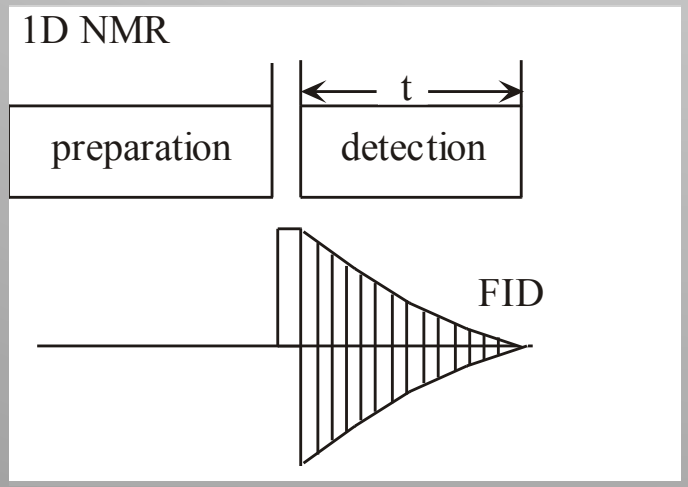
Elementary physics of multidimensional NMR

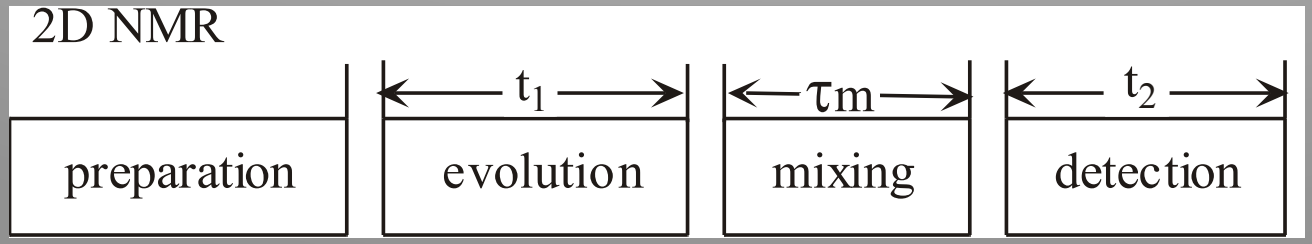
- Spin-magnetic field interaction (Zeeman interaction). $E = \gamma h m B_o$
- Indirect through bond coupling between spins (scalar coupling). $H_J = 2\pi J I_z S_z$. Geometry independent
- Direct dipolar interactions between spins. $H_D \sim(1 - 3\cos^2 \theta)I_zS_z$. Geometry dependent

1D vs 2D homonuclear NMR Spectroscopy



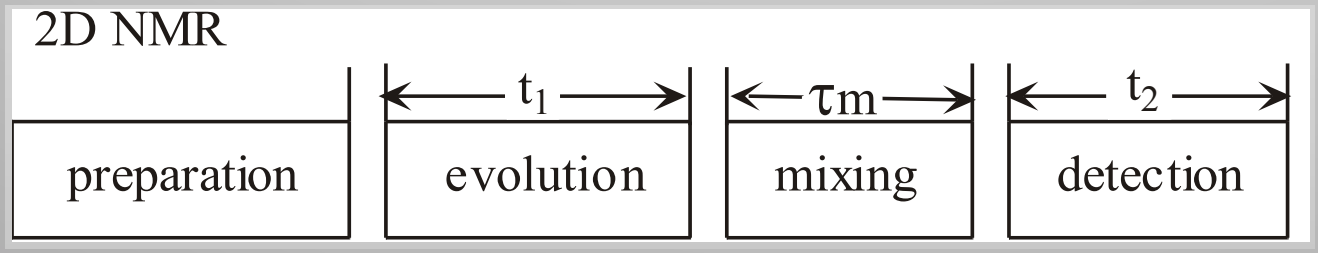
Basic structure of 1D experiment

Basic structure of 2D experiment (e.g. NOESY)

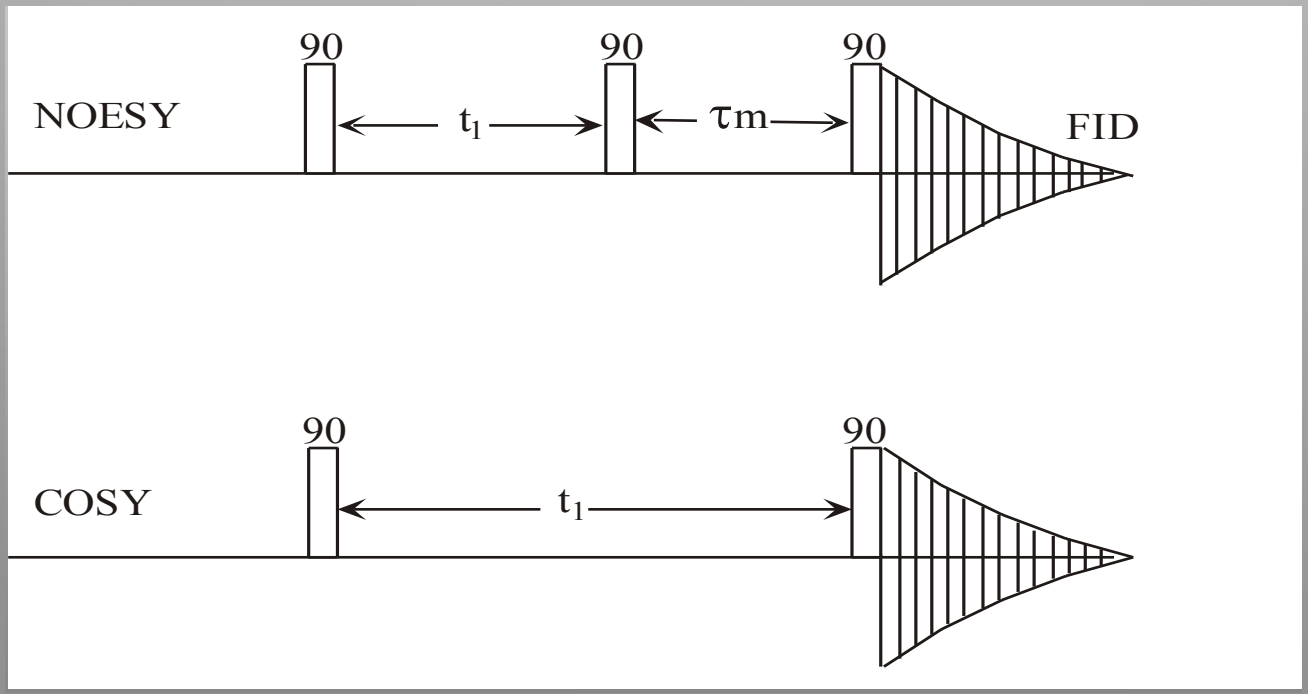


Jeener and Ernst, 1972

2D homonuclear NMR Spectroscopy

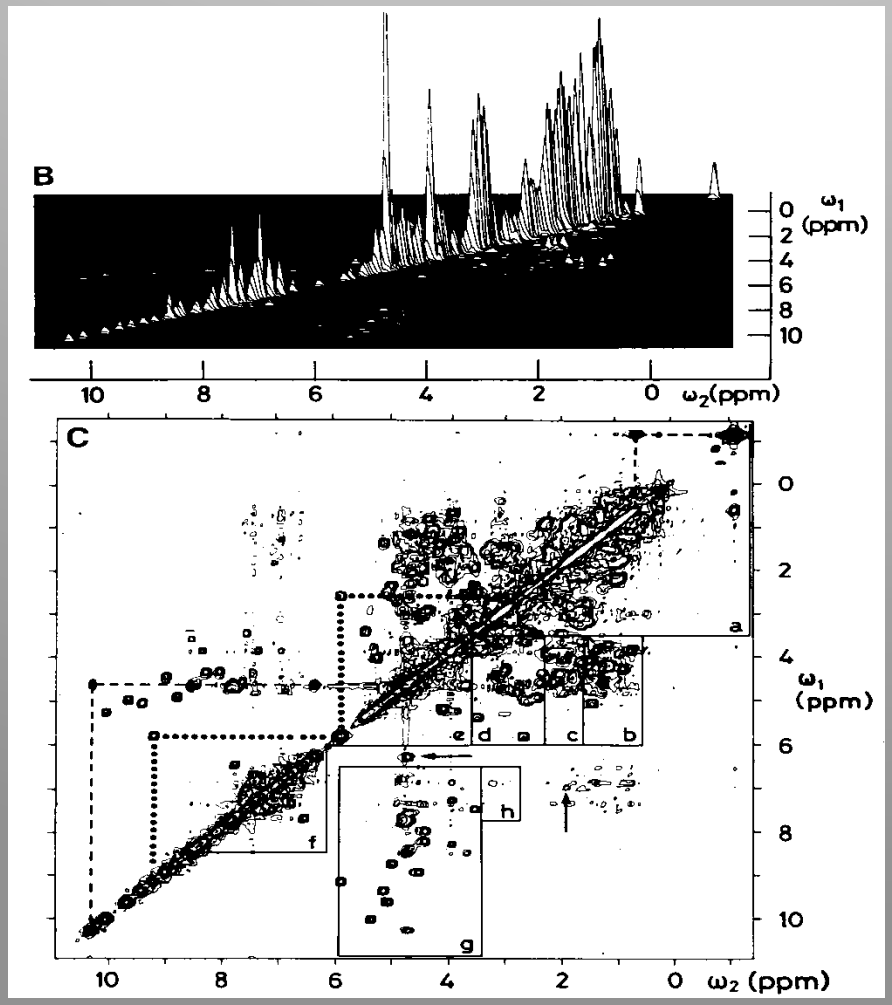


Basic structure



Two basic experiments: NOESY and COSY

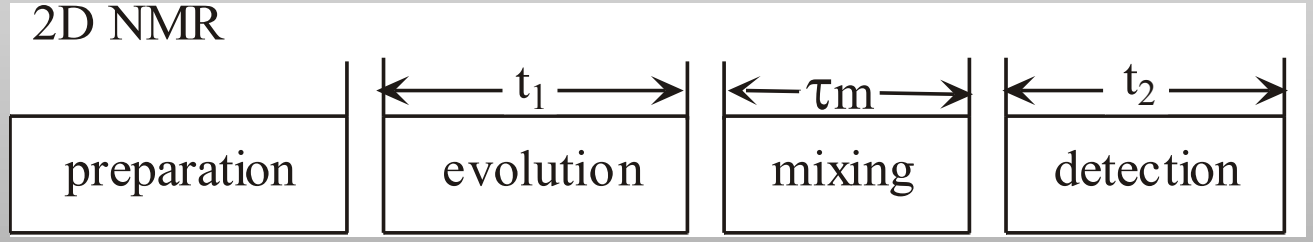
“cross-peaks” in multidimensional NMR carry structural information

