Protein-ligand interaction by NMR: Chemical shift analysis

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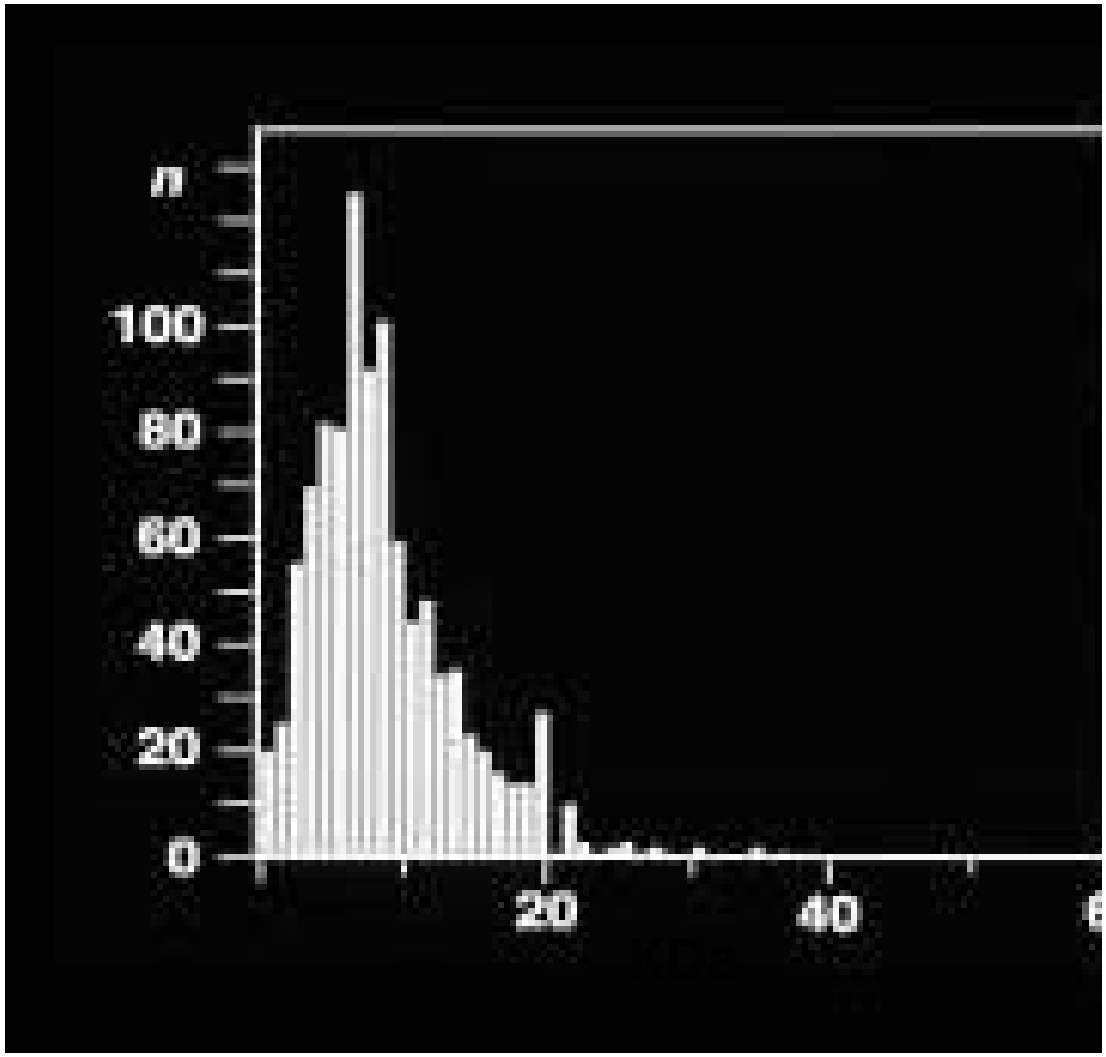
With NMR spectroscopy:

- structure of "small" (< 20 KDa) proteins with standard procedure (NOEs).
- higher molecular weight can be tackled using:
  - very high magnetic fields (900 MHz) and cryo-probes that increase sensitivity
  - Novel methodologies like RDC (residual dipolar couplings) and TROESY (transverse relaxation optimized spectroscopy)

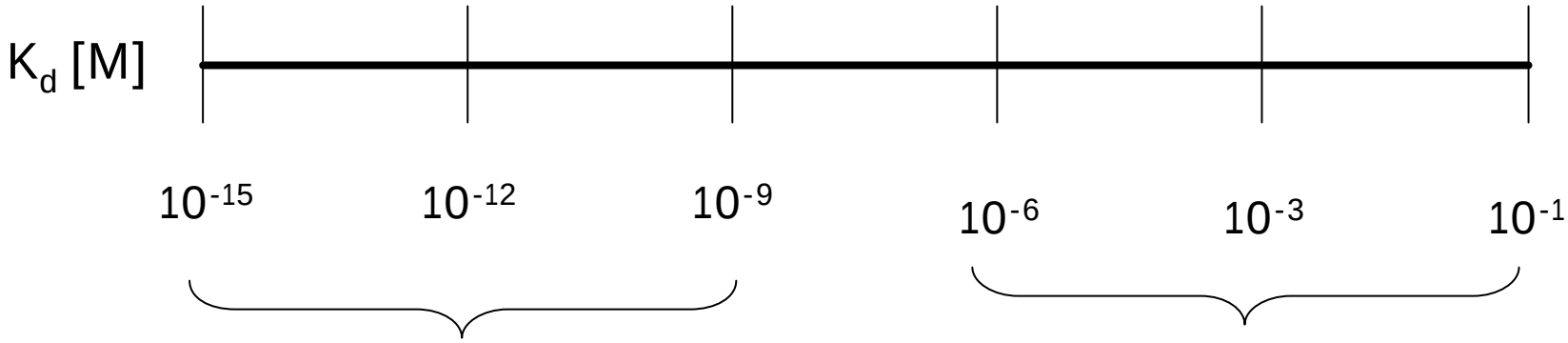


Wuthrich, Nobel lecture, Year 2000

interaction between macromolecules and ligands

- structure determination of protein-ligand complex
- selected aspect of the interaction on the ligand or on the protein
- determination of equilibrium and kinetic constants
- mapping of the interaction surface
- determination of the bound conformation of the ligand / protein

Complex stability and NMR



Stable complexes:
It is possible to determine the complete structure of the complex, provided the size is not too big, using NMR methods, alternatively by using X-ray crystallography

Labile complexes:
It is not possible to determine the complete structure at atomic resolution, but one can identify **interaction surfaces** and by using computational methods infer a plausible structure or to draw ad hoc mutagenesis experiments.

NMR

Acylphosphatase
small enzyme 103 residues
ferredoxin-like βαββαββ sandwich domain

total number of atoms : 1661

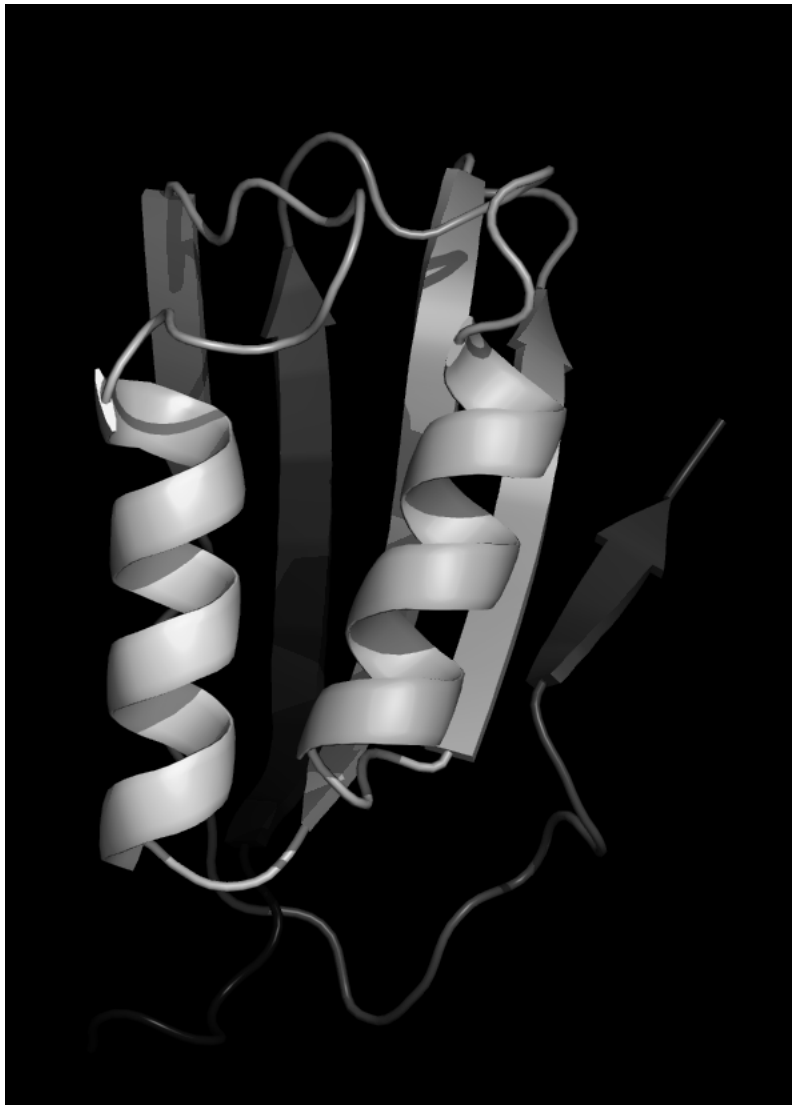
H: 830

N: 135

C: 537

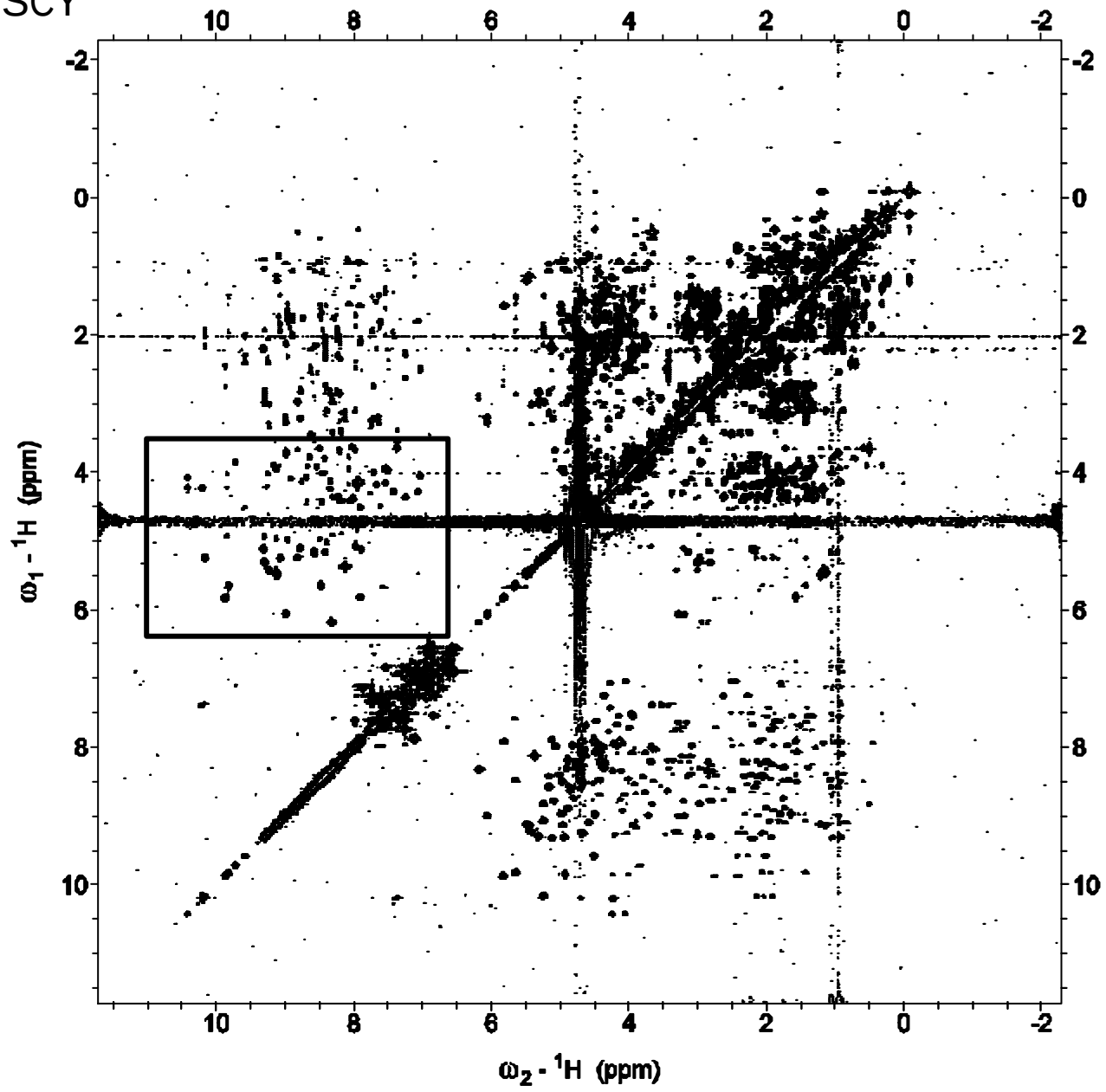
O: 157

S: 2



Corazza et al. Proteins (2006)
Pagano et al. J. Biomol NMR (2006)

