Class Today

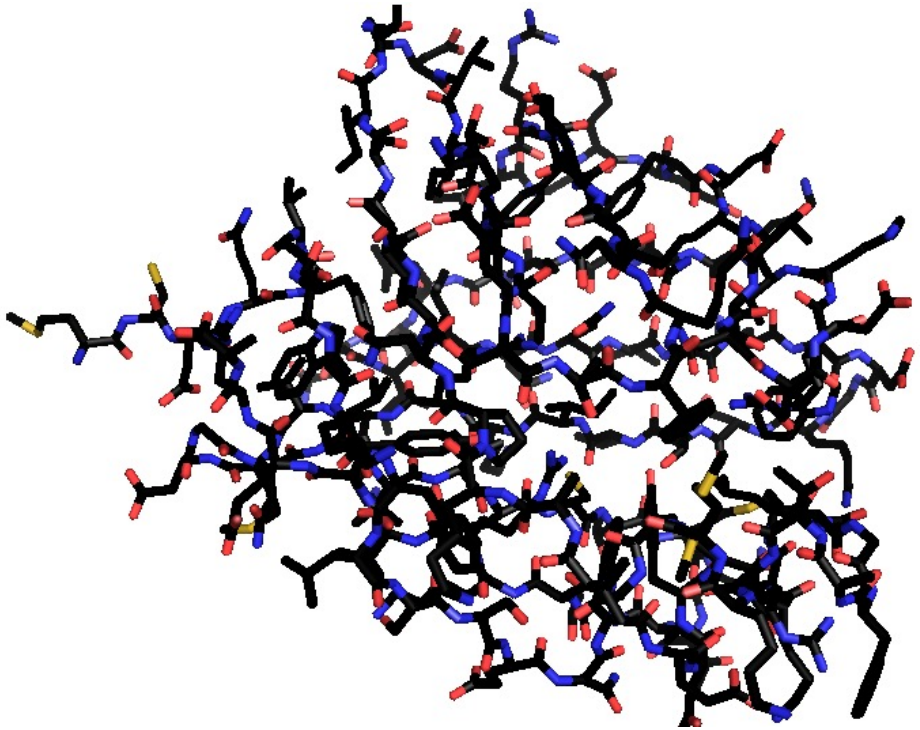
1. Finish up Lecture 9: X-ray Crystallography
2. Lecture 10-1: Nuclear Magnetic Resonance Spectroscopy
   - Basics of 1D, 2D and 3D NMR

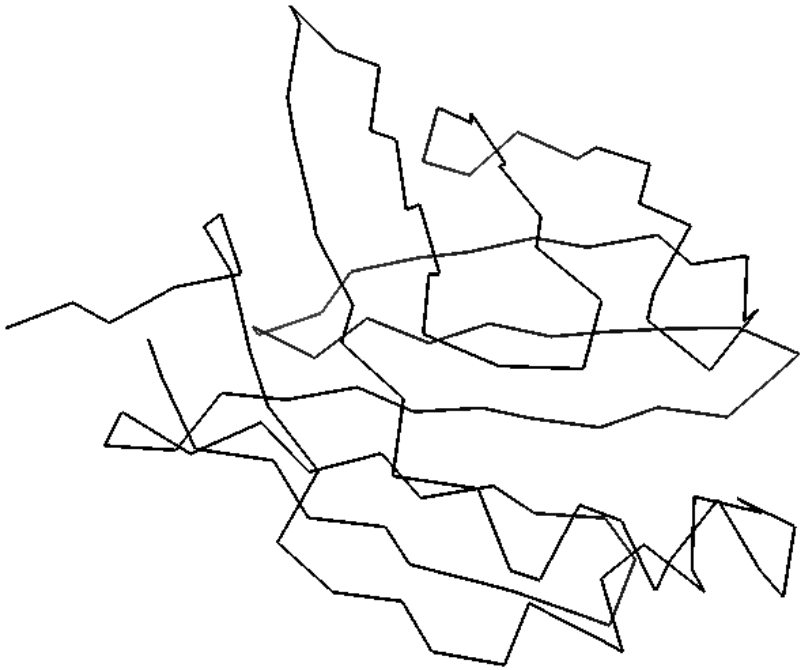
- Assignments:
  1. CW1 is due tomorrow(April 23rd) at 17:00.
  2. CW2 will be uploaded onto LMO April 23rd, it is due at 17:00 on May 7th.

A Protein Molecule Is Dynamic

- The electron density is a spatial average over all molecules in the crystal and a time average over the duration of the X-ray data measurement
- Multiple discrete conformations of a residue in different molecules are superimposed.
  - Some dynamical aspects evident in the electron density are lost in the pdb file
- Damage caused by X-rays may change the protein (mainly breaking of disulfide bonds)
- A crude description of dynamics is provided in the pdb file as the isotropic “B-factor”

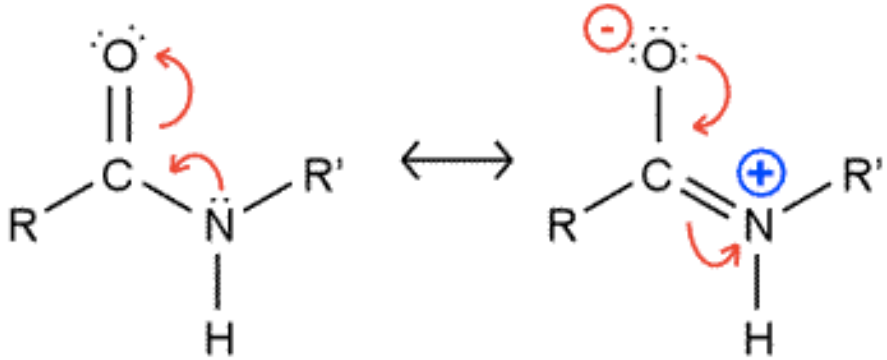
A protein’s folded shape can be roughly described by the path traced by its peptide backbone (ignoring side chains)



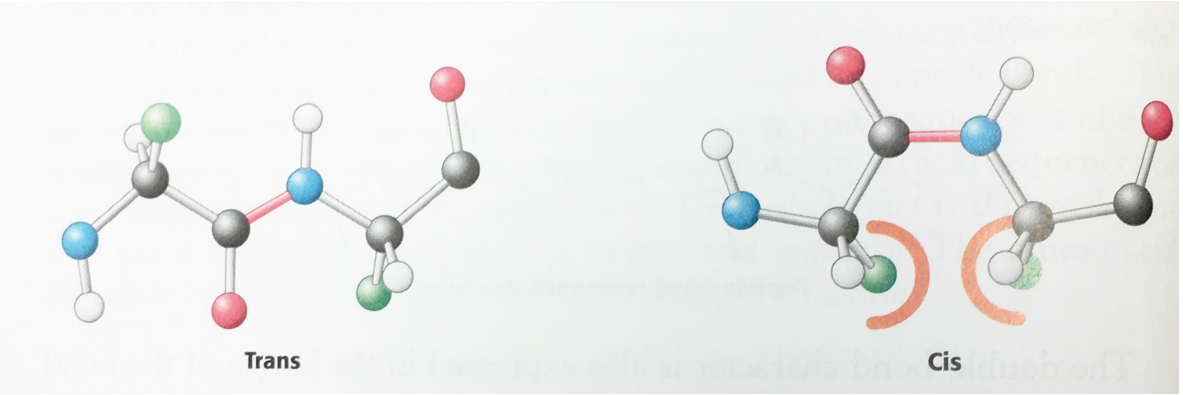


Peptide bonds have limited rotational freedom

The peptide bond
is planar:

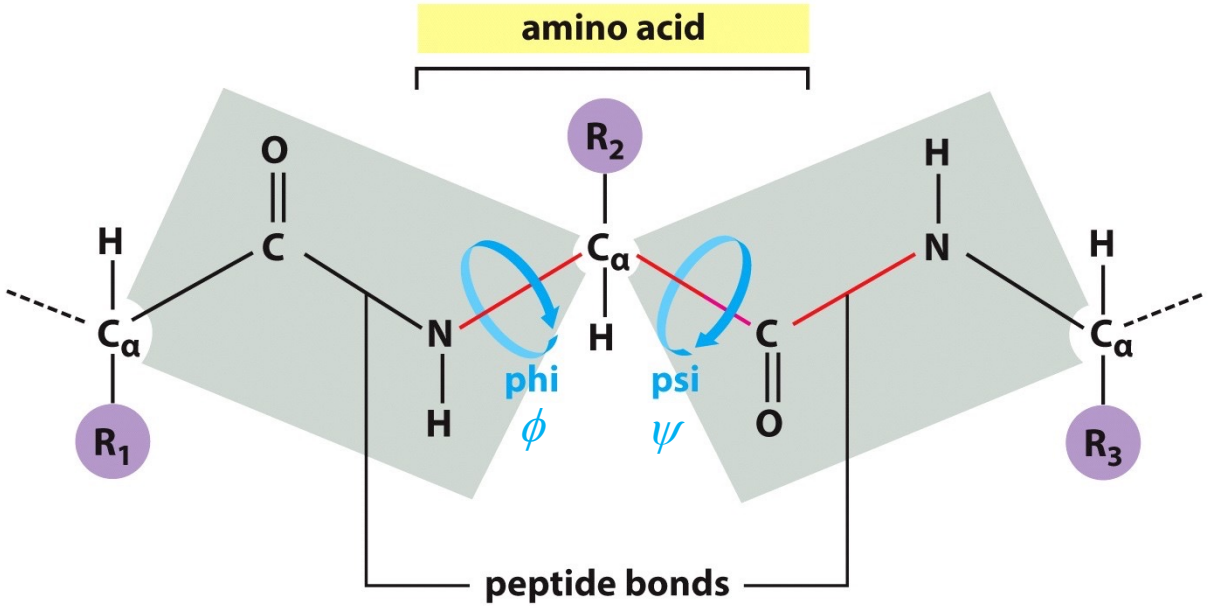


The peptide bond has more
than two conformations



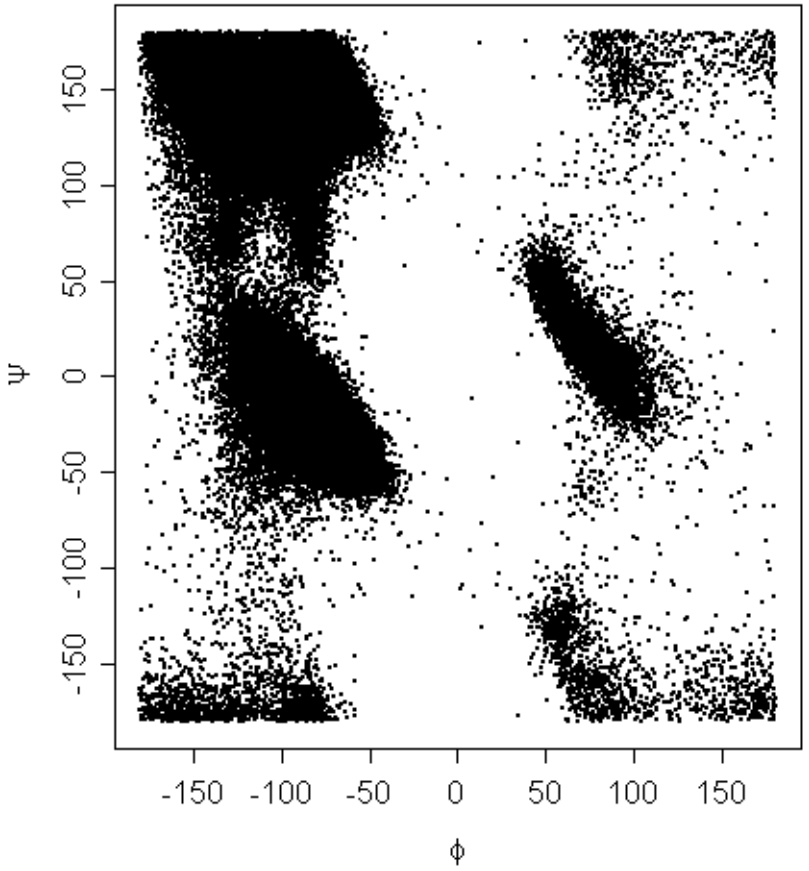
…but *cis* is less likely due
to steric clash

Ramachandran Plot



The torsional angles of each residue define the geometry of its attachment to its two adjacent residues, so the torsional angles determine the conformation of the residues and the peptide.

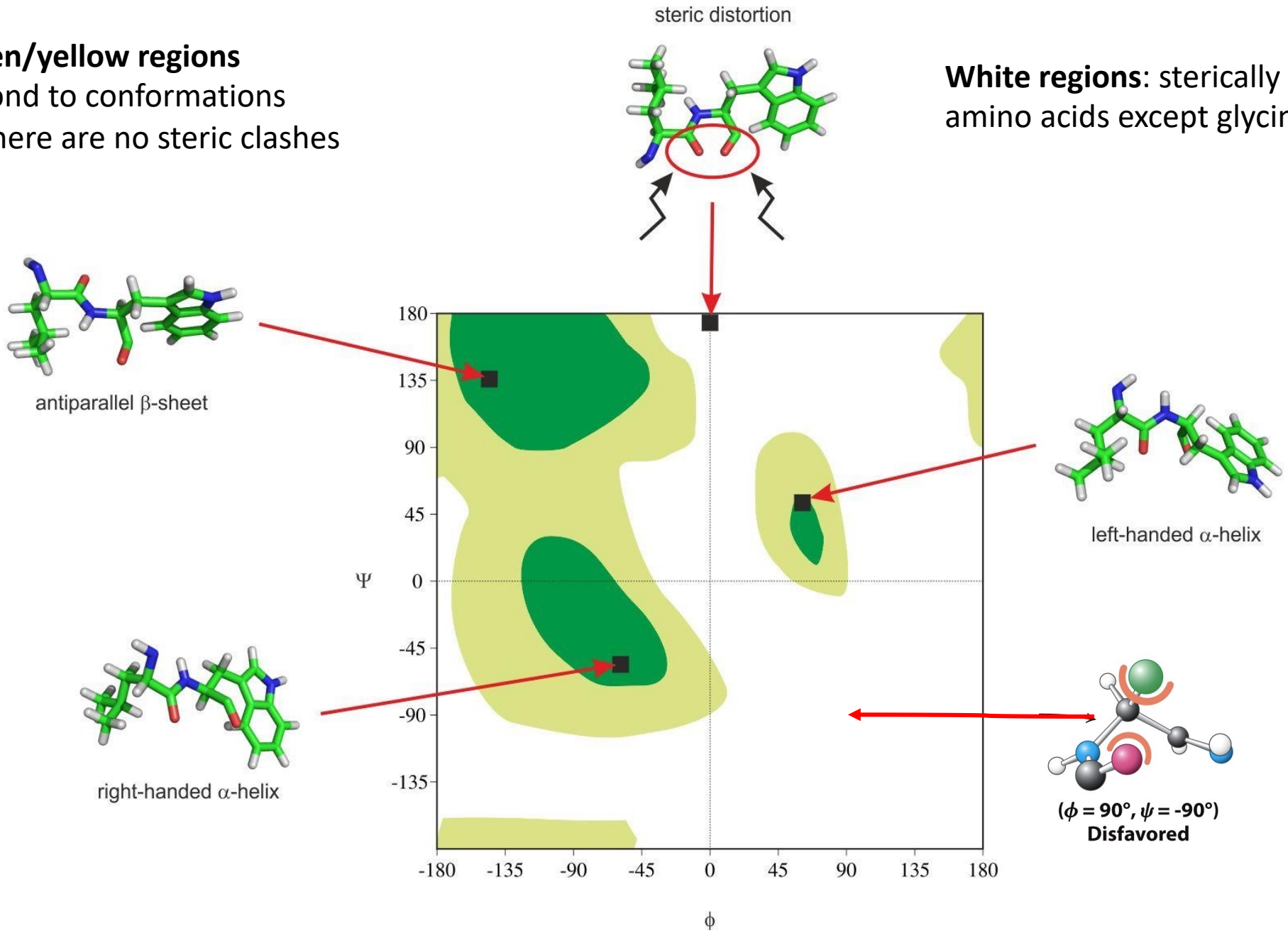
- Ramachandran plot (phi/psi plot) was the first **serious** **verification tool** for protein structures.
  - shows frequency of (ϕ, ψ) observed for residues in folded proteins



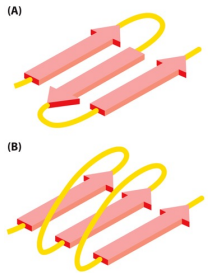
Each dot represents an amino acid

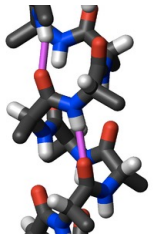
The **green/yellow regions** correspond to conformations where there are no steric clashes

