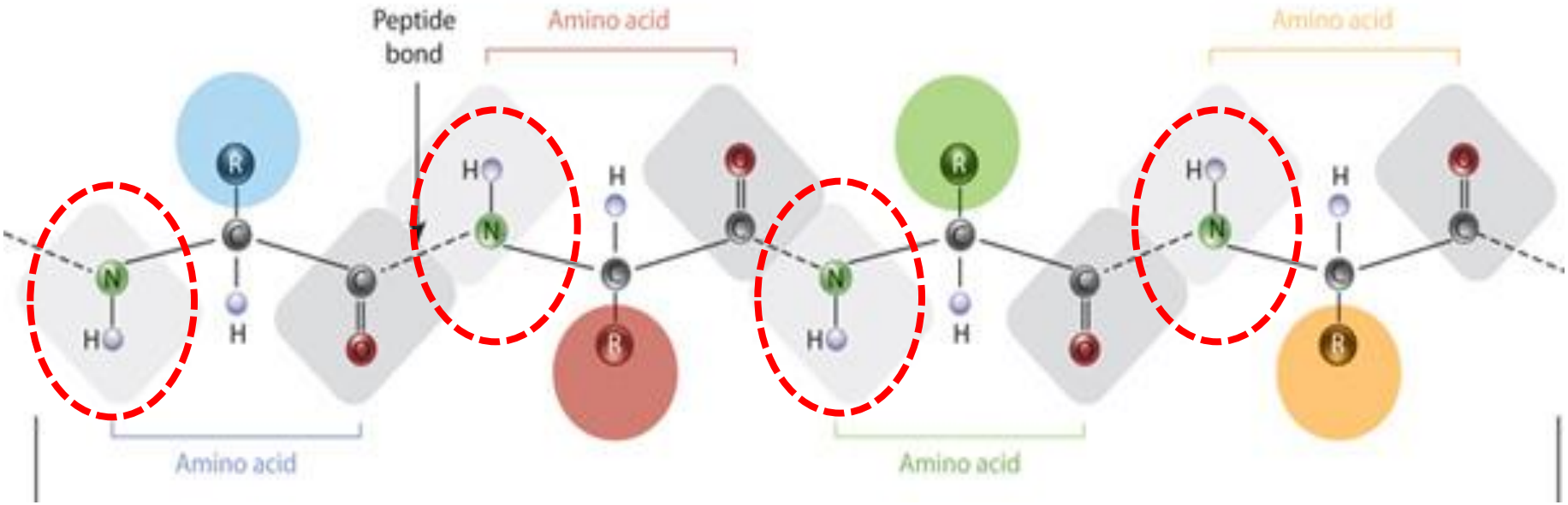
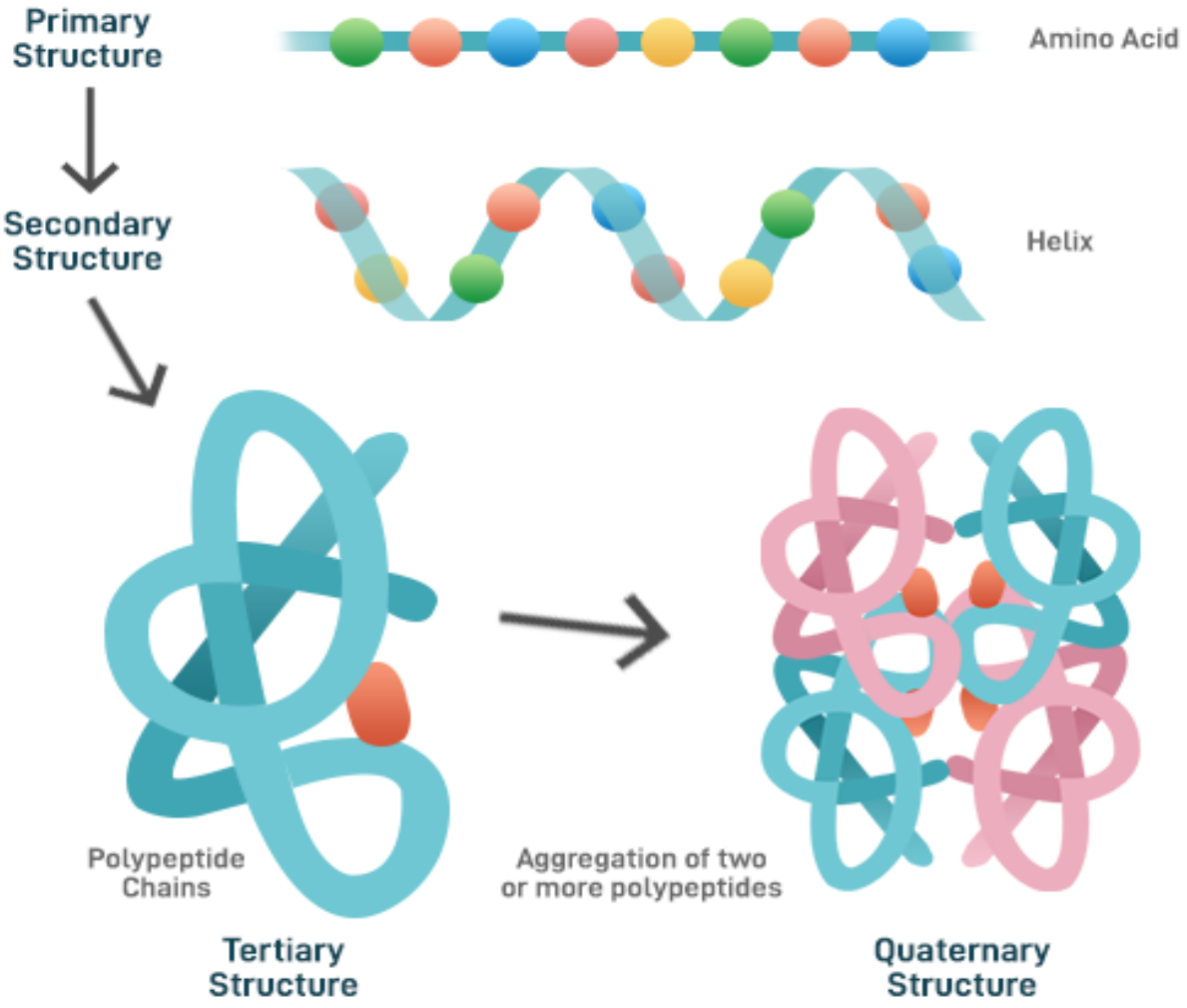
NMR Analysis of Protein Dynamics

NMR Meets Biology Workshop
Gokarna,
27 Jan 2026

Biomolecules – Proteins

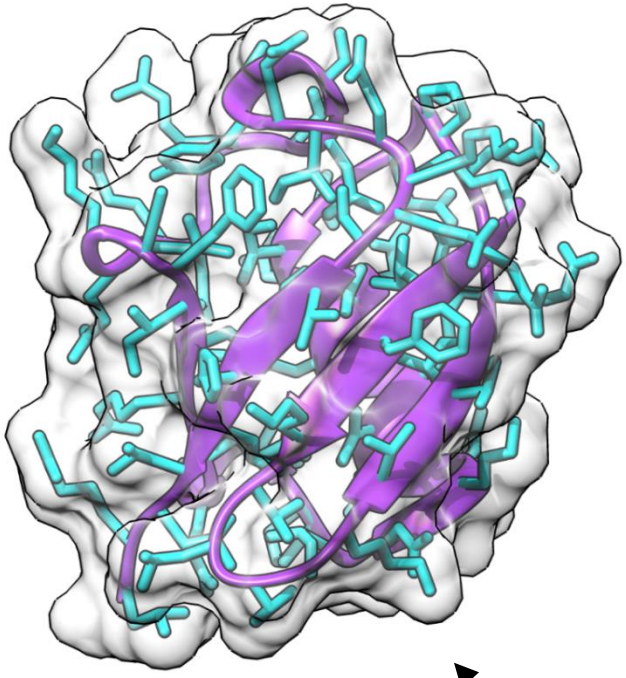


PROTEIN STRUCTURE

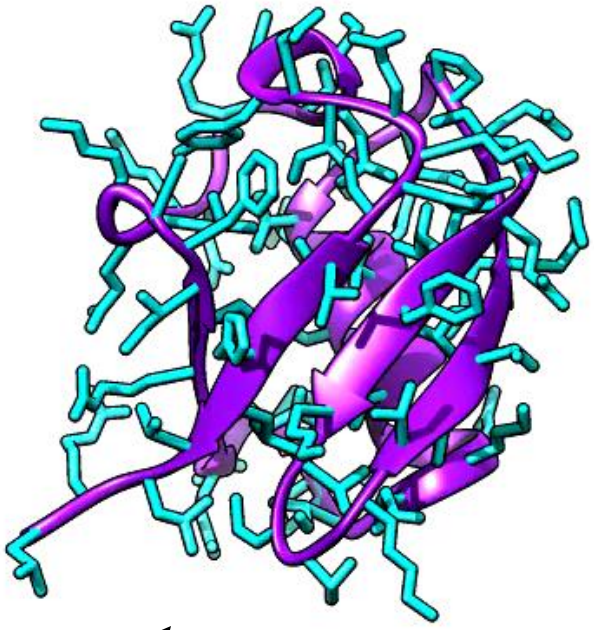


Proteins Structure and Dynamics

structure



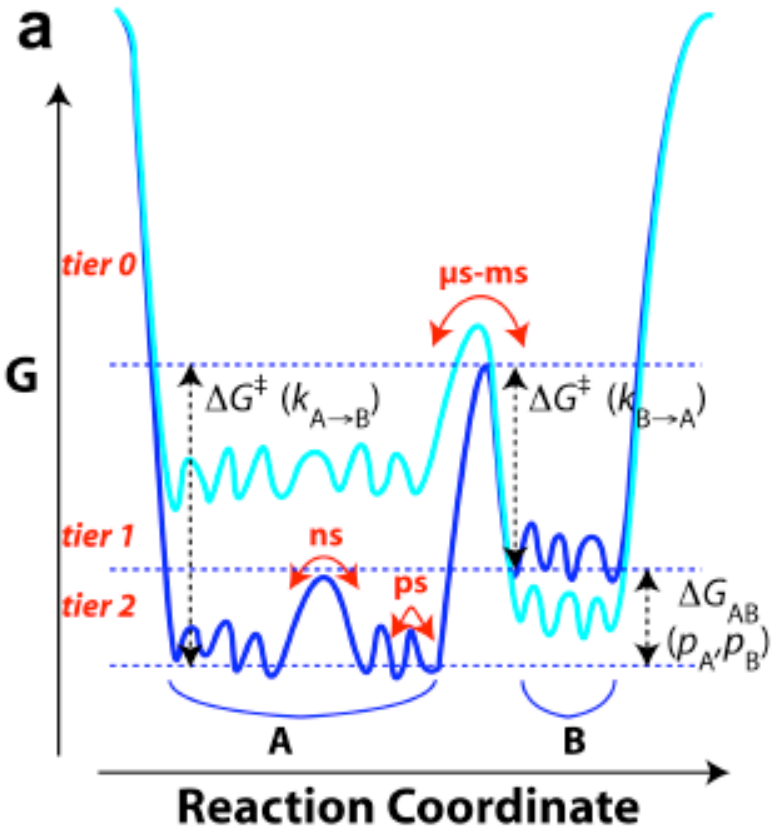
dynamics



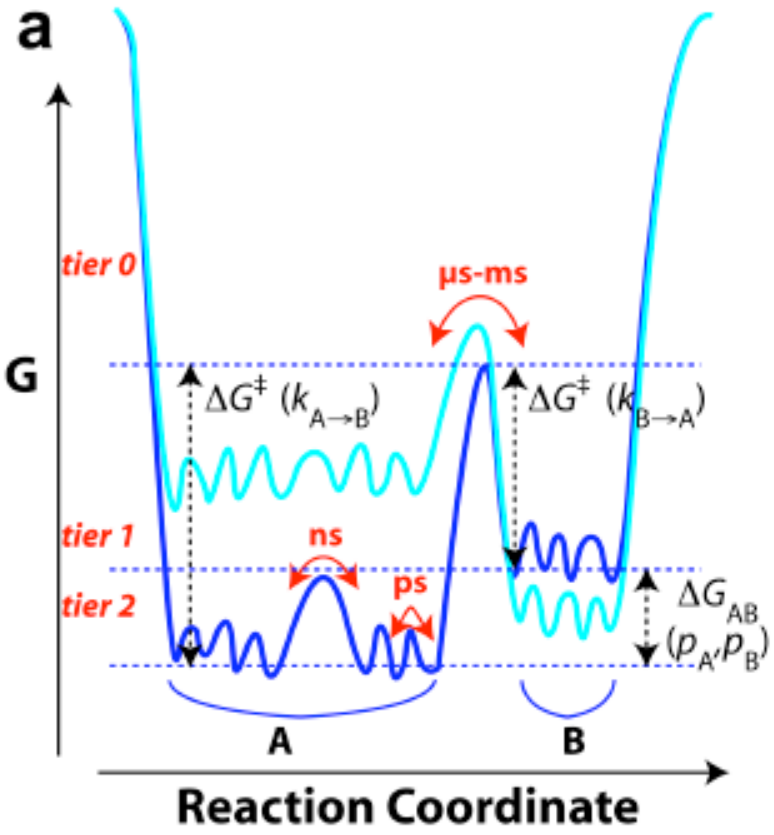
… A L V T S R K F M …

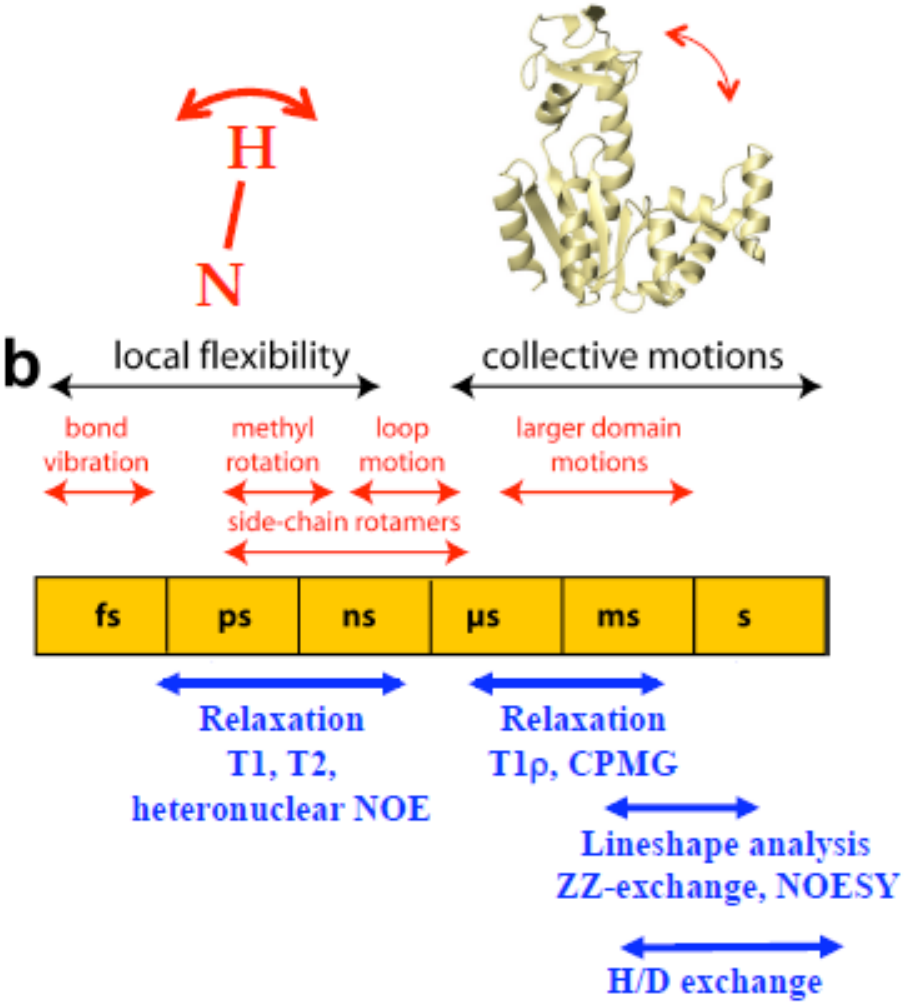
sequence

Protein Energy Landscape



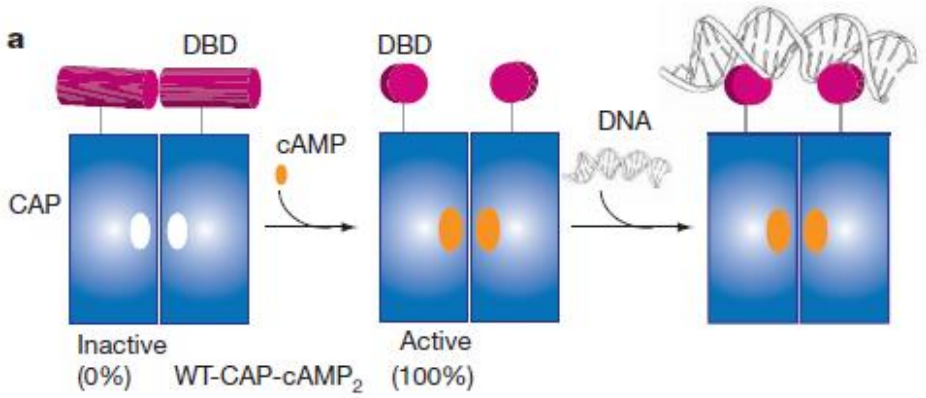
Protein Energy Landscape

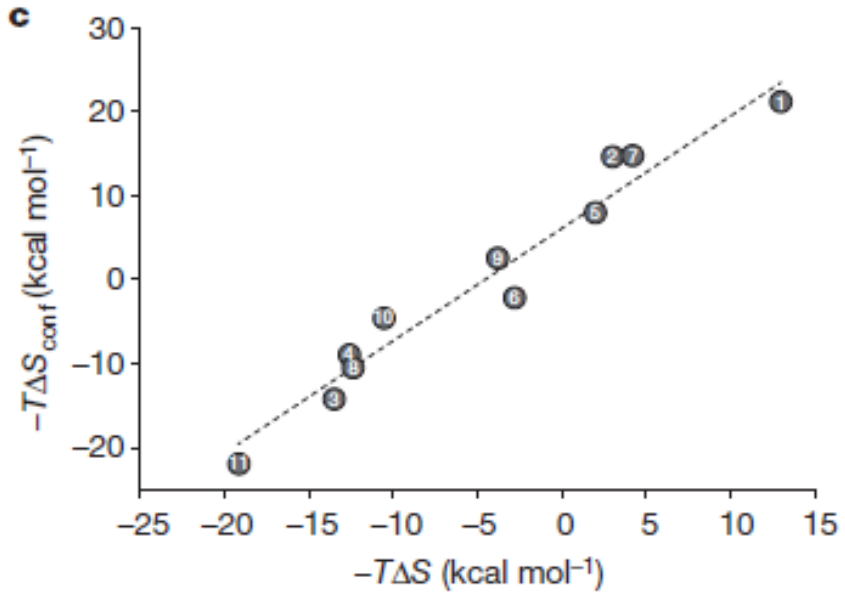




Why to measure fast protein dynamics?

