Universidade de São Paulo
Instituto de Química

Recent advances in Biomolecular NMR

Roberto K. Salinas
IQUSP

January/2014

1. Introduction to protein dynamics

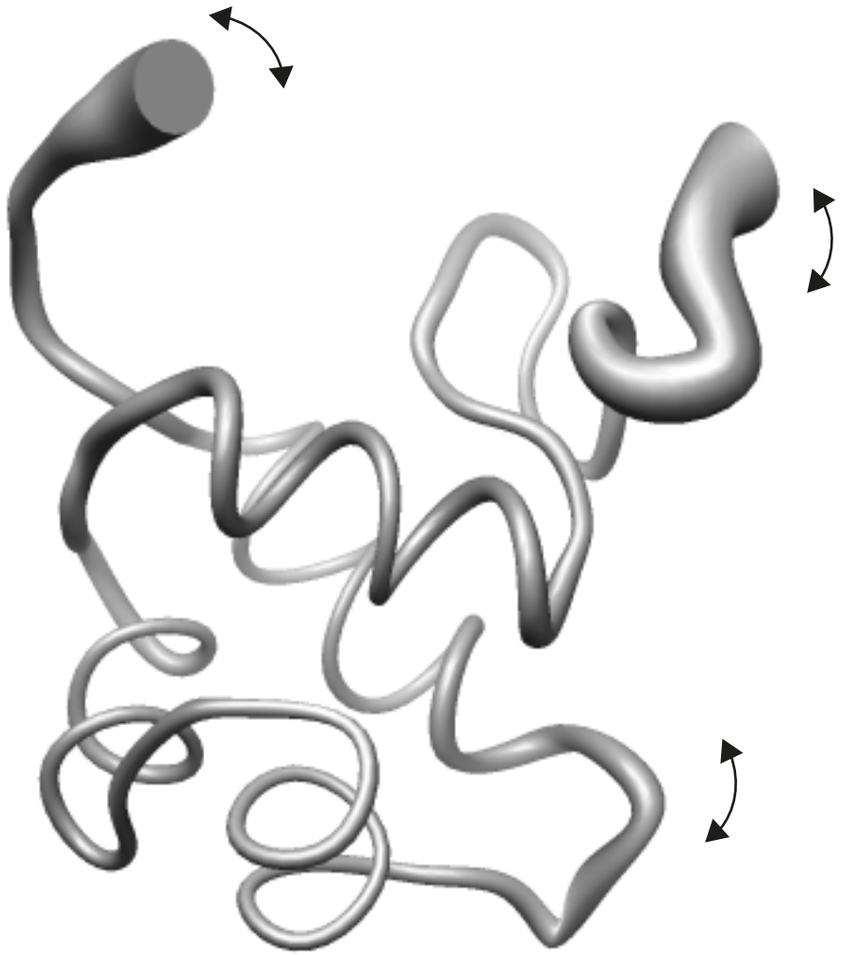
2. Example 1: VirB7

3. Example 2: Na/Ca exchanger

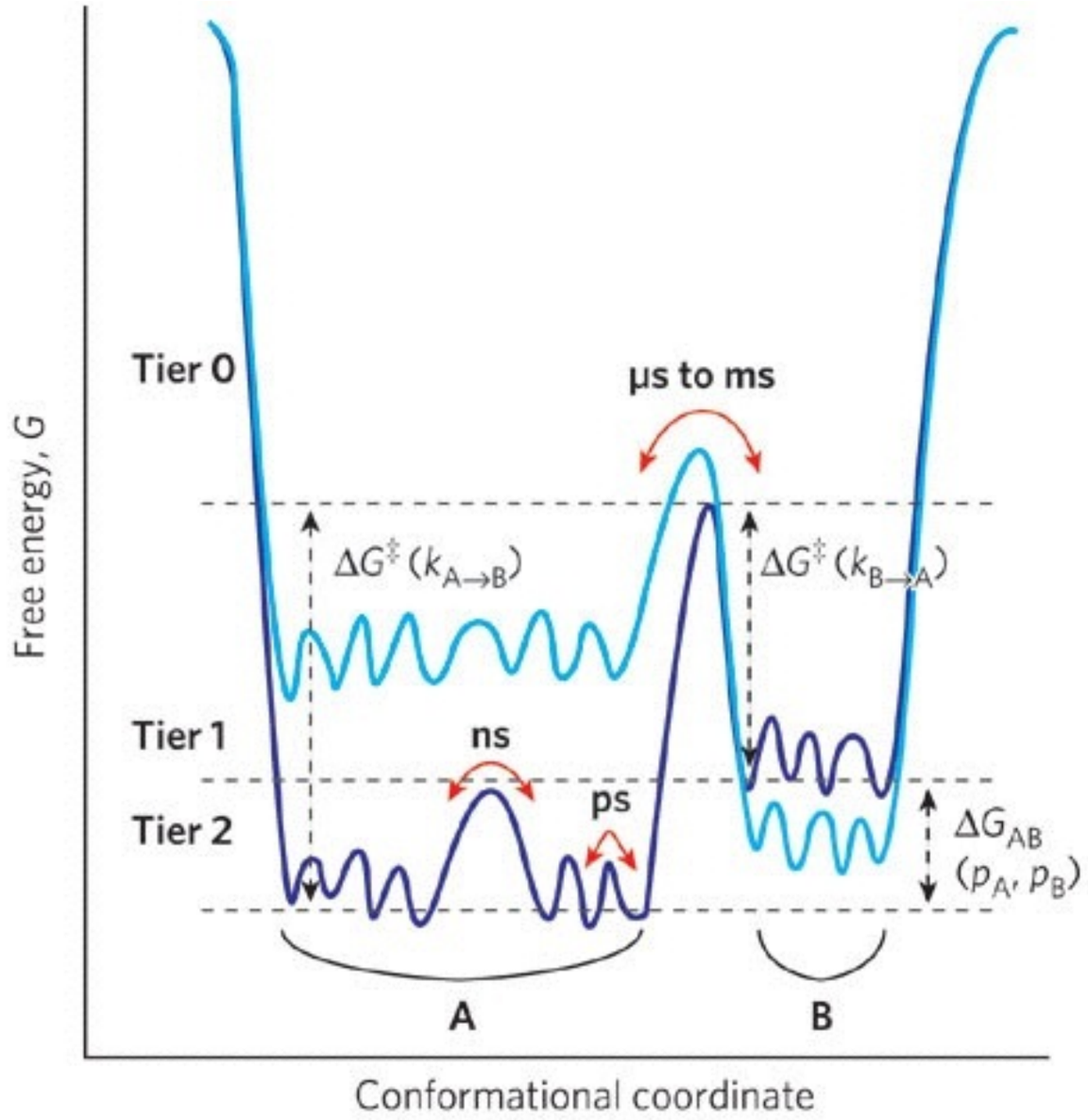
4. Example 3: Glucokinase

Protein dynamics

Proteins are not rigid, they consist of an ensemble of conformations that interconvert at different time scales



PDB 2JVL



Henzler-Wildman, K. and Kern, D. (2007) *Nature* **450**: 964-972

Protein motions

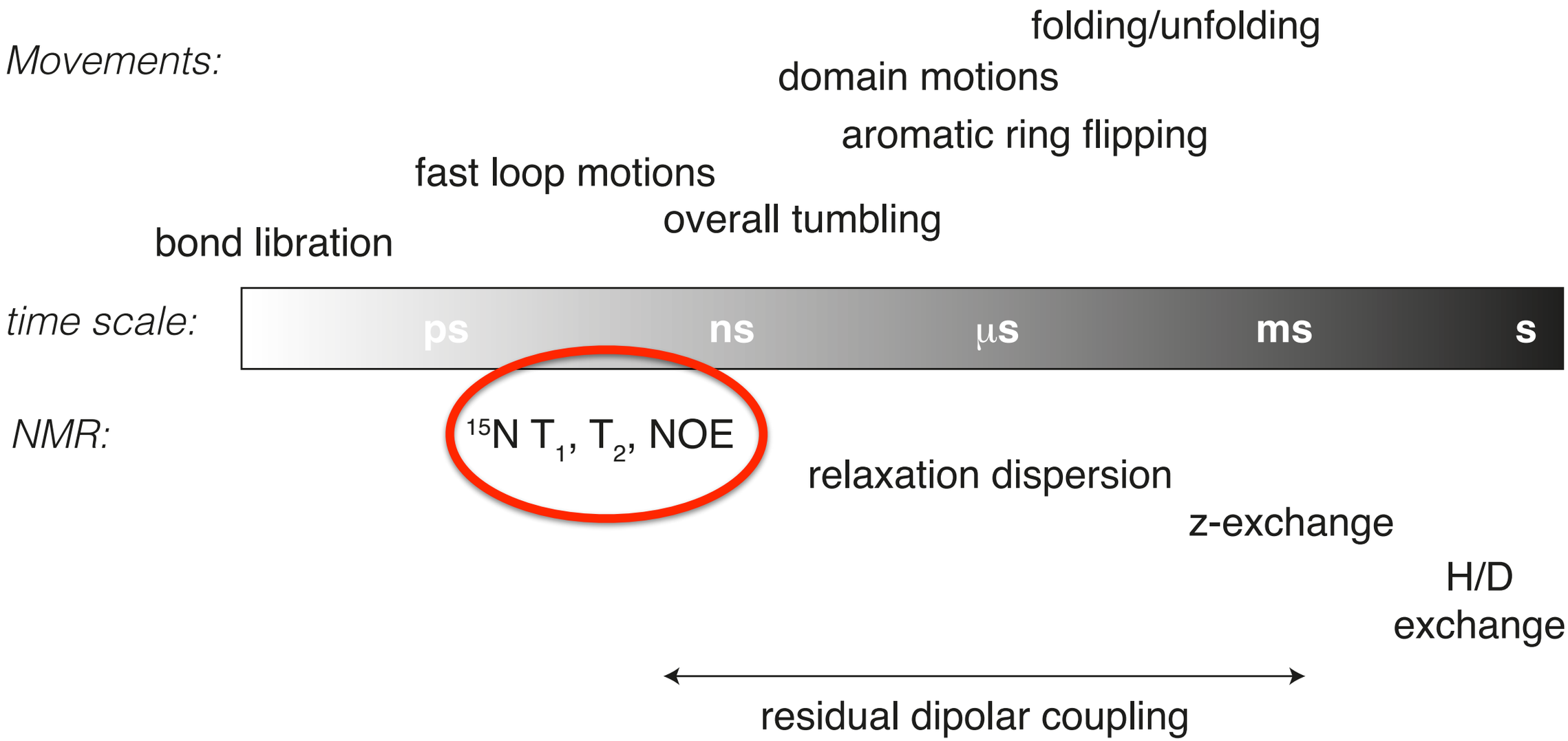
Proteins experience different kind of motions that are relevant in biological processes

- Fast backbone motion
- Side chain motion
- Aromatic ring flipping
- Inter-domain motion
- Overall tumbling

- Folding/unfolding
- Allosteric communication
- Ligand recognition
- Enzyme kinetics

NMR and dynamics

Different NMR observables are sensitive to motions at different time scales

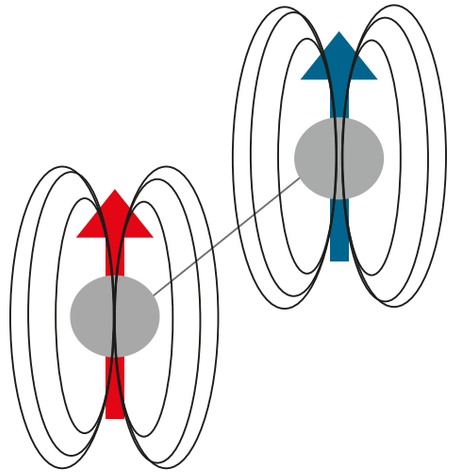


^{15}N relaxation

(Stochastic) time modulation of the dipolar interaction and chemical shielding cause relaxation of the ^{15}N spin

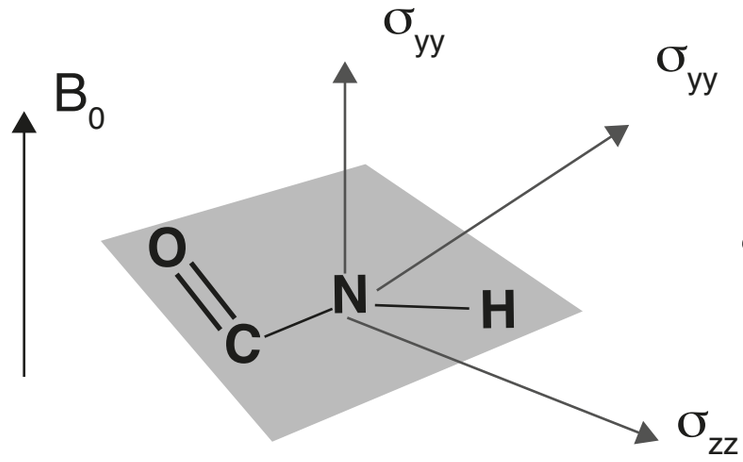
- dipolar interaction with the directly attached ^{1}H nuclei

$$\Delta\omega = \frac{\hbar\gamma_I\gamma_S}{r^3}\left(3\cos^2\theta(t) - 1\right)$$



- ^{15}N chemical shielding anisotropy (CSA)

$$\Delta\sigma = \sigma_{zz} - \left(\sigma_{xx} + \sigma_{yy}\right)/2$$



$$\vec{\sigma} = \begin{bmatrix} \sigma_{xx} & 0 & 0 \\ 0 & \sigma_{yy} & 0 \\ 0 & 0 & \sigma_{zz} \end{bmatrix}$$

- ^{15}N transverse relaxation is also sensitive to time modulation of the isotropic chemical shift. This effect can be exploited to obtain information on dynamics at microsecond to millisecond time scale

- **All dynamic information about the protein is contained within the spectral density function**

For isotropic tumbling:

$$J(\omega) = \frac{\tau_c}{1+\omega^2\tau_c^{\,2}} \qquad \tau_c = \frac{4\pi\eta a^3}{3kT}$$

- **The spectral densities determine the relaxation rates**

$$\frac{1}{T_{1,2}} = \sum_i a_i^{1,2} J(w_i)$$

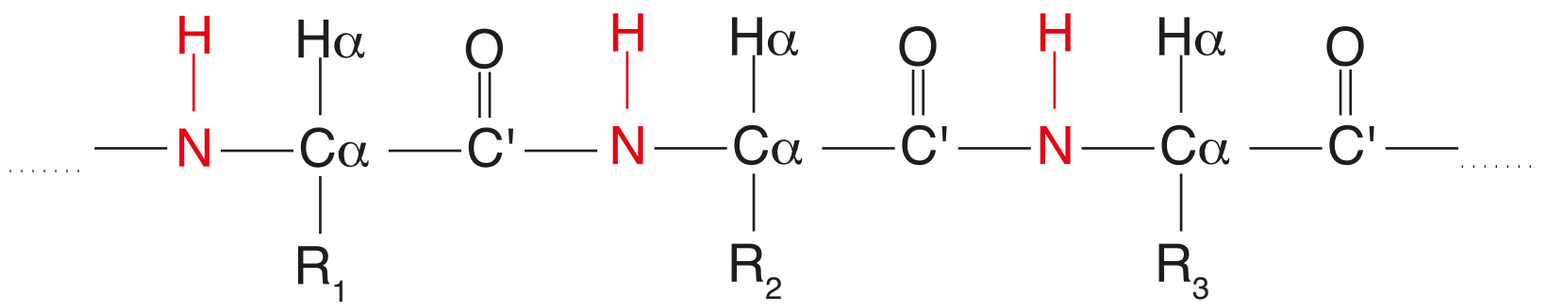
$$\frac{1}{T_1} = \frac{d^2}{10}\left[J(\omega_H-\omega_N)+3J(\omega_N)+6J(\omega_H+\omega_N)\right]+\frac{2}{15}\omega_N{}^2\Delta\sigma^2 J(\omega_N)$$

$$\frac{1}{T_2} = \frac{d^2}{20}\left[4J(0)+J(\omega_H-\omega_N)+3J(\omega_N)+6J(\omega_H)+6J(\omega_H+\omega_N)\right]+\frac{1}{45}\omega_N^2\Delta\sigma^2\left[4J(0)+3J(\omega_N)\right]+R_{ex}$$

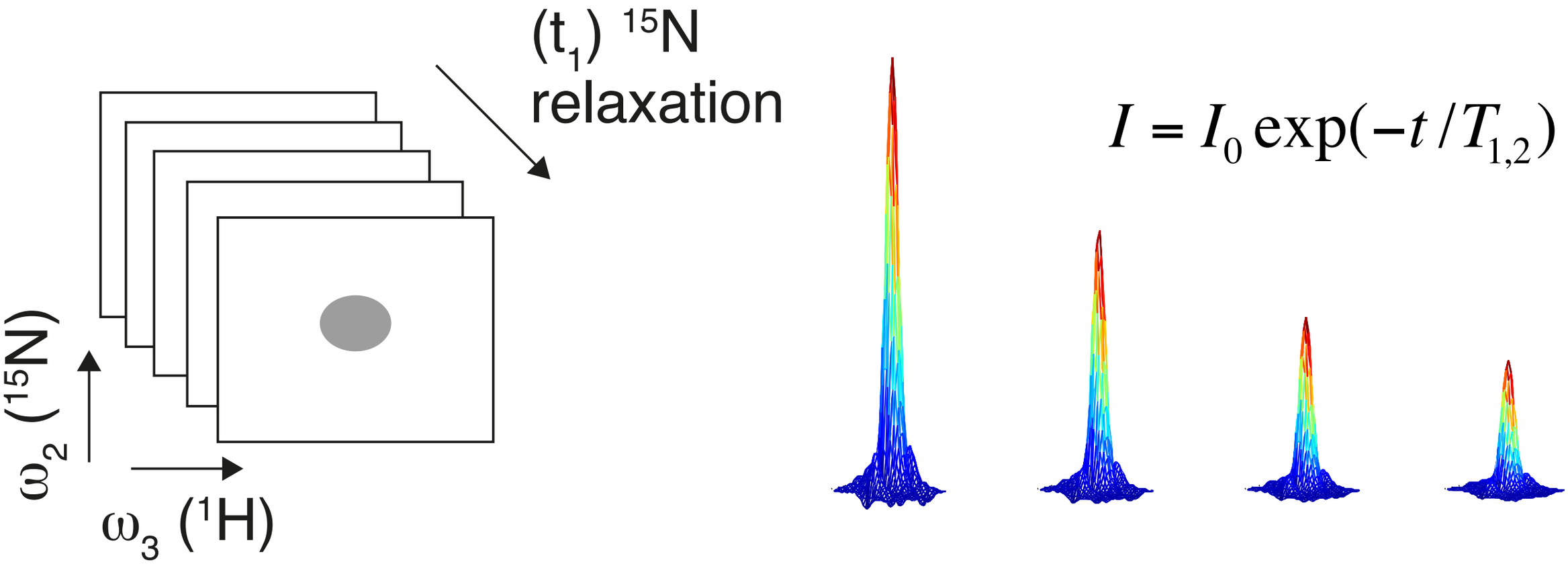
$$NOE = \frac{S_z}{S_z^0} = 1+\frac{\gamma_H}{\gamma_N}d^2\frac{1}{10}\left[6J(\omega_H+\omega_N)-J(\omega_H-\omega_N)\right]T_1^N$$

$$d^2 = \frac{\gamma_H^2\gamma_N^2\hbar^2}{r^6}$$

- **T1, T2 and NOE will give information about the overall tumbling and the fast time scale (ps to ns) internal motions experienced by each amide bond vector**



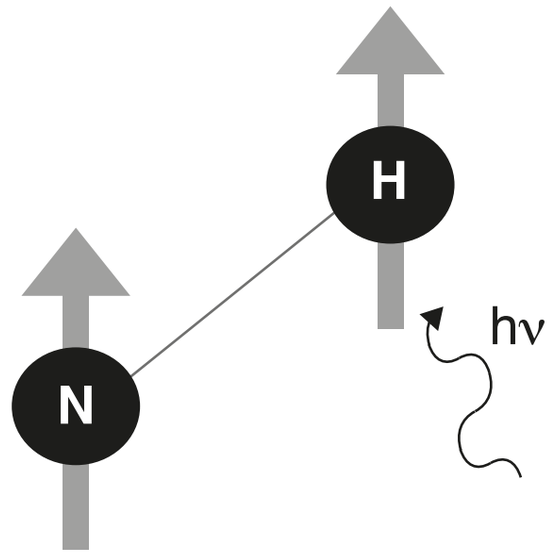
Measuring ^{15}N T_1 and T_2



- Nuclear spin polarization is excited by electromagnetic fields and the return of spin magnetisation to thermal equilibrium is monitored using HSQC-based experiments

- Peak intensity decay due to relaxation is fitted to an exponential decay function in order to extract the spin relaxation times T_1 and T_2

