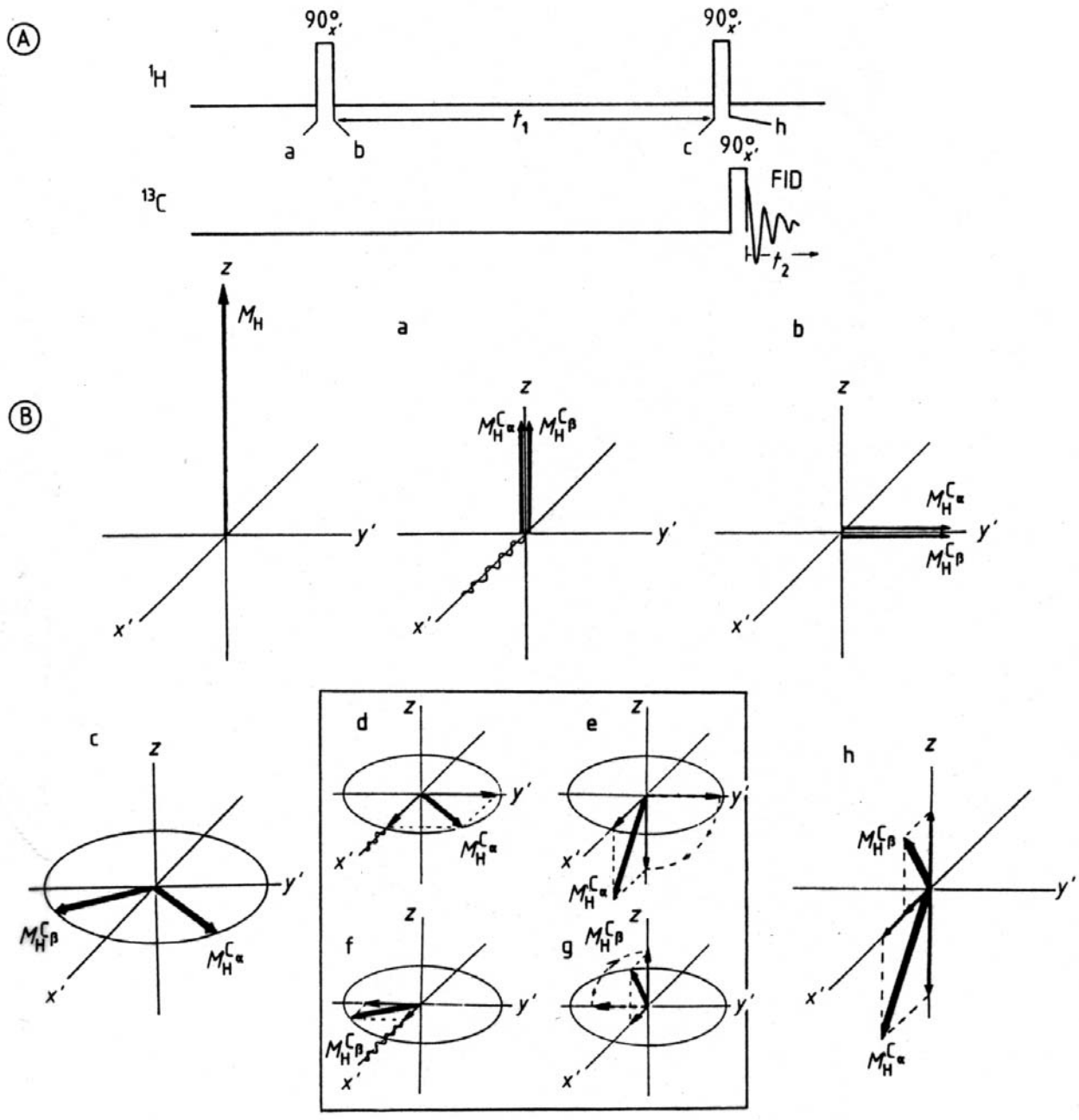
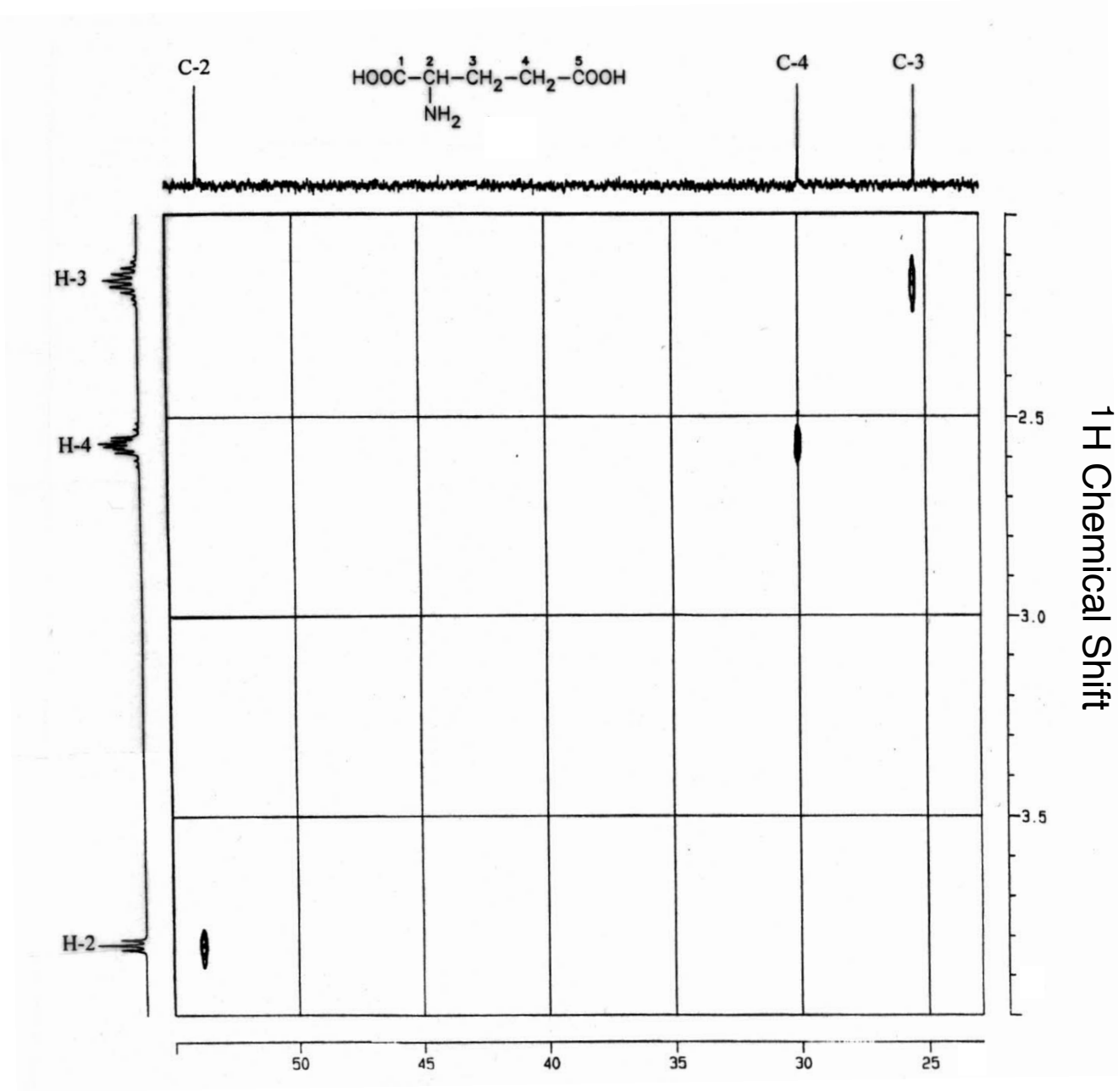
CHEM / BCMB 4190/6190/8189

