Using NMR to study Macromolecular Interactions

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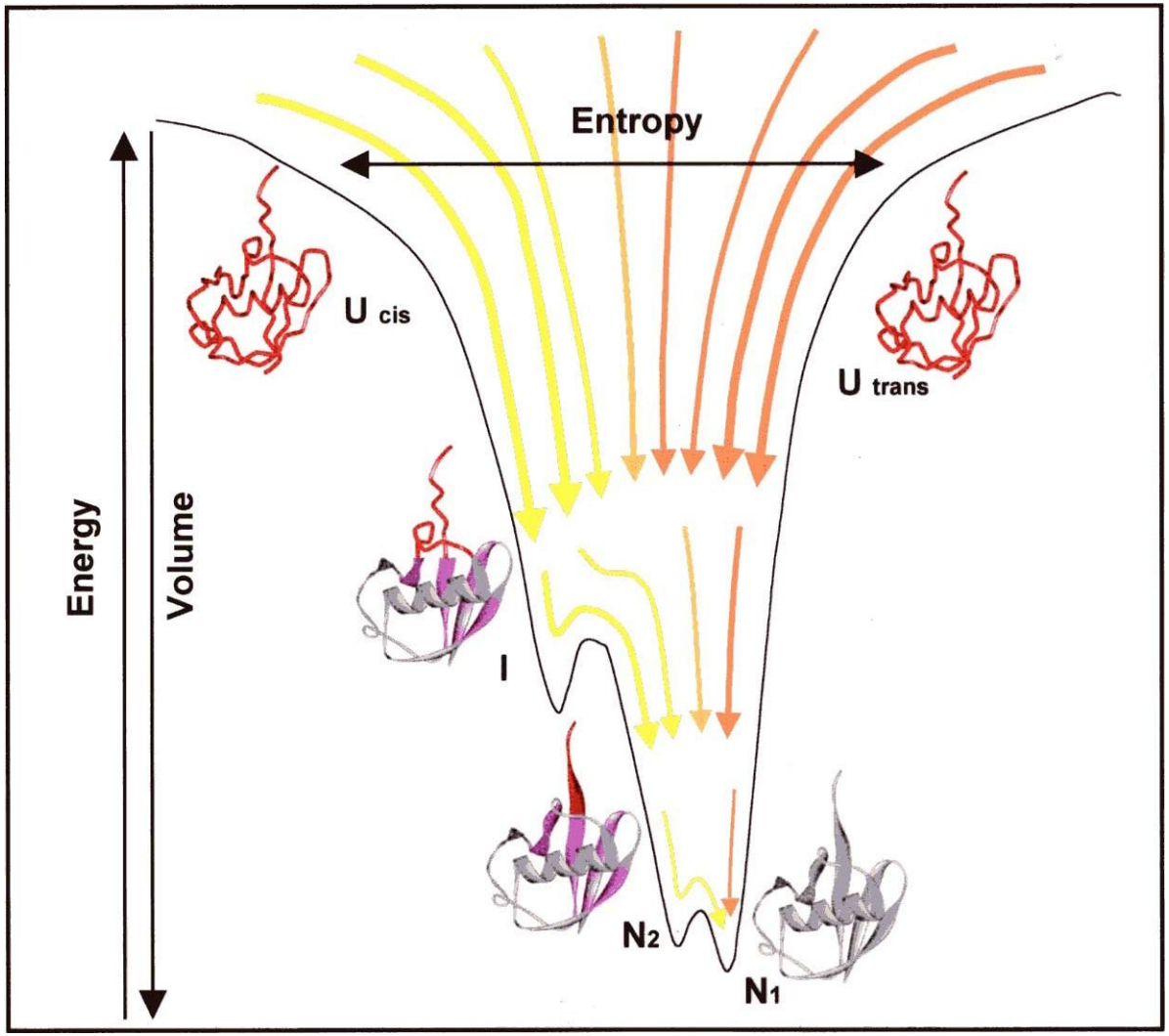
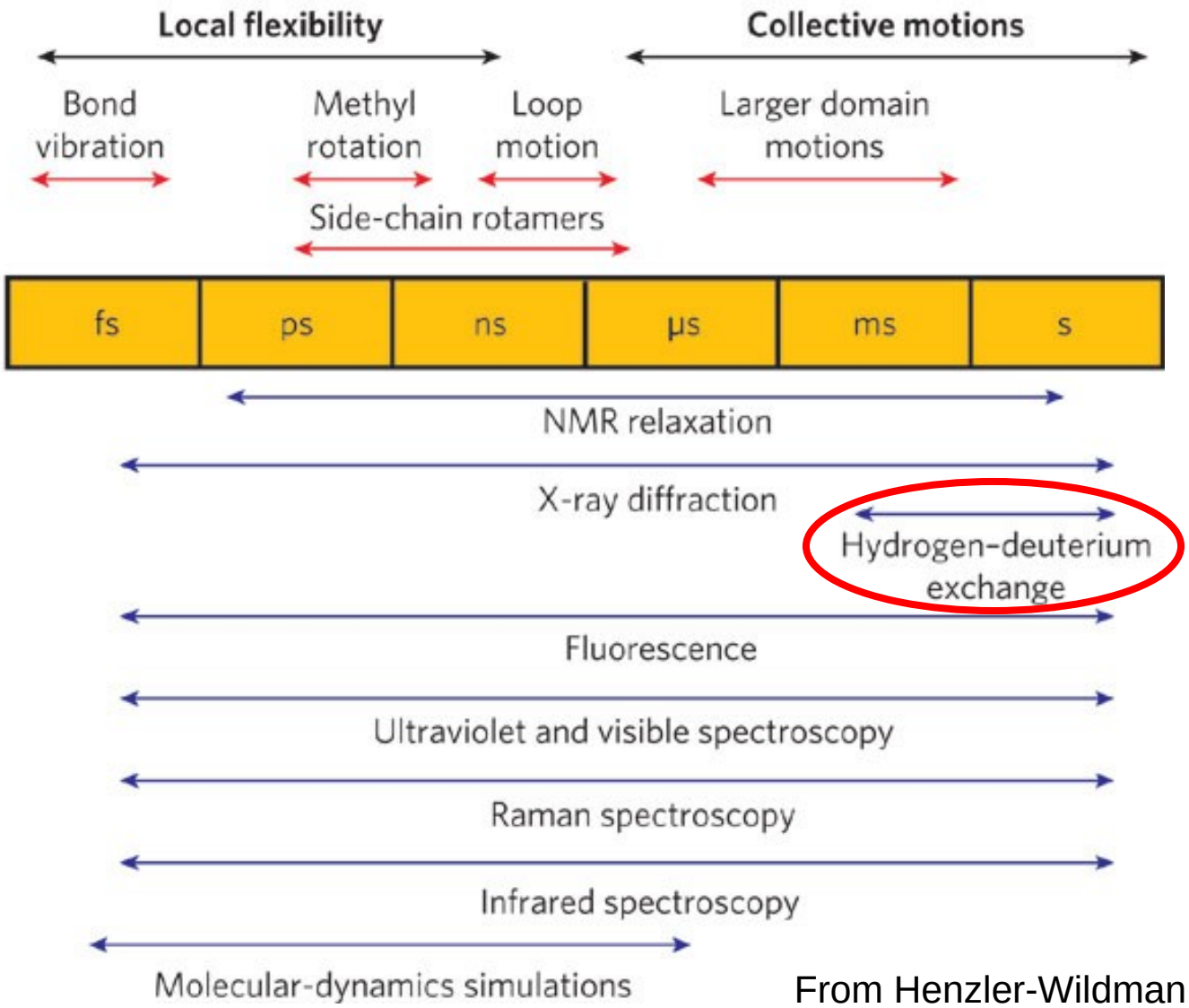
Outline

