



Structural Studies on Membrane Proteins by Solid-State NMR Spectroscopy

Daniel Huster

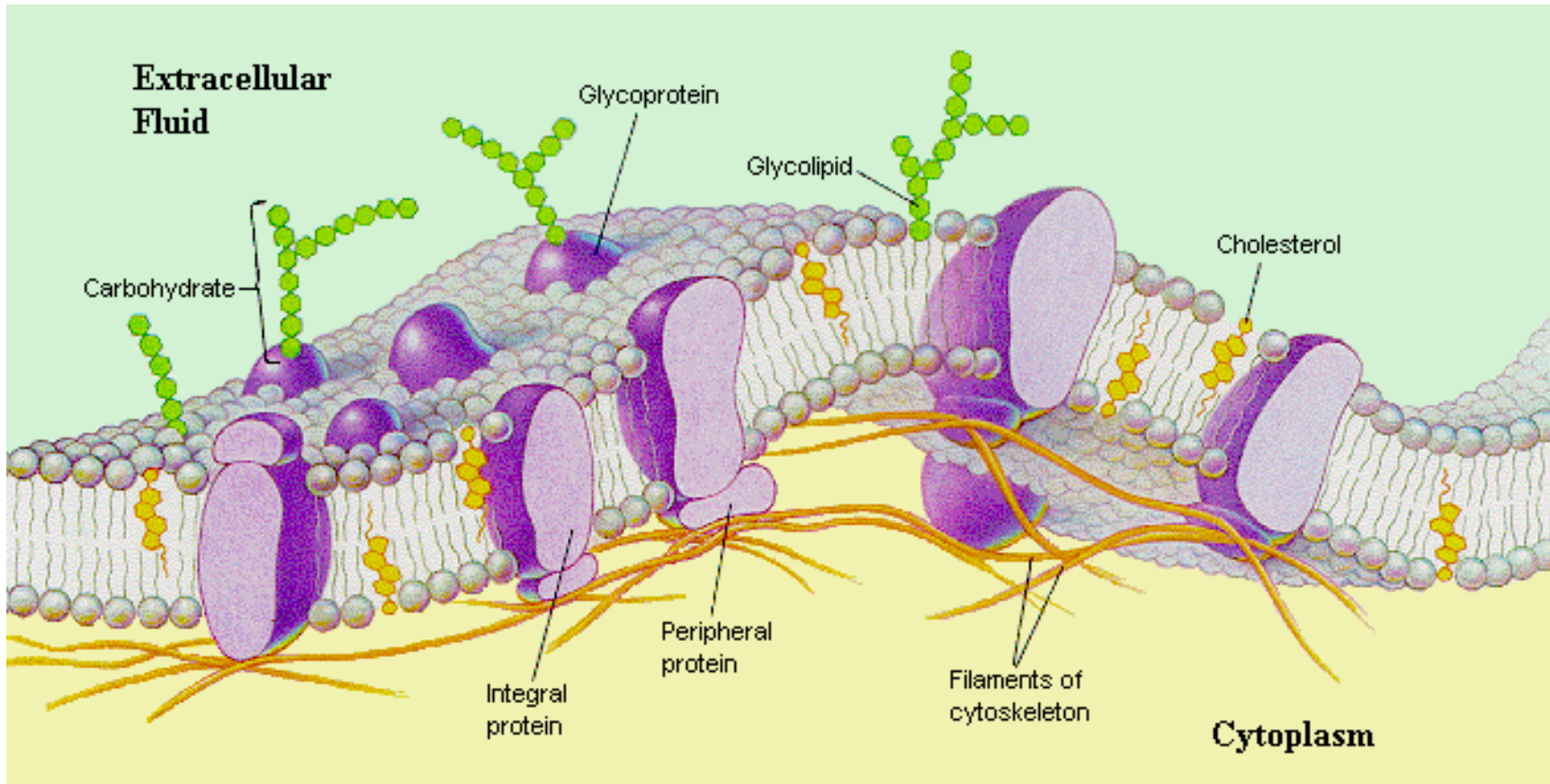
Junior Research Group

“Structural Biology of Membrane Proteins”

Institute of Biochemistry/Biotechnology

Martin-Luther-Universität Halle-Wittenberg

Membrane Proteins

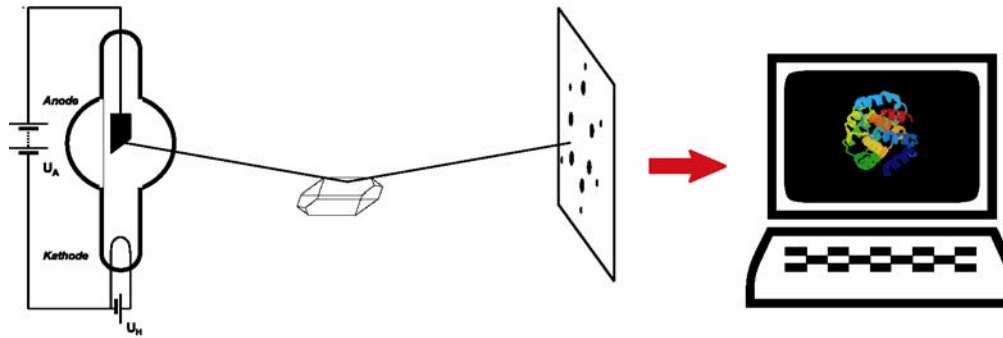


- About 30% of the human genes encode for membrane proteins.
- Membrane proteins play important roles as receptors and channels, in signal transduction and cell communication and many others.
- About 60% of all targets for newly developed drugs are membrane proteins.

Structure Determination of Membrane Proteins

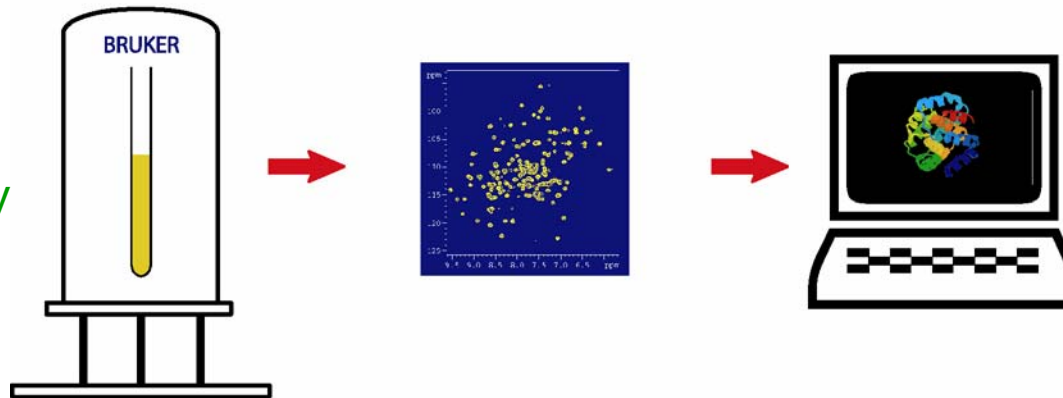
- By 2007 only ~100 structures of a few membrane proteins were known in strong contrast to ~35000 structures of soluble proteins
- Standard methods for structure determination fail for membrane proteins

X-Ray
Diffraktion



*Membrane proteins
do not easily form
highly ordered
crystals*

NMR-
Spektroskopie



*Membrane proteins
do not move
isotropically in
solution*

Why Solid-State NMR?



Review

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Membrane proteins: the 'Wild West' of structural biology

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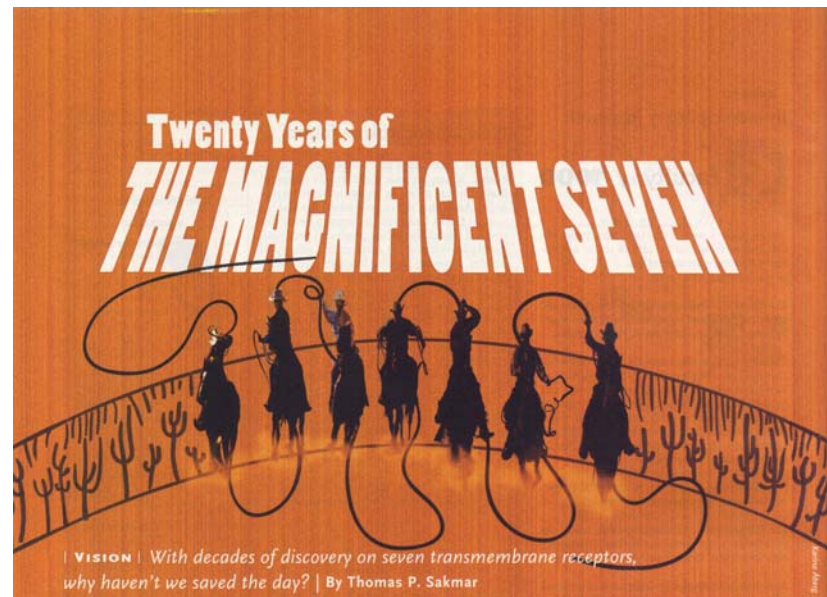
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³Wadsworth Center, New York State Department of Health, Albany, NY 12201-0509, USA and Department of Anesthesia, Brigham & Women's Hospital, Boston, MA 02115, USA

Concluding remarks

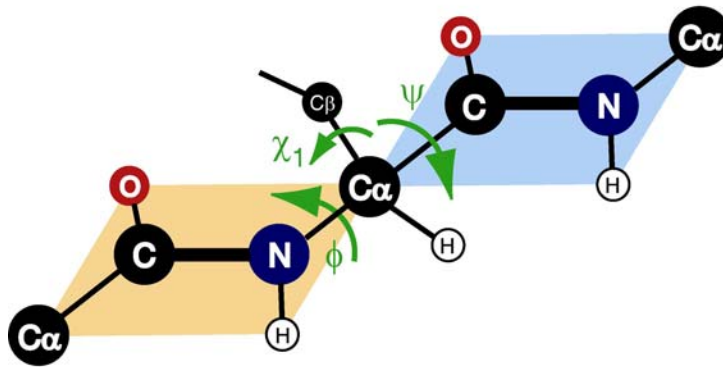
Membrane proteins remain a big challenge for the structural biologist. Because of experimental difficulties associated with their hydrophobic span, classical techniques such as X-ray crystallography and solution NMR, have been producing structures at a very slow pace. Therefore, alternative techniques are being sought. But because membrane proteins belong to a heterogeneous class of proteins, ranging from membrane-associated systems so big that can be studied with single-particle techniques, to single TM helices, this search has resulted in a plethora of new experimental approaches with various degrees of resolution. EM crystallography is clearly competing in resolution with X-ray diffraction, whereas single-particle and AFM techniques can detect conformational changes in large complexes. Progress using uniaxially oriented bilayers has been especially intense in the analysis of TM helix structure, particularly with the

lement the areas in which SDSL-EPR is weaker. Specifically, SSNMR has perhaps the brightest future because of the diversity of nuclei and nuclear spin interactions that can be observed, although use of complementary techniques will be crucial, especially taking into account the improvement in computational and prediction tools. We hope for progress in labeling strategies, hardware and development of experiments in the near future. However, we can expect that membrane proteins will still be beyond the reach of proteomics for the foreseeable future; some years left until the 'Wild West' is fully explored, then.

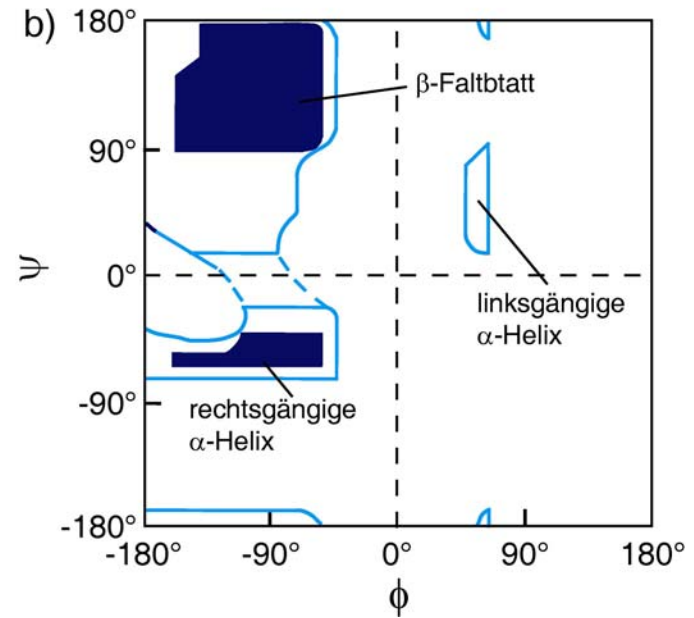


Structure Determination

a)

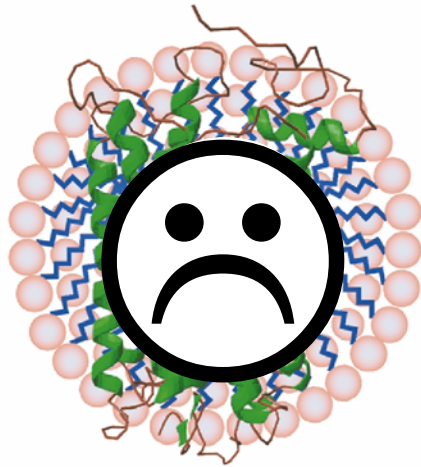


b)