Conformational Entropy regulates Protein Activity

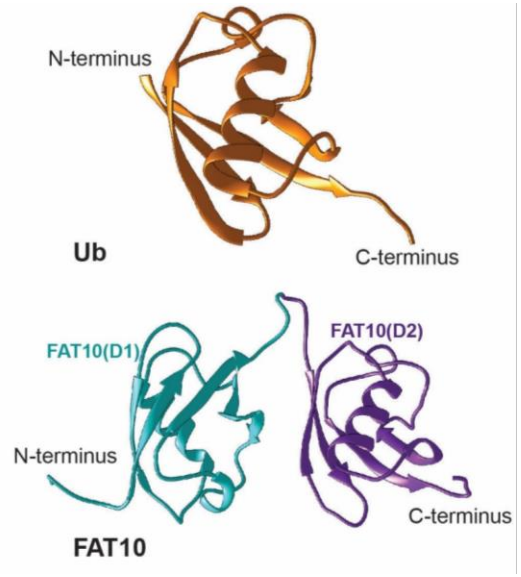


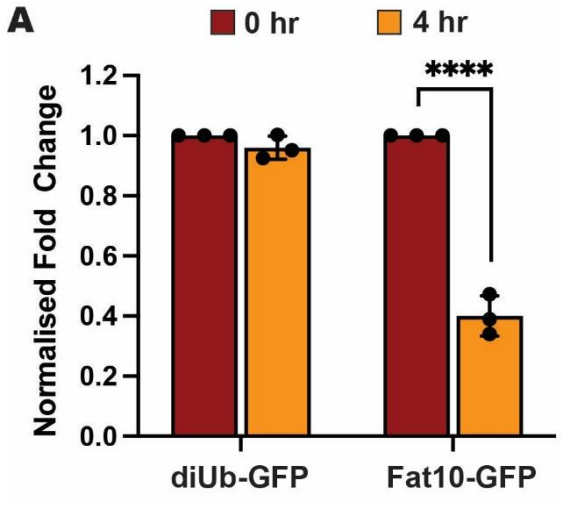


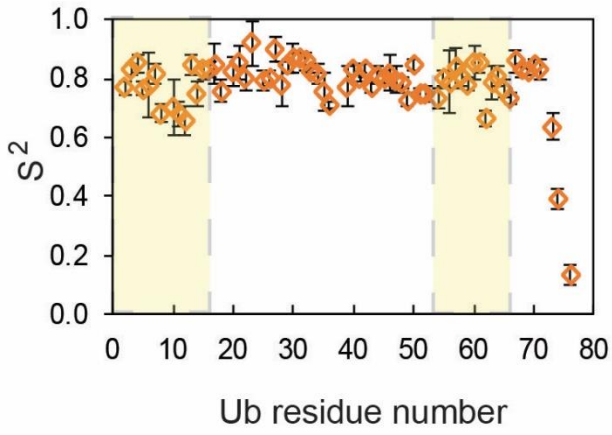
Conformational Entropy defines binding entropy

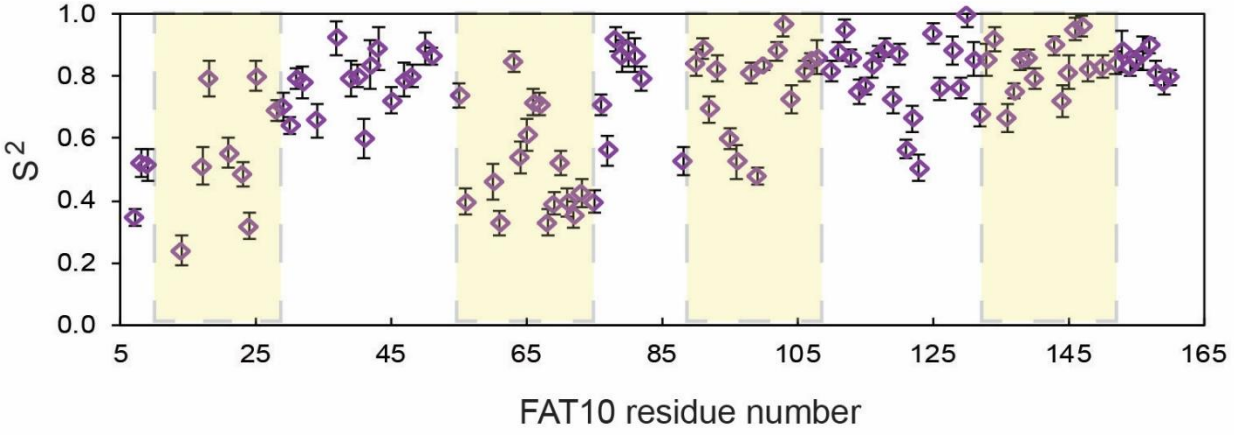
Kalodimos Nature (2012)

Conformational Entropy regulates Protein Degradation





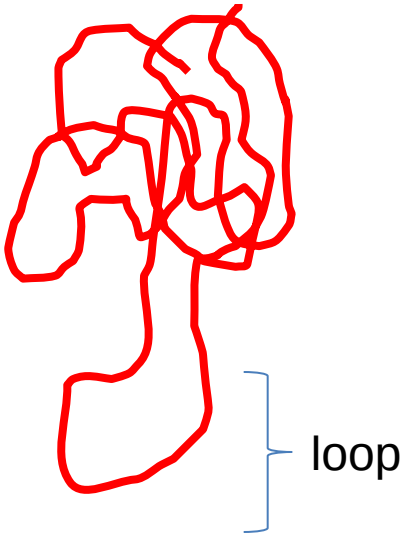




Negi et al Elife (2024)

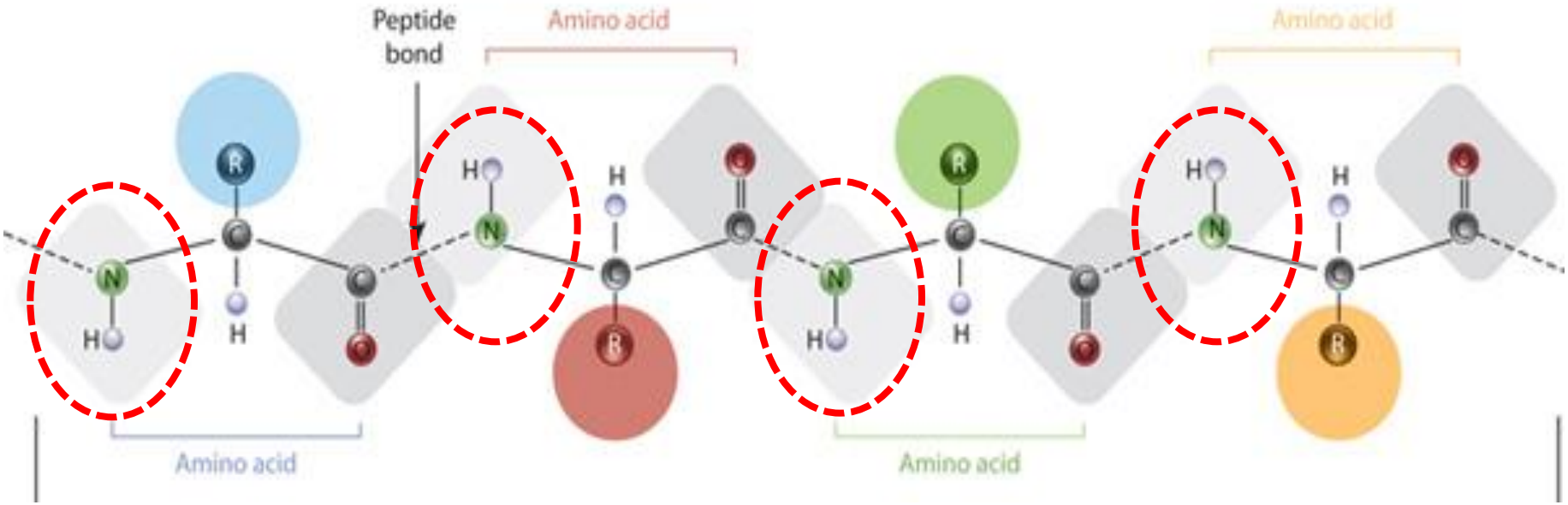
Why NMR to measure fast protein dynamics?

Measure Loop dynamics

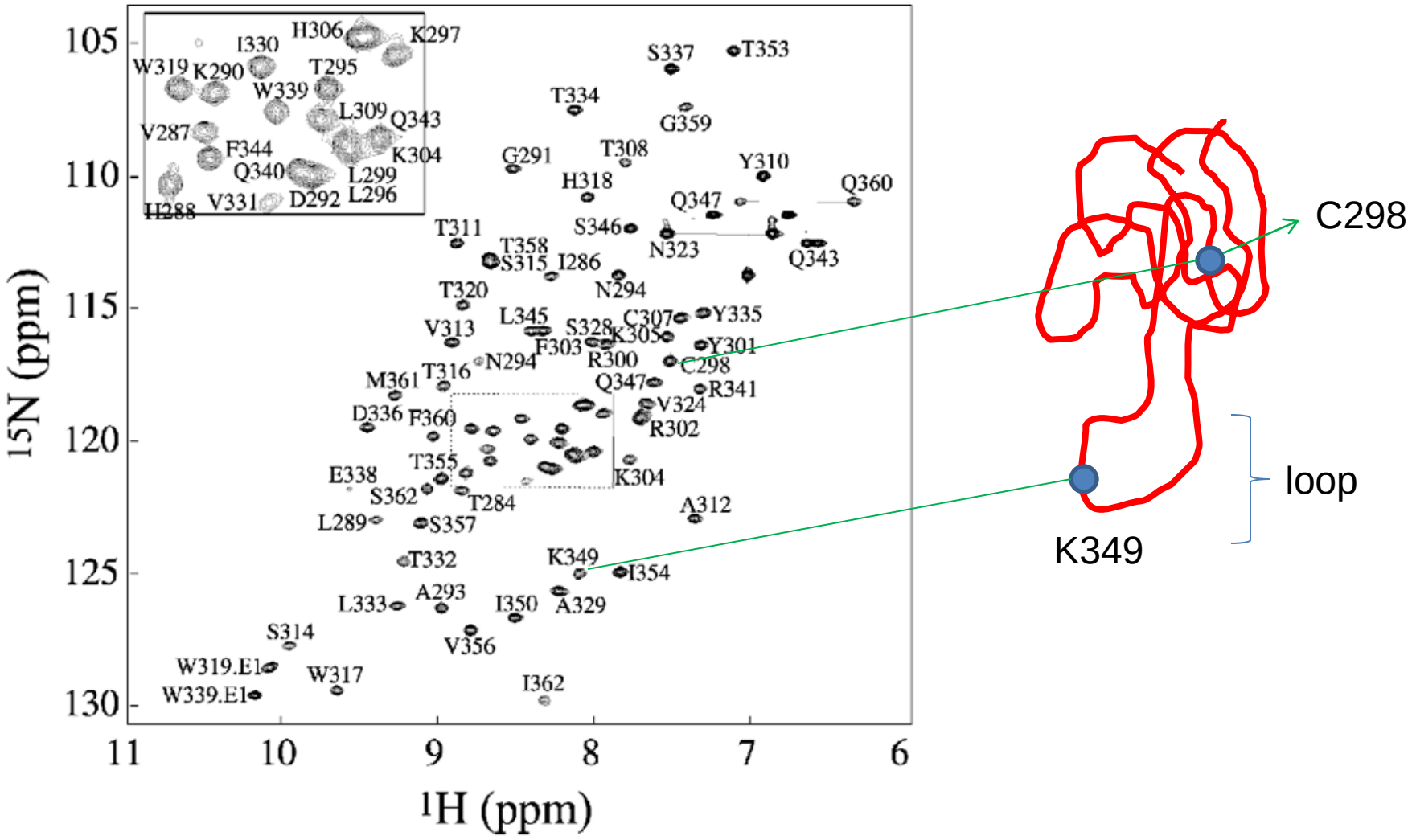


Other Techniques

Biomolecules – Proteins



Measure changes in dynamics (with solutes, co-factors, etc)

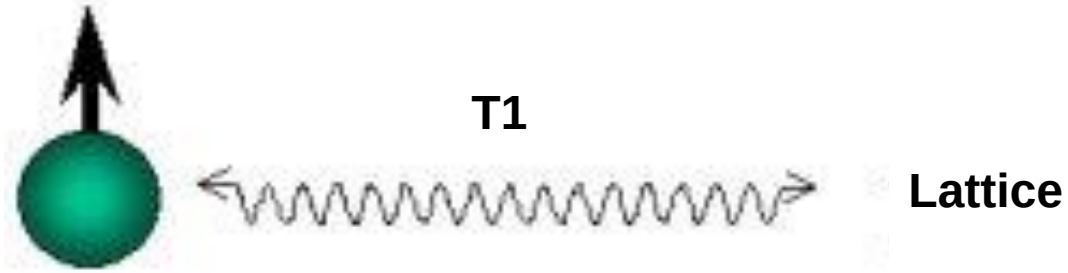


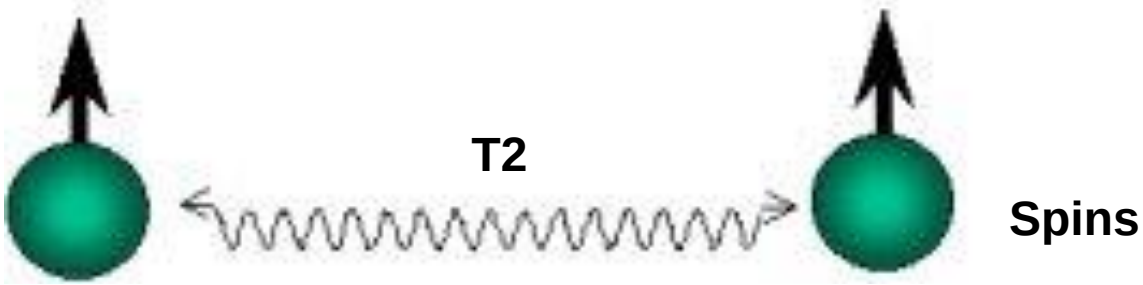
How to measure fast protein dynamics?

Calculate Order Parameter (S^2)
Conformational Entropy (S)

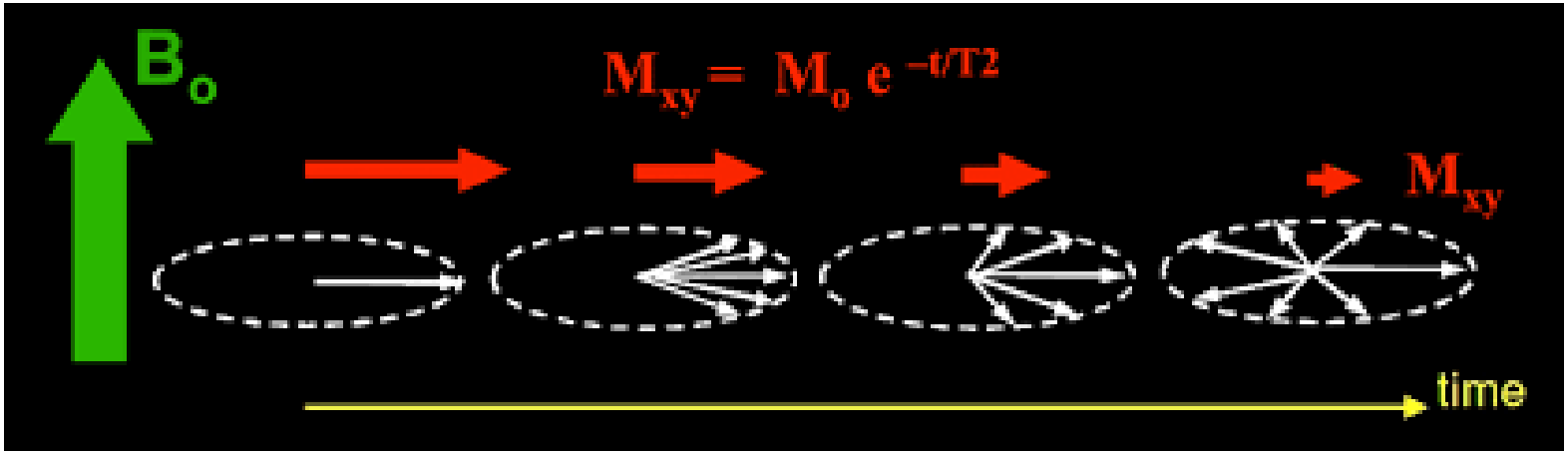
T1, T2 & het-NOE

Spin-Lattice (T1) and Spin-spin (T2) relaxation





T2 (spin-spin) relaxation



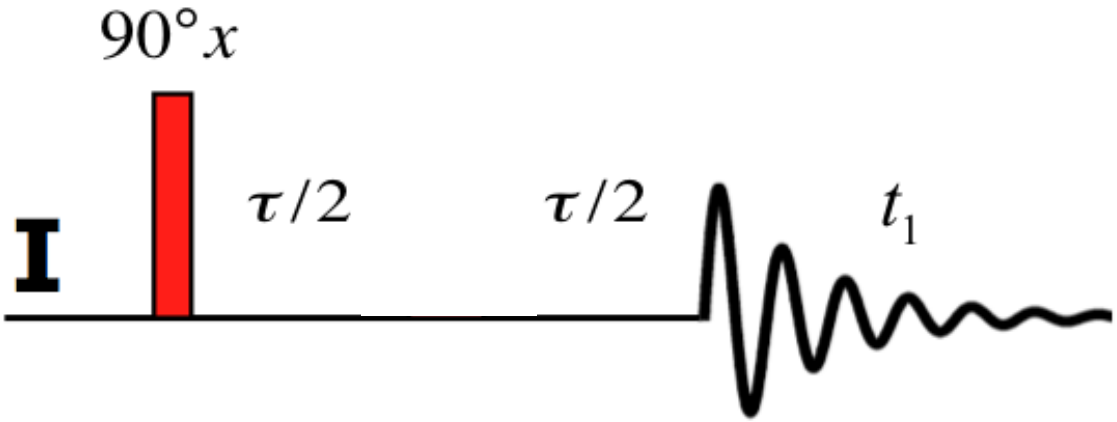
$$\frac{dM_{x(y)}}{dt} = -\frac{M_{x(y)}}{T_2}$$

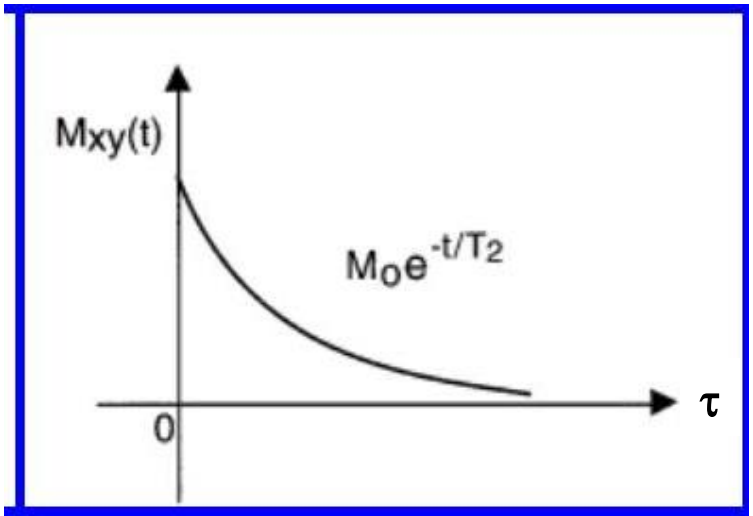
$$M_{xy} = M_o\, e(-t/T2)$$

Dephasing happens due to

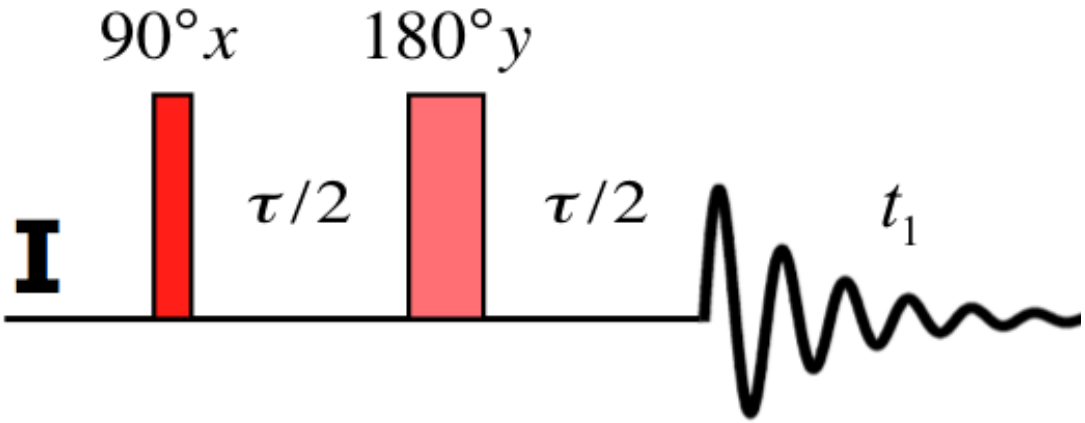
1) T2
2) Field inhomogeneity
3) Diffusion effect

