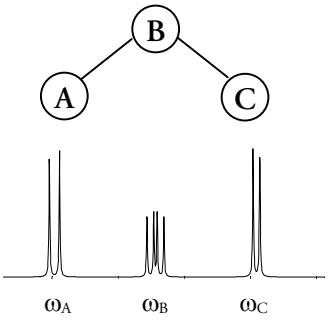
6
2D NMR

Lecture notes by Assaf Tal

The Concept of 2D NMR

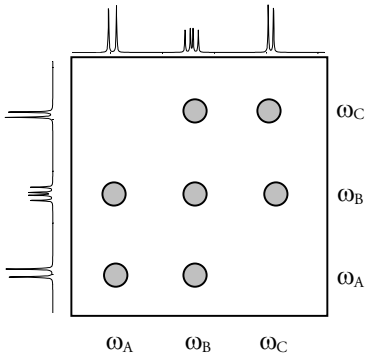
The Output

A tool or process can be understood on many levels. We'll start by understanding 2D NMR as a black box and examining its "input" and "output". Imagine a hypothetical J-coupled molecule such as the following:



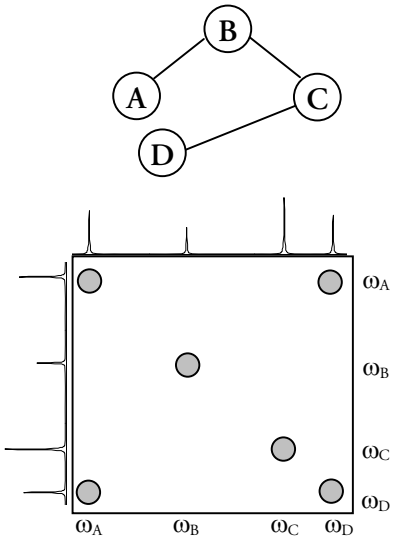
A, B and C resonate at frequencies ω_A, ω_B and ω_C. Even if I hadn't denoted them on the spectrum it would've been pretty obvious which peak corresponds to B (since it's J-coupled twice, and - since the J-couplings are not to be assumed equivalent, should have 4 peaks, i.e. a quartet). Telling ω_A and ω_C would be slightly more difficult but not too difficult if I had told you what chemical groups they were, so you could use your knowledge of where each group should resonate more-or-less to tell them apart.

This molecule, which we'll call ABC, is fairly easy to understand, but imagine more complex molecules with more complex spectra. How could we easily tell which molecule is coupled to which other molecule? The answer lies in 2D NMR. The "input" of 2D NMR is a molecule, and the "output" is a 2D spectrum, in which cross-peaks appear between all pairs of coupled molecules:



The peaks on the diagonal are uninteresting and an unavoidable artifact, but the cross peaks clearly show which molecule is connected to which other molecule. What we've just described is known as COrrelation SpectroscopY, or COSY, one of the earliest 2D NMR experiment which reveals which molecules are connected via J coupling.

There exist 2D NMR experiments which test for any kind of spin-spin interactions. For example, one very important experiment which tests for NOE cross-relaxation between adjacent spins is known as NOESY. Here, cross-peaks will stand for pairs of spins which are close to each other in space. For example (neglecting J-splittings):



The examples so far have been for homonuclear experiments, but can be extended easily (conceptually easily, that is ...) to account for heteronuclear couplings as well.

An Inefficient 2D Experiment

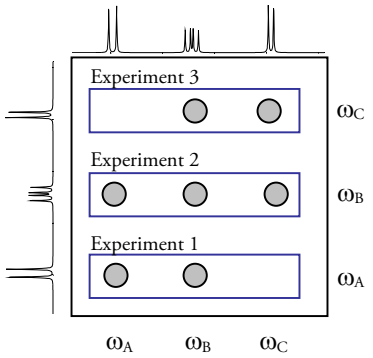
How could we get the above spectra? Here's a simple way. Let's say we have a "magic box", calling the "mixing box", the role of which is to let the interaction in question evolve while (hopefully) preventing all other interactions from happening. For example, for COSY, this would let J-coupling transfer magnetization between J-coupled nuclei. For NOESY, the box would let cross-relaxation occur between spatially adjacent nuclei.

For the ABC molecule above we would run three experiments:

(Excite ω_A) - (Mixing) - (Acquire)
(Excite ω_B) - (Mixing) - (Acquire)
(Excite ω_C) - (Mixing) - (Acquire)

Here, excite ω_A refers to applying a selective pulse which would excite only ω_A (this is possible, even though we haven't covered it in this course). As a result, we would only see the results of the mixing on the magnetization of ω_A, which, following mixing, would be transported to ω_B. Thus, when we acquire a signal, we would see the magnetization precessing at ω_B and acquire a peak at that position.

Similarly, in the second experiment, only ω_B would be excited and as a result of the mixing magnetization will be transferred to ω_A and ω_C, which would then appear in the spectrum. Thus, each experiment yields a different line in the 2D spectrum:



This experiment has two major drawbacks:

1. First, you need to know in advance where the peaks are so you can target them with your selective pulses. This is a practical but not a fundamental flaw.
2. Each experiment only excites one peak at a time, and therefore suffers from a major loss of sensitivity. This is a fundamental flaw.

To overcome issue (#2), we'd like to excite all peaks in each experiment. This, however, would prevent us from deciphering which peak during acquisition (after mixing) came from which peak before mixing. For example, if we'd see ω_C, we'd have no way of telling whether it came from ω_A or ω_B. To fix this, we will expand the basic scheme of 2D NMR in the next section.

