# Nuclear Magnetic Resonance

## Relaxation, Exchange & Multi-Dimensional NMR

# What we learned last week

- The Vector Model of Pulsed FT NMR

# Objectives

- Learn about multi-pulse NMR
- Determine the effect of molecular dynamics on NMR spectra

# What Happens After a Pulse? Relaxation

After a pulse, the system will oscillate under Larmor precession, but it must inevitably return to equilibrium.

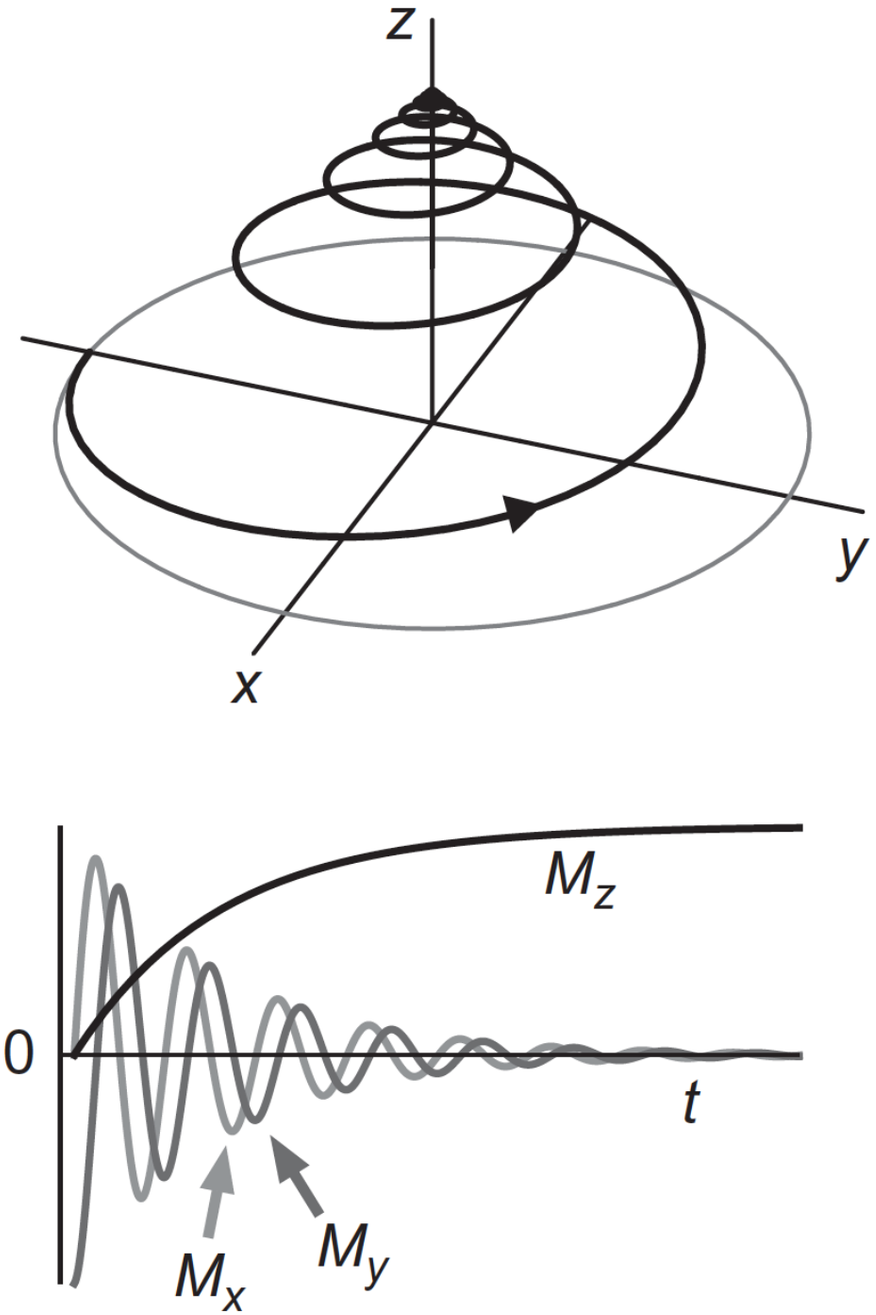
The longitudinal component (i.e. that aligned with the field (here z)) relaxes towards the equilibrium value (a non-zero value) $M_0$.

The transverse components both relax back to zero.

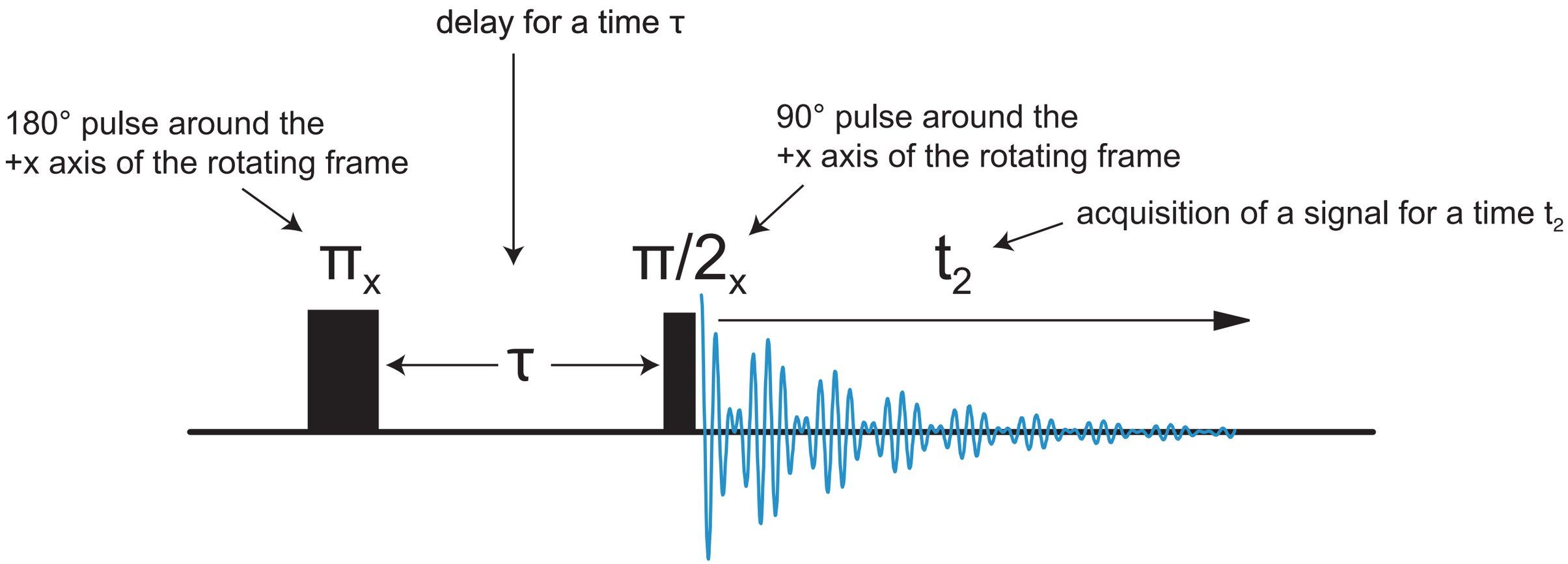
Evolution of $M_z$ towards equilibrium modifies the total energy of the system: there must be exchange of energy with the lattice.

Relaxation of the transverse components does not modify the total energy.

We thus distinguish ***longitudinal relaxation*** (or spin-lattice relaxation), $T_1$, from ***transverse relaxation*** (or spin-spin relaxation) relaxation, $T_2$.



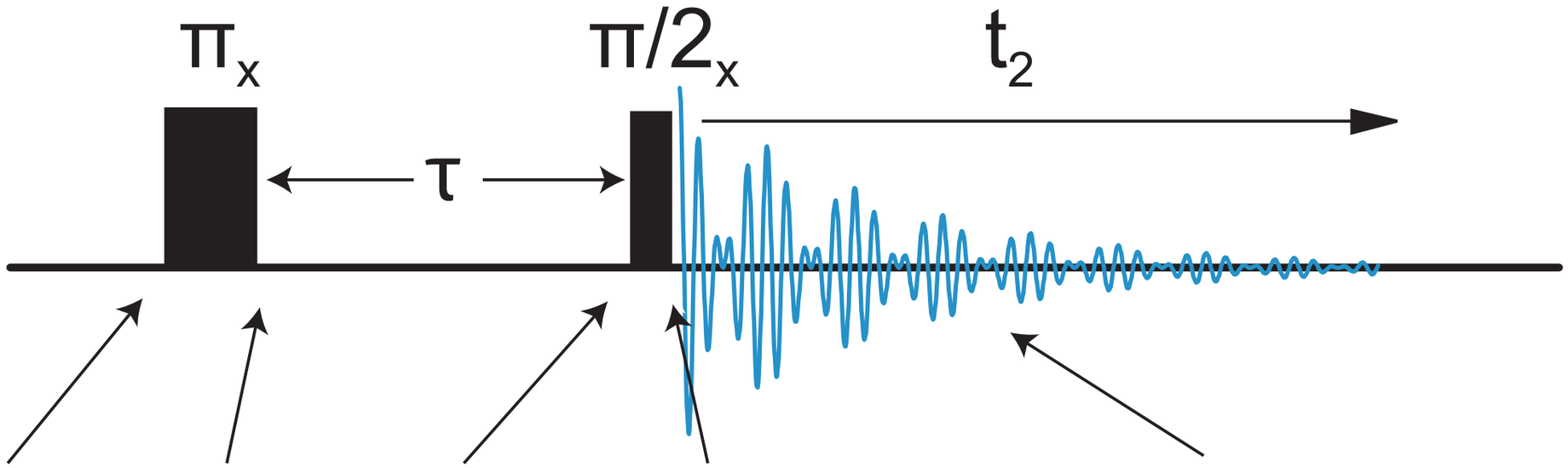
# Dances with Spins: Inversion-Recovery




In general a **pulse sequence** consists of multiple pulses and delays

[Note that these timing diagrams are usually not to scale: for example, in this case the radiofrequcency pulses are around 10 - 20 microseconds long, the delay τ can on the order of 0.1 to 10 seconds, and the signal is acquired for a time $t_2$ of between 0.1 and 1.0 second.]

# Dances with Spins: Inversion-Recovery




$\alpha_0 M_z$ $-\alpha_0 M_z$ $\alpha(\tau) M_z$ $\alpha(\tau) M_x$ $\alpha(\tau)(M_z \cos(\Omega t_2) + M_y \sin(\Omega t_2))$

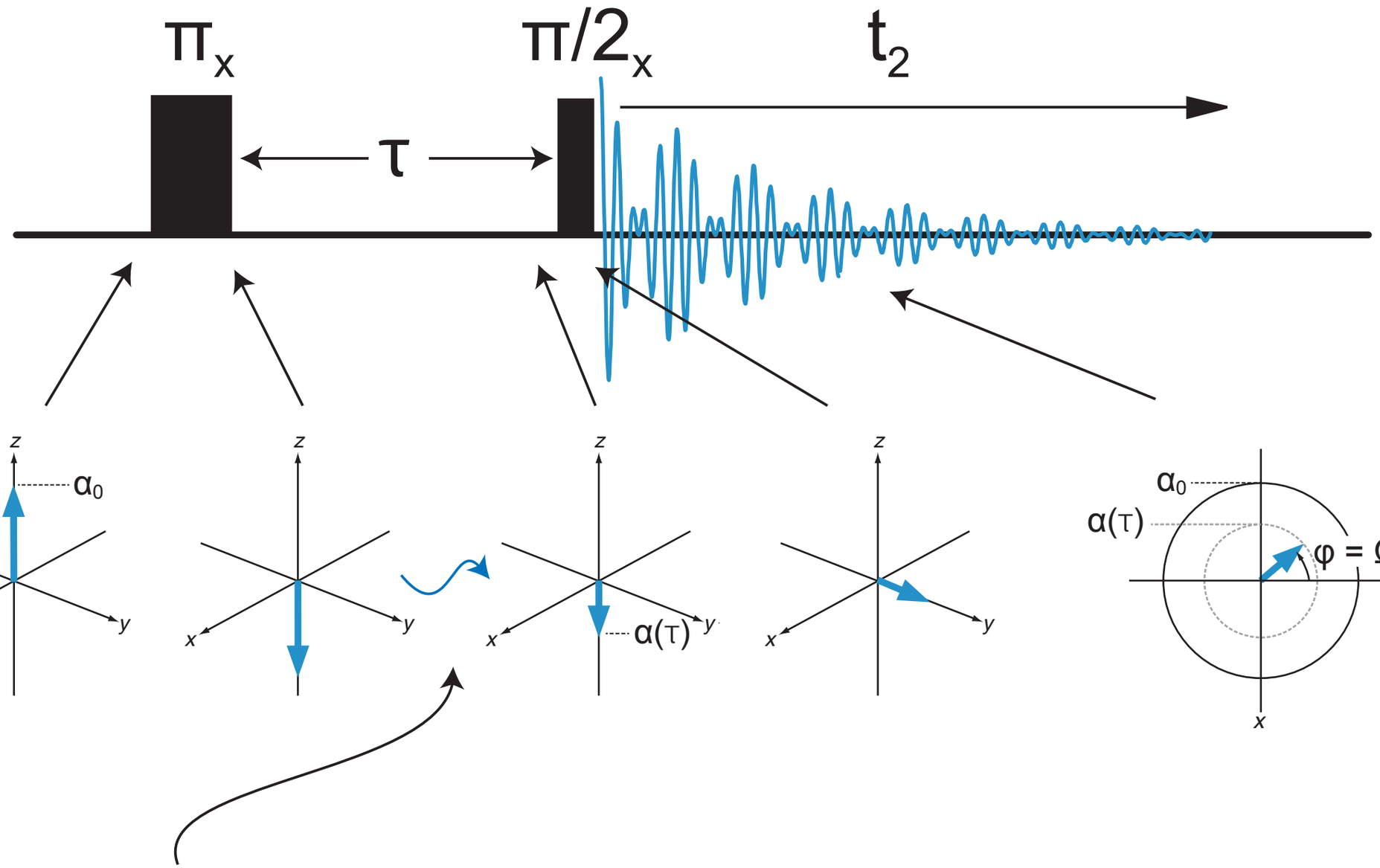
longitudinal relaxation ($T_1$)

if relaxation during $\tau$ follows a monoexponential

recovery towards equlibrium: $\alpha(\tau) = \alpha_0(1 - 2\exp(\tau/T_1))$

$\Omega = \omega_0 - \omega_{rf}$

# Dances with Spins: Inversion-Recovery

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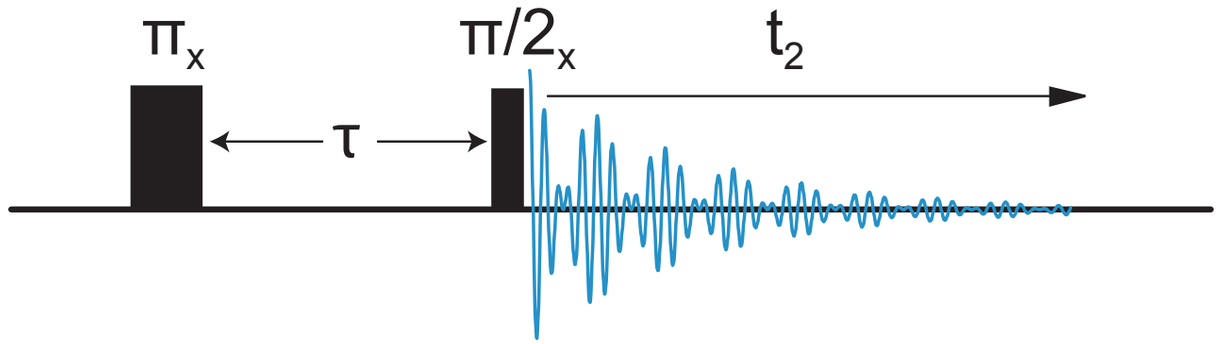
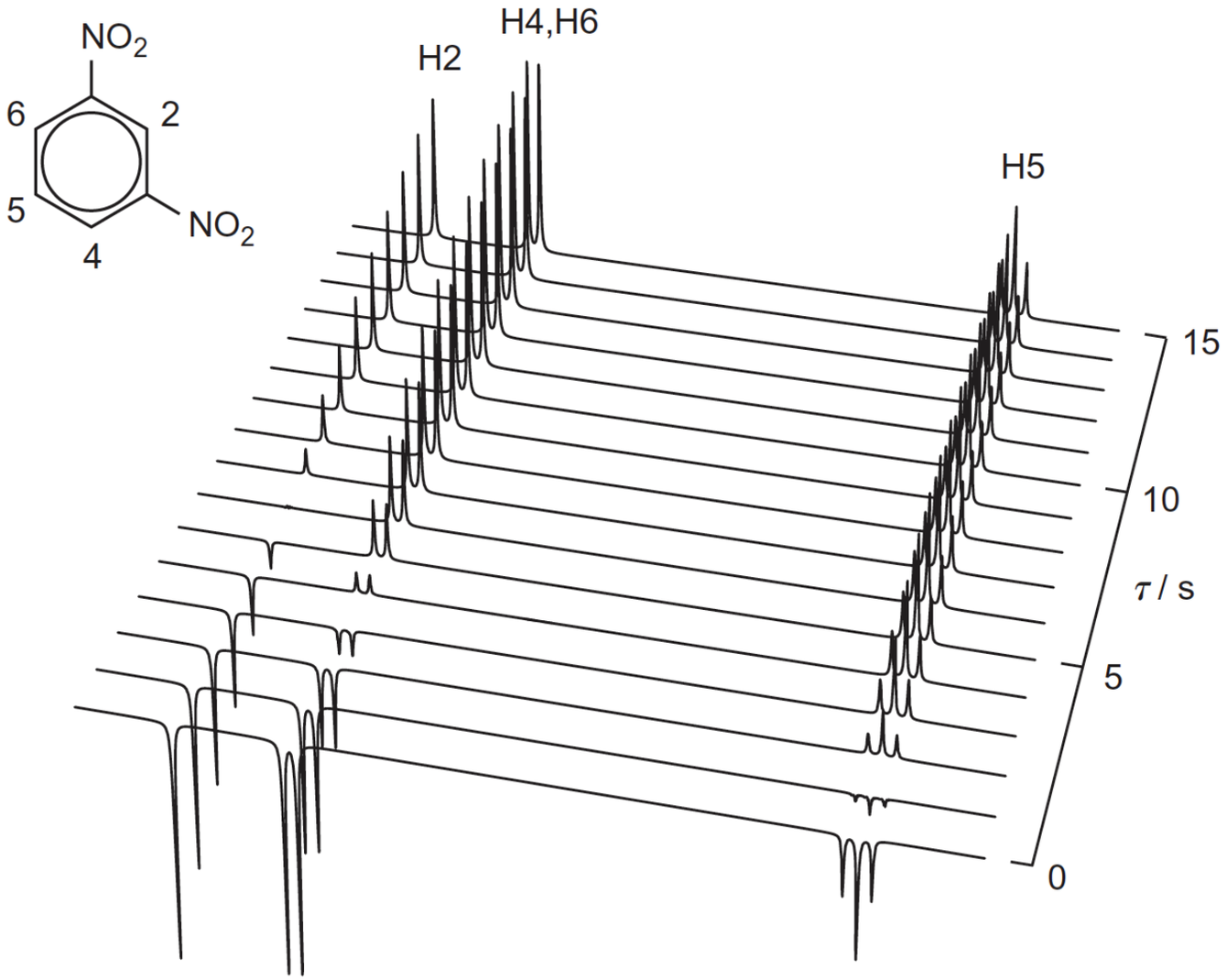
# Dances with Spins: Inversion-Recovery




Multiple-pulse NMR: Measurement of Longitudiunal Relaxation Times ($T_1$)



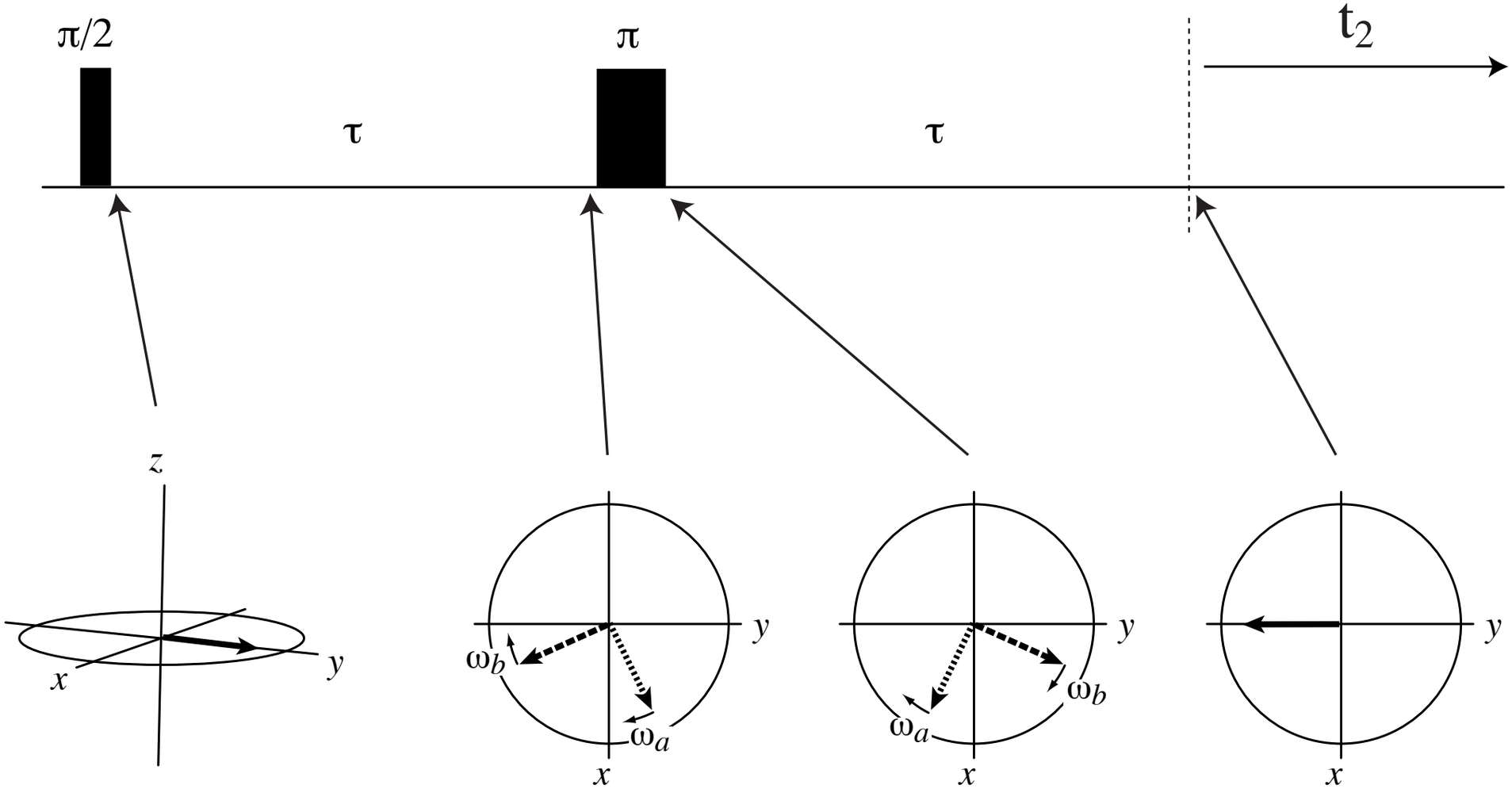

$^1H$ $T_1$ measurement for 1,3 dinitrobenzene.

For each peak, i, in the spectrum:
$I^i(\tau) = I^i_0(1 - 2\exp(\tau/T_1))$

The $T_1$ values found by fitting the measured intensities to $T_1$ are: 8.6 s (H2), 5.1 s (H4, H6), and 1.7 s (H5).

The principal source of relaxation is the $^1H$–$^1H$ dipolar interactions between adjacent protons on the ring. The relaxation rates are in the order H5 > H4, H6 > H2 because the numbers of nearest neighbour protons are, respectively, 2, 1, and 0.

# Dances with Spins: The Spin Echo



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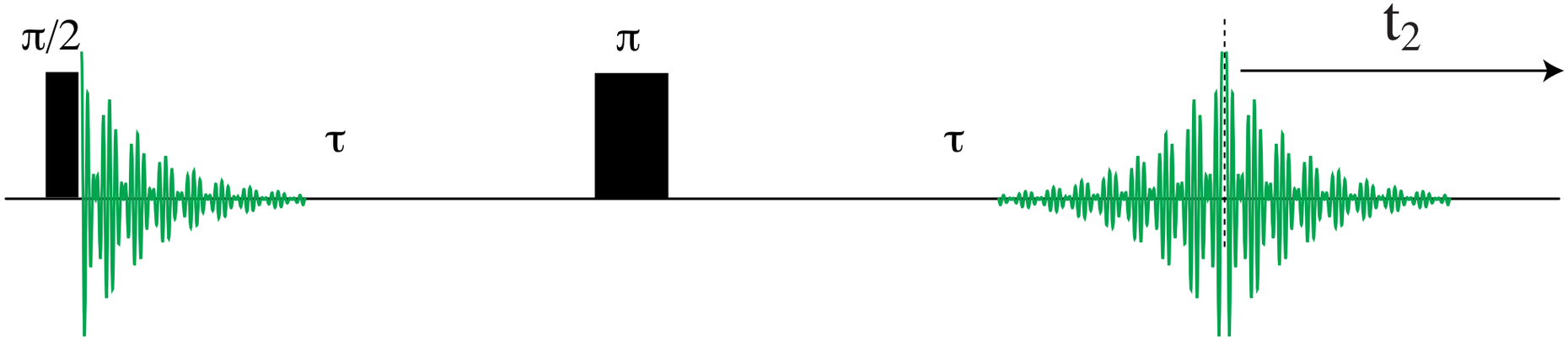
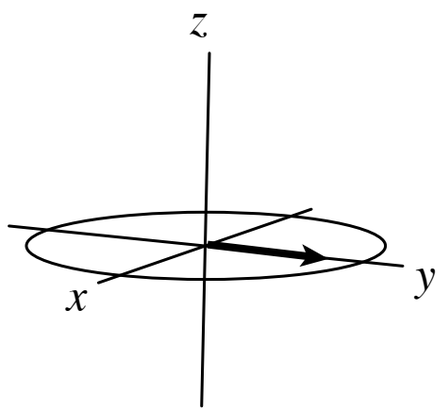
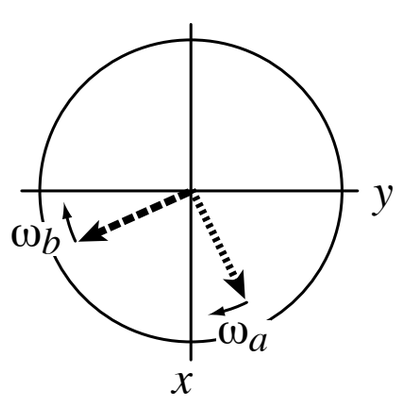
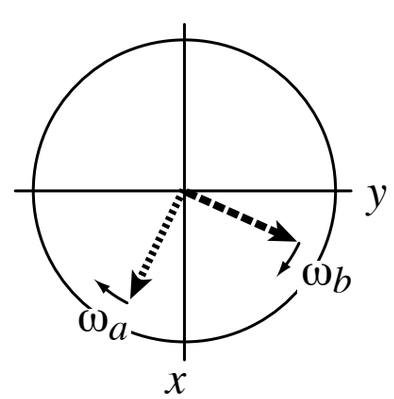
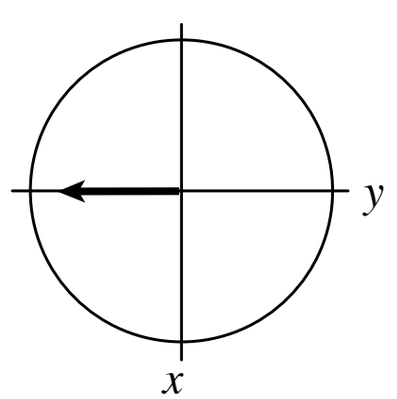


