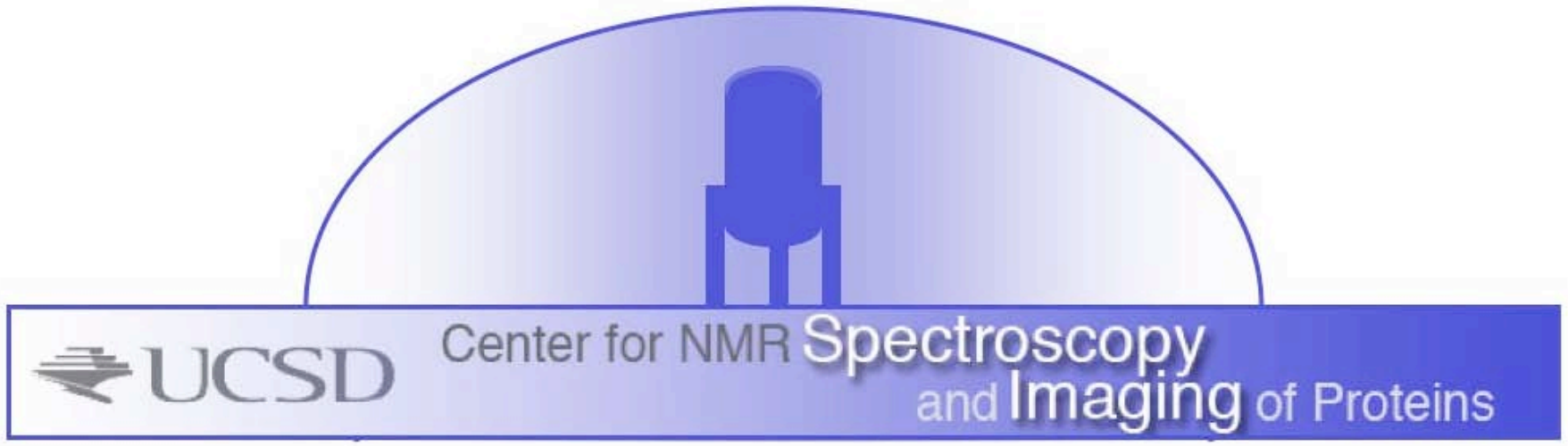
Winter School on Biomolecular Solid State NMR

January 20 - 25, 2008

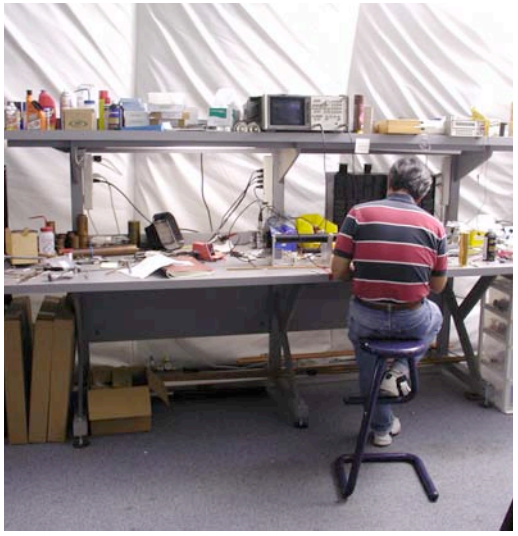
"Selected Topics Lecture"

Stanley J. Opella



University of California, San Diego

Center for NMR Spectroscopy and Imaging of Proteins



Solid-state NMR of membrane proteins in phospholipid bilayers.

bilayer samples

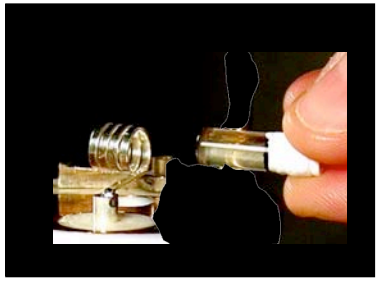
150 μl volume
q = 3.2 (DMPC:DHPC)
1 mg - 3 mg protein
28% lipid w/v
pH = 4 - 8
15 °C – 50 °C

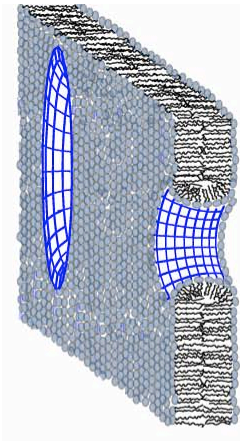
NMR probes

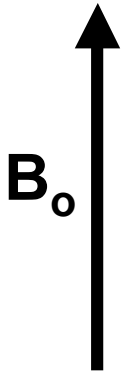
Outer modified Alderman-Grant
Inner 5 mm solenoid

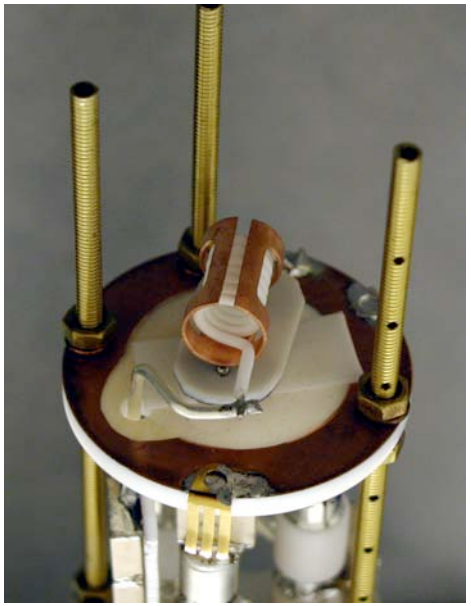
pulse sequences

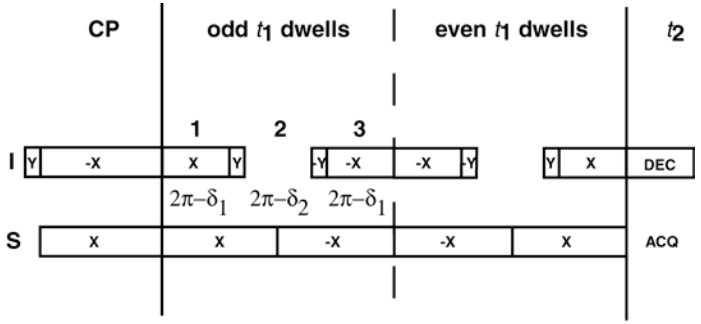
SAMPI4











Selected Topics

- **Part I: Recent developments in solid-state NMR of aligned samples.**
  - Lipid bilayers.
  - Double-resonance experiments.
  - Triple-resonance experiments.
- **Part II: Phospholipid bilayers are essential for biological relevance.**
  - Mercury transport membrane proteins.
  - Vpu from HIV.

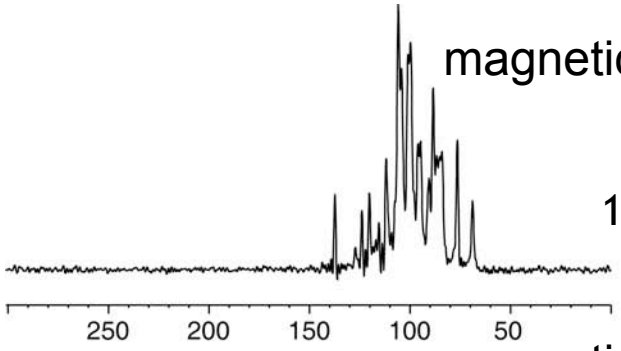
Part I: Recent developments in solid-state NMR of aligned samples.

Lipid bilayer samples.

Solid-state NMR spectra of Pf1 coat protein in phospholipid bilayers.

magnetically-aligned bilayers

14-O-PC/6-O-PC=3.2



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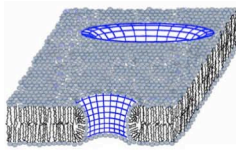
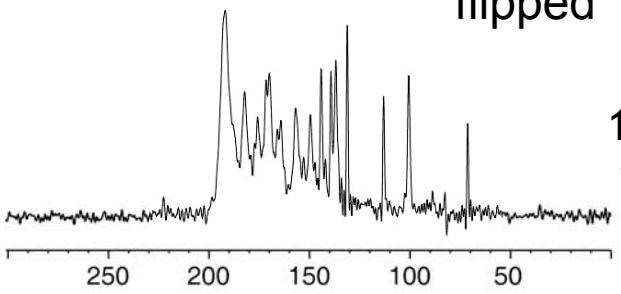
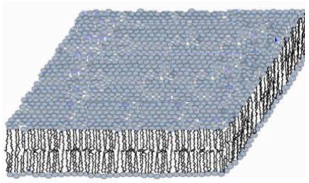


magnetically-aligned bilayers
'flipped' with lanthanide

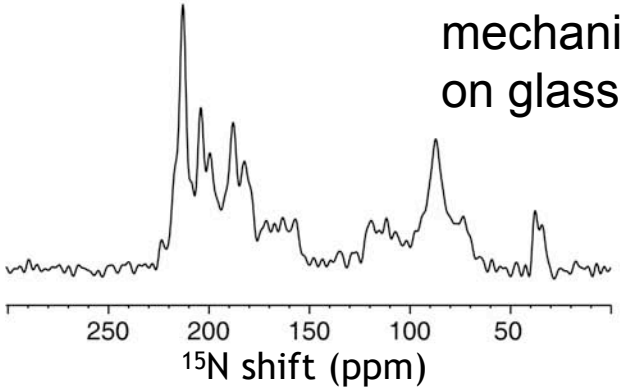
14-O-PC/6-O-PC=3.2
with Yb^{3+}





mechanically-aligned bilayers
on glass plates

DOPC/DOPG (9:1)