Introductory NMR

Lecture 17

Last Time:
-HETCOR or C, H-COSY




C-2
HOOC–CH–CH2–CH2–COOH
NH2
C-4
C-3
H-3
H-4
H-2
2.5
3.0
3.5
50
45
40
35
30
25
1H Chemical Shift
13C Chemical Shift

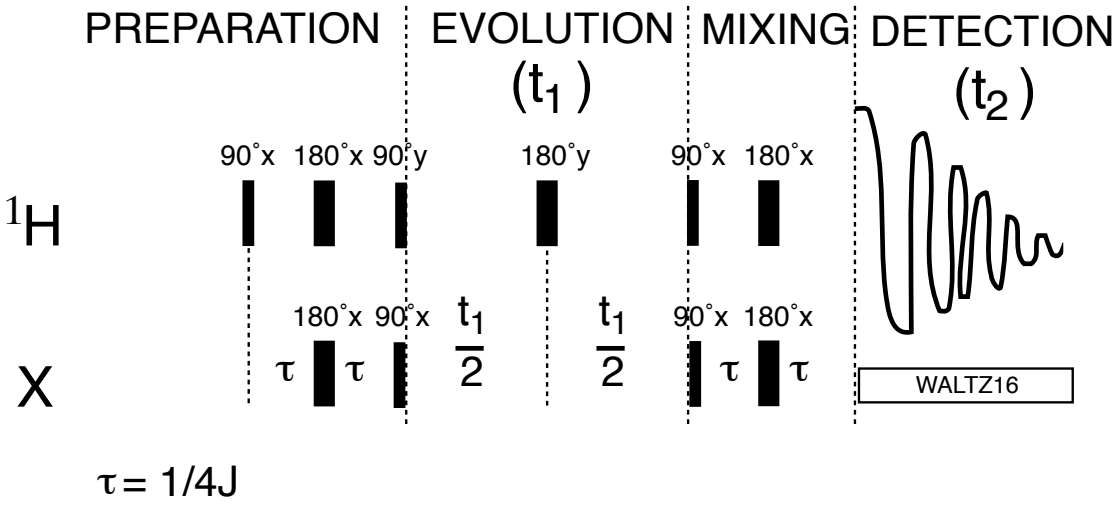
Two-dimensional Correlated NMR spectroscopy:
The HSQC Experiment

1. HETCOR (or C,H-COSY) Versus HSQC:

- **We have seen the C,H-COSY experiment where ^{1}H is detected in t1 and ^{13}C is detected in t2. Although there is population transfer from ^{1}H to ^{13}C, the sensitivity of this experiment is poor because ^{13}C (not ^{1}H) is detected in t2.**

- **Higher sensitivity can be achieved by doing the "reverse" experiment i.e. by detecting ^{1}H in t2 and ^{13}C in t1. The HSQC and HMQC are two experiments that achieve this heteronuclear H,C-correlation. Here, we will consider in more details the HSQC experiments.**

2. The HSQC Pulse Sequence:



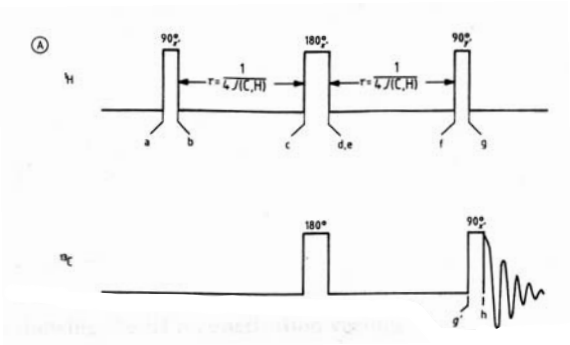
$\tau = 1/4J$

- **In the ^{1}H-^{13}C HSQC (X = ^{13}C):**

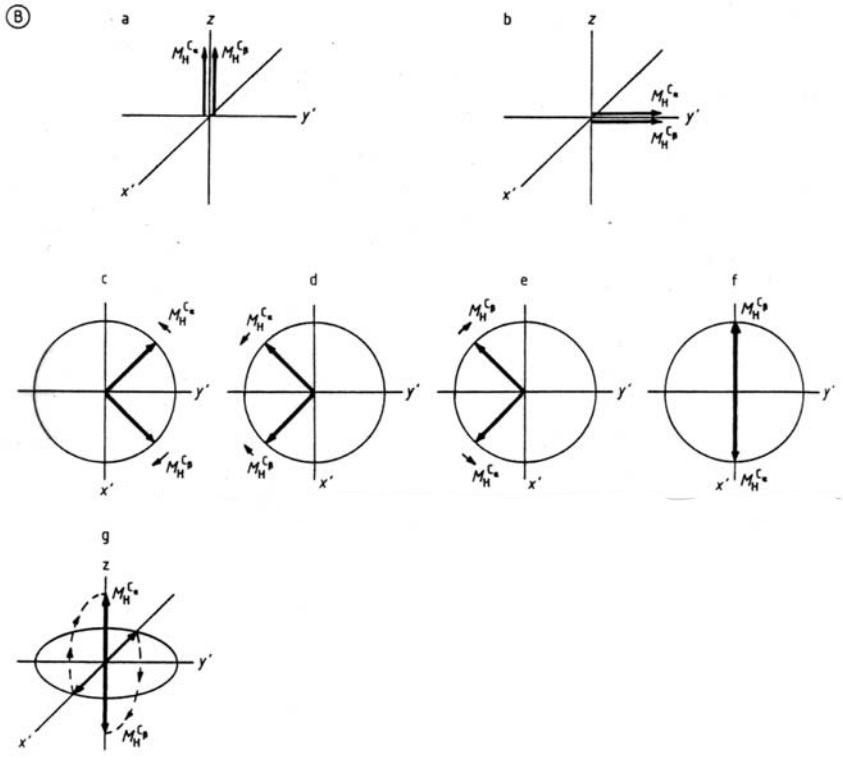
1) **The PREPARATION period is an INEPT sequence (^{1}H to ^{13}C).**
2) **The t1 EVOLUTION period allows for indirect ^{13}C chemical shift detection.**
3) **The MIXING period is a REVERSE INEPT sequence (^{13}C to ^{1}H).**
4) **The t2 EVOLUTION period allows for direct ^{1}H chemical shift detection.**

3. <u>Review of the INEPT experiment:</u>

A) Pulse sequence in the ^{1}H and ^{13}C channels



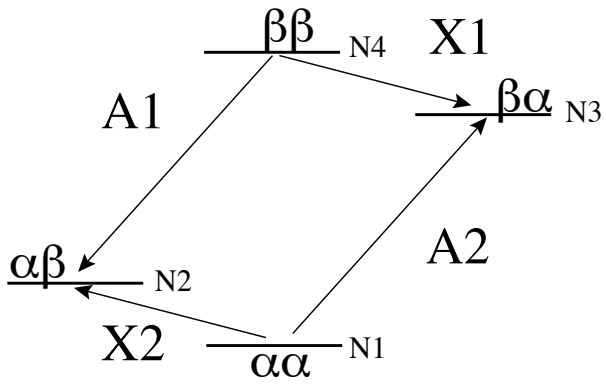
B) Vector diagrams showing the ^{1}H magnetization vectors ($^{13}CHCl_3$)



- **At point g: ^{1}H 90° pulse rotates $M_H^{C\alpha}$ to +z and $M_H^{C\beta}$ to –z**

The populations of N2 and N4 are inverted:

	before INEPT	after INEPT
N4	**N**	**N + ΔH**
N3	**N + ΔC**	**N + ΔC**
N2	**N + ΔH**	**N**
N1	**N + ΔC + ΔH**	**N + ΔC + ΔH**

