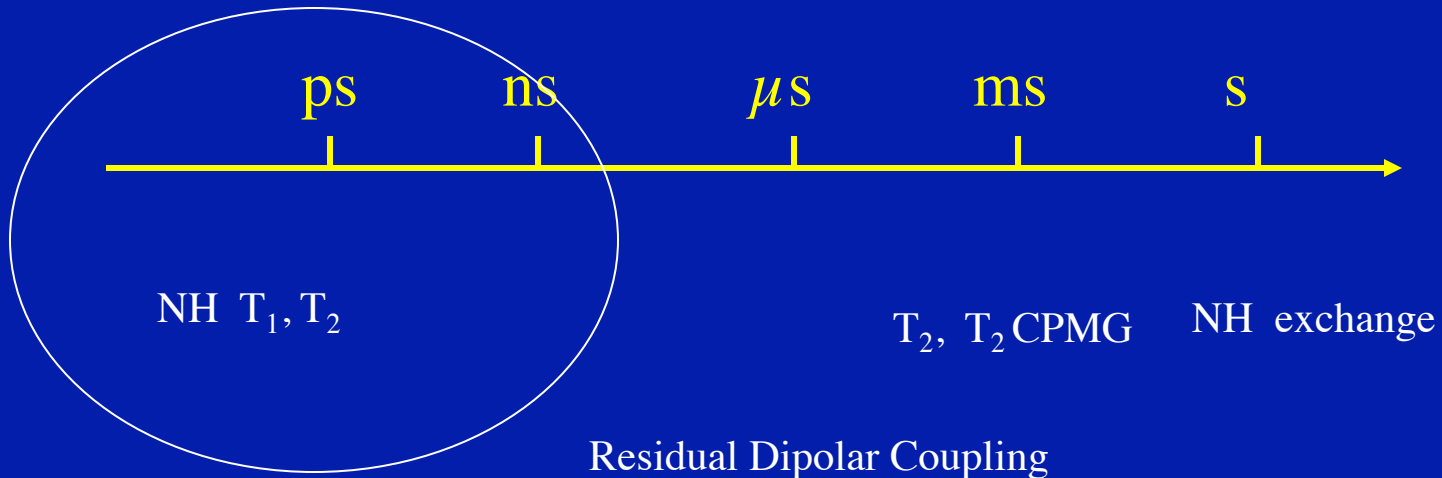
NMR relaxation detects pervasive motions in proteins on many timescales

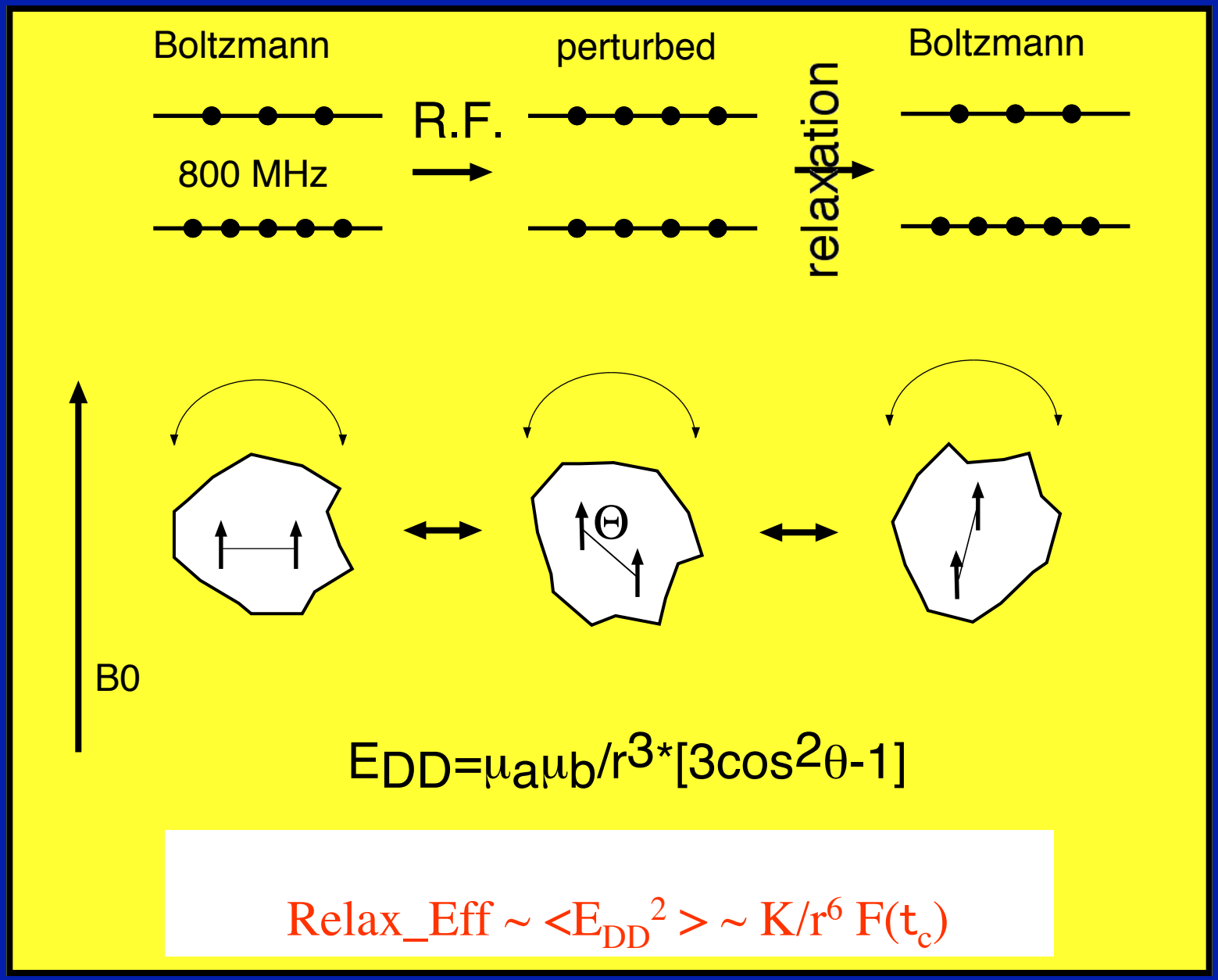
Dynamics research in the Zuiderweg Lab
1993-2010

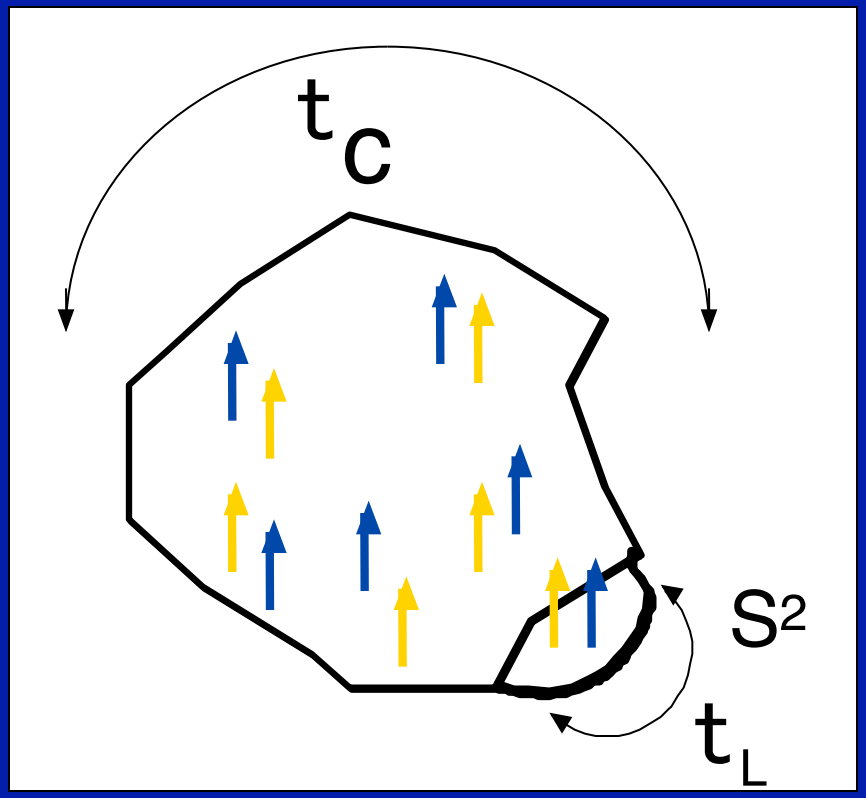
Common NMR relaxation

Hinge motion Allosteric motion

Residue motion Domain motion Protein Folding



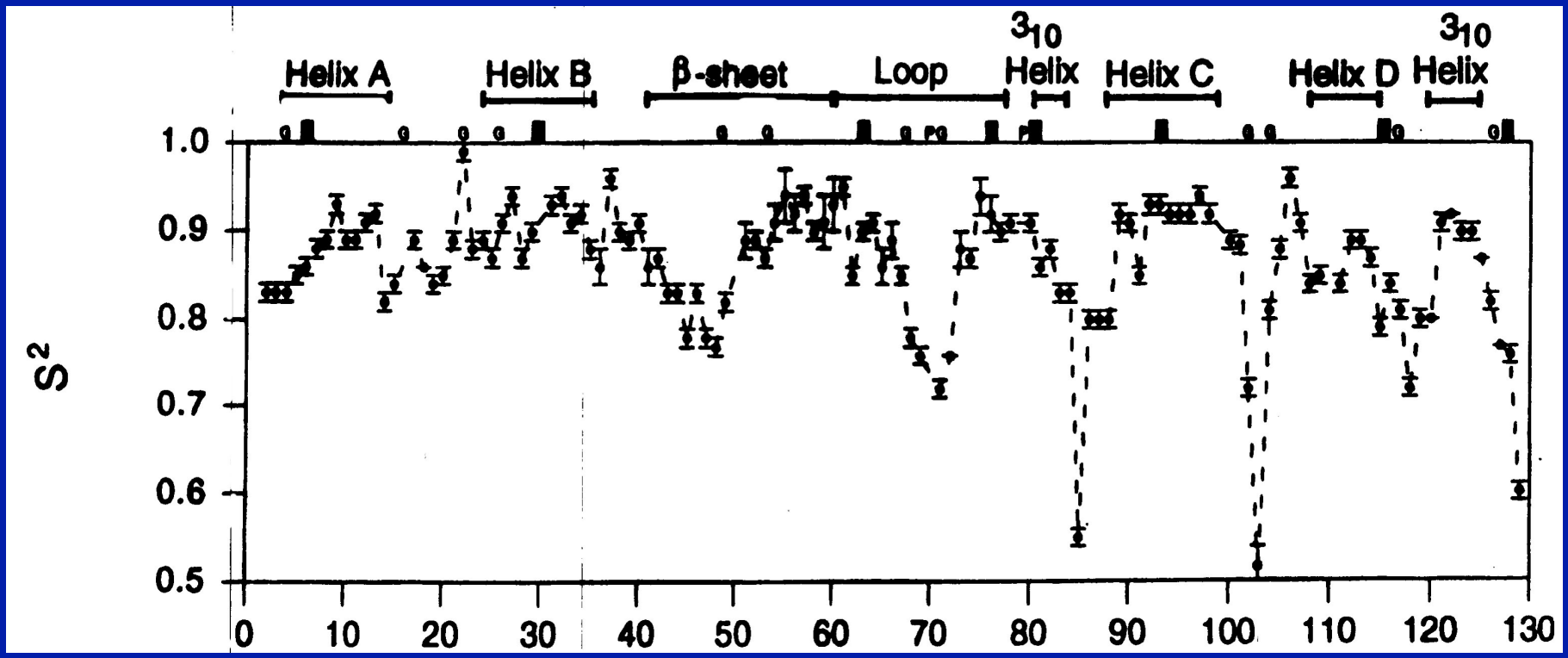

Boltzmann
perturbed
Boltzmann
R.F.
800 MHz
relaxation
B0
Θ
$E_{DD}=\mu_a\mu_b/r^3*[3\cos^2\theta-1]$
$Relax\_Eff \sim \langle E_{DD}^2 \rangle \sim K/r^6\ F(t_c)$



$$R \propto K \frac{1}{r_{ab}^{6}} F\left(\tau_{MOL}, \omega_{NMR}\right)$$

$$R \propto K \frac{1}{r_{ab}^{6}} \left[\frac{S^2 \tau_C}{1+\omega^2 \tau_C^2} + \frac{\left(1-S^2\right)\tau_L}{1+\omega^2 \tau_L^2} \right]$$

15N-1H vector (amide) Order parameters of Lysozyme



NMR Dynamics and Entropy

Model motion as a probabililty distribution P(θ)

and compute averaged spherical harmonics

$$\left\langle P_2\left(\cos\theta_{0-\infty}\right)\right\rangle = \frac{1}{4\pi}\int_0^{2\pi} P_2(\cos\theta)P(\theta)\sin\theta \; d\theta$$

