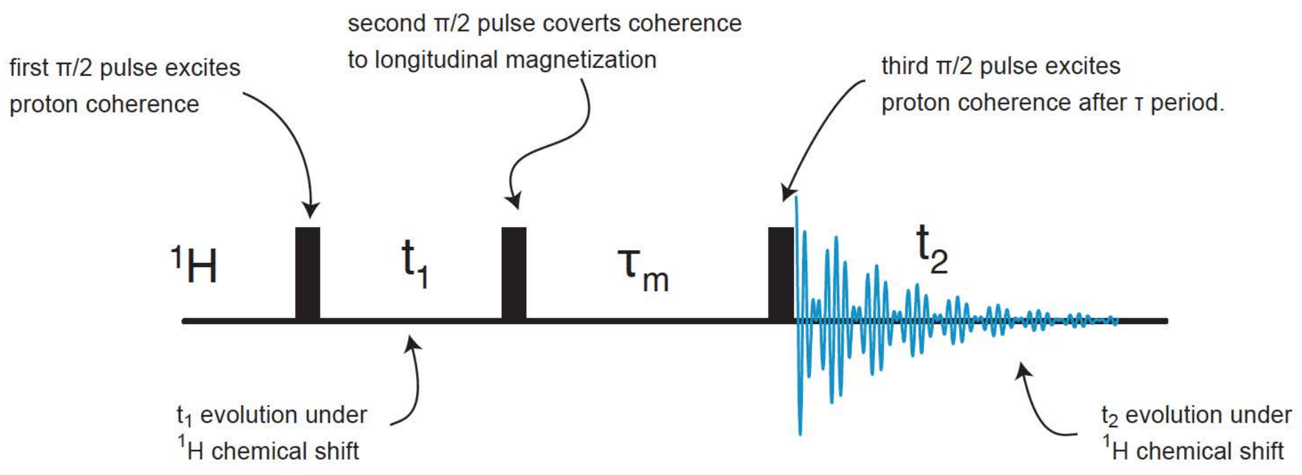
Two-Dimensional Exchange Spectroscopy (EXSY)



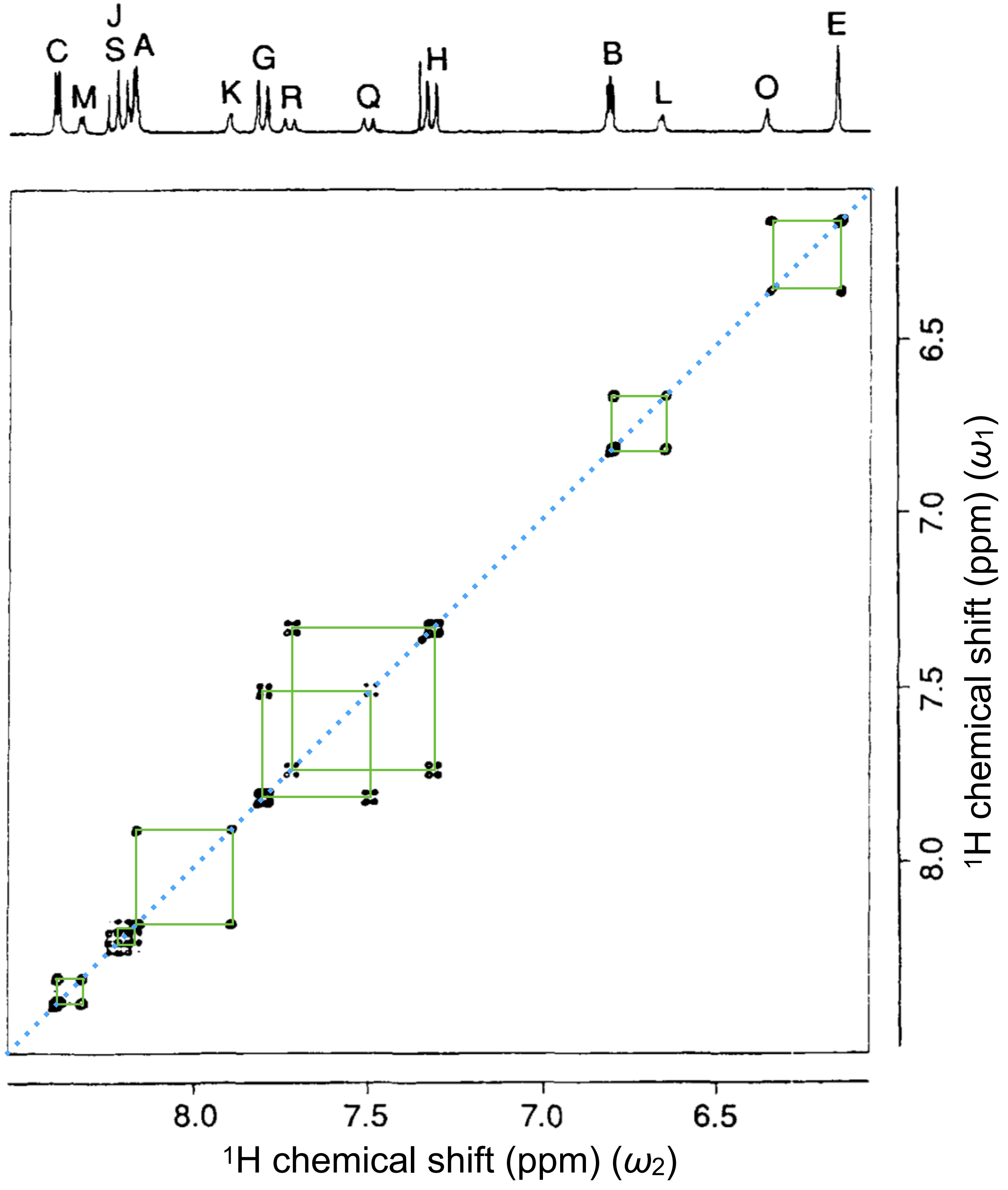
first π/2 pulse excites proton coherence

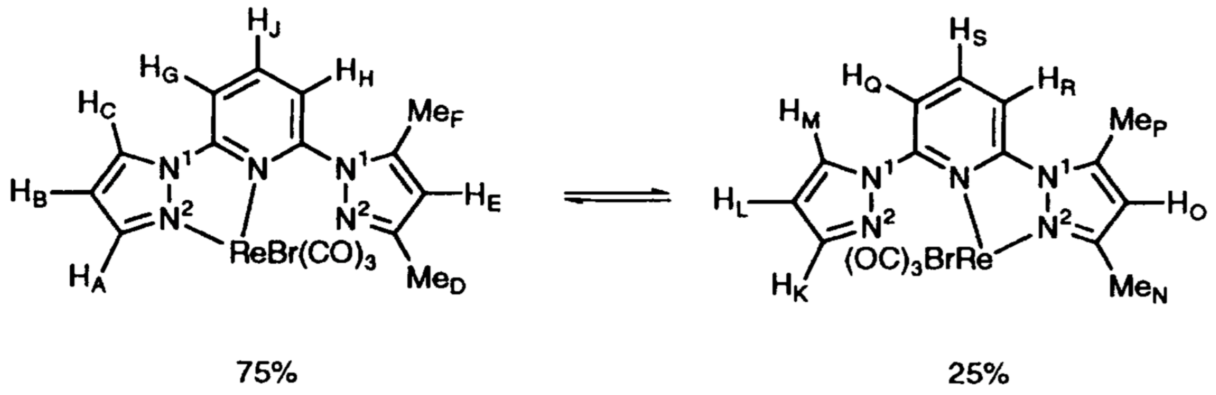
second π/2 pulse coverts coherence to longitudinal magnetization

third π/2 pulse excites proton coherence after τ period.

t_1 evolution under ^{1}H chemical shift

t_2 evolution under ^{1}H chemical shift





Proton two-dimensional exchange spectrum of the organometallic fluxional compound [ReBr(CO)3(Me2-bppy)], where bppy denotes 2,6-bis(pyrazol-1-yl)pyridine. The mixing interval was τ_m = 0.1 s. The off-diagonal peaks may be interpreted in terms of an exchange of the metal atom between two pairs of nitrogen binding sites.
Adapted from E. W. Abel, et al., J. Chem. Soc. Dalton Trans., 1079 (1994).

Two-Dimensional Correlation Spectroscopy

In the 2D EXSY spectrum, there will be cross peaks between the resonances of different atoms if they can be **interconverted by chemical exchange**.

It turns out that there are many other ways to generate correlations between resonance frequencies. To understand this, we will need the full quantum-mechanical treatment of coupled spins, that will be part of Advanced NMR.

For now, **we just note that pulse sequences can be designed to generate cross peaks between the resonances of different atoms if they are J-coupled**.

For analytical chemistry this is of great importance, since it allows us **to directly trace out connections between bonded nuclei**.

Two-Dimensional Correlation Spectroscopy

Correlations can be obtained for example between

J-coupled homonuclei: most often ^{1}H-^{1}H, but also ^{13}C-^{13}C, ^{31}P-^{31}P or ^{29}Si-^{29}Si… (sensitivity can be severely limited by the natural abundance of the isotope; e.g. ^{13}C is 1% abundant, so only 0.01% of molecules will contain a neighbouring ^{13}C-^{13}C pair.)

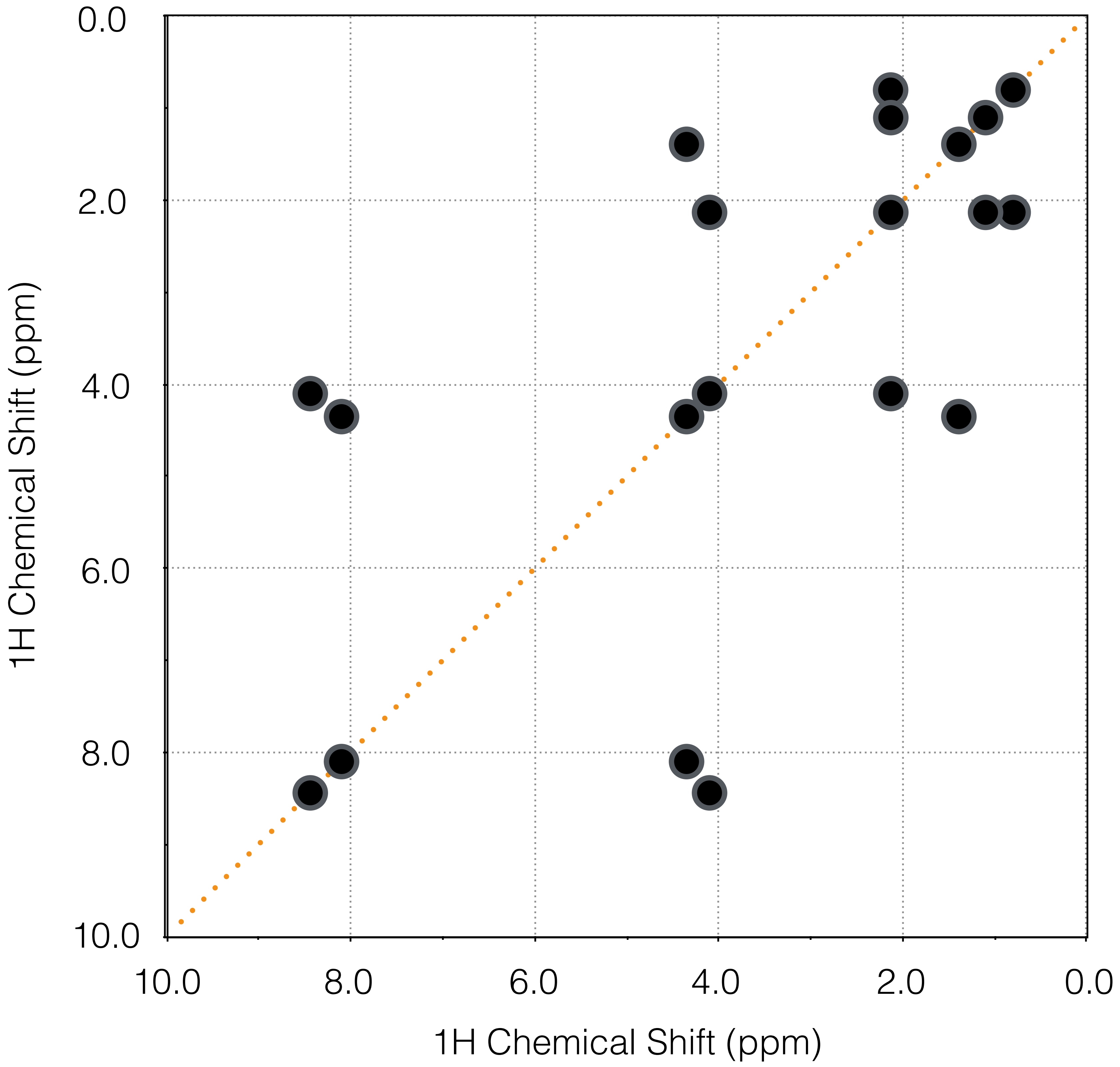
Between ^{1}H and a bonded (J-coupled) heteronucleus: e.g. ^{1}H-^{13}C, ^{1}H-^{15}N, ^{1}H-^{31}P, ^{1}H-^{29}Si, ^{1}H-^{27}Al, ^{1}H-^{11}B…

Between two different heteronuclei: e.g. ^{13}C-^{15}N (in proteins), ^{31}P-^{27}Al (e.g in aluminophosphates), ^{29}Si-^{27}Al (e.g. in silicates), ^{19}F-^{13}C (in pharmaceuticals)….

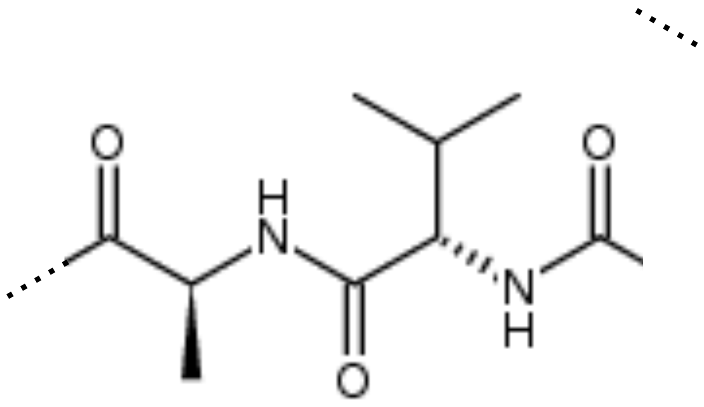
Summary of Cornerstone 2D NMR Experiments

Name:	Correlation by:
EXSY (NOESY)	^1H-^1H exchange
COSY (DQF-COSY)	^1H-^1H Scalar Couplings (directly coupled spins only)
TOCSY	^1H-^1H Scalar Couplings (whole spin system)
HSQC / HMQC	^1H-X $^1J_{HX}$ Scalar couplings (X = ^{13}C, ^{13}N, ^{13}P, ^{29}Si…)

Summary of Cornerstone 2D NMR Experiments

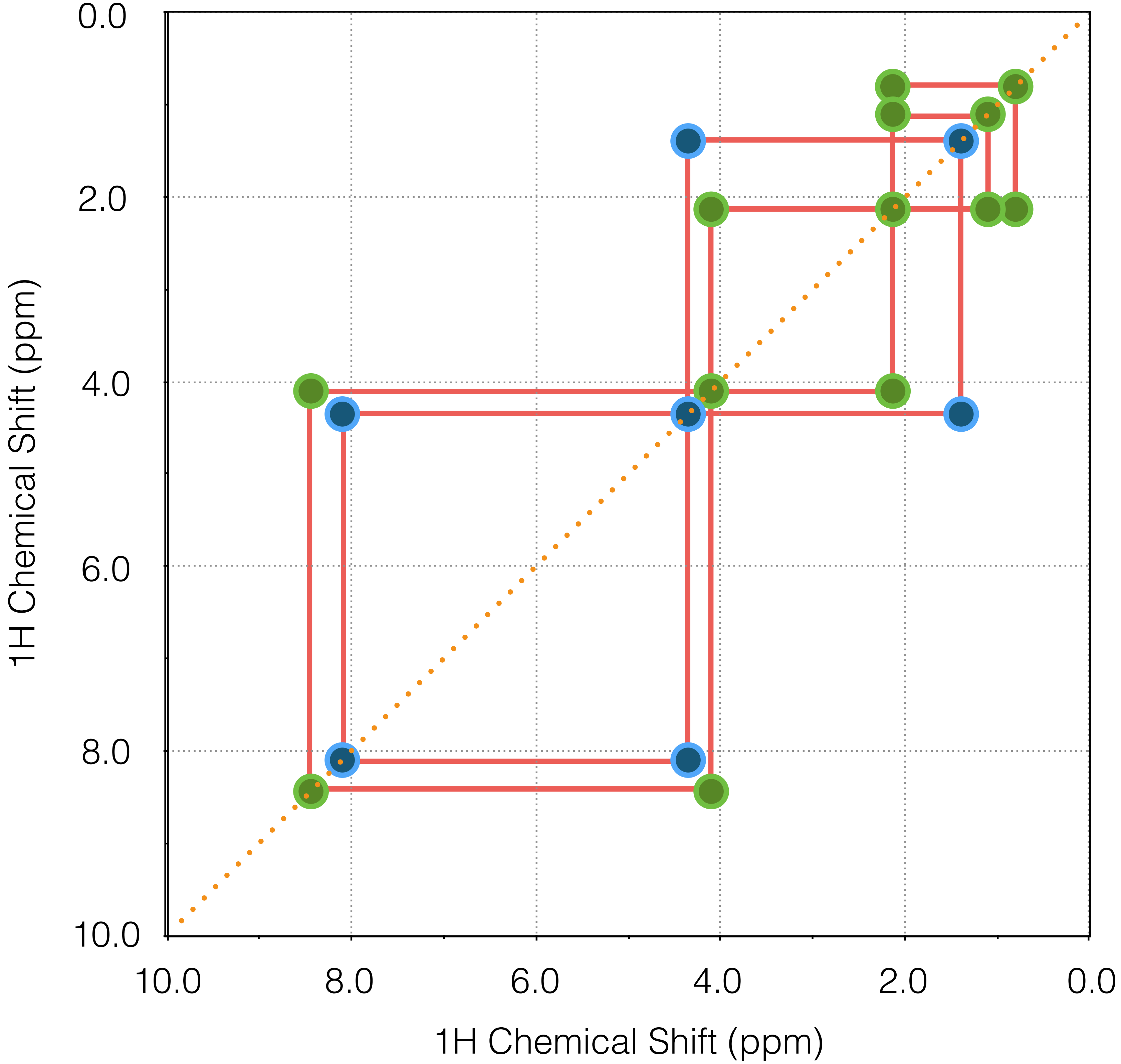


DQF-COSY
(directly J coupled
connections)

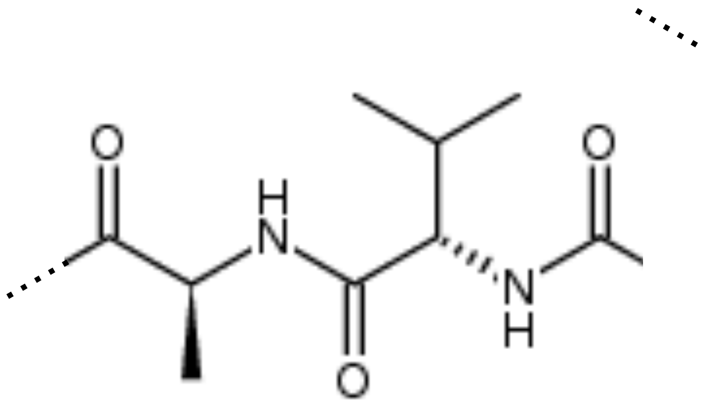


(example of schematic
connections expected for
a valine-alanine fragment
in a protein)

Summary of Cornerstone 2D NMR Experiments



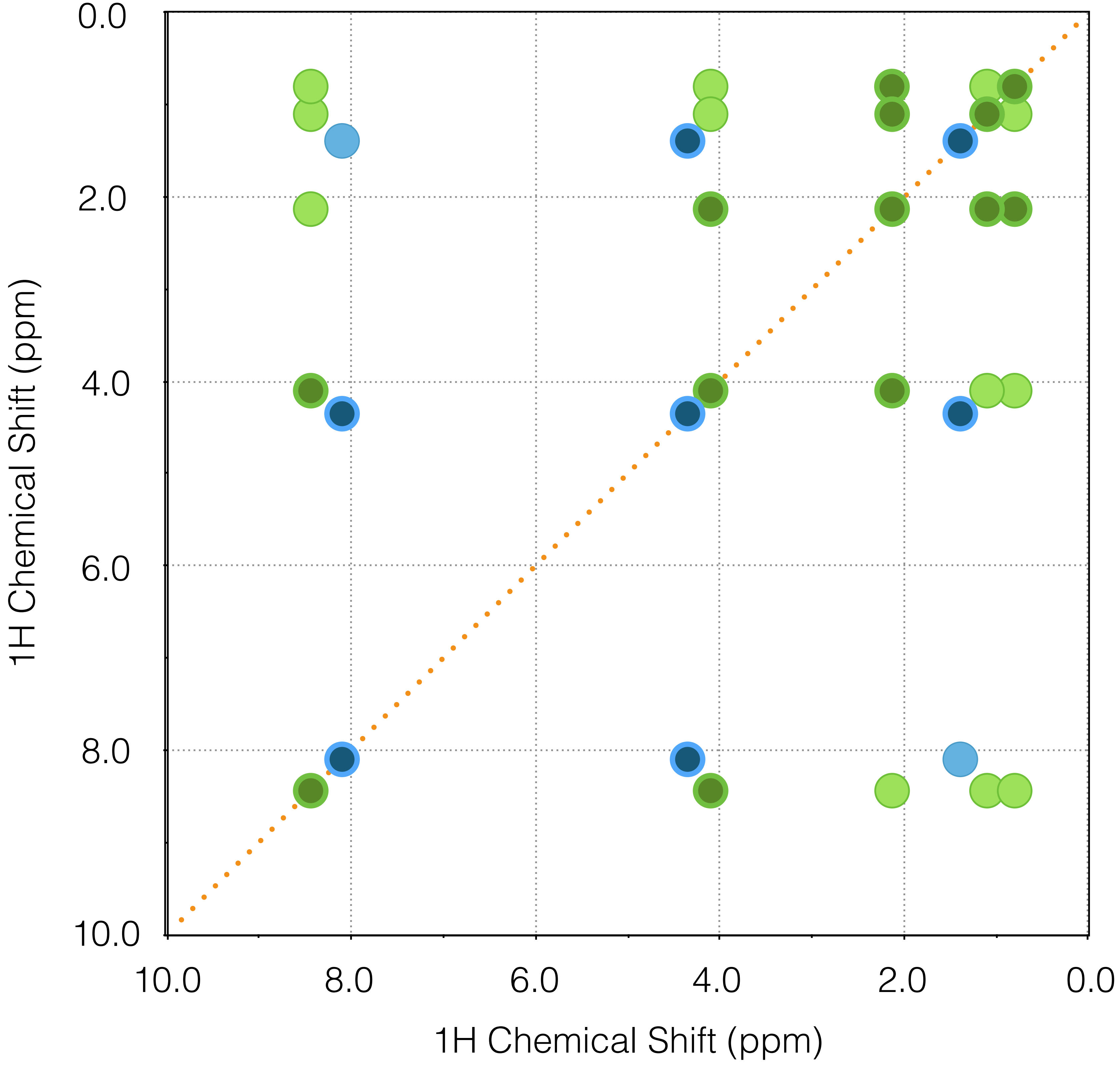
DQF-COSY
(directly J coupled
connections)



