PULSE SCHEMES FOR SOME COMMONLY USED 2-D EXPERIMENTS

(Nuclear Overhauser Effect Spectroscopy)
→ Through space dipolar effect
→ Determine NOE
→ Measuring distance
→ Assign resonances

(Correlated Spectroscopy)
→ Through bond J-coupling
→ Assign adjacent resonances

(Multiple Quantum Filtered Correlated Spectroscopy)
→ Through bond J-coupling similar to COSY
→ Assign adjacent resonances
→ More sensitive

(Homonuclear Hartman-Hahn spectroscopy)
(Total Correlated Spectroscopy) (TOC SY)
→ Through bond relayed J-coupling
→ Assign full spin system (residues type)
**COSY: (MQF-COSY; DQF-COSY)**
1. Off-diagonal resonances due to $^{1}J_{\text{NH}C\alpha}$ one bond J-coupling.
2. Assign adjacent resonances.
3. One can select a magnetization transfer pathway (efficiency) by varying the evolution time.

**TOCSY: (HOHAHA)**
1. Off-diagonal resonances due to relayed J-coupling.
2. Magnetization transfer thru Hartmann-Hahn cross polarization.
3. Assign long range correlated resonances (Whole a.a. system).

**NOESY:**
1. Off-diagonal resonances due to NOE.
2. Magnetization transfer thru energy transfer due to thru space dipolar effect.
   \[ I \propto R^{-6} \] \; \rightarrow \text{Determine distances.} 
3. Sequential resonance assignments.
1. NH-H\(\alpha\) only (Intra residue)
   同一胺基酸
2. Splitting \(\rightarrow^3J_{\text{HN-}}\)
TOCSY (Spin System Identification) RC-RNase

1. J-Coupling: HN $\rightarrow$ H$\alpha$ $\rightarrow$ H$\beta$ $\ldots$  
2. Identify Spin System (a.a. type)
TABLE 2.2. Side Chains R (see Fig. 2.1) and Three-Letter and One-Letter Symbols for the 20 Common Amino Acids, and Spin Systems of the Nonlabile Hydrogen Atoms in the Molecular Fragment H–αC–R^A

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Figure 7.1. Selected sequential and medium-range $^1H-^1H$ distances in polypeptide chains (from Wüthrich et al., 1984a).
Amide Proton Resonance Assignments of Thioesterase I
Multi-Dimensional NMR

Basic 3D Experiment

- Relaxation time $t_1$
- Evolution time $t_2$
- Acquisition $t_3$

2D

- Relaxation time $t_1$
- Evolution time $t_2$
- Acquisition $t_2$

3D

2D Spectrum

Frequency $f_1$

2D FID

Frequency $f_2$

Evolution time $t_2$

2D Fourier transform

Acquisition time $t_2$

$2D Spectrum$

$2D Spectrum$

$2D Spectrum$
Advantages of heteronuclear NMR:

1. Large chemical shift dispersion $\rightarrow$ Increased resolution.
2. Large coupling constant (Easy to transfer magnetization).
3. Thru bond connectivity $\rightarrow$ Easy assignments.
4. Permit easier analysis of protein dynamics.
5. Permit determining the structure of larger proteins (> 100 kDa).

Disadvantages of heteronuclear NMR:

1. Must label the protein with $^{13}C$ and/or $^{15}N$.
   a). Expensive.
   b). Time consuming.
2. Technically much more complicated.
4. Much larger data size.
圖5. 用在多維異核核磁共振實驗的單鍵異核偶合常數之概要圖。

圖4. 二氯紫酸還原酵素的三維$^{15}$N-區分後的NOESY-HSQC的部份光譜。
Thioesterase $^{15}$N-TOCSY HSQC

$^1H$ Chemical Shift (ppm)

$^1H$ Chemical Shift (ppm)
**J-coupling of backbone nuclei (Hz)**

$^3J(\text{HN-CA}) = 4 - 11 \text{ Hz}$ depends on secondary structure.

