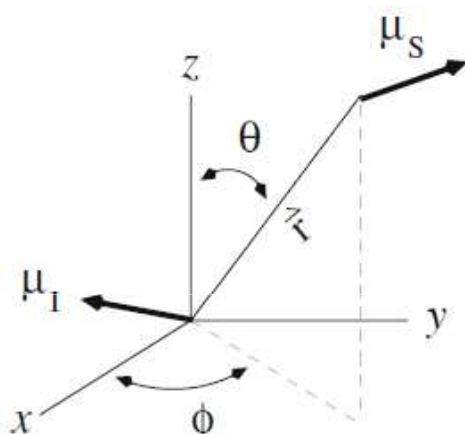


# The nuclear Overhauser effect

*Dipolar coupling* is a major source of information for structure determination by NMR spectroscopy.

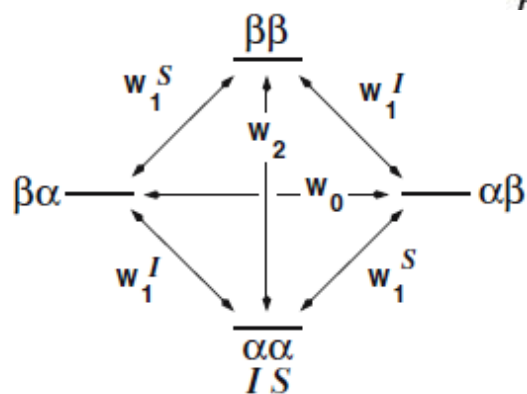


$$\mathcal{H} = \frac{\mu_I \cdot \mu_S}{r_{IS}^3} - \frac{3(\mu_I \cdot r_{IS})(\mu_S \cdot r_{IS})}{r_{IS}^5} \quad (67)$$

$$\mathcal{H}_{dipole} = DI_z S_z \quad (68)$$

$$\mathcal{H}_{II} = \underbrace{\frac{\gamma_I \gamma_S}{r^3} (3\cos^2\theta - 1)}_{\Delta} I_z S_z \quad (69)$$

The degree of splitting due to dipolar coupling:



$$\begin{aligned} E_{\alpha\alpha \rightarrow \beta\alpha} &= E_{\beta\alpha} - E_{\alpha\alpha} \\ &= \Delta [\langle \beta\alpha | I_z S_z | \beta\alpha \rangle - \langle \alpha\alpha | I_z S_z | \alpha\alpha \rangle] \\ &= \Delta [\langle \beta\alpha | \hbar^2 \left(\frac{1}{2}\right) \left(\frac{-1}{2}\right) | \beta\alpha \rangle - \langle \alpha\alpha | \hbar^2 \left(\frac{-1}{2}\right) \left(\frac{-1}{2}\right) | \alpha\alpha \rangle] \\ &= -\Delta \hbar^2 \frac{1}{2} \end{aligned} \quad (70)$$

$$E_{\alpha\beta \rightarrow \beta\beta} = +\Delta \hbar^2 \frac{1}{2}$$

# The nuclear Overhauser effect

Isotropic rotation of a single molecule in solution averages the dipolar coupling to zero.


The dipolar splitting  $\Delta\nu$  for amide groups in proteins:  $\Delta\nu = \Delta\omega/(2\pi) = 19.3 \text{ kHz}$

Rotational frequencies of typical proteins:  $10^8 \text{ Hz}$

NOE:

The rate of the stimulated zero- ( $k = 0$ ), single- ( $k = 1$ ), or double-quantum transitions ( $k = 2$ ) transitions:

$$W_k = \zeta_k \frac{\gamma^4 \hbar^2}{r^6} J(k\omega) \quad (71)$$

 inter-proton distances

$$\zeta_0 = 0.1, \zeta_1 = 0.15, \zeta_2 = 0.6$$

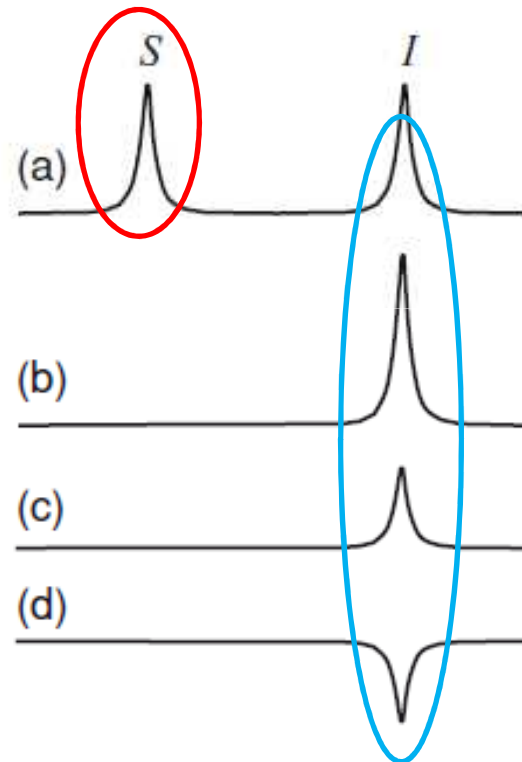
$$\text{spectral density function} = \frac{\tau_c}{1 + \omega^2 \tau_c^2}$$