Straigthforward substitution yields order

parameter

$$S^2_{NMR} \propto \left\langle \frac{3}{2}\cos^2\theta_{0-\infty} - \frac{1}{2}\right\rangle^2 \equiv \left\langle P_2\cos\theta_{0-\infty}\right\rangle^2$$

and relaxation rate

$$R \propto K\frac{1}{r_{ab}^6}\left[\frac{S^2\tau_C}{1+\omega^2\tau_C^2} + \frac{\left(1-S^2\right)\tau_L}{1+\omega^2\tau_L^2}\right]$$

Compare with experiment and adapt P(θ)

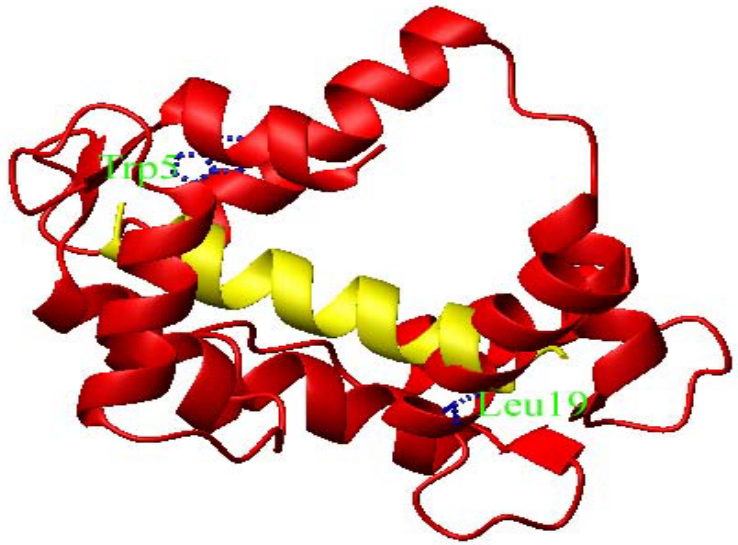
Take best P(θ) and compute entropy from basic

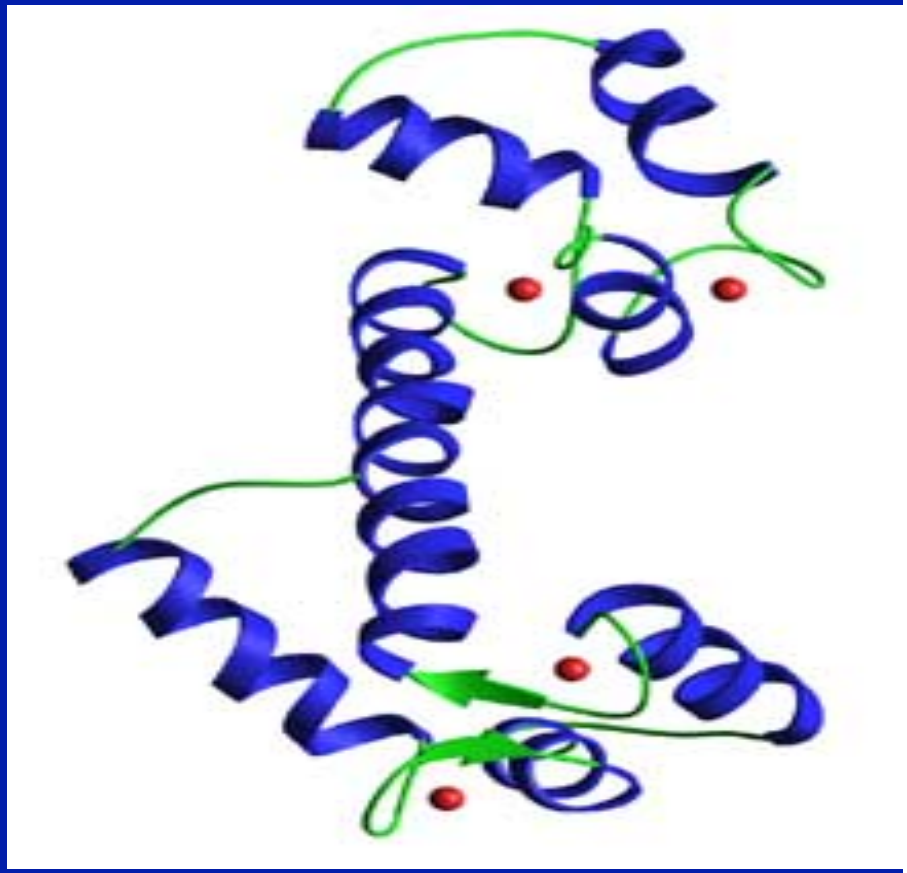
statistical mechanics

$$S_{CONFIG} = \int_0^{2\pi} P(\theta)\ln\left(P(\theta)\right)\sin\theta \; d\theta$$

Is the backbone really the best indicator of motion

Structure of Calmodulin with and without peptide





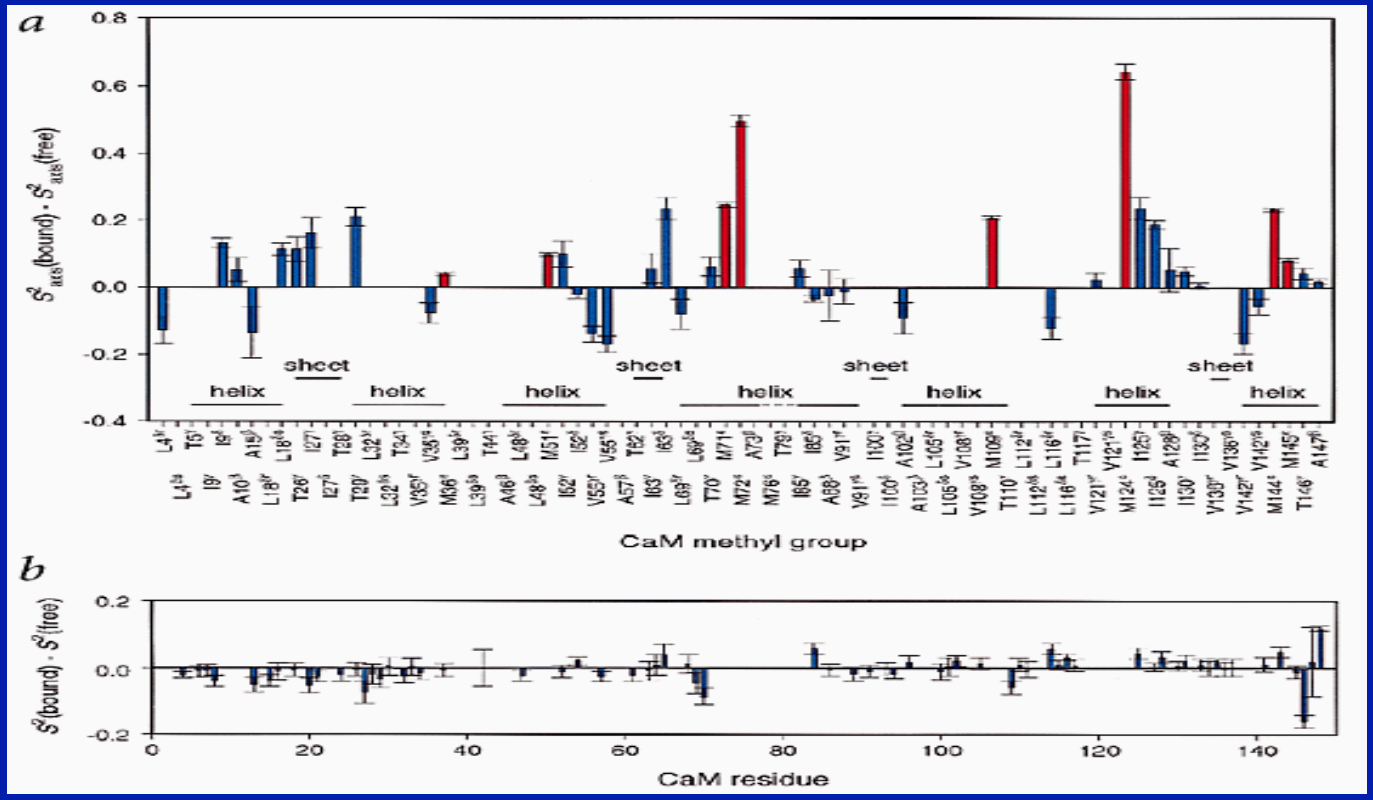
Smooth muscle myosin light chain kinase peptide

Dynamics of Calmodulin with and without peptide

Differences in order parameter (bound-free)