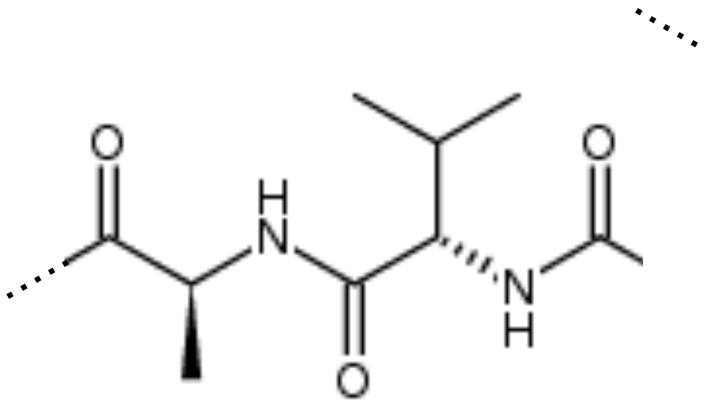
(example of schematic
connections expected for
a valine-alanine fragment
in a protein)

homework: assign the spectrum

Summary of Cornerstone 2D NMR Experiments



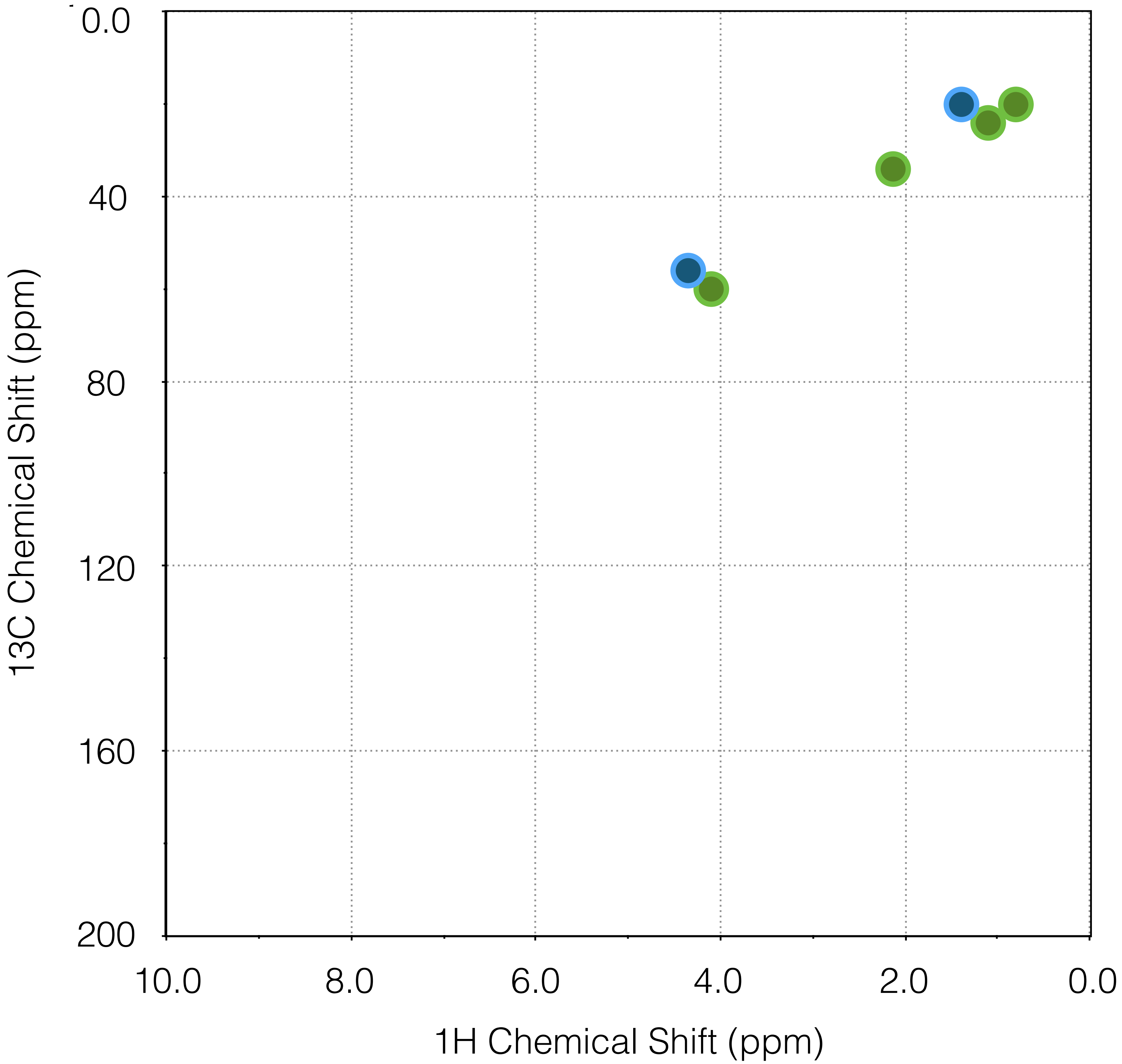
TOCSY
(all connections within a coupled spin system)



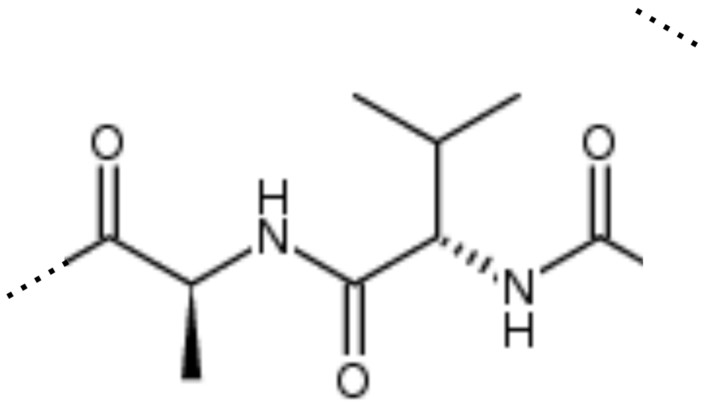
(example of schematic connections expected for a valine-alanine fragment in a protein)

homework: assign the spectrum

Summary of Cornerstone 2D NMR Experiments



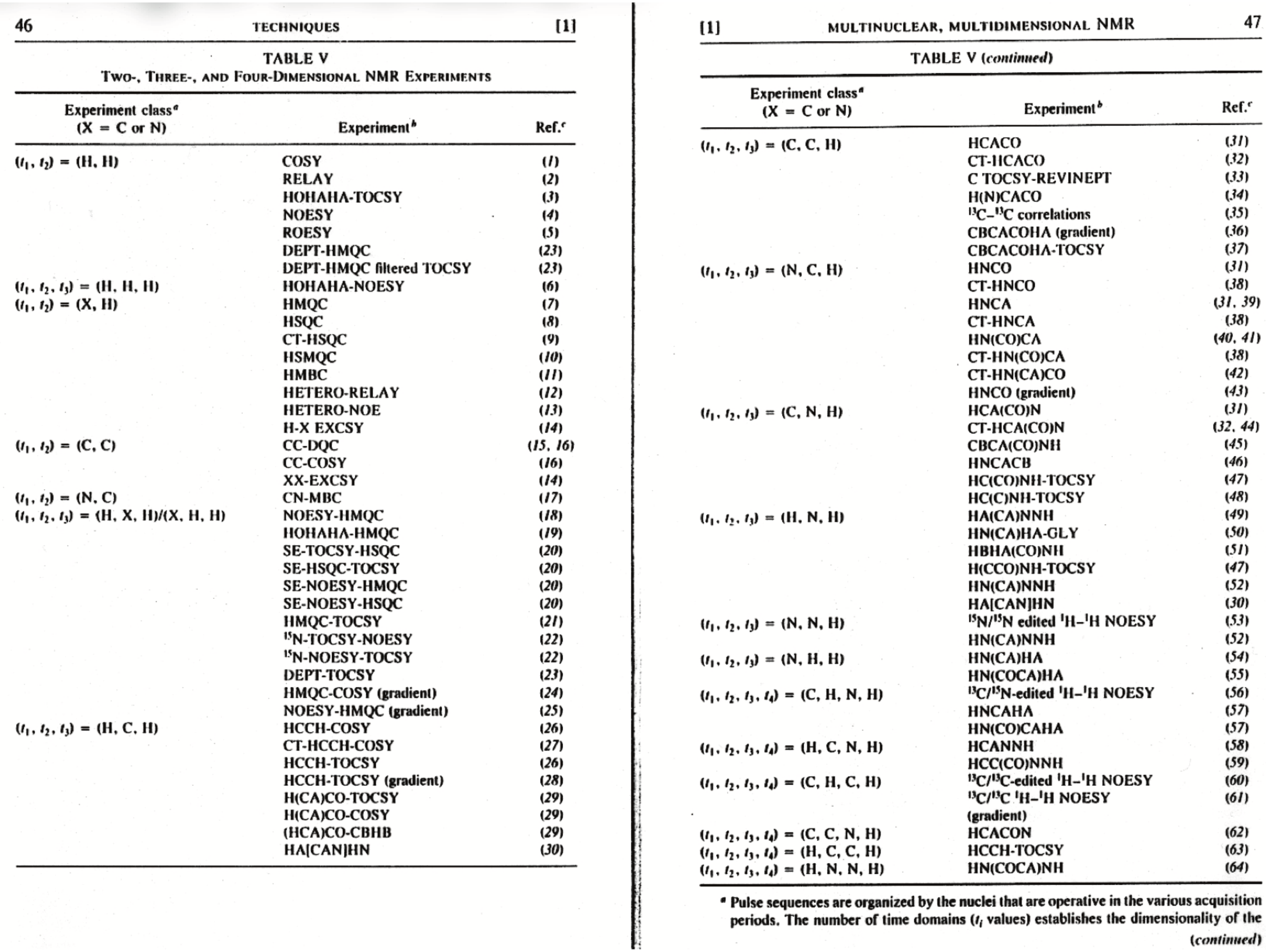
HSQC
(one-bond C-H
correlations)



(example of schematic connections expected for a valine-alanine fragment in a protein)

homework: assign the spectrum

The Tip of the Iceberg….



46 TECHNIQUES [1]

TABLE V
TWO-, THREE-, AND FOUR-DIMENSIONAL NMR EXPERIMENTS

Experiment class[a] (X = C or N)	Experiment[b]	Ref.[c]
$(t_1, t_2) = (H, H)$	COSY	(*1*)
	RELAY	(*2*)
	HOHAHA-TOCSY	(*3*)
	NOESY	(*4*)
	ROESY	(*5*)
	DEPT-HMQC	(*23*)
	DEPT-HMQC filtered TOCSY	(*23*)
$(t_1, t_2, t_3) = (H, H, H)$	HOHAHA-NOESY	(*6*)
$(t_1, t_2) = (X, H)$	HMQC	(*7*)
	HSQC	(*8*)
	CT-HSQC	(*9*)
	HSMQC	(*10*)
	HMBC	(*11*)
	HETERO-RELAY	(*12*)
	HETERO-NOE	(*13*)
	H-X EXCSY	(*14*)
$(t_1, t_2) = (C, C)$	CC-DQC	(*15, 16*)
	CC-COSY	(*16*)
	XX-EXCSY	(*14*)
$(t_1, t_2) = (N, C)$	CN-MBC	(*17*)
$(t_1, t_2, t_3) = (H, X, H)/(X, H, H)$	NOESY-HMQC	(*18*)
	HOHAHA-HMQC	(*19*)
	SE-TOCSY-HSQC	(*20*)
	SE-HSQC-TOCSY	(*20*)
	SE-NOESY-HMQC	(*20*)
	SE-NOESY-HSQC	(*20*)
	HMQC-TOCSY	(*21*)
	^{15}N-TOCSY-NOESY	(*22*)
	^{15}N-NOESY-TOCSY	(*22*)
	DEPT-TOCSY	(*23*)
	HMQC-COSY (gradient)	(*24*)
	NOESY-HMQC (gradient)	(*25*)
$(t_1, t_2, t_3) = (H, C, H)$	HCCH-COSY	(*26*)
	CT-HCCH-COSY	(*27*)
	HCCH-TOCSY	(*26*)
	HCCH-TOCSY (gradient)	(*28*)
	H(CA)CO-TOCSY	(*29*)
	H(CA)CO-COSY	(*29*)
	(HCA)CO-CBHB	(*29*)
	HA[CAN]HN	(*30*)

[1] MULTINUCLEAR, MULTIDIMENSIONAL NMR 47

TABLE V (*continued*)

Experiment class[a] (X = C or N)	Experiment[b]	Ref.[c]
$(t_1, t_2, t_3) = (C, C, H)$	HCACO	(*31*)
	CT-HCACO	(*32*)
	C TOCSY-REVINEPT	(*33*)
	H(N)CACO	(*34*)
	^{13}C–^{13}C correlations	(*35*)
	CBCACOHA (gradient)	(*36*)
	CBCACOHA-TOCSY	(*37*)
$(t_1, t_2, t_3) = (N, C, H)$	HNCO	(*31*)
	CT-HNCO	(*38*)
	HNCA	(*31, 39*)
	CT-HNCA	(*38*)
	HN(CO)CA	(*40, 41*)
	CT-HN(CO)CA	(*38*)
	CT-HN(CA)CO	(*42*)
	HNCO (gradient)	(*43*)
$(t_1, t_2, t_3) = (C, N, H)$	HCA(CO)N	(*31*)
	CT-HCA(CO)N	(*32, 44*)
	CBCA(CO)NH	(*45*)
	HNCACB	(*46*)
	HC(CO)NH-TOCSY	(*47*)
	HC(C)NH-TOCSY	(*48*)
$(t_1, t_2, t_3) = (H, N, H)$	HA(CA)NNH	(*49*)
	HN(CA)HA-GLY	(*50*)
	HBHA(CO)NH	(*51*)
	H(CCO)NH-TOCSY	(*47*)
	HN(CA)NNH	(*52*)
	HA[CAN]HN	(*30*)
$(t_1, t_2, t_3) = (N, N, H)$	^{15}N/^{15}N edited ^{1}H–^{1}H NOESY	(*53*)
	HN(CA)NNH	(*52*)
$(t_1, t_2, t_3) = (N, H, H)$	HN(CA)HA	(*54*)
	HN(COCA)HA	(*55*)
$(t_1, t_2, t_3, t_4) = (C, H, N, H)$	^{13}C/^{15}N-edited ^{1}H–^{1}H NOESY	(*56*)
	HNCAHA	(*57*)
	HN(CO)CAHA	(*57*)
$(t_1, t_2, t_3, t_4) = (H, C, N, H)$	HCANNH	(*58*)
	HCC(CO)NNH	(*59*)
$(t_1, t_2, t_3, t_4) = (C, H, C, H)$	^{13}C/^{13}C-edited ^{1}H–^{1}H NOESY	(*60*)
	^{13}C/^{13}C ^{1}H–^{1}H NOESY (gradient)	(*61*)
$(t_1, t_2, t_3, t_4) = (C, C, N, H)$	HCACON	(*62*)
$(t_1, t_2, t_3, t_4) = (H, C, C, H)$	HCCH-TOCSY	(*63*)
$(t_1, t_2, t_3, t_4) = (H, N, N, H)$	HN(COCA)NH	(*64*)

[a] Pulse sequences are organized by the nuclei that are operative in the various acquisition periods. The number of time domains (t_i values) establishes the dimensionality of the

(*continued*)

The Tip of the Iceberg….

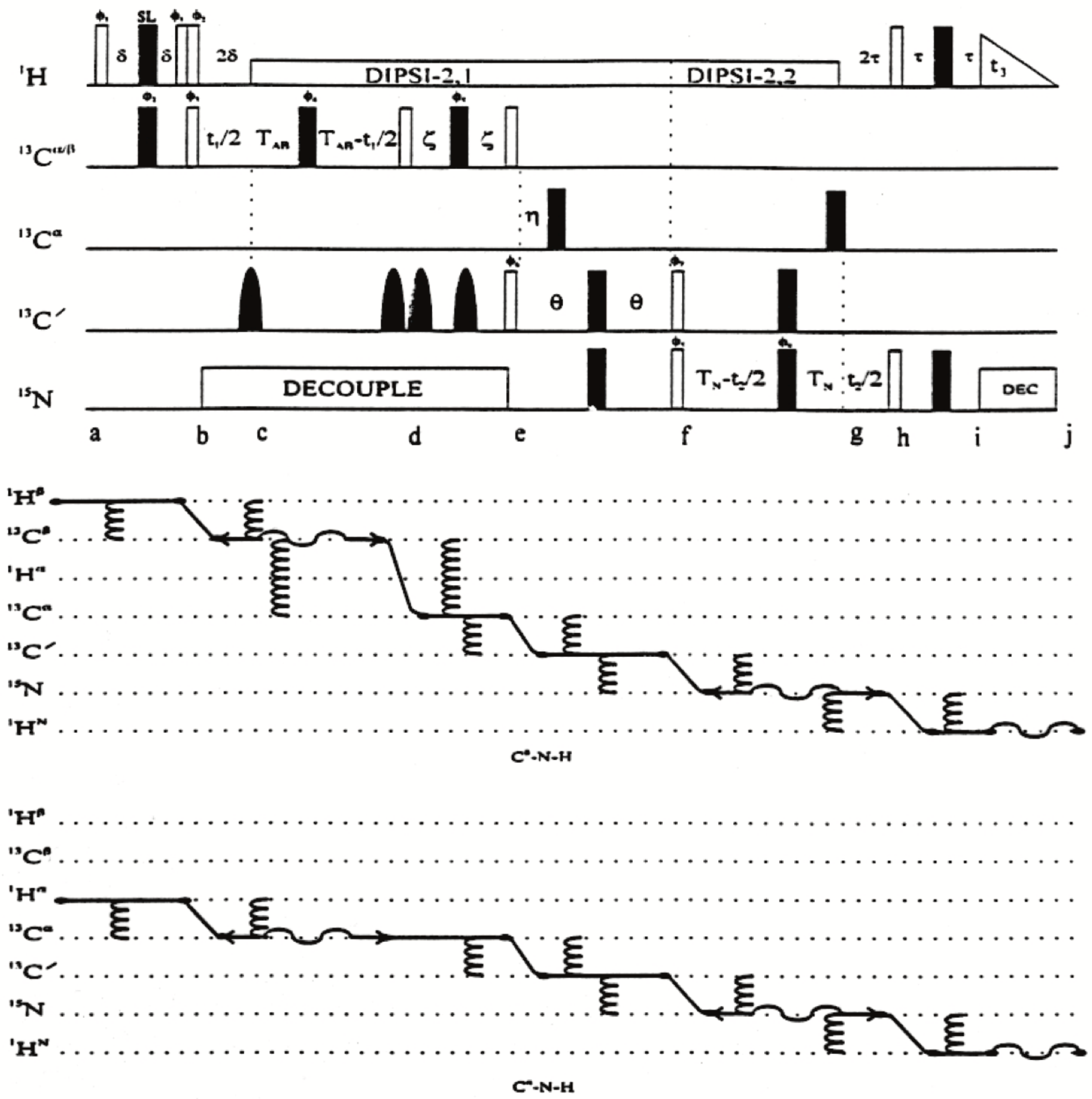


FIG. 14. Pulse sequence and CFN for 3D-CBCA(CO)NH.[11] The pulses and phases have the same convention described in Fig. 12. For values of the delays, consult the original work. The second and third selective (rounded) carbonyl pulses and the phase labeled ϕ_7 serve to remove nonresonant phase distortions (Section III.C). The CFN is best shown as two separate experiments, one originating from $^1H^\beta$ and the second originating from $^1H^\alpha$.

