

Spectroscopic Approaches to Protein Folding and Dynamics

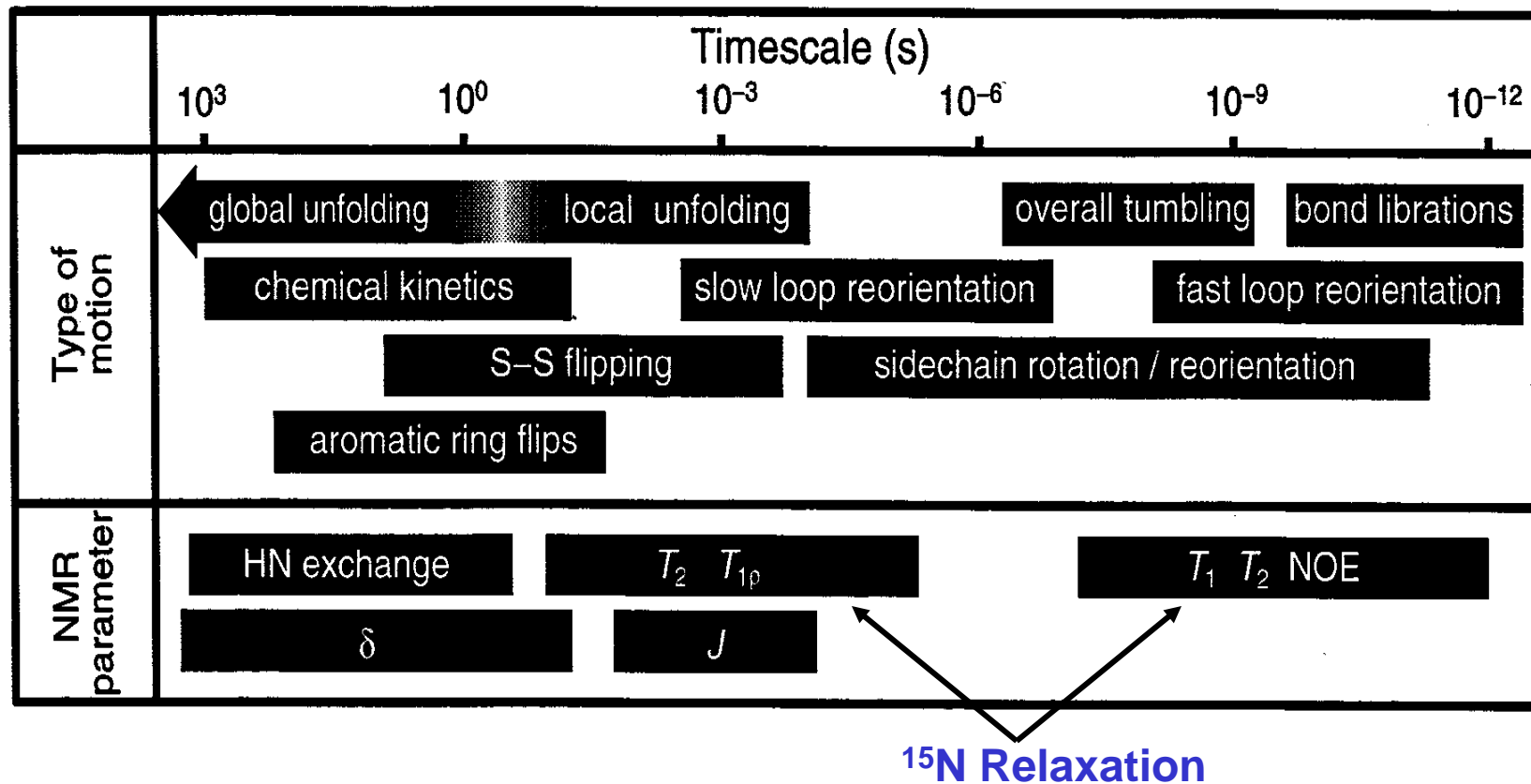
Sepideh Khorasanizadeh
September 29, 2008

Further Reading:

Henzler-Wildman, K. and Kern, D. (2007) Dynamic Personalities of Proteins. *Nature* 450, 964-72.

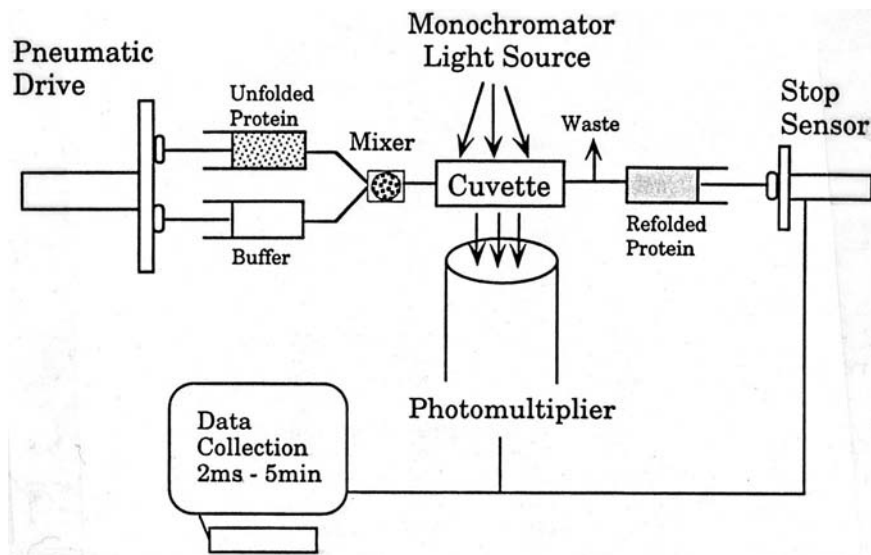
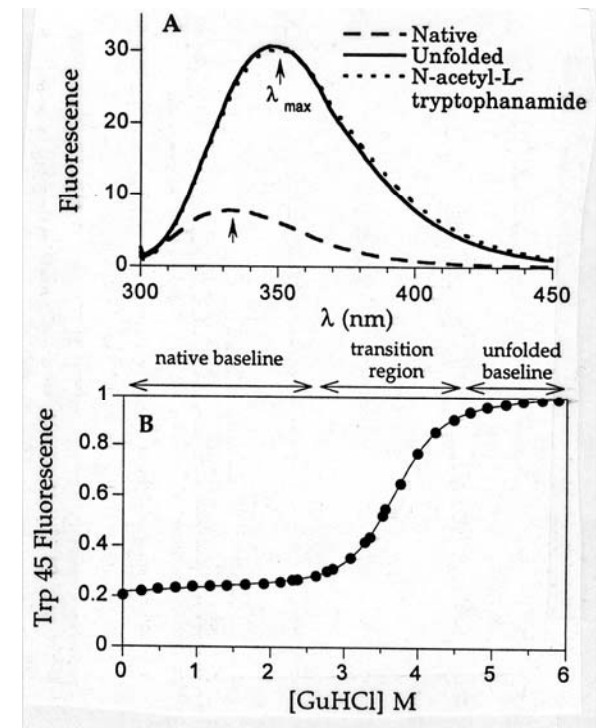
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18075575

