

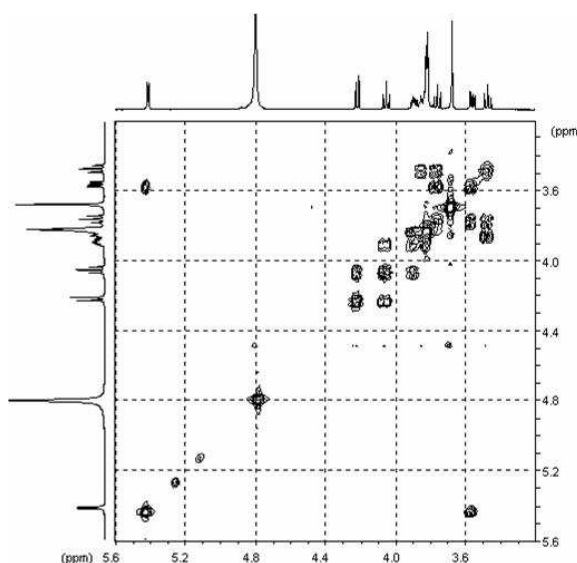
## Lecture 3

# Protein and NMR Structural Biology II

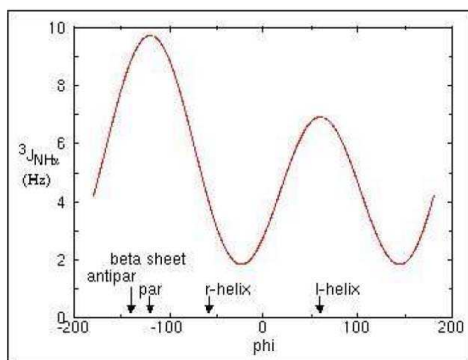
As mentioned in the last lecture, one-dimensional NMR techniques, which yield extremely useful information in small molecules, are of limited applicability to the complex, highly overlapped spectra of biological macromolecules. To effectively utilize the information available from NMR spectroscopy of biological macromolecules, we introduced the multidimensional NMR spectroscopy. It combines data from several kinds of spectra to establish the mappings for resonance assignments. In general, there are two types of multidimensional spectra, *homo-nuclear* spectra, in which each axis represents the same type of atom (usually protons), and *hetero-nuclear* spectra, in which each axis represents a different type of atom. In this lecture, we briefly introduce several essential multidimensional NMR experiments and techniques.

### 1 COSY

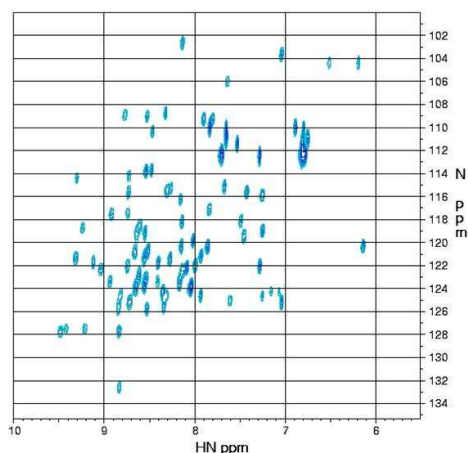
Homo-nuclear correlation spectroscopy (COSY) is the first two-dimensional spectroscopy, whose spectrum shows the frequencies for a single isotope (usually protons) along both axes of spectrum. As shown in Fig 1, the COSY spectrum consists of a series of peaks on the diagonal, and the peaks that appear off of the diagonal are called cross-peaks. These cross-peaks are symmetrical (above and below the diagonal) and indicate which protons are spin-spin coupled with each other. Therefore, though matching the center of a cross-peak with the center of each of two corresponding diagonal peaks, one can determine which atoms



**Figure 1:** COSY spectrum of proton-proton scalar couplings.



**Figure 2:** The Karplus relationship between the three-bond coupling constant  ${}^3J_{\text{HNH}\alpha}$  and the torsional angle  $\phi$ . The Karplus curve is calculated as  ${}^3J(\phi) = A \cos^2(\phi - 60) + B \cos(\phi - 60) + C$  where  $A$ ,  $B$  and  $C$  are empirically derived parameters.

