

Princípios de RMN (Teoria)

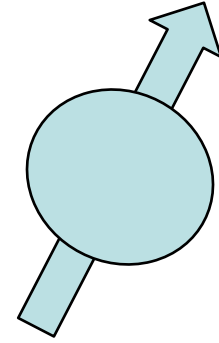
Roberto K. Salinas

IQUSP

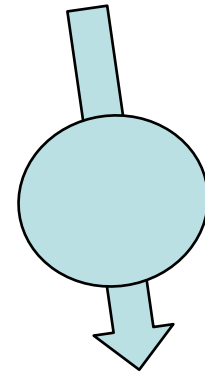
Spin nuclear

Número quântico de spin = I

Multiplicidade: $S = 2I + 1$



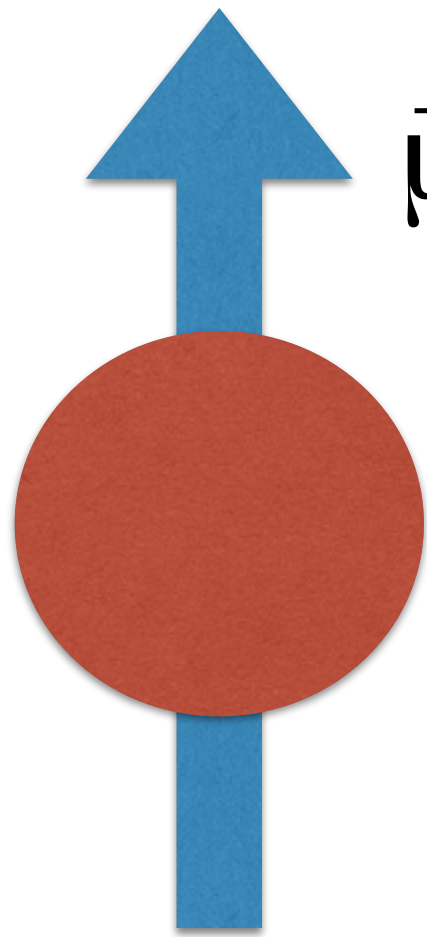
$$m_z = +\frac{1}{2}$$



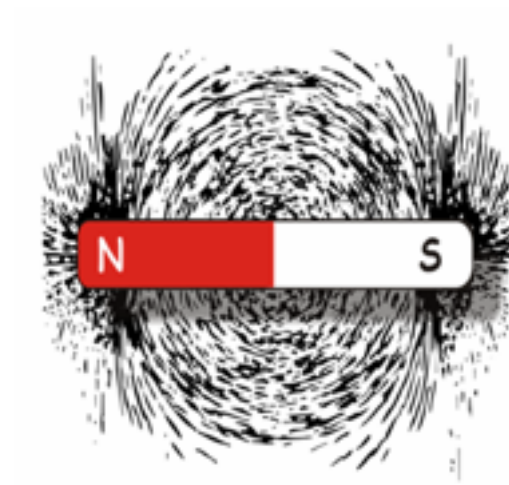
$$m_z = -\frac{1}{2}$$

Hidrogênio (^1H): $I = 1/2$

Magnetismo nuclear

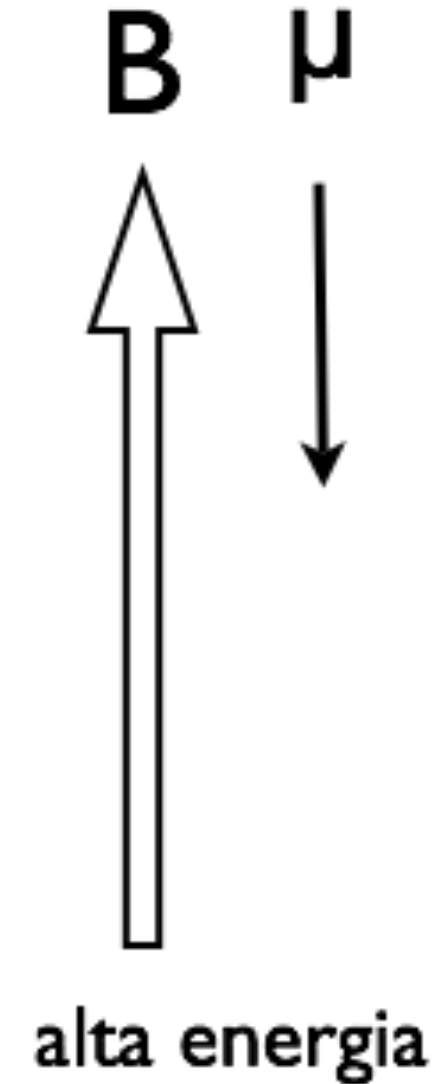
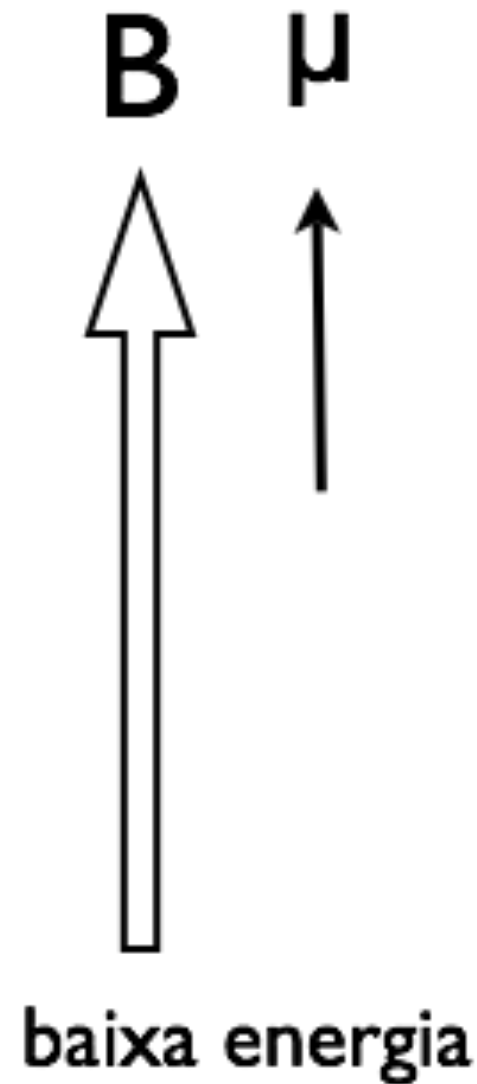


$$\vec{\mu} = \gamma \vec{I}$$



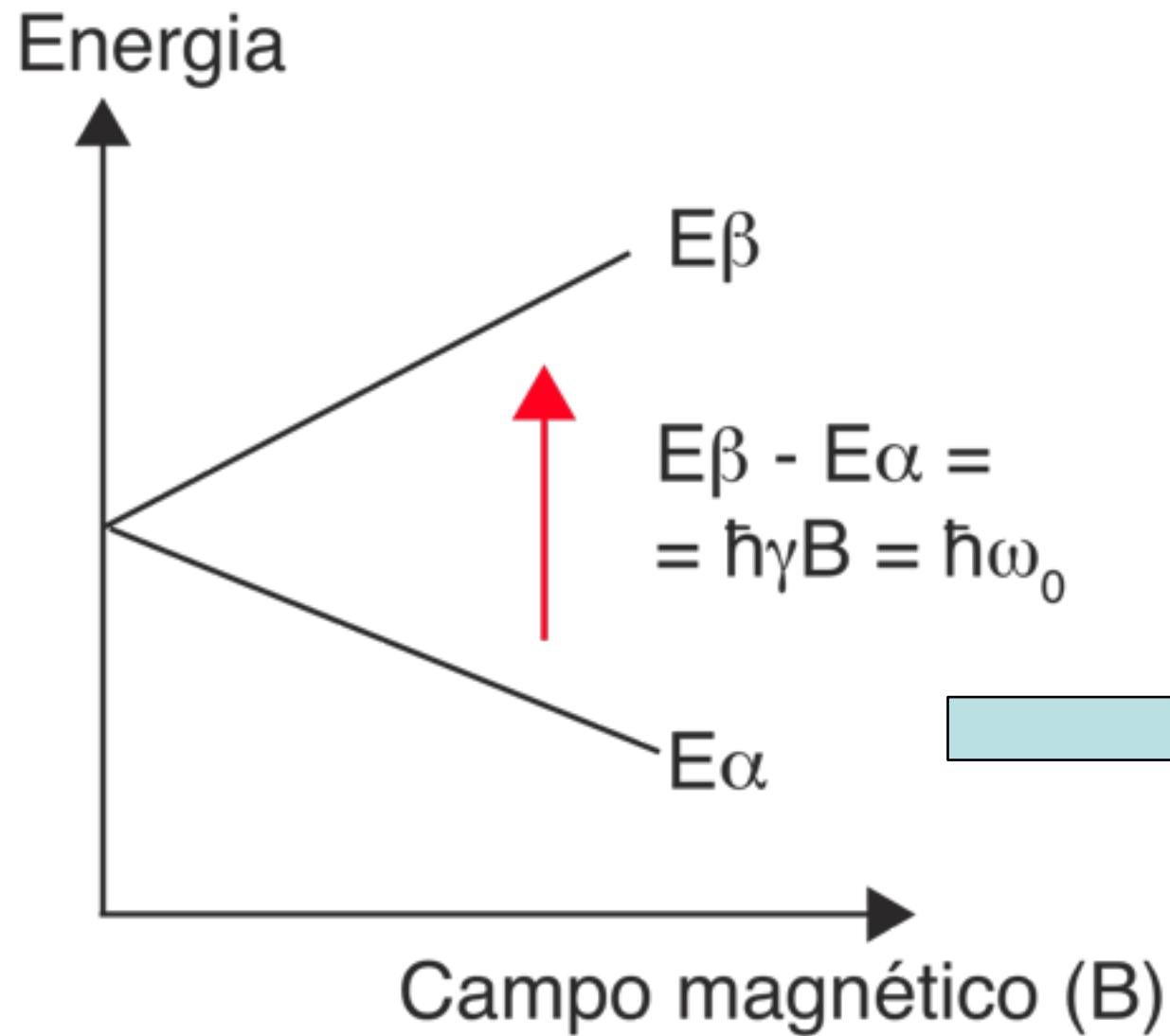
O momento magnético do núcleo interage com um campo magnético externo

$$E = - \vec{\mu} \cdot \vec{B}$$

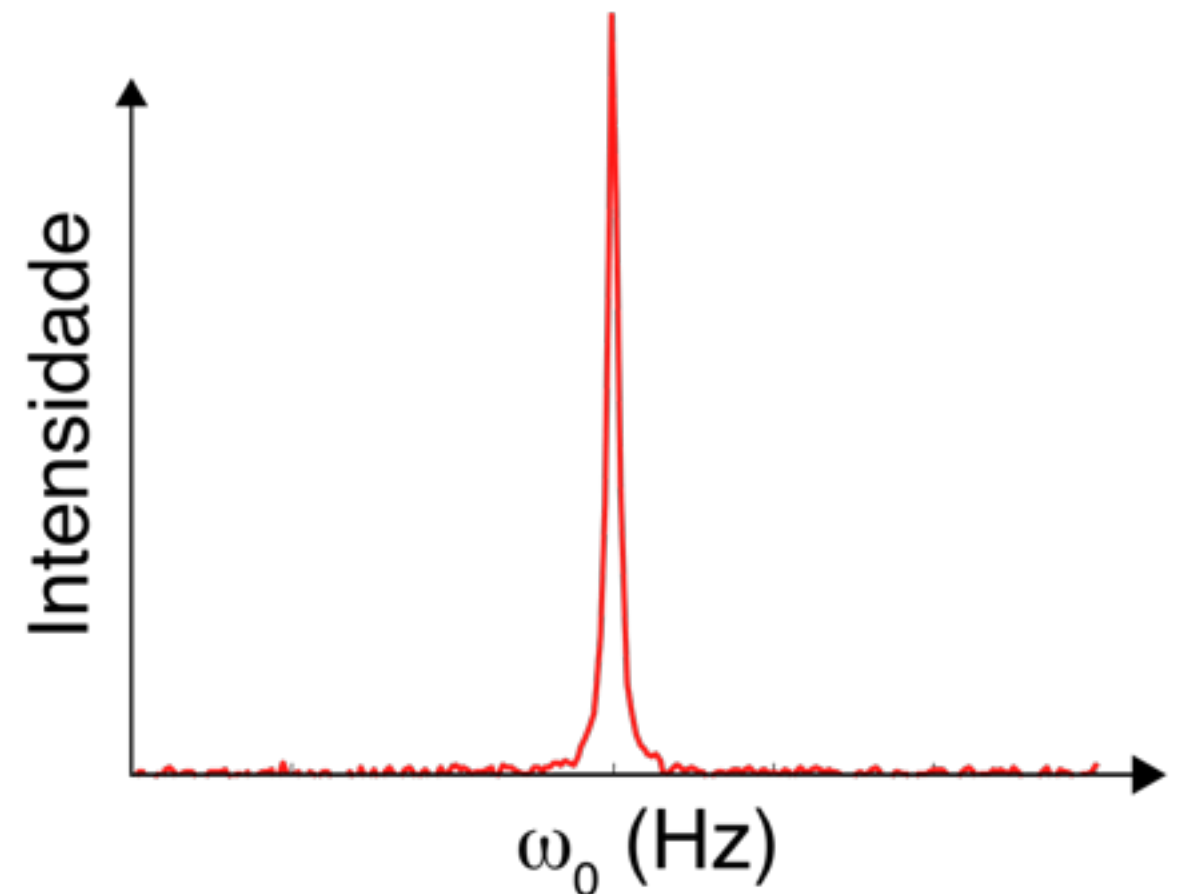


RMN detecta transições entre estados do spin nuclear com diferentes energias na presença de um campo magnético estático

ω_0 = Frequência de Larmor

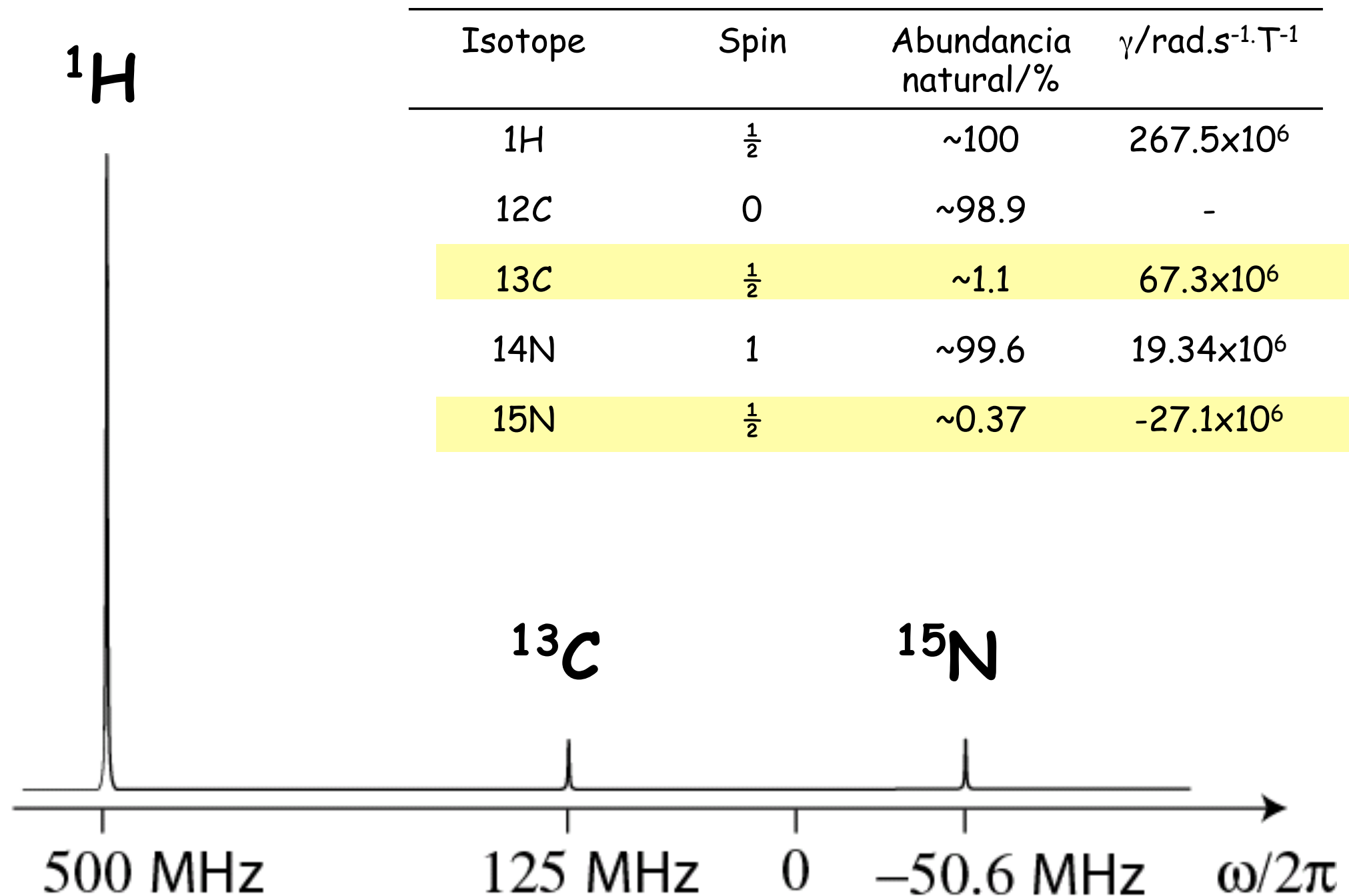


Sistema de spin 1/2

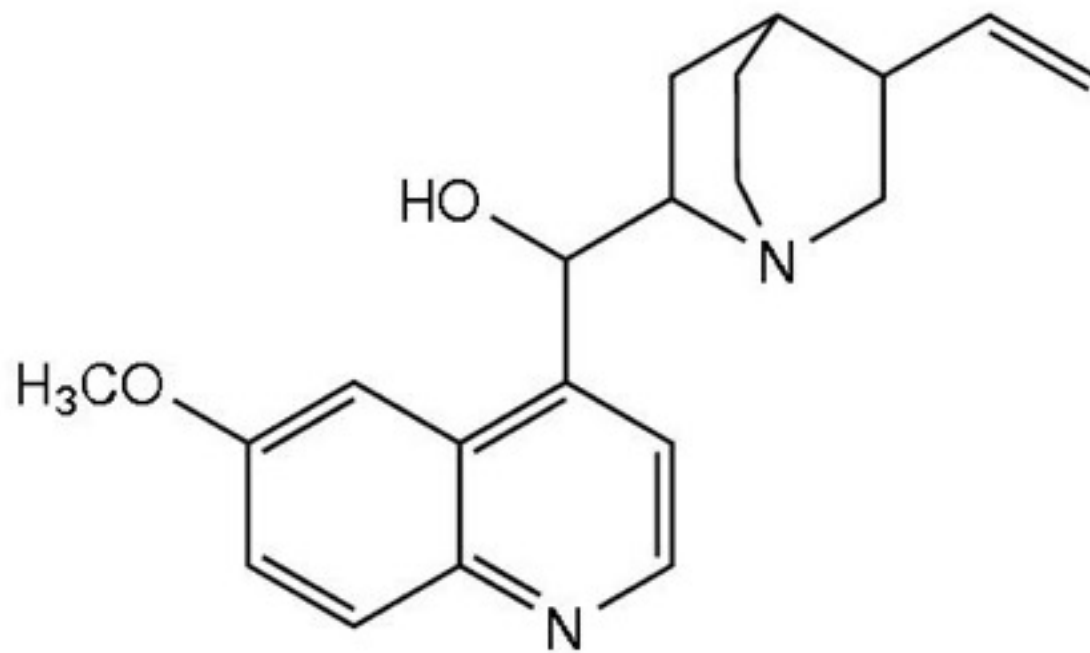


$$\text{ppm} = (\omega_0 - \omega_{\text{ref}}) / \omega_{\text{ref}}$$

A frequência de Larmor (ω_0) depende do campo e da razão giromagnética do núcleo

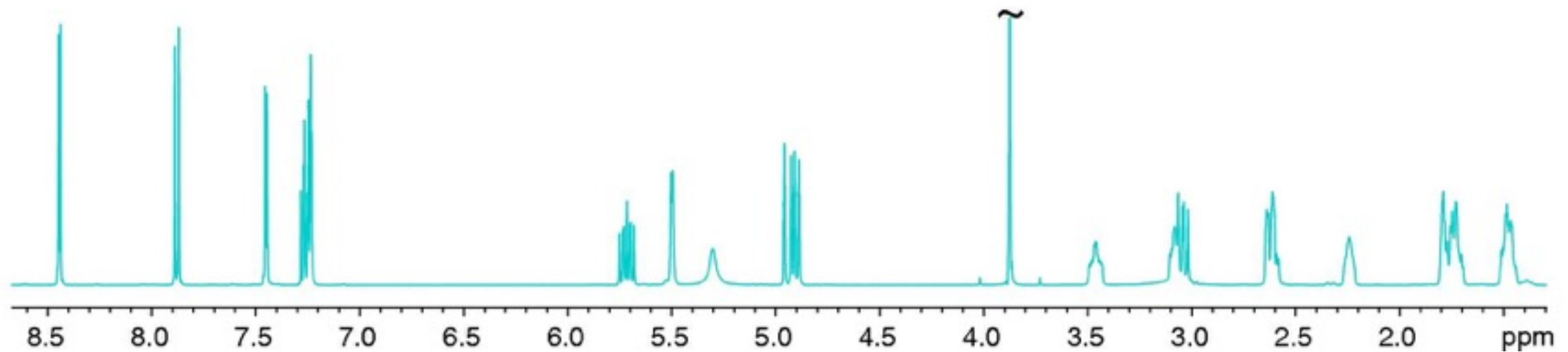


Deslocamento químico: a frequência de ressonância do spin nuclear depende da densidade eletrônica ao redor do núcleo



