

蛋白质核磁共振及其在生物医学中的应用

核磁组： 艾选军

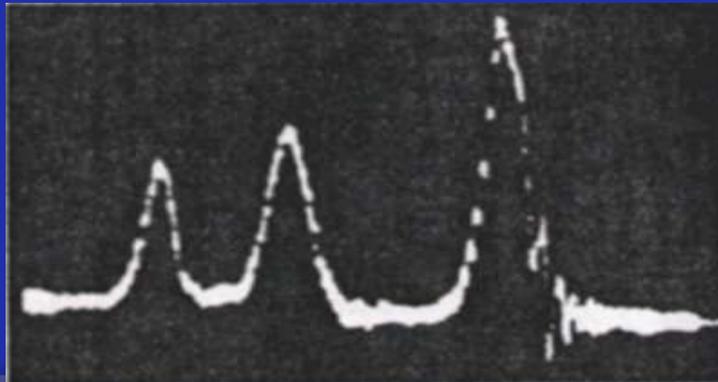
2018年9月29日

讲座主要内容

- (1) 核磁共振的历史
- (2) 核磁共振基础知识
- (3) 蛋白质核磁共振基础
- (4) 蛋白质核磁共振在生物学中的应用

核磁共振的历史

- 1922 Otto Stern发现质子磁矩
- 1939 哥伦比亚大学的Rabi等将射频作用于Stern-Gerlach装置产生的氢分子束，观测到可检测的分子束偏移
- 1946 哈佛大学的Purcell等人报道了石蜡中核的共振吸收现象
斯坦福大学的Bloch等发现了液态水中核的共振
- 1949 观察到化学位移现象 (Proctor, Yu)

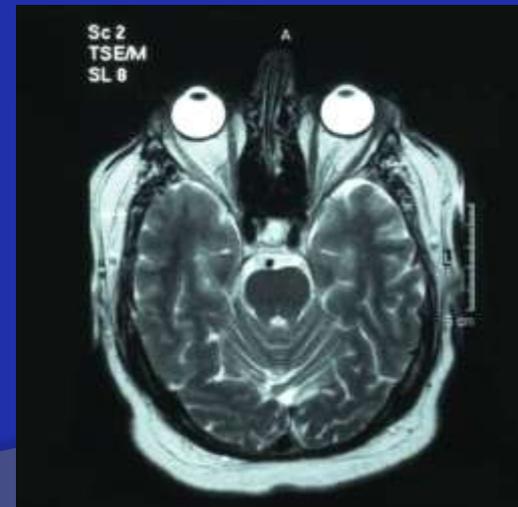


1955 Solomon等观察到NOE (Nuclear Overhauser Effect)

1966 ETH的Ernst和Anderson实现了
傅立叶变换核磁共振
(Fourier Transform NMR)

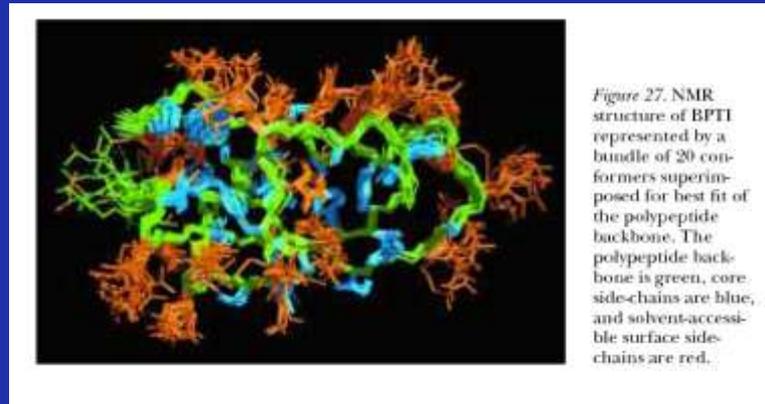


1973 Mansfield获得磁共振成像图



1970s Jeener, Ernst 提出/实现了二维核磁共振 (two-dimension NMR)

1985 Wüthrich由基于NOE的距离限制条件得到了第一个 (小) 蛋白BPTI的溶液结构



1987/8 三维核磁共振, ^{13}C , ^{15}N 同位素标记

1990s 残存偶极耦合 (Residual Dipolar Couplings)
 T_1/T_2 (各向异性扩散)
交叉相关弛豫 (Cross-correlated relaxation)
TROSY (Transverse Relaxation Optimized Spectroscopy)
CRINEPT (Cross-Correlated Relaxation N-Enhanced Polarization Transfer)

NMR Nobel Prize Laureates



Otto Stern
1943年物理奖



Isidor Isaac Rabi
1944年物理奖



Edward Mills Purcell
1952年物理奖



Felix Bloch
1952年物理奖



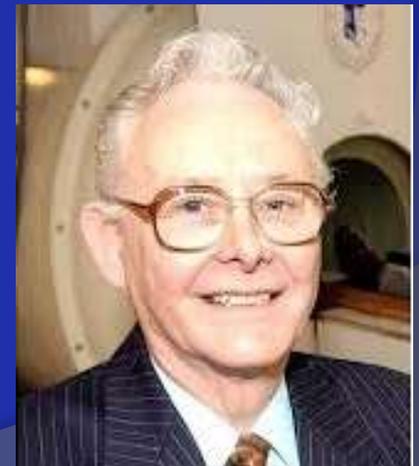
Richard R. Ernst
1991年化学奖



Kurt Wüthrich
2002年化学奖



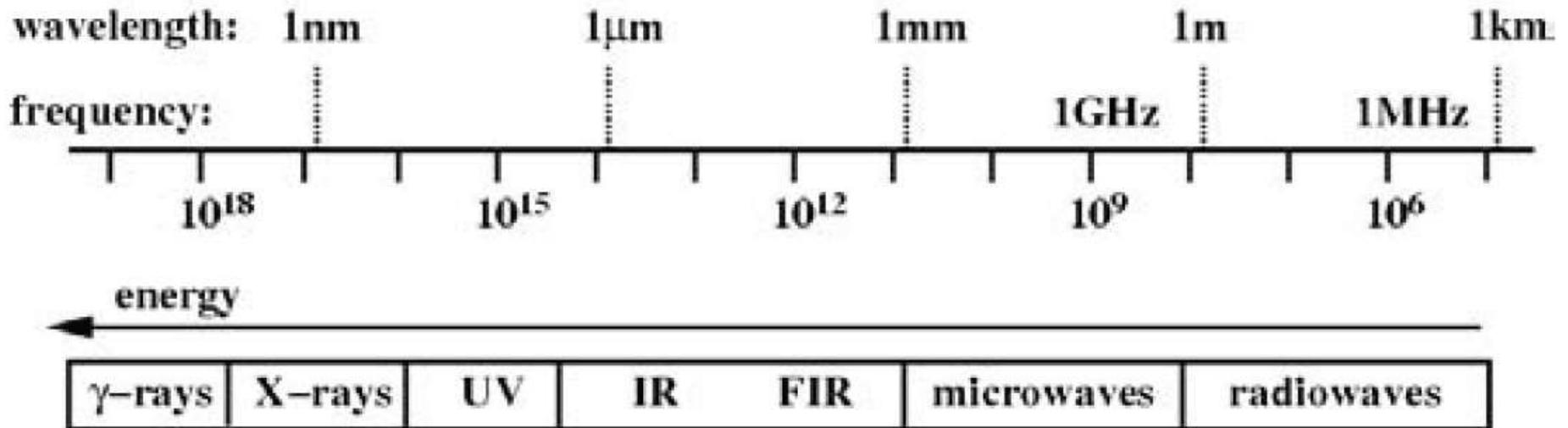
Paul Lauterbur
2003年医学奖



Peter Mansfield
2003年医学奖

核磁共振基础知识

核磁共振的频率范围



(核磁共振基本原理将另开讲座)

