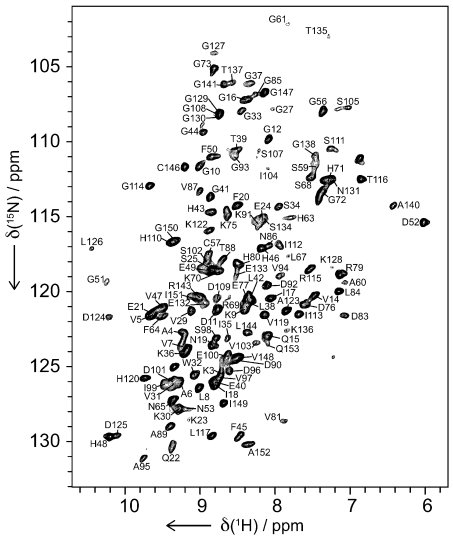
Introduction to biomolecular solid-state NMR



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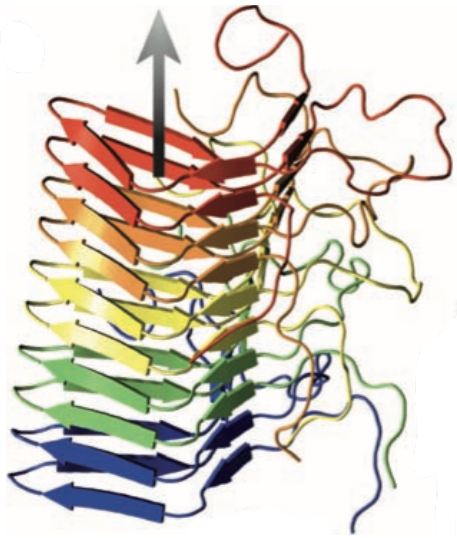

École de Biologie Structurale Intégrative RéNaFoBiS
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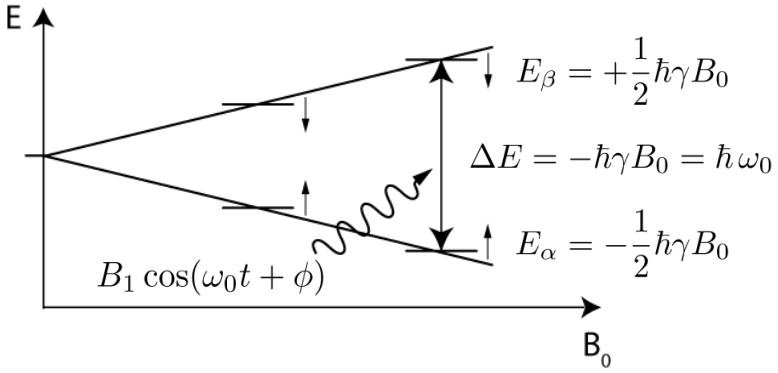
Overview

- Anisotropic interactions important in solid-state NMR
- Solid-state NMR techniques (and some general concepts as well)
- Applications: membrane proteins, protein fibrils, supramolecular assemblies

NMR: a primer (or reminder)

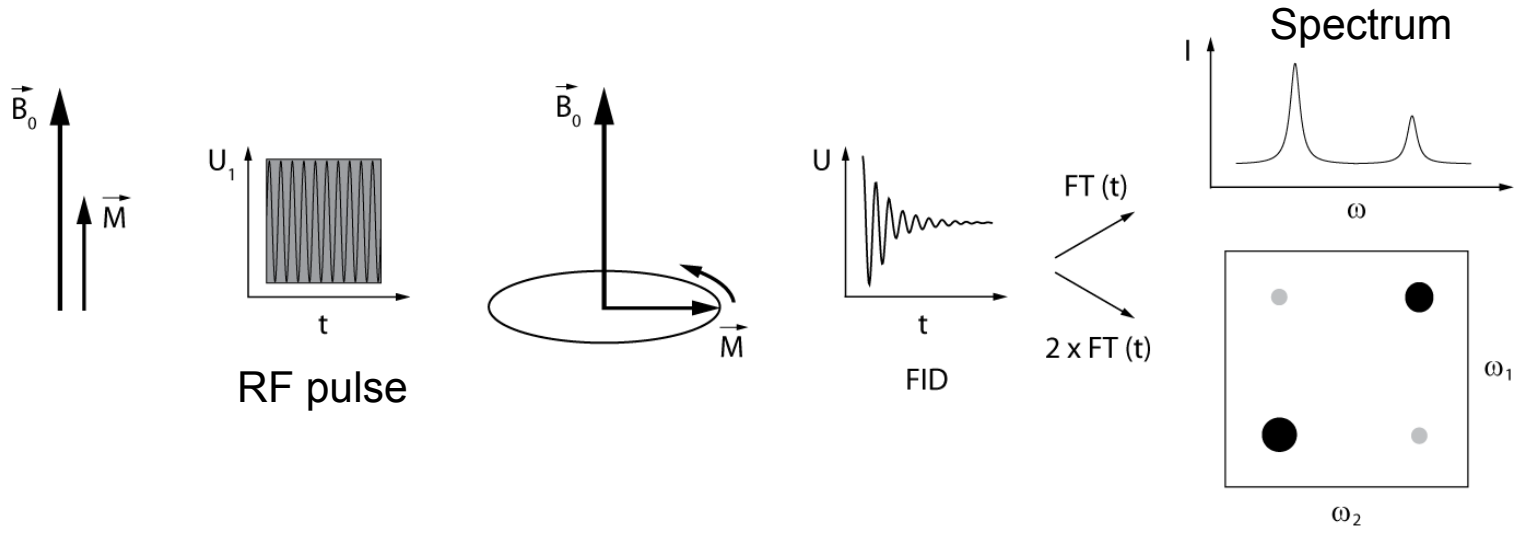
Zeeman splitting of nuclear spin energy states in a magnetic field B_0:



$$\hat{H}_Z = -\boldsymbol{\mu} \cdot \boldsymbol{B}_0 = -\gamma \hat{\boldsymbol{I}} \cdot B_0 \boldsymbol{e}_z = \omega_0 \hat{I}_z$$

B_0 up to ~ 23.5 T (ν_0 = 1 GHz for ^{1}H)

Perturbation via a pulsed oscillating magnetic field B_1:
Fourier transform NMR (Richard Ernst, Nobel prize 1991)

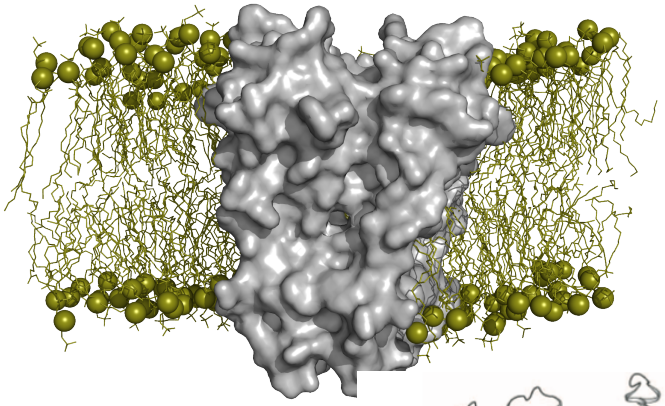


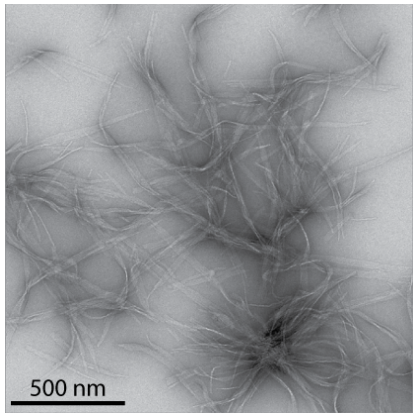
Solid-state NMR: a primer

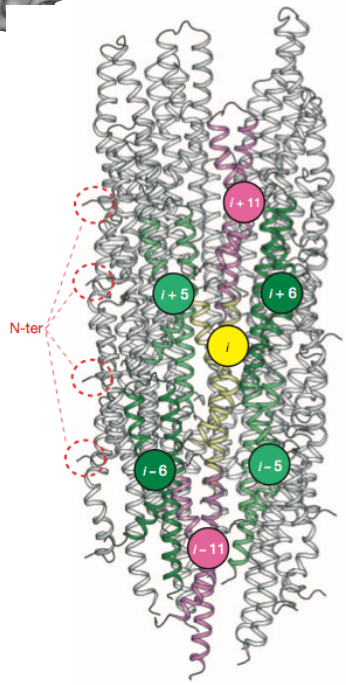
- NMR spectroscopy for systems that are
  - **insoluble**
  - (in principle, arbitrarily) **large**
  - **non-crystalline** (no long-range order)

  in a **native(-like) environment**

  such as:
  - membrane proteins
  - amyloid fibrils
  - large assemblies (viral capsids, secretion systems, pili, …)
- Access **structure, dynamics, interactions, ...** at atomic resolution







Loquet et al., Nature 486, 276, 2012

Anisotropic interactions

NMR interactions

NMR Hamiltonian:

$$\hat{H} = \hat{H}_{\mathrm{Z}} + \hat{H}_{\mathrm{RF}} + \hat{H}_{\mathrm{CS_i}} + \hat{H}_{\mathrm{J}} + \hat{H}_{\mathrm{CSA}} + \hat{H}_{\mathrm{D}} + \hat{H}_{\mathrm{Q}} + \ldots$$

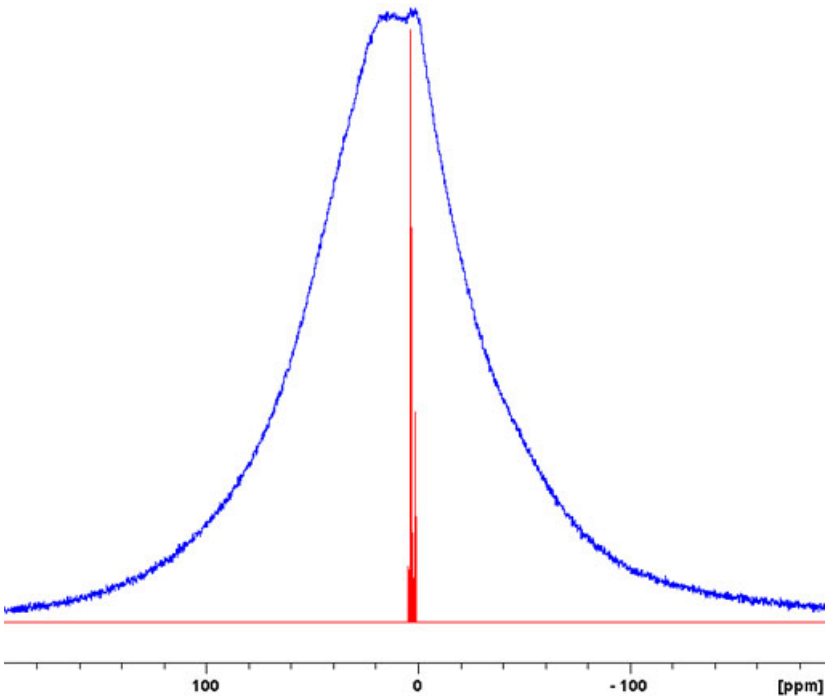
- in solution: **isotropic** interactions:
  - **Z**eeman interaction
  - **r**adio**f**requency irradiation
  - isotropic **c**hemical **s**hift
  - **J** coupling

  → **independent** of the orientation of a molecule with respect to the static B_0 field

- **Anisotropic** interactions:
  - chemical shift anisotropy
  - dipolar coupling
  - quadrupolar coupling (I > 1/2)

  are **orientation-dependent; averaged out** by molecular tumbling in solution, but not in a solid sample!

Anisotropic interactions: Result...



^{1}H spectra of isopropyl-β-D-thiogalactopyranose in solution (red) and solid (blue)

http://chem.ch.huji.ac.il/nmr/techniques/solid/solid.html

- Anisotropic interactions in solid samples lead to very **broad signals**, which may yield uninterpretable spectra!
- However, they contain valuable information (local environment, internuclear distances, ...) and can be used for spectroscopic purposes (polarization transfer).

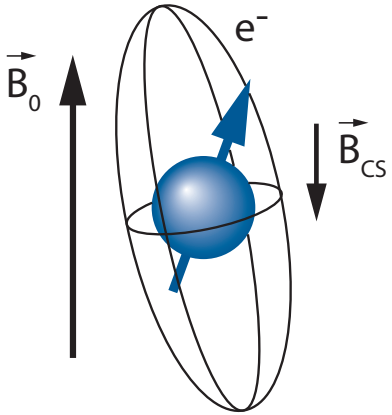
⇒ Challenge: obtain high-resolution spectra under these conditions, yet still take advantage of the information contained in anisotropic interactions.

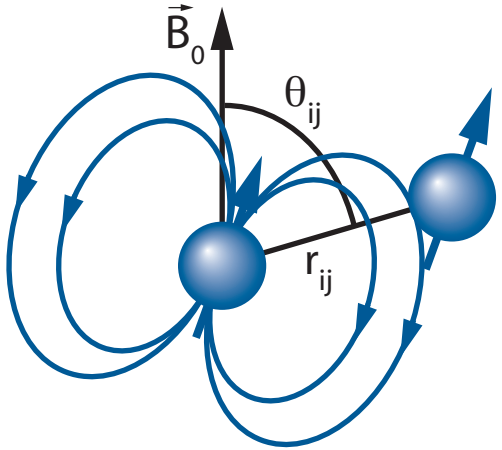
Anisotropic interactions

$$\hat{H} = \hat{H}_{\mathrm{Z}} + \hat{H}_{\mathrm{RF}} + \hat{H}_{\mathrm{CS_i}} + \hat{H}_{\mathrm{J}} + \hat{H}_{\mathrm{CSA}} + \hat{H}_{\mathrm{D}} + \hat{H}_{\mathrm{Q}} + \ldots$$

Chemical shift anisotropy

Dipolar coupling





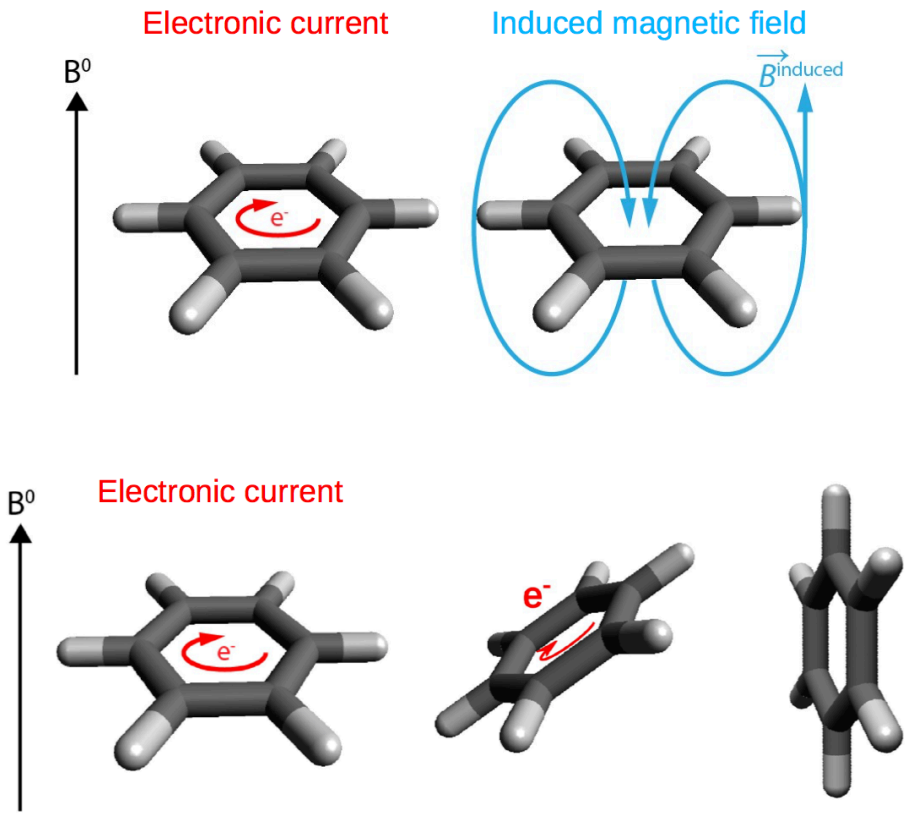
$$\hat{H}_{\mathrm{CS}} = -\gamma\, \hat{\boldsymbol{I}}\, \boldsymbol{\sigma}\, \boldsymbol{B}_0$$

$$\hat{H}_{\mathrm{D}} = \hat{\boldsymbol{I}}_i\, \mathbf{D}_{ij}\, \hat{\boldsymbol{I}}_j$$

- Spatial structure of electronic environment
- Orientation dependence
- Isotropic part visible in solution

- Interaction of magnetic moments of neighboring nuclei
- Dependence on orientation and internuclear distance
- No isotropic part – averaged out in solution

Chemical shift (anisotropy)