2D 1H TOSCY



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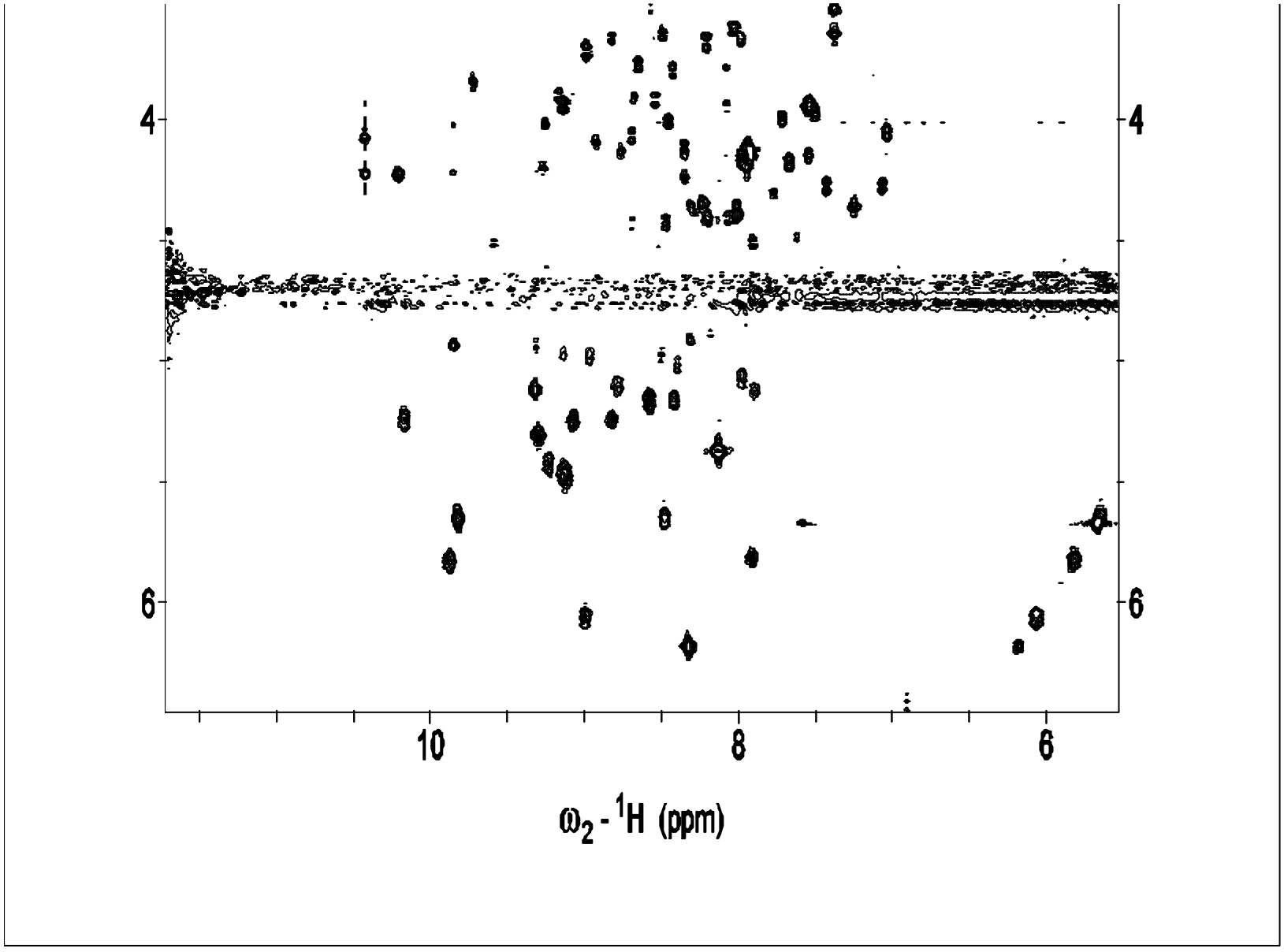
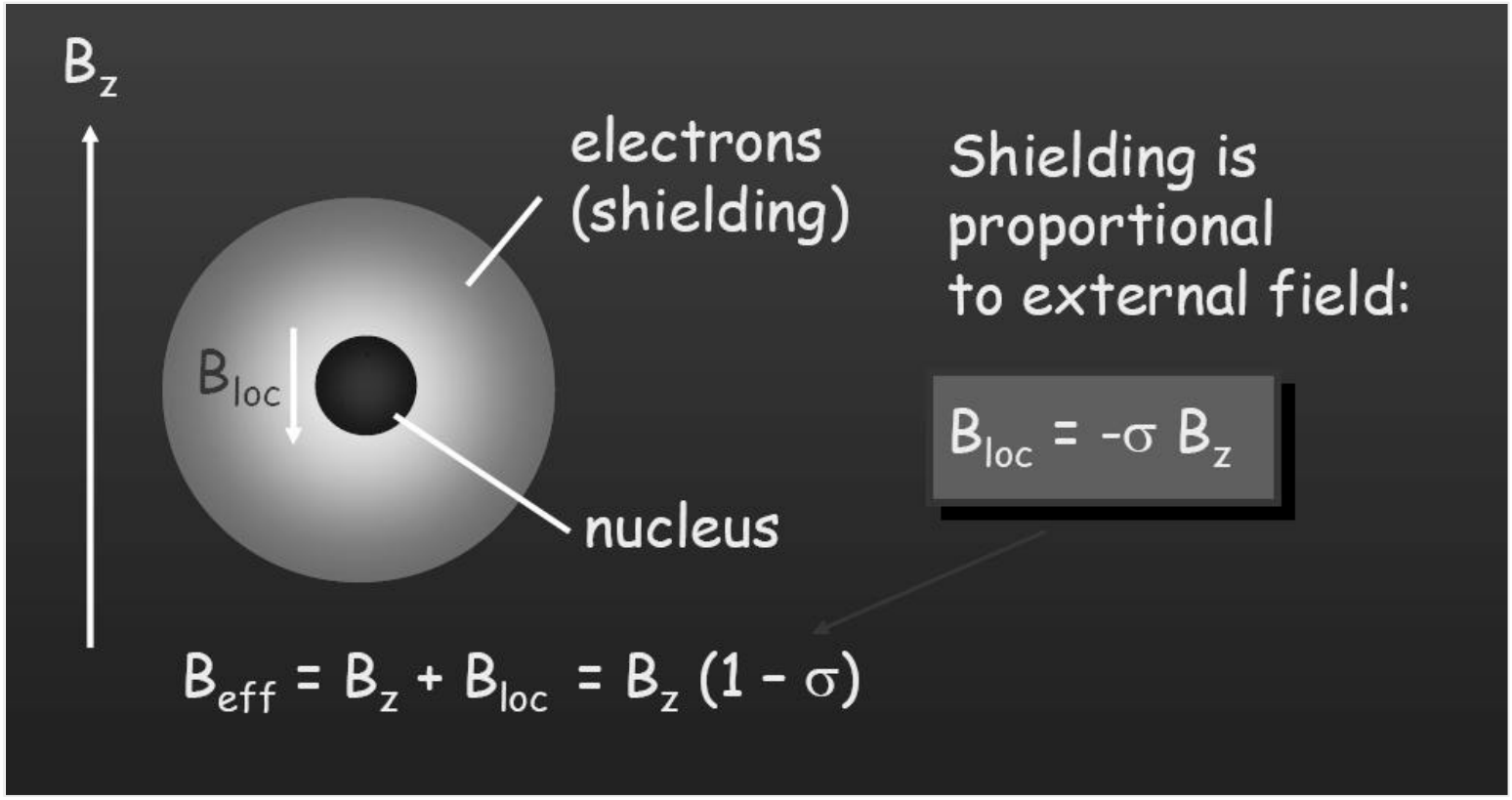


Chemical shift origin

The precise frequency absorbed by a nucleus in a sample depends on the chemical environment

or

the **chemical shift** describes the dependence of nuclear magnetic energy levels on the electronic environment in a molecule.

