NMR-lecture April 6th, 2009, FMP Berlin

Christian Freund

Outline:

- Basic understandings:
  - Relaxation
  - Chemical exchange
- Mapping interactions:
  - Chemical shift mapping (fast exchange)
    - Linewidth analysis (slow exchange)
  - Cross saturation transfer
  - Transfer NOE/STD NMR
  - Half filter exepriments/isotope labeling

Monitoring protein:protein interactions: Capitalizing on chemical shift and relaxation rate changes

In the absence of of an applied rf field, the Bloch equations are defined as:

$$\frac{dM_x(t)}{dt} = -\Omega M_y(t) - R_2 M_x(t)$$

$$\frac{dM_y(t)}{dt} = -\Omega M_x(t) - R_2 M_y(t)$$

$$\frac{dM_z(t)}{dt} = -\Omega\{M_z(t) - M_0\}$$

$\downarrow$

In the case of chemical exchange it is :

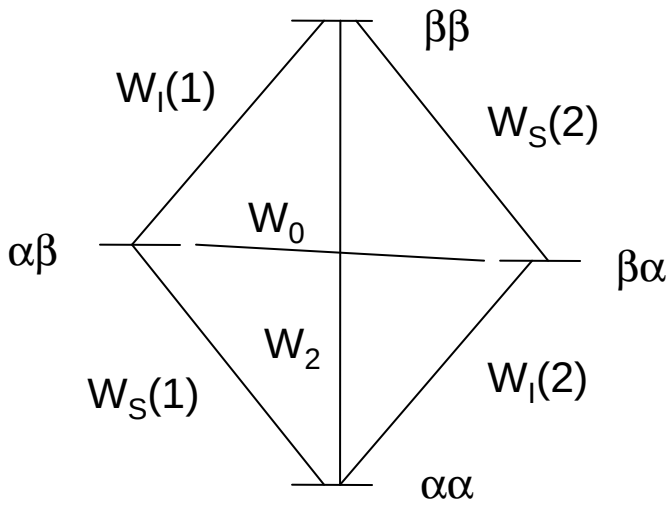
$$\frac{dM_{xy}(t)}{dt} = -(\Omega - R_2 + K)M_{xy}(t)$$

$$\frac{dM_z(t)}{dt} = -(\Omega + K)\Delta M_z(t)$$

Formal solution : $\Delta M_z(t) = e^{-Rt}\Delta M_z(0)$

$$R = \begin{bmatrix} \rho_I & \sigma_{IS} \\ \sigma_{IS} & \rho_S \end{bmatrix}$$

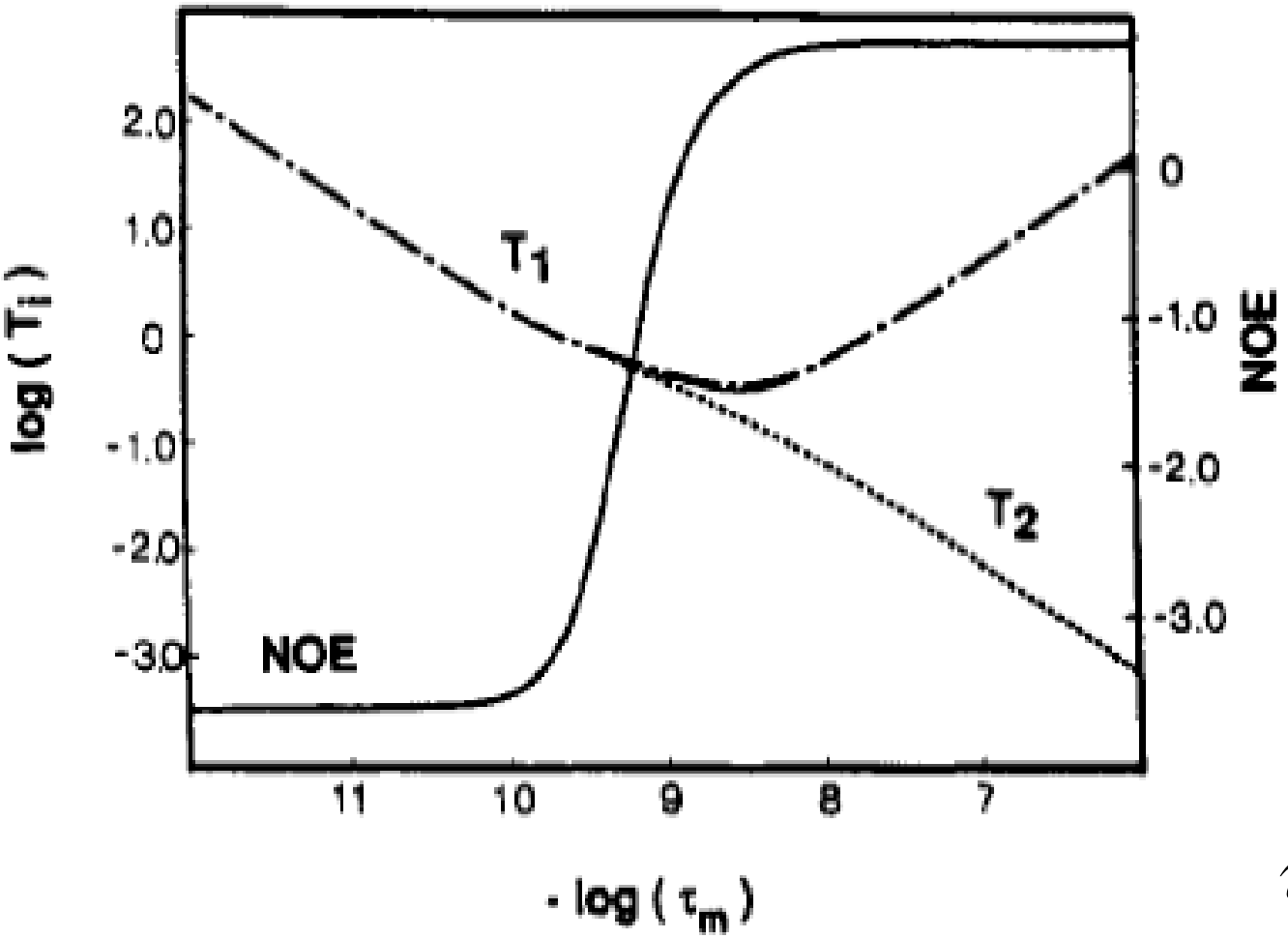
Transitions between states in a two-spin system



$$\sigma = W_2 - W_0$$

$$\sigma_{ij} \sim 1/r_{ij}^6 * [6J(2\omega_0) - J(0)]$$

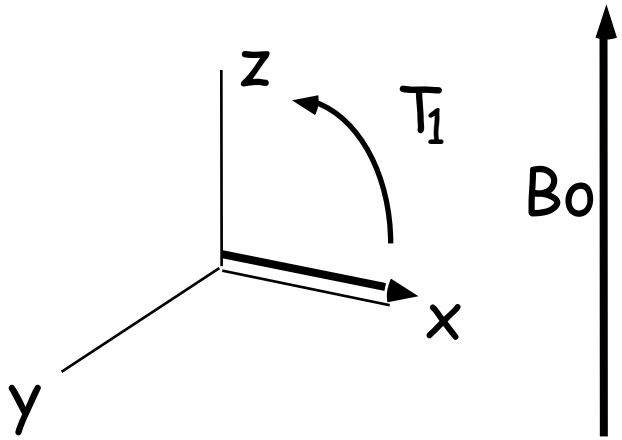
Relaxation times depend on the overall tumbling time of molecules which is proportional to molecular weight



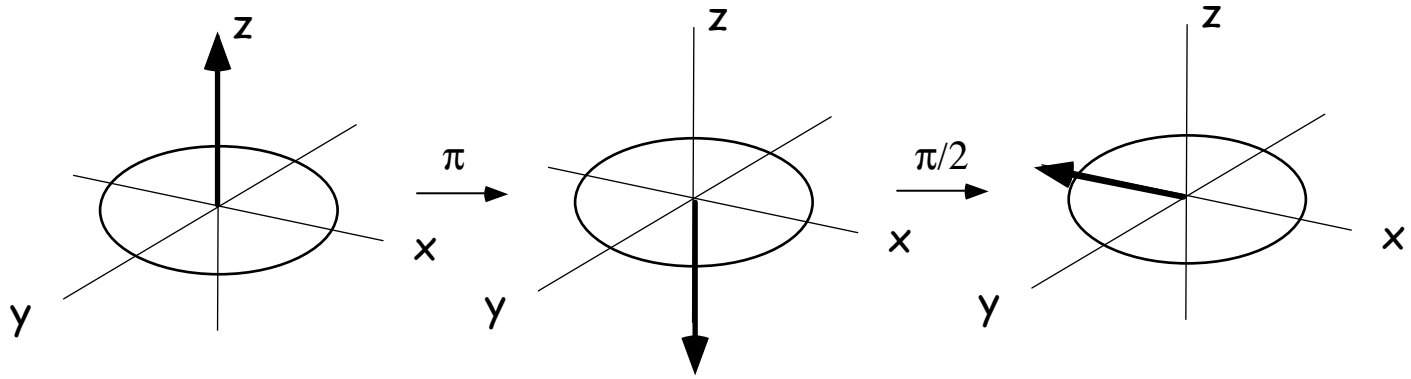
$$\tau_c = \frac{4\pi\eta r^3}{3kT}$$

Biochemistry, Vol. **28, No. 23, 1989**

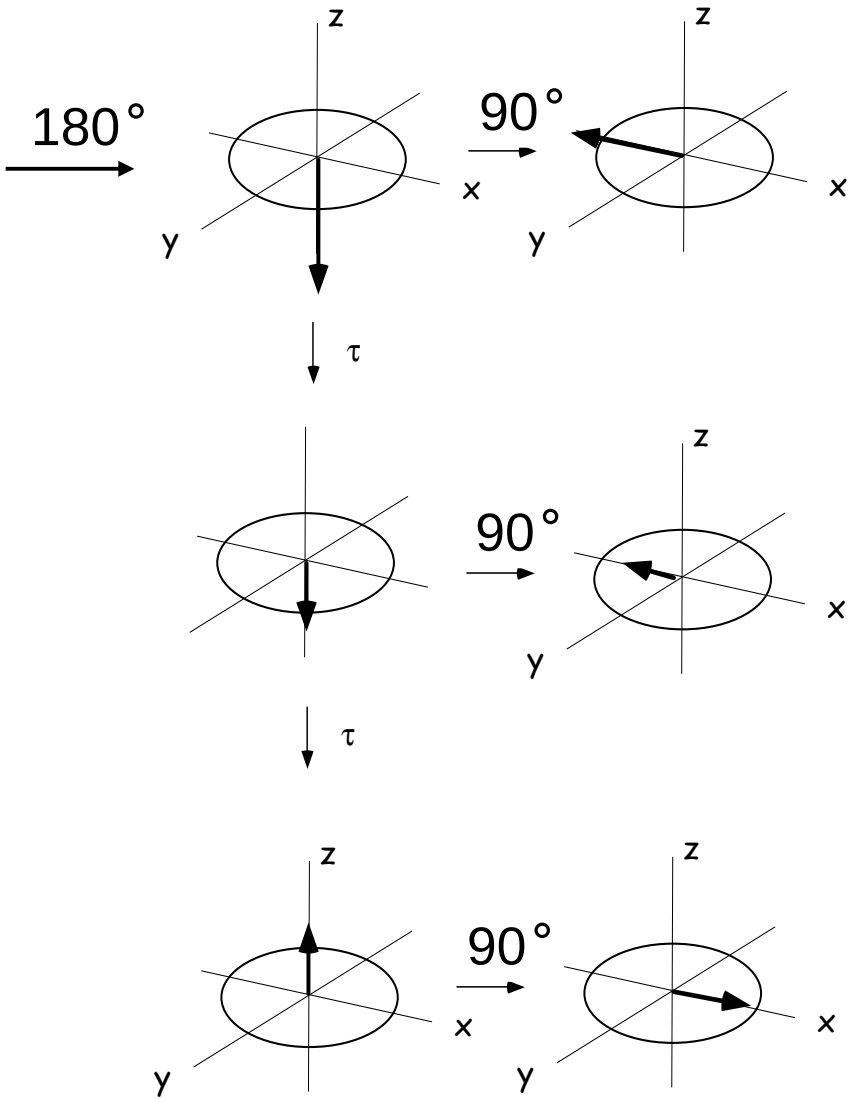
T_1 relaxation



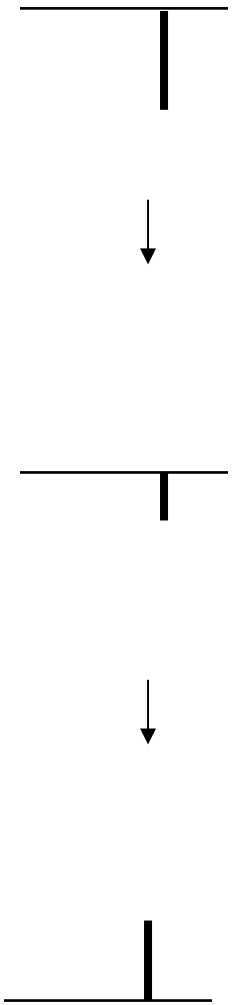
Measuring T1 from inversion recovery pulse sequence



