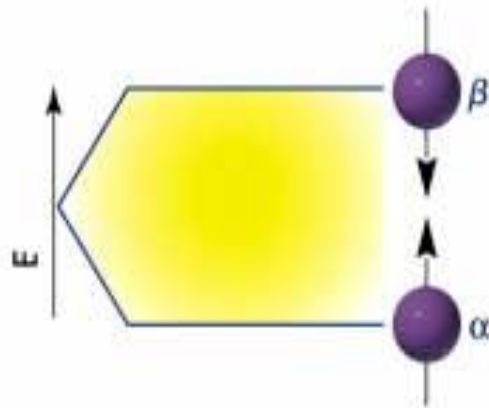
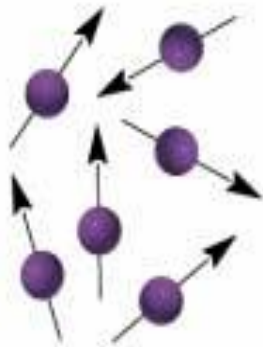


RMN Y RPE EN SISTEMAS BIOLÓGICOS

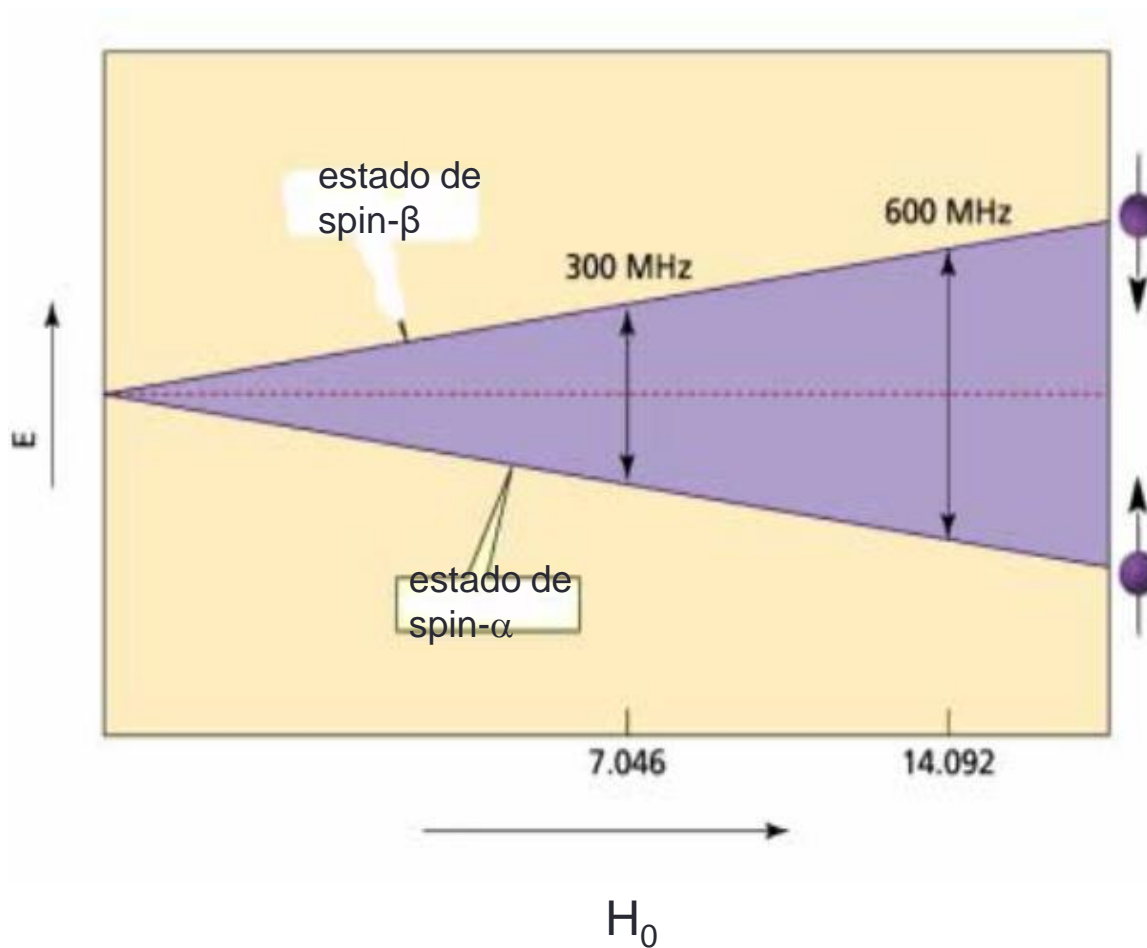
RMN

- Esta técnica espectroscópica se utiliza sólo para estudiar núcleos atómicos con un número impar de protones o neutrones (o de ambos).
- Este tipo de núcleos son magnéticamente activos, es decir poseen espín.
- En ausencia de campo magnético, los espines nucleares se orientan al azar.
- En un campo magnético los núcleos de spin positivo se orientan en la dirección del campo (α), sin embargo, aquéllos de spin negativo se orientan en contra del campo (β).



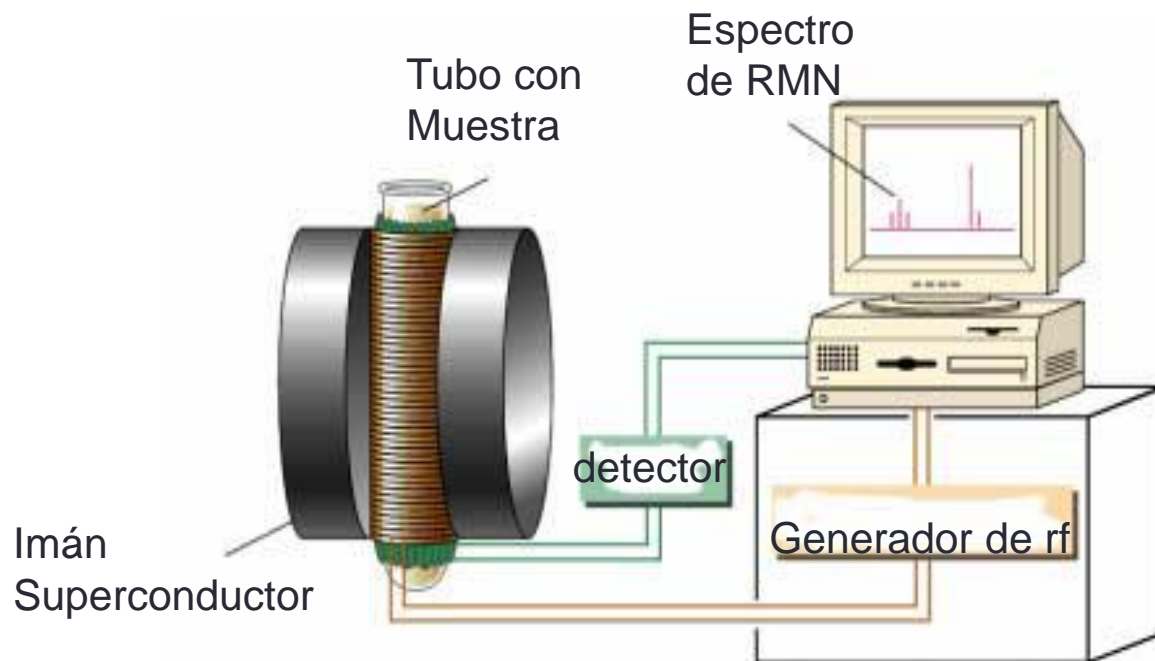
Estados de spin

La diferencia en energía entre los estado de spin depende de la fuerza del campo magnético (H_0).



$$\Delta E = h\nu = \frac{h\gamma}{2\pi} H_0$$

donde γ es el radio giromagnético



$$\delta \text{ (ppm)} = \frac{\nu \text{ muestra} - \nu \text{ referencia}}{\nu \text{ referencia}} \times 10^6$$

I = Momento angular de spin nuclear

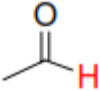
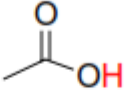
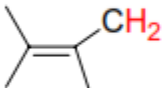
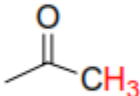
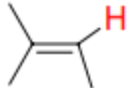
Valores de I según el número de protones y de neutrones del núcleo