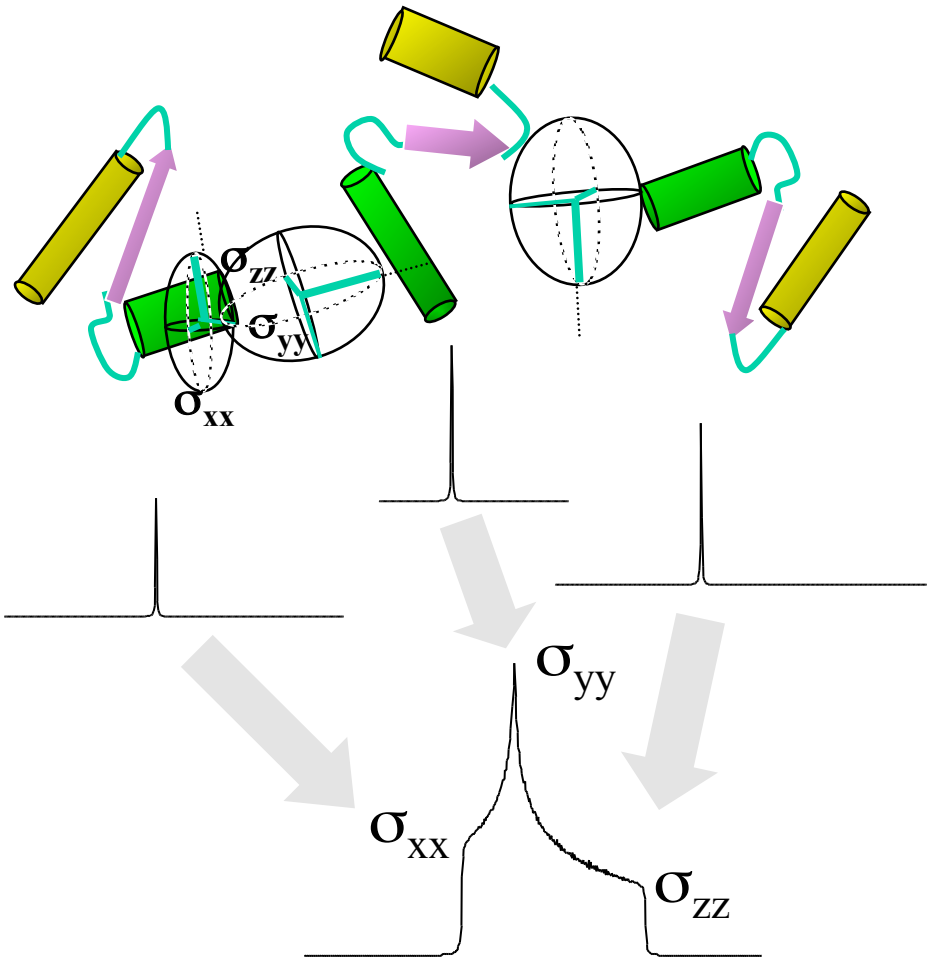


- B_0 field induces electron currents that generate secondary magnetic fields
- Total field felt by a nucleus results from the **superposition of B_0** with these **secondary fields**
- Generally, electron distribution around a nucleus is **not spherically symmetric**

⇒ chemical shift of a nucleus **depends on the orientation** of its molecule

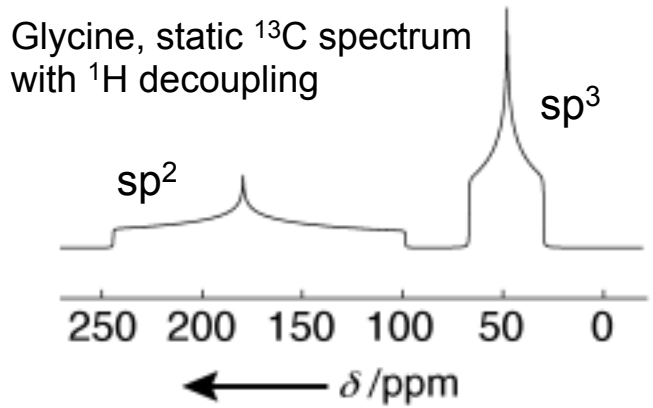
O. Lafon, MOOC NMR Univ. Lille
https://www.fun-mooc.fr/courses/lille1/54002S02/session02/about

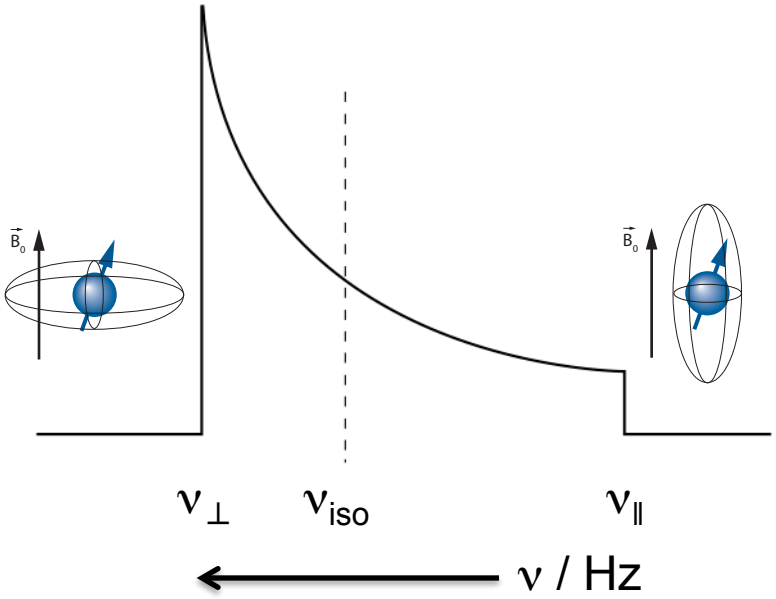
Chemical shift anisotropy



- Superposition of individual signals corresponding to different molecular orientations leads to the broad **“powder pattern”** observed in a static sample
- Gives information on **structure of electronic environment**

Chemical shift anisotropy



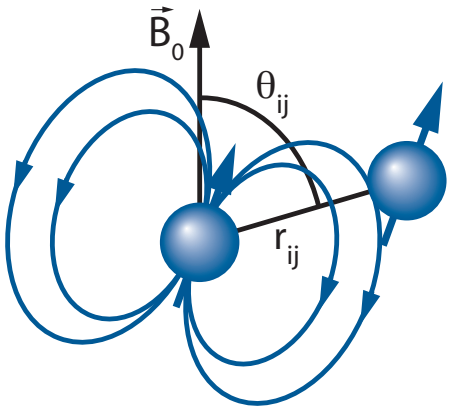


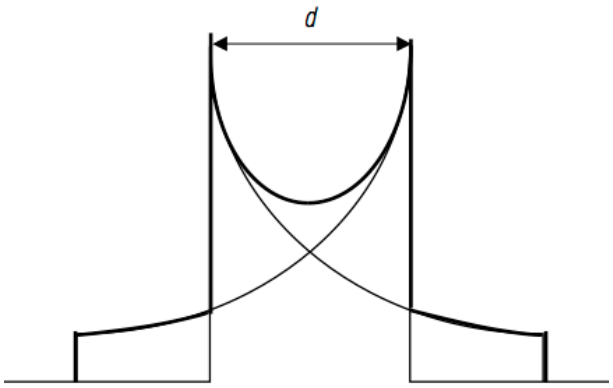
- CSA powder pattern reflects, e.g., on
  - symmetry
  - hybridization
  - bond lengths / angles
  - dihedral angles

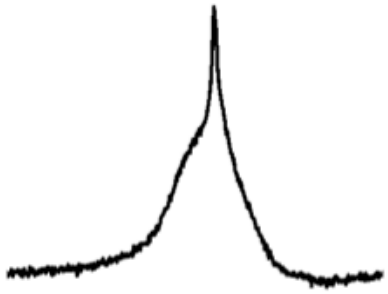
  of electronic environment.
- The **isotropic** chemical shift corresponds to the **barycenter** of the CSA pattern.

Laws et al., Angew. Chem. Int. Ed. 41, 3096, 2002
M. Duer: Solid-State NMR Spectroscopy. Oxford (Blackwell) 2002.

Dipolar coupling

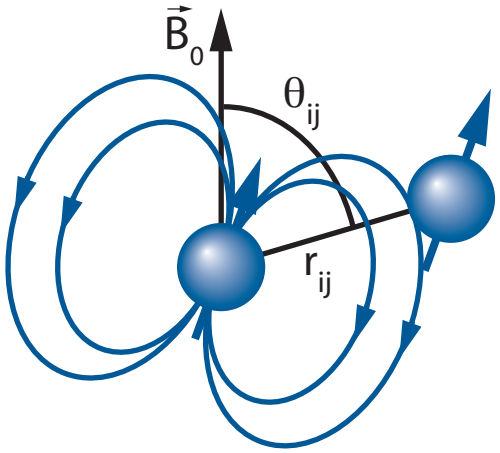






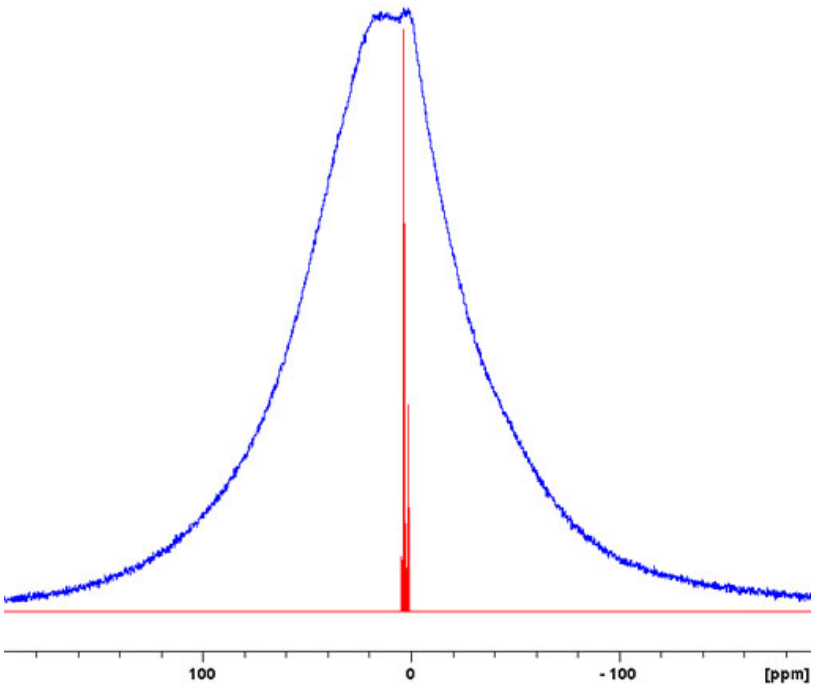
- Interaction between the **magnetic moments** of two spins (cf. bar magnets influencing each other)
- Depends on internuclear **distance** (as $1/r^3$) and **orientation** of internuclear vector with respect to B_0
- Gives a **doublet** (similar as for J coupling) for a **single crystal** (where all internuclear vectors have the same orientation)
- ... a **Pake pattern** (superposition of two powder lineshapes) for random orientations
- ... and a **broad hump** for a network of coupled nuclei (such as the many ^{1}Hs in biomolecules!)

Dipolar coupling



- Information about **distance** between nuclei
  (→ 3D structure!)
- Useful for **polarization transfer** (more efficient than J coupling!)
- Affected by molecular motion
  → information on **dynamics**!

Fair enough, but...



- … how do I get the resolution I need in order to be able to look at anything more complex, such as biomolecules?

http://chem.ch.huji.ac.il/nmr/techniques/solid/solid.html

Solid-state NMR techniques

Spin & space

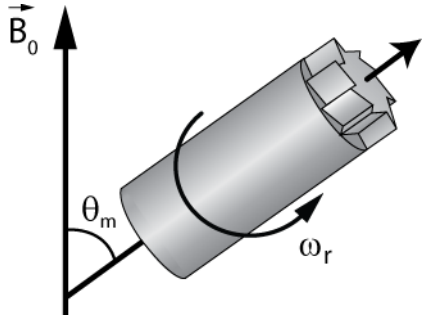
$$\hat{H} = \sum_{l=0}^{2} \hat{\boldsymbol{A}}_l \cdot \hat{\boldsymbol{T}}_l$$

space spin

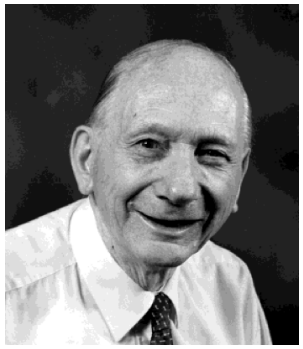
$$\hat{H}_{\mathrm{D}},\ \hat{H}_{\mathrm{CSA}} \propto (3\cos^2\theta - 1)$$

- The NMR Hamiltonian can be separated into a **space** and a **spin** part
- We can interfere with the spin system via either!
- The space part of CSA and dipolar coupling depends on orientation as $(3 \cos^2 \theta - 1)$
- In solution, rapid molecular tumbling averages out anisotropic interactions *via* this spatial dependence
- Can we do something similar for solid samples?

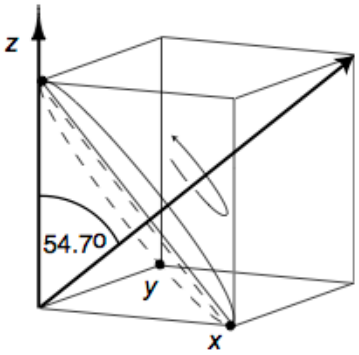
Magic Angle Spinning



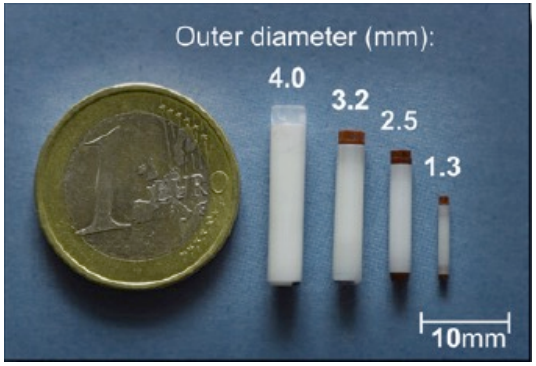
- Spin sample around an angle inclined 54.74° with respect to the B_0 axis ($3\cos^2\theta - 1 = 0$, space diagonal of a cube)
- by two airflows (bearing & drive) in a stator
- Need $\omega_r > 3\,\omega_D, \omega_{CSA}$ for efficient averaging



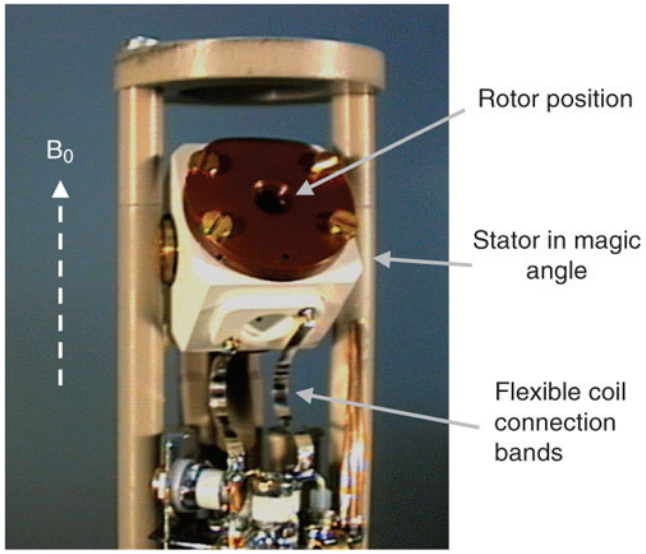
E. R. Andrew



Andrew et al., Nature 183, 1802, 1959

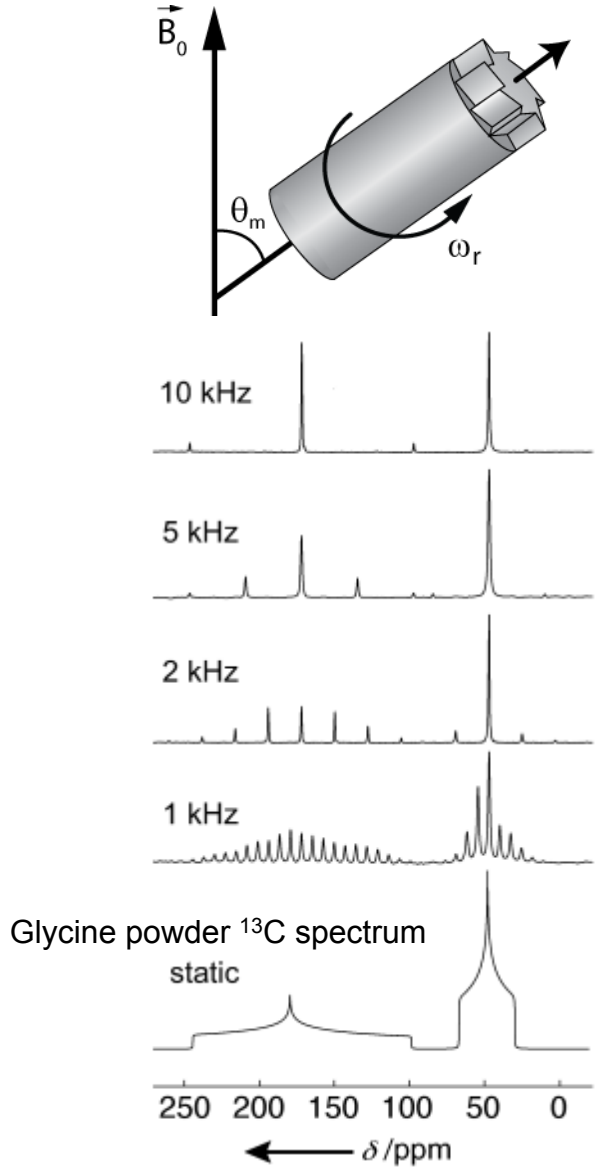


Demers et al., Solid State Nucl Magn Reson 40, 101, 2011



Beckonert et al., Nat Protoc 5, 1019, 2010

Magic Angle Spinning

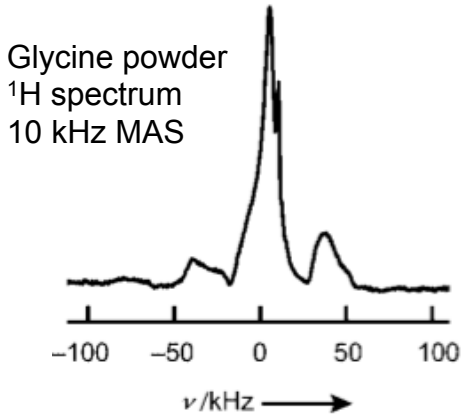


Laws et al., Angew. Chem. Int. Ed. 41, 3096, 2002

- Under MAS, CSA pattern "falls apart" into a series of **spinning sidebands** spaced at the spinning speed
- With increasing MAS speed, sidebands move out further and lose intensity until **only isotropic line remains**

⇒ resolution much improved!