Measuring the {^{1}H}^{15}N NOE

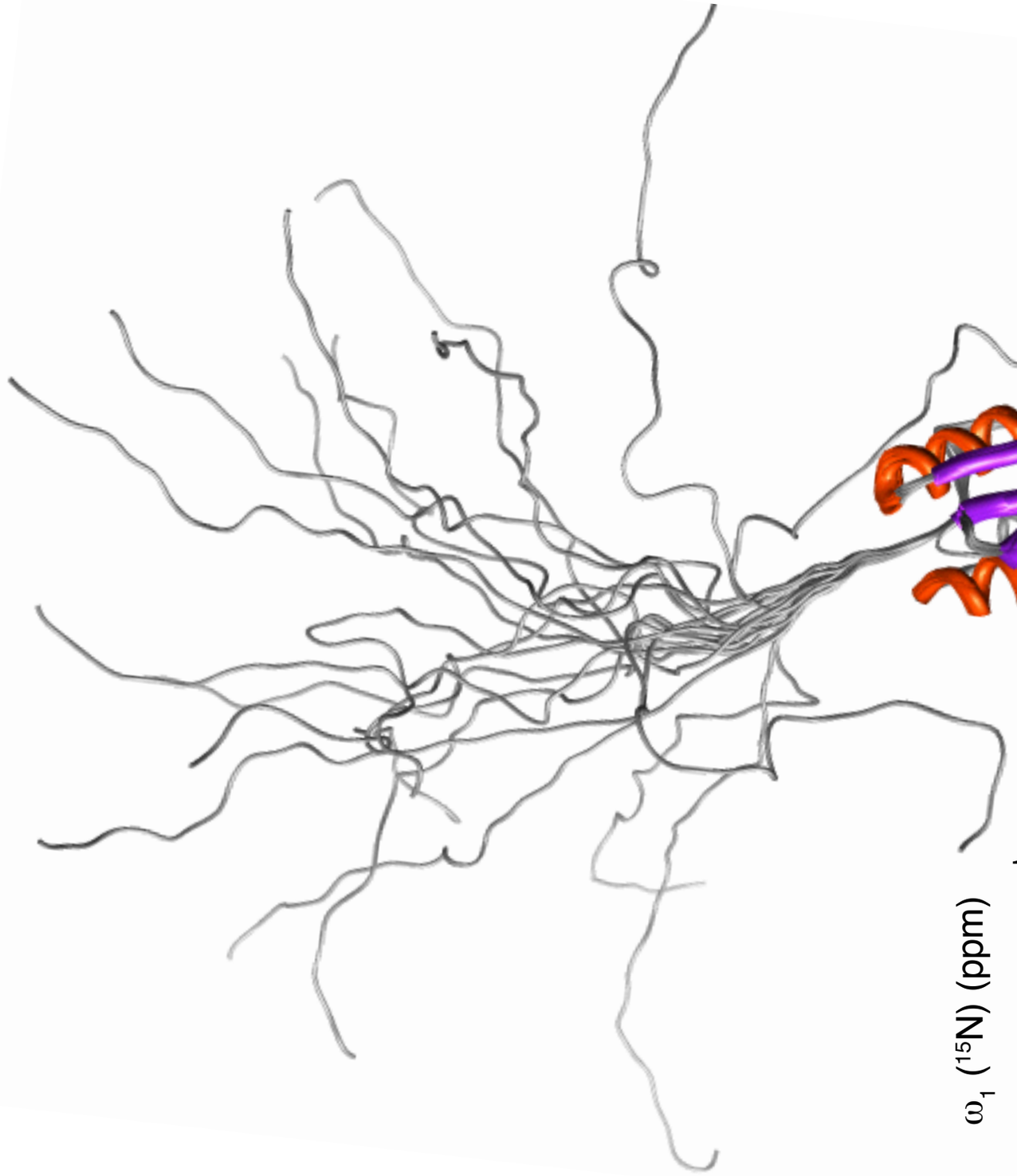


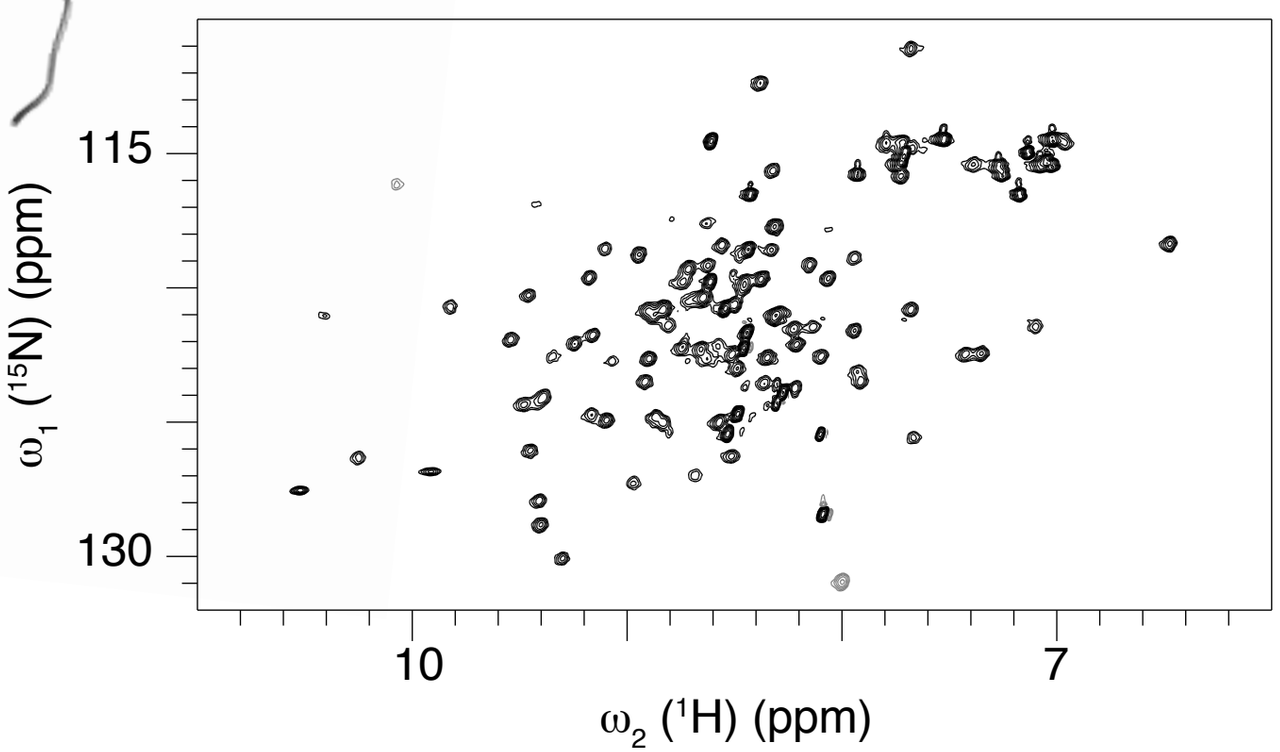
- The heteronuclear-NOE is given by ratio of the steady state ^{15}N spin polarisation recorded in the presence and absence of saturation of the directly attached proton spin transitions

$$NOE = \frac{S_z(sat)}{S_z^{\,0}}$$

Example 1: VirB7

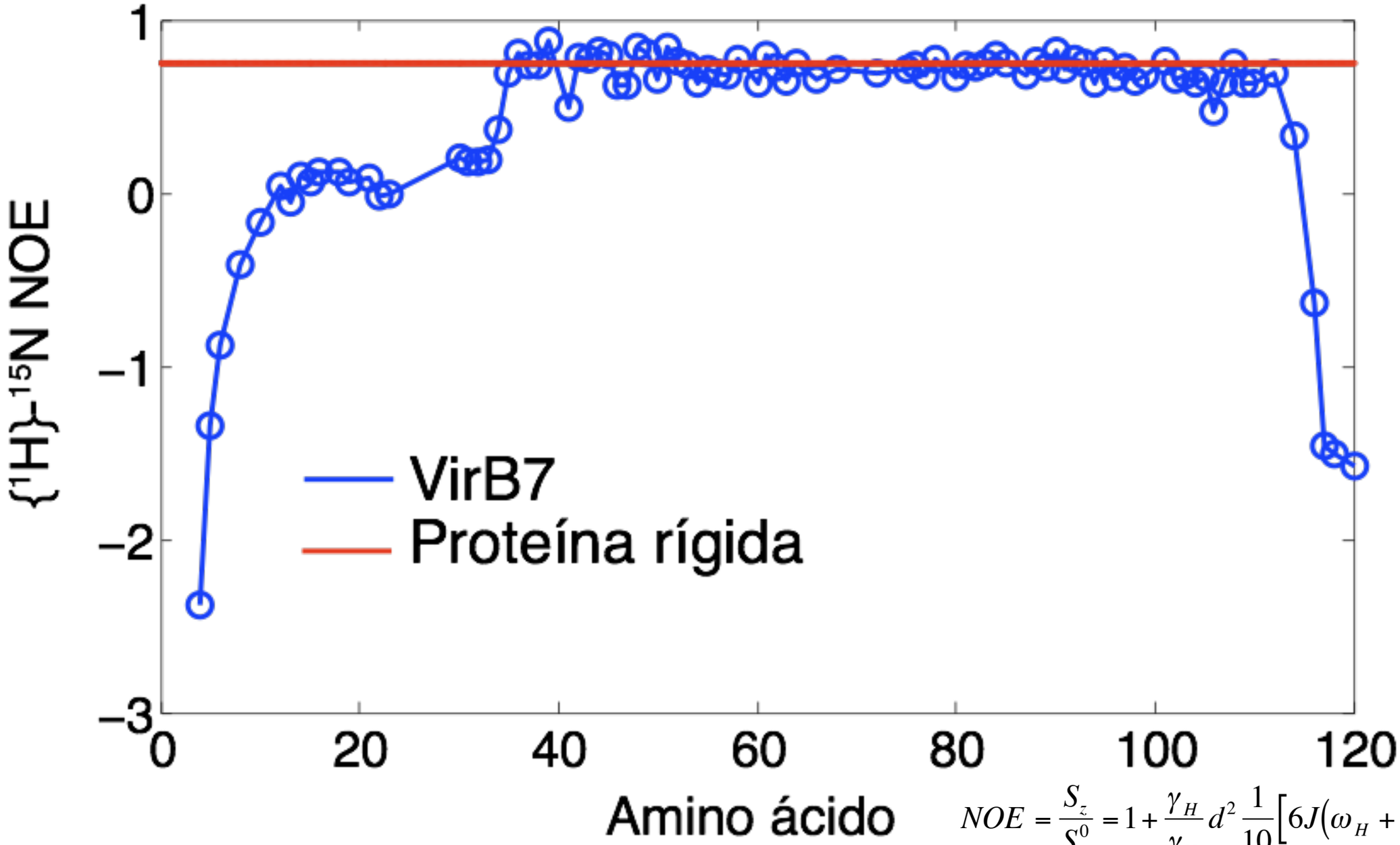
NMR structure of the N0 domain of VirB7





Souza et al. (2011) PLoS Pathogens

Measurements of {1H}-15N NOE confirm that VirB7 consists of a flexible N-terminal tail followed by a rigid protein core

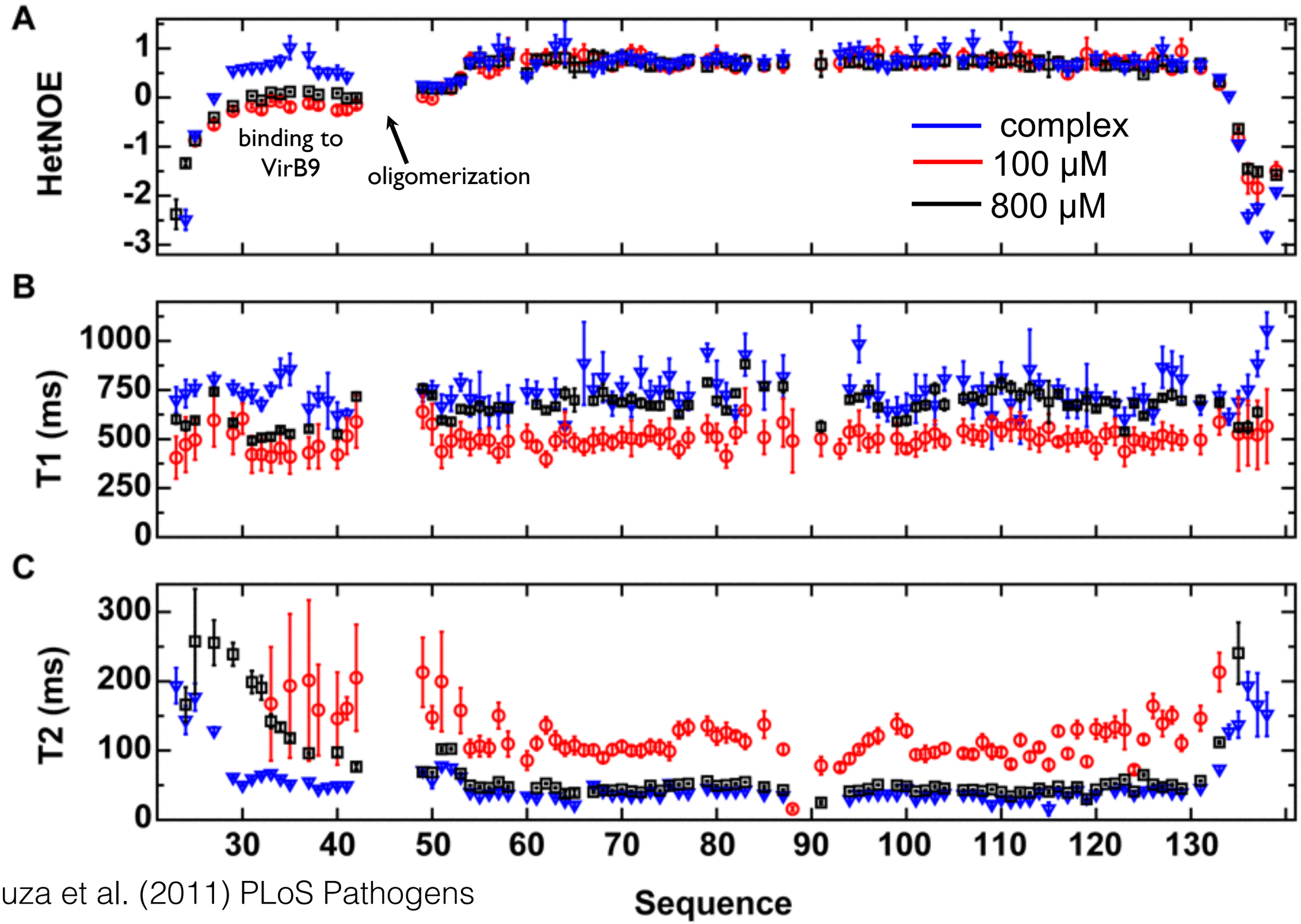


$$NOE = \frac{S_z}{S_z^0} = 1 + \frac{\gamma_H}{\gamma_N} d^2 \frac{1}{10}\left[6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)\right]T_1^N$$

$$d^2 = \frac{\gamma_H^2 \gamma_N^2 \hbar^2}{r^6}$$

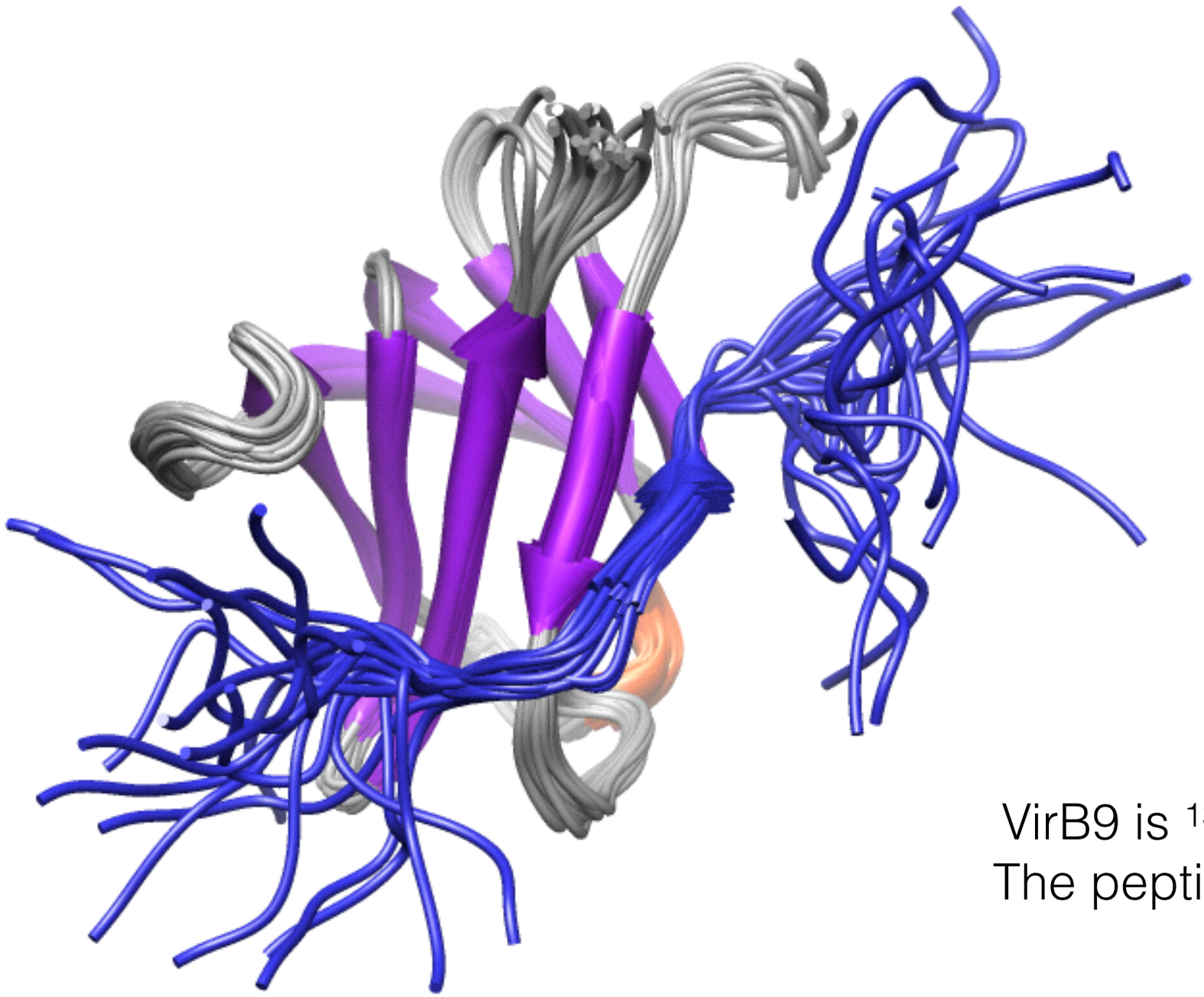
Souza et al. (2011) PLoS Pathogens

Measurements of 15N T1, T2 and NOE indicate that the flexible N-terminal tail becomes rigid upon ligand binding



Souza et al. (2011) PLoS Pathogens

A preliminar NMR structure shows that the N-terminal tail of VirB7 binds to VirB9 and folds into a beta-strand

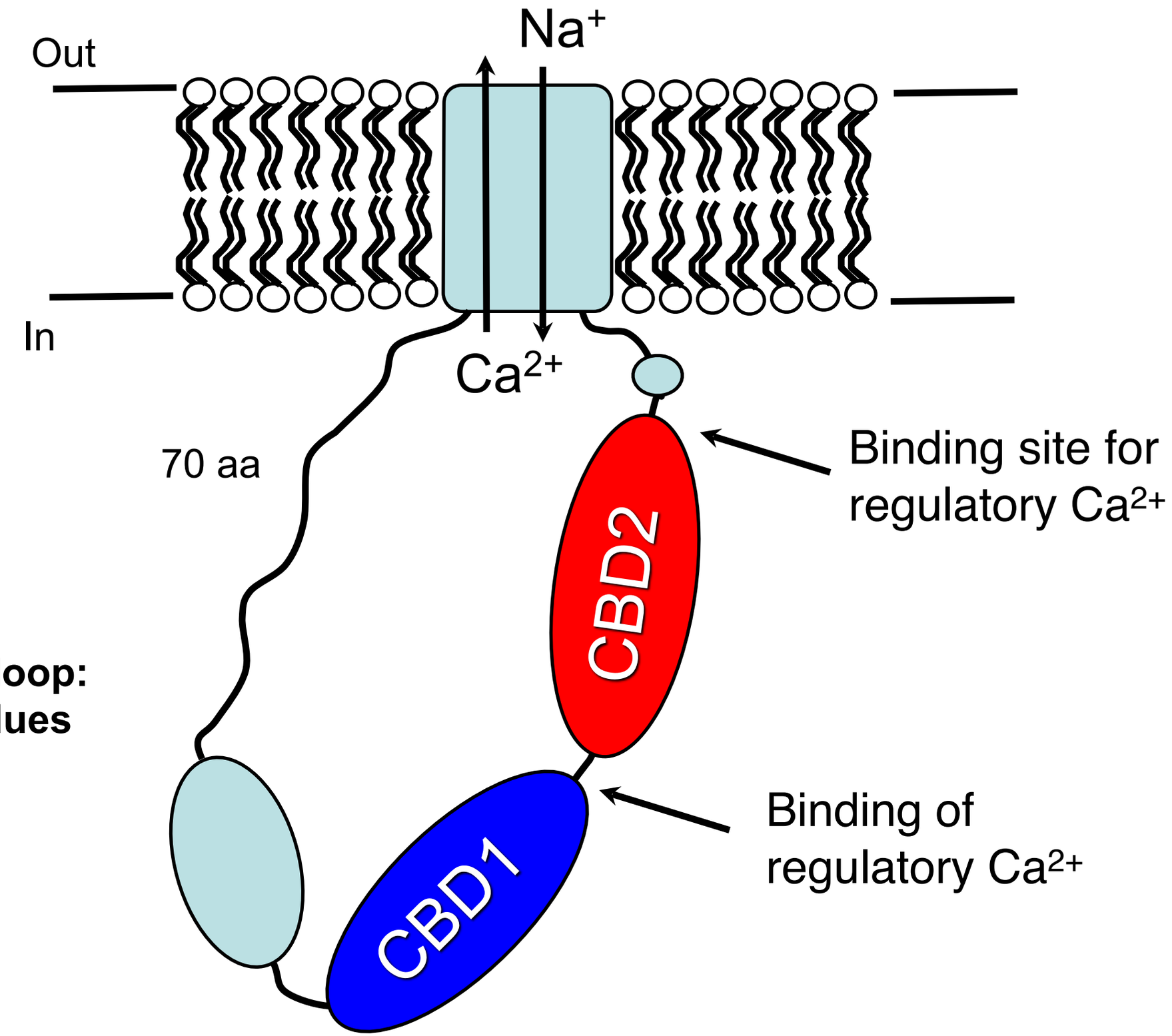


VirB9 is ^{13}C, ^{15}N labelled
The peptide is unlabelled

Obs: This structure is being calculated using Cyana, water refinement in CNS, protein-peptide NOEs were obtained from the analysis of filtered 2D NOE experiments, and ^{13}C separated NOESY

Example 2: The Na/Ca exchanger

How does the binding of regulatory Ca^{2+} to the cytoplasmic domain regulate the exchanger?



