Outline

1. History
2. Theory of 2D NMR
3. Applications of COSY
A brief history of 2D NMR

- In 1971, the idea of 2D NMR was proposed by Jean Jeener.
- The 1975 Ernst paper “Two-dimensional spectroscopy, application to nuclear magnetic resonance” utilized Jeener’s idea to produce spectra.
- 1980s-present: application of NMR to protein structures
- In 1991 Ernst won a Nobel Prize in Chemistry for his contributions to Fourier Transform NMR
Types of 2D NMR

- COSY: correlation spectroscopy
  - HETCOR: heteronuclear COSY
  - TOCSY: total correlation spectroscopy
- NOESY: Nuclear Overhauser effect spectroscopy
- EXSY: exchange spectroscopy
- J-Spectroscopy
A series of equally-spaced $t_1$ are used; 90° pulses occur at white circles.

Each spectrum measures the instantaneous state of the perturbed system after various $t_1$.
General 2D FTS experiment

1. At \( t = 0 \), the system is at \( \rho(0) \), an arbitrary nonequilibrium state.

2. During \( 0 < t < t_1 \) the system develops under the influence of the time-independent Hamiltonian \( H \).

3. At \( t = t_1 \) the density operator is rotated by an rf pulse.

4. At \( t > t_1 \) the system again develops under \( H \) and the spectrum is measured.

5. A Fourier transform is used to convert the time-dependent data to a 2D spectrum.
The motion of the system is described by the density operator eqn:

$$\frac{d\sigma}{dt} = -i[H(t), \sigma] - \mathbf{r}\{\sigma - \sigma_0(t)\}$$

where and $\mathbf{r}$ is the relaxation superoperator

At $t=t_1+t_2$ the solution is:

$$\sigma(t_1, t_2) = \sigma_0(2) + \exp\left(-iH(2)t_2 - rt_2\right)$$

$$\times \left[ \mathbf{r}\{\sigma_0(1) + \exp\left(-iH(1)t_1 - rt_1\right)[\sigma(0) - \sigma_0(1)]\} \right]$$

$$M_y(t_1, t_2) = N\gamma_h\text{Tr}\left(F_y\sigma(t_1, t_2)\right)$$

The observed spectrum is a 2D Fourier transform of the above.
A pair of projection superoperators with the properties

\[
\begin{align*}
d + n &= 1, \\
d^2 &= d, \quad n^2 = n, \\
dH(1) &= H(1), \\
[dA, H(1)] &= 0 \quad \text{for any operator } A.
\end{align*}
\]

are used to obtain an expression for the observed magnetization that is separated into two terms:

\[
M_y(t_1, t_2) = M_y^d(t_1, t_2) + M_y^n(t_1, t_2)
\]

\(M_y^n(t_1, t_2)\) consists of magnetization components which show oscillatory behavior during the evolution period, and will be responsible for cross-peaks and dia-peaks in the 2D spectrum.

The other term consists of components which do not oscillate and produces the axial peaks of the spectrum.
3 types of peaks

Dia peaks are related to only one transition and occur at the main diagonal of the 2D spectrum

Cross peaks correlate different transitions, are off-diagonal

Axial peaks give information about spin-lattice relaxation processes
Two pulse (90°) $^1$H-$^1$H experiment on 2,3-dibromothiophene. Absolute value spectrum shown.
Cross and dia peaks

- Give information about:
  - Connectivity of transitions in the energy level diagram:
    The rotation superoperator $r$ couples transitions
  - Transverse relaxation processes:
    Line shapes depend on transverse relaxation times $T_{2k1}$
  - The initial state of the spin system, $\rho(0)$:
    Conventional NMR only measures allowed transitions, while all matrix elements of $\rho(0)$ can be measured with 2D NMR
Uses

- Structural identification in organic and biological chemistry:
  - Since its creation, 2D NMR has been useful for elucidating the structure of small molecules
  - Advanced computing power now allows the structure of large, biological molecules to be solved

Figure from Proc Natl Acad Sci U S A. 1999 January 19; 96(2): 332–334.
Systematic application of two-dimensional $^1$H NMR techniques for studies of proteins, Wüthrich et al., Eur J of Biochem 114, 375-384 (1981)

- Used COSY and NOESY to obtain individual assignments for each proton in the protein backbone in the $\beta$-sheet secondary structure of pancreatic trypsin inhibitor
- COSY spectra taken in $^2$H$_2$O and H$_2$O were combined to obtain sequential resonance assignments
- Additional experiments were carried out with $t_1$ at different phases to cancel out axial peaks
2D COSY 1H NMR for pancreatic trypsin inhibitor at 360 MHz
• COSY NMR was used to determine the J connectivities on the protein backbone

• NOESY was used to determine distance-dependent coupling, since NOE can only occur at distances of <5-6 Å

• A “β-snail” structure was observed for the protein
Summary

- 2D NMR uses a sequence of two pulses with a series of different evolution times to determine which nuclear spins are coupled to one another.
- COSY spectra indicate through-bond coupling, and can be used to gain structural information about molecules of a wide range of sizes.