

# Using NMR to study Macromolecular Interactions

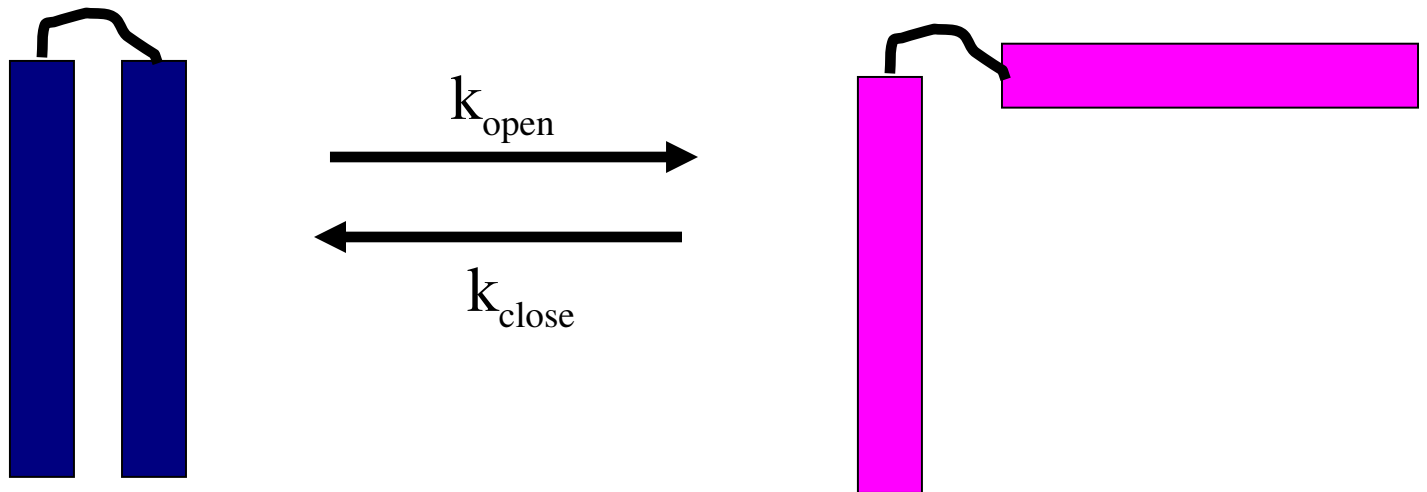
John Gross, BP204A  
UCSF

# *Outline*

- Multidimensional NMR
- Macromolecular Interactions
- Dynamics
- Dealing with large complexes
- Structure Determination

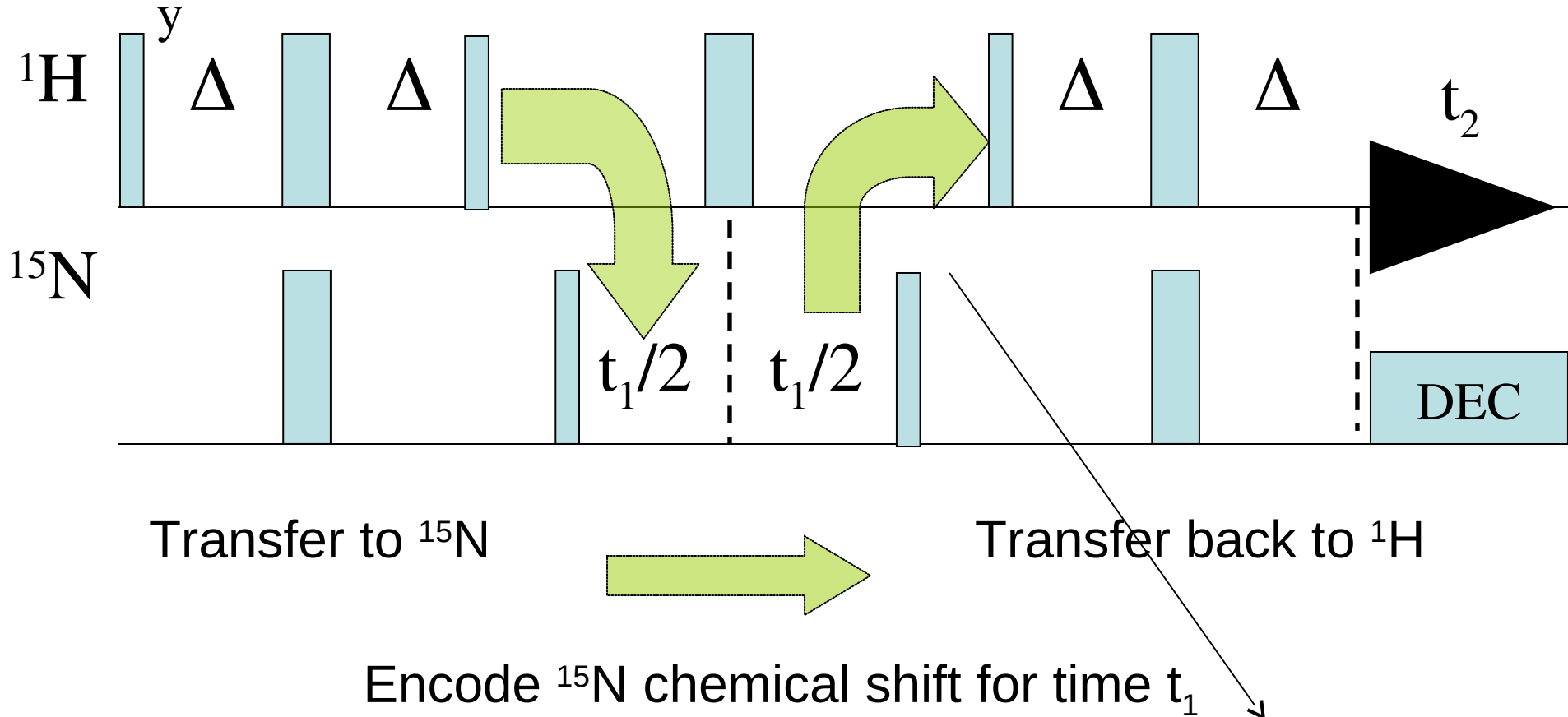
*Part IV- Methods to quantify slow  
dynamics in proteins*