Large cytosolic loop: ~500 residues

X-ray crystal structures of Ca^{2+}-bound forms of individual CBD1 & CBD1 domains

β-sandwich/
greek key
motif

$K_{d1} \approx 0.2\ \mu M$

C-terminal

N-terminal

FG-loop

CBD1 (2DPK)

N-terminal

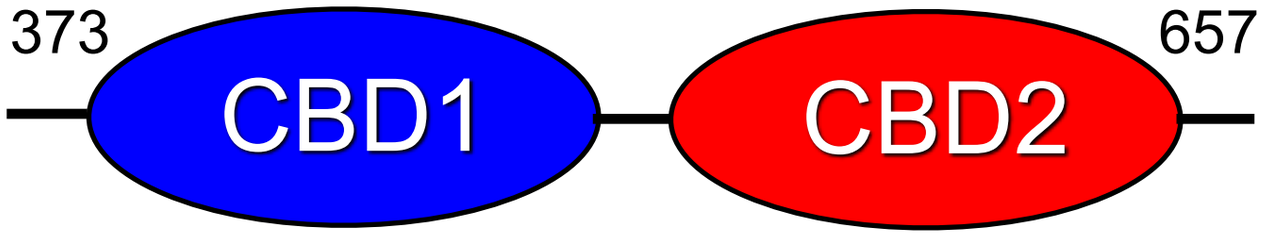
FG-loop

C-terminal

$K_{d2} \approx 14 - 45\ \mu M$

CBD2 (2QVM)

Characterization of cytosolic loop of the Na/Ca exchanger by solution NMR



= **CBD12 (32 kDa)**

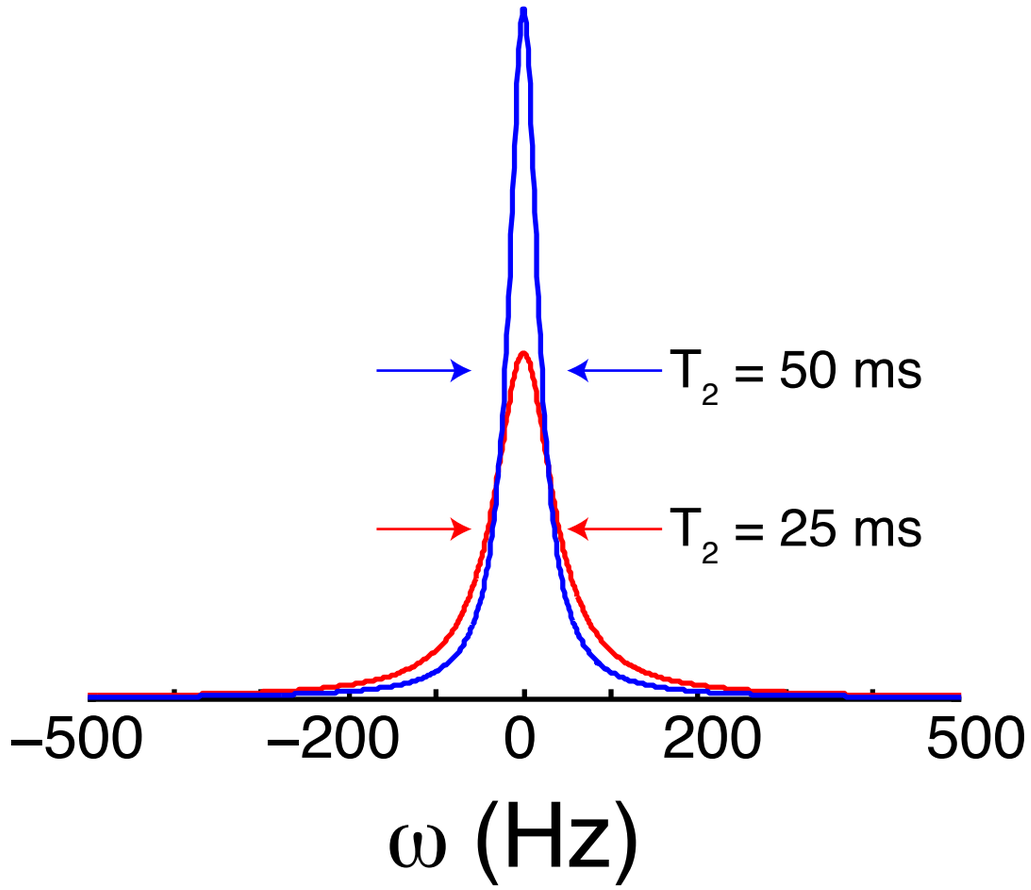
- How do CBD1 and CBD2 interact with each other?

- What is the effect of Ca^{2+}-binding on the structural dynamics of CBD12?

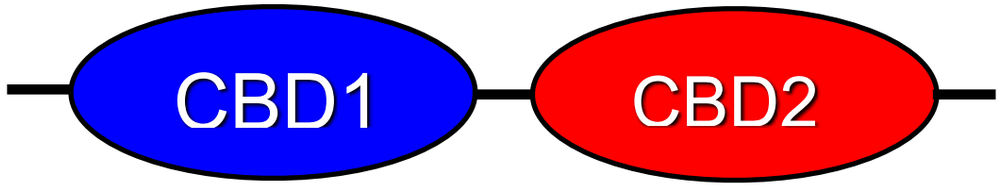
Characterization of the inter-domain structural dynamics of CBD12 by

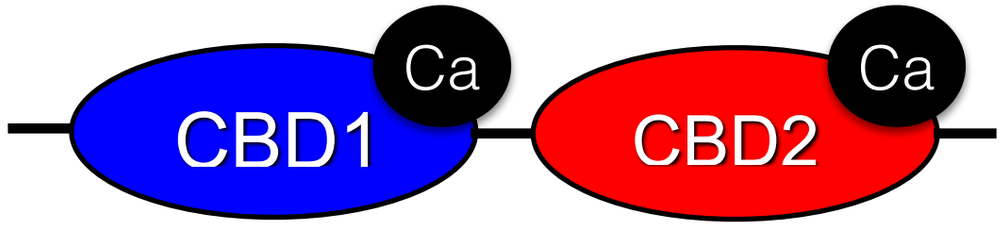
Chemical shift perturbation
^{15}N spin relaxation
Residual dipolar couplings (RDCs)

Larger proteins display shorter T_2 relaxation times, which turns out to be a problem for NMR

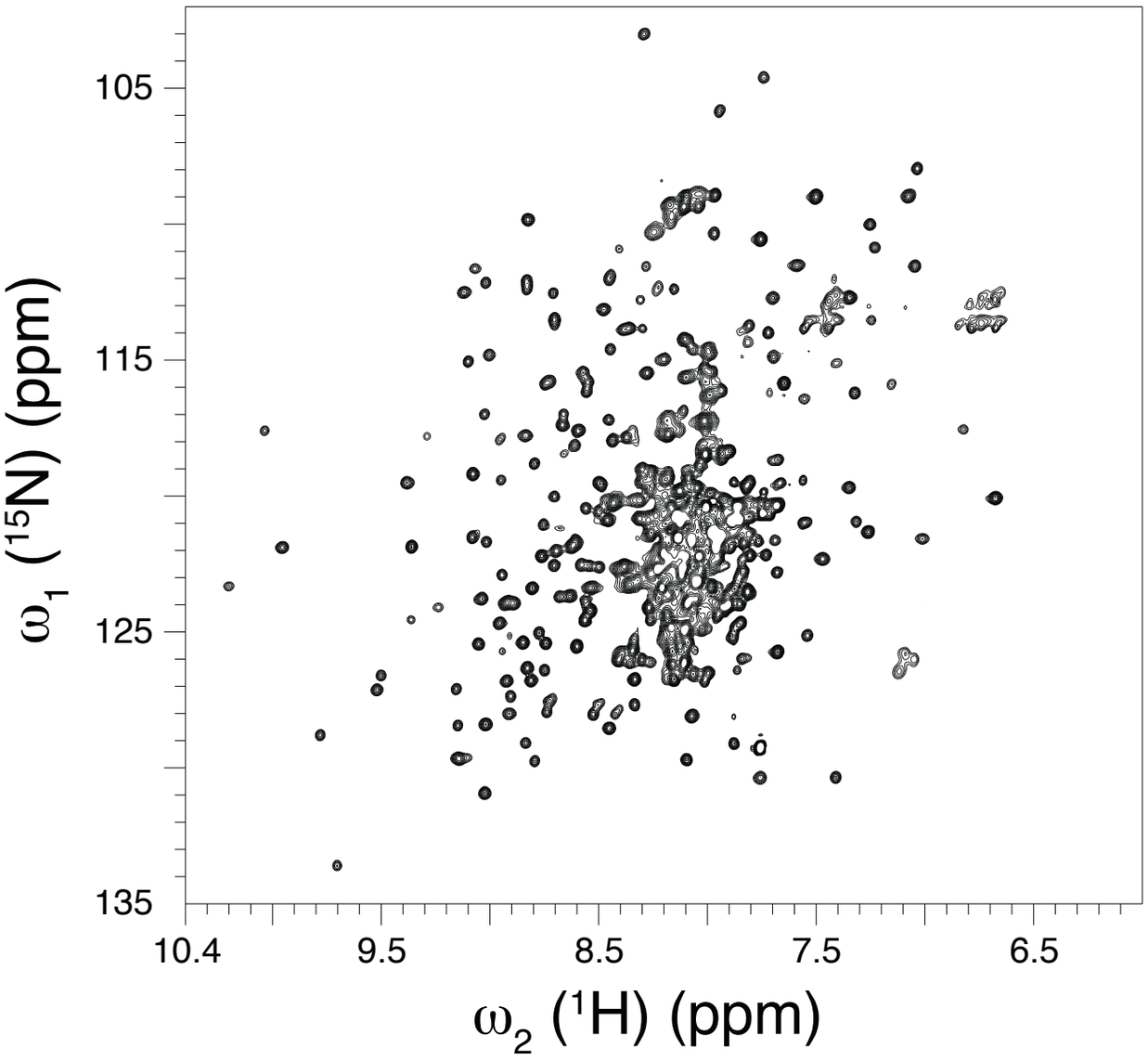


In order to increase T_2, CBD12 was randomly fractionally deuterated by growing *E. coli* cells in D_2O

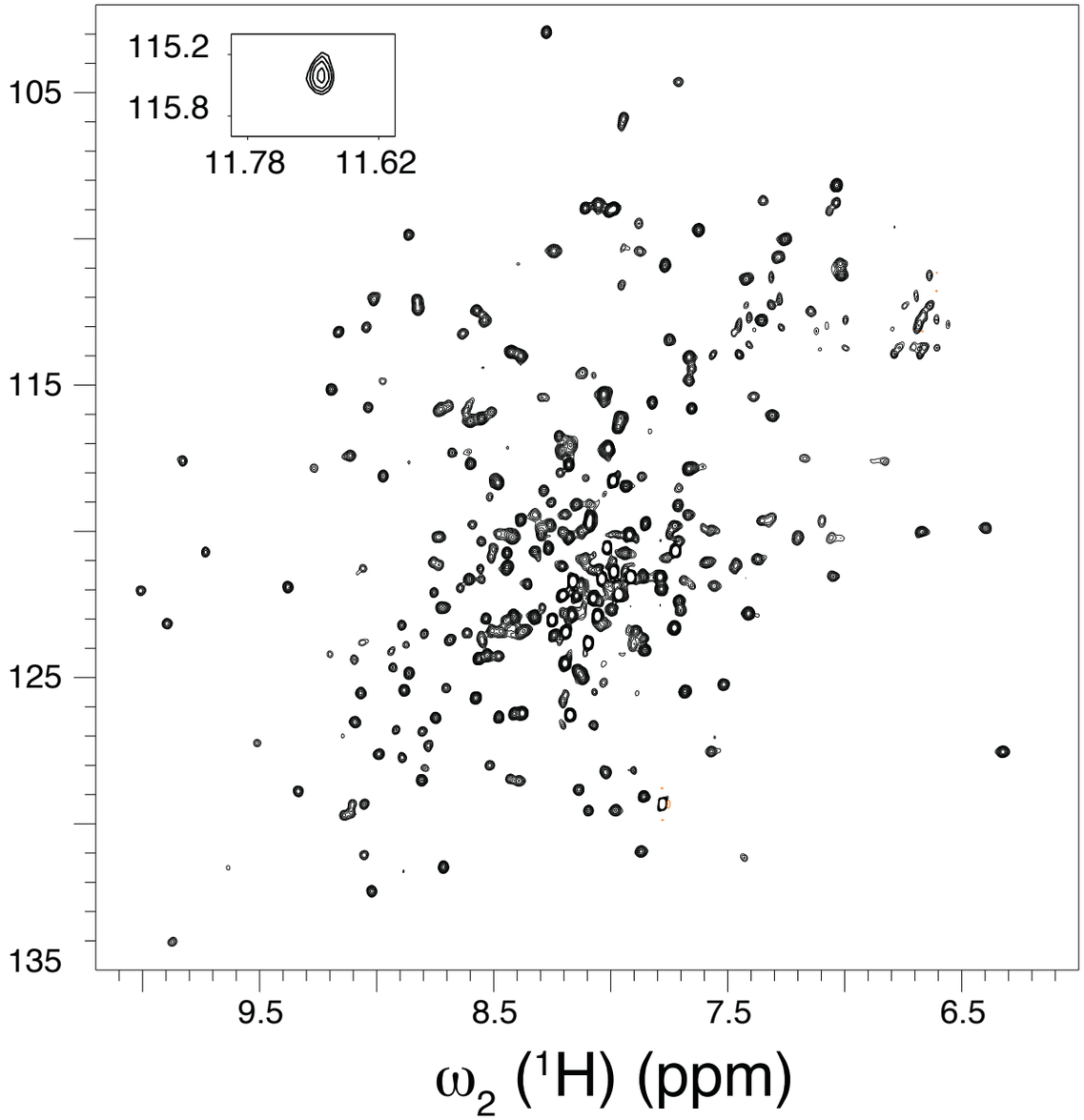




APO

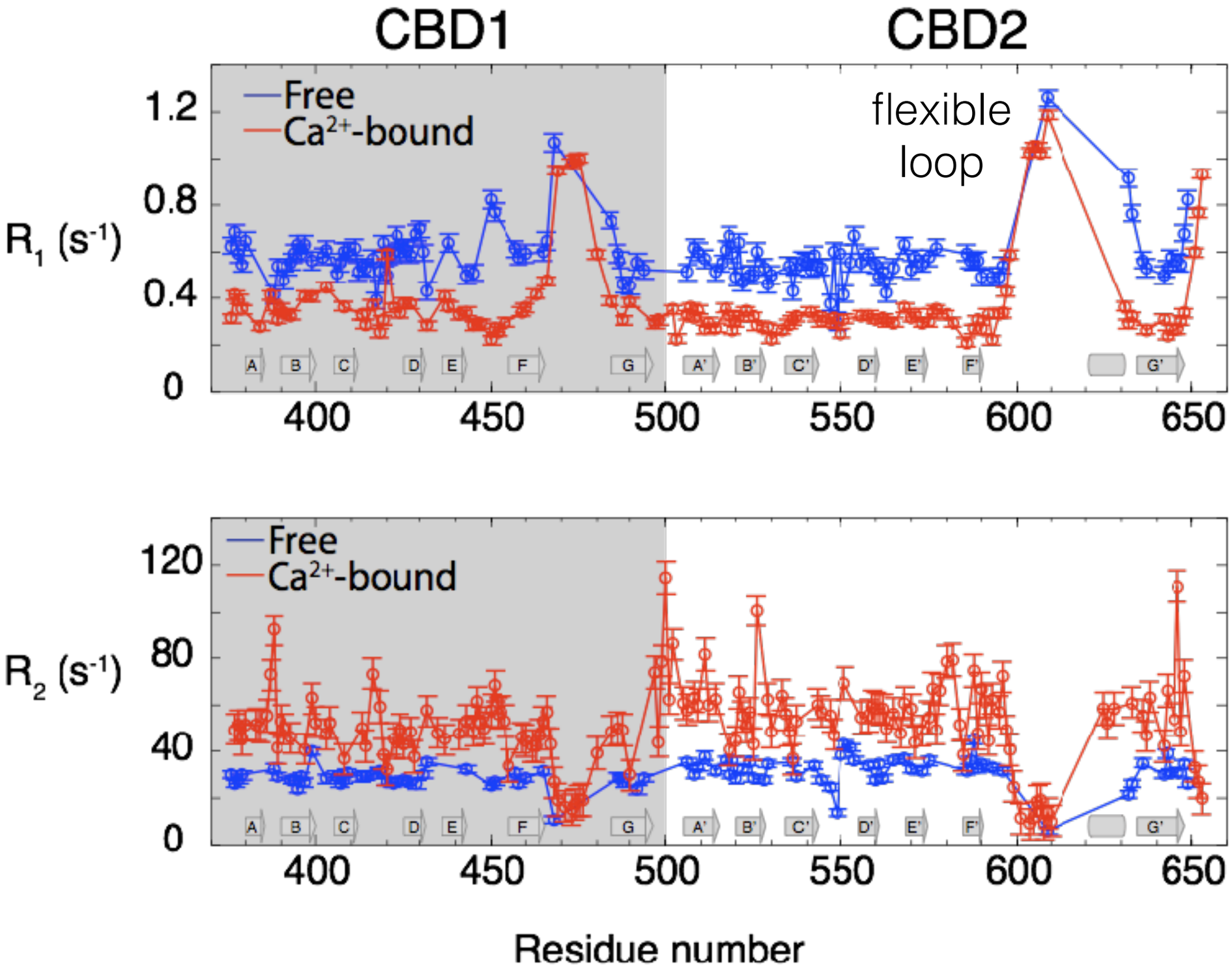


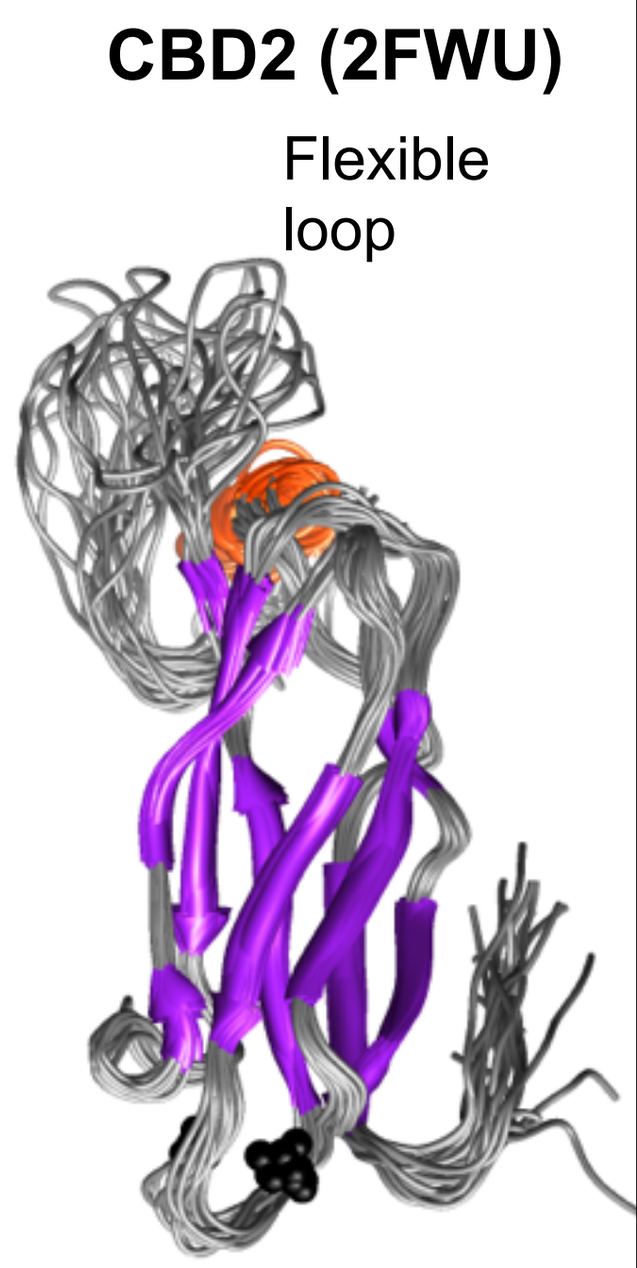
+ Ca^{2+}



Salinas et al. JBC (2011)

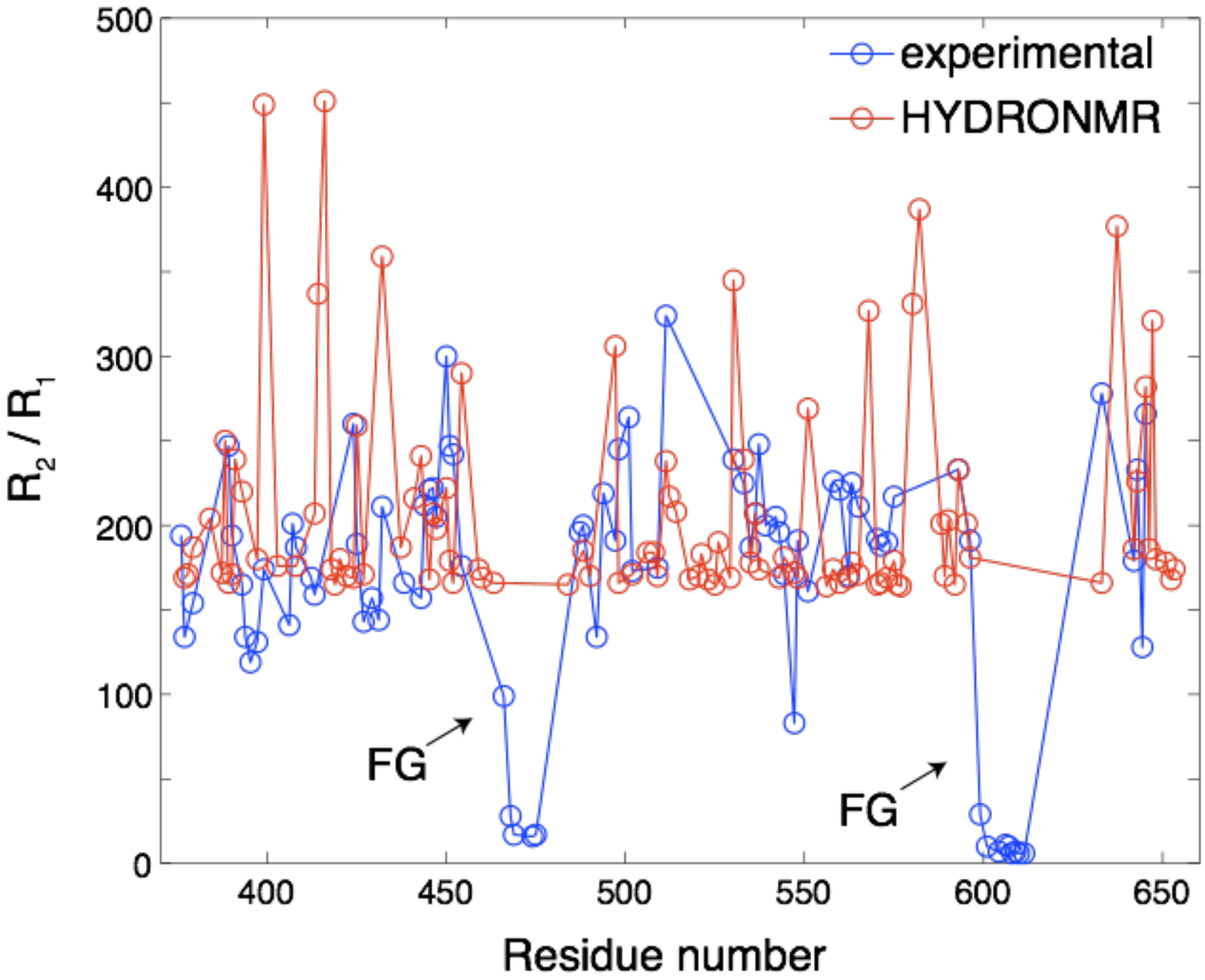
Backbone ^{15}N longitudinal (R_1) and transverse (R_2) relaxation rates





