Protein NMR

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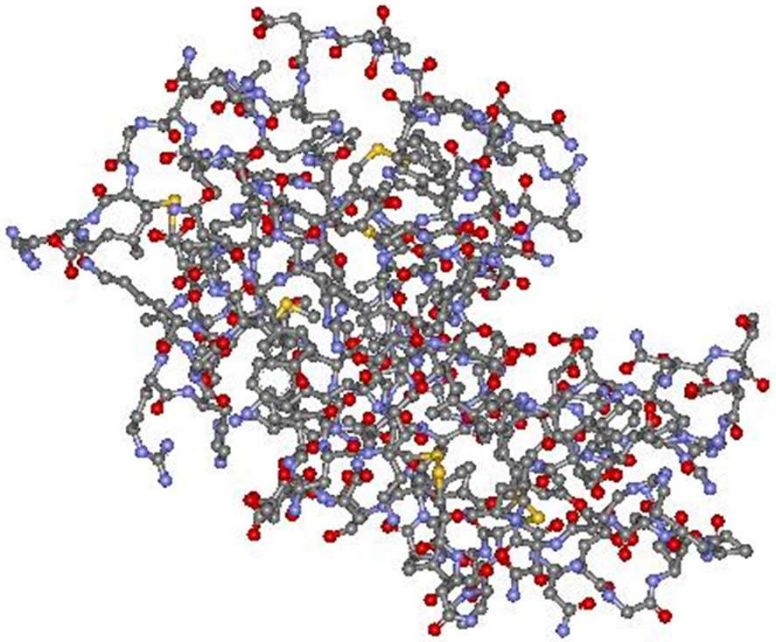
Overview

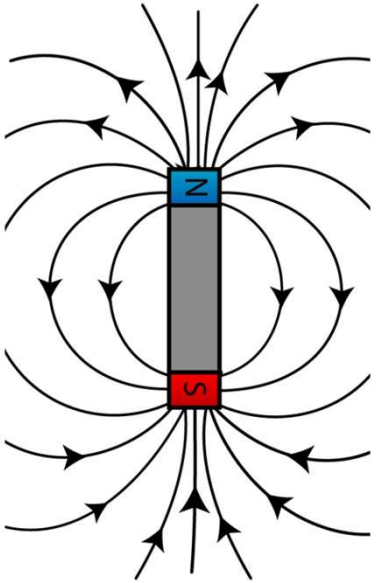
1. Principles of NMR
2. What is Protein NMR?
3. Two-dimensional NMR
4. Flowchart of determining structure of protein



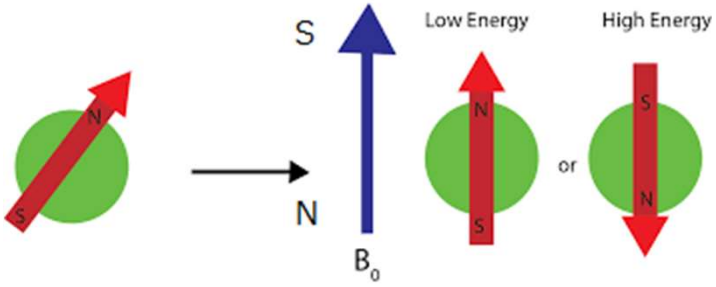
Chicken egg-white lysozyme structure, *lysozyme.co.uk*

Principles of NMR

- Every particle has its own characteristic "spin", I (I = 0, $\frac{1}{2}$, 1…).
- When particles spin, they generate a magnetic field.