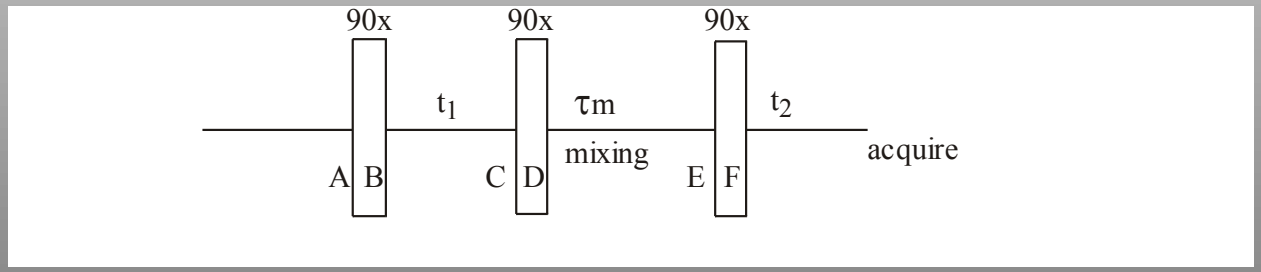


Diagonal is closely related to 1D spectrum

2D contour representation: peaks outside diagonal are called cross-peaks

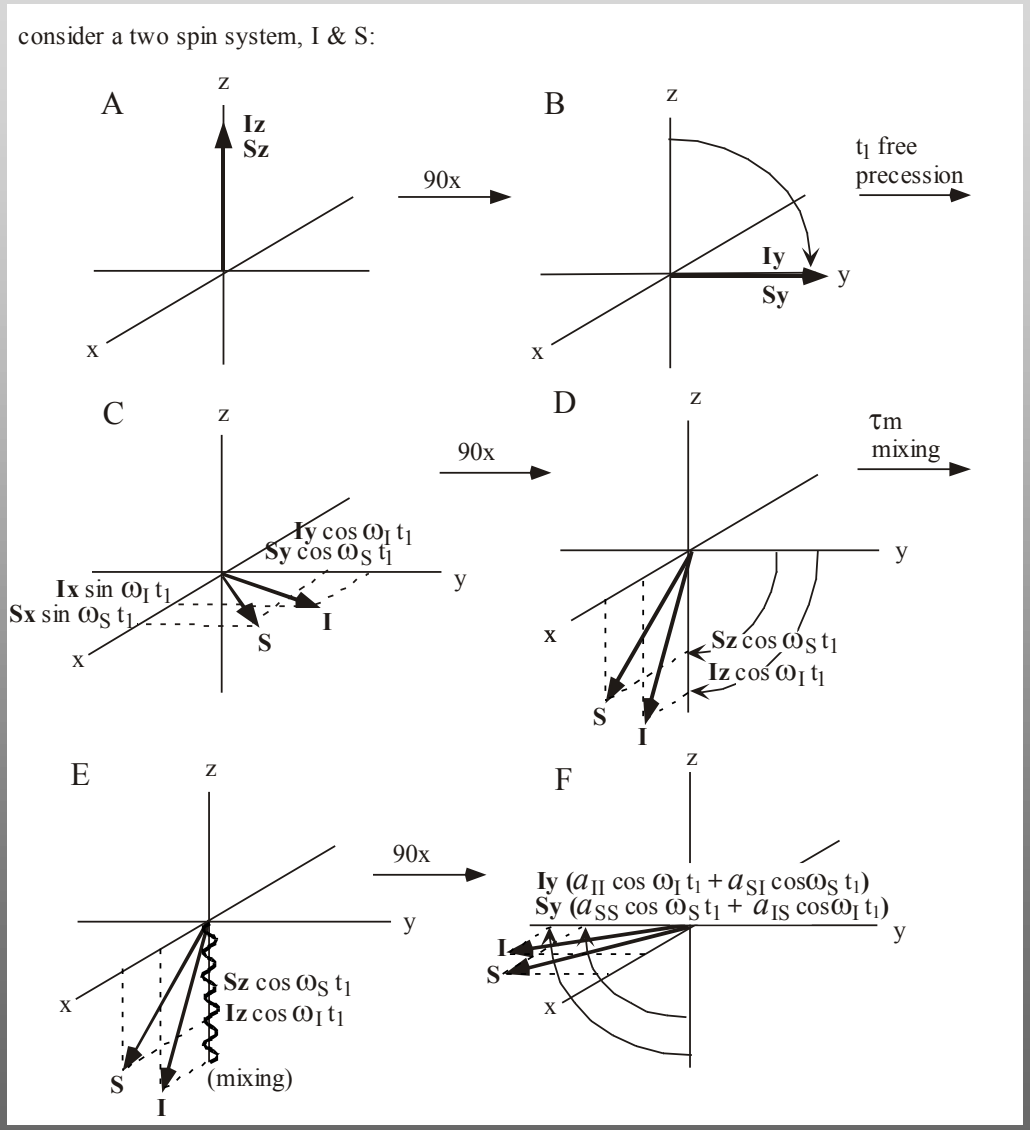
Nuclear Overhauser Effect SpectroscopY (NOESY)





The NOESY experiments detects interactions between spins that are close in space and dipolar coupled: the magnetization transfer mechanism is essentially the same as FRET in optical spectroscopy (fluorescence)

Nuclear Overhause Effect SpectroscopY (NOESY)



1. Excite with 90° pulse

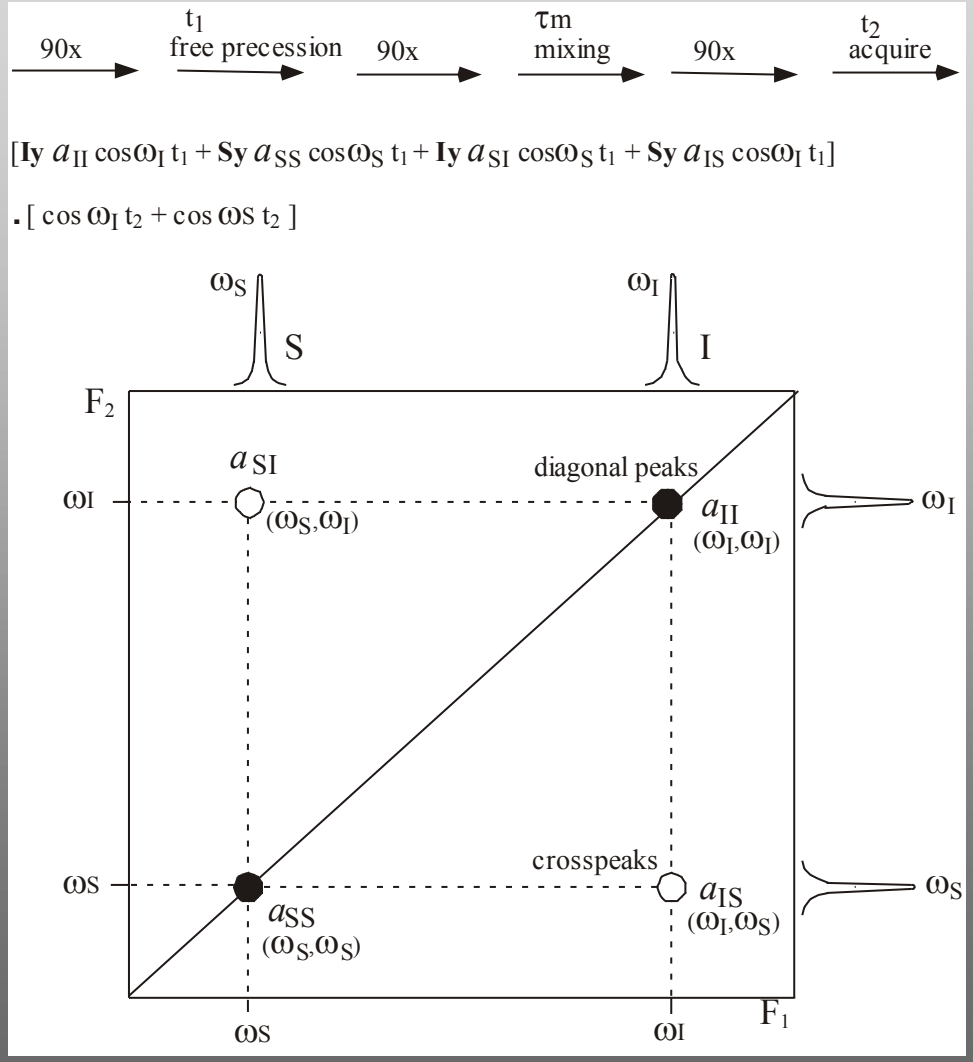
2. During t1, spins are labeled with their Larmor frequency