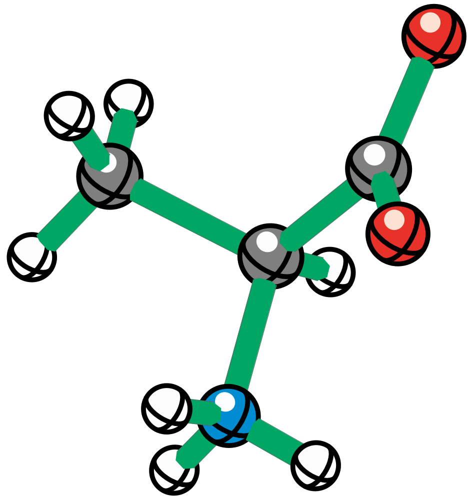
Nuclear Magnetic Resonance

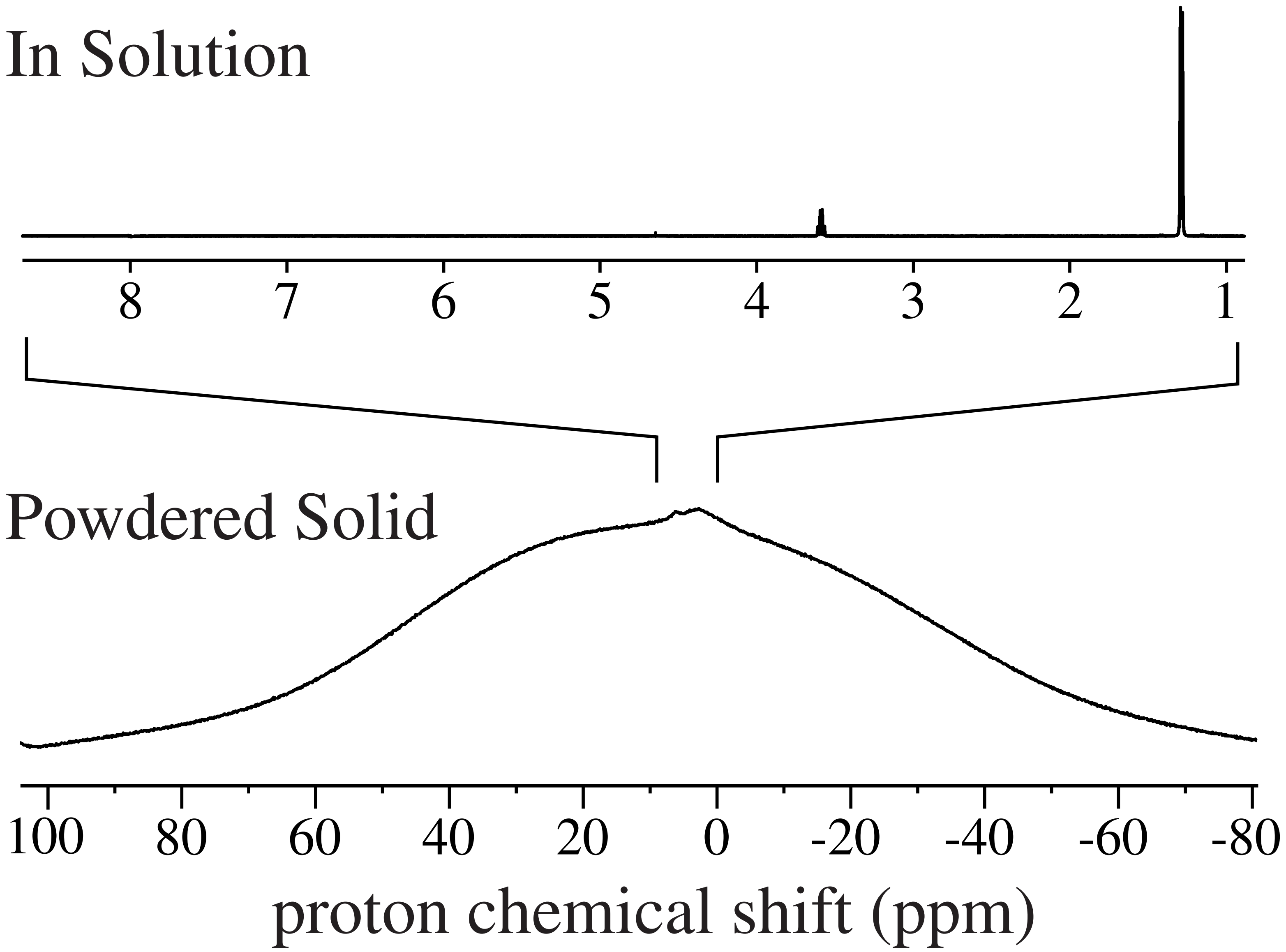
Week 4 NMR: Solid-state NMR

Objectives

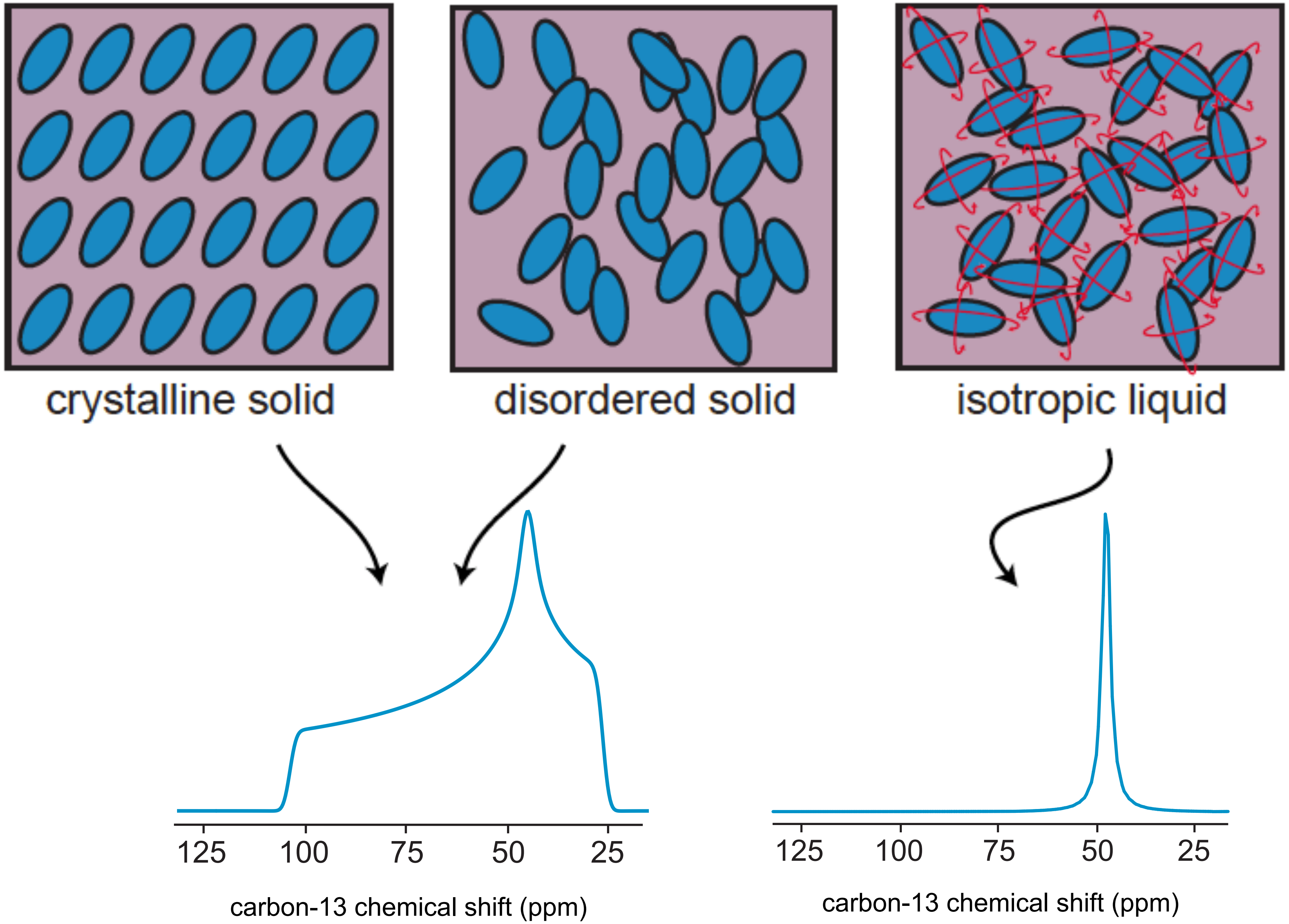
- Learn about anisotropy
- Learn about coherent averaging
- Know what MAS and spin decoupling are

What's the Problem?



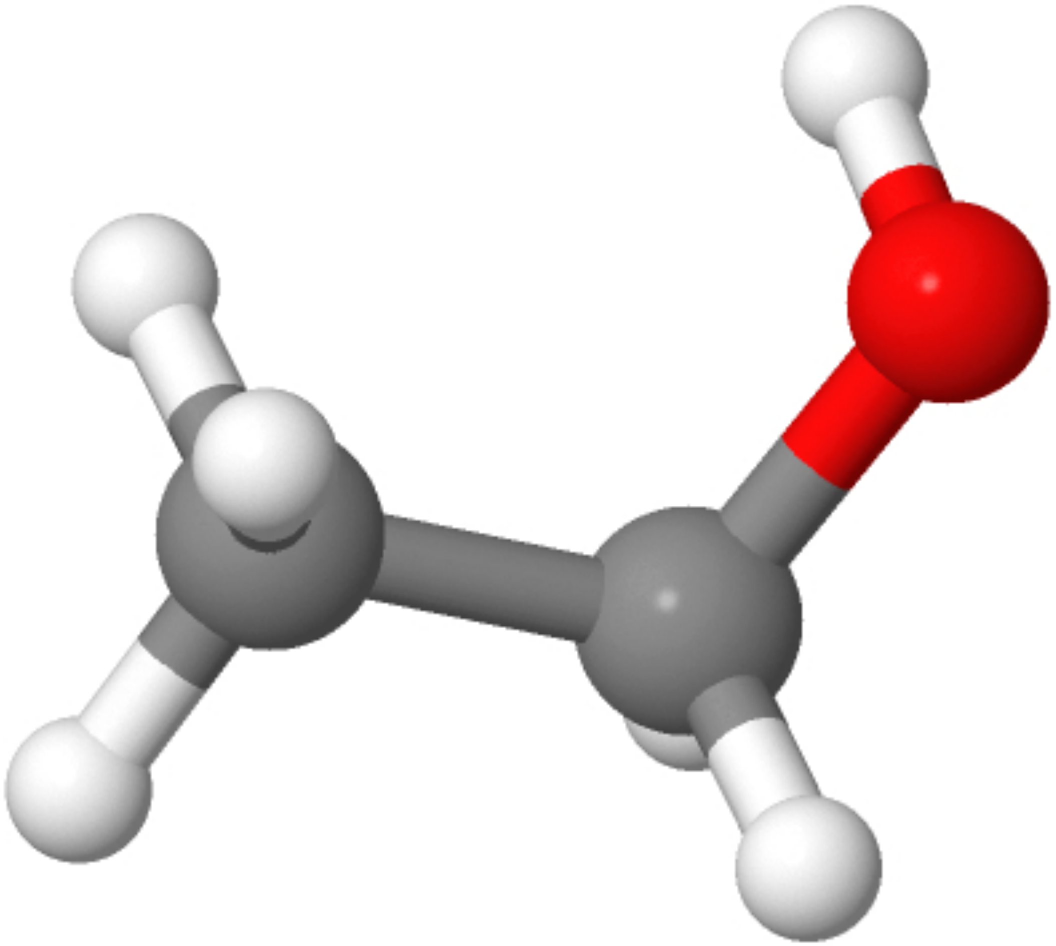


Solid-State NMR: Why is it different?



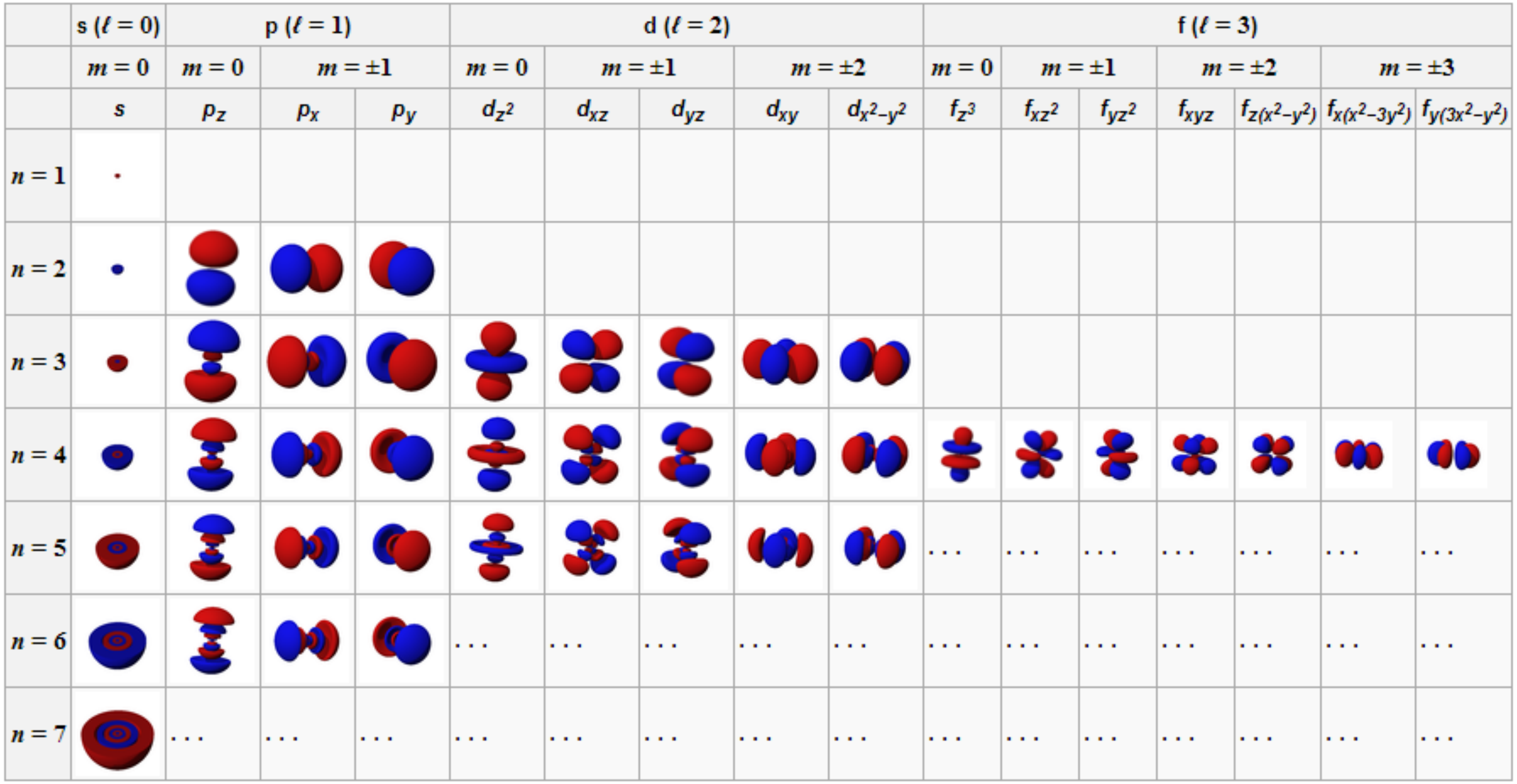
The only difference between solids and liquids, for NMR,
is the presence or absence of rapid molecular motion.

NMR interactions are anisotropic



Consider the chemical shift of the CH_2 carbon resonance in ethanol

NMR interactions are anisotropic



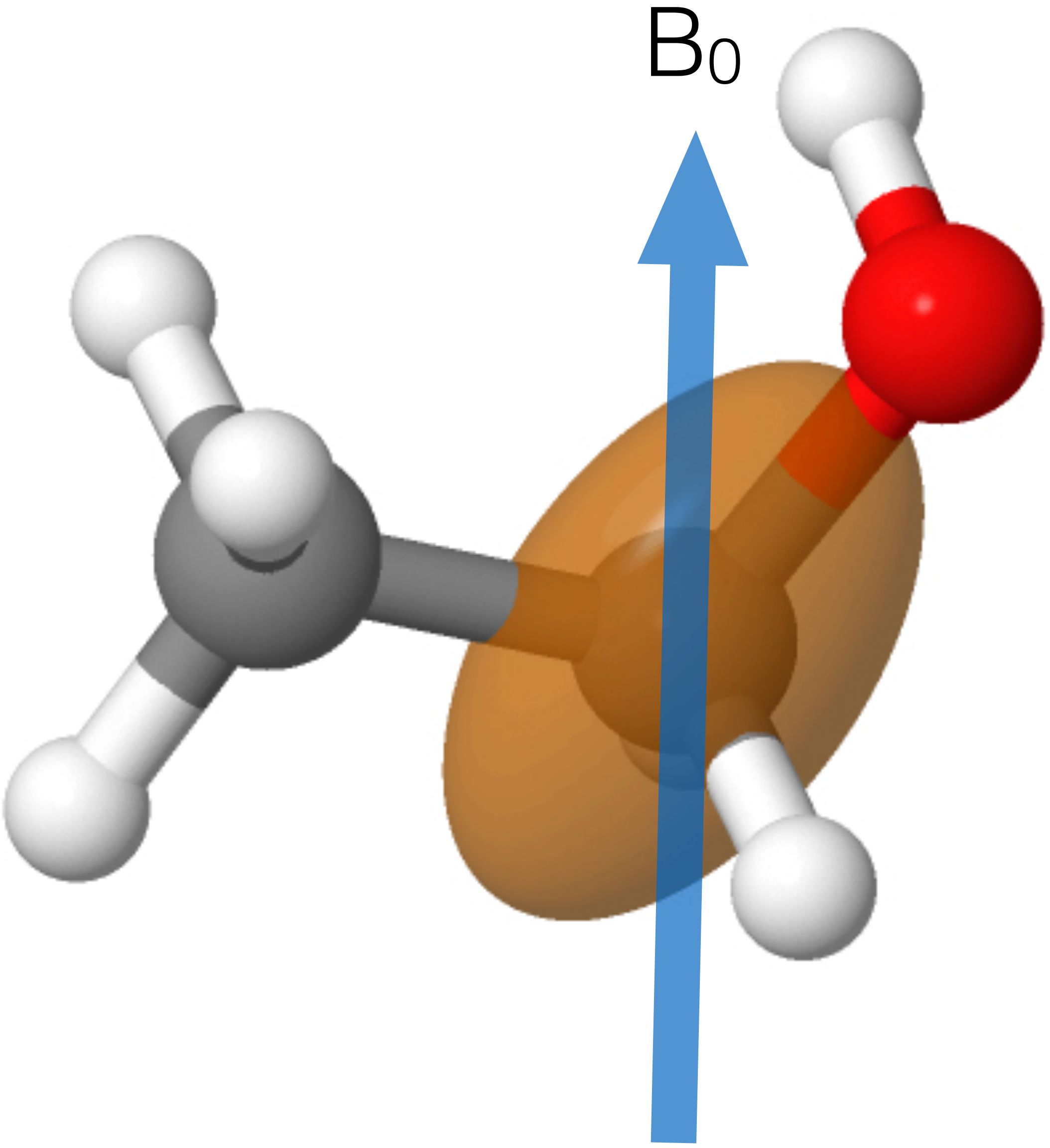
	s ($\ell = 0$)	p ($\ell = 1$)			d ($\ell = 2$)					f ($\ell = 3$)						
	$m = 0$	$m = 0$	$m = \pm 1$		$m = 0$	$m = \pm 1$		$m = \pm 2$		$m = 0$	$m = \pm 1$		$m = \pm 2$		$m = \pm 3$	
	s	p_z	p_x	p_y	d_{z^2}	d_{xz}	d_{yz}	d_{xy}	$d_{x^2-y^2}$	f_{z^3}	f_{xz^2}	f_{yz^2}	f_{xyz}	$f_{z(x^2-y^2)}$	$f_{x(x^2-3y^2)}$	$f_{y(3x^2-y^2)}$
$n = 1$																
$n = 2$																
$n = 3$																
$n = 4$																
$n = 5$										…	…	…	…	…	…	…
$n = 6$					…	…	…	…	…	…	…	…	…	…	…	…
$n = 7$		…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

The chemical shift can be thought of as the shielding of the nucleus from the external magnetic field by the electrons.

The magnetic field is a vector quantity (the magnetic field has a well defined direction). The electronic distribution around the nucleus is highly ***anisotropic***.

Therefore the chemical shift must depend on the orientation of the molecule with respect to the magnetic field.

NMR interactions are anisotropic



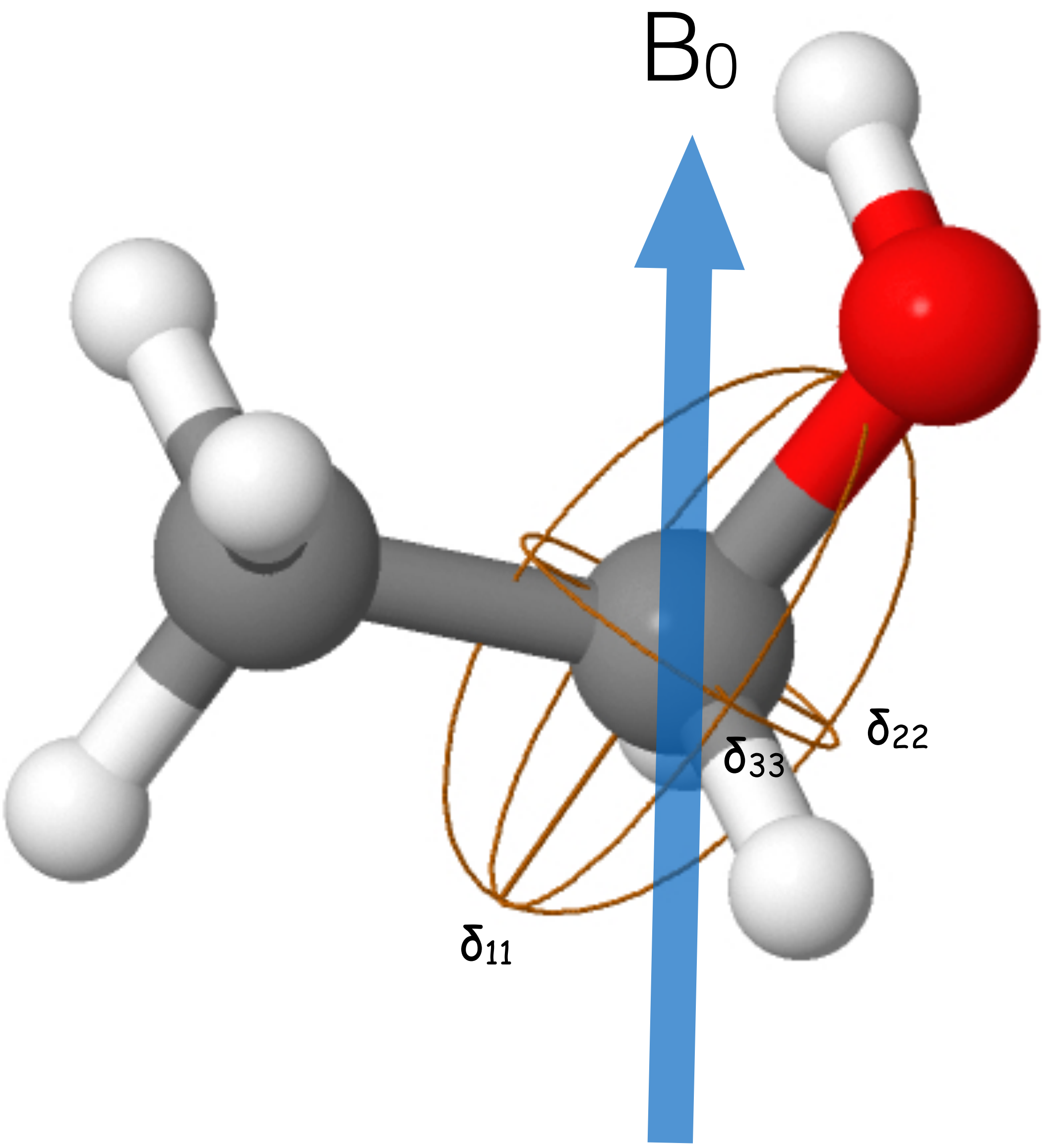
Here we represent the **chemical shift tensor** as (something like) an ellipsoid superimposed on the molecular structure.