White regions: sterically disallowed for all amino acids except glycine

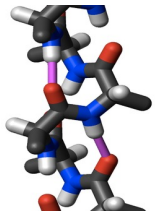


Ramachandran Plot

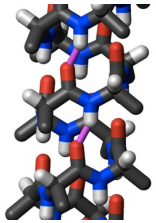




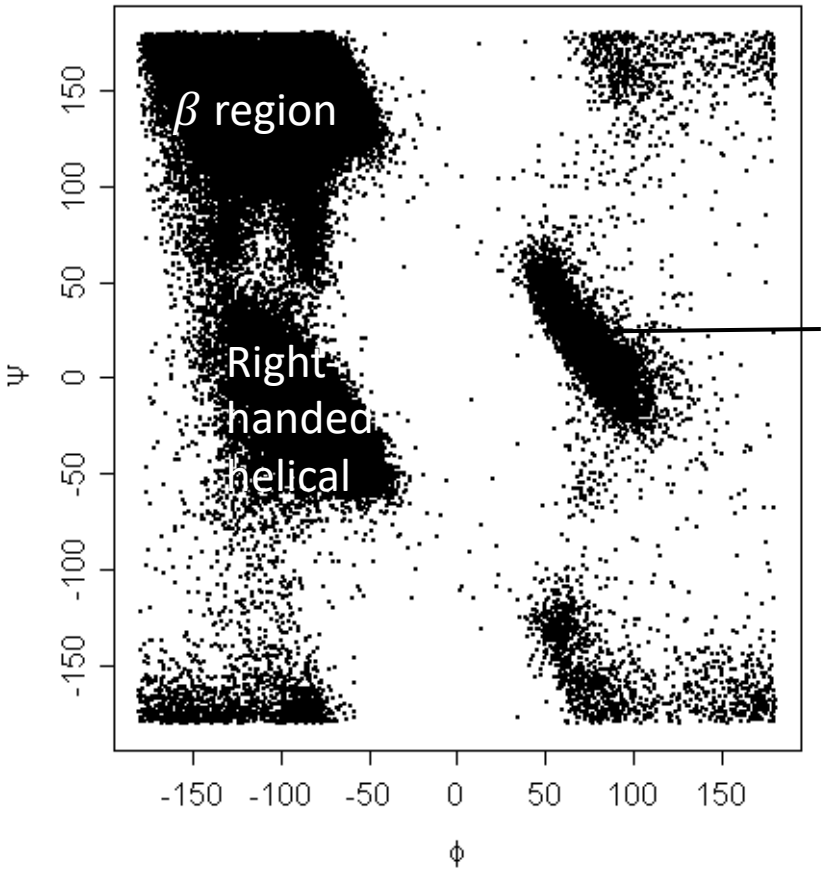
α helix



3_{10} helix



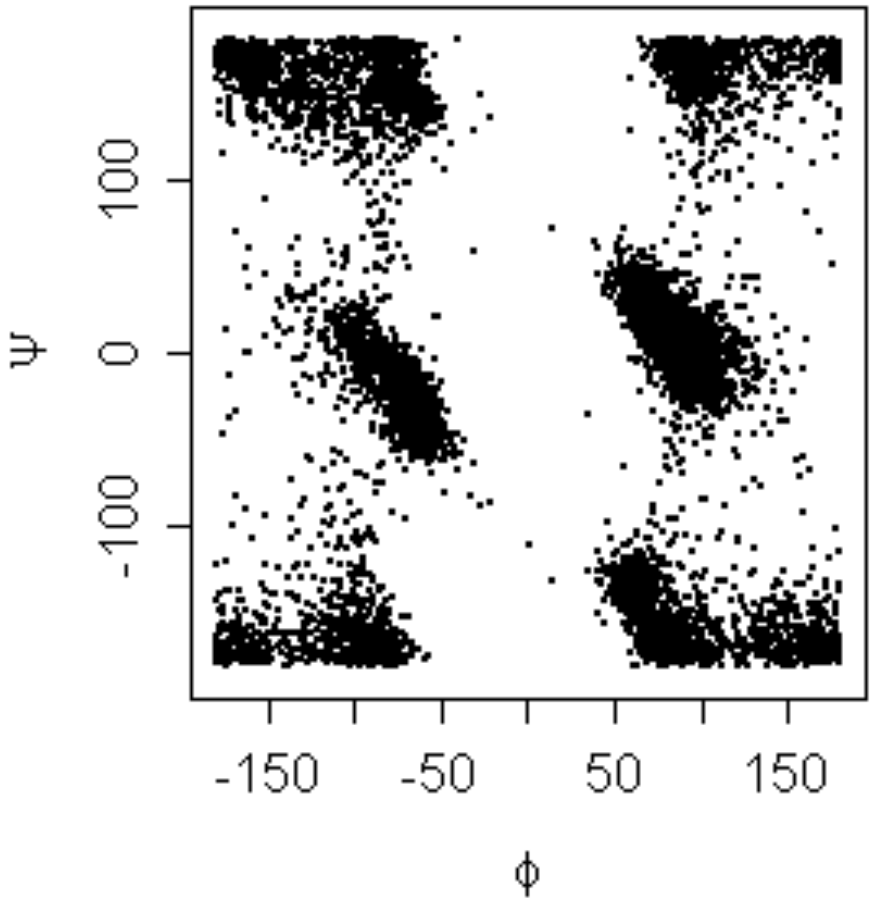
π helix

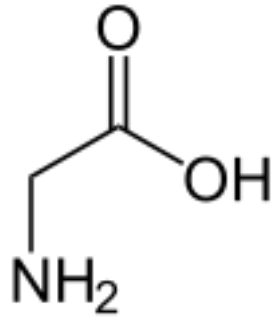


Each dot represents an amino acid

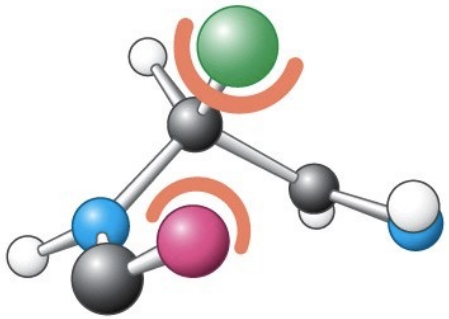
- Peptide backbone hydrogen bonding is the most common motif in folding
  - referred to as a protein's *secondary structure*
- Some side chains inhibit formation of certain secondary structures (e.g. proline/α helices)
- Another trend for consecutive amino acids: correlation in position

Glycine does adopt positive ϕ values

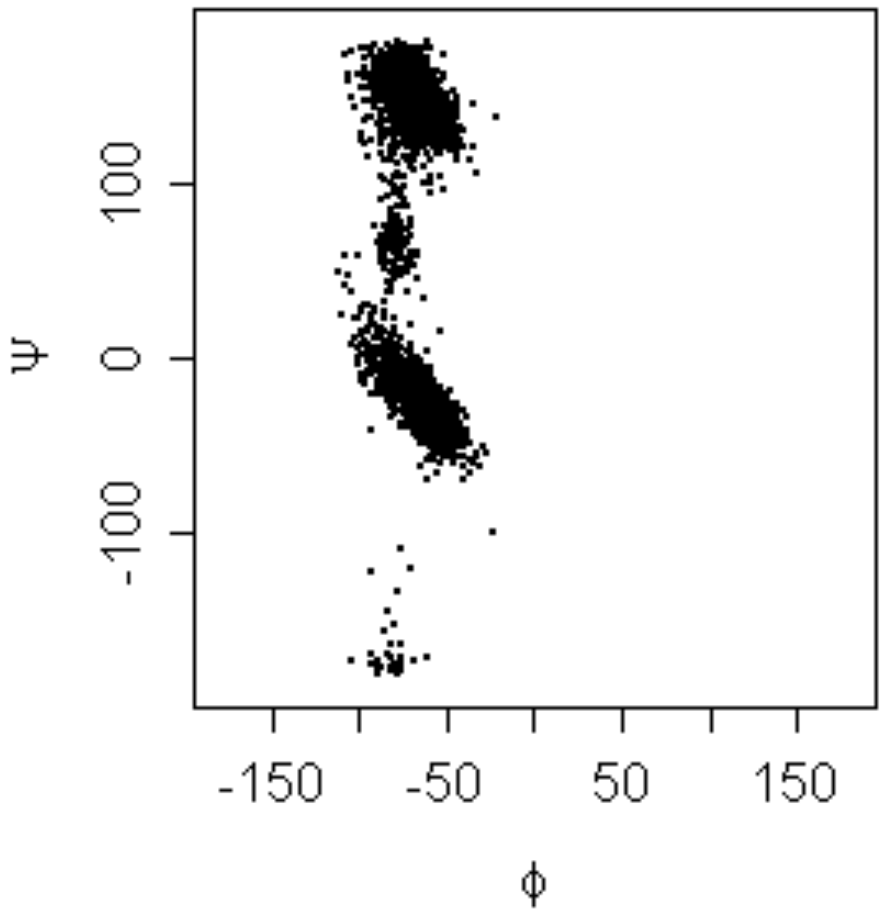


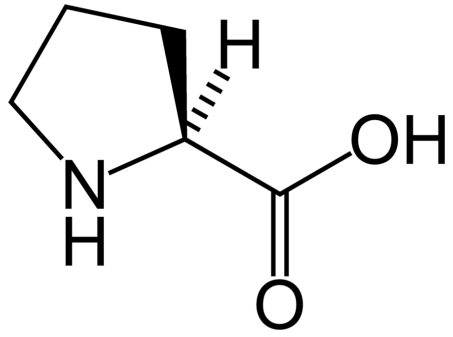


Less steric hindrance because side chain (green) is very small

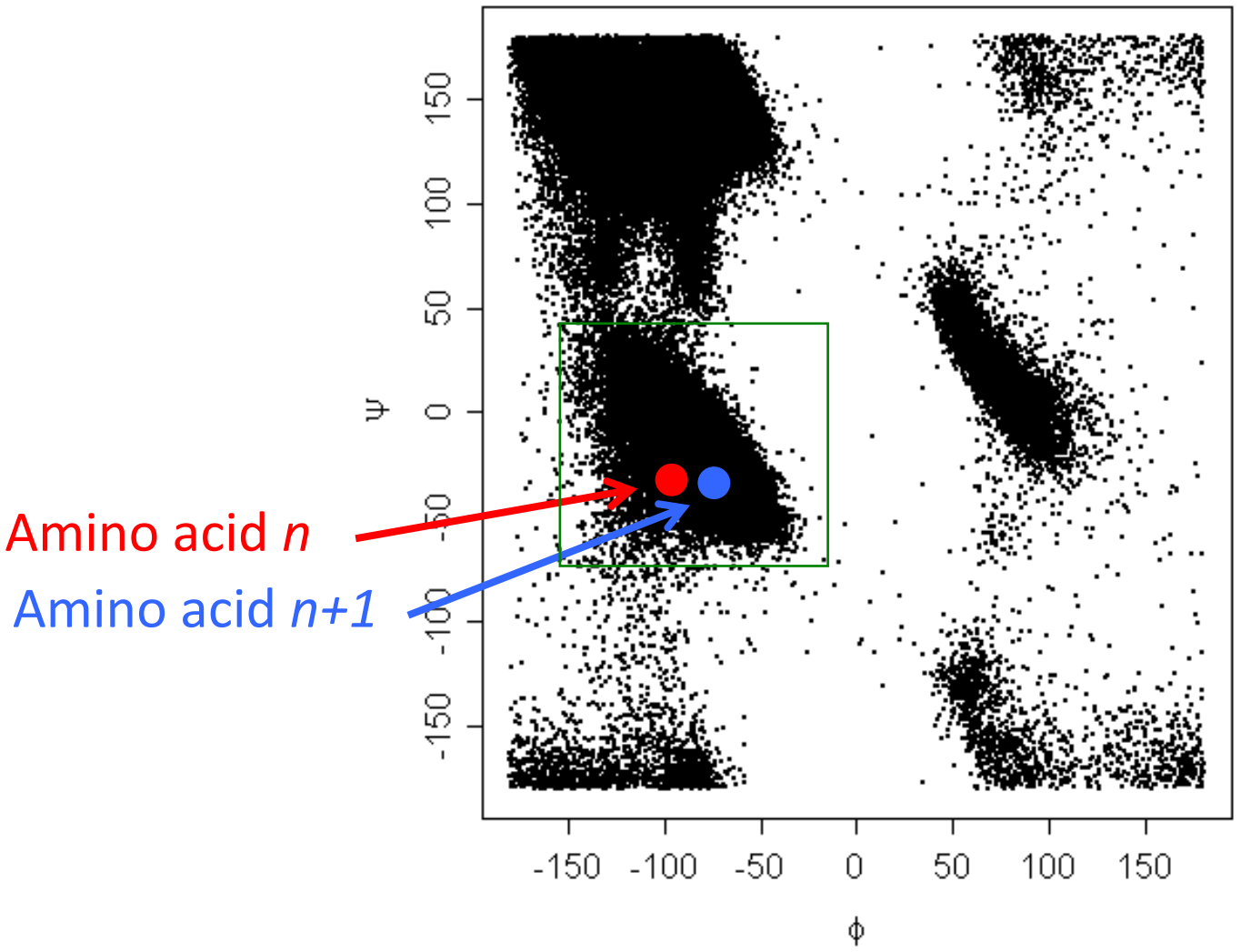


Proline's ϕ value is (somewhat) fixed by its side chain's bond to the backbone





This side chain can clash sterically with the preceding amino acid, so the"pre-proline" Ramachandran plot is also unique



Think: What do we get if we repeat the same torsion angles many times in a row?

Reading a crystallography paper$_1$:

Table 1. Crystallographic Data Collection and Refinement Statistics

	SwMppP	SwMppP·D-Arg
resolution (Å) (last shell)[a]	41.45–2.10 (2.14–2.10)	44.53–2.25 (2.29–2.25)
wavelength (Å)	0.97852	0.97856
no. of reflections		
observed	1532933 (60186)	256371 (12494)
unique	106188 (5236)	79816 (3984)
completeness (%)[a]	100.0 (100.0)	90.2 (91.2)
R_{merge} (%)[a,b]	0.106 (0.734)	0.106 (0.726)
multiplicity	14.4 (11.5)	3.2 (3.1)
$\langle I/\sigma(I)\rangle$[a]	30.7 (5.2)	10.0 (1.9)
Model Refinement		
no. of reflections in the working set	100876	76747
no. of reflections in the test set	5256	3015
R_{cryst} (R_{free})	0.148 (0.177)	0.162 (0.197)
no. of residues	1417	1404
no. of solvent atoms	903	693
no. of TLS groups	29	33
average B factor (Å^2)[c]		
protein atoms	32.0	34.1
ligands	30.9[d]	43.9[d]
solvent	35.6	36.6
root-mean-square deviation		
bond lengths (Å)	0.013	0.015
bond angles (deg)	1.395	1.565
coordinate error (Å)	0.17	0.22
Ramachandran statistics (favored/allowed/outliers) (%)	98.3/1.7/0	98.4/1.6/0

[a]Values in parentheses apply to the high-resolution shell indicated in the resolution row. [b]$R = \sum(||F_{obs}| - \text{scale} \times |F_{calc}||)/\sum|F_{obs}|$. [c]Isotropic equivalent B factors, including the contribution from TLS refinement. [d]In the unliganded SwMppP structure, "ligands" refers to the bound Cl ions, while in the D-Arg complex structure, it refers to the D-Arg-PLP unit.

Judge the quality of the data:

- R_{merge}: 0.05-0.10 good, 0.1-0.15 acceptable
- I/σ = signal/noise >2.0
- Completeness
- Redundancy
- R_{work}/R_{free}:
  - difference < 0.05,
  - R_{work}≈ resolution/10
- Deviations of known geometry

Reading a crystallography paper$_2$:

	SwMppP
resolution (Å) (last shell)[a]	41.45–2.10 (2.14–2.10)
wavelength (Å)	0.97852
no. of reflections	
observed	1532933 (60186)
unique	106188 (5236)
completeness (%)[a]	100.0 (100.0)
R_{merge} (%)[a,b]	0.106 (0.734)
multiplicity	14.4 (11.5)
$\langle I/\sigma(I)\rangle$[a]	30.7 (5.2)

Judge the quality of the data:

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- Completeness
- Redundancy
- R_{work}/R_{free}:
  - difference < 0.05,
  - R_{work}≈ resolution/10
- Deviations of known geometry

Reading a crystallography paper$_3$:

Model refinement of SwMppP

no. of reflections in the working set	100876
no. of reflections in the test set	5256
R_{cryst} (R_{free})	0.148 (0.177)
no. of residues	1417
no. of solvent atoms	903
no. of TLS groups	29
average *B* factor (Å^2)[c]	
protein atoms	32.0
ligands	30.9[d]
solvent	35.6
root-mean-square deviation	
bond lengths (Å)	0.013
bond angles (deg)	1.395
coordinate error (Å)	0.17
Ramachandran statistics (favored/allowed/outliers) (%)	98.3/1.7/0

Judge the quality of the data:

- R_{merge}: 0.05-0.10 good, 0.1-0.15 acceptable
- I/σ = signal/noise >2.0
- Completeness
- Redundancy
- R_{work}/R_{free}:
  - difference < 0.05,
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- Deviations of known geometry

Things to Know

1. What is X-ray Crystallography. Know the general process.
2. What are protein crystals? What's the common method used for making crystals? Why are protein crystals fragile?
3. Understand the protein phase diagram.
4. Phase problem. How to solve?
5. Resolution. (know high/low resolution)
6. Know how to assign atoms based on environment.
7. Occupancy; b factor
8. Understand Ramachandran Plot and know its application.
9. Common parameters used to judge the model.

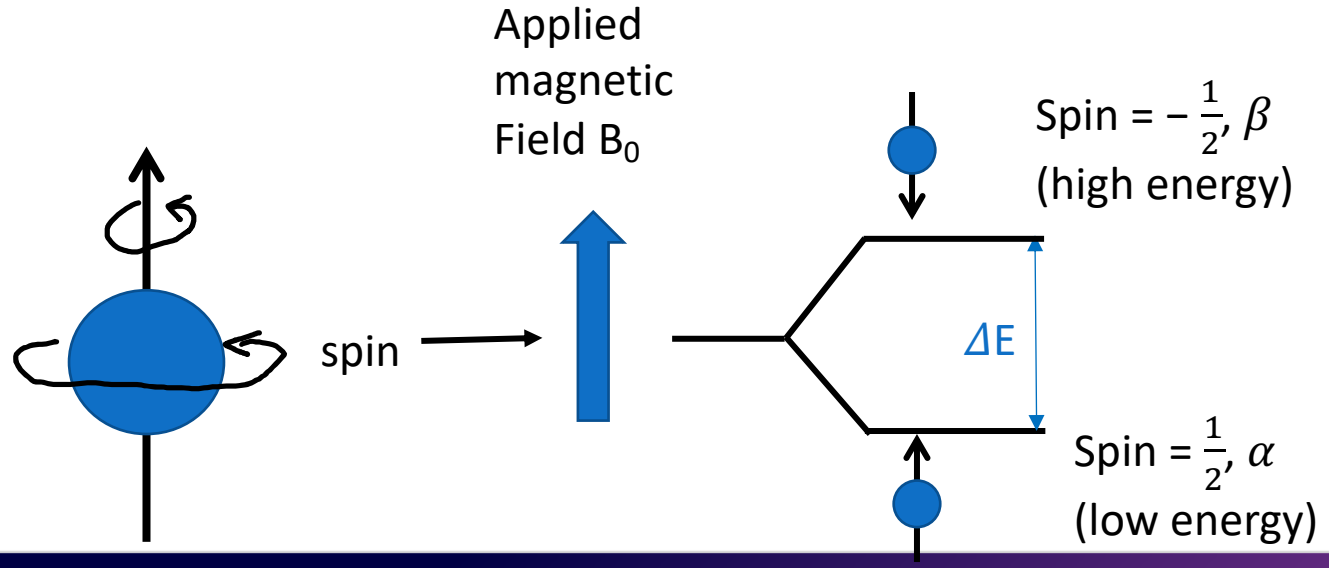
LECTURE 10-1: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

