Residual dipolar couplings and orientational effects

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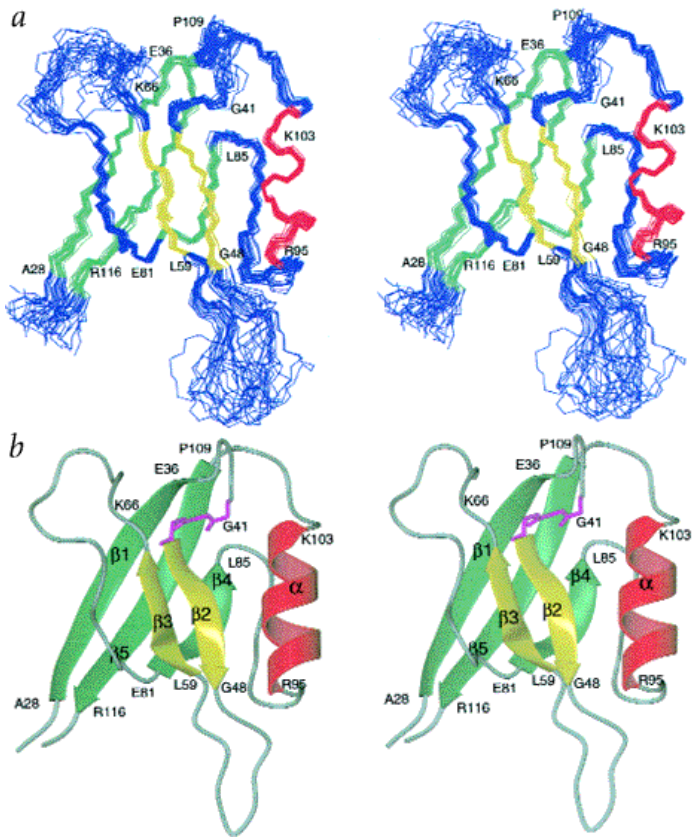
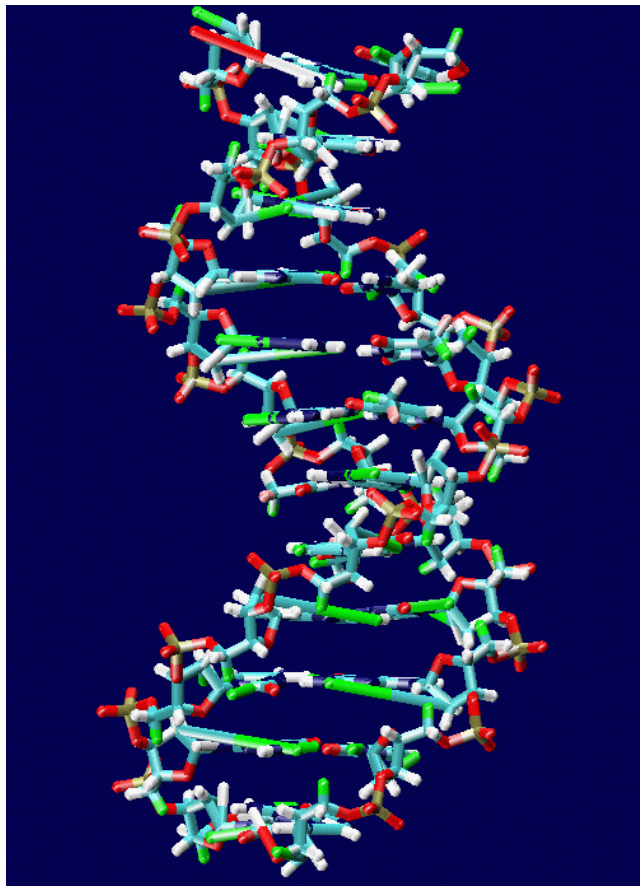
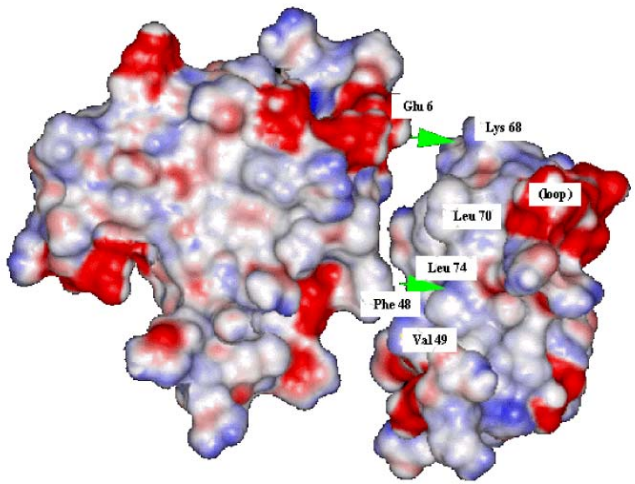
1) Why do we want to use dipolar couplings in solution NMR?



Nucleic acids – global structure

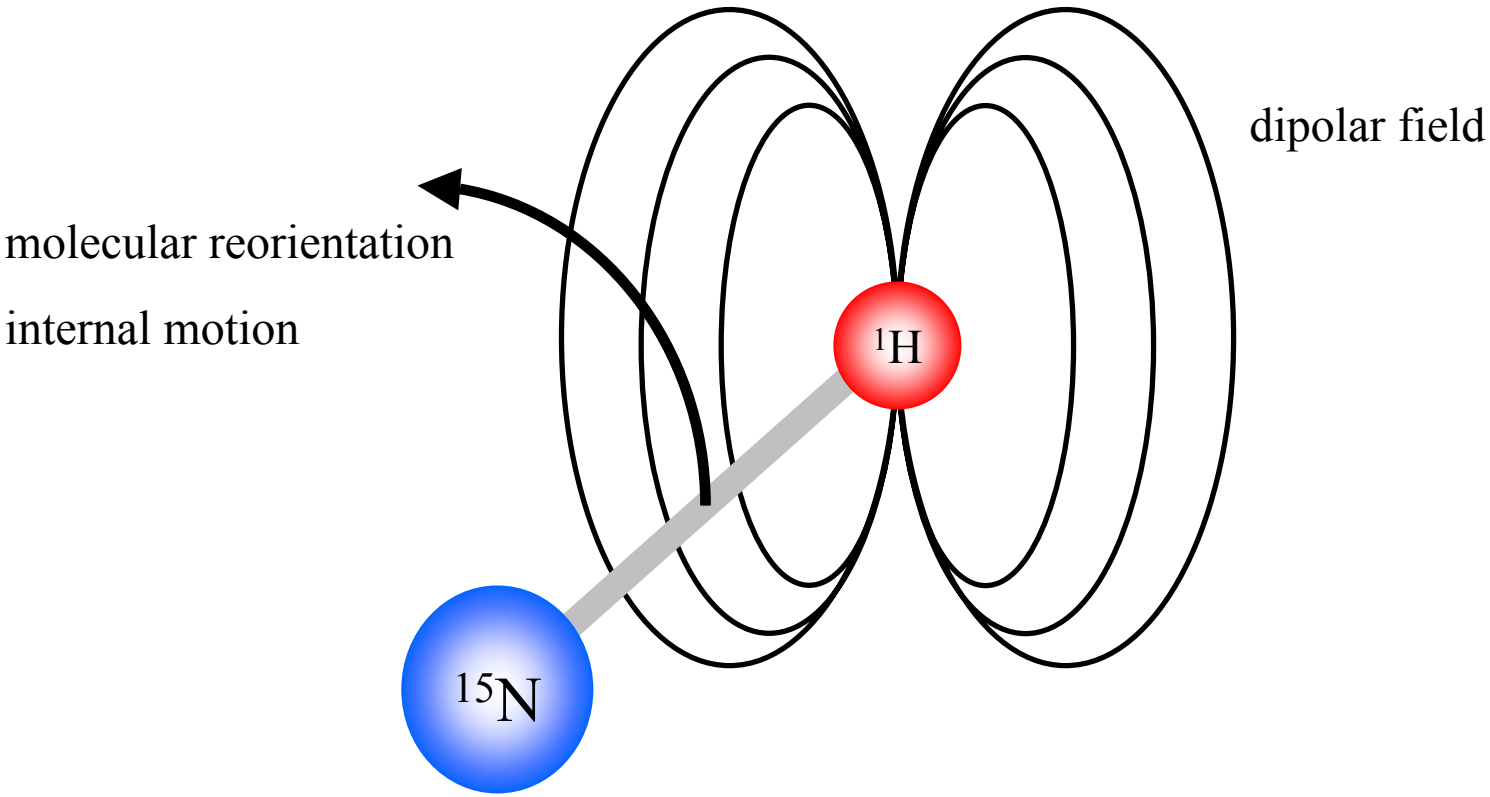


Protein-Protein complexes



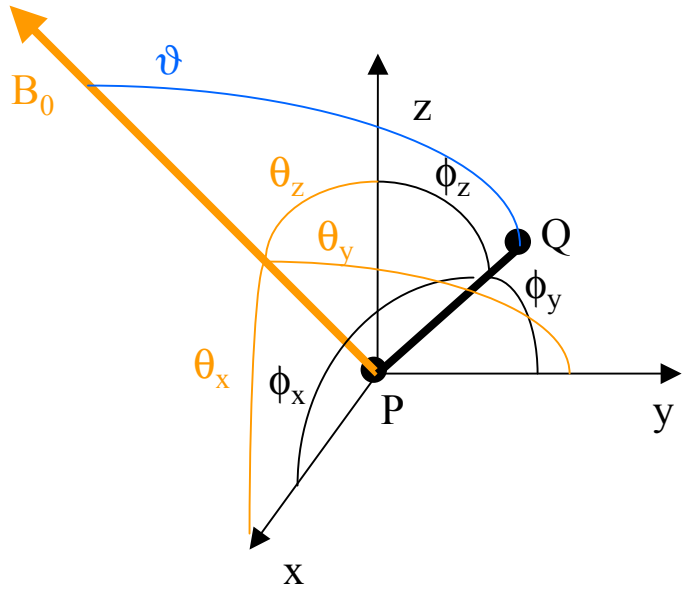
IGF-II mini-IGFBP-5

2) RDC theory


dipolar field
molecular reorientation
internal motion
^{1}H
^{15}N

$$D^{PQ} = D^{PQ}_{max} \langle P_2(\cos\vartheta)\rangle \quad \text{with } D^{PQ}_{max} = -\mu_0\gamma_p\gamma_q h/(8\pi^3\langle r_{pq}^3\rangle)$$

$$P_2(x) = \tfrac{1}{2}(3x^2 - 1)$$



$$D^{PQ} = D^{PQ}_{max} \sum_{ij} S_{ij} \cos\phi_i^{PQ} \cos\phi_j^{PQ} \quad \text{with}$$

$$S_{ij} = 1/2 \langle 3\cos\theta_i \cos\theta_j - \delta_{ij}\rangle$$

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

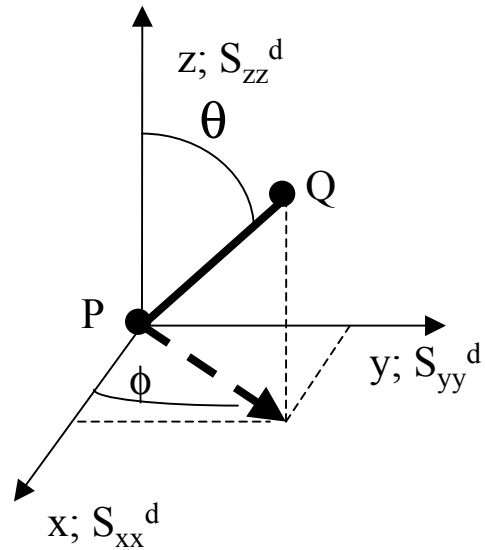
S: Saupe matrix, alignment tensor
Real, symmetric, traceless 3x3 matrix
➔ five independent elements

principal alignment frame, i.e. diagonalization of **S** ➔ $\mathbf{S}^d$

principal alignment frame, i.e. diagonalization of $\mathbf{S}$ → $\mathbf{S}^{d}$
eigenvectors of $\mathbf{S}^{d}$ are axes of alignment tensor

$$D^{PQ} = \tfrac{1}{2}\, D^{PQ}_{max} \left[A_a \left(3\cos^2\theta - 1\right) + \tfrac{3}{2} A_r \sin^2\theta \cos(2\phi)\right] \quad \text{with}$$

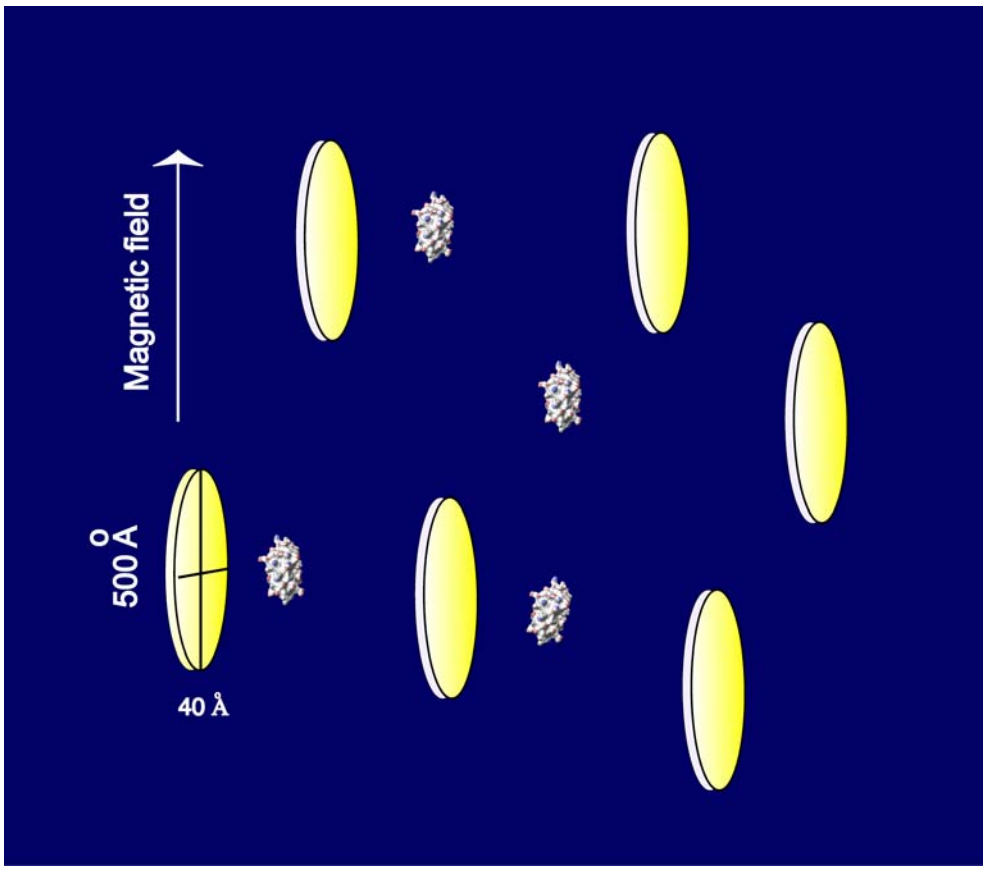
A_a and A_r the axial, S_{zz}^{d} , and rhombic, $2/3\ (S_{xx}^{d} - S_{yy}^{d})$,
components of the diagonalized alignment tensor $\mathbf{S}^{d}$ ($A_a \sim 10^{-3}$)