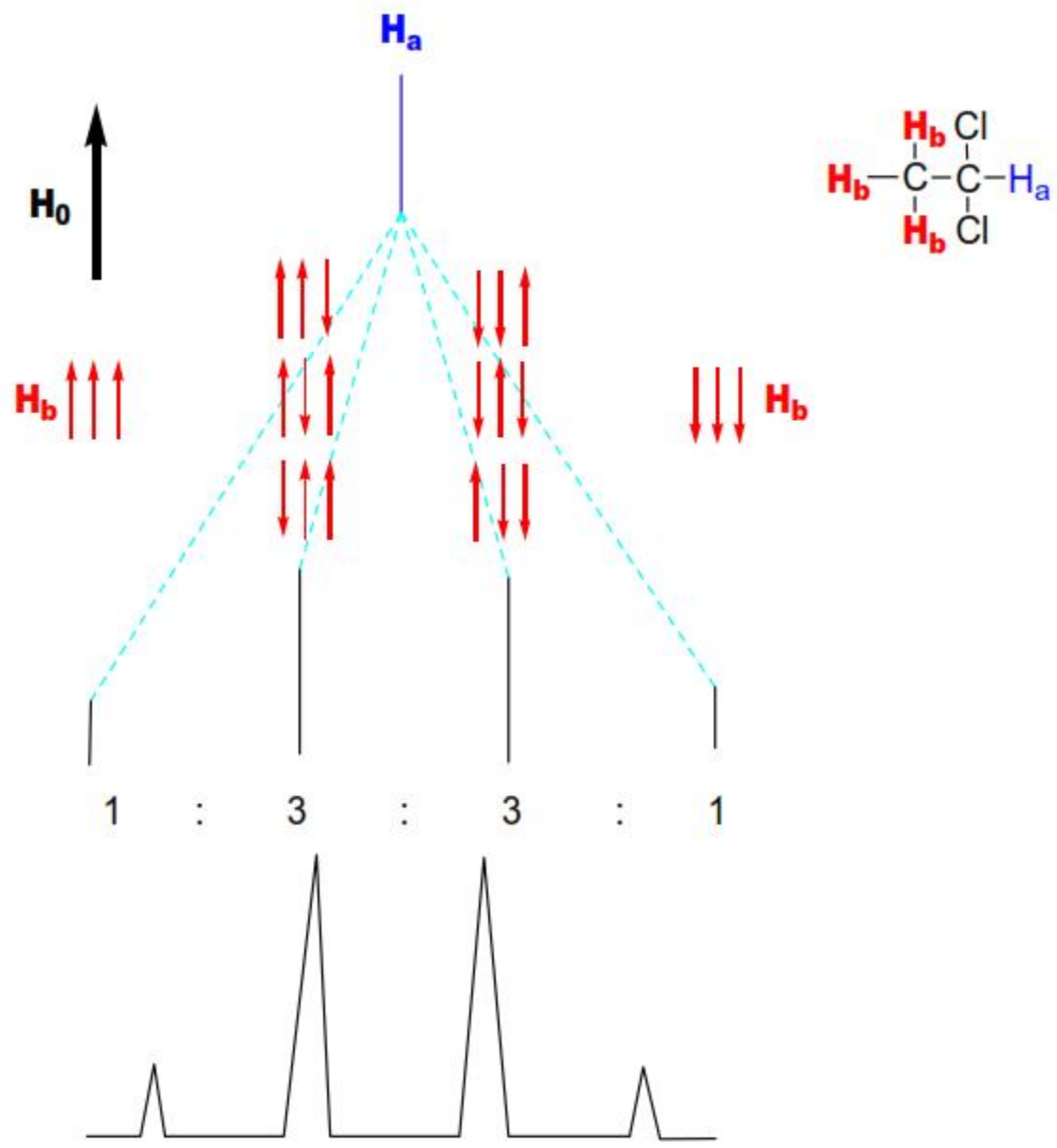
Número de protones	Número de neutrones	Valores de I
Par	Par	0
Impar	Impar	Entero (1, 2, 3...)
Par	Impar	Semientero (1/2, 3/2, 5/2,...)
Impar	Par	Semientero (1/2, 3/2, 5/2,...)

Propiedades magnéticas de diversos núcleos

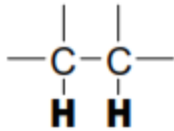
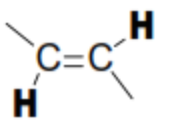
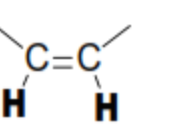
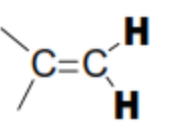
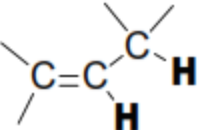
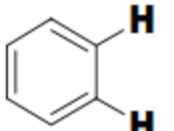
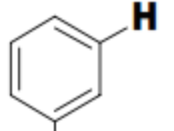
Núcleo	Abundancia isotópica %	Espín	g_N	$\gamma/(10^7 \text{ T}^{-1} \text{ s}^{-1}) \ddagger$	ν/MHz en un campo de 1T
^1H	99,985	1/2	5,5856948	26,7522	42,577
^2H	0,015	1	0,8574382	4,1066	6,536
^{13}C	1,10	1/2	1,4048236	6,7283	10,705
^{14}N	99,634	1	0,4037610	1,9338	3,076
^{15}N	0,366	1/2	-0,5663777	- 2,7126	4,315

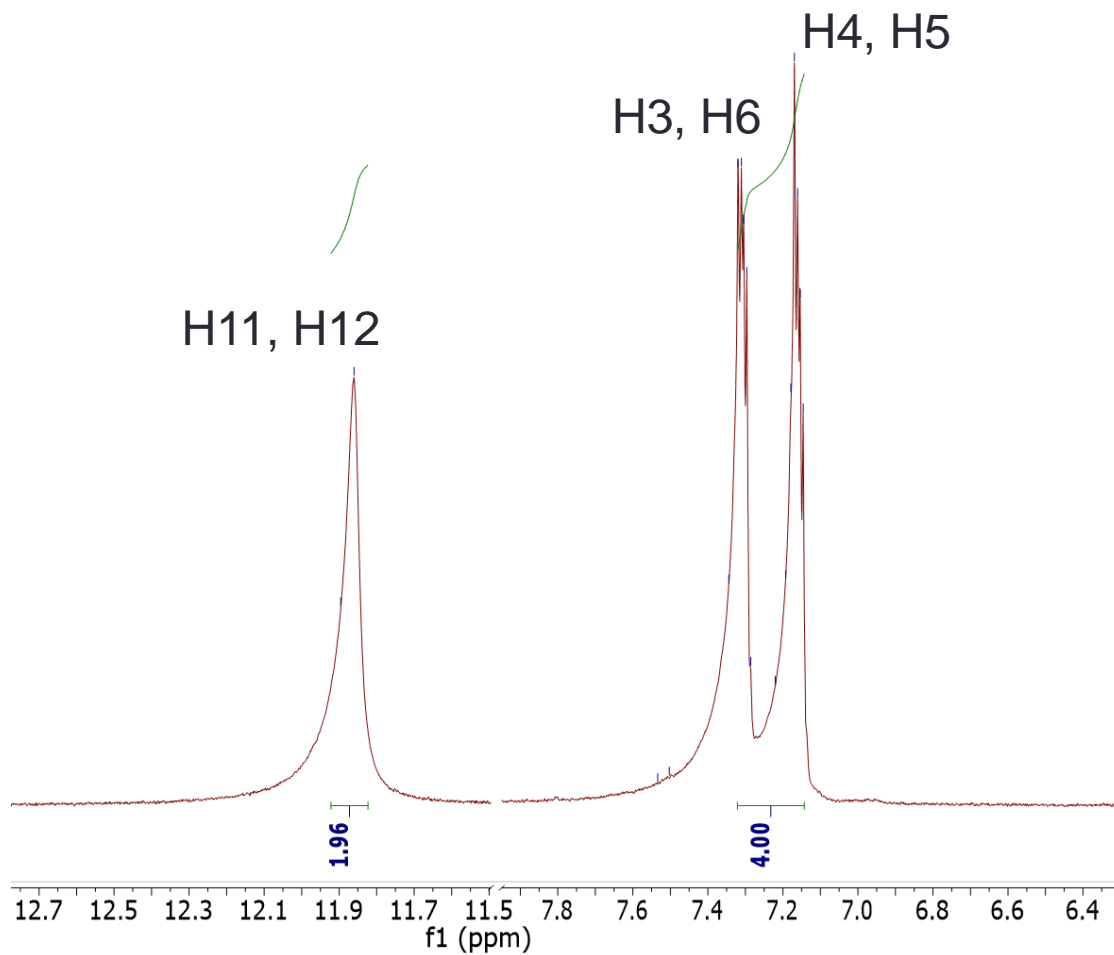
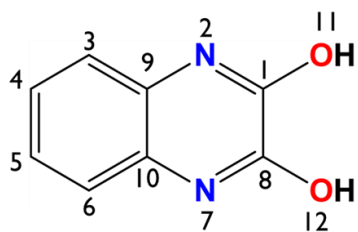
\ddagger 1T (Tesla) = 10^4 G (Gauss)

tipo de protón	Despl.químico (δ)	tipo de protón	Despl.químico (δ)
$-\text{CH}_3$	0.7-1.3		9.5-10.0
$-\text{CH}_2-$	1.2-1.4		10.0-12.0
$-\overset{ }{\text{C}}\text{H}$	1.4-1.7	$-\overset{ }{\text{C}}-\text{OH}$	1.0-6.0
	1.5-2.5	$\text{O}-\overset{ }{\text{C}}-\text{H}$	3.3-4.0
	2.1-2.6	$-\overset{ }{\text{C}}-\text{NH}_2$	1.5-4.0
$-\text{C}\equiv\text{C}-\text{H}$	2.5-3.1	$\text{Cl}-\overset{ }{\text{C}}-\text{H}$	3.0-4.0
$\text{Ar}-\text{H}$	6.0-9.0	$\text{Br}-\overset{ }{\text{C}}-\text{H}$	2.5-4.0
	4.5-6.5	$\text{I}-\overset{ }{\text{C}}-\text{H}$	2.0-4.0

