Institut de Biologie Structurale

ssNMR @ IBS Grenoble

Magic-angle spinning solid-state NMR spectroscopy and its applications in integrated structural biology

Paul Schanda
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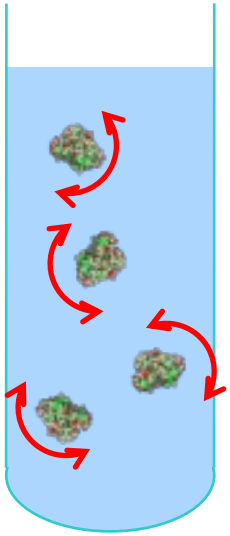
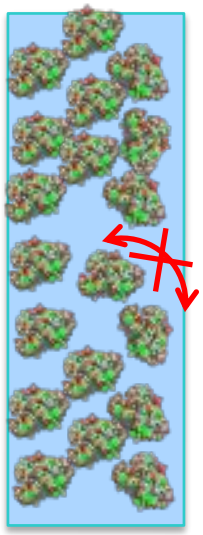
Solution-state and solid-state NMR

Solution-state NMR spectroscopy
Molecules tumble freely in solution



Soluble proteins
of rather small size
(ideally < 30 kDa)

Solid-state NMR spectroscopy
Molecules do **not** undergo overall tumbling

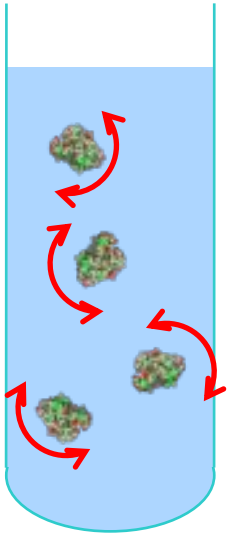


Folded globular proteins
Intrinsically disordered proteins
Membrane proteins solubilized in detergents
…

Solution-state and solid-state NMR

Solution-state NMR spectroscopy
Molecules tumble freely in solution

Solid-state NMR spectroscopy
Molecules do **not** undergo overall tumbling



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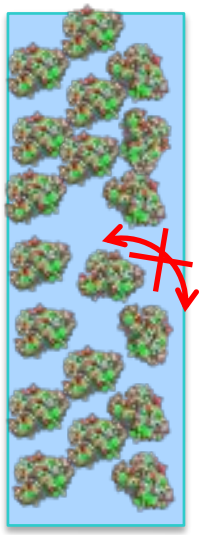
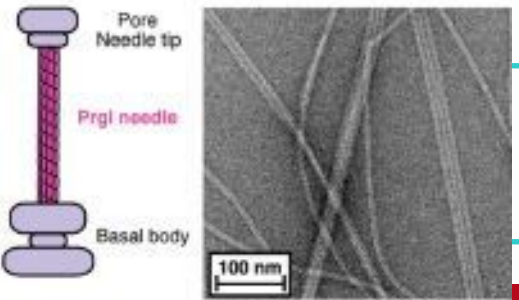
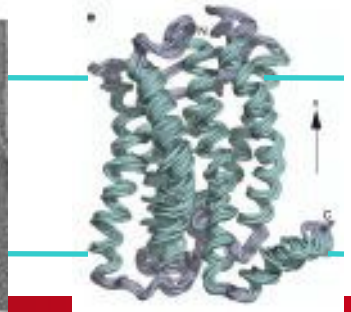


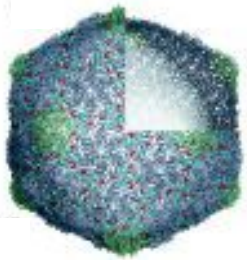
Amyloids, needles,…



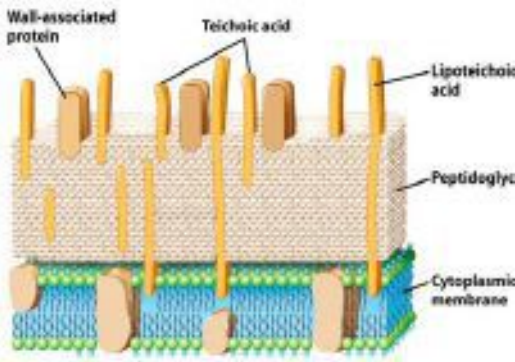
Membrane proteins in
(native) membranes



Large assemblies

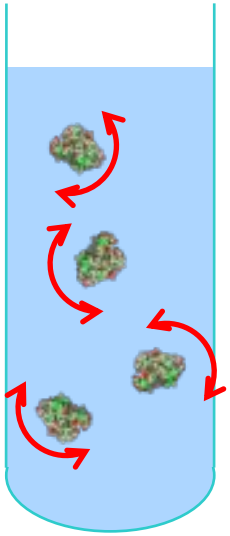


Entire cells, cell walls, …



Solution-state and solid-state NMR (magic-angle spinning NMR)

Solution-state NMR spectroscopy
Molecules tumble freely in solution



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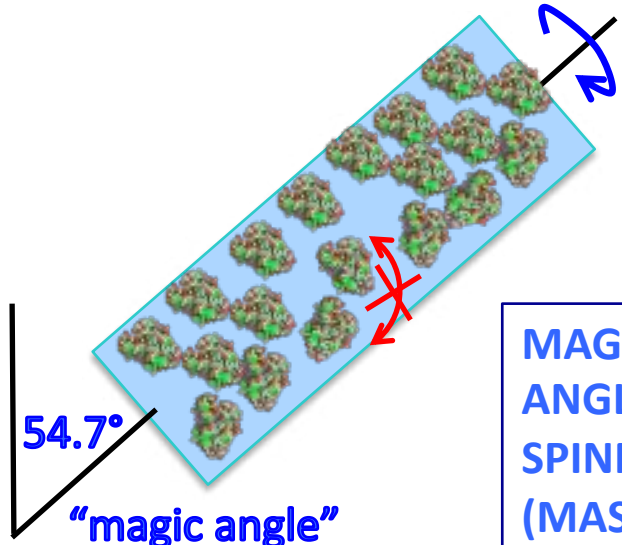
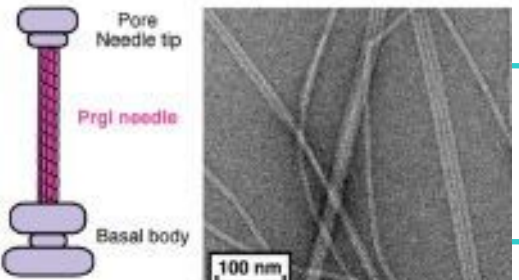
Solid-state NMR spectroscopy
Molecules do **not** undergo overall tumbling

WE SPIN THE SAMPLE

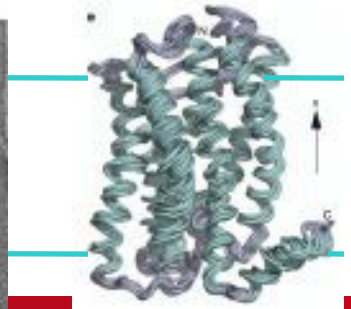


MAGIC
ANGLE
SPINNING
(MAS)

Amyloids, needles,…



Membrane proteins in
(native) membranes



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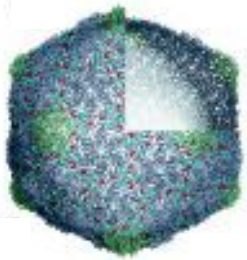
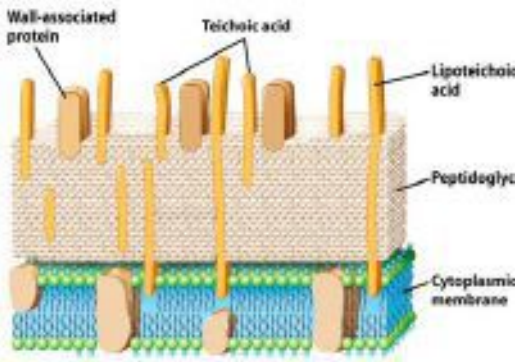
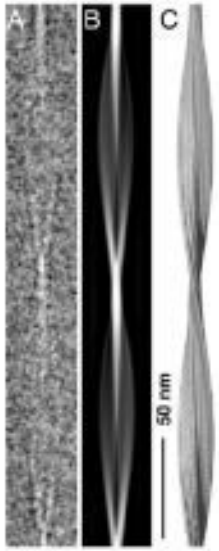


Entire cells, cell walls, …



Contributions of solid-state NMR in structural biology



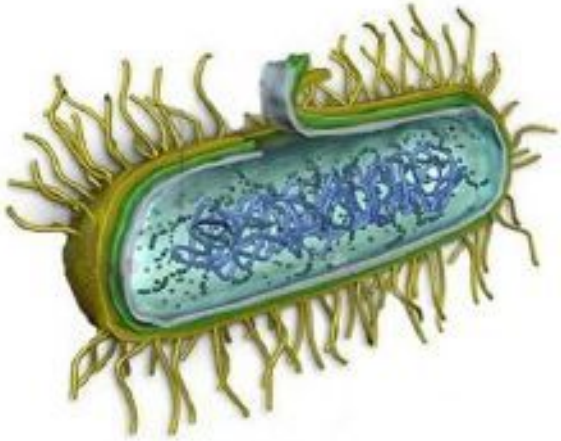
Amyloid fibers

- β-Amyloid (Alzheimer´s Disease)
- α-synuclein (Parkinson´s Disease)
- Huntingtin
- Prion diseases

Amyloid fiber structures are very hard to obtain at atomic resolution by any other method

Whole cells, cell walls,…

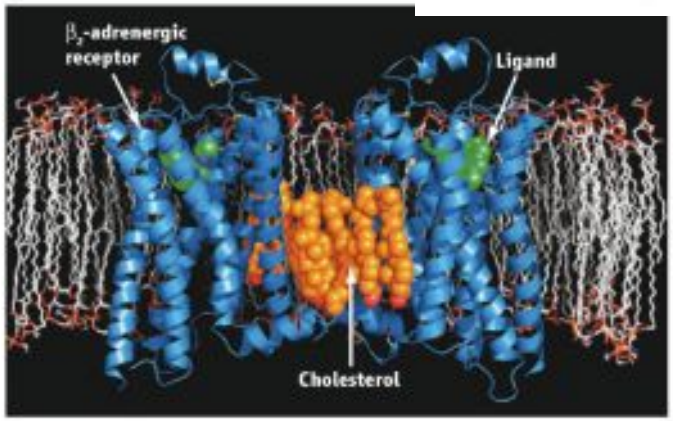
essentially impossible to study at atomic resolution by other techniques



Membrane Proteins

20-30% of open reading frames
60% of all drug targets

Structures may be obtained by crystallography / EM
Interactions can readily be studied by ssNMR <u>in lipid bilayer membranes</u>



Kobilka, Stevens, Schertler, *Science* 2008

Outline of this presentation

A brief reminder of **NMR basics**.

- What kind of information can we get from NMR?
- What is so special about solid-state NMR as compared to solution-NMR?
- Instrumentation for solid-state NMR.

Structure-determination from ssNMR.

- Approaches for structure determination.
- Where are we, what are the challenges

Monitoring **molecular interactions** and structural changes.

Insight into **dynamics** from ssNMR.

- Observable parameters. Amplitudes and time scales of motion.

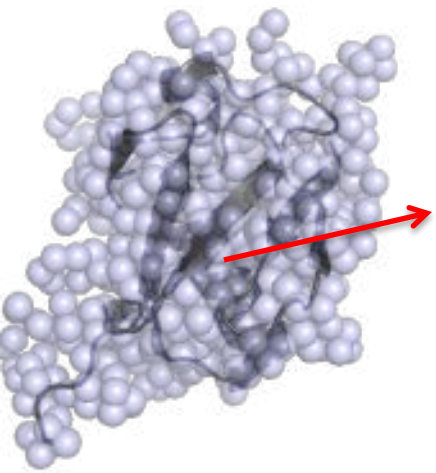
Hot topics / **new developments**.

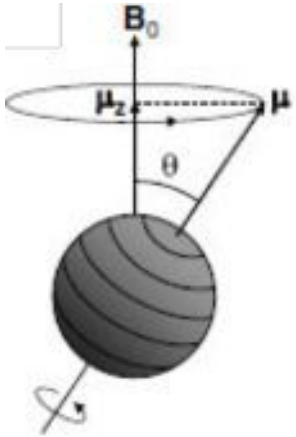
- Solid-state NMR on entire cells or cell compartments.
- Increasing NMR sensitivity by orders of magnitude: DNP.

Practical aspects

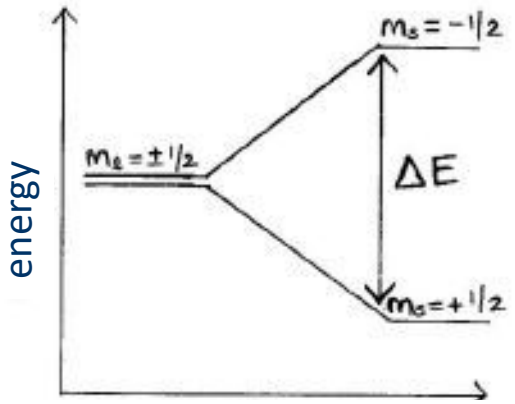
Application example: 500 kDa enzyme complex

NMR spectroscopy is an atomic-resolution technique

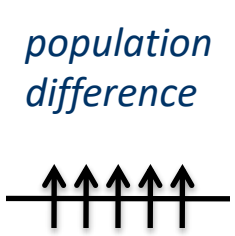




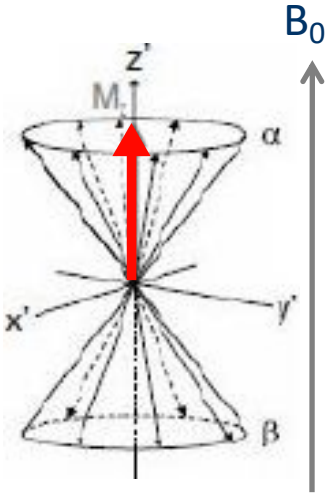
nuclear spin
(in each ^{1}H, ^{13}C, ^{15}N, ^{31}P, ^{19}F, ^{2}H,…)