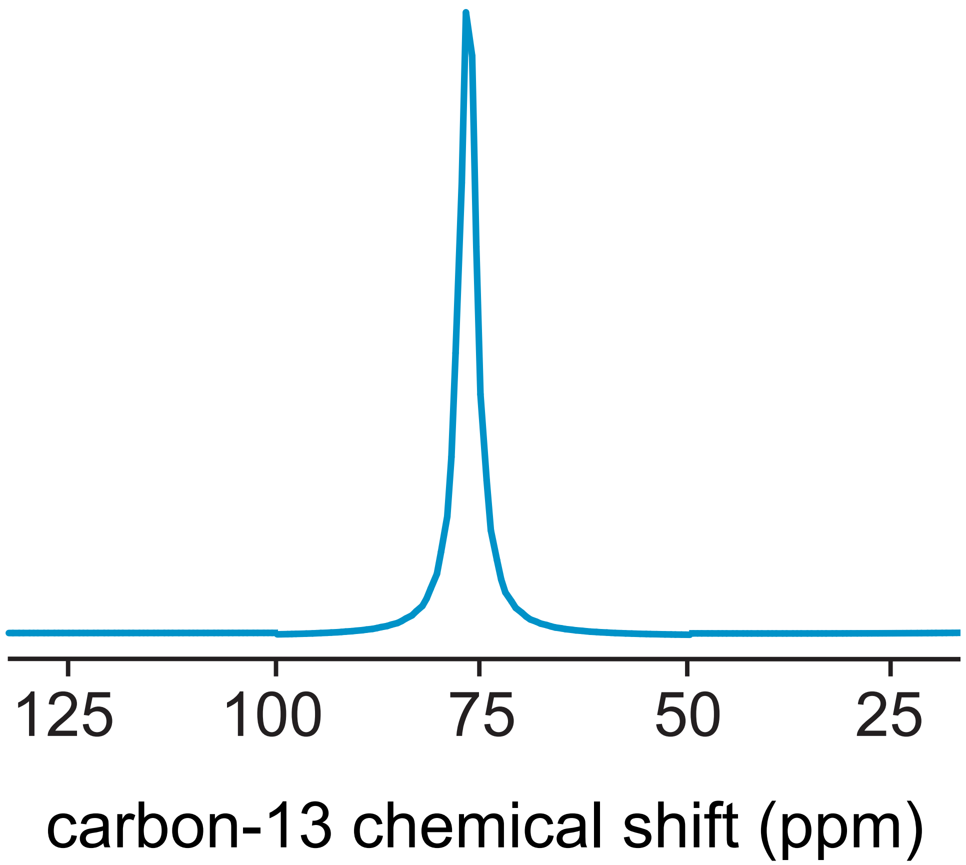
The shift tensor is fixed in the molecular frame.

Reminder: Shielding (σ) is related to chemical shift (δ) by:
$\delta \propto (1 - \sigma)$

The chemical shift is anisotropic. It is not described by a single number, but by a second rank spatial tensor, defined by the three orthogonal principal values of the tensor and the angles that define the orientation of the axes system of the principle values in the molecular reference frame.

NMR interactions are anisotropic





$\delta_{11} = 104$ ppm ; $\delta_{22} = 44$ ppm ; $\delta_{33} = 25$ ppm (by convention $\delta_{11} > \delta_{22} > \delta_{33}$)

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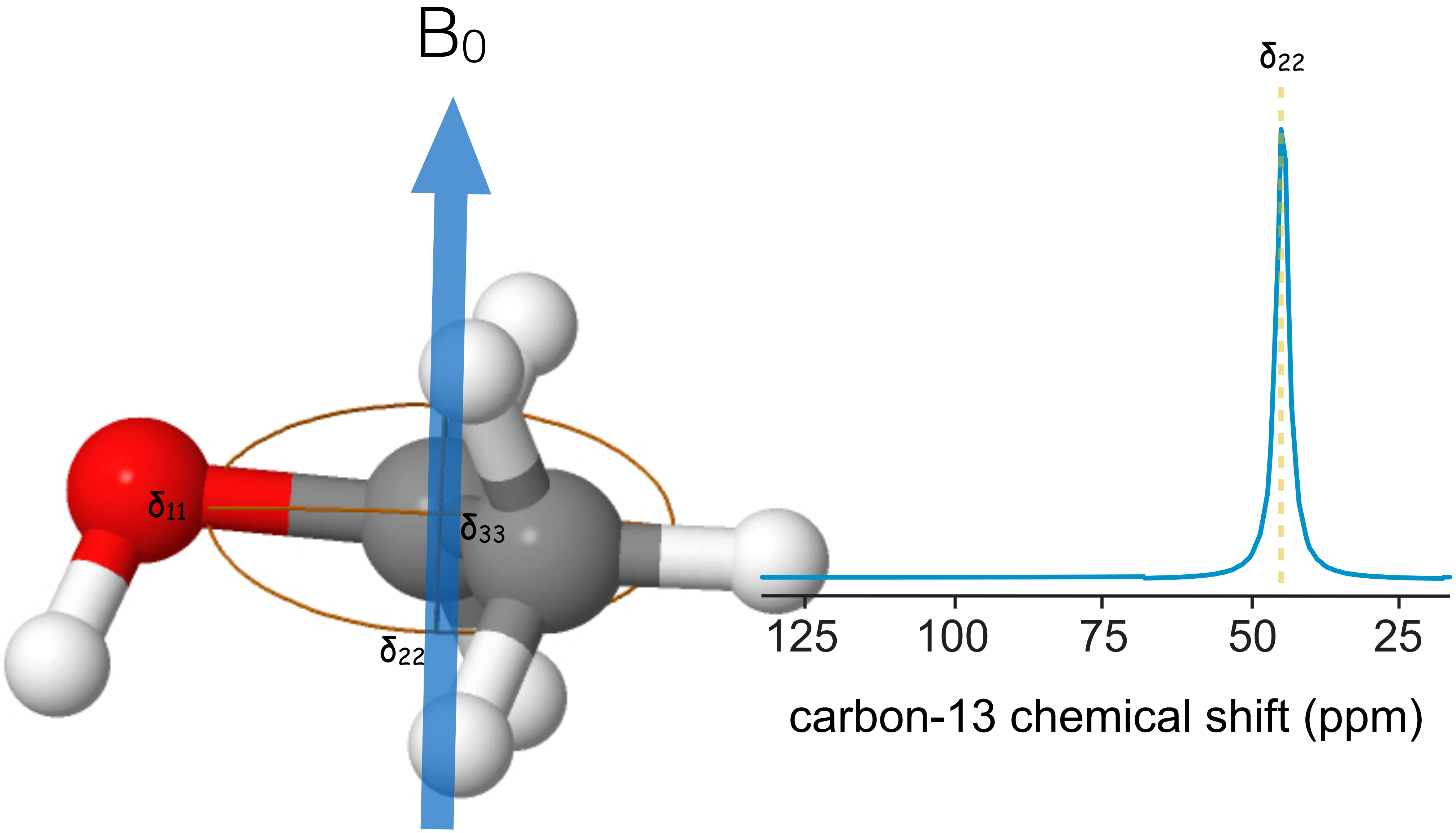
NMR interactions are anisotropic



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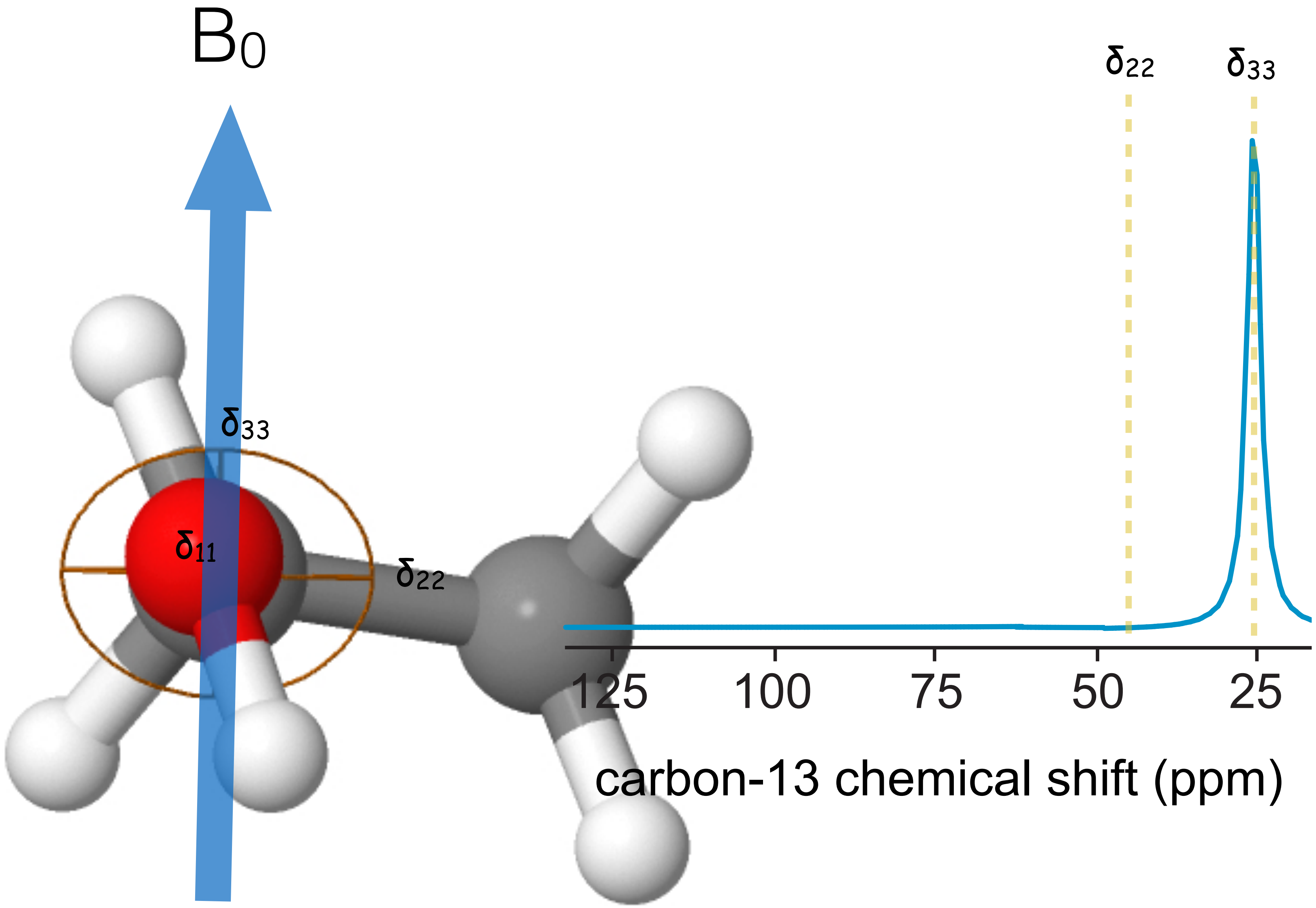

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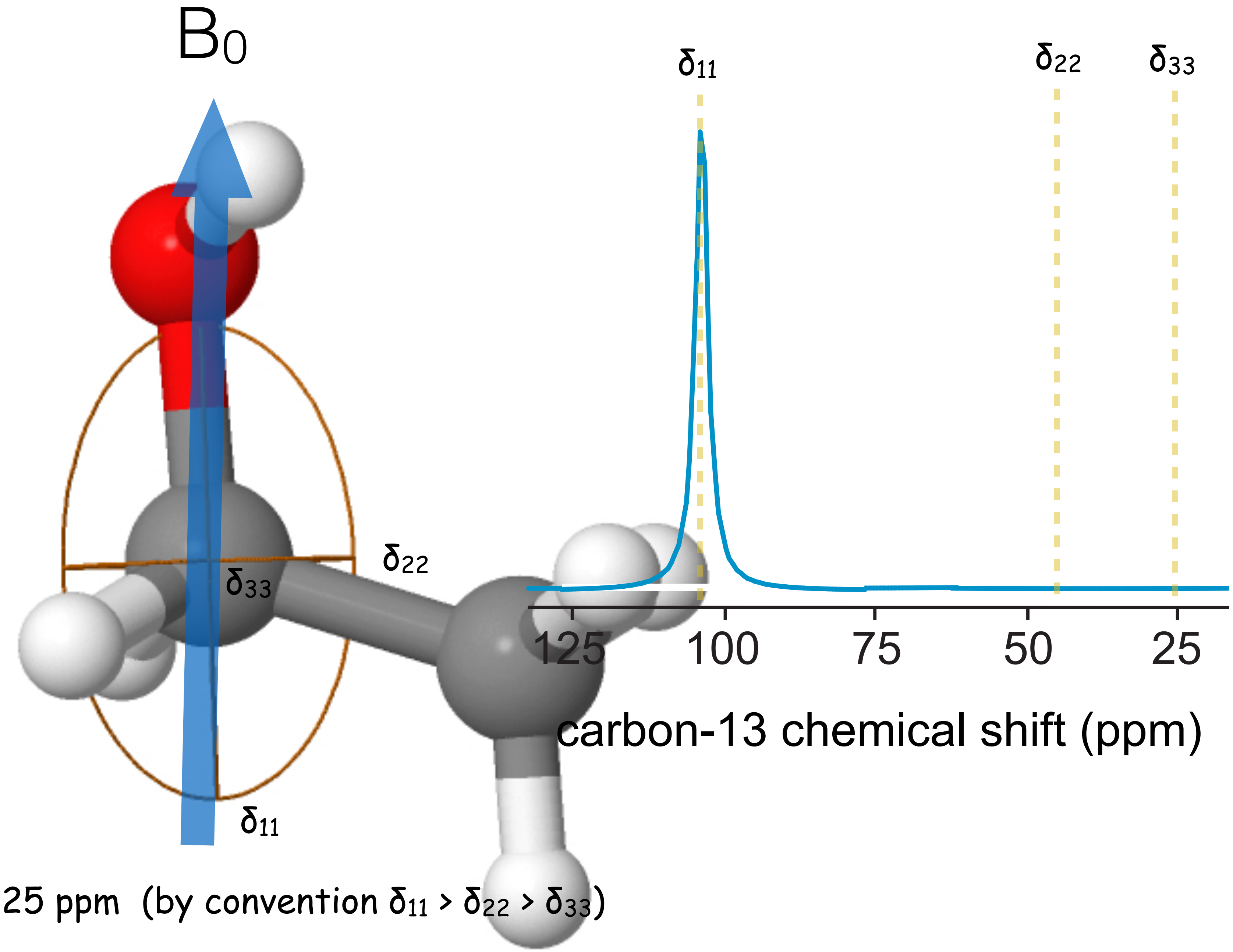

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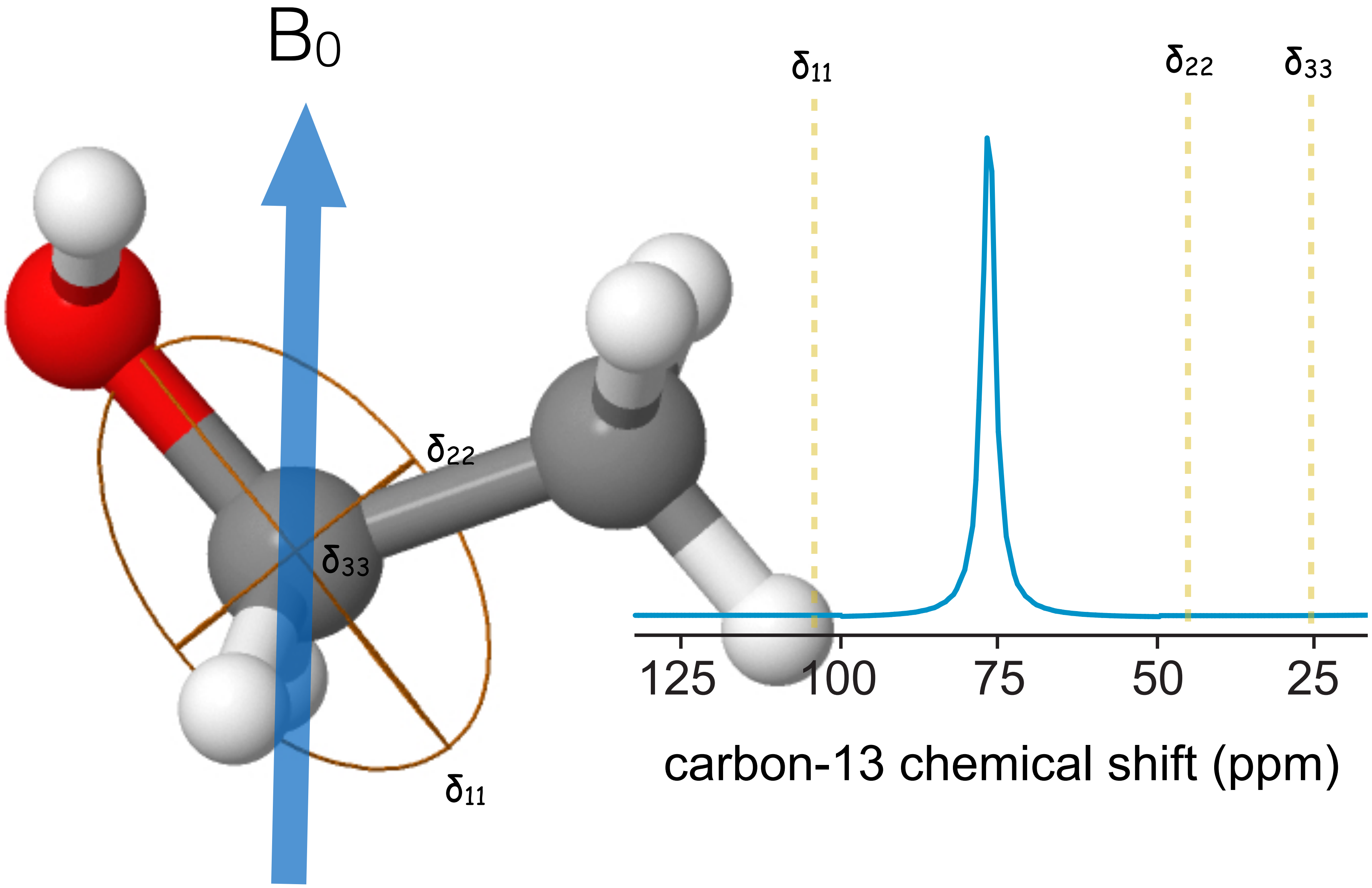
NMR interactions are anisotropic



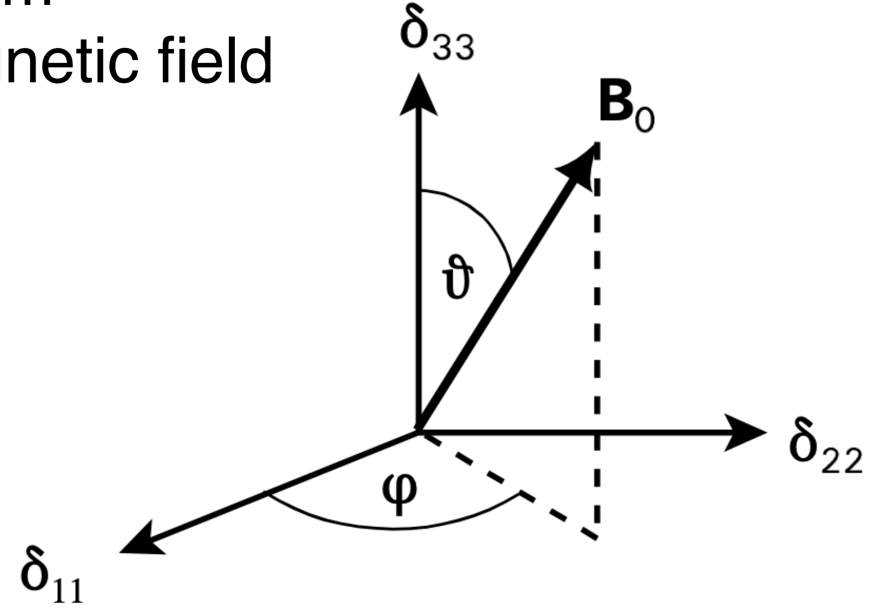
δ_{11} = 104 ppm ; δ_{22} = 44 ppm ; δ_{33} = 25 ppm (by convention $\delta_{11} > \delta_{22} > \delta_{33}$)

The chemical shift is anisotropic. It is not described by a single number, but by a second rank spatial tensor, defined by the three orthogonal principal values of the tensor and the angles that define the orientation of the axes system of the principle values in the molecular reference frame.