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

Part III: Dynamics by NMR



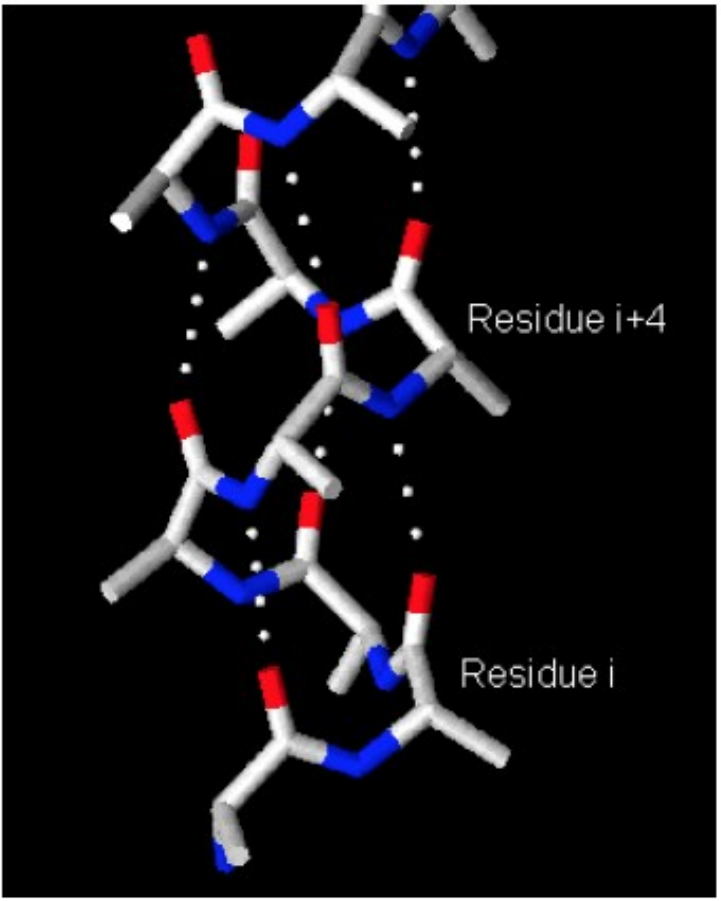
Timescales of Protein Dynamics

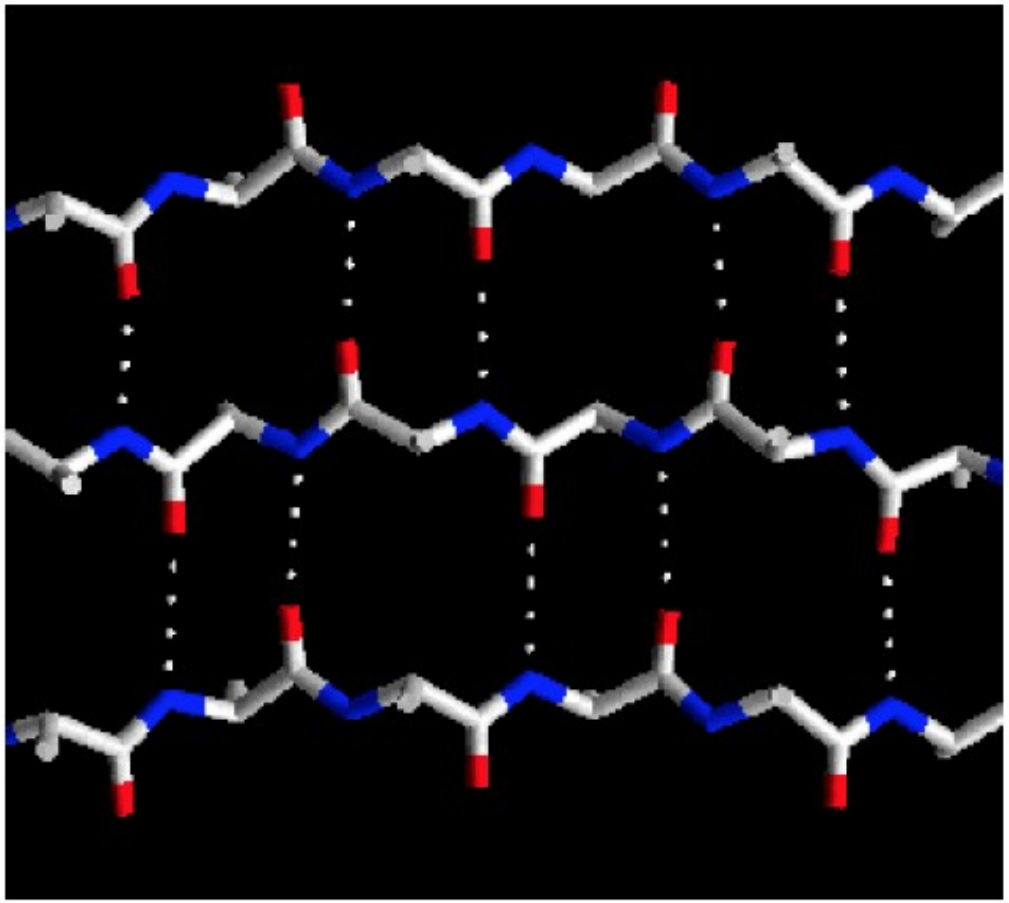


From Henzler-Wildman and Kern, Nature 2007

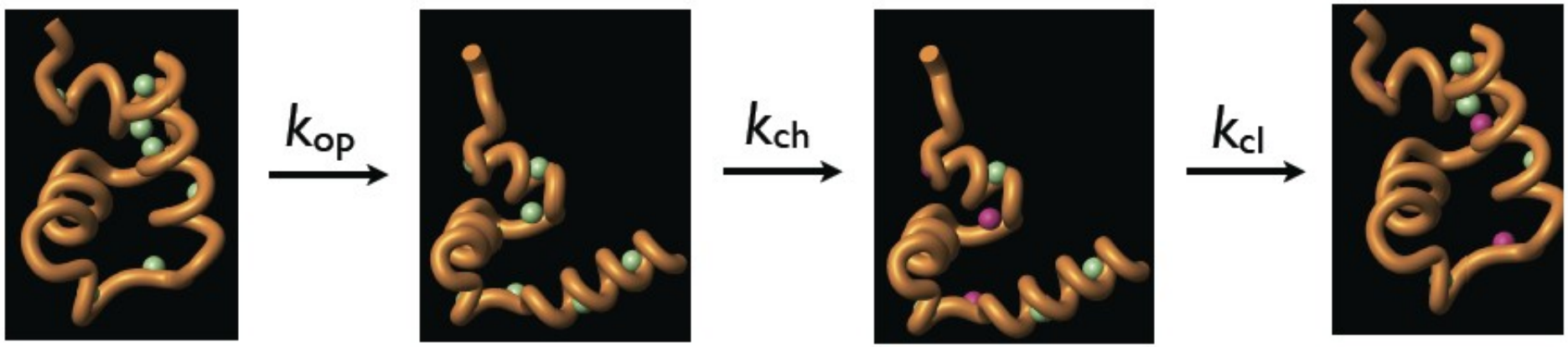
H/D exchange for measuring stability of H-bonds

Long lasting hydrogen bonds in proteins are typically part of secondary structure





Exchange of protons in the open conformation



EX1: $k_{cl} << k_{ch}$ $\quad\quad$ $k_{obs} = k_{op}$

EX2: $k_{cl} >> k_{ch}$ $\quad\quad$ $k_{obs} = k_{op}\, k_{ch}/(k_{cl}) = K_{op}\, k_{ch}$

