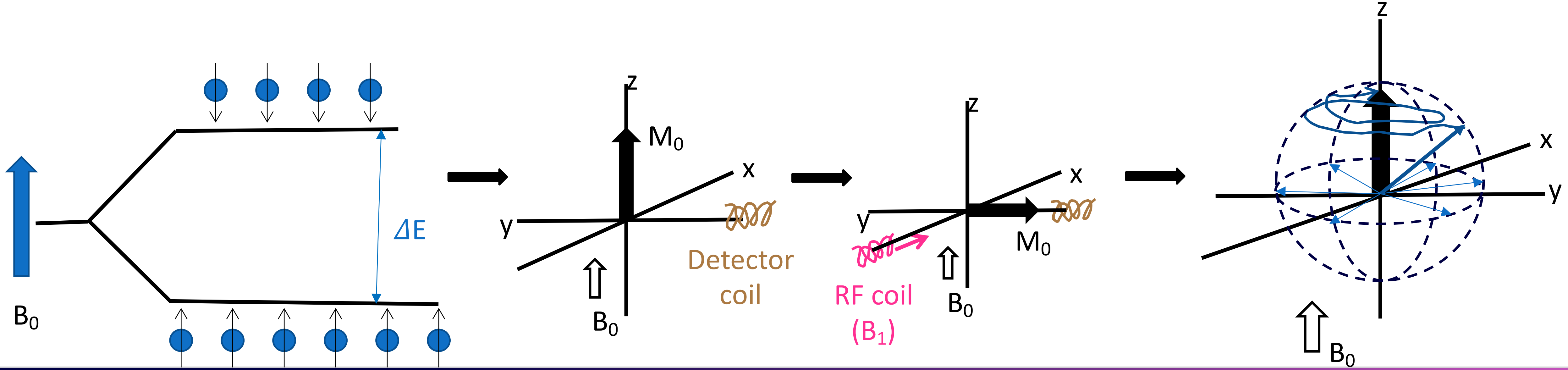
Tutorial: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

NMR Principle: Review

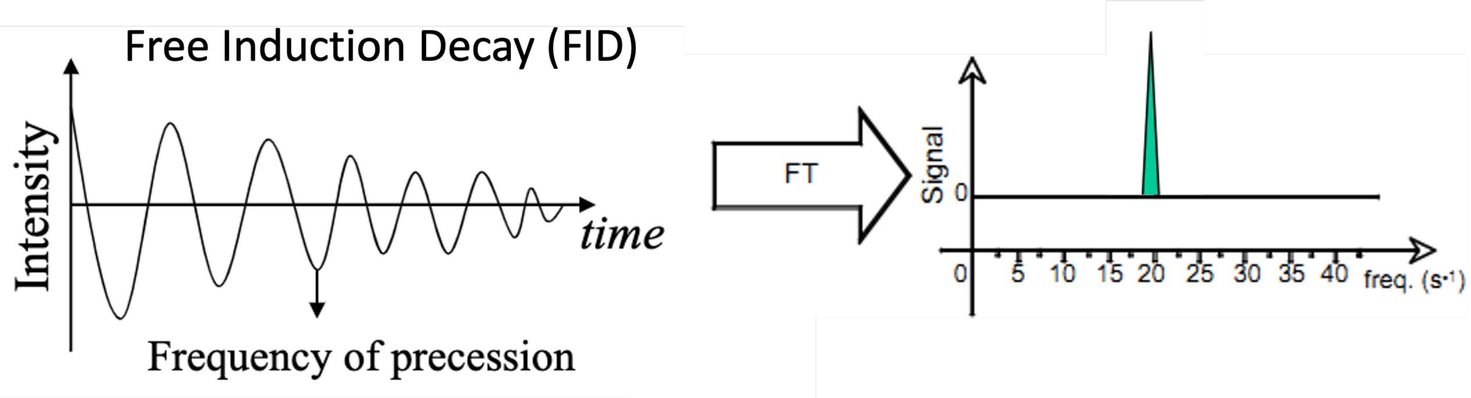
- NMR spectrometry is another form of absorption spectrometry.
- Absorption is a function of **certain nuclei** in the molecule.
  - Nuclei having an odd number of atomic number (e.g. ^{1}H, ^{13}C, ^{15}N, and ^{31}P)
- NMR spectrum: A plot of the frequencies (normalized into chemical shift)of absorption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

