structures

\[ E_{dip} = k_{dip}(D_{calc} - D_{meas})^2 \]

\( k_{dip} \) force constant ➔ adjust such that dipolar RMS is equal to measurement error)
**Critical Points**

**How to use the structural information obtained from molecular alignment**

1. In order to use the information one needs to know the direction and the size of the tensor (susceptibility, alignment, etc).
2. Minimization of the deviation between the measured quantity and its calculated value from a given structure and set of tensor parameters.
3. One has to be able to evaluate the outcome of the minimization.

**Minimization procedure**

1. Get a good estimate on the tensor parameters
2. Define structural constraints with respect to the arbitrary tensor coordinate system.
3. Turn on the force constant for a particular alignment potential such that the final RMS between the measured and calculated values reflect the experimental error.
4. During the calculation the tensor orientation will be automatically determined through global minimization with respect to the current structure.
5. Refine the initial estimate of the tensor parameters. This can be achieved through either grid search method or built into the minimization itself.
Define axis system representing the alignment tensor
From PALES to XPLOR

rDC(NH) measured aligned - isotropic (PALES convention)
(min(DC)=-16.2 Hz; max(DC)=12.7 Hz
==> estimated Da(NH)=-8.1*(-1) as correct measurement isotropic-aligned)

==> PALES gives: DATA Da -4.177638e-04
==> get correct Da by multiplying -4.177638e-04*-21585.19 = 9.0 Hz
(for XPLOR however use -9.0 Hz, i.e. sign of Szz/2)

rDC(NH) measured isotropic - aligned (SSIA convention)
(min(DC)=-12.7 Hz; max(DC)=16.2 Hz
==> estimated Da(NH)=16.2/2=8.1

==> PALES gives: DATA Da 4.177638e-04
==> get correct Da by multiplying 4.177638e-04*21585.19 = 9.0 Hz
(for XPLOR however use -9.0 Hz, i.e. (-1)*sign of Szz/2)
How to evaluate structures produced by minimizing against alignment data

1. The final dipolar RMS between measured vs. calculated values

2. Consistency between dipolar coupling data and NOE data (decrease in non-RDC energy terms)

3. If one has more than one class of dipolar couplings: **cross validation** with quality factor
   \[ Q = \frac{\text{rms} (D_{\text{obs}} - D_{\text{calc}})}{\text{rms} (D_{\text{obs}})} \]

4. Use programs such as Procheck to look at the overall quality of the structure (distribution in Ramachandran plot should improve)

5. In most cases where one obtains a high degree of consistency one would also gain in the overall RMSD of the family of calculated structures.
Quality measure of calculated/simulated RDCs

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

\[
\text{rms}(D_{\text{obs}}) = \left[2 \left(\frac{D_{a \text{norm}}}{\text{rms}}\right)^2 \left(4 + 3R^2\right)/5\right]^{1/2}
\]

\[
Q \sim 17\% \approx 1.8 \text{ Å X-ray}
\]
\[
O \sim 11\% \approx 1.1 \text{ Å X-ray}
\]

- use only for RDCs not included in structure determination!

- no translational validation
Impact of RDC refinement

a) without rdc
b) with rdc