核磁共振可检测核

Table 1.1 Nuclei of Major Interest to NMR Spectroscopists

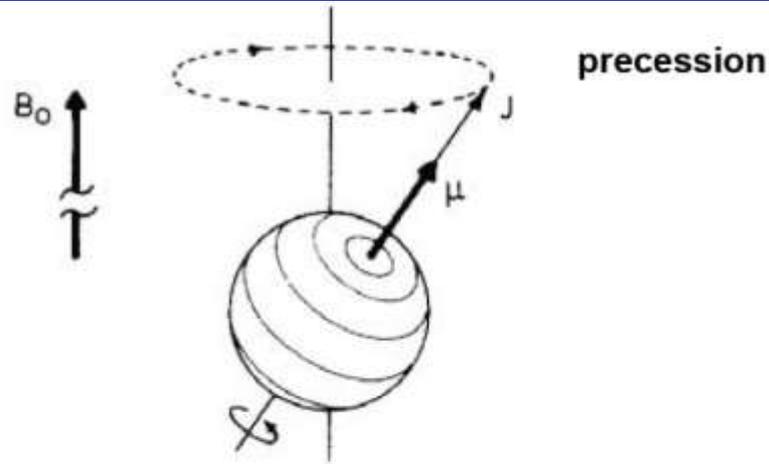
Iostope	Abundance (%)	Z	Spin	μ^2	$\gamma \times 10^{-8b}$	Relative ^c sensitivity	ν_0 at 1T(MHz)	At 7.04T
¹ H	99.9844	1	1/2	2.7927	2.6752	1.000	42.577	300
² H	0.0156	1	1	0.8574	0.4107	0.00964	6.536	46
¹⁰ B	18.83	5	3	1.8006	0.2875	0.0199	4.575	
¹¹ B	81.17	5	3/2	2.6880	0.8583	0.165	13.660	
¹³ C	1.108	6	1/2	0.7022	0.6726	0.0159	10.705	75.4
¹⁴ N	99.635	7	1	0.4036	0.1933	0.00101	3.076	
¹⁵ N	0.365	7	1/2	-0.2830	-0.2711	0.00104	4.315	30.4
¹⁹ F	100	9	1/2	2.6273	2.5167	0.834	40.055	282.3
²⁹ Si	4.70	14	1/2	-0.5548	-0.5316	0.0785	8.460	
³¹ P	100	15	1/2	1.1305	1.0829	0.0664	17.235	121.4

a Magnetic moment in units of the nuclear magneton, $eh/(\mu M_p c)$

b Magnetogyric ratio in SI units

c For equal numbers of nuclei at constant field

核自旋



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

$$|\mu| = \gamma \hbar \sqrt{I(I+1)}$$

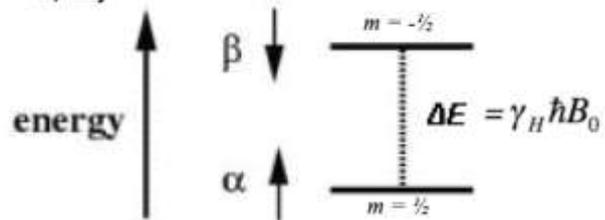
I = quantum number

$$\mu_z = \gamma \hbar m$$

$m = I, I-1, I-2 \dots -I$ = allowed states

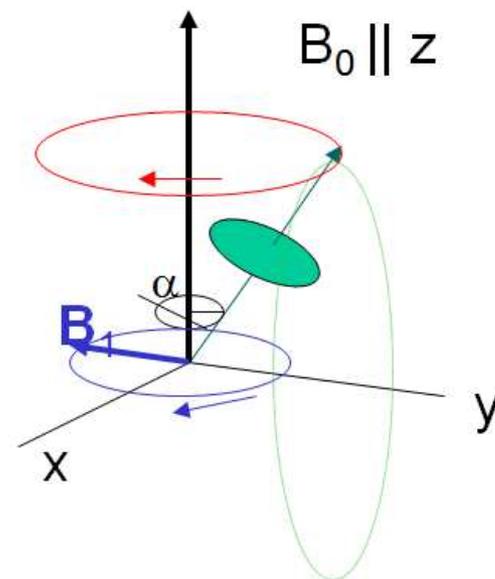
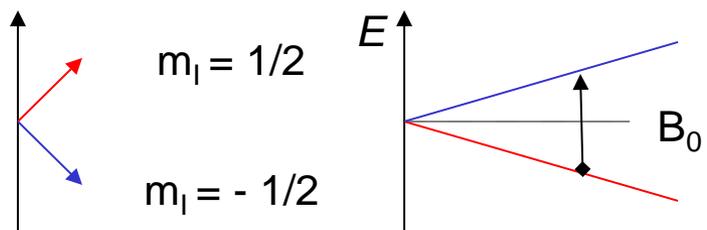
Larmor frequency、进动与张动

^1H ($I = 1/2$)



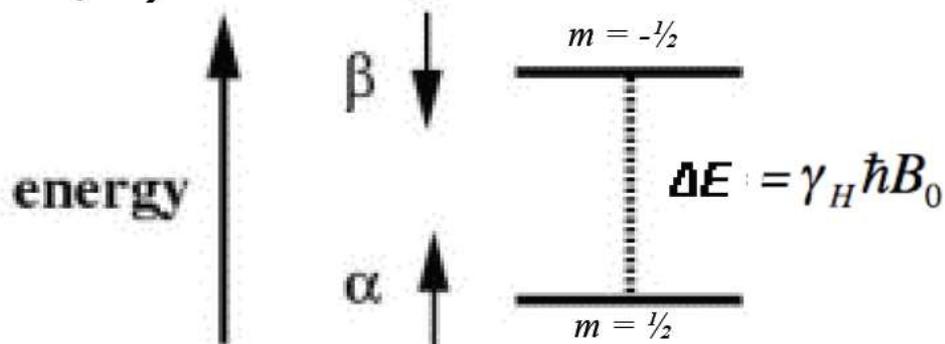
Larmor frequency

$$\omega_H = \gamma_H B_0 = 2\pi\nu_H$$



Boltzman分布（常规核磁共振的灵敏度）

^1H ($I = 1/2$)

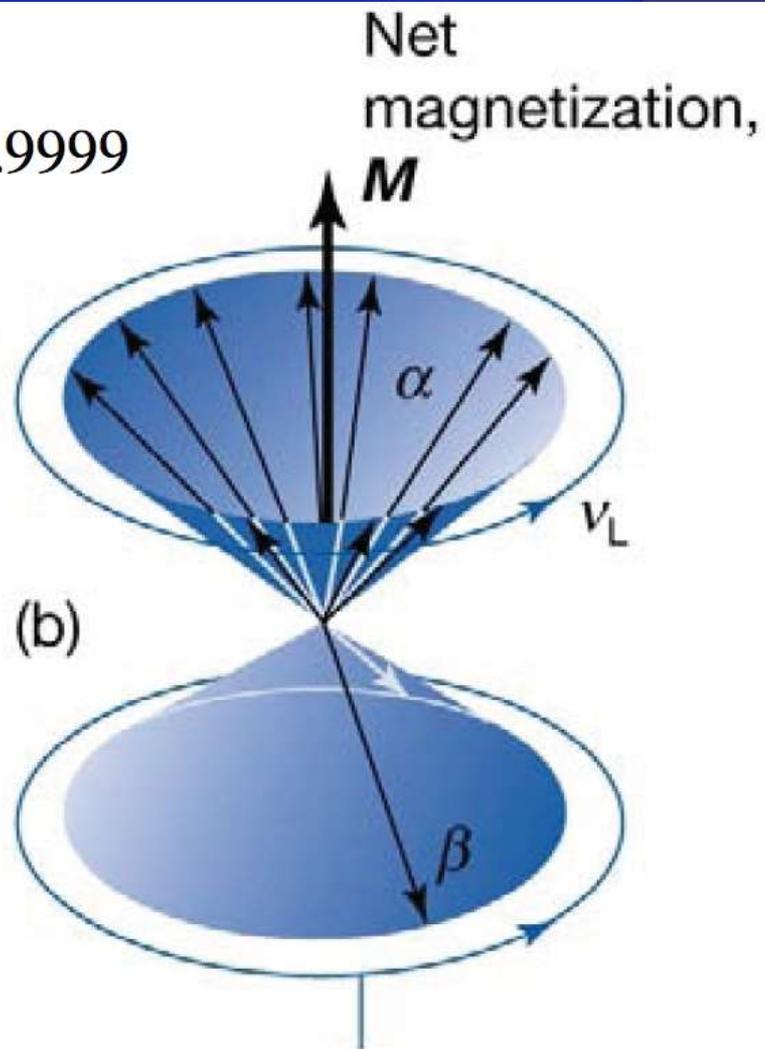
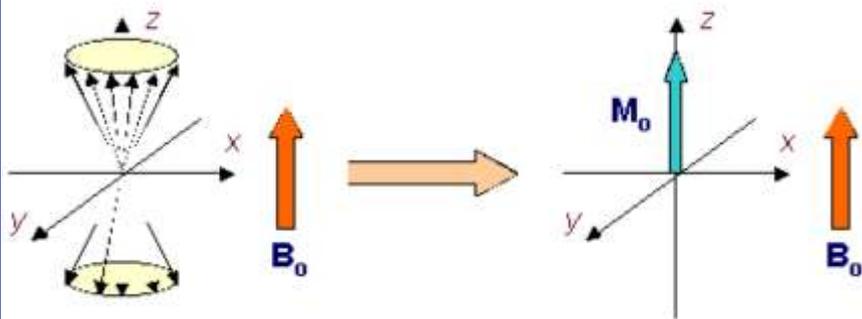


$$\frac{n_{\beta}}{n_{\alpha}} = \exp\left(-\frac{\Delta E}{k_B T}\right) = \exp\left(-\frac{\gamma_H \hbar B_0}{k_B T}\right) = 0.9999$$

体磁化率

$$\frac{n_{\beta}}{n_{\alpha}} = \exp\left(-\frac{\Delta E}{k_B T}\right) = \exp\left(-\frac{\gamma_H \hbar B_0}{k_B T}\right) = 0.9999$$