# *Slow Exchange Between Two States*



$$k_{\text{ex}} = k_{\text{open}} + k_{\text{close}}$$

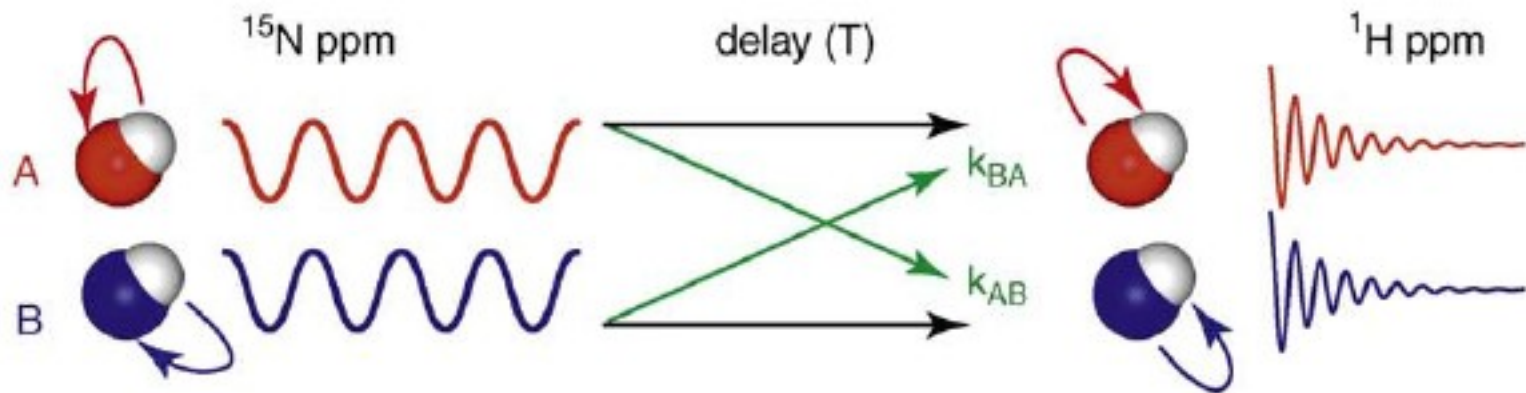
# Using HSQC to measure slow exchange



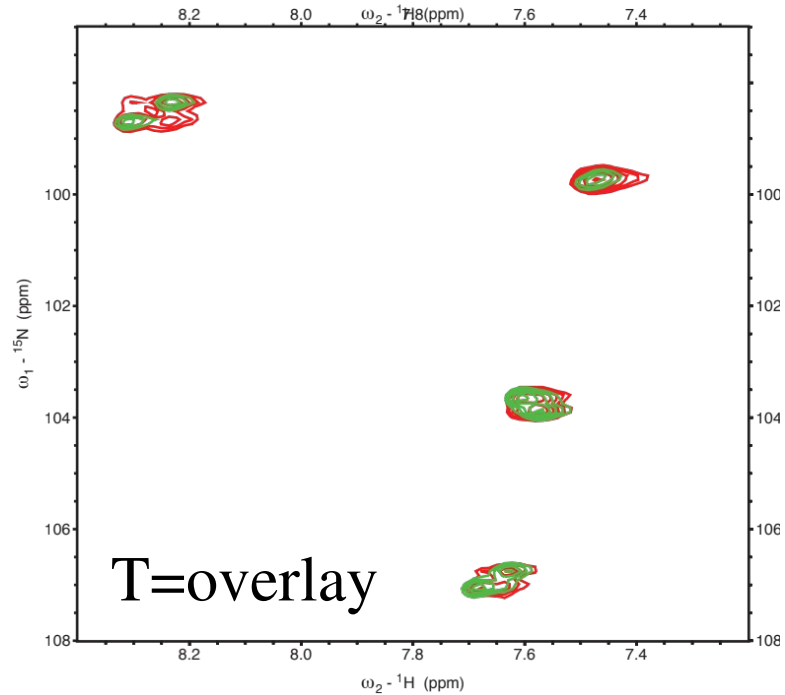
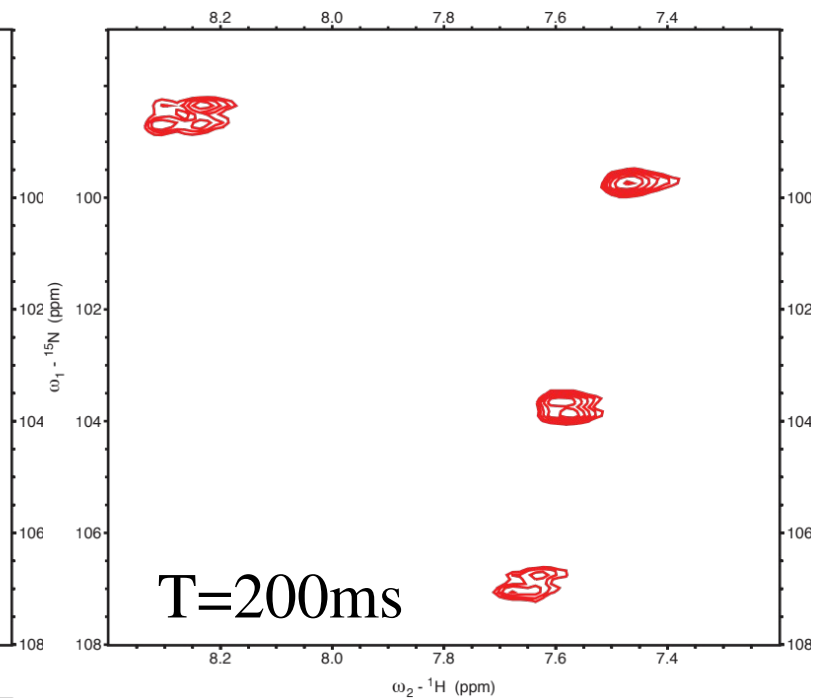
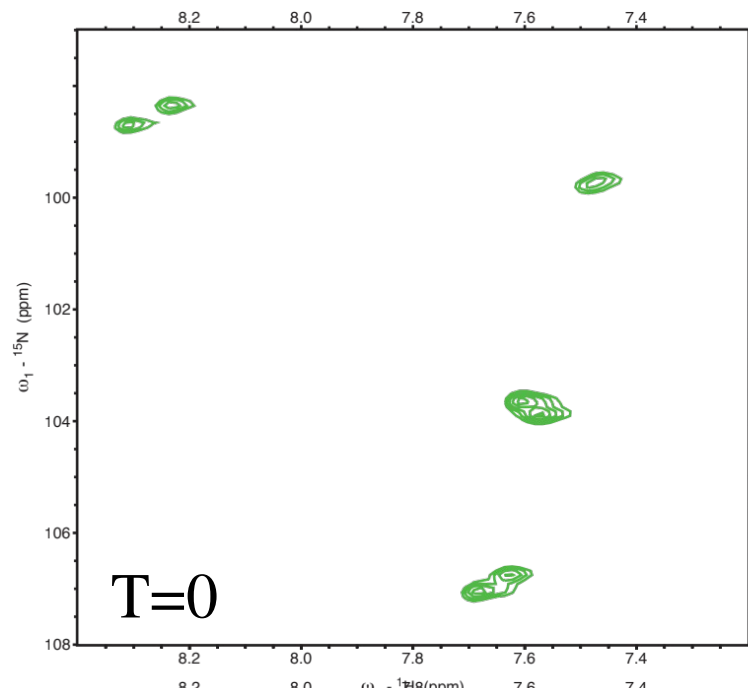
Insert delay  
to allow  
exchange