$$\omega = k \times \omega_s$$

$$\text{proteins: } \tau_c = 1 \text{ ns}$$

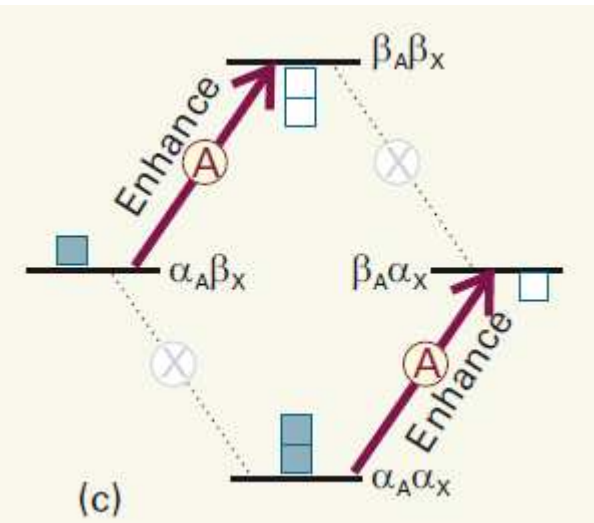
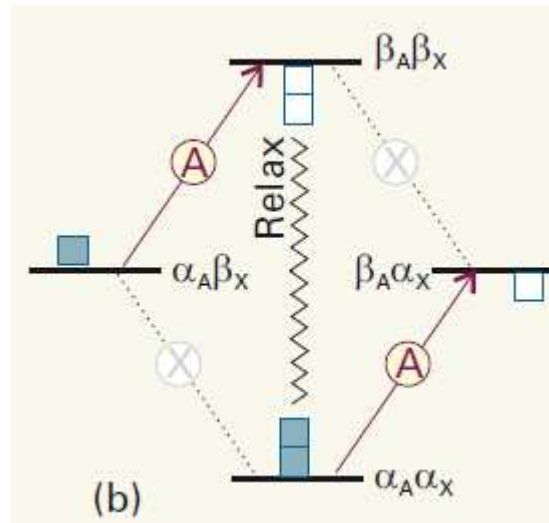
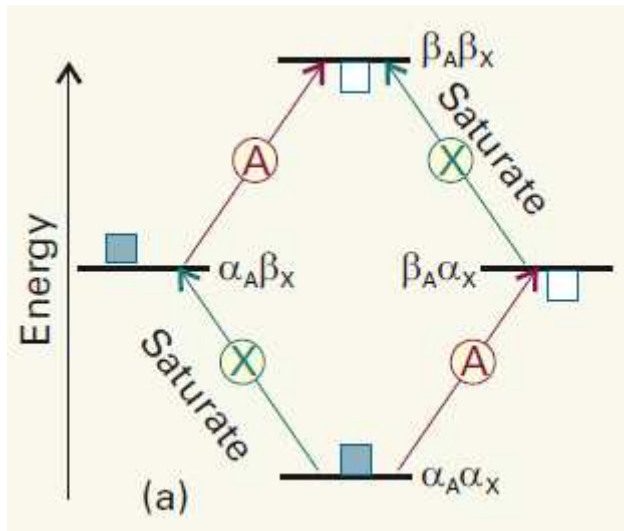
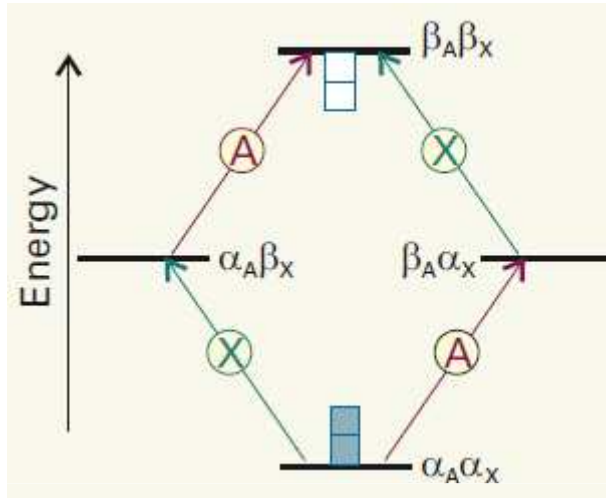
# The nuclear Overhauser effect