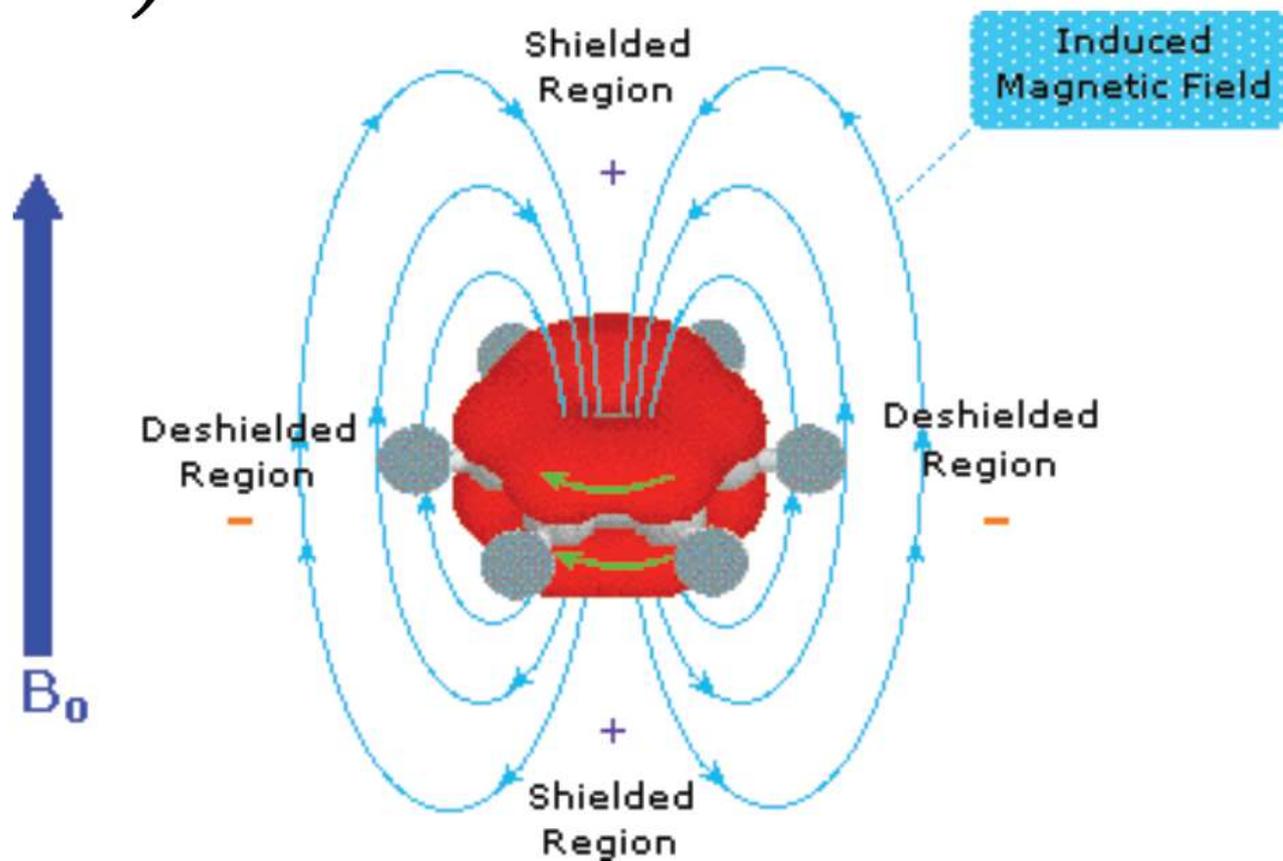
Classical view



化学屏蔽

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

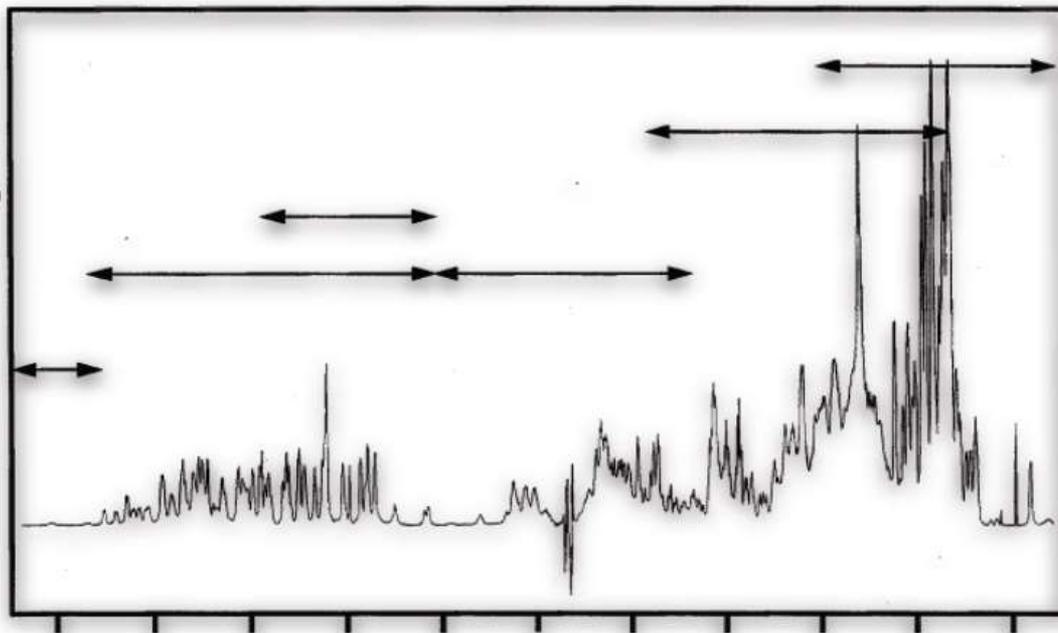
Chemical shielding



化学位移

Chemical shift:

$$-\delta\sigma_{\text{iso}} [\text{Hz}] = \nu_{\text{obs}} - \nu_0$$



$$-\delta\sigma_{\text{iso}} [\text{ppm}] = (\nu_{\text{obs}} - \nu_0) / (\nu_0 \cdot 10^{-6})$$

- ppm: parts per million
- ppm value is not field dependent

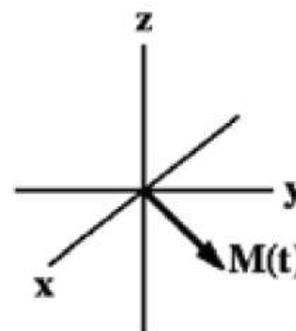
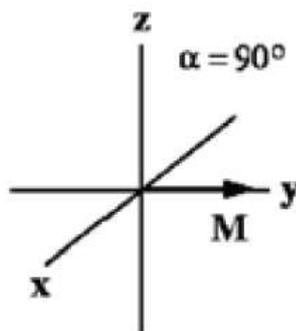
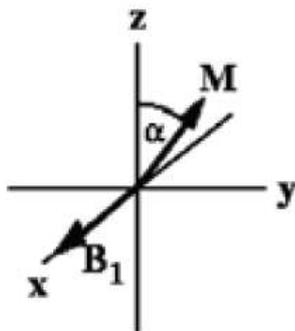
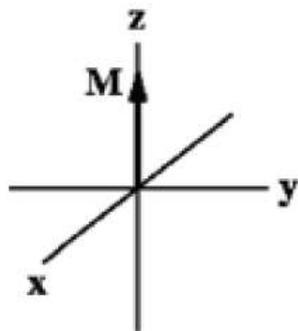
14 Tesla: $\nu_{\text{H}} = 600 \text{ MHz}$
→ 1 ppm = 600 Hz (^1H)

21 Tesla: $\nu_{\text{H}} = 900 \text{ MHz}$
→ 1 ppm = 900 Hz (^1H)

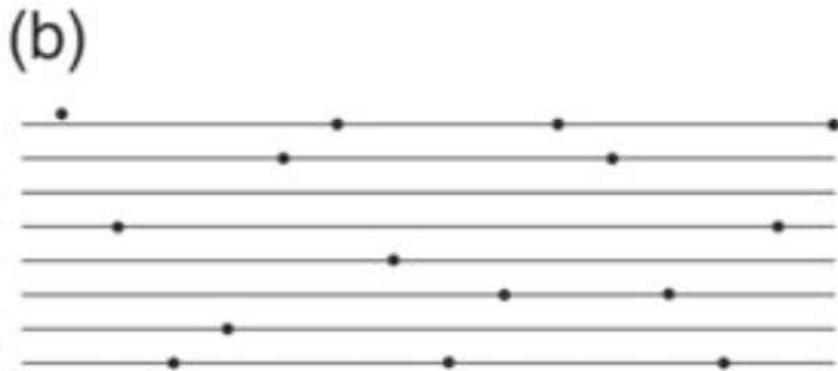
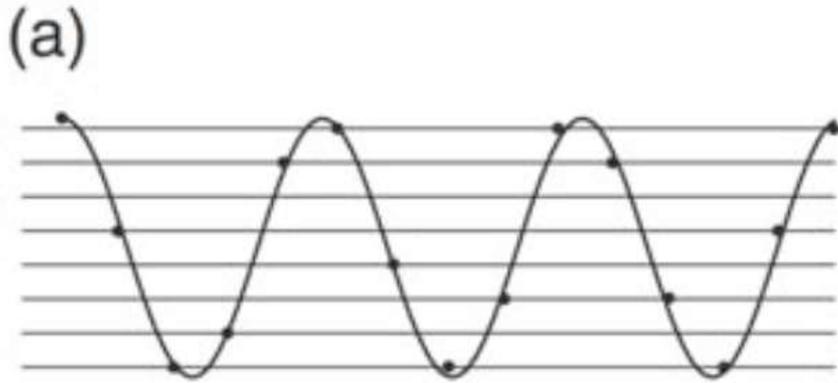
射频脉冲



