MARTIN-LUTHER-UNIVERSITÄT
HALLE-WITTENBERG

Mitteldeutsches
Zentrum für
Struktur und
Dynamik der
Proteine

Solid-State NMR Studies of the Structure of Membrane Bound Ras Proteins

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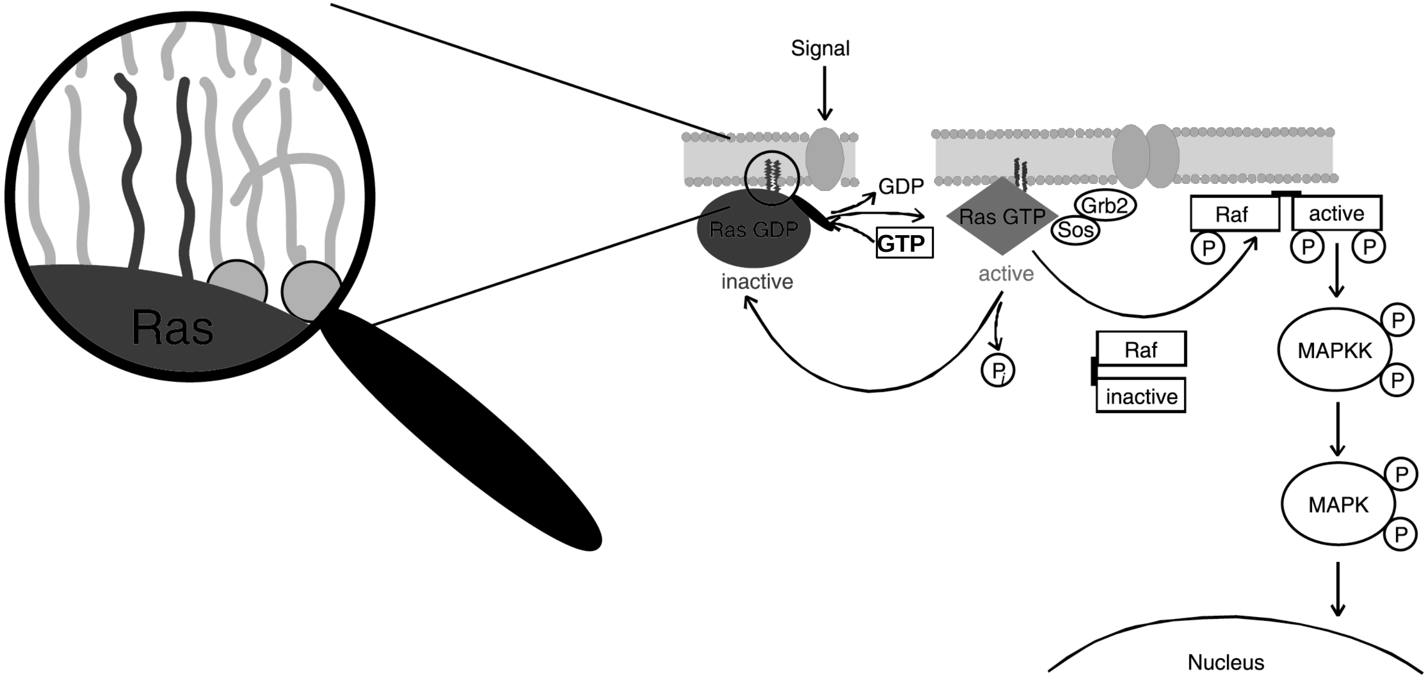
Junior Research Group “Structural Biology of Membrane Proteins”

Institute of Biotechnology

Martin Luther University Halle-Wittenberg

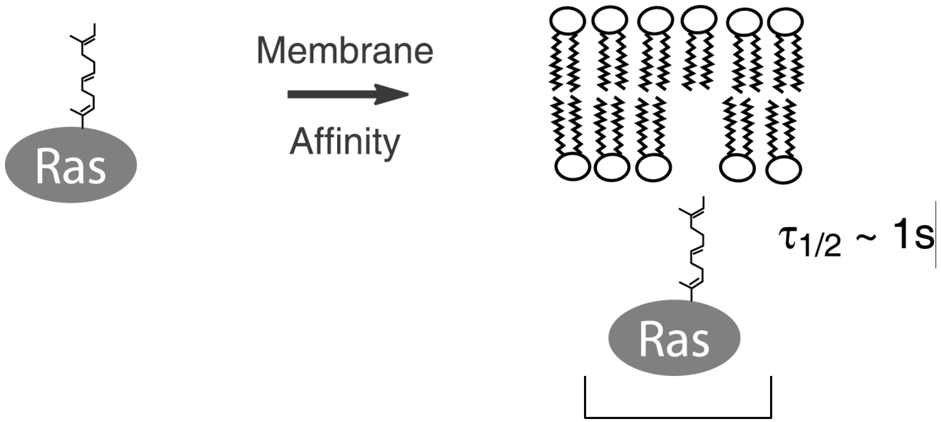
Interaction of the C-Terminus of N-ras with Membranes



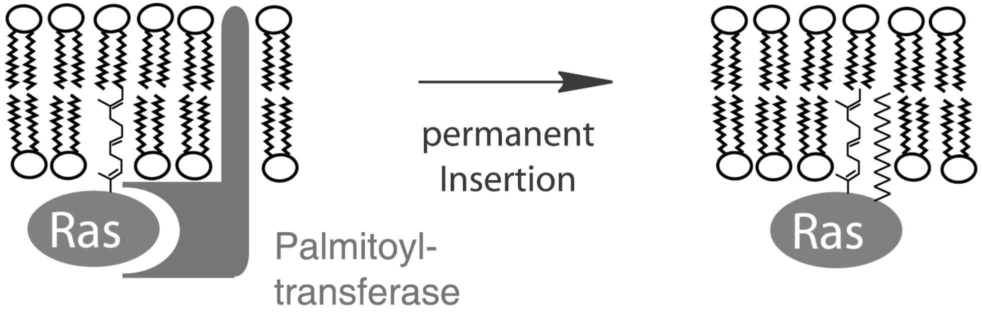
- Many proteins involved in signal transduction have posttranslational fatty acid modifications.
- Ras is an oncogene and therefore an important therapeuthic target.

Biophysics of Membrane Insertion of Ras

- Ras acquires a farnesylation during biosynthesis not sufficient to permanently anchor the protein in the plasma membrane.

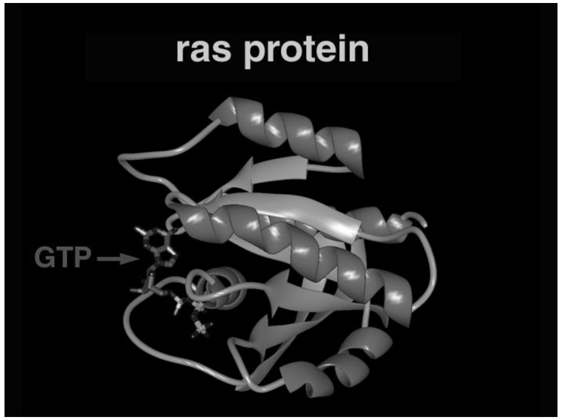


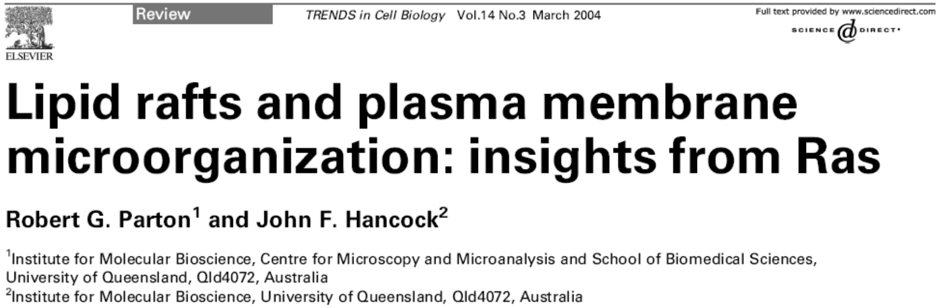
- Posttranslational palmitoylation provides sufficient hydrophobicity for permanent membrane insertion.



Why is the Membrane Anchor of Ras Important?

- The structure of the N-terminus (1-166) is known from x-ray and solution NMR
- No structural model of the lipidated membrane bound C-terminus exists




Review TRENDS in Cell Biology Vol.14 No.3 March 2004
Full text provided by www.sciencedirect.com

Lipid rafts and plasma membrane microorganization: insights from Ras

Robert G. Parton[1] **and John F. Hancock**[2]

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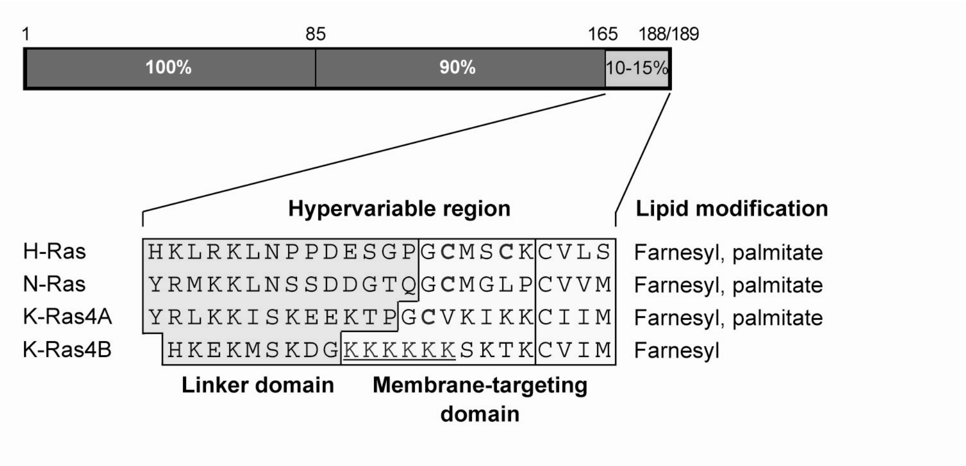
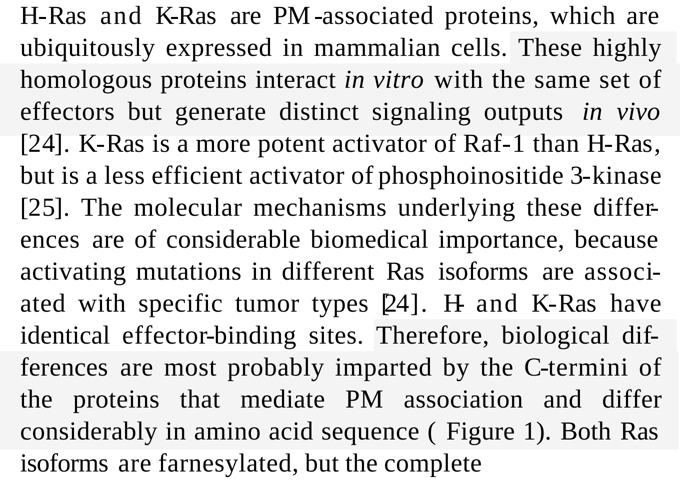
[2]Institute for Molecular Bioscience, University of Queensland, Qld4072, Australia

