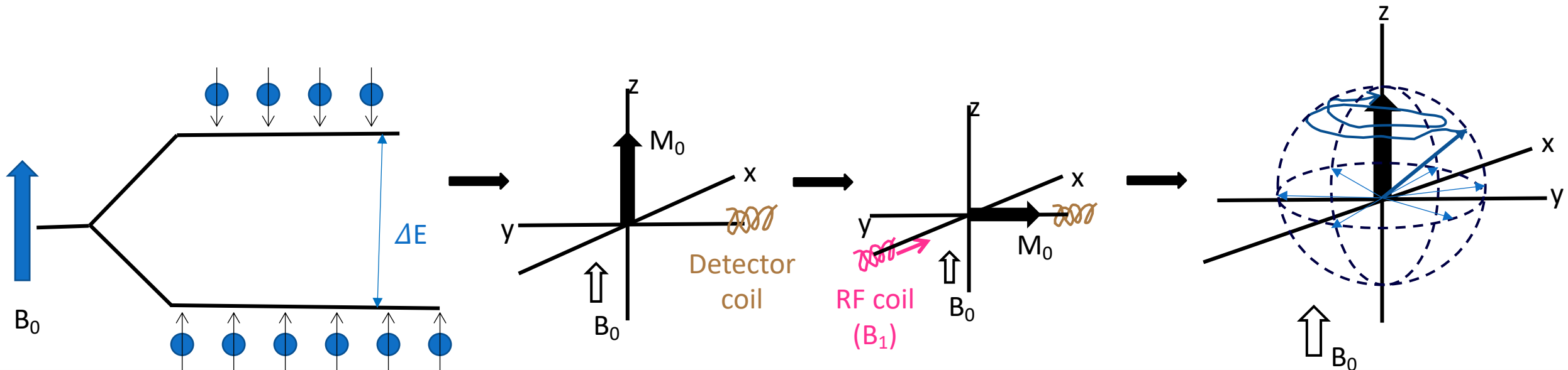


# Tutorial: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

# NMR Principle: Review

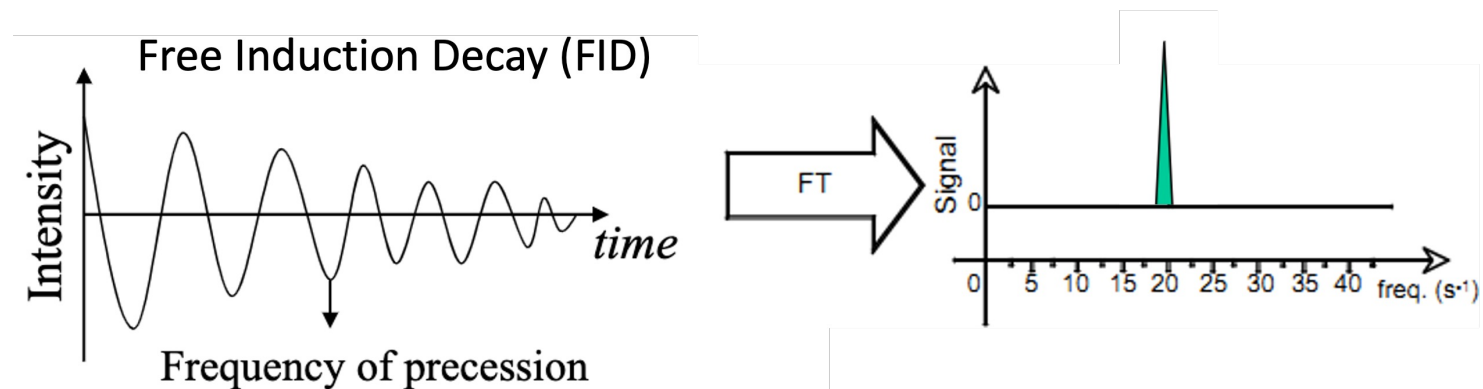
- NMR spectrometry is another form of absorption spectrometry.
- Absorption is a function of **certain nuclei** in the molecule.
  - Nuclei having an odd number of atomic number (e.g.  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{31}\text{P}$ )
- NMR spectrum: A plot of the frequencies (normalized into **chemical shift**) of absorption peaks *versus* **peak intensities**.



# NMR Principle: Review

- During relaxation, nuclei lose both the **excitation energy** and **phase coherence** (In other words, the magnetic vectors will lie at different points in the xy plane)
- For structure determination, we are most concerned with  $T_2$  (**decay constant/spin-spin relaxation**), since it is usually the shortest and determines the shape of resonance peaks
- A detector collects the radiated energy producing a **free induction decay (FID)** which is Fourier transformed into a readable spectrum, which is a function of **frequency**.

