

Unit 2: NMR Spectroscopy: 1-D and 2-D Techniques and Interpretation

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2.1 The 1-D experiments this unit builds on

One dimensional NMR was covered in MPC101T: the proton spectrum (chemical shift, integration, multiplicity from spin-spin coupling) and the proton decoupled carbon spectrum, in which each chemically distinct carbon gives one line. Two 1-D companions are assumed knowledge for everything that follows.

DEPT editing sorts carbon signals by the number of attached protons: in DEPT-90 only CH appears; in DEPT-135 CH and CH₃ point up and CH₂ points down; quaternary carbons are absent from all DEPT spectra and are found by subtraction from the broadband spectrum. Routine working values for carbon shifts: carbonyl carbons 160 to 220 ppm, aromatic and alkene carbons 100 to 160 ppm, carbons bearing oxygen or nitrogen 50 to 100 ppm, and ordinary alkyl carbons 10 to 50 ppm.

What 1-D spectra cannot do is tell the analyst which signal belongs next to which. A proton spectrum of a moderately complex drug molecule is a crowd of overlapping multiplets: shifts say what kind of environment each proton sits in, couplings hint at neighbours, but the map of connections is missing. Two dimensional NMR exists to draw that map.

2.2 Chemical shift maps for proton and carbon

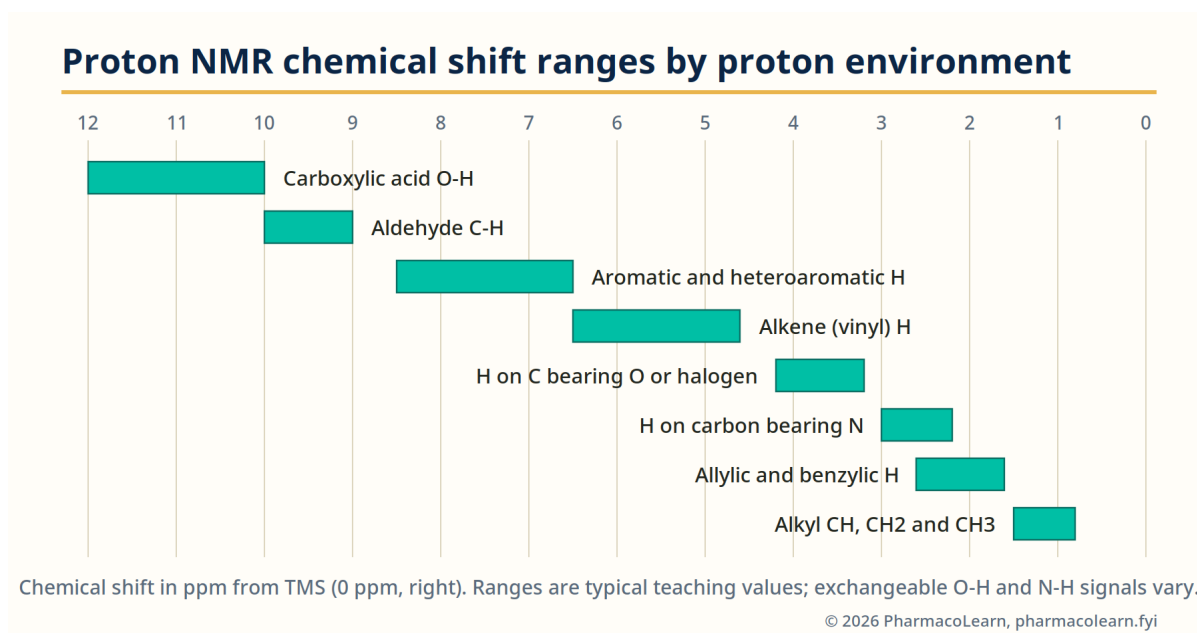


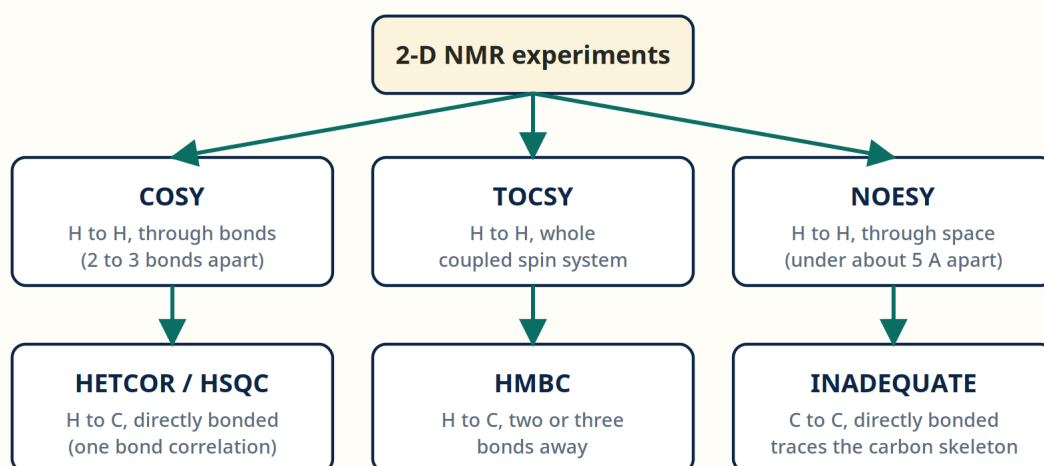
Figure: Proton shift ranges. A signal's position is the first hypothesis about its environment; 2-D experiments then test the hypothesis.

The chart is used actively, not decoratively. A three proton singlet at 3.9 ppm is a methoxy group on an aromatic ring until proved otherwise; a one proton signal near 9.8 ppm that vanishes on deuterium oxide shake was an aldehyde or an exchangeable proton, and the shake distinguishes them (an aldehyde C-H does not exchange). Exchangeable protons (O-H, N-H, S-H) give broad, concentration dependent signals that disappear on D₂O exchange, while carbon bound protons keep fixed shifts: this single experiment resolves whole classes of assignment doubt, and it costs one drop of heavy water.

2.3 Why a second dimension: the 2-D experiment

