Advanced NMR Training Course: February 27th, 2004

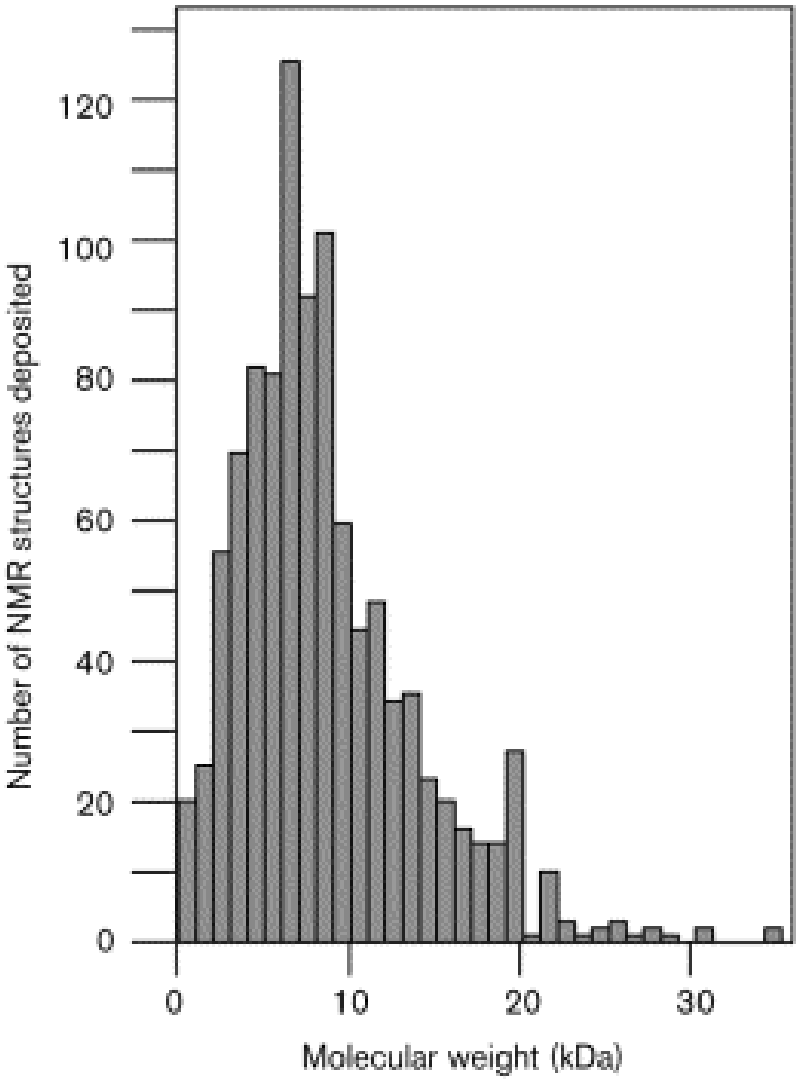
National Program for Genomic Medicine High-Field NMR Core Facility,
The Genomic Research Center, Academia Sinica

Course Topic: TROSY and NMR of Large Proteins

by

Wen-Jin Wu, Ph.D.

Size Limit on Solution NMR



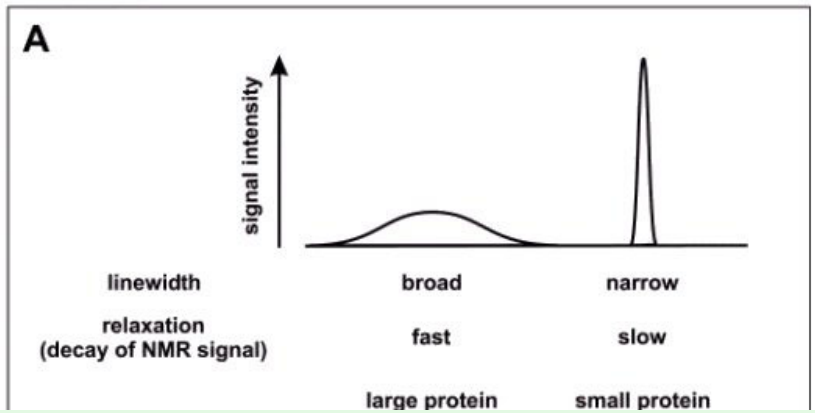
Molecular weight distribution of the NMR structures deposited in the PDB as 12/1997

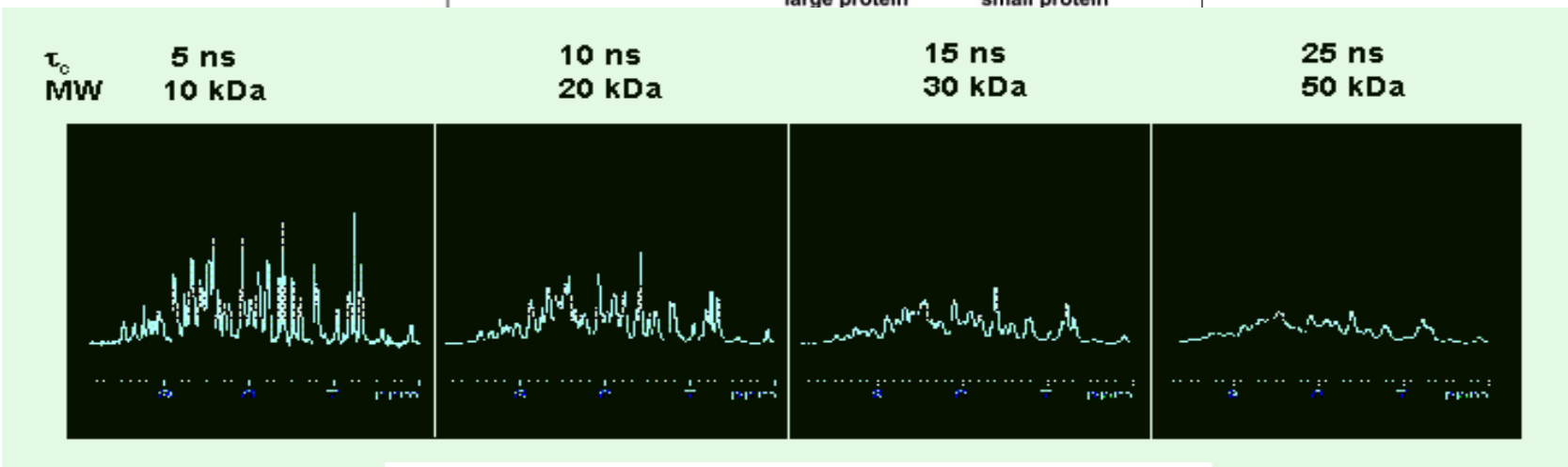
P. Gunter, *Q. Rev. Biophys*, 1998, v31, p145

Intrinsic Problems of Solution NMR of Large Molecules

- Many more signals → spectral overlap
- Slow tumbling → fast transverse relaxation rate (short T_2, fast decay of signal) → poor signal to noise (s/n)

Linewidth $\Delta\upsilon_{1/2} = 1/(\pi T_2)$





Pictures from M. Sattler, http://www.embl-heidelberg.de/nmr/sattler/teaching/

Current Techniques Designed to Attack the Size Limit

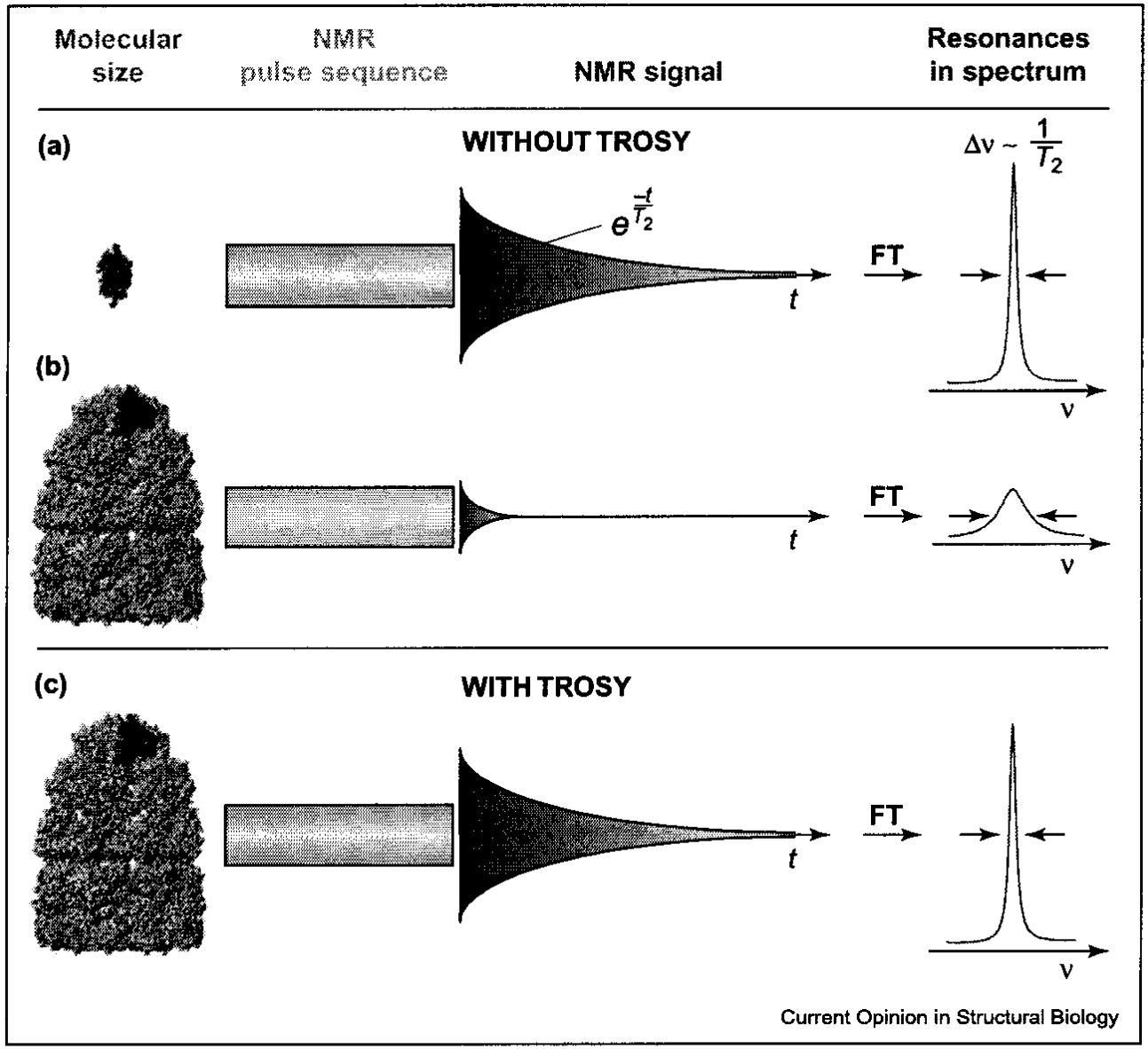
NMR hardware, new NMR methods, advanced in molecular biology

- Isotope labeling (^{15}N, ^{13}C), 3D, 4D triple resonance experiments: overlap
- NMR hardware: bigger magnets (overlap, s/n)
- Cryogenic probe: s/n (optimal 4-fold increase)

- TROSY, CRINPT: overlap, s/n
- Deuteration: overlap, s/n
- Selective isotope labeling: overlap

- Line-narrowing by low viscosity solvent: overlap, s/n
- Segmental Labeling: overlap
- Residual dipolar coupling: extra angle and long distance information
- Cross saturation: identify binding surface

Impact of TROSY on Solution NMR



Fernandex and Wider, *Current Opinion in Structural Biology* 2003, 13:570-580

Application of TROSY NMR on Biological Systems

Figure from K. Pervushin in *EMBO Practical NMR Course 2003*
http://www.embl-heidelberg.de/nmr/sattler/embo/coursenotes.html

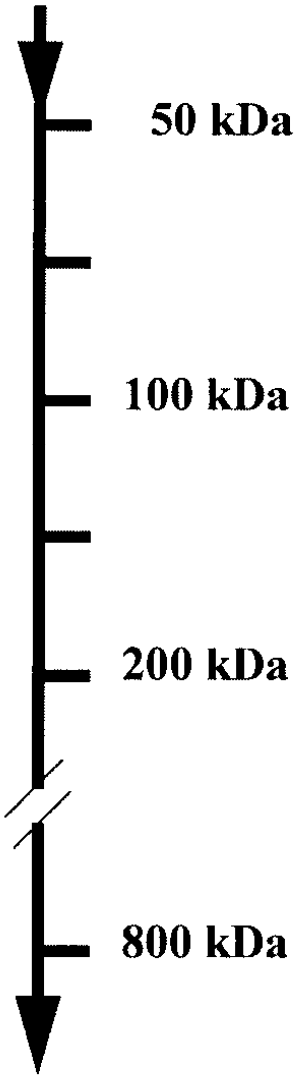
Nucleic acids and small proteins
(fine detailes of 3D structures, dynamics)

Oligomeric proteins
Large single chain proteins

β-barrel membrane proteins
Polytopic α-helical membrane proteins
(bb and sc assignment, 3D folds, dynamics)

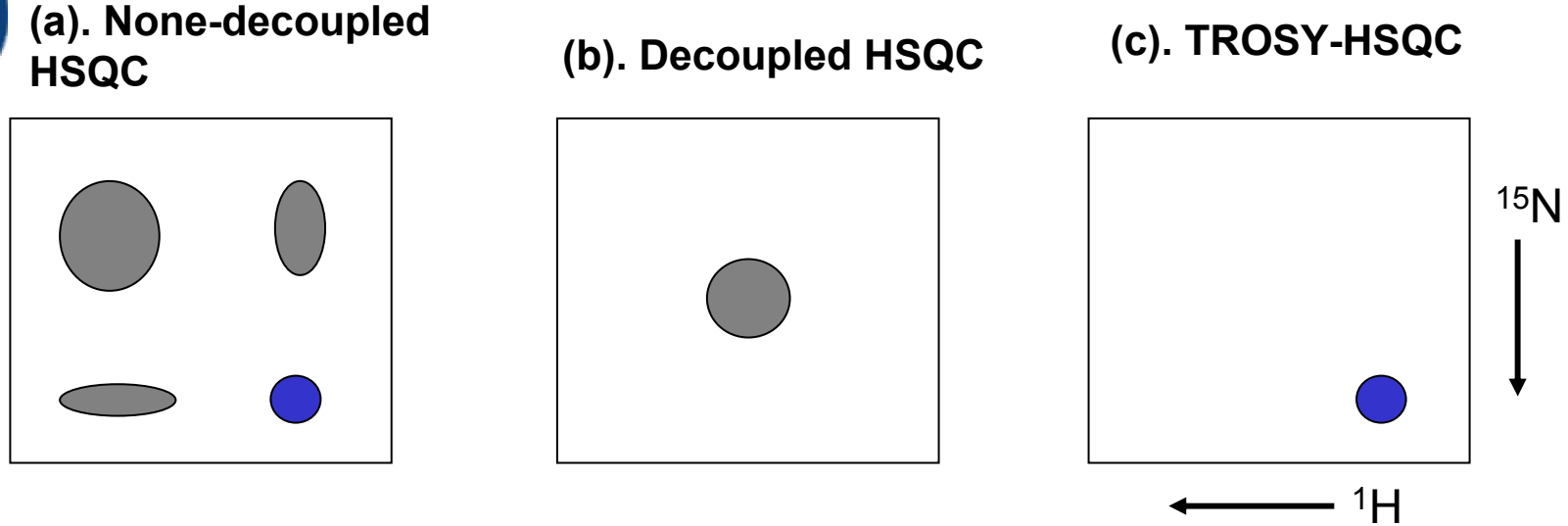
Protein/protein and
protein/nucleic acid interactions
(chem. shift mapping, surface mapping,
drug discovery)

Large supramolecular complexes
(detection of individual resonances)



The estimated range of molecular weights of biological systems the most effectively studied by TROSY NMR

Transverse Relaxation-Optimized Spectroscopy (TROSY)



1. Main relaxation source for ^{1}H and ^{15}N: dipole-dipole (DD) coupling and, at high magnetic fields, chemical shift anisotropy (CSA).