- Energy in the form of radio frequency can be supplied to change the spin state (from low to high).
- Absorption spectrum can then be plotted, which gives information on:
  1) Identity
  2) Chemical environment



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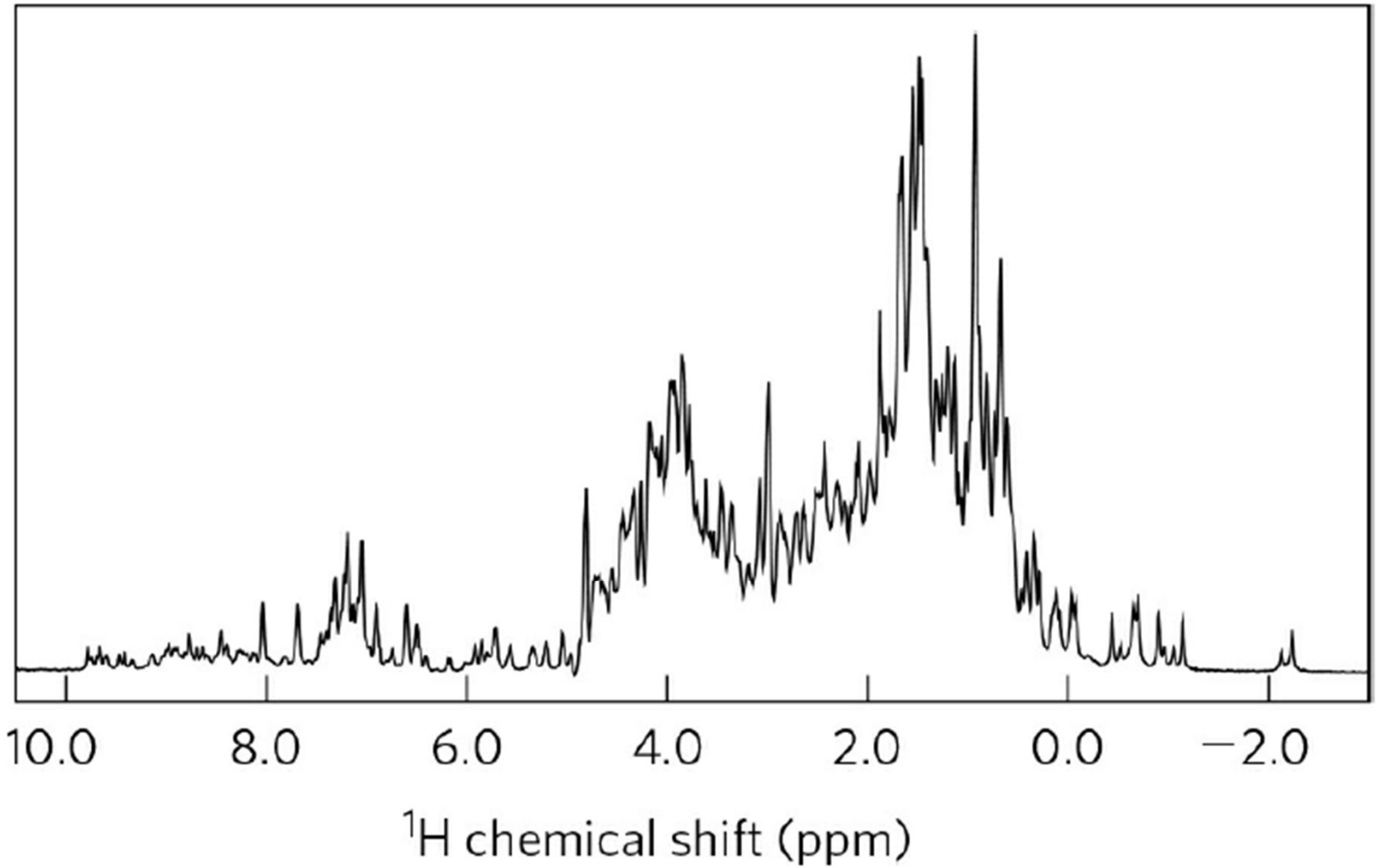

10.0
8.0
6.0
4.0
2.0
0.0
−2.0
^{1}H chemical shift (ppm)

What is Protein NMR?

