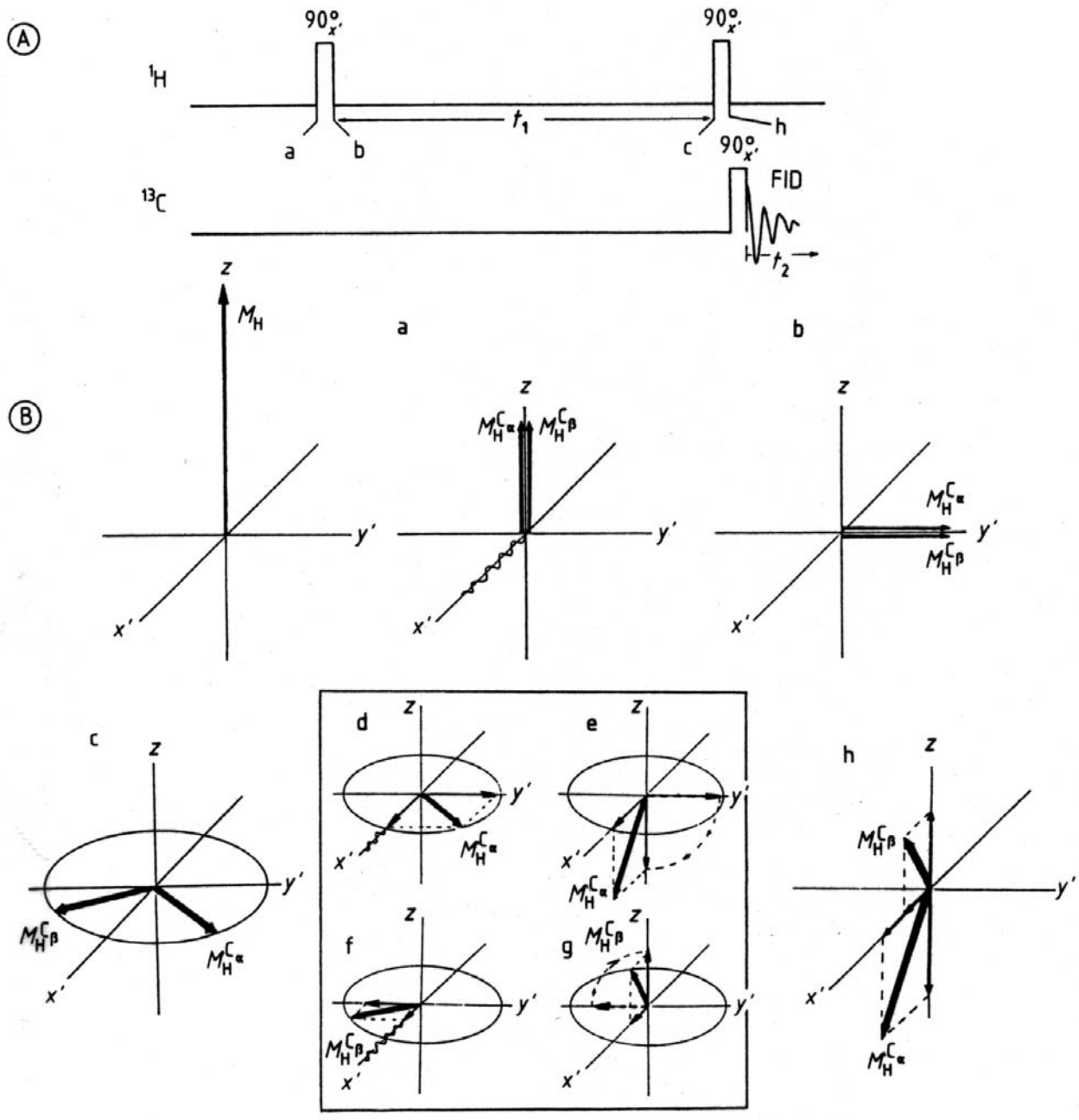
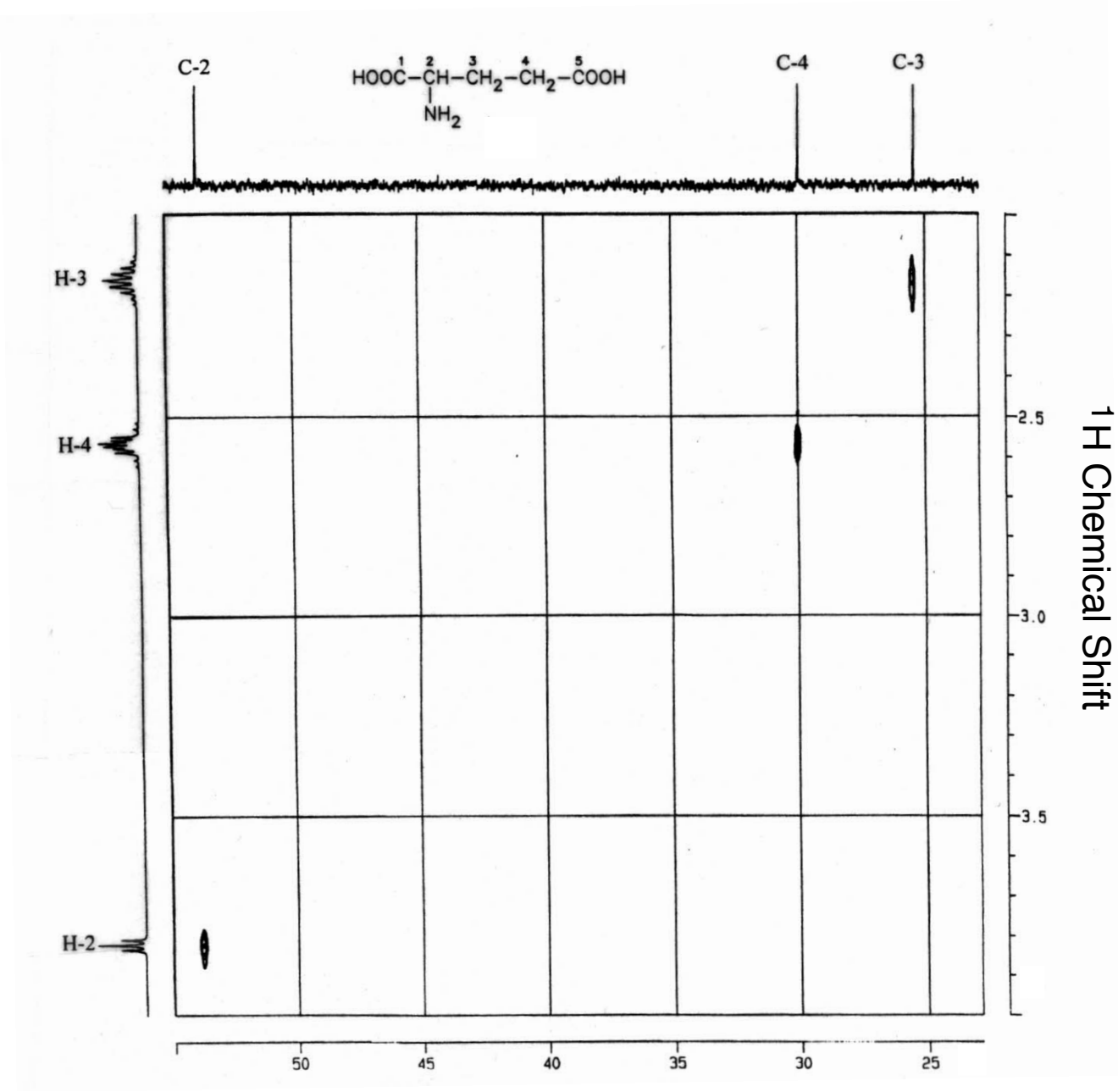
# CHEM / BCMB 4190/6190/8189