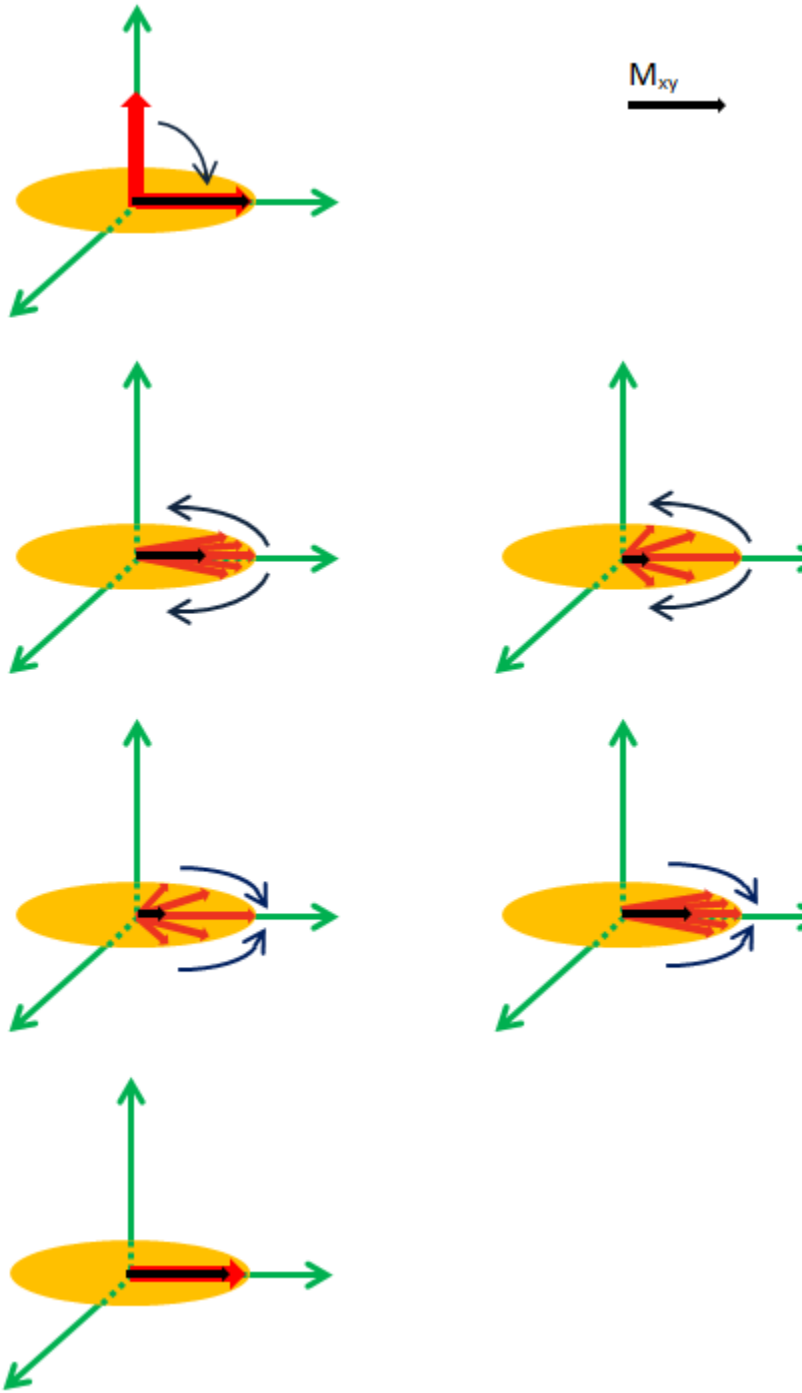
T2 (spin-spin) relaxation



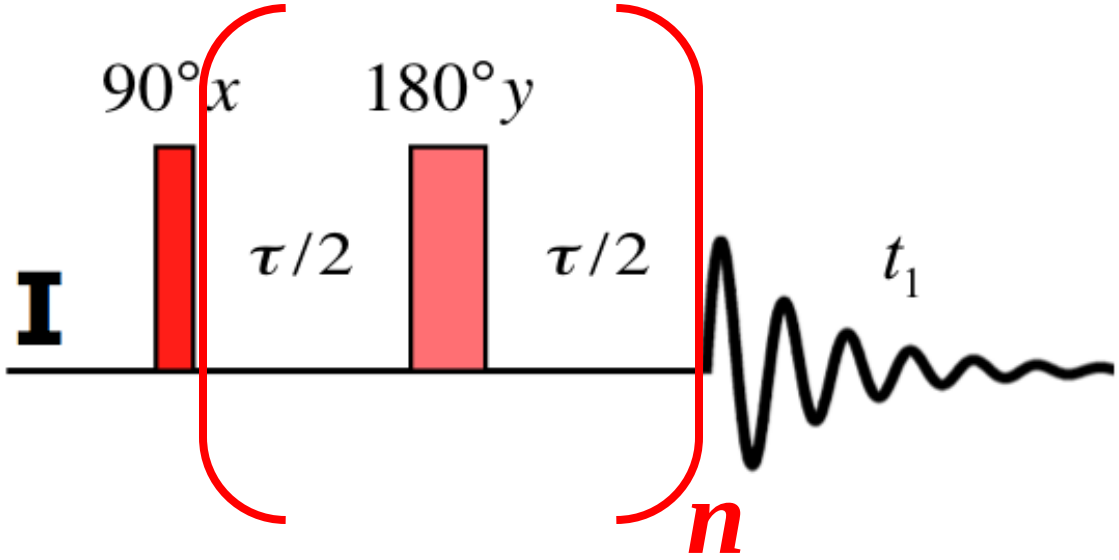


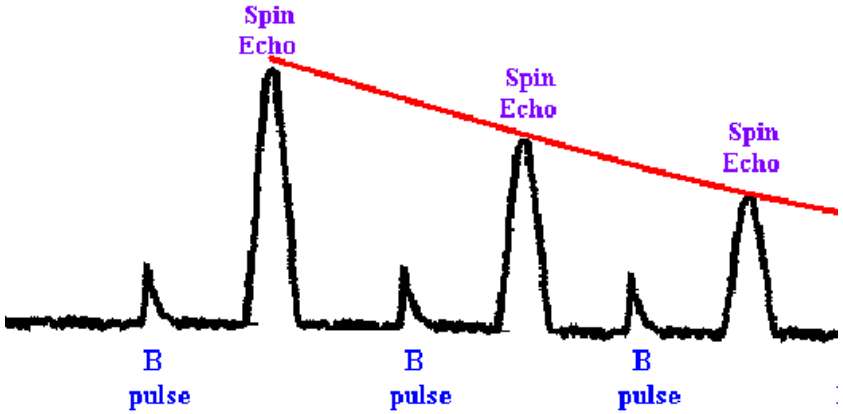
Spin-Echo



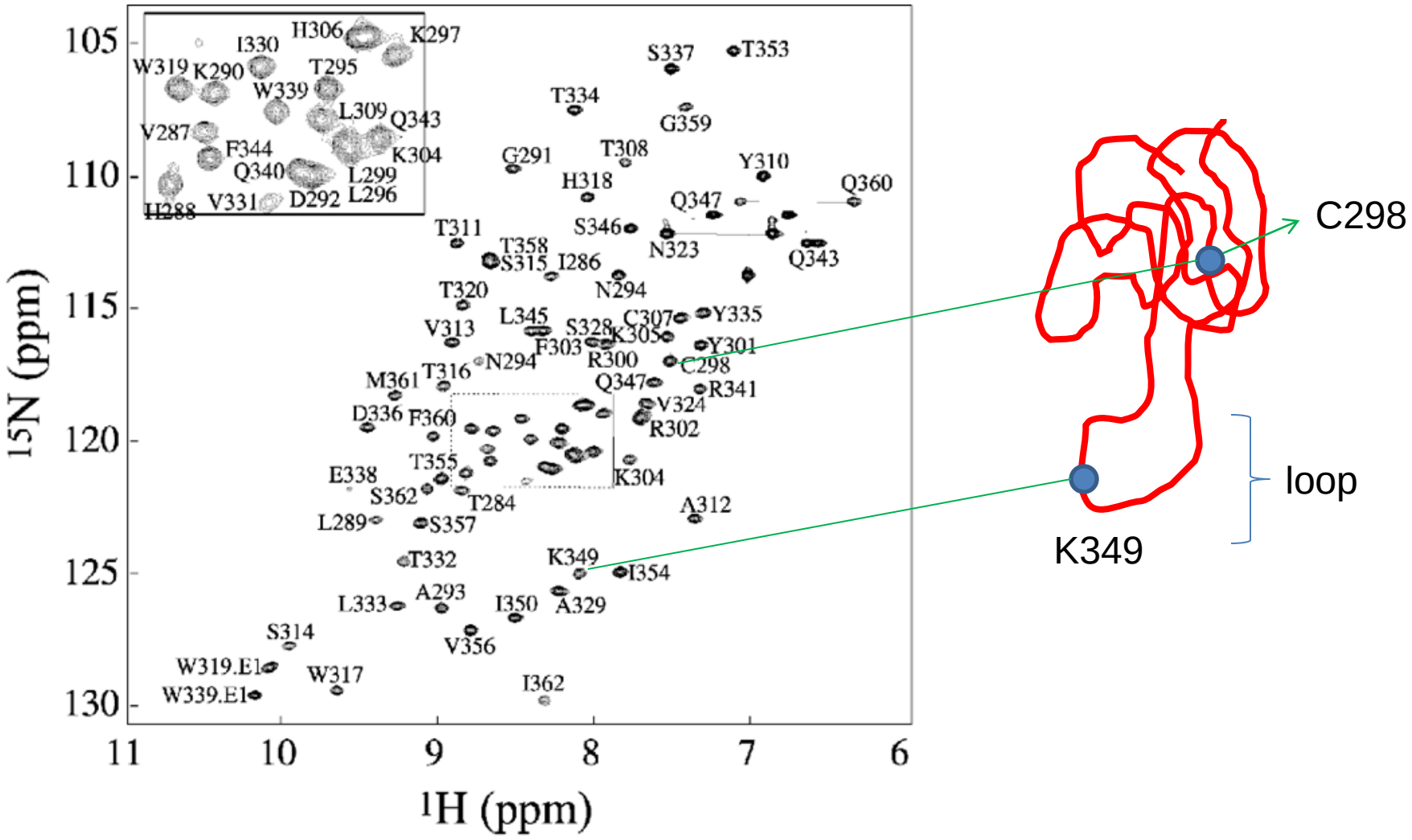


Spin-Echo

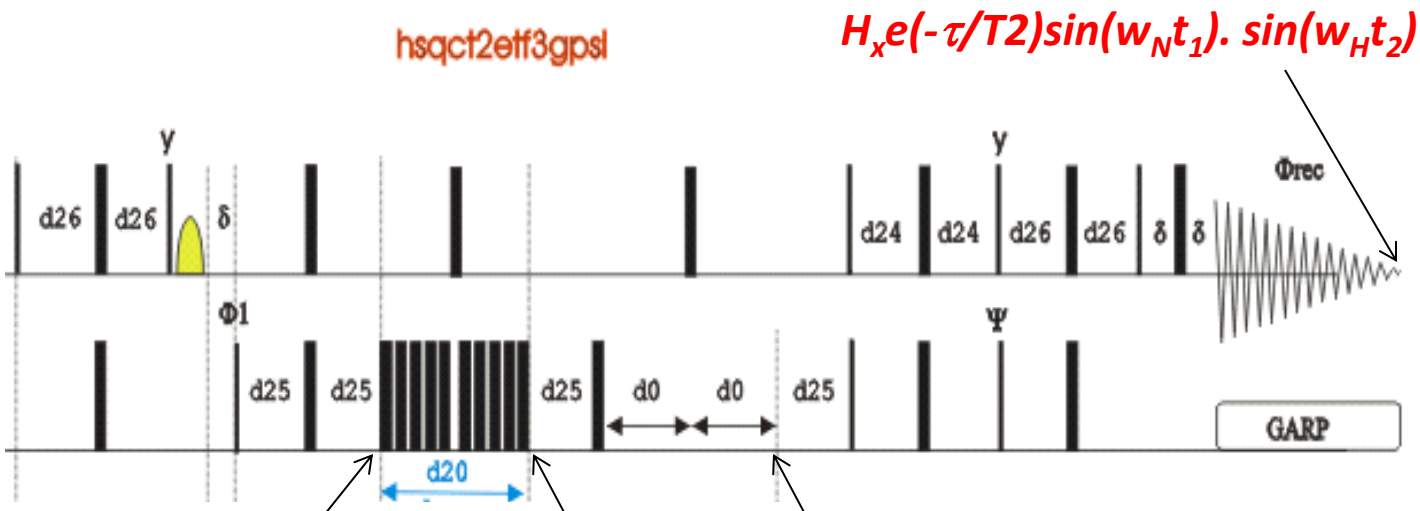

90°x
180°y
τ/2
τ/2
I
t1
n


Spin Echo
Spin Echo
Spin Echo
B pulse
B pulse
B pulse

Measure 15N-T2

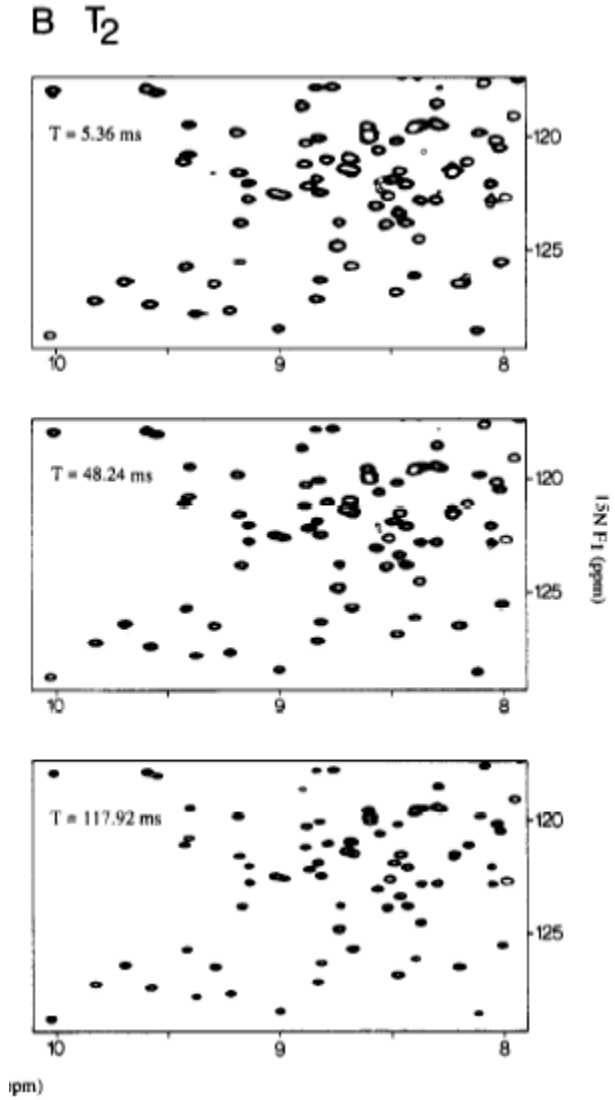


2D T2 spin-echo experiment



NMR Analysis of Protein Dynamics

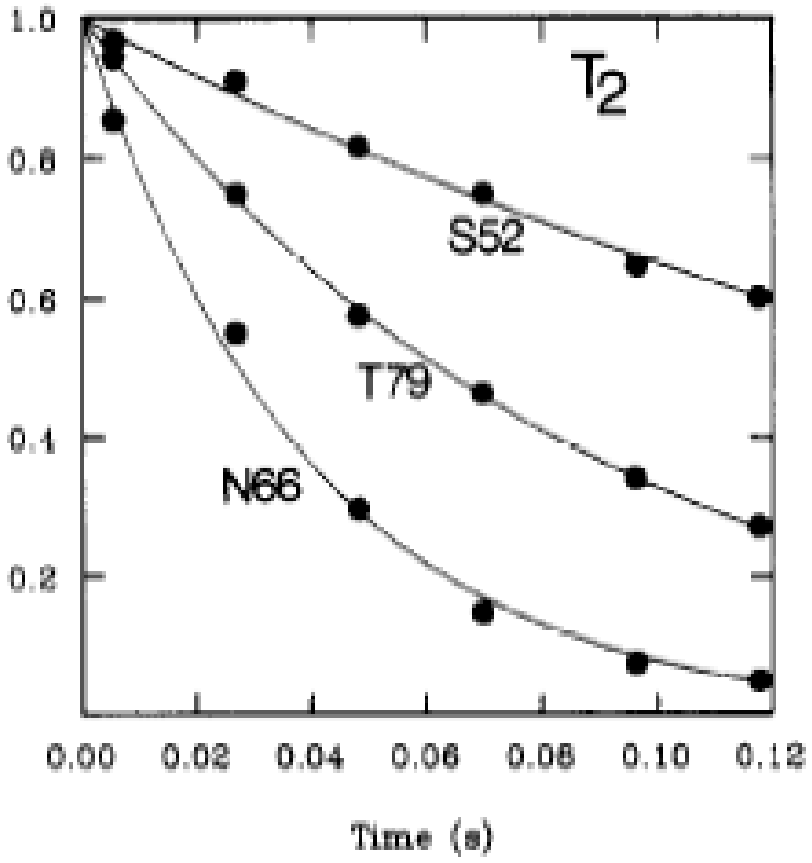
Typical T_2 data for a Protein



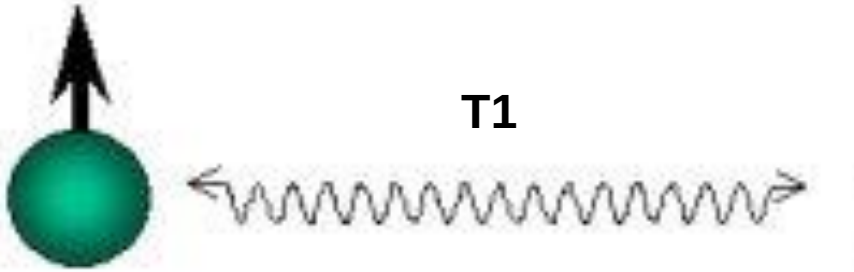
Biochemistry **1990,** ***29,*** 7387-7401

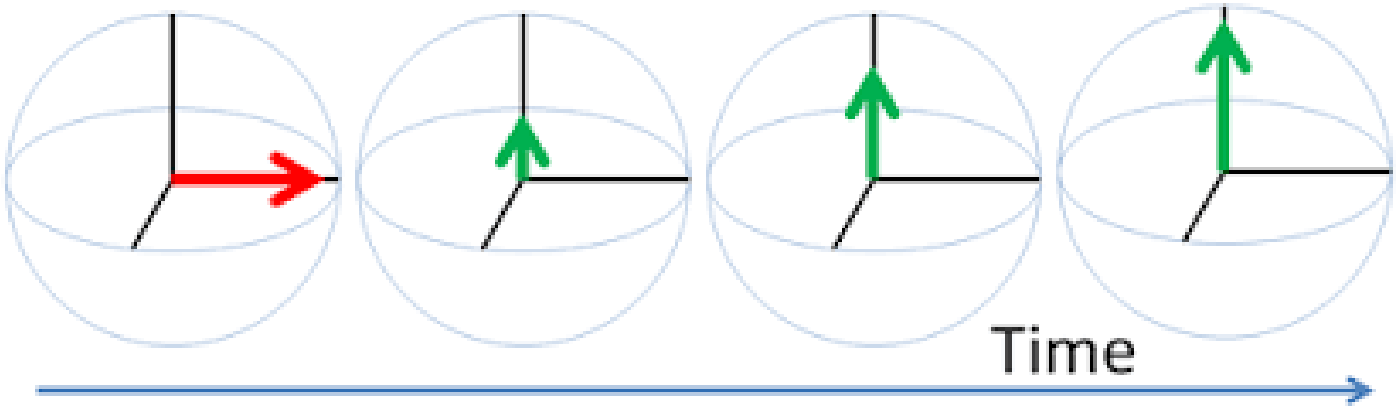
NMR Analysis of Protein Dynamics

Typical Quality of Fits for T_1 and T_2 2D ^{1}H-^{15}N HSQC Data



Spin-Lattice relaxation (T1)

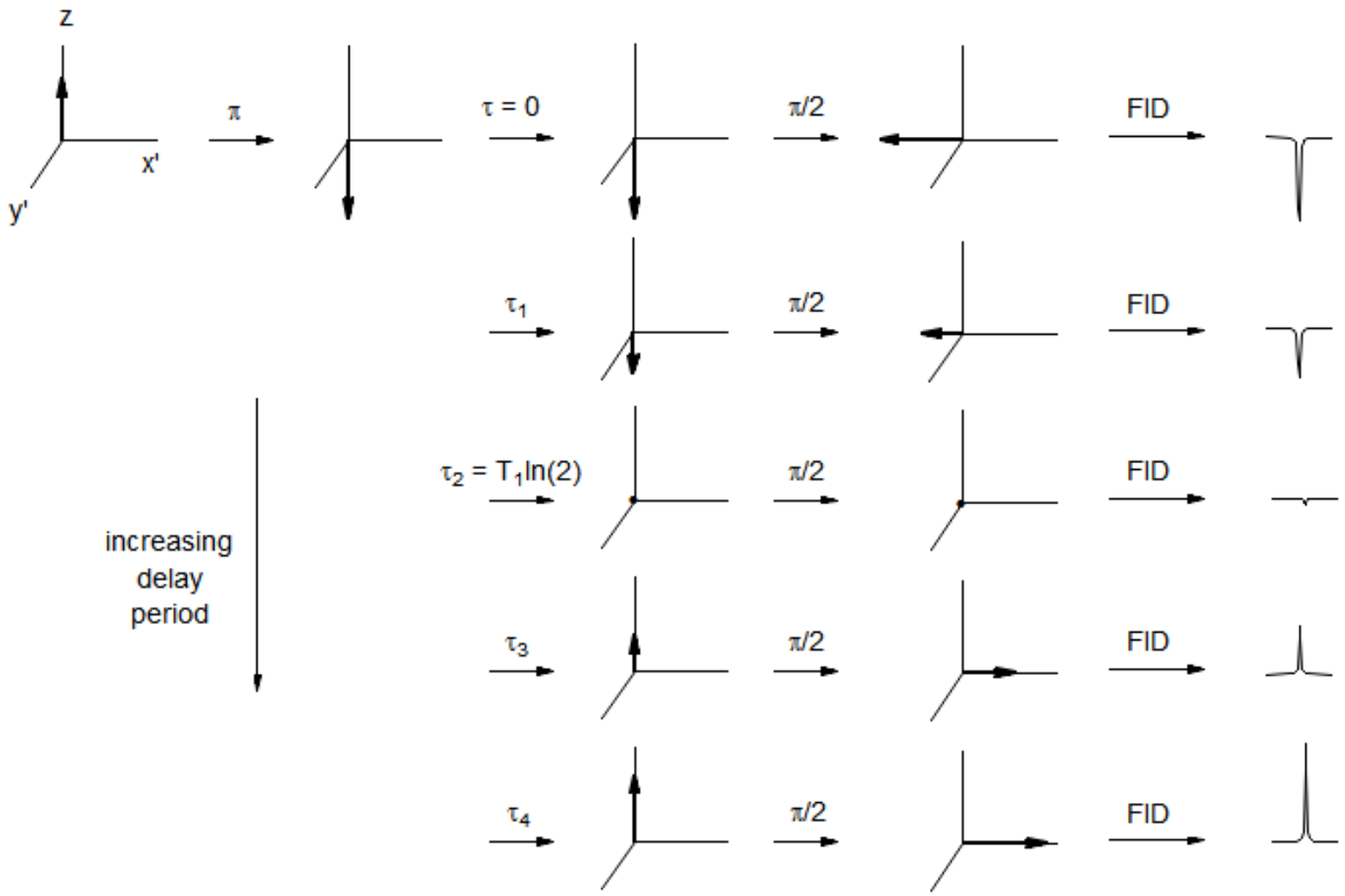




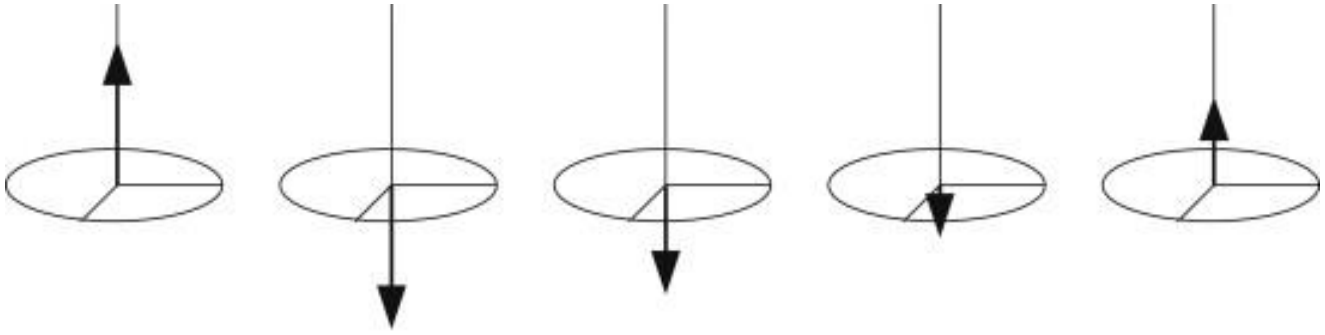
$$\frac{dM_z}{dt} = \frac{M_0 - M_z}{T_1}$$

