Advanced NMR & Imaging

Week 7: Multi-Dimensional Correlation Spectroscopy

Organisational Issues

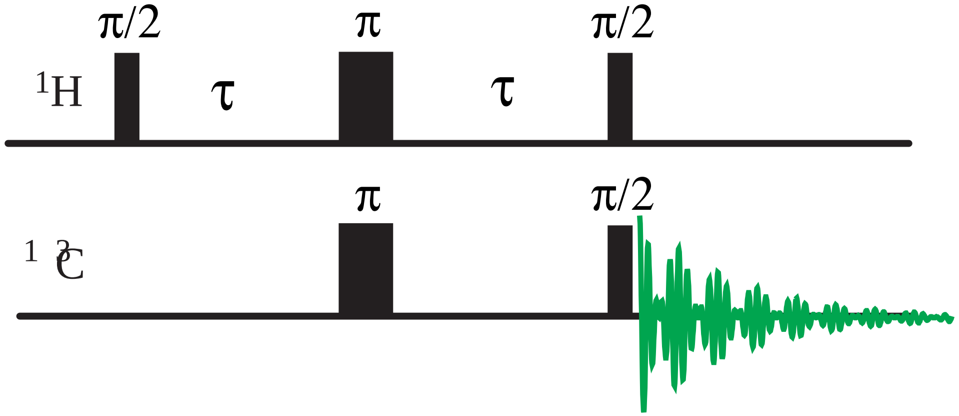
- Practical MRI session at the CHUV on tuesday 20th May, 13:00 to 15:00.
- Attendance is mandatory!
- Meet at CHUV at 12:30. More details on the meeting point will follow.
- Preparation: Read the papers in the folder "Preparation for Imaging Practical at CIBM" on Moodle which describe the sequences we will be using.

What we learned last week

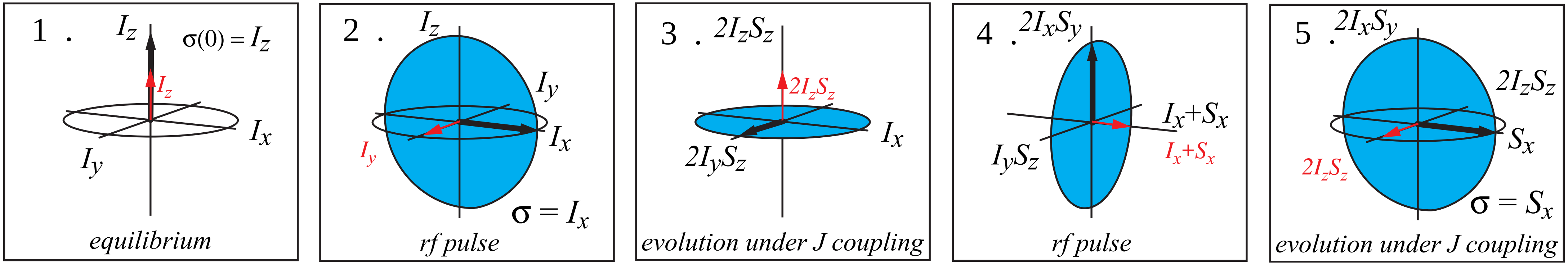
- Coherence Transfer

Polarisation Transfer: INEPT

coherence transfer from protons to carbon-13

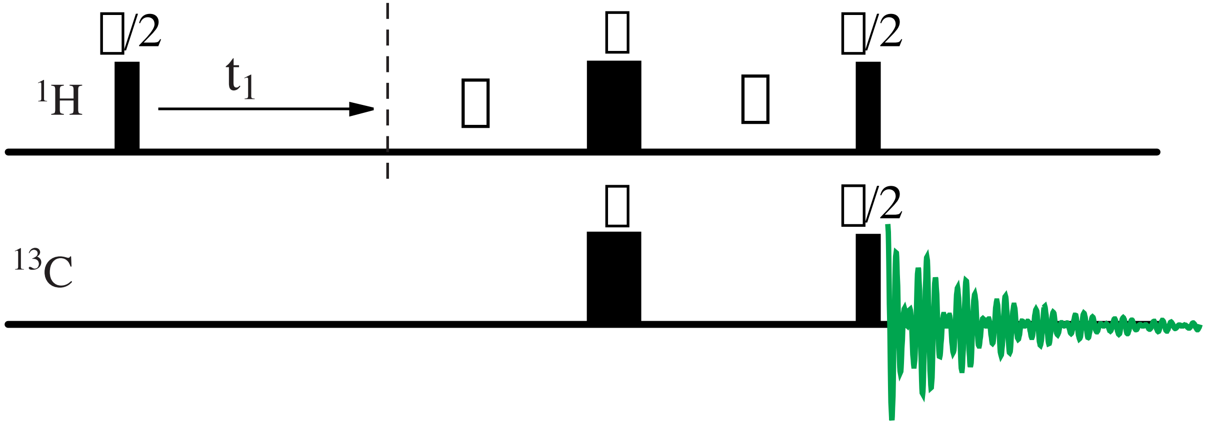


Evolution under different Hamiltonians is often confined to distinct three-dimensional subspaces.



Proton-Carbon Correlation Spectroscopy?

multi-dimensional NMR



couldn't we add another acquisition time on protons to find out which proton is connected to which carbon?

Objectives

- Can we use coherence transfer to make connections?

- Principles of two-dimensional NMR

- HSQC/HMQC/COSY/EXSY

How can we include two detection periods?

How does the detection period work in the ordinary experiment?

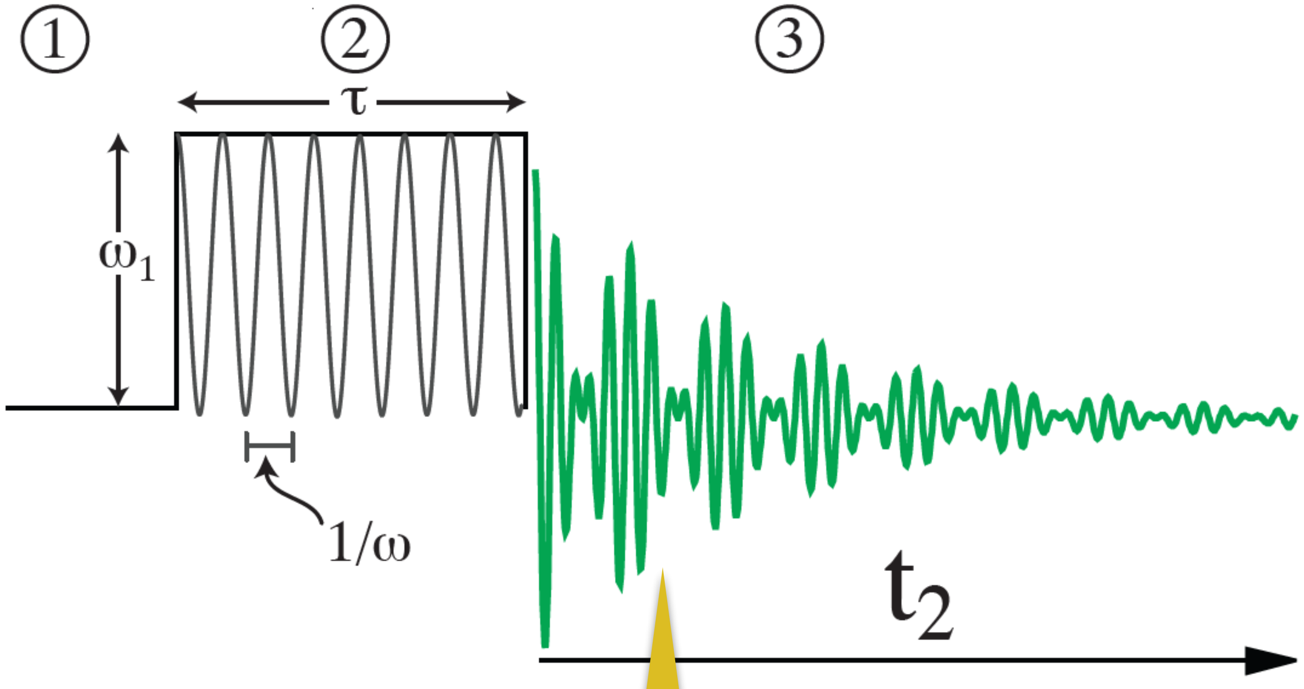
Pulsed FTNMR Spectroscopy

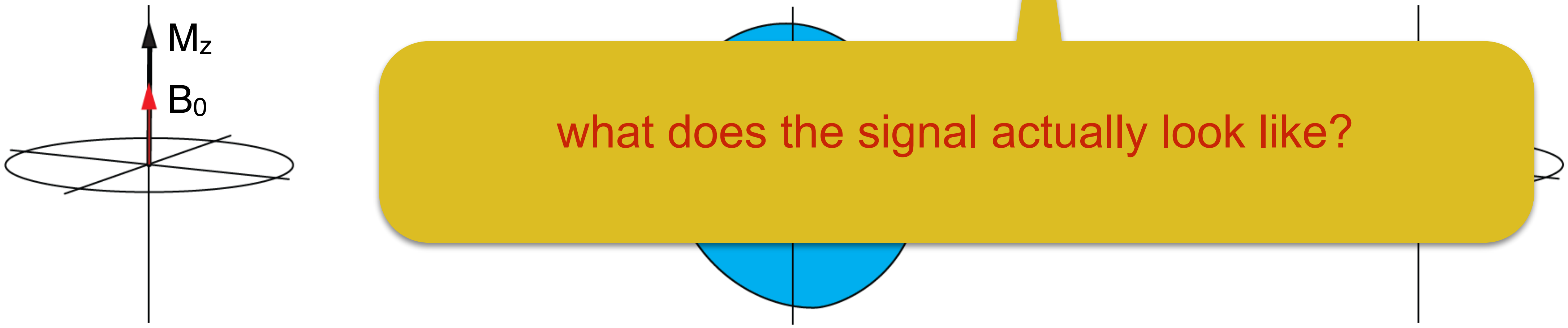
$\omega_1 = -\gamma B_1$

$\omega_1 \tau = \pi/2$

ω = carrier frequency, chosen by the operator to be near to the resonance frequencies

^{1}H





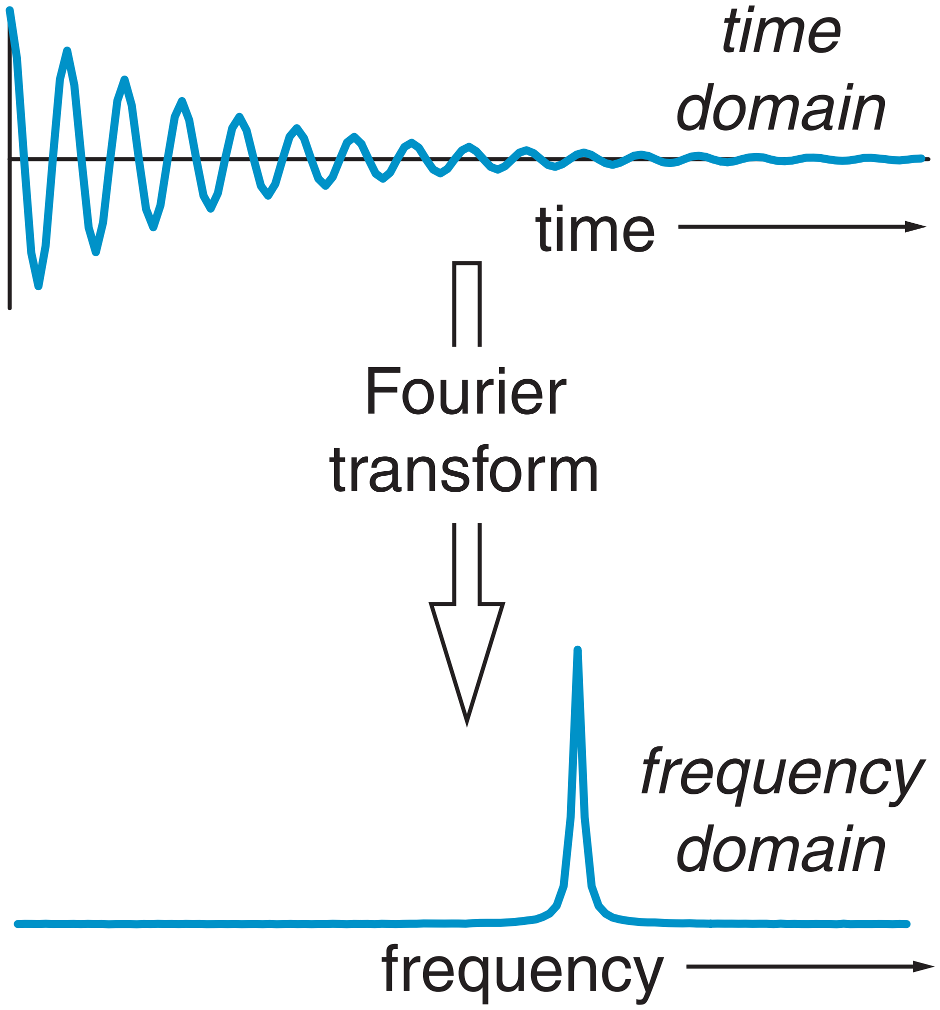
what does the signal actually look like?

1. Equilibrium. The net magnetization is aligned along the direction of the main field (z-axis).

2. A field is applied in the transverse plane. The magnetization of the ensemble precesses around the field.

3. The field is removed leaving a net transverse component of the ensemble magnetization. This ***coherence*** then starts to precess around the main field.

How does the detection period work in the ordinary experiment?



time domain

time

Fourier transform

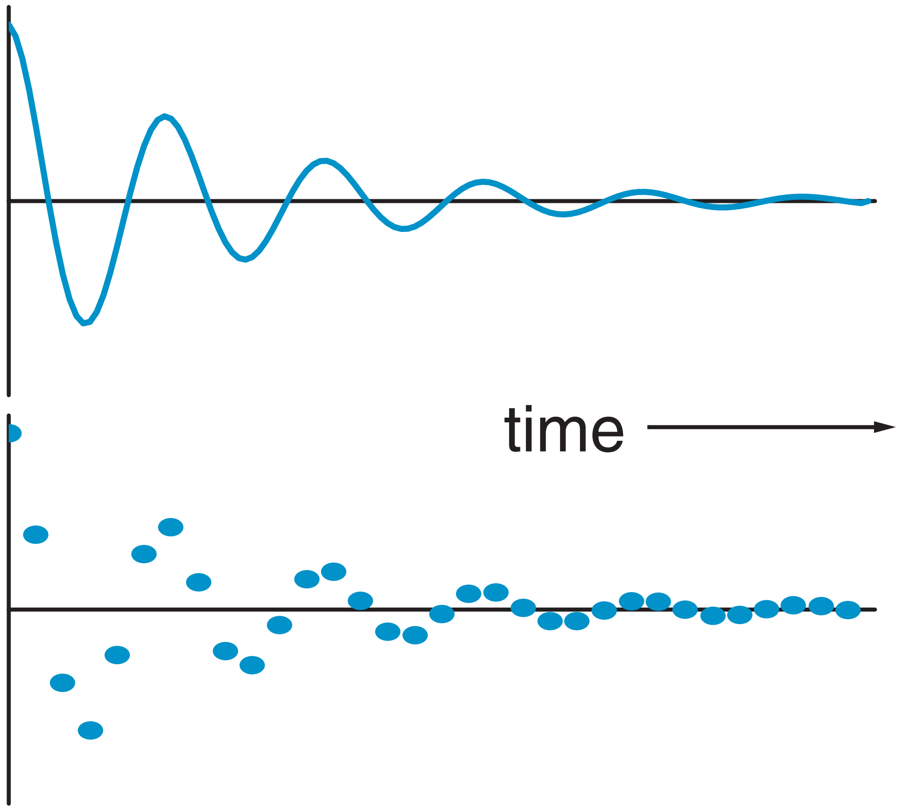
frequency domain

frequency

$$I(\omega) = \int S(t)\exp\{-i\omega t\}dt$$

The Fourier transform is a mathematical process which turns a time-domain signal, the FID, into a frequency-domain signal, the spectrum.

How does the detection period work in the ordinary experiment?

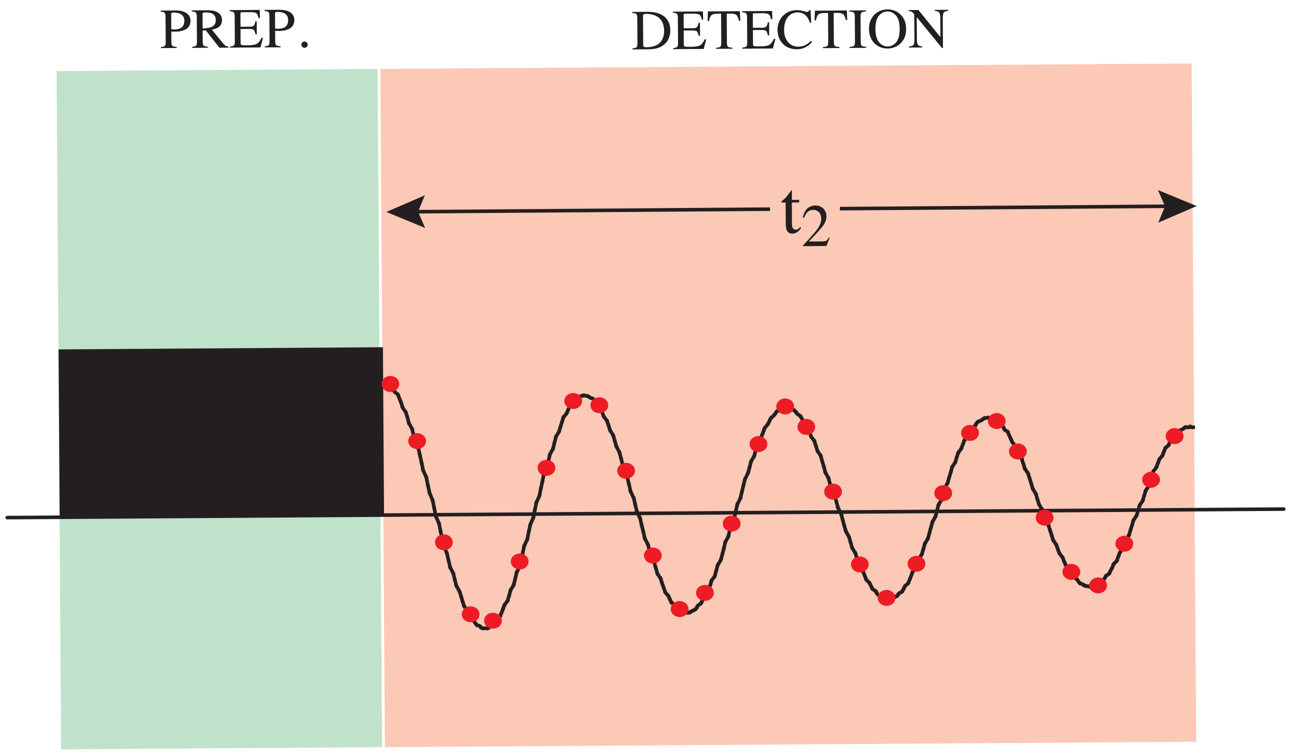


The amplitude of the FID varies smoothly as a function of time.

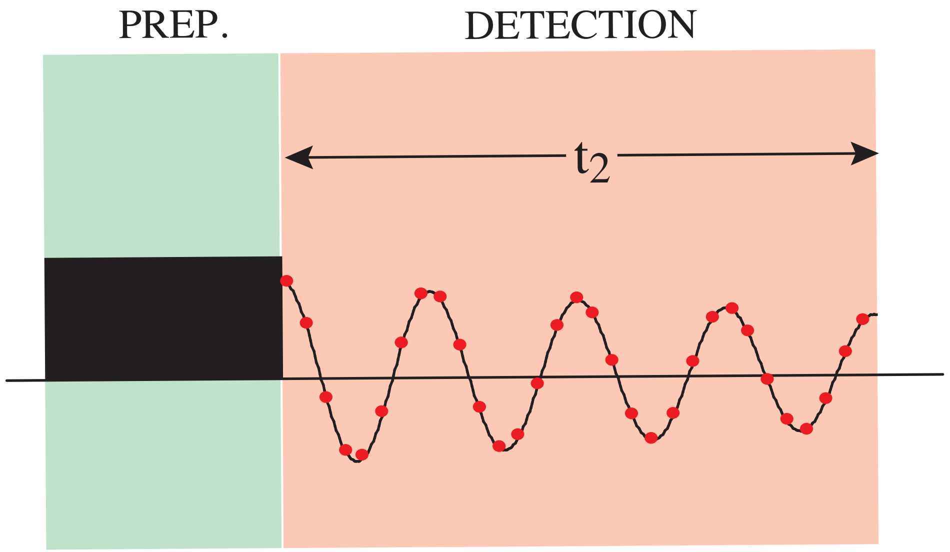
In order to be able to manipulate this time-domain signal in a computer, the signal is digitized at regular intervals.

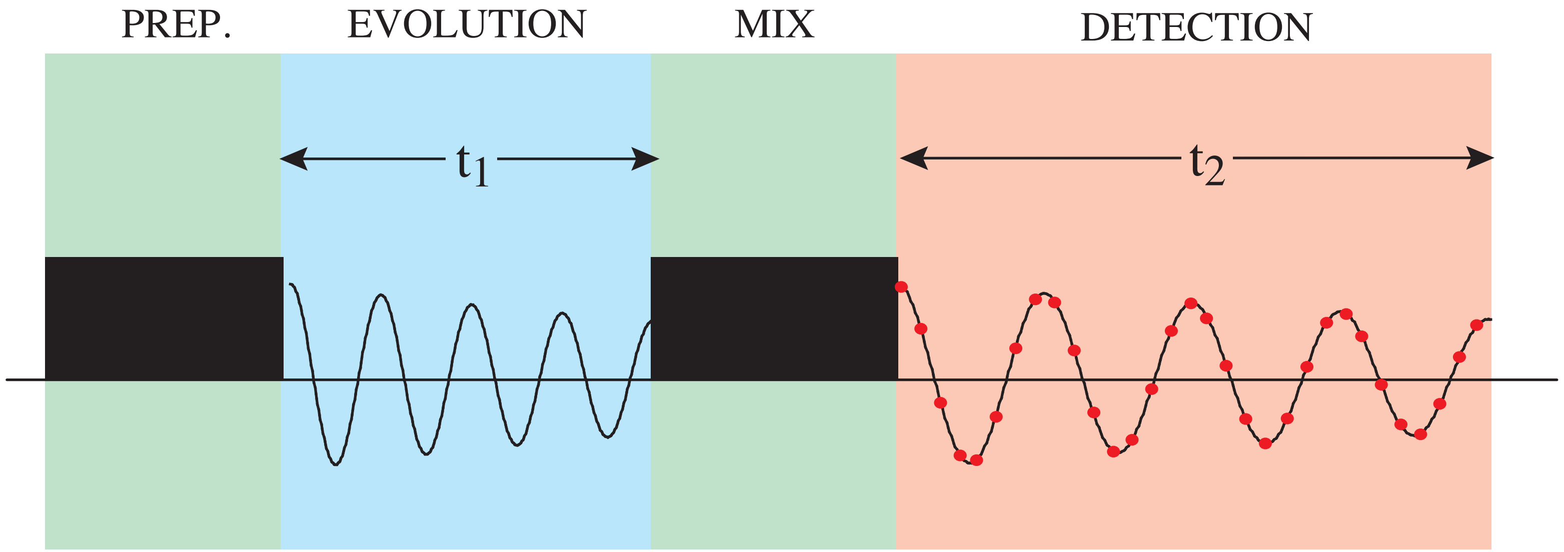
$$I(\omega) = \sum_{i=1}^{N} S(t_i)\exp\{-i\omega t_i\}dt$$

Principles of Multi-Dimensional Spectroscopy

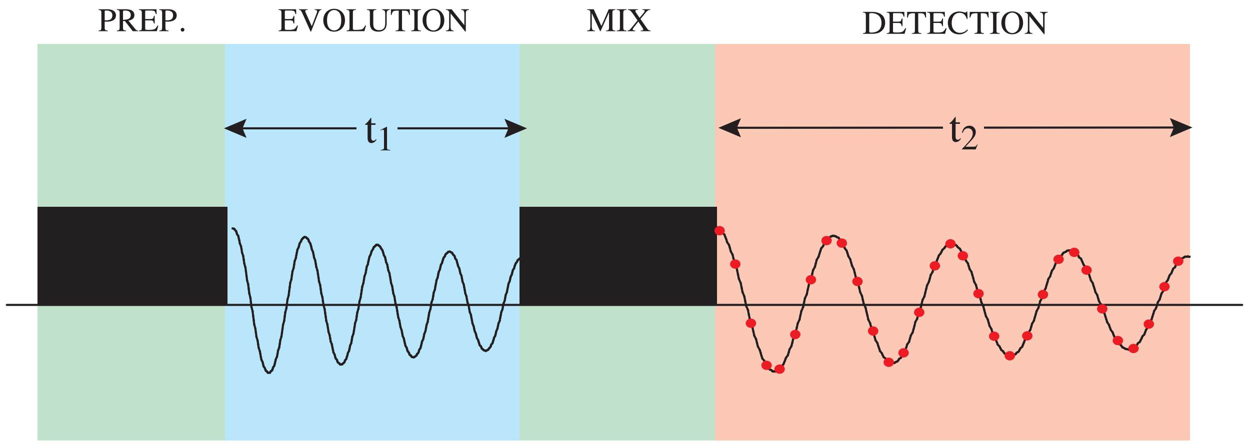


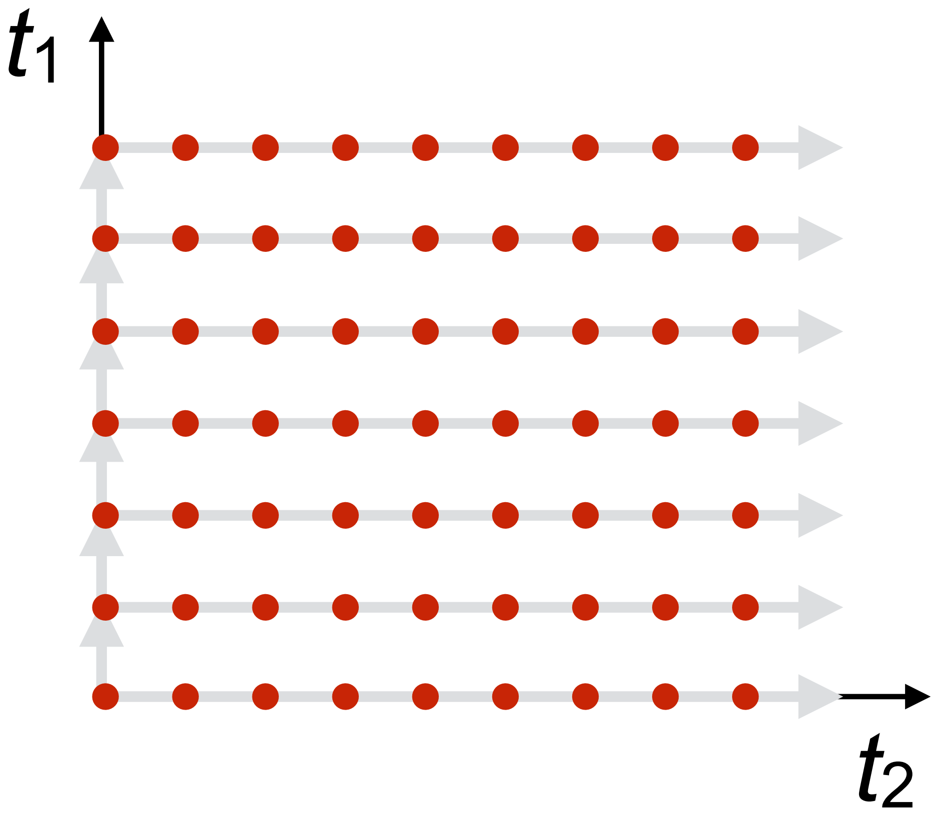
Principles of Multi-Dimensional Spectroscopy