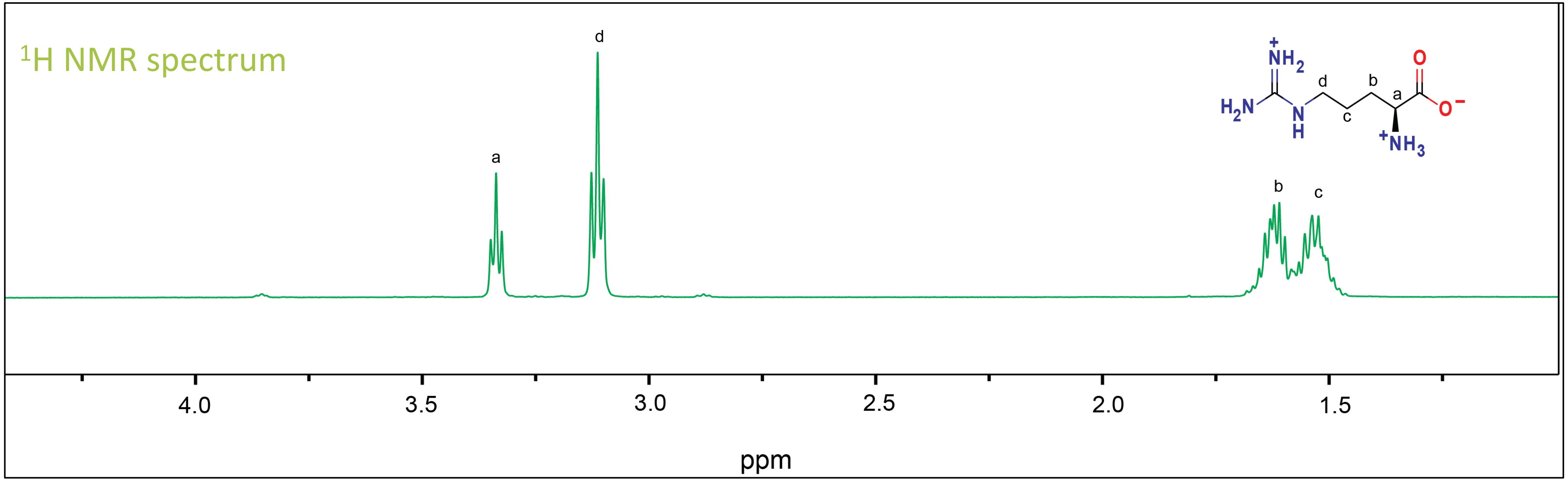
$$\nu_L = (\gamma/2\pi)B_{0,eff}$$

- γ is gyromagnetic ratio, which is a constant for a given isotope.
- $B_{0,eff}$ can change based on the environment.

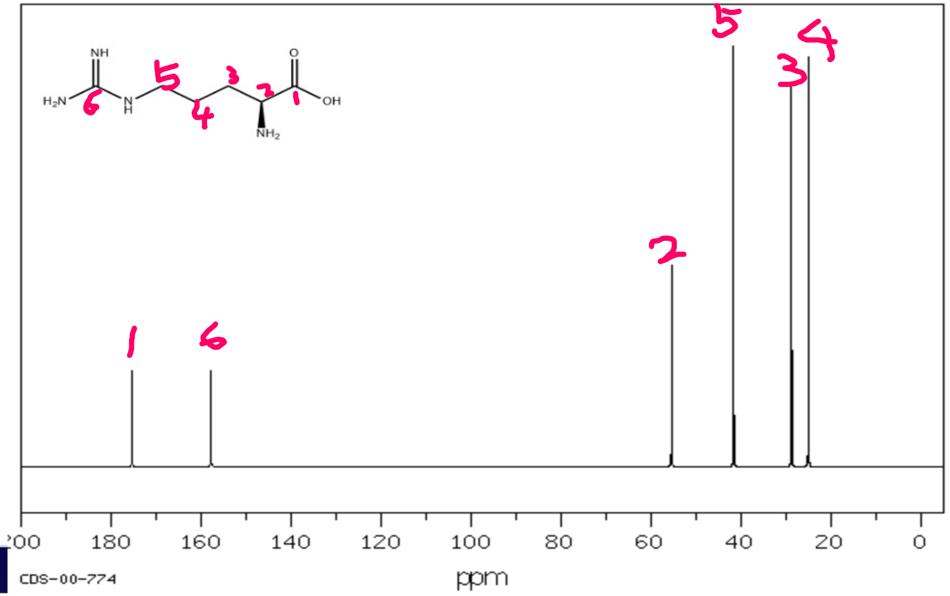
- The electrons create their own magnetic field which opposes the applied magnetic field B_0.
- A proton with a high electron density environment experiences a lower magnetic field and therefore a lower frequency (at the right side).