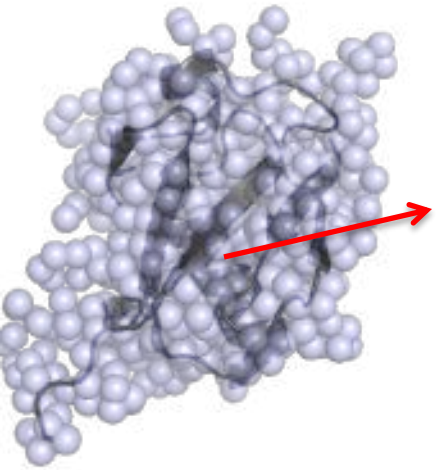
external magnetic field B_0

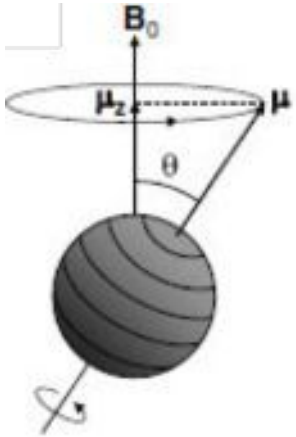


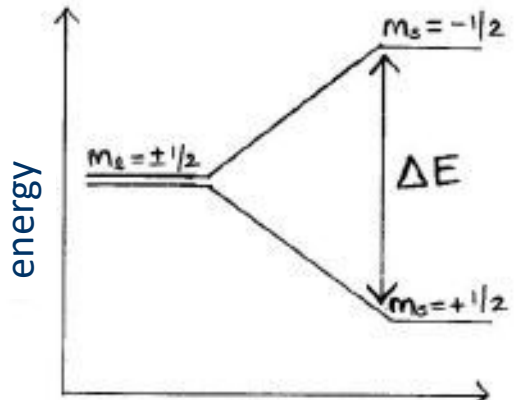
population difference

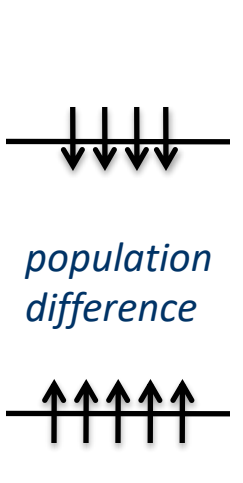


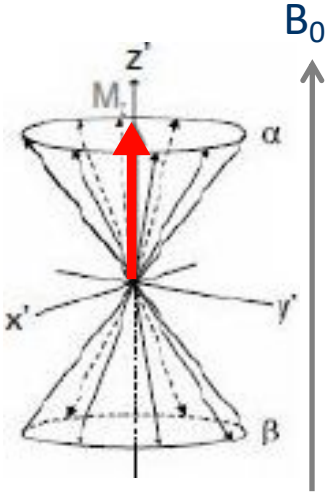
NMR spectroscopy is an atomic-resolution technique





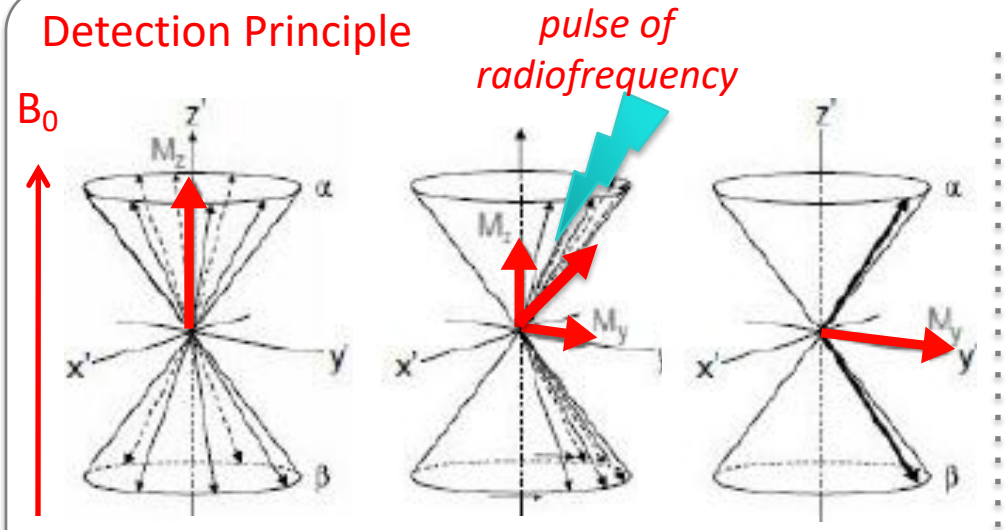




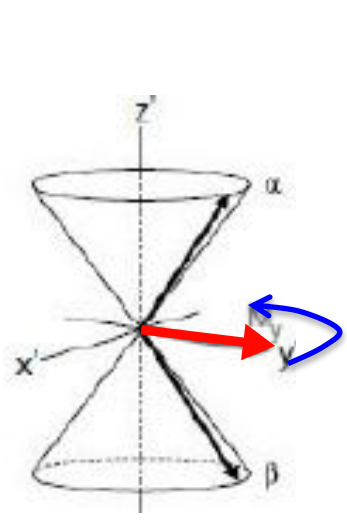


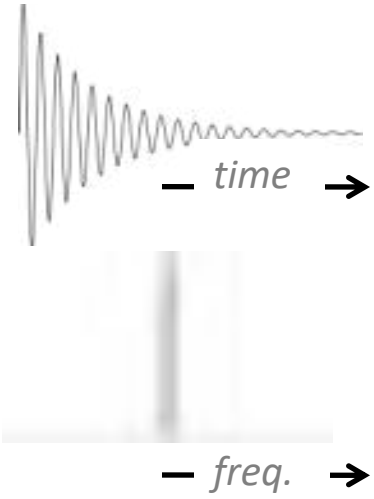
external magnetic field B_0

Detection Principle



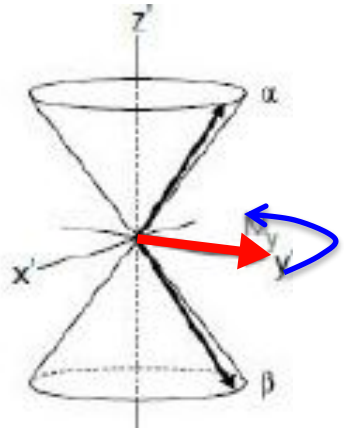
excite coherent superposition
(by radiofrequency irradiation)





observe precession of magnetization
(induction of current)

Nuclear spins act as “local spies”, reporting on their immediate environment



local field
chemical shift,
Spin-spin couplings.

micro-Tesla

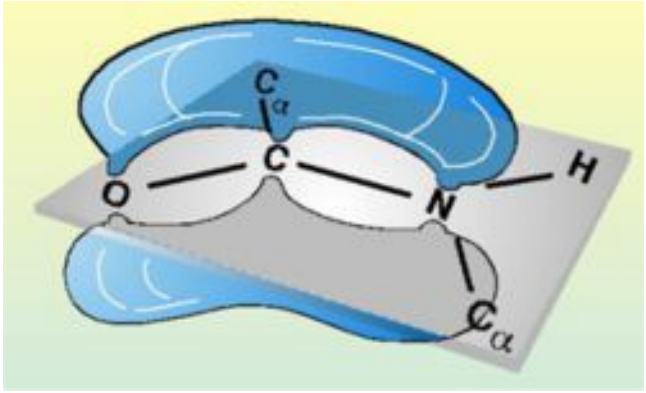
B_0
(external field)
10-20 Tesla
(10^6 x earth magnetic field)

The spins “see” the sum of

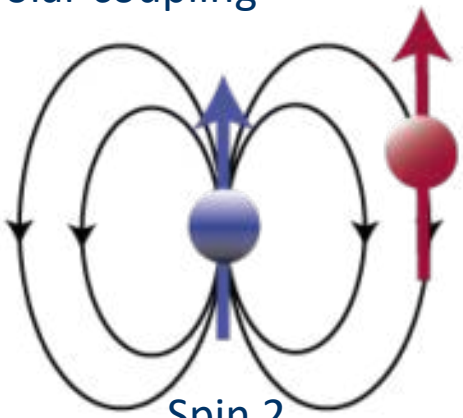
- the external field (our magnet)
- local magnetic fields in molecule

+

Electron density around nucleus
“chemical shift”



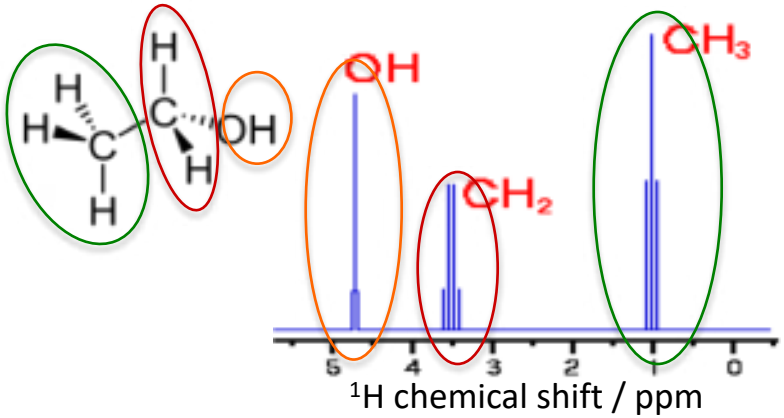
Local magnetic field induced by
neighboring spin
“dipolar coupling”



Physical interactions of a spin with its environment

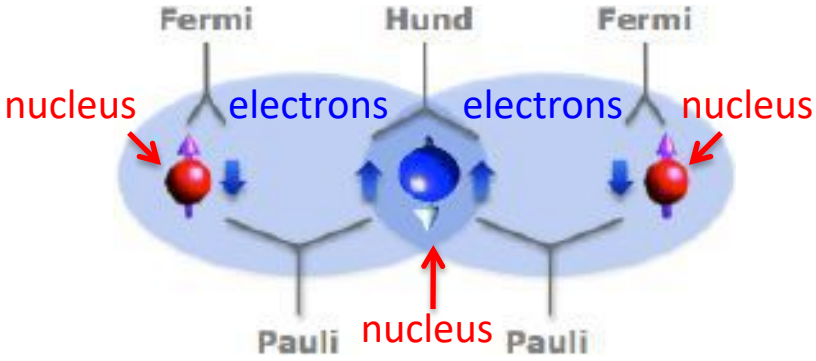
Interaction with electronic environment

Chemical shift (isotropic part)



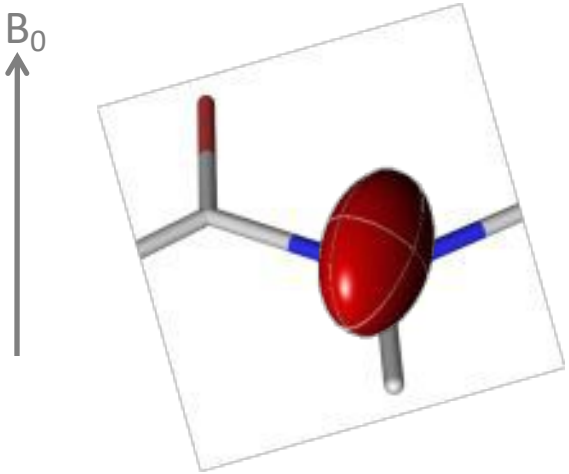
Spin-spin interactions

Scalar coupling (through-bond)

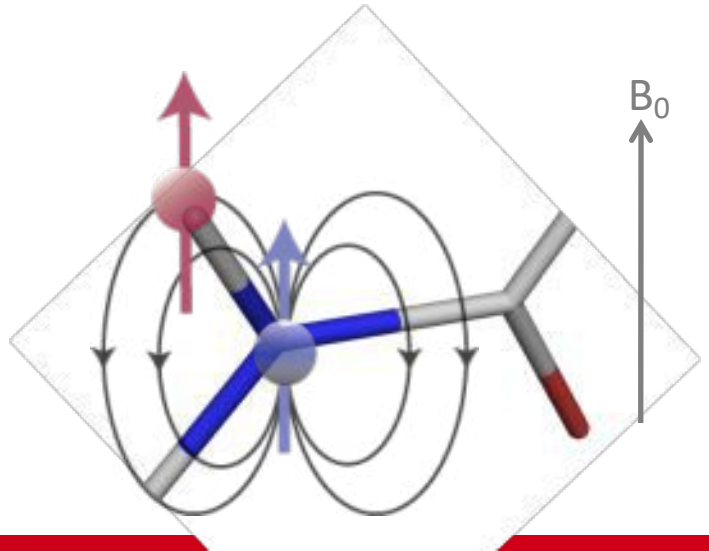


isotropic

Chemical shift (anisotropic part)



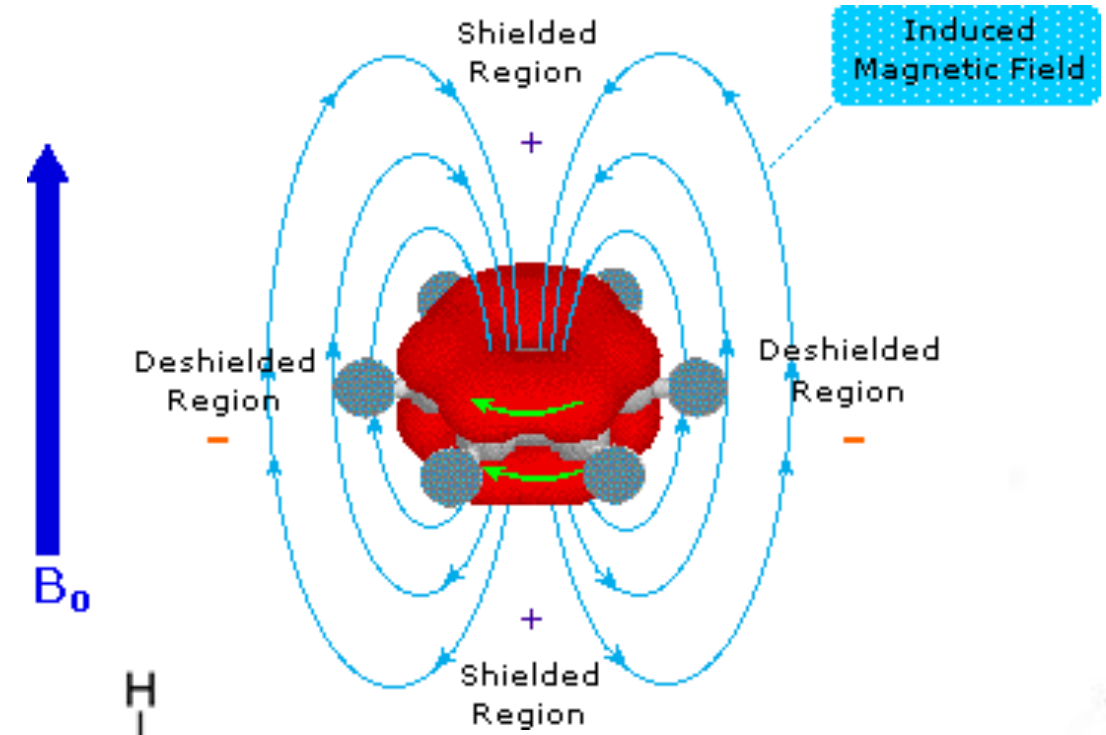
Dipolar coupling (through space)