FID is the sum of all nuclei radiating over time

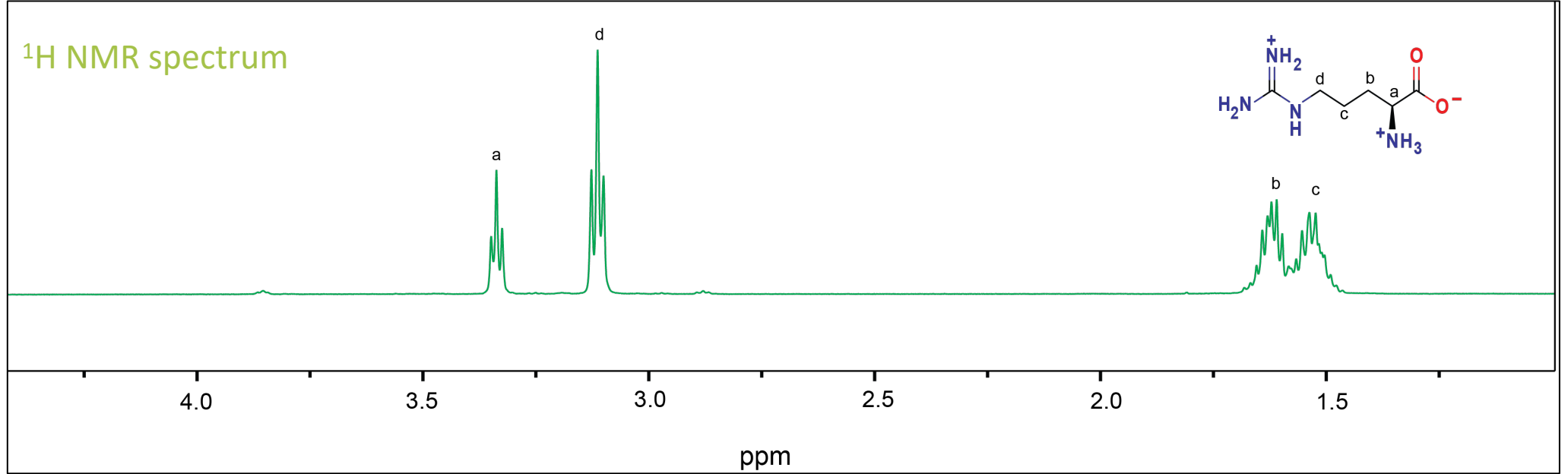


# NMR Principle: Review

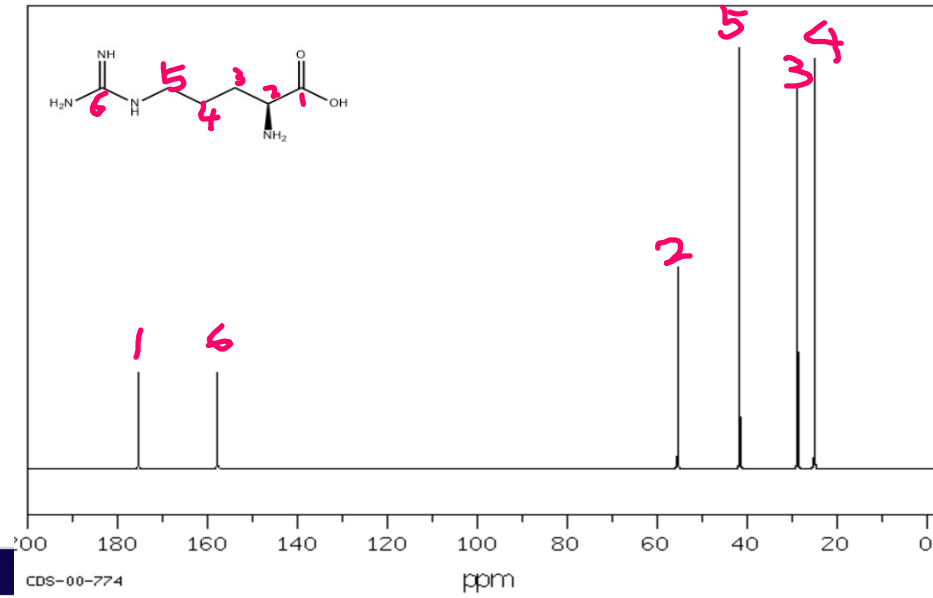
$$\nu_L = (\gamma/2\pi)B_{0,\text{eff}}$$

- $\gamma$  is gyromagnetic ratio, which is a constant for a given isotope.
- $B_{0,\text{eff}}$  can change based on the environment.
- The electrons create their own magnetic field which opposes the applied magnetic field  $B_0$ .
- A proton with a **high electron density environment** experiences a lower magnetic field and therefore a **lower frequency** (at the right side).
- **Atoms in the molecule affect one another by through-bond and through space.**