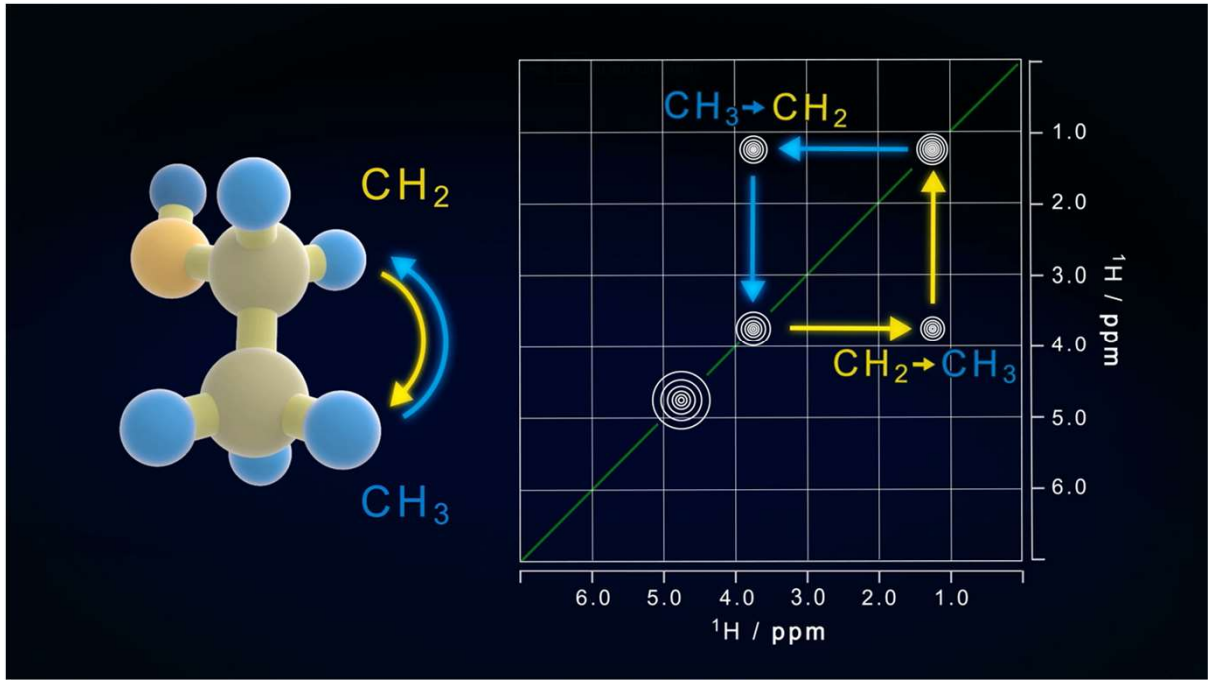
- Abbreviation for **nuclear magnetic resonance spectroscopy of proteins.**
- Allows the determination of three-dimensional structures of proteins molecules in the solution phase.

- ^{1}H is important in NMR due to:
  -> abundance (^{13}C and ^{15}N can be incorporated into protein samples as well)
  -> sensitivity

- However, for macromolecules, even a small protein would have hundreds of ^{1}H
  -> one dimensional NMR spectrum is too complex
  -> we turn to two-dimensional NMR techniques