K_{op} is referred to as the protection factor, P

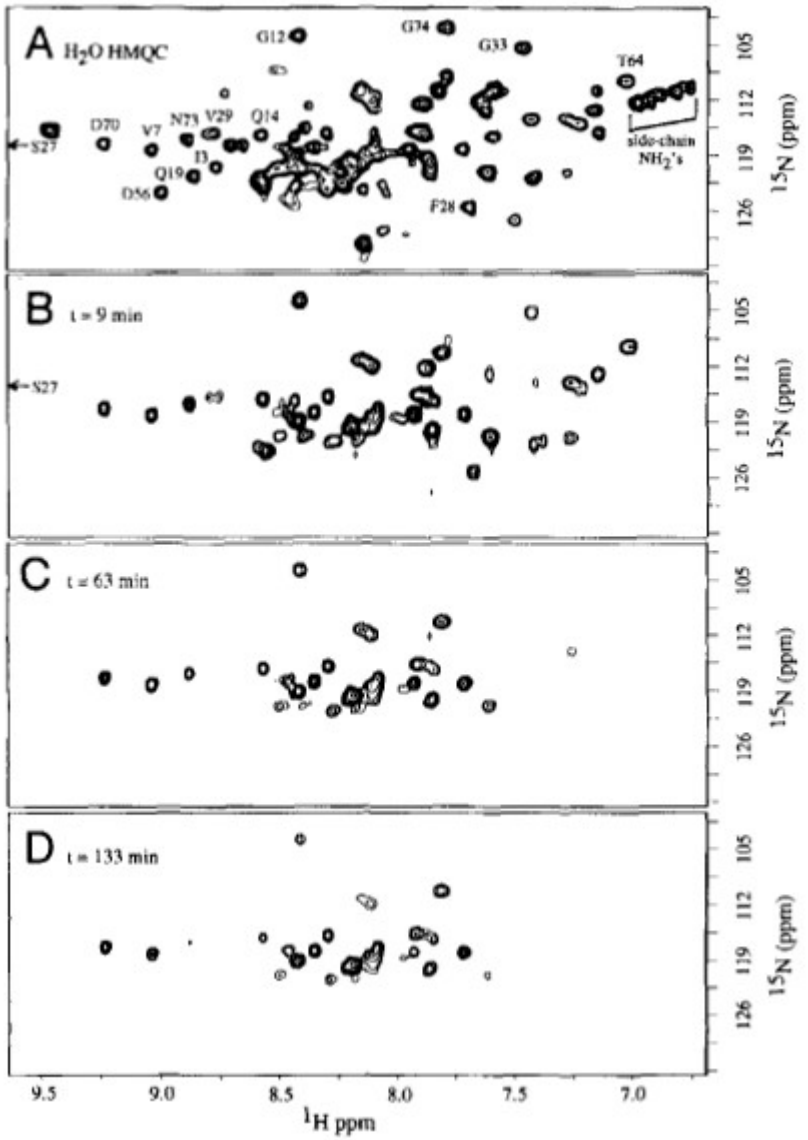
$$\Delta G_{op} = -RT \ln K_{op}$$

NMR Analysis of Protein Dynamics

Hydrogen-Deuterium Exchange

- As we saw before, slow exchanging NHs allowed us to identify NHs involved in hydrogen-bonds.
- Similarly, slow exchanging NHs are protected from the solvent and imply low dynamic regions.
- Fast exchanging NHs are accesible to the solvent and imply dynamic residues, especially if not solvent exposed.

Protein sample is exchanged into D_2O and the disappearance of NHs peaks in a 2D ^{1}H-^{15}NH spectra is monitored.

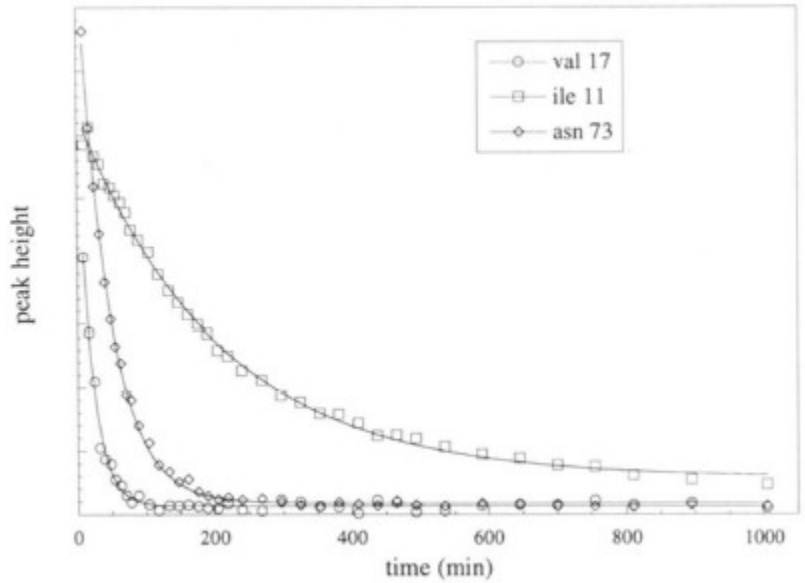


Protein Science (1995), 4:983-993.