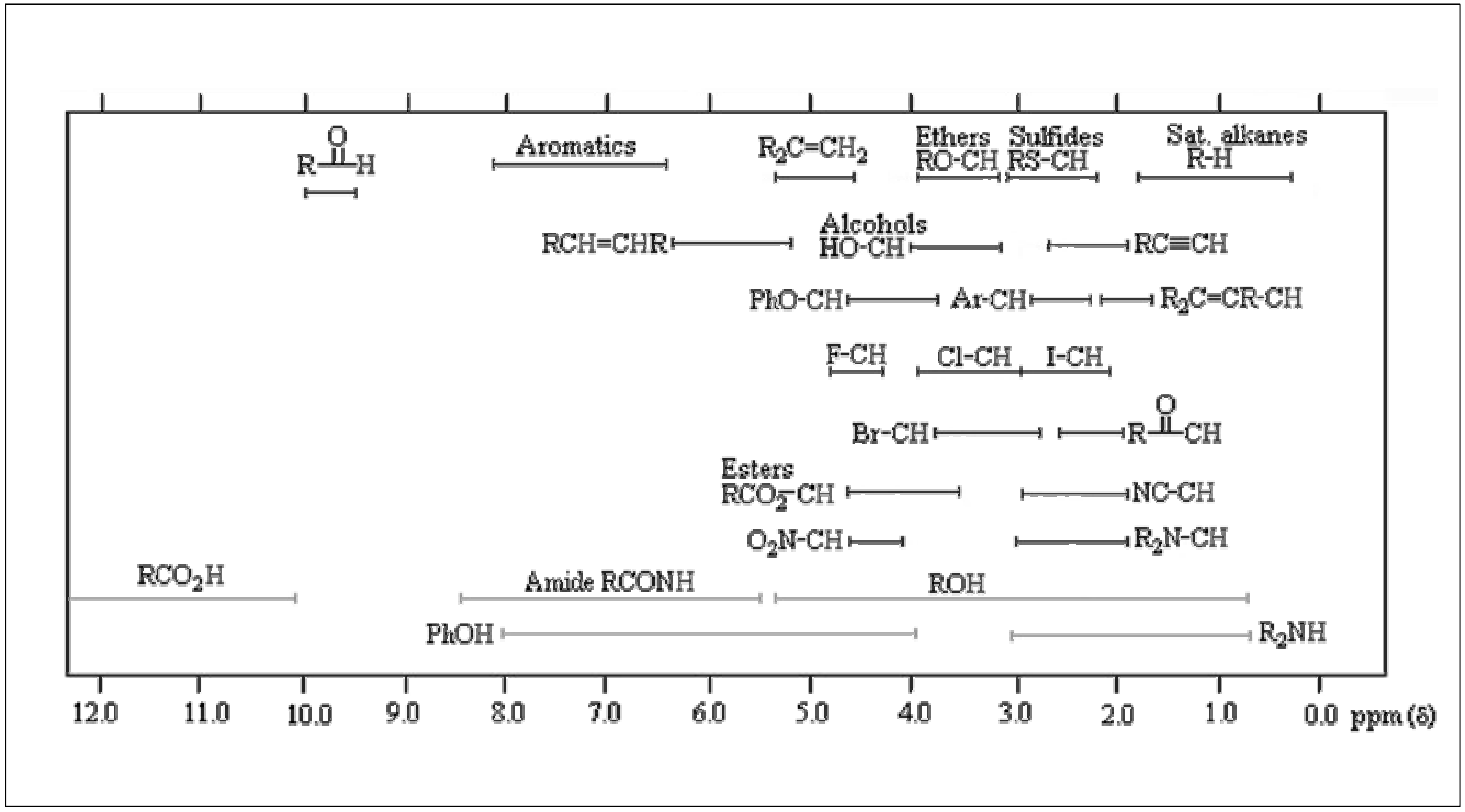


Chemical shift origin

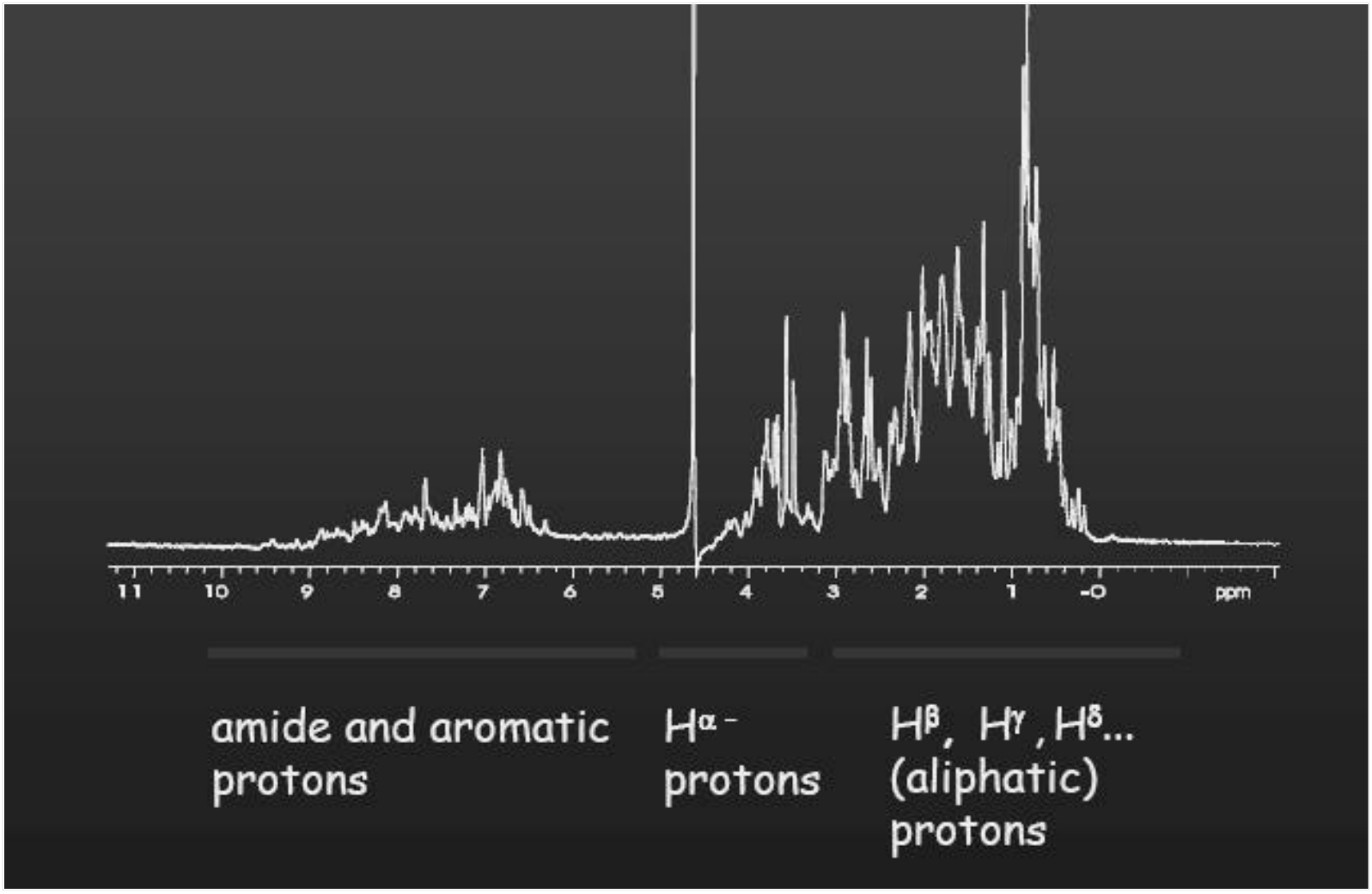
Factors influencing the chemical shift:

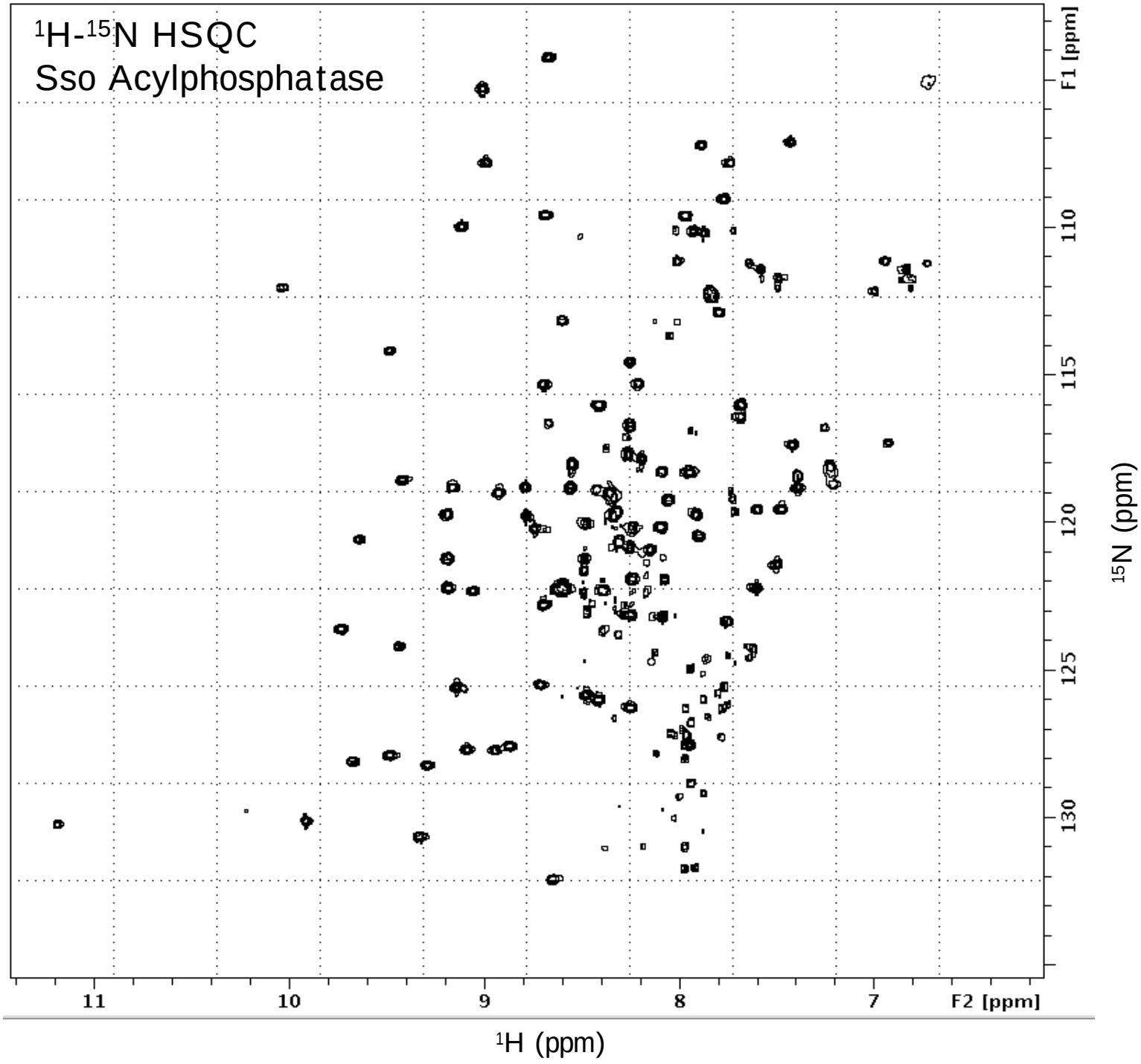
- nucleus shielding (electronegativity of the bound nuclei)
- presence of paramagnetic nuclei
- ring current effect (aromatic groups)
- chemical shift anisotropy (mediated in liquids)
- local electrostatic fields
- solvent

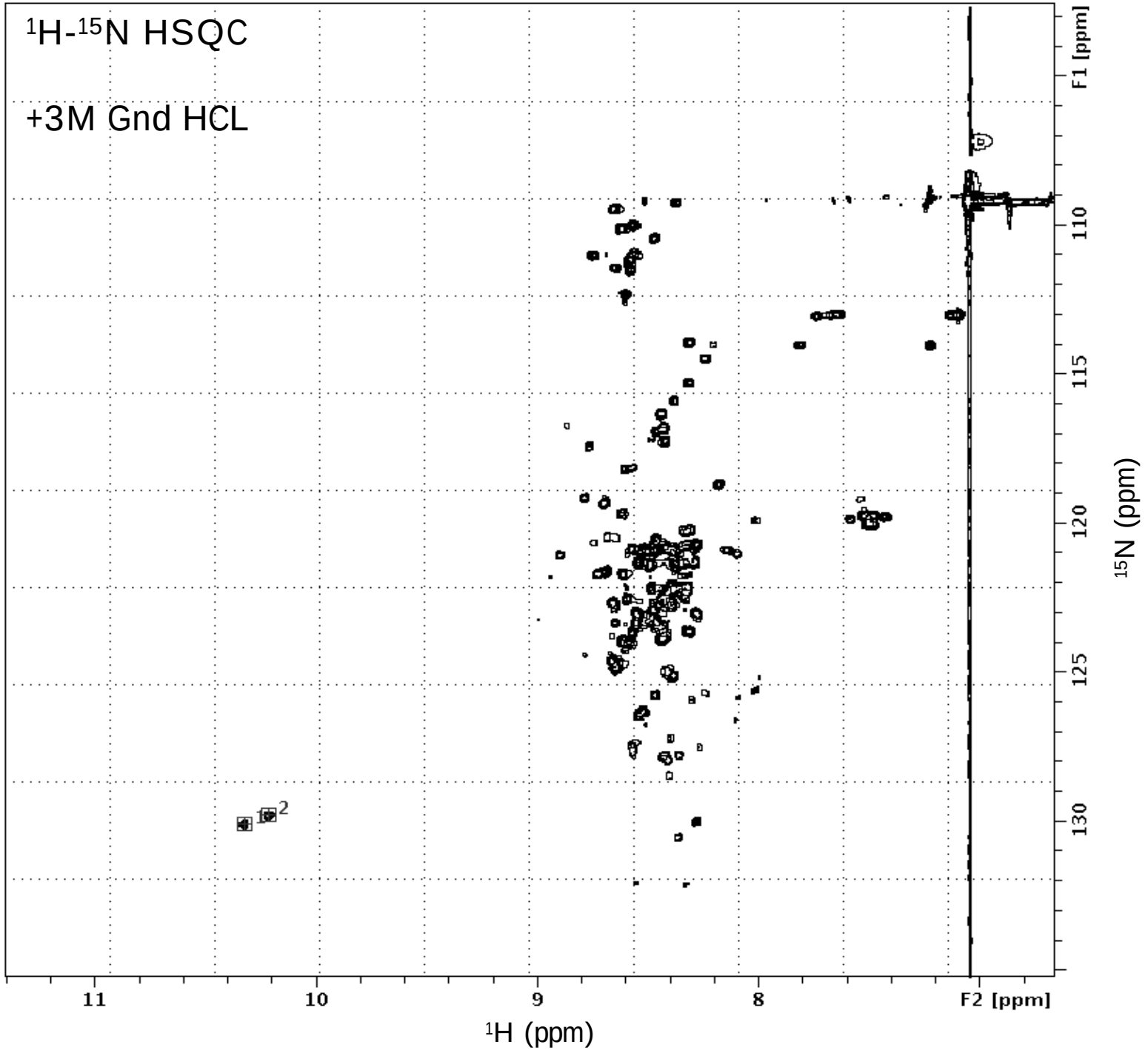
Typical Proton Chemical Shifts



1D-spectrum of a protein




^{1}H-^{15}N HSQC
Sso Acylphosphatase
F1 [ppm]
110
115
120
125
130
^{15}N (ppm)
11
10
9
8
7
F2 [ppm]
^{1}H (ppm)


^{1}H-^{15}N HSQC
+3M Gnd HCL
F1 [ppm]
110
115
120
125
130
^{15}N (ppm)
11
10
9
8
F2 [ppm]
^{1}H (ppm)

Chemical exchange

Chemical exchange in NMR refers to any process in which a nucleus changes between two or more environments in which its NMR parameters (chemical shift, scalar coupling, relaxation, …) differ.

intramolecular exchange processes include:

- motion of side chains in proteins
- unfolding of proteins
- conformational equilibria

intermolecular exchange processes include:

- binding of small molecules to macromolecules
- Protein- protein interactions
- DNA (RNA)-protein interactions
- protonation /deprotonation equilibria of ionizable groups
- isotope exchange processes
- enzyme catalysed reaction

In all these cases the effect of the exchange process

$$A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B$$

on the NMR spectrum depends upon its rate, relative to the magnitude of variation of the NMR parameters.