# 1D NMR: Review



<sup>13</sup>C NMR spectrum



## $^1\text{H}$ NMR of a Ubiquitin

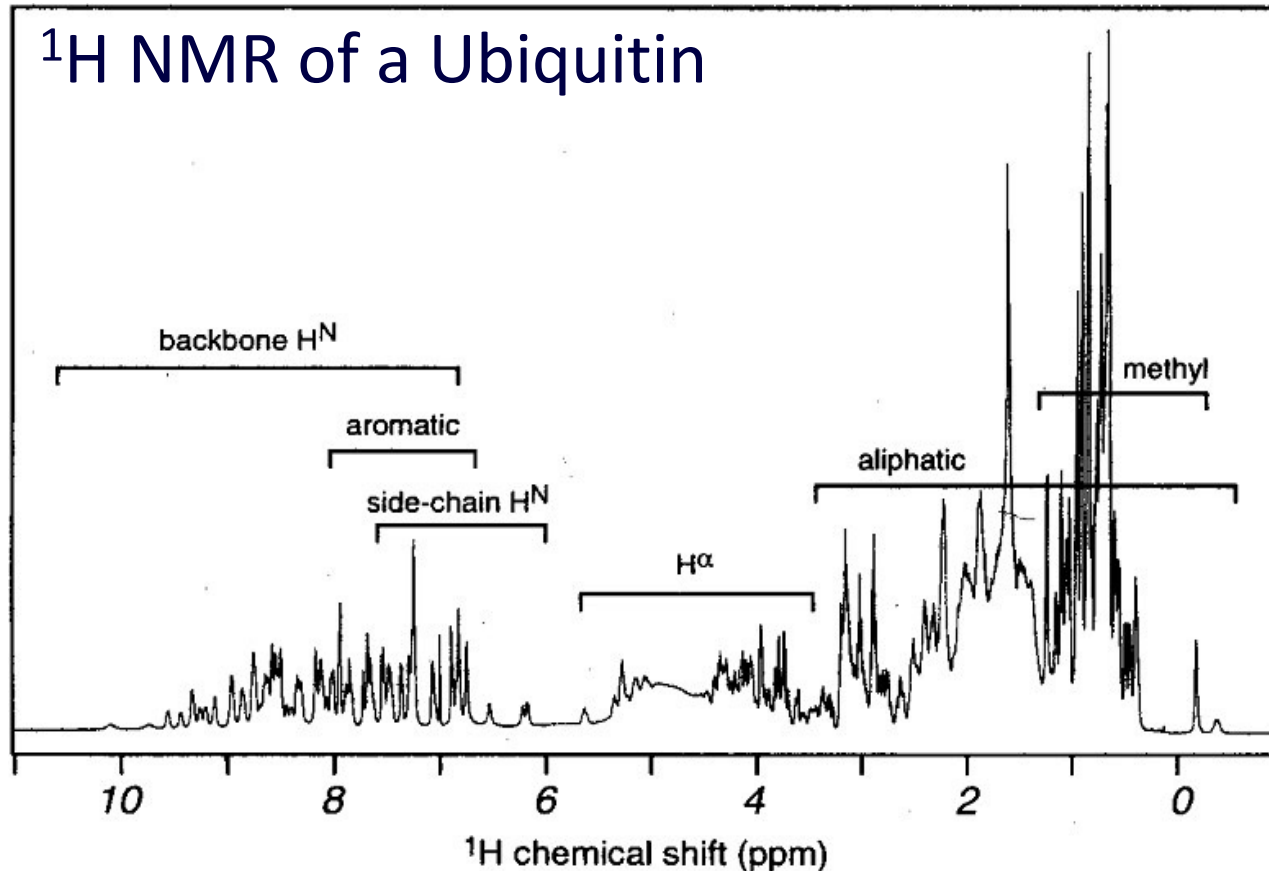


FIGURE 2.  $^1\text{H}$  chemical shift positions of chemical groups in ubiquitin <sup>1</sup> (from: Cavanagh *et al.*: Protein NMR Spectroscopy).

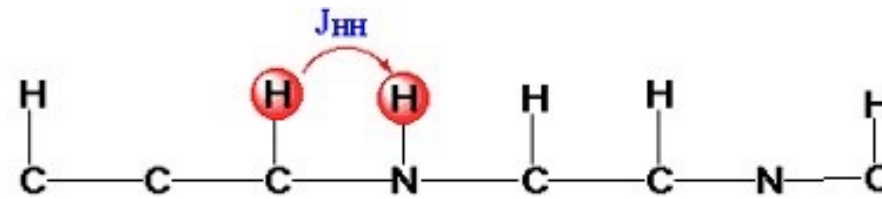
As the size of the molecule increases, because of the effect  $T_2$  on the resonance peaks, so you will get a large number of broad and smeary peaks, which make it difficult (impossible) to assign them to particular nuclei.



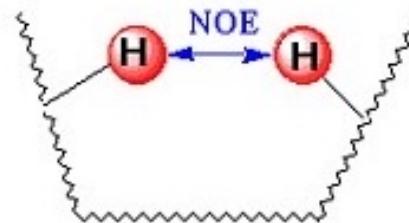
Multi-dimensional NMR

# 2D NMR: Review

- Magnetization is transferred between nuclei in two ways:
  1. **Scalar (or 'J') coupling** acts through bonds and gives information about nuclei connected by 3 or fewer bonds (COSY or  $^1\text{H}$ - $^1\text{H}$  correlated spectroscopy).



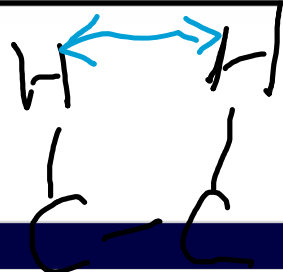
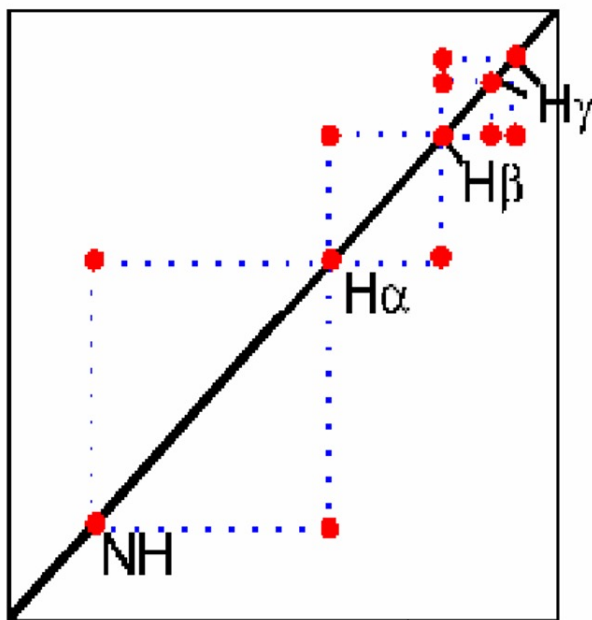
2. **Nuclear Overhauser Effect (NOE)**— nuclei affect each other through space (dipole-dipole interaction) and give information about how nuclei are arranged in space (must be within 5.5 Å of one another)