More conveniently expressed as **parts per million (ppm)** by comparison to a reference frequency:

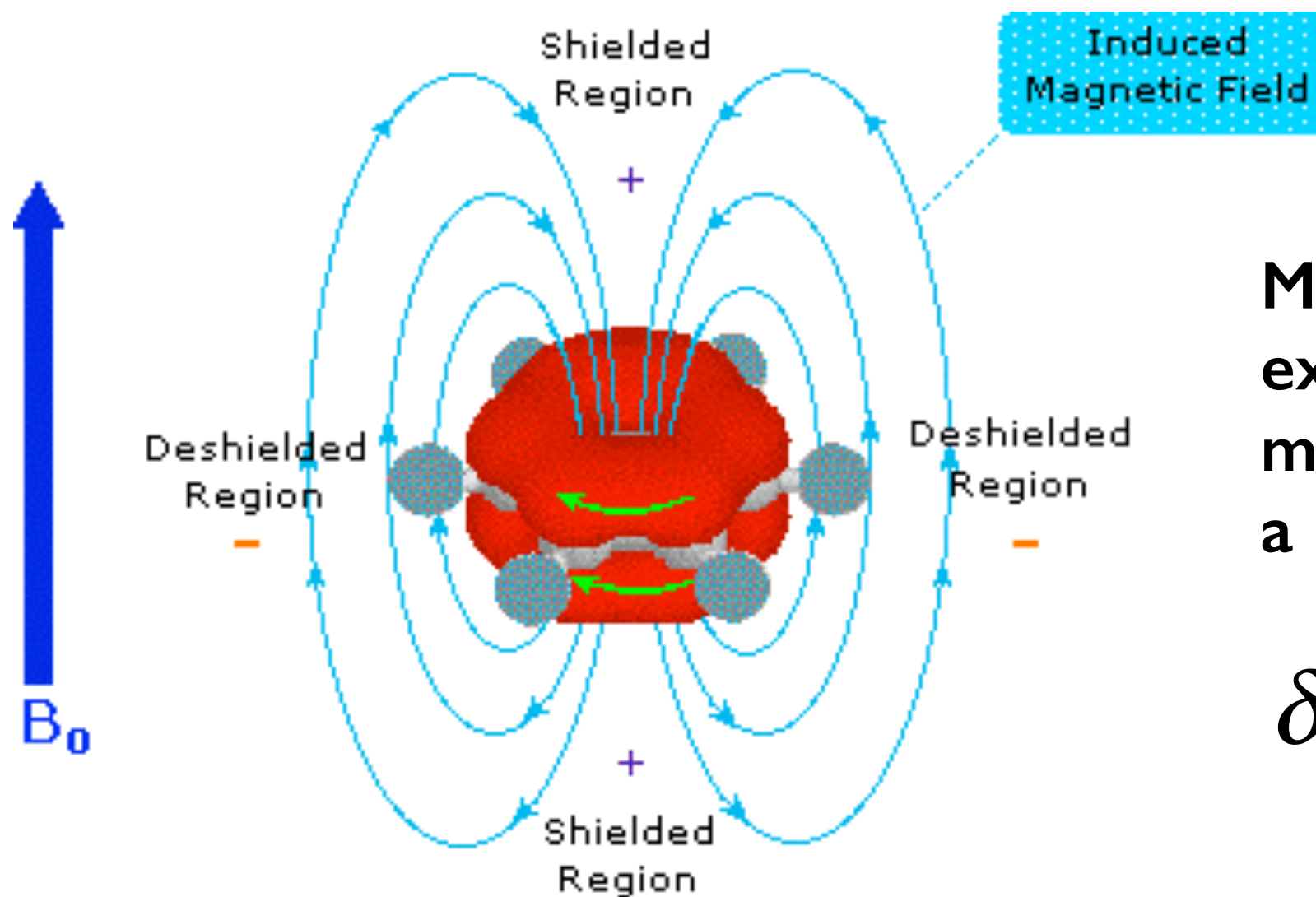
$$\delta = 10^6 \frac{\nu - \nu_{ref}}{\nu_{ref}}$$



Chemical shift

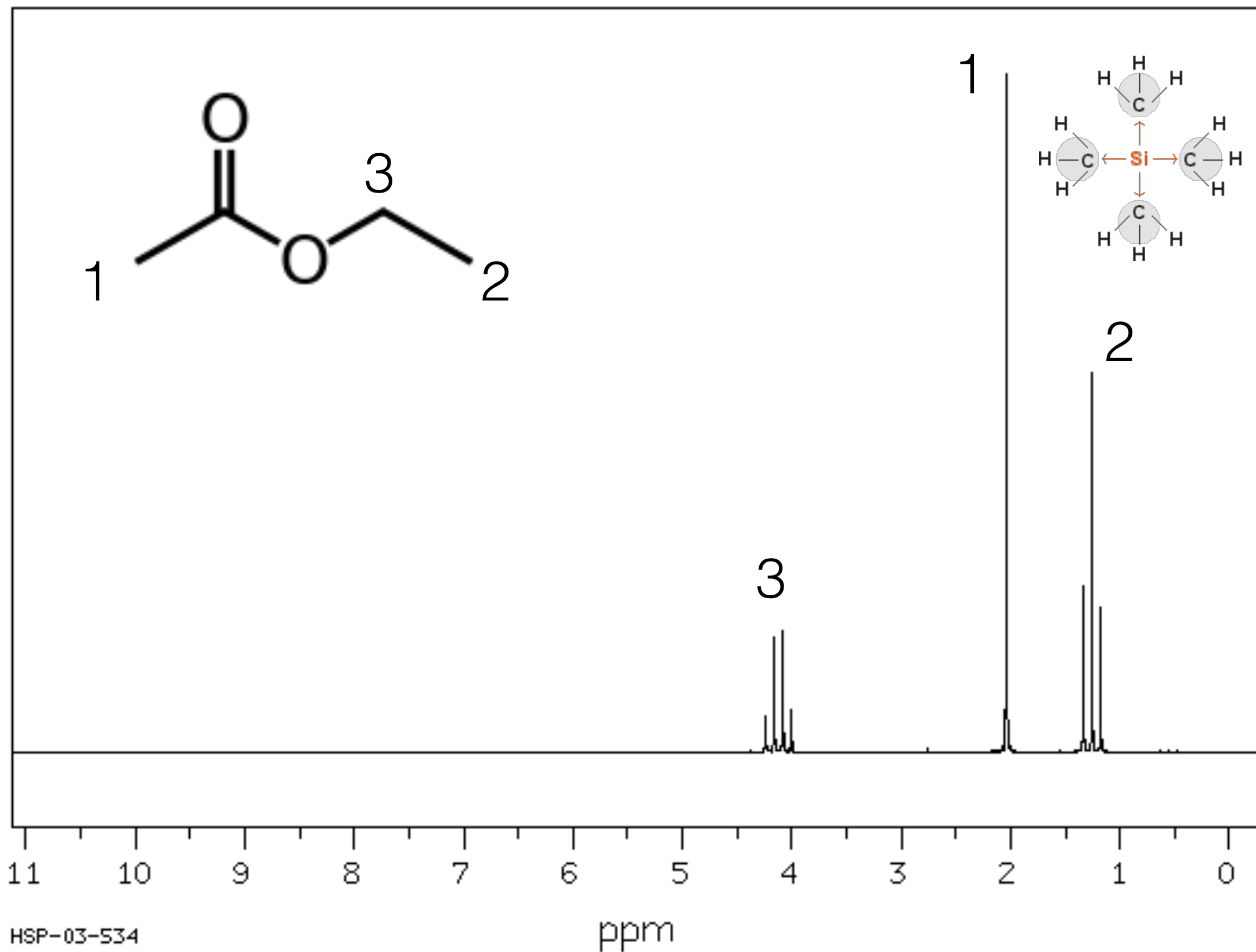
$$\nu = \frac{\gamma B_0}{2\pi} (1 - \sigma)$$

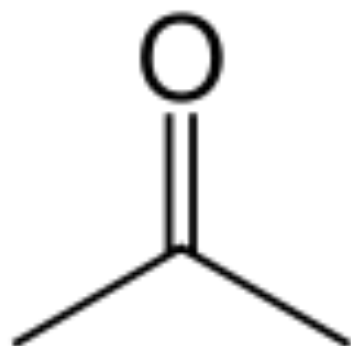
shielding constant



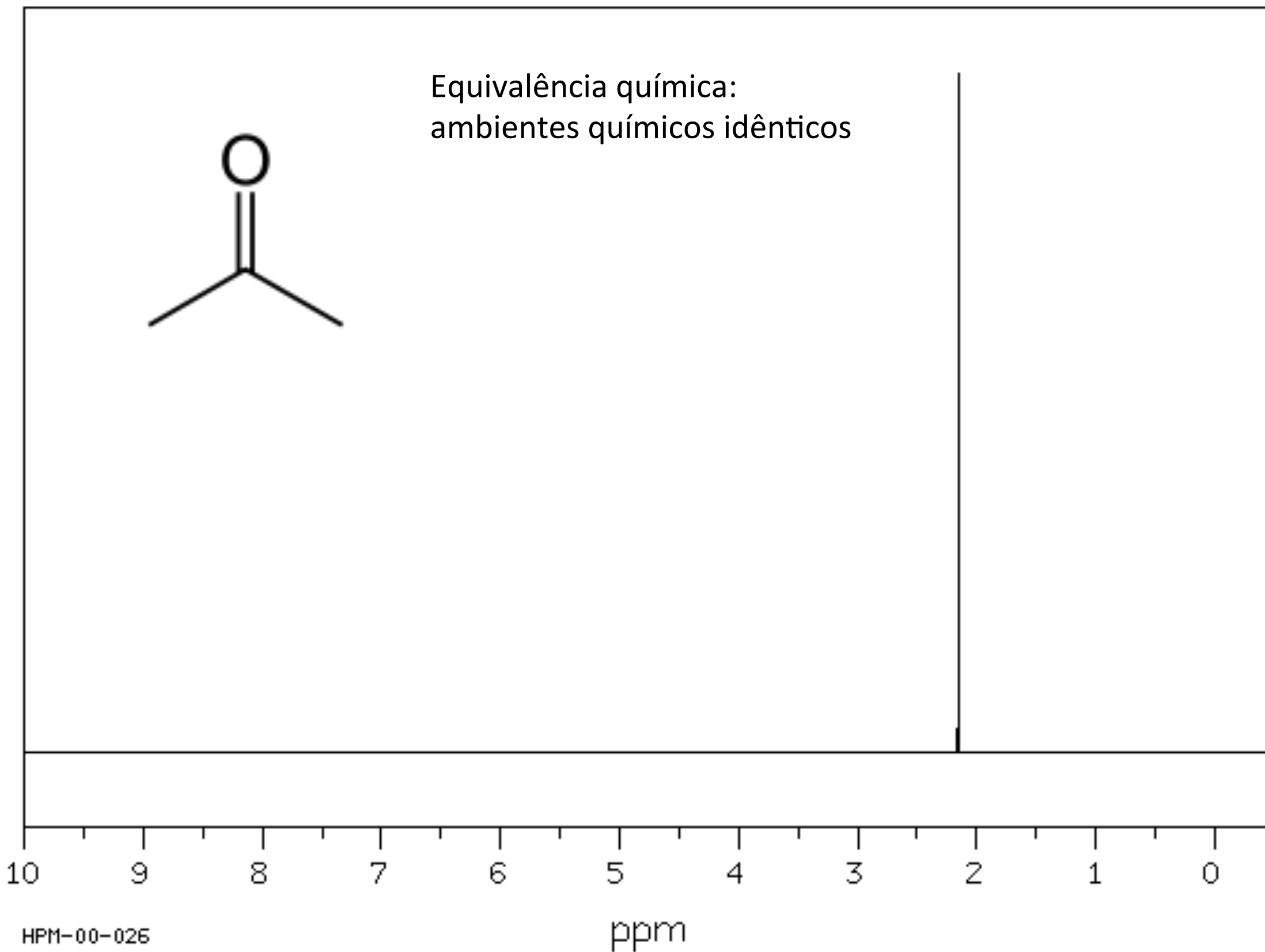
More conveniently expressed as part per million by comparison to a reference frequency:

$$\delta = 10^6 \frac{\nu - \nu_{ref}}{\nu_{ref}}$$

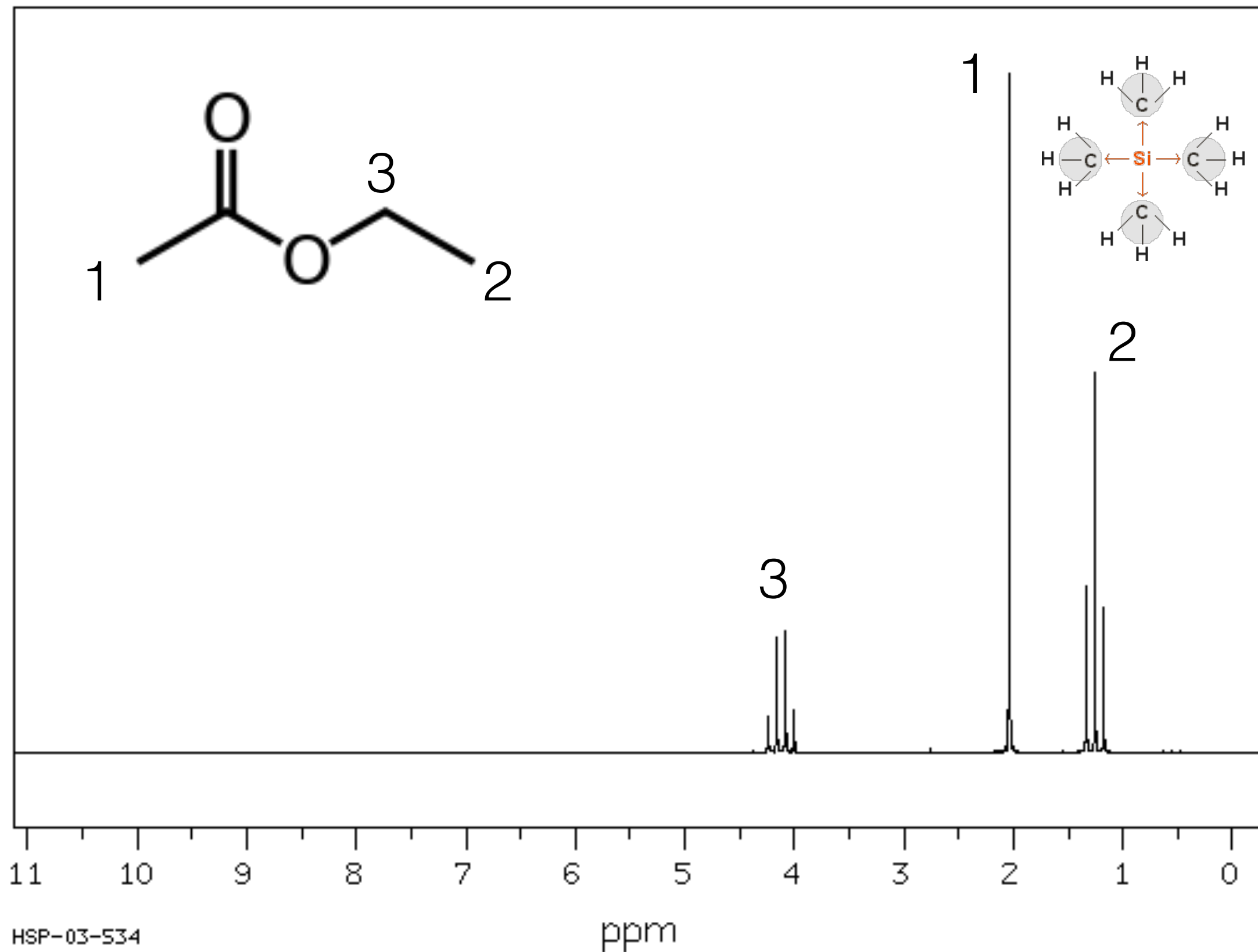




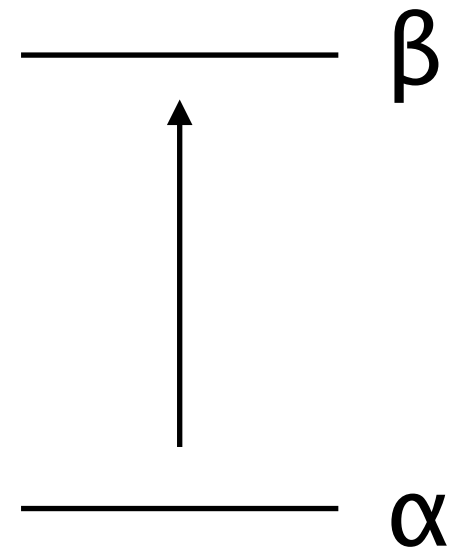
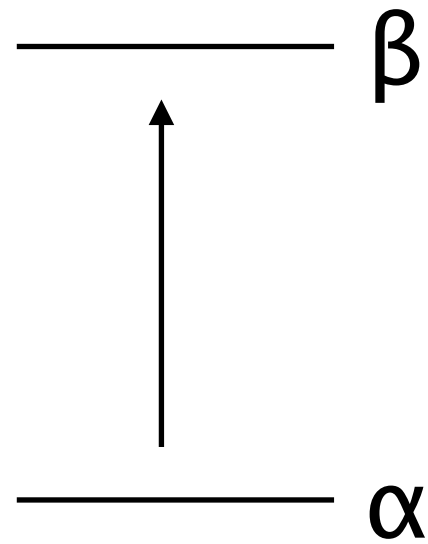
Equivalência química:
ambientes químicos idênticos



