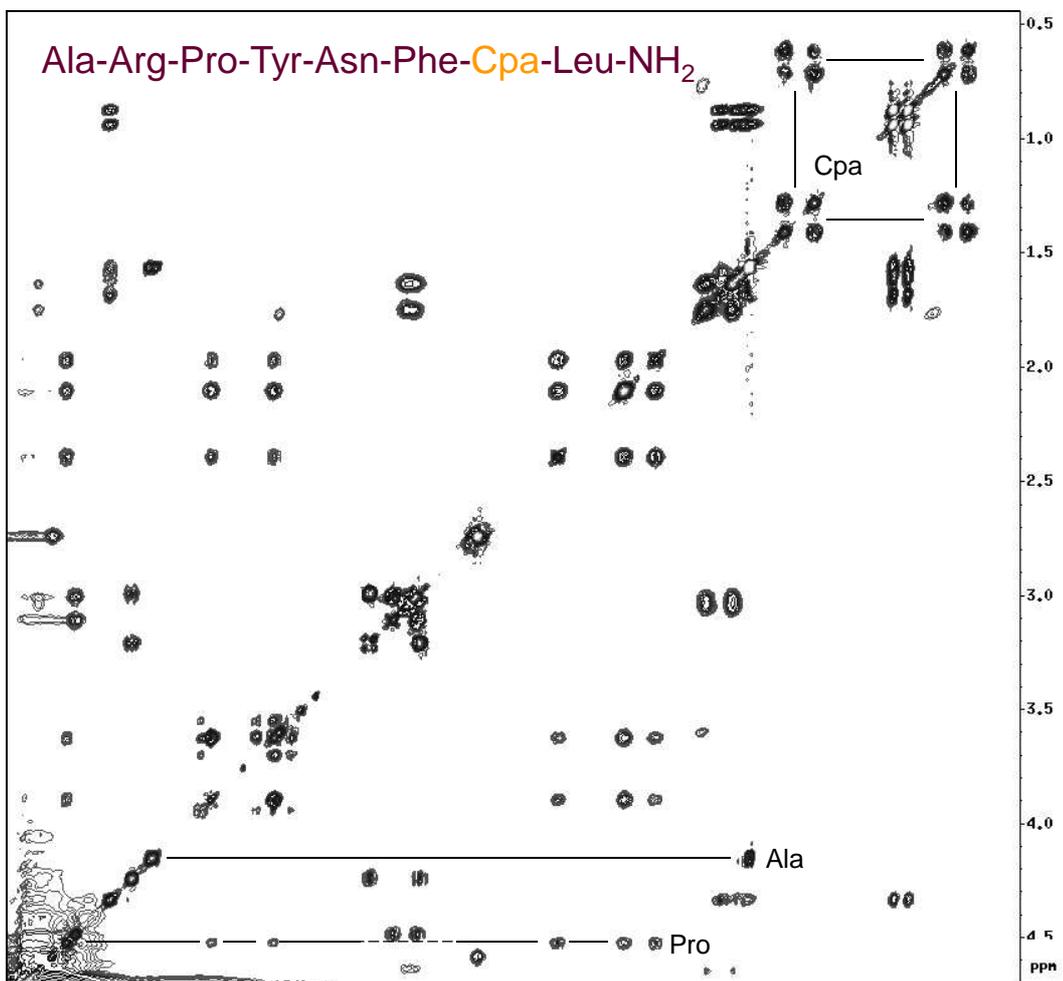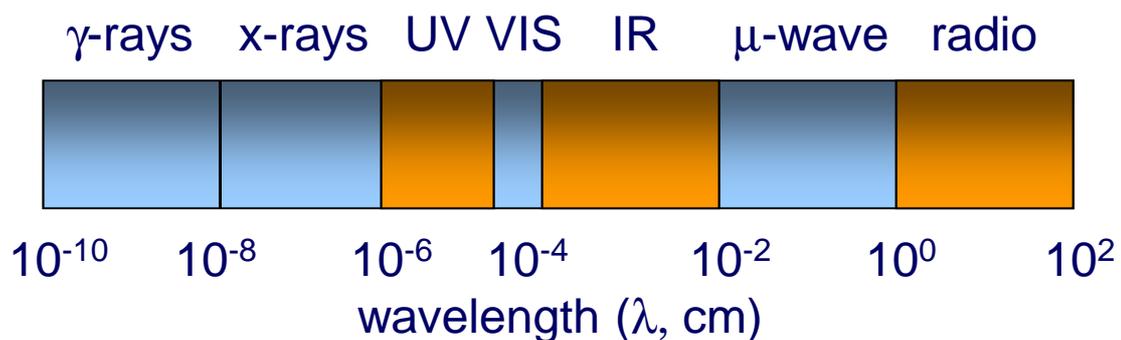# Applied Spectroscopy

Ala-Arg-Pro-Tyr-Asn-Phe-Cpa-Leu-NH$_2$

Cpa

Ala

Pro

Guillermo Moyna

What is Spectroscopy?