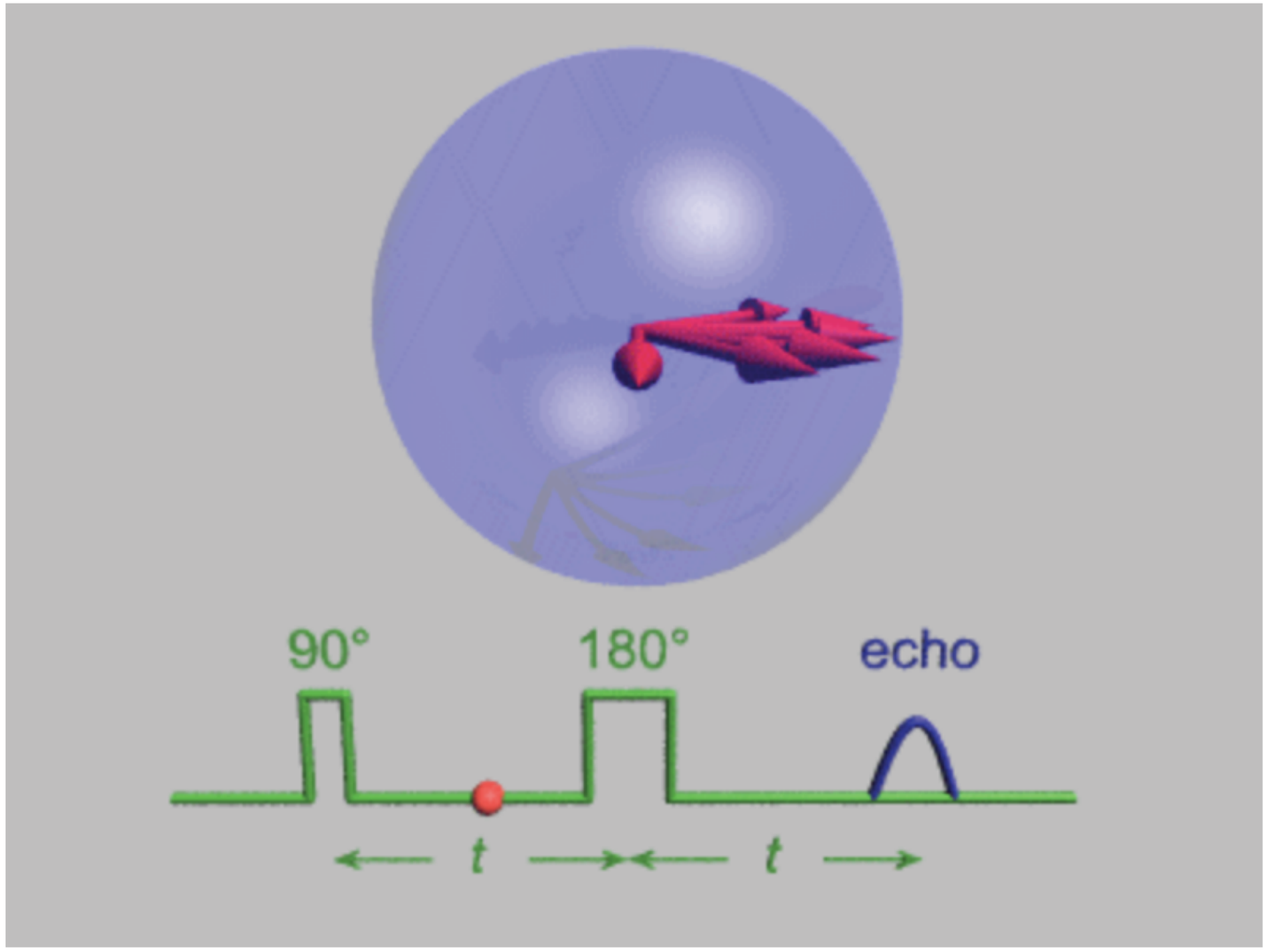



# The Hahn Echo

https://en.wikipedia.org/wiki/Spin_echo



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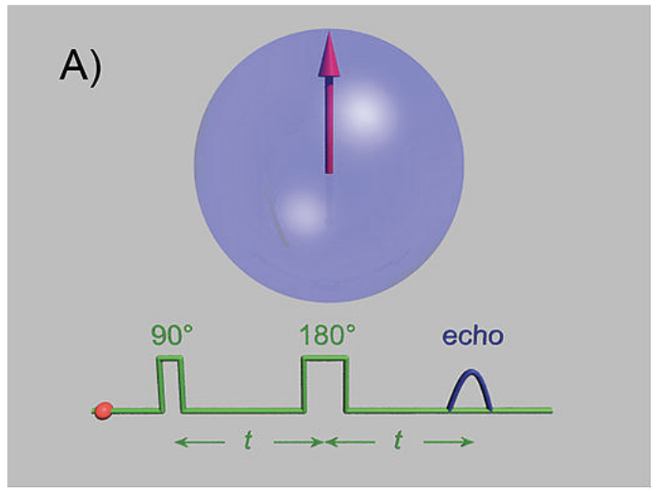
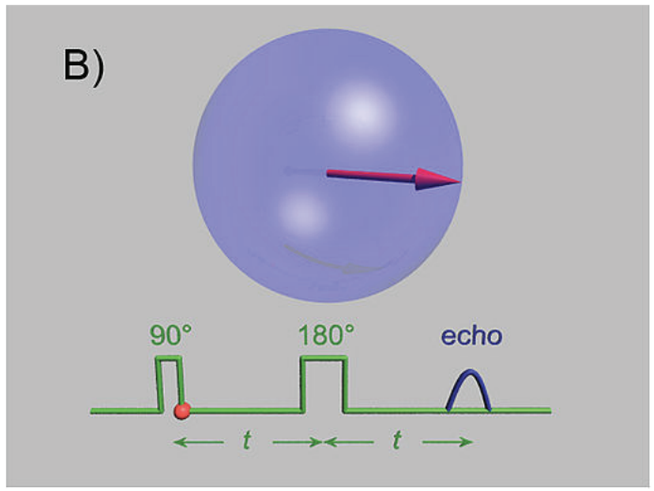
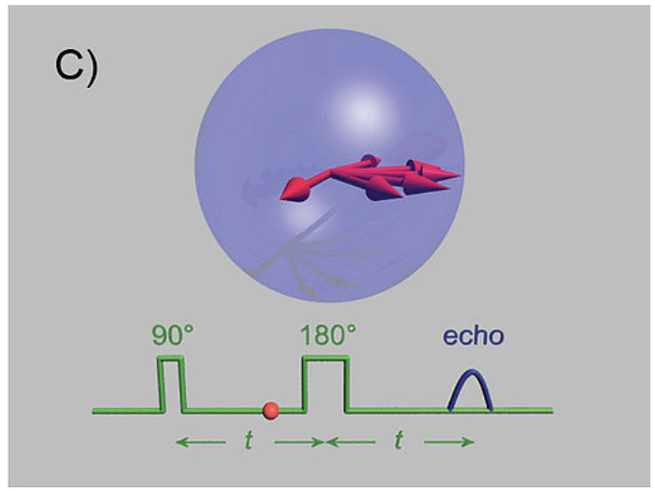
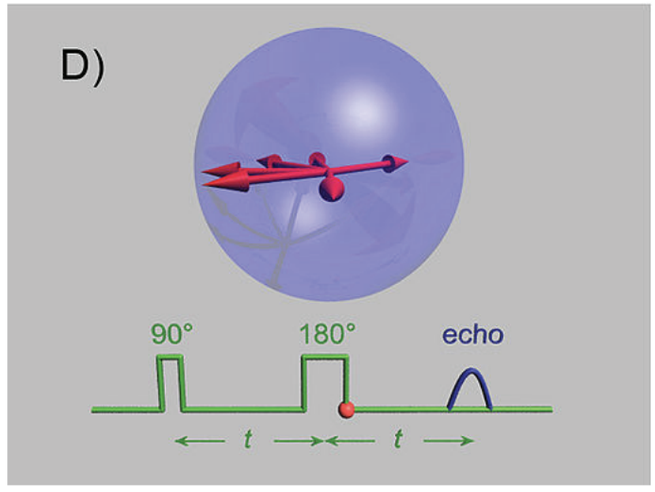
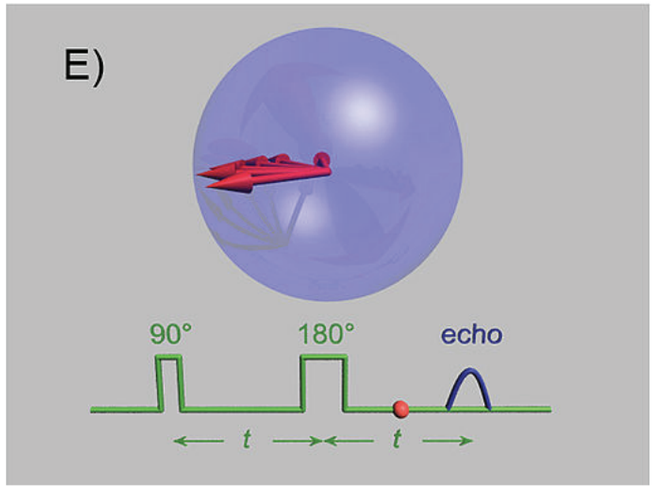
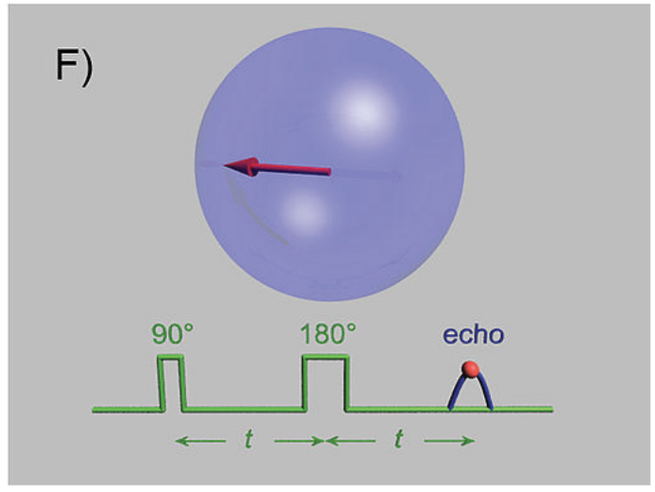


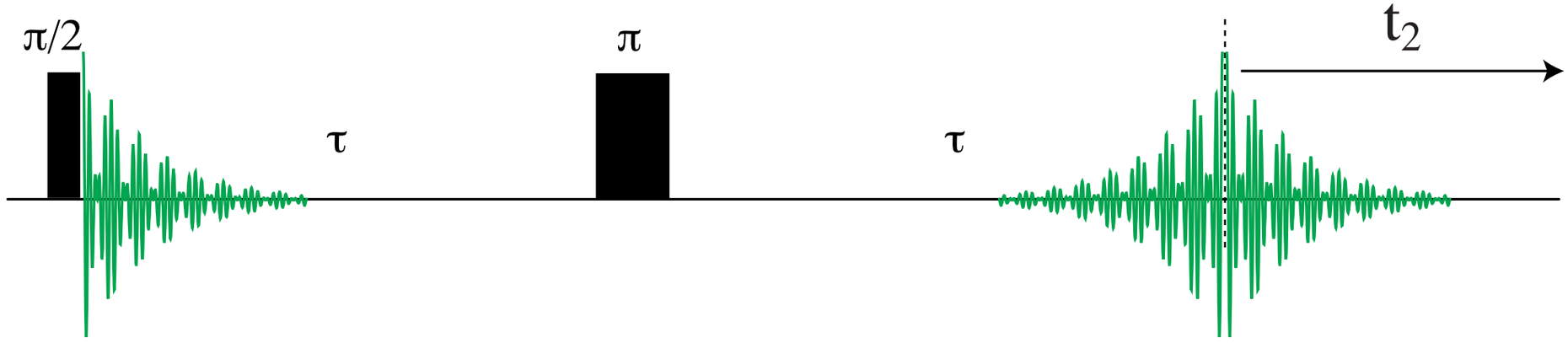



# Dances with Spins: The Spin Echo




***Multiple-Pulse NMR:***
***Measurement of Transverse Relaxation Rates ($T_2$)***

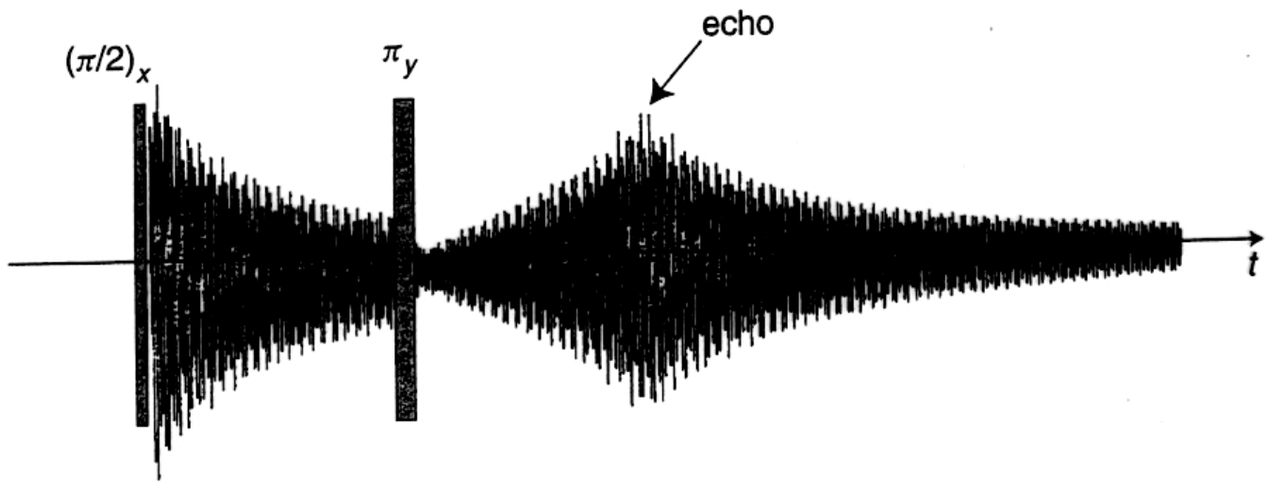
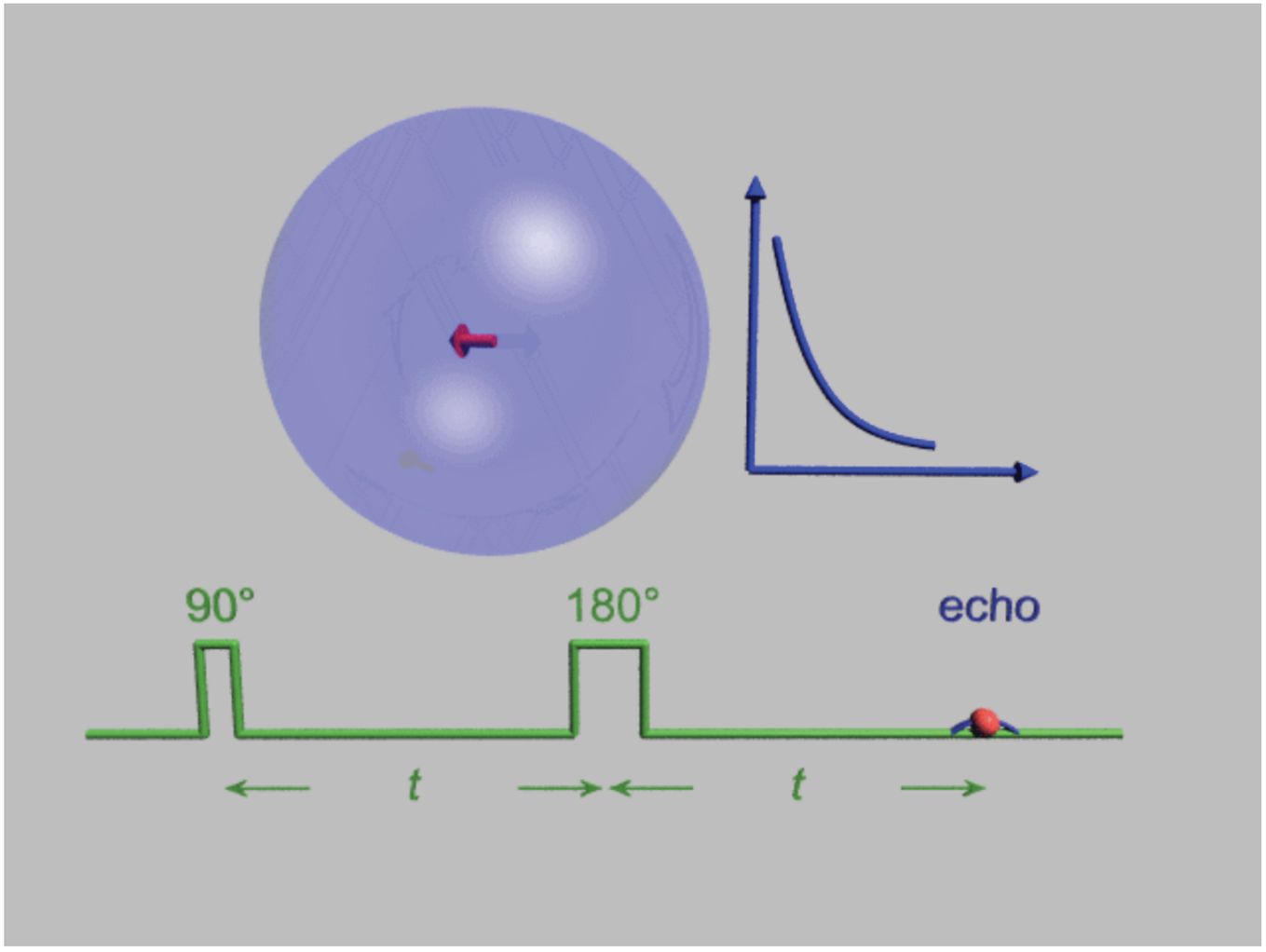



**Figure 11.19** Experimental echo signal. Adapted from A. E. Derome, *Modern NMR Techniques for Chemistry Research*, Pergamon Press, Oxford, 1987, p. 91. (Reproduced by permission of Elsevier Science).

# The Hahn Echo

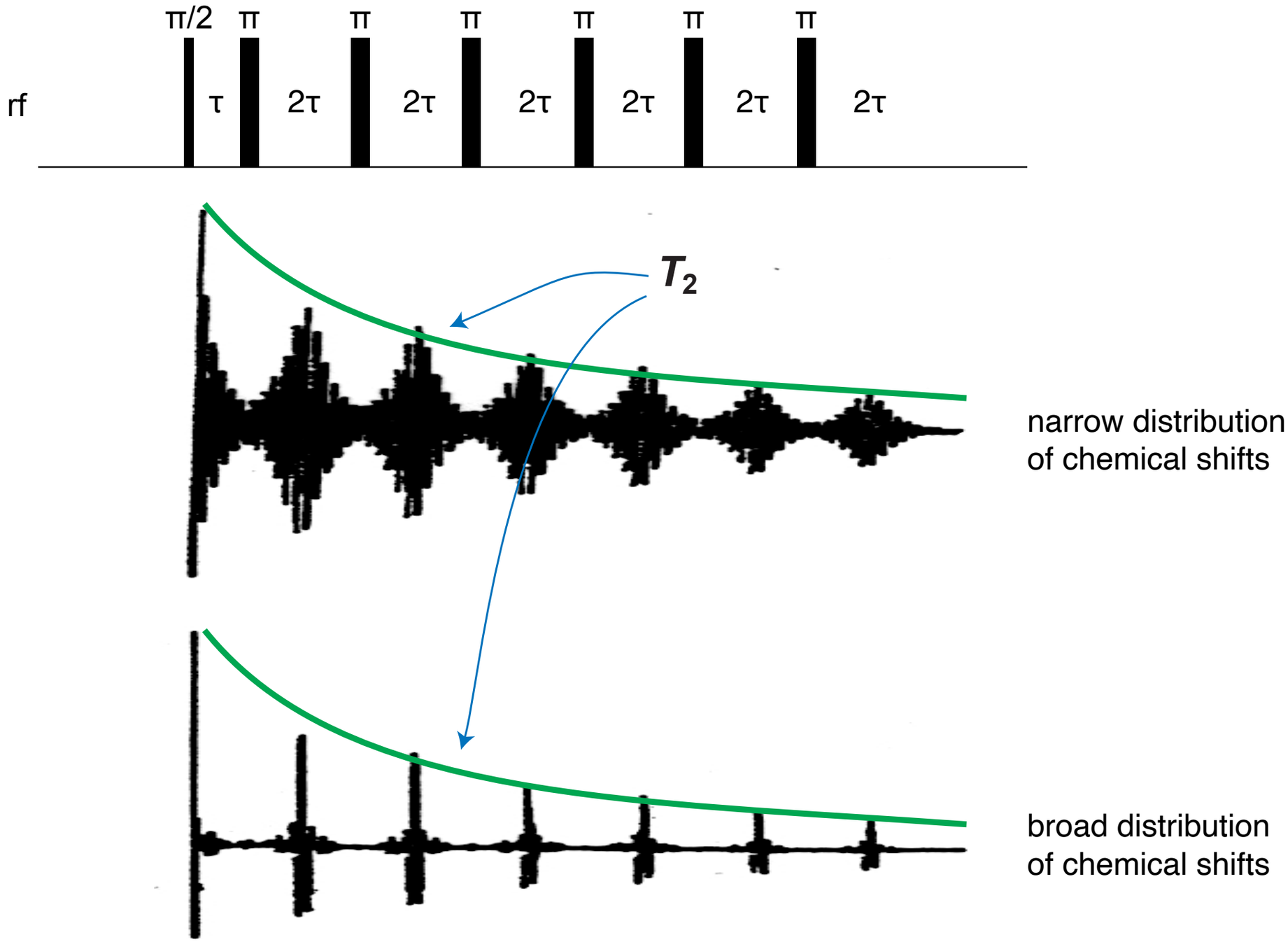
https://en.wikipedia.org/wiki/Spin_echo




The Hahn echo sequence can be used to measure the transverse relaxation time, $T_2$. The intensity of the echo formed at time 2t will decay as a function of t independently of any precesion frequencies present during t delays (i.e. different chemical shifts). A series of measurements with different 2t can then be fit to extract values of $T_2$ for each peak in the spectra (analogously to the way we measured $T_1$.)

# Dances with Spins: The Spin Echo

Multiple pulse NMR: Measurement of Transverse Relaxation Times ($T_2$)



# Conclusions: Part I

- $T_1$ and $T_2$ relaxation will tend to return the system to equilibrium

- Typically of the order of 10 ms —> 100 seconds

- The magnetisation dynamics is expressed by the Bloch equations

- $T_1$ can measured by the inversion-recovery pulse sequence

- $T_2$ can be measured by the spin-echo

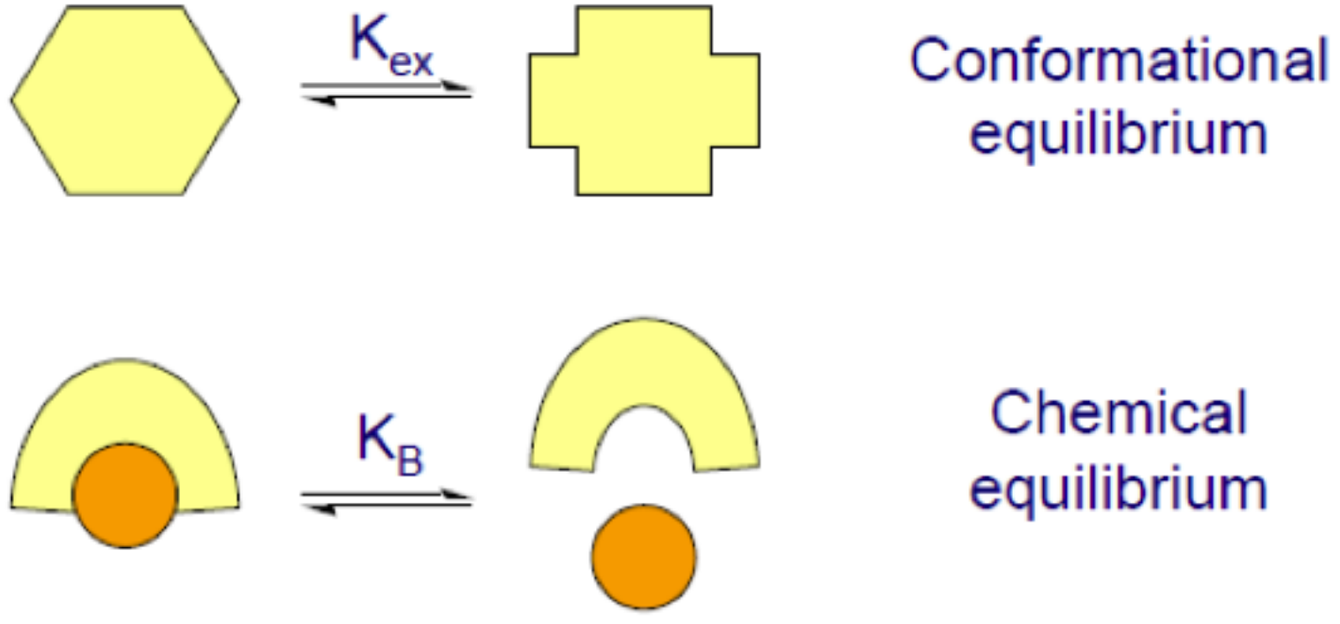
# Objectives

- Learn why exchange is a central idea in Chemistry.
- Determine how exchange can be observed in NMR spectra over different timescales.
- Provide a quantitative framework to describe exchange in NMR.

# Why is Exchange so Important?

More Generally, “Exchange” = Motion

Motion = Chemistry



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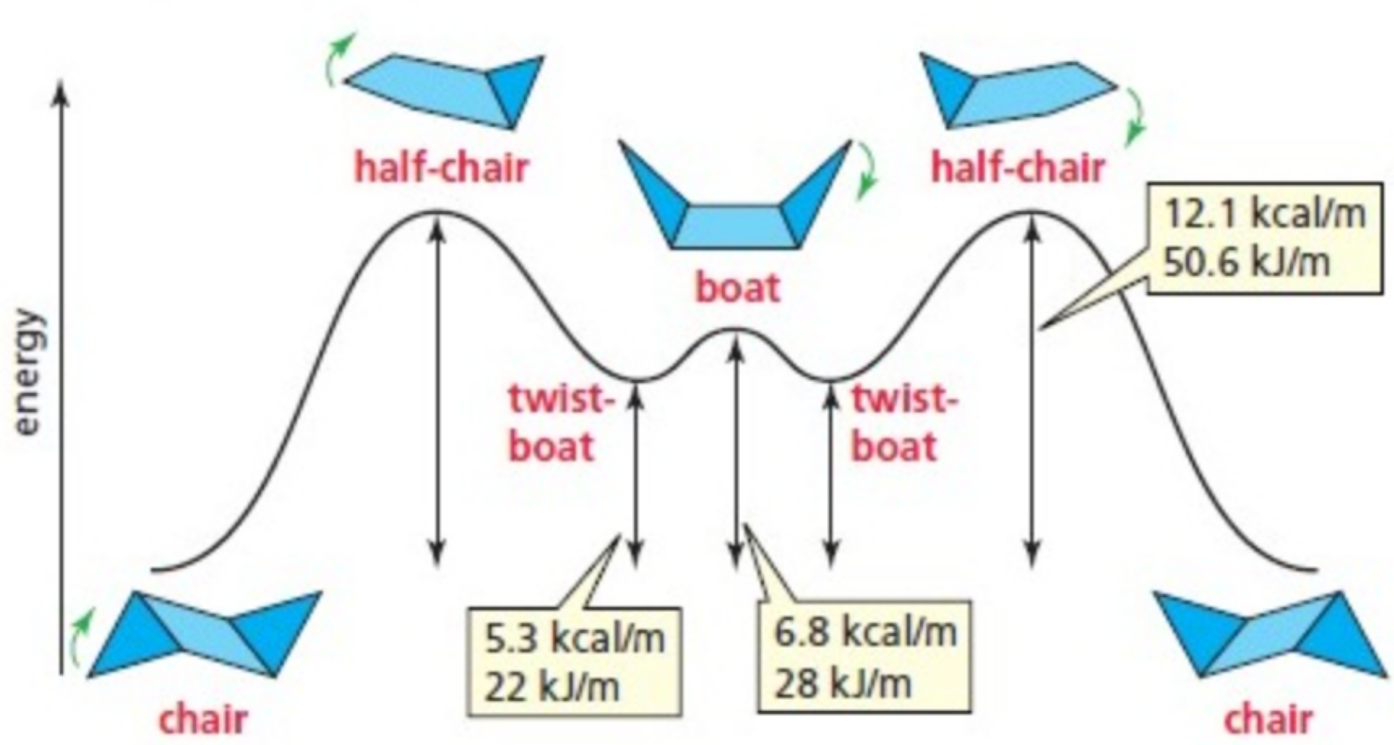

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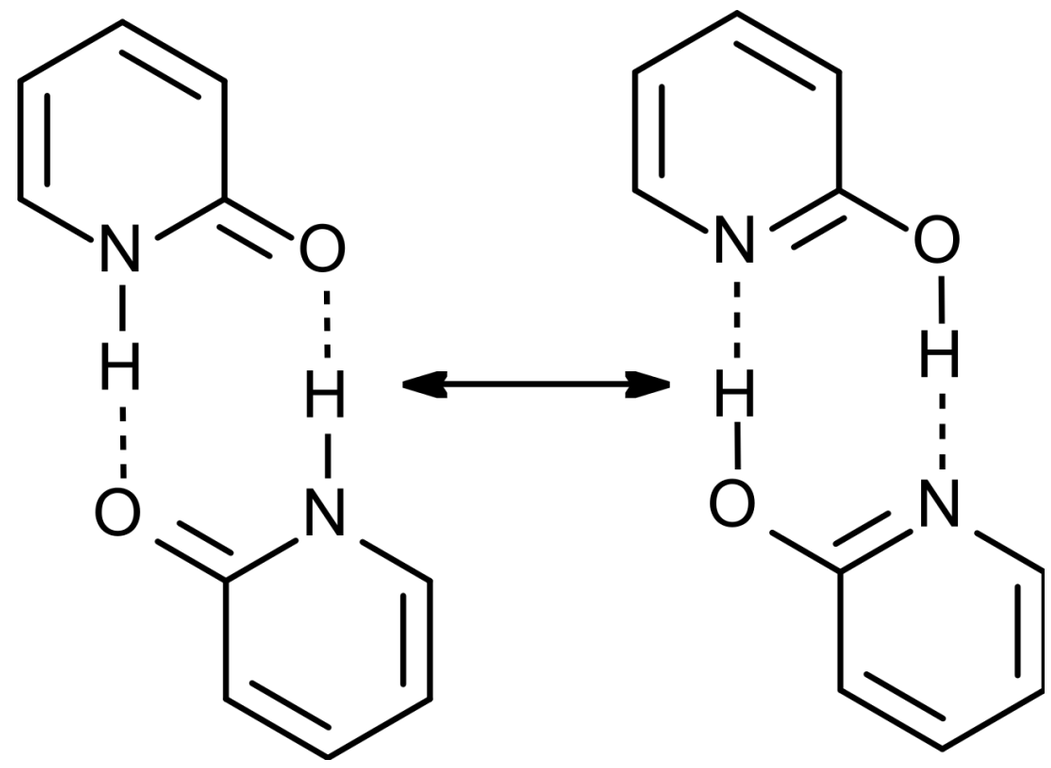