NMR Basics

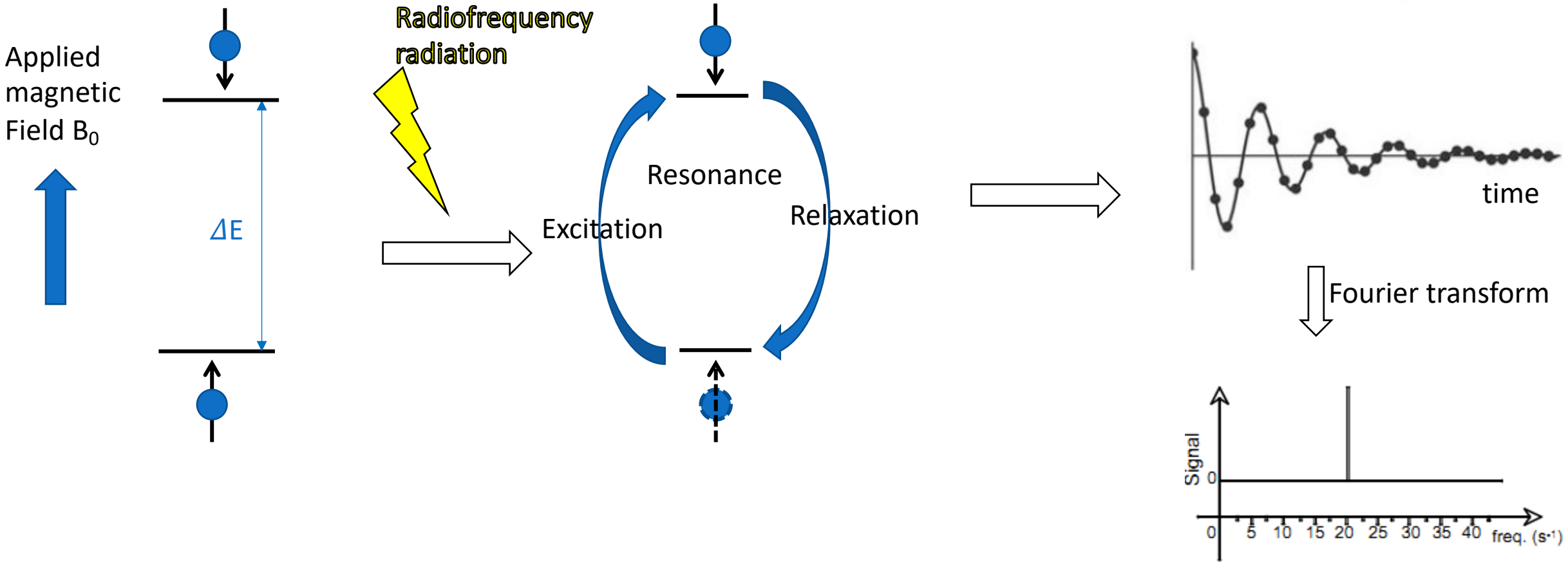
- NMR spectrometry is another form of absorption spectrometry.
- Absorption is a function of **certain nuclei** in the molecule.
  - Nuclei having an odd number of atomic number (e.g. ^{1}H, ^{13}C, ^{15}N, and ^{31}P)
- NMR spectrum: A plot of the frequencies of absorption peaks versus peak intensities.
- NMR can give information about dynamics – molecular motions on the ps to hour timescale and intermolecular interactions (binding events), even for very large proteins.

Magnetic Properties of Nuclei

- All nuclei carry a charge. This charge "spins" on the nuclear axis, and this circulation of nuclear charge generates a **magnetic dipole** along the axis. Different nuclei have different spins.
- NMR works because certain nuclei having an odd number of atomic number (e.g. ^{1}H, ^{13}C, ^{15}N, and ^{31}P) can *spin* and generate two spin states.

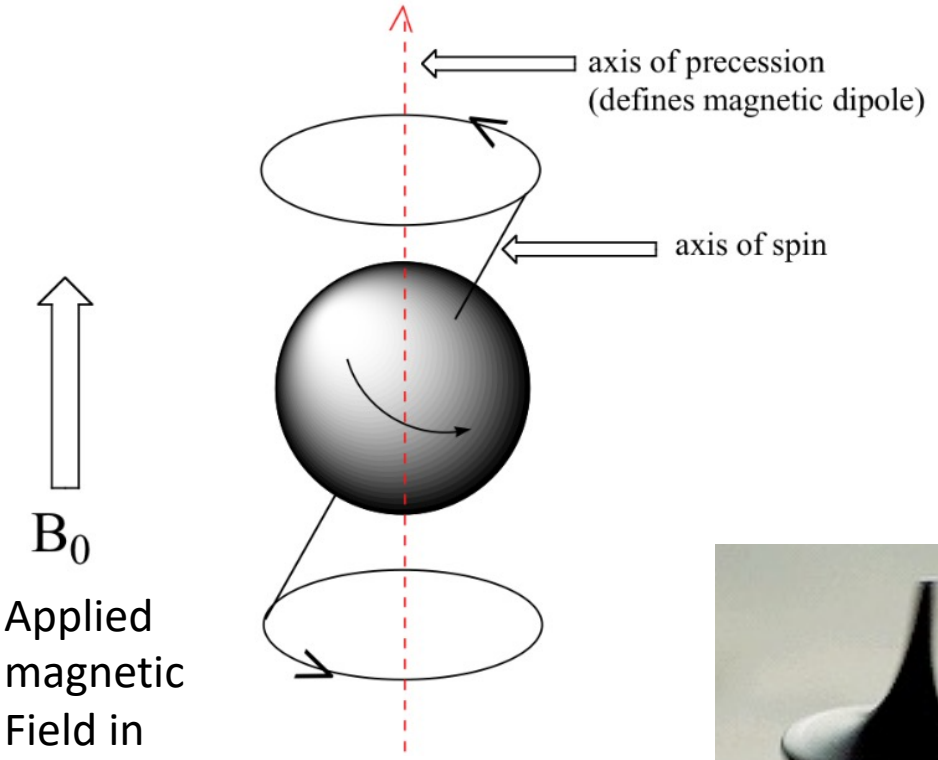


Magnetic field (B_0) strength ↑ → ΔE ↑ → larger difference b/w high and low populations (larger excess in low E state) ⟹ higher signal (sensitivity)



When we apply a radiofrequency radiation (RF) pulse, some of that energy is absorbed by spinning nuclei (must match ΔE): A corresponding frequency is needed to make the system in **resonance** state → bump the low energy nuclei to the excited state → signal derives from the nuclei returning to ground state

Proton Precessing in a Magnetic Field



Applied magnetic Field in NMR

- The magnetic axis of the proton precesses about the z axis of the stationary magnetic field B_0.
- The **frequency of precession** (ν_L) is simply the number of times per second that the proton precesses in a complete circle.

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

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

https://i.gifer.com/origin/6c/6c2404c82ee9589b2751440b1638cd2c.gif

Excitation of Bulky Spin ½ Nuclei

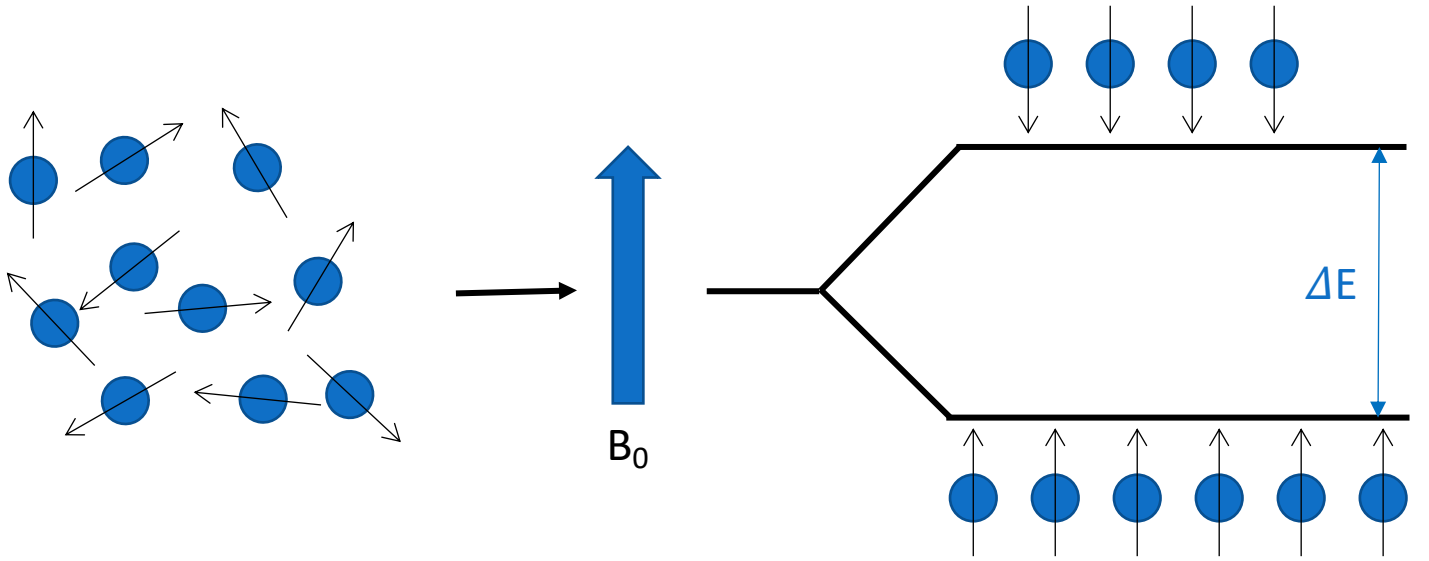
Atomic nuclei are oriented (spinning) randomly in solution

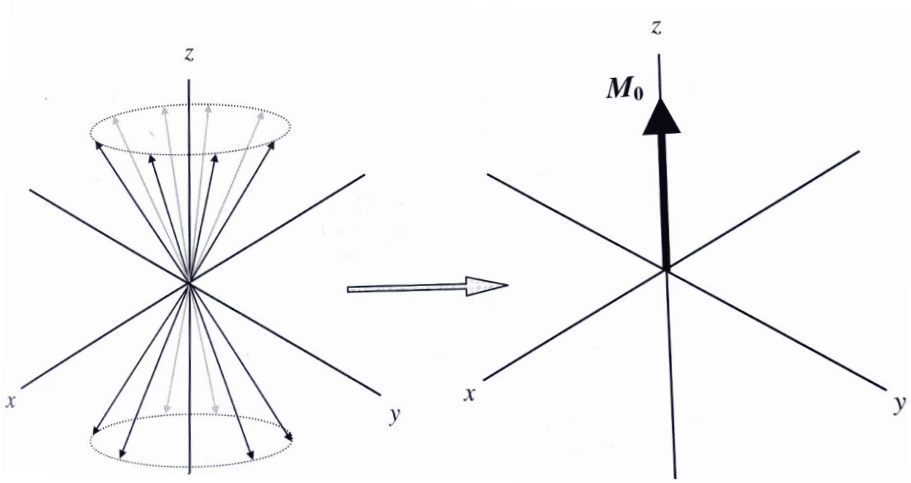
Applied magnetic field B_0 splits the population into Low and high E spins (BOLTZMAN Distribution)

- At RT, there is a tiny excess of spins in low E state

For bulk magnetization, assemblage of precessing nuclei generate the net macroscopic magnetization M_0 in the direction of B_0.

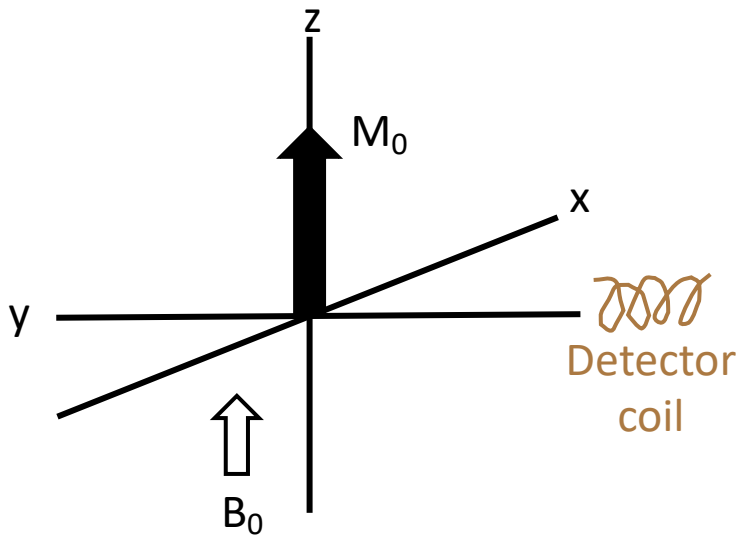
Sum of magnetic dipoles
-- **Magnetization vector**





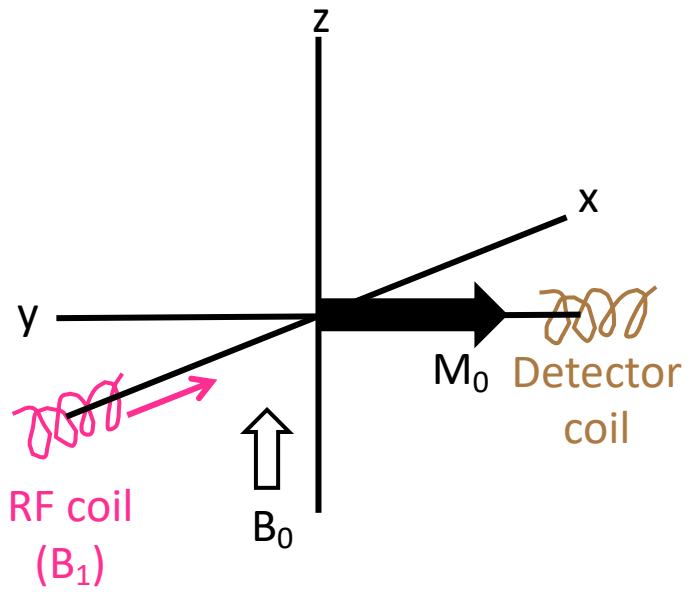
Excitation of Bulky Spin ½ Nuclei

Magnetism in line with B_0 (z by convention) is NOT detectable.



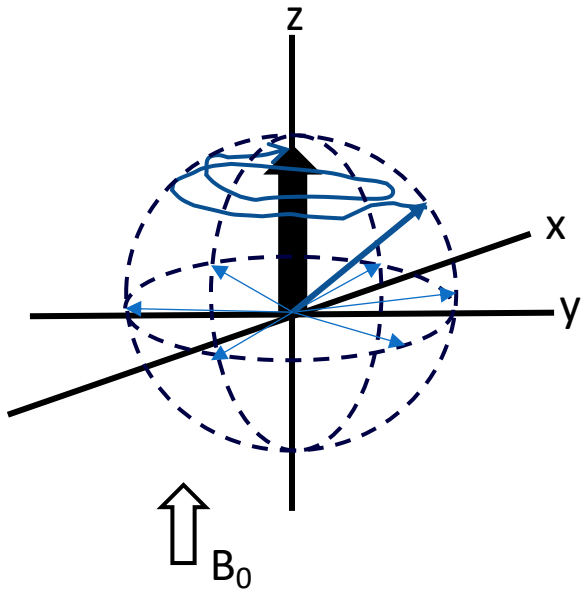
Radiofrequency (RF) pulse tips M_0 through 90° into xy plane and continue precess.

This transverse magnetism is detectable as current in a detector coil



Relaxation of Bulky Spin ½ Nuclei

This transverse magnetism then relaxes back to original orientation (along z).



- After a RF pulse is switched off, the nuclei relax to equilibrium conditions.

- 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)
  - Loss of energy is the non-radiative transition to ground state with release of heat and is described by a time constant T_1 (spin-lattice relaxation)
  - Loss of phase coherence means all magnetic vectors for the various nuclei fan out, returning spins to a random distribution of orientations. This process has a decay constant T_2 (spin-spin relaxation)

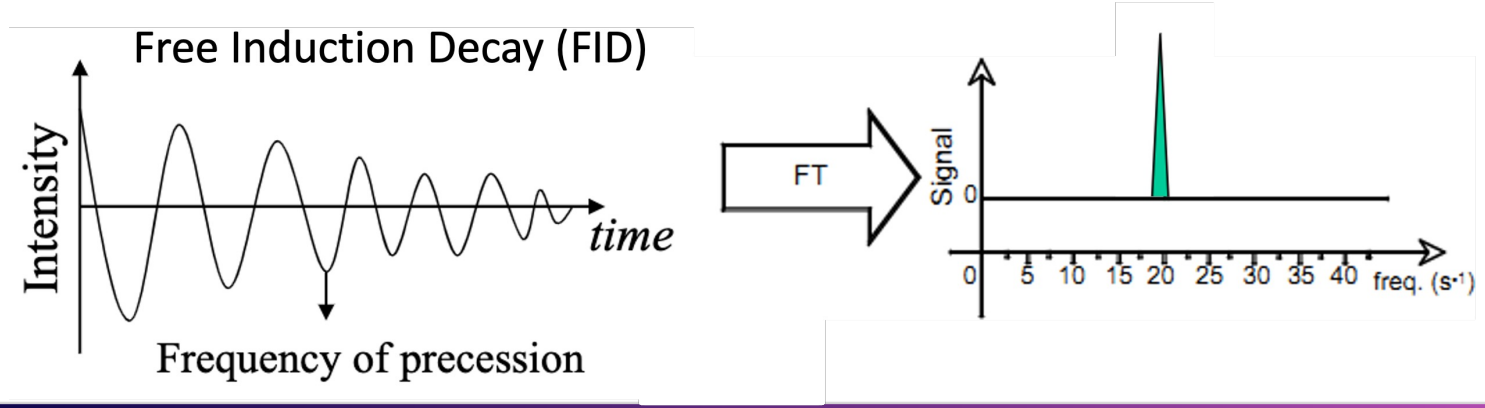
- The signal decays to zero in the xy plane due to T_2 relaxation
- The signal builds up along Z axis due to T_1 relaxation

Observing NMR Signal

- For structure determination, we are most concerned with T_2, since it is usually the shortest and determines the shape of resonance peaks
- T_2 is *inversely* proportional to the size of the molecule
  - Large, slow-moving molecules relax very quickly ⟹ Short T_2
  - Small, fast-moving molecules relax more slowly ⟹ Long T_2

- 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



Observing NMR Signal

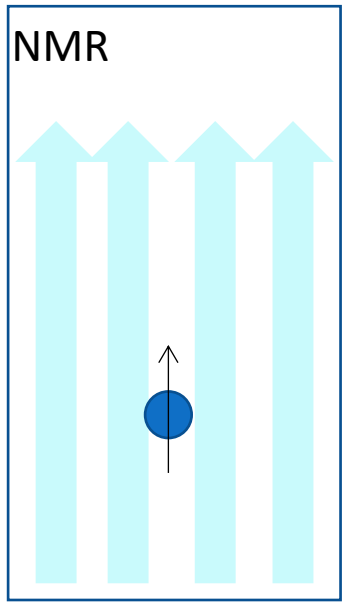
$$\nu_L = (\gamma/2\pi)B_{0,eff}$$

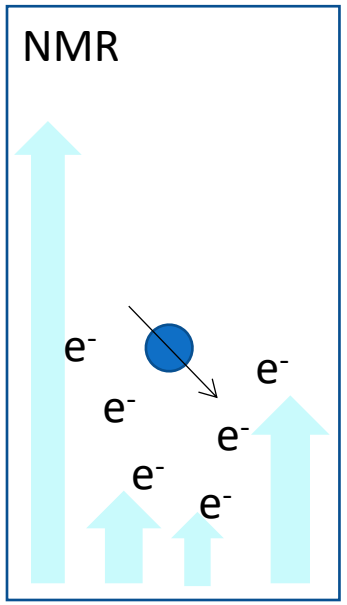
- γ is gyromagnetic ratio, which is a constant for a given isotope.
- $B_{0,eff}$ can change based on the environment.