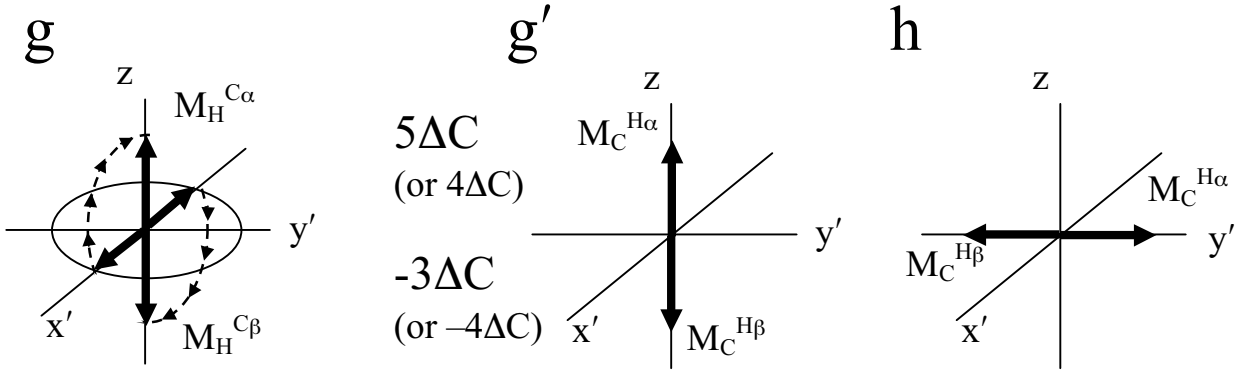


X1 transition: N3 – N4 = ΔC - ΔH = -3ΔC
X2 transition: N1 – N2 = ΔC + ΔH = 5ΔC

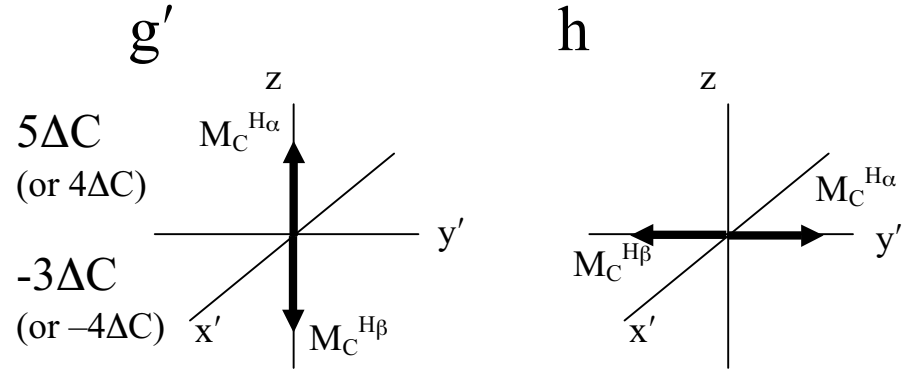
- **In the case of the HSQC, the contribution from the natural ^{13}C magnetization (ΔC) is unwanted and is removed using one of the selected methods that we have seen previously. The resulting populations and population differences are:**

N4 = N + ΔC/2 + ΔH
N3 = N + ΔC/2
N2 = N + ΔC/2
N1 = N + ΔC/2 + ΔH
X1 transition: N3 – N4 = -ΔH = -4ΔC
X2 transition: N1 – N2 = ΔH = 4ΔC

- **At point g': $MC^{H\alpha}$ is in its original position, but $MC^{H\beta}$ is inverted**

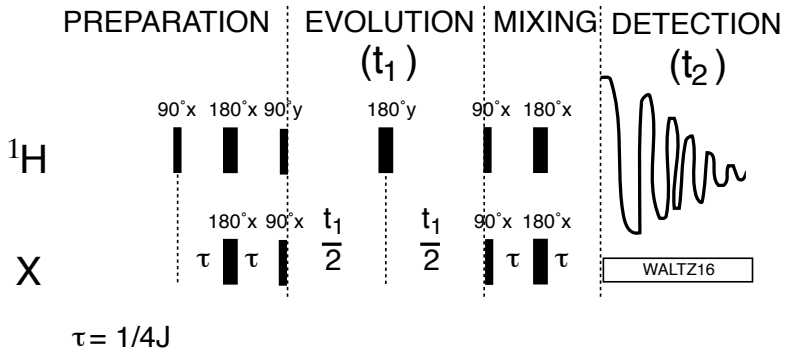


C) Vector diagrams showing the ^{13}C magnetization vectors

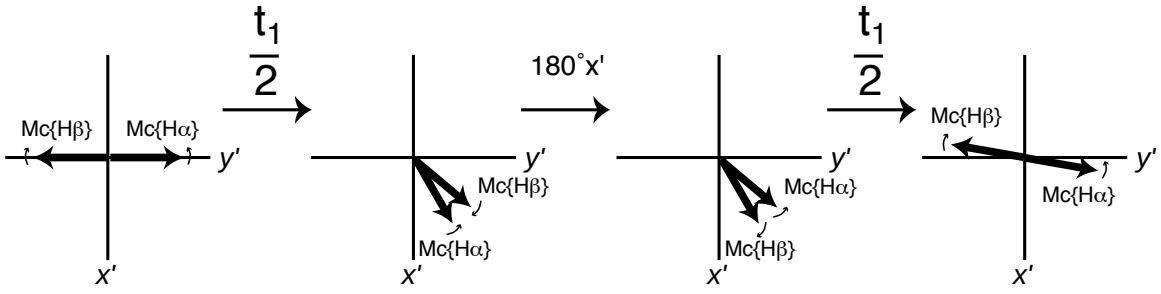


- **At point n: The 90˚x pulse on ^{13}C creates transverse magnetization components that evolve during t1. Note that these ^{13}C magnetization components are 180˚ out of phase with each others at the beginning of t1.**

4. <u>The t1 Evolution Period:</u>

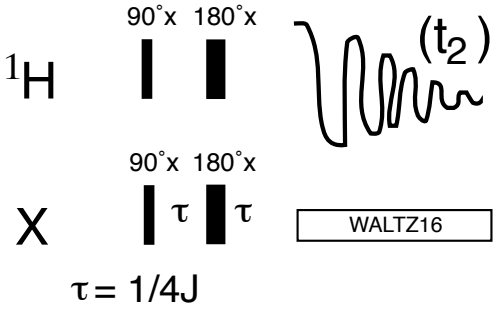


1) **The ^{13}C chemical shift evolves (to different points depending on the value of t1).**
2) **There is no net ^1H-^{13}C coupling evolution. Note that the ^{13}C magnetization components are 180˚ out of phase with each others at the beginning and the end of t1.**