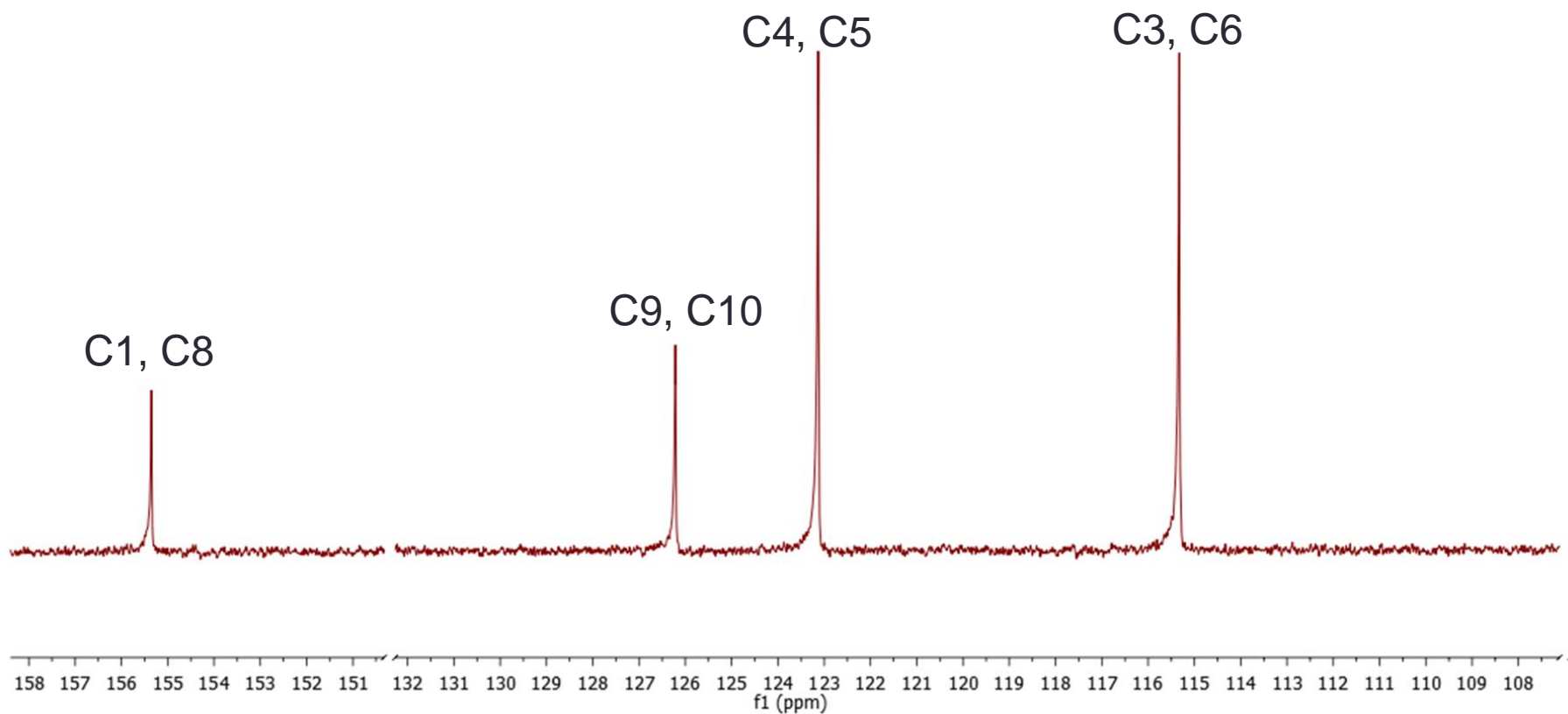
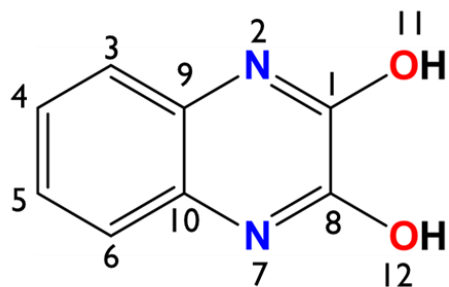


N. protones equivalentes	Número de picos (multiplicidad)	Relaciones de área (triángulo de Pascal)
0	1 (singulete)	1
1	2 (doblete)	1 1
2	3 (triplete)	1 2 1
3	4 (cuadruplete)	1 3 3 1
4	5 (quintuplete)	1 4 6 4 1
5	6 (sextuplete)	1 5 10 10 5 1

Estructura							
J (Hz)	7	15	10	2	6	8	2



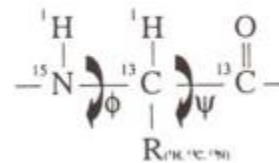
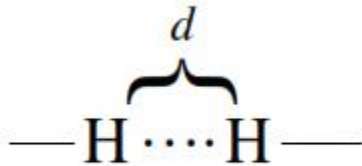
-Disolvente: DMSO-d₆ y DMF



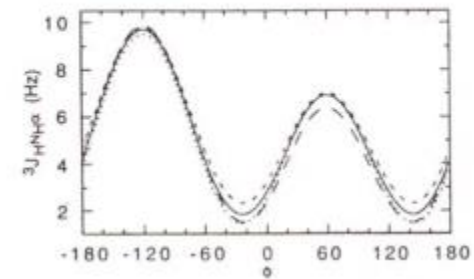
-Disolvente: DMSO- d_6 y DMF

Determinación de la Estructura de Proteínas mediante RMN

- el proceso para determinar la estructura en disolución mediante RMN implica medir muchas (cientos/miles) distancias cortas y ángulos protón-protón. Y restringir la estructura de la proteína mediante cálculos



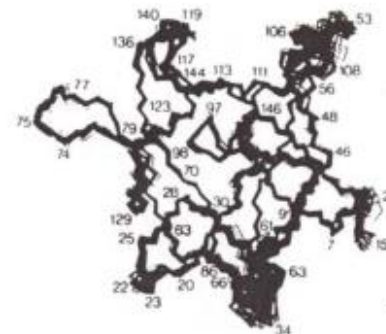
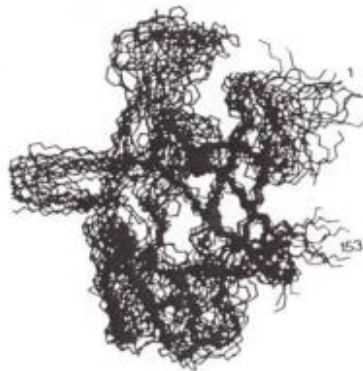
$$J = A \cos^2 \theta + B \cos \theta + C$$



interleukin 1β

536 distance restraints
RMSD (backbone atoms) = 2.0 angstroms

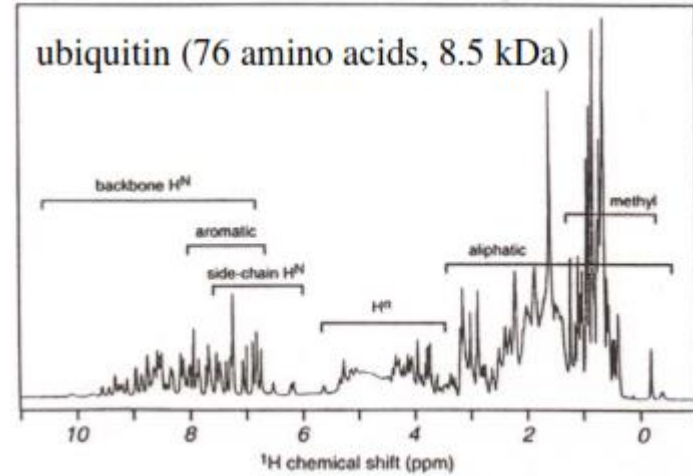
2780 distance restraints
RMSD (backbone atoms) = 0.4 angstroms