- Network of many strong ^{1}H-^{1}H dipolar couplings in biomolecules still problematic!



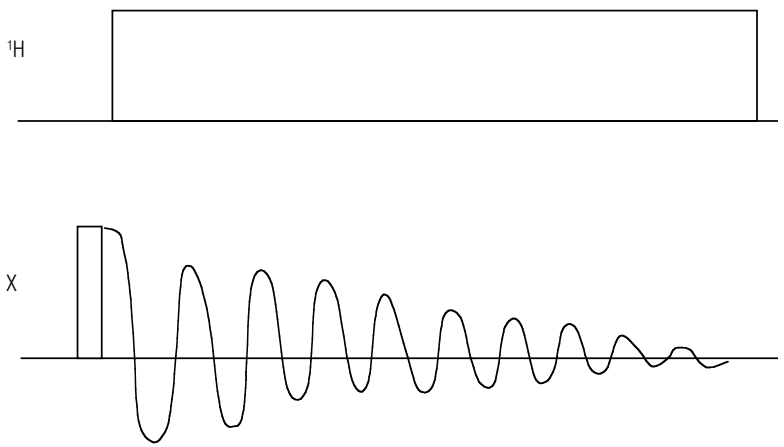
Heteronucleus detection and decoupling

- Strong ^{1}H dipolar coupling network precludes high-resolution ^{1}H spectra at “normal” MAS speeds

⇒ **detect** NMR signal on, e.g., **^{13}C**

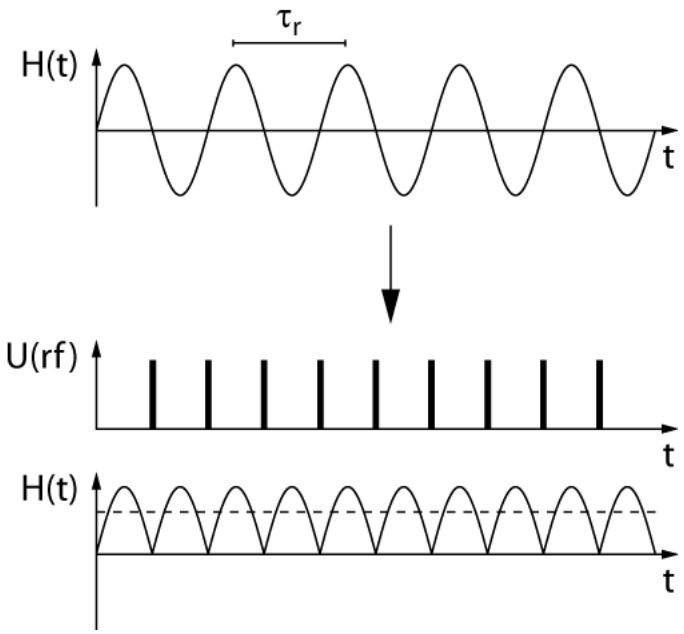
⇒ **decouple** ^{1}H using RF irradiation

- i.e. remove effect of ^{1}H-^{13}C coupling on ^{13}C spectrum by **continuously rotating** ^{1}H’s in **spin** space
- Same principle as used in solution state, but much higher RF power used!



M. Duer, Oxford (Blackwell) 2002

Correlation spectroscopy via recoupling



- We removed (to some extent) the interactions that broaden our spectra
- However, they are **useful** for **polarization transfer** (to enhance signal, obtain information about internuclear correlations, distances...)
- How to get them back − selectively?

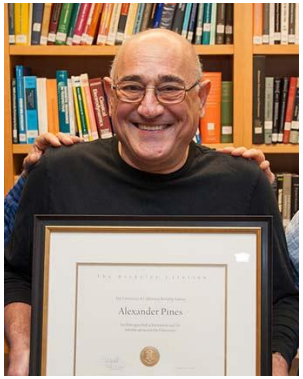
⇒ use **recoupling pulse sequences** to “switch on” desired interactions during “mixing time” of an NMR experiment!

Cross-polarization (CP)

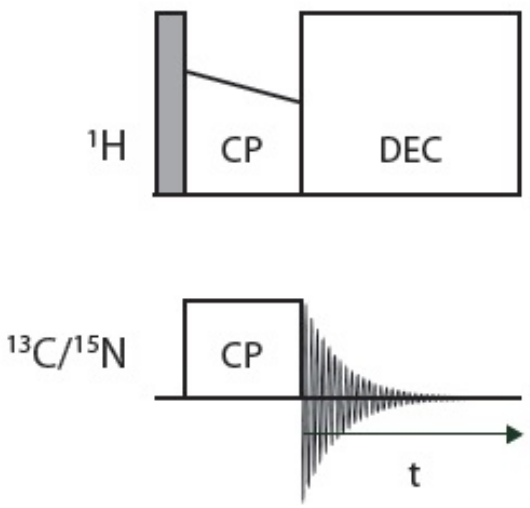
- Reintroduce, e.g., ^{1}H-^{13}C dipolar coupling by **simultaneous RF irradiation** at ^{1}H and ^{13}C Larmor frequencies
- RF amplitudes have to match the **Hartmann-Hahn condition**

⇒ obtain ^{1}H-^{13}C polarization transfer

⇒ **enhance ^{13}C magnetization** by a factor of 4!
(as for INEPT transfer in solution)



A. Pines



$$\omega_{1I} - \omega_{1S} = \pm\omega_r, \pm 2\omega_r$$

or

$$\omega_{1I} + \omega_{1S} = \omega_r, 2\omega_r$$

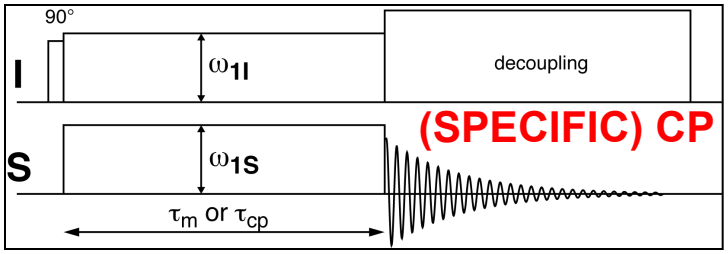
Pines et al., J Chem Phys 59, 569, 1973

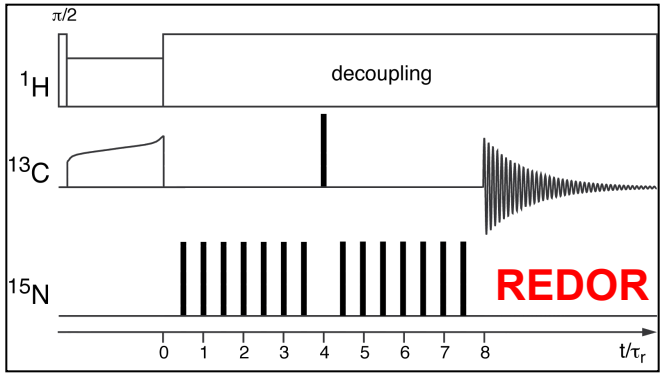
Recoupling pulse sequences

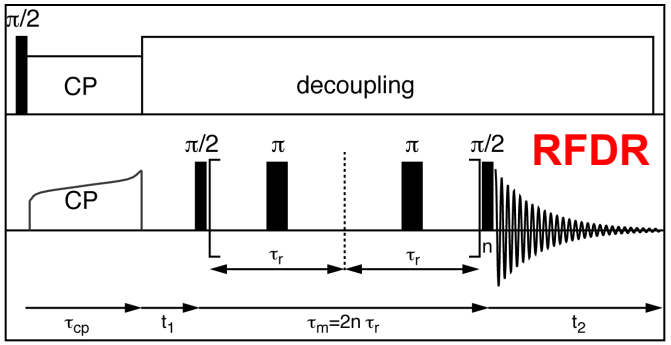
- A wide range of recoupling pulse sequences is available

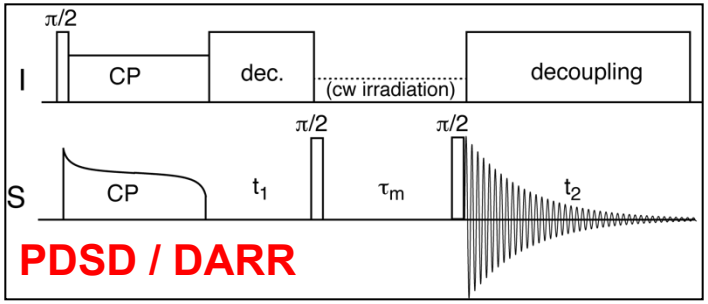
Choose your preference:

- dipolar coupling (→ distances) or chemical shift anisotropy (→ conformation)
- heteronuclear or homonuclear
- for shorter (filtering; dynamics) or longer distances (structure)
- broad-band or chemical-shift selective





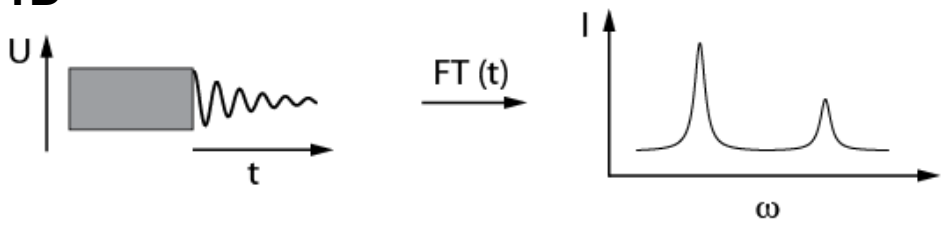




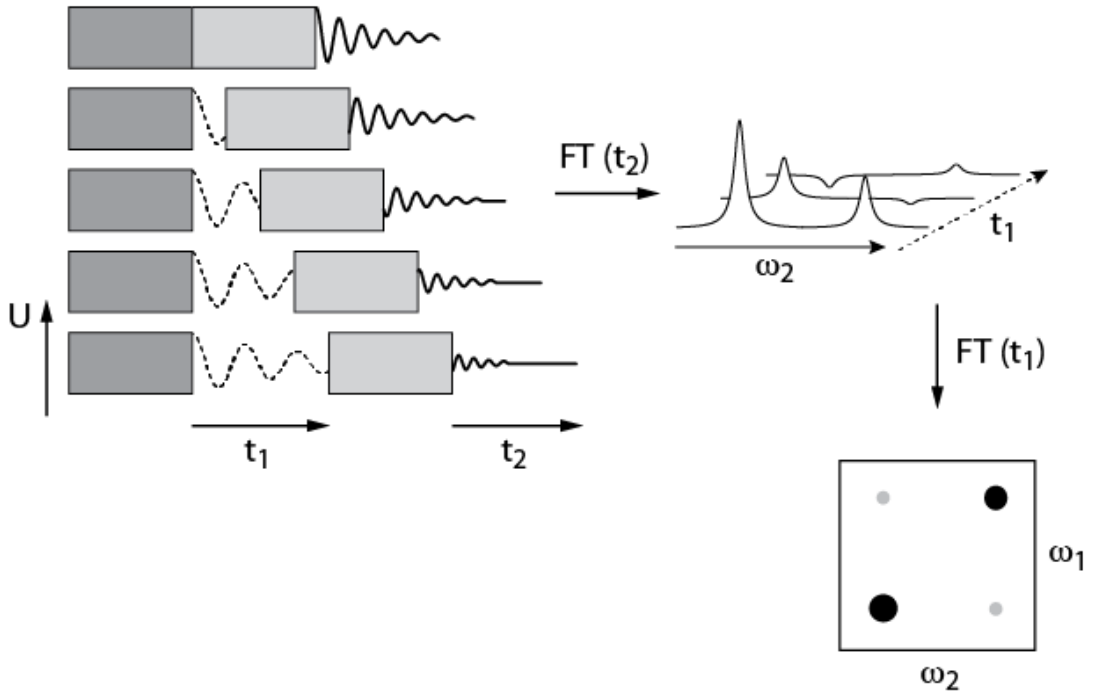
HORROR / DREAM, selective recoupling, R and C sequences, PAR / PAIN, ...

Parenthesis: From 1D to 2D

1D



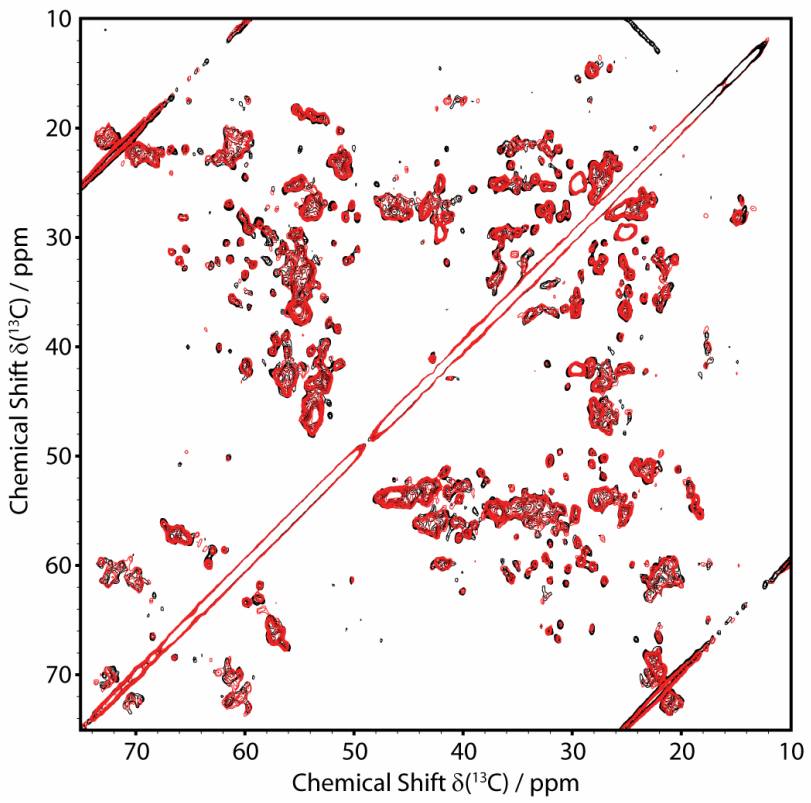
2D



- Additional frequency dimension is indirectly recorded via an **incremented delay**
- **Imprints** frequencies of **coupled nuclei** onto signals of the 1D spectrum

⇒ **2-dimensional Fourier transformation**

Solid-state fingerprint of a protein: ^{13}C-^{13}C correlation



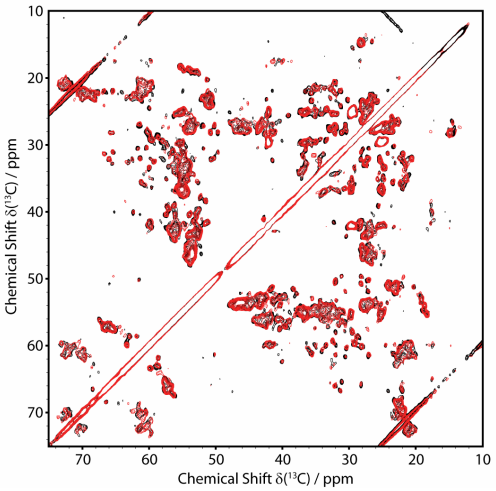
Schneider et al., Angew Chem Int Ed 49, 1882, 2010

- HSQC-type ^{15}N-^{1}H correlation spectrum as used in solution is typically **too broad** to yield useful information in the solid state!