Acoplamiento escalar



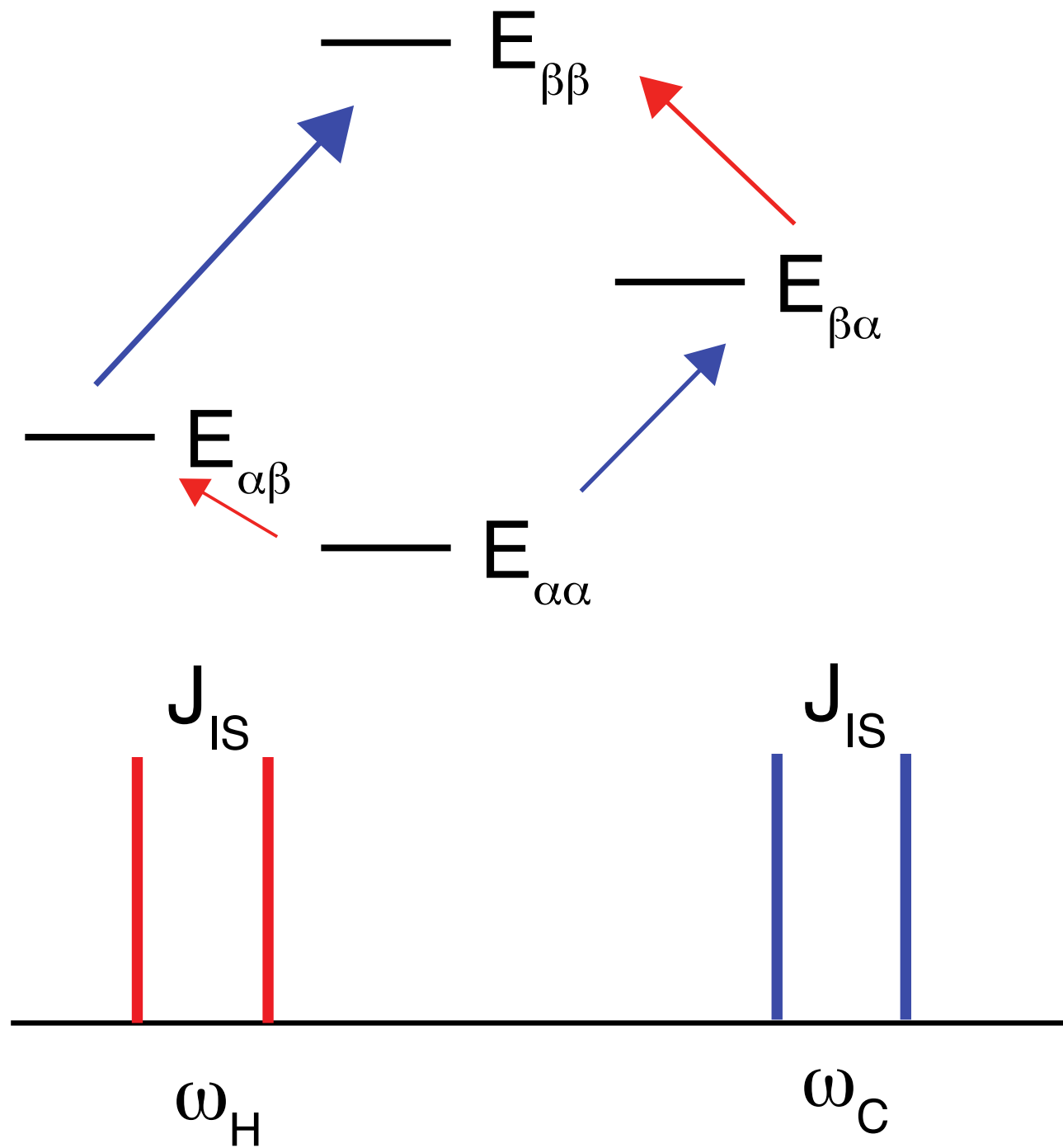
Sistema de 2 spins isolados



ex. 500 MHz

ex. 125 MHz

Estrutura fina devido ao acoplamento escalar



$$E_{\alpha\alpha} = \frac{1}{2}\hbar\omega_H + \frac{1}{2}\hbar\omega_C + \frac{1}{2}\hbar\pi J_{HC}$$

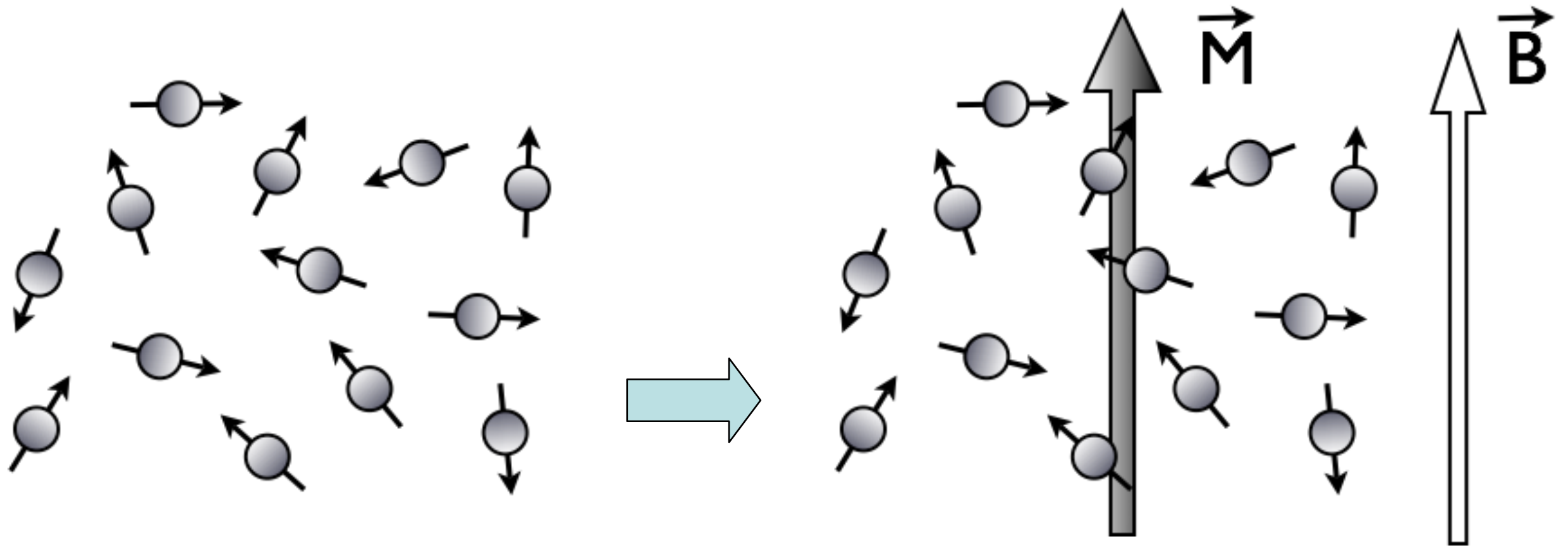
$$E_{\alpha\beta} = \frac{1}{2}\hbar\omega_H - \frac{1}{2}\hbar\omega_C - \frac{1}{2}\hbar\pi J_{HC}$$

$$E_{\beta\alpha} = -\frac{1}{2}\hbar\omega_H + \frac{1}{2}\hbar\omega_C - \frac{1}{2}\hbar\pi J_{HC}$$

$$E_{\beta\beta} = -\frac{1}{2}\hbar\omega_H - \frac{1}{2}\hbar\omega_C + \frac{1}{2}\hbar\pi J_{HC}$$

Como RMN funciona?

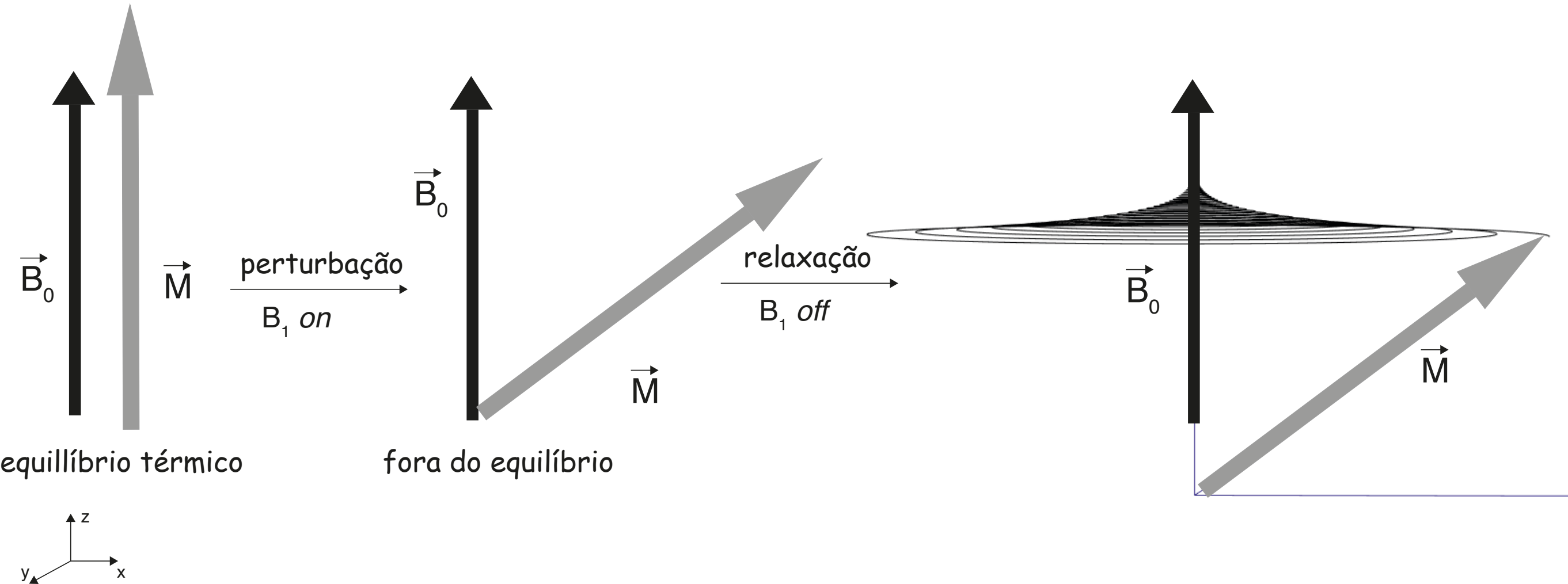
Magnetização macroscópica (M) no equilíbrio térmico



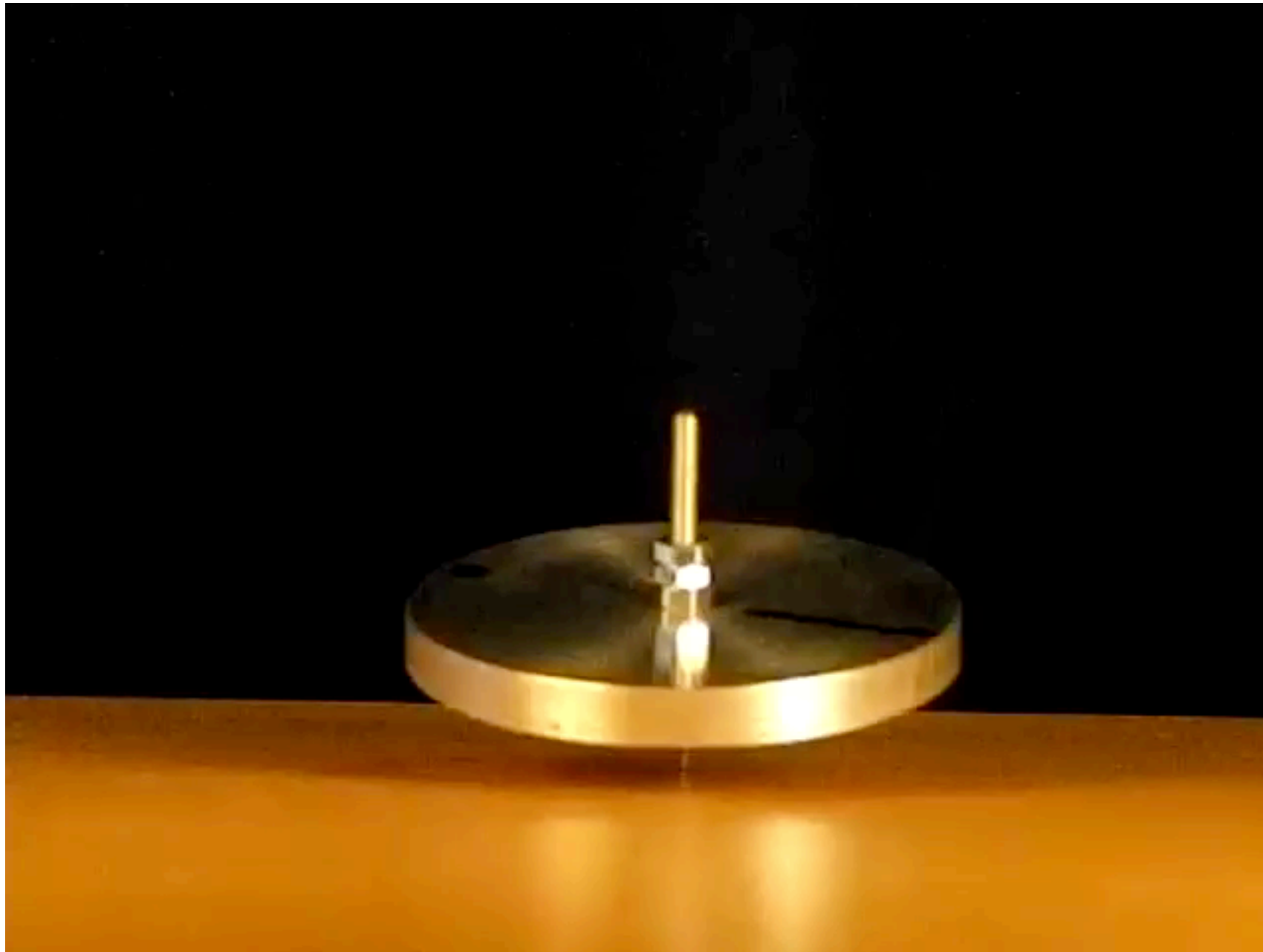
$\Delta E = 3.3 \cdot 10^{-25}$ J para ^1H a 11.74T
 $k_B T = 4.1 \cdot 10^{-21}$ J para Temp. ambiente

$$M_0 = N\gamma\hbar \left(\frac{1}{2} f_\alpha - \frac{1}{2} f_\beta \right) = \frac{N\hbar^2\gamma^2 B_0}{4k_B T} \quad \frac{p_\alpha}{p_\beta} = \exp \left(\frac{\Delta E}{k_B T} \right)$$