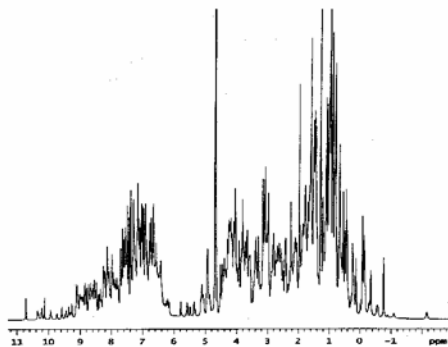


NMR-Methoden zur Untersuchung von Membranproteinen

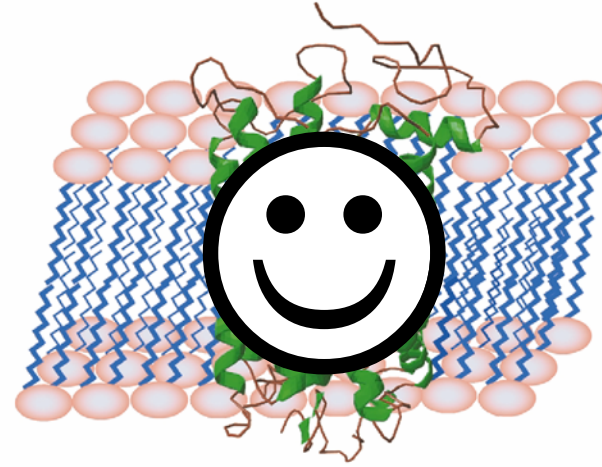
Lösungs-NMR



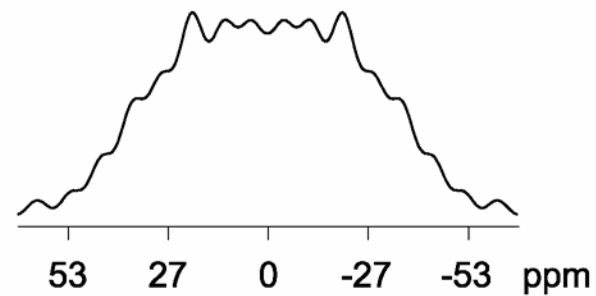
^1H NMR Spektrum



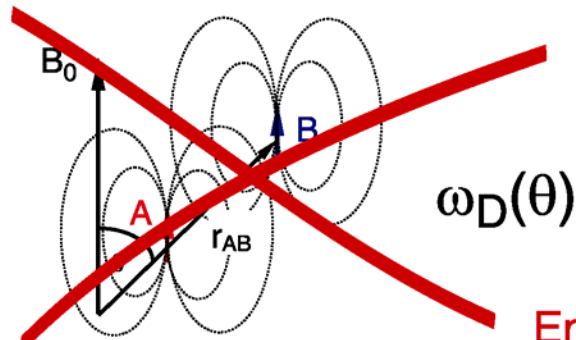
Festkörper-NMR



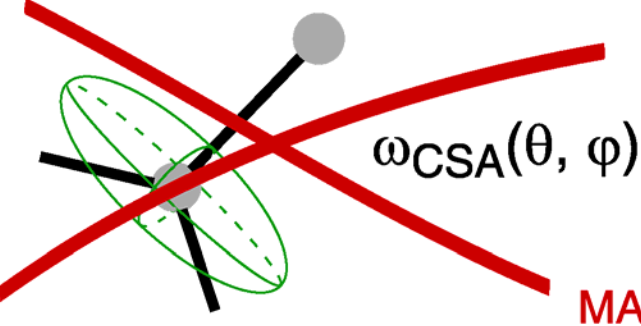
^1H NMR Spektrum



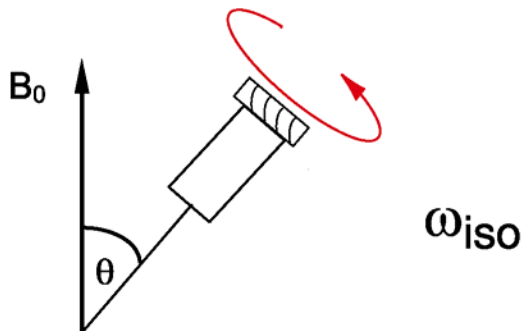
Magic Angle Spinning Techniques



Entkopplung

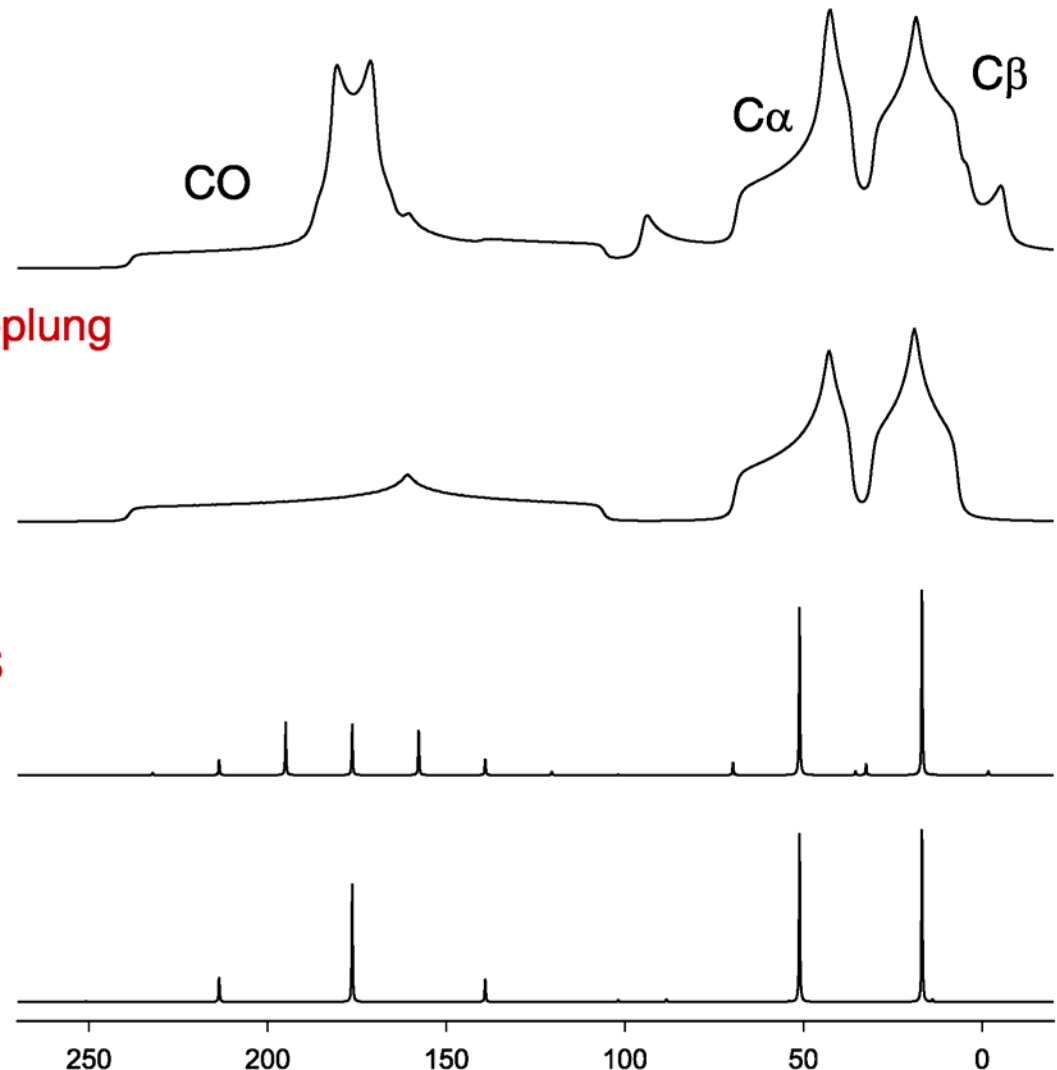


MAS



$$\frac{1}{2}(3\cos^2\theta - 1) = 0 \quad \theta = 54.7^\circ$$

Magic Angle Spinning (MAS)



^{13}C Chemische Verschiebung (ppm)

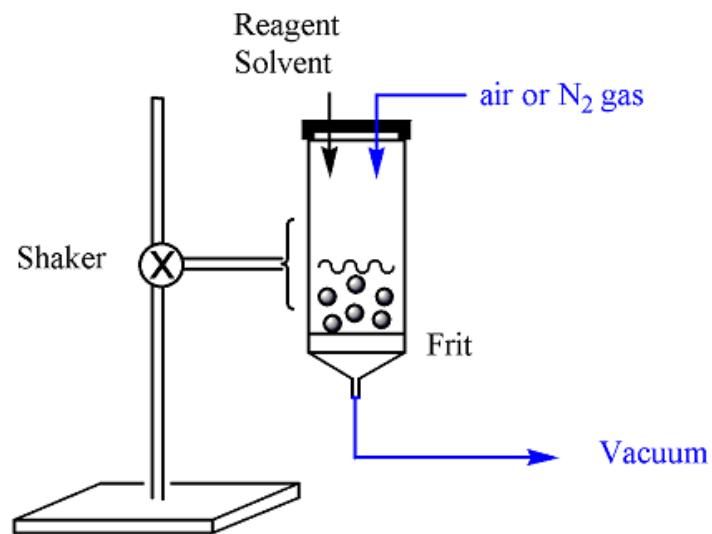
→ Verbesserung der spektralen Auflösung

Isotopic Labeling

➔ NMR active isotopes of spin $\frac{1}{2}$ that can be detected by solid state NMR methods are rare (^{13}C , ^{15}N), which calls for specific enrichment

1. Chemical synthesis of peptides at the solid phase

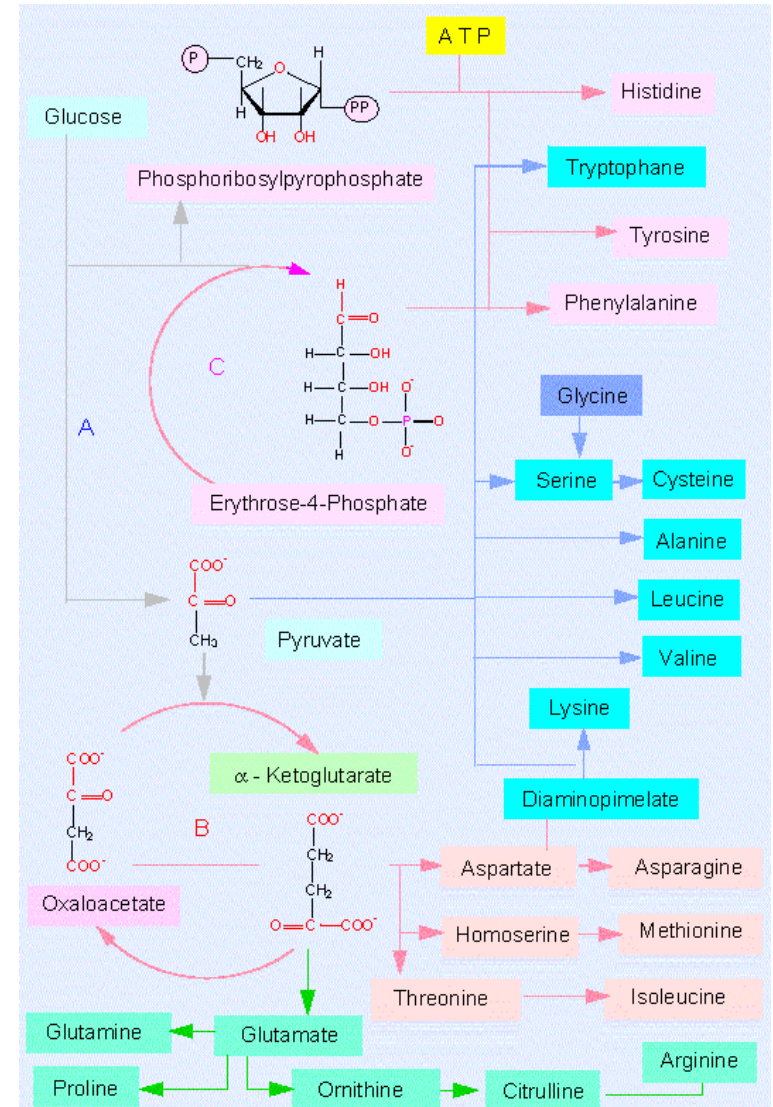
- ☺ Almost any given labeling scheme can be done, provided specifically labeled amino acids are available
- ☺ No scrambling of the label
- ☹ Expensive!!!
- ☹ Peptides should be smaller than ~50 residues



Isotopic Labeling

2. Biosynthesis with uniformly ^{13}C Glucose/ ^{15}N Ammoniumchloride

- ☺ Relatively cheap
- ☺ Works in principle for all recombinant proteins
- ☹ Many membrane proteins do not express well, are toxic for bacteria, or aggregate
- ☹ Poor resolution in solid-state NMR spectra
- ☹ ^{13}C spin diffusion
- ☹ Dipolar truncation



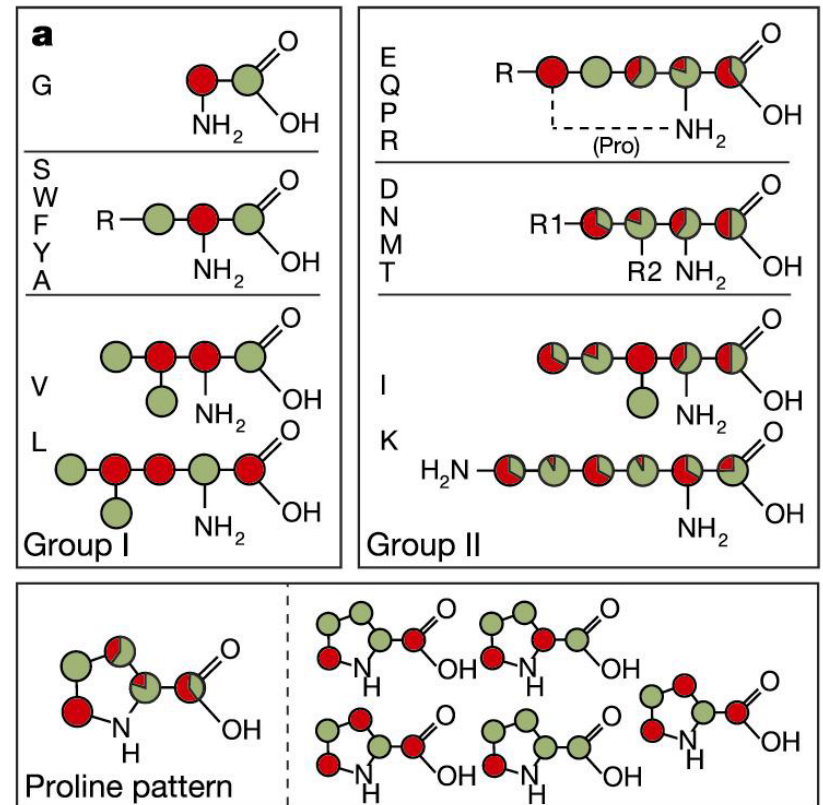
Isotopic Labeling

3. Biosynthesis with specifically labeled ^{13}C Glucose or ^{13}C Glycerol

- ☺ Moderate cost
- ☺ Works in principle for all recombinant proteins
- ☺ Better resolution in solid-state NMR spectra
- ☺ No ^{13}C spin diffusion
- ☺ No dipolar truncation
- ☹ Many membrane proteins do not express well, are toxic for bacteria, or aggregate

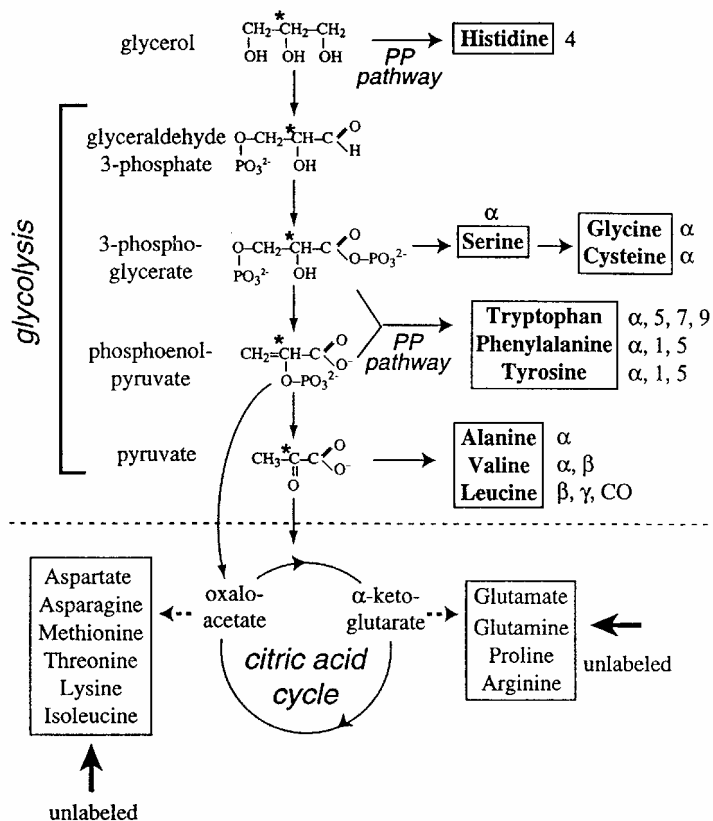
2- ^{13}C glycerol

1,3 ^{13}C glycerol



Specific and Extensive $^{13}\text{C}/^{15}\text{N}$ Labeling

- Ten-amino acid selective & extensive labeling (TEASE)



