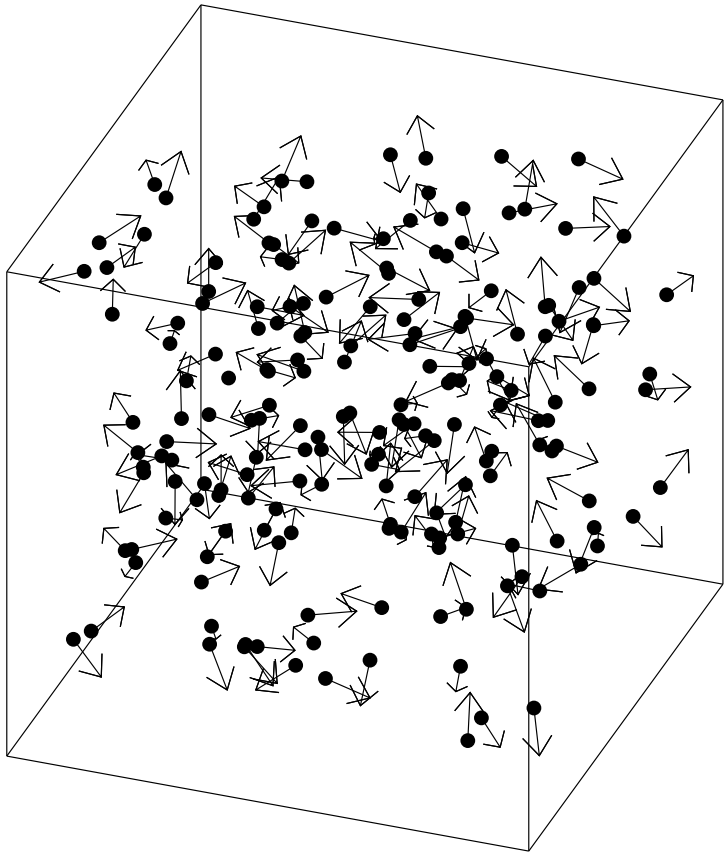
Nuclear Magnetic Resonance

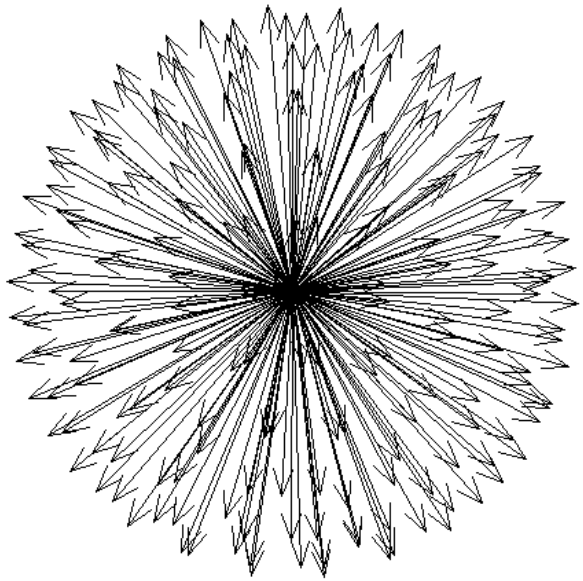
Lukáš Žídek

Principles of NMR

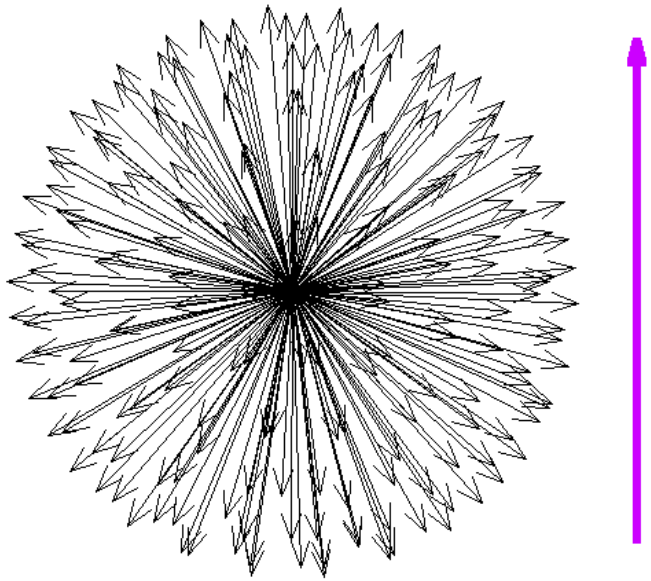
Molecular and magnetic interactions



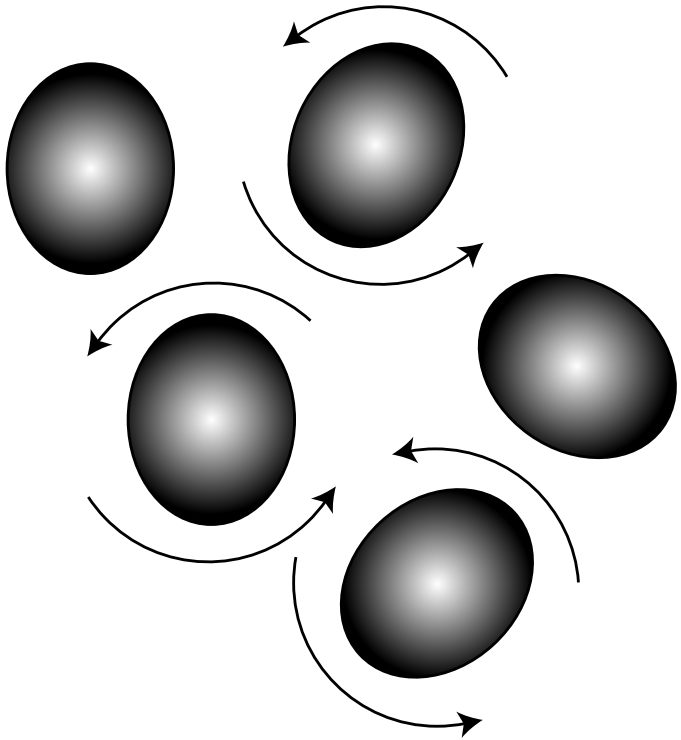
NMR sample outside magnet



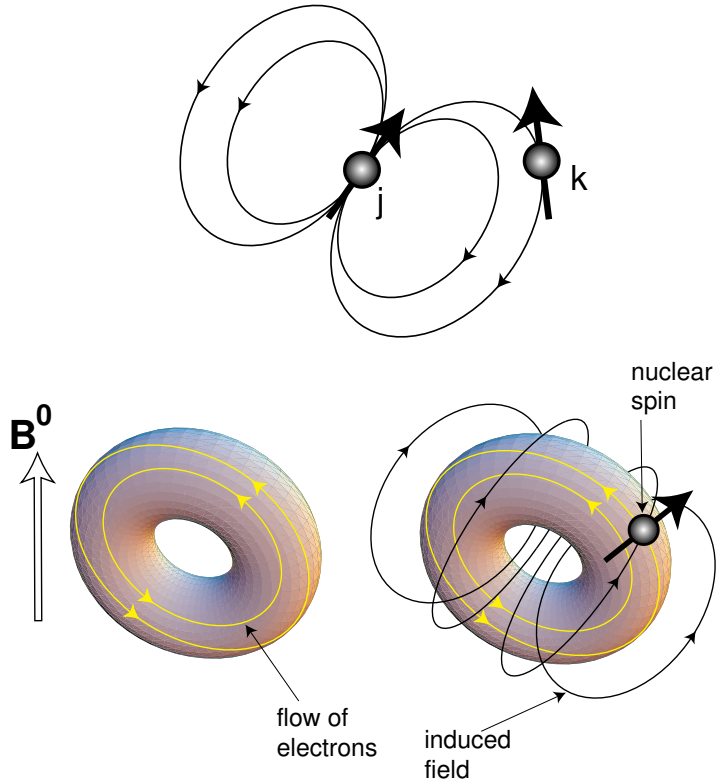
NMR sample inside magnet



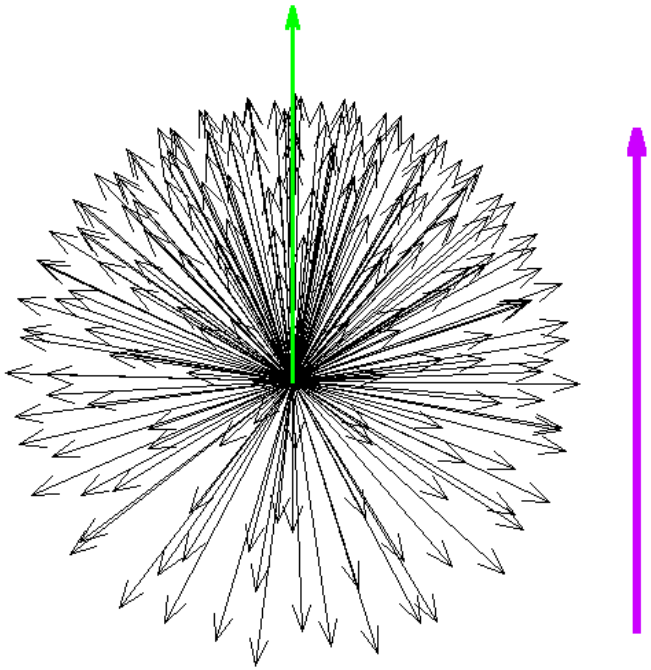
Relaxation via coupling with molecular rotation



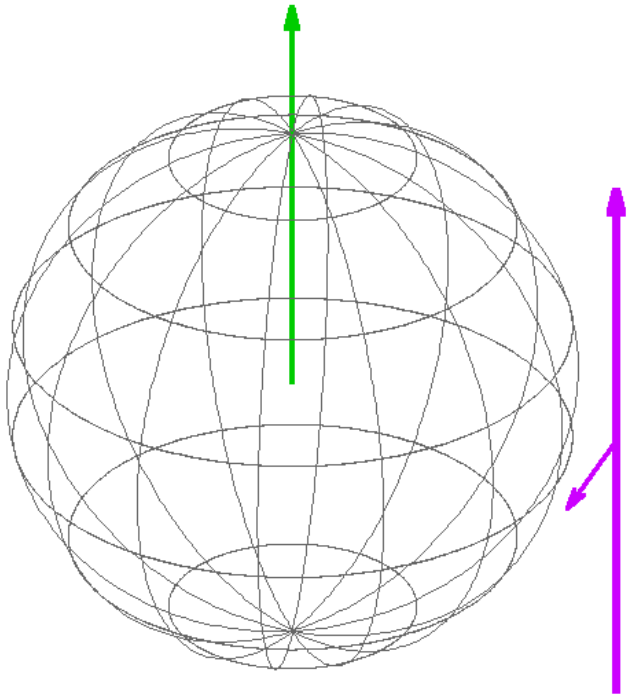
reproduced from M. H. Levitt: Spin Dynamics



Polarization



Excitation



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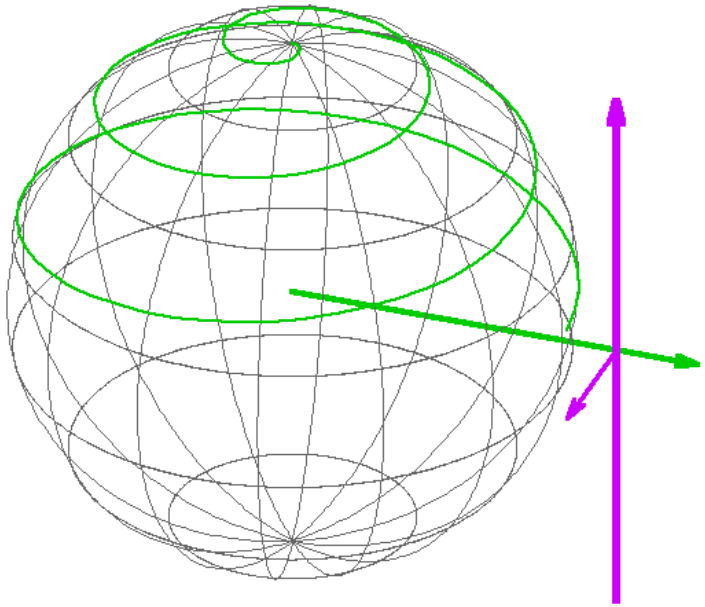
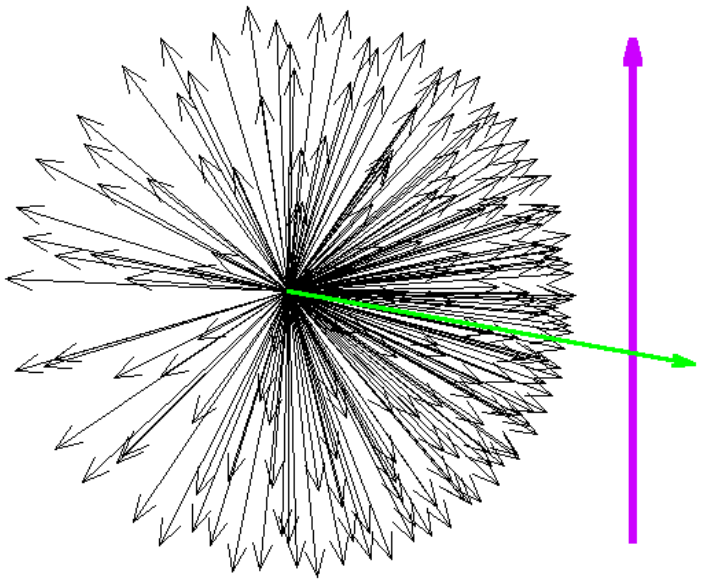
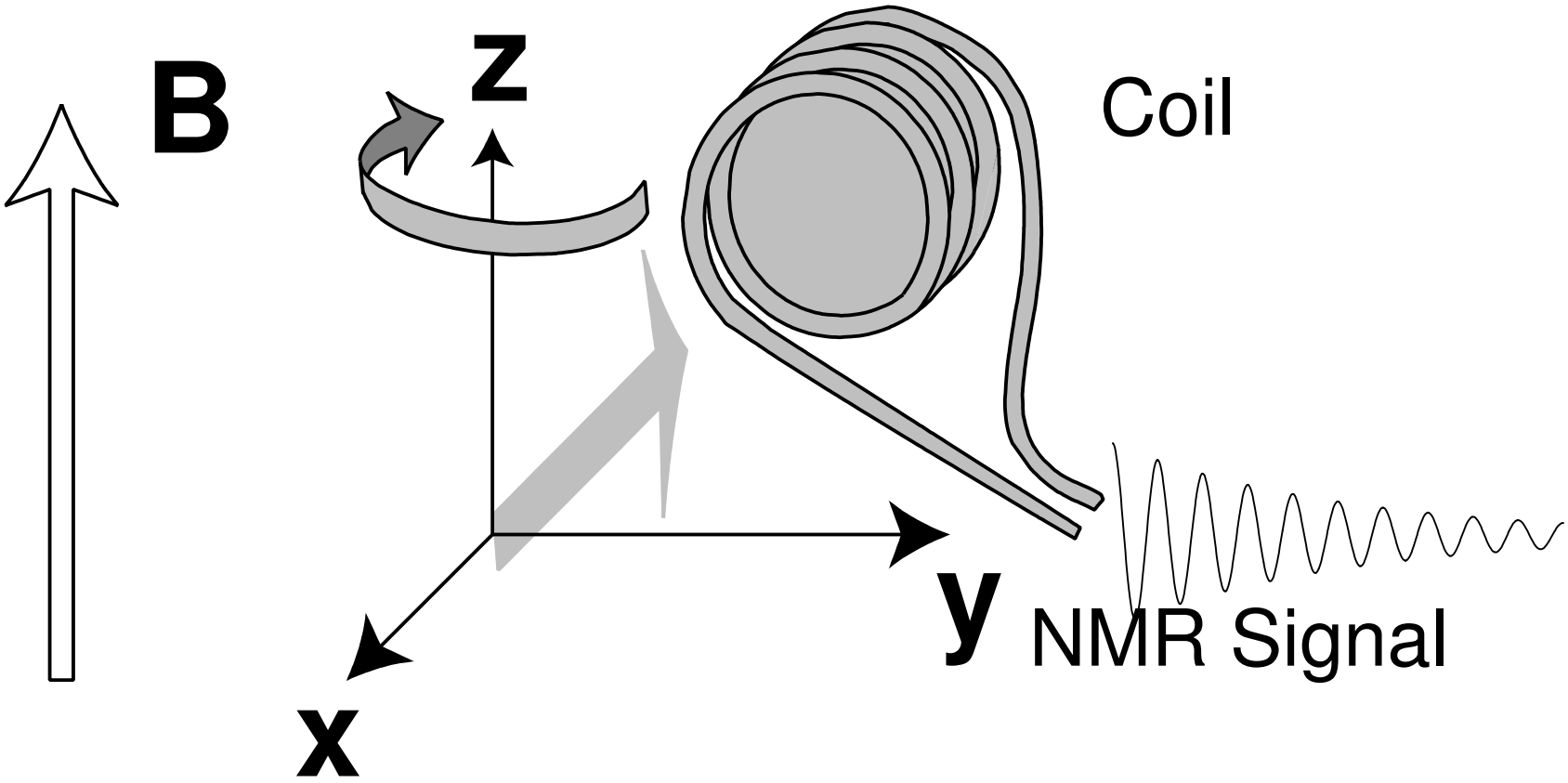


Coherent evolution

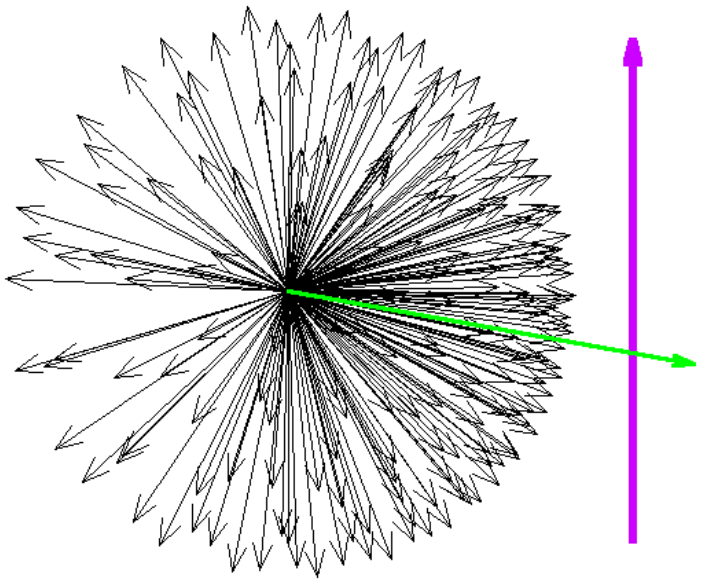


Signal detection

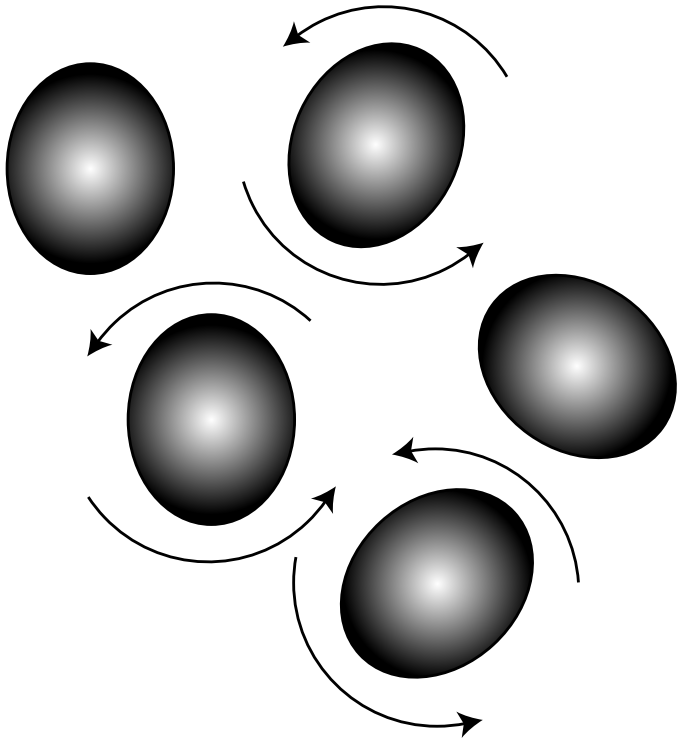


reproduced from M. H. Levitt: Spin Dynamics

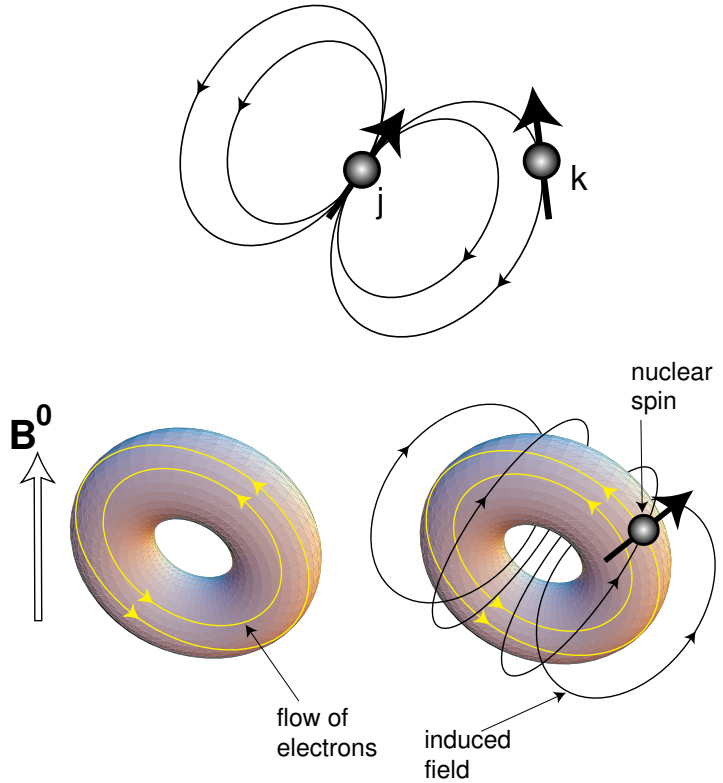
Non-equilibrium distribution of magnetic moments



