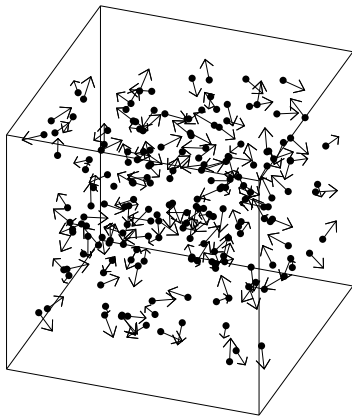


Nuclear Magnetic Resonance

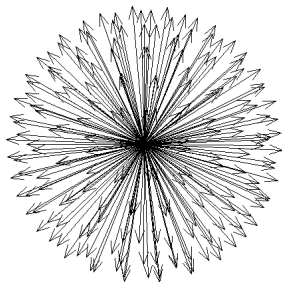
Lukáš Žídek

Principles of NMR

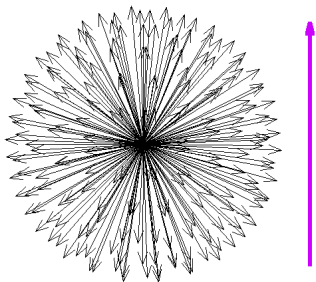
Molecular and magnetic interactions



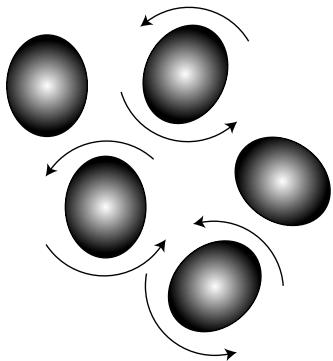
NMR sample outside magnet



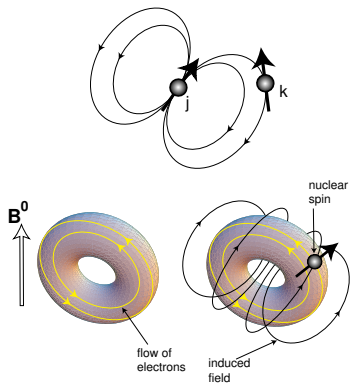
NMR sample inside magnet



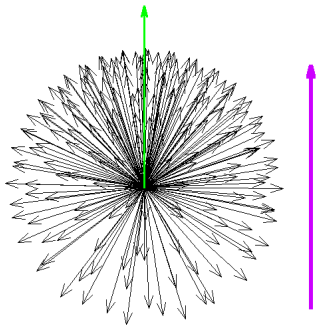
Relaxation via coupling with molecular rotation

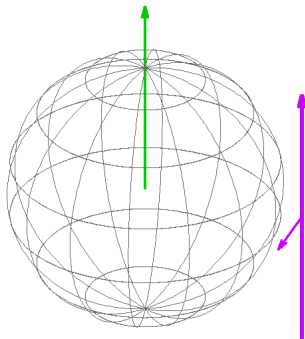


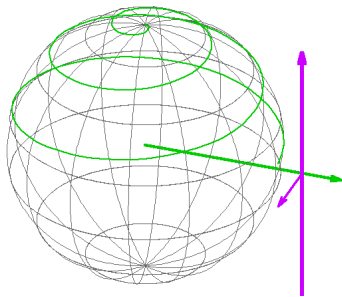
reproduced from M. H. Levitt: Spin Dynamics



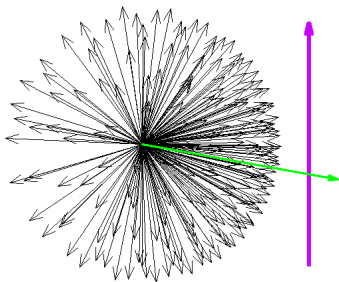
Polarization



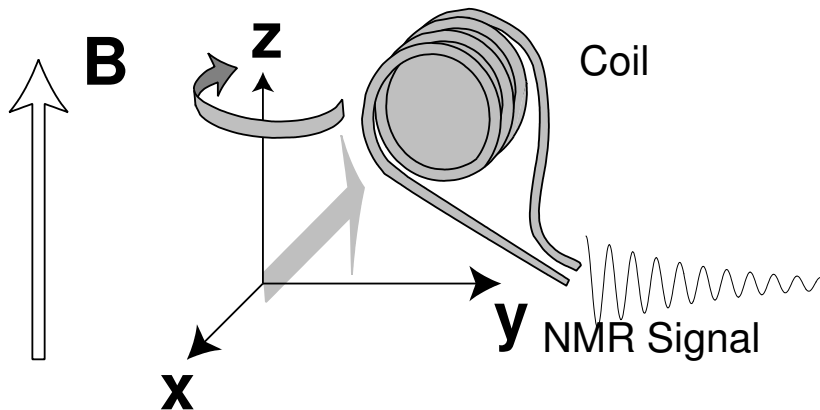




Coherent evolution

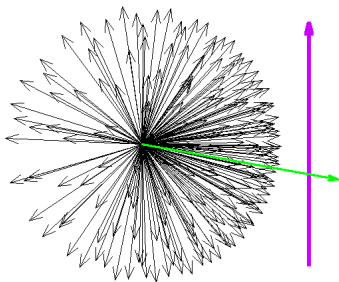


Signal detection

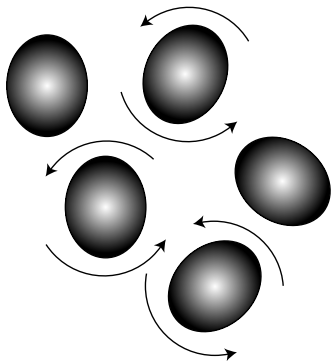


reproduced from M. H. Levitt: Spin Dynamics

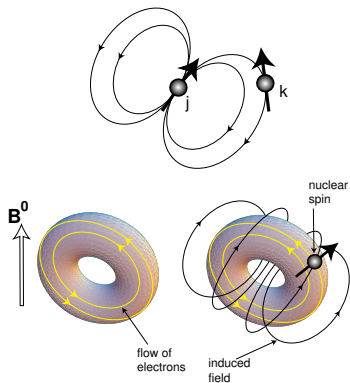
Non-equilibrium distribution of magnetic moments