An Efficient 2D Experiment

Modern 2D NMR experiments have this general outline:

$$\text{excite} - t_1 - \text{mixing} - t_2\,(\text{acquire})$$

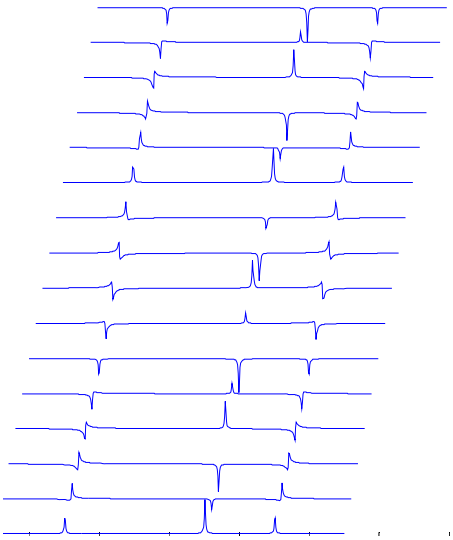
In the first step, all spins are excited. In the second step, they evolve for a time t_1. Then the magic mixing box is applied, and finally a signal is acquired. Let's follow a single chemical shift, say ω_B in our ABC molecule: after being excited, it evolves for a time t_1, acquiring a phase due to its chemical shift (neglecting J couplings and other interactions for simplicity):

$$M_{xy} = e^{i\omega_B t_1}\ .$$

Now mixing occurs, and magnetization is transferred to ω_A and ω_C.
where k_A, k_C are just two proportionality constants. If we were to Fourier transform this signal, we would get two peaks at ω_A, ω_C, but they would be weighted by the phase term prior to mixing, $e^{i\omega_B t_1}$. The basic scheme is repeated for many equally spaced values of t_1:

$t_1 = 0$
$t_1 = \Delta t_1$
$t_1 = 2\Delta t_1$
...
$t_1 = (N_1\text{-}1)\Delta t_1$

and the results plotted in a matrix:



The peaks we see in each experiment are those appearing **after** the mixing. However, they get modulated in successive experiments by their frequencies **before** mixing. A Fourier transform along the **indirect dimension** (t_1) will reveal the frequency before mixing, yielding our 2D spectrum.

This can be seen mathematically as well: our signal in each experiment is comprised from the contributions of each of the three peaks (neglecting J-coupling and T_2 relaxation):

If we treat t_1 as an independent variable, we see that a Fourier transform along t_1 will yield the frequencies before mixing. We saw that a Fourier transform turns the time domain data (sampled from t=0 to t=∞) into a spectrum with absorptive + dispersive components:

$$e^{i\omega_0 t} \rightarrow A(\omega-\omega_0)+iD(\omega-\omega_0)\,.$$

Thus, a 2D Fourier transform will yield, up to normalizing constants and neglecting couplings before and after mixing:

represents peaks on the diagonal, while terms such as

$$\overbrace{\left[A(\omega_1-\omega_A)+iD(\omega_1-\omega_A)\right]}^{\text{peak at } \omega_A \text{ along indirect domain}}\cdot\overbrace{\left[A(\omega_2-\omega_B)+iD(\omega_2-\omega_B)\right]}^{\text{peak at } \omega_B \text{ along direct domain}}$$

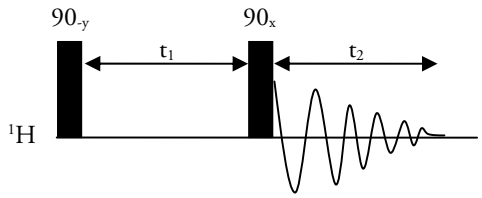
represent a cross-peak between A and B.

These ideas sum up the gist of 2D NMR. We now turn to some examples and practical considerations.

COSY

The Pulse Sequence

The simplest 2D experiment students encounter is called COSY, short for COrrelation SpectroscopY. It tests for homonuclear J-couplings between pairs of spins. The pulse sequence is:



For COSY, the mixing is a simple 90_x pulse. The phases of the pulses don't turn out to be very important, so we've picked them to simplify our calculations.

Analysis

We'll assume a weakly coupled homonuclear spin pair. Starting from thermal equilibrium, $\rho = S_{Az} + S_{Bz}$, the first pulse excites the spins onto the xy plane:

$$\rho = S_{Ax} + S_{Bx}\,.$$

As before, $S_{Ax} = S_x \otimes I$. We are using A and B to avoid confusion with the indirect (t_1) and direct (t_2) time evolution subscripts. During t_1, the system evolves according to both the chemical shift and the J-coupling interaction. We first apply the chemical shift evolution:

$$\begin{aligned}\rho = {} & S_{Ax}\cos(\omega_A t_1) - S_{Ay}\cos(\omega_A t_1) \\ & + S_{Bx}\cos(\omega_B t_1) - S_{By}\sin(\omega_B t_1)\end{aligned}$$

Next comes J-coupling during t_1:

$$\begin{aligned}\rho = & S_{Ax}\cos(\omega_A t_1)\cos(\pi J t_1) + 2S_{Ay}S_{Bz}\cos(\omega_A t_1)\sin(\pi J t_1)\\ & -S_{Ay}\sin(\omega_A t_1)\cos(\pi J t_1) + 2S_{Ax}S_{Bz}\sin(\omega_A t_1)\sin(\pi J t_1)\\ & +S_{Bx}\cos(\omega_B t_1)\cos(\pi J t_1) + 2S_{Az}S_{By}\cos(\omega_B t_1)\sin(\pi J t_1)\\ & -S_{By}\sin(\omega_B t_1)\cos(\pi J t_1) + 2S_{Az}S_{Bx}\sin(\omega_B t_1)\sin(\pi J t_1)\end{aligned}$$

At this point, the second 90° pulse acts in a similar manner to that of an INEPT experiment, and transfers polarization from A to B or from B to A, assuming of course that J≠0:

$$\begin{aligned}\rho = & S_{Ax}\cos(\omega_A t_1)\cos(\pi J t_1) - 2S_{Az}S_{By}\cos(\omega_A t_1)\sin(\pi J t_1)\\ & +S_{Az}\sin(\omega_A t_1)\cos(\pi J t_1) + 2S_{Ax}S_{By}\sin(\omega_A t_1)\sin(\pi J t_1)\\ & +S_{Bx}\cos(\omega_B t_1)\cos(\pi J t_1) - 2S_{Ay}S_{Bz}\cos(\omega_B t_1)\sin(\pi J t_1)\\ & +S_{Bz}\sin(\omega_B t_1)\cos(\pi J t_1) + 2S_{Ay}S_{Bx}\sin(\omega_B t_1)\sin(\pi J t_1)\end{aligned}$$

Now all we have to do is to calculate the time evolution of each term and take the trace with M_{xy} to calculate the FID. This is quite a bit of hassle, so we'll try to cut down on the amount of work. First, we can neglect all longitudinal (S_{Az}, S_{Bz}) terms, as well as all terms of the form $S_{Ax}S_{Bx}$, $S_{Ax}S_{By}$, $S_{Ay}S_{Bx}$, $S_{Ay}S_{By}$, since they are non-observable and will not become observable during t_2. This leaves us with:

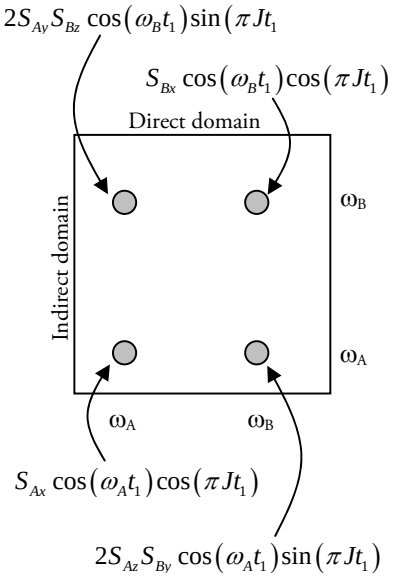
$$\begin{aligned}\rho = & S_{Ax}\cos(\omega_A t_1)\cos(\pi J t_1) - 2S_{Az}S_{By}\cos(\omega_A t_1)\sin(\pi J t_1)\\ & +S_{Bx}\cos(\omega_B t_1)\cos(\pi J t_1) - 2S_{Ay}S_{Bz}\cos(\omega_B t_1)\sin(\pi J t_1)\end{aligned}$$

The experiment will be repeated for many values of t_1. Formally there is no difference between $t_1$and t_2: they are both discrete variables with their own dwell time and maximum acquisition time.

The four terms represent the four groups of peaks we will see in the final spectrum:

S_{Ax}	Magnetization that started out in ω_A and did not get transferred to ω_B, resulting in a diagonal peak at (ω_A, ω_A)
$2S_{Az}S_{By}$	Magnetization that started out in ω_A but after mixing will resonate at ω_B (cross-peak at (ω_A, ω_B))
S_{Bx}	Magnetization that started out in ω_B and did not get transferred to ω_A, resulting in a diagonal peak at (ω_B, ω_B)
$2S_{Ay}S_{Bz}$	Magnetization that started out in ω_B but after mixing will resonate at ω_A (cross-peak at (ω_B, ω_A))

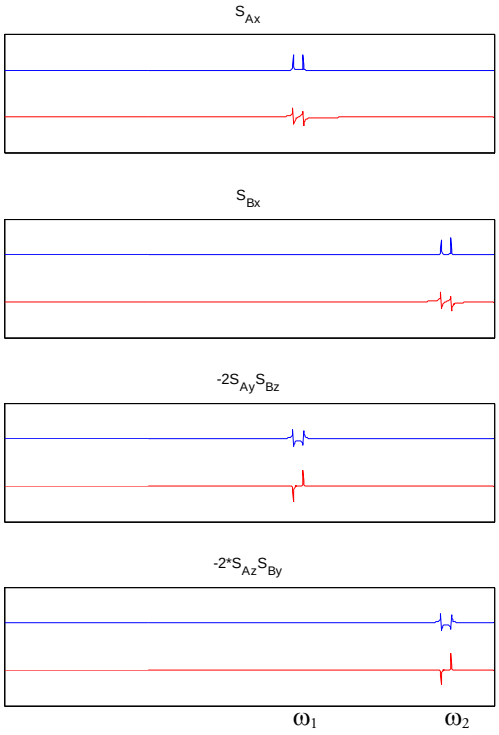
Naively, we would expect our spectrum to look like the toy spectra shown in the beginning:



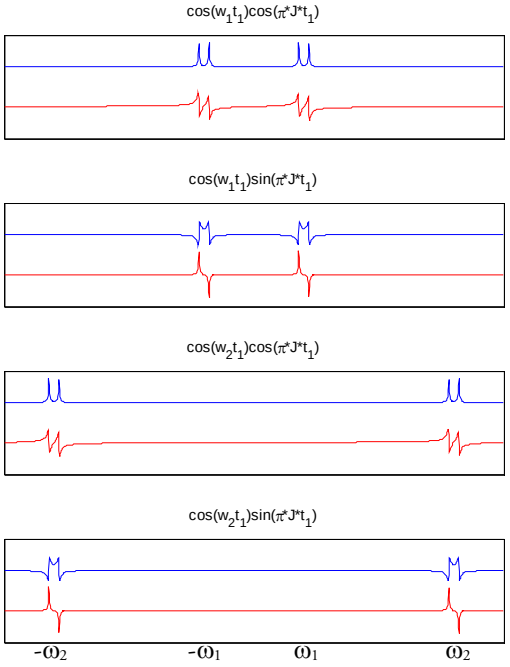
While the above naive picture is approximately correct, it does not account for some of the finer details of the 2D spectrum such as J-splittings and other issues which we address next, as we examine how the full two-spin spectrum looks like.

The Spectrum

We also don't really have to calculate the time evolution of the different parts of ρ because we've already discussed their appearance when talking about INEPT in lecture 5: S_{Ax} and S_{Bx} will give rise to an "in-phase" doublets at ω_A and ω_B, respectively; $-2S_{Az}S_{By}$ and $-2S_{Ay}S_{Bz}$ will give rise to out-of-phase doublets at ω_A and ω_B, respectively:



The splitting between the peaks is given by J. The above four lineshapes will be seen along the "direct domain" of the 2D spectrum, after a Fourier transform along t_2. Along the indirect domain we will observe, following a Fourier transform (real parts shown in black, imaginary parts in red):

