Introduction to biomolecular solid-state NMR

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École de Biologie Structurale Intégrative RéNaFoBiS
Oléron, June 2017

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Overview

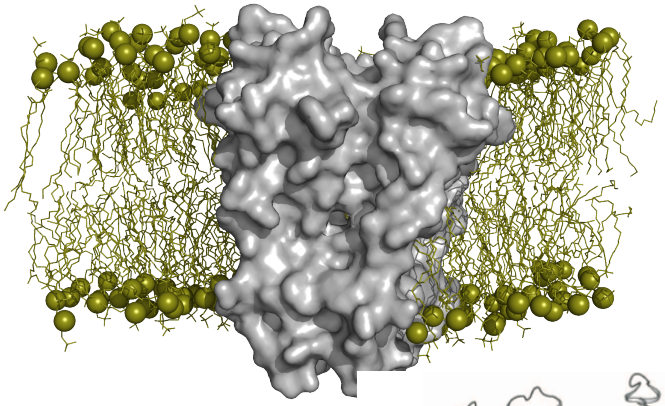
- Anisotropic interactions important in solid-state NMR
- Solid-state NMR techniques
- Applications: membrane proteins, protein fibrils, supramolecular assemblies

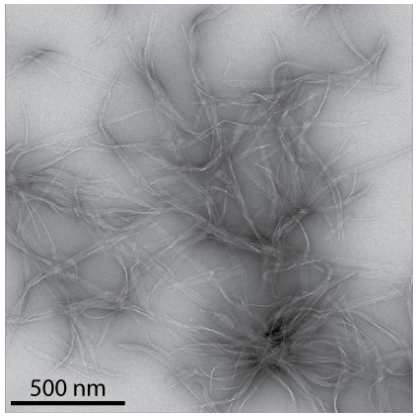
Solid-state NMR: a primer

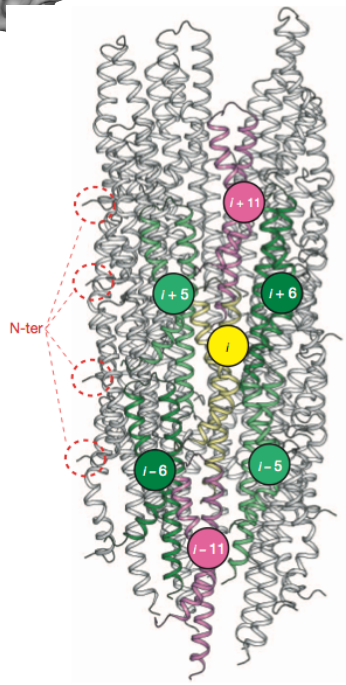
- NMR spectroscopy for systems that are
  - **insoluble**
  - (in principle, arbitrarily) **large**
  - **non-crystalline** (no long-range order)

  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

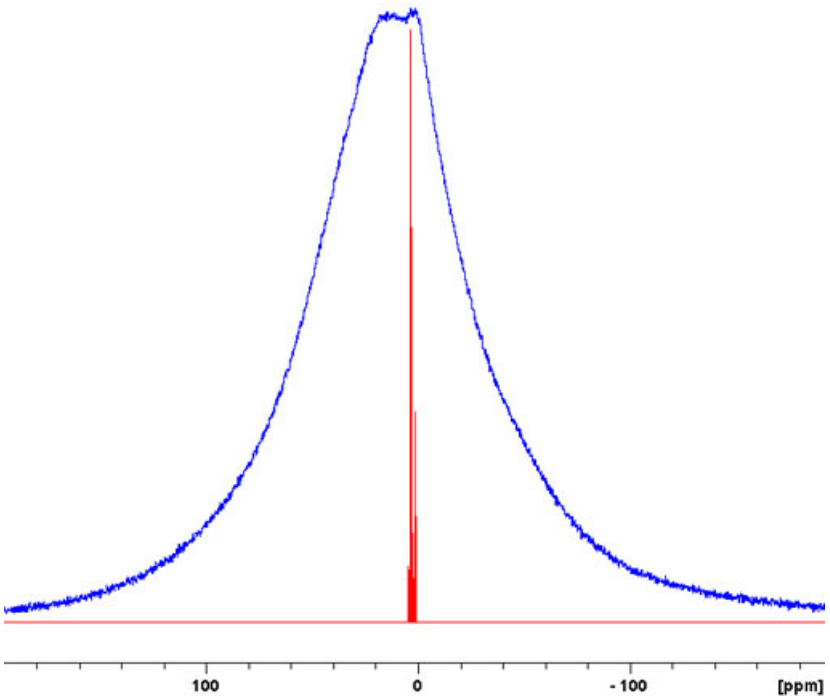
NMR Hamiltonian:

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

- 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).

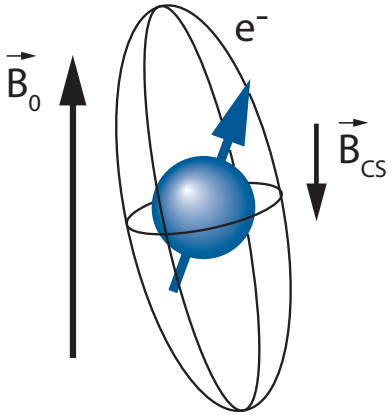
⇒ Challenge: obtain high-resolution spectra under these conditions, yet still take advantage of the information contained in anisotropic interactions.

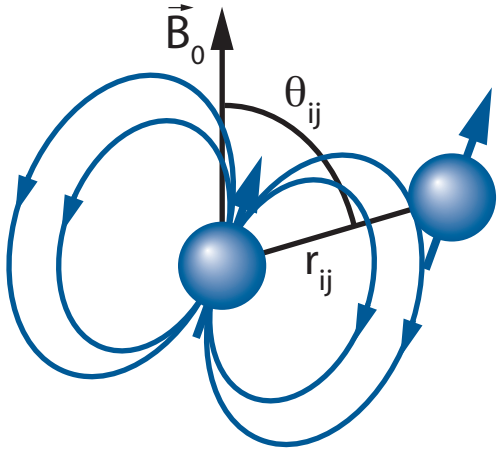
Anisotropic interactions

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

Chemical shift anisotropy

Dipolar coupling





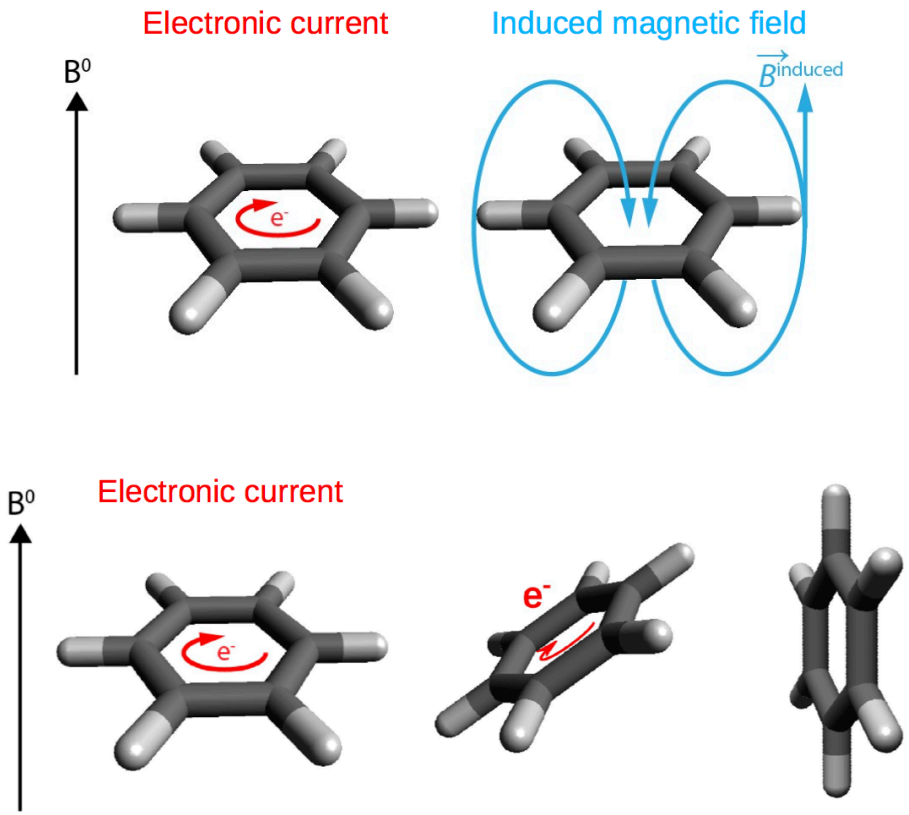
$$\hat{H}_{\mathrm{CS}} = -\gamma\, \hat{\boldsymbol{I}}\, \boldsymbol{\sigma}\, \boldsymbol{B}_0$$

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

- 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)

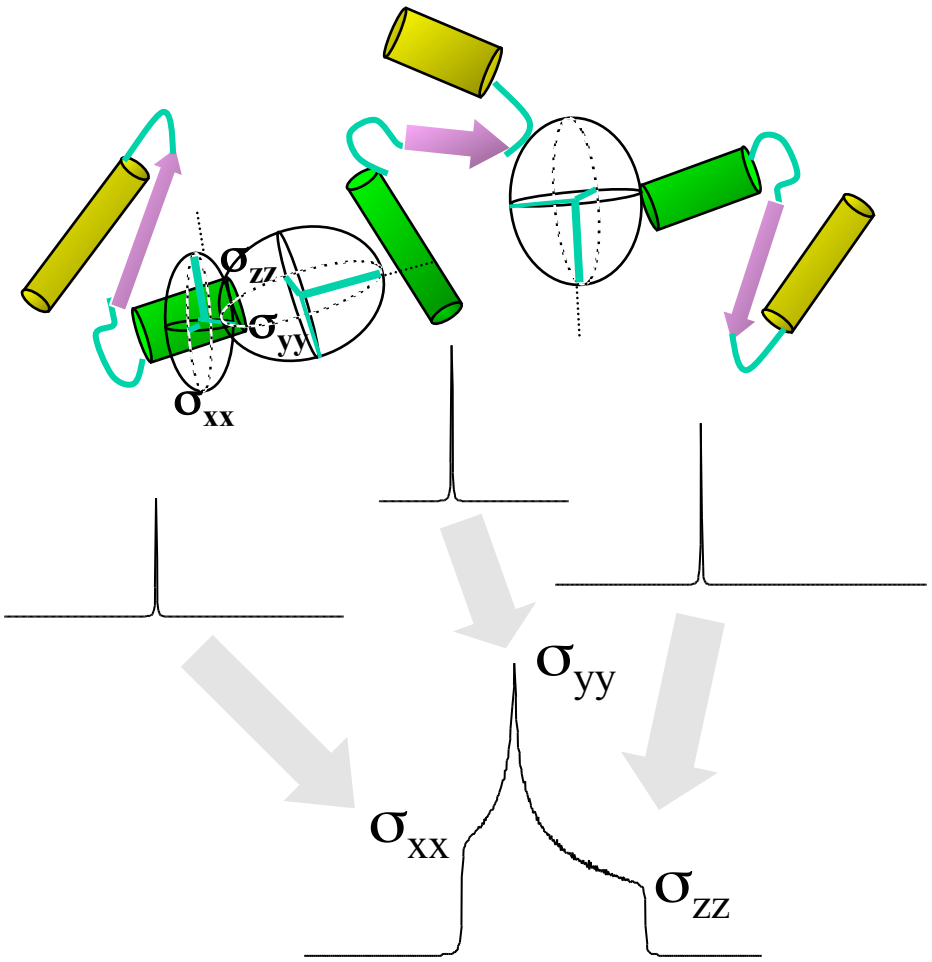


- B_0 field induces electron currents that generate secondary magnetic fields
- Total field felt by a nucleus results from the **superposition of B_0** with these **secondary fields**
- Generally, electron distribution around a nucleus is **not spherically symmetric**

⇒ 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

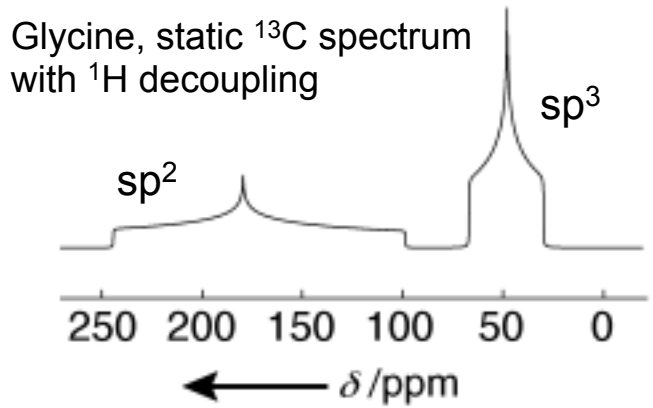
Chemical shift anisotropy

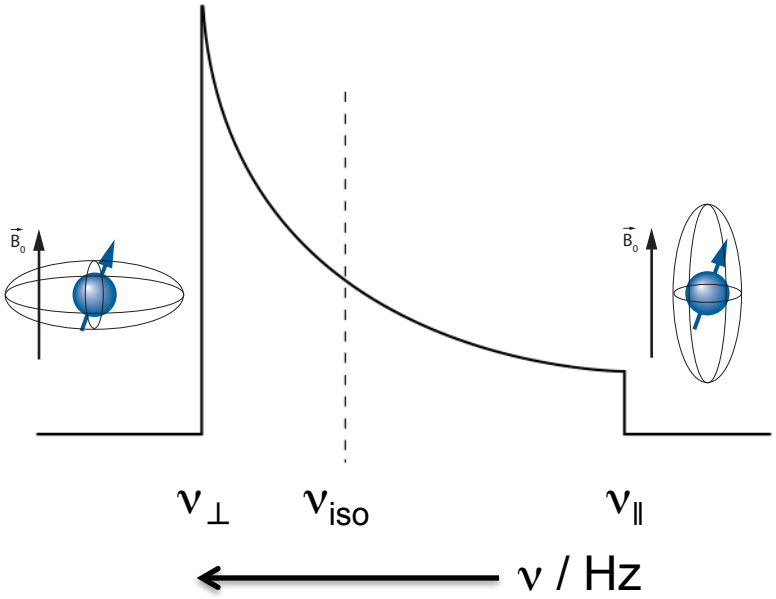


- Superposition of individual signals corresponding to different molecular orientations leads to the broad **“powder pattern”** observed in a static sample
- Gives information on **structure of electronic environment**