NMR Analysis of Protein Dynamics

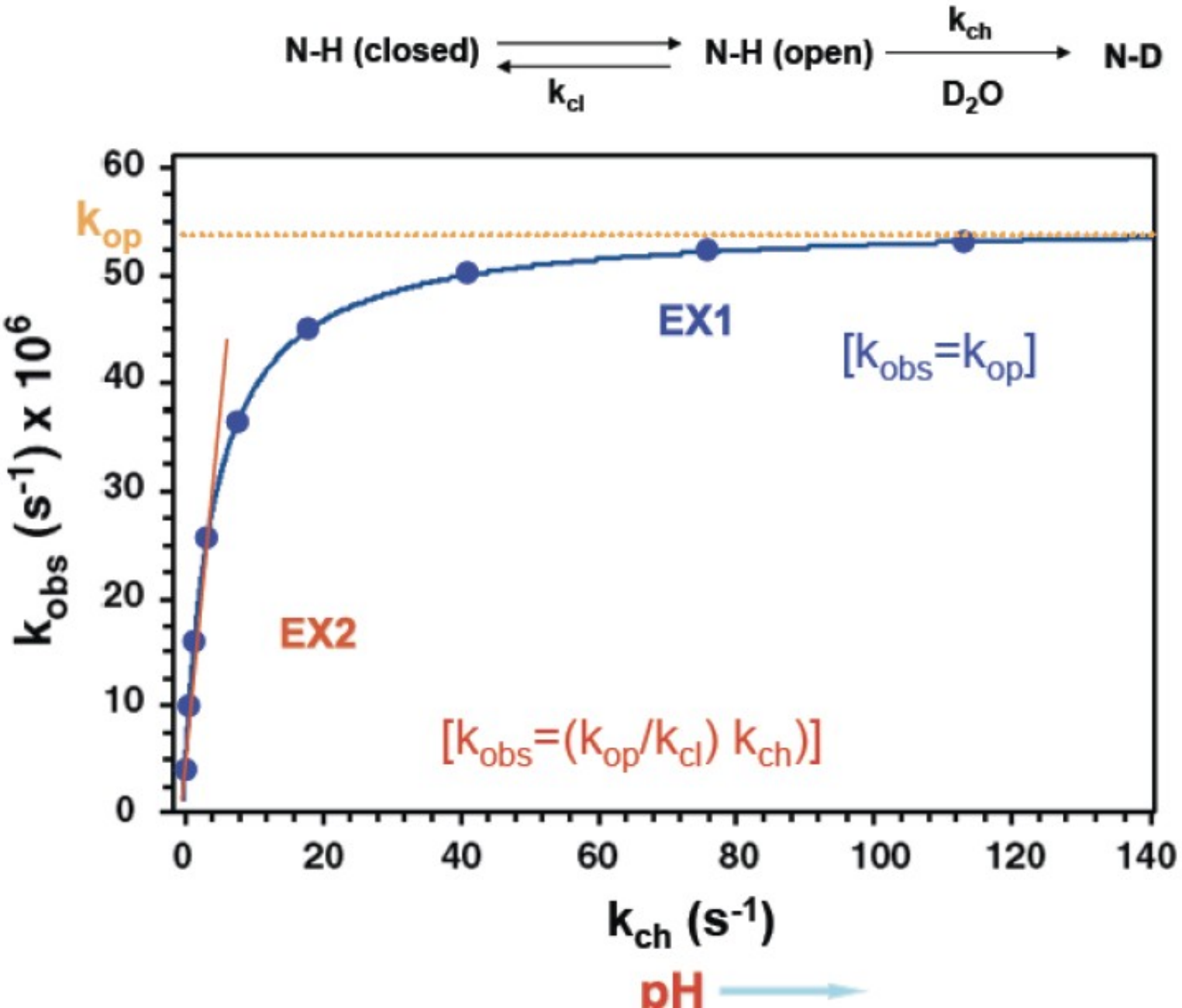
Hydrogen-Deuterium Exchange

- The observed NH intensity loss can be fit to a simple exponential to measure an exchange rate (k_{ex})
- These exchange rates may range from minutes to months!
  - NHs with long exchange rates indicate stable or low mobility regions of the protein
  - NHs with short exchange rates indicate regions of high mobility in the protein