Characterizing Protein Dynamics: Parameters/Timescales



Trp fluorescence signal to measure folding or unfolding at equilibrium or kinetically (in a stopped-flow apparatus).

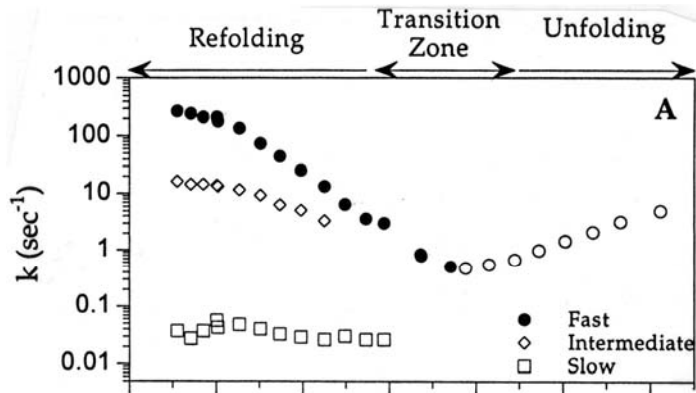
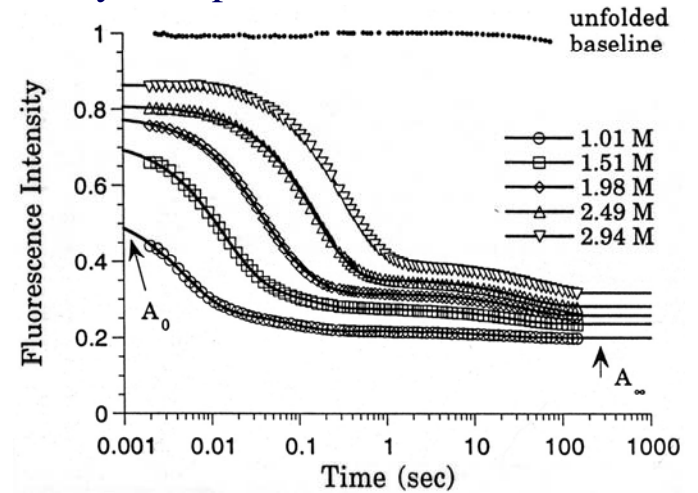
Relative fluorescence of native and unfolded protein are shown in comparison with free tryptophan. Equilibrium unfolding transition monitored by detection of fluorescence at 353 nm. Solid line is a fit based on a two-state model.



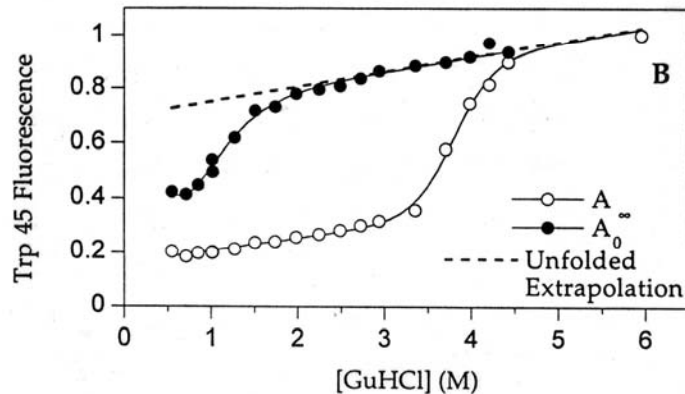
Multiphasic (fluorescence data in this case) kinetics can generally be represented as a sum of exponential contributions according to the equation:

$$A(t) = A_{\text{final}} - \sum A_i \exp(-t/\tau)$$

Where $A(t)$ is the change in signal detected during folding from 2 ms to ~ 100s.



V plot



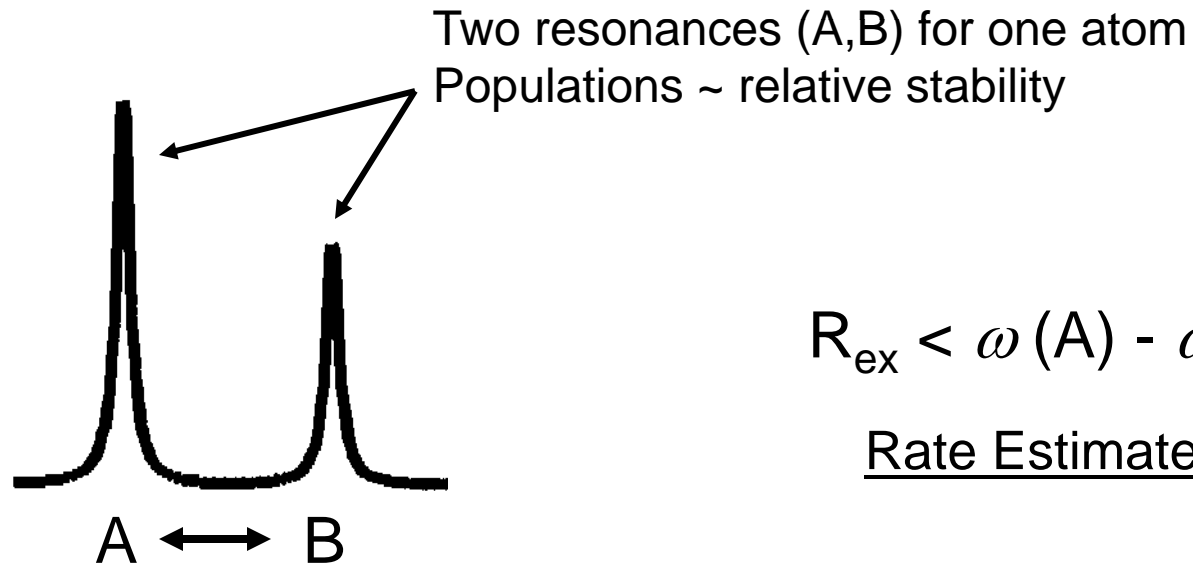
Folding equilibrium and kinetics can also be followed by CD Spectroscopy by measuring the secondary Structure signal.