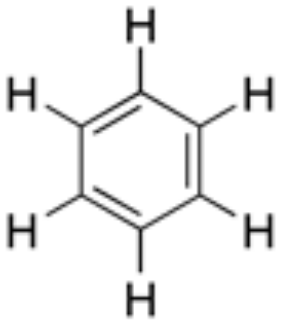


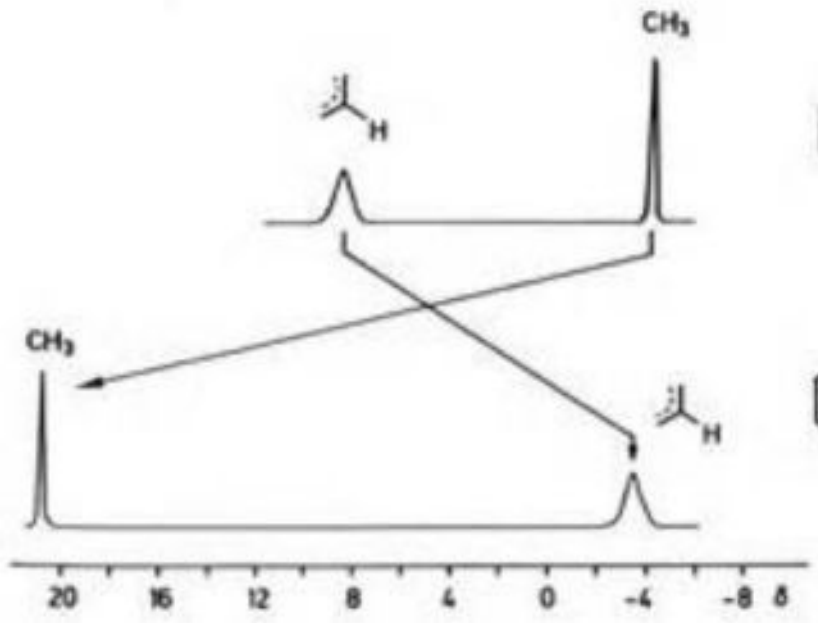
orientation-dependent

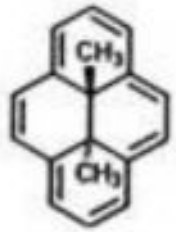
The chemical shift, a reporter of the local electronic environment

Chemical shift (isotropic part)

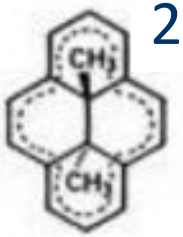






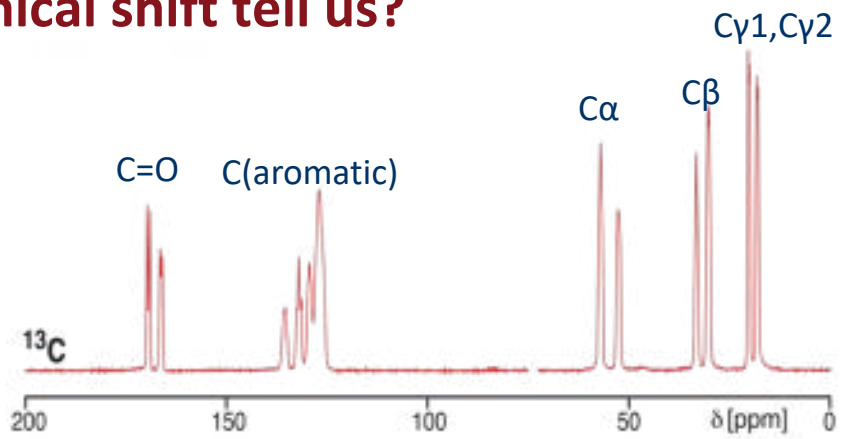


2-



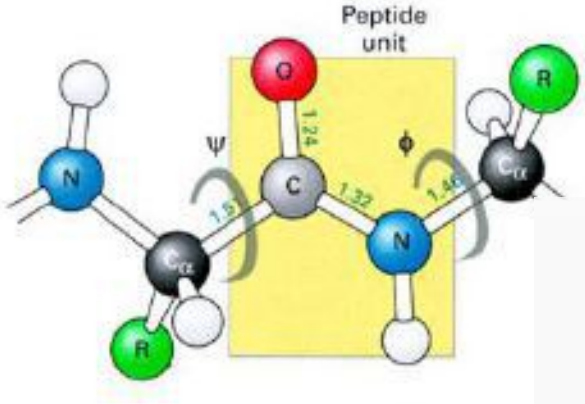
What does the chemical shift tell us?

Chemical environment around a nucleus

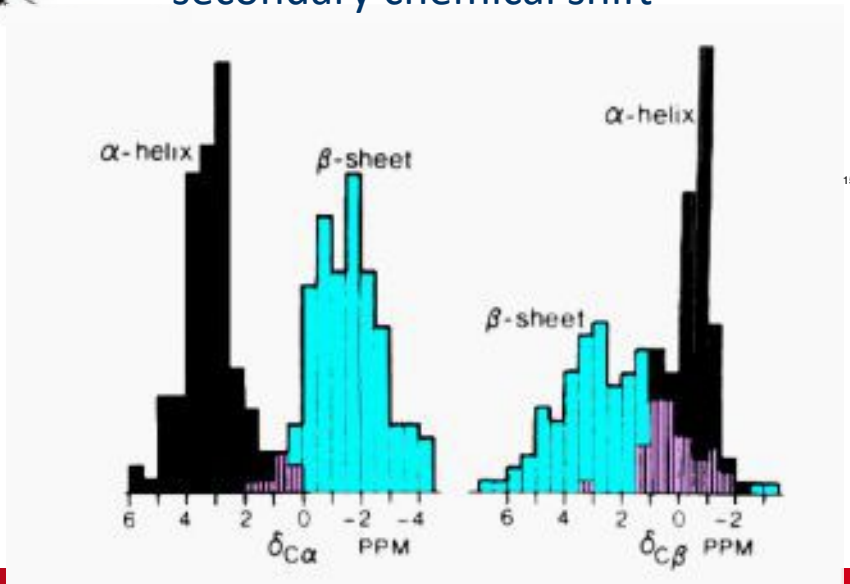


^{13}C resonance frequency / ppm

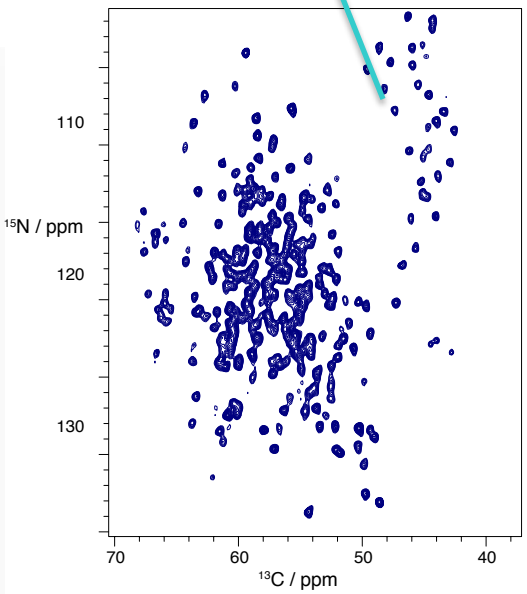
Local dihedral angles: e.g. backbone conformation



"secondary chemical shift"

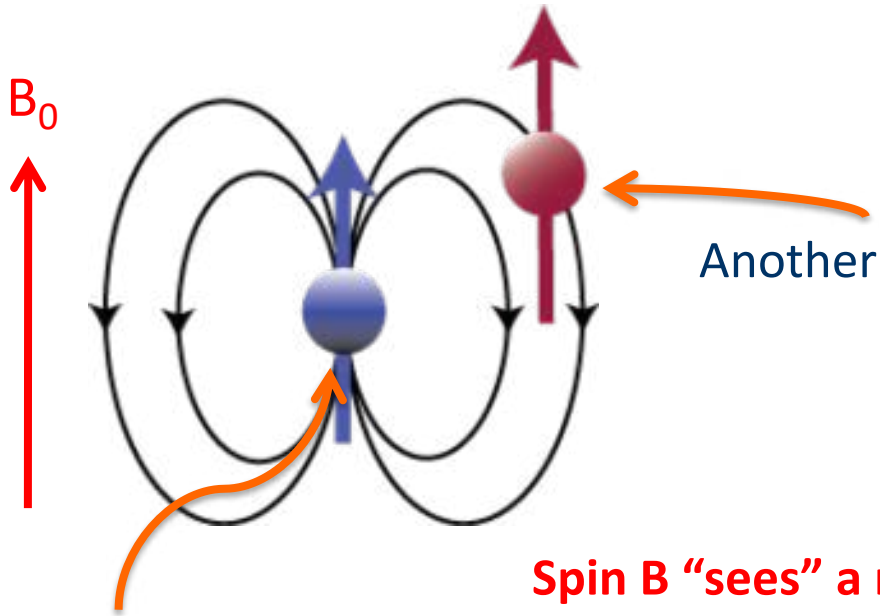


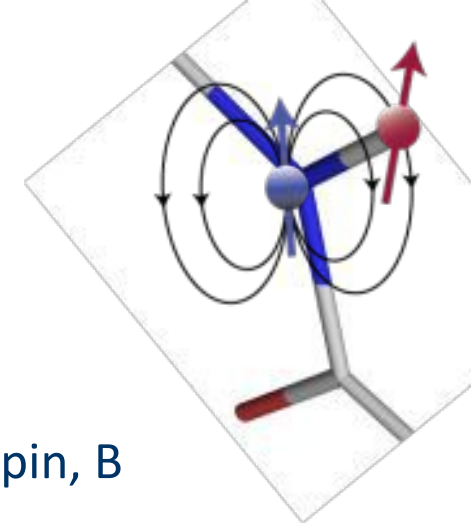
"Cα of Gly 57 has a chem shift of 47.2ppm hence it has a β-sheet conformation"



The spin-spin interactions (dipolar and scalar couplings)

Analogy:
A bar magnet

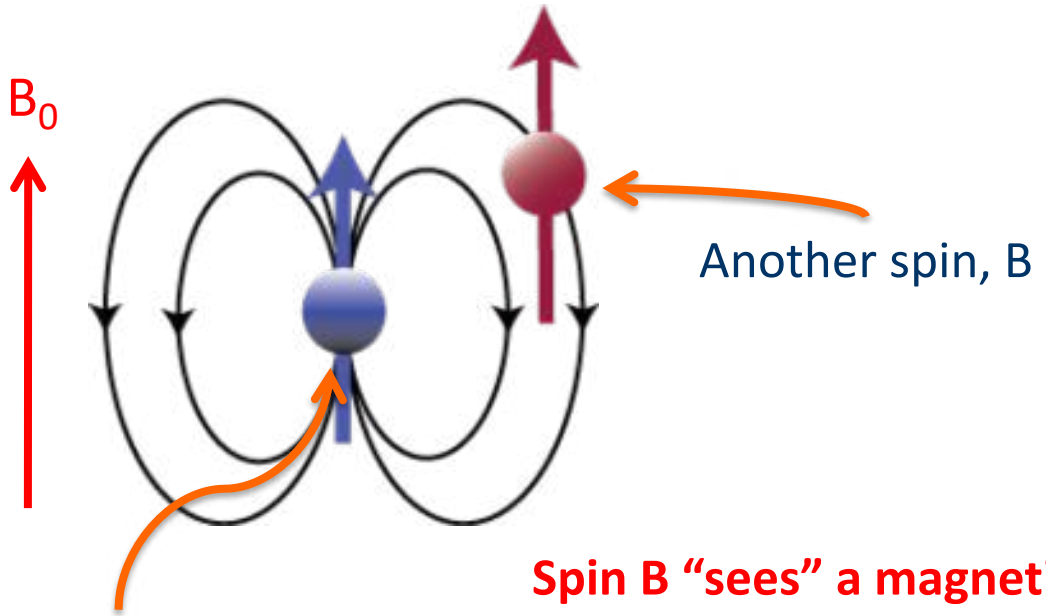




Spin B "sees" a magnetic field that is, in part, due to the presence of spin A

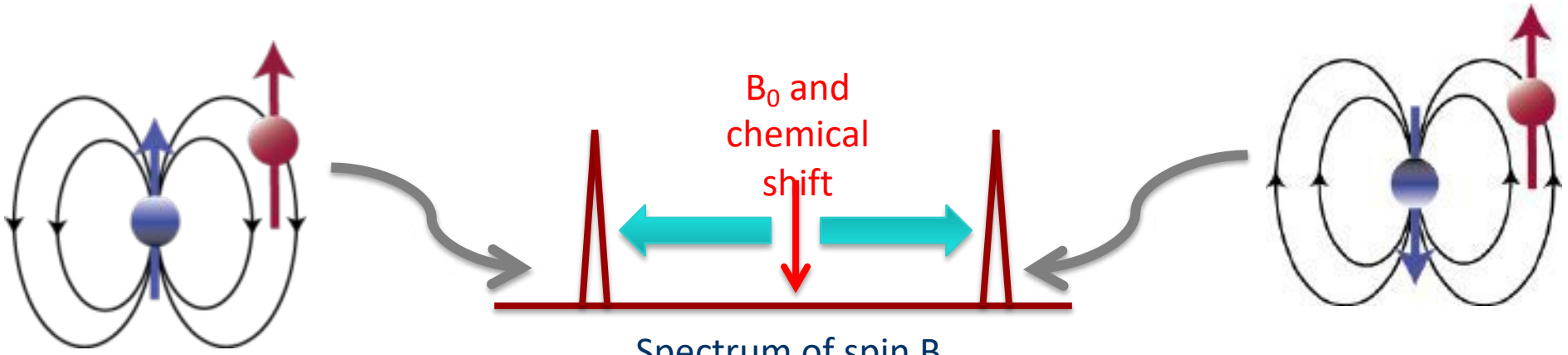
The spin-spin interactions (dipolar and scalar couplings)

Analogy:
A bar magnet



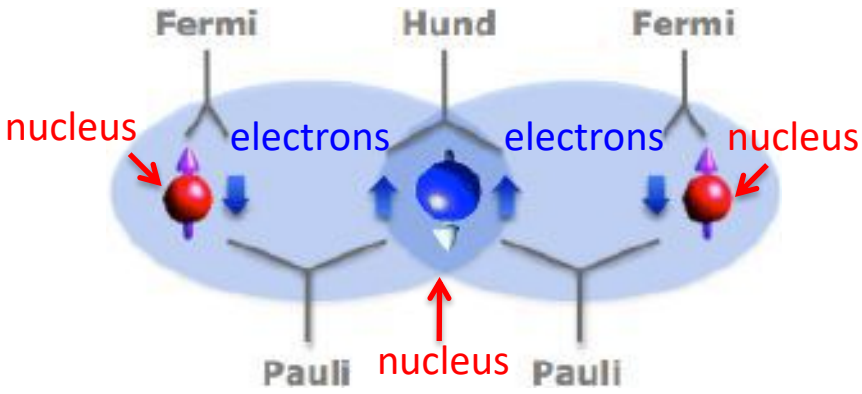
(e.g. a ^{1}H nucleus or electron)

Spin B "sees" a magnetic field that is, in part, due to the presence of spin A



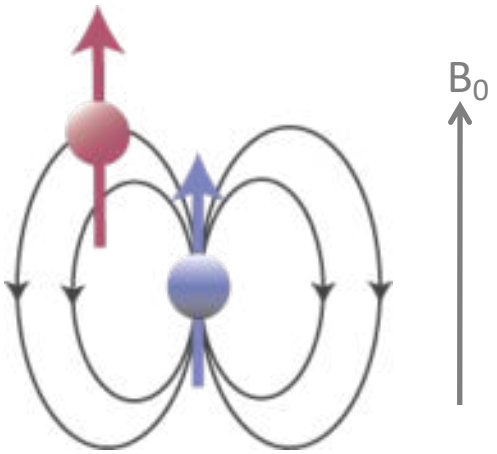
Spin-spin couplings: through bonds or through space