Principles of Multi-Dimensional Spectroscopy





nth experiment, $t_1 = (n-1)\Delta t$

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•
•
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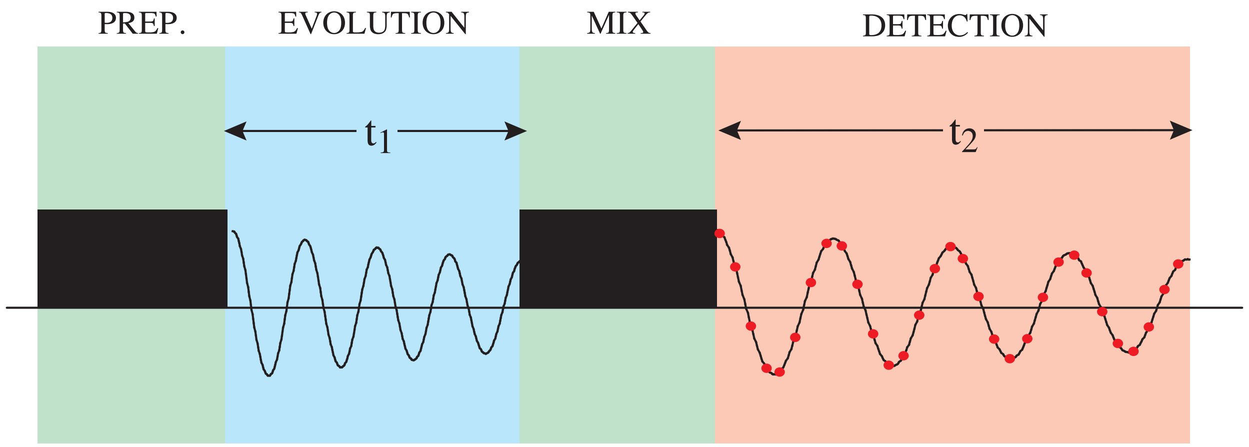
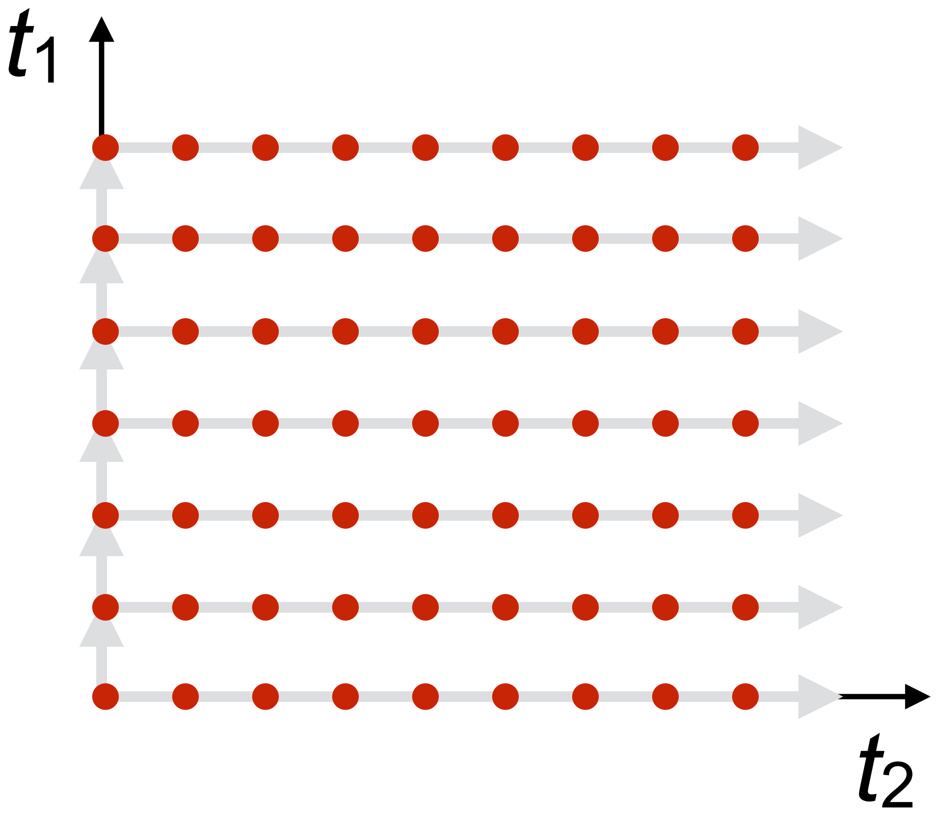
3rd experiment, $t_1 = 2\Delta t$

2nd experiment, $t_1 = \Delta t$

1st experiment, $t_1 = 0$

Principles of Multi-Dimensional Spectroscopy





nth experiment, $t_1 = (n-1)\Delta t$

⋮

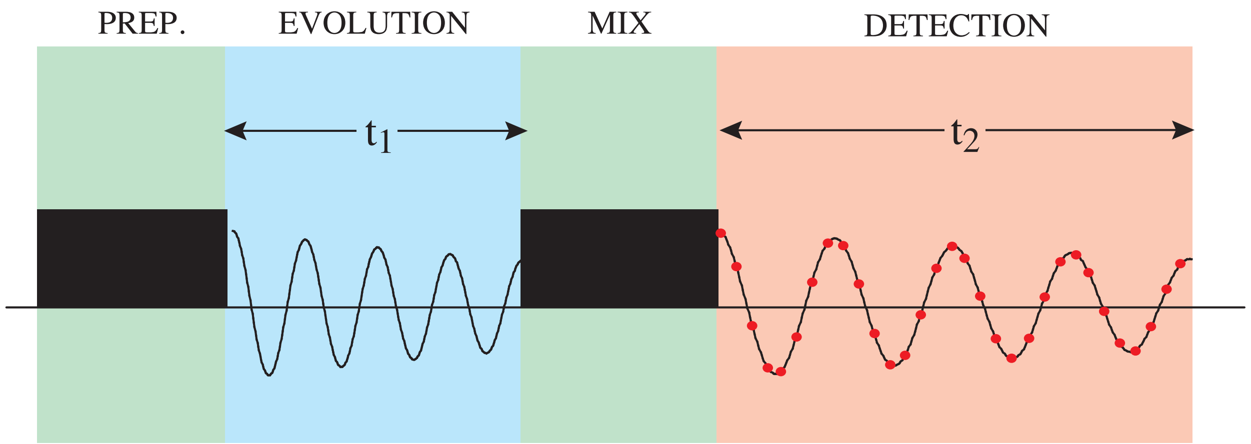
3rd experiment, $t_1 = 2\Delta t$

2nd experiment, $t_1 = \Delta t$

1st experiment, $t_1 = 0$

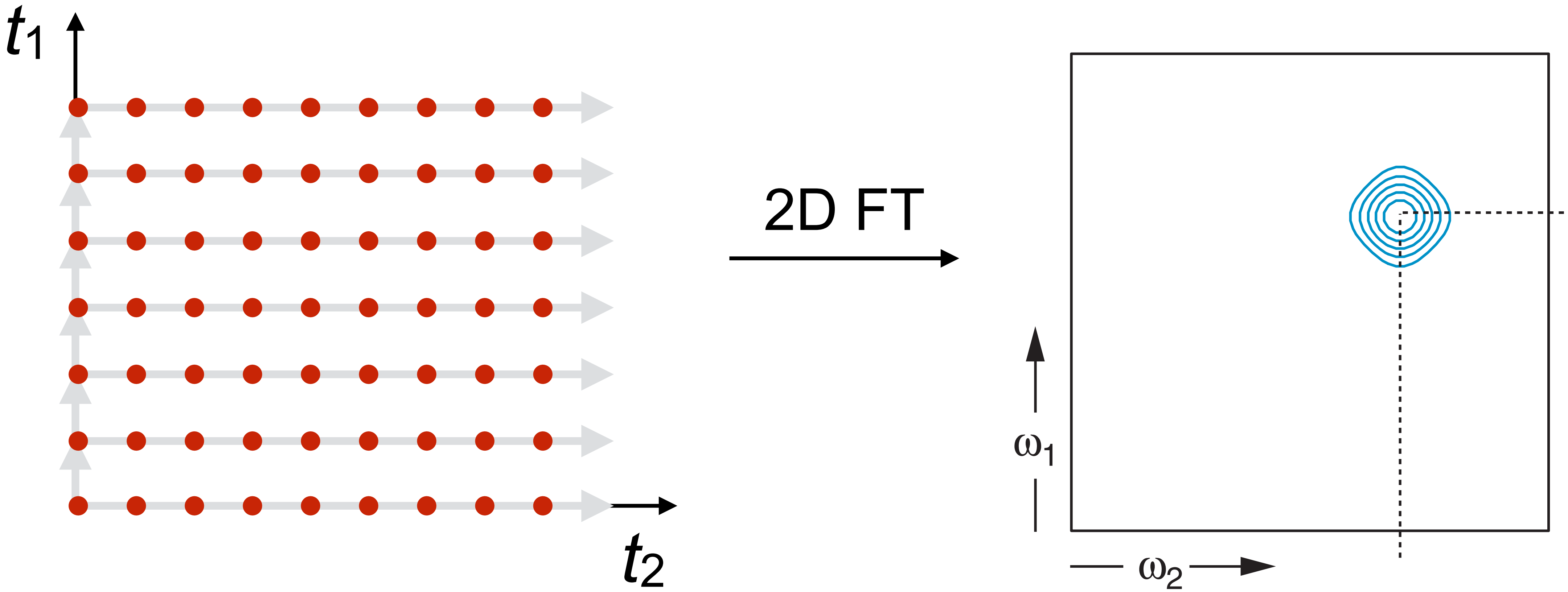
We can fill a two-dimensional time domain with data by repeating the experiment, with acquisition along t_2 in each experiment, and with t_1 being incremented progressively from one experiment to the next.

Principles of Multi-Dimensional Spectroscopy



A two-dimensional frequency domain (ω_1, ω_2), can be obtained by two-dimensional Fourier transformation of the data with respect to t_1 and t_2.

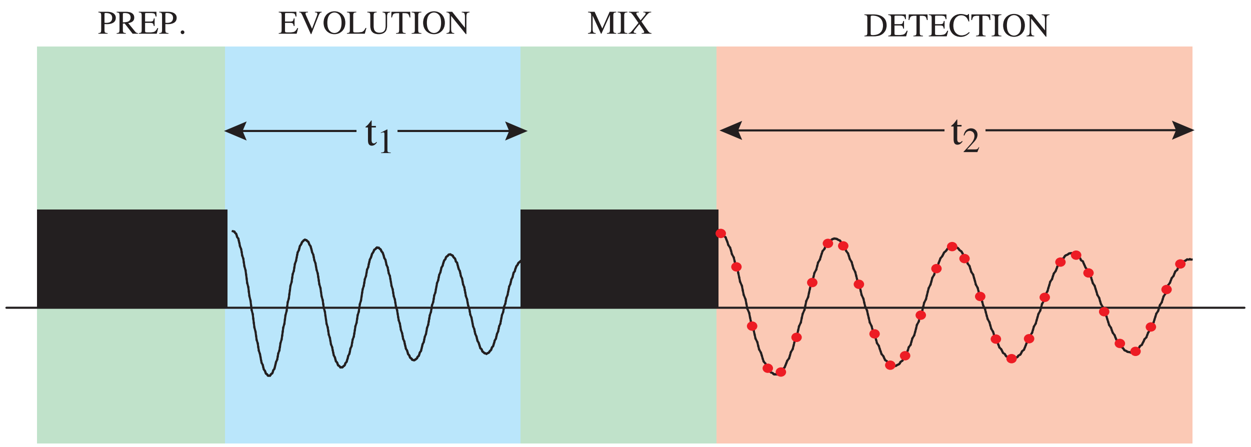
$$I(\omega_1,\omega_2) = \int\int s(t_1,t_2)\exp\{-i(\omega_1 t_1 + \omega_2 t_2)\}dt_1 dt_2$$



In two-dimensional NMR each peak has two frequency coordinates, measured along the ω_1 and ω_2 axes, corresponding to the precession frequencies experience during the periods t_1 and t_2 respectively.

Two-dimensional NMR spectra are usually presented as contour plots in which points of equal intensity are joined by lines, just as in a topographic map.

Principles of Multi-Dimensional Spectroscopy



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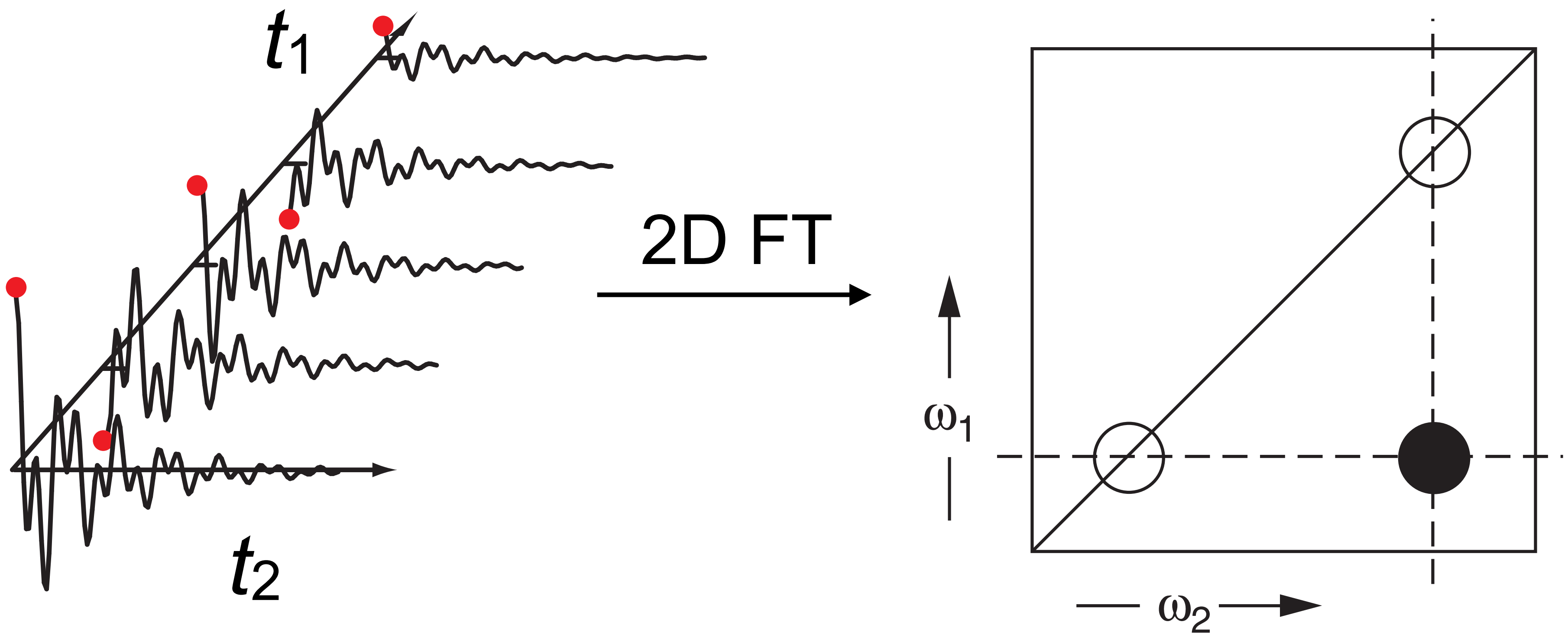
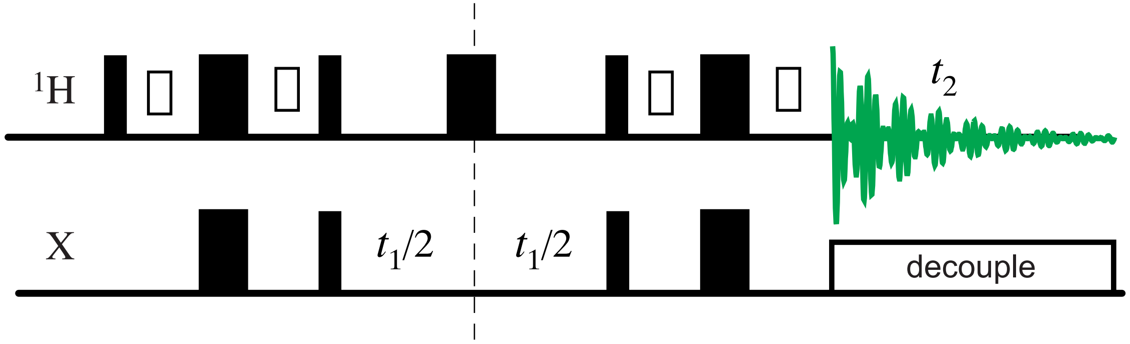
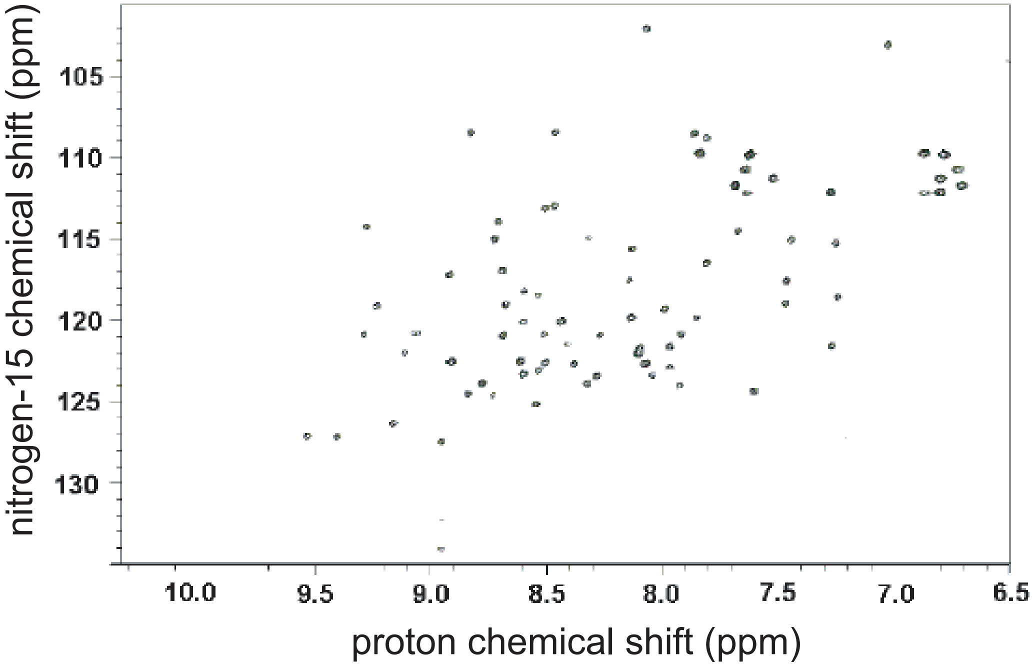


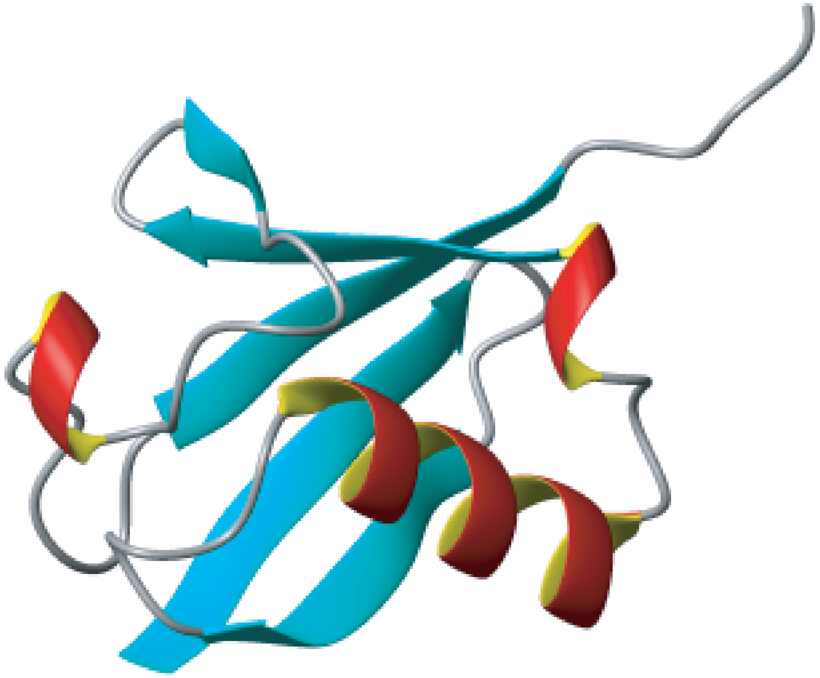
2D NMR spectra typically consist of "diagonal peaks" at $\omega_1 = \omega_2$ and "cross peaks" at $\omega_1 \neq \omega_2$ such that cross peaks occur at frequencies that link the resonance frequencies of different nuclei in the spectrum.

Cornerstone NMR Experiments: HSQC

(Heteronuclear Single Quantum Correlation)

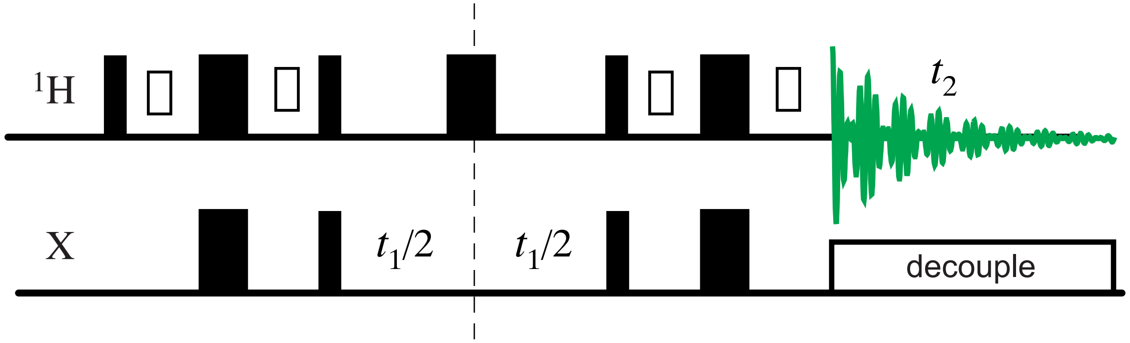


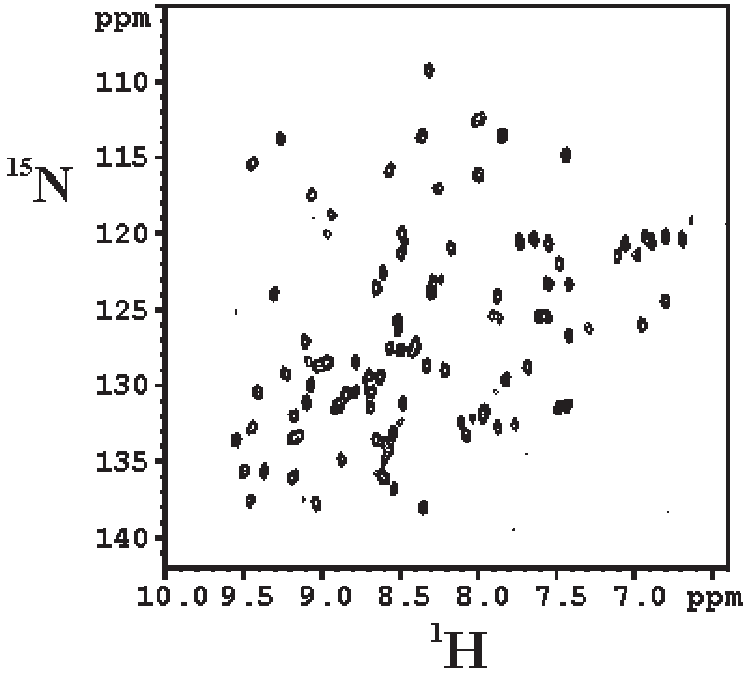




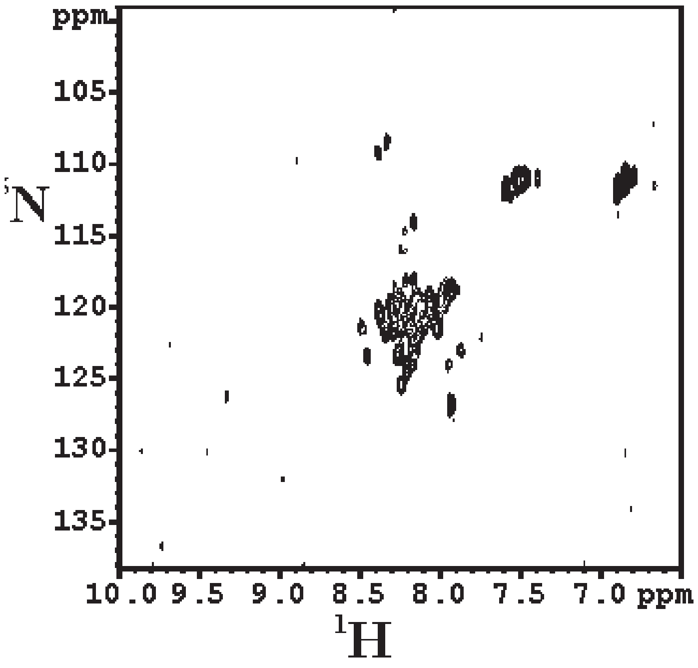
Cornerstone NMR Experiments: HSQC

(Heteronuclear Single Quantum Correlation)