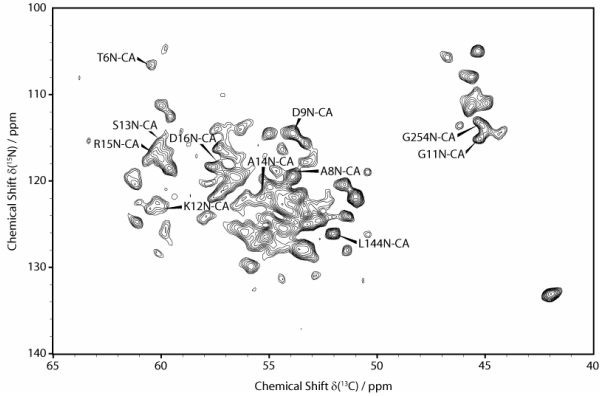
→ use a ^{13}C-^{13}C correlation map e.g. via spin diffusion / DARR

- Shorter mixing times → **intraresidue** correlations
- Longer mixing times → **interresidue**, **through-space** correlations

The toolbox



^{13}C-^{13}C

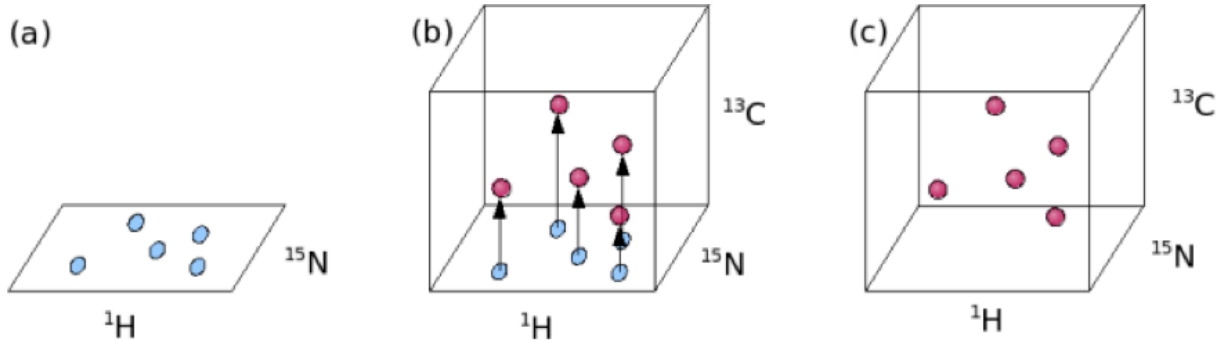


^{15}N-^{13}C

- High(er) resolution ^{15}N, ^{13}C detection using MAS and decoupling
- Polarization transfer ^{1}H-^{15}N, ^{1}H-^{13}C, ^{13}C-^{13}C, ^{15}N-^{13}C ...
- 2D, 3D, ... spectroscopy
- ... for structural analysis of biomacromolecules

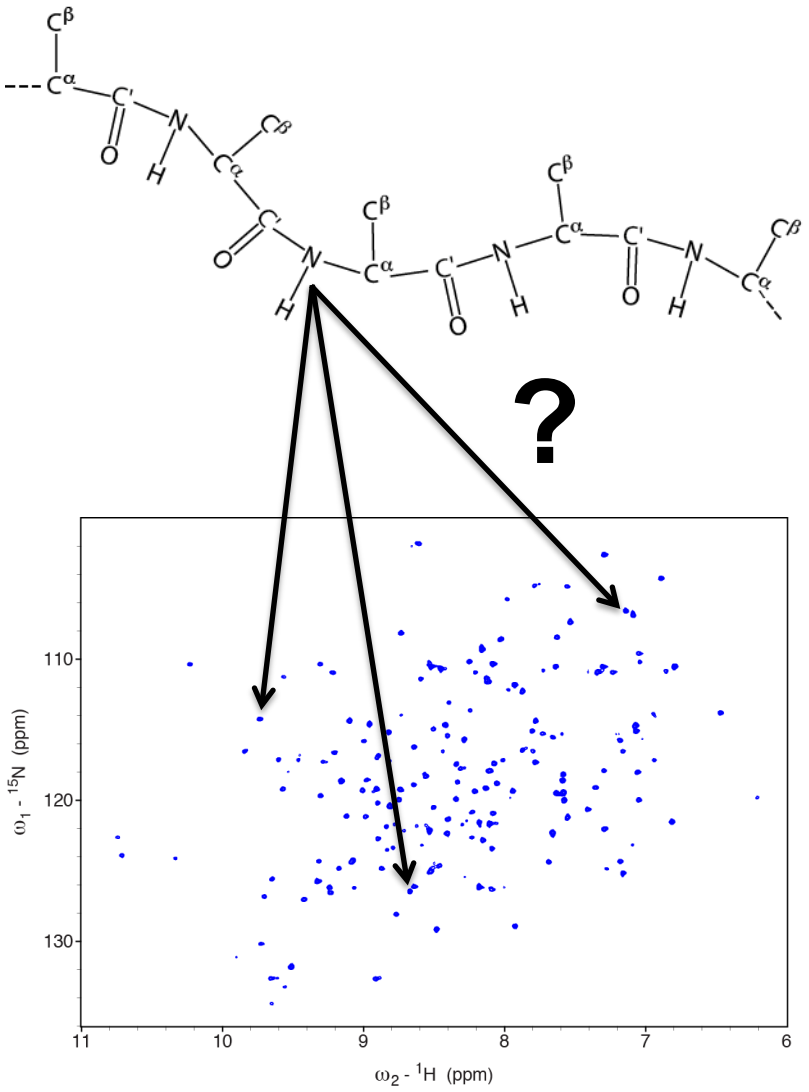
Schneider et al., Angew Chem Int Ed 49, 1882, 2010

http://www.protein-nmr.org.uk/solution-nmr/assignment-theory/visualising-3d-spectra/



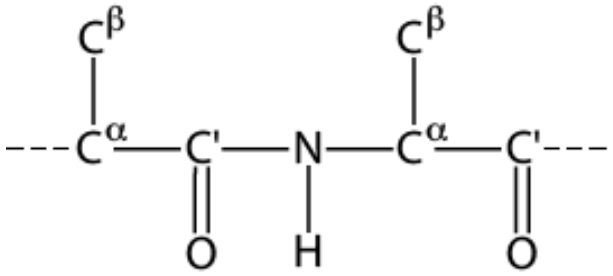
3Ds

Parenthesis: Resonance assignment



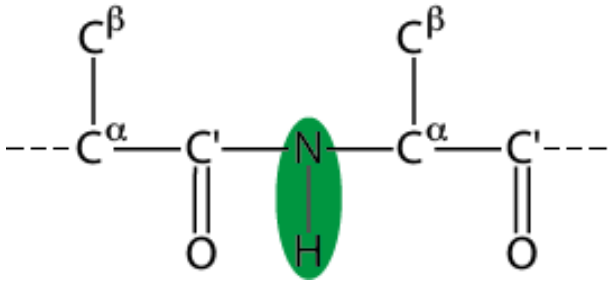
- How do I identify the **nuclei in my protein** a peak in my spectrum comes from?

Parenthesis: Resonance assignment



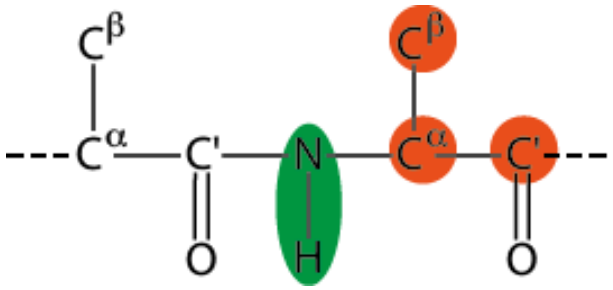
- Most common method for **protein backbone resonance assignment** in ^{15}N-, ^{13}C-labeled proteins:
- record a set of spectra that, ...

Parenthesis: Resonance assignment



- Most common method for **protein backbone resonance assignment** in ^{15}N-, ^{13}C-labeled proteins:
- record a set of spectra that, for **each amide group**, ...

Parenthesis: Resonance assignment



- Most common method for **protein backbone resonance assignment** in ^{15}N-, ^{13}C-labeled proteins:
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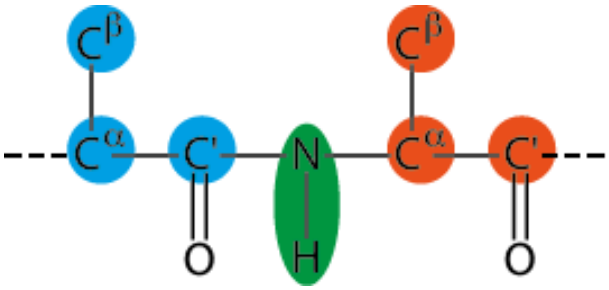
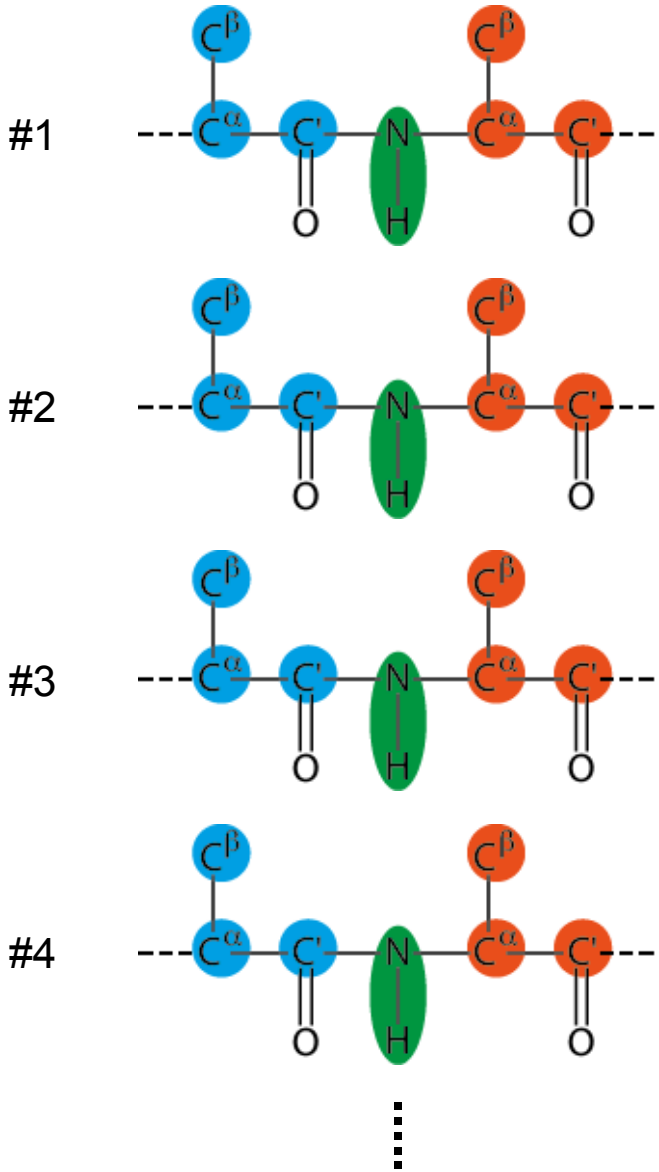
Parenthesis: Resonance assignment



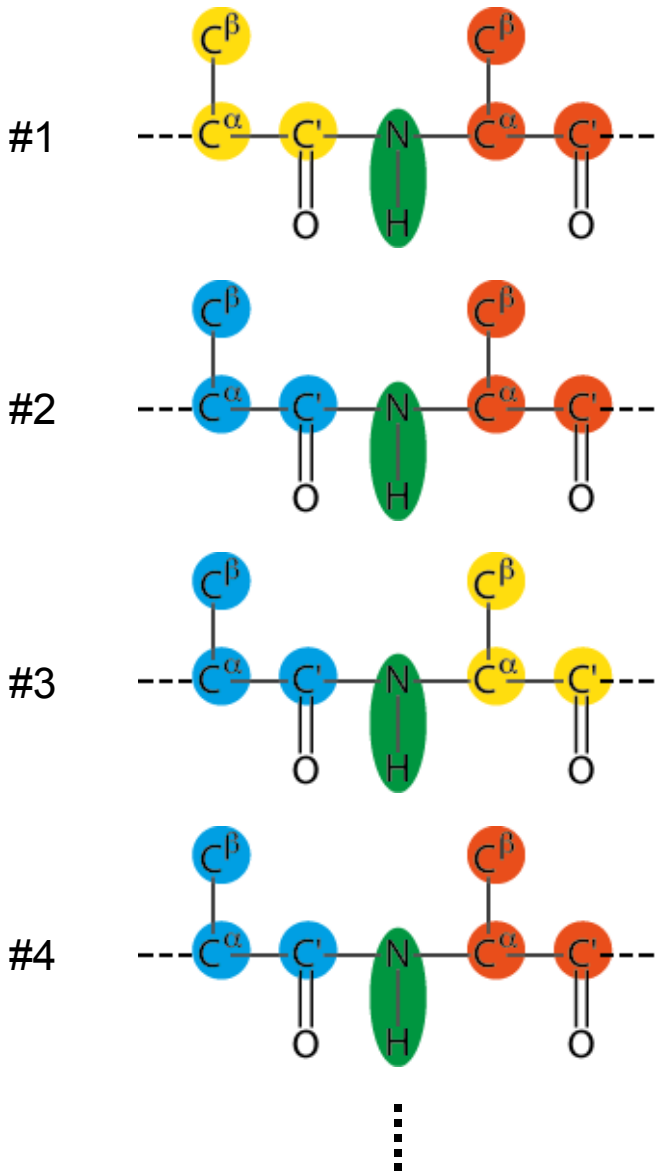
- Most common method for **protein backbone resonance assignment** in ^{15}N-, ^{13}C-labeled proteins:
- record a set of spectra that, for **each amide group**, give the resonance frequencies (=chemical shifts) of Cα, Cβ, CO nuclei in **its own amino acid residue**...
- ...as well as in the **preceding residue**!

Parenthesis: Resonance assignment



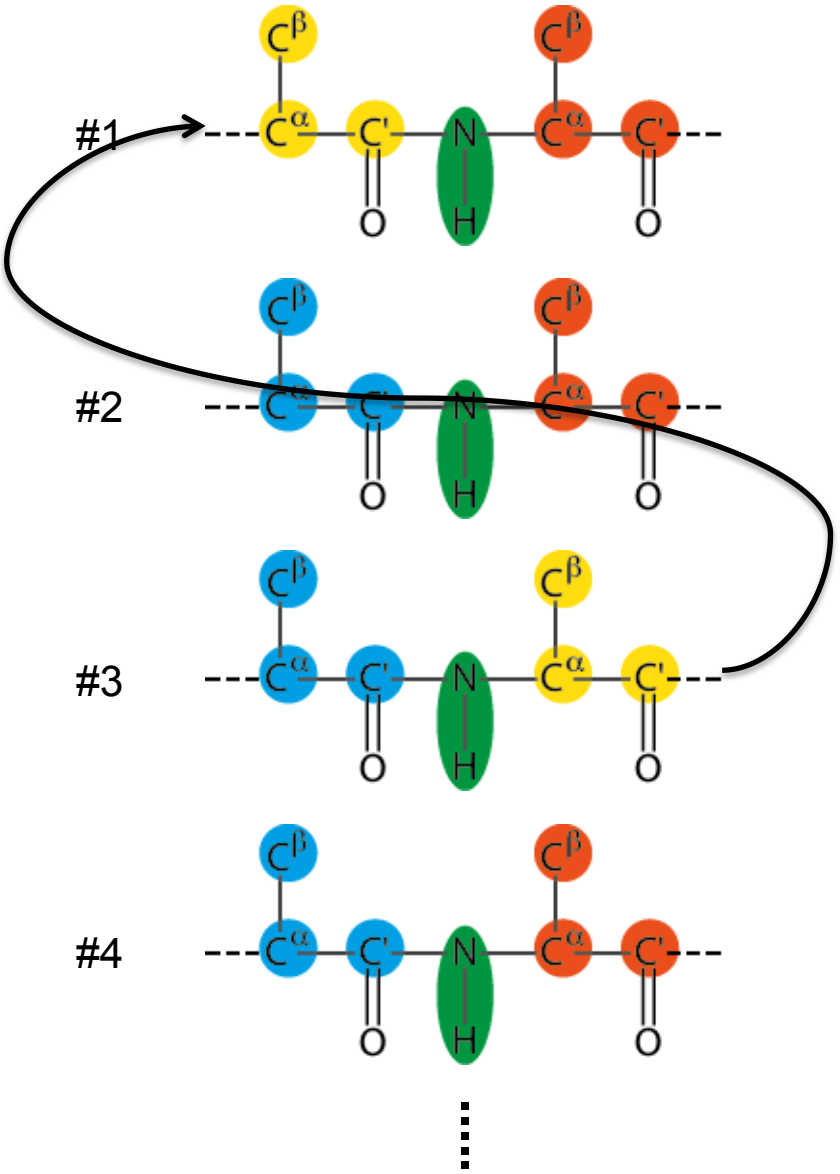
- This way, obtain a list of the chemical shifts of all amide "**spin systems**" in the protein, randomly numbered

Parenthesis: Resonance assignment



- This way, obtain a list of the chemical shifts of all amide "**spin systems**" in the protein, randomly numbered
- Find pairs of spin systems for which the **intraresidue (*i*) ^{13}C chemical shifts** of **one** match the **interresidue (*i-1*)** ones of the **other**

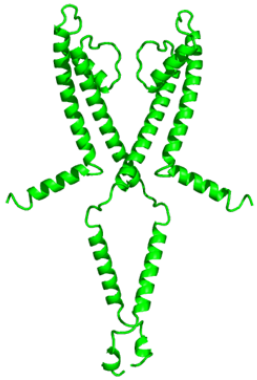
Parenthesis: Resonance assignment

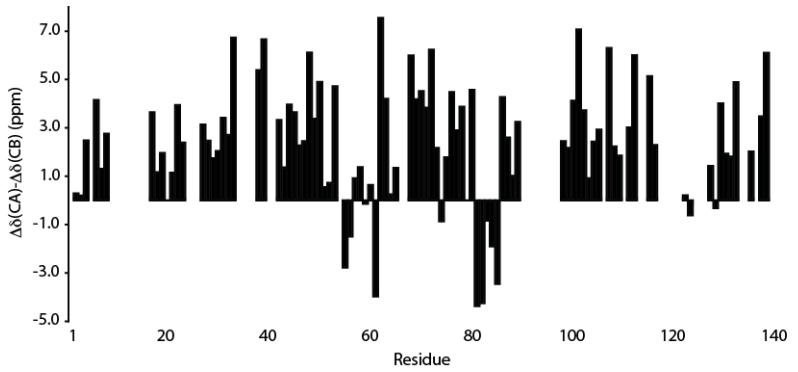


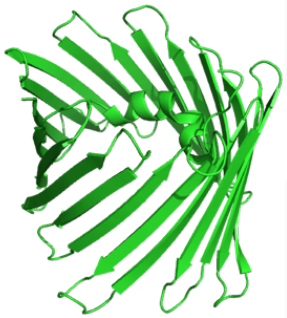
- This way, obtain a list of the chemical shifts of all amide "**spin systems**" in the protein, randomly numbered
- Find pairs of spin systems for which the **intraresidue (*i*) ^{13}C chemical shifts** of **one** match the **interresidue (*i-1*)** ones of the **other**

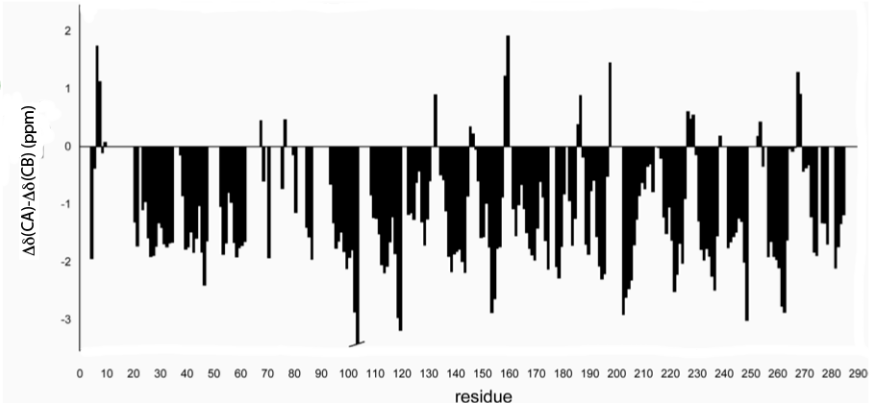
⇒ these spin systems must correspond to **neighboring residues** in the protein sequence!