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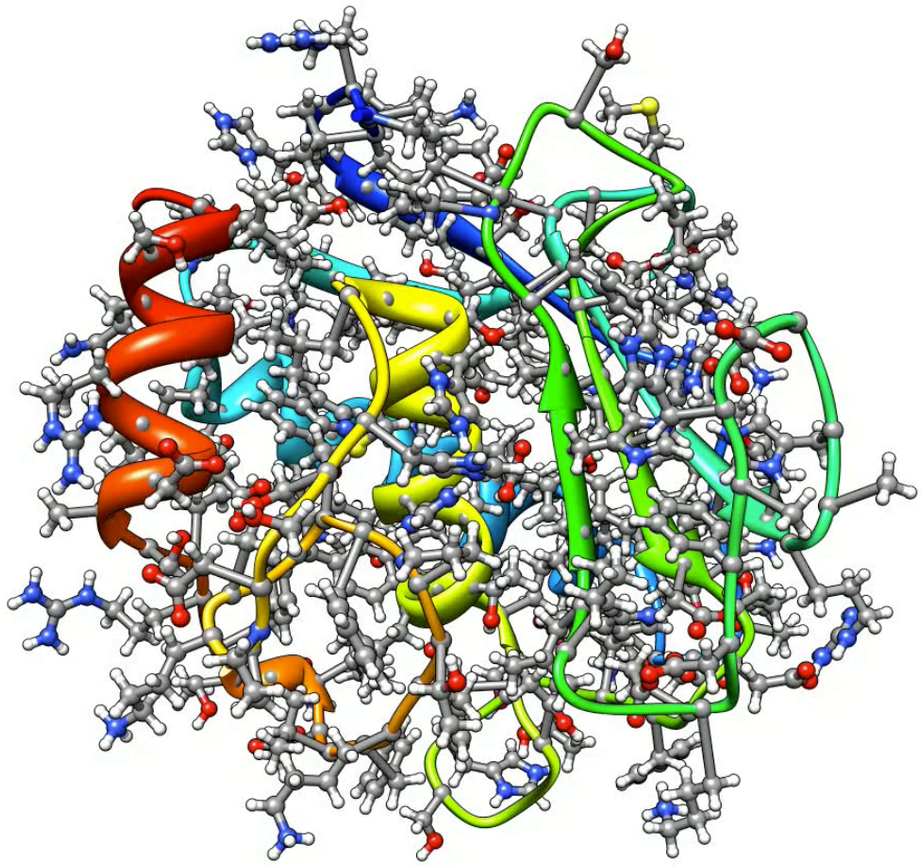

# Why is Motion so Important?

PROTEIN DYNAMICS

## Direct observation of hierarchical protein dynamics

Józef R. Lewandowski,[1*†] Meghan E. Halse,[1‡] Martin Blackledge,[2*] Lyndon Emsley[1,3*]

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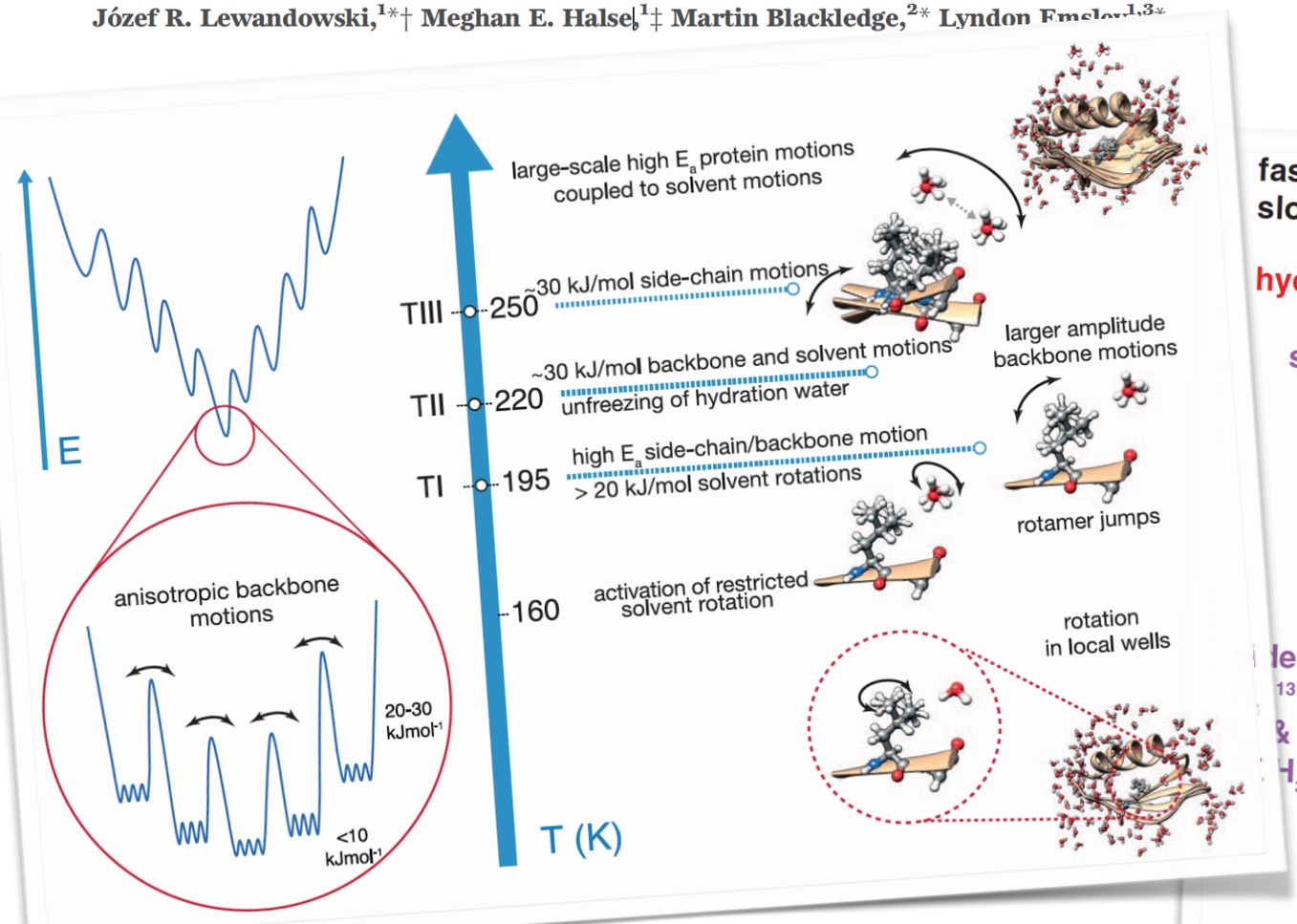


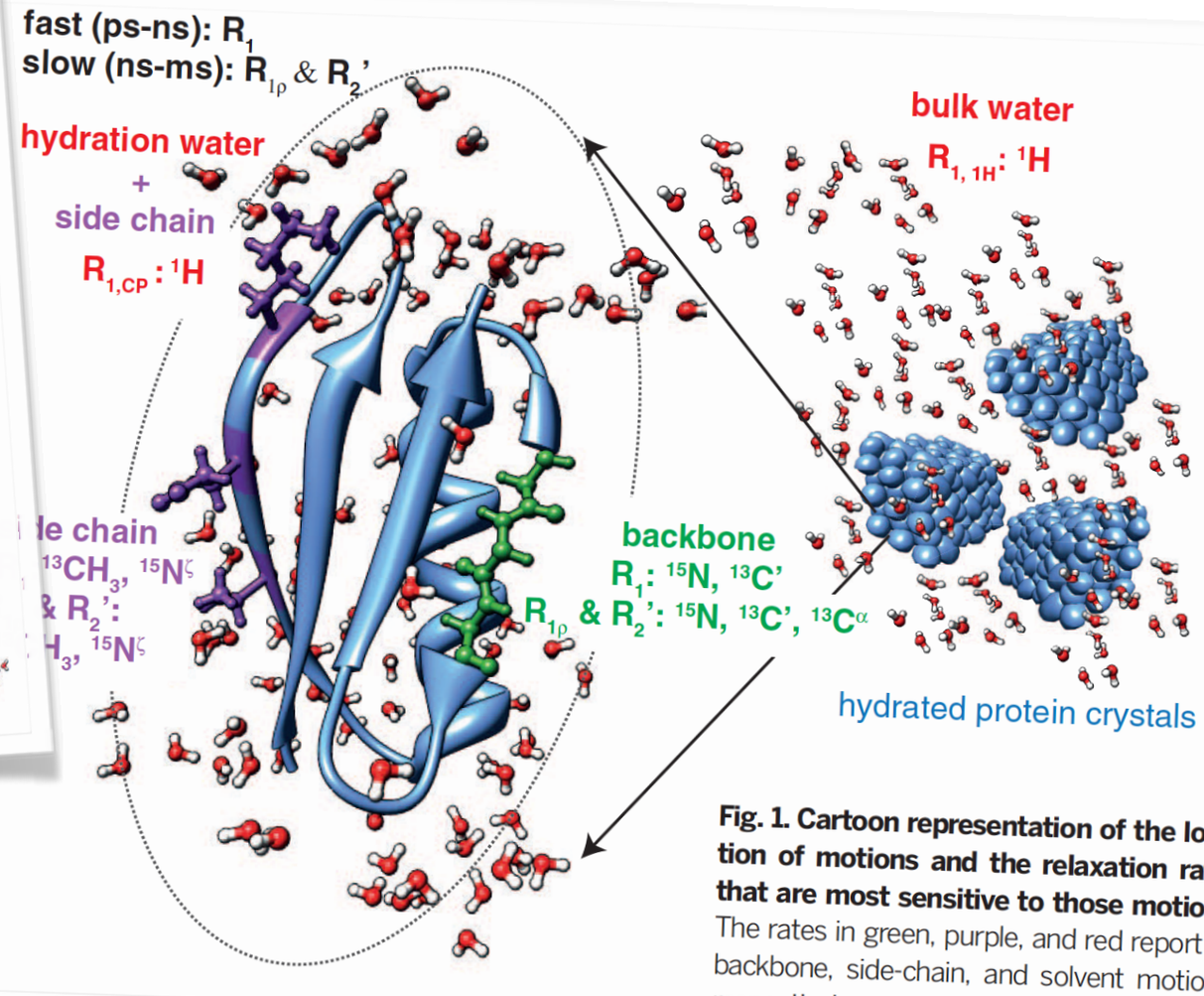
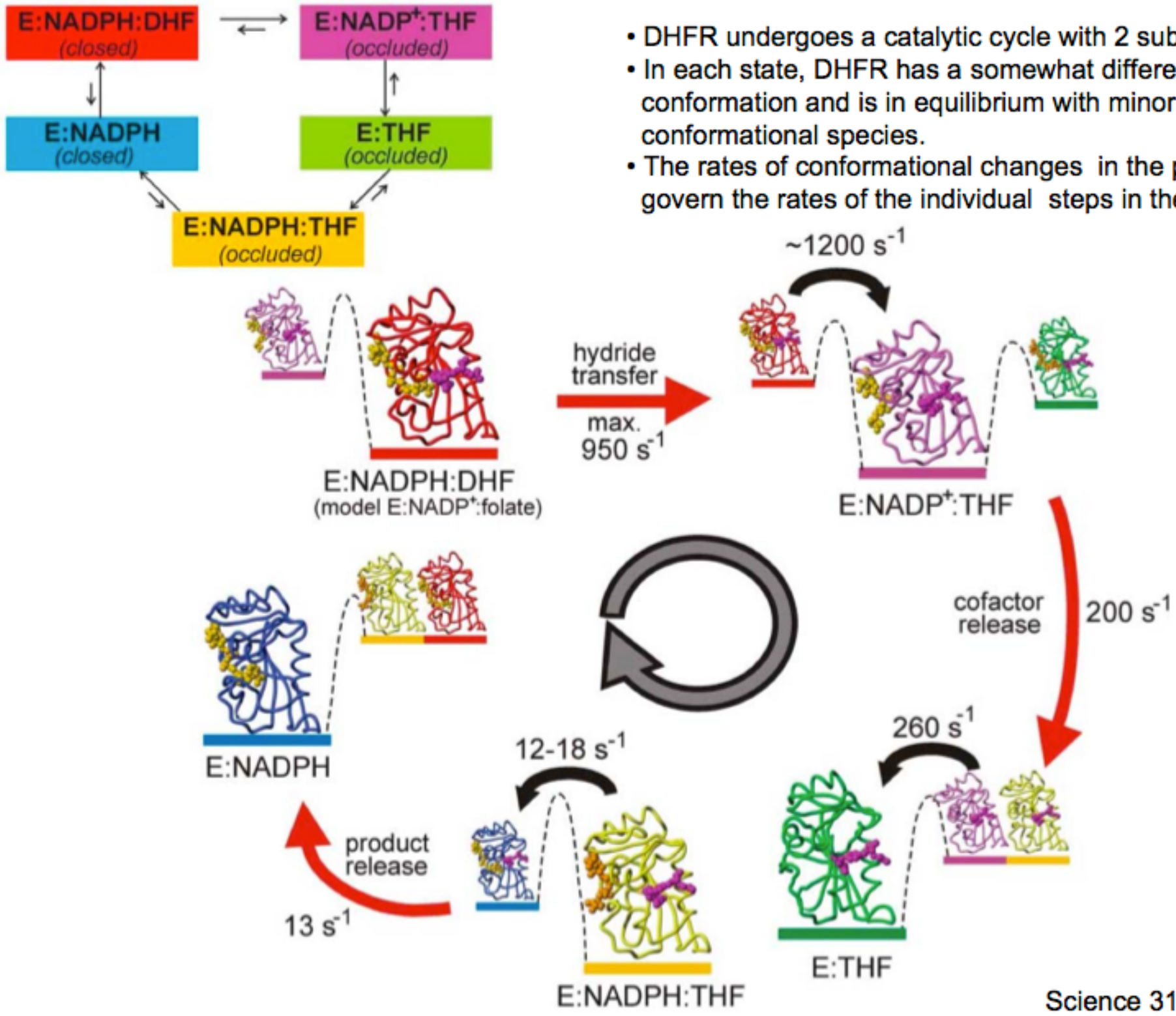




**Fig. 1. Cartoon representation of the location of motions and the relaxation rates that are most sensitive to those motions.** The rates in green, purple, and red report on backbone, side-chain, and solvent motions, respectively.

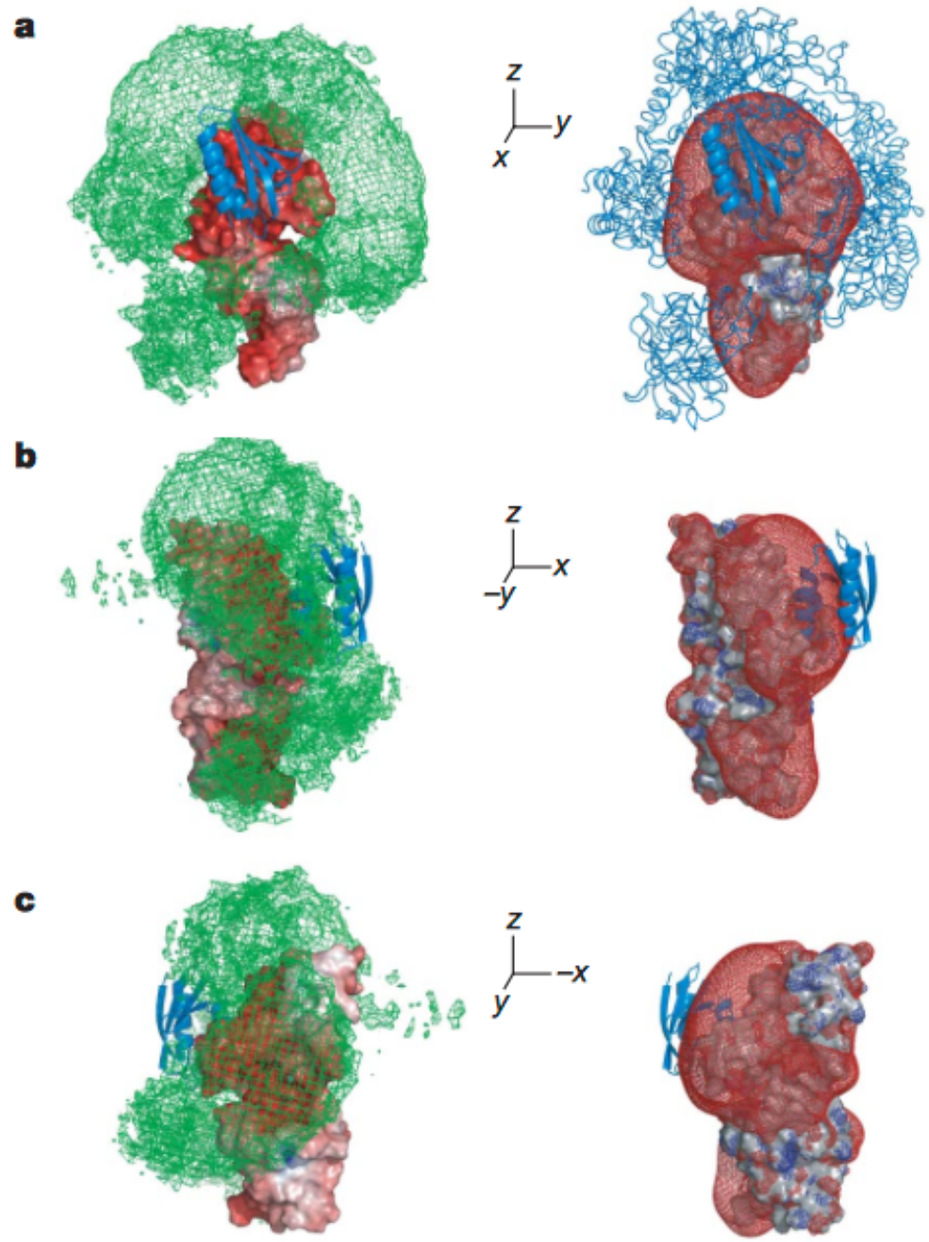



- DHFR undergoes a catalytic cycle with 2 substrates.
- In each state, DHFR has a somewhat different conformation and is in equilibrium with minor conformational species.
- The rates of conformational changes in the protein govern the rates of the individual steps in the cycle.

Science 313, 1638 (2006)

# Visualization of transient encounter complexes in protein–protein association

Chun Tang[1], Junji Iwahara[1] & G. Marius Clore[1]




nature
Vol 444|16 November 2006|doi:10.1038/nature05201


https://www.youtube.com/watch?v=ckTqh50r_2w

# Timescales of Motion

librational motions

overall tumbling

collective (domain) motions

ps ns µs ms s

spin relaxation

lineshape/exchange

relaxation dispersion

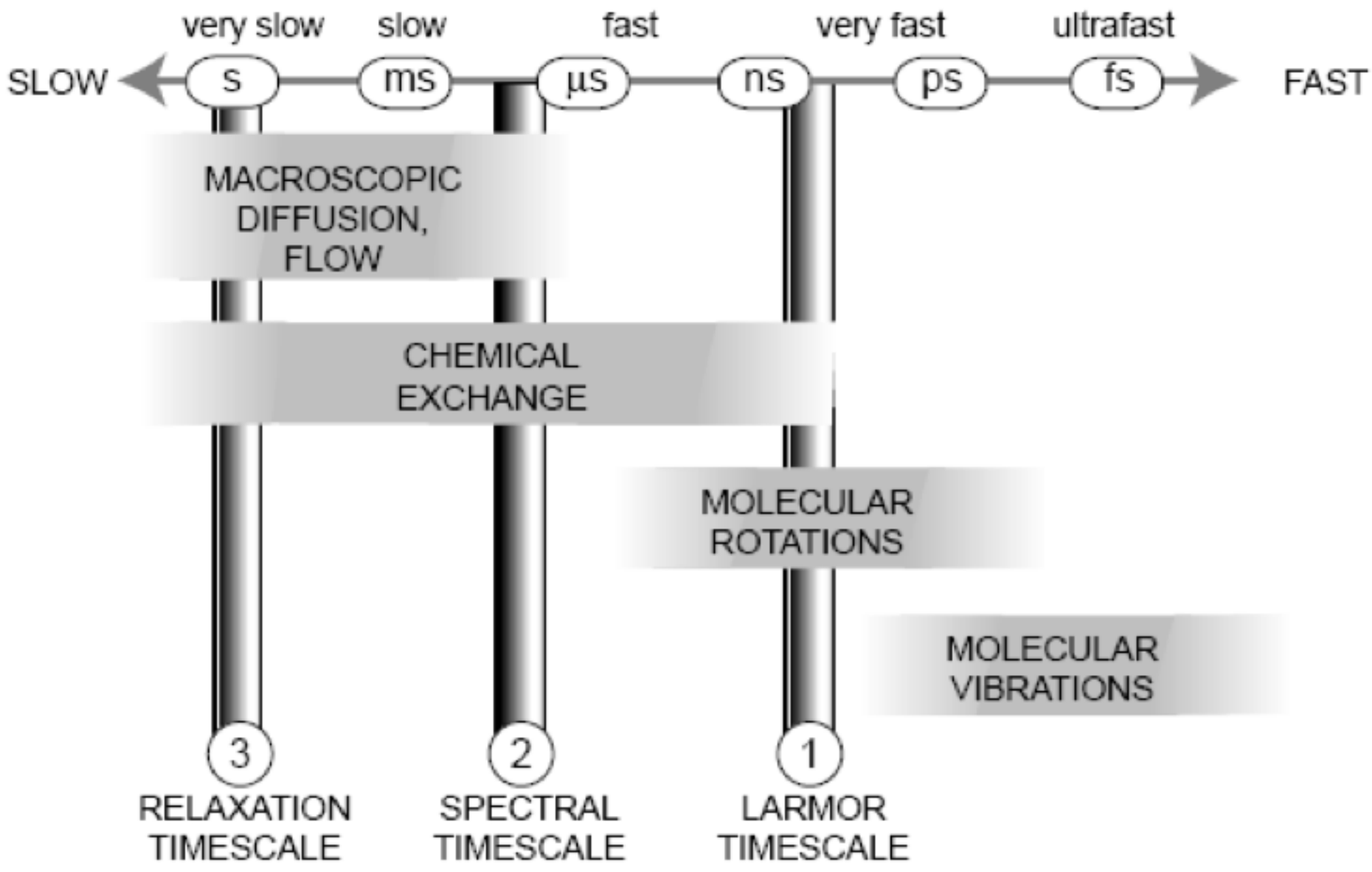
real-time NMR

dipolar, scalar couplings, chemical shifts

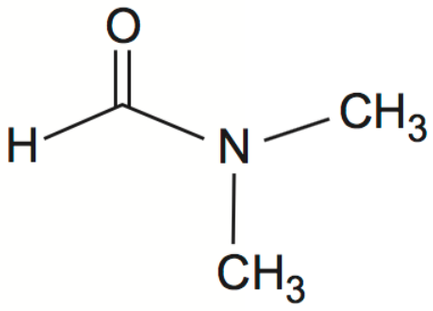
enzyme catalysis
signal transduction
ligand binding

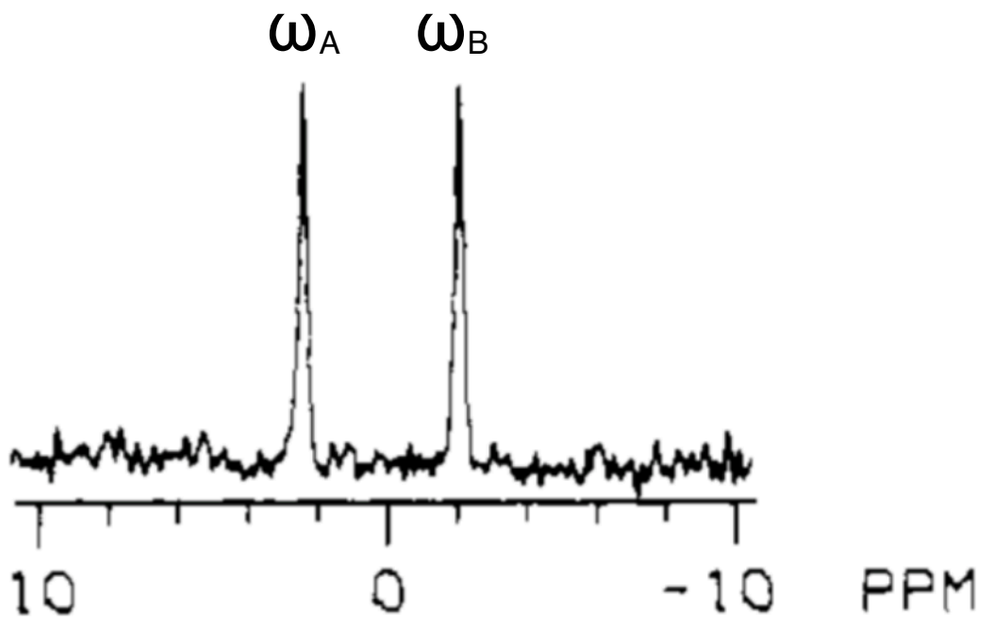
protein folding
hinge bending

# Timescales of Motion



# Determining Exchange by NMR

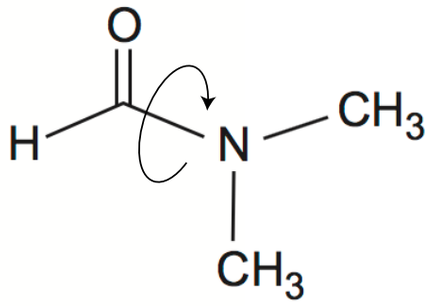


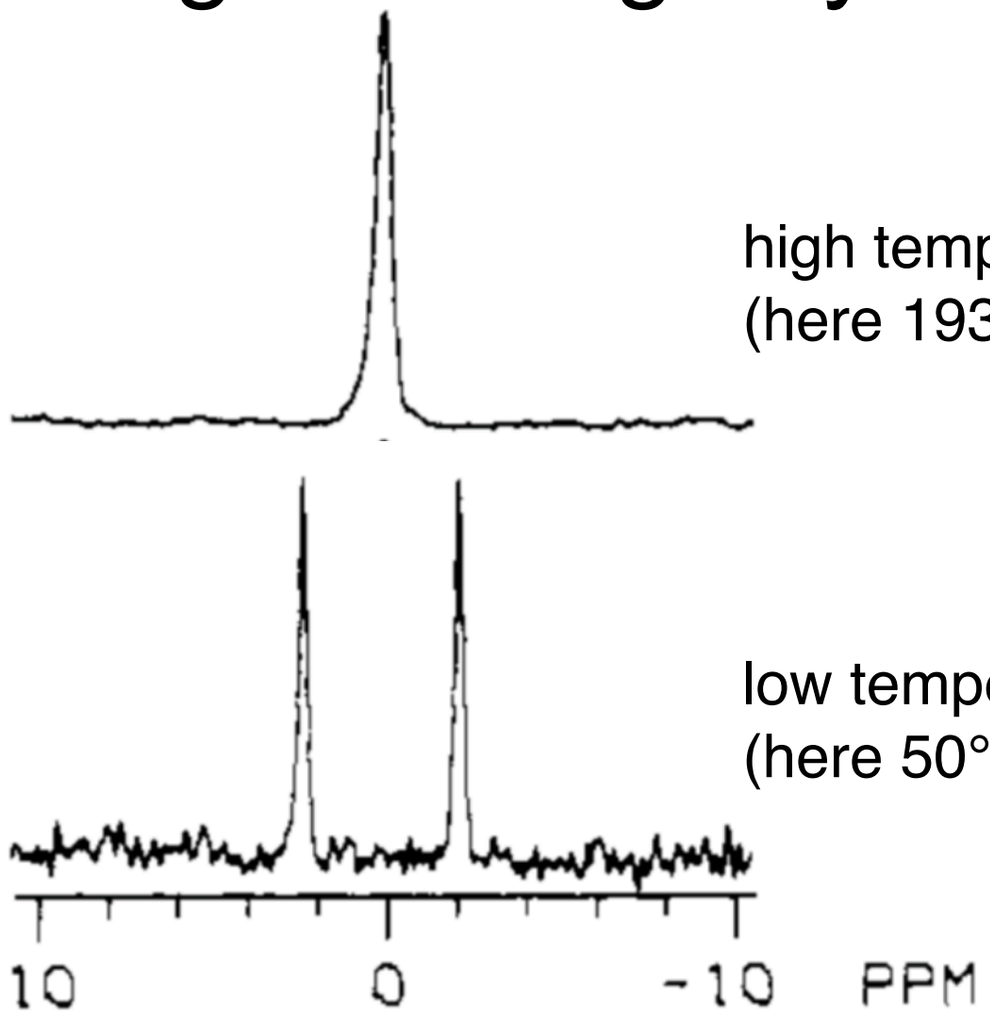




Consider a spectrum consisting of two singlets belonging to the two inequivalent methyl carbon nuclei in the molecule shown.
In the absence of exchange it yields the above spectrum.
(This is often referred to as the low temperature spectrum. (Here 50 °C))

1. If the exchange rate $k_{AB} << \Delta\omega$ (the **slow motion** regime): there is no effect on the spectrum.

# Determining Exchange by NMR



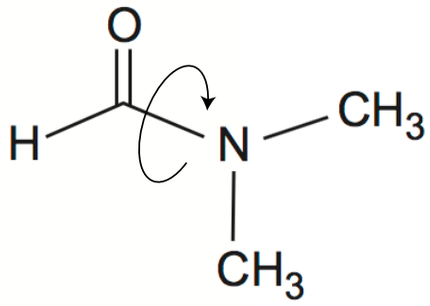

high temperature spectrum
(here 193 °C)

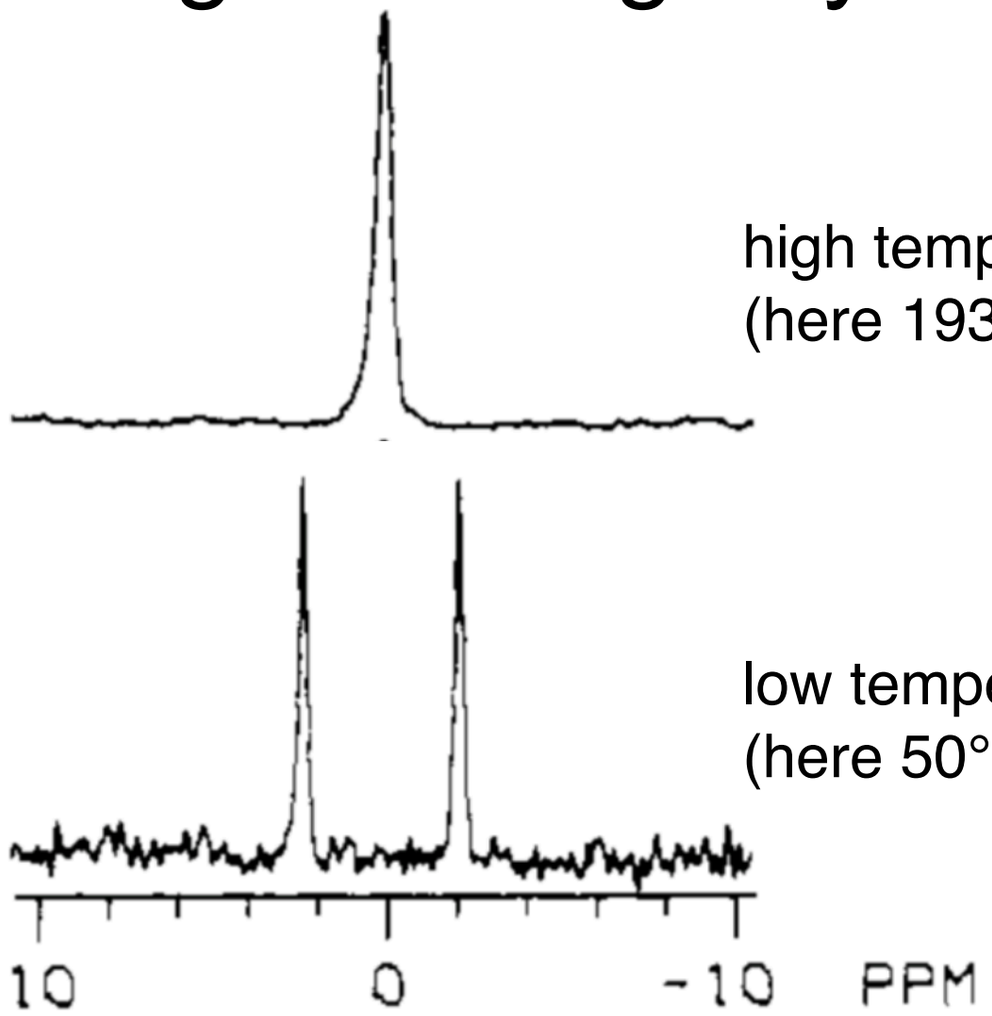
low temperature spectrum
(here 50°C)

Consider a spectrum consisting of two singlets belonging to the two inequivalent methyl carbon nuclei in the molecule shown.
If the temperature is increased, rotation around the C-N is activated.