M. Baldus

Chemical shift anisotropy



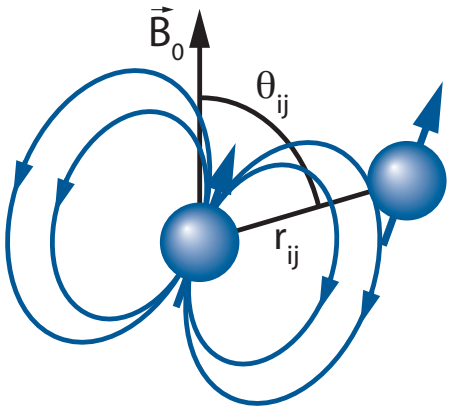


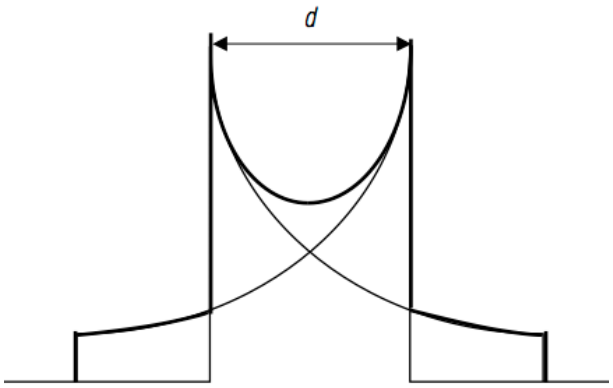
- CSA powder pattern reflects, e.g., on
  - symmetry
  - hybridization
  - bond lengths / angles
  - dihedral angles

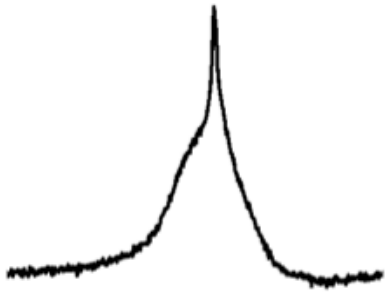
  of electronic environment.
- The **isotropic** chemical shift corresponds to the **barycenter** of the CSA pattern.

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

Dipolar coupling

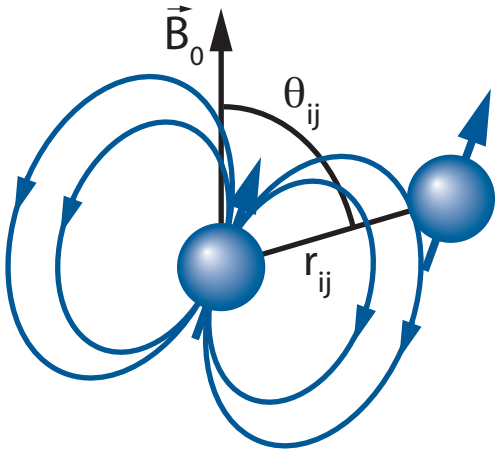






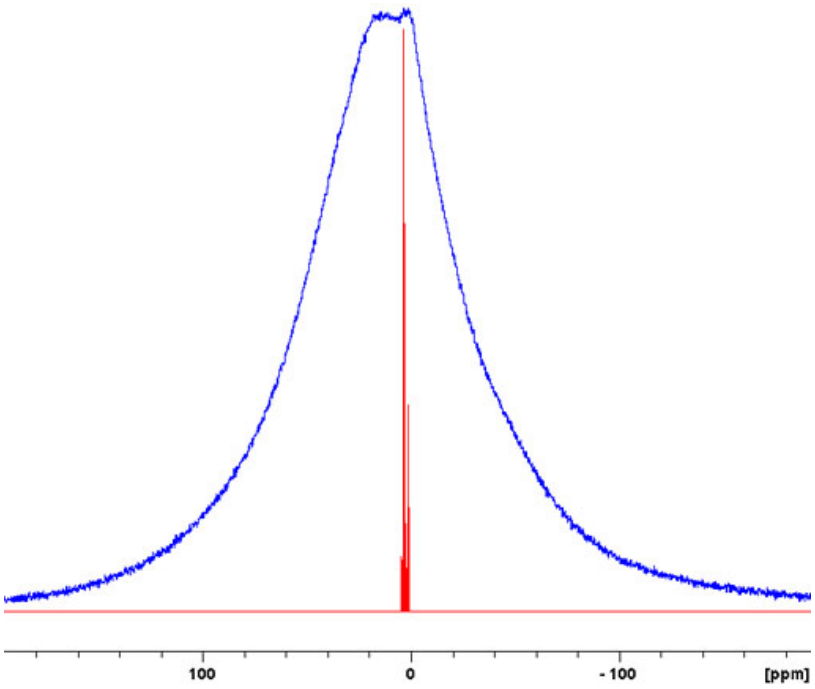
- Interaction between the **magnetic moments** of two spins (cf. bar magnets influencing each other)
- Depends on internuclear **distance** (as $1/r^3$) and **orientation** of internuclear vector with respect to B_0
- Gives a **doublet** (similar as for J coupling) for a **single crystal** (where all 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

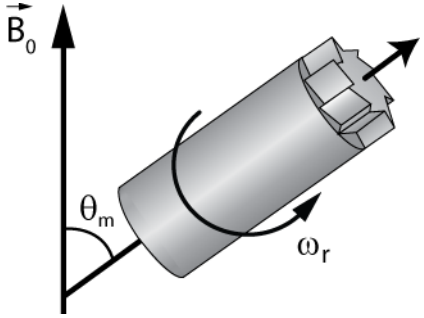
$$\hat{H} = \sum_{l=0}^{2} \hat{\boldsymbol{A}}_l \cdot \hat{\boldsymbol{T}}_l$$

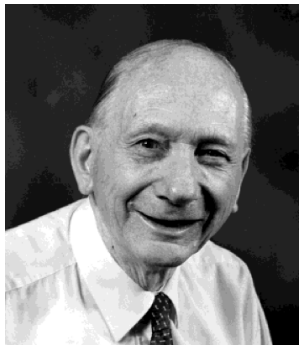
space spin

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

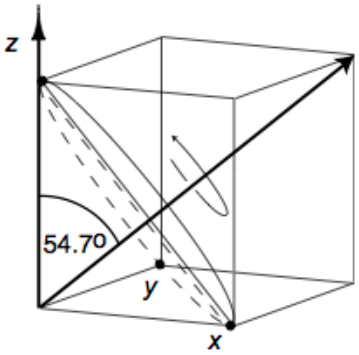
- The NMR Hamiltonian can be separated into a **space** and a **spin** part
- We can interfere with the spin system via either!
- The space part of CSA and dipolar coupling depends on orientation as $(3 \cos^2 \theta - 1)$
- In solution, rapid molecular tumbling averages out anisotropic interactions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

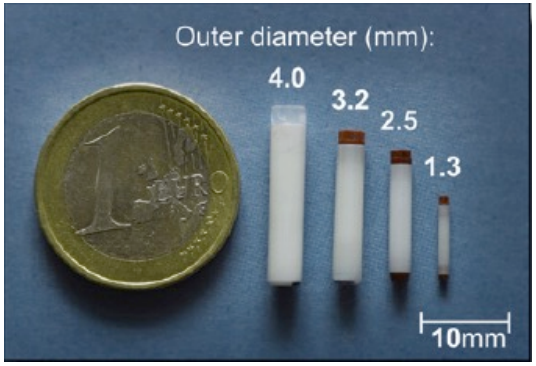
Magic Angle Spinning





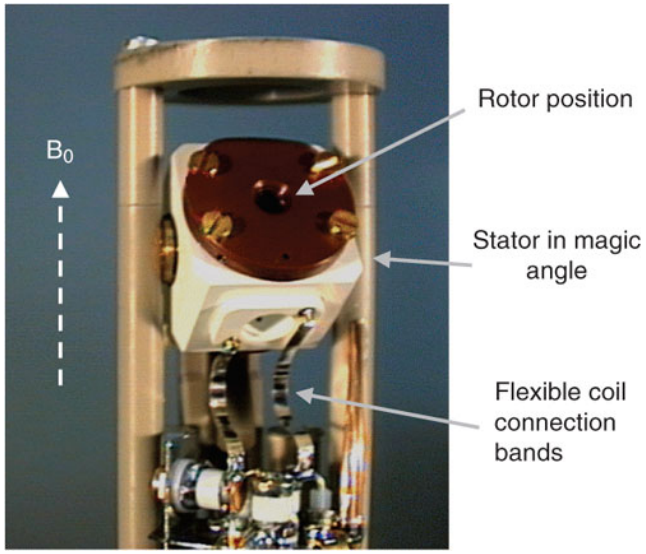
E. R. Andrew





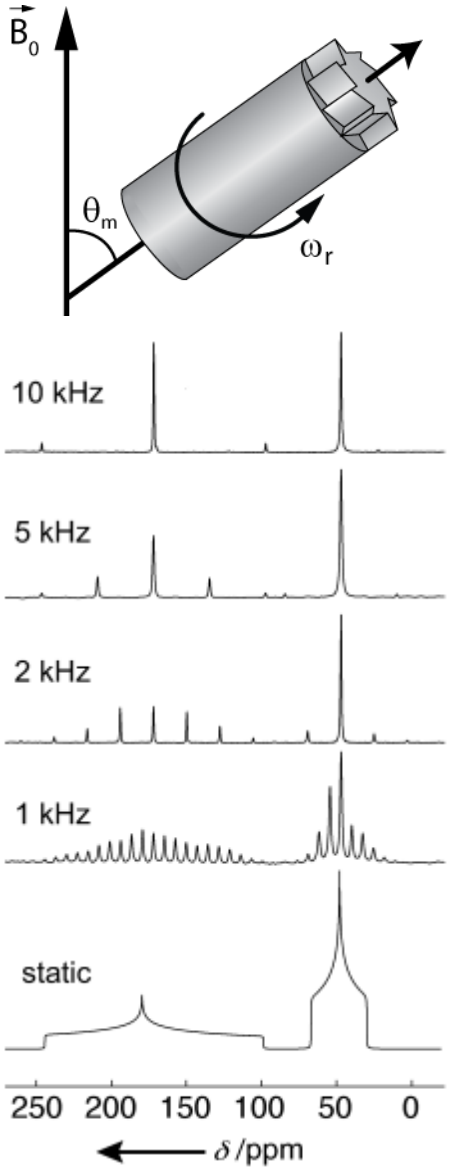
Demers et al., Solid State Nucl Magn Reson 40, 101, 2011

- 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



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

Magic Angle Spinning

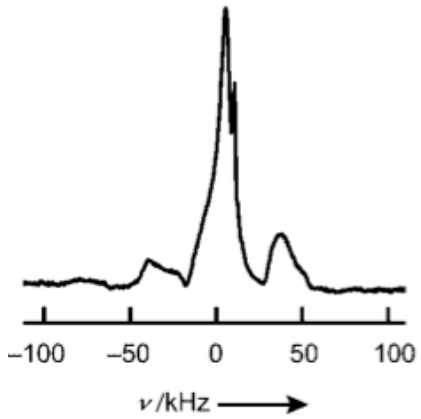


Laws et al., Angew. Chem. Int. Ed. 41, 3096, 2002

- 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!



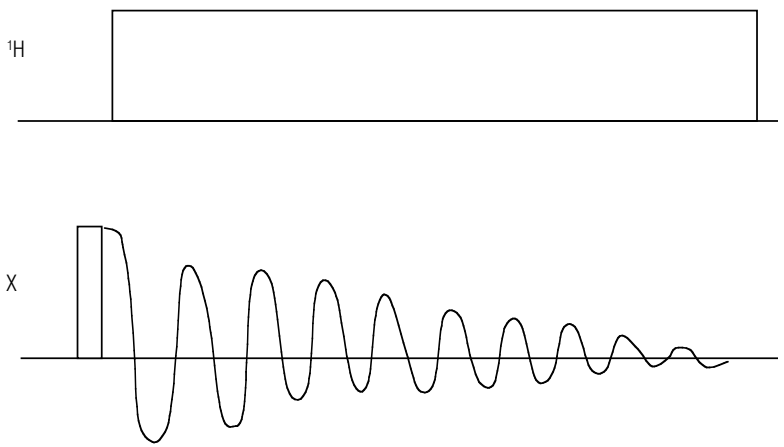
Heteronucleus detection and decoupling

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

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

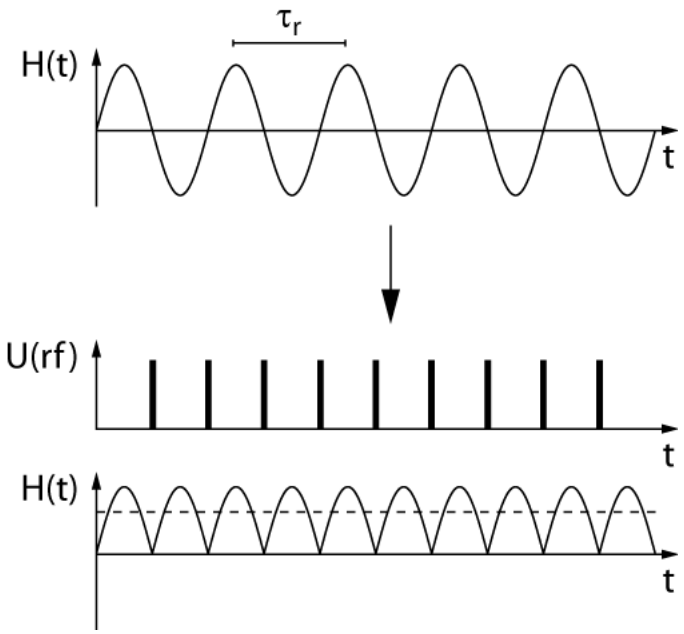
⇒ **decouple** ^{1}H using RF irradiation

- 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?