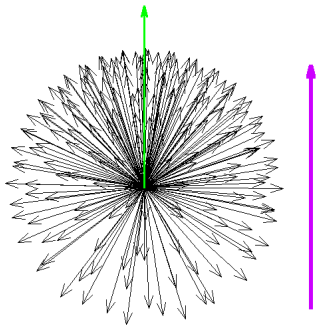


Relaxation via coupling with molecular rotation

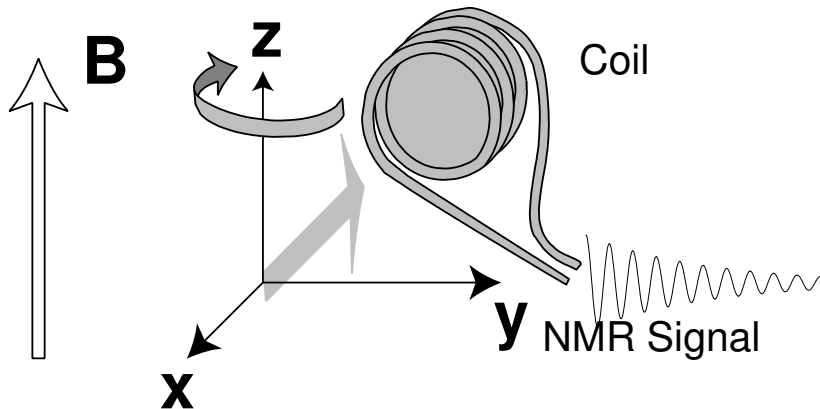


reproduced from M. H. Levitt: Spin Dynamics



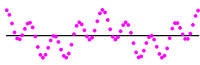
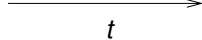
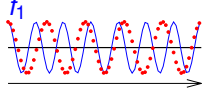
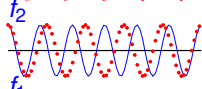
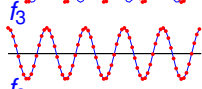
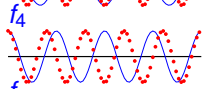
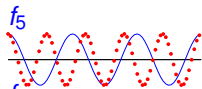
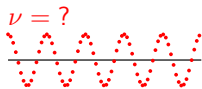


Signal decay

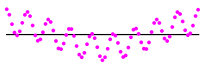


reproduced from M. H. Levitt: Spin Dynamics

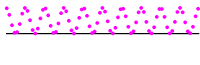
Fourier transformation



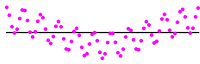
$$\Sigma = 0$$



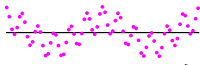
$$\Sigma = 0$$



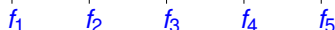
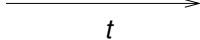
$$\Sigma = 50$$



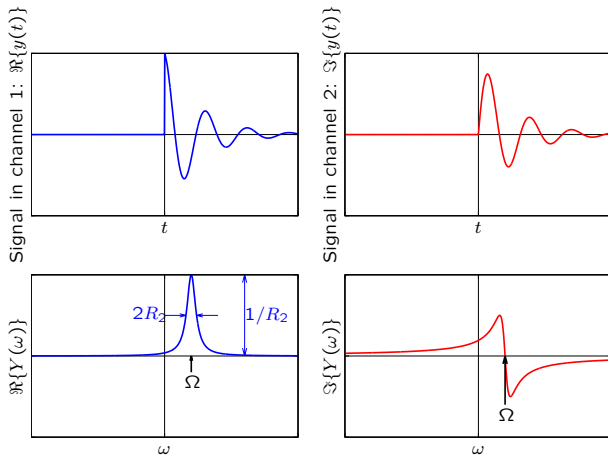
$$\Sigma = 0$$



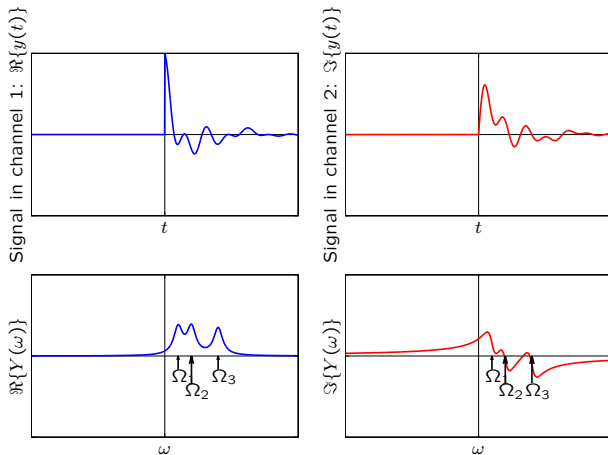
$$\Sigma = 0$$



$\nu = f_3$

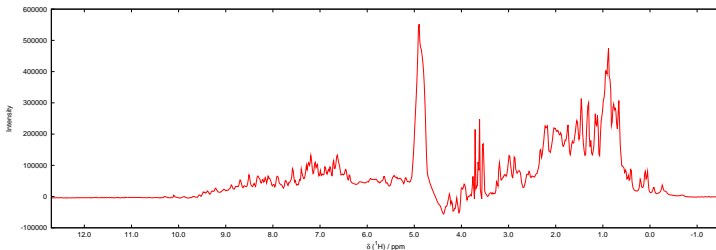
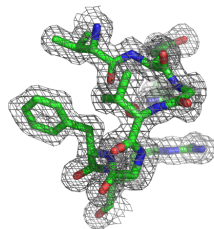
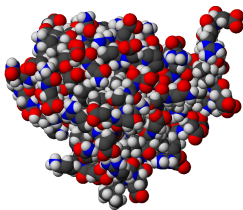
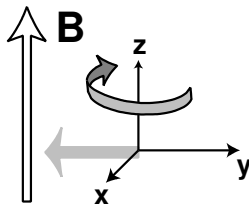


Fourier transformation of ideal signal.

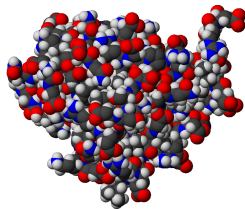
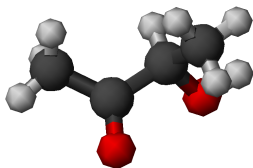


Three Larmor frequencies.