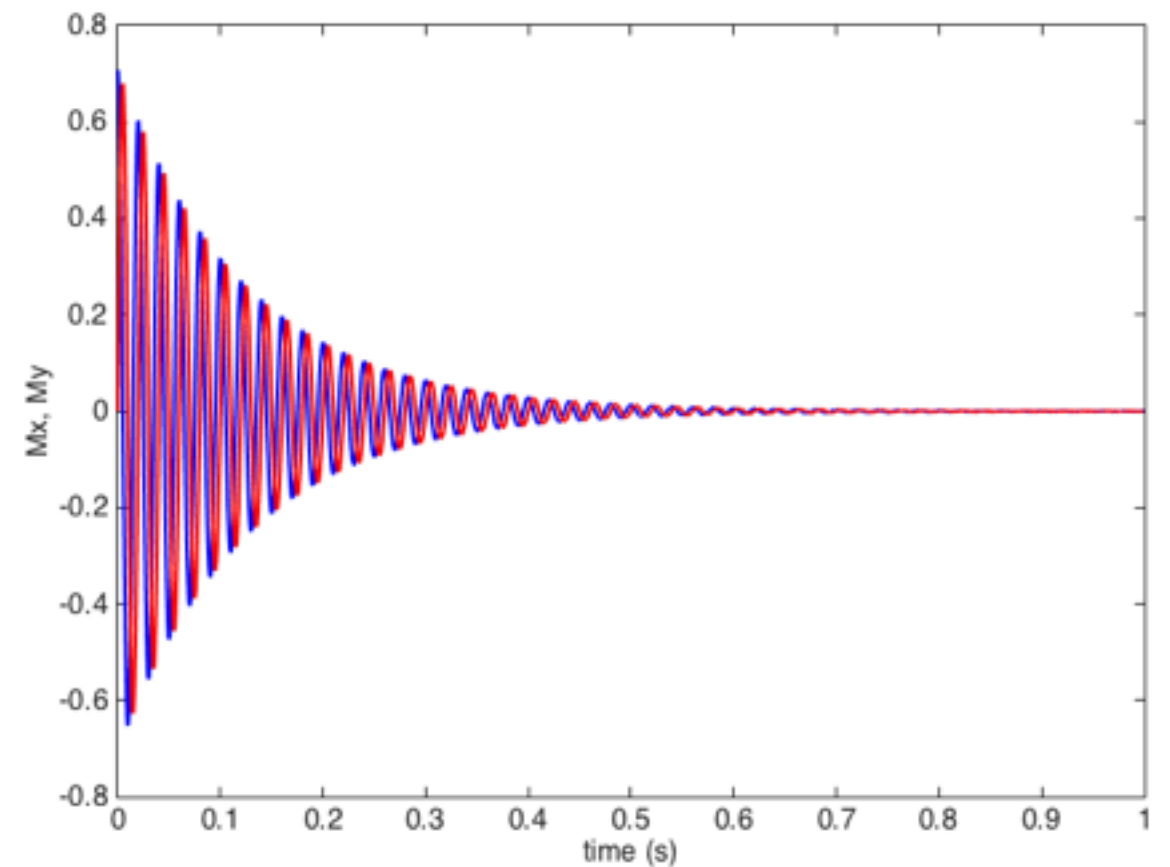
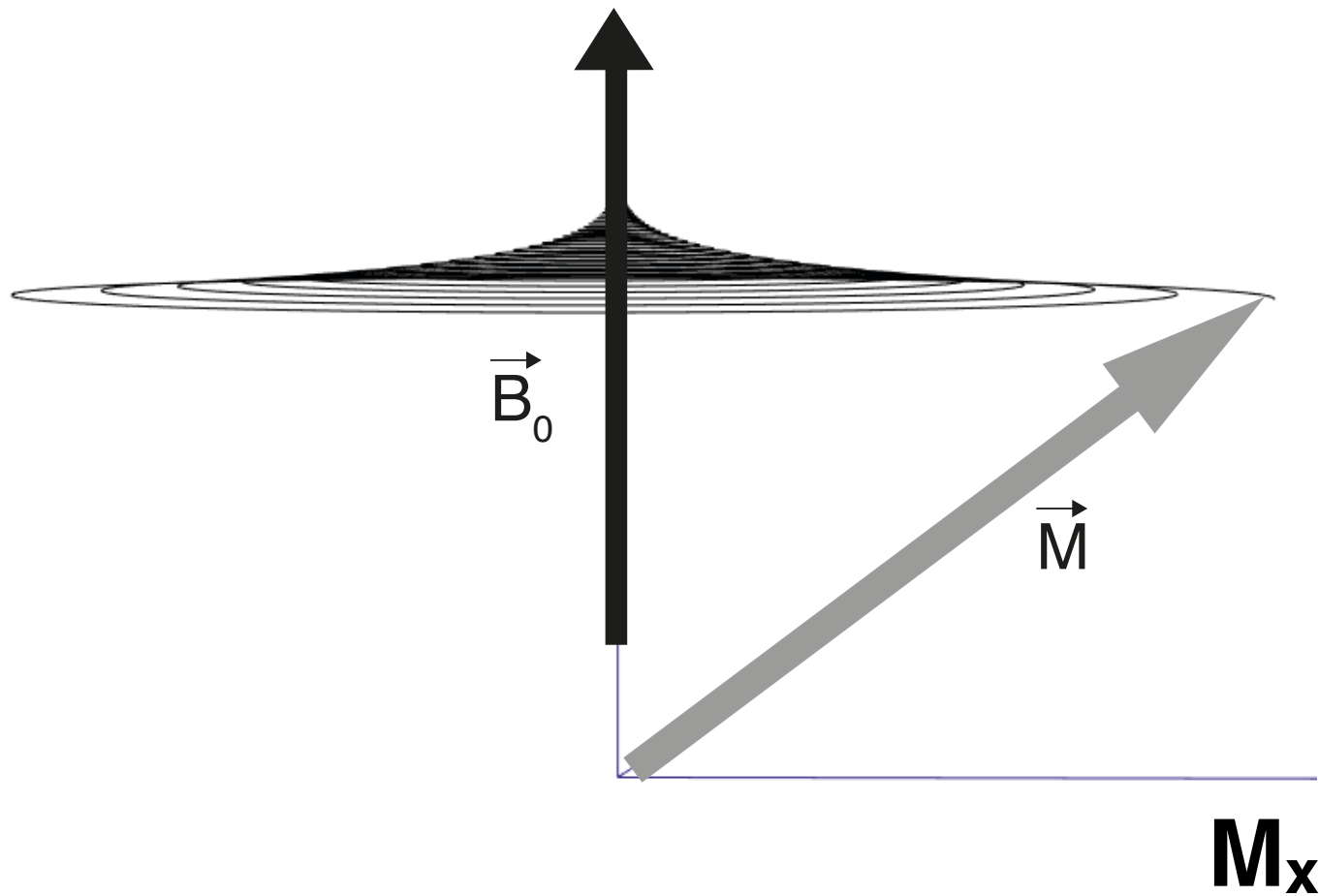
Como podemos detectar M ?



Movimento de precessão



O sinal é detectado à medida em que \vec{M} precessa ao redor de B_0 com velocidade angular ω_0

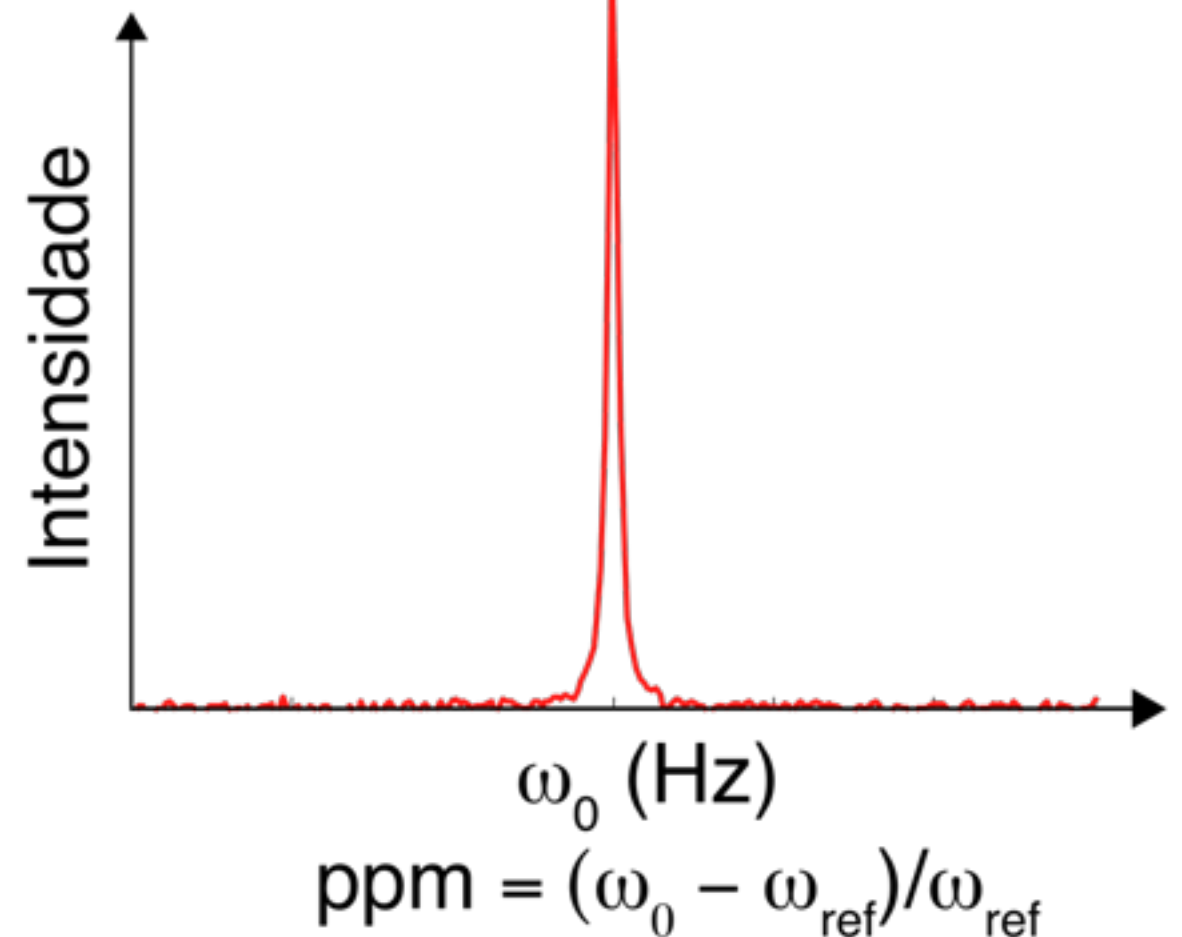
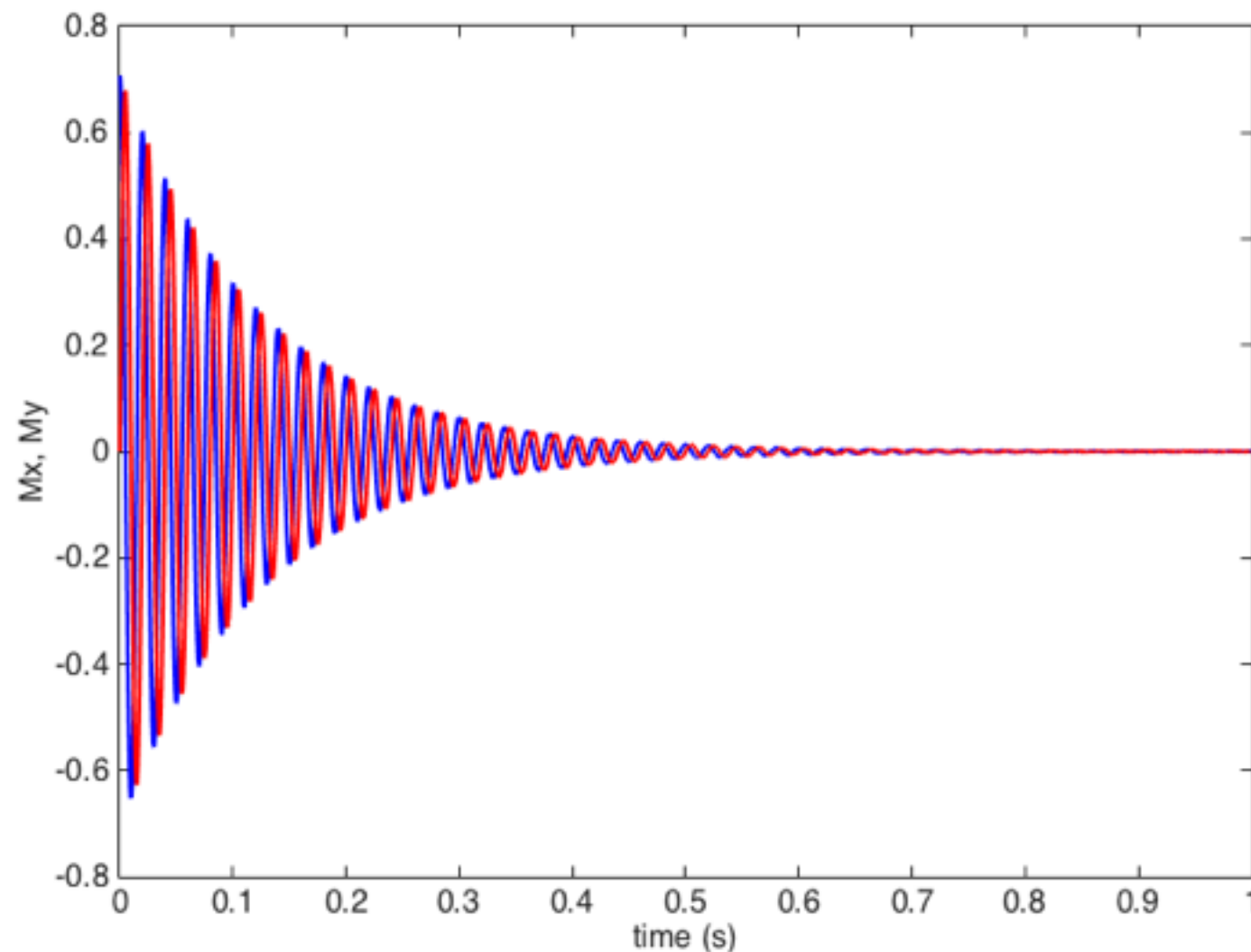


$$M_x(t) = M_0 \cos(\omega_0 t) \exp(-R_2 t)$$

$$M_y(t) = M_0 \sin(\omega_0 t) \exp(-R_2 t)$$

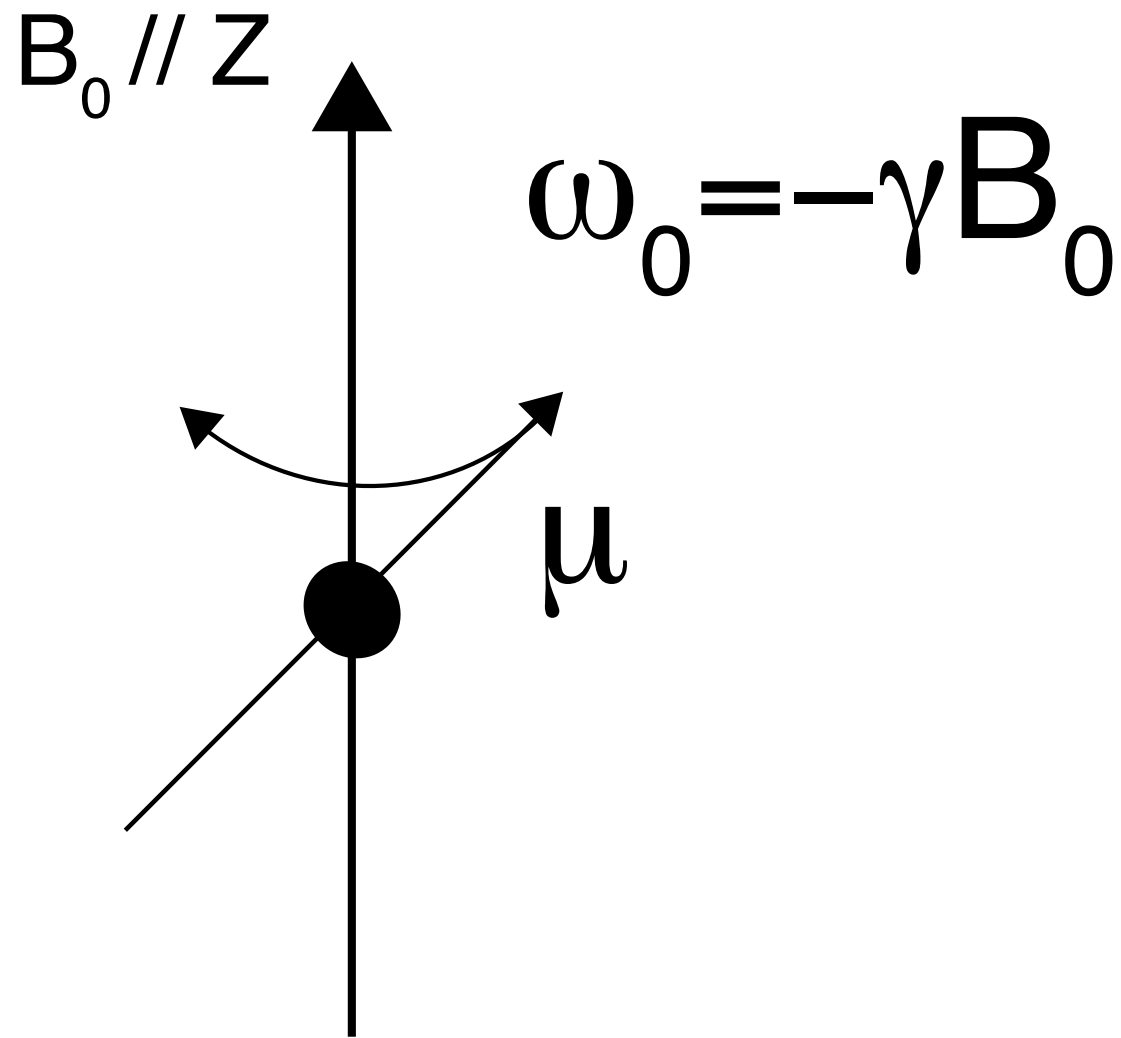
Sinal

O espectro é obtido a partir da transformada de Fourier do sinal detectado



R_2 é a velocidade de decaimento da magnetização transversal (spin spin) ($R_2 = 1/T_2$)

Frequência de Larmor



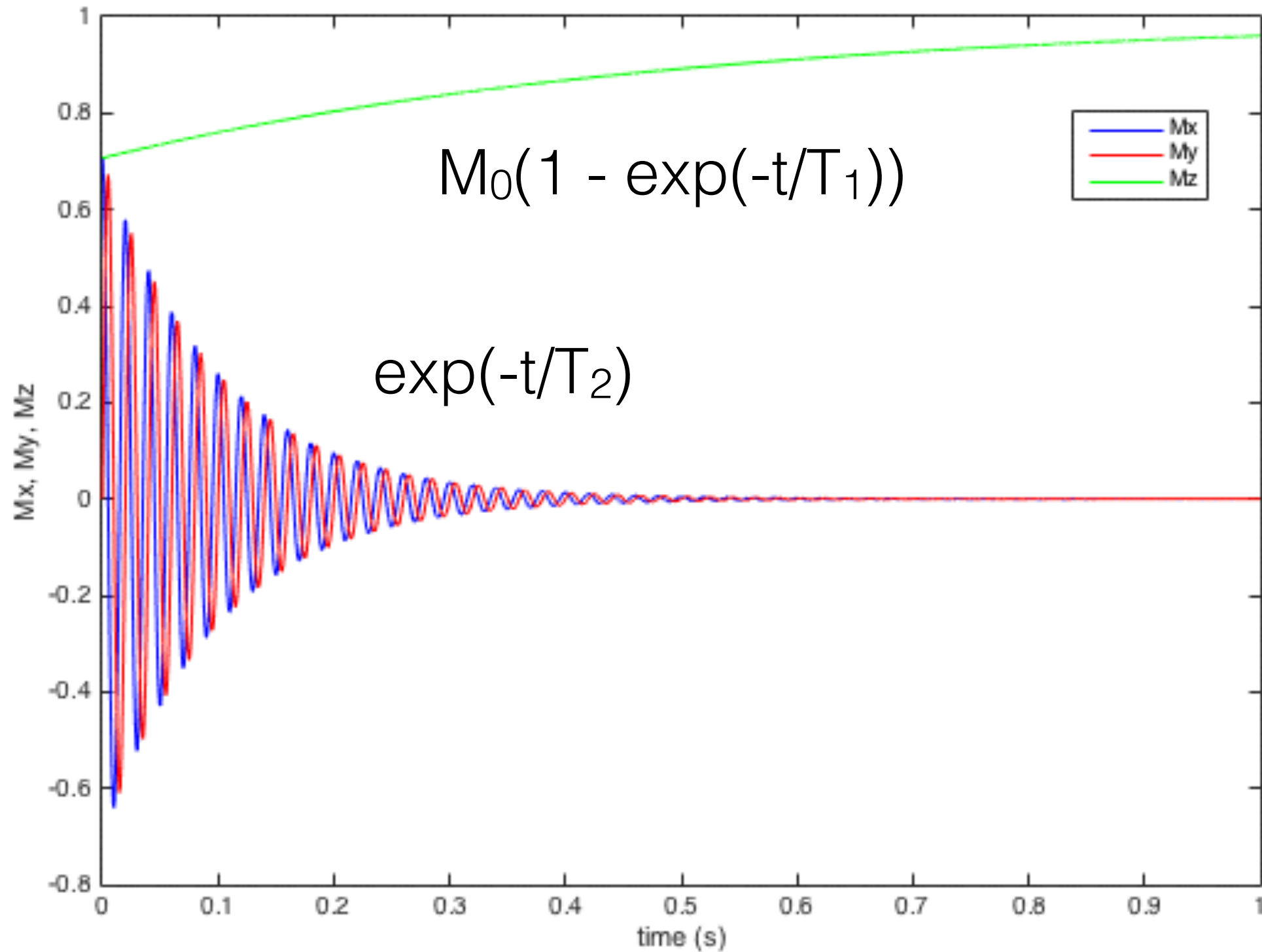
$$E_\alpha = -\frac{1}{2} \hbar \gamma B = \frac{1}{2} \hbar \omega_0$$
$$E_\beta = +\frac{1}{2} \hbar \gamma B = -\frac{1}{2} \hbar \omega_0$$