ZZ-exchange, Montelione and Wagner

# *Exchange Cross-Peaks*

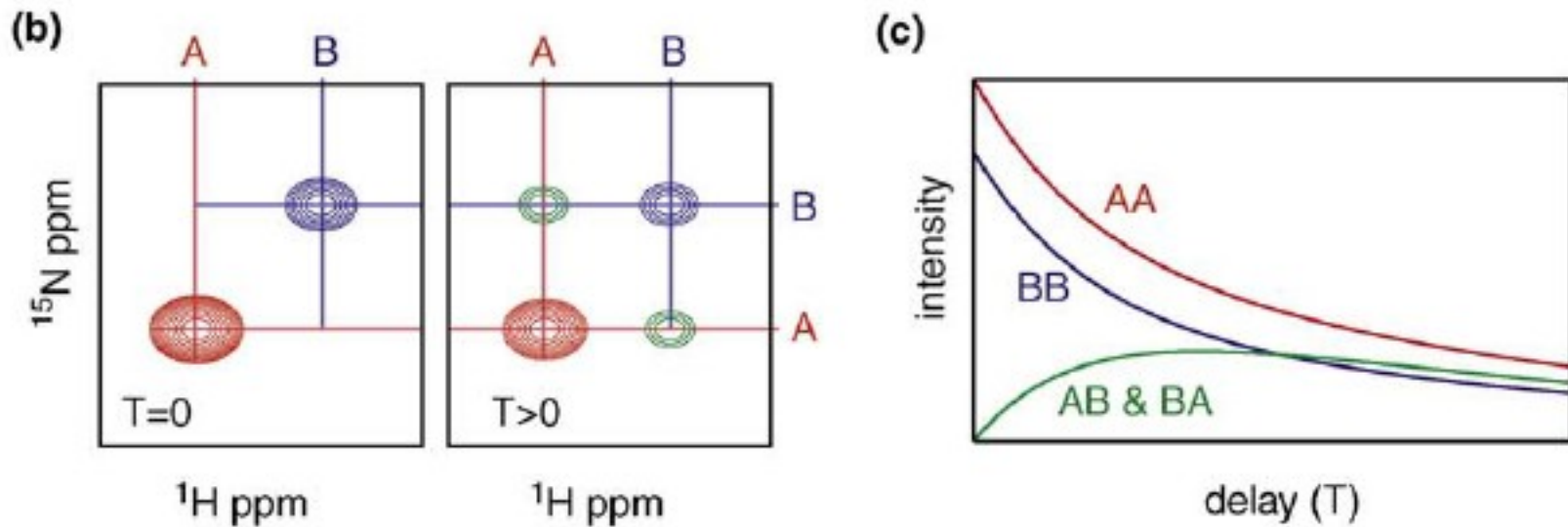


Cross-peaks from a conformational change during delay  
e.i.-red to blue



GR-DBD undergoing slow exchange while bound to DNA

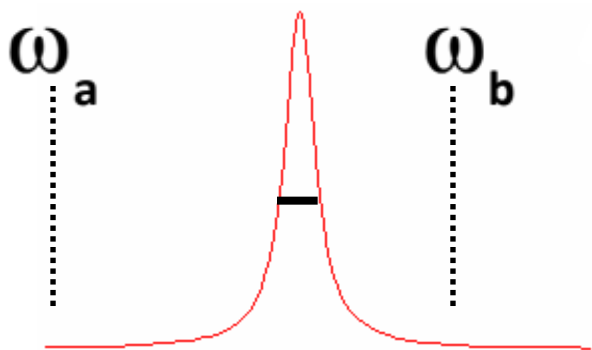
*ZZ-exchange peak intensity dependence on delay*



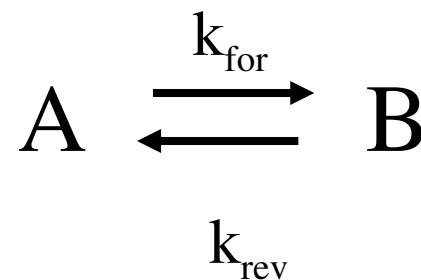
Fit to obtain populations and rate constants



*Expressions for the linewidth  
in the Fast Exchange Limit ( $k_{ex} > \Delta\omega$ )*