- Without going into latin or greek, spectroscopy is the study of the interactions between *light* and *matter*.

- Here *light* refers to any sort of electromagnetic radiation, such as visible light, UV, IR, and radiowaves.

- Depending on the *frequency* or *wavelength* of the radiation involved we will have different types of interactions with matter (molecules).

- The following chart shows the ranges (wavelengths), for different types of spectroscopies.
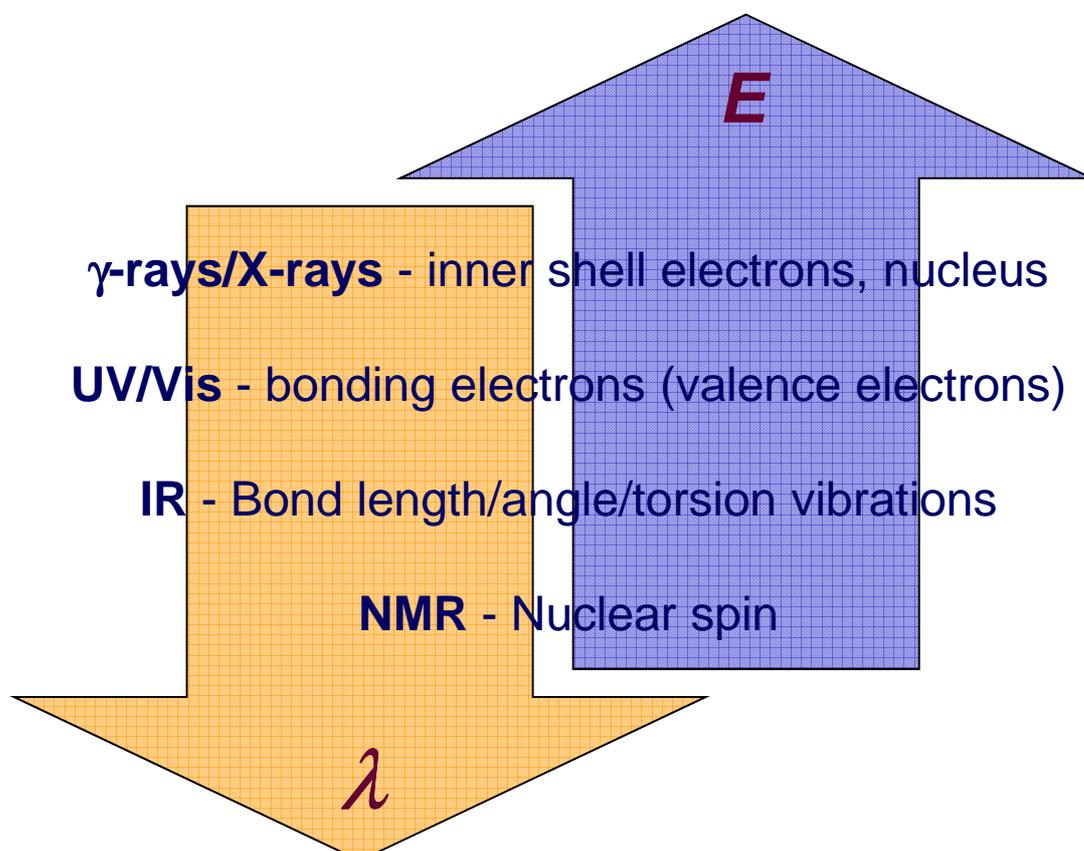
γ-rays	x-rays	UV VIS	IR	μ-wave	radio

$$10^{-10} \quad 10^{-8} \quad 10^{-6} \quad 10^{-4} \quad 10^{-2} \quad 10^{0} \quad 10^{2}$$

wavelength (λ, cm)

- As you know, wavelength and frequency are inversely proportional, so higher frequencies mean shorter wavelength.

Some background

- Also to remember throughout the course is the relationship between energy and frequency. The two are related by one of the fundamental equations of quantum mechanics:

$$\Delta E = h\,\nu$$

- Therefore, the higher the frequency, the higher the energy. In addition, and as said before, depending on the frequency and wavelength we'll have different interactions with matter (molecules). The following is a brief list of these:

E

γ-rays/X-rays - inner shell electrons, nucleus

UV/Vis - bonding electrons (valence electrons)

IR - Bond length/angle/torsion vibrations

NMR - Nuclear spin

λ

Why bother learning NMR?