- **Atoms in the molecule affect one another by through-bond and through space.**

1D NMR: Review



^{13}C NMR spectrum



^{1}H NMR of a Ubiquitin

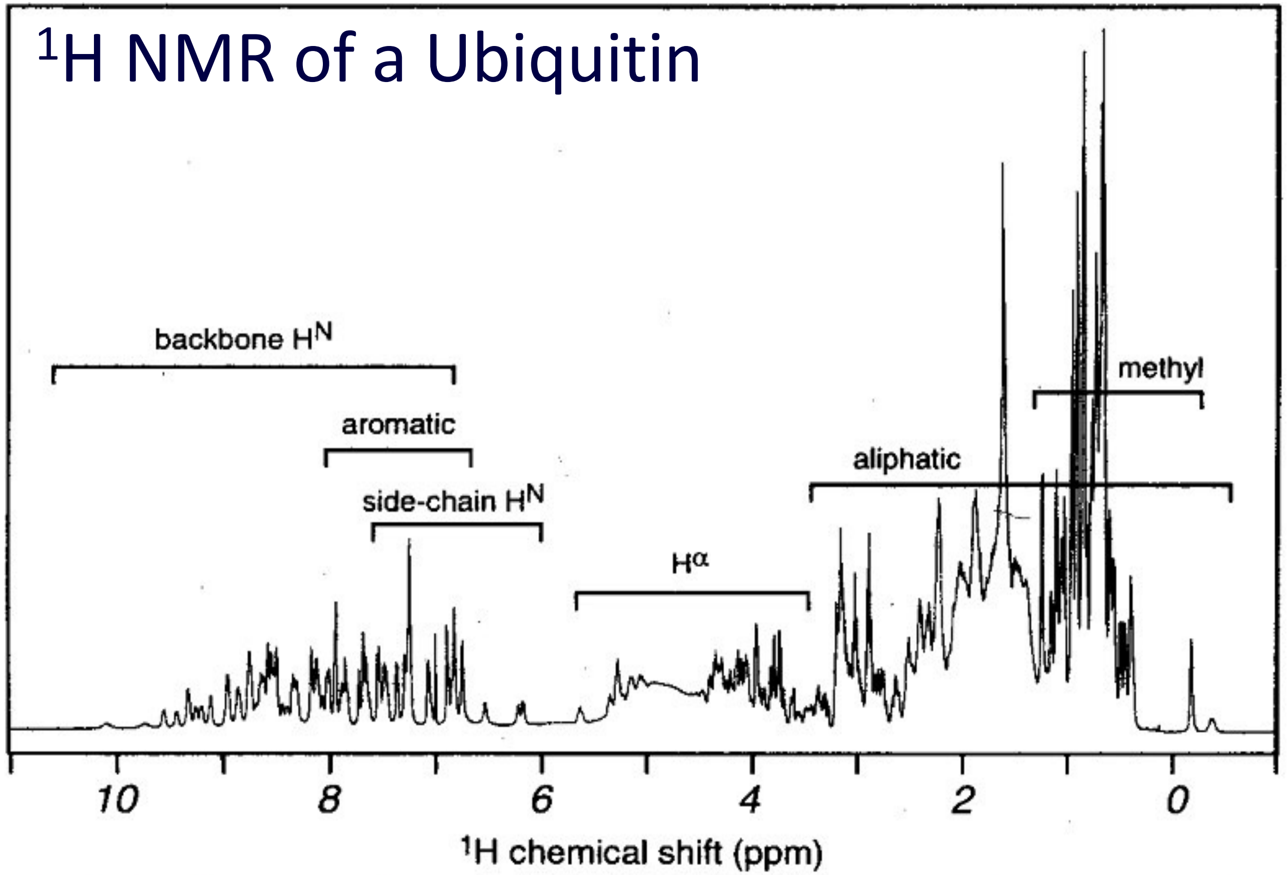


FIGURE 2. ^{1}H chemical shift positions of chemical groups in ubiquitin [1] (from: Cavanagh *et al.*: Protein NMR Spectroscopy).

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.

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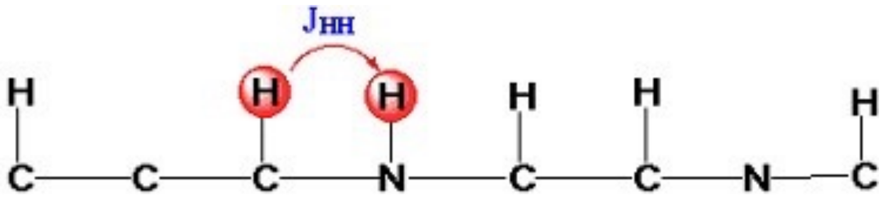
Multi-dimensional NMR

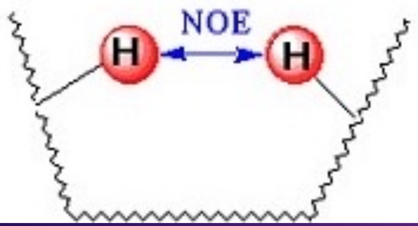
2D NMR: Review

- 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 ^1H-^1H correlated spectroscopy).



