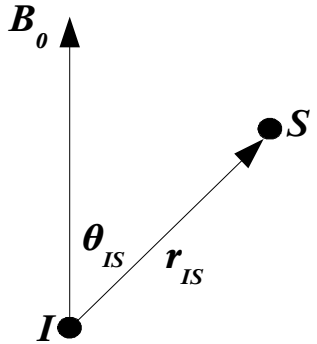
The dipole-dipole interaction

The dipolar Hamiltonian is given by $$\boldsymbol{H}_{ij}^{D}=\frac{\mu_0 \gamma_i \gamma_j h}{8\pi^2 r_{ij}^3}[\boldsymbol{I}_i \boldsymbol{I}_j - 3\frac{1}{r_{ij}^2}(\boldsymbol{I}_i \boldsymbol{r}_{ij})(\boldsymbol{I}_j \boldsymbol{r}_{ij})]$$

for a heteronuclear spin system in the high field approximation the following simplification can be obtained

$$\boldsymbol{H}_{IS}^{D}(t)=-\frac{\gamma_I \gamma_S \mu_0 h}{8\pi^3 \langle r_{IS}^3(t)\rangle}\cdot\frac{\langle(3\cos^2\theta_{IS}(t)-1)\rangle}{2}\boldsymbol{I}_z \boldsymbol{S}_z$$



in analogy to the scalar coupling (relevant spin operator is also $\boldsymbol{I}_z\boldsymbol{S}_z$) the dipolar coupling results in a dublett splitting with $$D_{IS}=-\frac{\gamma_I \gamma_S \mu_0 h}{8\pi^3 \langle r_{IS}^3(t)\rangle}\cdot\frac{\langle(3\cos^2\theta_{IS}(t)-1)\rangle}{2}$$

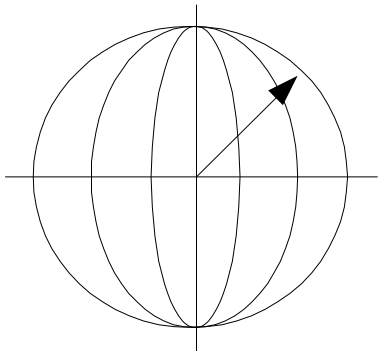
but coupling is a function of orientation relative to the external magnetic field !

Averaging of Dipolar Coupling by molecular tumpling in isotropic solution

molecular tumpling modulates orientation of internuclear vector relative to the static magnetic field in a random manner
time scale for modulation of D_{ij} is significant faster ($\tau_c \approx$ 1-50 ns) than the time scale for signal detection (µs – ms)

→ All orientations have the same probability
Average value of D_{ij} is obtained by integrating orientational dependence over surface of a sphere

$$D_{ij} = -\frac{\gamma_i \gamma_j \mu_0 h}{8\pi^3 \langle r_{ij}^3 \rangle} \cdot \frac{\langle (3\cos^2\theta_{ij} - 1) \rangle}{2}$$



$$\langle 3\cos^2\theta - 1 \rangle = \frac{1}{4\pi} \int_0^{2\pi} d\phi \int_0^{\theta} (3\cos^2\theta - 1)\sin\theta \, d\theta$$

$$= \frac{1}{2} \int_0^{\theta} (3\cos^2 - 1)\sin\theta \, d\theta$$

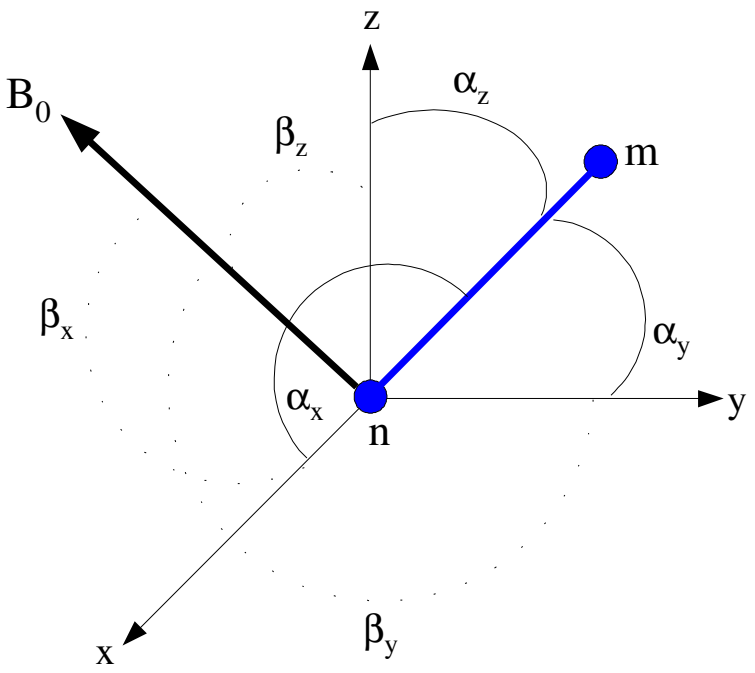
$$= \frac{1}{2} \left[3 \int_0^{\theta} \cos^2\theta \sin\theta \, d\theta - \int_0^{\theta} \sin\theta \, d\theta \right]$$

$$= \frac{1}{2} \left[\left[-3\cos^3\theta \right]_0^{\pi} + \left[\cos\theta \right]_0^{\pi} \right]$$

$$= 0$$

→ random molecular tumpling averages D_{ij} to zero

Description in an arbitrary frame



$$\cos\theta_{mn} = \begin{pmatrix}\cos\alpha_x\\ \cos\alpha_y\\ \cos\alpha_z\end{pmatrix}\cdot\begin{pmatrix}\cos\beta_x\\ \cos\beta_y\\ \cos\beta_z\end{pmatrix} = \cos\alpha_x\cos\beta_x + \cos\alpha_y\cos\beta_y + \cos\alpha_z\cos\beta_z$$

$$\left\langle \frac{3\cos^2\theta_{mn}-1}{2}\right\rangle = \frac{3}{2}\langle(\cos\alpha_x\cos\beta_x+\cos\alpha_y\cos\beta_y+\cos\alpha_z\cos\beta_z)^2\rangle - \frac{1}{2}$$

$$= \frac{3}{2}[\langle c_x^2\rangle C_x^2 + \langle c_y^2\rangle C_y^2 + \langle c_z^2\rangle C_z^2$$
$$+ 2\langle c_x c_y\rangle C_x C_y + 2\langle c_x c_z\rangle C_x C_z + 2\langle c_y c_z\rangle C_y C_z]$$

$$C_i = \cos\alpha_i\,;\; c_i = \cos\beta_i$$

fixed orientation of (m,n) vector relative to molecular frame, averaging only occurs by overall molecular tumbling

The average orientation of the molecule relative to the magnetic field can then be described by a symmetric order matrix ***A*** (alignment tensor), which is traceless (sum of diagonal elements = 0):

$$A_{ij} = \frac{3}{2}\langle\cos\beta_i\cos\beta_j\rangle - \frac{1}{2}\delta_{ij}$$