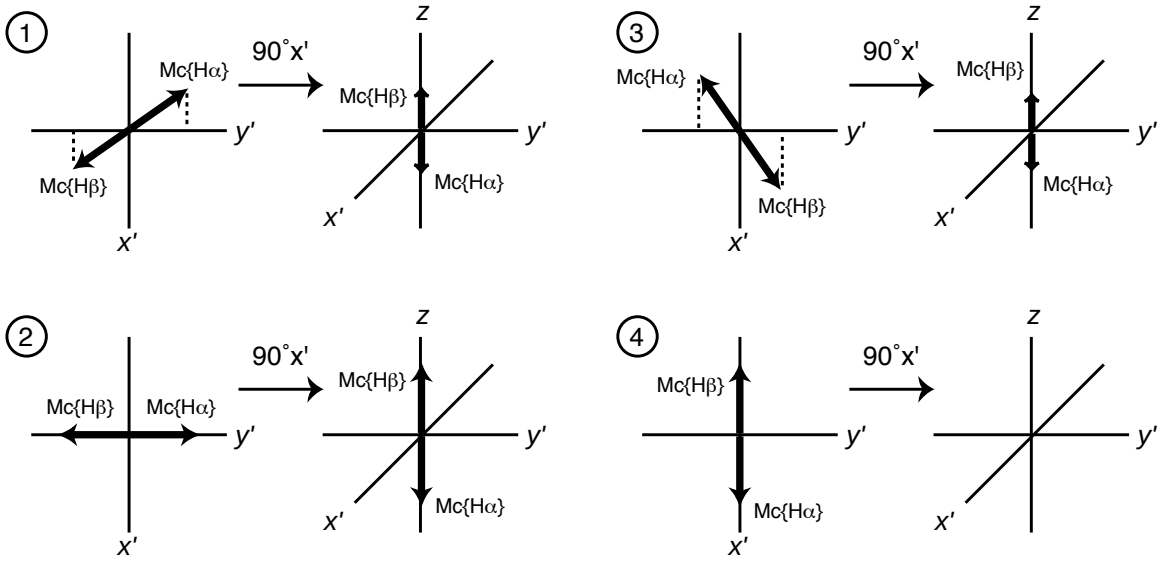


5. The Reverse-INEPT experiment:

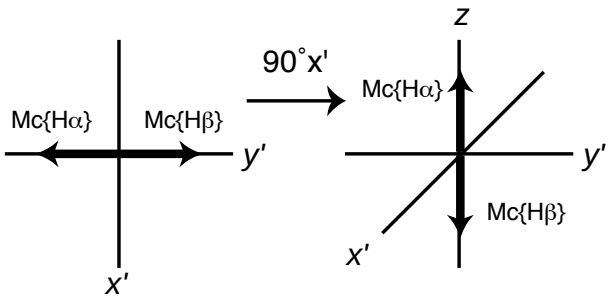
A) Pulse sequence in the ^{1}H and ^{13}C channels



B) Vector diagrams showing the effect of the first ^{13}C 90˚x' pulse on the ^{13}C magnetization vectors (Ex.: $^{13}CHCl_3$) for various t1 values. Four cases are shown below:

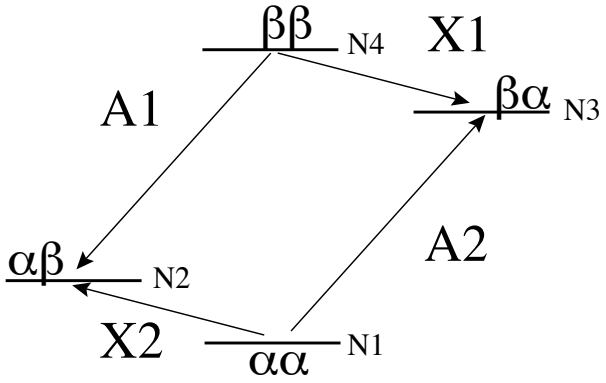


We will only consider the following case, where after the first ^{13}C 90˚x pulse, $MC^{H\beta}$ is in its original position, but $MC^{H\alpha}$ is inverted.



The populations are described as followed (same as after first ^{13}C 90˚x):

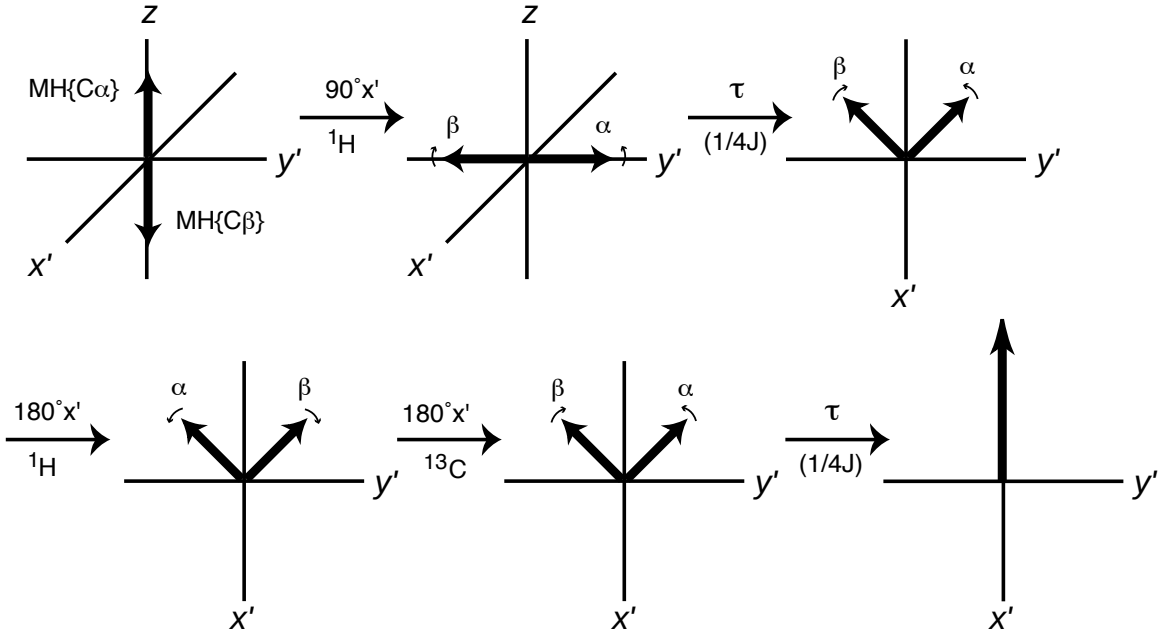
N4 = N + ΔC/2 + ΔH
N3 = N + ΔC/2
N2 = N + ΔC/2
N1 = N + ΔC/2 + ΔH



A1 transition: N2 - N4 = -ΔH
A2 transition: N1 - N3 = ΔH
X1 transition: N3 – N4 = -ΔH = -4ΔC
X2 transition: N1 – N2 = ΔH = 4ΔC

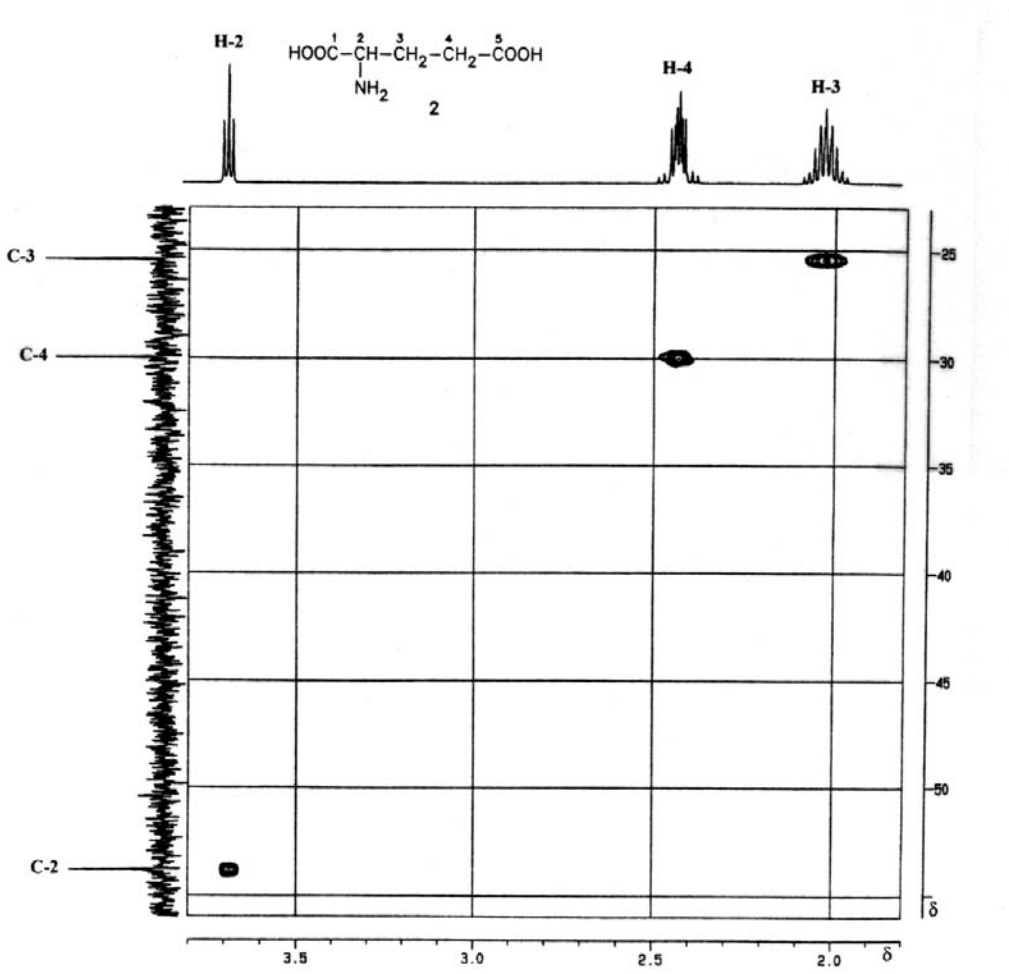
- **The antiphase ^{1}H magnetization is refocused during the 2*τ period. The two 180˚x' pulse (on ^{1}H and ^{13}C) in the middle of the 2*τ period allows J coupling evolution but refocuses the ^{1}H chemical shift evolution.**