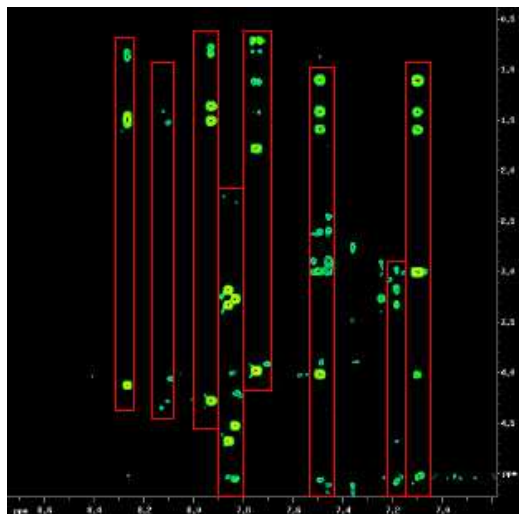


**Figure 3:** The NMR spectrum of  $\text{H}^{\text{N}}\text{-}^{15}\text{N}$  HSQC.

are connected to one another by chemical bonds. The peaks on the diagonal and the matched cross-peaks are coupled to each other.

## 2 ${}^3J_{\text{HNH}\alpha}$

The three-dimensional HNHA ( ${}^3J_{\text{HNH}\alpha}$ ) experiment is designed to accurately determine three-bond  $\text{H}^{\text{N}}\text{-H}^{\alpha}$  J-coupling constants. Similar to the NOE which provides information of the distance between two nuclei, the J-coupling constant is dependent on the structures. Hence, the coupling constant provides information about the dihedral angles subtended by the coupled atoms. For example, we can derive dihedral angle  $\phi$  by mapping J-coupling constants in the Karplus curve as shown in Fig 2.



**Figure 4:** The NMR spectrum of  $^{15}\text{N}$  TOCSY. Each strip indicates atoms grouped by the spin system.

### 3 $\text{H}^{\text{N}}\text{-}^{15}\text{N}$ HSQC

Heteronuclear Single Quantum Correlation (HSQC) is one of the most important two-dimensional NMR experiments. It correlates the nitrogen atom of an NH group with the directly attached proton, and each signal in a HSQC spectrum represents a proton that is bound to a nitrogen atom. Since every residue has a unique  $\text{H}^{\text{N}}\text{-}^{15}\text{N}$  pair on the protein backbone and ideally has distinct frequency signals, the HSQC spectrum can serve as the identification of each residue, and reference interaction for all other spectra. The HSQC spectrum also contains signals from the NH groups of the side chains, such as Asn and Gln and the aromatic  $\text{H}^{\text{N}}$  protons of Trp and His. As shown in Fig 3, the HSQC spectrum has no diagonal like a homo-nuclear spectrum, because different nuclei are observed during the relaxation T1 and T2.

### 4 $^{15}\text{N}$ TOCSY:

Total Correlation Spectroscopy (TOCSY) is a three-dimensional hetero-nuclear NMR experiment. In contrast to the  $\text{H}^{\text{N}}\text{-}^{15}\text{N}$  HSQC, TOCSY experiments indicate the side-chain protons as the third axis in the spectrum. Fig 4 shows a TOCSY spectrum by fixing the  $^{15}\text{N}$  atoms from 3D data, in which the peaks are from side-chain protons and  $\text{H}\alpha$  and the strips group atoms by the spin system. Given the fact that the chemical shifts of protons for different amino acids are characteristically different, we can regard these strips (number and position of peaks) as the "fingerprints" of the protons to identify probable amino acid types.

Having constrained the amino acid type for each peak in HSQC from TOCSY experiments, in order to obtain actual assignments, we still need to establish the sequential connectivities between spin systems. Generally, we combine information from different NMR experiments by cross-referencing peaks to reveal the sequential connectivities, e.g., HN(CO)CA and HNCA. Note that Prolines will not generate peaks in any spectrum which involves the backbone amide, e.g., HSQC, NH(CO)CA, HNCA,  $^{15}\text{N}$  TOCSY.