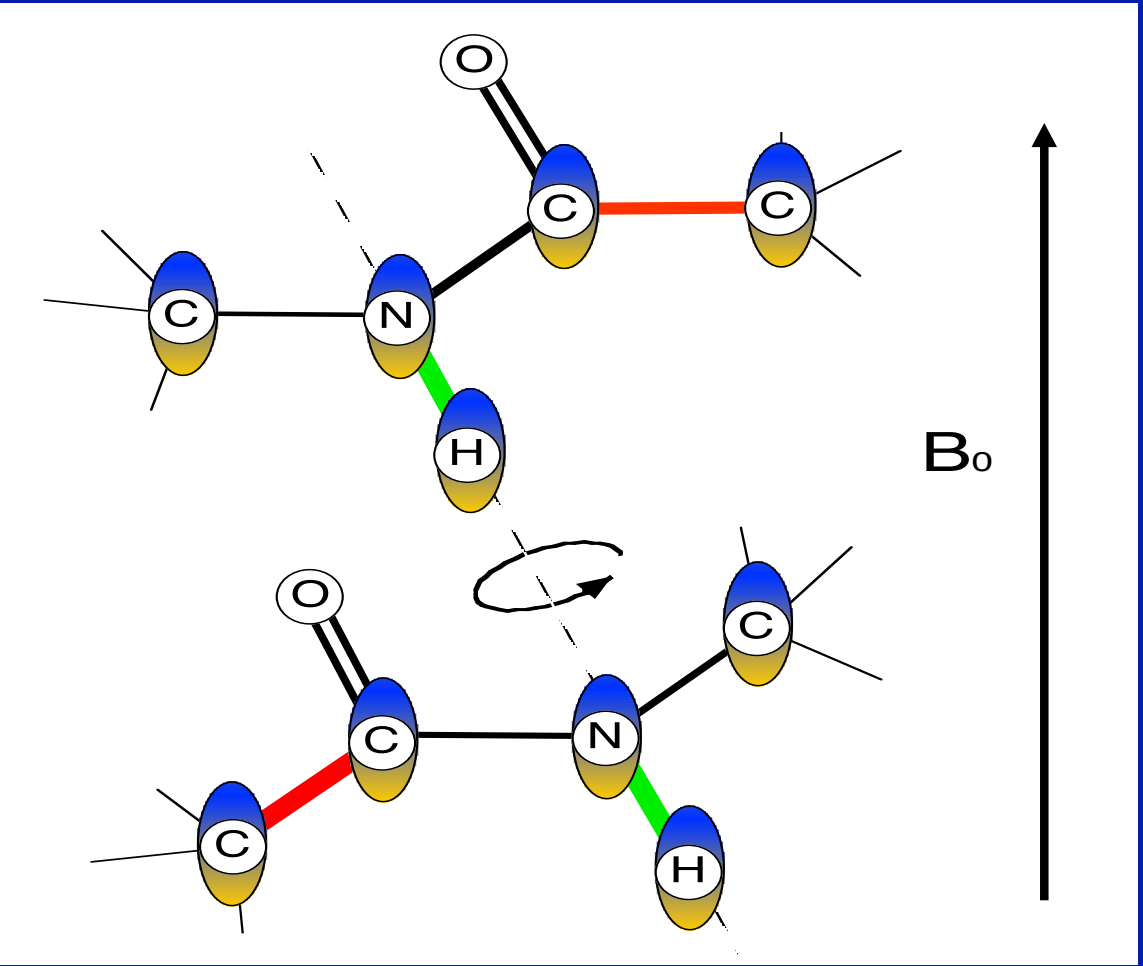
CH_3

NH



Lee, Kinnear, Wand, Nat. Struct. Biol. 7, 2000:72

The NH may not see motions the C-C can

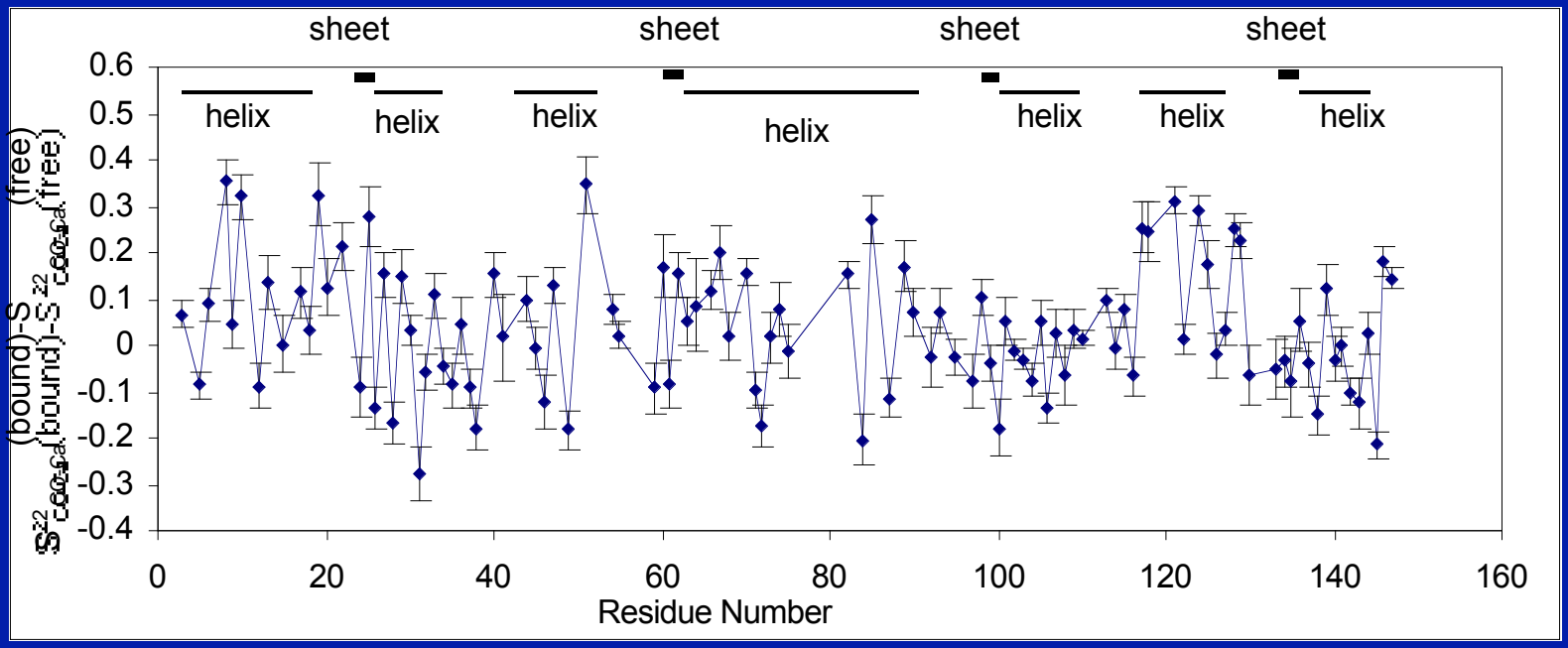


Zeng, L., Fischer, M.W.F. and Zuiderweg, E.R.P. Study of Protein Dynamics in Solution by Measurement of 13Ca-13CO NOE and 13CO longitudinal relaxation. J. Biomol. NMR, 1996; 7, 157-162

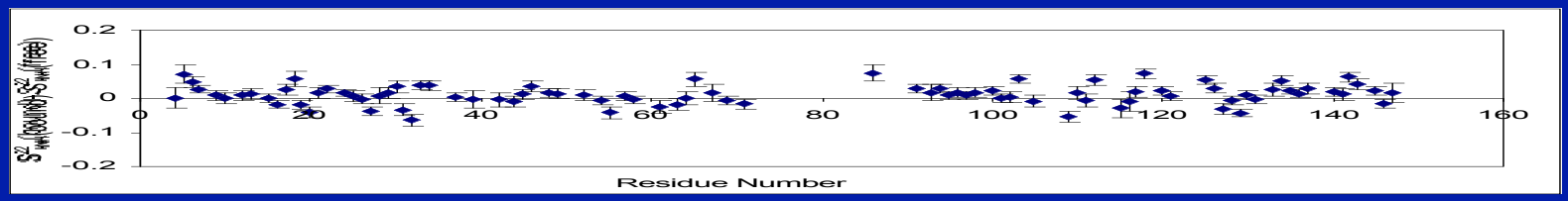
Dynamics of Calmodulin with and without peptide

Differences in order parameter (bound-minus-free)

CO-Ca

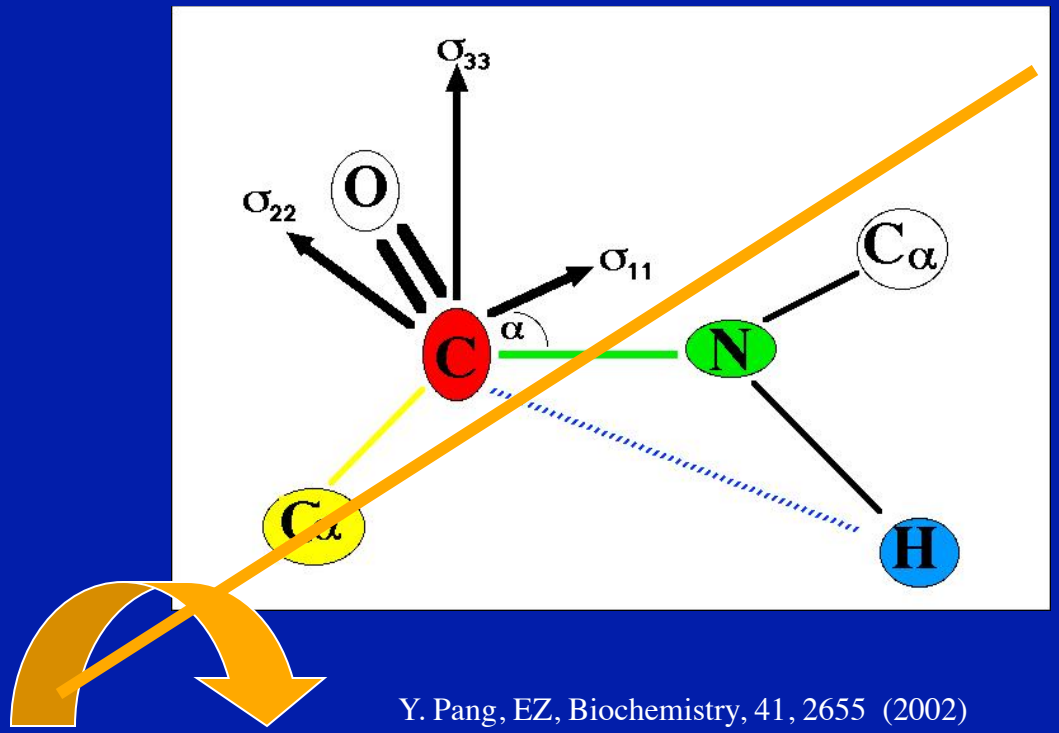


NH



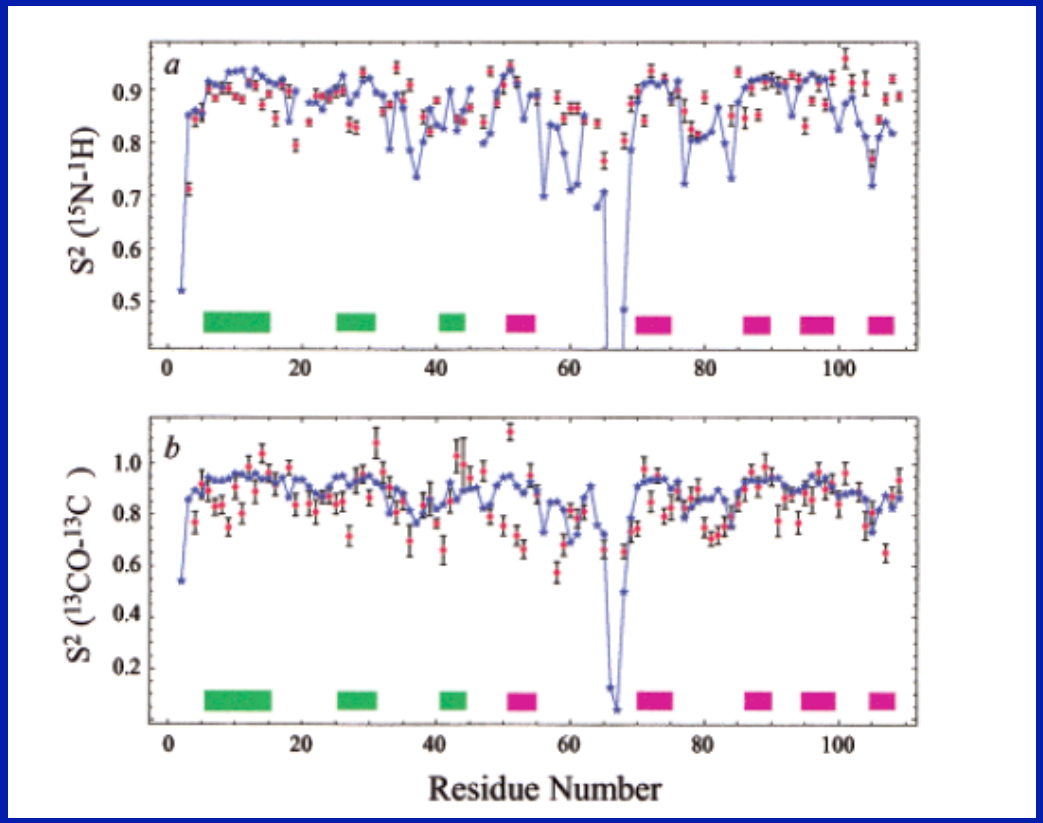
Wang, T, King Frederick, K., Igumenova, T.I., Wand, A.J. and Zuiderweg, E.R.P.
Changes in Calmodulin backbone dynamics upon ligand binding revealed by cross-correlated NMR relaxation measurements, J. Am.Chem.Soc. 127, 828-829 (2005)

MD: peptide-plane Dynamics is dominated by "crank-shaft" motions around the CA-CA direction. This would imply that CO-CA order parameter should be larger than N-H order parameters



Y. Pang, EZ, Biochemistry, 41, 2655 (2002)

However we generally find the average CO-Ca order parameter to be smaller than the NH order parameter



Data points are experimental
Blue line is a 1.6 ns MD simulation using the CHARMM-param22 forcefield, binase in water.

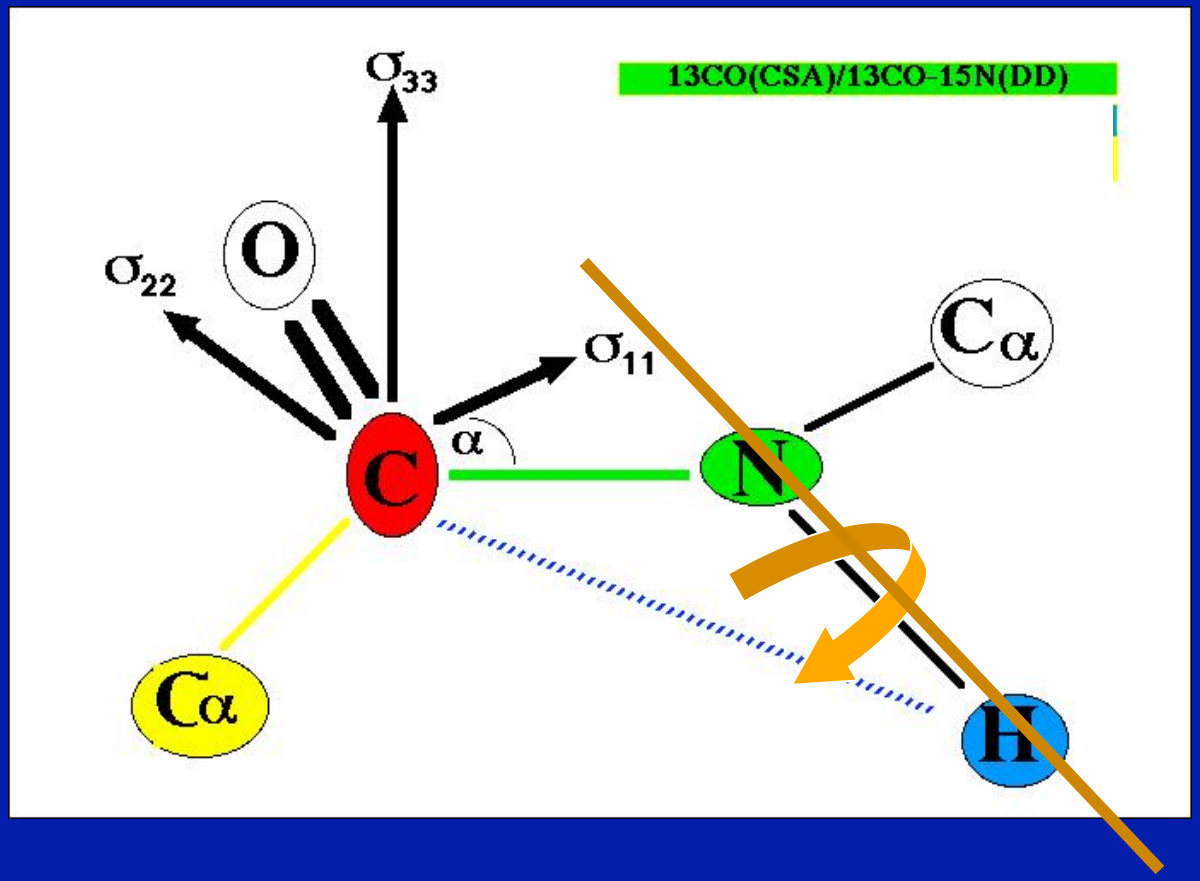
Pang, A, Buck, M., and Zuiderweg, E.R.P. Backbone Dynamics of the Ribonuclease Binase Active Site Area using Multinuclear (^{15}N and ^{13}CO) NMR Relaxation and Computational Molecular Dynamics, Biochemistry, 41, 2655-2666 (2002)

and the COCA order parameter decreases faster than the NH order parameter upon increase in temperature

	Ratio $S^2_{NH}(303) / S^2_{NH}(278)$	Ratio $S^2_{COCa}(303) / S^2_{COCa}(278)$
Binase	0.98	0.90
Ubiquitin	0.97	0.87
Cyt-b5	0.99	0.89

Wang, T., Cai, S. and Zuiderweg, E.R.P. Temperature dependence of anisotropic protein backbone dynamics J. Am. Chem. Soc. 125, 8639-8643 (2003).