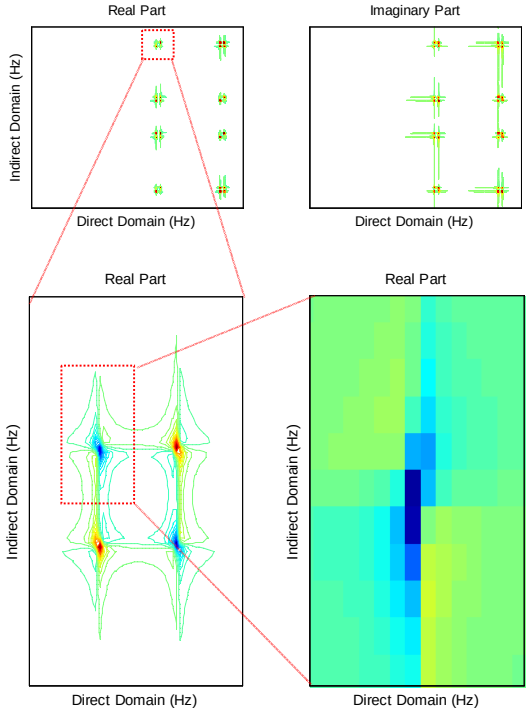


These shapes along the indirect domain can be deduced by expressing each term using complex exponentials and the identities $\cos(x)=\frac{1}{2}\left(e^{ix}+e^{-ix}\right)$, $\sin(x)=\frac{1}{2i}\left(e^{ix}-e^{-ix}\right)$. For example:

$$\begin{aligned}&\cos(\omega_B t_1)\sin(\pi J t_1)\\&=\tfrac{1}{2}\left(e^{i\omega_B t}+e^{-i\omega_B t}\right)\tfrac{1}{2i}\left(e^{i\pi Jt}-e^{-i\pi Jt}\right)\\&=-\tfrac{i}{4}\left(e^{i(\omega_B+\pi J)t_1}-e^{i(\omega_B-\pi J)t_1}-e^{i(-\omega_B+\pi J)t_1}+e^{i(-\omega_B-\pi J)t_1}\right)\end{aligned}$$

When T_2 relaxation is added, each of the terms of the form $e^{i\omega t}$ will turn into an absorptive line at frequency ω in the real part of the spectrum, along a dispersive line at ω in the imaginary part of the spectrum. A minus sign will mirror the lineshape about the y-axis, and an *i* will switch between the absorptive and dispersive parts.

The final 2D COSY spectrum will appear as the product of the indirect and direct lineshapes:



The spectrum has several features of interest:

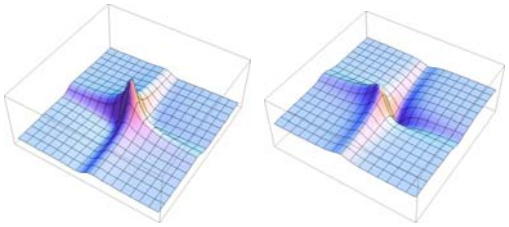
1. The 2D spectrum is reflected along zero frequency in the indirect domain, a

consequence of the cos modulation (as opposed to $e^{i\omega_t}$) along the t_1 domain.

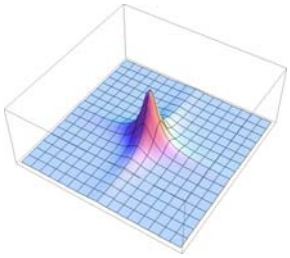
2. The diagonal peaks correspond to the case where magnetization was not transferred and are "uninteresting", since all frequencies have a component of the magnetization which they don't transfer.
3. The lineshapes are quite interesting: instead of a single diagonal/cross peak there are four, some of which are out of phase. Furthermore, each peak in each group of four is **distorted**. This is a direct consequence of what we've mentioned before: both along the direct and indirect domain one obtains lineshapes of the form $A(\omega)+iD(\omega)$. Their products give:

$$\begin{aligned}&\left[A(\omega_1)+iD(\omega_1)\right]\left[A(\omega_2)+iD(\omega_2)\right]\\&=\left[A(\omega_1)A(\omega_2)-D(\omega_1)D(\omega_2)\right]\\&+i\left[A(\omega_1)D(\omega_2)+D(\omega_1)A(\omega_2)\right]\end{aligned}$$

Thus, both the real and imaginary parts of the lineshape are distorted. We will discuss ways of avoiding this distortion in part 4. The real and imaginary parts are plotted below:



On the left we see $A(\omega_1)A(\omega_2)$-$D(\omega_1)D(\omega_2)$. On the right you see $A(\omega_1)D(\omega_2)$+$D(\omega_1)A(\omega_2)$. Although the real part (left) is not very distorted, it involves the dispersive components which decay very slowly and cause the line to be much broader than a puer lorentzian. Compare this to just $A(\omega_1)A(\omega_2)$:



The imaginary part looks completely unusable.

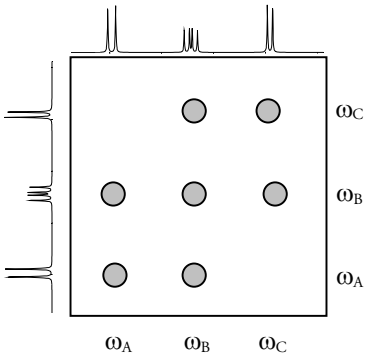
More Complex Systems

Systems can be more complex in the following senses:

1. Have more spins.
2. Have more inter-spin couplings.
3. Have strong J-couplings.

We discuss each case briefly:

1. Having more spins is the easiest scenario. The evolution of our spin system is linear in the spin operators. That is, if we had some other spin S_C it would be excited and evolve irrespective of the other two (A and B). The real question is, what happens when a spin has more than one J-coupling? This we address in #2.
2. The answer here can be grasped by looking at a simple A-B-C system, where a dash represents J-coupling. When analyzing spin B during t_1 we can apply the J-evolution between B-A and B-C in any order we choose. In either case, following excitation from S_{Bz} into S_{Bx}, we will end up with terms such as $S_{By}S_{Cz}$ and $S_{By}S_{Az}$. The mixing pulse will then convert these to $S_{Bz}S_{Cy}$ and $S_{Bz}S_{Ay}$, meaning we will get resonances at both ω_A and ω_B. Thus we will get something along the lines of the spectrum we've drawn earlier during the lecture:



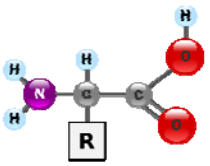
3. Strong coupling can complicate the COSY spectrum. However, even with strong coupling, coherences will only "jump" to neighbors a single J-coupling bond away.

Other 2D Sequences

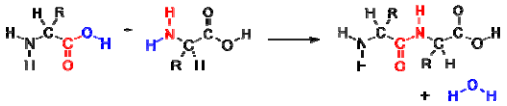
We briefly outline some important 2D experiments and their possible uses.