- Structural (chemical) elucidation

    - Natural product chemistry.

    - Organic chemistry. Analytical tool of choice for synthetic chemists.

- Study of dynamic processes

    - Reaction kinetics.

    - Study of equilibrium (chemical or structural).

- Structural (three-dimensional) studies

    - Proteins.

    - DNA/RNA. Protein complexes with DNA/RNA.

    - Polysaccharides

- Drug design

    - Structure Activity Relationships (SAR) by NMR

- Medicine - Magnetic Resonance Imaging (MRI)

- Finally, it's the biggest, meanest, most expensive piece of equipment you'll see in your career at USP, and this is a great time to get your hands on it...

The gory details

- Absorption (or emission) spectroscopy, as IR or UV. Detects the absorption of radiofrequencies (electromagnetic radiation) by certain nuclei in a molecule.

- Unfortunately, some quantum mechanics are needed to understand it (a lot to really understand it…).

- As opposed to the atomic mass or charge, the spin has no macroscopic equivalent. It exists, period...

- Only nuclei with **spin number** (I) $\neq$ **0** can absorb/emit electromagnetic radiation.

    - Even atomic mass & number $\Rightarrow I = 0$ (^{12}C, ^{16}O)
    - Even atomic mass & odd number $\Rightarrow I = $ **whole integer** (^{14}N, ^{2}H, ^{10}B)
    - Odd atomic mass $\Rightarrow I = $ **half integer** (^{1}H, ^{13}C, ^{15}N, ^{31}P)

- The **spin states** of the nucleus (m) are **quantized**:

$$m = I, (I - 1), (I - 2), \ldots , -I$$

- Properly, m is called the **magnetic quantum number**.

Background (continued)

- For ^{1}H, ^{13}C, ^{15}N, ^{31}P (biologically relevant nuclei with $I = ^1/_2$):

$$m = {}^1/_2, -{}^1/_2$$

- This means that only two states (energy levels) can be taken by these nuclei.

- Another important parameter of each particular nuclei is the **magnetic moment** (μ), which can be expressed as:

$$\vec{\mu} = \gamma I\, h\, /\, 2\pi$$

- It is a vector quantity that gives the direction and magnitude (or strength) of the 'nuclear magnet'

  - **h** is the Planck constant

  - γ is the **gyromagnetic ratio**, and it depends on the nature of each nuclei.

- Different nuclei have different magnetic moments.

- The energy of a spin in a magnetic field will depend on the magnetic field, which we call **B$_o$**, and μ:

Magnetic energy and populations

- When the B_o field is applied, spins have to possible energy limits. In one we are in favor of the field, and in the other one we are against it. The energy is the dot product of the corresponding vectors:

$$E = - \mu \cdot B_o$$

B_o μ B_o μ

$$E_\alpha = - \gamma h \, B_o / 4\pi \qquad E_\beta = \gamma h \, B_o / 4\pi$$

- The energy difference of the two levels, α and β, is:

$$\Delta E = \gamma h \, B_o / 2\pi$$

- The bigger B_o, the larger the energy difference. Also, the *population ratio* between the two levels depends on ΔE, and we can calculate it as a Boltzmman distribution.

- The ΔE for ^1H's at 400 MHz (B_o = 9.4 T) is 4 x 10^{-5} Kcal / mol.

$$N_\alpha / N_\beta = e^{\Delta E / RT}$$

- The N_α / N_β ratio is only **1.000064**.

- In one million spins we have a difference of just 64: NMR is very insensitive when compared to UV or IR...

Magnetic energy, sensitivity, and frequency

• Nuclei with larger γ will absorb/emmit more energy, and will therefore be more sensitive. Sensitivity is proportional to μ, to N_α / N_β, and to the 'magnetic flux of the coil,' all of which depend on γ. In sum, sensitivity is proportional to γ^3.
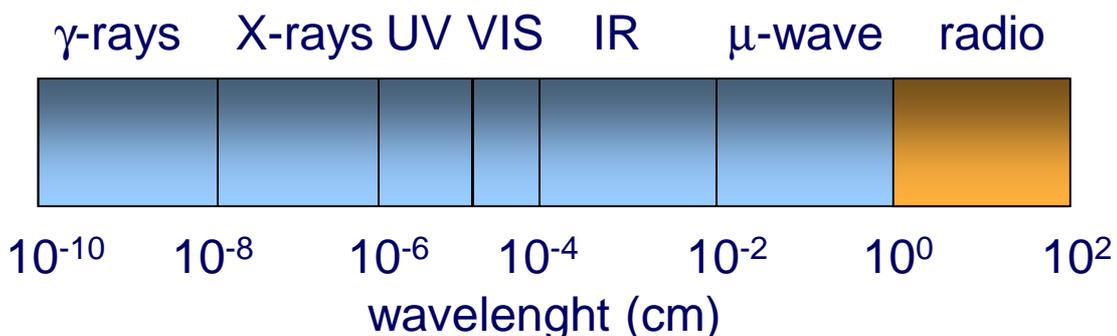
$$\gamma^{13}C = 6,728 \text{ rad / G}$$