The dipolar coupling between m, n is then $$D_{mn} = -\frac{\gamma_m\gamma_n\mu_0 h}{8\pi^3\langle r_{mn}^3\rangle}\sum_{kl}A_{kl}\cos\alpha_k\cos\alpha_l = D_{mn}^{max}\sum_{kl}A_{kl}\cos\alpha_k\cos\alpha_l$$

if the alignment tensor become collinear with the molecular frame, the order matrix will be of diagonal form:

$$A=\begin{pmatrix} A_{xx} & 0 & 0 \\ 0 & A_{yy} & 0 \\ 0 & 0 & A_{zz} \end{pmatrix} \qquad |A_{zz}|>|A_{yy}|>|A_{xx}|$$

The dipolar coupling becomes then $D_{mn}^{max}(\alpha_x,\alpha_y,\alpha_z)=\sum_{i=x,y,z} A_{ii}\cos^2\alpha_i$

transforming into polar coordinates results in $D_{mn}(\theta,\phi)=D_{mn}^{max}[A_{zz}\cos^2\theta+A_{xx}\sin^2\theta\cos^2\phi+A_{yy}\sin^2\theta\sin^2\phi]$

$$\cos\alpha_z=\cos\theta$$
$$\cos\alpha_x=\sin\theta\cos\phi$$
$$\cos\alpha_y=\sin\theta\sin\phi$$

with $D_a=\frac{1}{2}A_{zz}D_{mn}^{max}$ $D_r=\frac{1}{3}(A_{xx}-A_{yy})D_{mn}^{max}$ $R=\frac{D_r}{D_a}$

axial component rhombic component rhombicity

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

Description of dipolar coupling in the principal frame of the alignment tensor

Diagonalization of the Alignment tensor

$$D = V^{-1} A V \qquad \text{and} \qquad A = V D V^{-1}$$

V is rotation matrix, describing the rotation of the molecular frame to coincide with the alignment tensor

Diagonalization	Solving the Eigenvalue problem $\boldsymbol{A}\, v = \lambda\, v$ Eigenvalues are $\boldsymbol{D}_{ii}$ (i = x, y, z) Eigenvectors describe the rotation matrix $\boldsymbol{V}$

Rotation of frames is often given by three Euler angles (α, β, γ):

1. Rotation around z-axis with angle α
2. Rotation around y-axis with angle β
3. Rotation around z-axis with angle γ

Warning: Check convention, whether rotation of the Alignment tensor or the rotation of the molecular frame is meant

Determination of the Alignment tensor with given dipolar couplings, D_i, and a known structure

→ the direction cosini, cos α_{in}, for the different vectors can be calculated from the structure
the couplings D_i are measured:

$$D_{nm}(\alpha_x, \alpha_y, \alpha_z) = D_{nm}^{max} \sum_{i,j=x,y,z} A_{ij} \cos\alpha_i \cos\alpha_j$$

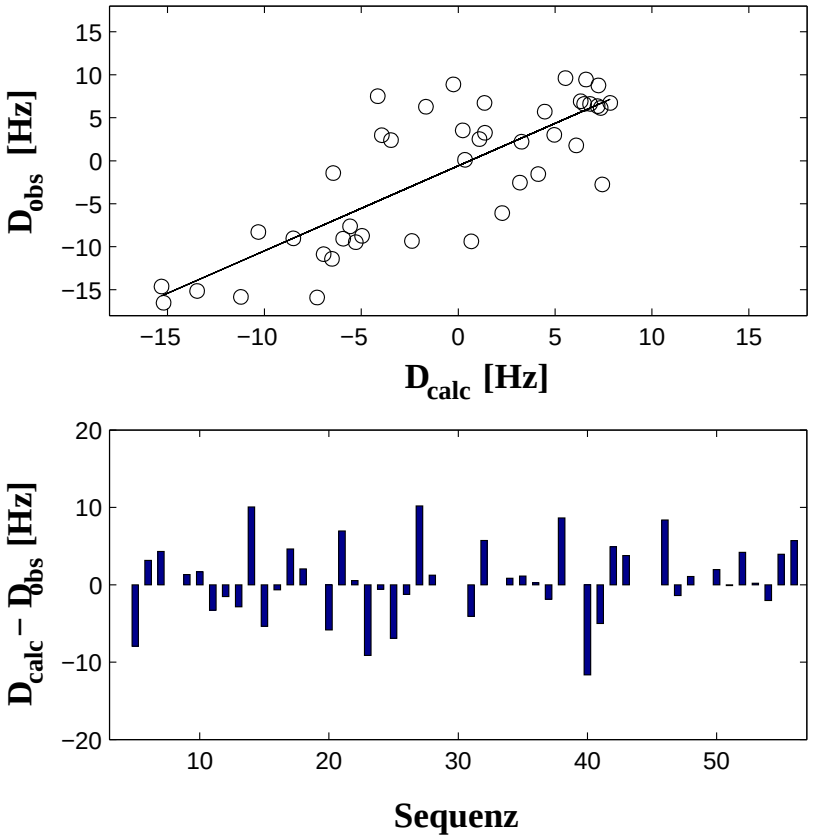
$$\begin{pmatrix} c_{y1}^2 - c_{x1}^2 & c_{z1}^2 - c_{x1}^2 & 2c_{x1}c_{y1} & 2c_{x1}c_{z1} & 2c_{y1}c_{z1} \\ c_{y2}^2 - c_{x2}^2 & c_{z2}^2 - c_{x2}^2 & 2c_{x2}c_{y2} & 2c_{x2}c_{z2} & 2c_{y2}c_{z2} \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ c_{yn}^2 - c_{xn}^2 & c_{zn}^2 - c_{xn}^2 & 2c_{xn}c_{yn} & 2c_{xn}c_{zn} & 2c_{yn}c_{zn} \end{pmatrix} \begin{pmatrix} A_{yy} \\ A_{zz} \\ A_{xy} \\ A_{xx} \\ A_{yz} \end{pmatrix} = \begin{pmatrix} D_1 \\ D_2 \\ \cdots \\ \cdots \\ D_n \end{pmatrix}$$

$$\cos\alpha_{in} = c_{in} \quad i = x, y, z$$

overdetermined system of linear equations (n > 5), can be solved by numerical methods
(e.g. Singular Value Decomposition SVD)