PET-15b vector

M**GSSHHHHHHH** **SSGLVPRGSH** MLEDP

451 INF**TTEFL** K**SVSEKYGAK** AEQLAREMAG
 481 Q**AKG**KKIRNV EE**AL**KTYEKY RAD**INKKINA**
 511 KDR**AAIAAAL** E**SVKLSDISS** NLNRF**SRGLG**
 541 **YAGK**F**TS**LAD W**ITE**FG**KAVR** TENWR**PL**LF**VK**
 571 TETII**AG**NAA TAL**VAL**VFSI LTGS**ALGIIG**
 601 YGLLM**AV**TGA LID**ESL**VEKA NK**FW**GI

Labeled amino acids:

Ala: 25 α

Phe: 8 α, 1, 5

Leu: 19 β, γ, CO

His: 7 4

Gly: 17 α

Tyr: 5 α, 1, 5

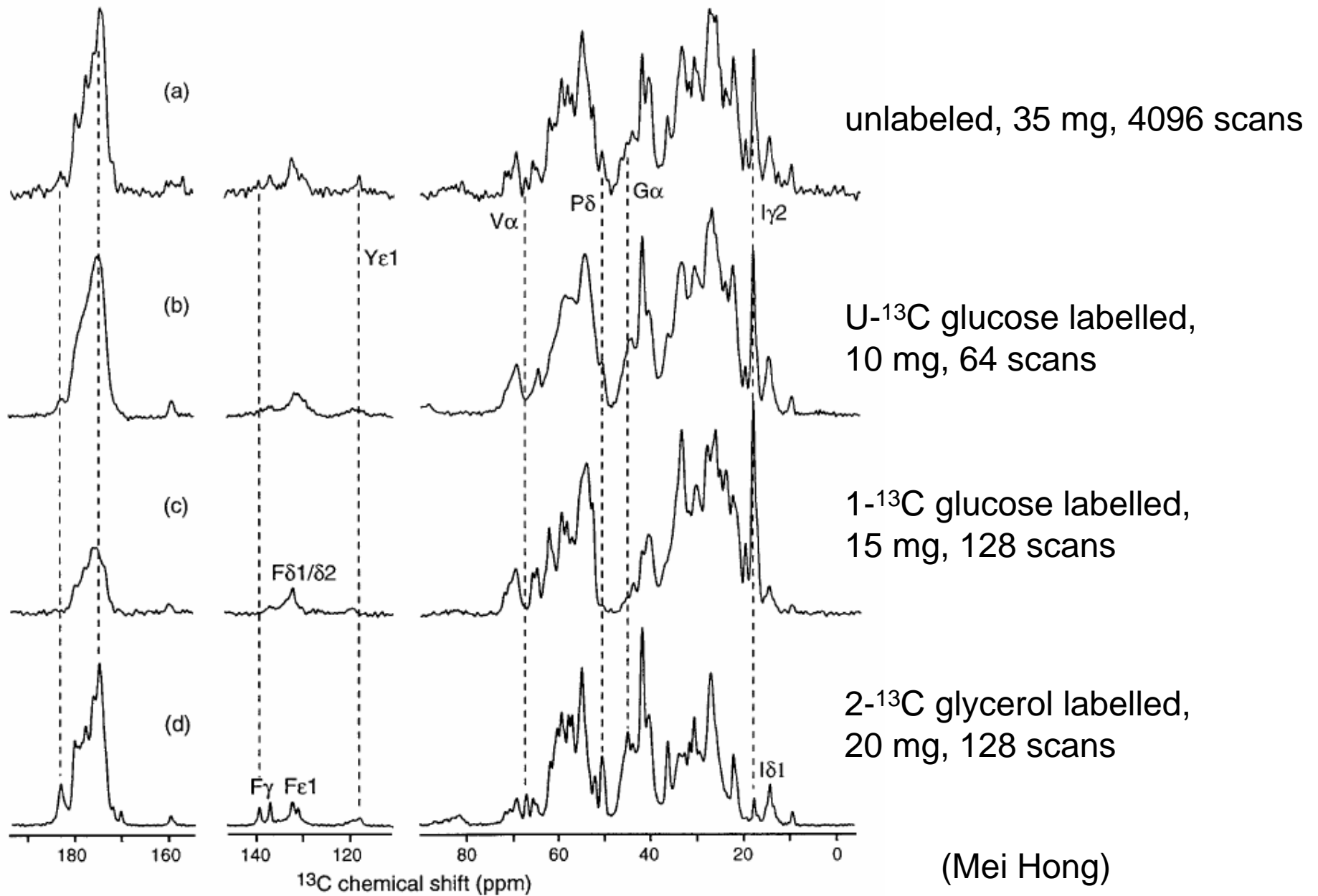
Ser: 16 α

Trp: 3 α, 5, 7, 9

Val: 10 α, β

Total: 110 out of 198 for Colicin Ia

¹³C Labeling on ubiquitin



Magic Angle Spinning Techniques

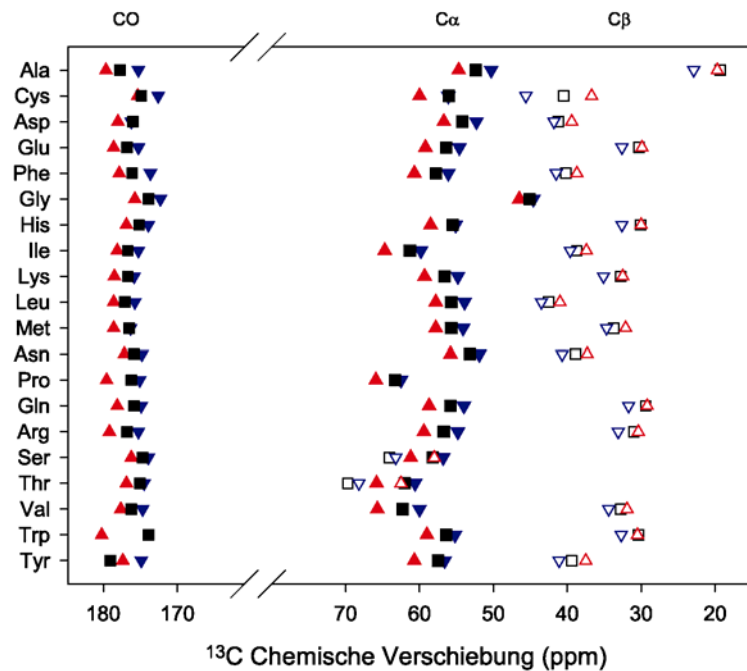
1st Isotropic Chemical Shifts

Structural Information from Isotropic Chemical Shifts

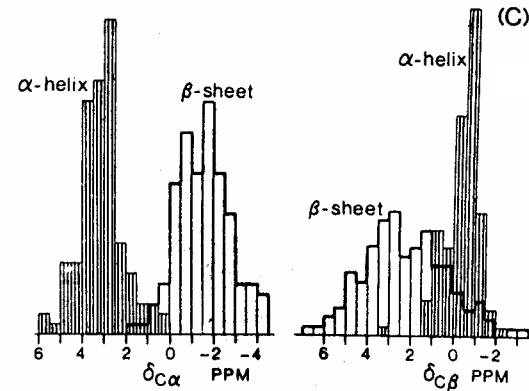
- ^{13}C $\text{C}\alpha$ chemical Shifts show a strong correlation with the backbone conformation of a protein

α -helix: $\text{C}\alpha \uparrow \text{C}\beta \downarrow$

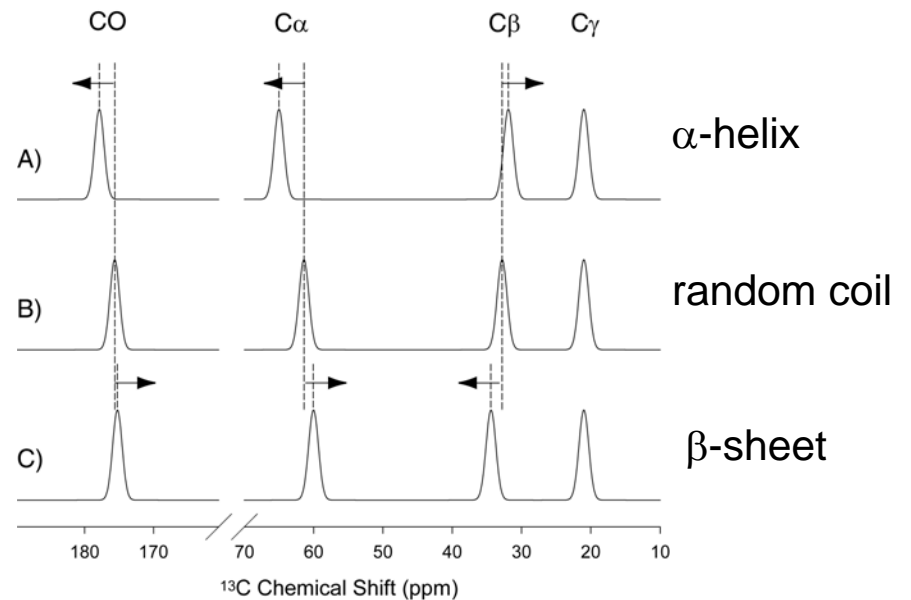
β -sheet: $\text{C}\alpha \downarrow \text{C}\beta \uparrow$



random coil
 α -helix
 β -sheet

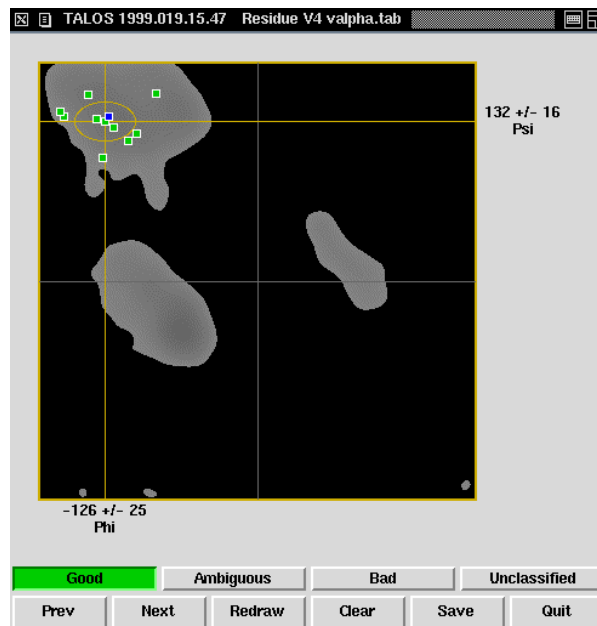
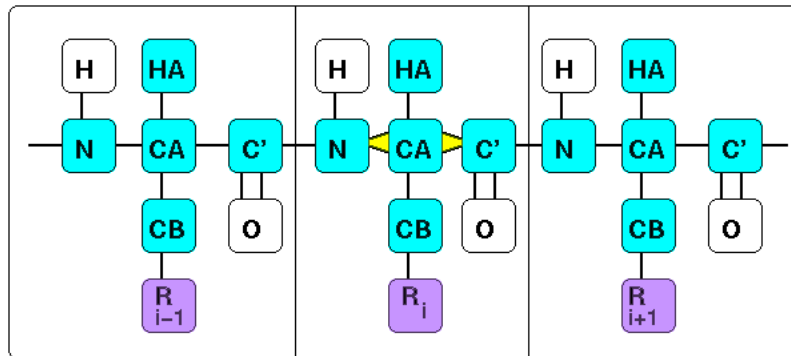


Spera & Bax, 1991



TALOS – Torsion Angle Prediction

Torsion **A**nge **L**ikelihood **O**btained from **S**hift and sequence similarity



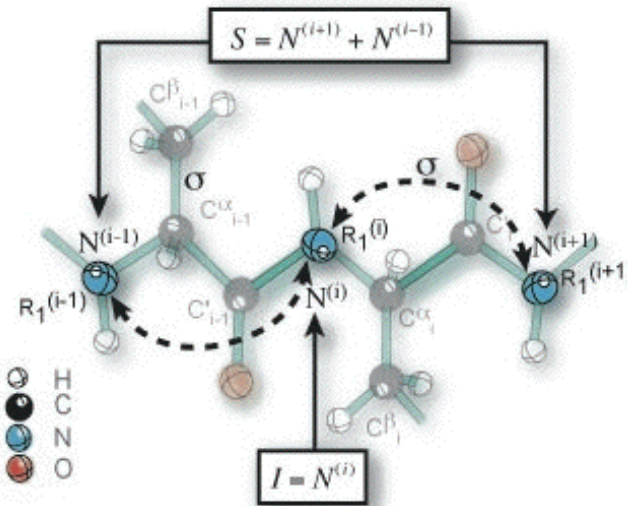
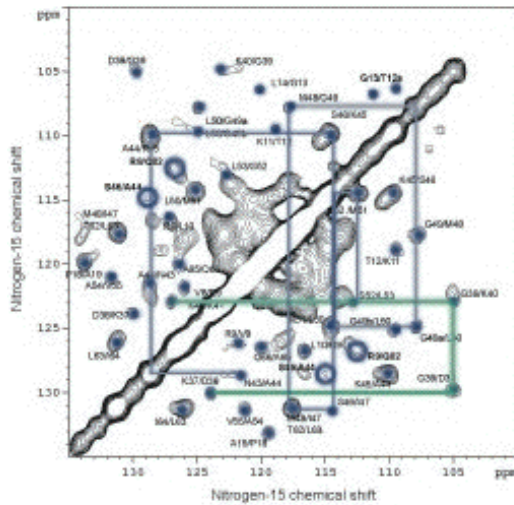
Conclusions/Remarks

- ☺ Isotropic chemical shifts are useful tools to determine secondary structure elements and even full structural models of membrane proteins
- ☺ Secondary chemical shifts are solely determined by the proteins secondary structure, there are no differences between chemical shifts in solution and in the solid state (Luca et al., 2001)
- ☺ With the lack of internal standards in solid-state NMR, chemical shift differences between $C\alpha$ and $C\beta$ are independent of referencing!
- ☺ Experiments can be carried out at any temperature
- ☹ For the determination of highly resolved NMR structures, additional constraints are necessary! Isotropic chemical shifts are not unique