Protein secondary structure





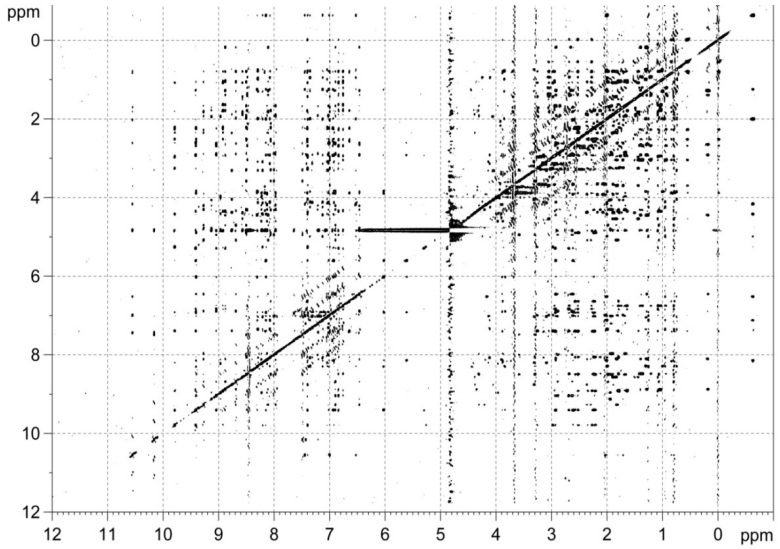




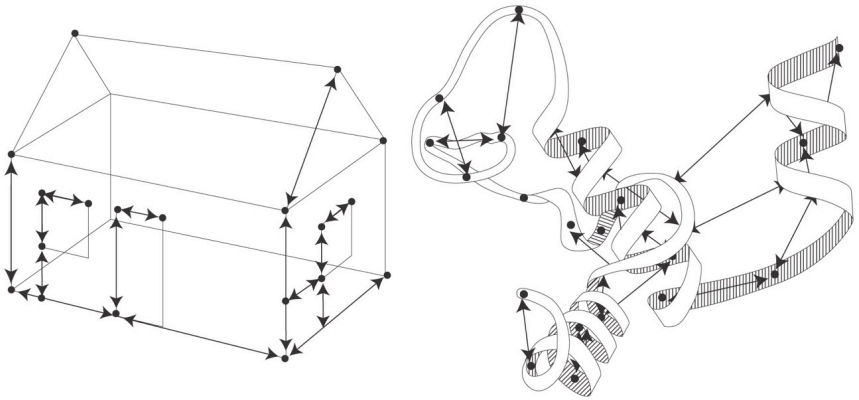
- As in solution, especially ^{13}C **chemical shifts** are indicative of **secondary structure**
- Compare assigned values in protein of interest to **reference** / **random coil** values to obtain **secondary chemical shift**
- Identify α-helices, β-strands, turns directly from resonance assignments!

Schneider et al., JACS 130, 7427, 2008
Hiller et al., Science 321, 1206, 2008

Parenthesis: Protein structures from NMR?



C. Smet-Nocca

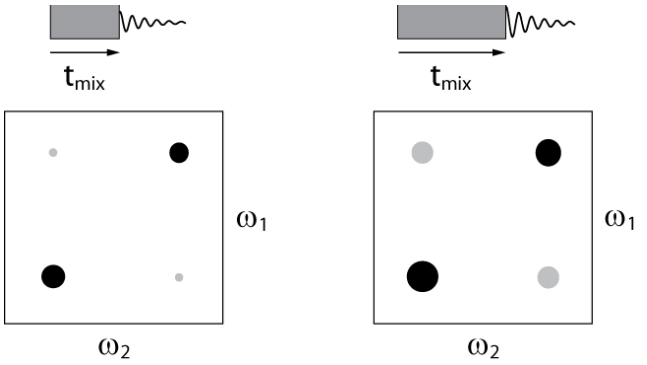


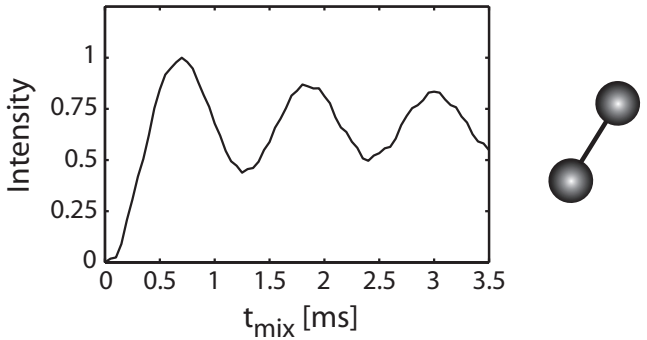
- Detect which nuclei are close in space via **through-space correlation spectra** making use of the **dipolar coupling**! (solution: NOESY; solid: spin diffusion, RFDR, ...)
- **Signal intensity** in those spectra encodes **distance** (albeit often imprecisely), usually up to about 6 Å
- Assemble a **model** that fulfils as many of these **distance restraints** as possible! (*in silico*, using a minimization algorithm)

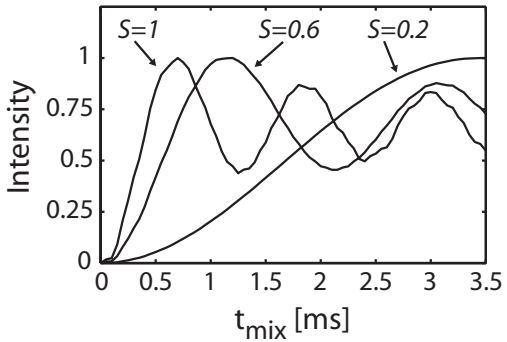
  (Kurt Wüthrich, Nobel prize 2002)

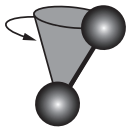
http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2002/popular.html

Dynamics





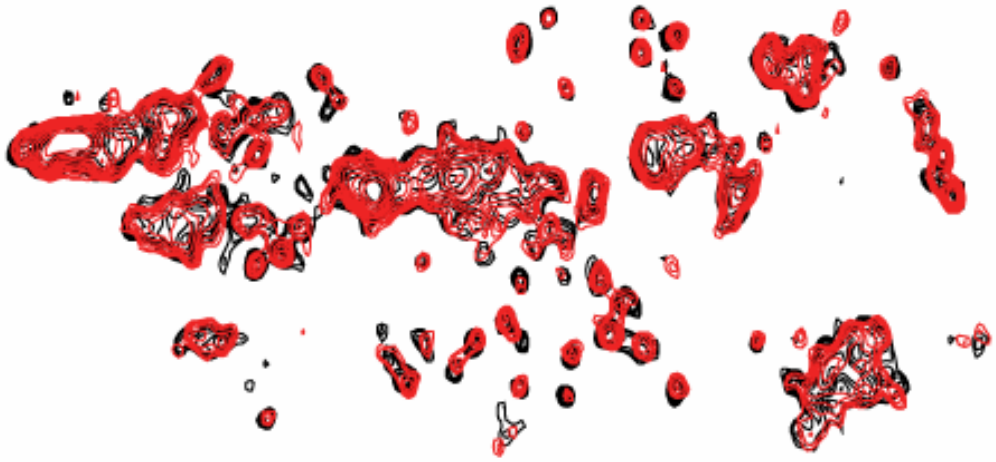




- NMR is sensitive to **molecular dynamics** on a **wide range of time scales** (ps − h)
- **Quantify** motional **amplitudes** and **time scales** in a **site-specific** manner
- In solids, **anisotropic interactions** are affected by dynamics on all time scales **faster** than the **inverse of the coupling strength** (e.g. up to ~ µs for dipolar coupling)

→ obtain motional amplitudes by measuring **build-up** of signal intensity in spectrum with **varying duration** of a **recoupling** pulse sequence!

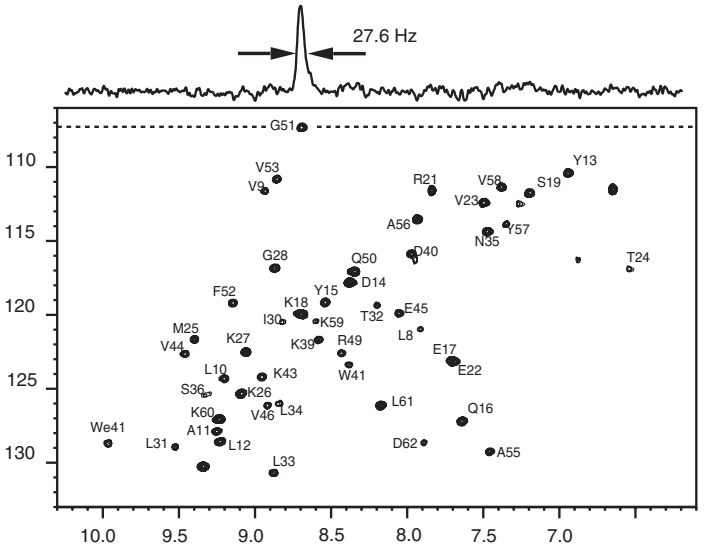
But still...

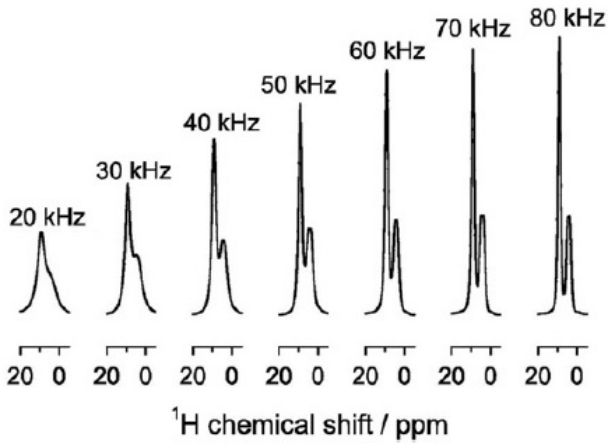


- Rather large linewidths
- Rather low signal to noise

limit what we can do with (classical) solid-state NMR!

Recent breakthroughs: deuteration and fast MAS



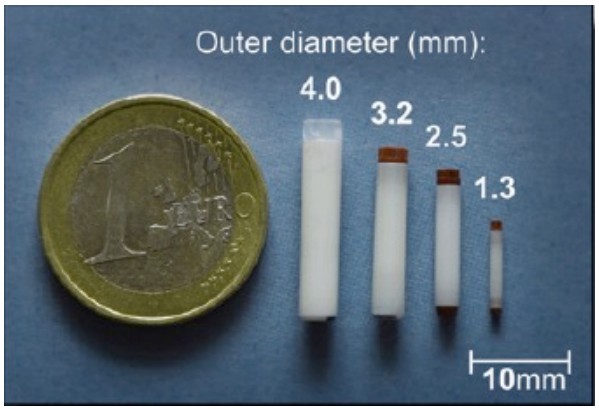


- **Strong dipolar couplings** between the many **protons** present in biomolecules are a main reason for line broadening

→ use **(per)deuteration** to obtain very high resolution spectra already at 10 − 20 kHz MAS

→ and / or **fast MAS** above about 45 kHz ("fast spinning" regime)

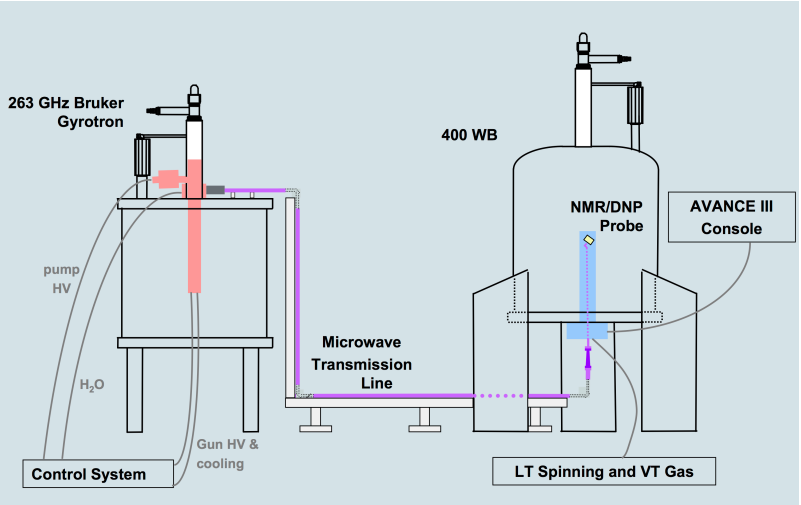
- can do **proton detection** (higher sensitivity), use **low RF power**, **small sample amounts**

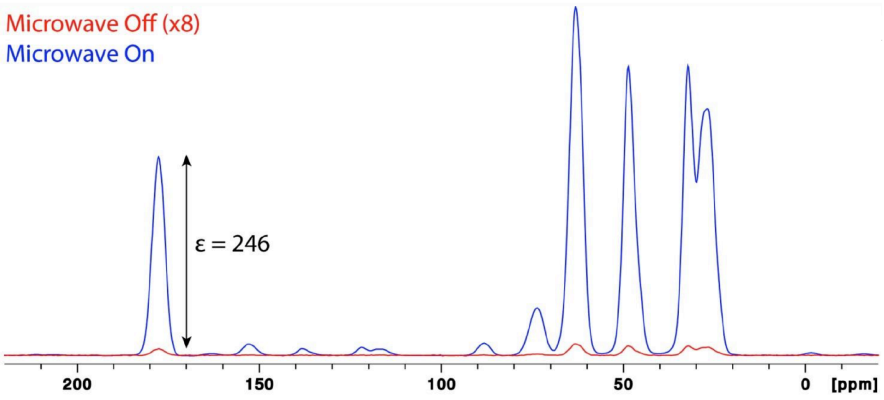


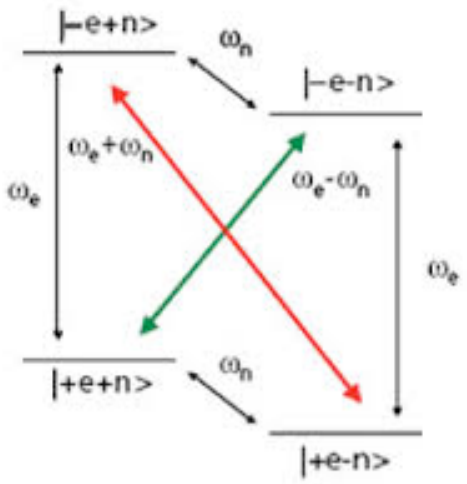
Chevelkov et al., Angew Chem Int Ed 45, 3878, 2006
Demers et al., Solid State Nucl Magn Reson 40, 101, 2011

Recent breakthroughs: DNP

- **Dynamic nuclear polarization**: obtain sensitivity enhancements up to, in theory, **660-fold** by transferring **electron polarization** to nuclei using **microwave** irradiation
- requires a **gyrotron**, **radicals**, **cryogenic temperatures**