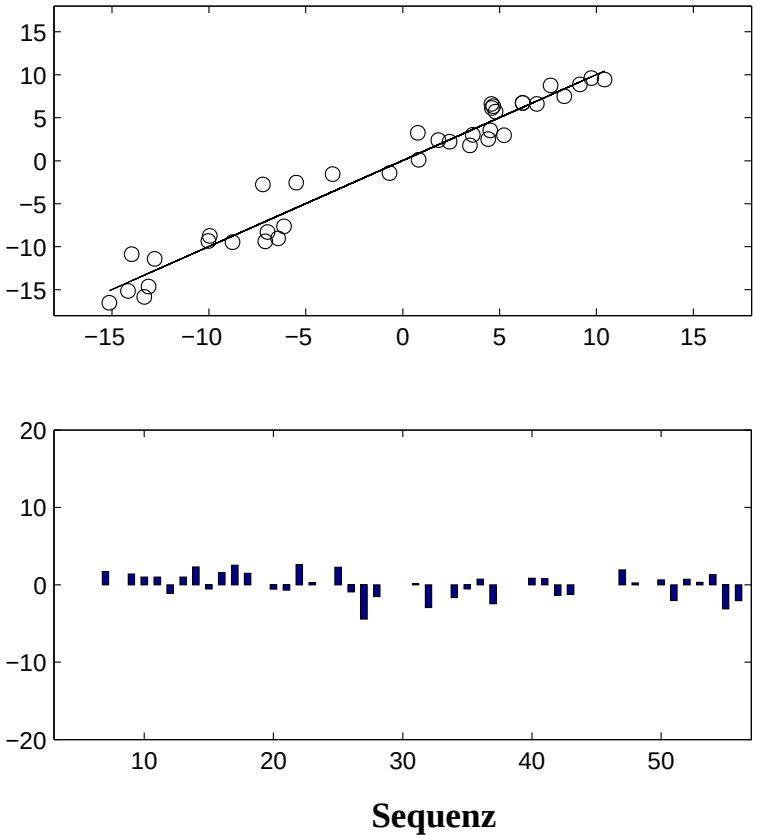
NMR Struktur (1DX8, keine RDC's)

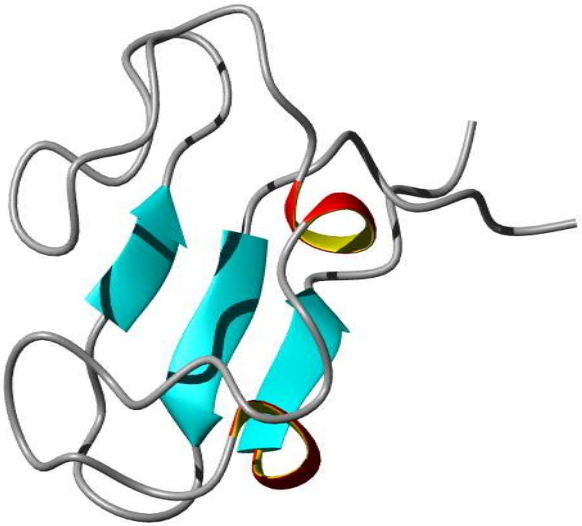
rmsd = 4.99 Hz



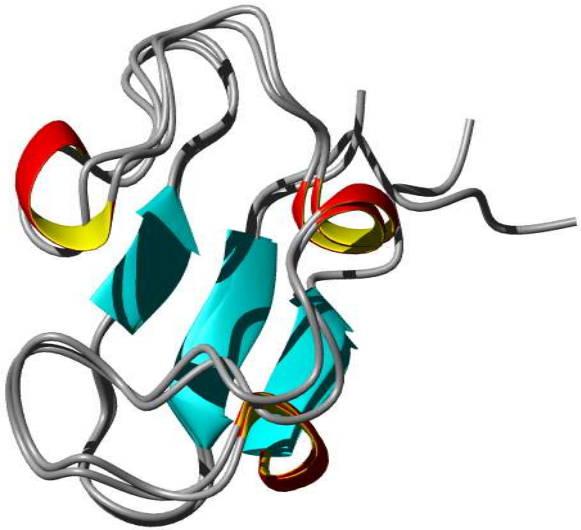
Kristallstruktur (1RDG, 1.4 A)

rmsd = 1.69 Hz

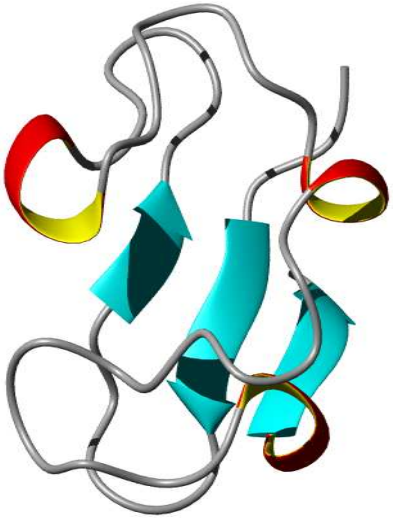




NMR structure of
Guillardia theta rubredoxin



overlay of both
backbone rmsd : 1.0 Å



1.4 Å crystal structure of
Desulfovibrio gigas rubredoxin

Quality parameter for least-square fit of the Alignment tensor

root mean square deviation (rmsd) of observed and calculated couplings

$$rmsd = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(D^{i}_{meas} - D^{i}_{calc}\right)^{2}}$$

Problem: no normalization to the absolute values of measured values

Q-factor: Normalization of the rmsd to the absolute value of measured couplings

$$Q = \frac{rmsd}{D^{rms}_{meas}}$$

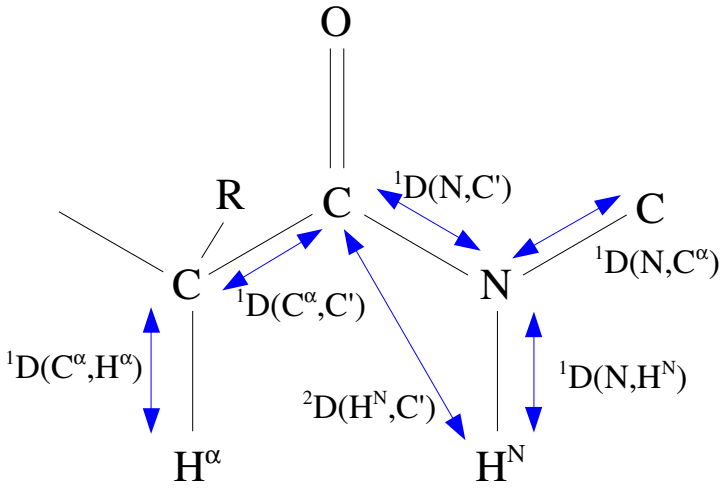
$$D^{rms}_{meas} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(D^{i}_{meas}\right)^{2}}$$

for isotropic distribution of the couplings

$$D^{rms}_{meas} = \left\{D_a^2\left(4+3R^2\right)/5\right\}^{1/2}$$

Remark: If couplings are used for structure calculation, the 'quality factors' do not serve for an independent measure of quality !