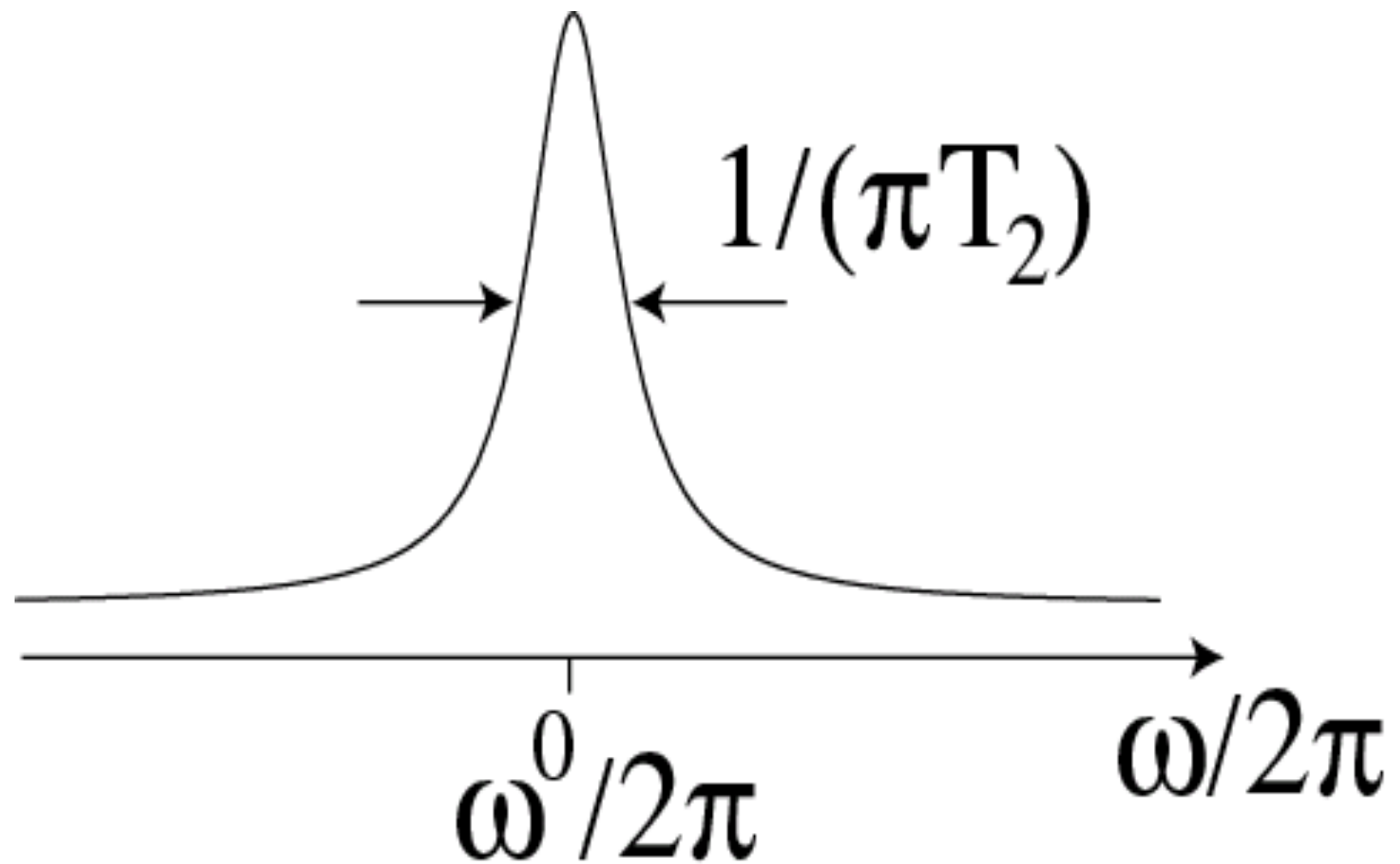
— E_β



— E_α

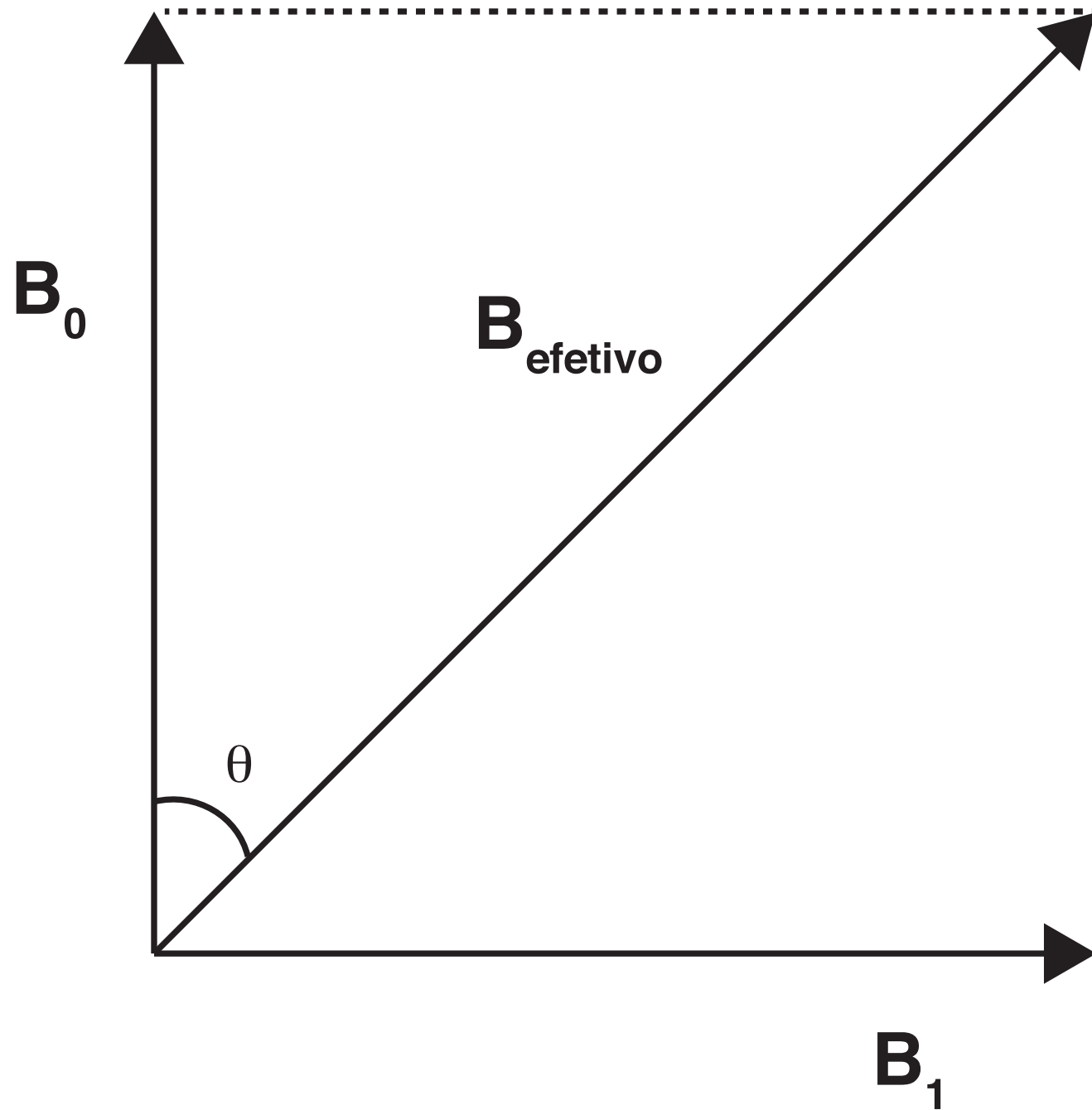
Dois mecanismos de relaxação diferentes: relaxação spin-rede (T_1 ou R_1), e relaxação spin-spin (T_2 ou R_2)





R_2 é a velocidade de decaimento da magnetização transversal (spin spin) ($R_2 = 1/T_2$)

Como retiramos M do equilíbrio?

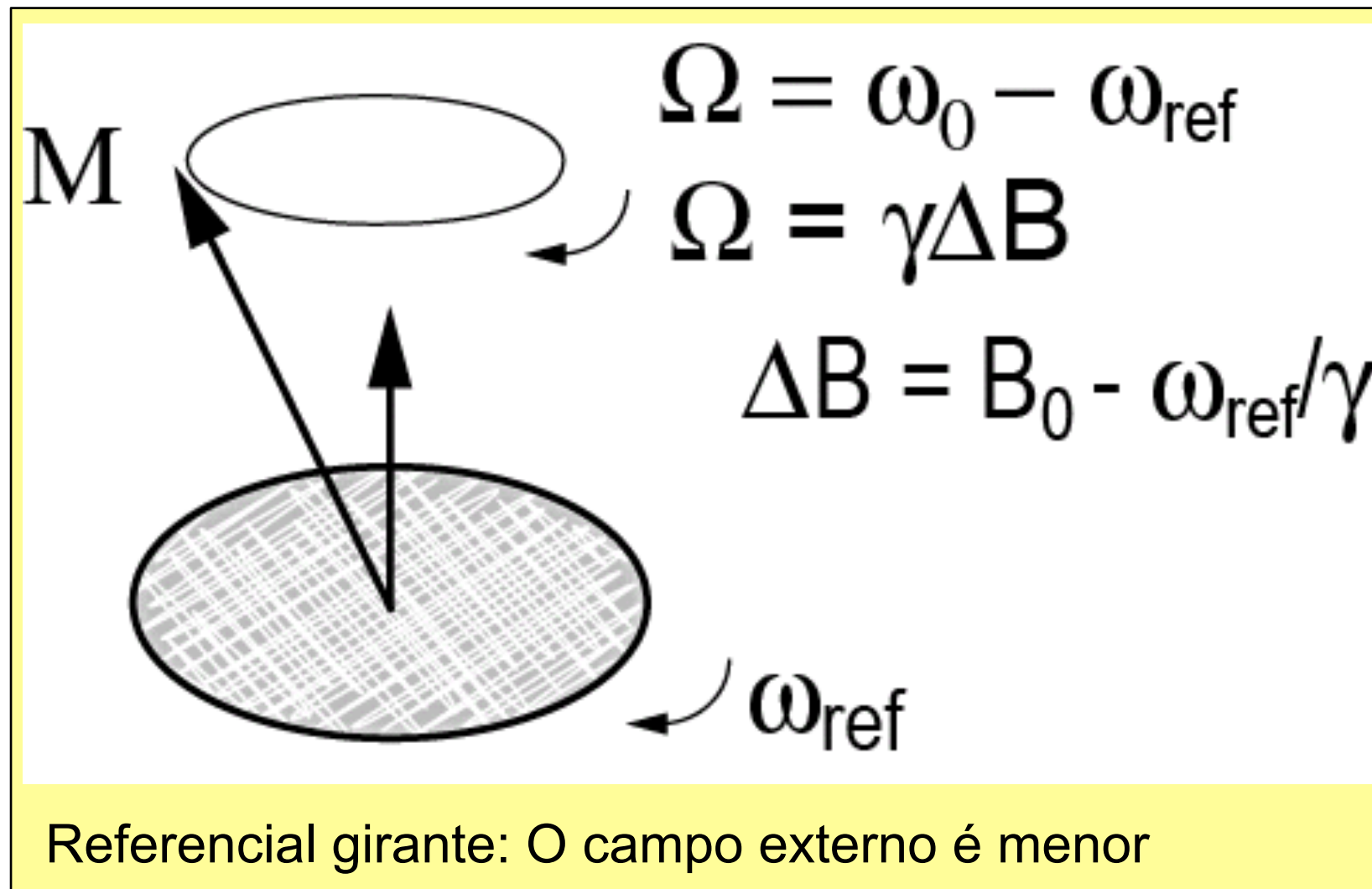


Neste caso M assume um movimento de precessão ao redor de B_{efetivo}

B_1 é modulado por uma frequência ω_{rf}

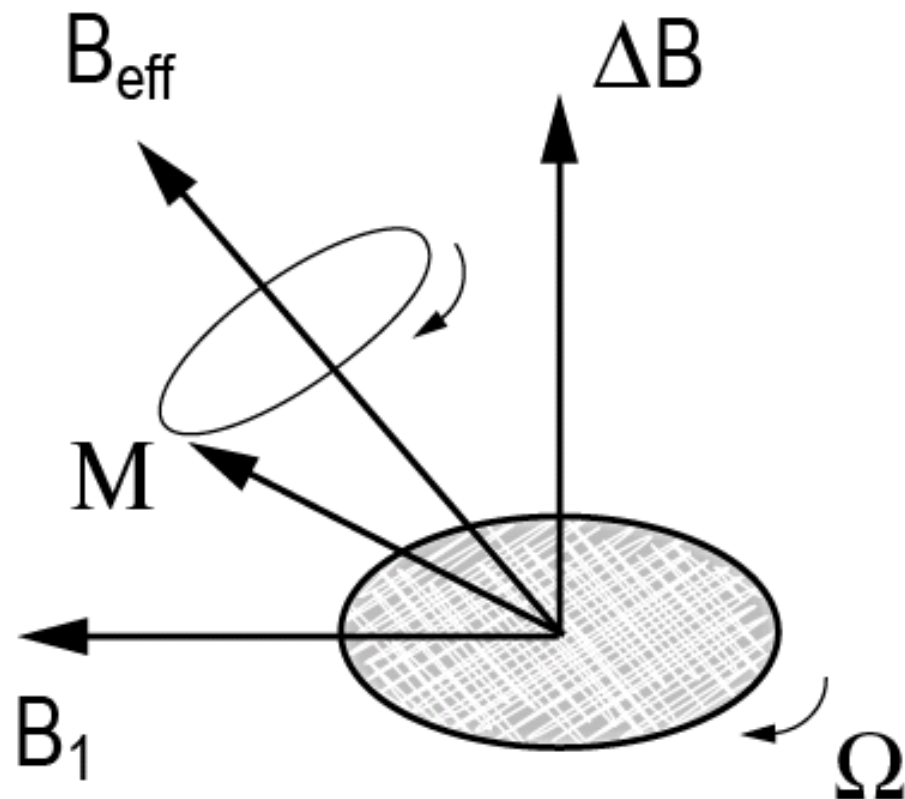
$$\vec{B}_{rf}(t) = 2B_1 \cos(\omega_{rf}t + \phi) \hat{i},$$

$\omega_{rf} \sim \omega_0$ (condição de ressonância)



Campo efetivo

B_1 está oscilando com frequência ω_{ref}

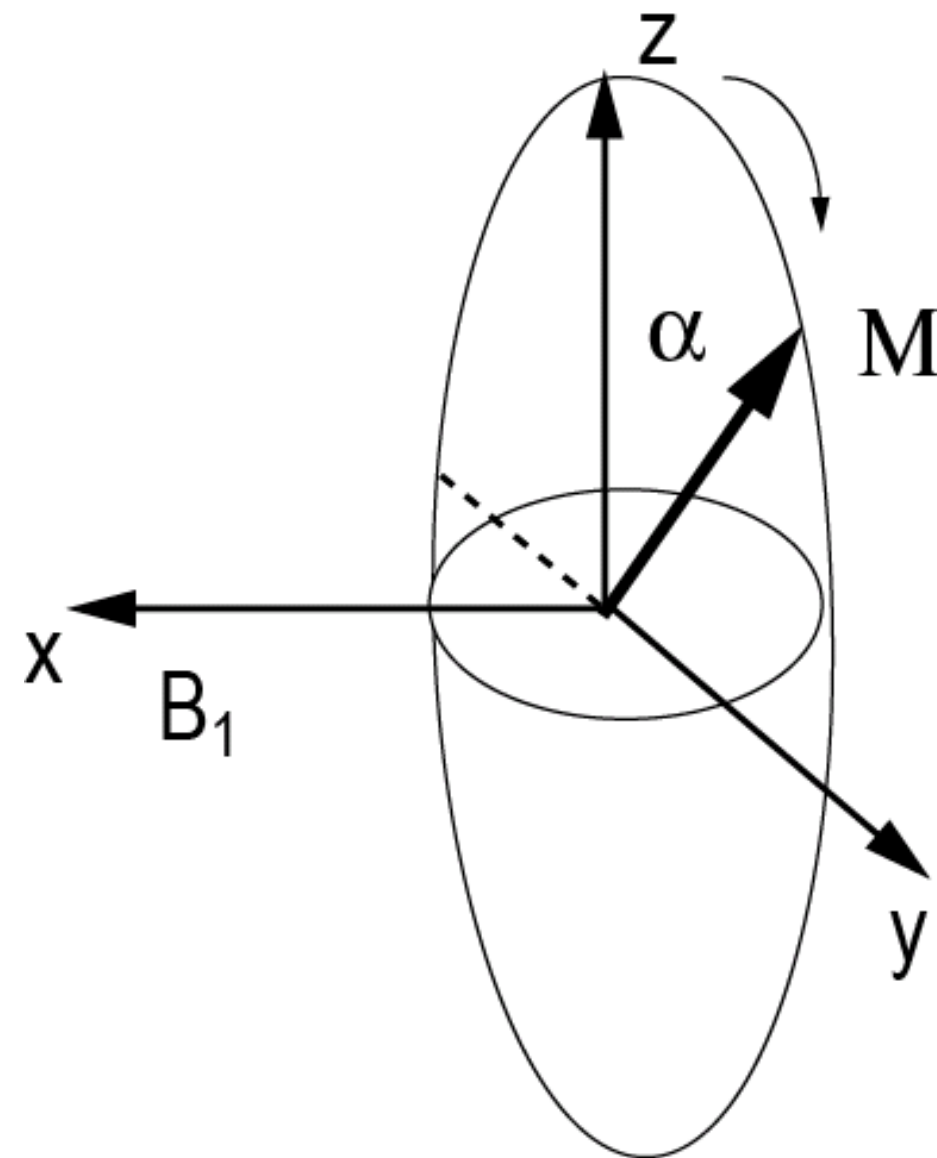


Off-resonance

$$B_{eff} = \sqrt{B_1^2 + \Delta B^2}$$

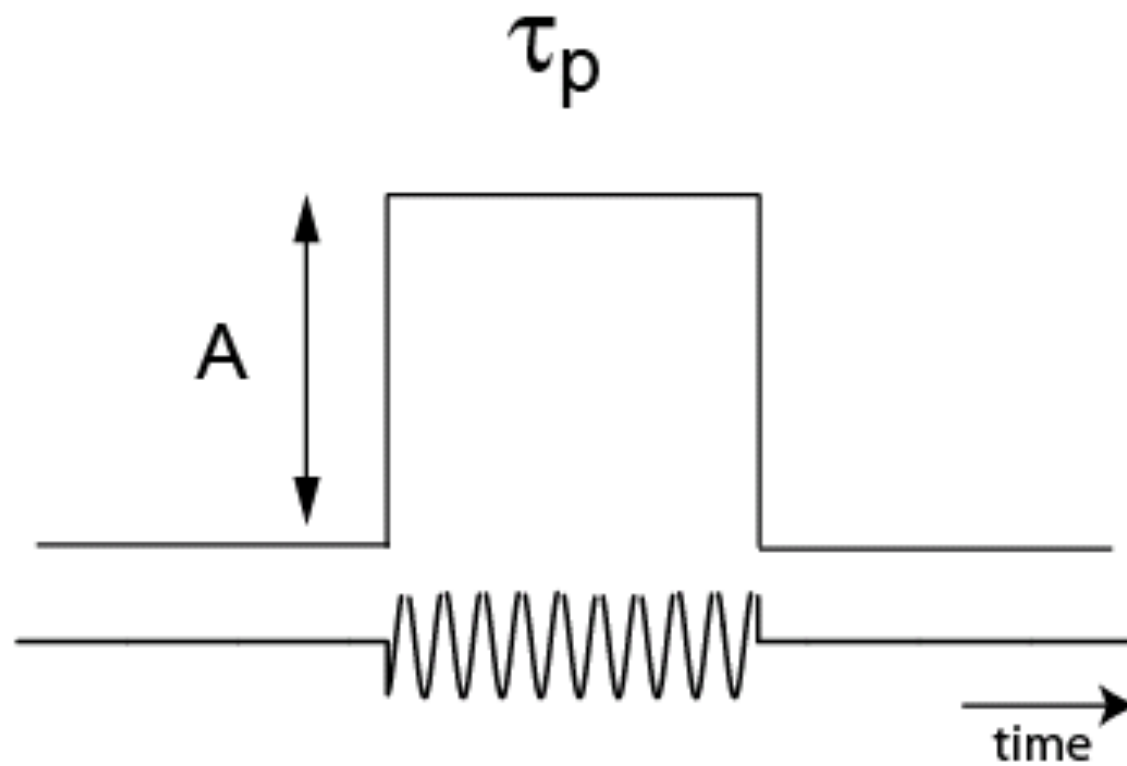
$$\omega_{eff} = \sqrt{\omega_1^2 + \Omega^2}$$