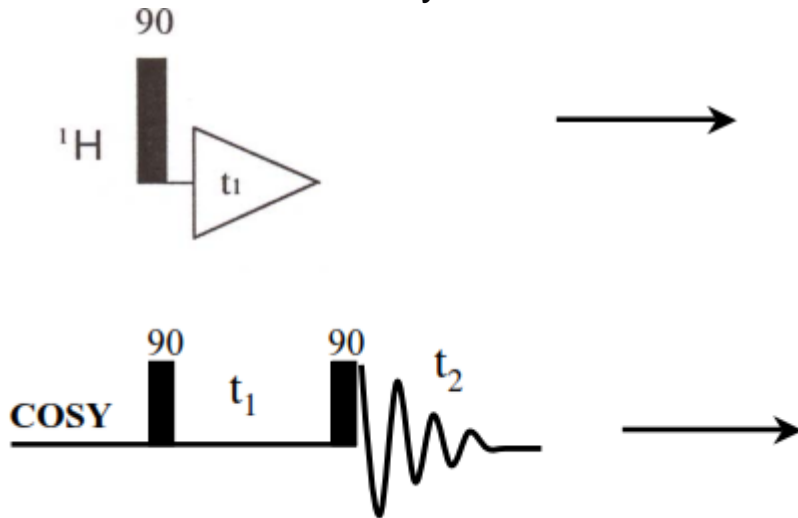
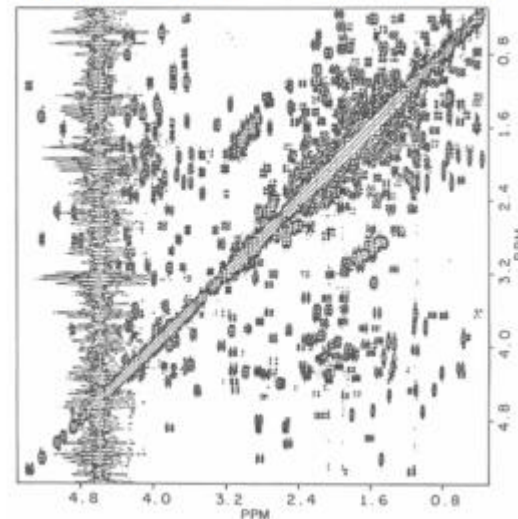
RMN ^1H de Proteínas

Los espectros RMN ^1H aún de proteínas pequeñas son imposibles de interpretar en forma completa. Por lo general solamente se puede hablar en forma aproximada de las estructuras secundarias y terciarias.

Experimento RMN ^1H en 1D



Experimento COSY ^1H cytochrome c, 12.5 kDa

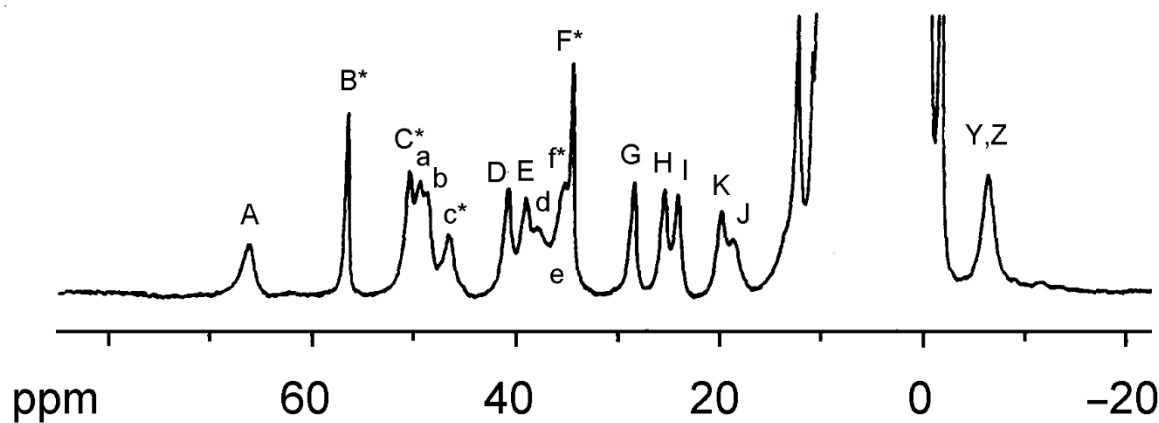
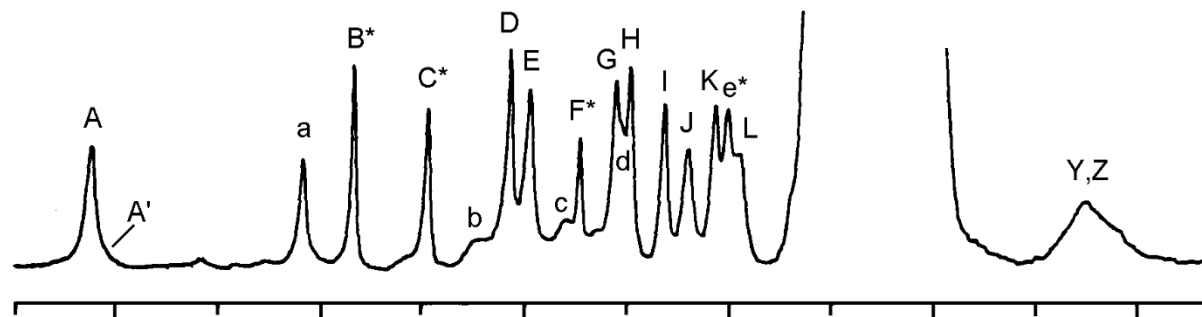
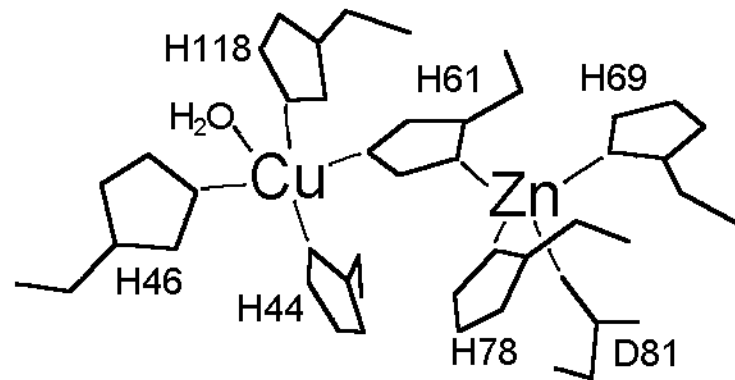


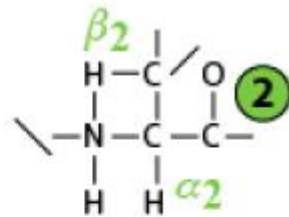
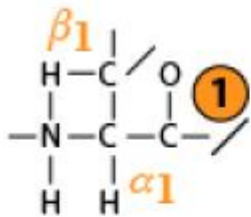
- Aun para proteínas de peso medio, incluir experimentos en 2 D no resuelve el problema

Aproximación utilizando Resonancia Triple

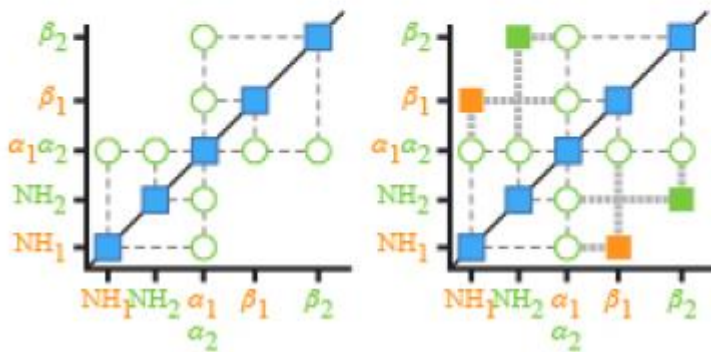
- Es aplicable a proteínas enriquecidas isotópicamente aplicable ^{13}C , ^{15}N : spin $1/2$
 - tres núcleos C, N y H
- Basada en la transferencia de la magnetización principalmente a través de los acoplamientos J a un enlace
 - proteínas de tamaño moderado (~ 20 kDa)
 - proporciona correlación selectiva del desplazamiento químico
 - se minimiza la degeneración espectral

Superóxido dismutasa de Cu-Zn

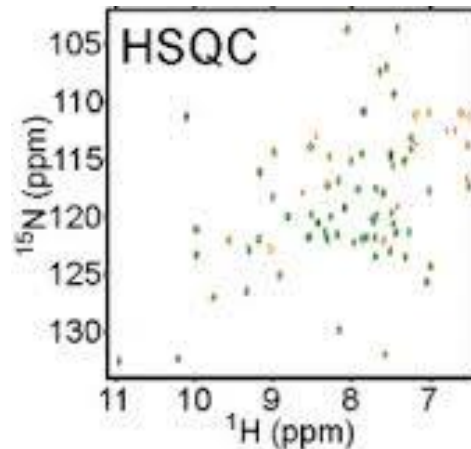




Espectro COSY (Correlation Spectroscopy)