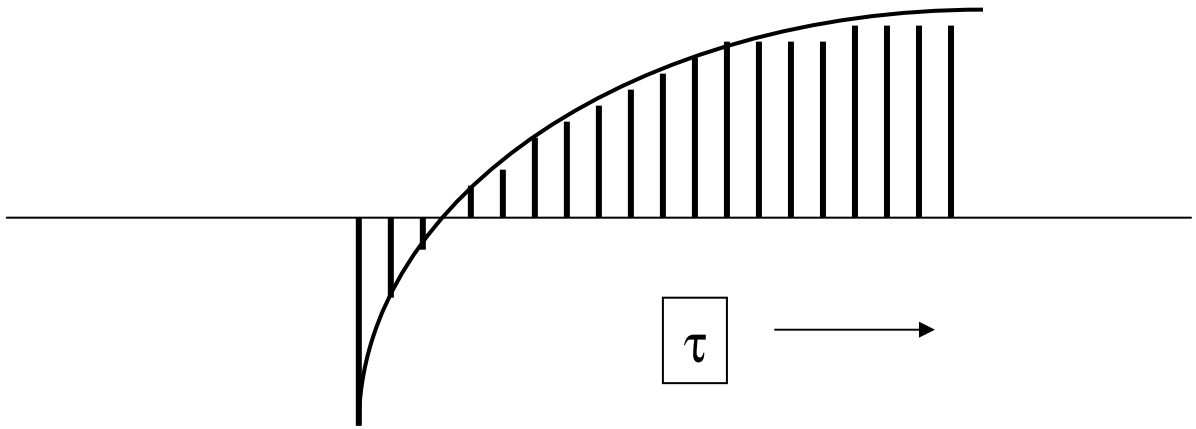
Measuring T1 relaxation times



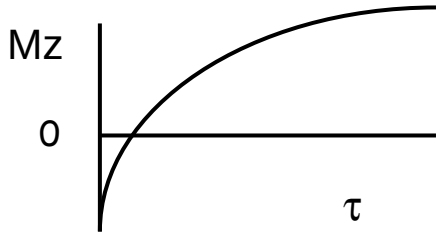
Inversion Recovery



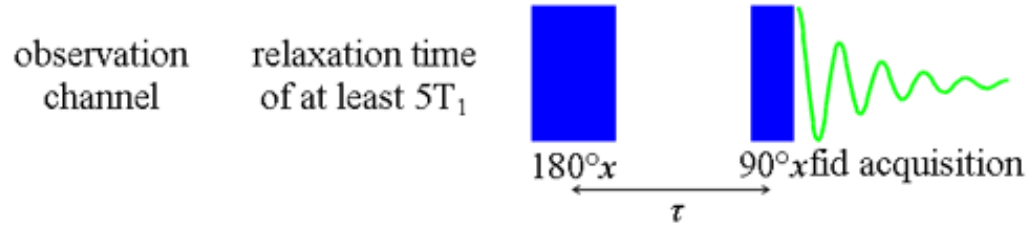
Inversion Recovery - Measure NMR Intensity as a function of the delay time τ and fit to an exponential function



$$Mz = Mo\,(1 - 2e^{-\tau/T1})$$

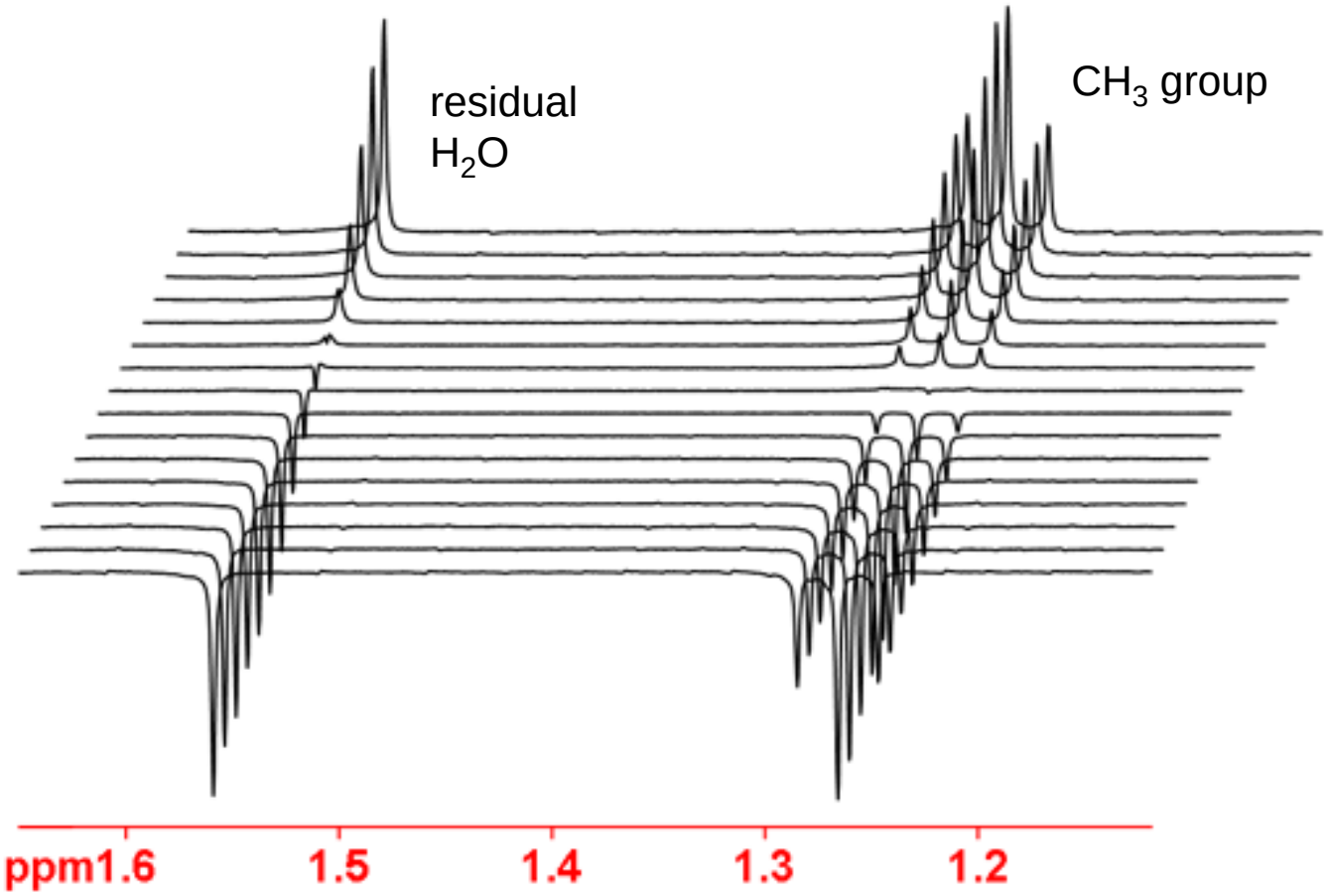


Inversion recovery pulse sequence for measuring T_1



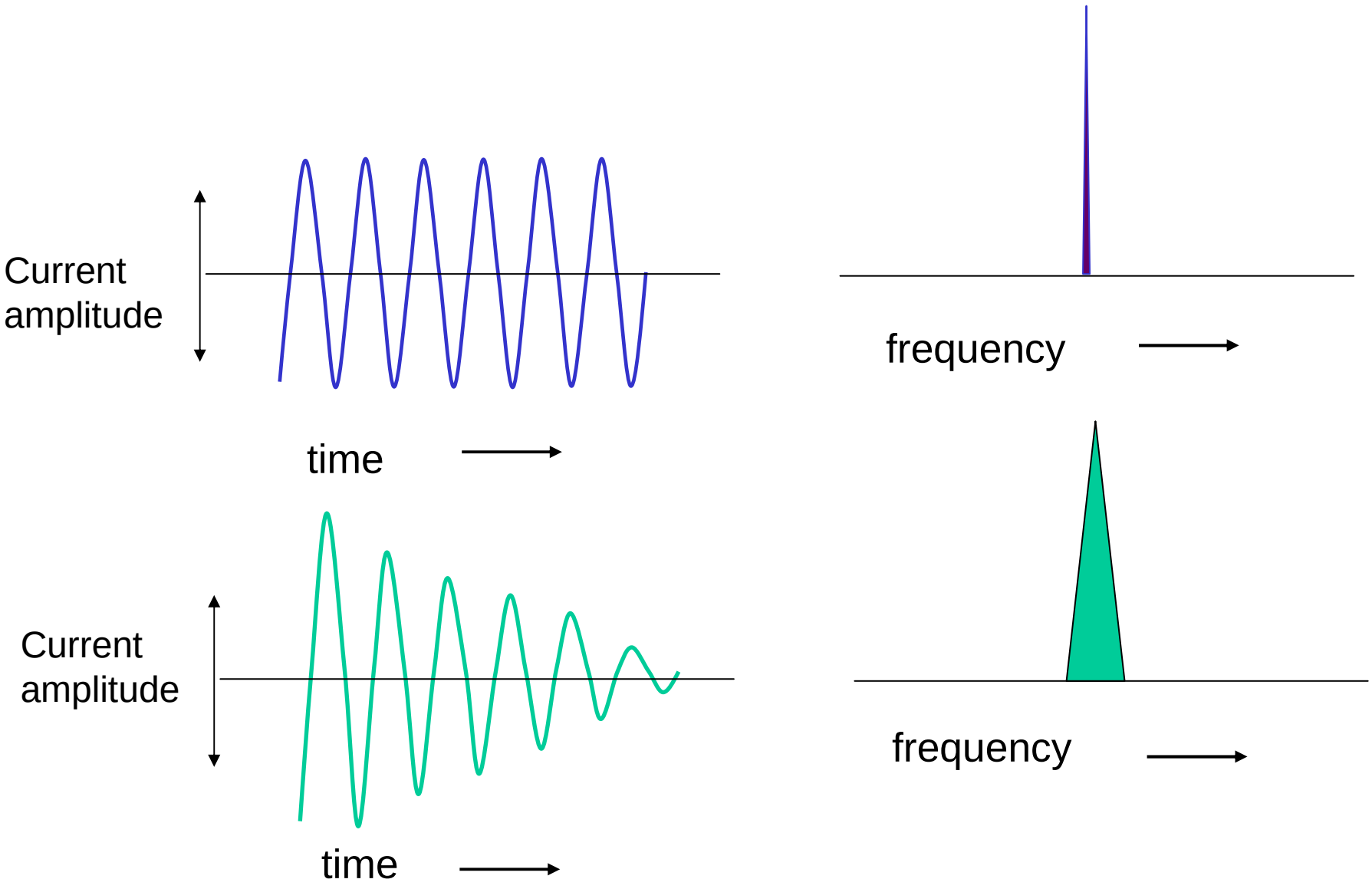
Adopted from Roy Hofmann, Hebrew University

The inversion recovery experiment yields T1 values for different signals that may have different relaxation times

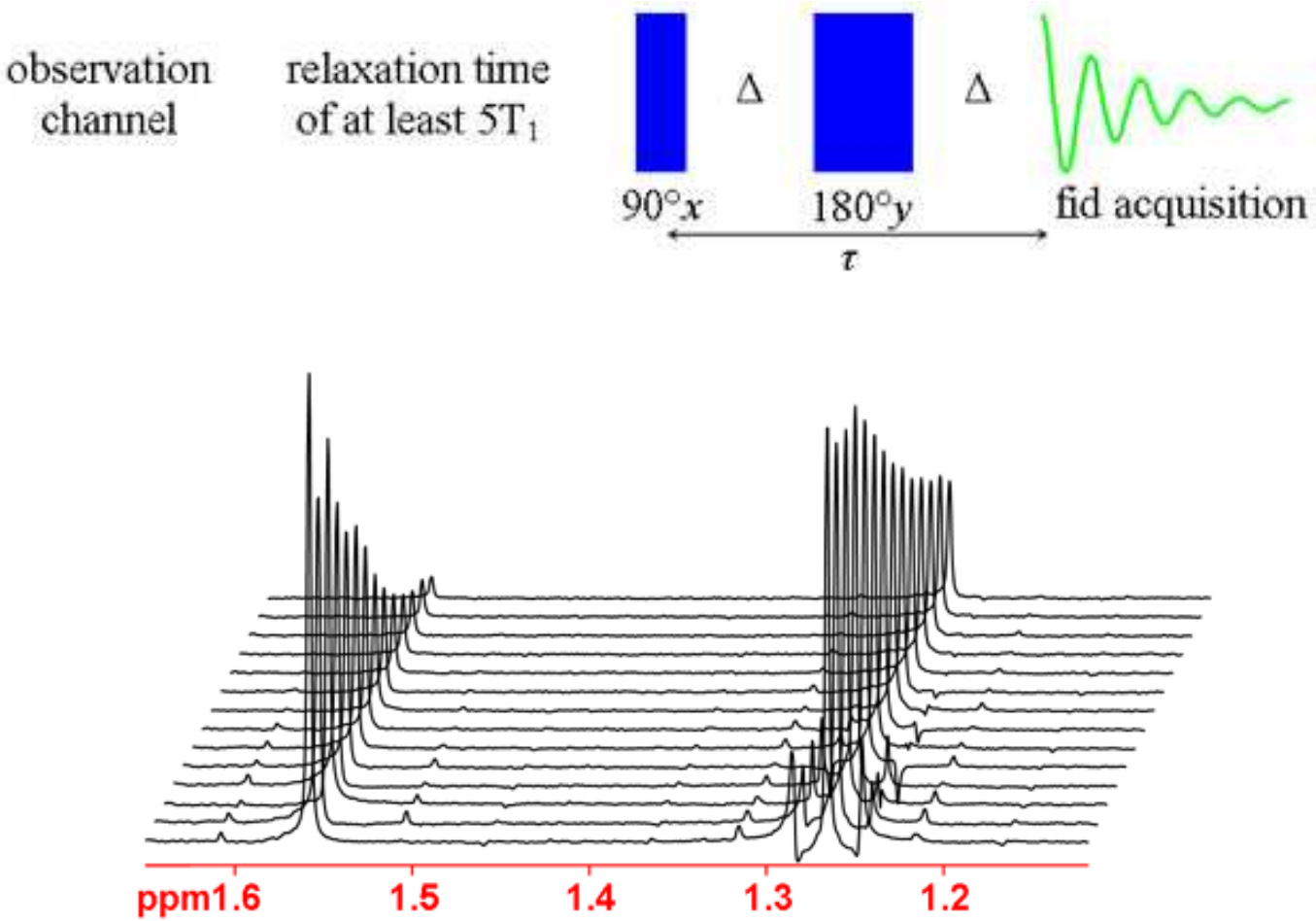


Example: Ethylbenzene in $CDCl_3$

T_2 relaxation leads to line-width broadening



Spin-echo pulse sequence for measuring T_2



Example: Ethylbenzene in $CDCl_3$

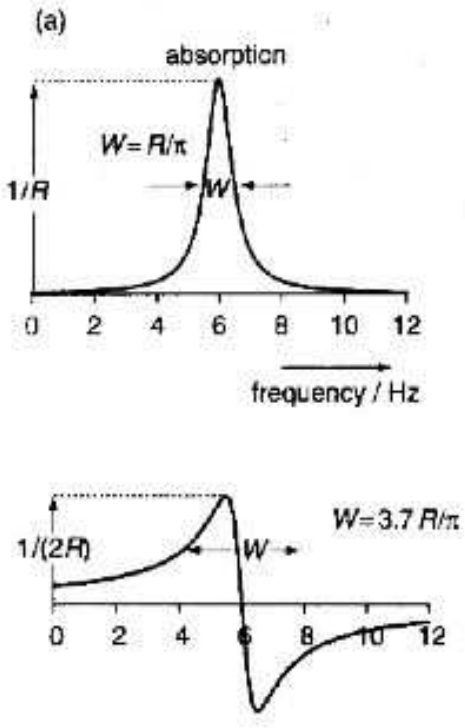
Linewidth depends on transverse relaxation

$$S(\omega) = \mathrm{Re}\int_0^\infty s^+(t)\exp(-i\omega t)dt$$

$$= \nu(\omega) + i\upsilon(\omega)$$

$$\nu(\omega) = \lambda M_0 \frac{R_2}{R_2^2 + (\Omega-\omega)^2}$$

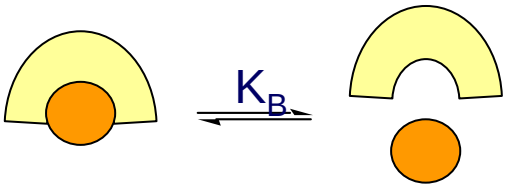
$$\upsilon(\omega) = \lambda M_0 \frac{\Omega-\omega}{R_2^2 + (\Omega-\omega)^2}$$