HSQC

Heteronuclear Single-Quantum Correlation is a 2D experiment which forms cross-peaks between J-coupled hydrogen and X-nuclei (usually ^{15}N, but could be anything really). Most nitrogen atoms in nature are ^{14}N (~99.6%), which has a spin of 1 and quadrupole moment, making it difficult to observe in high resolution liquid state NMR. What one usually does is use bacteria of some sort to express a protein of interest after providing an ^{15}N enriched substrate. The protein is made out of amino acids which have this general structure:

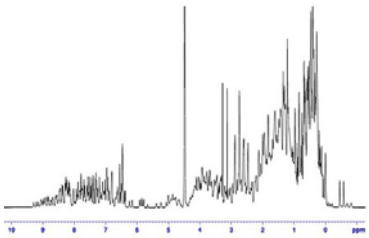


Amino acids are chained together to form a protein via peptide bonds:



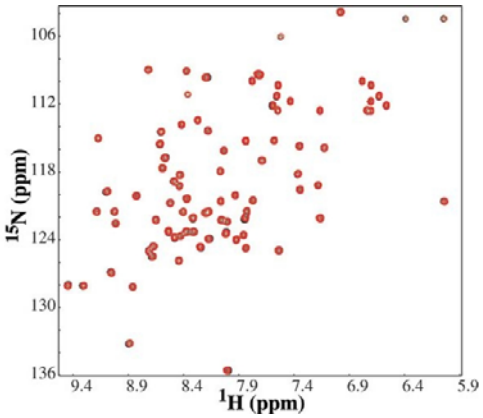
that is, proteins are basically long chains that look like NCCNCCNCCNCC etc... To each N, a hydrogen H is attached. The N-H bond is called an **amide bond**. When exploring protein NMR, a major simplification can be had by understanding which of the ^{1}H resonances we see come from the backbone of the protein.

Let's take as an example the simple protein ubiquitin. This is a small protein weighing about 8.5 kDa, where 1 Dalton is the weight of about a single proton (so a water H_2O molecule would weigh about 18 Da). It generally attaches to other proteins which starts a cascade that sends them to the cell's recycle bin. Although small, its 1D NMR spectrum is still extremely complicated:



(Courtesy of Dr. Maayan Gal)

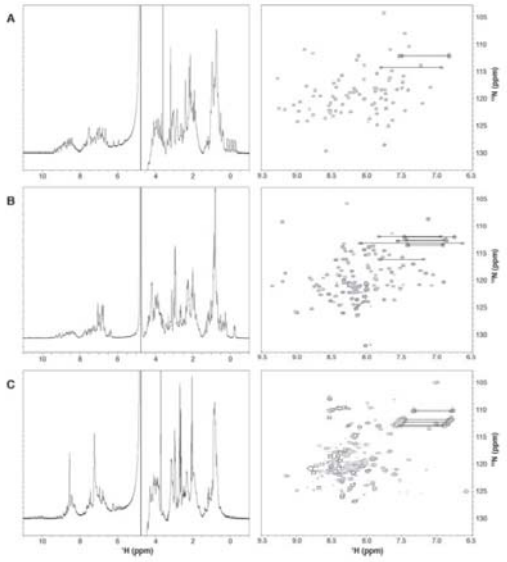
The amide protons resonate in the 6-10 ppm range to the left of the water, constitute the protein's backbone, and knowing their spatial positions determines the protein's structure. A 2D HSQC spectrum of ubiquitin will look like this:



(Courtesy of Dr. Maayan Gal)

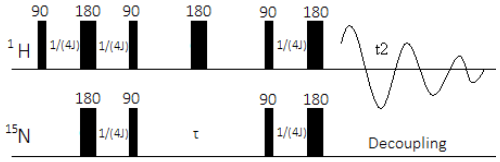
HSQC gives you the least complicated experiment that clearly resolves the various proton resonances and gives you a **fingerprint** of the protein. It is the "1D experiment of proteins".

Of course some cross-peaks belong to H-N pairs not in the backbone, and a good experimentalist will be able to weed those out either by examination or by further experiments. An HSQC experiment can be used to track how proton resonances appear/disappear in the spectrum, when, for example, a reactant is added and exchange occurs (we'll talk more about that in the next chapter, too). We can also use HSQC to determine how structured the protein is. Below, 1D ^{1}H-NMR (left) and 2D ^{1}H-^{15}N (right) spectra are shown for ASC2 (top), PEA-15 (middle) and a protein from a macrophage (bottom):

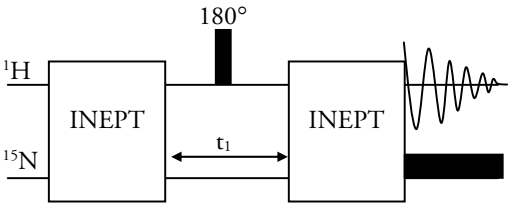


The dispersed signals of the hydrogens in the top and middle spectra indicate a **folded** protein, while the smaller dispersion in the bottom spectrum around 8.0-8.5 ppm is a strong indicator of a disordered protein in which the backbone is in a random coil configuration. The reason the chemical shifts bunch around 8-8.5 ppm is that disordered proteins perform fast conformational changes which affect their local chemical environments; the proton chemical shifts then tend to average up in the 6-10 ppm range and end up around 8 ppm.

The HSQC experiment looks like this:



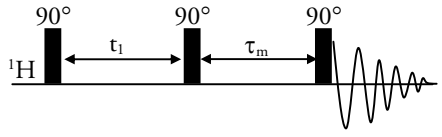
It might look daunting at first but it's really straightforward once you realize that it's just:



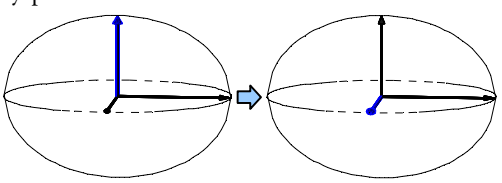
You start with hydrogen magnetization, which you transfer to the nitrogens, let evolve for a time t_1 and then transfer back to the hydrogens for greater efficient in detection, with decoupling on the 15N channel. The experiment is repeated many times for different values of t_1. Note also the 180° pulse on the hydrogen channel during t_1, to refocus the unwanted J-coupling evolution.