Time scale	Slow	Intermediate	Fast
Chemical shift	$k \ll \delta_A - \delta_B$	$k = \delta_A - \delta_B$	$k \gg \delta_A - \delta_B$
Coupling constant	$k \ll J_A - J_B$	$k = J_A - J_B$	$k \gg J_A - J_B$
T_2 relaxation	$k \ll T_{2,A}^{-1} - T_{2,B}^{-1}$	$k = T_{2,A}^{-1} - T_{2,B}^{-1}$	$k \gg T_{2,A}^{-1} - T_{2,B}^{-1}$

Lifetimes

$$E + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EL$$

For a nucleus in the ligand:

$$t_{EL} = \frac{1}{k_{off}}$$

$$t_L = \frac{1}{(k_{on}[E])}$$

For a nucleus in the protein:

$$t_{EL} = \frac{1}{k_{off}}$$

$$t_E = \frac{1}{(k_{on}[L])}$$

Equilibrium constant

$$E + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EL$$

$$[E_T] = [EL] + [E]$$
$$[L_T] = [EL] + [L]$$

$$K_d = \frac{k_{off}}{k_{on}} = \frac{[E][L]}{[EL]}$$

Lifetime and appearance of the NMR spectrum

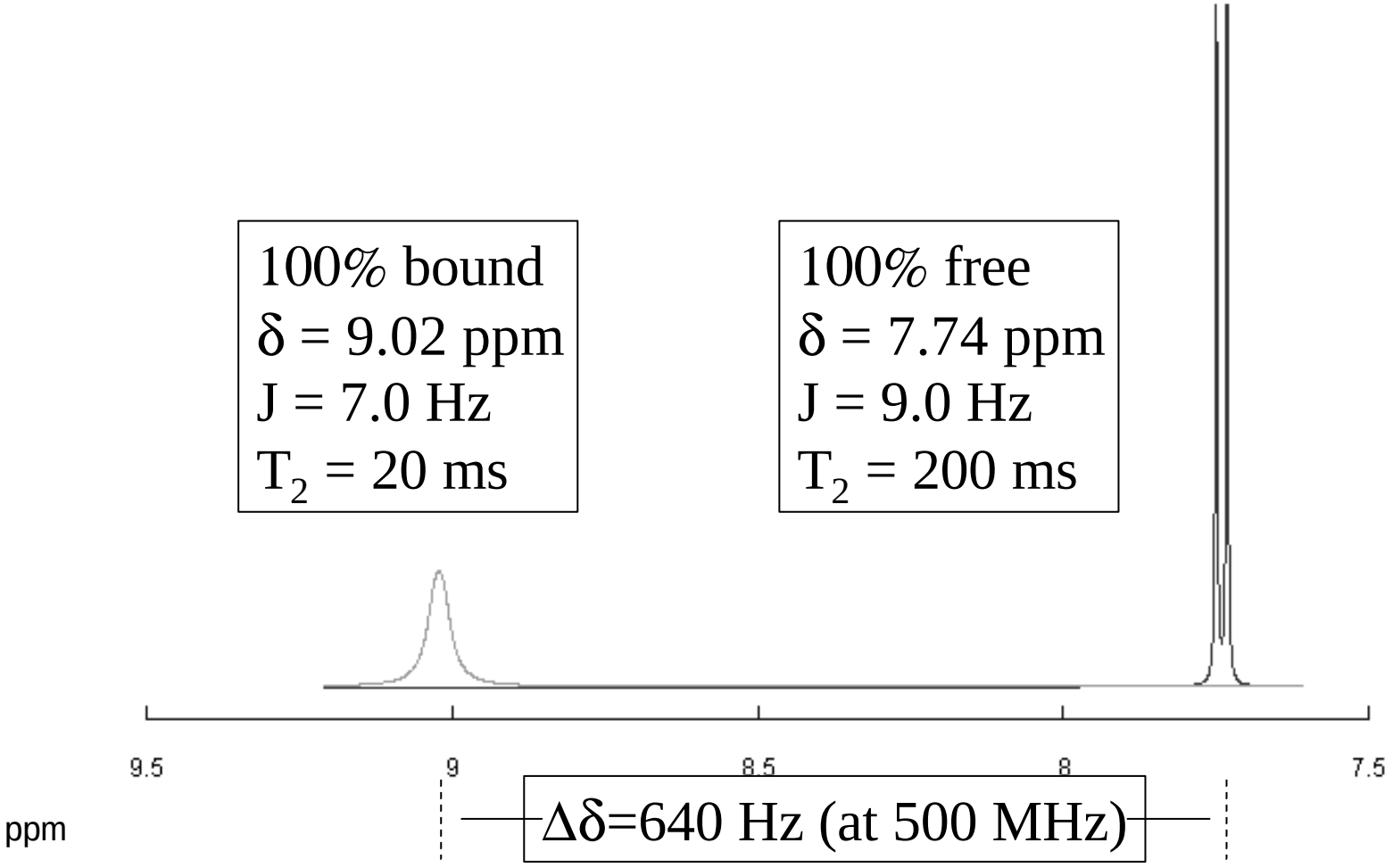
$$c_{LE} = [EL]/[L_T] \qquad c_L = [L]/[L_T]$$

$$1/t = 1/t_{EL} + 1/t_L = k_{off} + k_{on}[E] =$$

$$k_{off}(1 + [EL]/[E][L]) = k_{off}(1 + c_{LE}/c_L)$$

The relationship between the lifetime, τ, and the NMR parameters of chemical shift, relaxation time, and scalar coupling constants determines the appearance of the resulting spectrum.

Identification of Exchange Regions

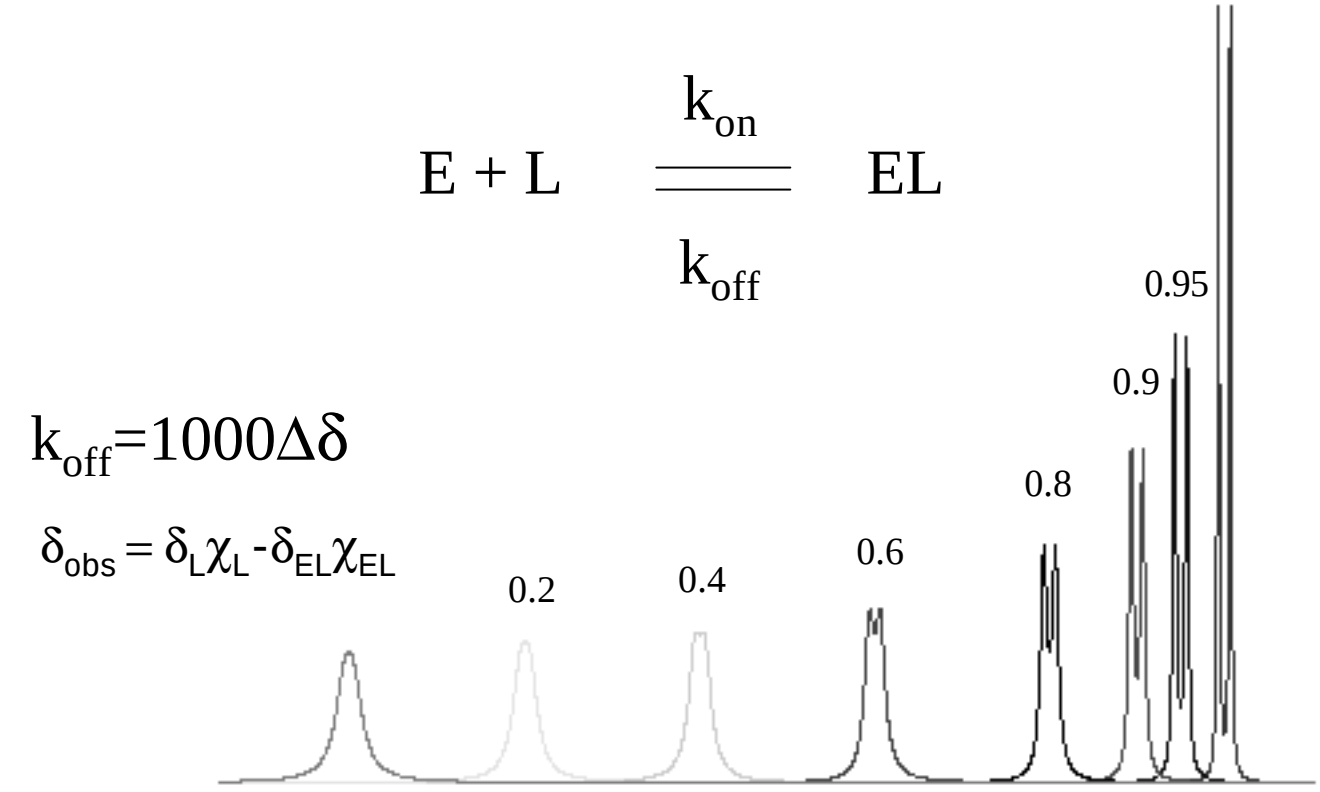


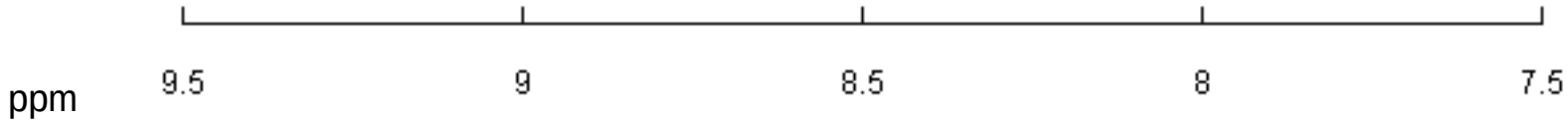
Very fast exchange: $k_{off} >> \Delta\delta$