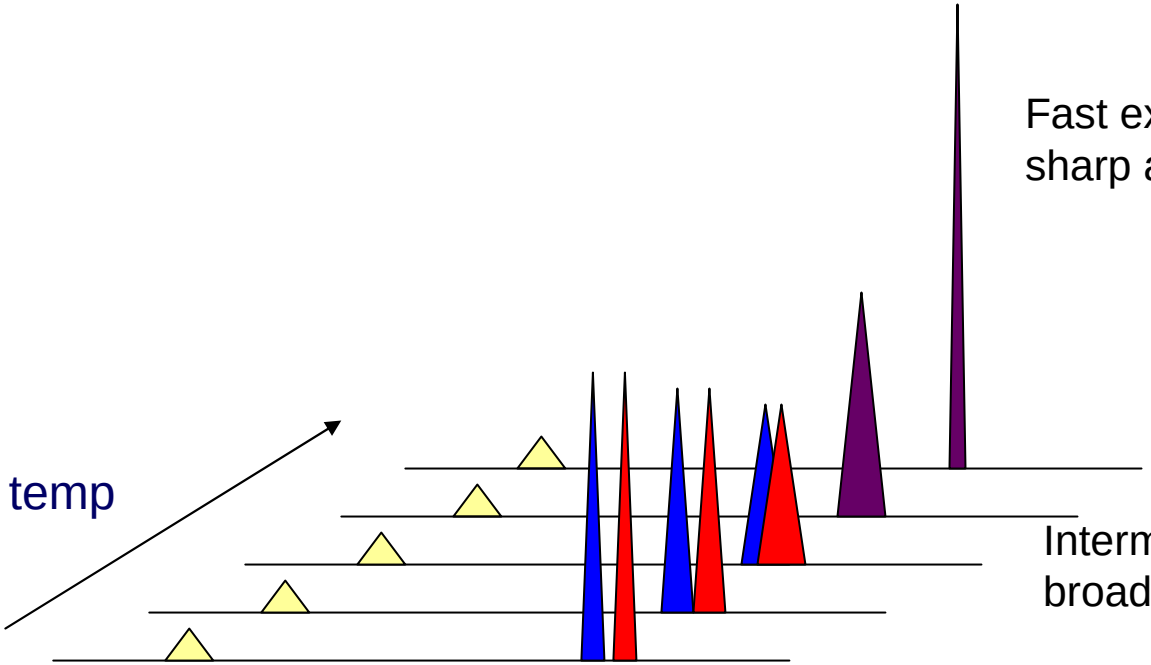
@ „Understanding NMR spectroscopyx by J. Keeler"

Chemical exchange complicates matters

Conformational equilibrium



Chemical equilibrium

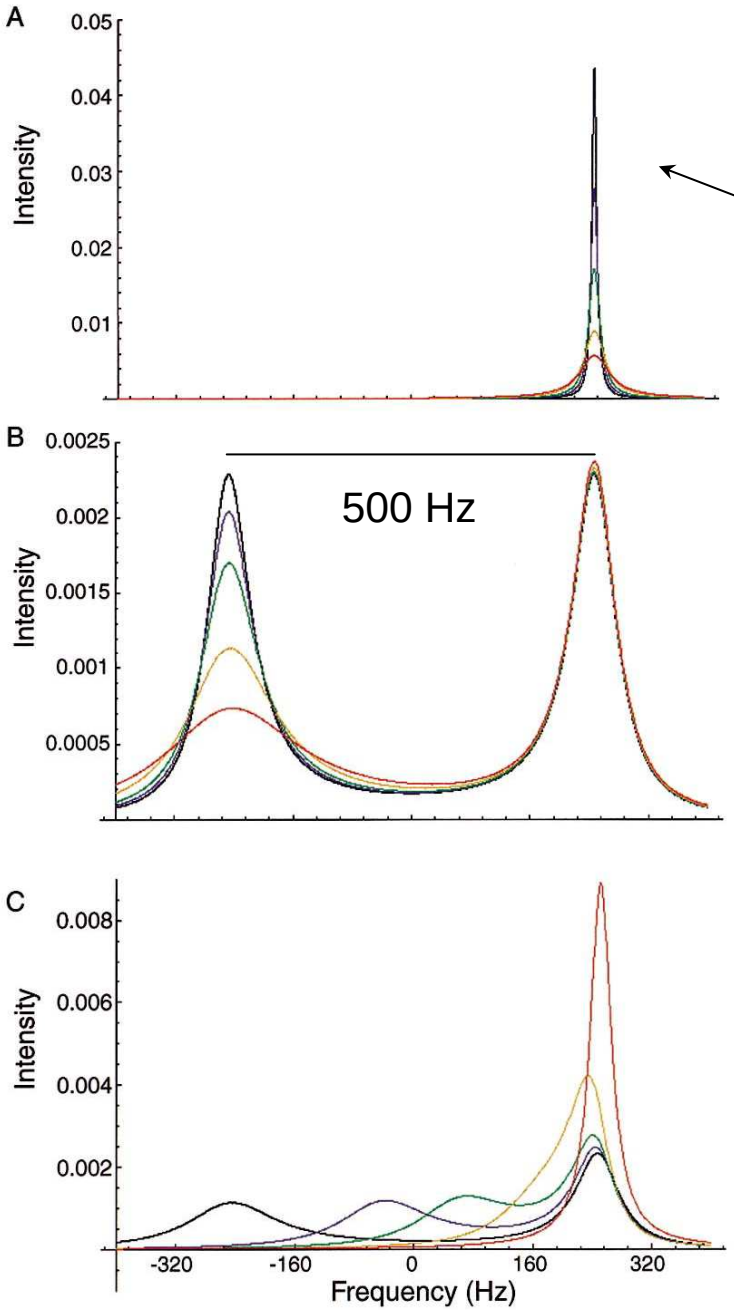


Fast exchange - one sharp average resonance

Intermediate exchange - one broad resonance

Slow exchange - two distinct resonances

Linewidth simulations for slow exchange interactions



$I(\omega) \sim Re \{W * A^{-1} * 1\}$

$A = i(\Omega - \omega E) + K + R$

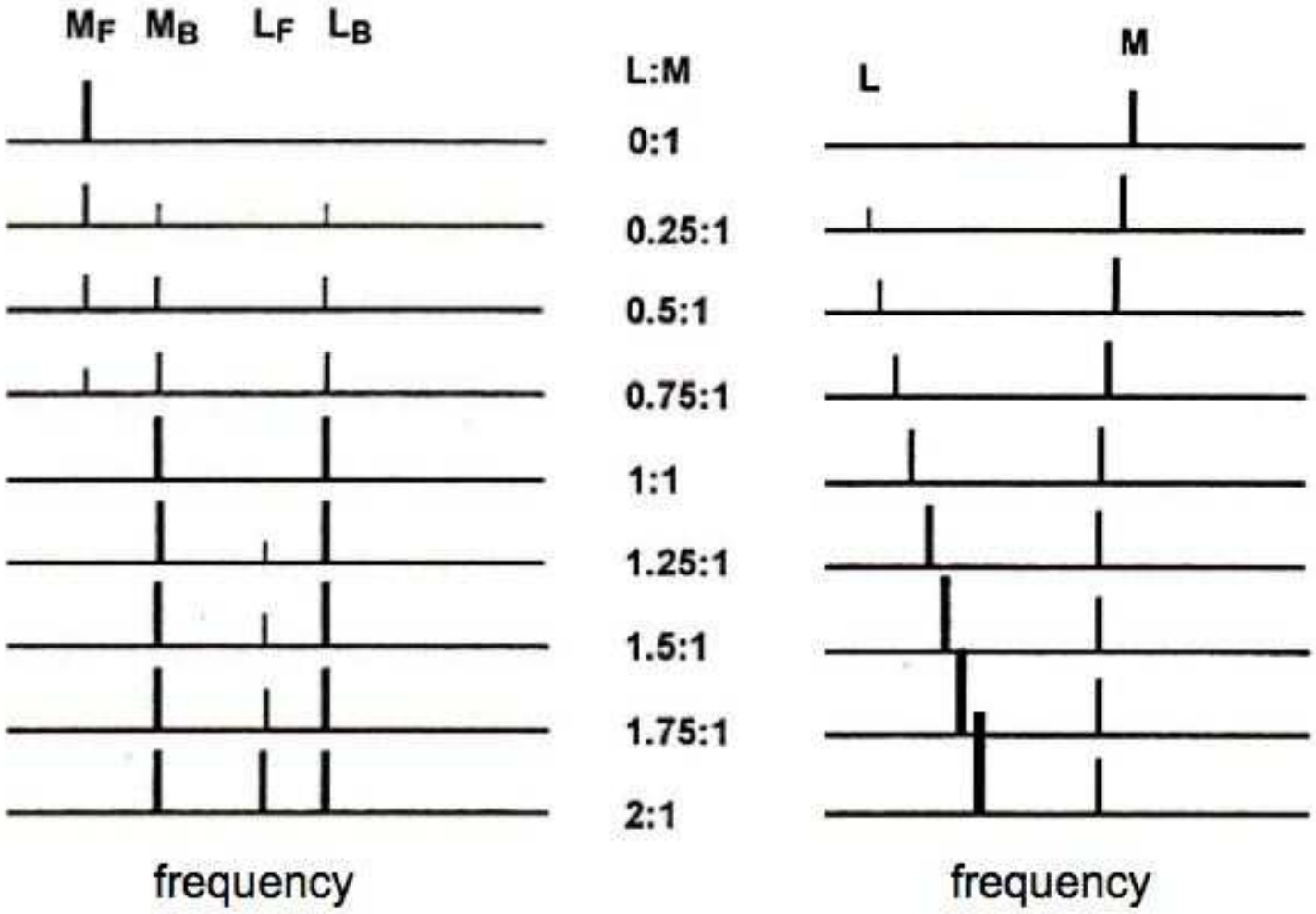
Changing R_2 of the bound state: 500, 250,100,50 and 23 s^{-1}. No chemical shift difference of free and bound state

Same parameters used as above. R_2 (free) is 23 s^{-1} and k_{off} = 200 s^{-1}. The fraction of free protein is 0.5.

R_2 (bound) is set to 250 s^{-1} and chemical shifts are varied : 500, 250, 100, 50 and 23 Hz

Published in: Hiroshi Matsuo; Kylie J. Walters; Kenta Teruya; Takeyuki Tanaka; George T. Gassner; Stephen J. Lippard; Yoshimasa Kyogoku; Gerhard Wagner; *J. Am. Chem. Soc.* **1999,** 121, 9903-9904.

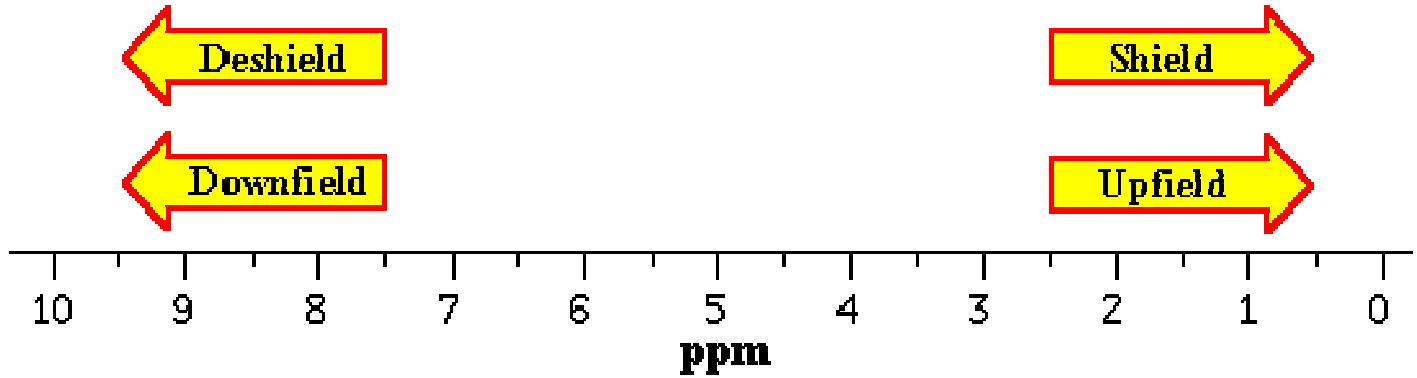
What type of exchange ?