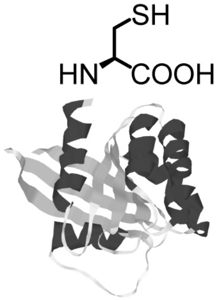




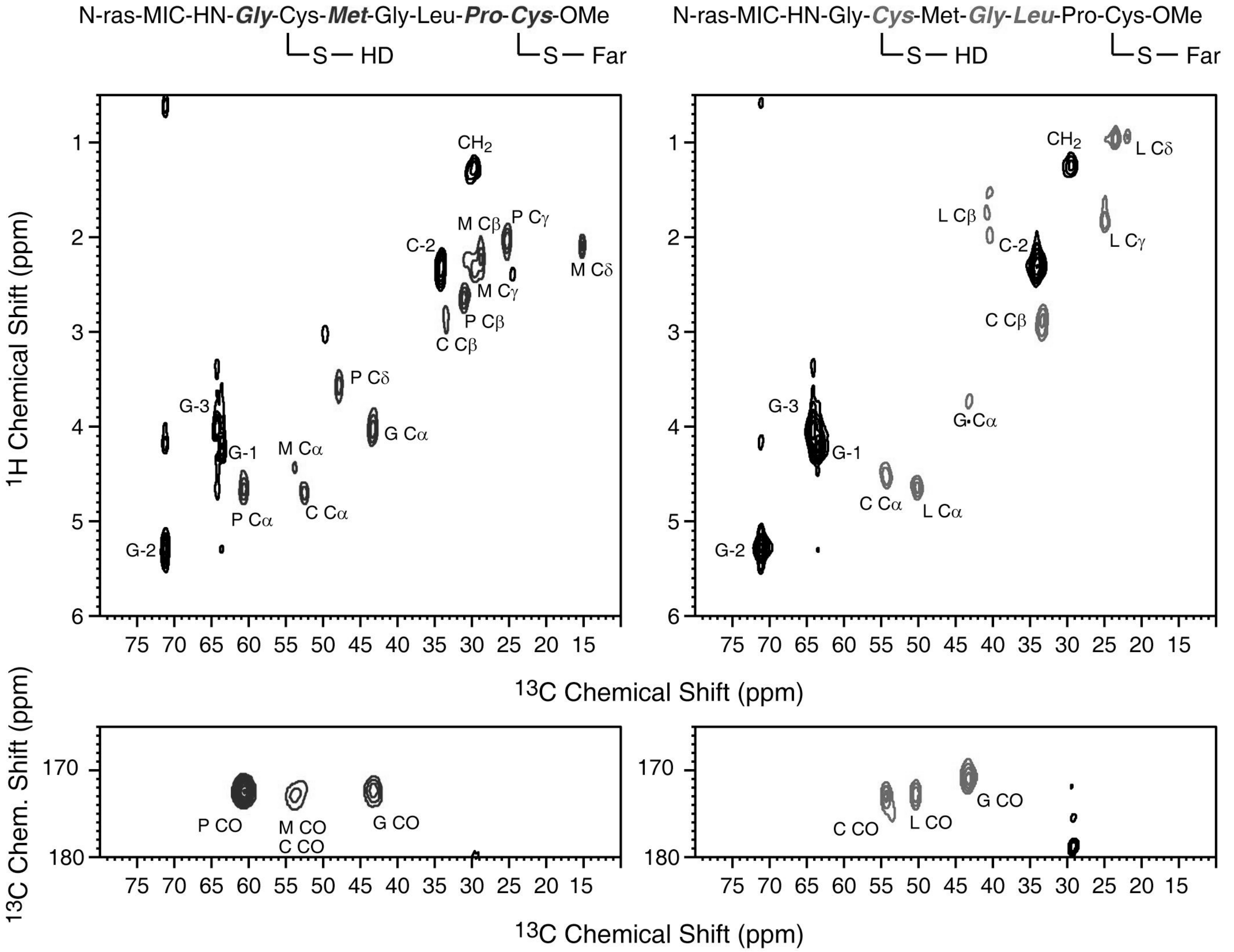
H-Ras and K-Ras are PM-associated proteins, which are ubiquitously expressed in mammalian cells. These highly homologous proteins interact *in vitro* with the same set of effectors but generate distinct signaling outputs *in vivo* [24]. K-Ras is a more potent activator of Raf-1 than H-Ras, but is a less efficient activator of phosphoinositide 3-kinase [25]. The molecular mechanisms underlying these differences are of considerable biomedical importance, because activating mutations in different Ras isoforms are associated with specific tumor types [24]. H- and K-Ras have identical effector-binding sites. Therefore, biological differences are most probably imparted by the C-termini of the proteins that mediate PM association and differ considerably in amino acid sequence (Figure 1). Both Ras isoforms are farnesylated, but the complete

Chemical Synthesis of Full-Length Ras Protein

recombinant
N-Ras protein



^{1}H-^{13}C and ^{13}C-^{13}C Correlation Experiments



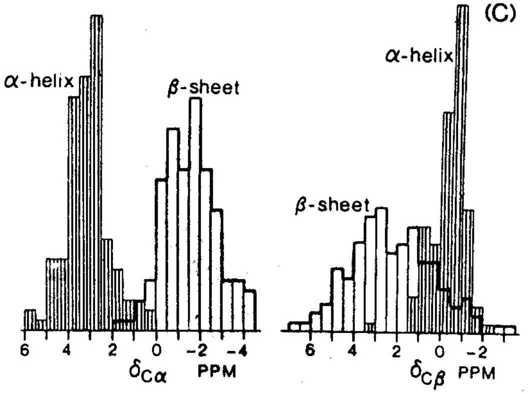
22 kDa Ras Protein in DMPC-d_{67} membrane (1:150), T = 303 K

Structural Information from Isotropic Chemical Shifts

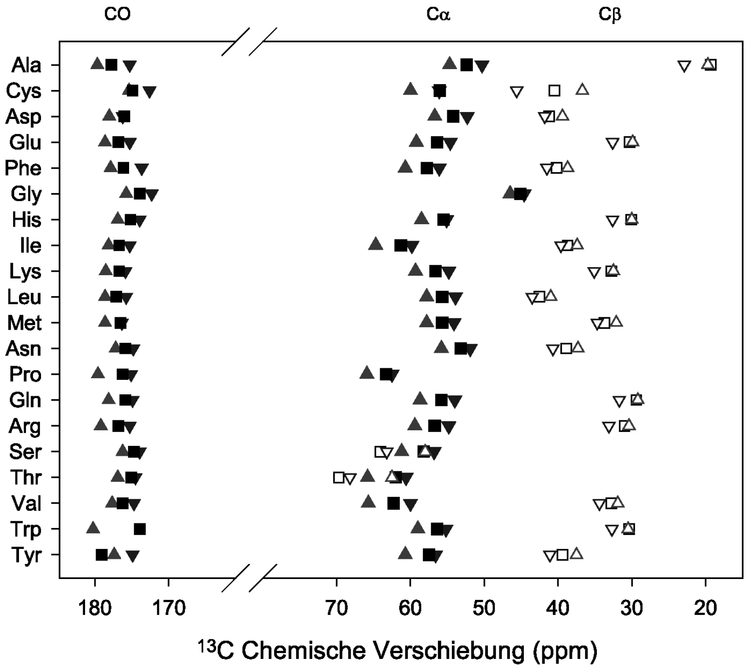
- ^{13}C Cα chemical shifts show a strong correlation with the backbone conformation of a protein