The equation suggests that all nuclei of a given type should have the same resonance frequency. Why is this not so?

- **Atoms in the molecule affect one another by through-bond and through space interactions that alter the local magnetic field, which in turn alters the resonance frequency.**

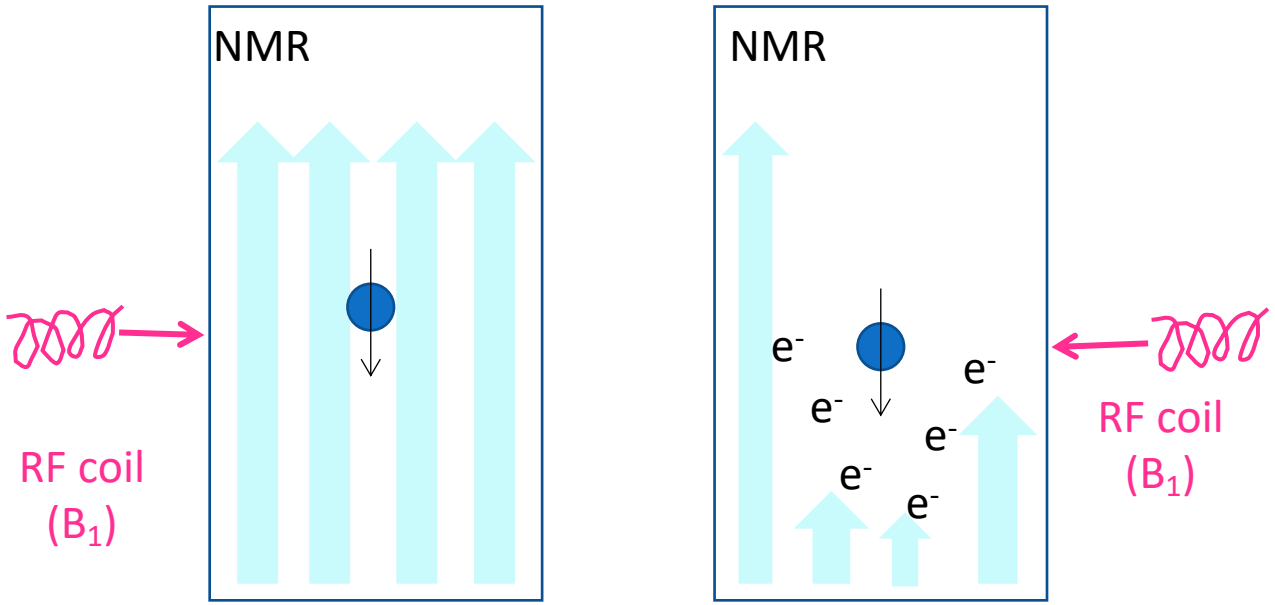
Observing NMR Signal





Diamagnetic shielding

- The electrons create their own magnetic field which opposes the applied magnetic field B_0.



De-shielded
High energy

Shielded
Low energy

- A proton with a high electron density environment experiences a lower magnetic field and therefore a lower frequency (at the right side).

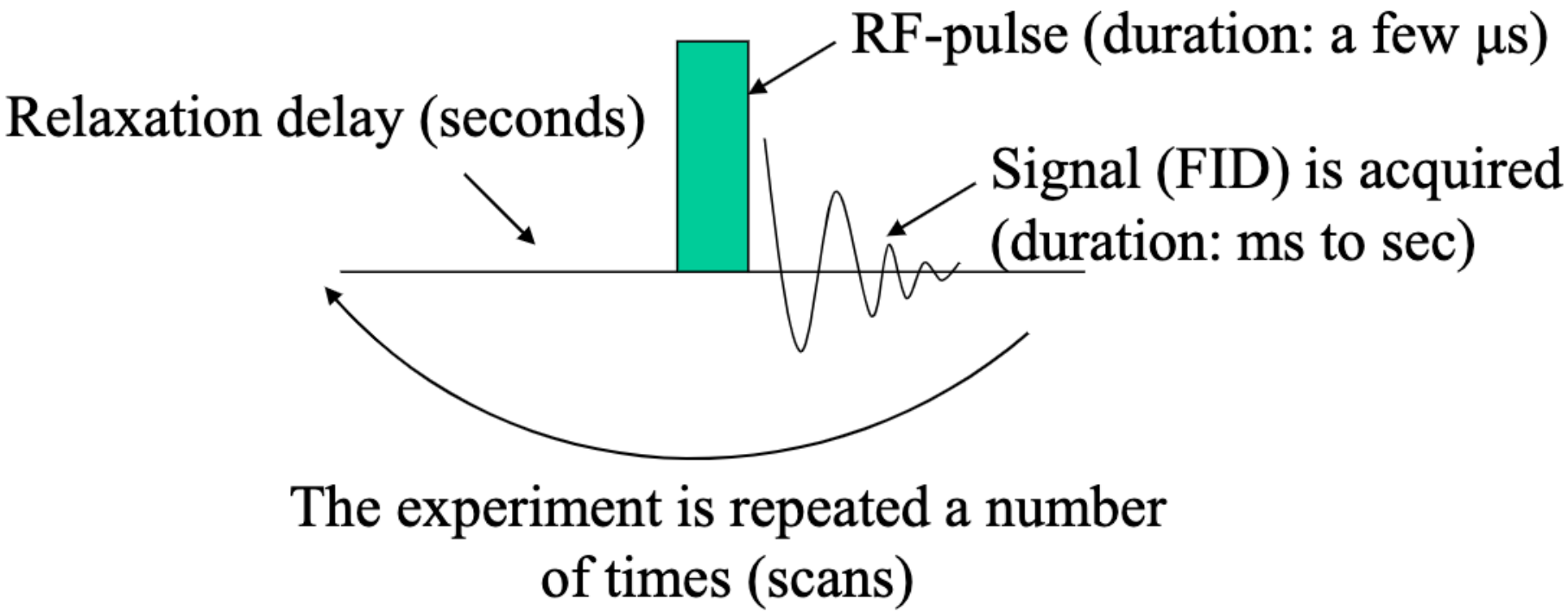
Chemical Shift

- The degree of shielding depends on the density of the circulating electrons in the **environment**.
- Because the exact resonance frequency depends on the field, and no two magnets are exactly same, it's not convenient to talk about resonance frequencies—This is why NMR data are reported as **chemical shift**.
- The frequency of each nucleus is normalized by comparison to a reference compound

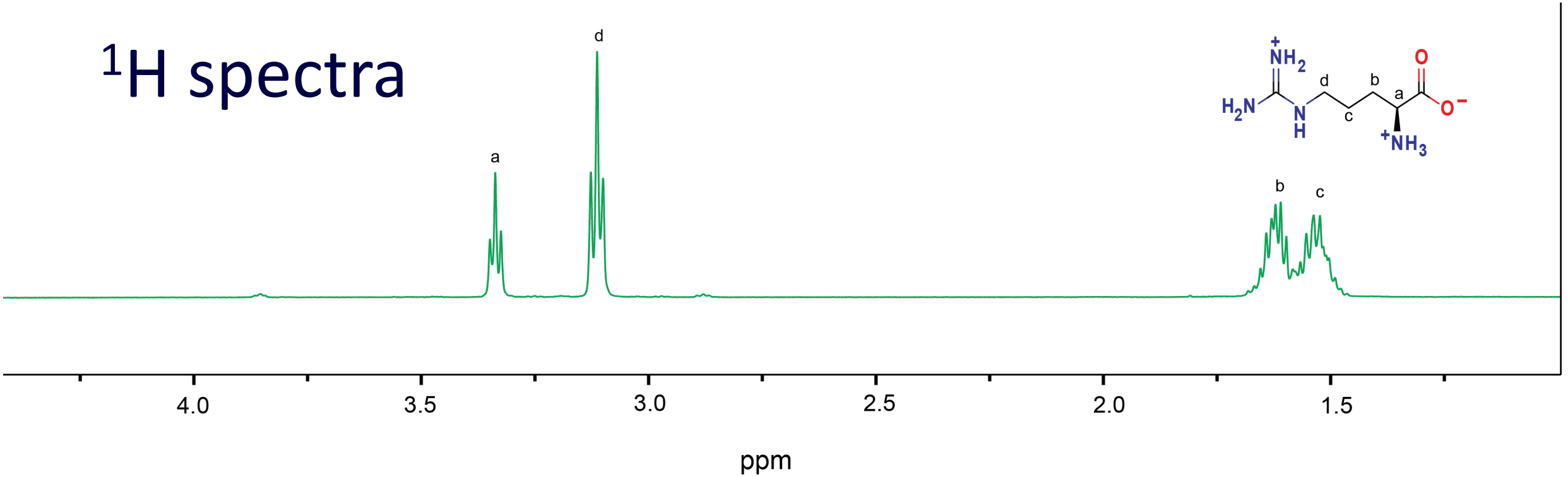
$$\delta_n = \left(\frac{\nu_n - \nu_{ref}}{\nu_{ref}}\right) * 10^6$$

  - The chemical shift (δ) of nucleus n is expressed by **parts per million (ppm)**

1D Pulse Sequence

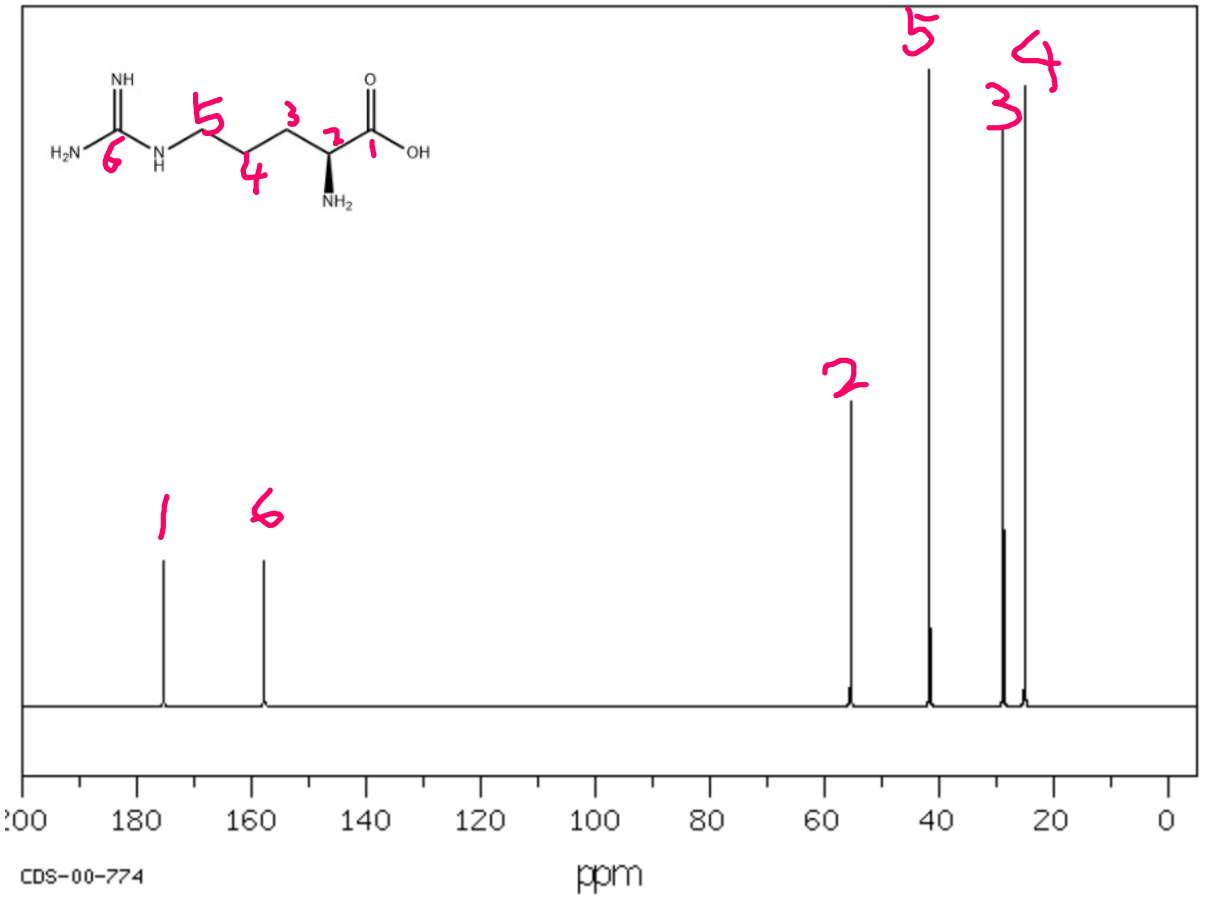


^{1}H spectra



- **Position of signals**: indicates what type of H the molecule contain
- **Number of signals**: indicates the number of different types of H in the molecule
- **Intensity of signals**: indicates the relative amounts of each kind of H in the molecule
- **Spin-spin splitting of signals**: gives further information about the neighboring environment for the various H in the molecule
  - In many cases, spin-spin splitting is given by the N+1 rule.

^{13}C Spectra



- ^{13}C spectra are **easier** to analyze than ^{1}H spectra because the signal splitting can be avoided.
- Each type (non-equivalent) of carbon atom appears as a single peak with ^{1}H-decoupling.
- NMR absorptions occur a broader range (0-220 ppm) than ^{1}H spectra (usually 0-10 ppm).

Example： 1D Proton NMR of a Ubiquitin

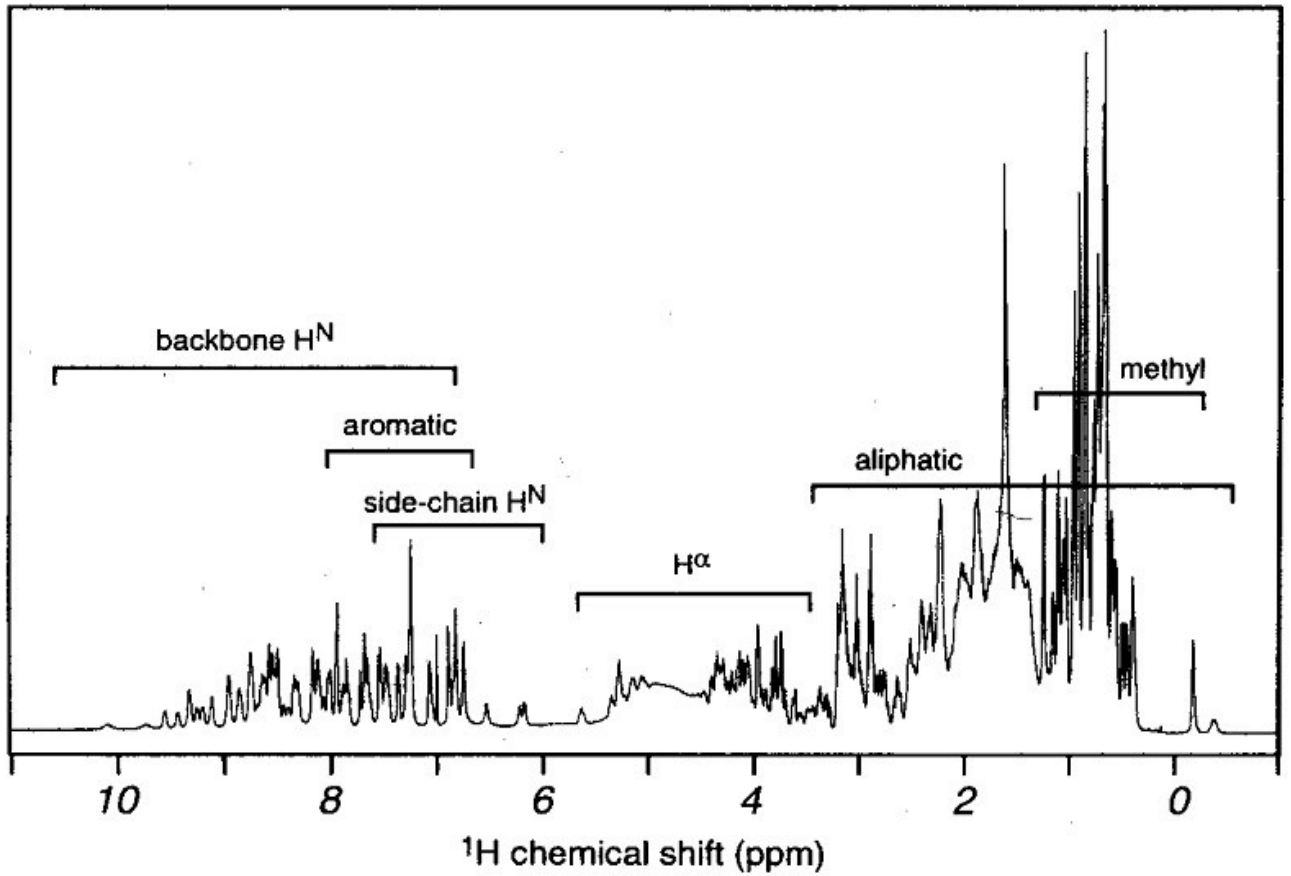


FIGURE 2. ^{1}H chemical shift positions of chemical groups in ubiquitin [1] (from: Cavanagh *et al.*: Protein NMR Spectroscopy).