Magic Angle Spinning Techniques

2nd Dipolar Coupling Measurements
by Proton Driven Spin Diffusion

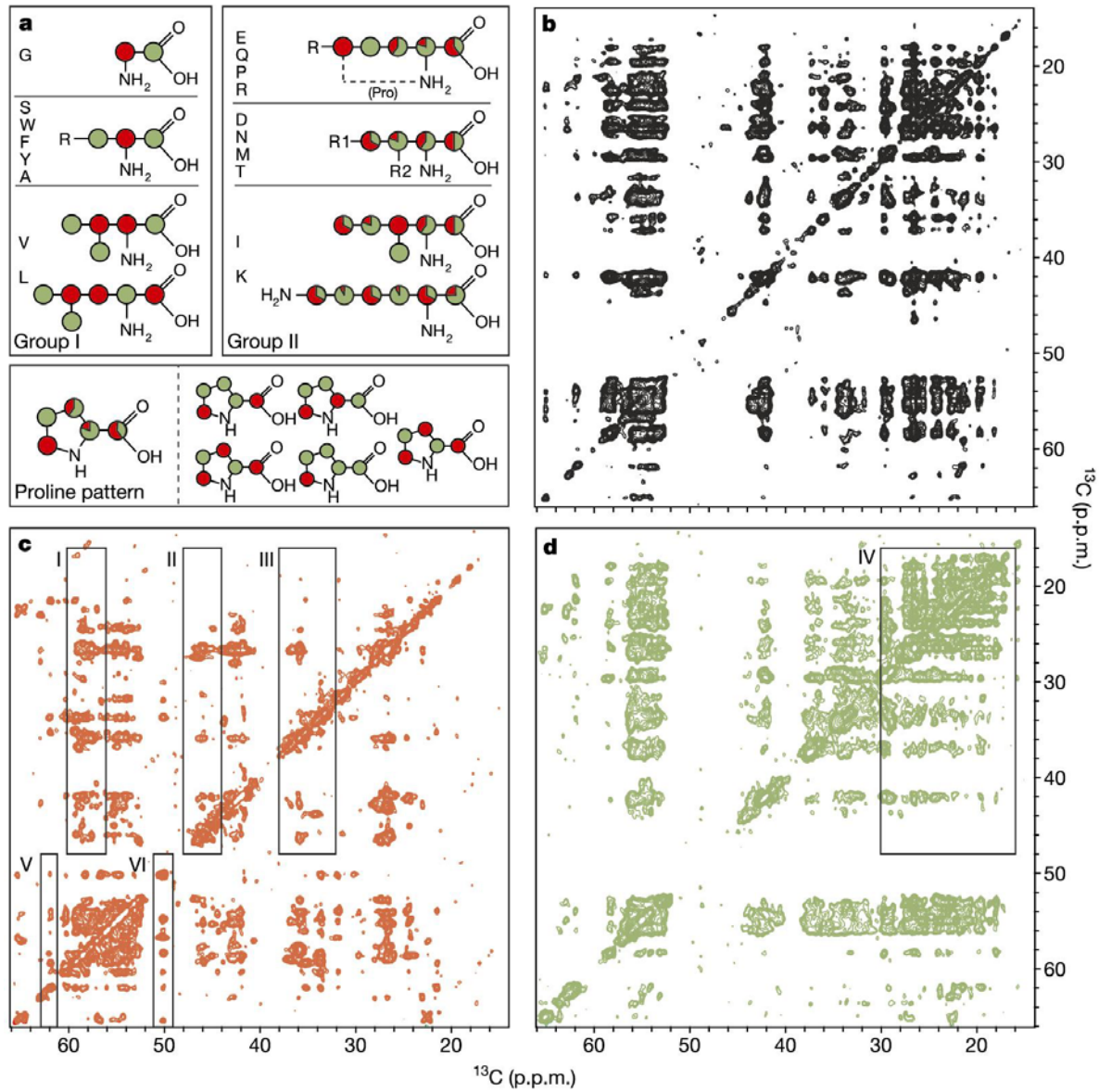
Proton Driven Spin Diffusion



Crosspeaks are due to dipolar coupling $D \sim r^{-3}$

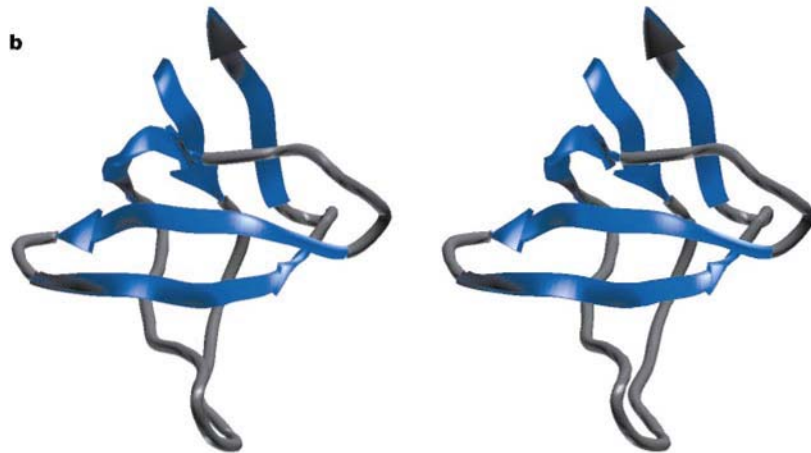
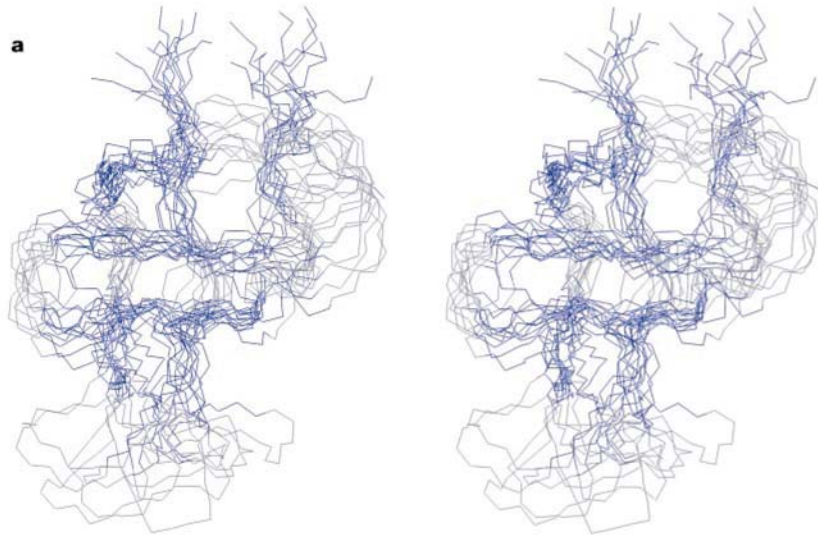
- ➔ Strong cross peak: 2.5 – 4.5 Å
- ➔ Medium cross peak: 2.5 – 5.5 Å
- ➔ Weak cross peak: 2.5 – 6.5 Å
- ➔ Very weak cross peak: 2.5 – 7.5 Å

PDSP



(Hartmut Oschkinat)

PDSP



- ☺ High precision, redundancy
- ☺ Simple experiment, low rf heating
- ☺ good sensitivity
- ☺ Peptide structure is provided
- ☹ number of ^{13}C labelled residues is resolution dependent

(Hartmut Oschkinat)

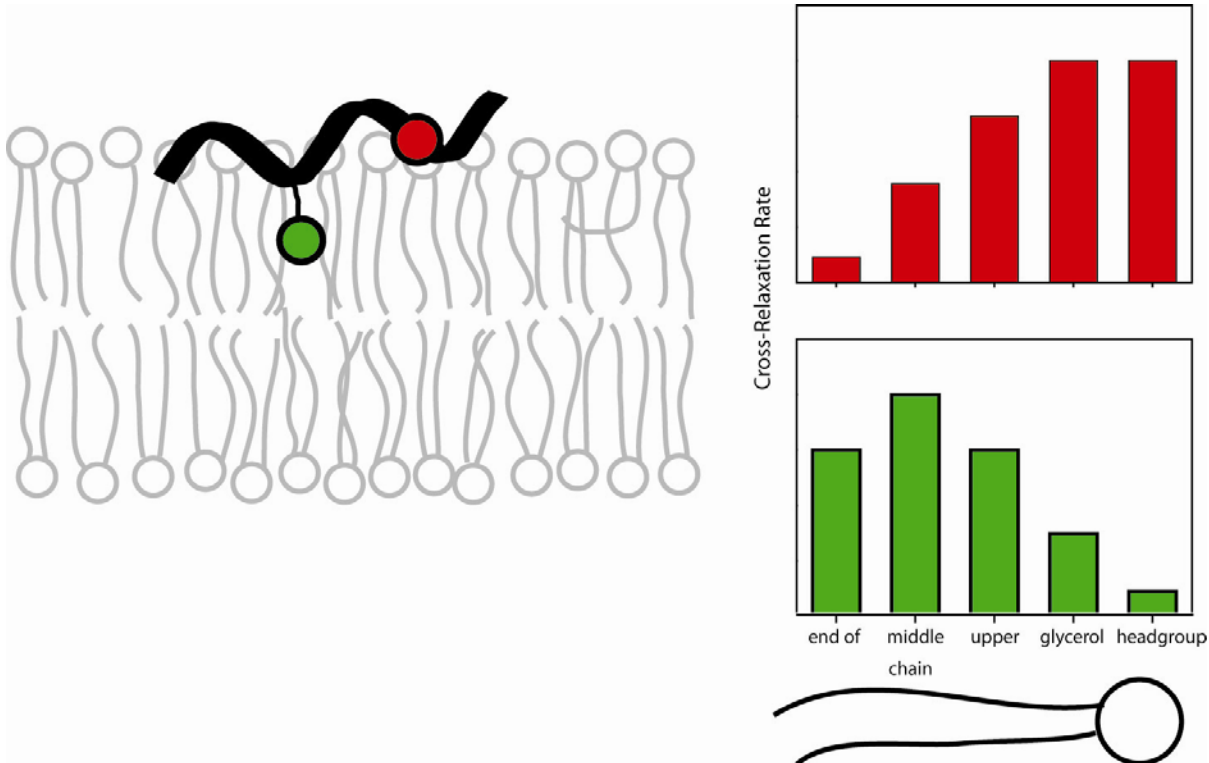
Magic Angle Spinning Techniques

3th Nuclear Overhauser Enhancement

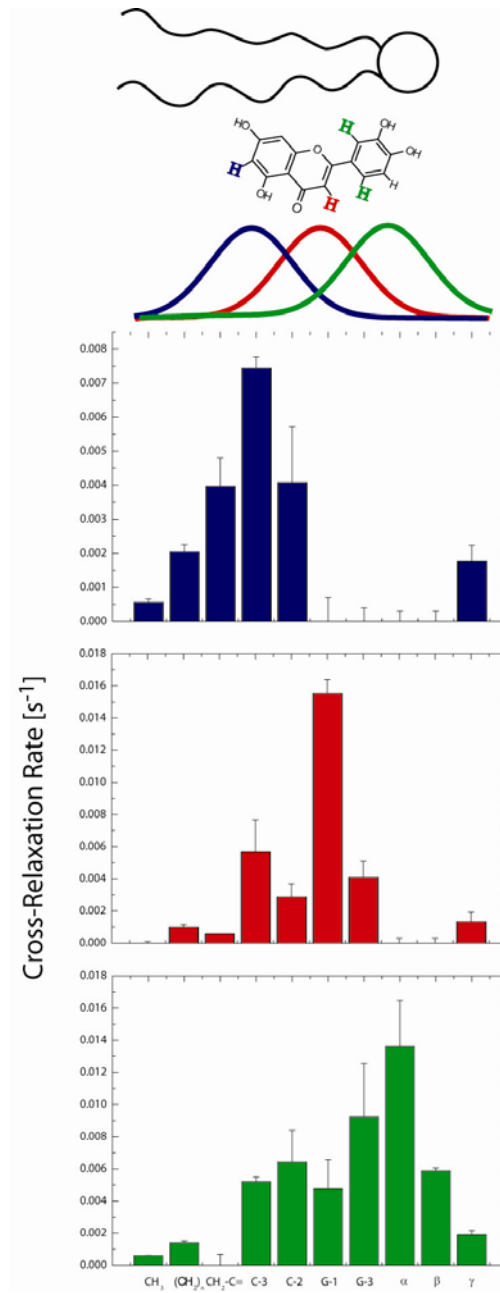
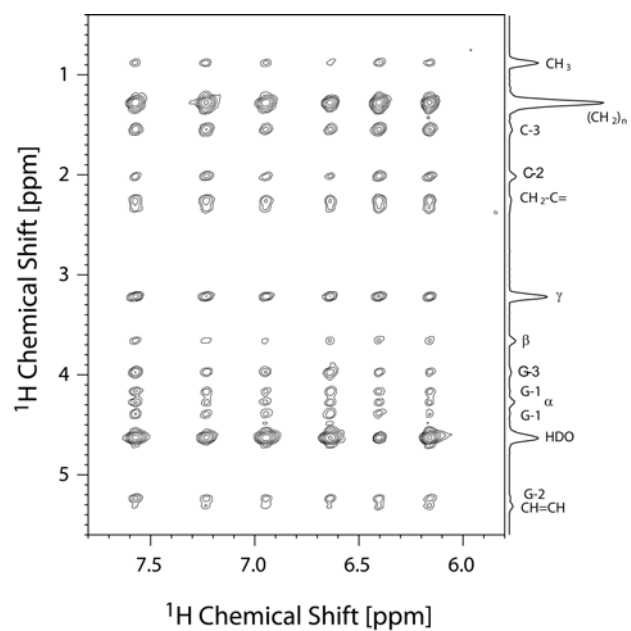
- Protein-Lipid-Interaction
- Protein Structure

C-Terminus of the Human N-ras Protein

- ^{13}C - ^{13}C dipolar coupling measurement is not feasible for sensitivity reasons
- Additional structural constraints may come from protein-membrane interactions
 - ☺ Intermolecular cross-relaxation rates provide this information
 - ☹ ^1H MAS NOESY only works for small peptides



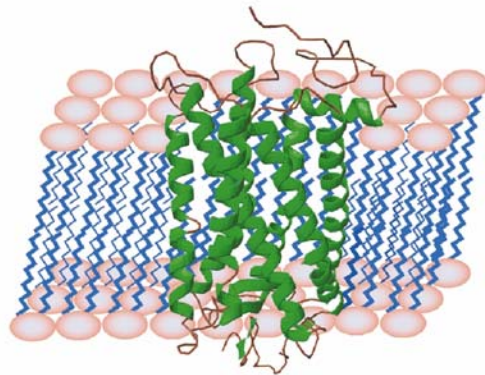
¹H NOESY MAS



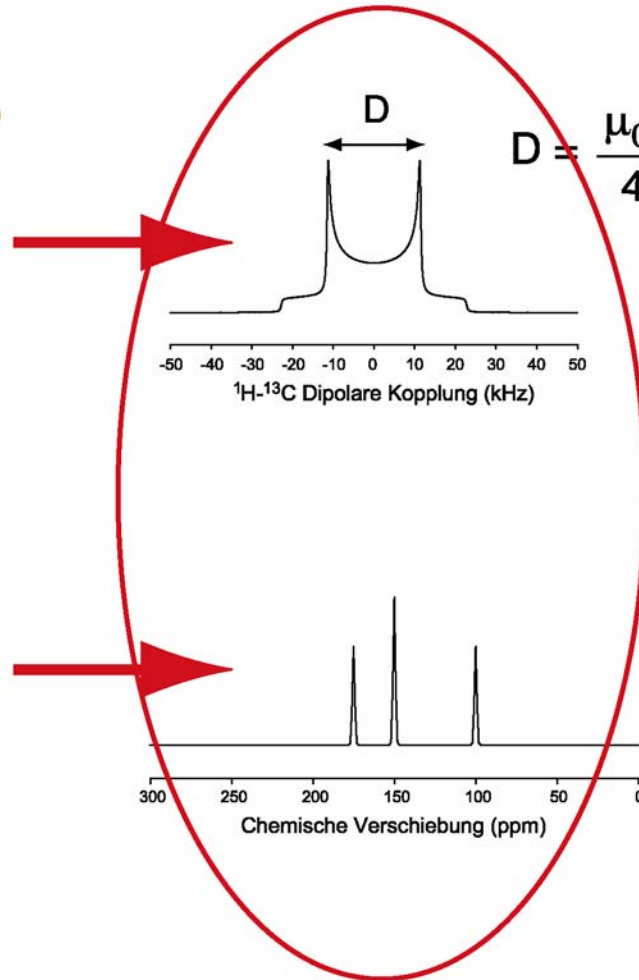
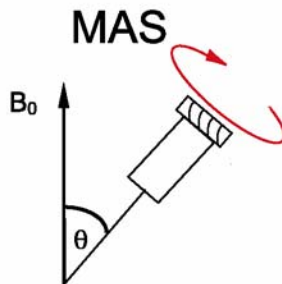
Magic Angle Spinning Techniques