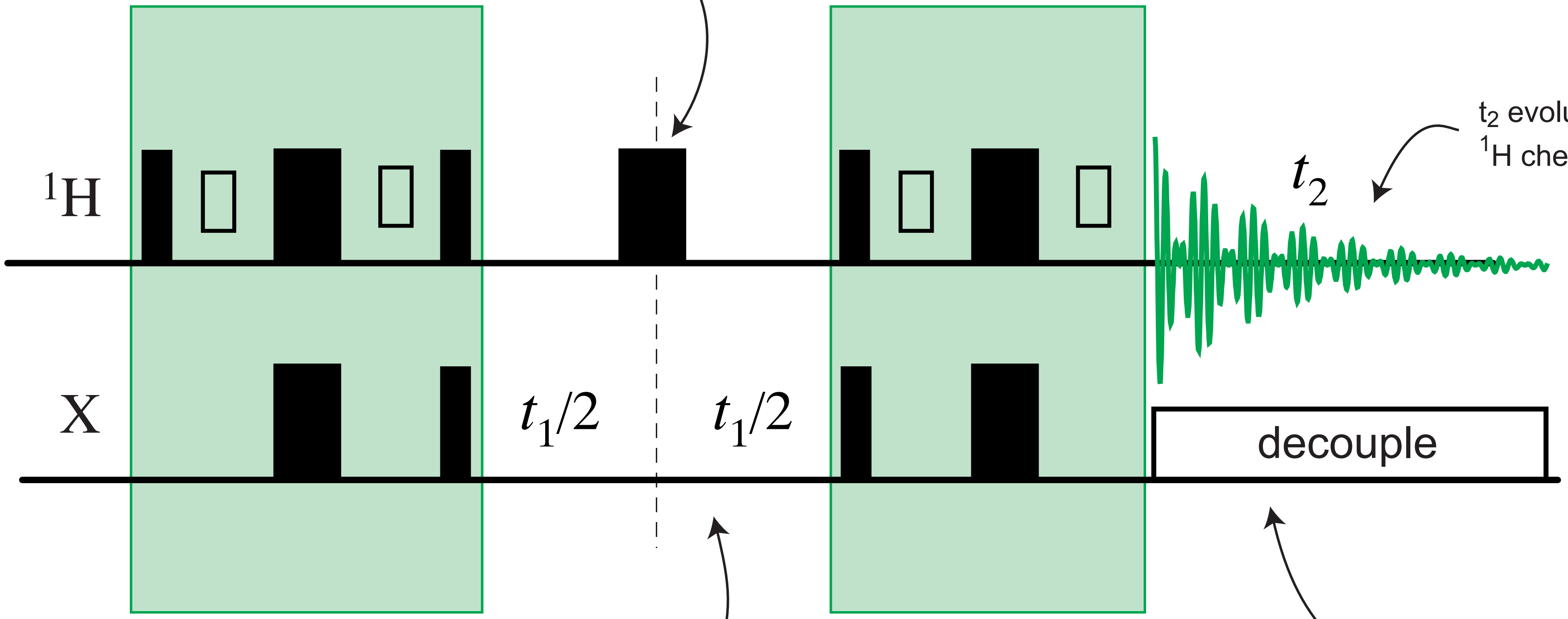
folded protein



unfolded protein

Cornerstone NMR Experiments: HSQC

(Heteronuclear Single Quantum Correlation)



first INEPT block
transfers polarisation to X

□-pulse refocuses
H-X J coupling
during t_1

second INEPT block
transfers magnetization back to ^{1}H

t_2 evolution under
^{1}H chemical shift

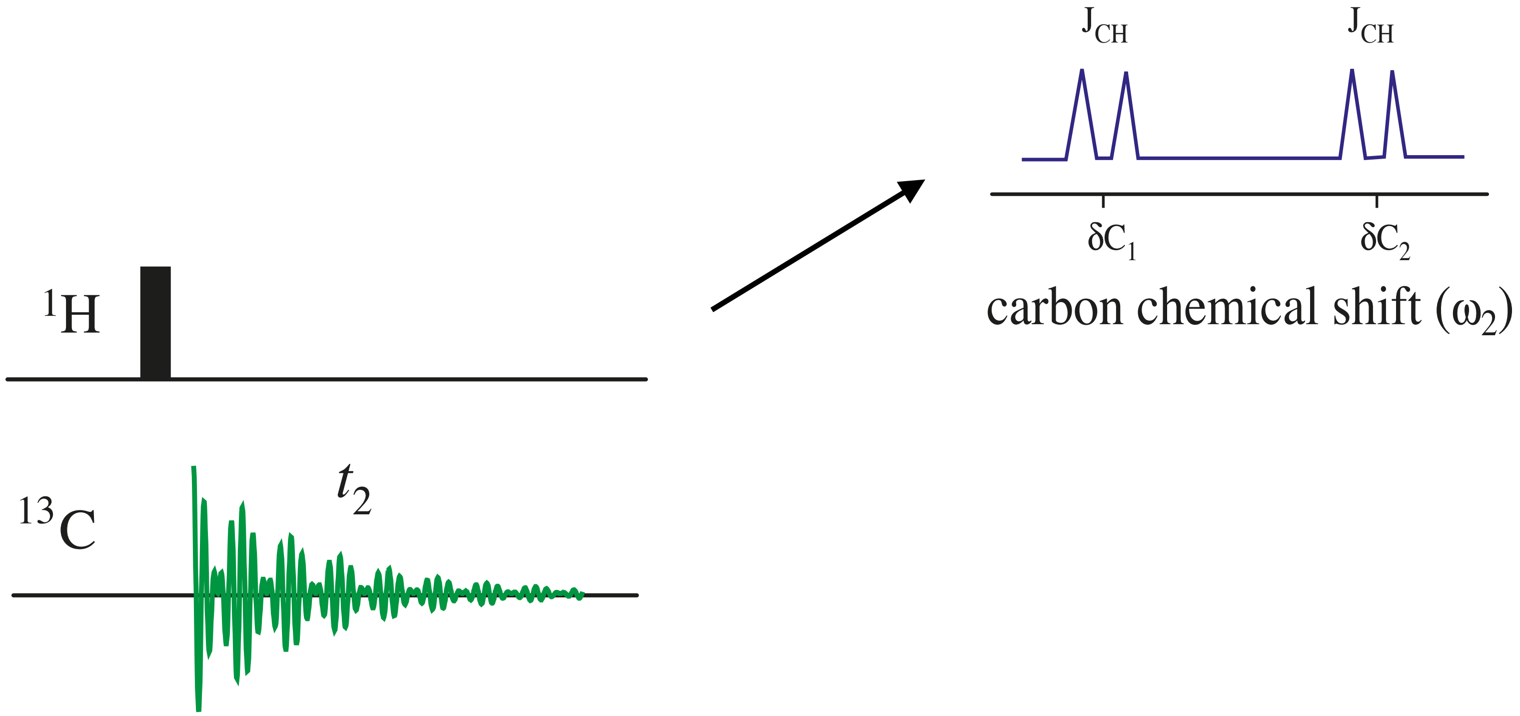
t_1 evolution under
X chemical shift

X spin irradiation
decouples H-X
J coupling during t_2

The HSQC experiment yields a correlation between proton resonances and the resonances of directly bonded X nuclei. The experiment has the sensitivity of protons. It uses heteronuclear J couplings for coherence transfer, to establish the correlations, but the couplings are decoupled during both acquisition periods.

X is typically ^{15}N or ^{13}C.

Heteronuclear Decoupling

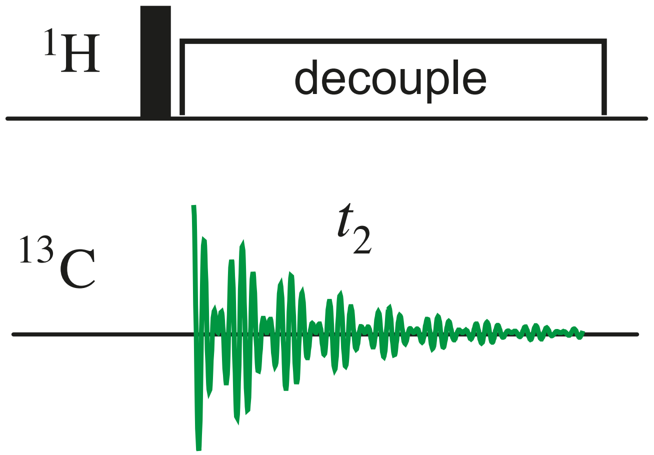


without decoupling

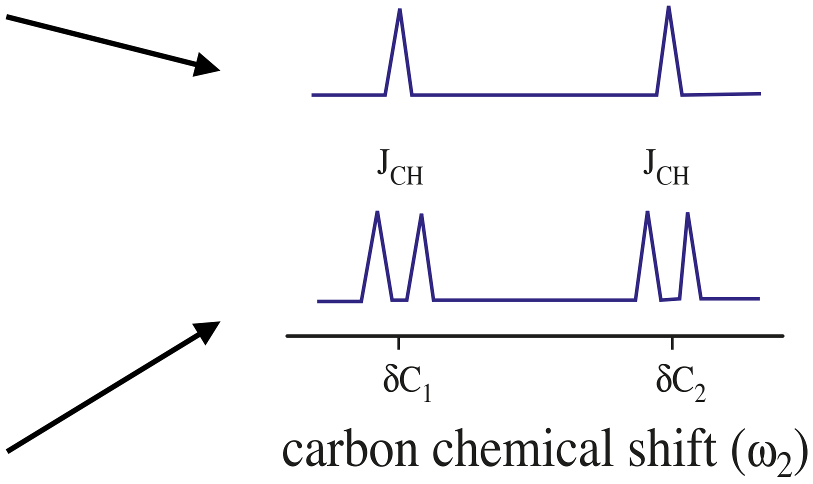
$$\mathscr{H} = \delta_{C_1} I_{1z} + \delta_{C_2} I_{2z} + 2\pi J_{CH} I_{1z} S_{1z} + 2\pi J_{CH} I_{2z} S_{2z}$$

Heteronuclear Decoupling

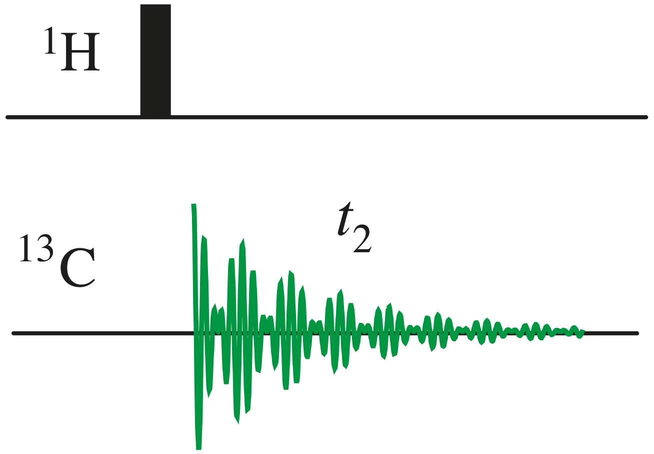
with decoupling



$$\overline{\mathcal{H}} = \delta_{C_1} I_{1z} + \delta_{C_2} I_{2z}$$

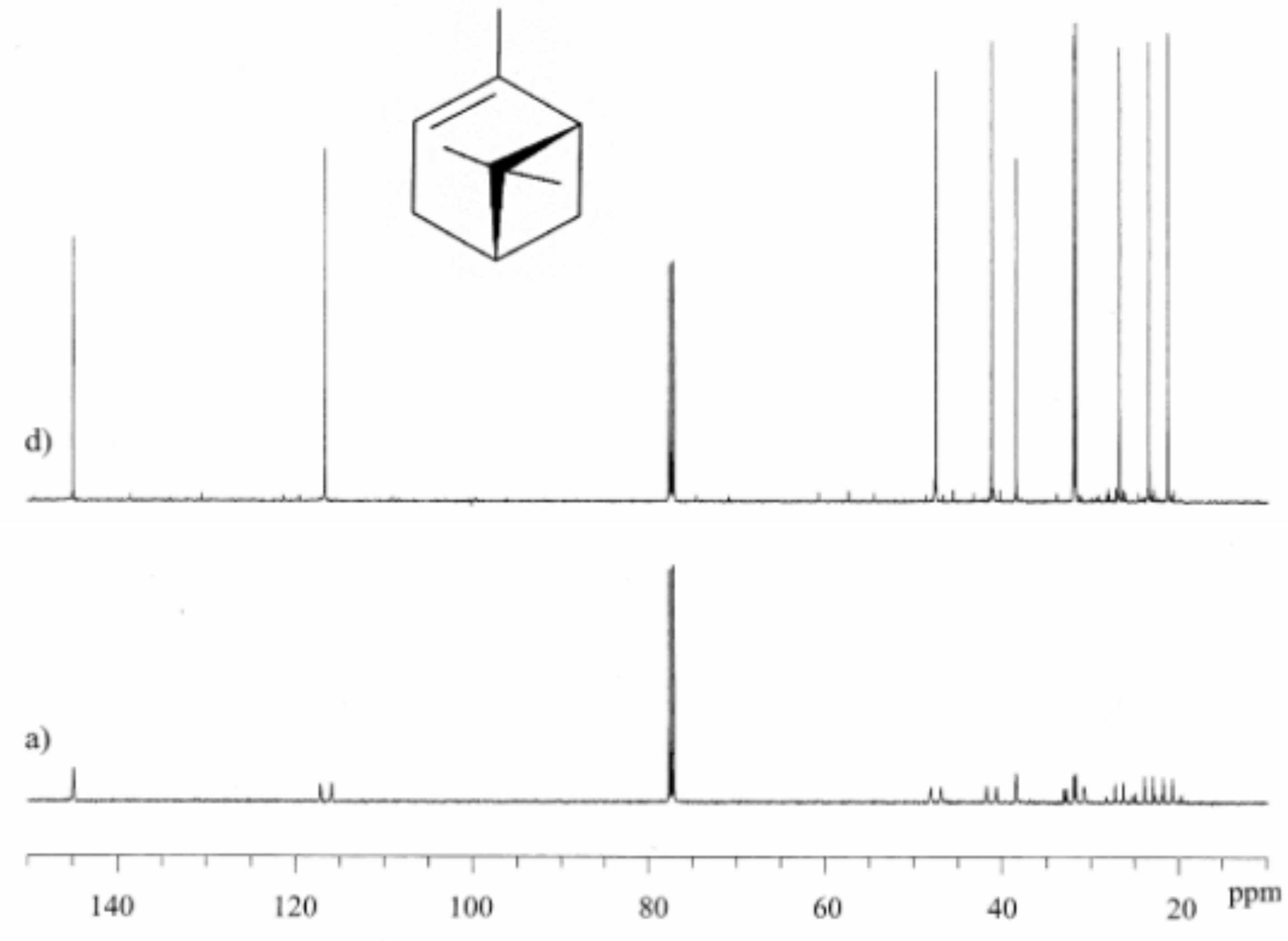


without decoupling



$$\mathcal{H} = \delta_{C_1} I_{1z} + \delta_{C_2} I_{2z} + 2\pi J_{CH} I_{1z} S_{1z} + 2\pi J_{CH} I_{2z} S_{2z}$$

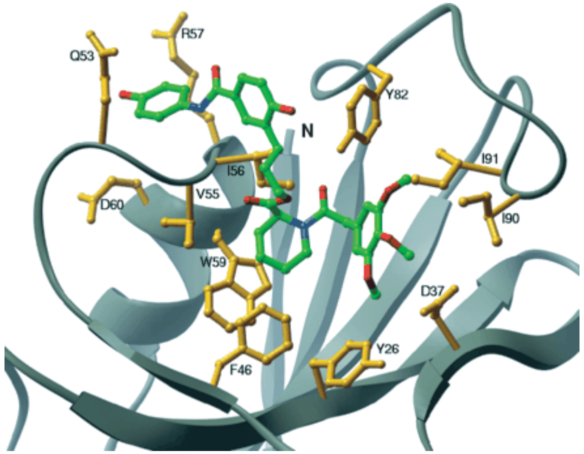
Heteronuclear Decoupling

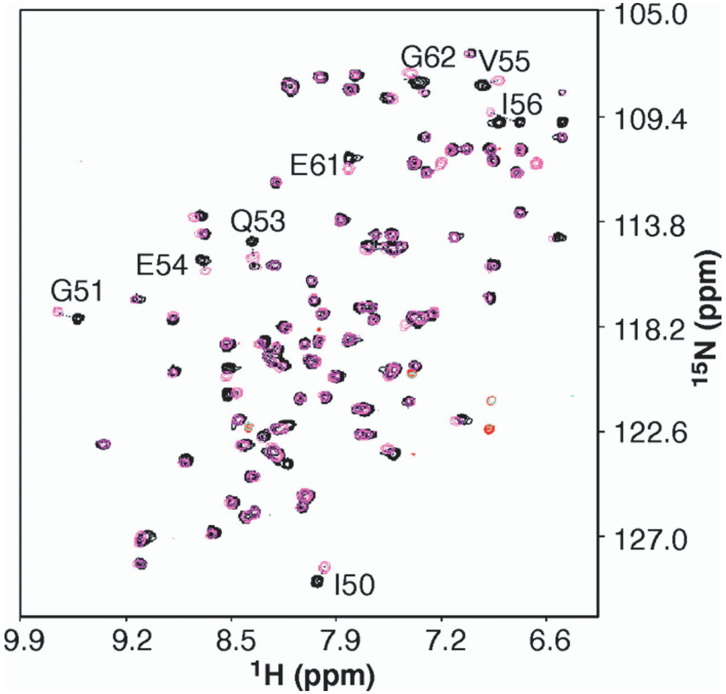


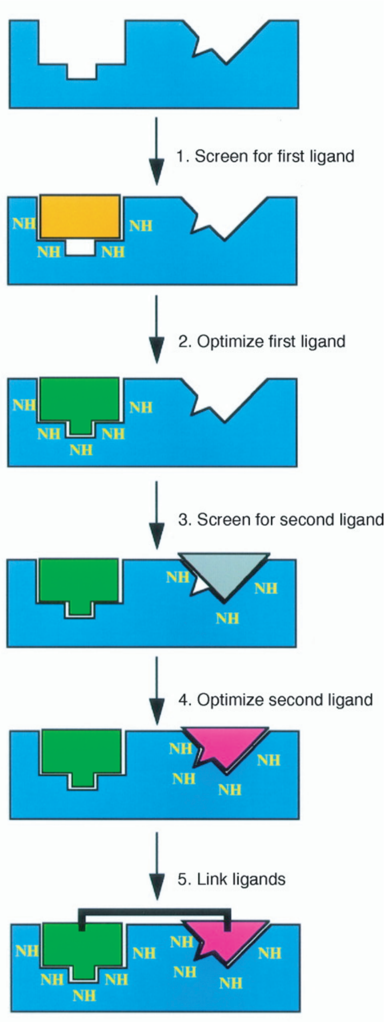
Discovering High-Affinity Ligands for Proteins: SAR by NMR

Suzanne B. Shuker, Philip J. Hajduk, Robert P. Meadows, Stephen W. Fesik *
Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064, USA.

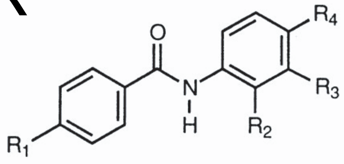
Science, Vol 274, Issue 5292, 1531-1534 , 29 November 1996

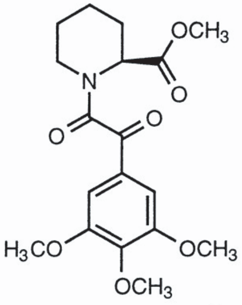






1. Screen for first ligand
2. Optimize first ligand
3. Screen for second ligand
4. Optimize second ligand
5. Link ligands

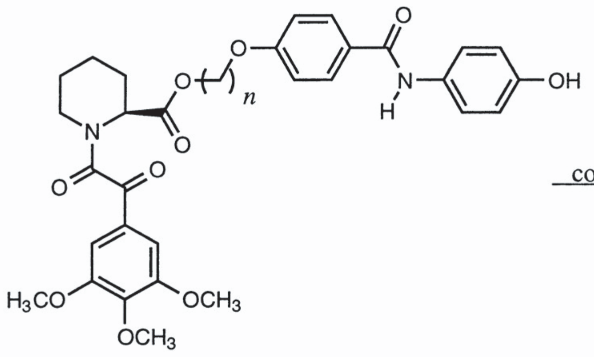




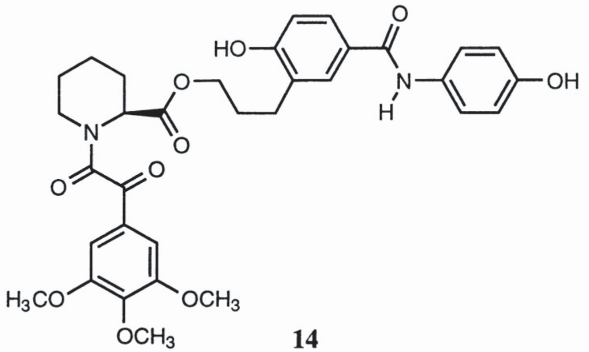
2

$K_d = 2\ \mu M$

compound	R_1	R_2	R_3	R_4	K_d
3	OH	OH	H	H	0.8 mM
4	H	OH	H	H	2.1 mM
5	OH	H	H	H	0.6 mM
6	H	H	OH	H	1.4 mM
7	H	H	H	OH	0.5 mM
8	H	H	H	H	9.5 mM
9	OH	H	H	OH	0.1 mM



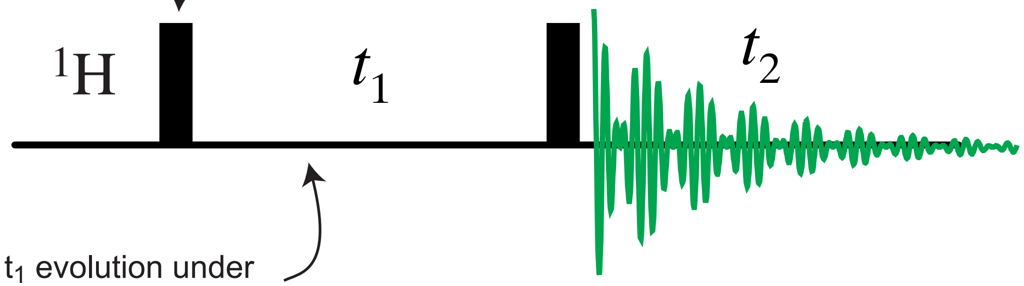
compound	*n*	K_d
10	3	19 nM
11	4	32 nM
12	5	49 nM
13	6	228 nM
14	-	49 nM



14

Cornerstone NMR Experiments: COSY

(Homonuclear Correlation Spectroscopy)

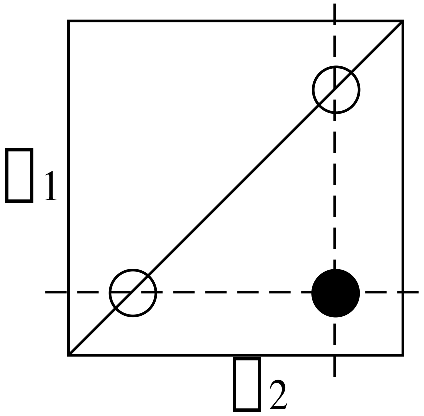


first π/2 pulse excites proton coherence

second π/2 pulse transfers coherence to J-coupled neighbors

t_1 evolution under ^1H chemical shift

The COSY experiment yields a correlation between resonances of J coupled protons.



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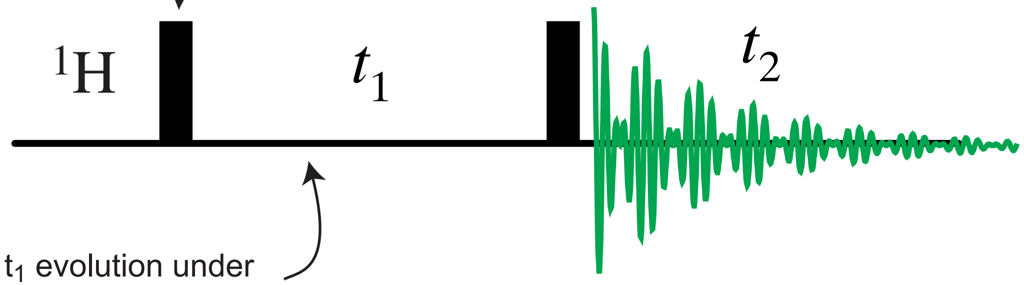


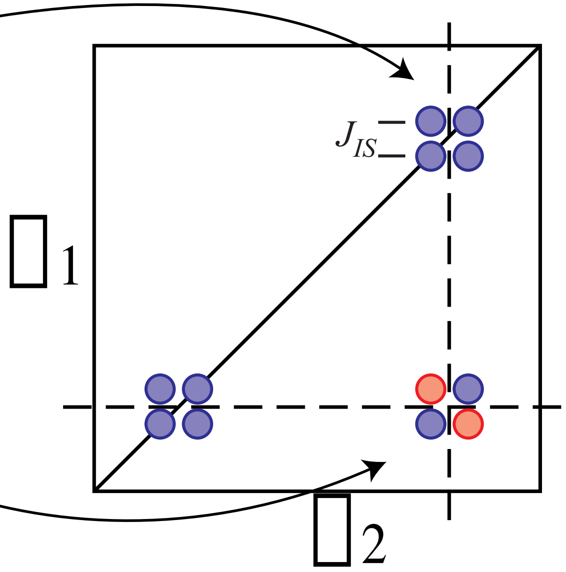
first π/2 pulse excites proton coherence

second π/2 pulse transfers coherence to J-coupled neighbors

t_1 evolution under ^{1}H chemical shift

A product operator analysis of this sequence yields

$$\sigma(t_1,t_2) = -I_x \sin(\Omega_I t_1)\cos\pi J_{IS}t_1 \sin(\Omega_I t_2)\cos\pi J_{IS}t_2$$
$$-S_x \sin(\Omega_I t_1)\sin\pi J_{IS}t_1 \sin(\Omega_S t_2)\sin\pi J_{IS}t_2$$
$$+\ldots.$$



Cornerstone NMR Experiments: COSY

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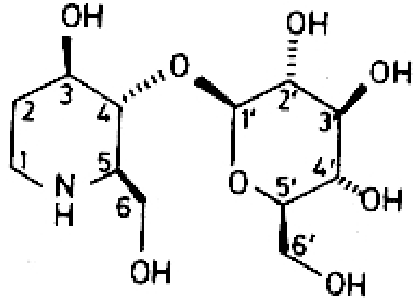


How can we assign this spectrum?

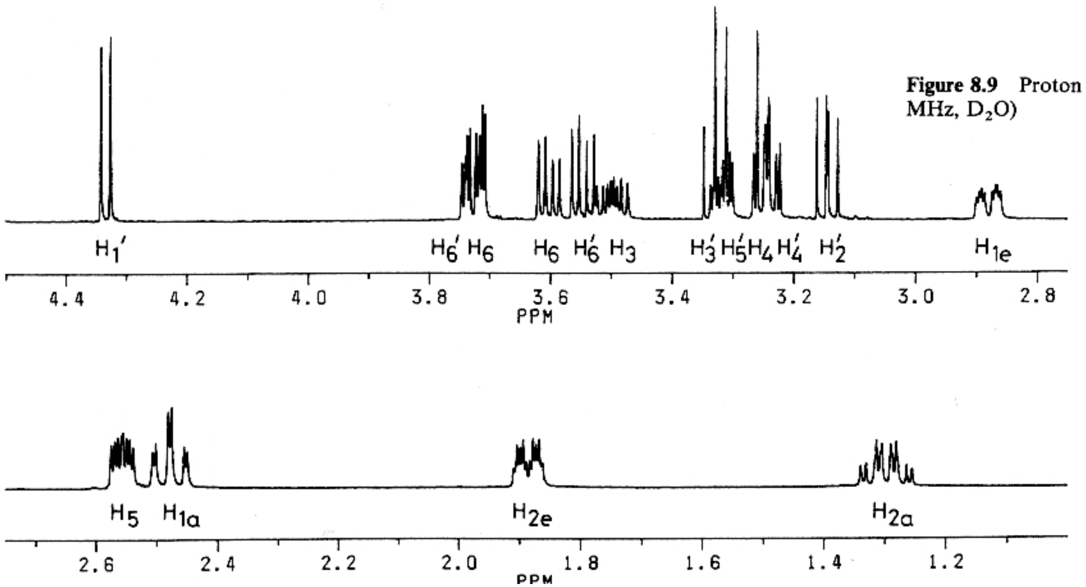


Figure 8.9 Proton spectrum of **1** (500 MHz, D_2O)

The COSY experiment yields a correlation between resonances of J coupled protons.