NMR Parameters That Report On Dynamics of Molecules

- **Number of signals per atom:** multiple signals for slow exchange between conformational states
- **Linewidths:** narrow = faster motion, wide = slower; **dependent on MW** and conformational states
- **Exchange of NH with solvent:** requires local and/or global unfolding events → slow timescales
- **Heteronuclear relaxation measurements**
 - R_1 ($1/T_1$) spin-lattice- reports on fast motions
 - R_2 ($1/T_2$) spin-spin- reports on fast & slow
 - Heteronuclear NOE- reports on fast & some slow

Dynamics From NMR chemical shift

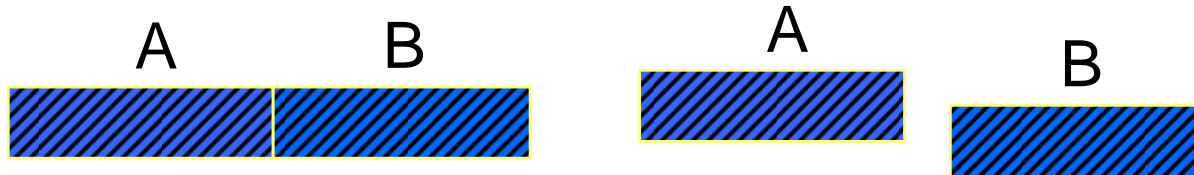
- **Number of signals per atom: multiple signals for slow exchange between conformational states**



$$R_{\text{ex}} < \omega(A) - \omega(B)$$

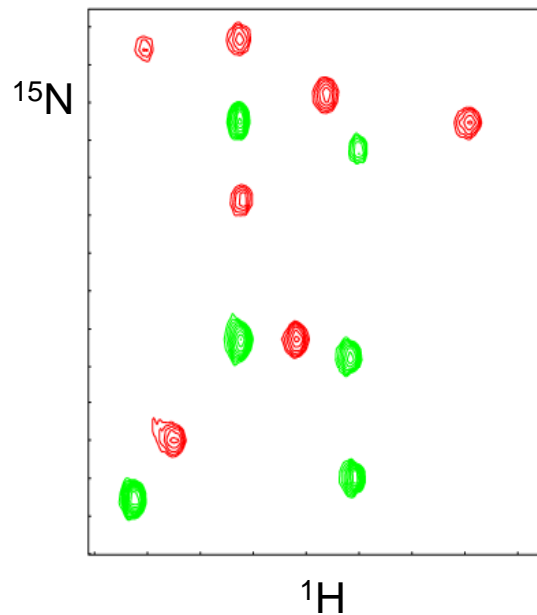
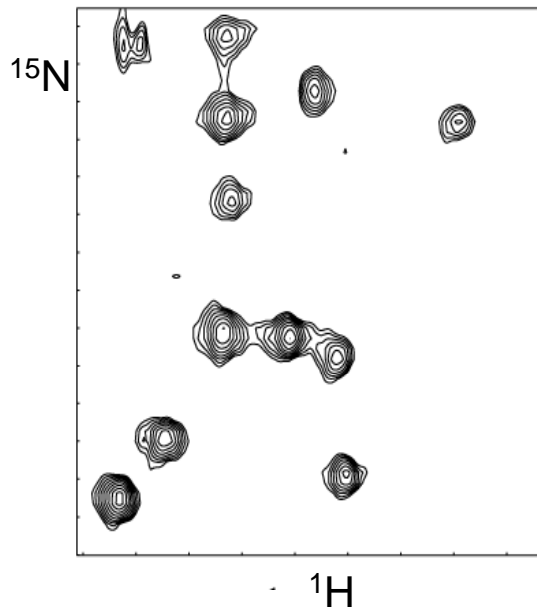
Rate Estimates