$$\bar{\omega} = p_a \omega_a + p_b \omega_b$$



Populations  $p_a, p_b$

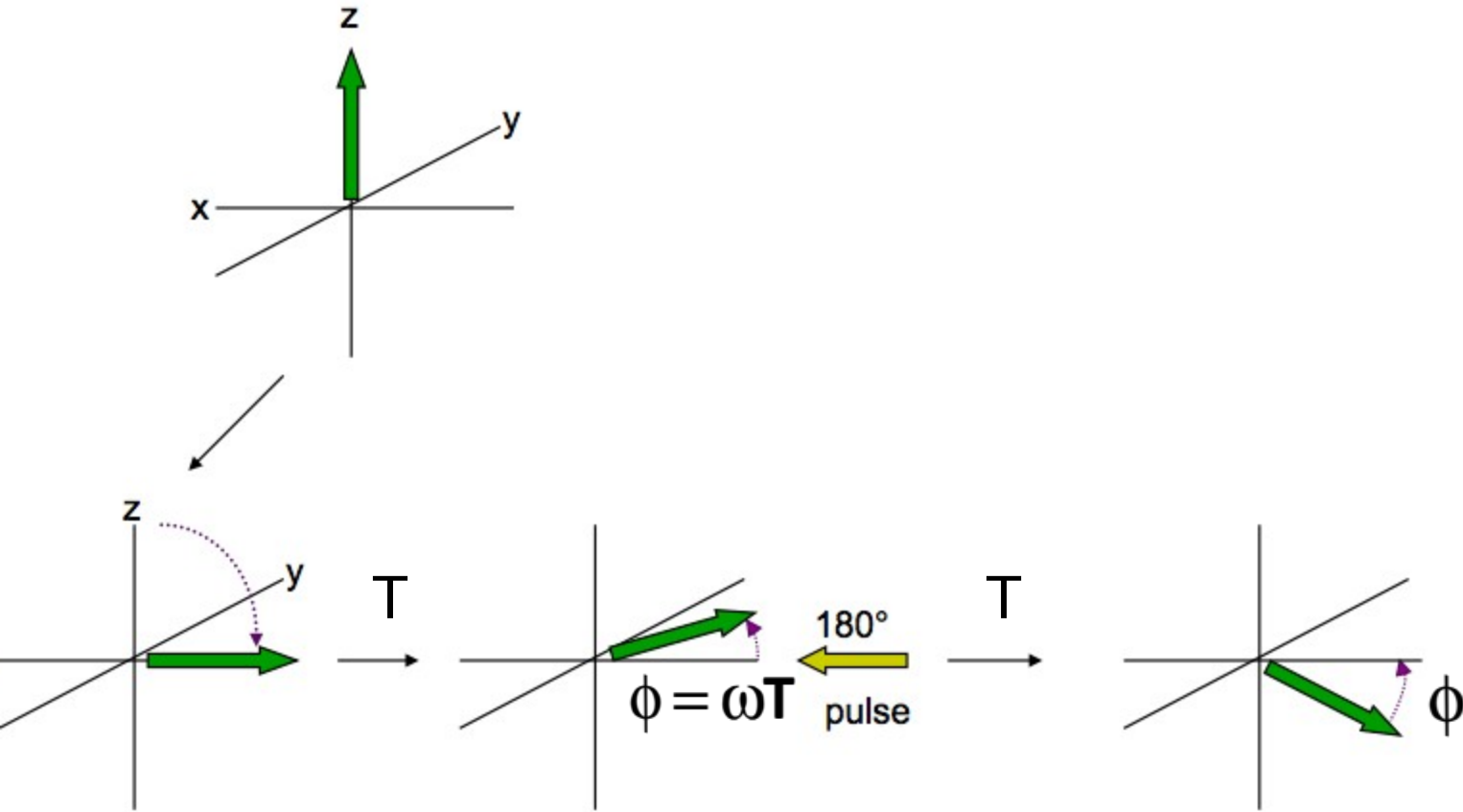
$$k_{ex} = k_{for} + k_{rev}$$

$$R_2 = \bar{R}_{2(ns-ps)} + \left. \frac{p_a p_b \Delta\omega^2}{k_{ex}} \right\} R_{ex}$$