2. If the exchange rate $k_{AB} >> \Delta\omega$ (the **fast motion** regime): we observe only the average of the two lines, which yields a single narrow line at $(\omega_A + \omega_B)/2$.

# Determining Exchange by NMR




high temperature spectrum
(here 193 °C)

low temperature spectrum
(here 50°C)

3. If the exchange rate $k_{AB} \sim \Delta\omega$ (the **intermediate motion** regime): what happens?

# Determining Exchange by NMR

NMR is sensitive to exchange processes on three distinct timescales:

1. **FAST** ($10^{-12} < t_c < 10^{-5}$ s)
2. **INTERMEDIATE** ($10^{-5} < t_c < 10^{-2}$ s)
3. **SLOW** ($10^{-2} < t_c < 10^{2}$ s)

And we use three different techniques to access dynamic information in the three regimes:

1. **FAST : relaxation times**
2. **INTERMEDIATE : lineshapes/positions**
3. **SLOW : 2D Exchange Spectroscopy**

# Determining Exchange by NMR

**Lineshapes and Exchange.**

$$A_j \leftrightharpoons A_r$$

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

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

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

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

# Modified Bloch Equations

The effects on the NMR spectrum can be described using a set of modified Bloch equations (often called the McConnell equations).

Recall: the ordinary Bloch equations describe the motion of the magnetization of spin *j*:

$$\frac{d}{dt}\mathbf{M}_j(t) = \gamma(1-\sigma_j)\mathbf{M}_j(t)\times\mathbf{B}(t) - \mathbf{R}_j\left(\mathbf{M}_j(t) - \mathbf{M}_j(0)\right) \qquad (2)$$

with the relaxation matrix

$$R_j = \begin{pmatrix} 1/T_{2j} & 0 & 0 \\ 0 & 1/T_{2j} & 0 \\ 0 & 0 & 1/T_{1j} \end{pmatrix}.$$

A chemical reaction network with *J* species causes magnetization transfer between the various species and couples J equations of the type of equation 2. The chemical dynamics leads to the modified Bloch equations

$$\frac{d}{dt}\mathbf{M}_j(t) = \gamma(1-\sigma_j)\mathbf{M}_j(t)\times\mathbf{B}(t) - \mathbf{R}_j\left(\mathbf{M}_j(t) - \mathbf{M}_{j0}(t)\right) + \sum_r k_{jr}\mathbf{M}_r(t)$$

where

$$\mathbf{M}_{j0}(t) = \mathbf{M}_0 \frac{[A_j](t)}{\sum_k [A_k]}$$

(the z component in magnetic equilibrium, $\mathbf{M}_{j0}$, is proportional to the instantaneous concentration $[A_j](t)$).

# 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

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

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

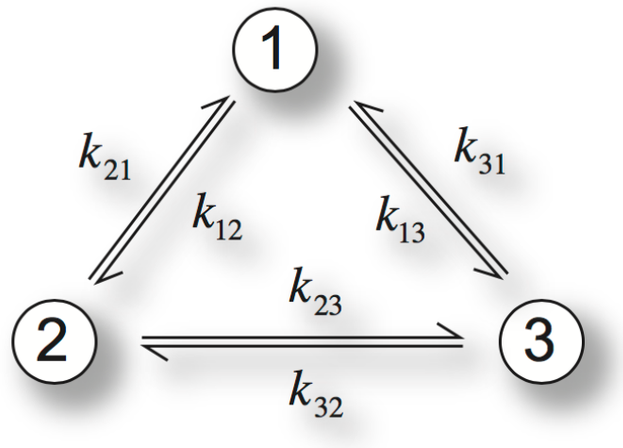
$\boldsymbol{\Omega}$ is a diagonal matrix containing the chemical shifts $\Omega_j$ .

$\boldsymbol{\Lambda}$ 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 $T_{1j}^{-1}$.

# Modified Bloch Equations

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

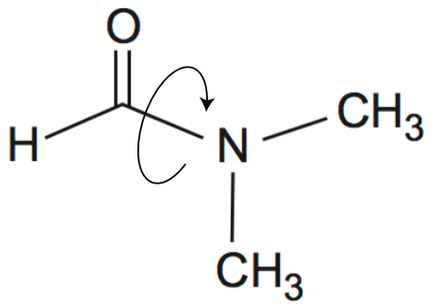


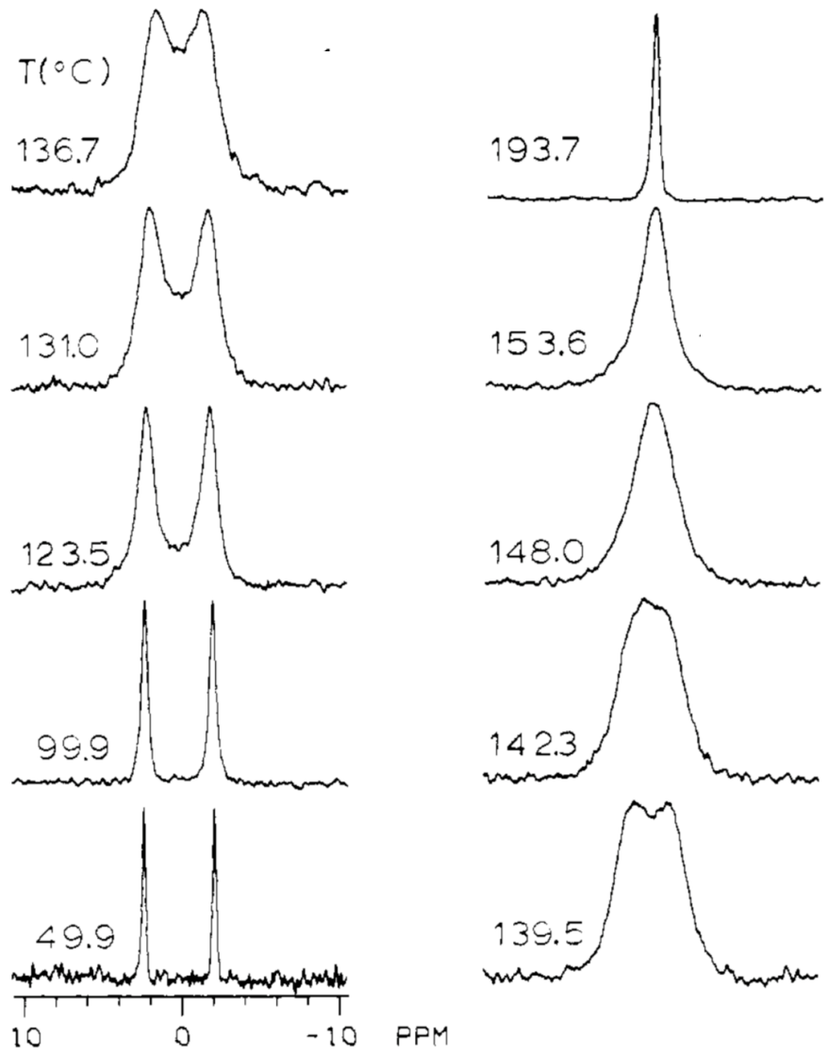

The time evolution of the transverse magnetization components is governed by

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

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

# Determining Exchange from Lineshapes

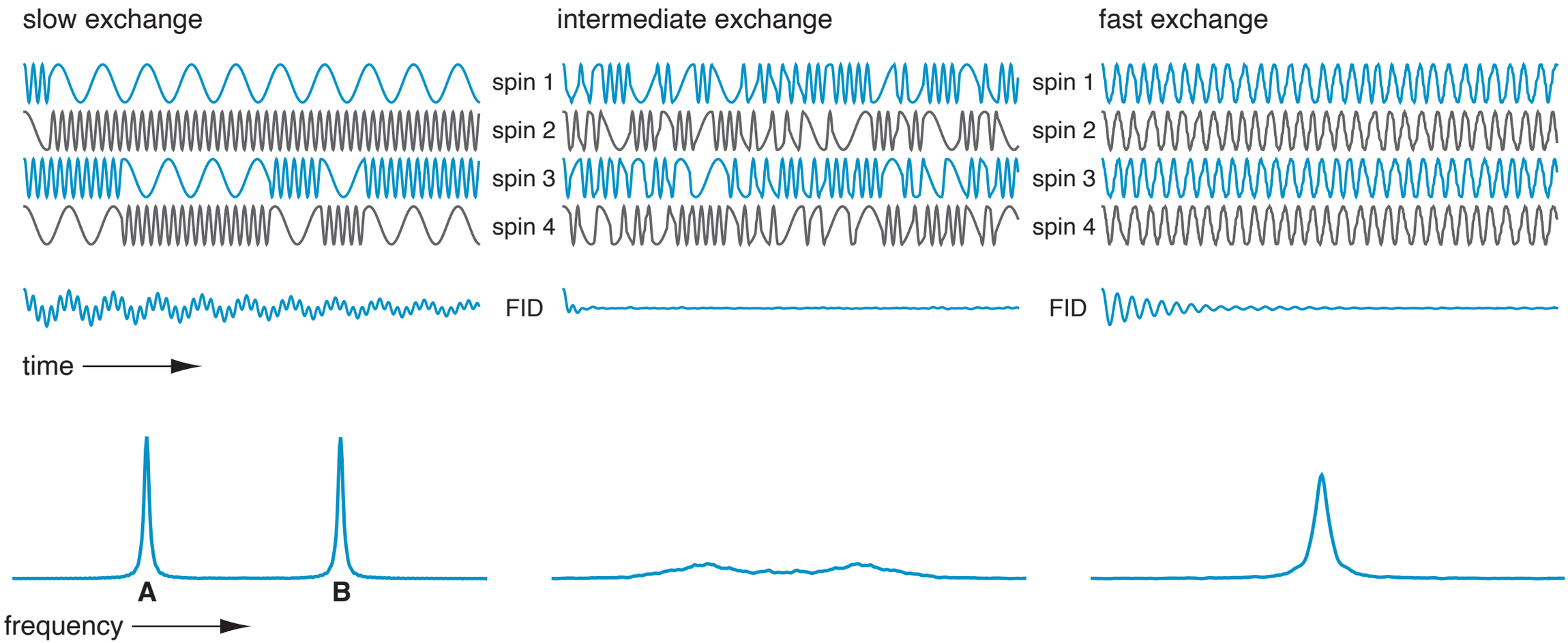



Experimental $^{13}C$ spectra of $^{13}C$-enriched N,N'-dimethylformamide gas at a set of different temperatures in a field of 4.7 T.
B. D. Ross and N. S. True, *J. Am. Chem. Soc.* **106**, 2451 (1984).

In the intermediate regime, as the rate increases (with increasing temperature) the lines first broaden, then they coalesce, and then the line narrows to a single resonance at the average frequency.

# Motional Broadening

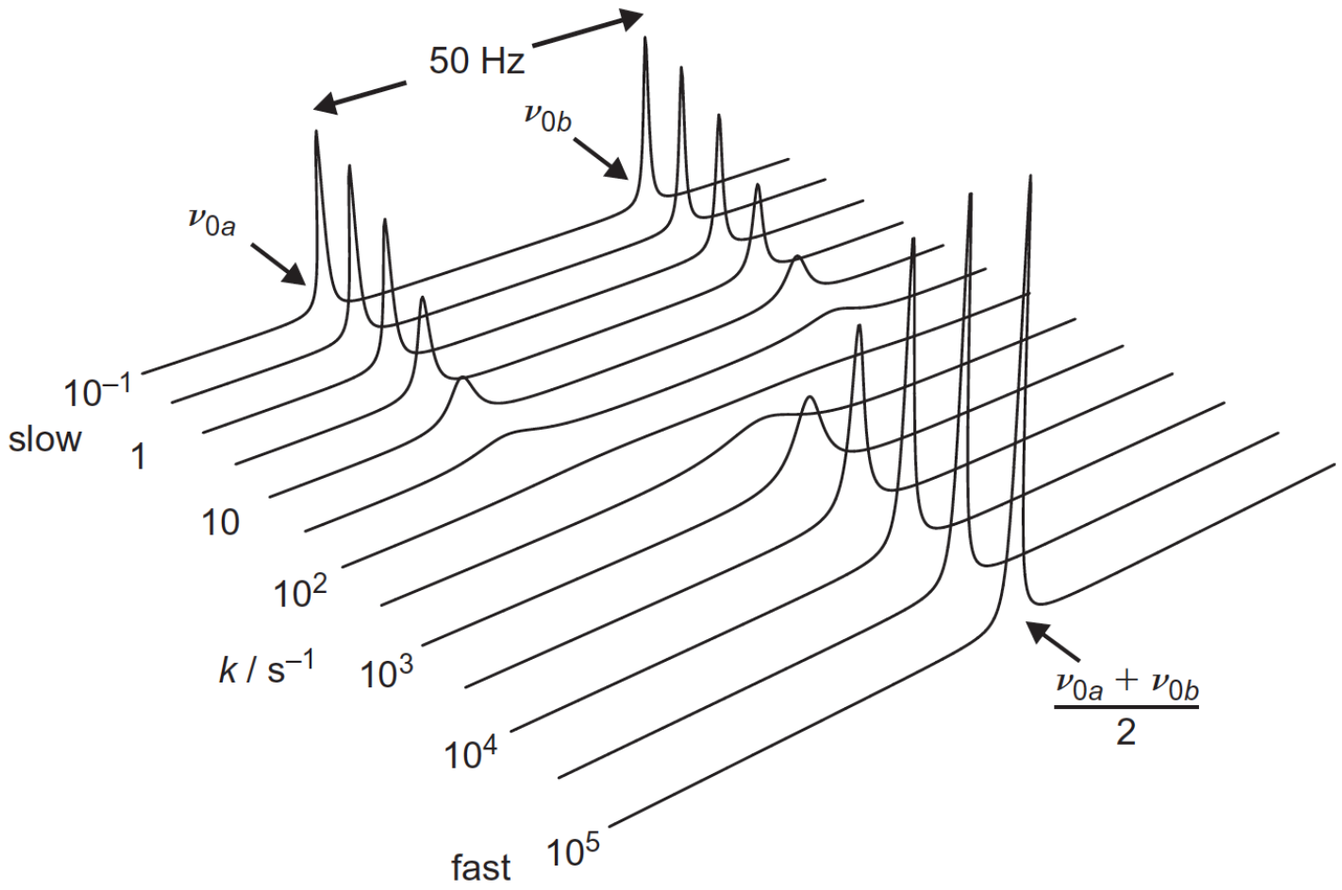



How the behaviour of individual spins accounts for the form of the spectra for an A ⇋ B exchange system. The top part shows the contributions from four typical spins. Underneath, labelled FID, is the sum of the contributions (from 1000 spins). Beneath each FID is shown the corresponding spectrum.

In slow exchange the number of transformations between A and B are rather few so distinct periods of oscillation at the two Larmor frequencies can be seen. The result is two clear lines in the spectrum. In intermediate exchange the transformations are more frequent, and as a result the contributions of the individual spins quickly cancel one another out, leading to a quickly decaying FID and broad lines in the spectrum. In fast exchange the transformations are so frequent that each spin appears to be evolving at the average frequency. The individual contributions do not significantly cancel, leading to a sharper line.

from Keeler

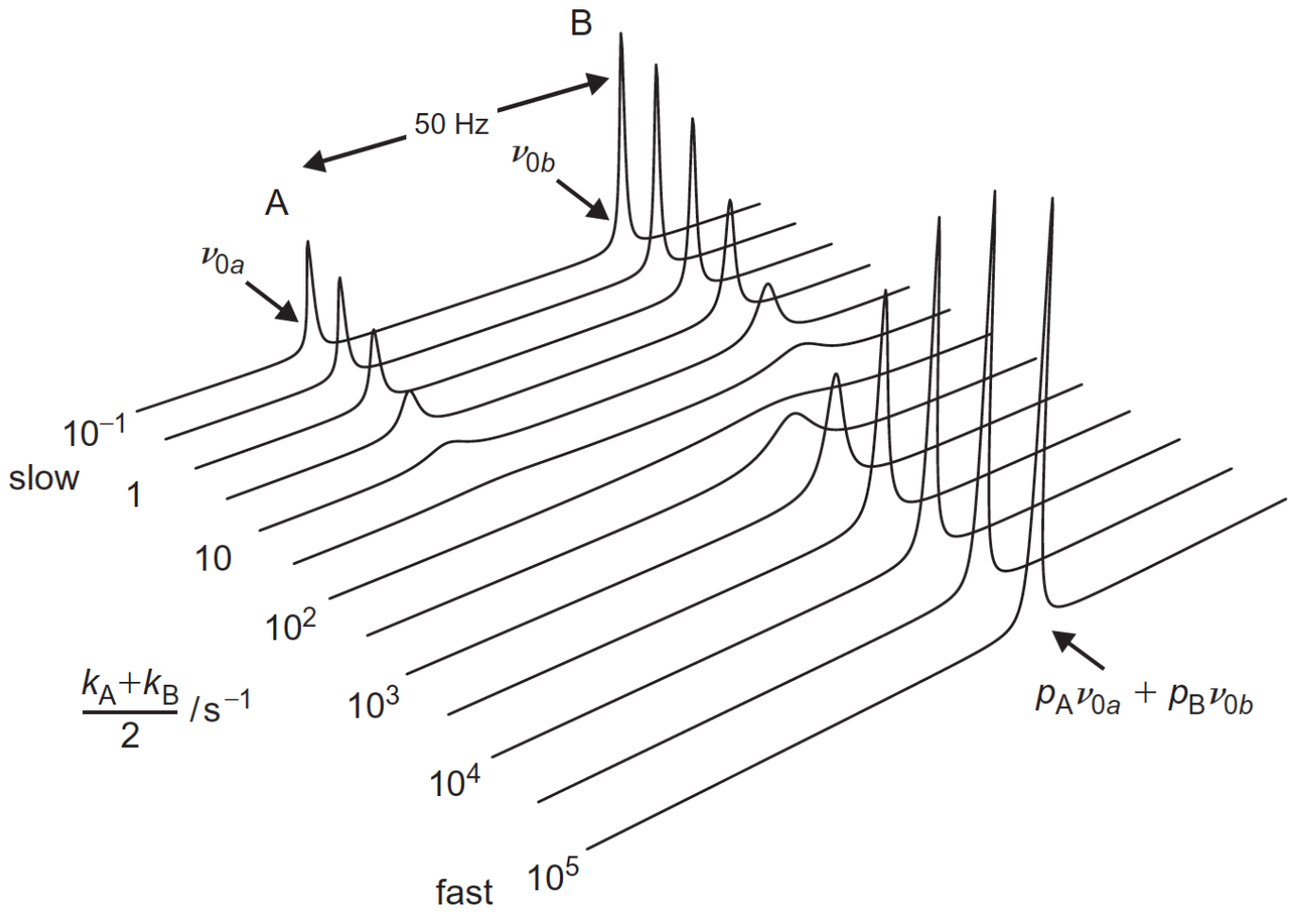
# Determining Exchange from Lineshapes




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.

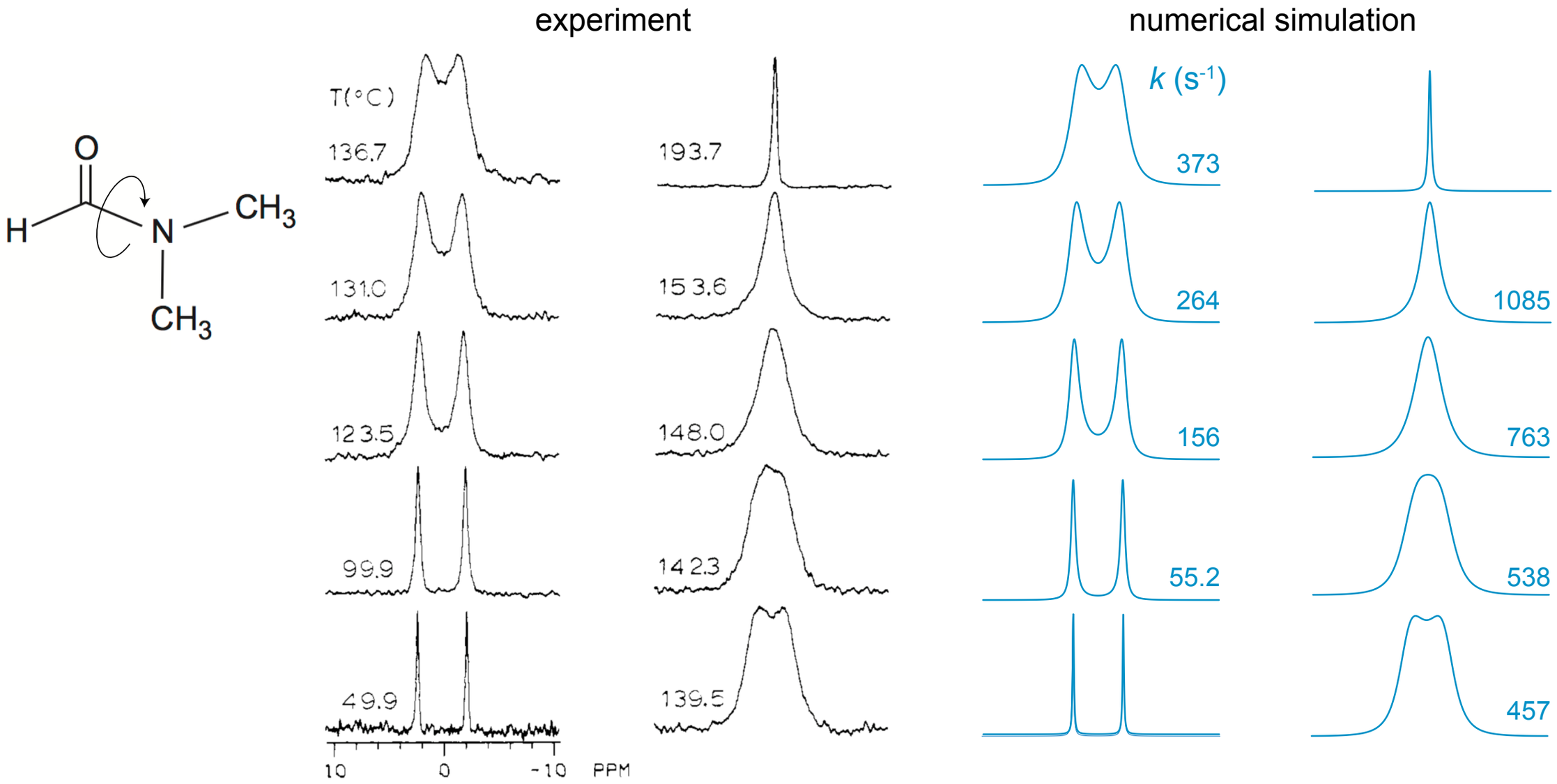
# Determining Exchange from Lineshapes




**Calculated NMR spectra** for a pair of nuclei exchanging between two sites A and B with populations in the ratio $p_B/p_A = 2$ (**unsymmetrical two-site exchange**). Spectra are shown for a range of values of the mean rate constant $(k_A + k_B)/2$. The difference in resonance frequencies of the two sites, δν, is 50 Hz. The linewidths in the absence of exchange are 1 Hz.

$$A \underset{k_B}{\overset{k_A}{\rightleftharpoons}} B \quad P_A = \frac{k_B}{k_A + k_B} \quad P_B = \frac{k_A}{k_A + k_B}$$

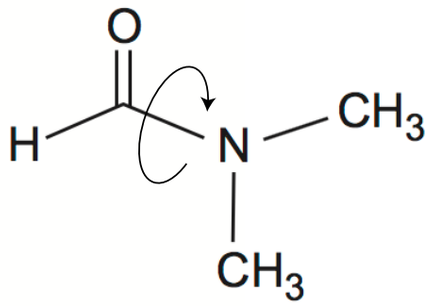
# Determining Exchange from Lineshapes



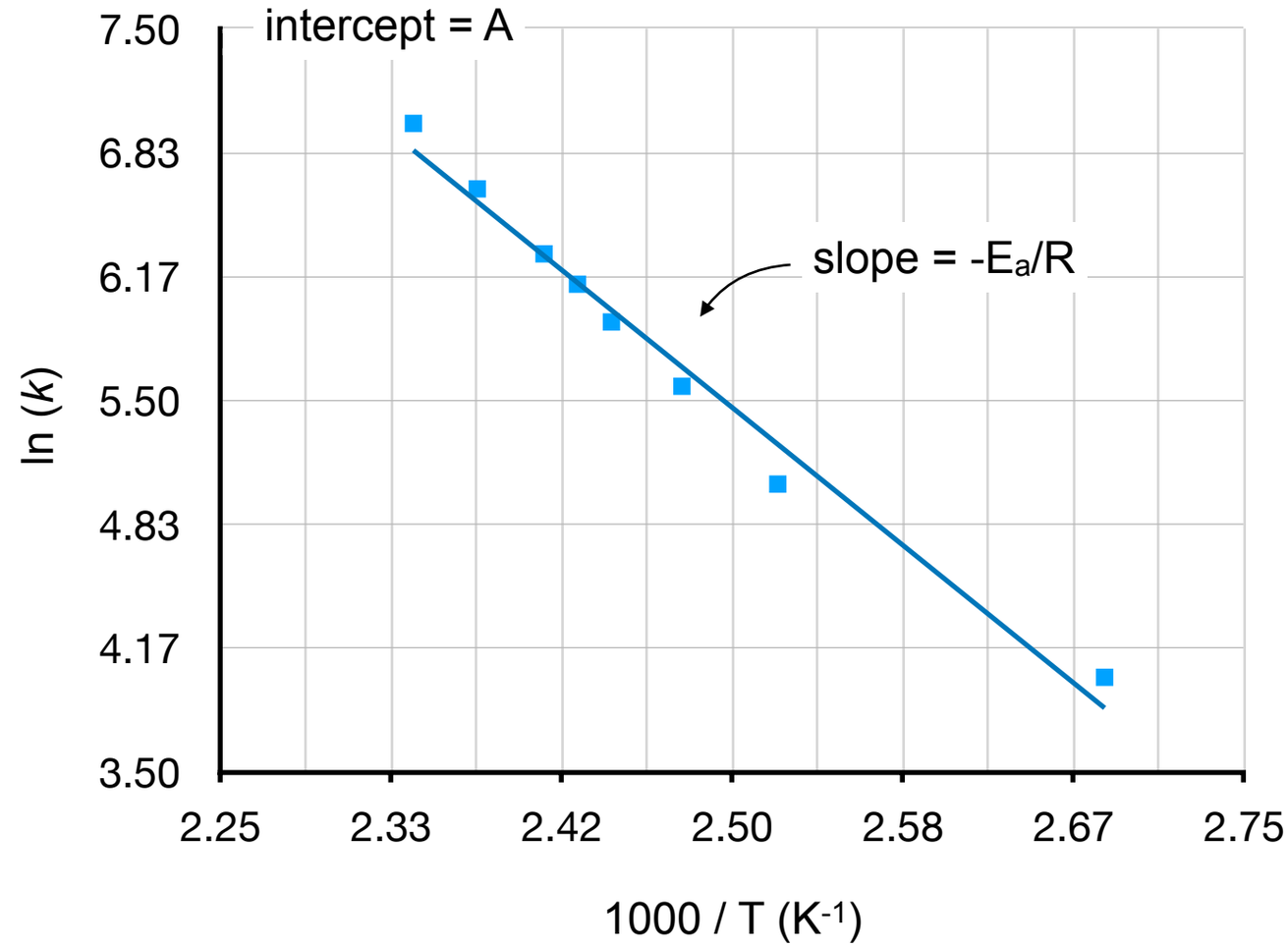

$^{13}$C spectra of N,N'-dimethylformamide gas at 4.7 T.
B. D. Ross and N. S. True, *J. Am. Chem. Soc,* **106** 2451 (1984).

Fitting each spectrum to a rate, using the modified Bloch equations and with the chemical shifts and intrinsic linewidths measured in the low temperature spectrum, yields the rate at each temperature

# Determining Exchange by NMR




Activation energy of C-N bond rotation in N, N'-dimethylformamide gas.
B. D. Ross and N. S. True,
J. Am. Chem. Soc. **106**, 2451 (1984).




For an activated process the Arrhenius equation is: $k = A \exp(E_a/RT)$ where A is a pre-exponential factor and $E_a$ is the activation energy for the process. $E_a$ can be determined from the slope of a plot of 1/T vs ln($k$) (called an Arrhenius plot).