$$\gamma^1 H = 26,753 \text{ rad / G}$$

• ^{1}H is ~ 64 times more sensitive than ^{13}C only due to γ.

• If we also take into account the natural abundance, ^{13}C (~1%) ends up being 6400 less sensitive than ^{1}H...

• Energy is related to frequency (quantum mechanics...):

$$\Delta E = h\, \nu_o$$

$$\Delta E = \gamma h\, B_o / 2\pi$$

$$\nu_o = \gamma B_o / 2\pi$$

• For ^{1}H's in normal magnets (2.35 - 18.6 T), the frequencies are between 100 and 800 MHz. For ^{13}C, 1/4 of this…

γ-rays   X-rays UV VIS   IR μ-wave   radio

10^{-10} 10^{-8} 10^{-6} 10^{-4} 10^{-2} 10^{0} 10^{2}

wavelenght (cm)

Precession

- To explain everything in NMR we have to refer to rotation, and Hz are not the best units to do this. We define the **precession** or **Larmor** frequency, ω_o, in radians:

$$\omega_o = 2\pi\nu_o \quad \Rightarrow \quad \boxed{\omega_o = \gamma B_o \text{ (radians)}}$$

- With what precession is ω_o related to? One thing we left out of the mix was the **angular momentum**, L, asociated will all nuclei (magnetic or not).

- We can think of nuclei as small magnetized tops that spin on their axis:

$$\vec{\mu}$$
$$\vec{L}$$
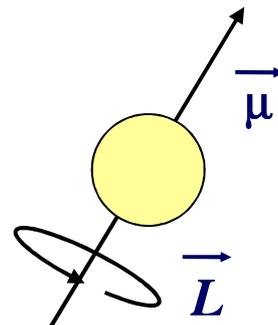
- After turning the magnet on we'll have two forces acting on the spins. One that tries to turn them towards B_o, and the other that wants to maintain their angular momentum. The net result is that the nuclei spins like a top:

$$\omega_o$$
$$B_o$$
$$\vec{\mu}$$
$$\vec{L}$$
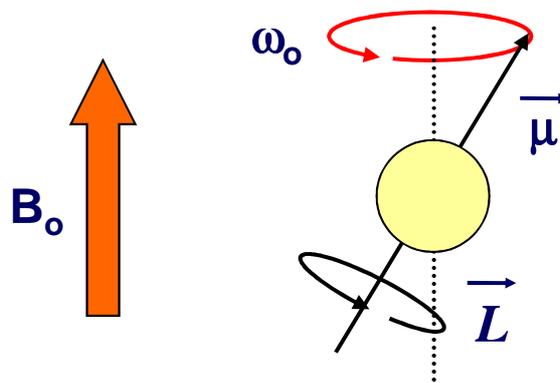
Precession (continued)

- Now we have to go against a concept used by lots of people to explain NMR: Spins **won't align** with B_o, no matter what their intiial orientation was. Spins pointing 'up' and 'down' **don't exist!**

- Spins will precess at the angle they were when we turned on the magnetic field B_o:
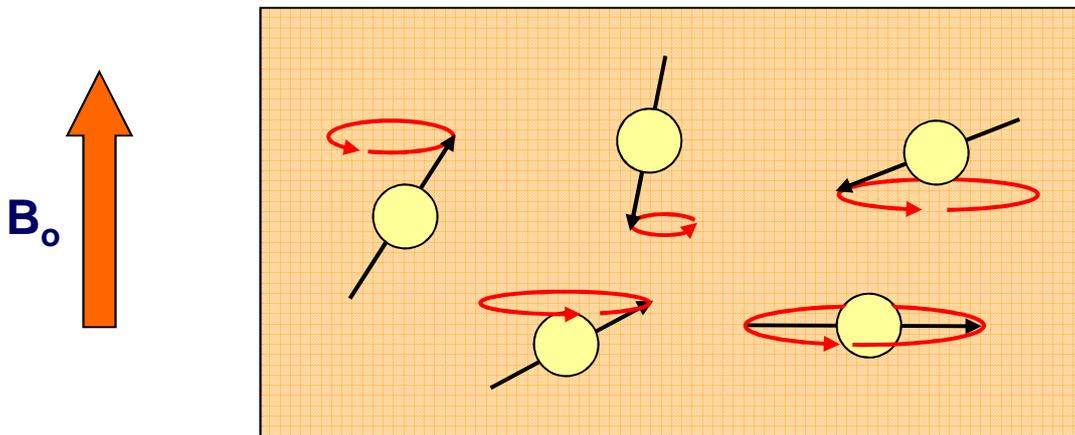
- There are several magnetic fields acting on the spins. One is B_o, which is constant in time and generates the precession at ω_o. The others are fluctuating due to the molecular anisotropy and its environment, and make the spins 'try' all the possible orientations with respect to B_o in a certain ammount of time.