irradiated to saturation

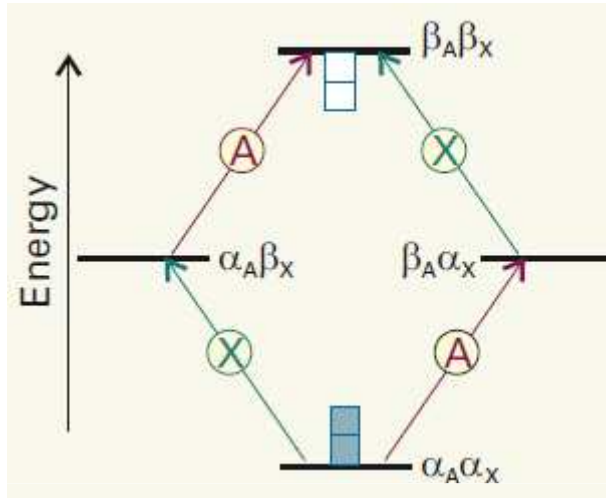


redistribution in the Zeeman population

# The nuclear Overhauser effect



# The nuclear Overhauser effect

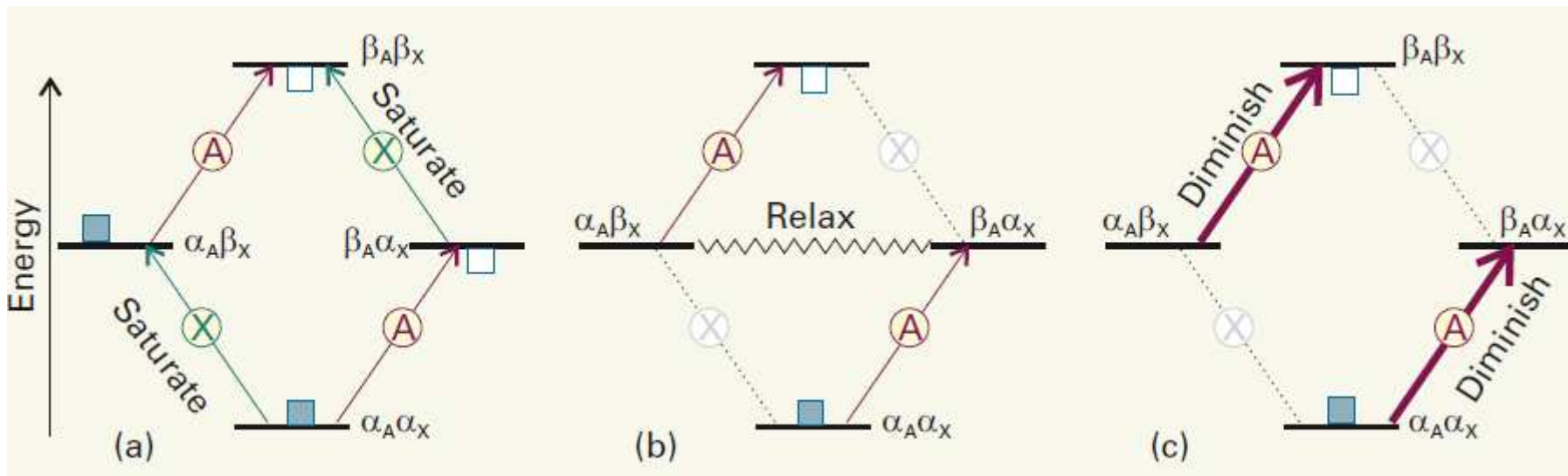


NOE enhancement:

$$\eta = \frac{I_A - I_A^0}{I_A^0} \quad (72)$$

$$\eta = \frac{\gamma_X}{2\gamma_A} \quad (73)$$

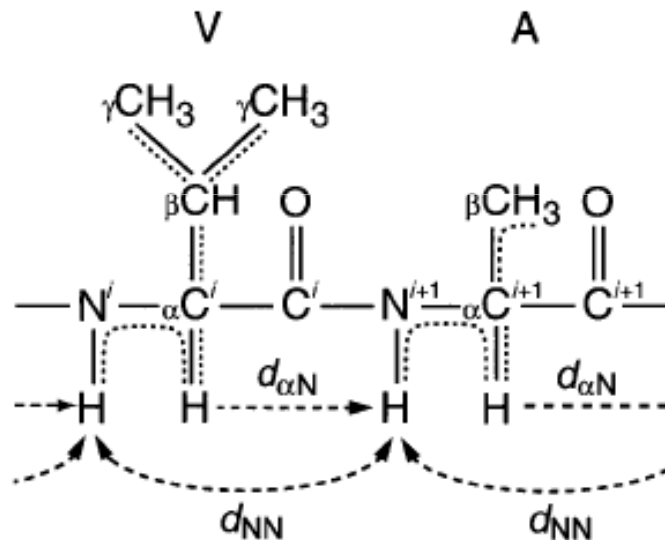
$^{13}\text{C}$  close to a saturated proton:  $\eta = 2$



# Nuclear Overhauser Enhancement (NOE)

NOE  $\sim r^{-6}$  is applicable only for very close neighbours (5 Å)

V-A polypeptide chain



----- inter-residue: through-bond (spin-spin ) coupling: max. 3 chem. bonds

----- intra-residue: short through-space interactions (NOE)

## 2D-NMR

Multidimensional NMR experiments:

- chemical shift labeling periods
- magnetization transfer (mixing) periods.

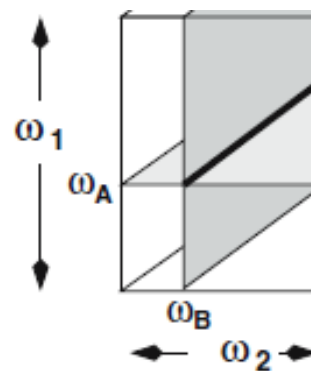
2D-NMR:  $A \longrightarrow B$

The final detected signal depends on two time domains:

$$S(t_1, t_2) = \eta e^{i(\omega_A t_1)} e^{i(\omega_B t_2)}$$

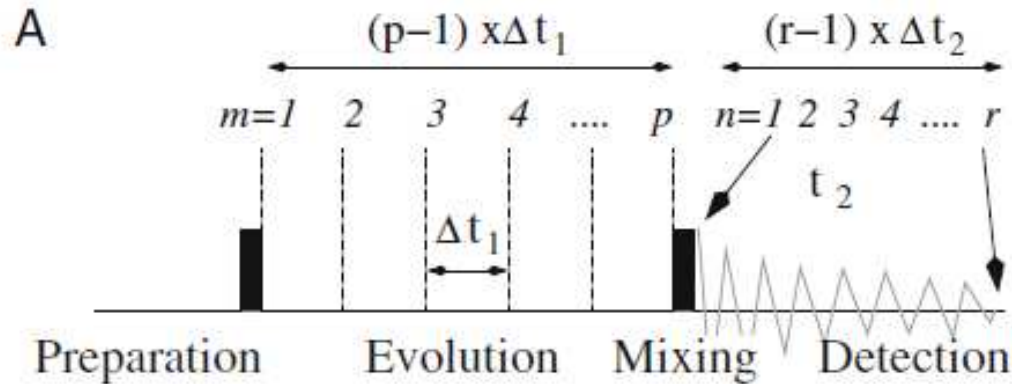
↓ FT

$$(\omega_A, \omega_B) \quad (\omega_A, \omega_A)$$



(74)

# 2D-NMR



Data structure of 2D experiment

	$t_2 \rightarrow$									
$t_1 \downarrow$	1	2	3	4	5	6	7	8	.	$r$
1	x	x	x	x	x	x	x	x	.	.
2	x	x	x	x	x	x	x	x	.	.
3	x	x	x	x	x	x	x	x	.	.
4	x	x	x	x	x	x	x	x	.	.
5	x	x	x	x	x	x	x	x	.	.
.	.	.	.	.	.	.	.	.	.	.
$p$	.	.	.	.	.	.	.	.	.	.

$$\Omega(\omega_1, \omega_2) = \int \int S(t_1, t_2) e^{i\omega t_1} e^{i\omega t_2} dt_1 dt_2$$

Classification of 2D-NMR after **K.Wüthrich**:

- through-bond interaction: **correlated NMR spectroscopy (COSY)**  
determines the characteristic „fingerprint“ spectrum of an amino acid side chain: **assignment**
- through-space interaction: **nuclear Overhauser enhanced spectroscopy (NOESY)**:  
**space information**





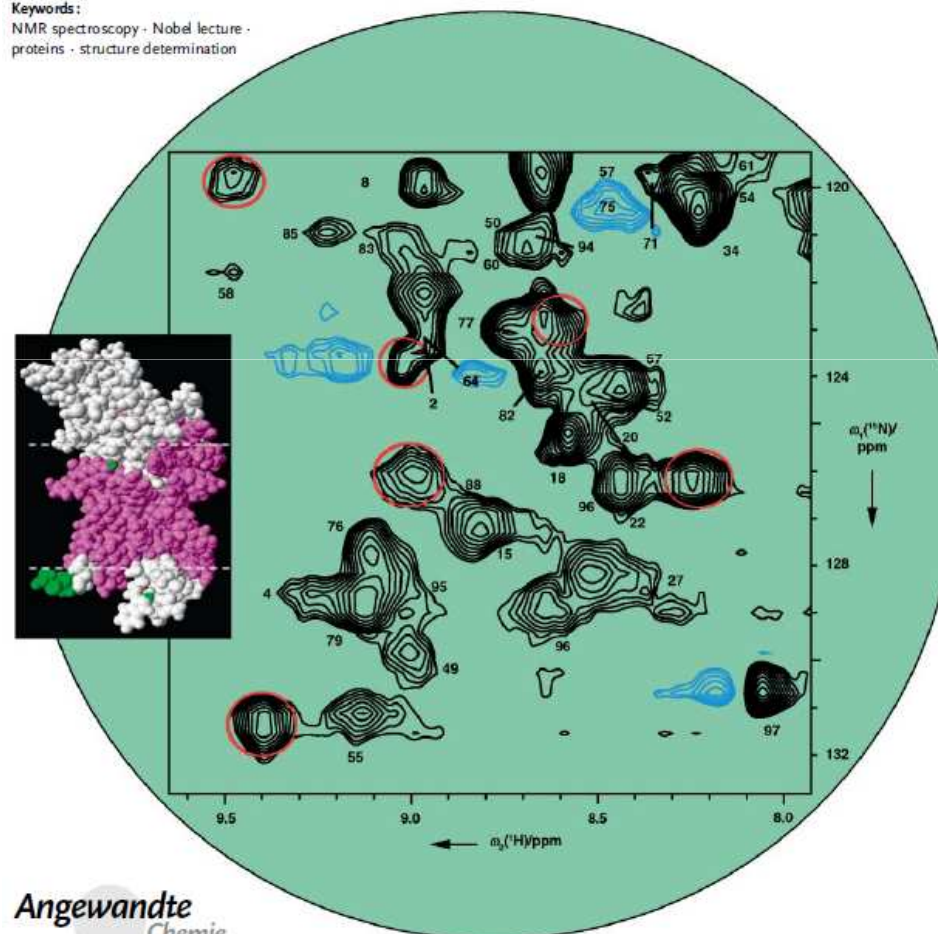
In 2002, **Kurt Wüthrich** was awarded the **Nobel Prize in Chemistry** for demonstrating that the NOE could be exploited using two-dimensional NMR spectroscopy to determine the **three-dimensional structures of biological macromolecules in solution.**