# 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

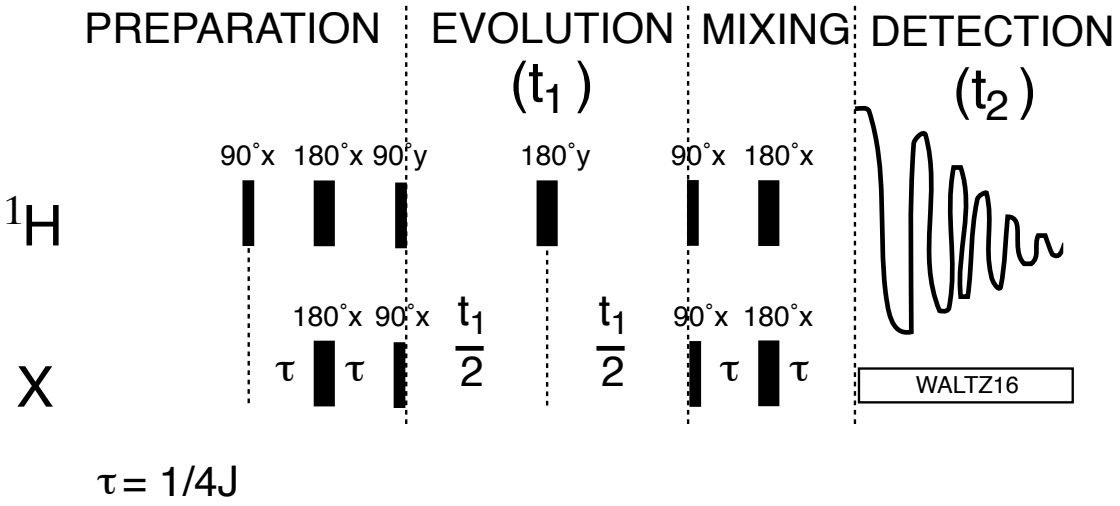
**Two-dimensional Correlated NMR spectroscopy:**
**The HSQC Experiment**

## 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:



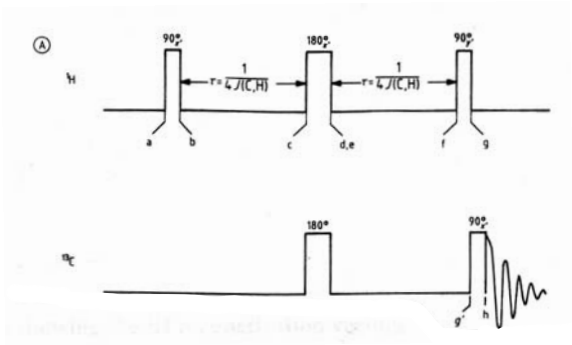

$\tau = 1/4J$

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

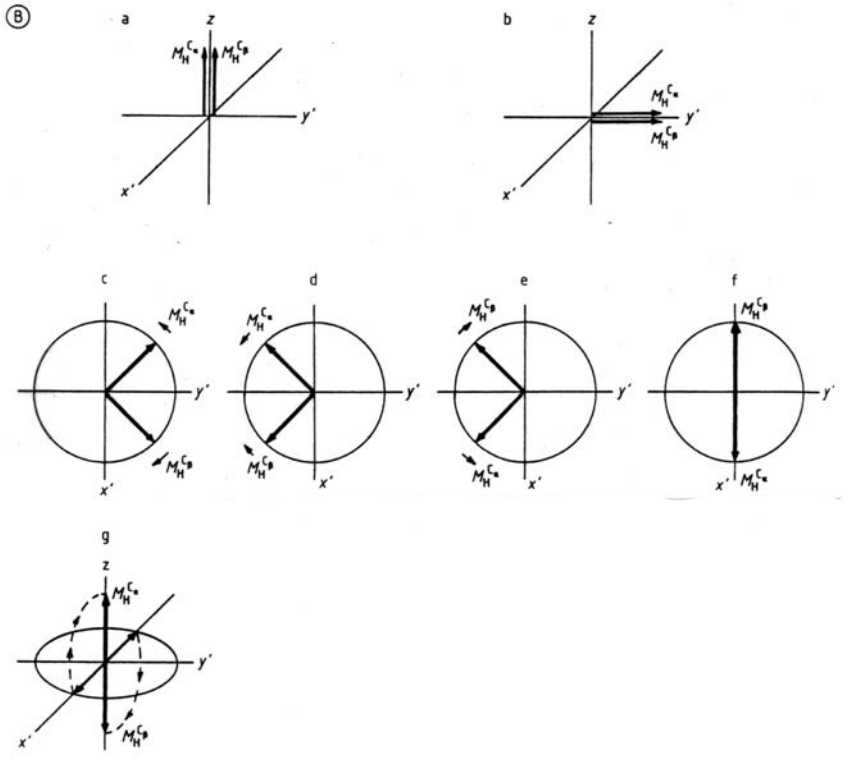
1) **The PREPARATION period is an INEPT sequence ($^{1}H$ to $^{13}C$).**
2) **The t1 EVOLUTION period allows for indirect $^{13}C$ chemical shift detection.**
3) **The MIXING period is a REVERSE INEPT sequence ($^{13}C$ to $^{1}H$).**
4) **The t2 EVOLUTION period allows for direct $^{1}H$ chemical shift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