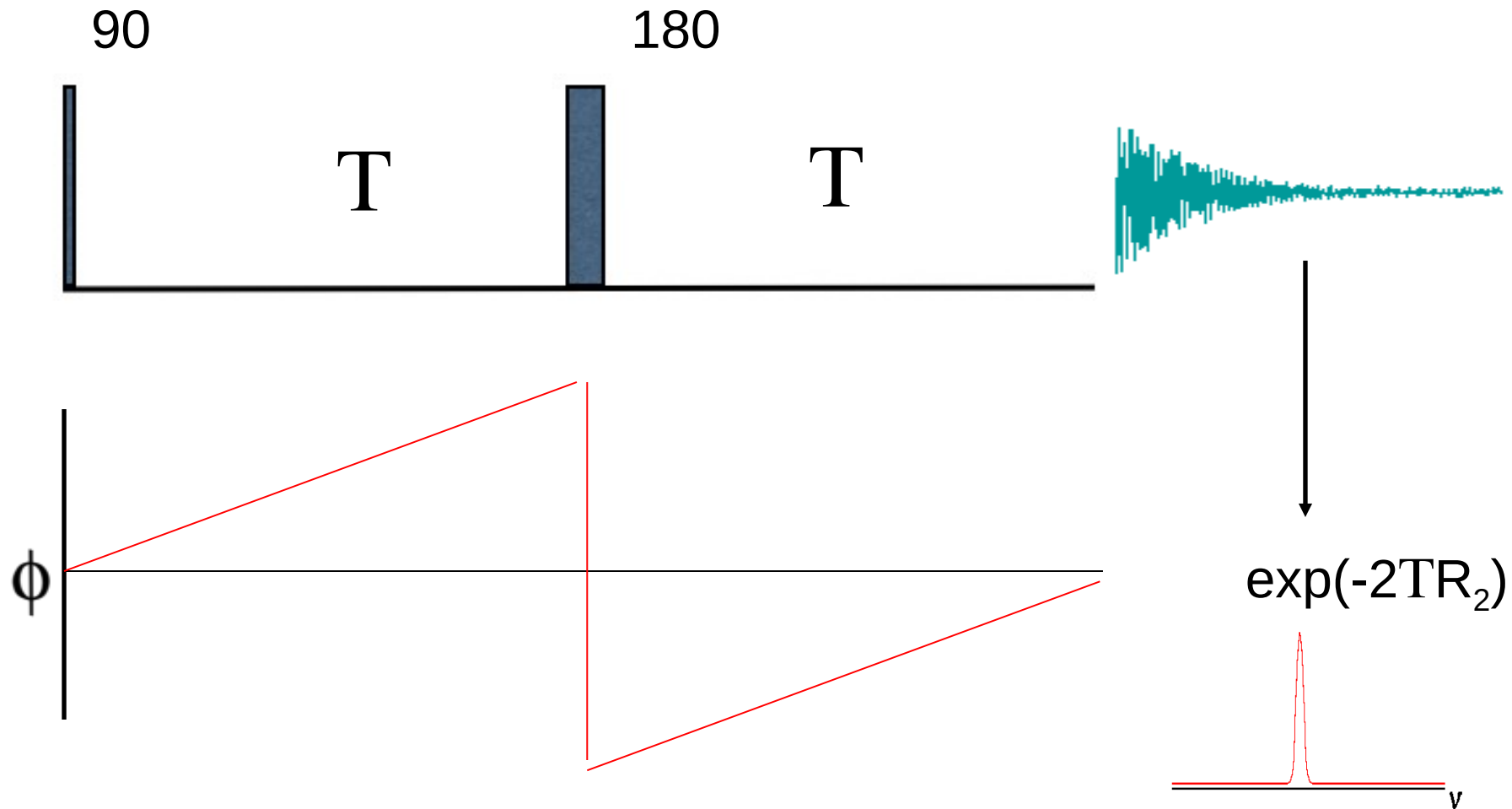
$$\Delta\omega = \omega_a - \omega_b$$

# Spin Echo

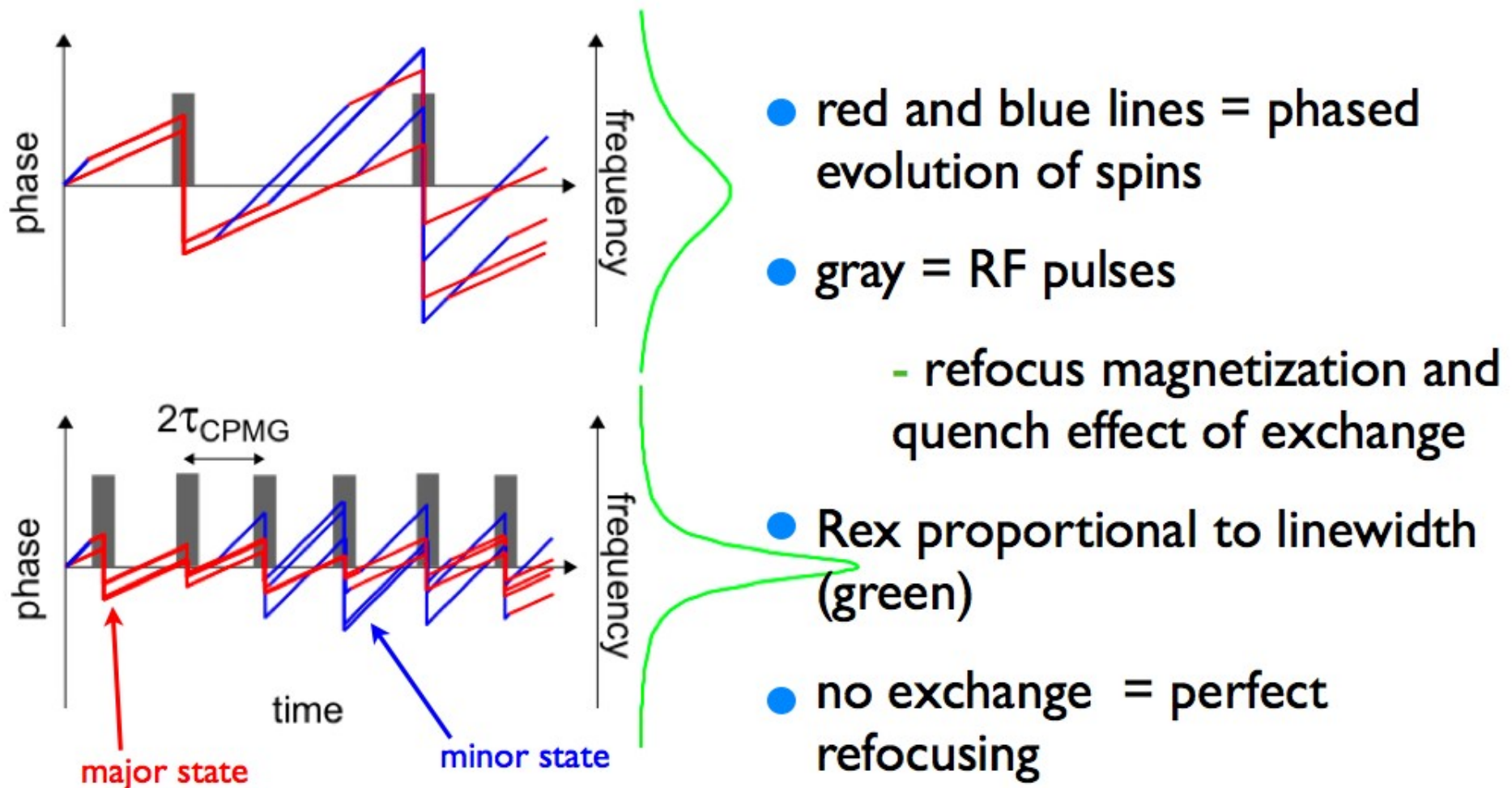
to measure ms-usec dynamics



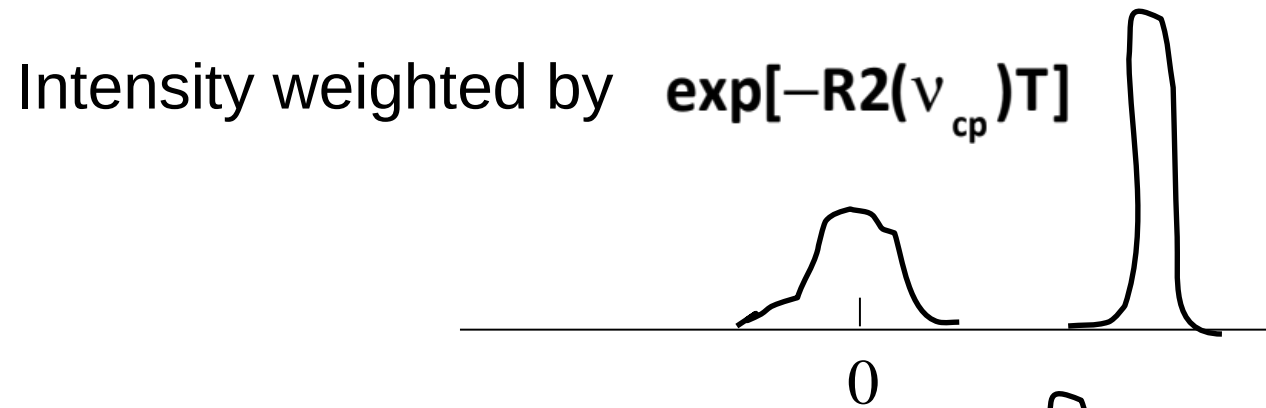
# *Spin Echo to Measure $R_2$*



# cpmg experiment

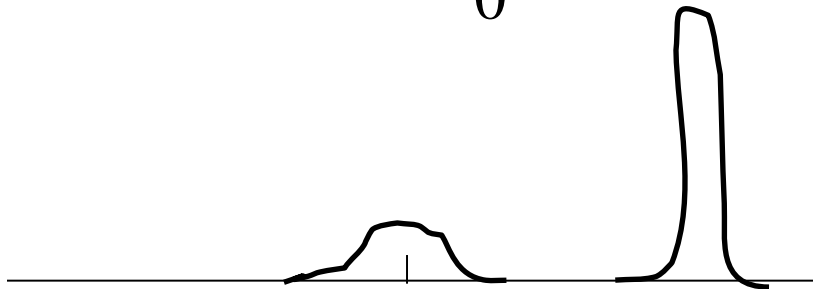


# *CPMG Protocol*

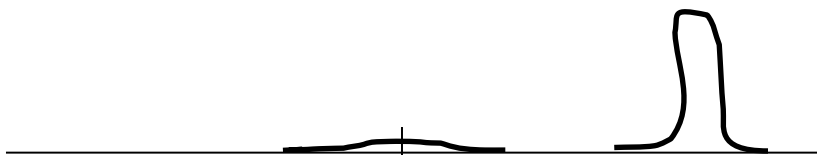


$$\nu_{cp} = \frac{1}{2\tau_{cp}}$$

$$\nu_{cp} = \mathbf{1000\text{Hz}}$$

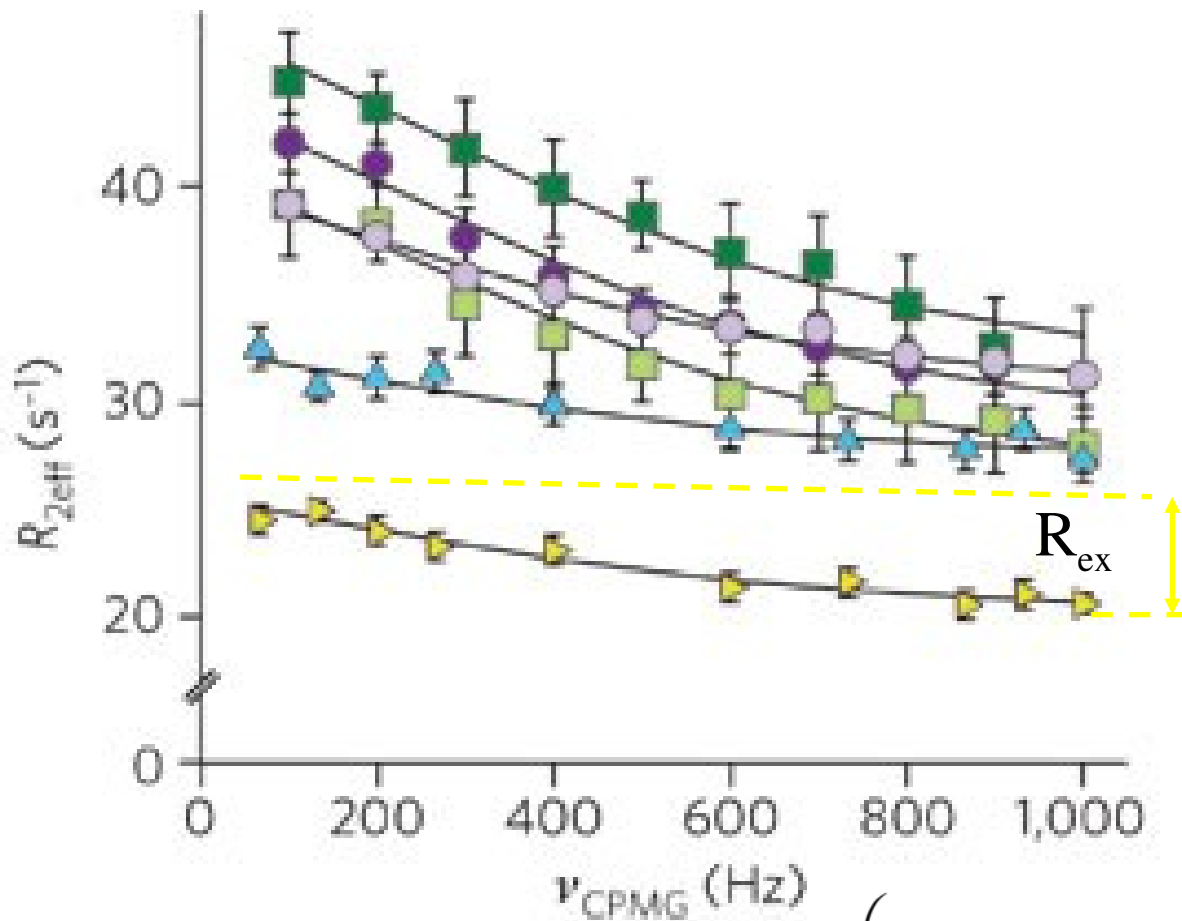