Cornerstone NMR Experiments: COSY

(Homonuclear Correlation Spectroscopy)

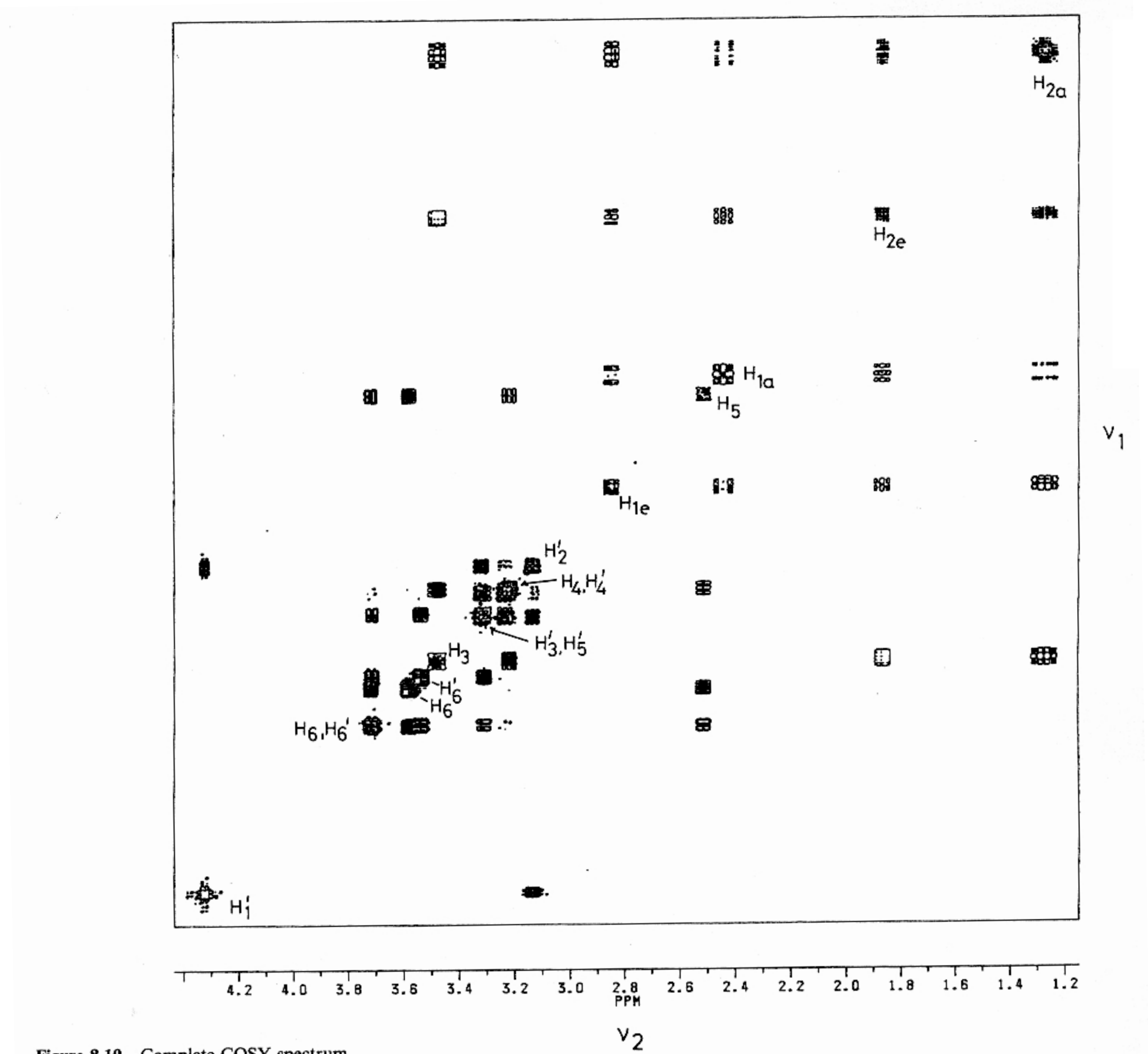
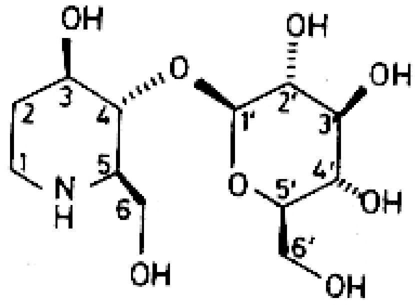


Figure 8.10 Complete COSY spectrum of **1**.



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Cornerstone NMR Experiments: COSY

(Homonuclear Correlation Spectroscopy)

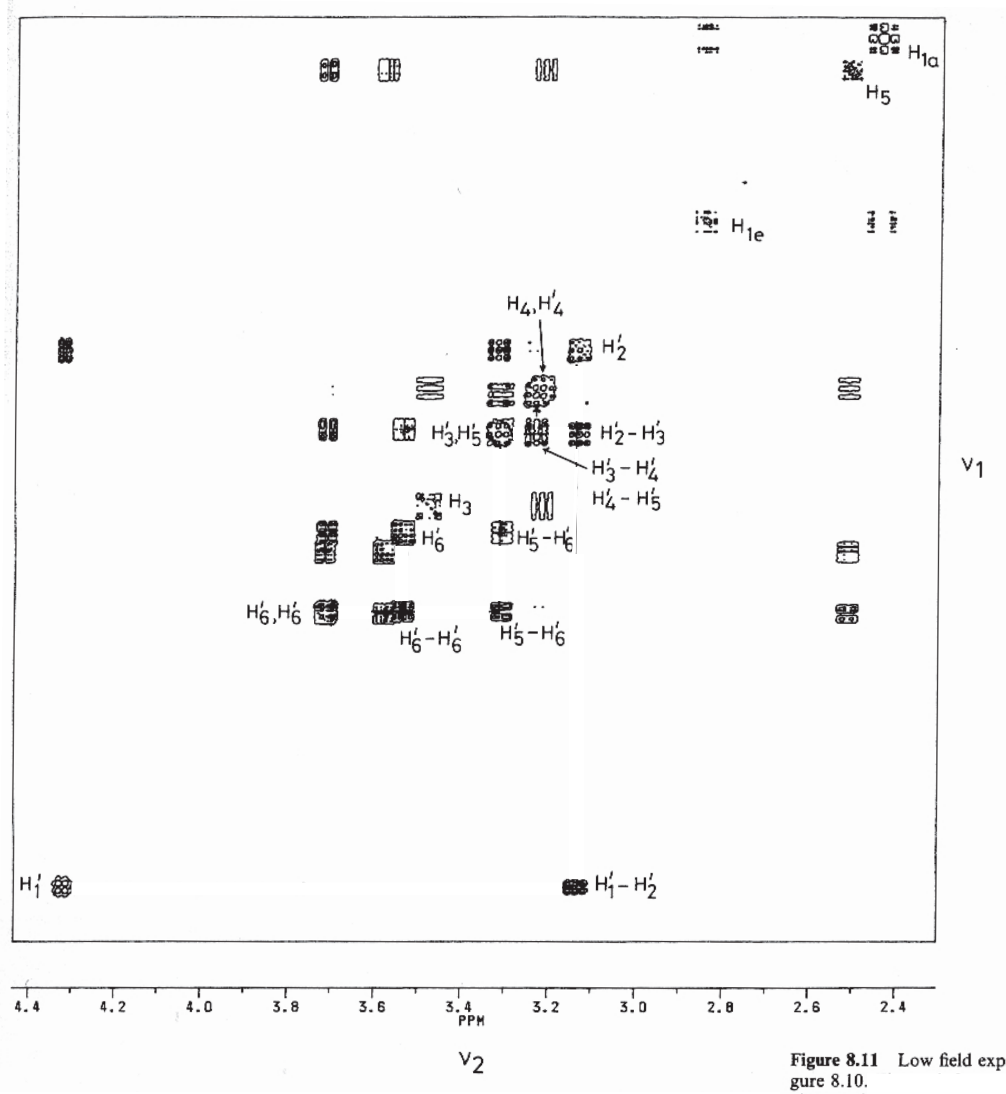
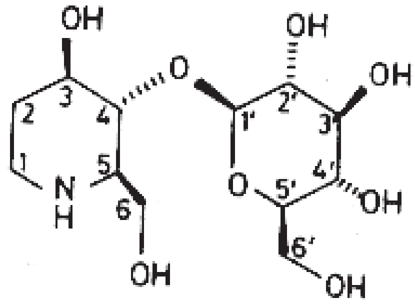


Figure 8.11 Low field expansion of Figure 8.10.



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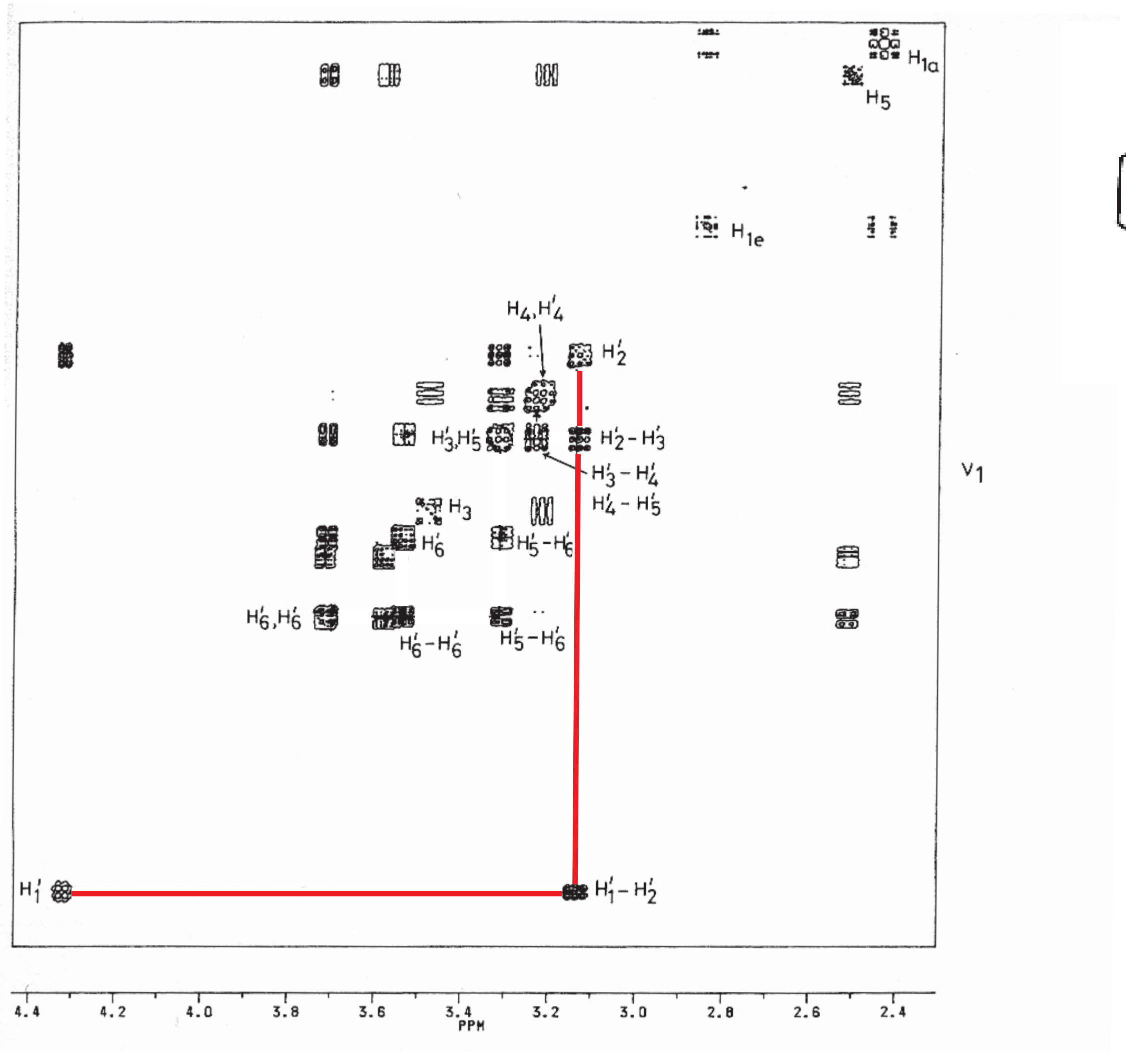
Cornerstone NMR Experiments: COSY

(Homonuclear Correlation Spectroscopy)



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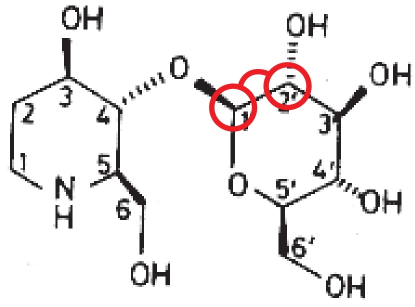

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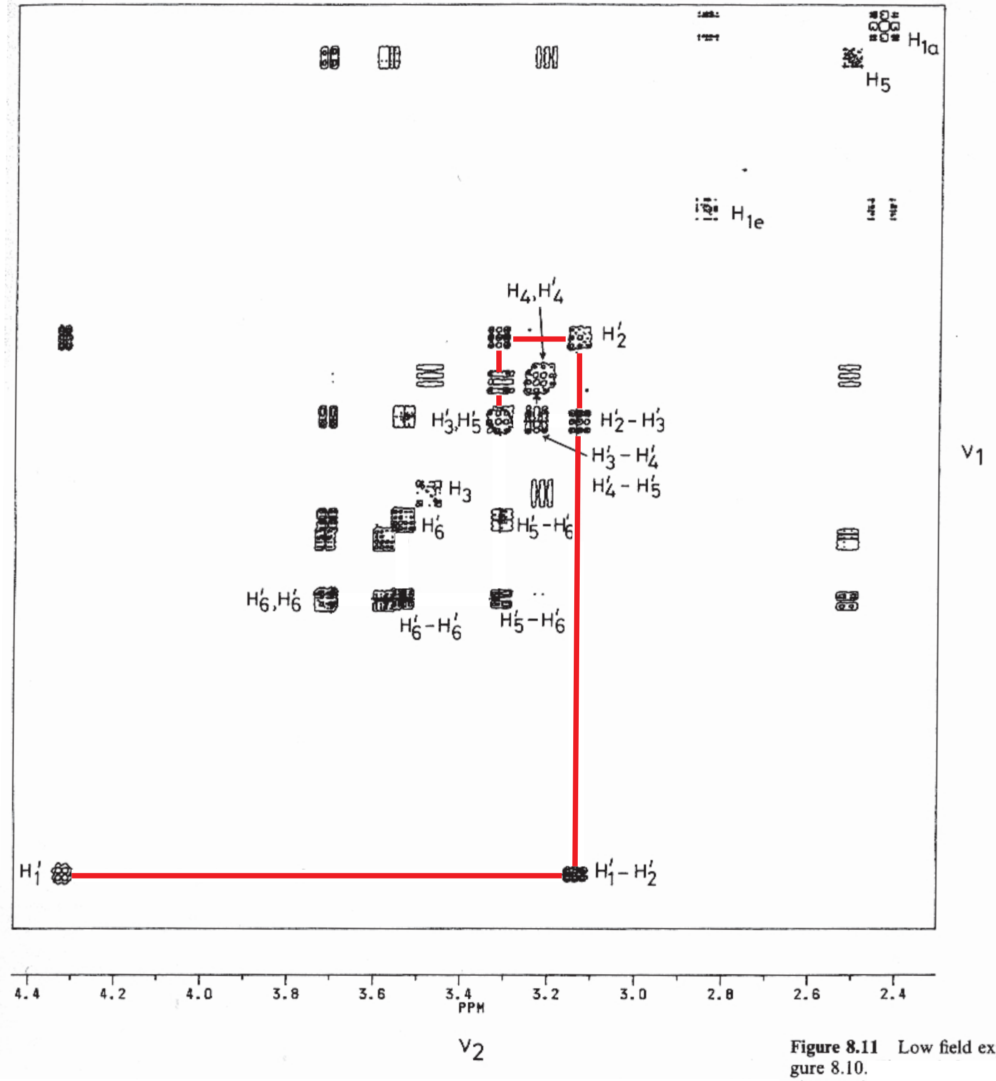
(Homonuclear Correlation Spectroscopy)



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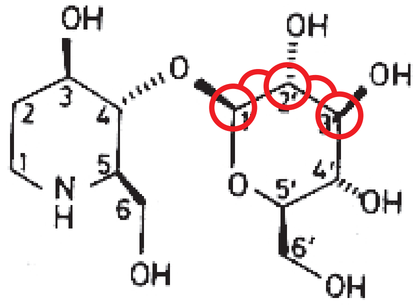

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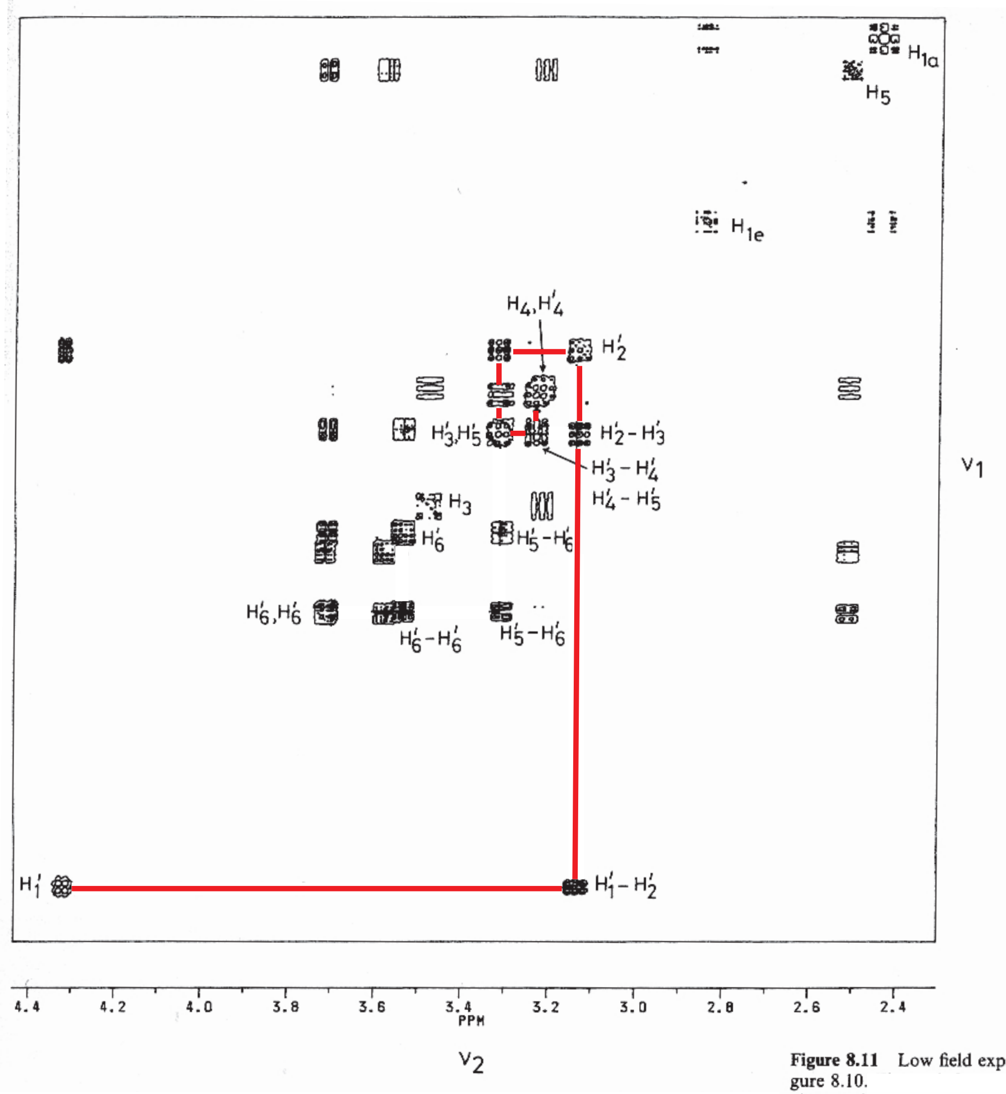

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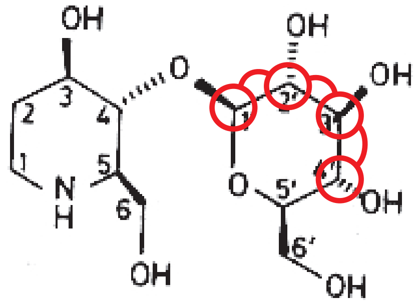
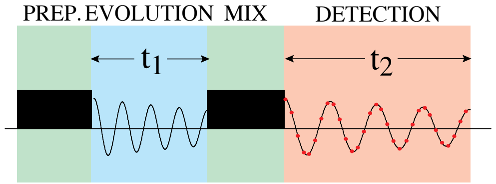
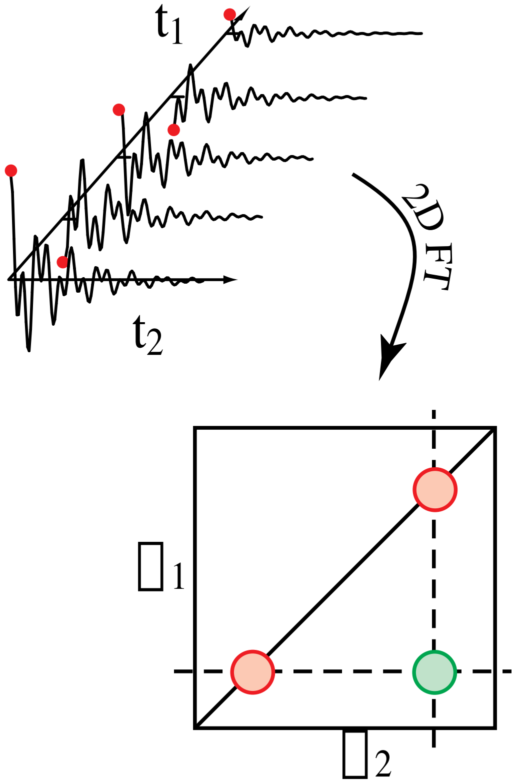


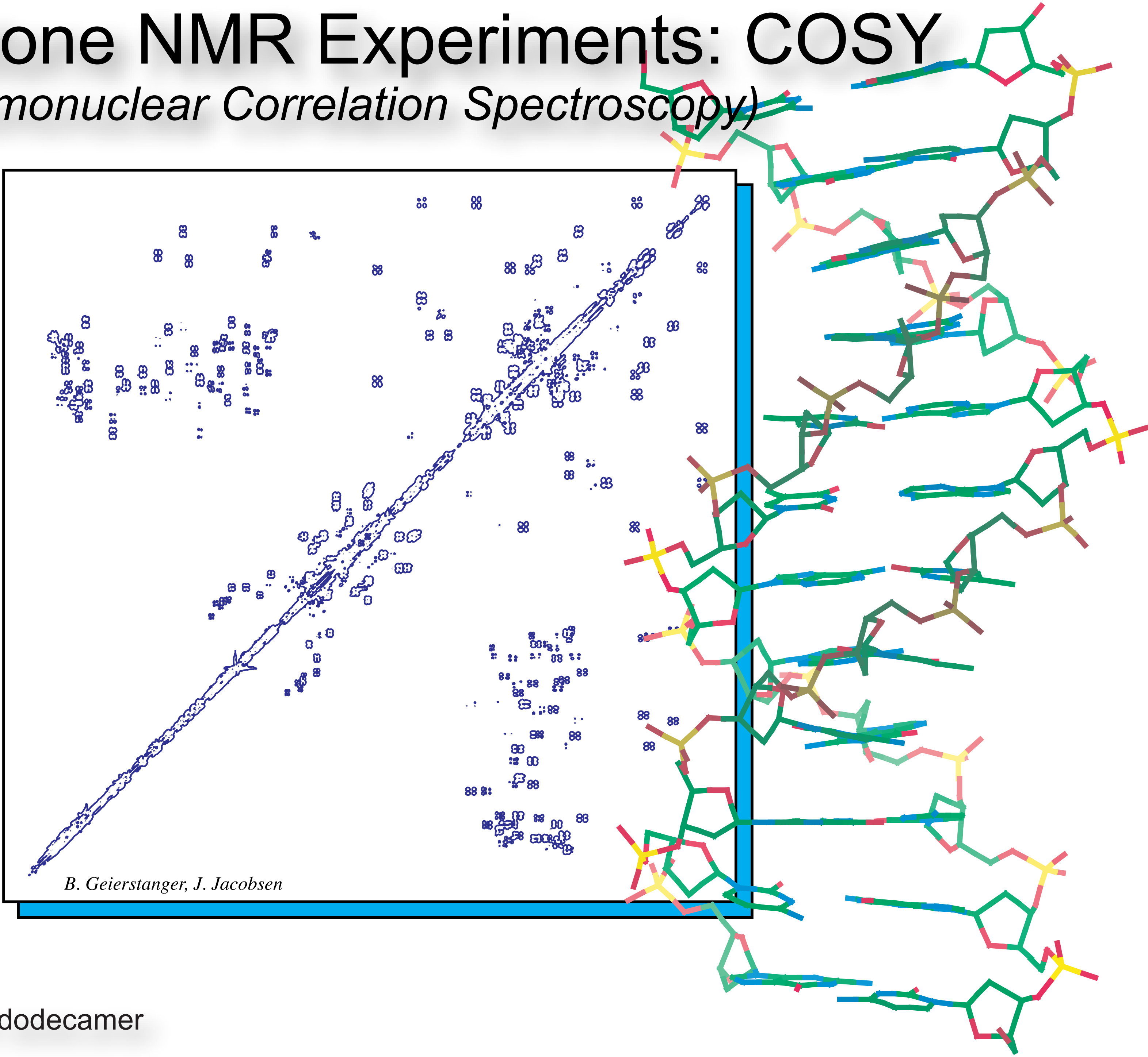
The COSY experiment yields a correlation between resonances of J coupled protons.

Cornerstone NMR Experiments: COSY

(Homonuclear Correlation Spectroscopy)



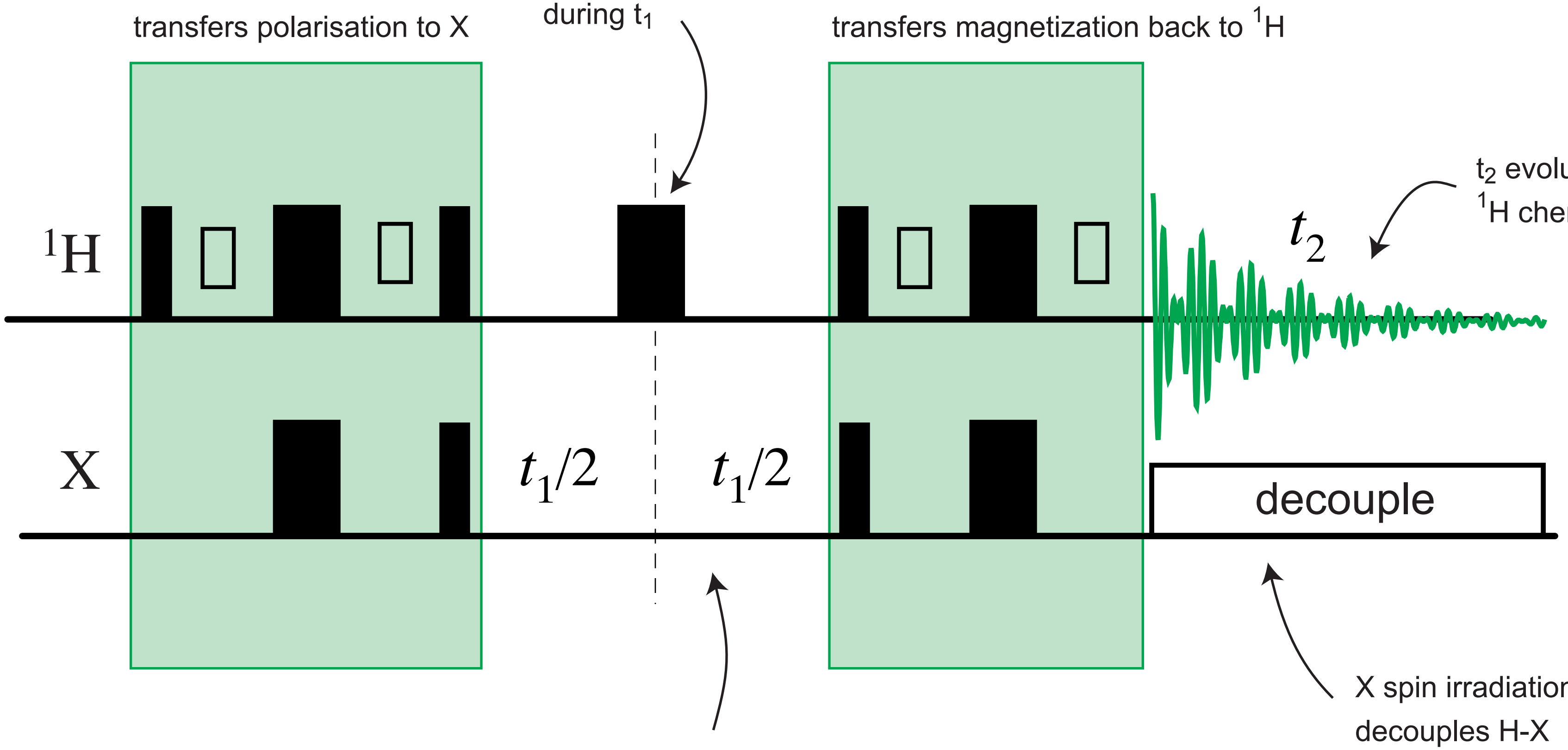



B. Geierstanger, J. Jacobsen

DQF-COSY Spectrum of a DNA dodecamer

Cornerstone NMR Experiments: HSQC

(Heteronuclear Single Quantum Correlation)



first INEPT block
transfers polarisation to X

□-pulse refocuses
H-X J coupling
during t_1

second INEPT block
transfers magnetization back to ^1H

t_2 evolution under
^1H chemical shift

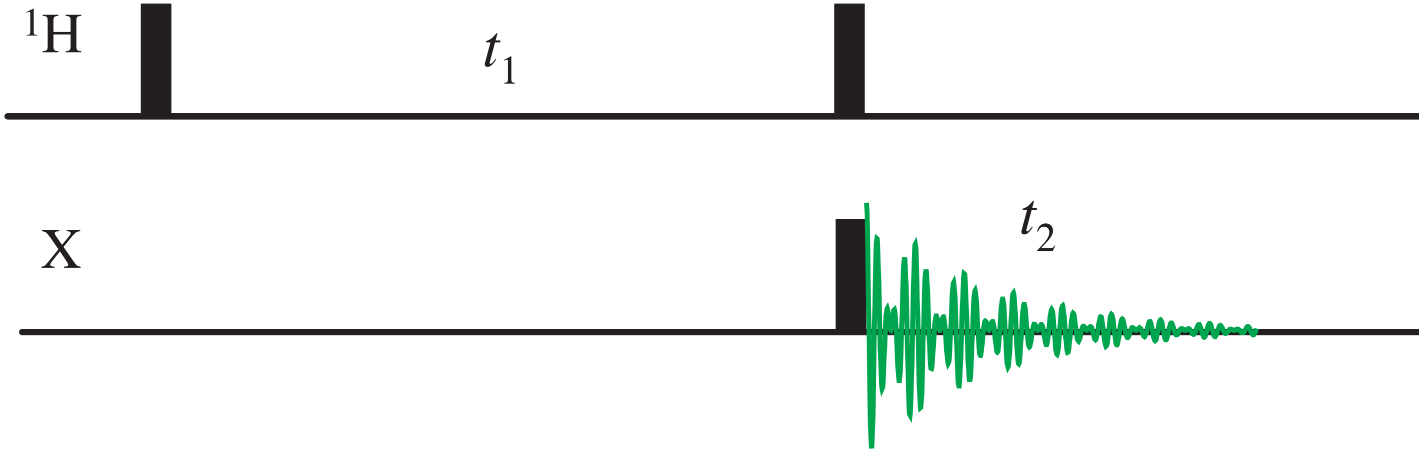
t_1 evolution under
X chemical shift

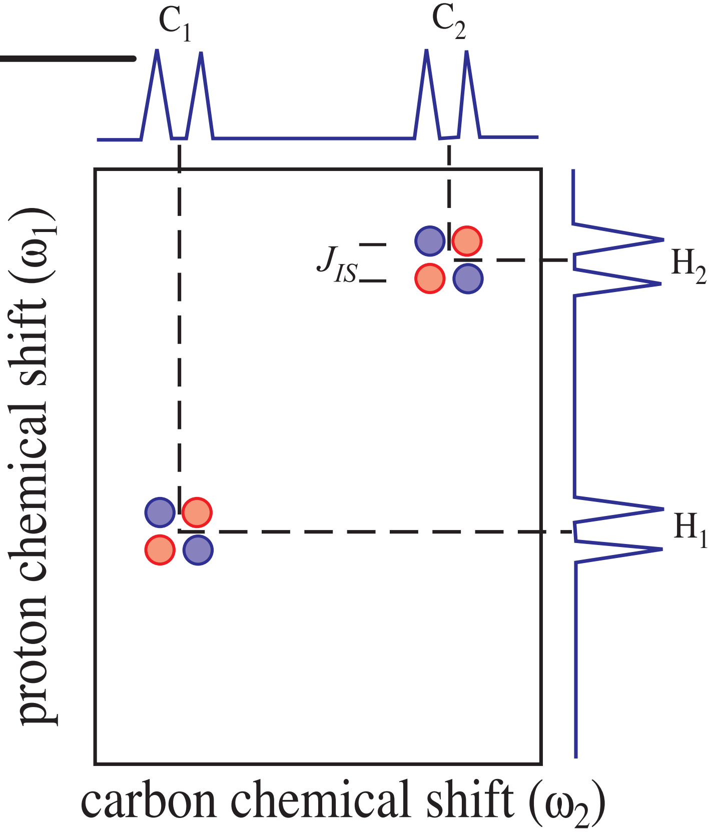
X spin irradiation
decouples H-X
J coupling during t_2