3. The second 90° pulse exchanges rotates magnetization to -z

4. Magnetization is exchanged between spin I and S during the mixing time

5. The final 90° pulse makes the signal observable and signal is acquired in t2

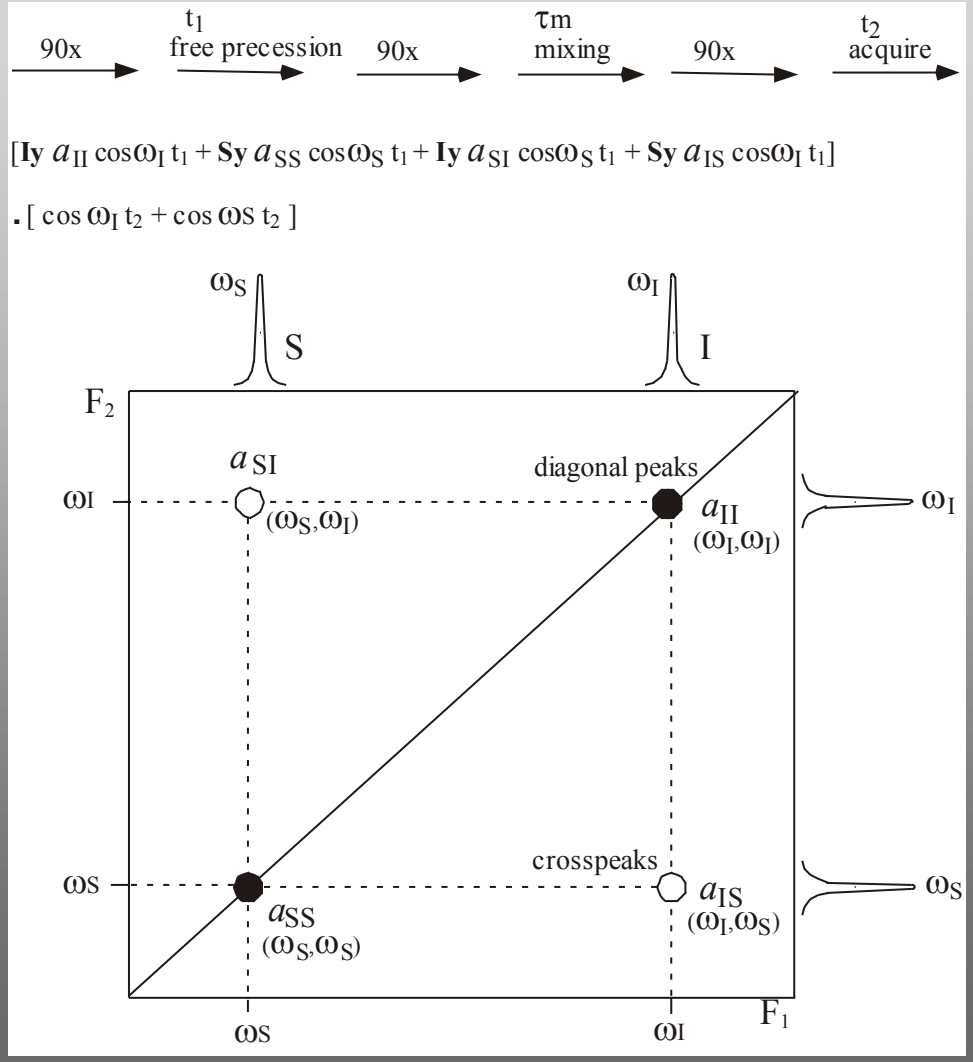
Nuclear Overhause Effect SpectroscopY (NOESY)



When magnetization is exchanged, spin I signal contains information on spin S and viceversa (cross-peaks)

Cross-peaks appear between spins which are close in space (<5-6 A) (assignments and structure)

Nuclear Overhause Effect SpectroscopY (NOESY)

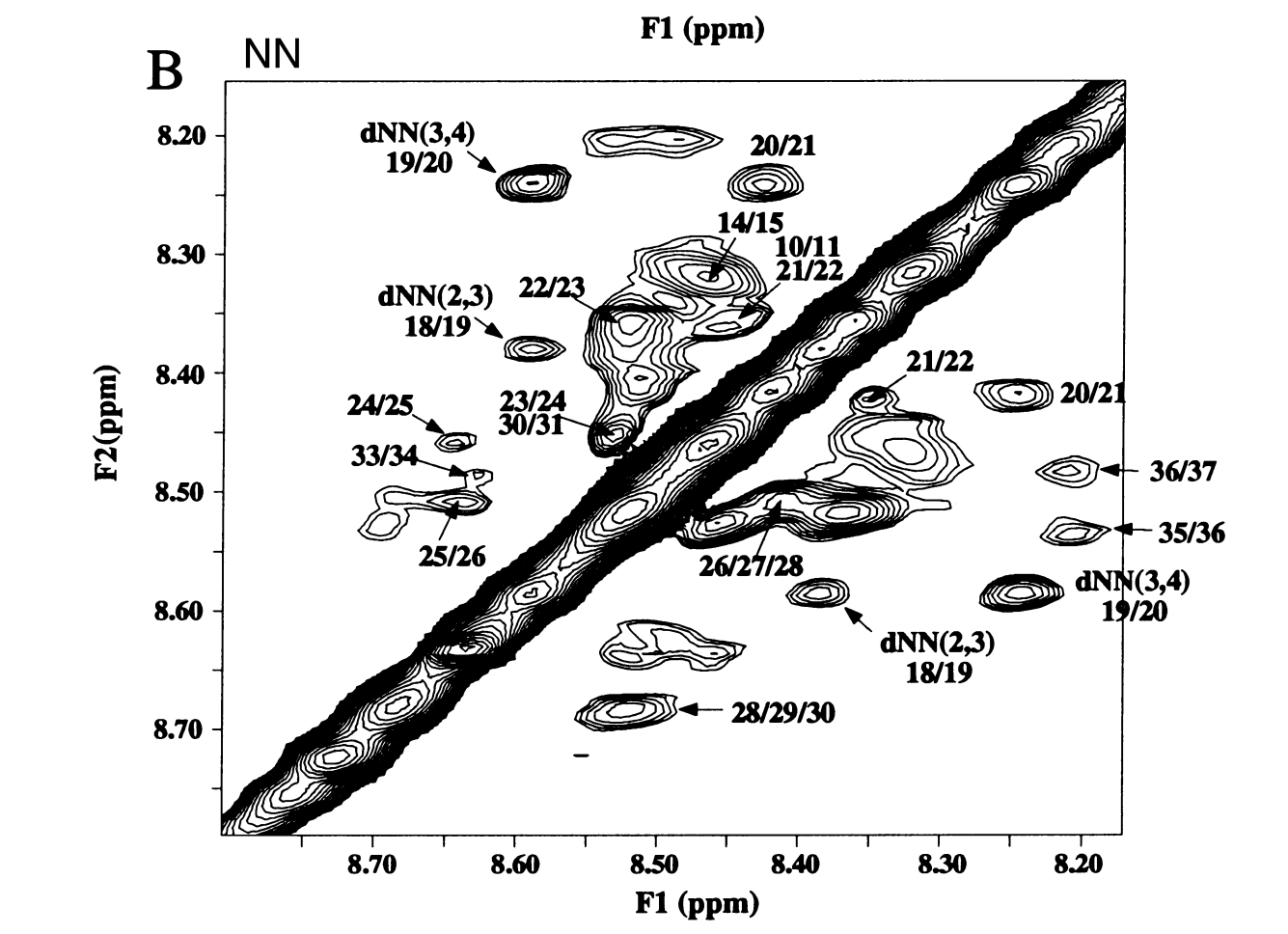


The mixing coefficients, a_{IS} = a_{SI} are proportional to the NOE between these two nuclei

The NOE is related to the distance *r* between the two spins and the correlation time t_c (the time for reorientation of the IS vector in the molecule): structure and motion

$$NOE \propto r_{IS}^{-6} f(t_c)\, \tau_m$$

A simple example: a small immunogenic peptide

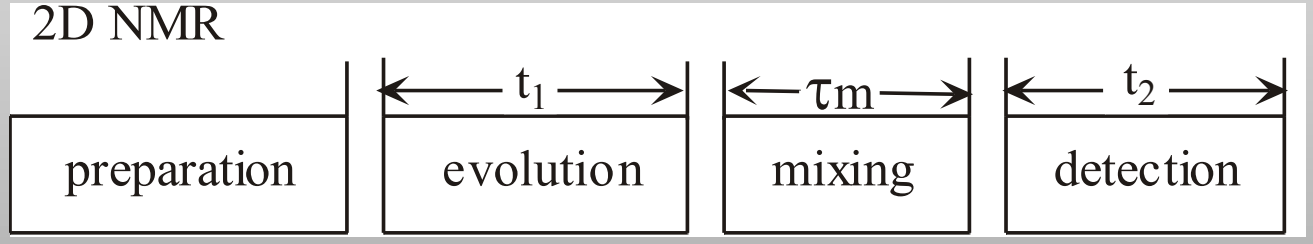


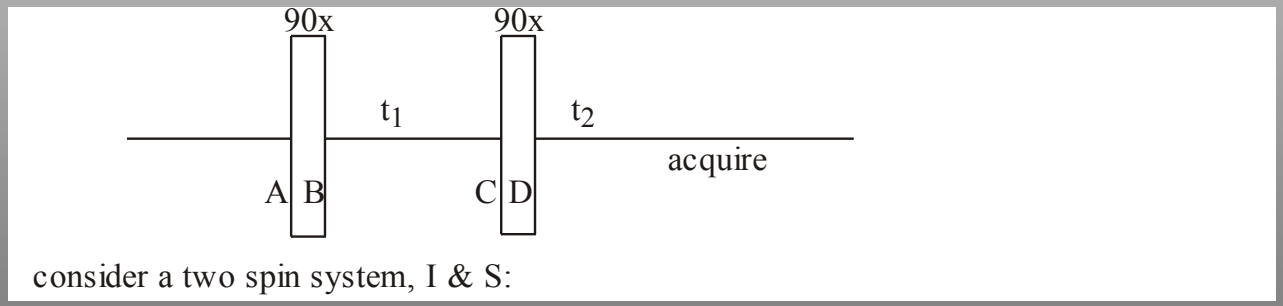
Patterns of NOE interactions define protein secondary structure