$$M = Mo(1\text{-}e^{(-t/T1)})$$

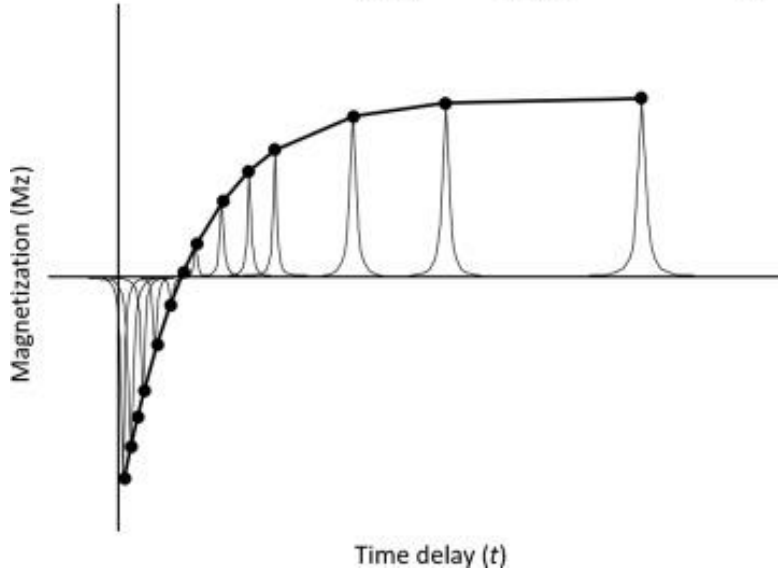
Spin-Lattice relaxation (T1)



Spin-Inversion Recovery

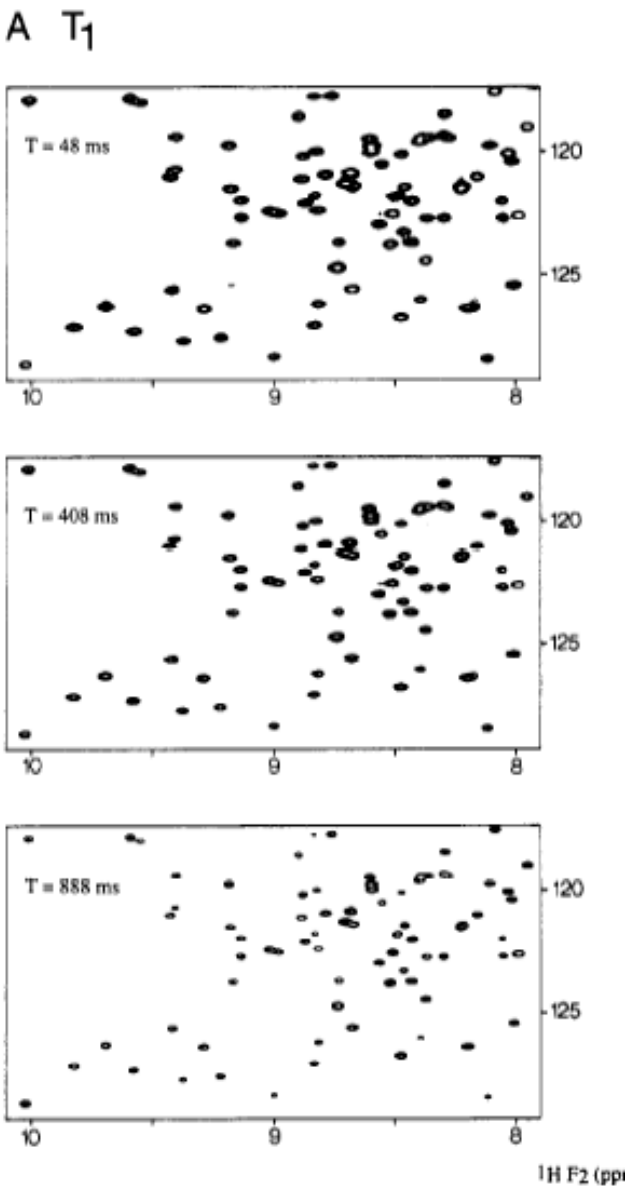


$$M_z(t) = M_{z,eq}(1 - 2e^{-t/T_1})$$



NMR Analysis of Protein Dynamics

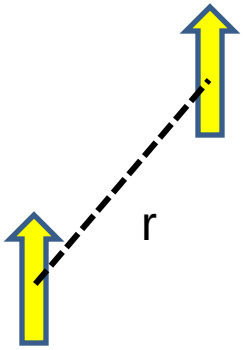
Typical T_1 data for a Protein

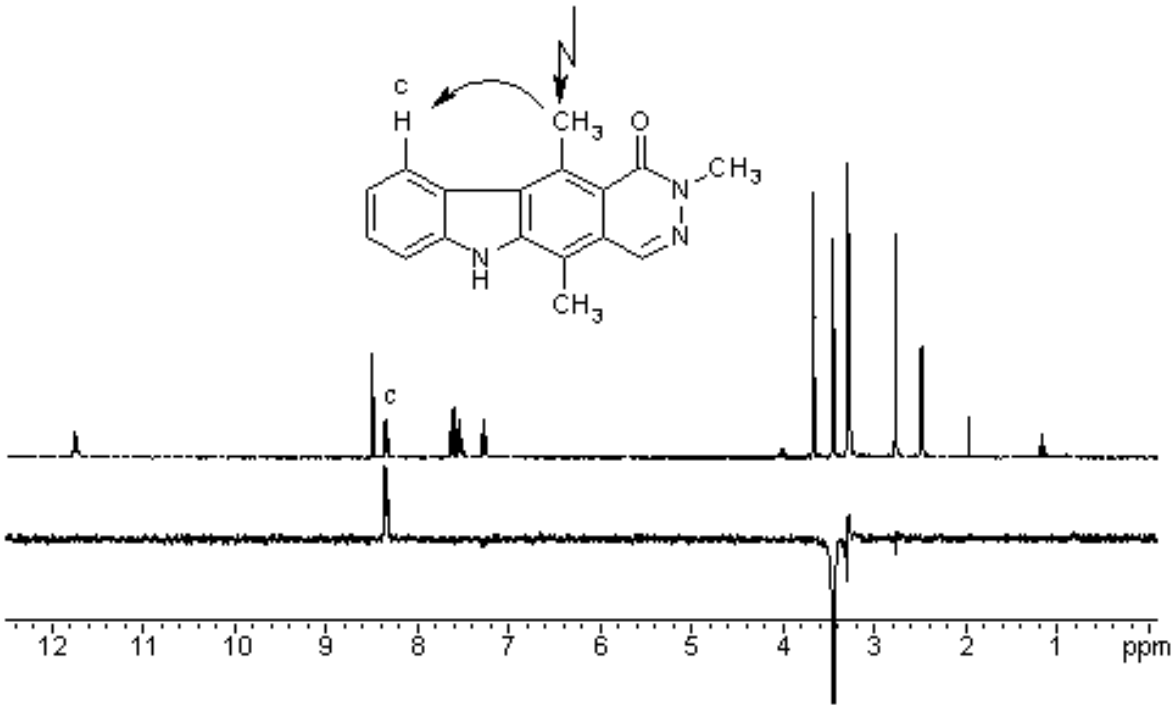


Biochemistry* 1990, *29, 7387-7401

Heteronuclear NOE

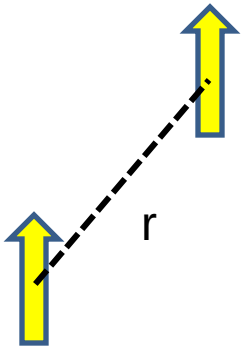
Nuclear Overhauser Effect – NOE : Effect of dipolar coupling through space
(distance dependent effect)

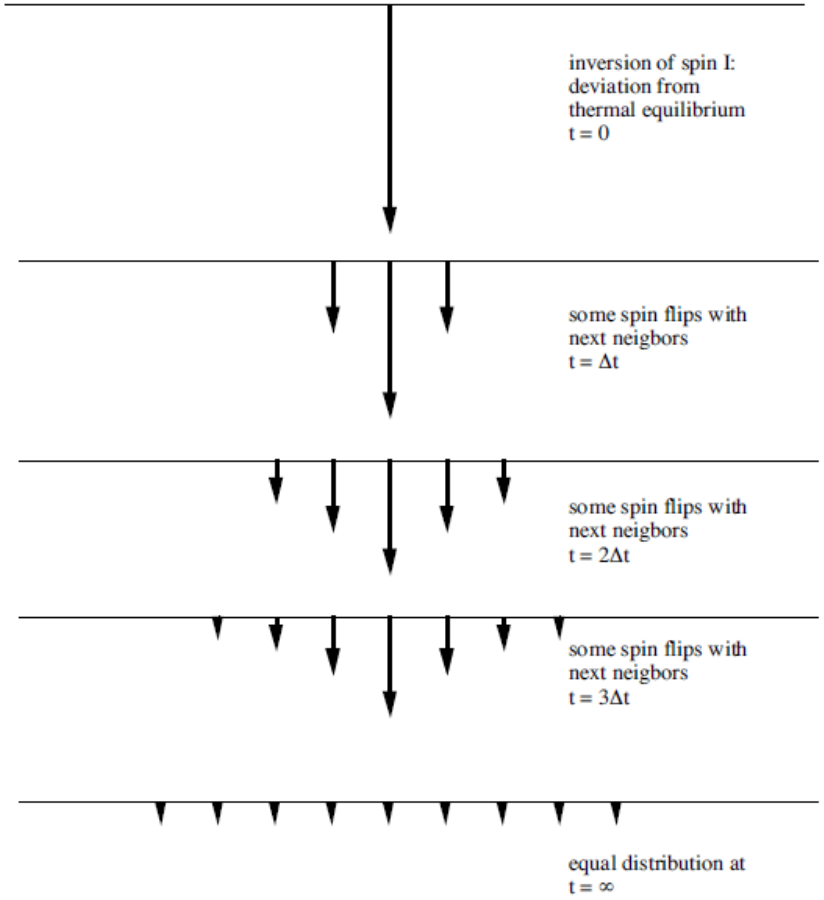




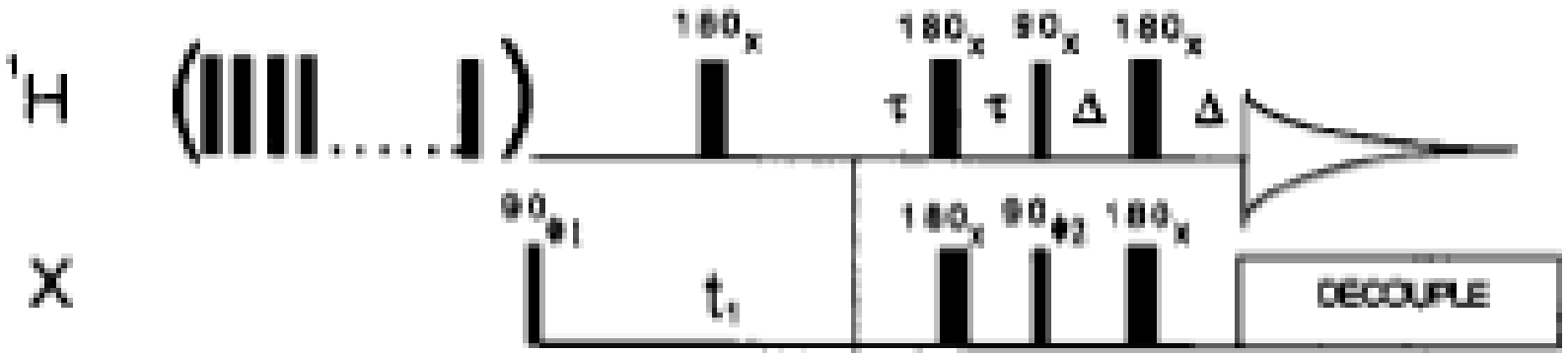
Heteronuclear NOE

Nuclear Overhauser Effect – NOE : Effect of dipolar coupling through space (distance dependance)





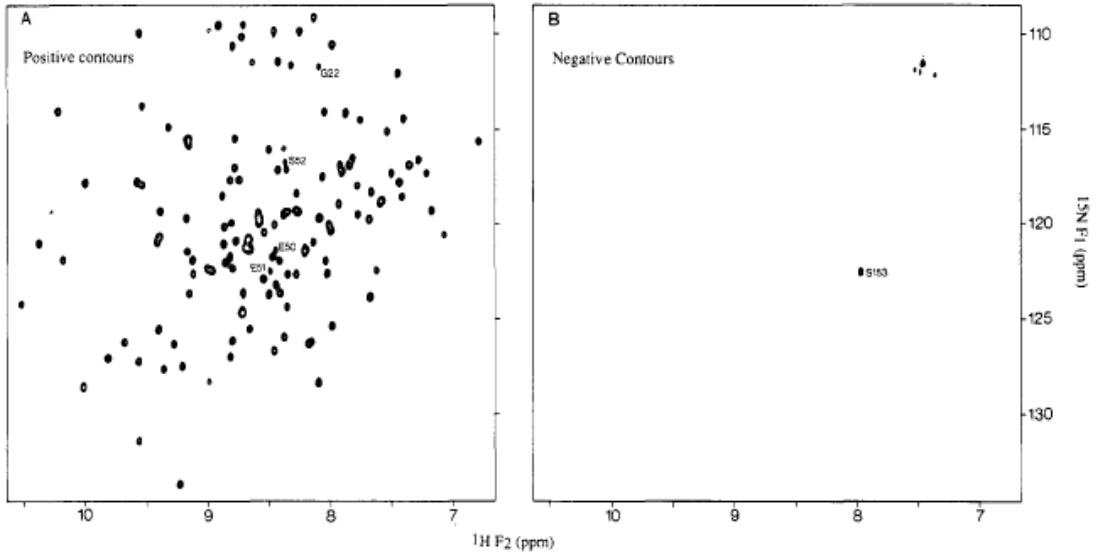
Heteronuclear NOE



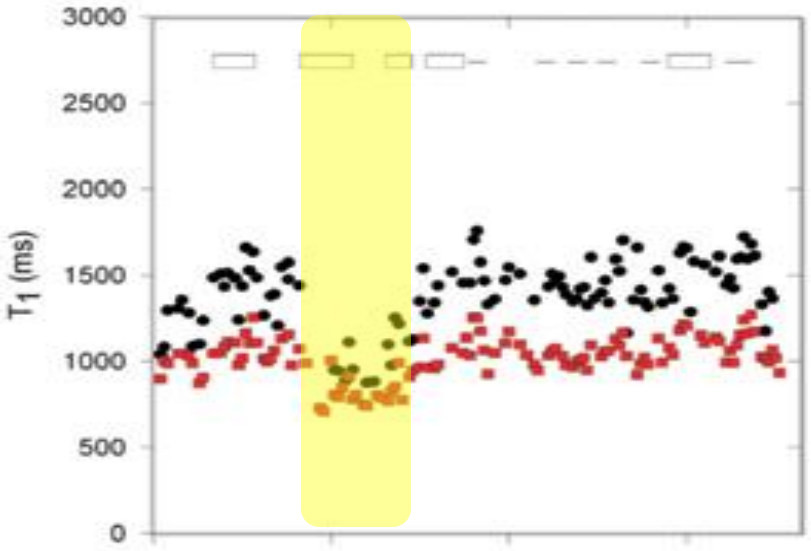
NMR Analysis of Protein Dynamics

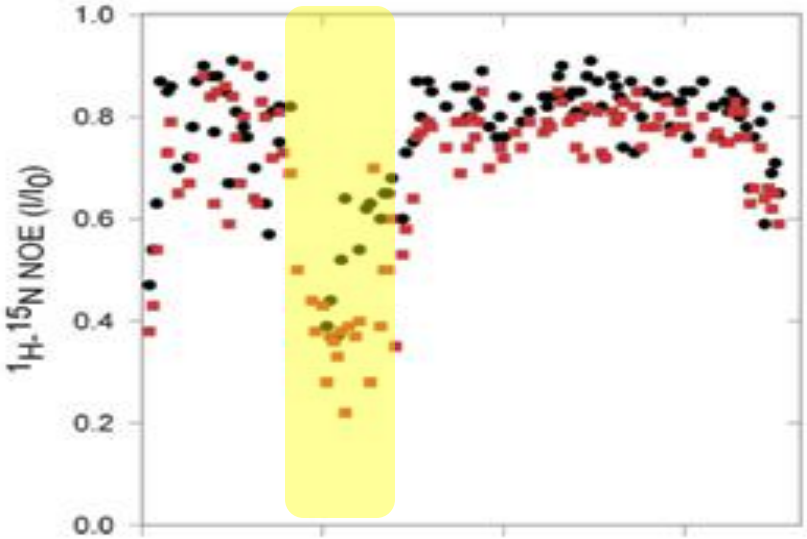
Positive (A) and Negative (B) contours for NOE data

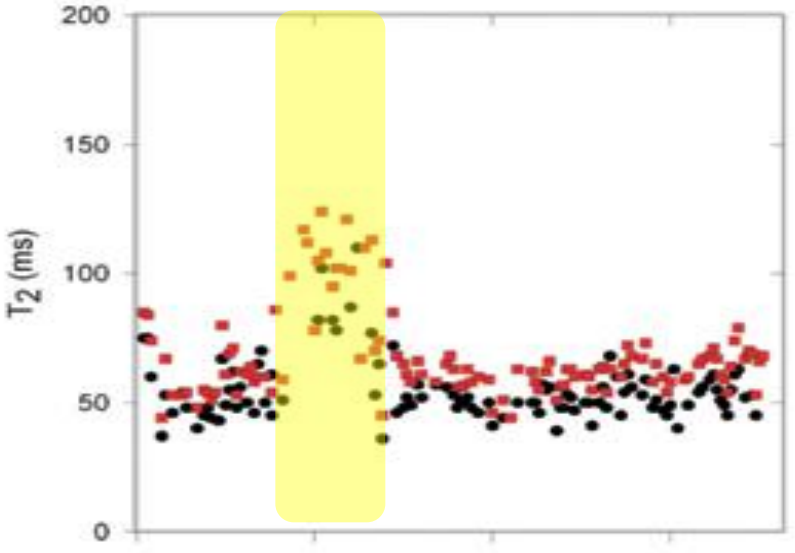
- negative NOEs indicate highly mobile residues



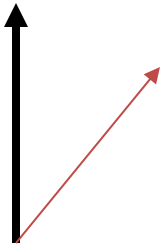
Lower T1, Higher T2, Lower het-NOE => Dynamic region







Why do NH spins relax?



Energy of magnetic dipole in magnetic field = $-\mu \cdot B$

$= -\mu B\cos(\theta)$

where μ is the magnetic dipole moment
and θ is the angle between μ and B

The relaxation occurs due to rotational diffusion motions of the nitrogen atom and orientation of the N-H bond vectors relative to the external field.