- $< 6 \text{ Hz} \rightarrow \alpha$-helix
- $> 8 \text{ Hz} \rightarrow \beta$-stand
圖6. 二項常用的三核共振實驗的脈衝圖譜。
Heteronuclear multidimensional NMR experiments for resonance assignments

Magnetization transfer pathway:

$^1\text{H} \rightarrow ^{15}\text{N} \rightarrow ^{13}\text{C} \rightarrow ^{15}\text{N}$

$\rightarrow ^1\text{H} \rightarrow ^1\text{H}$ Detection

$\rightarrow$ Detect $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$ resonances

$\rightarrow$ Permit sequential correlation of backbone $^1\text{H}$-$^{13}\text{C}$-$^{15}\text{N}$ resonances !!!
圖5. 用於多維異核核磁共振實驗的單鍵異核偶合常數之概要圖。
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Secondary Structure Identification

1. Medium range NOEs:
   - $d_{aN}(i, i + 4)$, $d_{aN}(i, i + 3)$, $d_{aN}(i, i + 3)$, $d_{N}(i, I + 2)$, $d_{aN}(i, I + 2)$, $d_{aN}$

2. Special NOE pattern for $\beta$-sheet structures.

3. Chemical shift index (CSI) for $H_a$, $^{13}C_a$, $^{13}C_\beta$, and $^{13}CO$
   - Compares to the chemical shift observed for random coil state. (Must be ± 0.1ppm to be significant)
   - Upfield (to the right) shift for $\alpha$-helix and down field (to the left) shift for $\beta$-sheets.

4. Exchange rates for the exchangeable (NH) protons
   - NH protons involved in secondary structure network usually have much slower exchange rates.
Figure 7.1. Selected sequential and medium-range $^1$H–$^1$H distances in polypeptide chains (from Wüthrich et al., 1984a).

(Define the secondary structure)
NOE cross peaks in $\beta$-sheet structure of RC-RNase
(B) RC-RNase

Diagram showing the structure of RC-RNase with labeled amino acid positions and arrows indicating secondary structures such as α-helices and β-sheet.

Key positions:
- 1-2, 29-30-31-32, 79-80
- α2, α3
- β-sheet

Amino acid positions are marked with numbers and arrows indicating the direction and type of secondary structure.
NMR Structural Constraints

1. Internuclear distances (Nuclear Overhauser Effect)
   \[ \text{NOE} \propto R^{-6} \]

2. Dihedral angles (J-coupling):
   \[ ^3J_{\text{NH}_a} = 6.4 \cos^2(F - 60) - 1.4\cos(F - 60) + 1.9 \]

3. Chemical Shift Index (CSI):
   Chemical shift difference between observed and random coil chemical shift values \( \rightarrow 2^{\text{nd}} \text{ structure determination} \)

4. Residual dipolar coupling:
   Partial orientation of protein molecules in liquid crystal media permits observation of residual dipolar coupling for assessing long range orientations of dipolar coupled bonds.
由 NOE 限制找出分子的三度空间结构。
Structure Calculation

1. Build a random structure of the given sequence.

2. Use molecular dynamics and simulated annealing to generate many structures with minimum violation of structure constraints and with minimal energy of the following energy term.

   \[ E_{\text{total}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{improper}} + E_{\text{VDW}} + E_{\text{cdih}} + E_{\text{NOE}} + E_{\text{RDC}} + \ldots \]

   \[ E_{\text{bond}} = \sum k_b (b-b_0)^2; \quad E_{\text{\varphi}} = \sum k_\varphi (\varphi - \varphi_0)^2; \]

   \[ E_{\text{improper}} = \sum k_{\text{impr}} (\omega - \omega_0)^2; \quad E_{\text{\varphi\varphi}} = \sum k_{\text{cdih}} (\varphi - \varphi_0)^2; \]

   \[ E_{\text{\varphi\varphi\varphi}} = \sum k_{\text{NOE}} (\varphi - \varphi_0)^2; \quad E_{\text{\varphi\varphi\varphi\varphi}} = \sum k_{\text{RDC}} (\varphi - \varphi_0)^2; \]