$$\nu_{cp} = \mathbf{500\text{Hz}}$$



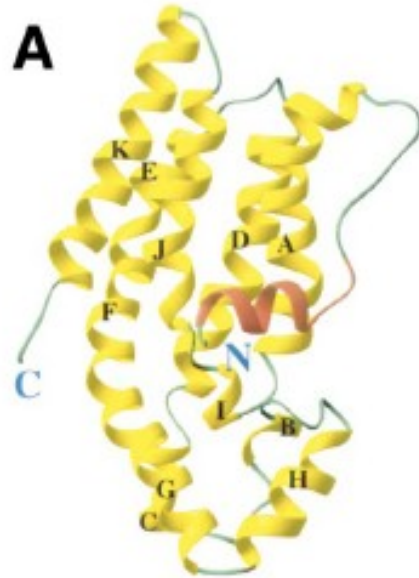
$$\nu_{cp} = \mathbf{50\text{Hz}}$$

# Dynamics Constants from Relaxation Dispersion Curves

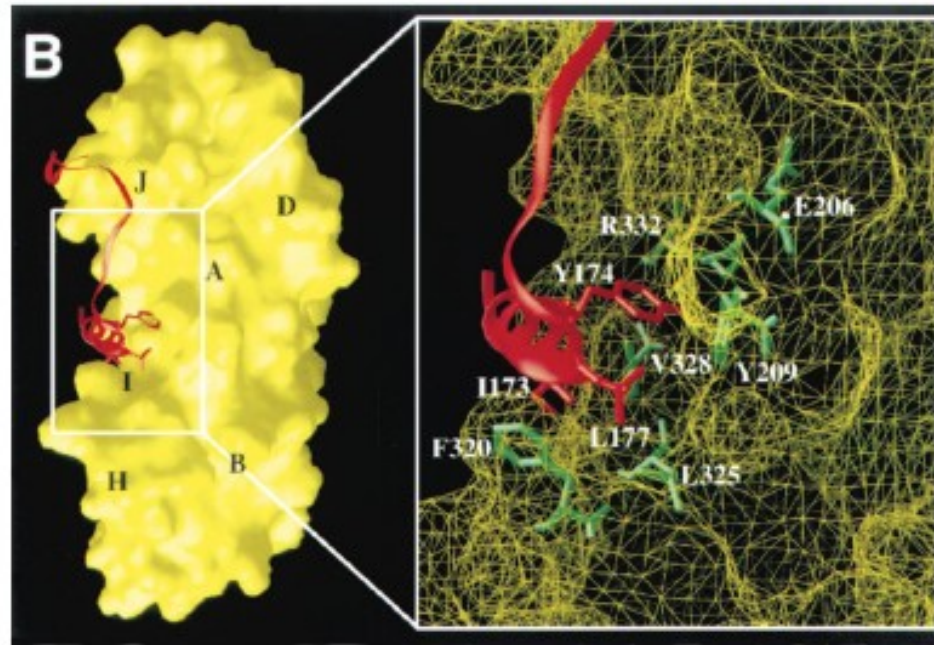


$$R_2^{\text{eff}}(\nu_{\text{CPMG}}) = R_2^a(ns-ps) + p_a p_b \frac{\Delta\omega^2}{k_{\text{ex}}} \left( 1 - \frac{4\nu_{\text{CPMG}} \tanh\left(\frac{k_{\text{ex}}}{4\nu_{\text{CPMG}}}\right)}{k_{\text{ex}}} \right)$$

# Regulation of Vav1 activity by autoinhibition



How is this GEF activated?