NMR interactions are anisotropic

For any given orientation of the principle axis system
of the chemical shift tensor with respect to the magnetic field
the chemical shift is:

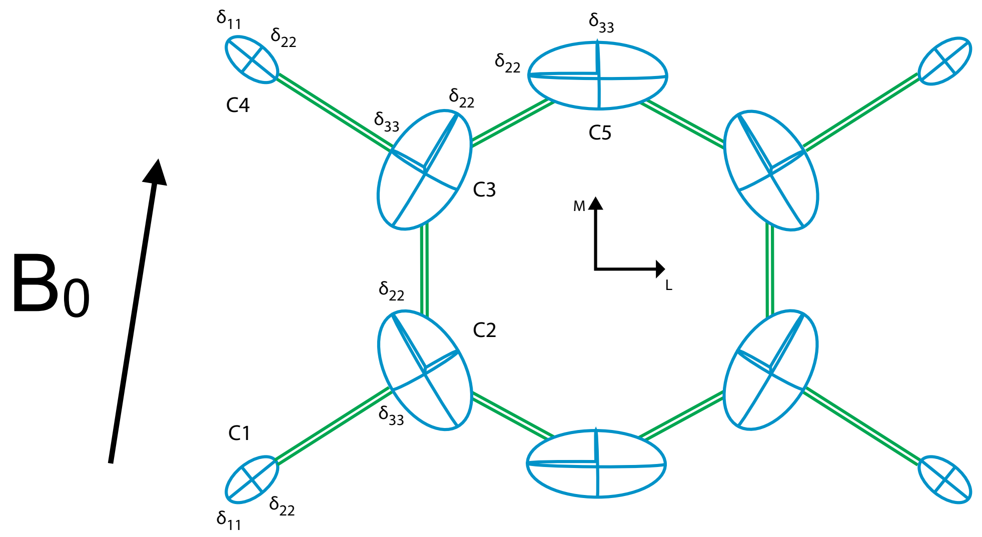
$$\delta(\theta,\phi) = \delta_{11}\sin^2\theta\cos^2\phi + \delta_{22}\sin^2\theta\sin^2\phi + \delta_{33}\cos^2\theta$$

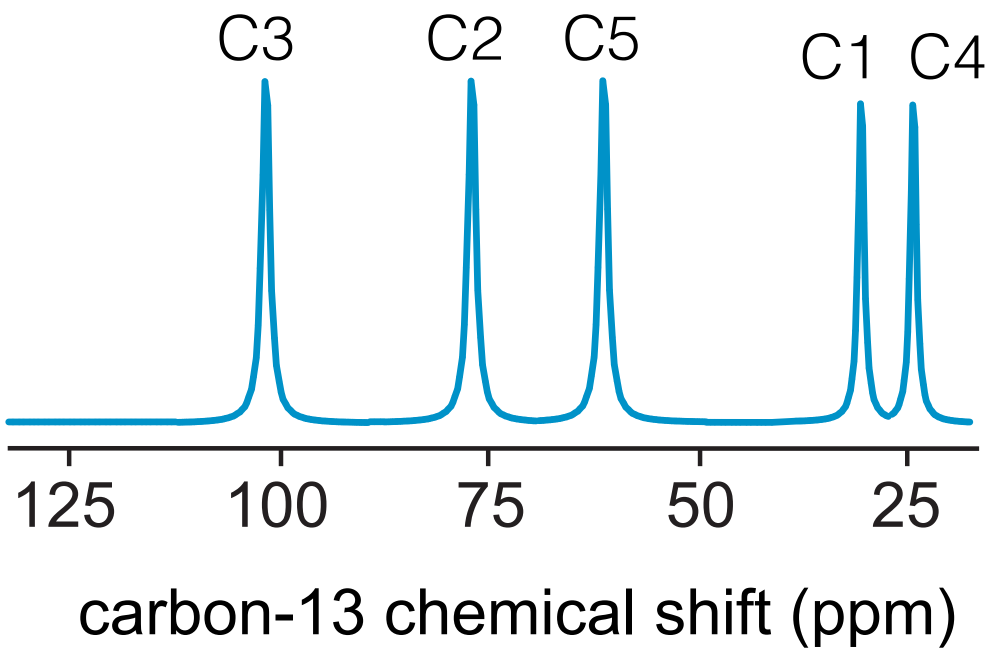


The chemical shift is anisotropic. It is not described by a single number, but by a second rank spatial tensor, defined by the three orthogonal principal values of the tensor and the angles that define the orientation of the axes system of the principle values in the molecular reference frame.

NMR of Single Crystals

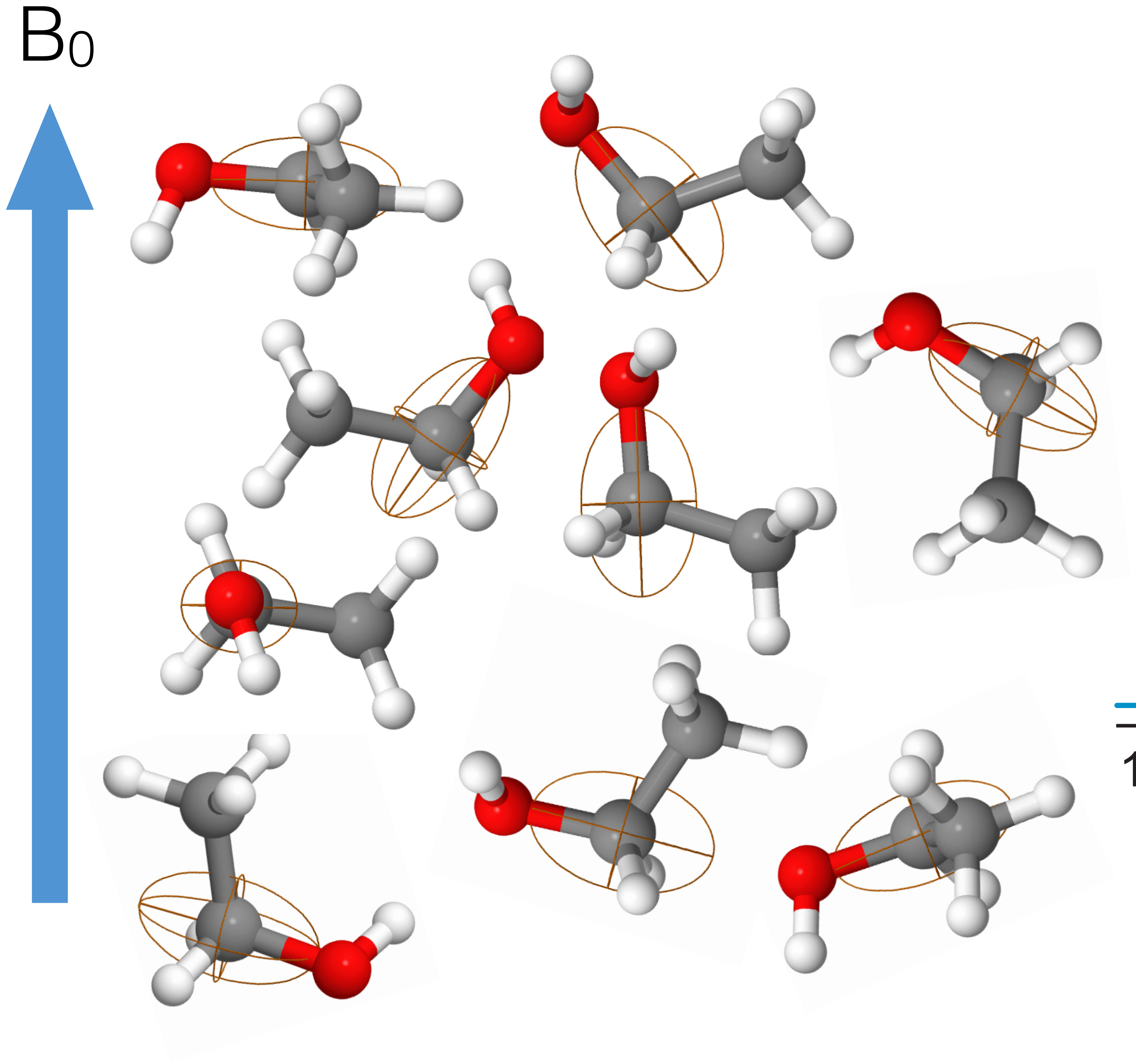
What are the chemical shifts for a single crystal of durene at a given orientation?

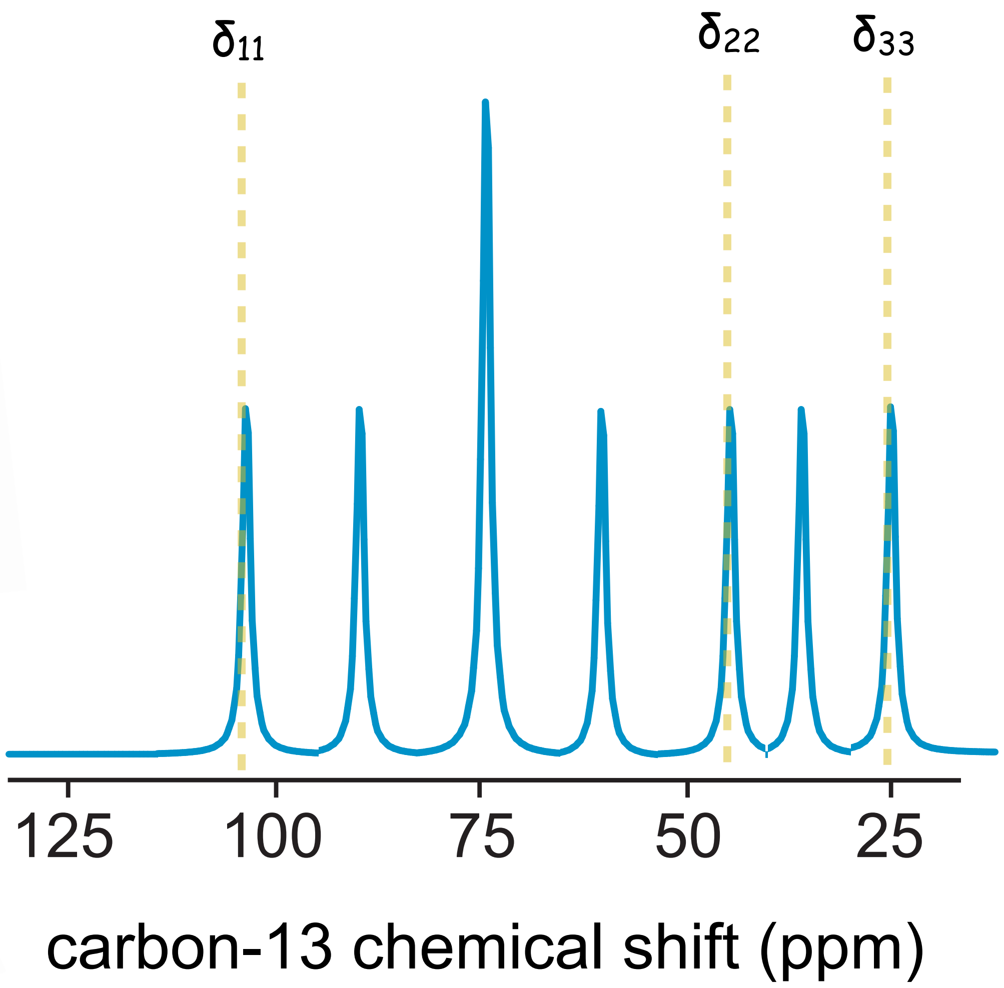




Powder Spectra

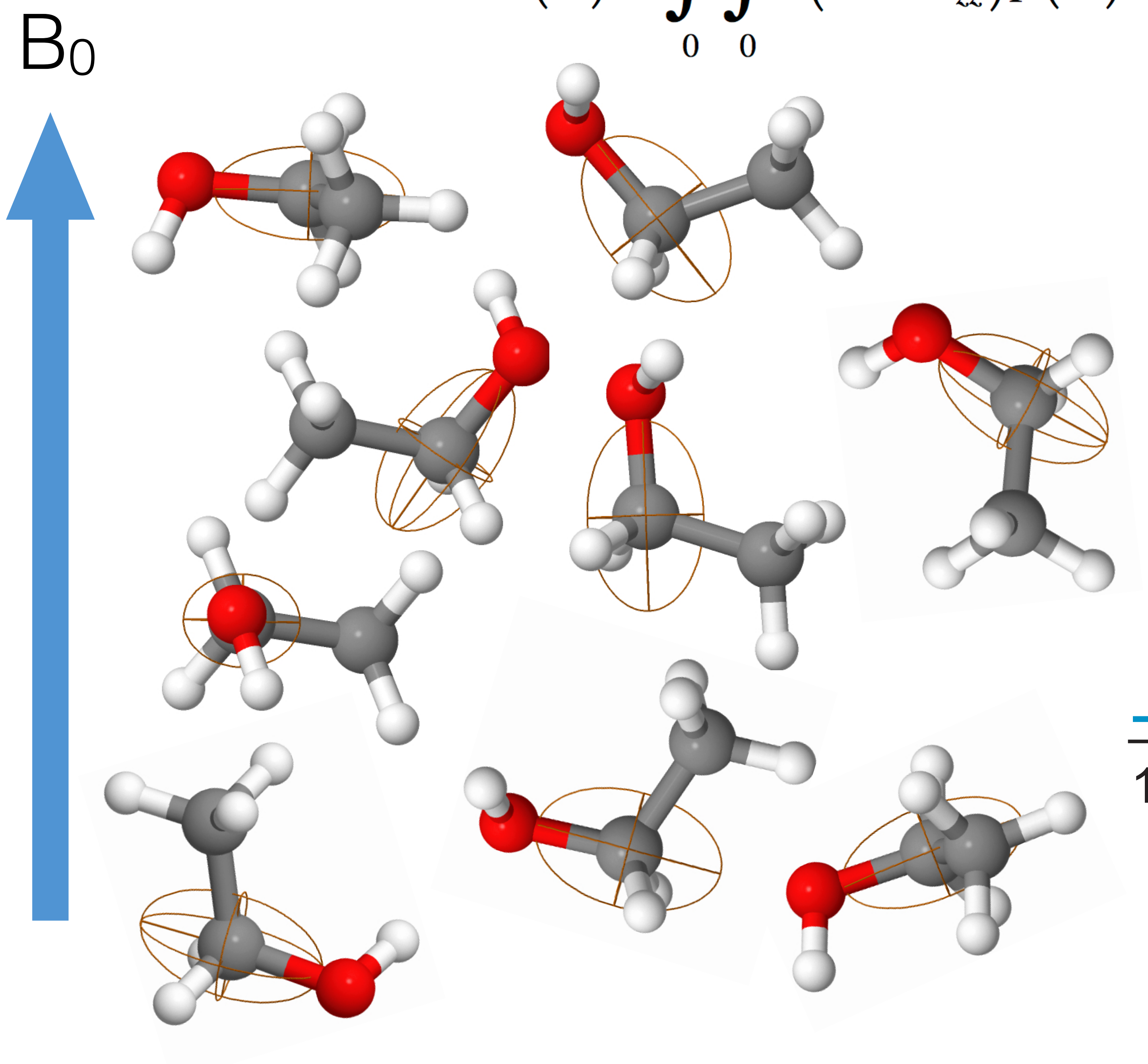
What does the spectrum of a powder look like?

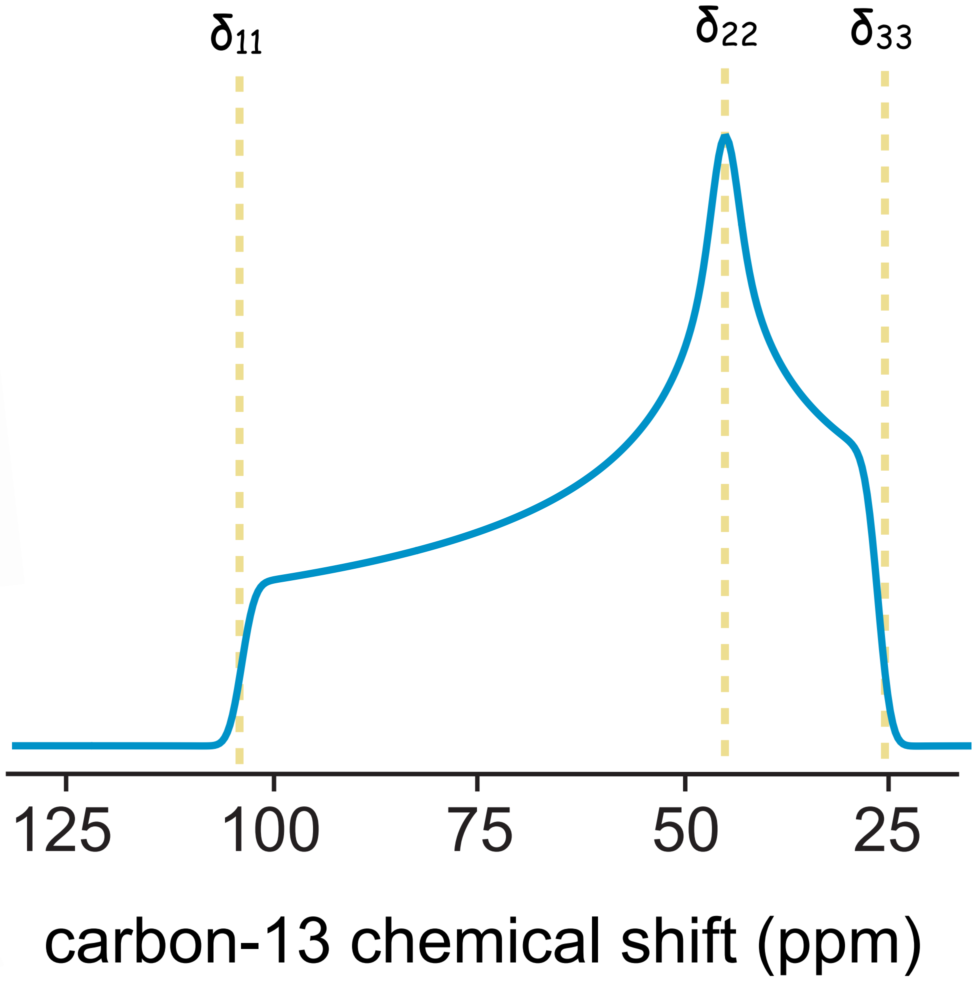




Powder Spectra

$$I(\omega) = \int_0^{2\pi}\int_0^{\pi} \delta(\omega - \omega_{zz})\, p(\Omega)\, d\alpha \sin\beta\, d\beta$$

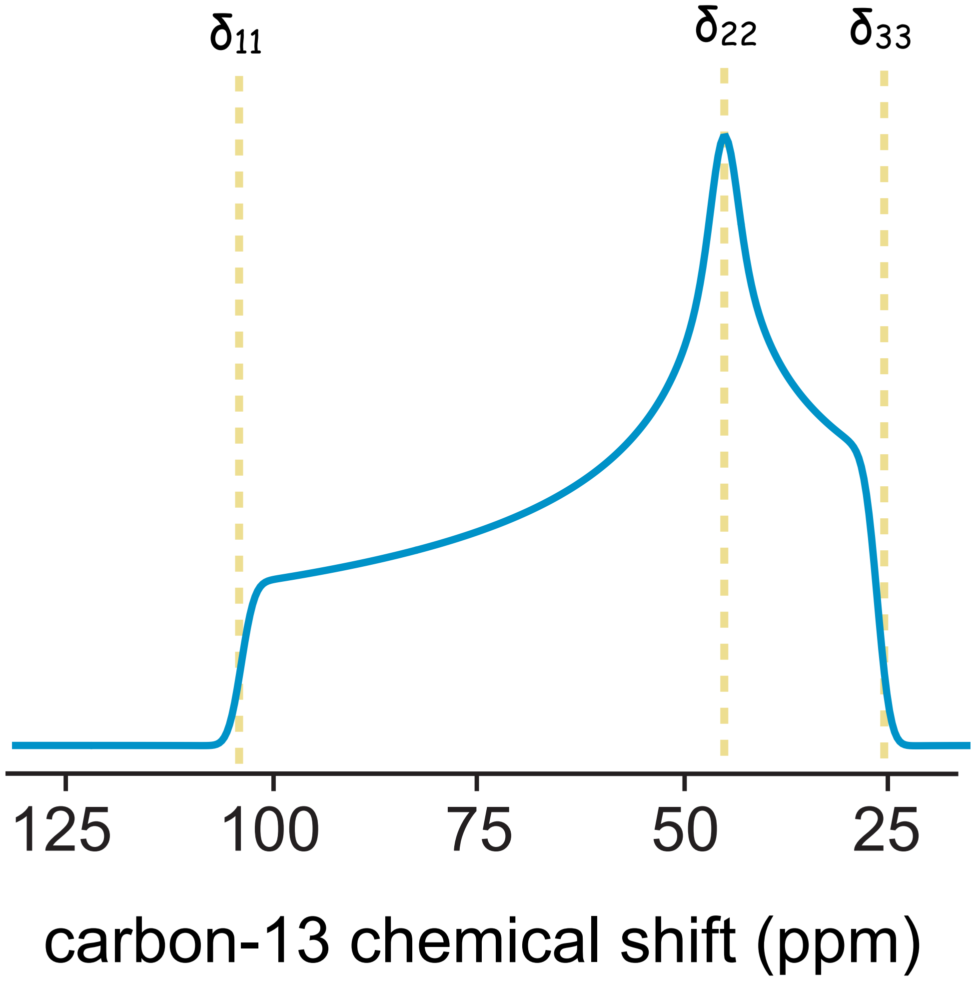




$p(\Omega)$ is the probability of finding a particular crystallite orientation and ω_{zz} is the observed frequency for that orientation. In a powder sample, all orientations are present with equal probability.
The spectrum of a powder is a sum of the spectra arising from each of the crystallites present in the sample.

Powder Spectra

$$I(\omega) = \int_0^{2\pi} \int_0^{\pi} \delta(\omega - \omega_{zz})\, p(\Omega)\, d\alpha \sin\beta\, d\beta$$



Chemical shift principle values (but not orientations) are available from powder spectra by simple inspection.

Powder Spectra

Chemical shift principle values (but not orientations) are available from powder spectra by simple inspection.

We refer to the special cases where $\delta_{11} = \delta_{22}$ or $\delta_{22} = \delta_{33}$ as **axially symmetric** tensors.