# Homonuclear 2D NMR: Review

- $^1\text{H}$ - $^1\text{H}$  COSY:

3 or less bonds correlation



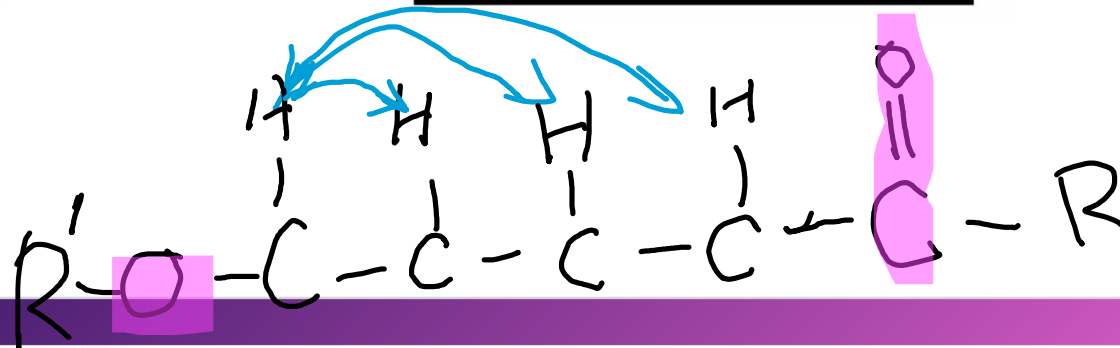
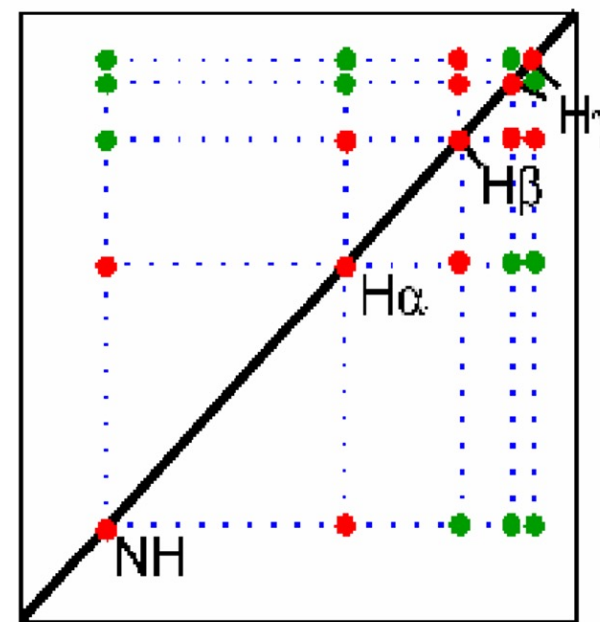
- Nuclear Overhauser Spectroscopy (NOSEY):

The intensity of the NOE is in first approximation proportional to  $1/r^6$ , with  $r$  being the distance between protons



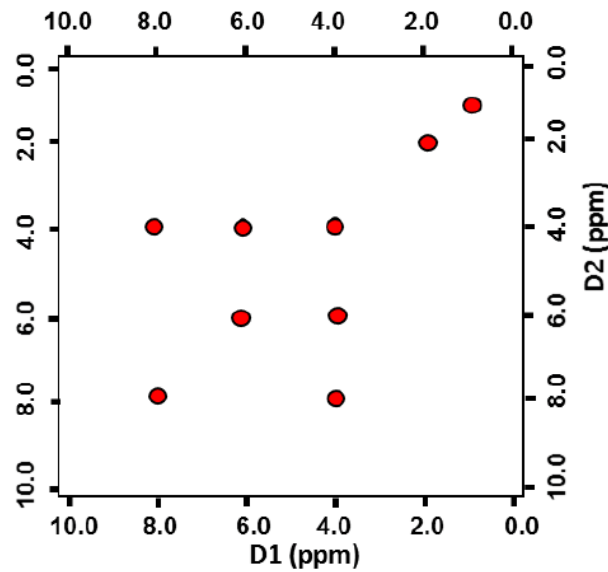
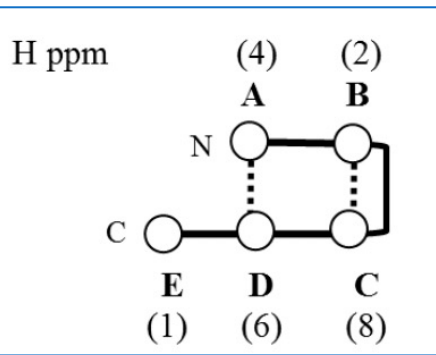
- TOCSY:

nuclei correlation within the same spin system

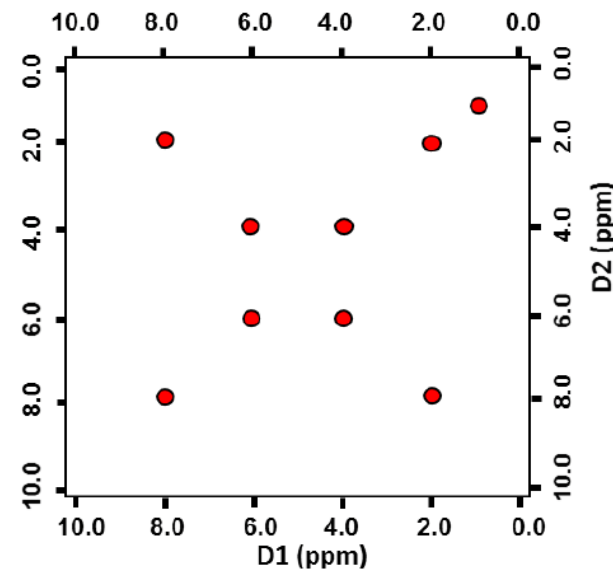




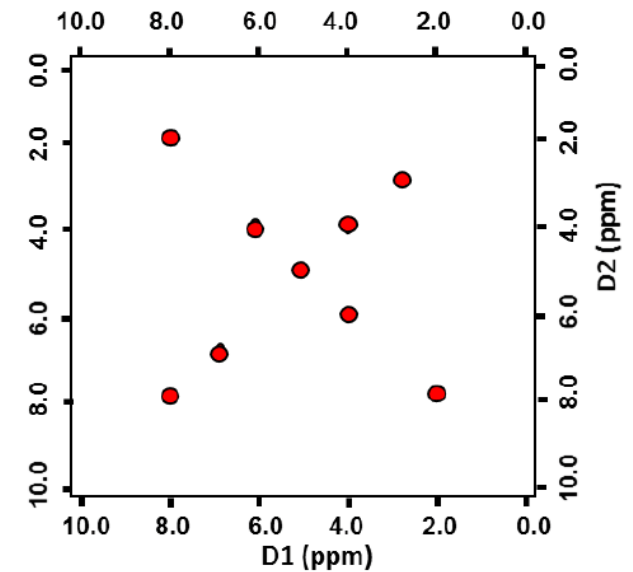
- Practice:** Below shows the structure of a fictitious molecule consisting of five atoms (A, B, C, D and E) as determined by 2D NOESY NMR. The  $^1\text{H}$  chemical shift numbers are in bracket. Based on the structure of the molecule, which spectrum (A, B or C) is most likely to be the NOESY spectrum of the molecule?