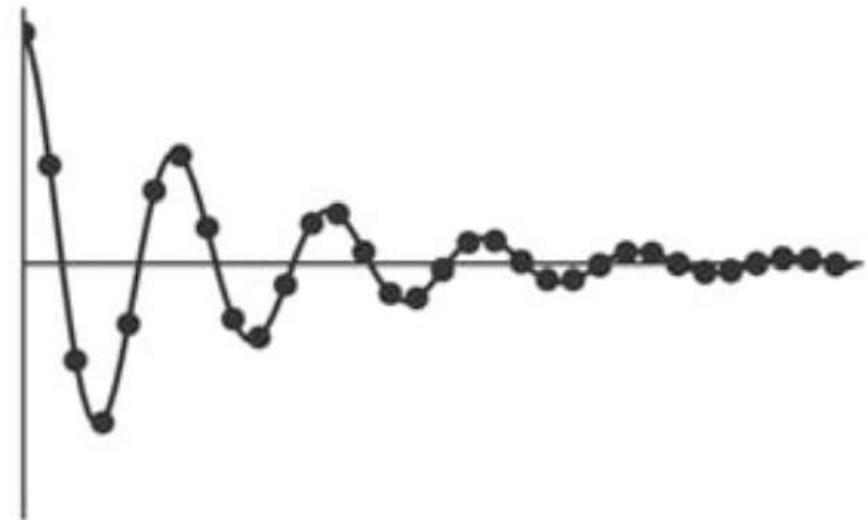
Observe with the Larmor frequency
→ "rotating frame"



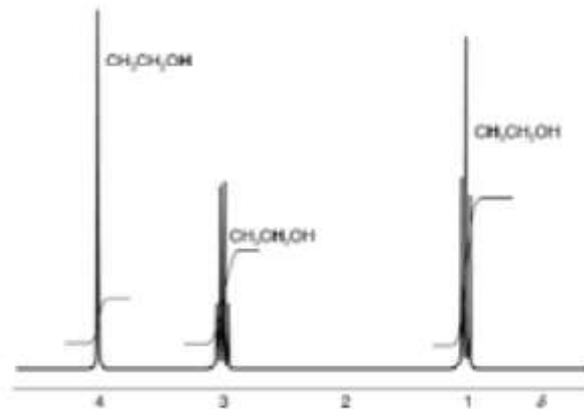
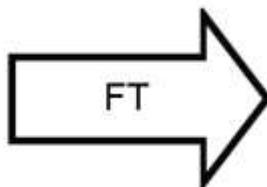
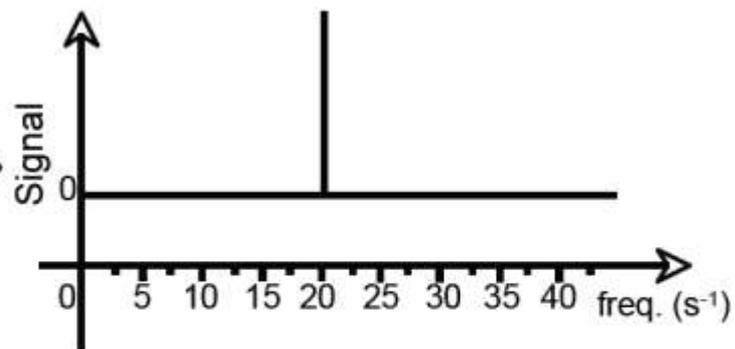
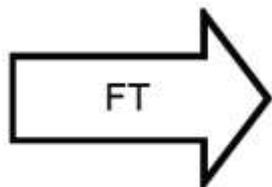
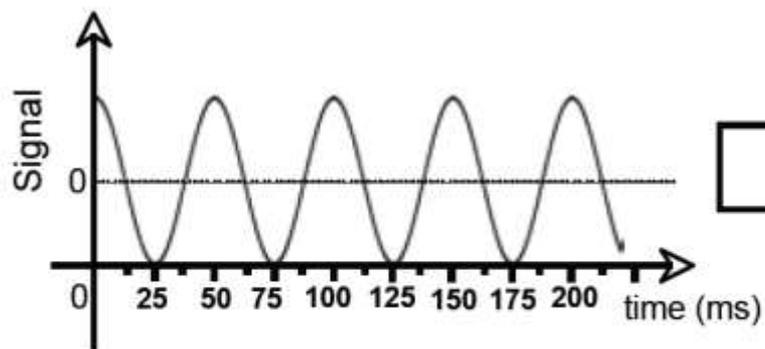
自由感应衰减、Nyquist采样定律



Free Induction Decay (**FID**)



傅立叶变换



- **NMR Relaxation**

- Restoring Boltzmann equilibrium

- **T2-relaxation**

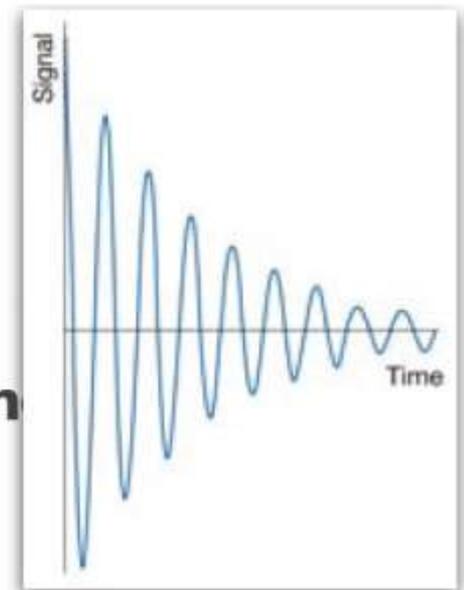
- disappearance of transverse (x,y) magnetization

- $1/T2 \sim$ signal line-width

- **T1-relaxation**

- build-up of longitudinal (z) magnetization

- determines how long you should wait for the next experiment



NMR谱质量

- **Sensitivity**

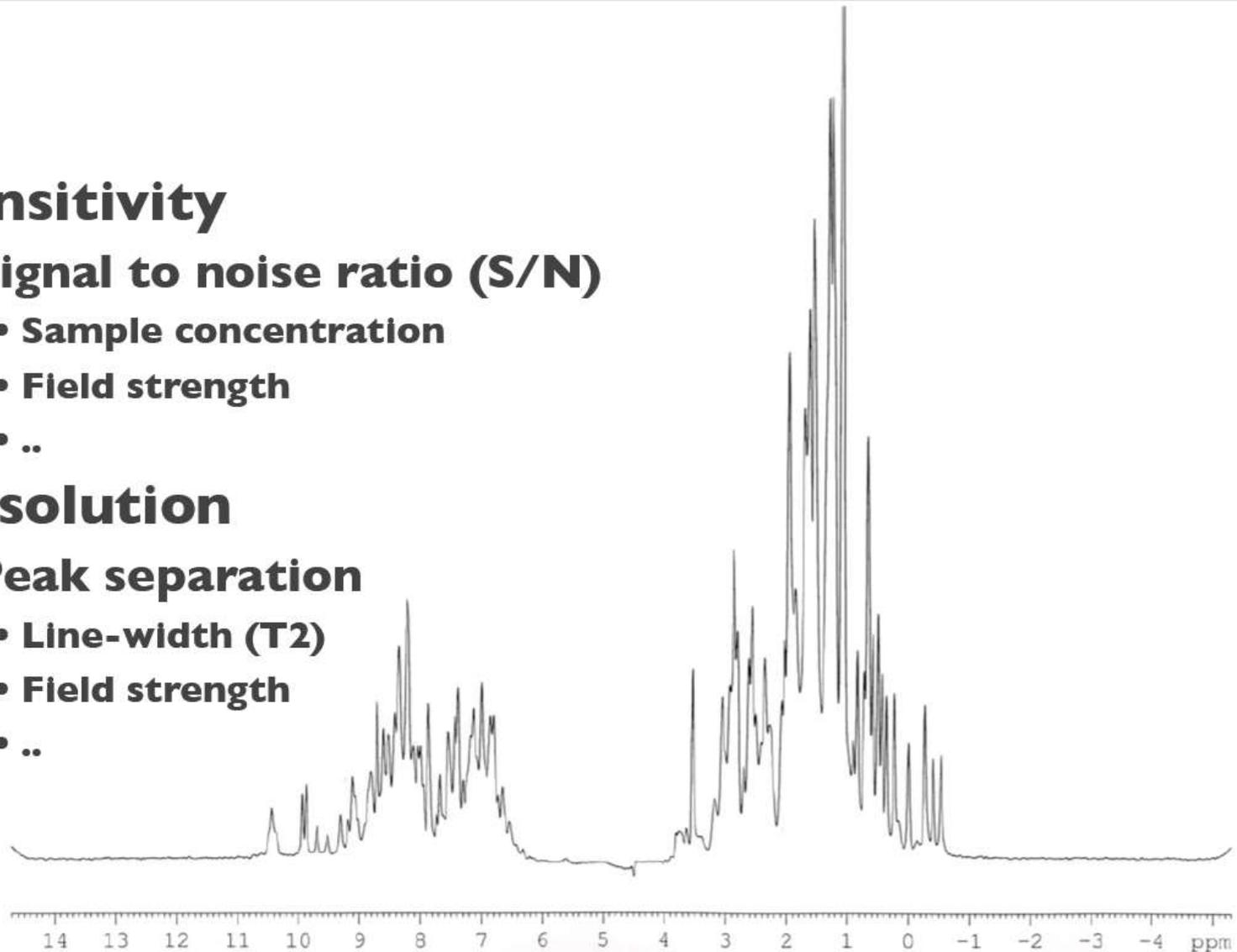
- **Signal to noise ratio (S/N)**

- Sample concentration
 - Field strength
 - ..