Chemical shift (= NMR frequency) and conformation

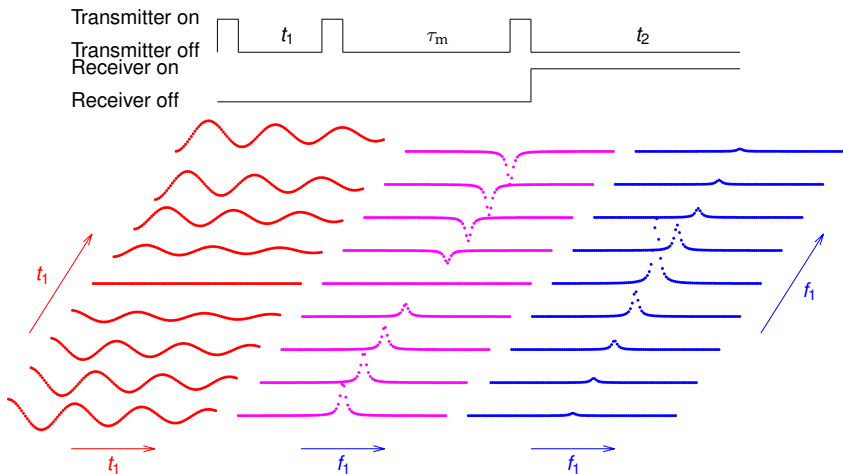


More advanced NMR experiments

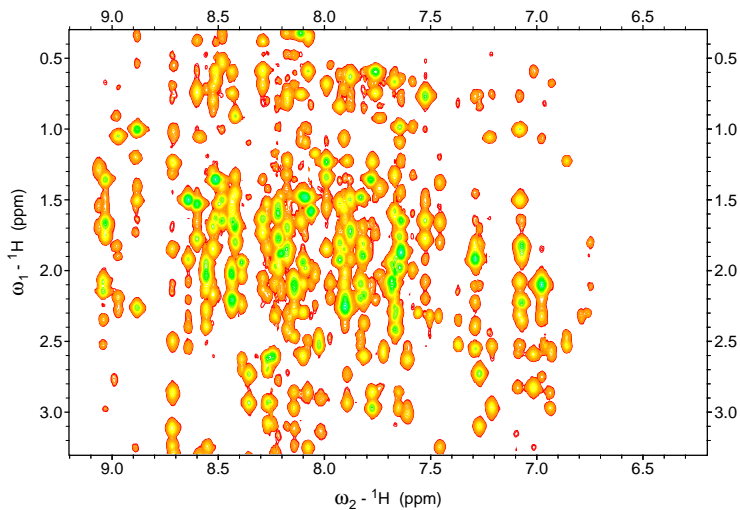


- Solvent (water) suppression
- Simplification of spectra
- Resolution improvement
- Obtaining chemical/biological information
structure, dynamics, interactions