The HSQC experiment yields a correlation between proton resonances and the resonances of directly bonded X nuclei. The experiment has the sensitivity of protons. It uses heteronuclear J couplings for coherence transfer, to establish the correlations, but the couplings are decoupled during both acquisition periods.

X is typically ^{15}N or ^{13}C.

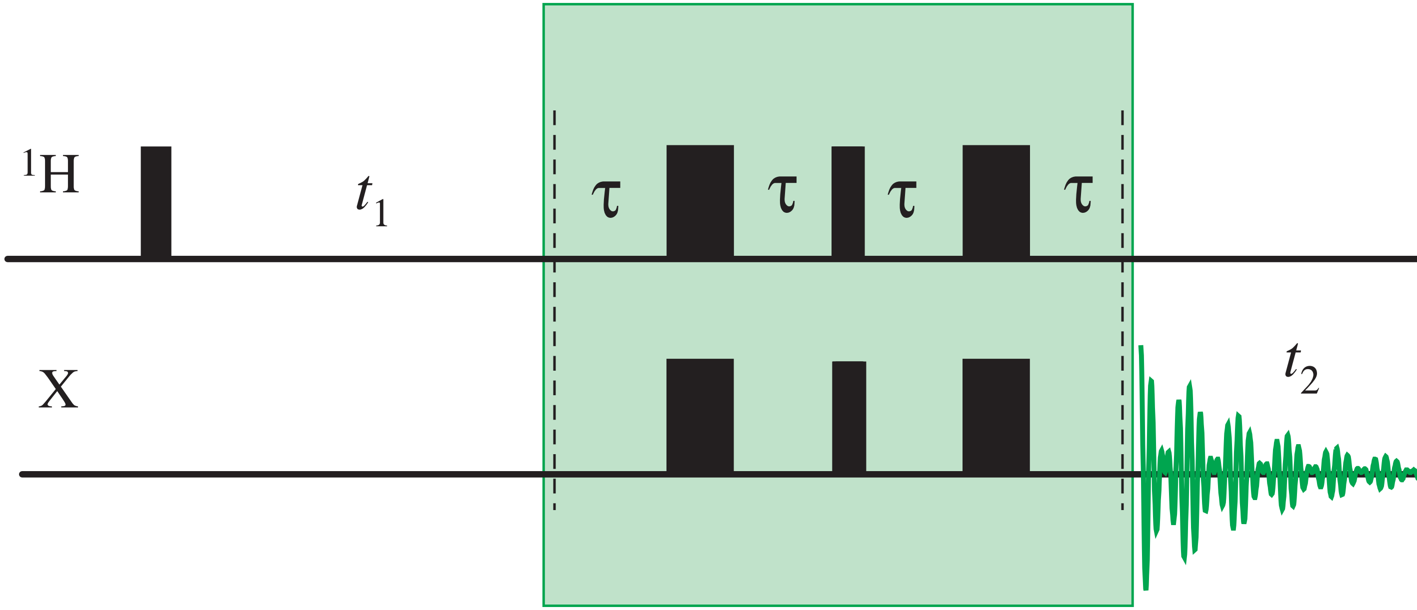
Building NMR Experiments:
Heteronuclear Correlations

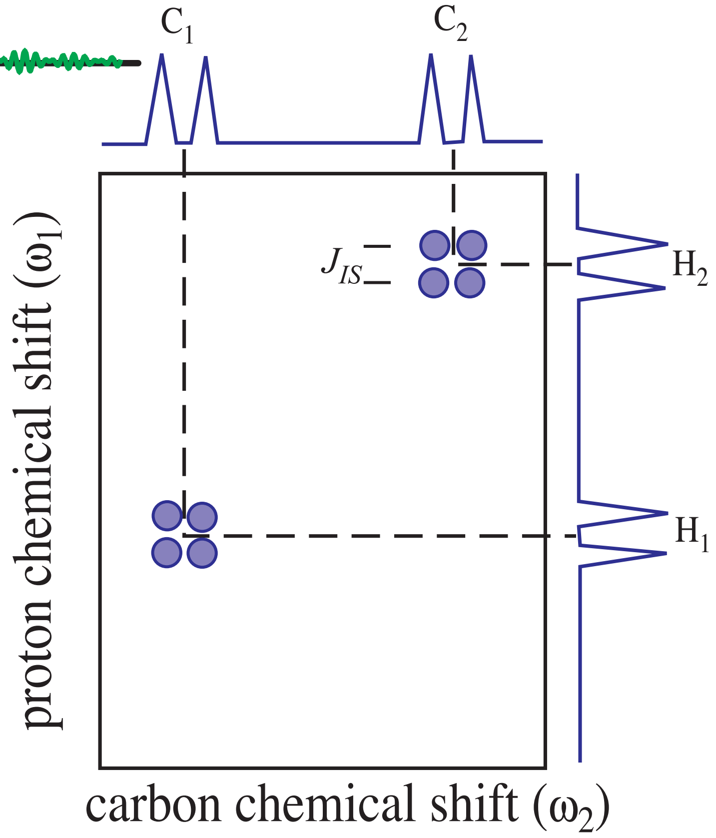




This can be seen as a heteronuclear COSY experiment, where only the cross-peak region of the spectrum, centered on the ^{1}H chemical shift in ω_1 and the (here) ^{13}C shift in ω_2.

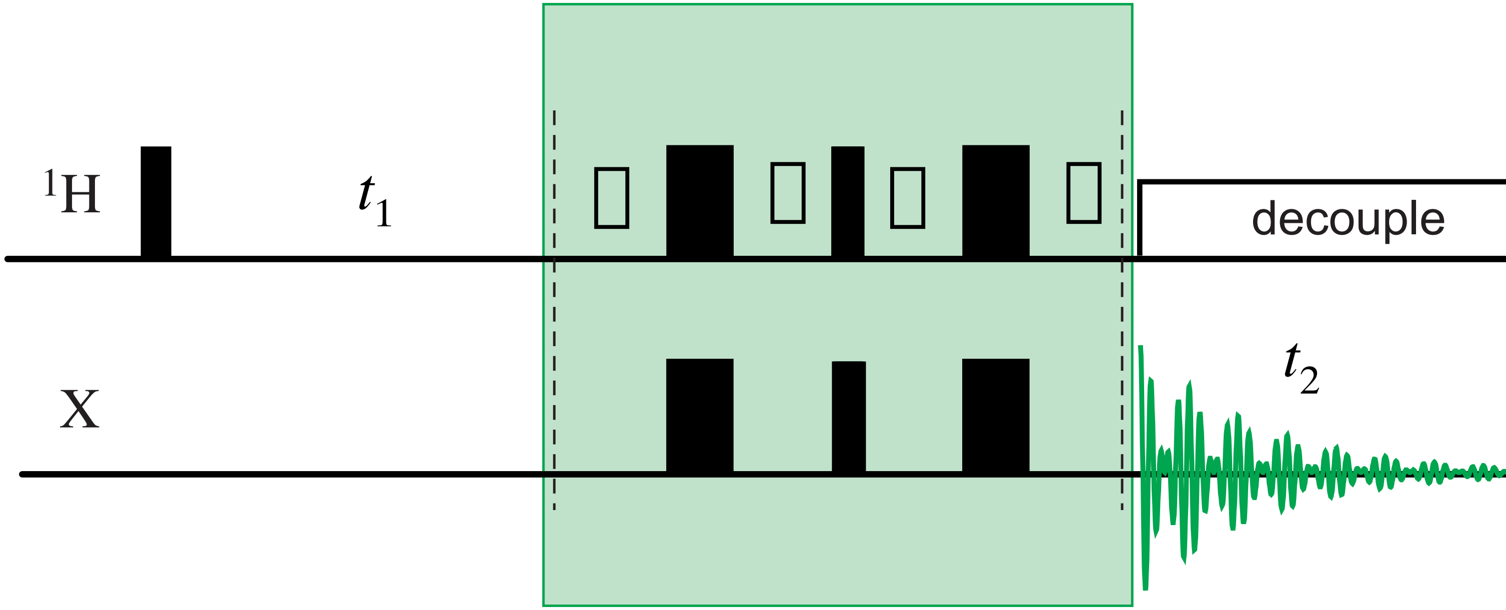
Building NMR Experiments:
Heteronuclear Correlations

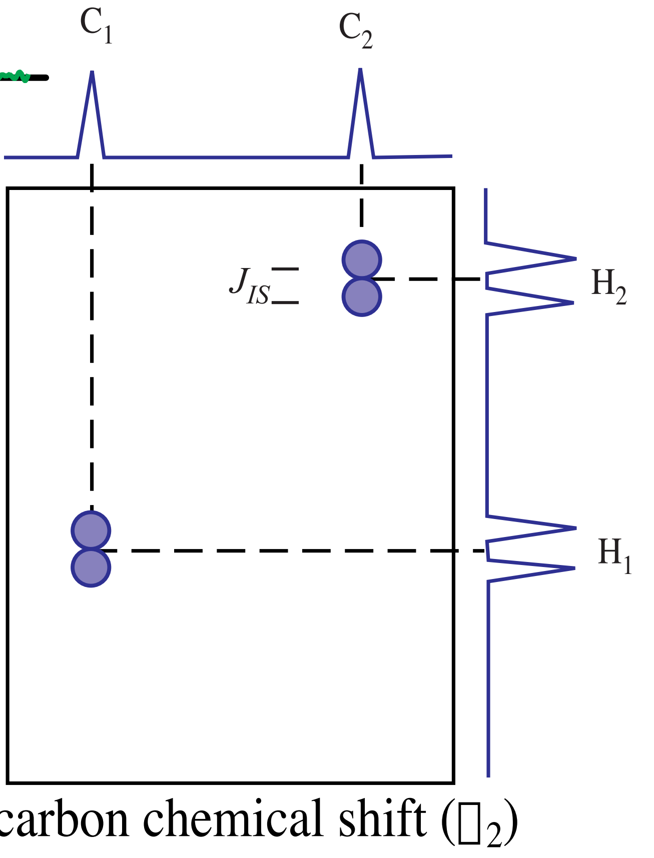




By adding a refocused INEPT block for polarization transfer, instead of a single pulse on both nuclei, the anti-phase peak structure is transformed into an in-phase structure.

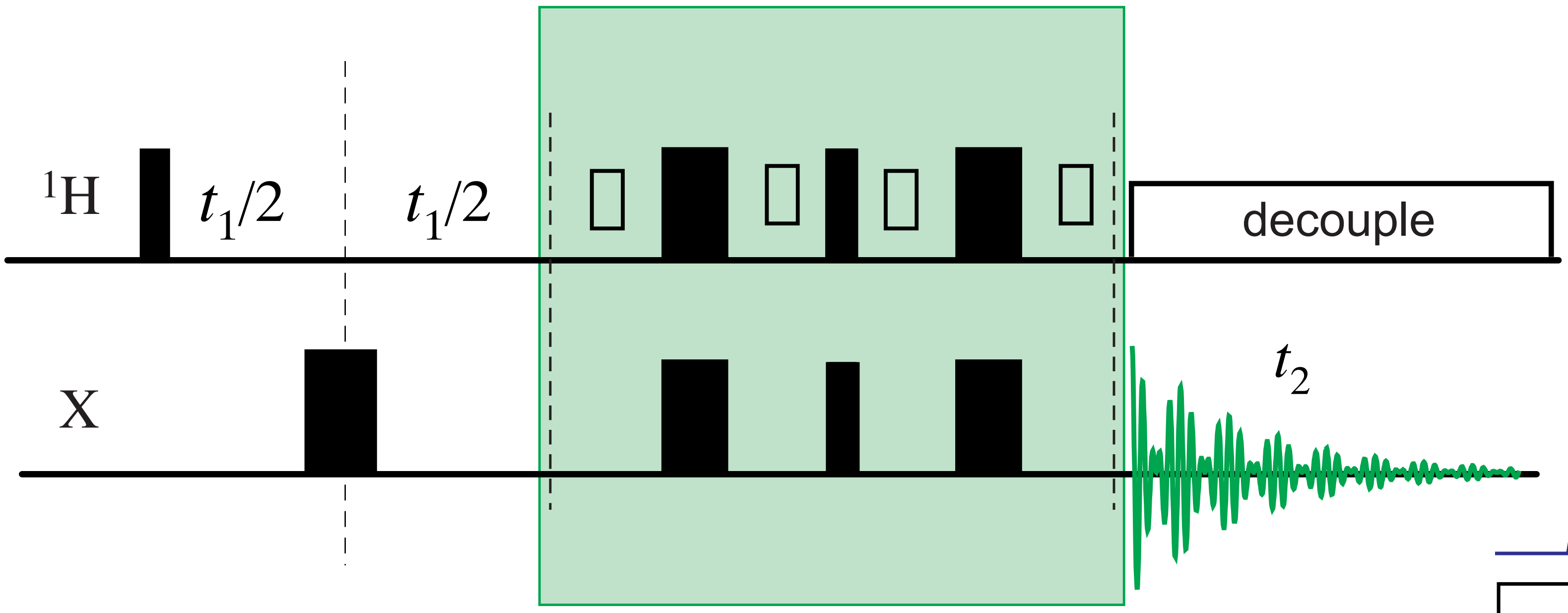
Building NMR Experiments: Heteronuclear Correlations





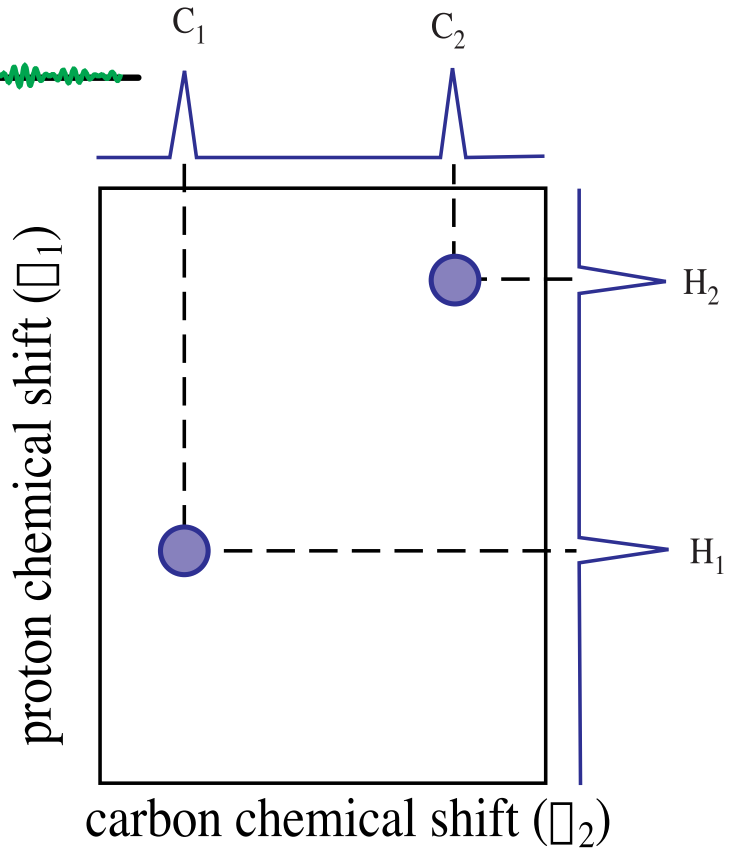
The in-phase peak structure now allows the addition of heteronclear decoupling during acquisition. This removes the splitting due to J couplings in ω_2. This simplifies the spectrum and doubles the signal intensity.

Building NMR Experiments: Heteronuclear Correlations

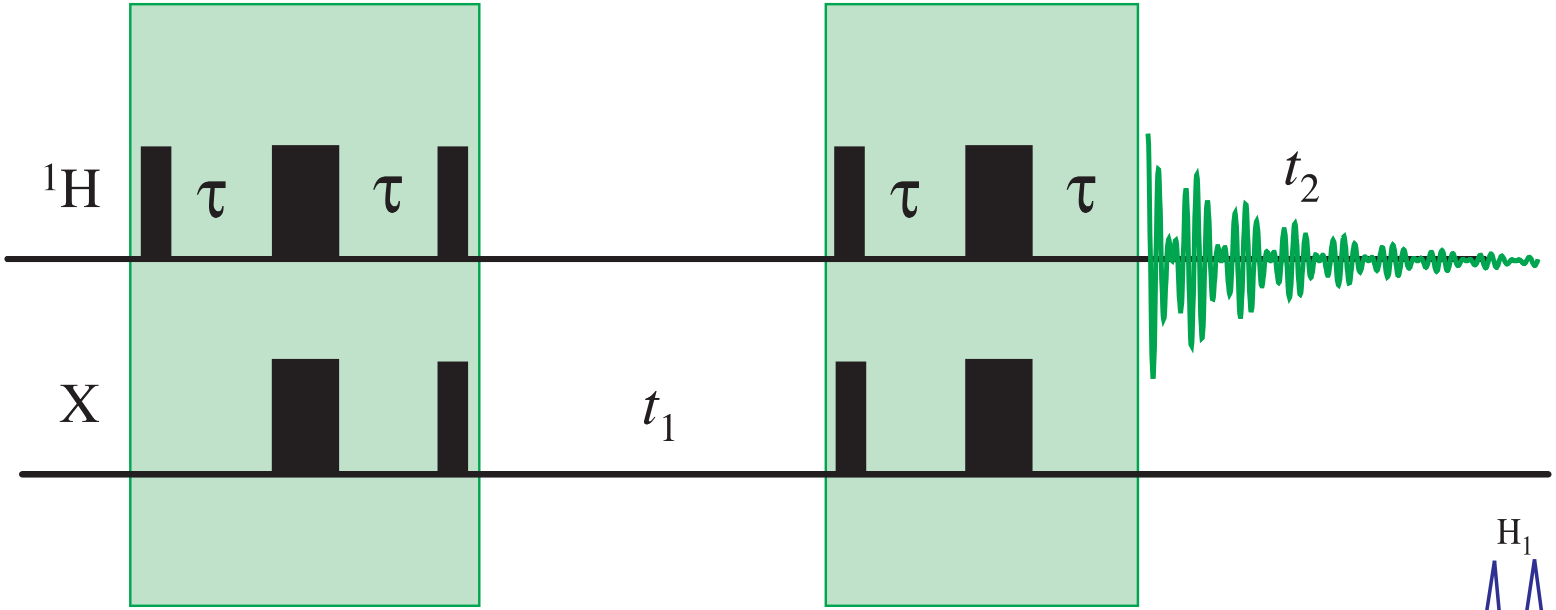


Heteronuclear decoupling in ω_1 can be achieved by simply adding a π pulse applied to the X nucleus in the center of t_1. This will not affect the ^{1}H shifts or ^{1}H-^{1}H couplings during t_1, but it will refocus the heteronuclear J couplings. Homework: Verify this with a calculation of the average propagator for the sequence.

This further simplifies the spectrum, and again doubles the signal intensity for an XH group. (What is the gain for a XH_2 or XH_3 group?)



Building NMR Experiments: Heteronuclear Correlations

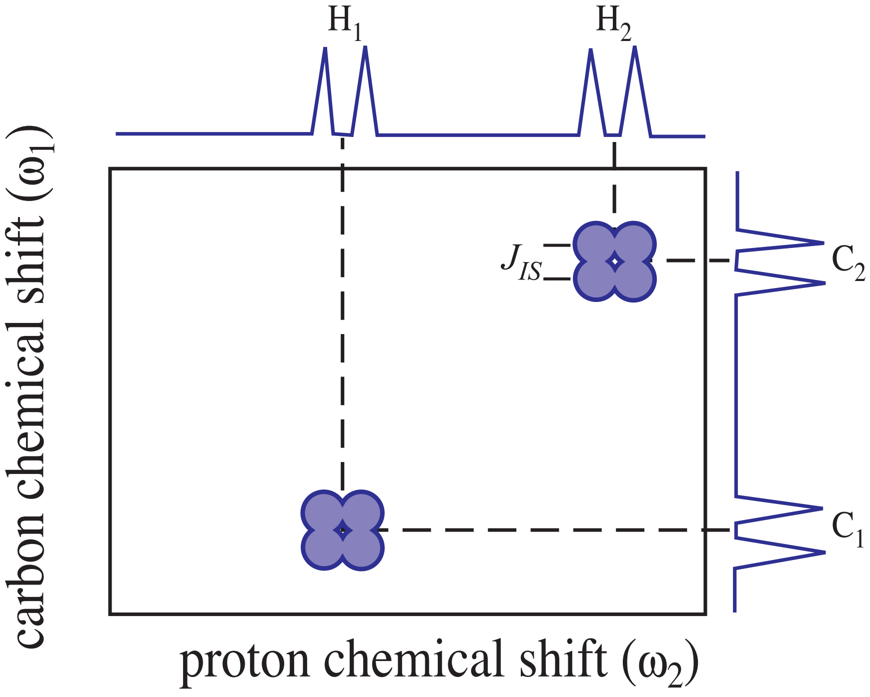


Detecting the signal in in t_2 on ^{1}H instead of ^{13}C is more sensitive because of the larger magnetic moment of ^{1}H. The gain is by a factor

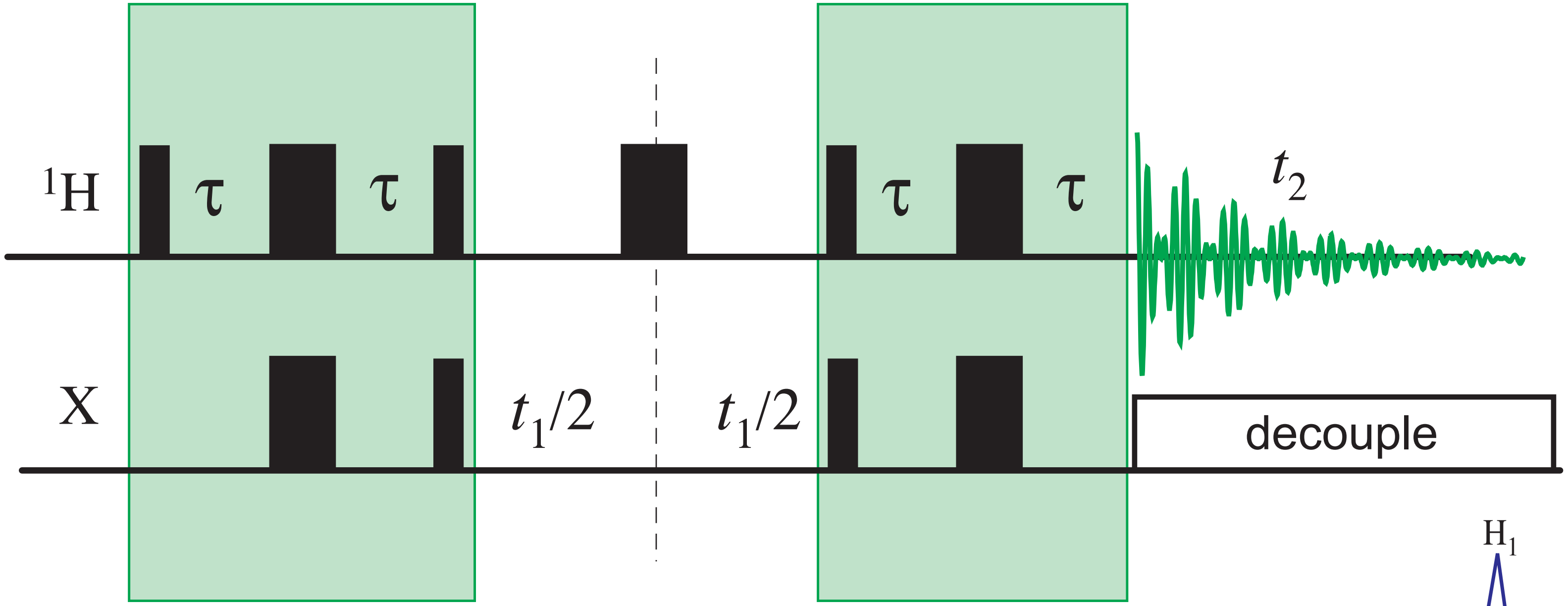
$$\left(\frac{\gamma_H}{\gamma_C}\right)^{3/2} = 8$$

(if the linewidths are the same for both nuclei.)