3. Check for wrong assignments and recalculate the structure.

4. Select 20 structures of least NOE violation (> 0.5 Å)

5. Criteria for good structures:
   a. No NOE violation
   b. RMSD < 0.5 Å
   c. No violation in dihedral angle (Inspect Ramanchandran diagram)(Atomic hindrance).
II. Dynamics
4-dimensional structure
Spin-lattice relaxation ($T_1$) and spin-spin relaxation ($T_2$) of nuclear spins. Figure shows the evolution of the magnetization after it has been flipped by 90° pulse.
Under current magnetic field strength the relaxation rates are dominated by dipolar interaction and chemical shift anisotropic interaction, and is related to the correlation time, $J(\omega)$, by the following equations:

$$ R_1 = 1/T_1 = (d^2/4)[J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)] + c^2 J(\omega_N) \quad ---------(1) $$

$$ R_2 = 1/T_2 = (d^2/8)[4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)] $$
$$ + \frac{(c^2/6)[4J(0) + 3J(\omega_N)]}{1} + R_{\text{ex}} \quad -----------------------------------(2) $$

where $d = (\mu_0 h \gamma_N \gamma_H / 8\pi^2)(r_{NH}^{-3})$, $c = \omega_N(s_\parallel - s_\perp) / \sqrt{3}$. 

$\mu_0$: permeability constant of free space; $h$: Planck constant; 
$\gamma_i$: magnetogyric ratio of spin $i$; $\omega_i$: Larmor frequency of spin $i$; 
$r_{NH} = 1.02$ Å: length of the NH bond vector; $R_{\text{ex}}$: exchange rate; 
$s_\parallel - s_\perp = -170$ ppm (size of the CSA tensor of the backbone amide nitrogen).
Nuclear Overhauser Effect (NOE)
(Energy transfer through dipolar effect)

\[
\text{XNOE} = 1 + \left(\frac{d^2}{4}\right)\left(\frac{\gamma_H}{\gamma_N}\right)\left[6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)\right]T_1
\]

where \[d = \left(\mu_0\hbar\gamma_N\gamma_H/8\pi^2\right)(r_{NH}^{-3})\]
The spins are assumed to be attached to a rigid macromolecule undergoing Brownian motion with a rotational correlation time $\tau_m$. In addition, the spins also undergo internal motion with rotational correlation time $\tau_s$. Under this assumption the spectral density function, $J(\omega)$ is given by:

$$J(\omega) = \frac{2}{5} \left[ \frac{S^2 \tau_m}{1 + (\omega \tau_m)^2} + \frac{(1-S^2)^2}{f} \right] + \frac{(S^2 - S^2) \tau_s'}{f} \frac{f}{1 + (\omega \tau_s')^2}$$

$S^2$: Order parameters (Magnitude of motion)
$\tau$: Correlation times (Speed of motion)
$R_{\text{ex}}$: Chemical exchange rate (Slow motion in ms or $\mu$s regime)

Fitting $T_1$, $T_2$ and NOE data to determine $S^2$, $\tau$ and $R_{\text{ex}}$ which contain the dynamics information of the protein.
1. Measured $T_1$, $T_2$ and ($^1$H, $^{15}$N) NOE at 500 and 600 MHz at 310 K.
2. Total of 128 resonances were measured at 500 MHz and 134 resonances were determined at 600 MHz.
3. Average:
   \[ R_1 = 1.108 \pm 0.056 \text{ S}^{-1} (1.506 \pm 0.096 \text{ S}^{-1}), \]
   \[ R_2 = 10.31 \pm 1.40 \text{ S}^{-1} (9.236 \pm 1.17 \text{ S}^{-1}) \]
   \[ X_{\text{NOE}} = 0.742 \pm 0.044 (0.705 \pm 0.039) \text{ at 14.09 T (11.74 T)}. \]
4. From the above data one can determine:
   a). Rotational diffusion constant.
   b). Order parameter, $S^2$ \(\Rightarrow\) Measure of the flexibility.
   c). Determine rate of local motion.
   d). Conformational exchange rates.

\(\Rightarrow\) At atomic resolution
Relaxation Data

Obtained in two fields:

- ○ : 500 MHz
- ● : 600 MHz
Order parameter – Flexibility

$S^2_{av} = 0.85$
$S = 1$ rigid
$S = 0$ random

Mostly rigid

Flexible region
Exchange rate – Residues with low motion
Radius of the sausage $\propto 1/S$
Large radius $\rightarrow$ disordered region