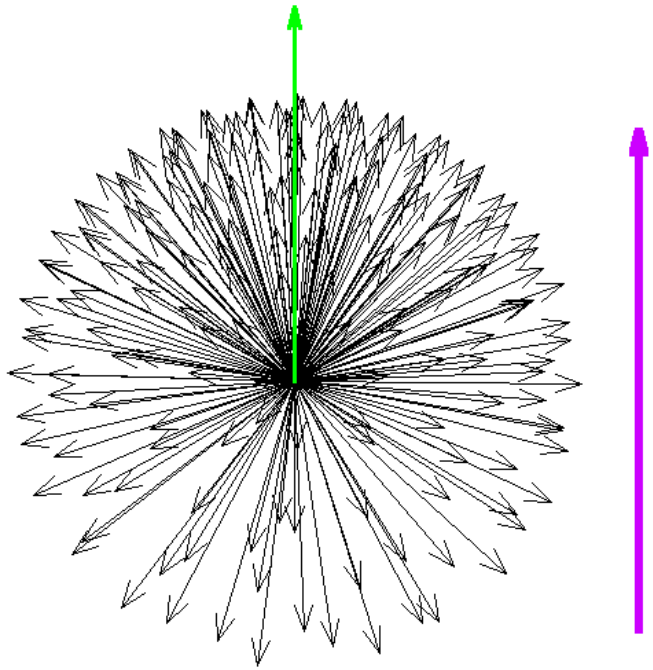
Relaxation via coupling with molecular rotation



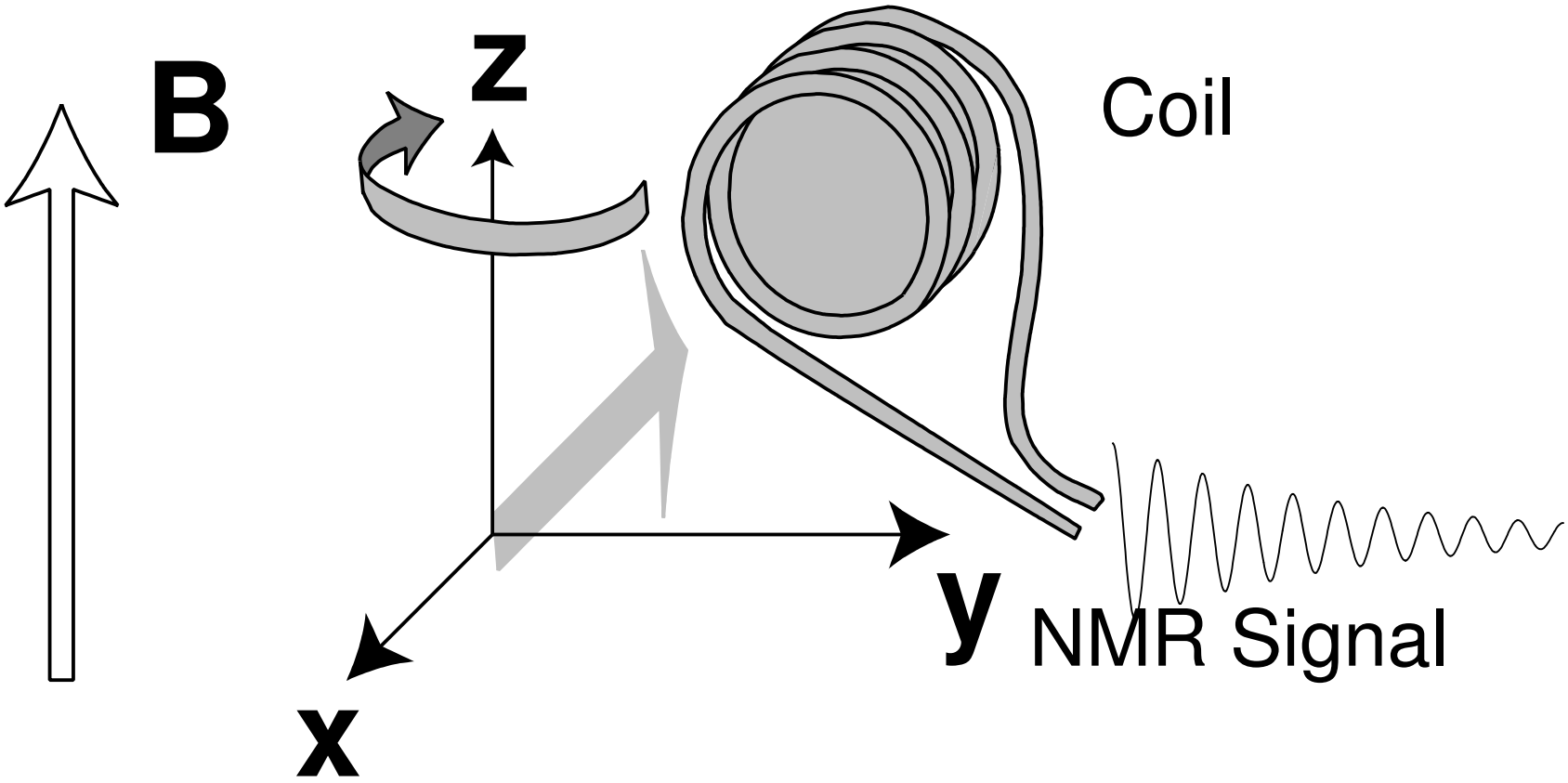
reproduced from M. H. Levitt: Spin Dynamics



Relaxation

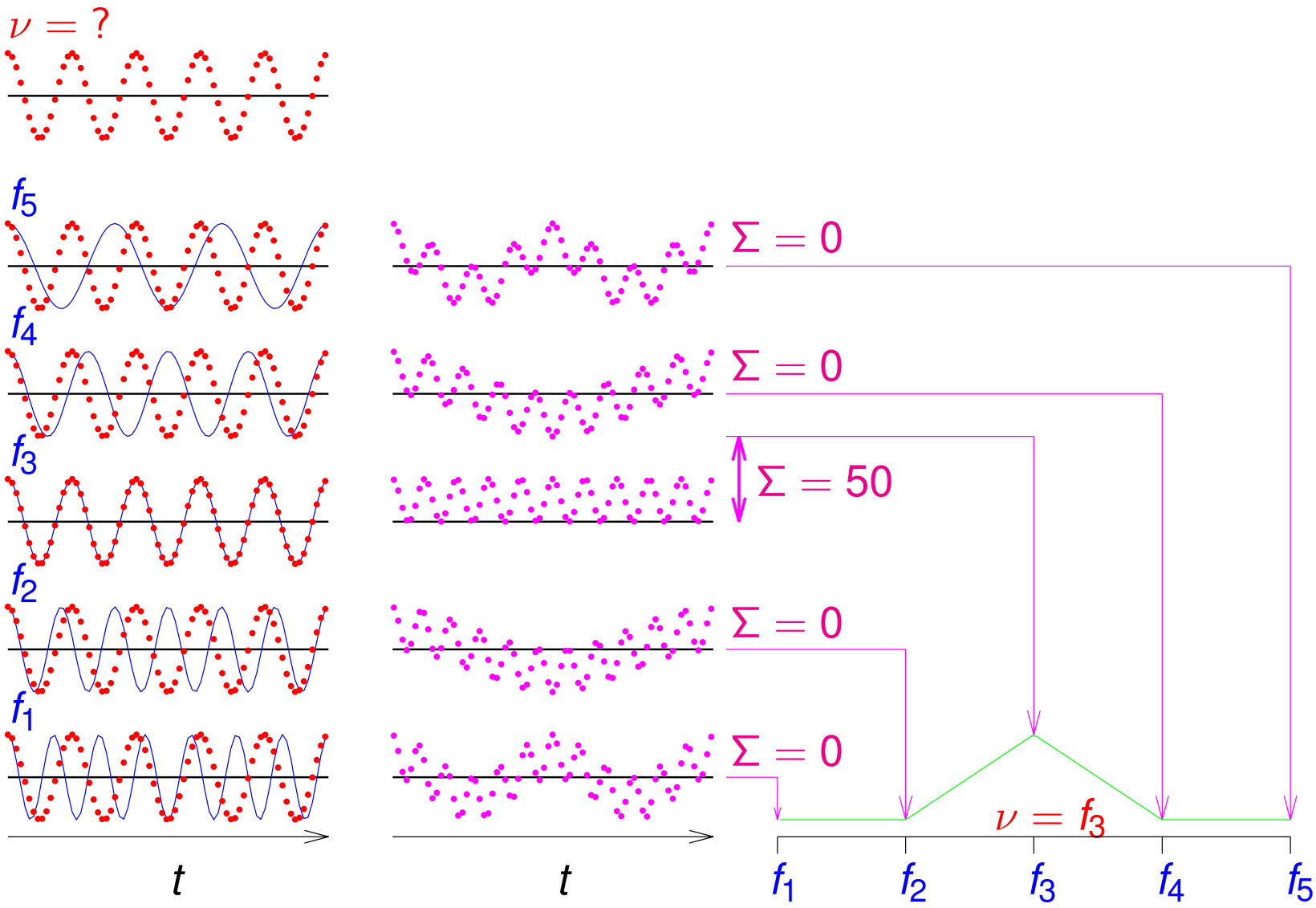


Signal decay



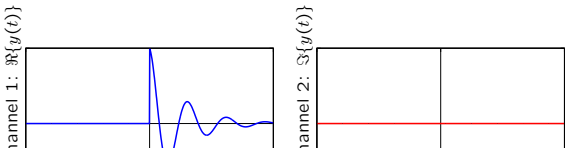
reproduced from M. H. Levitt: Spin Dynamics

Fourier transformation

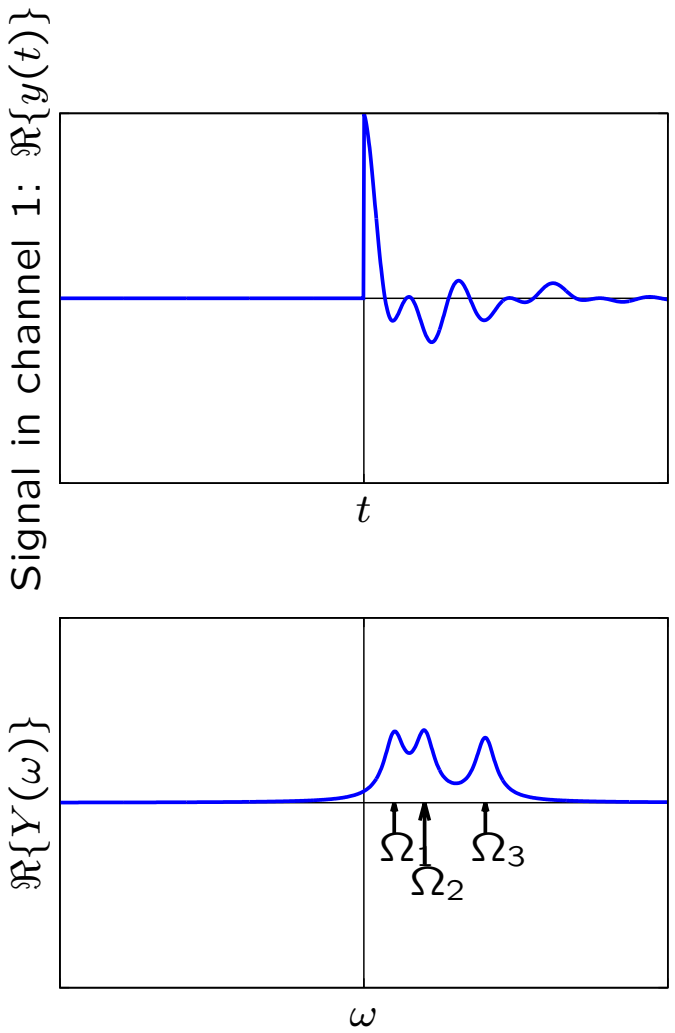


Signal processing

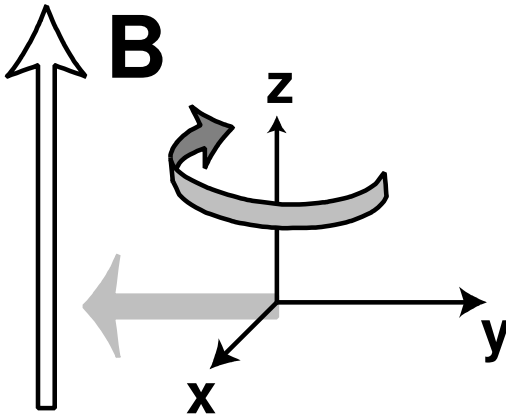
Fourier transformation of ideal signal.