Salinas et al. JBC (2011)

The ratios ^{15}N T_1/T_2 of rigid HN bond vectors are approximately independent of the internal dynamics and reflect the overall tumbling correlation time



Salinas et al. JBC (2011)

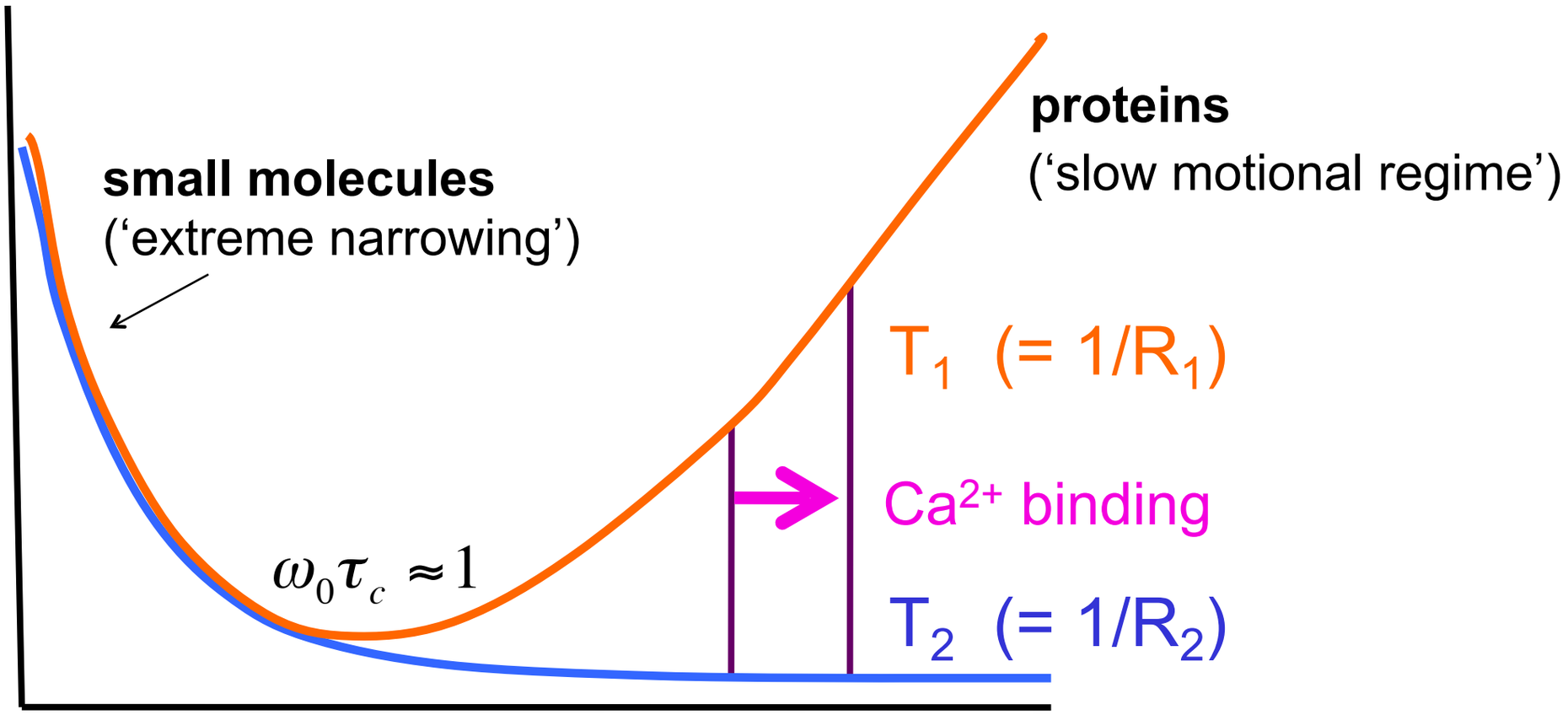
The ratio of R2/R1 for rigid HN bond vectors allows an estimation of the isotropic overall tumbling time

CBD12	CBD1: τ_c (ns)	CBD2: τ_c (ns)
apo state	16.9 ± 1.7	18.7 ± 1.4
Ca^{2+}-bound CBD12	30.2 ± 4.6	33.3 ± 3.8
Isolated domains (apo states)	11.03 ± 0.02	11.4 ± 2.3

➔ Overall tumbling correlation time of CBD12 ~doubles upon Ca^{2+} binding

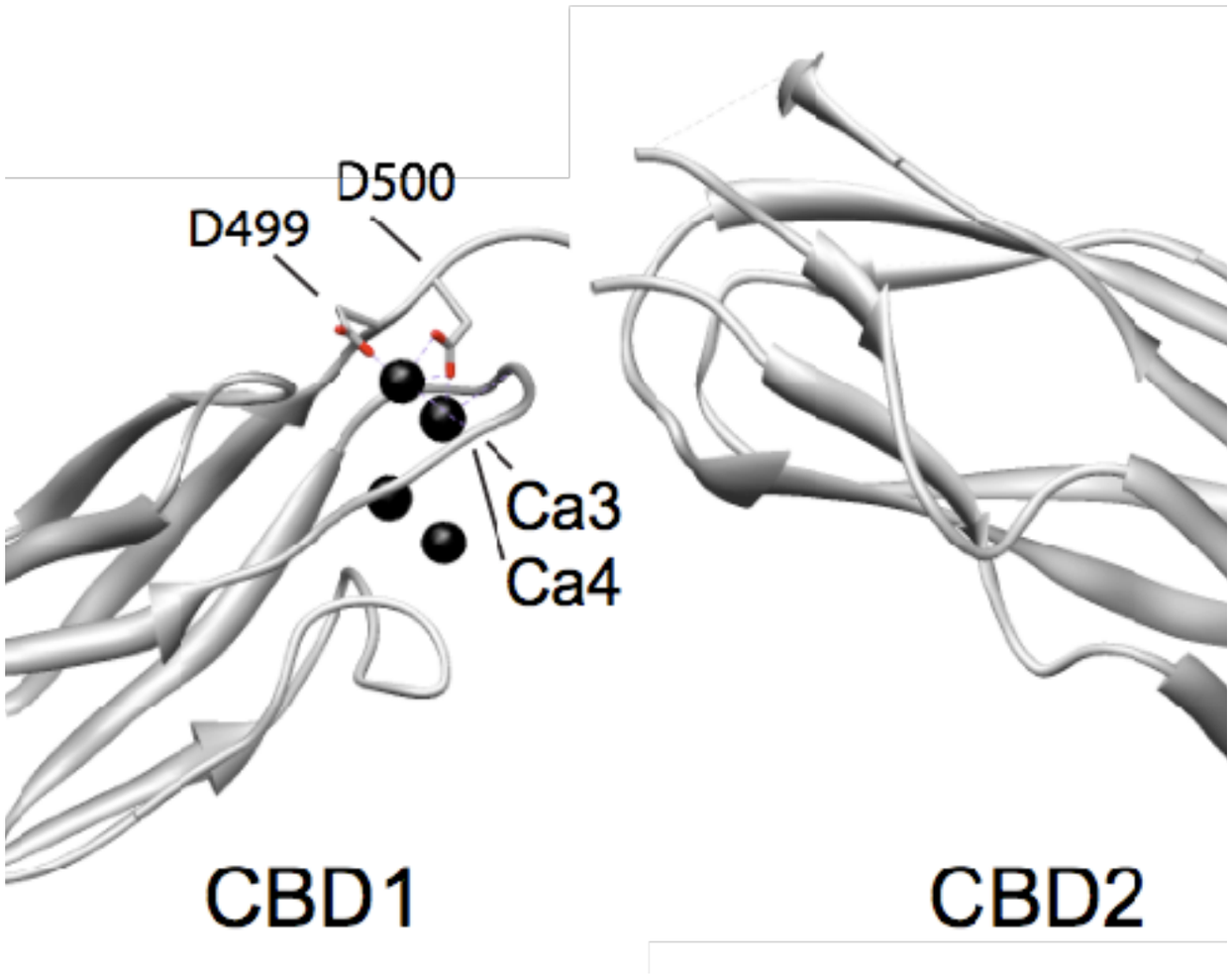
Salinas et al. JBC (2011)

Dependence of T_1 and T_2 on molecular size



Ca^{2+} binding substantially slows down overall tumbling dynamics

Ca^{2+}-binding rigidifies the linker and increases the dynamic coupling between CBD1 and CBD2



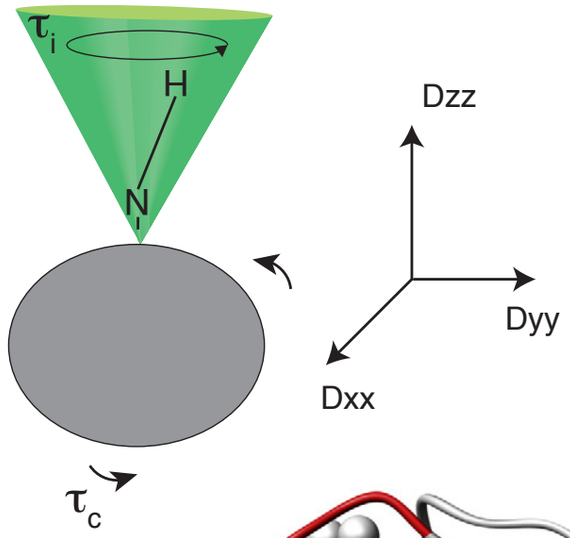
Quantitative analysis: The model-free formalism

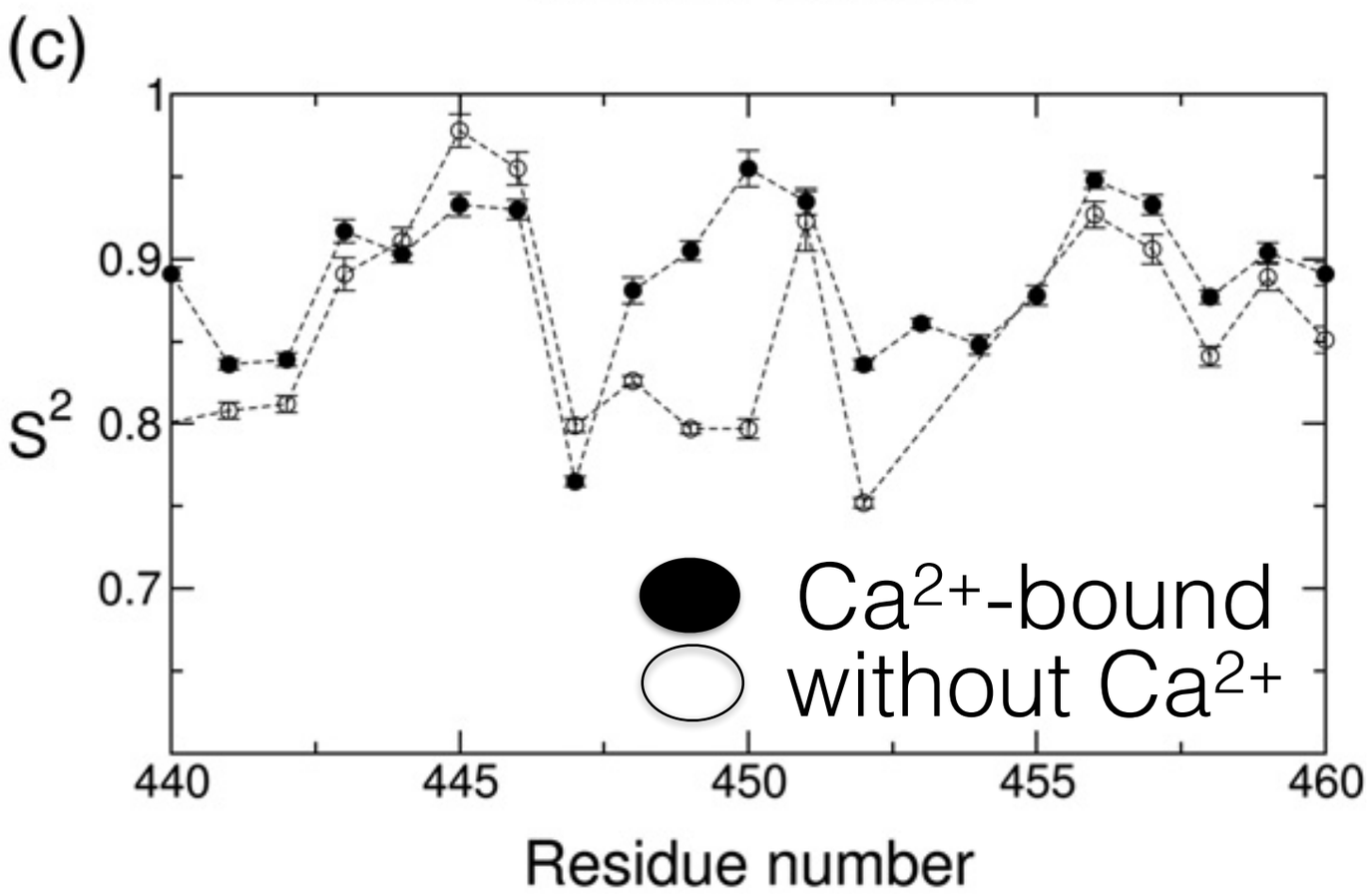
$$J(\omega) = \frac{2}{5}\left[S^2 \frac{\tau_c}{1+(\omega\tau_c)^2} + (1-S^2)\frac{\tau_i}{1+(\omega\tau_i)^2} \right]$$

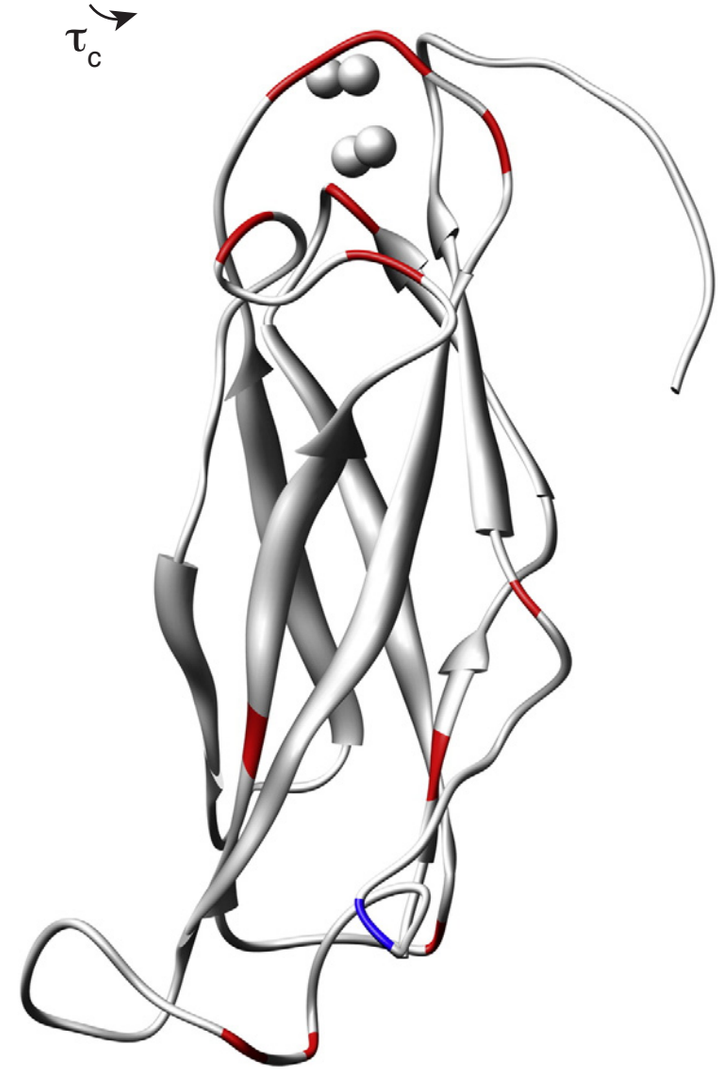
isotropic tumbling

$$\tau^{-1} = \tau_c^{-1} + \tau_i^{-1}$$

$$C_{total}(t) = C_{overall}(t).C_{Internal}(t)$$





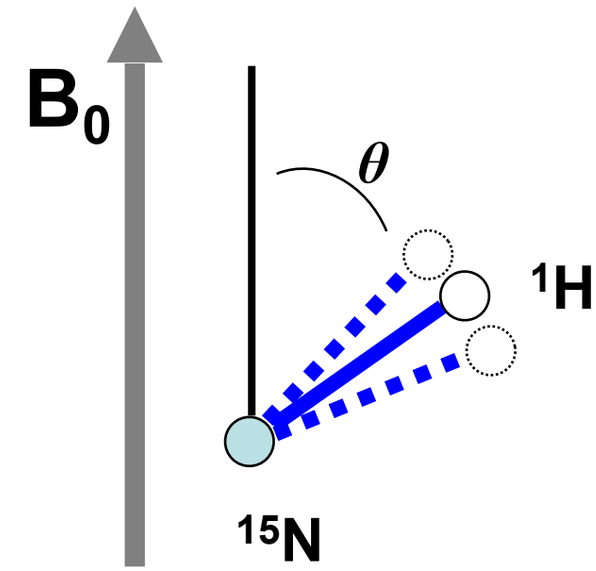


Johnson et al. (2008) J. Mol. Biol. 377: 945
Lipari and Szabo (1982) JACS 104: 4546

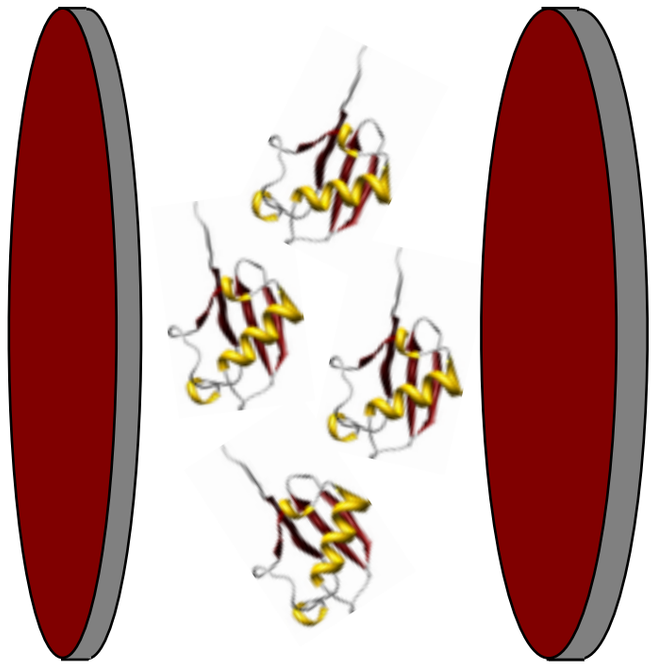
Residual dipolar couplings (RDCs)

$$\Delta\omega = \frac{\hbar\gamma_I\gamma_S}{r^3}\left(3\cos^2\theta(t)-1\right) = D_{\max}\frac{1}{2}\left(3\cos^2\theta(t)-1\right)$$

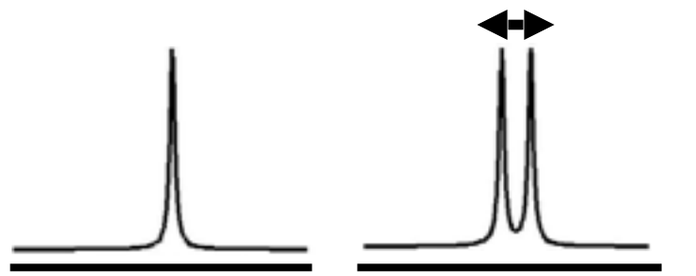
D_{max}~21 kHz for a $^1H^{15}N$ bond vector



$$D \propto \left\langle (3\cos^2\theta - 1)/2 \right\rangle$$



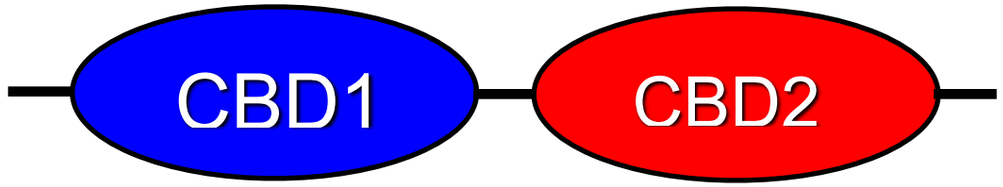
weakly aligned



Isotropic Solution ν **Weak Alignment** ν

- **RDCs reflect both average structure *AND* fast & slow motions:**
  - ps – ms motions
  - no explicit time-scale dependence