Here we find $E_a$ = 74 kJ/mol for C-N bond rotation in N, N'-dimethylformamide gas.

# Conclusions

- NMR is sensitive to exchange in different ways depending on the exchange timescale.

- Intermediate exchange directly affects line shapes in the spectra. This can be evaluated quantitatively using the Bloch-McConnell Equations for a network of *N* exchanging sites.

- As $k_{ex}$ increases, averaging of the exchanging frequencies first produces line broadening ($k_{ex} < \Delta_{AB}$) and then leads to line narrowing ($k_{ex} > \Delta_{AB}$).

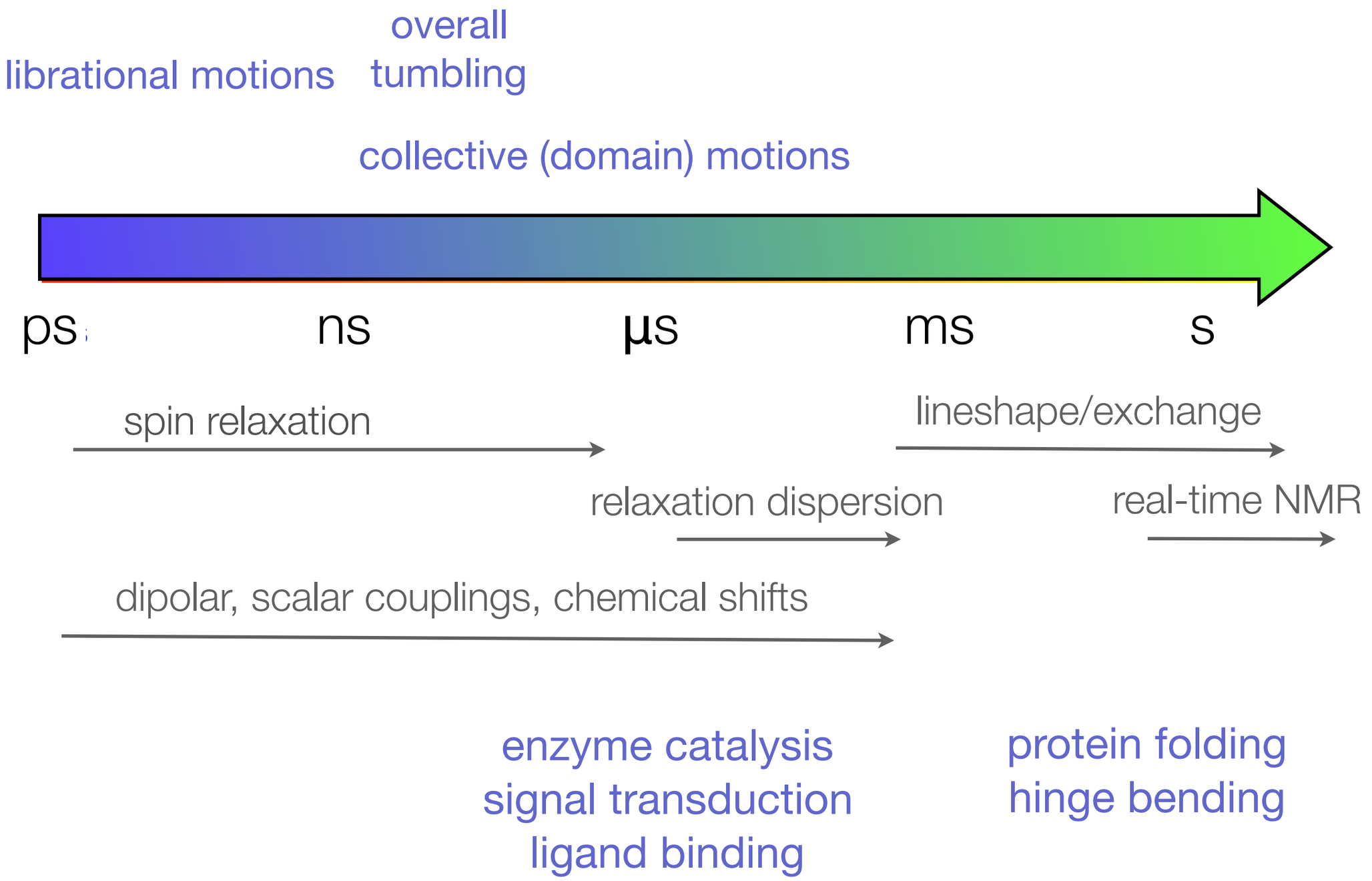
- Measuring the rate as a function of temperature yields the *activation energy* for the exchange process.

- NMR can measure exchange rates in samples at equilibrium.

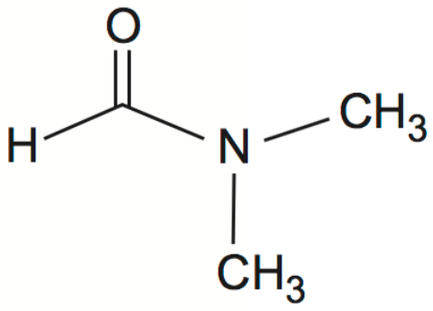
# Objectives

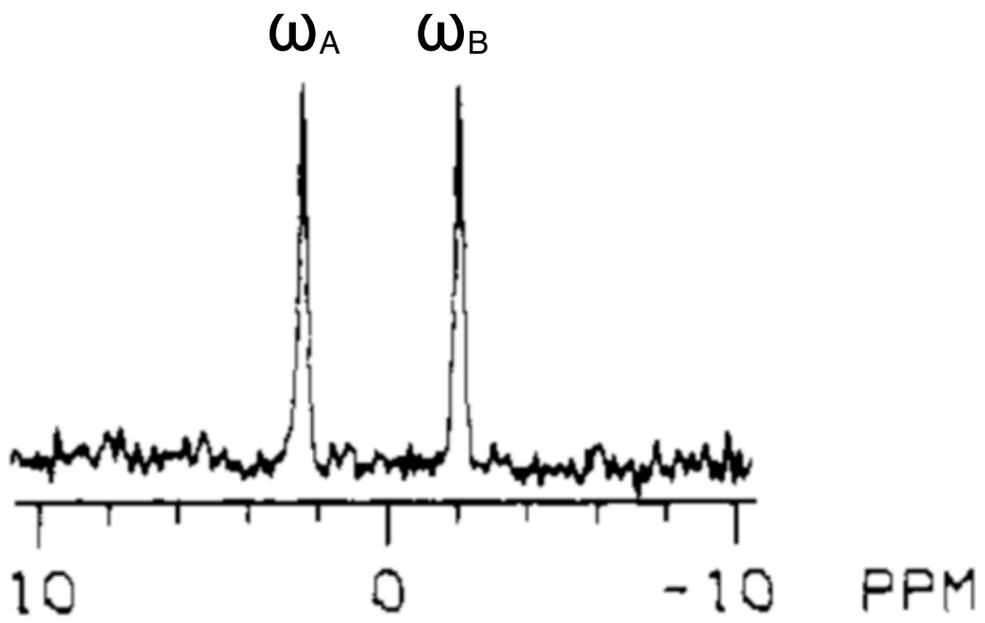
- How can we measure slow exchange processes?
- Principles of two-dimensional NMR
- Two-dimensional Exchange Spectroscopy (EXSY)

# Timescales of Motion



# Determining Exchange by NMR




1. If the exchange rate $k_{AB} << \Delta\omega$ (the **slow motion** regime): there is no effect on the spectrum.

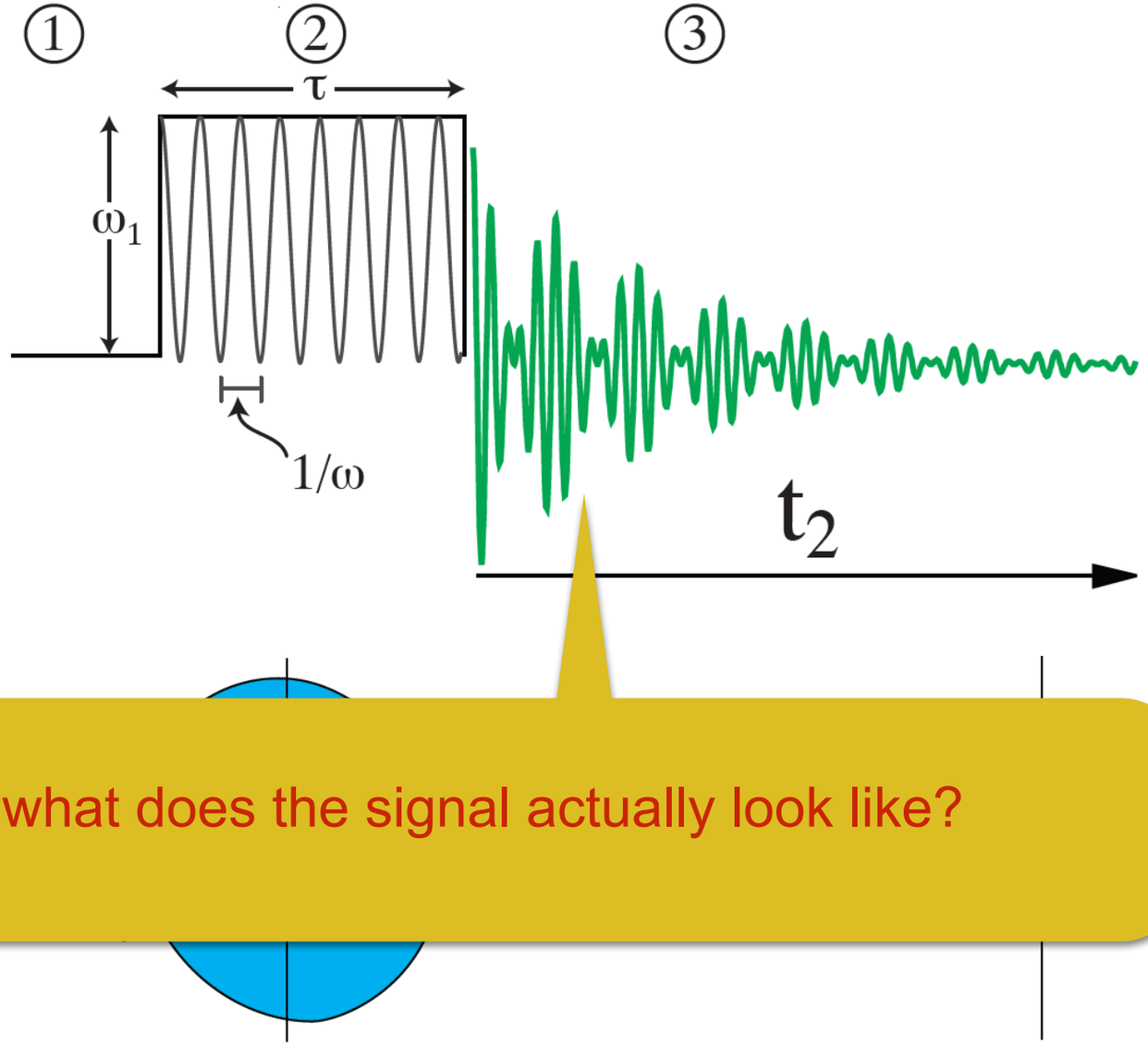
# Pulsed FTNMR Spectroscopy

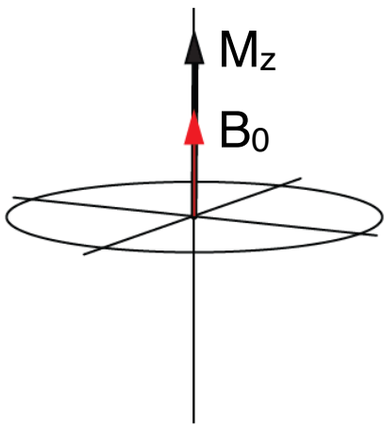
$\omega_1 = -\gamma B_1$

$\omega_1 \tau = \pi/2$

$^1\mathbf{H}$

$\omega$ = carrier frequency, chosen by the operator to be near to the resonance frequencies



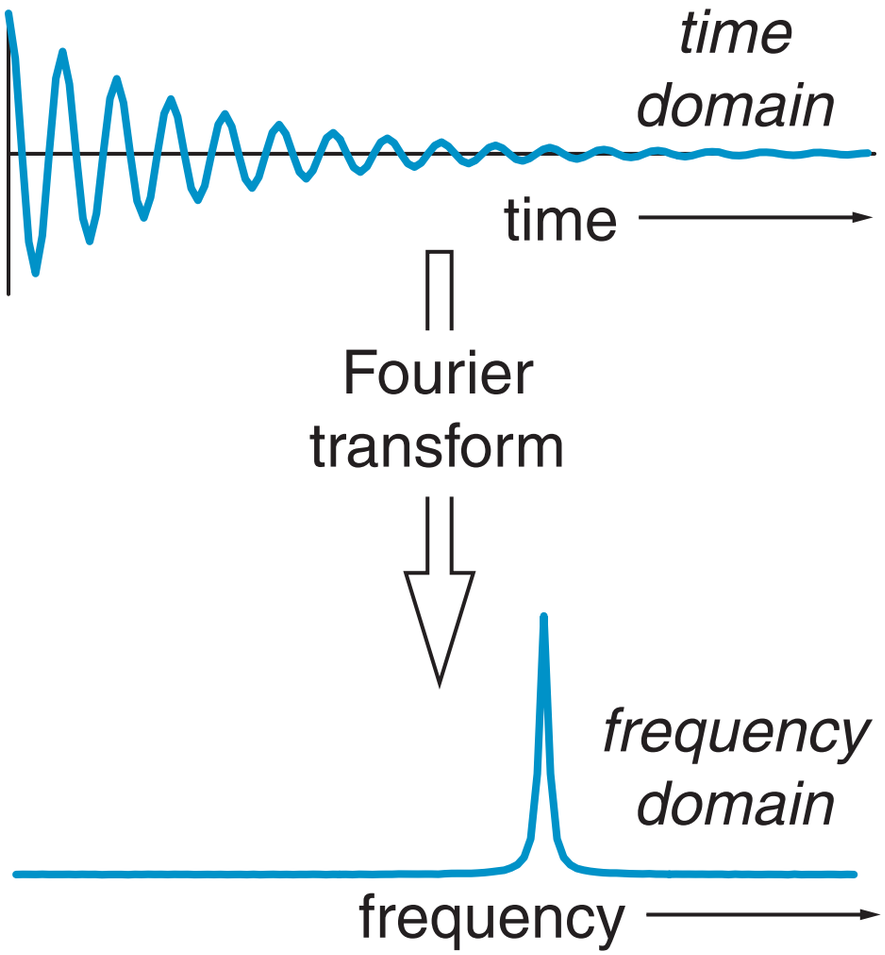




1. Equilibrium. The net magnetization is aligned along the direction of the main field (z-axis).

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.

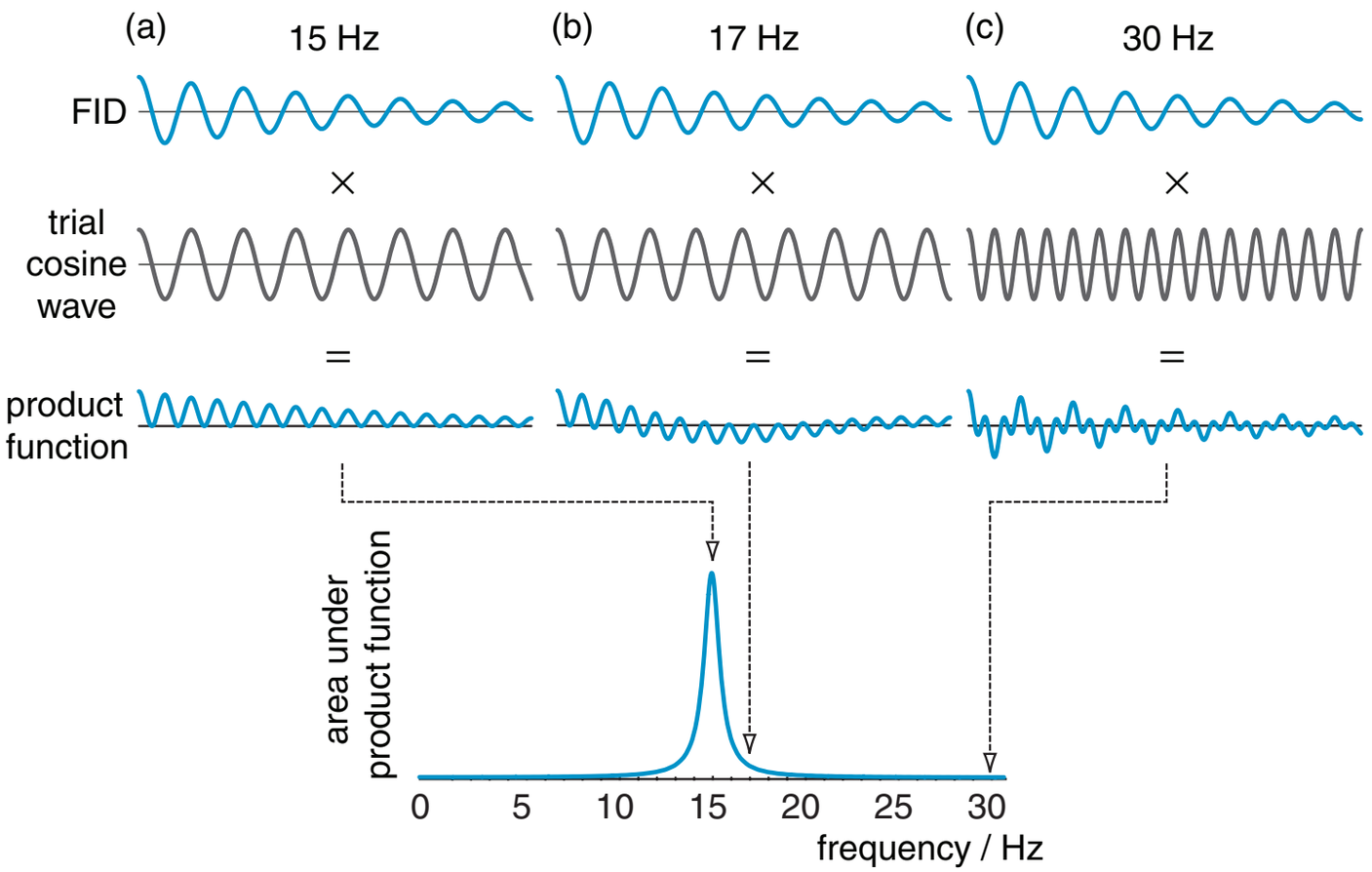
# How does the detection period work in the ordinary experiment?




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

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

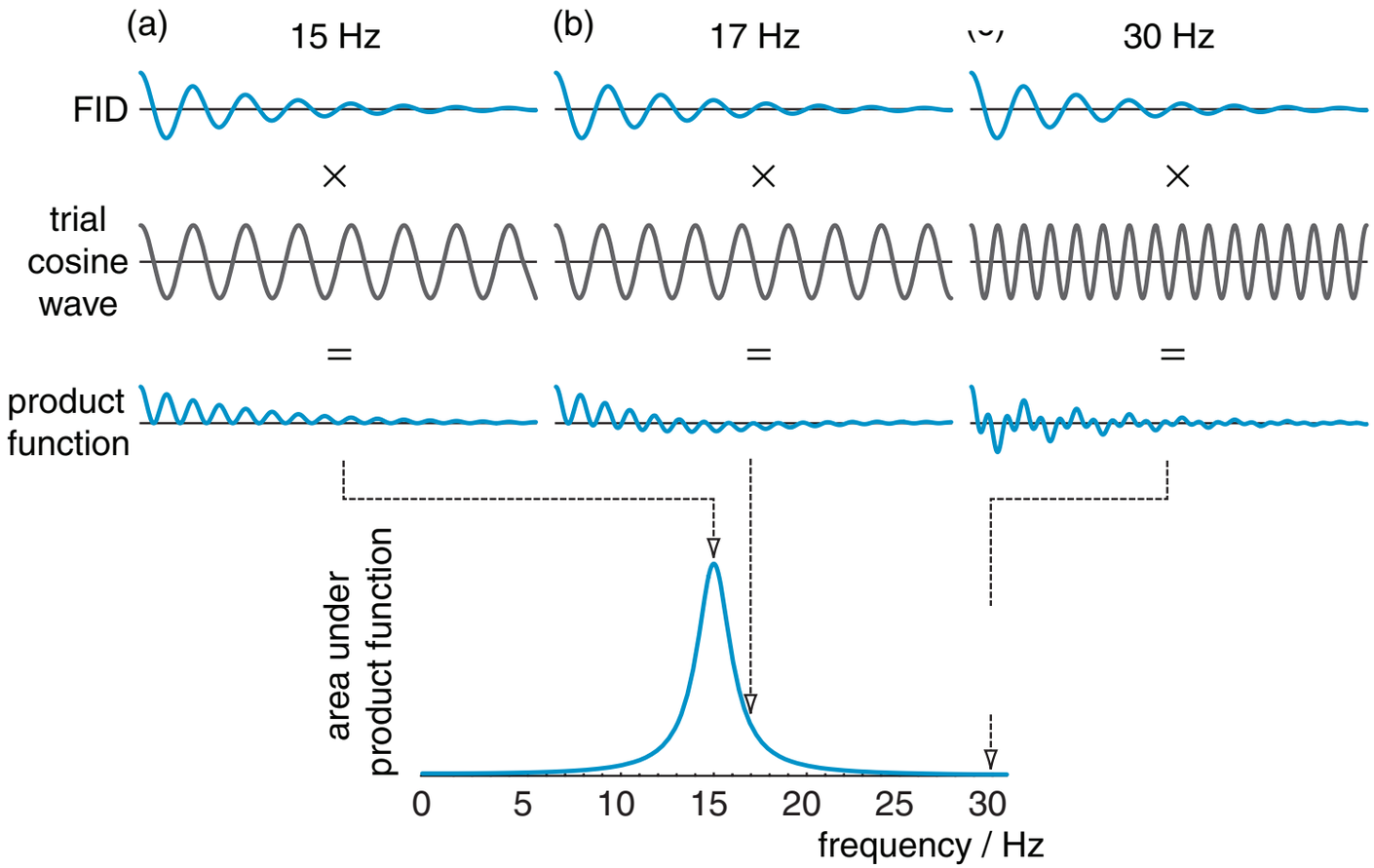
# How does the Fourier Transform work?




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

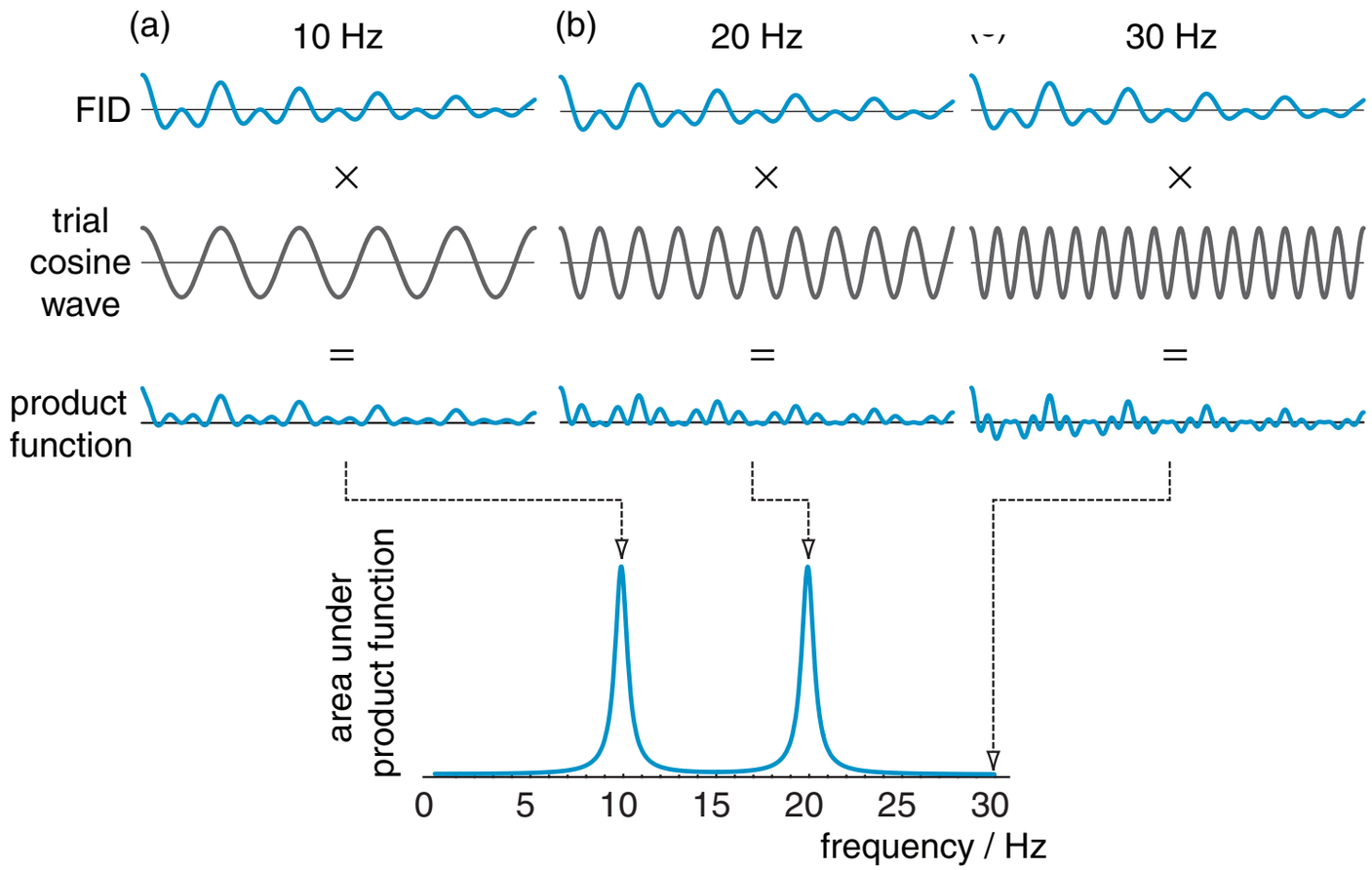
The FID, shown along the top, is multiplied by trial cosine functions of known frequency to give a product function. The area under this product function corresponds to the intensity in the spectrum at the frequency of the cosine wave. Three cases are shown. In (a) the trial cosine is at 15 Hz which matches the oscillation in the FID; as a result the product function is always positive and the area under it is a maximum. In (b) the trial frequency is 17 Hz; now the product function has positive and negative excursions, but on account of the decay of the FID, the area under the trial function is positive, although smaller than the area in case (a). The intensity of the spectrum at 17 Hz is therefore less than at 15 Hz. Finally, in (c) the trial frequency is 30 Hz; the product function oscillates quite rapidly about zero so that the area under it is essentially zero. As a result, the intensity in the spectrum at this frequency is zero. The spectrum is generated by plotting the area under the product function against the frequency of the corresponding trial cosine wave

# How does the Fourier Transform work?



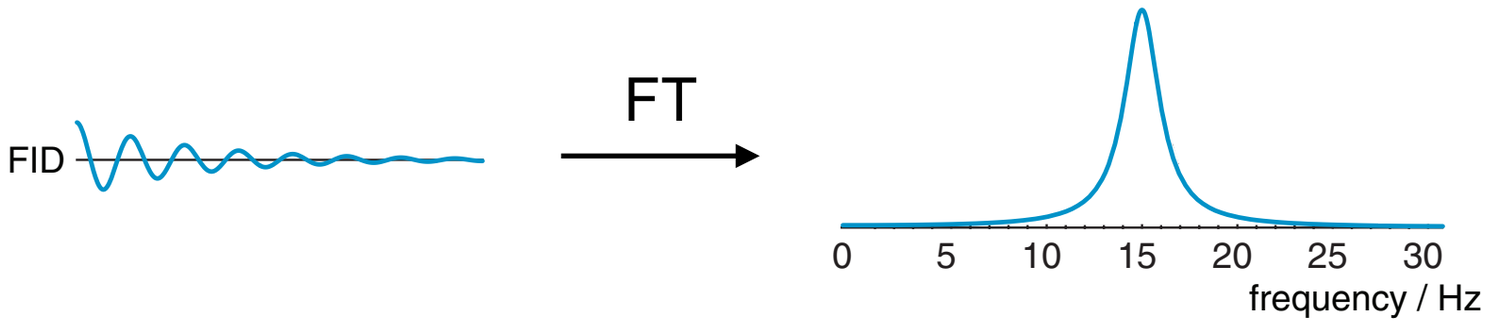

$$I(\omega) = \int S(t)\exp\{-i\omega t\}dt$$

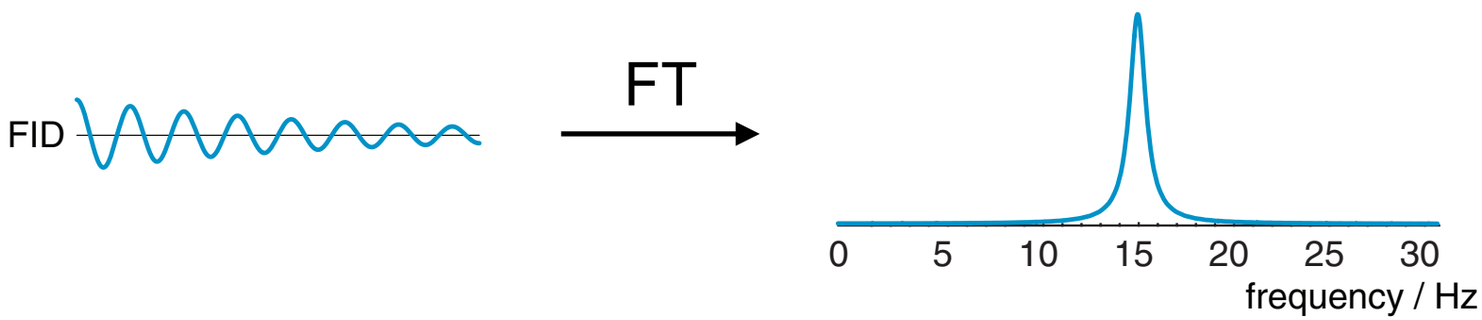
# How does the Fourier Transform work?