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

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



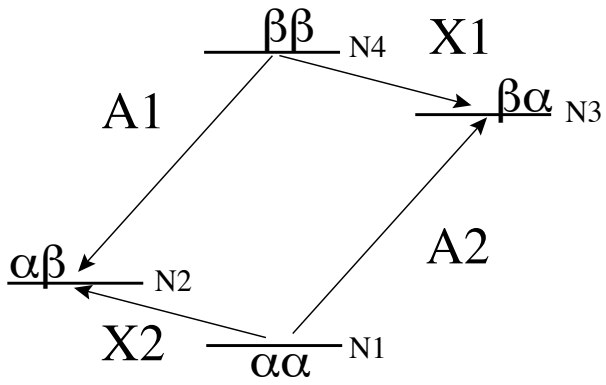

**B) Vector diagrams showing the $^{1}H$ magnetization vectors ($^{13}CHCl_3$)**




- **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** |

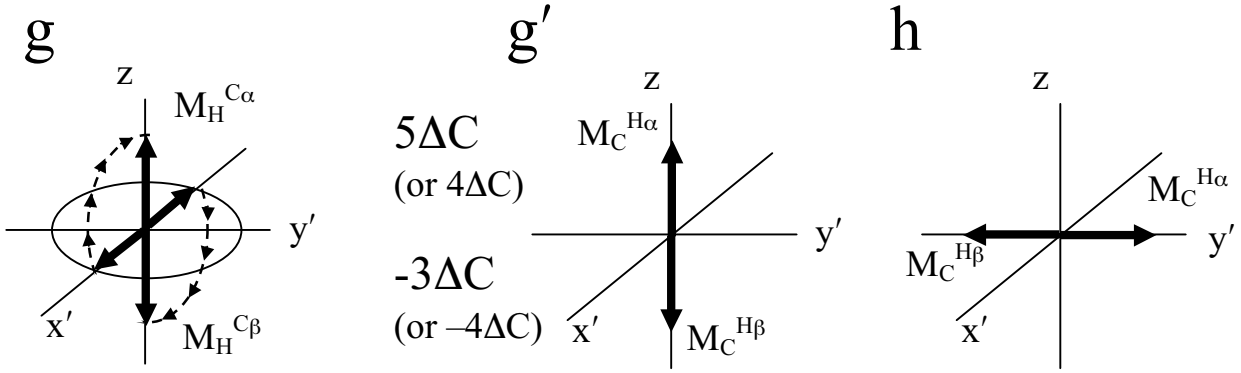



**X1 transition: N3 – N4 = ΔC - ΔH = -3ΔC**
**X2 transition: N1 – N2 = ΔC + ΔH = 5ΔC**

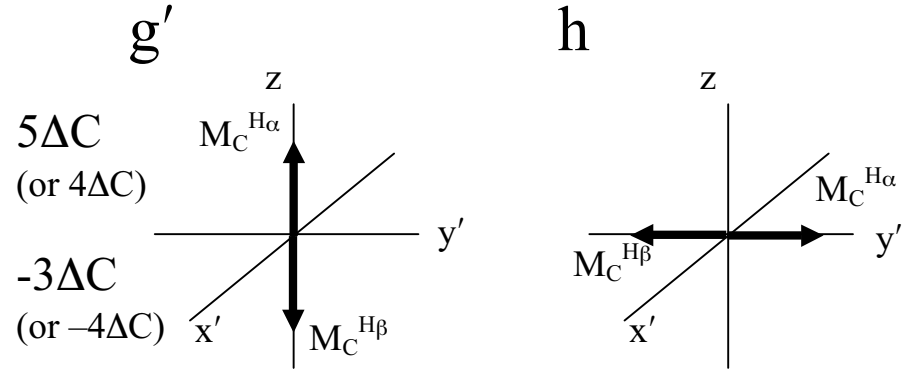
- **In the case of the HSQC, the contribution from the natural $^{13}C$ magnetization (ΔC) is unwanted and is removed using one of the selected methods that we have seen previously. The resulting populations and population differences are:**

**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**

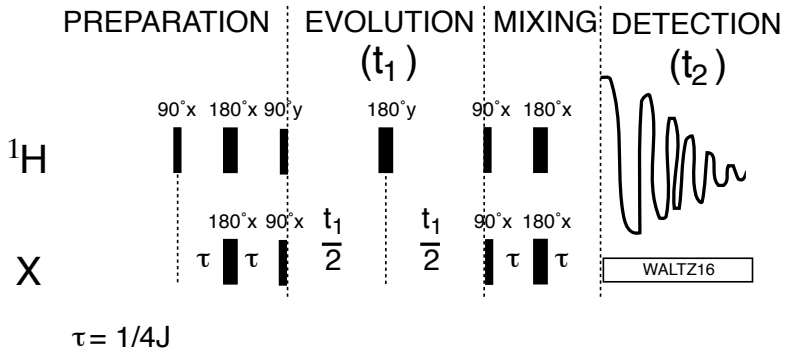


**C) Vector diagrams showing the $^{13}C$ magnetization vectors**

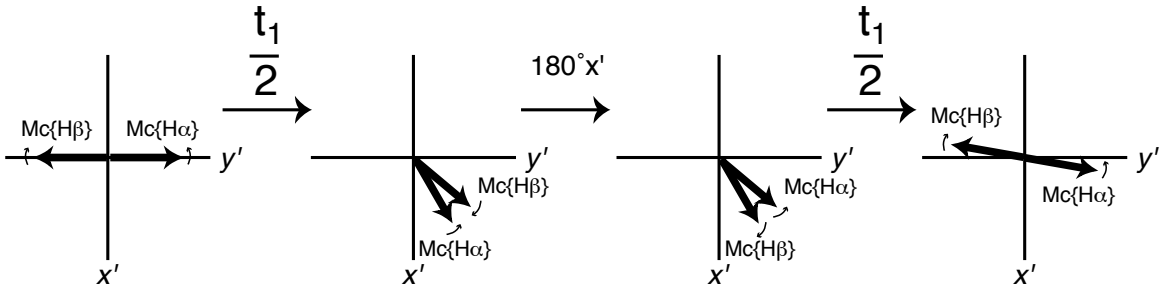



- **At point n: The 90˚x pulse on $^{13}C$ creates transverse magnetization components that evolve during t1. Note that these $^{13}C$ magnetization components are 180˚ out of phase with each others at the beginning of t1.**

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

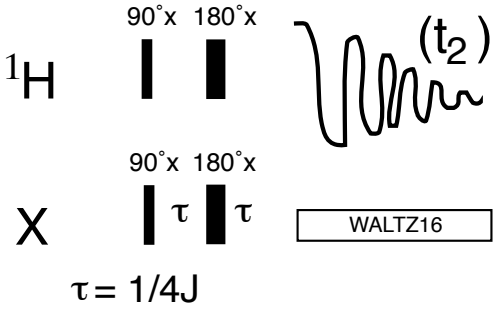



1) **The $^{13}C$ chemical shift evolves (to different points depending on the value of t1).**
2) **There is no net $^1H$-$^{13}C$ coupling evolution. Note that the $^{13}C$ magnetization components are 180˚ out of phase with each others at the beginning and the end of t1.**