Thus, there must be significant motion affecting the CO-CA order more than the NH order parameter – in a rigid peptide plane, only a motion around the NH vector would do this



However, such a correlated motion is unlikely.
We currently favor the idea that there is sufficient flexibility in the peptide plane that re-orientational motions affecting the Ca atom would not necessarily affect the N-H bond vector.

There is QM and IR evidence that such may indeed be the case
(Mannfors, B. E.; Mirkin, N. G.; Palmo¨, K.; Krimm, S. *J. Phys. Chem.* ***2003, A107, 1825-1832.)***

Better QM-based forcefields for MD simulations are needed to settle this issue

In subsequent research, we have taken a more practical approach: how to get proper order parameters for the motions that affect the CO?

Effective Lipari–Szabo order parameters and local correlation times for relaxation vectors of protein 13CO nuclei are extracted from a ^{13}CO-R_1 experiment, a transverse ^{13}CO CSA/^{13}CO-^{13}Ca CSA/dipolar cross correlation and a transverse ^{13}CO CSA/^{13}CO–^{15}N CSA/dipolar cross correlation experiment.
Given the global rotational correlation time from ^{15}N relaxation experiments, the program COMFORD fits the ^{13}CO data to an effective order parameter S^2 CO, an effective local correlation time and the orientation of the CSA tensor with respect to the molecular frame.

Wang, T., Weaver, D.S., Cai, S., Zuiderweg, E.R.P.
Quantifying Lipari-Szabo modelfree parameters from 13CO NMR relaxation experiments. J Biomol NMR. 36, 79-102. (2006)

We are trying to formulate a NMR relaxation paradigm that works for larger proteins.

The standard 15N-based paradigm does not work very well for proteins with tc > 15 ns.
The most problematic is the 1H→ 15N NOE experiment, which when properly performed, may take a month of instrument time.
There also problems with the R_2 experiments – for the larger proteins one uses higher-field instruments which exacerbate R_{ex} and the variations in 15N CSA relaxation.

A ^{15}N paradigm suitable for larger proteins we try to develop uses

^{15}N R_1

R_2 ^{15}N-CSA/ ^{15}N-^{1}H DD cross correlation

R_1 ^{15}N-CSA/ ^{15}N-^{1}H DD cross correlation

Model computations suggest that we can reliably fit for τ_c, S^2, τ_{loc} and the ^{15}N CSA out of of these upto τ_c 30 ns.

What is holding us back?

Lousy funding climate.

Experimental

^{15}N $R_{1:}$ easy to run, easy on the probe, easy to interpret

R_2 ^{15}N-CSA/ ^{15}N-^{1}H DD cross correlation:
easy to run, easy on the probe, easy to interpret

R_1 ^{15}N-CSA/ ^{15}N-^{1}H DD cross correlation:
easy to run, easy on the probe, difficult to interpret

R_1 ^{15}N-CSA/ ^{15}N-^{1}H DD cross correlation:
Is difficult to interpret because 1H-1H NOE effects enter into the cross-relaxation matrix.

Earlier, we have tried to measure the NOEs and back-fit these in the relaxation matrix

Wang, L. Kurochkin, A.V. and Zuiderweg, E.R.P. An iterative fitting procedure for the determination of longitudinal NMR cross-correlation rates. J. Magn. Reson., 144, 175-185 (2000)

More recently, we have been able to separate the effects using symmetric reconversion

Weaver, D.S and Zuiderweg, E.R.P. a TROSY NMR experiment measuring longitudinal relaxation interference. J Chem Phys,128 155103 (14 pg) (2008)

We thus now have available the R_1 ^{15}N-CSA/ ^{15}N-^{1}H DD cross correlation rates, and are able to carry out the ^{15}N relaxation on larger proteins.

What started as a nasty interference, the ^{1}H-^{1}H relaxation rates extracted from the R_1 ^{15}N-CSA/ ^{15}N-^{1}H DD can be interpreted in terms of dynamics themselses:

Weaver DS, Zuiderweg ER. Protein proton-proton dynamics from amide proton spin flip rates. J Biomol NMR. 45, 99-119. (2009)

The general applicability of this still needs to be further validated with MD runs.

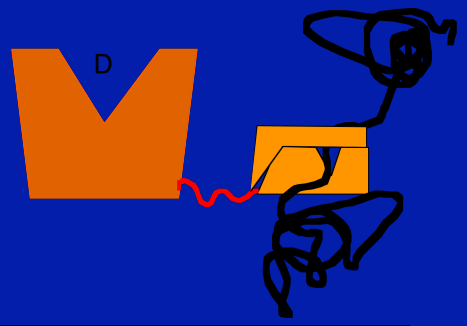
Another approach to measure dynamics in proteins in a semi-quantitavive way, is to just measure te intensity of cross peaks in HSQC, TROSY or HNCO.

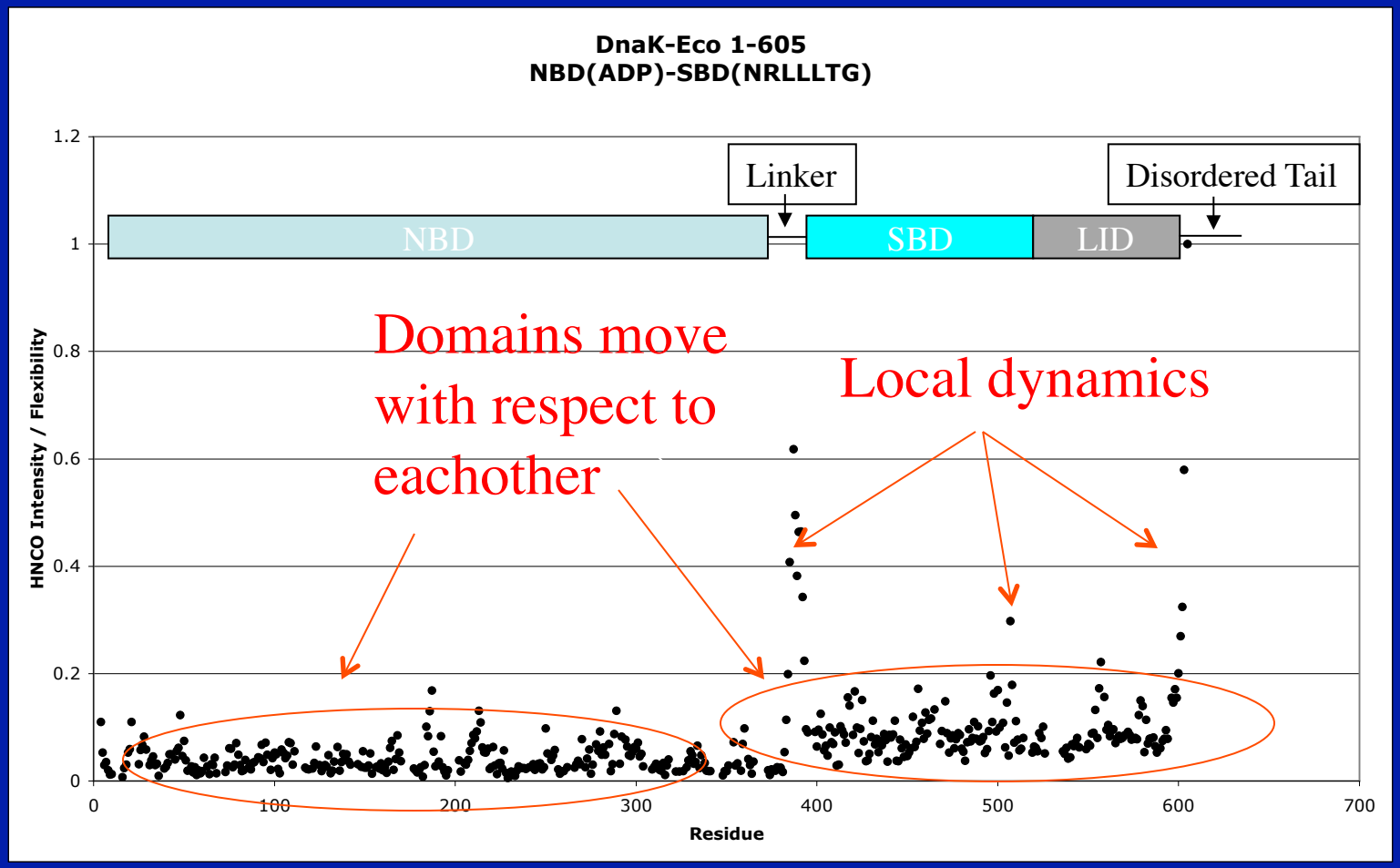
Areas with low order parameters stand out with high peak intensities, areas with R_{ex}, stand out with low peak intensities.

This is almost exlusively due to variations in amide proton R_2 rates. Simulations show that dynamical effects on these rates have a much larger effect than variations in the local environment, provided one choses an appropriate perdeuteration level.

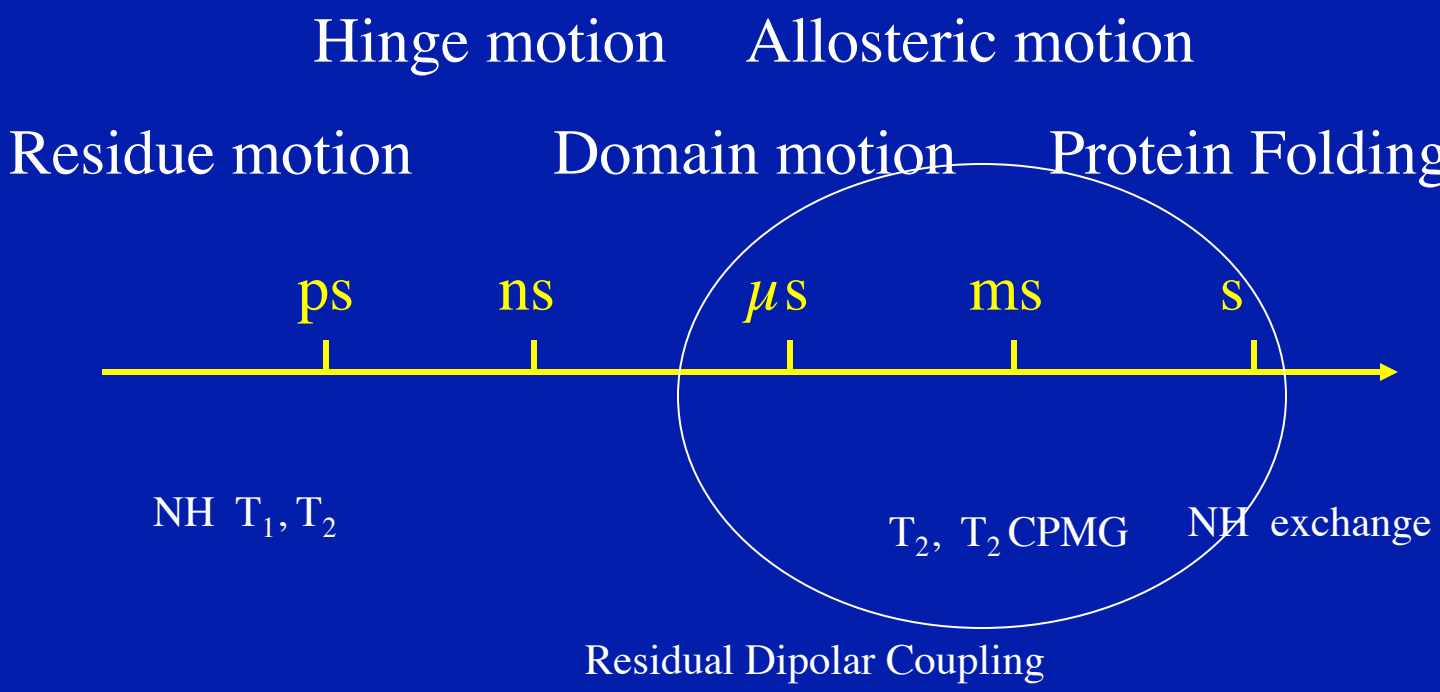
An example is shown for the dynamical properties of a large protein
(to be submitted)