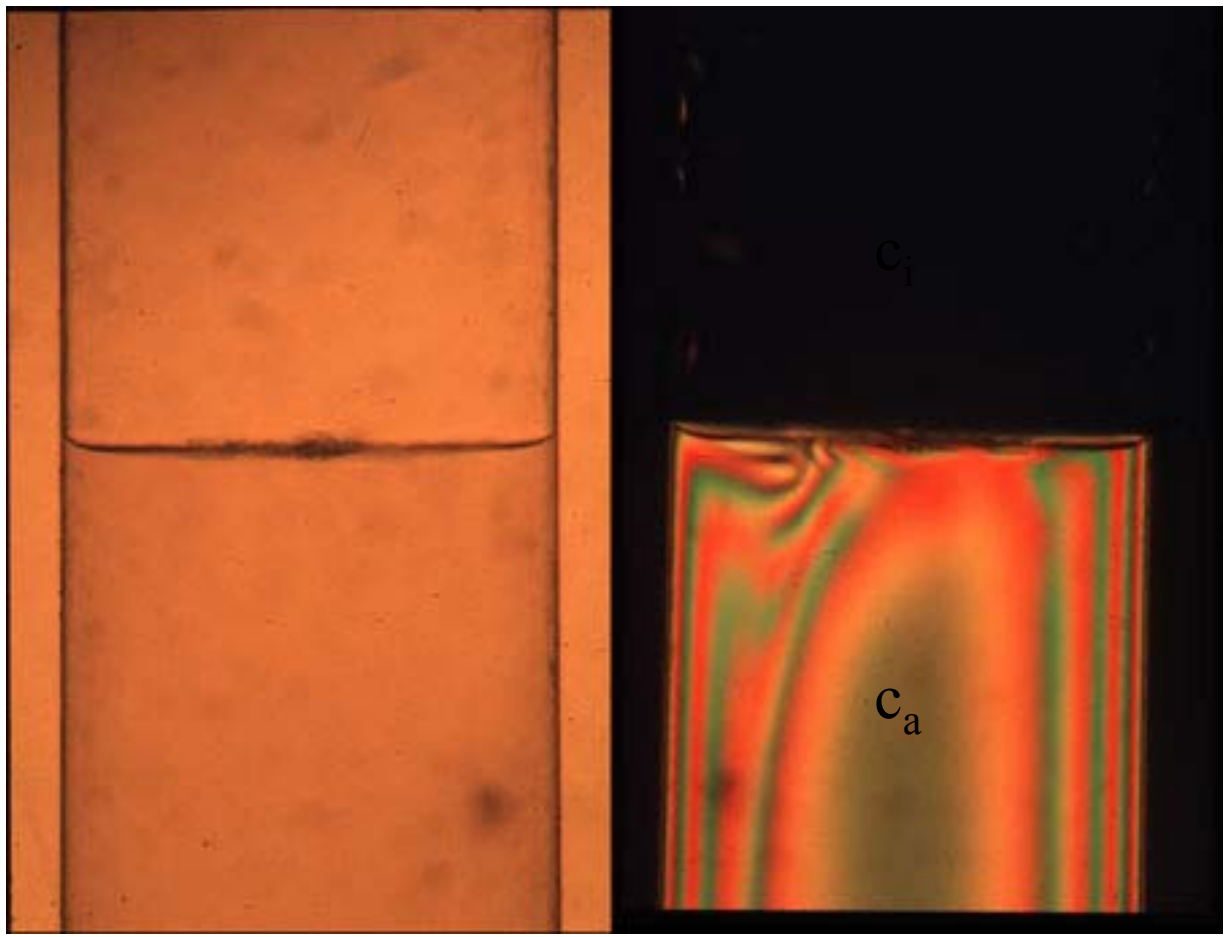
$$\boxed{D^{PQ} = D_a^{PQ} \left[\left(3\cos^2\theta - 1\right) + \tfrac{3}{2} R \sin^2\theta \cos(2\phi)\right]}$$

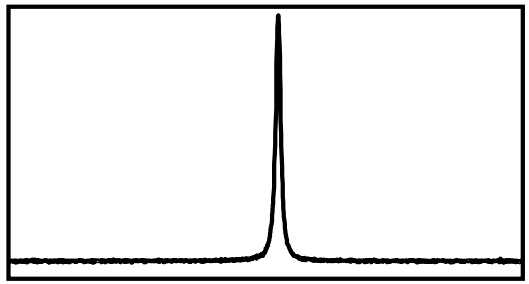
$D_a^{PQ} = \tfrac{1}{2}\, D^{PQ}_{max} A_a$: magnitude of alignment tensor ($D_a^{NH} \sim 10$ Hz)
$R = A_a/A_r$: rhombicity of alignment tensor; $R \in [0; 2/3]$
θ,ϕ: polar coordinates of vector PQ relative to alignment tensor

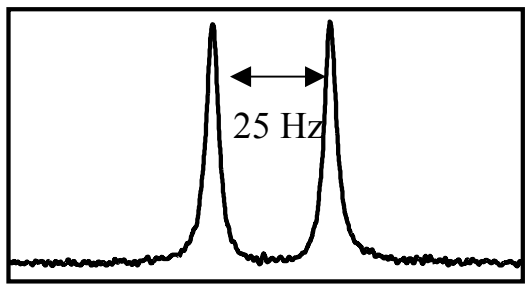
3) How to get partial alignment of biomolecules



Dilute nematic liquid crystals





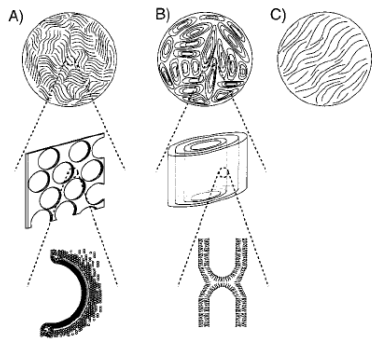


$B_2 c_p > c_a$

$B_2 = \pi D_{eff} L^2/4$

Alignment media

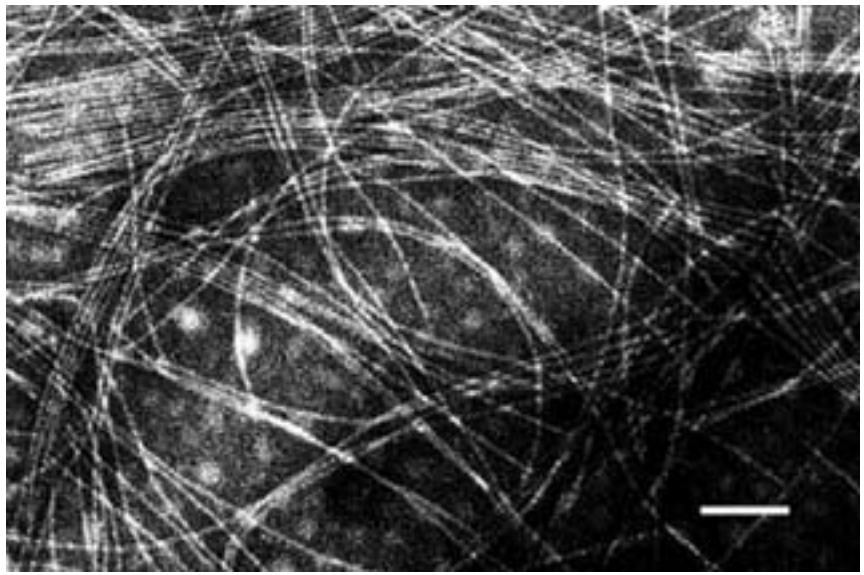
requirement: liquid crystalline at < 10% w/v
➔order of biomolecules: ~ 0.002
(aqueous, stable at different ionic strength,
not too strongly charged < 0.5 e/nm^2)



- 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, ...

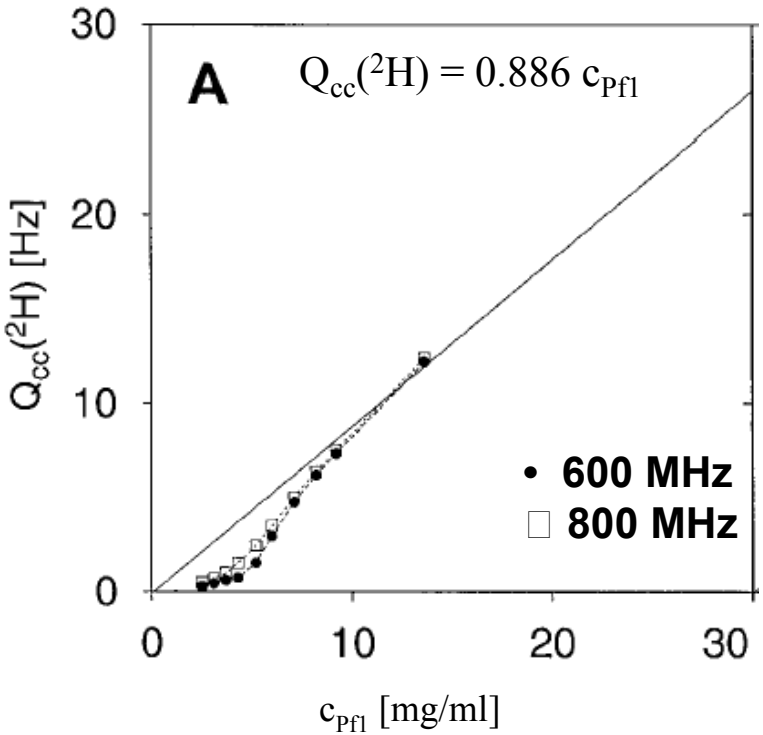
Gaemers & Bax
JACS 2001

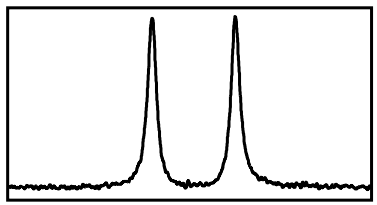
Pf1 bacteriophage

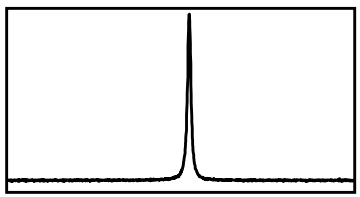


http://www.asla-biotech.com/asla-phage.htm

Pf1: magnetic field induced order





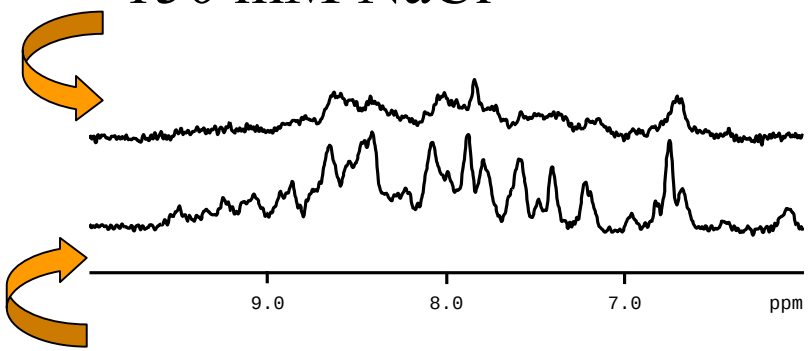


Zweckstetter & Bax, JBNMR 2001

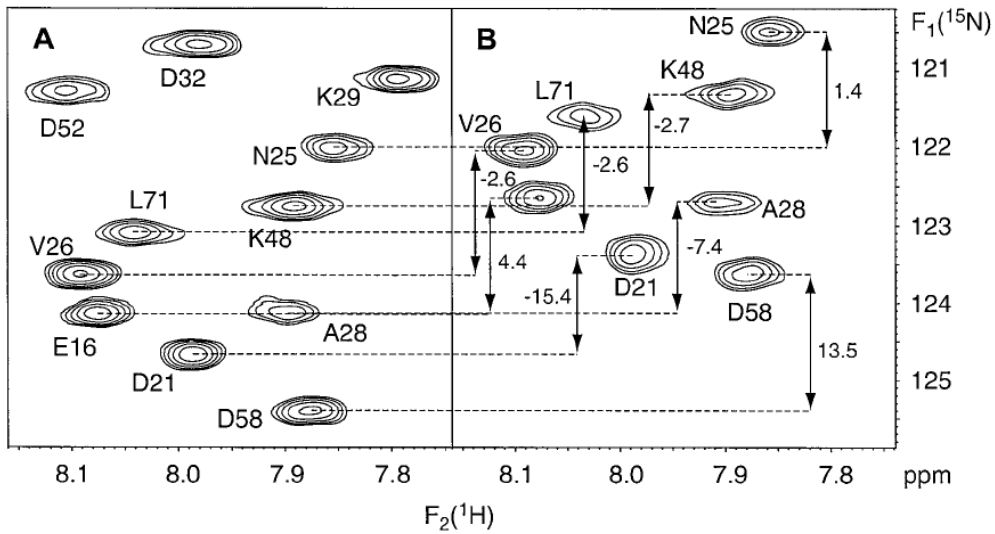
Attenuation of alignment strength by increasing the ionic strength