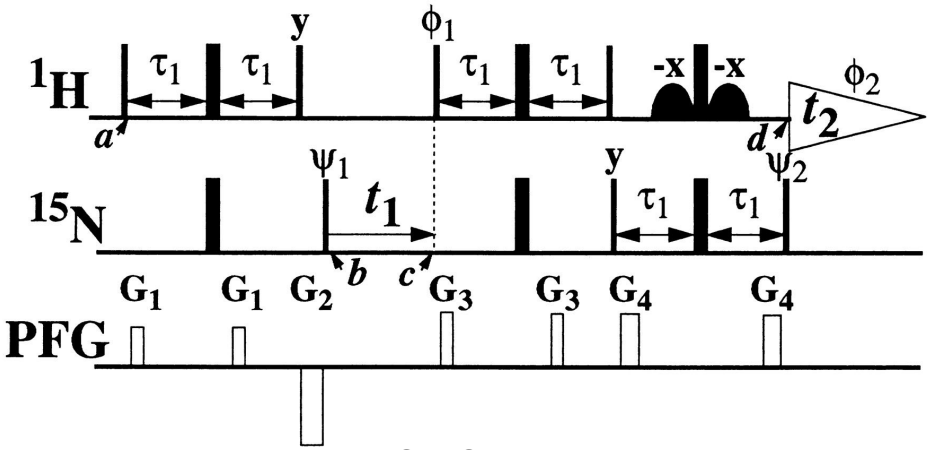
2. Different relaxation rates (line width) for each of the four components of ^{15}N-^{1}H correlation.

3. The narrowest peak (the blue peak) is due to the constructive canceling of transverse relaxation caused by chemical shift anisotropy (CSA) and by dipole-dipole coupling at high magnetic field.

4. TROSY selectively detect only the narrowest component (1 out of 4).

TROSY-HSQC and Conventional HSQC

TROSY-HSQC



Pervushin et al. *PNAS USA,* v94, p12366 (1997).

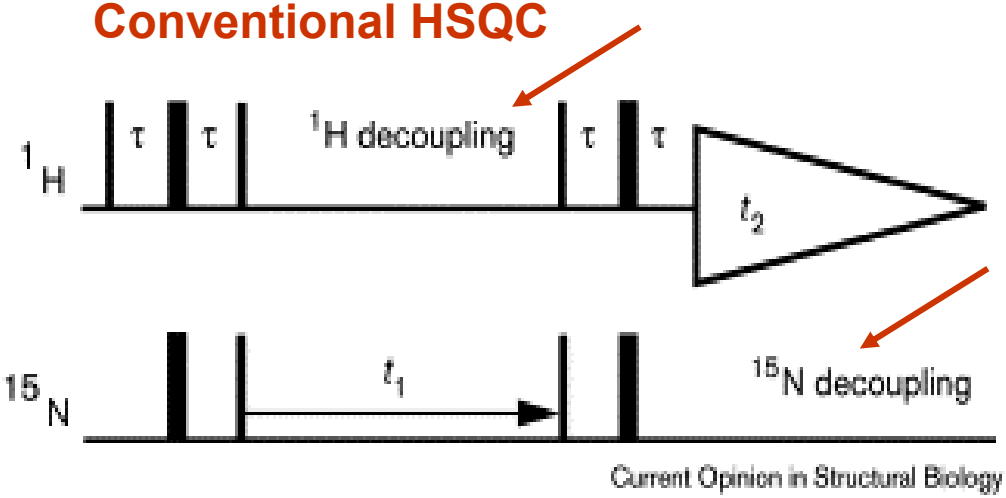
TROSY-HSQC

(1) No ^{1}H decoupling during ^{15}N evolution.

(2) No ^{15}N decoupling during ^{1}H acquisition.

(3) Use the TROSY-HSQC pulse sequence to selectively observe the most slowly relaxing component.

Conventional HSQC

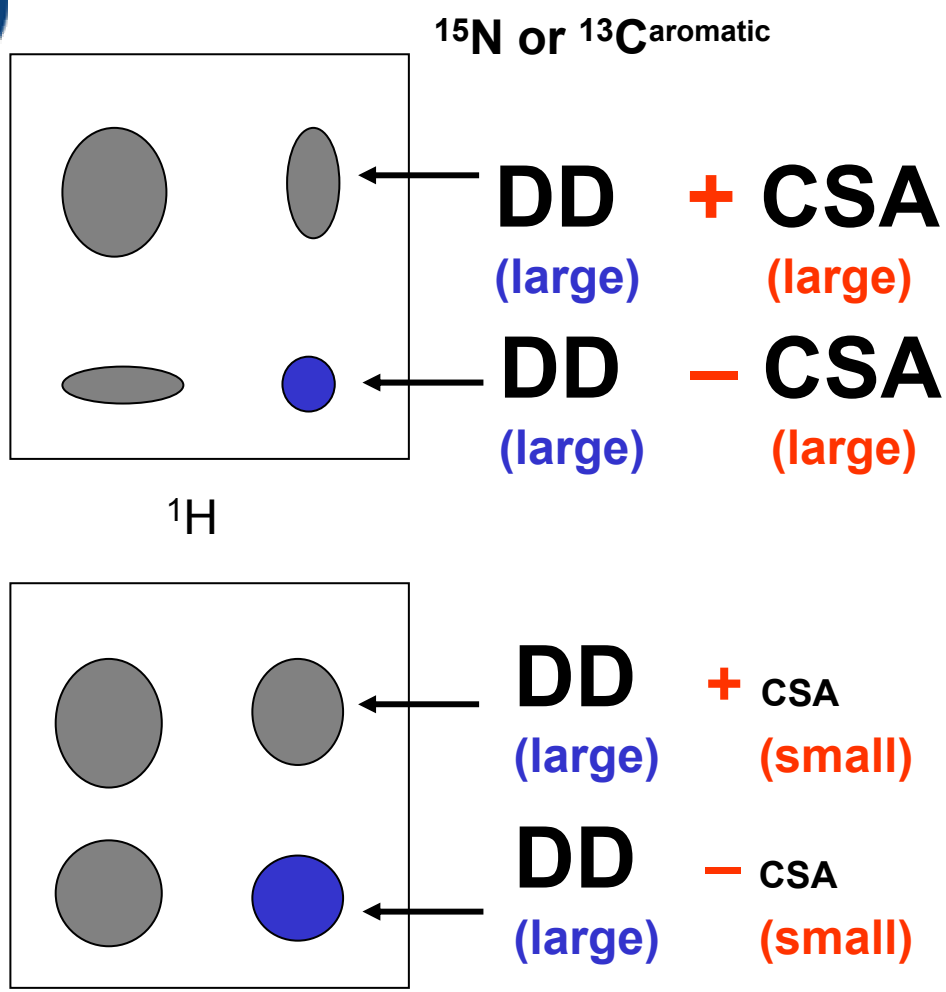


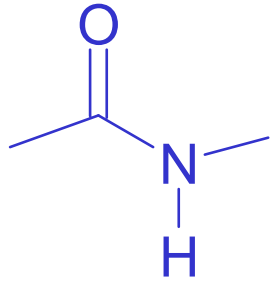
HSQC

In both ^{1}H and ^{15}N dimensions, both J-split components are mixed by hetero-nuclear decoupling. This collapses each ^{15}N and ^{1}H doublet into a single peak in each dimension.

Wider and Wuthrich, *Current Opinion in Structural Biology*,1999, 9:594-601

Interference of Relaxation between DD and CSA Relaxation





(A) At High Magnetic Field (TROSY line-narrowing effect)

(B) At Low Magnetic Field (almost no TROSY line-narrowing effect)

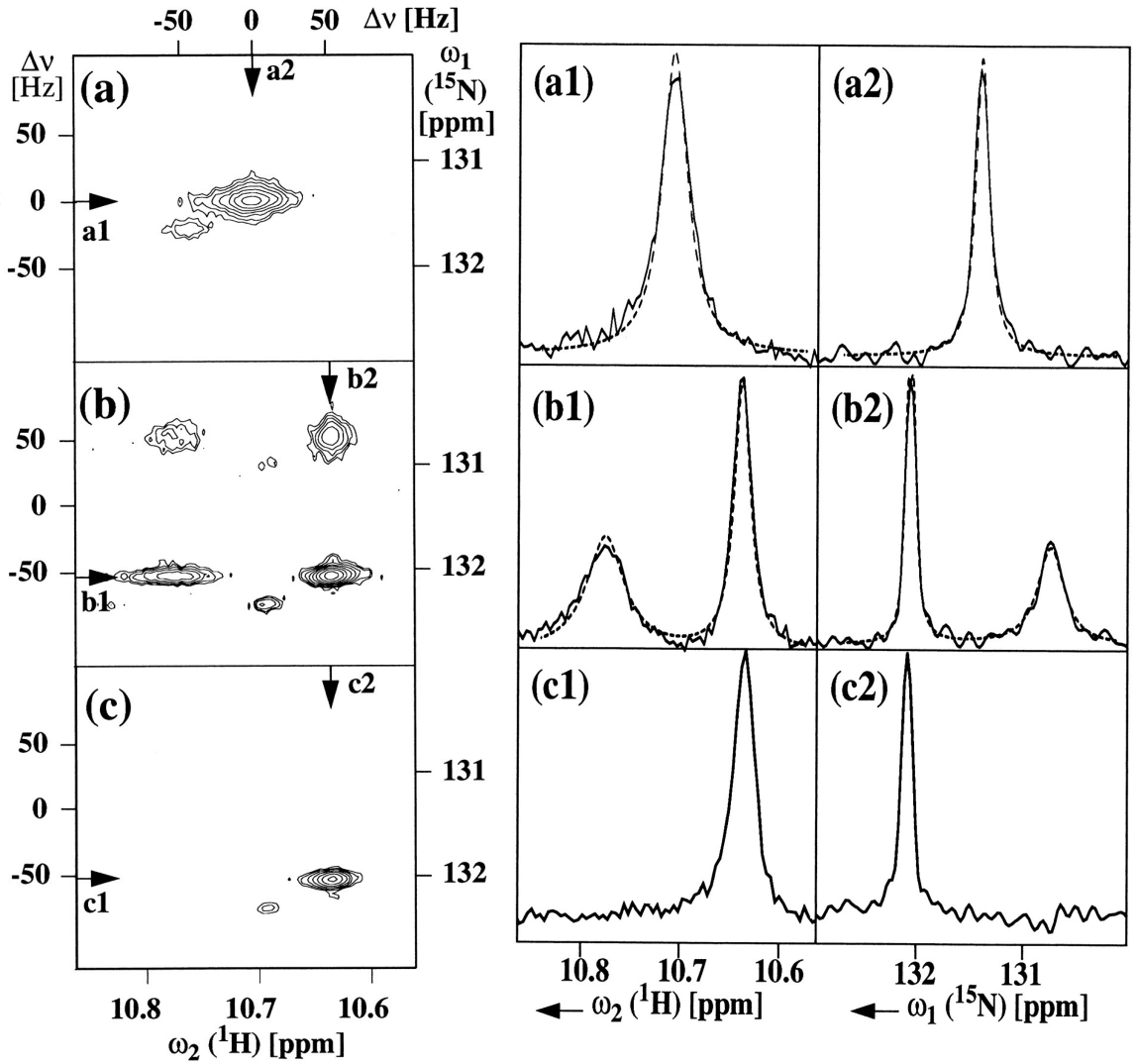
- **DD relaxation is field-independent. However, CSA$\propto B_0^2$, therefore at high magnetic fields, CSA relaxation can be comparable to DD relaxation, and the interference effect on relaxation can be observed.**

TROSY, 40 kDa, 750 MHz

Decoupled HSQC (during t1 &t2)

Non-decoupling HSQC

TROSY-HSQC

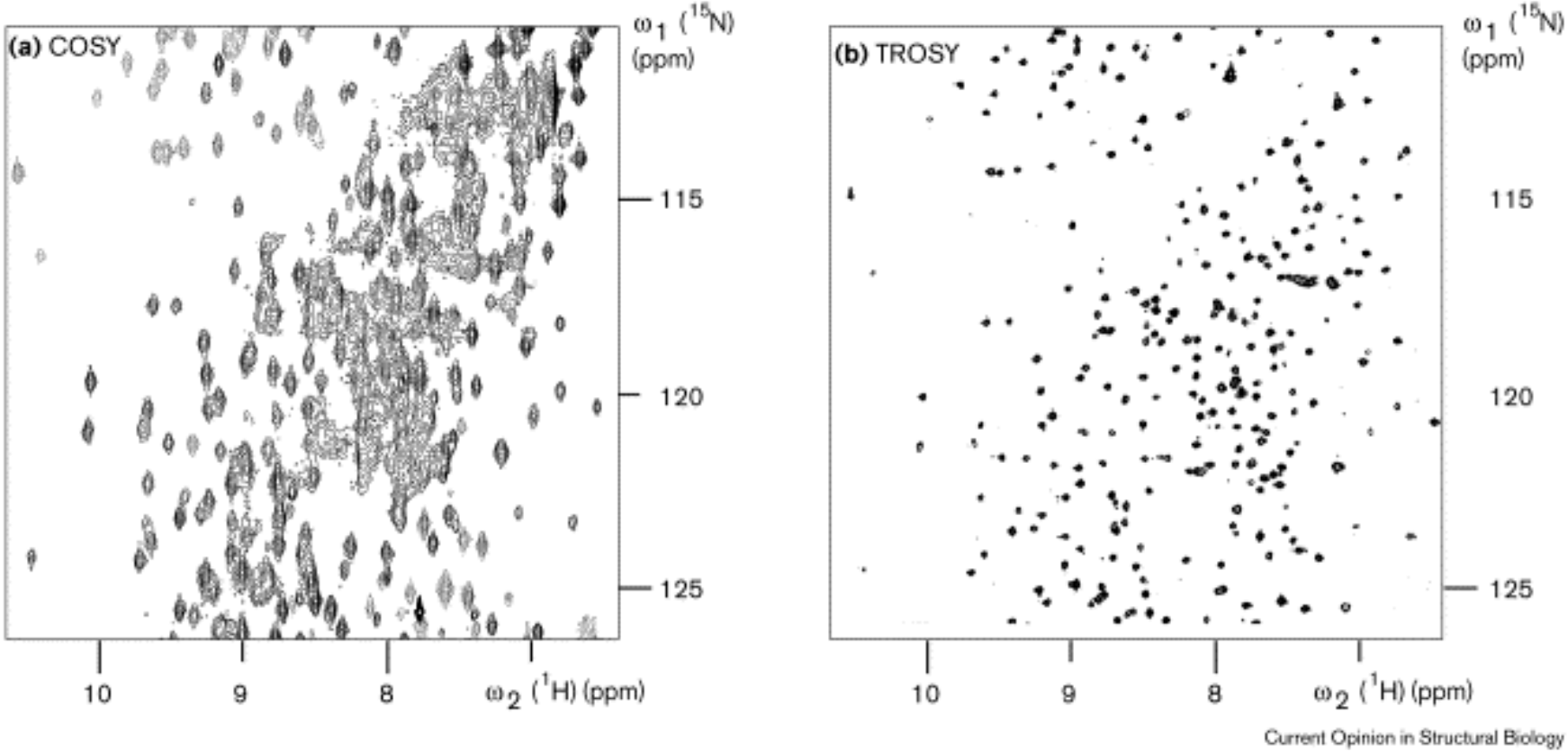


Linewidrh:
60% reduction in ^{1}H, 40%reduction in ^{15}N

If perdeuterated:
Expected reduction 40-fold for ^{1}H & 10-fold for ^{15}N

Pervushin et al. *PNAS USA*, v94, p12366 (1997)