An optimum sequence thus starts with the high ^{1}H polarization, transfers to ^{13}C for t_1, and transfers back to ^{1}H for t_2.

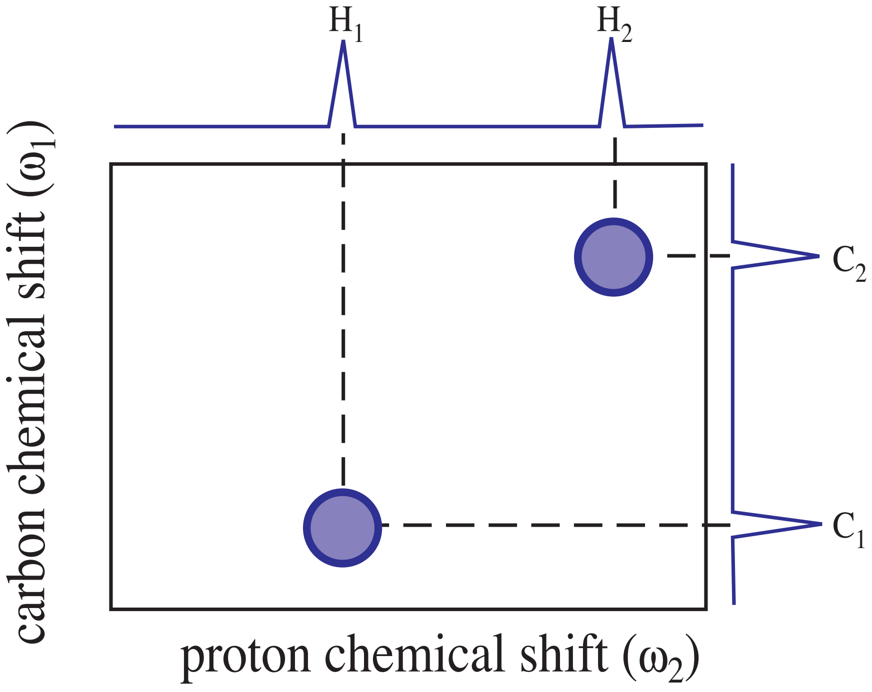


Building NMR Experiments: Heteronuclear Correlations



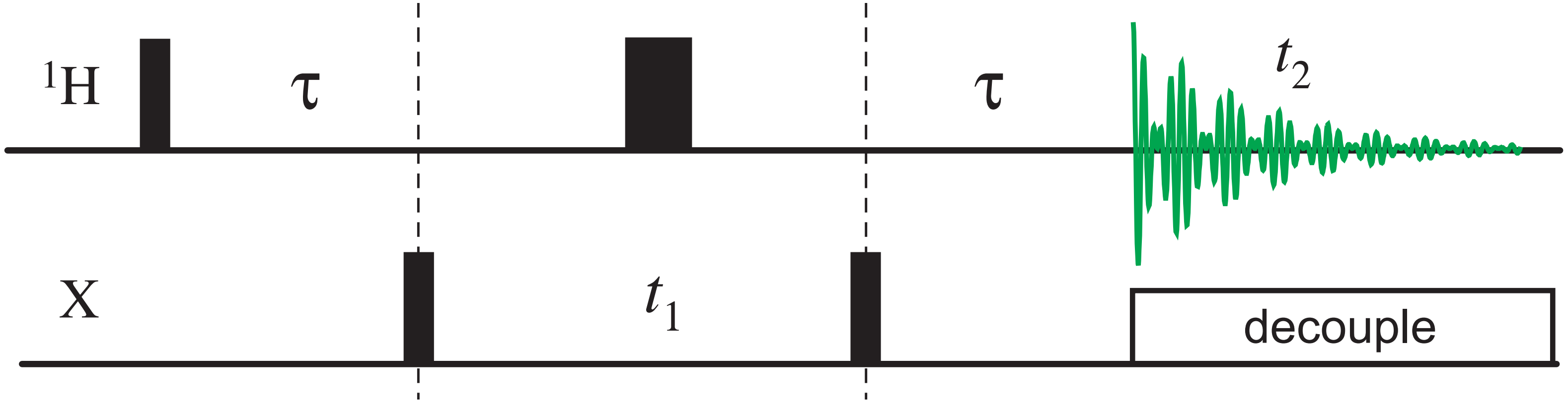
Heteronuclear J-decoupling can be added in both dimensions to simplify the spectrum and increase the sensitivity, as before.

This sequence is 8 x 2 x 2 = 32 times more sensitive for a CH group than the original heteronuclear COSY experiment we started with. That yields a factor 322 = 1024 in s/n per unit time. (Or overnight instead of 1.5 years)

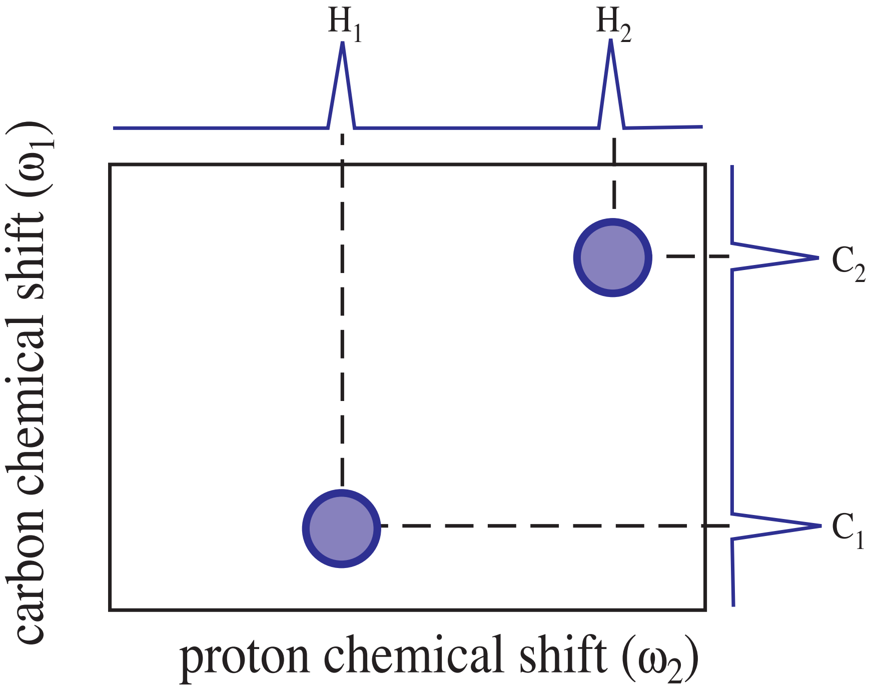


Building NMR Experiments:

HMQC (Heteronuclear Multiple-Quantum Coherence)



This very simple sequence also yields a carbon-proton chemical shift correlation.... how?



Building NMR Experiments

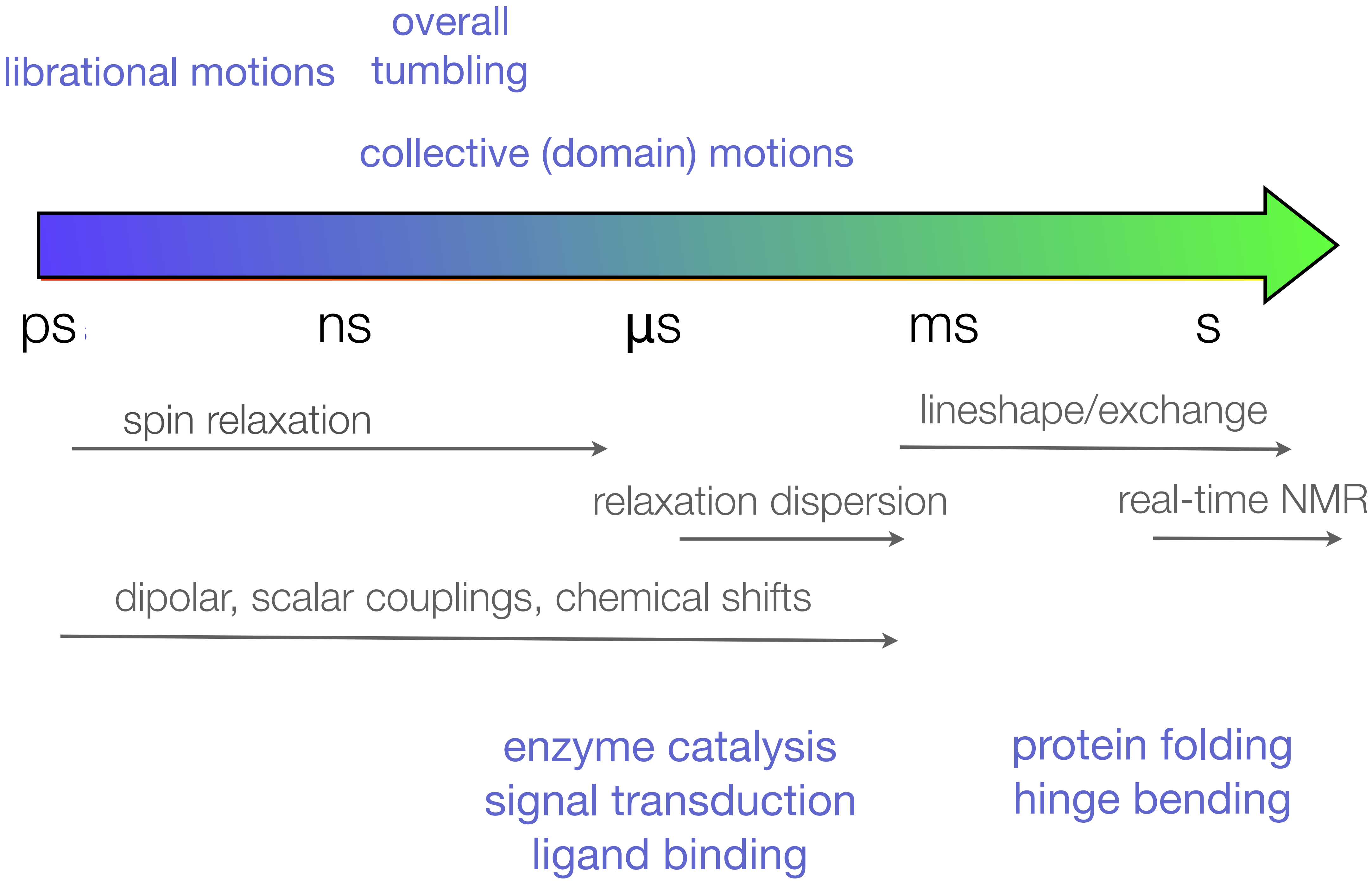
Some open questions for the curious scholar...

- What is the observable in t_1 in HSQC?
  (The signal is in-phase in t_1. What operator is present at the beginning of t_1? Is it "observable"? Can anything be observed then?)

- What is the observable in t_1 in HMQC?

- What is the difference between the pulse sequence for DQF-COSY and EXSY/NOESY?
  (Why/how do these two experiments give completely different results from the same pulse sequence?)

Recap Ba3: Timescales of Motion



librational motions

overall tumbling

collective (domain) motions

ps

ns

µs

ms

s

spin relaxation

lineshape/exchange

relaxation dispersion

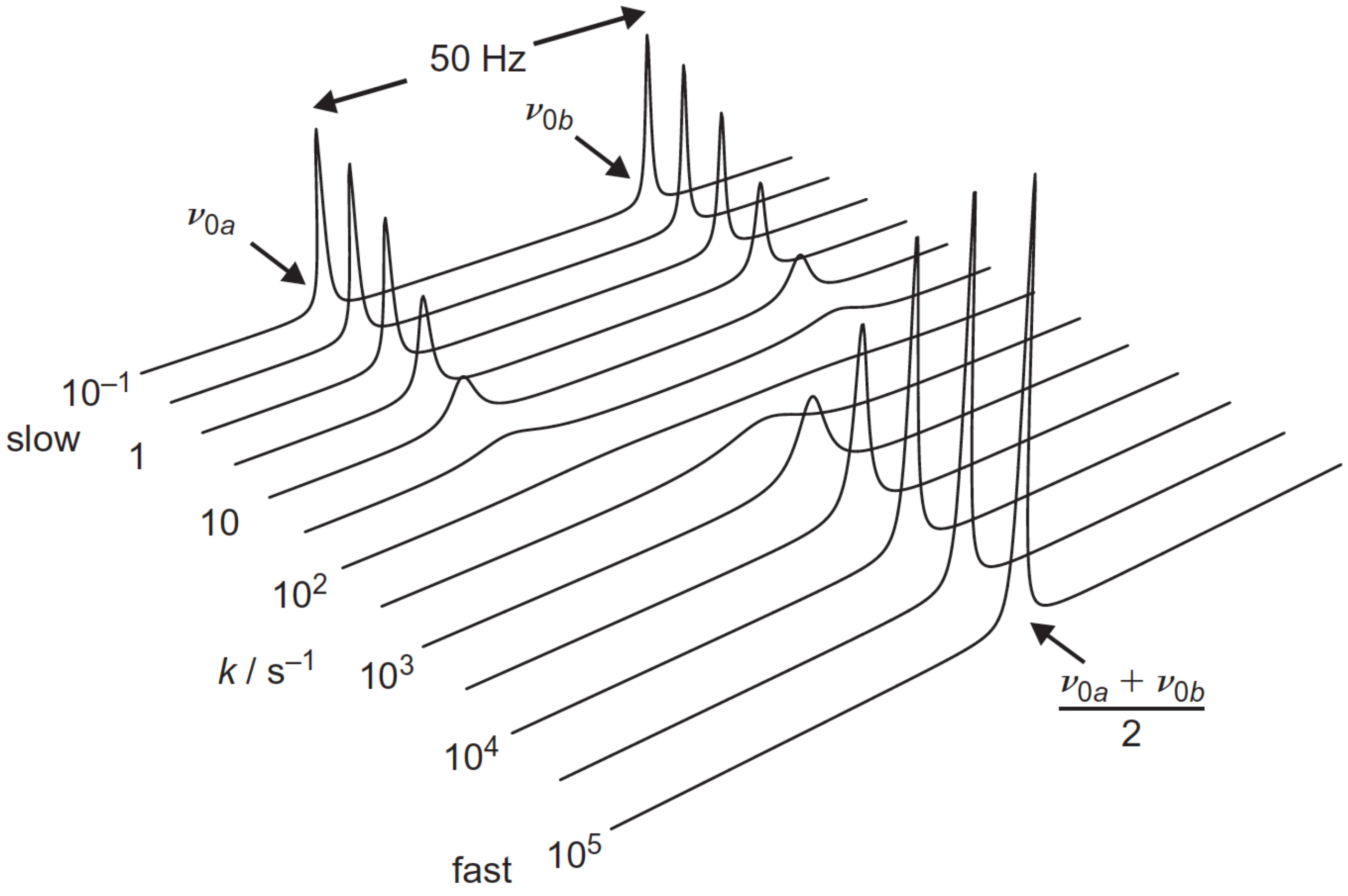
real-time NMR

dipolar, scalar couplings, chemical shifts

enzyme catalysis
signal transduction
ligand binding

protein folding
hinge bending

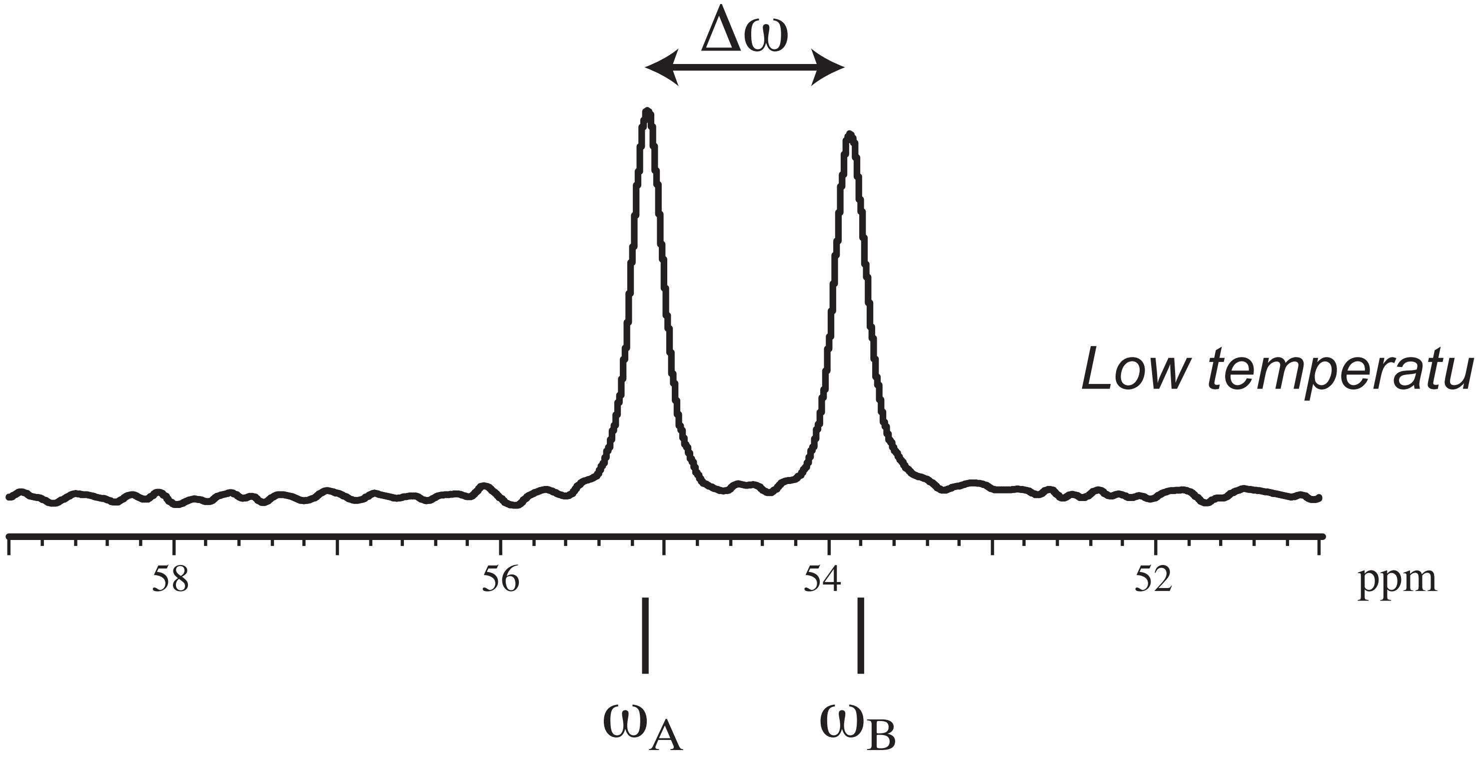
Recap Ba3: Determining Exchange from Lineshapes



Rates can be determined by numerically solving the modified Bloch equations with parameters that match the experiment, and by finding the values of the rate that produce a matching calculated NMR spectrum.

Here we show calculated NMR spectra for a pair of nuclei exchanging between two sites with equal populations (**symmetrical two-site exchange**). Spectra are shown for a range of values of the exchange rate constant k. The difference in resonance frequencies of the two sites, δv, is 50 Hz. The linewidths in the absence of exchange are 1 Hz.

Recap Ba3: Determining Exchange by NMR



Low temperature spectrum

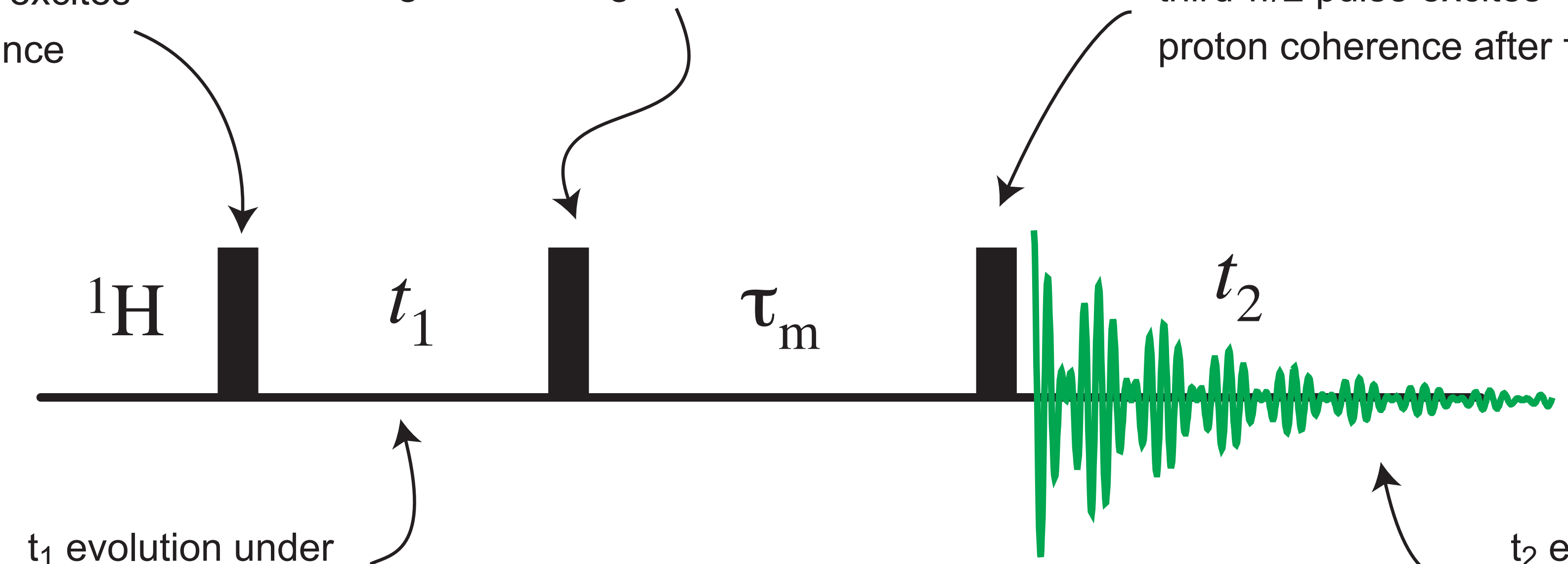
Consider a spectrum consisting of two singlets belonging to two protons. In the absence of exchange it yields the above spectrum. This is the low temperature spectrum.

As we increase the temperature, exchange processes may start to occur which interchange the two protons.

1. If the exchange rate $k << \Delta\omega$ (the SLOW MOTION regime):
There is no effect on the spectrum.

How Could We Measure Slow Exchange?

Two-Dimensional Exchange Spectroscopy (EXSY)



first π/2 pulse excites
proton coherence

second π/2 pulse converts coherence
to longitudinal magnetization

third π/2 pulse excites
proton coherence after τ period.

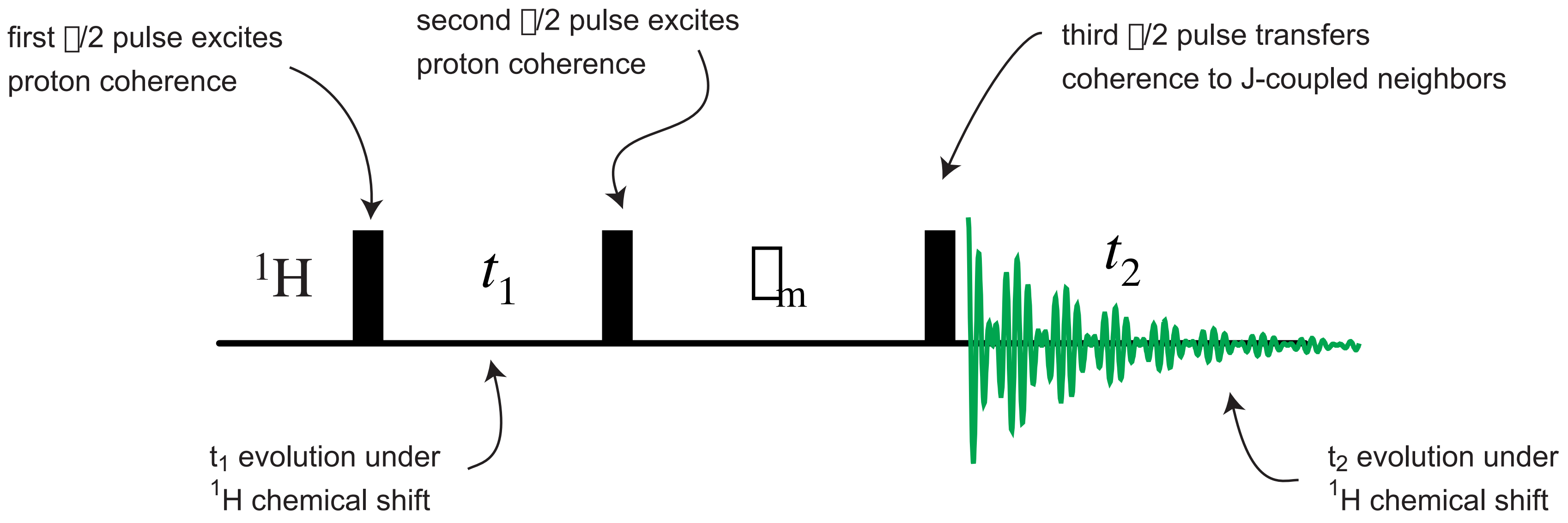
t_1 evolution under
^{1}H chemical shift

t_2 evolution under
^{1}H chemical shift

The EXSY experiment yields a correlation between resonances of protons that are exchanging on a timescale comparable to τ_m