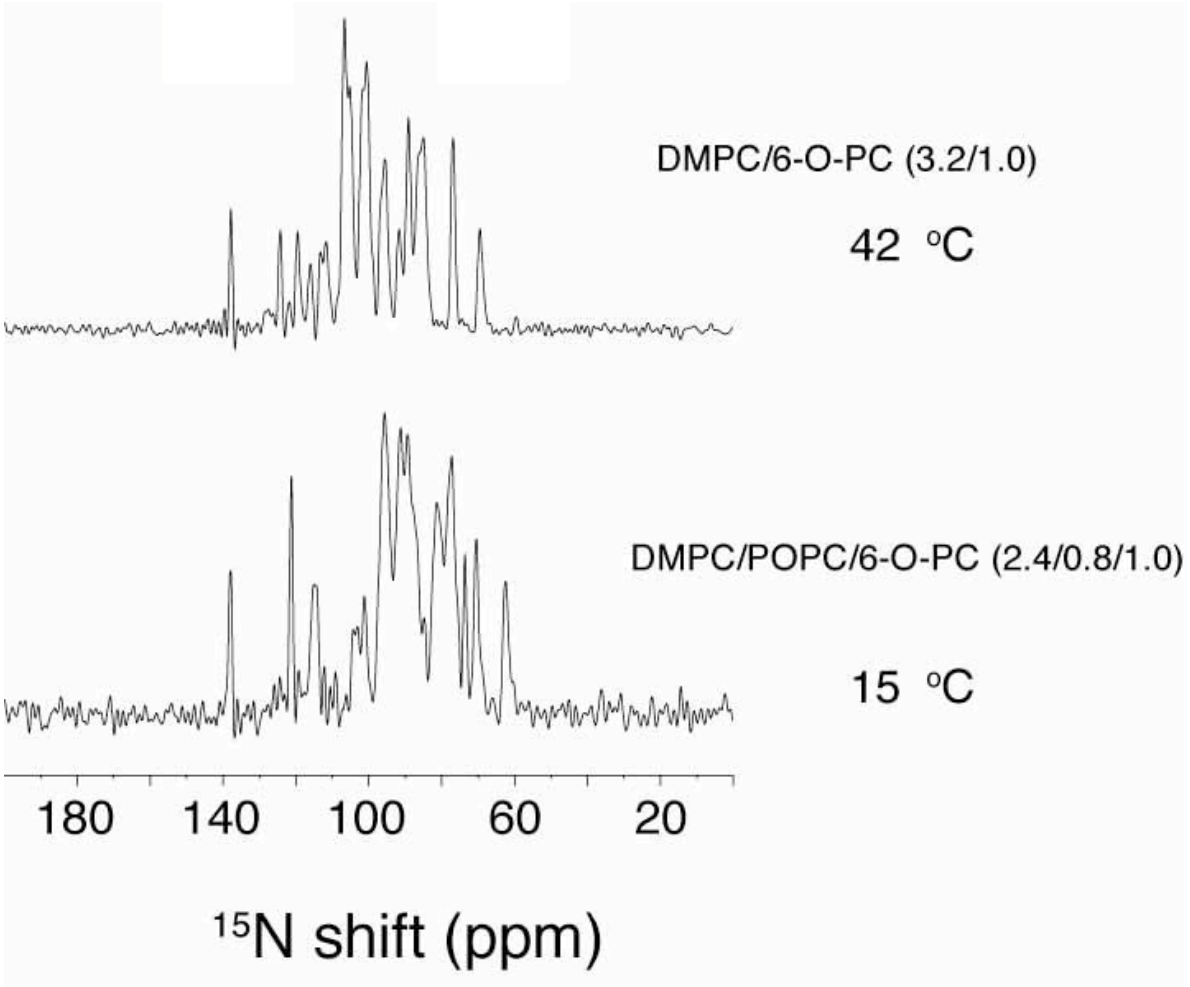


Pf1 coat protein

Magnetic alignment of bilayers at low temperatures without loss of resolution.

Effects of Lipid Chain Length and Unsaturation on Bicelles Stability. A Phosphorus NMR Study

Mohamed N. Triba, Philippe F. Devaux, and Dror E. Warschawski
Unité Mixte de Recherche No. 7099, Centre National de la Recherche Scientifique, Institut de Biologie Physico-Chimique, Paris, France

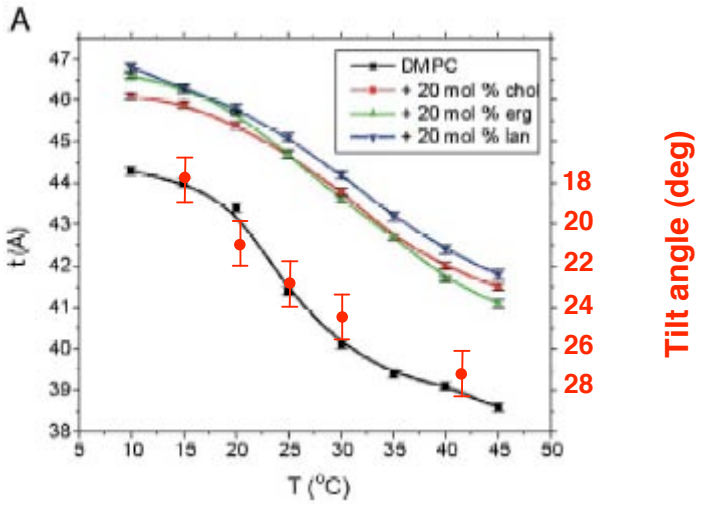


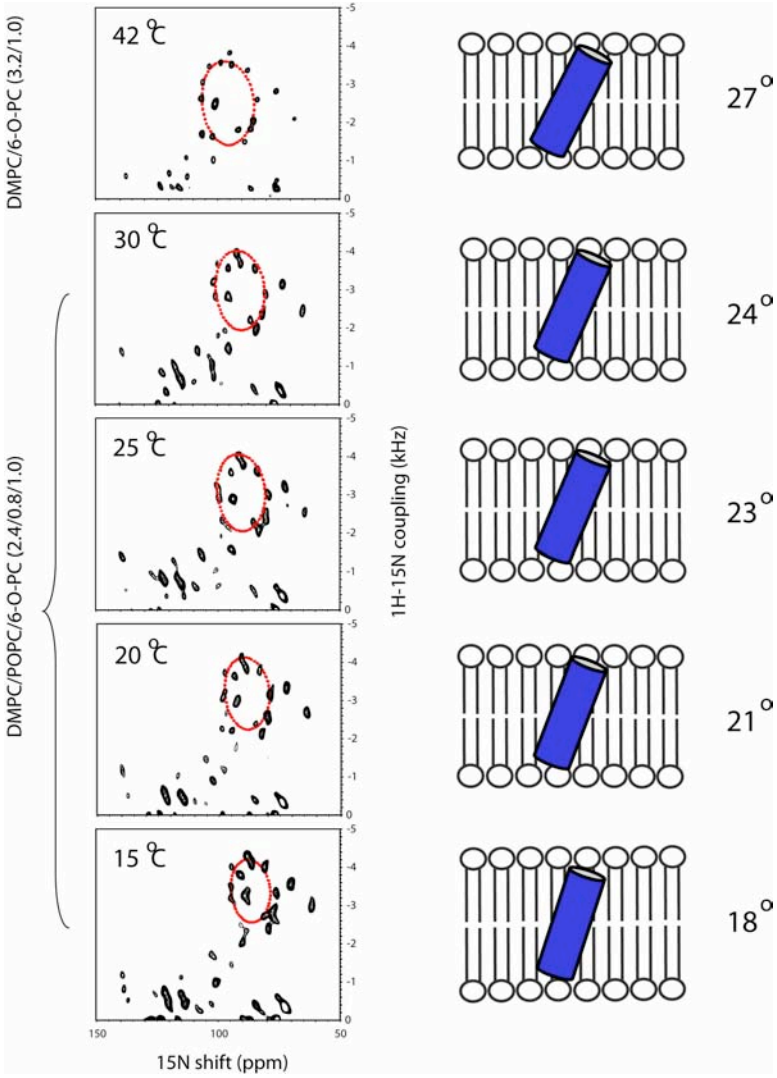
Vpu TM domain

2006

TM helix tilt angle determined by bilayer thickness.

bilayer thickness vs. temperature





Temperature: Pencer et al 2005
Addition of POPC: Triba et al 2006

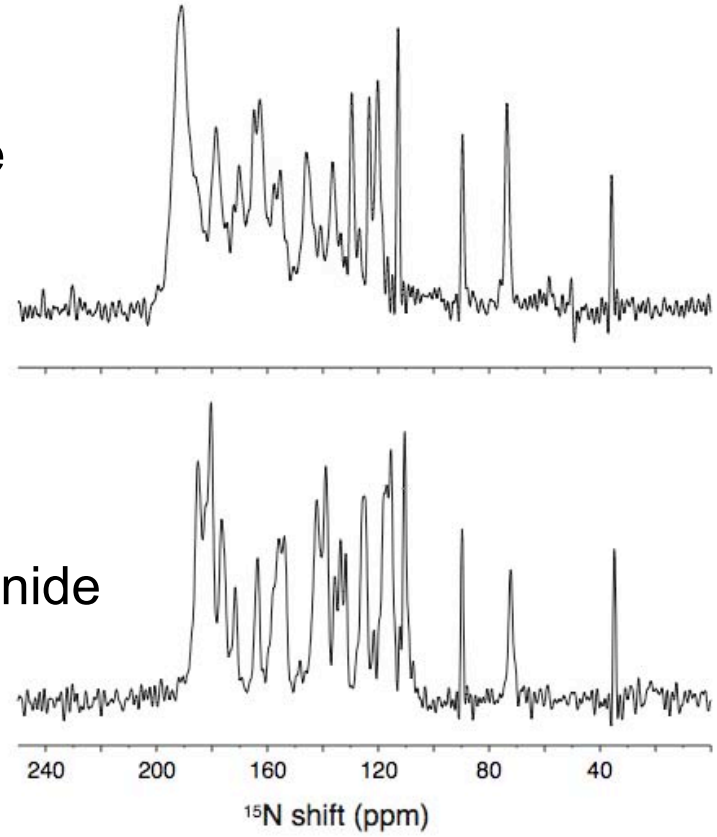
Parallel alignment without lanthanides.