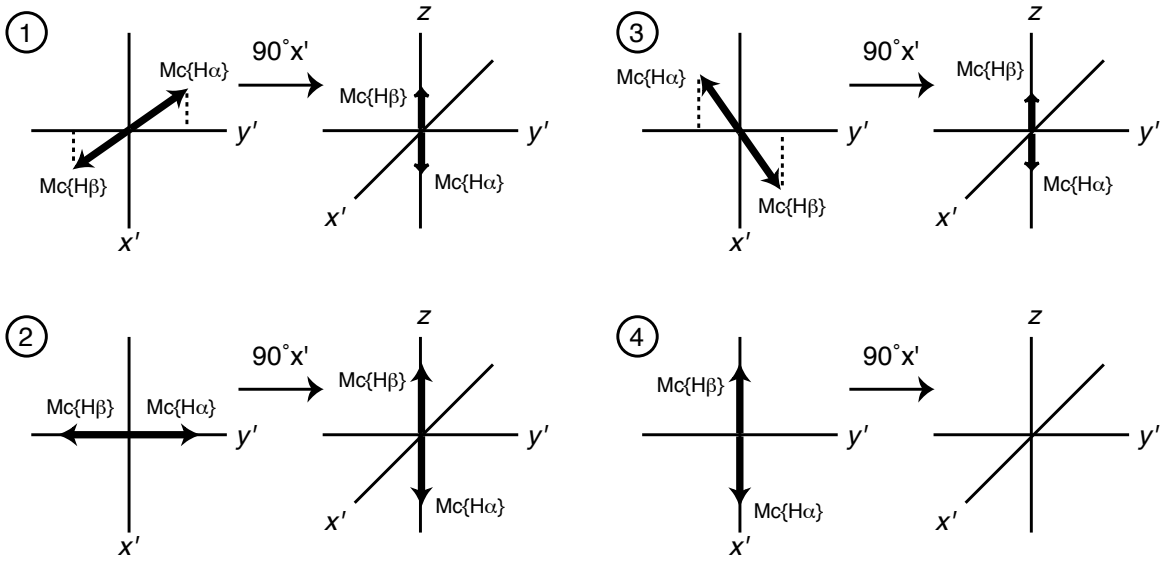


## 5. The Reverse-INEPT experiment:

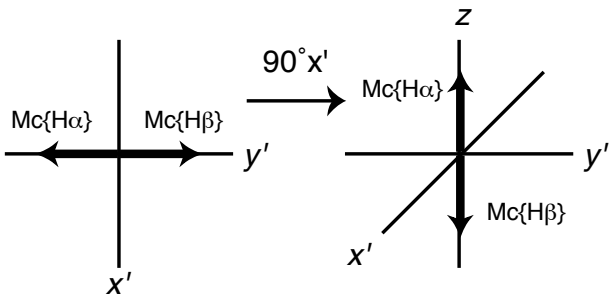
### A) Pulse sequence in the $^{1}H$ and $^{13}C$ channels




### 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:

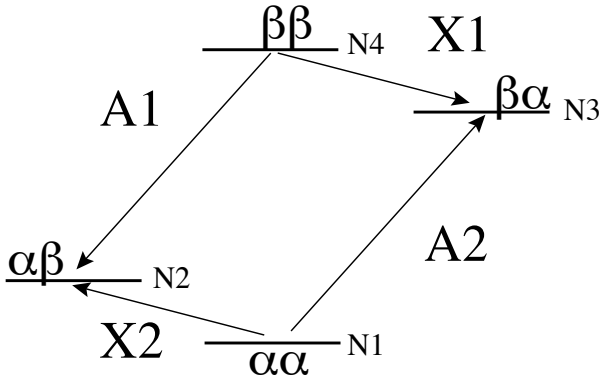



**We will only consider the following case, where after the first $^{13}C$ 90˚x pulse, $MC^{H\beta}$ is in its original position, but $MC^{H\alpha}$ is inverted.**



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

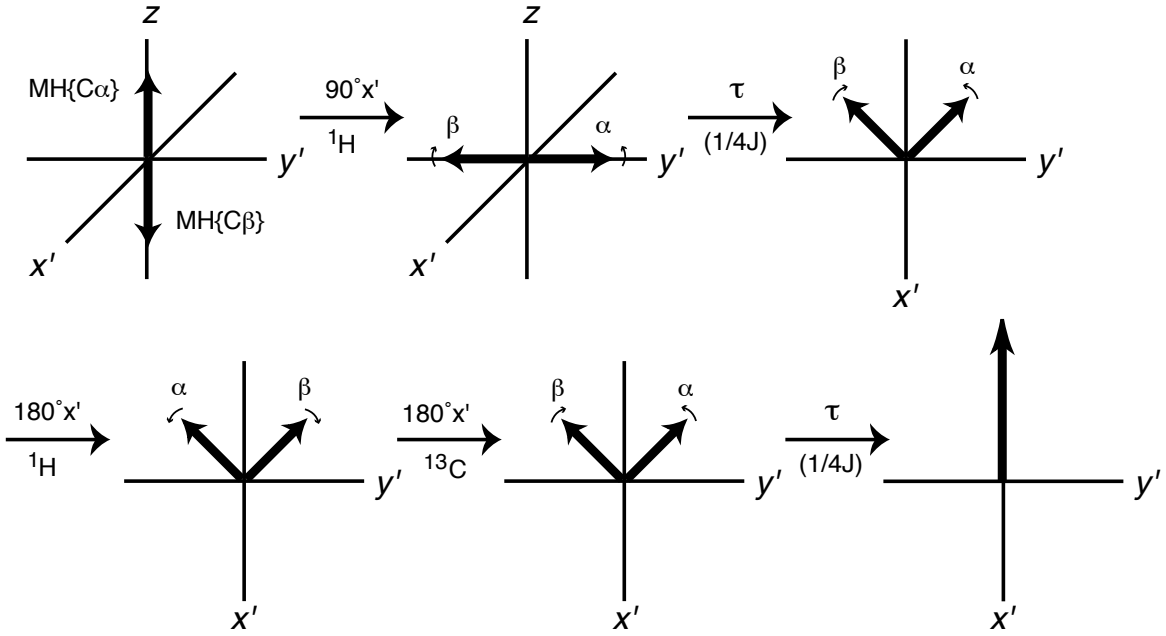
**N4 = N + ΔC/2 + ΔH**
**N3 = N + ΔC/2**
**N2 = N + ΔC/2**
**N1 = N + ΔC/2 + ΔH**




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

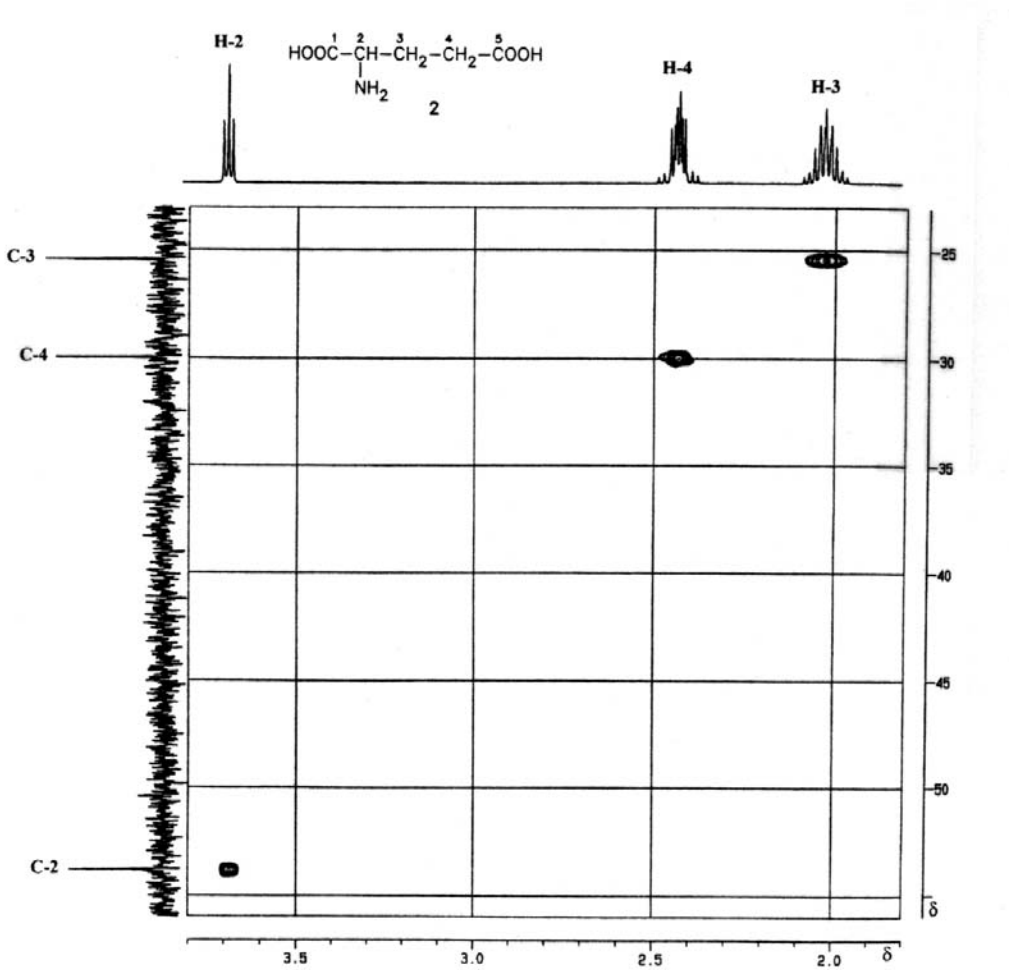
- **The antiphase $^{1}H$ magnetization is refocused during the 2*τ period. The two 180˚x' pulse (on $^{1}H$ and $^{13}C$) in the middle of the 2*τ period allows J coupling evolution but refocuses the $^{1}H$ chemical shift evolution.**