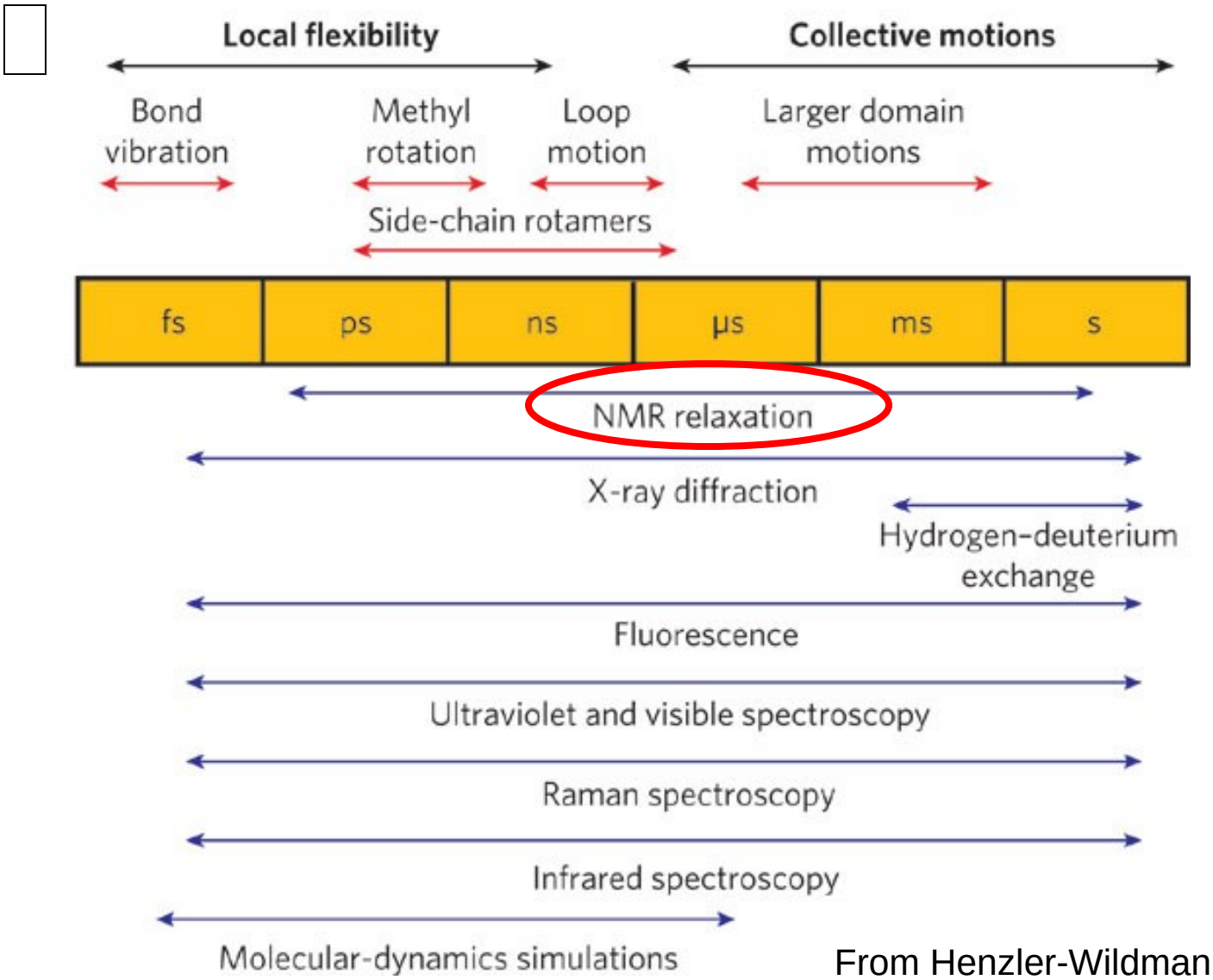


$$I = \alpha e^{-k_{obs}t}$$

The exchange mechansim depends on pH

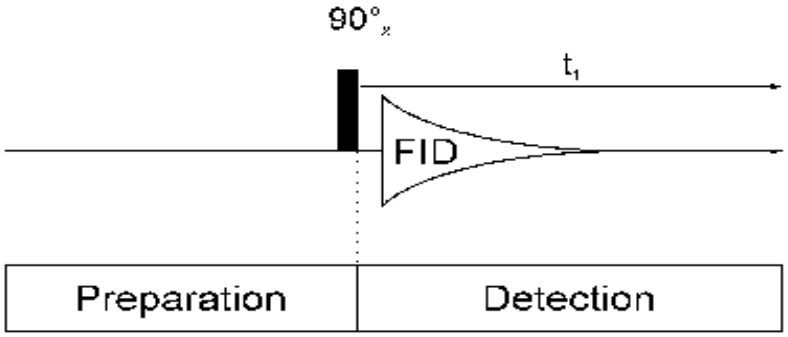


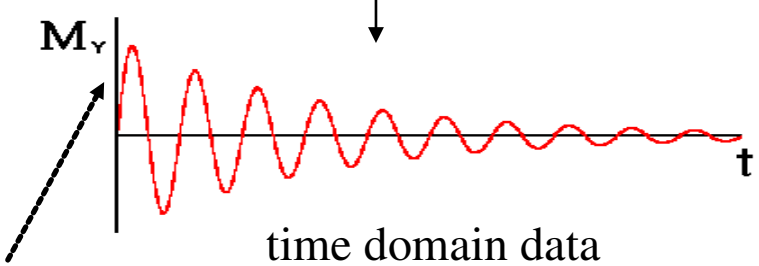
Timescales of Protein Dynamics



From Henzler-Wildman and Kern, Nature 2007

Summary of 1D Experiment





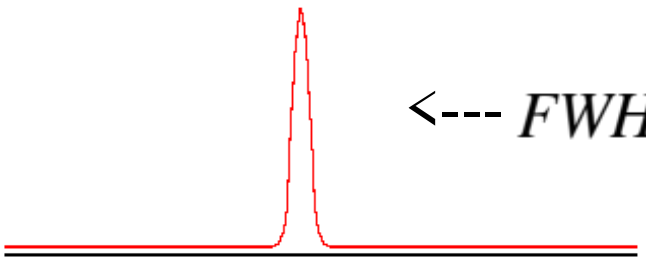
time domain data

Transverse Magnetization
Decay constant T2

$$R_2 = \frac{1}{T_2}$$

Amplitude propotional to amount magnetization prior to pulse

Fourier Transform (FT)