- α-helix: Cα ↑ Cβ ↓

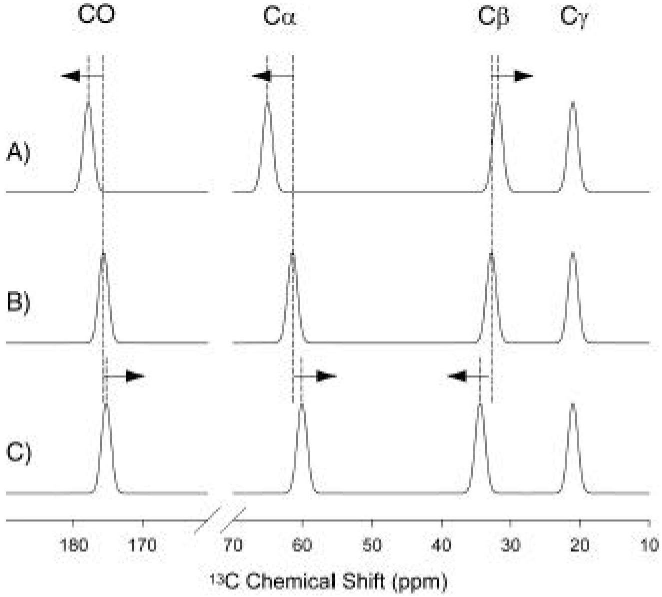
  β-sheet: Cα ↓ Cβ ↑



Spera & Bax, 1991



■ random coil ▲ α-helix ▼ β-sheet



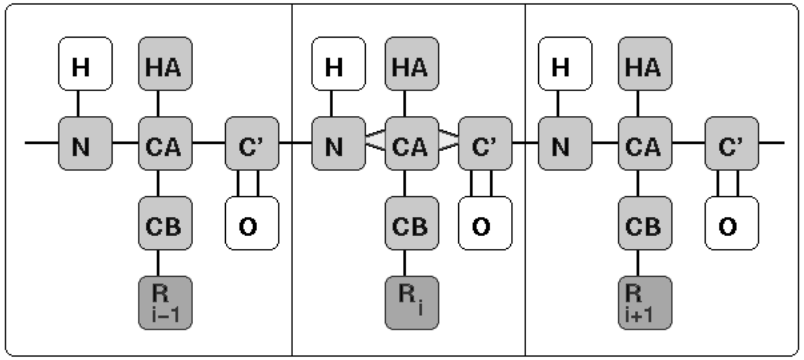
α-helix

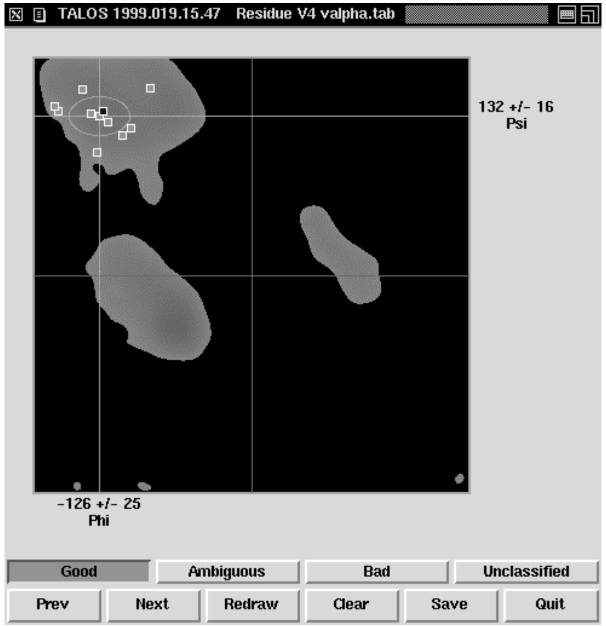
random coil

β-sheet

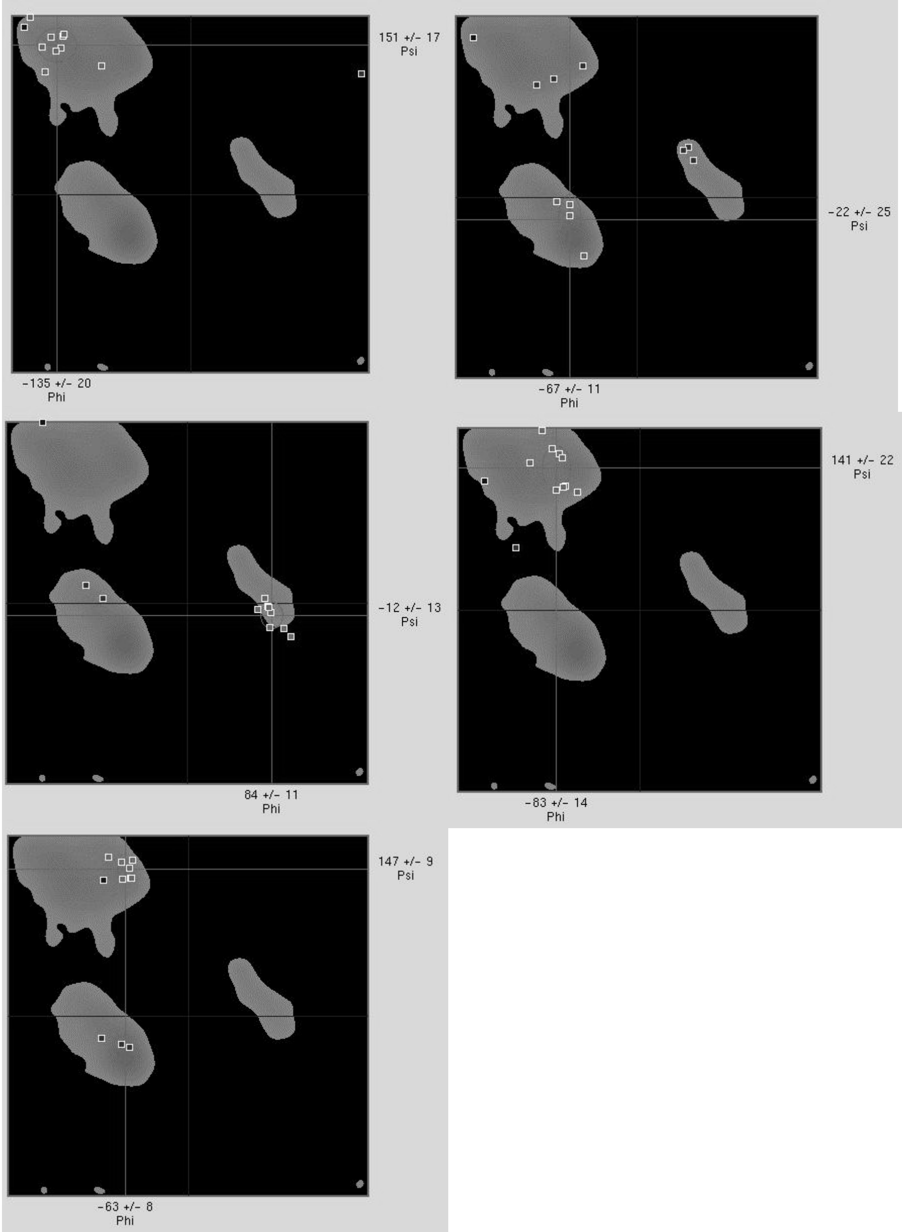
TALOS – Torsion Angle Prediction

Torsion Angle Likelihood Obtained from Shift and sequence similarity





Structural Results for Membrane Bound Ras Protein



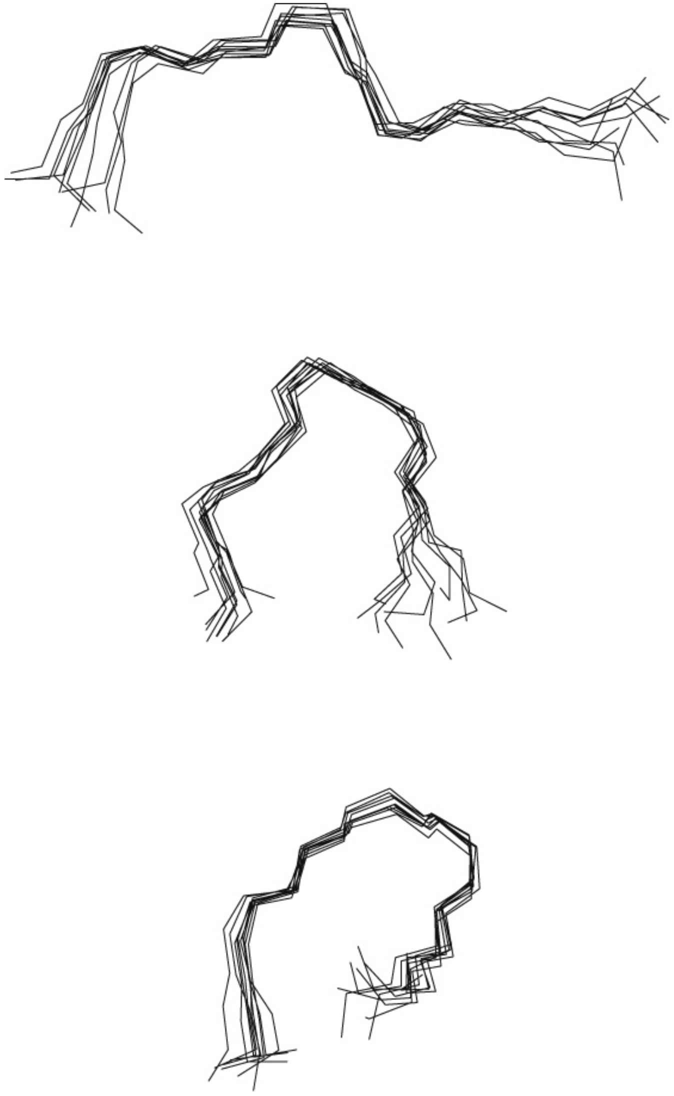
Residue	ϕ	ψ
cysteine 181	-135° ± 20°	151° ± 17°
methionine 182	-67° ± 11° -79° ± 23° 51° ± 5°	-22° ± 25° 121° ± 10° 45° ± 7°
glycine 183	84° ± 11°	-12° ± 13°
leucine 184	-83° ± 14°	141° ± 22°
proline 185	-63° ± 8°	147° ± 9°

\+ Pro is in *trans* conformation

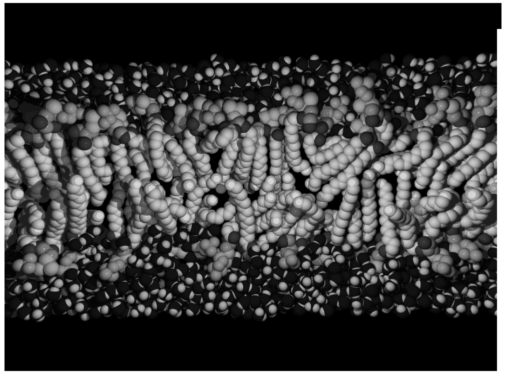
Σ 11 structural constraints

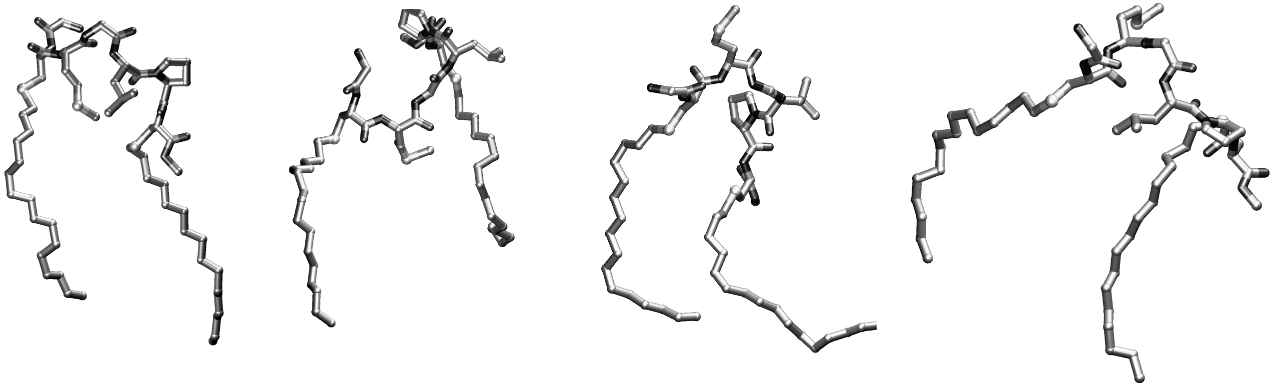
➔ TALOS prediction is not unique!