Cornerstone NMR Experiments: NOESY/EXSY

(Determining Exchange and Internuclear Distances)



first π/2 pulse excites proton coherence

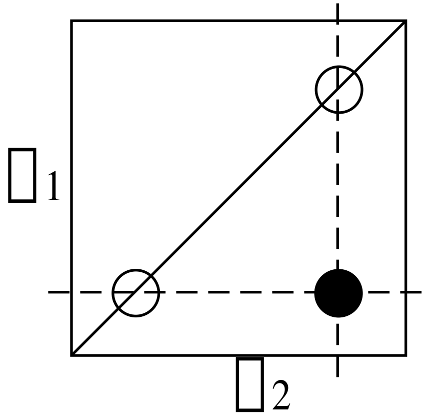
second π/2 pulse excites proton coherence

third π/2 pulse transfers coherence to J-coupled neighbors

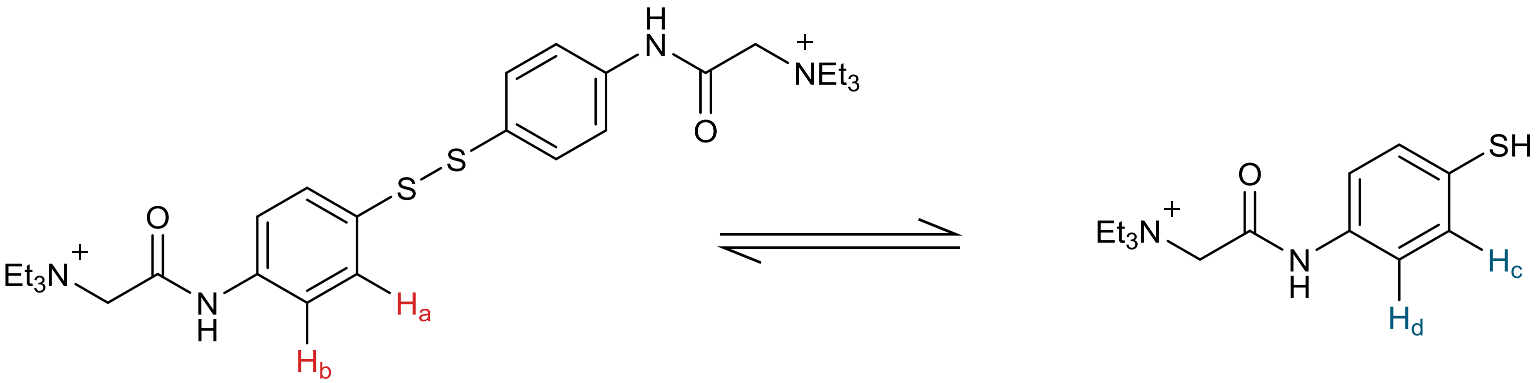
t_1 evolution under ^{1}H chemical shift

t_2 evolution under ^{1}H chemical shift

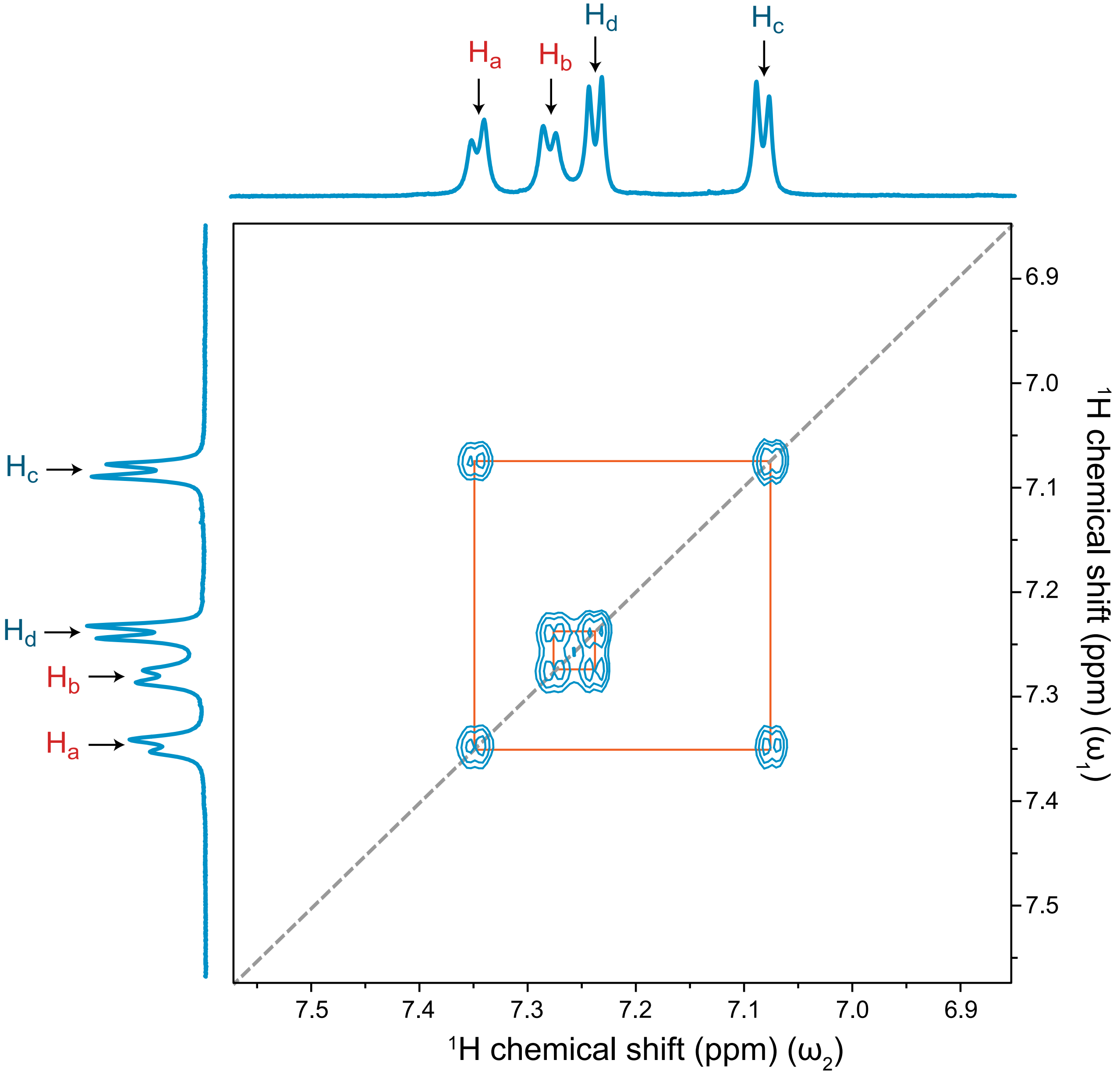
$$I_x(t_1) \xrightarrow{\pi/2} I_z(t_1) \xrightarrow{exchange} S_z(t_1,\tau_m) \xrightarrow{\pi/2} S_x(t_1,\tau_m,t_2)$$



Two-Dimensional Exchange Spectroscopy (EXSY)

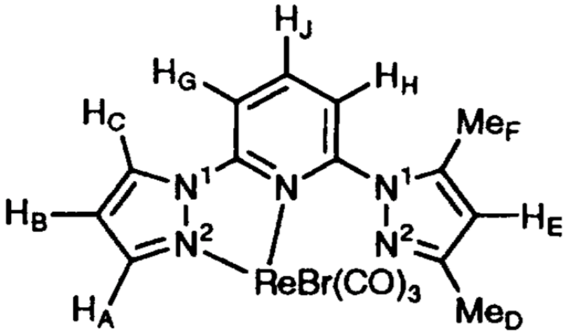


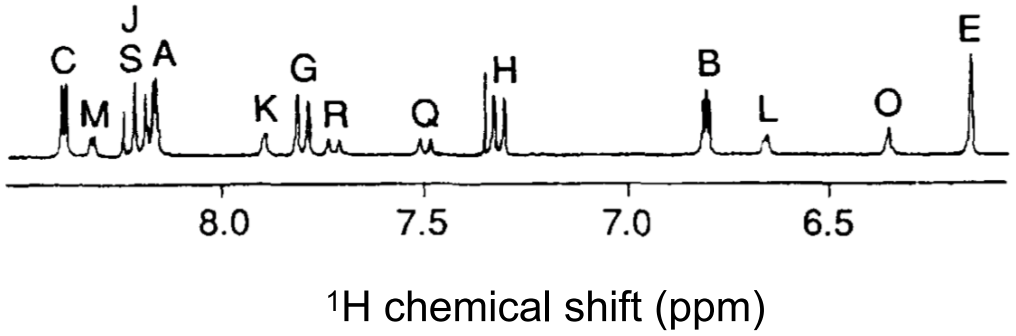
Contour plot of the expanded ‘aromatic region’ of a 2D EXSY ^{1}H NMR spectrum (700 MHz, D$_2$O, pH 6.5) with τ_m = 100 ms of a 1:1 mixture of the disulfide and the thiol shown (30 mM total concentration). A 1D spectrum of the mixture is shown on the vertical and horizontal projections.



adapted from Bracchi & Fulton, “Orthogonal breaking and forming of dynamic covalent imine and disulfide bonds in aqueous solution.” Chem. Commun., **51**, 11052 (2015).

Two-Dimensional Exchange Spectroscopy (EXSY)



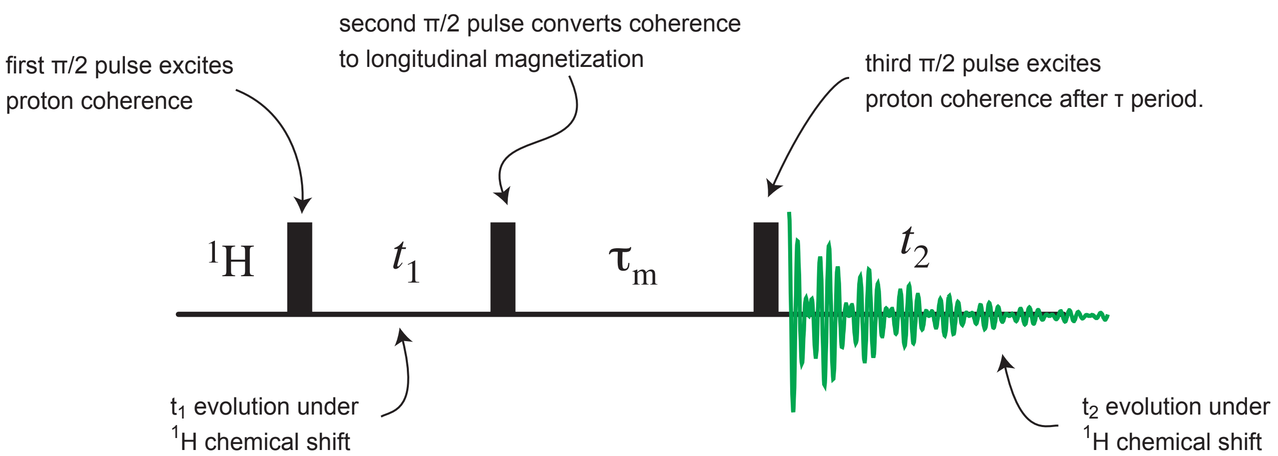


One-dimensional proton NMR spectrum of the organometallic compound [ReBr(CO)3(Me2-bppy)], where bppy denotes 2,6-bis(pyrazol-1-yl)pyridine.

Twice as many peaks as there are types of different protons in the molecule! Must be a second species?

Intensities suggest a 3:1 ratio in concentration between the major and the minor species. Is the second species an impurity, or is it a second product in slow exchange?

Two-Dimensional Exchange Spectroscopy (EXSY)



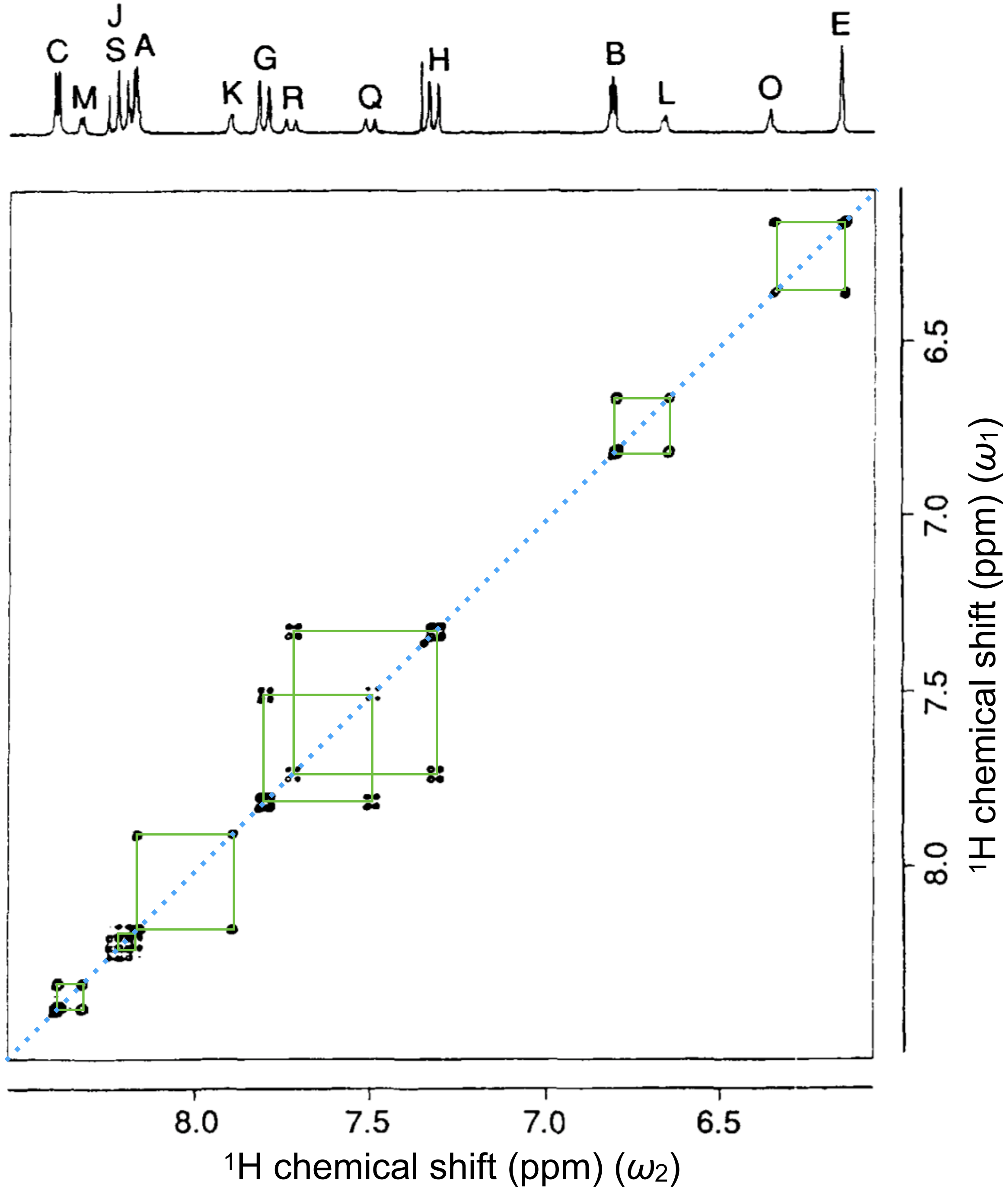
first π/2 pulse excites proton coherence

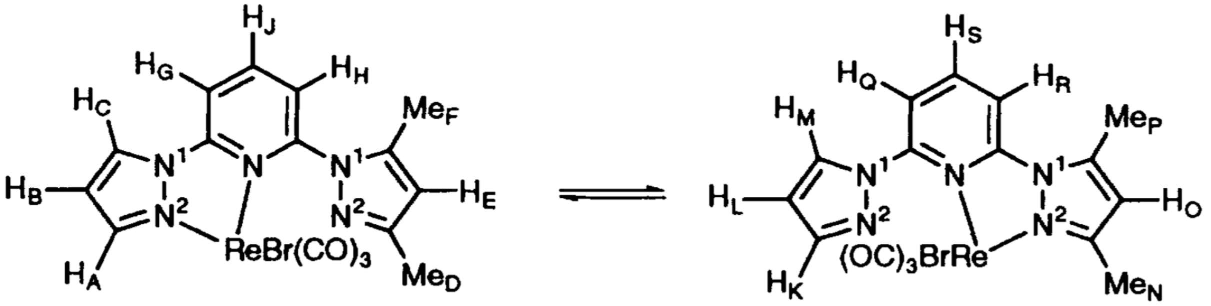
second π/2 pulse converts coherence to longitudinal magnetization

third π/2 pulse excites proton coherence after τ period.

t_1 evolution under ^1H chemical shift

t_2 evolution under ^1H chemical shift



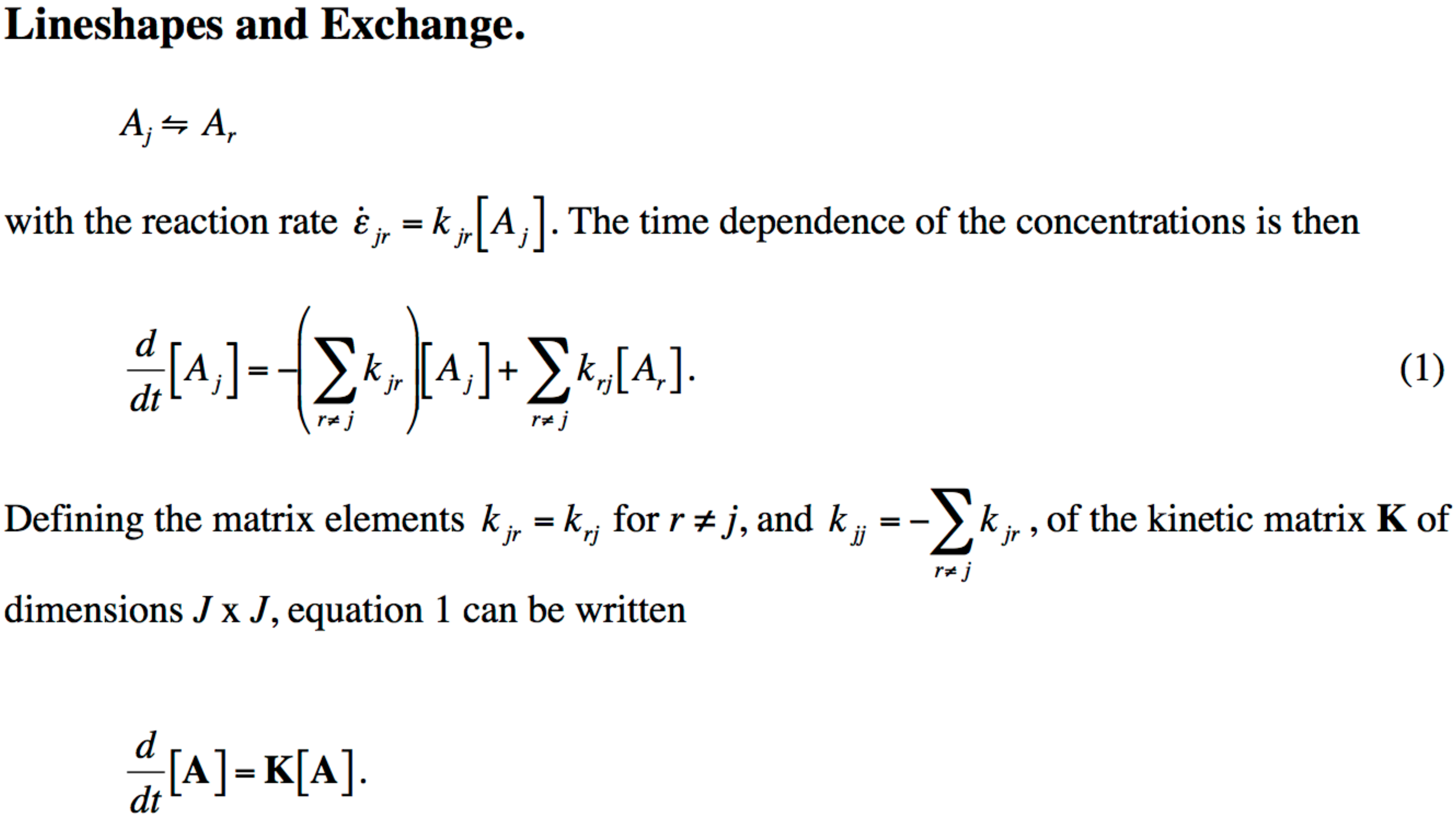


75%

25%

Proton two-dimensional exchange spectrum of the organometallic fluxional compound [ReBr(CO)3(Me2-bppy)], where bppy denotes 2,6-bis(pyrazol-1-yl)pyridine. The mixing interval was $\tau_m = 0.1$ s. The off-diagonal peaks may be interpreted in terms of an exchange of the metal atom between two pairs of nitrogen binding sites.
Adapted from E. W. Abel, et al., J. Chem. Soc. Dalton Trans., 1079 (1994).

Recall: Determining Exchange by NMR



Lineshapes and Exchange.

$$A_j \leftrightarrows A_r$$

with the reaction rate $\dot{\varepsilon}_{jr} = k_{jr}\left[A_j\right]$. The time dependence of the concentrations is then

$$\frac{d}{dt}\left[A_j\right] = -\left(\sum_{r \neq j} k_{jr}\right)\left[A_j\right] + \sum_{r \neq j} k_{rj}\left[A_r\right]. \tag{1}$$

Defining the matrix elements $k_{jr} = k_{rj}$ for $r \neq j$, and $k_{jj} = -\sum_{r \neq j} k_{jr}$, of the kinetic matrix **K** of dimensions J x J, equation 1 can be written

$$\frac{d}{dt}\left[\mathbf{A}\right] = \mathbf{K}\left[\mathbf{A}\right].$$

Recall: Modified Bloch Equations

Normally we observe the rate process in the absence of rf fields during free precession periods. Thus, transverse and longitudinal components evolve separately.

$$\frac{d}{dt}\mathbf{M}_j^+ = \left(i\Omega_j - \frac{1}{T_2}\right)\mathbf{M}_j^+ + \sum_r k_{jr}\mathbf{M}_r^+$$

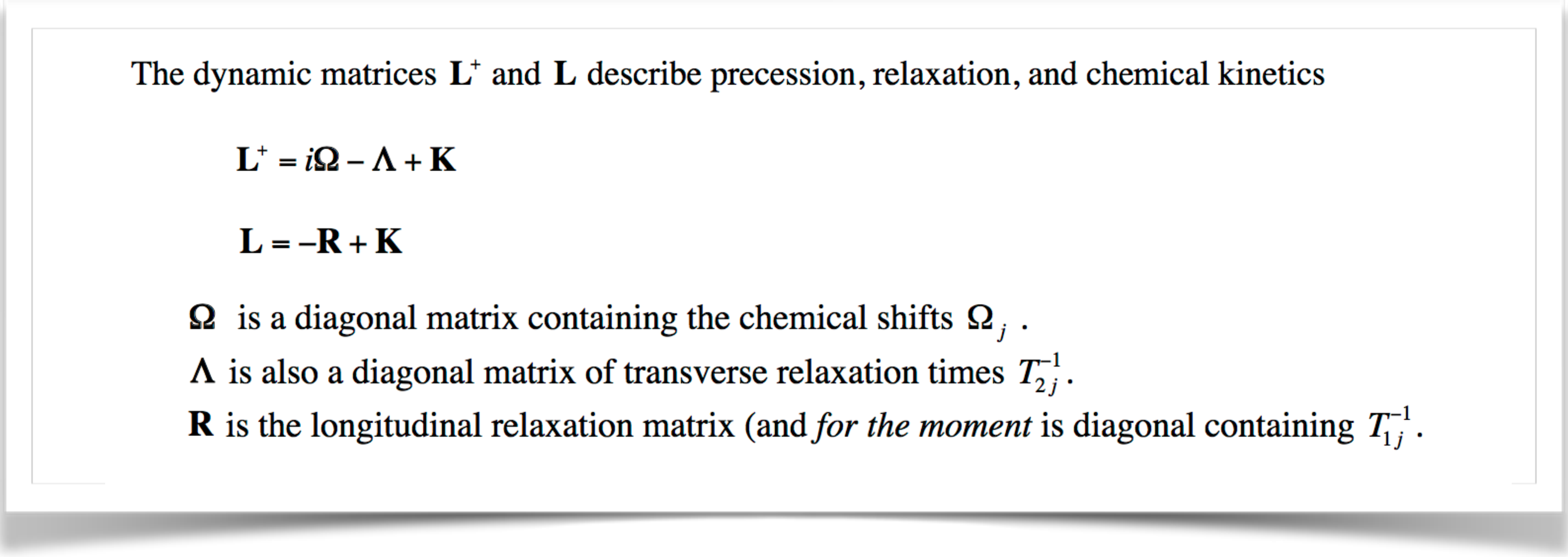
$$\frac{d}{dt}\mathbf{M}_{jz} = -\frac{1}{T_1}\left(\mathbf{M}_{jz} - \mathbf{M}_{j0}(t)\right) + \sum_r k_{jr}\mathbf{M}_{rz}$$

and these equations are conveniently written in matrix form (at equilibrium)

$$\frac{d}{dt}\mathbf{M}^+ = \mathbf{L}^+\mathbf{M}^+ \qquad (3)$$

$$\frac{d}{dt}\mathbf{M}_z = \mathbf{L}\{\mathbf{M}_z - \mathbf{M}_0\} \qquad (4)$$

where $\mathbf{M}^+$, $\mathbf{M}_z$ and $\mathbf{M}_0$ contain the magnetization vectors for all J chemical species.



The dynamic matrices $\mathbf{L}^+$ and $\mathbf{L}$ describe precession, relaxation, and chemical kinetics

$$\mathbf{L}^+ = i\boldsymbol{\Omega} - \boldsymbol{\Lambda} + \mathbf{K}$$

$$\mathbf{L} = -\mathbf{R} + \mathbf{K}$$

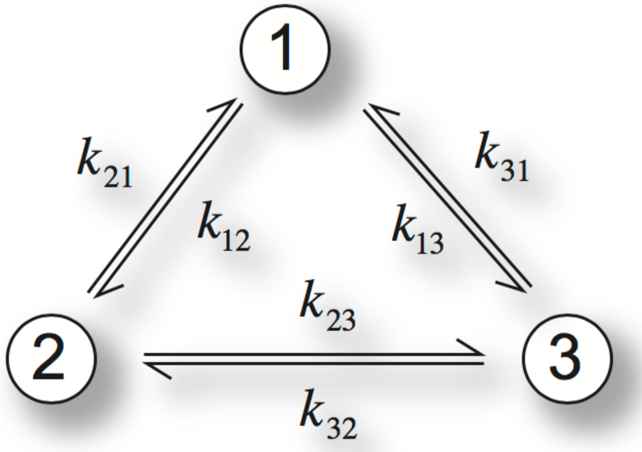
$\boldsymbol{\Omega}$ is a diagonal matrix containing the chemical shifts Ω_j .

$\boldsymbol{\Lambda}$ is also a diagonal matrix of transverse relaxation times T_{2j}^{-1}.

$\mathbf{R}$ is the longitudinal relaxation matrix (and *for the moment* is diagonal containing T_{1j}^{-1}.

Recall: Modified Bloch Equations

Consider for example a system with three chemical species exchanging by first order reactions (with $k_{ij} = k_{ji}$).



The time evolution of the transverse magnetization components is governed by

$$\frac{d}{dt}\begin{pmatrix} M_1^+ \\ M_2^+ \\ M_3^+ \end{pmatrix} = \left\{ i\begin{bmatrix} \Omega_1 & 0 & 0 \\ 0 & \Omega_2 & 0 \\ 0 & 0 & \Omega_3 \end{bmatrix} - \begin{bmatrix} T_{2(1)}^{-1} & 0 & 0 \\ 0 & T_{2(2)}^{-1} & 0 \\ 0 & 0 & T_{2(3)}^{-1} \end{bmatrix} + \begin{bmatrix} -k_{12}-k_{13} & k_{21} & k_{31} \\ k_{12} & -k_{21}-k_{23} & k_{32} \\ k_{13} & k_{23} & -k_{31}-k_{32} \end{bmatrix} \right\} \begin{pmatrix} M_1^+ \\ M_2^+ \\ M_3^+ \end{pmatrix}.$$

These equations can be solved to yield the time-domain signals observed for various k_{ij}.

Longitudinal Exchange in EXSY

EXSY can be used to follow relatively complex reactions, and one may think it difficult to obtain quantative information on the rates, bu the dynamics of the longitudinal magnetization during τ_m are controlled by equation (4).

$$\frac{d}{dt}\mathbf{M}_z = \mathbf{L}\left\{\mathbf{M}_z - \mathbf{M}_0\right\} \tag{4}$$

which under conditions of dynamic chemical equilibrium (i.e. a stationary state) can be simplified to (net exchange of M_0 is zero)

$$\frac{d}{dt}\Delta\mathbf{M} = \mathbf{L}\Delta\mathbf{M}.$$

This has a formal solution

$$M_z(\tau_m) = M_0 + \exp\left\{\mathbf{L}\tau_m\right\}\Delta M_z(\tau_m = 0).$$

Which implies that the magnetization components recover during τ_m towards equilibrium. We find that the signal during t_2 is then given by

$$M^+(t_1,\tau_m,t_2) = -\exp\left\{\mathbf{L}^+ t_2\right\}\exp\left\{\mathbf{L}\tau_m\right\}\exp\left\{\mathbf{L}^+ t_1\right\}M_0.$$

Longitudinal Exchange in EXSY

$$M^{+}(t_1,\tau_m,t_2) = -\exp\{\mathbf{L}^{+}t_2\}\exp\{\mathbf{L}\tau_m\}\exp\{\mathbf{L}^{+}t_1\}M_0 .$$

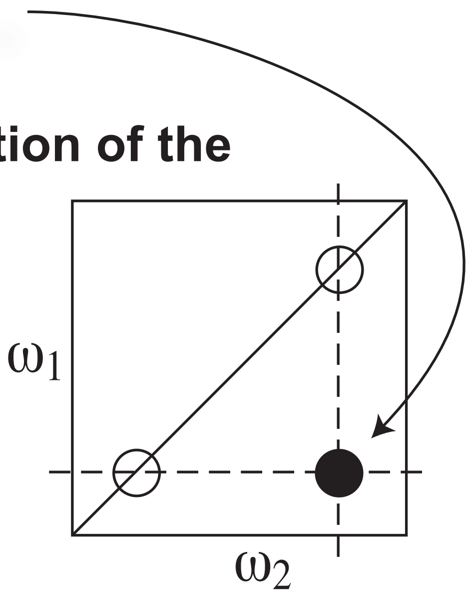
For slow exchange, lineshapes in t_1 and t_2 are not affected by contributions of $\mathbf{K}$ to $\mathbf{L}^{+}$ and they may be neglected. As a result the time-domain signal simplifies to

$$s(t_1,\tau_m,t_2) = -\sum_k \sum_l \exp\{-i\Omega_k t_2 - \lambda_k t_2\}\left[\exp\{\mathbf{L}\tau_m\}\right]_{k,l} \exp\{-i\Omega_l t_1 - \lambda_l t_1\}M_{l0} .$$

and after 2D FT the integrated amplitude of a signal with frequency coordinates $(\omega_1,\omega_2) = (\Omega_l,\Omega_k)$ is

$$I_{kl}(\tau_m) = a_{kl}(\tau_m)M_{l0} \text{ and } a_{kl}(\tau_m) = \left[\exp\{\mathbf{L}\tau_m\}\right]_{kl} .$$

The 2D spectrum amounts to a pictorial representation of the exponeneital mixing operator.



