Magnetic and chemical equivalence

- Before we get deeper into analysis of coupling patterns, let's pay some more attention to naming conventions, as well as to some concepts regarding chemical and magnetic equivalence.

- Our first definition will be that of a **spin system**. We have a spin system when we have a group of \( n \) nuclei (with \( I = \frac{1}{2} \)) that is characterized by no more than \( n \) frequencies (chemical shifts) \( \nu_i \) and \( n \left( n - 1 \right) / 2 \) couplings \( J_{ij} \). The couplings have to be within nuclei in the spin system.

- We start by defining **magnetic equivalence** by analyzing some examples. Say that we have an ethoxy group (-O-CH\(_2\)-CH\(_3\)).

- As we saw last time, we can do a very simple first order analysis of this spin system, because we assumed that all CH\(_2\) protons were ‘equal’, and all CH\(_3\) protons were ‘equal’. Is this true?

- We can easily see that they are chemically equivalent. Additionally, we have free rotation around the bond, which makes their chemical shifts and couplings equal.
Magnetic equivalence (continued)

• Since the $^1$Hs can change places, they will alternate their chemical shifts (those bonded to the same carbon), and we will see an average.

• The same happens for the $\mathbf{J}$ couplings. We’ll see an average of all the $J_{HH}$ couplings, so in effect, the coupling of any proton in CH$_2$ to any proton in the CH$_3$ will be the same.

• If we introduce some notation, and remembering that $\delta($CH$_2$) is $\gg \delta($CH$_3$), this would be an $\mathbf{A}_2\mathbf{X}_3$ system: We have 2 magnetically equivalent $^1$Hs on the CH$_2$, and 3 on the CH$_3$.

• The $^2J_{HH}$ coupling (that is, the coupling between two nuclei bound to the same carbon) is zero in this case, because the energies for any of the three (or two) protons is the same.

• Finally, we use $\mathbf{A}$ to refer to the CH$_2$ protons, and $\mathbf{X}$ to refer to the CH$_3$ protons because they have very different $\delta$s. We usually start with the letter $\mathbf{A}$ for the most deshielded spin.

• Difluoromethane is another example of an ‘$\mathbf{AX}$’ type system:

![Diagram of Difluoromethane](image)

• In this case, the $^1$Hs and the $^{19}$Fs are equal not due to rotation, but to symmetry around the carbon. It’s an $\mathbf{A}_2\mathbf{X}_2$ system.
Magnetic equivalence (…)

- For CH₂F₂, we can also compare the couplings to check that the ¹Hs and ¹⁹Fs are equivalent: \( J_{H₁F₁} = J_{H₁F₂} = J_{H₂F₁} = J_{H₂F₂} \). All due to their symmetry...

- Now, what about the ¹Hs and ¹⁹Fs in 1,1-difluoroethene?

![Diagram of CH₂F₂ molecule with Hₐ, H₅, H₆, and Fₐ, F₅, F₆ labeled]

- Here we also have symmetry, but no rotation. The two ¹Hs and the two ¹⁹Fs are chemically equivalent, and we can easily see that \( \delta H_a = \delta H_b \) and \( \delta F_a = \delta F_b \).

- However, due to the geometry of this compound, \( J_{HₐFₐ} \neq J_{HₐF₅} \). Analogously, \( J_{H₅Fₐ} \neq J_{H₅F₅} \).

- Furthermore, since the couplings are different, the energy levels for \( H_a \) and \( H_b \) are different (not degenerate anymore as in CH₃), and we have \( J_{HₐHₕ} \neq 0 \).

- If we consider all the possible couplings we have, we have three different couplings for each proton. For \( H_a \), we have \( J_{HₐFₐ}, J_{HₐFₙ}, \) and \( J_{HₐHₕ} \). For \( Hₕ \), we have \( J_{HₕFₐ}, J_{HₕFₙ}, \) and \( J_{HₕHₕ} \). This means more than the eight possible transitions \((2*2*2)\) in the energy diagram, and an equal number of possible lines in the spectrum!
Magnetic equivalence (…)

- These are representative spectra (only the 1H spectrum is shown) of CH$_2$F$_2$ and F$_2$C=CH$_2$:

- A system like this is not an $A_2X_2$, but an $AA'XX'$ system. We have two A nuclei with the same chemical shift but that are not magnetically equivalent. The same goes for the X nuclei.