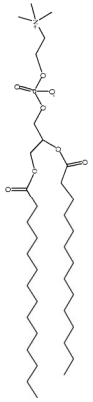
Biphenyl Bicelle Disks Align Perpendicular to Magnetic Fields on Large Temperature Scales: A Study Combining Synthesis, Solid-State NMR, TEM, and SAXS

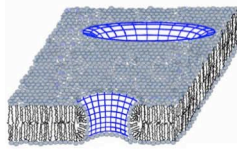
Cécile Loudet,* Sabine Manet,* Stéphane Gineste,† Reïko Oda,* Marie-France Achard,† and Erick J. Dufourc*
*UMR 5248 CBMN, CNRS-Université Bordeaux 1-ENITAB, Institut Européen de Chimie et Biologie, Pessac, France; and
†UPR 8641, Centre de Recherche Paul Pascal, CNRS, Pessac, France

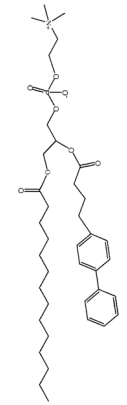
q = 3.2 bicelles

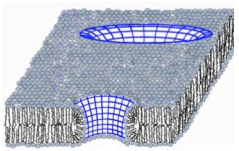
DMPC with lanthanide











B_0

TBBPC <u>without</u> lanthanide

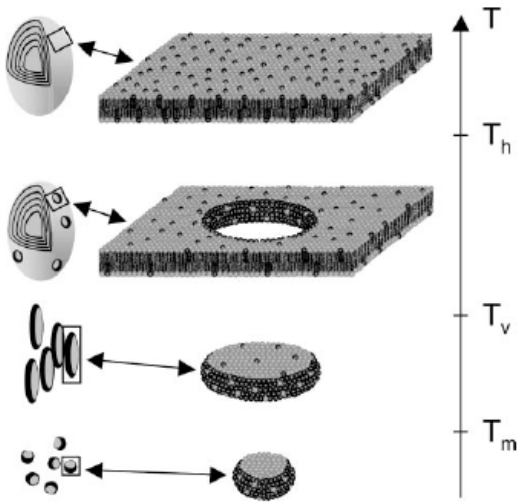
Biphenyl group confers positi
magnetic anisotropy

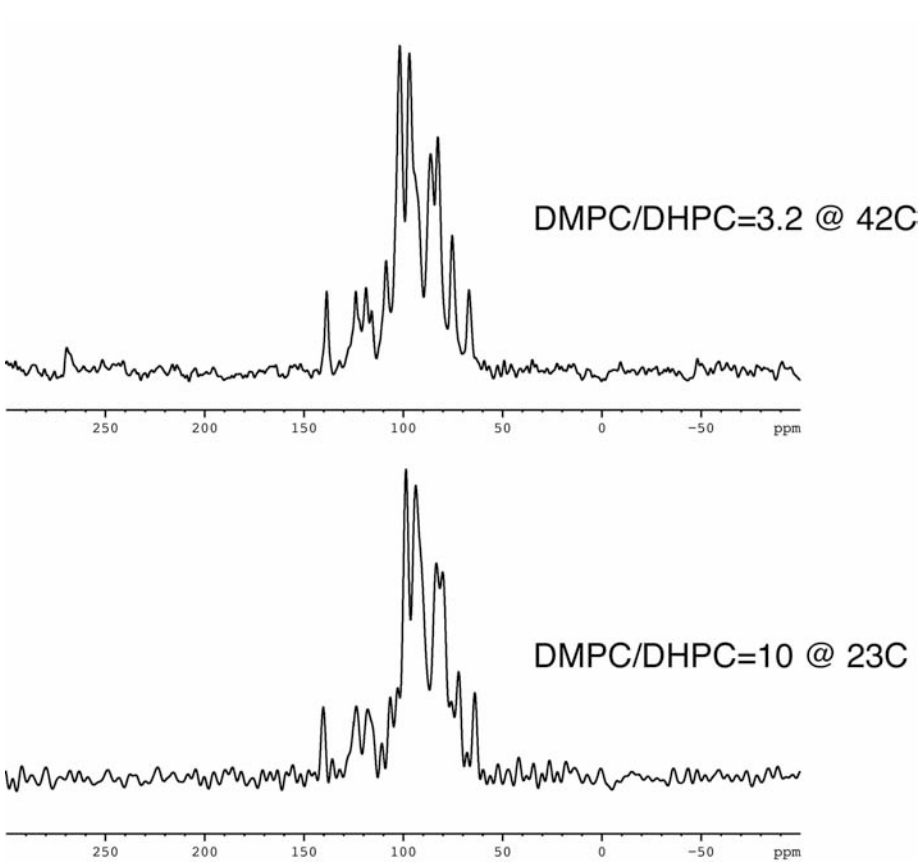
Pf1 coat protein

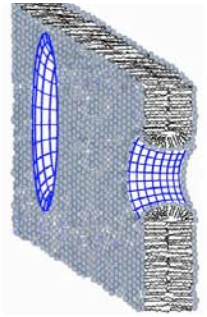
Sang Ho Park, <u>Cecille Loudet</u>, Francesca Marassi and Erick Dufourc

Reinvestigation by Phosphorus NMR of Lipid Distribution in Bicelles

Mohamed N. Triba, Dror E. Warschawski, and Philippe F. Devaux
Unité Mixte de Recherche No. 7099, Centre National de la Recherche Scientifique, Institut de Biologie Physico-Chimique, Paris, France







B_0

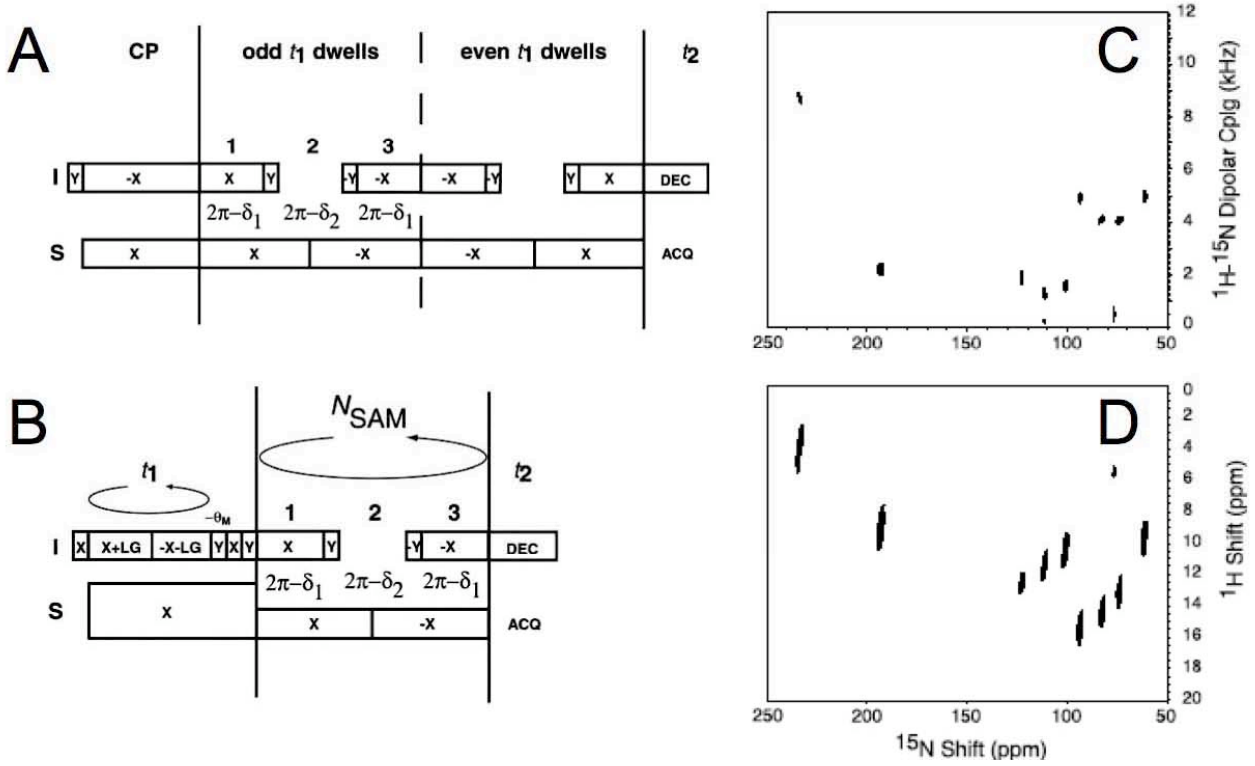
2005

Double resonance experiments.