A 2-D NMR spectrum spreads the information onto a plane. One axis (F2) is the ordinary chemical shift scale of the detected nucleus; the second axis (F1) is the shift scale of a second nucleus, or of the same nucleus in an earlier part of the experiment. Peaks on the **diagonal** of a homonuclear experiment correspond to the 1-D spectrum itself. The **cross peaks** off the diagonal are the new information: a cross peak at coordinates (shift A on F1, shift B on F2) states that nucleus A and nucleus B are connected in a defined way, by coupling through bonds in COSY, by closeness in space in NOESY, or by a direct carbon-proton bond in HETCOR. Choosing an experiment is choosing which kind of connection to interrogate.

The 2-D NMR family: what each experiment correlates



Every 2-D spectrum plots one frequency axis (F2) against another (F1); cross peaks carry the structural information.

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Figure: The 2-D family. Every experiment answers exactly one kind of connectivity question; interpretation is largely a matter of asking them in the right order.

2.4 Coupling constants as structural evidence

Before the 2-D experiments, one 1-D quantity deserves its own section because the 2-D spectra lean on it: the coupling constant J , measured in hertz and independent of the spectrometer's field strength. Standard working values:

Coupling situation	Typical J (Hz)	Structural message
Geminal, two protons on one sp^3 carbon (2J)	12 to 15	A CH_2 group with diastereotopic protons
Vicinal, protons on adjacent carbons (3J)	6 to 8	Neighbouring CH groups; value follows dihedral angle
Alkene cis (3J)	6 to 12, about 10	Cis configured double bond
Alkene trans (3J)	12 to 18, about 17	Trans configured double bond
Aromatic ortho (3J)	7 to 10	Adjacent ring protons
Aromatic meta (4J)	2 to 3	Ring protons with one carbon between
Aromatic para (5J)	0 to 1	Opposite ring protons, often unresolved
Allylic (4J)	1 to 3	Proton allylic to a double bond

The vicinal value carries stereochemistry through the Karplus relationship: a dihedral angle near 180 degrees (antiperiplanar, as in axial-axial protons of a cyclohexane chair) gives a large 3J of 10 to 13 Hz, while a gauche angle near 60 degrees gives 2 to 5 Hz. Sugars and steroidal systems are routinely assigned