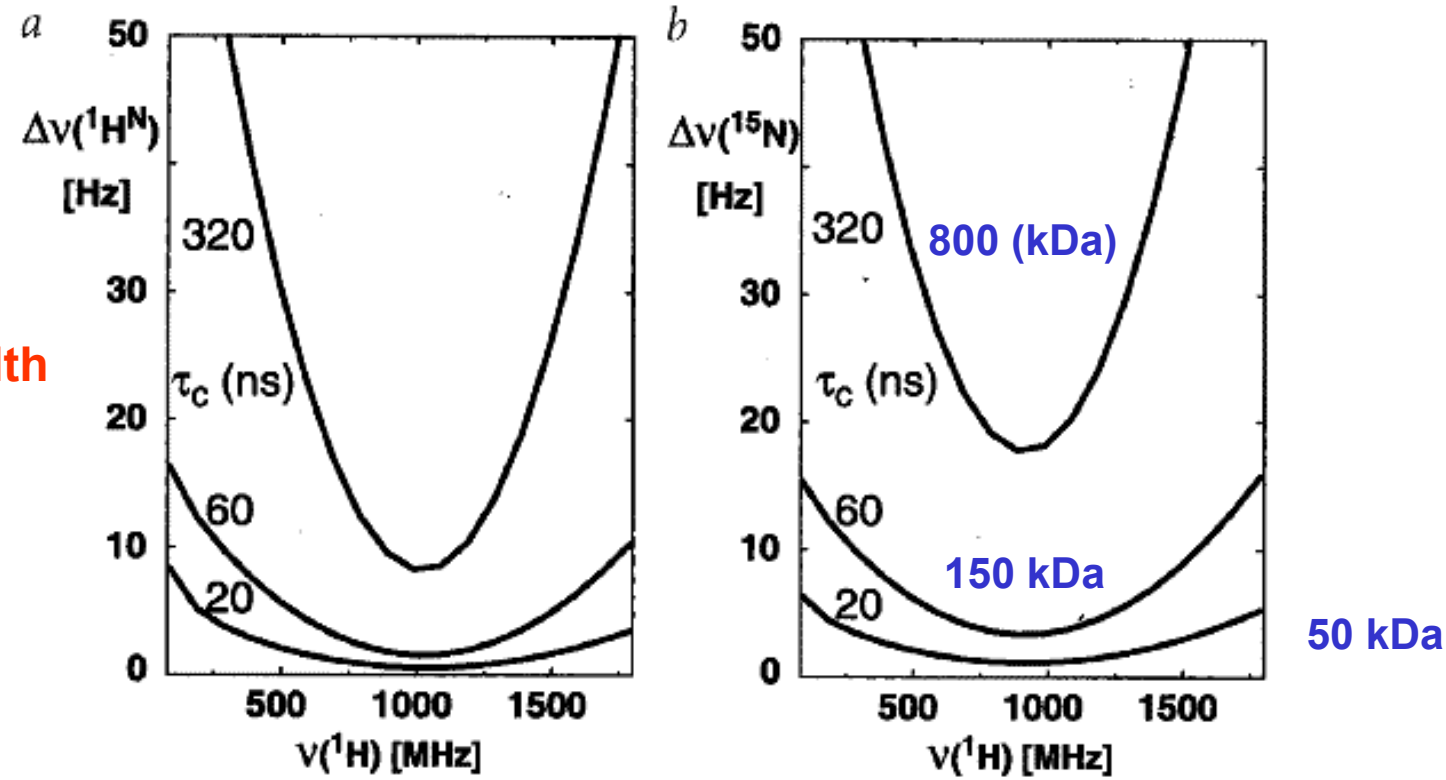
The Sensitivity and Resolution Gain by TROSY and Deuteration



u-^{2}H,^{15}N-Gyrase-45 (45 kDa), 750 MHz

Wider and Wuthrich, *Current Opinion in Structural Biology*, 1999, 9:594-601

TROSY Effect is Field Dependent and Motion Dependent



Linewidth

Magnetic field strength

•**Optimal field strength:** 1 GHz for amide NH;
600 MHz for CH in aromatic moieties (500-800 MHz applicable).

•Relaxation rate for the narrowest component of the doublet:

$$R_{1212} = (p-\delta_S)^2(4J(0)+3J(\omega_S)) + p^2(J(\omega_I-\omega_S)+3J(\omega_I)+6J(\omega_I+\omega_S))+3\delta_I^2 J(\omega_I),$$

TROSY Application

- The implication of TROSY technique is revolutionary and wide spreading for large molecules (>20 KDa). Virtually all ^{1}H,^{15}N-HSQC-based double resonance and triple resonance experiments gain sensitivity and spectral resolution via TROSY.

- Salzmann et al., "TROSY in triple-resonance experiments: New perspectives for sequential NMR assignment of large proteins" *PNAS USA*, v95, 13585-13590 (1998).

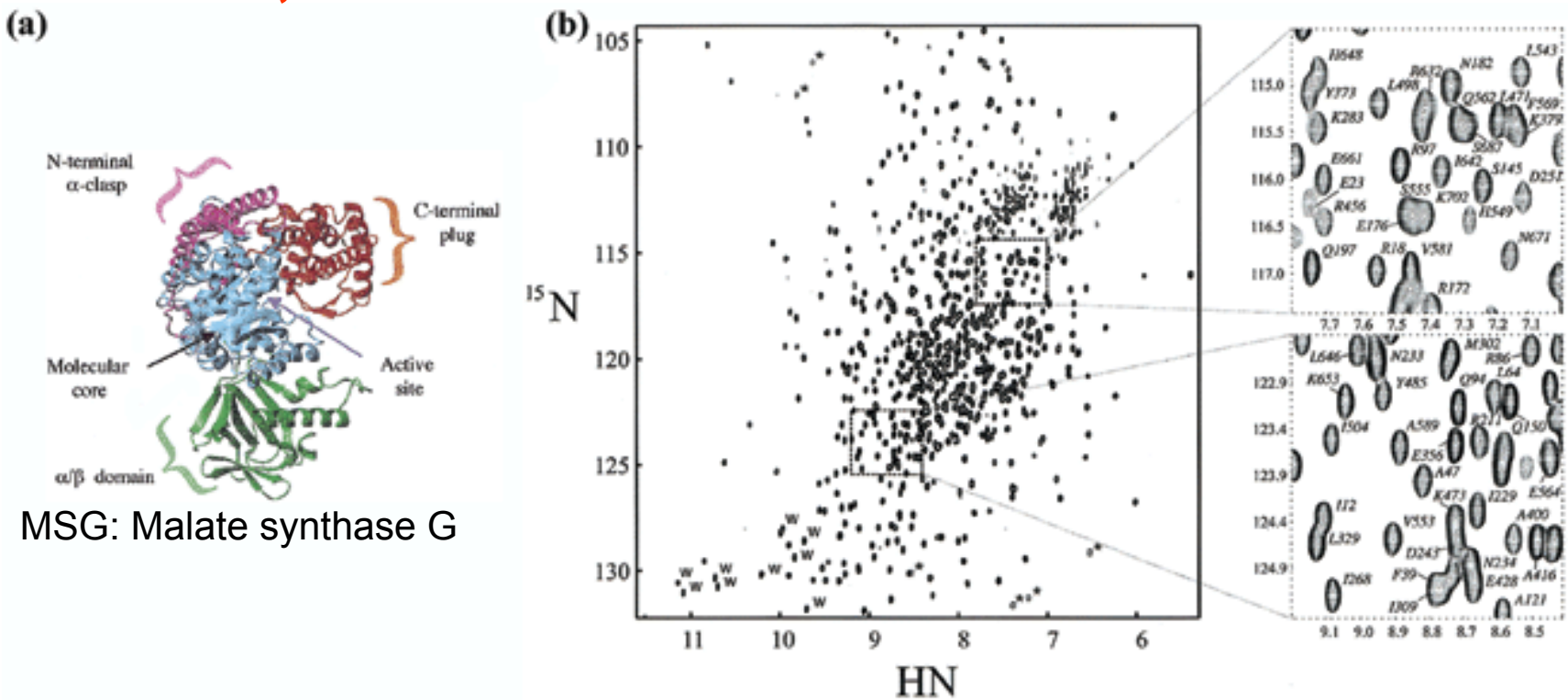
- D. Yang and L.E. Kay, *JACS*, "TROSY Triple-Resonance Four-Dimensional NMR Spectroscopy of a 46 ns Tumbling Protein "

- Salzmann et al., "NMR Assignment and Secondary Structure Determination of an Octameric 110 kDa Protein Using TROSY in Triple Resonance Experiments", *JACS*, 122, 7543-7548 (2000).

Pushing the Size Limit by TROSY ,Deuteration, and Selective Isotope Labeling

723 a.a., 81.4 kDa

TROSY-HSQC of U-[^{2}H,^{15}N]-MSG at 800 MHz



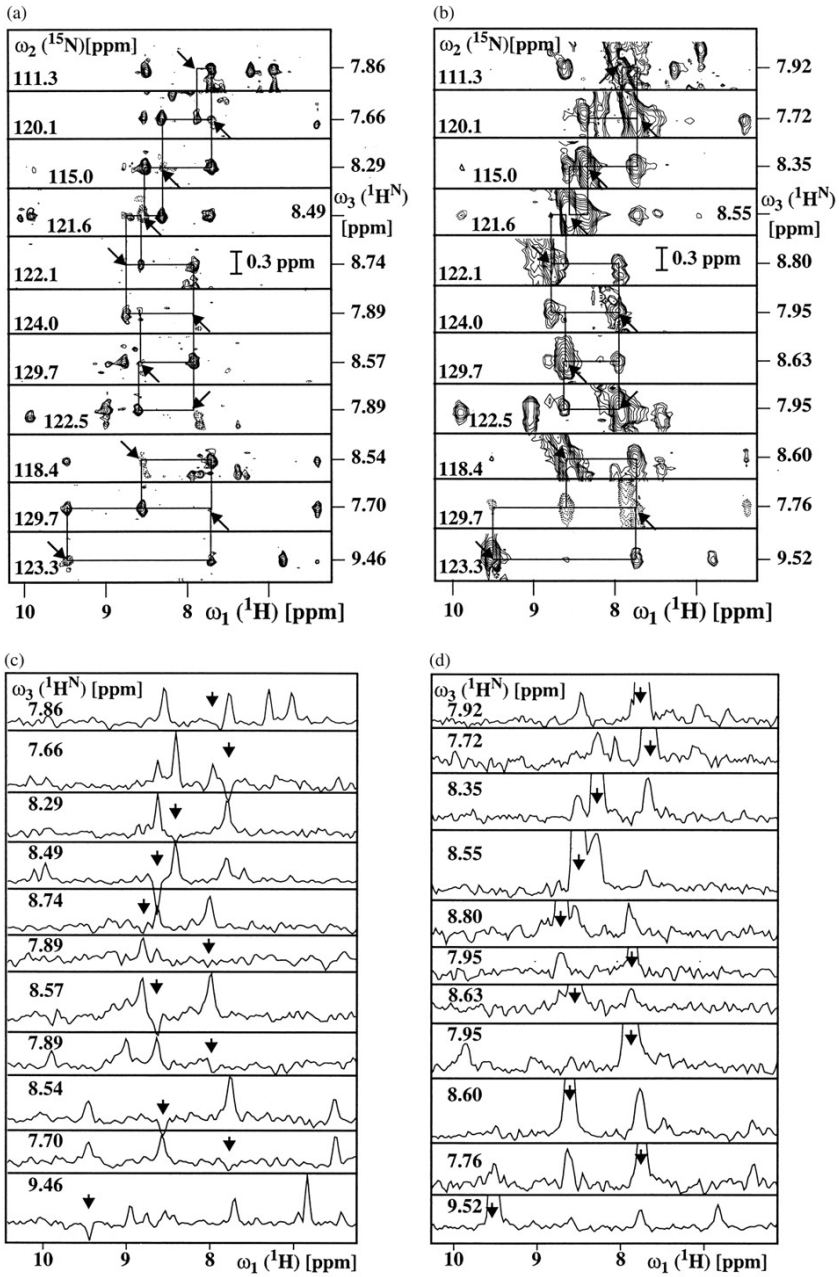
MSG: Malate synthase G

>95% ^{1}H, ^{15}N, $^{13}C_{\alpha}$, ^{13}CO, $^{13}C_{\beta}$ assigned !!!

Tugarinov et al, ***JACS***, 2002, 124, 10025-10035

3D NOESY-[^{1}H, ^{15}N, ^{1}H]-ZQ-TROSY

NOESY-[^{1}H, ^{15}N, 1H]-ZQ-TROSY



110 kDa,
[70% ^{2}H, U-^{15}N]-DHNA

Diagonal peaks in NOESY are suppressed !

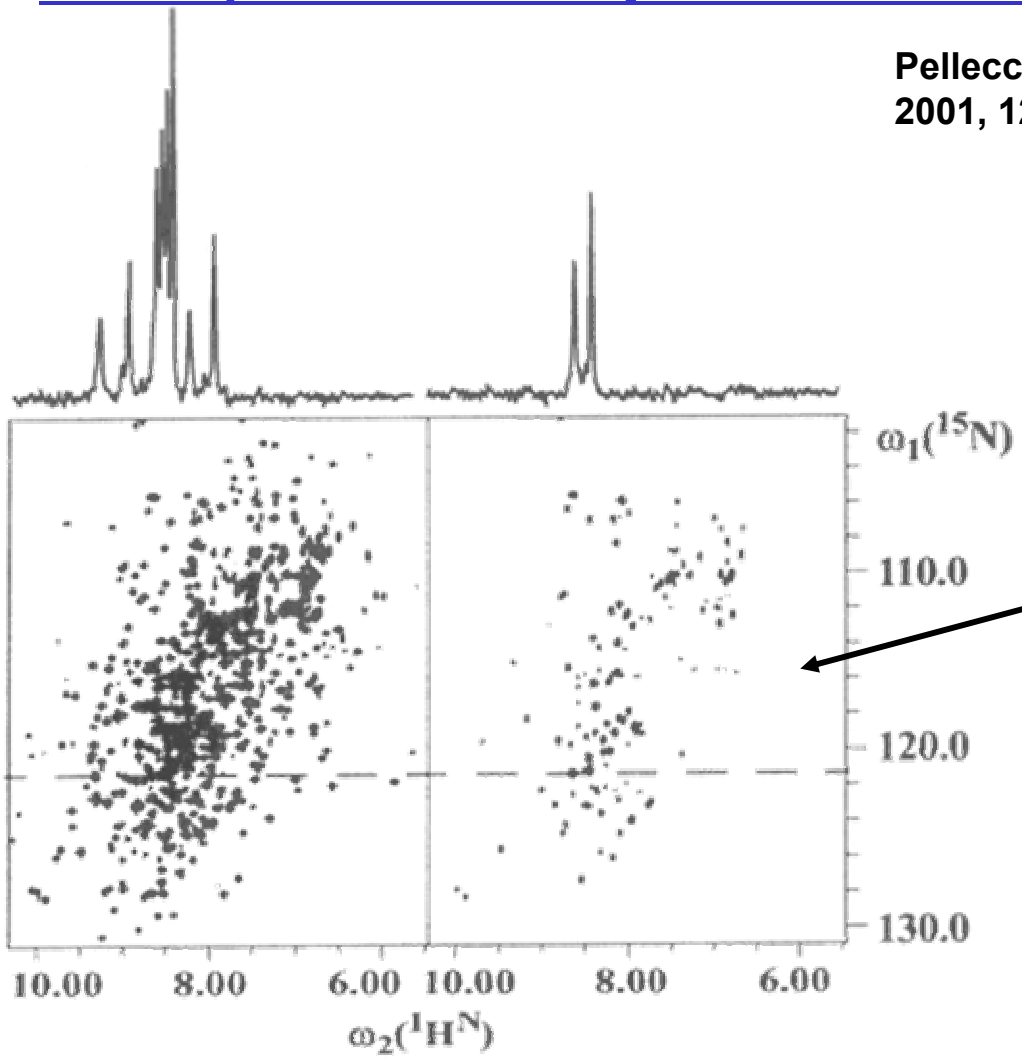
Pervushin et al.
***PNAS USA*, V96, P9607, (1999)**

SEA (Solvent Exposed Amides)-TROSY

Pellecchia et al., *J. Am. Chem. Soc.* 2001, 123, 4633-4634

71 kDa, [U-^{2}H, ^{15}N]- P450

TROSY



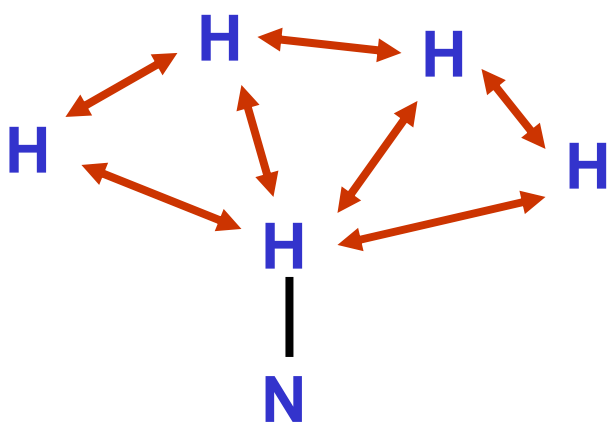
SEA-TROSY (only the solvent exposed NH are observed)

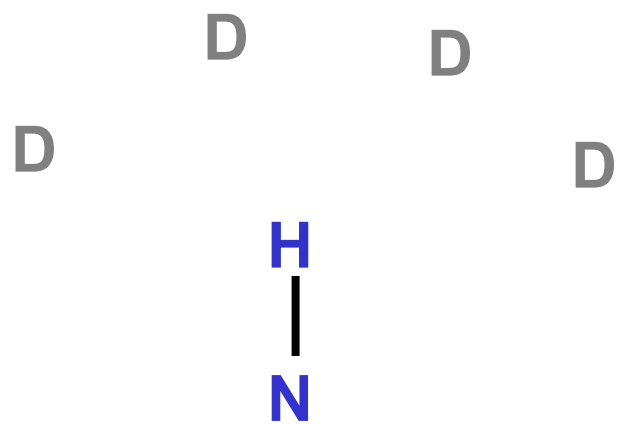
This technique has become very popular for drug screening since binding mainly occurs on the surface of a protein.