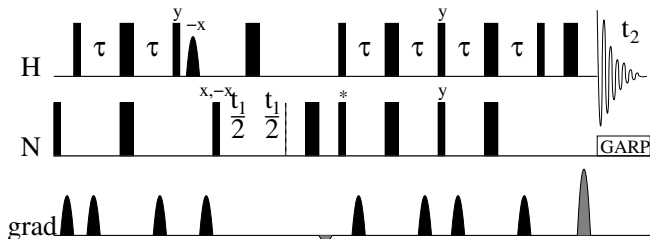
2D spectroscopy: NOESY



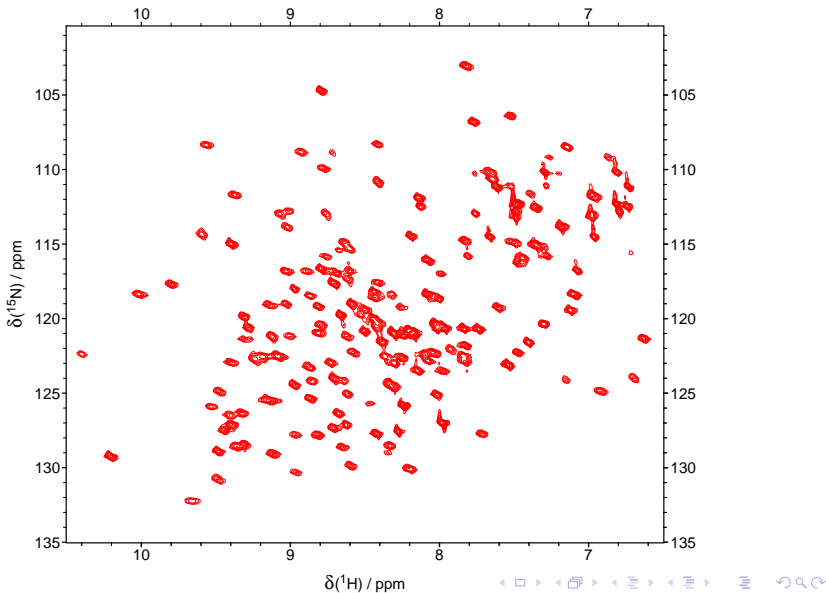
2D NOESY spectrum



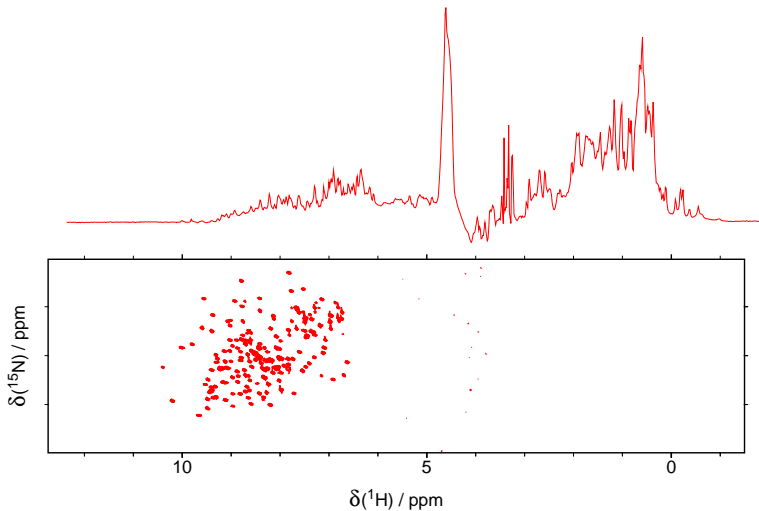
HSQC



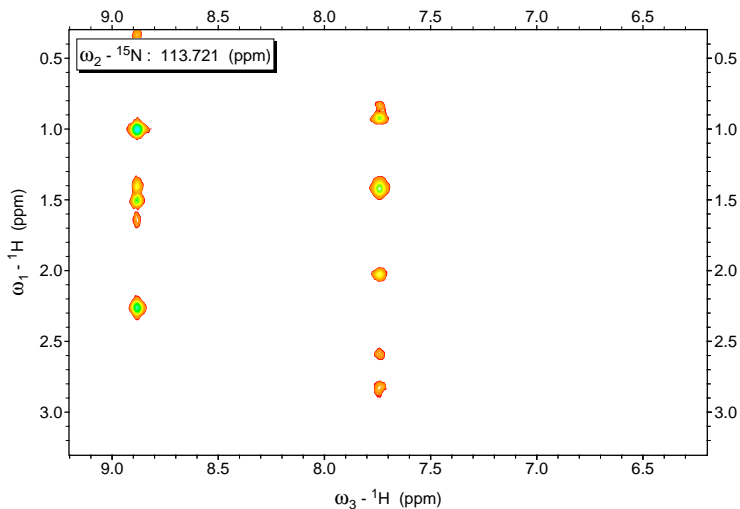
Correlated multidimensional NMR experiments



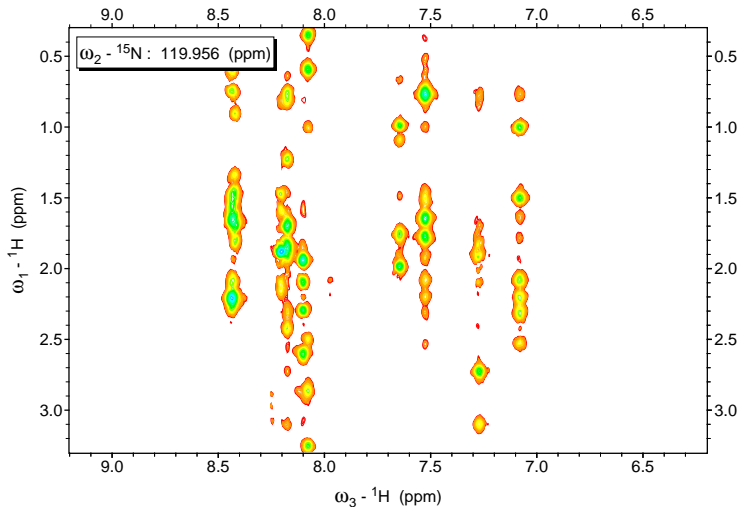
Correlated multidimensional NMR experiments



3D NOESY spectrum



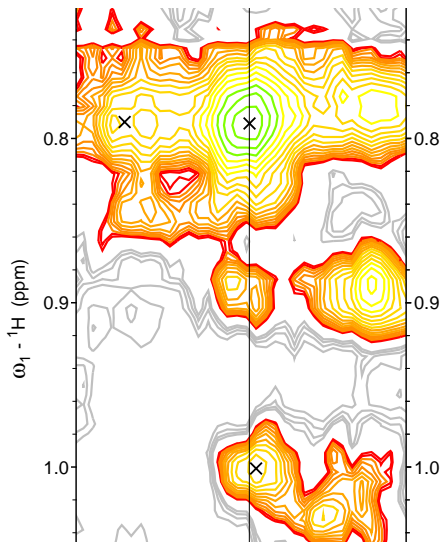
3D NOESY spectrum



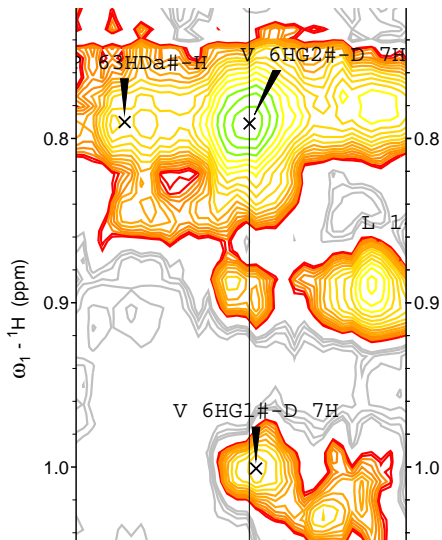
Biomolecular applications

- No special sample requirements
- Low energy
non-destructive × low sensitivity
dynamic nuclear polarization (by electrons)
paramagnetic relaxation enhancement (by electrons)
- Atomic resolution
- Many atoms described by single measurement
high information content × complexity of data
correlated spectroscopy, selective labeling
Assignment of spectra is demanding

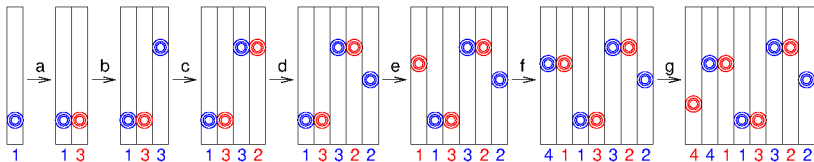
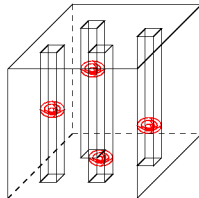
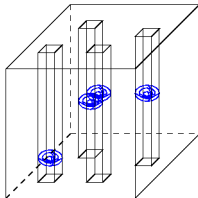
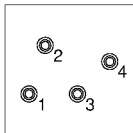
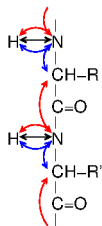
Assignment of spectra