Dynamical Properties of a 70 kDa protein

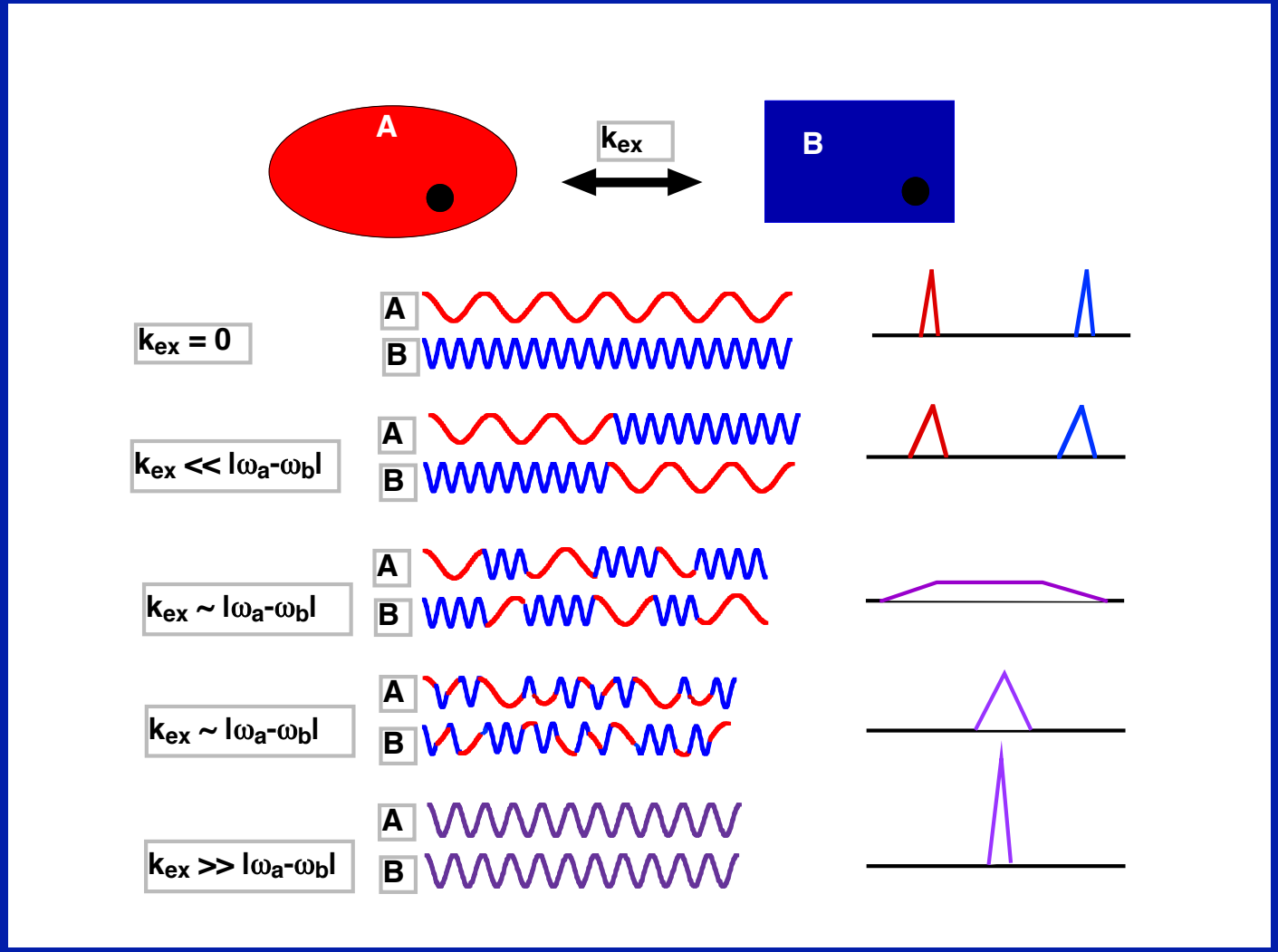




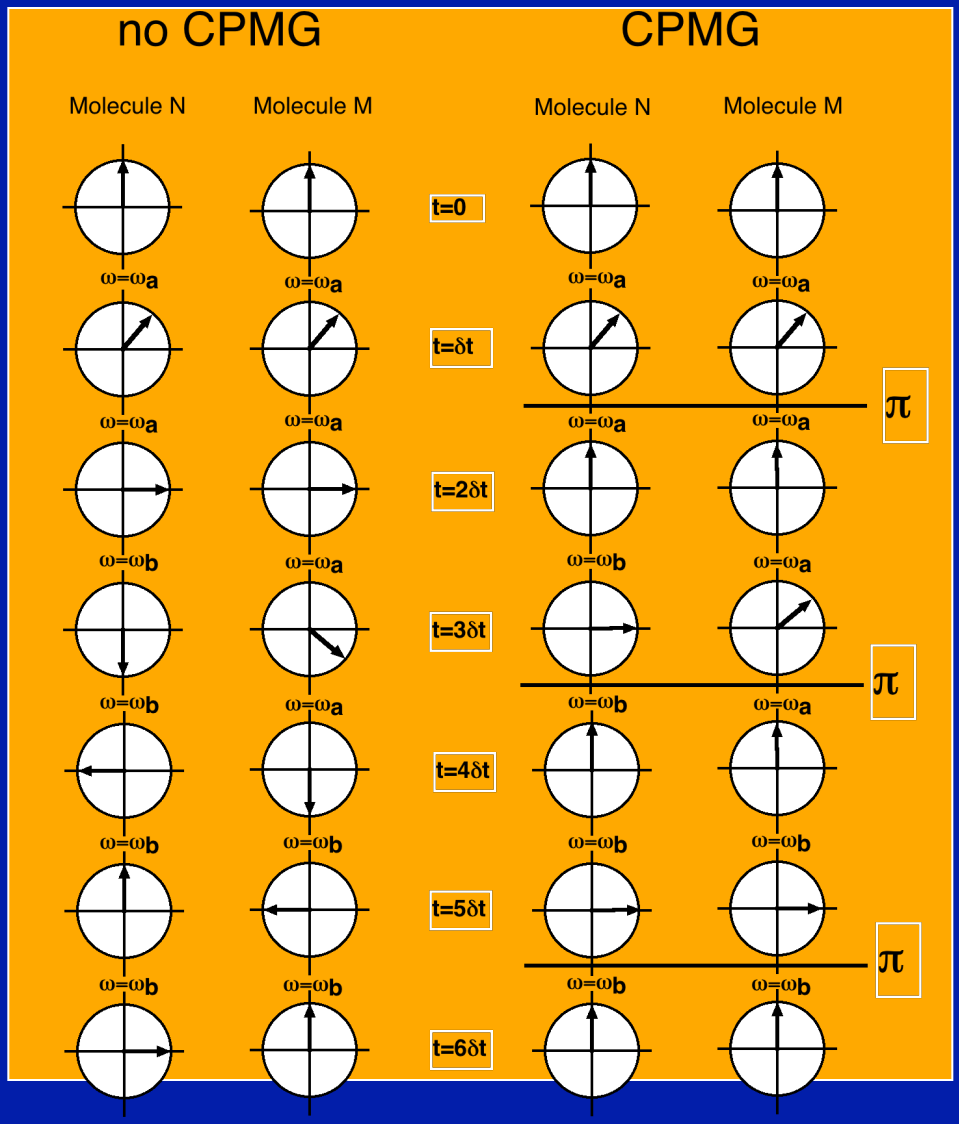
Common NMR relaxation



Exchange Broadening and dynamics



How to detect milli/micro second dynamics

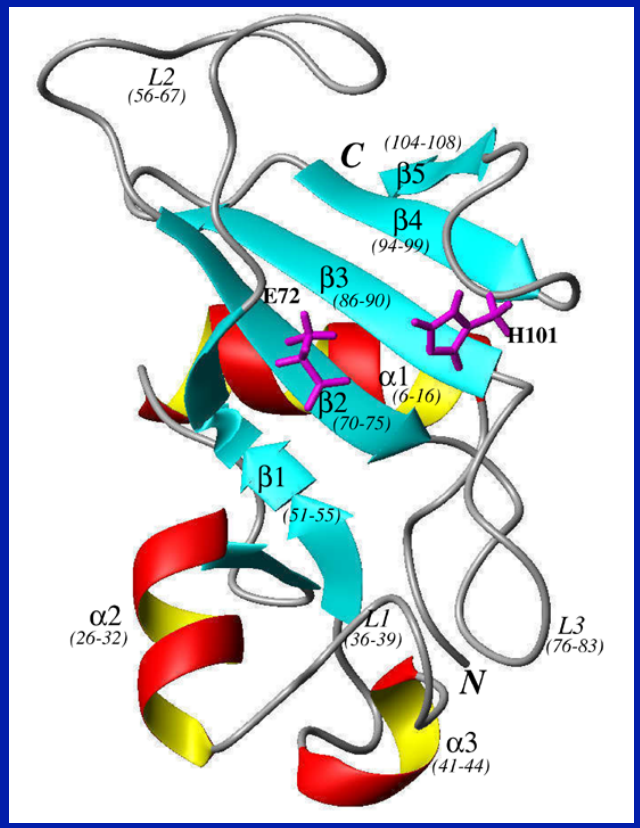


Functional dynamics in the active site of the ribonuclease Binase

Wang, L., Pang, Y., Holder, T., Brender, J.R., Kurochkin, A, Zuiderweg, E.R.P.

Proc. Natl. Acad. Sci. USA, 98, 7684-7689 (2001)