Residual dipolar couplings in the protein backbone peptide bond geometry results in rigid distances !



normalization of RDC's to ^1D(N, H^N)

$$D^r_{OP} = D_{OP}\frac{\gamma_N \gamma_H r^3_{OP}}{\gamma_O \gamma_P r^3_{NH}}$$

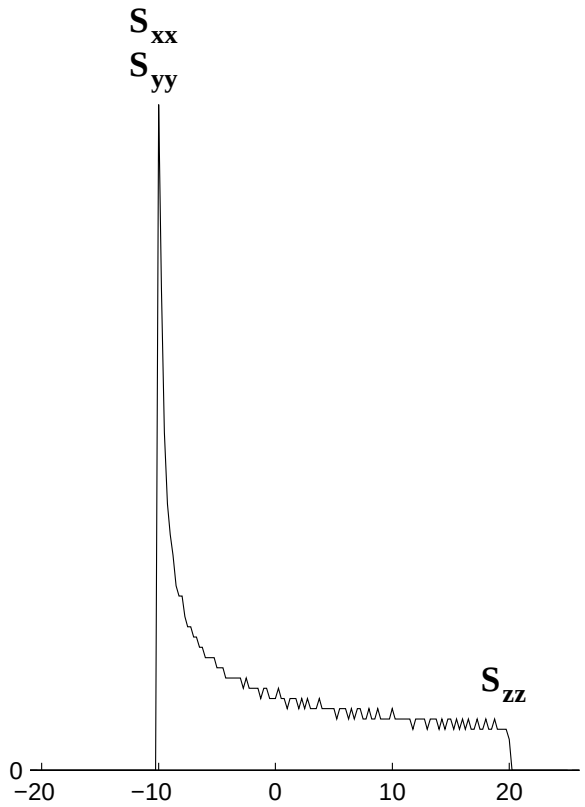
$$\frac{^1\text{D(N,H}^N\text{)}}{^1\text{D(C}^\alpha\text{,H}^\alpha\text{)}} = -0.5$$