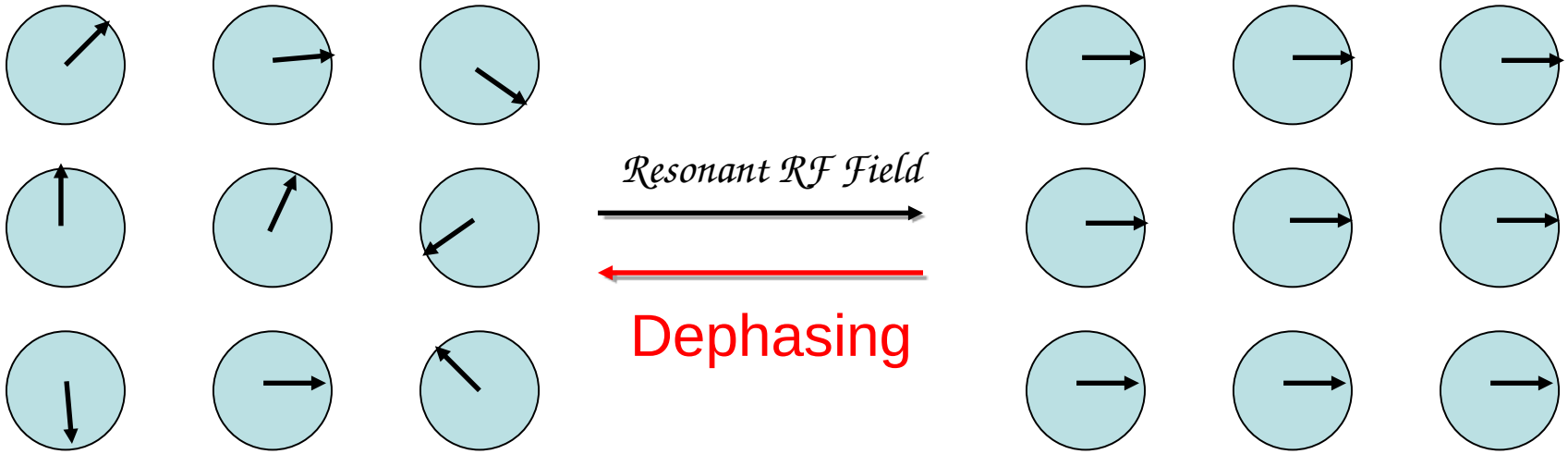
<--- $FWHM = \frac{R_2}{\pi}$

frequency domain data ω

Position of resonance ---> local magnetic environment

R_2 is a Measure of Dephasing

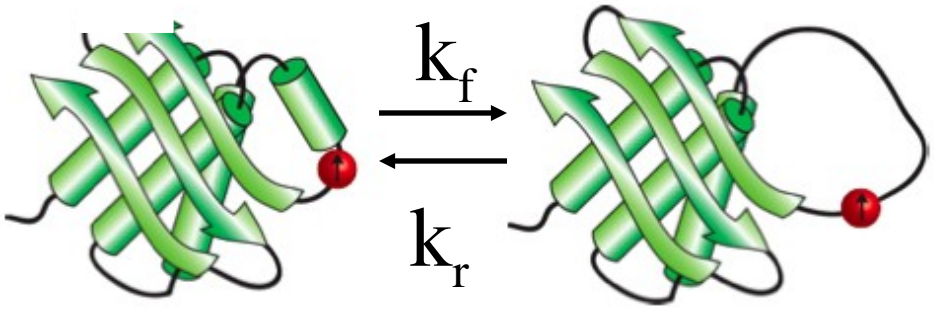
Ensemble of Nuclear Spins

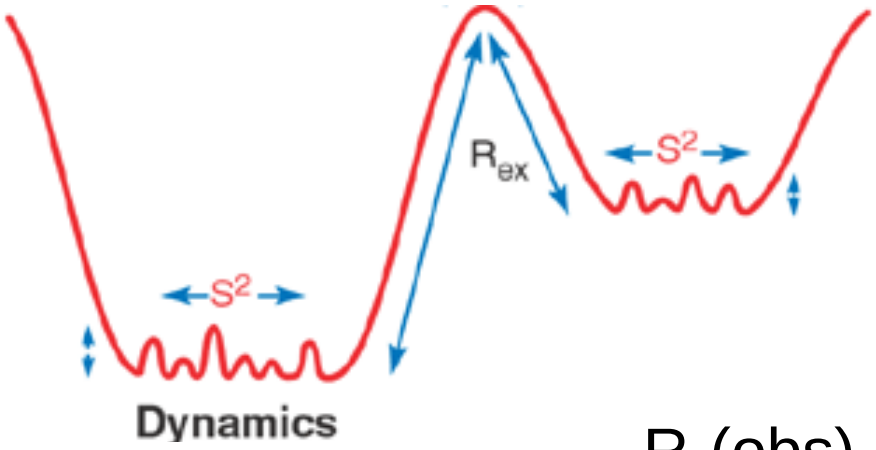


Random Phase
No NMR Signal

Phase Synchronization
NMR Signal!

Contributions to R_2 from Conformational Dynamics on the Chemical Shift timescale



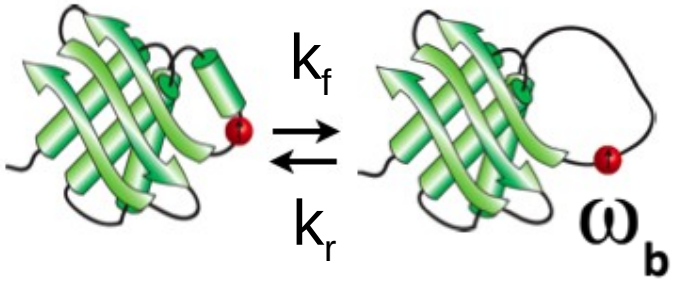


Tumbling,
Vibration,
Libration

Binding,
Conf Change,
Allostery

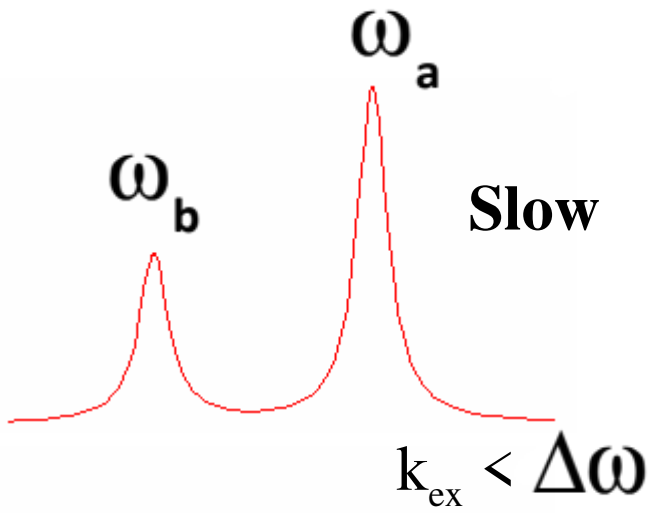
$R_2(obs) = R_2(ns\text{-}ps) + R_{ex}(ms\text{-}\mu s)$