Linewidth is Dependent on MW



RPA70-AB

RPA70-A+B

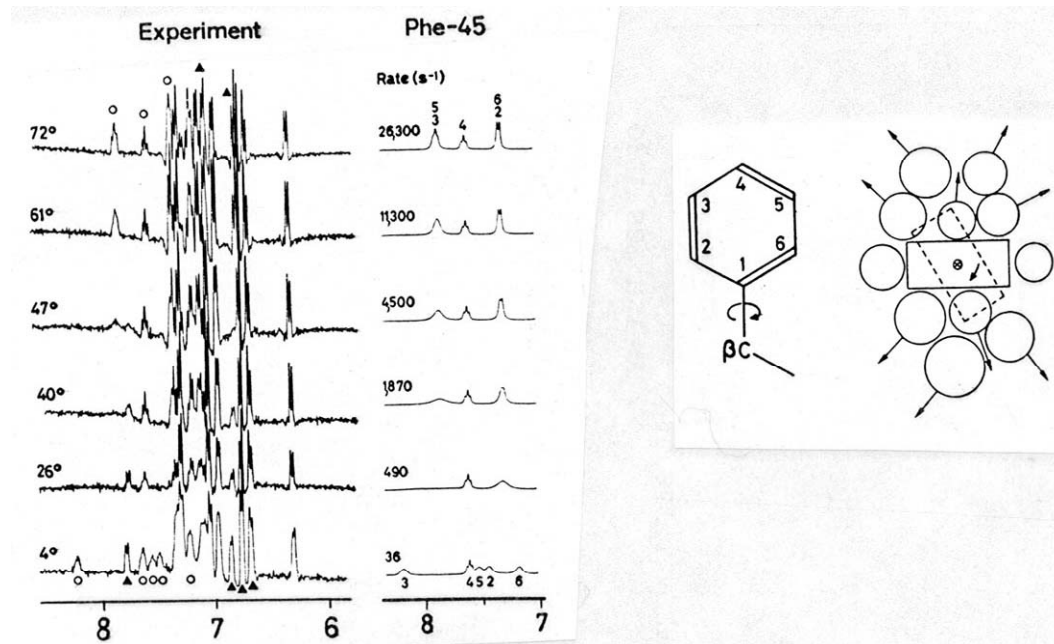


- Linewidth determined by size of particle
- Fragments have narrower linewidths

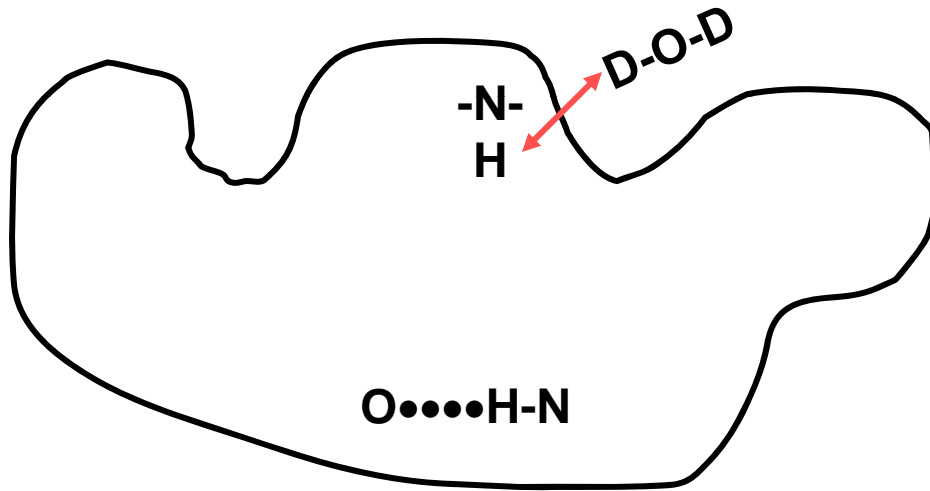
An example of the use of 1D proton NMR

Dynamics of aromatic ring flips in proteins are direct evidence for structural fluctuations in the interior of globular proteins

Temperature dependence of the aromatic ^1H NMR resonances of a Phenylalanine in BPTI manifests 180° ring flips with the flipping frequencies of slow (low temperature), intermediate and fast (high temperature).



Amide Proton Exchange



- Peptides/unfolded proteins exchange rapidly
- Folded proteins protected: solvent accessibility, H-bonds

➤ *H-bonded amides: exchange occurs via local or global unfolding events*

Hydrogen Exchange Experiment

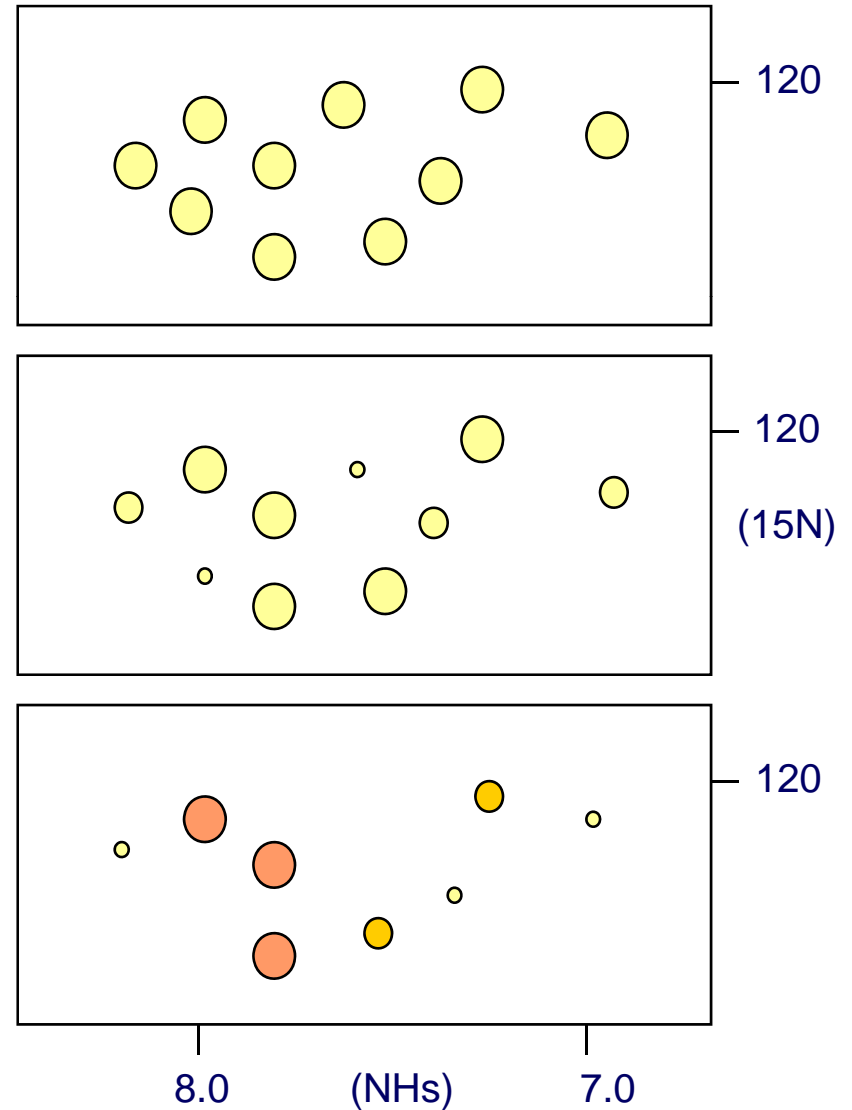
We add D_2O to our H_2O solution and take spectra at different times, we'll see that signals from different amide protons will decrease in size at different rates on the 1H ^{15}N HSQC

$t = 0$ - No D_2O

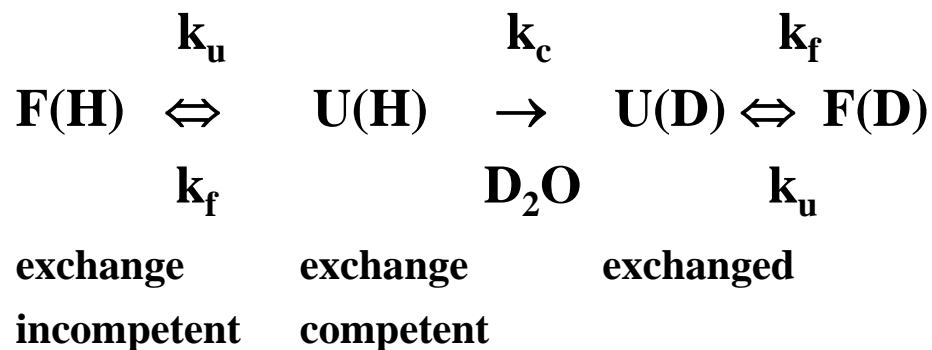
Add D_2O

$t = t_1$

$t = t_2$



Mechanism of Hydrogen Exchange



Exchange-competence is associated with an opening transition with the equilibrium constant K_{op} ($K_{op} = k_u/k_f$).