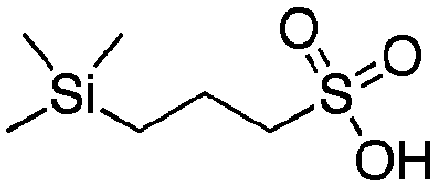
Mapping protein interactions:

Chemical shift as a measure of chemical environment :

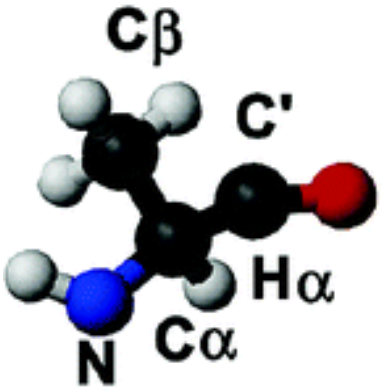
$$\delta = \frac{\text{difference in precession frequency between two nuclei}}{\text{operating frequency of the magnet}}$$



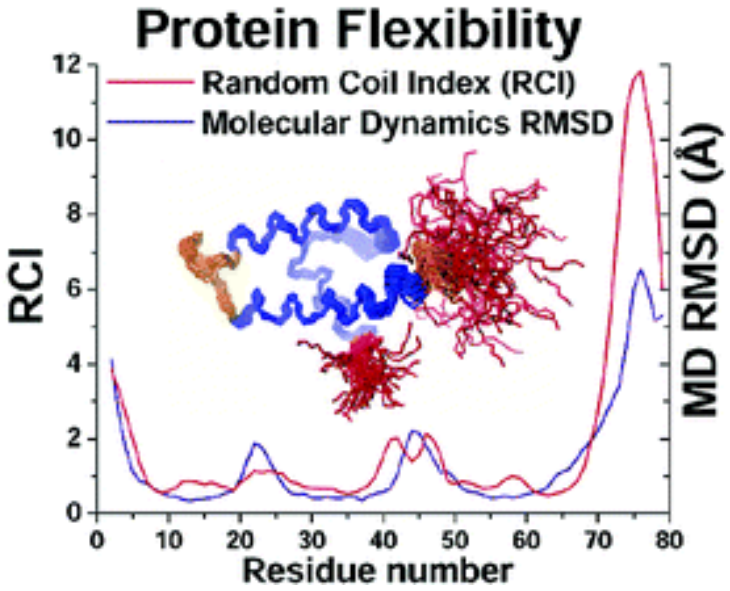
Reference against: 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS)



Predicting protein chemical shifts via CSI

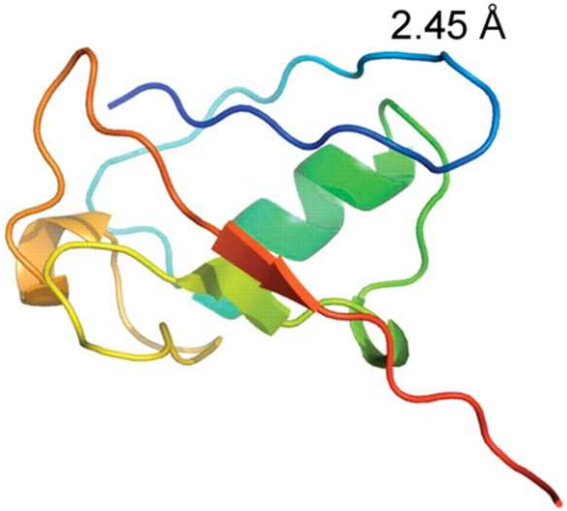


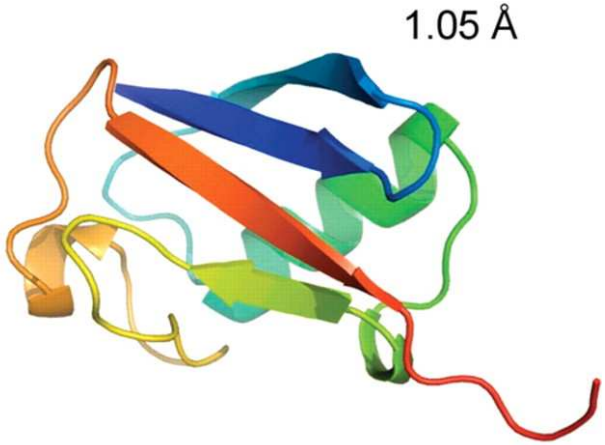
NMR



Berjanskii & Wishart,
Nature Protocols 1, - 683 - 688 (2006)

Structure refinement via CSI

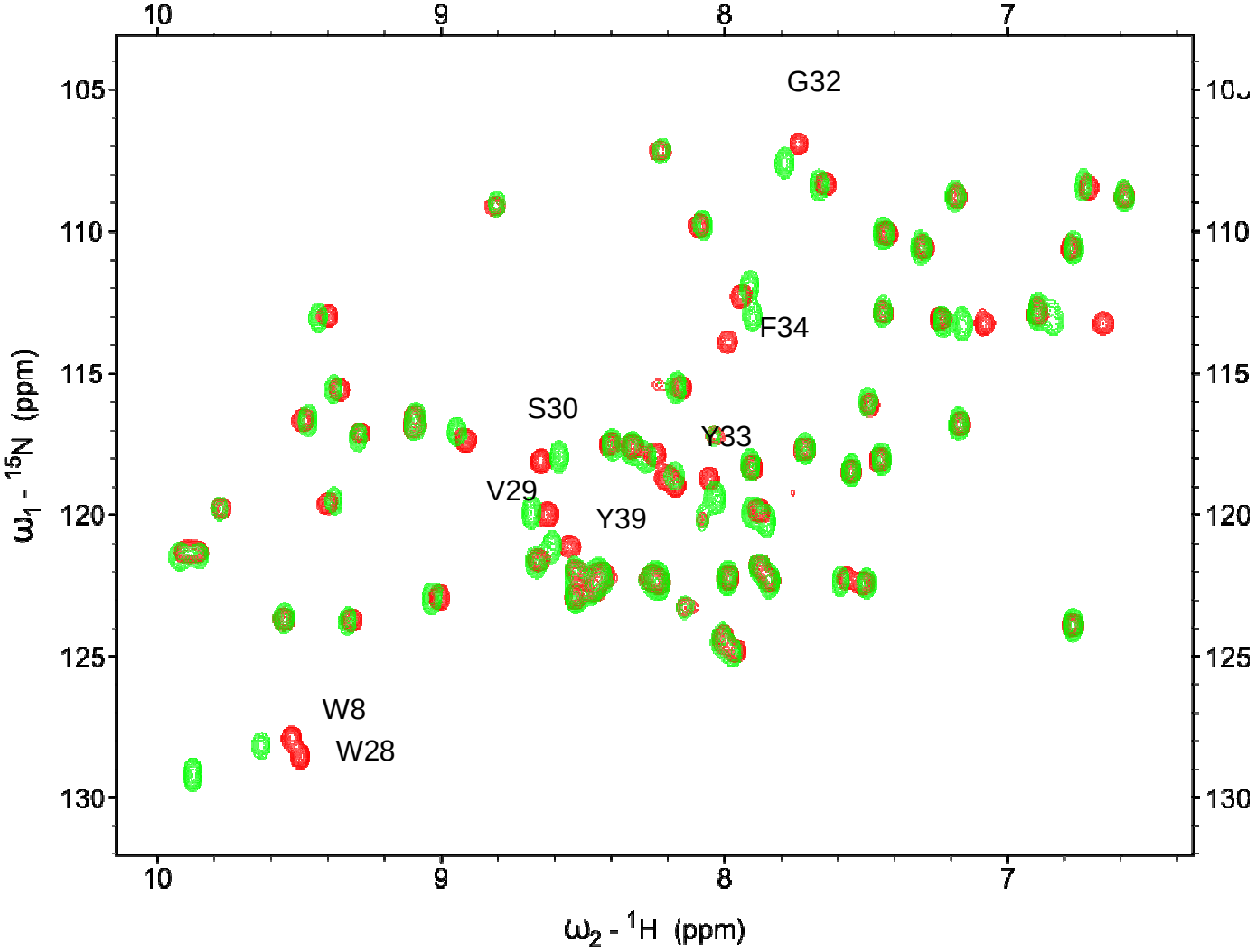


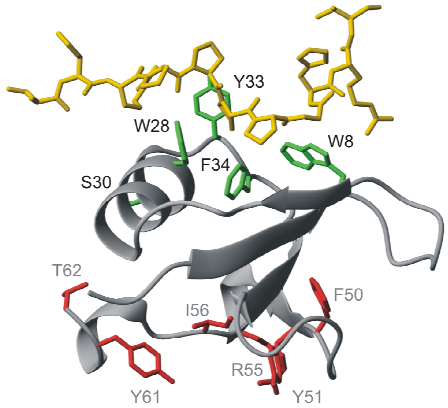


http://www.cs23d.ca/

Chemical shift changes : a single non-disruptive mutation

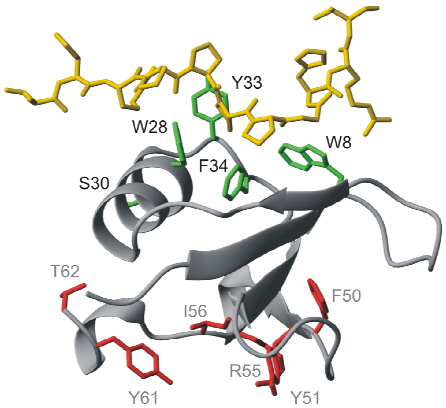
Wt(rot)/Y33A(grün)



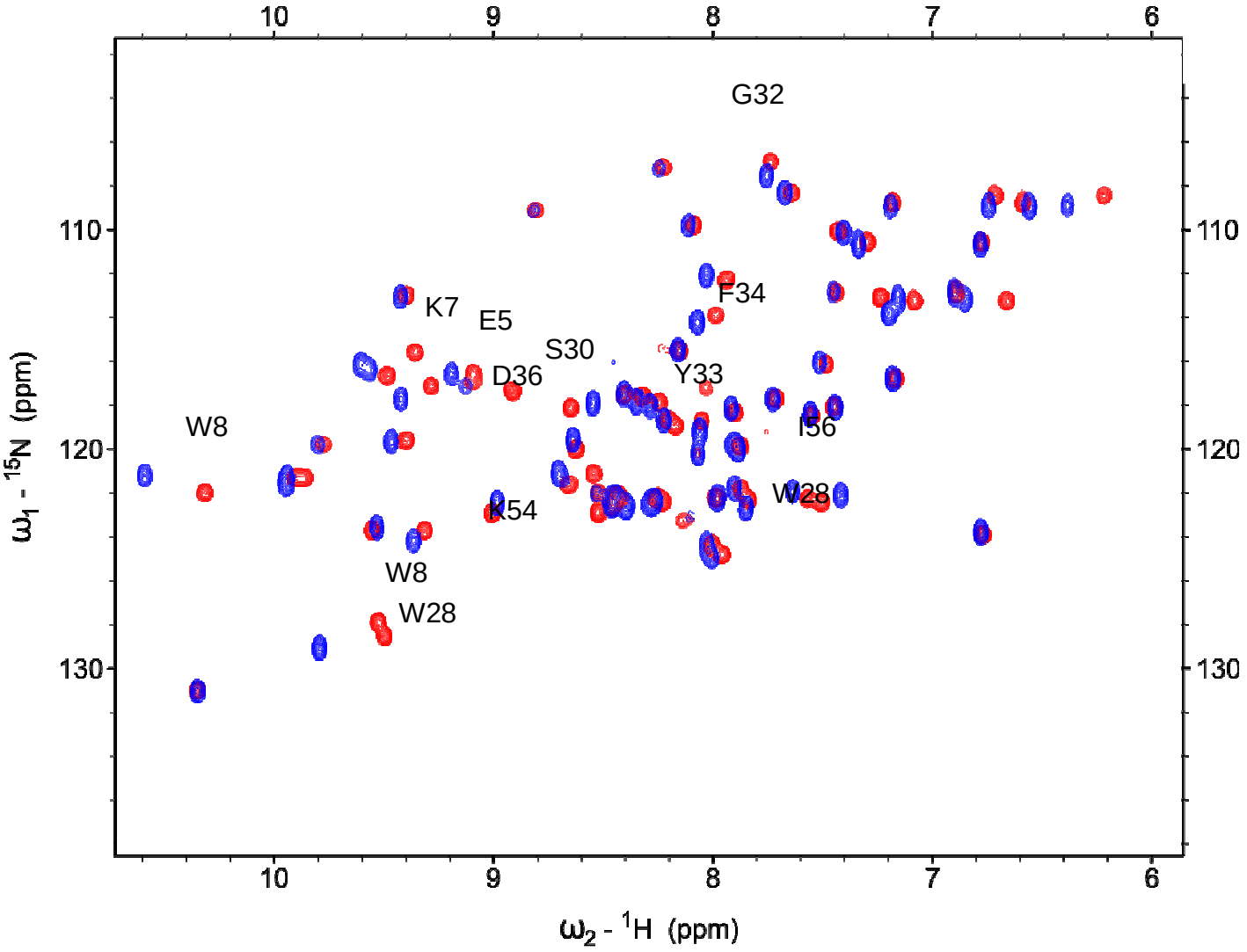


GYF domain

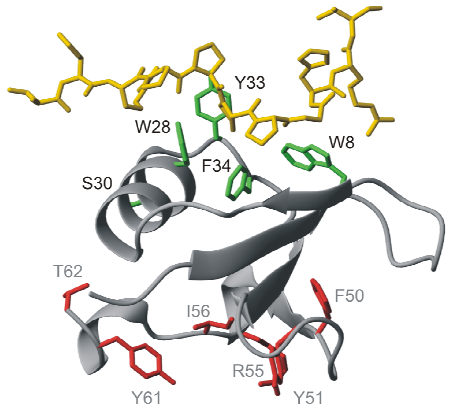
Chemical shift changes : two non-disruptive mutations



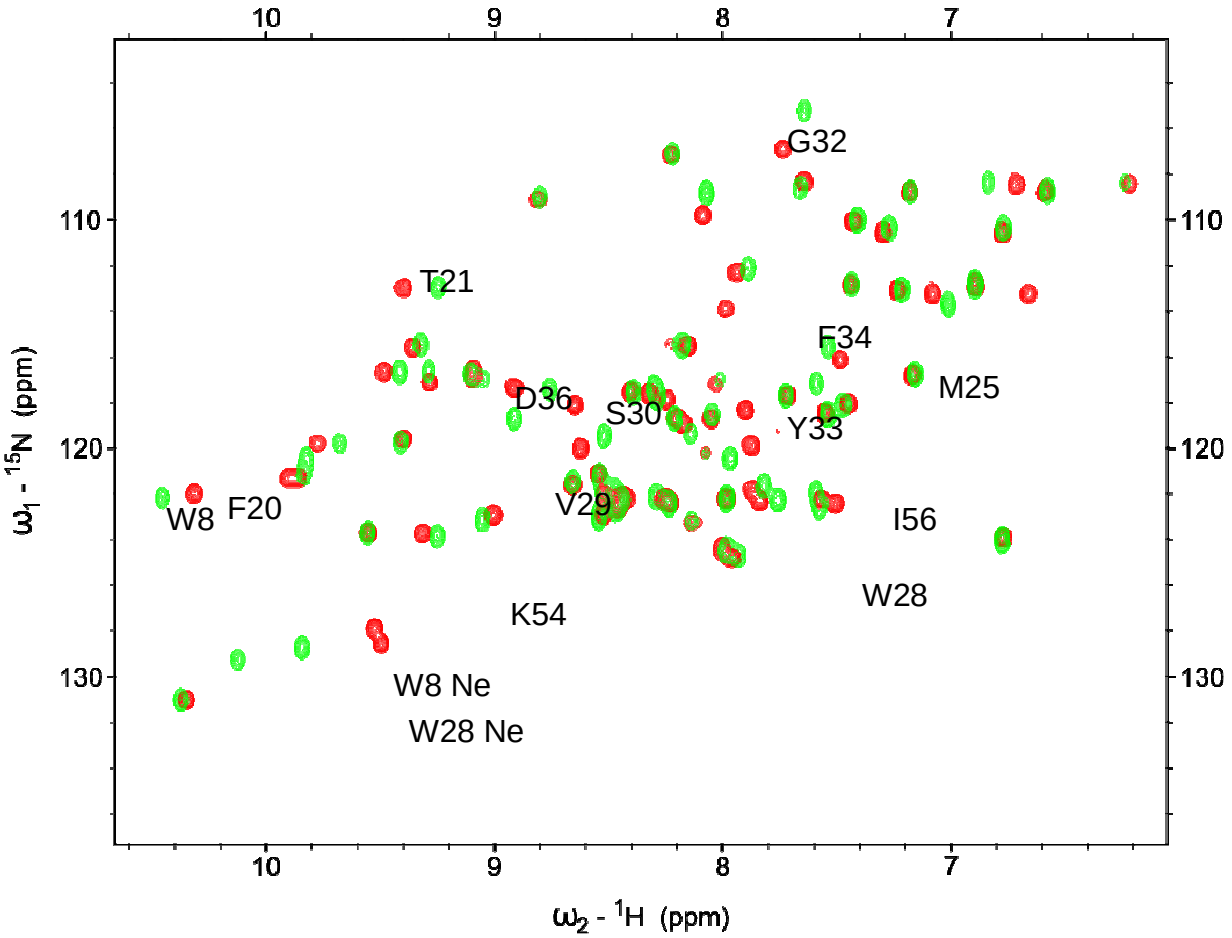
Wt(rot)/Y33AW8R(blau)