Distance	α-helix	3_{10}-helix	β	β_P	turn I[a]	turn II[a]
$d_{\alpha N}$	3.5	3.4	2.2	2.2	3.4 3.2	2.2 3.2
$d_{\alpha N}(i,i+2)$	4.4	3.8			3.6	3.3
$d_{\alpha N}(i,i+3)$	3.4	3.3			3.1-4.2	3.8-4.7
$d_{\alpha N}(i,i+4)$	4.2					
d_{NN}	2.8	2.6	4.3	4.2	2.6 2.4	4.5 2.4
$d_{NN}(i,i+2)$	4.2	4.1			3.8	4.3
$d_{\beta N}$[b]	2.5-4.1	2.9-4.4	3.2-4.5	3.7-4.7	2.9-4.4 3.6-4.6	3.6-4.6 3.6-4.6
$d_{\alpha\beta}(i,i+3)$[b]	2.5-4.4	3.1-5.1				

Observable NOE interactions (<5 A) in regular protein secondary structures

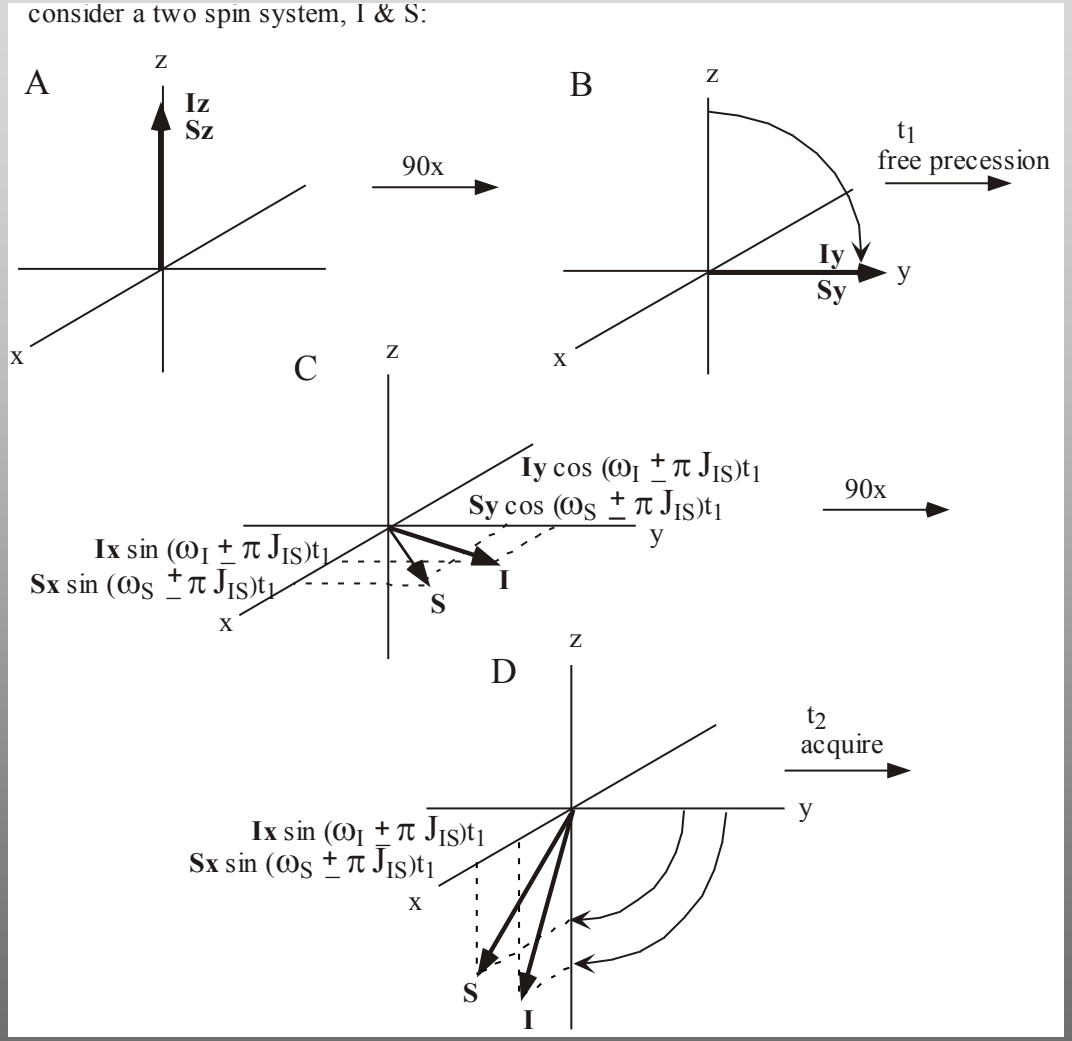
COherence transfer SpectroscopY (COSY)





The COSY experiments detects interactions (correlation) between spins that are scalar coupled: beware, it can only be understood through quantum mechanics

COherence transfer SpectroscopY (COSY)



1. Excite with 90° pulse

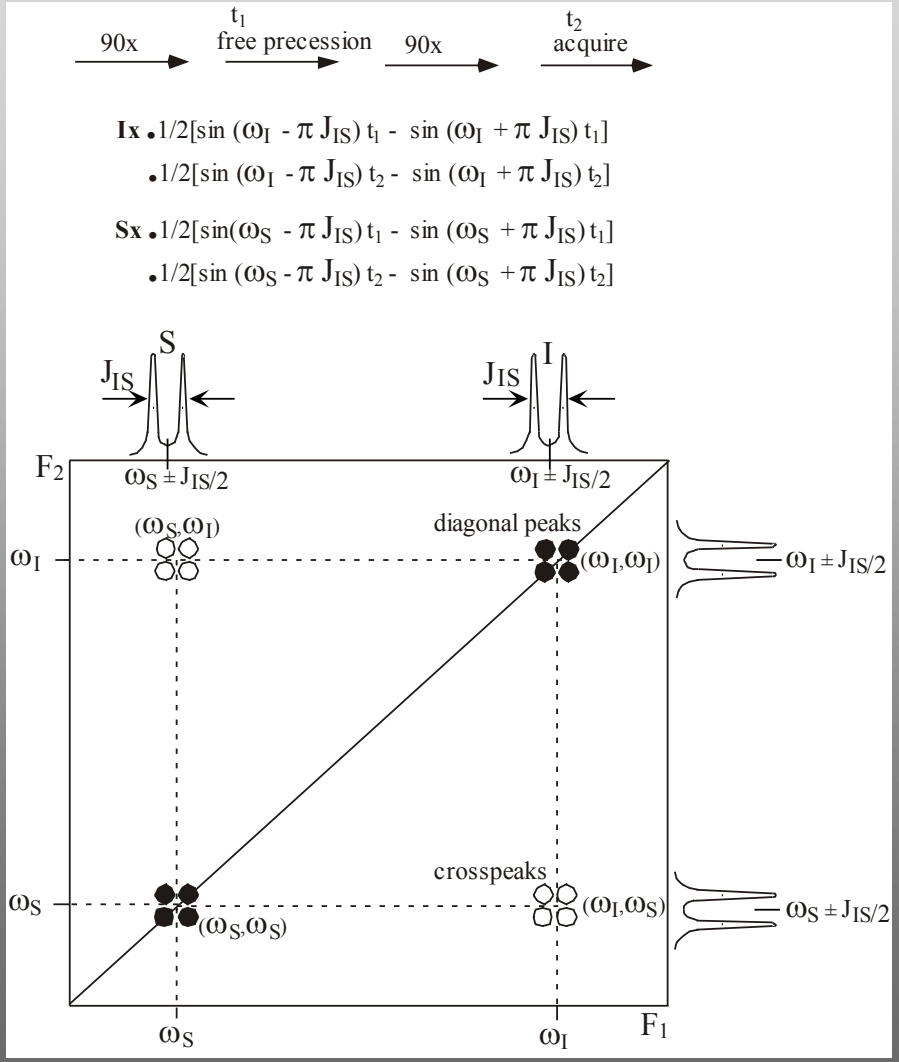
2. During t1, spins are labeled with their Larmor frequency

3. During t1, if spins are scalar coupled, the signal encodes this information as well

4. The second 90° pulse exchanges magnetization between spins: spin I now has memory of spin S and viceversa

5. Signal is acquired in t2

COherence transfer SpectroscopY (COSY)

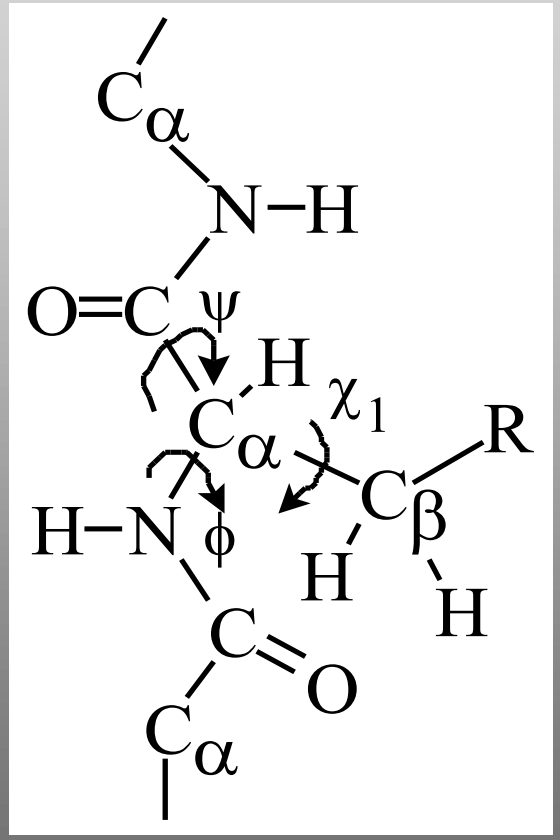


When magnetization is exchanged, spin I signal contains information on spin S and viceversa (cross-peaks)