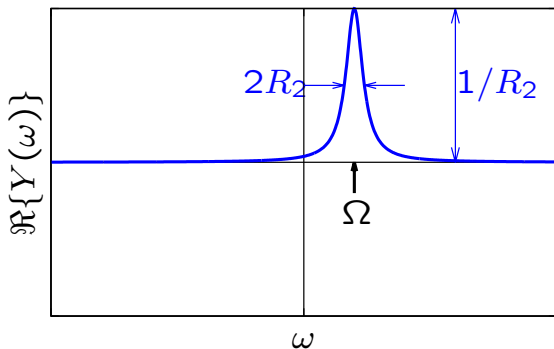
Assignment of spectra



Assignment of spectra

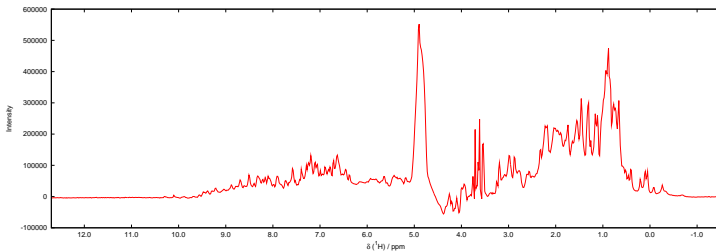
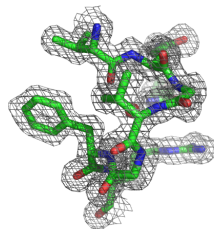
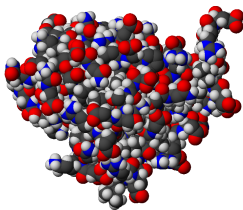
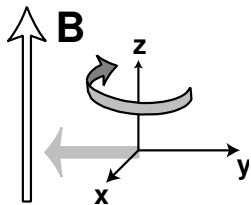


Chemical/biological information in NMR spectrum

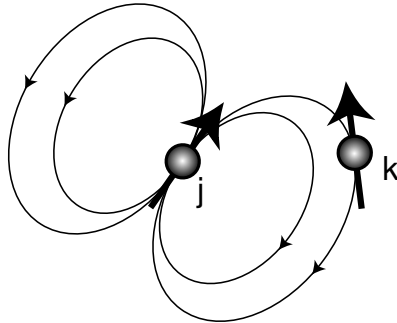


- $\Omega \rightarrow$ structure
- $R_2 \rightarrow$ dynamics
- Area \rightarrow (relative) concentration

Structure from chemical shift

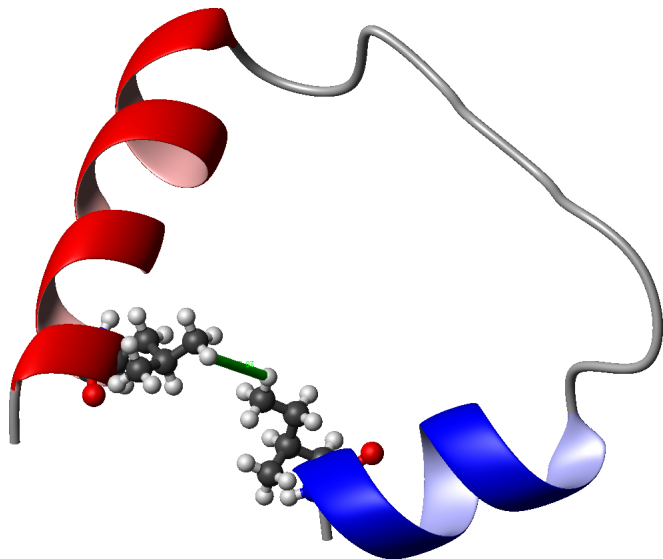


Structure from nuclear Overhauser effect

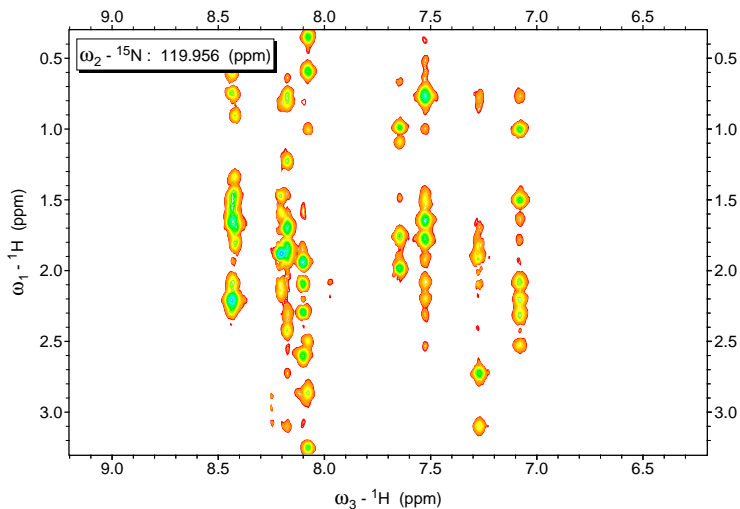


reproduced from M. H. Levitt: Spin Dynamics

Structure from nuclear Overhauser effect



Structure from nuclear Overhauser effect



Structure from nuclear Overhauser effect

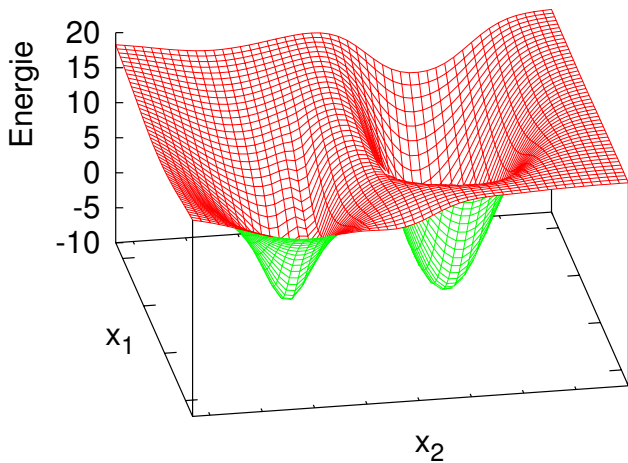
$$\frac{S}{S_{\text{ref}}} = \left(\frac{r_{\text{ref}}}{r}\right)^6 \quad (1)$$