$$\frac{^1\text{D(N,H}^N\text{)}}{^1\text{D(N,C')}} = 8.2$$

$$\frac{^1\text{D(N,H}^N\text{)}}{^2\text{D(H}^N\text{,C')}} = -3.2$$

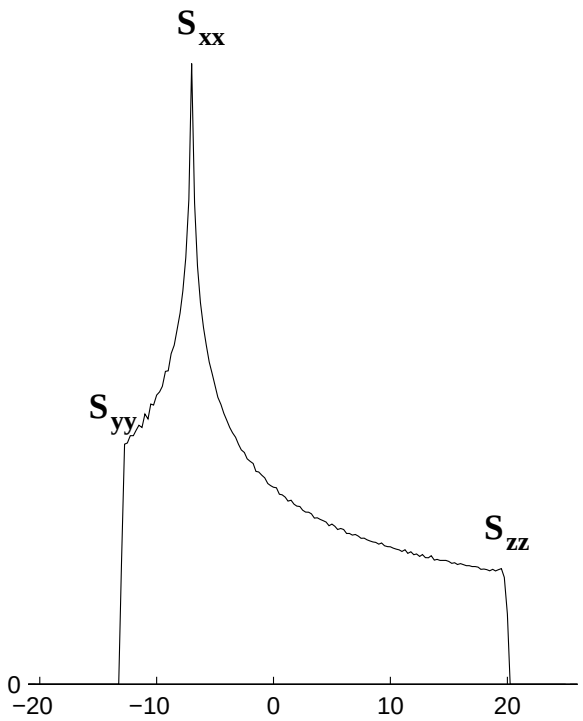
isotropic distribution of RDC's

→ powder pattern like distribution



axialsymmetrischer Tensor

D_a = 10 Hz, R = 0

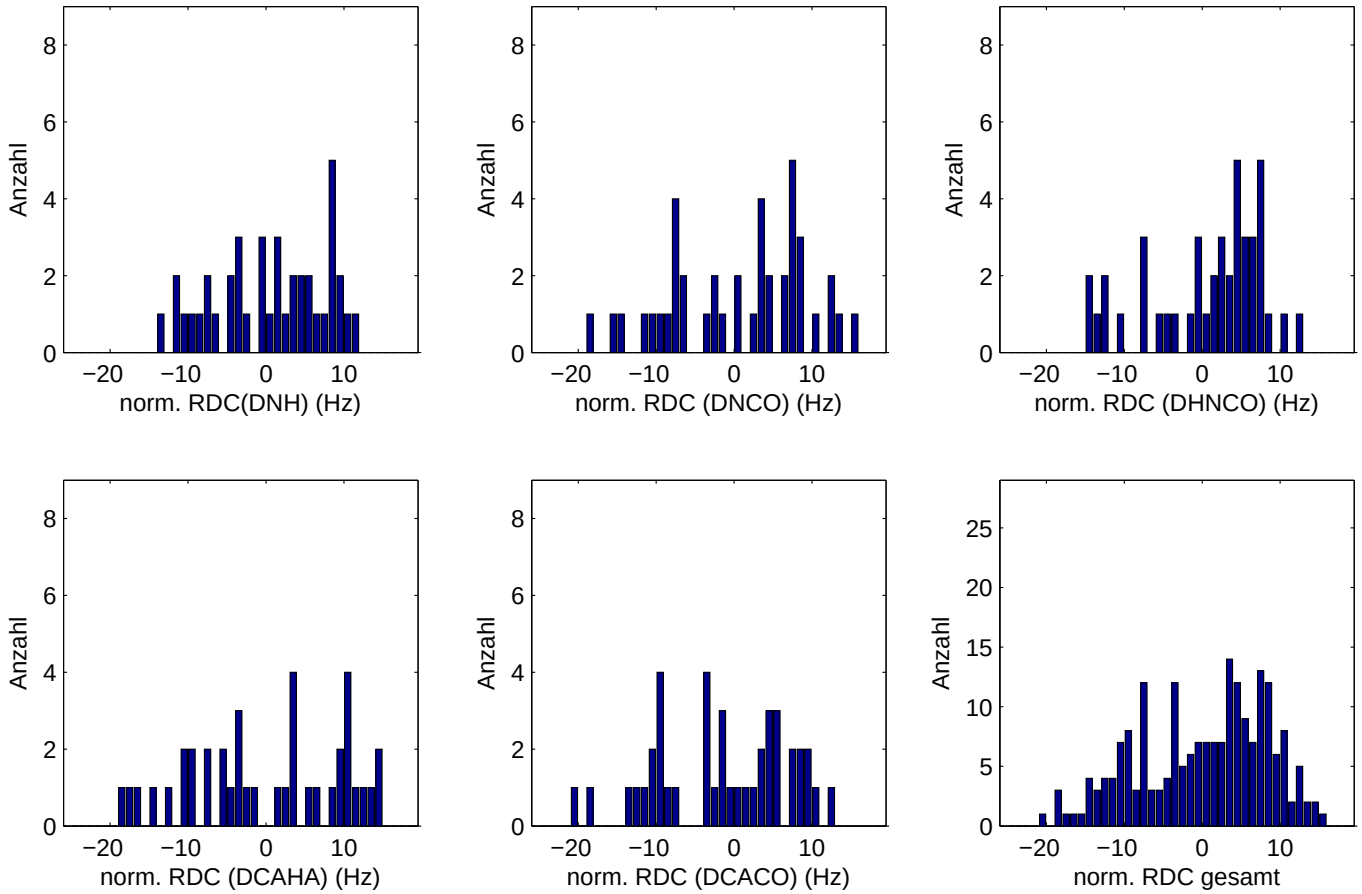


rhombischer Tensor

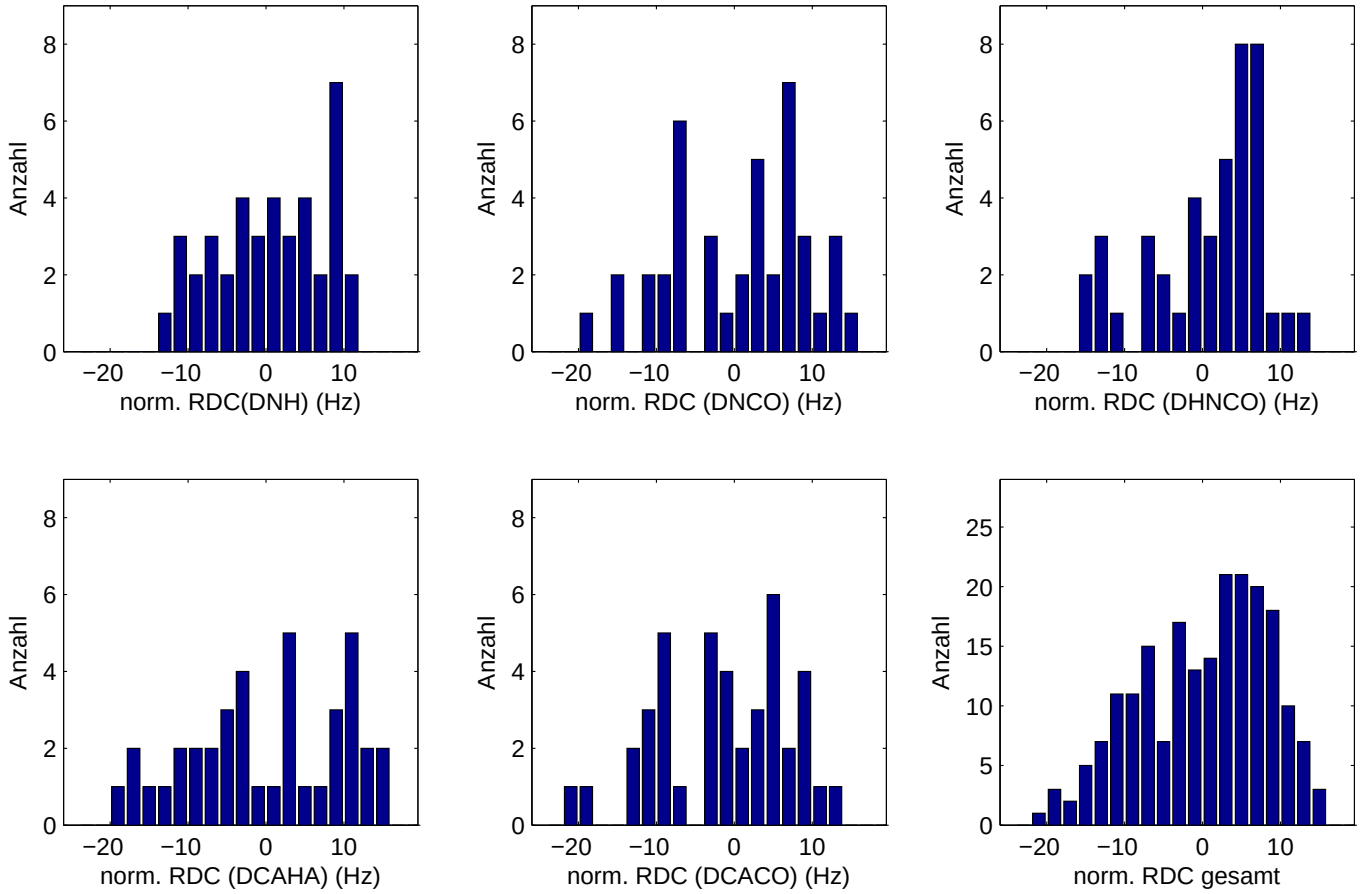
D_a = 10 Hz, R = 0.3

$S_{zz} = 2\ D_a$ $\quad S_{yy} = -D_a(1 + 1.5R)$ $\quad S_{xx} = -D_a(1-1.5R)$

Distribution of couplings in a real protein
hostrogram methods for estimating the alignment tensor