20 mg/ml Pf1

150 mM NaCl

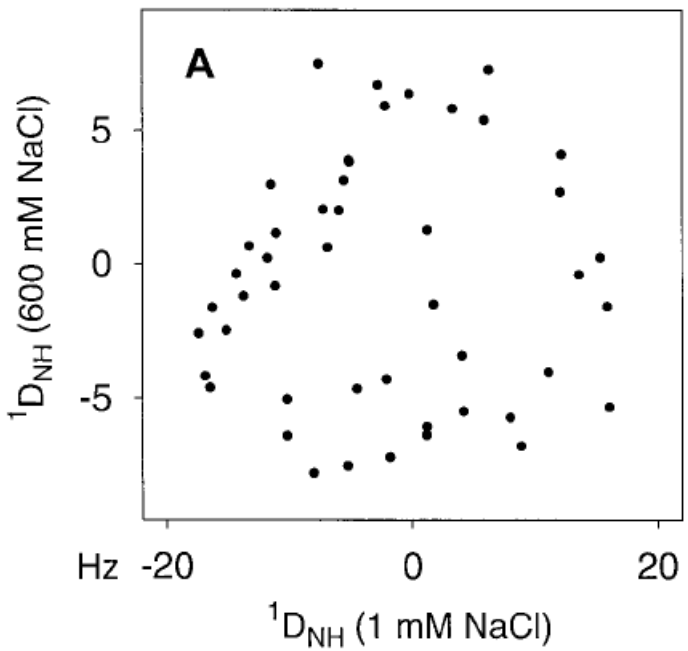


450 mM NaCl



ubiquitin at 450 mM NaCl
in 20 mg/ml Pf1

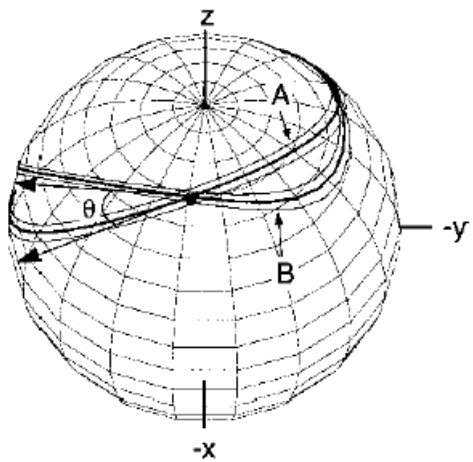
Modulation of alignment tensor orientation by ionic strength changes



GB1

Orientational degeneracy of RDC – use of multiple media

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

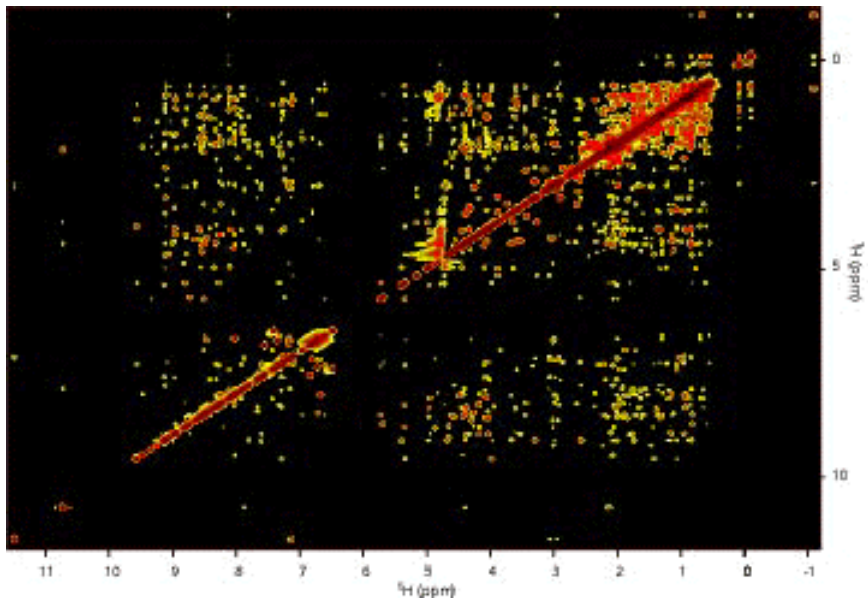


Ramirez & Bax

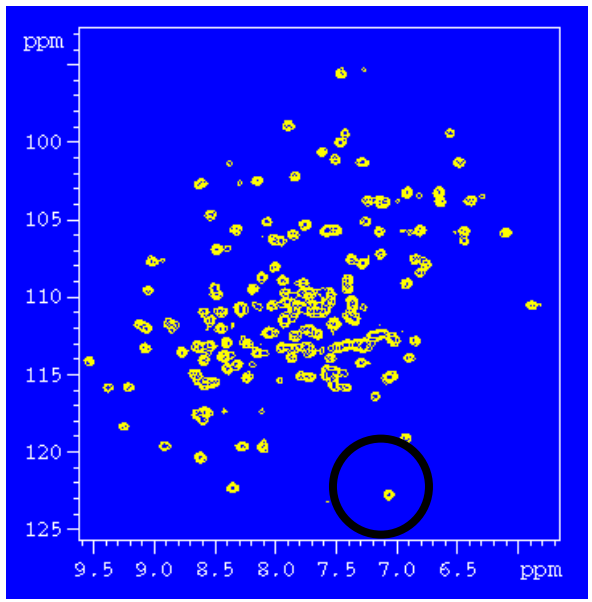
JACS, 1998

4) RDC measurement

NOESY



HSQC



Accuracy of measured splitting: $\Delta J = LW/SN$

required accuracy < 5% * Da

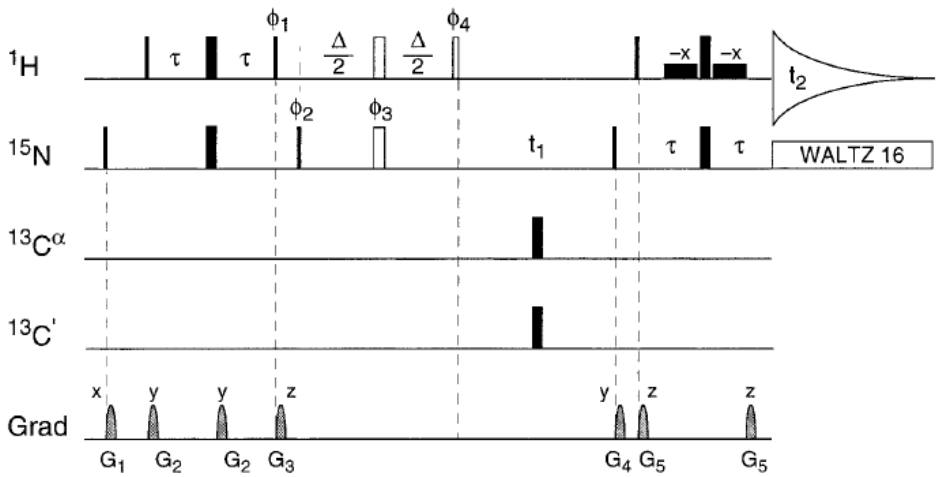
$^{1}J_{HN}$ [1]: IPAP-HSQC, DSSE-HSQC, 3D HNCO
$^{1}J_{C'C\alpha}$ [5]: 3D HNCO (CSA(C') ➔~ 500 MHz optimum)
$^{1}J_{C'N}$ & $^{2}J_{C'HN}$ [8.3]: 2D HSQC, 3D TROSY-HNCO
$^{1}J_{C\alpha H\alpha}$ [0.5]: 2D J_{CH}-modulated HSQC, (HA)CANH, HN(CO)CA
$^{1}J_{CH}$ (side-chain): 2D J_{CH}-mod. HSQC, CCH-COSY, SPITZE-HSQC
^{1}H-^{1}H: COSY, CT-COSY, HNHA, 3D SS-HMQC2 (long-range)

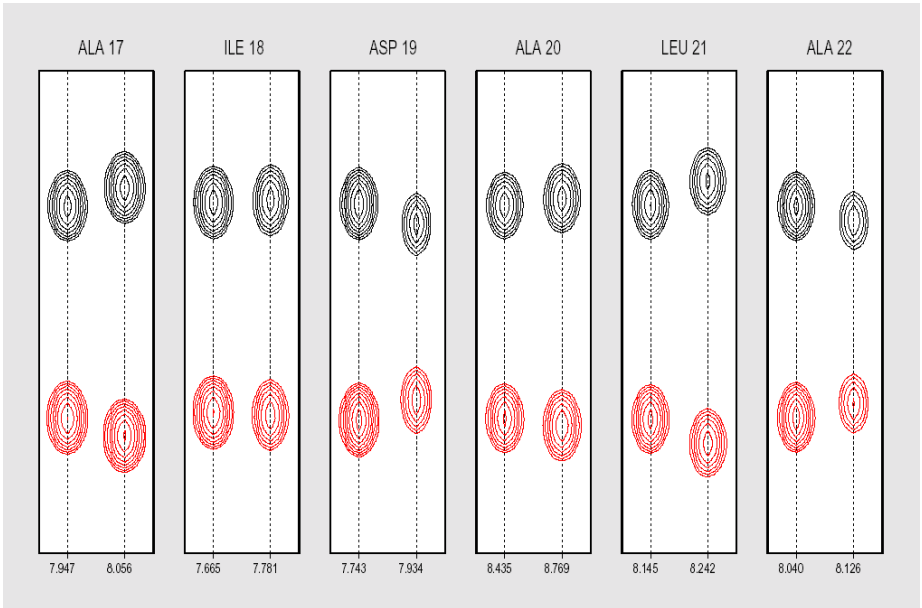
Bax, Kontaxis & Tjandra Method Enzymol. 339, 127-174, 2001;

Chou & Bax JBNMR, 2001; Delaglio et al. JMR 2001; Wu & Bax, JACS, 2002;

RDC measurement: J splitting ($^1J_{HN}$)

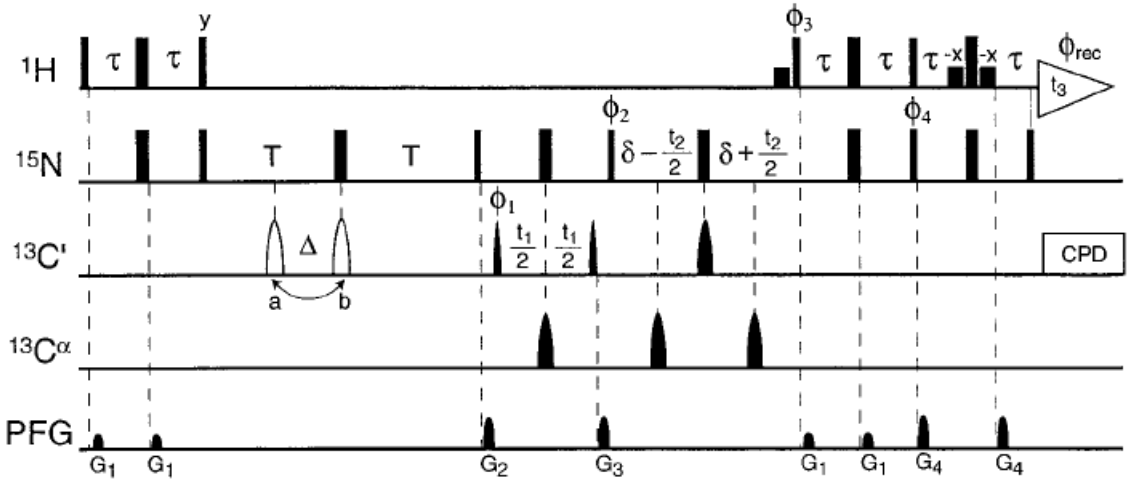
IPAP-HSQC

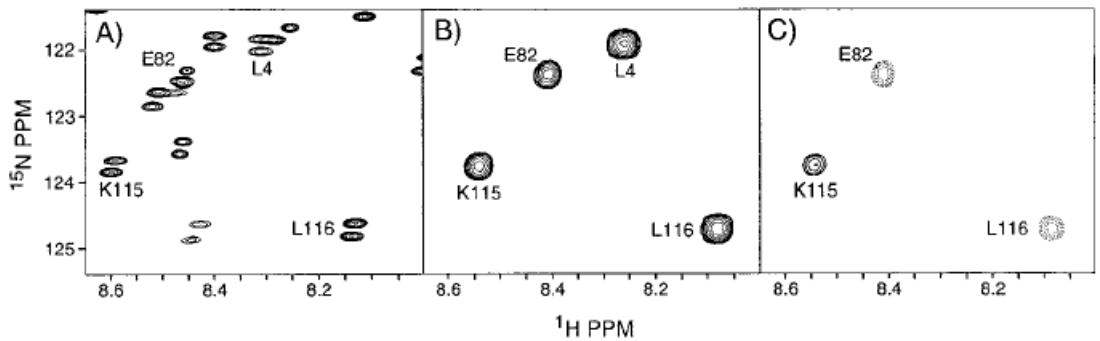




Ottiger et al. JMR, 1998

RDC measurement: Quantitative J correlation ($^1J_{C'N}$)



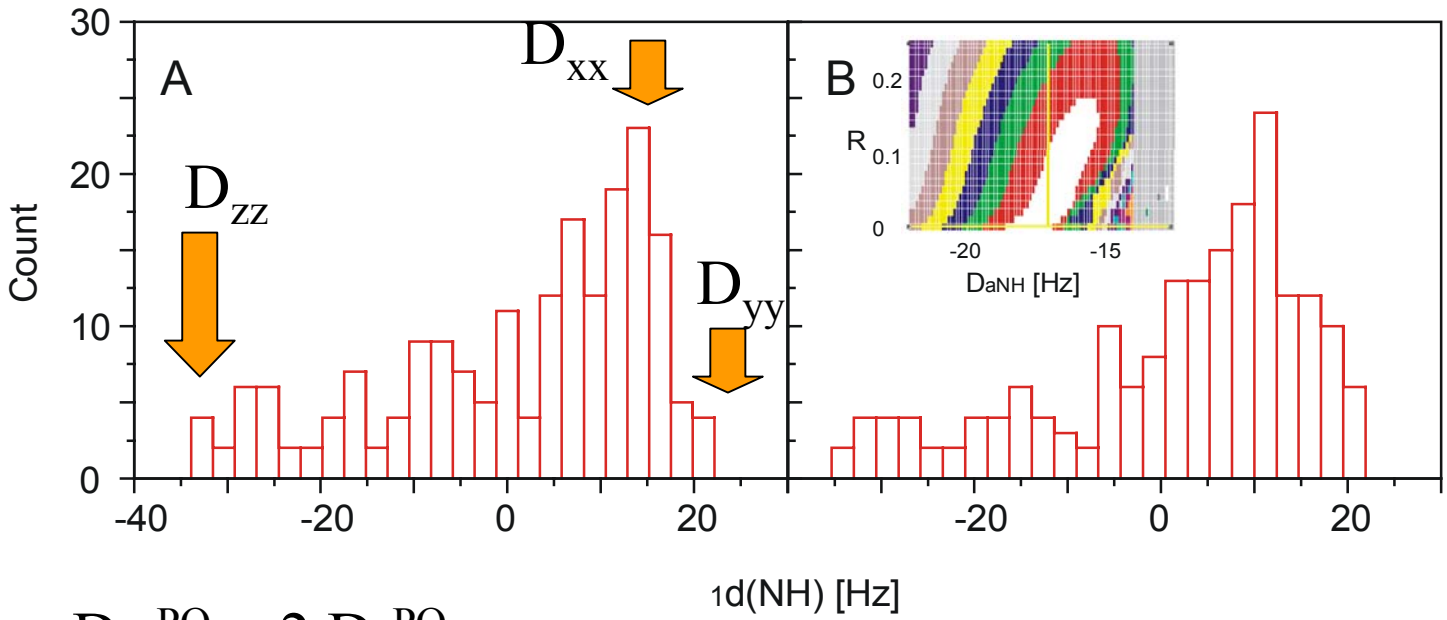


Chou & Bax
JBNMR, 2001

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



$$D_{zz}^{PQ} = 2\, D_a^{PQ}$$

$$D_{yy}^{PQ} = -D_a^{PQ}\,(1 + 1.5\, R)$$

$$D_{xx}^{PQ} = -D_a^{PQ}\,(1 - 1.5\, R)$$

with $D_{ii}^{PQ} = D^{PQ}_{max}\, S_{ii}^{d}$

no structure necessary !