Scalar coupling (*via* bond electrons)



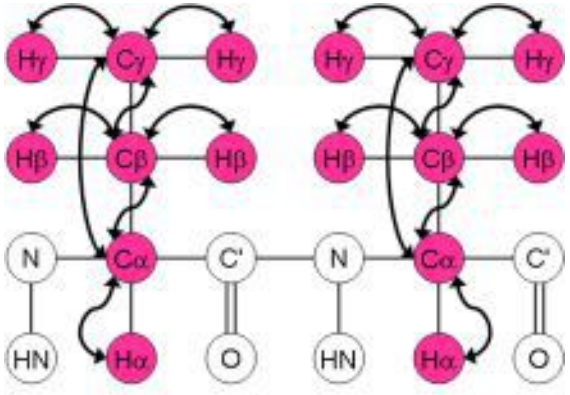
isotropic

Dipolar coupling (through space)



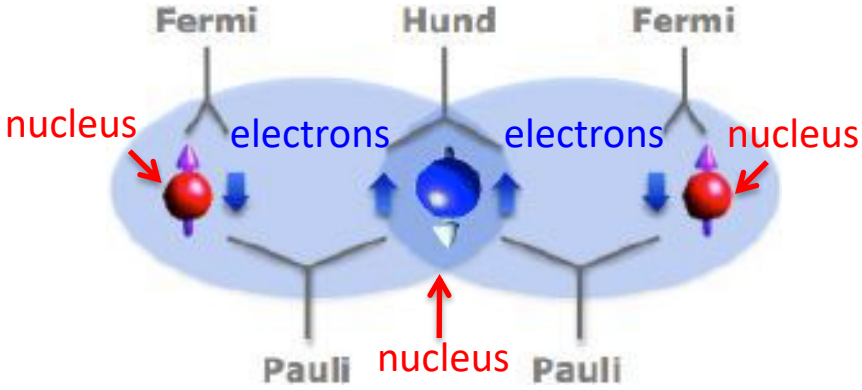
orientation-dependent

Spin-spin couplings: through bonds or through space

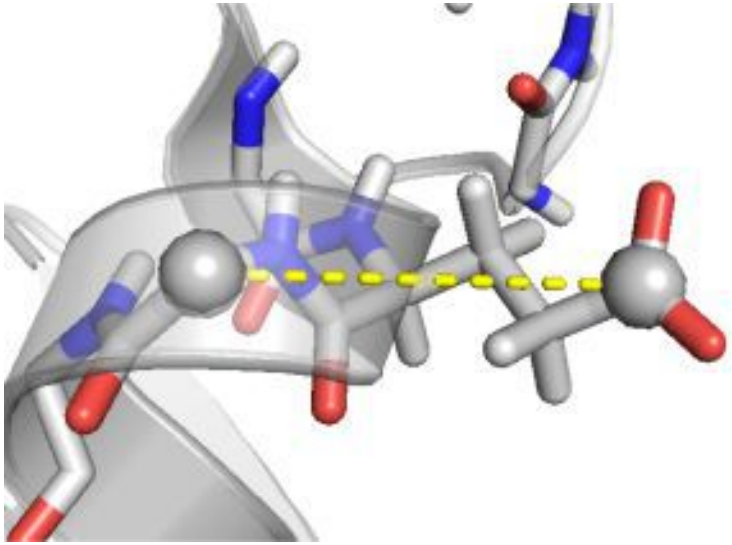


"which atom is bonded to which atom"
-> establish sequential connections

Scalar coupling (*via* bond electrons)

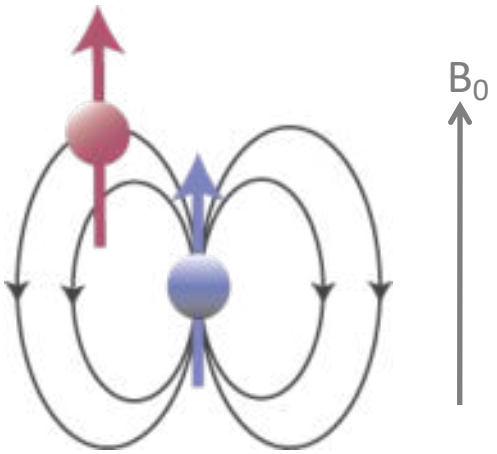


isotropic



"which atom is close in space to which atom"
-> extremely useful for structure determination

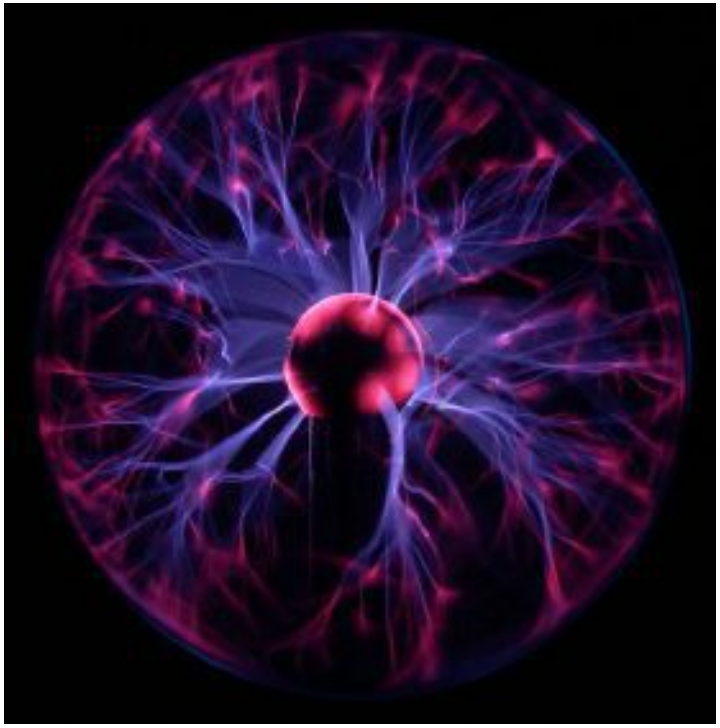
Dipolar coupling (through space)



orientation-dependent

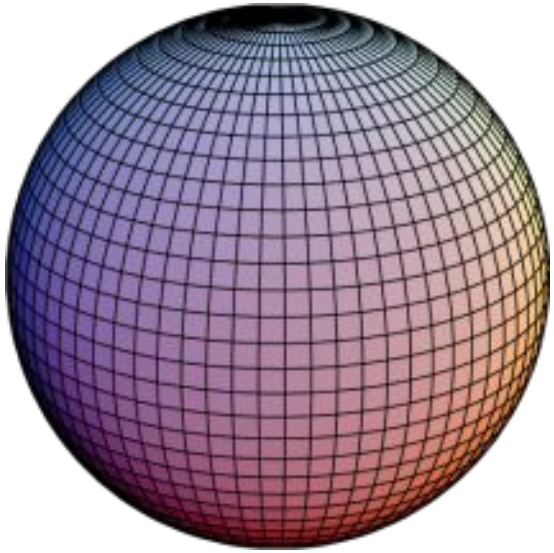
Anisotropy (i.e., orientation dependence)

A highly anisotropic object



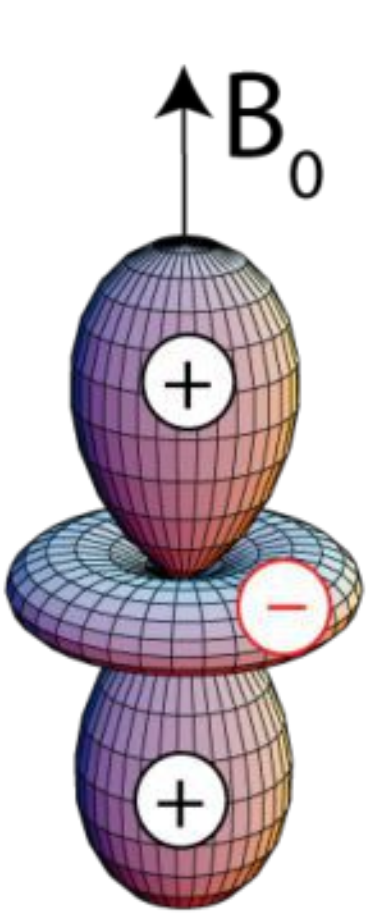
Plasma lamp

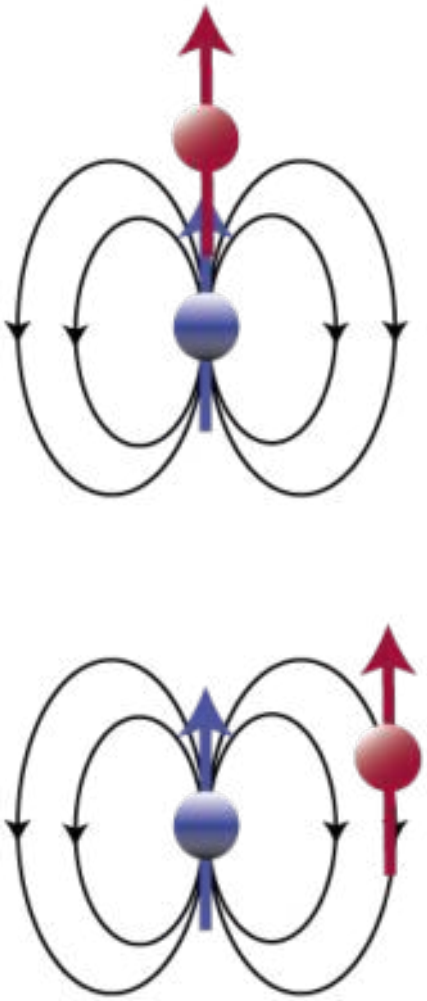
isotropic object

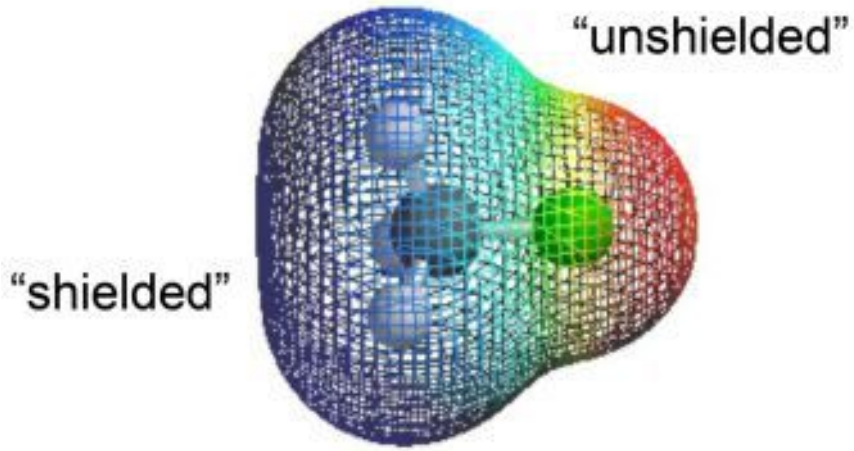


Perfect sphere

Anisotropic interactions in NMR spectroscopy

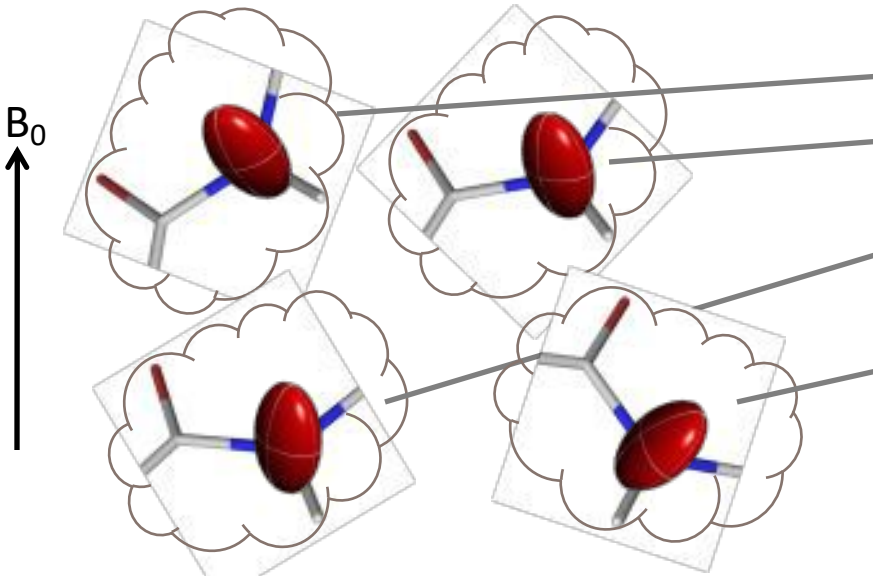


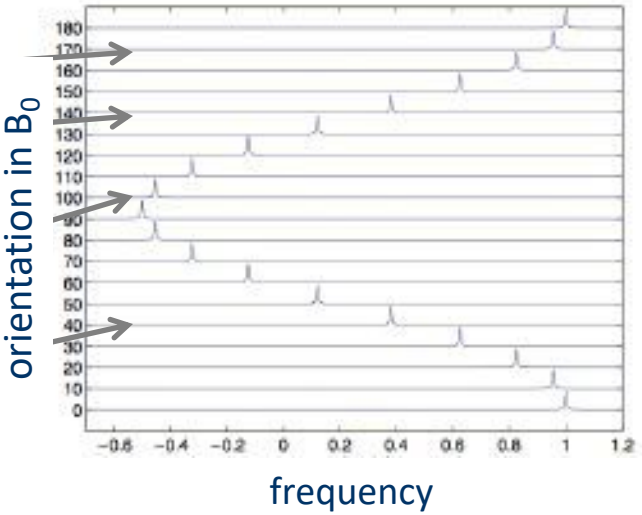




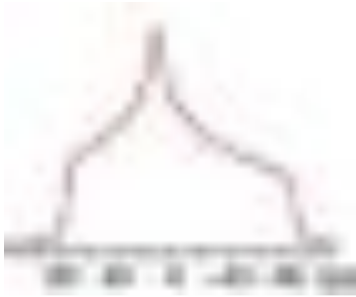
Orientation-dependent interactions in NMR

chemical shift anisotropy



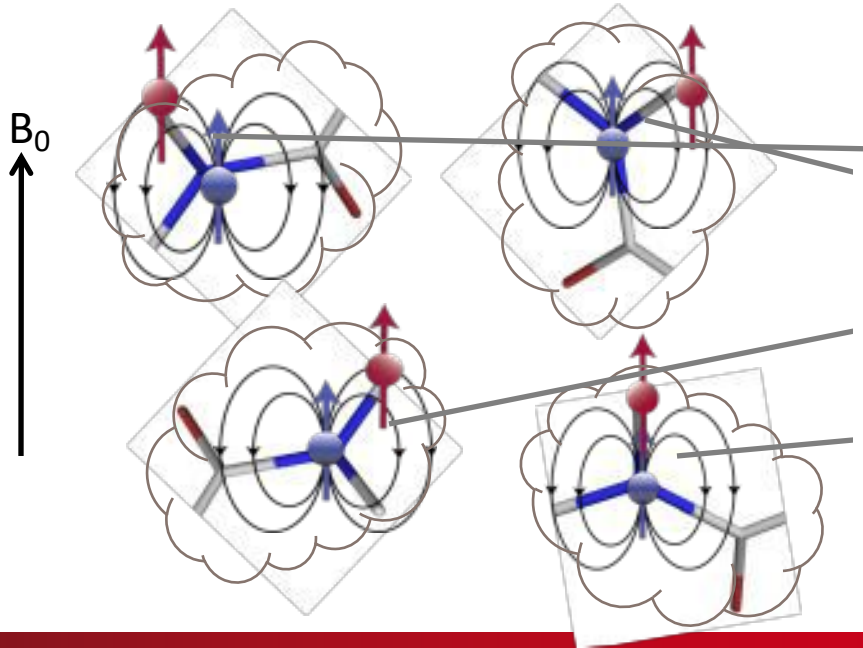


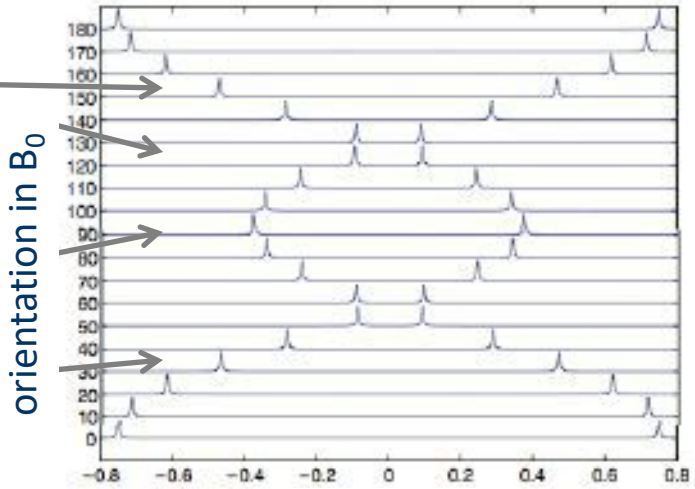
Resulting:

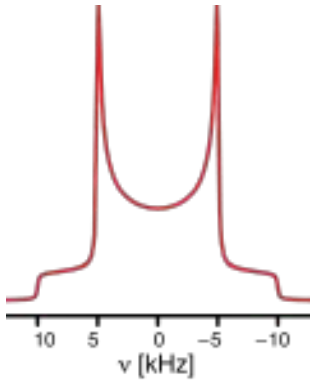


broad pattern!

dipolar coupling







broad pattern!

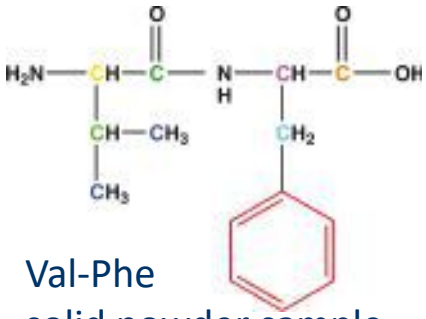
Orientation-dependent interactions in NMR

chemical shift anisotropy

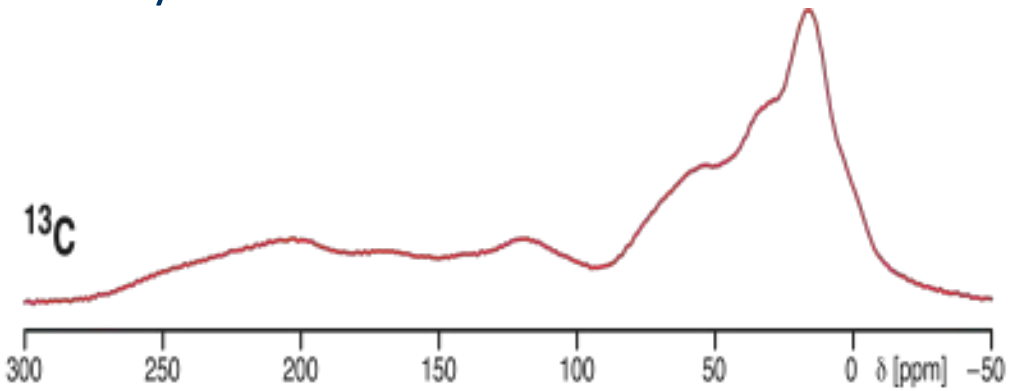
Resulting:

B_0

Very broad lines and essentially no atomic resolution



Val-Phe
solid powder sample



^{13}C chemical shift / ppm

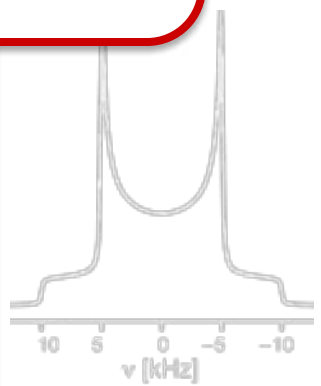
dipolar

ttern!

B_0

orientation in B_0

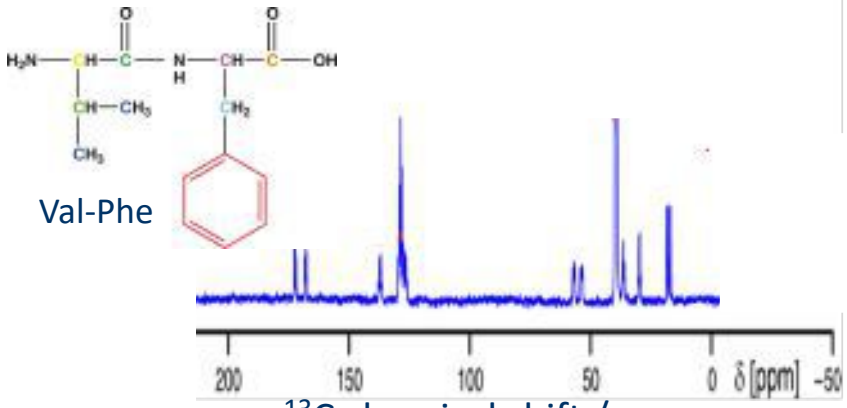
frequency



broad pattern!

Molecular tumbling in solution averages anisotropic interactions

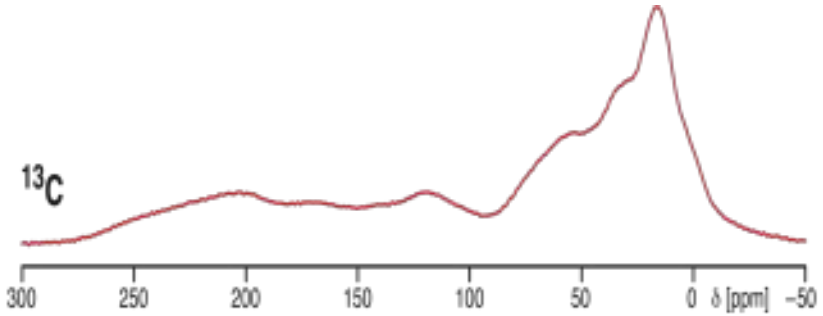
Solution-state



^{13}C chemical shift / ppm

Solid-state

(static powder of randomly oriented crystallites)

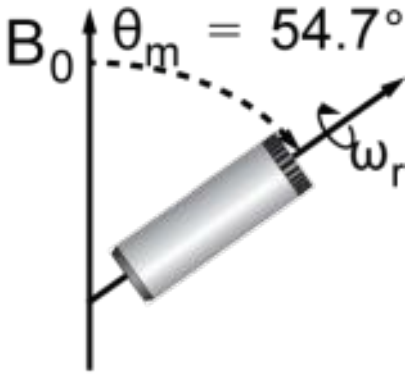


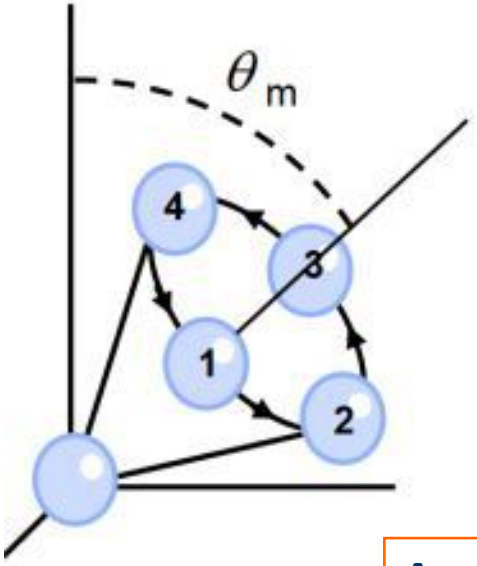
^{13}C chemical shift / ppm

Rapid Brownian motion
➔ high-resolution spectra

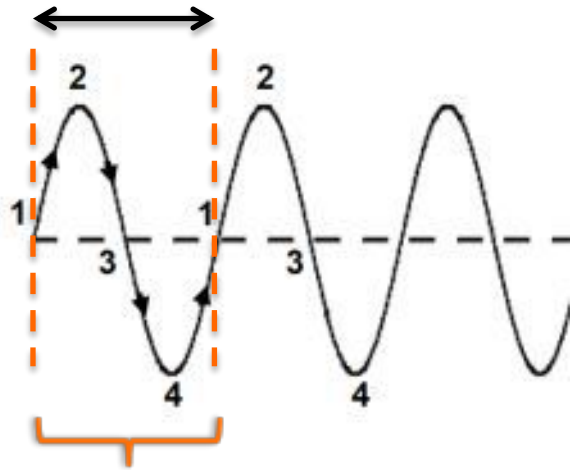
Static, randomly oriented molecules
➔ broad lines

High-resolution solid-state NMR by “magic-angle spinning”

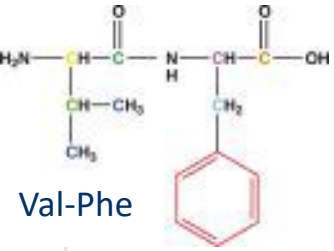




One sample rotation

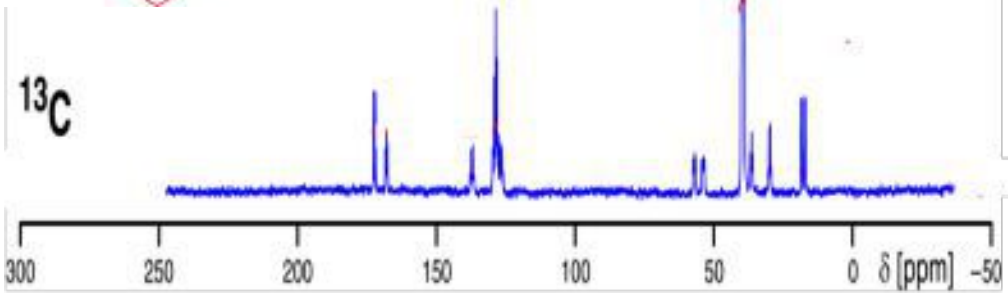


Average interaction is zero !

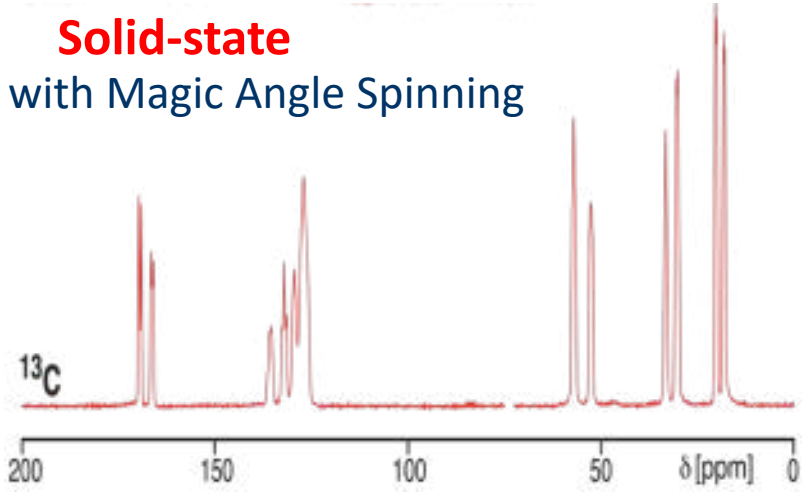


Val-Phe

Solution-state



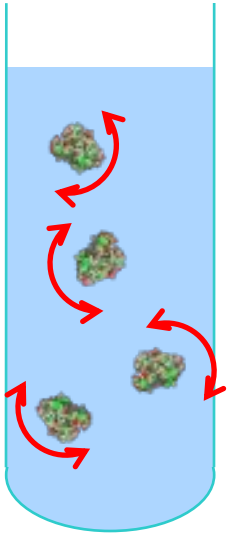
Solid-state
with Magic Angle Spinning



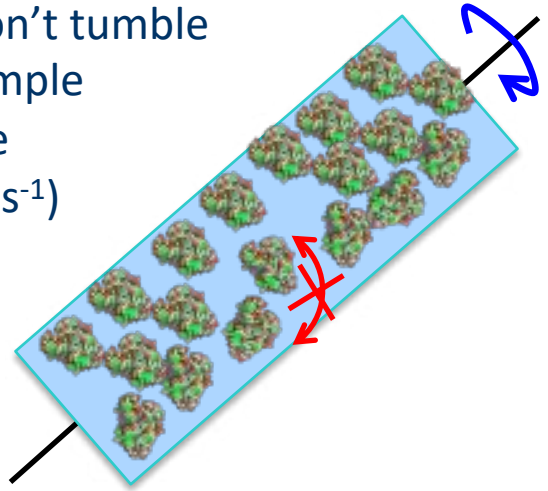
^{13}C chemical shift / ppm

High-resolution solid-state NMR by “magic-angle spinning”