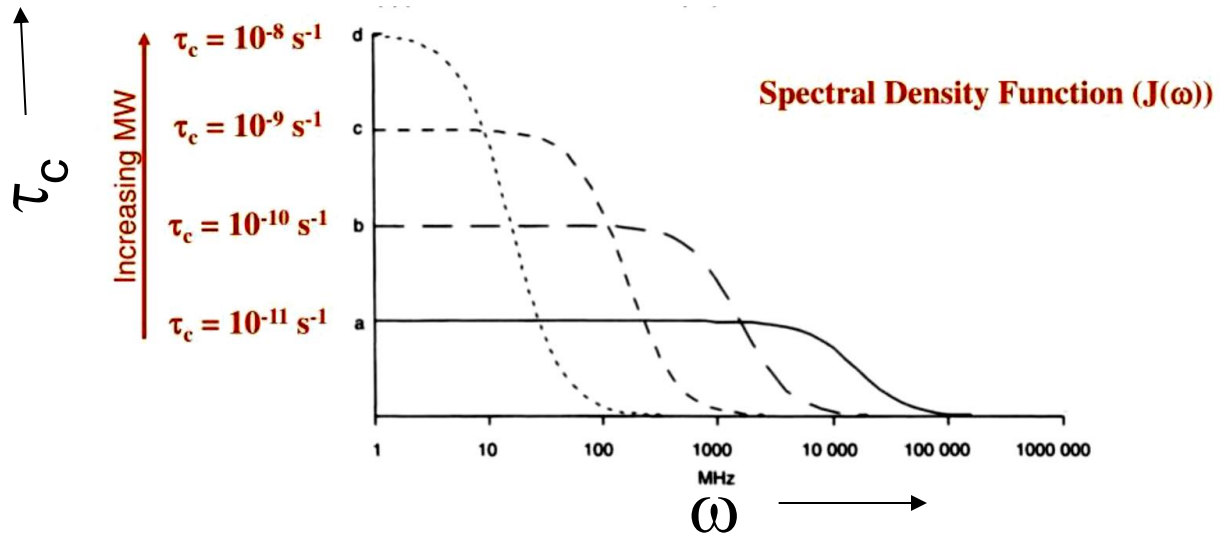
The molecular motions cause ^{15}N nucleus to create energy fluctuation, inducing transitions amongst the Zeeman energy levels and resulting in relaxation

Spectral density functions

The frequency distribution of the motion of a randomly tumbling molecule is expressed in terms of its spectral density **J(ω,tau$_c$)**

Spectral density function **J(ω,τ_c)** = Probablity of finding the frequency (energy) ω in the thermal bath that is provided by the motion of the molecule.

For a spherical object $$J(\omega,\tau_c) = \frac{\tau_c}{1+\omega^2\tau_c^2}$$



Rotational correlation time or Tumbling time (tauc)=

Time taken to rotate by 1 radian = $360/2\pi$

For spherical objects

$$\tau_c = \frac{4\pi a^3 \eta}{3kT}$$

T_1, T_2, and the NOE defined in terms of spectral density function

$$1/T_1 = d^2\{J(\omega_A - \omega_X) + 3J(\omega_X) + 6J(\omega_A + \omega_X)\} + c^2 J(\omega_X)$$

$$1/T_2 = 0.5d^2\{4J(0) + J(\omega_A - \omega_X) + 3J(\omega_X) + 6J(\omega_A) + 6J(\omega_A + \omega_X)\} + \tfrac{1}{6}c^2\{3J(\omega_X) + 4J(0)\}$$

$$\mathrm{NOE} = 1 + [(\gamma_A/\gamma_X)d^2\{6J(\omega_A + \omega_X) - J(\omega_A - \omega_X)\}T_1]$$

where:

$$d^2 = 0.1\gamma_A^2\gamma_X^2\hbar^2/(4\pi^2)\langle 1/r_{AX}^3\rangle^2$$

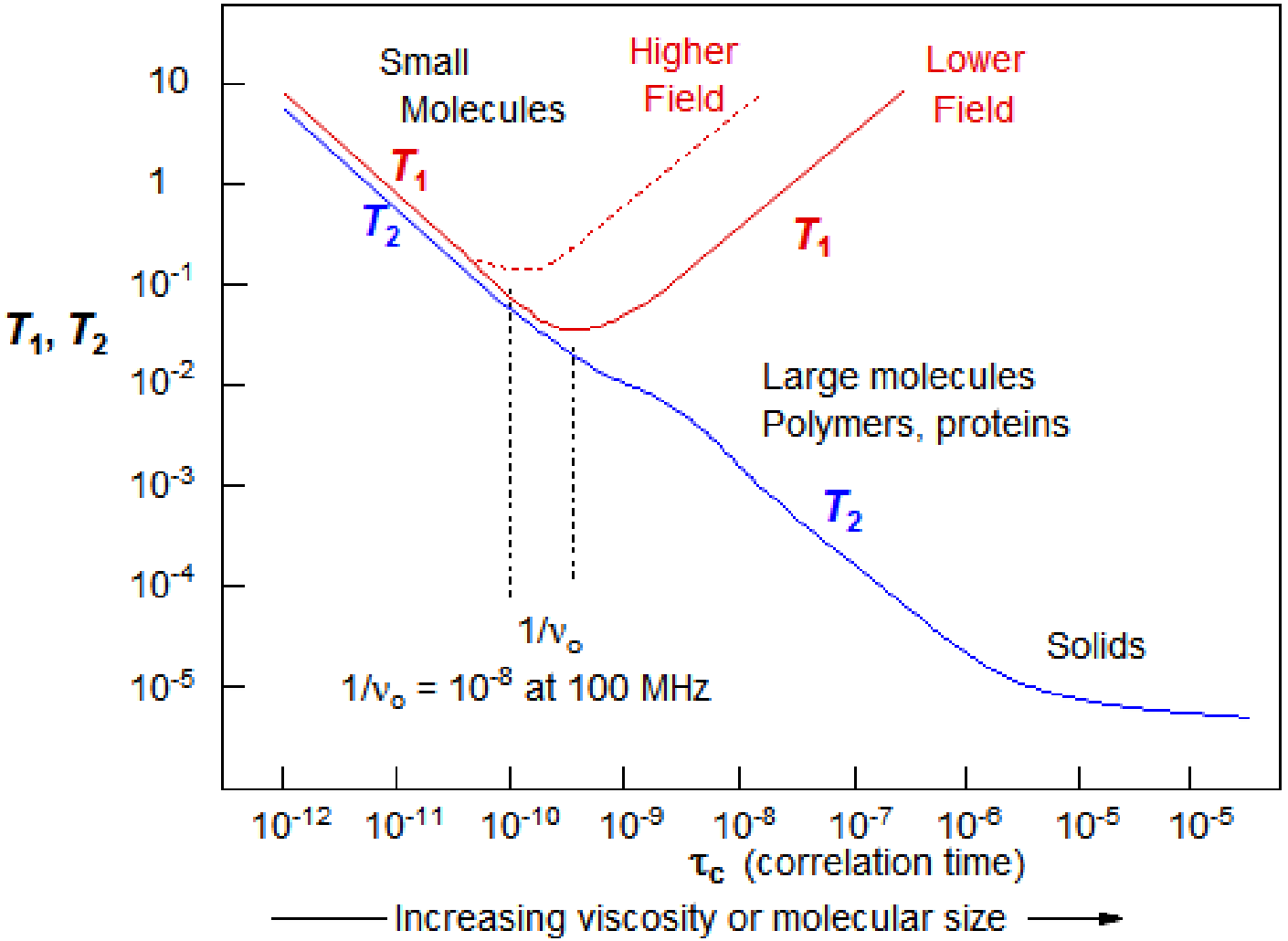
$$c^2 = (\tfrac{2}{15})\gamma_X^2 H_O^2(\sigma_\parallel - \sigma_\perp)^2$$

$$\sigma_\parallel - \sigma_\perp = -160 \text{ (peptide bonds)}$$

r_{AX} – ^{1}H-^{15}N bond distance
H_o – magnetic field strength
$\sigma_\parallel - \sigma_\perp$ - ^{15}N chemical shift tensors

Some concepts of Motion

Both T1 and T2 depend on spectral density functions (ω and tauc)



Quantifying Protein Dynamics From NMR Data

- For a Protein in Solution, $J(\omega_i)$ depends on:
  - overall motion of the protein as a whole
  - internal motion of the ^{1}H-^{15}N bond vector

Lipari-Szabo Model-Free Formulism

$$J(\omega) = \frac{2}{5}\left[\frac{S^2\tau_m}{1+\omega^2\tau_m^2} + \frac{(1-S^2)\tau}{1+\omega^2\tau^2}\right] \qquad \tau^{-1} = \tau_e^{-1} + \tau_m^{-1}$$

where: τ_m is the overall motion of the protein
τ_e is the ^{1}H-^{15}N internal motion
S^2 is the spatial restriction of internal motion (order parameter)

If the internal motion is very rapid, τ_e approaches zero.
If the internal motion is not present, S^2 approaches one.

Sometimes it is necessary to add an exchange contribution (R_{ex}) to the predicted R_2 (T_2) to account for the experimentally observed R_2

***Journal of Biomolecular NMR*, 18: 83–100, 2000.**

Quantifying Protein Dynamics From NMR Data

- T_1, T_2 and NOE can then be described in terms of:
  - order parameters (S^2, S_s^2, S_f^2)
  - correlation time (τ_m,τ_e)

$$1/T_1 = S^2(1/T_1)_{isot}[1 + (10 + \delta)/(3 + \delta) \times \{(1 - S^2)/S^2\}(\tau_e/\tau_m)(\omega_X\tau_m)^2]$$

$$1/T_2 = S^2(1/T_2)_{isot}[1 + |10 + (7/6)\delta|/|2 + (2/3)\delta| \times \{(1 - S^2)/S^2\}(\tau_e/\tau_m)]$$

$$NOE = NOE_{isot} - 50/(3 + \delta)[\{(1 - S^2)/S^2\}(\tau_e/\tau_m)(\omega_X\tau_m)^2]$$

Table 1: Expressions of Spectral Density Functions for the Five Models

model	spectral density functions	optimized parameters
1	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2)]$	S^2
2[a]	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2) + (1 - S^2)\tau_e'/(1 + \omega^2\tau_e'^2)]$	S^2, τ_e
3	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2)]$ $R_{2(obs)} = R_2 + R_{ex}$	S^2, R_{ex}
4	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2) + (1 - S^2)\tau_e'/(1 + \omega^2\tau_e'^2)]$ $R_{2(obs)} = R_2 + R_{ex}$	S^2, τ_e, R_{ex}
5[b]	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2) + S_f^2(1 - S_s^2)\tau_s'/(1 + \omega^2\tau_s'^2)]$	S_f^2, S_s^2, τ_e

[a] $\tau_e' = \tau_m\tau_e/(\tau_m + \tau_e)$. [b] $\tau_s' = \tau_m\tau_s/(\tau_m + \tau_s)$; $S^2 = S_f^2S_s^2$.

***Biochemistry*, 29: 7387-7401, 1990**
***Biochemistry*, 31:9150-9157,1992**

Quantifying Protein Dynamics From NMR Data

- For a Protein in Solution, $J(\omega_i)$ depends on:
  - overall motion of the protein as a whole
  - internal motion of the ^{1}H-^{15}N bond vector

Extended Model-Free Approach

$$J(\omega) = \frac{2}{5} S_f^2 \left[\frac{S_s^2 \tau_m}{1 + \omega^2 \tau_m^2} + \frac{(1 - S_s^2)\tau}{1 + \omega^2 \tau^2} \right] \qquad \tau^{-1} = \tau_e^{-1} + \tau_m^{-1}$$

where: τ_m is the overall motion of the protein
τ_e is effective correlation time for the slow motion
S_f^2 is the order parameter for fast internal motion
S_s^2 is the order parameter for slow internal motion

The effective correlation time for the fast motion is assumed to be zero.

Sometimes it is necessary to invoke internal motions on two widely different time scales

Quantifying Protein Dynamics From NMR Data

- If you assume the only motion present in the protein is the overall molecular tumbling then:
  - ♦ spectral density function is only dependent on S^2 and τ_m
  - ♦ModelFree (RELAX) – software program generally used to analyze NMR T1,T2 and NOE data to extract dynamic parameters (τ_m,τ_e,S^2,S_f^2,S_s^2)

Mandel, A. M.,Akke, M. & Palmer, A. G. (1995) *J. Mol. Bio 246*, 144-163.
Palmer, A. G.,Rance, M. & Wright, P. E. (1991) *J. Am. Chem. Soc. 113*, 4371-4380.

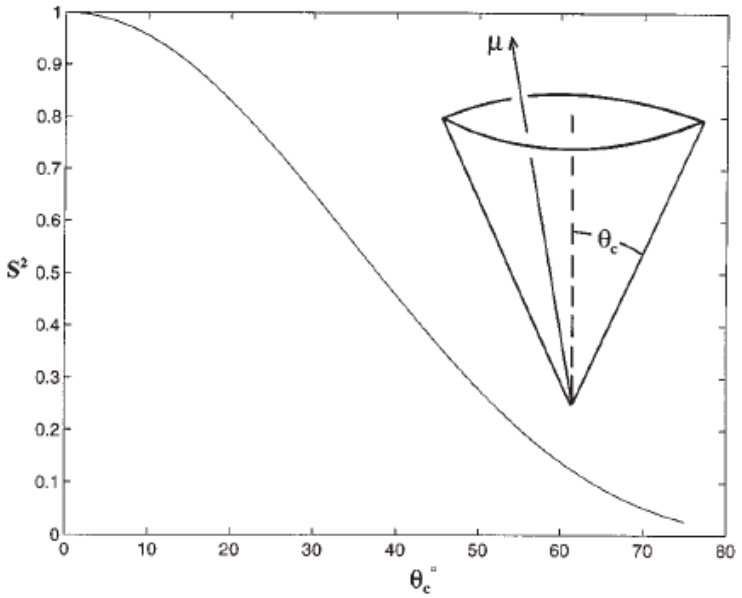
Quantifying Protein Dynamics From NMR Data

- ModelFree (RELAX) – software program generally used to analyze NMR T1,T2 and NOE data to extract dynamic parameters (τ_m,τ_e,S^2,S_f^2,S_s^2)

- Given the overall rotational correlation time τ_m for the protein, can determine how well each residues T_1,T_2 and NOE data can be explained by only this motion
  - Does the data fit better by adding:
    - exchange (R_{ex})
    - single internal motion (τ_e)
    - fast (S_f^2) and slow (S_s^2,τ_e) internal motion
  - Using ModelFree, τ_m and the individual T_1,T_2 and NOE data calculate dynamic parameters for each residue in the protein.

Relationship between S^2 and the angle (θ) between the bond vector (μ) and the cone the bond vector traces.

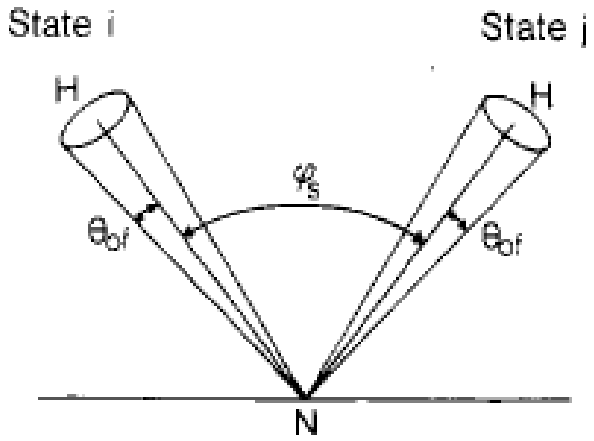
Smaller θ angle → smaller motion → larger S^2
Larger θ angle → larger motion → smaller S^2



nature structural biology • volume 7 number 9 • september 2000

Quantifying Protein Dynamics From NMR Data

- Model for system with two distinct internal motions
  - motions on time scale of <20-50 ps and 0.5-4 ns
  - slower motion is represented by a jump between two states (i and j)
  - faster motion is represented as free diffusion within two axially symmetric cones centered about the two I and j states
    - θ_{of} is the semiangle of the cone
    - ϕ is the angle between the NH vectors in the two states (i and j)



$$S_f^2 = [0.5 \cos \theta_{of} (1 + \cos \theta_{of})]^2$$

$$S_s^2 = (1 + 3 \cos^2 \varphi_s)/4$$

$$S^2 = S_f^2 S_s^2$$

Quantifying Protein Dynamics From NMR Data

- Relationship between entropy (S) and NMR order-parameter (S^2_{NMR})

$$S = k_B M\left[A + Bf(1 - S^2_{NMR})\right]$$

Table 1. Amino Acid-Specific Parametrizations of Side-Chain and Backbone Entropies versus S^2_{NMR} According to Equation 2

amino acid[c]	no. of data points	error/M (k_B)	R^d	M	A^e	B^e
VST[a]	30	0.13	0.93	1	2.19	1.32
IL[a]	34	0.09	0.96	2	1.95	1.55
M[a,g]	4	0.02	0.98	3	2.73	0.77
N[af]	7	0.06	0.99	2	2.06	2.08
Q[af]	11	0.17	0.93	3	2.16	1.60
FHY[a,g]	18	0.10	0.96	2	2.07	1.51
P[a]	10	0.05	0.89	1	1.90	1.15
K[a]	21	0.06	0.93	4	2.20	1.22
R[a]	10	0.07	0.98	5	2.22	1.23
D[b]	11	0.12	0.93	2	3.69	0.44
E[b]	21	0.09	0.99	3	3.66	0.64
backbone[b]	206	0.15	0.88	2	3.42	0.50

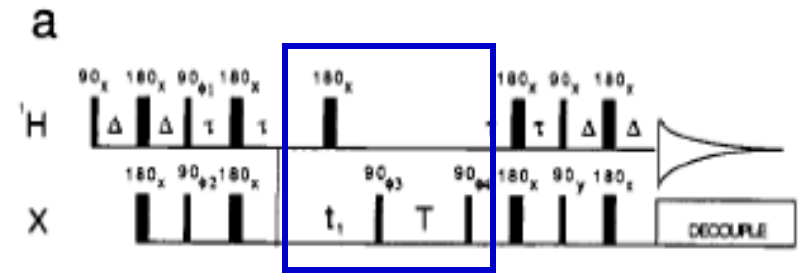
D. W. Li & R. Bruschweiler (2009) *J. Am. Chem. Soc. 131*, 7226.

NMR Analysis of Protein Dynamics

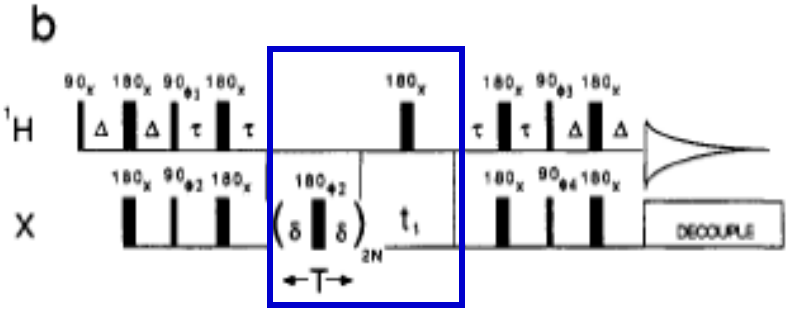
How Do We Measure T_1, T_2 and NOE data For a Protein?

- Modified 2D ^{1}H-^{15}N HSQC Spectra
  - Standard 1D T1, T2, and NOE experiments are incorporated into the HSQC experiment

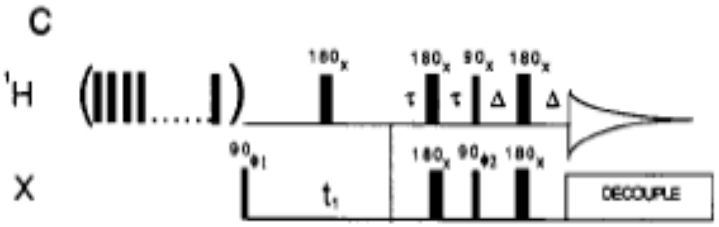
T_1 experiment: generate –Z magnetization that relaxes as exp(-T/T_1)



T_2 experiment: generate XY magnetization that relaxes as exp(-T/T_2) with re-focusing of field inhomogeniety (CPMG)



NOE experiment: data sets are collected with/without ^{1}H presaturation. NOE is measured from the ratio of the peak intensity in the two experiments.