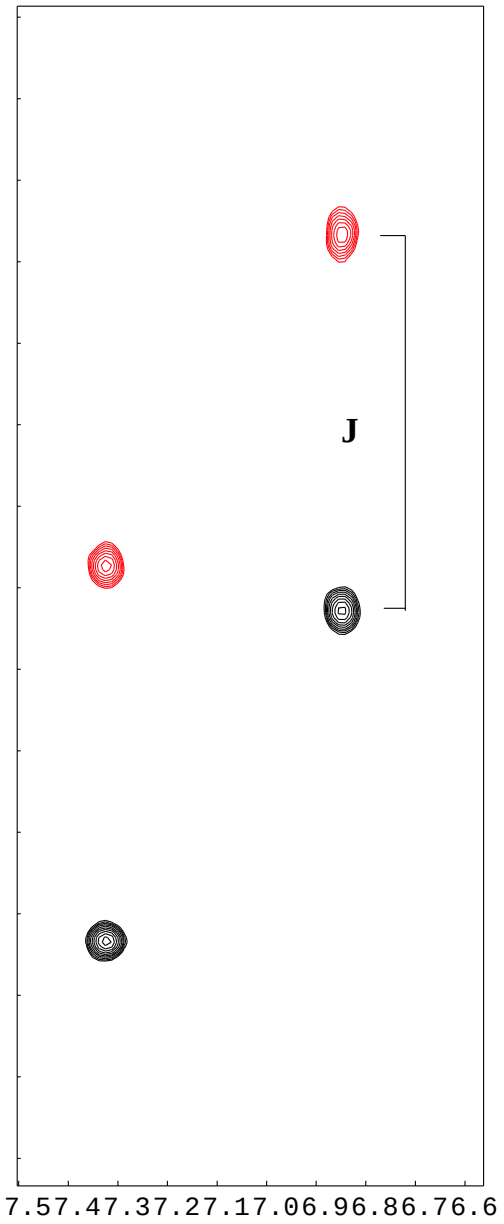


step width in histogram : 1 Hz

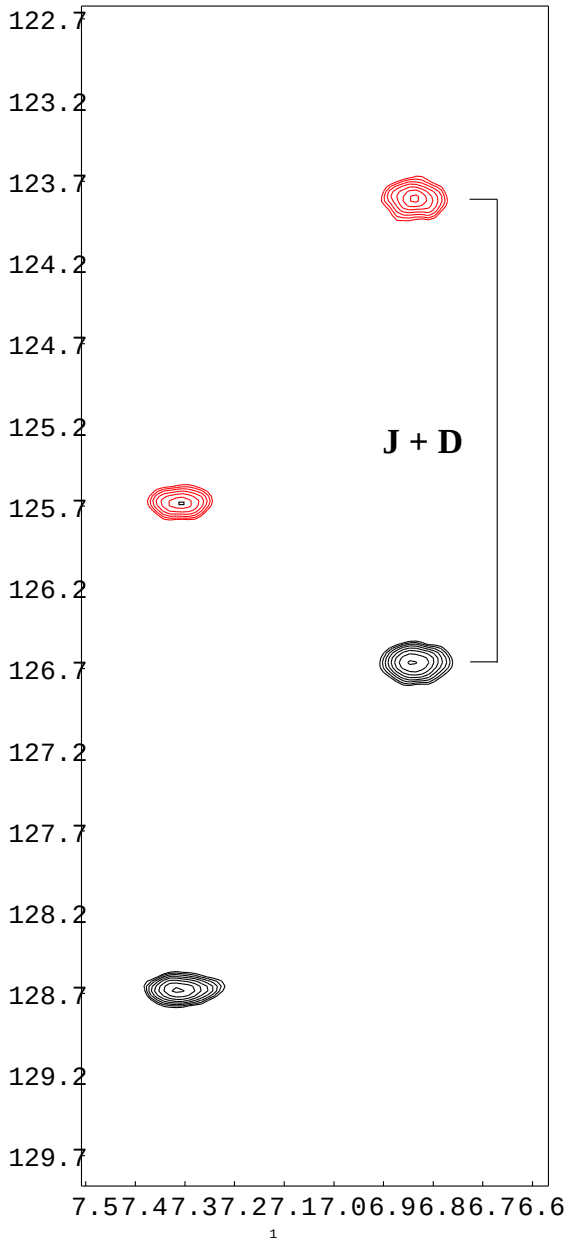


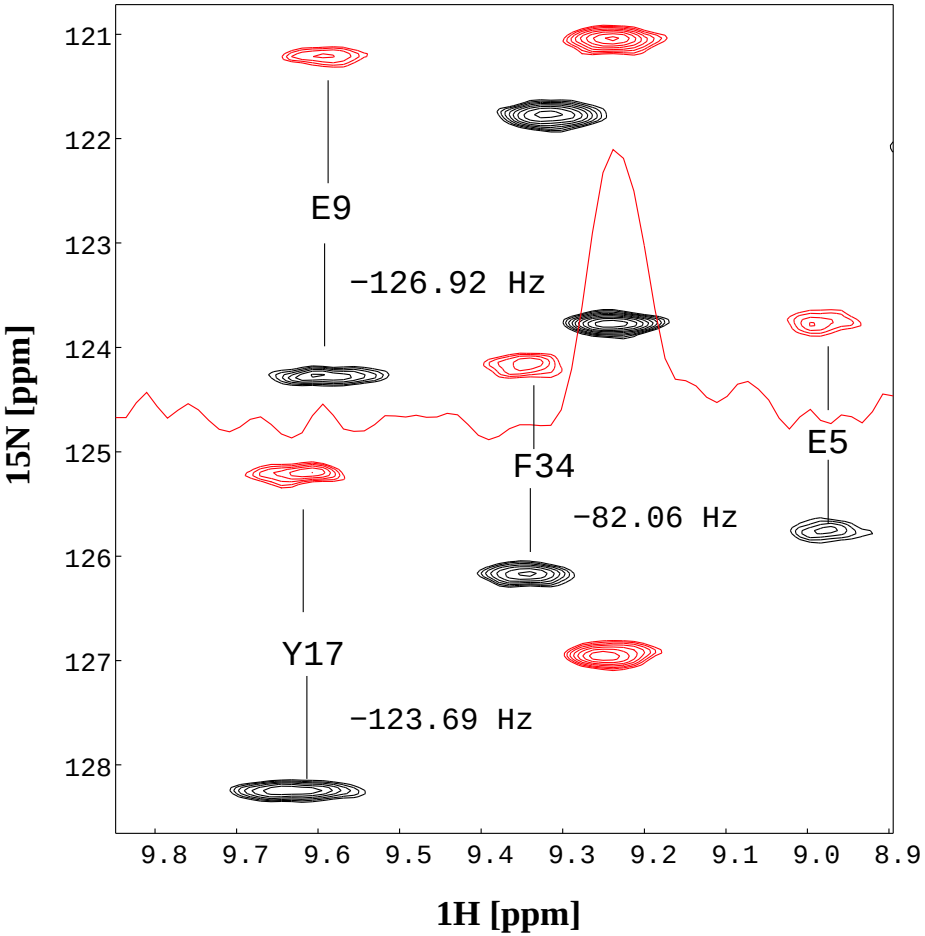
step width in histogram: 2 Hz

unorientiert

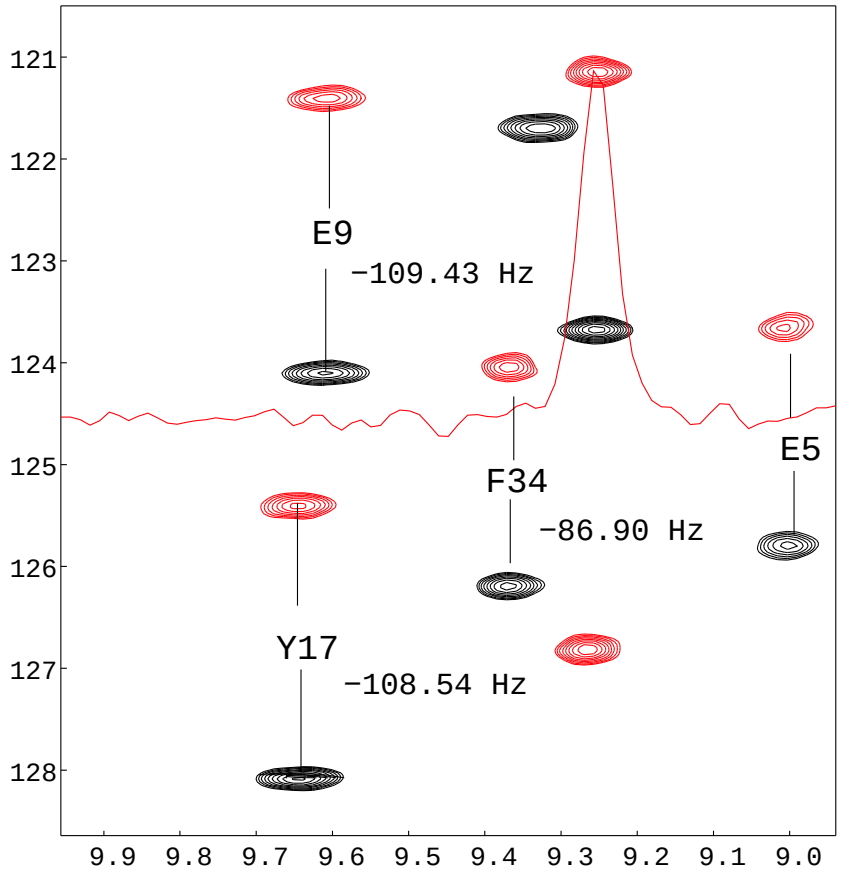


orientiert





G. theta rubredoxin in Pf1 phage solution (25 mg/ml)



G. theta rubredoxin in Pf1 phage solution (18 mg/ml)

Practical aspects for determination of RDC's

orientation to large	inefficient coherence transfer because of large variation of ($^{1}J + {}^{1}D$) passive ^{n}D (e.g. between protons) increase line width
orientation to small	RDC's are small, especially $^{1}D(N, C)$ cannot be determined
optimal orientation	D_a ($^{15}N, {}^{1}H^{N}$) approx. 8-15 Hz, that means maximal $^{1}D(H^{N}, N)$ approx. 16-30 Hz