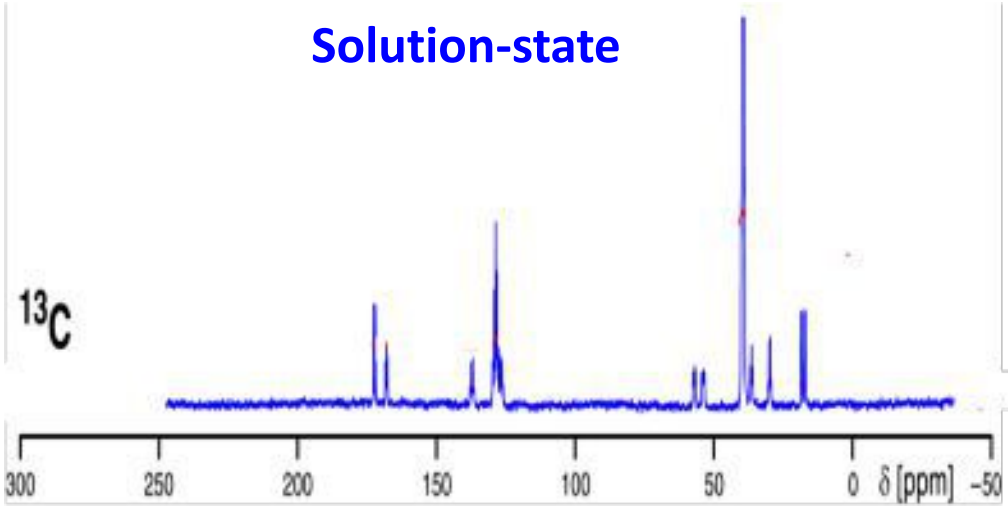
the molecules tumble stochastically



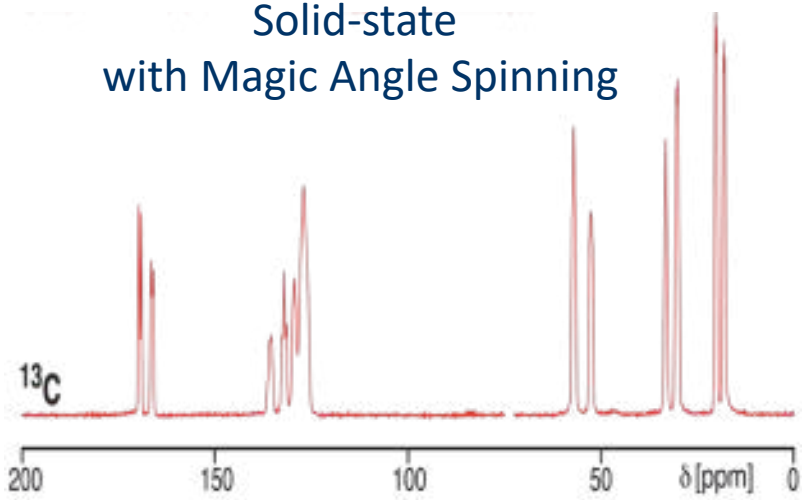
the molecules don’t tumble
we rotate the sample
at a constant rate
(10.000-100.000 s^{-1})



Solution-state



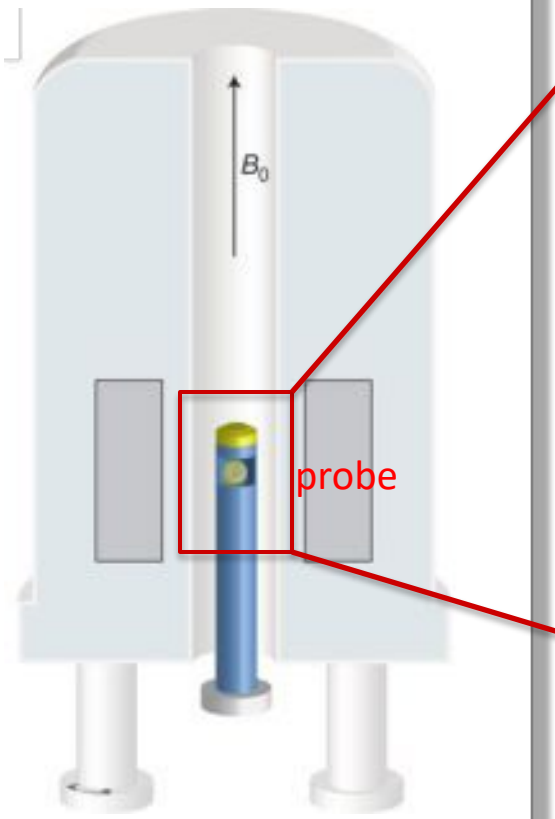
Solid-state
with Magic Angle Spinning



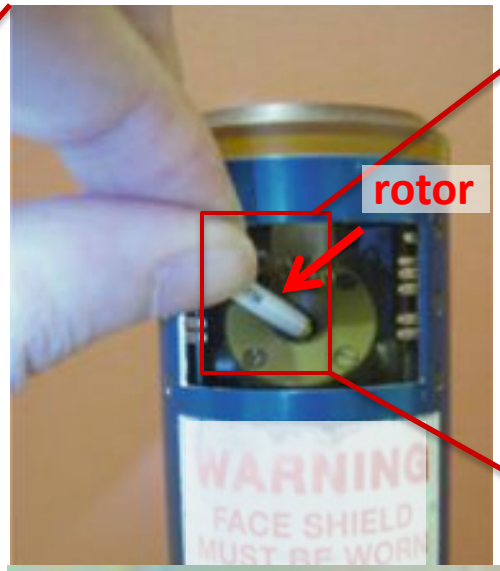
^{13}C chemical shift / ppm

Instrumentation for Magic-Angle-Spinning ssNMR

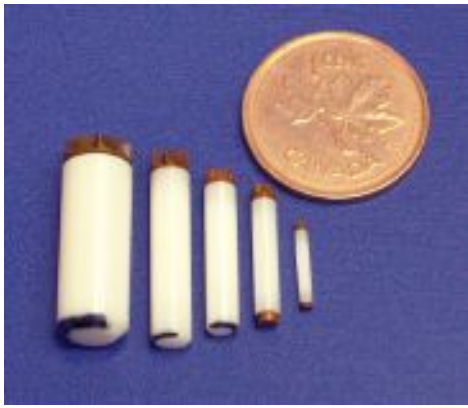
NMR magnet



probe



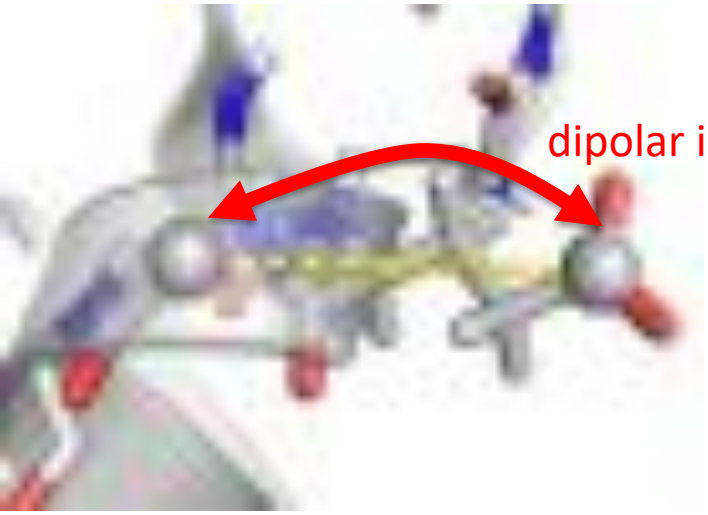
sample container
("rotor")



diameter	max. speed	sample volume
4 mm	15 kHz	70 µL
3.2 mm	25 kHz	30 µL
1.6 mm	40 kHz	8 µL
1.3 mm	67 kHz	1.7 µL
0.7 mm	111 kHz	0.7 µL

rotation driven by gas flows

ssNMR techniques can “turn on and off” the interactions as needed

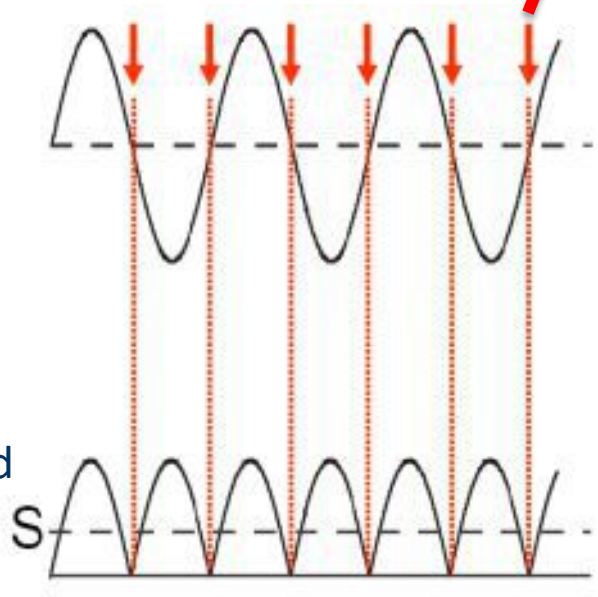


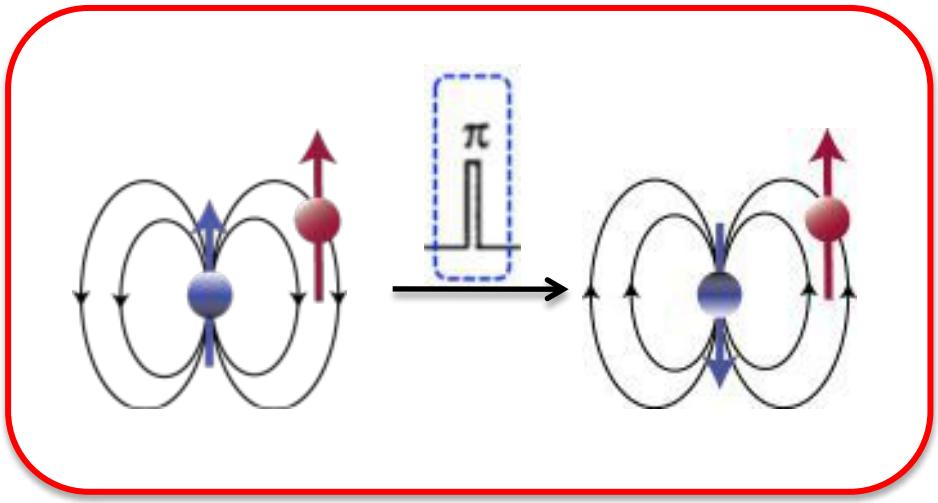
Magic-angle spinning averages out the dipolar interaction
-> distance information is lost

But we can turn them “on” again for selected time periods to get the distance information.

MAS only

MAS and Synchronized RF pulses

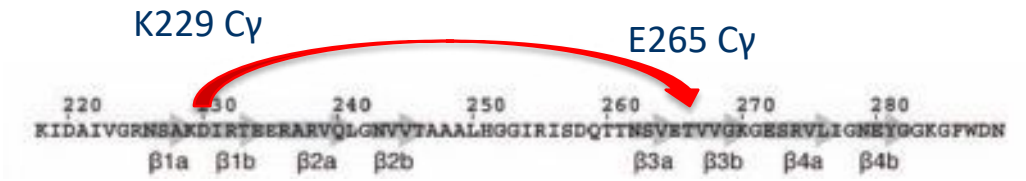


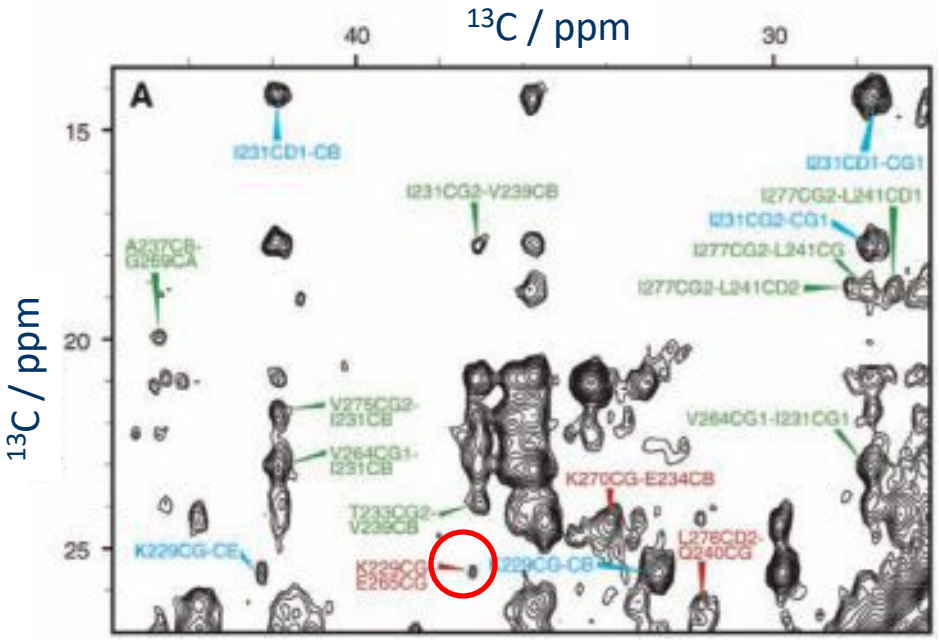


Structure determination from measurement of atom-atom distances

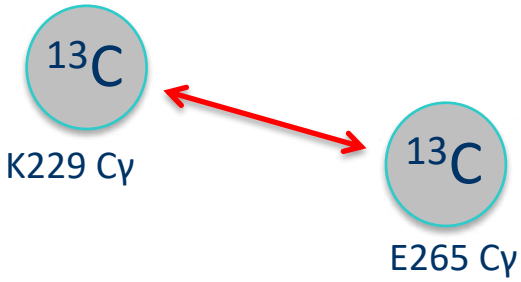
Structure determination is based on (many) **local atomic distances**

➔ reconstruction of global structure.