- Orientations in favor of B_o will have lower magnetic energy, and will be slightly favored. After a certain time (the **longitudinal relaxation**, more later), a **net magnetizacion** (M_o) pointing in the direction of B_o will develop.
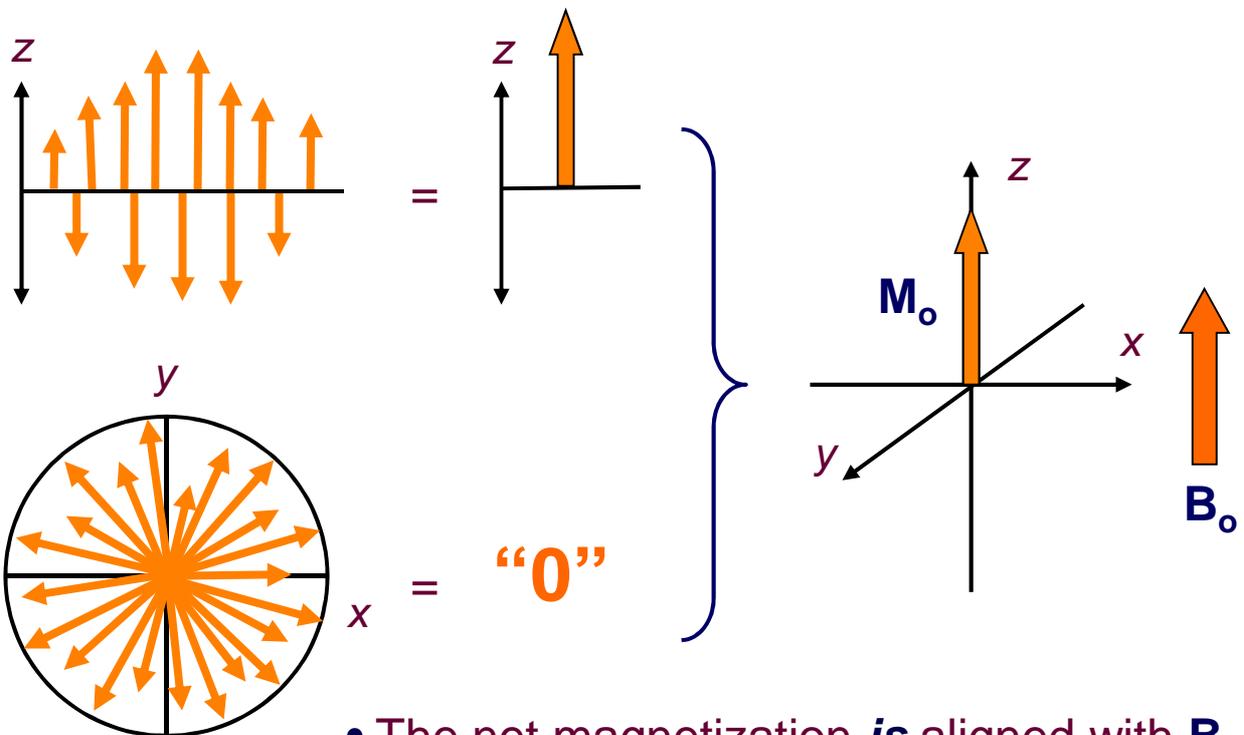
Net magnetization

- Where does the net magnetization comes from? In order to figure it out we translate all the spins to the origin of the coordinate sysmem. We'll see something like this:
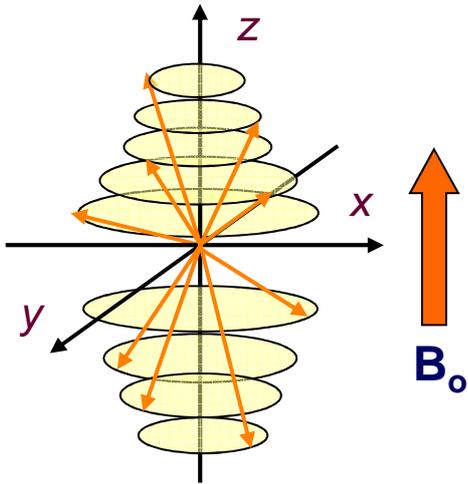
- We'll have a slight excess of spins aligned with B_o, but at any angle with respect to **z**. The distribution is proportional to N_α / N_β.

