

Protein NMR.

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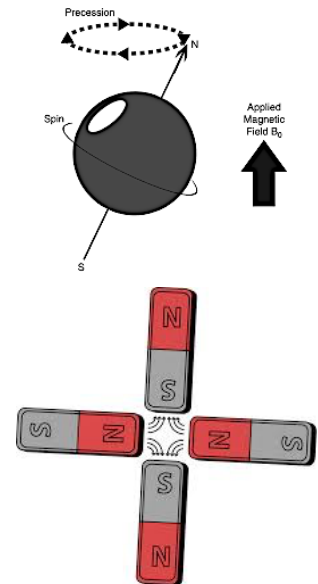
Overview

- Do you remember?
- Protein consists of hundreds amino acids.
- Amino acids are made mainly from organic compounds which consists of C, N, O and H.
- Meaning: these compounds can be characterized by NMR nuclear magnetic resonance **spectroscopy** as same as any other organic compounds.

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NMR phenomena

- At NMR phenomenon: the property of all atomic nuclei becomes 'spin'.
- Spin describes the nature of a magnetic field surrounding a nucleus and is characterized by a spin number.



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What happen if you apply a magnetic field on atoms.

- At equilibrium, the magnetization is along the B_0 field (z axis).
- To produce transitions between the energy levels, we apply a magnetic field (B_1) in the x-y plane.
- When we apply a magnetic field to electrons, they will align either with magnetic field direction or against it. Why?

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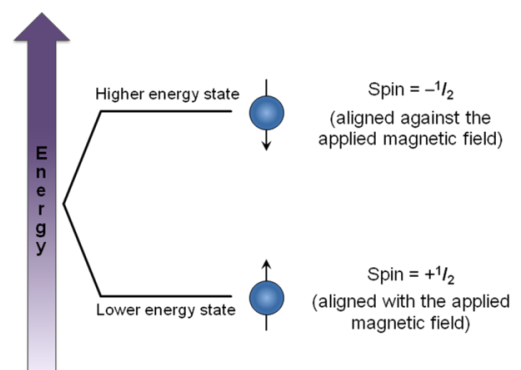
Magnetization

- The alignment of the magnetic field is due to the active magnetic field of the element.
- How magnetic field of the element can be active or silent?
- Spin 1/2 nuclei represent the simplest situation and arise when the number of neutrons plus the number of protons is an odd number:
- $N+P=\text{odd number } 1,3,5,\dots$
- ^{12}C the most common isotope is NMR 'silent' and the 'active' spin 1/2 nucleus (^{13}C).

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Energy of spinning

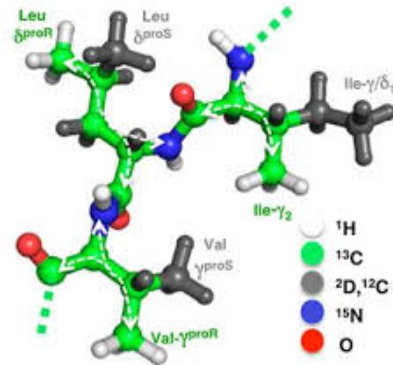
- Spins aligned parallel with external magnetic fields are of slightly lower energy than those aligned in an antiparallel orientation.
- The concept of two energy levels allows one to predict transitions between lower and higher energy level.



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Isotopes

- ^1H proton is not an isotope but it is active.
- ^2D Deuterium is an isotope but it is silent.
- ^{12}C proton is not an isotope but it is silent.
- ^{13}C proton is an isotope but it is active.
- ^{14}N proton is not an isotope but it is silent.
- ^{15}N proton is an isotope but it is active.

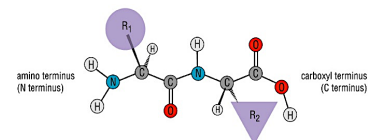


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What we need to get an active protein in the NMR?



- What we need to get an active protein in the NMR?
- So, the protein must have expressed in isotopes such as ^{15}N and ^{13}C .
- HOW?
- By growing up the bacteria in an isotope media instead of LB media (normal) to produce labeled protein.
- Bacteria eat the isotope N or C to express protein.