- **For simplicity, one can ignore the effect of chemical shift, which is refocused during the reverse-INEPT period.**



- **At the end of the reverse-INEPT α and β are in phase, therefore we can turn on BB ^{13}C decoupling during acquisition.**

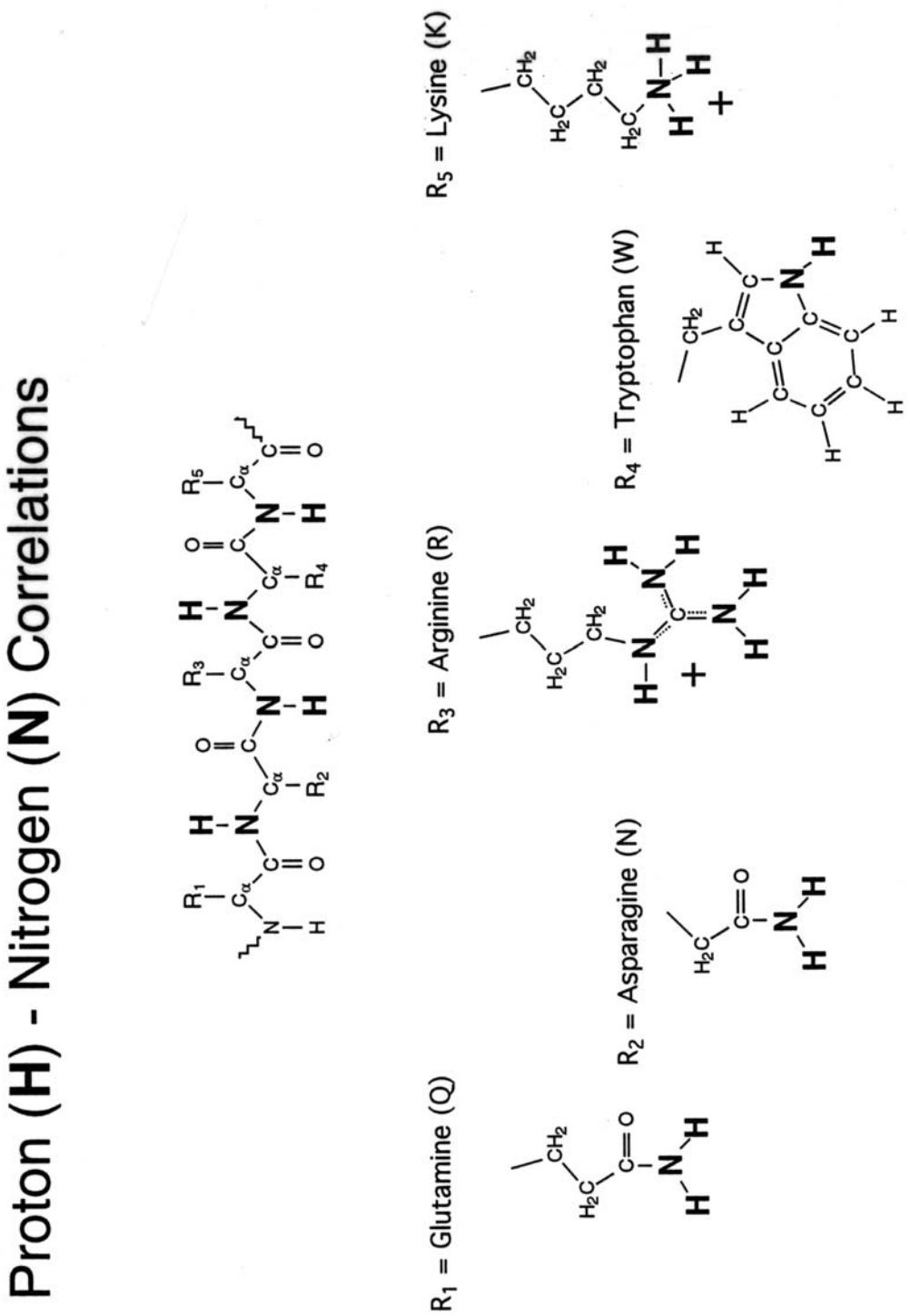
6) <u>HSQC Spectrum</u>:
Example: ^{1}H-^{13}C HSQC of Glutamic Acid



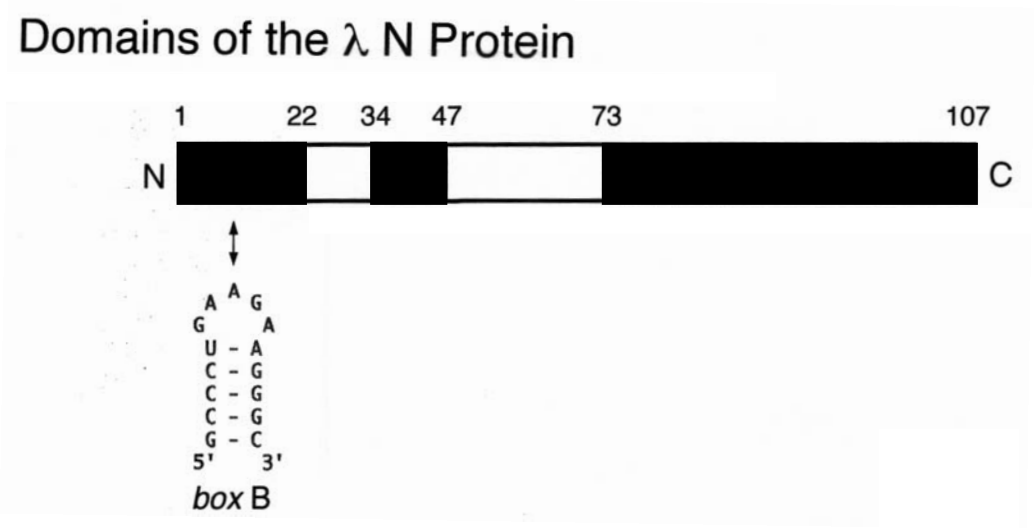
- **Signals <u>not</u> obtained for ^{1}H that are <u>not</u> bound to ^{13}C**
- **Comparison with C,H COSY:**
    1) **Axes are interchanged (in HSQC, ^{1}H is detected, in HETCOR, ^{13}C is detected)**
    2) **HSQC is more sensitive and a good spectrum can be recorded quickly.**