Cross-peaks appear between spins which are scalar coupled (assignments)

The cross-peak fine structure contains information on scalar coupling (structure)

Structural information: scalar couplings directly gives you the torsion angles that define protein or n.a. structure



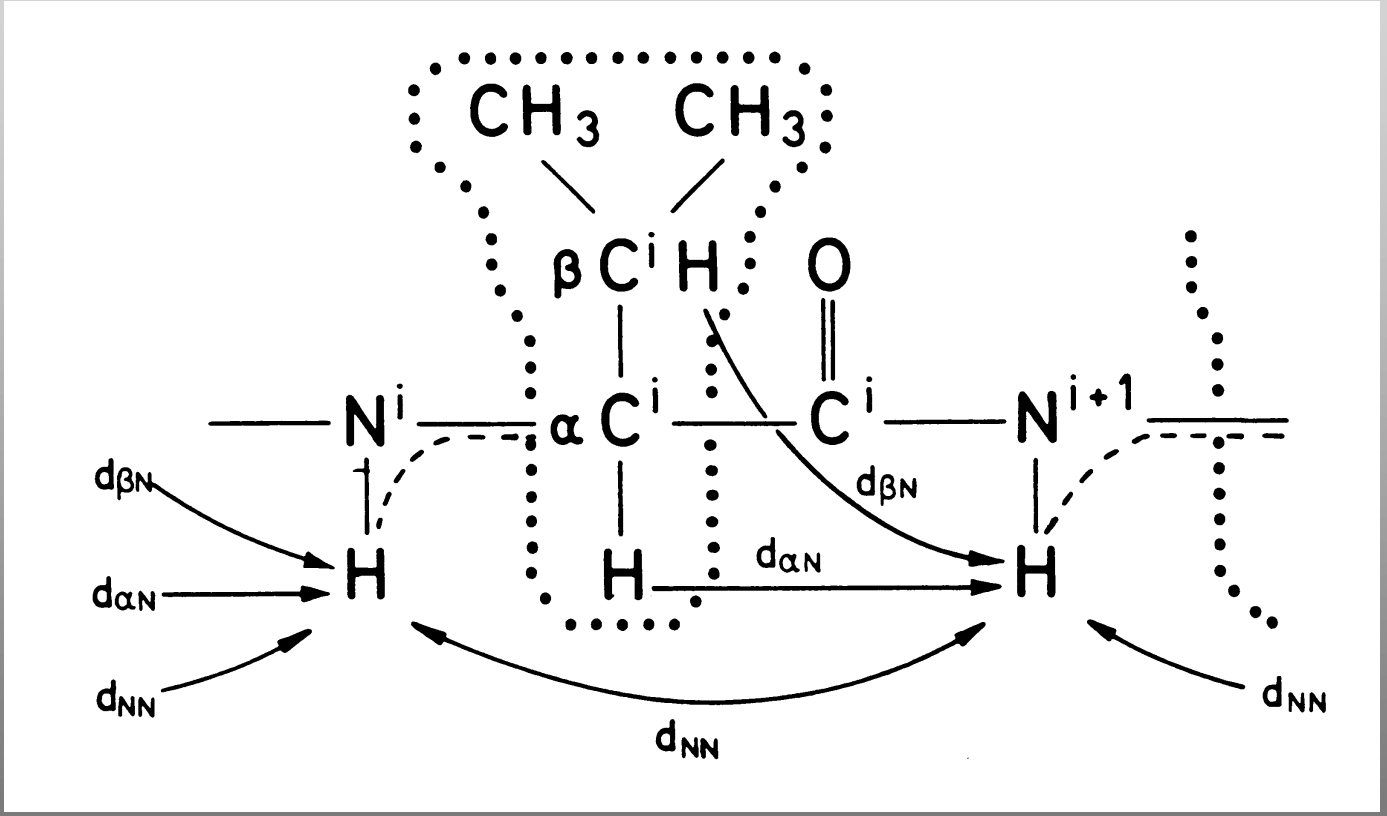
$^3J_{H\alpha N}=5.9\cos^2\phi-1.3\cos\phi+2.2$

$^3J_{\alpha\beta}=9.5\cos^2\chi_1-1.6\cos\chi_1+1.8$

(Karplus, 1958)

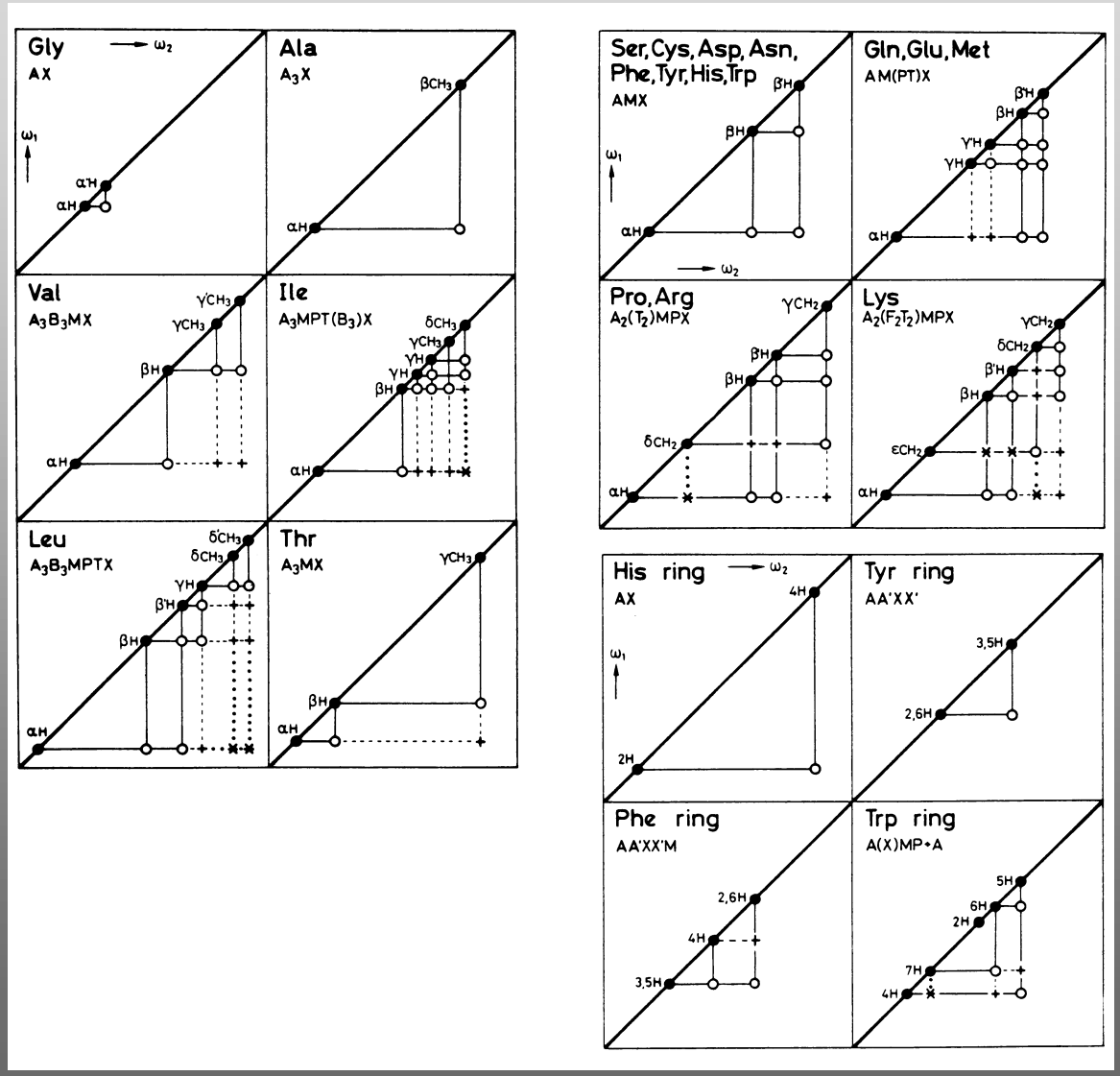
Cross-peaks in COSY experiments occur only between residues that are scalar coupled; in turns, these couplings can be measured in COSY experiments

COSY and NOESY connectivities in the polypeptide unit



COSY (broken lines) and NOESY (continuous lines)