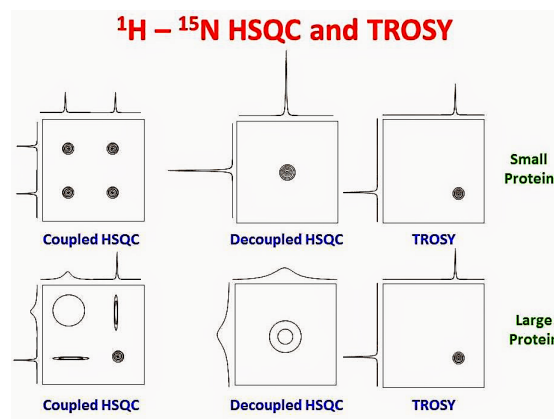


Polypeptide backbone

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What is the 2D spectra of NMR?

- The 2D spectra experiments are two frequency axes representing a chemical shift. It shows the correlation of atoms with other.
- The overview spectra is as you look at the peaks from the top.



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Types of the 2D experiments

- There are many experiments of 2D. E.g:
 1. COSY: it is used for small organic compounds to show the correlation between H and other in the compound.
 2. HSQC and TROSY: it is used for proteins to show the correlation between H and other N of the compound.
 3. NOE: : it is used to show the correlation between any two H in the compound.

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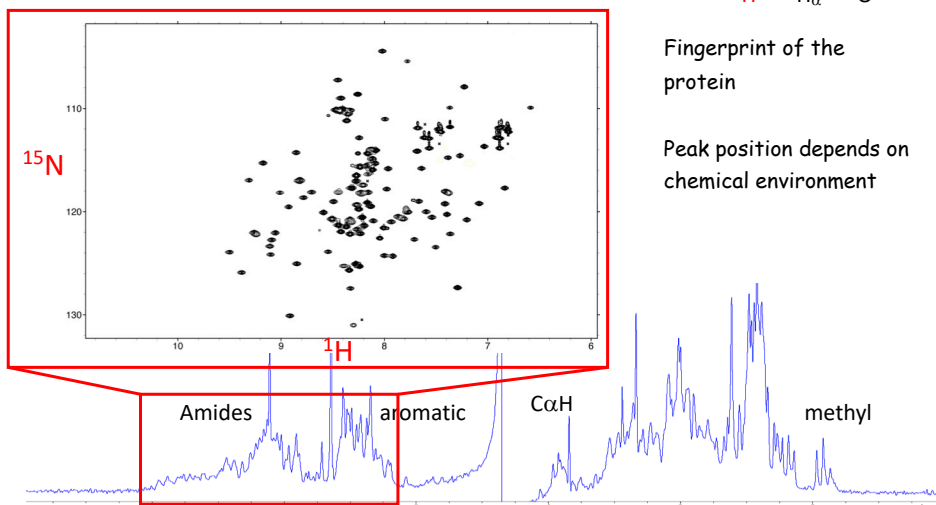
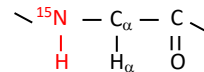
What do 2D HSQC spectra of protein show?

- HSQC show only the labelled atoms such as (^{13}C and ^{15}N) on the protein.
- Therefore, from HSQC spectra we can calculate exactly the number of amino acids in the protein.
- Also, we can assign each amino acid and find the sequence of all.
- Sequence means: the order of the amino acids in the protein.