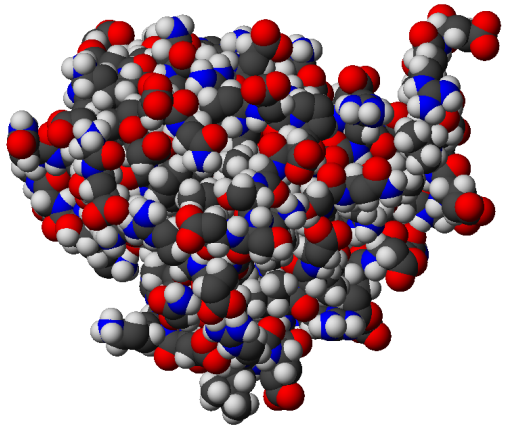


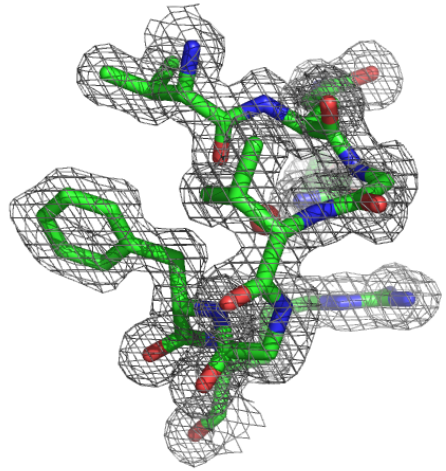
Signal processing

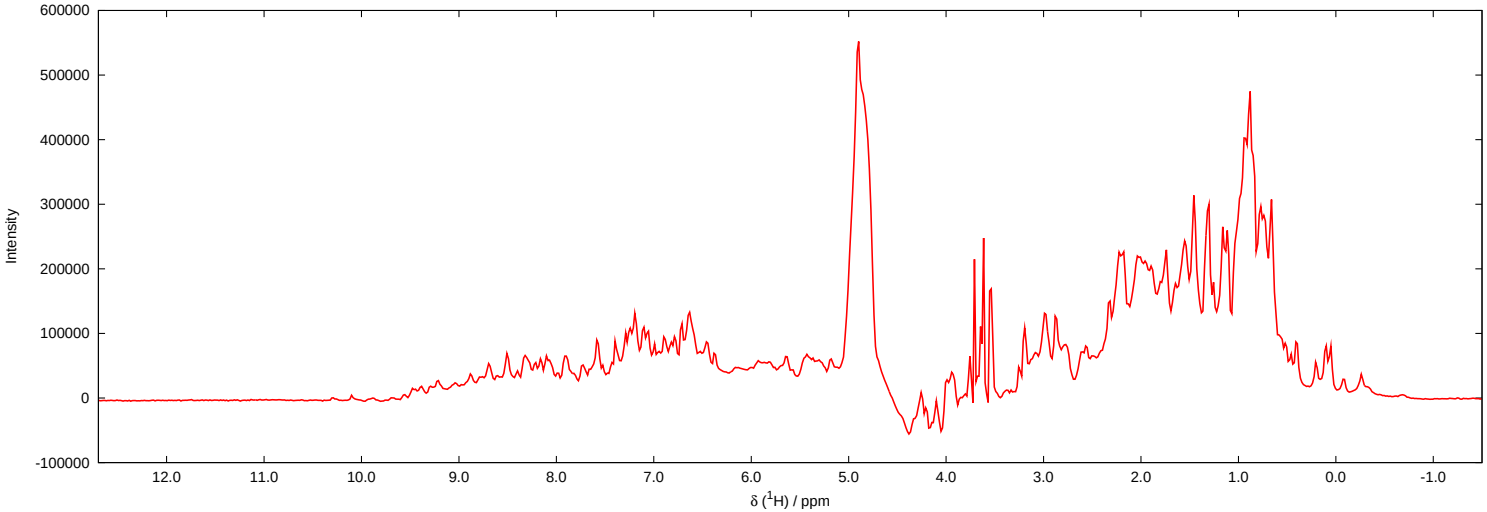


Chemical shift: influence of electrons

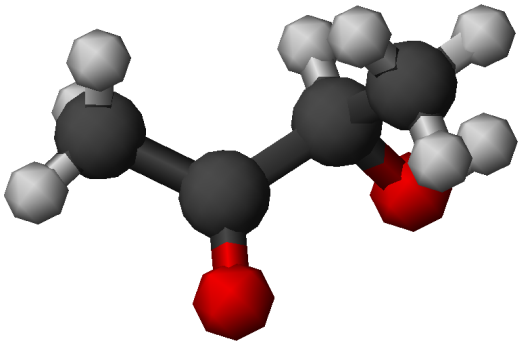


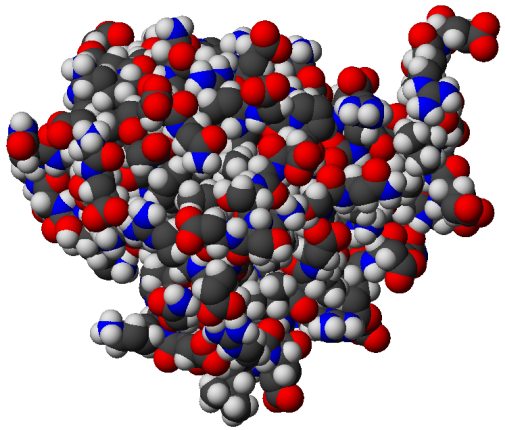






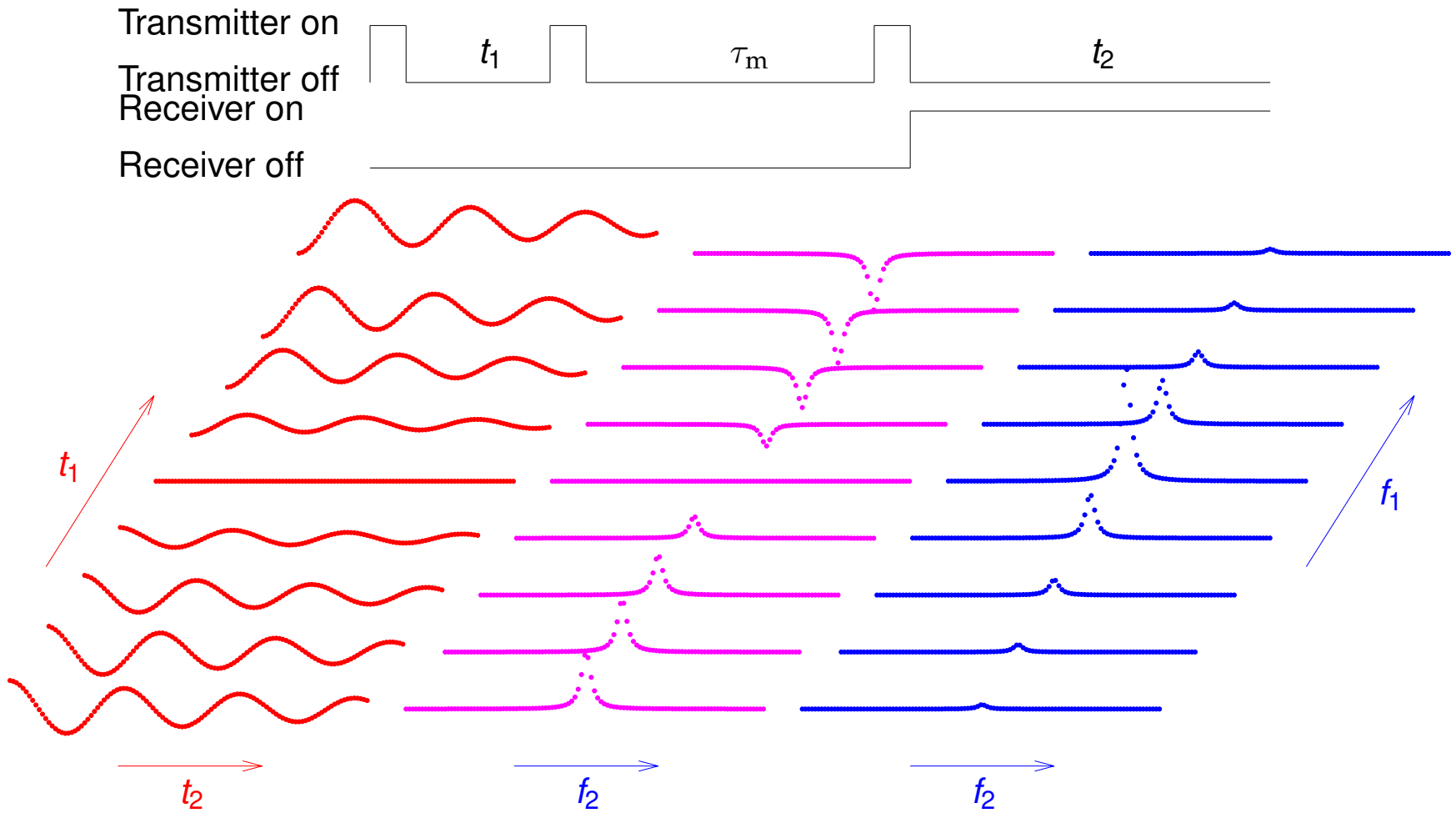
More advanced NMR experiments



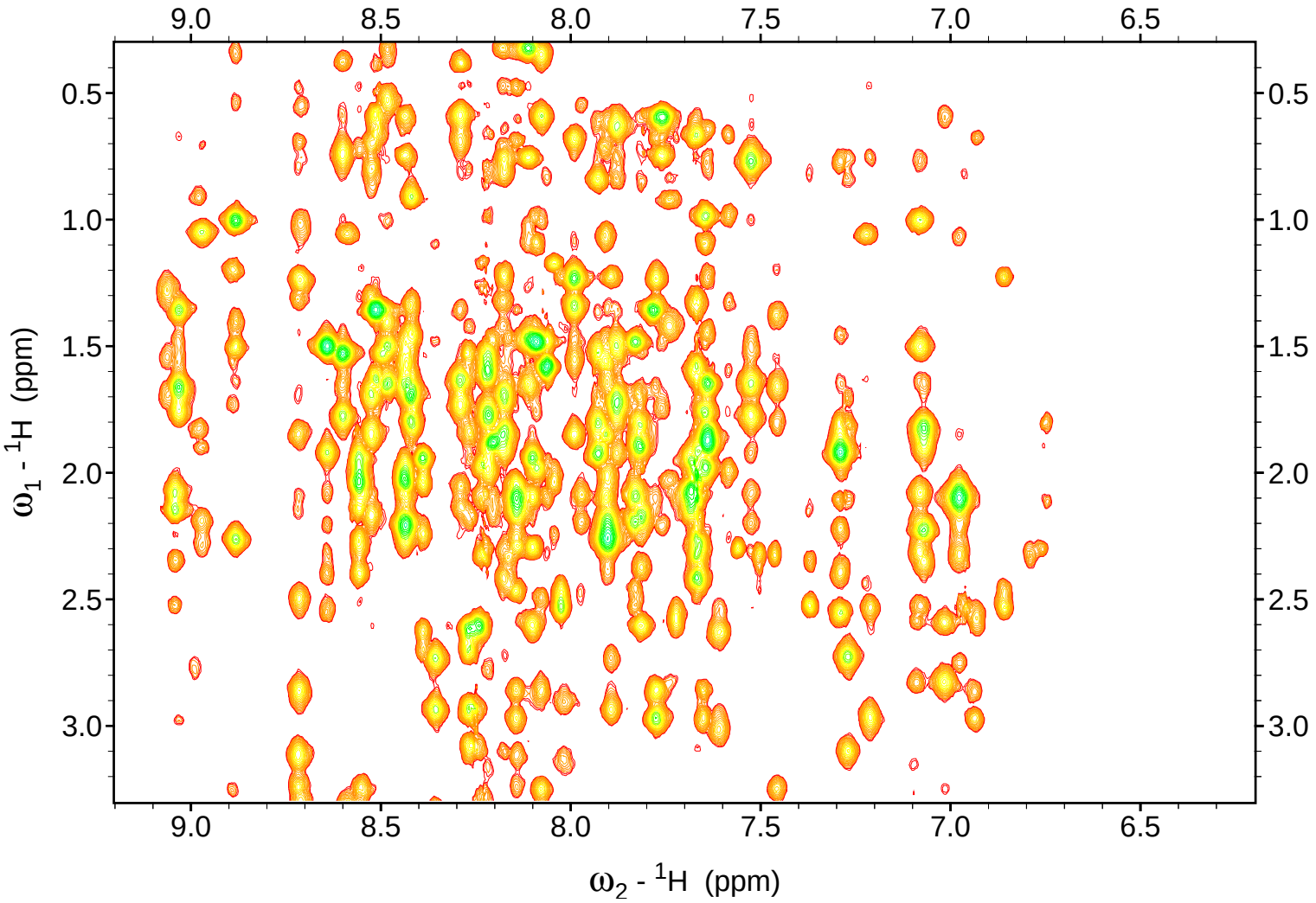


- Solvent (water) suppression
- Simplification of spectra
- Resolution improvement
- Obtaining chemical/biological information
  structure, dynamics, interactions

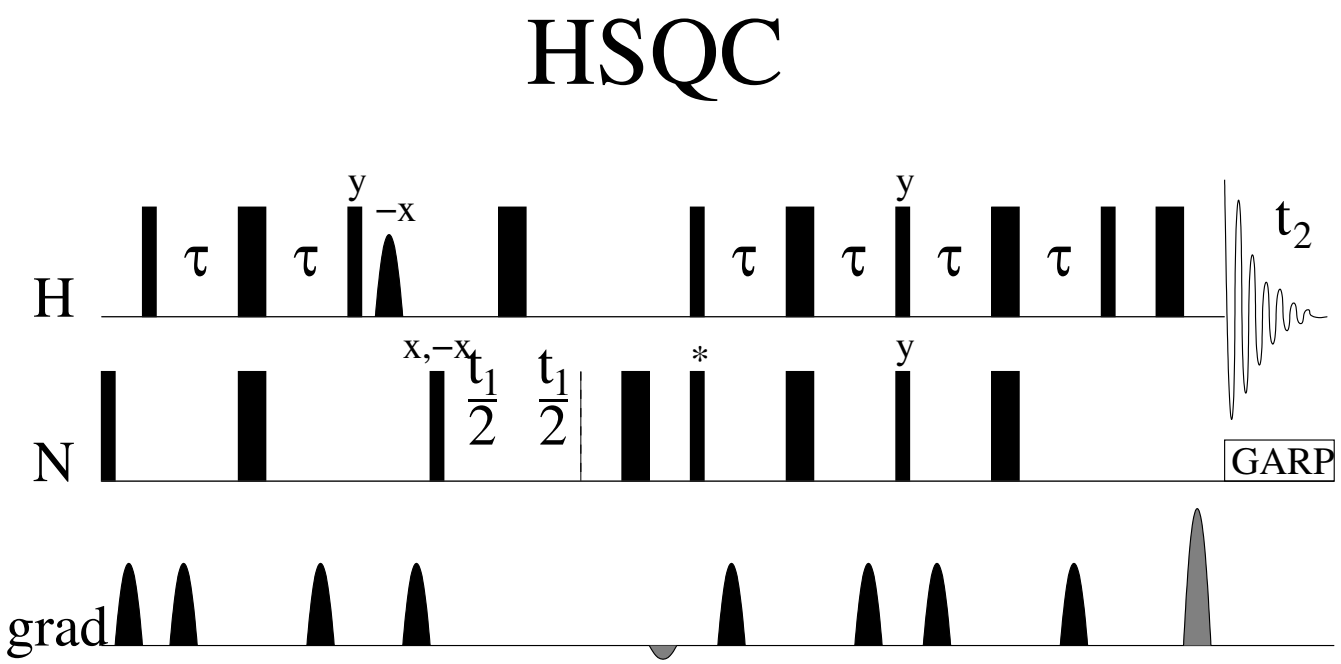
2D spectroscopy: NOESY



2D NOESY spectrum



Correlated multidimensional NMR experiments



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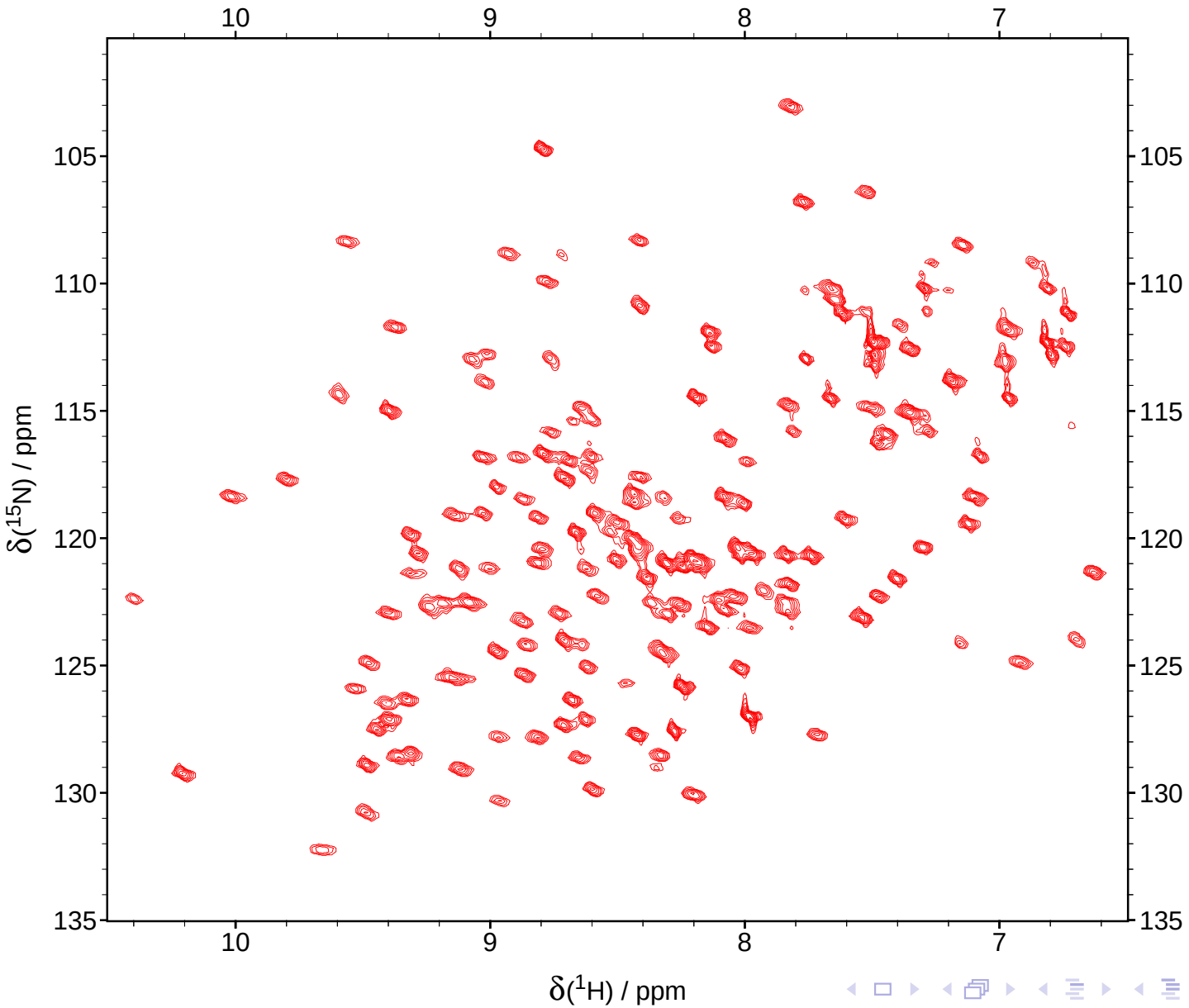
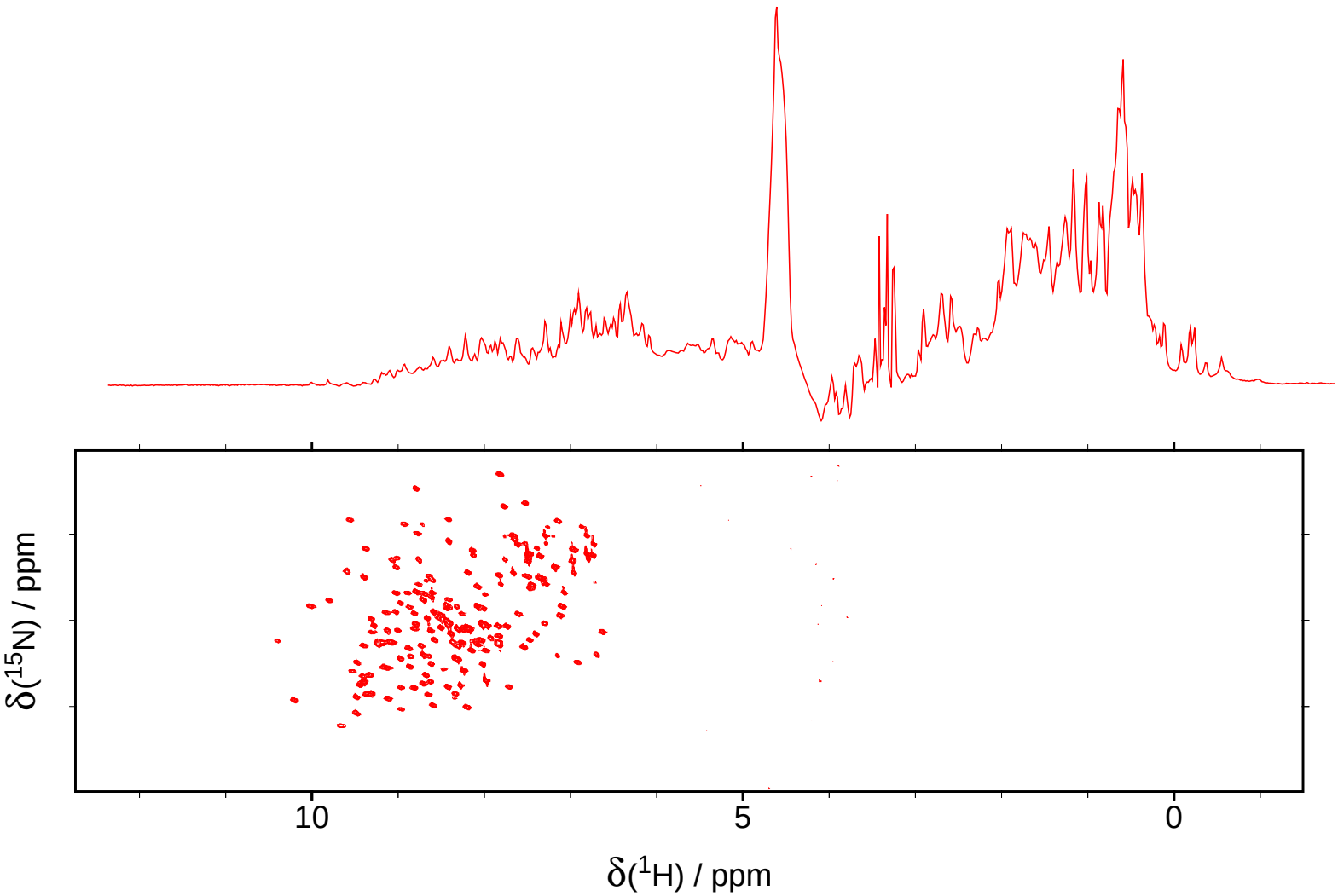
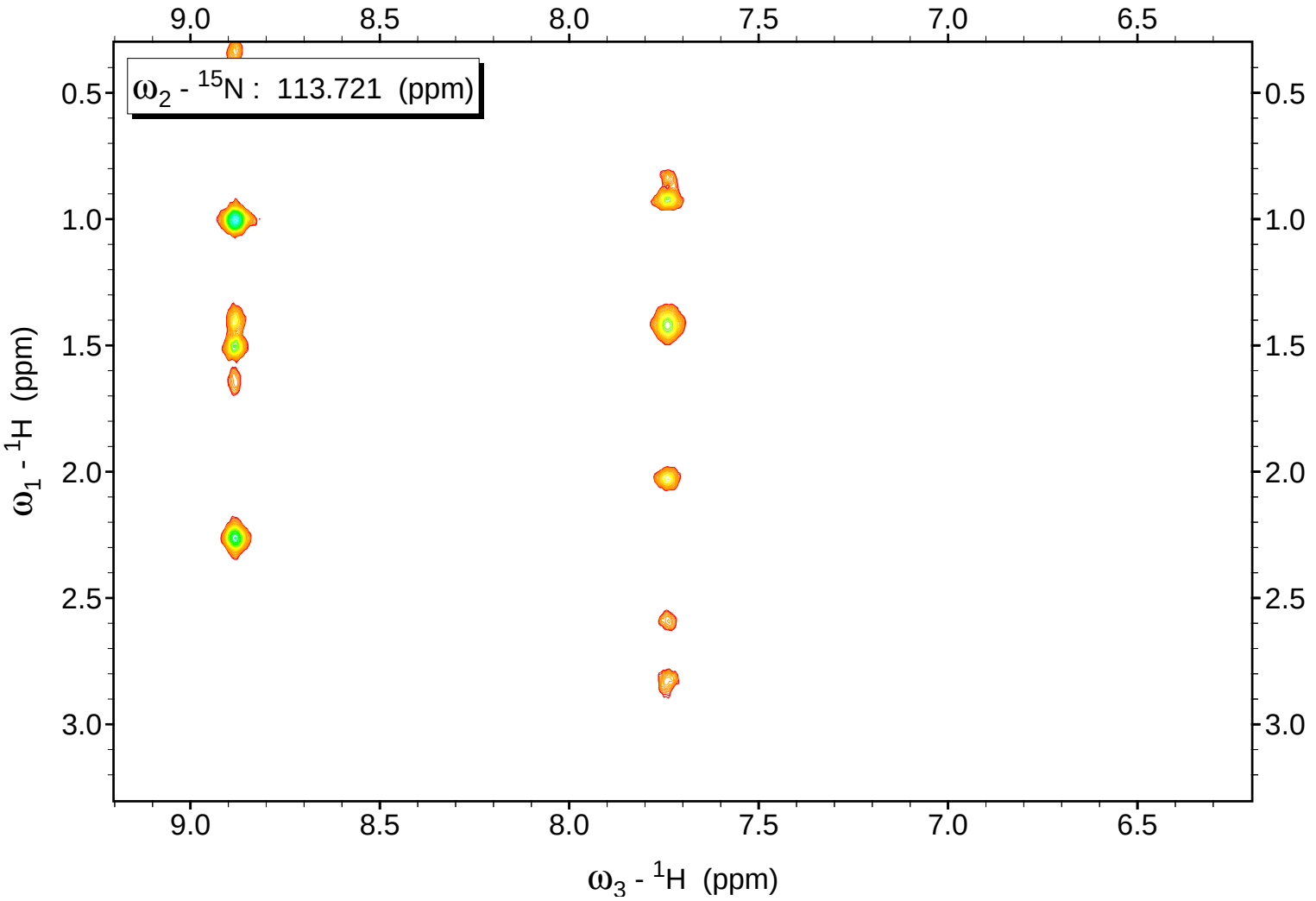