After the transient formation of the U state the amide site becomes accessible to the solvent and hydrogen-deuterium exchange occurs at an intrinsic chemical exchange rate k_c . Exchange with solvent hydrogen is practically irreversible, because hydrogen concentration in the solvent is far greater than that in the protein molecule.

The intrinsic exchange rate of the exposed amide, k_c , depends on pH, nearest neighbor side chains (due to inductive effects) and temperature. These effects have been calibrated for model peptides, and empirical rules for the prediction of k_c for each amino acid with particular right and left neighboring residues have been published.

Under typical experimental conditions where $k_f \gg k_u$, the observed exchange rate k_{ex} is described by

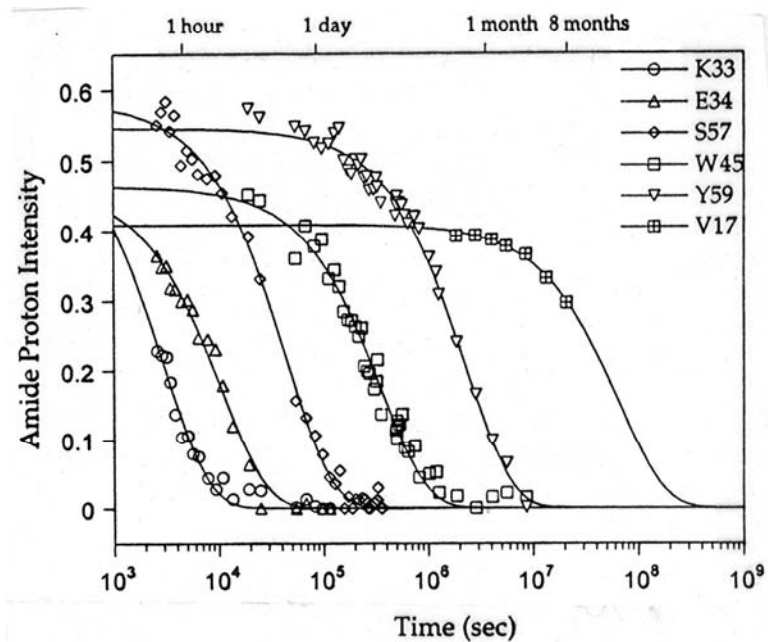
$$k_{ex} = k_u k_c / (k_f + k_c)$$

Under stabilizing conditions, where structural fluctuations are far more rapid than the intrinsic exchange ($k_f \gg k_c$):

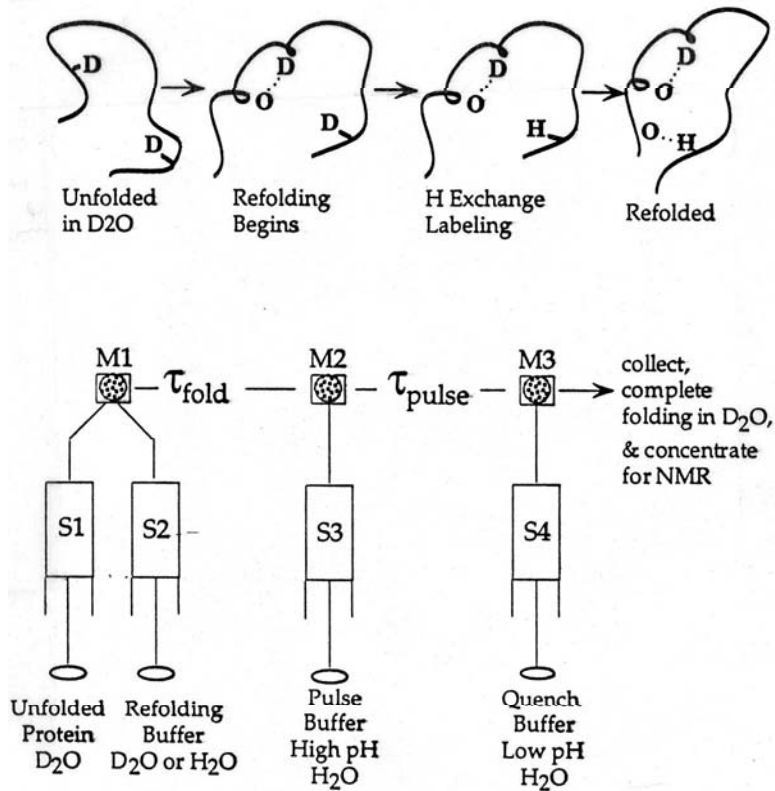
$$k_{ex} = K_{op} k_c$$

By determining K_{op} from hydrogen exchange measurements, information about the free energy can be obtained:

$$\Delta G_{op} = -RT \ln K_{op} = -RT \ln (k_{ex}/k_c)$$



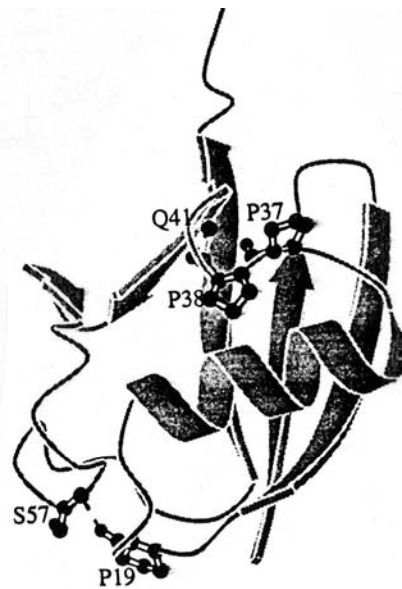
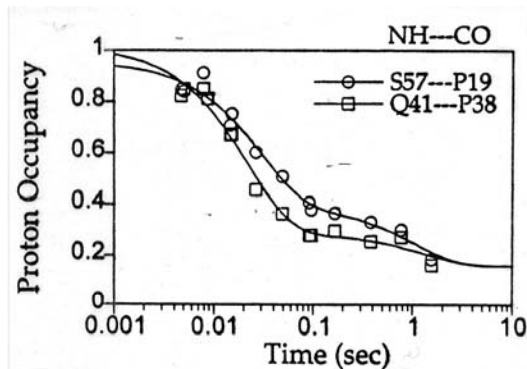
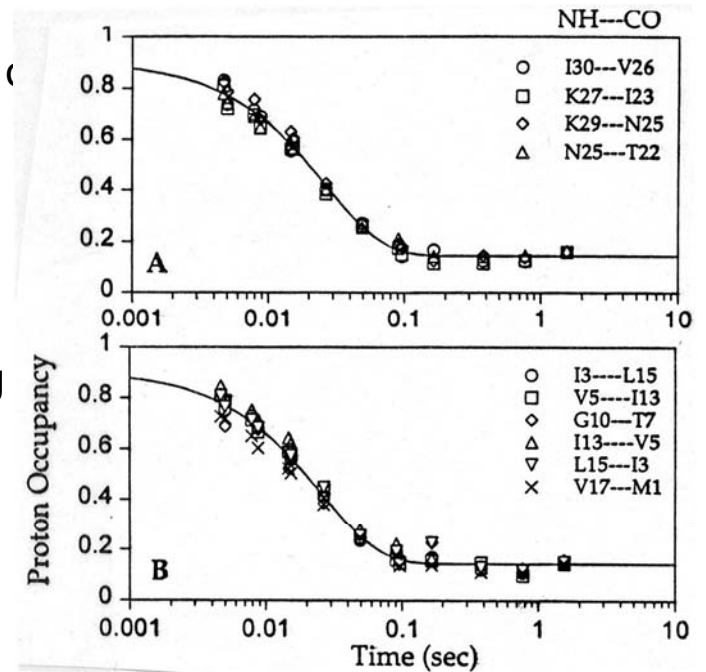
A powerful approach that combines amide proton exchange with rapid mixing apparatus is the **pulse labeling** strategy. This allows the study of the time course of hydrogen bond formation for individual amide groups during folding (millisecond to minutes).



The rate of protection from exchange may be single or multi-exponential depending on whether the folding kinetics occurs in two-state or it occurs through intermediate states.

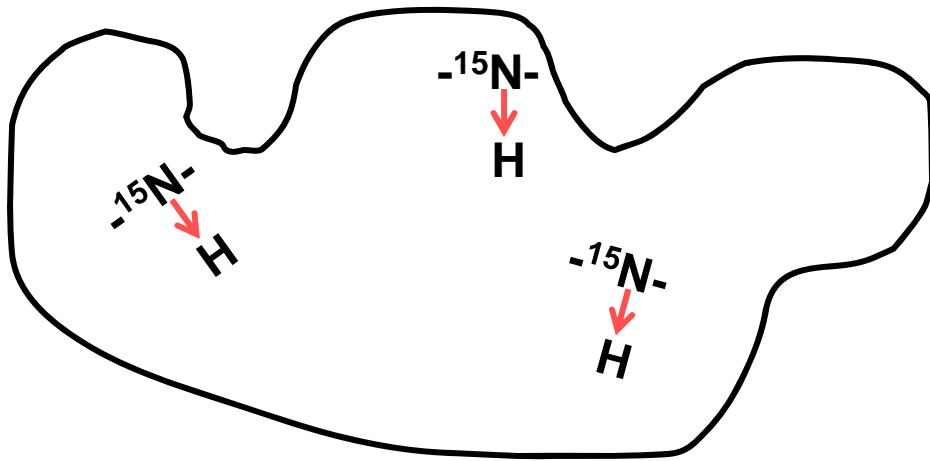
In Ubiquitin, protection from exchange during folding shows a two-state behavior (single exponential) for the majority of the amide protons.

However, a proline isomerization-dependent slower rate of protection is detected for the sites that make specific hydrogen bond to the proline residues.



Heteronuclear Relaxation

(psec \rightarrow nsec & μ sec \rightarrow msec)



- ^{15}N relaxation dominated by ^1H
- N-H distance fixed, variation in relaxation due to differences in motional properties
- Overall tumbling, internal motions

Measurements of T1, T2 and $\{^1\text{H}\}$ - ^{15}N NOE in proteins yield

Detailed information about the motion of the polypeptide in the time range of ps- μ s:

Ratio of T1 and T2 rates allows calculation of correlation time of the protein (τ_c),

τ_c is the time constant for the overall rotational diffusion of the entire protein.