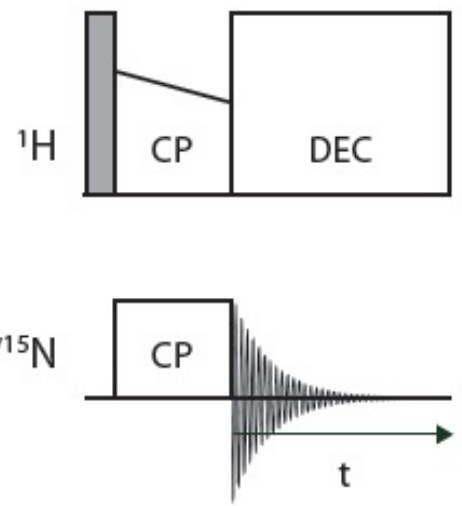
⇒ use **recoupling pulse sequences** to “switch on” desired interactions during “mixing time” of an NMR experiment!

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

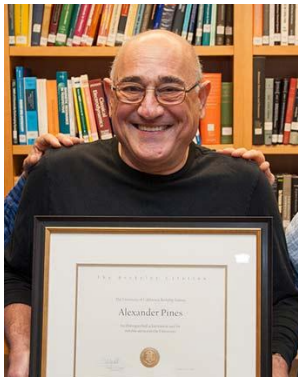
⇒ **enhance ^{13}C magnetization** by a factor of 4!
(as for INEPT transfer in solution)



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

or

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



A. Pines

Communications

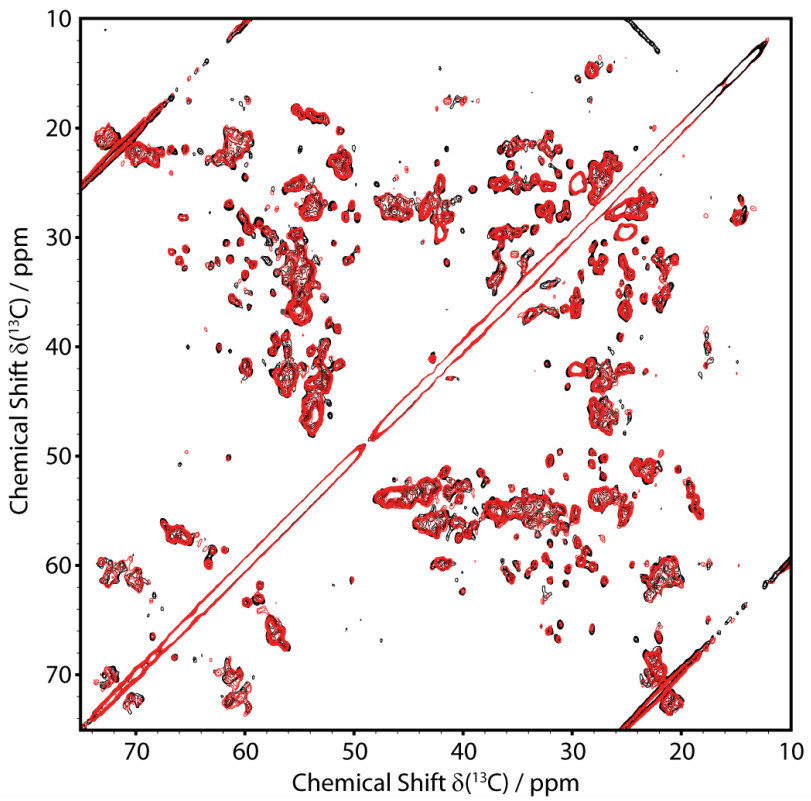
THE JOURNAL OF CHEMICAL PHYSICS VOLUME 56, NUMBER 4 15 FEBRUARY 1972

Proton-Enhanced Nuclear Induction Spectroscopy. A Method for High Resolution NMR of Dilute Spins in Solids*

A. PINES, M. G. GIBBY,† AND J. S. WAUGH
Department of Chemistry and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

(Received 18 November 1971)

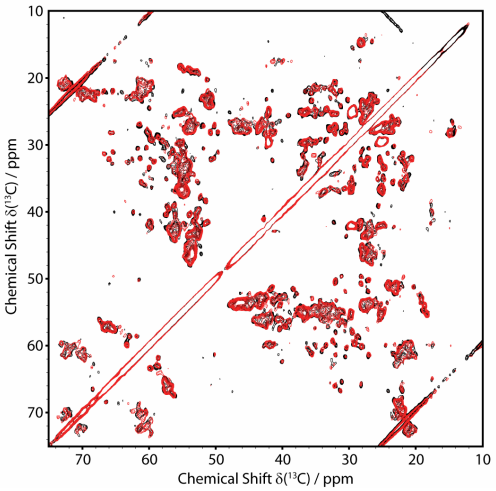
Solid-state fingerprint: ^{13}C-^{13}C correlation



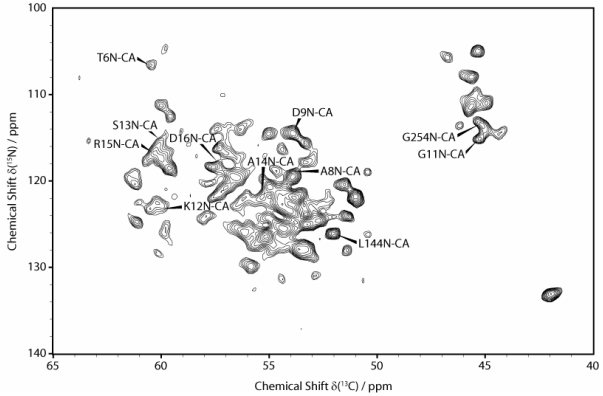
Schneider et al., Angew Chem Int Ed 49, 1882, 2010

- Different recoupling techniques available
- Obtain correlation map of ^{13}Cs in a protein
- Shorter mixing times → **intraresidue** correlations
- Longer mixing times → **interresidue**, **through-space** correlations

The toolbox



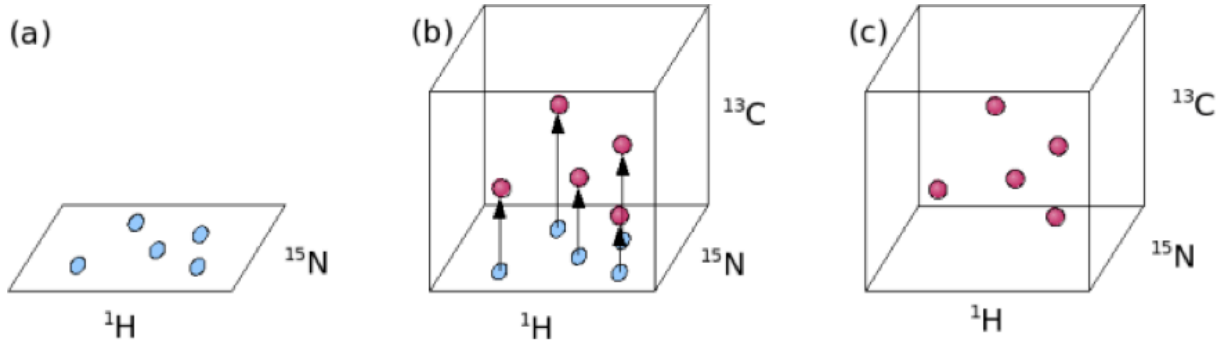
^{13}C-^{13}C



^{15}N-^{13}C

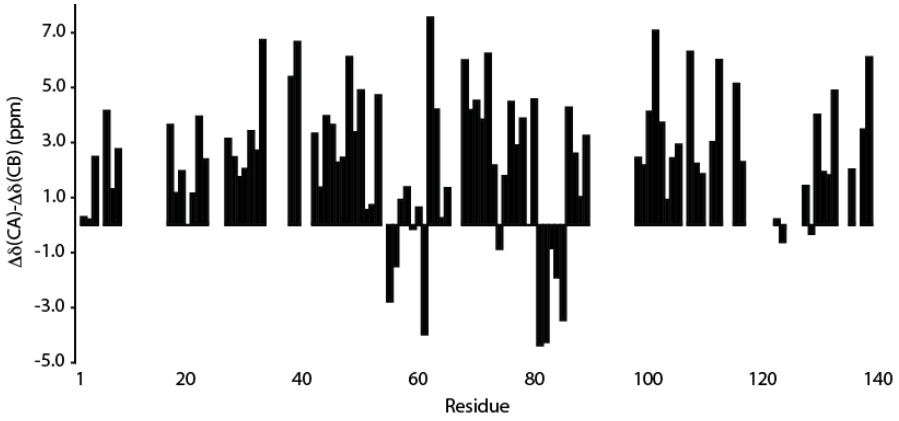
- High(er) resolution ^{15}N, ^{13}C detection by MAS and decoupling
- Polarization transfer ^{1}H-^{15}N, ^{1}H-^{13}C, ^{13}C-^{13}C, ^{15}N-^{13}C ...
- 2D, 3D, ... spectroscopy
- ... for structural analysis of biomacromolecules

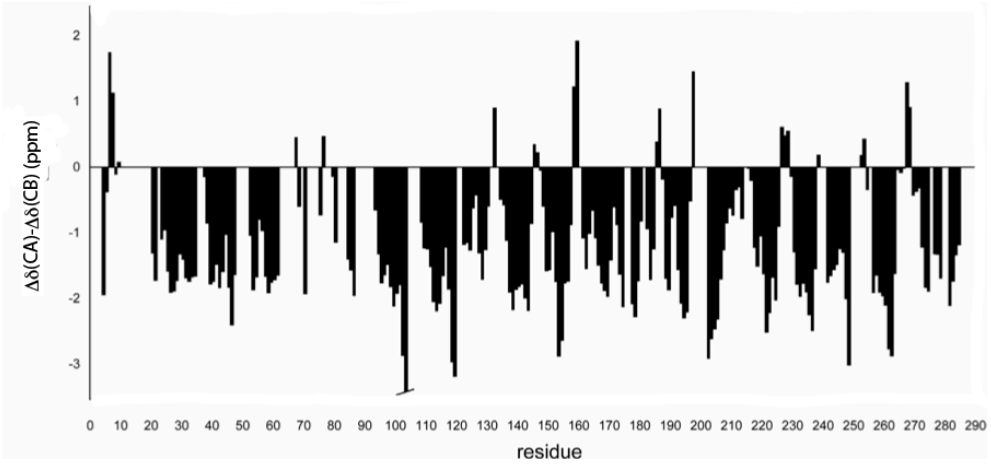
Schneider et al., Angew Chem Int Ed 49, 1882, 2010
http://www.protein-nmr.org.uk/solution-nmr/assignment-theory/visualising-3d-spectra/



3Ds

Protein secondary structure

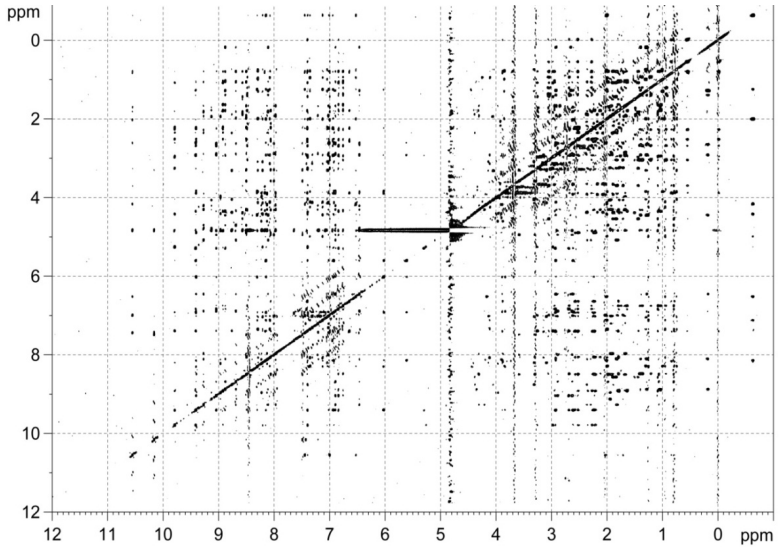




- As in solution, especially ^{13}C chemical shifts are indicative of secondary structure
- Compare assigned values in protein of interest to **reference** / **random coil** values to obtain **secondary chemical shift**
- Identify α-helices, β-strands, turns directly from resonance assignments!

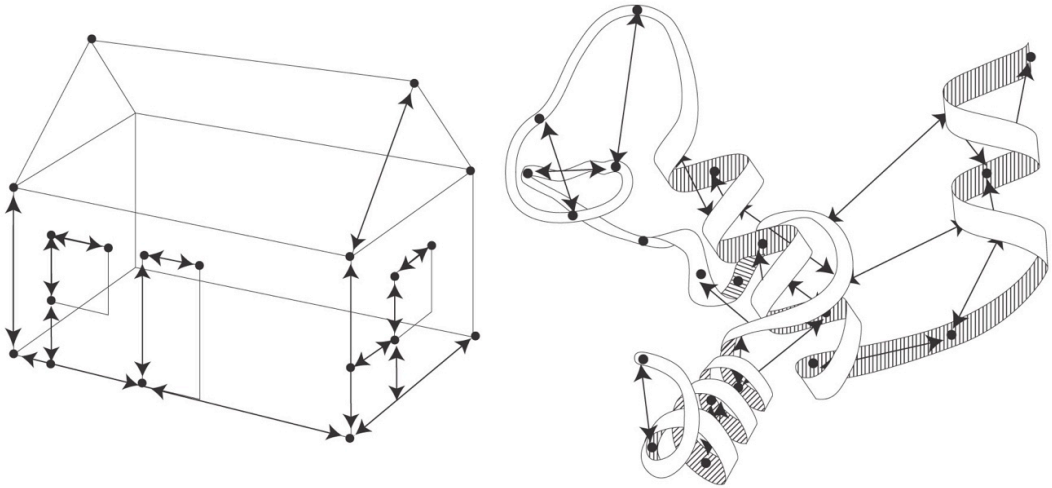
Schneider et al., JACS 130, 7427, 2008
Hiller et al., Science 321, 1206, 2008

Parenthesis: Protein structures from NMR?