Condição de ressonância
 $\Delta B=0$, e $B_{eff}=B_1$

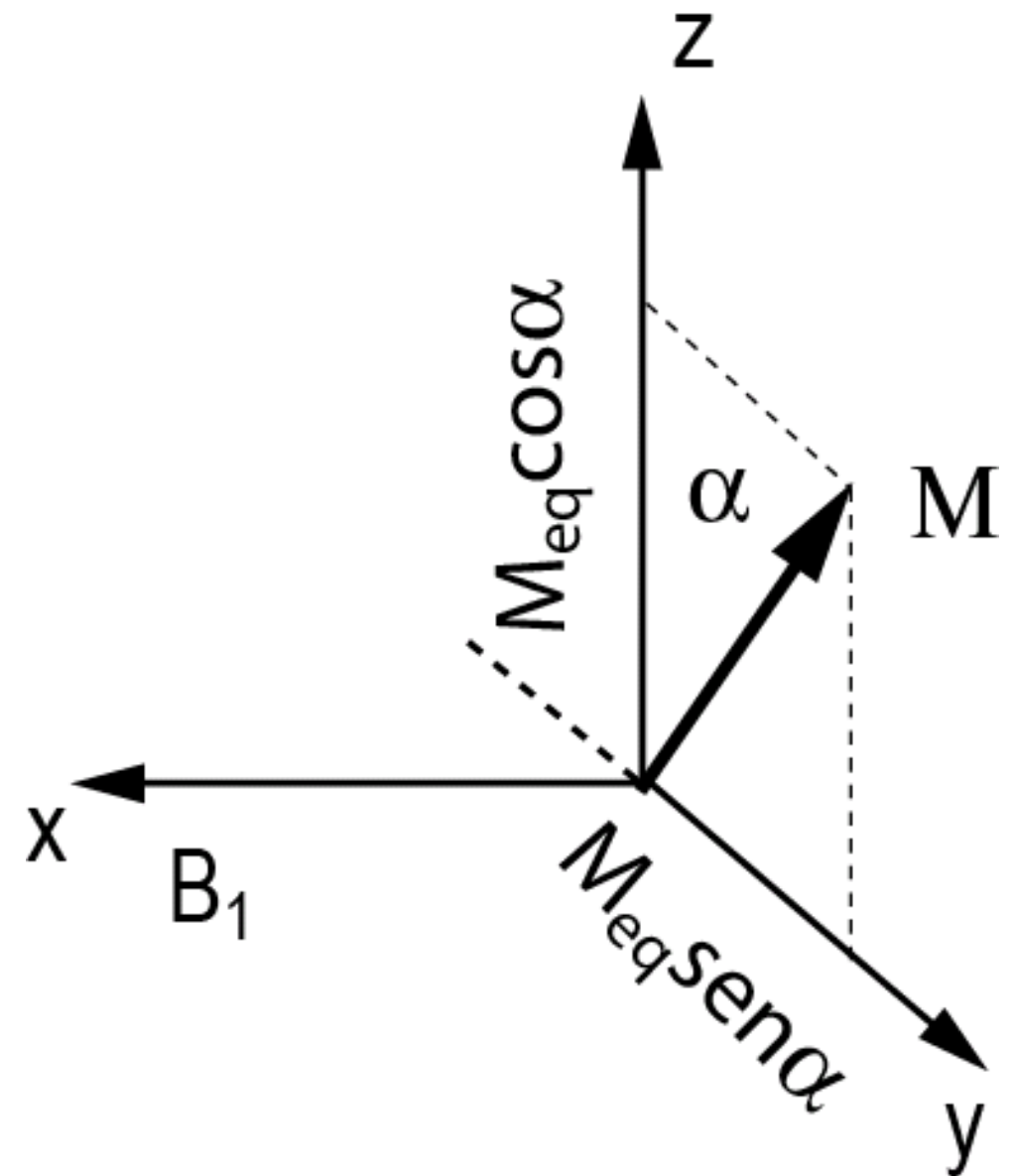


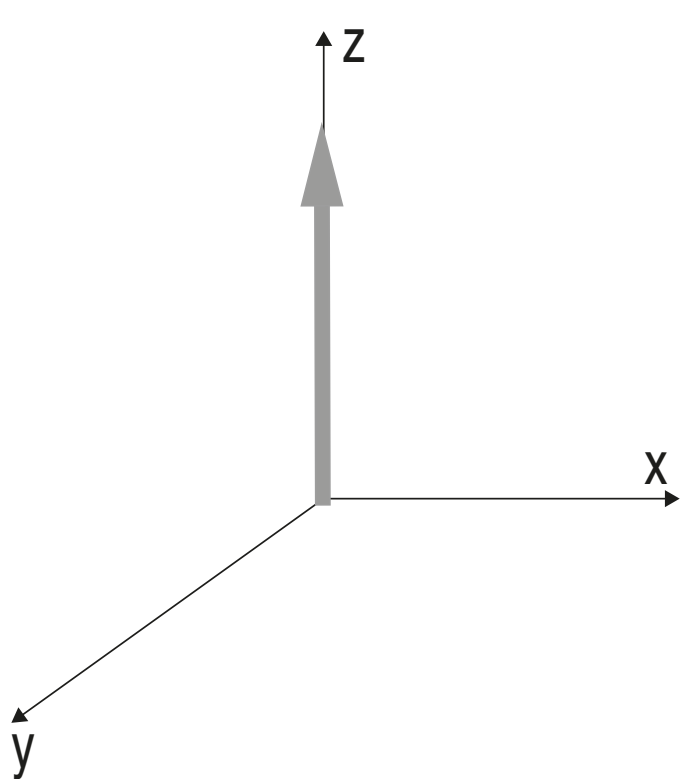
Pulsos de RF

$$\alpha = \gamma B_1 \tau_p = \omega_1 \tau_p$$



ϕ , fase

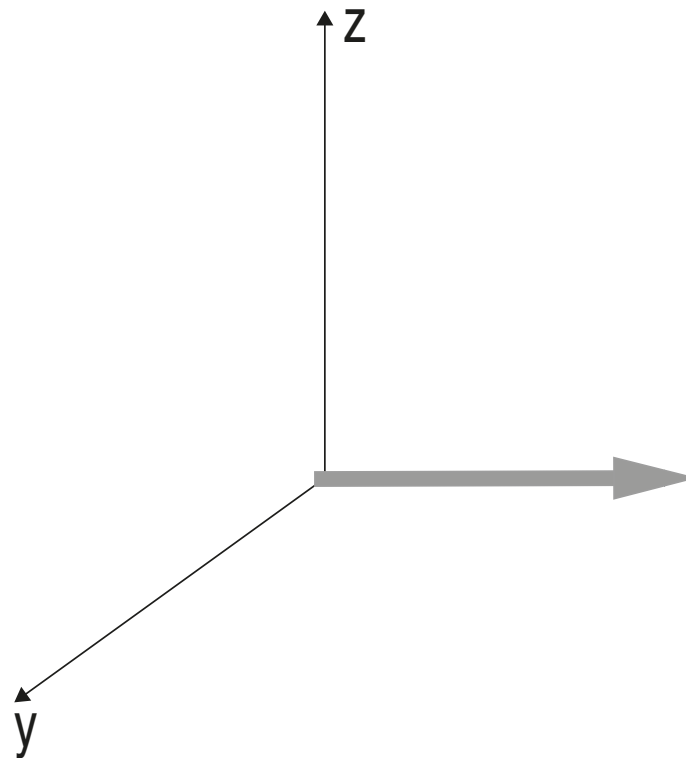




β $N - \Delta/2$

α $N + \Delta/2$

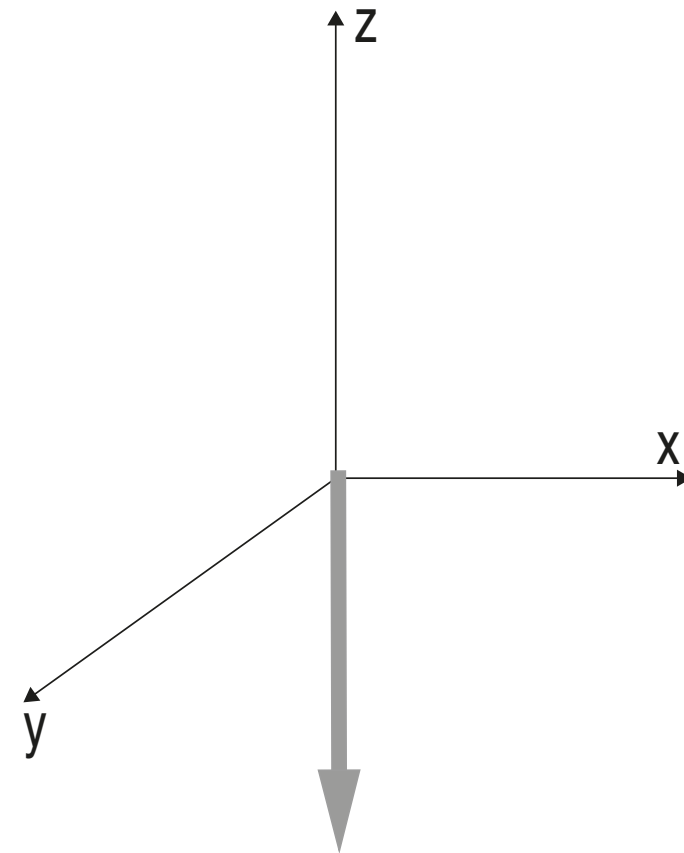
equilíbrio



β N

α N

excitação



β $N + \Delta/2$

α $N - \Delta/2$

inversão

Equação de Bloch

$$\frac{d\vec{M}}{dt} = \vec{M} \times \gamma \vec{B} + \hat{R}(\vec{M} - \vec{M}_{eq})$$

$$\vec{M} = \begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} \quad \vec{M}_{eq} = \begin{pmatrix} 0 \\ 0 \\ M_0 \end{pmatrix} \quad \vec{B} = \begin{pmatrix} 0 \\ 0 \\ \Delta B \end{pmatrix} \quad \hat{R} = \begin{pmatrix} -R_2 & 0 & 0 \\ 0 & -R_2 & 0 \\ 0 & 0 & -R_1 \end{pmatrix}$$

$$M^+ = M_x + iM_y \quad \frac{dM^+}{dt} = i(\Omega - R_2)M^+$$

$$M^+(t) = e^{i\Omega t} e^{-R_2 t} M^+(0)$$

RMN multidimensional

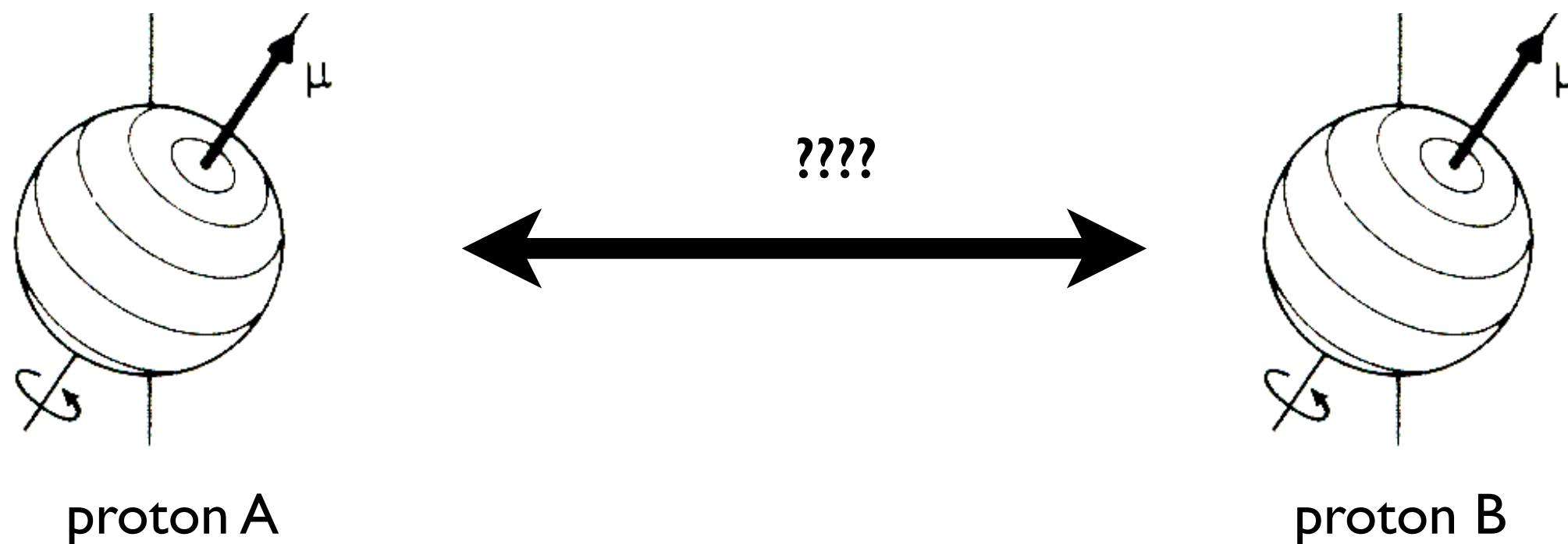


Richard R. Ernst



Encoding information

- mixing/magnetization transfer



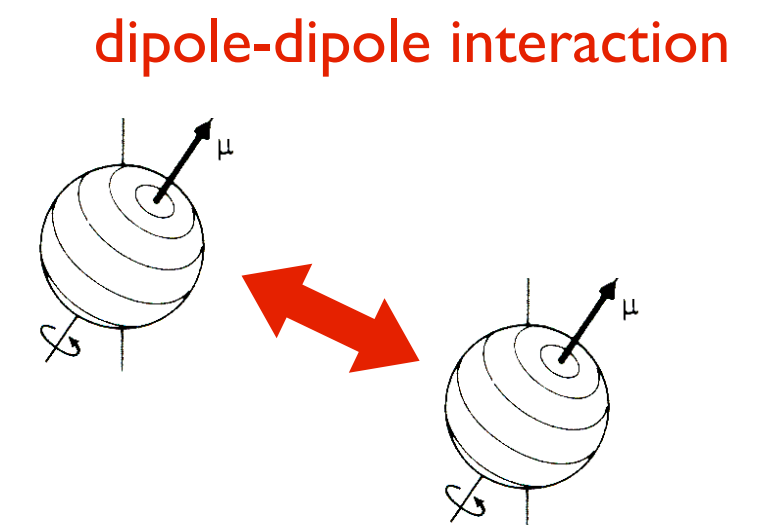
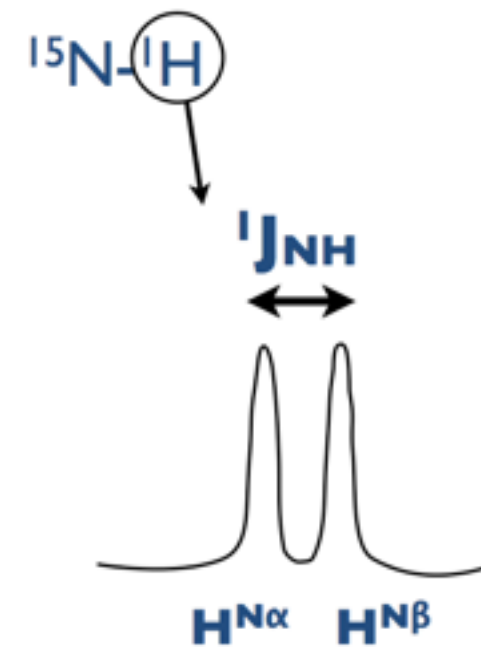
spin-spin interactions