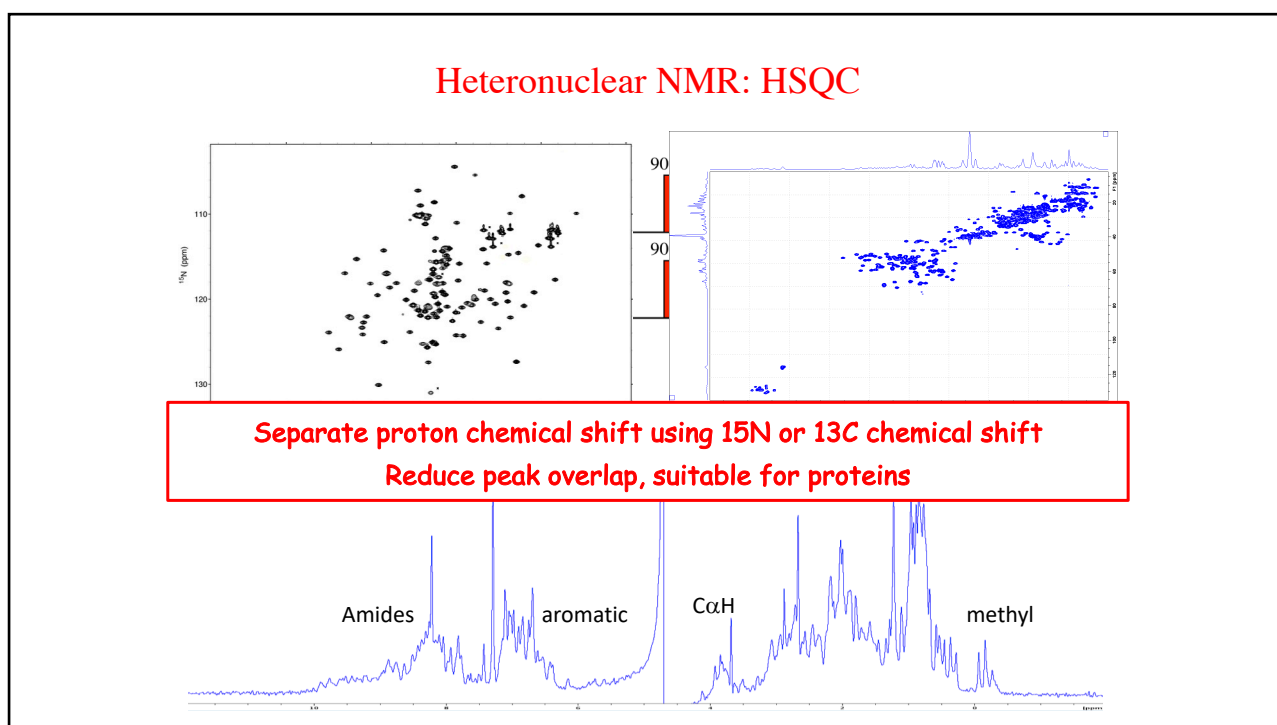
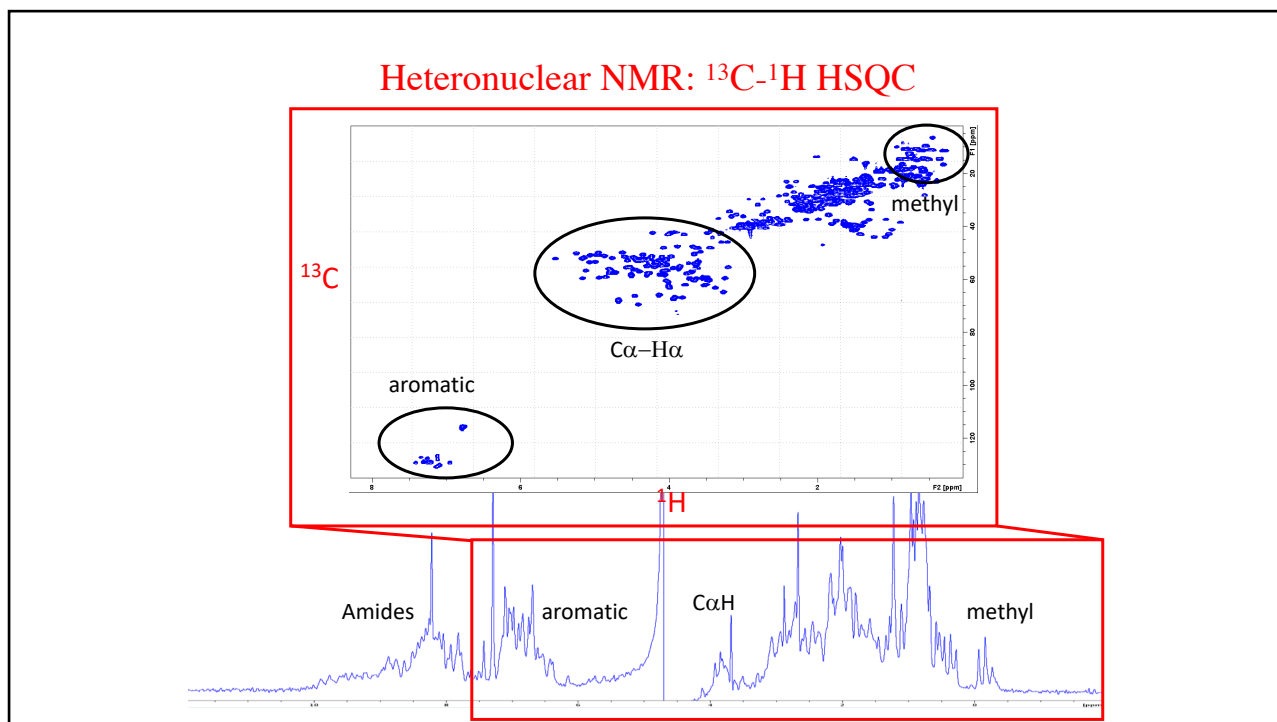
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Heteronuclear NMR: ^{15}N - ^1H HSQC