Chemical shift changes:Fast exchange: GYF binding to spliceosomal SmB

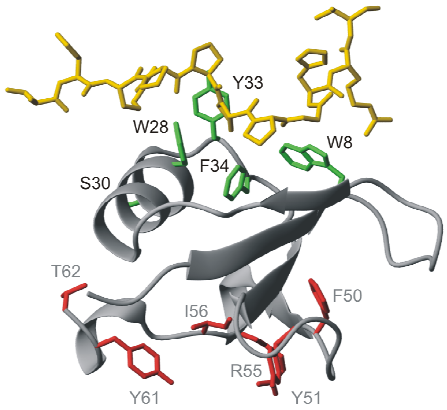


Wt(rot)/Wt_SmB(grün)

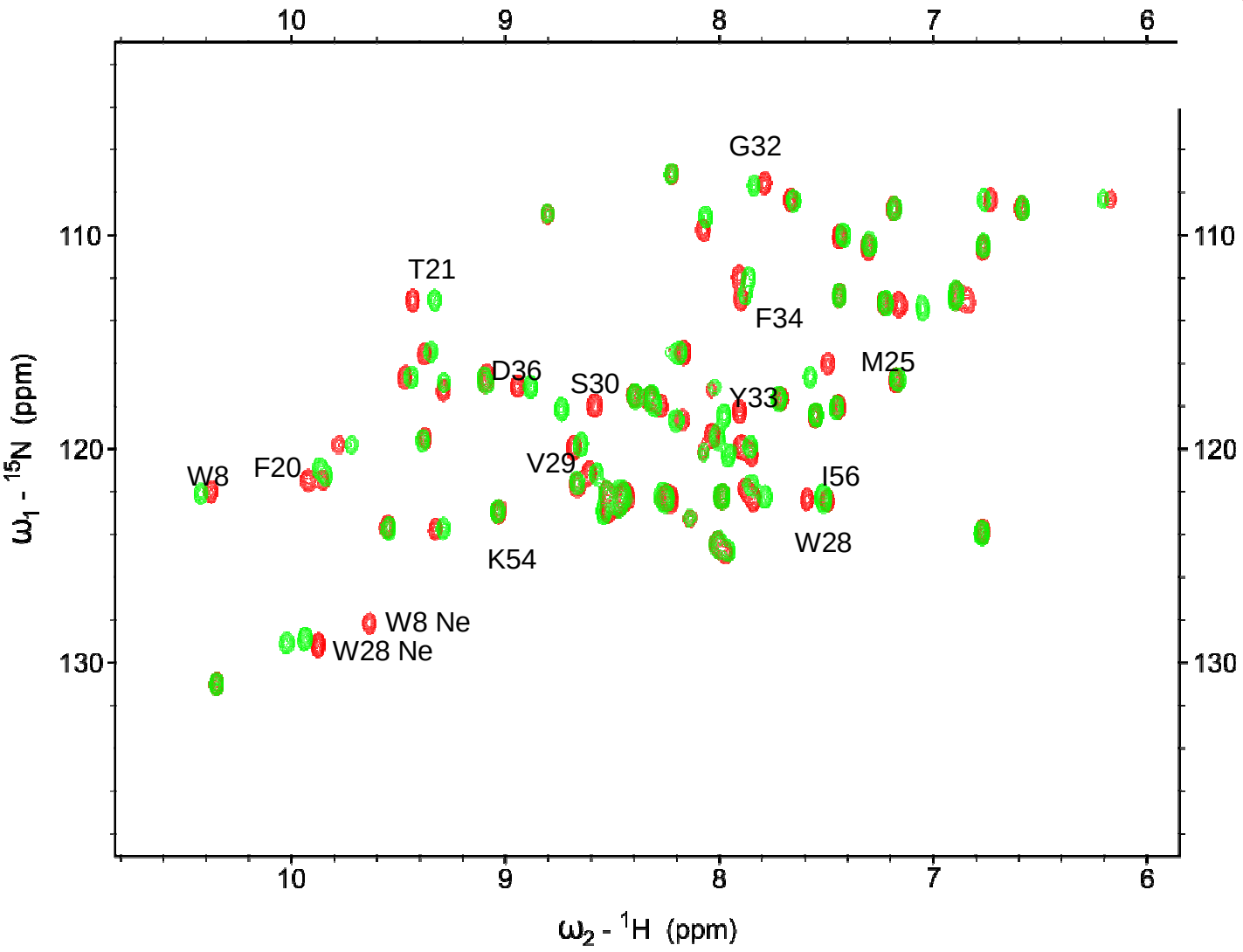


$$[PL] = 1/2(K_D + [P]_0 + [L]_0) - \sqrt{1/4(K_D + [P]_0 + [L]_0)^2 - [L]_0\,[P]_0}$$

Binding of the single-site mutant

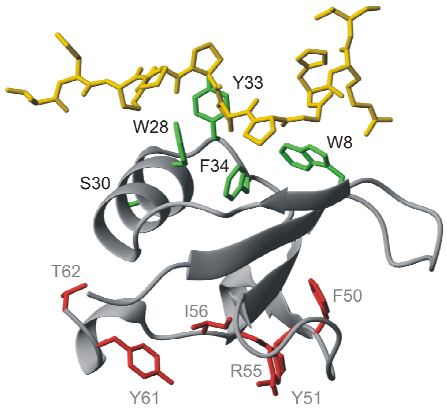


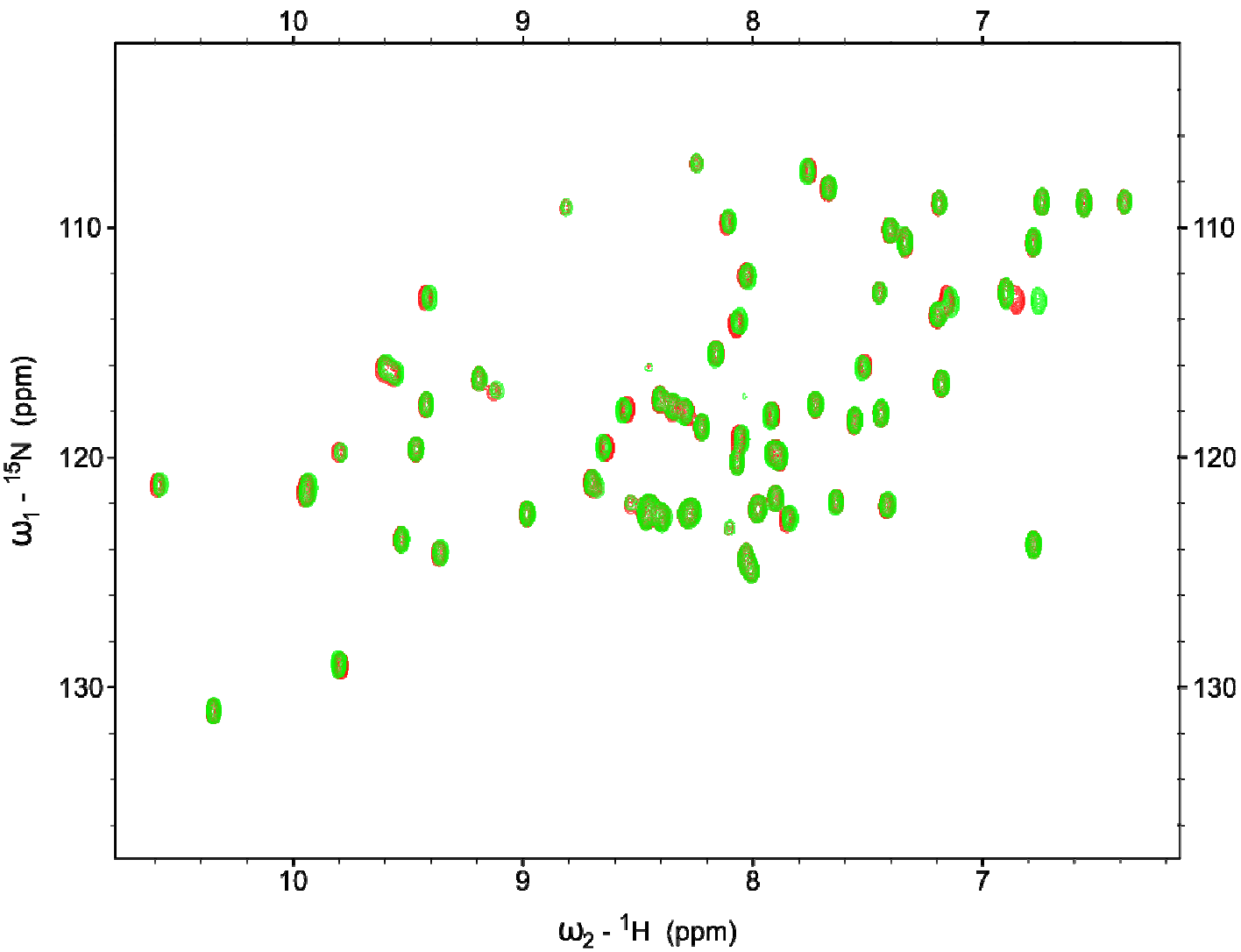
Y33A(rot)/Y33A_SmB(grün)



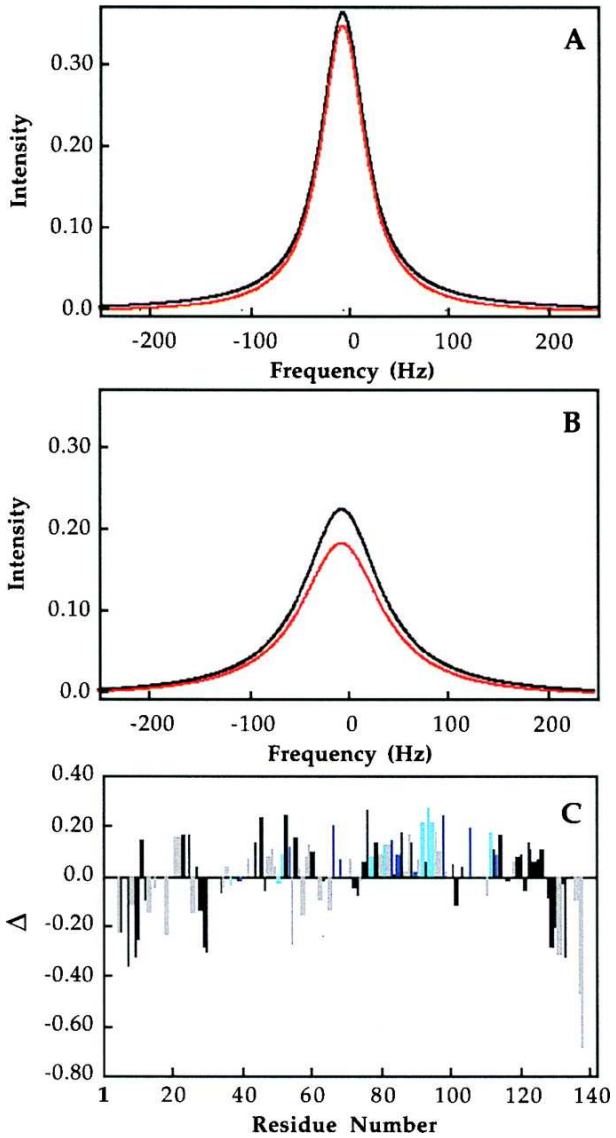
(Non-)Binding of the double mutant

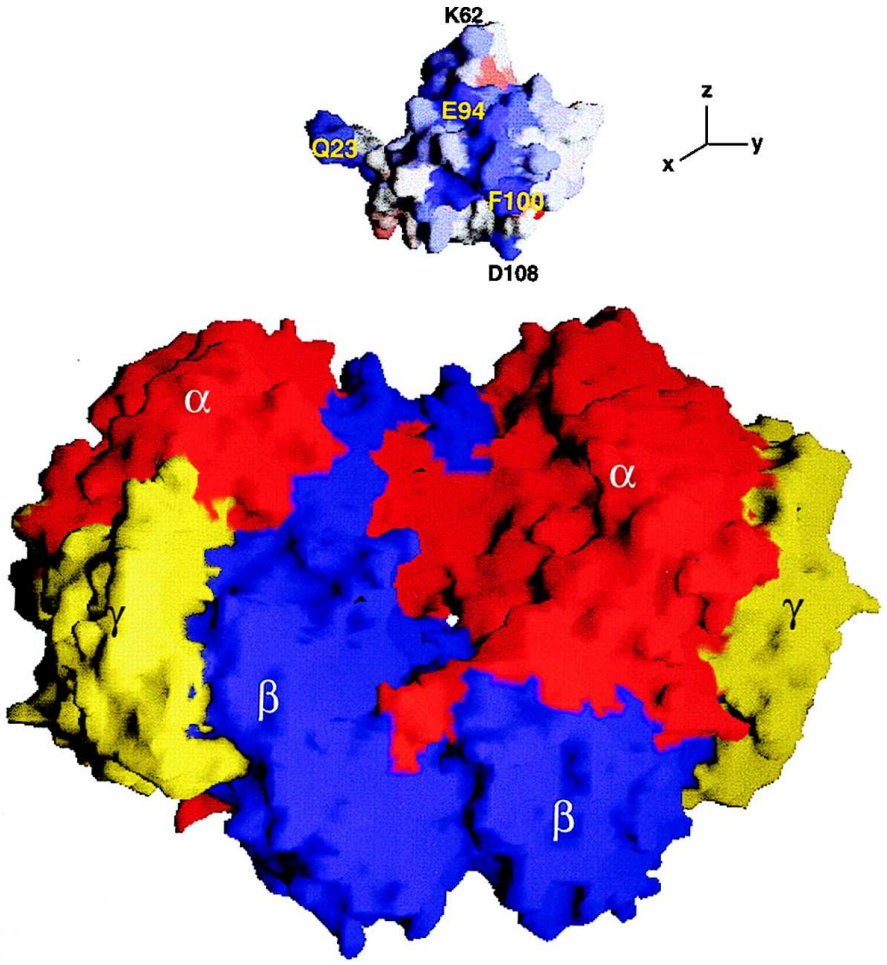
Y33AW8R(rot)/Y33AW8R_SmB(grün)