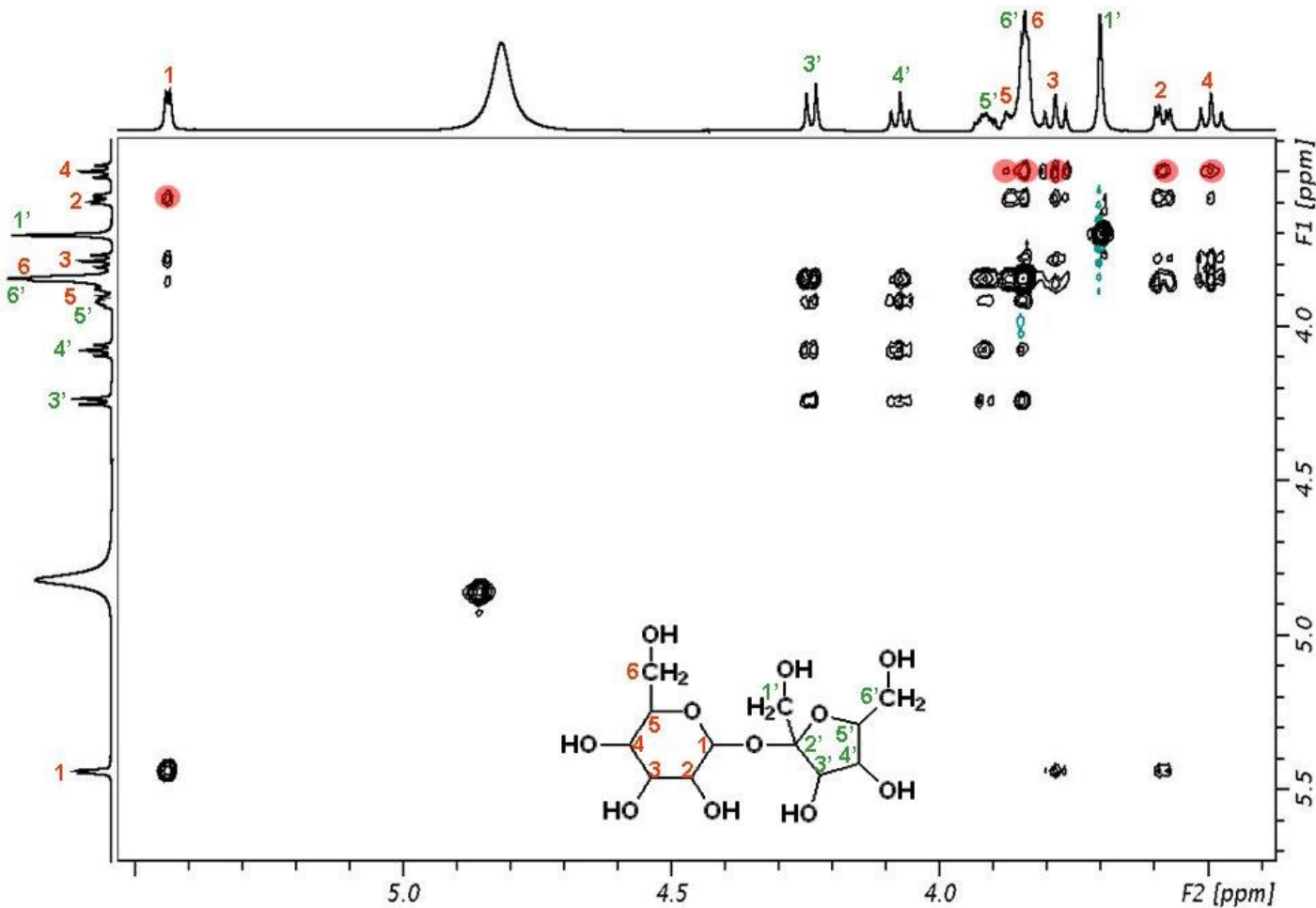
Espectro HSQC (Heteronuclear Single Quantum Correlation)



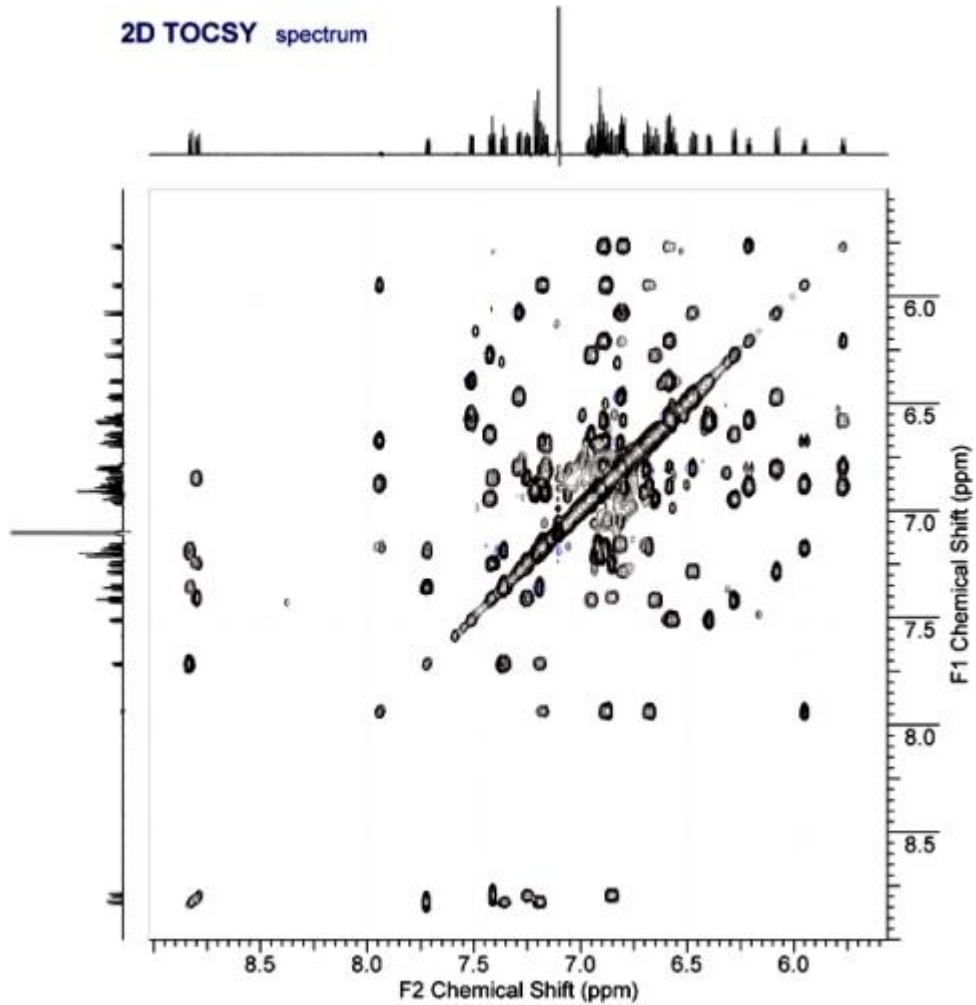
Metalloprotein Structure and Dynamics with Single Amino Acid Resolution

TOCSY (Total Correlation Spectroscopy)

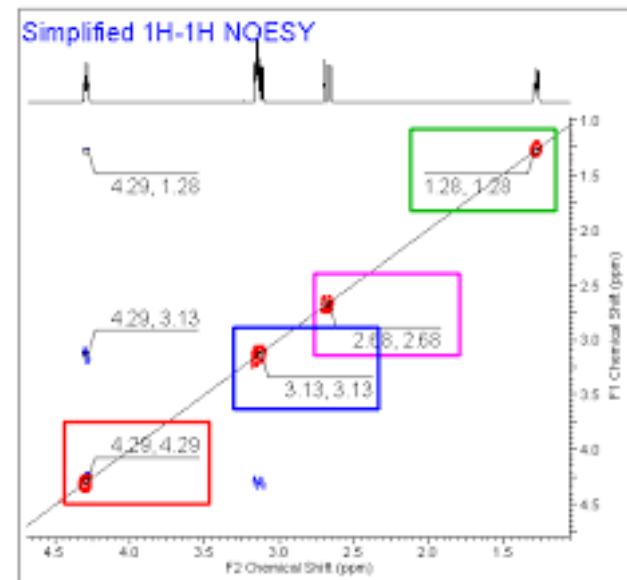
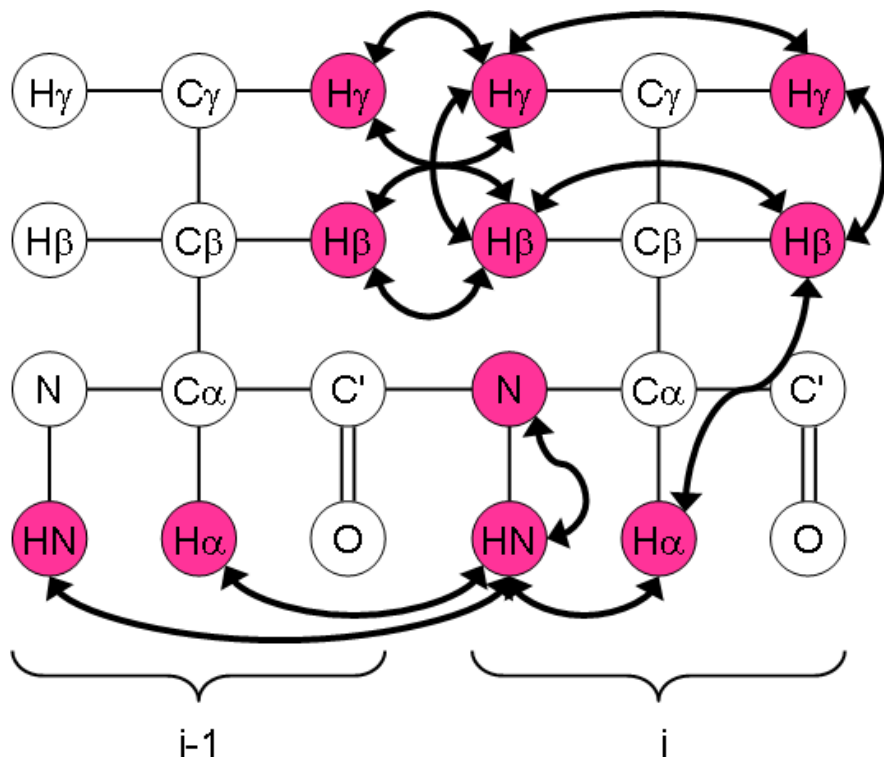
La magnetización se transfiere sucesivamente a través de hasta 5 ó 6 enlaces siempre que haya protones sucesivos acoplados.