Binase = Barnase

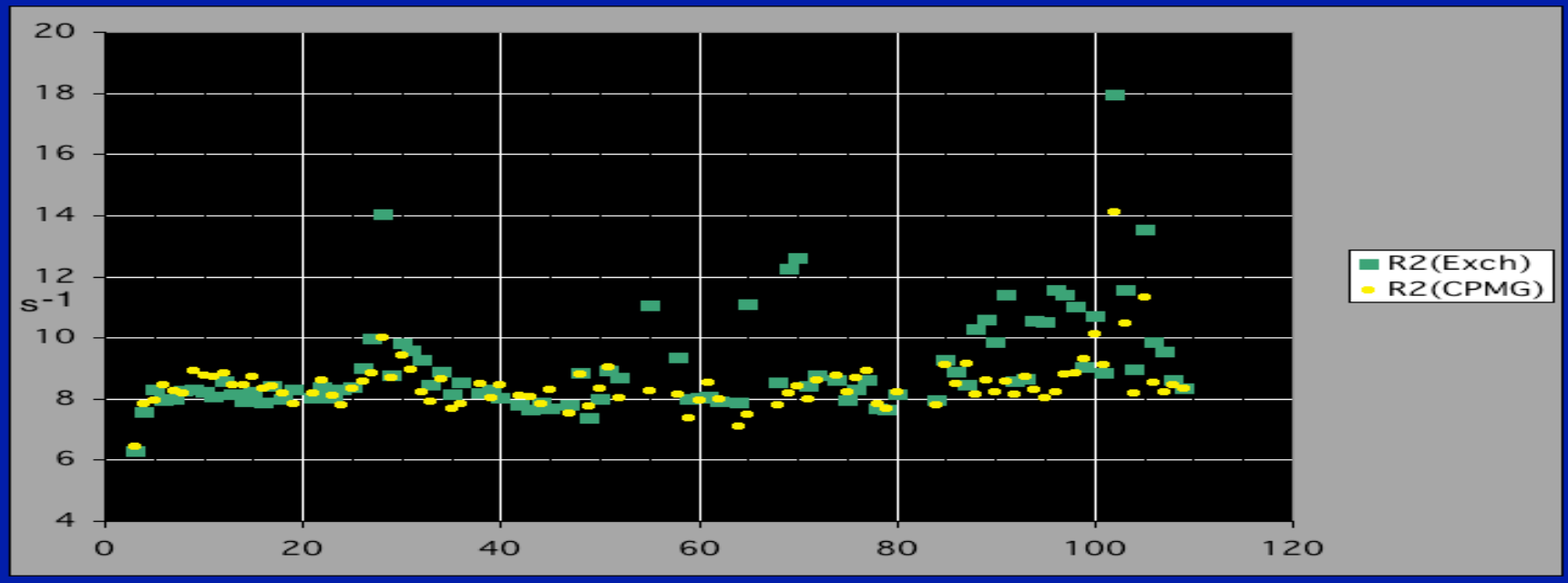


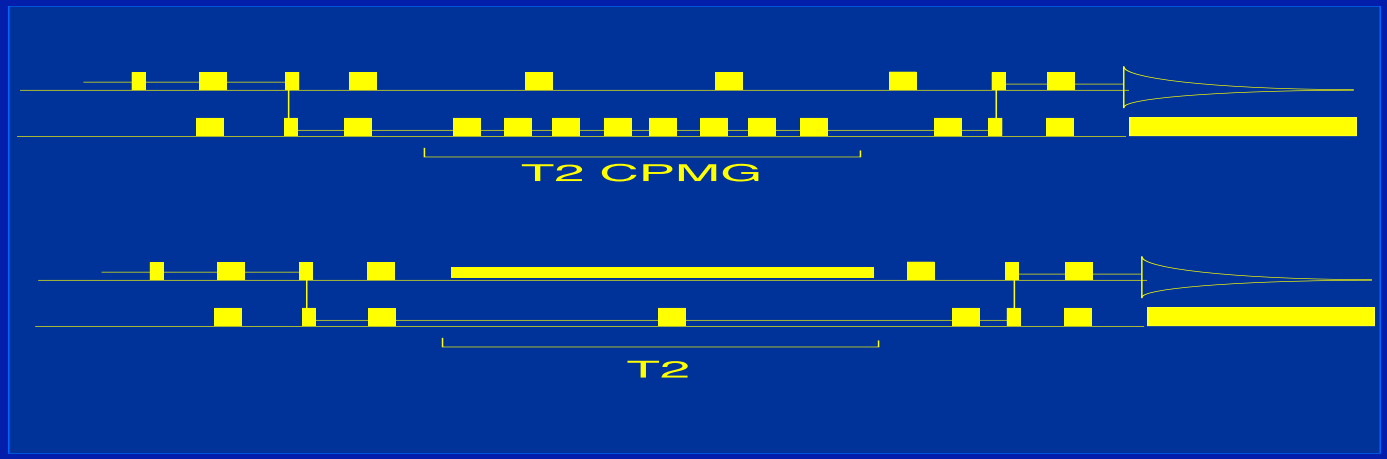
109 residues

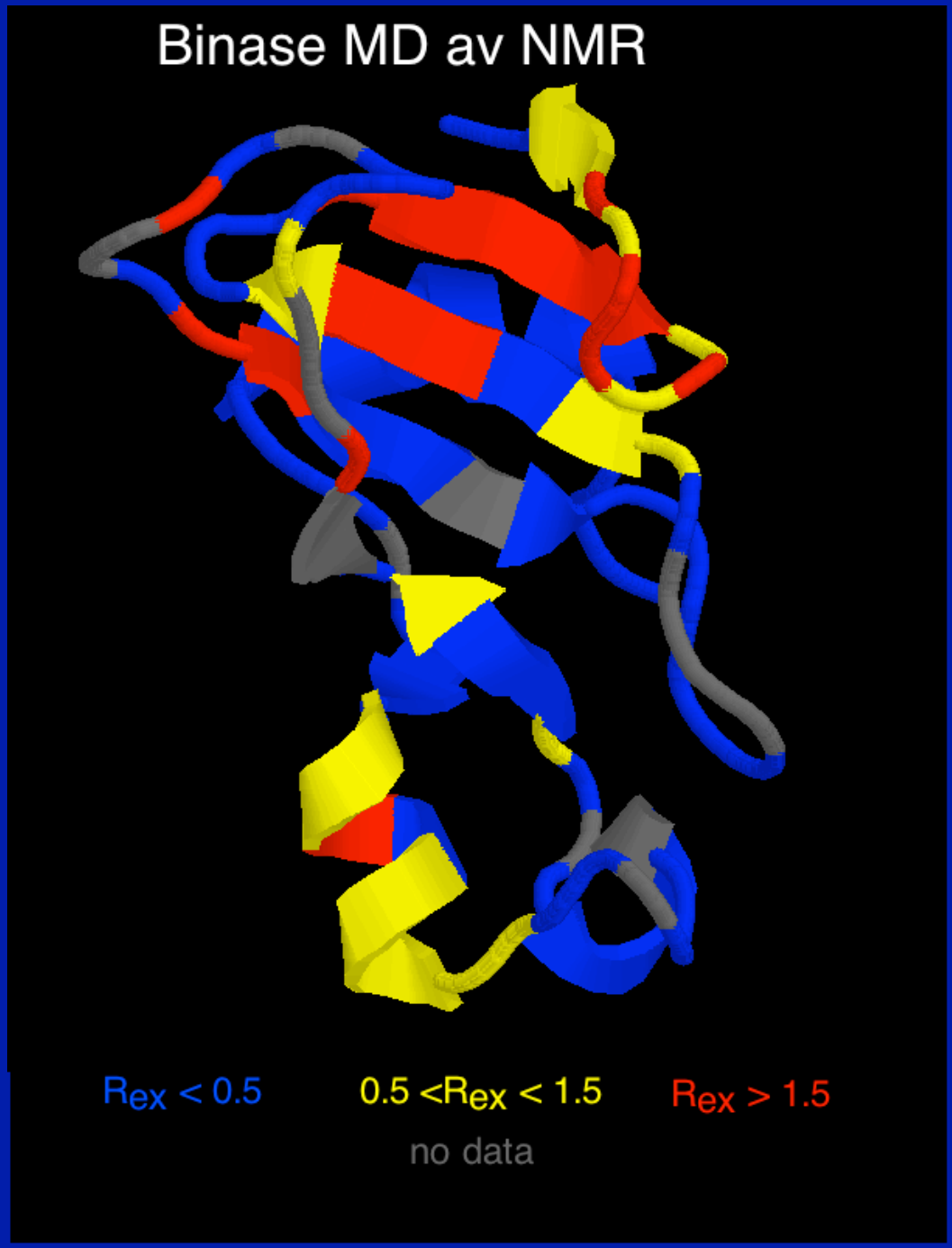
Guanyl-specific ribonuclease

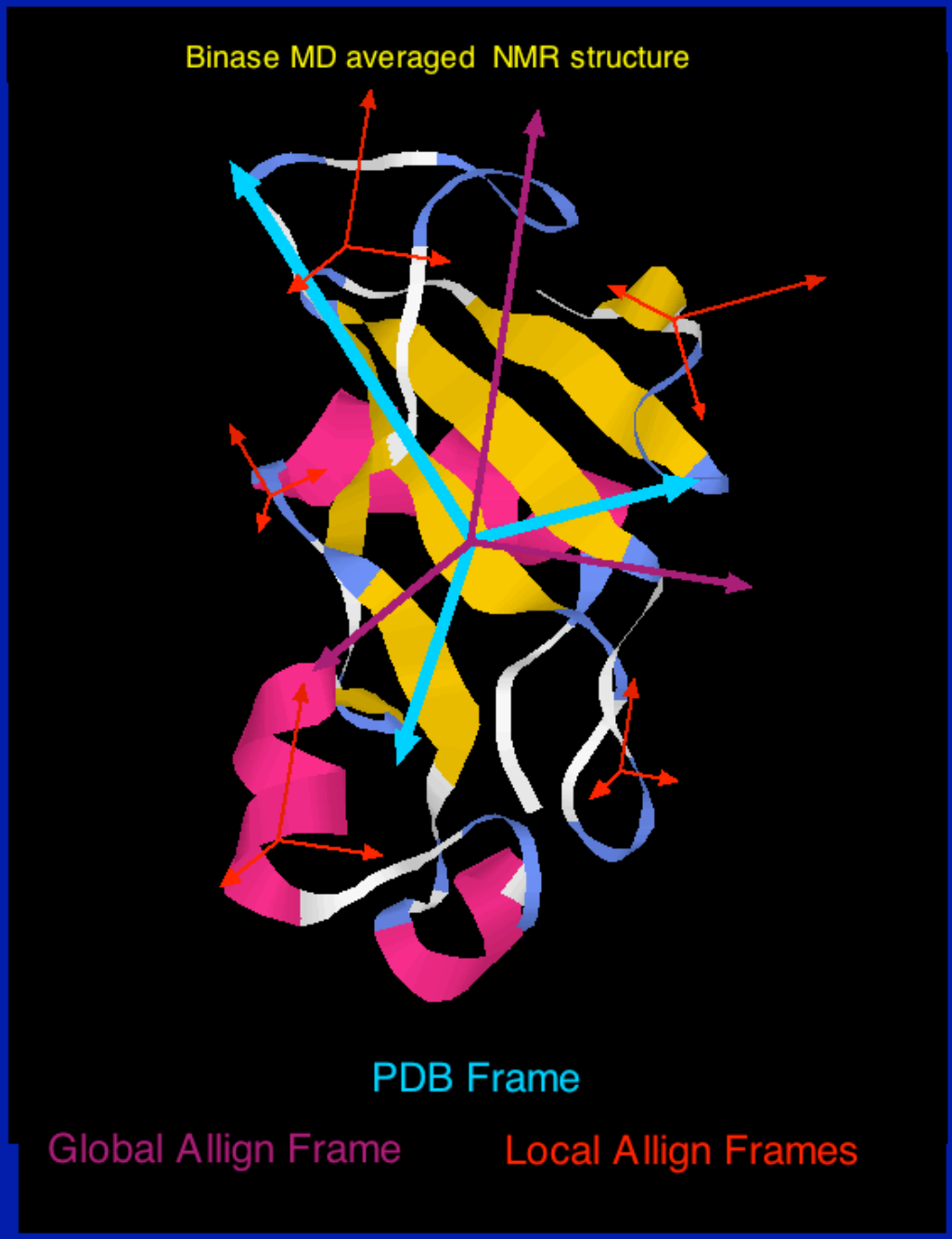
17 a.a. difference with barnase

Conformational exchange broadening in Binase

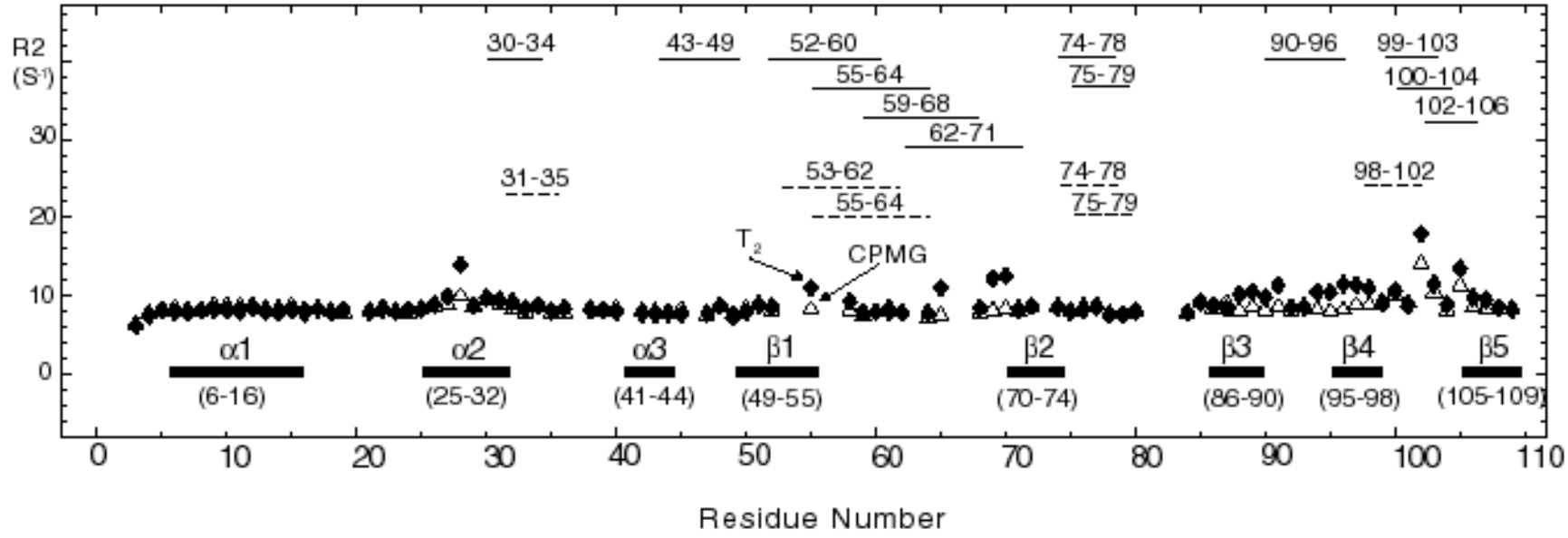





Binase MD av NMR
Rex < 0.5
0.5 <Rex < 1.5
Rex > 1.5
no data

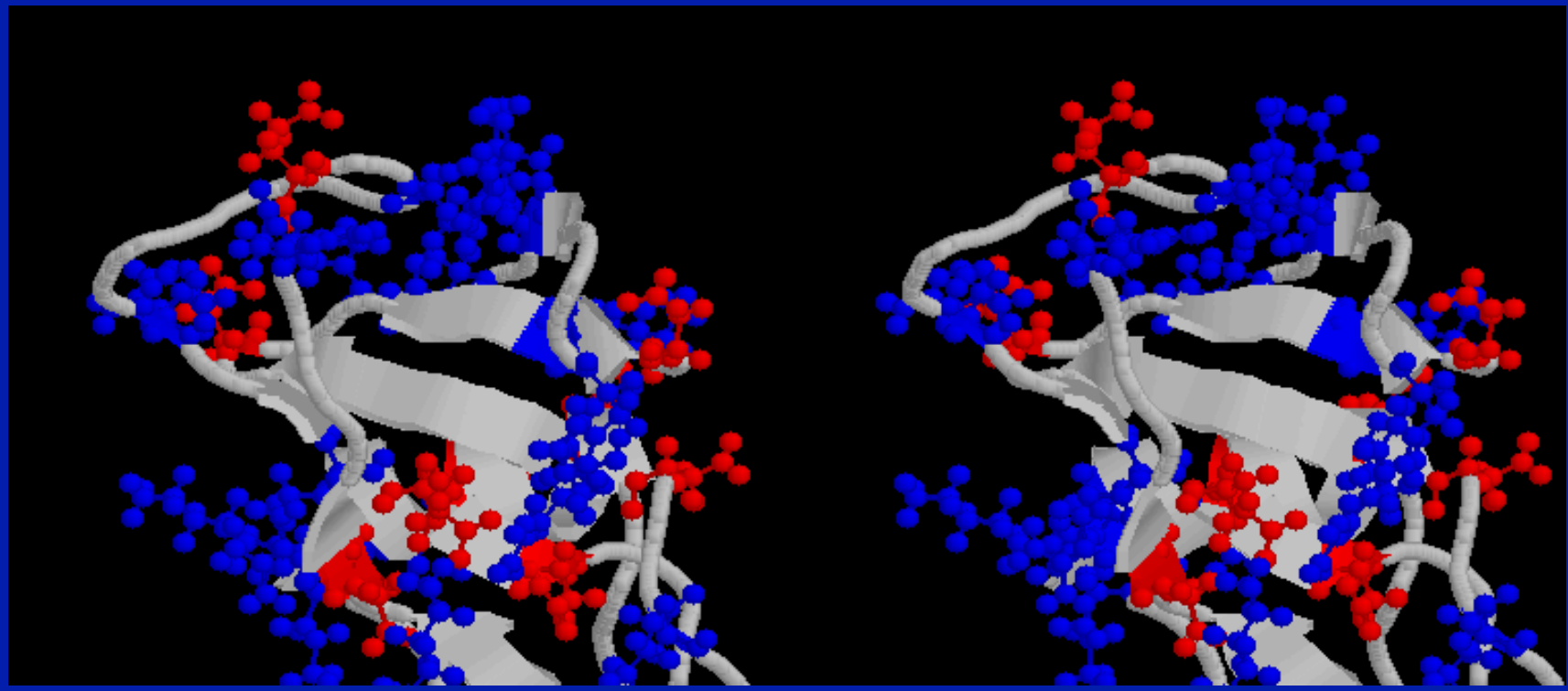

Binase MD averaged NMR structure
PDB Frame
Global Allign Frame
Local Allign Frames

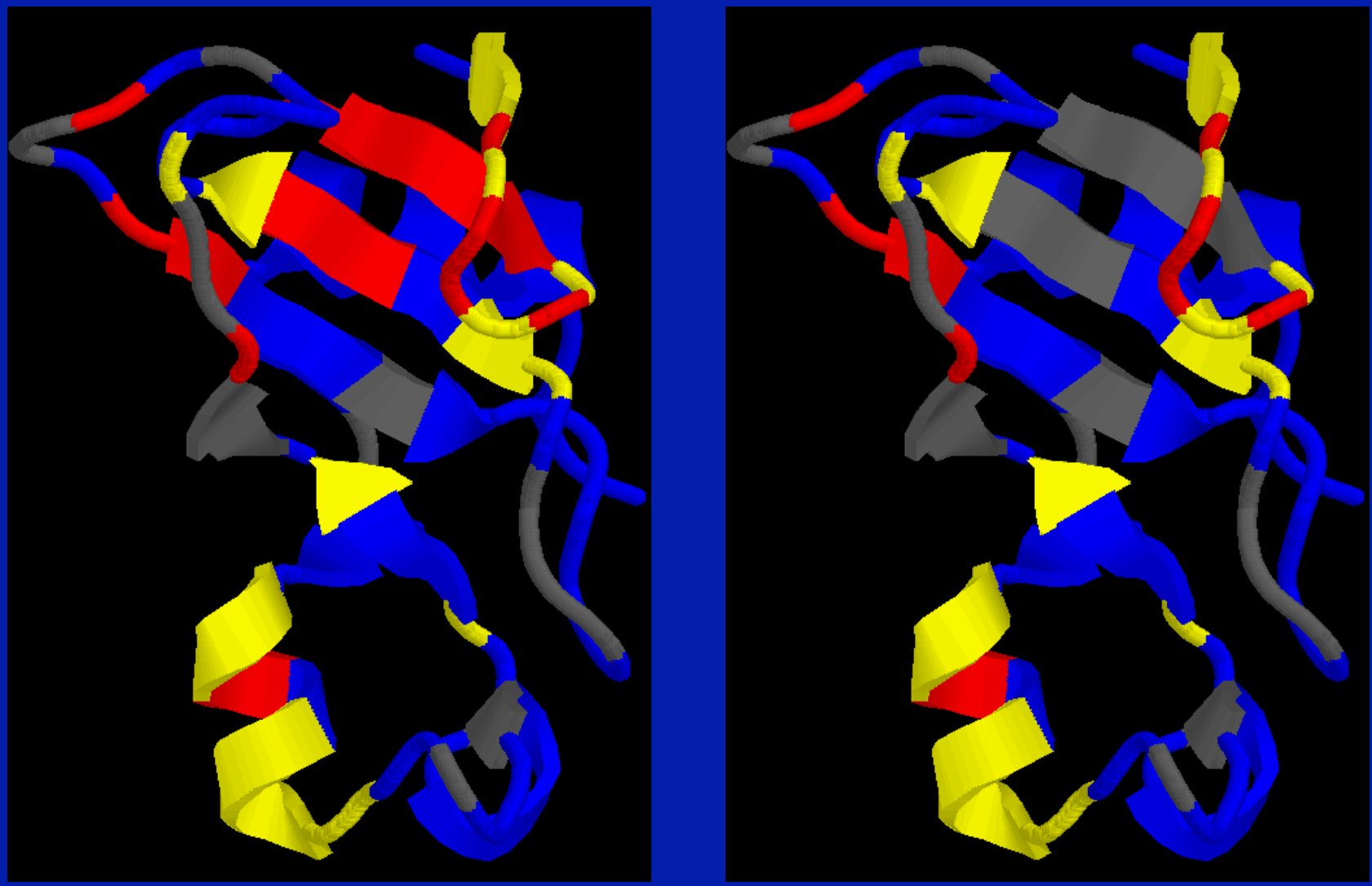