Richard Ernst e Jean Jeener

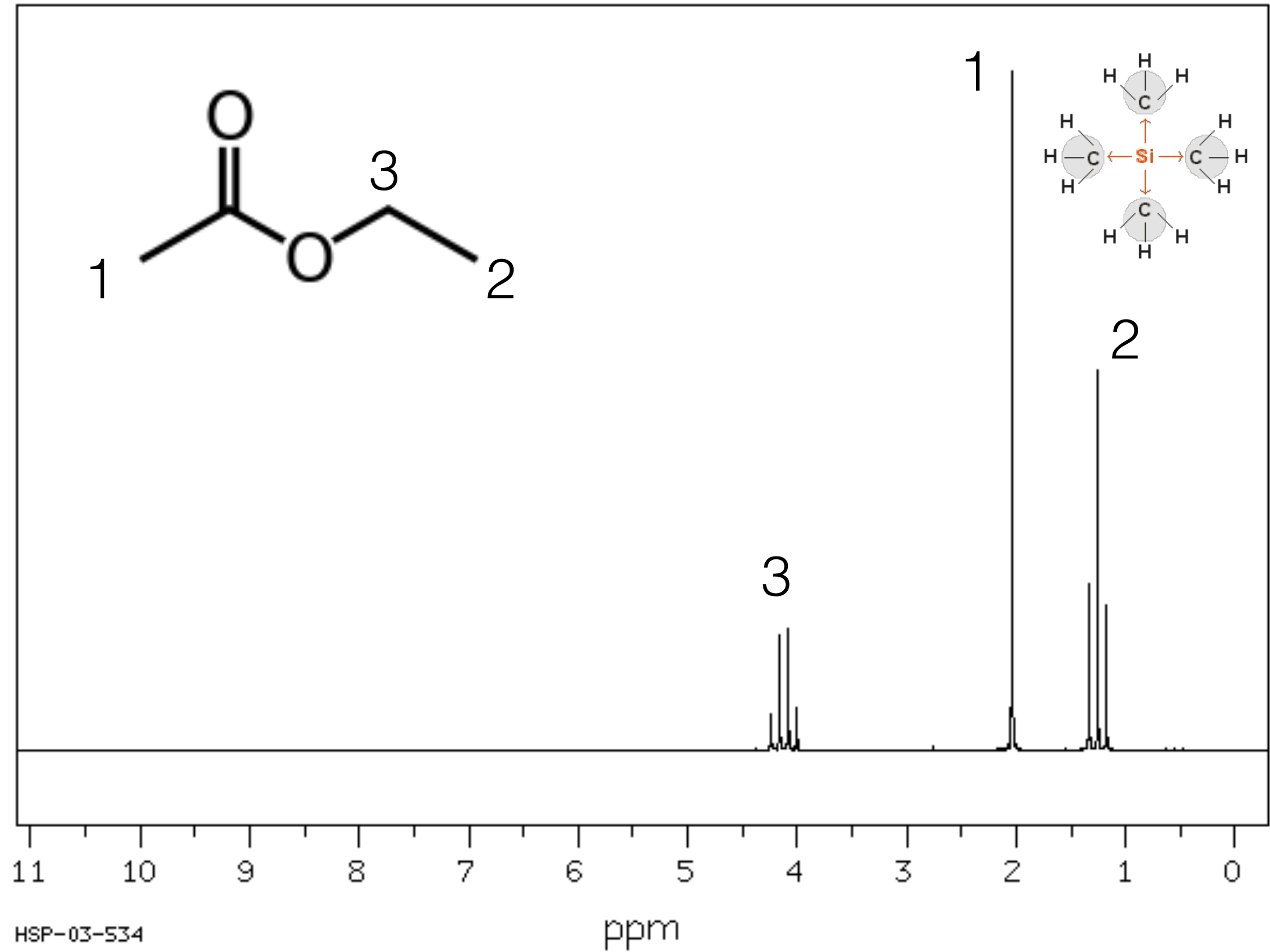
Magnetization transfer

- **J-coupling interaction**
 - through 3-4 bonds max.
 - chemical connectivities
 - assignment
 - also conformation dependent

- **Magnetic dipole interaction (NOE)**
 - Nuclear Overhauser Effect
 - through space
 - distance dependent ($1/r^6$)
 - NOESY -> distance restraints



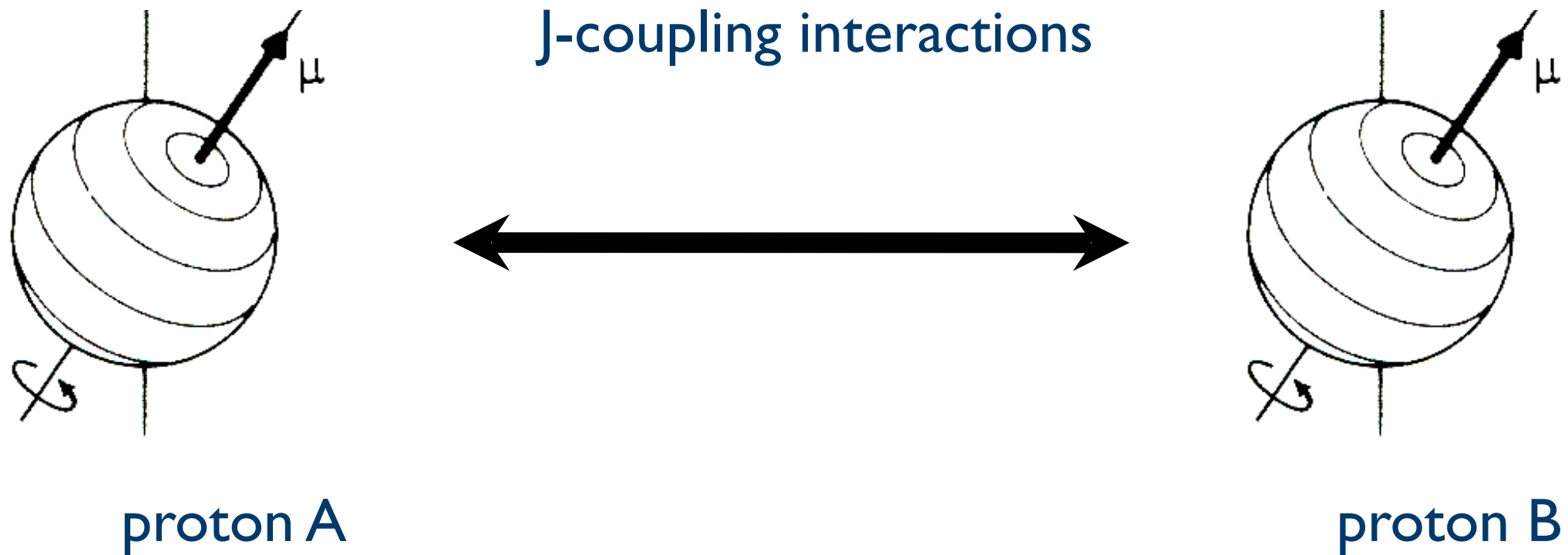
Quem acopla com quem?



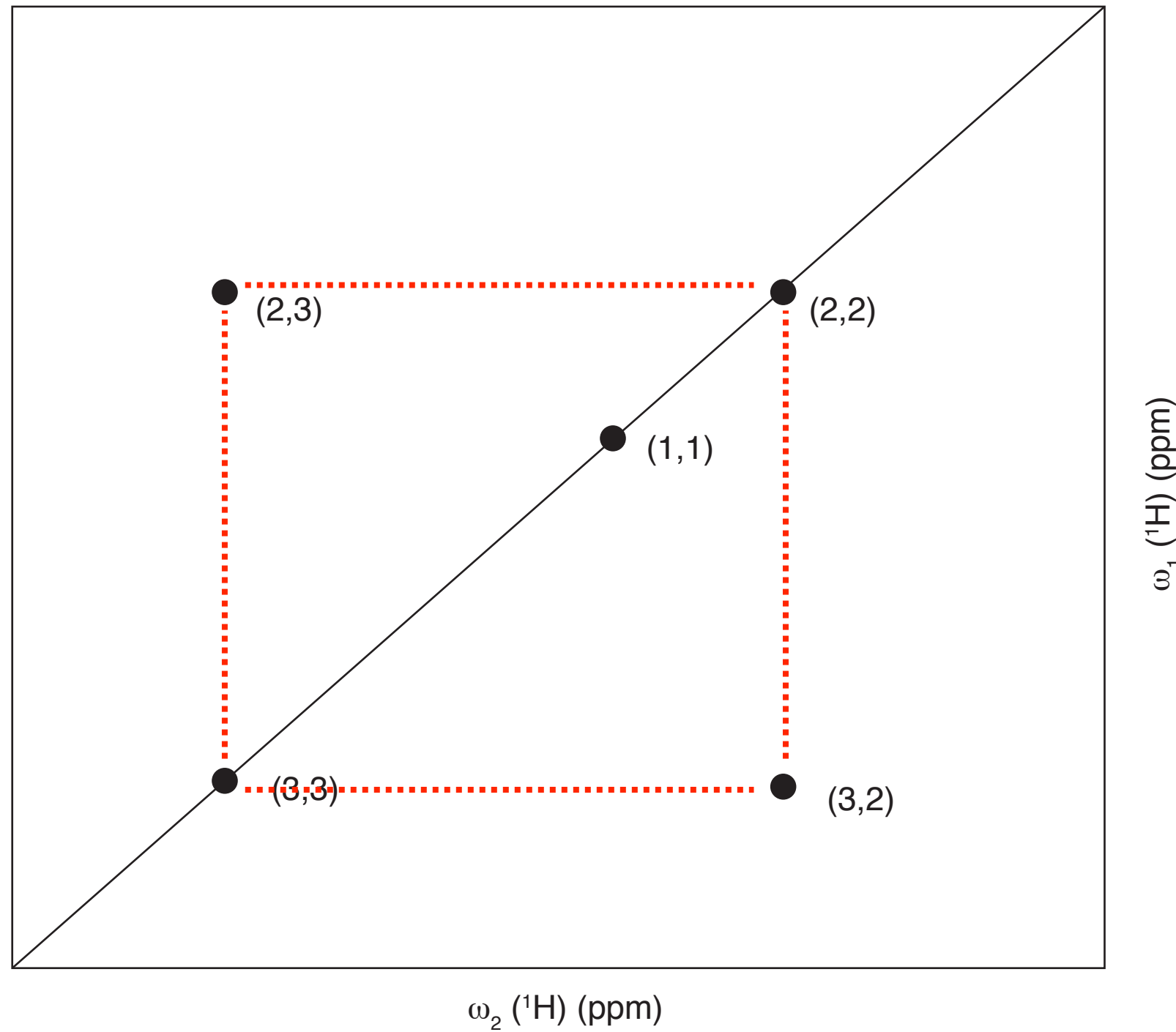
2D NMR

Encodes information between spins

- Type of information depends on transfer mechanism



COSY (Correlated Spectroscopy)