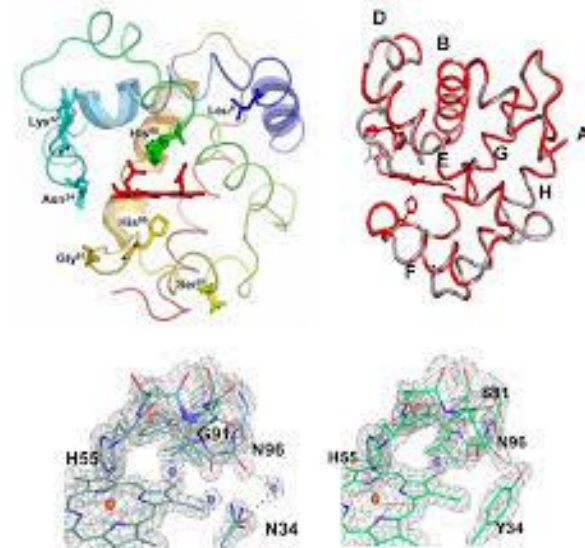
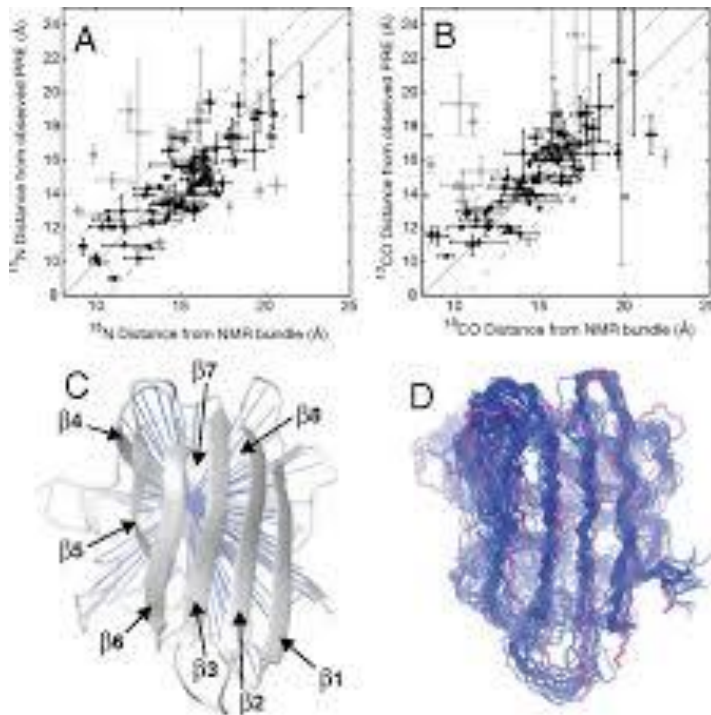
TOCSY (Total Correlation Spectroscopy)



NOESY (Nuclear Overhauser Effect Spectroscopy)



Estructura de las proteínas

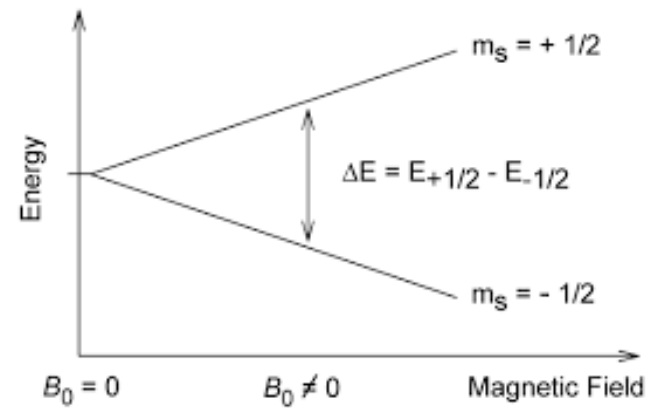
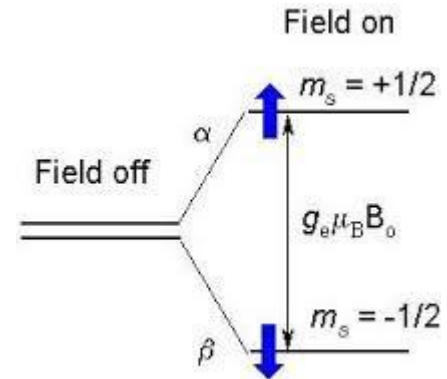
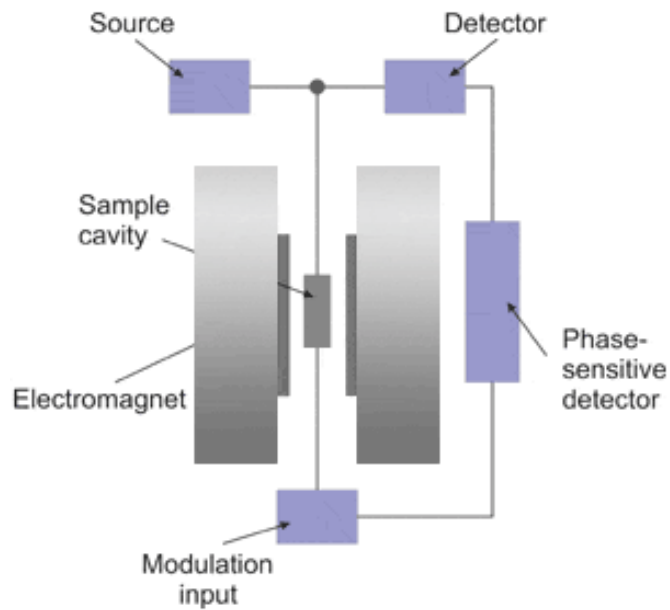


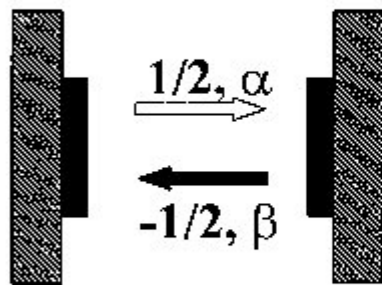
Resumen

- RMN vs cristalografía de rayos X para la determinación de estructuras de proteínas
- ¿Por qué usar RMN?
- no se pueden obtener cristales/ se desea trabajar en disolución
- se desea estudiar otras proteínas o moléculas a las que se enlaza
- se desea conocer la estabilidad
- se desean medir procesos dinámicos rápidos