- **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.**

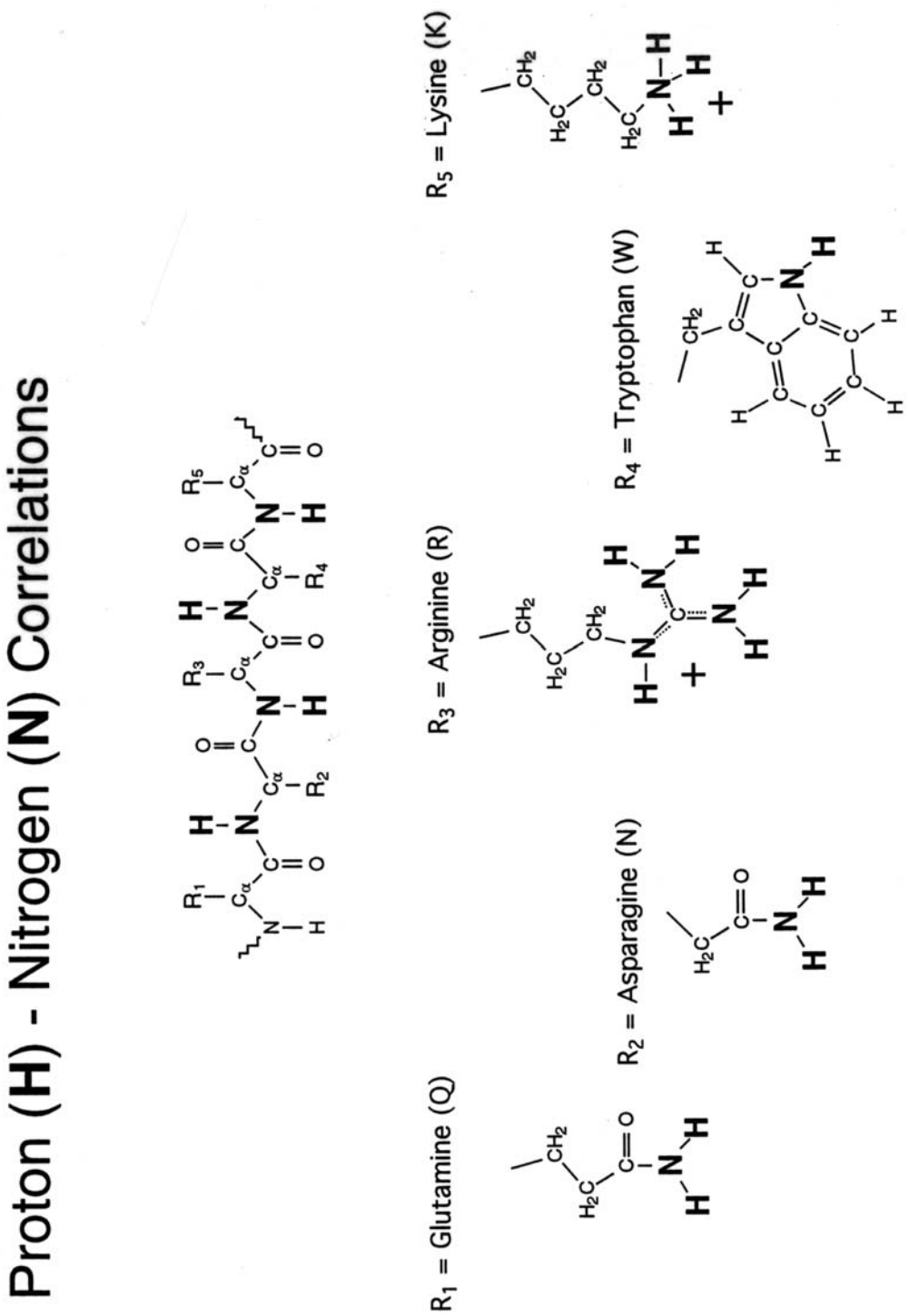
**6) <u>HSQC Spectrum</u>:**
**Example: $^{1}H$-$^{13}C$ HSQC of Glutamic Acid**



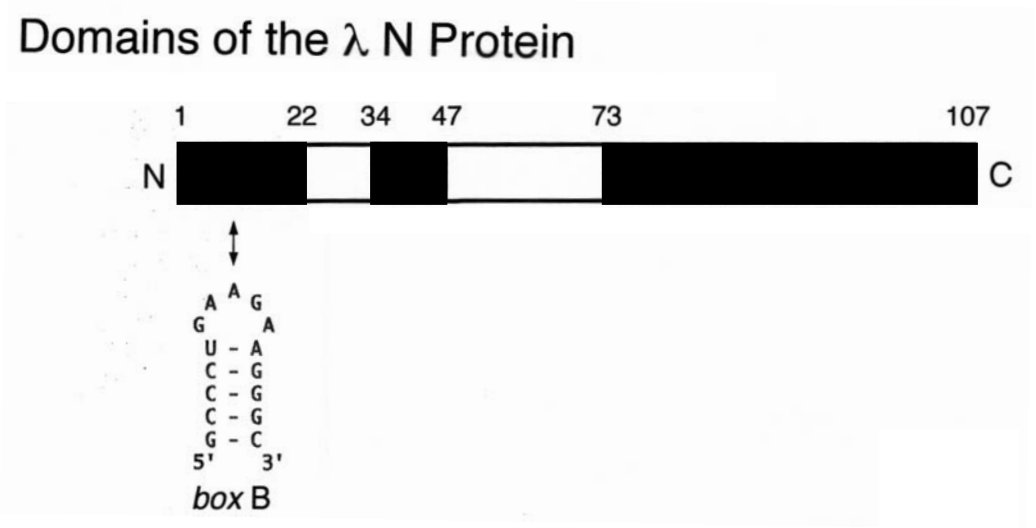

- **Signals <u>not</u> obtained for $^{1}H$ that are <u>not</u> bound to $^{13}C$**
- **Comparison with C,H COSY:**
    1) **Axes are interchanged (in HSQC, $^{1}H$ is detected, in HETCOR, $^{13}C$ is detected)**
    2) **HSQC is more sensitive and a good spectrum can be recorded quickly.**