2. Atom-atom distances



edit frequency of first nucleus

⇩

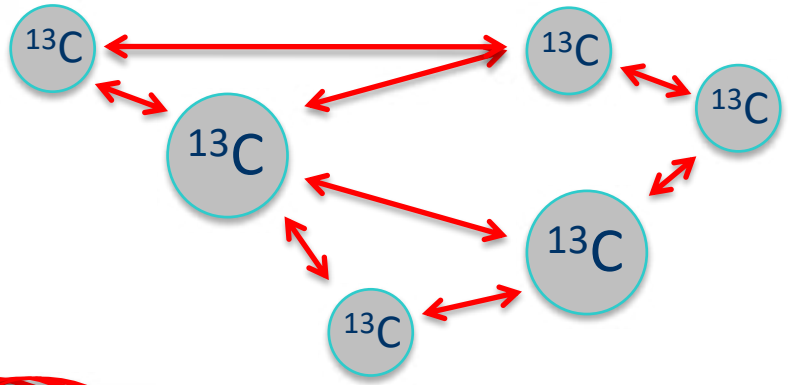
transfer magnetization

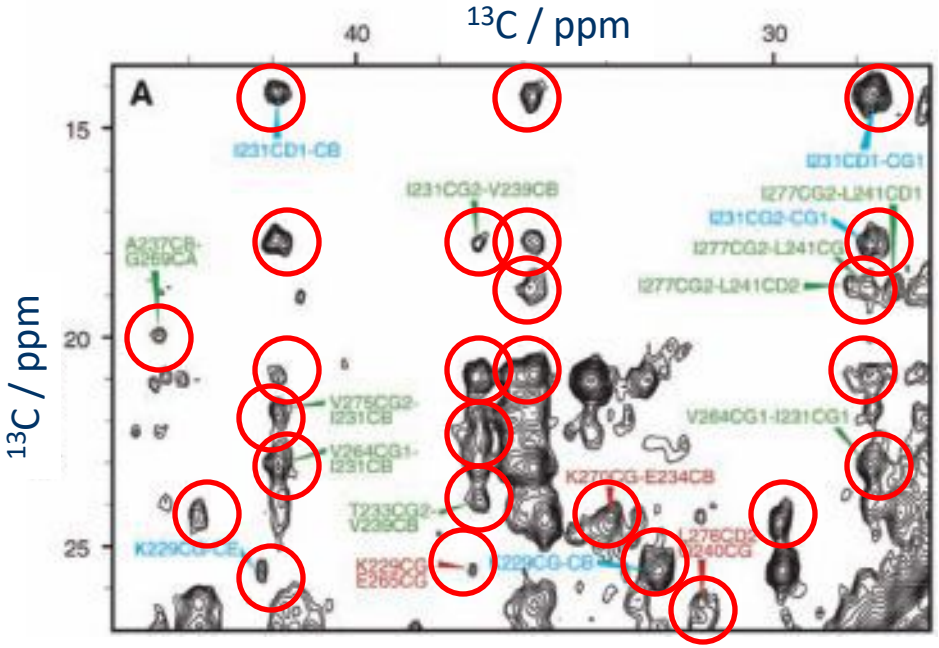
⇩

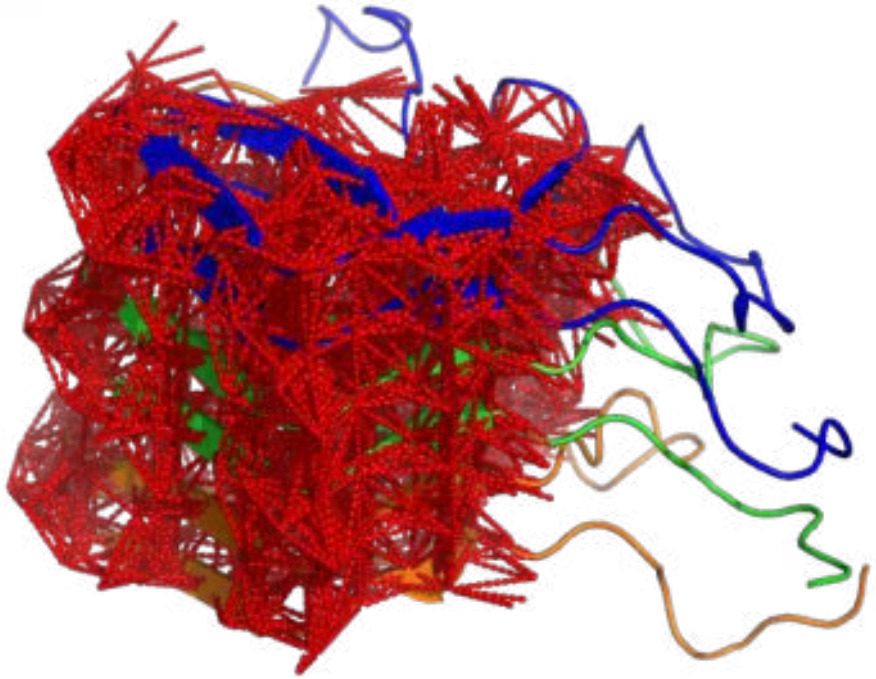
detect spin second nucleus

Structure determination is based on local observables

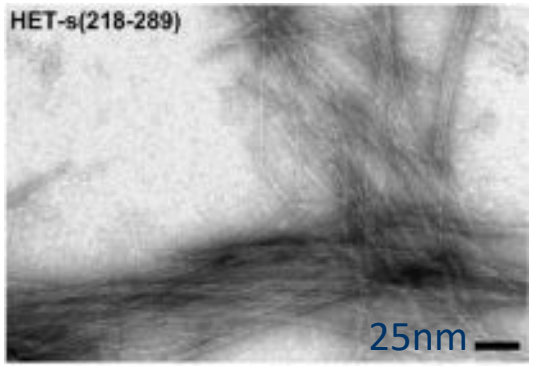
semi-quantitative treatment of distances

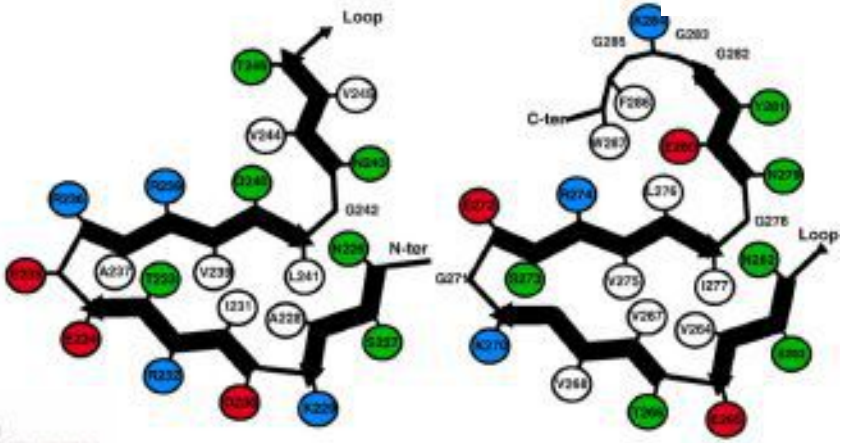


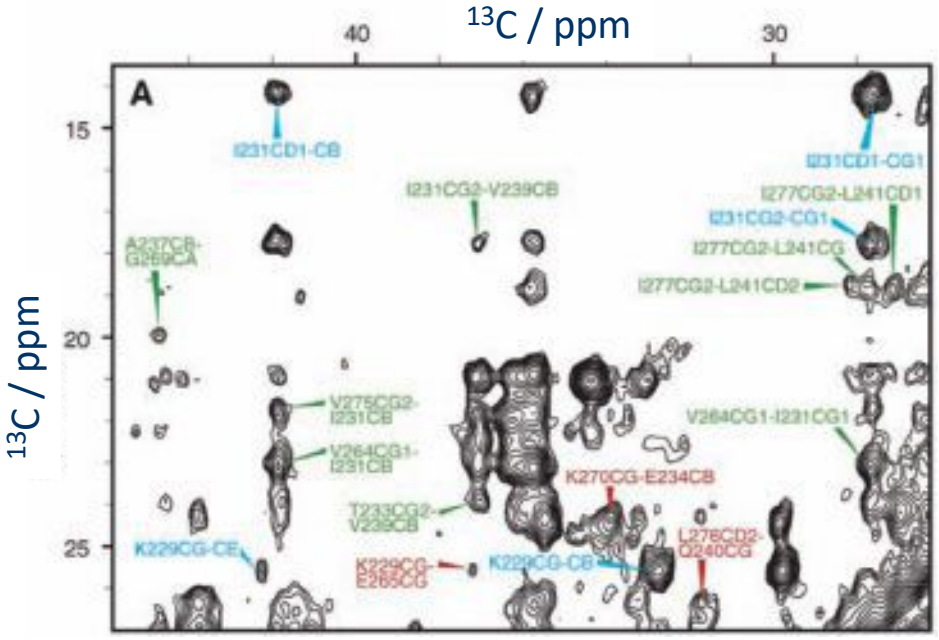


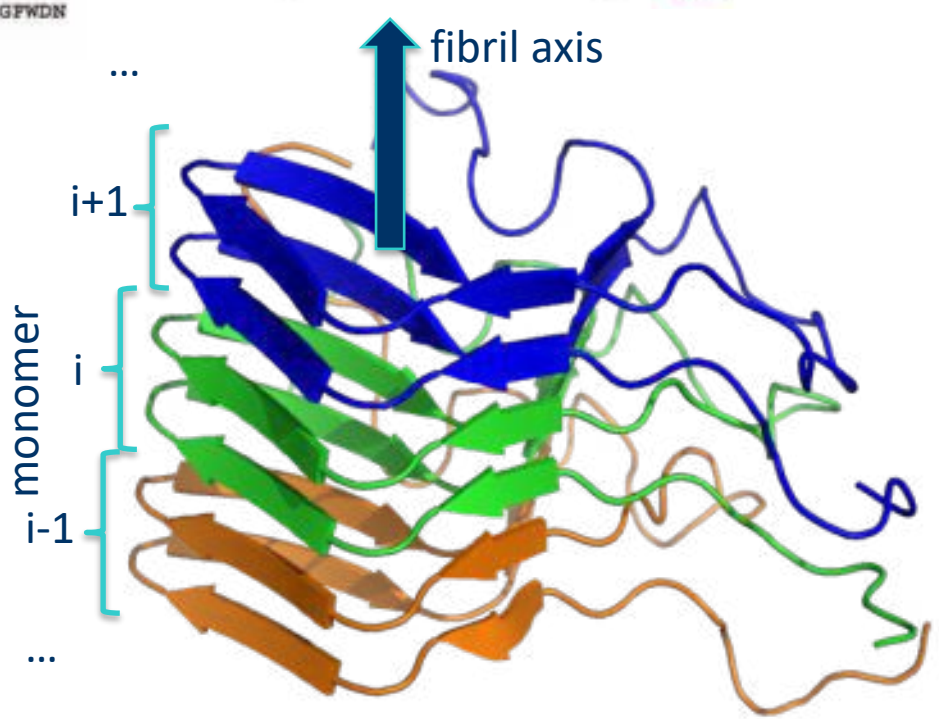


Structure determination is based on local observables









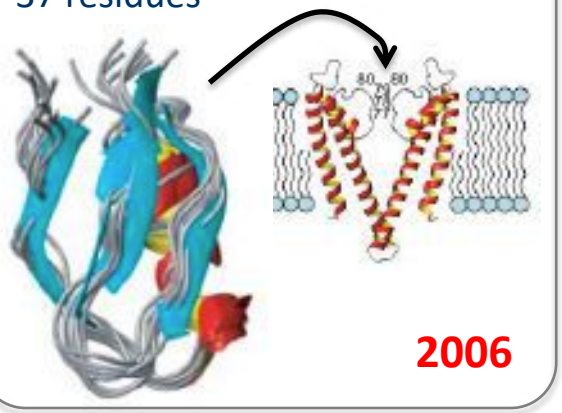
Wasmer et al, Science (2008) 319: 1523-26

De novo structure determination from MAS ssNMR: status quo

2002

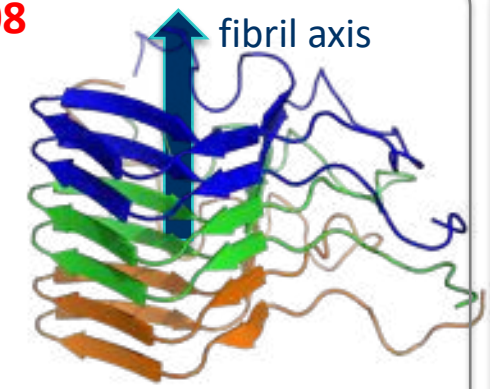
H3 crystal
2 residues

Kaliotoxin bound to
membrane protein KcsA
37 residues



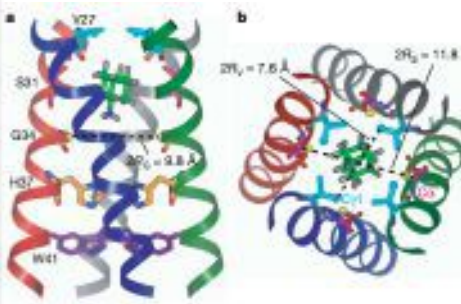
2006

2008



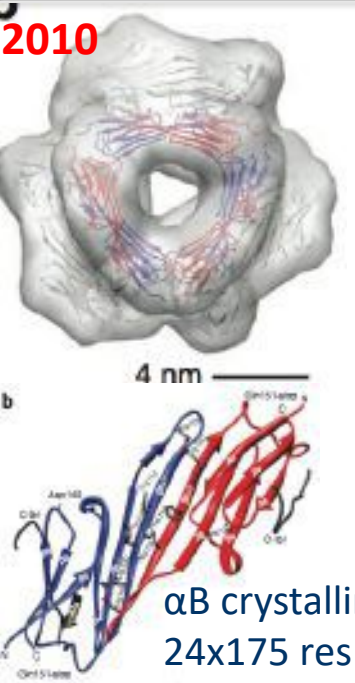
HET-s amyloid fibers
69 residues/monomer

2010



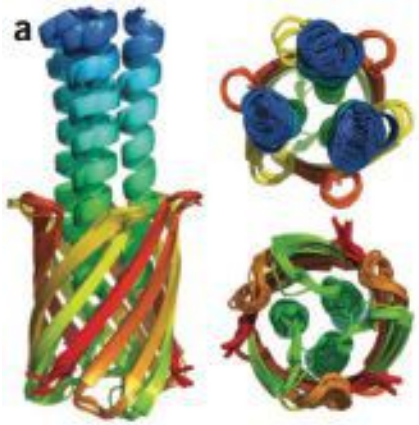
Influenza M2 channel
with drug in lipid
membrane, 4x30 res.

2010

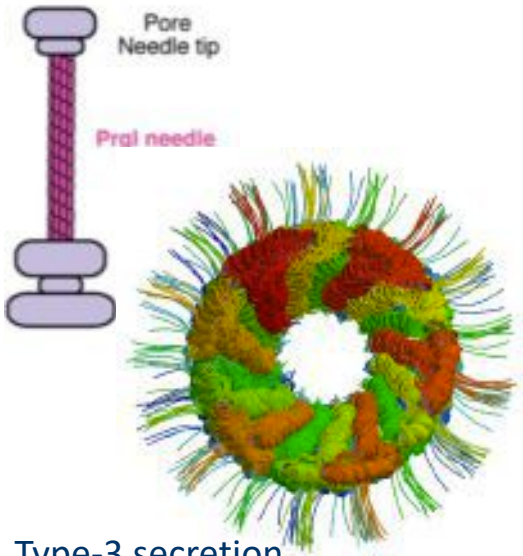


αB crystallin
24x175 res

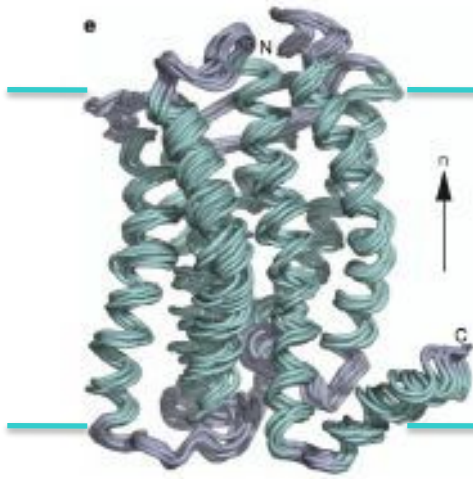
2012



YadA autotransporter
membrane protein
3 x 105 residues



Type-3 secretion
system needle
81 res/monomer

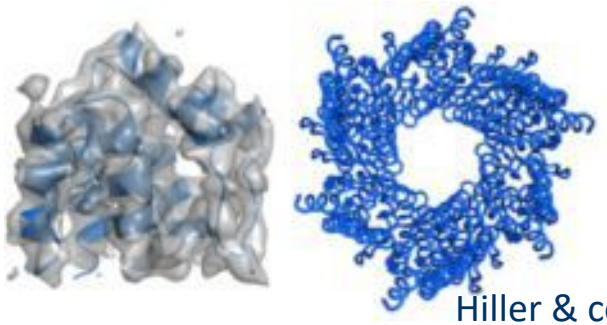


CXCR1 chemokine receptor
in lipid membrane
328 residues (GPCR)

De novo structure determination from MAS ssNMR: status quo

2015

Inflammasome (CARD domain fibers)