A

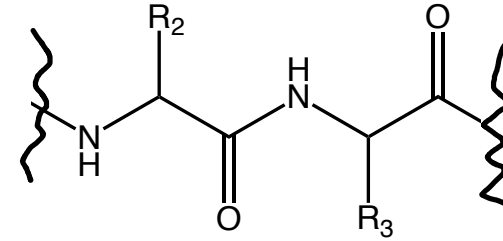


B

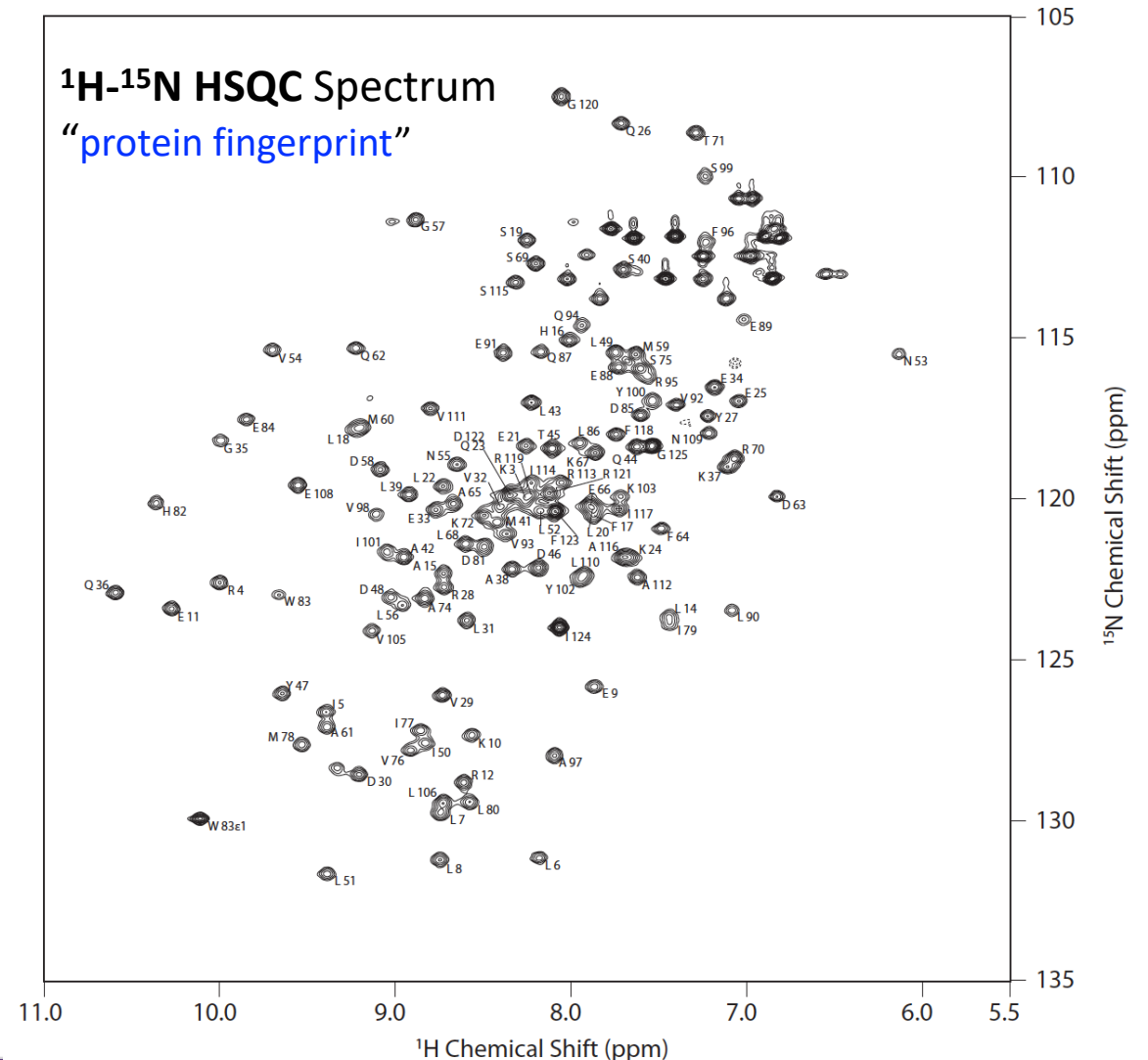


C

# Heteronuclear 2D NMR: Review



- Heteronuclear Single Quantum Coherence (HSQC) experiment is one of the fundamental in multidimensional heteronuclear NMR experiments.
- HSQC determines the correlations between two different types of nuclei (commonly  $^1\text{H}$  with  $^{13}\text{C}$  or  $^{15}\text{N}$ ), which are separated by one bond (J coupled).
  - only one peak will be obtained per pair of coupled atoms.

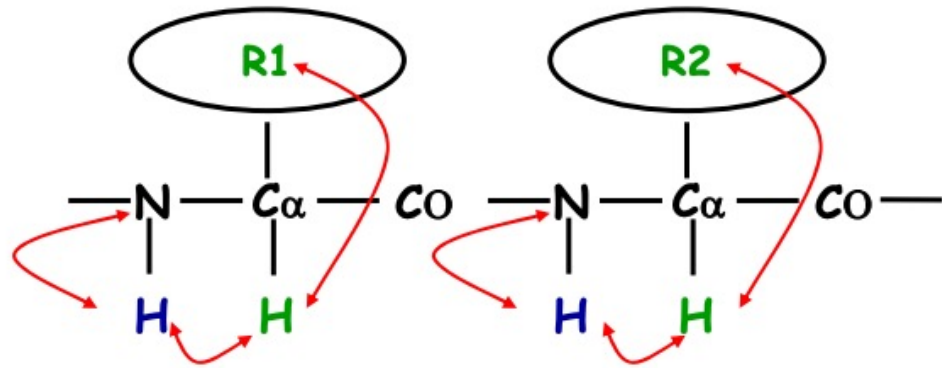


# 3D NMR Experiments: Review

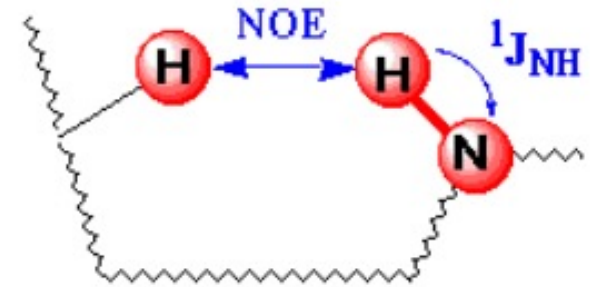
- There are two principal classes of 3D NMR experiments:
  1. Experiments consisting of **two 2D NMR experiments**  
NOSEY-HSQC, TOCSY-HSQC
  2. The **triple resonance experiments**
    - Sequential assignment of larger proteins (> 150 AA)
    - Three nuclei ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ) are correlated
    - Performed on doubly labelled ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ) proteins

# Two 2D NMR Experiments

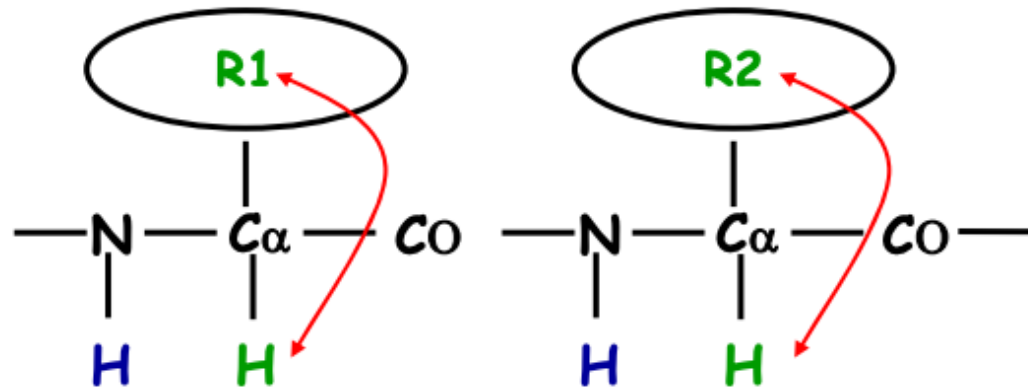
- $^1\text{H}$ - $^{15}\text{N}$ - $^1\text{H}$ : 3D TOCSY-HSQC