Longitudinal Exchange in EXSY

For a 2 spin system, the 2x2 dynamic matrix leads to an analytical solution, and for a symmetrical case we obtain:

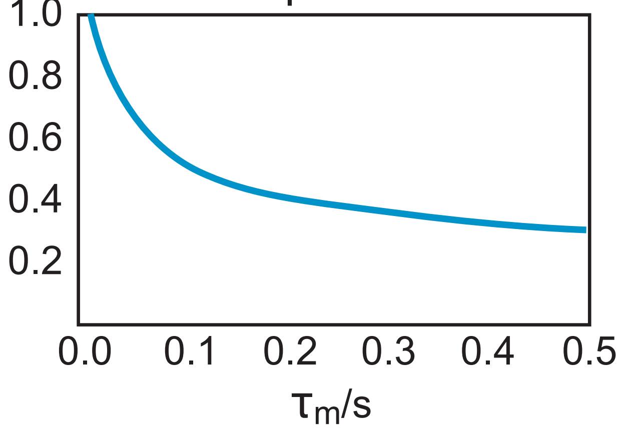
$$I_{AA}(\tau_m) = \tfrac{1}{2}\left[1 + \exp\{-2k\tau_m\}\right]\exp\{-\tau_m/T_1\}\,M_{A0}$$

$$I_{BB}(\tau_m) = \tfrac{1}{2}\left[1 + \exp\{-2k\tau_m\}\right]\exp\{-\tau_m/T_1\}\,M_{B0}$$

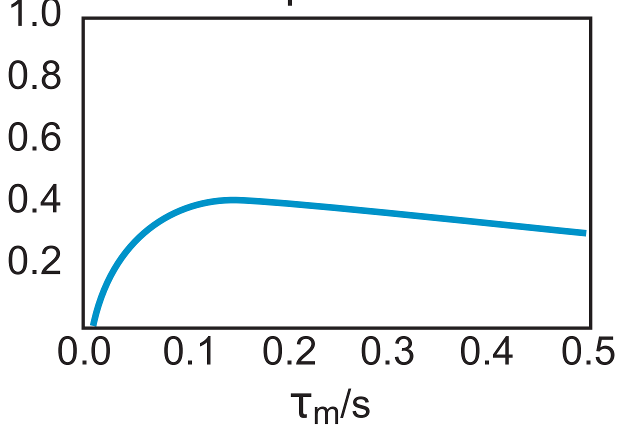
$$I_{AB}(\tau_m) = \tfrac{1}{2}\left[1 - \exp\{-2k\tau_m\}\right]\exp\{-\tau_m/T_1\}\,M_{B0}$$

$$I_{BA}(\tau_m) = \tfrac{1}{2}\left[1 - \exp\{-2k\tau_m\}\right]\exp\{-\tau_m/T_1\}\,M_{A0}$$

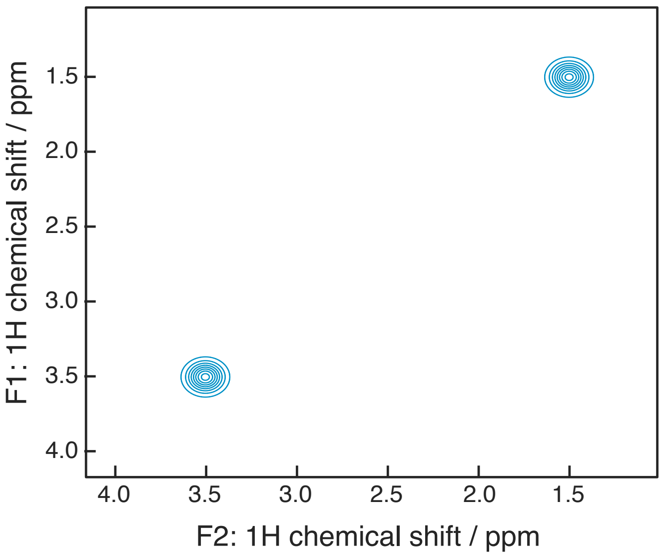
Diagonal peak amplitudes



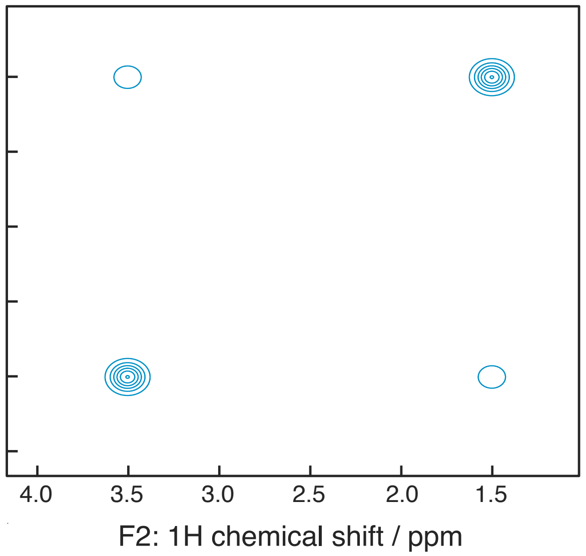
Cross peak amplitudes



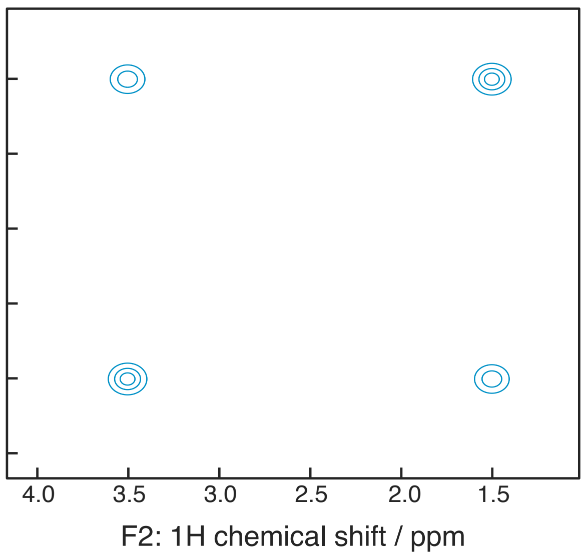
$\tau_m = 0$



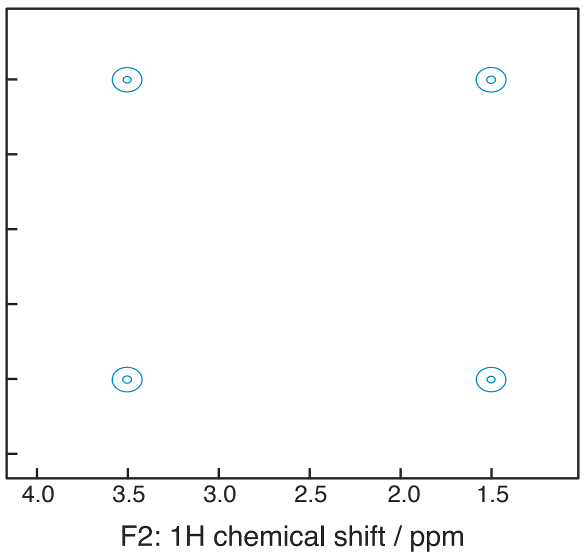
$k\tau_m < 1$



$k\tau_m \sim 1$



$k\tau_m > 2$



Quantitative NMR spectroscopy of supramolecular complexes: Dynamic side pores in ClpP are important for product release

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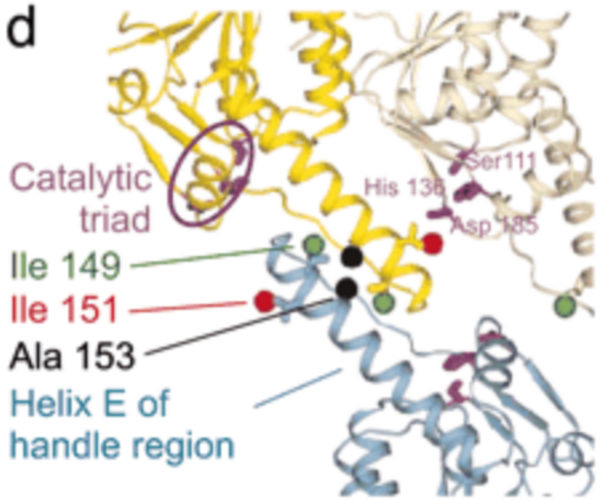
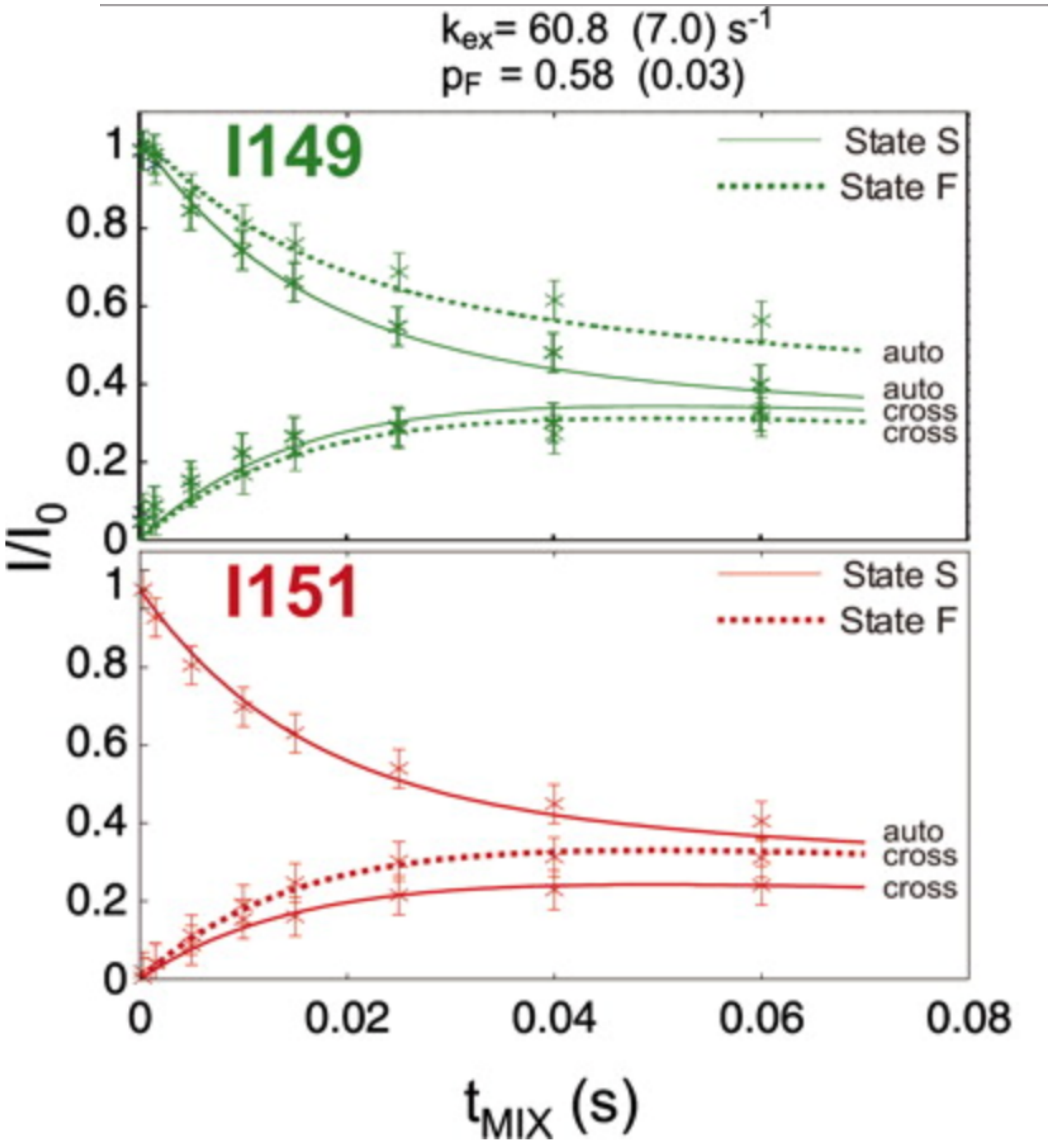


Abridged Abstract: The 300-kDa cylindrical protease ClpP is an important component of the cellular protein quality machinery. It consists of 14 subunits arranged into two ***heptameric rings*** that enclose a large chamber containing the protease active sites. ClpP associates with ClpX and ClpA ATPases that unfold and translocate substrates into the protease catalytic chamber through ***axial pores located at both ends of the ClpP cylinder***. Although the pathway of substrate delivery is well established, ***the pathway of product release is unknown***. Here, we show that ***the interface between the heptameric rings exchanges between two structurally distinct conformations***. ***The conformational exchange process has been quantified by magnetization exchange experiments recorded between 0.5°C and 40°C, so that the thermodynamic properties for the transition could be obtained.*** Restriction of the observed motional freedom in ClpP through the introduction of a cysteine linkage results in a protease where substrate release becomes significantly slowed relative to the rate observed in the reduced enzyme, suggesting that the observed motions lead to the formation of transient side pores that may play an important role in product release.



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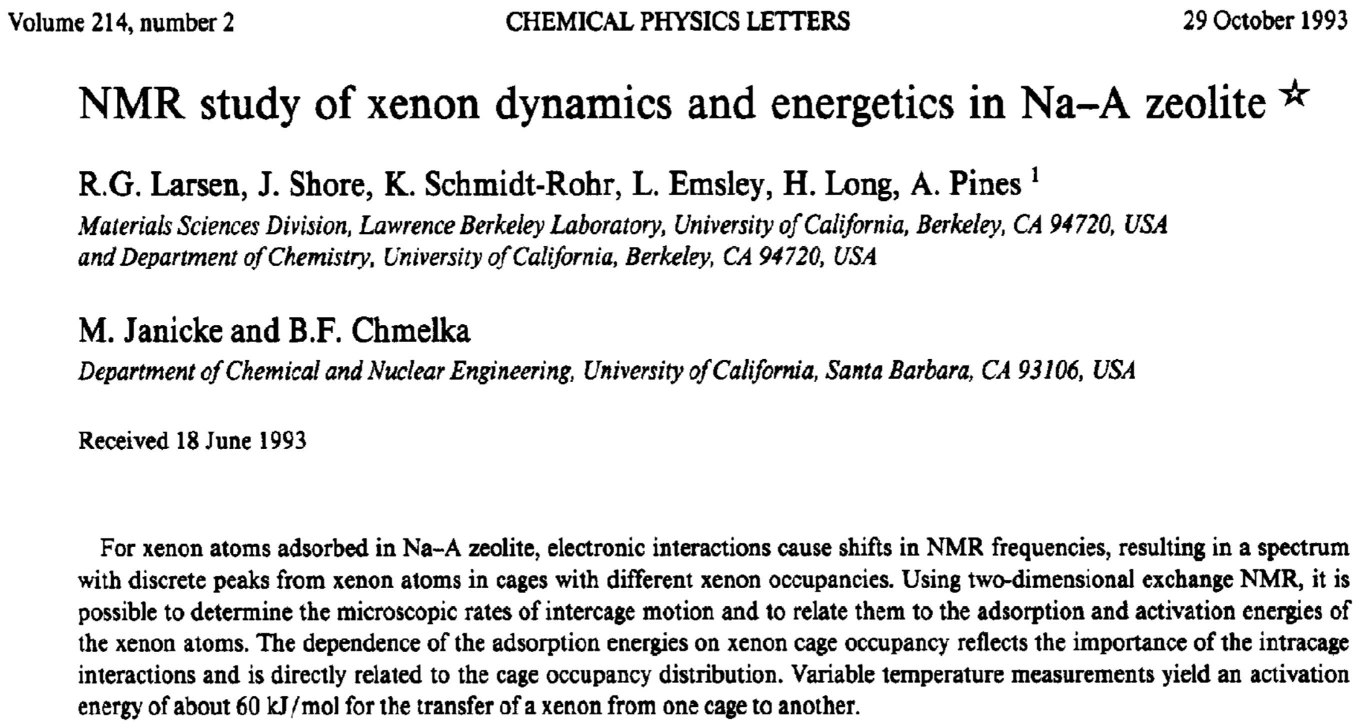
Quantitative Analysis of EXSY Spectra



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NMR study of xenon dynamics and energetics in Na–A zeolite ☆

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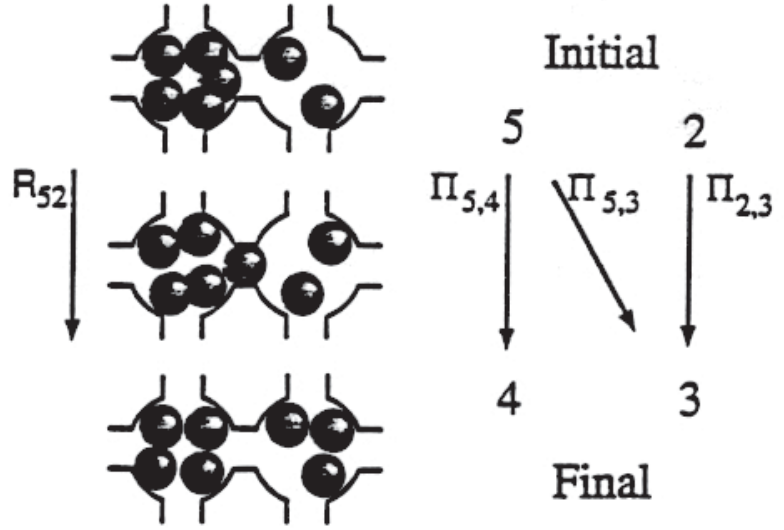
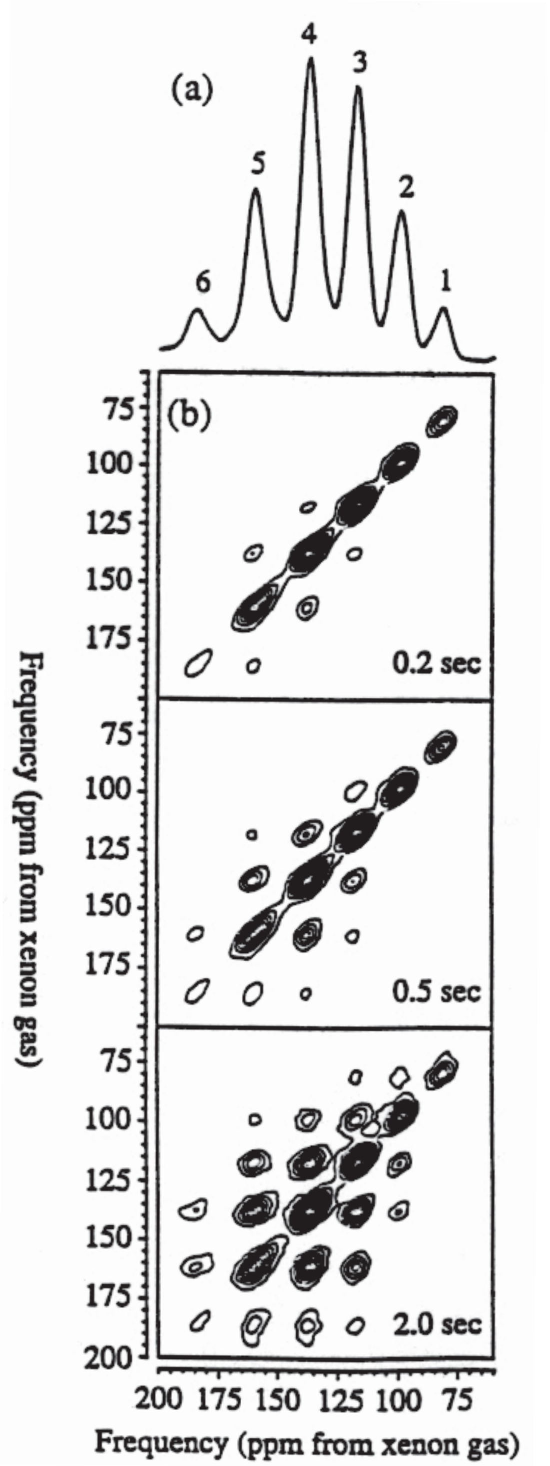
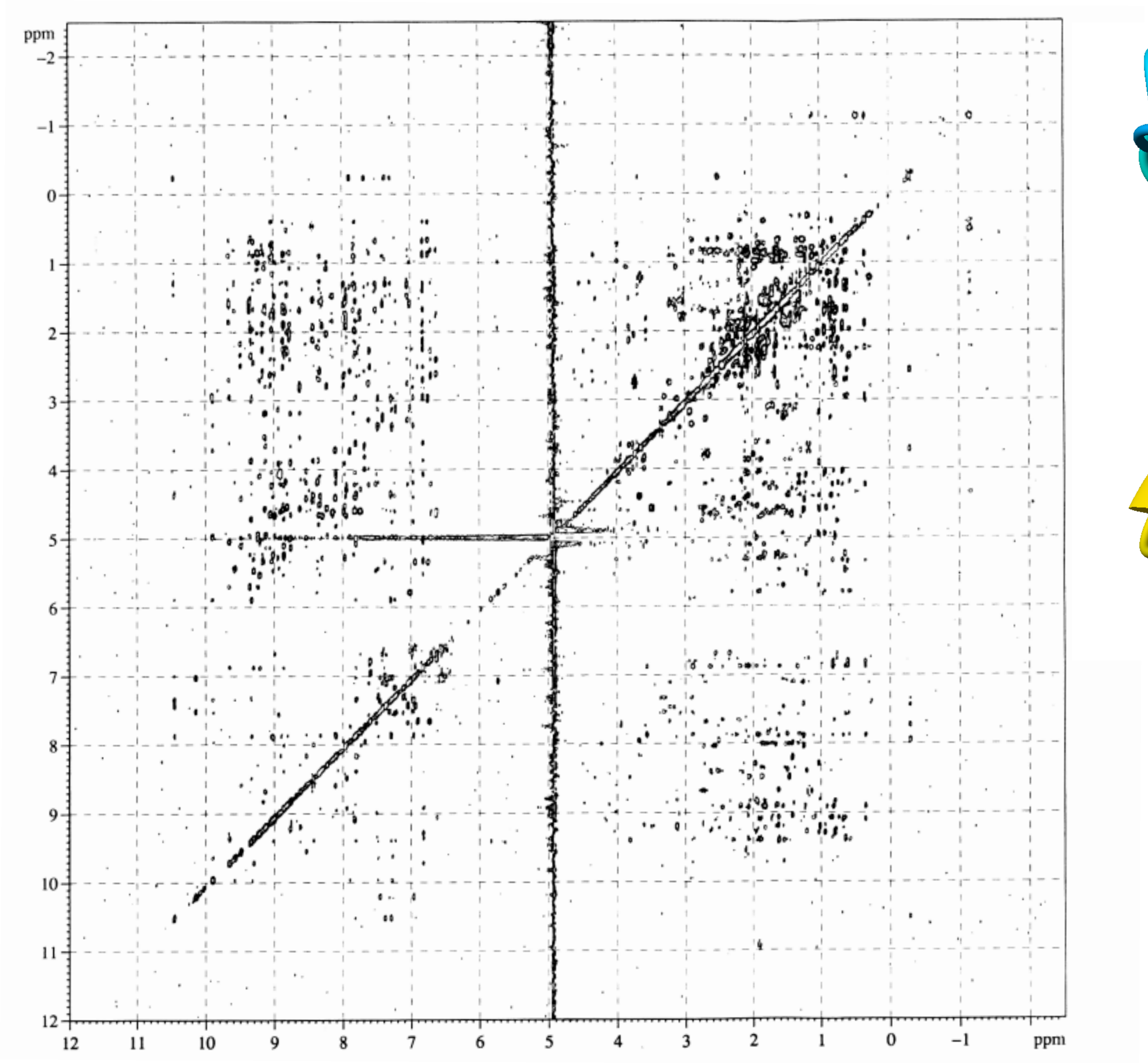
For xenon atoms adsorbed in Na–A zeolite, electronic interactions cause shifts in NMR frequencies, resulting in a spectrum with discrete peaks from xenon atoms in cages with different xenon occupancies. Using two-dimensional exchange NMR, it is possible to determine the microscopic rates of intercage motion and to relate them to the adsorption and activation energies of the xenon atoms. The dependence of the adsorption energies on xenon cage occupancy reflects the importance of the intracage interactions and is directly related to the cage occupancy distribution. Variable temperature measurements yield an activation energy of about 60 kJ/mol for the transfer of a xenon from one cage to another.

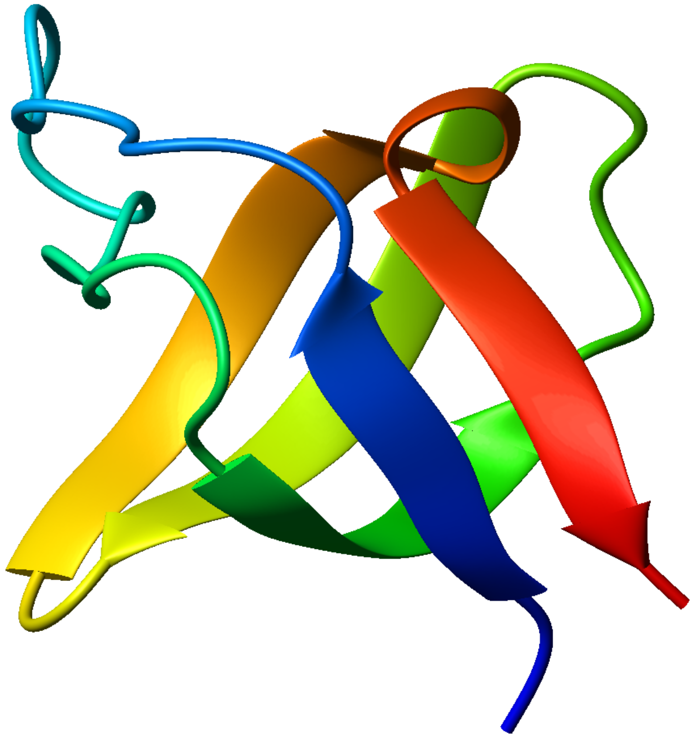




Homework: Read & Understand from this paper (pdf on Moodle) how one can go from the 2D exchange spectra to the activation energies

Exchange spectroscopy: something deeply wrong?





The ^1H-^1H 2D EXSY spectrum of the SH3 domain (60 amino acids) seems to contain many cross peaks between protons that cannot reasonably be in chemical exchange…?

Conclusions

- We can add another dimension to the time-domain to obtain a spectrum with frequencies correlated between t_1 and t_2: a “preparation - evolution - mixing - detection” approach.

- Acquisition of the two time domains is achieved by repeating the experiment for different values of t_1.

- 2D heteronuclear correlation yields through-bond ^{1}H-X (X =^{13}C, ^{15}N, ^{31}P, ^{29}Si…) correlations.

- 2D ^{1}H-^{1}H correlation (COSY) yields through-bond H↔H connectivity.

- 2D Exchange Spectroscopy can used to observe slow chemical exchange. 2D EXSY spectra can be analysed quantitatively to obtain exchange rates and thermodynamic parameters.

Homework

Prepare for the practical MRI session: Read the papers in the folder "Preparation for Imaging Practical at CIBM" on Moodle which describe the sequences we will be using.

Learn the course material. Try to answer the open questions.

Read & understand from the paper (pdf on Moodle) on how one can go from the 2D exchange spectra to the activation energies for Xe diffusion inside Zeolites.