- If we decompose the μ vectors in **z** and **<xy>**, we get:

- The net magnetization *is* aligned with B_o, and this is what we use in NMR.

NMR excitation

- So far nothing happened. We have a little tube spinning in the magnet. To see something we have to move the system away from equilibrium. That is, we have to **perturb its populations**.

- We need the system to absorb energy. The energy source is an oscillating electromagnetic radiation generated by an alternating current:
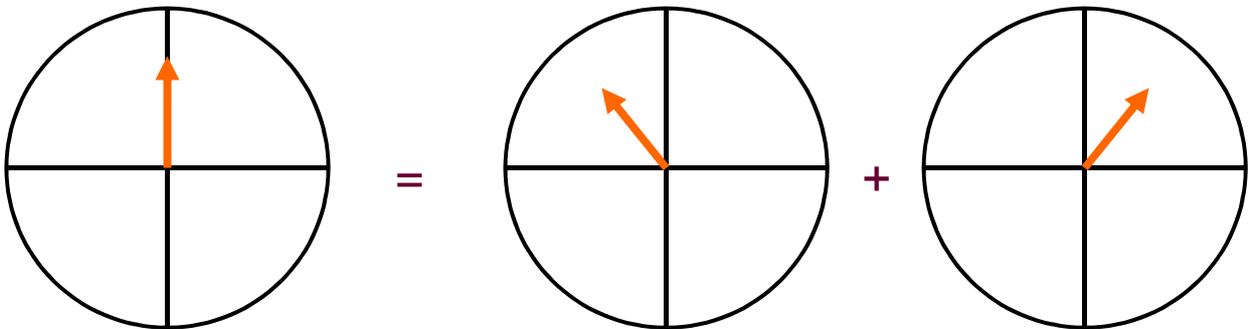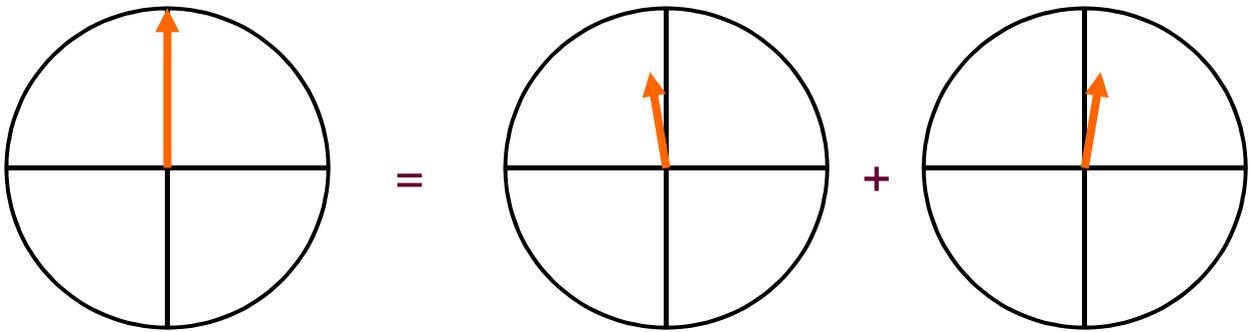
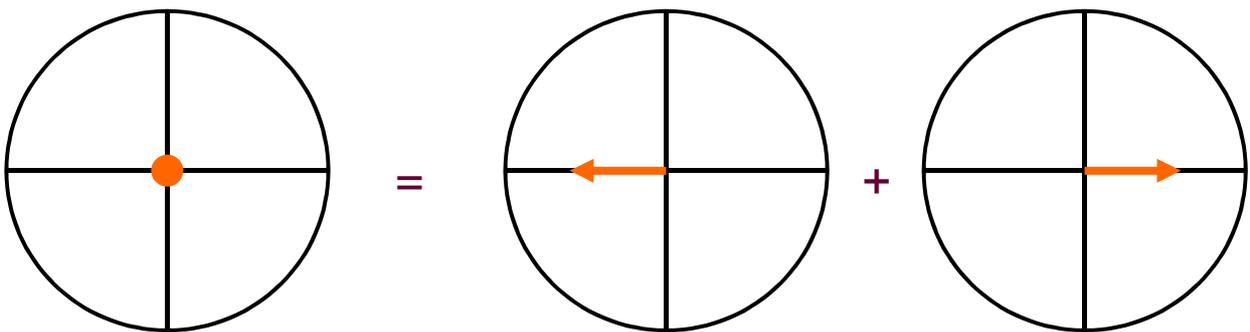$$B_1 = C * cos (\omega_o t)$$
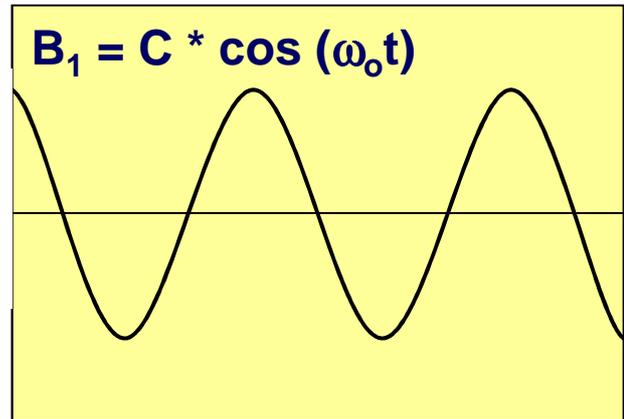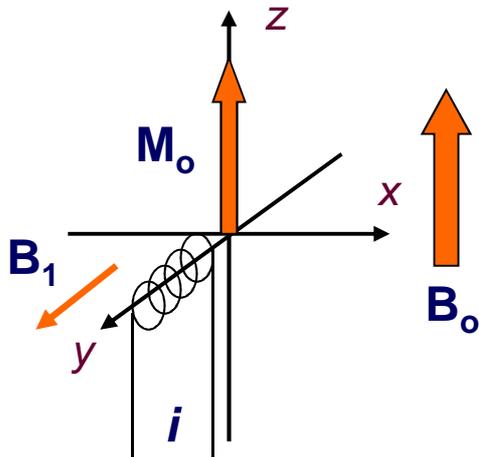
Transmitter coil (y)