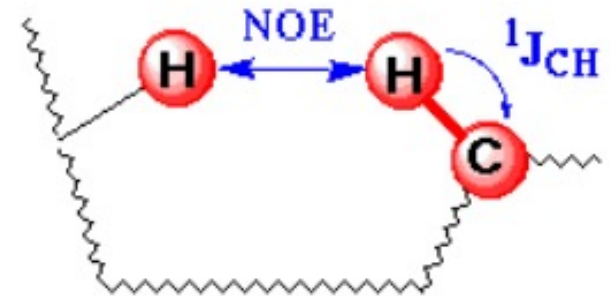
- 3D  $^{15}\text{N}$ -edit NOESY-HSQC



- $^1\text{H}$ - $^{13}\text{C}$ - $^1\text{H}$ : 3D TOCSY-HSQC

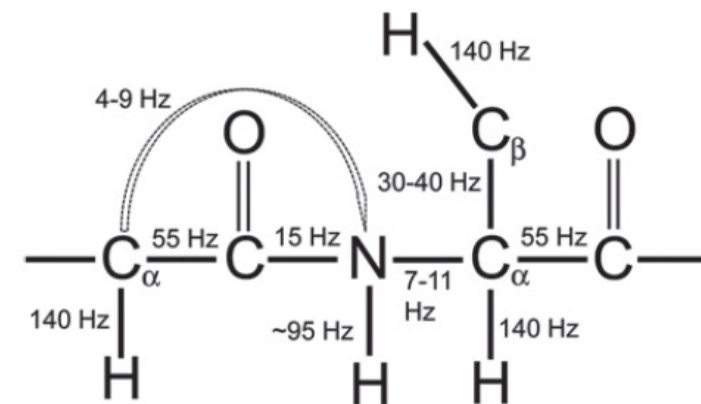
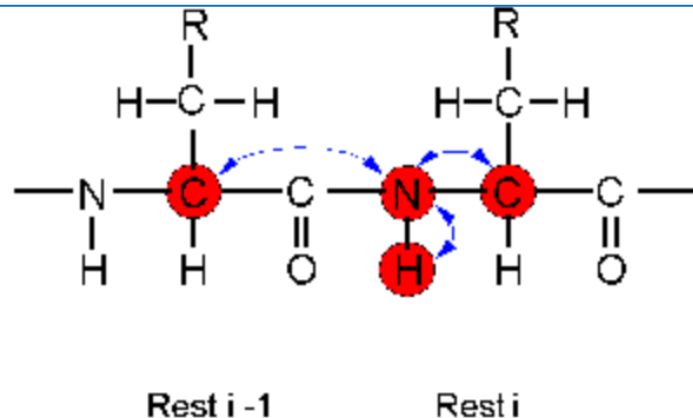
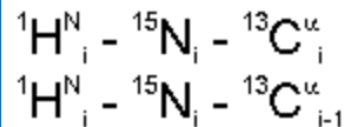


- 3D  $^{13}\text{C}$ -edit NOESY-HSQC



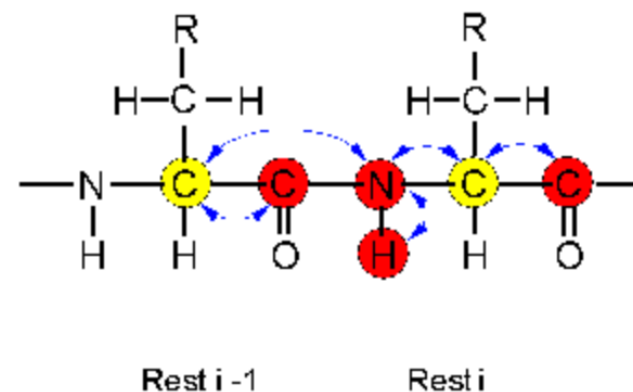
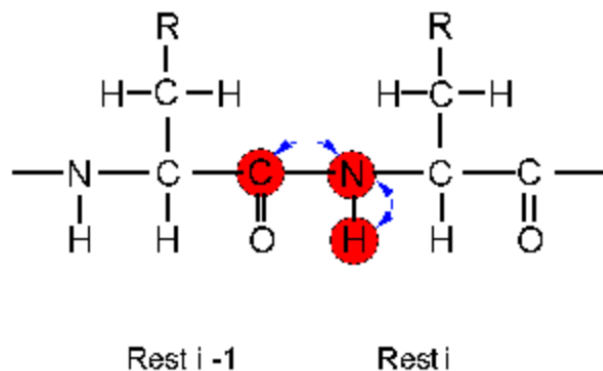
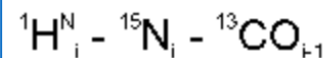
# Triple Resonance Experiments

## HNCA

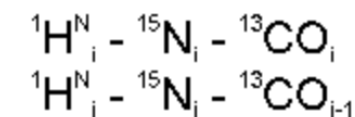


*Summary of one- and two-bond couplings important for triple resonance experiments used for assignment.*