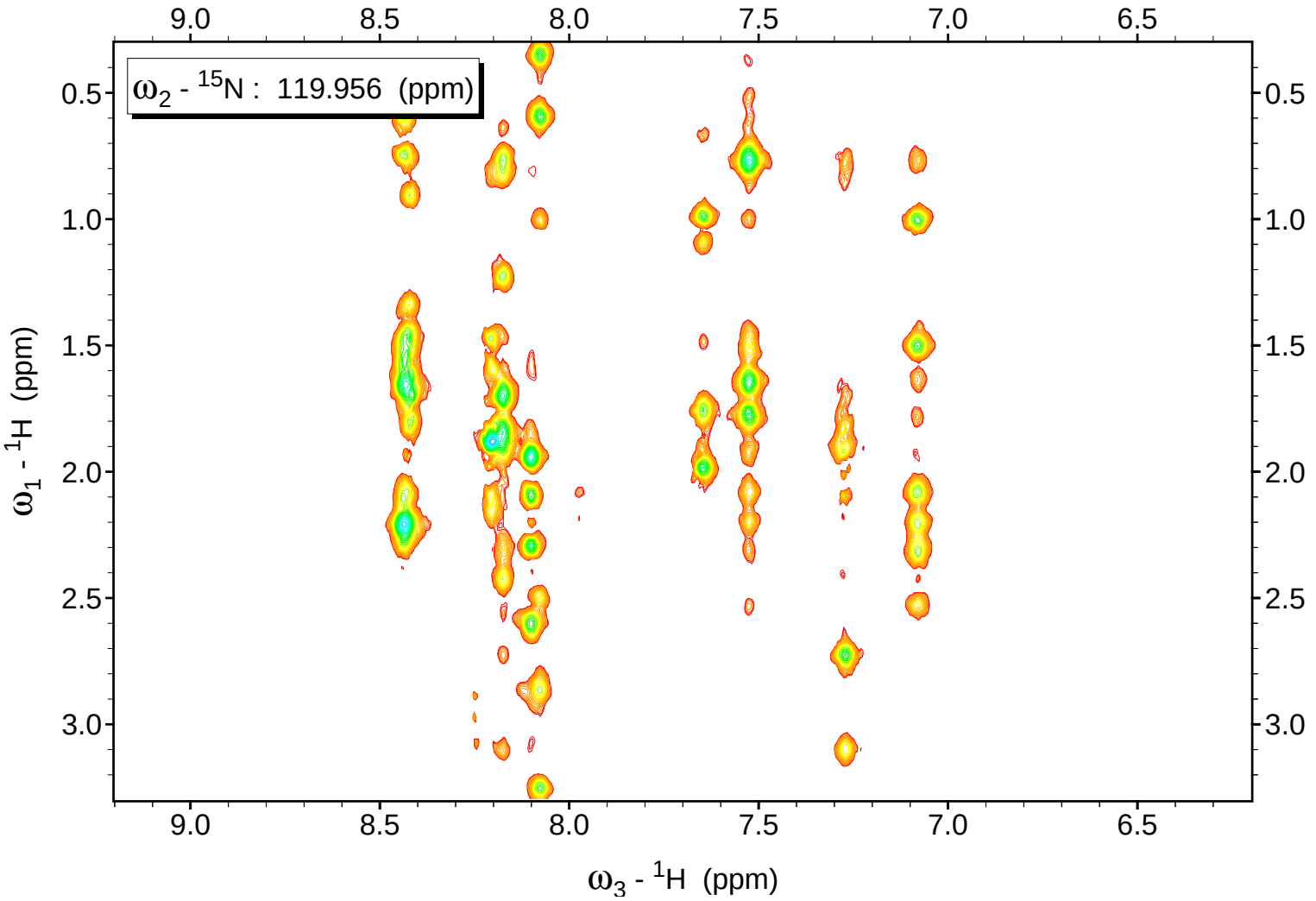
Correlated multidimensional NMR experiments



3D NOESY-HSQC spectrum



3D NOESY-HSQC spectrum

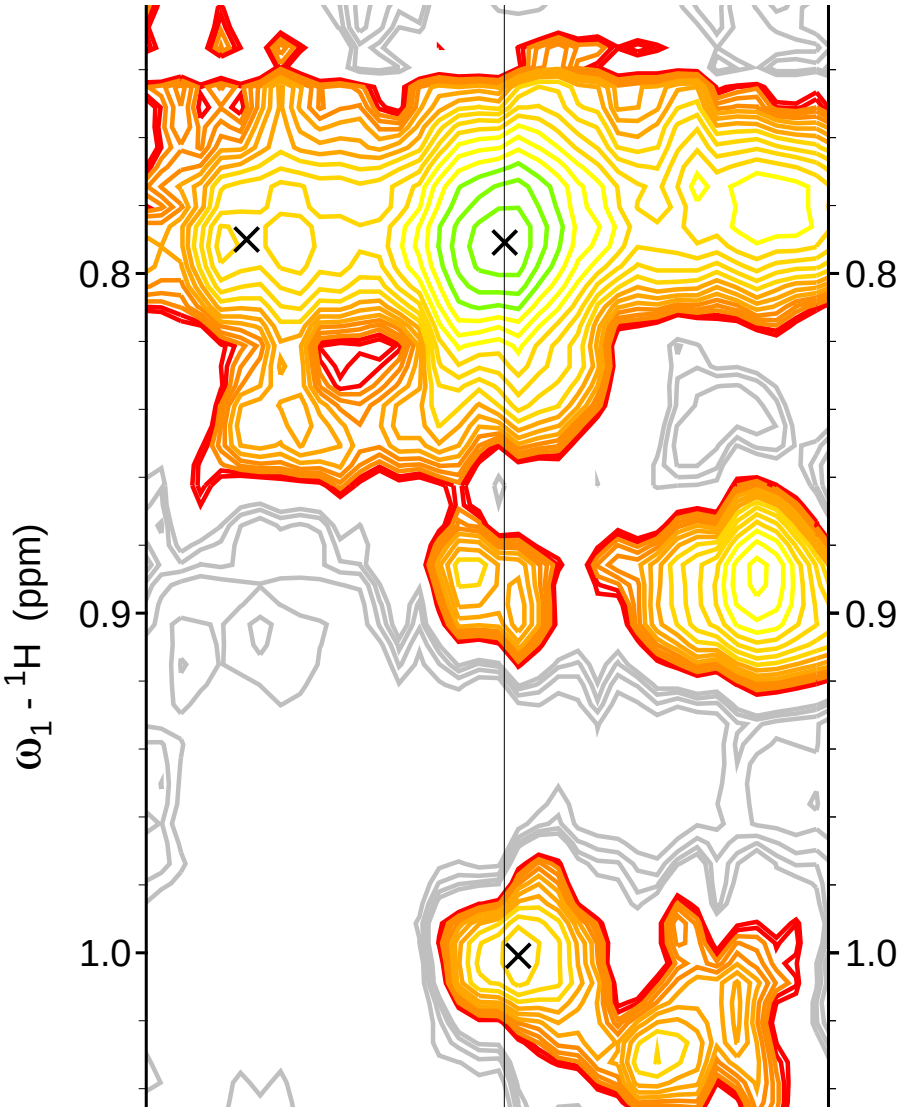


Biomolecular applications

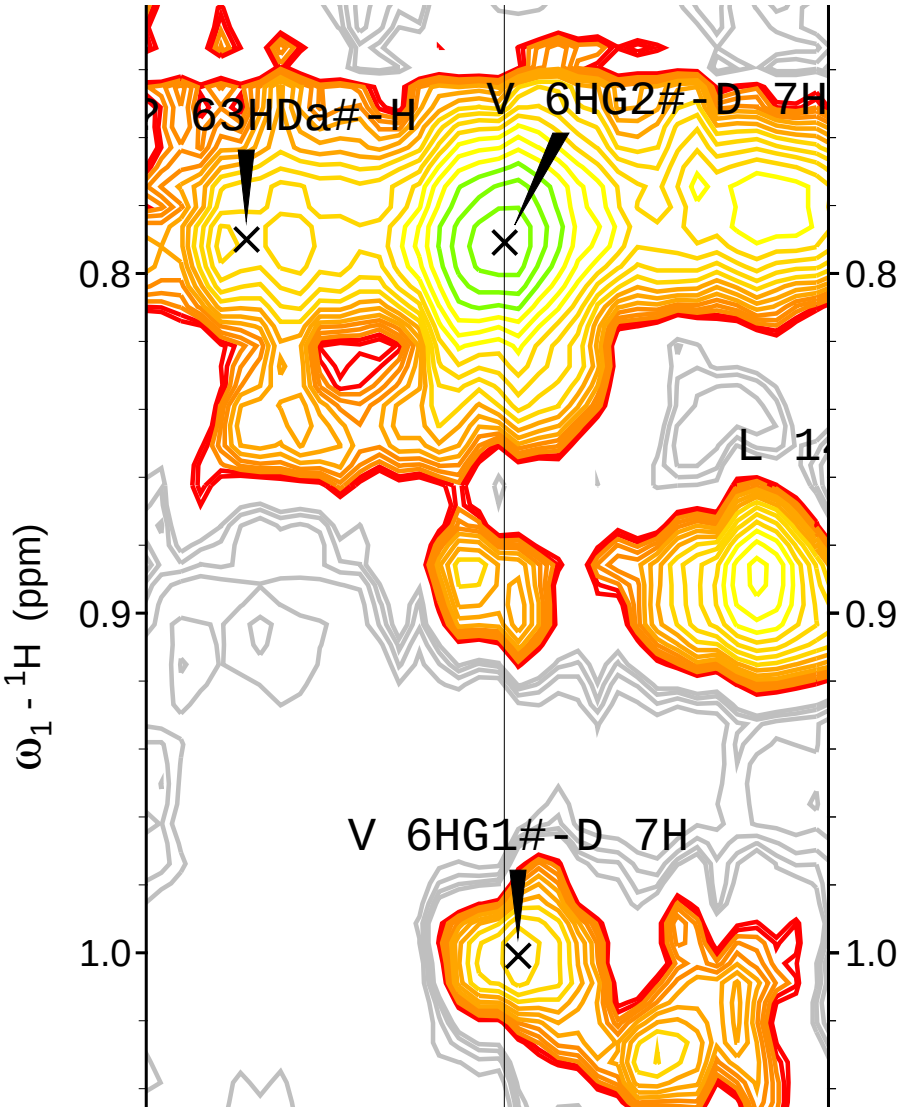
Pros and cons of NMR

- No special sample requirements
- Low energy
  non-destructive $\times$ low sensitivity
  high concentration, high magnetic field, isotope labeling
  long measurement time
- Atomic resolution
- Many atoms described by single measurement
  high information content $\times$ complexity of data
  correlated spectroscopy, selective labeling
  Assignment of spectra is demanding

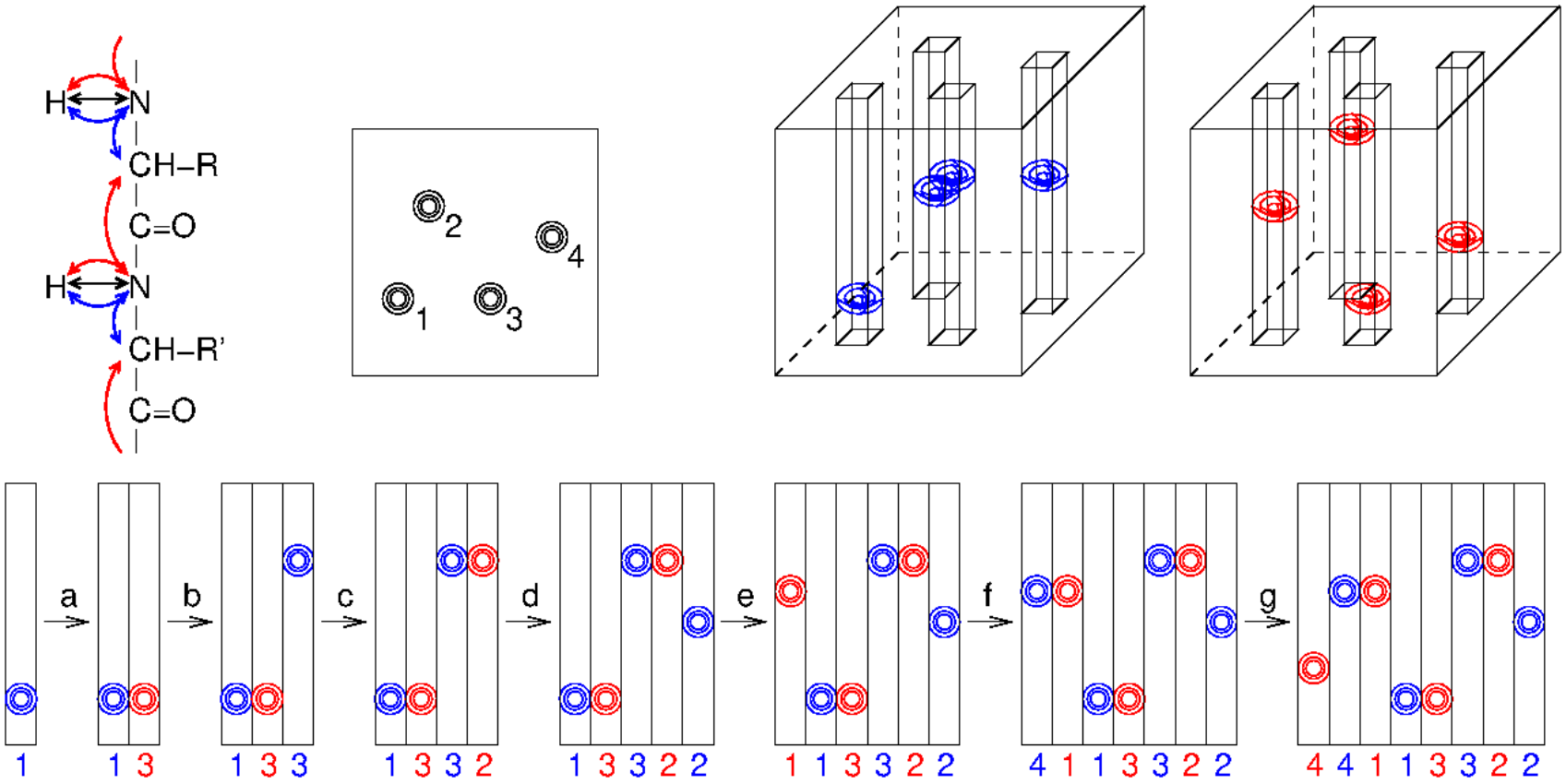
Assignment of spectra



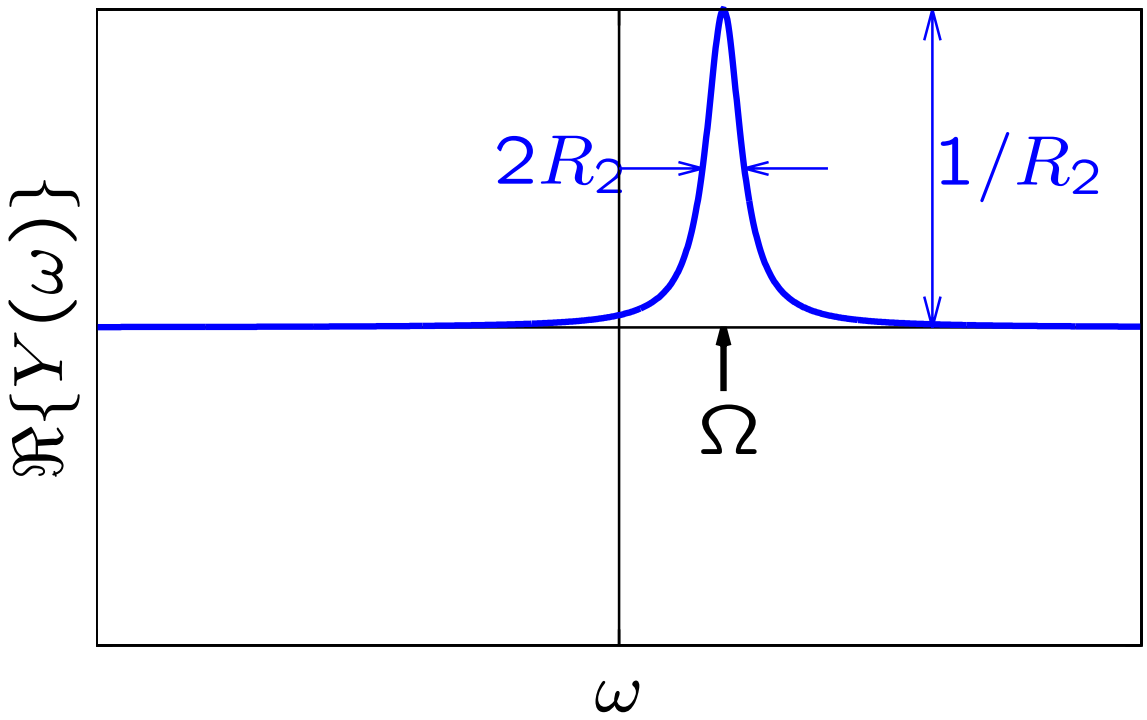
Assignment of spectra



Assignment of spectra

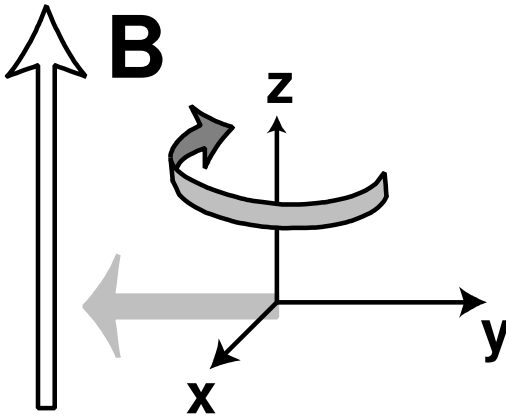


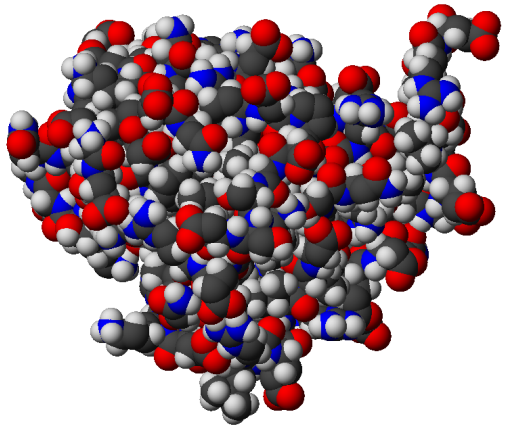
Chemical/biological information in NMR spectrum

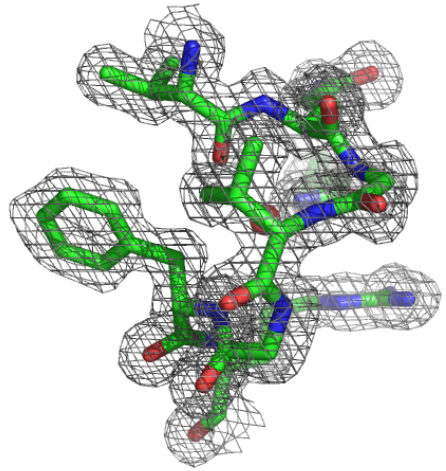


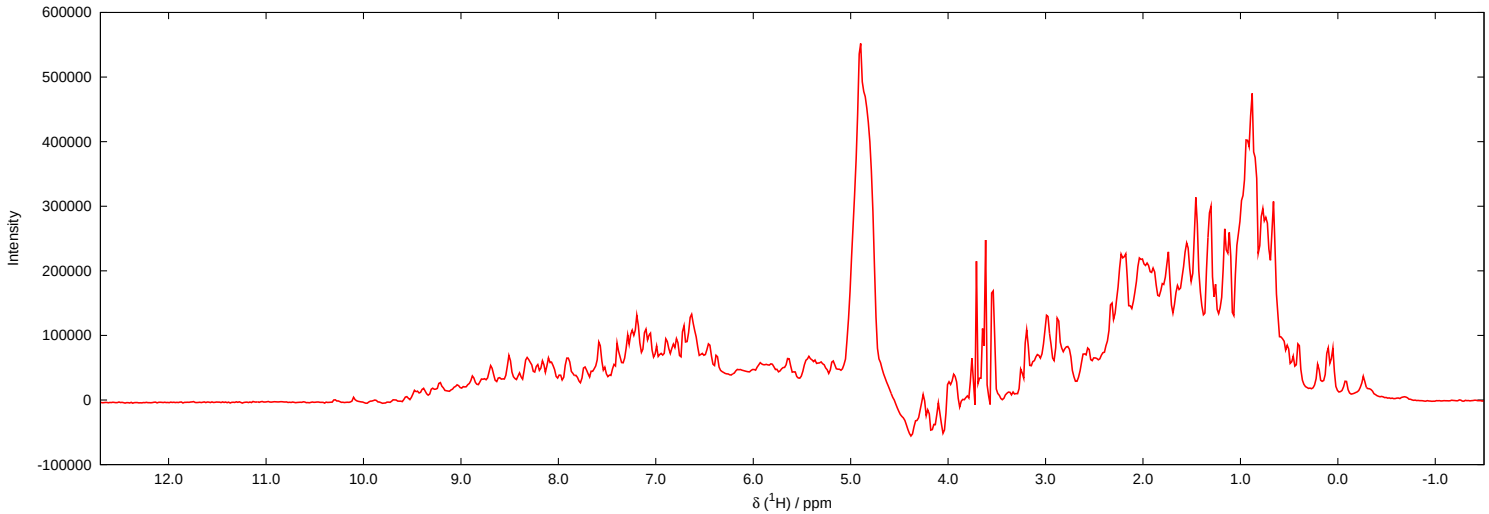
- $\Omega \longrightarrow$ structure
- $R_2 \longrightarrow$ dynamics
- Area $\longrightarrow$ (relative) concentration