$$E + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EL$$

$k_{off}=1000\Delta\delta$

$\delta_{obs} = \delta_L\chi_L - \delta_{EL}\chi_{EL}$



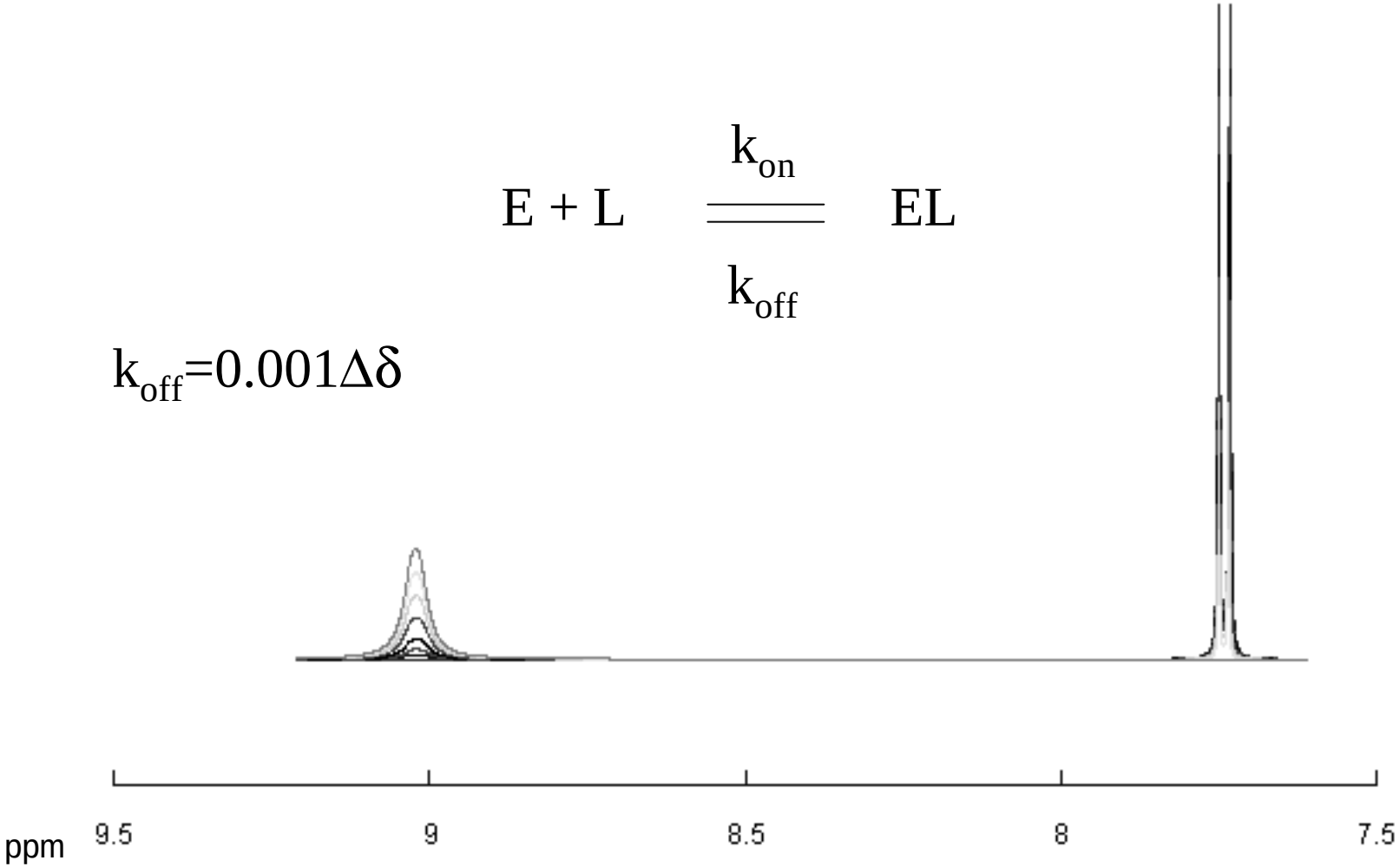


D. Cicero

Very slow exchange:
$k_{off} \ll \Delta\delta$

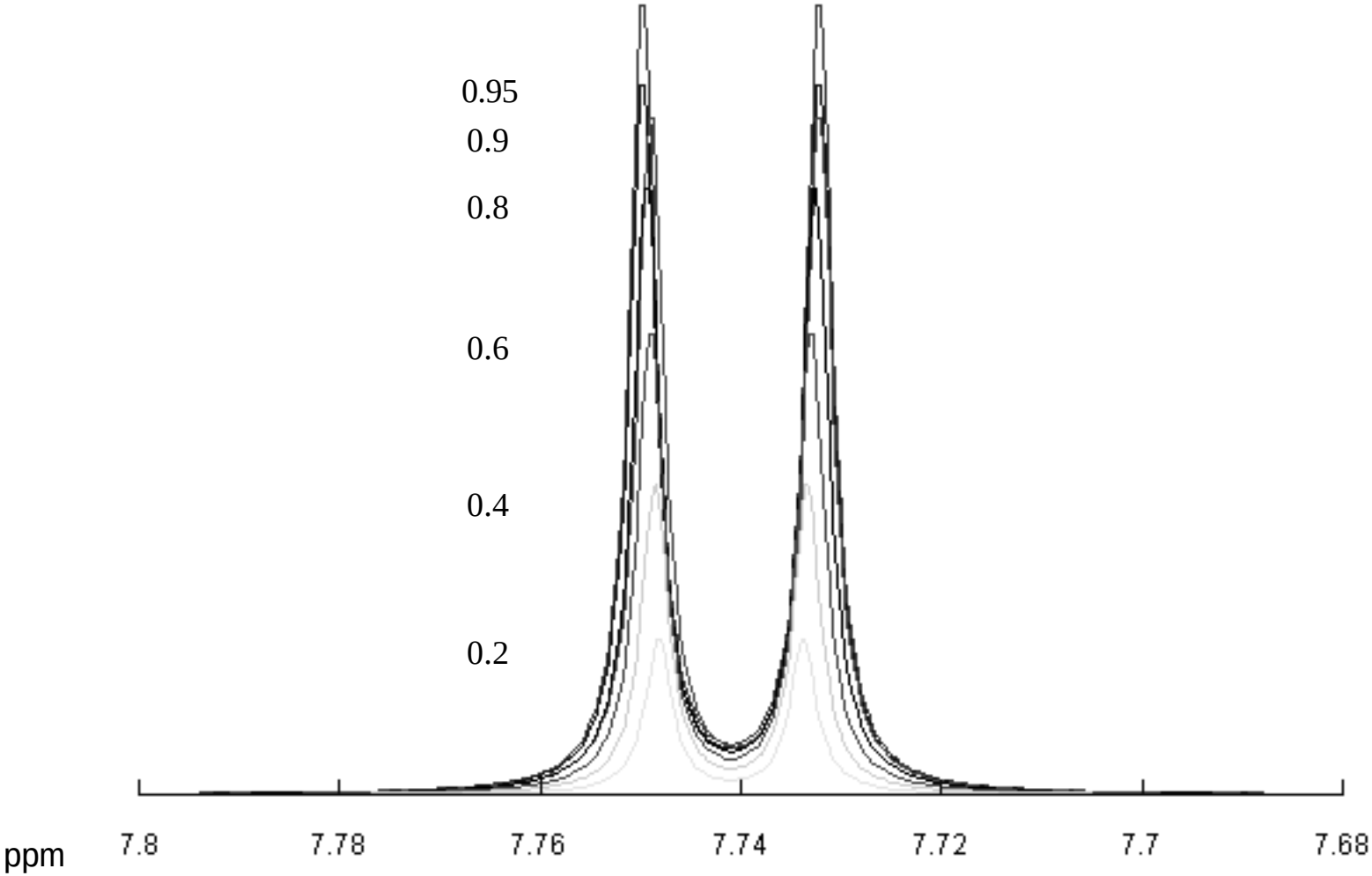
$$E + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EL$$

$k_{off}=0.001\Delta\delta$



D. Cicero

Very slow exchange:
$k_{off} \ll \Delta\delta$



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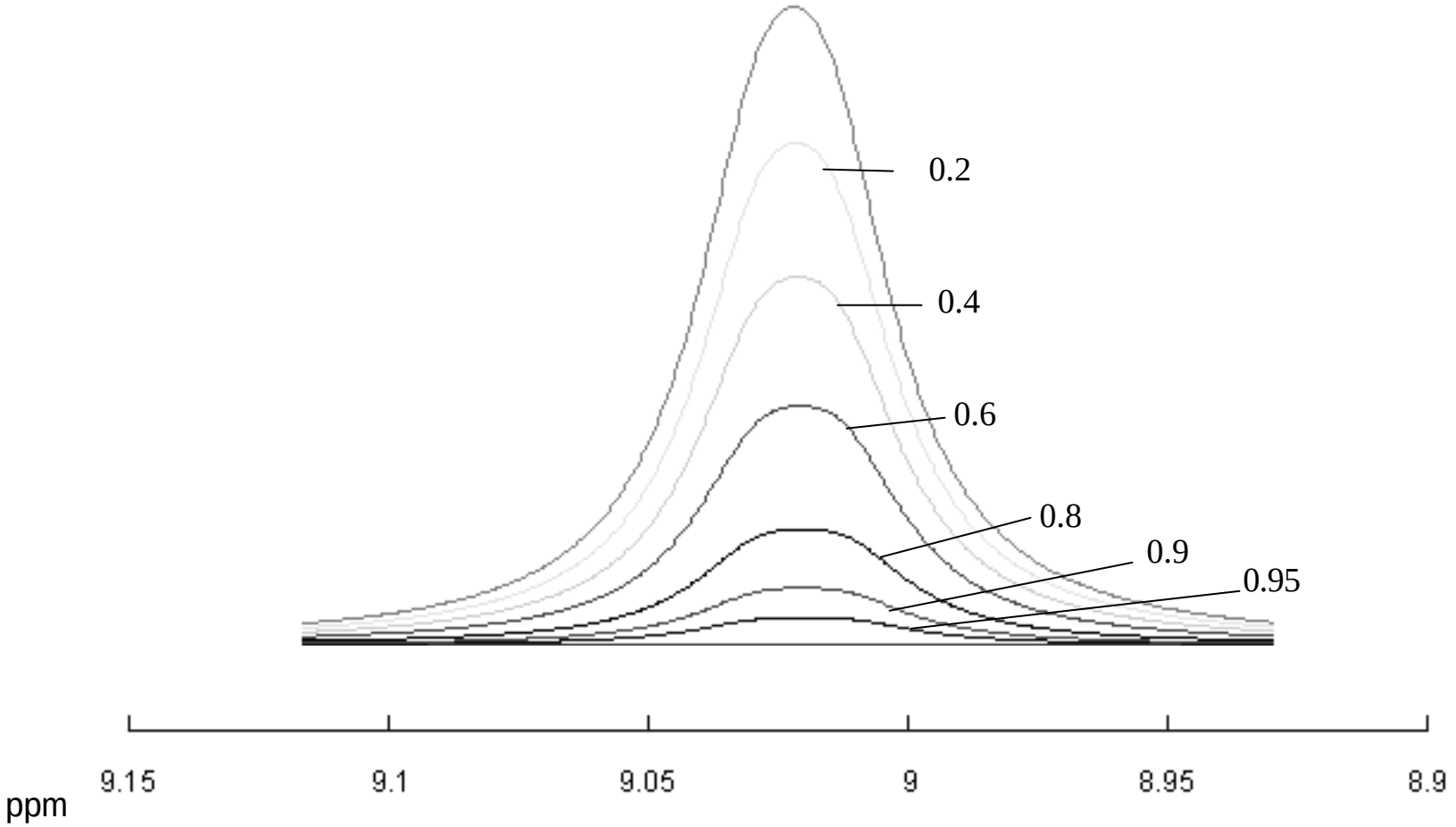
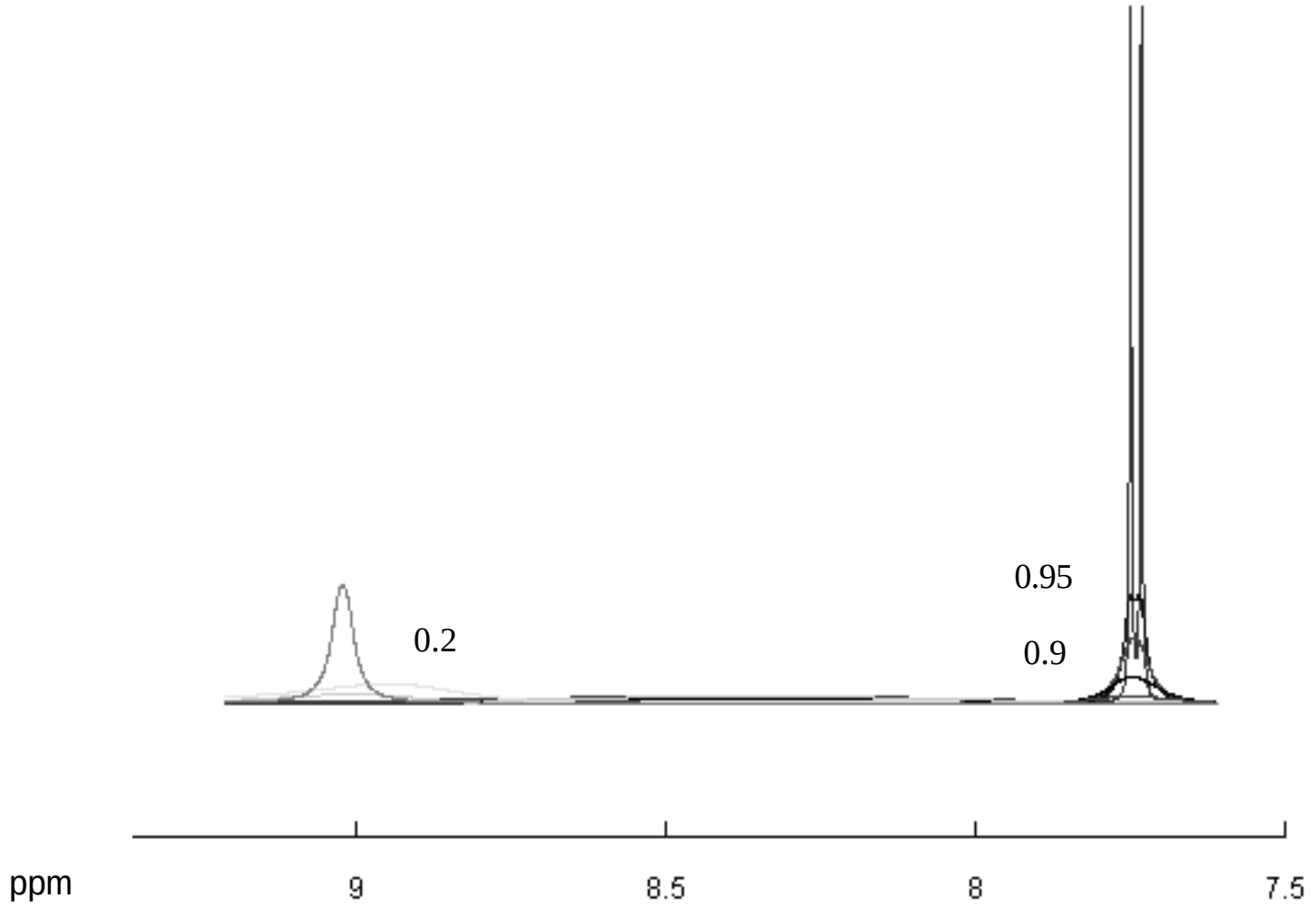
Very slow exchange:
$k_{off} \ll \Delta\delta$