Deuteration

- Reduce relaxation ($\gamma_{D/}/\gamma_H = 1/6.5$). (a maximal 16 fold reduction).
- Reduce number of signals.
- Suppress spin-spin diffusion

^1H ↕ ^{13}C Dipole/dipole relaxation





Impact of Deuteration on Relaxation and Sensitivity

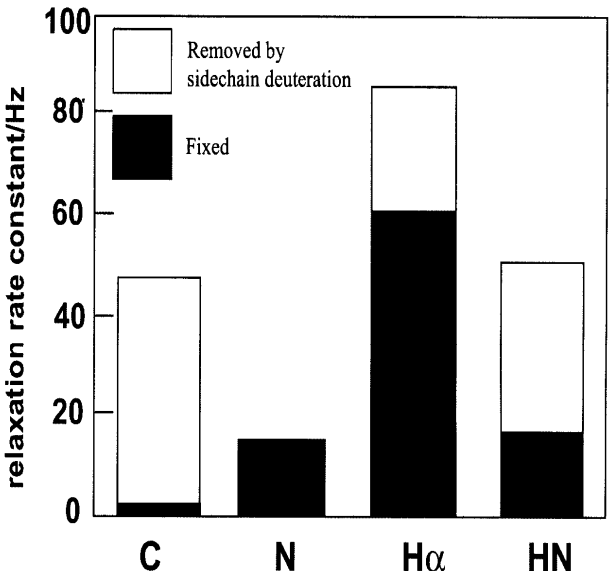


Figure from D. Nietlispach in " EMBO NMR course, 2003".

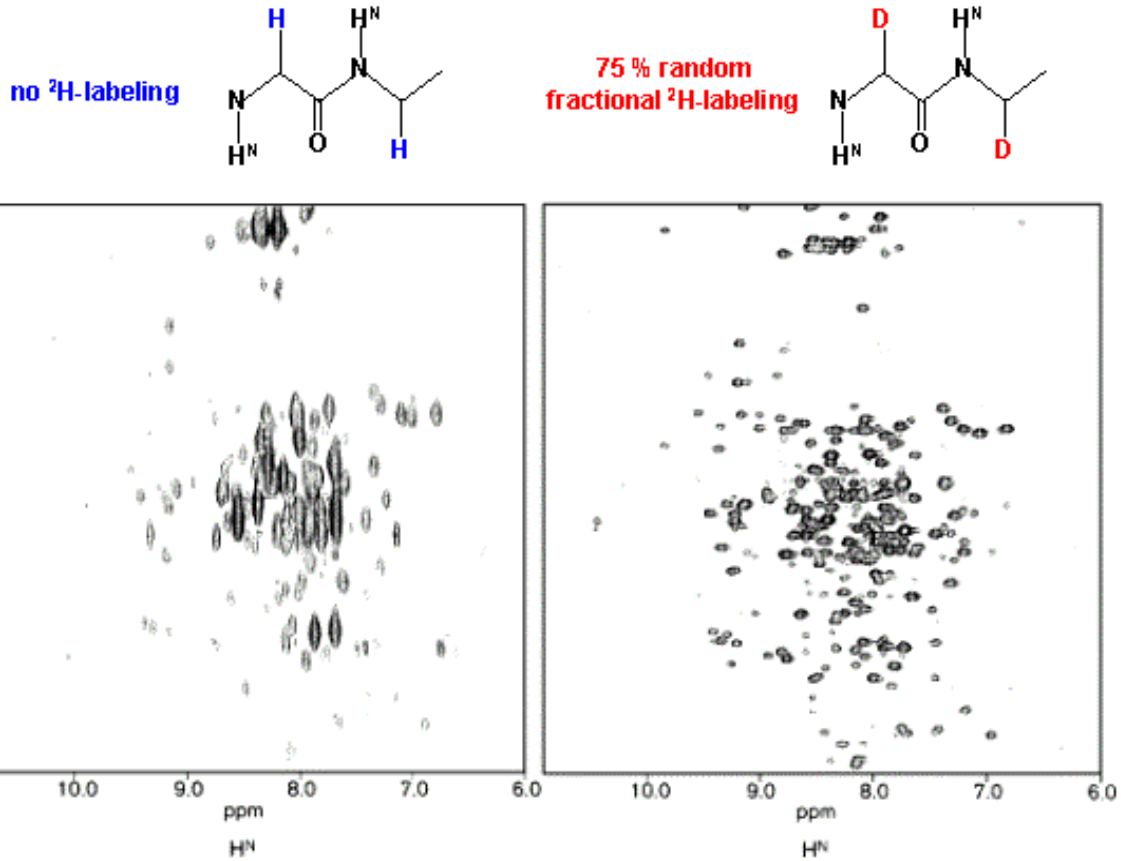


Figure from M. Sattler, http://www.embl-heidelberg.de/nmr/sattler/teaching/

Some Notes on TROSY

- **Intrinsic 50% loss in sensitivity (the sensitivity improved version*) due to rejection of some coherence pathway. However, for large proteins (>20 kDa) at high magnetic fields, the detection of the most slowly relaxing peak compensates for the loss of sensitivity. Instead sensitivity and resolution are both gained.**

- **TROSY effect is field strength dependent. Optimal field strength: 1 GHz for amide NH; 600 MHz for aromatic moieties (500-800 MHz).**

- **Minimal of phase cycling need to be complete for the selection of the narrowest component. (1 out of 4).**

- **The larger a protein (< 200 kDa), the more pronounced line-narrowing effect by TROSY.**

***Pervushin et al., J. Biomol. NMR, 1998, 12:345-348**

TROSY via INEPT Transfer Becomes Ineffective for Very Large Molecule (>200 kDa)

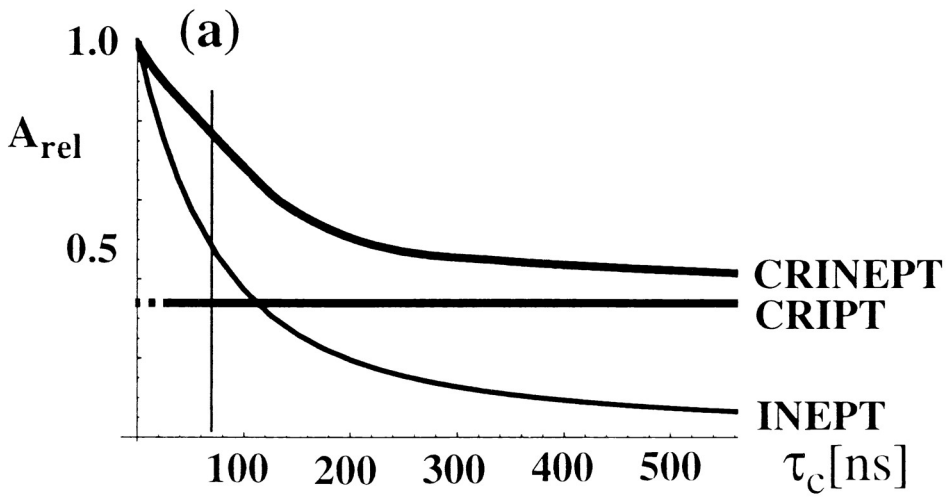
- Due to very fast transverse (T2) relaxation for large proteins (>200 kDa). TROSY via INEPT through-bond scalar coupling transfer becomes inefficient.

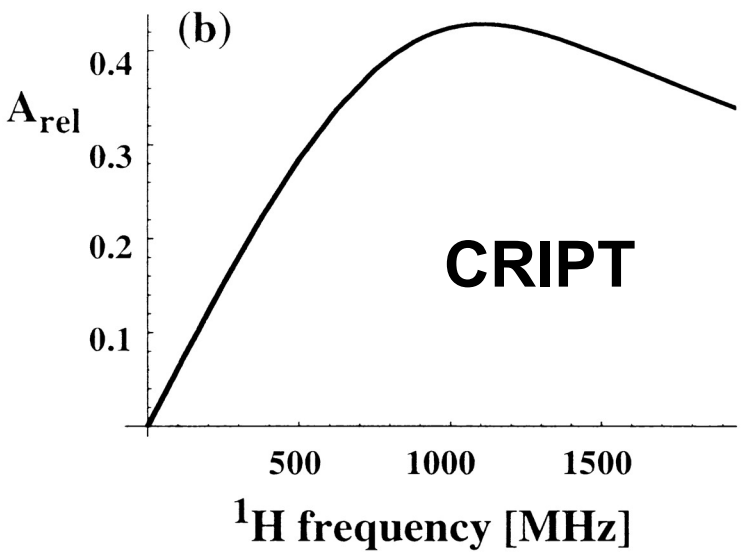
- For M.W. >200 kDa, use cross correlation between dipole-dipole coupling and CSA relaxation to transfer in-phase ^{1}H coherence to ^{15}N coherence in ^{15}N-^{1}H moieties (CRIP).

- Cross relaxation enhanced polarization transfer (CRINEPT=CRIP+INENP) become more effective for very large molecules.

- Riek et al. "Polarization transfer by cross-correlated relaxation in solution NMR with very large molecules", PNAS USA V96, 4918-4923, 1999.

CRIPT or CRINEPT Offers Higher Sensitivity for Very Larger Molecules (>200 kDa)





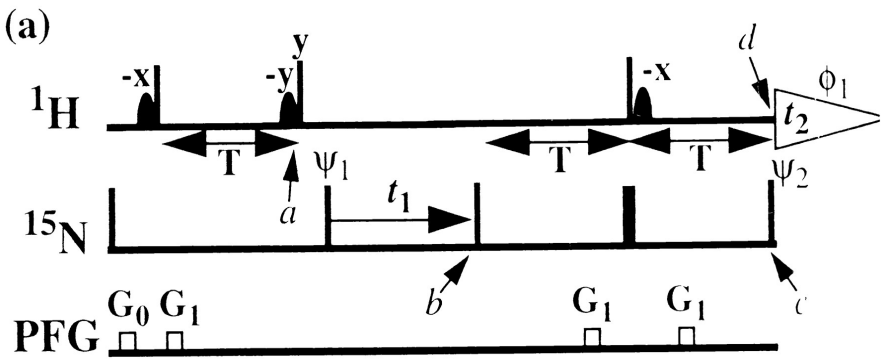
INEPT: **Insensitive nuclei enhanced by polarization transfer**

CRIPT: **Cross relaxation-induced polarization transfer (between DD coupling and CSA relaxation).**

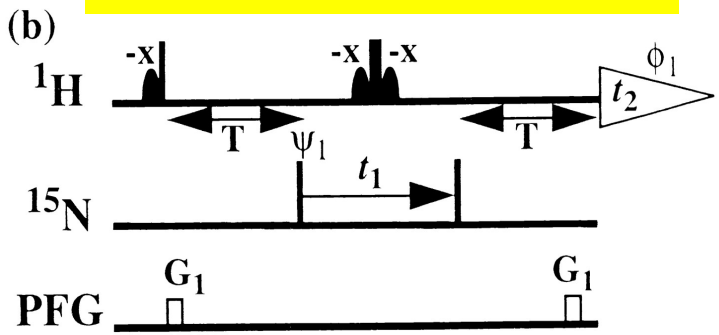
CRINEPT: **Cross relaxation-enhanced polarization transfer (CRIPT+INEPT)**

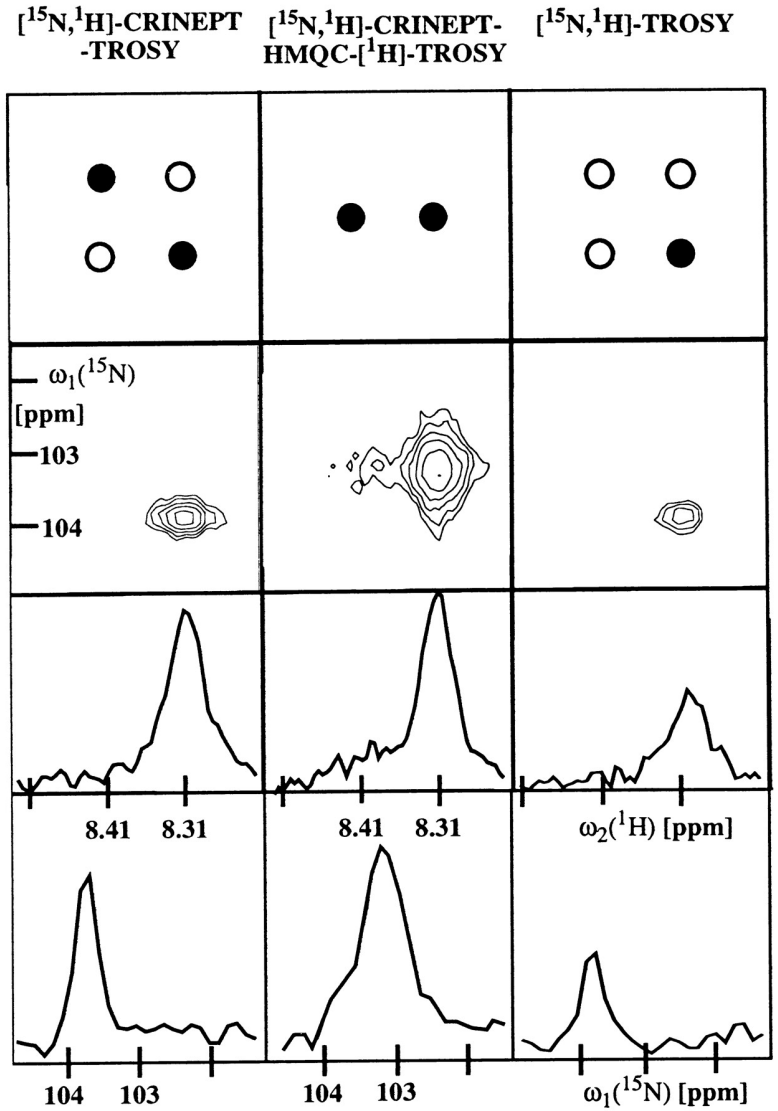
CRINEPT-TROSY for Very Larger Molecules (>200 kDa)