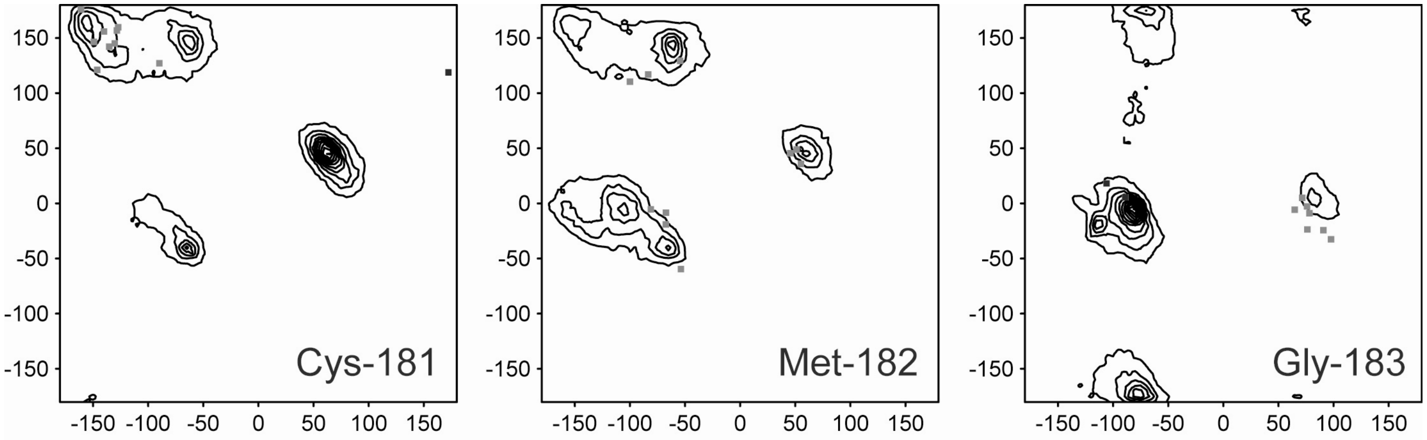
Structure of the C-Terminus of Membrane Bound Ras Protein

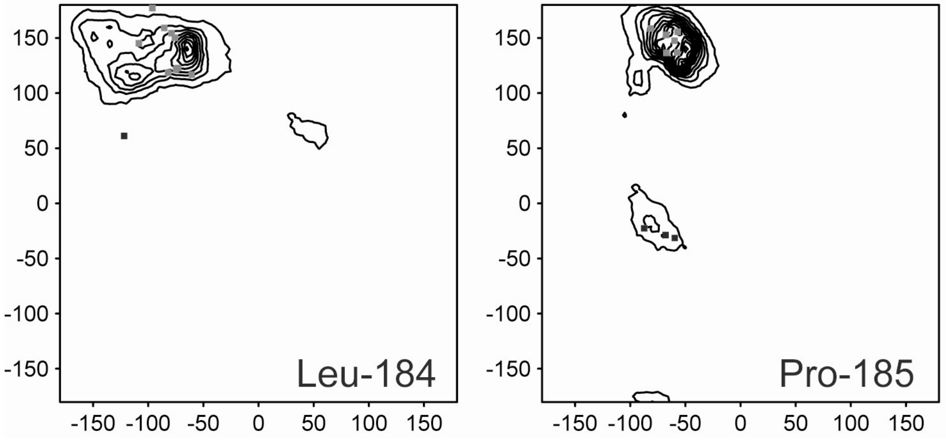


Comparison between simulated and measured torsion angles







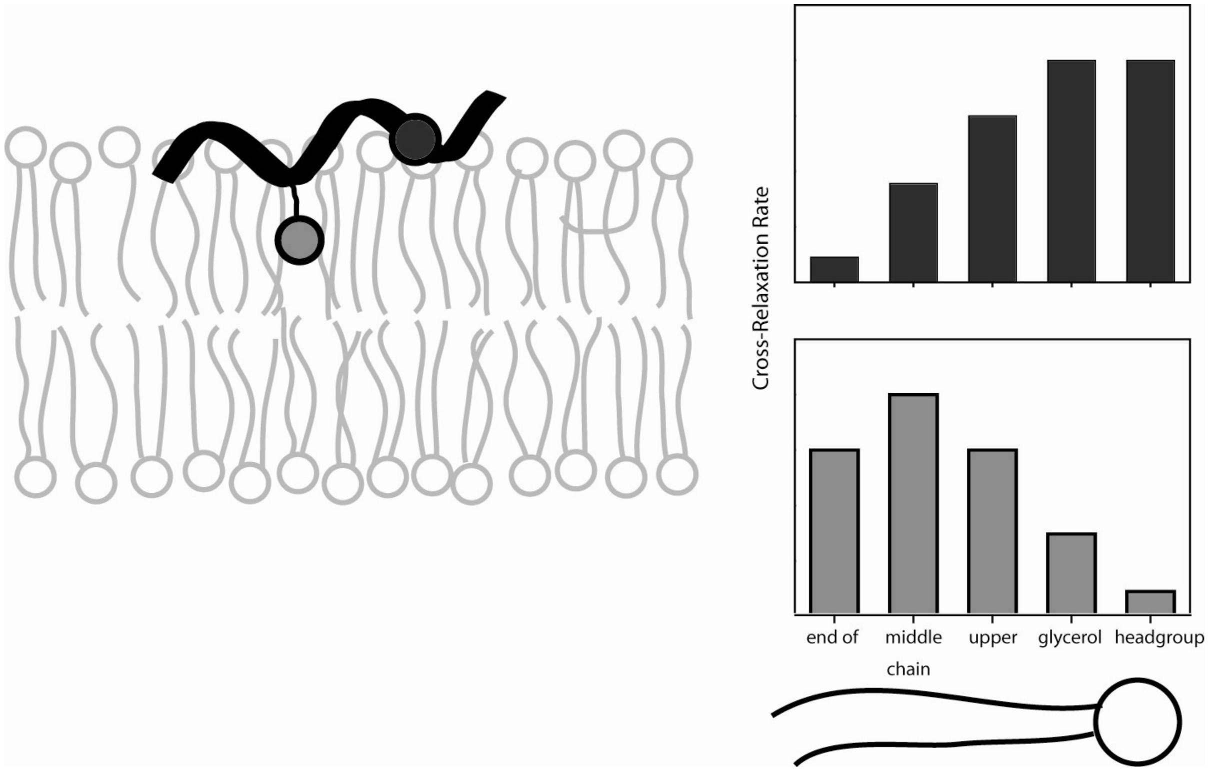


100 ns MD simulation of 4 ras peptides in a matrix of 60 DMPC molecules

S. Feller, Wabash College, Crawfordsville, USA

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
  - ☹ ^{1}H MAS NOESY only works for small peptides



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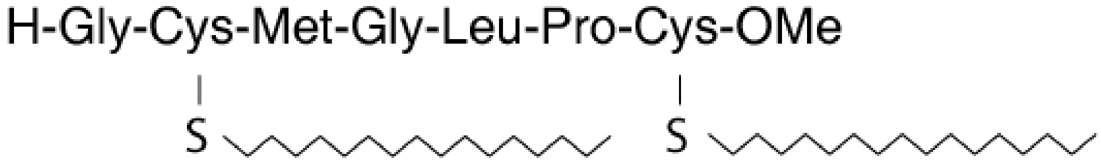
➔ Synthesis of a model peptide:

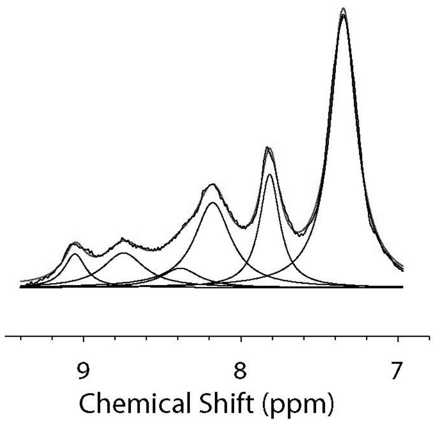


H-Gly-Cys-Met-Gly-Leu-Pro-Cys-OMe

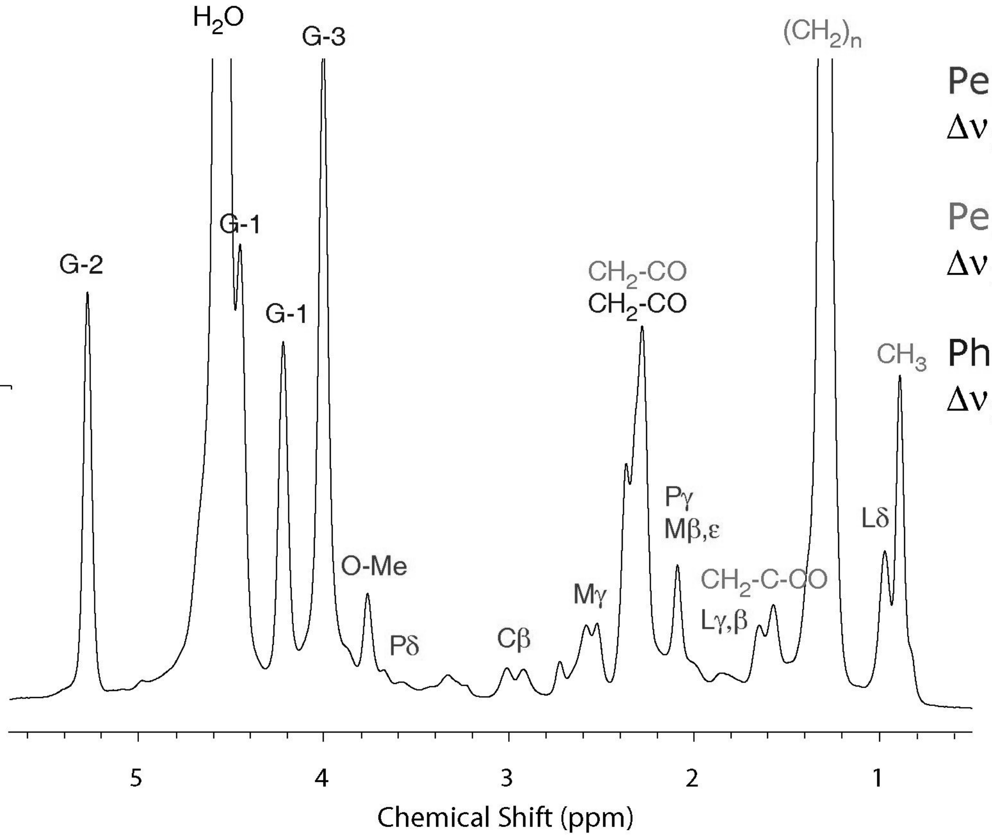
16:0 16:0

➔ 1:10 molar mixing ratio with DMPC , ~50 wt% H_2O

^1H MAS NMR of DMPC/ras Membranes



Amide Signals
$\Delta\nu_{1/2}$ ~ 0.2 ppm



Peptide Side Chains
$\Delta\nu_{1/2}$ ~ 0.1 ppm

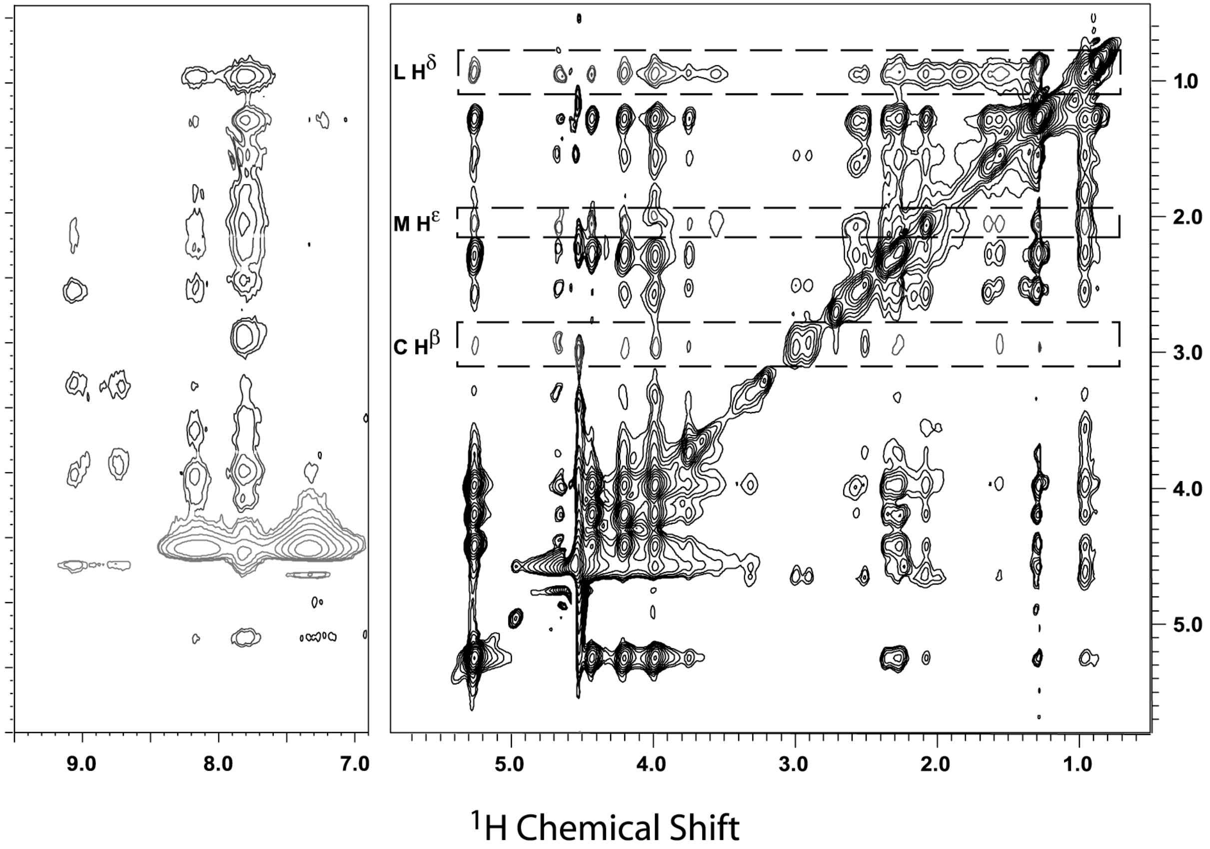
Peptide Lipid Chains
$\Delta\nu_{1/2}$ ~ 0.05 ppm

Phospholipid Signals
$\Delta\nu_{1/2}$ ~ 0.05 ppm

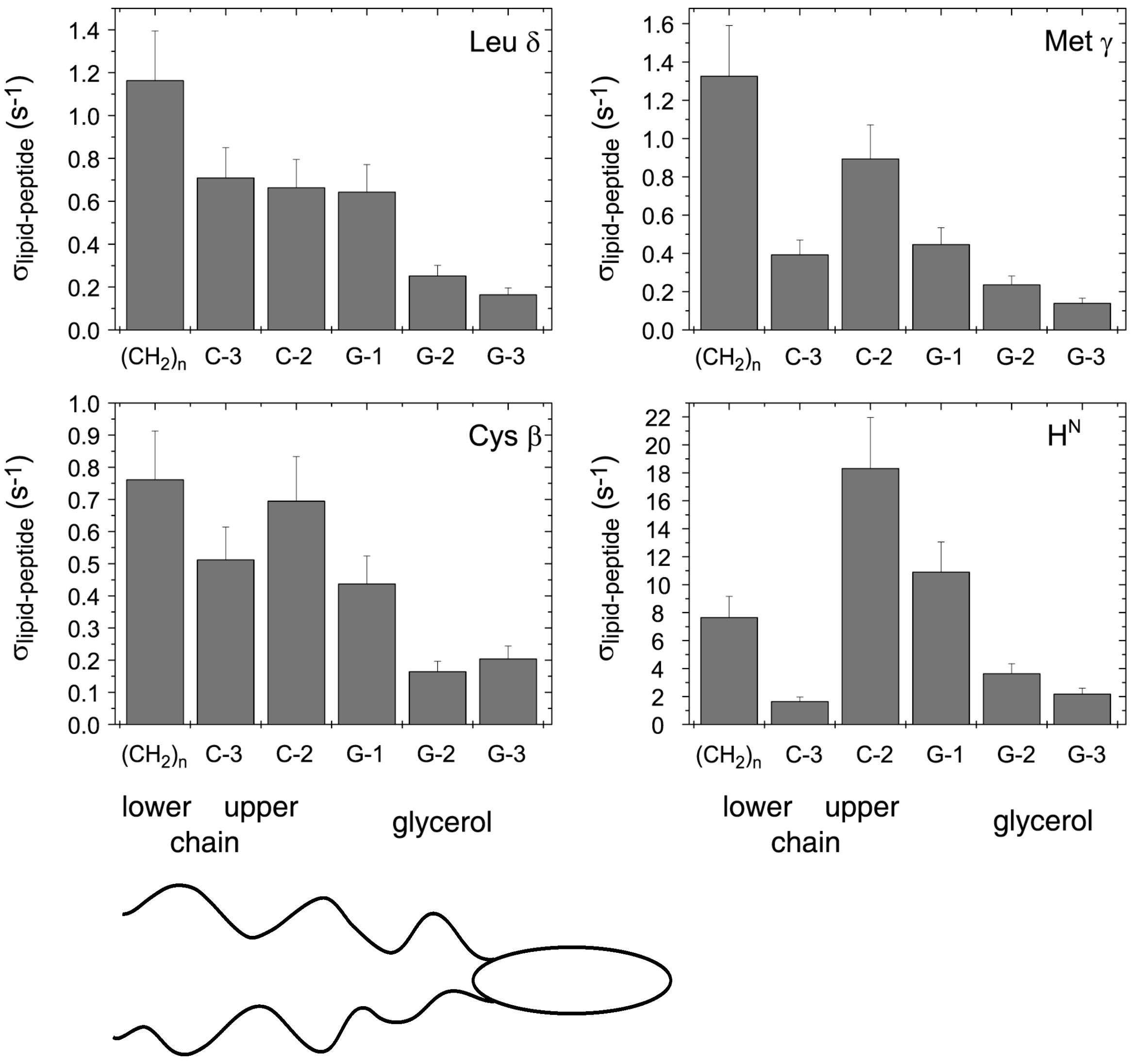
Huster et al., *Angew. Chem. Int. Ed.* **40** (2001) 1056-1058

^{1}H MAS NOESY

- Magnetization exchange is monitored by the appearance of non-diagonal crosspeaks that can be due to
    - ➛ intermolecular lipid-peptide contacts
    - ➛ intramolecular peptide-peptide and lipid-lipid contacts
    - ➛ exchange of labile NH protons with H_2O