- The following are other examples of $AA'XX'$ systems:
Energy diagrams for 2nd order systems

• From what we’ve seen, most cases of magnetic non-equivalence give rise to 2nd order systems, because we will have two nuclei with the same chemical environment and the same chemical shift, but with different couplings (AA’ type…).

• Last time we analyzed qualitatively how a 2nd order AB looks like. In an AB system we have two spins in which $\Delta \delta \sim J_{AB}$. The energy diagram looks a lot like a 1st order AX system, but the energies involved (frequencies) and the transition probabilities (intensities) are such that we get a not so clear-cut spectrum:

• Some examples of AB systems:
Transition from 1\textsuperscript{st} order to 2\textsuperscript{nd} order.

- The following is a neat experimental example of how we go from a 1\textsuperscript{st} order system to a 2\textsuperscript{nd} order system. The protons in the two compounds have the same ‘arrangement’, but as $\Delta \delta$ approaches $J_{AB}$, we go from, in this case, $A_2X$ to $A_2B$:

![Diagram of two compounds with protons labeled $H_A$, $H_B$, and $H_X$.](image)

- Most examples have ‘ringing’ and are obtained at relatively low fields (60 MHz) because at these fields 2\textsuperscript{nd} order effects are more common...
Now, let’s analyze 2nd order systems with more than 2 spins. We already saw an example, the $A_2X$ system and the $A_2B$ system. The $A_2X$ system is 1st order, and is therefore easy to analyze.

- The $A_2B$ is 2nd order, and energy levels includes transitions for what are known as symmetric and antisymmetric wavefunctions. They are related with the symmetry of the quantum mechanical wavefunctions describing the system.

In any case, we have additional transitions from the ones we see in a $A_2X$ system:

- We now have 10 lines in the $A_2B$ system, instead of the 5 we have in the $A_2X$ system (we have to remember that in the $A_2X$ many of the transitions are of equivalent energy…).
More than 2 spins (continued)

- An $A_2B$ (or $AB_2$) spectrum will look like this:

- Another system that we will encounter is the $ABX$ system, in which two nuclei have comparable chemical shifts and a third is far from them. The energy levels look like this:
More than 2 spins (…)

- In a **ABX** spectrum, we will have 4 lines for the **A** part, 4 lines for the **B** part, and 6 lines for the **X** part:

  ![ABX Spectrum Diagram]

  - **ABX** spin systems are very common in trisubstituted aromatic systems.
  - The last system we will discuss is the **AA’BB’** system, that we saw briefly at the beginning (actually, we saw **AA’XX’**…).
More than 2 spins (…)

- In an AA′BB′/AA′XX′ system we have 2 pairs of magnetically non-equivalent protons with the same chemical shift. The energy diagram for such a system is:

- We see that we can have a total of 12 transitions for each spin (the AA′ part or the BB′ part). However, some of the energies are the same (they are degenerate), so the total number of lines comes down to 10 for each, a total of 20!

- In an AA′XX′ we see two sub-spectra, one for the AA′ part and one for the XX′ part. In a AA′BB′ we see everything on the same region of the spectrum.
More than 2 spins (…)

- Some examples of spin systems giving rise to AA’XX’ and AA’BB’ patterns are given below.

A typical AA’BB’ spectrum is that of ODCB, orthodichloro benzene. There are so many signals and they are so close to each other, that this compound is used to calibrate instrument resolution.
Common cases for 2nd order systems

- So what type of systems we will commonly encounter that will give rise to 2nd order patterns? Most of the times, aromatics will give 2nd order systems because the chemical shift differences of several of the aromatic protons will be very close (0.1 to 0.5 ppm), and $J_{HH}$ in aromatics are relatively large (9 Hz for $^3J$, 3 Hz for $^4J$, and 0.5 Hz for $^5J$).