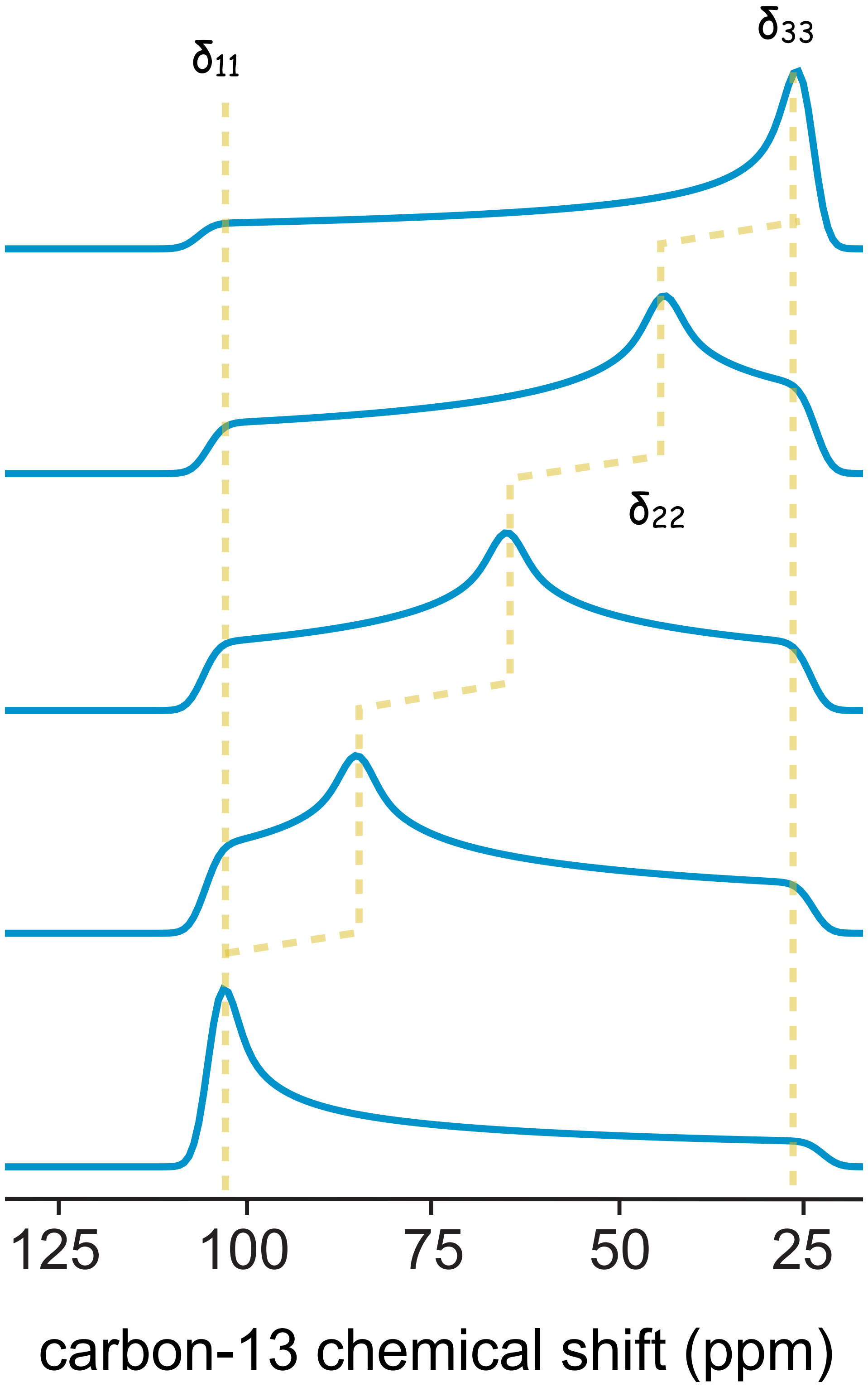
The CSA tensor principle values are often expressed in terms of the isotropic shift, δ_{iso}, span, Ω, and skew, κ :

$$\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3$$

$$\Omega = \delta_{11} - \delta_{33}$$

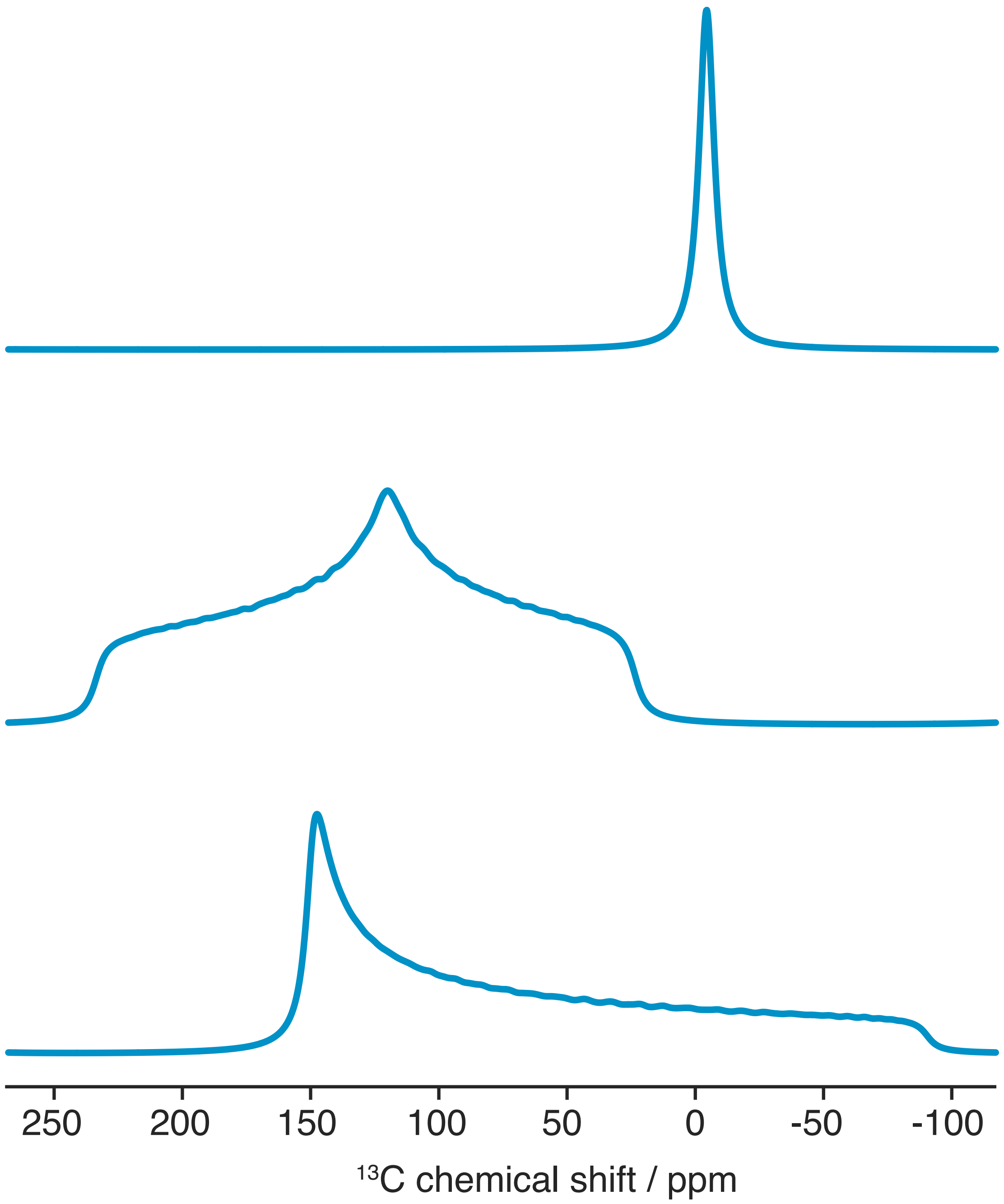
$$\kappa = 3(\delta_{22} - \delta_{iso})/\Omega$$

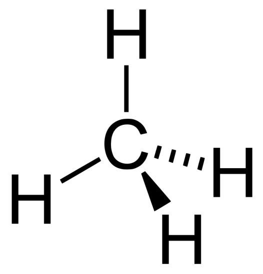
Ω will always be larger than or equal to 0. It measures the overall extent of anisotropy. κ will range from -1 to 1 and it measures the deviation from axial symmetry.



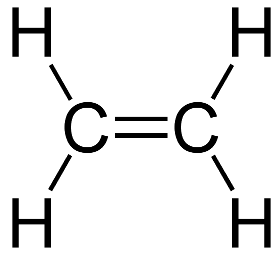
Chemical Shift Anisotropy

Consider carbon-13 chemical shift anisotropy (CSA) in some simple cases:





highly symmetric environment
shielding is very similar in all directions
very small CSA

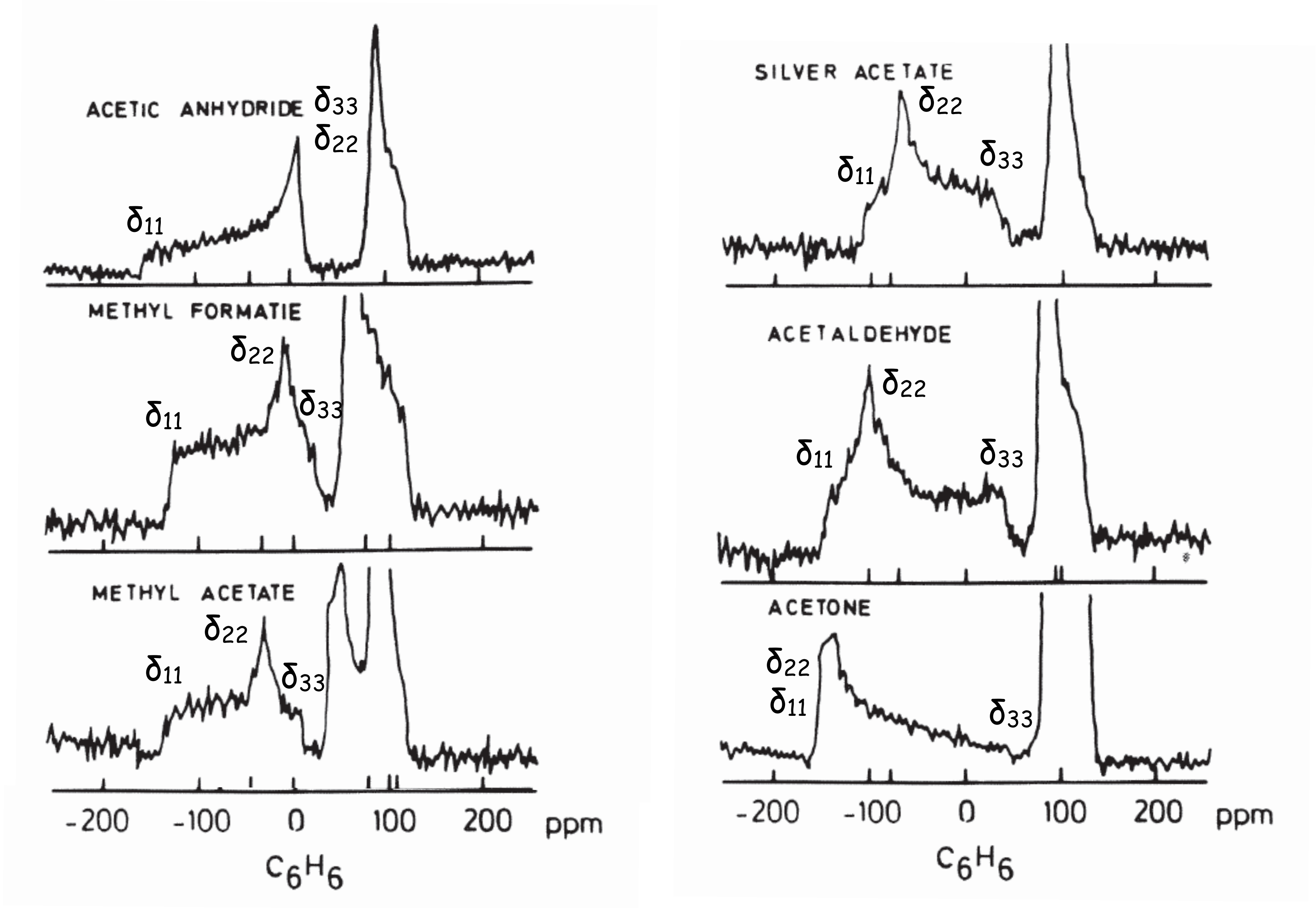


asymmetric environment
shielding is quite different in all three directions
large CSA, large asymmetry parameter

$H-C\equiv C-H$

axially symmetric environment
shielding is quite different in two directions (⊥ and ∥ to the molecular axis)
large CSA, no asymmetry

Chemical Shift Anisotropy



carbon-13 chemical **shielding** from C_6H_6 (ppm)

Chemical shift anisotropy is a sensitive reporter of electronic structure.

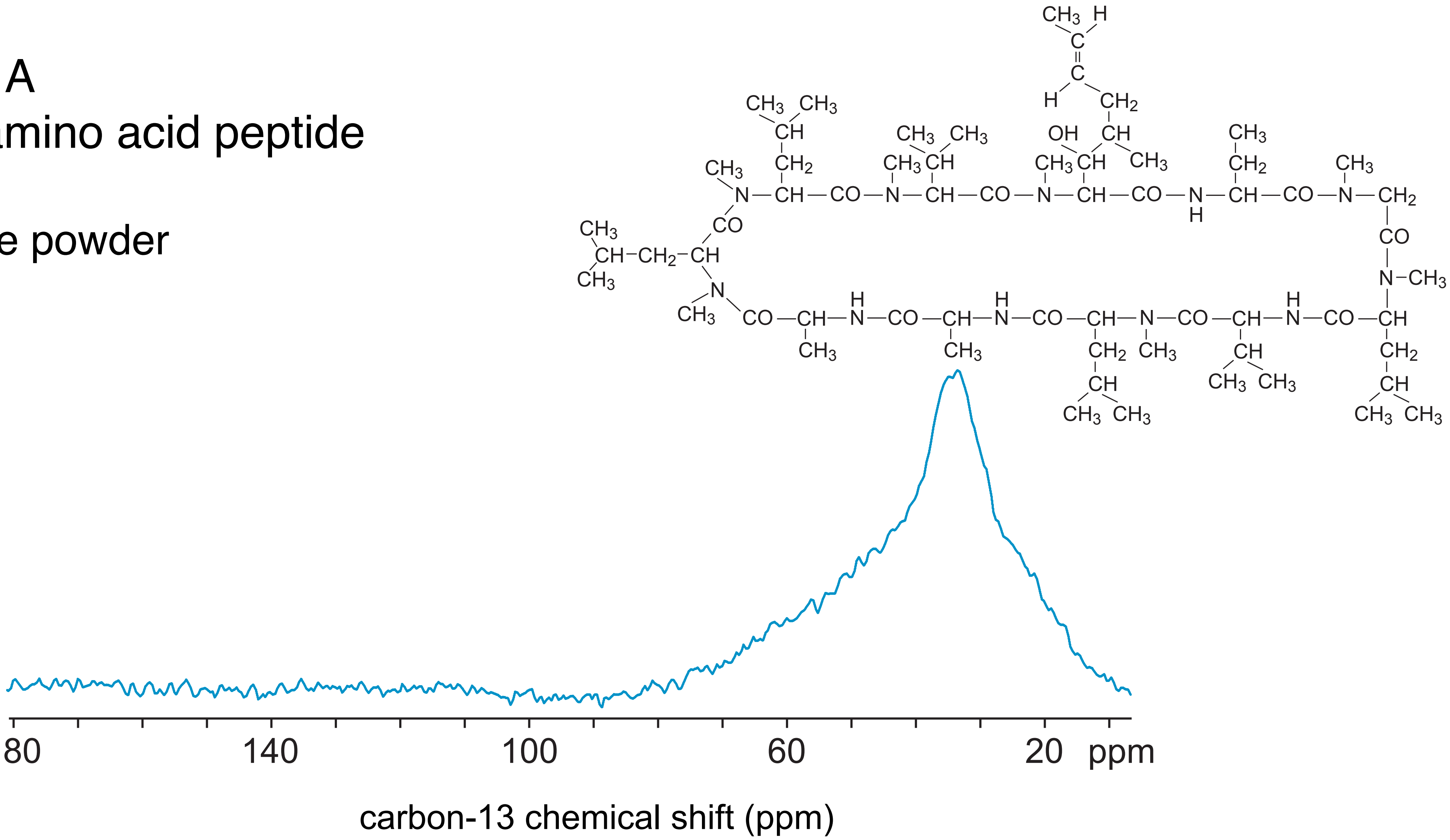
homework: work out the chemical basis for the difference in CSA between acetic anhydride and acetone

Figure: adapted from M. Mehring, "High Resolution NMR Spectroscopy in Solids," Springer, 1976

Powder Spectra Have Low Resolution

Cyclosporin A
a cyclic 11 amino acid peptide

polycrystalline powder



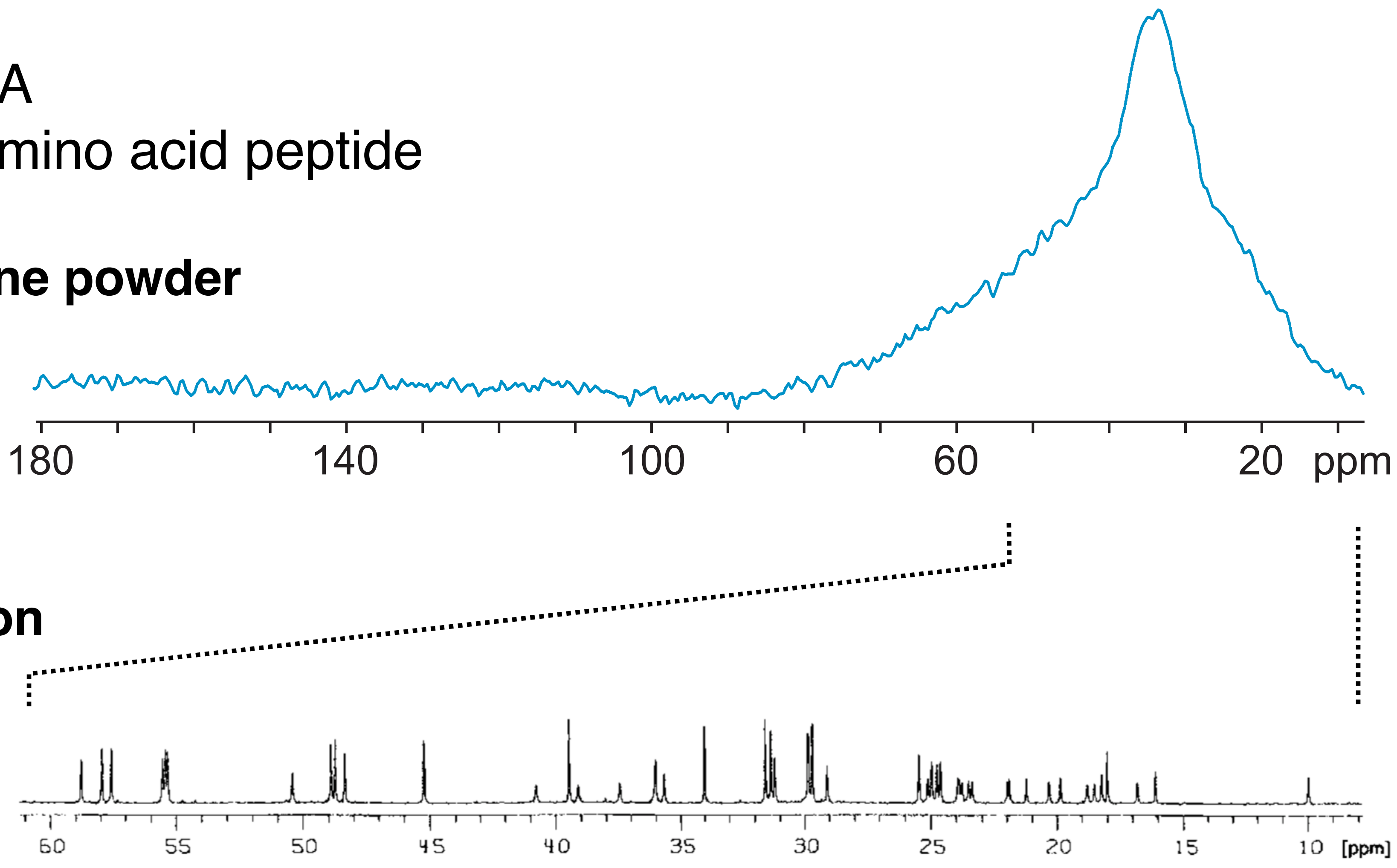
In molecules with many different nuclei, powder spectra will overlap, and resolution is lost.
Many overlapping powder patterns make spectra unreadable.
No access to pertinent chemical information.

How can we regain high-resolution?

Cyclosporin A
a cyclic 11 amino acid peptide

polycrystalline powder

CDCl$_3$ solution



carbon-13 chemical shift (ppm)

One simple way to gain resolution is by dissolving the sample in a liquid. ***Why***?

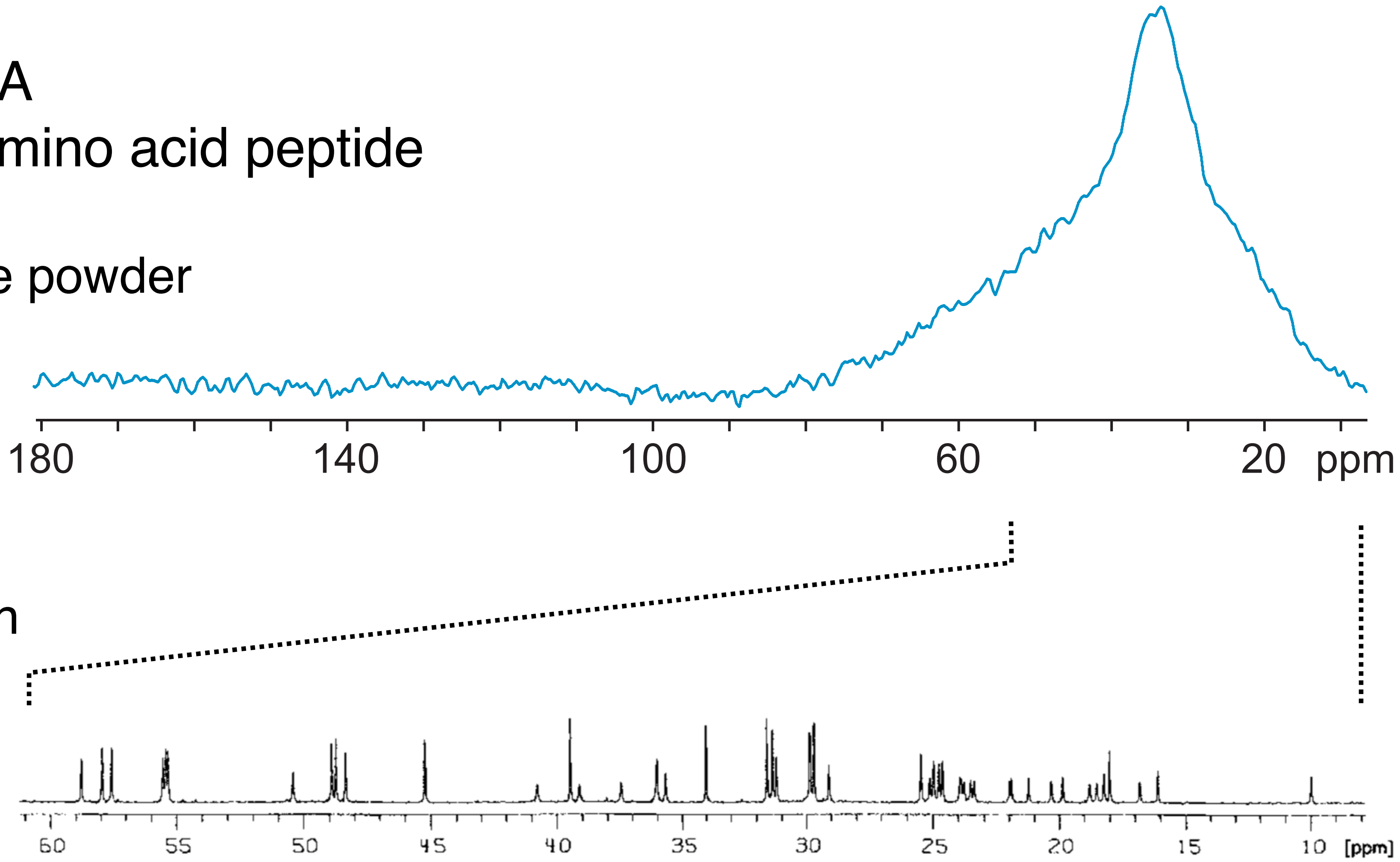
Solution spectrum from Helv. Chim. Acta **68**, 661 (1985)

How can we regain high-resolution?