Structure from chemical shift

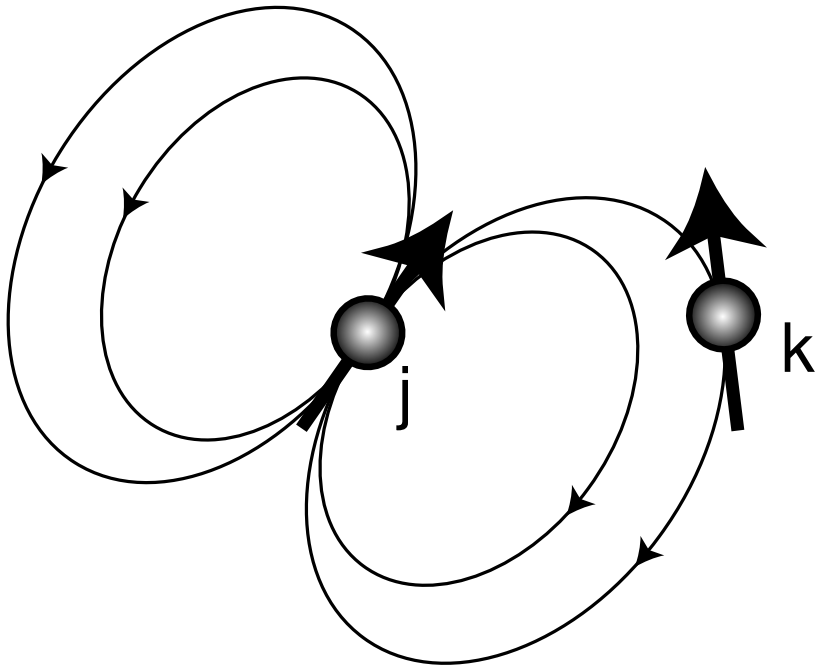








Structure from nuclear Overhauser effect



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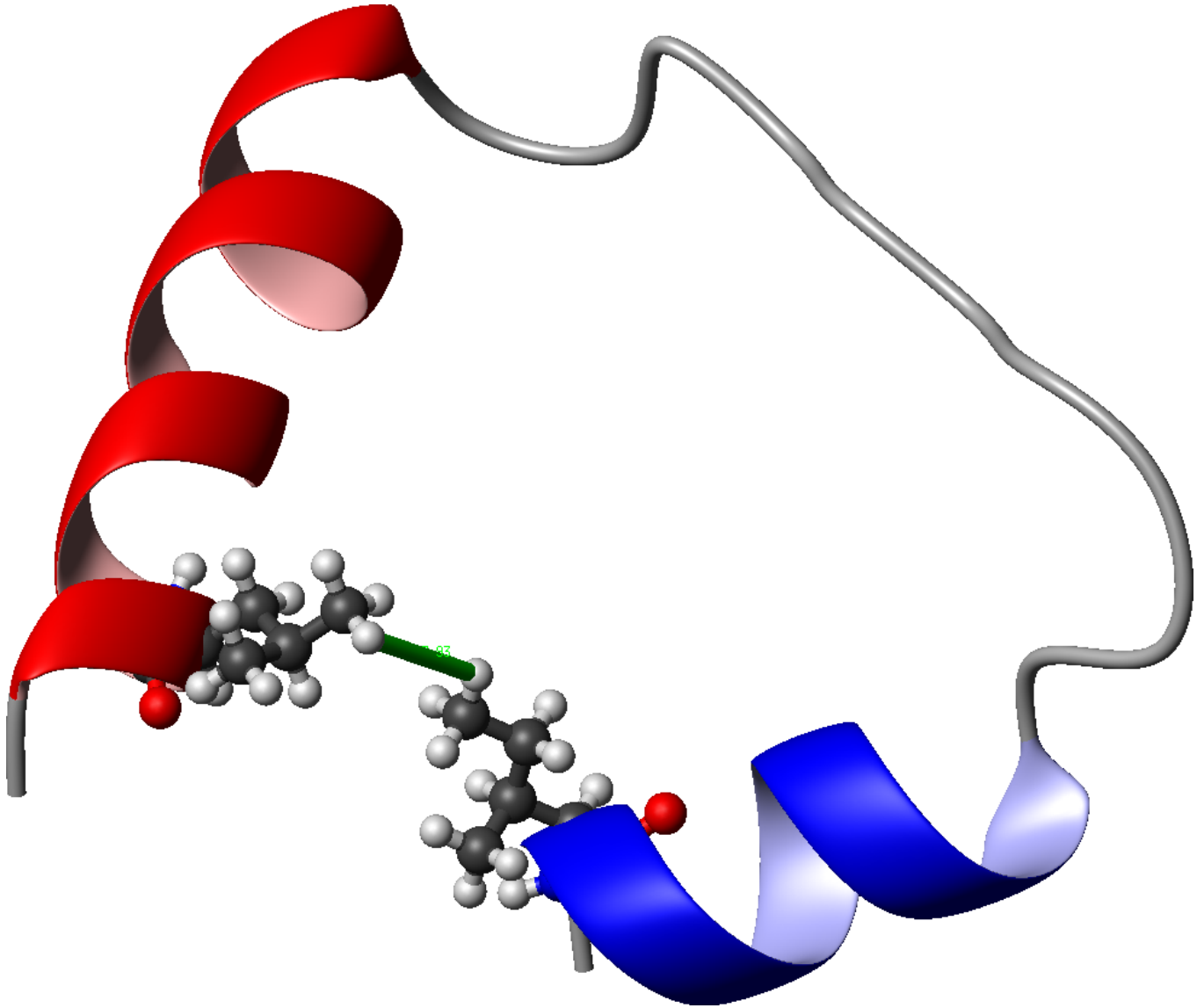

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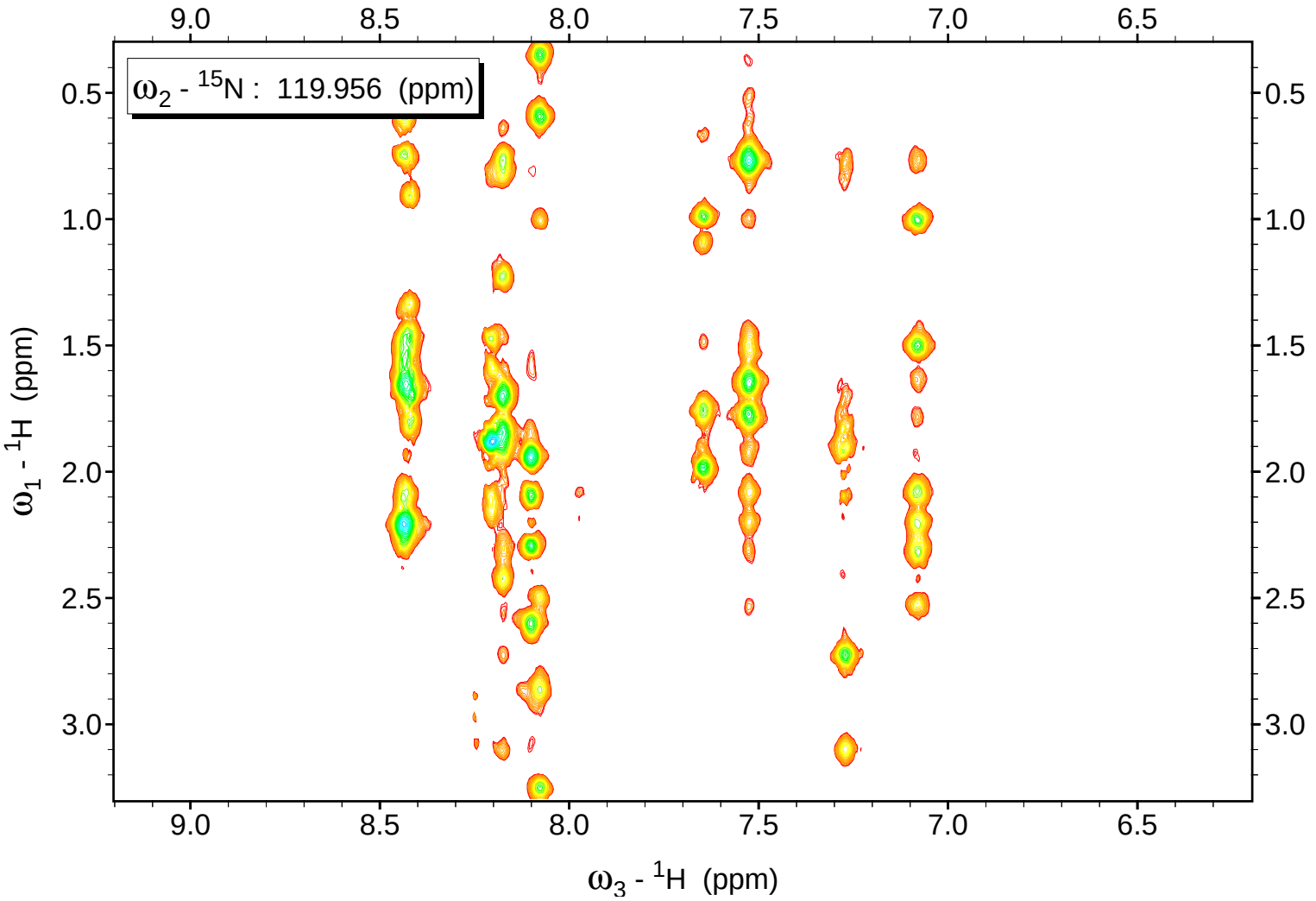


Structure from nuclear Overhauser effect

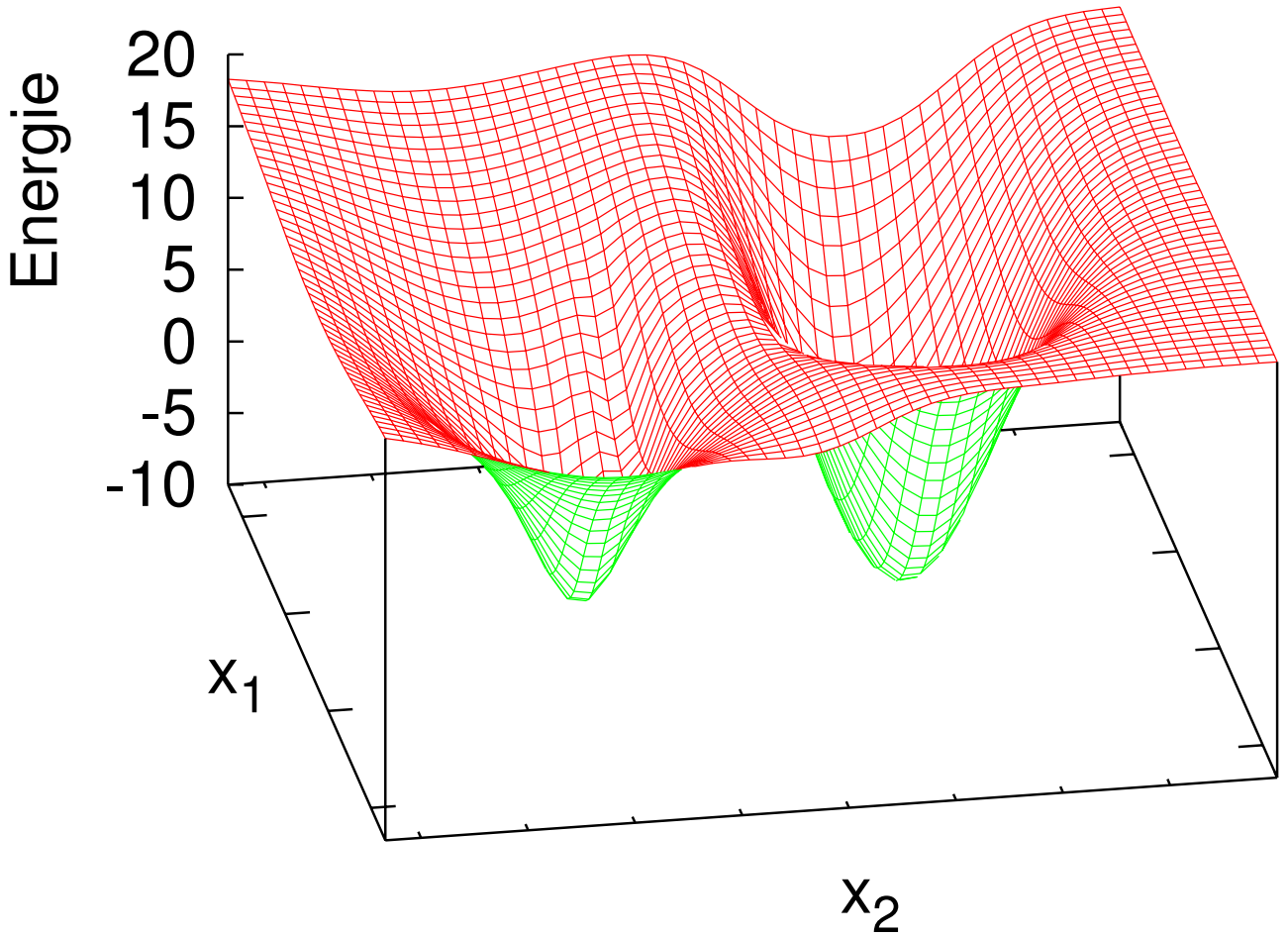
$$\frac{S}{S_{\text{ref}}} = \left(\frac{r_{\text{ref}}}{r}\right)^6 \tag{1}$$

$$r = r_{\text{ref}} \sqrt[6]{\frac{S_{\text{ref}}}{S}} \tag{2}$$

Calibration:

Reference protons		distance
geminal in methylene	H–C–H	0.17 nm
vicinal in an aromatic ring	H–C=C–H	0.25 nm
meta in an aromatic ring	H–C=CH–C–H	0.42 nm

NMR structure calculation



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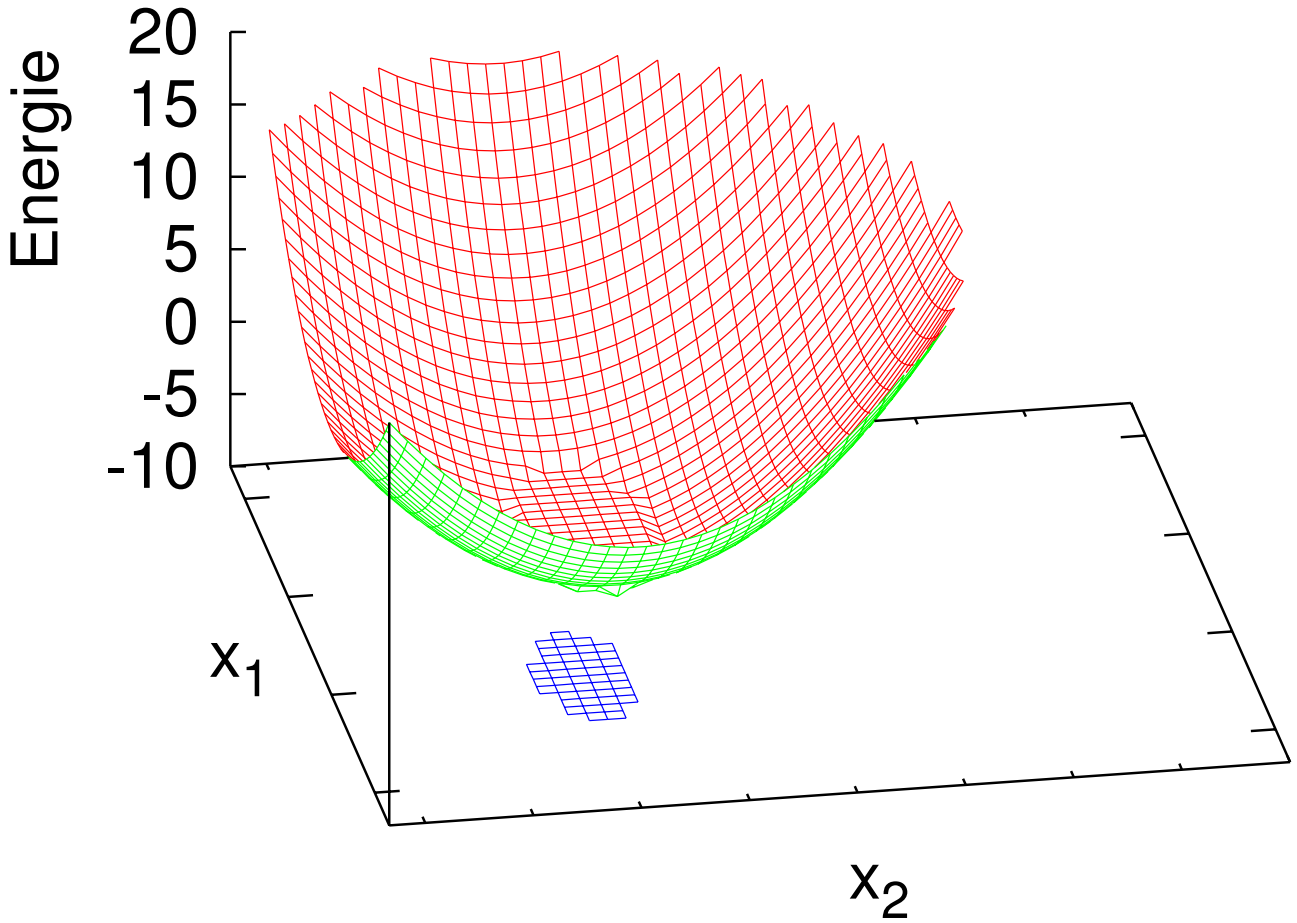
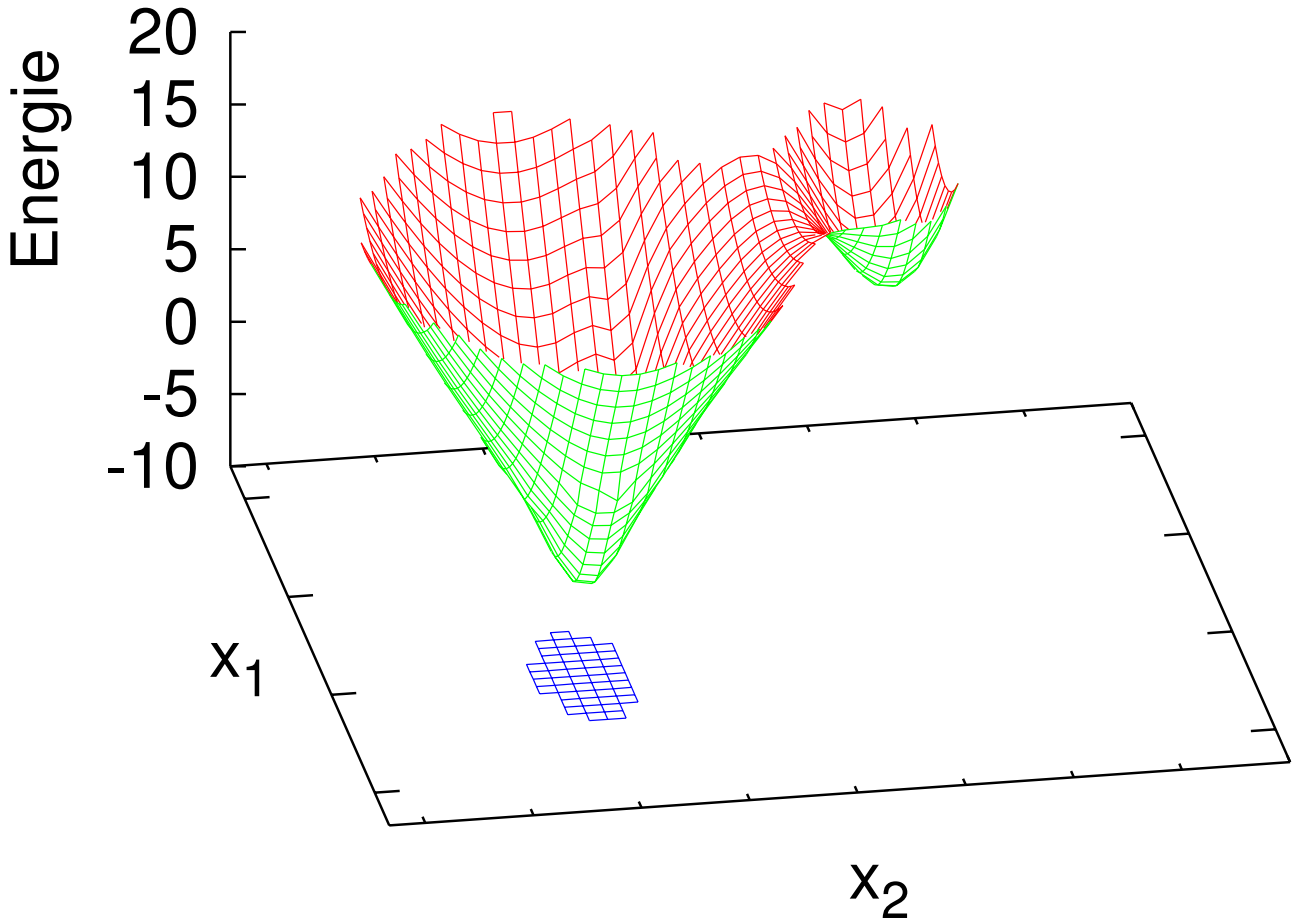