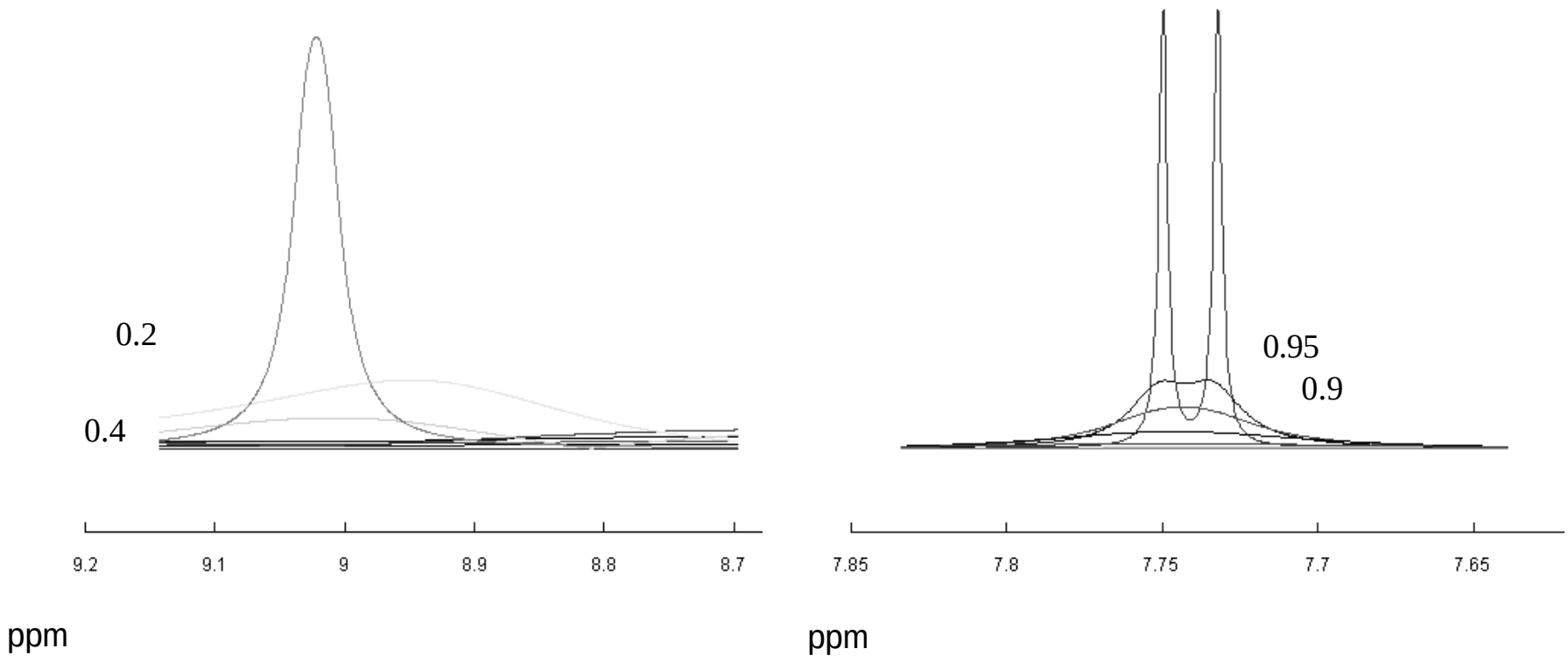
D. Cicero

Intermediate exchange:
$k_{off} = \Delta\delta$



D. Cicero

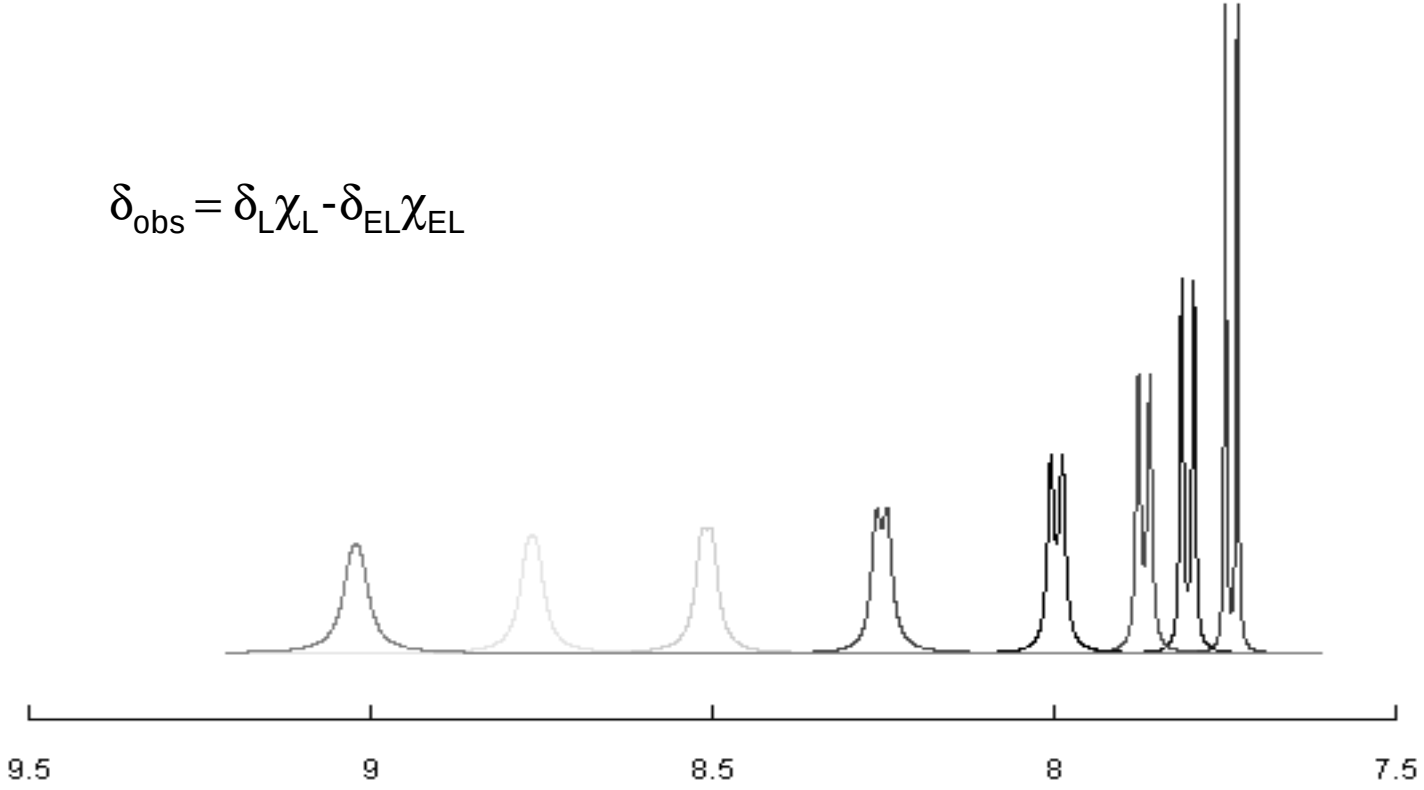
Intermediate exchange:
$k_{off} = \Delta\delta$



D. Cicero

Fast exchange

$\delta_{obs} = \delta_L\chi_L - \delta_{EL}\chi_{EL}$



$$\delta_{obs} - \delta_L = \frac{(\delta_{EL} - \delta_L)\left\{(E_T + L_T + K_d) - \sqrt{(E_T + L_T + K_d)^2 + 4E_T L_T}\right\}}{2L_T}$$

Example of fast exchange – determination of the Kd

$d = d_f c_f - d_b c_b$ SPM + ATP $\rightleftharpoons$ SPM-ATP

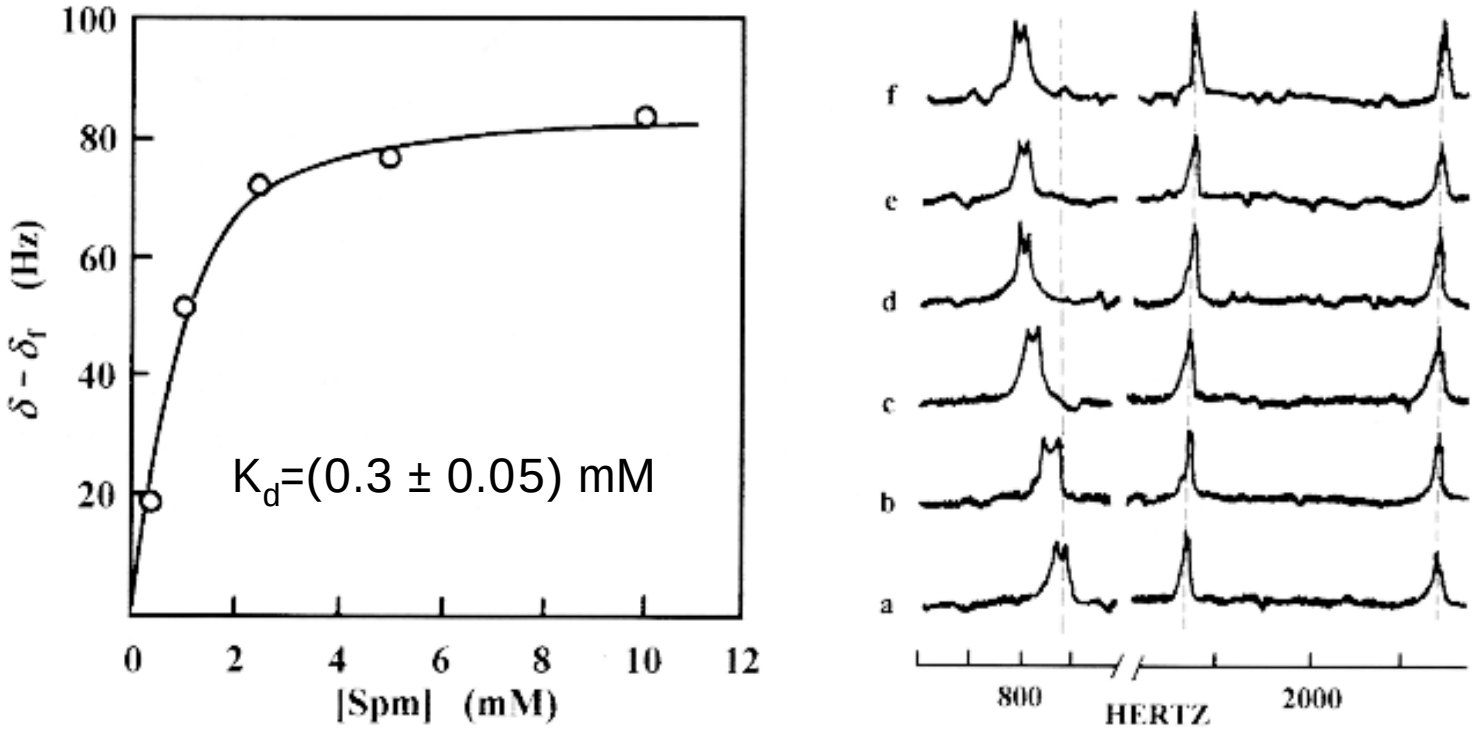
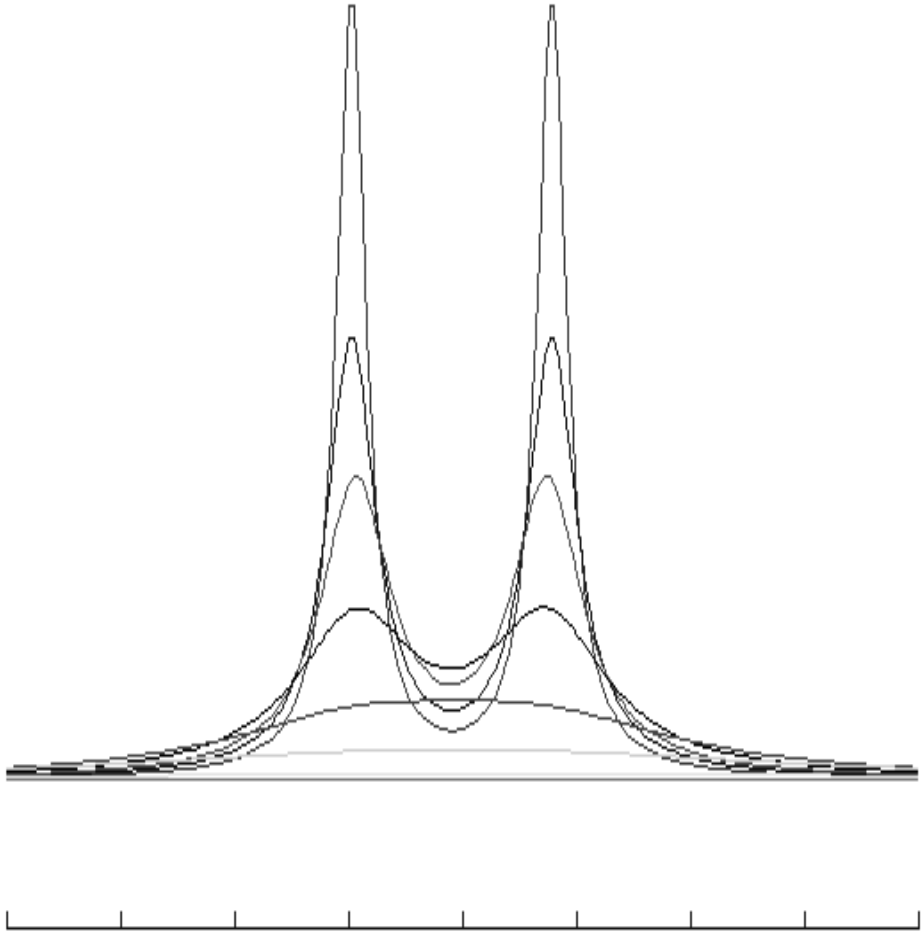


Fig. 1. Chemical shift dependence of ^{31}P of the γ-phosphate group of ATP on spermine concentration. The measurements were carried out in 10 mM Hepes, pH 7.0, 27°C, 1 mM ATP. NaCl was added to obtain 90 mM ionic strength. $(\delta - \delta_f)$ represents the difference between the chemical shift of ^{31}P observed in the presence and in the absence of polyamine and the chemical shift of the free phosphate group. Phosphoric acid was used as chemical shift reference. Insert: NMR spectra of ATP in the presence of various concentrations of SPM: **a** – 0, **b** – 0.4, **c** – 1, **d** – 2.5, **e** – 5, and **f** – 10 mM.