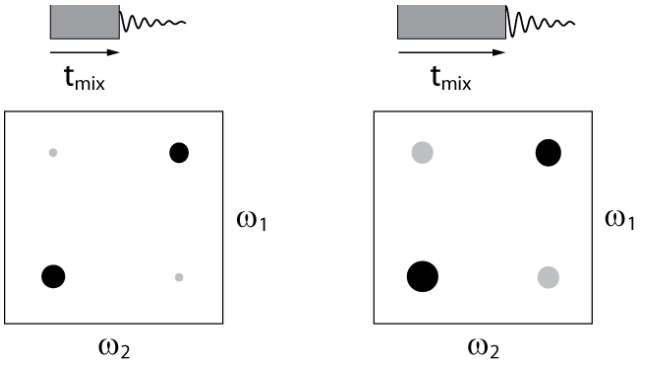
C. Smet-Nocca

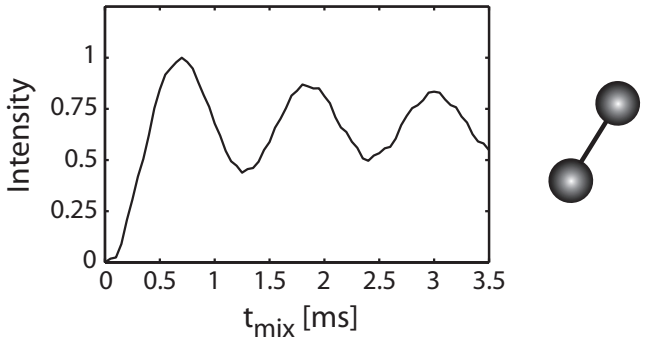
- Detect which nuclei are close in space via **through-space correlation** spectra (solution: NOESY; solid: spin diffusion, DARR, PAR, CHHC, RFDR, ...)
- Assemble a **model** that fulfils as many of these (short-range and rather imprecise) **distance restraints** as possible!

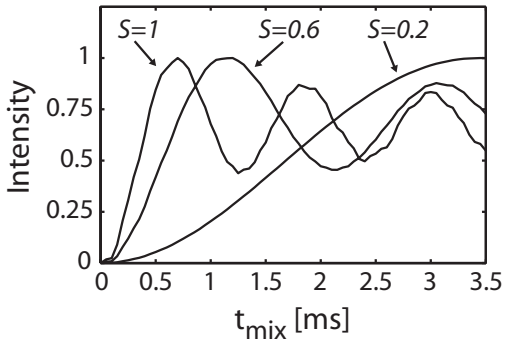


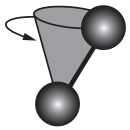
http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2002/popular.html

Dynamics



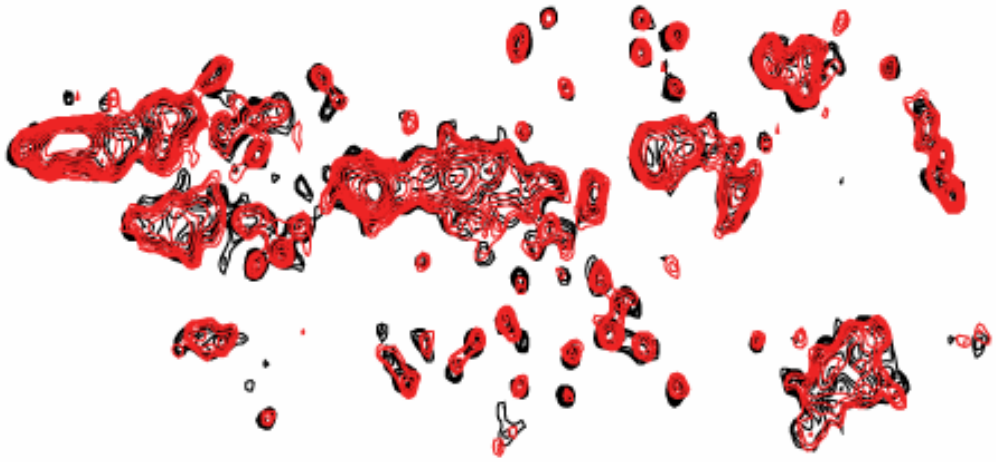






- NMR is sensitive to **molecular dynamics** on a **wide range of time scales** (ps − h)
- **Quantify** motional **amplitudes** and **time scales** in a **site-specific** manner
- In solids, **anisotropic interactions** are affected by dynamics on all time scales **faster** than the **inverse of the 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!

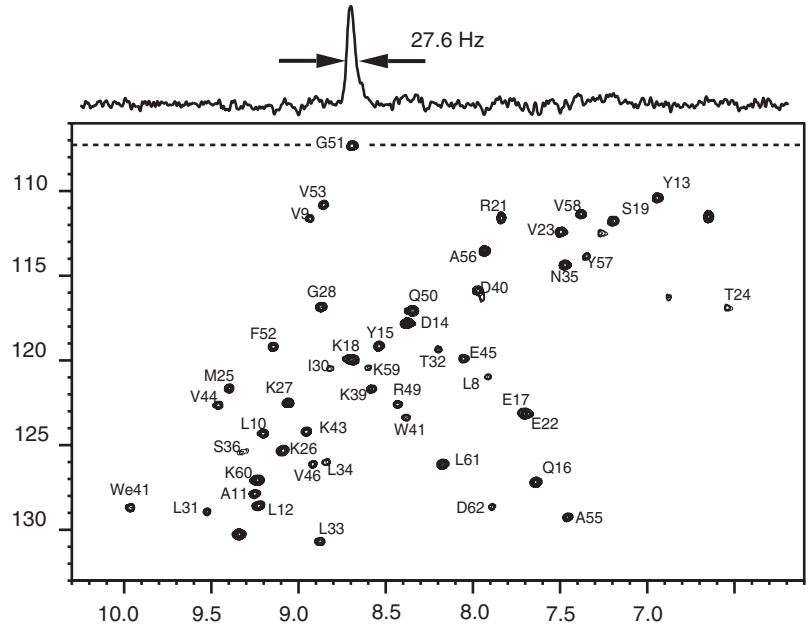
But still...



- Rather large linewidths
- Rather low signal to noise

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

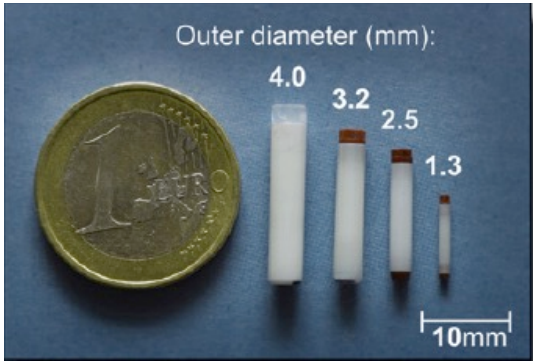
Breakthrough 1: Deuteration

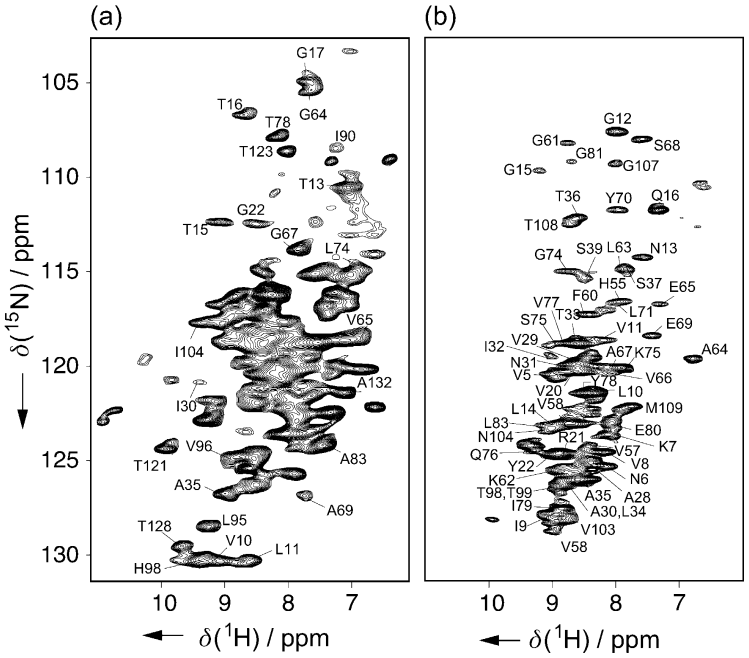


Chevelkov et al., Angew Chem Int Ed 45, 3878, 2006

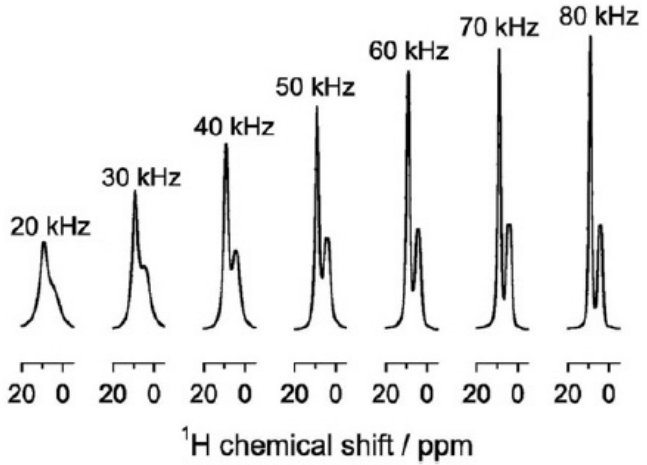
- (Per)Deuteration with (partial) back-exchange of protons yields very high resolution spectra already at 10 − 20 kHz MAS
- Permits **proton detection** as in solution state, leading to higher sensitivity! (proportional to $\gamma^{3/2}$)

Breakthrough 2: (Very) Fast MAS



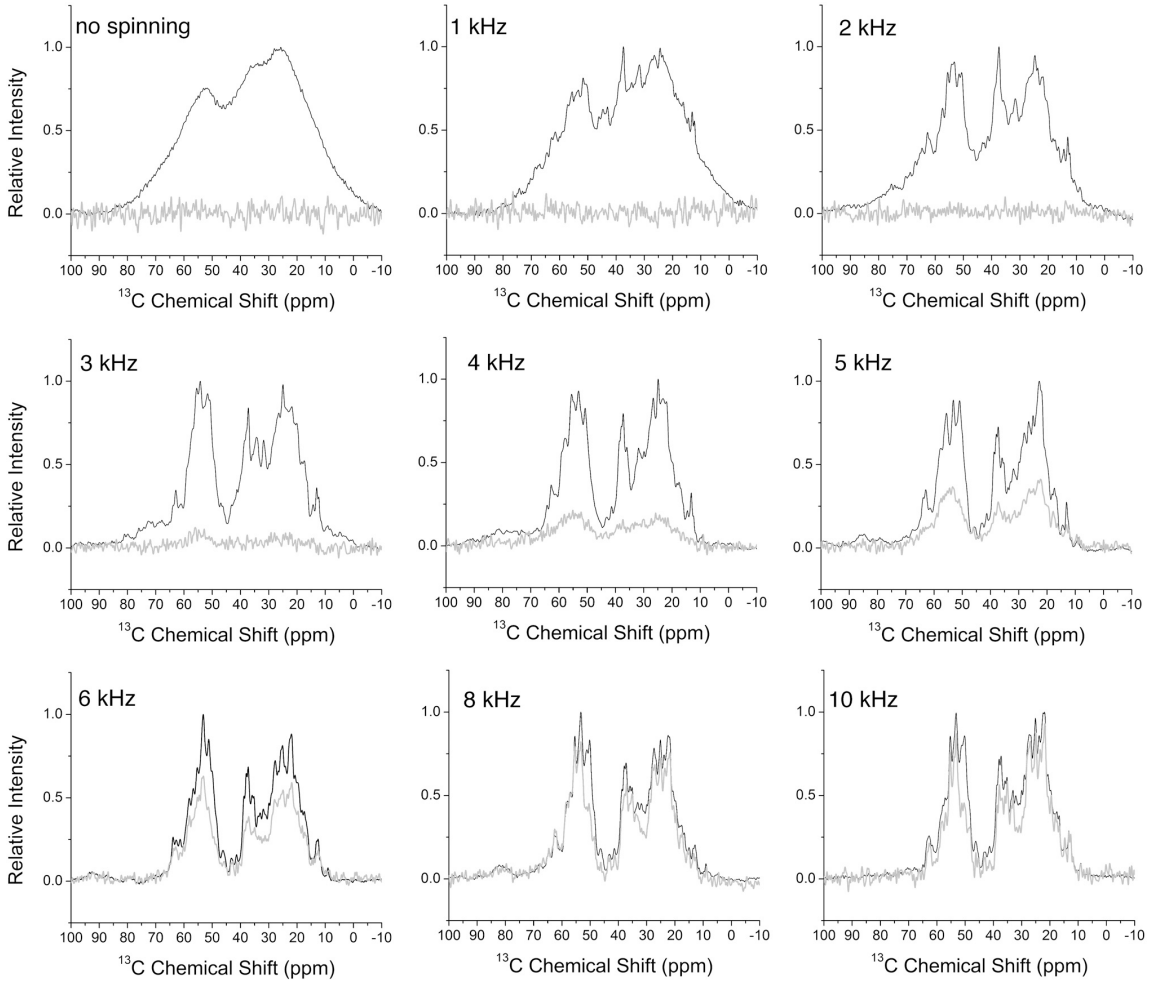


- Above about 45 kHz MAS, high-resolution spectra with ^{1}H detection become possible even for protonated proteins!
- New type of pulse sequences using **low RF power**
- Site-specific **relaxation** measurements for **dynamics** studies