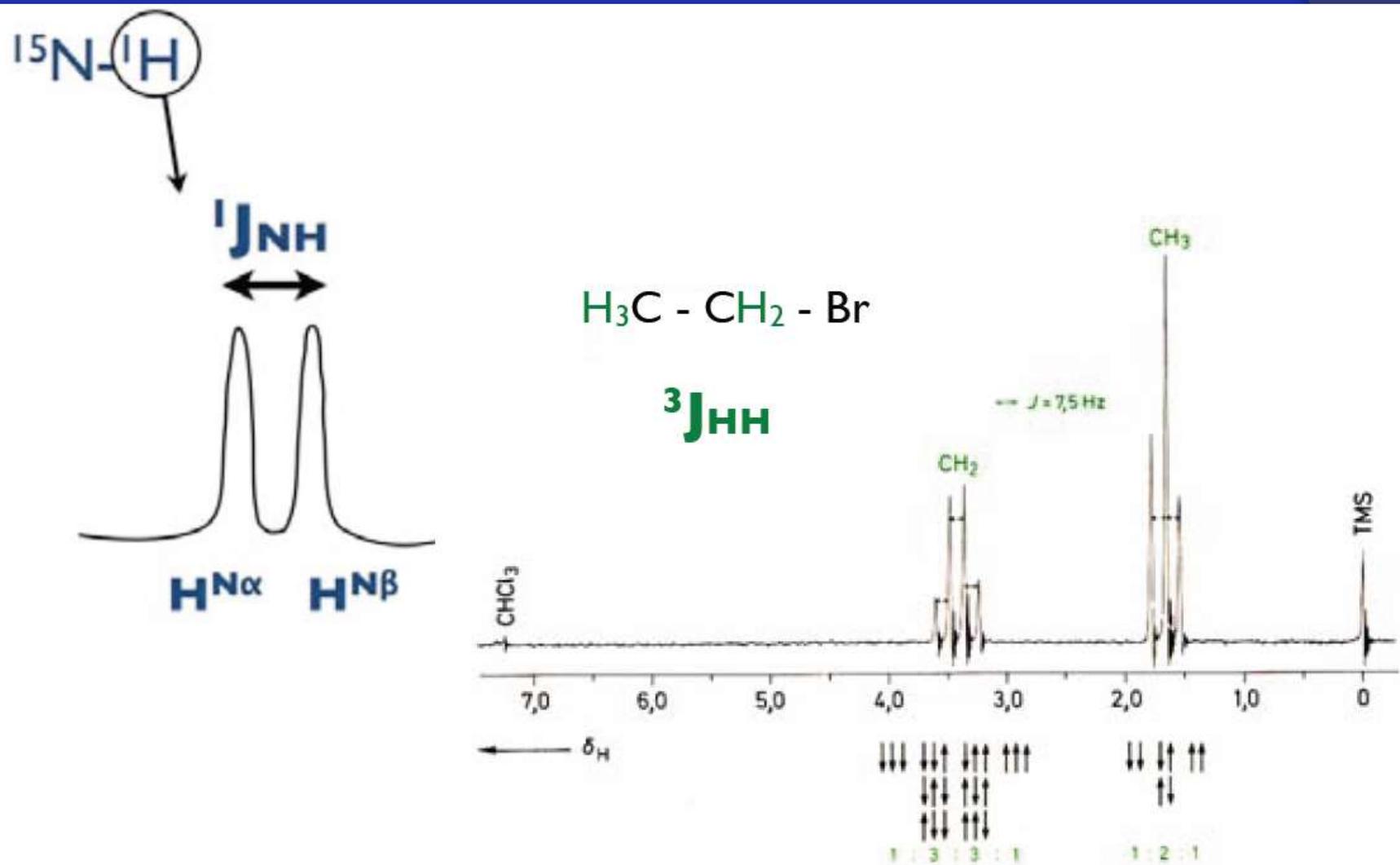
- **Resolution**

- **Peak separation**

- Line-width (T2)
 - Field strength
 - ..