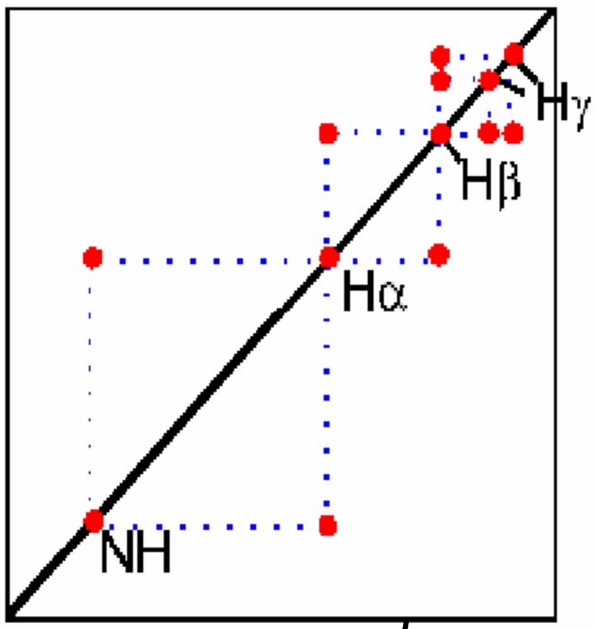
2. **Nuclear Overhauser Effect** (NOE)– nuclei affect each other through space (dipole-dipole interaction) and give information about how nuclei are arranged in space (must be with 5.5 Å of one another)

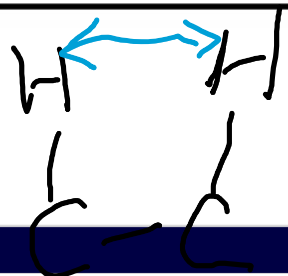


Homonuclear 2D NMR: Review

- ^1H-^1H COSY:

3 or less bonds correlation



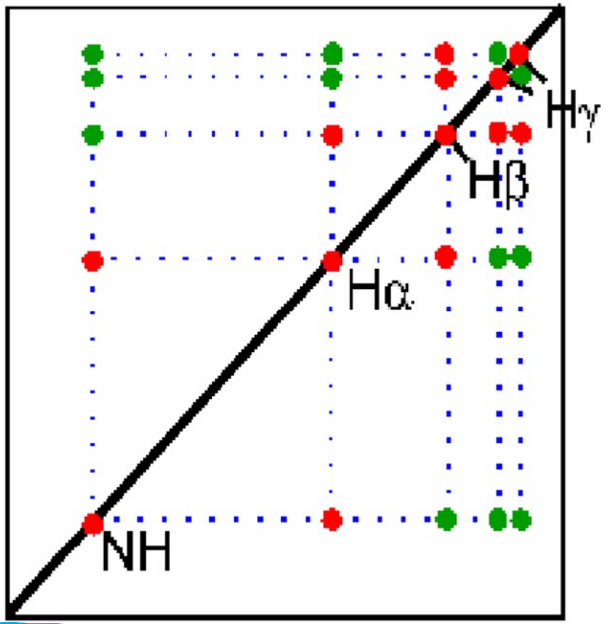


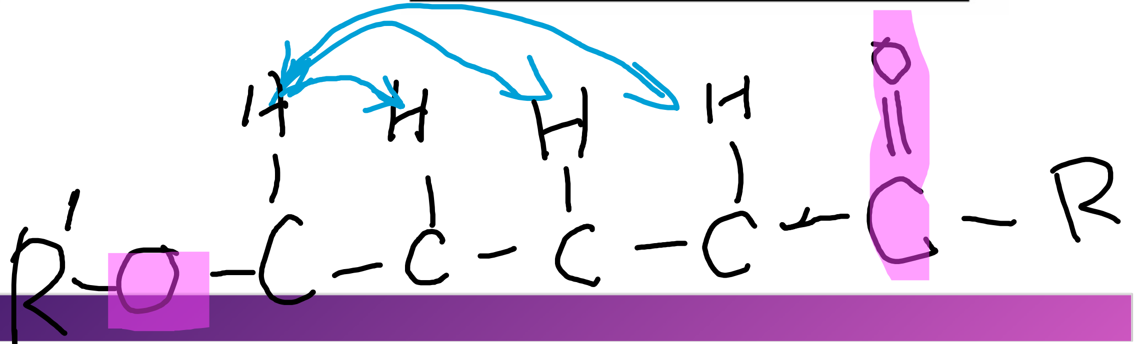
- Nuclear Overhauser Spectroscopy (NOSEY):

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

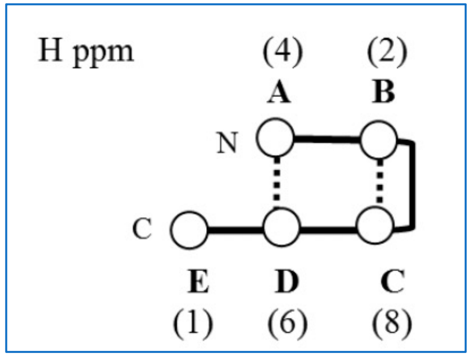
- TOCSY:

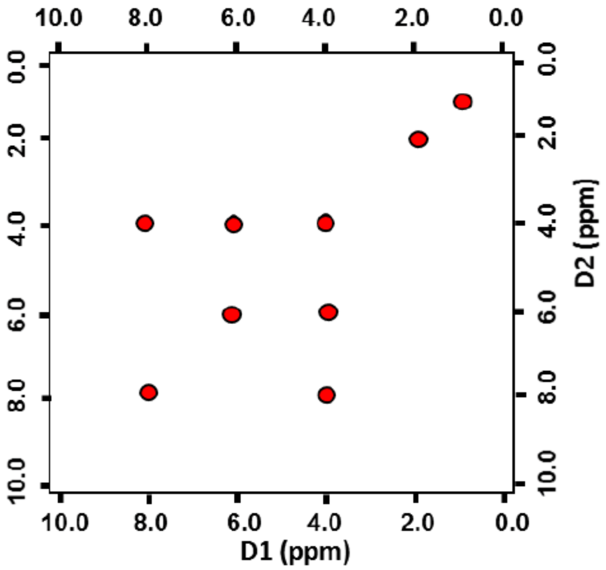
nuclei correlation within the same spin system



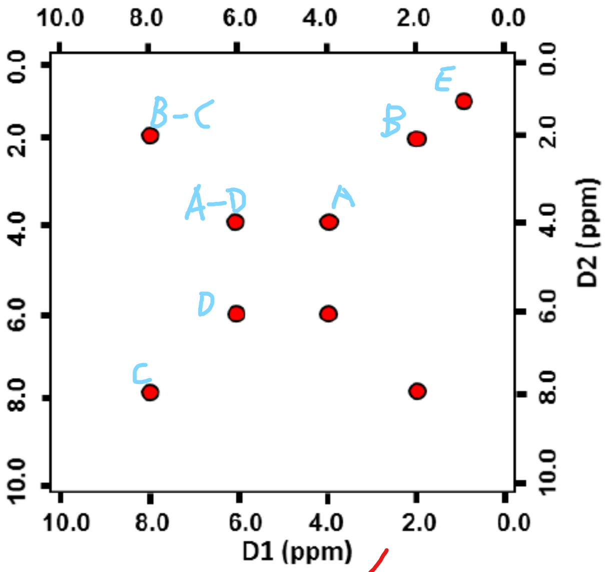


- **Practice**: Below shows the structure of a fictitious molecule consisting of five atoms (A, B, C, D and E) as determined by 2D NOESY NMR. The ^{1}H chemical shift numbers are in bracket. Based on the structure of the molecule, which spectrum (A, B or C) is most likely to be the NOESY spectrum of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