Demers et al., Solid State Nucl Magn Reson 40, 101, 2011
Marchetti et al., Angew Chem Int Ed 51, 10756, 2012

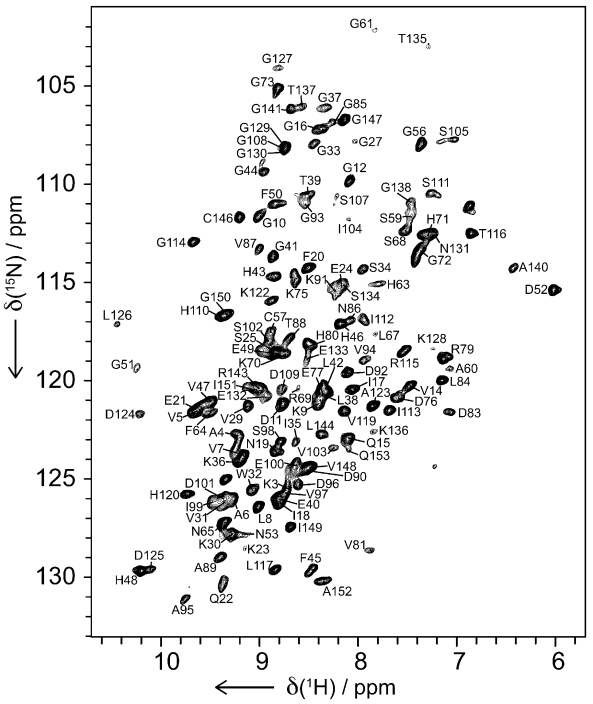
Sedimentation from solution at fast MAS



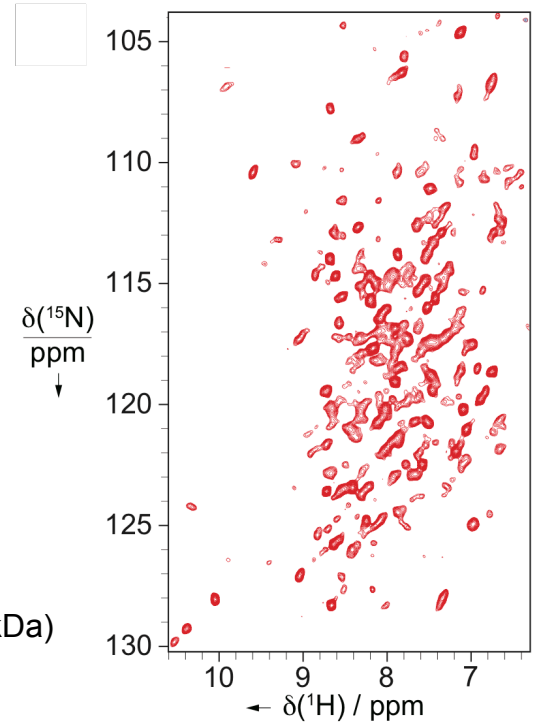
- Centrifugal forces during MAS can be one order of magnitude larger than in ultracentrifuges

⇒ Depending on molecular size and spinning speed, proteins can be **reversibly sedimented from solution in the MAS rotor** for solid-state NMR experiments!

Deuteration and fast MAS combined



SOD
(2 x 16 kDa)

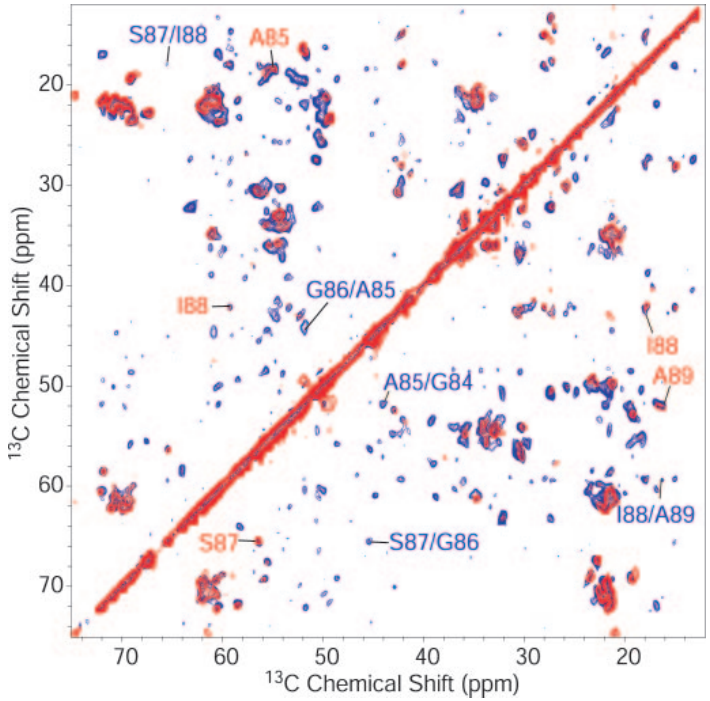


MeV capsids
(n x 43.5 kDa; MDa-size assembly!)

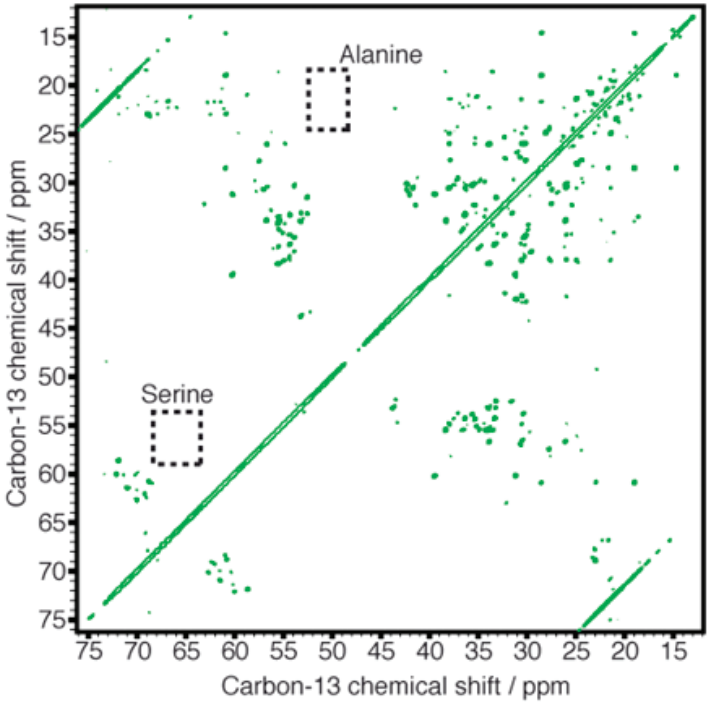
- Combine (per)deuteration and high MAS speeds for best results
- (However: missing signals due to lack of back-exchange; lack of sidechain protons important for structure determination!)
- As opposed to solution-state NMR, **linewidth** does **not depend** on **molecular size**, i.e. can in principle access arbitrarily large molecules!
- Small rotors − **small amounts of sample** required

Knight et al., Angew Chem Int Ed 50, 11697, 2011; Schneider et al., unpublished

...and of course, sample preparation!



Heise et al., PNAS 102, 15871, 2005



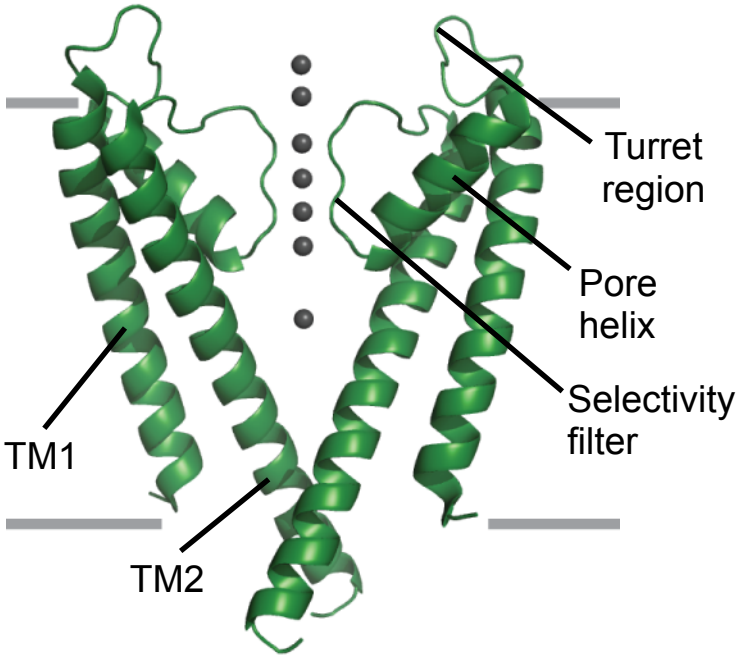
Loquet et al., JACS 132, 15164, 2010

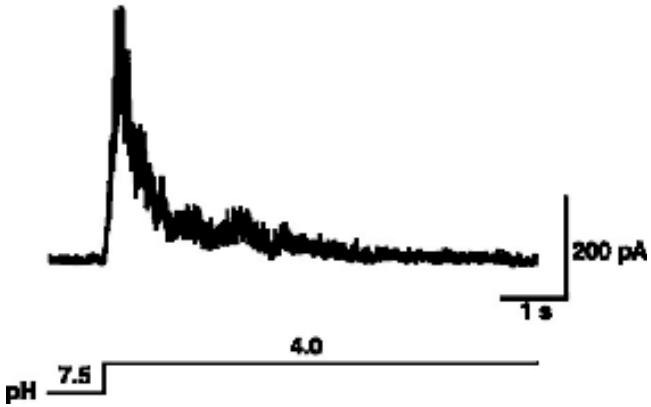
- Careful sample preparation to achieve optimal **local homogeneity** is essential!
- (and: use of **alternative isotope labeling schemes**)
- Has proven crucial especially for studies of amyloid fibrils
- However, this kind of optimization remains some kind of black magic...

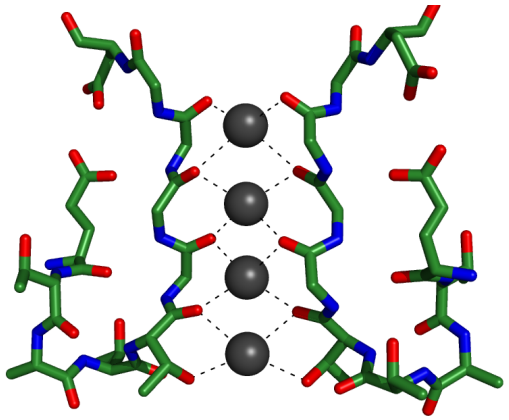
Applications I:
The potassium channel KcsA-Kv1.3