# Example from literature

Internal dynamics control activation and activity of the autoinhibited Vav DH domain

Pilong Li<sup>1,3</sup>, Ilídio R S Martins<sup>1-3</sup>, Gaya K Amarasinghe<sup>1,3,4</sup> & Michael K Rosen<sup>1</sup>

Nature Structural and Molecular Biology, 15:6 (2008)



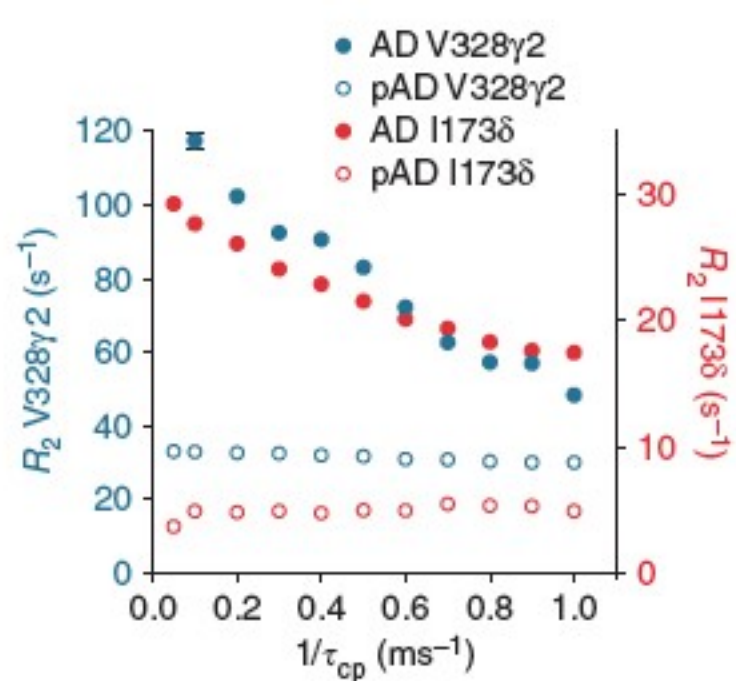
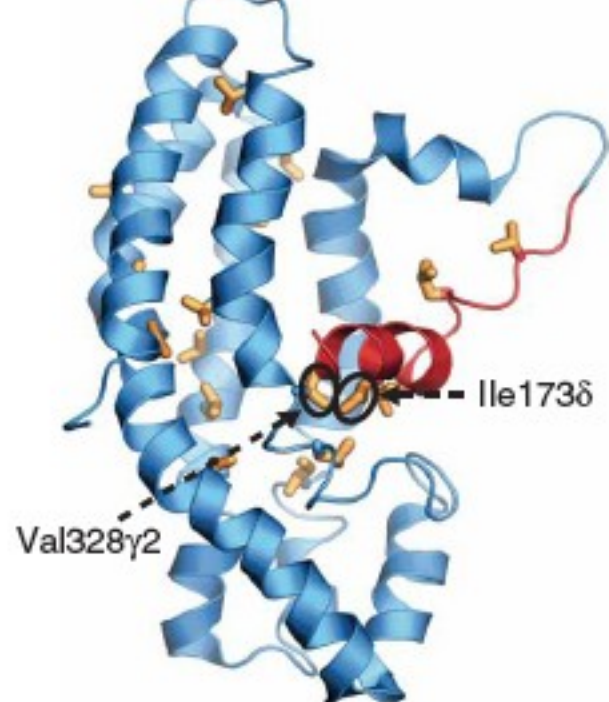
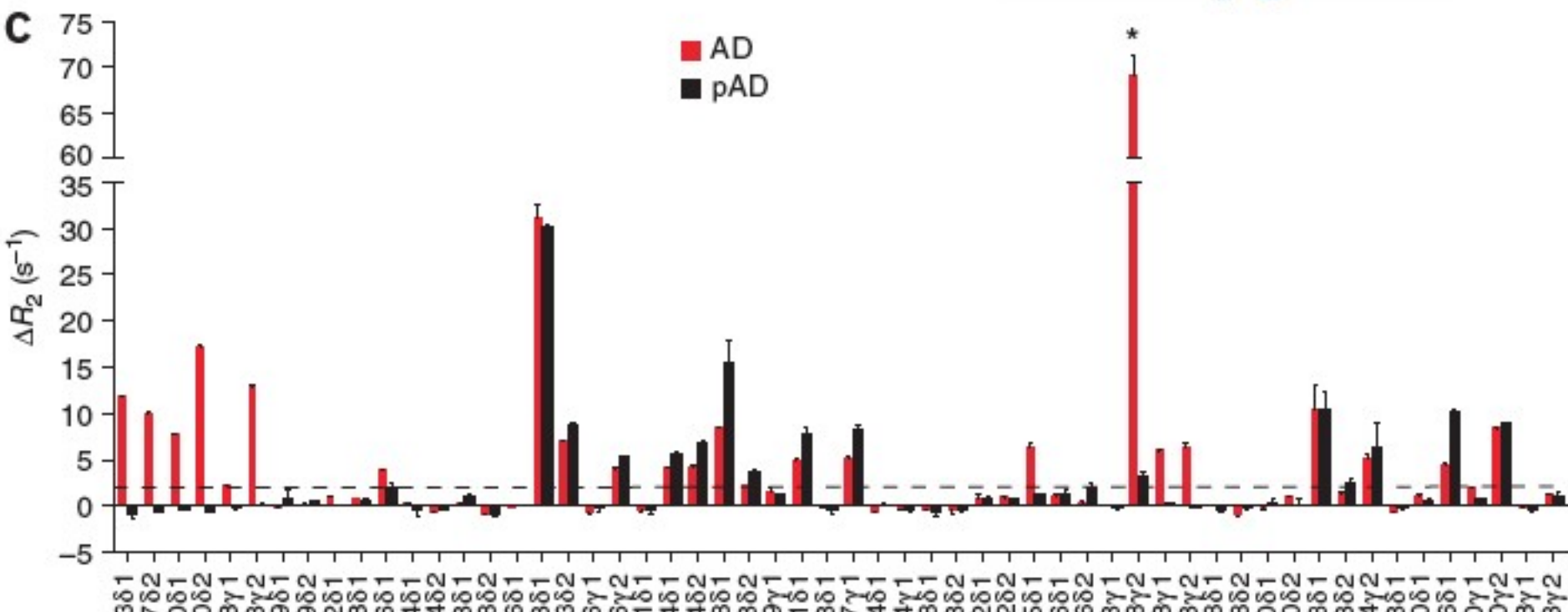
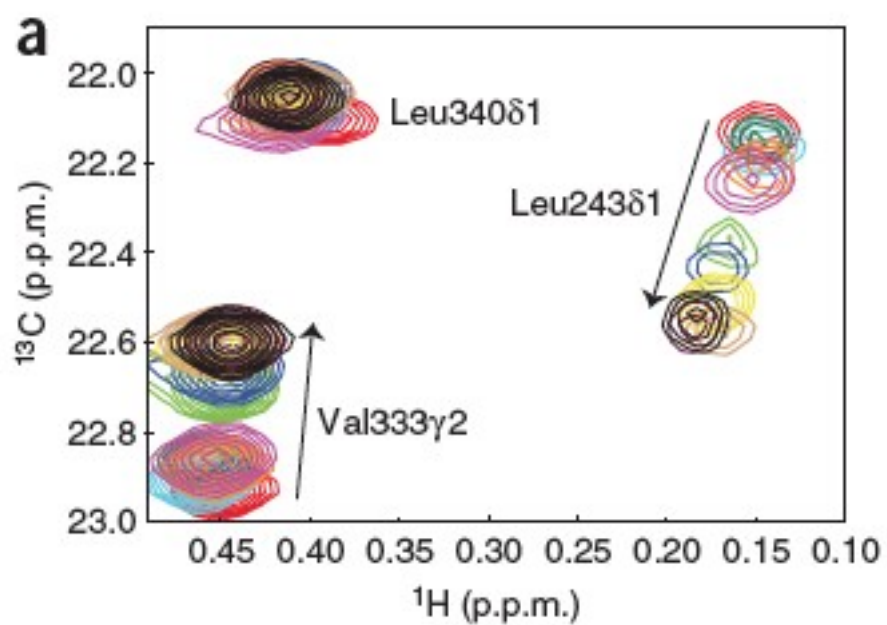
**a****b****c**