Slow exchange: Linewidth analysis can be used to map binding sites

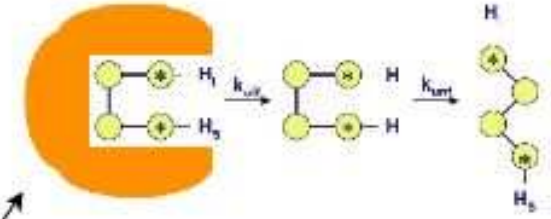




Walters et al., PNAS 1999, 96, 7877-7882

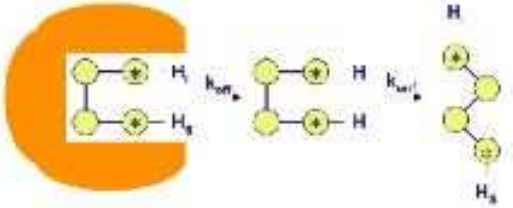
k_{off} values of 3.2 s^{-1} (A) or 25.6 s^{-1} (B) for chemical shift differences
of 0 Hz (black) and 500 Hz (red). R 1, R 2, and a are 23 Hz, 250 Hz, and 0.8, respectively

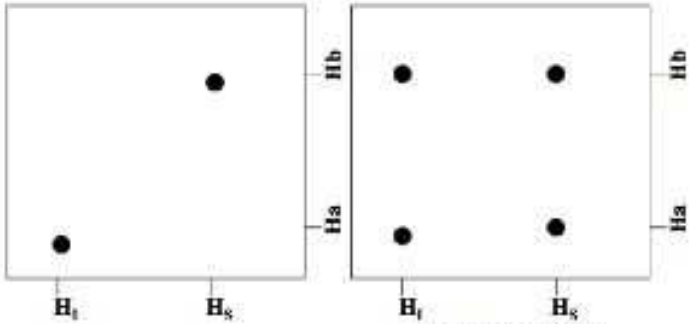
Transferred NOEs for determination of bound ligand structures



Selective irradiation
of protein signals

Saturation transfer
difference spectroscopy





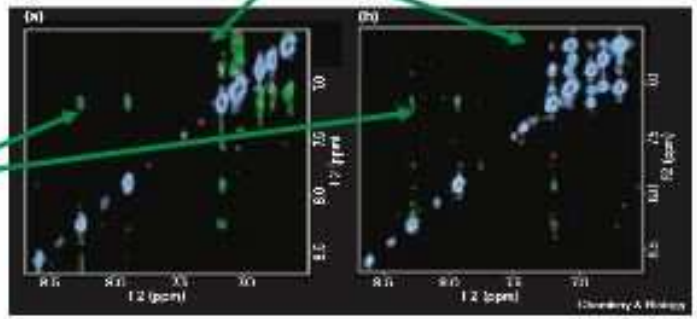
2D NOESY spectrum
before addition of receptor

2D NOESY spectrum
after addition of small amount
of receptor (a few percent)

Change in sign of cross peak indicates binding

2D NOESY spectra
Positive peaks –cyan
Negative peaks - green

No change in
sign, no
binding

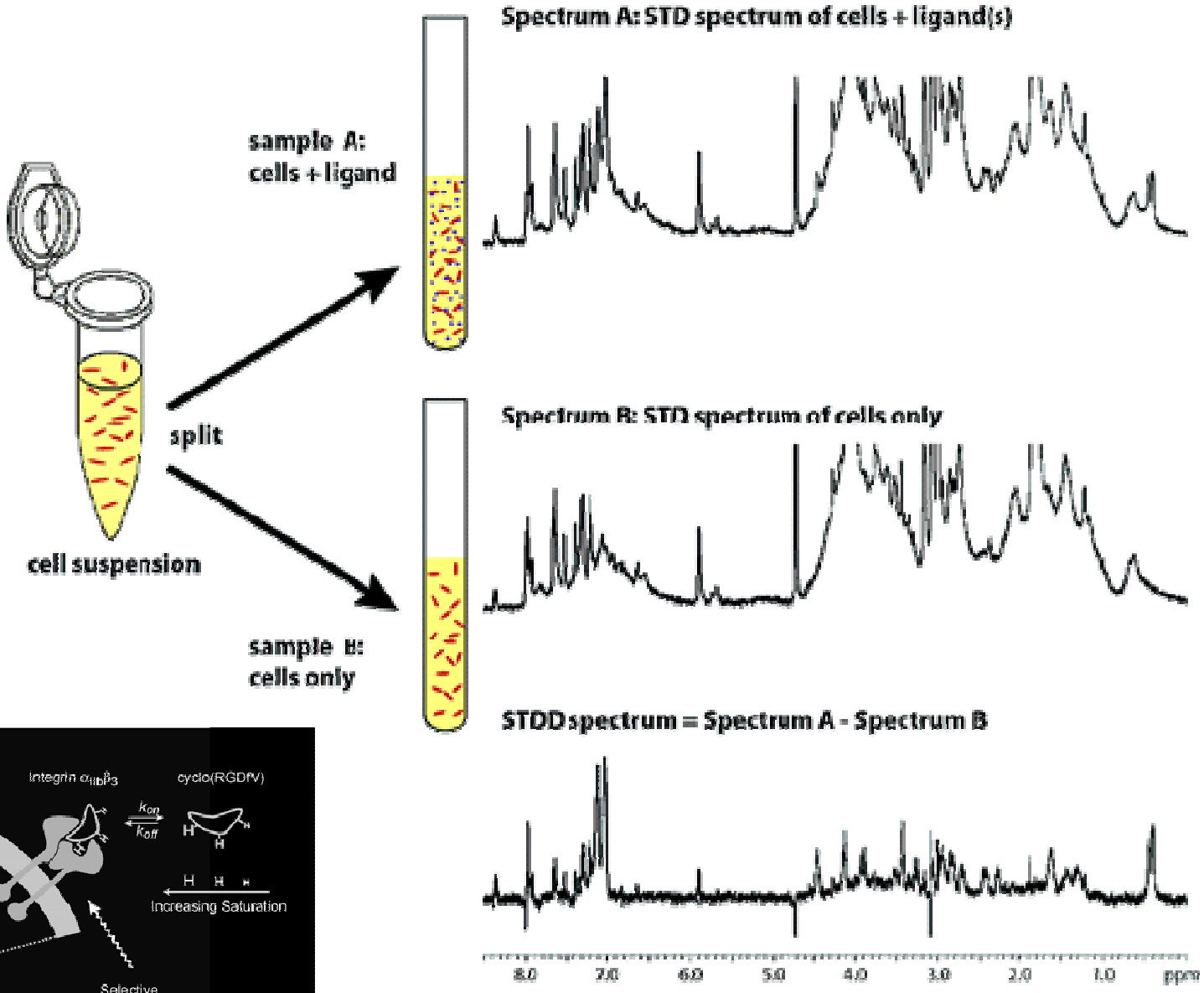


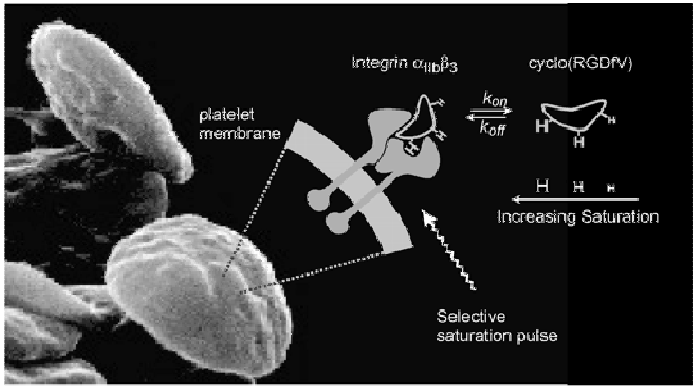
Free Ligands

Ligands + Protein

Adopted from Mark Girvin,
Albert-Einstein College , NY

STD-NMR can be used to observe binding in complex mixtures

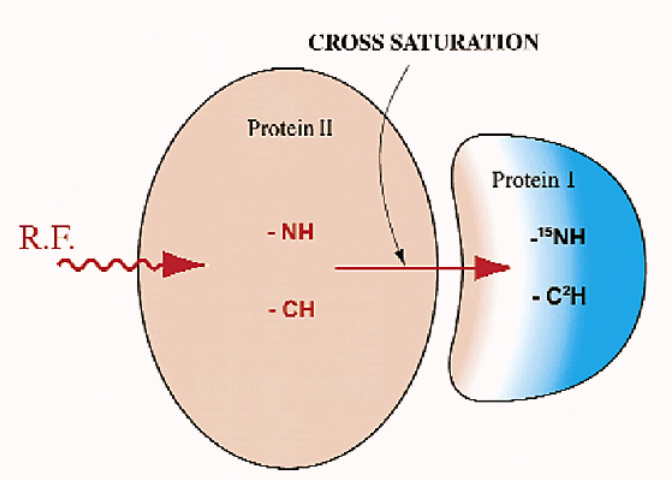


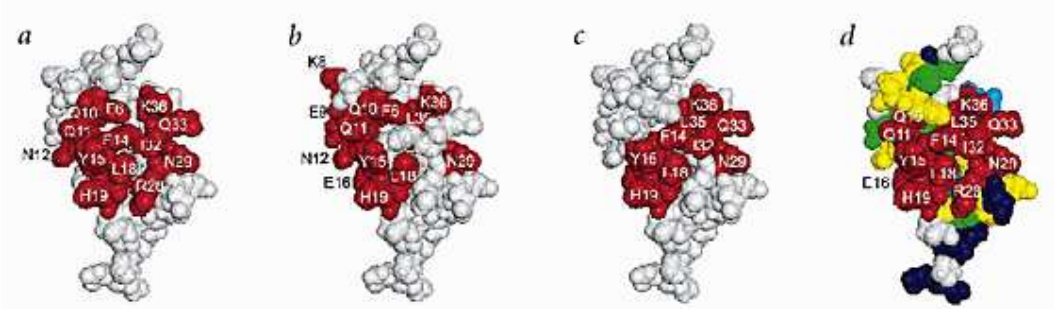


J. Am. Chem. Soc., 2005, 127 (3), pp 916-919

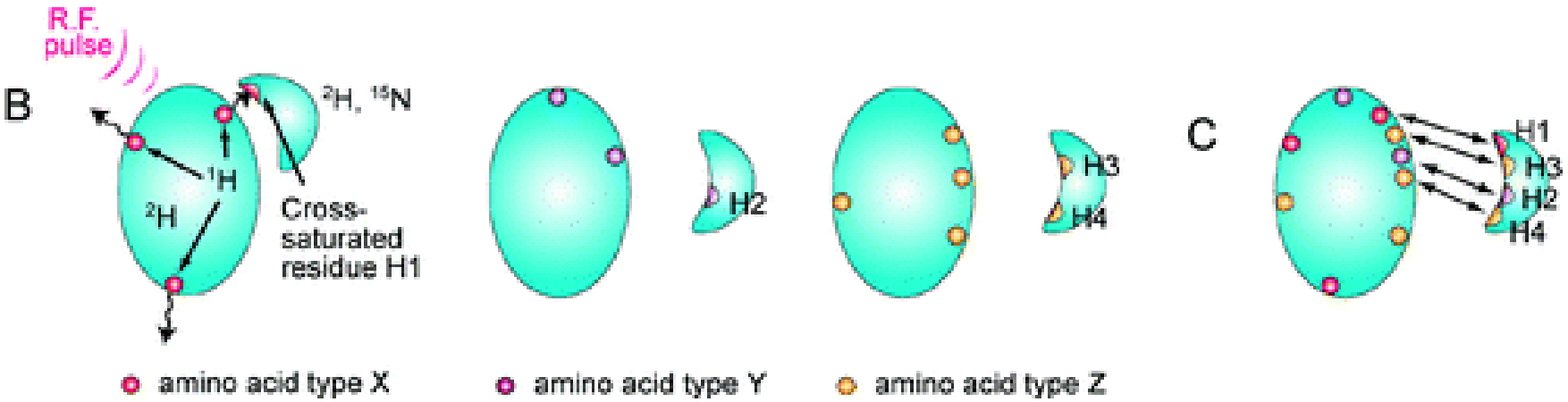
Cross saturation: detection of ^{15}NH groups of a deuterated acceptor protein

Takahashi et al., Nat. Struct. Biol.(2000)