- How is that something that has a linear variation can be thought as circular field? A linear variation in **y** is the linear combination of two counter-rotating circular fields:

$-\omega_o$ $+\omega_o$
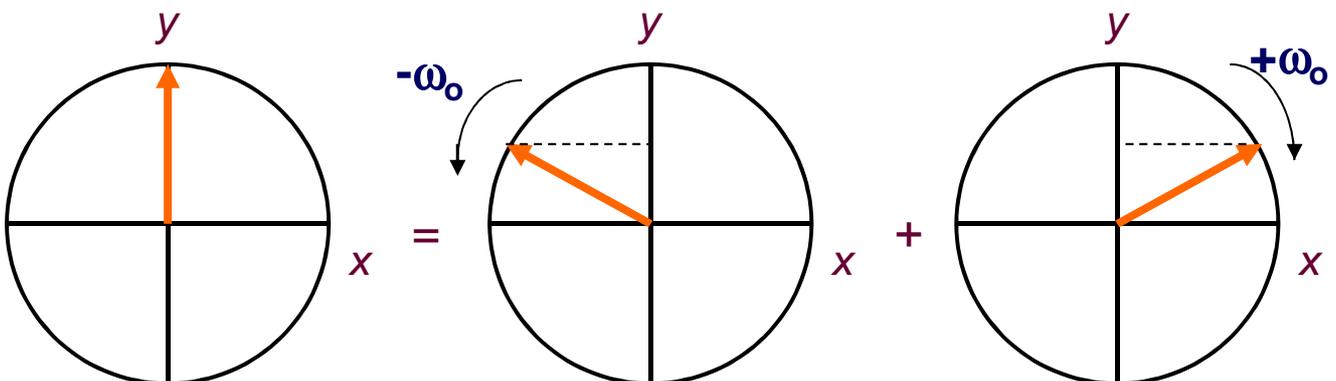
For part of the period of oscillation:


- We go through zero and then it repeats…

- Only the one vector that rotates at $+\omega_o$ (in the same direction of the precession of M_o) interacts with the bulk magnetization. $-\omega_o$ is the one normally used, but it's just a convention...

Now we throw M_o on the mix...