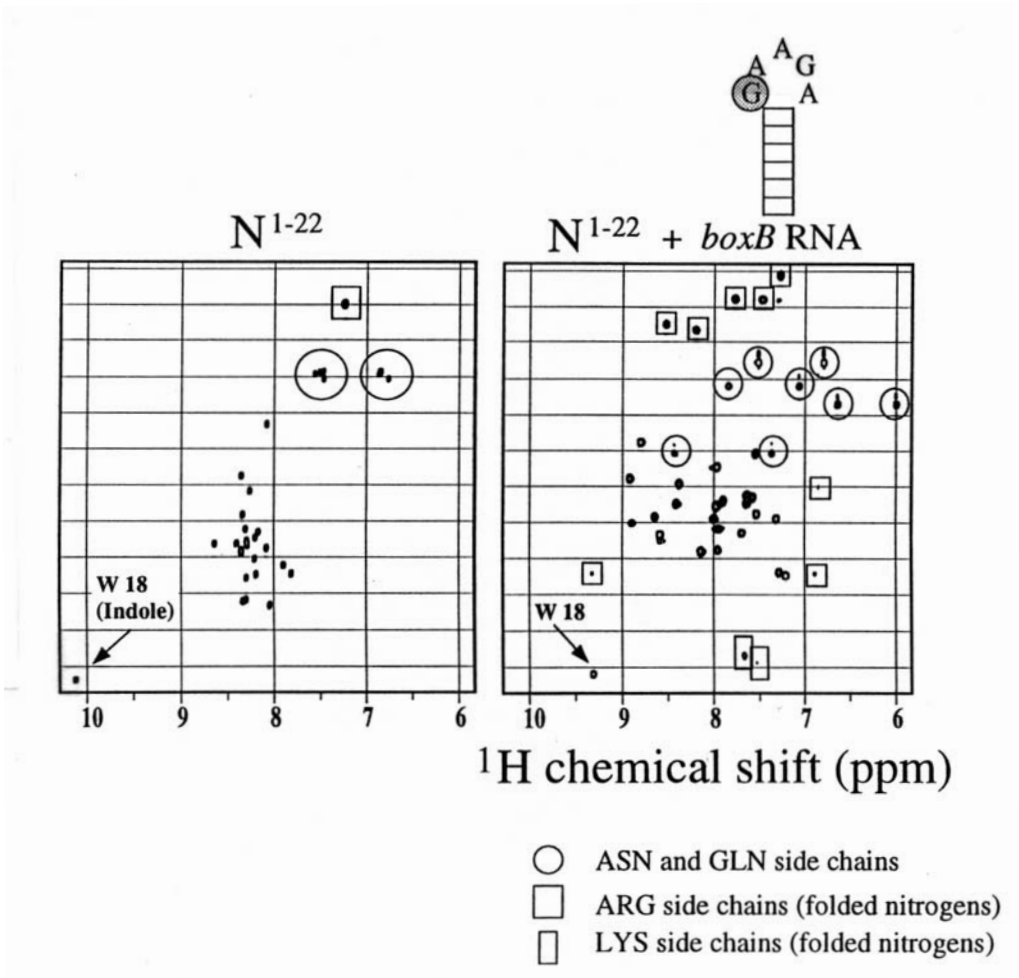
        -Example: for 1 mM uniformly ^{13}C- (or ^{15}N-) isotopically labeled samples, typical recording times are:
            - 15-30 minutes for HSQC
            - 24 hours for C, H COSY

7) <u>Important "Biologically Biased" Applications</u>: ^{1}H-^{15}N HSQC for Analysis of Ligand Binding in Protein

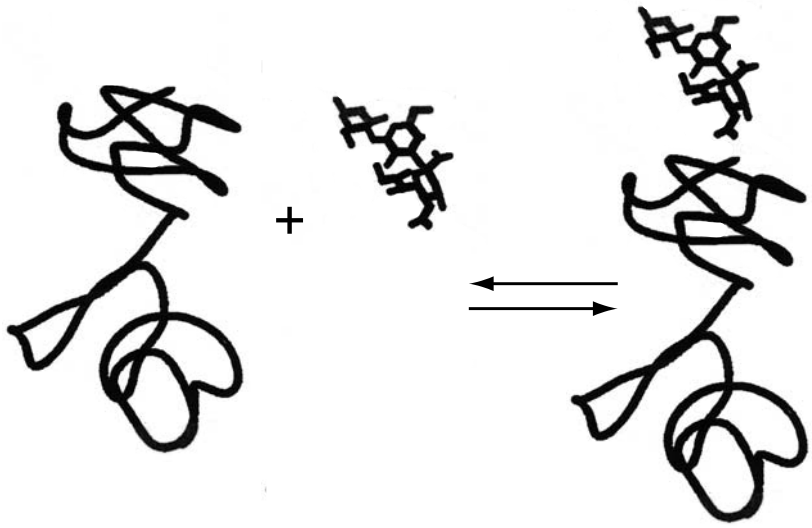


CASE 1: Protein Folding Upon Ligand Binding

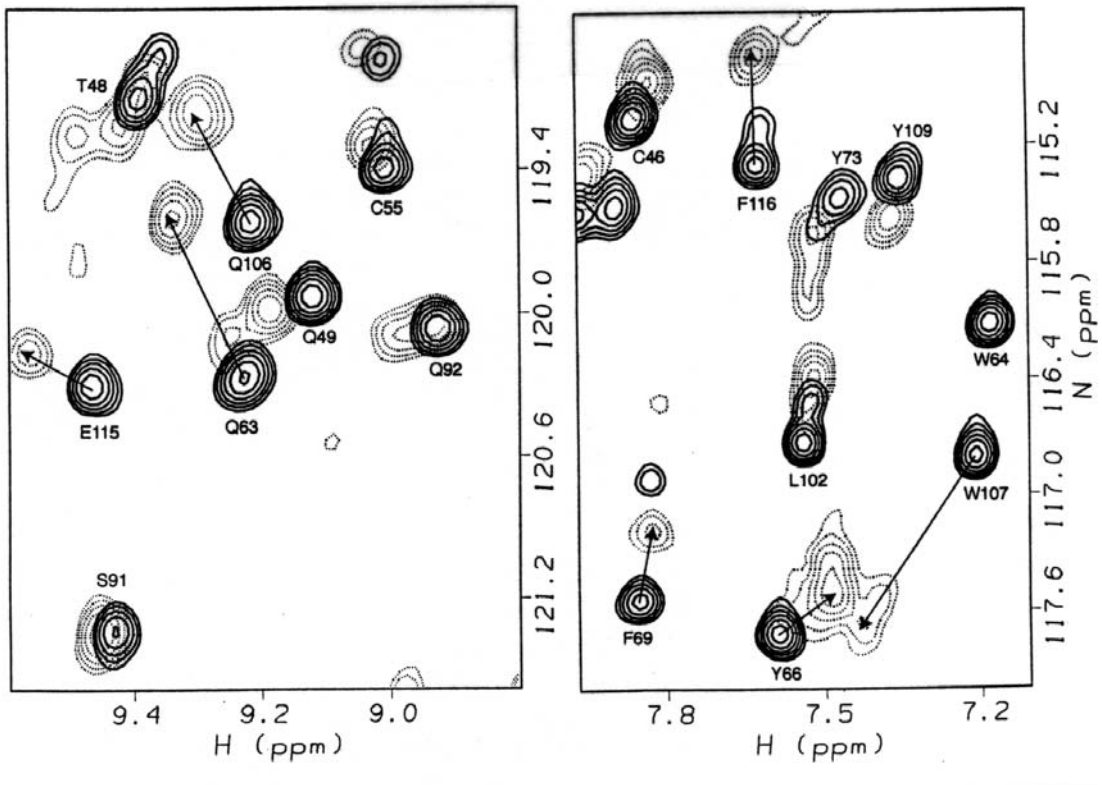




CASE 2: Mapping Ligand Binding Sites in Folded Proteins



- **Chemical shifts of ligand binding site are perturbed:**



The TOCSY Experiment

TOCSY: Total Correlation SpectroscopY
Also known as HOHAHA (HOmonuclear HArtmann-Hahn)