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



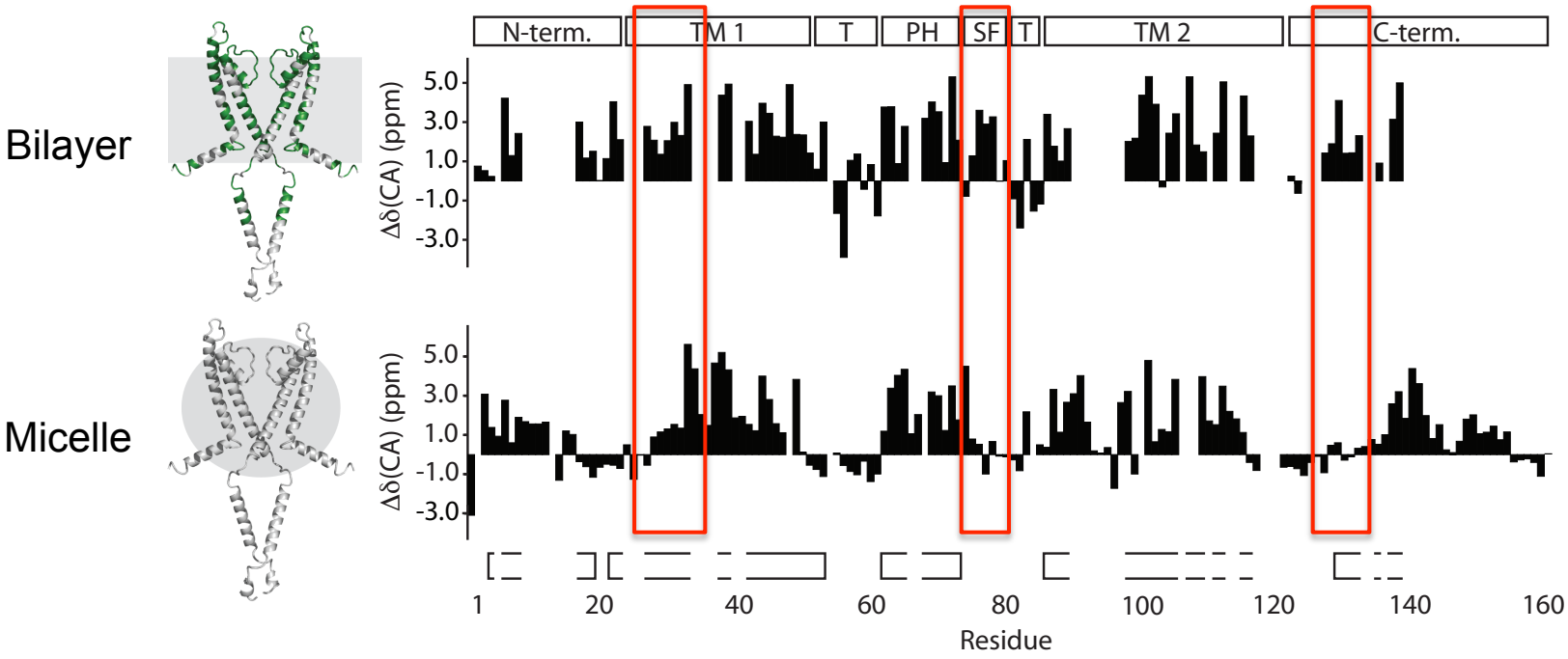




Ader, Schneider et al., Nat. Struct. Mol. Biol. 15, 605, 2008

Zhou et al., Nature 414, 43, 2001

Secondary structure in lipid bilayers

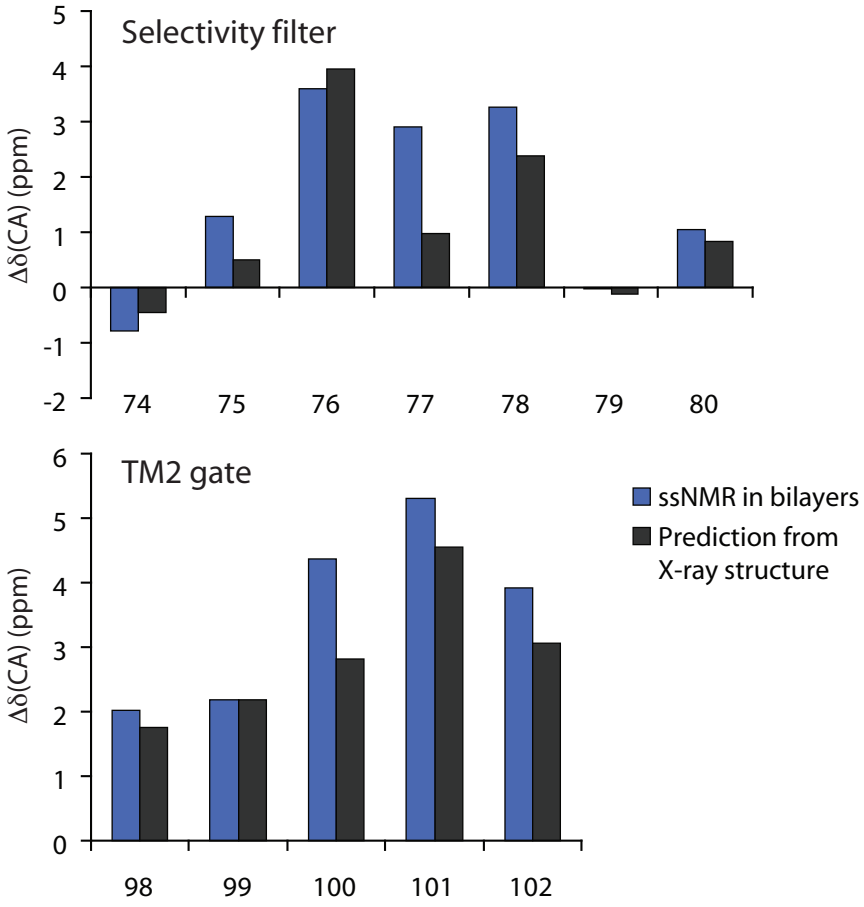


- Longer helices
- Different conformation in the selectivity filter

in lipid bilayers compared to micelles!

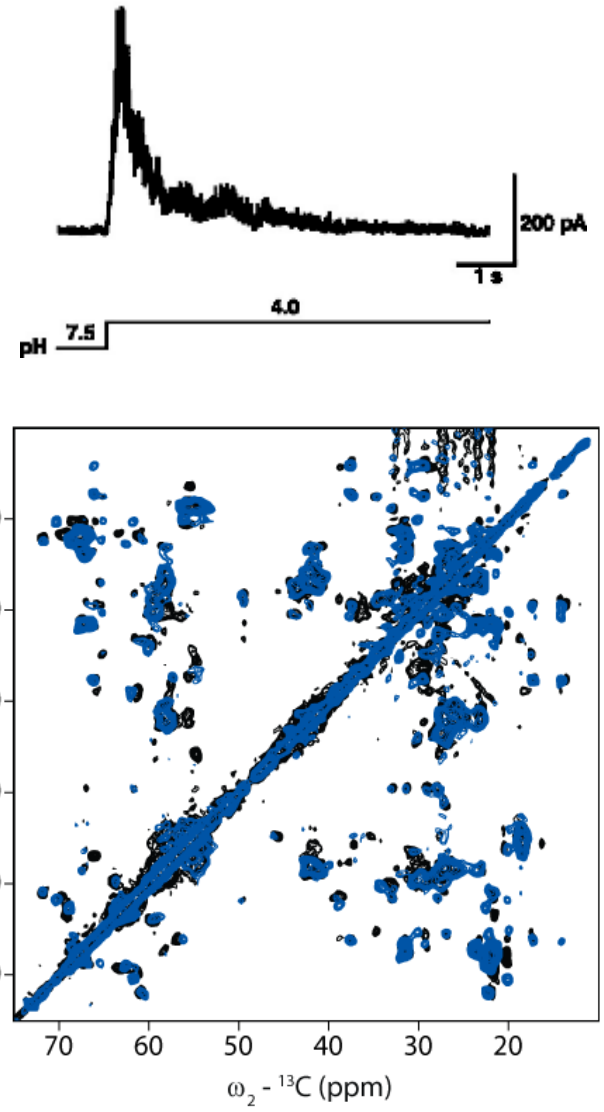
Schneider et al., JACS 130, 7427, 2008
Baker et al., Nat. Struct. Mol. Biol. 14, 1089, 2007

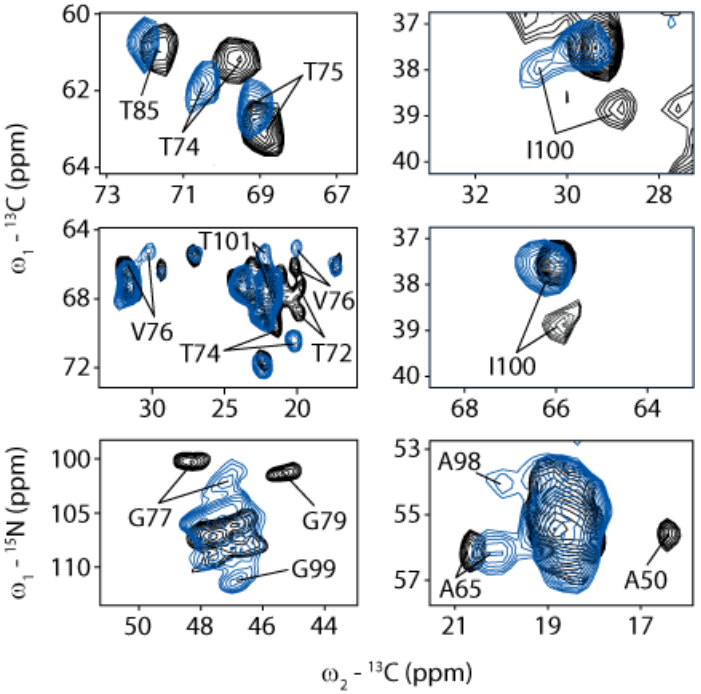
Solid-state NMR data correspond to crystal structure



- (Secondary) chemical shifts in selectivity filter and transmembrane helix 2 obtained on KcsA-Kv1.3 at neutral pH correspond to expectations from the KcsA crystal structure

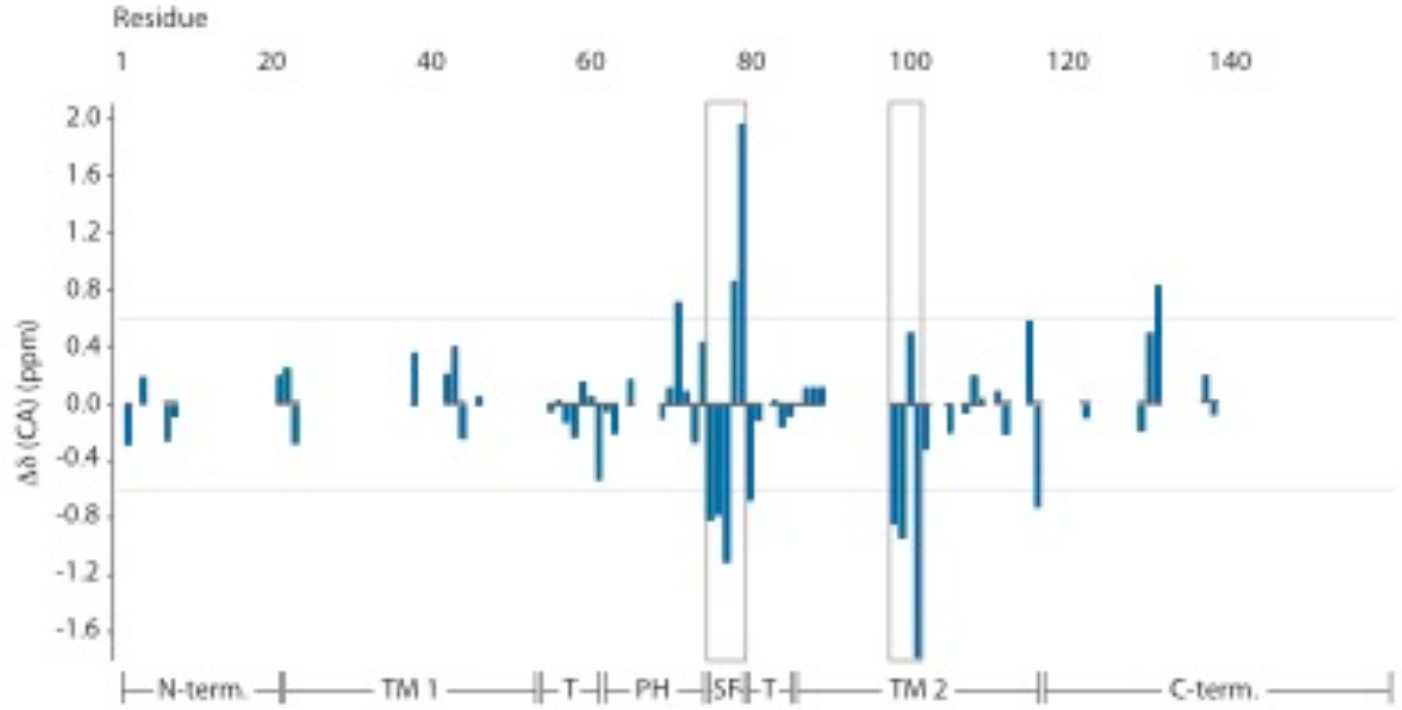
Transition to pH 4.0





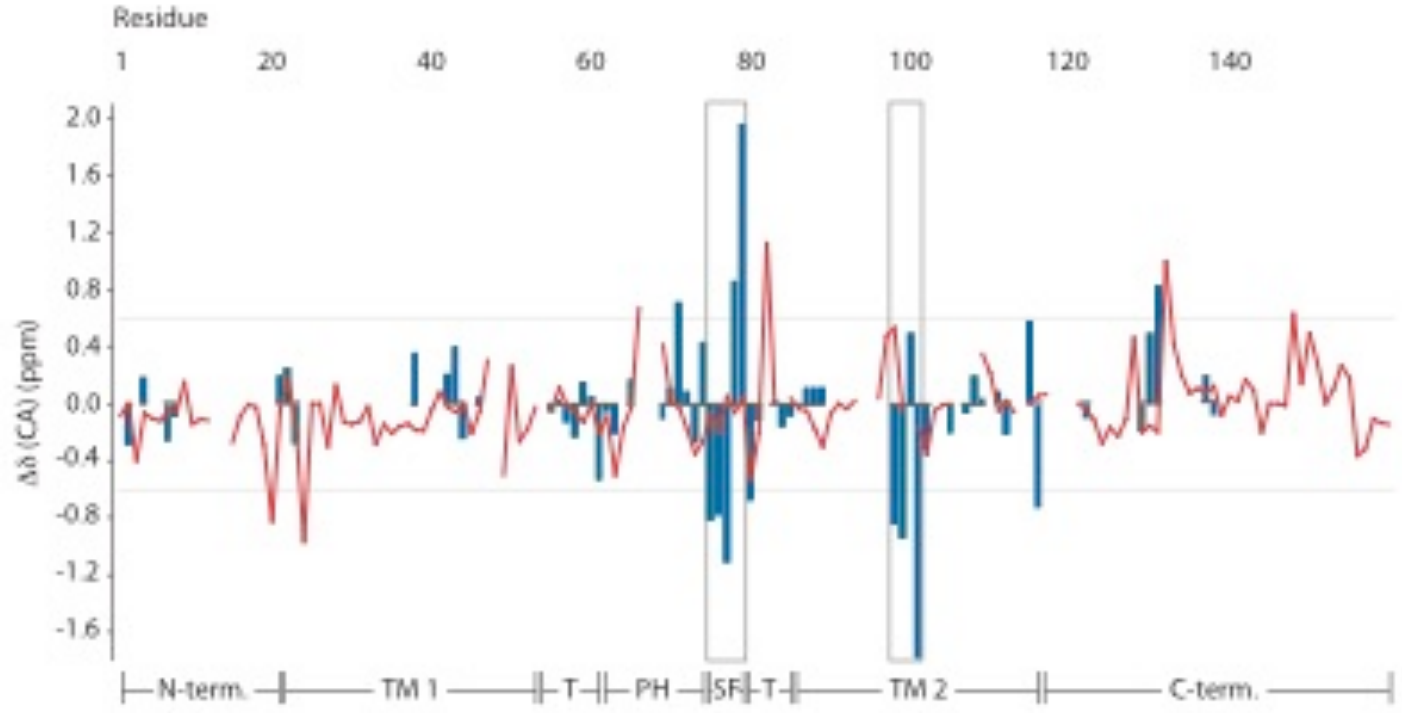
- Global structure preserved
- However, localized chemical shift changes are clearly observed