## 5 NOESY:

Nuclear Overhauser Effect (NOE) is caused by dipolar coupling between nuclei, that is, the local field at one nucleus is affected by the presence of another nucleus. The intensity of the interactions is a function of the distance between the nuclei according to the following equation

$$I = A(1/r^6),$$

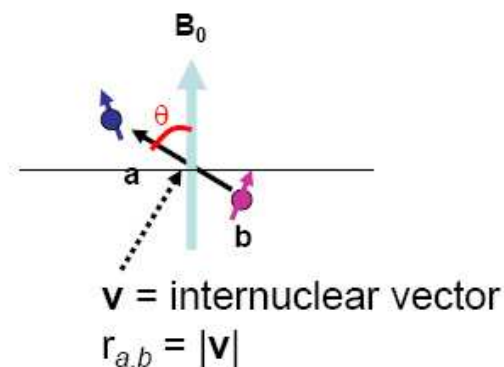
where  $I$  is the intensity which is measurable,  $A$  is a scaling constant, and  $r$  is the distance between nuclei. In other words, NOE provides a link between an experimentally measurable quantity,  $I$ , and internuclear distance. NOEs are measured with NOE spectroscopy (NOESY) experiments. For example, 3D  $^{15}\text{N}$ -HSQC NOESY, a 3D hetero-nuclear NMR experiment, measures the through-space NOE between an amide proton  $\text{H}^{\text{N}}$  and its neighboring  $^1\text{H}$ , which are within a distance less than 6 Å. In a NOESY experiment, the volume of the peak is related to the distance between the two atoms: bigger peaks indicate close distance, and weak peaks indicate that the atoms are further apart and/or moving.

## 6 RDC

Residual Dipolar Coupling (RDC)[2], which was first proposed in 1980s, has emerged as an important tool in NMR to study macromolecular structure and function in a solution environment. In the typical NMR experiments, the protein is in water (or deuterium) and it tumbles isotropically. However, if the molecules in solution exhibit a partial alignment, such as adding an alignment agent to the solution, leading to an incomplete averaging of spatially anisotropic dipolar couplings, RDCs between two spins will occur.

RDCs are complementary to NOEs; they provide orientational information as well as both short range and long range. RDCs also contain distance information as well as angles which are formed by a vector connecting the two atoms within a tensor axis system. In addition, both NOEs and RDCs can be utilized as restraints in molecular dynamics calculations.

RDCs are defined in terms of a coordinate system called the principal order frame (POF). The general formula to extract bond orientations using RDCs for two spins  $a$  and  $b$ , as



**Figure 5:** Extracting bond orientations using RDCs: spins  $a$  and  $b$  are in the  $B_0$  magnetic field.  $v$  and  $r_{a,b}$  indicate the orientation and the distance between the spins  $a$  and  $b$ , respectively.

described in Fig 5, is as follows:

$$D = \frac{\mu_0 \gamma_a \gamma_b \hbar}{4\pi^2 r_{a,b}^3} \left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle,$$

where  $D$  is the residual dipolar coupling constant, measured in units of Hertz,  $\hbar$  is the Planck's constant,  $\gamma$  is the gyromagnetic ratio,  $r$  is the inter-spin distance,  $\theta$  is the angle between the inter-spin vector and the external magnetic field, and  $a$  and  $b$  are spin operators. Then, we can simplify the equation as (see [3] for details)

$$D = D_{\max} \mathbf{v}^T \mathbf{S} \mathbf{v},$$

where  $D_{\max}$  is a physical constant,  $v$  is the orientation of the bond vector in the POF, and  $S$  is a  $3 \times 3$  symmetric and traceless matrix, which contains 5 degrees of freedom, called the alignment tensor (or Saupe matrix). In practice, the dipolar coupling  $D$  is measured directly, and the matrix  $S$  and the vector  $v$  are initially unknown. However, given a putative model, we can solve for  $S$  (via SVD) and back-compute RDCs. The difference between the computed and observed RDCs ( $RDC_o - RDC_c$ ) can be used to refine the model.

## 7 Summary

### Multidimensional NMR experiments

- ◇ [HN(CO)CA+HNCA] ⇒ Sequential connectivity via  $C_\alpha$  shifts.
- ◇ HSQC, [HN(CO)CA+HNCA] ⇒ Sequential connectivity of HSQC peaks.
- ◇ HSQC, TOCSY ⇒ Amino acid types.
- ◇  $^3J_{\text{HNHA}}$  ⇒ J-coupling ( $\phi$  angles).
- ◇  $^{15}\text{N}$  NOESY ⇒  $\text{H}^{\text{N}}\text{-}^1\text{H}$  distance ( $\leq 6\text{\AA}$ ).

### Data type and information content

◇ Chemical shifts	⇒	Resonant frequencies
◇ Scalar Couplings	⇒	Angular constraints
◇ NOEs	⇒	Distance constraints
◇ Residual dipolar couplings	⇒	Orientation Constrains

**More applications of NMR:** Solvent Accessibility, Dynamics, Simple Folding Assays, Protein Interaction Assay, and etc.

### References

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