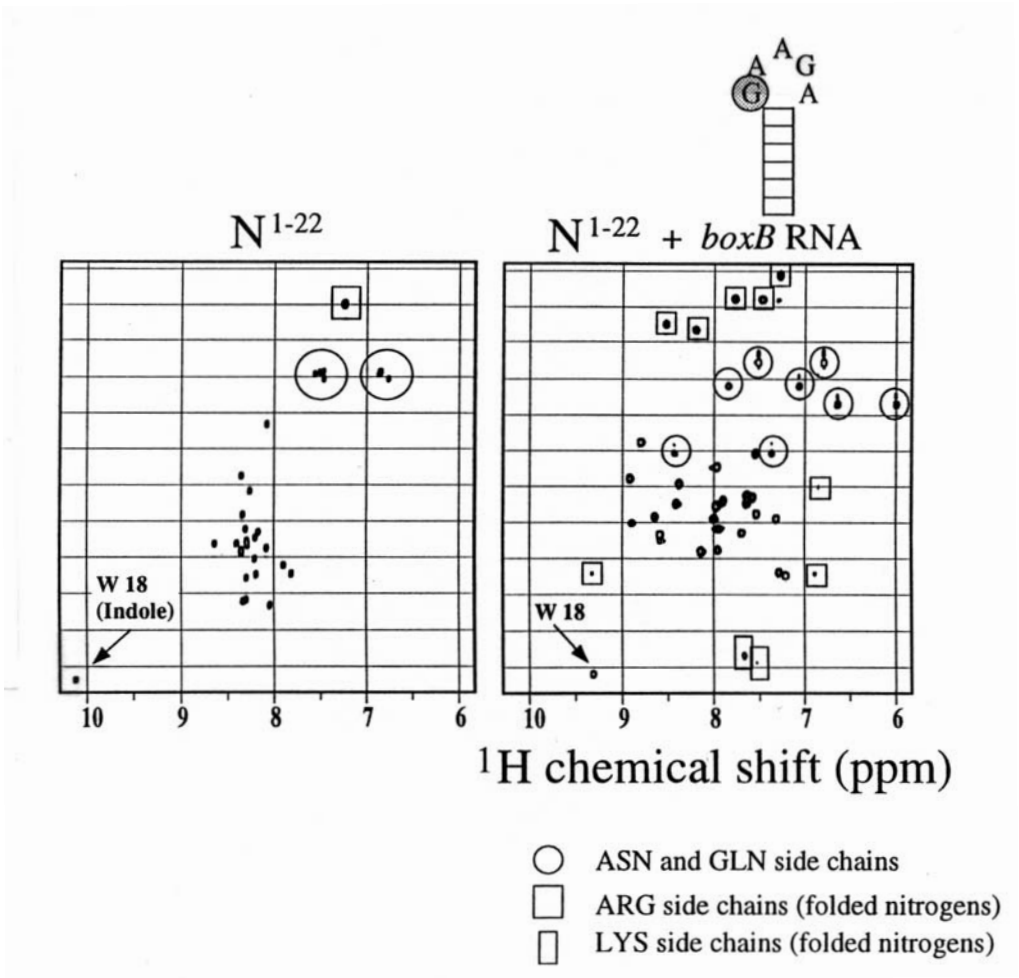
        **-Example: for 1 mM uniformly $^{13}C$- (or $^{15}N$-) isotopically labeled samples, typical recording times are:**
            **- 15-30 minutes for HSQC**
            **- 24 hours for C, H COSY**