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

# Signal Lifetimes vs. Linewidths

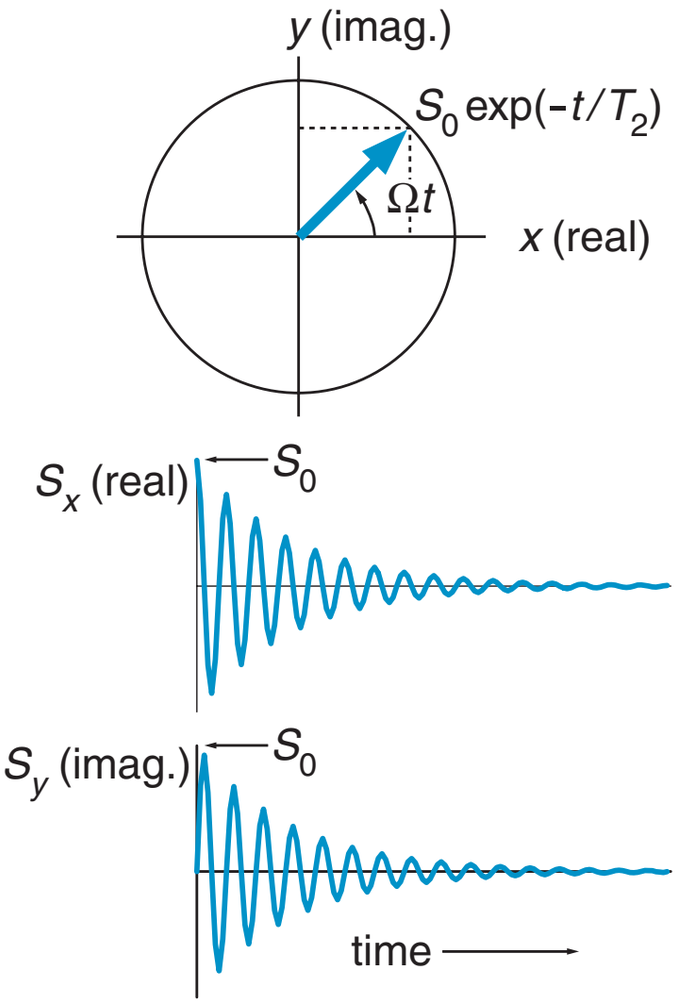



$$\Delta = 1/\pi T_2^*$$

The line width in the spectrum (Δ) is inversely proportional to the decay time of the signal ($T_2$*). (This is a manifestation of the uncertainty principle)

# Lineshapes in NMR




A 90°(y) pulse rotates the equilibrium magnetization onto the x-axis; from there it precesses in the transverse plane, creating x- and y-components $S_0\cos(\omega t)$ and $S_0\sin(\omega t)$. The diagram assumes that the offset Ω is positive.

The signal will decay over time as it returns to equilibrium. We model this by assuming that the signal decays exponentially:

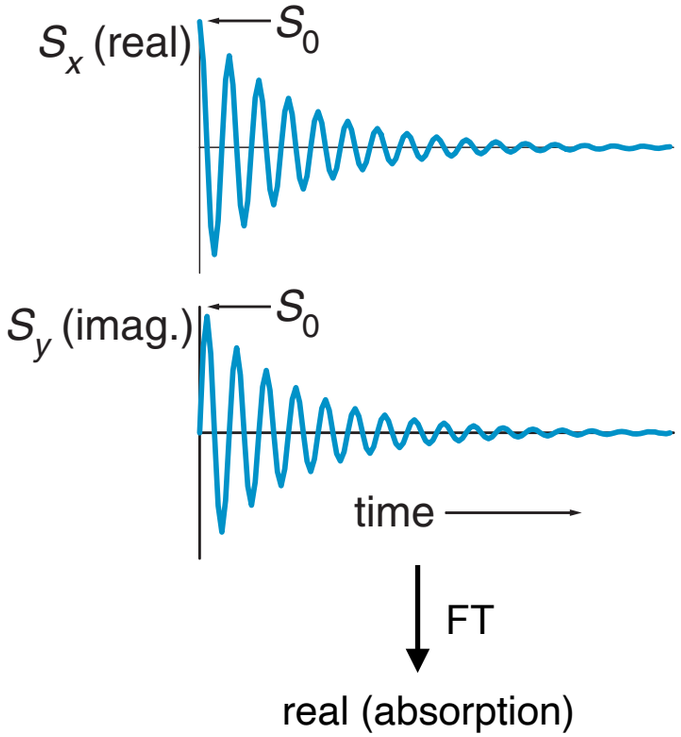
$$S_x = S_0\cos(\Omega t)\exp(-t/T_2)$$

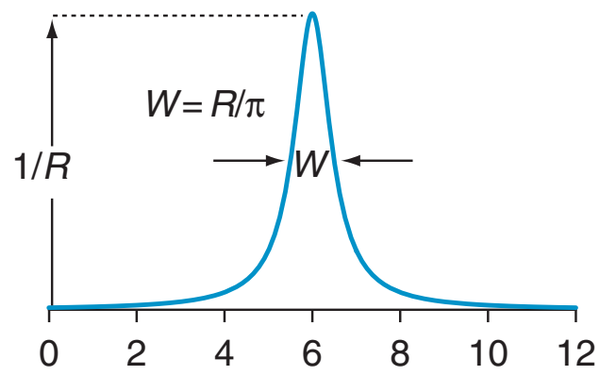
$$S_y = S_0\sin(\Omega t)\exp(-t/T_2)$$

Rather than dealing with the x- and y-components separately, it is convenient to bring them together as a complex signal, with the x-component becoming the real part and the y-component the imaginary part.

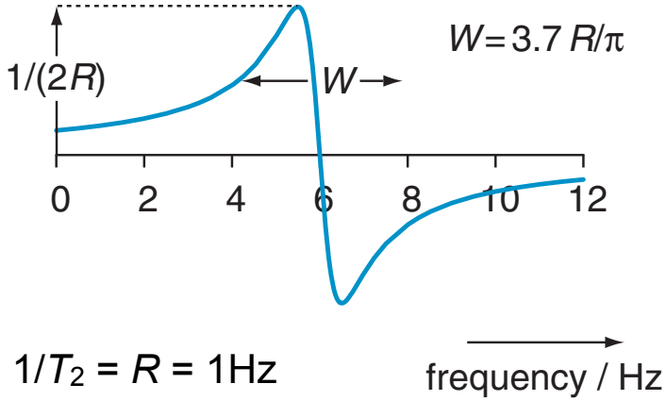
$$\begin{aligned} S(t) &= S_x + \mathrm{i}S_y \\ &= S_0\exp(\mathrm{i}\Omega t)\exp(-t/T_2) \end{aligned} \qquad (1)$$

# Lineshapes in NMR




imaginary (dispersion)




Not surprisingly, if we start with a complex time-domain signal, Fourier transformation gives a complex frequency-domain signal or spectrum. Normally, the software on the spectrometer only displays the real part of this complex spectrum, but it is important to realize that the imaginary part exists, even if it is not displayed.

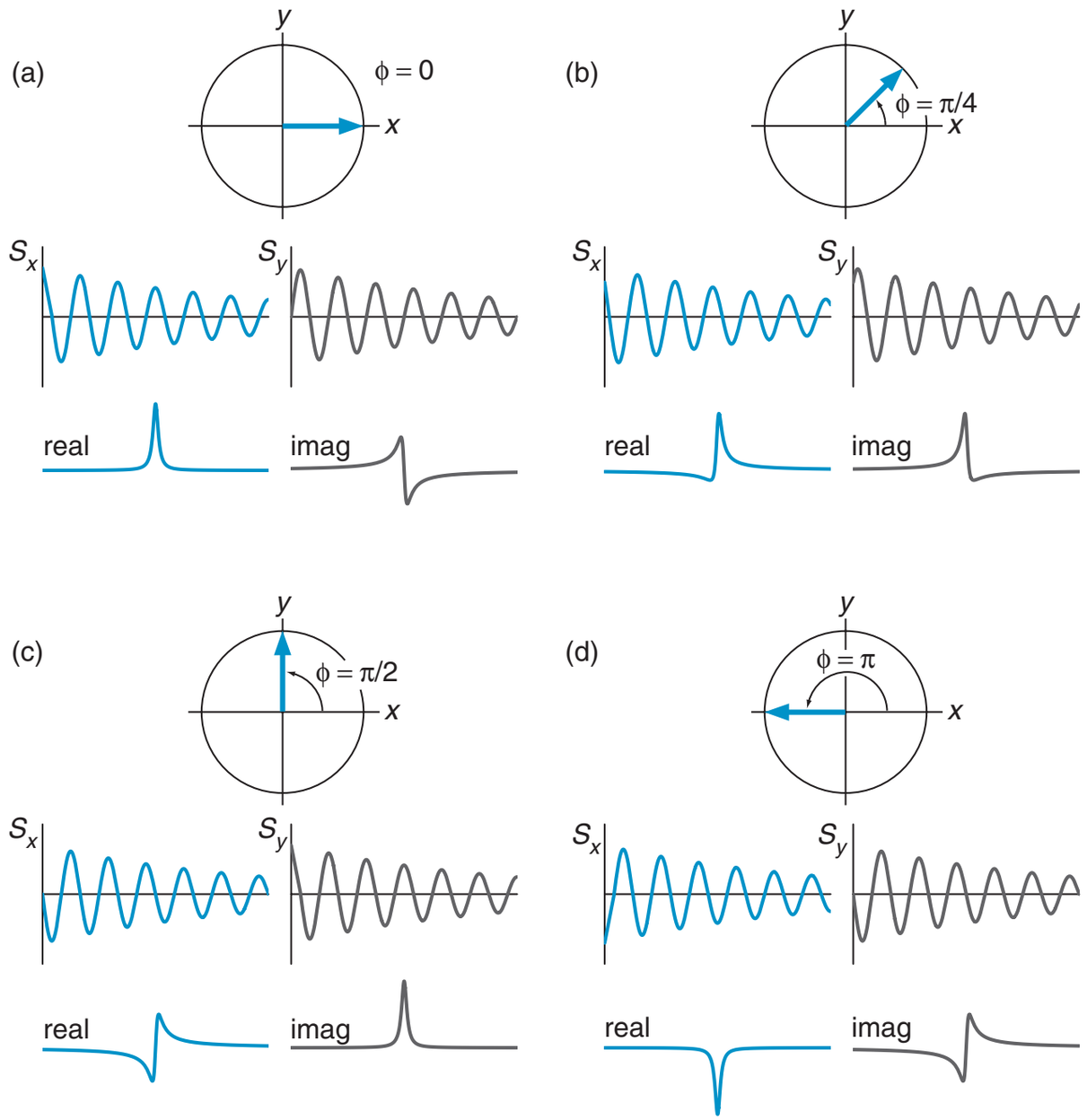
Fourier transformation of the complex time-domain signal of Eq. 1 on the previous slide gives a complex frequency-domain signal, or spectrum $S(\omega)$:

$$S(t) \overset{FT}{\rightarrow} S(\omega)$$

$$S_0 \exp(i\Omega t)\exp(-t/T_2) \overset{FT}{\rightarrow} S_0 \left[ \underbrace{\frac{1/T_2}{(1/T_2)^2 + (\omega - \Omega)^2}}_{real} - i \underbrace{\frac{(\omega - \Omega)}{(1/T_2)^2 + (\omega - \Omega)^2}}_{imaginary} \right]$$

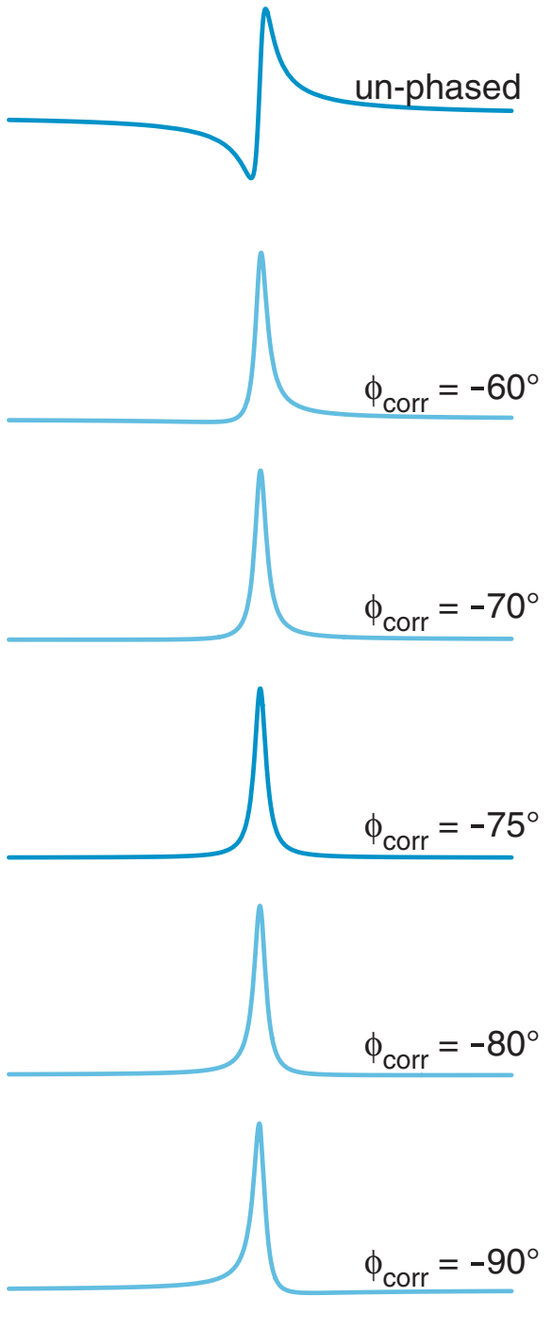
The real part of the spectrum is a peak with the absorption mode Lorentzian lineshape, whereas the imaginary part has the dispersion mode Lorentzian lineshape

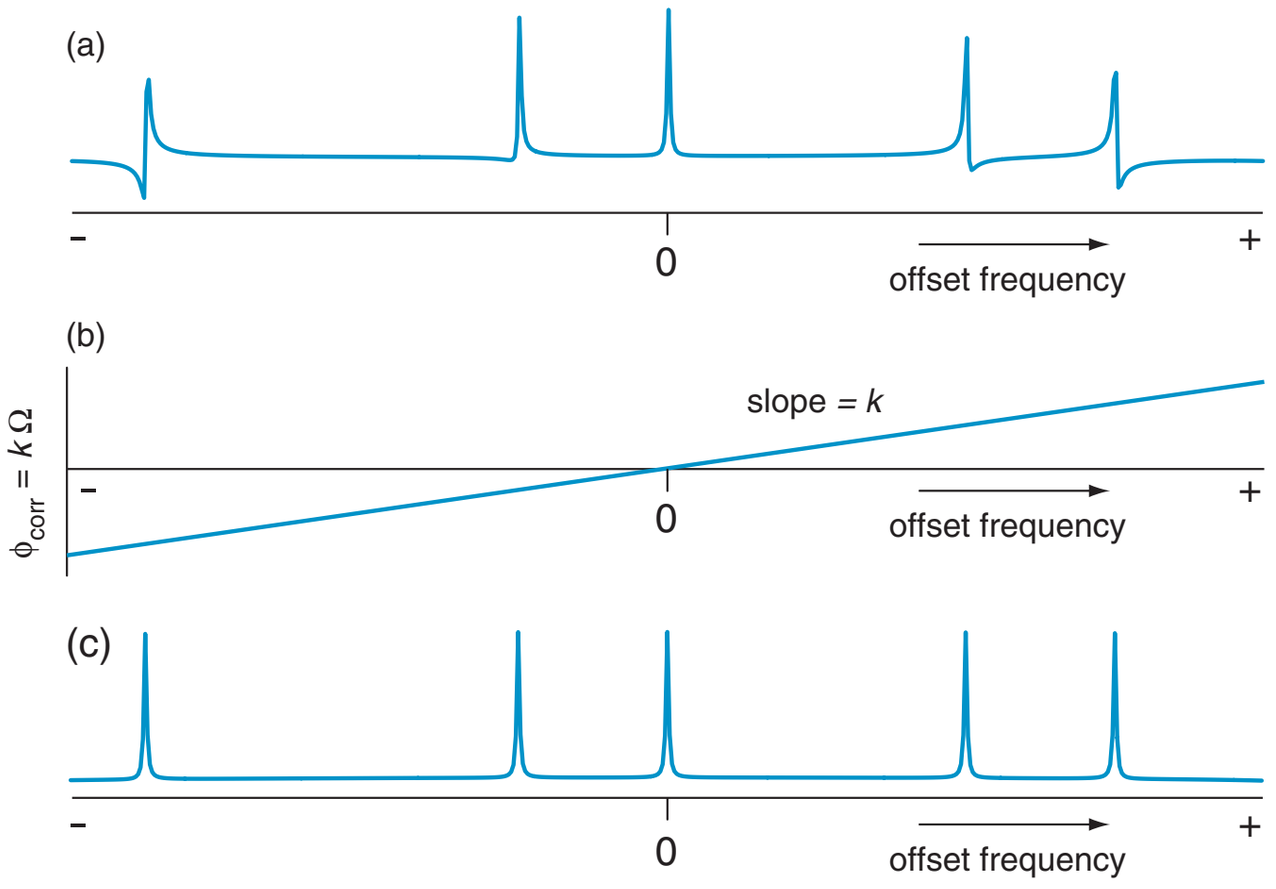
# Phase of the NMR Signal




Obviously the *x* and *y* components of the signal can be interchanged. For example, after $\pi/2_x$ pulse, the magnetisation will start at $M_y$ for $t = 0$, and the absorption signal will be found in the imaginary component. (A phase shift of $\pi/2$). After a $\pi/2_{-y}$ pulse the magnetisation will start at $-M_x$ for $t = 0$; the absorption signal will be in the real component, but it will be negative.

# Phase Corrections



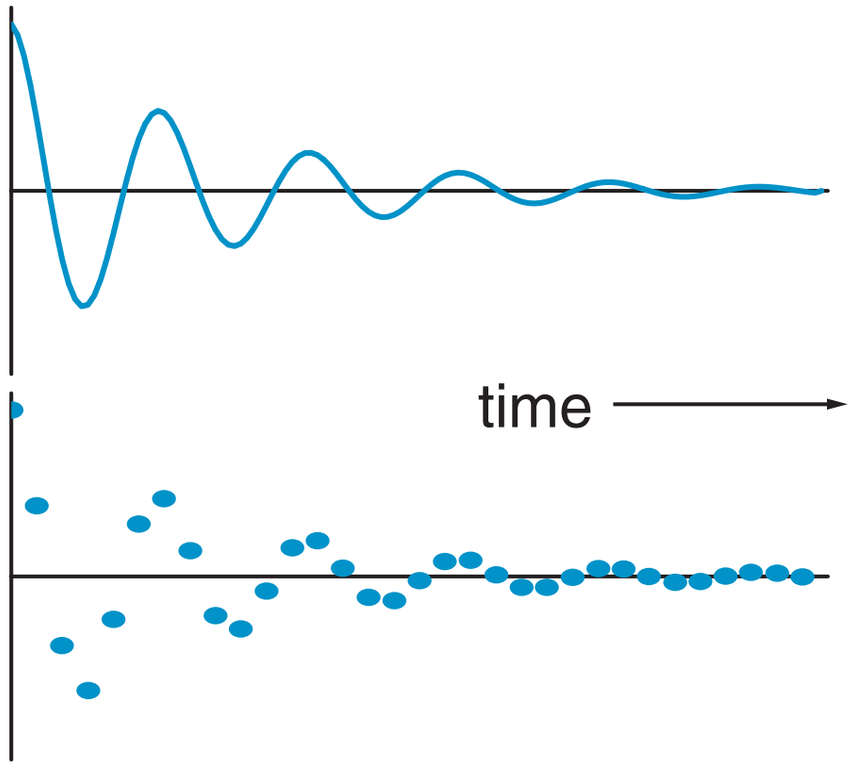




The phase can be “corrected” by multiplying the signal by $\exp(i\Phi_{corr})$.

In addition to an overall constant phase correction factor (*a zero-order phase correction*) The phase correction often varies (linearly) with offset, we then apply a phase correction that is proportional to offset (*a first-order phase correction*).

The result is a spectrum where the pure absorption mode is obtained for all the peaks in the real part of the spectrum.

# How does the detection period work in the ordinary experiment?

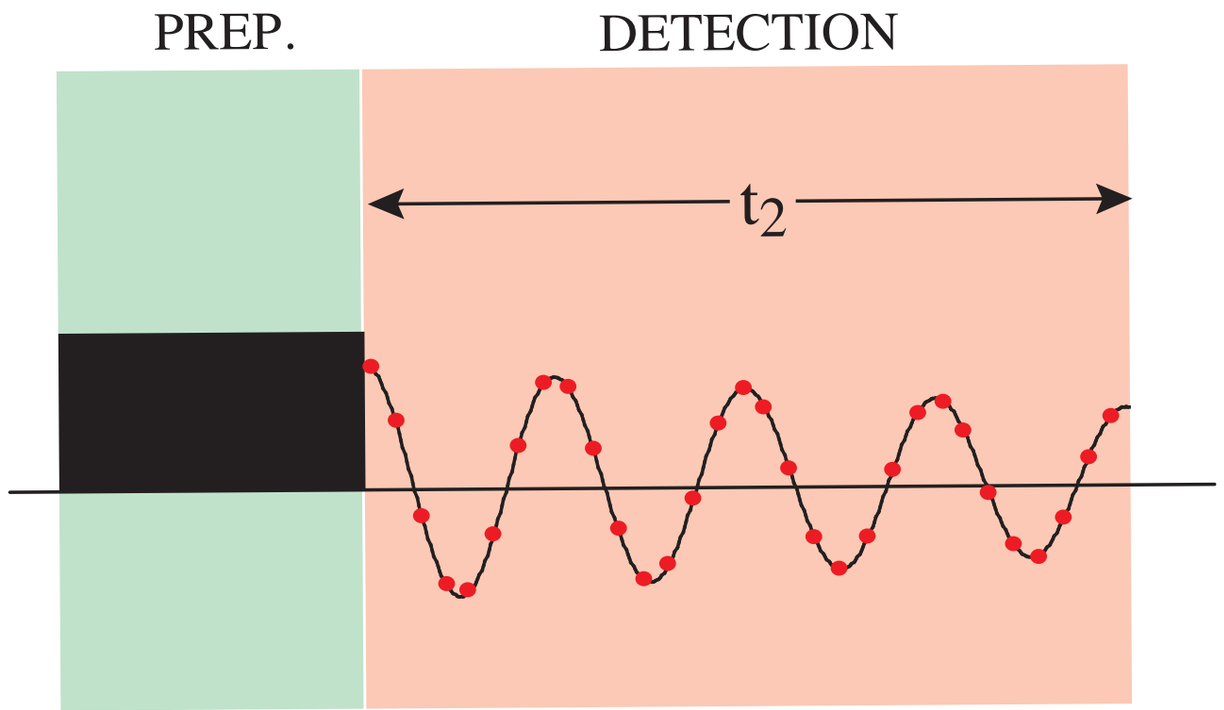



The amplitude of the FID varies smoothly as a function of time.

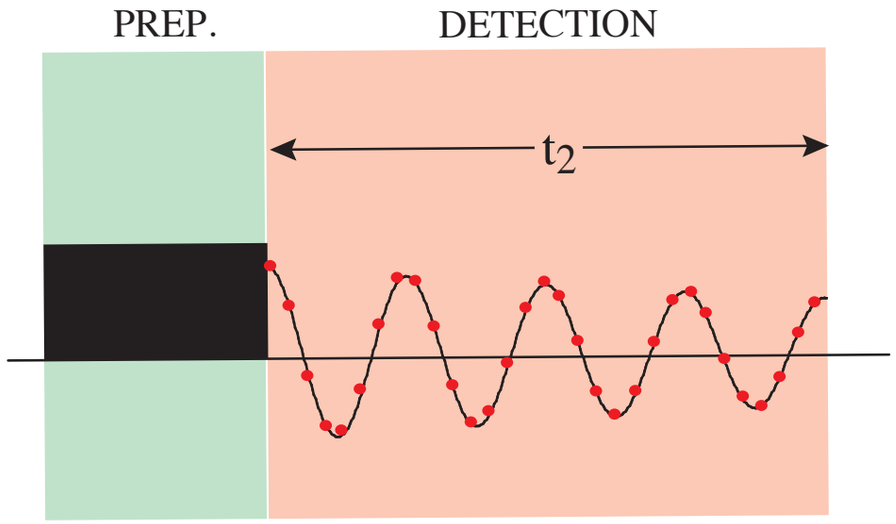
In order to be able to manipulate this time-domain signal in a computer, the signal is digitized at regular intervals.

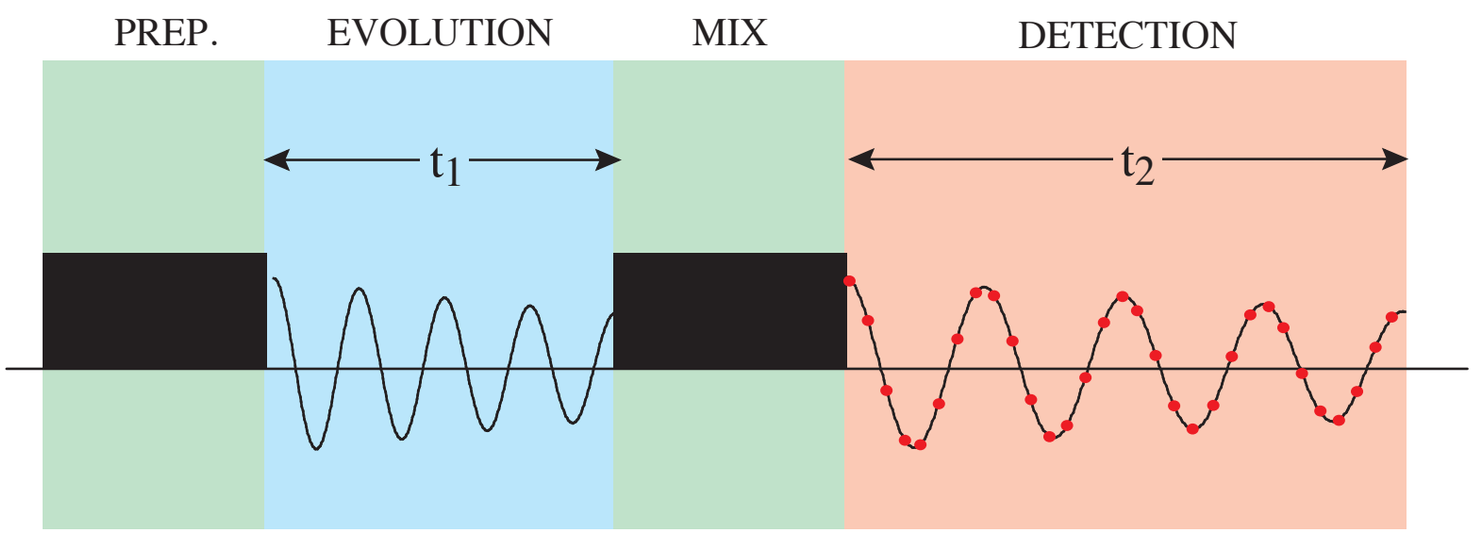
$$I(\omega) = \sum_{i=1}^{N} S(t_i) \exp\{-i\omega t_i\} dt$$

# Principles of Multi-Dimensional Spectroscopy

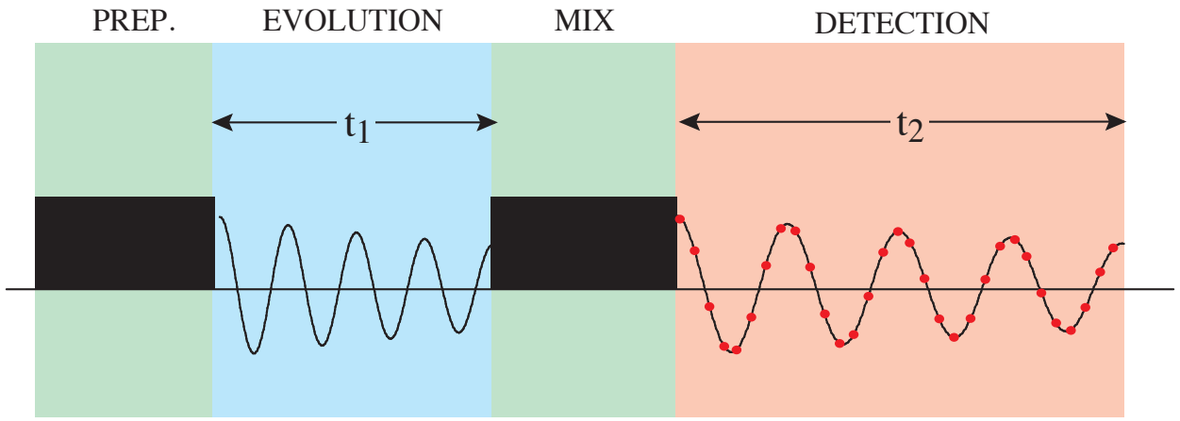


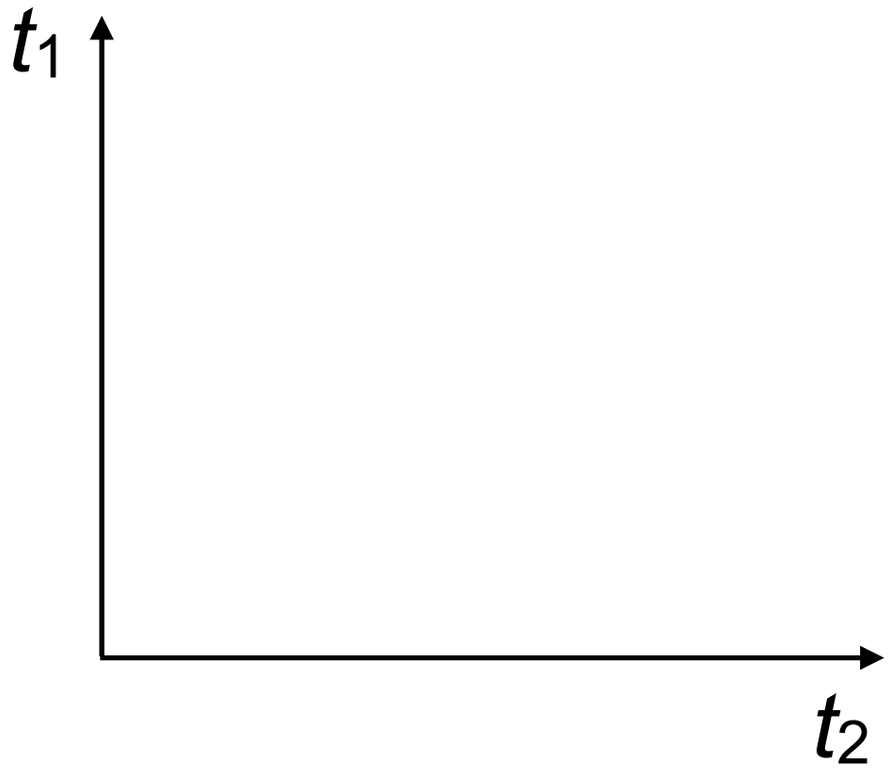
# Principles of Multi-Dimensional Spectroscopy