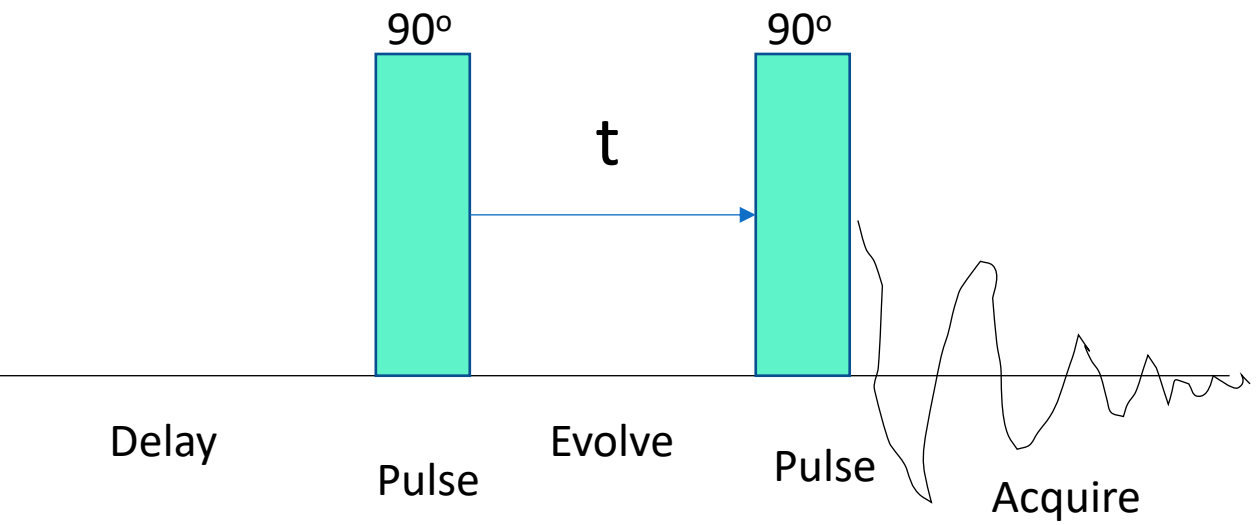
As the size of the molecule increases, because of the effect T_2 on the resonance peaks, so you will get a large number of broad and smeary peaks, which make it difficult (impossible) to assign them to particular nuclei.

↓

Multi-dimensional NMR

2D Pulse sequence

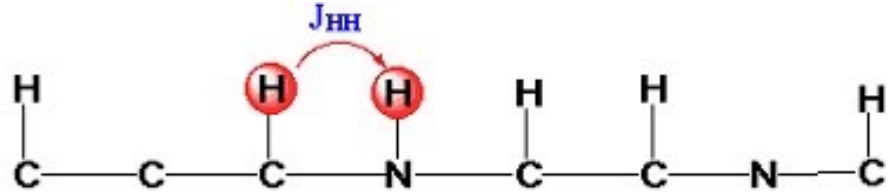
Generalized homonuclear 2D experiment



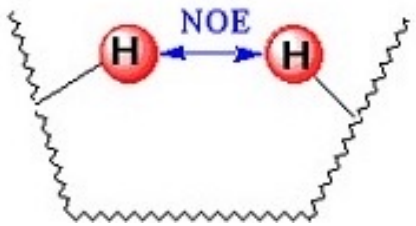
- In the simple homonuclear 2D NMR experiment, the precessing nuclei are allowed to relax for some time period (t) before being hit with a second RF pulse, during which magnetism is transferred between nuclei.
  - By collecting many spectra varying t from 0 to n, one can expand the peaks in the 1D spectrum out over a second dimension, allowing a 100-fold improvement in resolution.

- Magnetization is transferred between nuclei in two ways:

1. **Scalar (or 'J') coupling** acts <u>through bonds</u> and gives information about nuclei connected by 3 or fewer bonds (COSY or ^{1}H-^{1}H correlated spectroscopy).

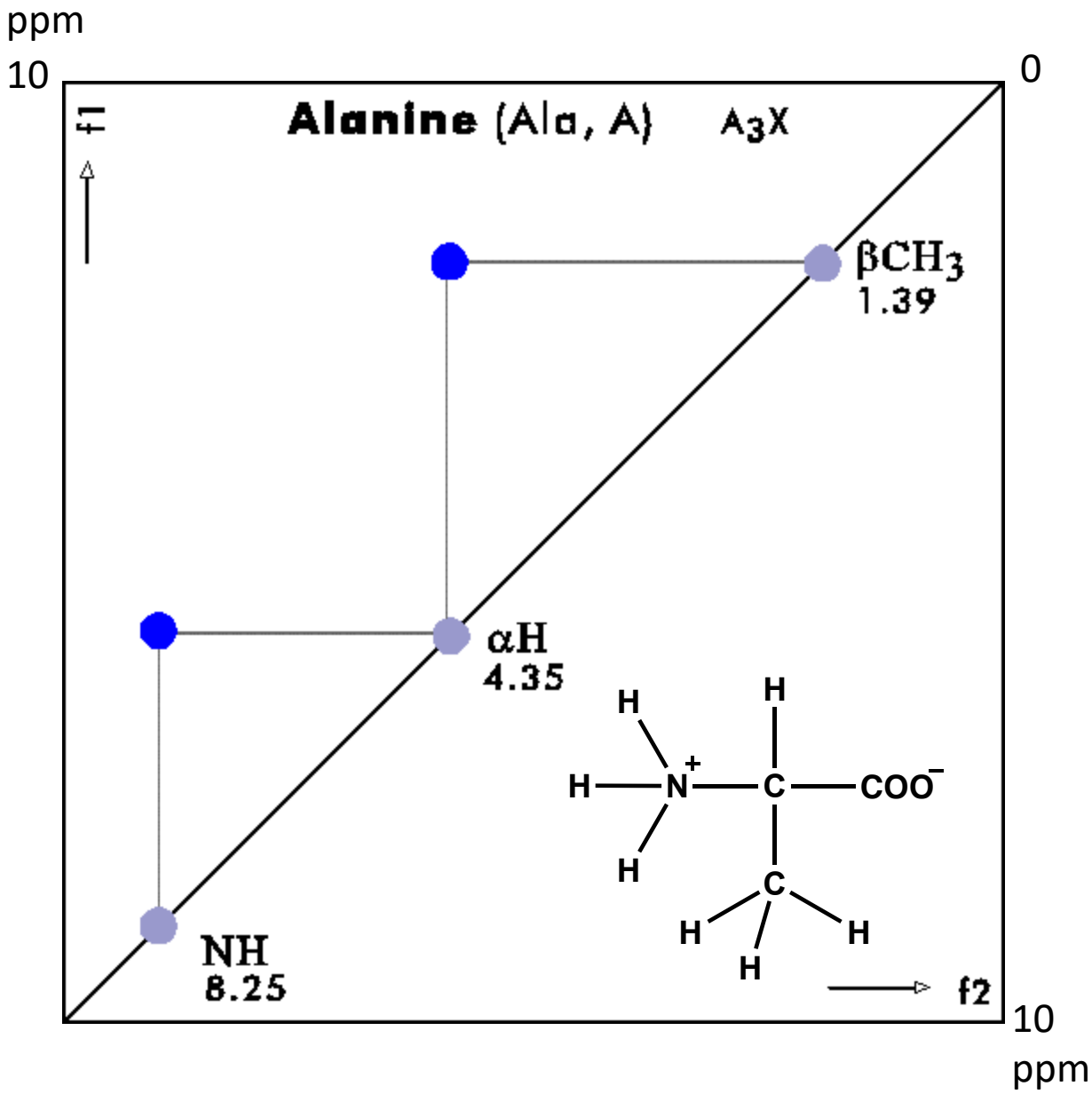


2. **Nuclear Overhauser Effect** (NOE)– nuclei affect each other <u>through space</u> (dipole-dipole interaction) and give information about how nuclei are arranged in space (must be with 5.5 Å of one another)

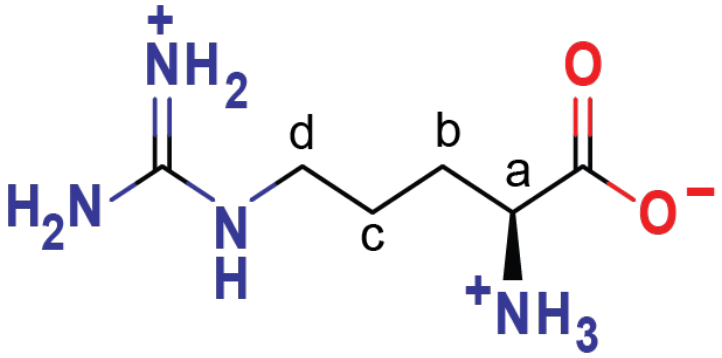


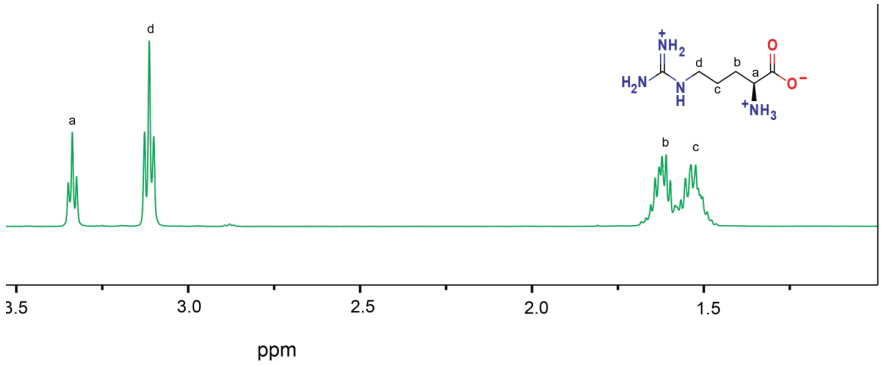
COSY

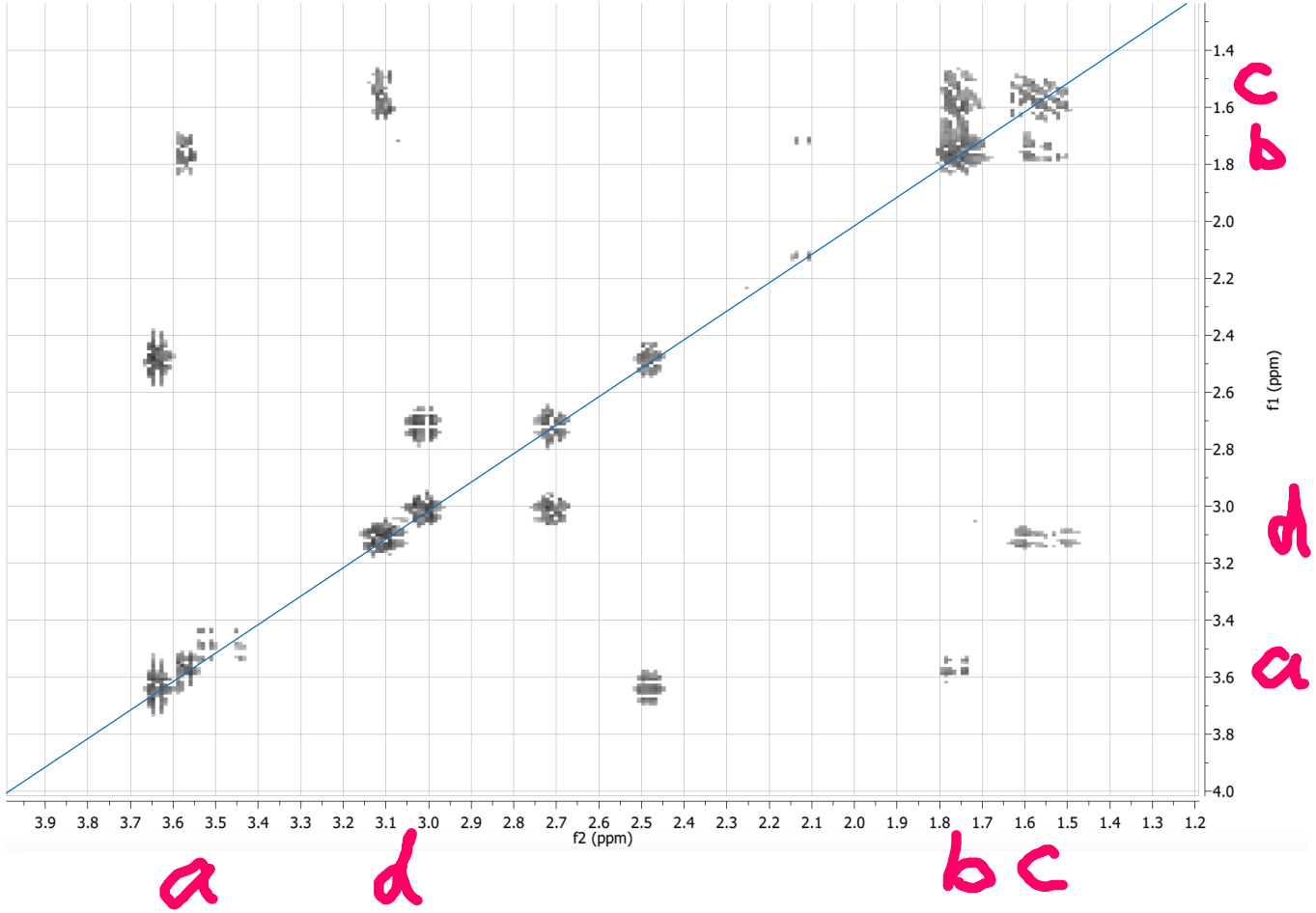
- Magnetization is transferred by scalar (J) coupling
- Only signals of protons which are two or three bonds apart are visible
- Phi torsion angle of the protein backbone can be derived from the ^3J coupling constant between $\mathbf{H_N}$-$\mathbf{H_\alpha}$ ^3J coupling.



COSY spectrum of a mixture containing Arg.

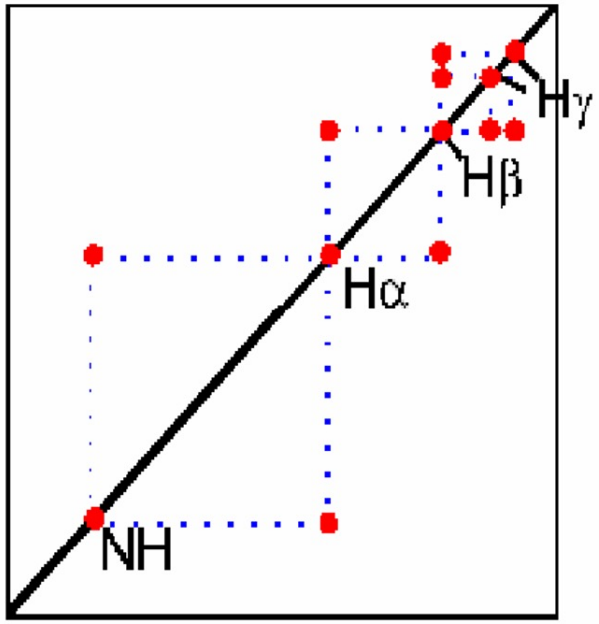






Homonuclear NMR

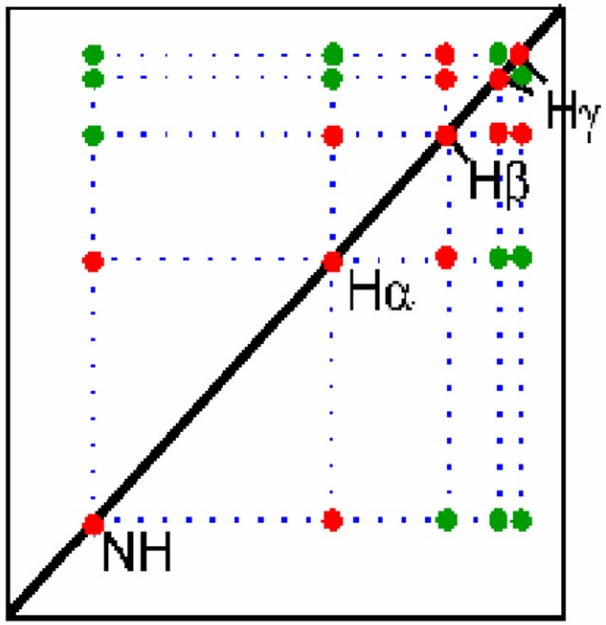
- ^{1}H-^{1}H COSY:



- NOSEY:

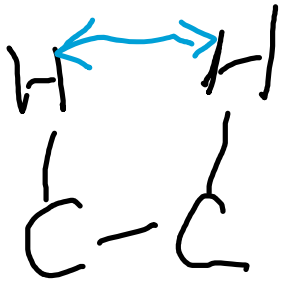
  The intensity of the NOE is in first approximation proportional to $1/r^6$, with r being the distance between protons

- TOCSY:

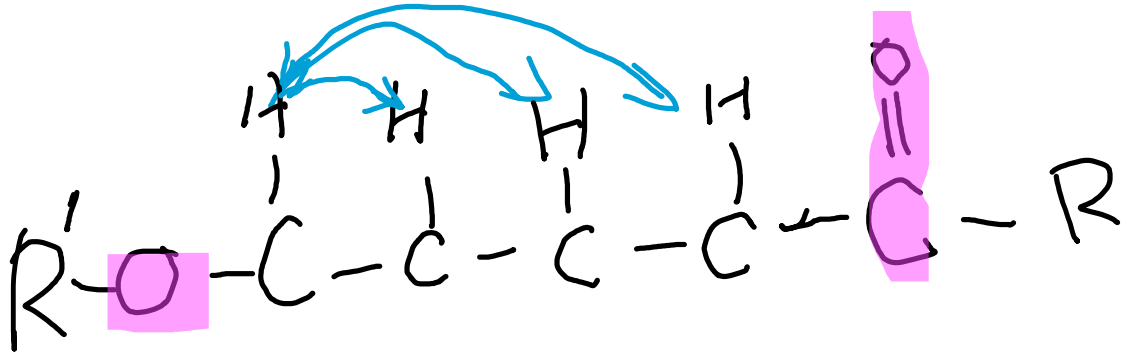


TOCSY (**TO**tally **C**orrelated **S**pectroscop**Y**)

- Goal: to relay or transfer magnetization beyond the directly coupled spins thus enabling us to see correlations among nuclei that are not directly coupled but within the same spin system.
- The 2D-TOCSY resembles all aspects of COSY, but the different is the cross peaks arise from relayed coherence transfer.