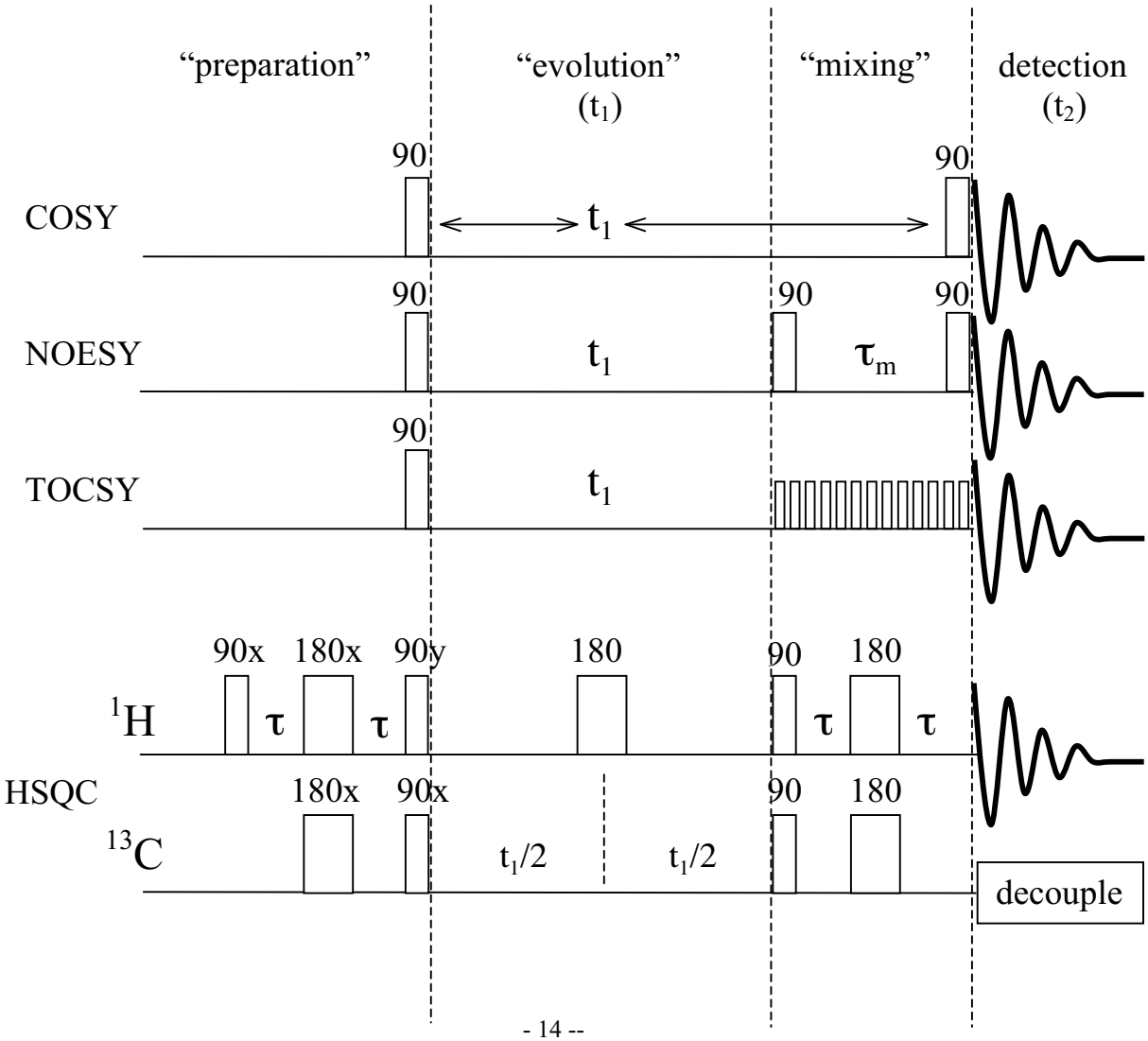
- **Very useful experiment for determining the structures of oligosaccharides and peptides as well as many other organic compounds.**

1. Pulse sequence:

The pulse sequence is similar to that of the COSY experiment except that the second ^{1}H 90°x' pulse is replaced by a spin-lock pulse train:

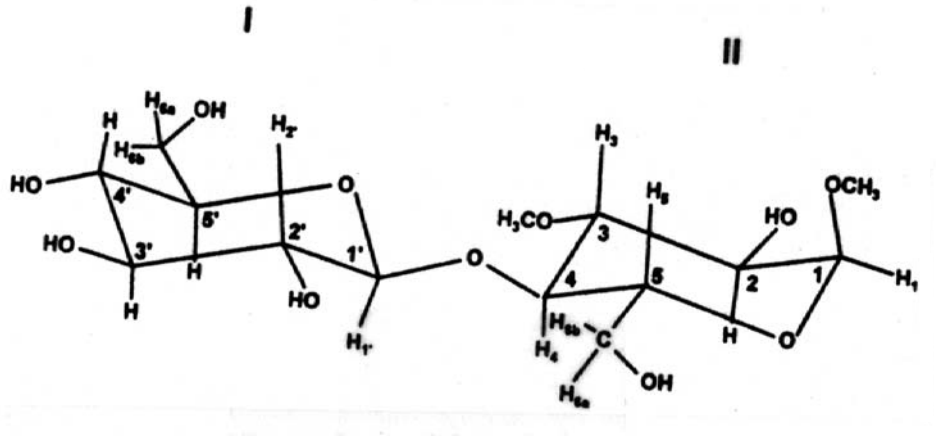
$$90^\circ - t_1 - \text{spin-lock}(t_m) - \text{FID}$$

-The spin-lock allows polarization transfer from one selected proton to all protons within a coupled system (a.k.a. spin system).



2. Spin sytems

Lets take α-methyl-3-O-methylcellobioside for example.



- **In sugar I:**
  - **H1' is coupled to H2' (i.e. $J \neq 0$)**
  - **H2' is coupled to H3'**
  - **H3' is coupled to H4'**
  - **H4' is coupled to H5'**
  - **H5' is coupled to H6'a and H6'b ($J_{H5'\text{-}H6'a} > J_{H5'\text{-}H6'b}$)**
  - **H6'a is coupled to H6'b**

  H1', H2', H3', H4', H5', H6'a, and H6'b form a spin system

- **In sugar II:**
  - **H1 is coupled to H2 (i.e. $J \neq 0$)**
  - **H2 is coupled to H3**
  - **H3 is coupled to H4**
  - **H4 is coupled to H5**
  - **H5 is coupled to H6a and H6b ($J_{H5\text{-}H6a} > J_{H5\text{-}H6b}$)**
  - **H6a is coupled to H6b**

  H1, H2, H3, H4, H5, H6a, and H6b form a spin system.

- **Because there is no ^{1}H-^{1}H *J* coupling between sugar I and sugar II, they both form independent spin systems.**