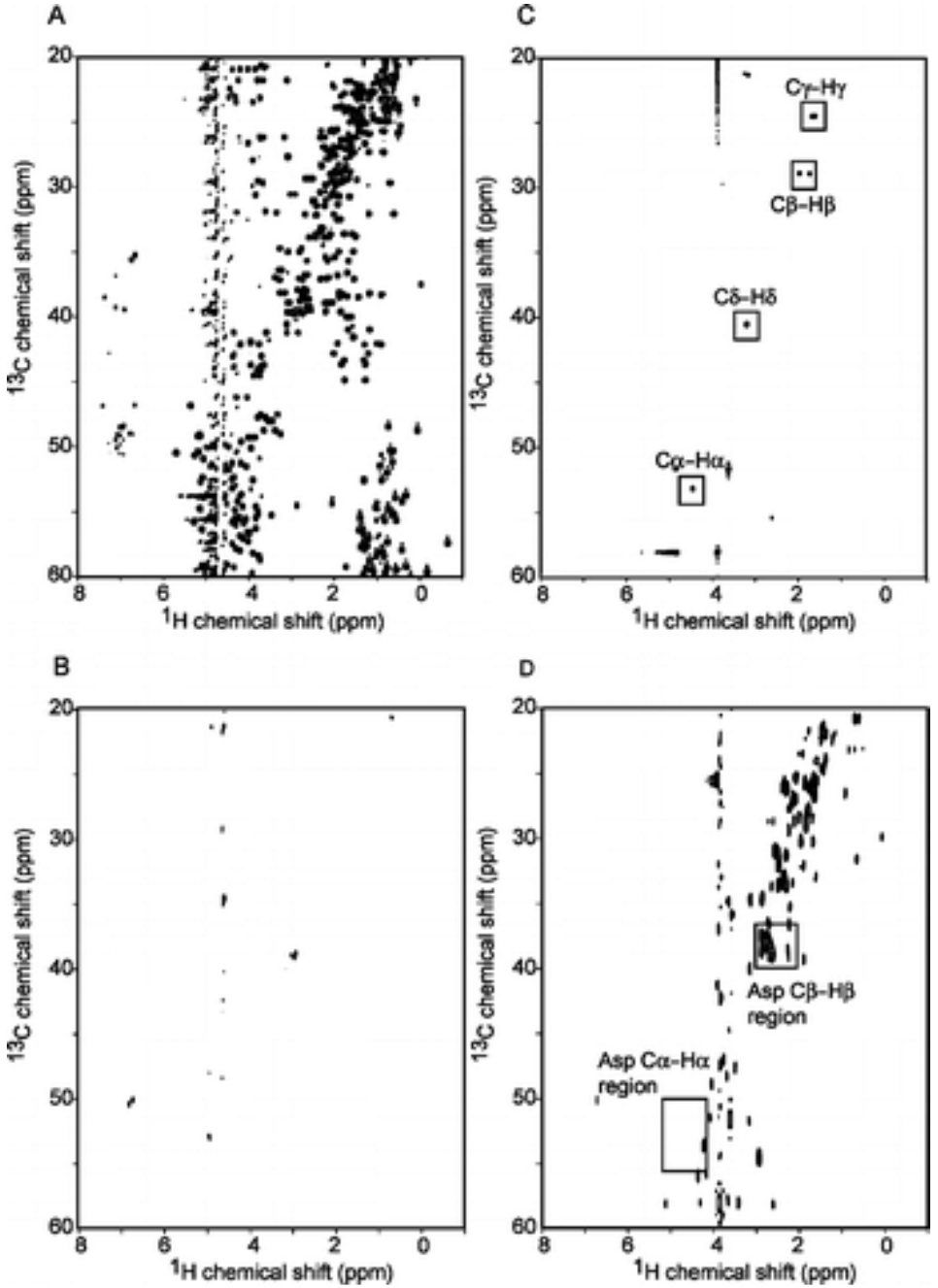


Variant: Selective protonation of an otherwise deuterated donor protein



Igarashi et al., JACS 2008

Selective protonation of amino acids with different efficiencies

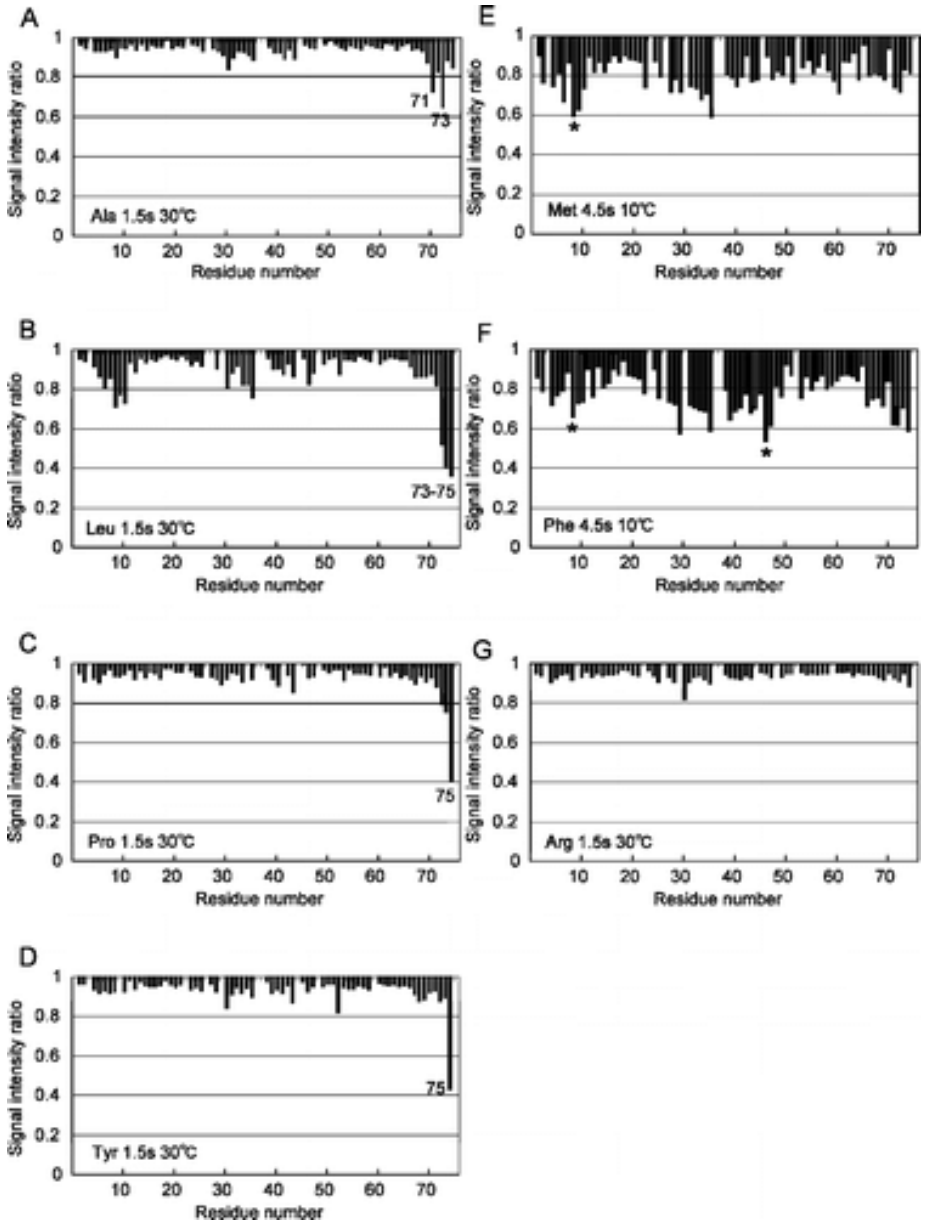


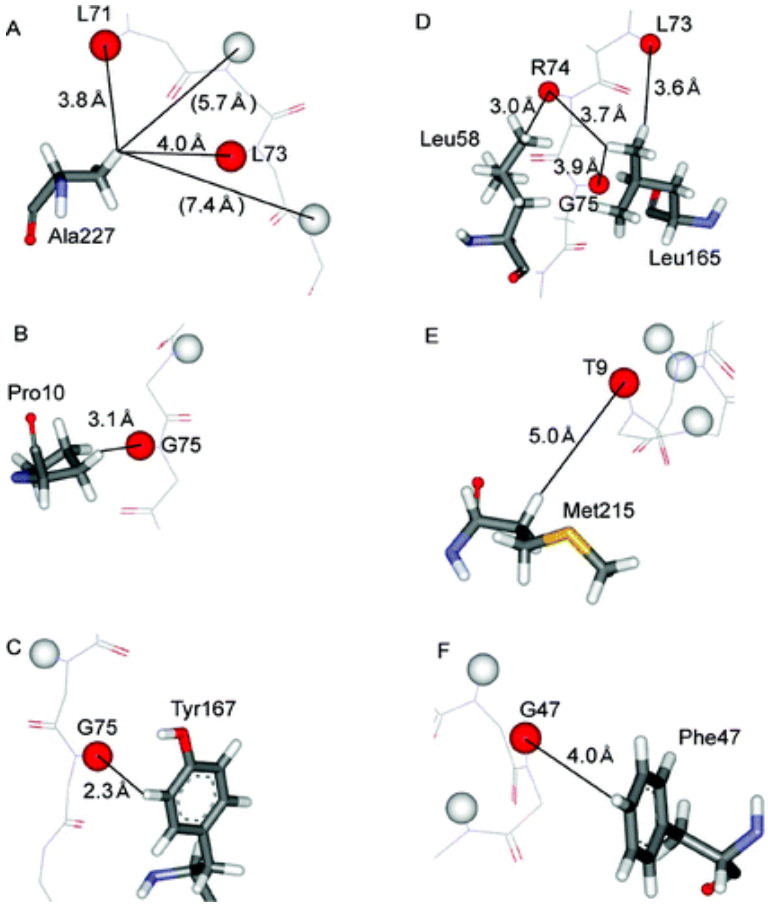
Arginine

Feasible for: Ala, Arg, Cys, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Trp, and Tyr

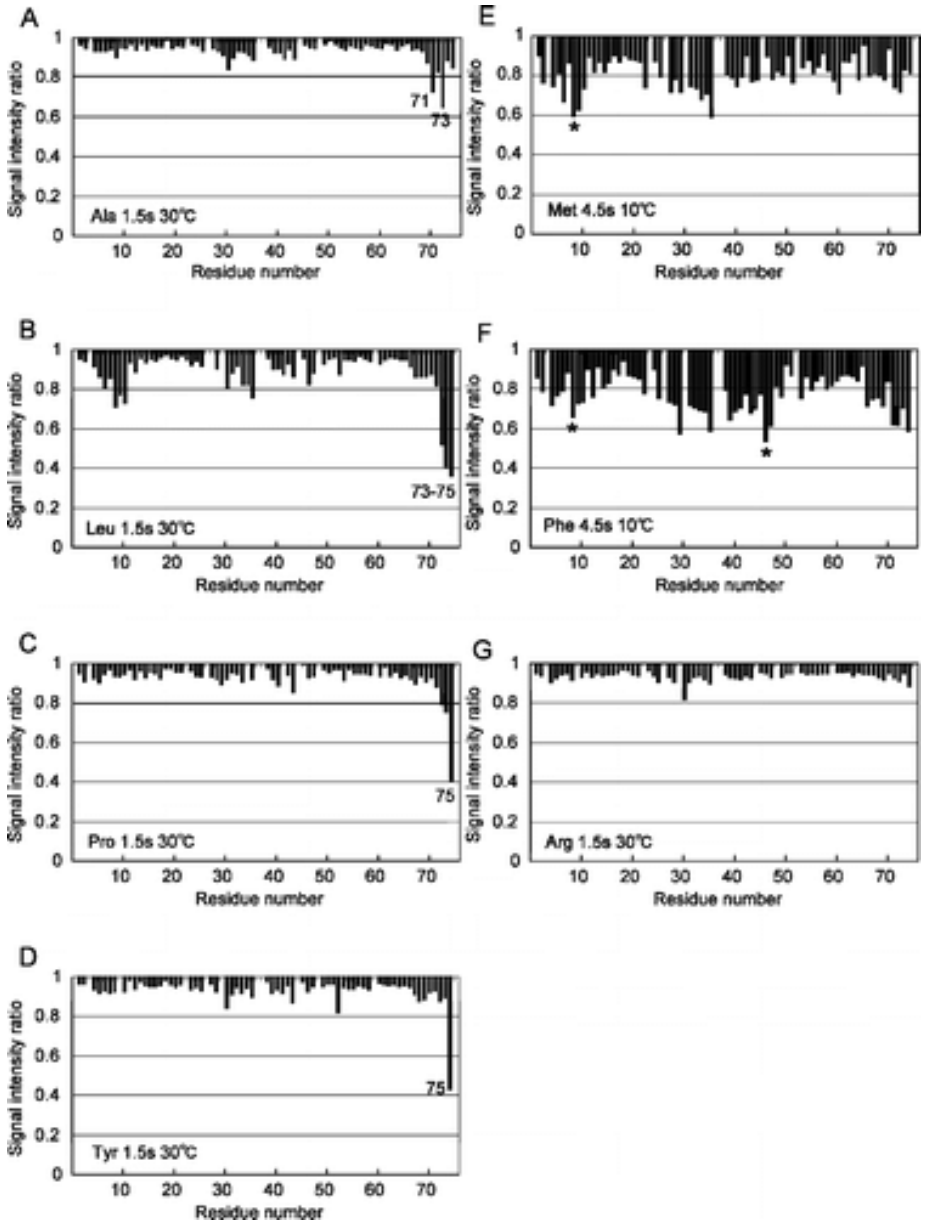
Asparate

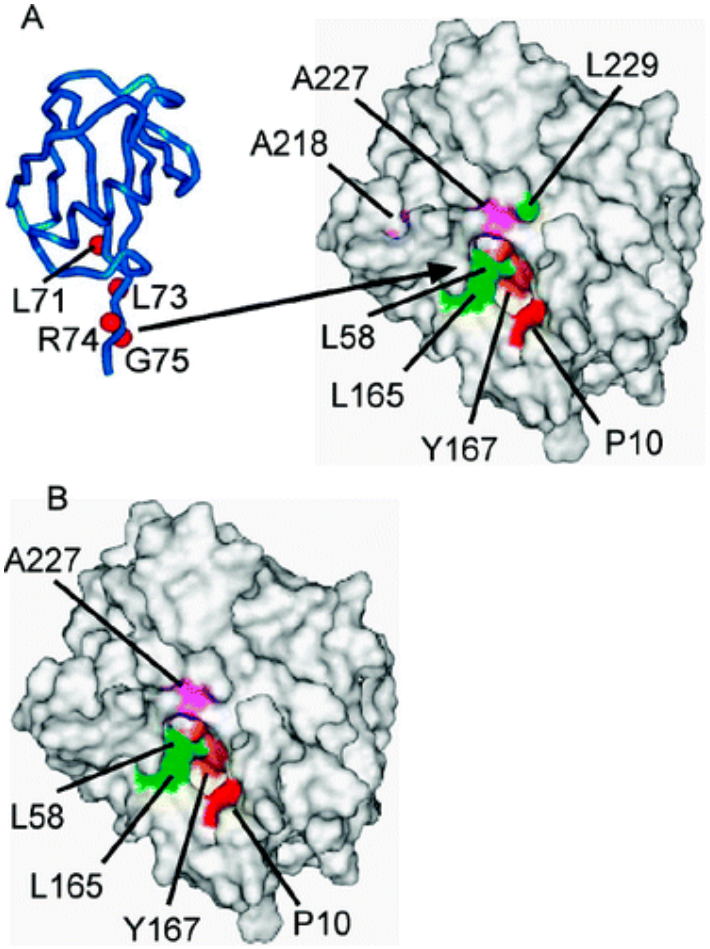
Identification of donor residues close in space to affected acceptor amides





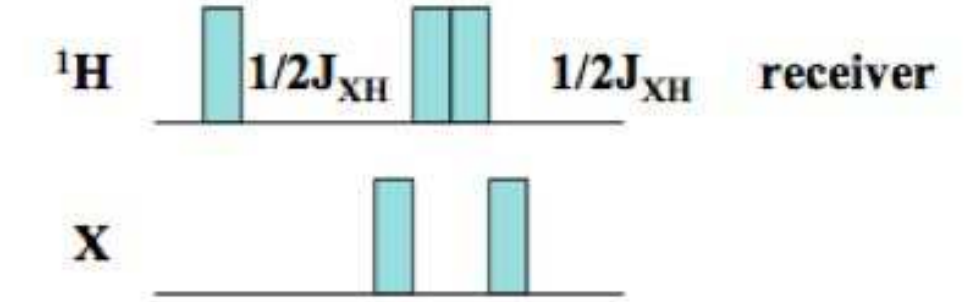
Identification of donor and acceptor interfaces