Corazza et al. 1994

Slow exchange



$$1/T_{2L,obs} = 1/T_{2L} + k_{off}x_{EL}/x_L$$

Intermediate exchange

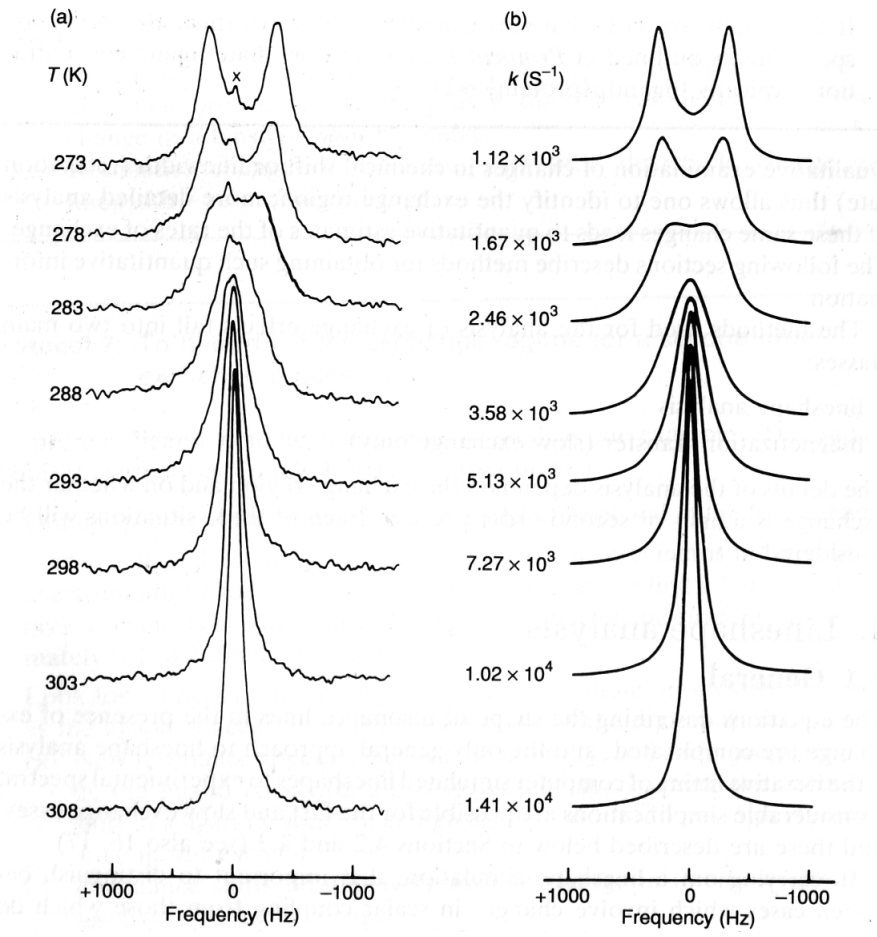
Lineshape analysis

$$G(n) = \frac{iC[2x_A x_B t - t^2(x_A a_A + x_B a_A)]}{x_A x_B - t^2 a_A a_B}$$

$$a_A = 2pi(n_A - n) + (1/2T_{2A}) + x_B/t$$

$$a_B = 2pi(n_B - n) + (1/2T_{2B}) + x_A/t$$

McConnell modification of the Bloch equation takes into account lifetimes, free and bound molar fractions and transverse relaxation rate of both free and bound species.



In this experiment all the diflоromethotrexate is bound to the enzyme.
The experiment is done by changing the temperature and the process observed is monomolecular.

Observed (a) and calculated (b) 188.3 MHz ^{19}F NMR spectra of 3',5'-difluoromethotrexate bound to *L. casei* dihydrofolate reductase.
Clore *et al*. *Biochem. J.* **217**, 659 (1984)

By lowering the temperature one can obtain, in a slow regime, resonating frequencies and relaxation rates and initial estimates of population and lifetime by the appearance of the spectrum

Determination of the exchange regime

- Obtain spectra of the protein without the ligand and of the free ligand
- Add the ligand to the protein sample by small increments and record a spectrum after each addition
- Look for changes in the spectrum; the nature of these progressive changes as the ligand concentration is increased reveals the kind of exchange

Estimating k_{off}

- Fast exchange:

  Find the signal showing the largest difference in d between free and bound state:

  $K_{off} >> \Delta\delta$

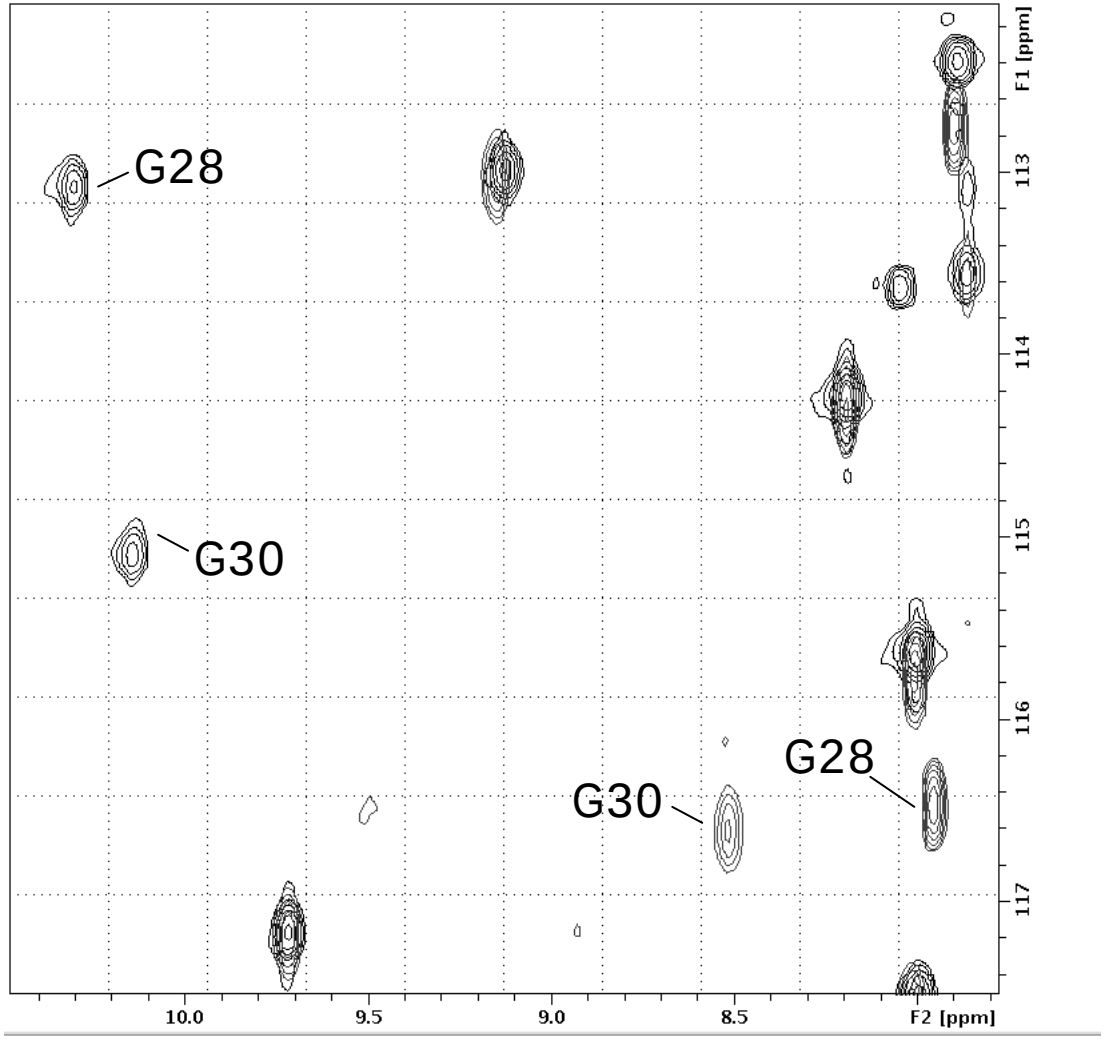
- Slow exchange:

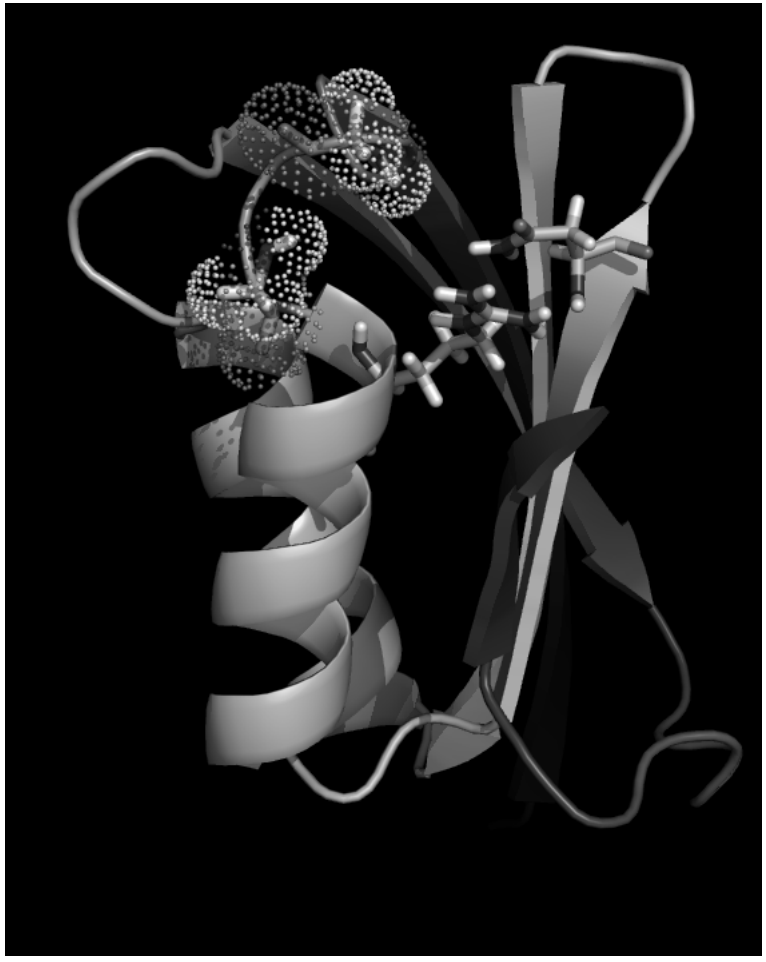
  Find the signal showing the smallest difference in d between free and bound state:

  $K_{off} << \Delta\delta$

Determination of the binding site

Sulfolobus solfataricus acylphosphatase in the presence (black) and in the absence (blue) of Pi