NMR structure calculation



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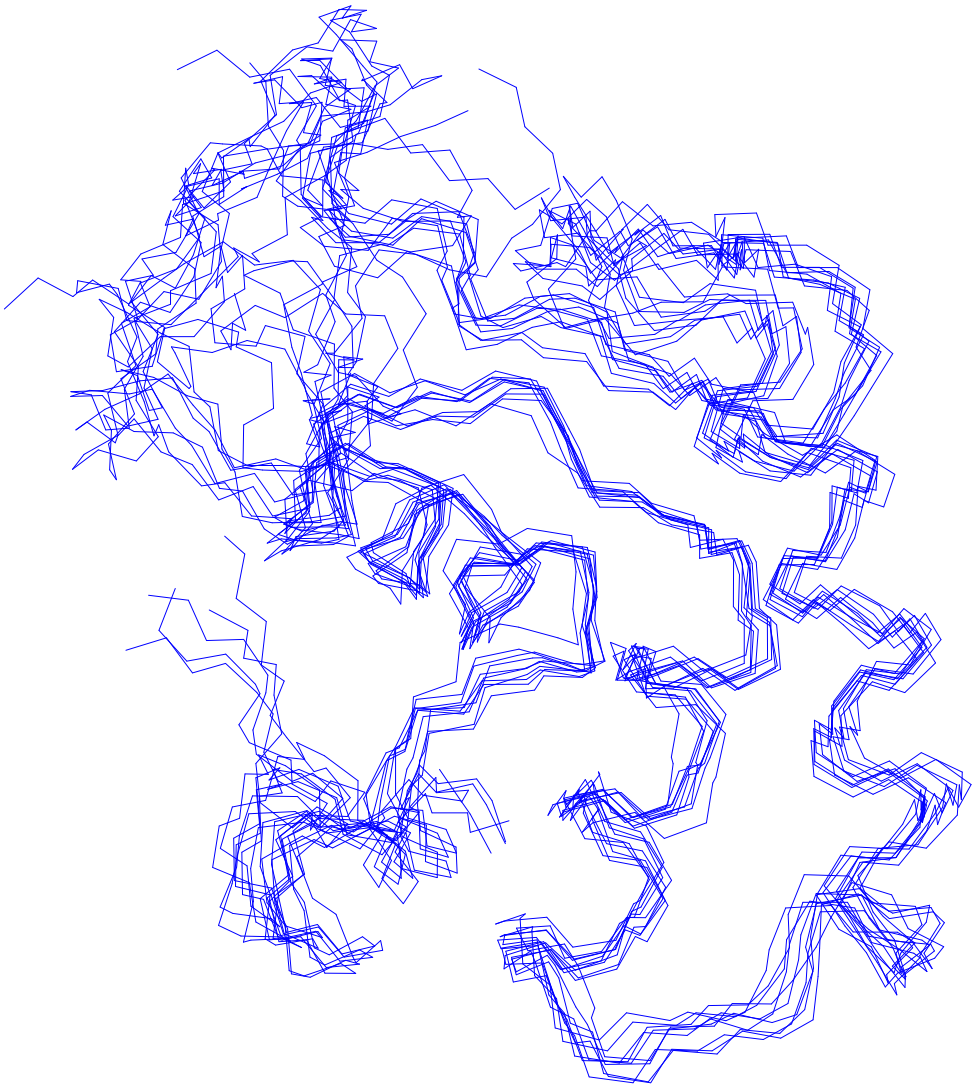

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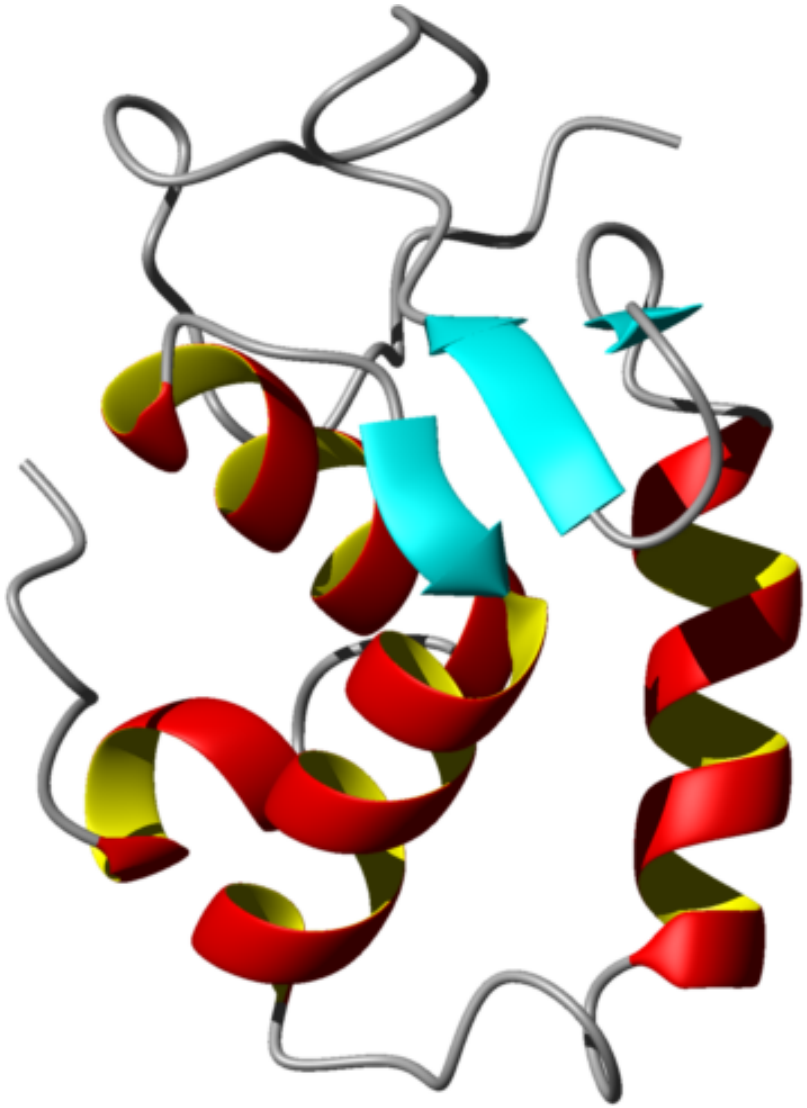
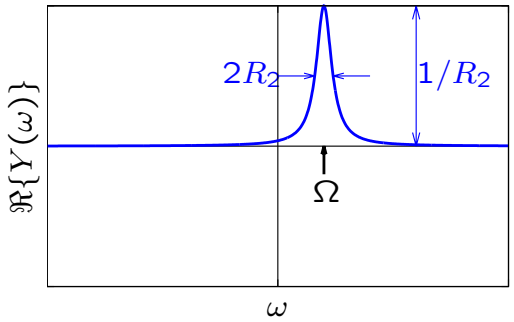
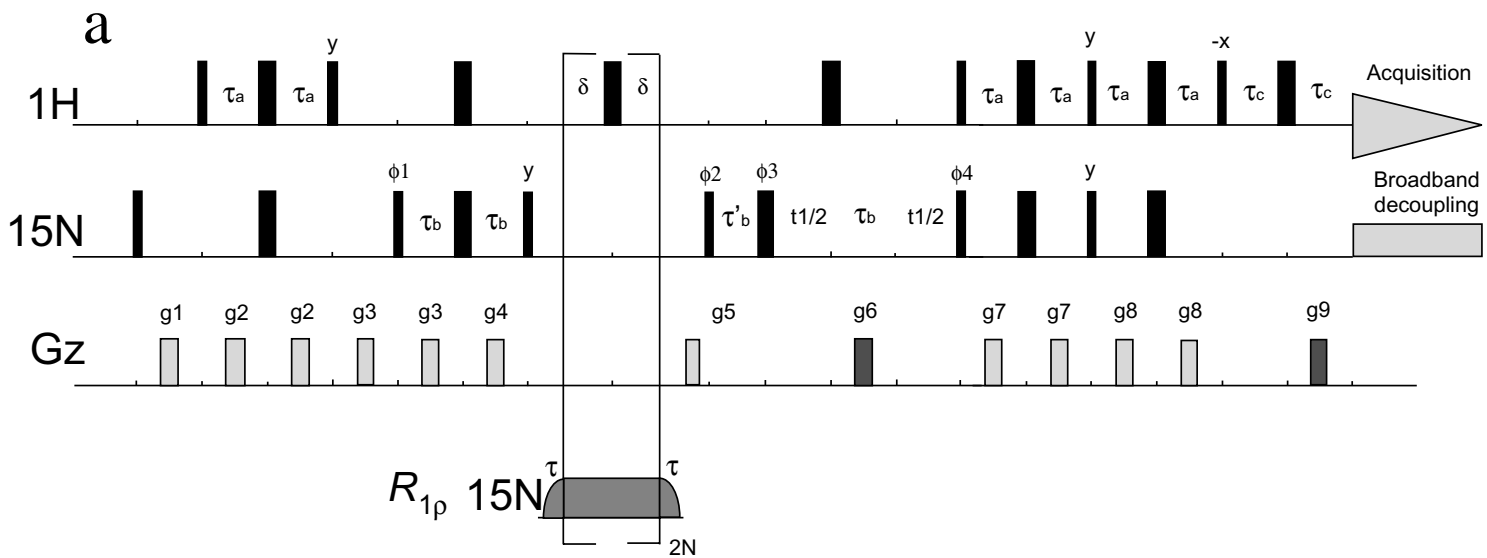
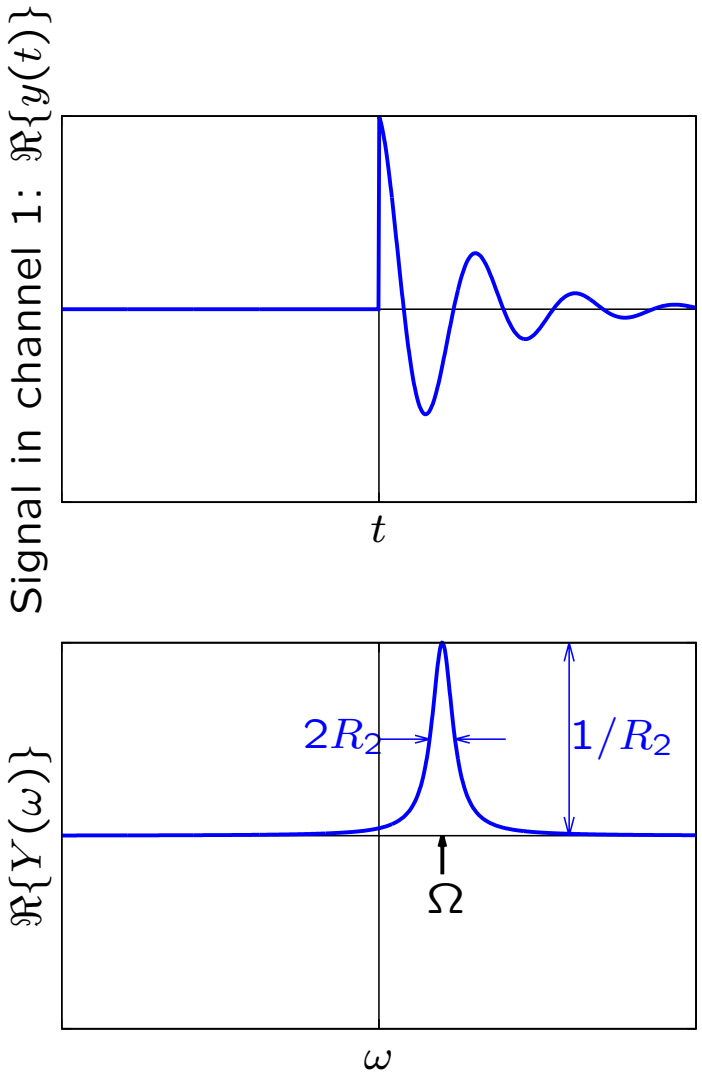


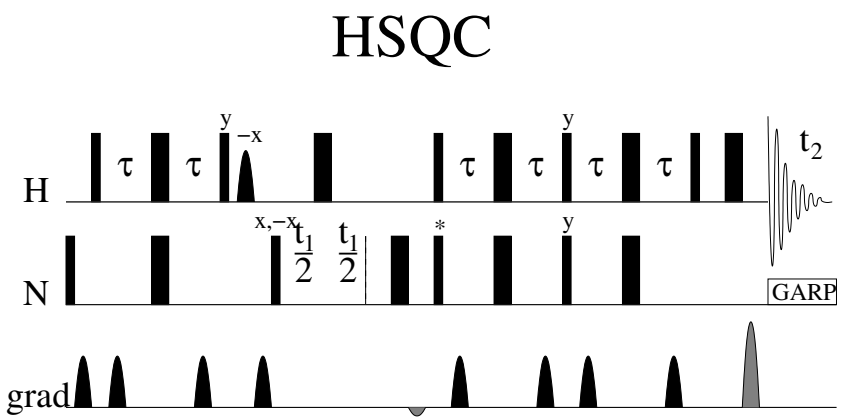
Relaxation rates from special experiments





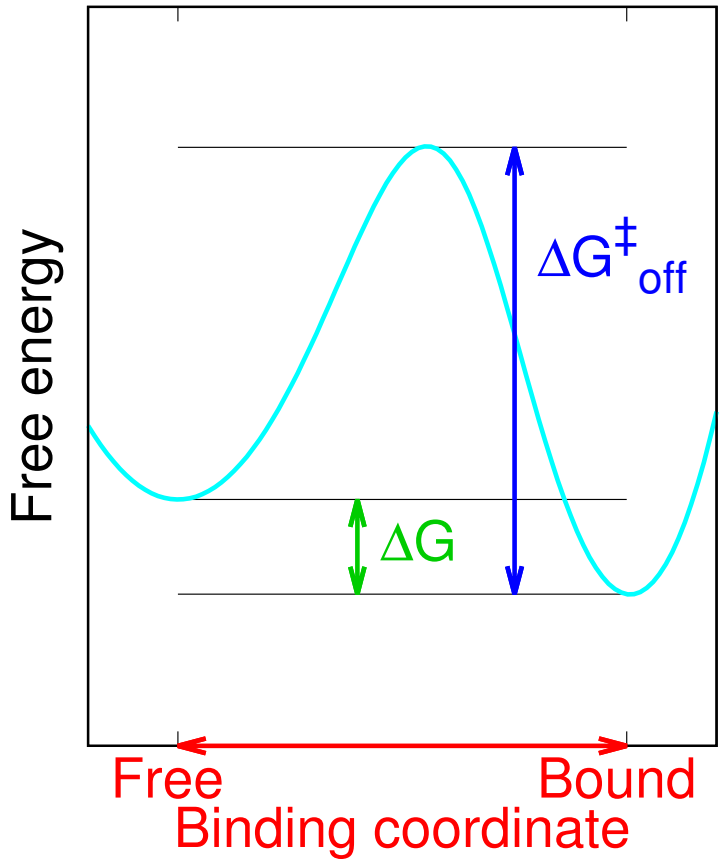
Peak area and relative concentration





Biomolecular interactions

- Does it bind?
- How many molecules?
  Stoichiometry
- In how many steps?
  Mechanism
- Where?
  Structure
- How strongly?
  Affinity
- How fast?
  Kinetics

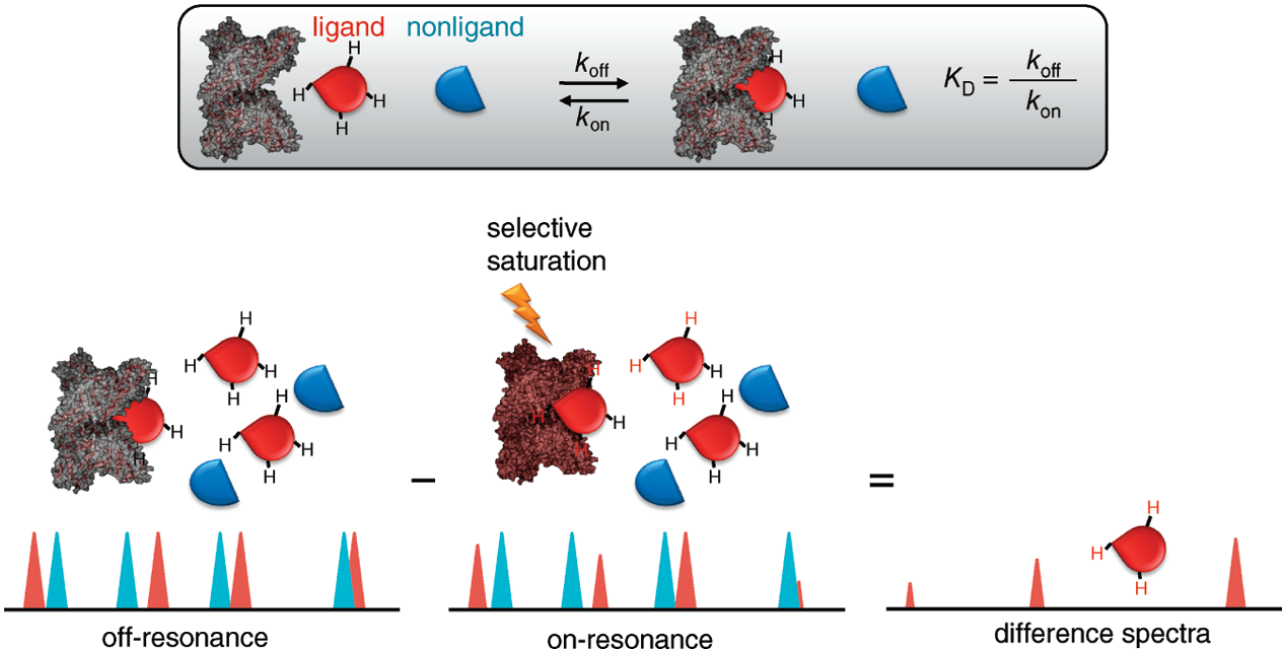


Protein or ligand?