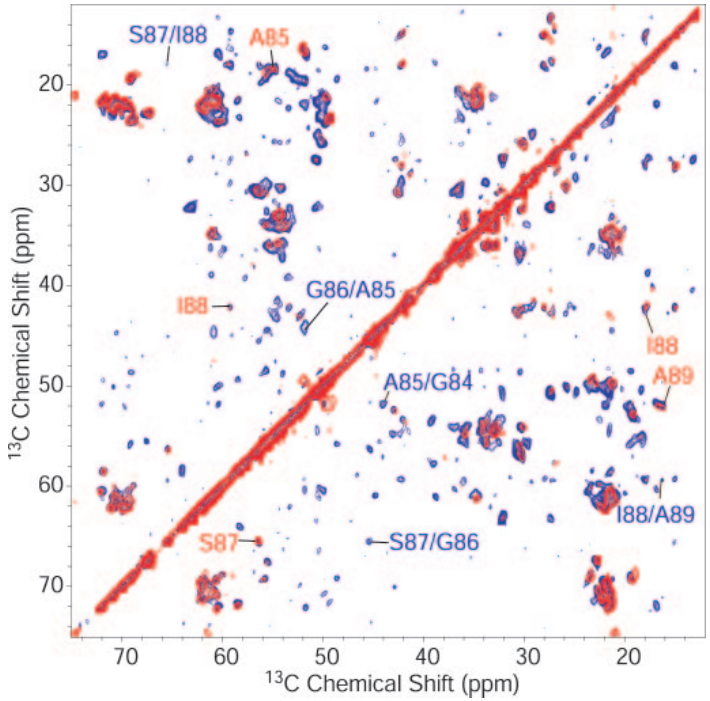




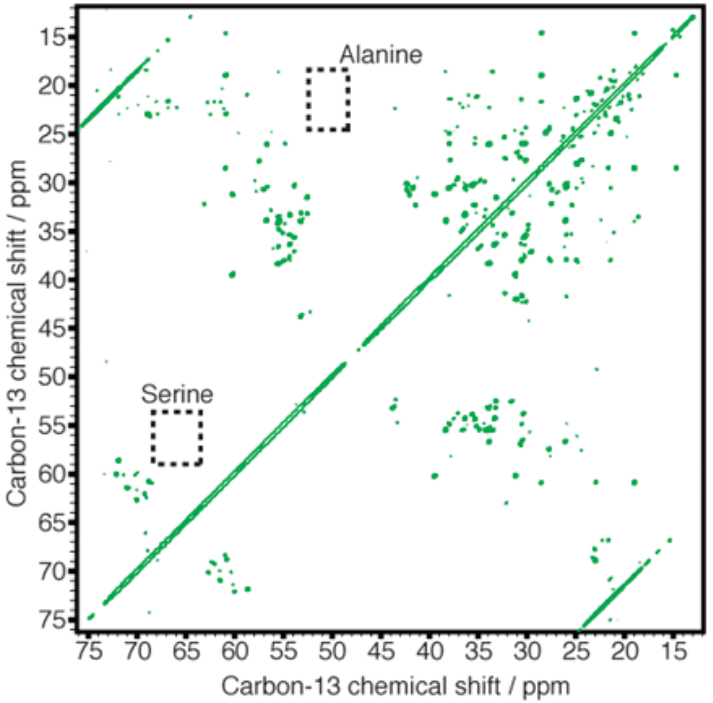


Bruker Biospin (https://www.bruker.com/service/education-training/webinars/nmr-webinars/introduction-to-solid-state-dnp-nmr.html)

...and of course: sample preparation!



Heise et al., PNAS 102, 15871, 2005



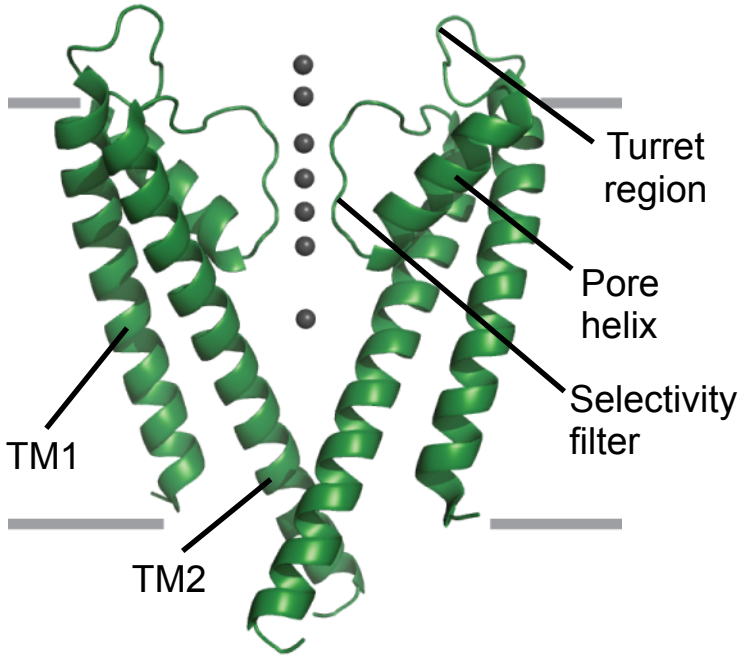
Loquet et al., JACS 132, 15164, 2010

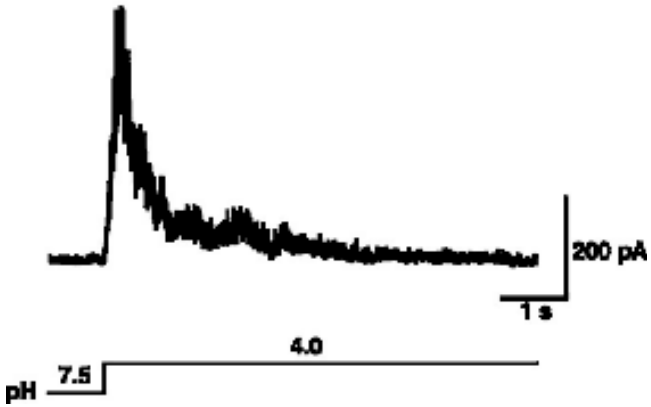
- Careful sample preparation to achieve optimal **local homogeneity**, as well as sufficient **water content**, is essential!
- and: use of **alternative / reduced isotope labeling schemes**
- Has proven crucial especially for studies of amyloid fibrils
- However, this kind of optimization remains some kind of black magic...

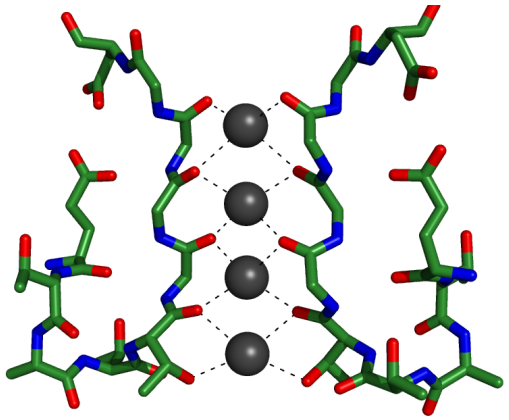
Applications I:
The potassium channel KcsA-Kv1.3

The potassium ion channel KcsA(-Kv1.3)

- 4 x 160 AA tetramer
- **Selectivity filter** coordinates K+ ions via carbonyl groups
- Opening / closing (**„gating“**) can be induced by pH change
- **Inactivation** process upon prolonged opening



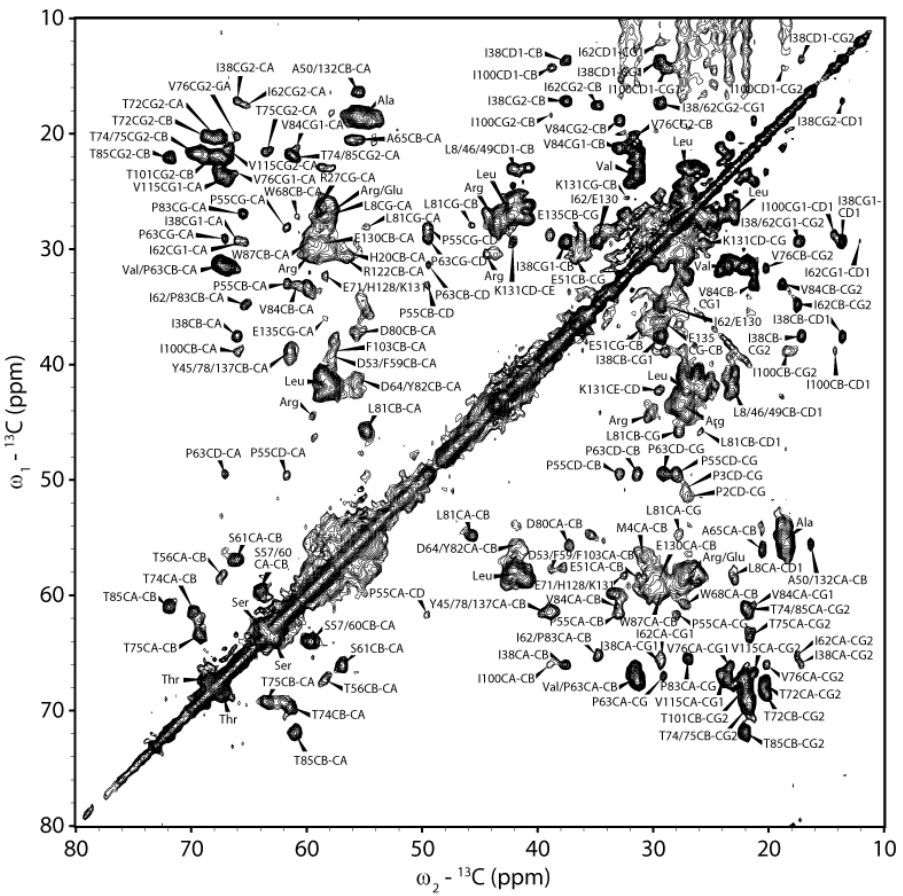




Ader, Schneider et al., Nat. Struct. Mol. Biol. 15, 605, 2008

Zhou et al., Nature 414, 43, 2001

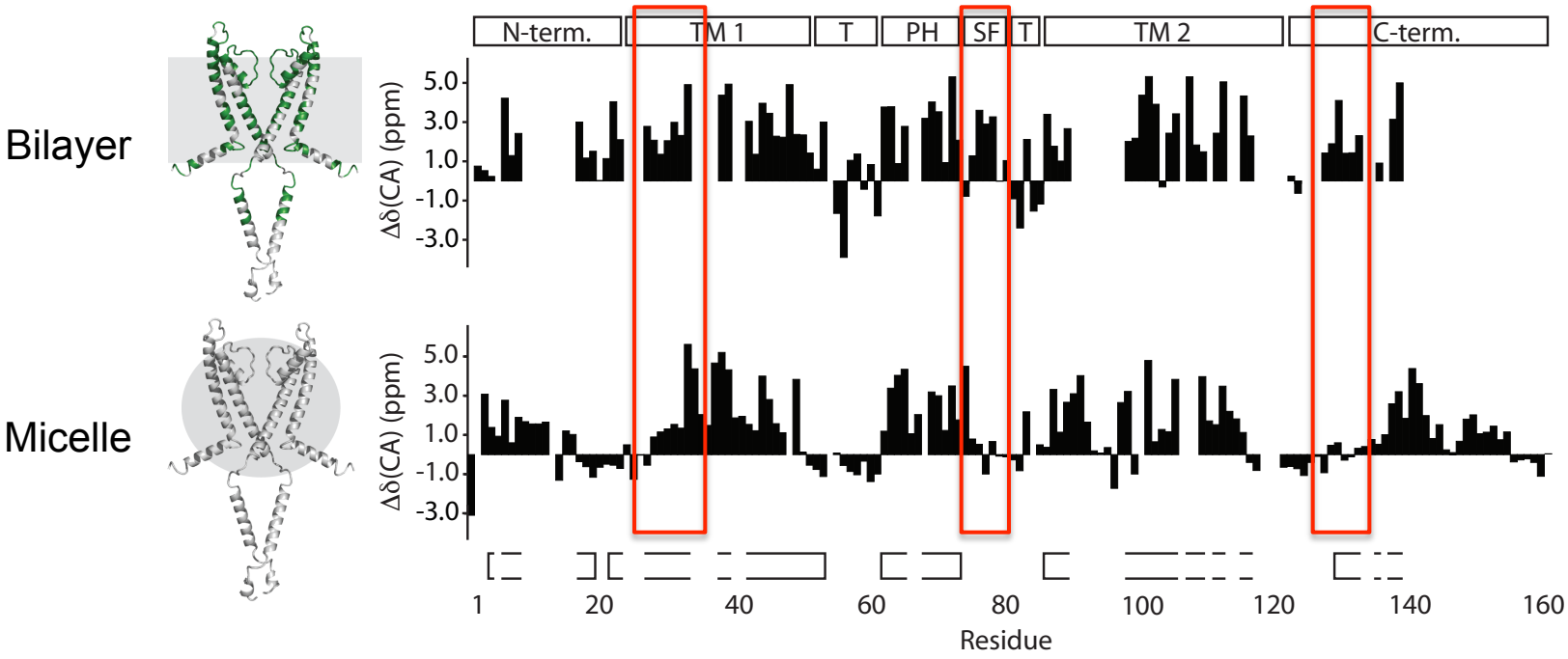
Study by solid-state NMR in liposomes



Schneider et al., JACS 130, 7427, 2008

- Channel reconstituted in **liposomes** (i.e. **lipid bilayers**!)
- **Functional** preparation, used in parallel for **electrophysiology** measurements!
- Obtain spectra of good quality (at the time, at least); resonance assignments for 60% of the protein's residues
- Fortunately, residues in functionally important regions (selectivity filter and gating region) fall outside of crowded spectral regions!

Secondary structure in lipid bilayers

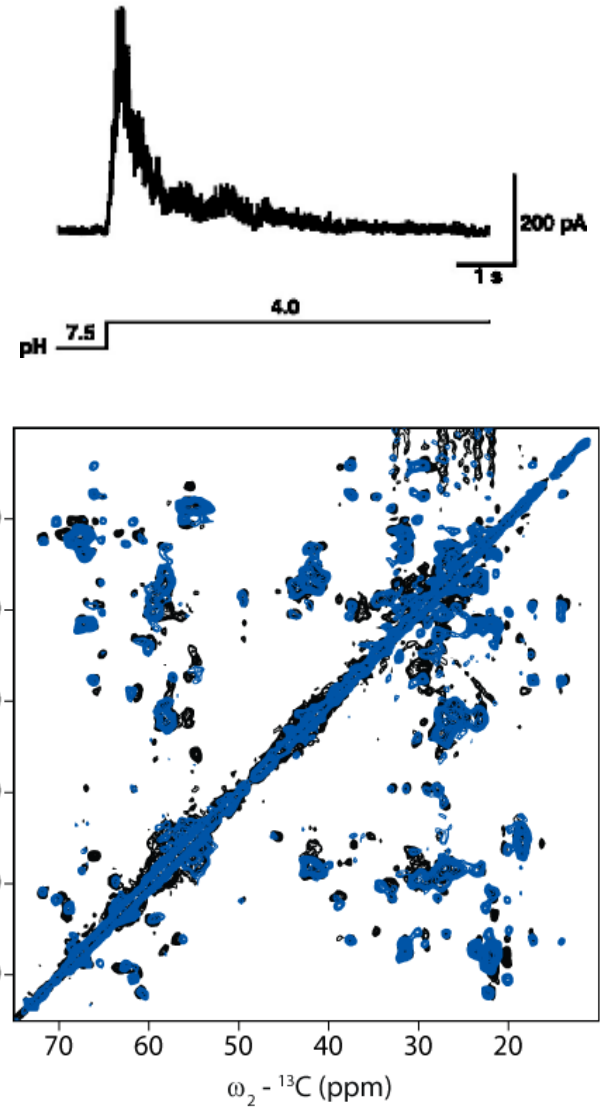


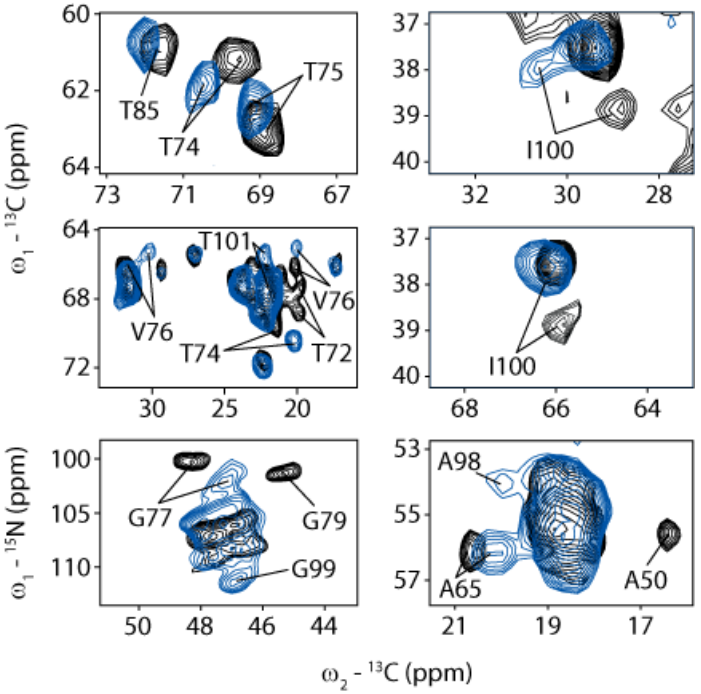
- Longer helices
- Different conformation in the selectivity filter

in lipid bilayers compared to micelles!