Back-calculation of alignment tensor

- singular value decomposition (SVD)

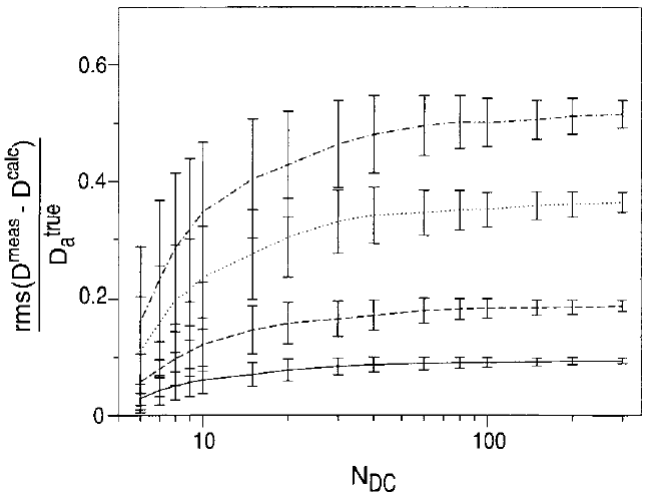
➔very stable & with a minimum
of five RDCs possible

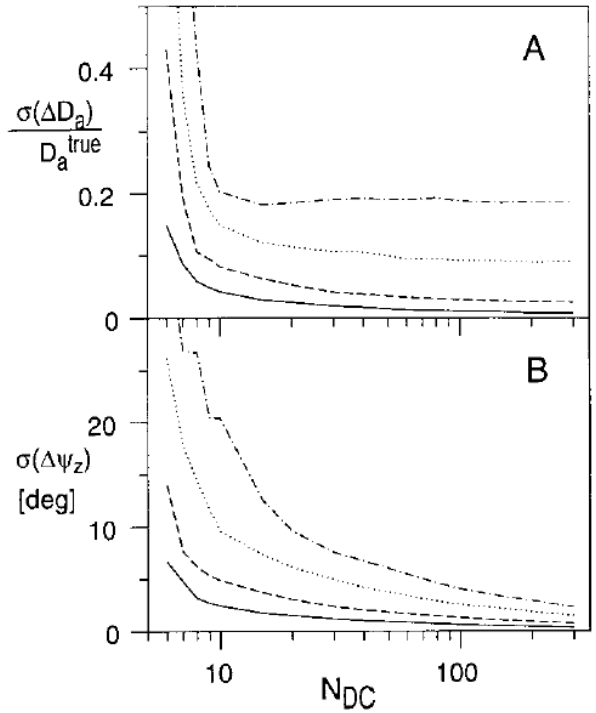
- iterative least squares procedure (Levenberg-Marquardt minimization)

$$\chi^2 = \sum_{i=1,..,N} [d_i^{PQ}(exp) - d_i^{PQ}(calc)]^2/(\sigma_i^{PQ})^2$$

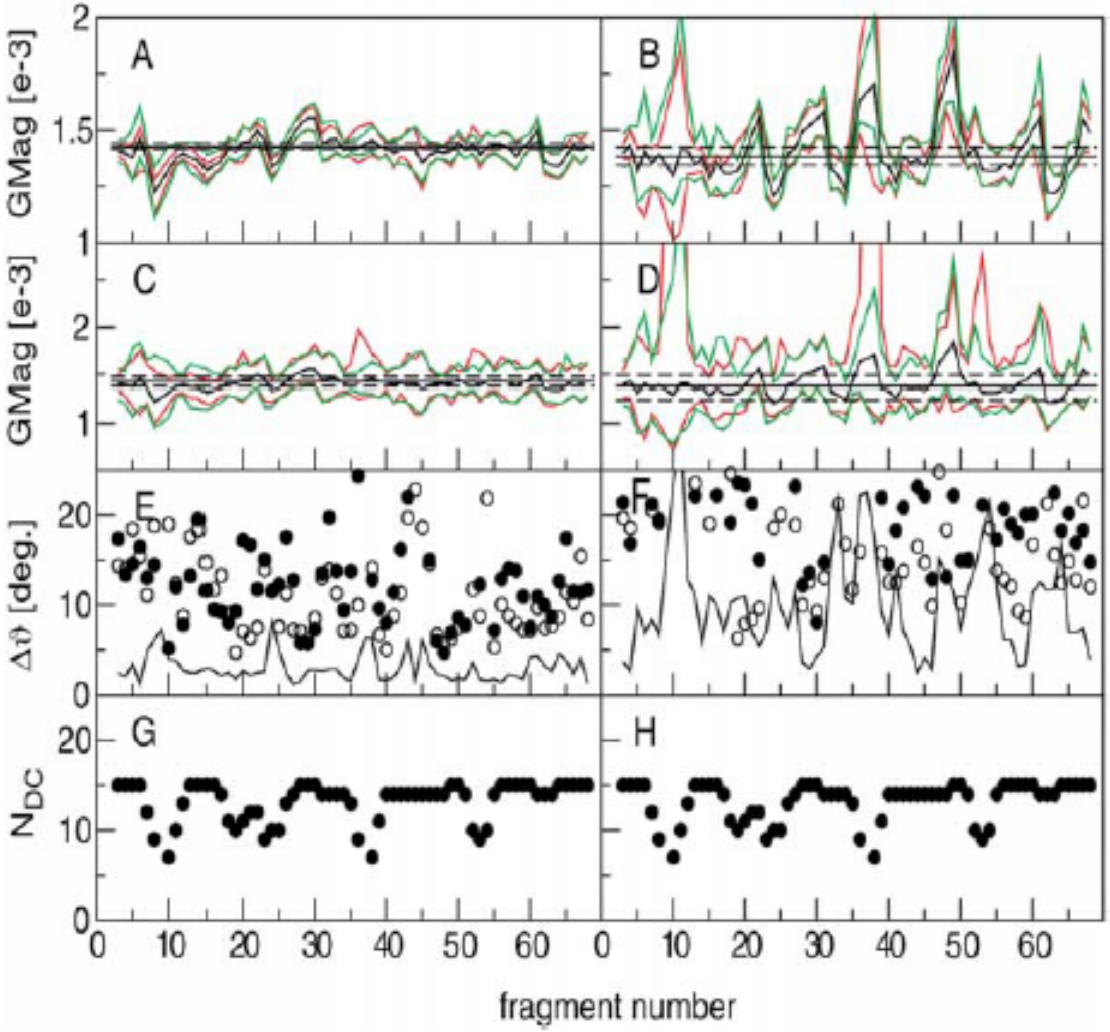
➔ fixing of alignment parameters (e.g. rhombic component zero due to three-fold or higher symmetry)