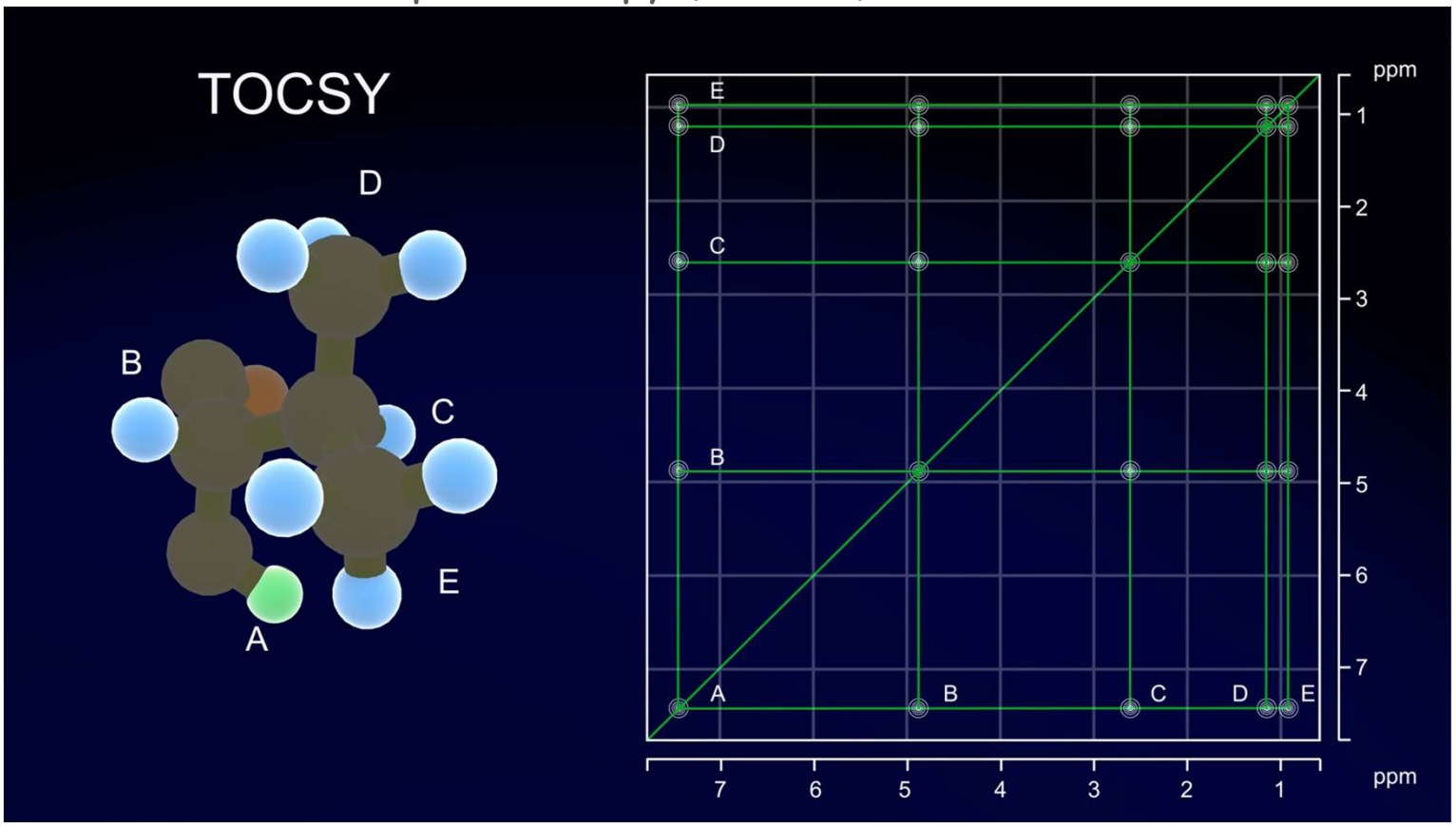
Two-dimensional NMR

- Rotates the 1-D spectrum 90°, then creating a two-dimensional spectrum out of the combination of the original and rotated spectrum.
- Formation of "cross peaks" gives information about chemical bonds and structure of the molecules.