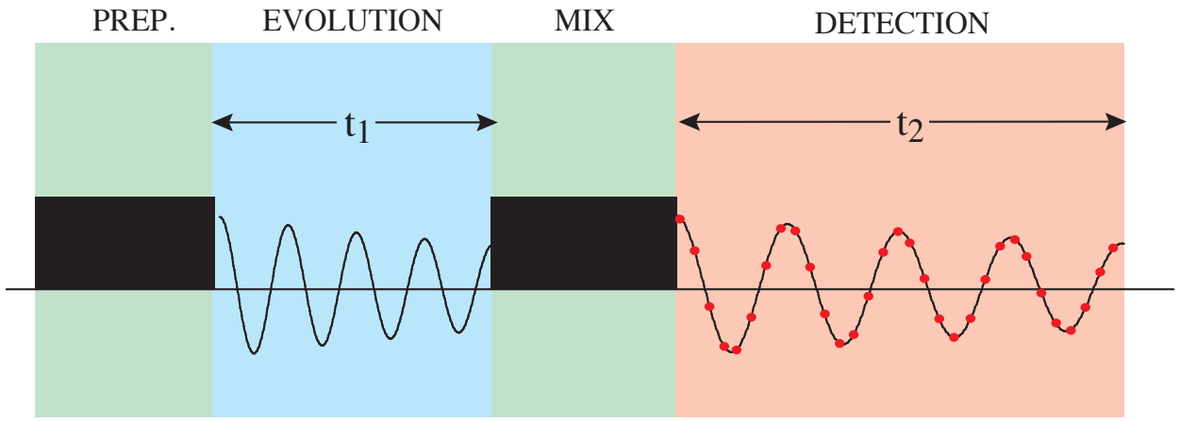



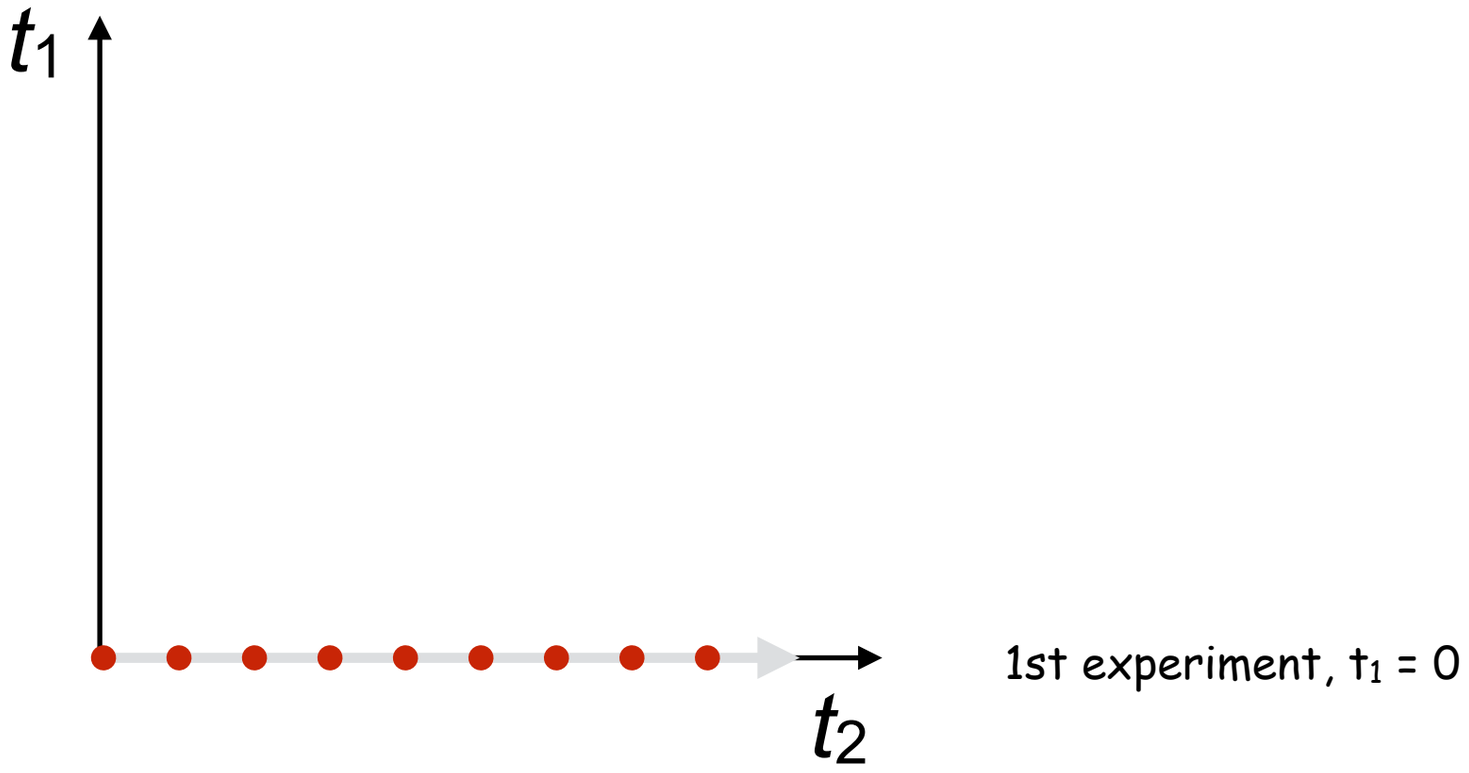
# Principles of Multi-Dimensional Spectroscopy



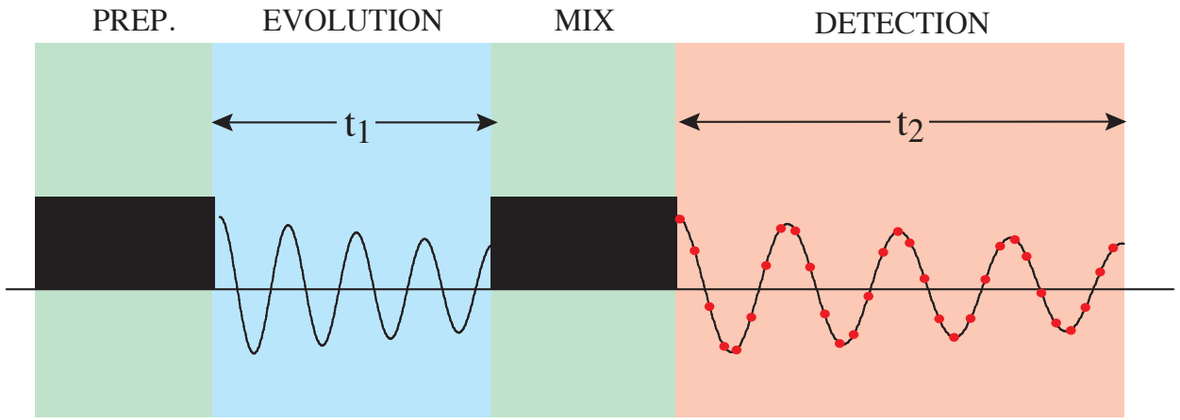



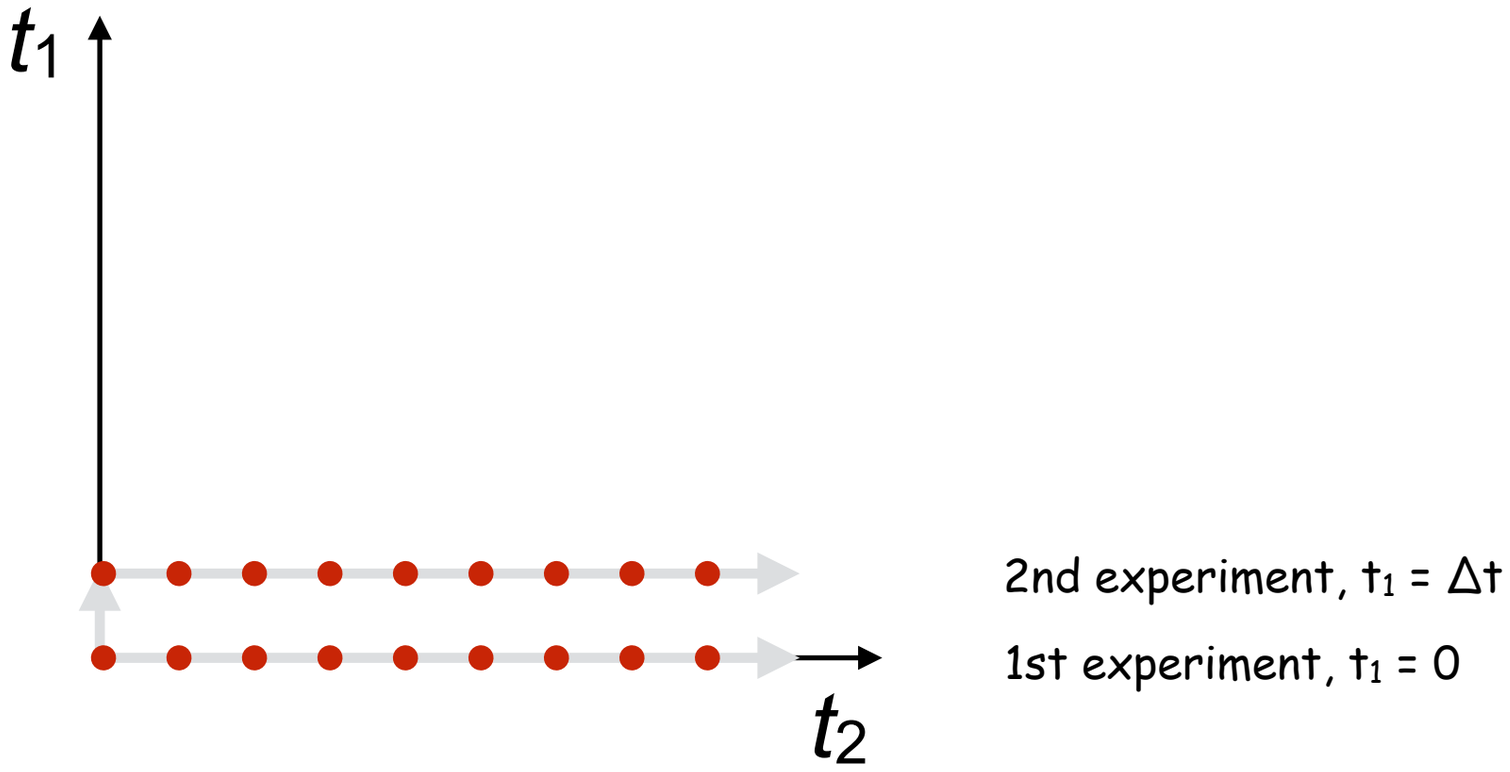
# Principles of Multi-Dimensional Spectroscopy



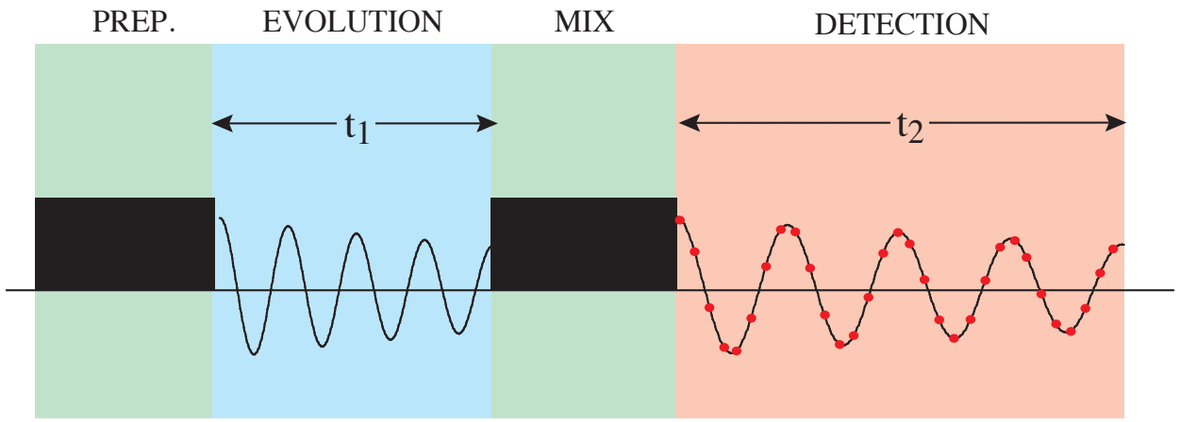



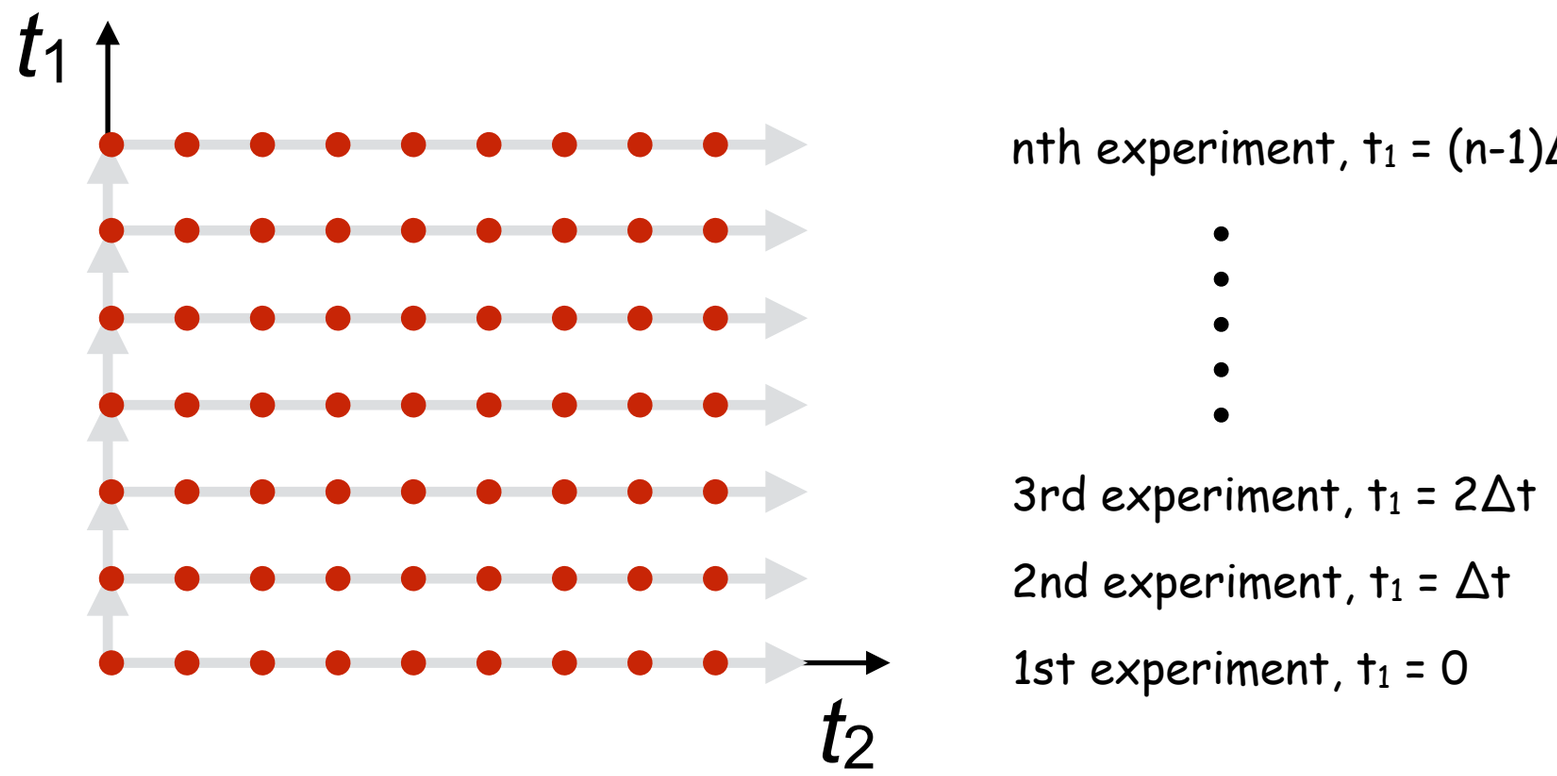
# Principles of Multi-Dimensional Spectroscopy



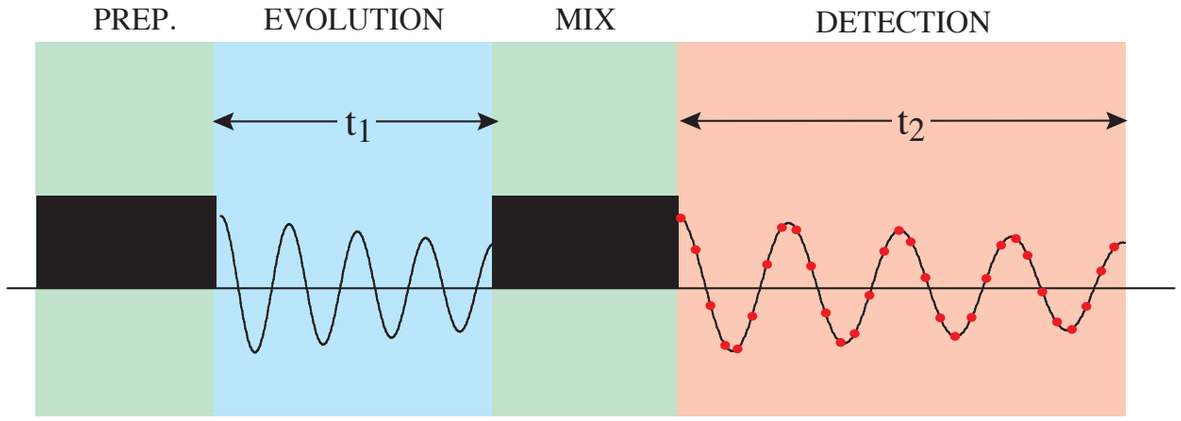
# Principles of Multi-Dimensional Spectroscopy



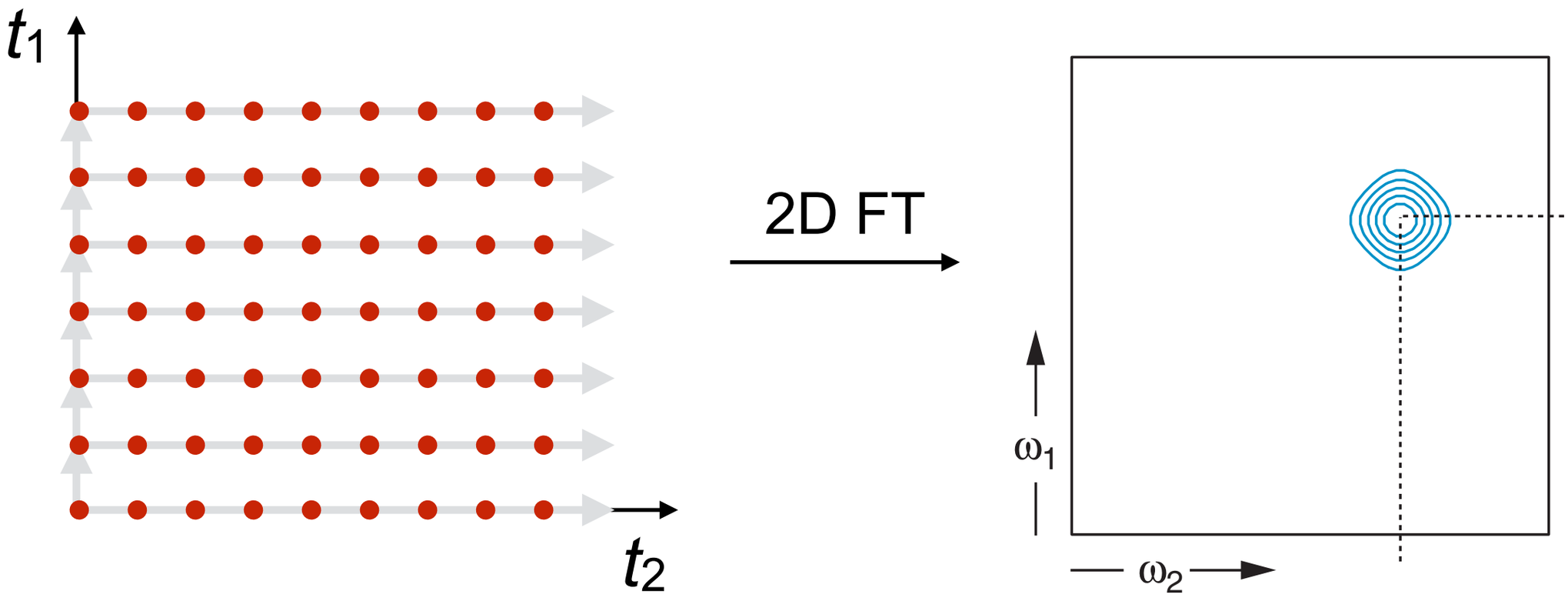

We can fill a two-dimensional time domain with data by repeating the experiment, with acquisition along $t_2$ in each experiment, and with $t_1$ being incremented progressively from one experiment to the next.

# Principles of Multi-Dimensional Spectroscopy




A two-dimensional frequency domain ($\omega_1$, $\omega_2$), can be obtained by two-dimensional Fourier transformation of the data with respect to $t_1$ and $t_2$.

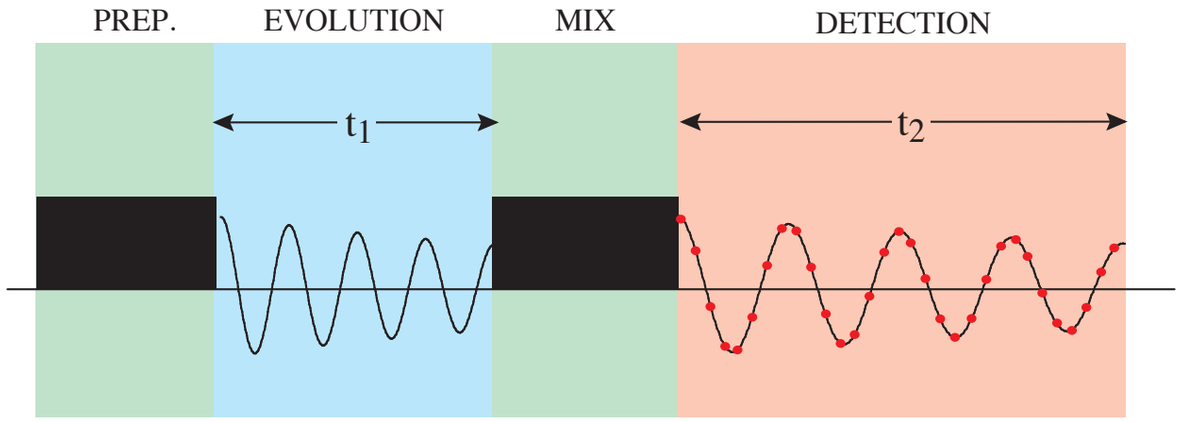
$$I(\omega_1,\omega_2) = \int\int s(t_1,t_2)\exp\{-i(\omega_1 t_1 + \omega_2 t_2)\}dt_1 dt_2$$




In two-dimensional NMR each peak has two frequency coordinates, measured along the $\omega_1$ and $\omega_2$ 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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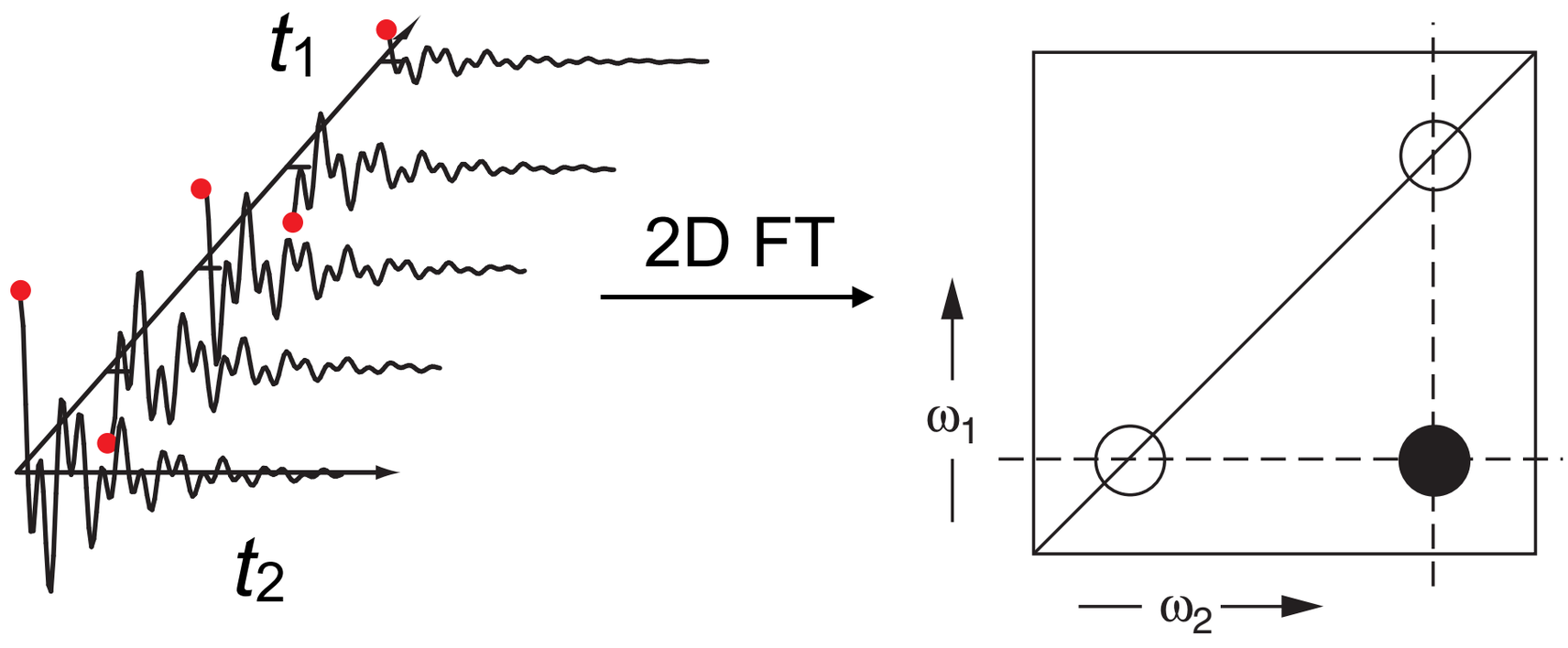
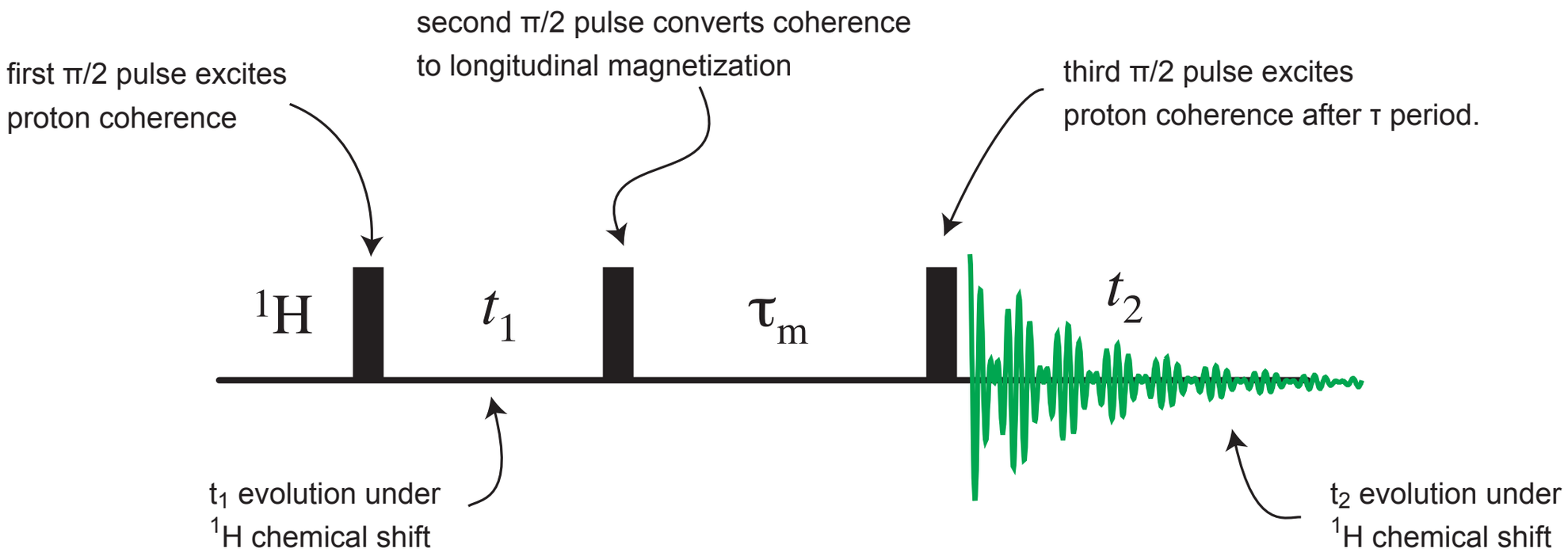


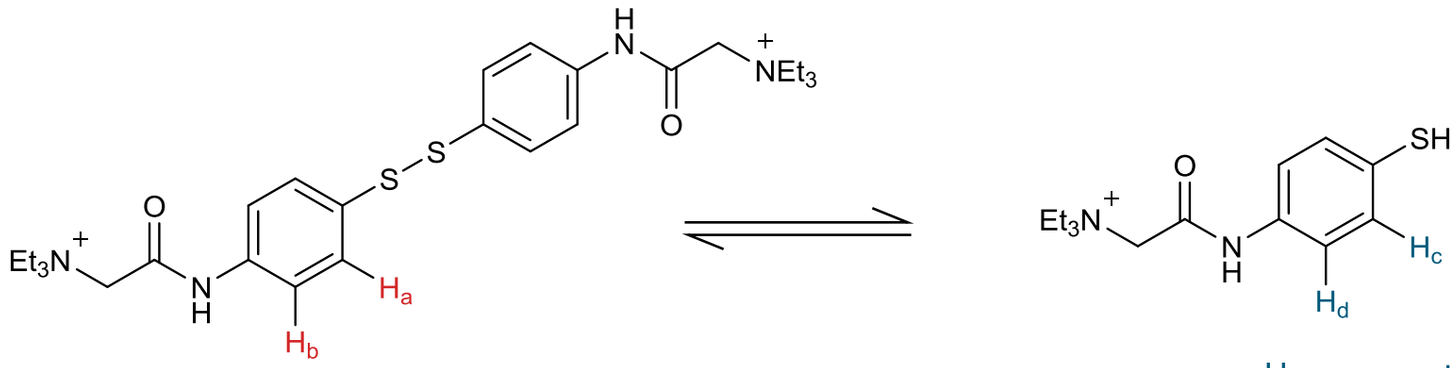

2D NMR spectra typically consist of “diagonal peaks” at $\omega_1 = \omega_2$ and “cross peaks” at $\omega_1 \neq \omega_2$ such that cross peaks occur at frequencies that link the resonance frequencies of different nuclei in the spectrum.