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

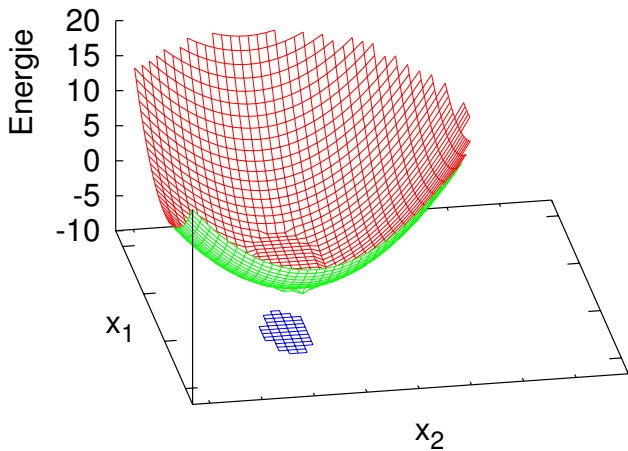
Calibration:

Reference protons		distance
geminal in methylene	$\text{H}-\text{C}-\text{H}$	0.17 nm
vicinal in an aromatic ring	$\text{H}-\text{C}=\text{C}-\text{H}$	0.25 nm
<i>meta</i> in an aromatic ring	$\text{H}-\text{C}=\text{CH}-\text{C}-\text{H}$	0.42 nm

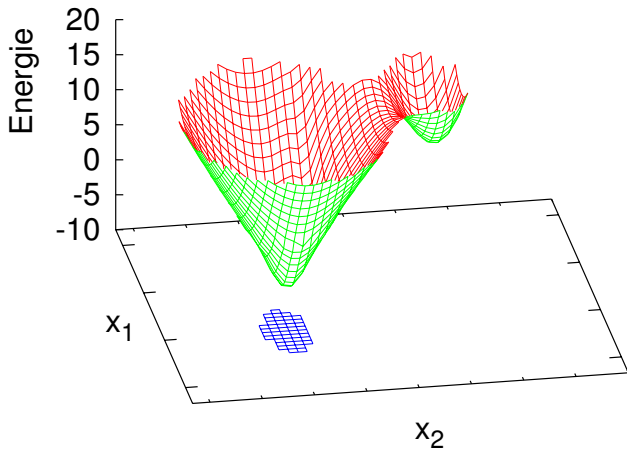
NMR structure calculation



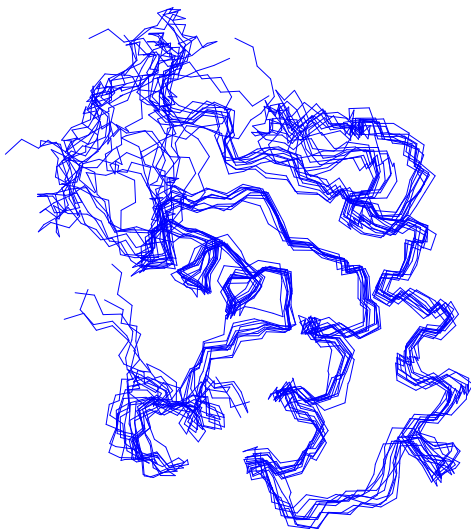
NMR structure calculation



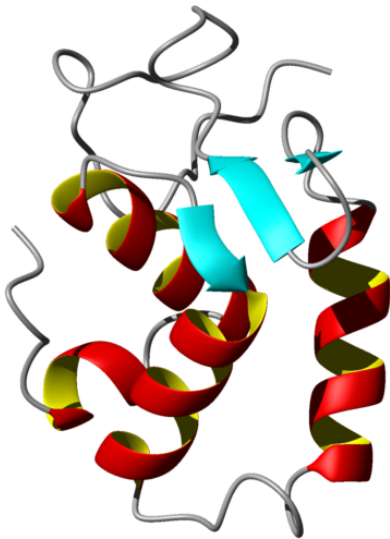
NMR structure calculation



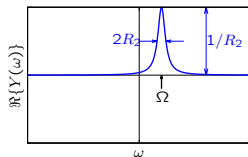
NMR structure calculation



NMR structure calculation

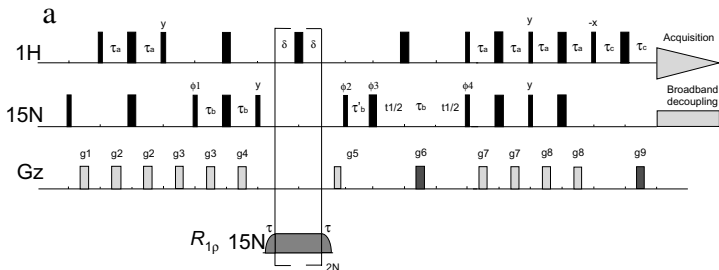


Relaxation rates from special experiments



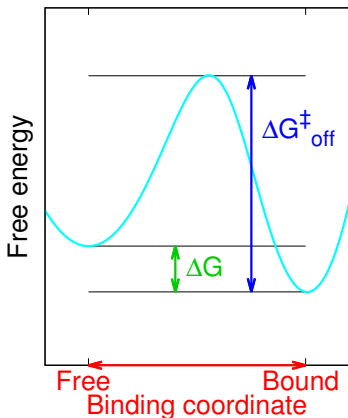
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D.M. Korzhnev et al. / Progress in Nuclear Magnetic Resonance Spectroscopy 38 (2001) 197–266



Biomolecular interactions

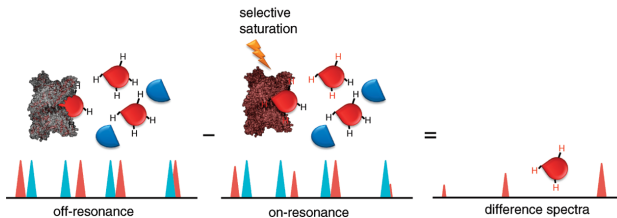
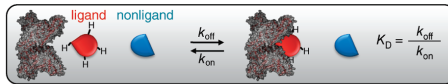
- Does it bind?
- How many molecules?
Stoichiometry
- In how many steps?
Mechanism
- Where?
Structure
- How strongly?
Affinity
- How fast?
Kinetics