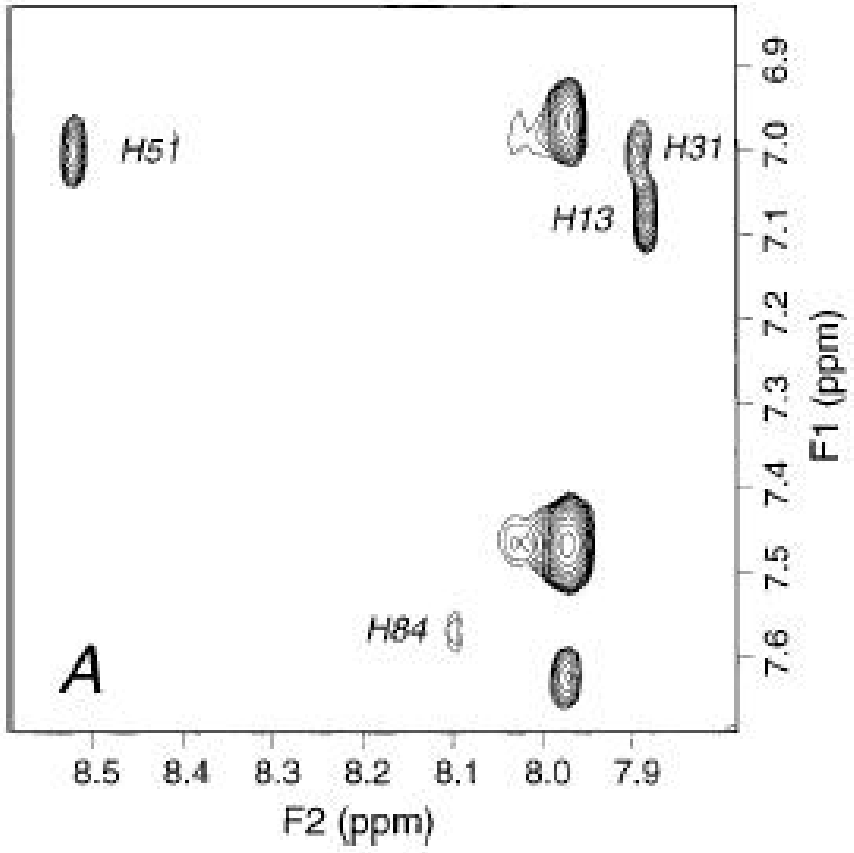
Corazza et al. in preparation

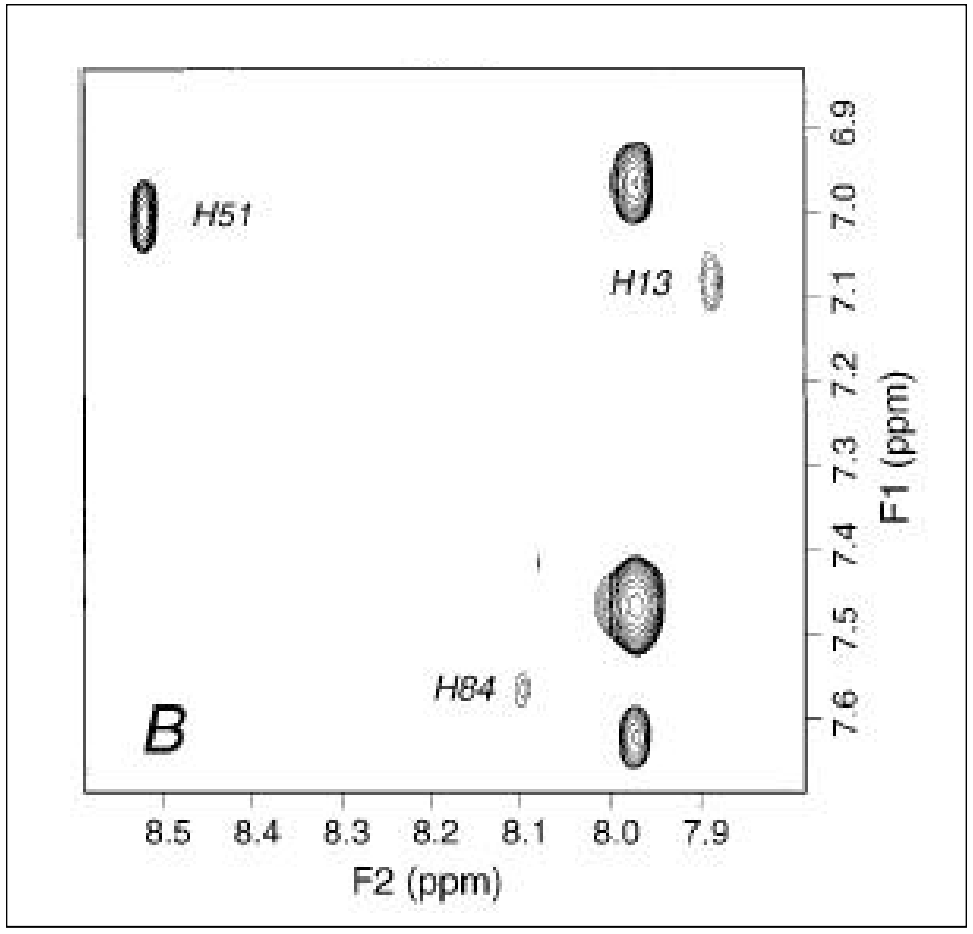
1H 2D TOCSY

Determination of the binding site

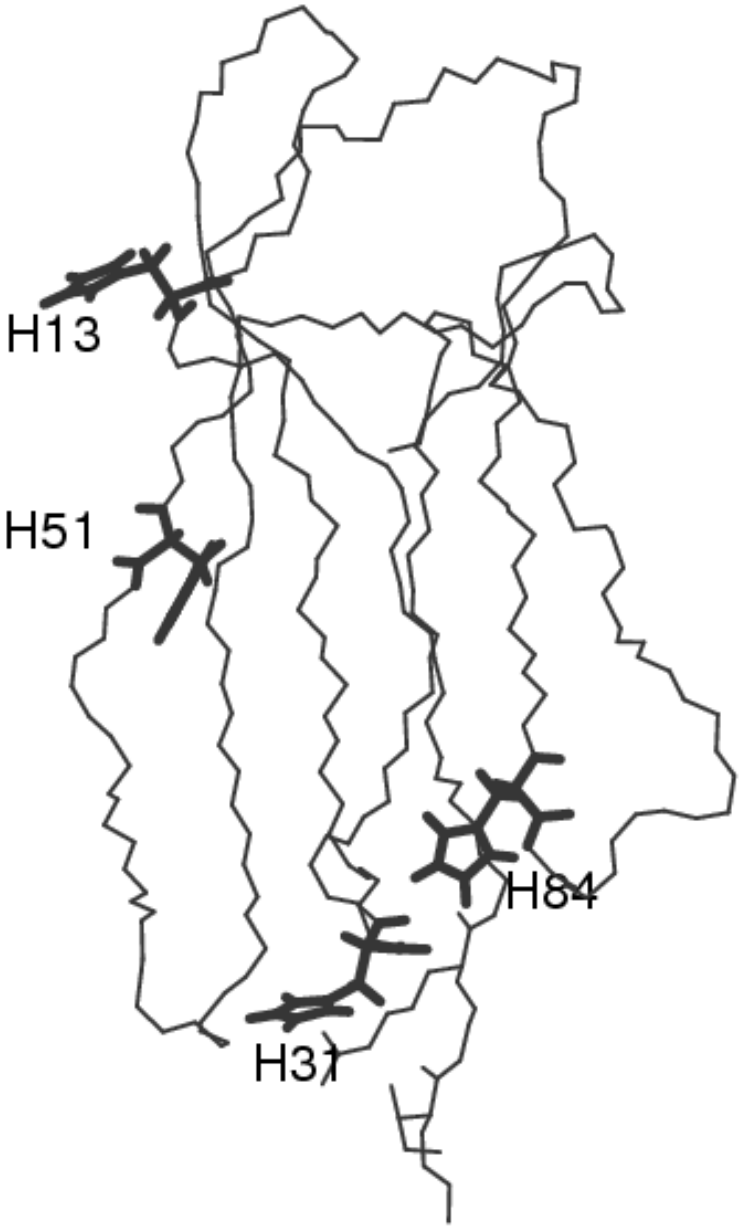
β2–m

1:3= Cu^{2+}:β2m





Verdone et al. Prot. Sci. 2000


H13
H51
H84
H31

Chemical shift perturbation to map interaction surfaces

Titration experiments can be used to monitor the magnitude in chemical shift changes upon binding of different regions of the interacting molecules

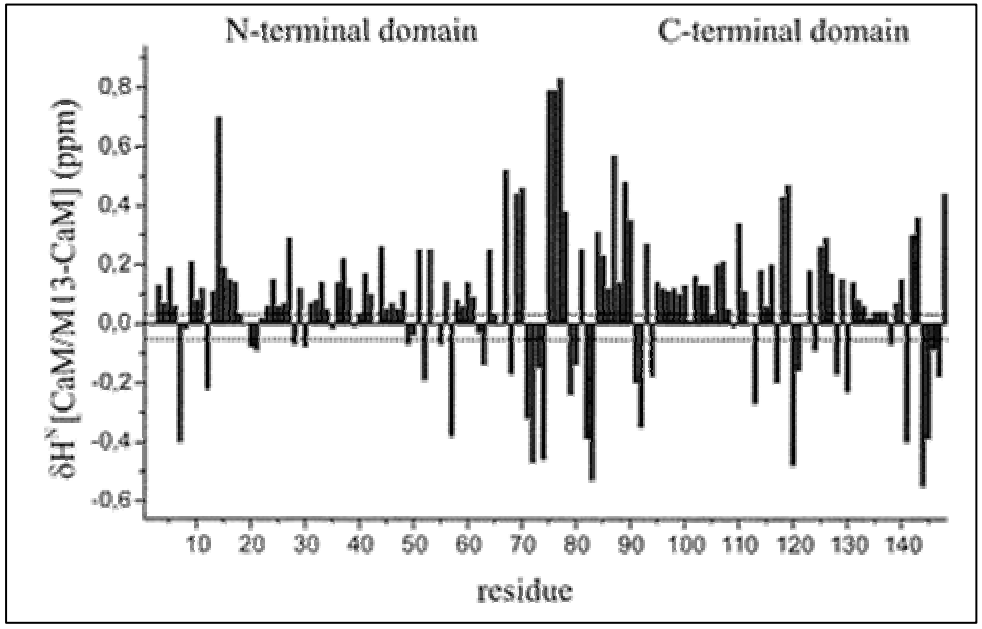
The regions showing the largest effects are candidate to define the interaction surface

Using chemical shift perturbation to map interaction surfaces

- sk-MLCK (Miosin Light Chain Kinase) M13:
  KRRWKKNFIAVSAANRFKKI

- Ca^{2+} pump C20W:
  LRRGQILWFRGLNRIQTQIK

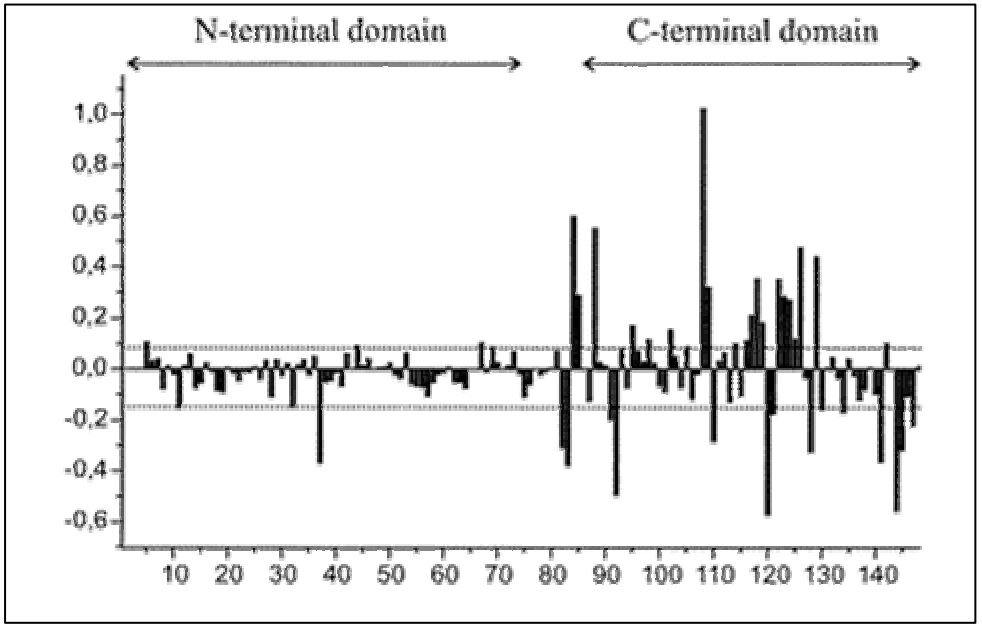
Using chemical shift perturbation to map interaction surfaces

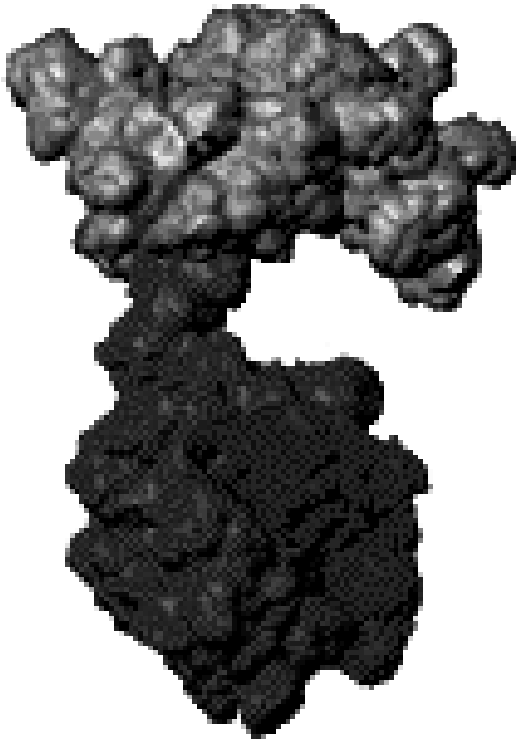


M. Ikura et al., *Science.* **256**,632 (1992)

CaM/M13

Using chemical shift perturbation to map interaction surfaces





B. Elshorst et al., *Biochemistry* **38**, 12320 (1999)

CaM/C20W