Evaluation of uncertainty in back-calculated alignment tensors

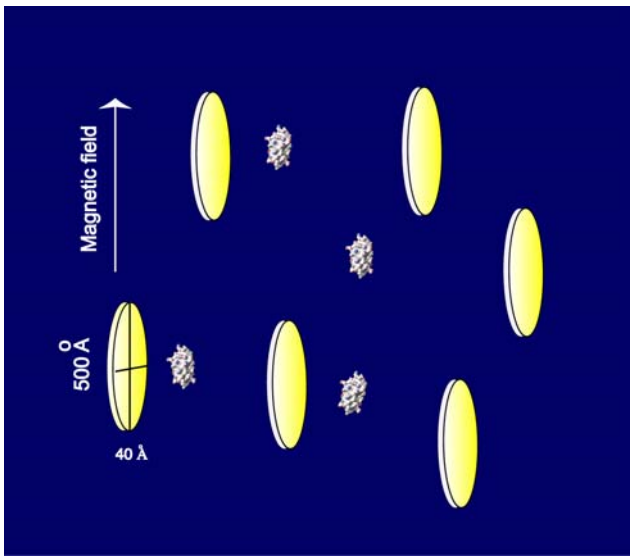


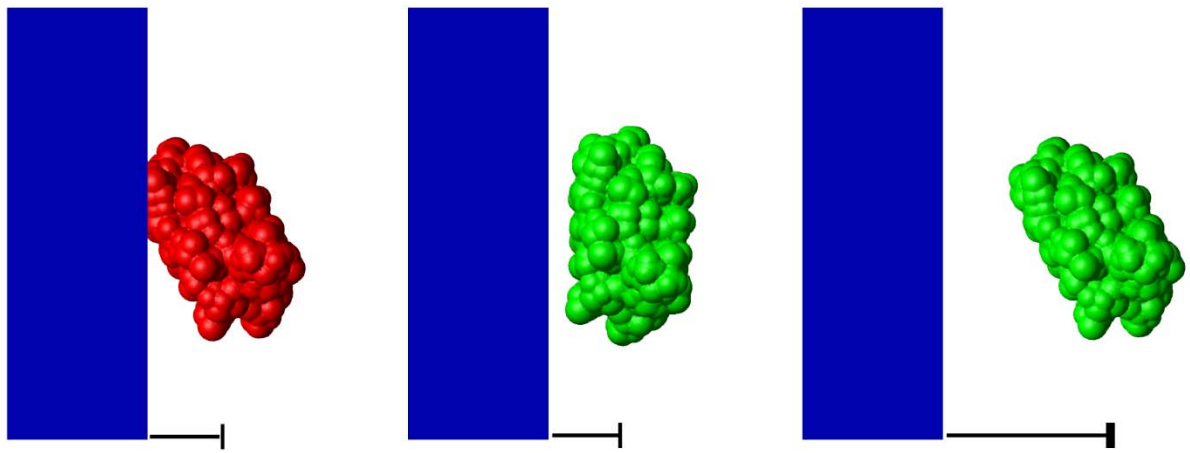


Zweckstetter & Bax, JBNMR 2002


A
B
C
D
E
F
G
H
GMag [e-3]
GMag [e-3]
Δϑ [deg.]
N_DC
fragment number

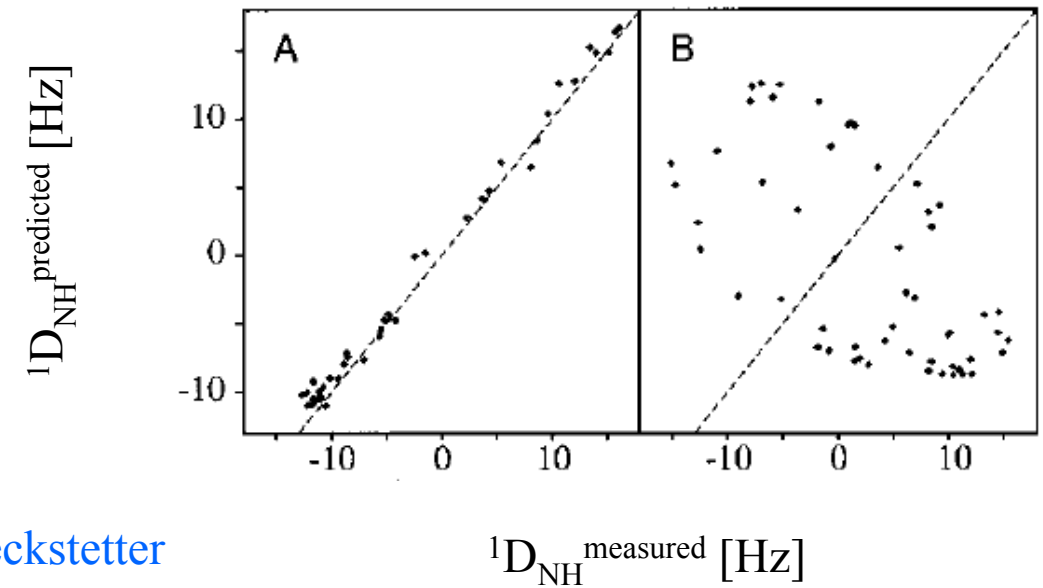
Steric model of alignment





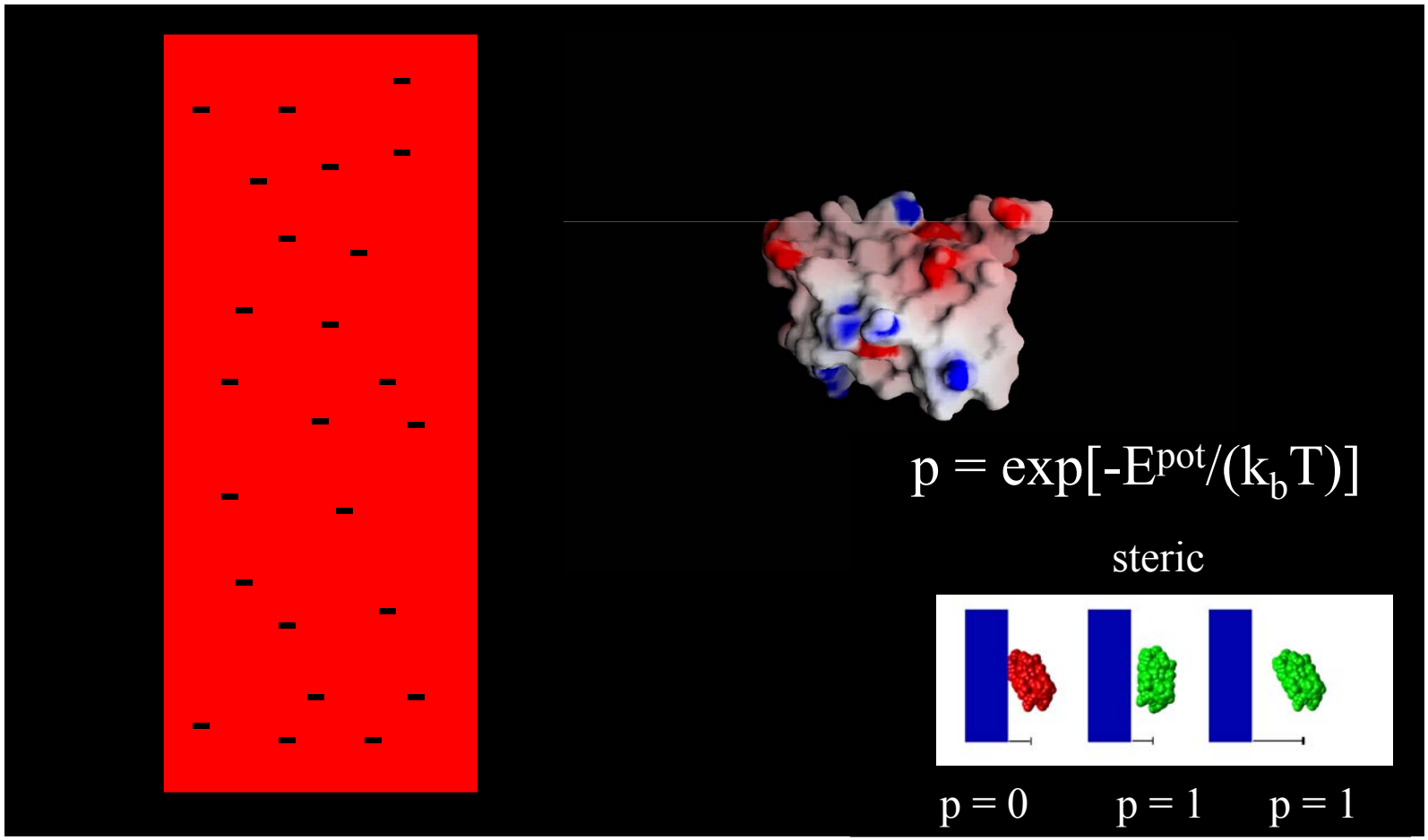
no RDCs necessary !