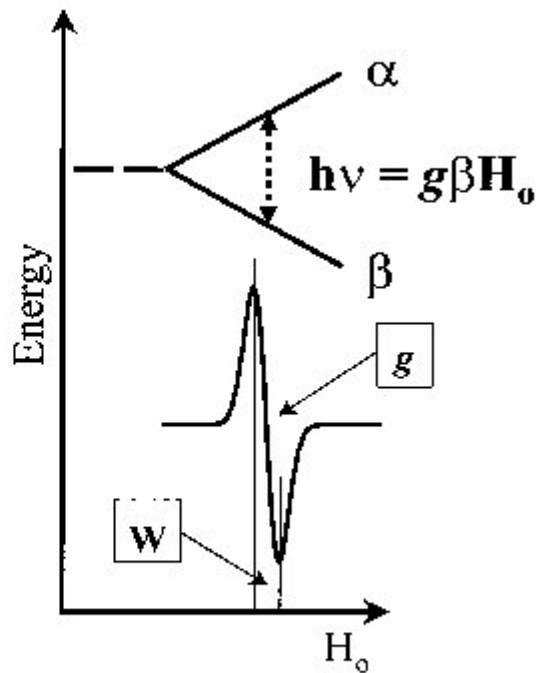


RPE



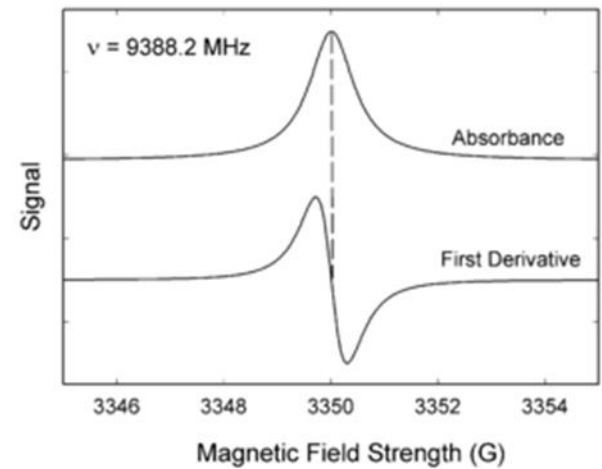


a



b

Condición de resonancia

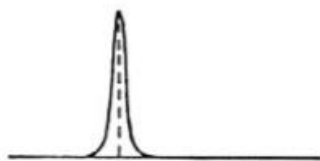


(a) ISOTROPIC

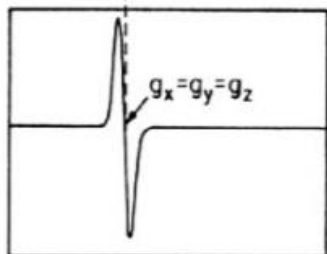
$$g_x = g_y = g_z$$



ABSORPTION

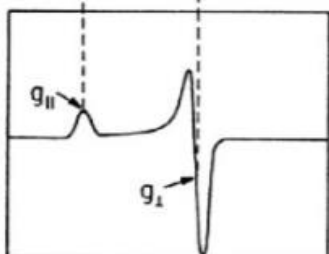
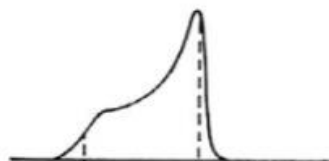


ABSORPTION
DERIVATIVE



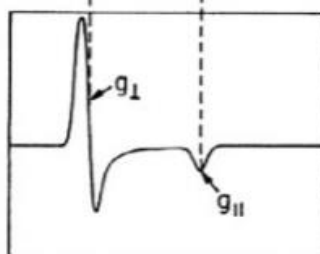
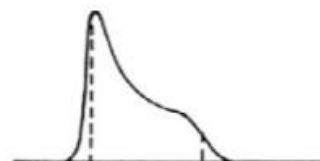
(b) AXIAL

$$g_x = g_y < g_z$$



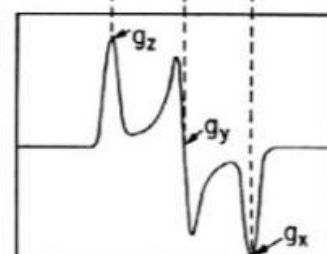
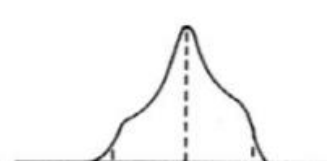
(c) AXIAL

$$g_x = g_y > g_z$$



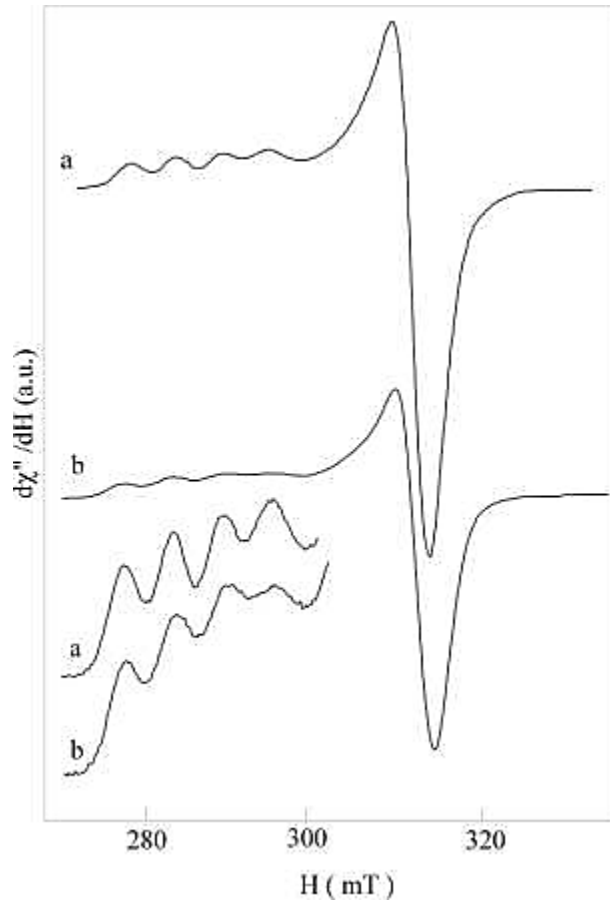
(d) RHOMBIC

$$g_x \neq g_y \neq g_z$$



MAGNETIC FIELD →

Espectro de EPR



$$\# \text{ señales} = 2(nI) + 1$$

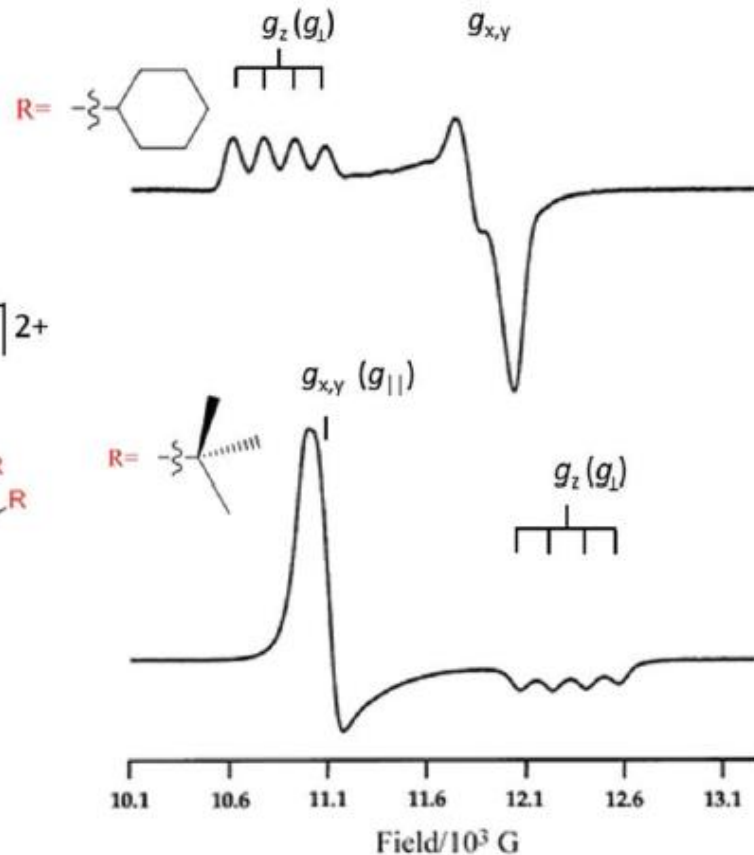
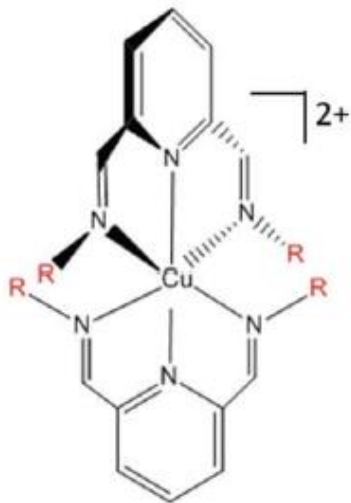
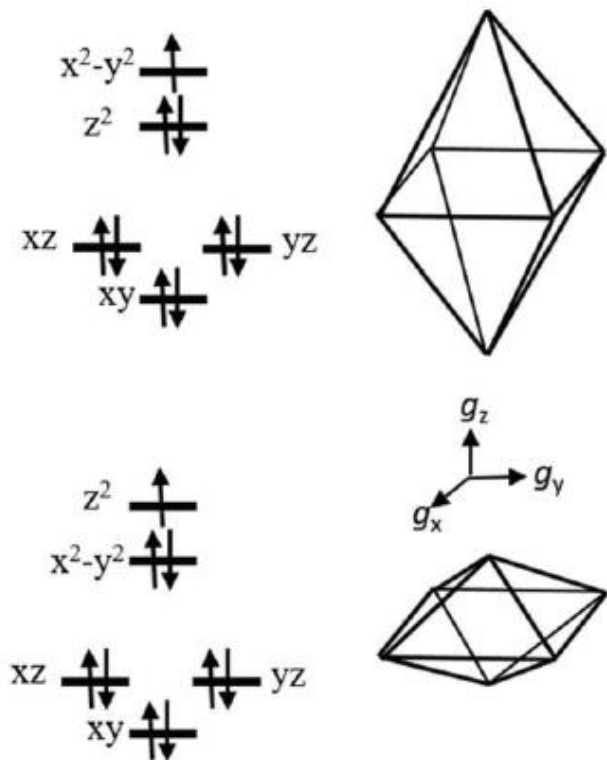
n = número de vecinos