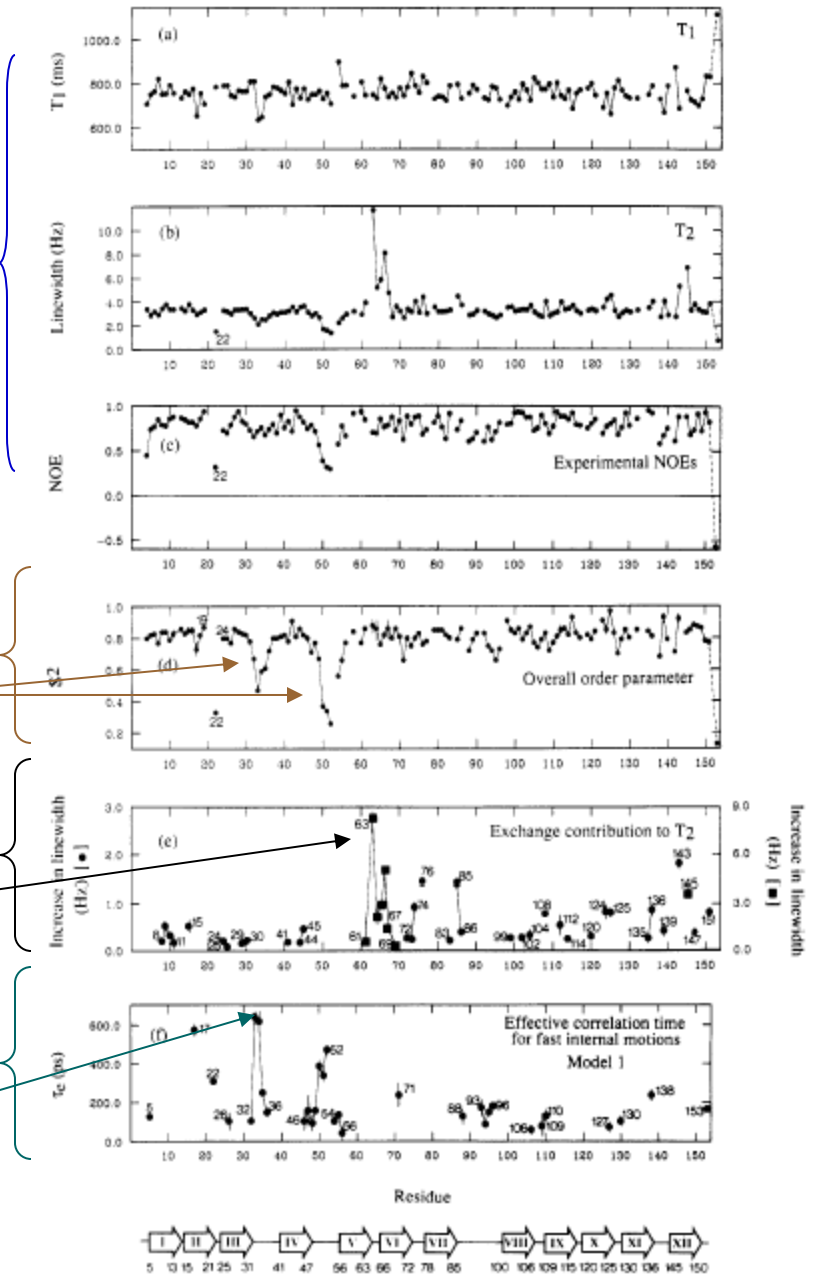
NMR Analysis of Protein Dynamics

Experimental parameters plotted as a function of sequence

Calculated order parameters (S^2) as a function of sequence. Regions of high mobility are inferred from low S^2 values

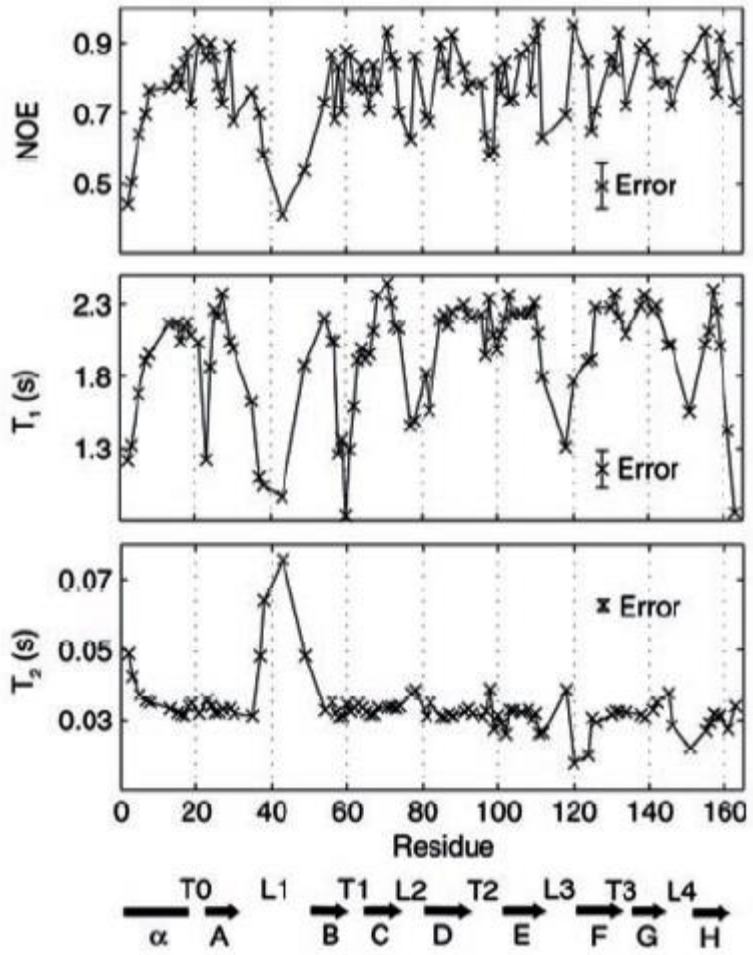
Residues with exchange contribution (R_{ex}) to T_2 → slow conformational exchange (msec to sec)

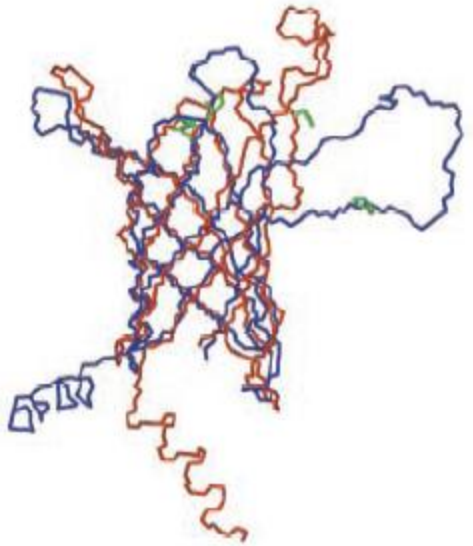
Residues that exhibit fast internal motions (τ_e)

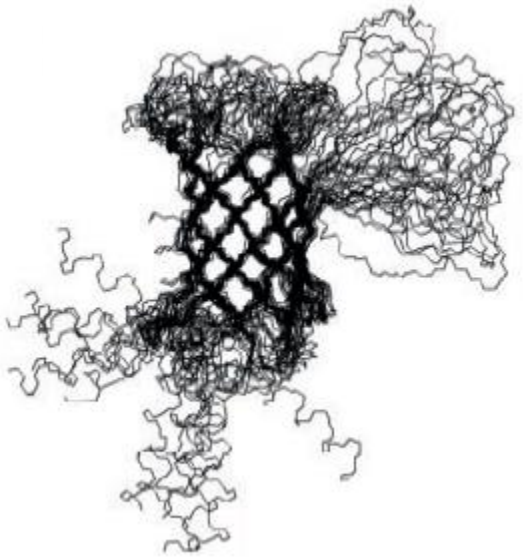


NMR Analysis of Protein Dynamics

In general, regions of secondary structure show low mobility while turns, loops and N-,C-terminus exhibit high mobility



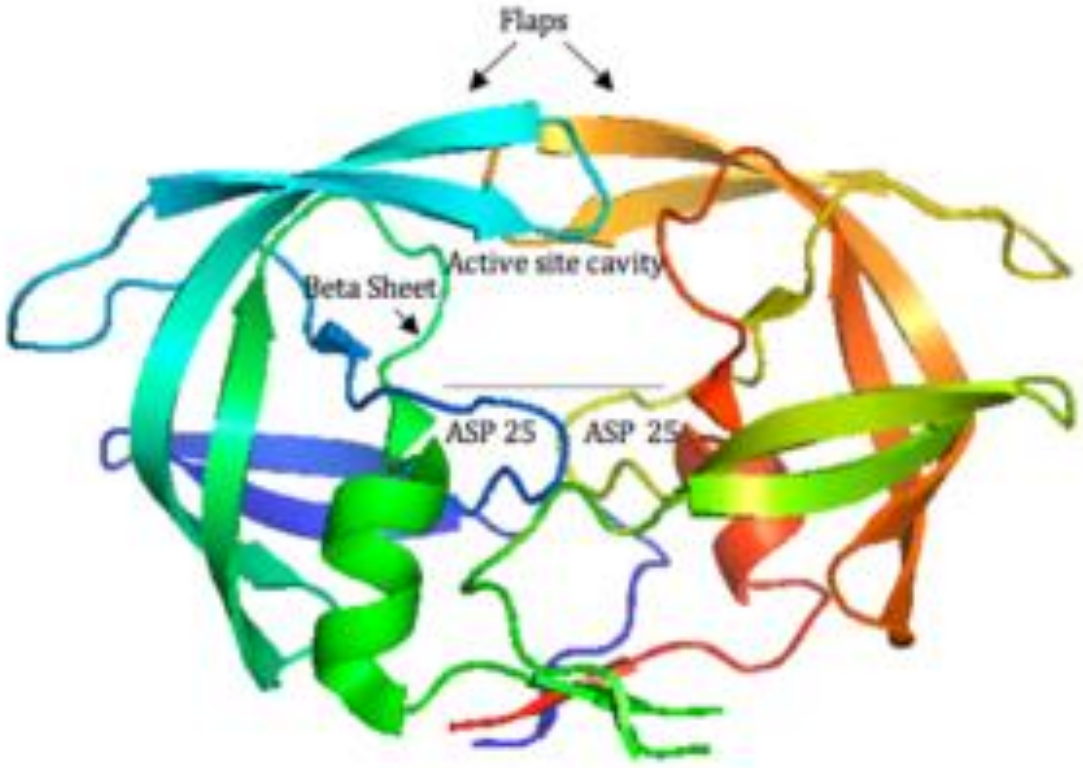




PNAS **2002** vol. 99 no. 21 13560-13565

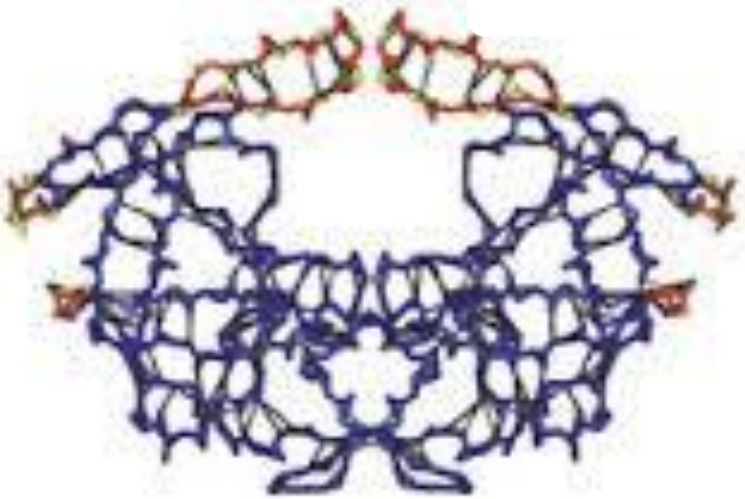
HIV-protease

The **HIV**-1 **Protease** (PR) hydrolyzes viral poly-proteins into functional protein products that are essential for viral assembly and subsequent activity

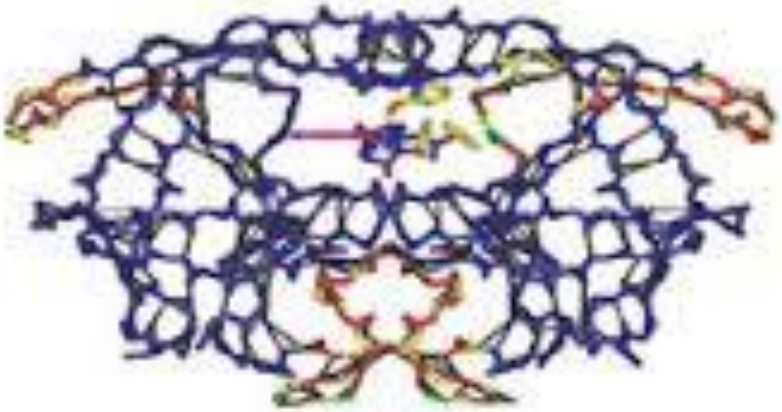


HIV-protease

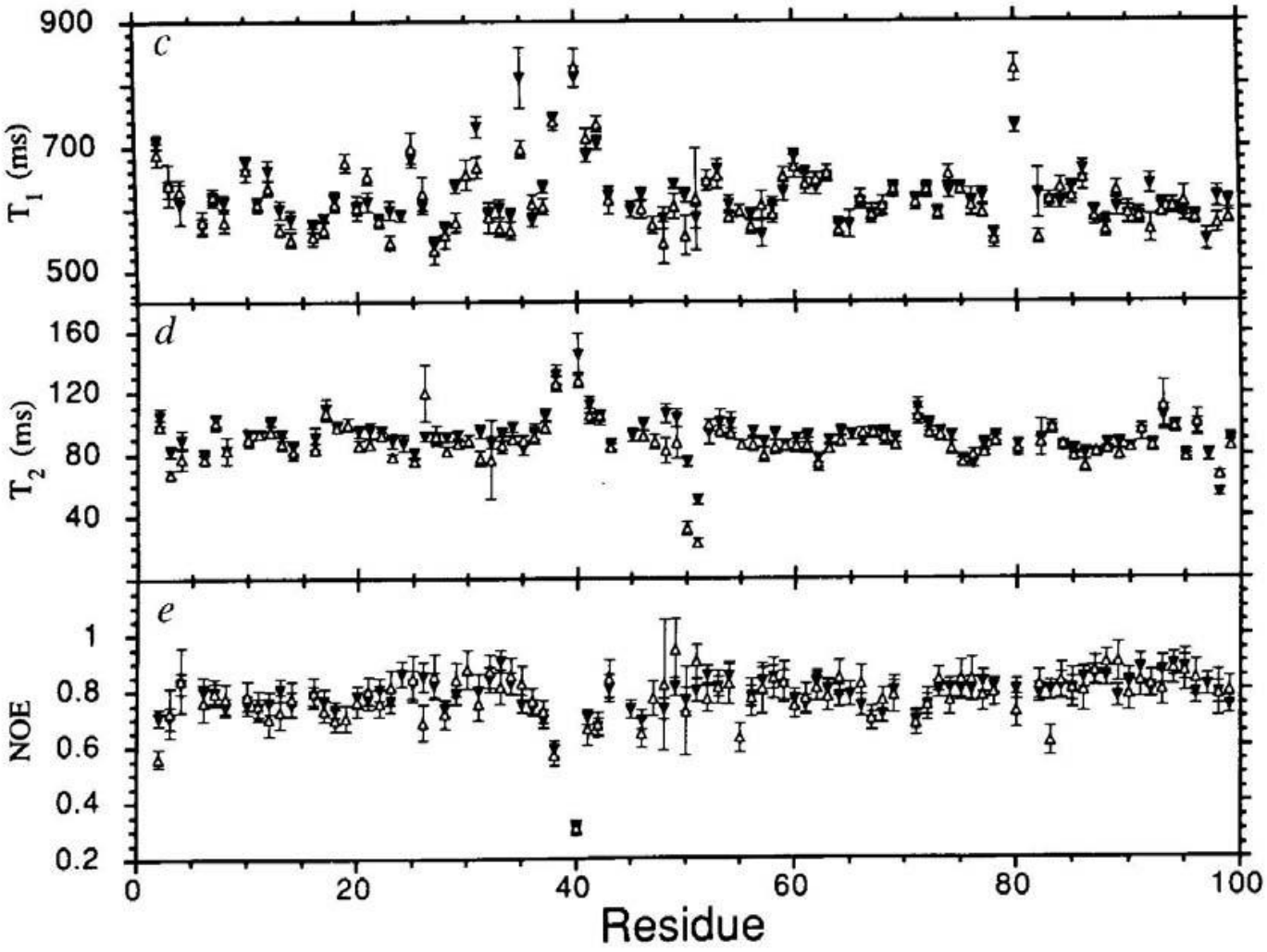
Open



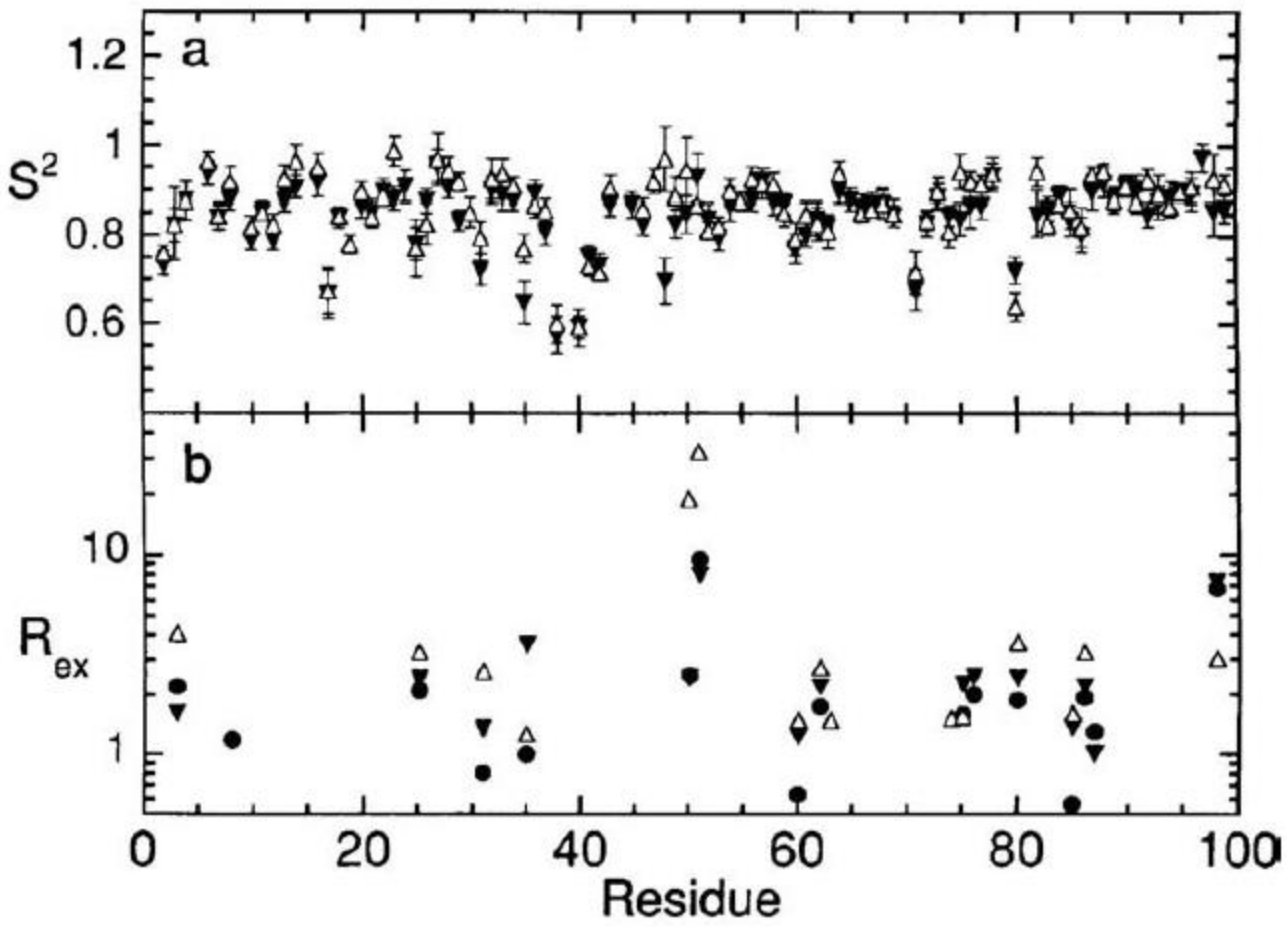
Closed



HIV-protease



HIV-protease



These information can lead to better drug-design

Thank You

How to obtain motional parameters ?

One can keep measuring multiple relaxation experiments

OR

Make an assumption of an analytical model of the spectral density function that has only 3 parameters and can be used to fit the measured relaxation data

Lipari-Szabo model

$$J(\omega) = S^2 \frac{\tau_m}{1 + (\omega \tau_m)^2} + \left(1 - S^2\right) \frac{\tau}{1 + (\omega \tau)^2}$$

$$\text{with } \tau^{-1} = \tau_m^{-1} + \tau_e^{-1}$$

τ_e = correlation time for internal motions

τ_m = overall correlation time

S^2 = Order parameter (a quantitative measure of internal motions)

How to obtain motional parameters ?

Lipari-Szabo model

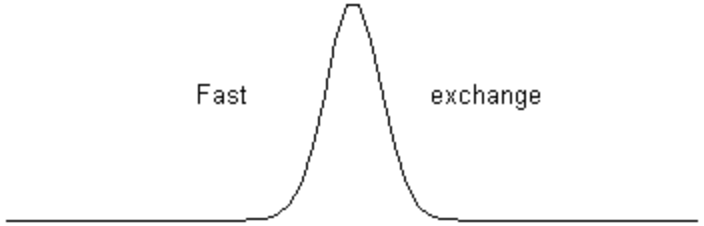
$$J(\omega) = S^2 \frac{\tau_m}{1+(\omega\tau_m)^2} + \left(1 - S^2\right) \frac{\tau}{1+(\omega\tau)^2}$$

$$\text{with } \tau^{-1} = \tau_m^{-1} + \tau_e^{-1}$$

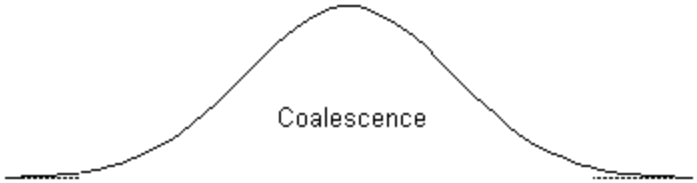
Fit to obtain tau_e, tau_m, S^2 and a by-product Rex !!