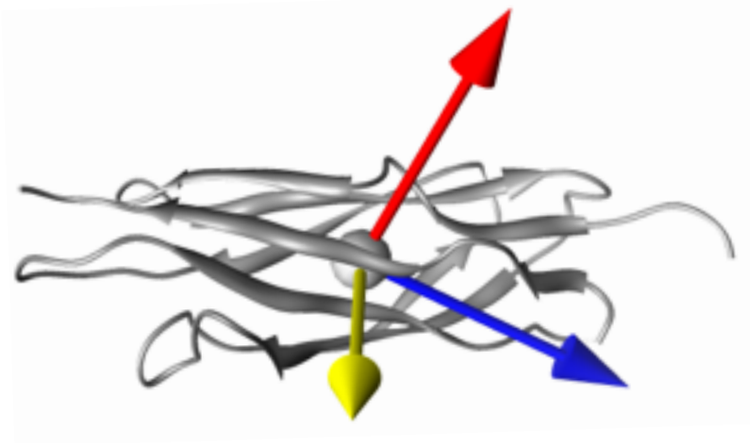
One-bond backbone ^{1}H-^{15}N residual dipolar couplings (RDCs) of CBD12 partially aligned in compressed polyacrylamide gel

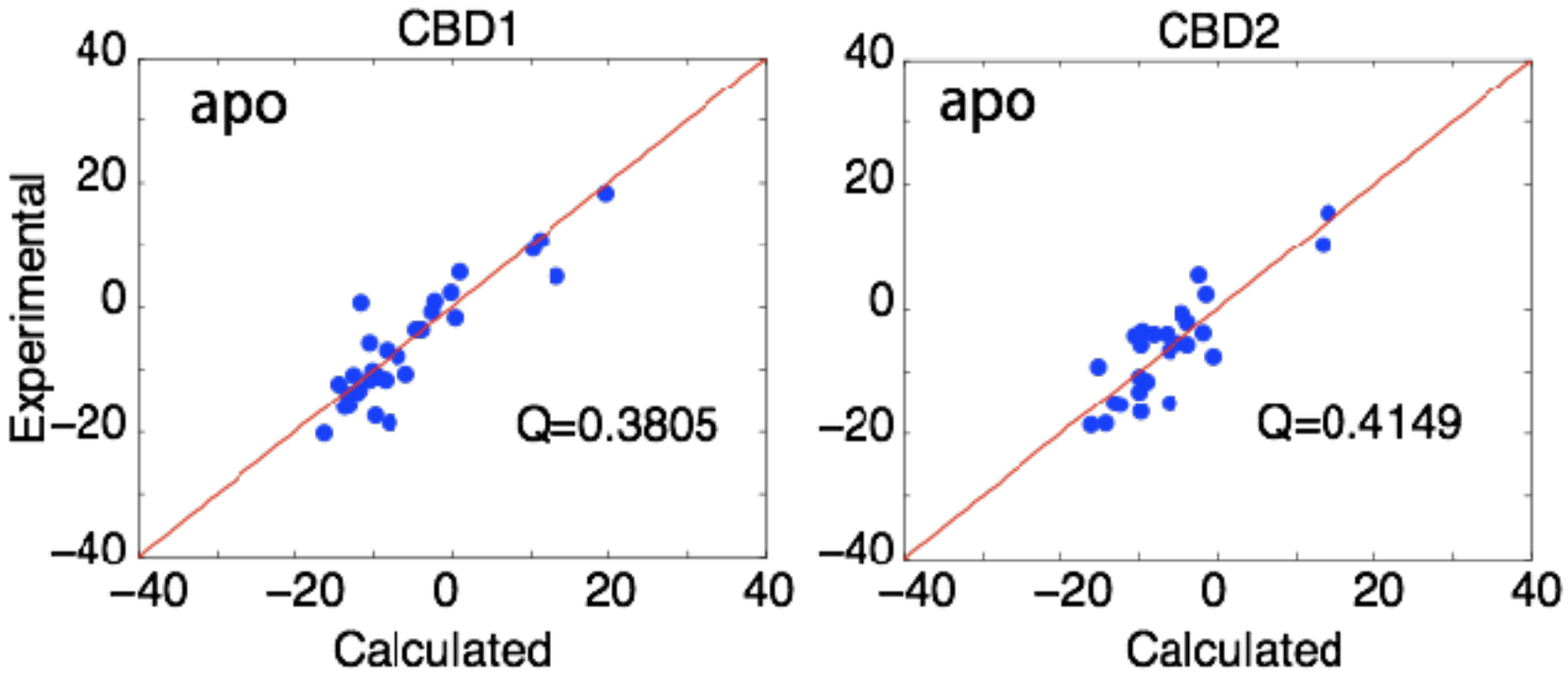


$$D^{NH} = D_{\max}\left[A_a \frac{1}{2}\left(3\cos^2(\theta) - 1\right) + A_r \frac{3}{4}\sin^2(\theta)\cos(2\phi)\right]$$

$$A_a = \frac{3}{2}A_{zz}$$

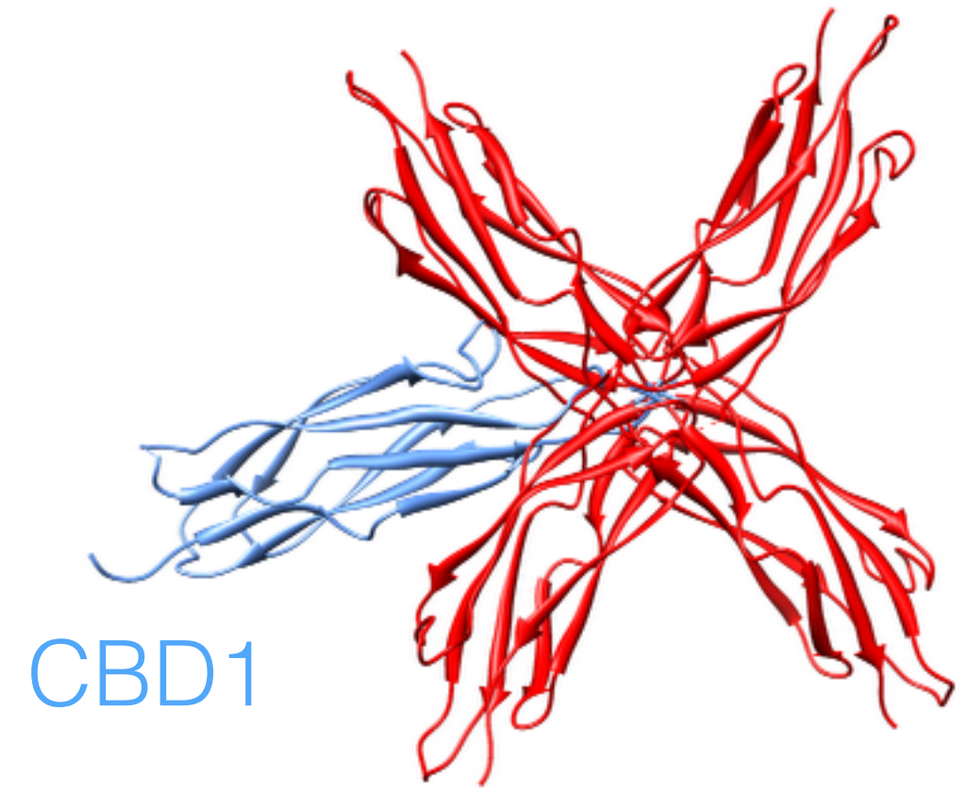
$$A_r = A_{xx} - A_{yy}$$





The ^{1}H-^{15}N dipolar couplings were measured on the two-domain construct, CBD12, and fitted on the crystal structures of isolated CBD1 and CBD2 in order to obtain the alignment tensor

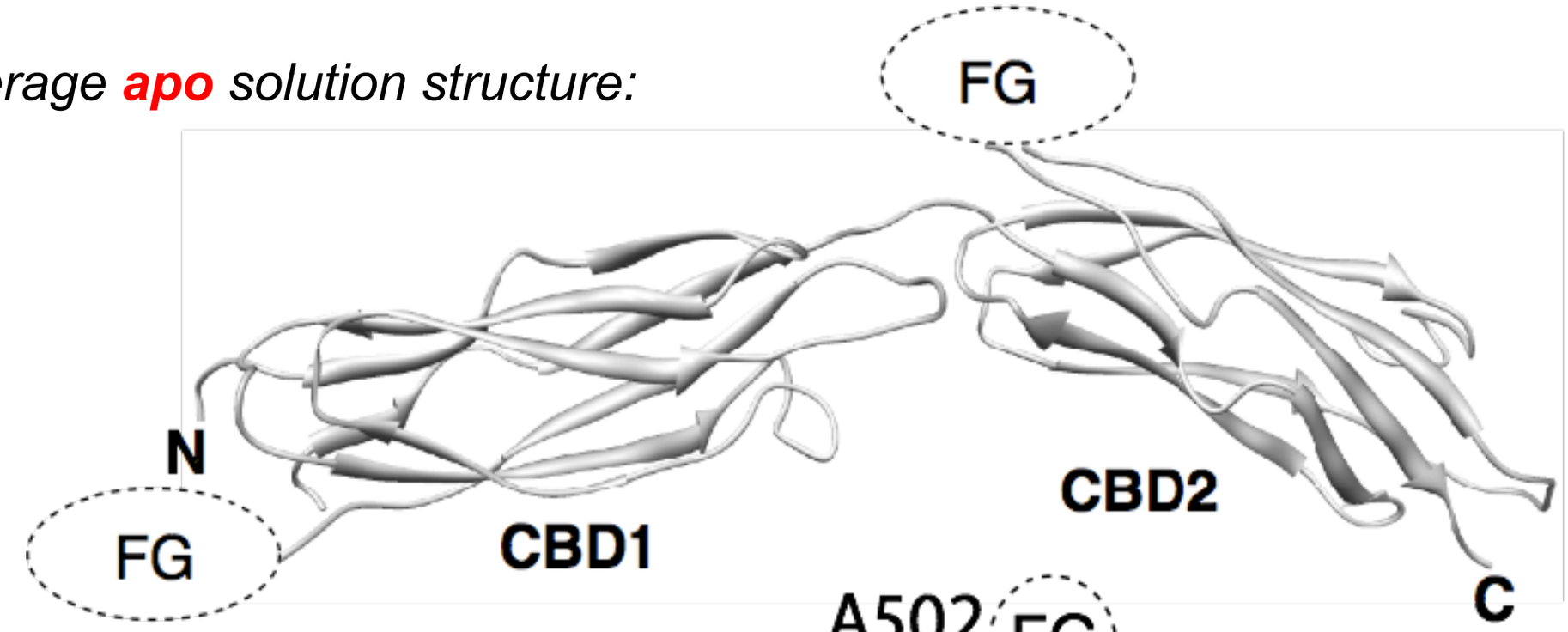
Rotation of CBD1 and CBD2 to the reference frame of the alignment tensor and translation of one with respect to the other generates 4 symmetric solutions



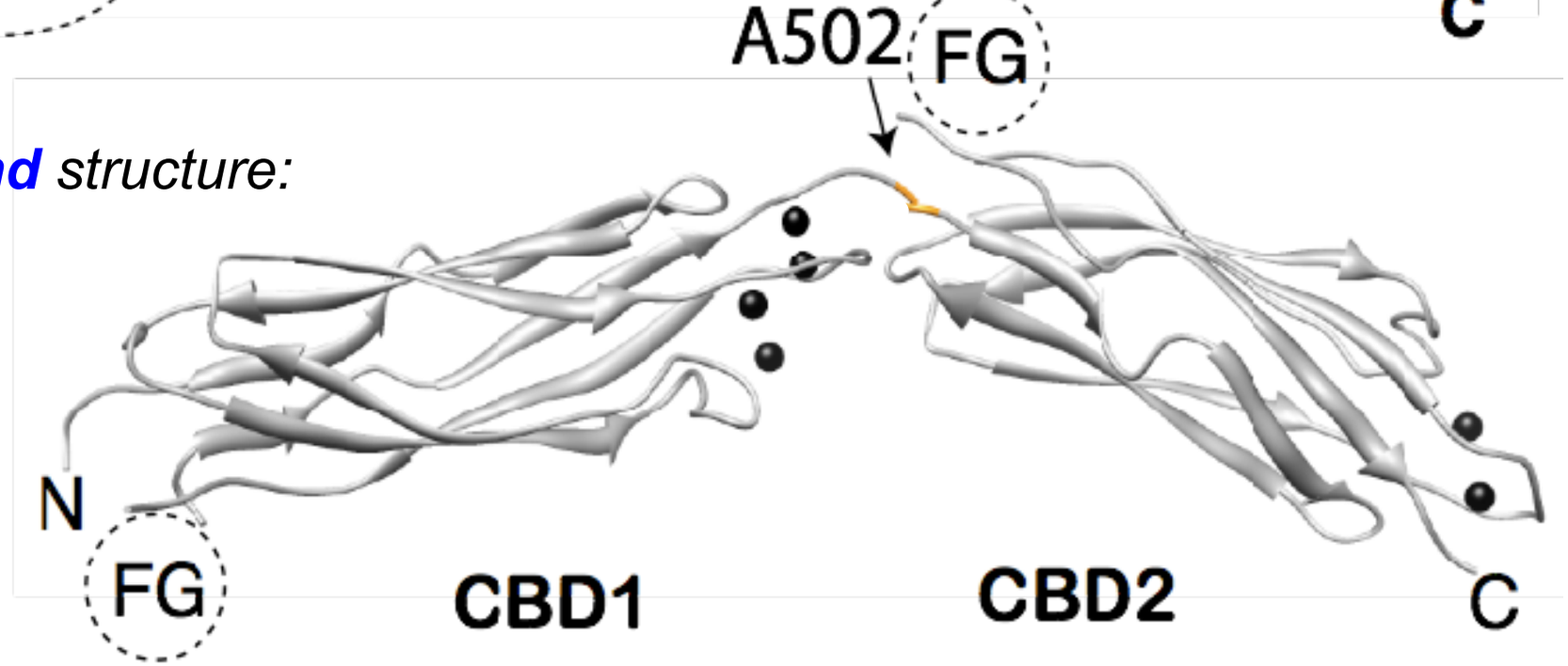
In the absence of data obtained with different alignment tensors we used a steric criteria to decide for the best solution

The time and ensemble average orientation between CBD1 and CBD2 is elongated in the absence and presence of Ca^{2+}

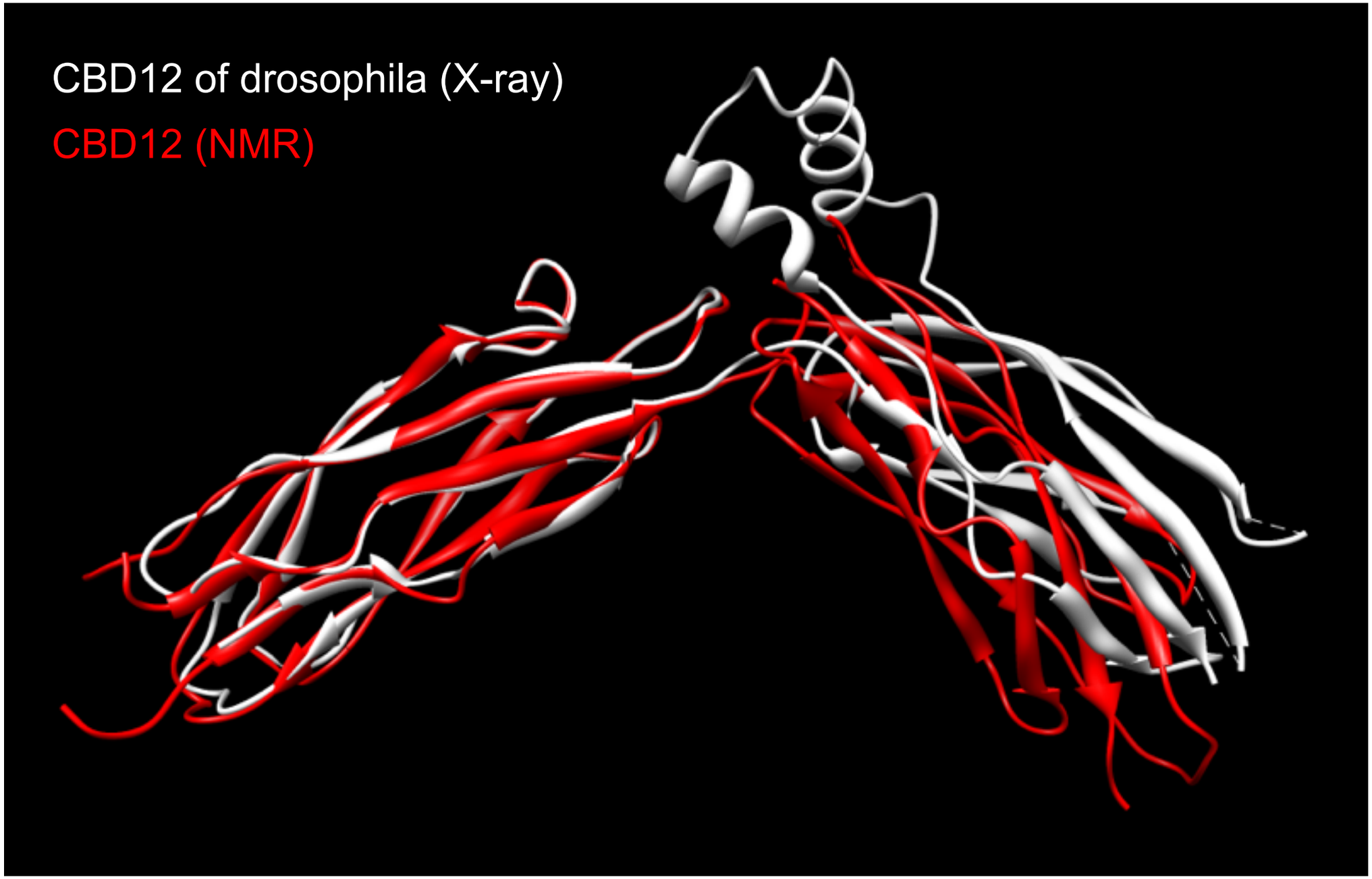
*Average **apo** solution structure:*



***Ca^{2+}-bound** structure:*



Comparison between canine CBD12 and X-ray crystal structure of drosophila CBD12 (both Ca^{2+} bound)



Drosophila CBD12 X-ray crystal structure: Wu *et al.* Structure 19, 1509–1517

RDC alignment tensors in compressed polyacrylamide gel

CBD12	CBD1	CBD2
apo state	Q = 0.38 D_a = 12.23 Hz	Q = 0.41 D_a = 12.04 Hz
Ca^{2+}-bound state	Q = 0.18 D_a = 24.05 Hz	Q = 0.41 D_a = 25.29 Hz

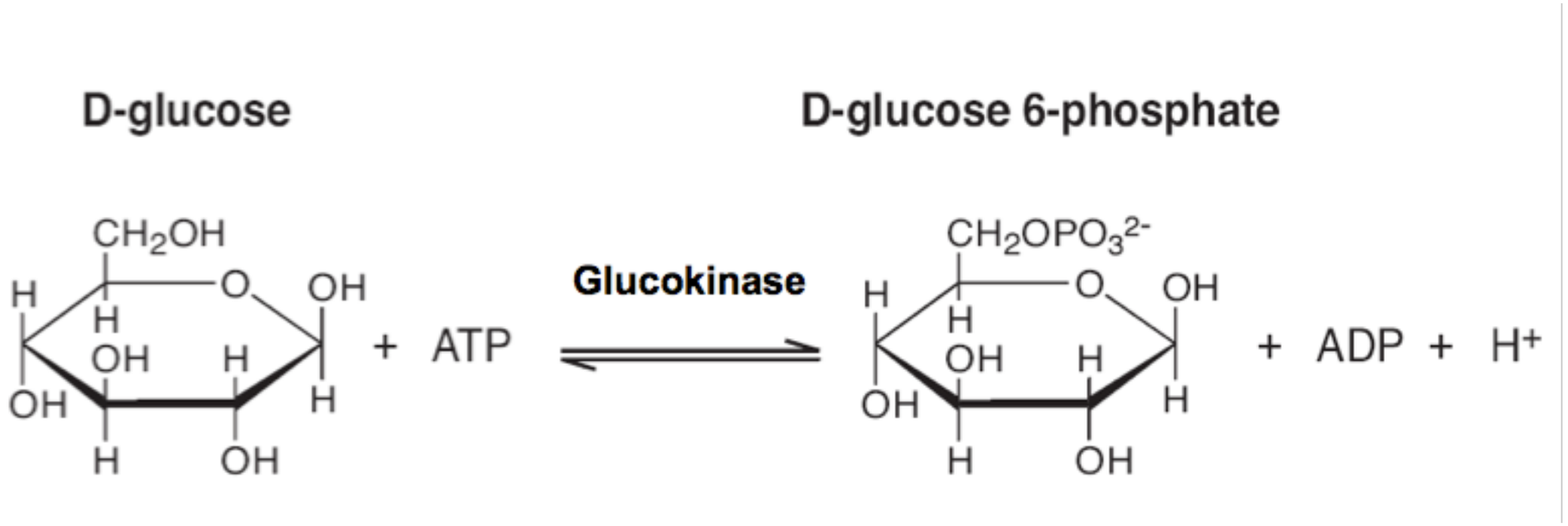
➔ Larger alignment tensor reflects a bulkier protein shape