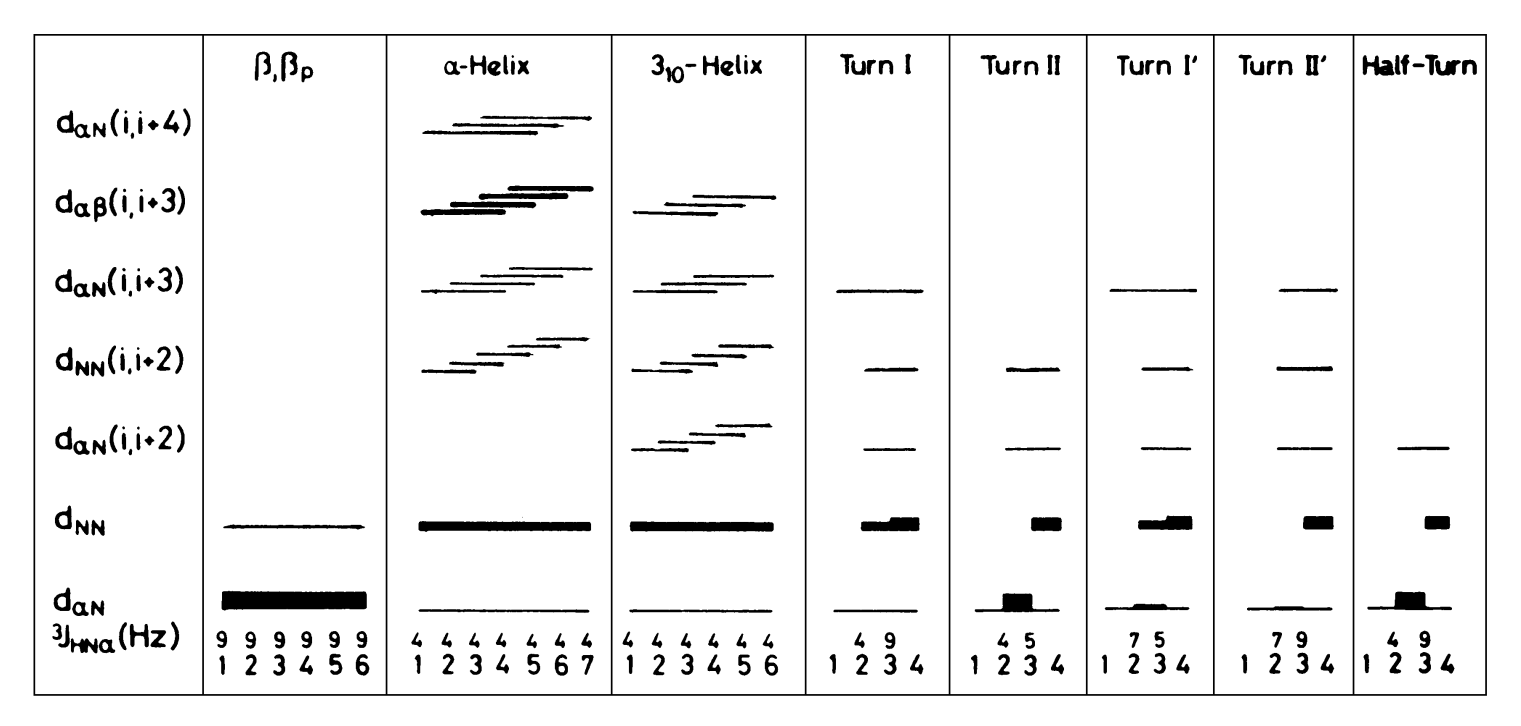
Amino acid identification from scalar coupling patterns



Different pattern of scalar couplings allows amino acid type identification in correlated spectra (COSY, 2QF-COSY, TOCSY)

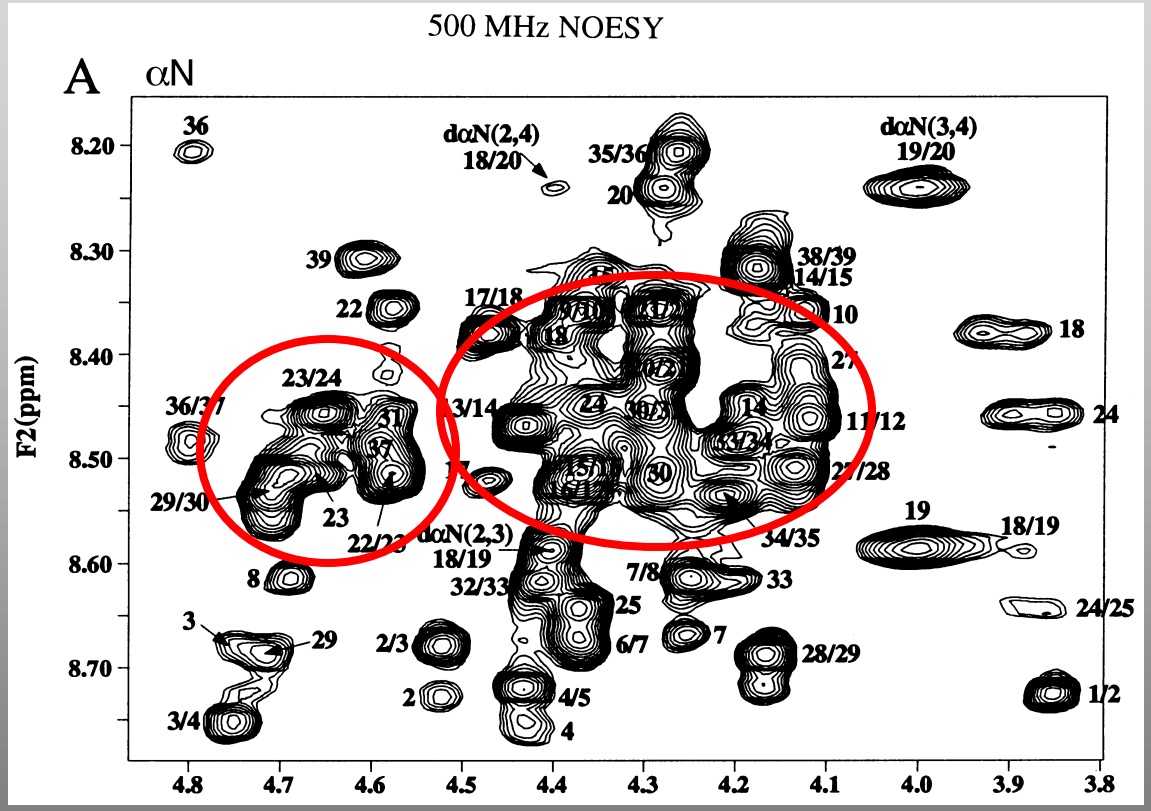
This is the first step towards complete spectral assignments of a protein spectrum (at least before heteronuclear NMR)

NOE interactions, scalar couplings (and chemical shifts) can be combined to define protein secondary structure



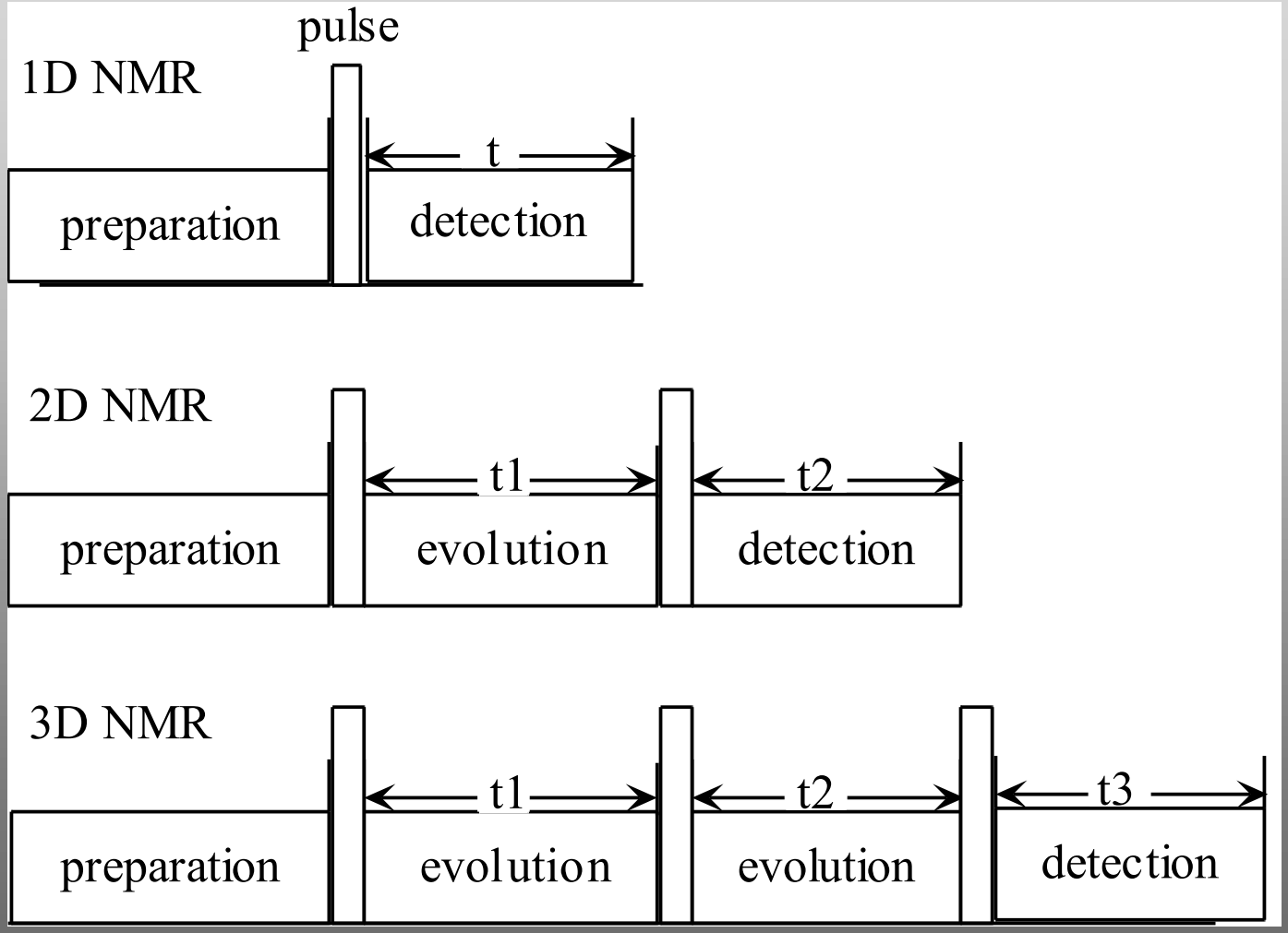
NOE interactions (<4.5 A) and scalar coupling patterns in regular protein secondary structures

Even 2D spectra can be (and indeed are) very crowded



Realistic limit of homonuclear NMR: proteins of 100-120 amino acids; spectra of larger proteins are too crowded

3D Heteronuclear NMR



Useful nuclei such as ^{15}N, ^{13}C are rare

Isotope	Spin (I)	Natural abundance	Magnetogyric ratio $\gamma/10^7$ rad $T^{-1}s^{-1}$	NMR frequency MHz (2.3 T magnet)
^{1}H	1/2	99.985 %	26.7519	100.000000
^{2}H	1	0.015	4.1066	15.351
^{13}C	1/2	1.108	6.7283	25.145
^{14}N	1	99.63	1.9338	7.228
^{15}N	1/2	0.37	-2.712	10.136783
^{17}O	5/2	0.037	-3.6279	13.561
^{19}F	1/2	100	25.181	94.094003
^{23}Na	3/2	100	7.08013	26.466
^{31}P	1/2	100	10.841	40.480737
^{113}Cd	1/2	12.26	-5.9550	22.193173