Exchange Broadening and RDC Tensor Deviations



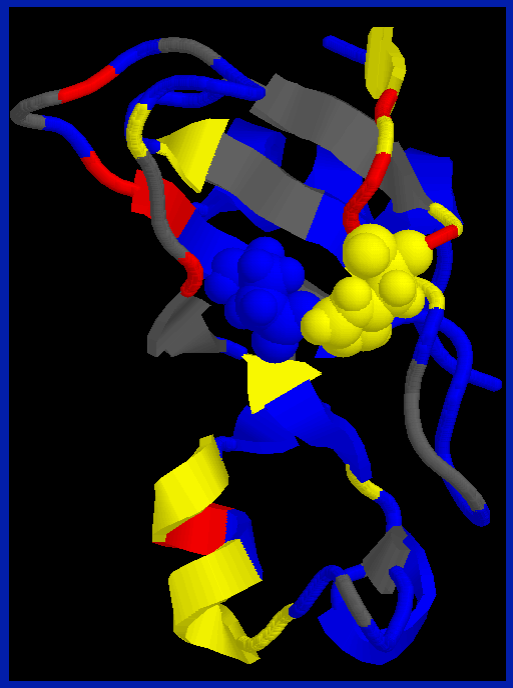
RDC tensors deviate in all areas where we see exchange broadening, except for beta 3. This indictes that b3 is not moving.

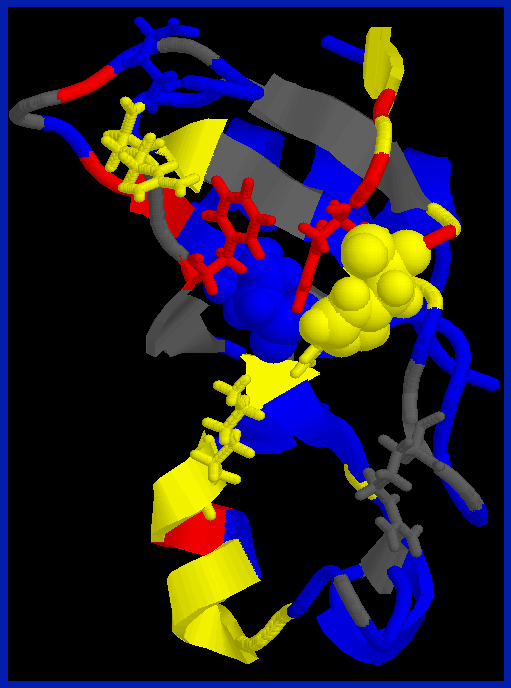
Exchange broadening on the beta sheet is an induced effect

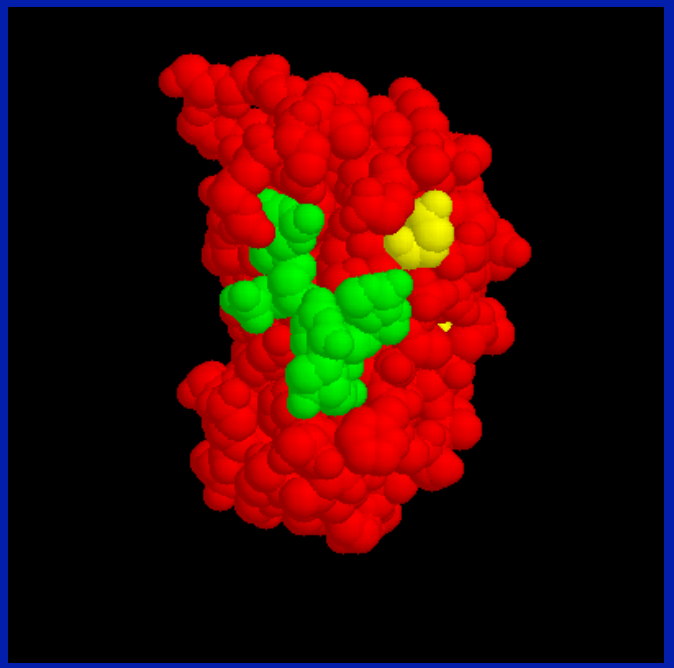




Eliminating the induced broadening from the picture







Catalytic residues

Residues that interact with substrate

i.e. residues important to the protein function move

Where it matters it moves!