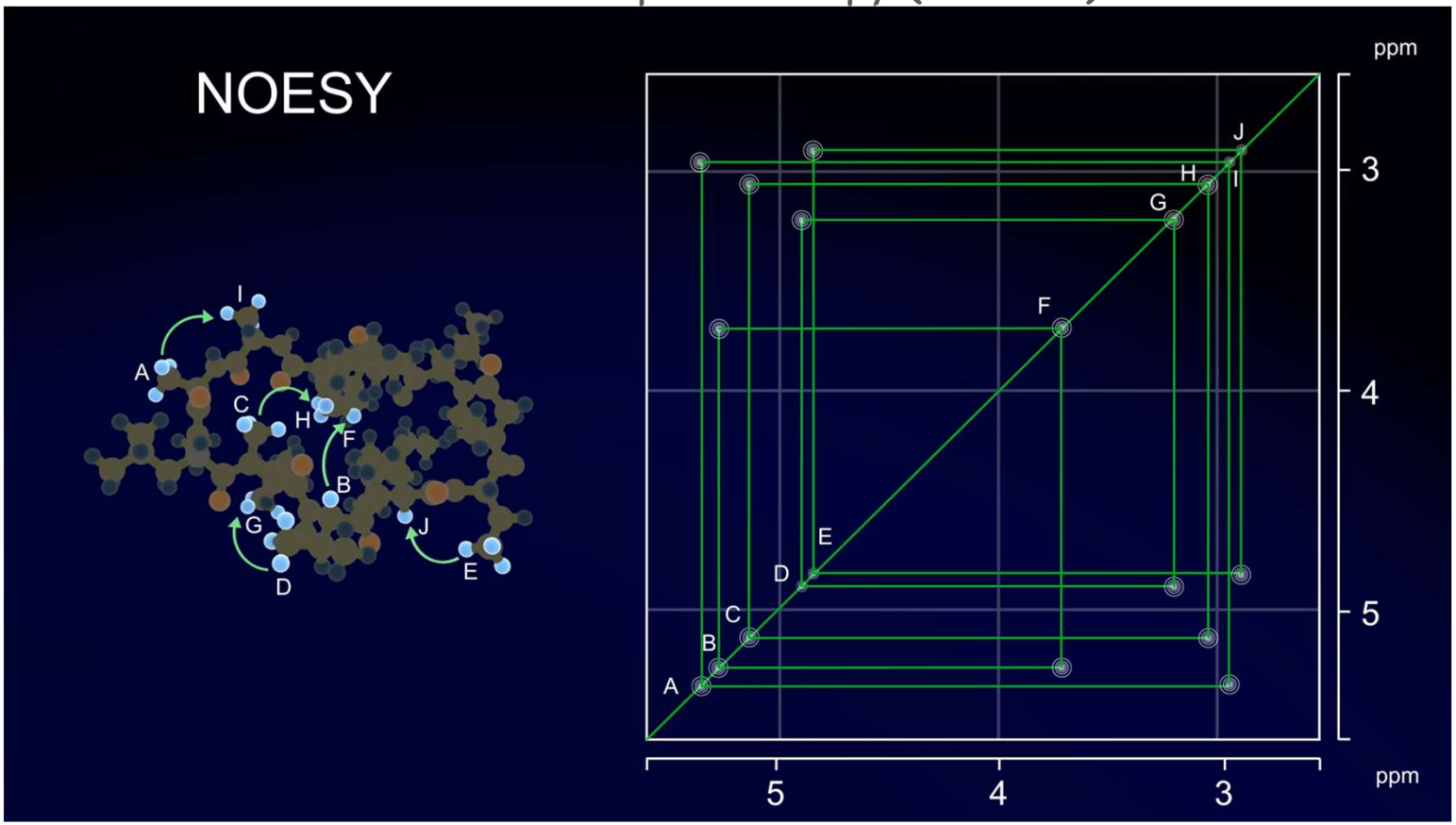
- Examples:

  1) Total Correlation Spectroscopy (TOCSY)



-> Correlates nuclear particle spins at long covalent distances.

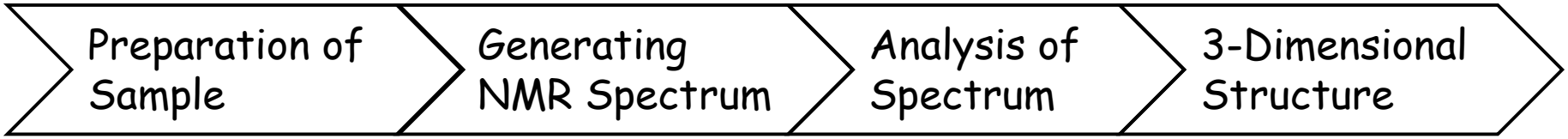
- 2) Nuclear Overhauser Effect Spectroscopy (NOESY)



-> Correlates nuclear spins that are close in space but are not necessarily covalently bound (allows to measure distances between particles).

A simplified flowchart

Determining 3-dimensional Structure of a Protein



Aqueous solution of highly purified protein; natural or genetic engineering

Short pulse of electromagnetic energy of resonance frequency applied

Using NOESY & TOCSY techniques; utilizing isotopes ^{13}C ^{15}N; knowledge of polypeptide sequence

Yay

Feed data to computer, to generate closely related structures that are consistent with distance constraints

THE END

Thank you