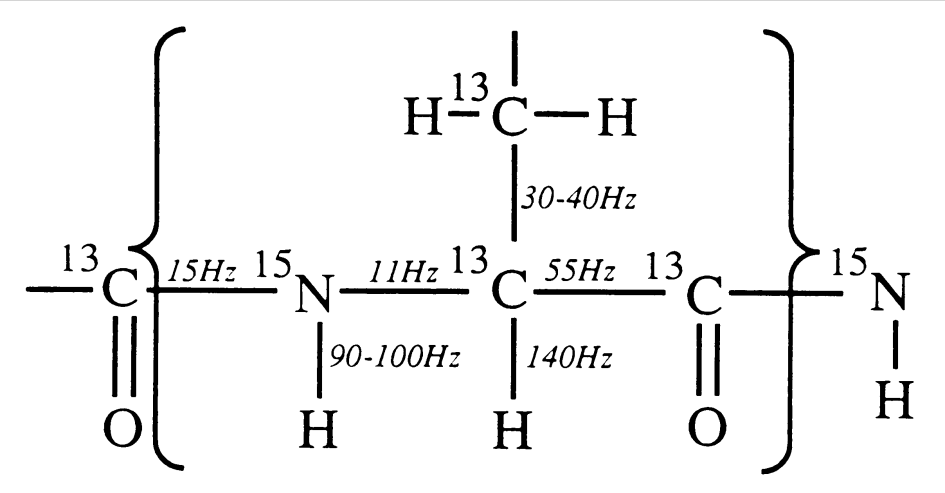
Requirements for Heteronuclear NMR: isotope labeling

- **Isotopically labelled proteins can be prepared straightforwardly in *E. coli* by growing cells in minimal media (e.g. M9) supplemented with appropriate nutrients (^{15}NH4Cl, ^{13}C-glucose)**

- **Metabolic pathways can be exploited and appropriate auxotrophic strains of *E. coli* can also be used for selective labelling: e.g. use acetate instead of glucose and obtain selective labeling of certain side chain CH_3**

- **Isotopic labelling of protein expressed in eukaryotic cells is expensive but can be done (post-translational modifications can be studied but you need $$$$)**

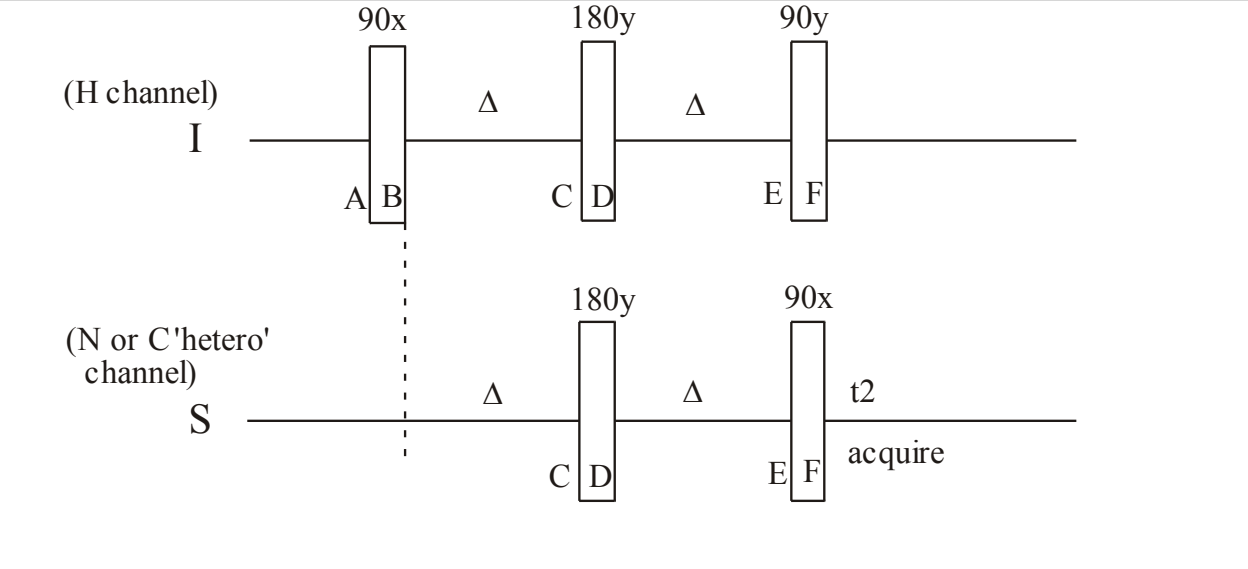
Heteronuclear NMR exploits 1-bond scalar couplings



- 1-bond couplings are large (20-150 Hz) compared to HH couplings (1-10 Hz)

- They are independent of conformation: no structural insight but wonderful for assignments

The basic building block of Heteronuclear NMR (INEPT)

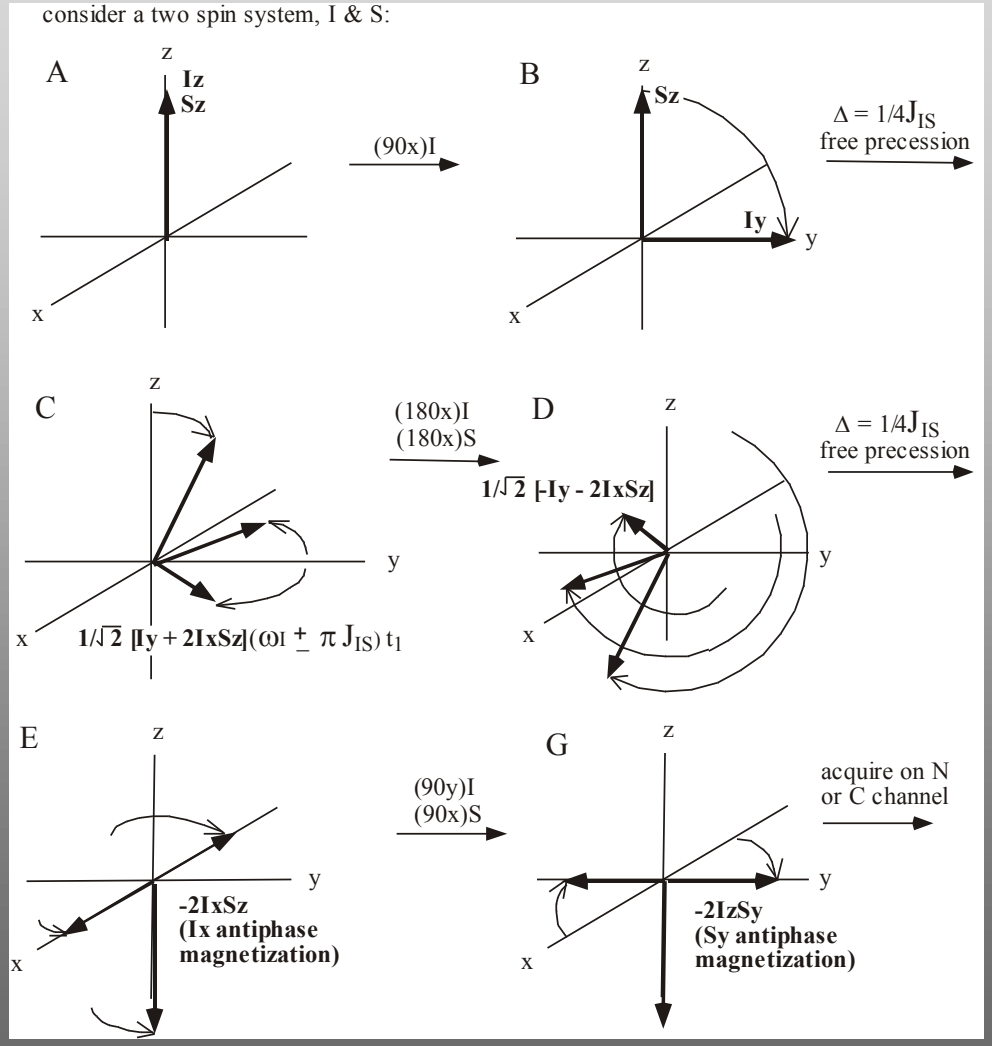


- **Polarization of ^{13}C and ^{15}N is low: start with ^{1}H polarization**

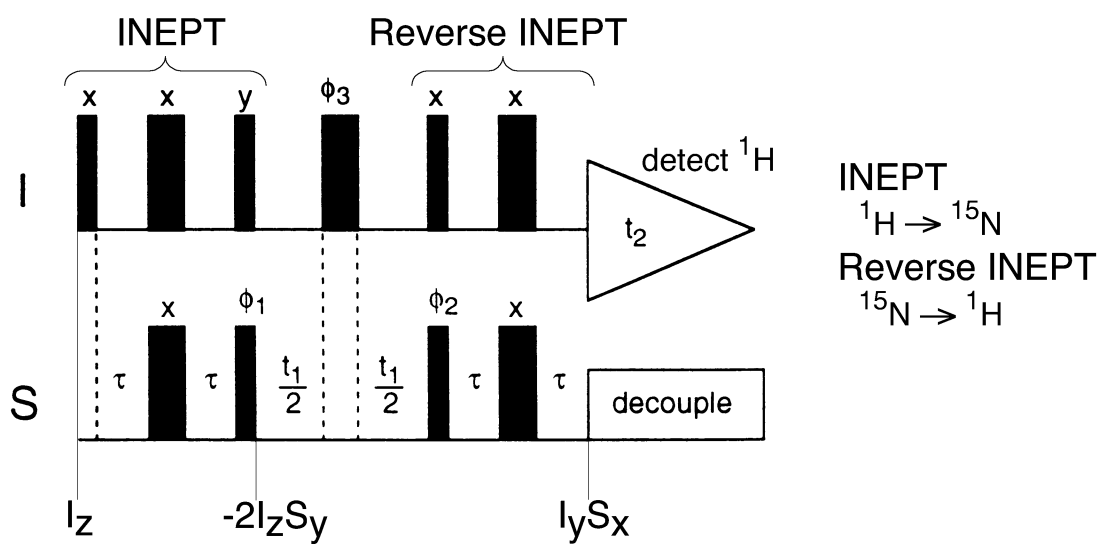
- **Use 1-bond scalar couplings to transfer magnetization from ^{1}H to the nucleus of interest**