Recoupling

Festkörper- NMR zur Untersuchung von Membranproteinen



Membranprotein in sphärischem Liposom

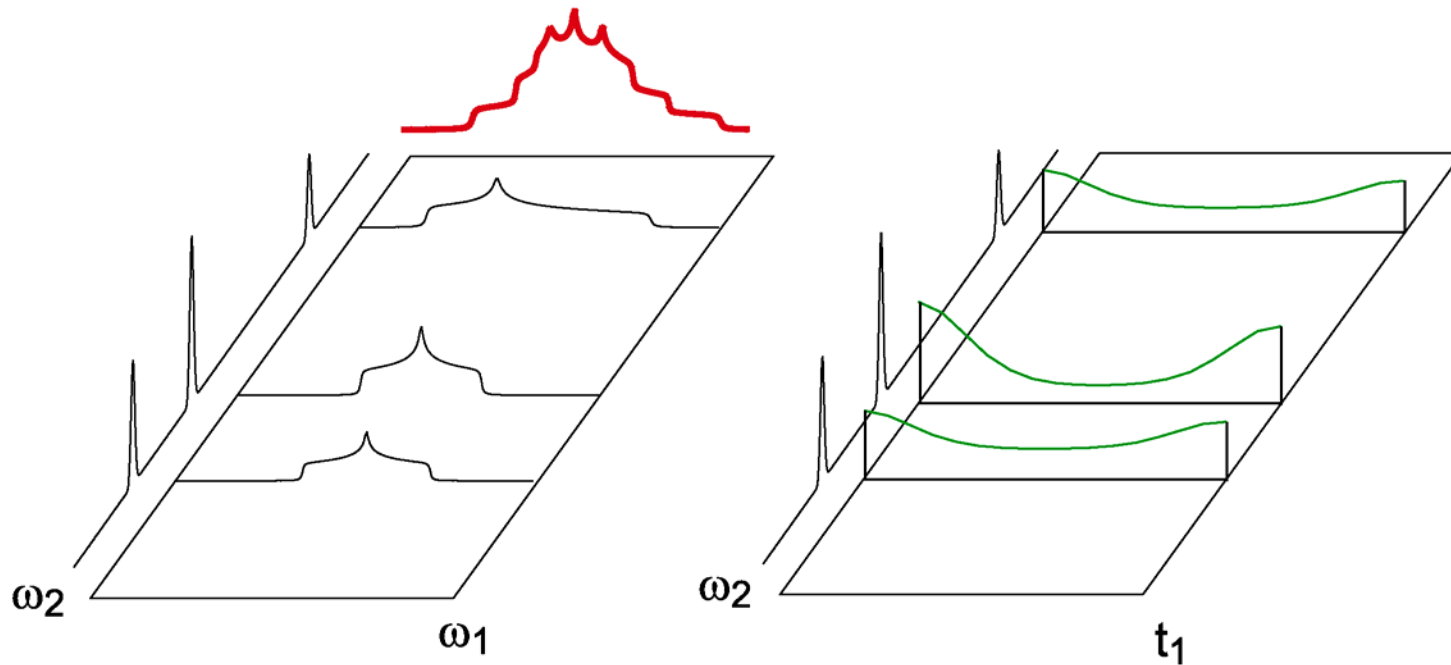


$$D = \frac{\mu_0 \gamma_H \gamma_C \hbar}{4\pi r_{HC}^3}$$

- Rückkopplung anisotroper Wechselwirkungen
- Separation isotroper und anisotroper Spektren

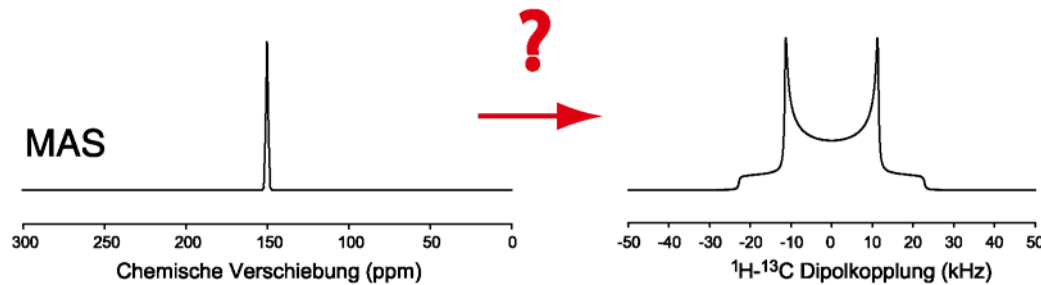
Separation isotroper und anisotroper NMR-Signale

- In t_1 wird die anisotrope Wechselwirkung evolviert und seitenspezifisch in t_2 das MAS-Spektrum detektiert.



Separation von: Pulverspektren
MAS-Seitenbandspektren
MAS-Zeitsignalen

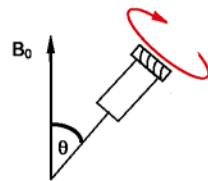
Rückkopplung anisotroper Wechselwirkungen



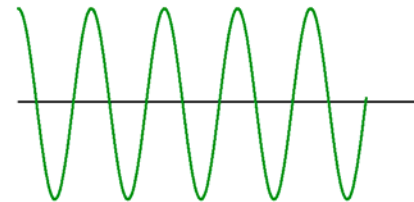
$$\omega_D(t) = C_1 \cos(\gamma + \omega_r t) + C_2 \cos(2\gamma + 2\omega_r t)$$

$$C_1 = -\delta \frac{\sqrt{2}}{2} \sin(2\beta) \left(1 + \frac{1}{3} \eta \cos(2\alpha) \right) \quad C_2 = \delta \left\{ \frac{1}{2} \sin^2 \beta - \frac{\eta}{6} (1 + \cos^2 \beta) \cos(2\alpha) \right\}$$

MAS



$\omega_D(t)$

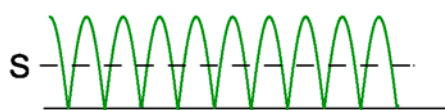


$$\bar{\omega}_D(t) = 0$$

rf-Pulse



$\omega_D(t)$

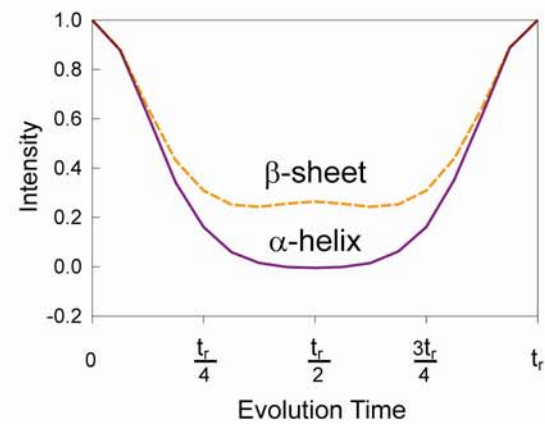
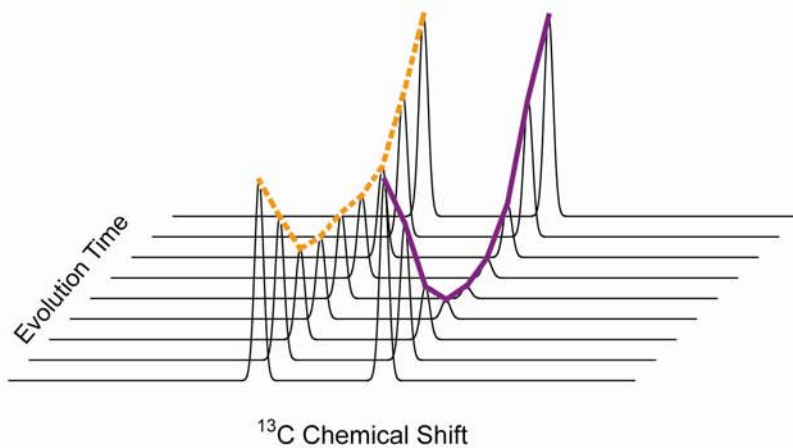
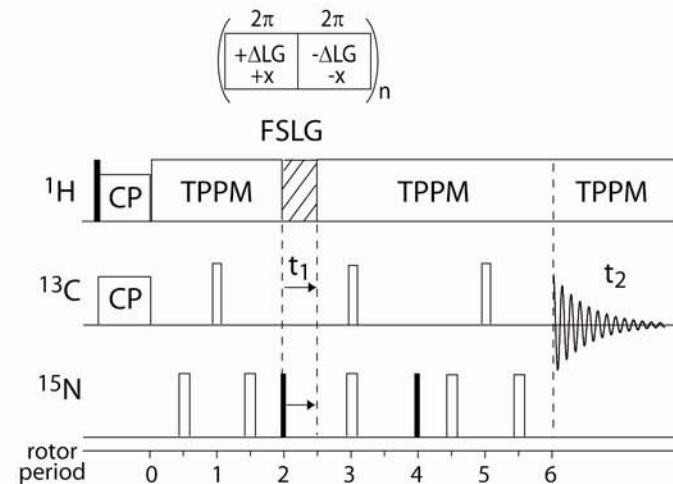
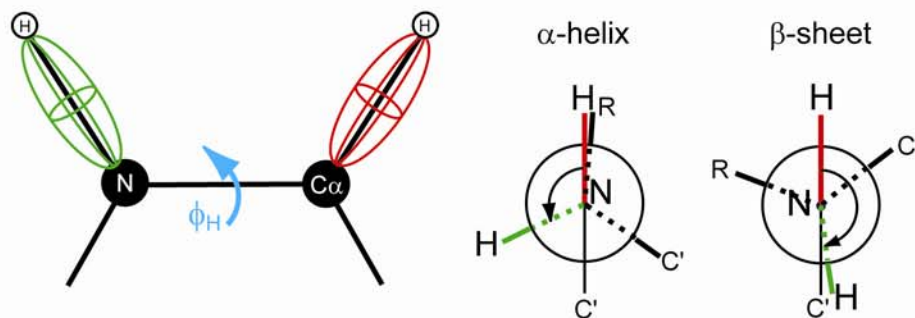


$$\bar{\omega}_D(t) = S \omega_D$$

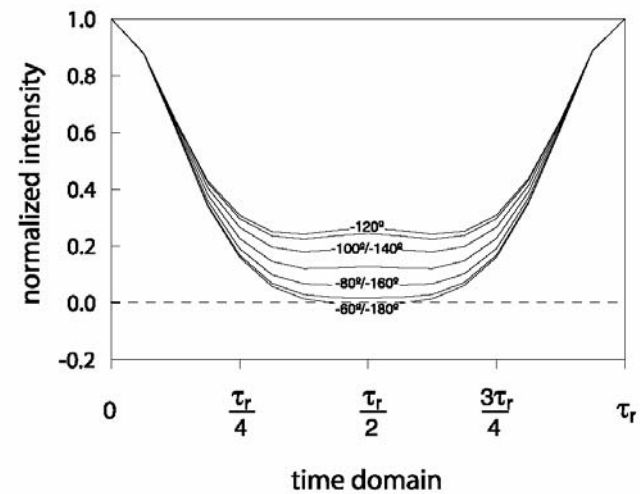
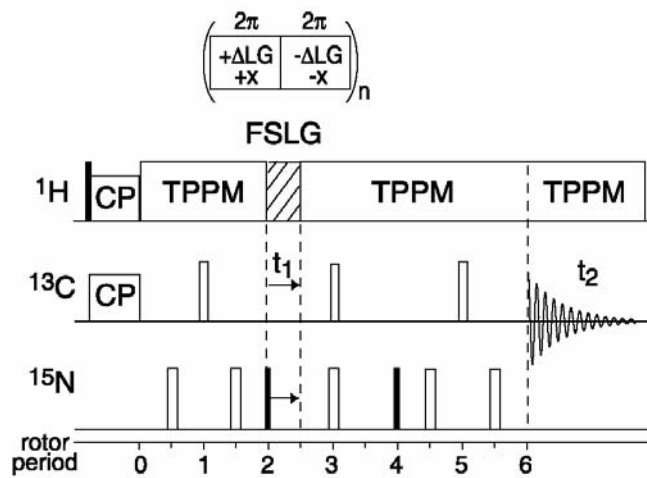
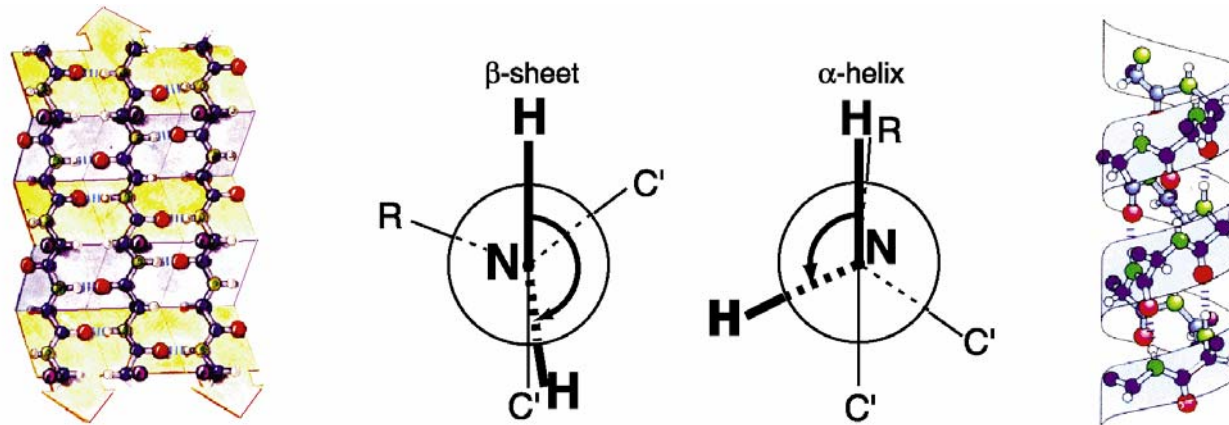
Magic Angle Spinning Techniques