Observe:

- **Ligand**
  saturation transfer difference (STD), transferred NOE
  features of bound-ligand reflected in free-ligand spectra
  not limited by the size of the protein
- **Protein**
  usually more structural details

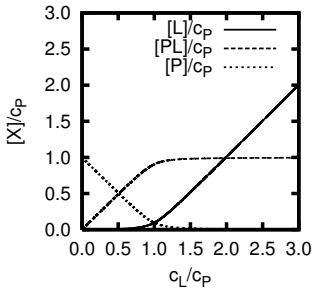
Saturation transfer difference



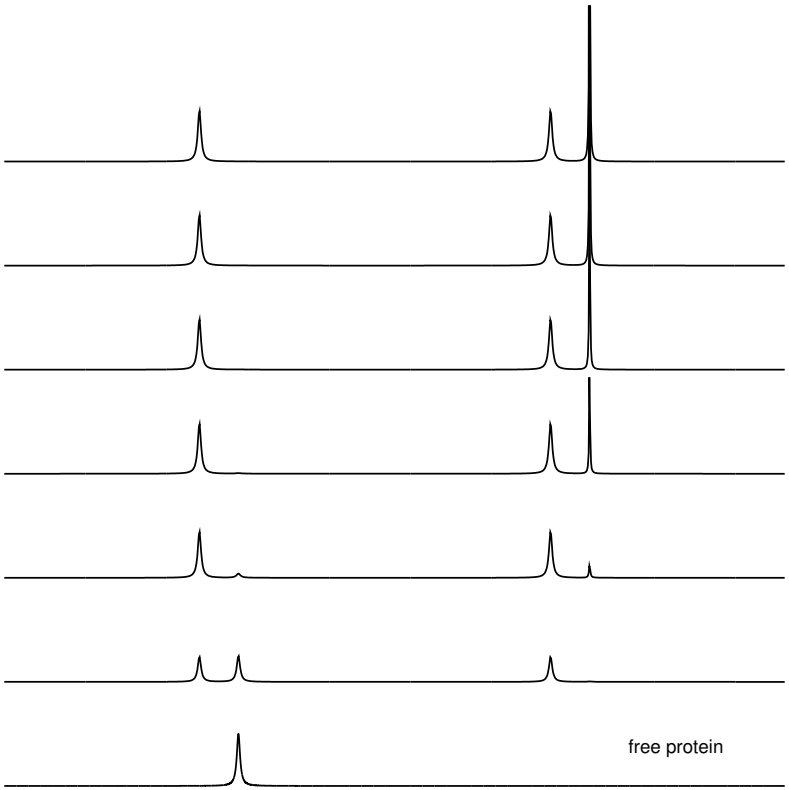
Titration

$k_{off} = 0.2\ s^{-1}$
$K_d = k_{off}/k_{on} = 0.01\ c_P$

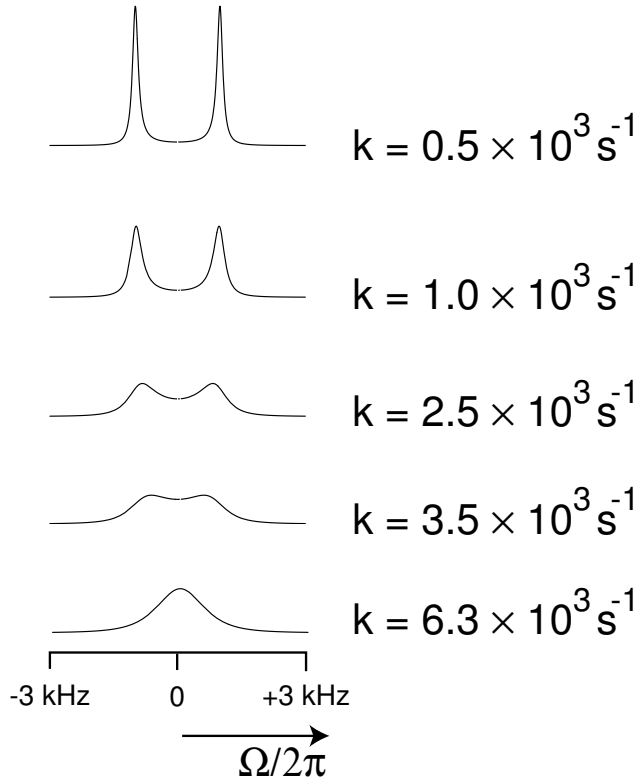
$\Delta\omega$(ligand) = 200 rad s^{-1}
$\Delta\omega$(protein) = 200 rad s^{-1}
R_2(free ligand) = 2 s^{-1}
R_2(bound ligand) = 10 s^{-1}
R_2(free protein) = 10 s^{-1}
R_2(bound protein) = 10 s^{-1}



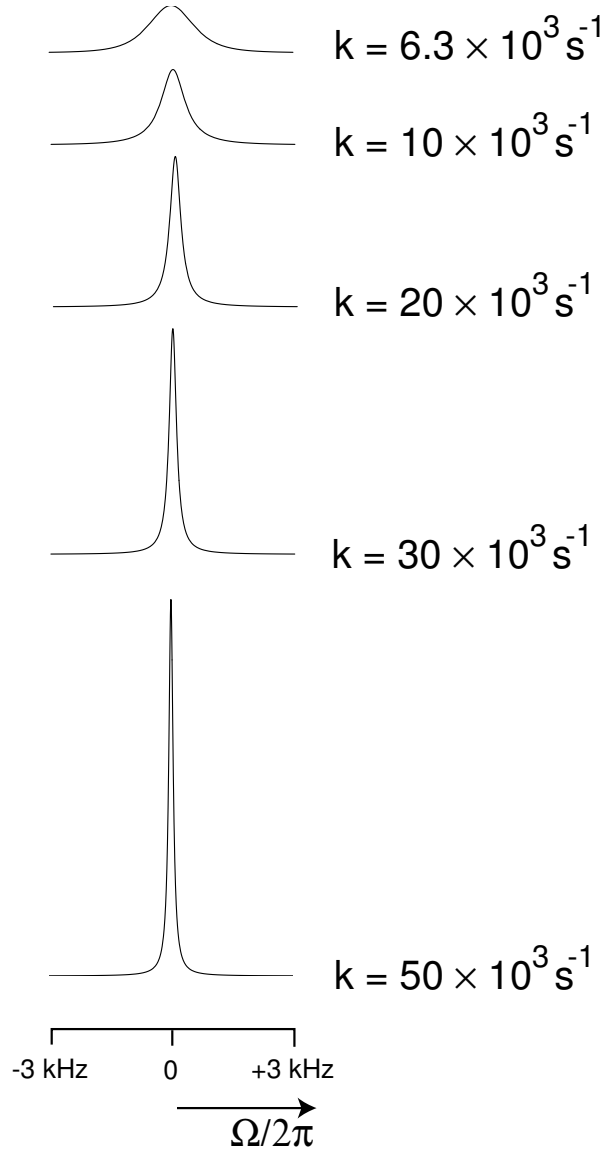
Titration with ligand aliquots of $c_L = 0.5\ c_P$:



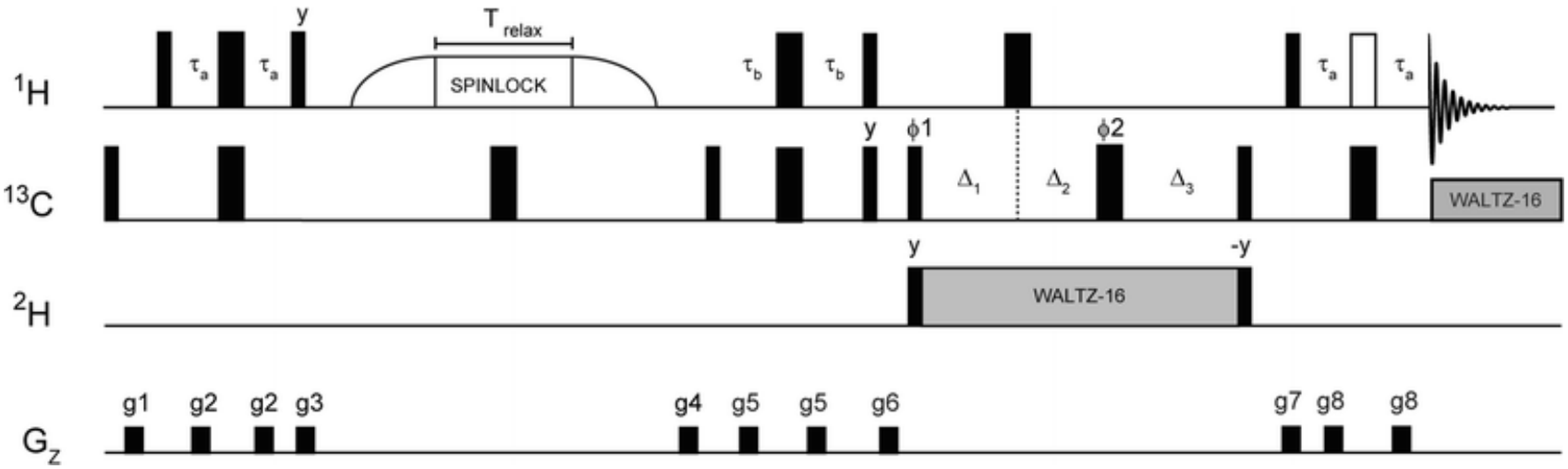
Rate of dissociation



reproduced from M. H. Levitt: Spin Dynamics



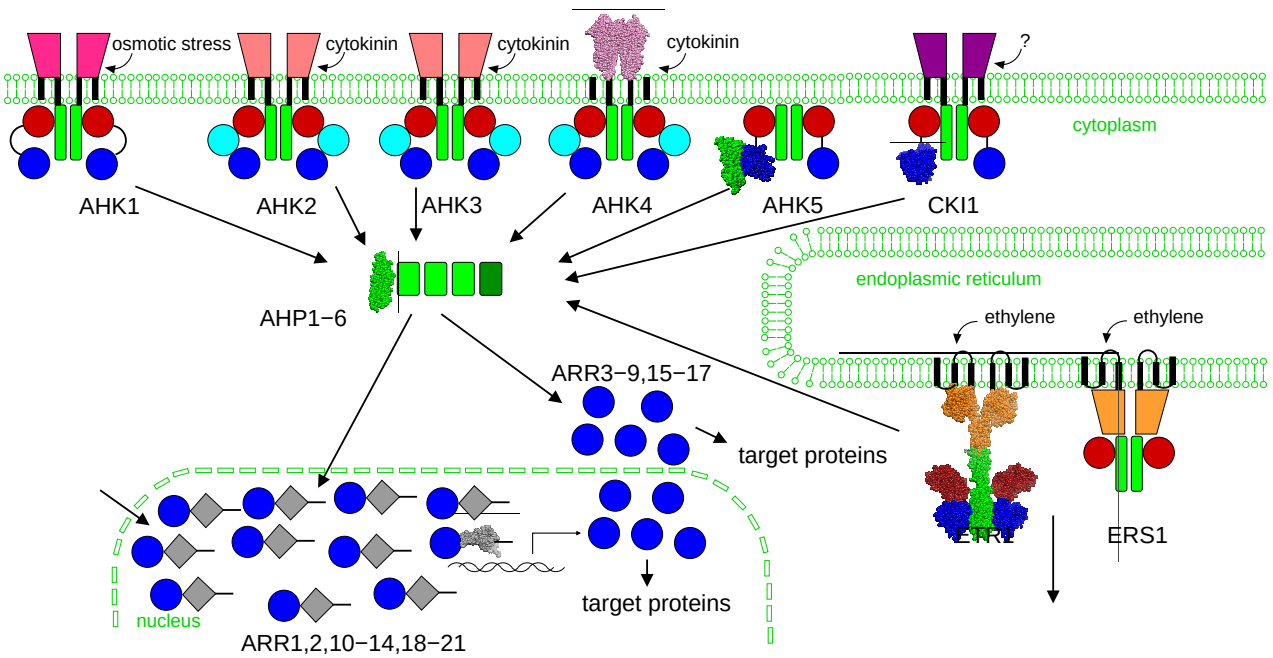
Kitetics from relaxation dispersion experiments



Example 1: fast exchange

Interacting molecules:

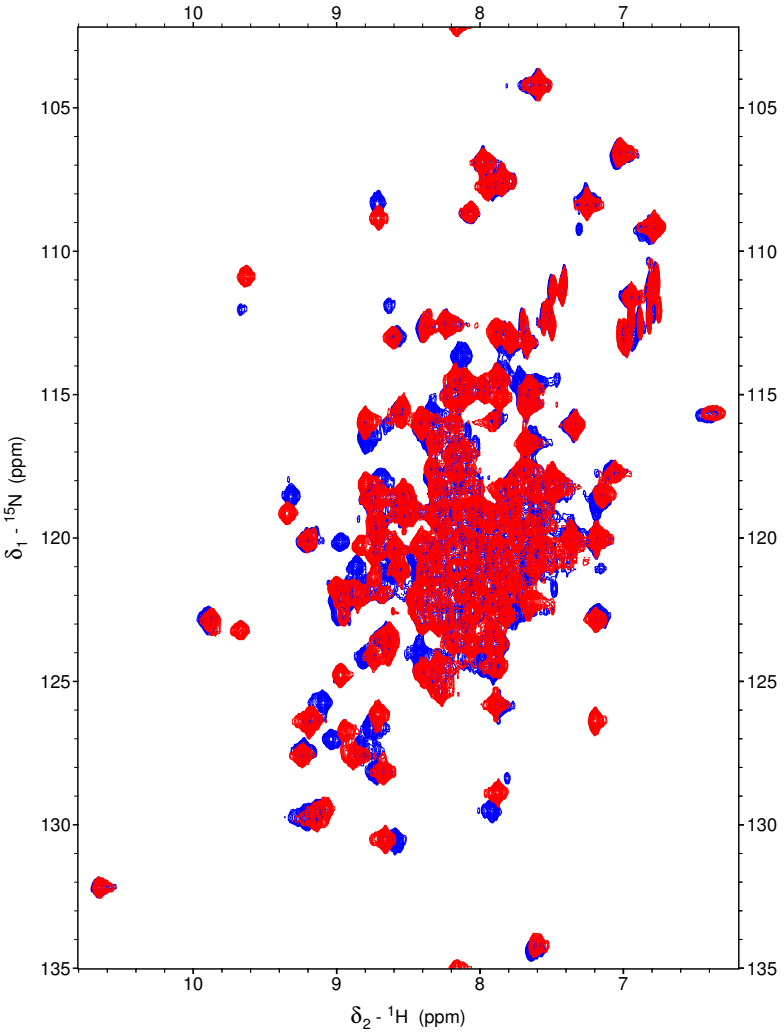
- Receiver domain of plant sensory histidine kinase CKI1 (from *Arabidopsis thaliana*)
- Mg^{2+} ions



Pekarova et al., *Plant J.* **67** (2011) 827

Otrusinova et al., *J. Biol. Chem.* **292** (2018) 17525

Does it bind?

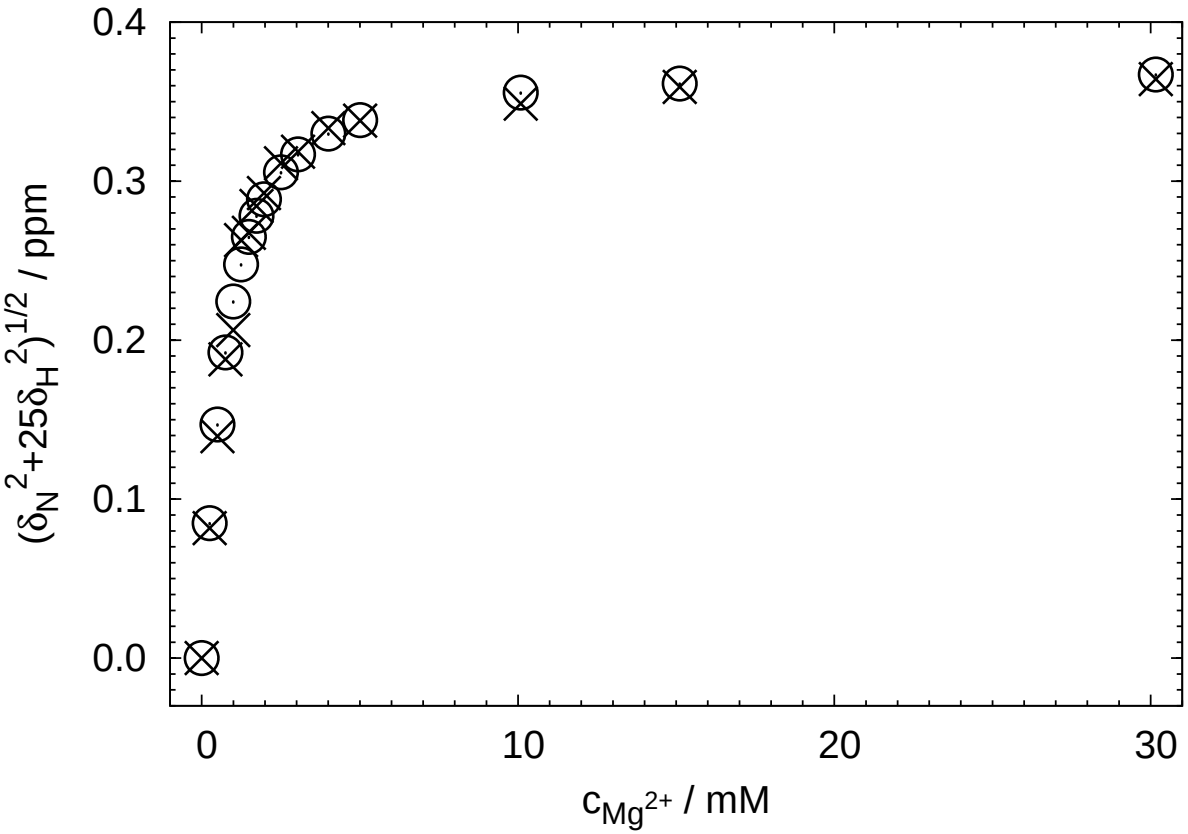


free

Mg^{2+}-bound

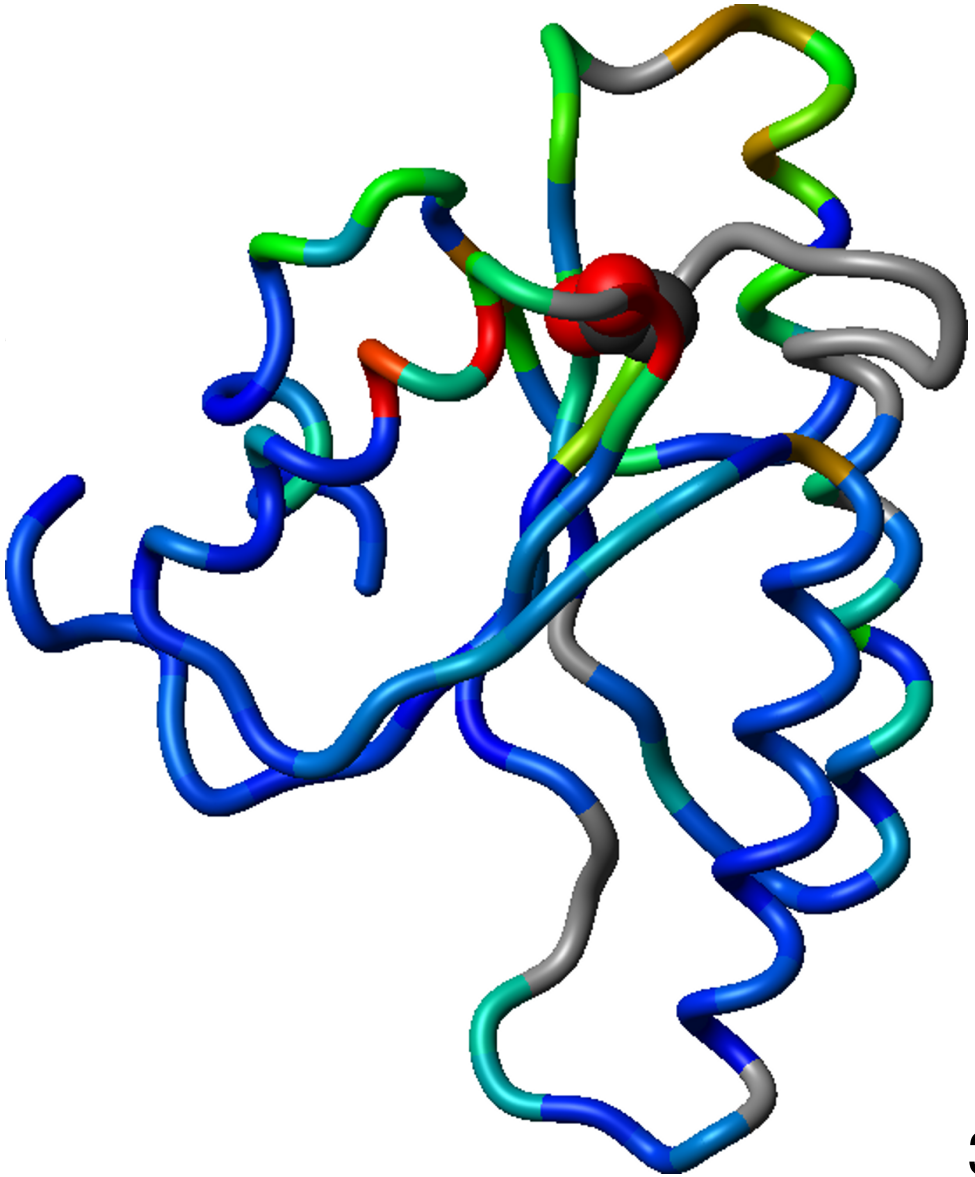
How fast? How strongly?

$$\text{Free} + Mg^{2+} \rightleftharpoons \text{Bound}$$



$K_d = 0.43 \pm 0.06\text{mM}$ (data for Q92)

Where? From chemical shifts

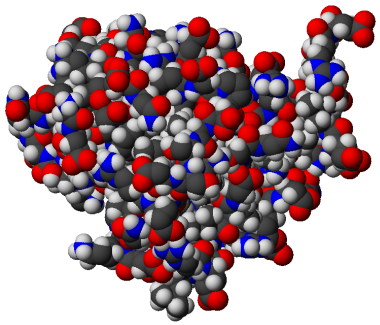


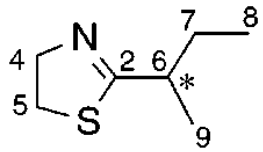
3MM4.pdb

Example 2: slow exchange

Interacting molecules:

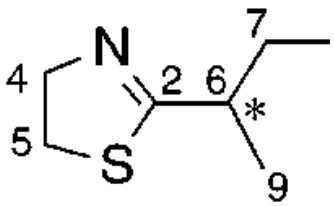
- Mouse major urinary protein I
- male pheromone 2-*sec*-butyl-4,5-dihydrothiazole (estrus synchrony and puberty acceleration in females)