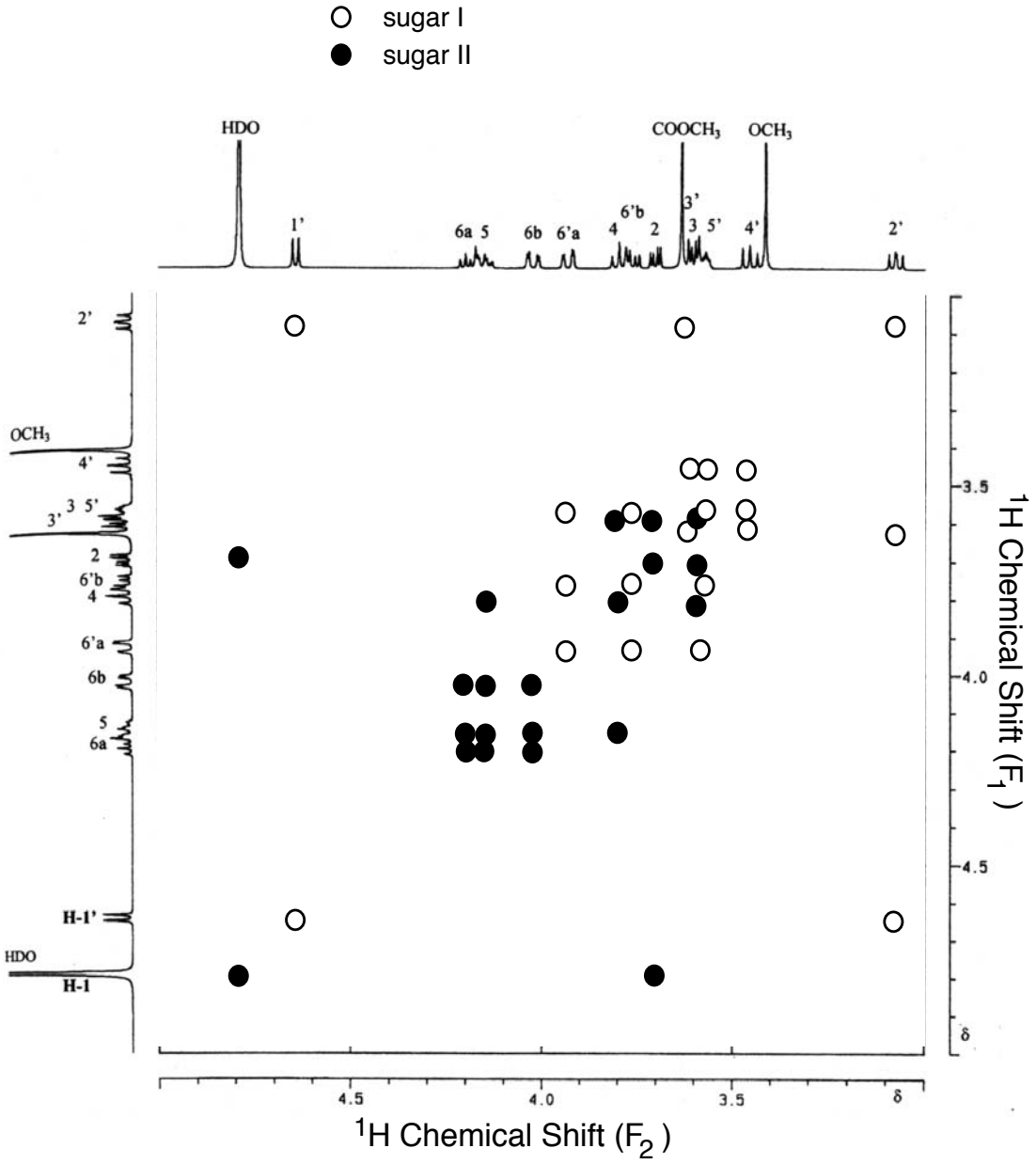
3. Differences between COSY and TOCSY

- **In a COSY spectrum, we observe one crosspeak for H1' (F2):**

  H1' (F2)-H2' (F1)

- **We also observed the symmetrical peak: H2'(F2) -H1' (F1))**

- **Simulated COSY spectrum of α-methyl-3-O-methylcellobioside:**

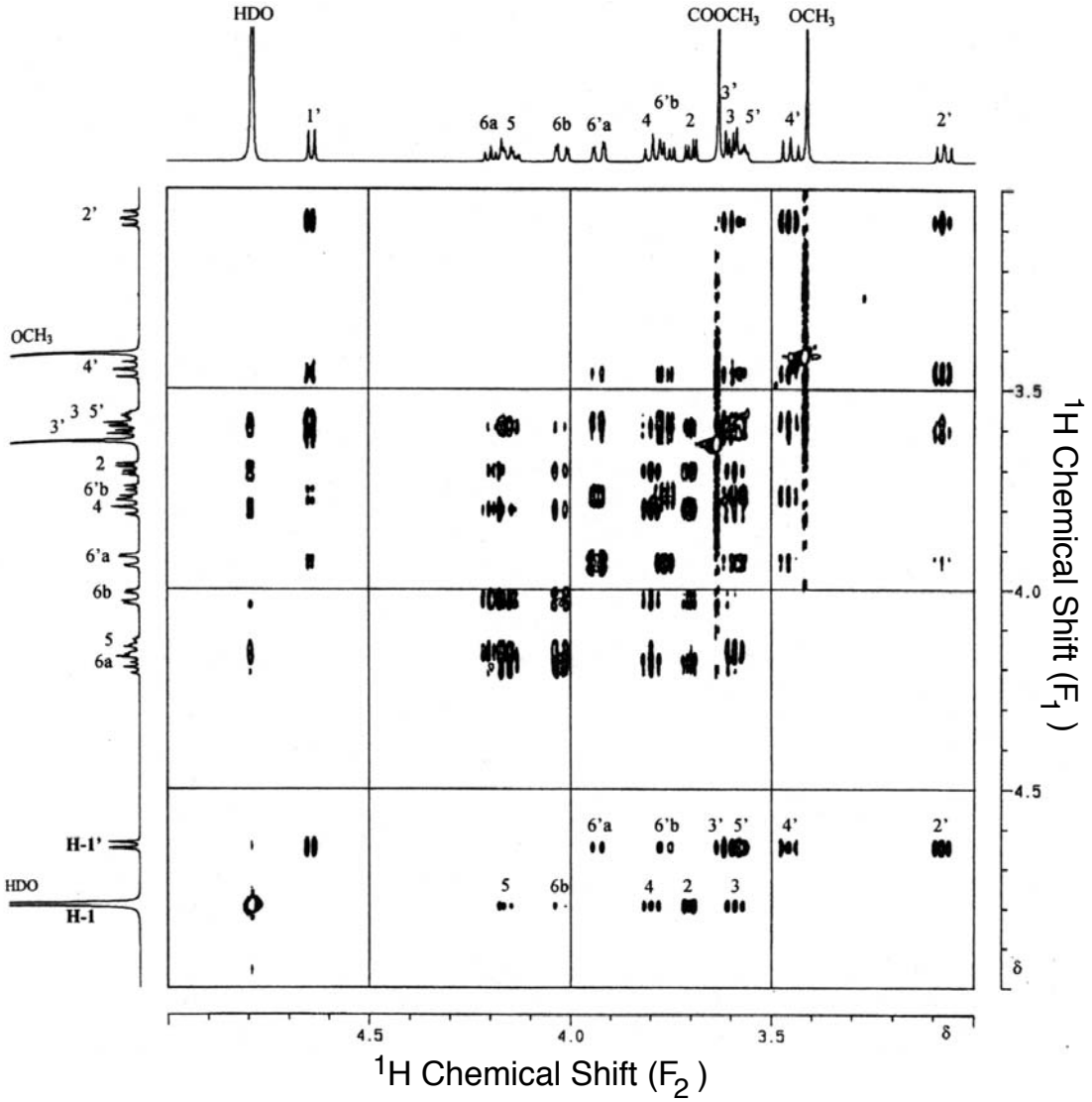


- **In a TOCSY spectrum, we observe multiple crosspeaks for H1'**

F2	F1
H1'	H2'
H1'	H3'
H1'	H4'
H1'	H5'
H1'	H6'a
H1'	H6'b

- **We also observed the symmetrical peaks across the diagonal.**

- **TOCSY spectrum of α-methyl-3-O-methylcellobioside:**



4. Importance of the spin-lock mixing period

- **We also observe multiple crosspeaks for H1 in the TOCSY spectrum:**

F2	F1
H1	H2
H1	H3
H1	H4
H1	H5
H1	H6b

But not H1 to H6a !!!! and H1 to H6b is very weak!

- **The range to which crosspeaks can be detected depends on the length of the mixing time (τ_m) where the spin-lock is applied. τ_m usually varies from 10 ms - 200 ms.**
- **Very short τ_m gives few crosspeaks (more like the COSY) and very long τ_m gives a total correlation spectrum.**