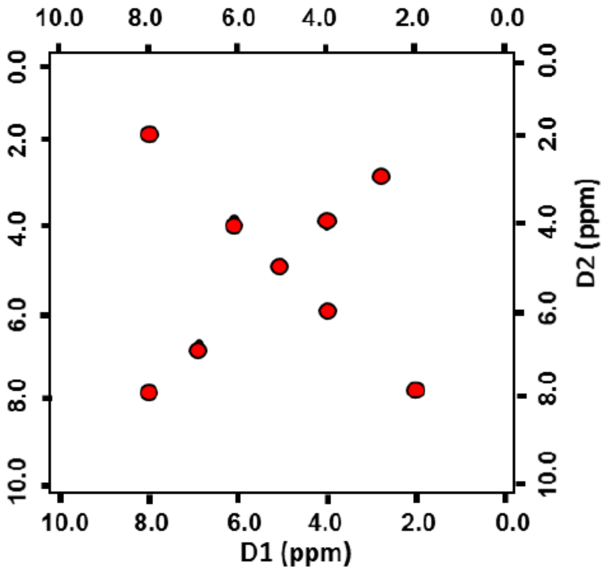




A

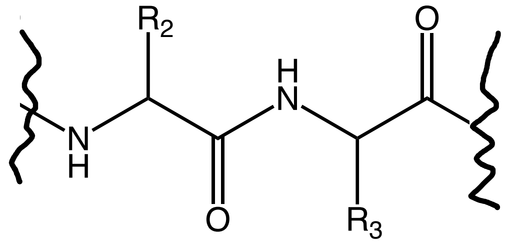


B

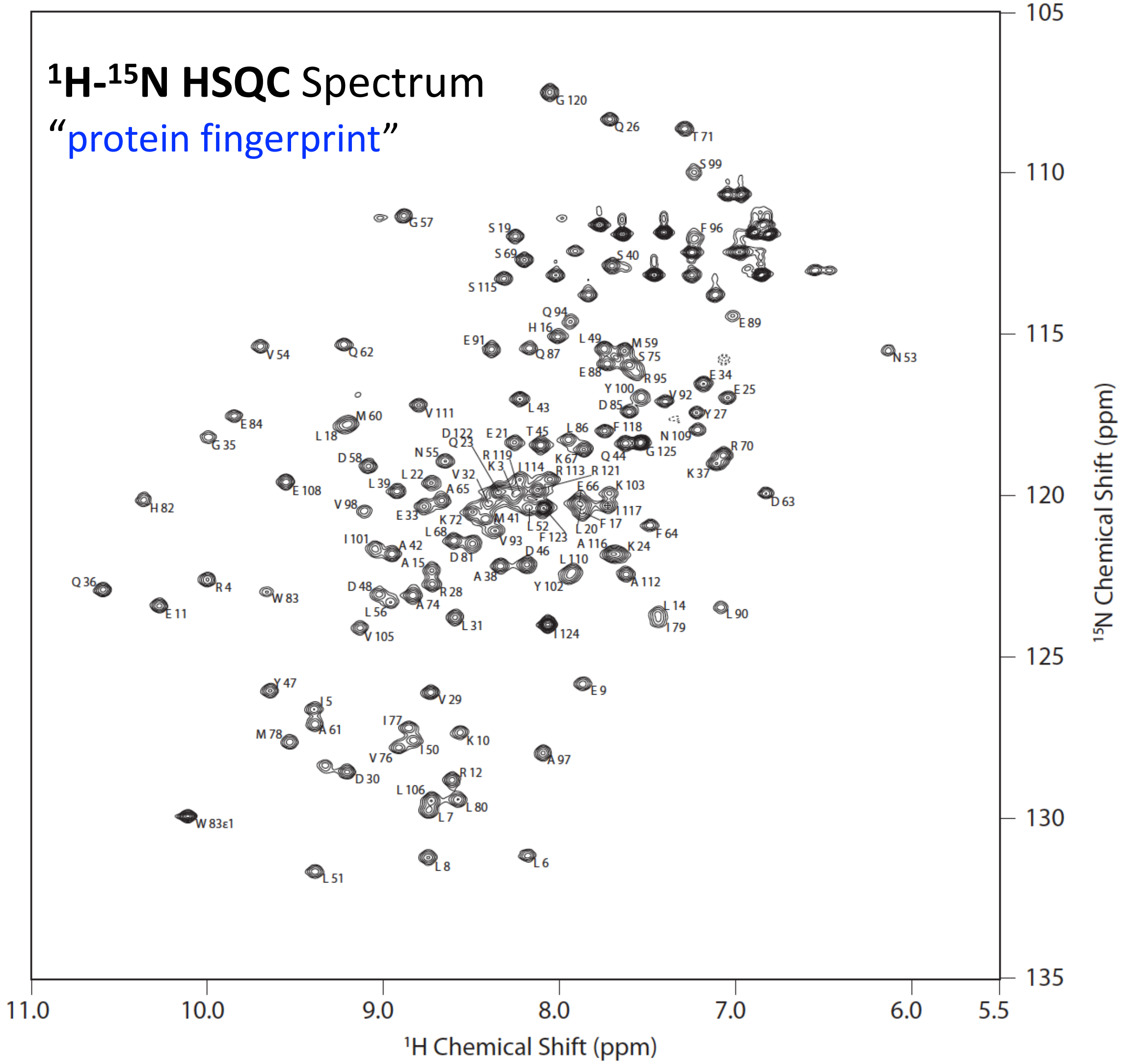


C

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 ^1H with ^{13}C or ^{15}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**

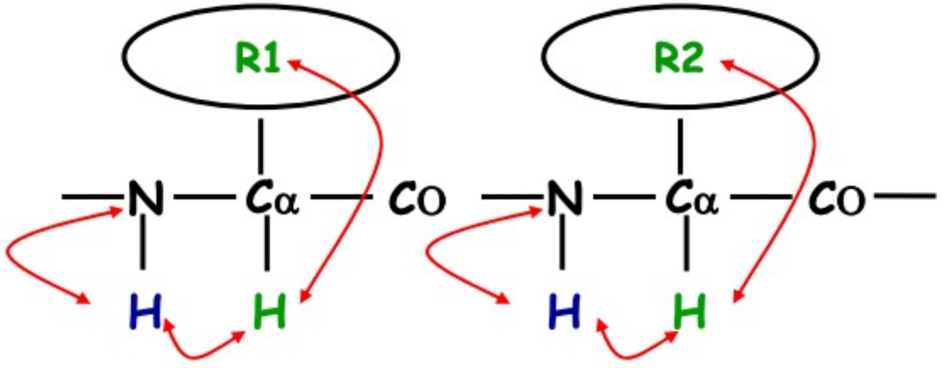
     -- Sequential assignment of larger proteins (> 150 AA)

     -- Three nuclei (^{1}H, ^{13}C, ^{15}N) are correlated

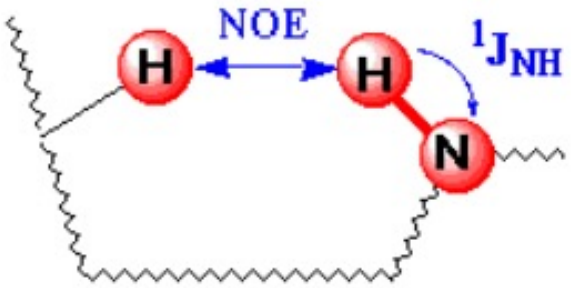
     -- Performed on doubly labelled (^{13}C, ^{15}N) proteins