CRINEPT-TROSY



CRINEPT-HMQC-TROSY



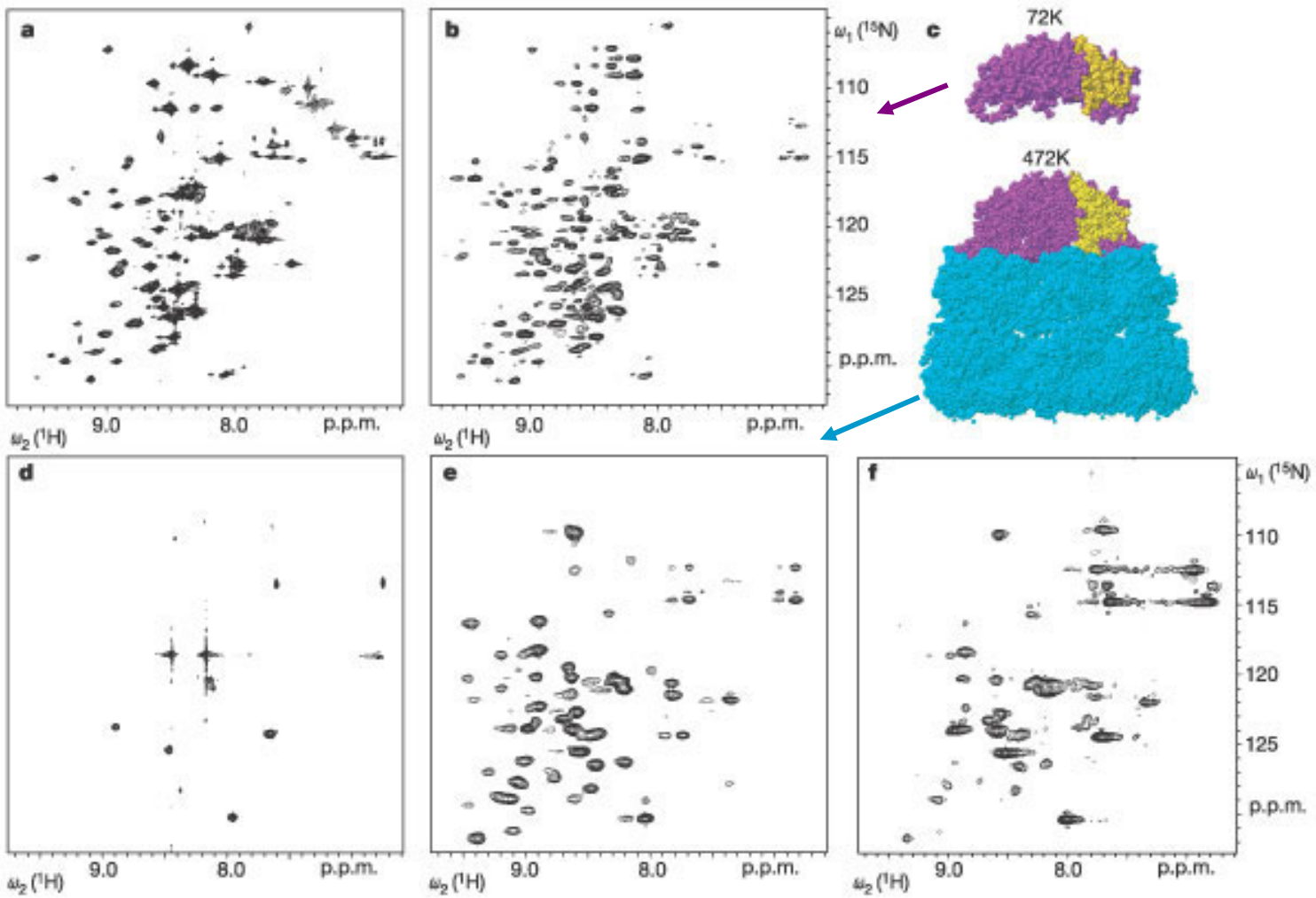


In (b): No splitting in the ^{15}N dimension since $^1J_{NH}$ is decoupled during the ^{15}N evolution.

A 470 kDa Complex by Solution NMR

TROSY CRIPT-TROSY

72 kDa



472 kDa

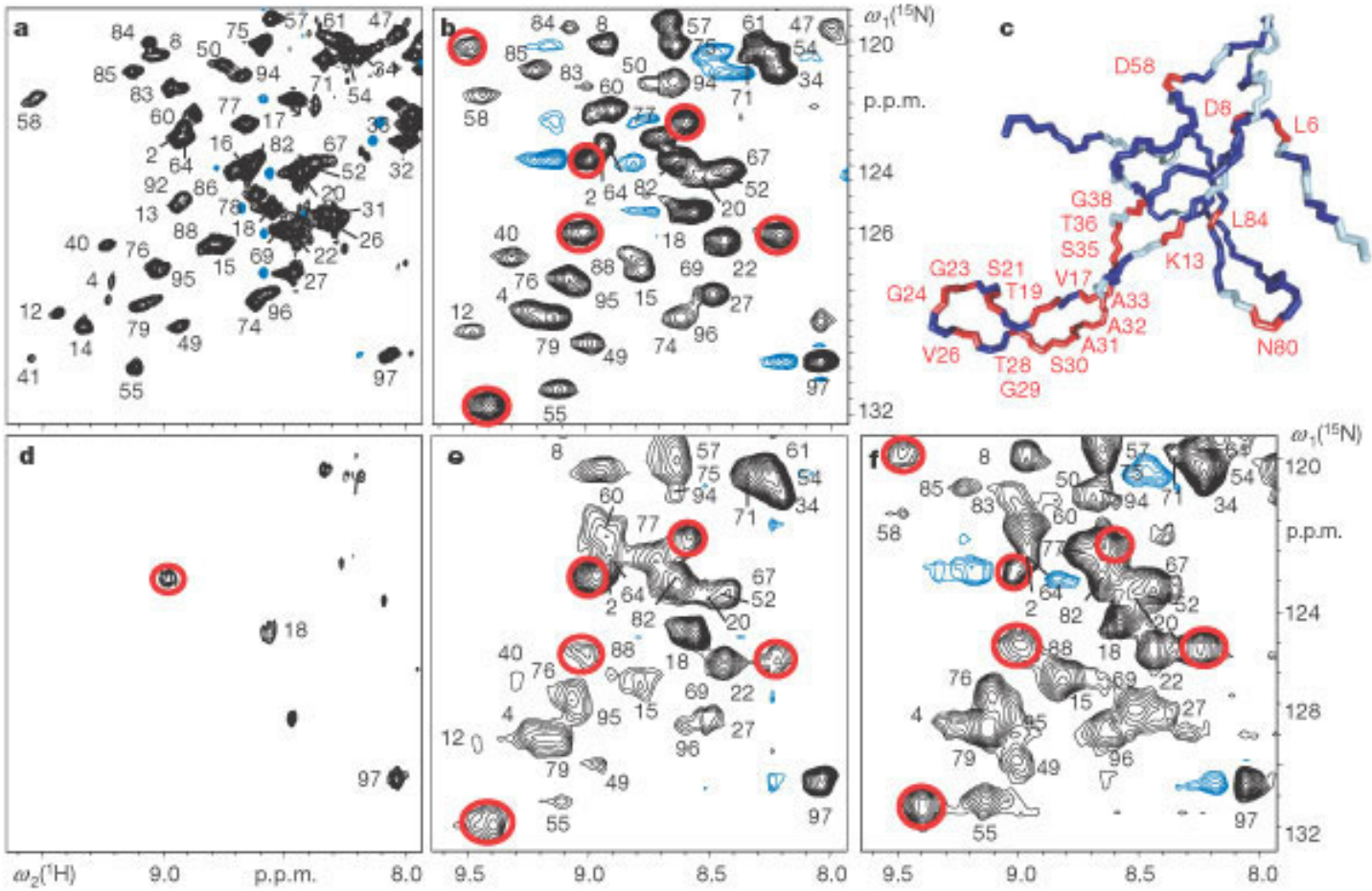
TROSY CRIPT-TROSY CRINEPT-TROSY

Fiaux et al. (2002) **Nature,** v418, p207

900 kDa Complex by Solution NMR

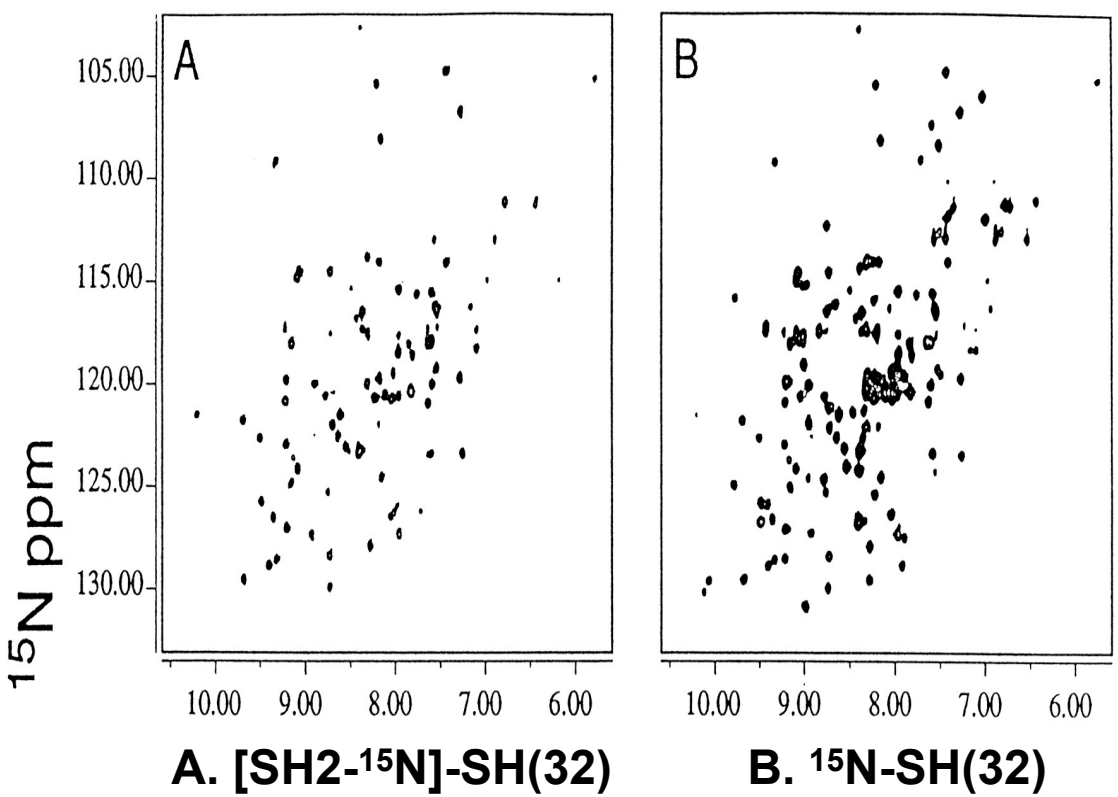
72 kDa 472 kDa

900 kDa

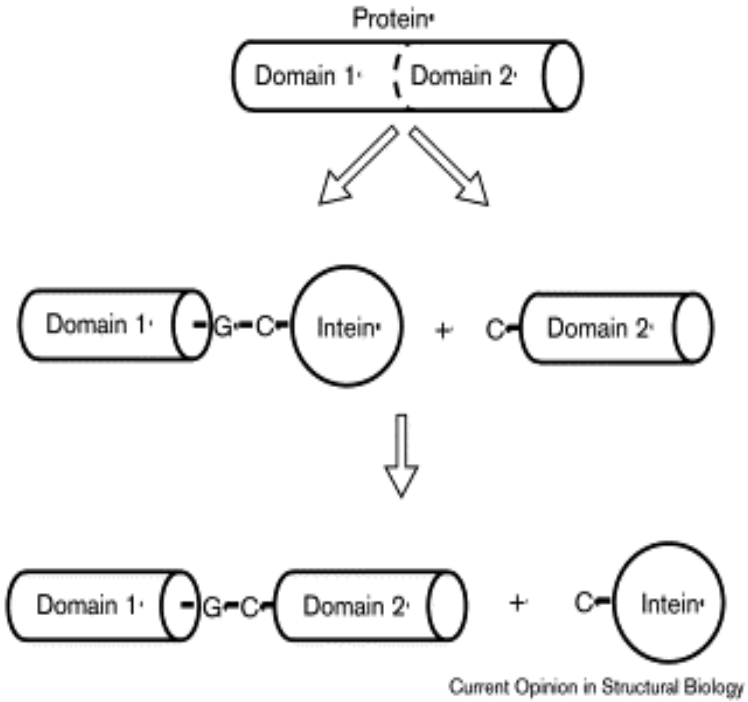


TROSY CRIPT-TROSY

Simplify Spectra by Segmental Labeling



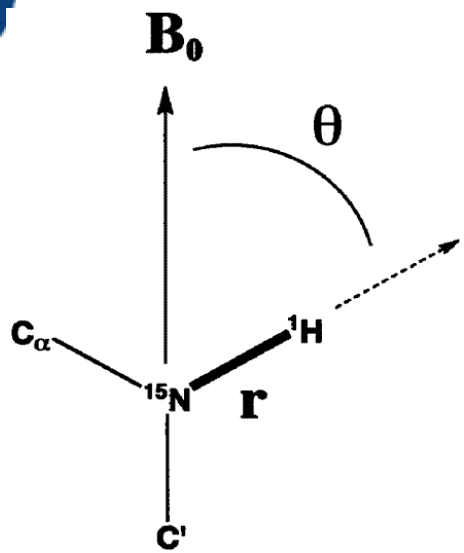
A. [SH2-^{15}N]-SH(32) **B. ^{15}N-SH(32)**

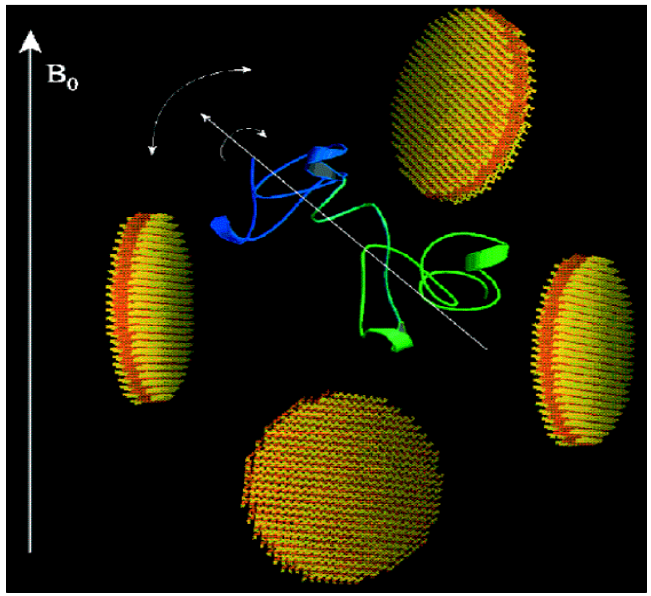


Only one out of several domains is isotope labeled. Spectra are therefore simplified.