4th Torsion Angle Measurement

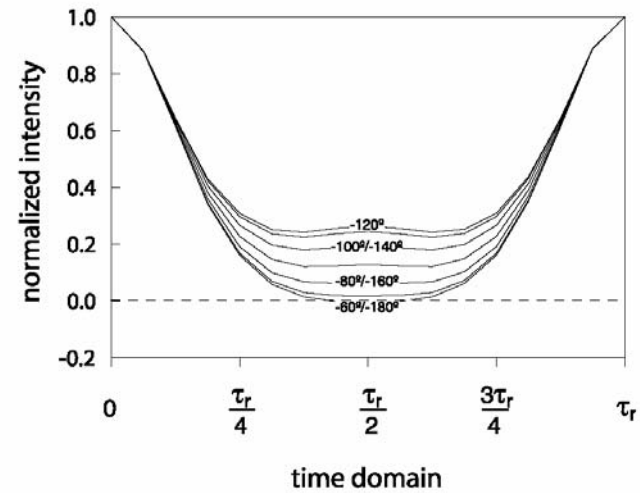
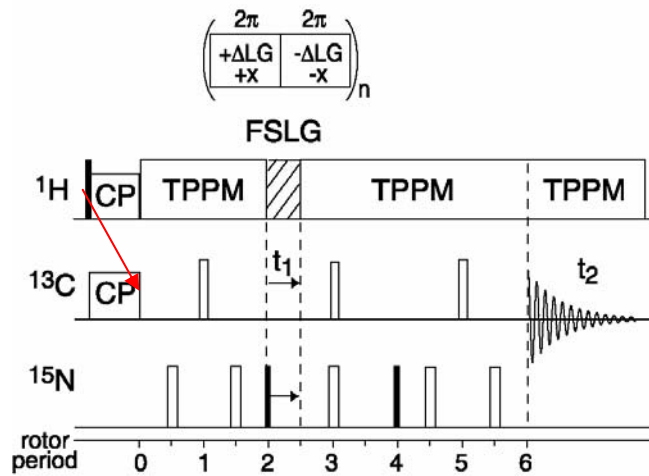
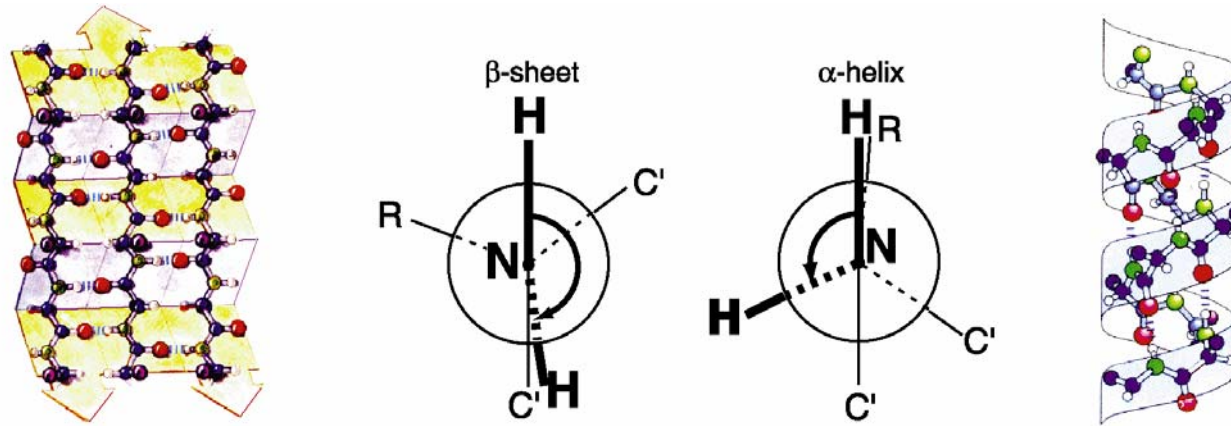
Torsion Angle Measurement



Torsion Angle Measurement

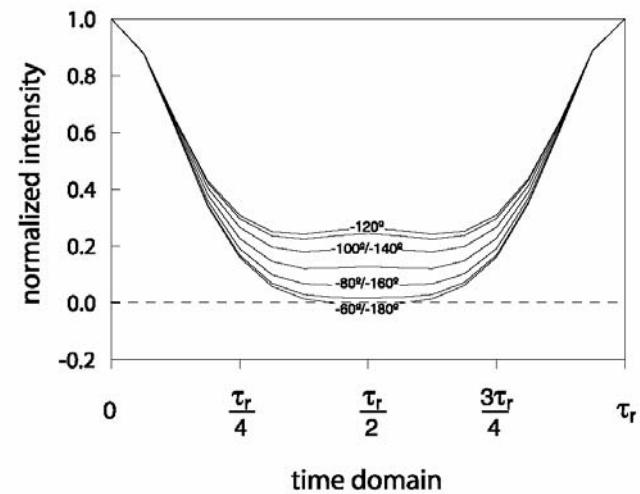
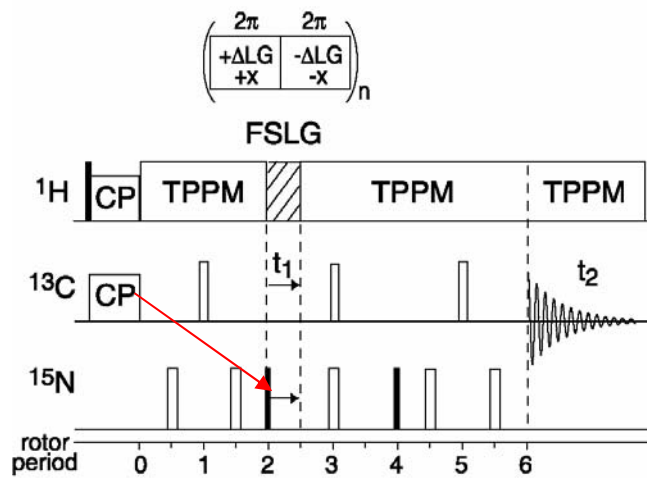
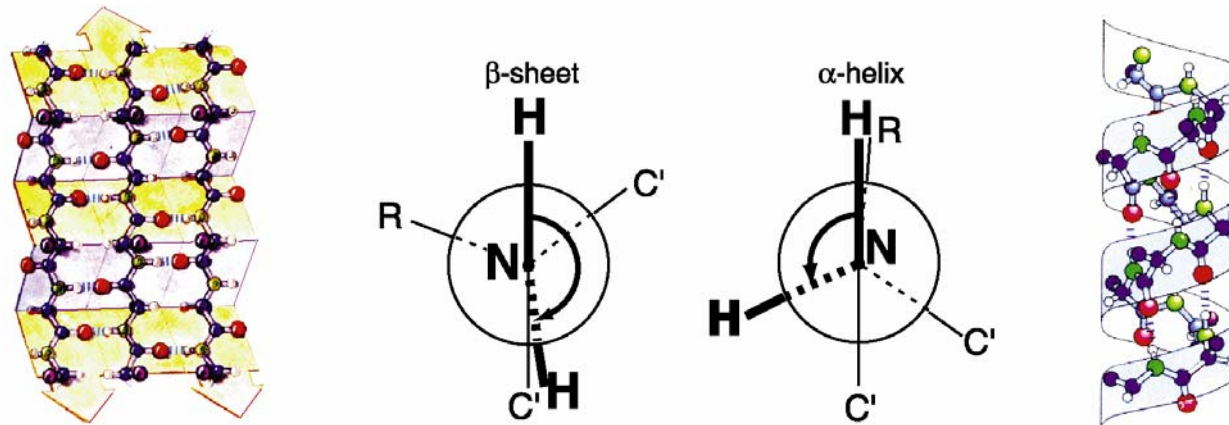


Torsion Angle Measurement



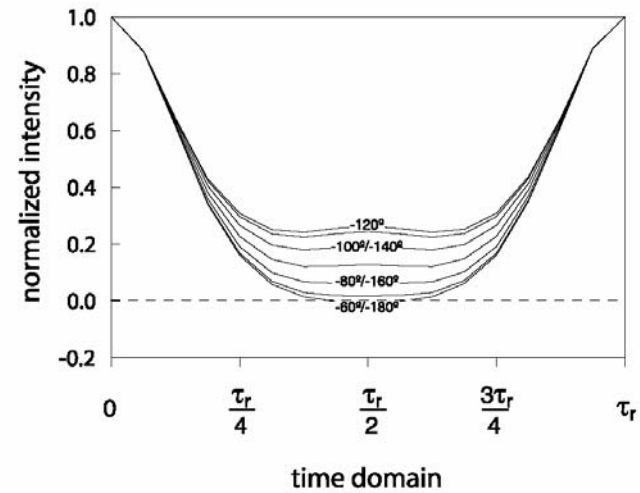
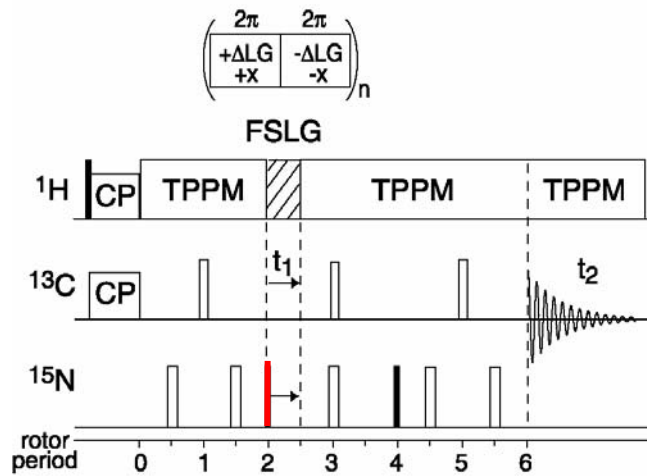
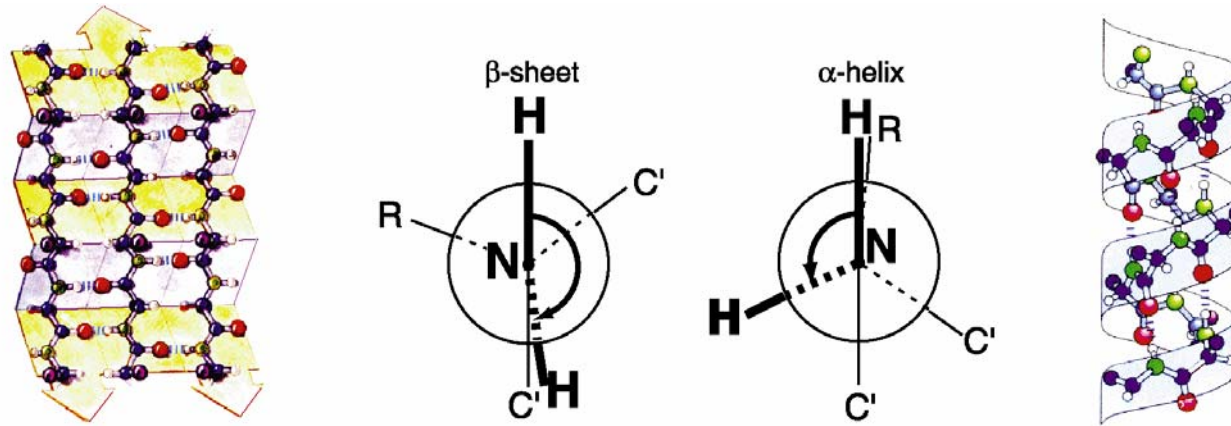
CP: C_x

Torsion Angle Measurement



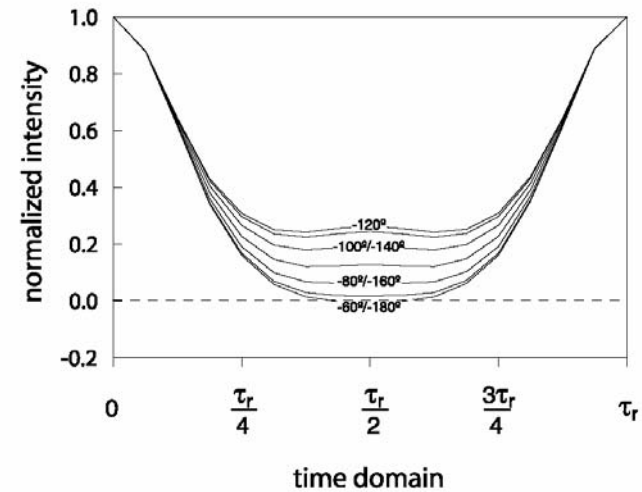
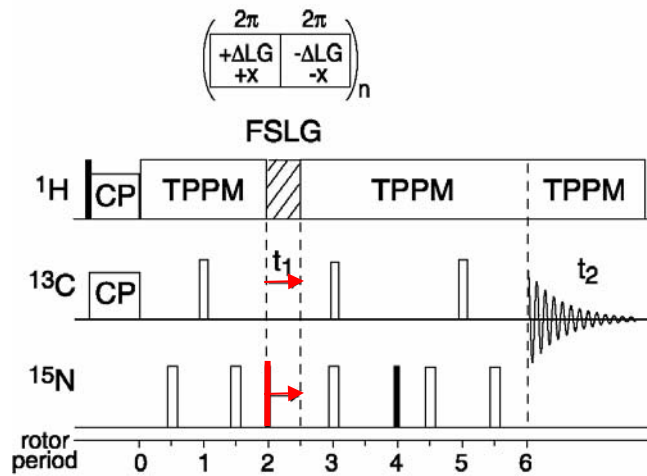
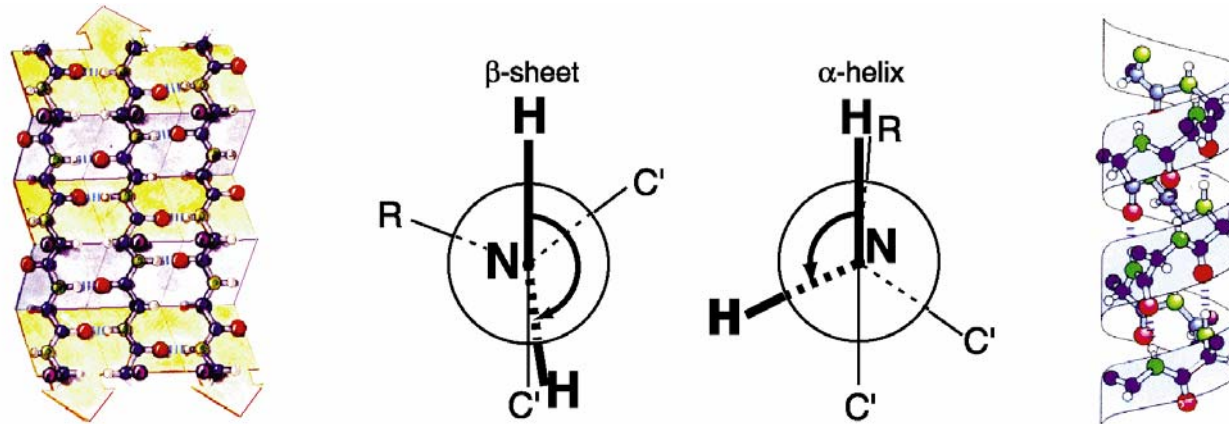
REDOR: $C_y N_z$