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

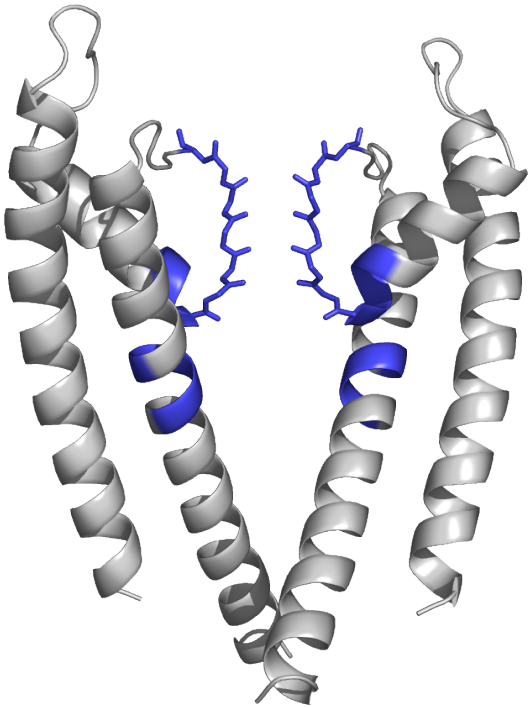
Chemical shift changes at pH 4.0



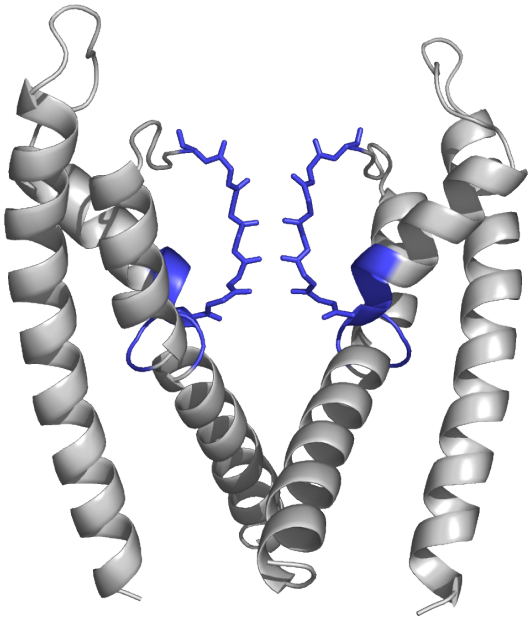
- Largest chemical shift changes localized to selectivity filter and region around Gly99 in TM2 known as “gating hinge” in other channels
- Very different results compared to micelles!

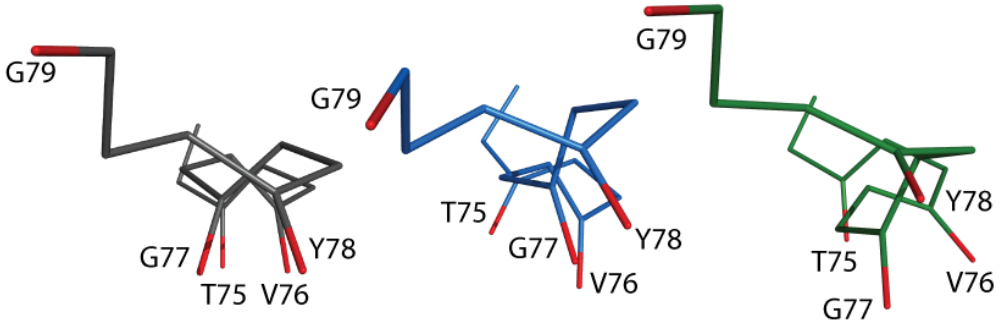
Ader, Schneider et al., Nat. Struct. Mol. Biol. 15, 605, 2008
Baker et al., Nat. Struct. Mol. Biol. 14, 1089, 2007

pH4 analysis: Results



pH4 analysis: Results





X-ray structure conductive

ssNMR model pH 4.0

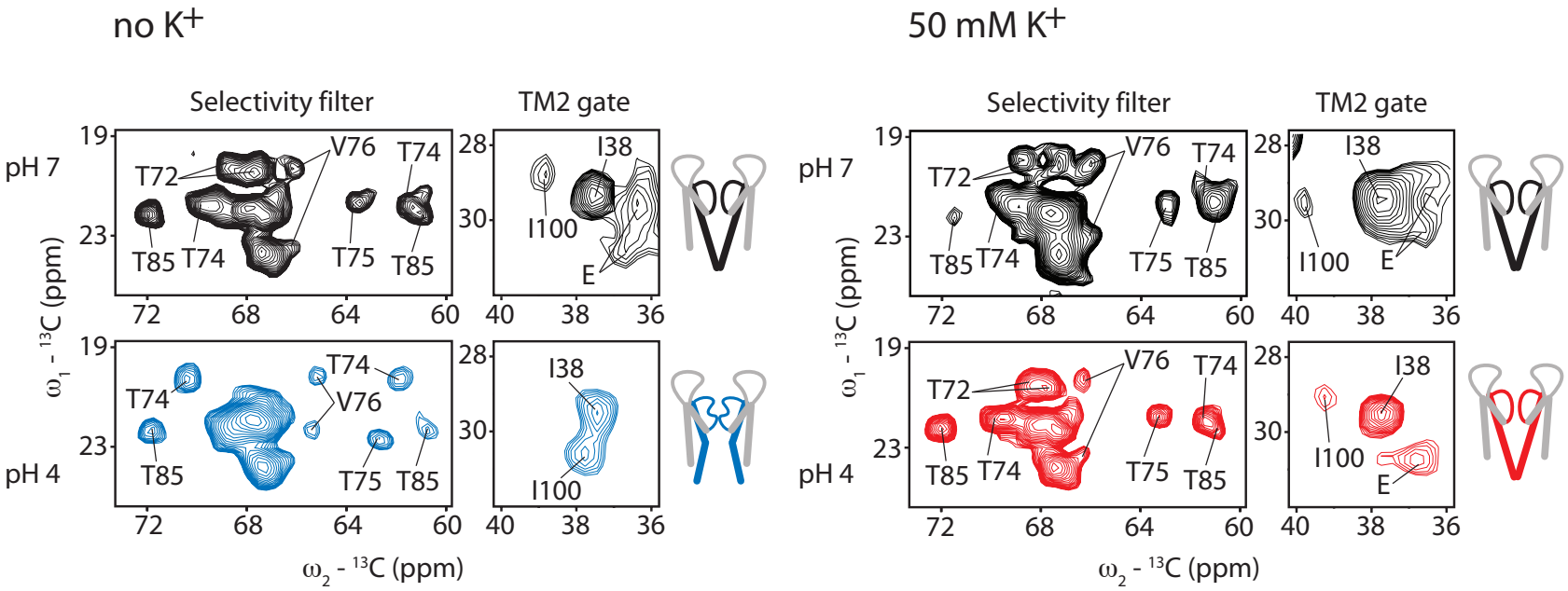
X-ray structure collapsed

- 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

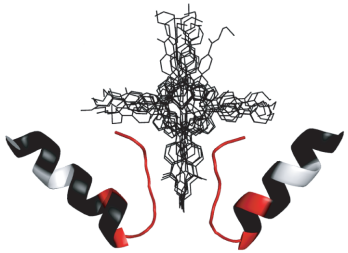
Open probability depends on K^+



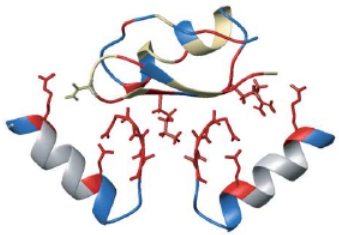
- Open probability at pH 4 **depends on K^+ concentration**
- In presence of K+, the conformation with **closed TM gate** and **conductive selectivity filter** dominates **even at pH 4**!

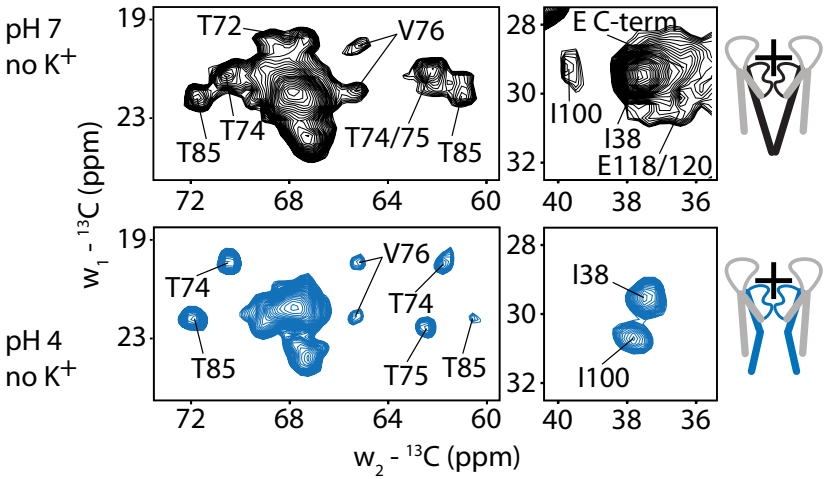
Selectivity filter and gate are coupled

Porphyrin

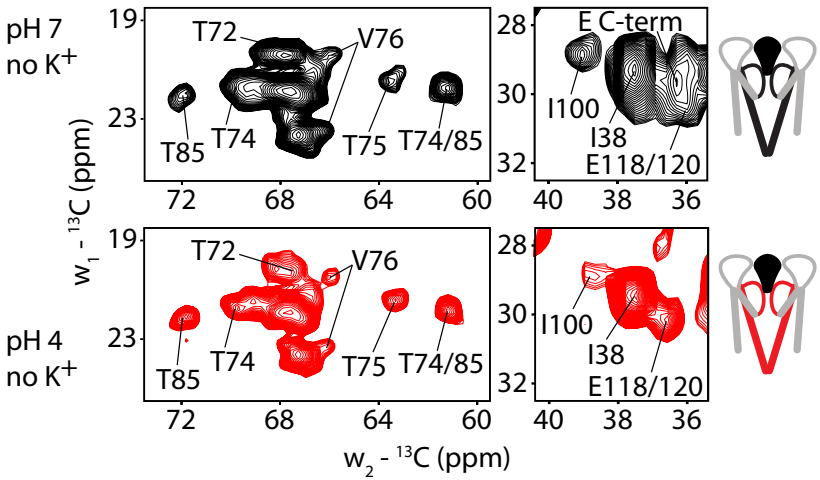


Kaliotoxin





open-inactivated



closed-conductive

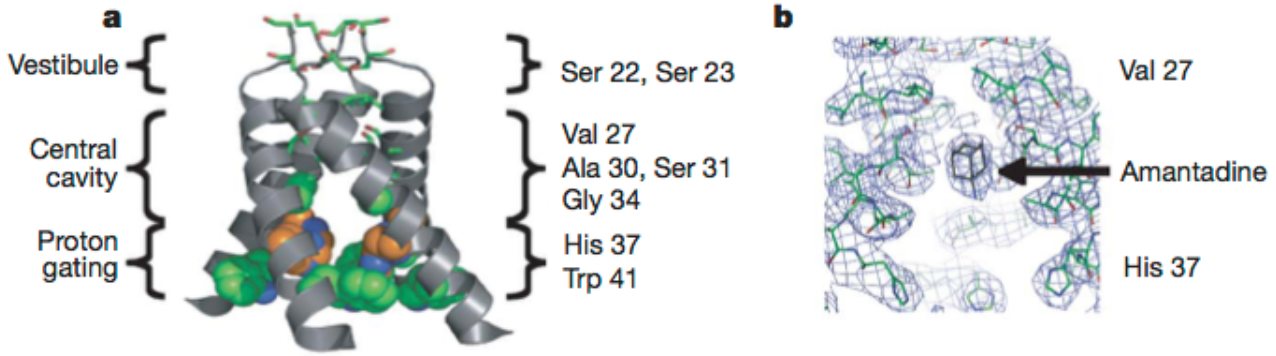
- Kaliotoxin binding **enforces conductive selectivity filter** even without K^+
- Conductive selectivity filter **keeps TM2 gate closed** even at pH 4

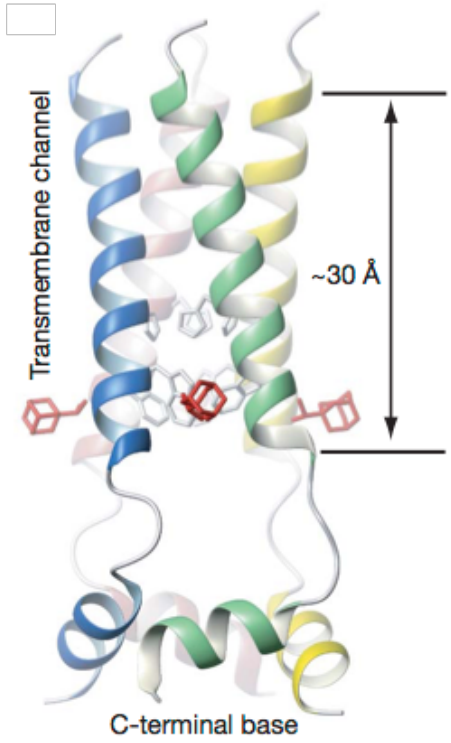
⇒ selectivity filter and TM2 gate are **coupled**!