Selective averaging for high-resolution solid-state NMR spectroscopy of aligned samples

Alexander A. Nevzorov, Stanley J. Opella *

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0307, USA



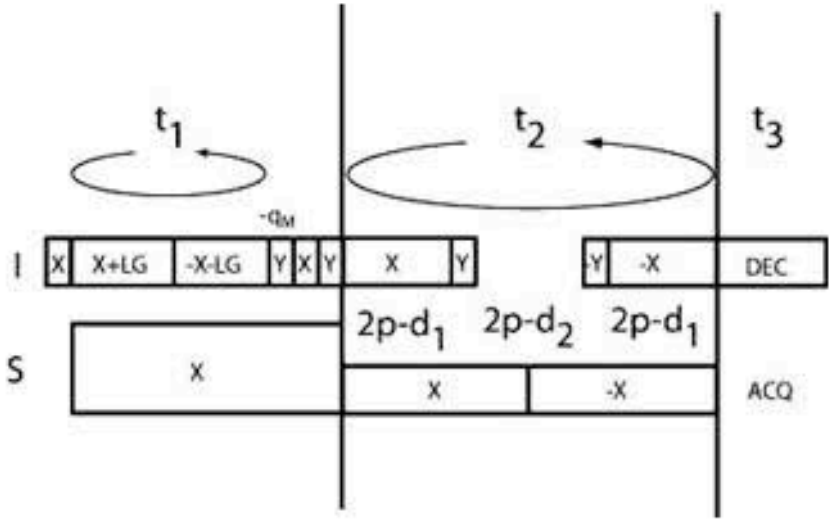
SLF

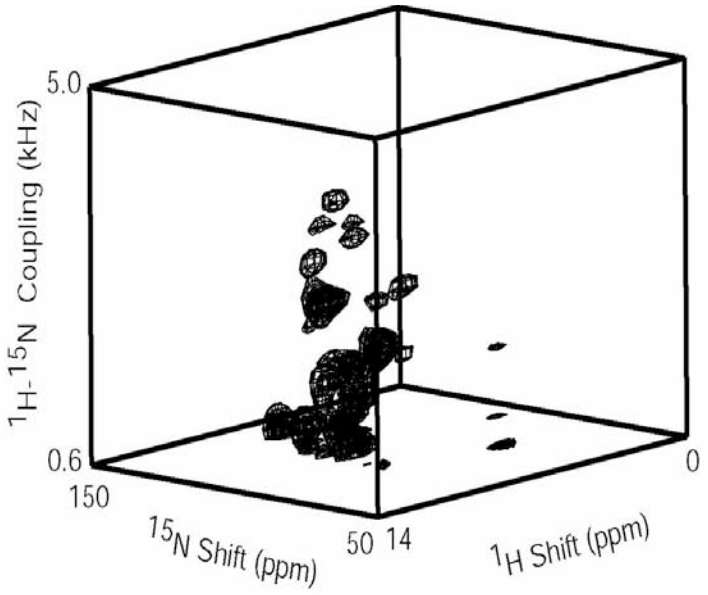
HETCOR

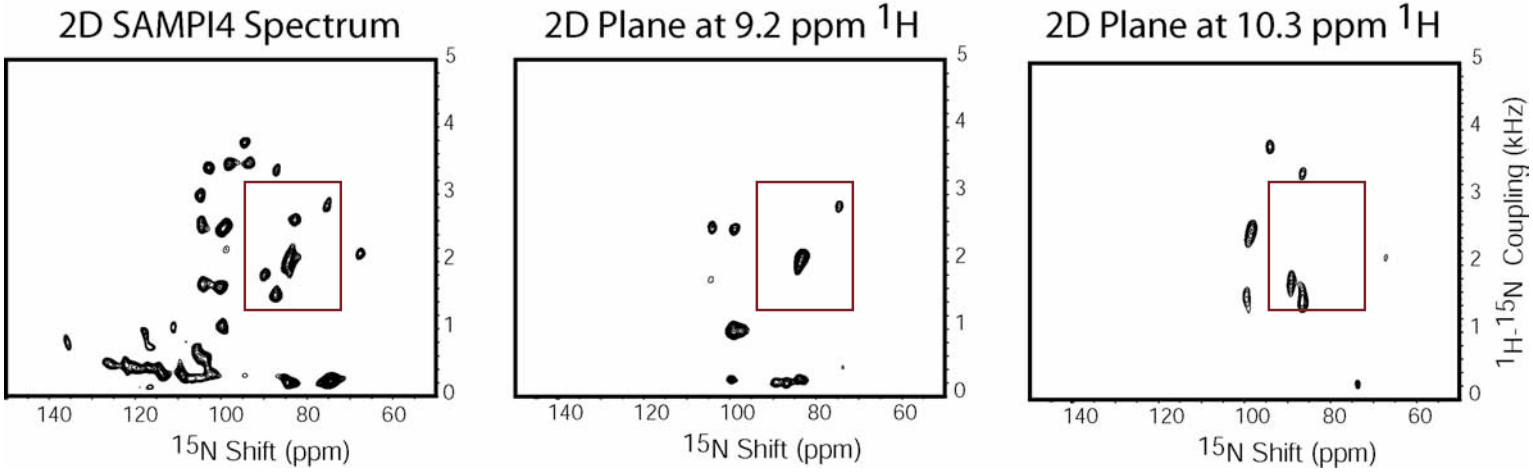
at 900 MHz ^{1}H shift spans 14 kHz and ^{15}N shift spans 16 kHz

2007

SAMPI4 based ^{1}H Shift/^{1}H-^{15}N Coupling/^{15}N Shift experiment at 900 MHz on a magneticaly aligned bicelle sample.







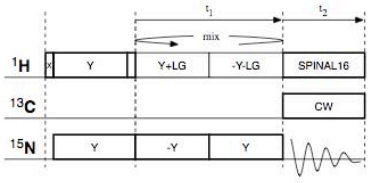
Proton-detected separated local field spectroscopy

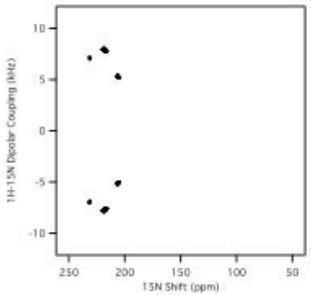
Chin H. Wu, Stanley J. Opella *

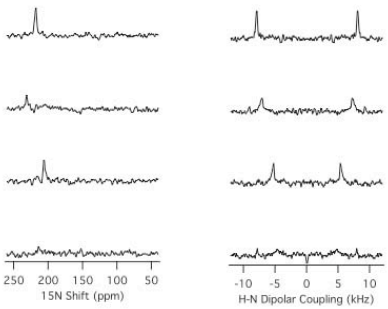
Department of Chemistry and Biochemistry, 9500 Gilman Drive, University of California, San Diego, La Jolla, CA 92093-0307, United States

2008

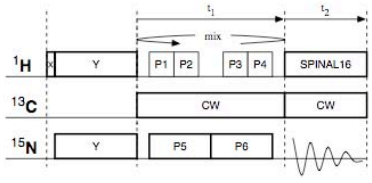
^{15}N-detected PISEMA

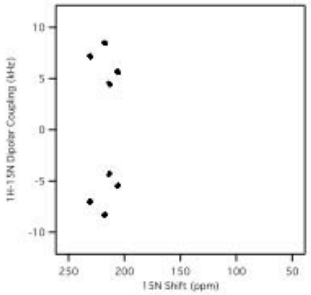


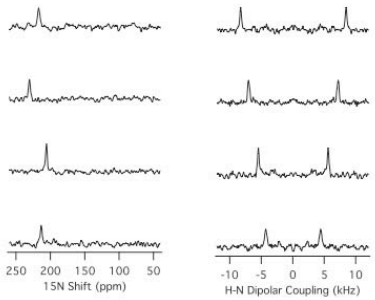




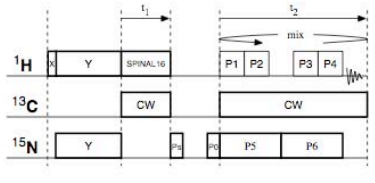
^{15}N-detected PISEMO

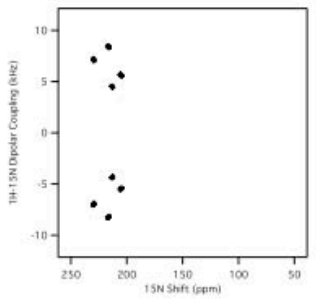


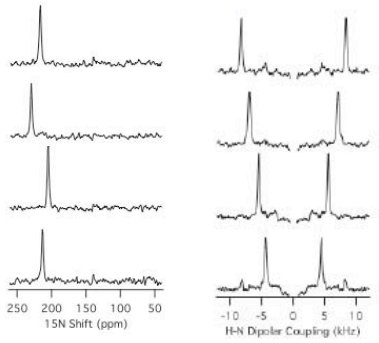




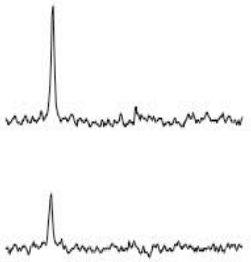
^{1}H-detected PISEMO







^{1}H vs 15N



Triple resonance experiments.