## HNCO

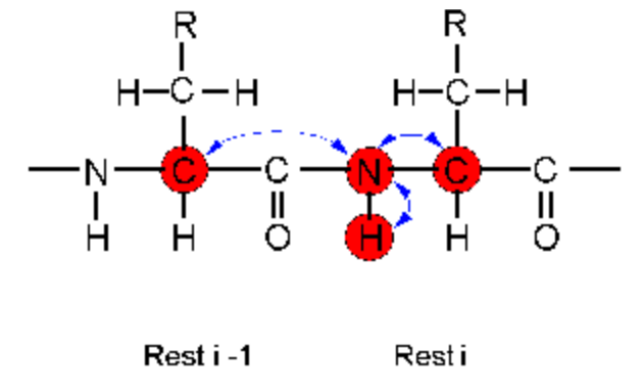
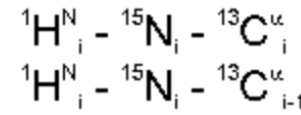


## HN(CA)CO



# Triple Resonance Experiments<sub>1</sub>: HNCA Experiment

HNCA

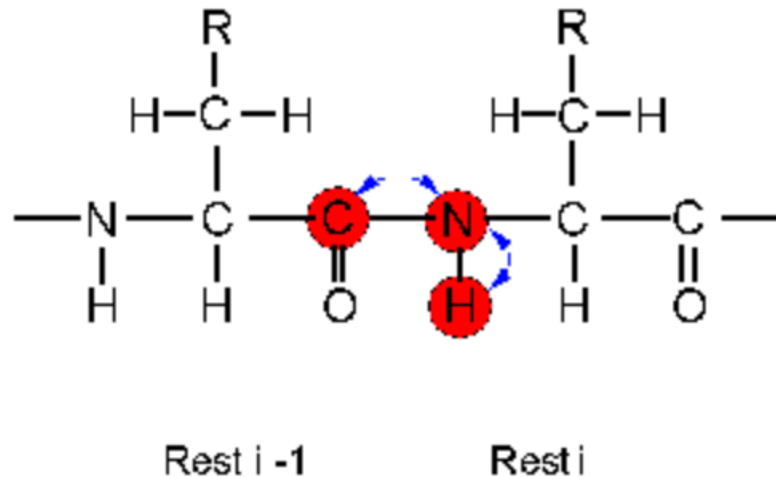
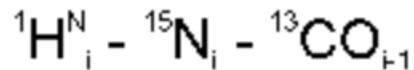


- The HNCA experiment is the prototype of all triple resonance experiments.
  - 1<sup>st</sup> dimension: The magnetization of  $\text{H}_\text{N}$  is transferred to  $\text{N}$
  - 2<sup>nd</sup> dimension: magnetization is transferred from  $\text{N} \rightarrow \text{C}_\alpha$  (on both  $i$  and  $i-1$ )
  - 3<sup>rd</sup> dimension: magnetization is transferred back to the amide proton  $\text{H}_\text{N}$
- The N atom of a given amino acid is correlated with both  $\text{C}_\alpha$  – its own and the preceding residue, so it's possible to **assign the backbone exclusively with HNCA**
- But usually more triple resonance experiments are needed because the cross signal of the preceding residue has to be identified and the degenerate resonance frequencies have to be resolved

# Triple Resonance Experiments<sub>2</sub>: HNCO Experiment

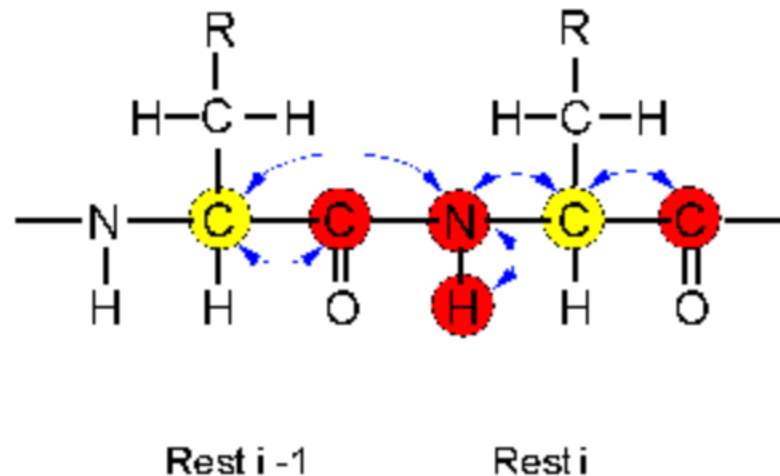
- In the HNCO experiment, the magnetization is transferred from  $H_N(i)$  proton via the  $N(i)$  atom to the directly attached  $CO(i-1)$  carbon atom and **return** the same way to  $H_N(i)$  nucleus which is directly detected
- The amide proton is correlated with the CO atom of the preceding residue

HNCO



# Triple Resonance Experiments<sub>3</sub>: HN(CA)CO experiment

- In the HN(CA)CO experiment, the magnetization is transferred from the  $H_N(i)$  proton via the N(i) atom and the CA nucleus ( $C_\alpha(i)$ ) to the CO(i) carbon atom and back the same way.
- Only the frequencies of  $H_N$ , N, and CO (red part) are detected.
  - The  $C_\alpha$  atom (yellow) acts only as relay nucleus, its frequency is not detected
- The amide proton  $H_N$  is correlated with the CO carbon atom of both the preceding residue and its own residue.



HN(CA)CO