- For other compounds, the general rule is that if protons in similar environments are ‘fixed’ (that is, restricted rotation), we will most likely have 2nd order patterns.

- A typical example of this, generally of an $ABX$ system, are pro-$R$ and pro-$S$ protons of methylenes a to a chiral center:

- In this case, we will have two protons that are coupled to one another (because they are different, $A$ and $B$), they will have very similar chemical shifts, and can be coupled to other spins. For example, the oxetane protons in taxol:
1D Pulse sequences

• We now have most of the tools to understand and start analyzing pulse sequences. We’ll start with the most basic ones and build from there. The simplest one, the sequence to record a normal 1D spectrum, will serve to define notation:

**Vectors:**

- According to the direction of the pulse, we’ll use $90_x$ or $90_y$ (or $90_\phi$ if we use other phases) to indicate the relative direction of the $B_1$ field WRT $M_o$ in the rotating frame.

**Shorthand:**

- The acquisition period will always be represented by an FID for the nucleus under observation (the triangle).
Inversion recovery

- Measurement of $T_1$ is important, as the relaxation rate of different nuclei in a molecule can tell us about their local mobility. We cannot measure it directly on the signal or the FID because $T_1$ affects magnetization we don’t detect.

- We use the following pulse sequence:

![Pulse sequence diagram]

- If we analyze after the $\pi$ pulse:

- Since we are letting the signal decay by different amounts exclusively under the effect of longitudinal relaxation ($T_1$), we’ll see how different $t_D$’s affect the intensity of the FID and the signal after FT.
Inversion recovery (continued)

Depending on the $t_D$ delay we use we get signals with varying intensity, which depends on the $T_1$ relaxation time of the nucleus (peak) we are looking at.
Inversion recovery (continued)

- If we plot the intensity versus time we get the following curve:

\[ I(t) = I_\infty \cdot (1 - 2 \times e^{-t/T_1}) \]

- It is an exponential with a time constant equal to the \(T_1\) relaxation time.

- In principle, measuring \(T_2\) would just involve calculating the envelope of the FID, since the signal in \(M_{xy}\) decays only due to transverse relaxation.

- The problem is that the decay we see on \(M_{xy}\) is not only due to proper relaxation, but also to the inhomogeneity of \(B_0\) (the fanning out or dephasing of the signal). The decay constant of the FID is called \(T_2^*\). To measure \(T_2\) properly we have to use spin-echoes.
Spin-echoes

- The pulse sequence is the following:

  $90_y$  $t_D$  $180_y \text{ (or } x\text{)}$  $t_D$

- We do the analysis after the $90_y$ pulse:
Spin-echoes (continued)

- We now go back to the <xyz> coordinates:

- If we acquire the FID right after the spin-echo sequence, the intensity of the signal after FT will only be affected by $T_2$ relaxation and not by dephasing due to $B_o$ imperfections.

- Upon repetition for different $t_D$ values, we plot the intensity versus $2 * t_D$ and get a graph similar to the one we got for inversion recovery, but in this case the decay rate will be equal to $T_2$. 
Heteronuclear polarization transfer

- An heteronuclear $^{13}$C - $^1$H spin system:

  - The population differences between the energy levels reflect that we have a 1 to 4 ratio between $^{13}$C and $^1$H due to the differences in the gyromagnetic ratios. Here is where we start seeing why it may be useful…

- One thing that we have wrong in the drawing is the relative intensities. Here we are drawing using only the ratios of the gyromagnetic ratio, not the abundance…
Now we will apply Selective Population Transfer and SP Inversion on this spin system, and see what happens. First SPT…

After we saturate, say, the $1,2$ transition we get the following populations in the energy diagram:

The signals of both spins change accordingly, but now we have a 3-fold increase for one of the $^{13}$C transitions - Now we are talking.