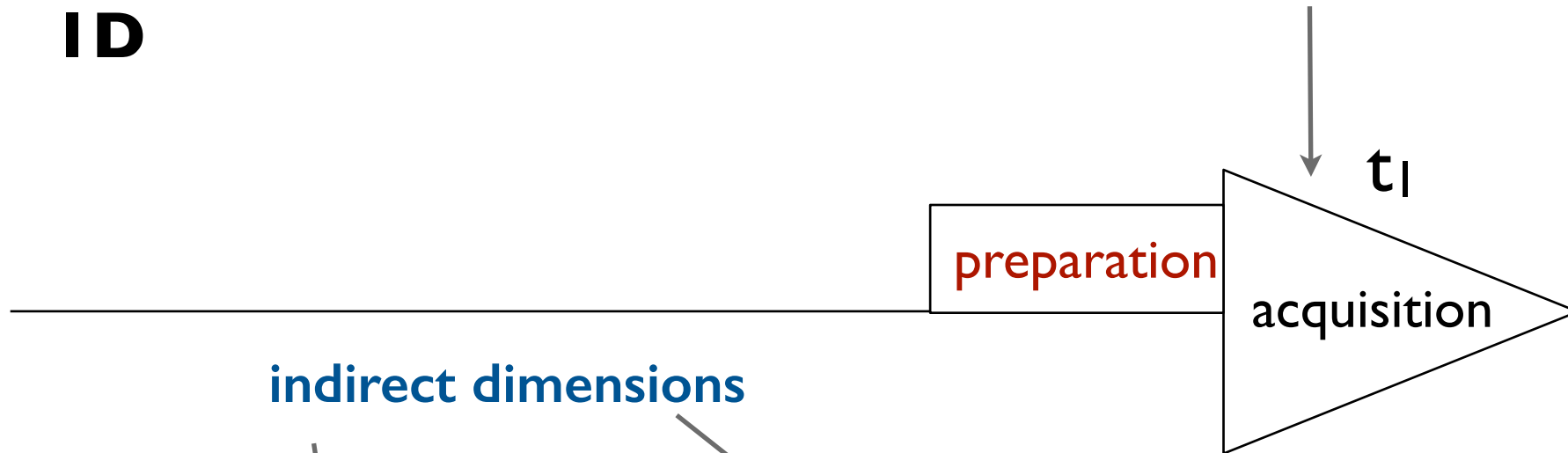


nD experiment

1D

direct dimension

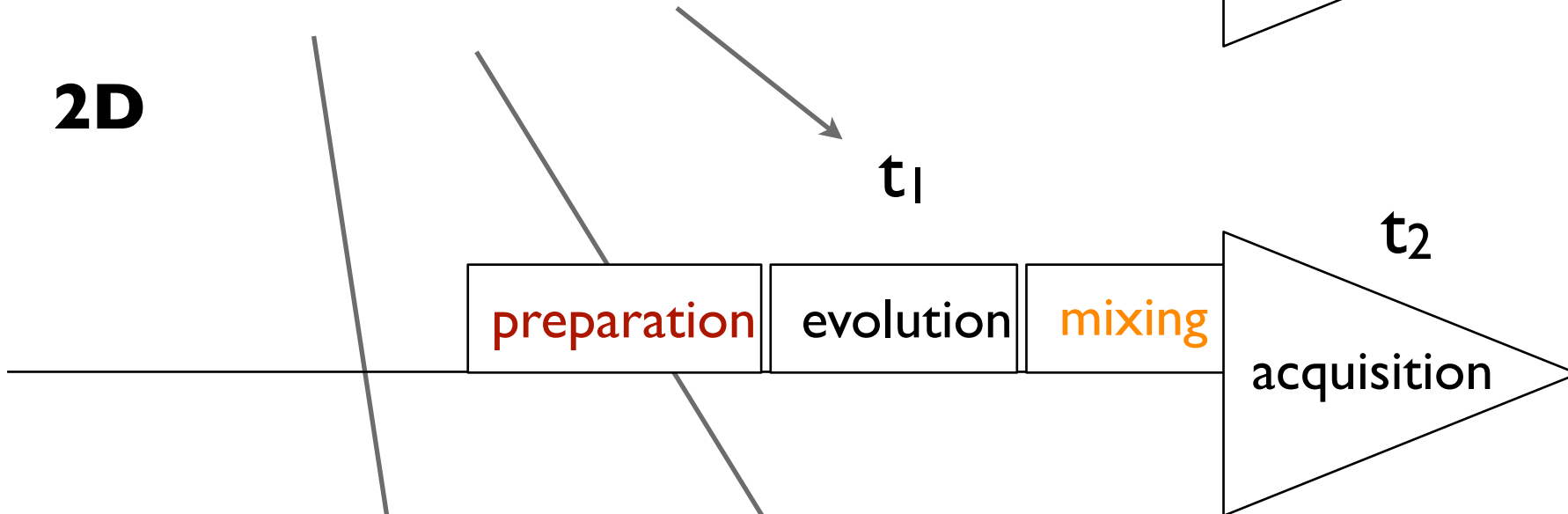
1 FID of N points



2D

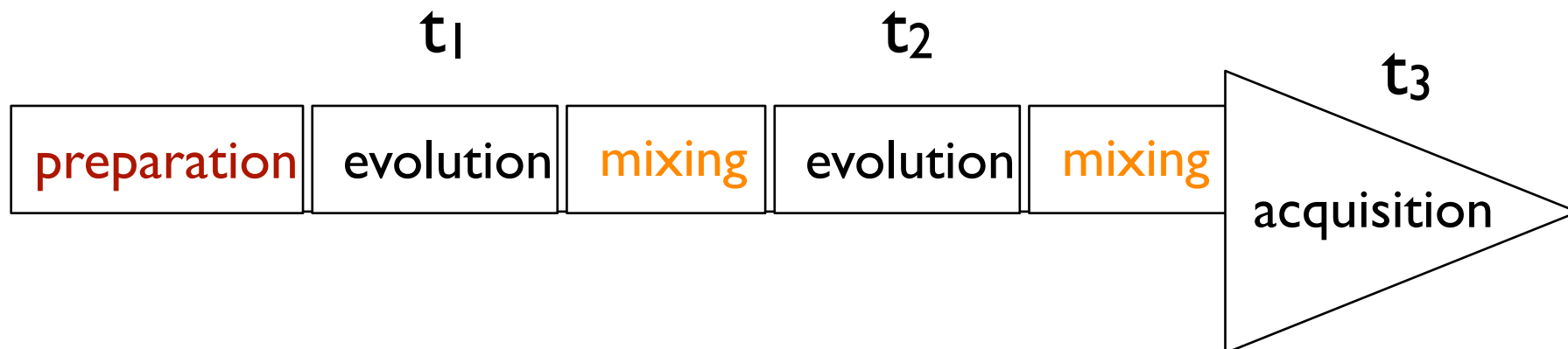
N FIDs of N points

indirect dimensions



3D

$N \times N$ FIDs of N points



3D NMR

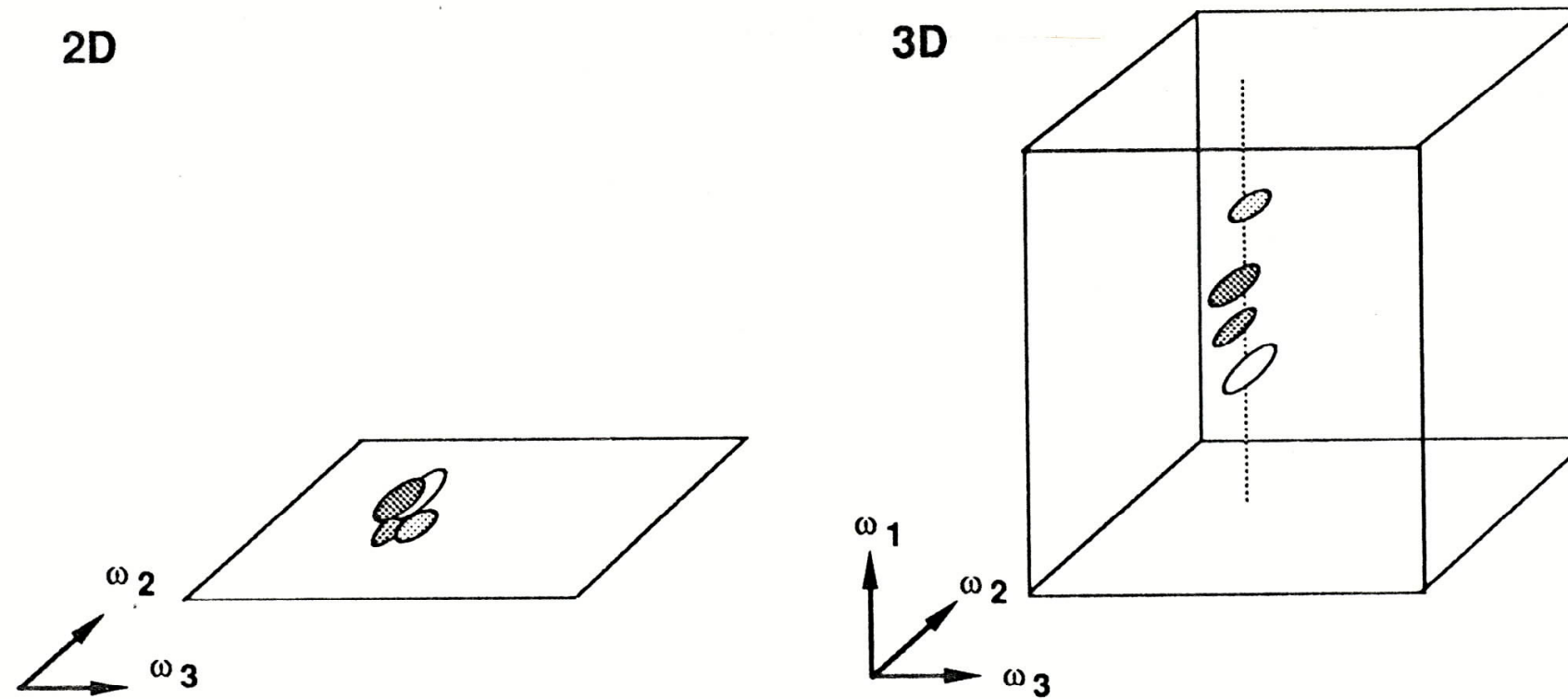
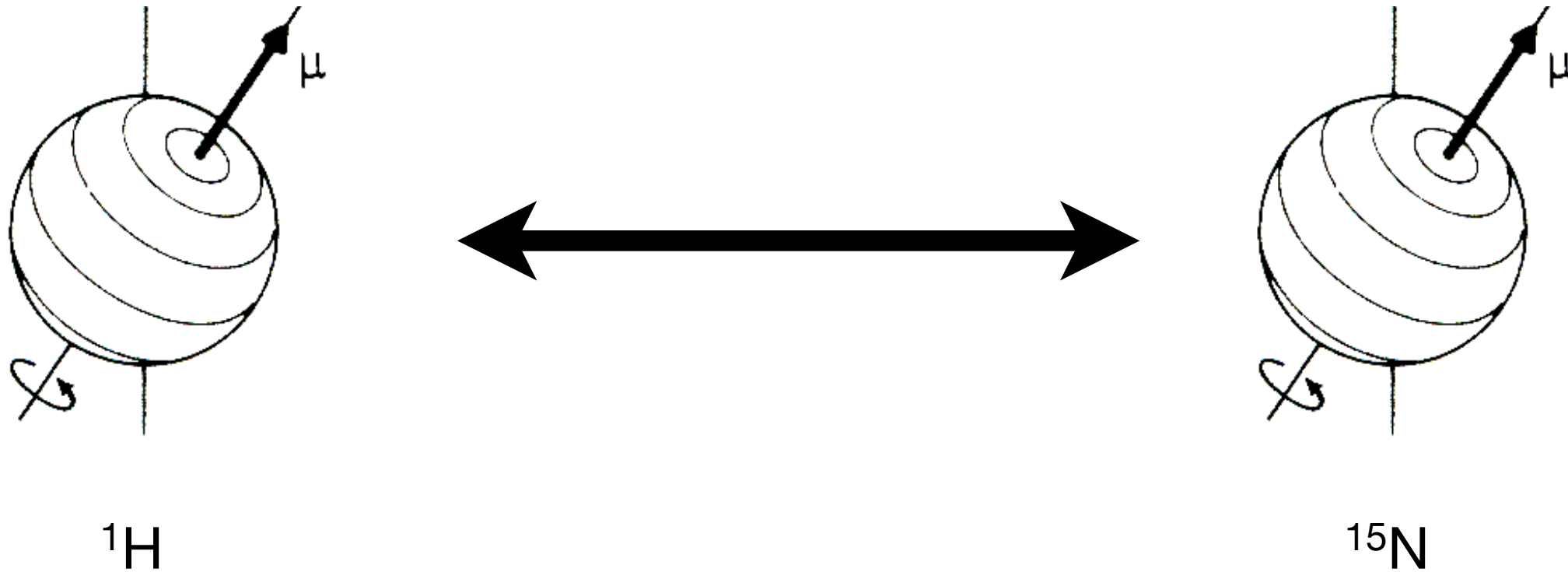


Figure 1. Illustration of the increase in resolution afforded by the increase in dimensionality. In the 2D spectrum, four cross-peaks overlap. By correlation with a third resonance frequency, each cross-peak obtains a different position along a line in the 3D spectrum, thus resolving the overlap problem.

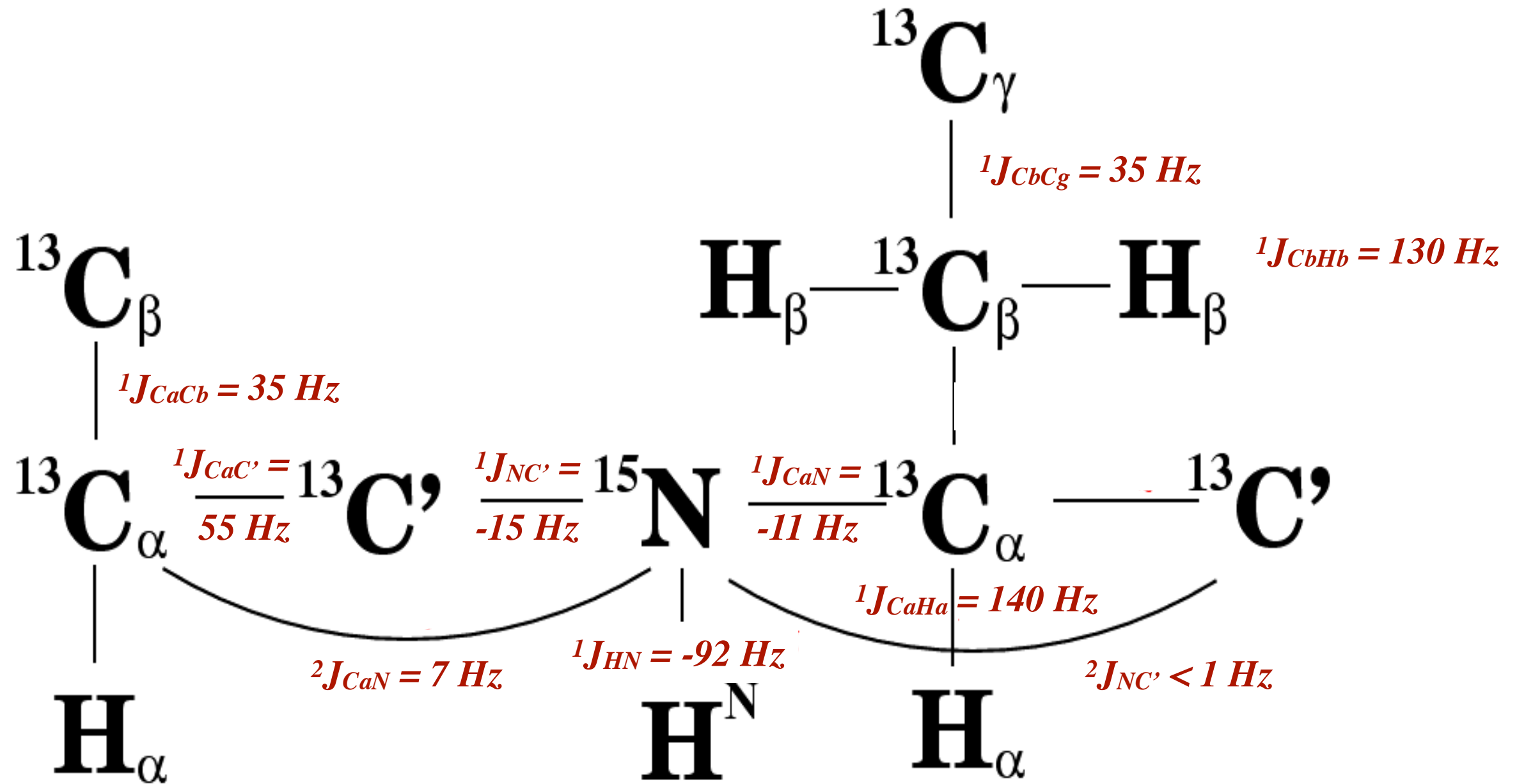
(Unister, Ph. D. Thesis, 1991)

heteronuclear NMR

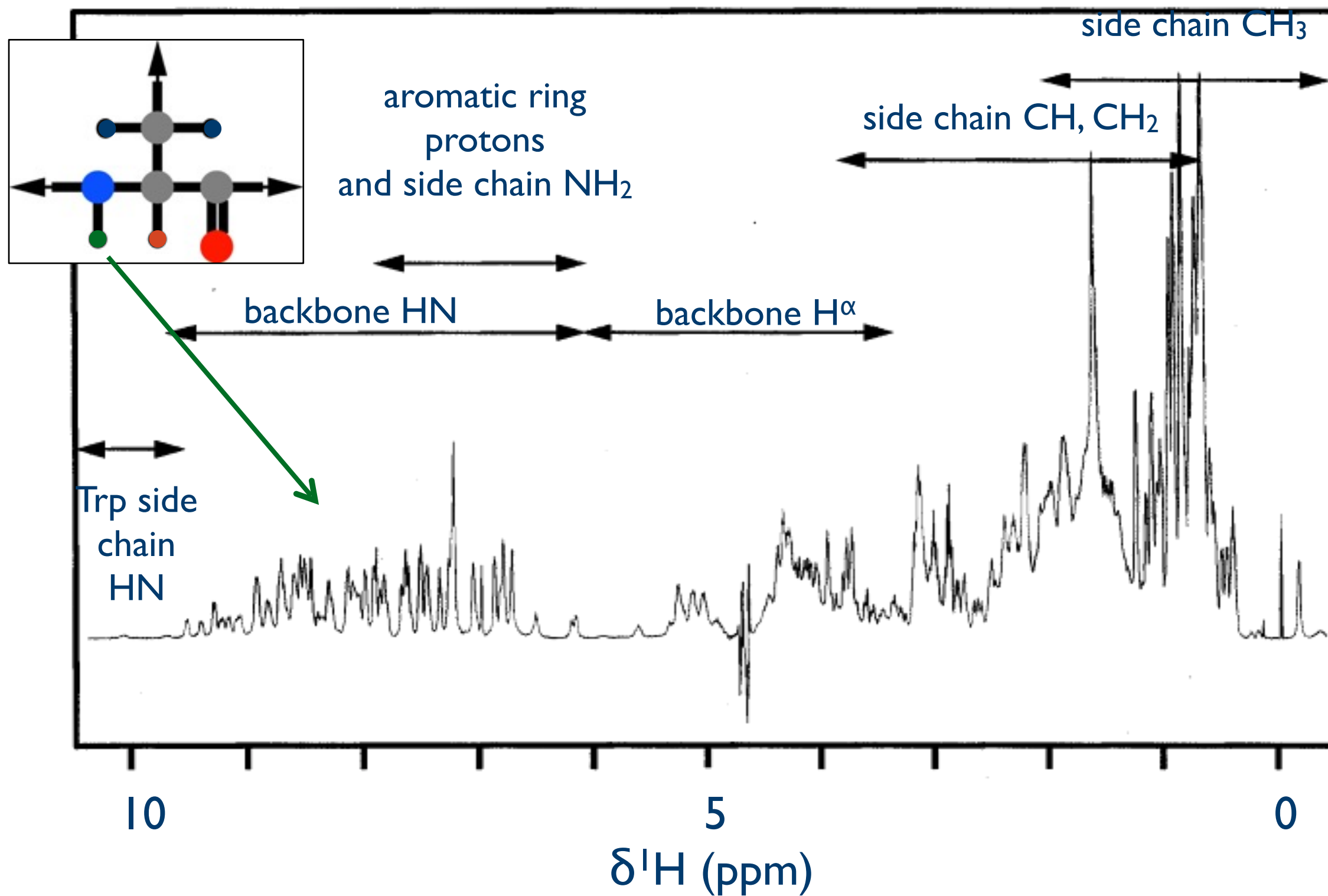


- measure frequencies of different nuclei; e.g. ^1H , ^{15}N , ^{13}C
- no diagonal peaks
- mixing not possible using NOE, only via J

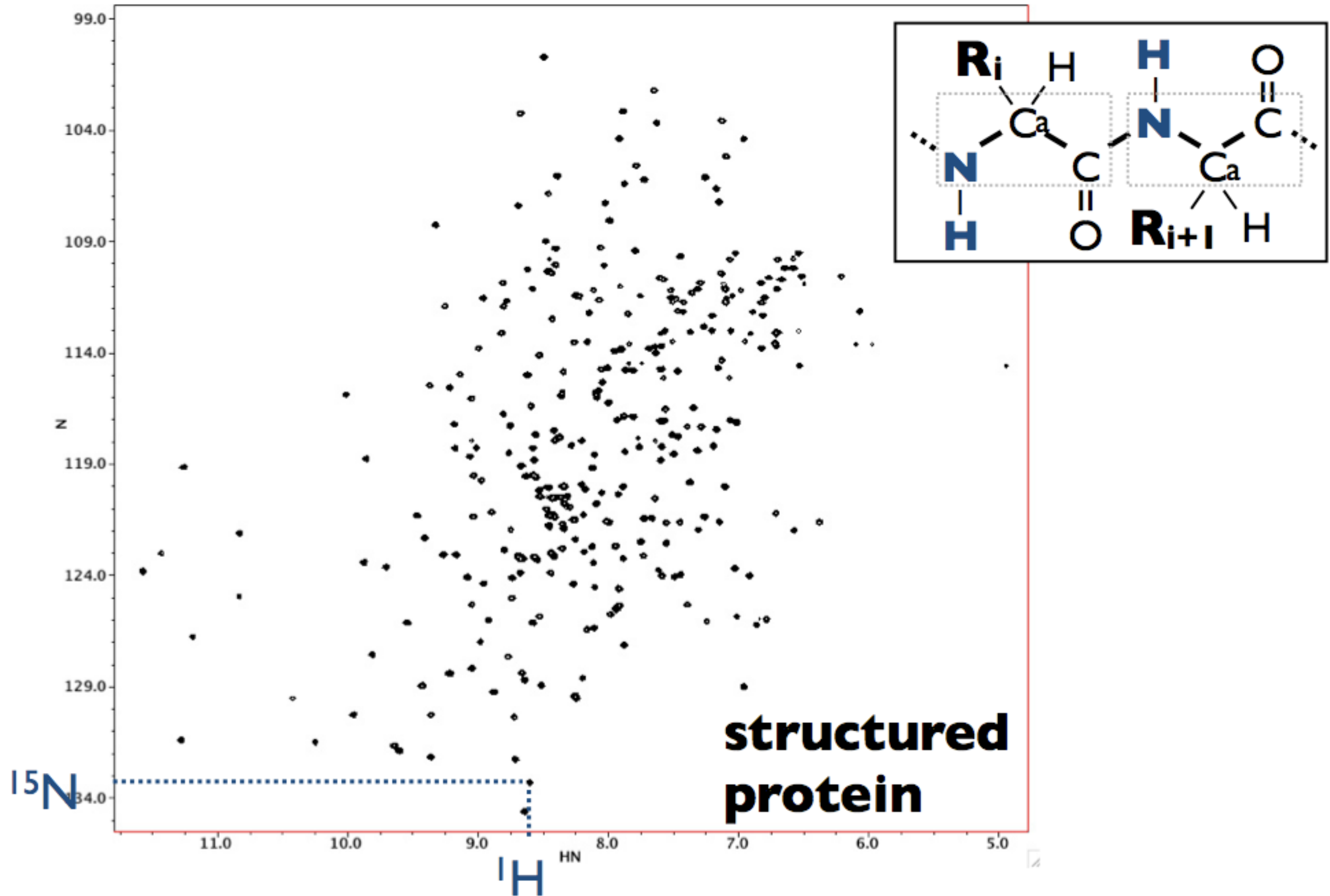
J coupling constants



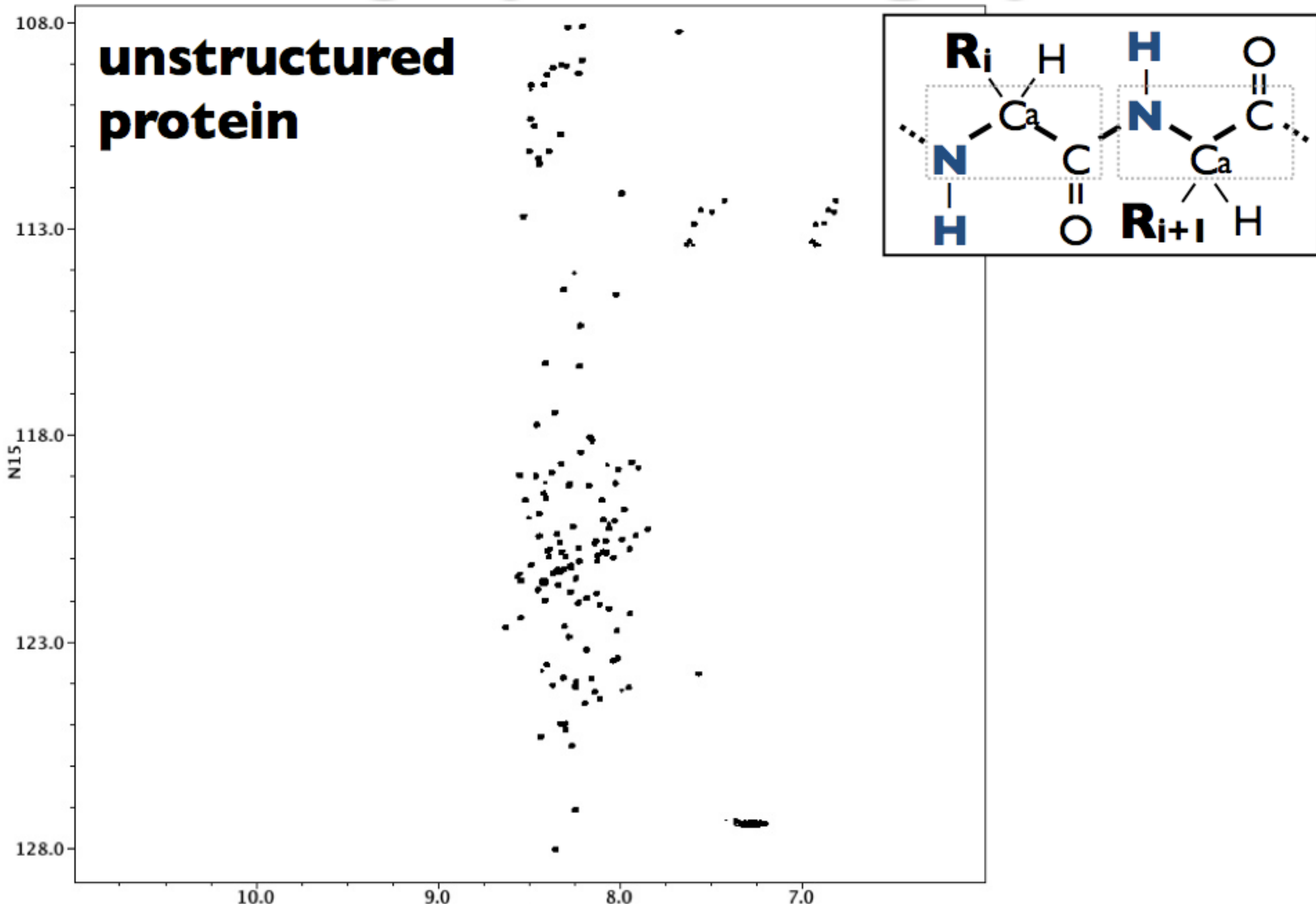
^{15}N -filter - selecting a subspectrum



^{15}N -HSQC: fingerprint



^1H - ^{15}N HSQC: '*protein fingerprint*'



Peaks not resolved in 2D

- The third frequency dimension will likely resolve all peaks