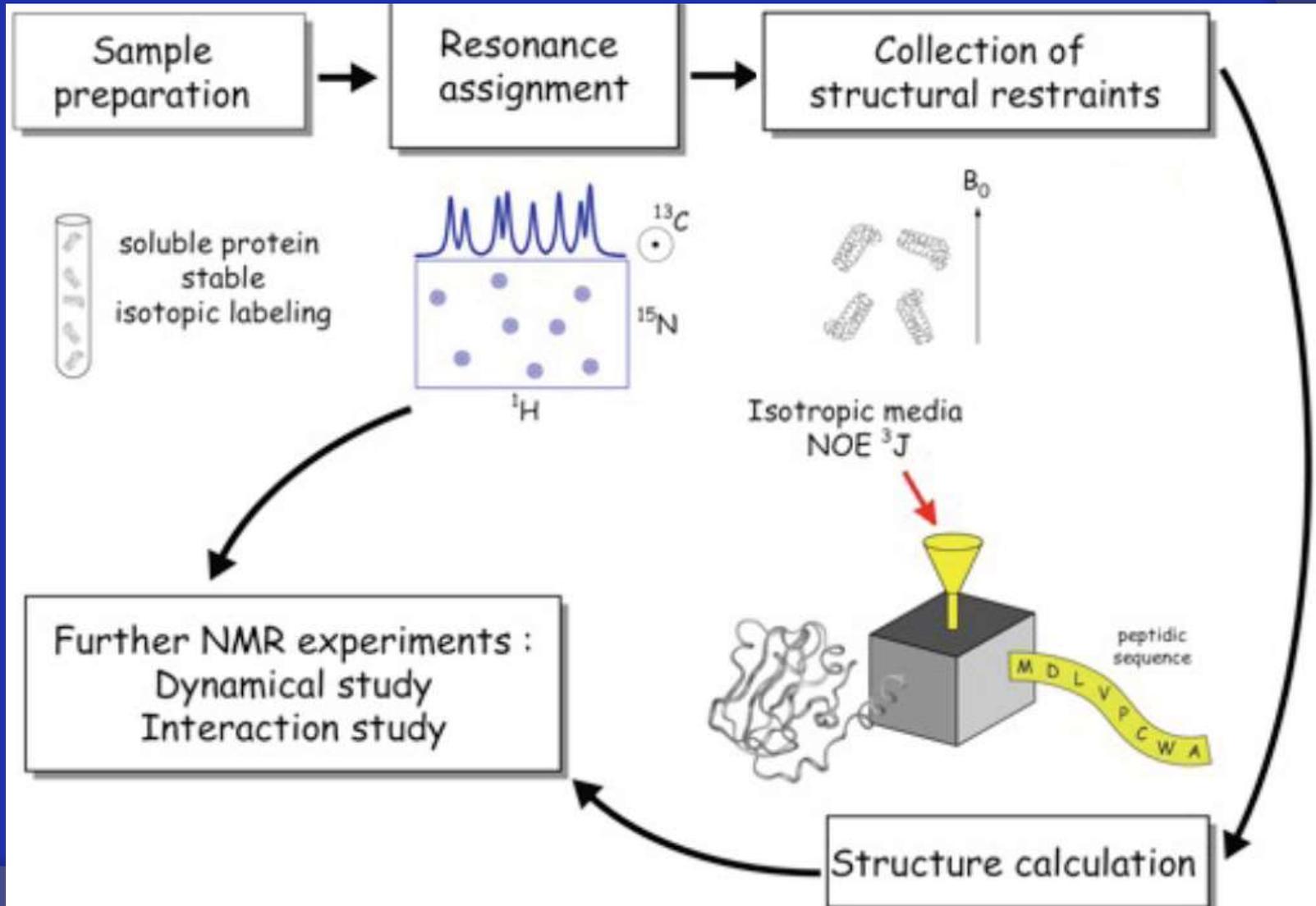


标量耦合/J耦合



蛋白质核磁共振基础

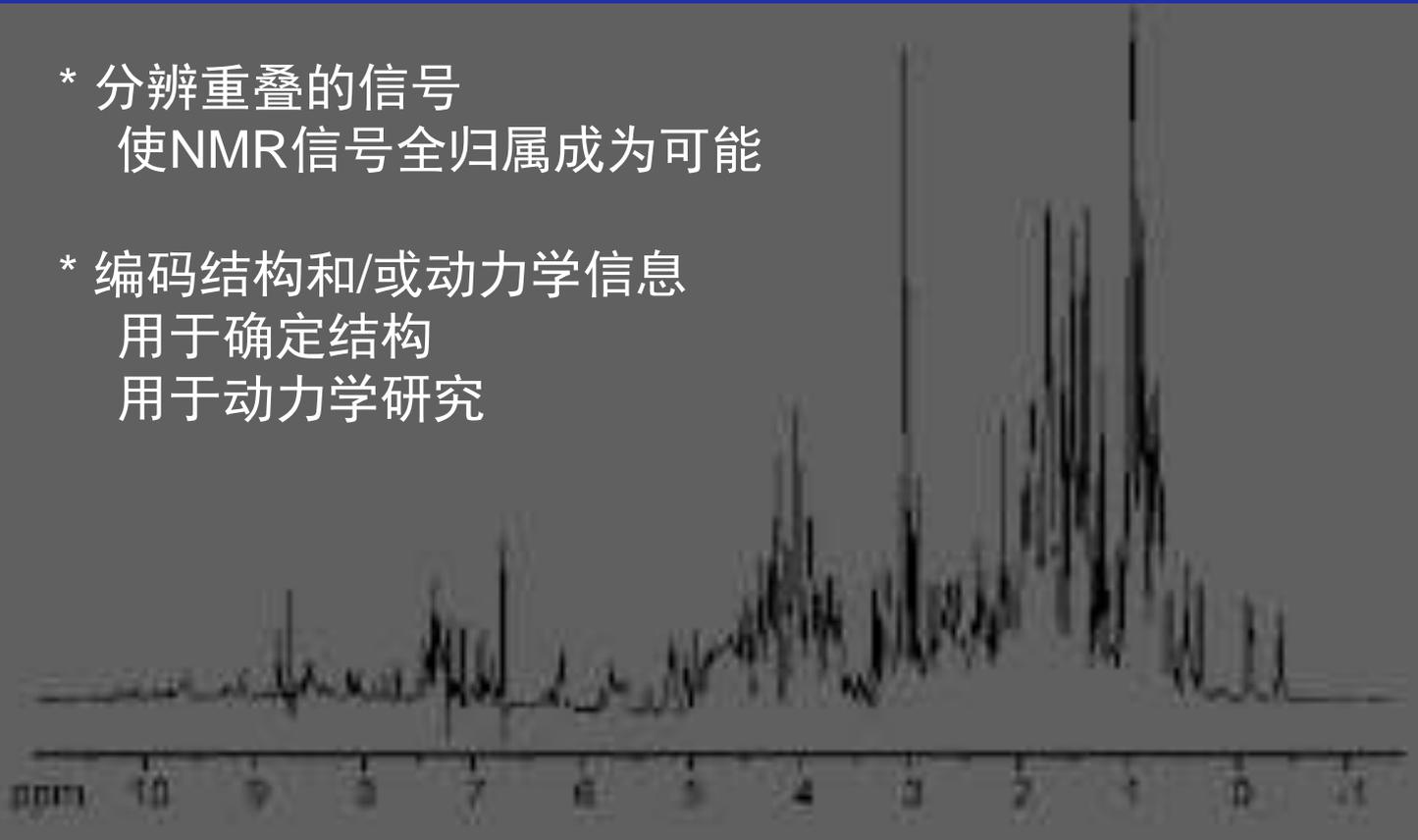
蛋白质核磁共振流程



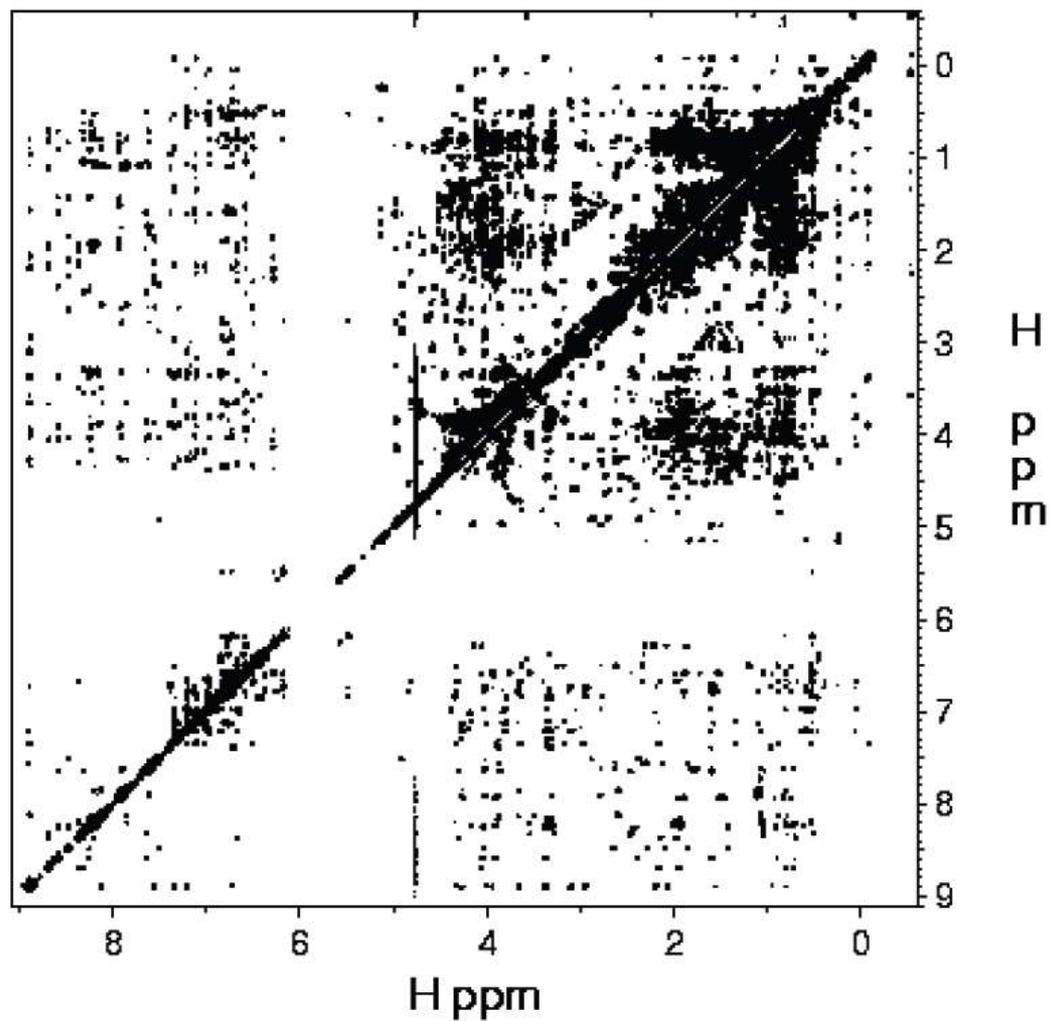
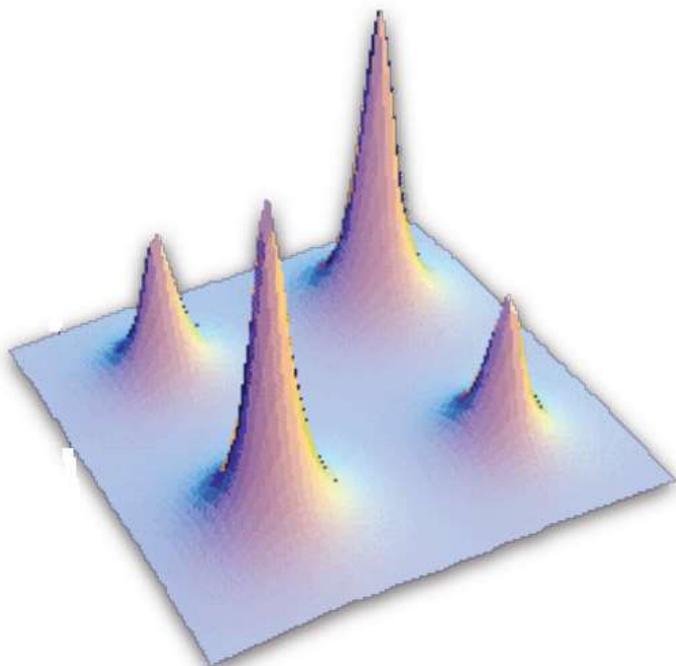
为什么需要多维NMR