Shape prediction of magnitude and orientation of alignment

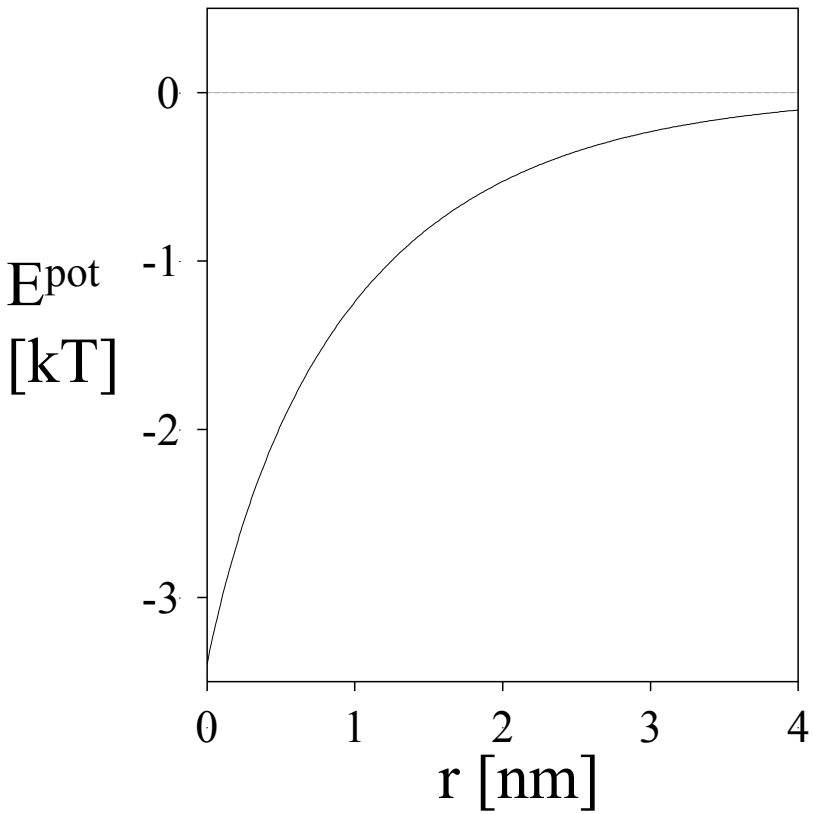


Zweckstetter & Bax JACS, 2000

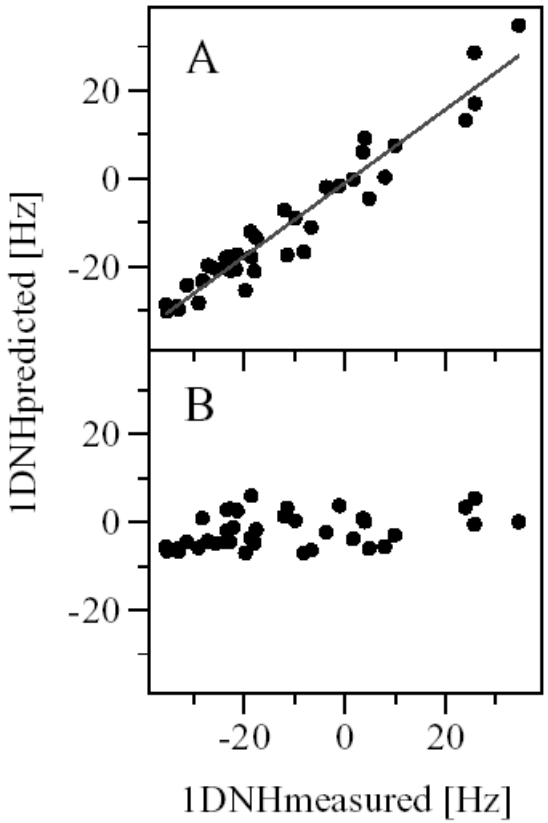
Electrostatic model of alignment



Electrostatic potential



Shape & charge prediction of alignment tensor

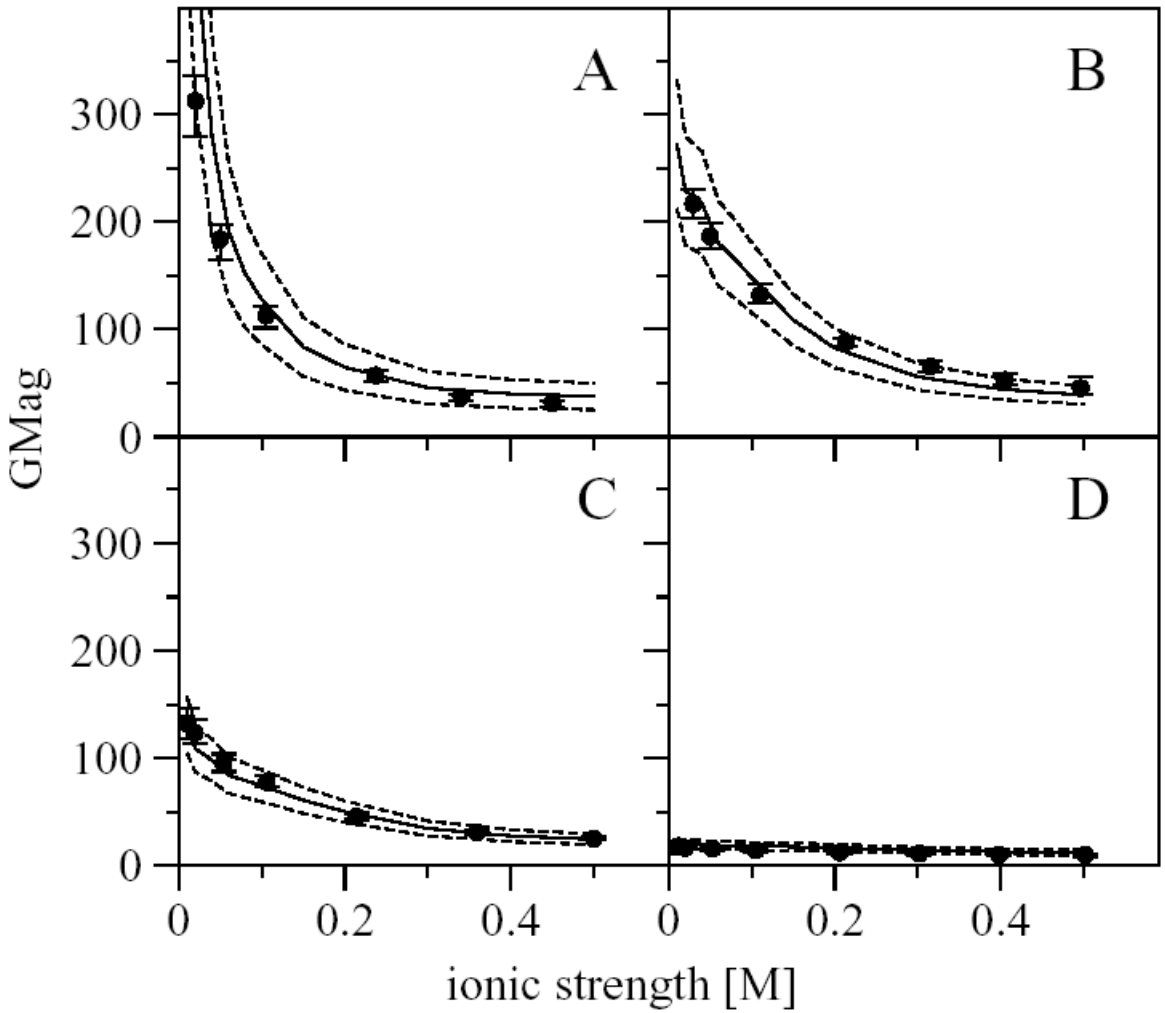


electrostatic

steric

ubiquitin

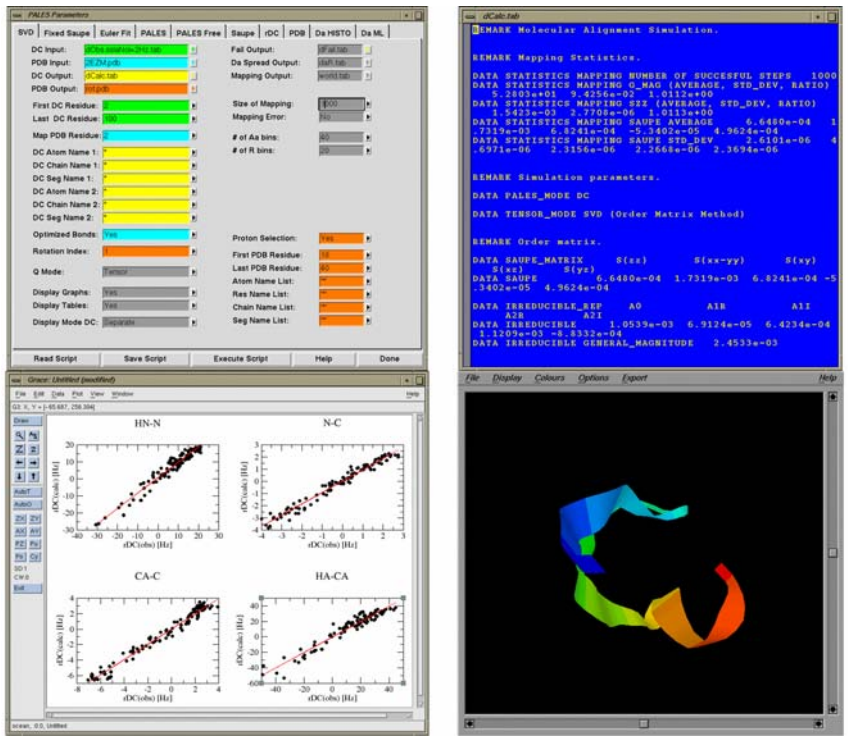
DinI



GB3

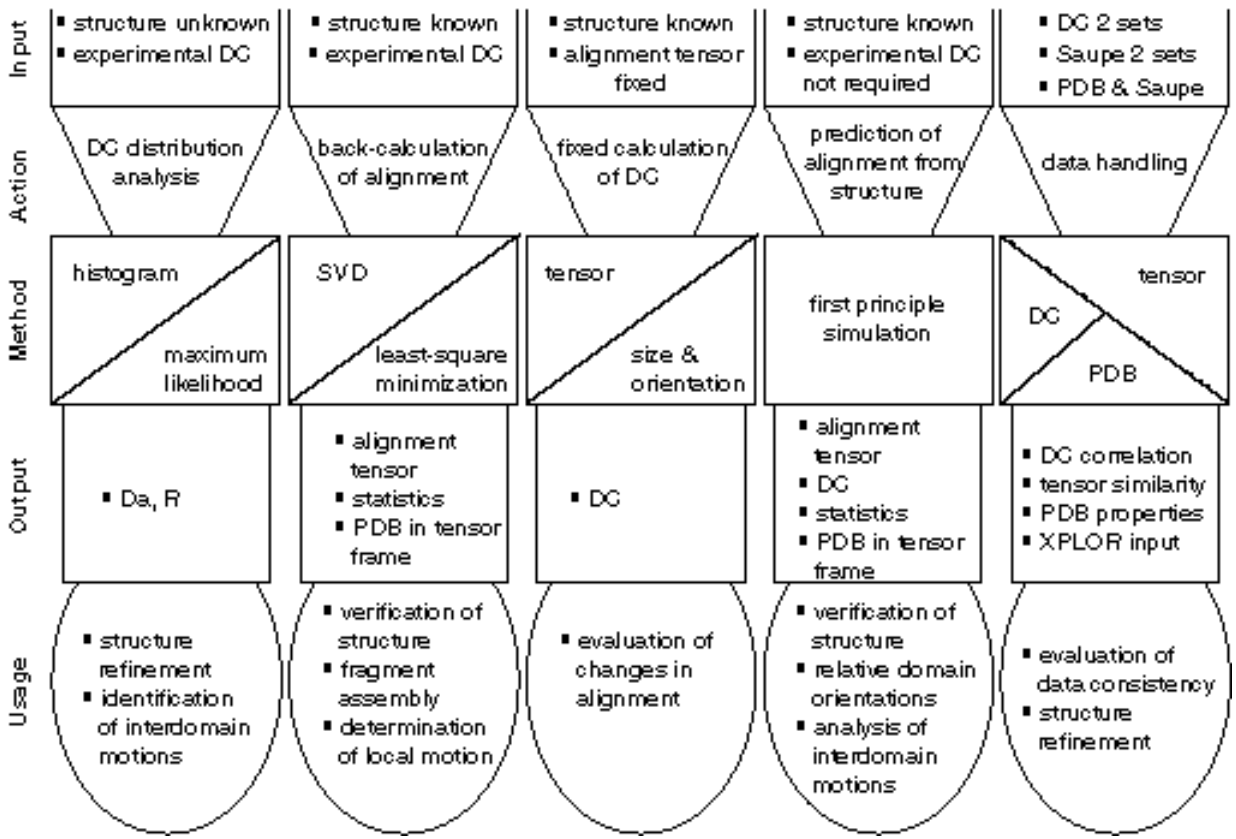
GB1

PALES – software for analysis of RDC



http://spin.niddk.nih.gov/bax

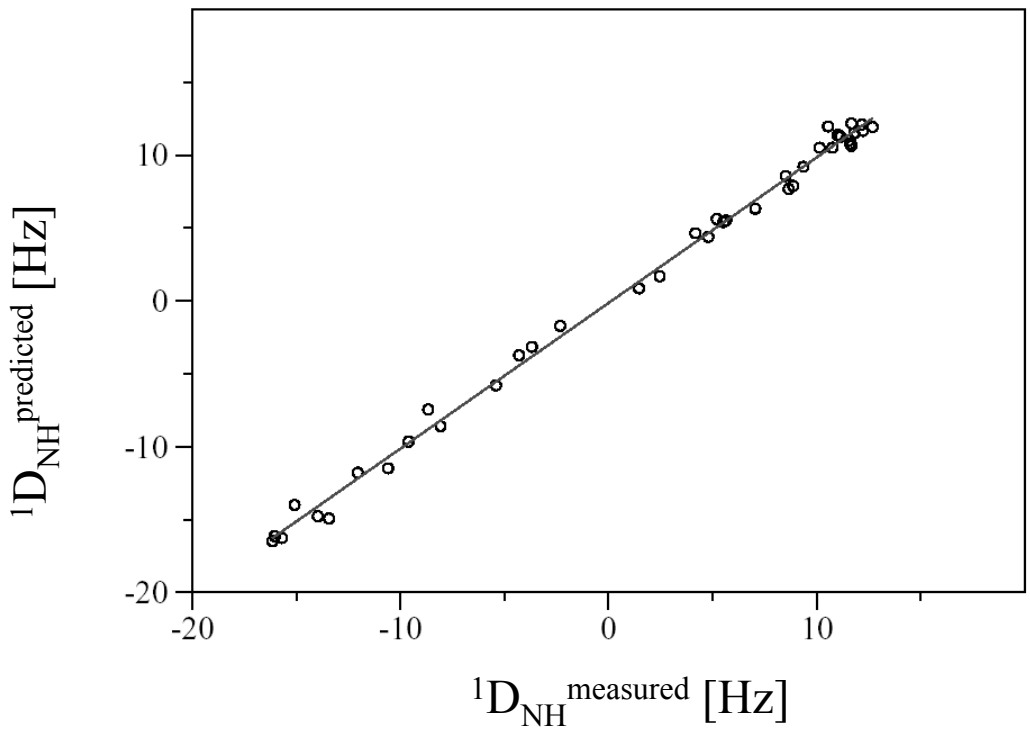
PALES


Input
Action
Method
Output
Usage
structure unknown
experimental DC
structure known
experimental DC
structure known
alignment tensor fixed
structure known
experimental DC not required
DC 2 sets
Saupe 2 sets
PDB & Saupe
DC distribution analysis
back-calculation of alignment
fixed calculation of DC
prediction of alignment from structure
data handling
histogram
maximum likelihood
SVD
least-square minimization
tensor
size & orientation
first principle simulation
tensor
DC
PDB
Da, R
alignment tensor
statistics
PDB in tensor frame
DC
alignment tensor
DC
statistics
PDB in tensor frame
DC correlation
tensor similarity
PDB properties
XPLOR input
structure refinement
identification of interdomain motions
verification of structure
fragment assembly
determination of local motion
evaluation of changes in alignment
verification of structure
relative domain orientations
analysis of interdomain motions
evaluation of data consistency
structure refinement

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



$$Q = \frac{\text{rms}\,(D^{obs} - D^{calc})}{\text{rms}\,(D^{obs})}$$

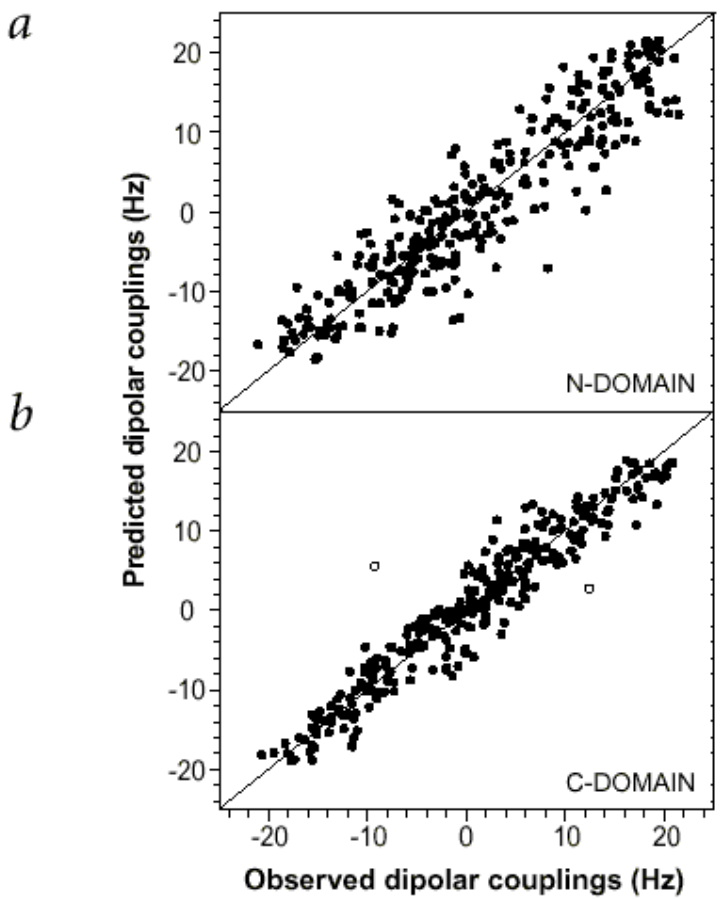
$Q \sim 0.2 \approx 1.5$ Å X-ray

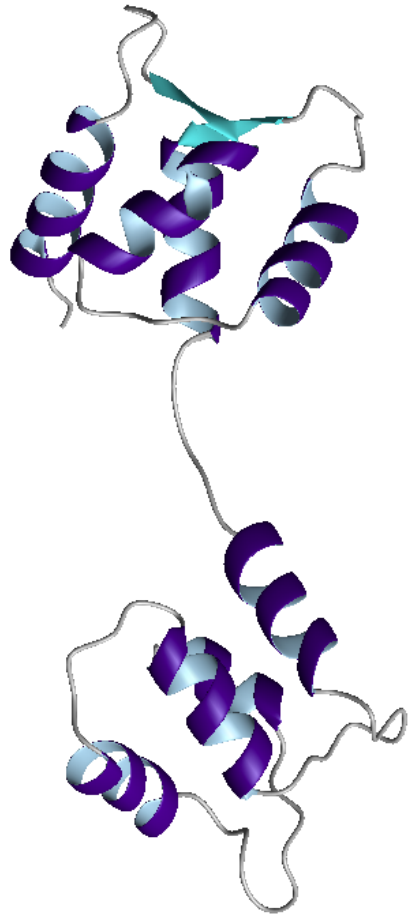
- use only for RDC not included in structure determination !
- no translational validation

Conformational differences in solution: calmodulin

Q = 41%

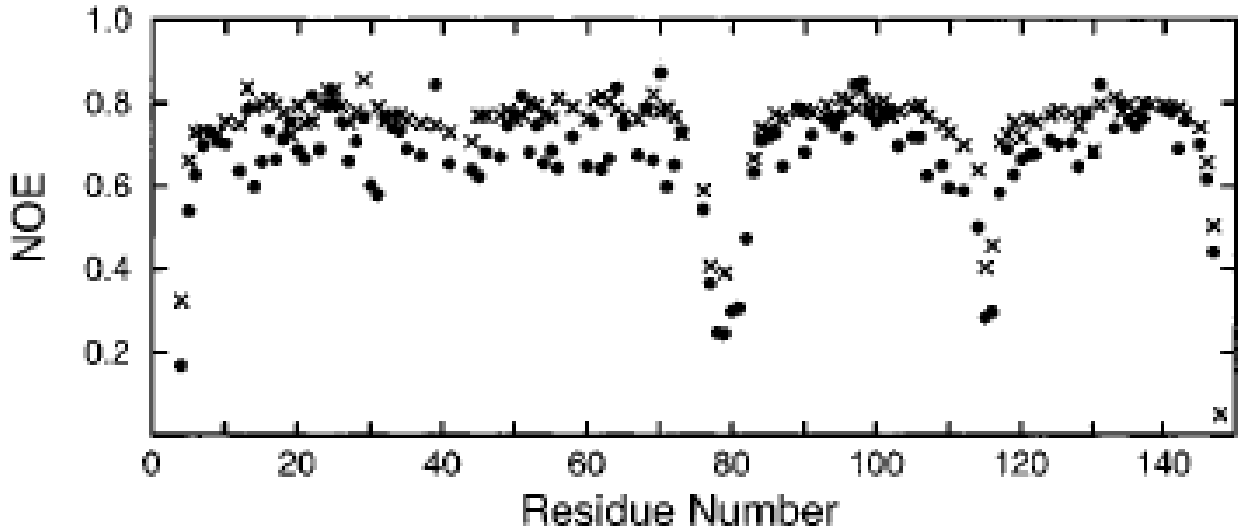
Q = 25%





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

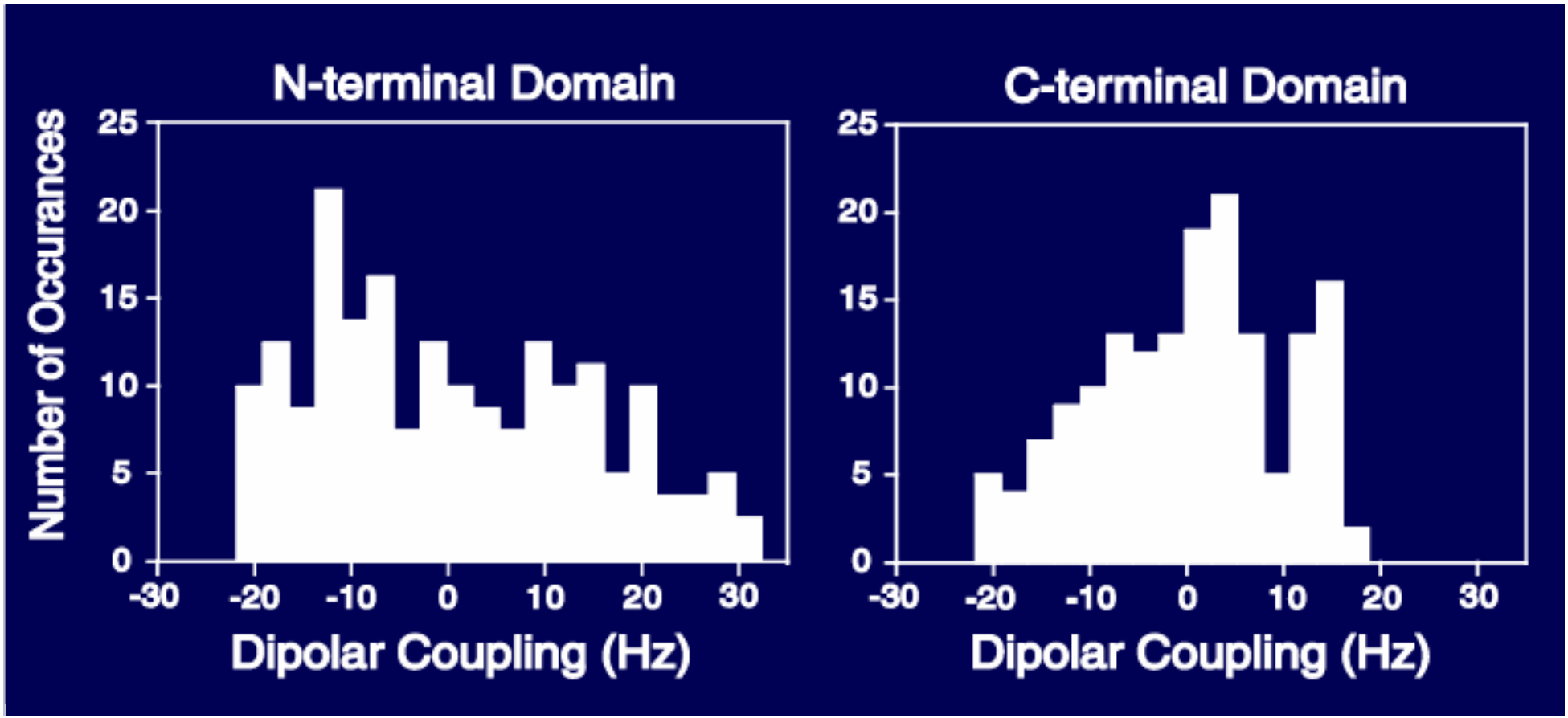
Flexibility of the inter-domain linker in solution