- When the frequency of the alternating current is ω_o, the frequency of the right vector of B_1 is ω_o and we achieve a *resonant condition*. The alternating magnetic field and all the μ's interact, there's a torque generated, and they rotate. Since they all rotate the same ammount, the macroscopic effect is that M_o rotates around the **y** axis (in this case...), and we generate *transverse magnetization* (M_{xy}):
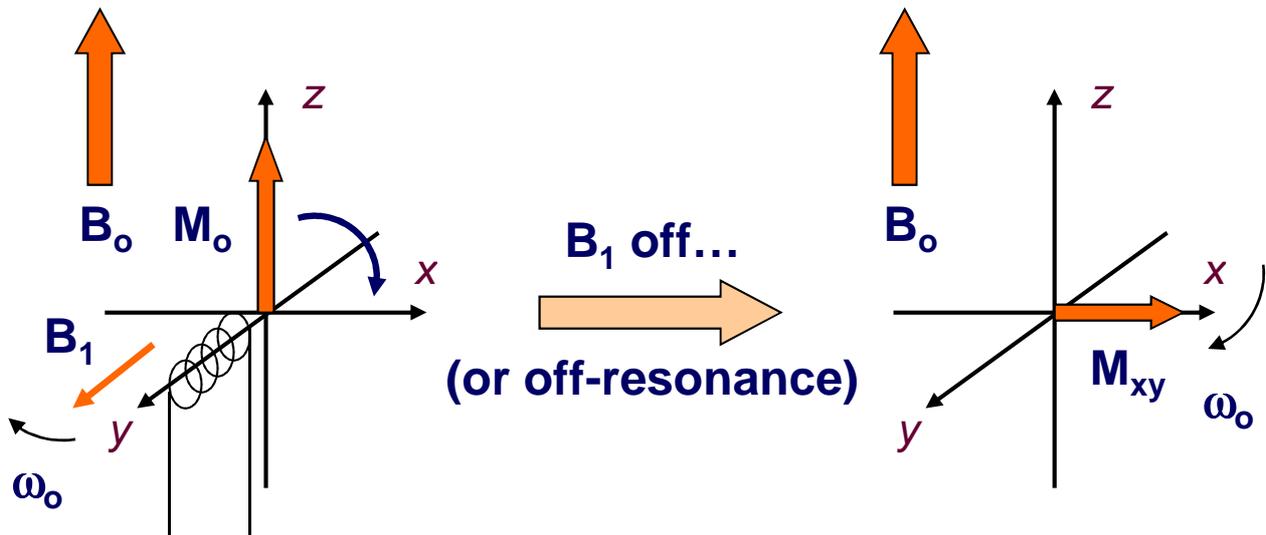
- Since we altered the population ratio between energy levels (i.e., N_α / N_β), the system absorbed energy and we altered the equilibrium...

- Since the individual spins keep precessing under the effect of B_o, the transverse magnetization M_{xy} will rotate around the **z** axis at the precession frequency, ω_o.

Detection of M_{xy} and return to equilibrium

- In the absence of the external B_1, M_{xy} will try to go back to the **z** axis (M_o, equilibrium) by restoring the original N_α / N_β. distributiuon. We'll see the physics that rule this phenomenon (*relaxation*) later.

- M_{xy} returns to the **z** axis precessing on the **\<xy\>** plane (to damned hard to draw...):

equilibrium...
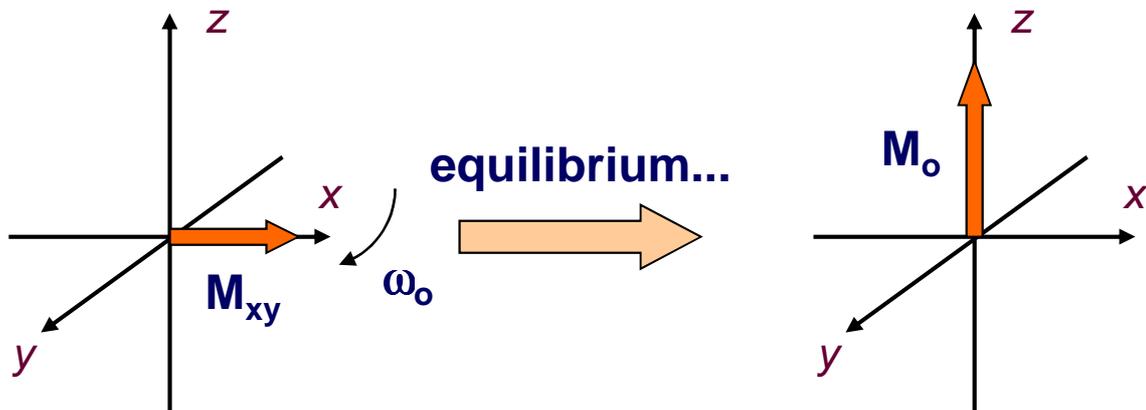
- The oscillation of M_{xy} generates a fluctuating magnetic field which can be used to generate a current in a coil:

Receiver coil (x) $\Rightarrow$ **NMR signal**