Media for orienting biological macromolecules

general: The degree of alignment of the protein (nucleic acid) should be ca. 0.001 (-> D_a^{HN} ca. 10 Hz)

liquid crystalline media have a degree of alignment of ca. 0.5 -0.85

→ very weak interaction between protein (nucleic acid) and alignment medium

typical alignment media

very large particles (> 500 Å)

large free spaces between the particles allow weak orientation

together with rotational freedom comparable to isotropic solution

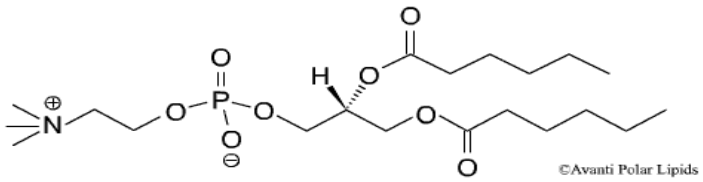
only small contributions to transverse relaxation

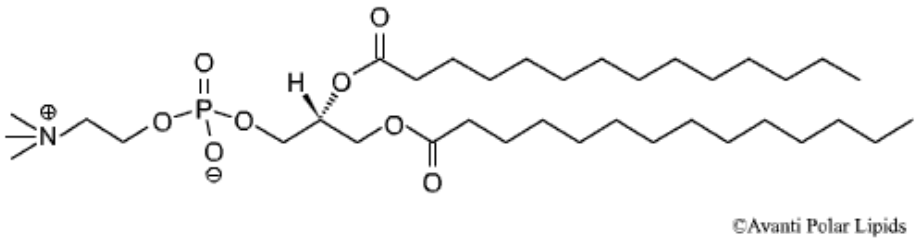
Bicelles

first liquid crystalline medium for the weak alignment of proteins and DNA

consists of usual phospholipids, they form rod-like micelles that orient in the magnetic field

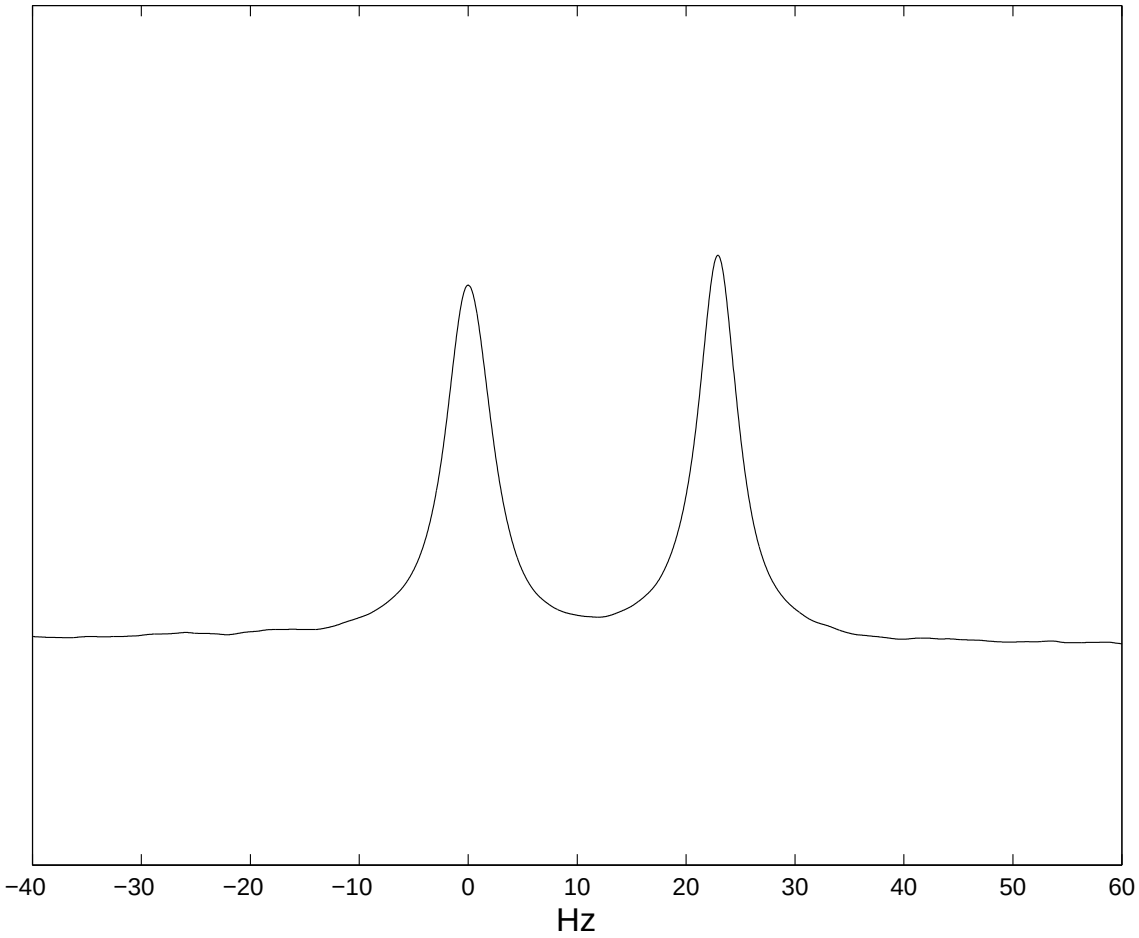
examples: mixtures of DMPC (Dimyristoyl-phosphatidylcholine)
and DHPC (Dihexanoylphosphatidylcholine)
molar ratio DMPC:DHPC 3:1, 5% w/v lipids in water (5 g / 100 ml)
liquid crystalline phase in temperature range 30° - 45° C
bicelles can be stabilized with ionic detergents (SDS or CTAB)





filamentous phages (fd, Pf1)	contour length of 2 μm, diameter 6.5 nm liquid crystalline at low concentrations of few mg / ml ionic strength reduces alignment at concentrations < 15 mg / ml in praxis: interaction between protein and phage is electrostatic !
further media	ternary mixtures of cetylpyridimiumchloride (bromide), hexanol and NaCl (NaBr) in water
	purple membrane fragments (cellular membrane from *Halobacterium salinarum*)
	Alkyl-polyethylene glycol based media e.g. C10E5 : hexanol (molar ratio 0.95), 3% (wt) C10E5 in water

^{2}H quadrupolar splitting in 25 mg/ml Pf1 phage solution



Structure calculation with RDC's

XPLOR refinement

1. Calculation of a structure ensemble with 'classical' restraints (NOE's, J-couplings, hydrogen bonds)
2. Determination of the alignment tensor (D_a, R)

   either by analysis of RDC-distribution (histograms)

   or least square fit to preliminary structures

then ' restrained molecular dynamics' refinement with additional potential $E_{dip} = k_{dip}(D_{obs} - D_{calc})^2$

The orientation relative to a external coordinate system will be varied.