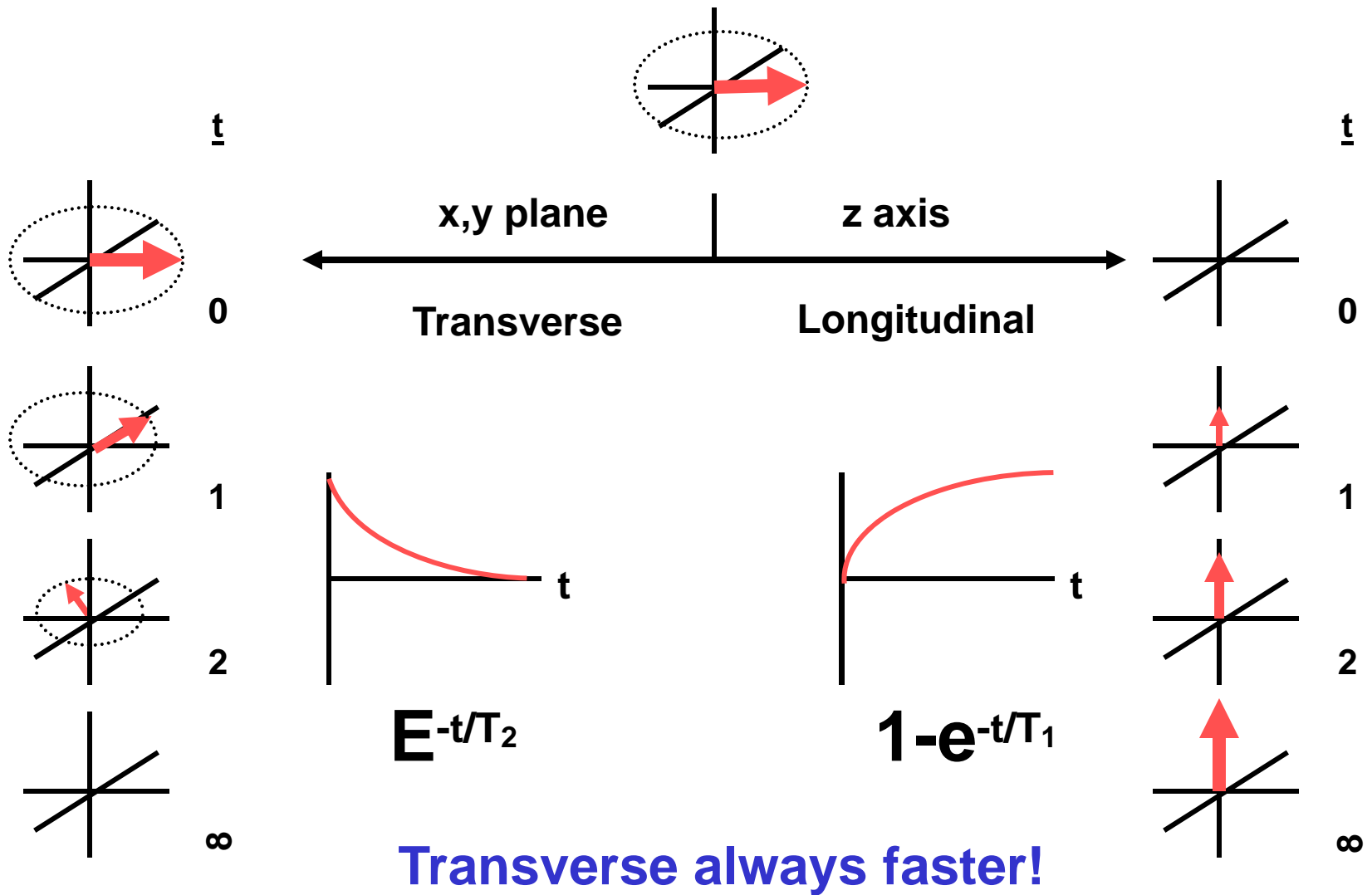
For proteins of 5,000 to 20,000 Da, it is typically in the range of 3 to 12 ns.

Segments of fast internal motions (faster than the overall tumbling of the protein) can be identified. This kind of motion is typically found in flexible polypeptide tails, linkers and surface loops.

Heteronuclear NOE ($\{^1\text{H}\}$ - ^{15}N NOE) can be measured to determine the amplitude of the flexibility of each amide (i.e., the angle space sampled by the N-H vector due to the internal motion). It is an indication of how much the N-H vector bends and wiggles relative to the overall motion of the protein.

Fit these relaxation parameters to the Lipari-Szabo model to calculate “order parameter” (S^2)
For every amide.

Relaxation: Return to Equilibrium



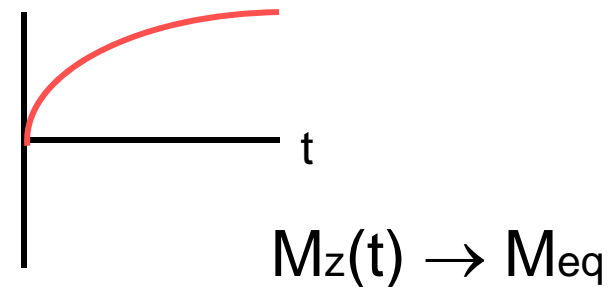
Longitudinal (T_1) Relaxation

MECHANISM

- Molecular motions cause the nuclear magnets to fluctuate relative to a fixed point in space
- Fluctuating magnetic fields promote spins to flip between states
- Over time, spin flips cause a return to equilibrium

$$dM_z/dt = M_{eq} - M_z/T_1$$

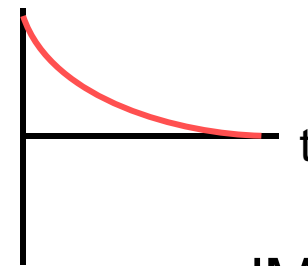
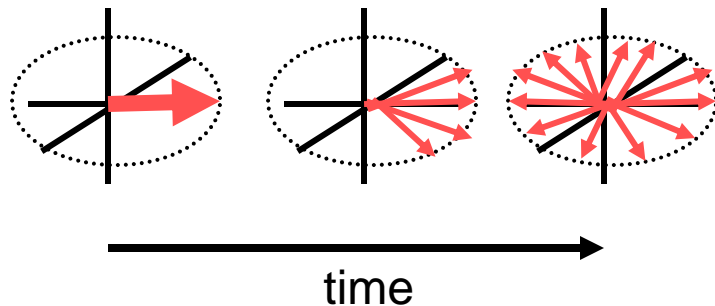
$$M_z(t) = M_{eq} (1 - e^{-t/T_1})$$