Two 2D NMR Experiments

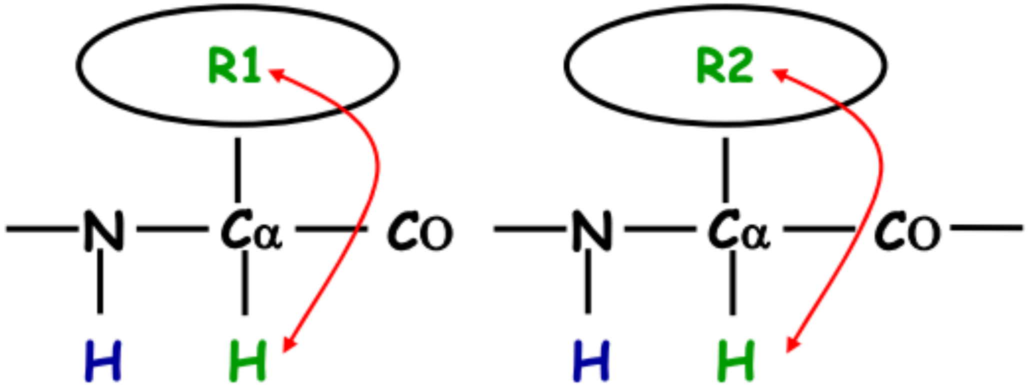
- ^1H-^{15}N-^1H: 3D TOCSY-HSQC



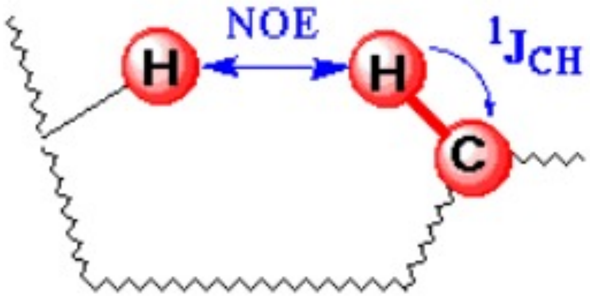
- 3D ^{15}N-edit NOESY-HSQC



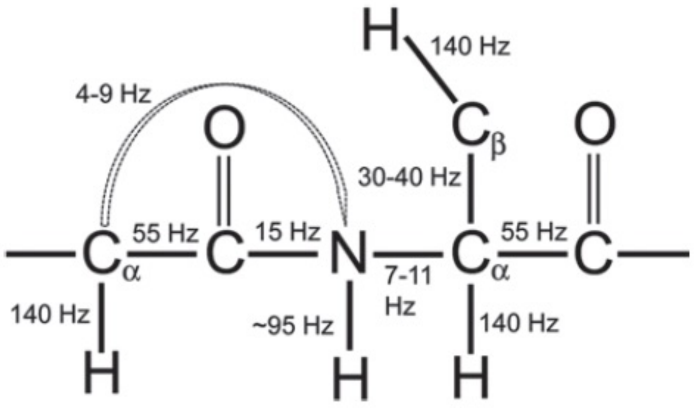
- ^1H-^{13}C-^1H: 3D TOCSY-HSQC



- 3D ^{13}C-edit NOESY-HSQC



Triple Resonance Experiments

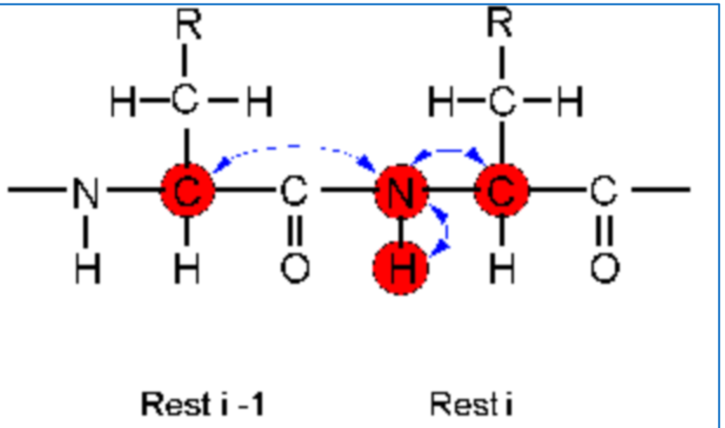


Summary of one- and two-bond couplings important for triple resonance experiments used for assignment.