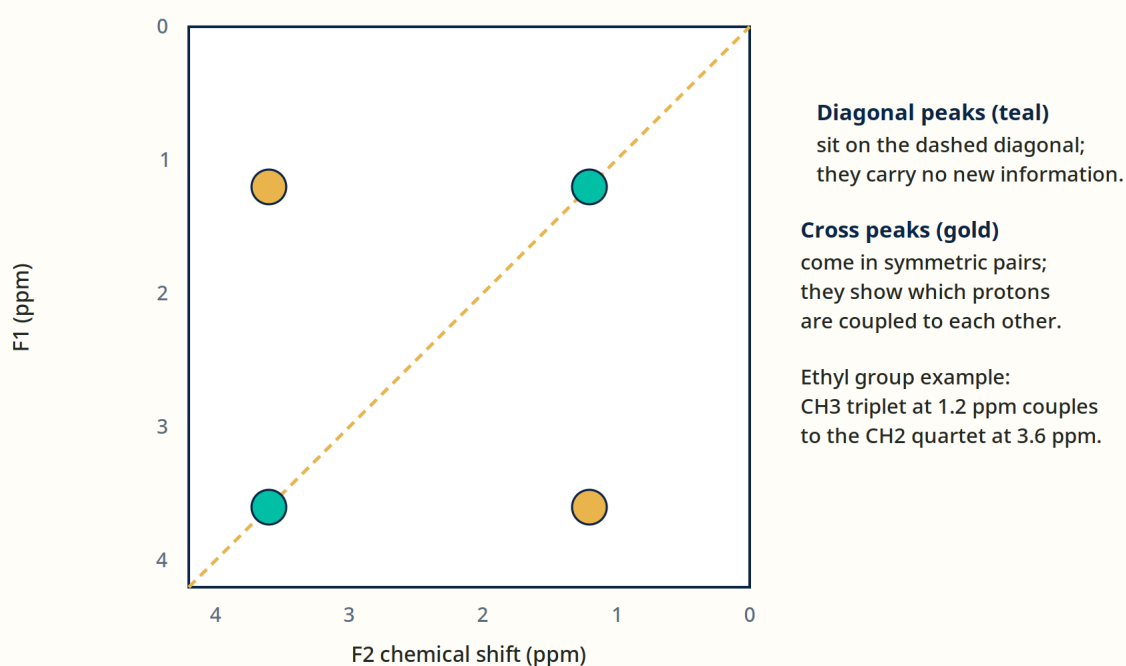
from these numbers alone. The cis and trans alkene values settle double bond geometry in one glance, a question no other routine spectrum answers as cheaply.

2.5 COSY: tracing coupled protons

In a COSY (correlation spectroscopy) experiment, both axes are the proton shift scale. A cross peak joins two proton signals that share a coupling: they are geminal partners or, far more usefully, vicinal neighbours on adjacent carbons. The spectrum is symmetric about the diagonal, so every connection appears twice.

Reading COSY is a walking exercise. Start at an unmistakable signal, an aldehyde proton, an isolated methyl singlet's neighbour, a distinctive downfield proton. Find its cross peaks: each one names a coupled partner. From that partner, find the next cross peaks, and so on, walking along the chain of coupled protons until the walk stops at a heteroatom, a quaternary carbon or a terminal methyl. Each uninterrupted walk traces one spin system, one connected run of CH and CH₂ groups.

Reading a COSY spectrum: diagonal peaks and cross peaks



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Figure: COSY logic in miniature. The ethyl group's two signals share exactly one pair of cross peaks; that pair is the entire proof that CH₃ and CH₂ are neighbours.

The common errors are worth naming. Long range couplings (allylic, aromatic meta) also give weak cross peaks, so a cross peak proves coupling, not always direct neighbourhood; intensity and the 1-D multiplicities arbitrate. And COSY cannot cross a break in the coupling network: an ether oxygen, an ester linkage or a quaternary carbon stops every walk, which is precisely why the heteronuclear experiments of section 2.7 exist.

2.6 TOCSY and NOESY: whole spin systems, and through space contacts

TOCSY (total correlation spectroscopy) extends COSY's idea. During its mixing period, magnetisation is relayed from proton to coupled proton along the whole network, so every proton of a spin system shows cross peaks to every other proton of the same system, not only to its immediate neighbours. In a sugar ring or an amino acid side chain, one TOCSY row displays the entire residue's protons at once, which is why

TOCSY is the workhorse of peptide and carbohydrate assignment.