Maximum turn over rate for these enzymes is 1400 s^{-1}

	substrate	k_{cat}	K_M	k_{cat}/K_M
		s^{-1}	uM	$M^{-1}s^{-1}$
Binase	GpU	0.4	230	1.7e3
Barnase	GpU	4.3	150	2.9e4
Binase	Poly-I	141	80	1.8e6
Barnase	Poly-I	1413	130	1.1e7

from Schulga et al, Prot. Engin. 11, 775, 1998

NMR-detected dynamics is at rougly the same time scale

- **Conformational exchange rates in Binase (s-1).**
- Gln 28 $3.8 \times 10^3 \pm 600$
- Phe 55 $2.4 \times 10^3 \pm 120$
- Trp 70 $2.7 \times 10^3 \pm 200$
- Leu 97 $2.7 \times 10^3 \pm 700$
- Tyr 102 $4.7 \times 10^3 \pm 800$
- Ala 103 $6.1 \times 10^3 \pm 1200$

Functional Dynamics?

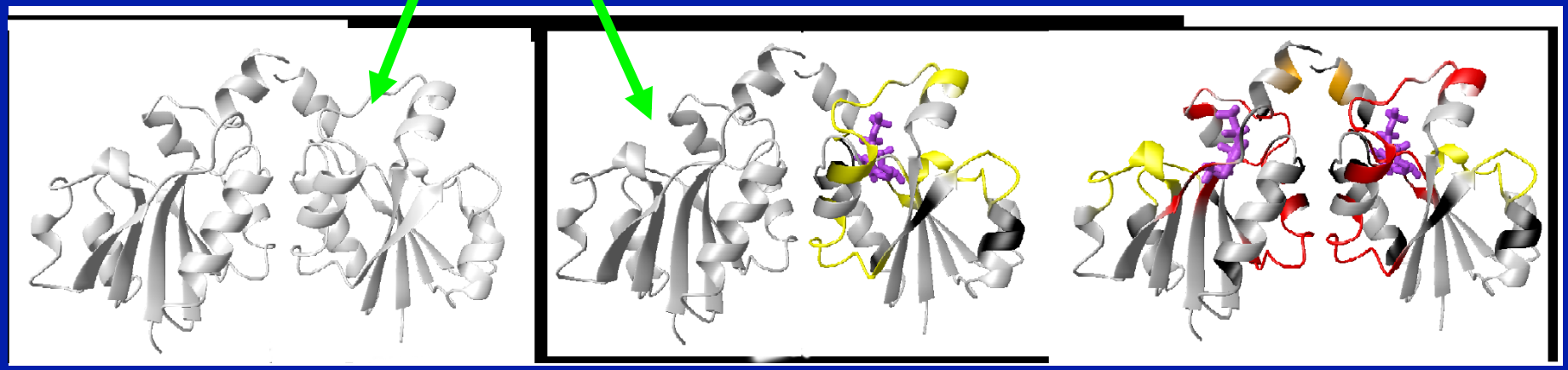
- Apparently, k_{cat} max corresponds to the flap dynamics rate
- Apparently, k_{cat} max reflects product release rate (exit rate)

GCTase, a negatively cooperative enzyme

Binding sites identical,
Expect identical local
binding free energies

Thus:

2.7 Kcal/M of binding
free energy lost on
interface



First CTP

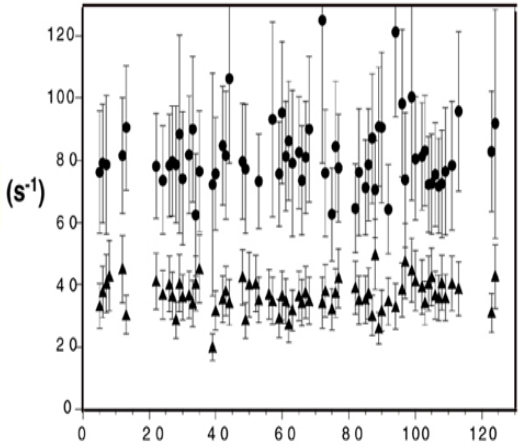
ΔG = -7.3 Kcal/M

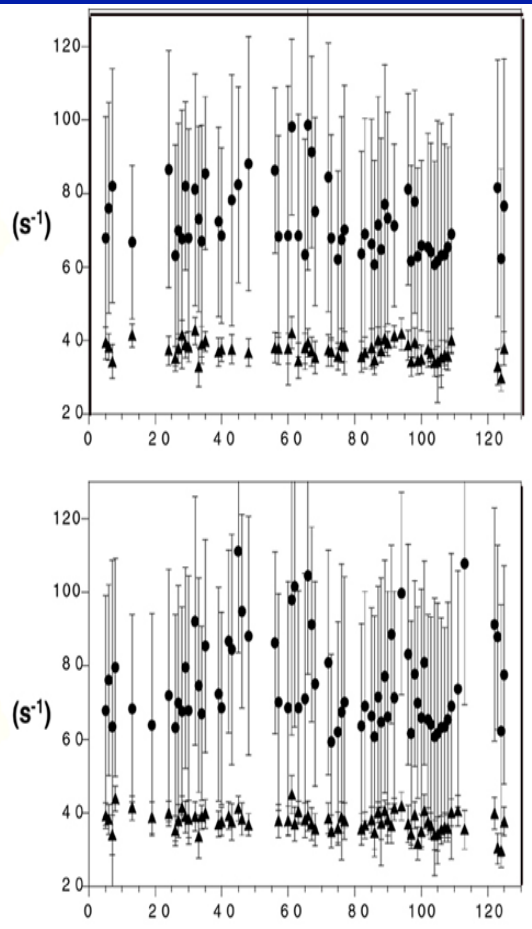
Second CTP

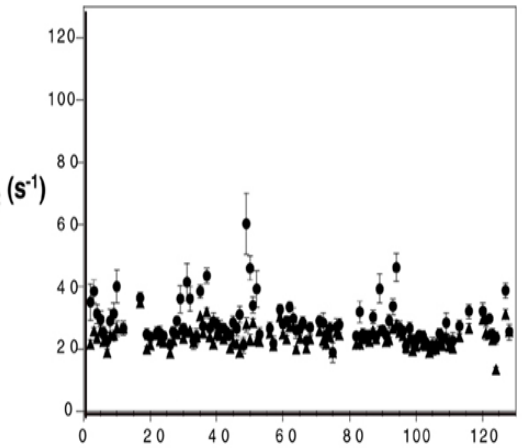
ΔG = -4.6 Kcal/M

Stevens, S.Y., Sanker, S., Kent, C. and Zuiderweg, E.R.P. Delineation of the allosteric mechanism for a cytidylyltransferase exhibiting negative cooperativity, Nature Structural Biology 8, 947-952 (2001)

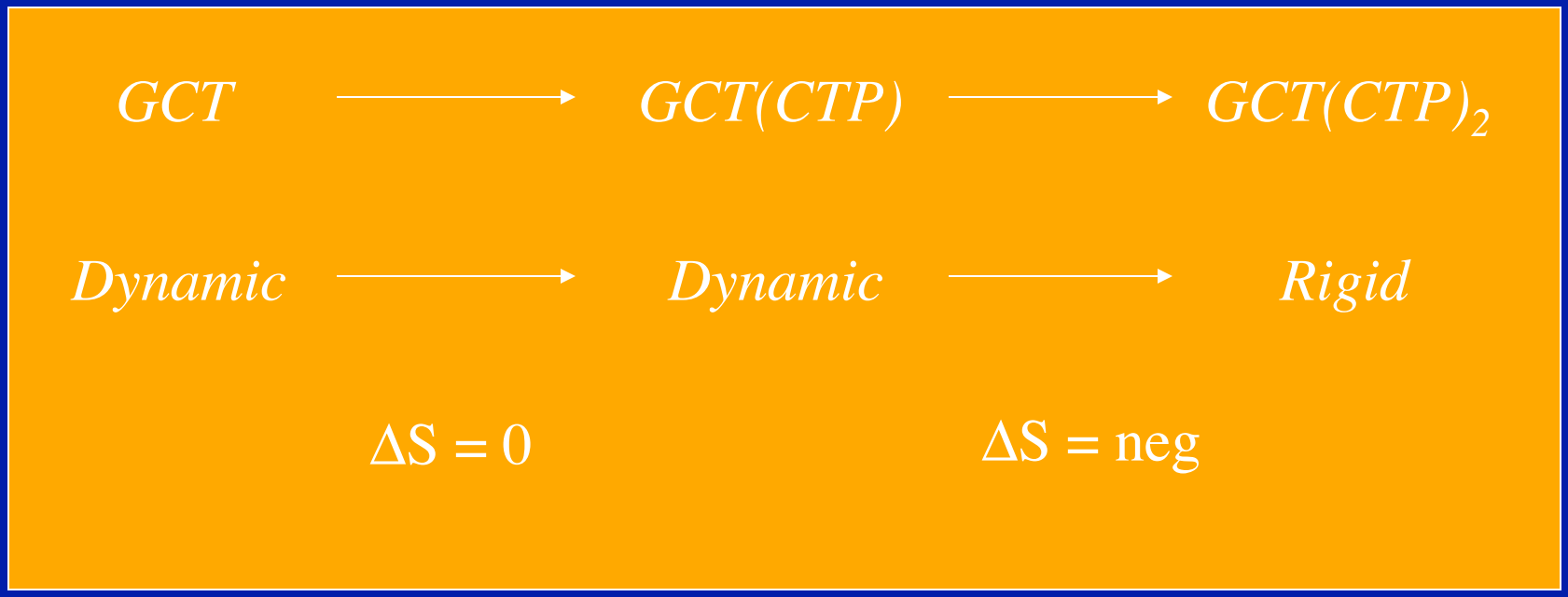
^{15}N R_2 relaxation with and without exchange broadening suppression







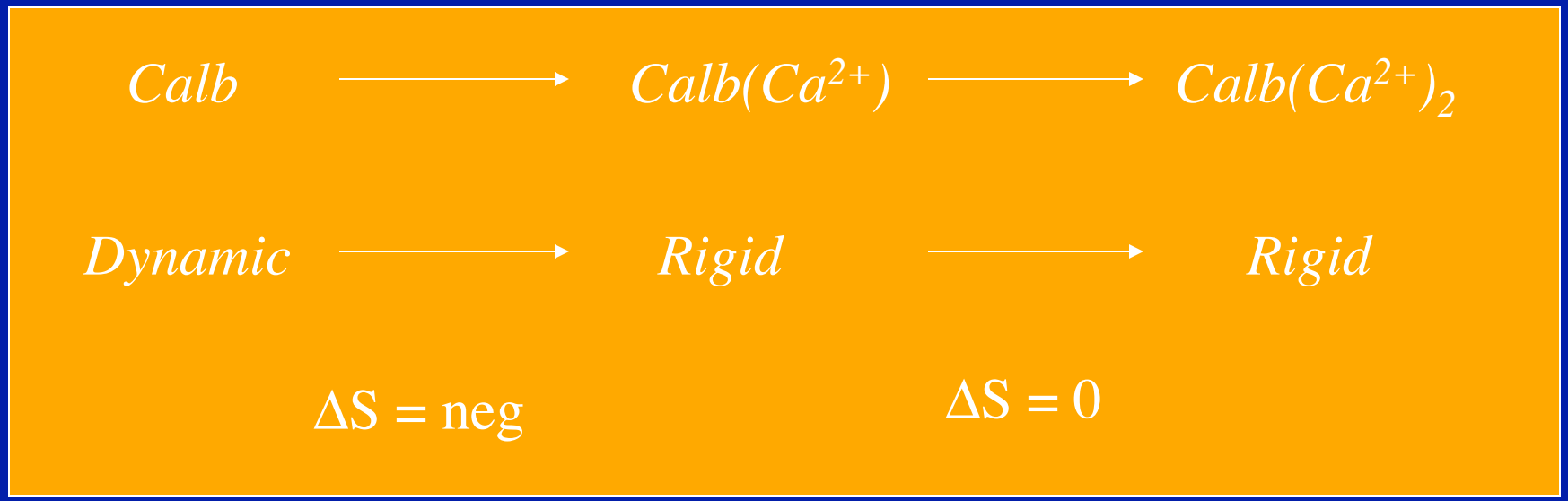
GCT *GCT(CTP)* *GCT(CTP)$_2$*



Therefore, the allosteric free energy of negative cooperativity has an entropic component.

Compare dynamic NMR studies of Calbindin

Mäler, L., Blankenship, J., Rance, M. & Chazin, W. J. *Nature Struct. Biol.* **7**, 245 – 250 (2000).



The allosteric free energy of *positive* cooperativity has an entropic component.

Students associated with dynamics research in the Zuiderweg lab:

Mark Fischer (1992-1998 Ph.D.)
Yuxi Pang (1997-2001 Ph.D.)
Daniel Weaver (2004-2009 Ph.D.)

Weidong Hu, Ph.D. (1994-1997)
Lei Zheng, Ph.D. (1995-1997)
Ananya Majumdar, Ph.D. (1997)
Maurizio Pellecchia, Ph.D. (1998-1999)
Shawn Stevens, Ph.D. (1997-2003)
Lincong Wang, Ph.D. (1998-2001)
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