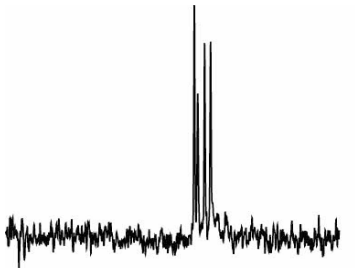
^{13}C vs ^{15}N detection of ^{15}N, $^{13}C\alpha$ labeled peptides.

single crystal of N-acetyl-leucine

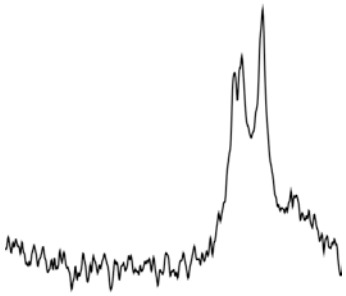
aligned sample of Leu (4 sites) labeled Pf1 bacteriophage

1H →13C (detect)

8 scans



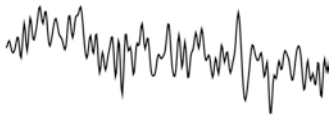
128 scans



1H →15N (detect)

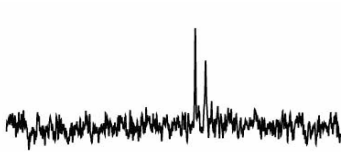
8 scans

128 scans

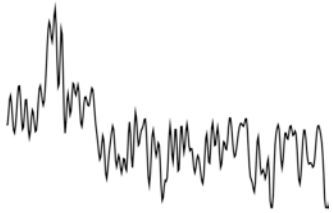


1H →15N→13C (detect)

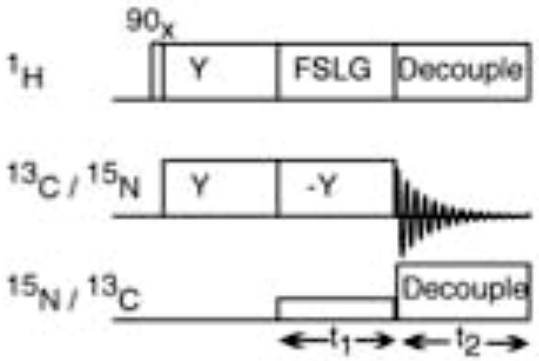
8 scans

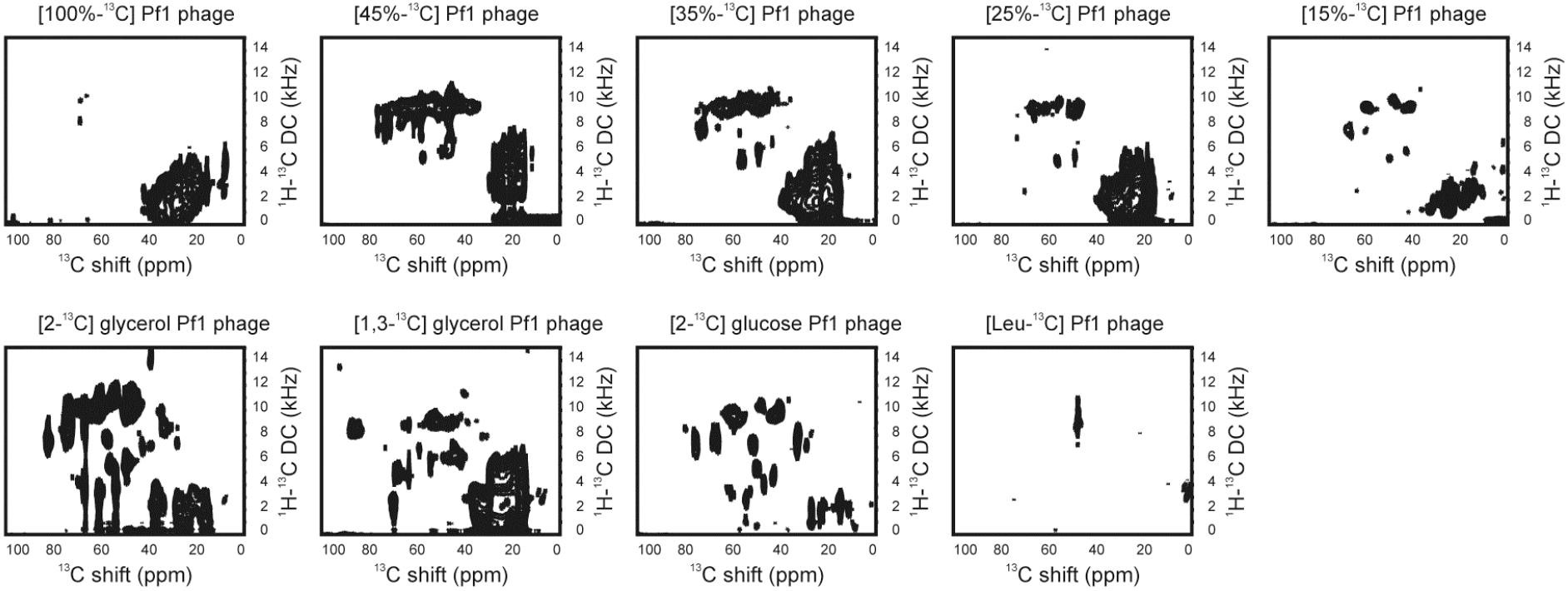


500 scans

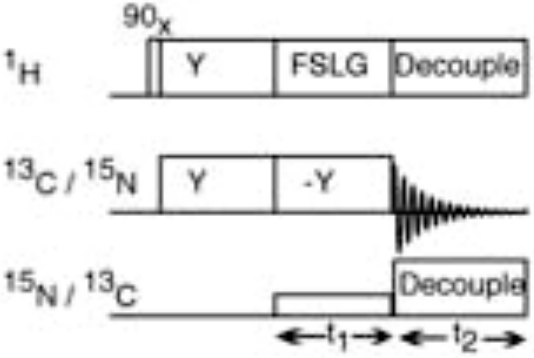


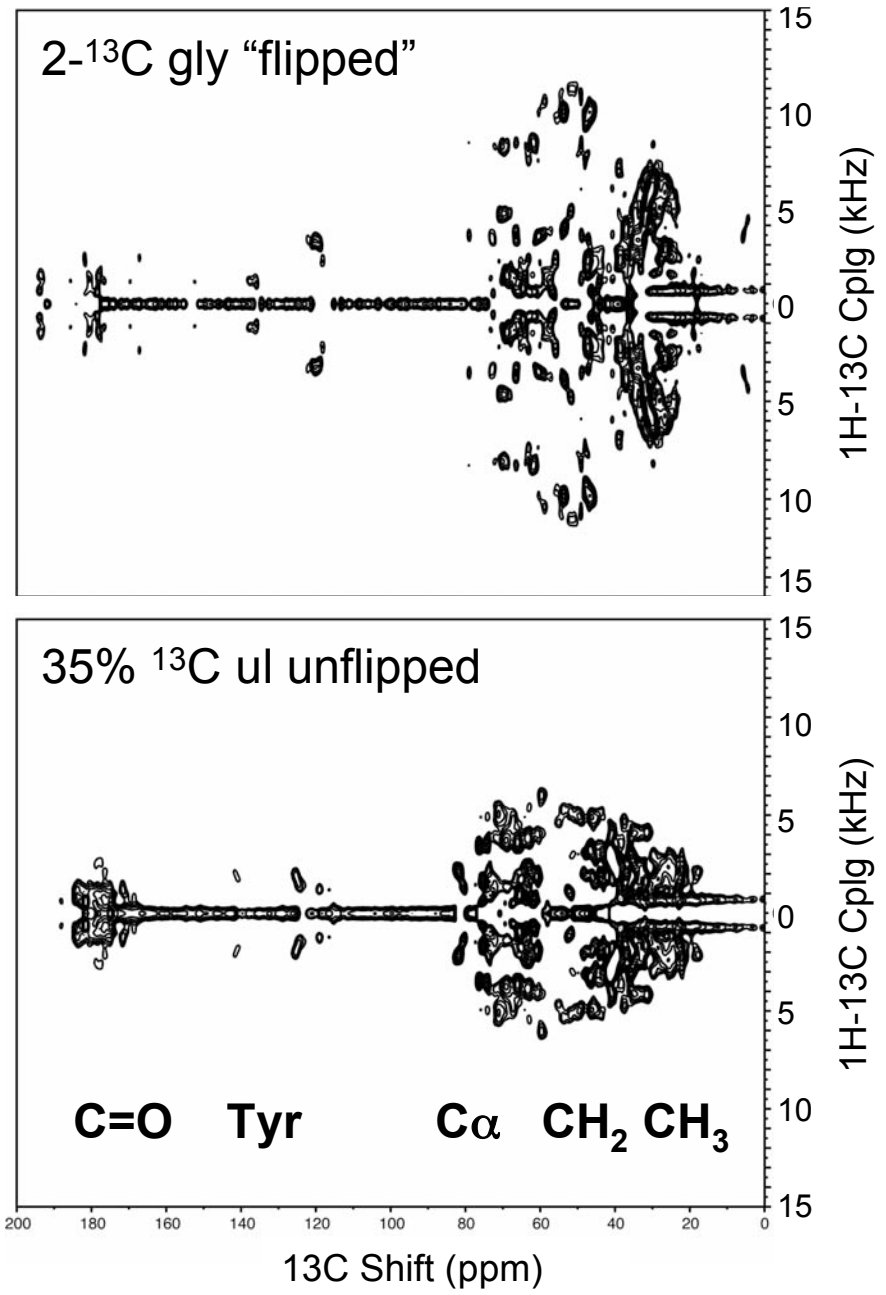
^{1}H-^{13}C PISEMA of ^{13}C and ^{15}N labeled Pf1 coat protein in aligned bacteriophage.

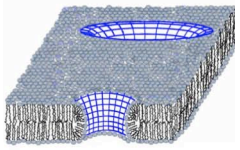




^{1}H-^{13}C PISEMA of Pf1 coat protein in bicelles.