Xu et al, **PNAS USA,** 1999, V96, P388

Residual Dipolar Coupling





Figures borrowed from J.H. Prestegard in "**Nature Structural Biology**, NMR Supplement, 1998, v5, p517-522

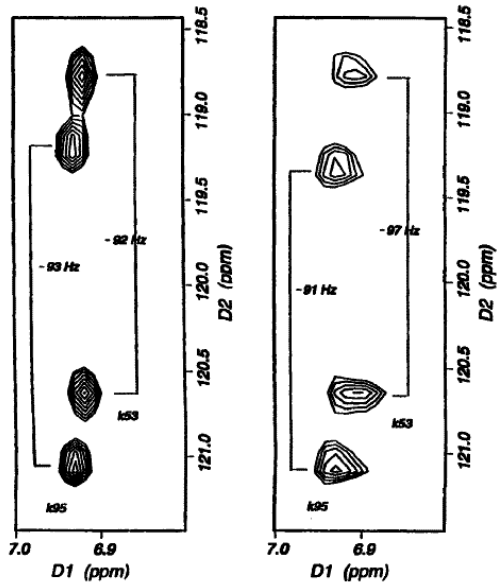
- **Measure θ from residual dipolar coupling to provide additional structural constrain.**

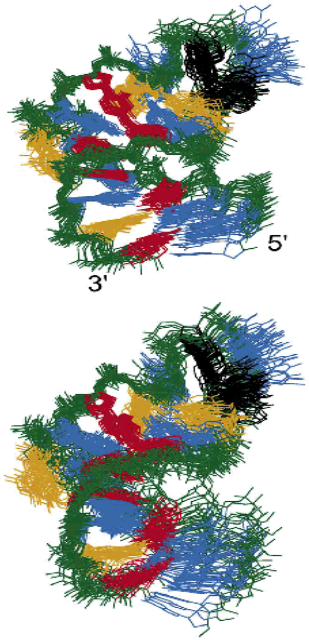
- **Usually residual dipolar coupling is averaged to zero due to the isotropic motion of a protein in solution.**

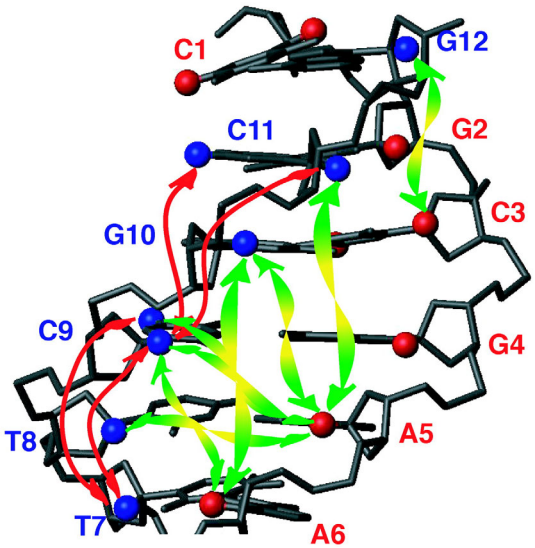
- **Align a paramagnetic protein by high magnetic field strength.**
(Tolman et al. (Prestegard's lab), ***PNAS USA***, 1995, V. 92, P9279).

- **Introduce (enhance) anisotropy by orienting a protein in liquid crystalline medium. (**Tjandra and Bax, ***Science***, 1997, V 278, p1111)

Residual Dipolar Coupling



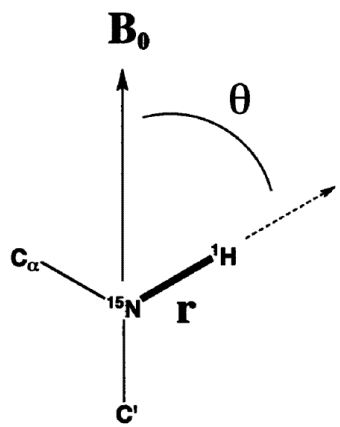




- **Residual dipolar coupling introduces an extra splitting in the ^{15}N dimension in a non-^{1}H decoupled HSQC spectra.**

Tolman et al. *PNAS USA*, (1995), V. 92, P9279

Angle: Structural Refinement ; relative orientation



Long Distance Measurement:
Inter-nuclear distance up to 12 A was measured by residual dipolar coupling.

Boisbouvier et al. *PNAS USA*, (2003) vol. 100, 11333-11338

NMR of Encapsulated Proteins Dissolved in Low-Viscosity Fluids

$\tau_c = \eta v/kT$

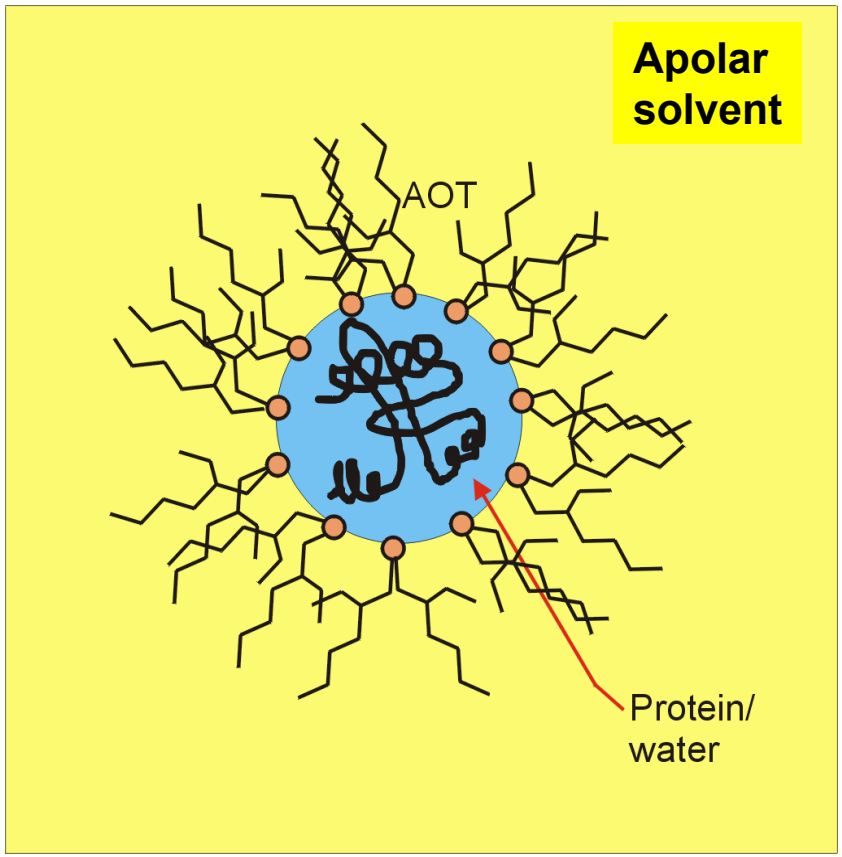
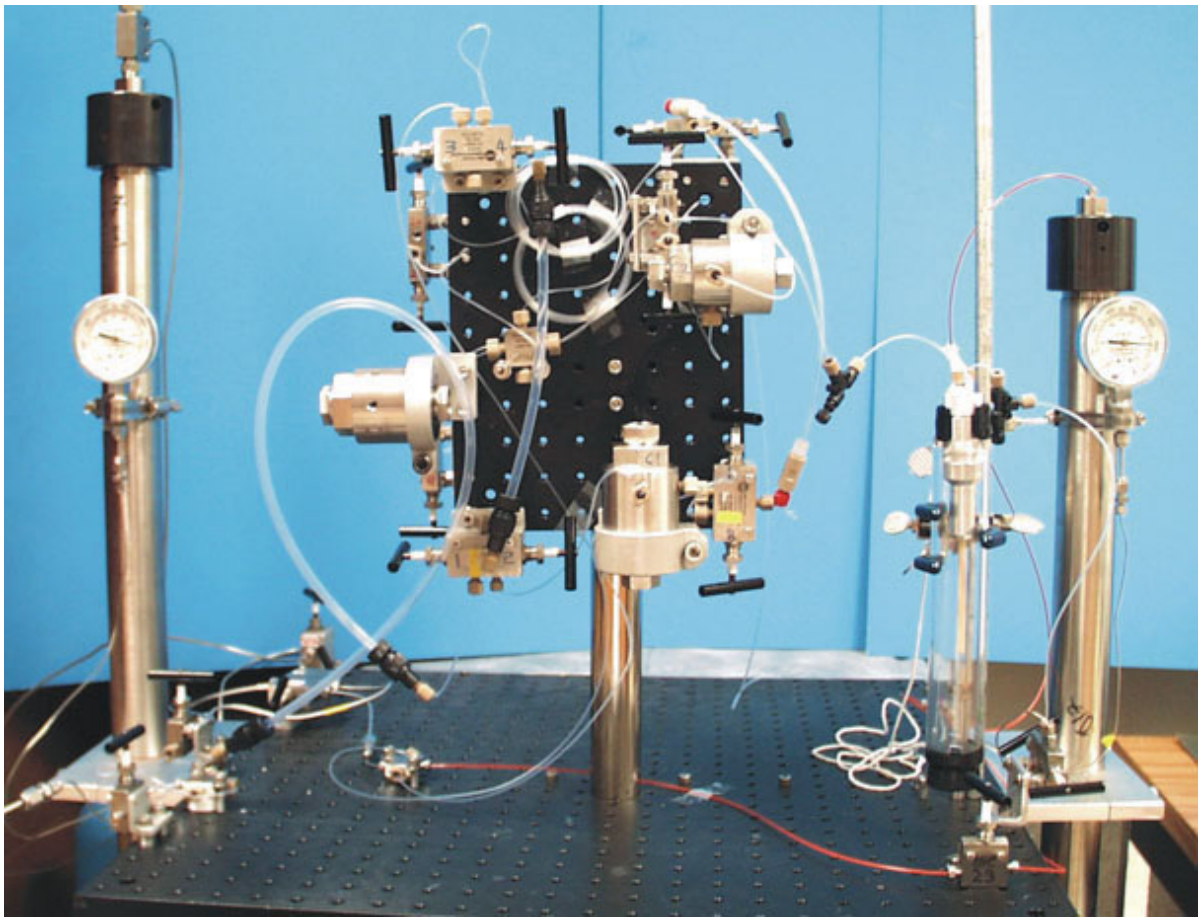


Figure from W. Westler at NMRFAM

- **To arrange for a protein to tumble at a faster rate (smaller t_c) by reducing the viscosity of the bulk solvent.**
- $\eta^{propane}=0.1*\eta^{H2O}$
- **Use the reverse micelle technique to solublize proteins.**
- **Pioneered by J. Wand's lab.**

Wand et al., **PNAS USA**, 1998, V 95, P15299

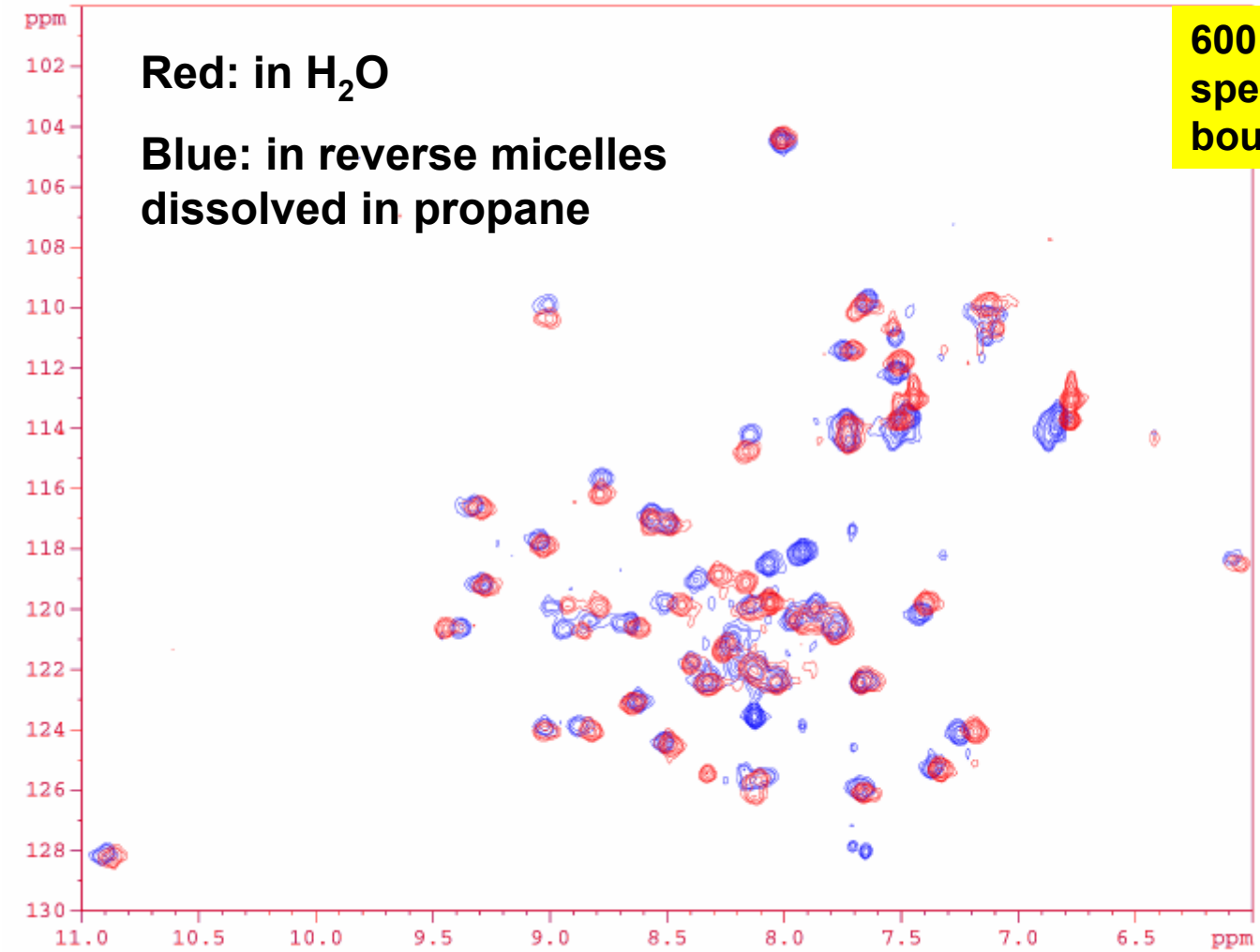
High Pressure Mixing Apparatus for Preparing Protein Sample Under Pressure



Designed for preparing protein samples in liquid propane

W.-J. Wu, G. Vidugiris , E. S. Mooberry, W. M. Westler, J. L. Markley, "Mixing apparatus for preparing NMR samples under pressure," *J. Magn. Reson.* 164, 84-91 (2003)

Native-Like Structure for Chymotrypsin-Bound ^{15}N-OMTKY3 in Reverse Micelles Dissolved in Propane



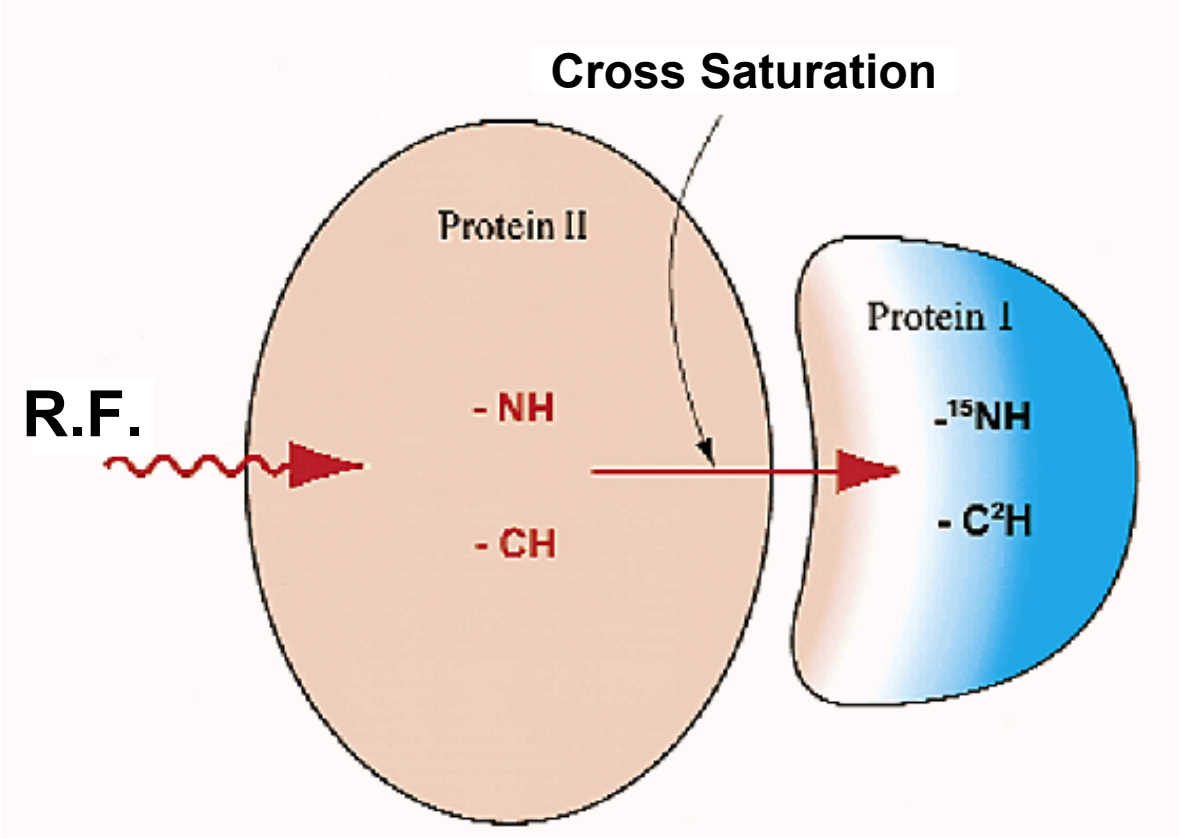
Red: in H_2O

Blue: in reverse micelles dissolved in propane

600 MHz ^{1}H, ^{15}N-HSQC spectra of chymotrypsin-bound ^{15}N-OMTKY3

W.-J. Wu, *J. Magn. Reson.* 164, 84-91 (2003)

Cross Saturation Method for Determining the Interfaces of Large Protein–Protein Complexes



The residues of protein I at the interfaces acquires change in ^{1}H, ^{15}N chemical shifts due to saturation transfer from protein II.