These coordinate system represents the alignment tensor.

general problems: additional potential term creates an energy surface with a large number of local minima, this results in bad convergence during structure calculation.

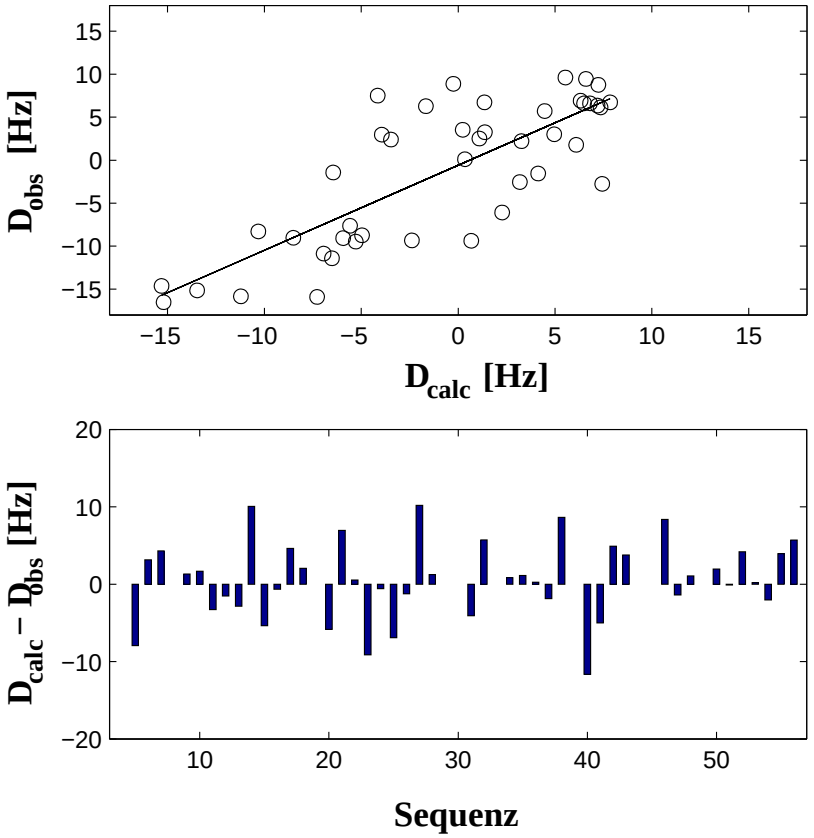
Improvements: several independent repetitions of individual refinements

increasing simulation time (very slow cooling)

Inclusion of RDC's into structure calculation

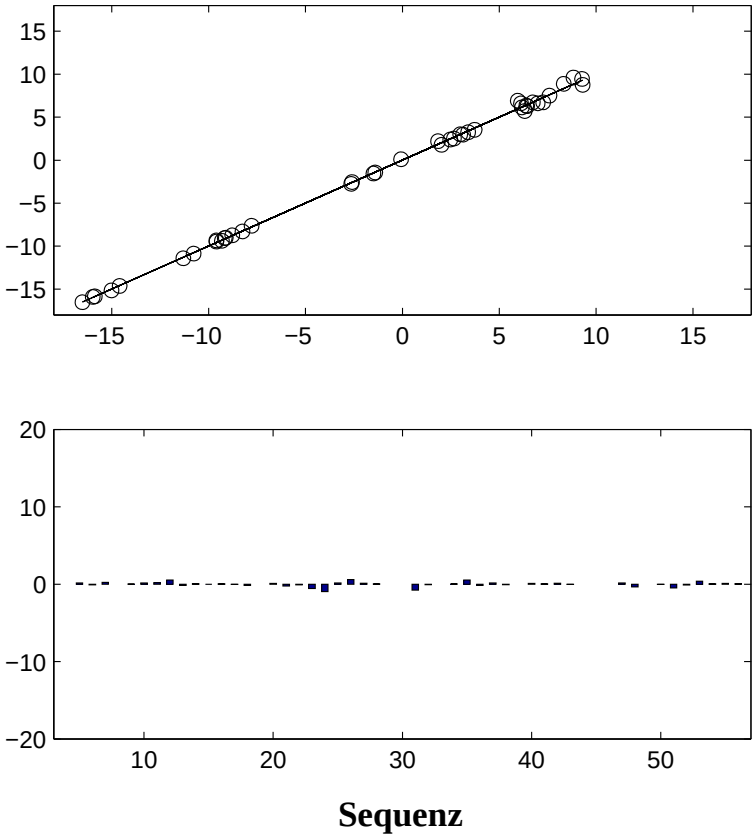
NMR Struktur (1DX8, keine RDC's)

rmsd = 4.99 Hz

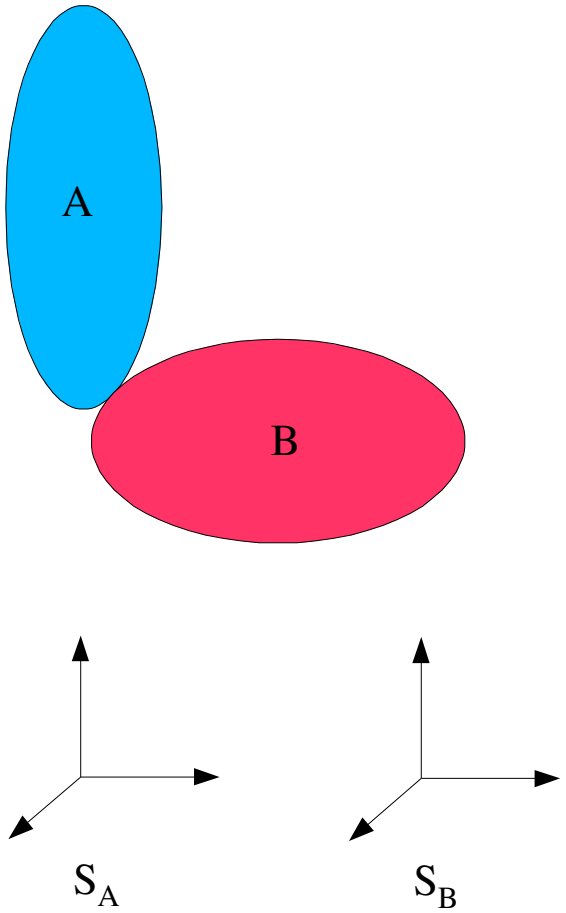


NMR Struktur nach RDC Refinement

rmsd = 0.3 Hz



Domain orientation



only few NOE's define the domain interface

therefore ususally bad definition of domain orientation

with 'classical' NMR restraints

rigid orientation: $S_A = S_B$

one alignment tensor describes the complete alignment

of the complete molecule (both domains)

relative flexibility: $S_A \neq S_B$

two different alignment tensors (each for one domain)

are necessary for describing the alignment

relative flexibilityt in multidomain proteins
(Braddock et al. JACS 123 (2001), 8634.)