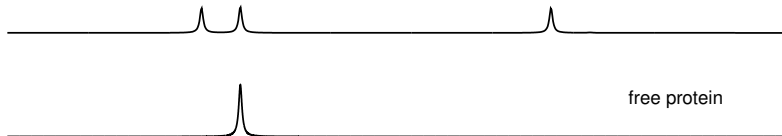
Observe:

- **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



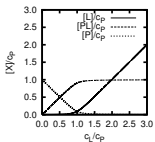
Spectrum of a complex with slow dissociation



Titration

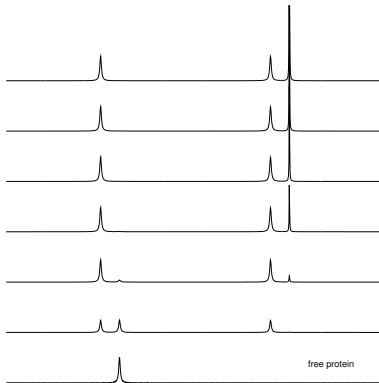
$$k_{off} = 0.2 \text{ s}^{-1}$$
$$K_D = k_{off}/k_{on} = 0.01 c_P$$

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



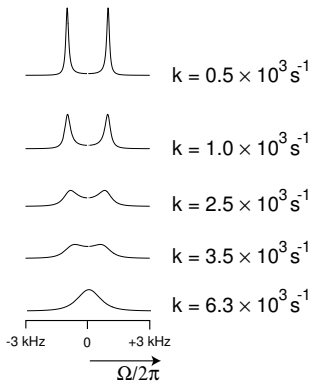
free ligand

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

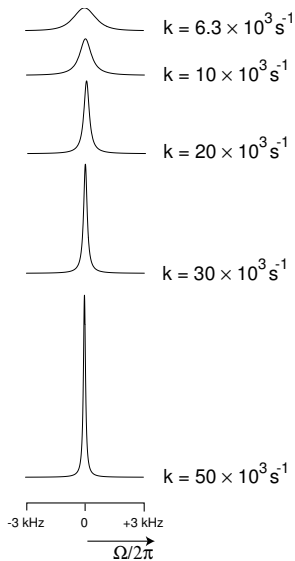


free protein

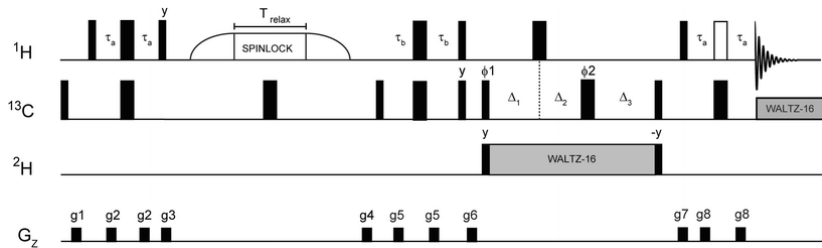
Rate of dissociation



reproduced from M. H. Levitt: Spin Dynamics



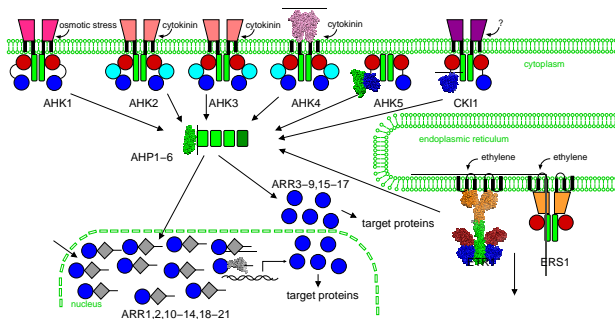
Kinetics from relaxation dispersion experiments



Example 1: fast exchange

Interacting molecules:

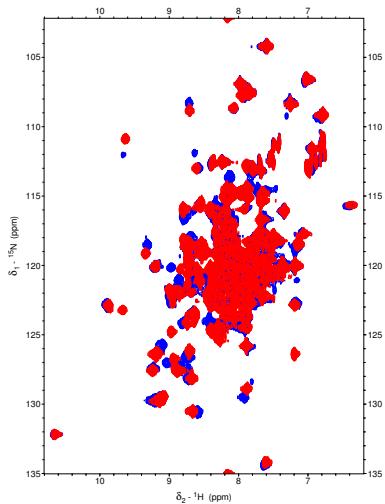
- Receiver domain of plant sensory histidine kinase CKI1 (from *Arabidopsis thaliana*)
- Mg^{2+} ions



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

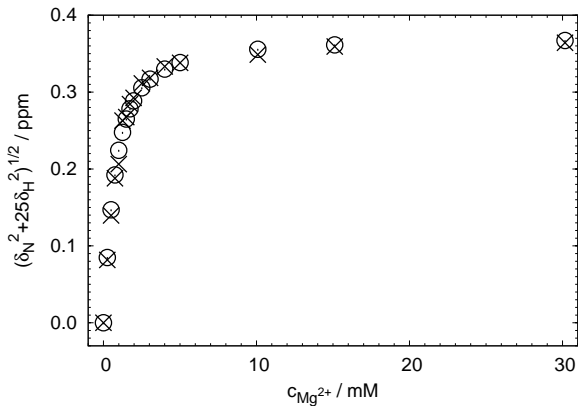
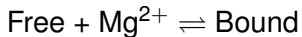
Otrusinova et al., *J. Biol. Chem.* **292** (2018) 17525

Does it bind?



free
 Mg^{2+} -bound

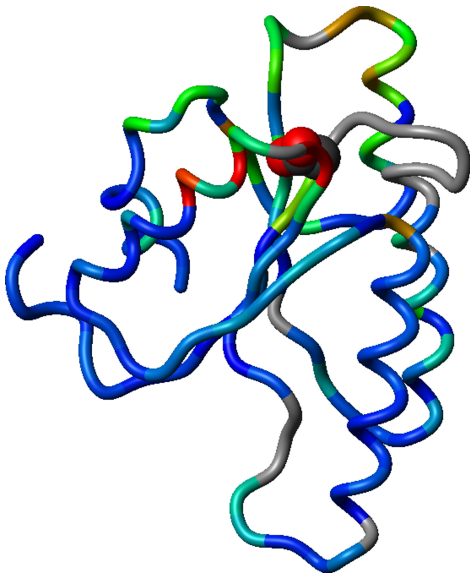
How fast? How strongly?