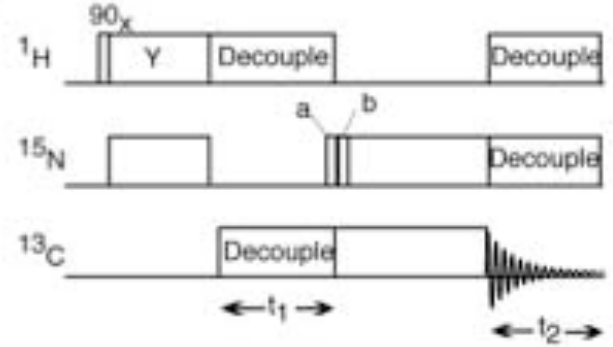


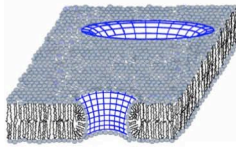


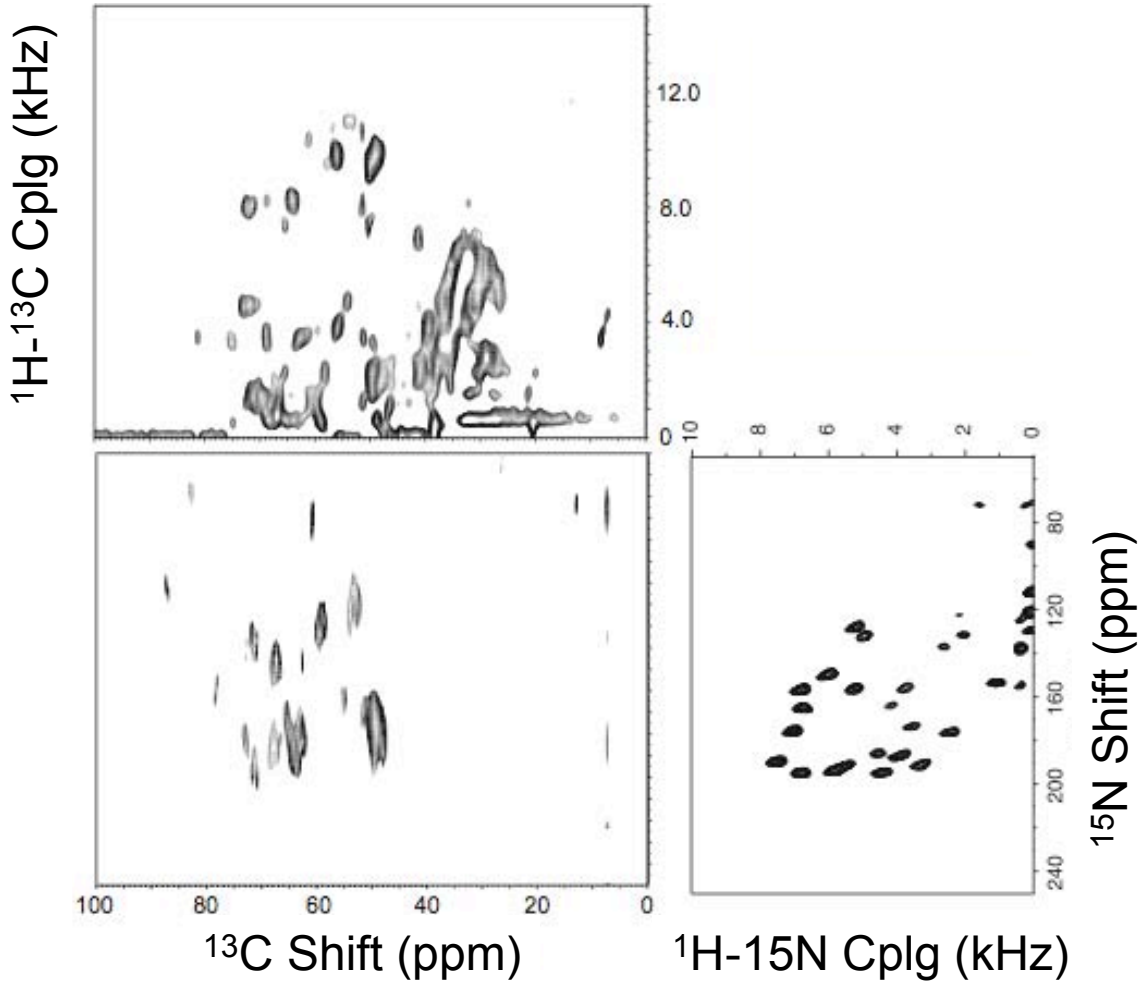


^{1}H-^{13}C PISEMA / ^{1}H-^{15}N PISEMA / ^{13}C/^{15}N HETCOR.

2-^{13}C glycerol/ul ^{15}N labeled Pf1 coat protein in “flipped” bicelles.

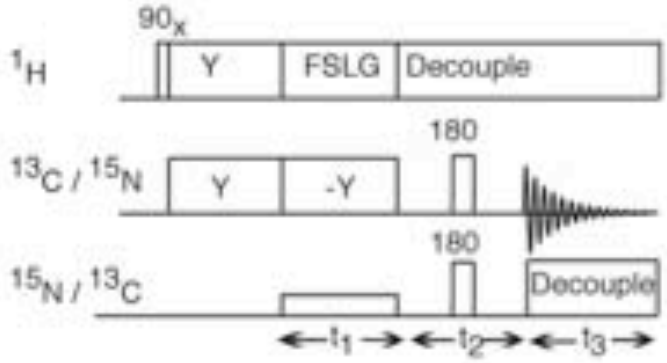


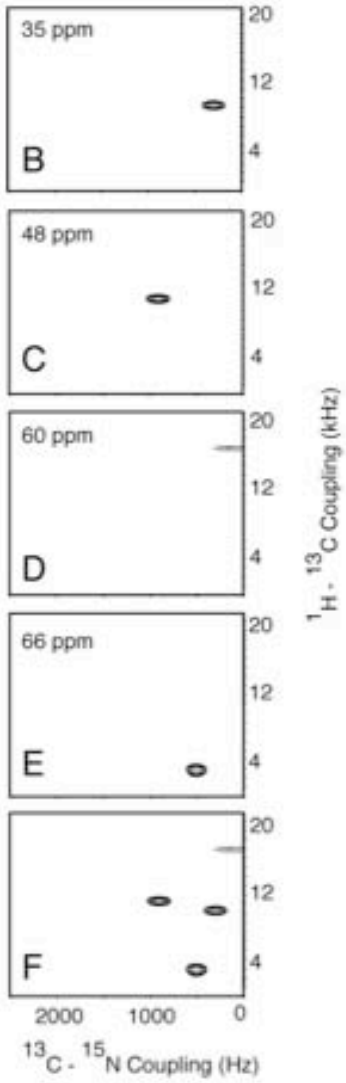


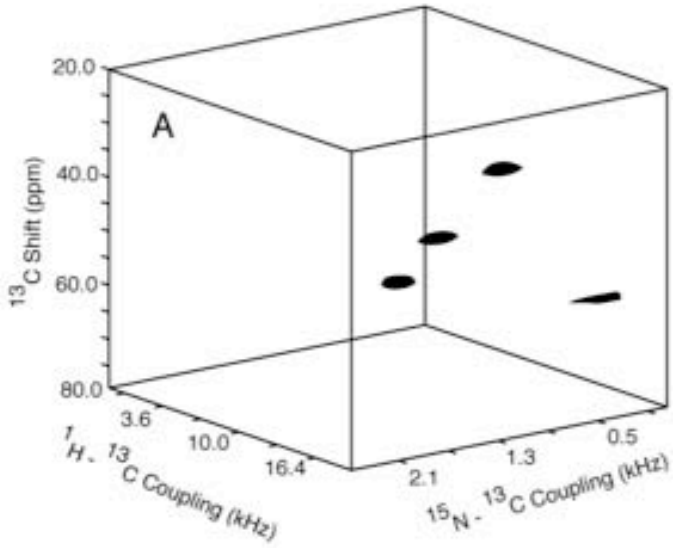


Three-dimensional triple-resonance spectrum of a $^{13}C\alpha$, ^{15}N labeled model peptide crystal.

^{13}C shift separated ^{1}H-^{13}C/^{13}C-^{15}N dipolar spectra



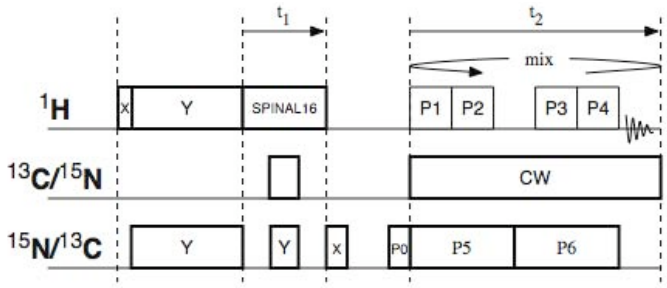




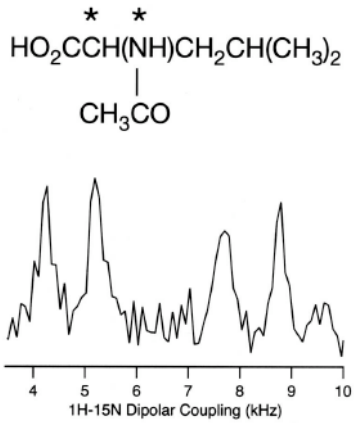
Shiftless nuclear magnetic resonance spectroscopy

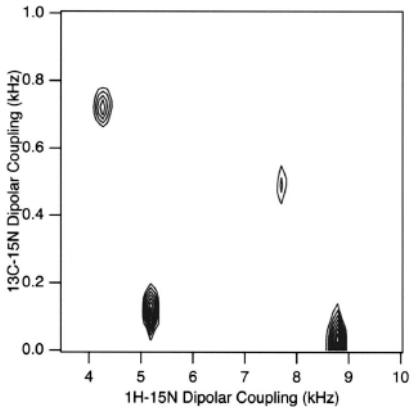
Chin H. Wu and Stanley J. Opella[a)]
Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0307, USA