多维核磁共振实验

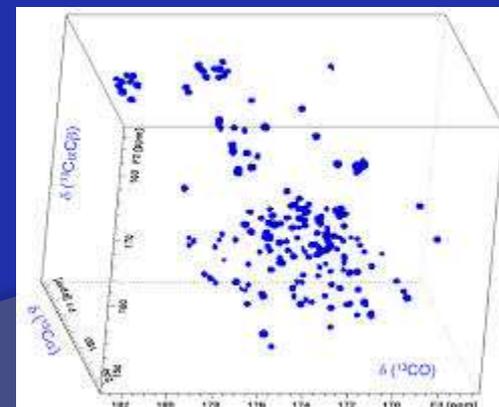
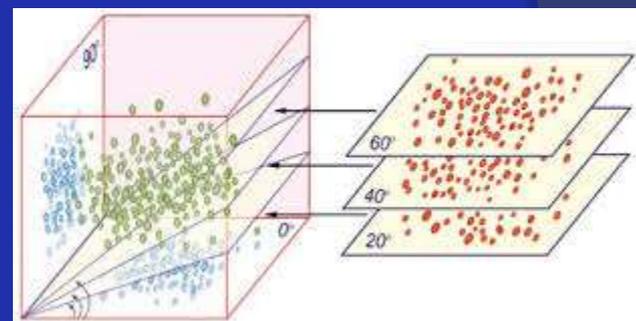
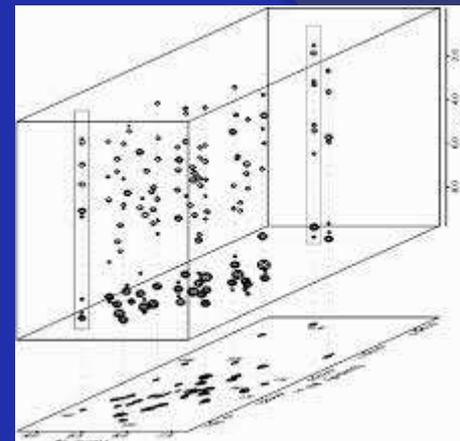
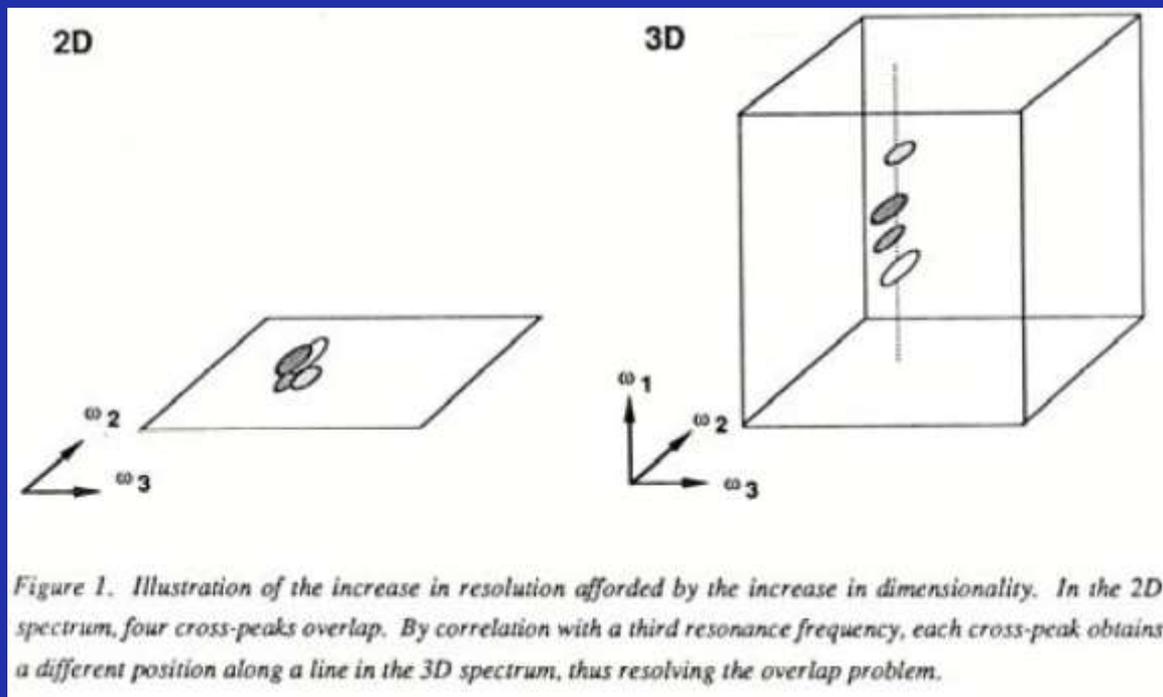
- * 分辨重叠的信号
使NMR信号全归属成为可能
- * 编码结构和/或动力学信息
用于确定结构
用于动力学研究



二维核磁共振谱 (2D NMR)

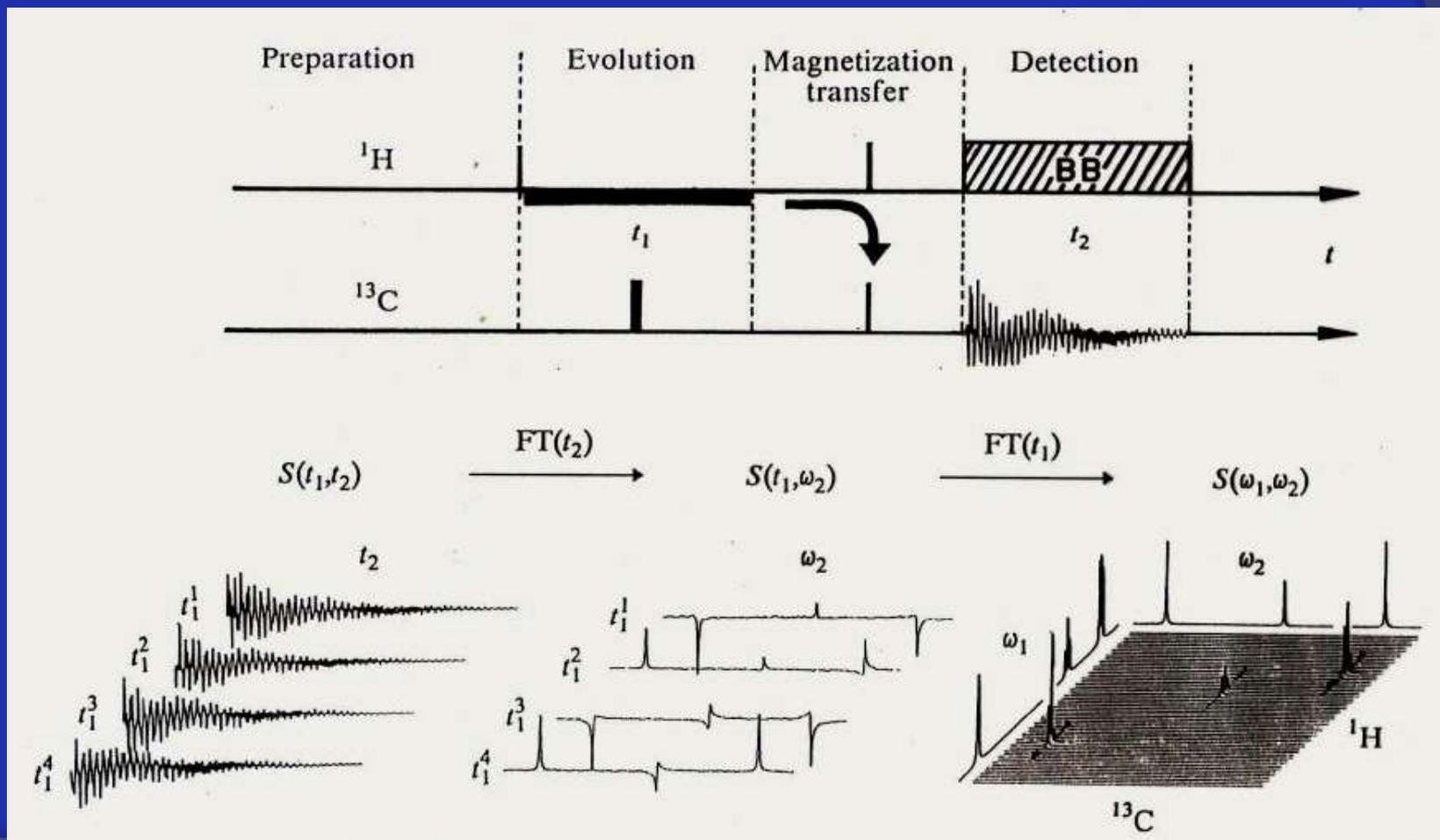


三维核磁共振谱 (3D NMR)