HNCA

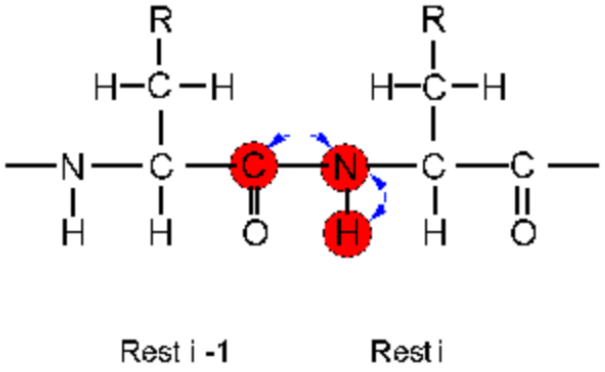
$^{1}H^{N}_{i} - {}^{15}N_{i} - {}^{13}C^{\alpha}_{i}$

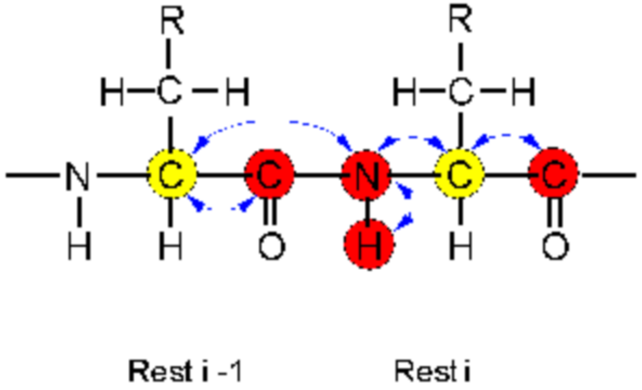
$^{1}H^{N}_{i} - {}^{15}N_{i} - {}^{13}C^{\alpha}_{i-1}$



HNCO

$^{1}H^{N}_{i} - {}^{15}N_{i} - {}^{13}CO_{i-1}$





HN(CA)CO

$^{1}H^{N}_{i} - {}^{15}N_{i} - {}^{13}CO_{i}$

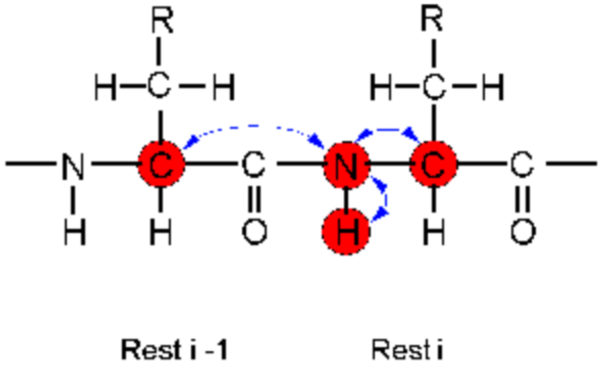
$^{1}H^{N}_{i} - {}^{15}N_{i} - {}^{13}CO_{i-1}$

Triple Resonance Experiments$_1$: HNCA Experiment

HNCA

$^{1}H^{N}_{i}$ - $^{15}N_{i}$ - $^{13}C^{\alpha}_{i}$

$^{1}H^{N}_{i}$ - $^{15}N_{i}$ - $^{13}C^{\alpha}_{i-1}$



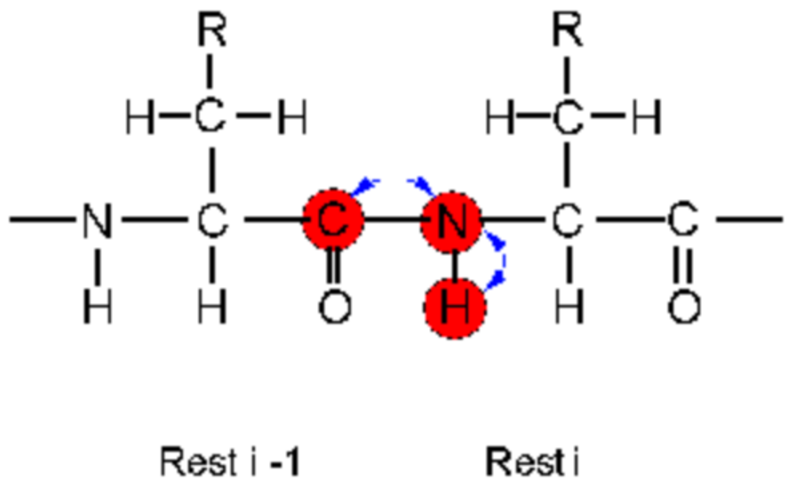
- The HNCA experiment is the prototype of all triple resonance experiments.
  - 1st dimension: The magnetization of H_N is transferred to N
  - 2nd dimension: magnetization is transferred from N→ C_α (on both i and i-1)
  - 3rd dimension: magnetization is transferred back to the amide proton H_N
- The N atom of a given amino acid is correlated with both C_α – 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$_2$: 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

$^{1}H^{N}_{i}$ - $^{15}N_{i}$ - $^{13}CO_{i-1}$



Triple Resonance Experiments$_3$: 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_α(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_α 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.