NMR Analysis of Protein Dynamics

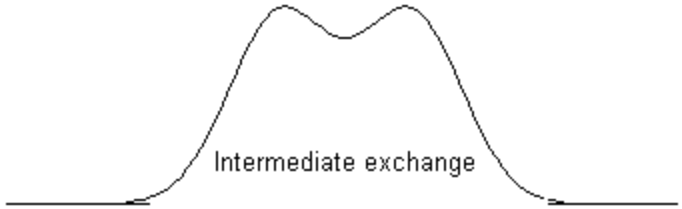
$k = \pi \Delta\nu_o^2 / 2(h_e - h_o)$



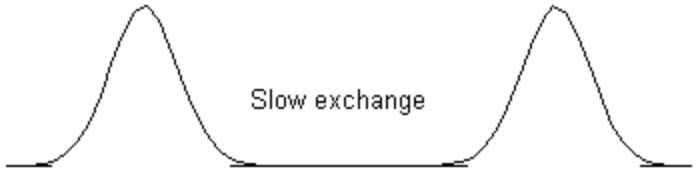
$k = \pi \Delta\nu_o / 2^{1/2}$



$k = \pi (\Delta\nu_o^2 - \Delta\nu_e^2)^{1/2} / 2^{1/2}$



$k = \pi (h_e - h_o)$



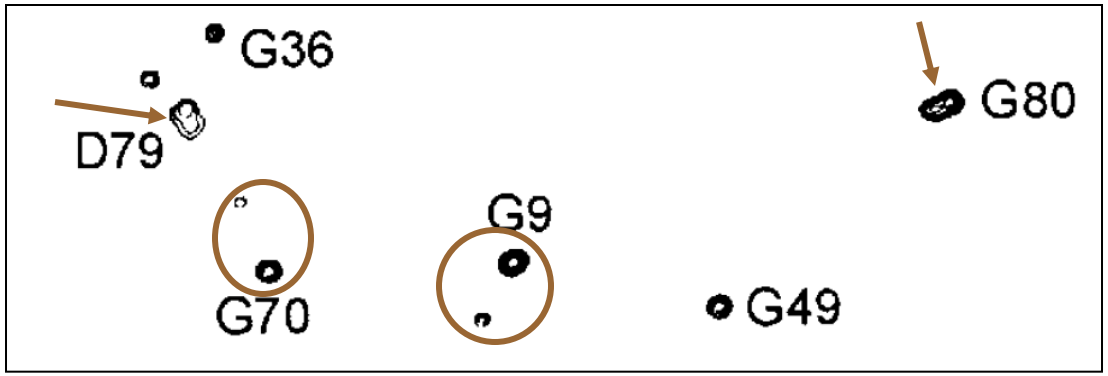
k – exchange rate
ν – peak frequency
h – peak-width at half-height
e – with exchange
o – no exchange

NMR Analysis of Protein Dynamics

For Protein Samples, Typically Monitor Exchange Using 2D NMR Experiments

- Need resolution and chemical shift dispersion to identify exchange peaks
  - ♦ presence of slow exchange effectively increases the number of expected peaks based on the sequence
  - ♦ typically in the range of milusecond to second time range

Expanded Region of 2D ^{1}H-^{15}N HSQC Showing Major and Minor Conformational Exchange Peaks



Biochem. J. (2002) *364*, 725±737

NMR Analysis of Protein Dynamics

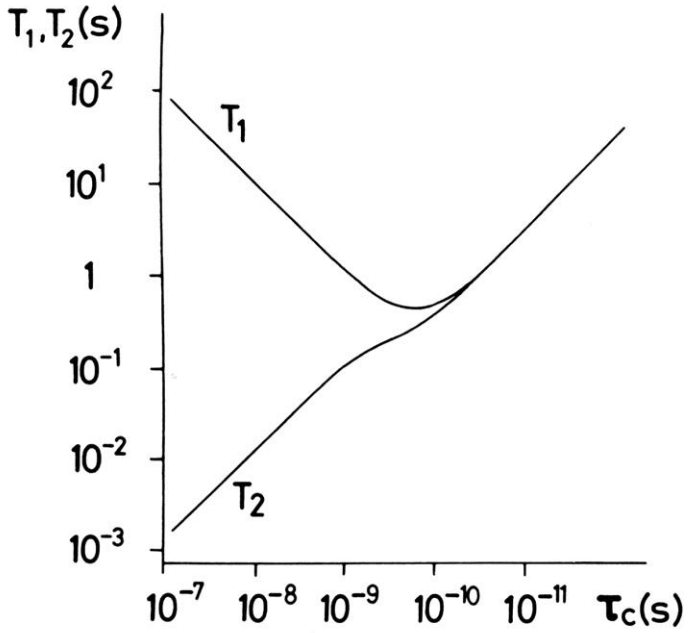
Line-Widths Are Indicative of Overall Tumbling Times of the Molecule

- Rotational Correlation Time (τ_c)
  - related to MW
  - time it takes a molecule to rotate one radian ($360^o/2\pi$)
  - typically in the nanosecond time range

$$\tau_c = \frac{4\pi\eta r^3}{3kT}$$

where:

r = radius
k = Boltzman constant
η = viscosity coefficient



NMR Analysis of Protein Dynamics

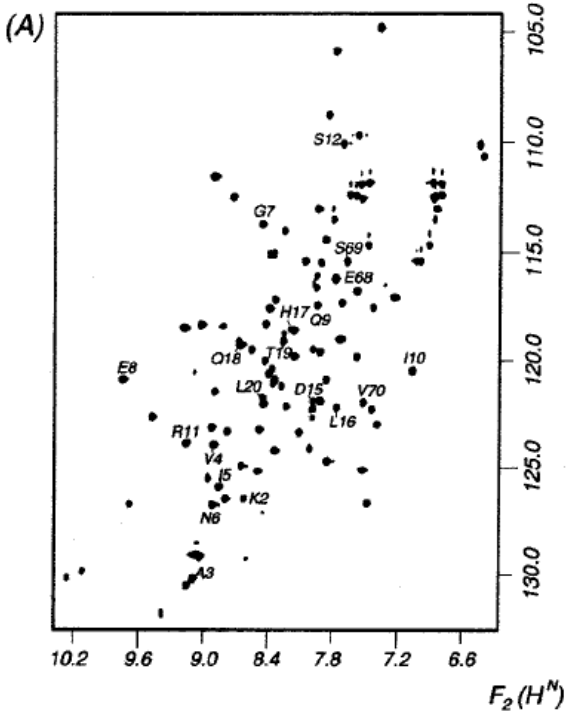
The MW of the Protein Would Imply an Expected NMR Line-Widths

- Broader than expected line-widths in the 2D ^{1}H-^{15}N HSQC may imply:
  - multimer formation (dimer, tetramer, etc)
  - aggregation
  - unfolded/denatured

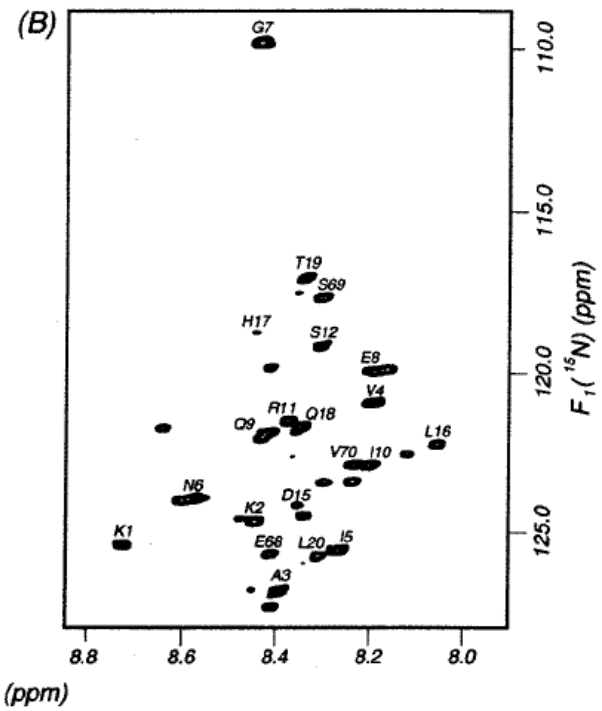
Can estimate τ_c for a spherical protein:

$\tau_c \approx$ MW/2400 (ns)

Barstar pH 6.8



Barstar pH 2.7

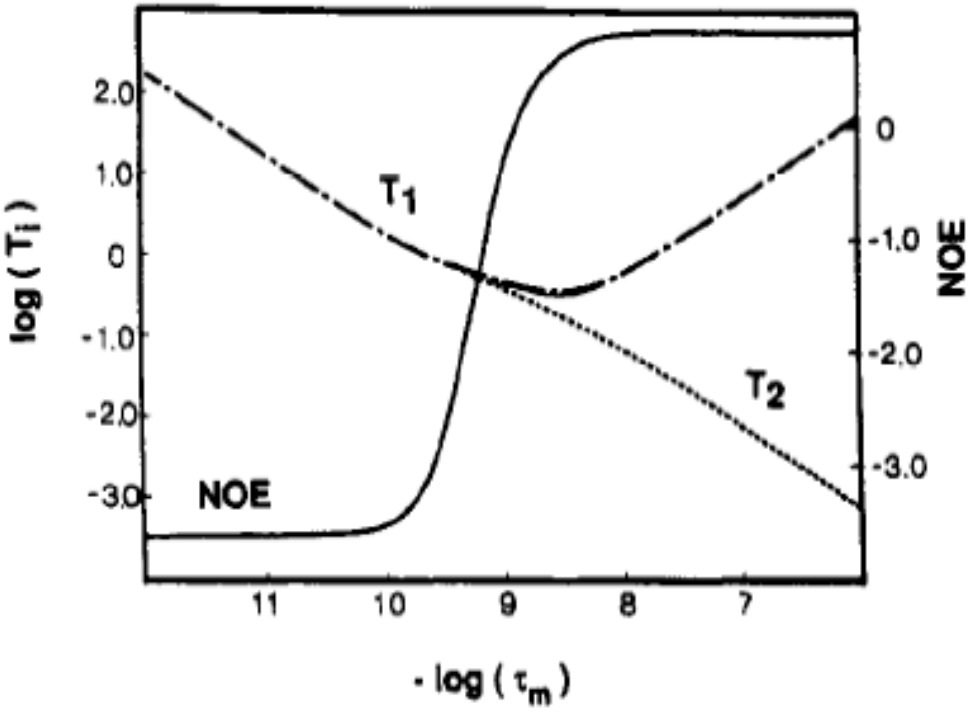


Biochemistry, Vol. 41, No. 31, 2002

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- T_1 and T_2 relaxation and the NOE are related to dynamics
  - correlated to the rotational correlation time of the protein



Biochemistry, Vol. 28, No. 23, 1989

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- T_1, T_2 and the NOE defined in terms of spectral density function
  - total "power" available for relaxation is the total area under the spectral density function

$$1/T_1 = d^2\{J(\omega_A - \omega_X) + 3J(\omega_X) + 6J(\omega_A + \omega_X)\} + c^2 J(\omega_X)$$

$$1/T_2 = 0.5d^2\{4J(0) + J(\omega_A - \omega_X) + 3J(\omega_X) + 6J(\omega_A) + 6J(\omega_A + \omega_X)\} + \tfrac{1}{6}c^2\{3J(\omega_X) + 4J(0)\}$$

$$\mathrm{NOE} = 1 + [(\gamma_A/\gamma_X)d^2\{6J(\omega_A + \omega_X) - J(\omega_A - \omega_X)\}T_1]$$

where:

$$d^2 = 0.1\gamma_A^2\gamma_X^2\hbar^2/(4\pi^2)\langle 1/r_{AX}^3\rangle^2$$

$$c^2 = (\tfrac{2}{15})\gamma_X^2 H_O^2(\sigma_\parallel - \sigma_\perp)^2$$

$$\sigma_\parallel - \sigma_\perp = -160 \text{ (peptide bonds)}$$

r_{AX} – ^{1}H-^{15}N bond distance
H_o – magnetic field strength
$\sigma_\parallel - \sigma_\perp$ - ^{15}N chemical shift tensors

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- For a Protein in Solution, $J(\omega_i)$ depends on:
  - overall motion of the protein as a whole
  - internal motion of the ^{1}H-^{15}N bond vector

Lipari-Szabo Model-Free Formulism

$$J(\omega) = \frac{2}{5}\left[\frac{S^2\tau_m}{1+\omega^2\tau_m^2} + \frac{(1-S^2)\tau}{1+\omega^2\tau^2}\right] \qquad \tau^{-1} = \tau_e^{-1} + \tau_m^{-1}$$

where: τ_m is the overall motion of the protein
τ_e is the ^{1}H-^{15}N internal motion
S^2 is the spatial restriction of internal motion (order parameter)

If the internal motion is very rapid, τ_e approaches zero.
If the internal motion is not present, S^2 approaches one.

Sometimes it is necessary to add an exchange contribution (R_{ex}) to the predicted R_2 (T_2) to account for the experimentally observed R_2

***Journal of Biomolecular NMR*, 18: 83–100, 2000.**

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- For a Protein in Solution, $J(\omega_i)$ depends on:
  - overall motion of the protein as a whole
  - internal motion of the ^{1}H-^{15}N bond vector

Extended Model-Free Approach

$$J(\omega) = \frac{2}{5} S_f^2 \left[\frac{S_s^2 \tau_m}{1 + \omega^2 \tau_m^2} + \frac{(1 - S_s^2)\tau}{1 + \omega^2 \tau^2} \right] \qquad \tau^{-1} = \tau_e^{-1} + \tau_m^{-1}$$

where: τ_m is the overall motion of the protein
τ_e is effective correlation time for the slow motion
S_f^2 is the order parameter for fast internal motion
S_s^2 is the order parameter for slow internal motion

The effective correlation time for the fast motion is assumed to be zero.

Sometimes it is necessary to invoke internal motions on two widely different time scales

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- T_1, T_2 and NOE can then be described in terms of:
  - order parameters (S^2, S_s^2, S_f^2)
  - correlation time (τ_m,τ_e)

$$1/T_1 = S^2(1/T_1)_{isot}[1 + (10 + \delta)/(3 + \delta) \times \{(1 - S^2)/S^2\}(\tau_e/\tau_m)(\omega_X\tau_m)^2]$$

$$1/T_2 = S^2(1/T_2)_{isot}[1 + \{10 + (7/6)\delta\}/\{2 + (2/3)\delta\} \times \{(1 - S^2)/S^2\}(\tau_e/\tau_m)]$$

$$\mathrm{NOE} = \mathrm{NOE}_{isot} - 50/(3 + \delta)[\{(1 - S^2)/S^2\}(\tau_e/\tau_m)(\omega_X\tau_m)^2]$$

Table 1: Expressions of Spectral Density Functions for the Five Models

model	spectral density functions	optimized parameters
1	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2)]$	S^2
2[a]	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2) + (1 - S^2)\tau_e'/(1 + \omega^2\tau_e'^2)]$	S^2, τ_e
3	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2)]$ $R_{2(obs)} = R_2 + R_{ex}$	S^2, R_{ex}
4	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2) + (1 - S^2)\tau_e'/(1 + \omega^2\tau_e'^2)]$ $R_{2(obs)} = R_2 + R_{ex}$	S^2, τ_e, R_{ex}
5[b]	$J(\omega) = {}^2/_5[S^2\tau_m/(1 + \omega^2\tau_m^2) + S_f^2(1 - S_s^2)\tau_s'/(1 + \omega^2\tau_s'^2)]$	S_f^2, S_s^2, τ_e

[a] $\tau_e' = \tau_m\tau_e/(\tau_m + \tau_e)$. [b] $\tau_s' = \tau_m\tau_s/(\tau_m + \tau_s)$; $S^2 = S_f^2S_s^2$.

Biochemistry, 29: 7387-7401, 1990
Biochemistry, 31:9150-9157,1992

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- If you assume the only motion present in the protein is the overall molecular tumbling then:
  - spectral density function is only dependent on S^2 and τ_m

$$J'(\omega_i) = S^2\tau_m / [1 + (\omega_i\tau_m)^2]$$

  - correlation time can then be determined from the ratio of experimental T_1/T_2 ratios
  - determined by minimizing the difference between the left and right side of the following equation for each T_1/T_2 pair for each residue in the protein.

$$T_1/T_2 \sim [d^2\{J'(\omega_A - \omega_X) + 3J'(\omega_X) + 6J'(\omega_A + \omega_X)\} + c^2J'(\omega_X)] / [0.5d^2\{4J'(0) + J'(\omega_A - \omega_X) + 3J'(\omega_X) + 6J'(\omega_A) + 6J'(\omega_A + \omega_X)\} + \tfrac{1}{6}c^2\{3J'(\omega_X) + 4J'(0)\}] \quad ($$

  - ModelFree – software program generally used to analyze NMR T1,T2 and NOE data to extract dynamic parameters (τ_m,τ_e,S^2,S_f^2,S_s^2)

Mandel, A. M.,Akke, M. & Palmer, A. G. (1995) *J. Mol. Bio 246*, 144-163.
Palmer, A. G.,Rance, M. & Wright, P. E. (1991) *J. Am. Chem. Soc. 113*, 4371-4380.

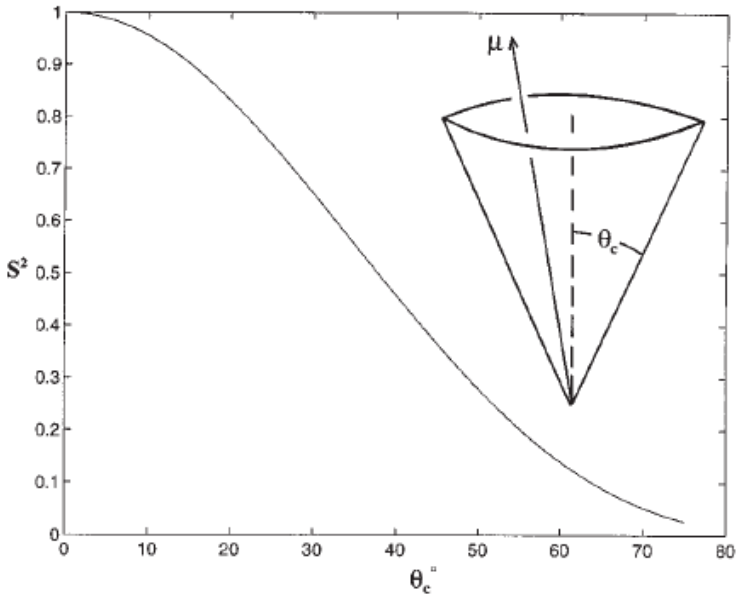
NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- Given the overall rotational correlation time τ_m for the protein, can determine how well each residues T_1,T_2 and NOE data can be explained by only this motion
  - Does the data fit better by adding:
    - exchange (R_{ex})
    - single internal motion (τ_e)
    - fast (S_f^2) and slow (S_s^2,τ_e) internal motion
  - Using ModelFree, τ_m and the individual T_1,T_2 and NOE data calculate dynamic parameters for each residue in the protein.

Relationship between S^2 and the angle (θ) between the bond vector (μ) and the cone the bond vector traces.