COSY

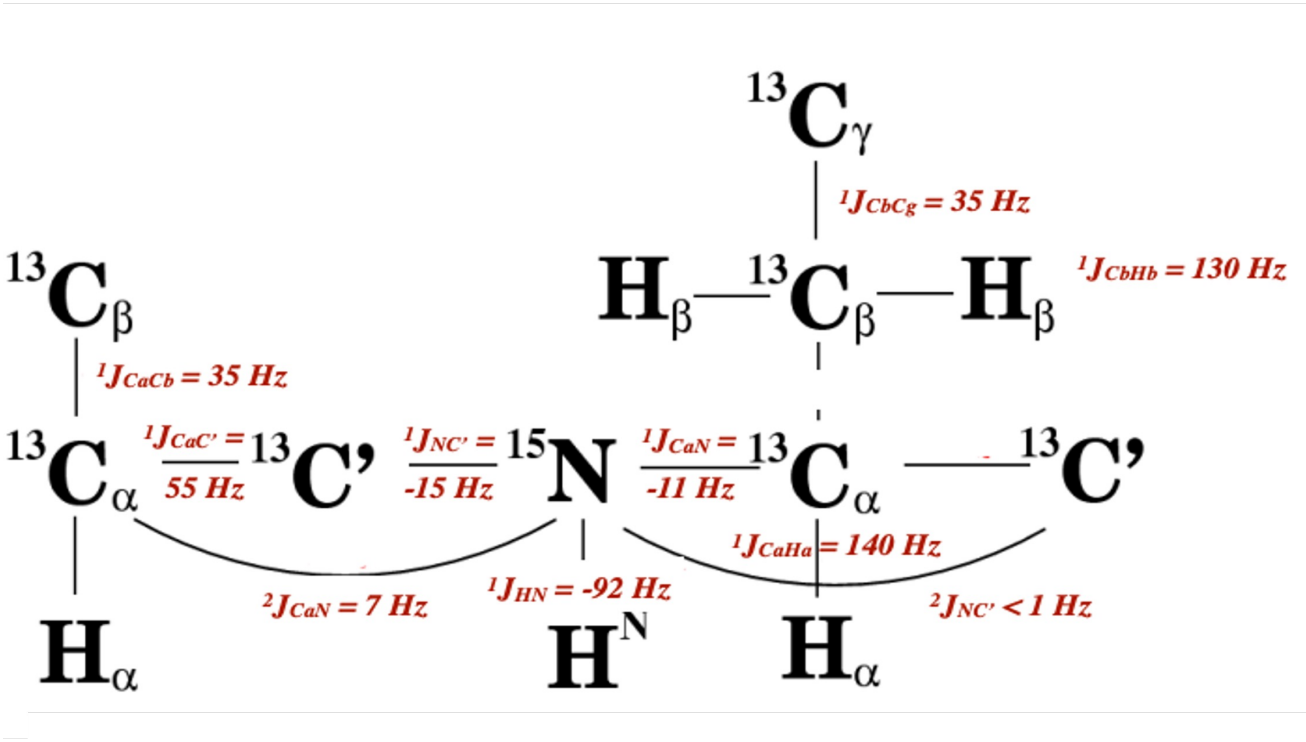


2D-TOCSY

Heteronuclear NMR

- In heteronuclear NMR, the much larger shifts associated with ^{1}J coupling help spreading the spectrum so that peaks can be resolved
  - ^{1}H coupling: 0-10 ppm
  - ^{15}N coupling: 100-135 ppm
  - ^{13}C coupling: 0-180 ppm

- Measure frequencies of different nuclei (^{1}H, ^{13}C, ^{15}N)
- No diagonal peaks
- Mixing not possible using NOE (through space), only via **J** (through bond)



Heteronuclear Single Quantum Coherence (HSQC)

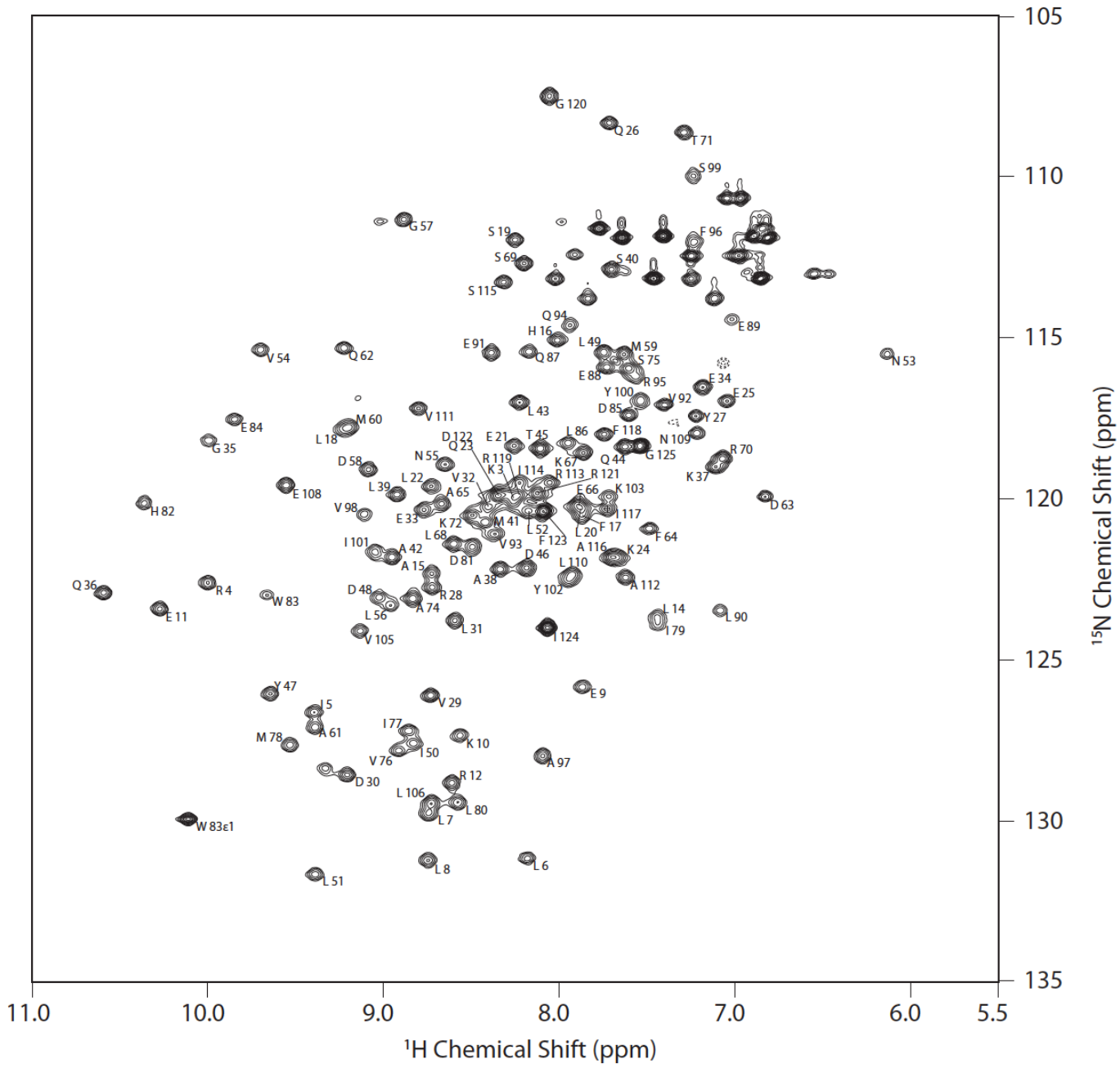
- HSQC experiment is one of the fundamental in multidimensional heteronuclear NMR experiments.
- The HSQC experiment correlates chemical shift of one nuclei to another through bond (J coupled)

- ^{1}H-^{13}C HSQC
- ^{1}H-^{15}N HSQC

- **^{1}H-^{15}N HSQC** Spectrum: "protein fingerprint"

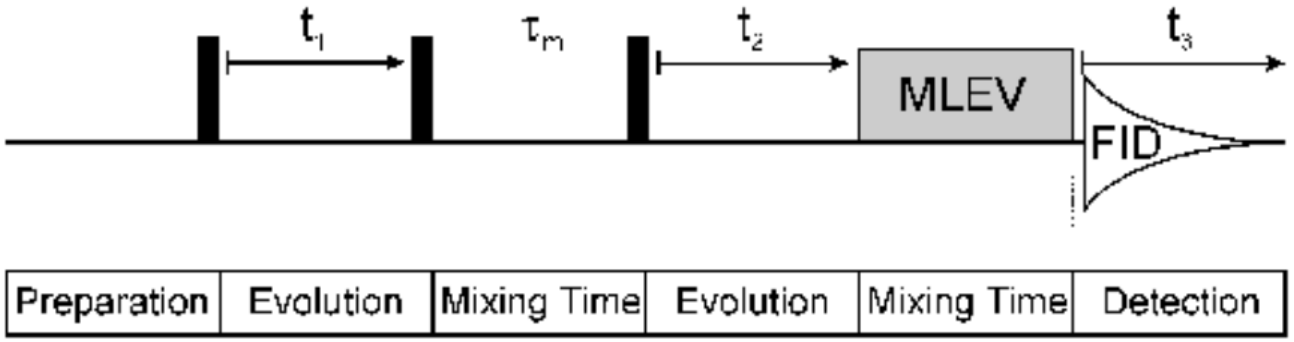
  -- can be used to monitor structural changes due to ligand binding or solution conditions, etc.

  For high sensitivity, protein should be isotope labeled with ^{15}N.



2D spectra (like NOESY or TOCSY) of larger proteins are often crowded with signals. Therefore, these spectra are spreading out in a third dimension (usually ^{15}N or ^{13}C), so that the signals are distributed in a cube instead of a plane.

3D NMR

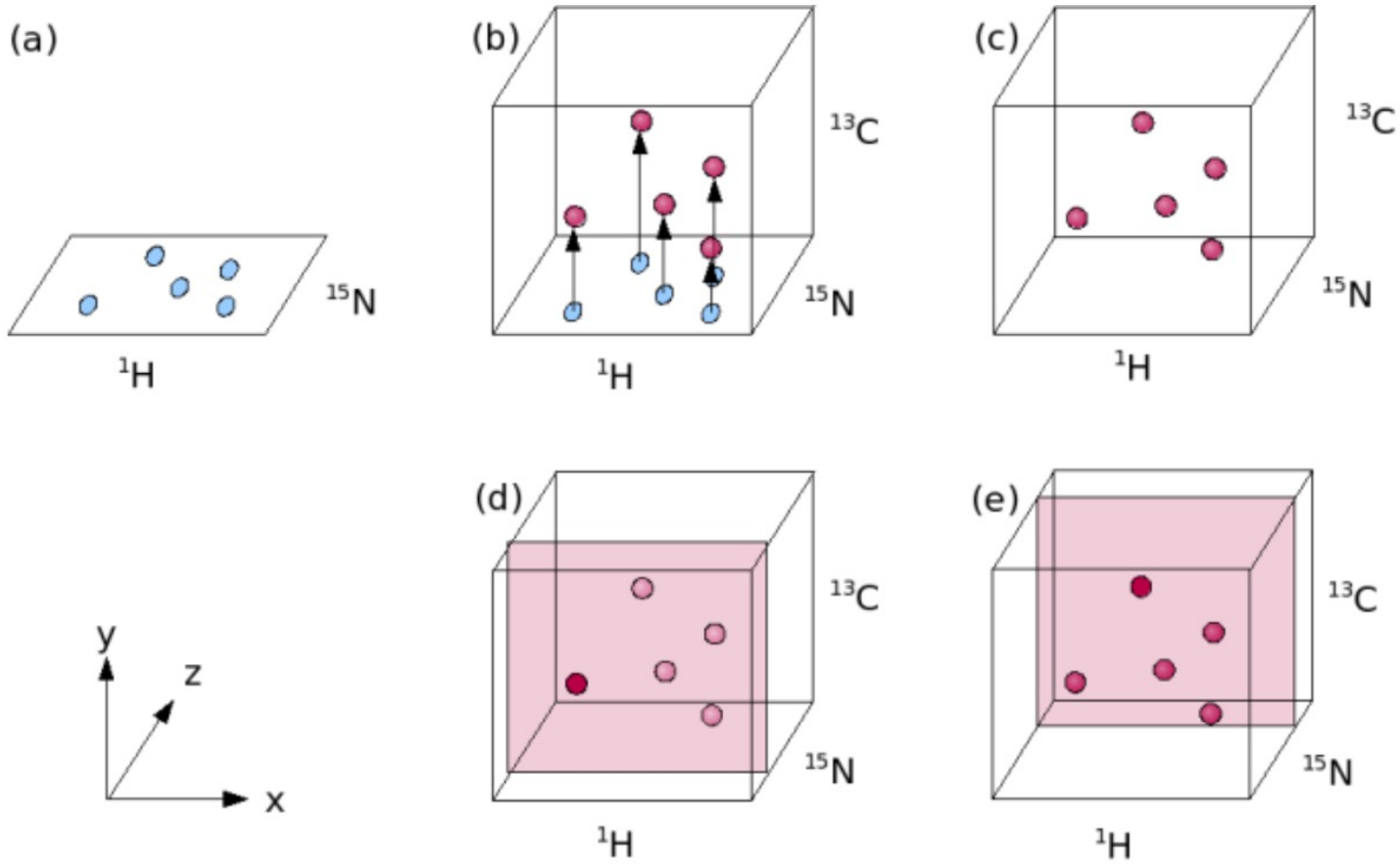


- In 3D experiments, two different 2D experiments are combined so that three frequency coordinates are involved.
- In general, the 3D experiment may be made up of the preparation, evolution (t_1), and mixing periods of the first 2D experiment, combined with the evolution (t_2), mixing, and detection (t_3) periods of the second 2D experiment.
  - t1 and t2 are incremented separately
- The 3D signals are, therefore, recorded as a function of *two* variable evolution times, t_1 and t_2, and the detection time t_3.

Visualizing 3D Spectra

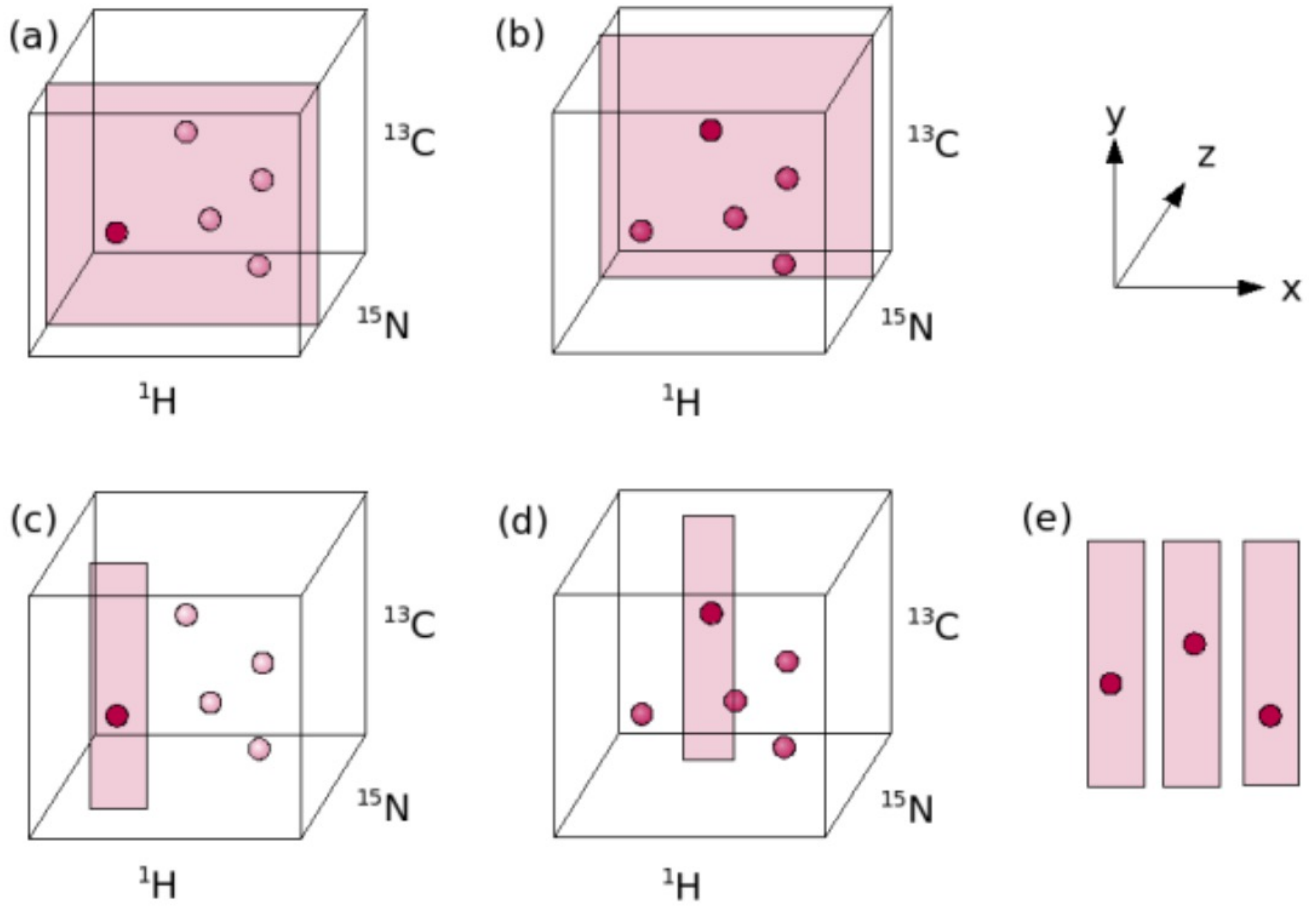
- 3D experiments are generally based upon 2D experiments and so the easiest way to think of a 3D is of a 2D which is then extended into a third dimension.

- For example,
  - x-dimension: ^{1}H
  - y-dimension: ^{13}C
  - z-plane: ^{15}N
- So essentially you end up looking at a ^{1}H-^{13}C 2D spectrum at varying places along the ^{15}N dimension



Visualizing 3D Spectra

- A common way of visualizing 3D spectra is as so-called **Strips**.
- If you start with an HSQC where each peak is defined by a ^{1}H and ^{15}N value, you can then pick out strips for each HSQC peak and then lay them next to one another for easy comparison (e). For protein backbone assignment this is particularly useful.



