Homonuclear 2D experiments: Principles and Applications

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Introduction to Homonuclear 2D NMR Experiments

- Homonuclear two-dimensional (2D) NMR experiments are powerful techniques for probing interactions between nuclei of the same type—most commonly protons (^{1}H).
- Unlike 1D spectra, which can suffer from severe signal overlap in complex molecules, 2D methods spread the information across two frequency dimensions, improving resolution and enabling detailed structural insights.
- Key homonuclear 2D experiments include:

COSY (Correlation Spectroscopy): Reveals scalar (J) couplings between protons, helping establish connectivity within spin systems.

TOCSY (Total Correlation Spectroscopy): Extends coupling information across entire spin networks, useful for identifying complete fragments.

NOESY (Nuclear Overhauser Effect Spectroscopy): Provides through-space correlations, critical for determining spatial proximity and 3D structure.

- All these techniques are essential for confirming molecular frameworks, assigning resonances, and studying conformational dynamics. Their application is particularly valuable in complex mixtures, natural products, and biomolecules where 1D analysis alone is insufficient.

How it Works

1. Basic Concept
- In 1D NMR, all resonances appear along a single frequency axis, often causing overlap.
- In 2D NMR, the experiment introduces an indirect dimension (F1) in addition to the direct dimension (F2), creating a 2D map of correlations.

2. Pulse Sequence Structure

A typical 2D experiment consists of three main periods:
- Preparation: Spins are excited by a 90° pulse.
- Evolution (t_1): Magnetisation evolves under chemical shifts and couplings during an incremented delay (t_1). This is the indirect dimension.
- Mixing: Additional pulses transfer coherence between spins (e.g., via J-coupling for COSY or NOE for NOESY). Detection (t_2): The signal is recorded as a function of time in the direct dimension.

3. Data Acquisition
- The experiment is repeated many times, incrementing t_1 each time.
- For each increment, a FID is collected in t_2.
- The result is a matrix of data points ($t_1 \times t_2$).

4. Fourier Transformation
- A double Fourier transform converts the time-domain data into a frequency-domain spectrum. F1 axis: Frequencies from t_1 evolution. F2 axis: Frequencies from t_2 detection.

5. Spectrum Interpretation
- Diagonal peaks: Correspond to the same nucleus (like 1D peaks).
- Cross-peaks: Indicate correlations between nuclei (through-bond or through-space, depending on the experiment).

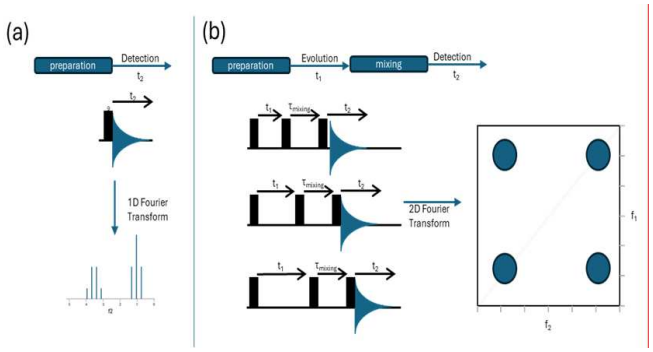

Figure 1: (a) General schematic of 1D NMR experiments, with an example given for a pulse-acquire sequence and spectrum shown. (b) General schematic of 2D NMR experiments.

Example Spectrum and Molecule Showing TOCSY Correlations

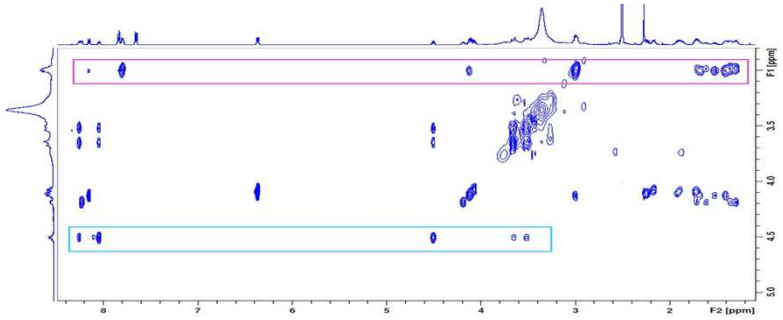

Figure 2: Partial TOCSY spectrum showing correlations between different signals

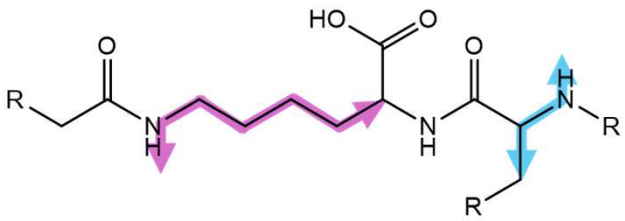

Figure 3: Example molecule with arrows showing correlations observed in TOCSY spectrum (Fig. 2)

Brief Comparison of TOCSY and NOESY Experiments

Advantages

TOCSY
- Excellent for mapping connectivity within fragments.
- Helps resolve overlapping signals by linking all protons in a residue.

NOESY
- Enables structure determination via inter-proton distances.
- Detects long-range interactions (not limited to covalent connectivity).

Disadvantages

TOCSY
- Does not provide spatial information
- Limited by relaxation losses in large molecules (signal decay during mixing).

NOESY
- Interpretation can be complex due to spin diffusion.
- Requires careful calibration of mixing time to avoid artifacts.

Key Experimental Parameters

TOCSY
- Mixing Time (τm): Too short → incomplete transfer (missing correlations). Too long → relaxation losses and artifacts. Typical range: 60–120 ms for small molecules.
- Pulse Sequence: Optimise isotropic mixing (MLEV-17 or DIPSI-2).
- Temperature & Solvent: Higher temperature can reduce viscosity and improve transfer.

NOESY
- Mixing Time: Too short → weak NOE signals. Too long → spin diffusion dominates (false long-range correlations). Typical range: 200–400 ms for small molecules; shorter for large proteins.
- Field Strength: Higher fields reduce NOE efficiency for small molecules (due to correlation time).
- Sample Conditions: Adjust viscosity and temperature for optimal correlation time.

Troubleshooting Tips

TOCSY

Issues: Weak correlations → increase τm slightly or check pulse calibration. Overlapping peaks → consider higher resolution or selective TOCSY.

NOESY

Issues: Missing NOEs → increase τm or reduce temperature. Spin diffusion artifacts → shorten τm or use ROESY for intermediate-size molecules.

Key Applications in Pharma

COSY
- Structural confirmation of small molecules
- Detecting impurities and related substances. Fragment connectivity in synthetic intermediates

TOCSY
- Assigning residues in peptides and oligonucleotides
- Characterising complex excipients or natural products
- Mixture analysis in formulations

NOESY
- 3D structure determination of APIs and peptides
- Conformational analysis of drug candidates
- Studying ligand–protein interactions