- **Delay Δ must be set to 1/4J for optimal transfer: in the absence of relaxation, magnetization transfer is 100% efficient**

The basic building block of Heteronuclear (INEPT-1D)



The basic building block of 2D Heteronuclear NMR (HSQC)

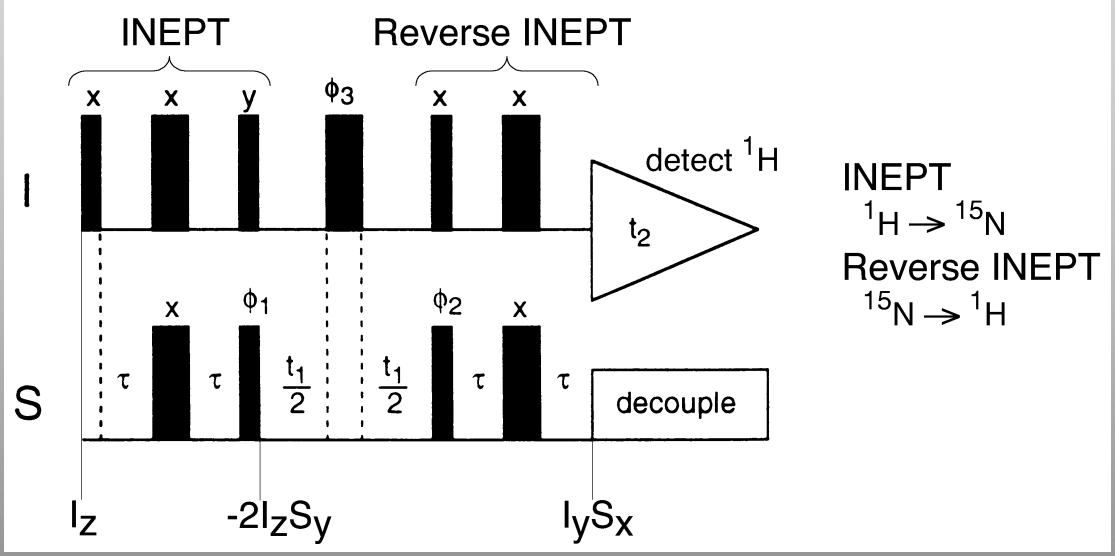


- **Polarization of ^{13}C and ^{15}N is low: start with ^{1}H, transfer to ^{1}H with INEPT (sensitivity increases by the ratios of γ, e.g. 10 for ^{15}N)**

- **Label magnetization with ^{15}N Larmor frequency in t1 and record ^{15}N evolution in the first dimension**

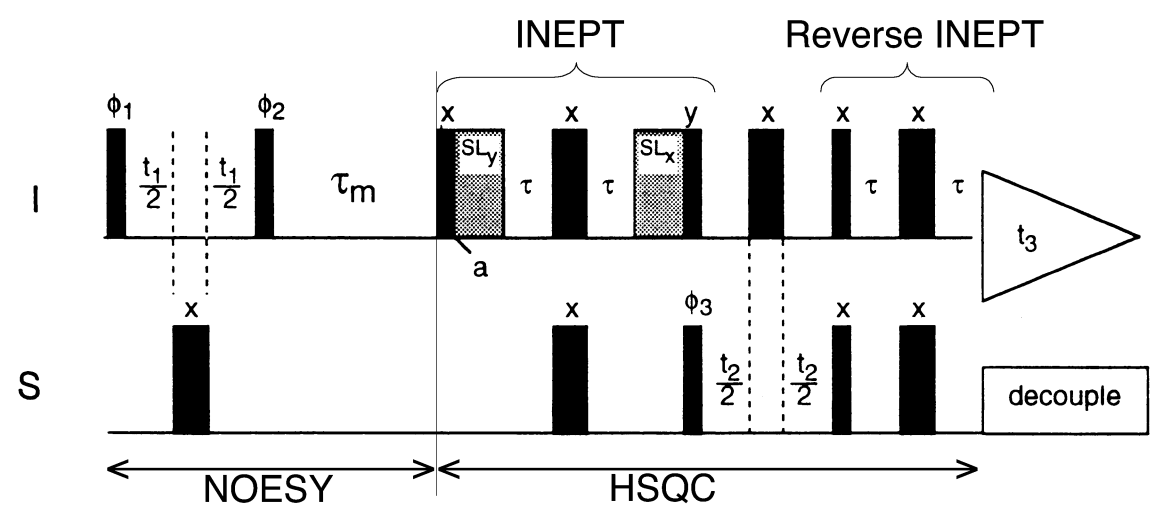
- **Go back to ^{1}H for detection with a reverse INEPT, i.e. from ^{15}N to ^{1}H and record ^{1}H evolution in the direct dimension (high s/n)**

HSQC is the building block and foundation for very many heteronuclear NMR experiments



- **Measurements of relaxation properties (motion)**
- **Spectral assignments**
- **3D versions of NOESY and COSY spectra (structure)**

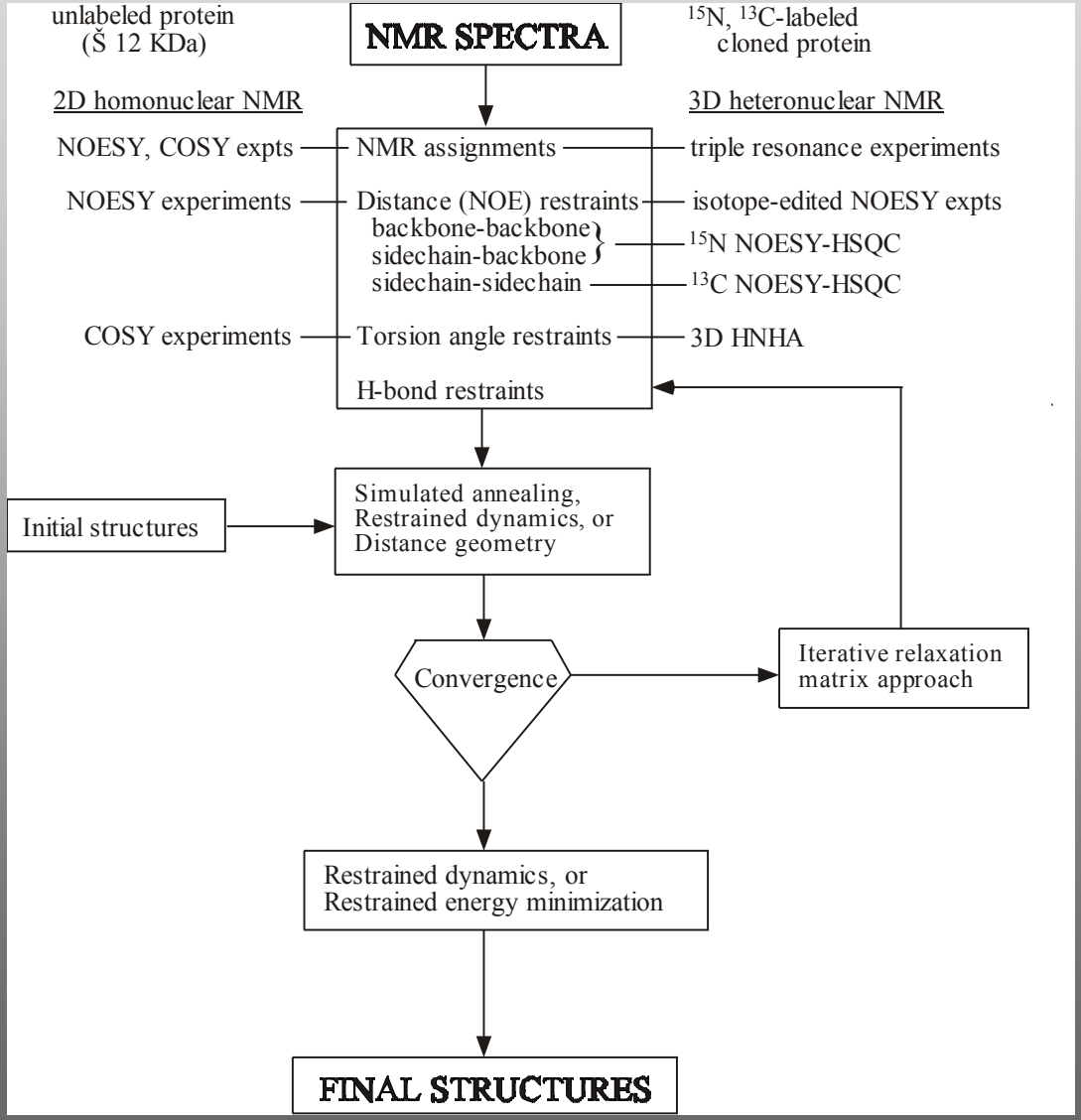
The most important experiment for protein structure determination: 3D NOESY HSQC



- **Measurements of ^{1}H-^{1}H distances (as in 2D NOESY)**

- **Resolution is spread in a third dimension (usually ^{15}N but also ^{13}C; for nucleic acids mostly ^{13}C; for protein/nucleic acid complexes you can observe only the protein, only the RNA or only the contact from one to the other)**

Protein and nucleic acid 3D structure generation from NMR



Typical protocol for 3D structure generation from NMR data (with 3D data)