### NMR of Biomacromolecules

## NMR Studies of Structure and Function of Biological Macromolecules (Nobel Lecture)\*\*

Kurt Wüthrich\*

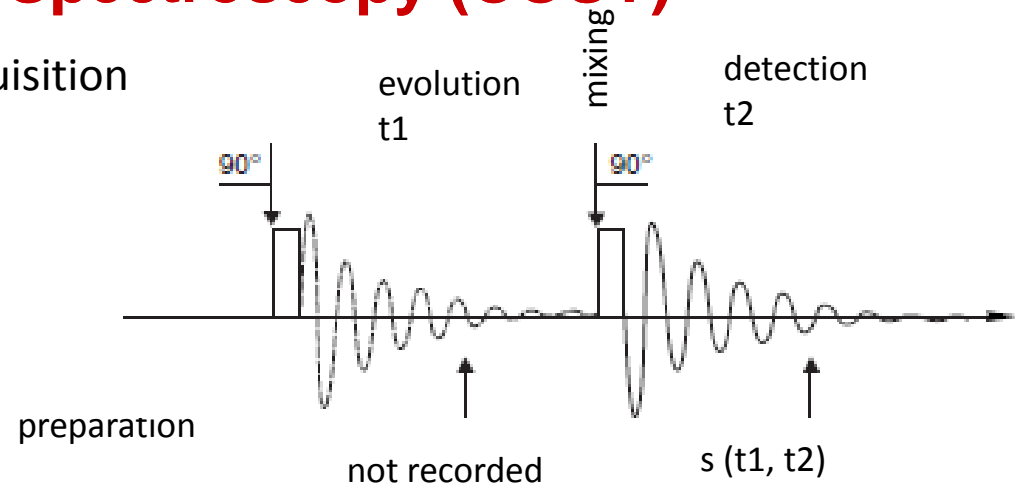
Keywords:  
NMR spectroscopy · Nobel lecture ·  
proteins · structure determination



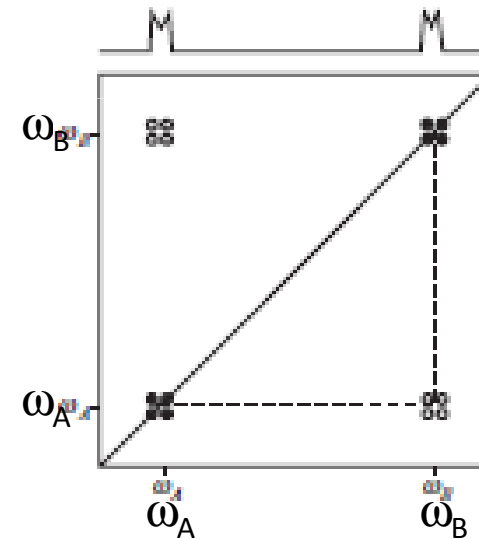
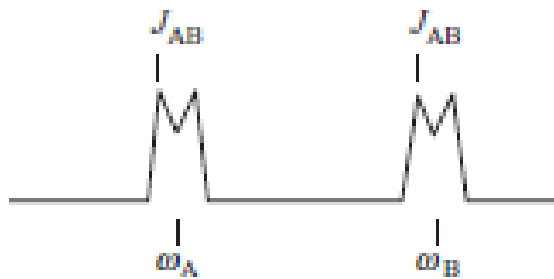
Angewandte  
Chemie

# Correlation Spectroscopy (COSY)

pulse sequence:  $90^\circ$ - $k t_1$ - $90^\circ$ -data acquisition  
 $k = 0, 1, 2, \dots, 2^n$

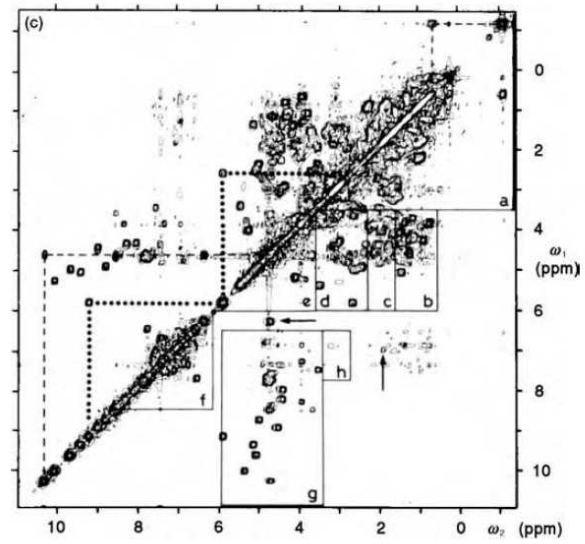
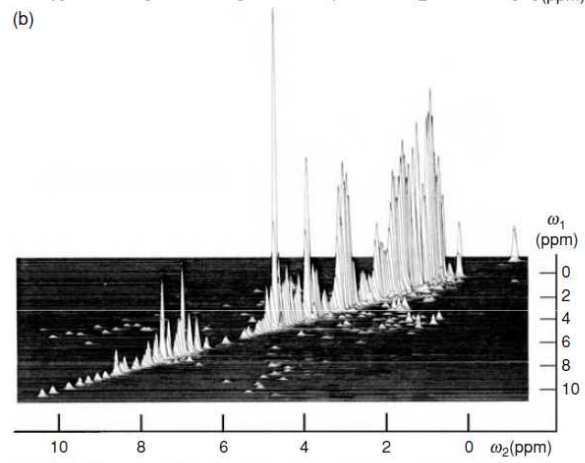
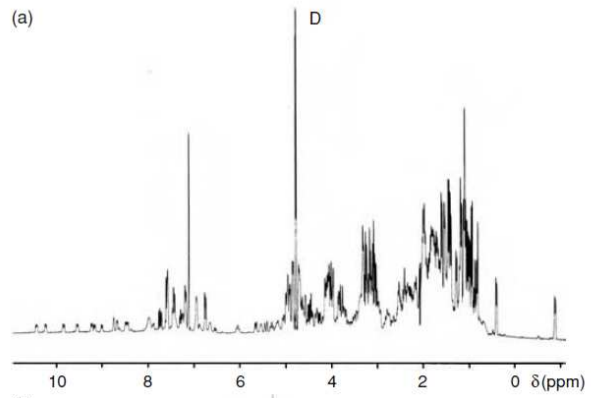


two coupled spins A, B:

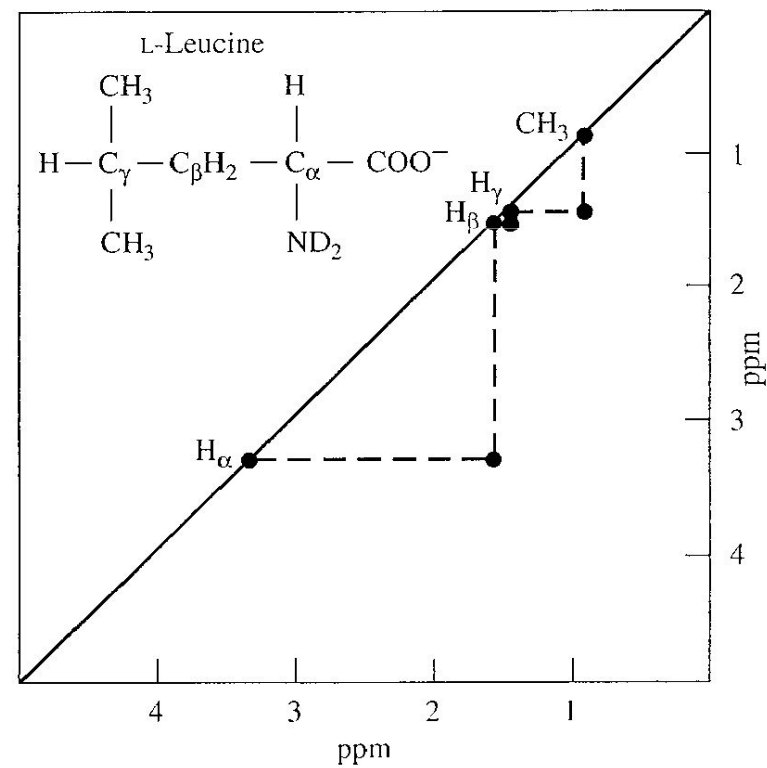
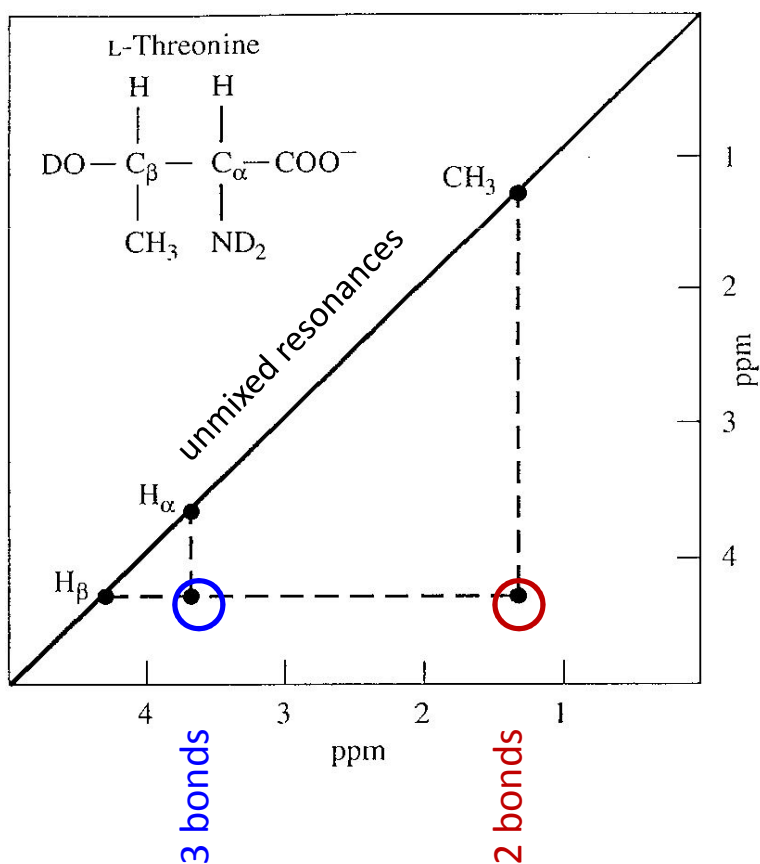