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

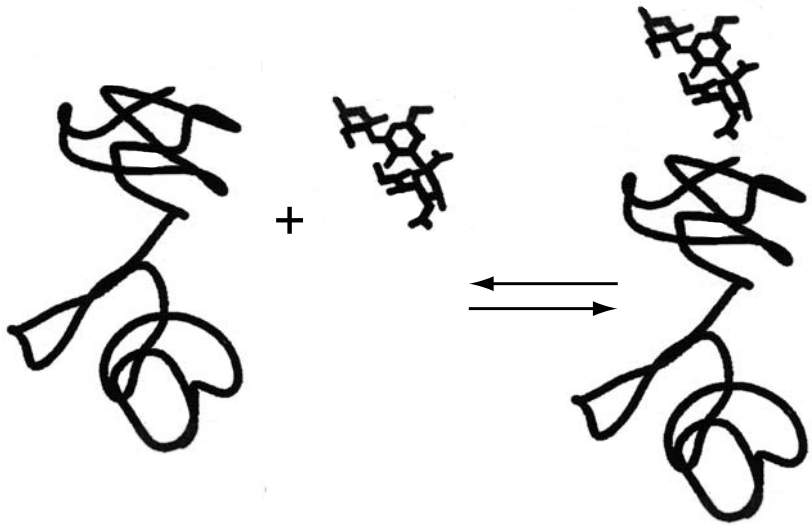


## CASE 1: Protein Folding Upon Ligand Binding

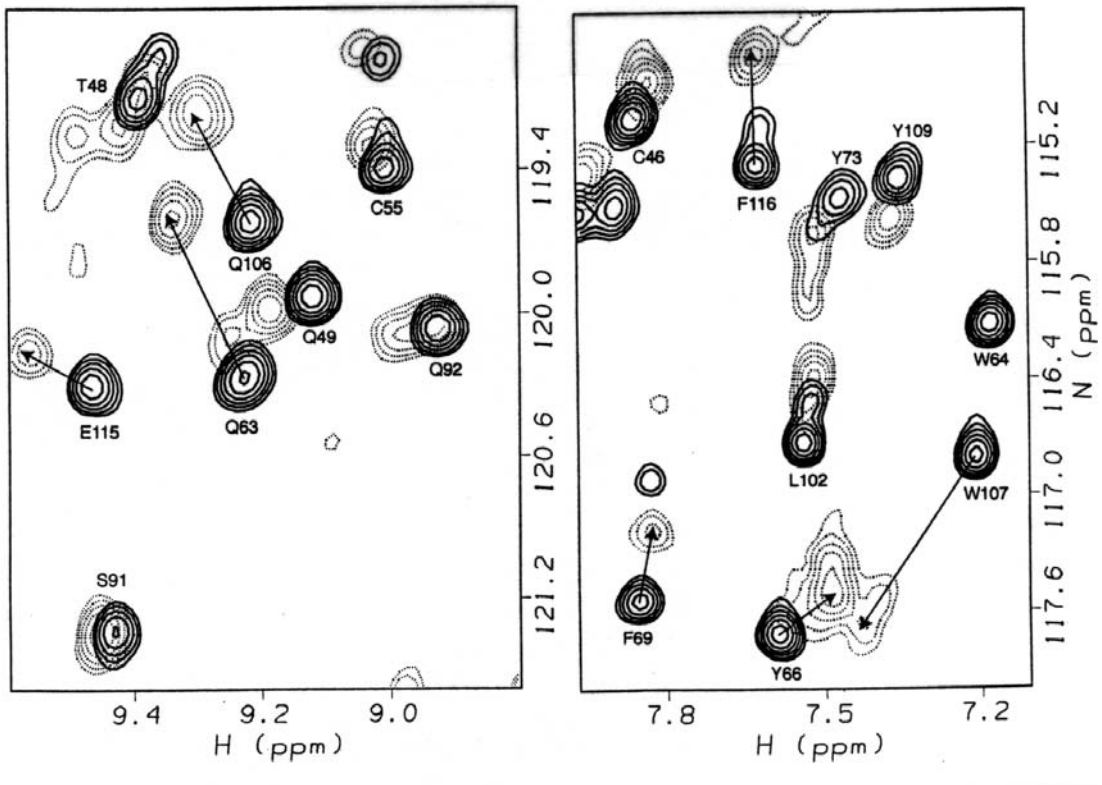


## CASE 2: Mapping Ligand Binding Sites in Folded Proteins



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



## The TOCSY Experiment

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

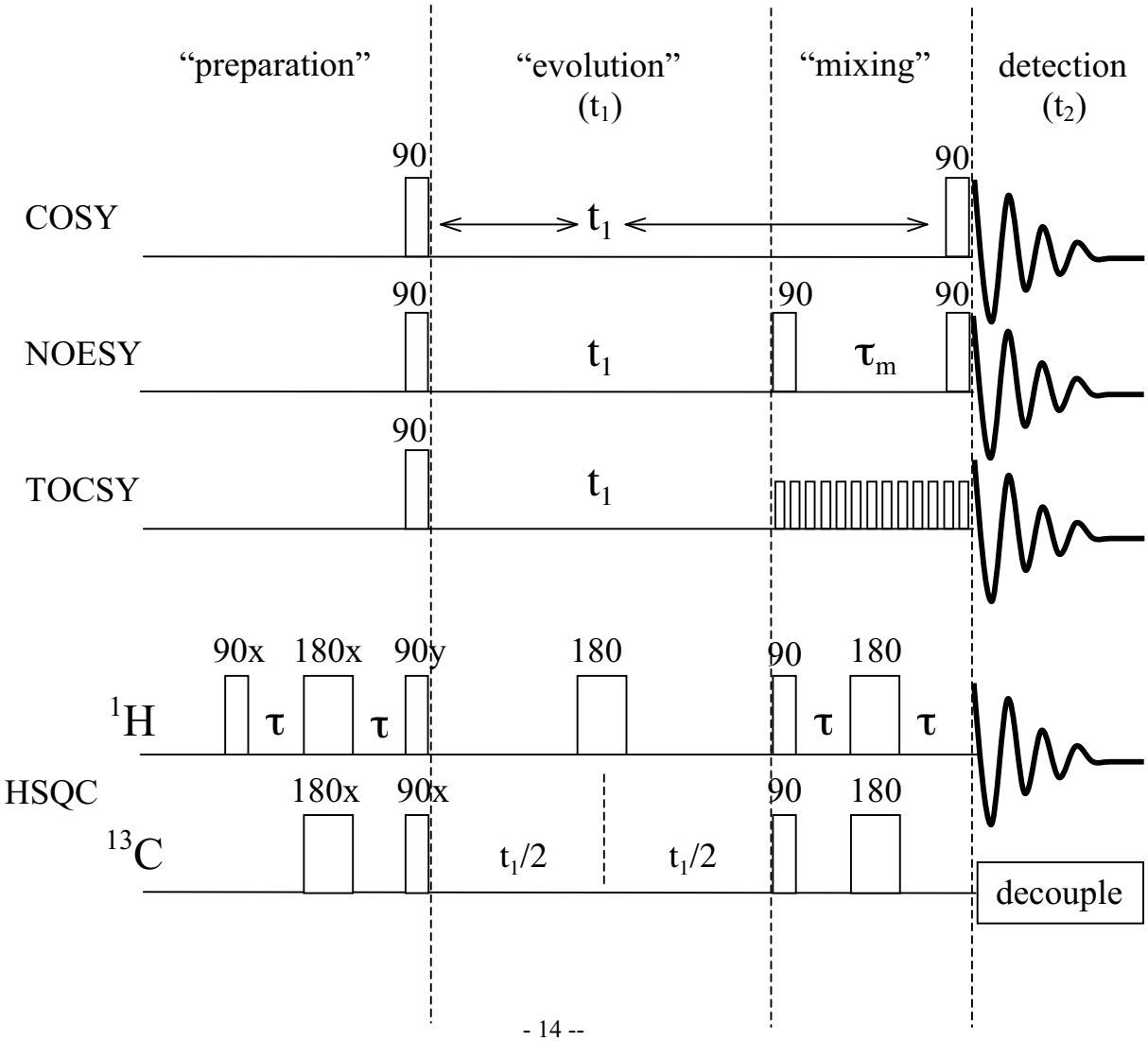
- **Very useful experiment for determining the structures of oligosaccharides and peptides as well as many other organic compounds.**

**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:**

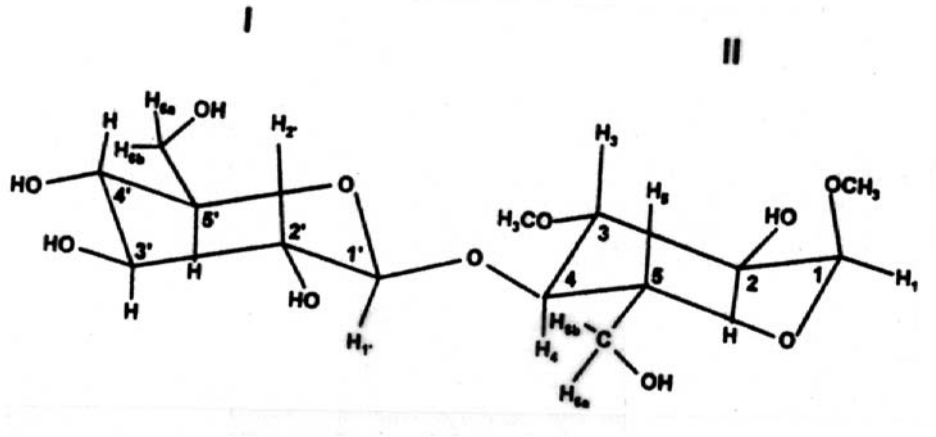
$$90^\circ - t_1 - \text{spin-lock}(t_m) - \text{FID}$$