If we consider the absolute values of the signal, we have twice as much signal as in the original spectrum…
Heteronuclear polarization transfer - SPI

Now we do the same analysis for SPI. If we invert selectively the populations of 1,2, we get the following:

Now \textbf{THAT} was pretty cool, if we consider that we had started with a $^{13}$C signal that looked like this:

By manipulating the polarization of the protons, we obtain an enhancement of 4 in the $^{13}$C signal (considering positive and negative signals).
Non-selective polarization transfer - INEPT

- INEPT (*Insensitive Nuclei Enhancement by Polarization Transfer*) is an important pulse sequence building block found throughout multiple pulse sequences.

- It is used to increase the sensitivity (polarization) of nuclei such as $^{13}$C and $^{15}$N. It looks like this:

- Here $X$ is either $^{13}$C or $^{15}$N.
DEPT (Distortionless Enhancement by Polarization Transfer) is a sequence that takes advantage of the surplus $^1H$ population to see $^{13}C$ signals. Furthermore, it can edit the signals in order to obtain response from CH, CH$_2$, and CH$_3$ according to the settings of the sequence:

Unfortunately, it relies on the creation and manipulation of multiple quantum magnetization (the $^{13}C\pi/2$ pulse) which we cannot see or represent with vectors.

However, we’ll describe the results for different phases of $\phi$. 
DEPT (continued)

- If we plot the responses for different carbons versus the tip angle $\phi$ of the $^1$H pulse, we get:
DEPT results for different $\phi$ values

- Using pulegone as an example (real data…)

- For $\phi = \pi / 2$ (90), we edit the CH carbons

- For $\phi = 3\pi / 4$ (135), we can distinguish CH, CH$_2$, and CH$_2$ carbons.
So far we have been dealing with multiple pulses but a single dimension - that is, 1D spectra. We have seen, however, that a multiple pulse sequence can give different spectra which depend on the delay times we use.

The ‘basic’ 2D spectrum would involve repeating a multiple pulse 1D sequence with a systematic variation of the delay time $t_D$, and then plotting everything stacked. A very simple example would be varying the time before acquisition:

We now have two time domains, one that appears during the acquisition as usual, and one that originates from the variable delay.
2D NMR basics

- There is some renaming that we need to do to be more in sync with the literature:
  - The first perturbation of the system (pulse) will now be called the **preparation** of the spin system.
  - The variable $t_D$ is renamed the **evolution time**, $t_1$.
  - We have a **mixing** event, in which information from one part of the spin system is relayed to other parts.
  - Finally, we have an **acquisition period** ($t_2$) as with all 1D experiments.

- Schematically, we can draw it like this:

```
Preparation  Evolution  Mixing  Acquisition
```

- $t_1$ is the variable delay time, and $t_2$ is the normal acquisition time. We can envision having $f_1$ and $f_2$, for both frequencies...

- We’ll see that this format is basically the same for all 2D pulse sequences and experiments.
A rudimentary 2D experiment

- We’ll see how it works with the backbone of what will become the **COSY** pulse sequence. Think of this pulses, were $t_1$ is the preparation time:

- We’ll analyze it for an off-resonance ($\omega_o$) singlet for a bunch of different $t_1$ values. Starting after the first $\pi/2$ pulse:
The rudimentary 2D (continued)

- The second $\pi / 2$ pulse acts only on the $y$ axis component of the magnetization of the $<xy>$ plane.
- The $x$ axis component is not affected, but its amplitude will depend on the frequency of the line.

$$A(t_1) = A_o \cdot \cos(\omega_o \cdot t_1)$$
The rudimentary 2D (…)

- If we plot all the spectra in a **stacked plot**, we get:

Now, we have frequency data in one axis ($f_2$, which came from $t_2$), and time domain data in the other ($t_1$).

- Since the variation of the amplitude in the $t_1$ domain is also periodic, we can build a pseudo FID if we look at the points for each of the frequencies or lines in $f_2$.