Torsion Angle Measurement



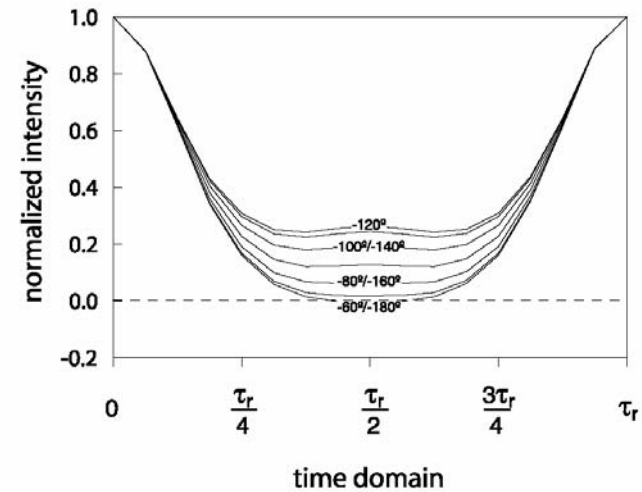
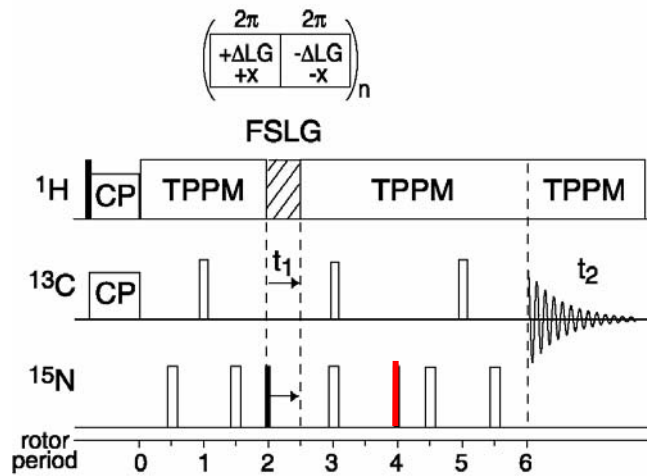
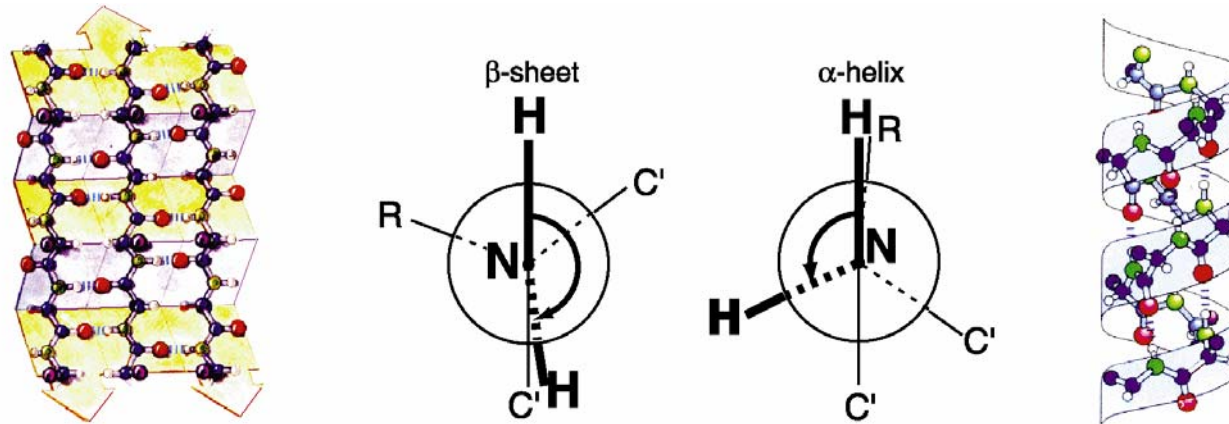
90° $^{15}\text{N}_y$: $C_y N_x$

Torsion Angle Measurement



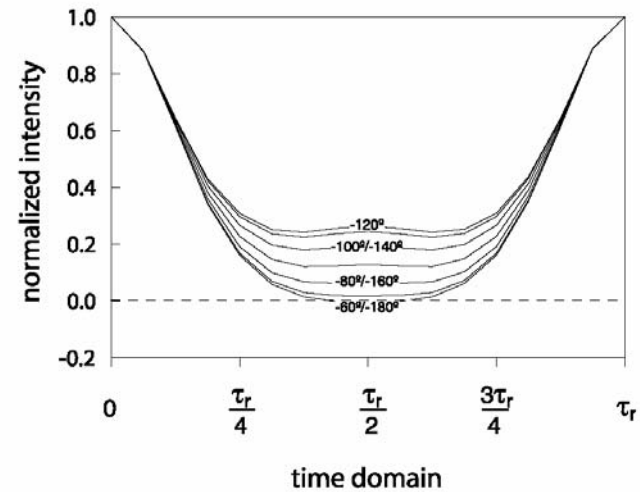
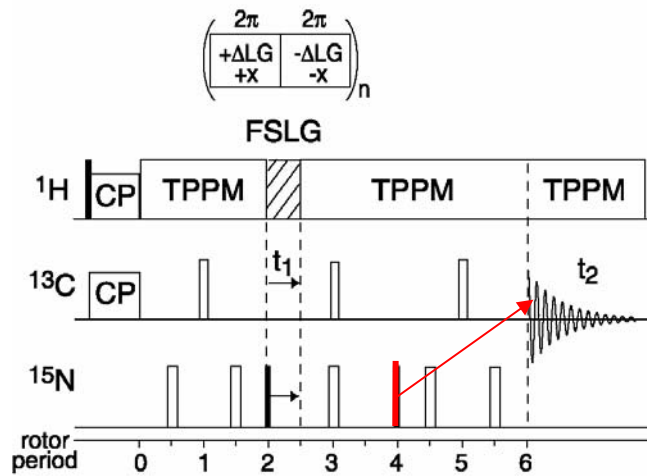
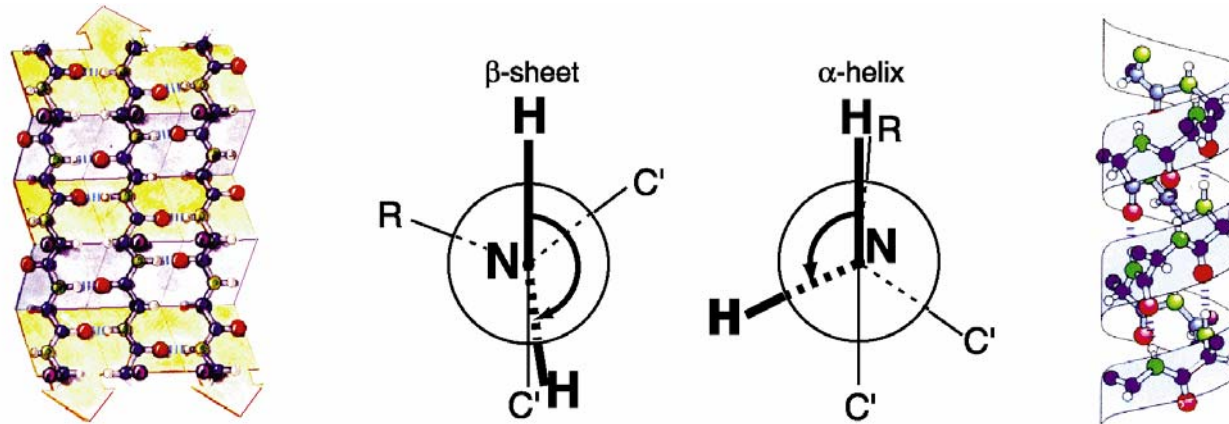
t_1 ev.: $C_y N_x \cos(\Psi_{CH}(t_1)) \cos(\Psi_{NH}(t_1)) =$
 $C_y N_x \cos((\Psi_{CH}(t_1) + \Psi_{NH}(t_1))/2) \cos((\Psi_{CH}(t_1) - \Psi_{NH}(t_1))/2)$

Torsion Angle Measurement



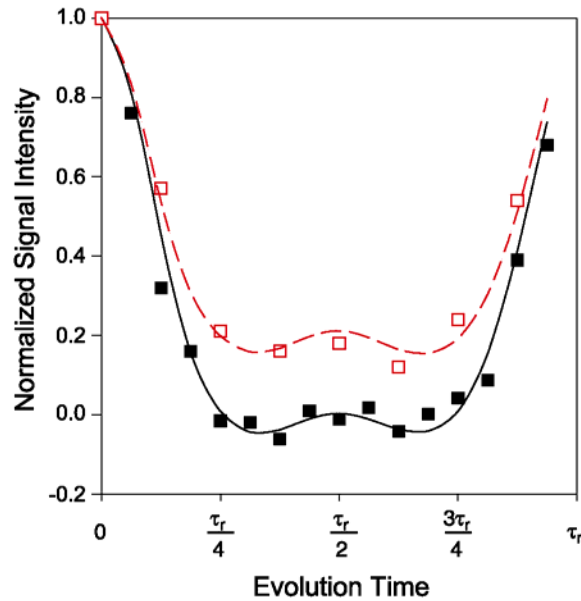
$$90^\circ \text{ } ^{15}\text{N}_{-y} : C_y N_z \cos(\Psi_{CH}(t_1)) \cos(\Psi_{NH}(t_1)) = C_y N_z \cos((\Psi_{CH}(t_1) + \Psi_{NH}(t_1))/2) \cos((\Psi_{CH}(t_1) - \Psi_{NH}(t_1))/2)$$

Torsion Angle Measurement



REDOR: $C_x \cos(\Psi_{CH}(t_1)) \cos(\Psi_{NH}(t_1)) =$
 $C_x \cos((\Psi_{CH}(t_1) + \Psi_{NH}(t_1))/2) \cos((\Psi_{CH}(t_1) - \Psi_{NH}(t_1))/2)$

Torsion Angle Measurement



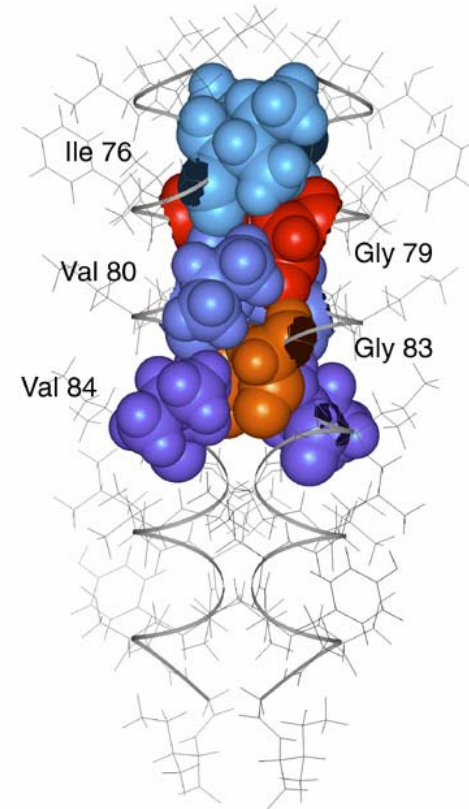
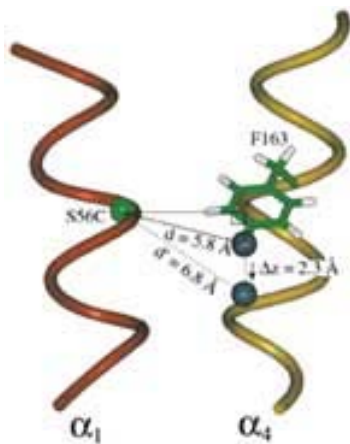
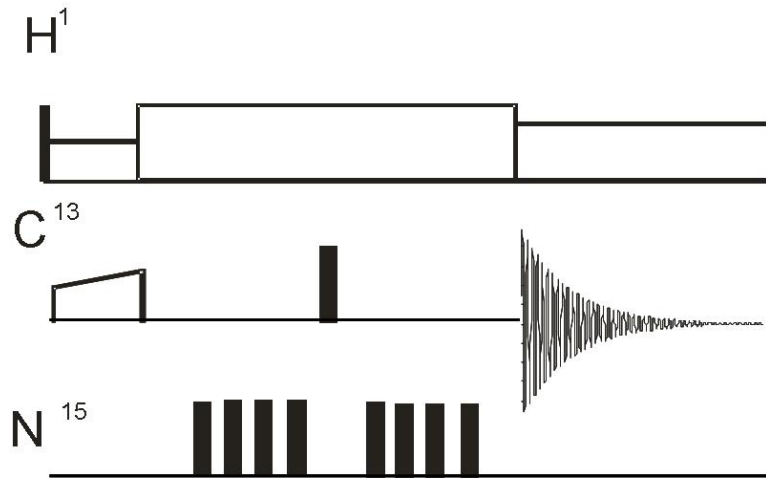
	Crystalline	POPC/Zn ²⁺ (5 mM)
B18 L-4	-80°	-120°
B18 L-8	-80°	-110°
B18 L-15	-90°	-120°

- ☺ High precision ($\pm 5^\circ$)
- ☹ Complicated experiment, high rf heating
- ☹ Low sensitivity
- ☹ unique labelling pattern, 1D resolution

5th Explicit Distance Measurements

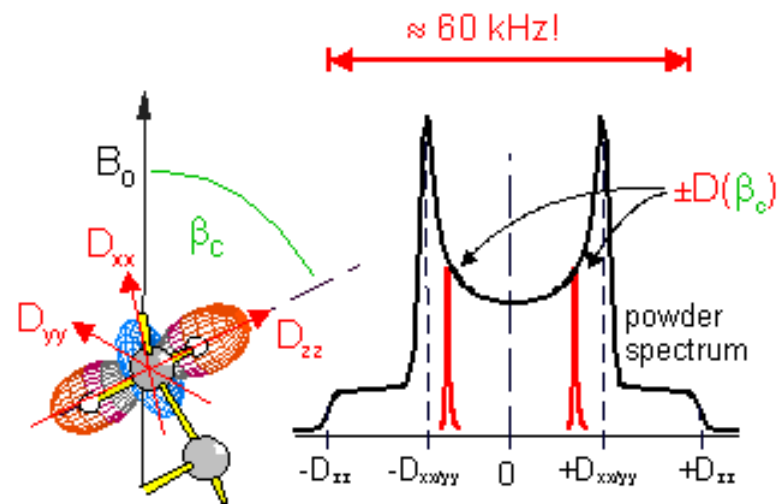
Magic Angle Spinning Techniques

REDOR and rotational resonance



- ☺ High precision (0.1 Å)
- ☺ Unique distance constraint
- ☹ Low sensitivity, high rf heating
- ☹ Needs isolated spin pair
- ☹ Experiments at low temperature