**-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.**

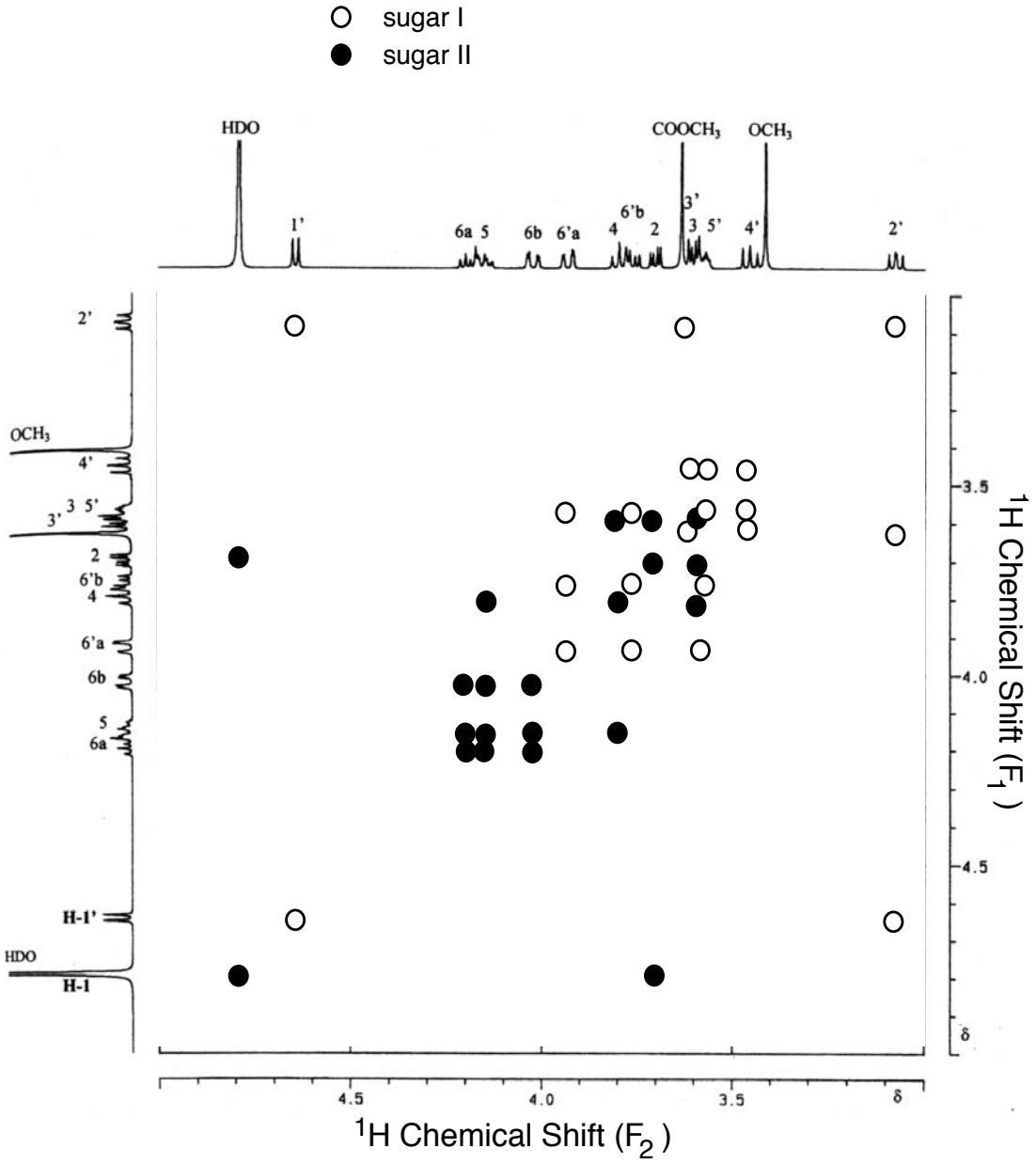
## 3. Differences between COSY and TOCSY

- **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:**

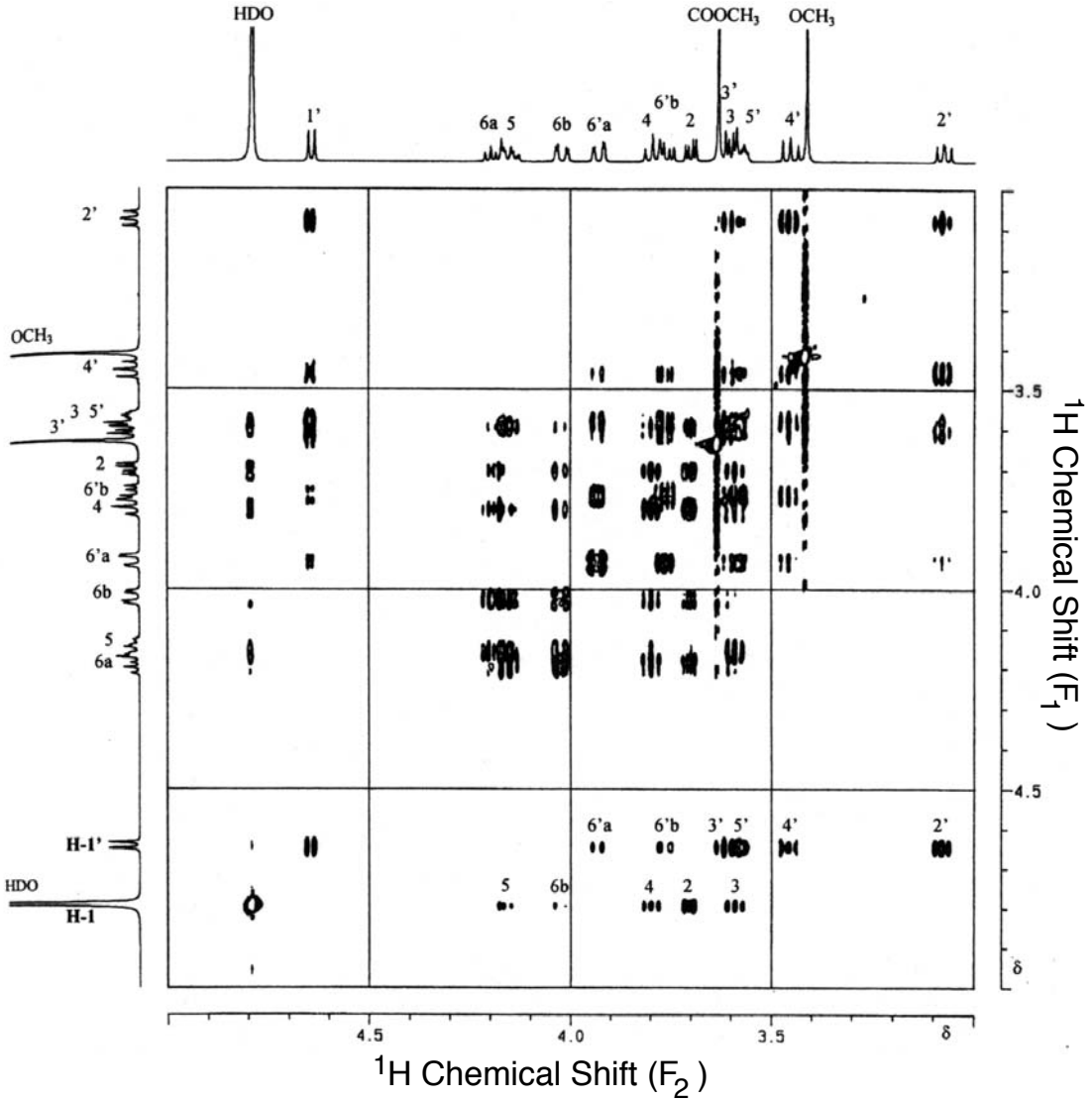


- **In a TOCSY spectrum, we observe multiple crosspeaks for H1'**

| 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 ($\tau_m$) where the spin-lock is applied. $\tau_m$ usually varies from 10 ms - 200 ms.**
- **Very short $\tau_m$ gives few crosspeaks (more like the COSY) and very long $\tau_m$ gives a total correlation spectrum.**