H. Takahashi et al., Nature Structural Biology, (2000) V7, pp 220 - 223

Cross Correlation and TROSY Effect

- **Cross correlation:** **Two different types of interactions interacts with each other. DD-DD, DD-CSA, CSA-CSA cross correlation. (compared to "Cross relaxation: NOEs")**

- **TROSY takes advantage of the interference relaxation between chemical shift anisotropy (CSA) and dipole-dipole coupling (DD-coupling) for large molecules at high magnetic fields.**

How does the "line-narrowing" (reduction in line width) occur?

Chemical Shift Anisotropy

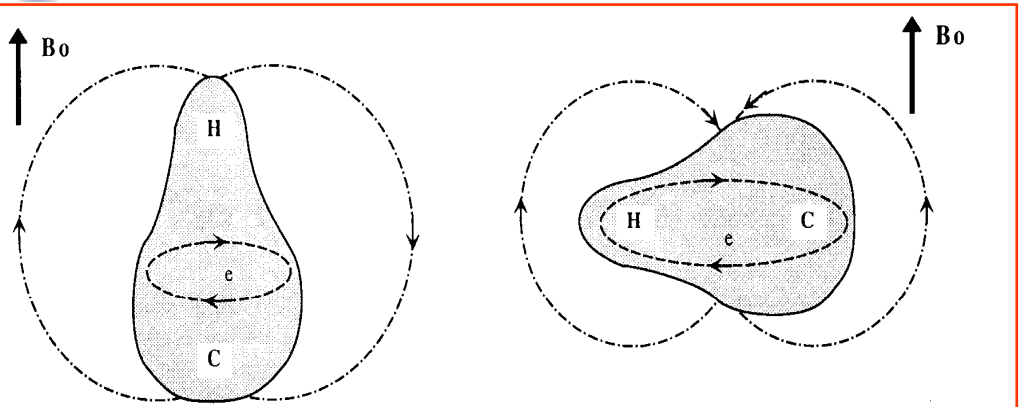
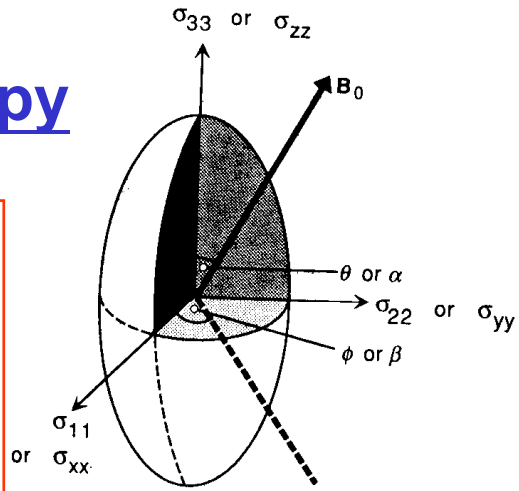
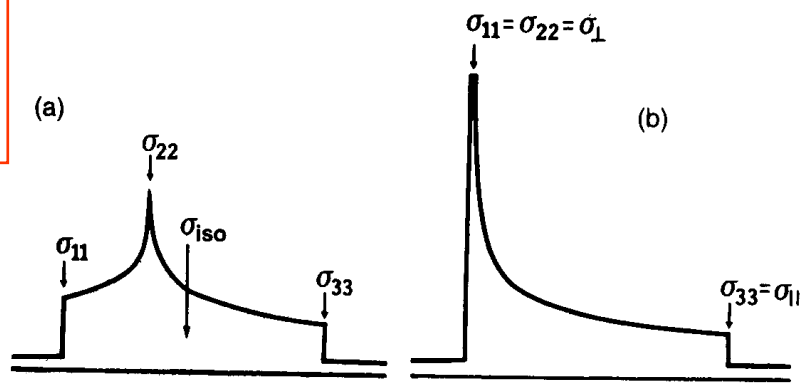


Fig. 1.38 Origins of chemical-shift anisotropy in a C—H bond. The circulation of the electrons in a plane perpendicular to the bond describes a smaller area than circulation in a plane containing the bond. (Redrawn from ref. 12 with permission.)



$B_{eff}=B_0 (1-\sigma)$

σ: shielding constant or chemical shift tensor.



(a) Asymmetric (b) Axially symmetric

1. σ is directly related to the electron density at a distance r from the nucleus by Lamb's equation.
2. There are three principle components of the tensor: σ_{11}, σ_{22}, σ_{33} (or σ_{xx}, σ_{yy}, σ_{zz}).
3. Isotropic shift tensor: $\sigma_{iso}=1/3\ (\sigma_{11}+\sigma_{22}+\sigma_{33})$.
4. The shift tensor σ is related to the Larmor frequency ω_0: $\omega_0=B_0(1-\sigma)\ \gamma/2\pi$

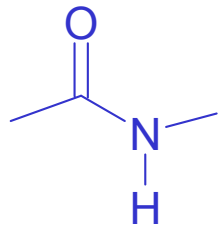
Figures taken from p34 and p40 of "Biomolecular NMR Spectroscopy" by Jeremy N.S. Evans

Transverse Relaxation and TROSY

Consider two scalar coupled spines 1/2 , I and S

Let I-S = H-N

$$p = \frac{1}{2\sqrt{2}}\gamma_I\gamma_S\hbar/r^3_{IS}$$



DD coupling between spin I and spin S

$$\delta_I = \frac{1}{3\sqrt{2}}\gamma_I B_0 \Delta\sigma_I \qquad \delta_s = \frac{1}{3\sqrt{2}}\gamma_s B_0 \Delta\sigma_s$$

Chemical shift anisotropy of I and S

γ_I, γ_S : gyromagnetic ratios of I and S; $\hbar$: Plank constant / 2π ;

r_{IS}: inter-nuclear distance between I and S; B_0: magnetic field strength

$\Delta\sigma_I, \Delta\sigma_s$: the differences between the axial and the perpendicular principle components of the axially symmetric chemical shift tensors of I and S.

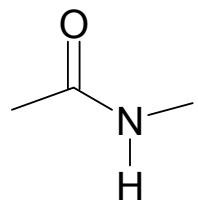
$\gamma^{1H} \sim 10 * \gamma^{15N}$, $\gamma^{1H} \sim 4 * \gamma^{13C}$, $\gamma^{1H} \sim 6.5 * \gamma^{2H}$

Transverse Relaxation and Interference Effect by Cross Correlation between DD and CSA

$$R_{1212} = (p - \delta_S)^2(4J(0) + 3J(\omega_S)) + p^2(J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)) + 3\delta_I^2 J(\omega_I),$$

$$R_{3434} = (p + \delta_S)^2(4J(0) + 3J(\omega_S)) + p^2(J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)) + 3\delta_I^2 J(\omega_I),$$

- **R_{1212} and R_{3434} are the transverse relaxation rates of the individual components of the "S" doublet (i.e. ^{15}N, and let $1H^N$ be the "I" spin) in a single quantum spectrum.**



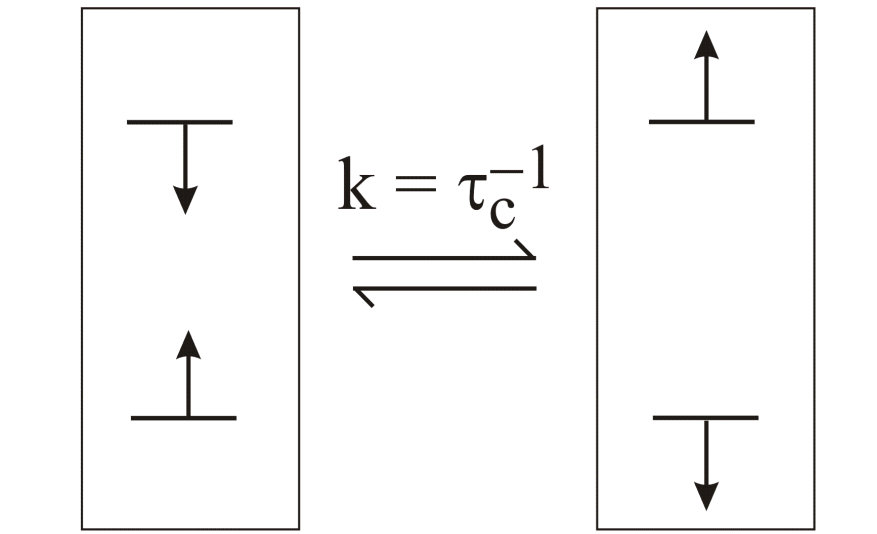
- **J(w) represent the spectral density functions at the frequencies indicated.**

- **In the slow-tumbling limit, only terms in the J(0) need to be considered.**

- **Recall that J(w) is the power (energy) available to bring about relaxation as a function of molecular tumbling.**

- **If CSA and DD coupling are comparable, i.e. $p \approx \delta_s$, The relaxation rate of R_{1212} becomes small (slow relaxation), and the resonance at ω_s^{12} relaxes slowly even for large molecules.**

Relaxation of Transverse Magnetization:



Fast Exchange limit

$$\frac{(\Omega_1 - \Omega_2)^2}{k} = \Delta\Omega^2 \tau_c$$

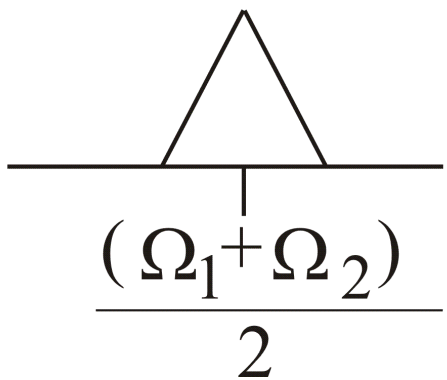
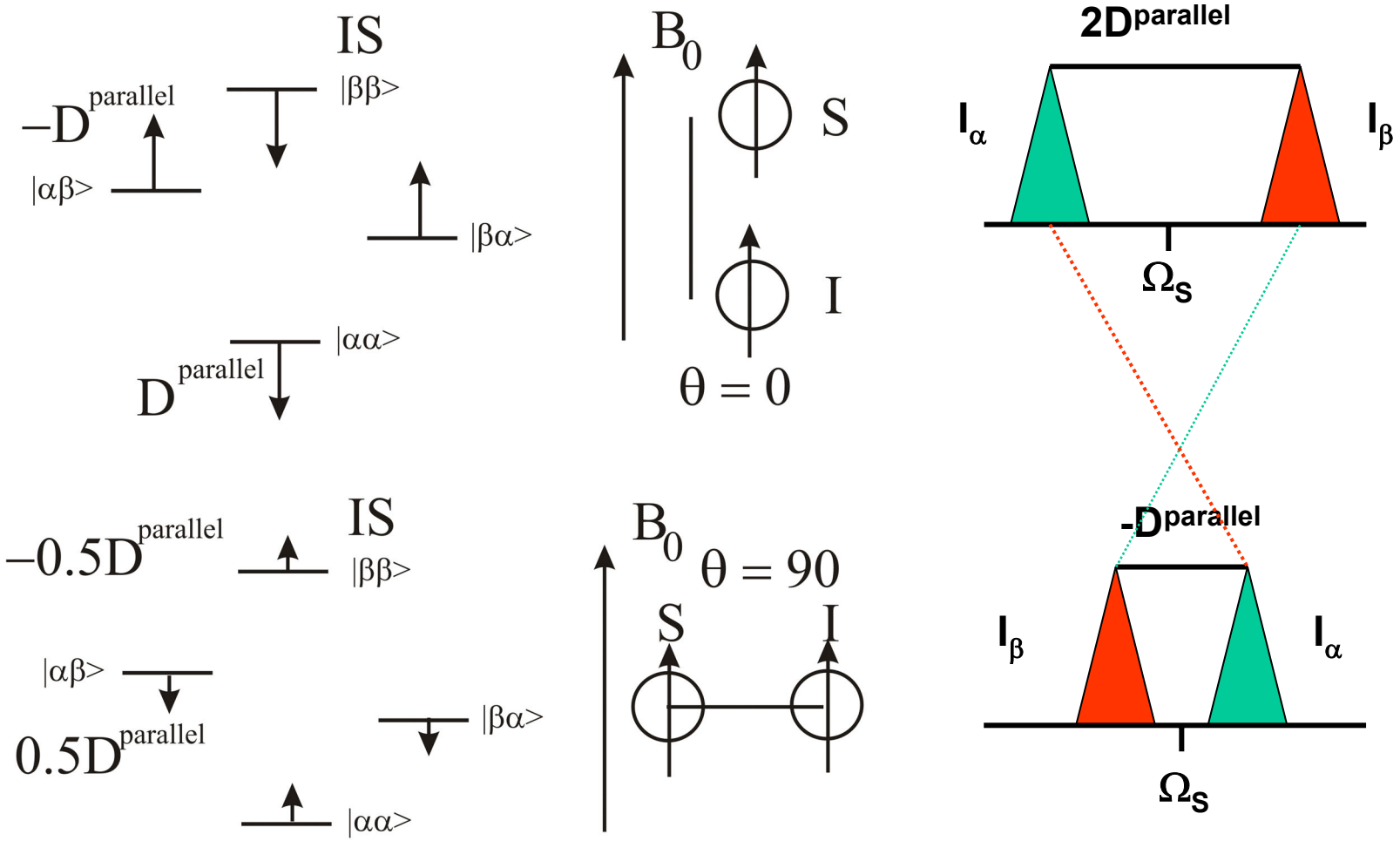


Figure modified from that by C. Griesinger with permission.
http://www.embl-heidelberg.de/nmr/sattler/embo/handouts/griesinger_lecture_ccr.pdf