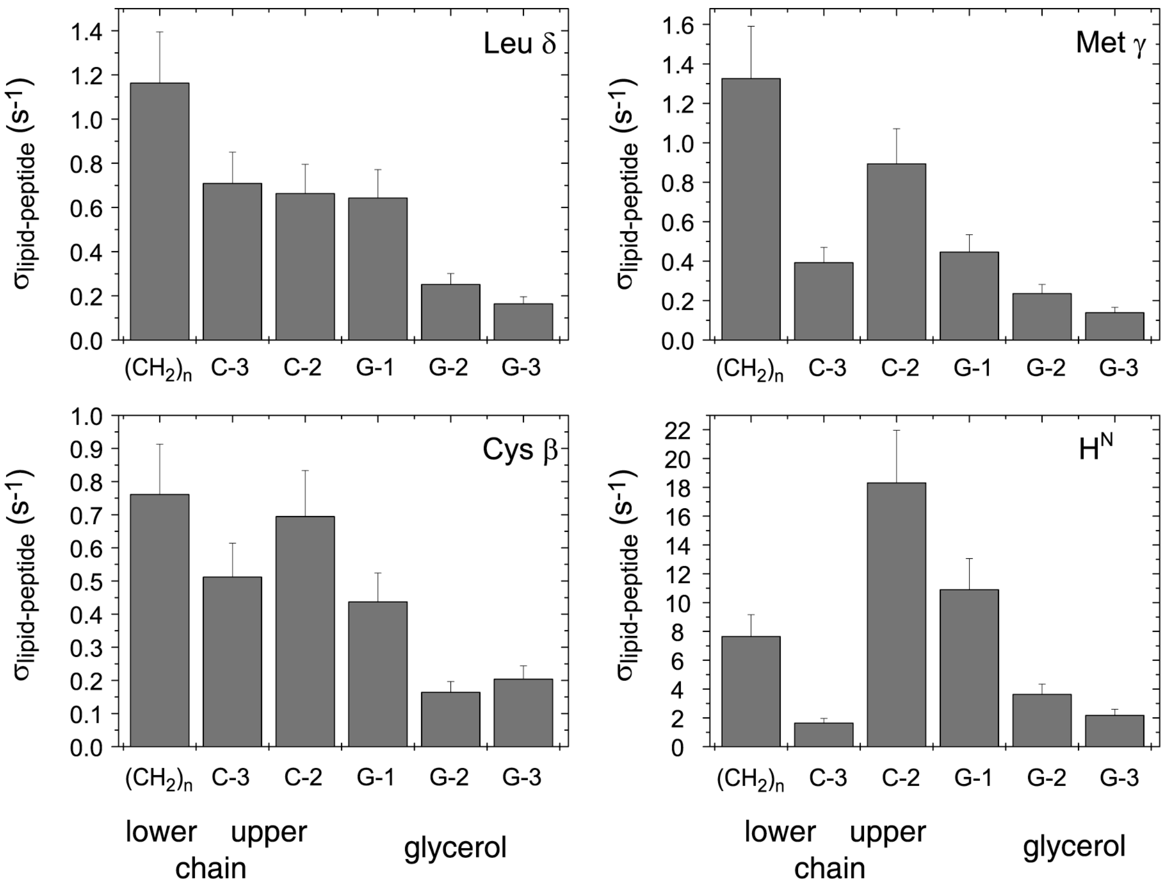
NOESY Cross-relaxation Rates

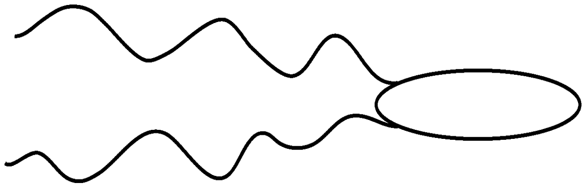


- Ras backbone and sidechains are membrane inserted

Huster et al., *J. Am. Chem. Soc.* **125** (2003) 4070-4079

NOESY Cross-relaxation Rates





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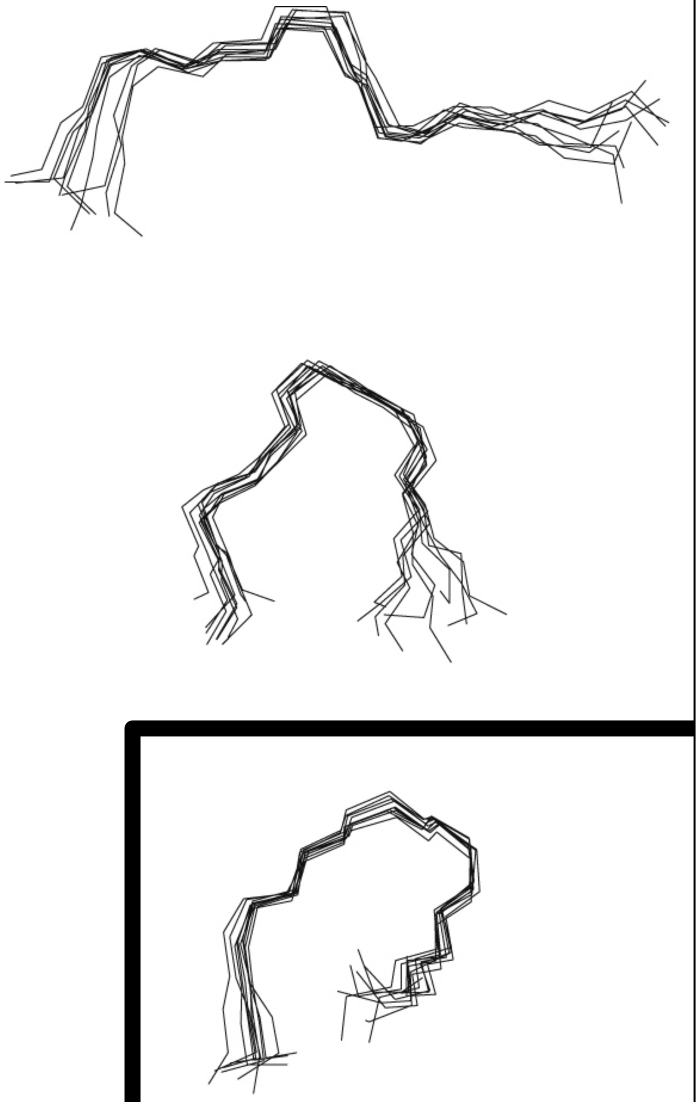
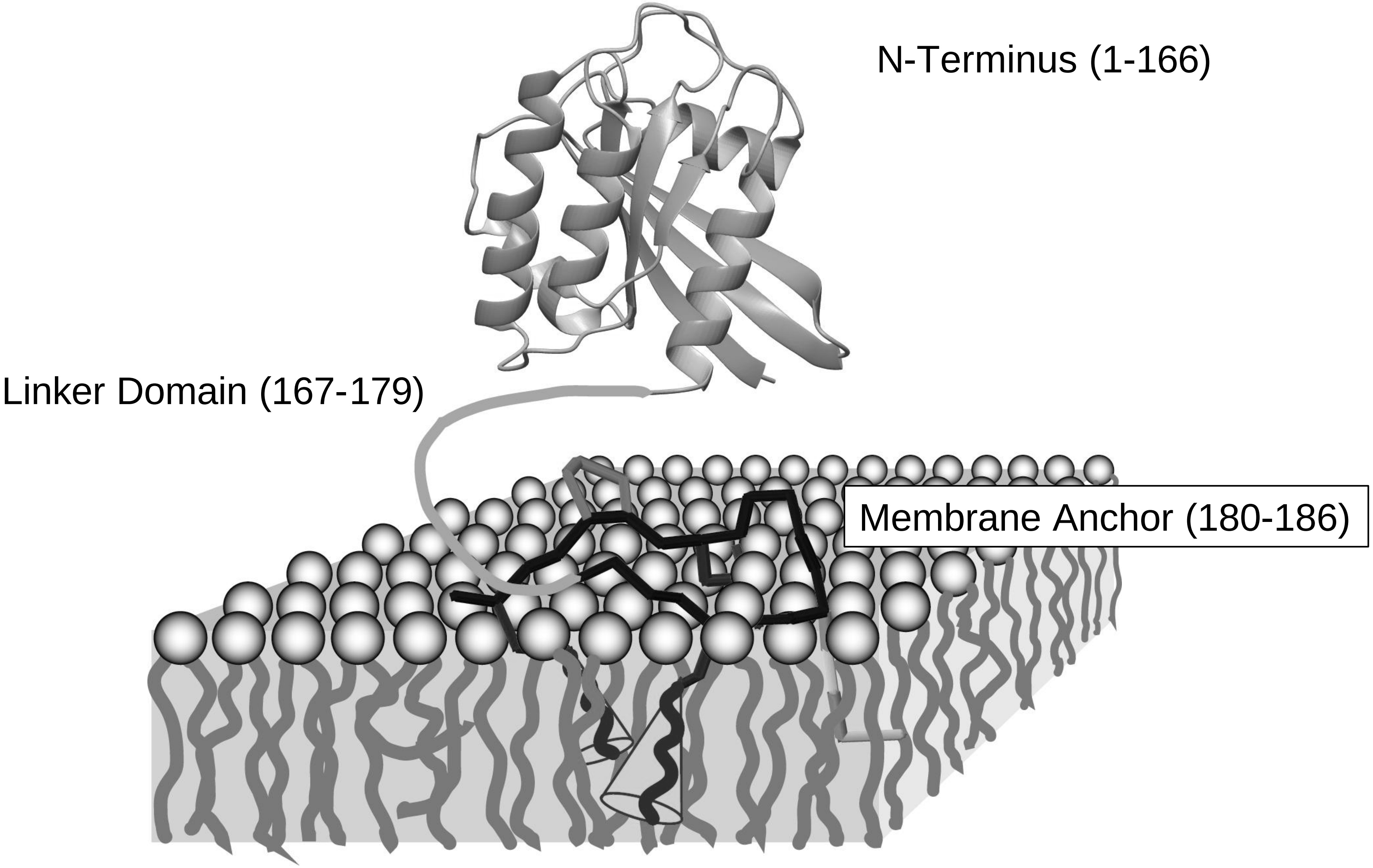
Structure of the C-Terminus of Membrane Bound Ras Protein