Schneider et al., JACS 130, 7427, 2008
Baker et al., Nat. Struct. Mol. Biol. 14, 1089, 2007

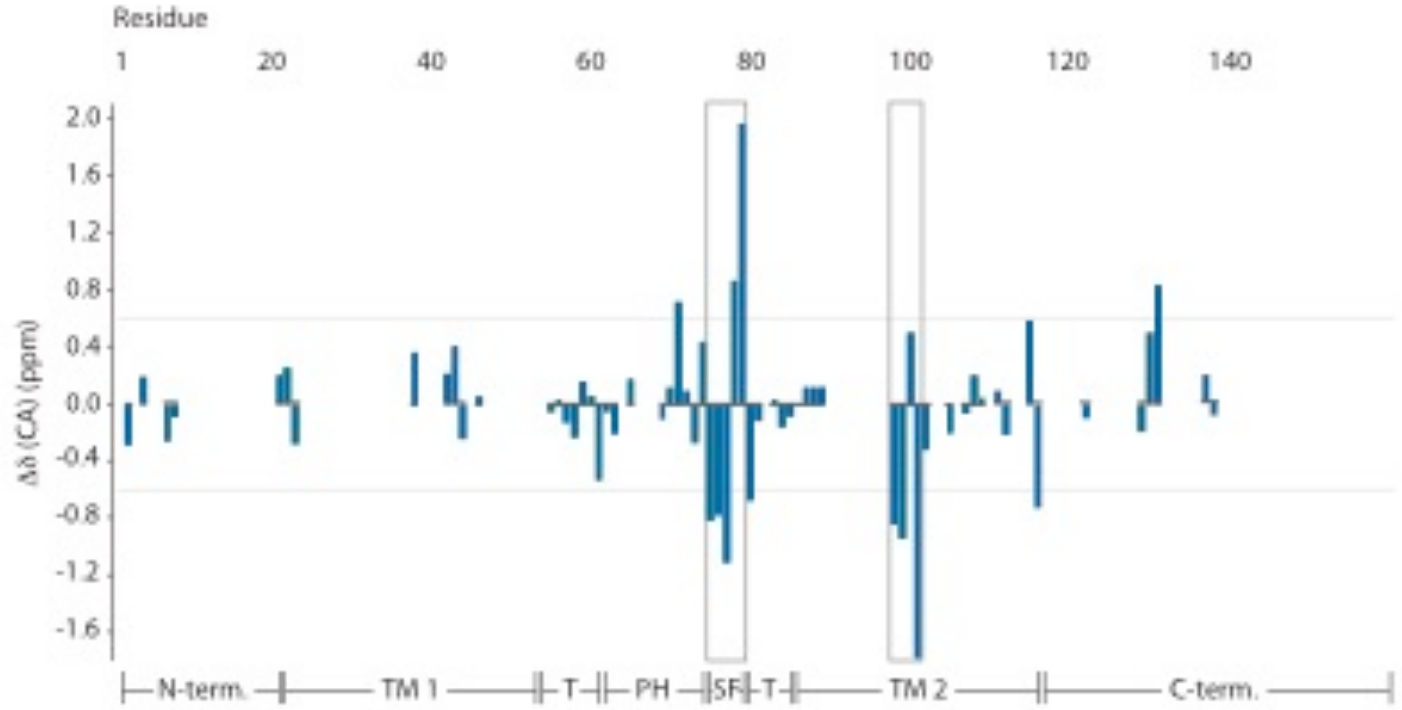
Transition to pH 4.0





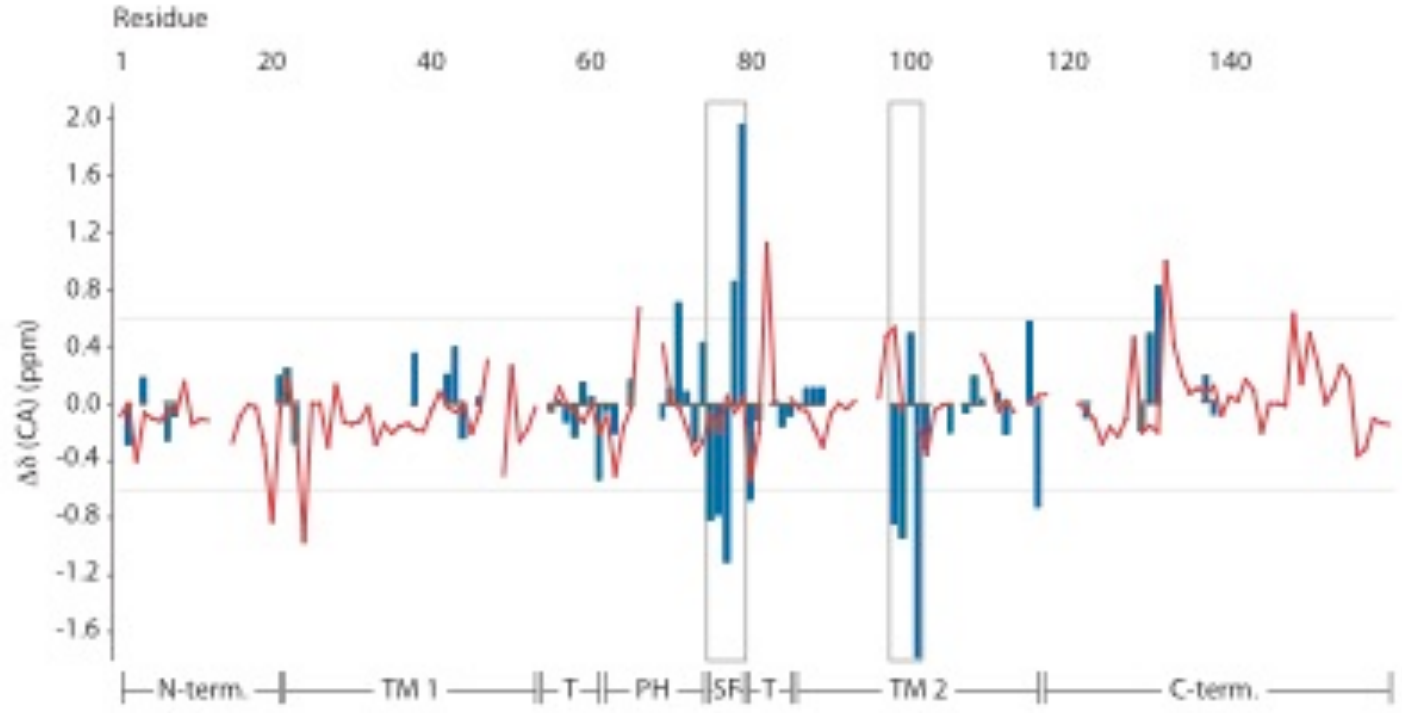
- Global structure preserved
- However, localized chemical shift changes are clearly observed

Chemical shift changes at pH 4.0



- Largest chemical shift changes localized to selectivity filter and region around Gly99 in TM2 known as “gating hinge” in other channels

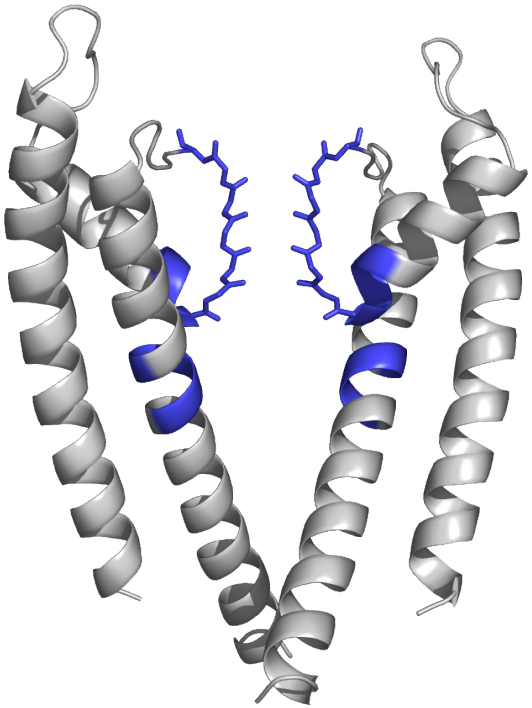
Chemical shift changes at pH 4.0



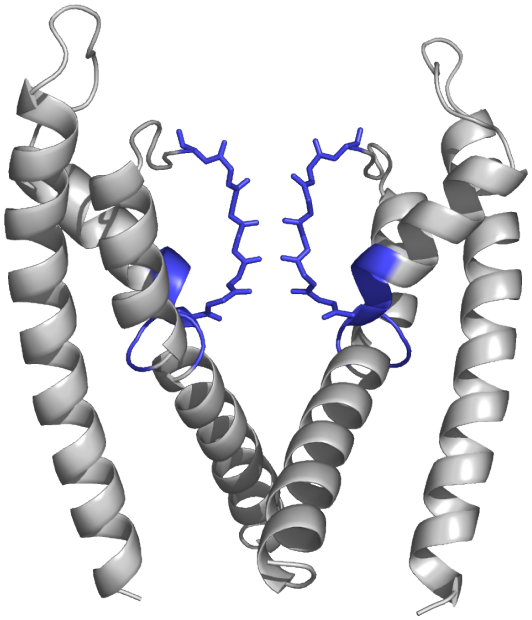
- Largest chemical shift changes localized to selectivity filter and region around Gly99 in TM2 known as “gating hinge” in other channels
- Very different results compared to micelles!

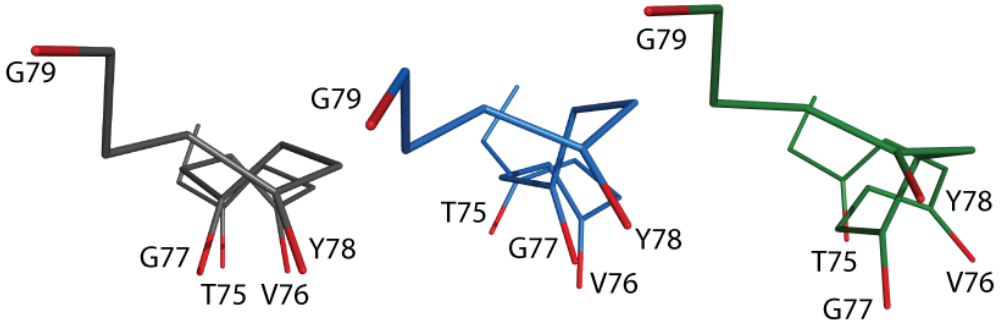
Ader, Schneider et al., Nat. Struct. Mol. Biol. 15, 605, 2008
Baker et al., Nat. Struct. Mol. Biol. 14, 1089, 2007

pH4 analysis: Results



pH4 analysis: Results





X-ray structure conductive

ssNMR model pH 4.0

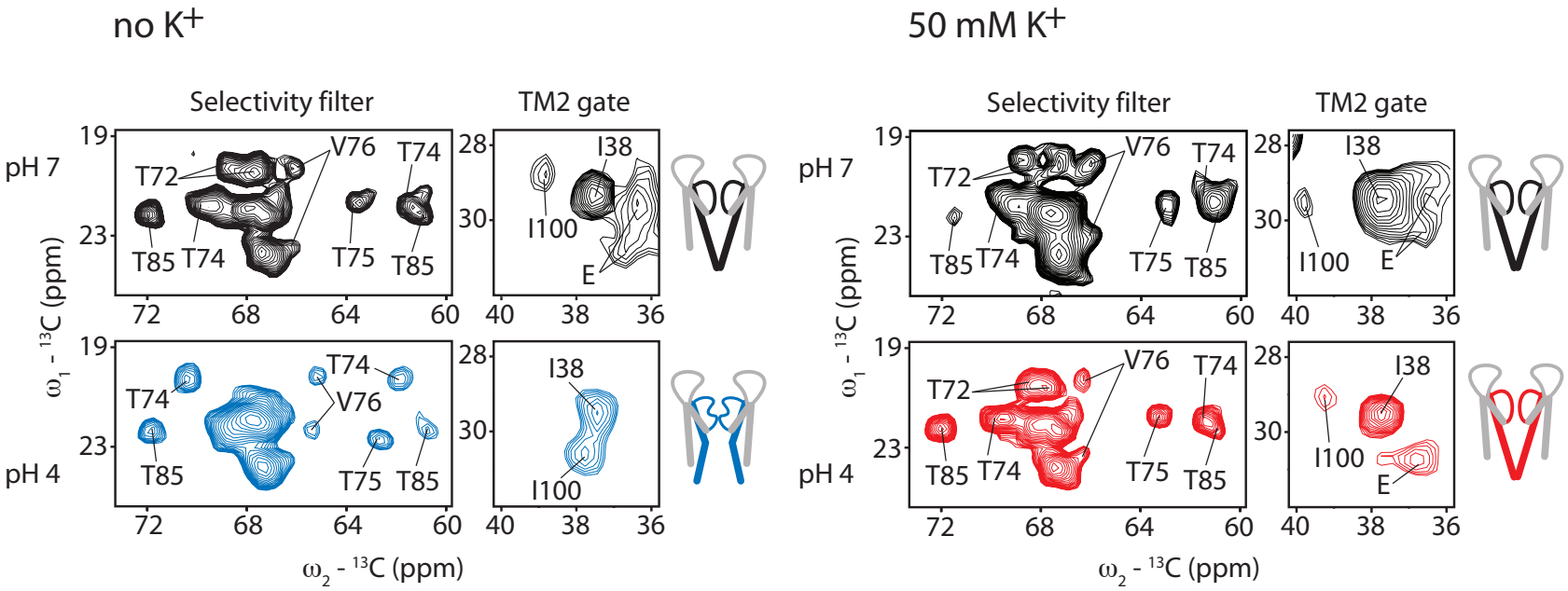
X-ray structure collapsed

- TM2 helix bundle („gate“) open
- Selectivity filter non-conductive

⇒ *open-inactivated* state at pH 4

Ader, Schneider et al., Nat. Struct. Mol. Biol. 15, 605, 2008
Zhou et al., Nature 414, 43, 2001

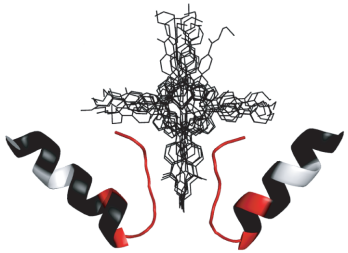
Open probability depends on K^+



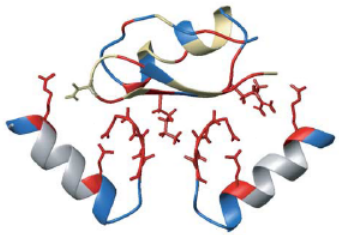
- Open probability at pH 4 **depends on K^+ concentration**
- In presence of K+, the conformation with **closed TM gate** and **conductive selectivity filter** dominates **even at pH 4**!

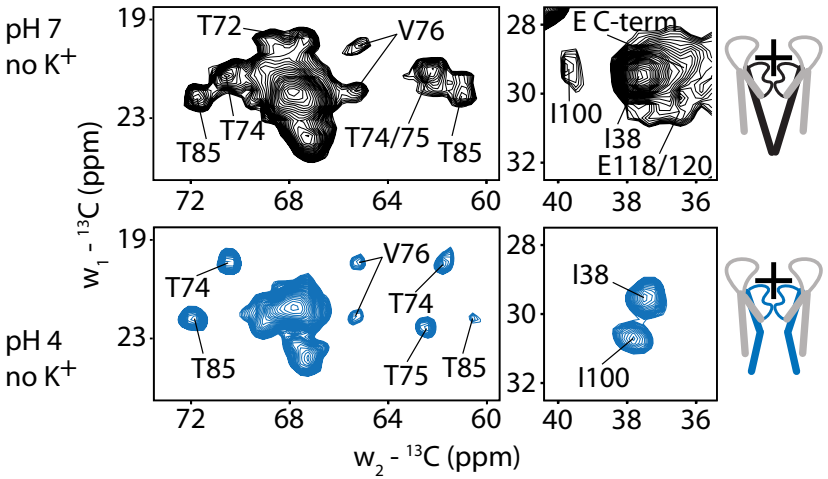
Selectivity filter and gate are coupled

Porphyrin

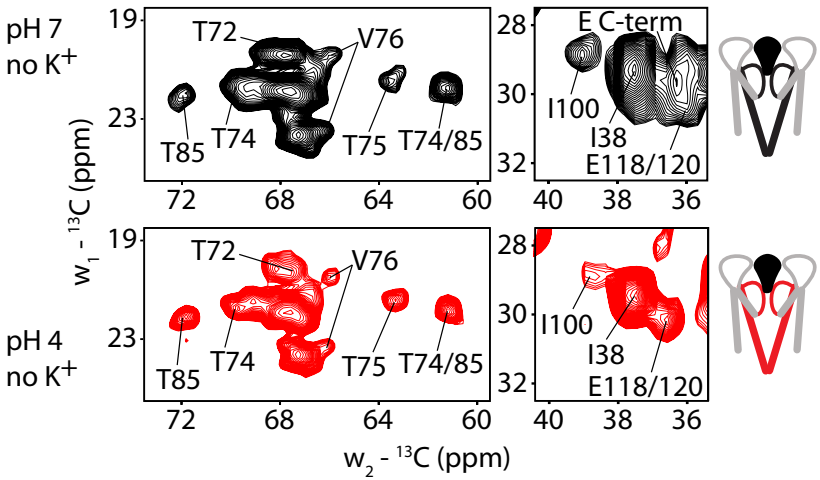


Kaliotoxin





open-inactivated



closed-conductive

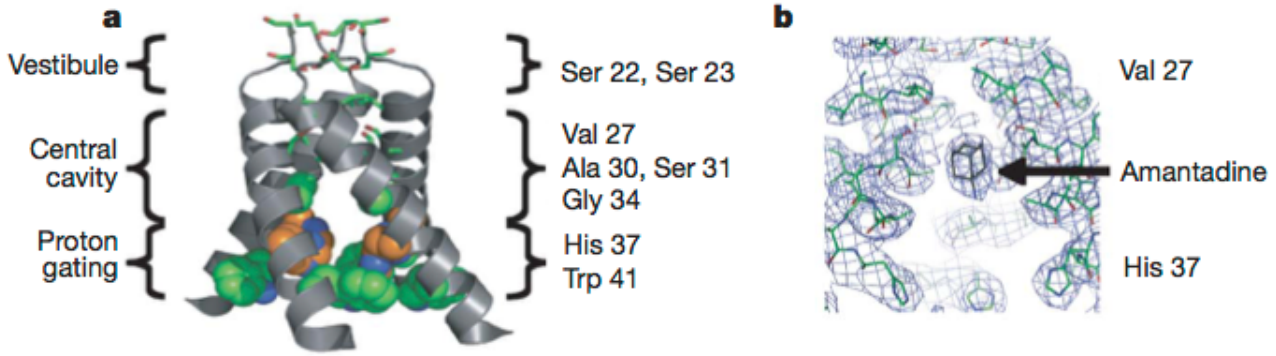
- Kaliotoxin binding **enforces conductive selectivity filter** even without K^+
- Conductive selectivity filter **keeps TM2 gate closed** even at pH 4

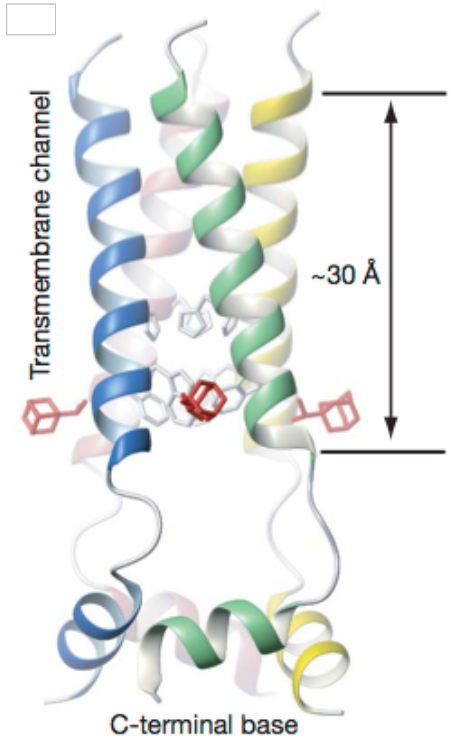
⇒ selectivity filter and TM2 gate are **coupled**!