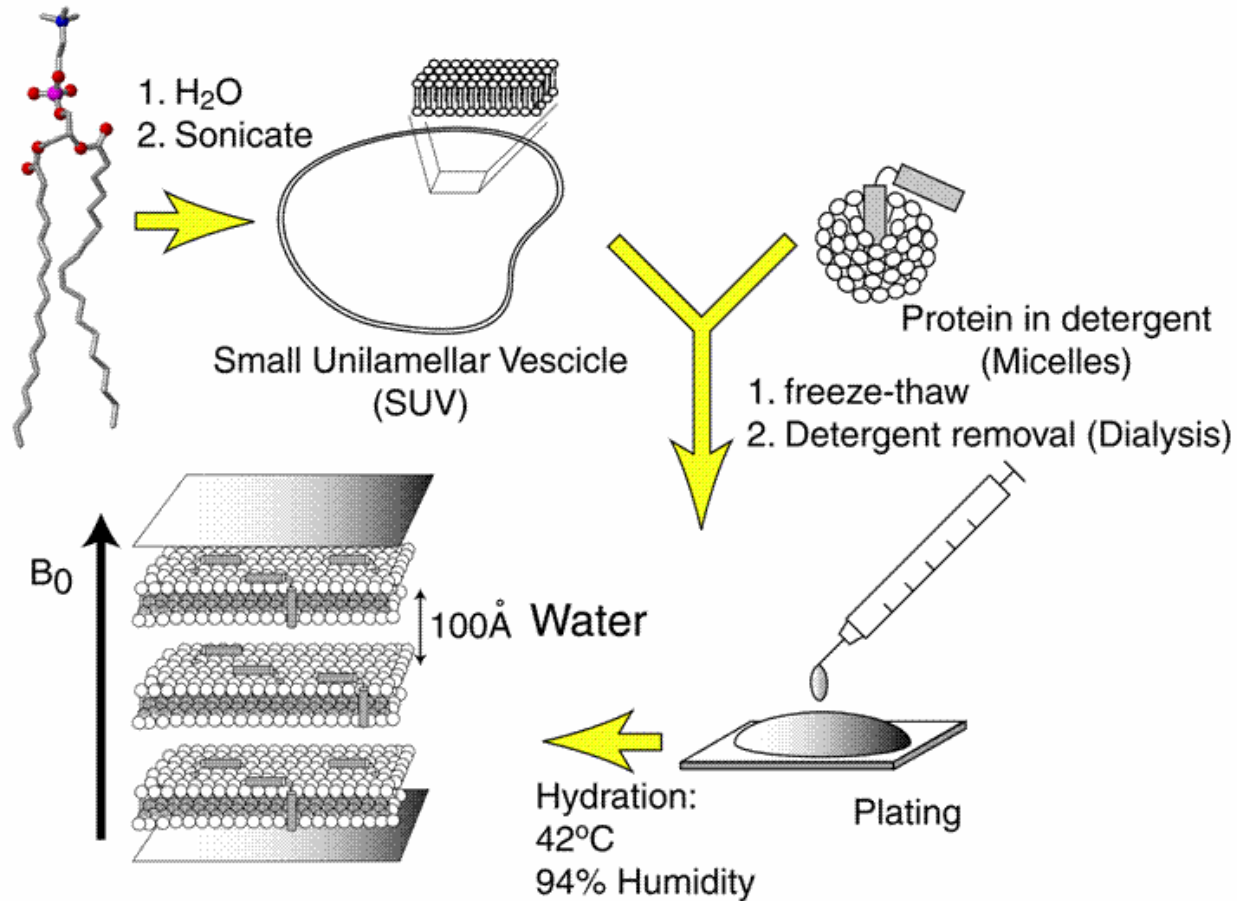
Static Solid Techniques



Static Solid Techniques

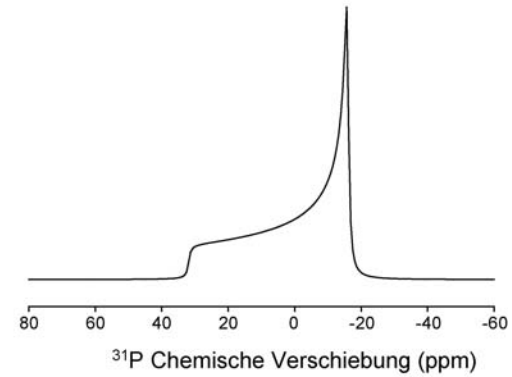
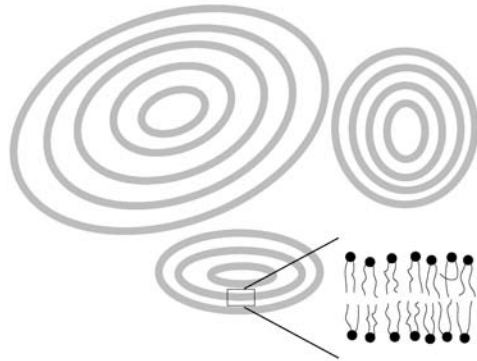
6th Oriented Membrane Stacks

Oriented Membrane Stacks

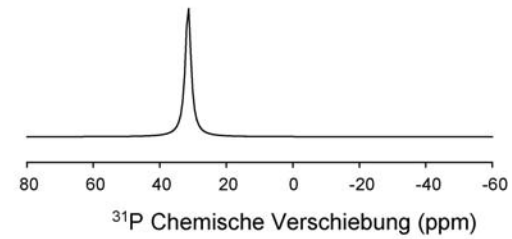
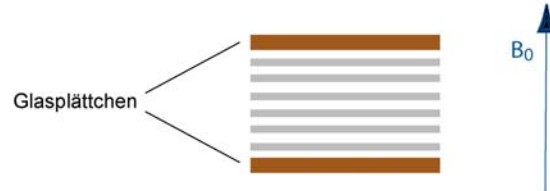


Oriented Membrane Stacks

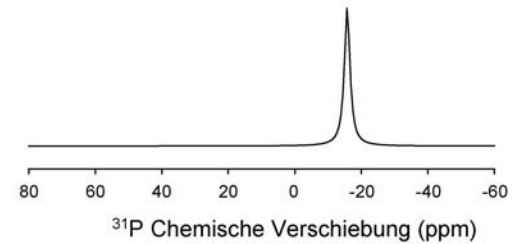
a) Multilamellare Lipidvesikel



b) Orientierte Membranen parallel zum Magnetfeld

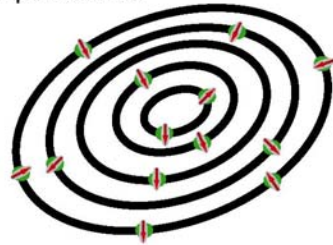


c) Orientierte Membranen orthogonal zum Magnetfeld

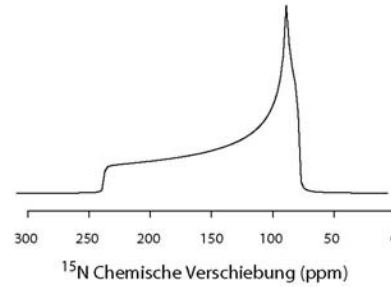


Oriented Membrane Stacks

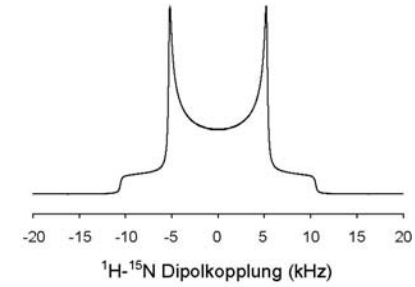
(a) Membranprotein in multilamellaren Lipidvesikeln



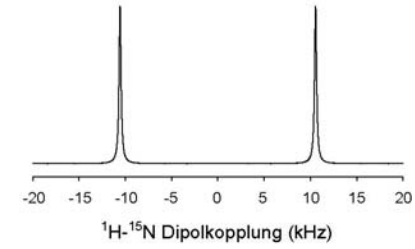
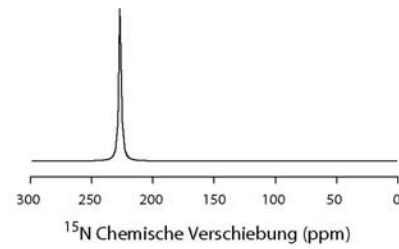
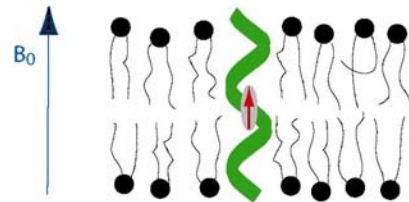
Chemische Verschiebung



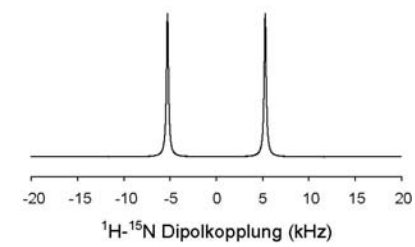
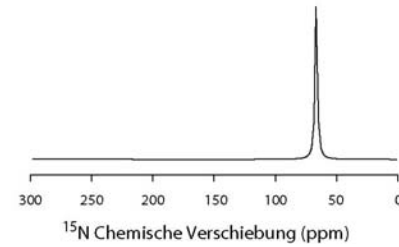
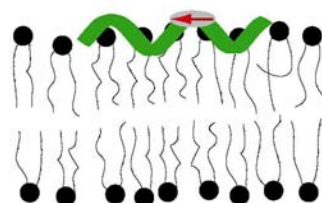
Dipolare Kopplung



(b) Transmembrane α -Helix

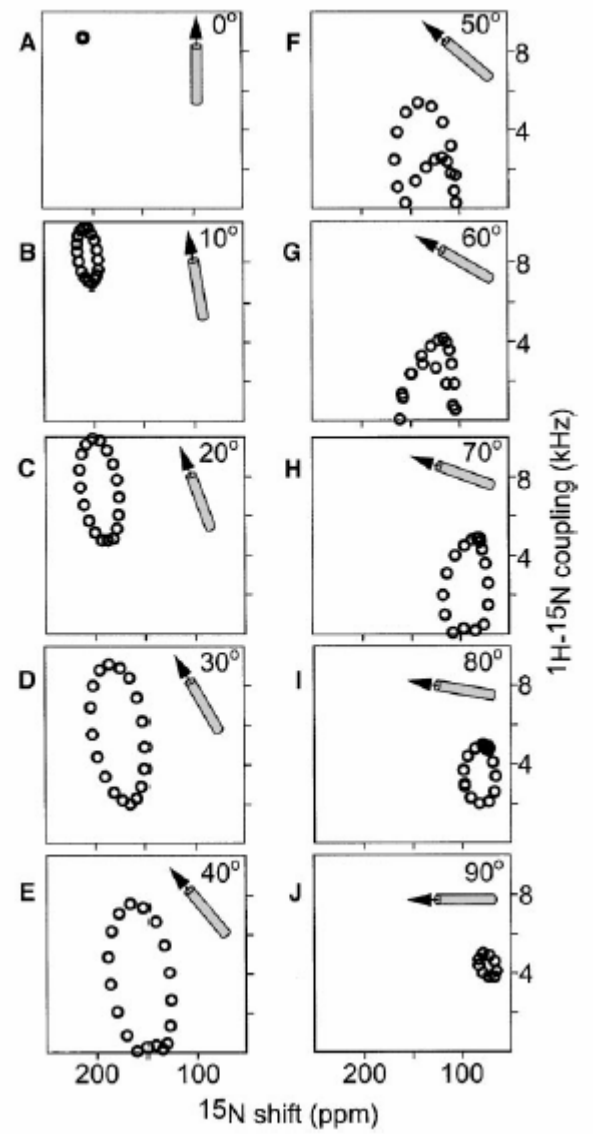
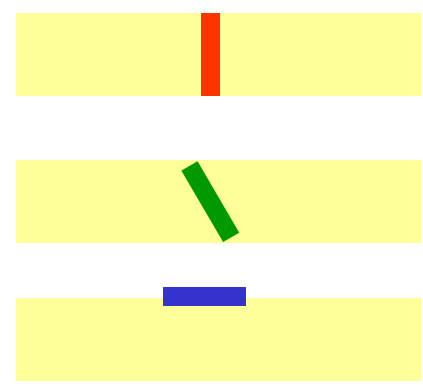
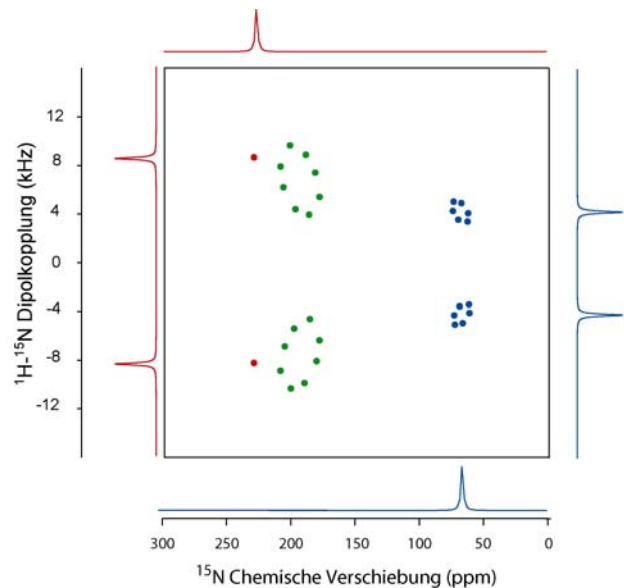


(c) Oberflächenassoziierte α -Helix

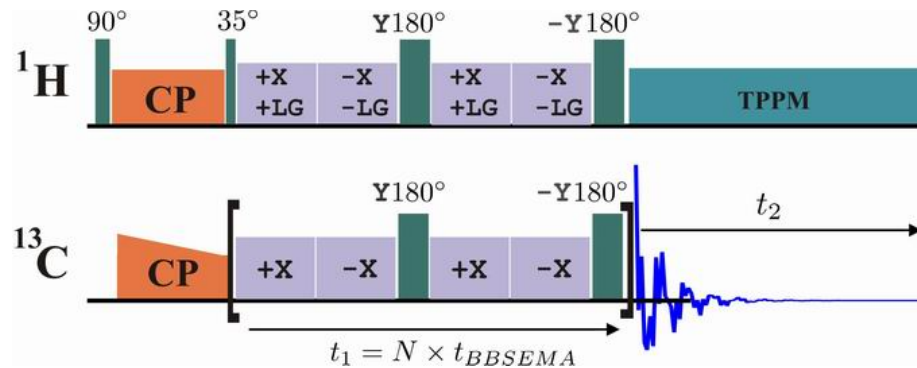


Oriented Membrane Stacks

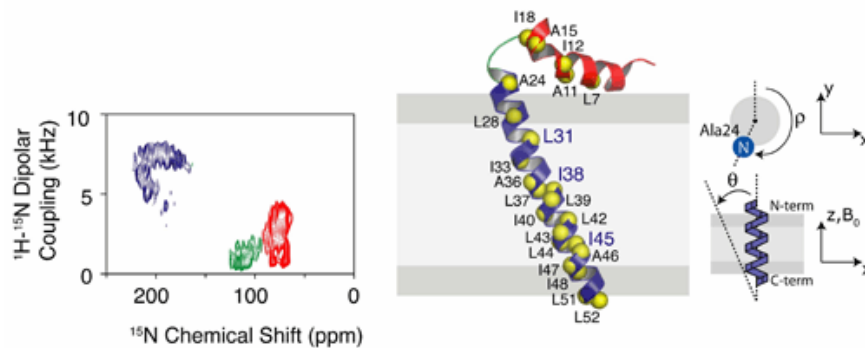
PISEMA: Correlation of ^{15}N anisotropic chemical shift and ^{15}N - ^1H dipolar coupling



Oriented Membrane Stacks



- ☺ High precision
- ☺ Peptide topology
- ☹ Complicated experiment, high rf heating
- ☹ Low sensitivity
- ☹ Very complicated sample preparation (large membrane proteins are very difficult to orient)
- ☹ No peptide structure
- ☹ Flat coil probe



Outlook

- The limitations of solid-state NMR for structure determination are defined by **sensitivity** and **resolution**.

Sample preparation:

5.5 mg protein

29 mg lipid

35 wt% H₂O

4 amino acids ¹³C-labeled (~30 µg each)

≅ 0.25% of the rotor volume