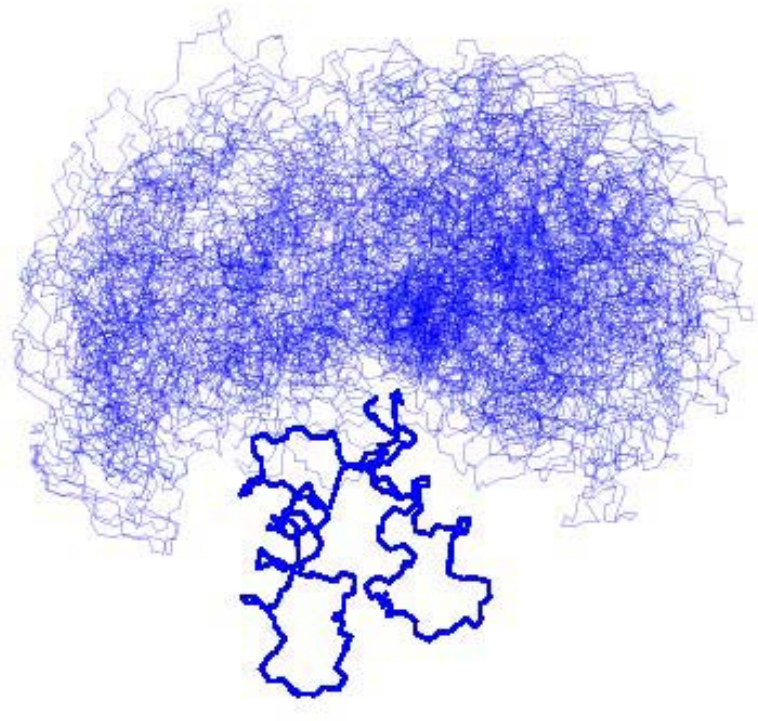
Baber et al. JACS, 2000

NH-dynamics from RDC: Peti et al. JACS 2002

Qualitative analysis of inter-domain motion



Quantitative analysis of interdomain motion

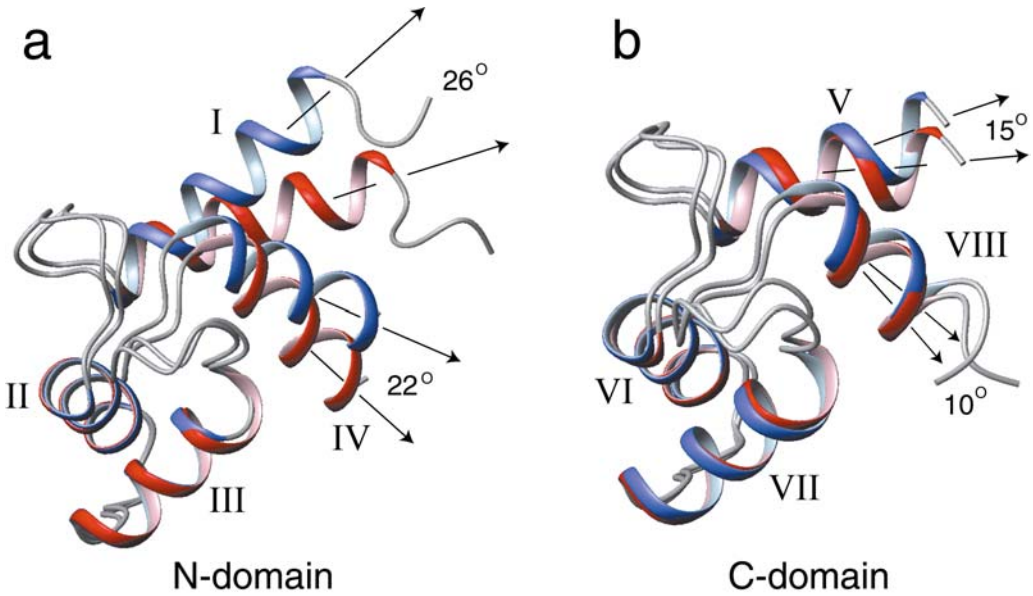


Structure refinement

$$E_{dip} = k\,(D^{calc}_{PQ} - D^{obs}_{PQ})^2$$

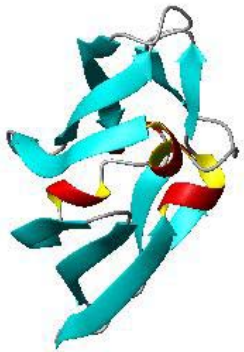
k: 10^{-4} ➔ 1 kcal/Hz^2

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

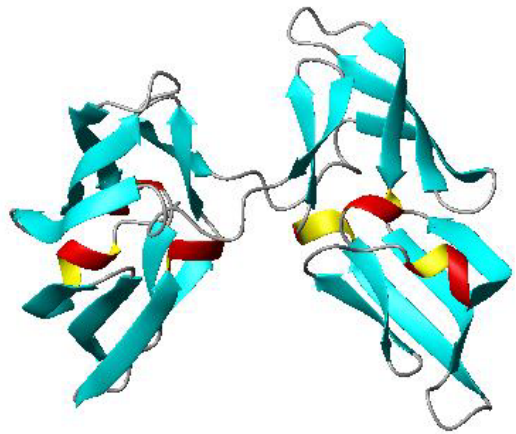


Chou, Li, Klee & Bax, Nature Struc Biol 2001

Determination of multi-module structures



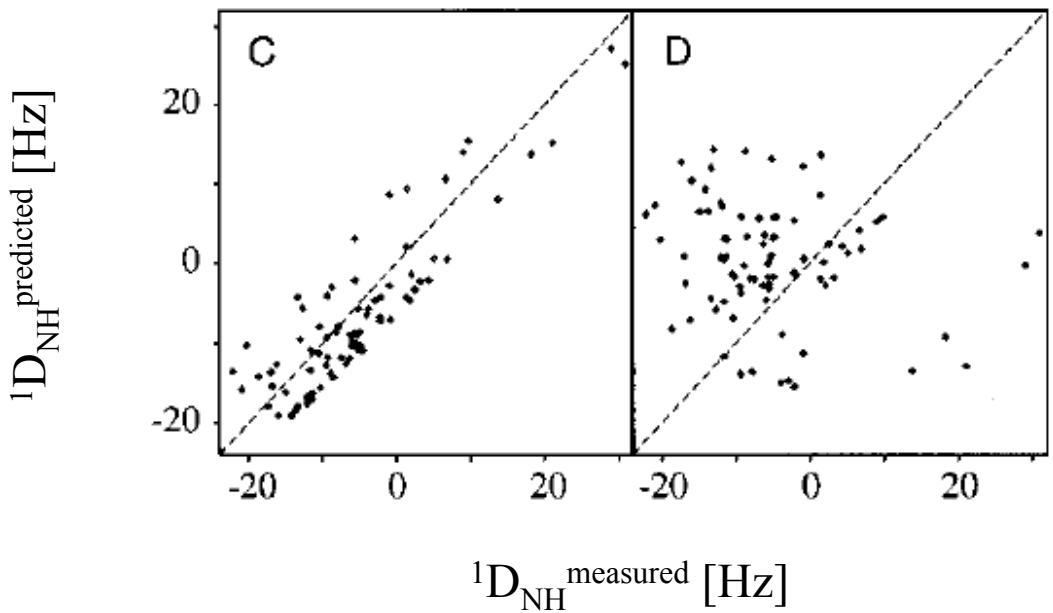
in Lösung

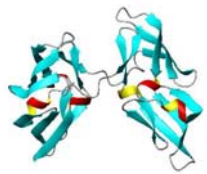


im krystallinen Zustand

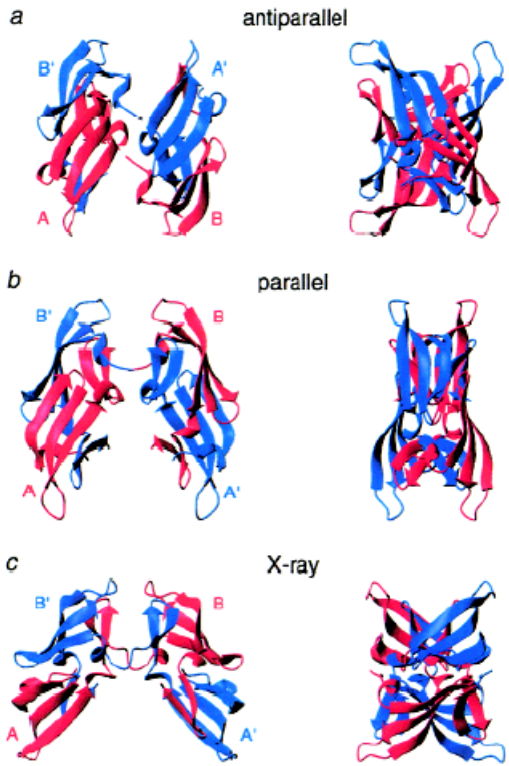
cyanovirin-N

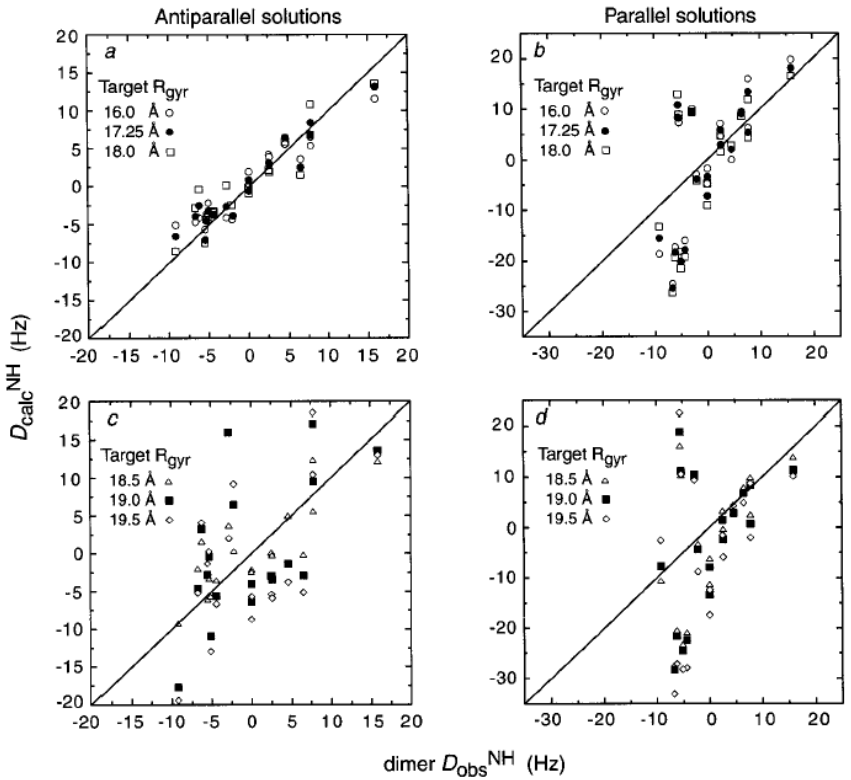
Monomeric versus multimeric structures





Translational information from shape-prediction





Bewley & Clore JACS, 2000

Rapid structure determination

assignment

```
MGSSHHHHHHSSGLVPRGSHMNNS
LDIKDVTTFYEEDKHLIFGYTPTC
GTCKVSERMLDIANEILQLPLLKI
DLNFYPQFCKDMQIMSTPILLLMN
KDKEVKRIYAFKSVTDLLENLK
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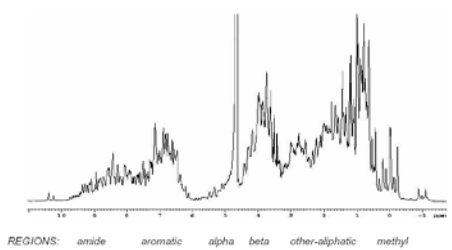