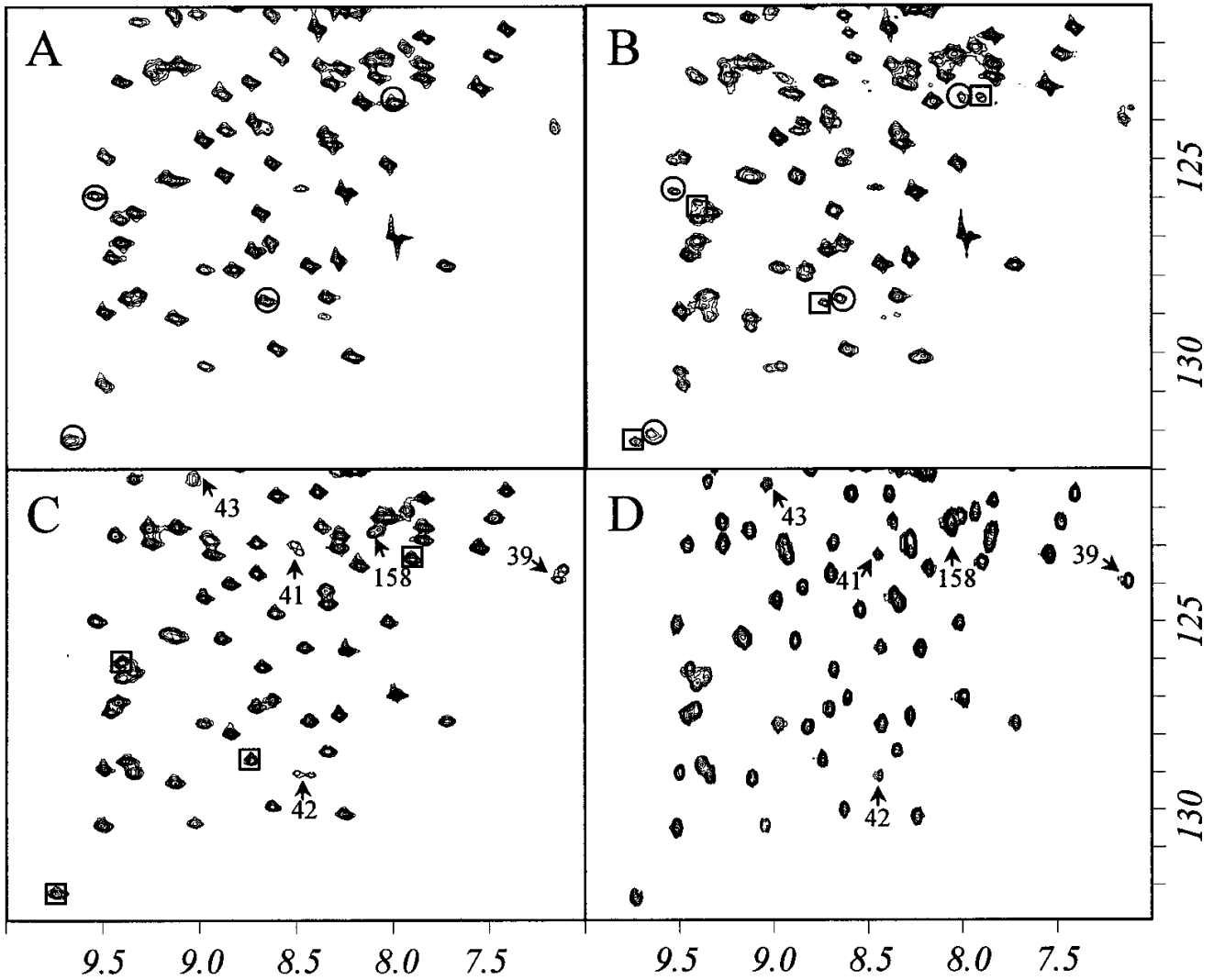




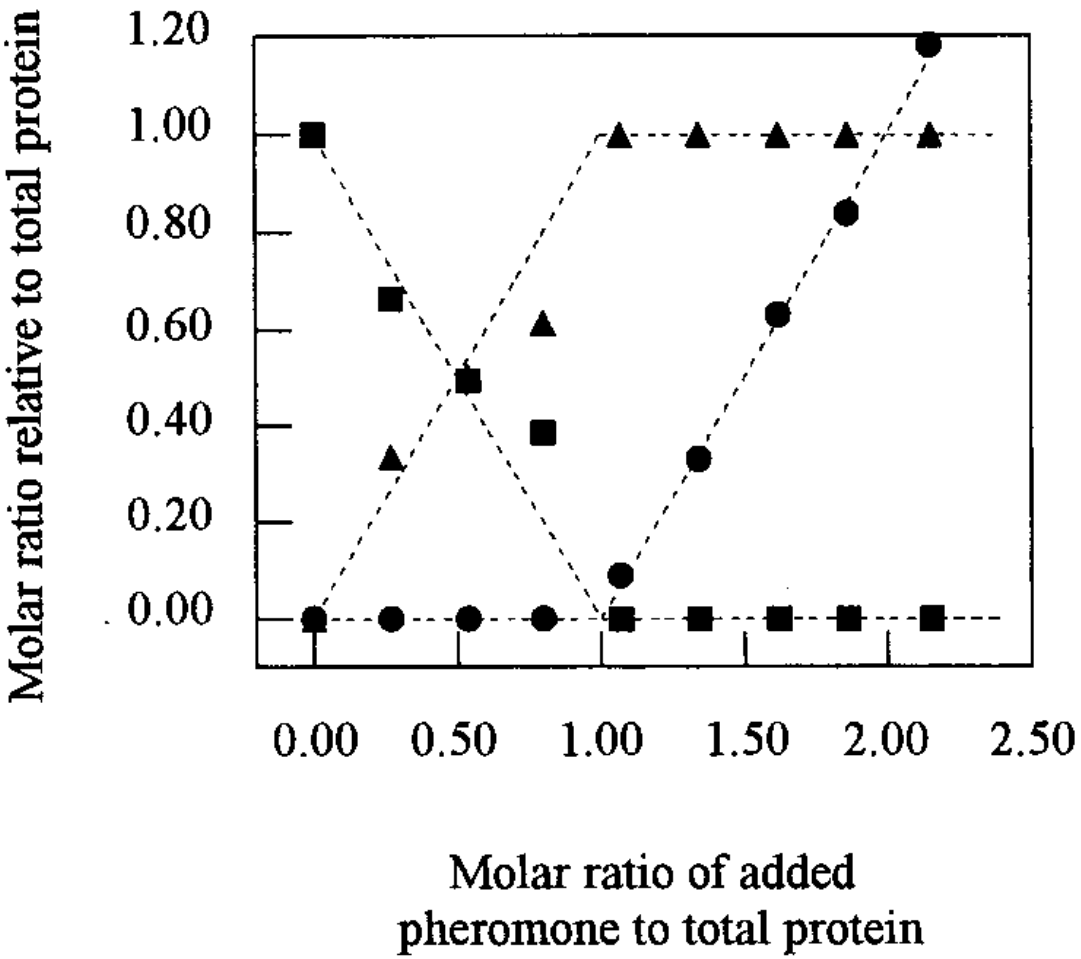
Zidek et al., *Biochemistry* **38** (1999) 9850

Does it bind? How fast?





How much? How strongly?

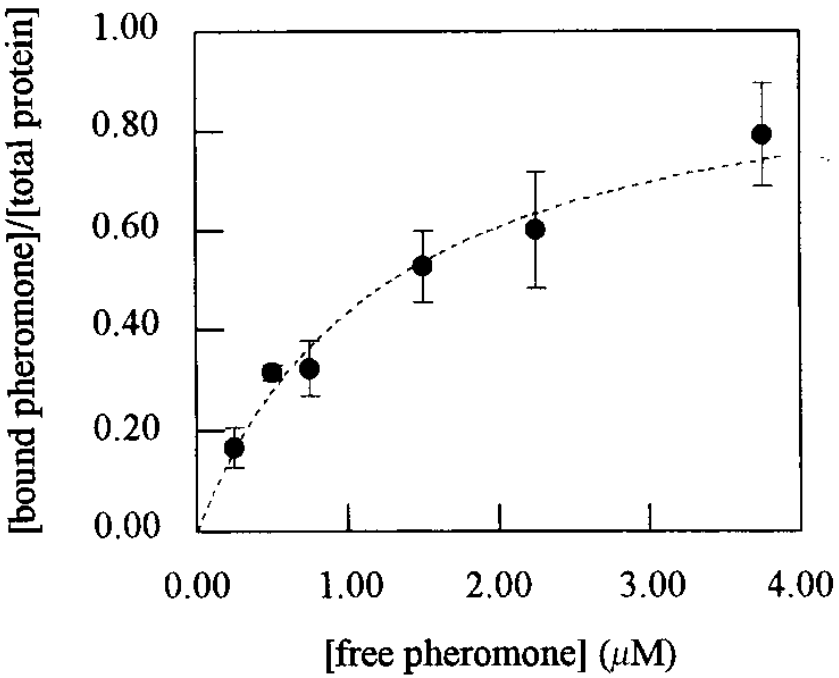


stoichiometry $= 1.0 \pm 0.1 \mu$M

How strongly?

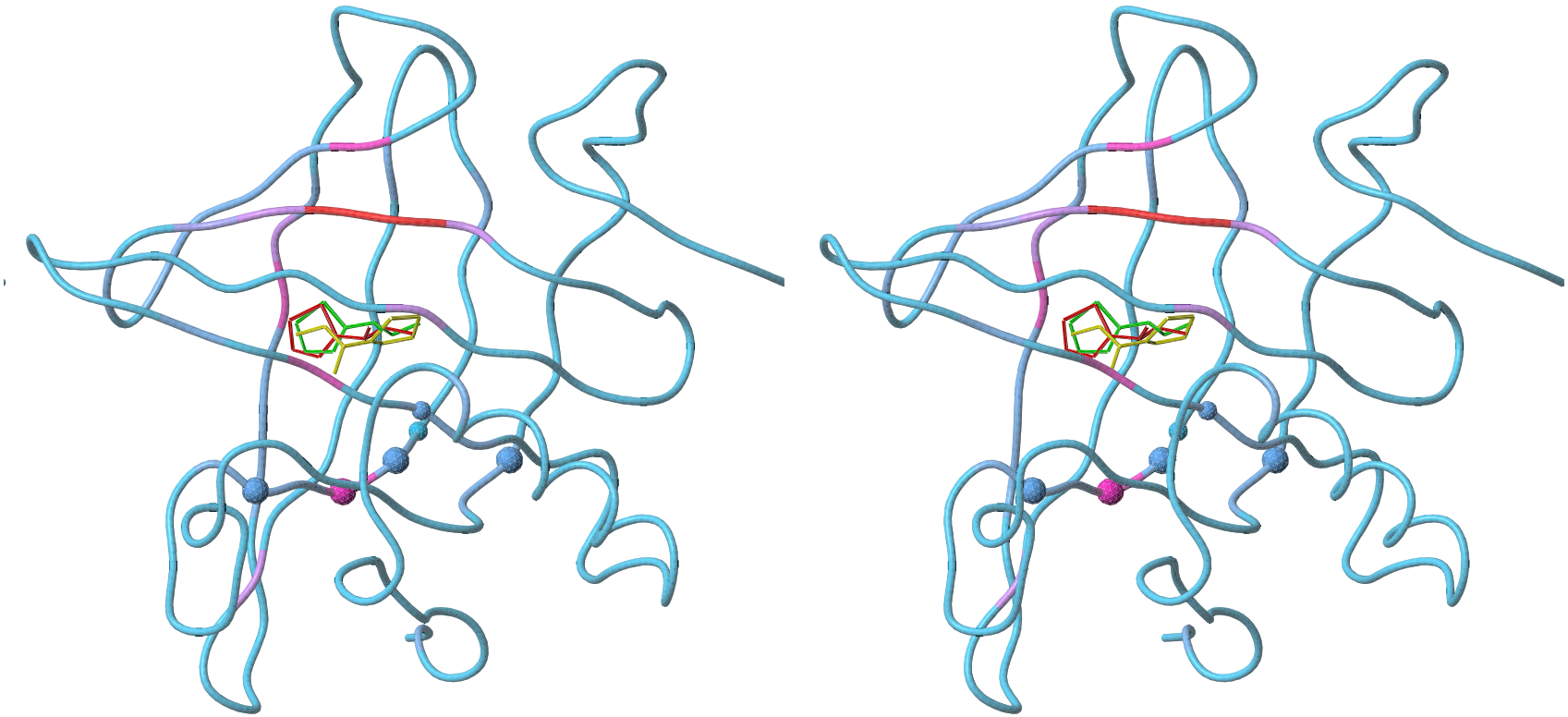
Too strong for NMR

Determined by equilibrium diffusion/gas chromatography



$K_d = 1.3 \pm 0.1 \mu M$

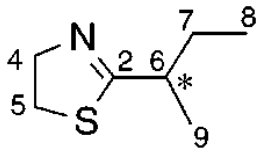
Where? From chemical shifts

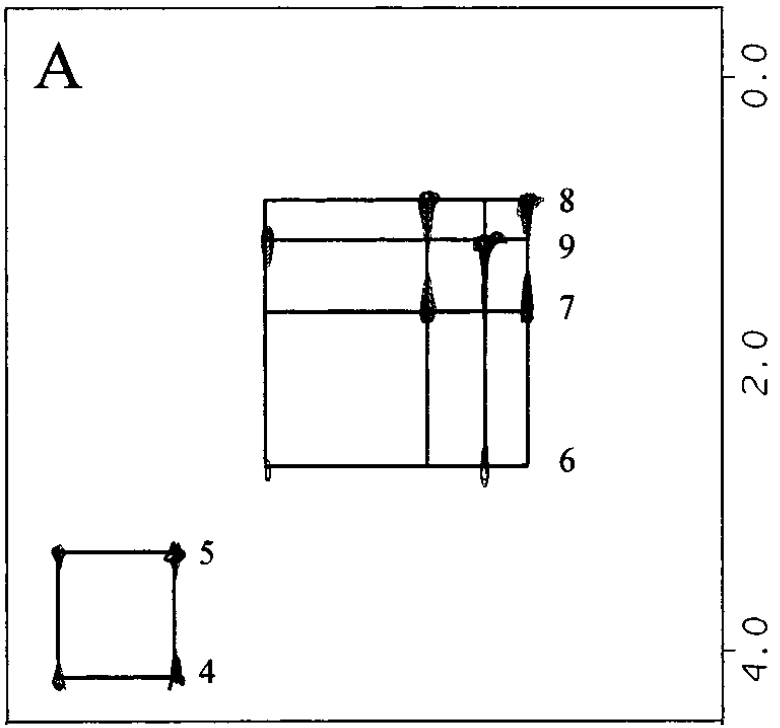


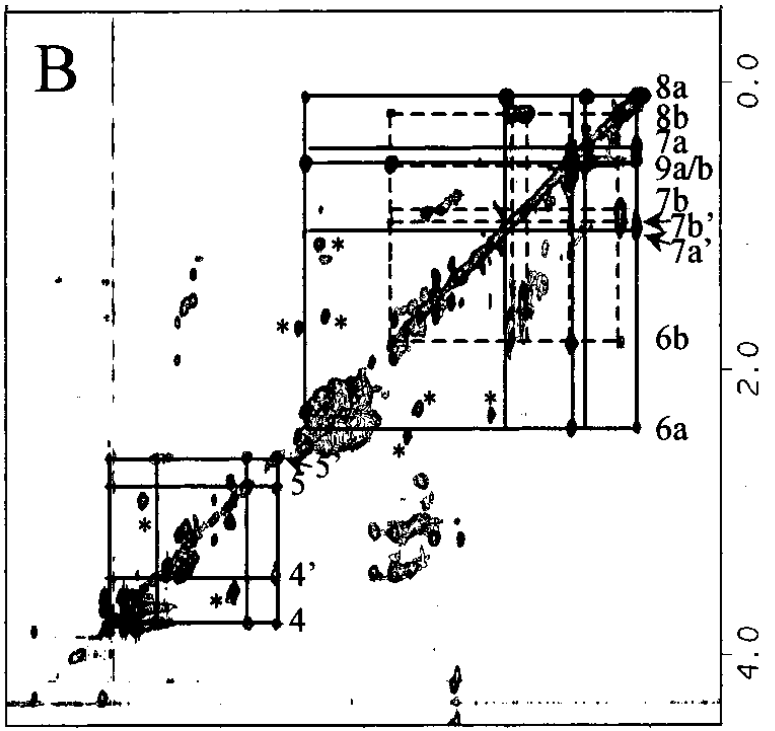
MUP1.pdb

Where? From isotope-filtered NOE

^{12}C-$^{1}H \longleftrightarrow {}^{1}H$-$^{12}C$







Where? From isotope-filtered NOE

^{13}C-$^{1}H \longleftrightarrow {}^{1}H$-$^{12}C$

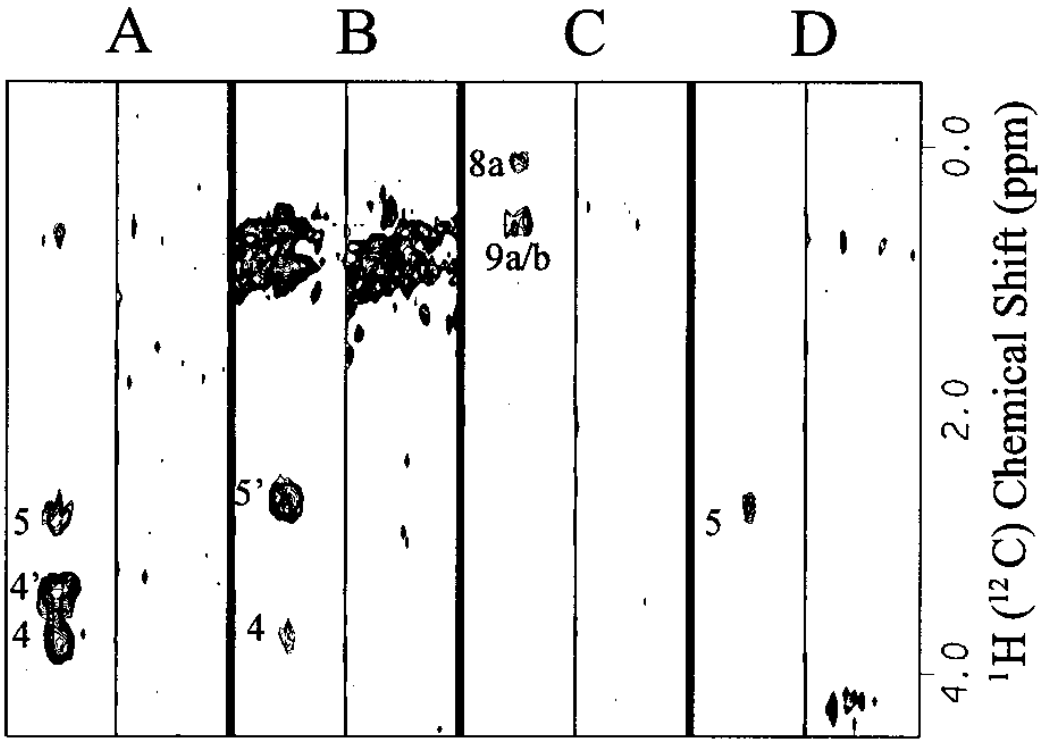


FIGURE 7: Representative strips from ^{13}C F1-filtered, F3-edited NOESY−HSQC spectra of free (right strips) and 2-*sec*-butyl-4,5-dihydrothiazole-complexed (left strips) rMUP-I. The strips were taken from the 3D spectra at chemical shift values corresponding to (A) Leu 40δ_1, (B) Leu 105δ_1, (C) Tyr 120ϵ_2, and (D) Tyr 84δ_2. The NOE cross-peaks are labeled with the corresponding ligand proton numbers.

Where? From isotope-filtered NOE

Table 3: Intermolecular NOEs between 2-*sec*-Butyl-4,5-dihydrothiazole and MUP-I[a]

	ligand									
	sec-butyl chain protons[b]						dihydrothiazole ring protons[b]			
protein	9a/b	8a	8b	7a	7b	7a′/7b′	5	5′	4	4′
Leu 42δ_1	w[c]	x	x	m	x	m[d]				
Leu 42δ_2	m	s				m				
Ala 103β	vs[d]	s[d]	w[c]	w[c]	x	x				
Leu 54δ_1	x	w[c]	s[d]	x	m[c]	m				
Leu 54δ_2	x	w[c]	s[d]	x	x	x				
Tyr 120ϵ_2	m	m								
Phe 90δ_2	m			w[c]						
Phe 90ϵ_2						m				
Phe 56ϵ_2	w				m[d]	m				
Phe 56ζ	m			w[c]		m	m[d]		m[d]	
Leu 105δ_1	x			x	x	x	w	m	m	w
Leu 40δ_1	x			x			m	m	s	s
Val 82γ_1	x	x	x	x	x	x	m	m		
Met 69ϵ	x	x	x	x	x	x	s	w	w	m
Tyr 84δ_2							m	w[c]		

[a] Strength of the NOEs is expressed in a semiquantitative manner (vs, very strong; s, strong; m, medium; w, weak; and x, obscured by background). [b] The symbols a and b in the proton labels refer to individual *sec*-butyl spin systems and diastereotopic protons are distinguished with a prime as indicated in Figure 6B. [c] Possible weak signal obscured by a close intense NOE peak. [d] Medium or intense peaks close to an area of high background.

Where? From isotope-filtered NOE