➔ Bulky shape pertains up to millisecond time scale

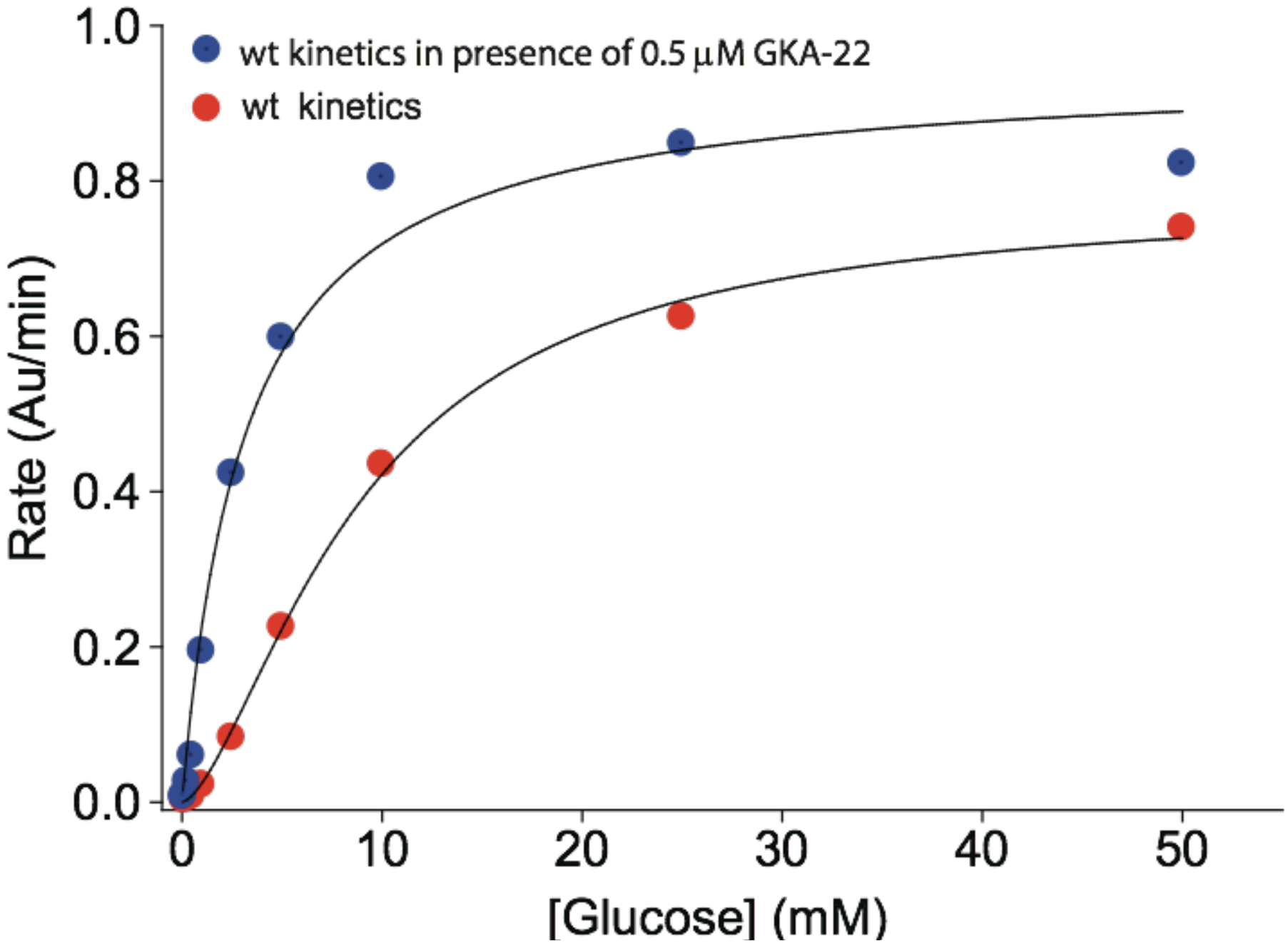
Example 3: Glucokinase

Working with larger proteins: glucokinase

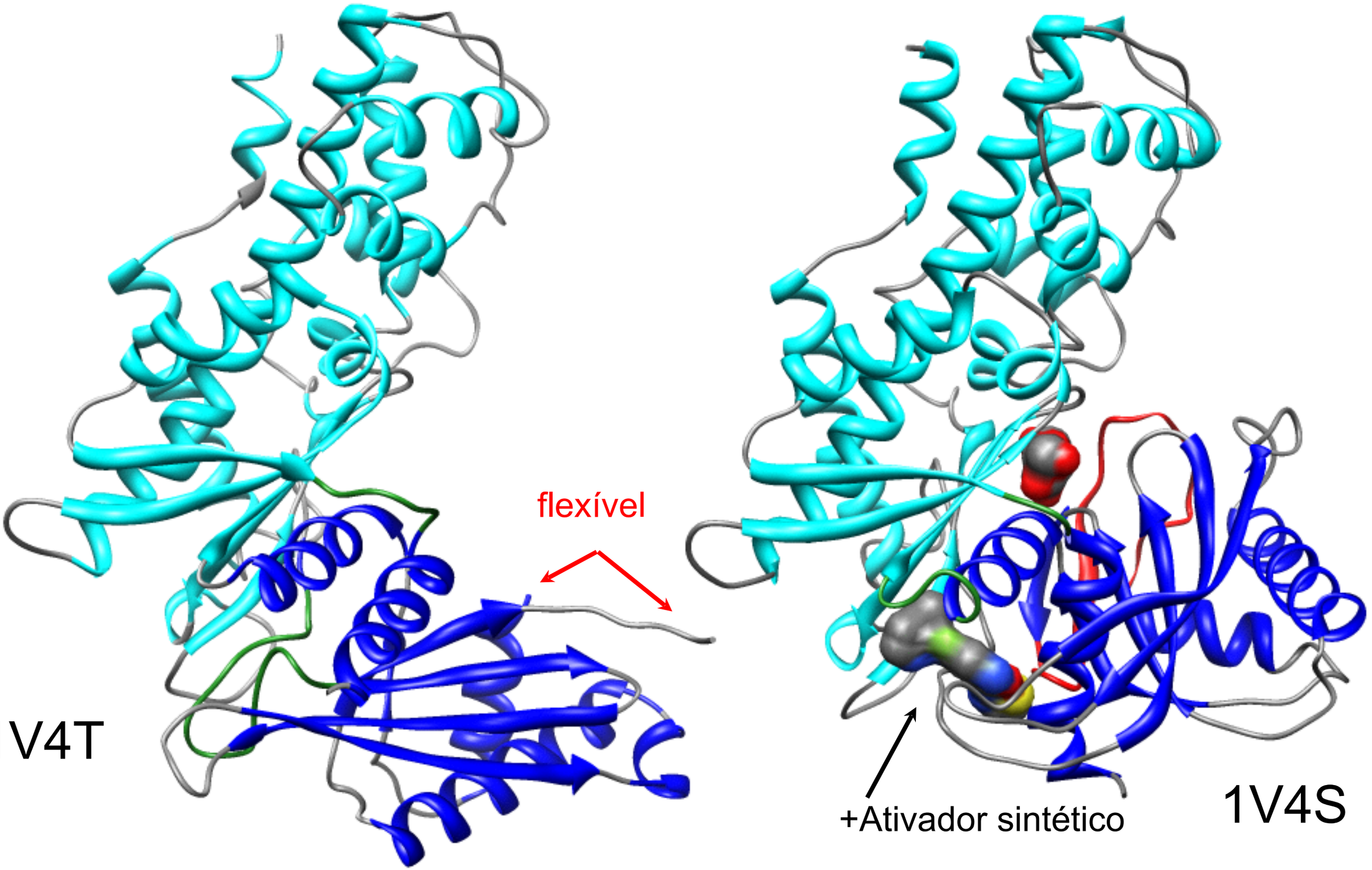
Glicokinase (hexokinase IV) is a key enzyme that catalyses the phosphorylation of glucose in the pancreas and liver



Glucokinase is a monomeric protein that displays kinetic cooperativity

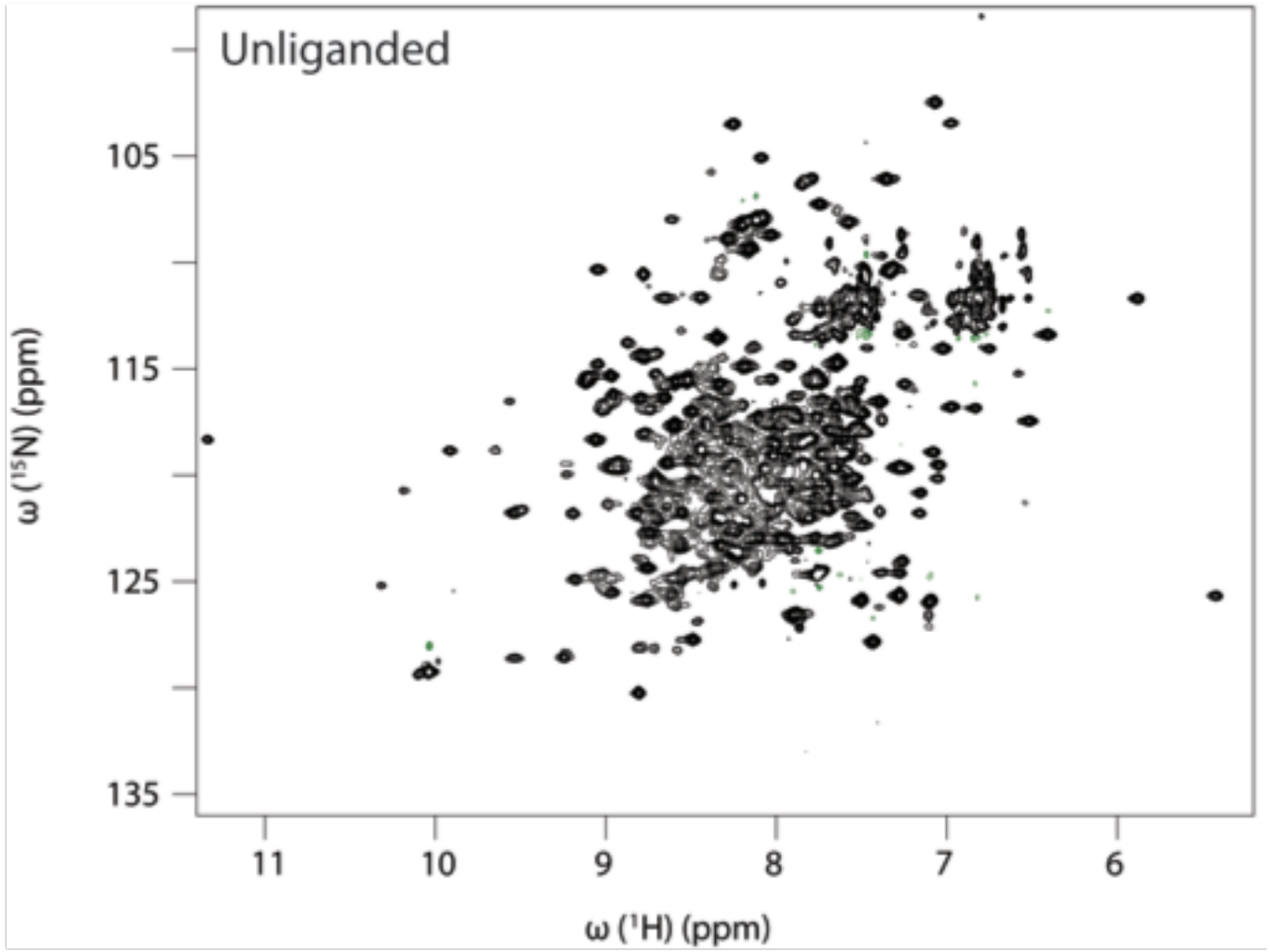


Crystallographic structures show that glucokinase undergoes considerable conformational changes upon glucose binding but this does not fully explain the kinetic cooperativity

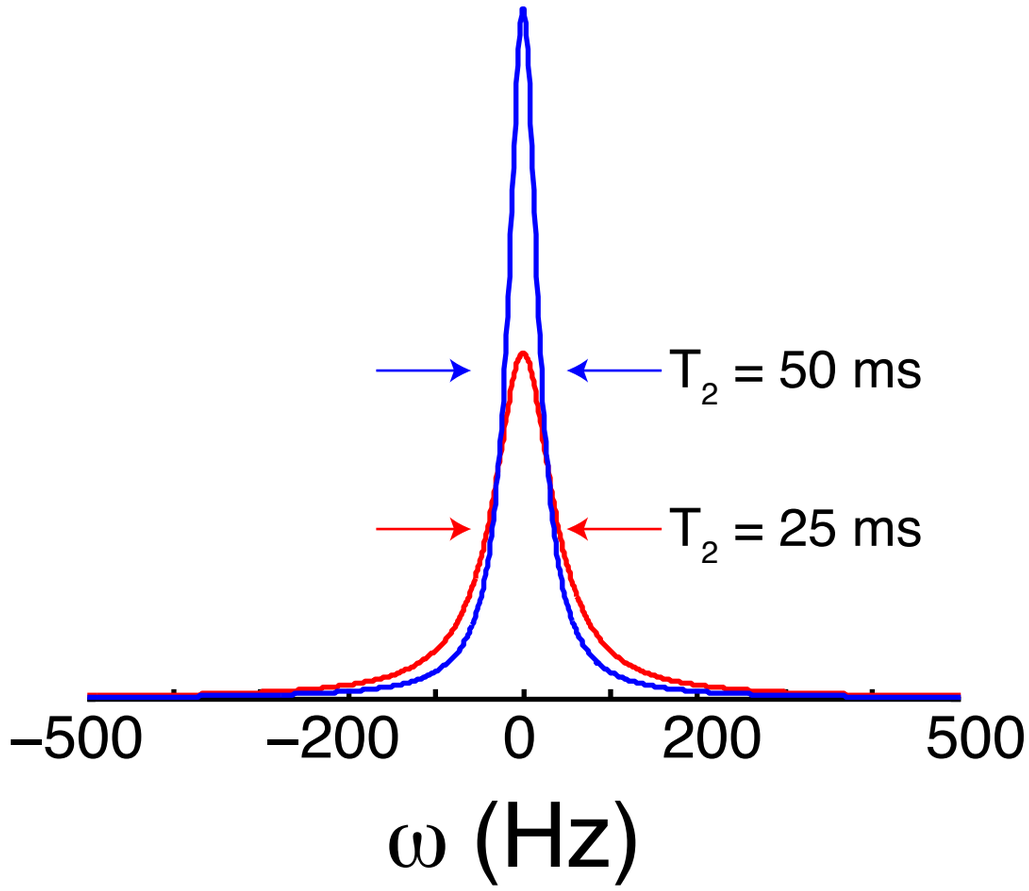


Kamata et al. (2004) *Structure* **12**: 429-438

NMR studies of large proteins such as glucokinase (52 kDa) suffer from severe spectral overlapping in addition to faster T2 relaxation

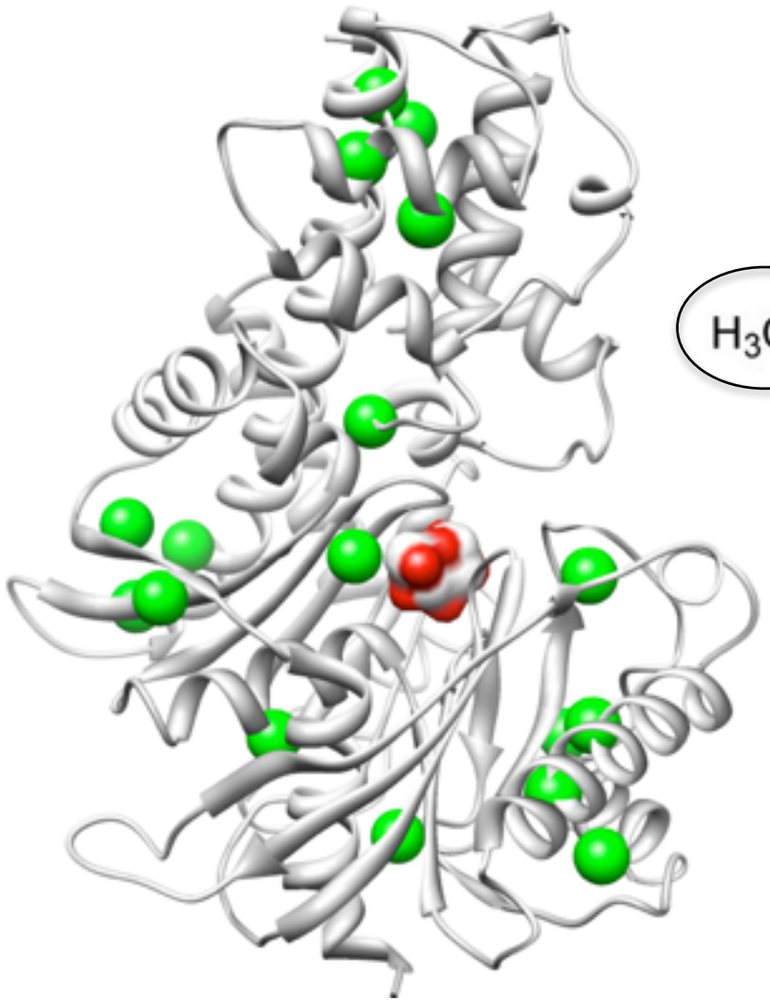


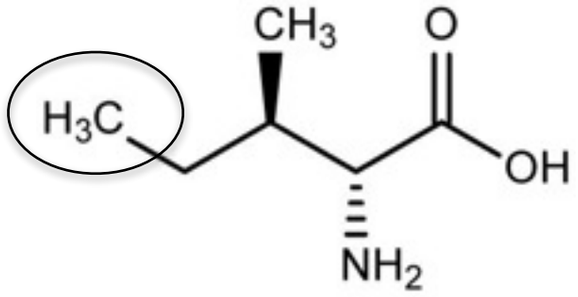
Glucokinase (Apo) ^{15}N-HSQC

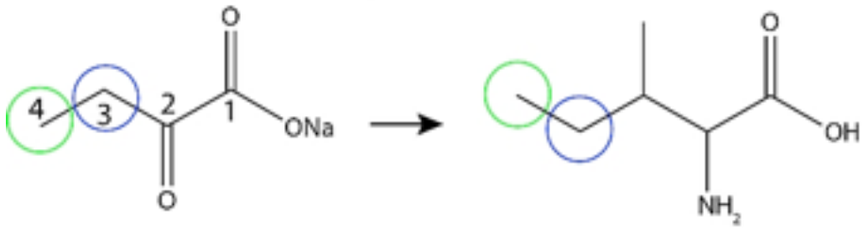


Larion, Salinas, Bruschweiler, Brüschweriler e Miller (2010) *Biochemistry* **49**: 7969-7971

The spectral complexity can be alleviated by introducing selective labels

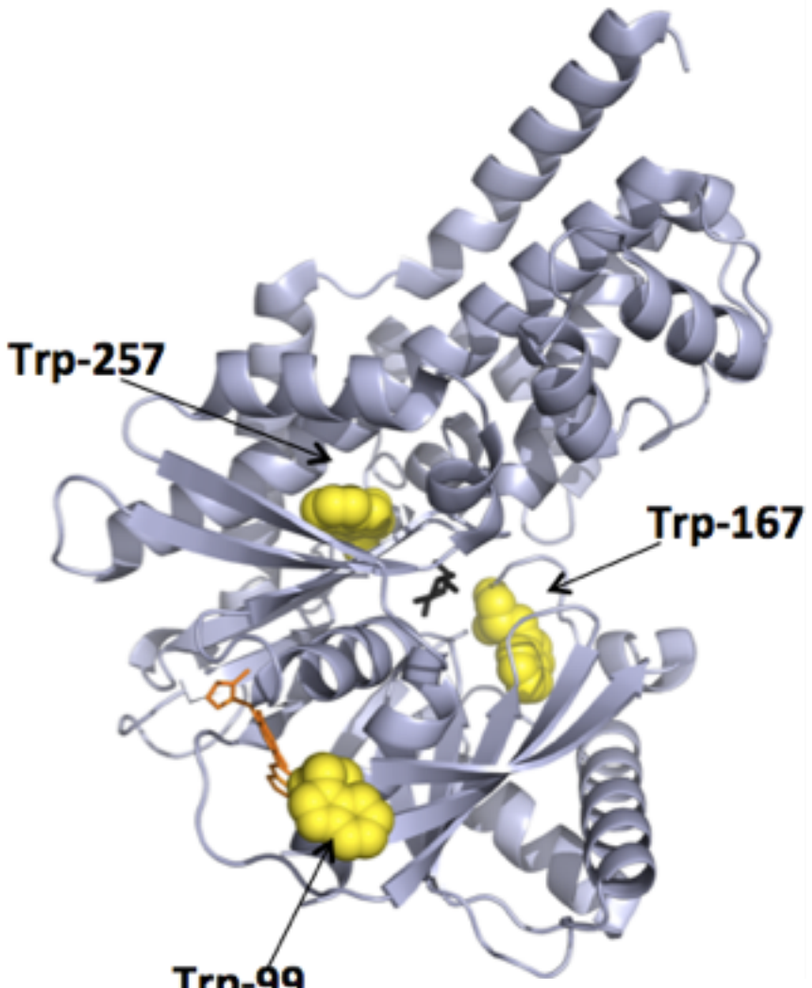


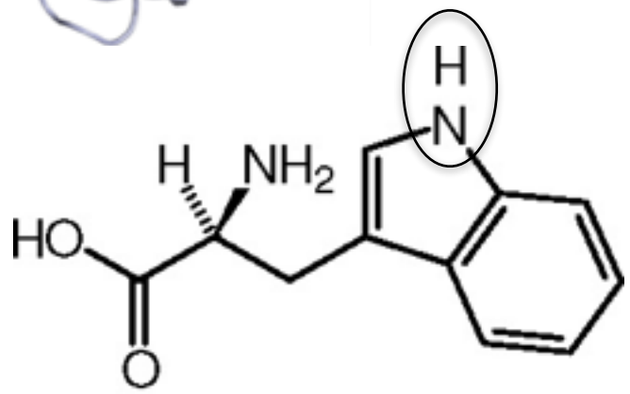




2-Ketobutyric acid Isoleucine

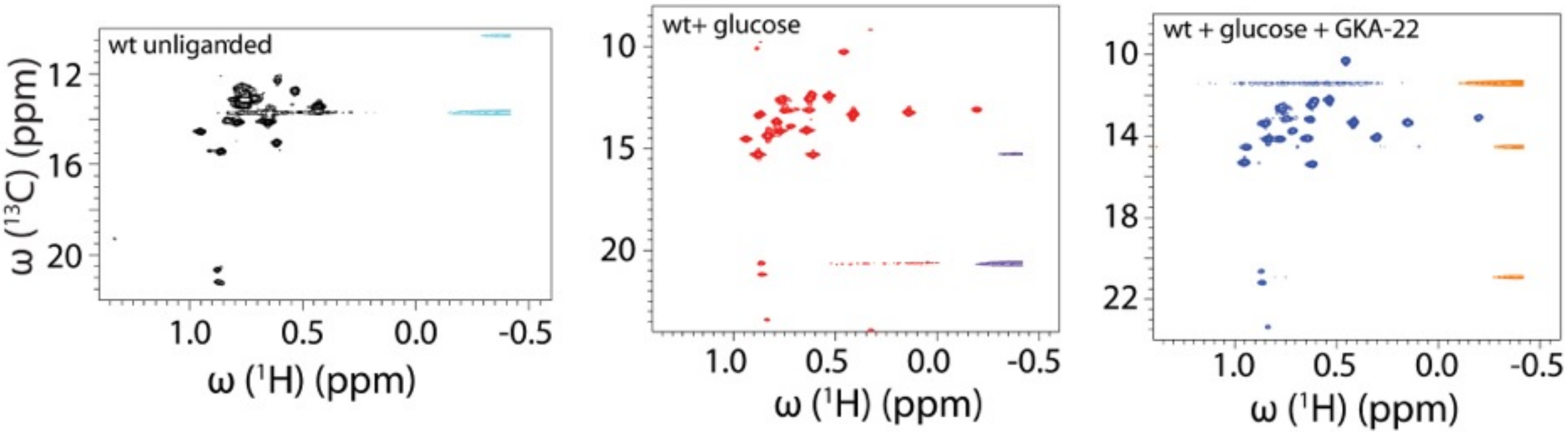
The use of alpha-keto acids, such as alpha-keto butyric acid labeled with ^{13}C at position 4, is a good strategy to label specifically methyl groups