NOESY

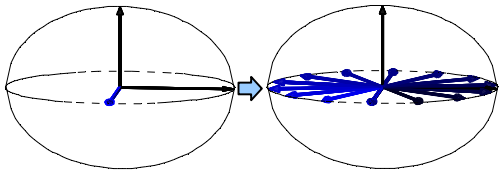
In NOESY, mixing occurs due to NOE effect: magnetization is transferred between spins that are close in space (~ 5Å or less). Thus, we get powerful distance constraint on our molecule's structure. The NOESY sequence is:



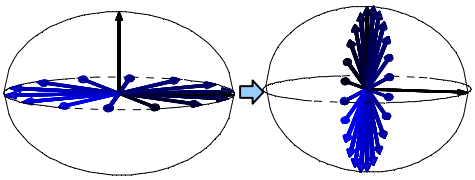
The first pulse excites the spins (blue line) onto the xy-plane:



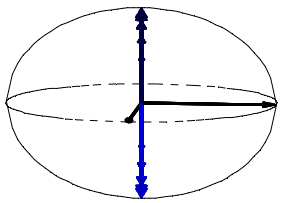
Following the t_1 period, the spins will all precess with their own frequency in the xy plane (neglecting J-coupling):



The second 90° pulse will "store" the spins along the z-axis:

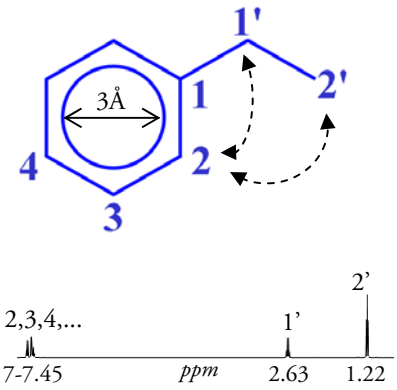


For simplicity, let's assume $\tau_m >> T_2$. As a result, the magnetization in the xy-plane will die out before the next excitation pulse, leaving us only to worry about the longitudinal component:

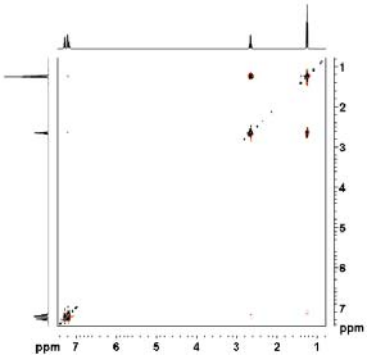


During the mixing period, these longitudinal spins will relax with a time constant T_1, and also exchange magnetization with nearby spins with the NOE effect. After we excite, we measure the usual spectrum along the direct (t_2) domain.

As an example, consider ethyl-benzene:



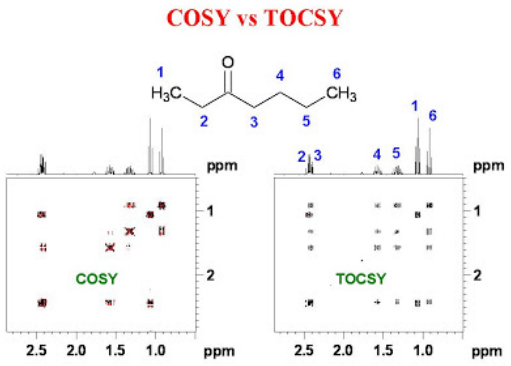
and its 2D NOESY spectrum:



The cross-peaks between H1' and H2' are due to unfiltered COSY correlations that contaminate the NOESY spectrum. The "basic" NOESY experiment can be modified to filter out these cross-peaks, but we will not address this here. A weak through-space cross-peak however appears between H1' and H2, as well as H2' and H2.

TOCSY

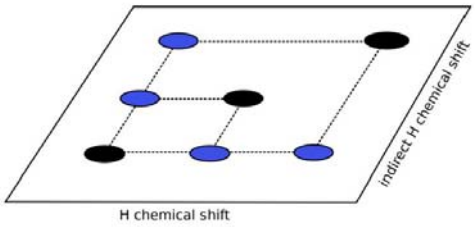
COSY yields cross-peaks between two spins that are directly J-coupled. In a molecule A-B-C-D you would only get cross peaks between (A,B), (B,C) and (C,D). However, one can also think in terms of "J-coupled spin networks": A,B,C and D are all part of the same "network" and are coupled, even though via multiple J-coupling "jumps". TOCSY reveals spin networks and creates cross-peaks between all spins in a network, as shown in the example below of 3-heptanone (aka butyl-ethyl-ketone):



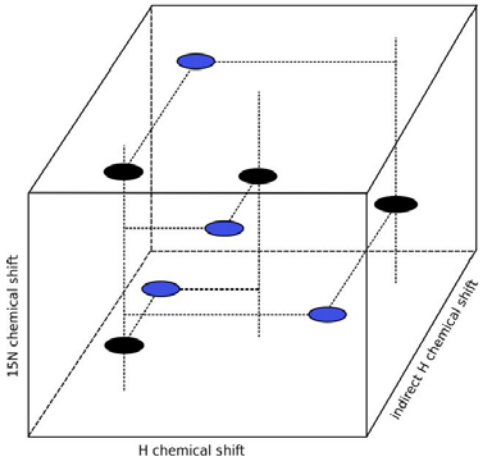
As a standalone experiment it is not as useful, but in nD experiments it can be very powerful, as discussed below.

nD NMR

The concept of 2D NMR can be extended in a straightforward way. For example, a 3D NMR experiment would reveal cross-peaks (in 3D space) between spin triplets. A good example of that is a 2D NOESY experiment in a protein. Here, a cross peak between two protons doesn't tell us if the two protons have come from the same ^{15}N backbone protein:

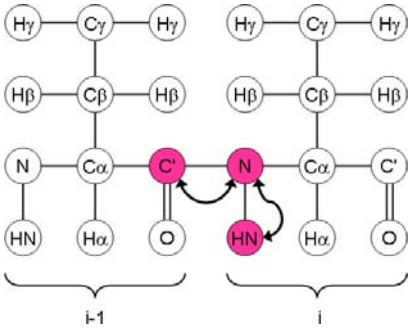


The solution is to add a third heteronuclear dimension for resolving the ^{15}N chemical shift:



Now the third dimension lets us acquire a separate 2D NOESY data set for each nitrogen in the backbone of the protein, making our distance constraint a lot more exact.