2008

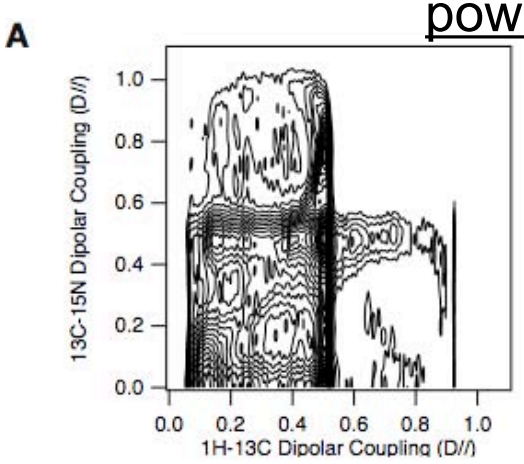


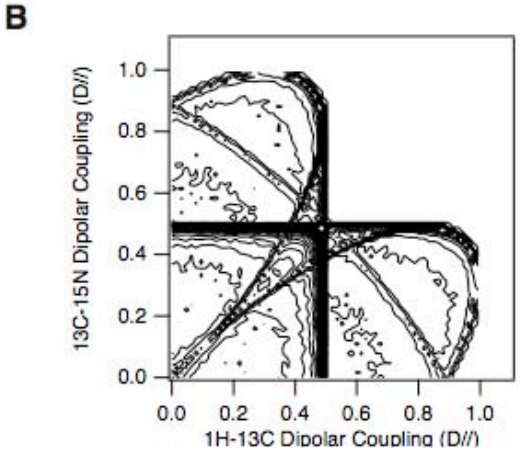
single crystal

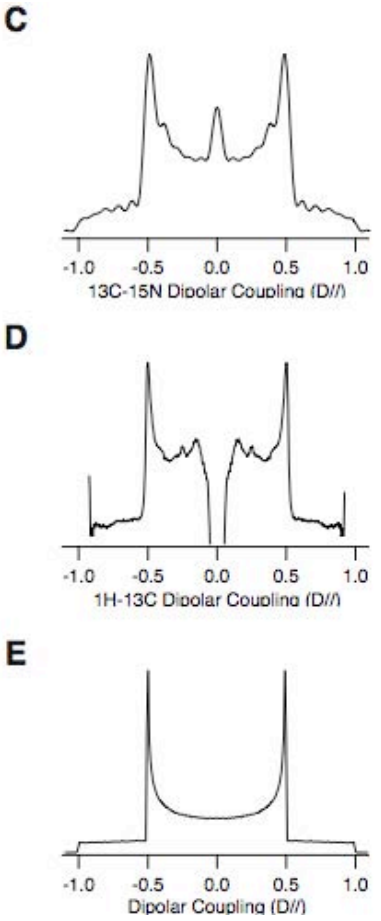




powder



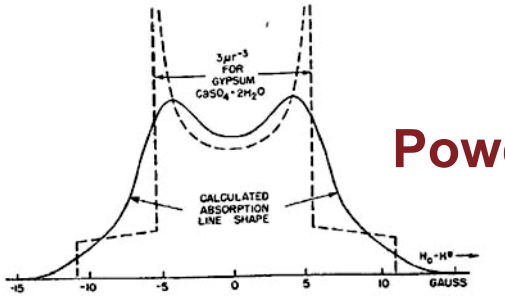




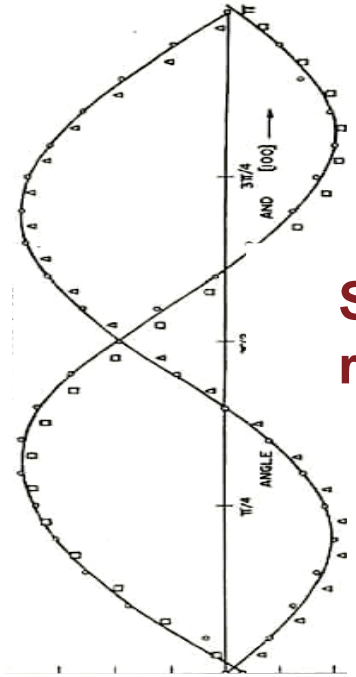
The dipole-dipole interaction is anisotropic.

Nuclear Resonance Absorption in Hydrated Crystals: Fine Structure of the Proton Line

G. E. PAKE*



Powder pattern.

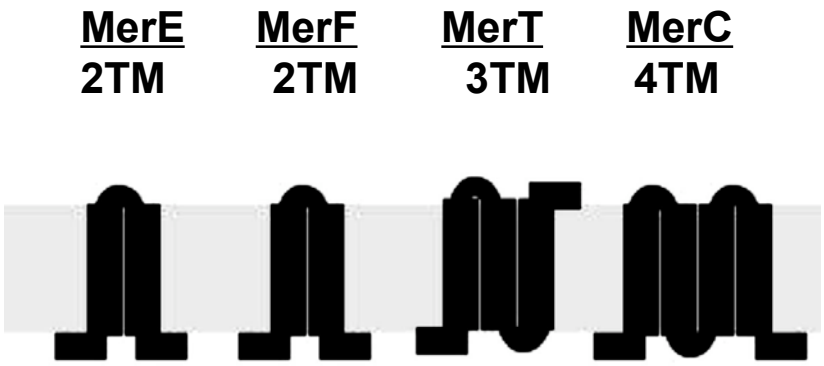


Single crystal rotation pattern.

1948

Part II: Phospholipid bilayers are essential for biological relevance.

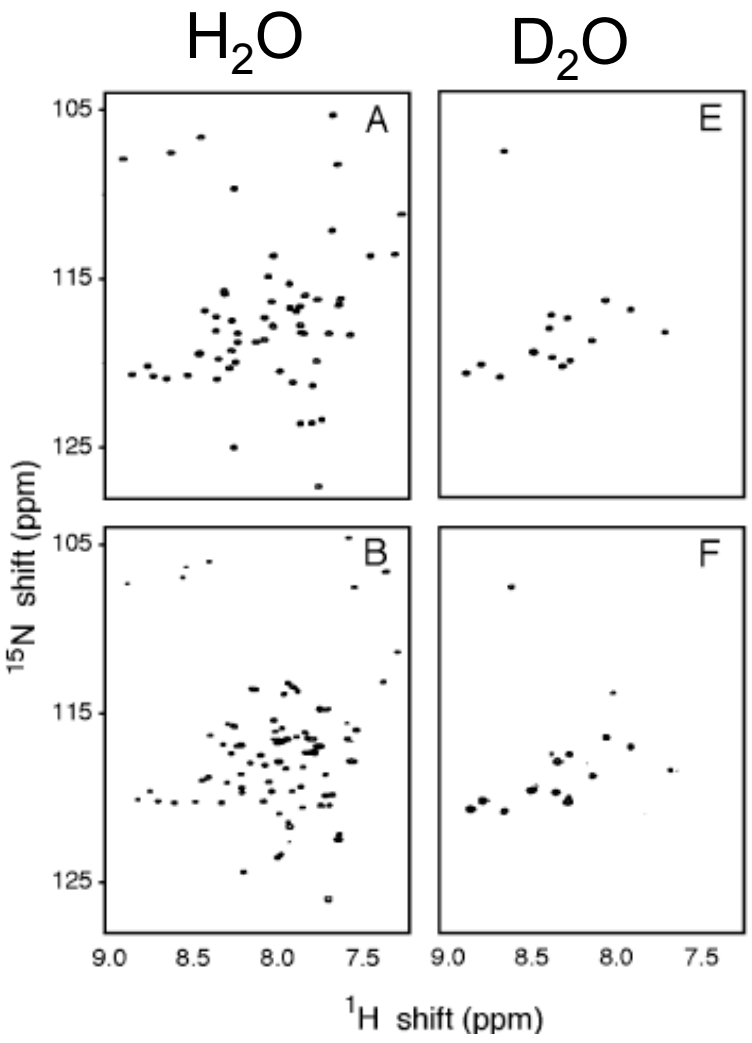
Mercury transport membrane proteins with 2, 3, and 4 trans-membrane helices.