- One thing that we are overlooking here is that during all the pulsing and waiting and pulsing, the signal will also be affected by $T_1$ and $T_2$ relaxation.
The rudimentary 2D (…)

- Now we have FIDs in $t_1$, so we can do a second Fourier transformation in the $t_1$ domain (the first one was in the $t_2$ domain), and obtain a two-dimensional spectrum:

- We have a cross-peak where the two lines intercept in the 2D map, in this case on the diagonal.

- If we had a real spectrum with a lot of signals it would be a royal mess. We look it from above, and draw it as a contour plot - we chop all the peaks with planes at different heights.

- Each slice is color-coded depending on the height of the peak.
The same with some real data

- This is data from a COSY of pulegone...

- time - time
- time - frequency
- frequency - frequency
The same with some real data

- Now the *contour-plot* showing all the *cross-peaks*:

  ![Contour plot](image)

- OK, were the heck did all the *off-diagonal* peaks came from, and what do they mean?

- I’ll do the best I can to explain it, but again, there will be several black-box events. We really need a mathematical description to explain COSY rigorously.
Homonuclear correlation - COSY

- **COSY** stands for **COrrelation SpectroscopY**, and for this particular case in which we are dealing with homonuclear couplings, **homonuclear correlation spectroscopy**.

- In our development of the 2D idea we considered an isolated spin not coupled to any other spin. Obviously, this is not really useful.

- What COSY is good for is to tell which spin is connected to which other spin. The off-diagonal peaks are this, and they indicate that those two peaks in the diagonal are coupled.

- With this basic idea we’ll try to see the effect of the COSY \(90_y - t_1 - 90_y - t_1\) pulse sequence on a pair of coupled spins. If we recall the 2 spin-system energy diagram:

![2D diagram](image)

- We see that if we are looking at \(I\) and apply both \(\pi/2\) pulses, (a pseudo \(\pi\) pulse) we will invert some of the population of spin \(S\), and this will have an effect on \(I\) (polarization transfer).
Since the \( I \) to \( S \) or \( S \) to \( I \) polarization transfers are the same, we’ll explain it for \( I \) to \( S \) and assume we get the same for \( S \) to \( I \). We first perturb \( I \) and analyze what happens to \( S \).

After the first \( \pi / 2 \), we have the two \( I \) vectors in the \( x \) axis, one moving at \( \omega_I + J / 2 \) and the other at \( \omega_I - J / 2 \). The effect of the second pulse is that it will put the components of the magnetization aligned with \( y \) on the \(-z\) axis, which means a partial inversion of the \( I \) populations.

For \( t_1 = 0 \), we have complete inversion of the \( I \) spins (it is a \( \pi \) pulse and the signal intensity of \( S \) does not change. For all other times we will have a change on the \( S \) intensity that depends periodically on the resonance frequency of \( I \).

The variation of the population inversion for \( I \) depends on the cosine (or sine) of its resonance frequency. Considering that we are on-resonance with one of the lines and if \( t_1 = 1 / 4 J \):
Homonuclear correlation (…)

• If we do it really general (nothing on-resonance), we would come to this relationship for the change of the $S$ signal (after the $\pi/2$ pulse) as a function of the $I$ resonance frequency and $J_{IS}$ coupling:

$$A_S(t_1, t_2) = A_0 \times \sin(\omega_I \times t_1) \times \sin(J_{IS} \times t_1) \times \sin(\omega_S \times t_2) \times \sin(J_{IS} \times t_2)$$

• After Fourier transformation on $t_1$ and $t_2$, and considering also the $I$ spin, we get:

• This is the typical pattern for a doublet in a phase-sensitive COSY. The sines make the signals dispersive in $f_1$ and $f_2$. 
Summary of COSY

- The 2D spectrum has cross peaks on the diagonal as well as off the diagonal.

- Everything is doubled, because we have I to S as well as S to I polarization transfer.

- Exactly on the diagonal we see the normal 1D spectrum. Off the diagonal we see all connected or coupled transitions.