An example of a 3D experiment is the HNCO, in which magnetization is transfered between H to N to C, and then back to H (via N) for detection. The protein needs to be ^{13}C and ^{15}N labeled for this to work. The resonances we end up seeing are mostly from the backbone's CO-N-HN elements:



This is a good experiment for assigning the carbon chemical shifts in the C=O bonds in the backbone (i.e. we can use this to spread out the carbon chemical shifts). It also provides constaints on which carbon chemical shifts are connected to which nitrogen chemical shifts, providing us with constaints that help determine protein structure.

The general structure of a 3D NMR experiment is:

Excite-(t_1)-(mixing #1)-(t_2)-(mixing #2)-(t_3)

where t_3 is now the direct time along which we acquire our spectrum, and t_1 and t_2 need each to be

incremented in steps, each step requiring its own experiment:

Exp. 1:	$t_1=0$	$t_2=0$
Exp. 2:	$t_1=\Delta t$	$t_2=0$
Exp. 3:	$t_1=2\Delta t$	$t_2=0$
...		
Exp. N:	$t_1=(N-1)\Delta t$	$t_2=0$
Exp. N+1:	$t_1=0$	$t_2=\Delta t$
Exp. N+2:	$t_1=\Delta t$	$t_2=\Delta t$
Exp. N+3:	$t_1=2\Delta t$	$t_2=\Delta t$
...		
Exp. 2N:	$t_1=(N-1)\Delta t$	$t_2=\Delta t$
Exp. 2N+1:	$t_1=0$	$t_2=2\Delta t$
...		
Exp N^2:	$t_1=(N-1)\Delta t$	$t_2=(N-1)\Delta t$

Here it was assumed the two dimensions share the same dwell time Δt and number of points N, but this does not have to be so.

Practical Considerations

Experimental Time

The major drawback of 2D experiments is time! A 2D experiment will often need as many points along the indirect domain as along the direct domain, a figure in the hundreds – say, 256. If each scan takes 1 seconds as we wait for the magnetization to return to thermal equilibrium, then a 2D experiment will take

$$256\times1 \text{ sec} \sim 4 \text{ mins}$$

Most 2D experiments employ some form of phase cycling. A simple HSQC will have a minimum of 4 scans, bringing this up to:

$$256\times4 \text{ sec} \sim 17 \text{ mins}$$

In many experiments, however, the concentration of the protein you're studying is very low, so you end up averaging for a long amount of time anyway.

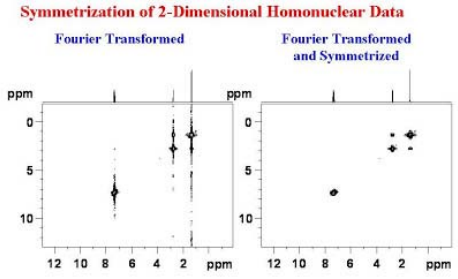
A 3D experiment will require ~ 256 points along the third time dimension, and will take

$$256\times256\times4 \text{ sec} \sim 3 \text{ days}$$

without even taking into account additional phase cycling for the third dimension! In reality these times can be decreased significantly by applying advanced sampling methodologies that allow us to acquire fewer points. Sometimes if we are interesting in a particular frequency along the indirect domain we can excite it selectively, effectively removing the requirement to sample the domain (e.g. if we know the offset of the particular ^{15}N in a 3D ^{15}N-resolved NOESY experiment). However, time remains the most daunting challenge nD-NMR is faced with (although in practice most of the time of protein structure labs is spent trying to synthesize the protein, not measuring its properties with NMR!).

t_1-Noise

As experimental times increase, instrument instabilities play an ever-increasing role. Tiny fluctuations in the main field, temperature and other factors will introduce artifacts into the spectrum. These will appear along the indirect "slow" domain, since the direct domain is read almost instantaneously (~ms per point), while the indirect domain is read a lot more slowly (~sec per point), making it much more susceptible to errors that accumulate over time. The classic appearance of such errors is in the form of streaks along the indirect domain, called **t_1-noise**:



A simple trick to removing t_1 noise is to force our spectrum to be symmetrical, since if A and B are "connected", we should get a cross peak at both (ω_A, ω_B) and (ω_B, ω_A). Thus, any non-symmetric component can be removed. This, however, leads to very unsatisfactory results in highly crowded spectra. On modern spectrometers this tends to be less of an issue it used to be in the past, although it still tends to appear to some degree in nD dimensional spectra.

2D Lineshapes

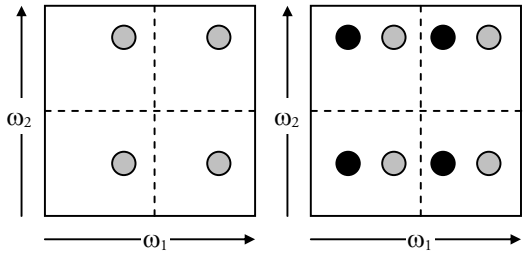
We've commented how distorted 2D lineshapes arise in COSY due to the appearance of dispersive and absorptive components along both the direct and indirect domains. This is a consequence of only acquiring for times $t_1,t_2>0$. Remember than the FT of $e^{i\omega_{cs}t-t/T_2}$ is given by

$$\begin{aligned} S(\omega) &= \int_{-\infty}^{\infty} e^{i\omega_{cs}t-t/T_2} e^{-i\omega t} dt \\ &= A(\omega-\omega_{cs}) + iD(\omega-\omega_{cs}) \end{aligned}$$

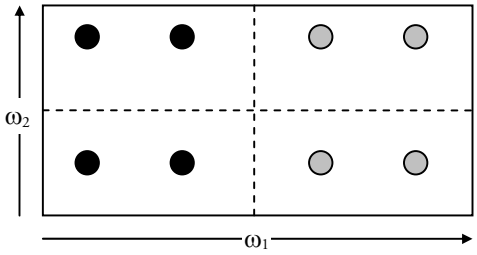
Another issue is the appearance of a "mirror spectra" along the indirect domain, since the signal there is modulated as

$$\cos(\omega_A t_1) = \tfrac{1}{2}\left(e^{i\omega_A t_1} + e^{-i\omega_A t_1}\right).$$

This is illustrated in the following schematic spectrum, in which the grey points represent the "true" spectrum from a two-spin system (say, in COSY) and the black points represent the mirror images (the dashed lines represent the $\omega_1=0$ and $\omega_2=0$ lines):



In the above diagram the mirror images don't interfere with other peaks, but in a real spectrum they very well could! To avoid this, we would have to (1) double our spectral width SW_1 along the indirect domain, and (2) shift our spectrum such that all frequencies start at $\omega>0$, and the mirror images fall outside the spectrum at $\omega<0$:



The main cost of this approach is doubling SW_1, which means halving the dwell time Δt_1 along the indirect domain, which means **doubling the number of acquisitions**. This is an unavoidable cost. This approach is called TPPI (Time Proportional Phase Increments). However, this approach does not deal with the distorted lineshapes. I also haven't explained how to shift the spectrum along ω_1, and I'm not going to because I'm going to present a better alternative.