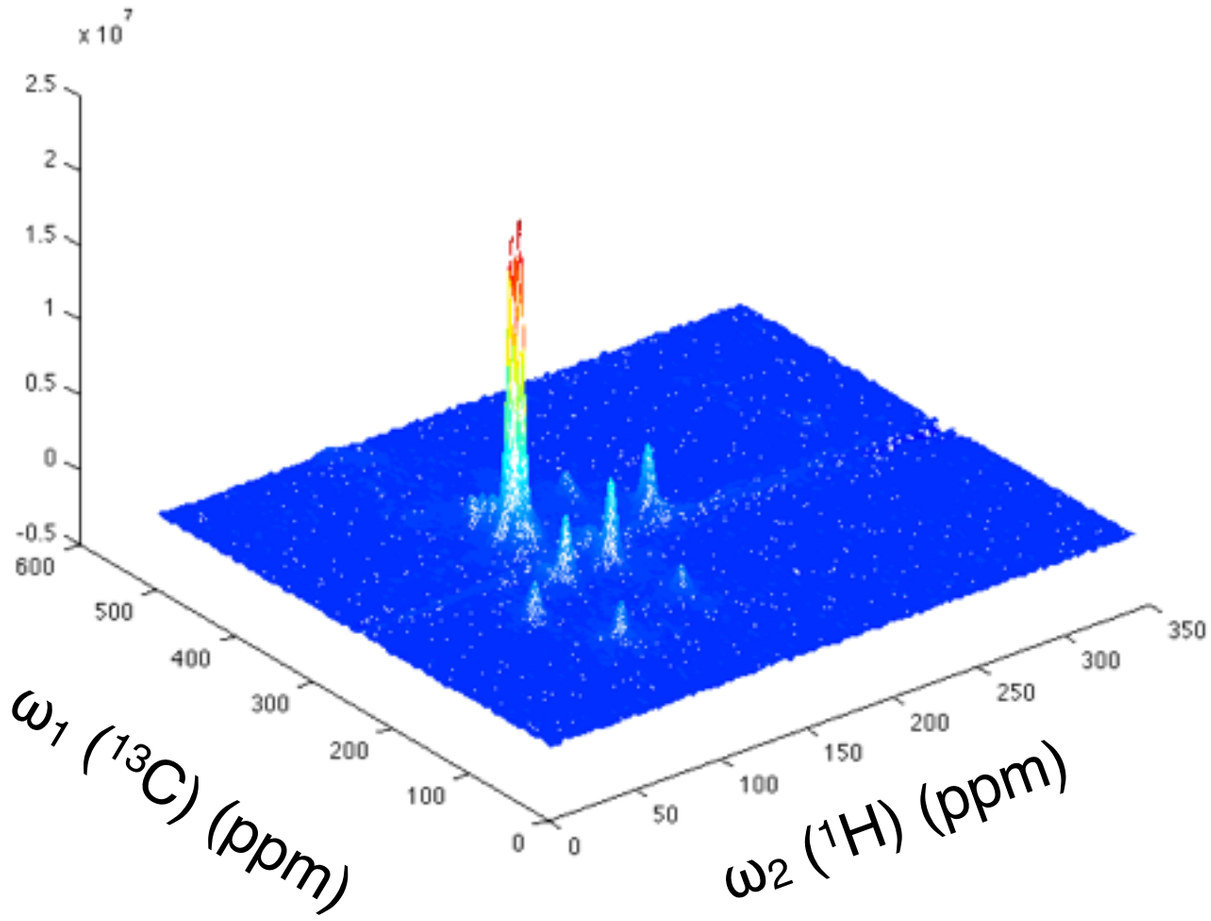


L-tryptophan

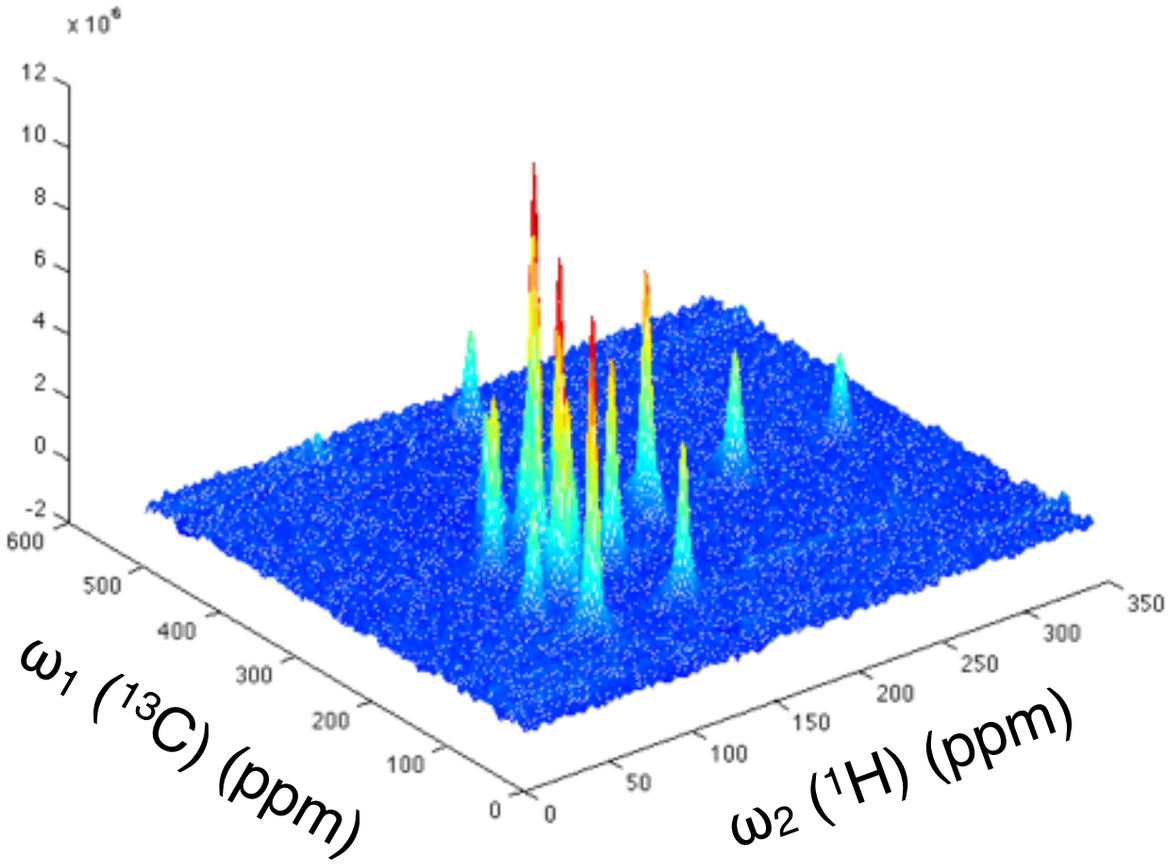
1H-13C HMQC spectra of glucokinase in the absence and presence of glucose and glucose + activator



The binding of glucose to glucokinase leads to a drastic change on the 1H-13C HMQC spectrum

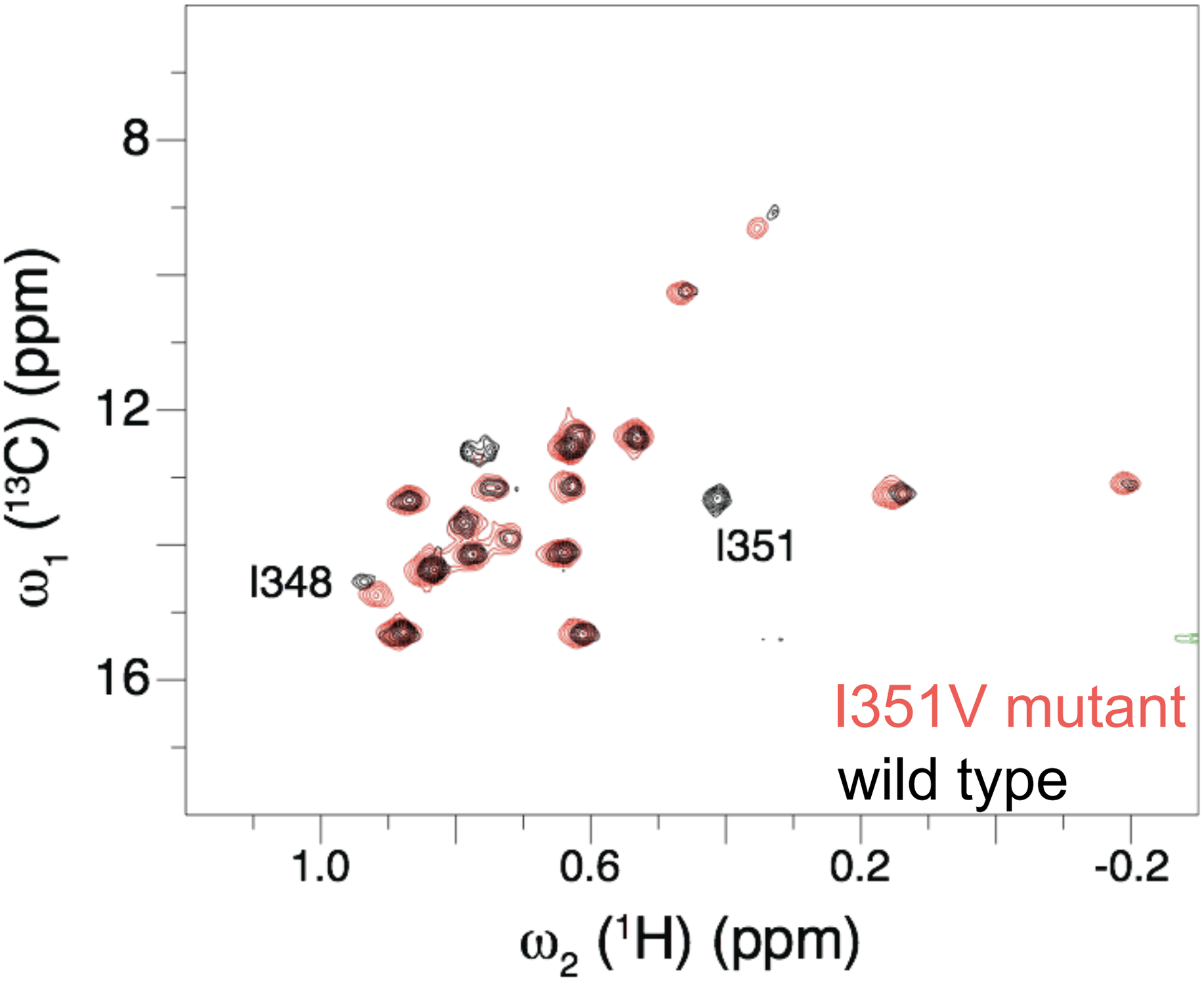


unliganded

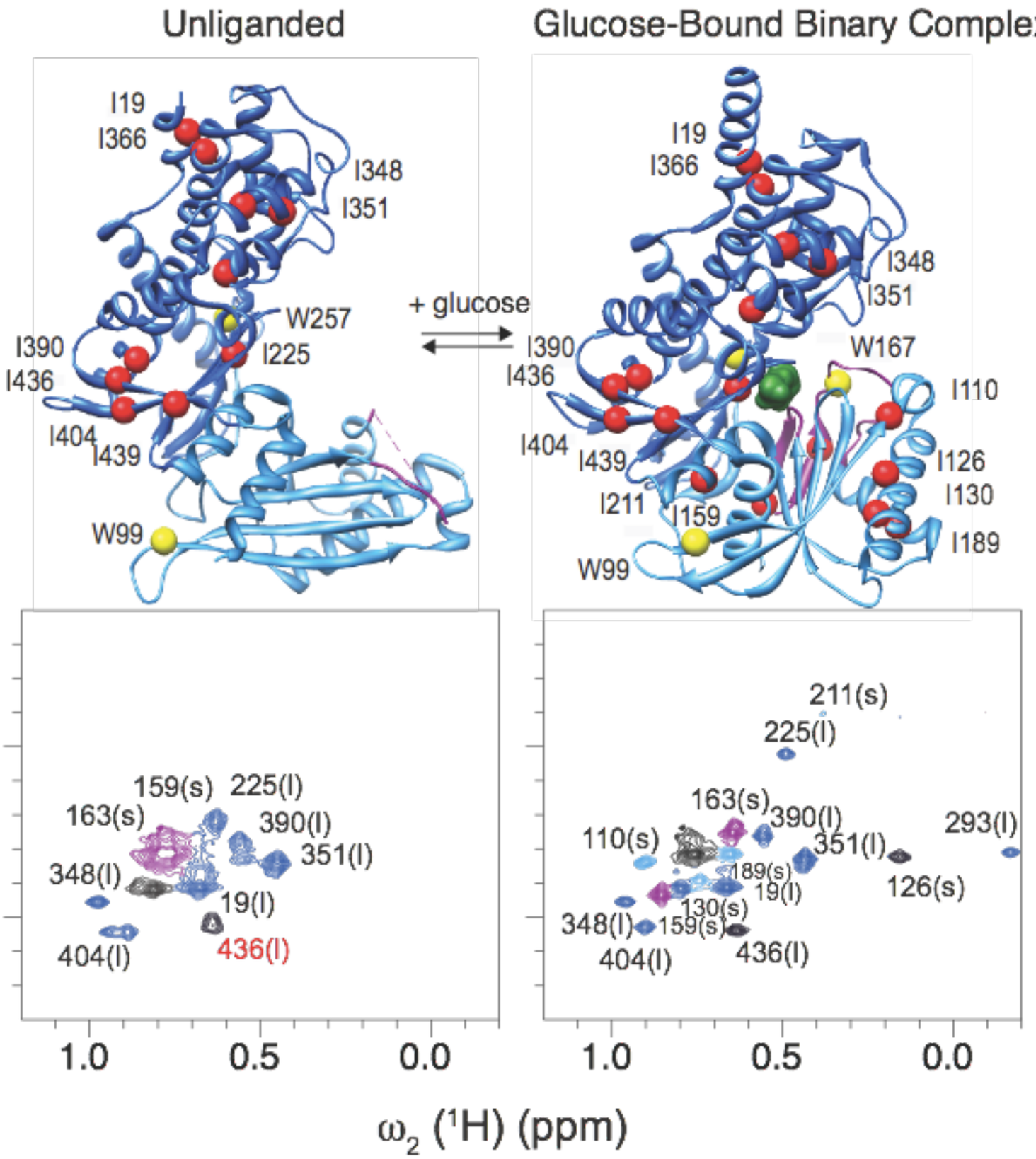


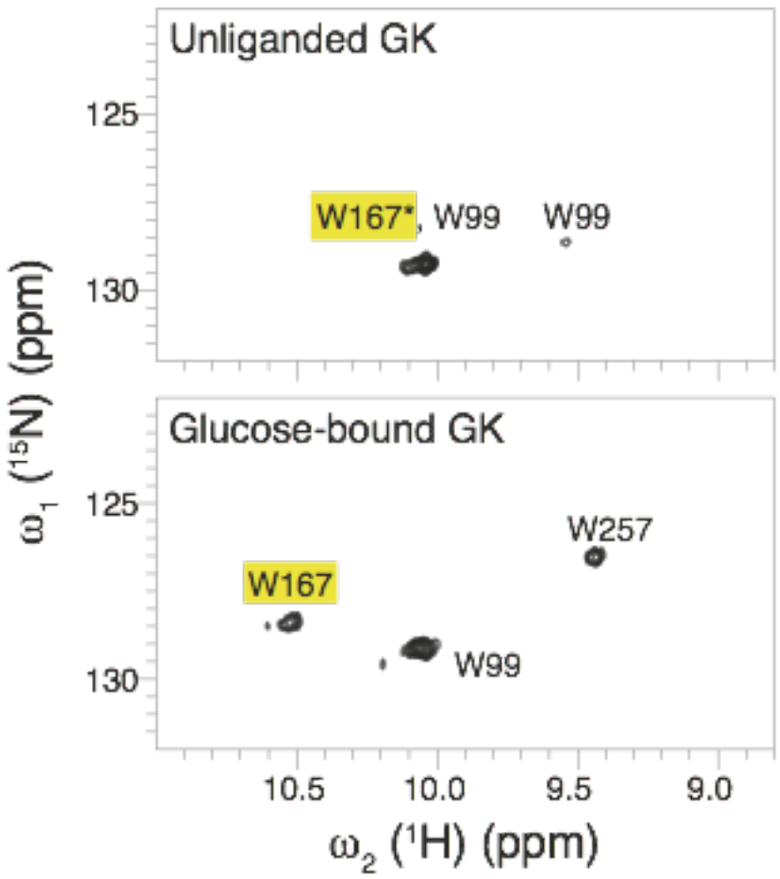
+glucose

Assignments for the cross-peaks of isoleucine methyl groups were obtained from site-directed substitutions to valine

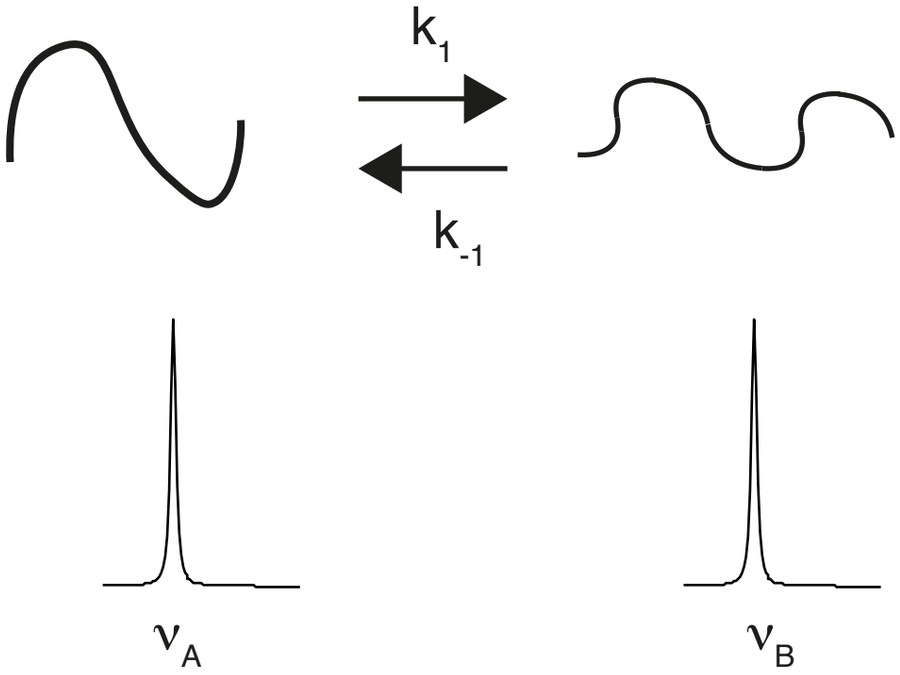


Glucose binding leads to the appearance of signals from isoleucines located at the small domain



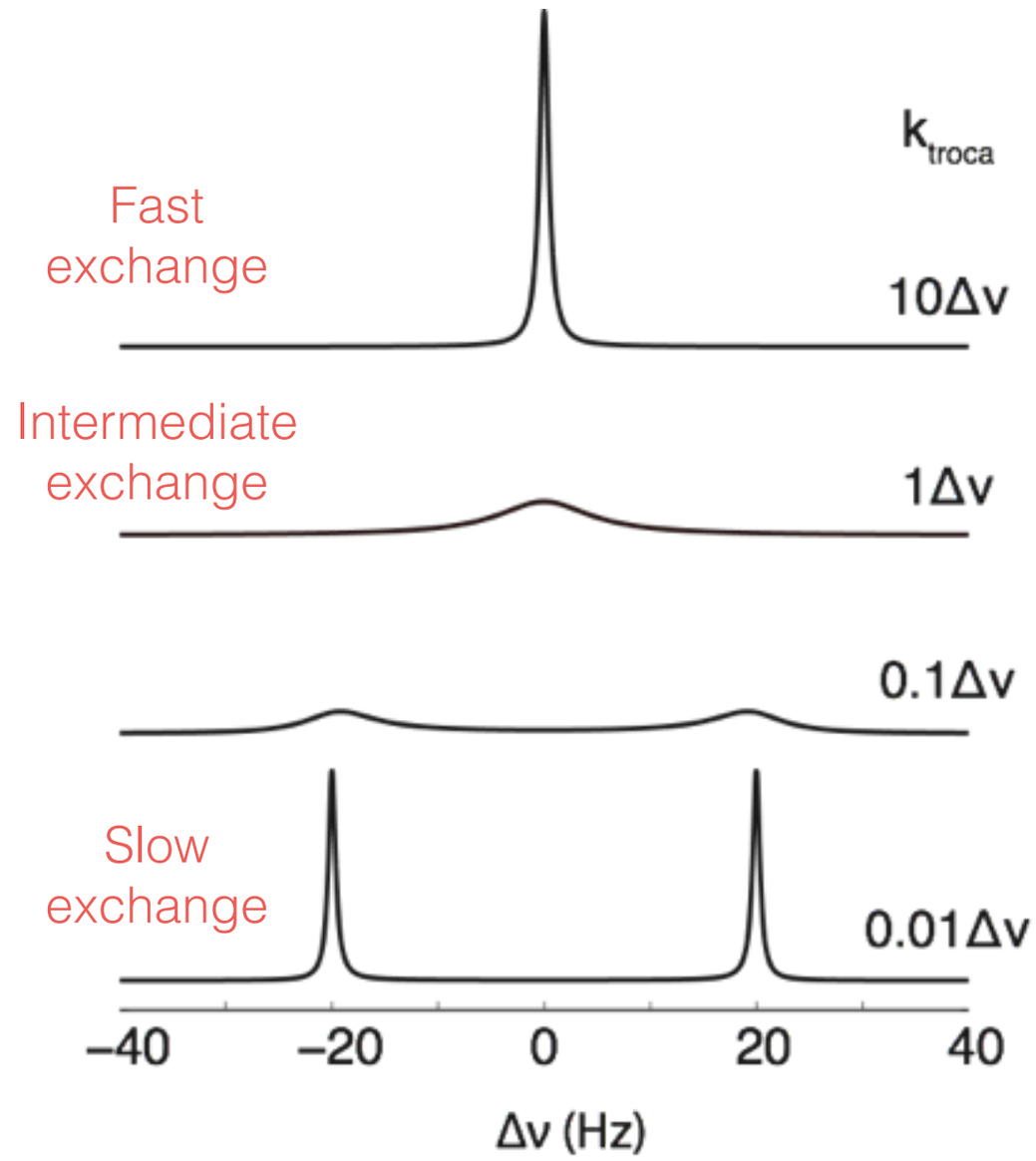


Conformational eExchange between two conformations will cause a time modulation of the chemical shift affecting the spectral line shape