- There are two principal classes of 3D NMR experiments:
  1. Experiments consisting of **two 2D NMR experiments**
     NOSEY-HSQC, TOCSY-HSQC

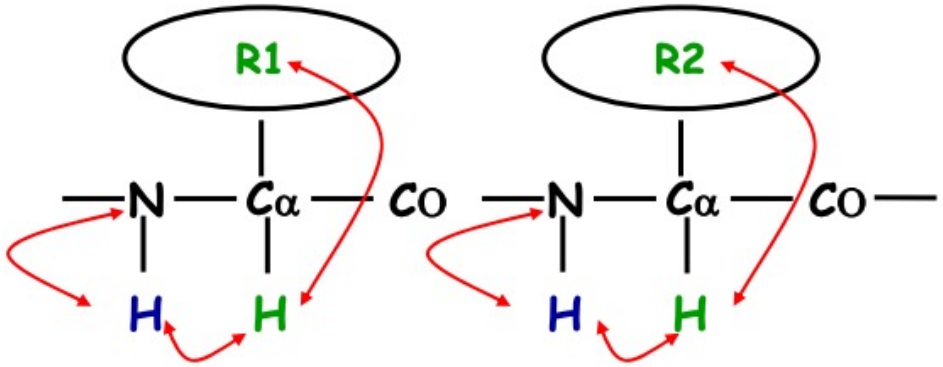
  2. The **triple resonance experiments**
     -- Sequential assignment of larger proteins (> 150 AA)
     -- Three nuclei are correlated
     -- Performed on doubly labelled (^{13}C, ^{15}N) proteins

- Advantages:
  - only a few signals on each frequency
  - high sensitivity which is caused by an efficient transfer of magnetization (^{1}J and ^{2}J coupling)

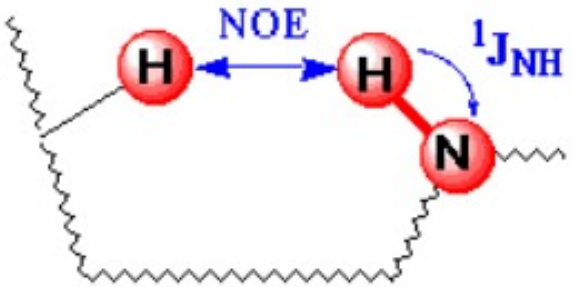
- Disadvantage:
  - Doubly labelled proteins $\Longrightarrow$ expensive

Two 2D NMR Experiments

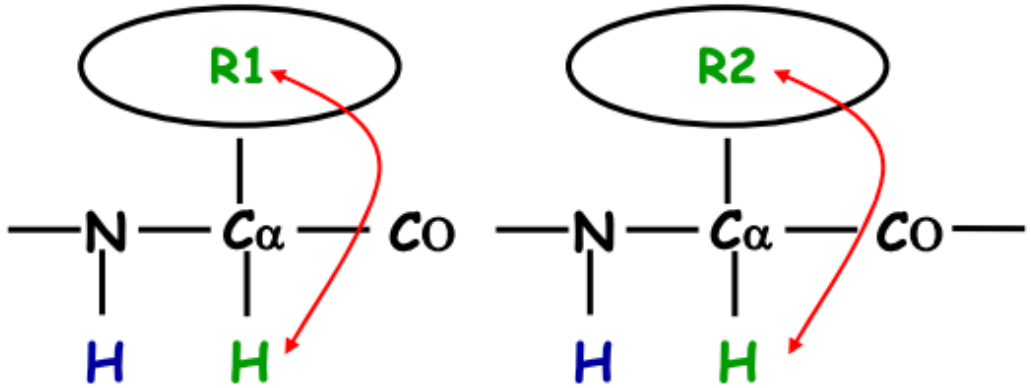
- ^{1}H-^{15}N-^{1}H: 3D TOCSY-HSQC



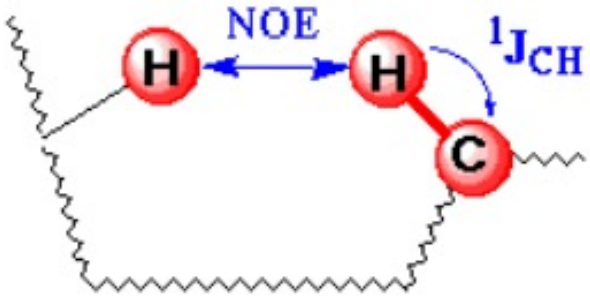
- 3D ^{15}N-edit NOESY-HSQC



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



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



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

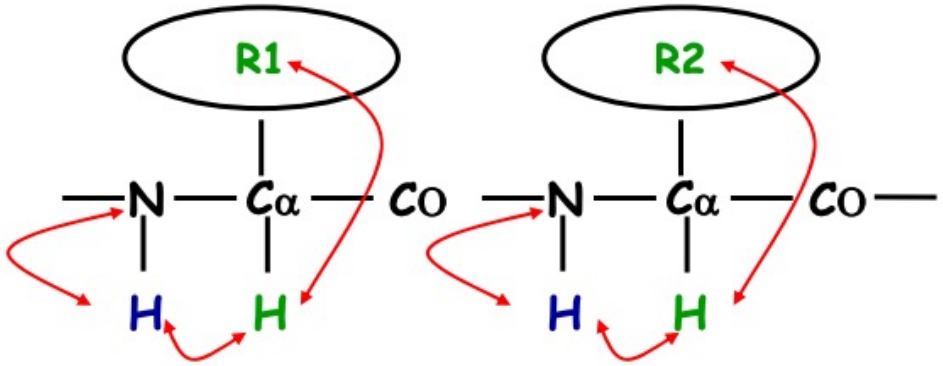
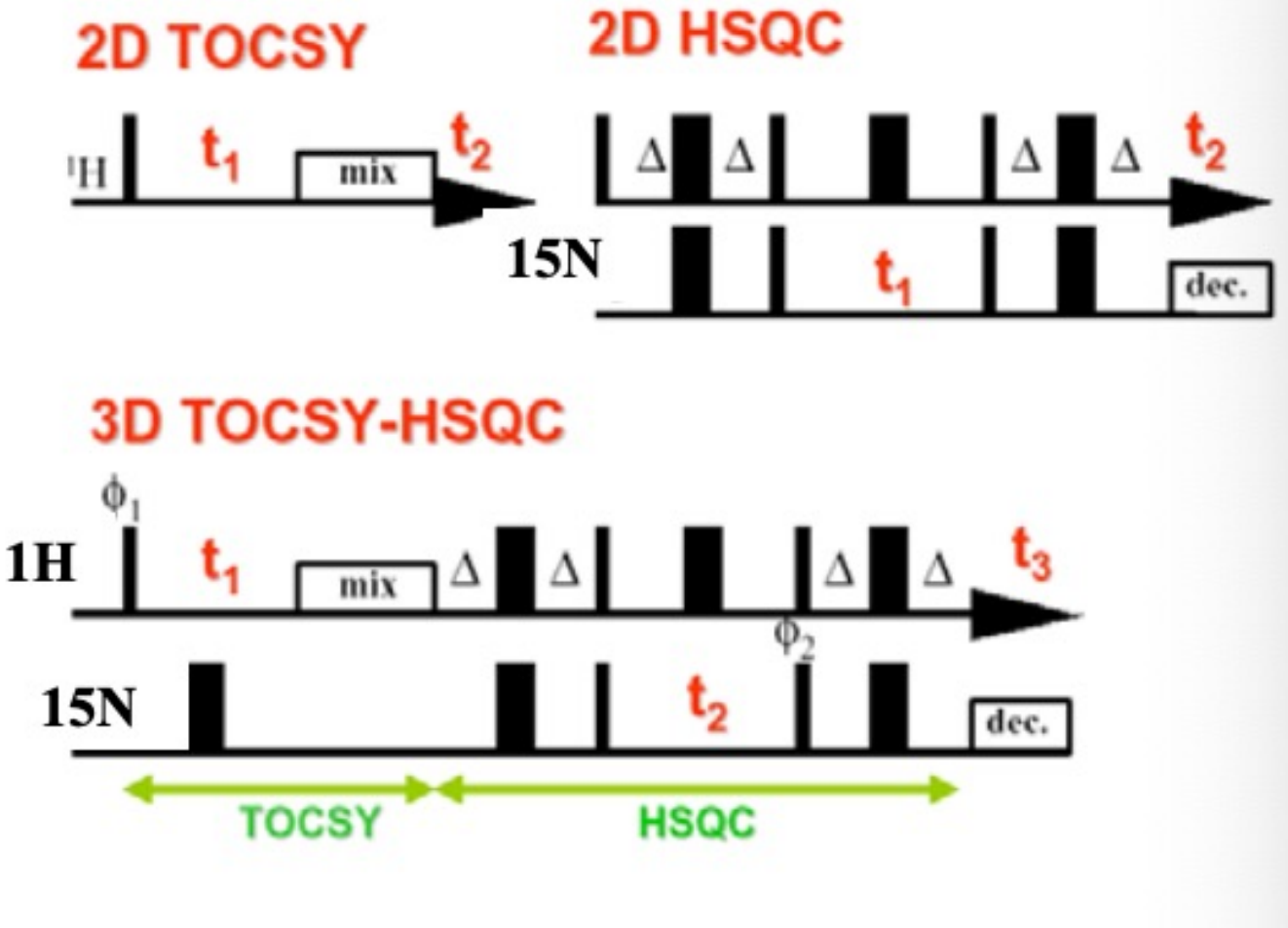


Fig. 1. The 3D pulse sequence.

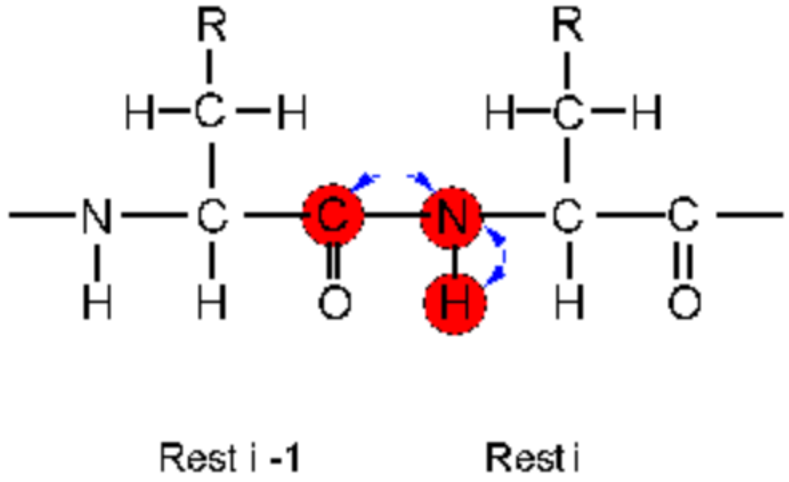


Triple Resonance Experiments: HNCO Experiment

- 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

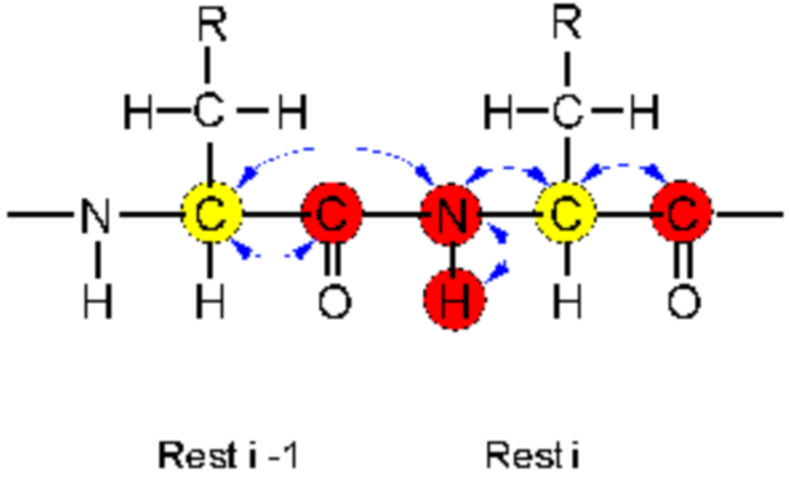
$^{1}H^{N}_{i}$ - $^{15}N_{i}$ - $^{13}CO_{i-1}$



Triple Resonance Experiments: 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) are detected.
  - The C_α atom (yellow) acts only as relay nucleus, its frequency is not detected

- The amide proton is correlated with the CO atom of both the preceding residue and the intra-residue.



HN(CA)CO

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

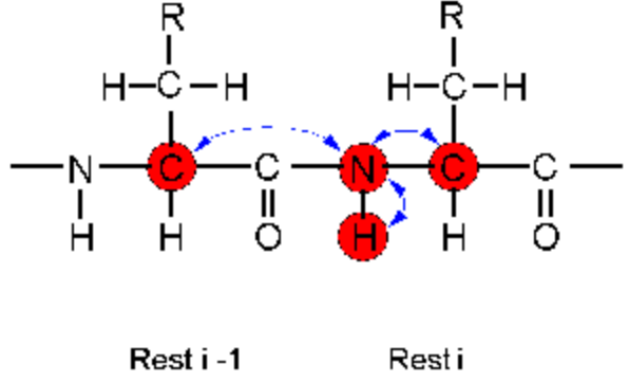
$^{1}H^{N}_{i}$ - $^{15}N_{i}$ - $^{13}CO_{i-1}$

Triple Resonance Experiments:
HNCA Experiment

HNCA

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

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



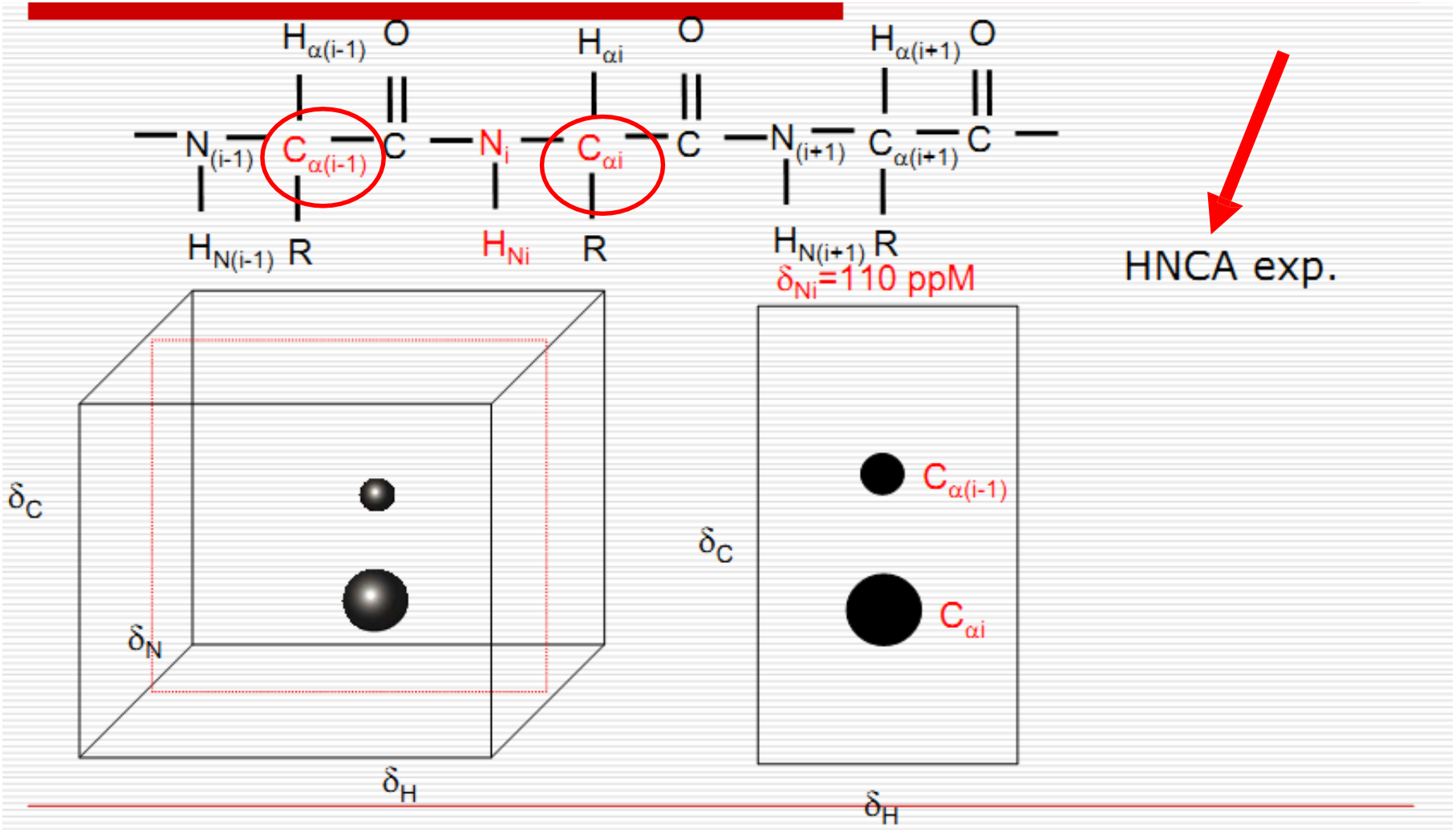
- The HNCA experiment is the prototype of all triple resonance experiments.
  - 1st dimension: The magnetization of H_N is transferred to N
  - 2nd dimension: magnetization is transferred from N→ C_α (on both i and i-1)
  - 3rd dimension: magnetization is transferred back to the amide proton H_N
- The 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

Assignment of spectra

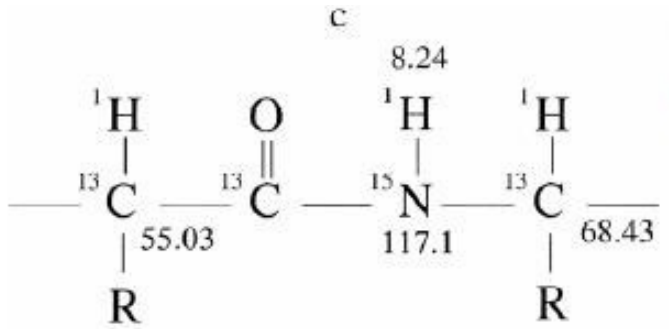
- The aim of the analysis of NMR spectra is to extract all available information about interatomic distances and torsion angles.
- Initial stage: Assign each resonance to a specific nucleus
  - Backbone assignment
  - Side chain assignment
  - NOE based assignments for structure determination

1. Backbone Assignment

- Let's start from: alpha-Carbon (CA)