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



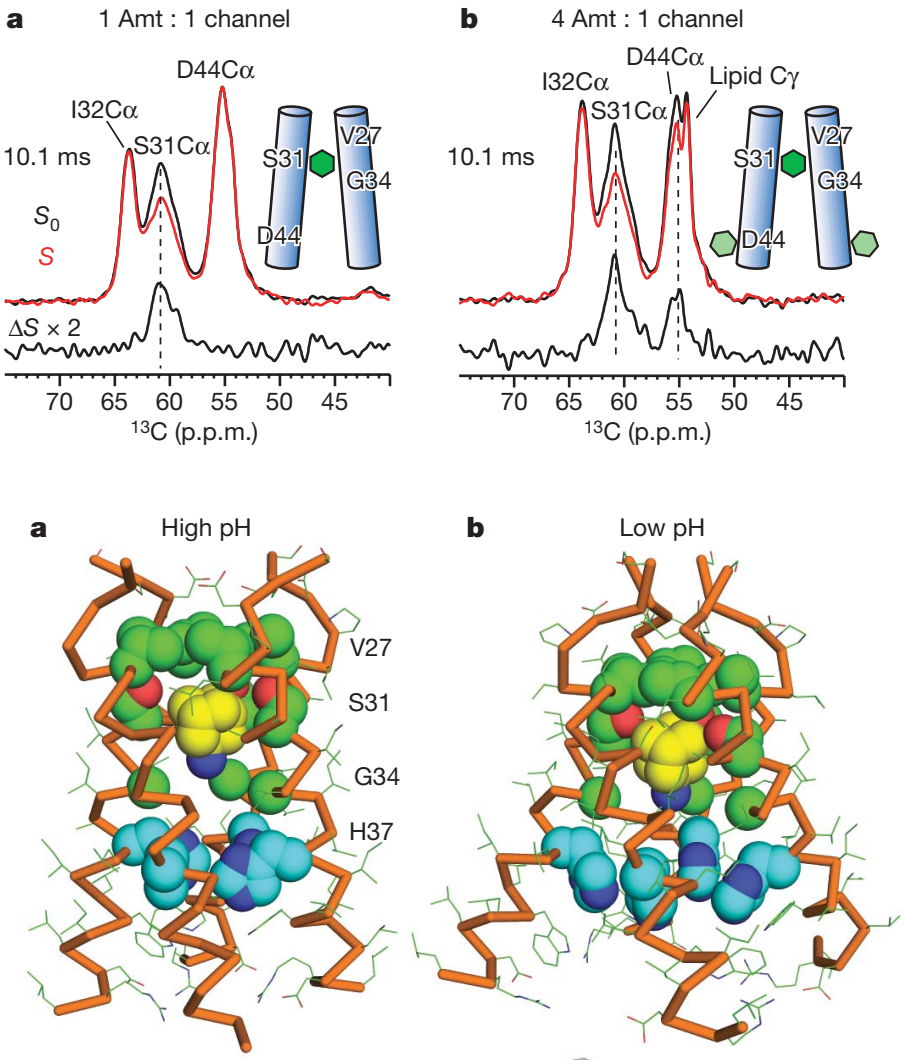


- pH-activated proton channel, involved in acidification and uncoating of virus particle as well as viral assembly
- Tetramer of four single transmembrane helices
- Targeted by adamantane-based antiviral drugs
- Crystal structure: one drug molecule binds in channel **lumen**
- Solution NMR structure: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



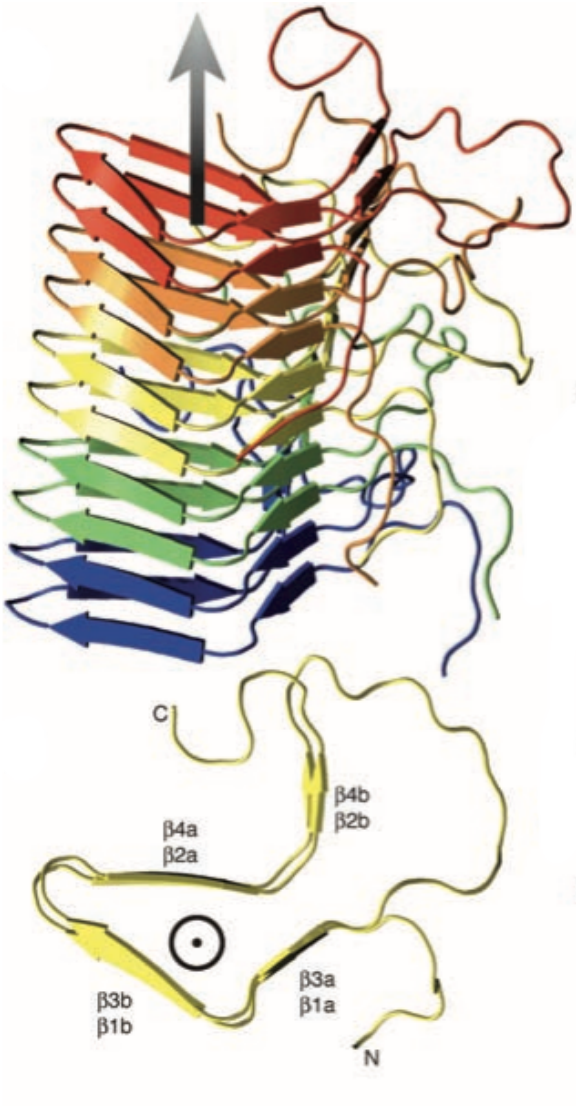
Solid-state NMR on M2 in lipid bilayers:

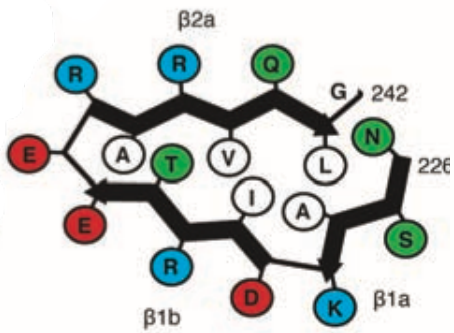
- channel selectively ^{13}C-labeled, amantadine deuterated
- Recouple ^{2}H-^{13}C interaction
- at **low drug:protein** ratio, find drug in channel **lumen**; when drug in **excess**, find it **also** on the **membrane** side!
- Structure calculation based on ssNMR restraints: **helix bundle tighter**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

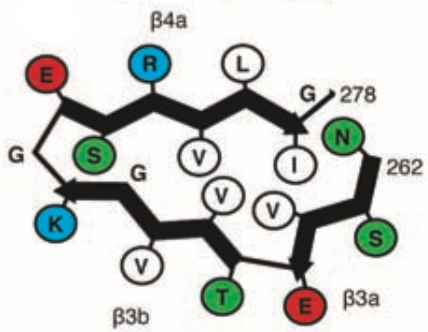
Cady et al., Nature 463, 689, 2010

Applications III:
Amyloid fibrils, supramolecular assemblies

The HET-s prion



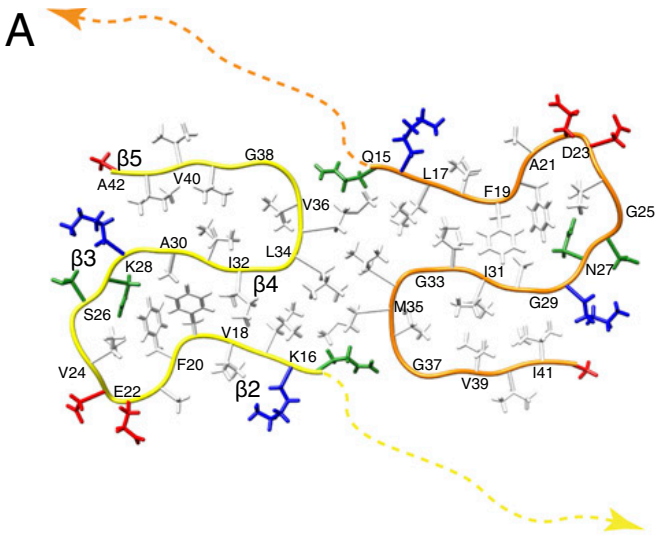


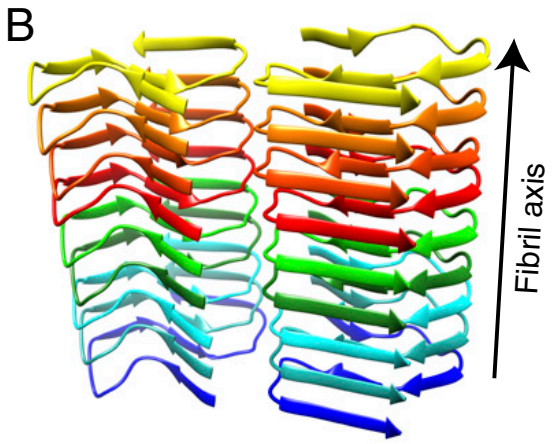


- Functional fungal prion involved in self/ nonself recognition
- Structure resolved by solid-state NMR as β-helical solenoid
- First structure of an amyloid fibril (apart from fibrils formed by short peptides)!
- Dry core formed by hydrophobic residues; stabilization by salt bridges and H bond ladders

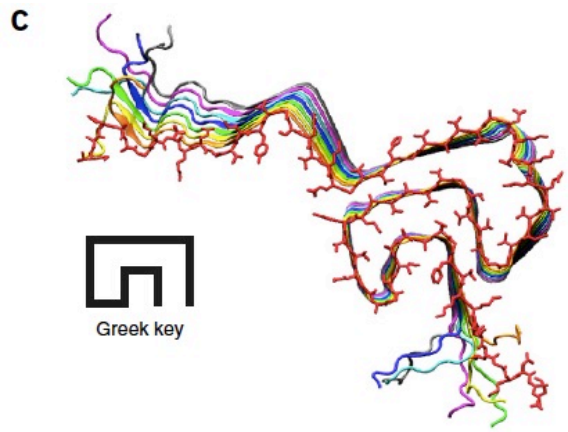
Wasmer et al., Science 319, 1523, 2008

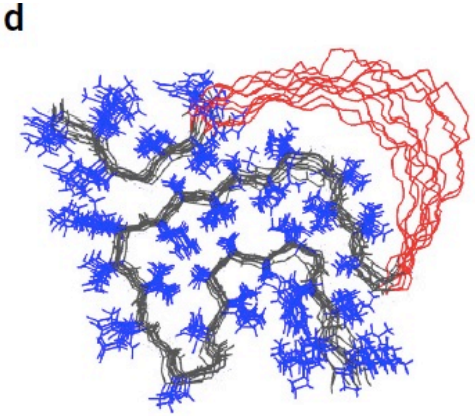
Amyloid-β and α-synuclein





- 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!

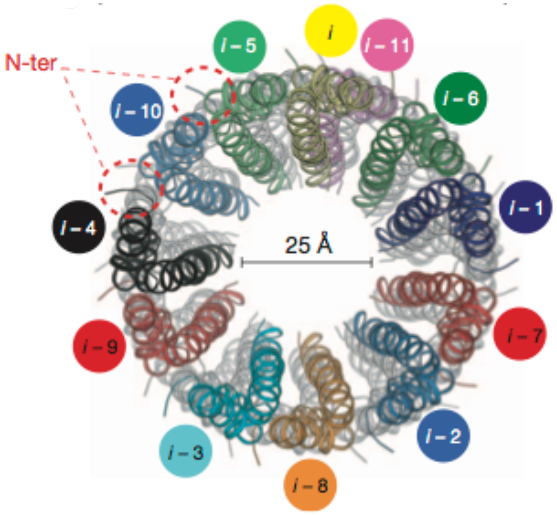


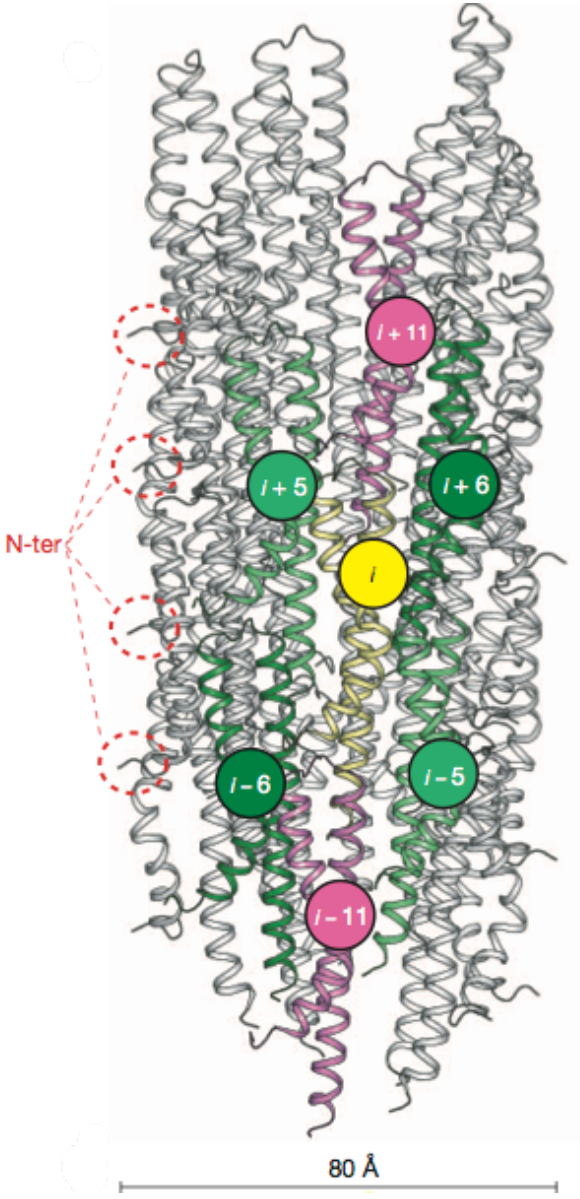


Wälti et al., PNAS 113, E4976, 2016
Tuttle et al., Nat Struct Mol Biol 23, 409, 2016

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!