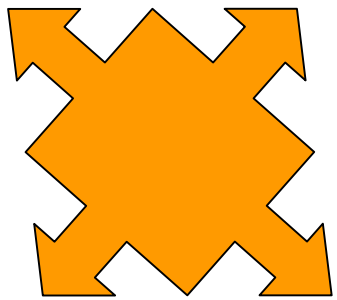
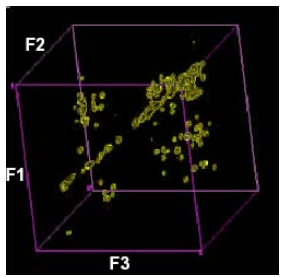
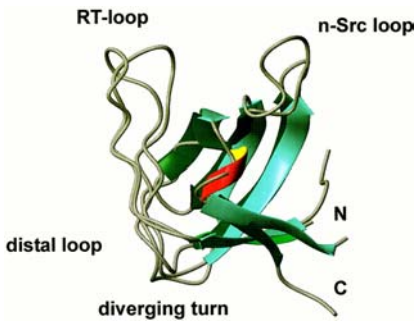


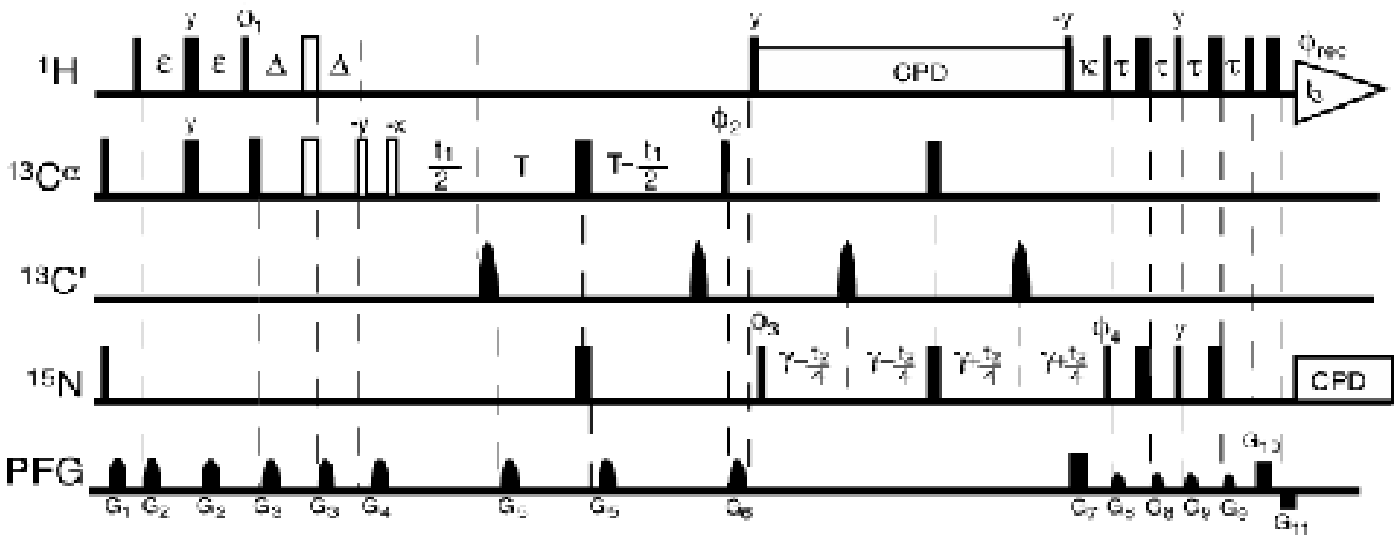


structure determination

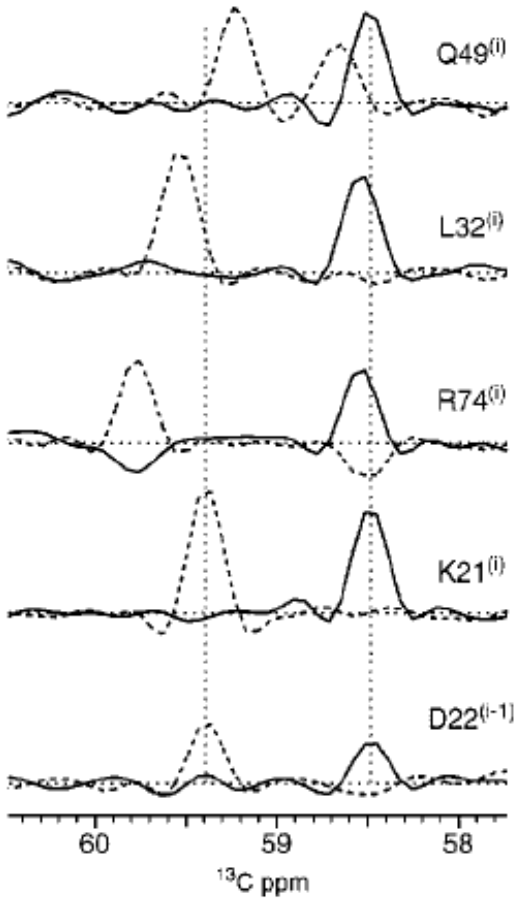




3D IPAP-(HA)CANH

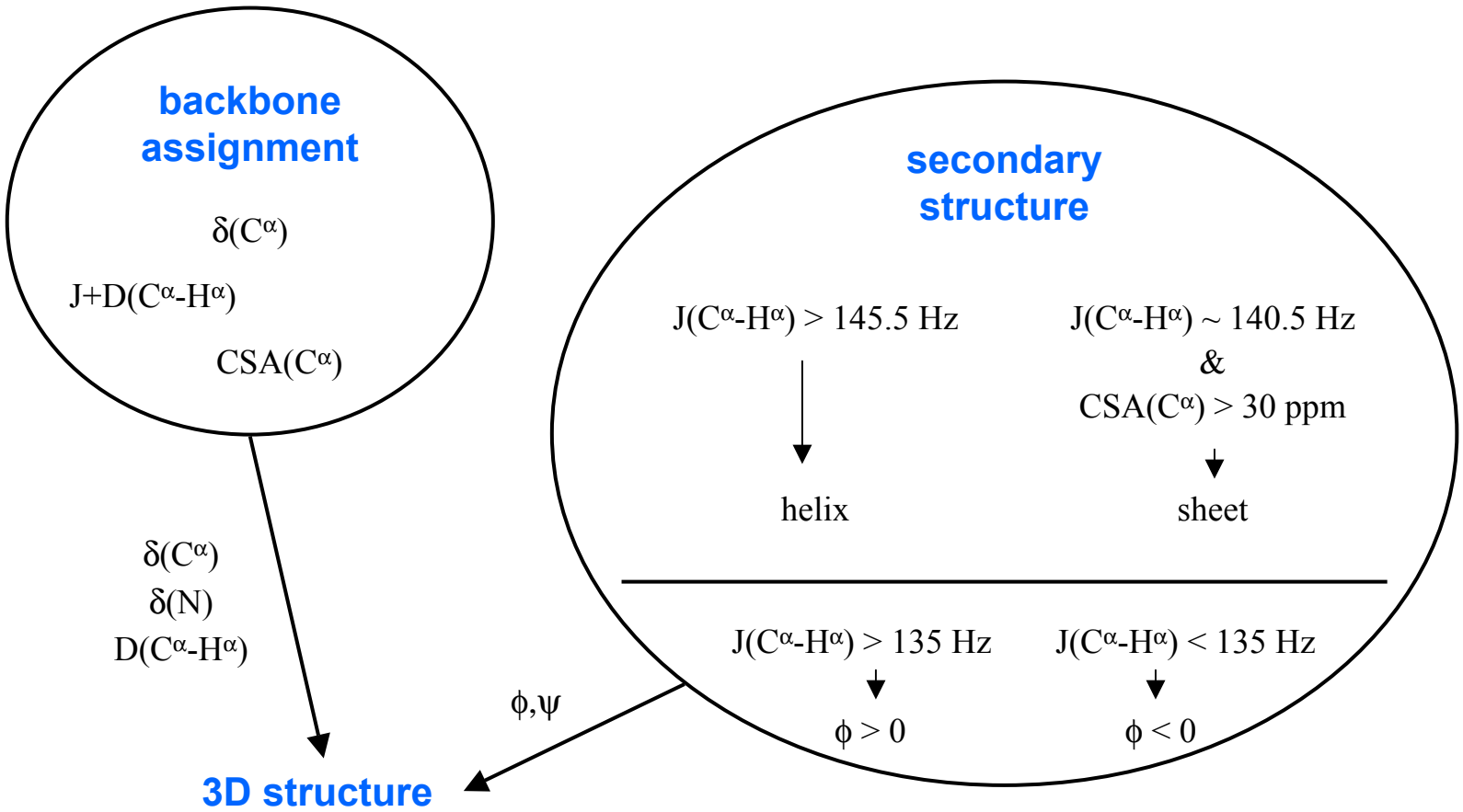


Improved NMR assignment with RDC

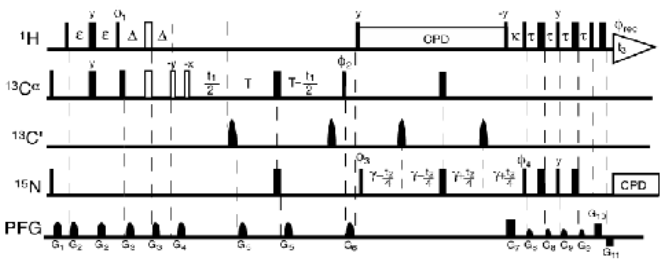


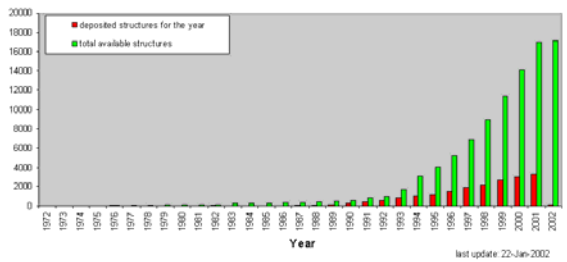
Zweckstetter & Bax JACS, 2001

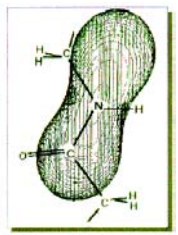
Secondary structure

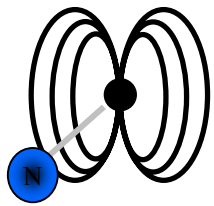


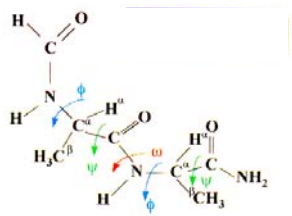
Molecular fragment homology search

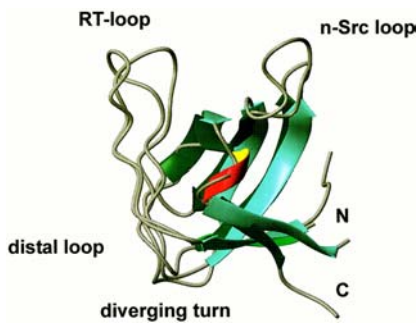




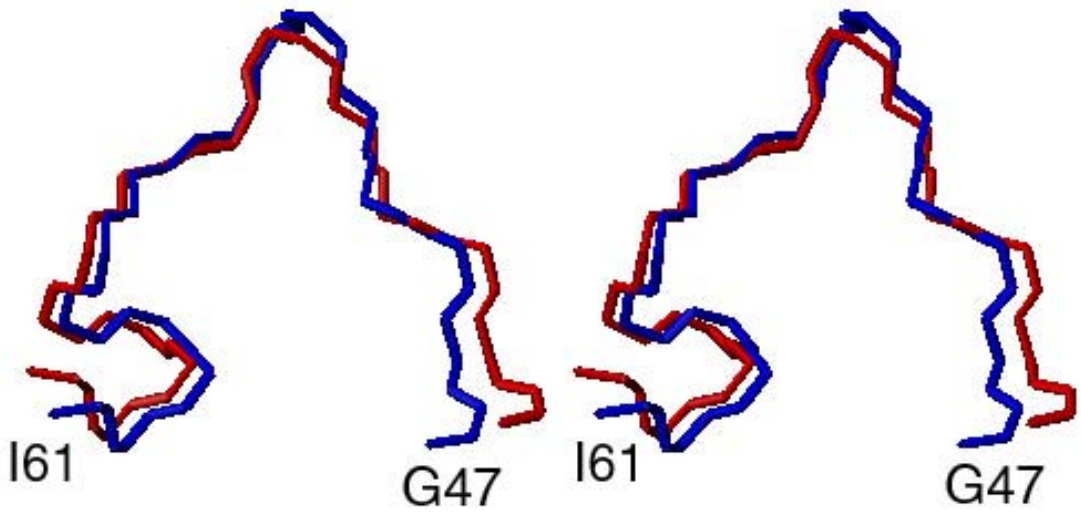




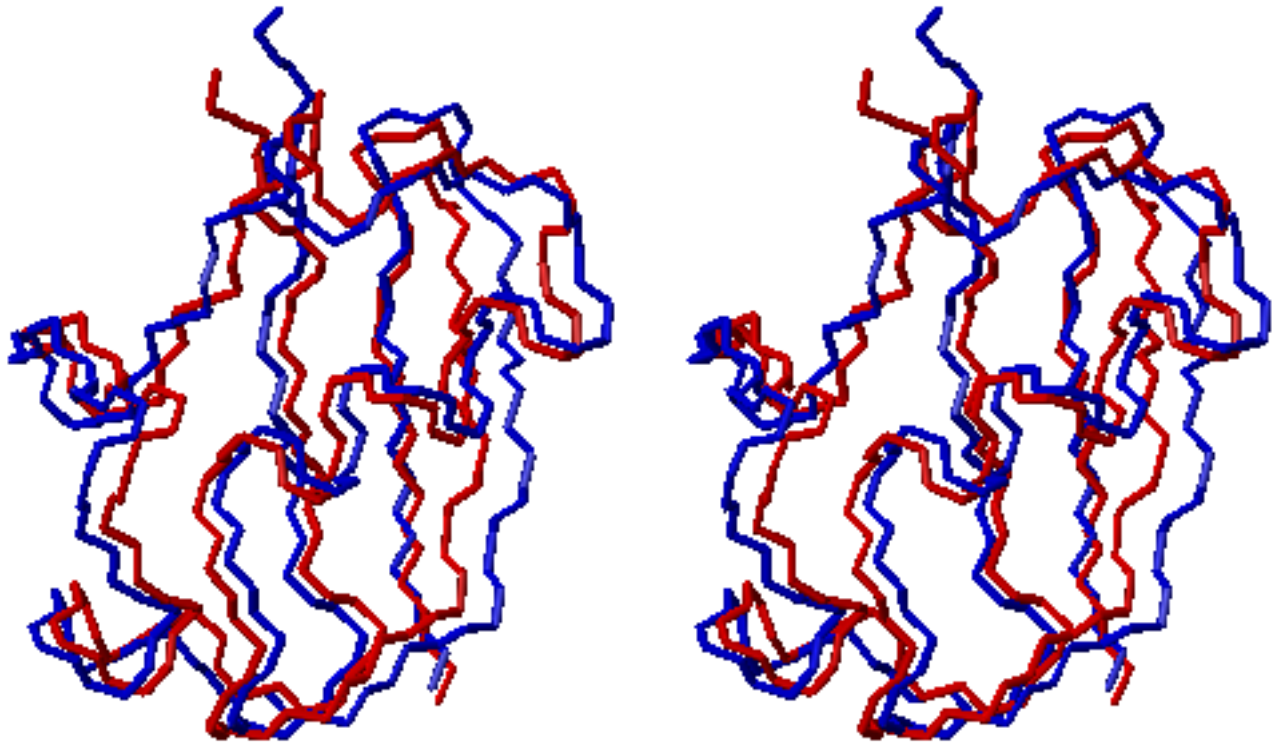




3D structure of molecular fragments



Zweckstetter
& Bax
JACS, 2001



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Bax, A., Kontaxis, G. & Tjandra, N., Method Enzymol 339, 127 (2001).
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Journal of American Chemical Society, Journal of Biomolecular NMR, Journal of Magnetic Resonance, ...