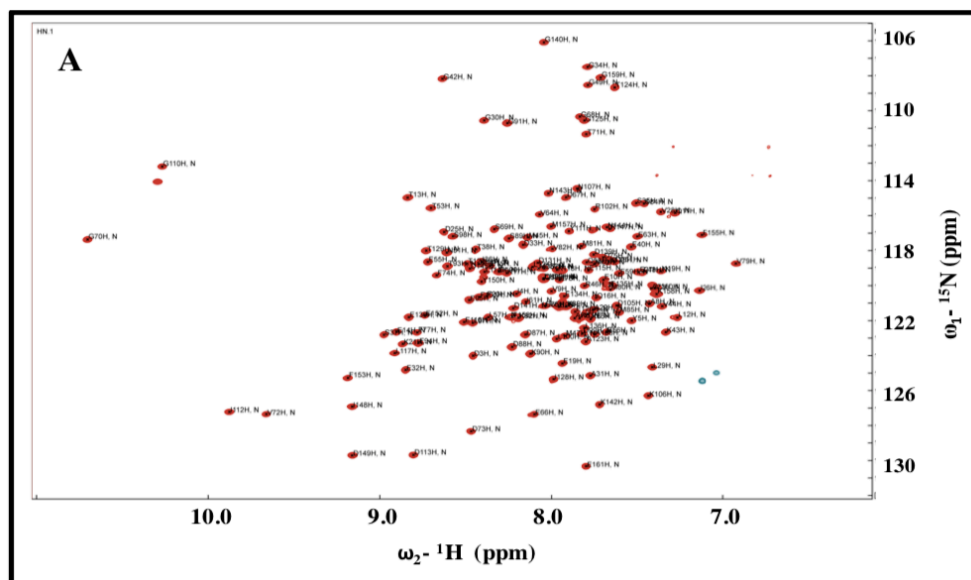
^1H - ^{15}N HSQC - one cross-peak per residue (except Pro)



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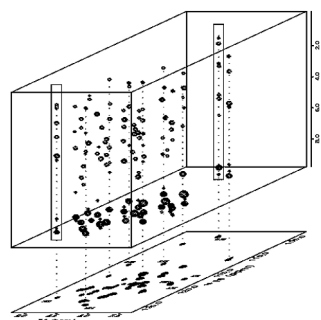
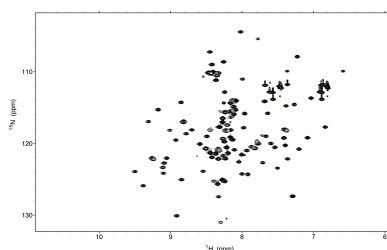
Just example: calculate the number of amino acids in the spectra?



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What we get from studying 3D and 4D?

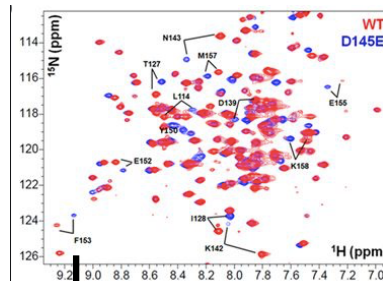
- We can get the 3D structure of the protein and biomolecules.
- We can measure the length of the bonds between any two atoms in the protein.



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What we do get from processing NMR spectra?

- We can get the 3D structure of the protein.
- There are many pdb for protein structure are available online. Visit [RCSB PDB: Homepage](#)
- What we can study on the 3D NMR structure of the protein?
 1. To study the protein-protein interactions.
 2. Drug design.
 3. Protein modeling.
 4. Protein function.



By software