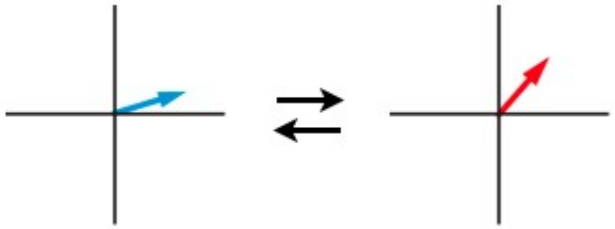
Spectral Manifestations of Exchange



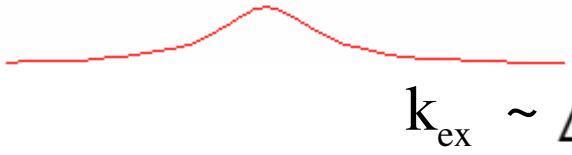
$$k_{ex} = k_f + k_r$$

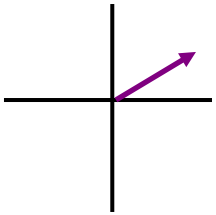
$$\Delta\omega = \omega_a - \omega_b$$



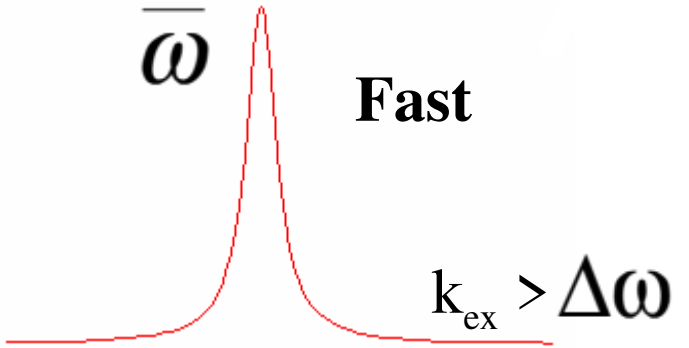


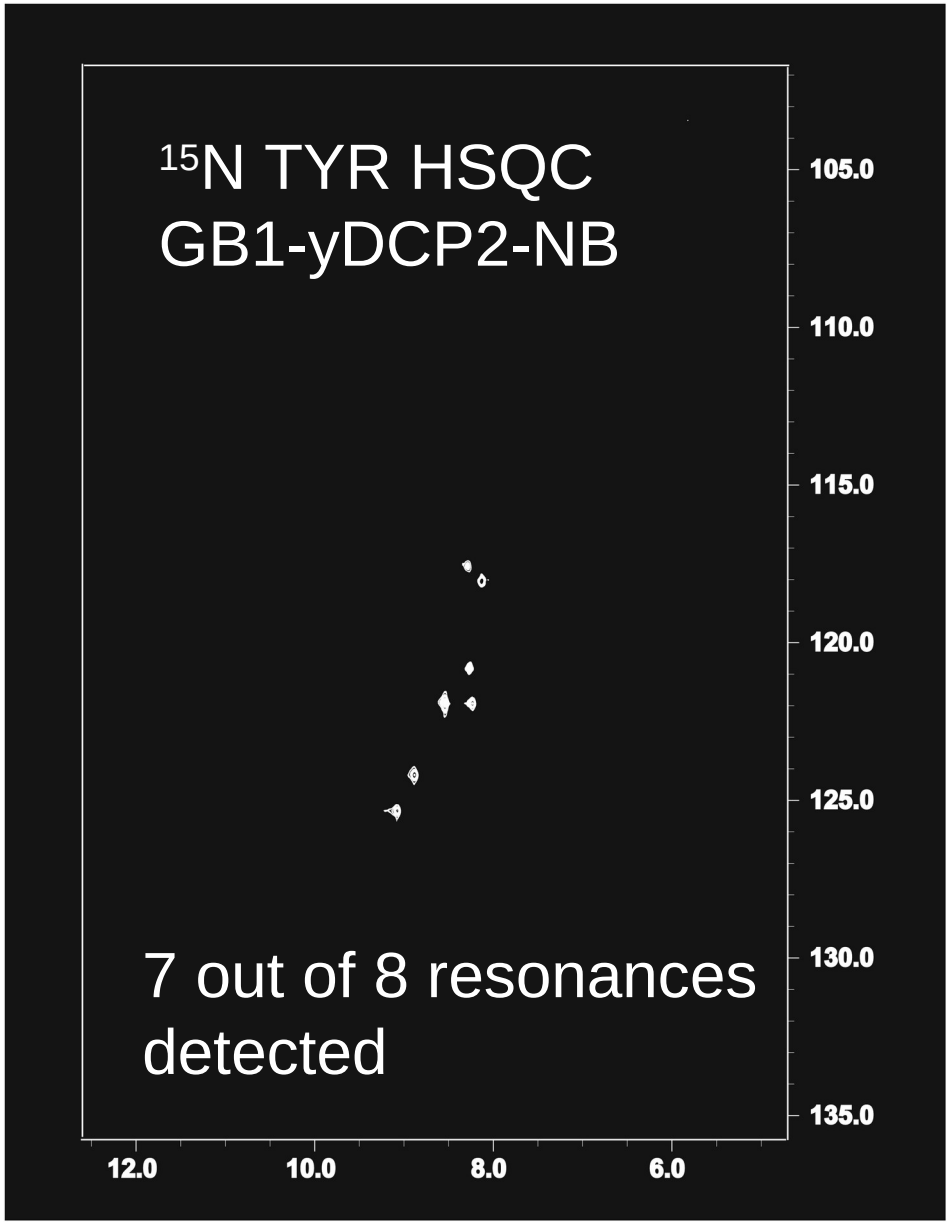
Intermediate



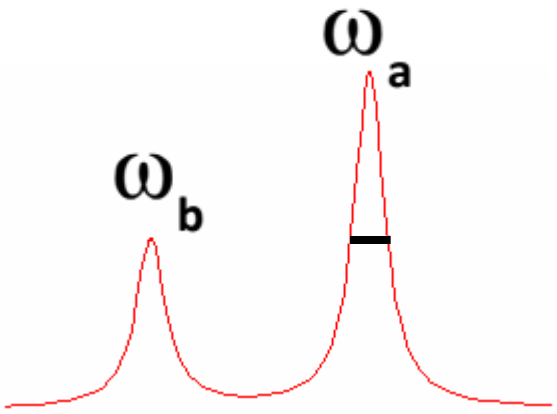


$$\overline{\omega} = p_a\omega_a + p_b\omega_b$$




15N TYR HSQC
GB1-yDCP2-NB
105.0
110.0
115.0
120.0
125.0
130.0
135.0
12.0
10.0
8.0
6.0
7 out of 8 resonances detected

Expressions for the Linewidth in the Slow Exchange Limit (k_{ex} <)$\Delta\omega$



$$A \underset{k_{rev}}{\overset{k_{for}}{\rightleftharpoons}} B$$

$$R_2^a = R_2^a(\text{ns} - \text{ps}) + p_b k_{for}$$

$$R_2^b = R_2^b(\text{ns} - \text{ps}) + p_a k_{rev}$$

R_{ex}

Populations p_a, p_b

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

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