TALOS Structural Model of the C-Terminus of Ras Protein

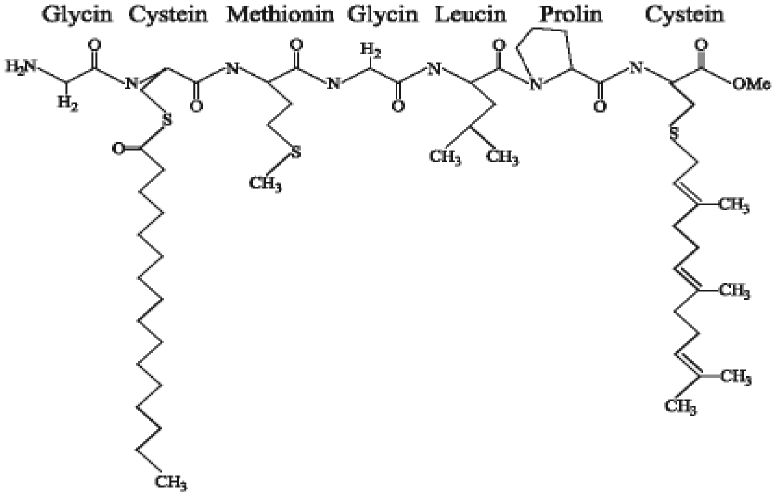


Reuther et al., *Angew. Chem. Int. Ed.* **45** (2006) 5387-5390

Structure Refinement

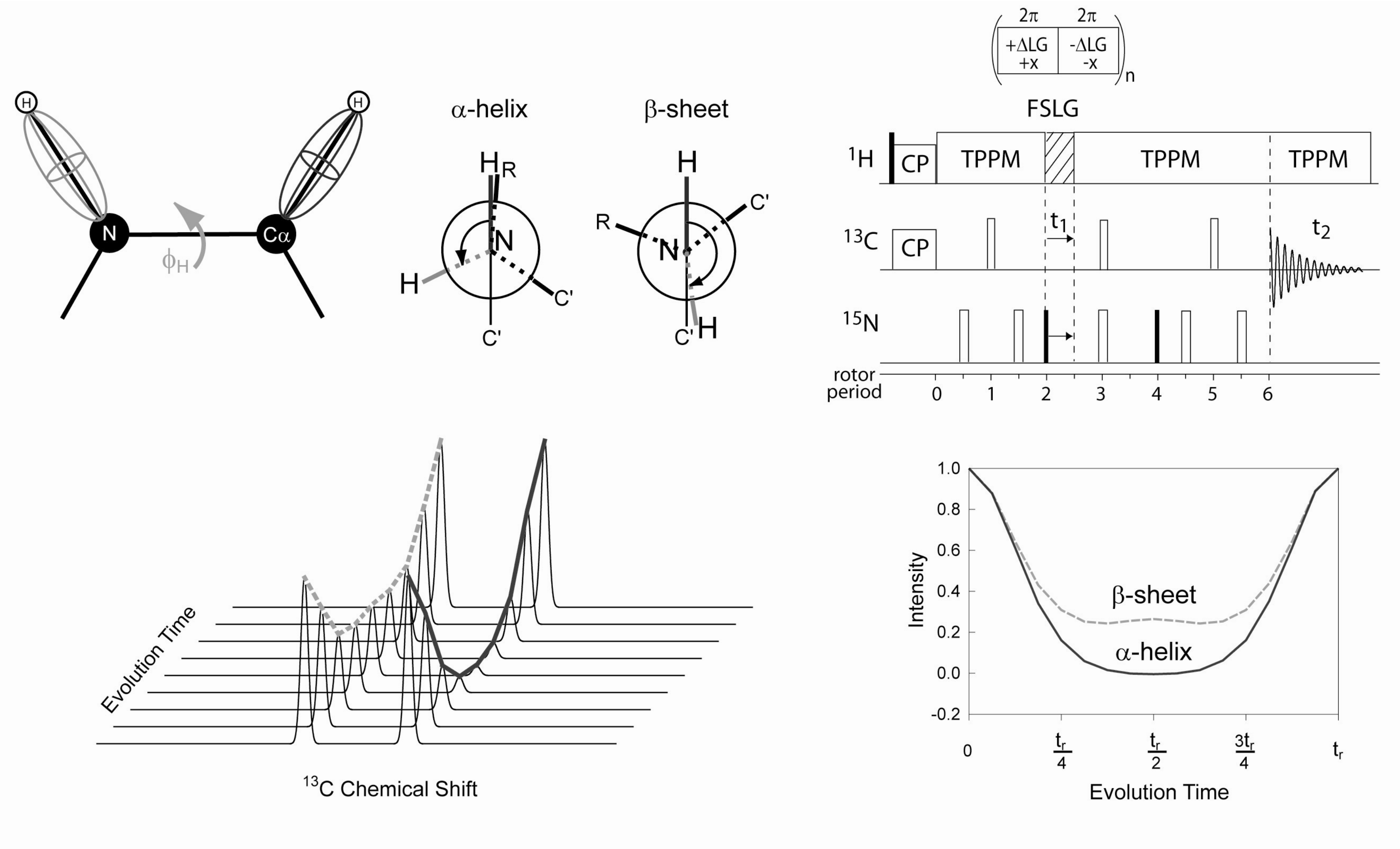
- TALOS prediction is not unique!
- No side chain information from isotropic chemical shifts

➔ Get more structural constraints from the ras peptide:

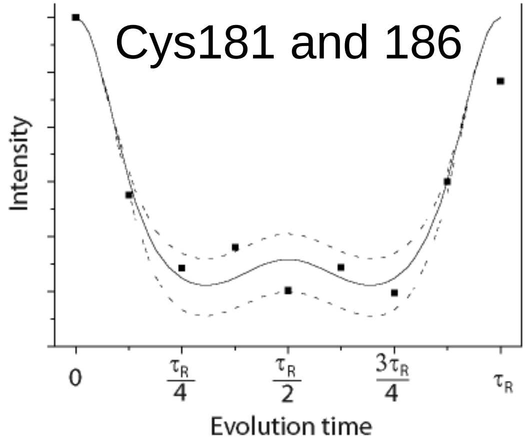


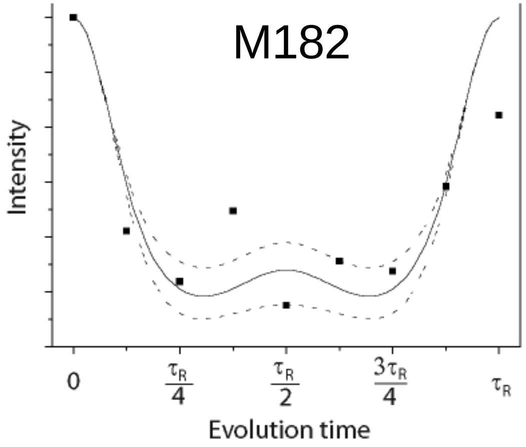
- fully $^{13}C/^{15}N$ labeled
- phospholipid / peptide ratio: 10:1