Determining the structure of protein:ligand complexes

Half-filter :

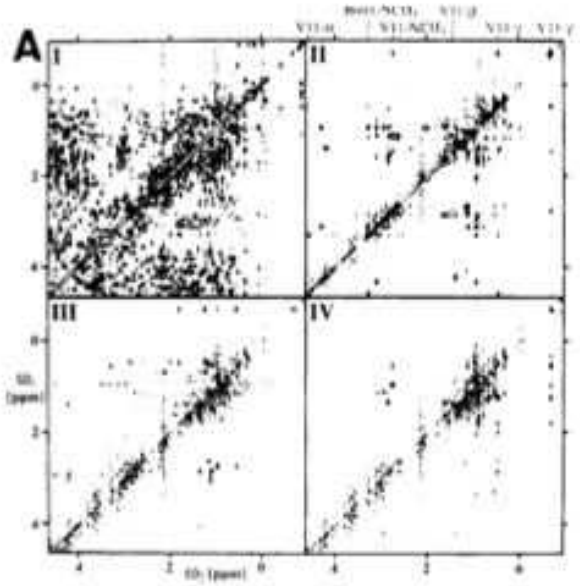


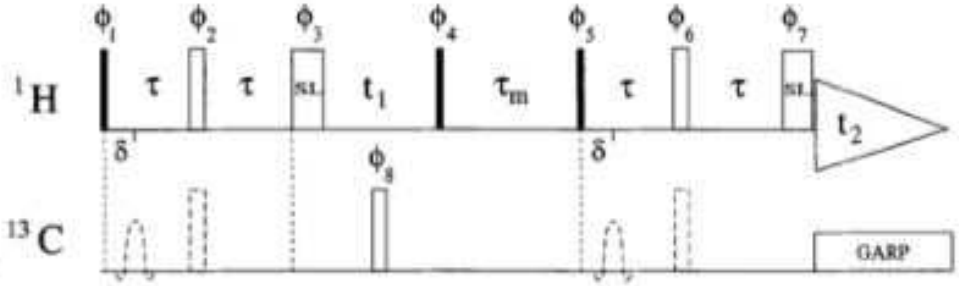
phase x x x x -x

x
-x
} selects X-^{1}H

or

x
x
} selects for ^{1}H that are not X-^{1}H



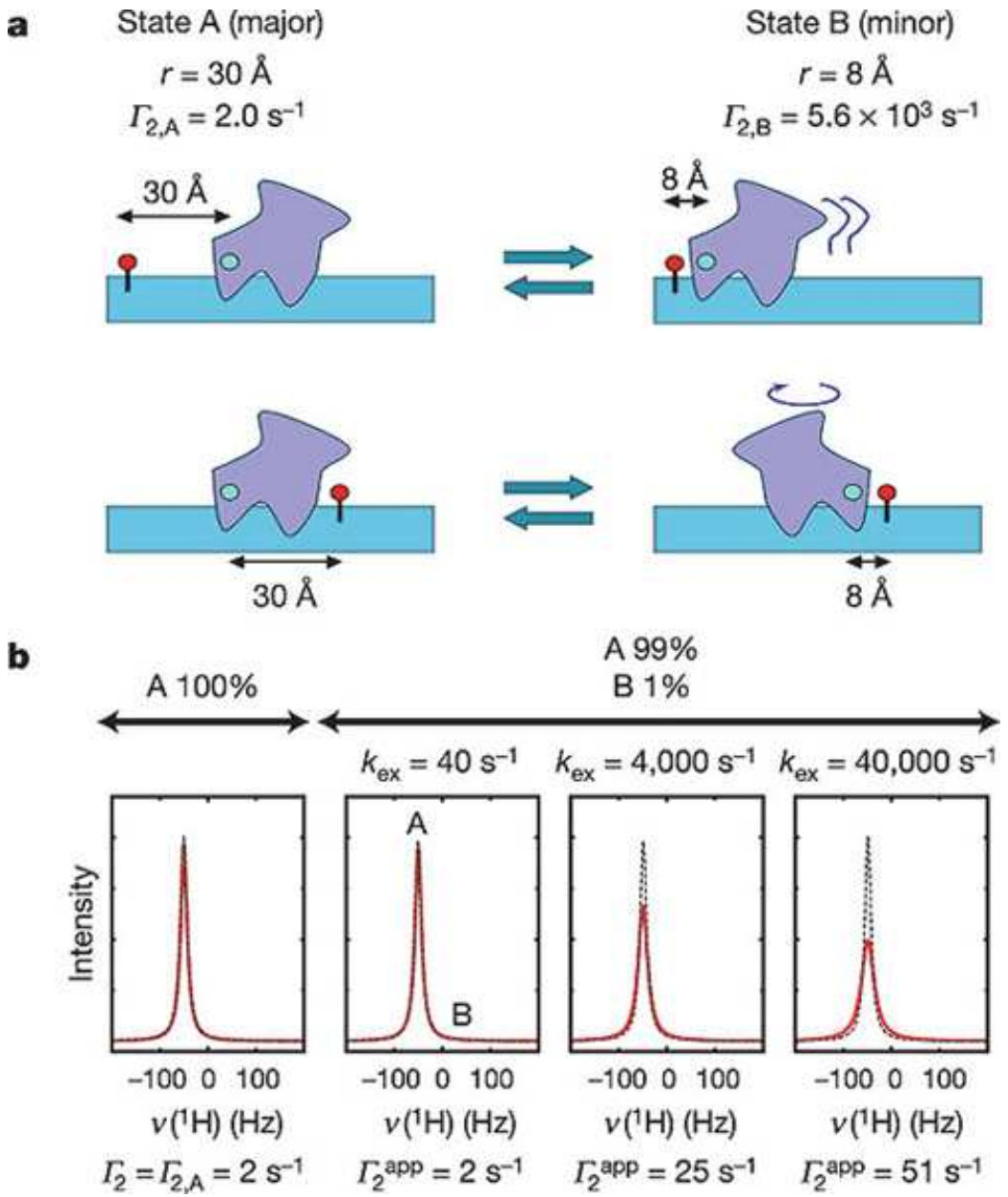


Wider et al., JACS, 1990

Folmer et al., J Biomol. NMR 1995

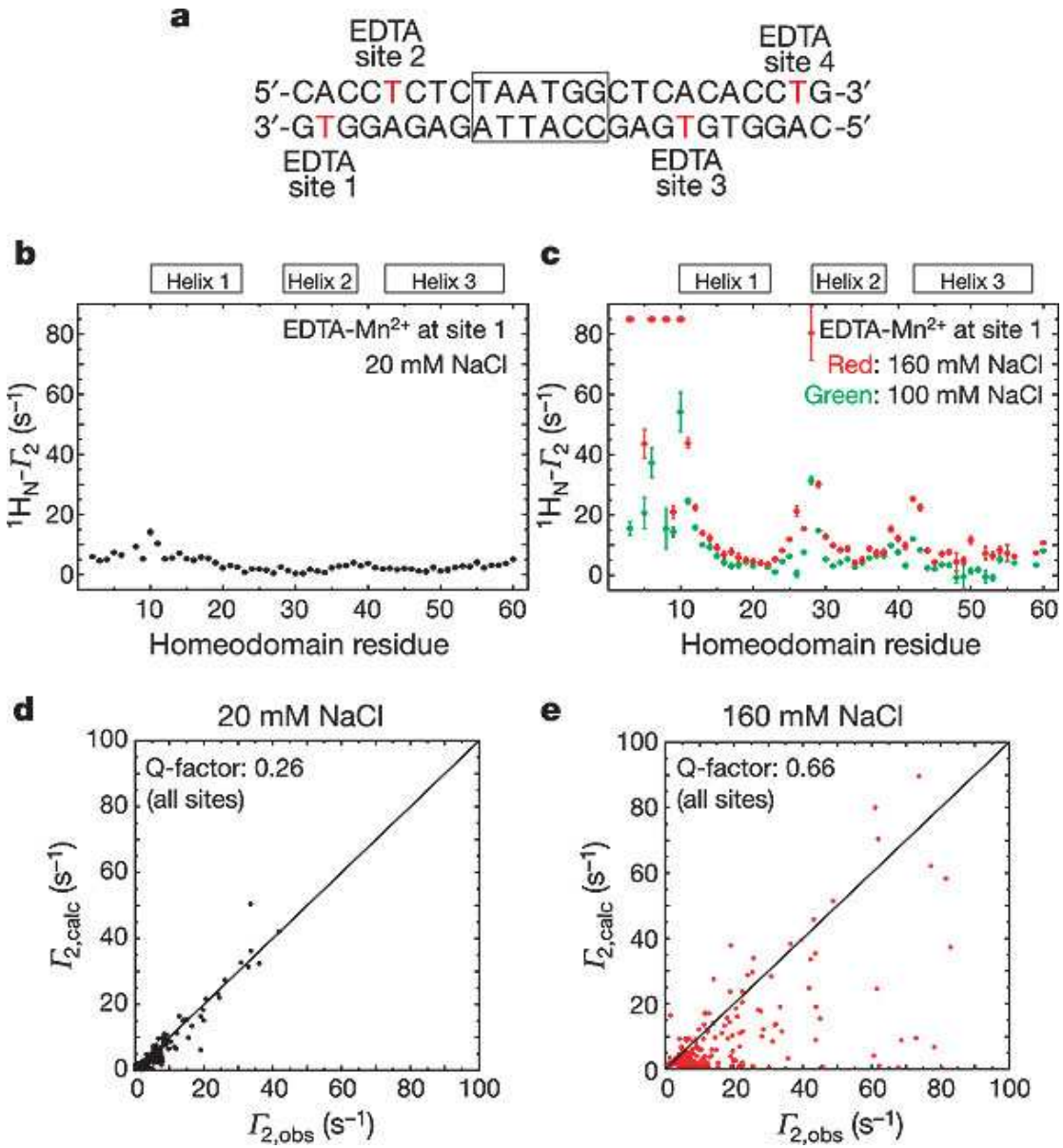
..to be continued in later sessions

Paramagnetic relaxation enhancement



Iwahara & Clore, Nature, 2006

At higher salt concentrations, T_2 relaxation data cannot be explained by the specific complex

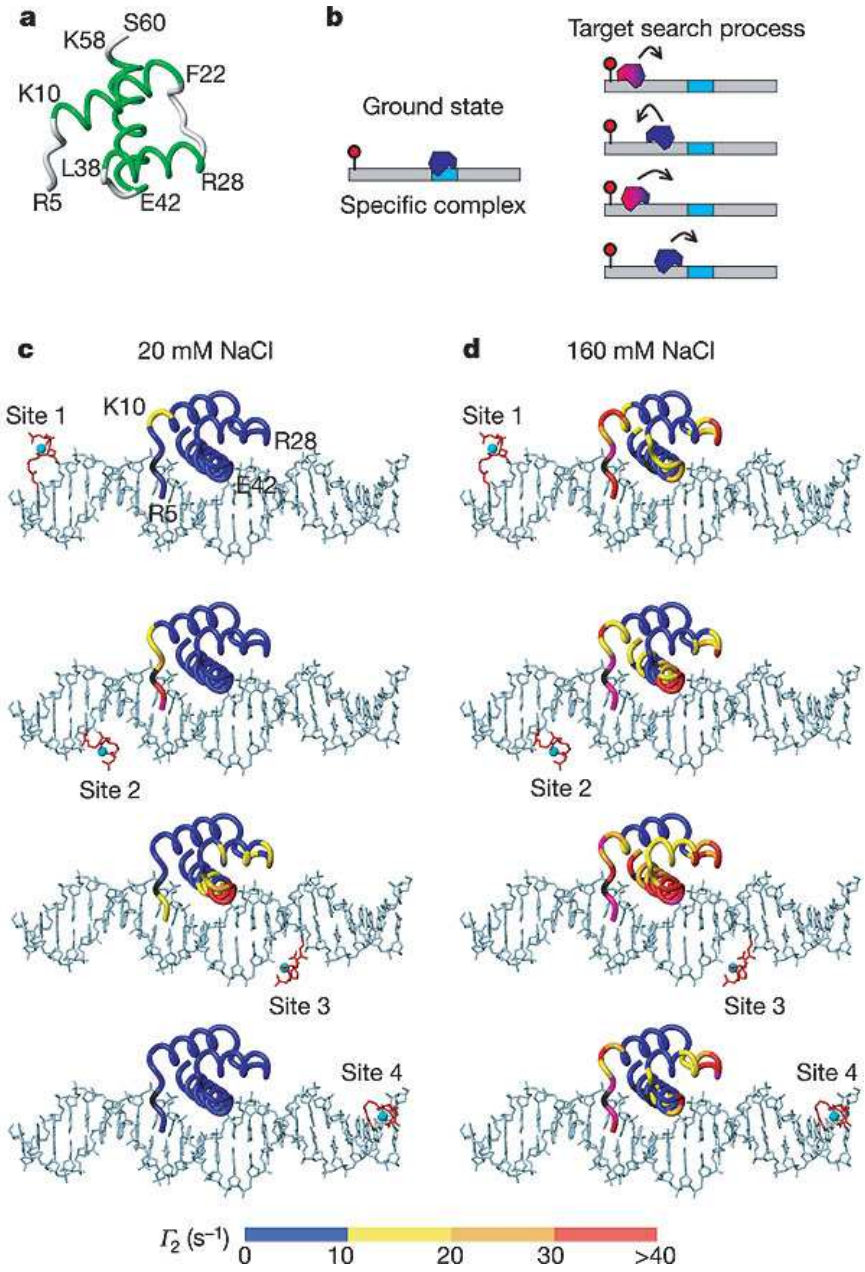


Model system:
Homeodomain of human HOXD9 in complex with a 24-base-pair DNA duplex

Incorporation of conjugated deoxy(d)T-EDTA-Mn^{2+} into the DNA

$$Q=\left(\frac{\Sigma\{\Gamma_2^{obs}(i)-\Gamma_2^{calc}(i)}{\Sigma\,\Gamma_2^{obs}(i)^2}\right)$$

Low-affine interactions likely contribute to formation of the specific complex



^{15}N-^{1}H-correlation spectra indicate that the structure of the specific complex does not change significantly when going from 20 mM to 160 mM salt!

Structural representations of the measured intermolecular PRE profiles.

At 20mM NaCl, the data is compatible with the structure of the complex bound to the specific site.

At 160 mM NaCl the observed PRE are interpreted as footprints of minor species that are in rapid exchange with the specific complex.