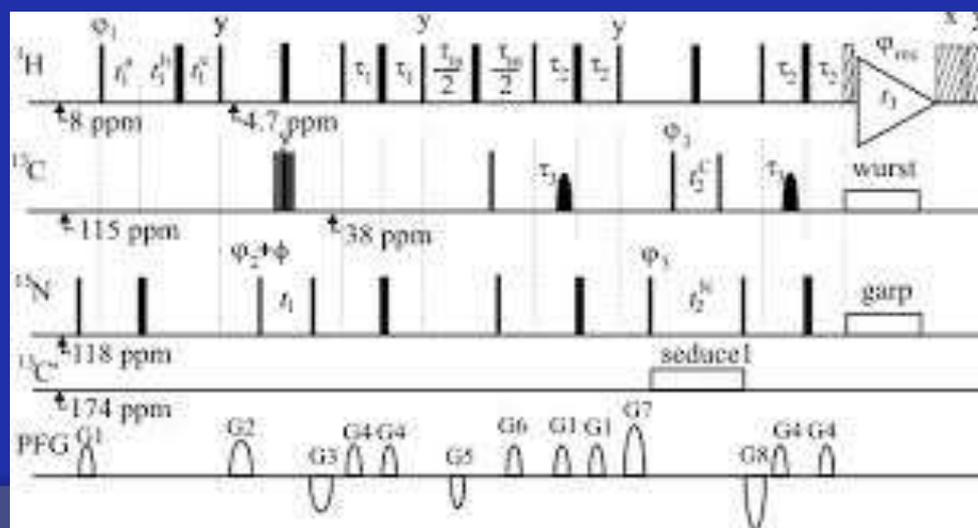
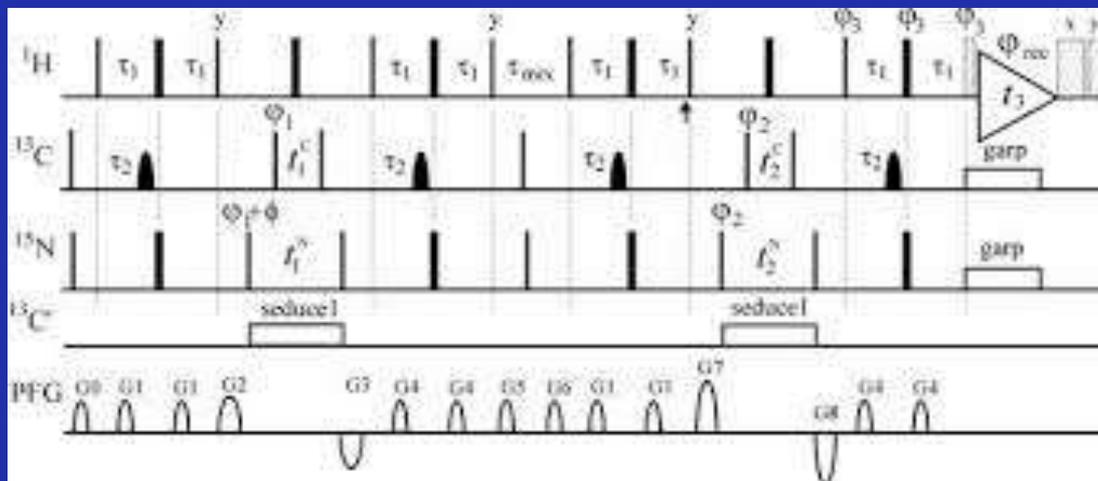
nD NMR的实现

2D NMR实验



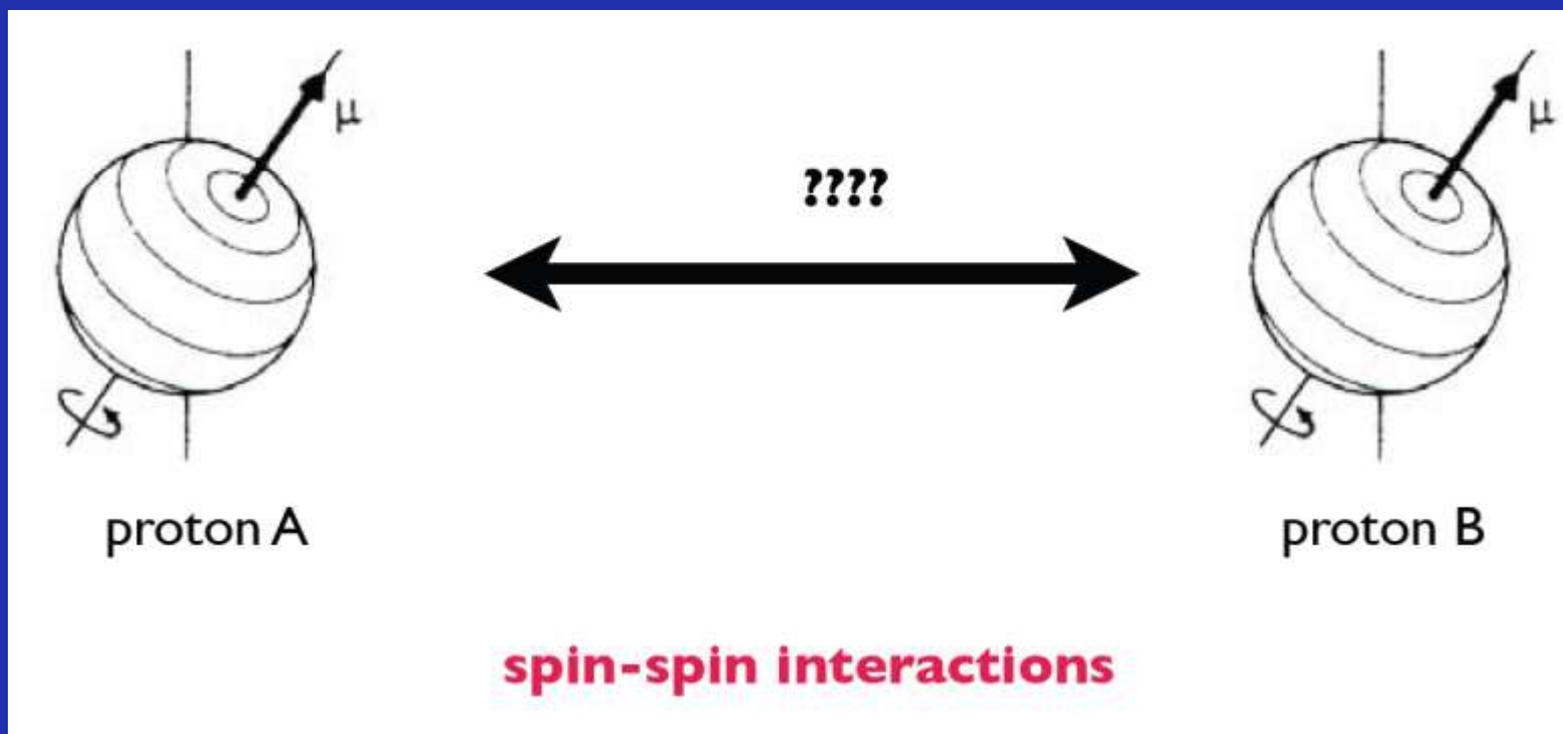
nD NMR的实现

3D NMR实验



编码信息

混合/磁化矢量转移



磁化矢量转移

磁偶极相互作用 (NOE)

核Overhauser 效应

through space

距离相关

由NOESY得到的距离限制条件

J耦合相互作用

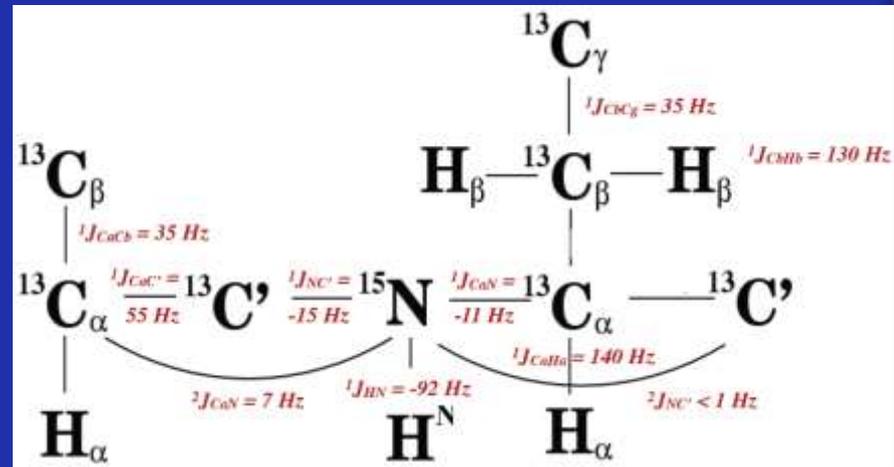
最多通过3-4键

化学连接

信号归属

蛋白质构型相关

J 耦合常数



蛋白质核磁共振主要相关核的性质

Properties of NMR friendly nuclides

Nuclide	Spin Number	Gyromagnetic Ratio (γ) [$10^7 \text{ rad T}^{-1} \text{ s}^{-1}$]	Natural Abundance [%]
^1H	1/2	26.7519	99.985
^2H	1	4.1066	0.015
^{13}C	1/2	6.7283	1.108
^{15}N	1/2	-2.7126	0.37
^{31}P	1/2	10.8394	100.0

Overview of NMR structure determination