Figure 2



**b**

Protein	$p_0$
pWT	$\sim 1$
K208E	$1.00 \pm 0.06$
Y174F	$0.99 \pm 0.03$
K208S	$0.86 \pm 0.06$
K208A	$0.77 \pm 0.12$
E169K K208A	$0.64 \pm 0.06$
K208V	$0.26 \pm 0.10$
E169K K208V	$0.14 \pm 0.08$
Wild type	$0.09 \pm 0.03$
E169K	$0.05 \pm 0.06$
E169K E207K	$\sim 0$

Figure 3

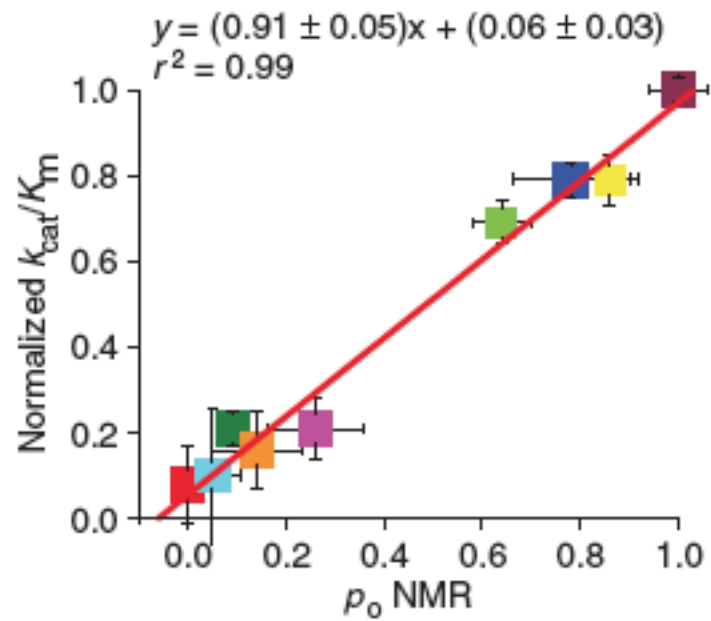
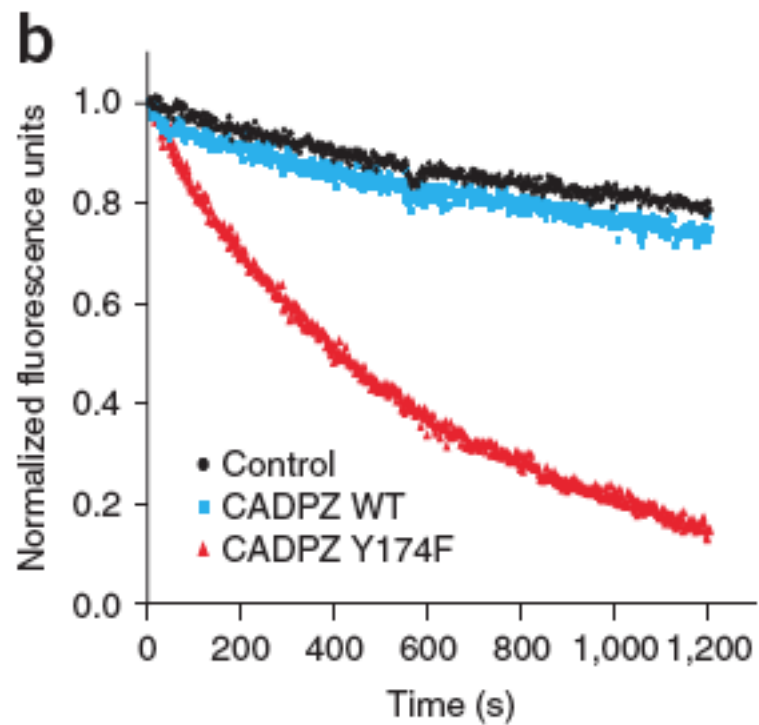
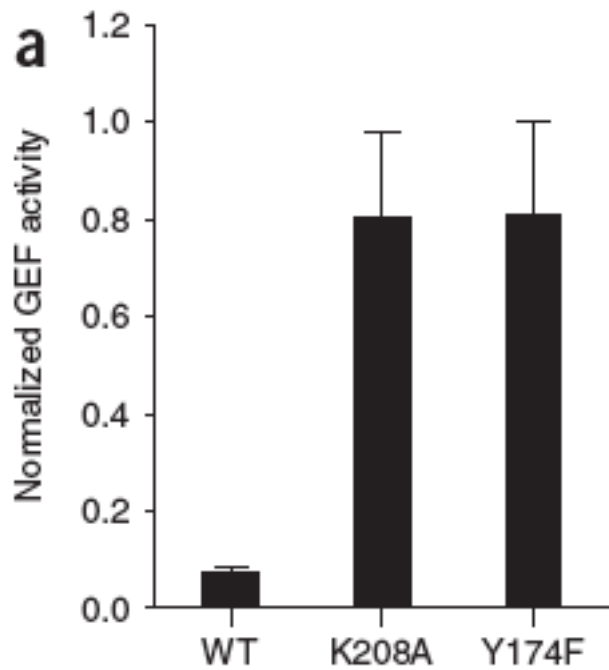


Figure 4



# Model