Cyclosporin A
a cyclic 11 amino acid peptide

polycrystalline powder

CDCl$_3$ solution



carbon-13 chemical shift (ppm)

Rotational molecular motion (usually referred to as rotational diffusion) with a correlation time τ_c much faster than $1/\Omega$ will result in a spectrum in which only the average value of the chemical shift is observed. (Think of it as rapid exchange between all orientations.) For each type of nucleus, it will be the same for all the molecules in the sample: δ_{iso}.

Idea: Impose motions on the sample, which will average the anisotropic component to zero. Do these motions have to be low symmetry? (SO(3)?)

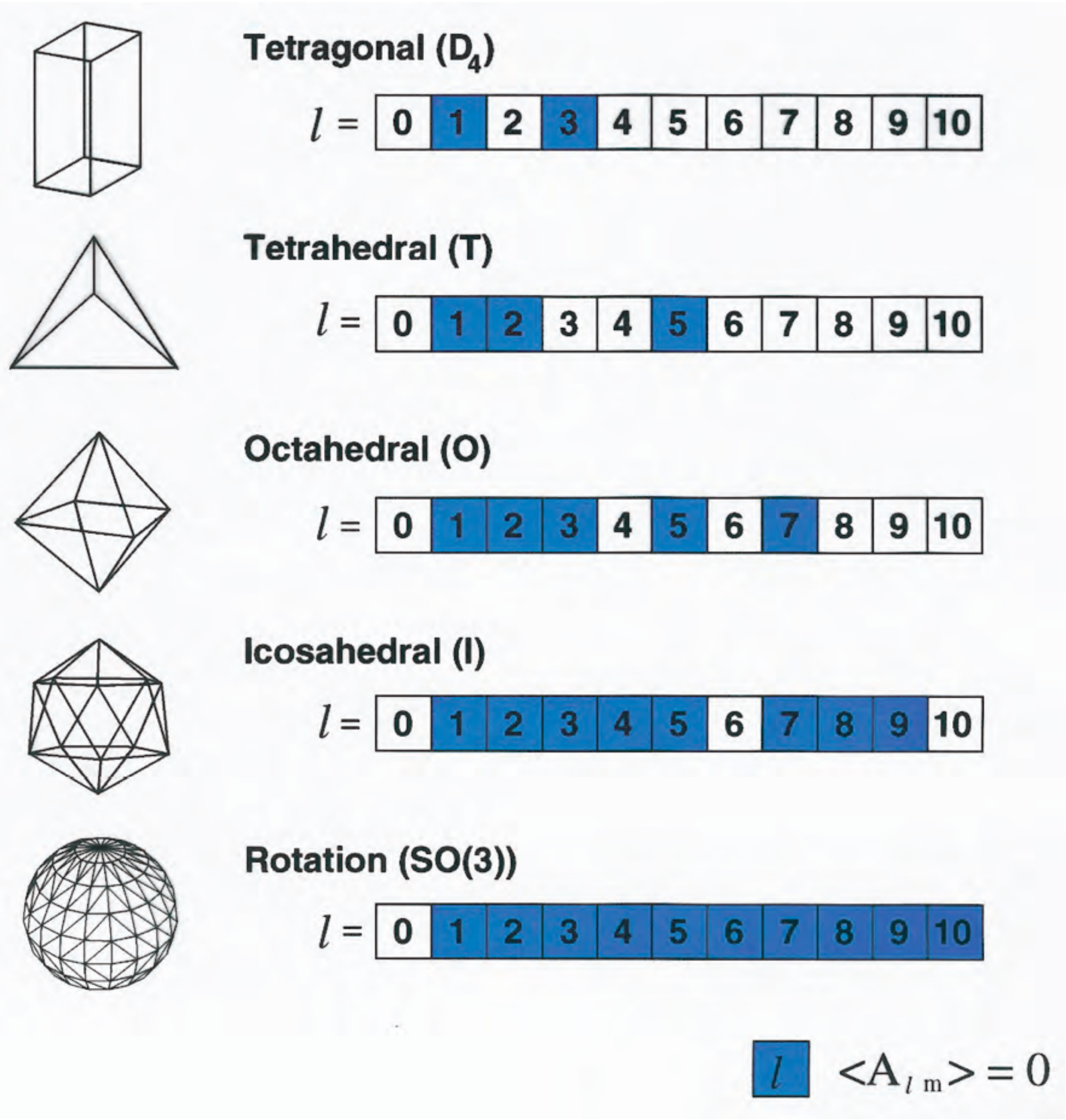
Coherent Averaging

What is the simplest motion that will remove the anisotropic part of the interaction? Does it have to be random isotropic rotational motion?

By comparing the symmetry of the interaction (here 2nd rank spherical harmonics) and the symmetry of a given motion, we can determine to what extent that motion will average the interaction. Group theory gives us the answer.

We find that motions with tetrahedral or octahedral symmetry are already sufficient to average interactions with a 2nd rank spatial dependence to zero.

For example, a motion with octahedral symmetry consists in jumping between the three orientations that point along the x, y and z axes of a cube.

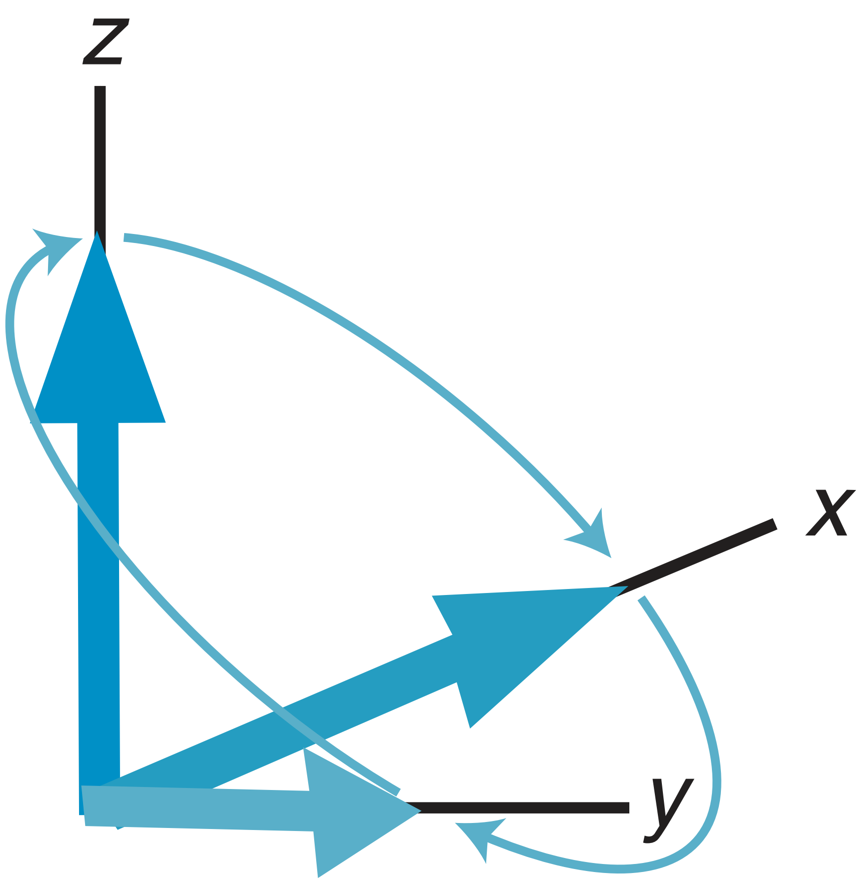


l =	0	1	2	3	4	5	6	7	8	9	10
Tetragonal (D_4)	0	🟦 1	2	🟦 3	4	5	6	7	8	9	10
Tetrahedral (T)	0	🟦 1	🟦 2	3	4	🟦 5	6	7	8	9	10
Octahedral (O)	0	🟦 1	🟦 2	🟦 3	4	🟦 5	6	🟦 7	8	9	10
Icosahedral (I)	0	🟦 1	🟦 2	🟦 3	🟦 4	🟦 5	6	🟦 7	🟦 8	🟦 9	10
Rotation (SO(3))	0	🟦 1	🟦 2	🟦 3	🟦 4	🟦 5	🟦 6	🟦 7	🟦 8	🟦 9	🟦 10

🟦 l $\langle A_{l\,m} \rangle = 0$

not required for the exam

Coherent Averaging: Magic Angle Hopping

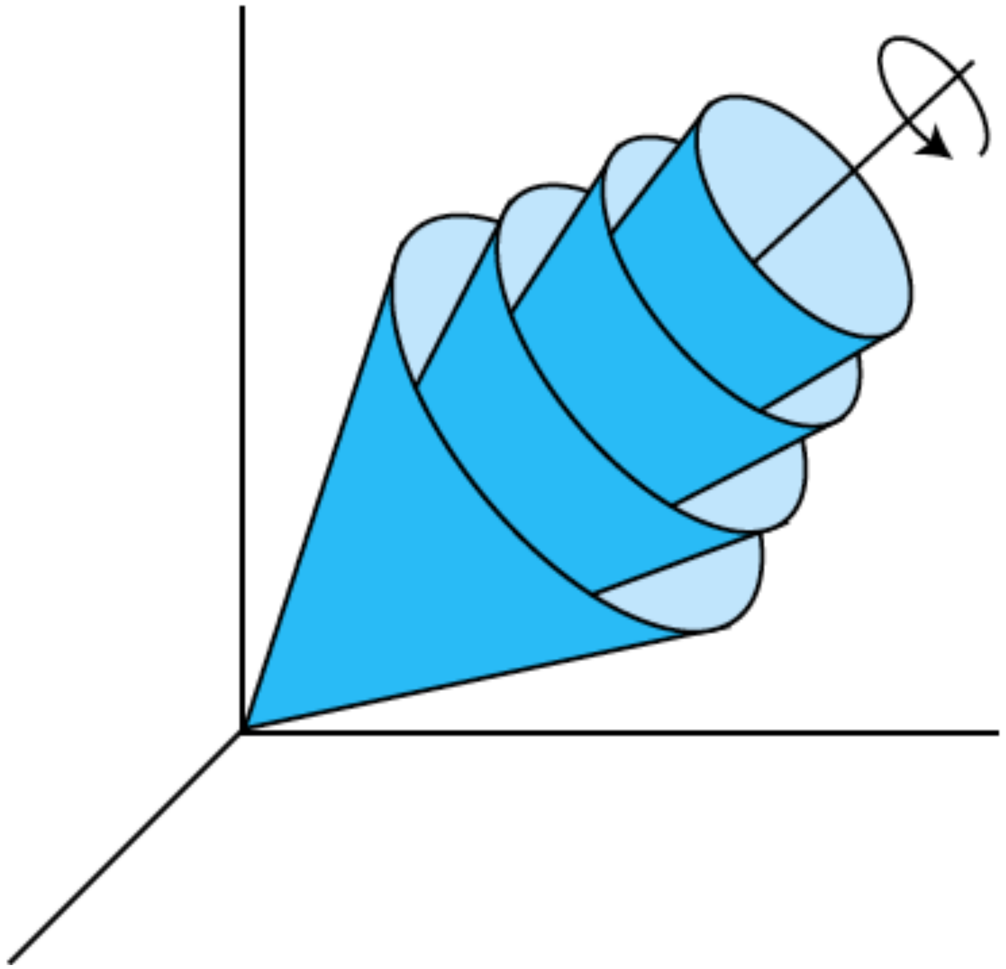


A motion with octahedral symmetry consists in the sample **jumping** between the three orientations that point along the x, y and z axes of a cube.

This can also be achieved by **spinning** the sample around the body diagonal of the cube (the 1,1,1 axis)

not required for the exam

Coherent Averaging: Magic Angle Spinning

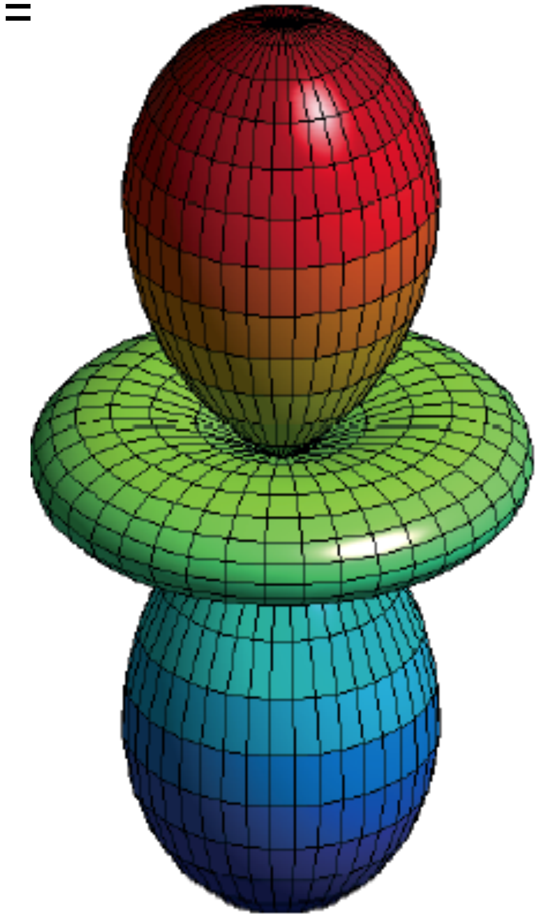


Another way to visualise this is to consider that physically spinning the sample produces an **average orientation** that is aligned with the spinning axes, for any given initial orientation of the interaction.

not required for the exam

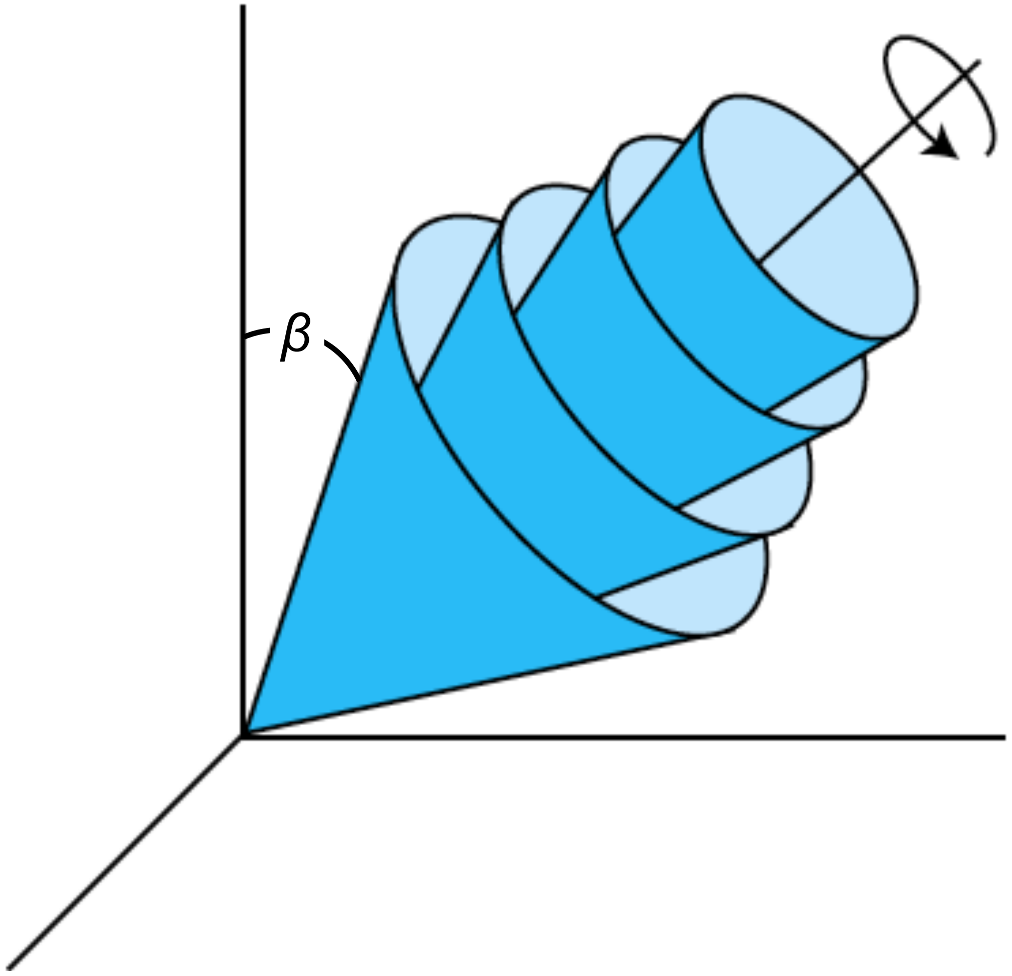
Coherent Averaging: Magic Angle Spinning

For an axially symmetric tensor (i.e. either with $\delta_{22} = \delta_{33} = \delta_{xx}$ and δ_{11} = or with $\delta_{11} = \delta_{22} = \delta_{xx}$ and $\delta_{33} = \delta_{zz}$) the chemical shift is given by:



$$\delta = \delta_{iso} + \quad (\delta_{zz} - \delta_{iso}) \left(\frac{3cos^2\beta - 1}{2} \right)$$

where β is the angle between the δ_{zz} axis and the magnetic field.

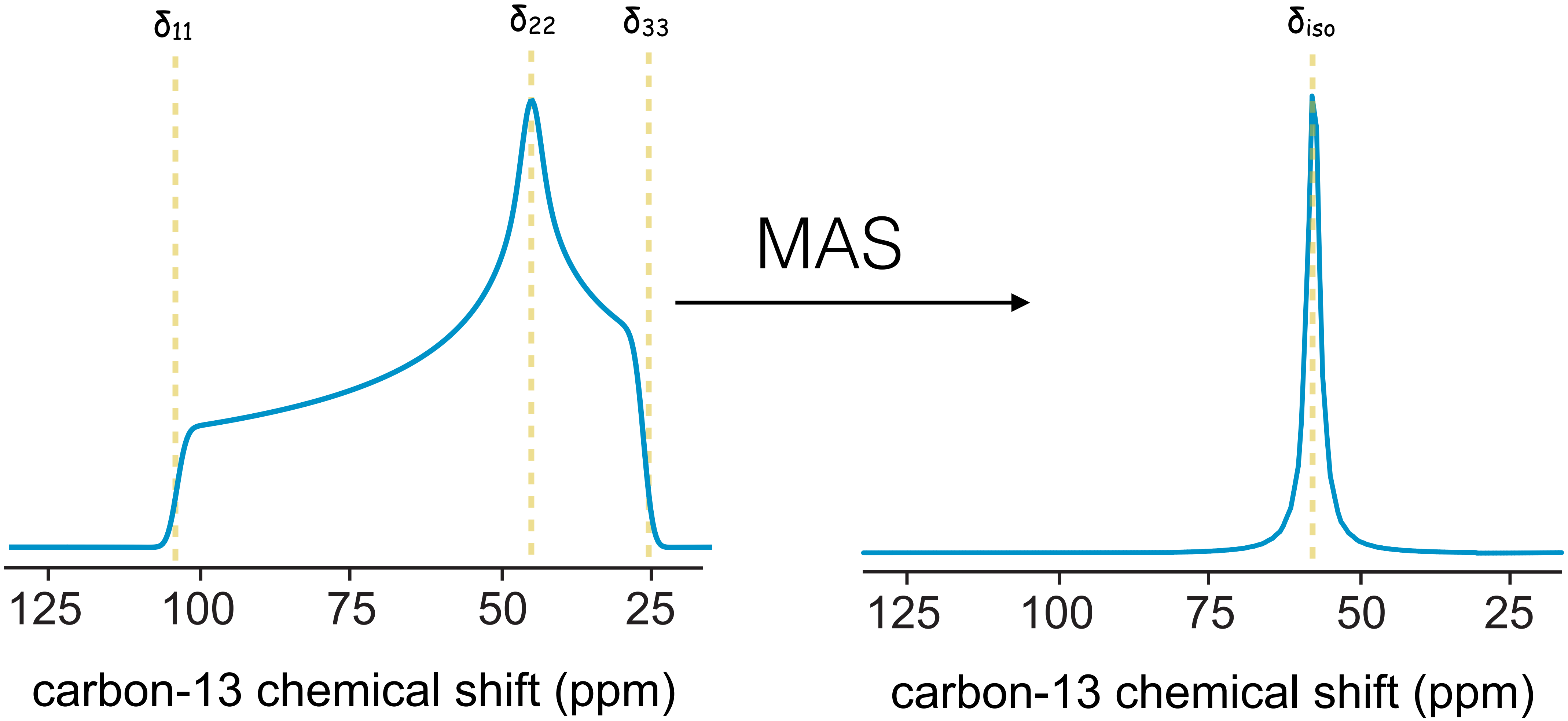


Spinning the sample makes all crystallites appear to have an average β angle $\langle\beta\rangle$ that is oriented along the spinning axis.