NOESY (nuclear Overhauser effect spectroscopy) is the odd one out: its cross peaks arise not from bonds but from the nuclear Overhauser effect, dipolar relaxation between protons close **in space**, effective only under about 5 angstroms. A NOESY cross peak therefore reports proximity, and its classic uses are stereochemical: cis substituents on a ring show NOE contacts that trans ones cannot; the geometry of a double bond, the fold of a peptide, and the relative configuration of a new stereocentre are all read from NOESY. Its rotating frame cousin ROESY serves medium sized molecules whose NOE passes through zero. The discipline is to keep the two correlation types separate in one's head: COSY edges are bonds, NOESY edges are distances, and confusing them builds impossible structures.

2.7 HETCOR, HSQC and HMBC: attaching protons to carbons

Heteronuclear correlation experiments put the proton scale on one axis and the carbon scale on the other. **HETCOR**, the experiment named in the syllabus, transfers magnetisation between directly bonded carbon and proton pairs, so each cross peak assigns a proton signal to the carbon it sits on. Its modern inverse detected form, **HSQC**, does the same job with far higher sensitivity (the proton, not the carbon, is detected) and, edited HSQC even reproduces DEPT's CH/CH₂/CH₃ distinction by peak sign. After an HSQC, every protonated carbon in the molecule has a name.

HMBC retunes the same idea to small long range couplings, so its cross peaks join protons to carbons **two or three bonds away**. This is the experiment that crosses the barriers COSY cannot: a methyl singlet showing an HMBC correlation to a carbonyl carbon three bonds away places an acetyl group; aromatic protons correlating to a quaternary carbon map ring substitution; a sugar's anomeric proton correlating across the glycosidic oxygen to the next residue's carbon proves the linkage position. Quaternary carbons, invisible in HSQC, acquire assignments through the HMBC web around them. Together the three experiments convert a list of shifts into a connected structure.

2.8 INADEQUATE: the carbon skeleton directly

INADEQUATE (incredible natural abundance double quantum transfer experiment) correlates directly bonded carbon pairs through their one bond carbon-carbon coupling. Its spectrum is, in principle, the perfect answer: walk the cross peaks and you trace the entire carbon skeleton bond by bond, with no inference. Its price is sensitivity. A carbon-carbon bonded pair requires two adjacent carbon-13 nuclei; at 1.1 percent natural abundance, only about one molecule in ten thousand contains a given adjacent pair, so the experiment demands concentrated samples and long acquisitions, often overnight. INADEQUATE is therefore reserved for precious structure problems, novel natural products and skeletons that defeat the indirect methods, but every student should know it exists as the court of last appeal in carbon connectivity.

2.9 Interpretation workflow: a worked assignment

QUESTION	EXPERIMENT
Which protons are neighbours?	--> COSY (walk the cross peaks)
Which protons share a system?	--> TOCSY (whole spin systems)
Which carbon carries each H?	--> HSQC / HETCOR (one bond)
What lies across O, N, C=O, Cq?	--> HMBC (two and three bonds)
Which groups are near in space?	--> NOESY (stereochemistry)
Skeleton still ambiguous?	--> INADEQUATE (C-C bonds direct)

Worked example: ethyl acetate. Proton spectrum: 1.26 ppm (triplet, 3H), 2.04 ppm (singlet, 3H), 4.12 ppm (quartet, 2H). Carbon spectrum: 171, 60.5, 21 and 14 ppm. Work it as an examiner would. The 171 ppm carbon is an ester or acid carbonyl; IR has already shown an ester band near 1740 cm⁻¹ and no O-H, so an ester. COSY shows one cross peak pair only, between 1.26 and 4.12 ppm: an ethyl group, and its

methylene at 4.12 ppm sits on oxygen. The 2.04 ppm singlet has no COSY partner: a methyl on a carbon with no protons beyond, and at 21 ppm its carbon is the acetyl methyl. HSQC pairs 1.26 with 14, 4.12 with 60.5, and 2.04 with 21 ppm, confirming the guesses. HMBC closes the proof: the methylene protons at 4.12 ppm correlate to the carbonyl carbon at 171 ppm across the ester oxygen (three bonds), and the acetyl methyl protons correlate to the same carbonyl (two bonds). Every signal assigned, every correlation explained: $\text{CH}_3\text{COOCH}_2\text{CH}_3$, with the ester linkage proved rather than assumed. That standard, no unexplained signal and no unproven connection, is what "interpretation of organic compounds" means in this unit.

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