One suggestion for addressing the lineshape issue is to acquire a second dataset which is **sine-modulated** along t_1. If we had two datasets, with signals:

$$\begin{aligned} S(t_1,t_2) &= \cos(\omega_A t_1) e^{i\omega_B t_2} \\ S(t_1,t_2) &= \sin(\omega_A t_1) e^{i\omega_B t_2} \end{aligned}$$

We are not writing the relaxation terms along both axes out of laziness, but they're there. We now take the first spectrum, perform a FT along t_2, discard the imaginary part, perform a FT along t_1 and discard the imaginary part again (this is equivalent to doing something called a *cosine transform* along both axes):

$$\begin{aligned} S(t_1,t_2) &= \cos(\omega_A t_1) e^{i\omega_B t_2} \\ &\xrightarrow{FT_2} \cos(\omega_A t_1)\left(A(\omega_2-\omega_B)+iD(\omega_2-\omega_B)\right) \\ &\xrightarrow{\text{discard}} \cos(\omega_A t_1) A(\omega_2-\omega_B) \\ &= \tfrac{1}{2}\left(e^{i\omega_A t_1}+e^{-i\omega_A t_1}\right) A(\omega_2-\omega_B) \\ &\xrightarrow{FT_1} \tfrac{1}{2}\begin{pmatrix}\left[A(\omega_1-\omega_A)+iD(\omega_1-\omega_A)\right] \\ +\left[A(\omega_1+\omega_A)+iD(\omega_1+\omega_A)\right]\end{pmatrix} A(\omega_2-\omega_B) \\ &\xrightarrow{\text{discard}} \tfrac{1}{2}\left(A(\omega_1-\omega_A)+A(\omega_1+\omega_A)\right) A(\omega_2-\omega_B) \end{aligned}$$

The same sort of dance is repeated with the second data set, yielding:

$$S(t_1,t_2)=\sin(\omega_A t_1)e^{i\omega_B t_2}$$
$$\rightarrow -\tfrac{i}{2}\left[A(\omega_1-\omega_A)-A(\omega_1+\omega_A)\right]A_2(\omega_2-\omega_B)$$

Combining the two experiments yields a purely absorptive peak:

$$(\text{first})+i(\text{second})=A(\omega_1-\omega_A)A_2(\omega_2-\omega_B)$$

This solves both problems - the mirror peaks and the non-absorptive peaks - in one shot (but still requires two data sets). **But the cost is losing sensitivity**: we've performed two sets of experiments and ended up with the same signal intensity as we got from a single experiment. In the same amount of time we could have repeated the original experiment. So not only do we have to spend twice the amount of time, but we also don't get any extra signal out of it.

How does one acquire a second sine-modulated data set? This will depend on the sequence at hand. For a COSY spectrum, the phase of the second pulse is shifted by 90°. Remember that just before the second pulse, our density matrix is:

$$\begin{aligned}\rho = {} & S_{Ax}\cos(\omega_A t_1)\cos(\pi J t_1)+2S_{Ay}S_{Bz}\cos(\omega_A t_1)\sin(\pi J t_1)\\ & -S_{Ay}\sin(\omega_A t_1)\cos(\pi J t_1)+2S_{Ax}S_{Bz}\sin(\omega_A t_1)\sin(\pi J t_1)\\ & +S_{Bx}\cos(\omega_B t_1)\cos(\pi J t_1)+2S_{Az}S_{By}\cos(\omega_B t_1)\sin(\pi J t_1)\\ & -S_{By}\sin(\omega_B t_1)\cos(\pi J t_1)+2S_{Az}S_{Bx}\sin(\omega_B t_1)\sin(\pi J t_1)\end{aligned}$$

Our calculation assumed the second pulse was a 90_x, leaving us with (after discarding the unobservable coherences):

$$\begin{aligned}\rho = {} & S_{Ax}\cos(\omega_A t_1)\cos(\pi J t_1)-2S_{Az}S_{By}\cos(\omega_A t_1)\sin(\pi J t_1)\\ & +S_{Bx}\cos(\omega_B t_1)\cos(\pi J t_1)-2S_{Ay}S_{Bz}\cos(\omega_B t_1)\sin(\pi J t_1)\end{aligned}$$

Had we applied a 90_y instead, we would have gotten:

$$\begin{aligned}\rho = {} & S_{Az}\cos(\omega_A t_1)\cos(\pi J t_1)-2S_{Ay}S_{Bx}\cos(\omega_A t_1)\sin(\pi J t_1)\\ & -S_{Ay}\sin(\omega_A t_1)\cos(\pi J t_1)-2S_{Az}S_{Bx}\sin(\omega_A t_1)\sin(\pi J t_1)\\ & +S_{Bz}\cos(\omega_B t_1)\cos(\pi J t_1)-2S_{Ax}S_{By}\cos(\omega_B t_1)\sin(\pi J t_1)\\ & -S_{By}\sin(\omega_B t_1)\cos(\pi J t_1)-2S_{Ax}S_{Bz}\sin(\omega_B t_1)\sin(\pi J t_1)\end{aligned}$$

Discarding the unobservable terms, we end up with:

$$\begin{aligned}\rho = {} & -S_{Ay}\sin(\omega_A t_1)\cos(\pi J t_1)-2S_{Az}S_{Bx}\sin(\omega_A t_1)\sin(\pi J t_1)\\ & -S_{By}\sin(\omega_B t_1)\cos(\pi J t_1)-2S_{Ax}S_{Bz}\sin(\omega_B t_1)\sin(\pi J t_1)\end{aligned}$$

Now we get a $\sin(\omega t_1)$ term along the indirect domain. Here is our sine modulated data set!