Contour plot of the COSY spectrum

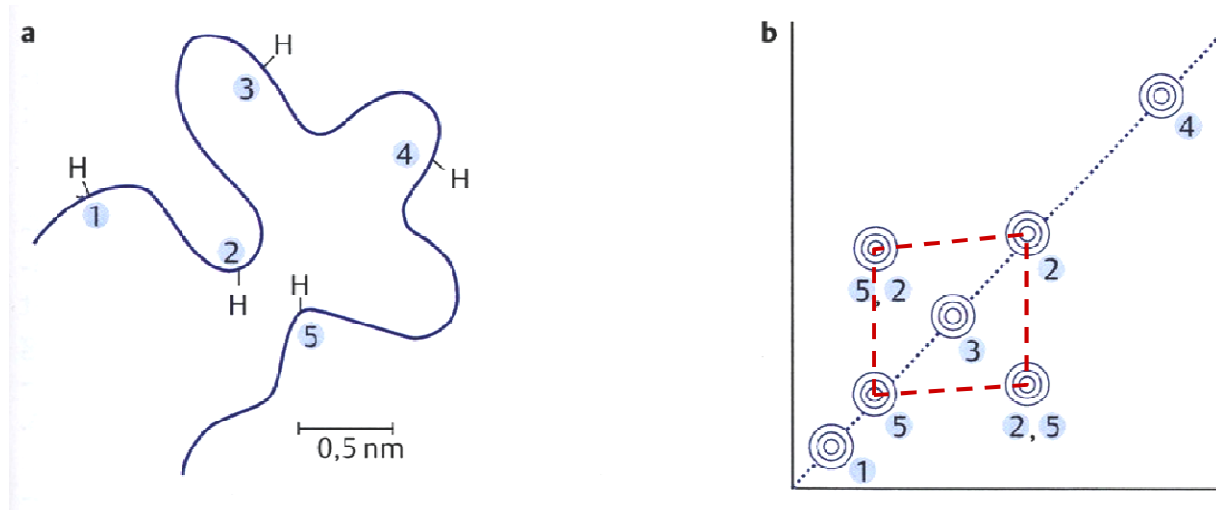
Cross peaks are caused by nuclei which could interchange magnetization during the mixing time. Cross peaks show interaction between two nuclei and contain the essential information of a 2D spectrum.



# (Homonuclear) Correlation Spectroscopy (COSY)



# Nuclear Overhauser enhanced spectroscopy (NOESY)

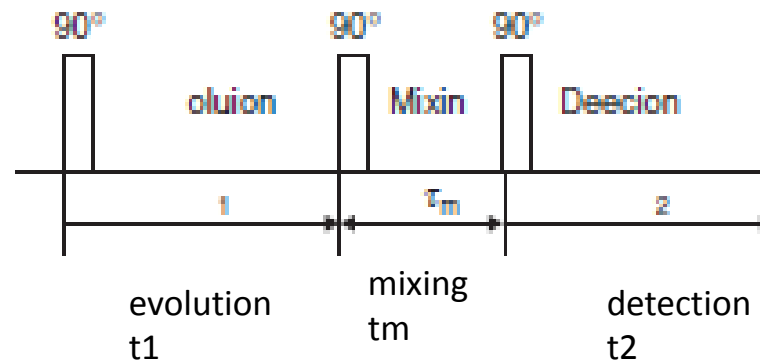


## NOESY analysis of a polypeptide chain

From the 5 highlighted protons 2 and 5 are within favorable distance: NOE

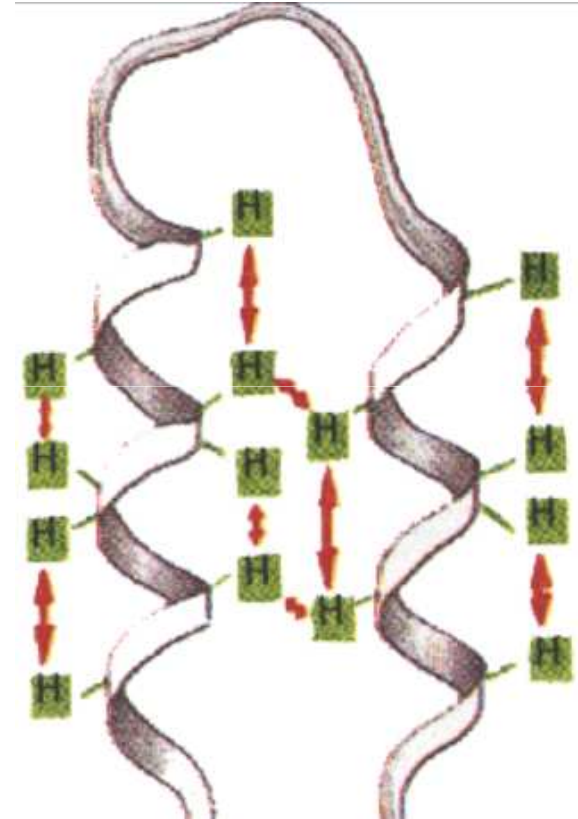
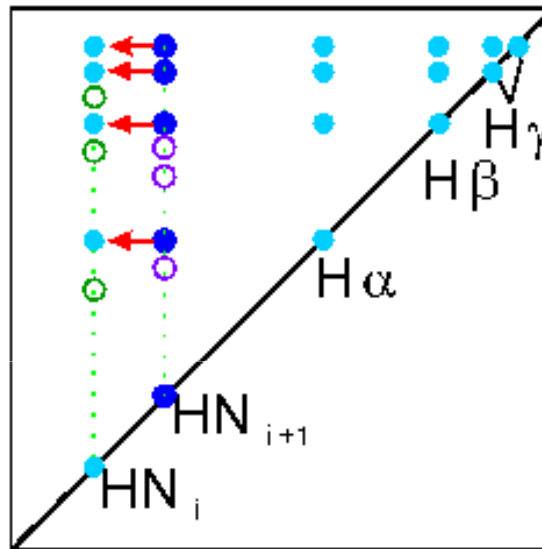
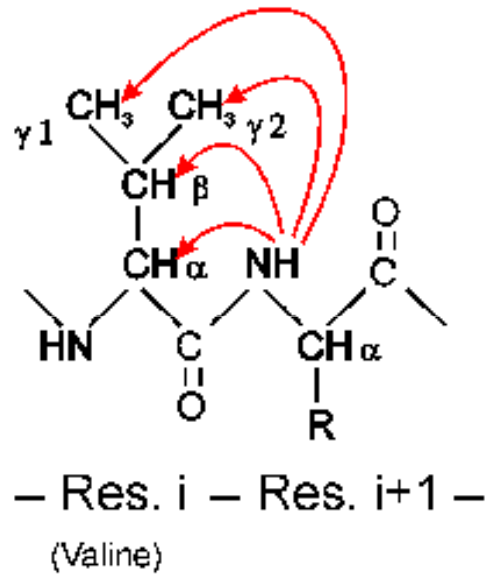
Diagonal peaks: the unmixed resonances of 1-5 protons.