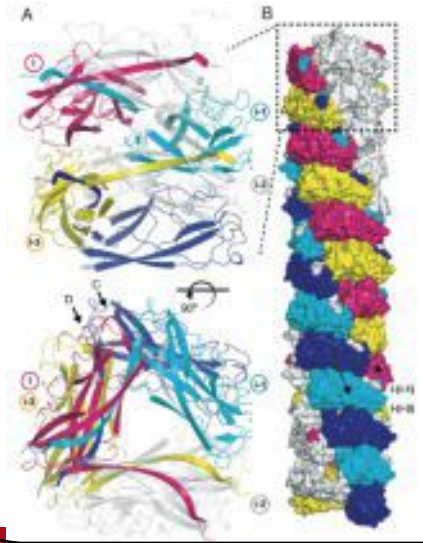


Hiller & co, PNAS

(combined with EM data)

mitochondrial antiviral signaling domain fibers

Ritter & co, PNAS



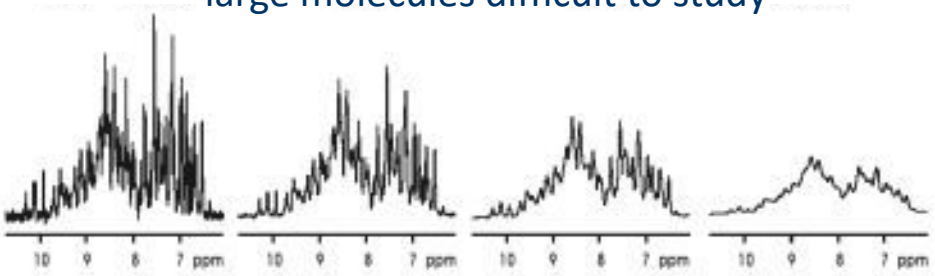
bacterial type 1 pilus

Lange & co, Angew Chem

ssNMR offers new possibilities (exceeding solution-state NMR)

Solution-state

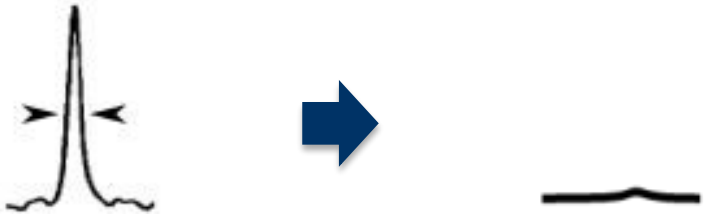
slow overall tumbling makes
large molecules difficult to study



8kDa 16kDa 24kDa 50kDa

fast overall rotation

slow overall rotation

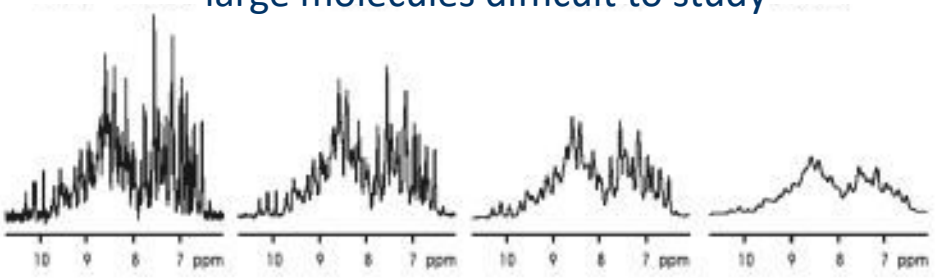


Solution-state NMR is severely challenged by high molecular weight (slow molecular tumbling)

ssNMR offers new possibilties (exceeding solution-state NMR)

Solution-state

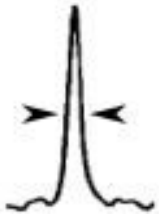
slow overall tumbling makes
large molecules difficult to study



8kDa 16kDa 24kDa 50kDa

fast overall rotation

slow overall rotation



Solid-state + MAS

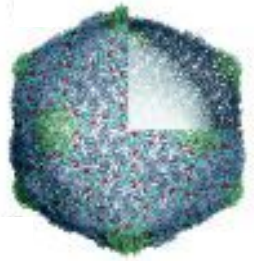
NO stochastic Brownian tumbling
but MAS sample spinning

size-independent line width

γS
19 kDa

MSG
82 kDa

Ribosome
2.3 MDa



Virus capsid
≈11 MDa

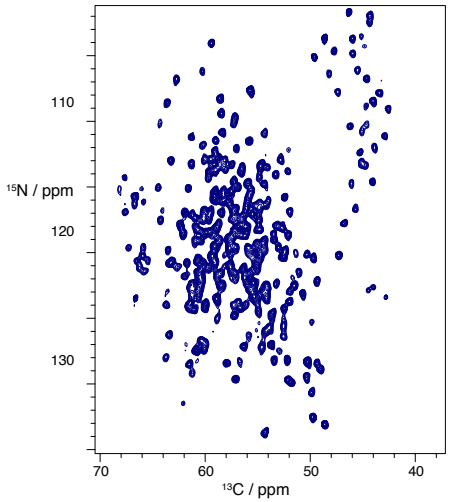
microcrystalline
8kDa ubiquitin

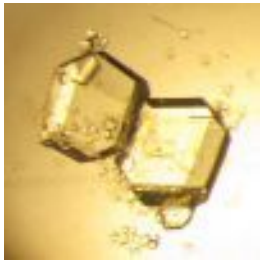
HIV-1 capsid protein
11MDa = 420 x 26kDa

Samples for biological Magic-Angle-Spinning solid-state NMR

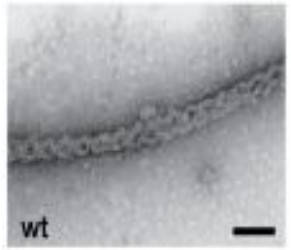
defining criterion: molecules are not rapidly tumbling in solution

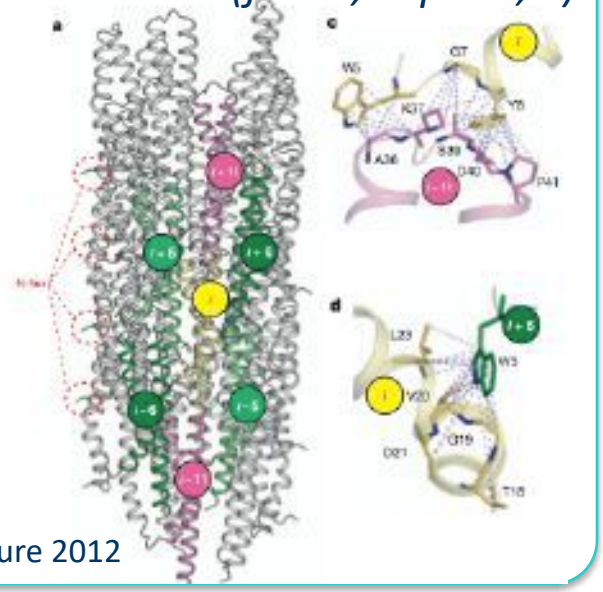
(micro-)crystalline proteins





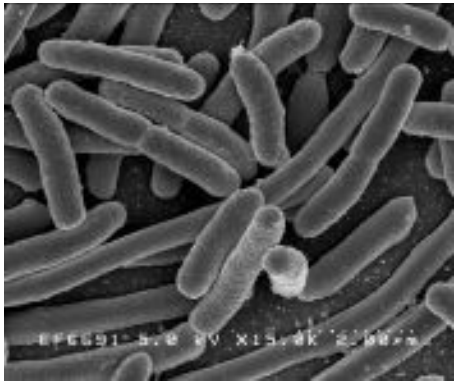
insoluble assemblies (fibrils, capsids,…)

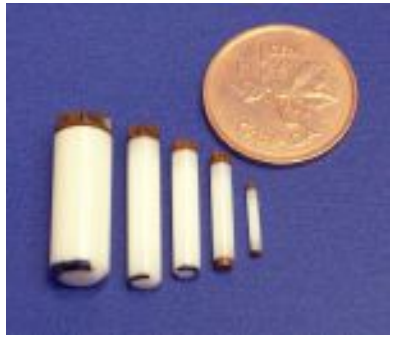




Loquet,…& Lange, Nature 2012

intact cell walls, entire cells





Practical aspects: bio-ssNMR

Labeling requirements

- minimum labeling: 13C, 15N
- for certain approaches: 2H, 13C, 15N

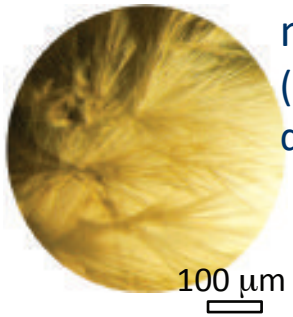
- easiest:
  E. coli, ^{13}C-glucose + $^{15}NH_4$
- also well established:
  - *P. pastoris* ^{13}C-methanol + $(^{15}NH_4)_2SO_2$
  - cell-free

Sample amounts and rotor diameters

diameter	max. speed	sample volume	sample amount
3.2 mm	25 kHz	30 µL	20-25 mg
1.6 mm	40 kHz	8 µL	5-8 mg
1.3 mm	67 kHz	1.7 µL	1-2 mg

Handling

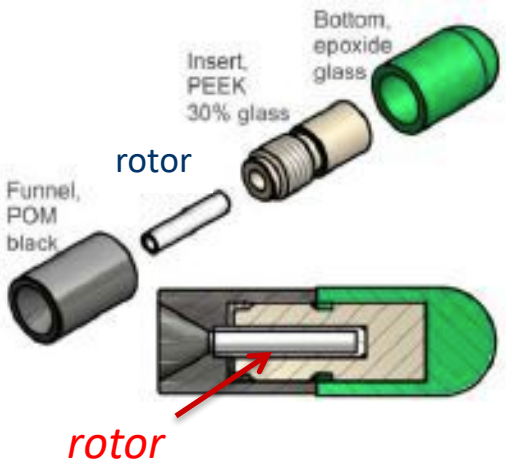
centrifuged cell walls



microcrystals (non-diffracting, doesn't matter)

pelleted proteoliposomes assemblies,…

centrifuge into rotor



Practical aspects: bio-ssNMR

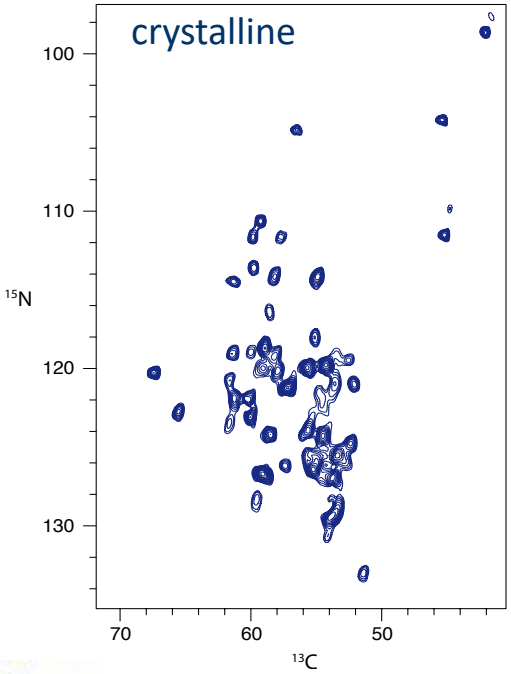
Size matter. Monomer size!

Types of proteins

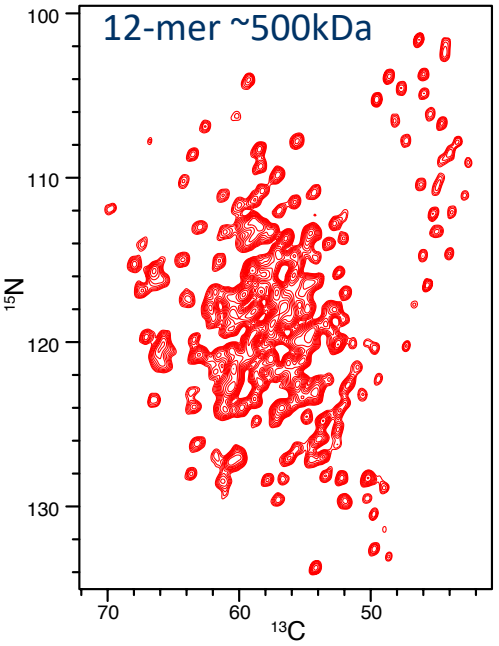
- Aggregates (fibers, capsids, needles…)
- Proteoliposomes
- Large (symmetric) assemblies
- Proteins in interaction with cellular component (cell wall, capsid, …)
- *… be creative*

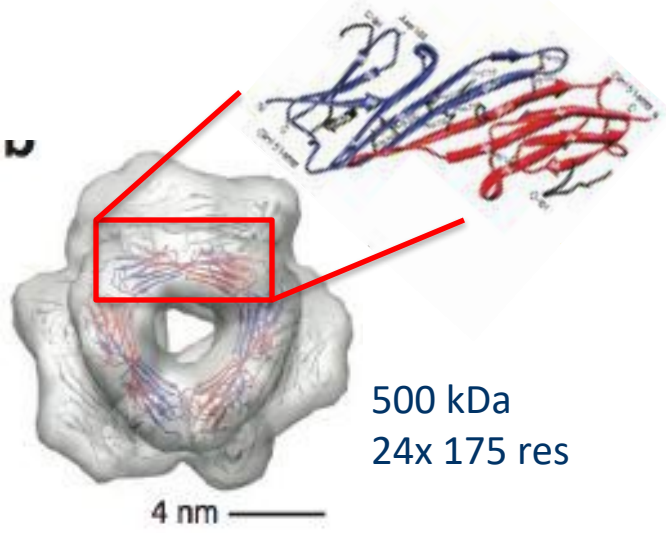
Size: Monomer preferable < 200 - 350 a.a.

8kDa

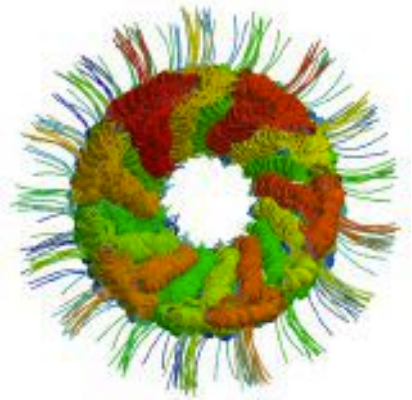


39kDa monomer

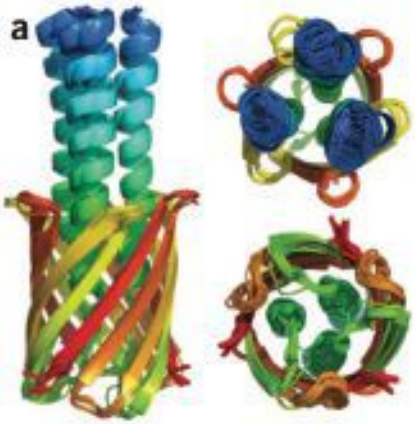




500 kDa
24x 175 res



~infinitely long
monomer: ~80 res



homotrimeric membr. prot.
monomer: ~110 res

Take-home message: solid-state NMR in structural biology

Atom-resolved information about

structure
(local structure, full 3D structures)

interactions
binding interfaces
water/lipid/small molecules

dynamics
local fluctuations
exchange between different states
ligand binding/release

highly complementary in particular to EM

Samples

Crystals
Assemblies/fibers
Membrane proteins
Very large proteins