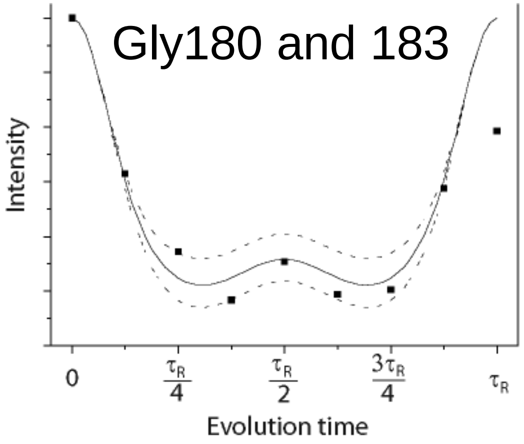
Torsion Angle Measurement

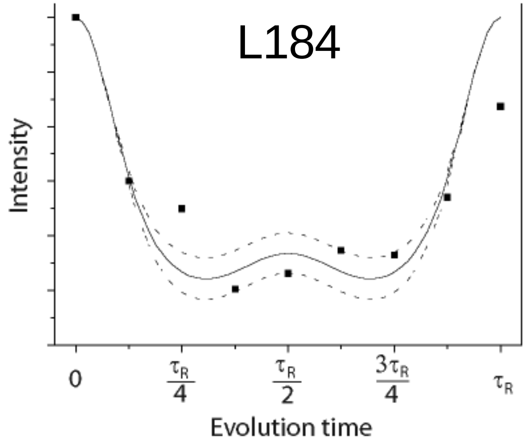


Torsion Angle Measurement


Cys181 and 186


M182


Gly180 and 183

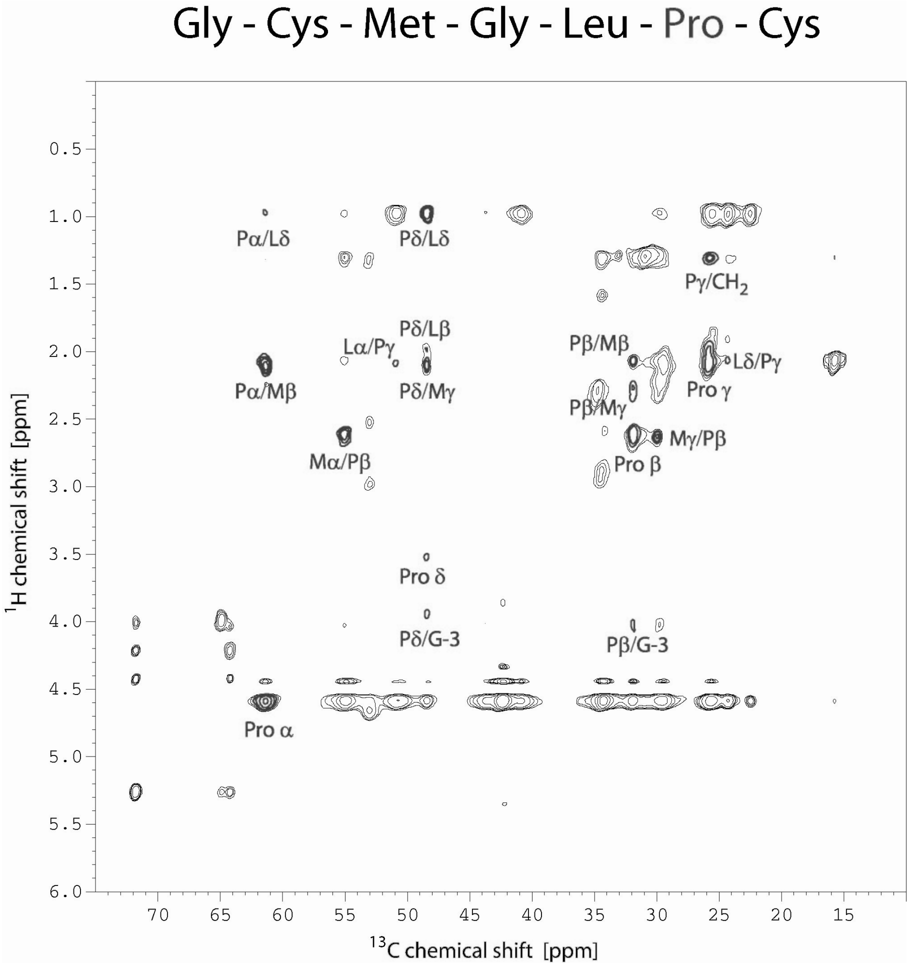

L184

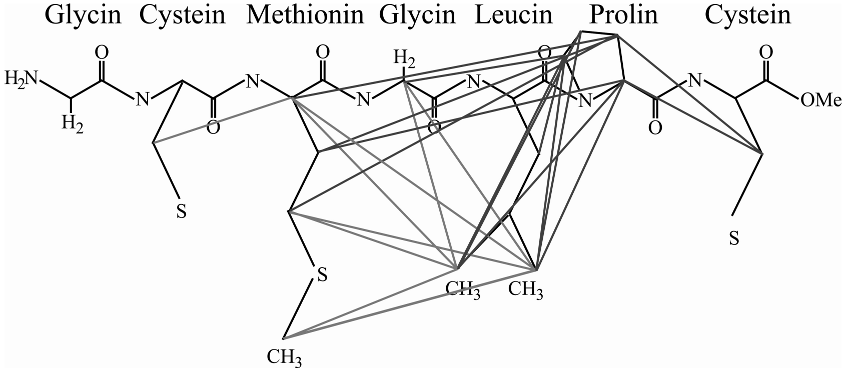
Residue	$\Phi_{experimentell}$	Φ_{Talos}
cysteine	-143° ± 31°	-122° ± 23°
methionine 182	-91° ± 31°	-75° ± 15°
glycine	-97° ± 28°	86° ± 8°
leucine 184	-100° ± 20°	-100° ± 23°

➔ Experimentally determined torsion angles confirm predictions by TALOS.

^{13}C Detected ^{1}H-^{1}H NOEs

- Measure intramolecular NOEs (for protons: distances ≤5 Å)
- ^{13}C detection for better resolution
- Mixing time of 100 ms

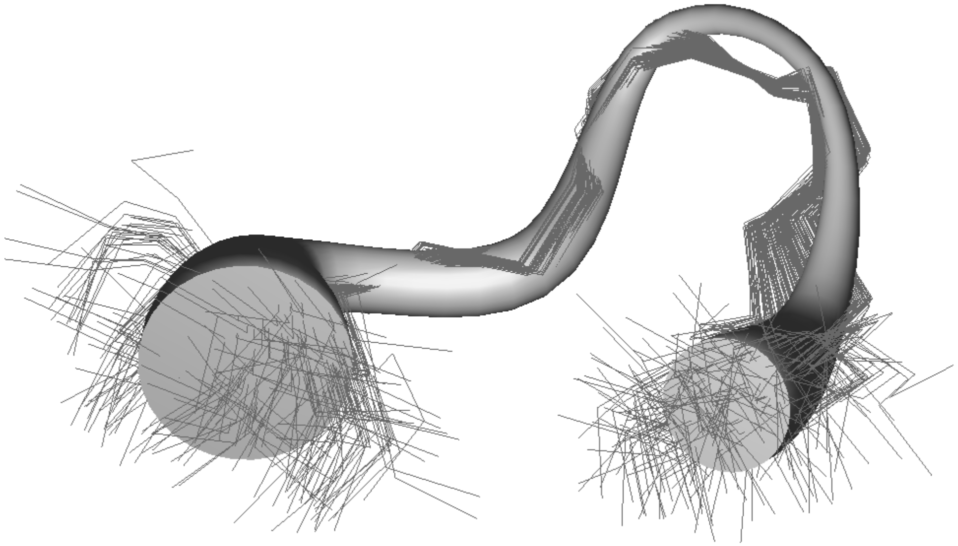




17 additional NOE constraints

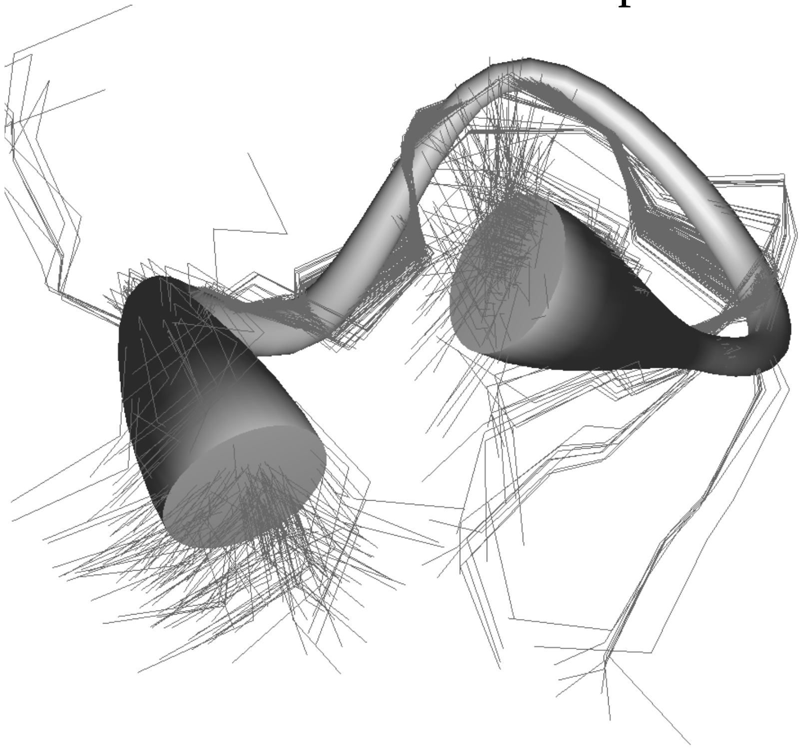
Backbone Structure of Ras

Ras peptide



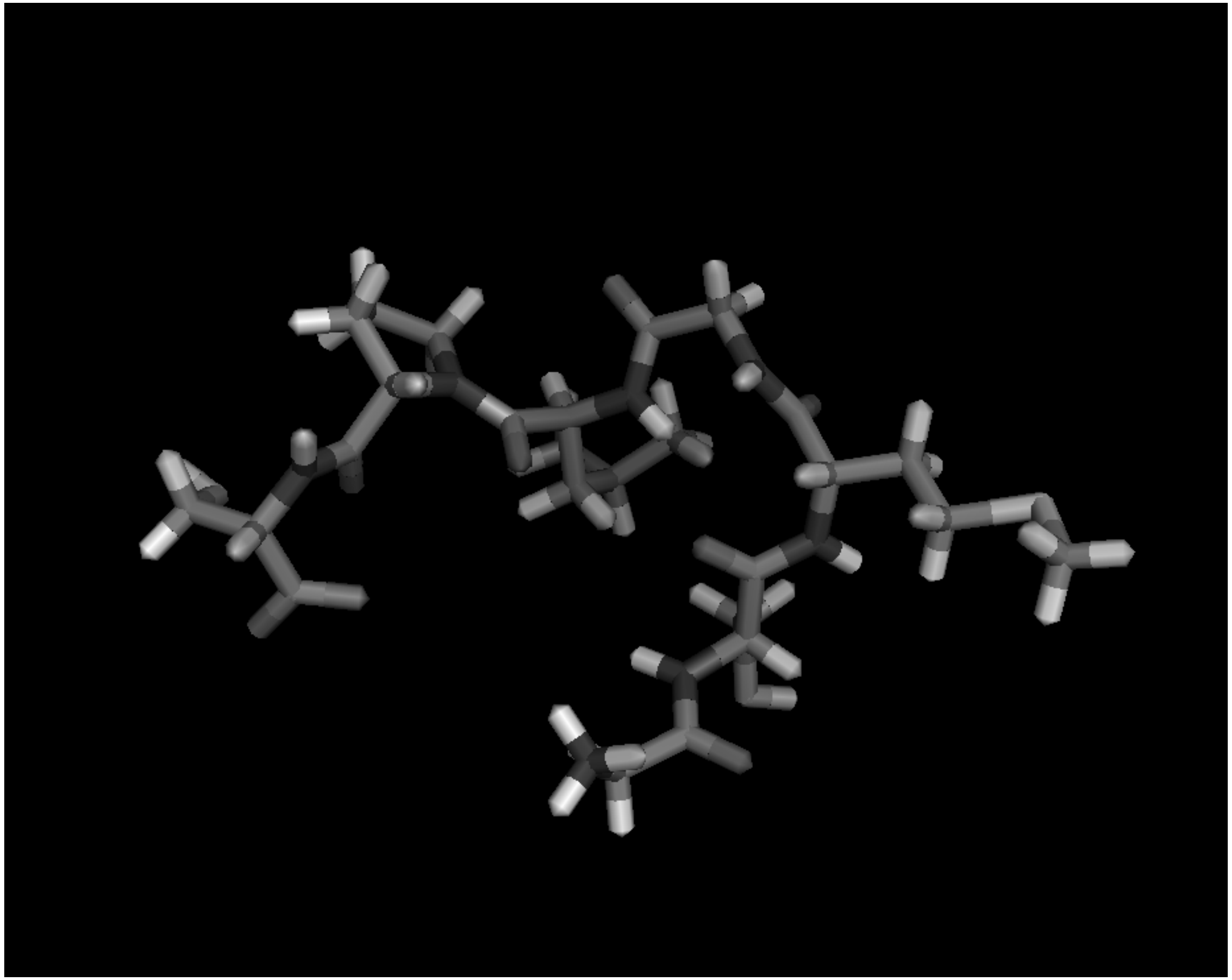
28 constraints

C-terminus of the Ras protein



11 constraints

Structural Model of Membrane-Associated Ras



Acknowledgements

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  Junior Research Group, Martin Luther University Halle-Wittenberg

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  Christine Nowak, Jürgen Kuhlmann,
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