Cross peaks: through-space coupling of protons 2 and 5.



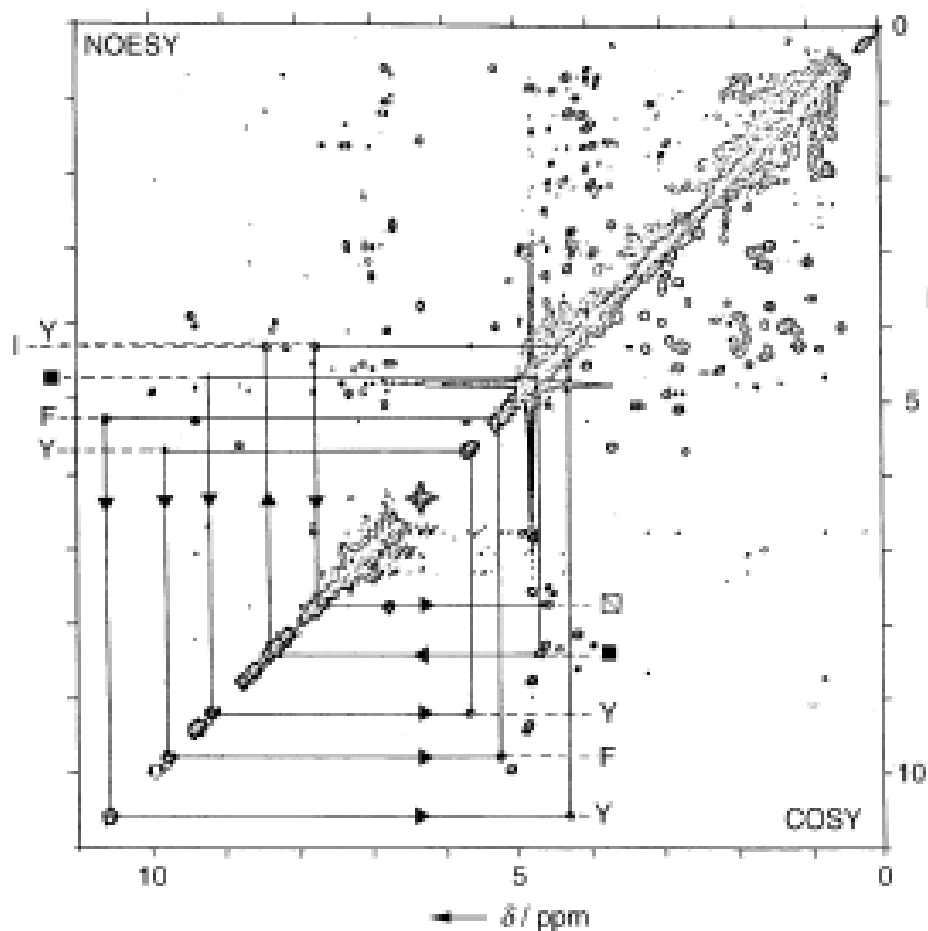
# NOESY sequential alignment

Dipeptide Fragment



- Through-space connections, as given by the NOESY experiment, are the key to solving a protein structure by NMR
- Long-range interactions give the fold of the protein chain

# Combining COSY and NOESY: structural information



Scalar couplings

Dipolar couplings

Through bonds

Through space

Strength  $\leq 100$  Hz

Strength depends on distance and nucleus (e.g. for N-H pair  $\sim 20$  kHz)

Isotropic

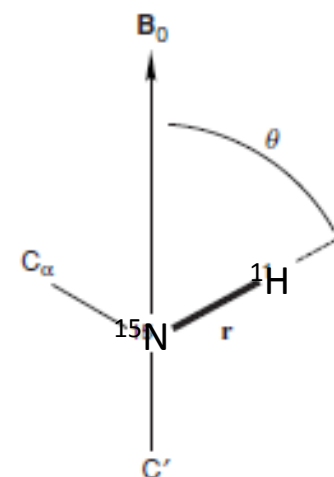
$3\cos^2\theta - 1$

Small for  $>3$  bonds

$1/r^3$

Produce splittings

Splittings when motion restricted



COSY-NOESY connectivity diagram of a protein