Transverse (T₂) Relaxation

MECHANISM

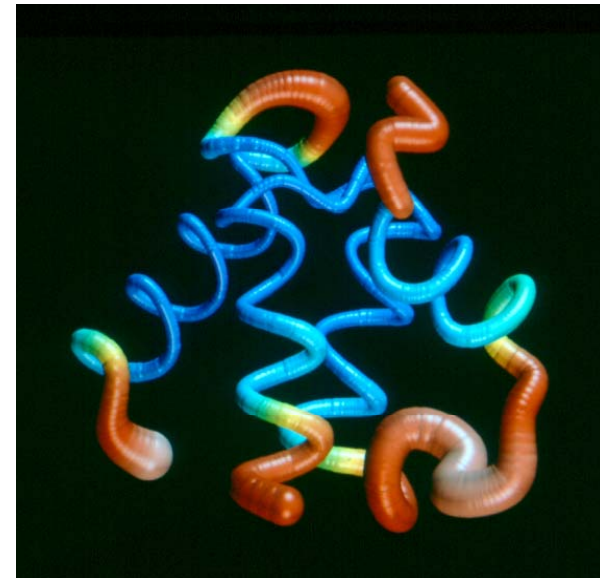
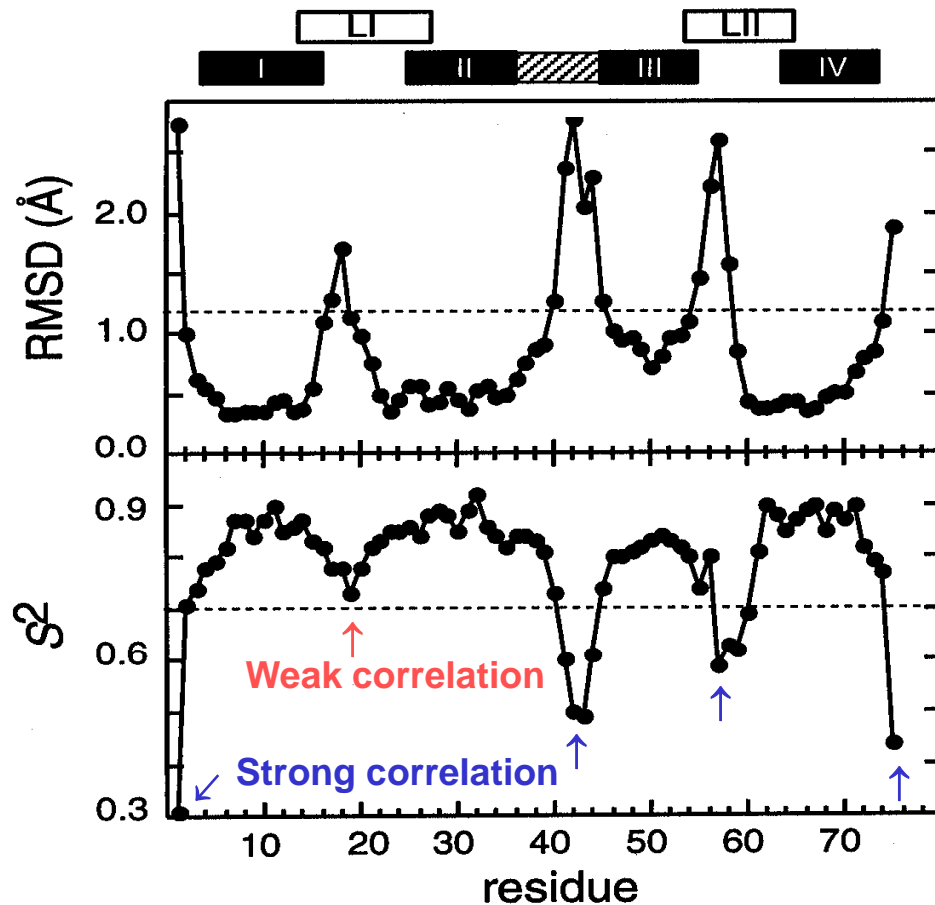
- Magnetic field is not homogenous to an infinite degree
- Each spin comprising the bulk magnetization will feel a slightly different field
- Over time, the spin fan out (lose coherence)



$$dM_{x,y}/dt = M_{x,y}/T_2$$

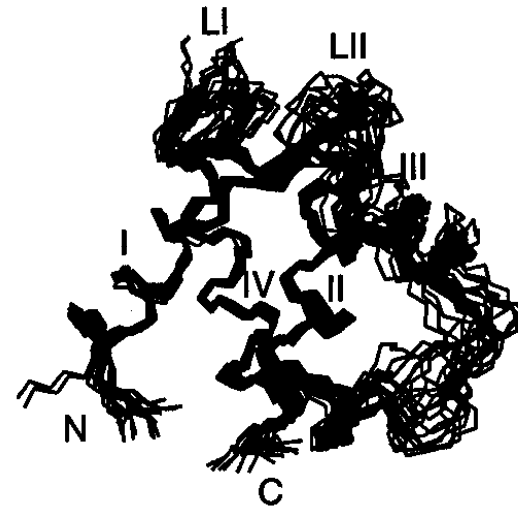
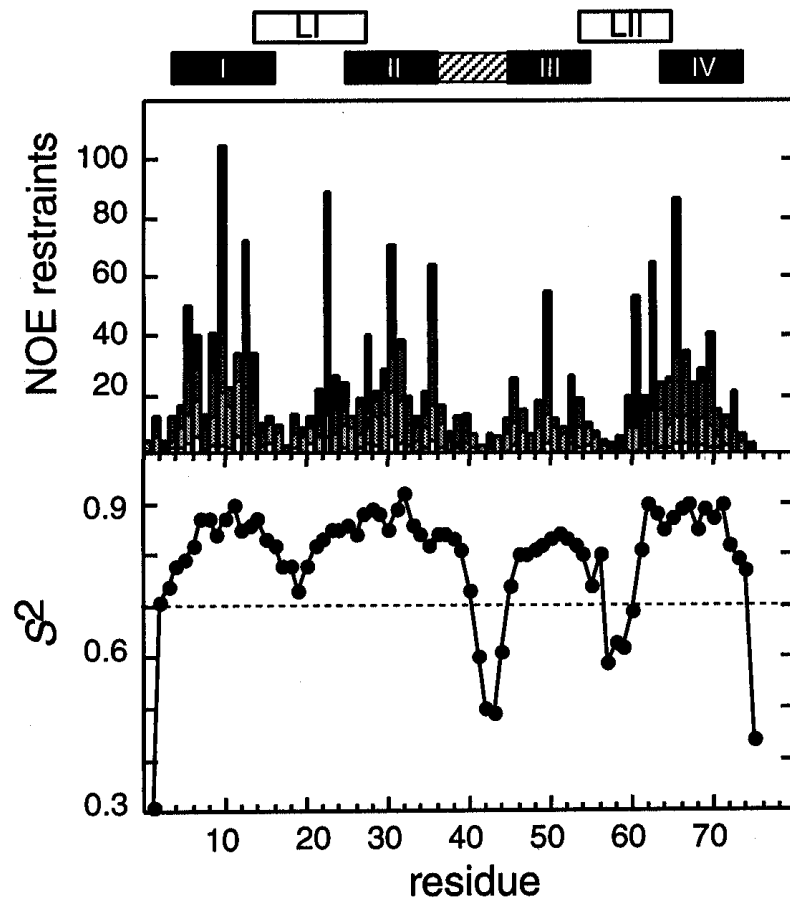
→ Linewidth

Dynamics To Probe The Origin Of Structural Uncertainty



➤ Measurements show if high RMSD is due to high flexibility (low S^2)

Flexibility In Structure Correlates With Lack Of NOE distances/restraints



➤ Regions with higher flexibility (low order, S^2) exhibit few NOEs