$$K_d = 0.43 \pm 0.06 \text{mM}$$

(data for Q92)

Where? From chemical shifts



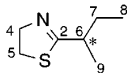
3MM4.pdb



Example 2: slow exchange

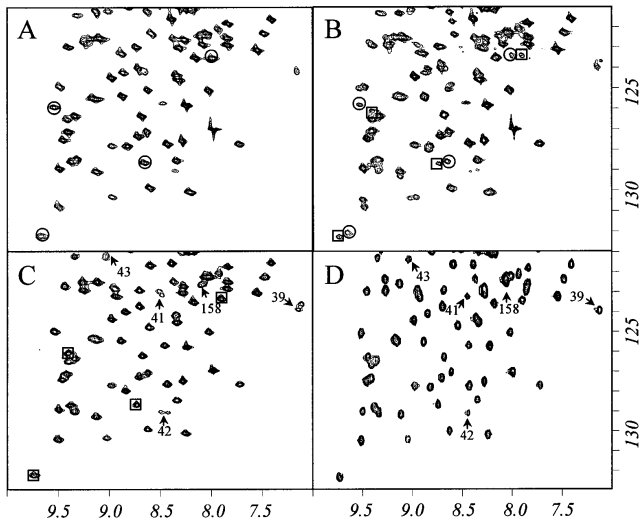
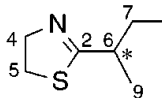
Interacting molecules:

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

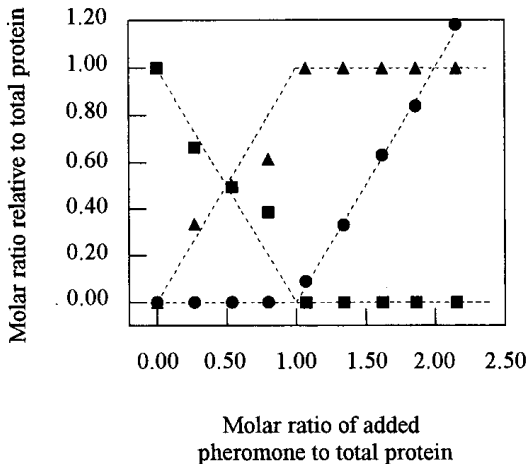


Zidek et al., *Biochemistry* **38** (1999) 9850

Does it bind? How fast?



How much? How strongly?

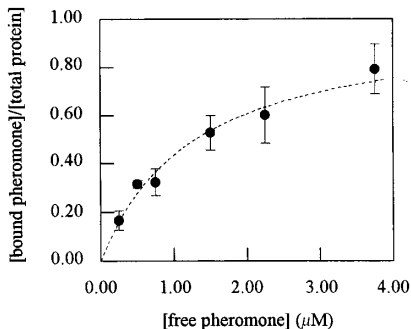


stoichiometry = $1.0 \pm 0.1 \mu\text{M}$

How strongly?

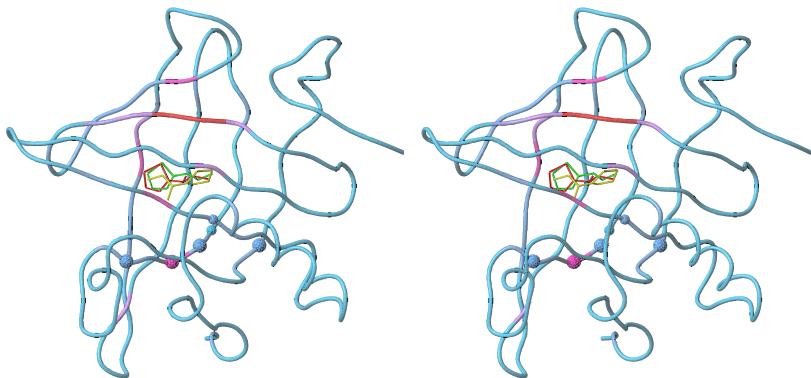
Too strong for NMR

Determined by equilibrium diffusion/gas chromatography



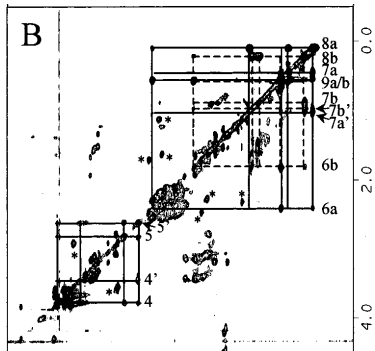
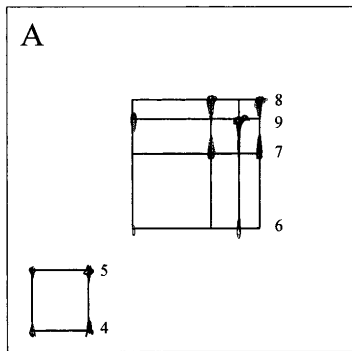
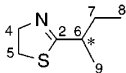
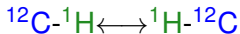
$$K_d = 1.3 \pm 0.1 \mu\text{M}$$

Where? From chemical shifts



MUP1.pdb

Where? From isotope-filtered NOE



Where? From isotope-filtered NOE

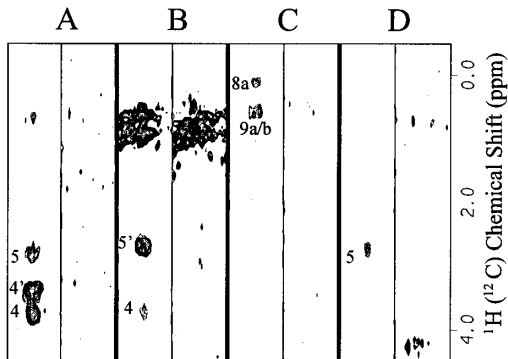
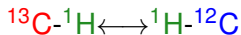


FIGURE 7: Representative strips from ^{13}C F1-filtered, F3-edited NOESY-HSQC spectra of free (right strips) and 2-*sec*-butyl-4,5-dihydrothiazole-complexed (left strips) rMUP-I. The strips were taken from the 3D spectra at chemical shift values corresponding to (A) Leu 40 δ_1 , (B) Leu 105 δ_1 , (C)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

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