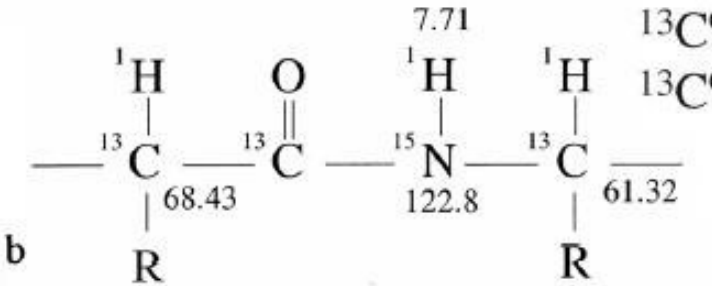


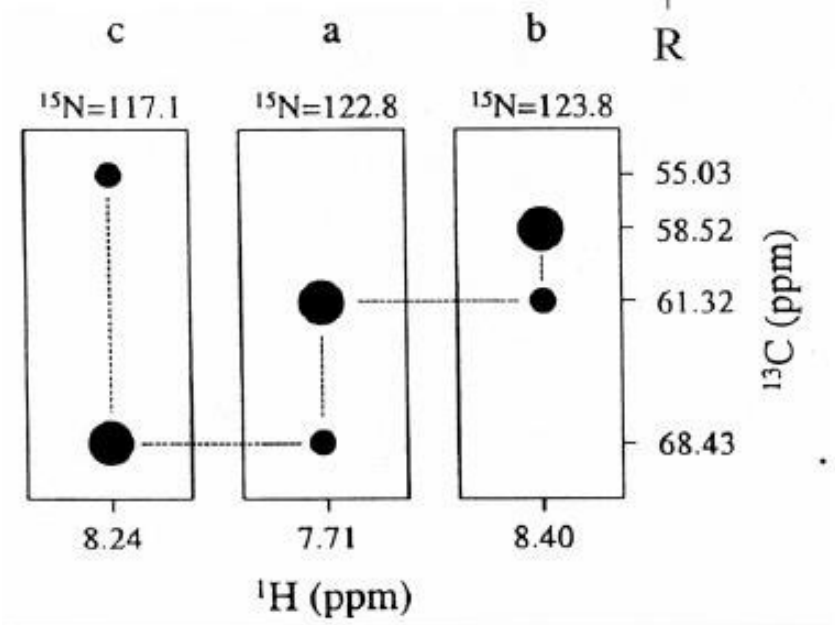
Linking the dots from every NMR

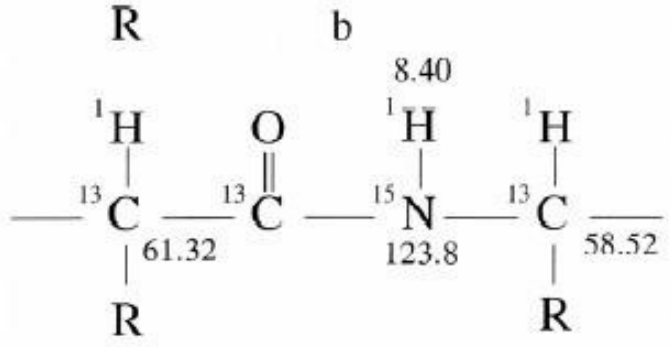


• link the correlated shifts numerically….

	c	a	b
$^{1}H^{N}_{i}$	8.24	7.71	8.40
$^{15}N_{i}$	117.1	122.8	123.8
$^{13}C^{\alpha}_{i}$	68.43	61.32	58.52
$^{13}C^{\alpha}_{i-1}$	55.03	68.43	61.32

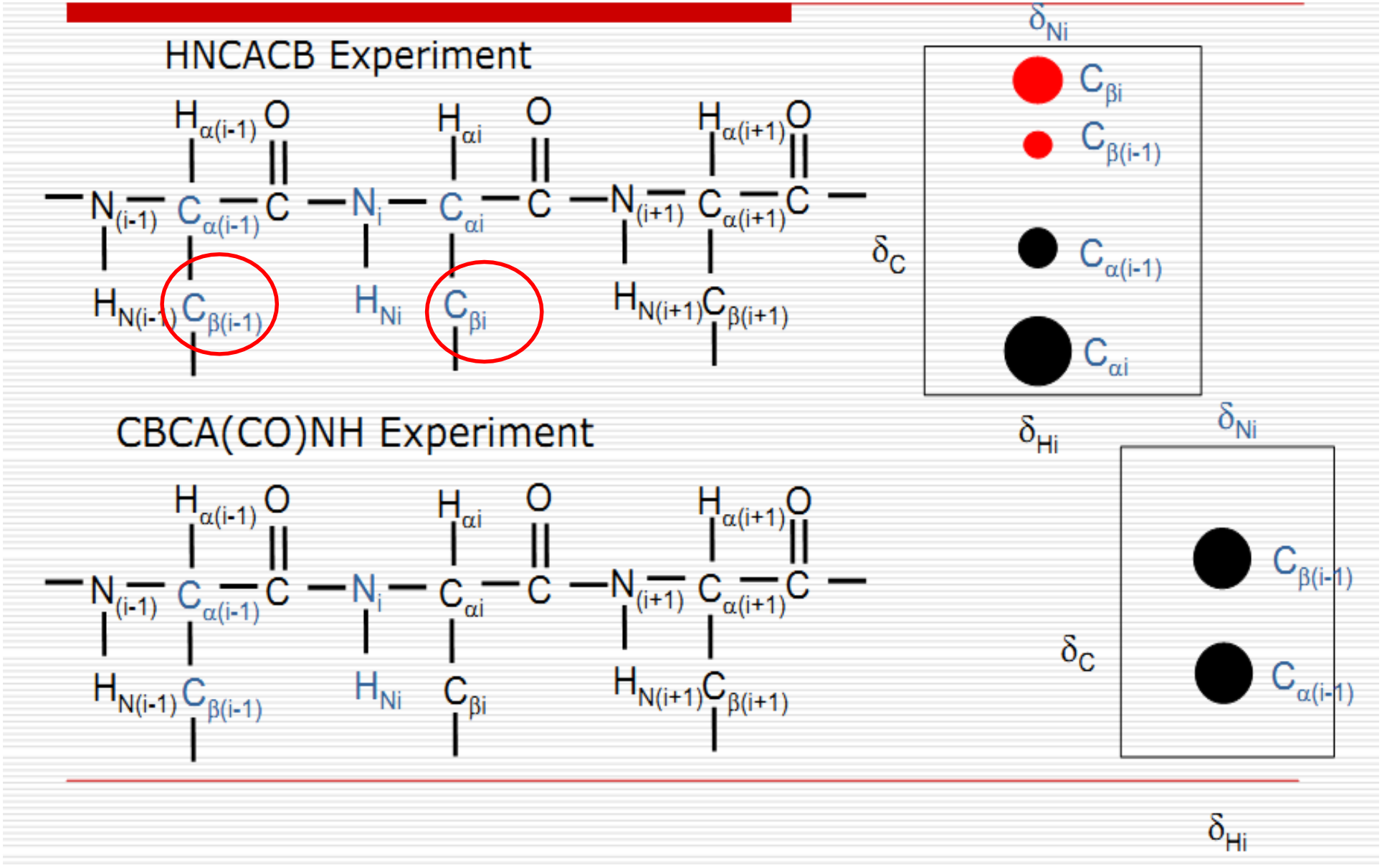






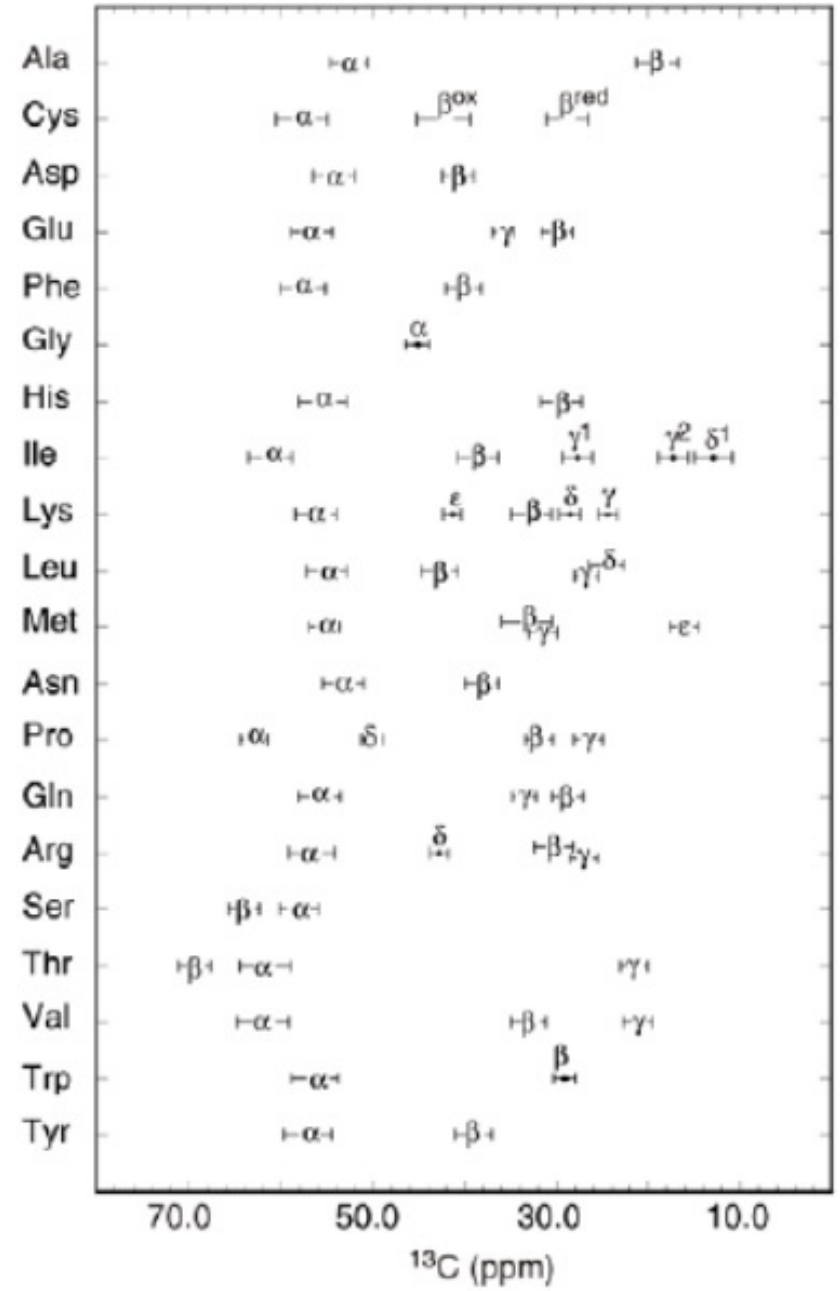
…. or visually

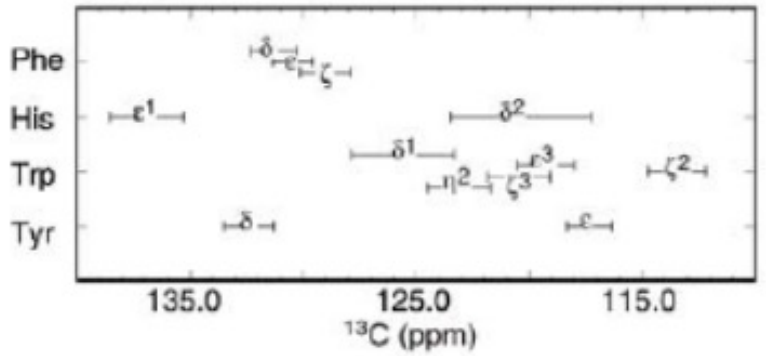
2. Side Chain Assignment



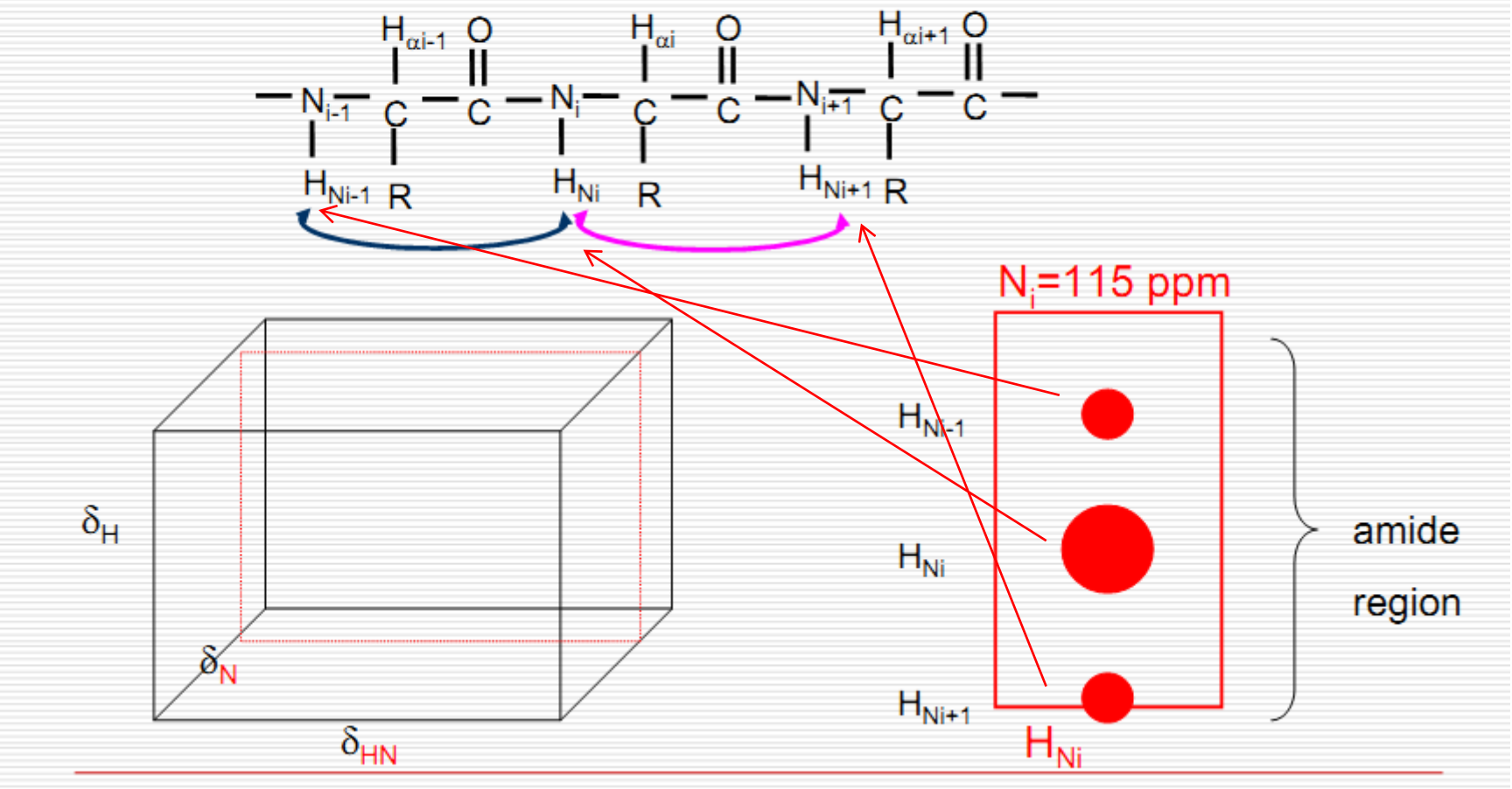
- The Cα and Cβ chemical shifts adopt values characteristic of the amino acid type.

- ^{13}C chemical shifts are amino acid specific





3. NOE-based Assignments for Structure Determination



Combining all the results found from above experiments protein 3D structure can be determined.

Step-by-Step: How to Construct a 3D structure (not necessary in sequence)

(1) **Assign each resonance in the ^{1}H-NMR spectrum to a specific hydrogen** nucleus by COSY, which established 'connections' between protons made through covalent bonds.

(2) **Assign peptide backbone** (**carbon**) by HNCO, HNCA and HN(CO)CA: used together to allow the assignment of all the carbon, hydrogen and nitrogen resonances in the backbone of a protein (**Remember to label protein with C13**!).

(3) **Assign peptide backbone** (**amide**) by HNCO, HNCA, HN(CO)CA: (**Remember to label protein with N15**!)

(4) **Determine the side chains** by NOESY (probes 'connections through space): identifies protons which are brought into close proximity to one another even though these protons may be quite distant in the linear sequence.

(5) Given sufficient NOE-derived '**distance constraints**', it then became possible to reconstruct the three dimensional shape of the protein.

(6) **Computer converts large number of NOE-derived distance restraints into energy terms**, and then **minimise the energy** of the system as a whole.

(7) Such an approach typically results in an **ensemble of structures**.

(8) These structures overlap in highly ordered regions of the interior of the protein, but there is less convergence in more disordered regions on the surface.

Secondary Structure

- the torsion angle phi calculated by the coupling constant $^{3}J\ H_N\text{-}H_\alpha$

right-handed alpha helix: phi = -57, $3JH_AH_N$ = 3.9 Hz
right handed 3.10 helix: phi = -60, $3JH_AH_N$ = 4.2 Hz
antiparallel beta sheet: phi = -139, $3JH_AH_N$ = 8.9 Hz
parallel beta sheet: phi = -119, $3JH_AH_N$ = 9.7 Hz
left-handed alpha helix: phi = 57, $3JH_AH_N$ = 6.9 Hz

- NOE: a number of short distances are fairly unique to 2^o structure elements
- Proton exchange

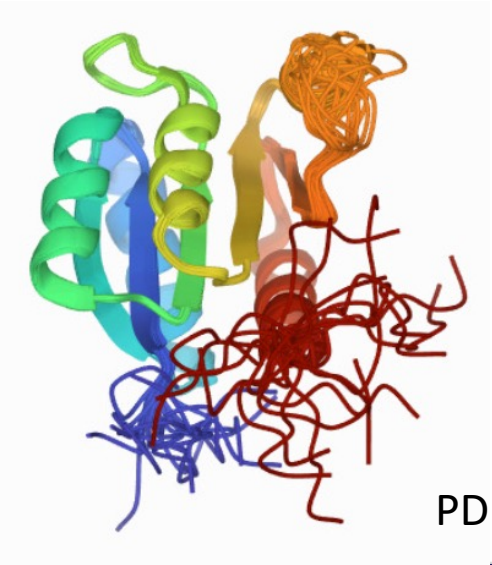
Tertiary Structure

- The idea of computer-aided structure calculation is to convert distance and torsion angles data (constraints) into a visible structure

- Usually a randomly folded start structure is calculated from the empirical data and the known amino acid sequence
- The computer program then tries to fold the starting structure in such a way, that the experimentally determined interproton distances are satisfied by the calculated structures.

NMR structures:

- The NMR structure of a protein is presented as a bundle of conformers
- Each conformer presents a good solution to the NMR restraints
- Typically, a bundle of 20 conformers are deposited in the PDB



PDB: 2N9U

Assessing the Quality of NMR Models

1. Data Quality
   - Number of restraints: the rule of thumb is 15-20 NOE restraints per residue. Even more important are the long range NOEs – a good NMR structure will have ≥3-5 restraints per residue
2. Model quality
   a. Root mean square deviation (RMSD) of the structure in the ensemble.
      a. Estimate of the precision of the model
      b. Values < 0.8 Å (backbone) and <1.5 Å (non-hydrogen) ⟹ high resolution
   b. Model geometry statistics
      a. There should be few/no violations of the restraints
      b. >90% of residues should be in the most favored region of the Ramachandran plot (with few/no outliers)
      c. Low deviation: <0.01 Å in bond length and <1.5 Å in bond angles
      d. Few/no unusual (strained) side chain rotamers