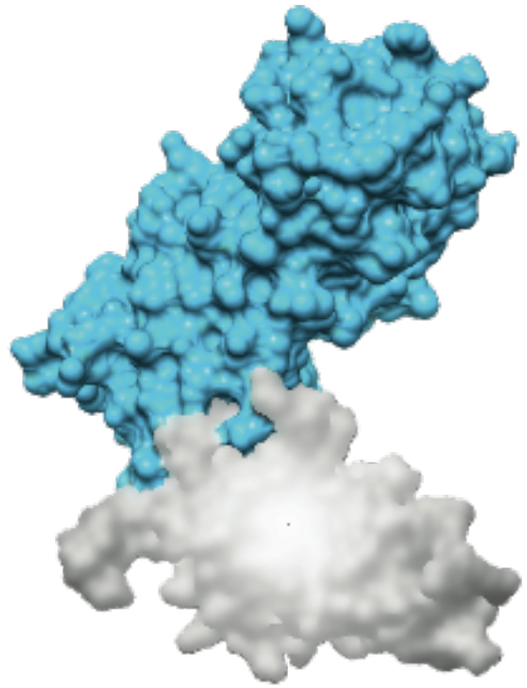


$$k_{ex} = k_1 + k_{-1}$$

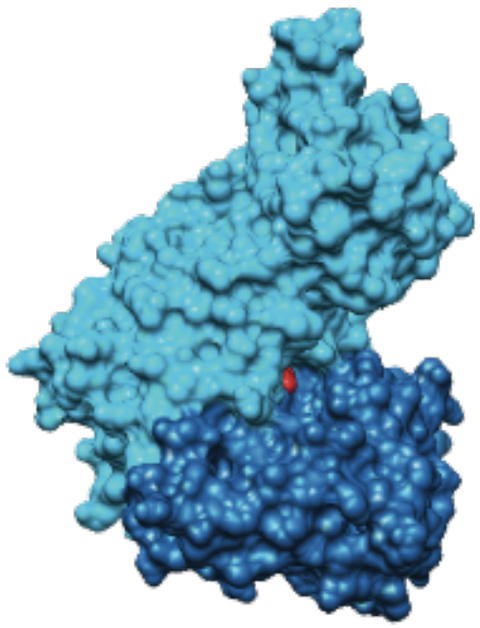
$$\Delta\nu = | \nu_A - \nu_B |$$

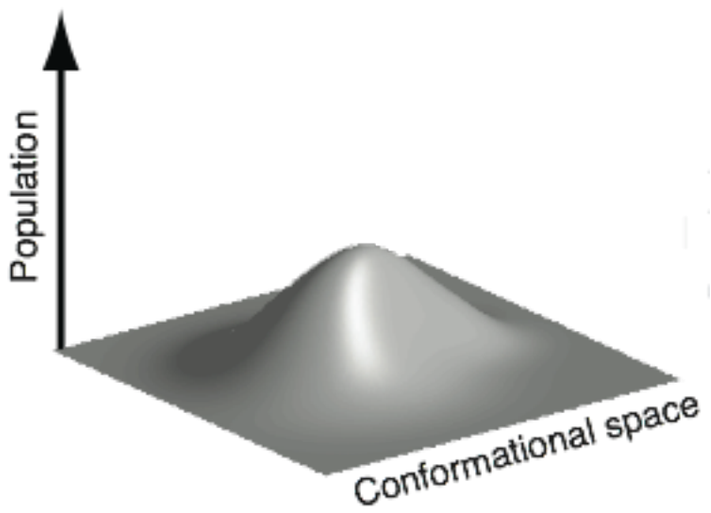


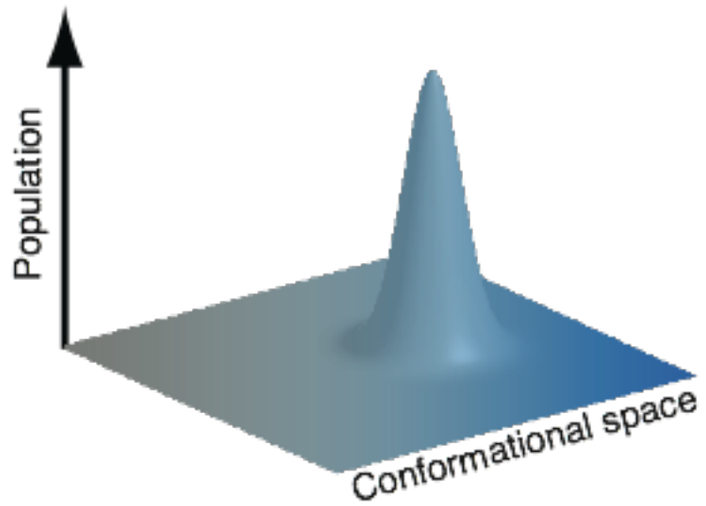
Qualitative interpretation of the data shows that binding of glucose to glucokinase modulates the slow-time scale dynamics at the small domain



glucose

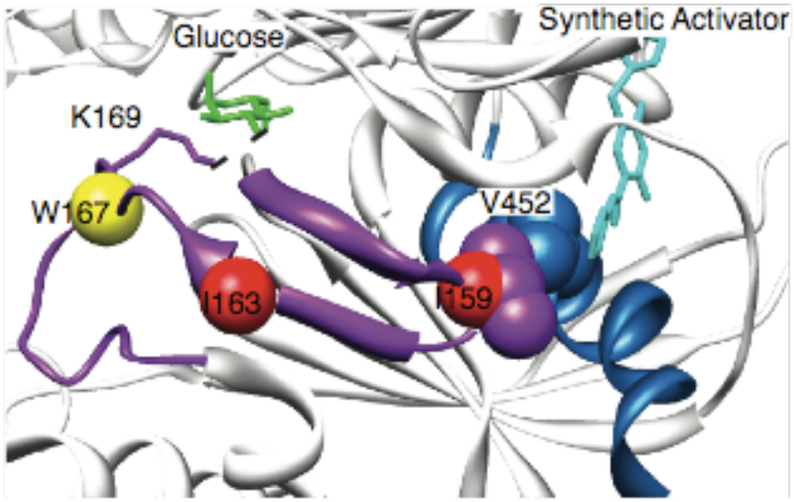


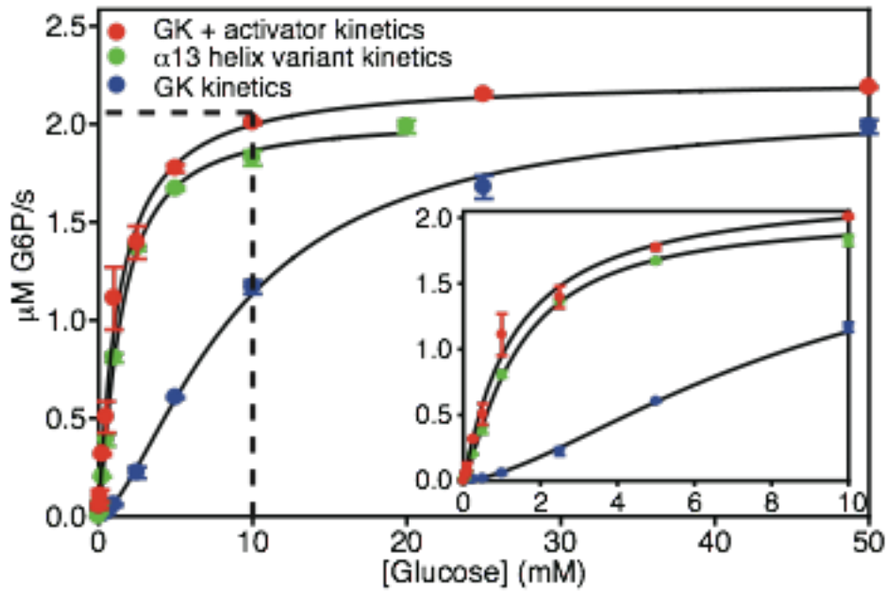


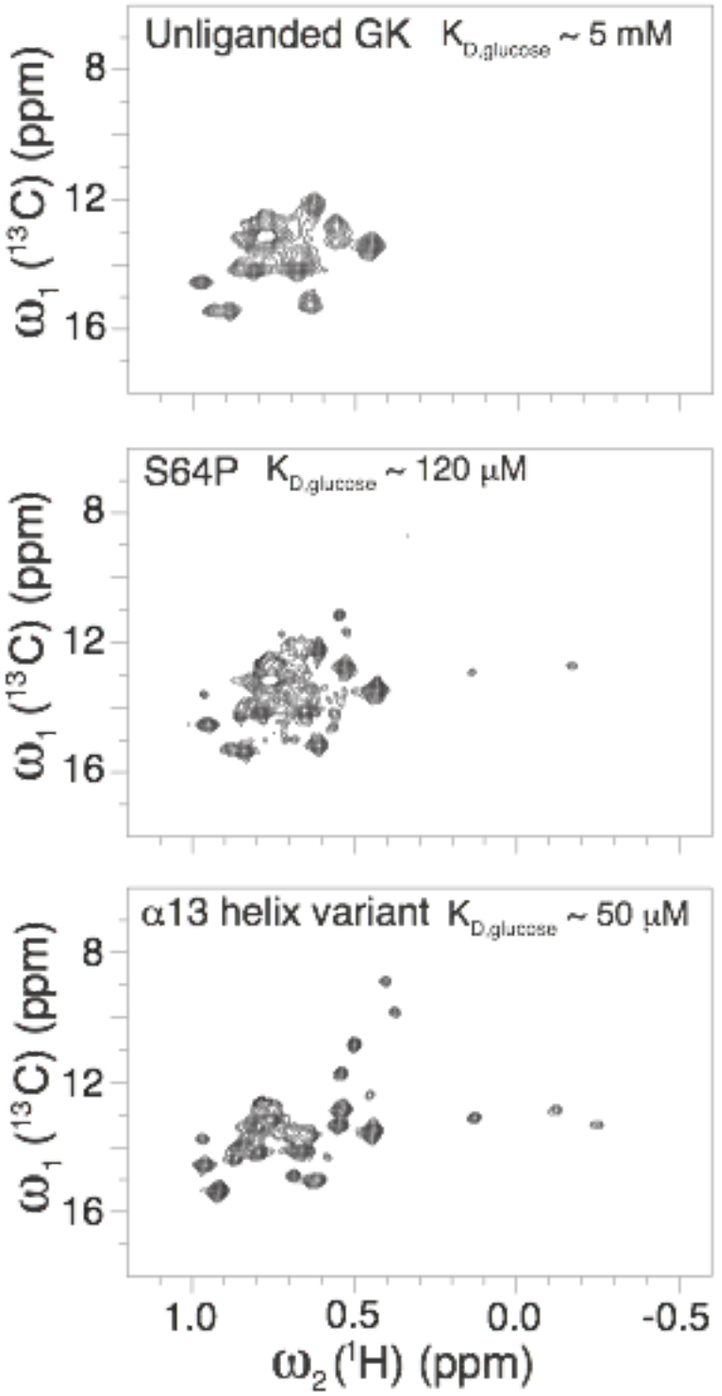


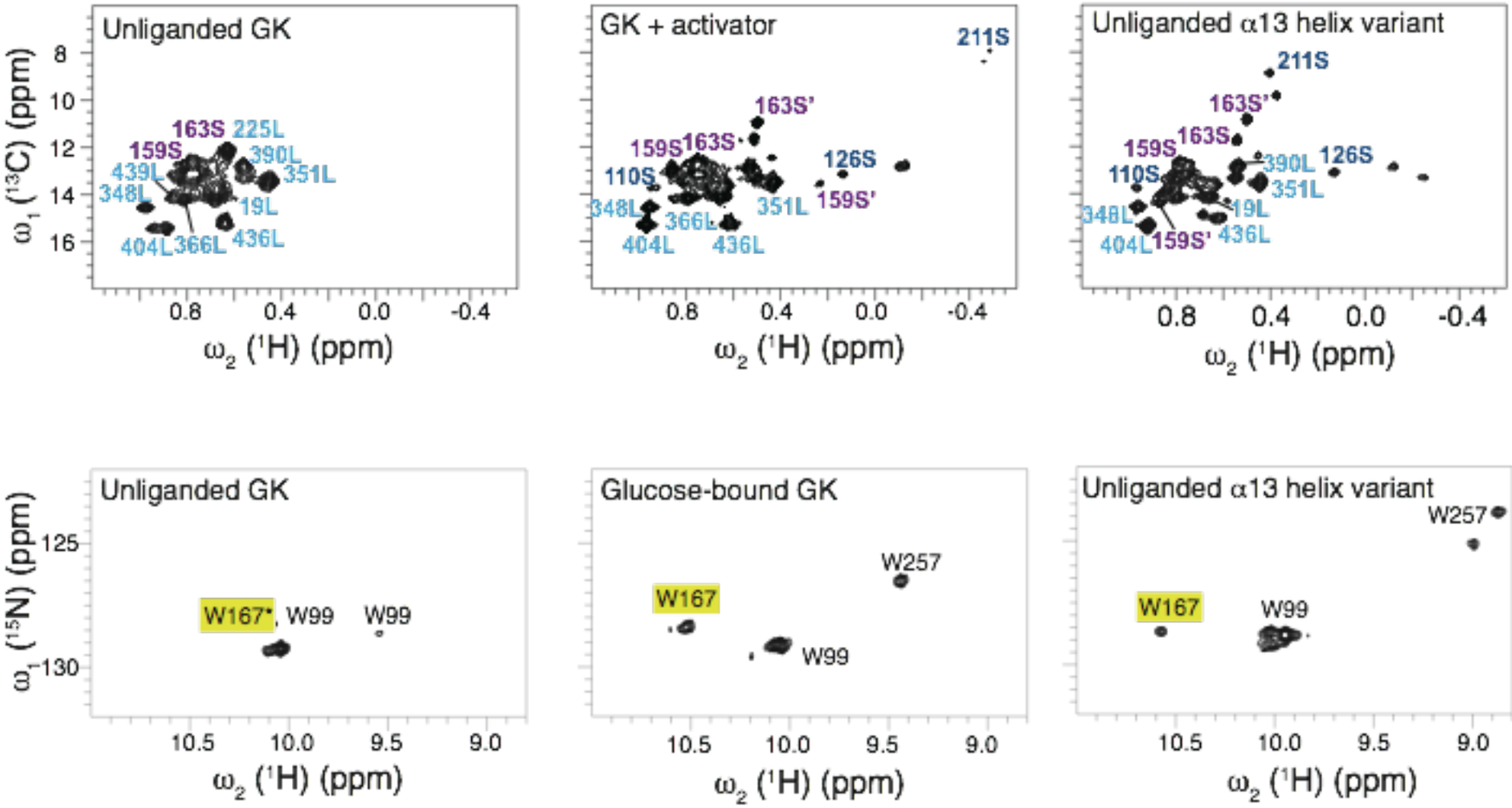
Larion et al. (2012) PLoS Biology

Introduction of mutations that cause hyperactivity or binding of the allosteric activator shift the conformational equilibrium towards more organised states

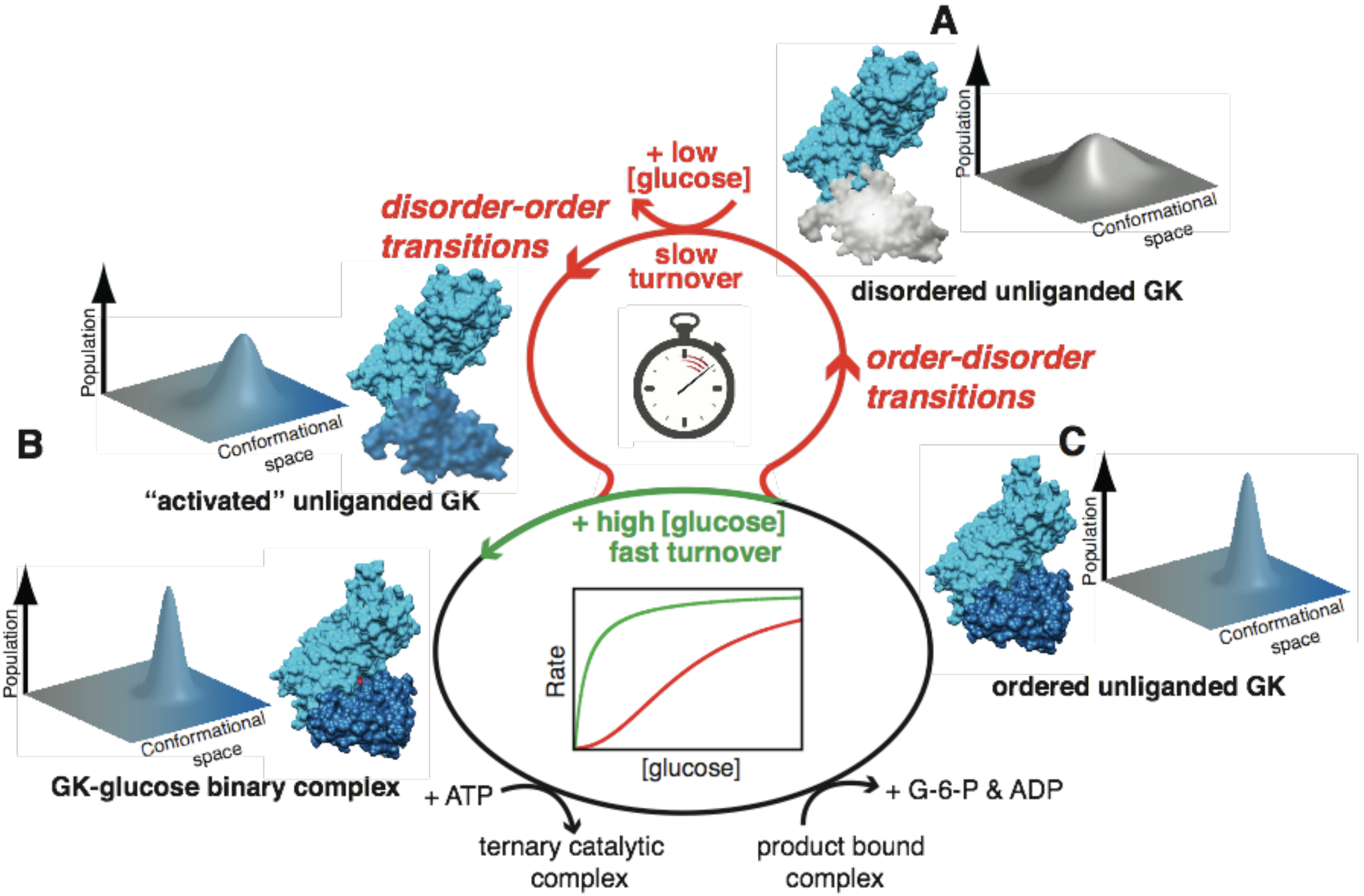








These observations lead to a model to explain glucokinase kinetic cooperativity



Larion et al. (2012) PLoS Biology

Summary

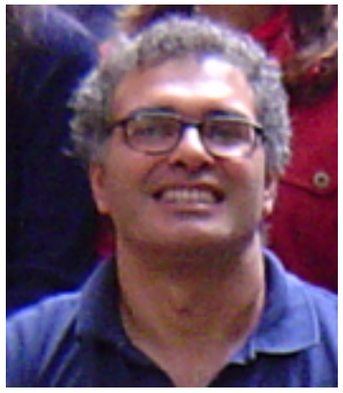
- ^{15}N relaxation can be used efficiently to investigate the protein backbone conformation dynamics at fast time scales

- The NMR study of larger proteins is affect by pronounced spectral overlap and faster T2 relaxation rates. Two ways to overcome these disadvantages are deuteration and amino acid specific labelling

- The study of side dynamics combined with amino acid specific labelling can give relevant insights into protein dynamics and function

Florida State University and National High Magnetic Field Laboratory

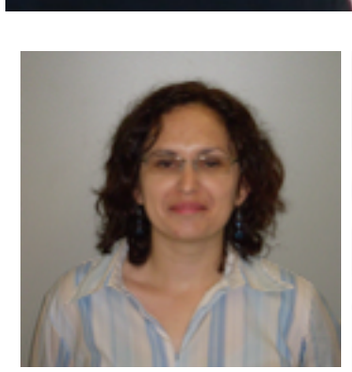
Prof. Rafael Brüschweiler
Dr. Lei Bruschweiler-Li
Dr. Miora Larion
Dr. Brian Miller





University of São Paulo

Prof. Shaker Chuck Farah (IQ)
Prof. Cristiano Oliveira (IF)
Dr. Diorge Paulo Souza
Luciana Coutinho de Oliveira
Layara Akemi Abiko
Denize Cristina Favaro



Funding: FAPESP, CNPq and CAPES (Brazil), AHA (USA)