Smaller θ angle → smaller motion → larger S^2
Larger θ angle → larger motion → smaller S^2

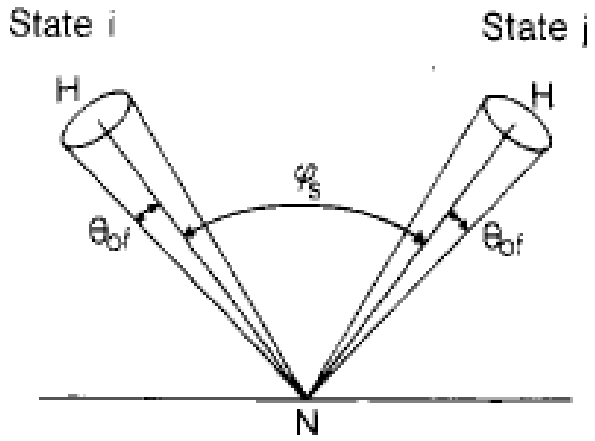


nature structural biology • volume 7 number 9 • september 2000

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- Model for system with two distinct internal motions
  - motions on time scale of <20-50 ps and 0.5-4 ns
  - slower motion is represented by a jump between two states (i and j)
  - faster motion is represented as free diffusion within two axially symmetric cones centered about the two I and j states
    - θ_{of} is the semiangle of the cone
    - ϕ is the angle between the NH vectors in the two states (i and j)



$$S_f^2 = [0.5 \cos \theta_{of} (1 + \cos \theta_{of})]^2$$

$$S_s^2 = (1 + 3 \cos^2 \varphi_s)/4$$

$$S^2 = S_f^2 S_s^2$$

NMR Analysis of Protein Dynamics

Quantifying Protein Dynamics From NMR Data

- Relationship between entropy (S) and NMR order-parameter (S^2_{NMR})

$$S = k_B M\left[A + Bf(1 - S^2_{NMR})\right]$$

.f is log (x) base e

Table 1. Amino Acid-Specific Parametrizations of Side-Chain and Backbone Entropies versus S^2_{NMR} According to Equation 2

amino acid[c]	no. of data points	error/M (k_B)	R^d	M	A^e	B^e
VST[a]	30	0.13	0.93	1	2.19	1.32
IL[a]	34	0.09	0.96	2	1.95	1.55
M[a,g]	4	0.02	0.98	3	2.73	0.77
N[af]	7	0.06	0.99	2	2.06	2.08
Q[af]	11	0.17	0.93	3	2.16	1.60
FHY[a,g]	18	0.10	0.96	2	2.07	1.51
P[a]	10	0.05	0.89	1	1.90	1.15
K[a]	21	0.06	0.93	4	2.20	1.22
R[a]	10	0.07	0.98	5	2.22	1.23
D[b]	11	0.12	0.93	2	3.69	0.44
E[b]	21	0.09	0.99	3	3.66	0.64
backbone[b]	206	0.15	0.88	2	3.42	0.50

D. W. Li & R. Bruschweiler (2009) *J. Am. Chem. Soc. 131*, 7226.

NMR Analysis of Protein Dynamics

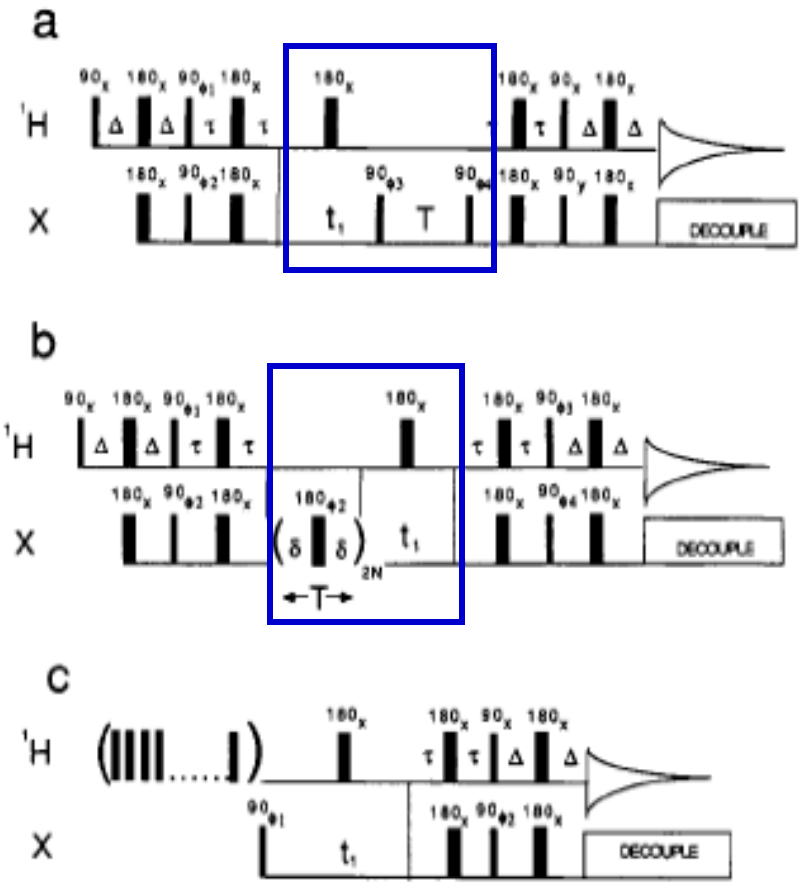
How Do We Measure T_1, T_2 and NOE data For a Protein?

- Modified 2D ^{1}H-^{15}N HSQC Spectra
  - Standard 1D T1, T2, and NOE experiments are incorporated into the HSQC experiment

T_1 experiment: generate –Z magnetization that relaxes as exp(-T/T_1)

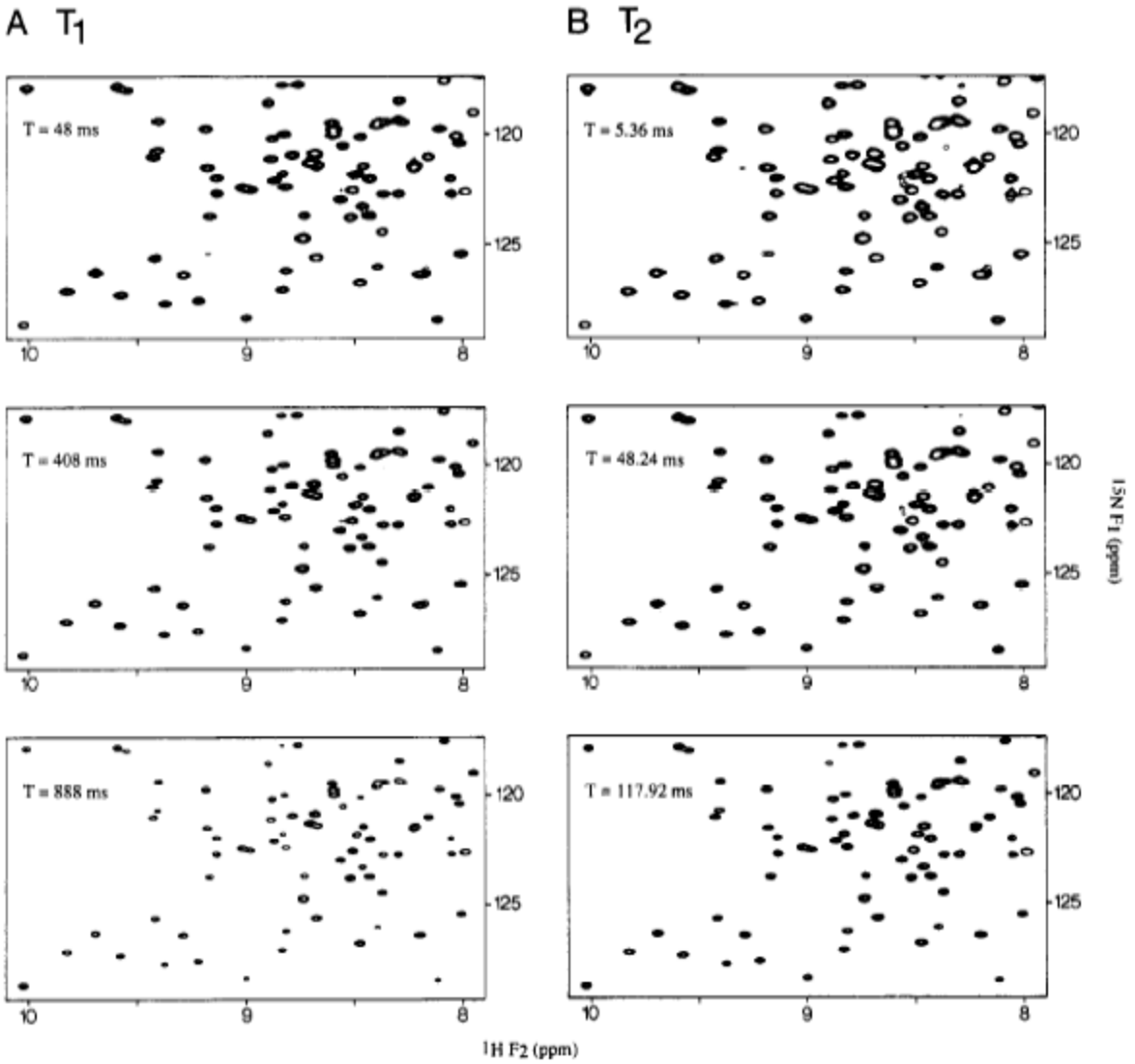
T_2 experiment: generate XY magnetization that relaxes as exp(-T/T_2) with re-focusing of field inhomogeniety (CPMG)

NOE experiment: data sets are collected with/without ^{1}H presaturation. NOE is measured from the ratio of the peak intensity in the two experiments.



NMR Analysis of Protein Dynamics

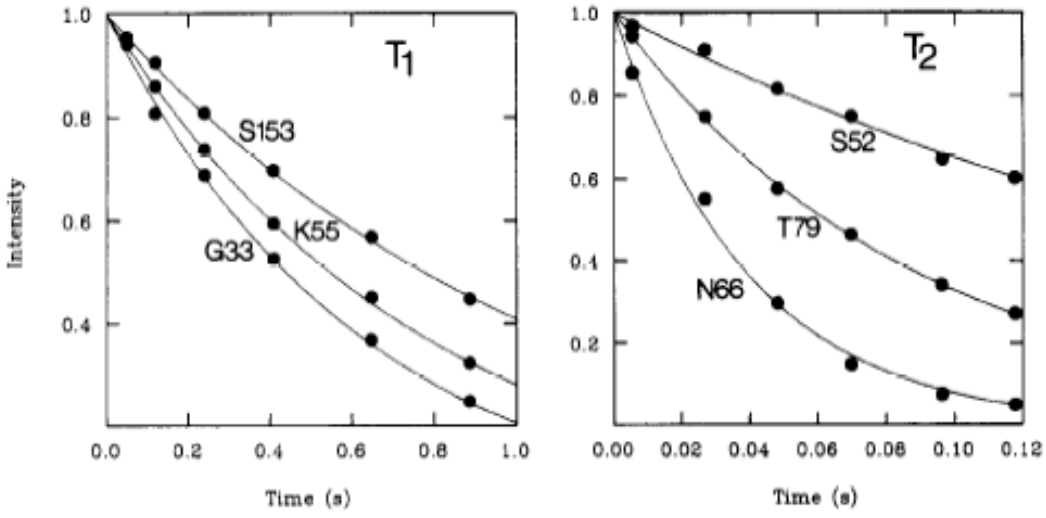
Typical T_1 and T_2 data For a Protein



Biochemistry **1990,** ***29,*** 7387-7401

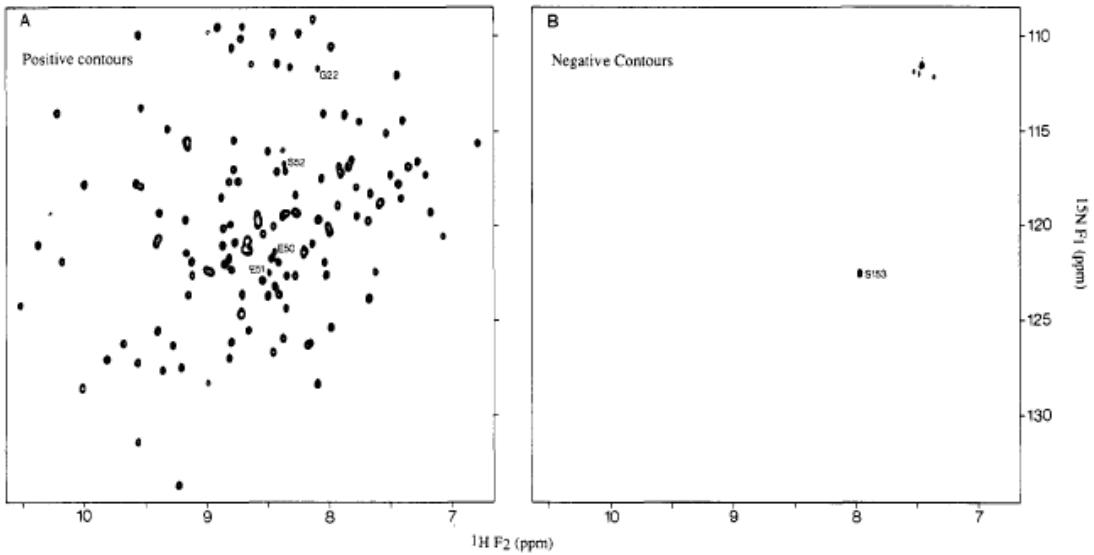
NMR Analysis of Protein Dynamics

Typical Quality of Fits for T_1 and T_2 2D ^1H-^{15}N HSQC Data



Positive (A) and Negative (B) contours for NOE data

- negative NOEs indicate highly mobile residues



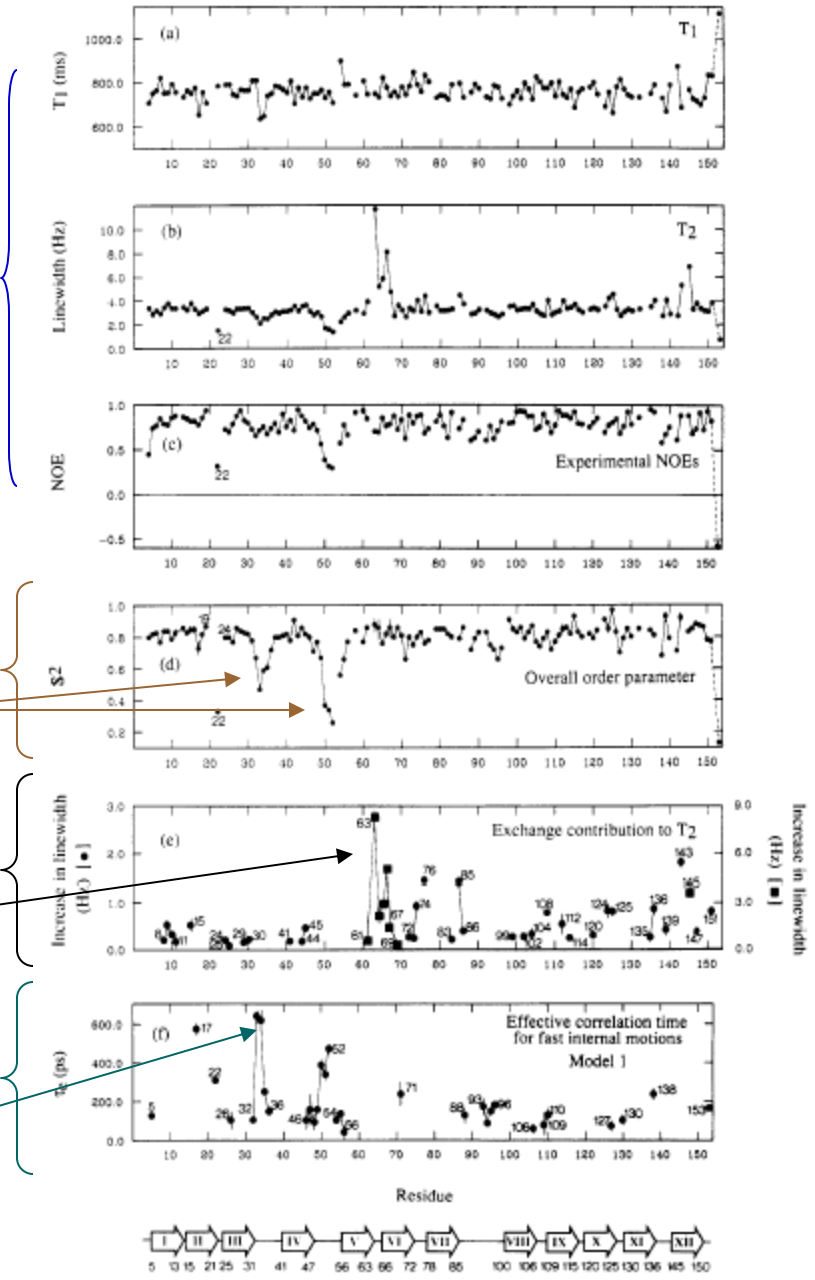
NMR Analysis of Protein Dynamics

Experimental parameters plotted as a function of sequence

Calculated order parameters (S^2) as a function of sequence. Regions of high mobility are inferred from low S^2 values

Residues with exchange contribution (R_{ex}) to T_2 → slow conformational exchange (msec to sec)

Residues that exhibit fast internal motions (τ_e)

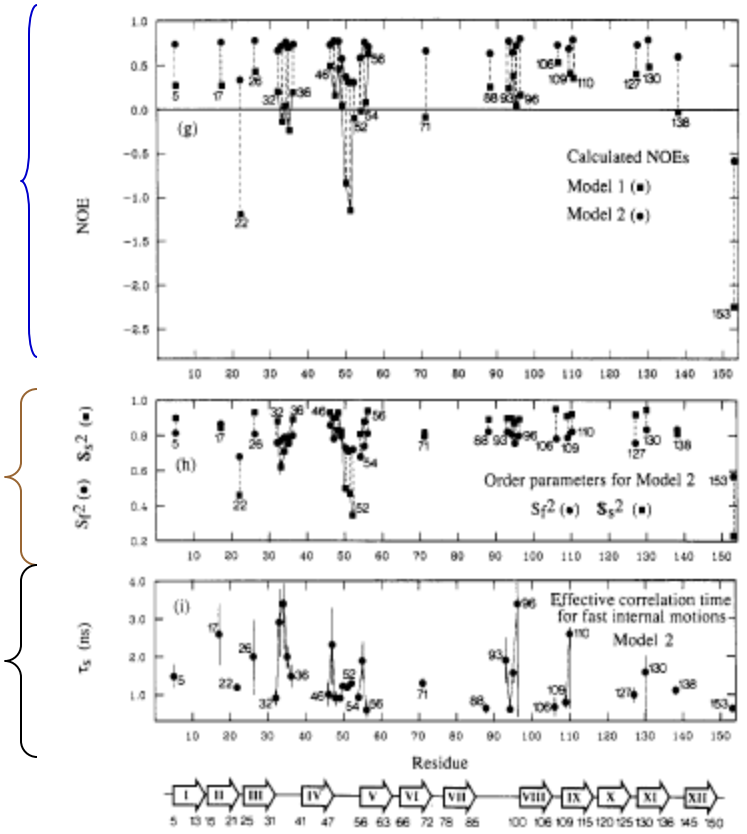


NMR Analysis of Protein Dynamics

Difference in calculated NOEs between models with one and two internal motions

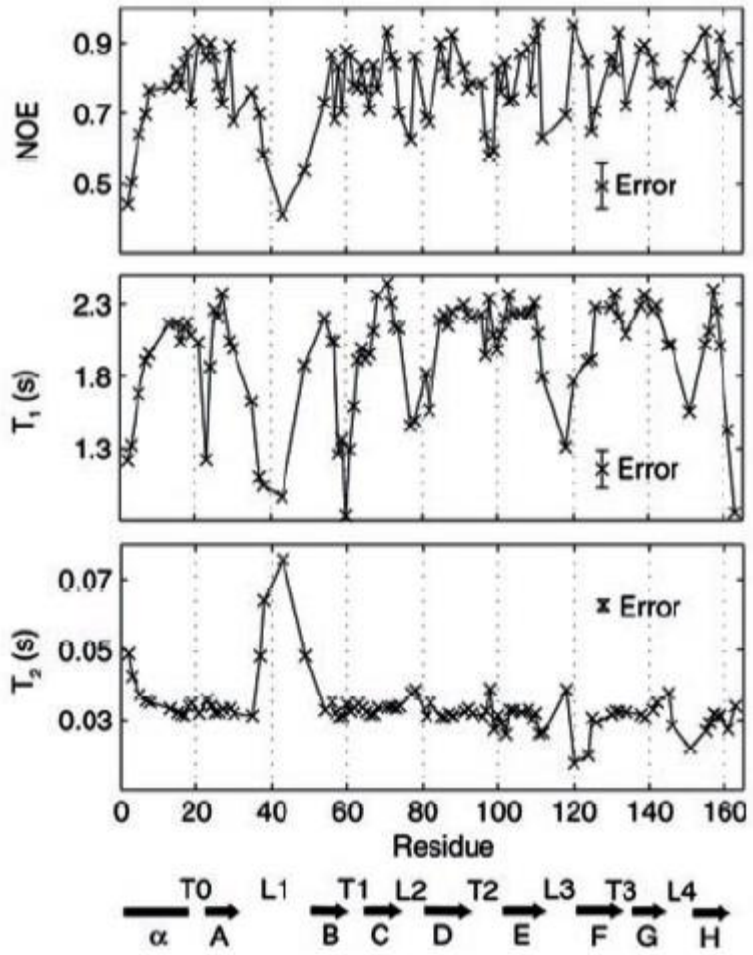
Calculated fast (S_f^2) and slow (S_s^2) order parameters for residues exhibiting both a fast (ps) and slow (ns) internal motion

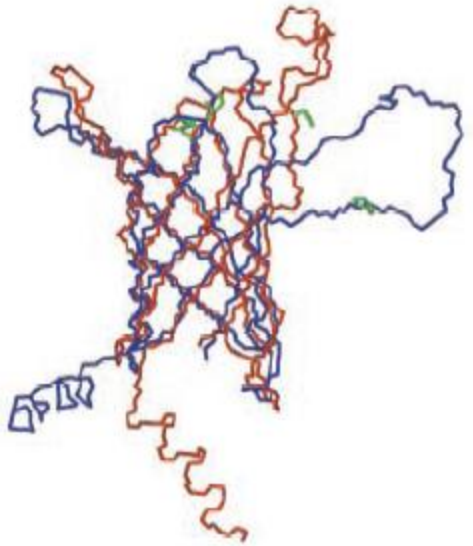
Slow internal motions (τ_s) for residues exhibiting both fast and slow internal motion ($\tau_e = 0$)

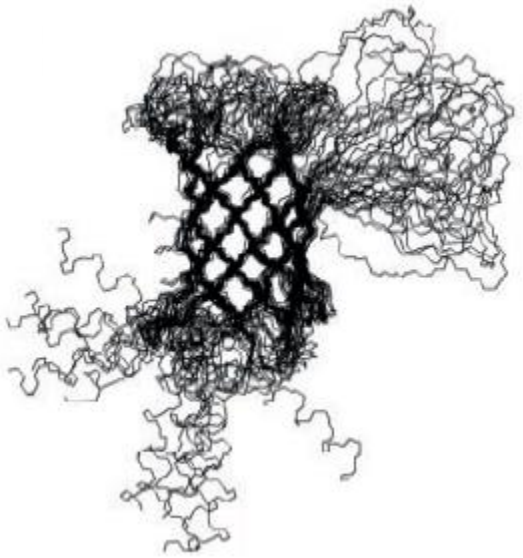


NMR Analysis of Protein Dynamics

In general, regions of secondary structure show low mobility while turns, loops and N-,C-terminus exhibit high mobility







PNAS **2002** vol. 99 no. 21 13560-13565