If the axis of spinning is 54.7°, then $\langle\beta\rangle = 54.7°$ and $3\cos^2 \langle\beta\rangle - 1 = 0$so that $\langle\delta\rangle = \delta_{iso}$

Magic Angle Spinning

If the angle of the spinning axes is set to 54.7° wrt to B_0, then
all orientations will have the same average frequency.
It is the center of gravity of the powder pattern: **the isotropic chemical shift**



$$\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3$$

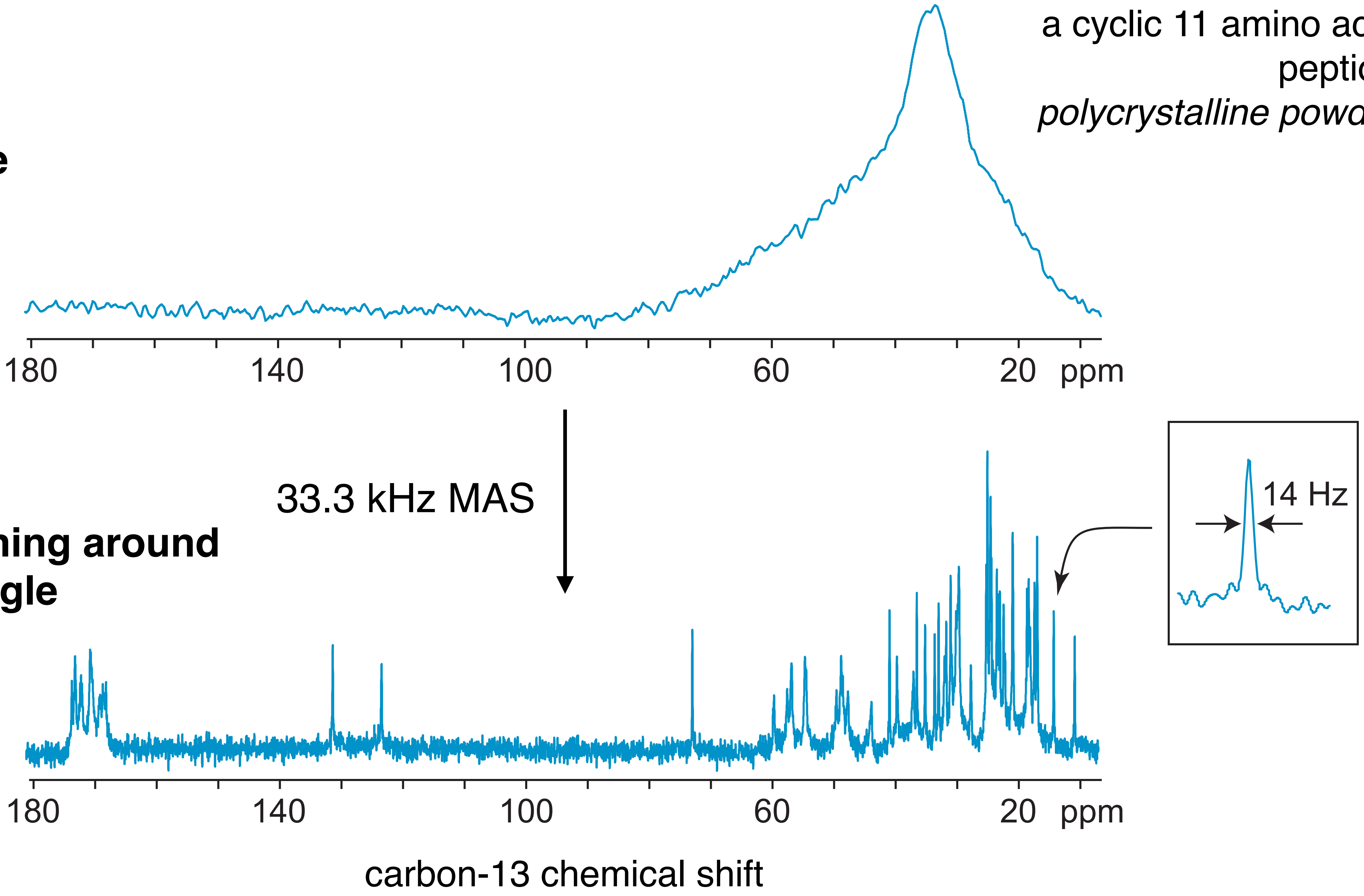
Magic Angle Spinning

Cyclosporin A
a cyclic 11 amino acid
peptide
polycrystalline powder

static sample

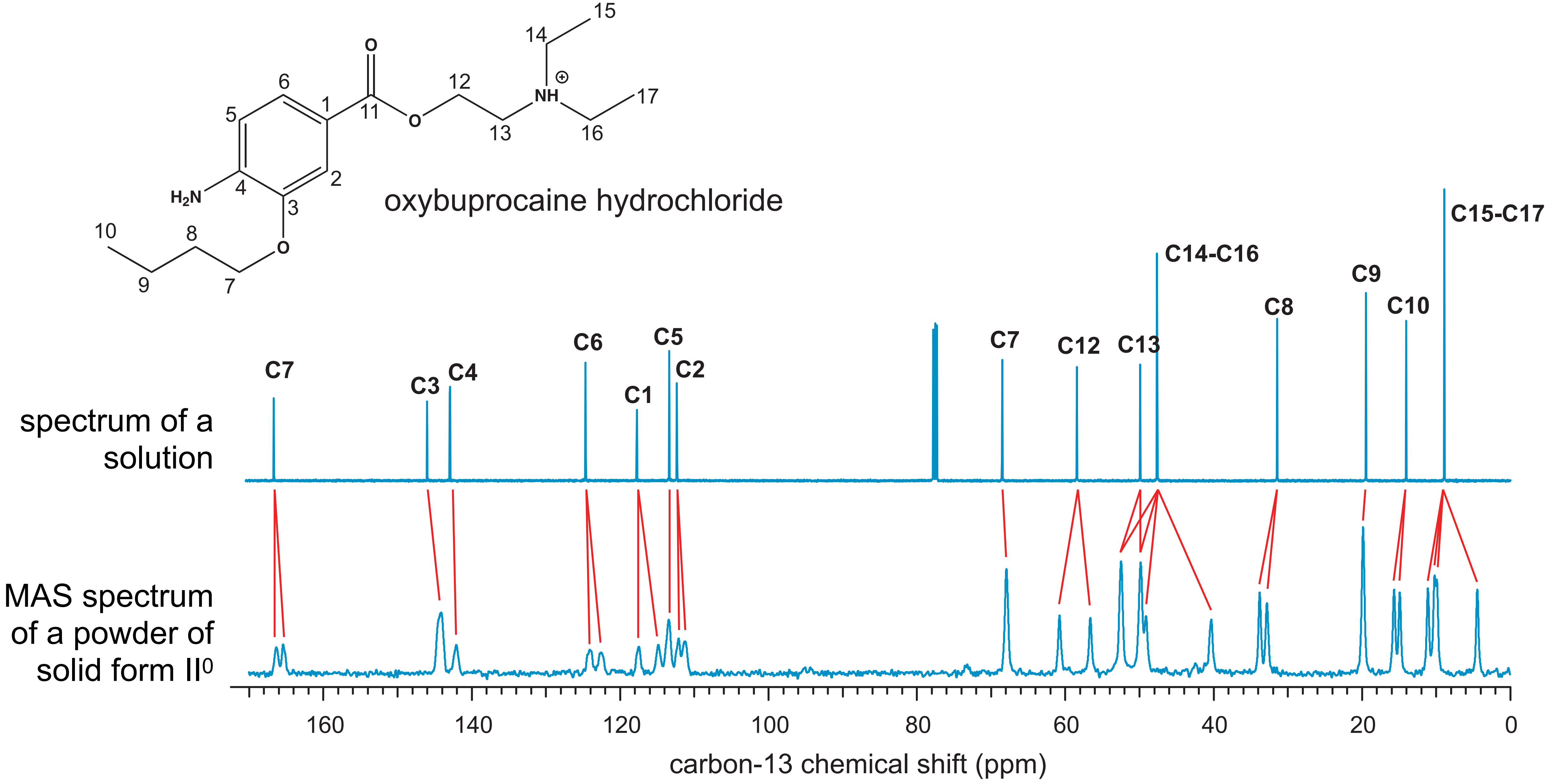
sample spinning around the magic angle

33.3 kHz MAS



Magic Angle Spinning

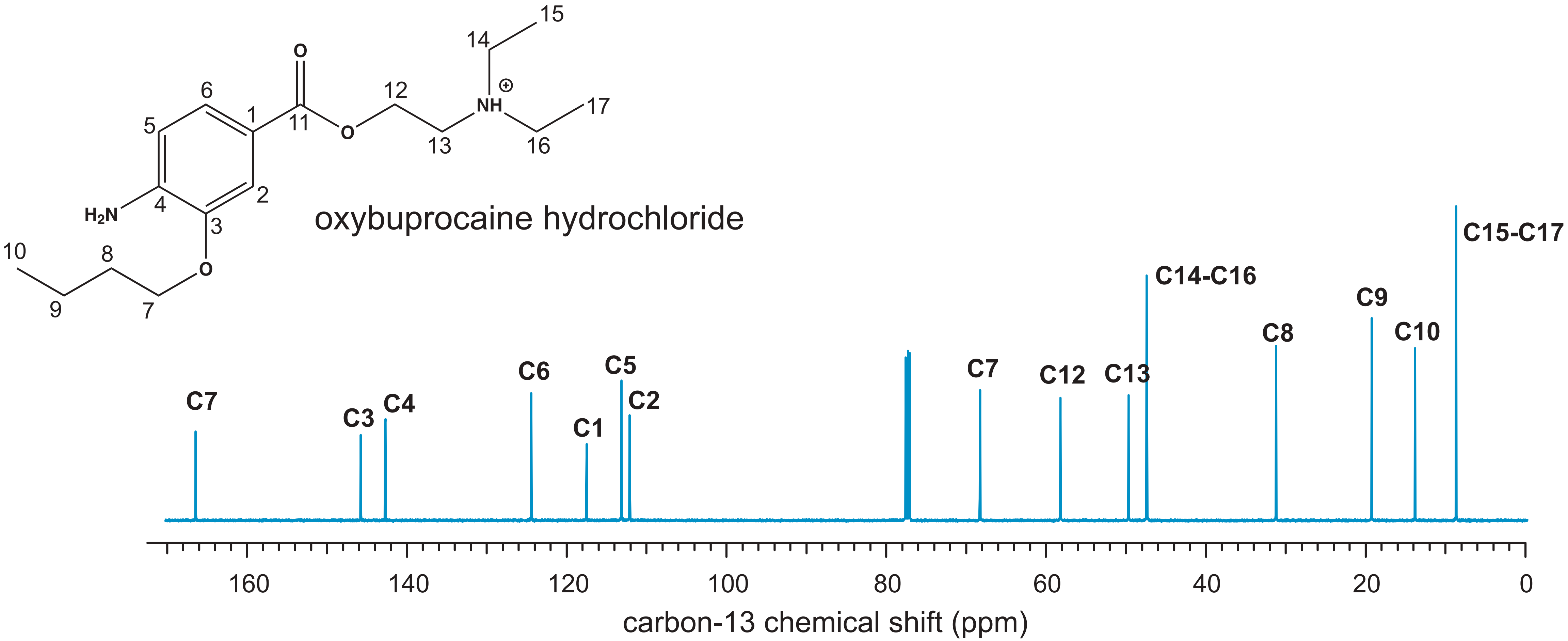
Note that the isotropic chemical shift can change between the solid and liquid phases



oxybuprocaine hydrochloride

Chemical shifts are exquisite reporters of chemical structure and dynamics

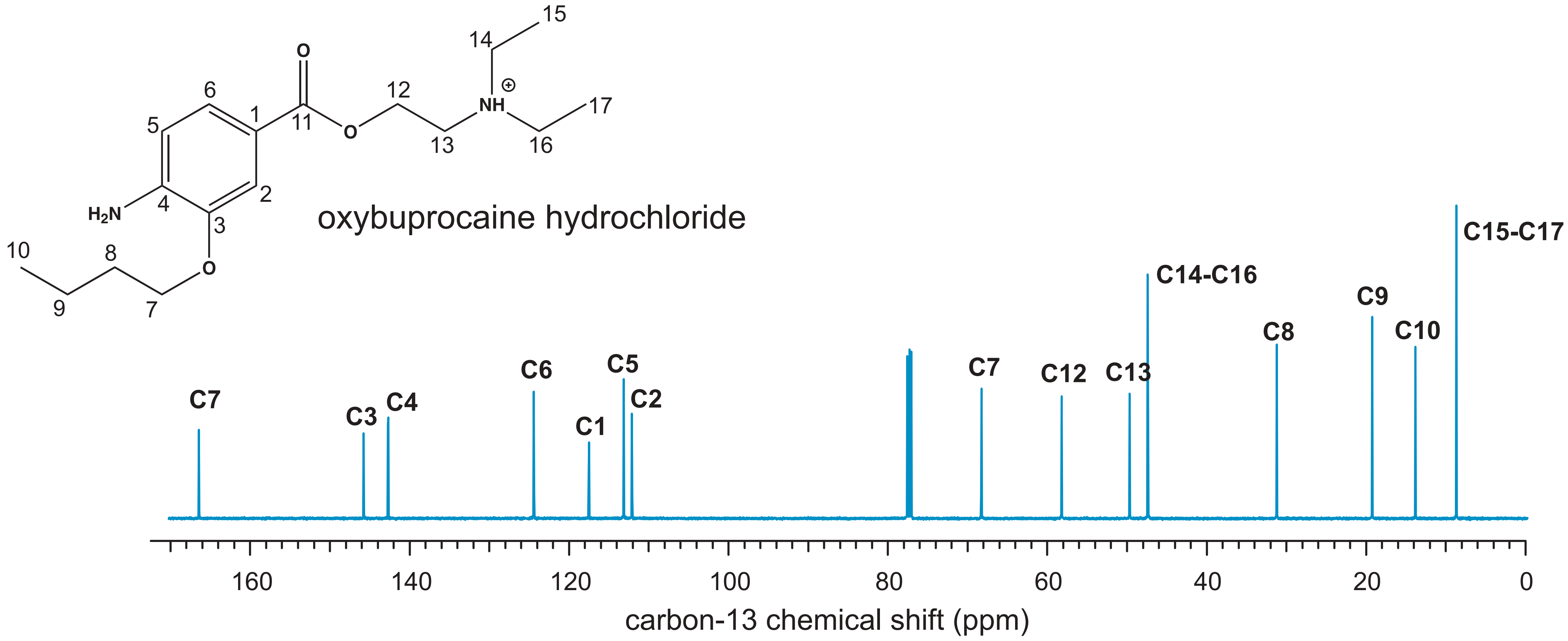
Coherent Averaging II: Take a closer look at solution-state carbon-13 spectra



oxybuprocaine hydrochloride

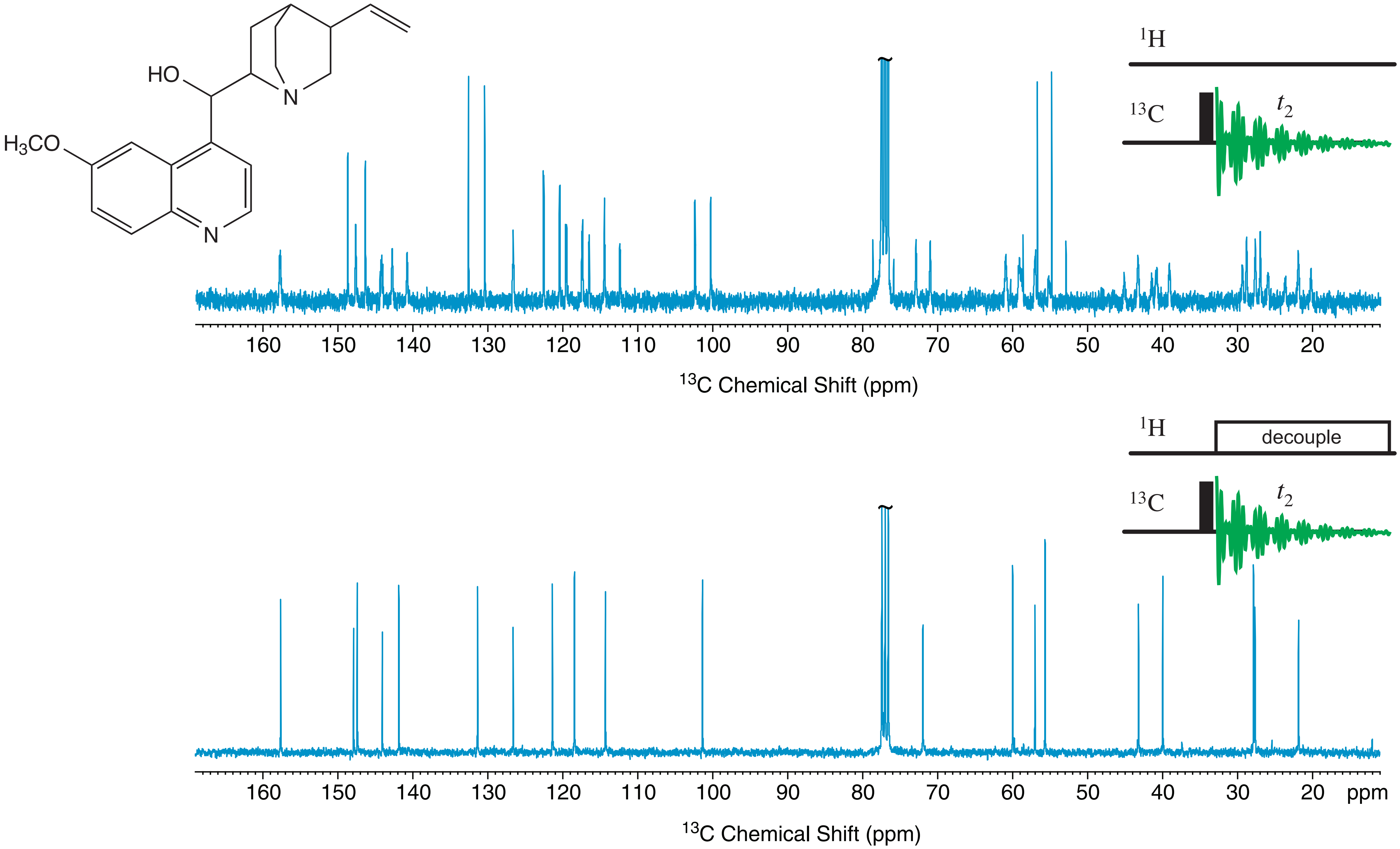
What is the fine structure of the peaks? ***Why***?

Coherent Averaging II: Take a closer look at solution-state carbon-13 spectra


oxybuprocaine hydrochloride

How could we remove the heteronuclear J couplings?

Coherent Averaging in Spin Space: Spin Decoupling



By applying a train of π pulses to the heteronucleus (usually ^{1}H), or by continuous irradiation of the heteronucleus, we can average the heteronucelar J coupling to zero.

The decoupled spectrum has better resolution & sensitivity. Carbon-13 NMR spectra are almost always recorded with decoupling.

Conclusions

- The chemical shift is anisotropic. The NMR frequency depends on the orientation of the molecule with respect to the magnetic field.

- Anisotropic interactions are described by a tensor. The CSA tensor is second rank, and has three principle values in an axes system described by three angles with respect to a reference frame fixed in the molecule.

- The full CSA tensor can be determined from NMR of single crystals.

- Powder spectra are the sum of spectra from all orientations. Line shapes are characteristic of the tensor principle values.

- By rapidly spinning the sample around an axes at an angle of 54.7° with respect to the main field, the anisotropy can be averaged to the isotropic value for any crystallite orientation. We refer to this as coherent averaging. Magic angle spinning yields a high-resolution isotropic spectrum from a powder.

- Coherent averaging can also be applied in spin space. By irradiating protons during acquisition of carbon-13, heteronuclear scalar couplings can be averaged to zero (decoupled).

Objectives

- Learn to read research articles
- Learn how to apply NMR to real-world problems

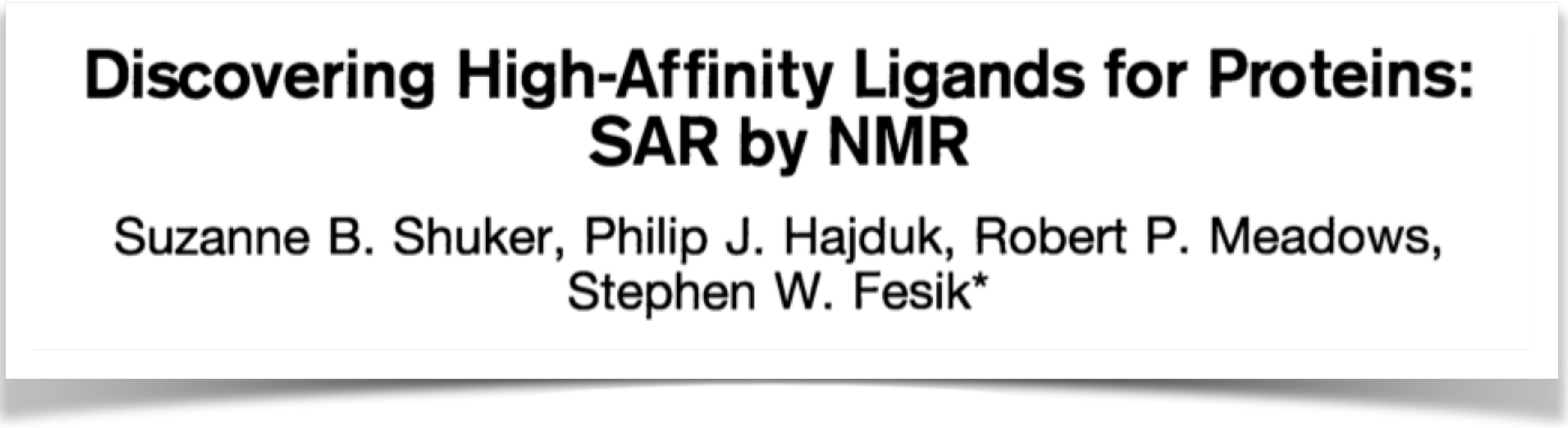
Case Studies

For each of the papers:

- What is the subject of the research & why is it interesting or important?
- What is the precise objective of this study?
- What is/are the approach(es) used?
- What role does NMR play?
- What are the conclusions? How do the NMR results support the conclusions?

Case Study 1

Drug Discovery by NMR



Discovering High-Affinity Ligands for Proteins: SAR by NMR

Suzanne B. Shuker, Philip J. Hajduk, Robert P. Meadows,
Stephen W. Fesik*

SCIENCE • VOL. 274 • 29 NOVEMBER 1996 1531

Homework

For next week, in the same groups as for the problem sets, choose one paper for each group from the papers on Moodle.

Prepare a **short (10 min) joint** (i.e. every member should say at least one thing) presentation (with slides) to the class for next week's course in which you present and analyse the paper using the 5 terms we have used this week.

Each presentation will be followed by a group discussion. **Every** member of the class will need to ask at least one question during the session.

[Tip: Try without using AI tools. If you do use AI tools, acknowledge their use, and make sure there are no mistakes. Remember, there will be a Q&A after your presentation!]