# Two-Dimensional Exchange Spectroscopy (EXSY)

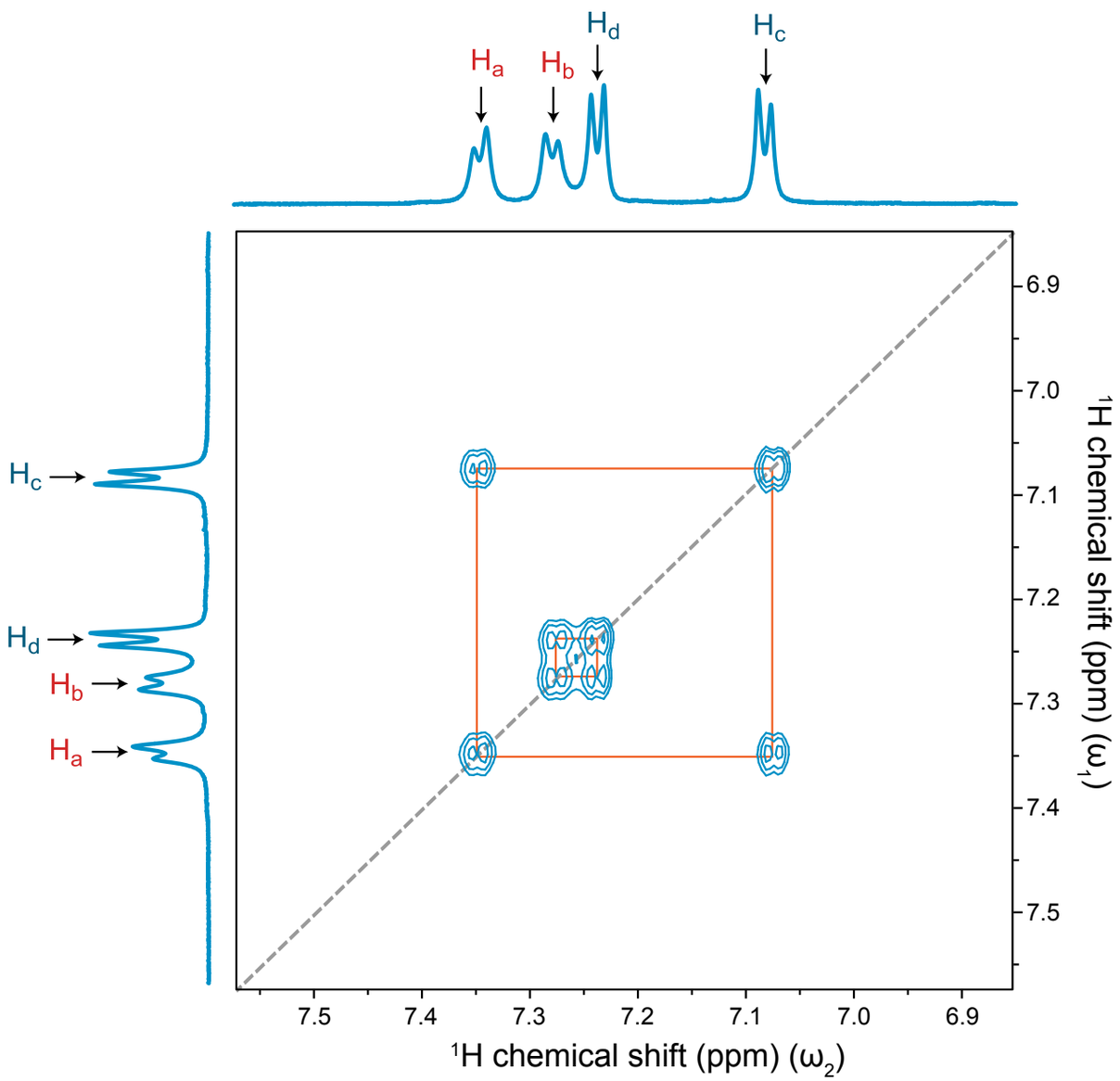



The EXSY experiment yields a correlation between resonances of protons that are exchanging on a timescale comparable to $\tau_m$

# Two-Dimensional Exchange Spectroscopy (EXSY)

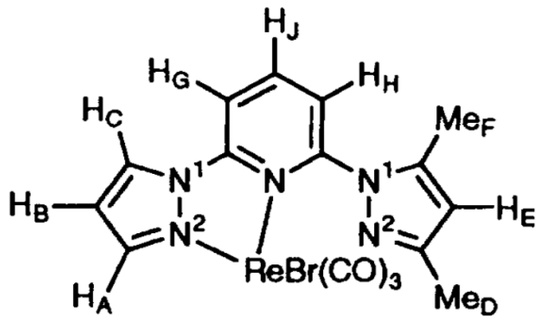


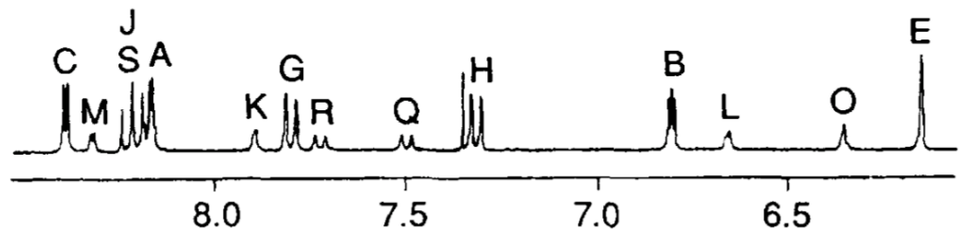

Contour plot of the expanded ‘aromatic region’ of a 2D EXSY $^1$H NMR spectrum (700 MHz, $D_2O$, pH 6.5) with $\tau_m$ = 100 ms of a 1:1 mixture of the disulfide and the thiol shown (30 mM total concentration). A 1D spectrum of the mixture is shown on the vertical and horizontal projections.




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)



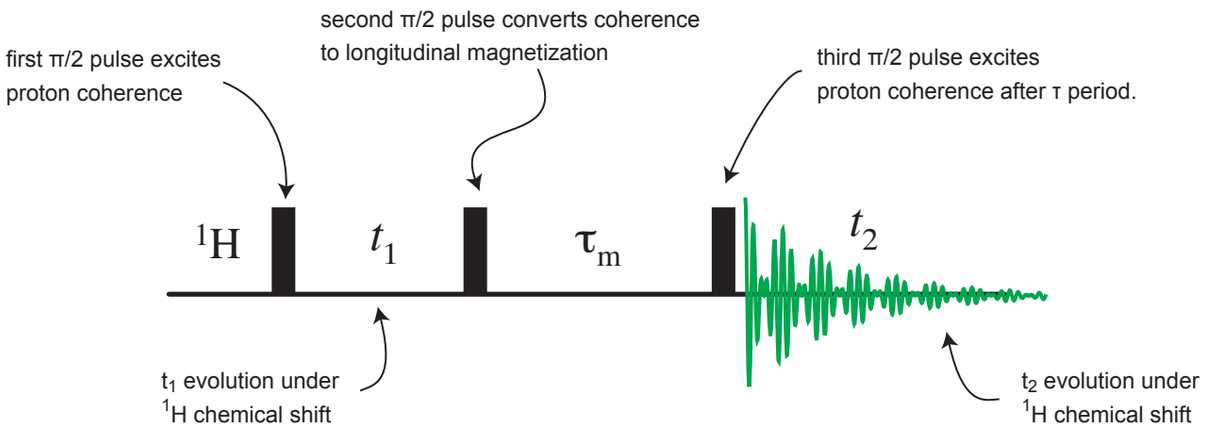



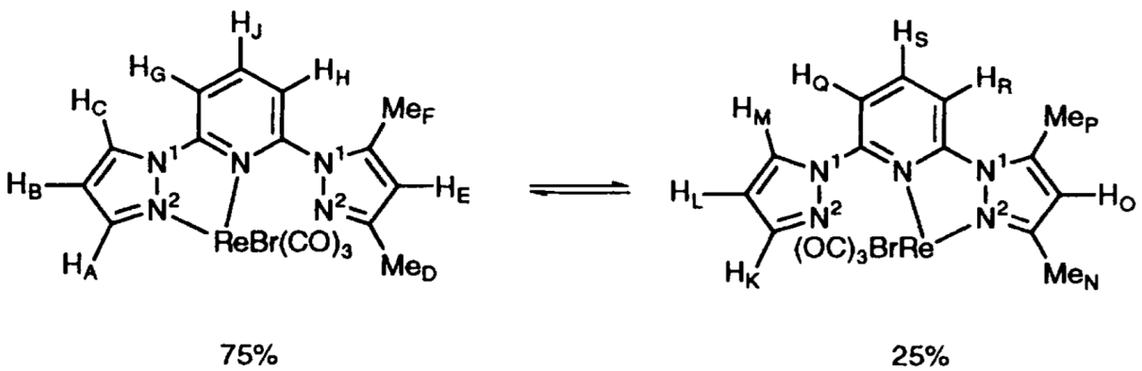

One-dimensional proton NMR spectrum of the organometallic compound [ReBr(CO)3(Me2-bppy)], where bppy denotes 2,6-bis(pyrazol-1-yl)pyridine.

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

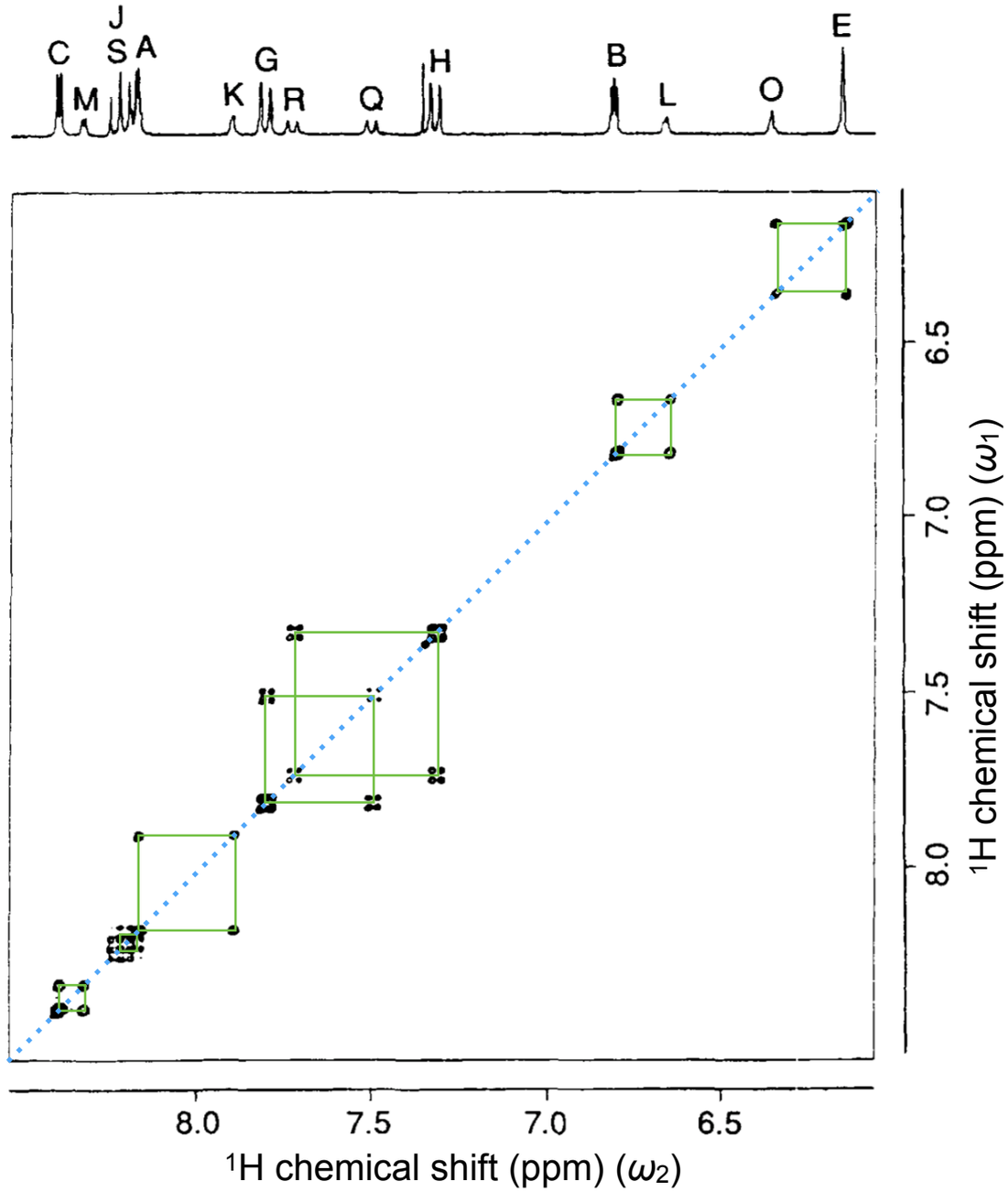
Intensities suggest a 3:1 ratio in concentration between the major and the minor species. Is the second species an impurity, or is it a second product in slow exchange?

# Two-Dimensional Exchange Spectroscopy (EXSY)




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: 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

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

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

$\mathbf{\Omega}$ is a diagonal matrix containing the chemical shifts $\Omega_j$ .
$\mathbf{\Lambda}$ 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 $T_{1j}^{-1}$.

# 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 $\tau_m$ are controlled by equation (4).

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

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

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

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

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

# Longitudinal Exchange in EXSY

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

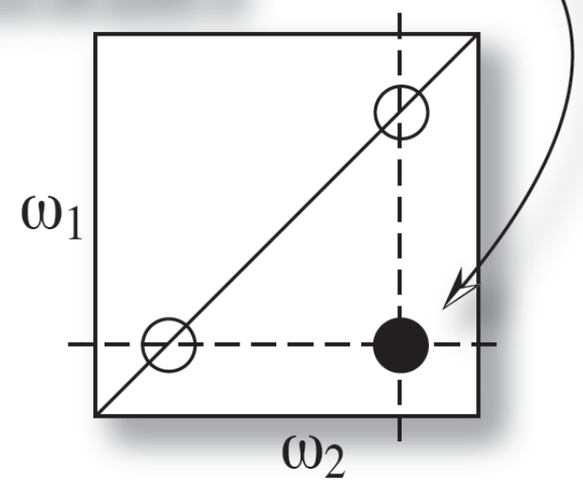
For slow exchange, lineshapes in $t_1$ and $t_2$ are not affected by contributions of $\mathbf{K}$ to $\mathbf{L}^{+}$ and they may be neglected. As a result the time-domain signal simplifies to

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

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 exponential mixing operator.***



# Longitudinal Exchange in EXSY

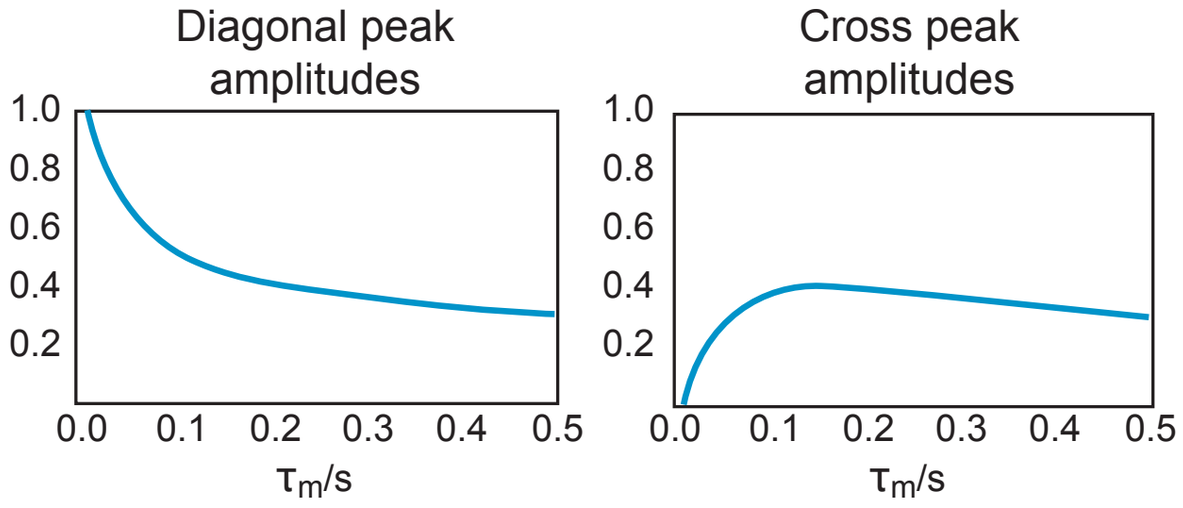
For a 2 spin system, the 2x2 dynamic matrix leads to an analytical solution, and for a symmetrical case we obtain:

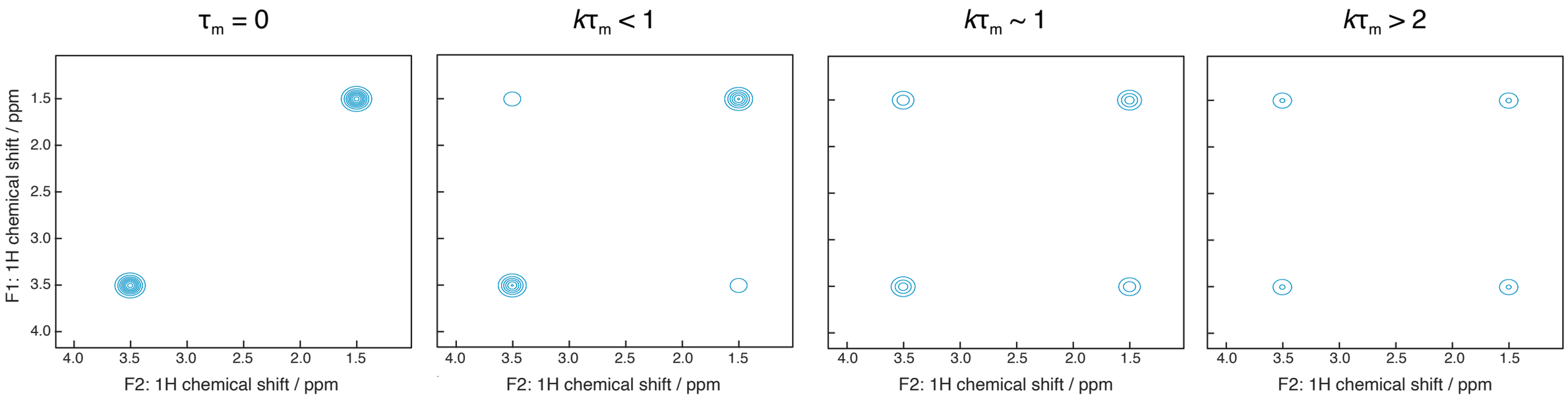
$$I_{AA}(\tau_m) = \tfrac{1}{2}\left[1+\exp\{-2k\tau_m\}\right]\exp\{-\tau_m/T_1\}M_{A0}$$

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

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

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



PNAS

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

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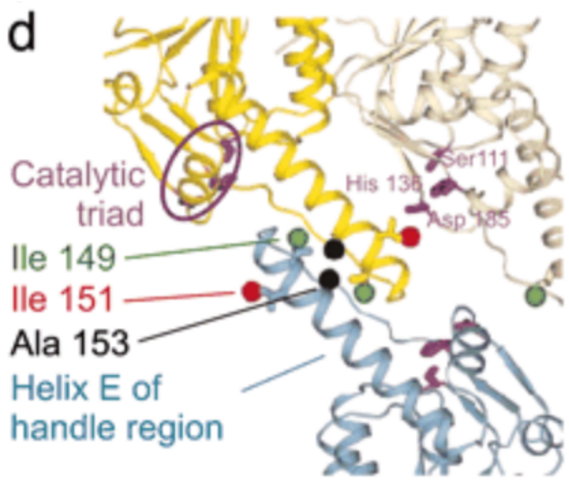
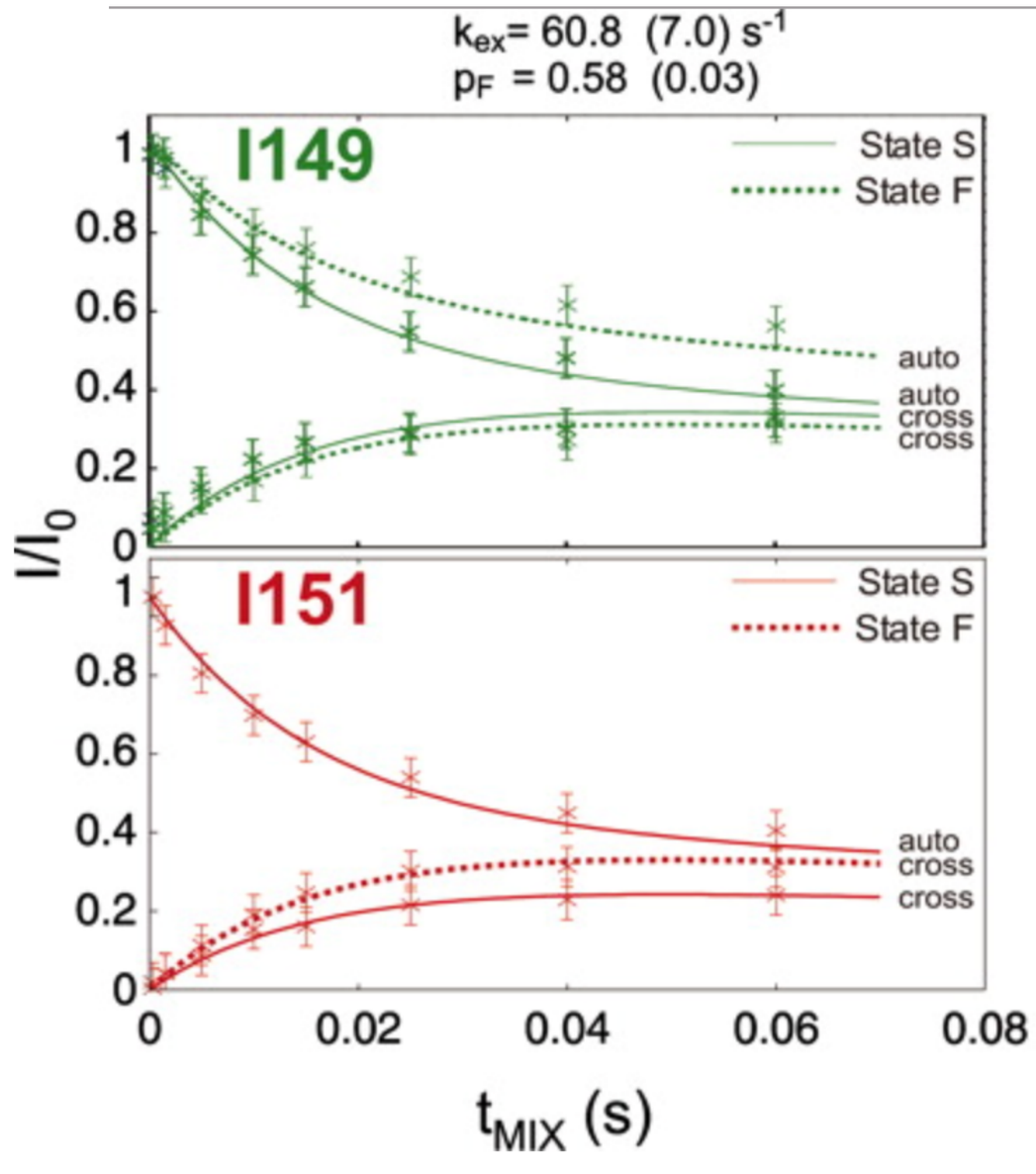
Edited by Alfred G. Redfield, Brandeis University, Waltham, MA, and approved September 30, 2005 (received for review August 23, 2005)




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.

# Quantitative Analysis of EXSY Spectra

Volume 214, number 2 CHEMICAL PHYSICS LETTERS 29 October 1993

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

R.G. Larsen, J. Shore, K. Schmidt-Rohr, L. Emsley, H. Long, A. Pines [1]
*Materials Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720, USA and Department of Chemistry, University of California, Berkeley, CA 94720, USA*

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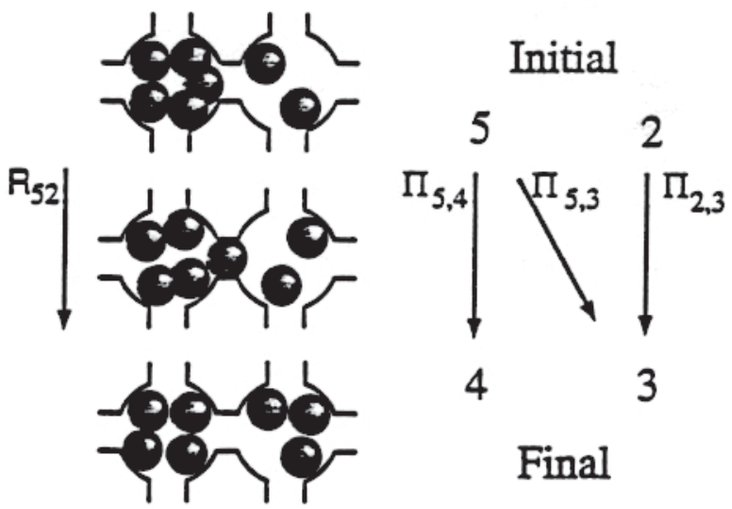
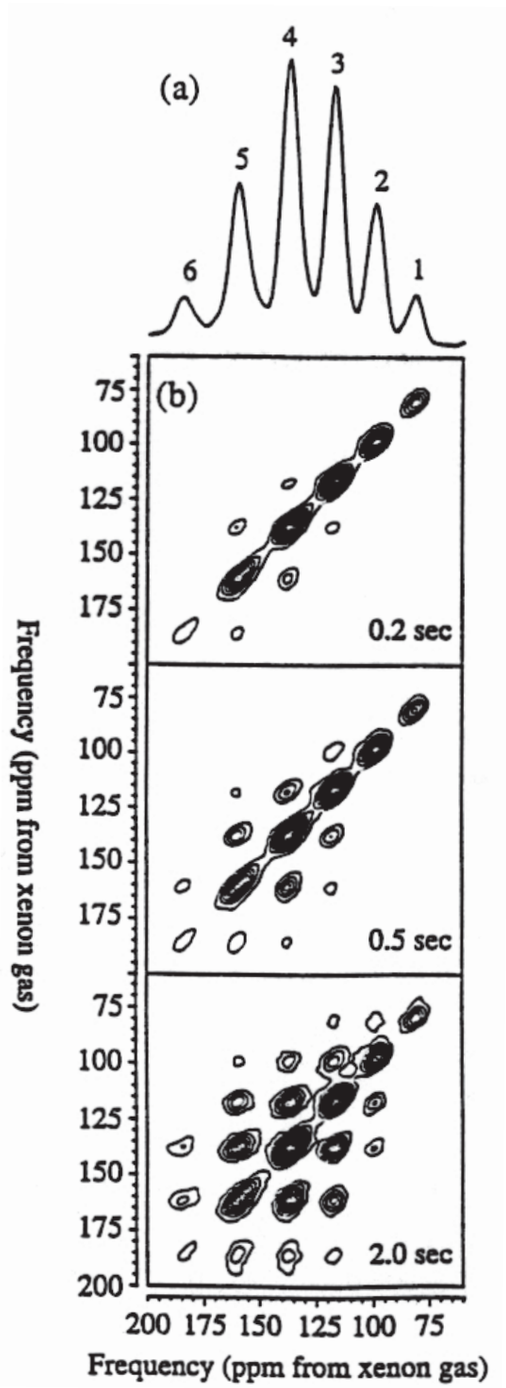
Received 18 June 1993

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**

# Conclusions

- The Fourier transform integrates the time-domain signal to provide the intensity of each frequency contained in the signal.
- The longer the signal lasts, the narrower the spectral line will be.
- The complex time domain signal yields a complex (real & imaginary) spectrum, in which the signals have a particular phase. The signal is digitised for storage in the computer.
- 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 Exchange Spectroscopy can used to observe slow chemical exchange.
- 2D EXSY spectra can be analysed quantitatively to obtain exchange rates and thermodynamic parameters.

# Homework

Each group should jointly finalise and upload their answers to their jigsaws.

***Learn the course material***