Dipolar Relaxation: $(3\cos^2\theta-1)/2$



Dipolar Coupling between I and S

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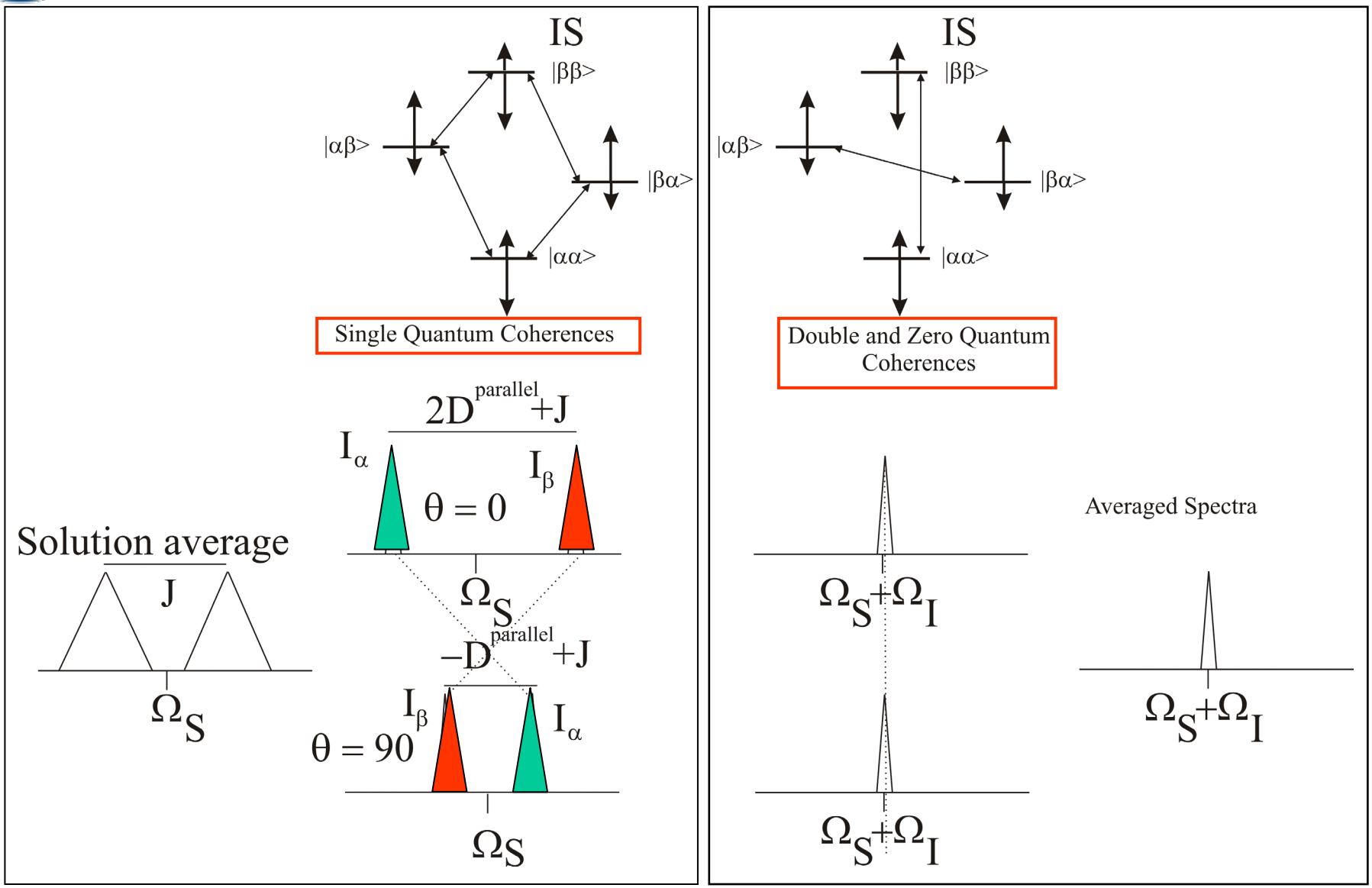
Dipolar Relaxation II



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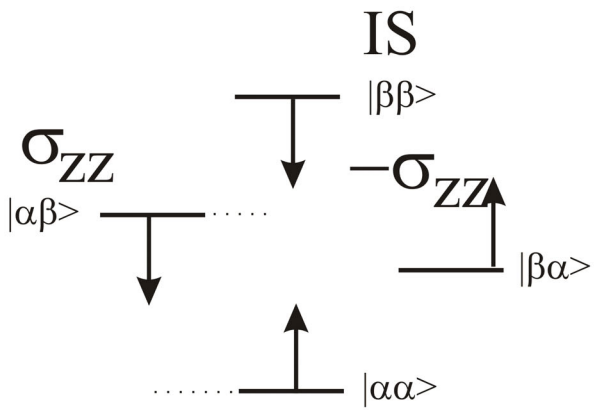
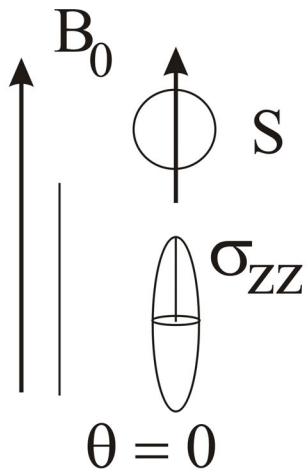
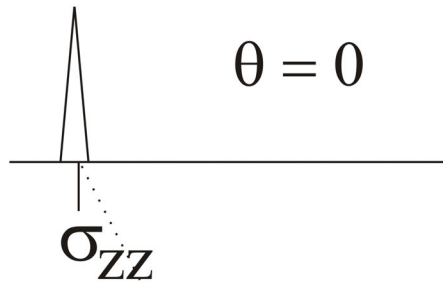
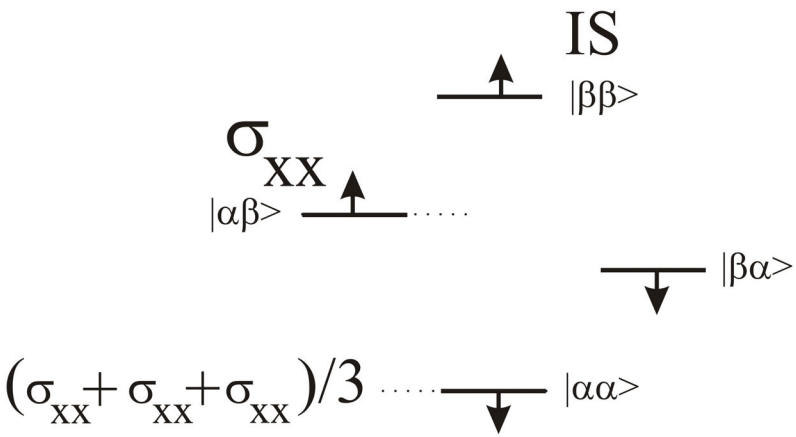
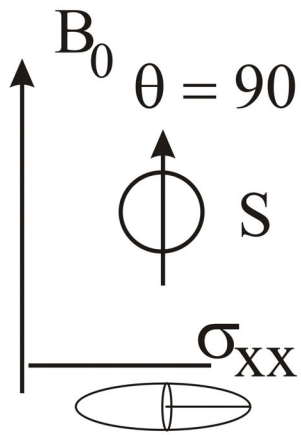
CSA Relaxation of S

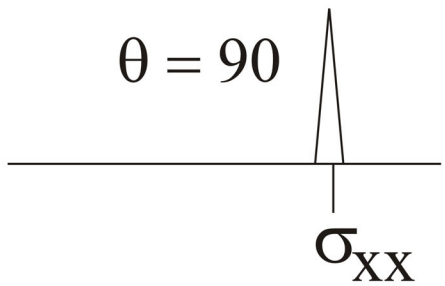


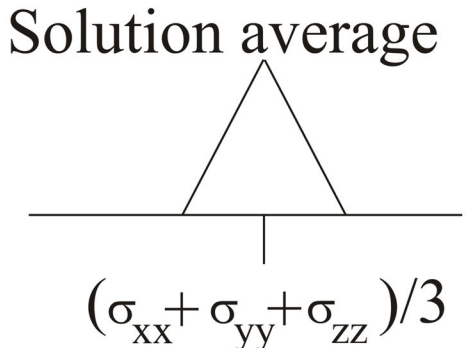












Chemical Shift Anisotropy of S

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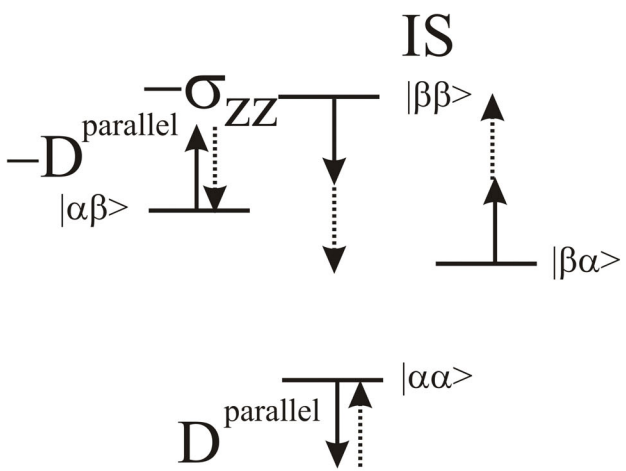
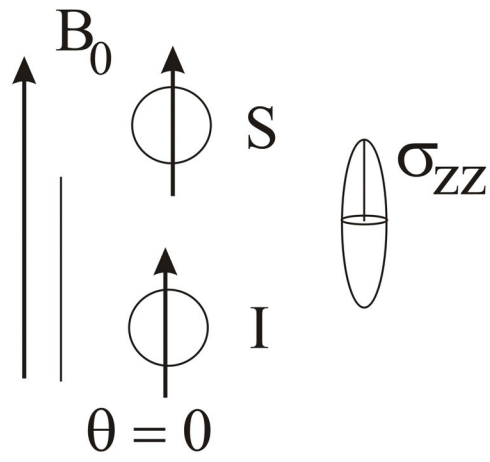
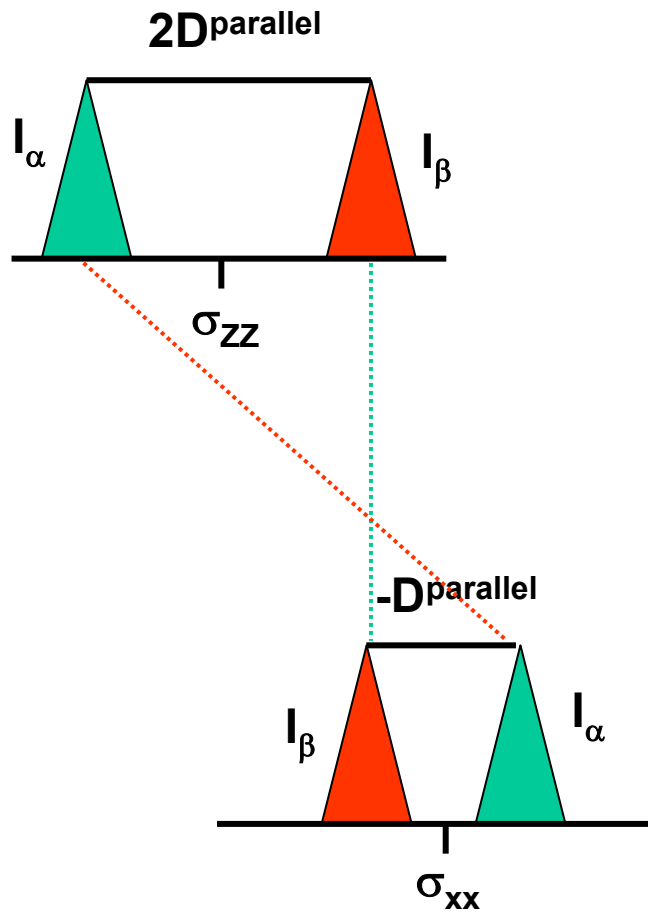
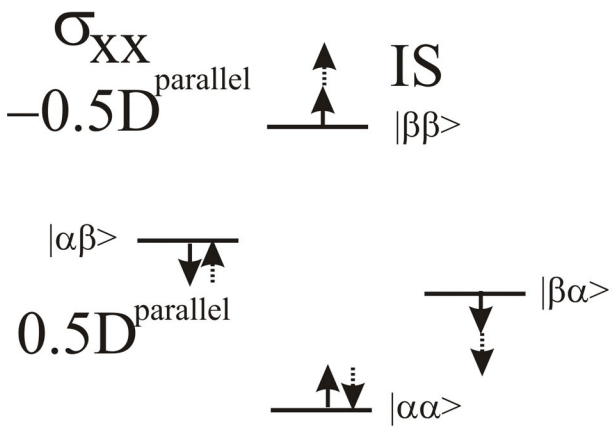
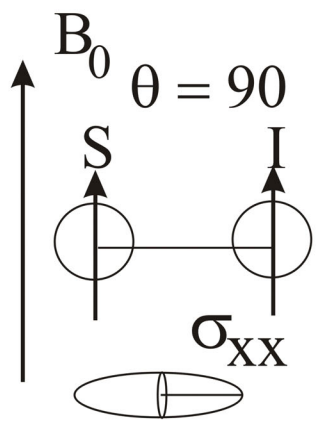
CSA Relaxation of S and Dipolar Coupling between I and S: Interferrence by Cross Correlation





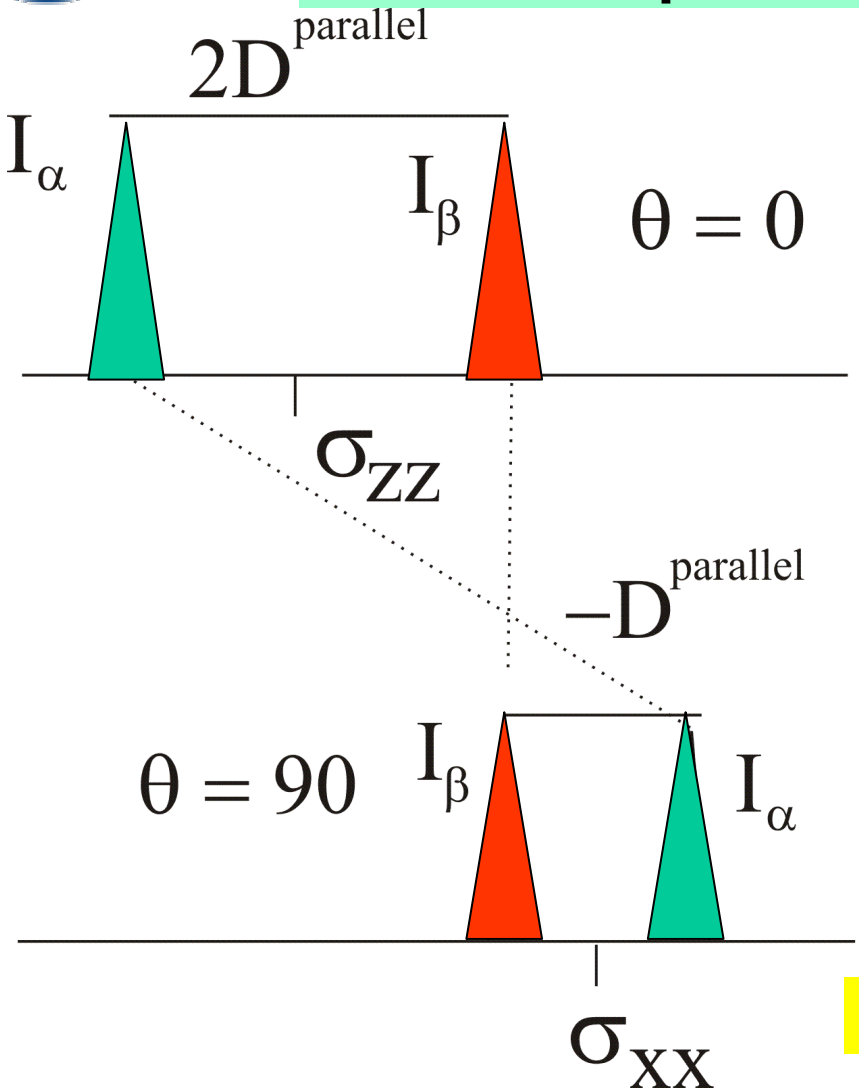




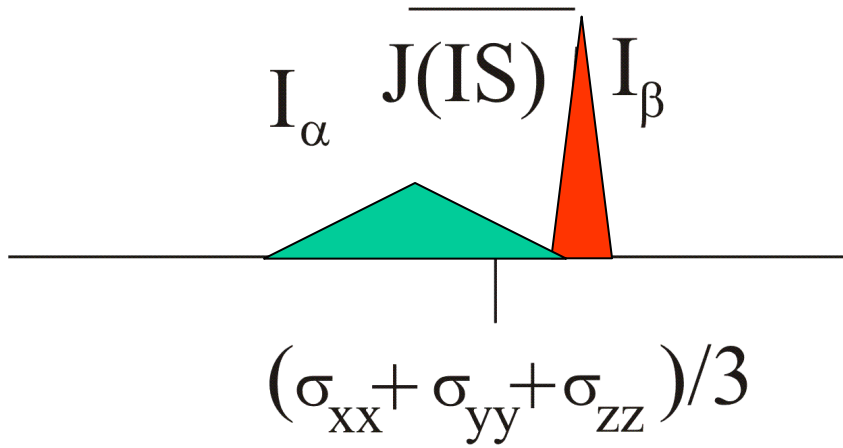


Dipolar Coupling between I and S and CSA of S

Interference of Relaxation Cross Correlation between Dipolar and CSA Relaxation



Solution average



TROSY selects the narrowest component !

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