I = spin nuclear

$${}^{63}\text{Cu} \quad I = 3/2$$

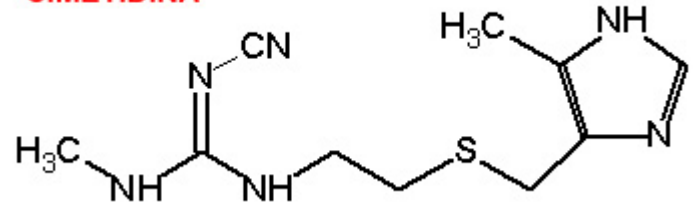
$${}^{65}\text{Cu} \quad I = 3/2$$

Espectros EPR de compuestos de cobre(II)



Disolución

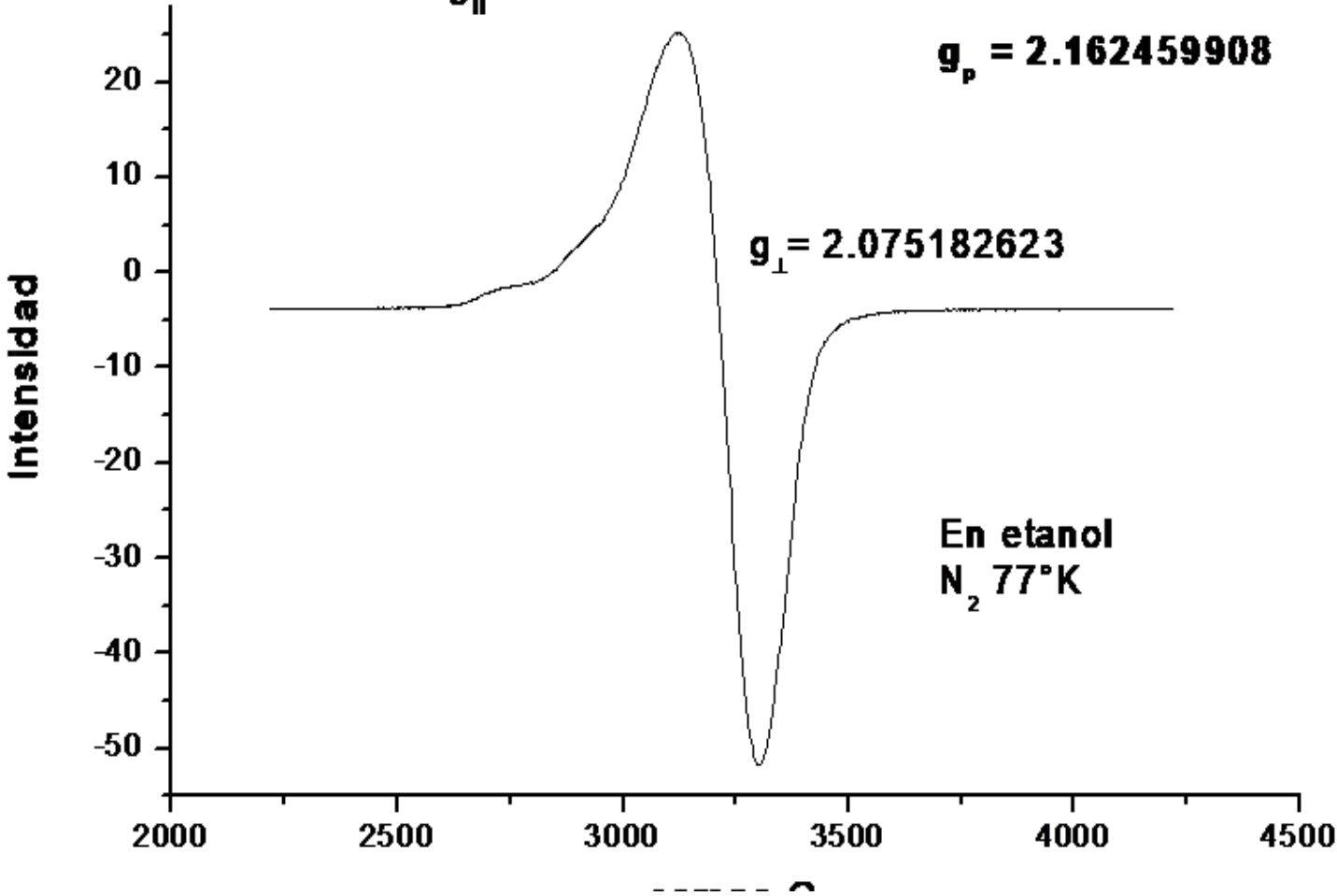
CIMETIDINA



$g_{||} = 2.249737193$

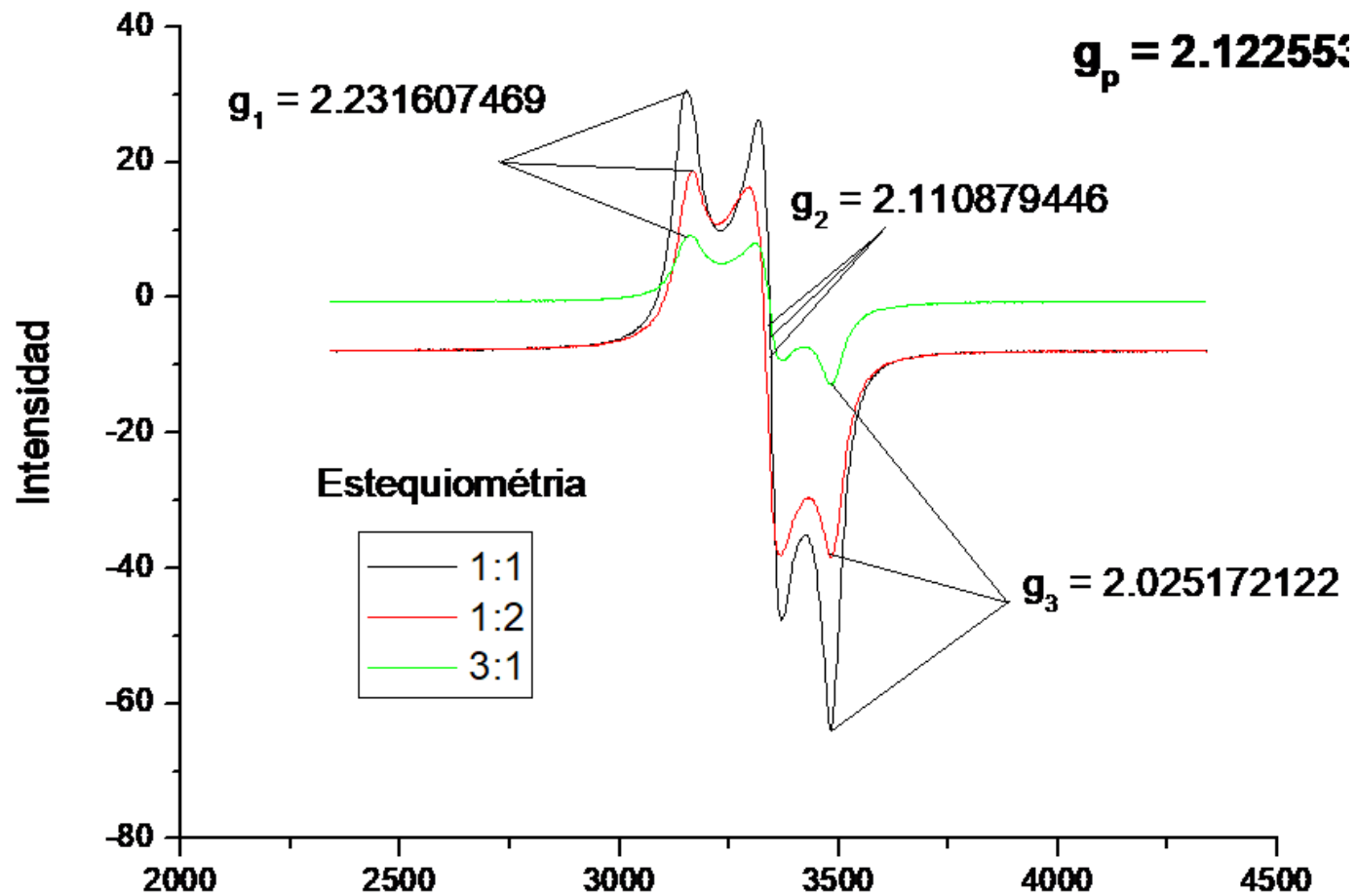
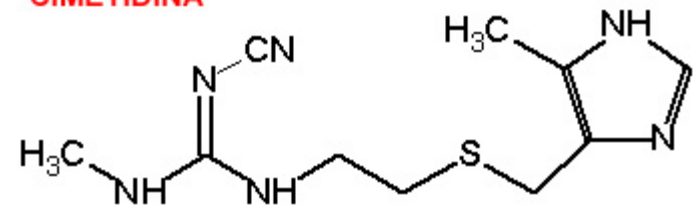
$g_p = 2.162459908$

$g_{\perp} = 2.075182623$



Estado sólido

CIMETIDINA



Espectro EPR de una ferredoxina [2Fe-2S] de espinaca