Lange et al., Nature 440, 959, 2006
Ader, Schneider et al., EMBO J. 28, 2825, 2009

Applications II:
The influenza M2 proton channel

The influenza M2 proton channel



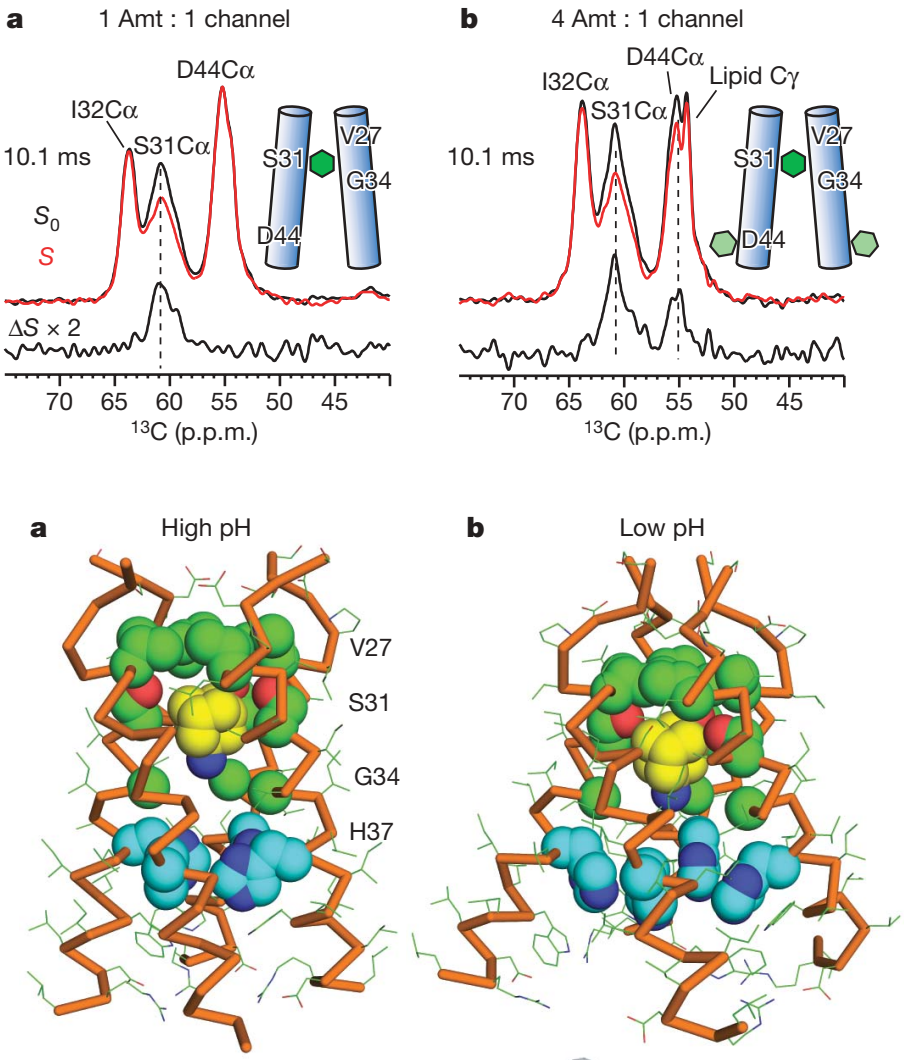


- pH-activated proton channel, involved in acidification and uncoating of virus particle as well as viral assembly
- Tetramer of four single transmembrane helices
- Targeted by adamantane-based antiviral drugs
- Crystal structure: one drug molecule binds in channel **lumen**
- Solution NMR structure: four drug molecules bind from the **membrane**

→ ?!?

Stouffer et al., Nature 451, 596, 2008
Schnell & Chou, Nature 451, 591, 2008

Dilemma resolved by solid-state NMR



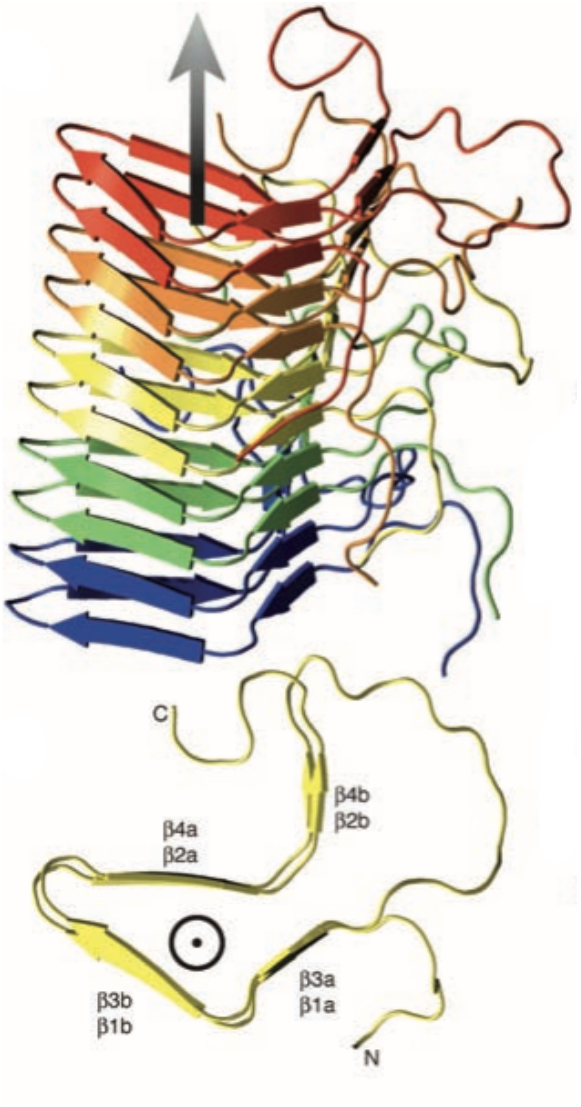
Solid-state NMR on M2 in lipid bilayers:

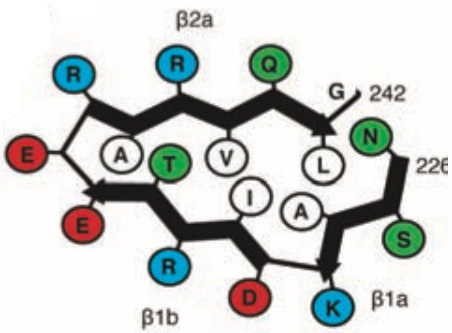
- channel selectively ^{13}C-labeled, amantadine deuterated
- Recouple ^{2}H-^{13}C interaction
- at **low drug:protein** ratio, find drug in channel **lumen**; when drug in **excess**, find it **also** on the **membrane** side!
- Structure calculation based on ssNMR restraints: **helix bundle tighter** in the C-terminal region − crucial His residues not protonated as in low-pH crystal structure!

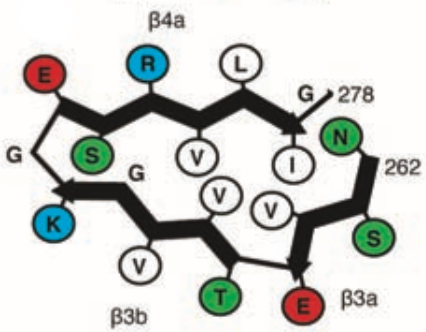
Cady et al., Nature 463, 689, 2010

Applications III:
Amyloid fibrils, supramolecular assemblies

The HET-s prion



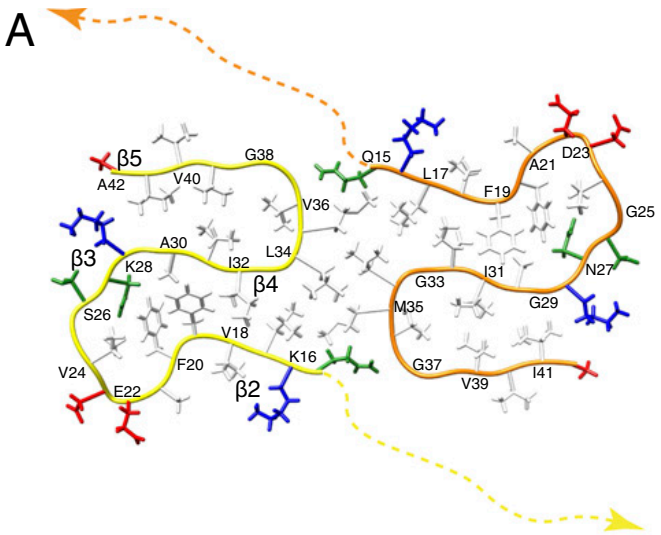


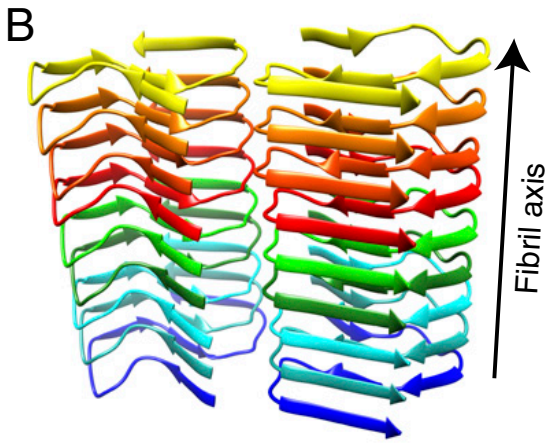


- Functional fungal prion involved in self/ nonself recognition
- Structure resolved by solid-state NMR as β-helical solenoid
- First structure of an amyloid fibril (apart from fibrils formed by short peptides)!
- Dry core formed by hydrophobic residues; stabilization by salt bridges and H bond ladders

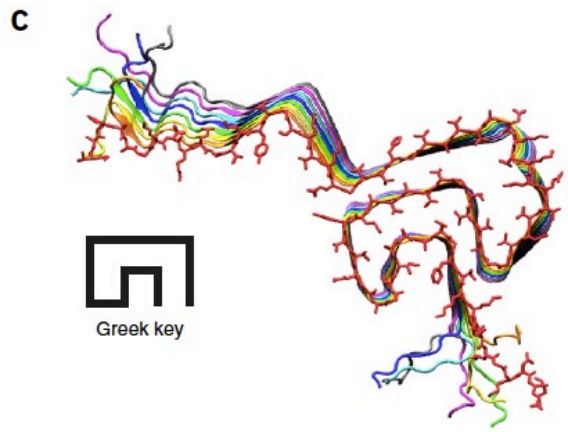
Wasmer et al., Science 319, 1523, 2008

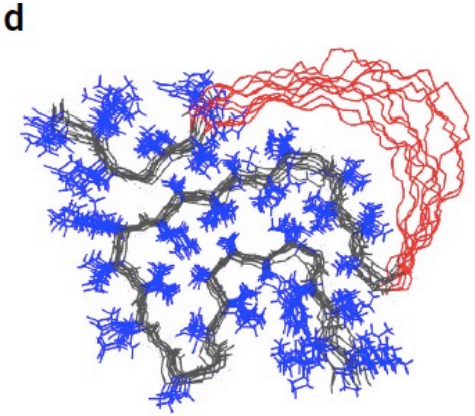
Amyloid-β and α-synuclein





- Amyloid fibrils formed by two proteins involved in two important neurodegenerative diseases (Alzheimer's, Parkinson's) have been resolved by ssNMR
- Fibril polymorphism was a major problem in those studies!

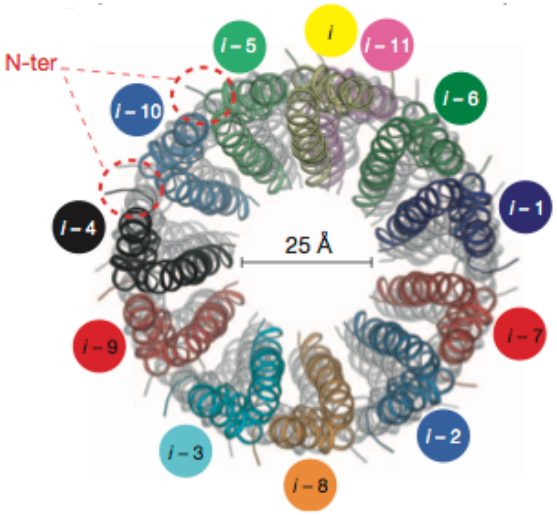


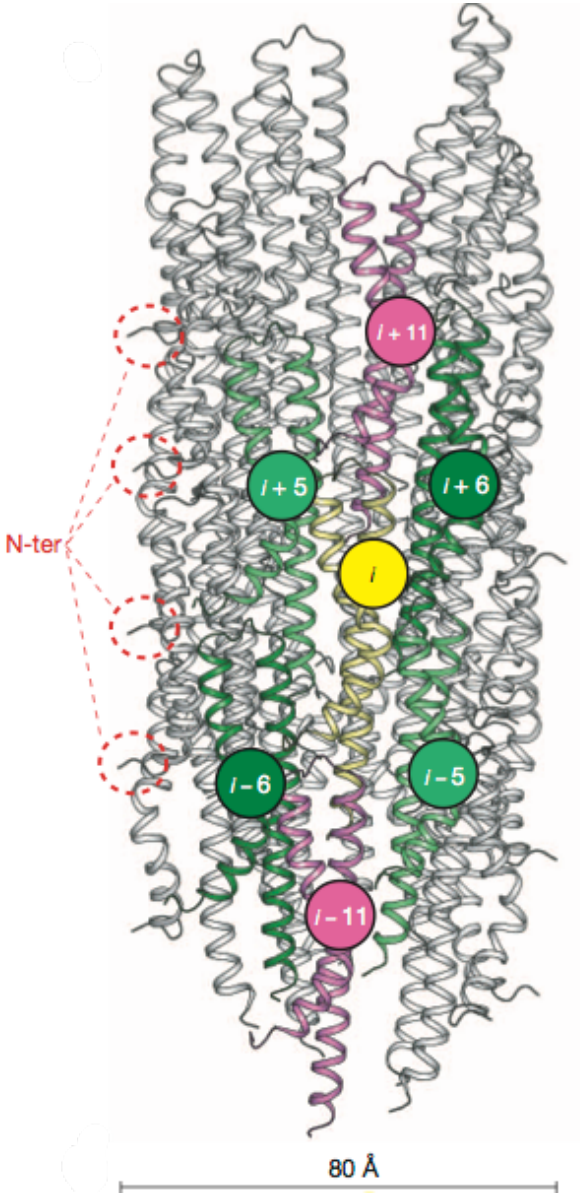


Wälti et al., PNAS 113, E4976, 2016
Tuttle et al., Nat Struct Mol Biol 23, 409, 2016

The *Salmonella* type-III secretion system

- Hollow needle formed from 80 AA PrgI protein, used for injection of effector proteins into host cells
- Combination of solid-state NMR data with mass-per-length measurements by STEM and Rosetta modeling allowed for calculation of a 3D structure





Loquet et al., Nature 486, 276, 2012

Merci!