NMR of Large Biomolecules
CHEM 6154 – Nuclear Magnetic Resonance

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2 | Learning Goals for Today

In this lecture, we will:

▶ Examine how the structure of large proteins are determined by NMR
▶ Discuss the basic working of some special pulse sequences for this purpose
▶ Discuss assignment strategies

At the end, you will

▶ know how 3D NMR spectral data is presented and interpreted;
▶ understand the principles of resonance assignment;
▶ be able to explain the principle of structure determination by distance constraints.
3 | Labelled Protein Samples

- Proteins can be expressed in uniformly $^{13}$C, $^{15}$N media (u-$^{13}$C glucose, $^{15}$NH$_4$Cl)
- $^1J$ and $^2J$ couplings in proteins are universal, and can be used for polarisation/coherence transfer
- $^1$H, $^{15}$N and $^{13}$C chemical shifts depend on residue, conformation, and environment
- $^3J_{HH}$ and $^4J_{HH}$ couplings are conformation dependent
- spatial proximity leads to H-H cross-relaxation
4 | Structure Determination Protocol

- Protein Expression
- Resonance Assignment
- Measure NOE and $J$ couplings
- Structure calculation
N-H NSQC: The Basis of It All

- N-H direct couplings are nearly universal
- $^{15}\text{N-}^{1}\text{H}$ chemical shifts are residue- and conformation-specific
- each residue gives rise to at least one peak (some amino acids contain a secondary NH$_2$ group and therefore give two)
- for a given protein, the chemical shift pair ($\delta_H, \delta_N$) can be used as a “marker” for a specific residue.
7 | Representing 3D Data

(a)  

(b)  

(c)  

(d)  

(e)  

https://www.protein-nmr.org.uk
8 | Interpreting 3D Data
9 | Sequential Assignment: CACBNH

10 | CACBNH Strips

[Diagram showing amino acid structures and NMR peaks]
CACBNH: Another example

NOESY: Distance constraints

cytochrome c, 12.5 kDa
3D NOESY-HSQC: Distance constraints

15N-NOESY-HSQC Example

Each strip contains NOEs from one NH group to all other hydrogen atoms close by.

NOEs to aliphatic hydrogens

NOEs to amide and aromatic hydrogens
15 | Structure Determination

- Distance constraints
- Dihedral angle constraints
- Chemical shift constraints

→ Optimisation → 3D Structure
Fig. 2. NMR structure of the dimeric Rcf1 in DPC micelles. (A) Backbone ribbon trace of the 15 lowest-energy structures determined by solution state NMR. (B) Cylindrical representation of the Rcf1 dimer structure. The five TM helices of monomer A (A1–A5), monomer B (B1–B5) and the short flexible soluble helices (ASH, BSH) are shown (see labels). (C) Top view (Upper) and bottom view (Lower) of the Rcf1 dimer.
Take-home messages from today:

- Multi-dimensional spectroscopy allows to determine *correlations* among spectral signals;
- Protein structures are obtained from $^{15}$N, $^{13}$C labelled samples;
- 3D NMR spectra are interpreted and displayed using strips;
- Resonance assignment through sequential correlation (CACBNH);
- NOESY provides distance constraints;
- Protein structure is obtained by computational optimisation, using distance (NOE), dihedral ($J$), and chemical shift constraints.