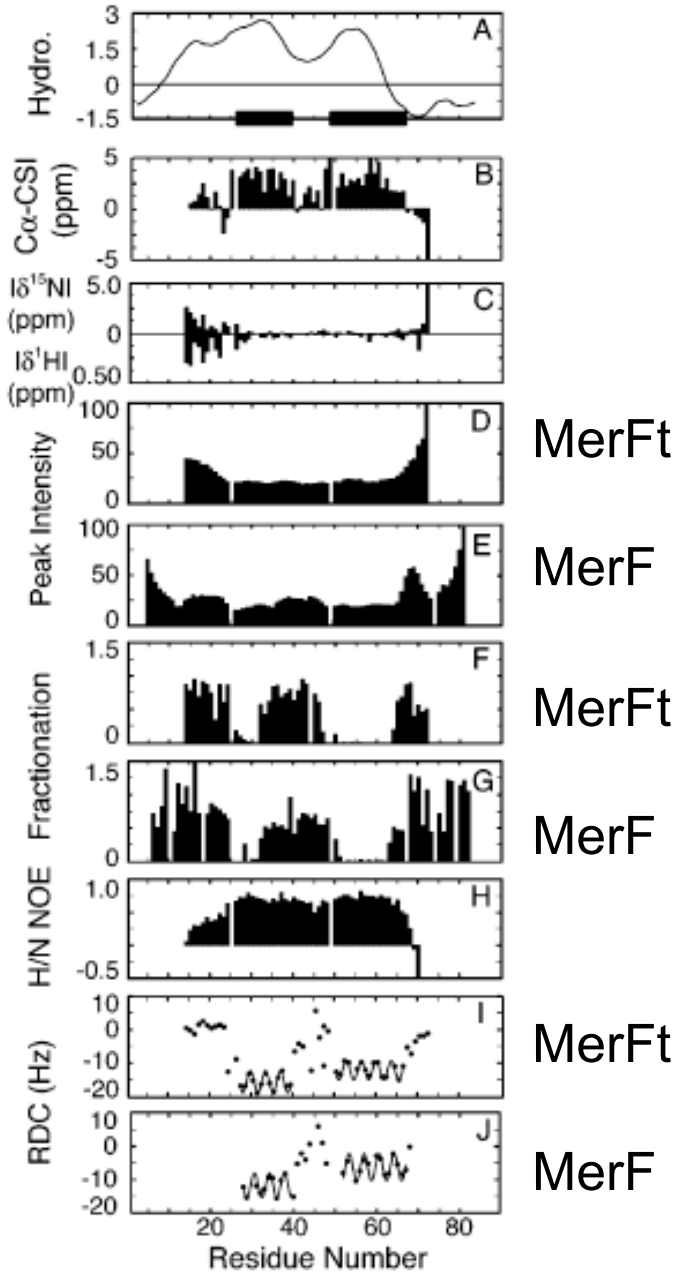


Solution NMR of the mercury transport membrane protein MerF in micelles.

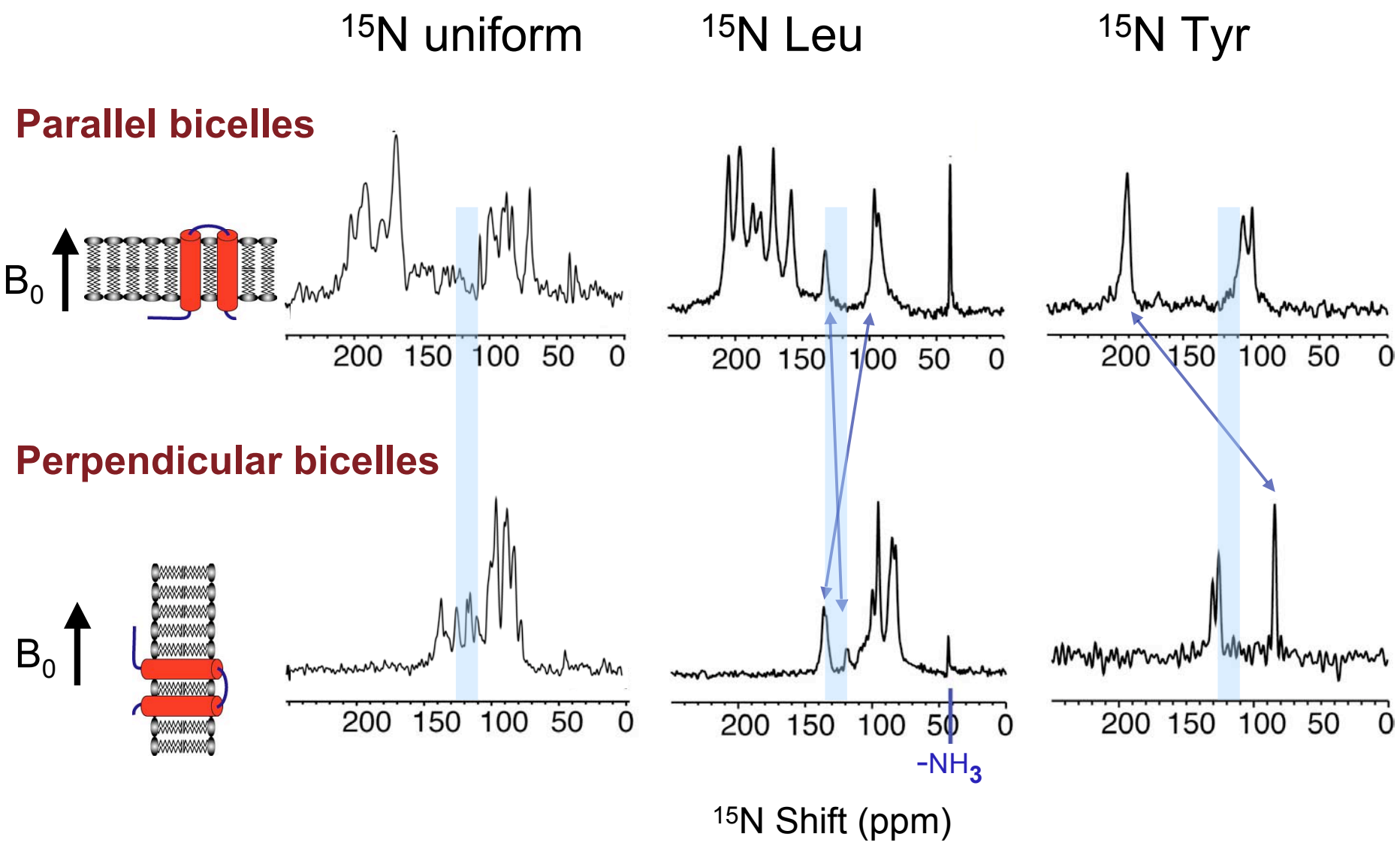
MerFt
60 residues
(truncated)

MerF
80 residues
(full length)





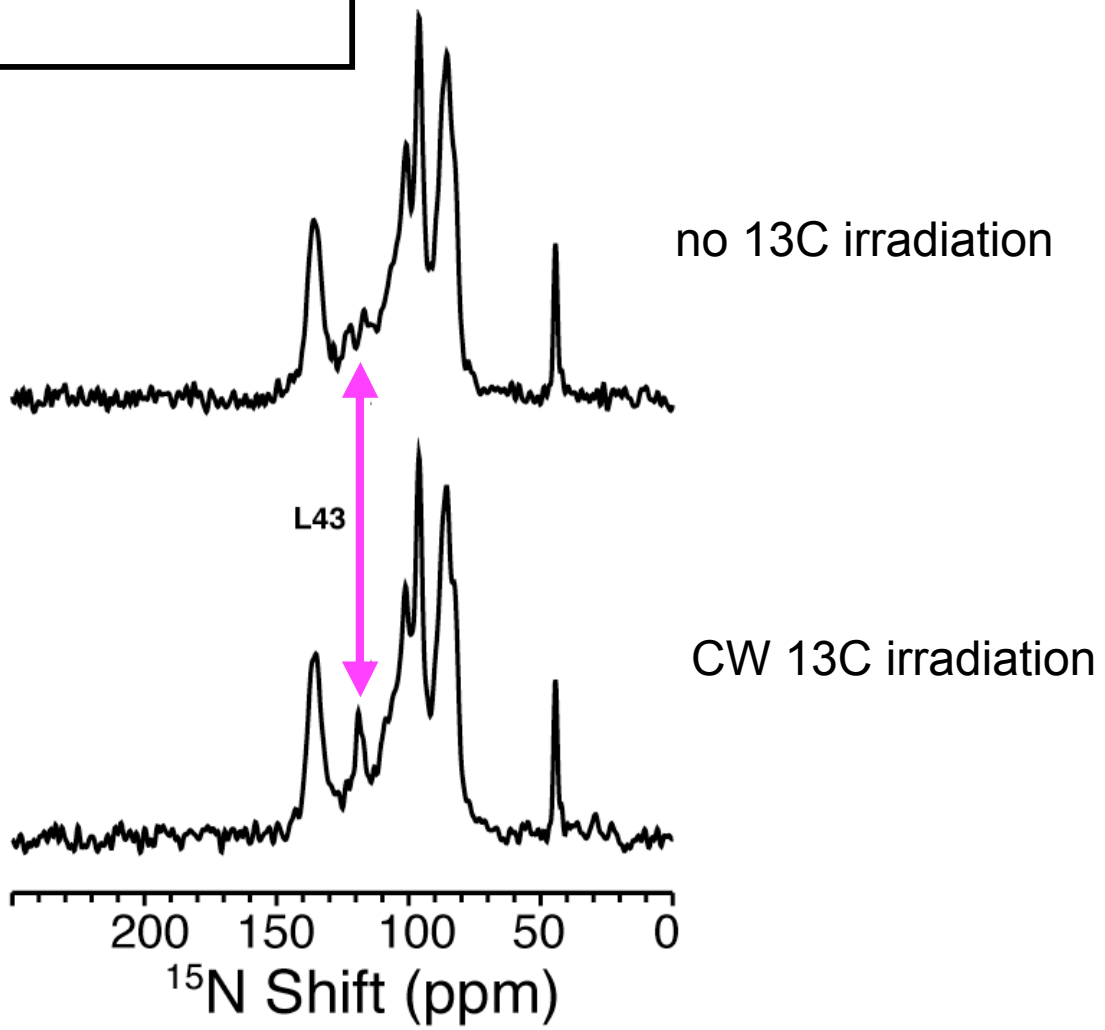
Effect of "flipping" on MerFt (60aa, 2TM) spectra.



^{13}C decoupling of a selectively ^{15}N Leu (13 sites) and ^{13}C' Tyr (3 sites) labeled membrane protein.

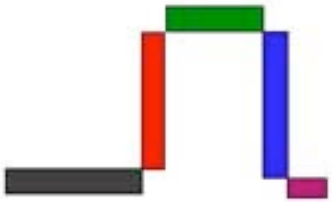
only one ^{13}C-^{15}N bond in the protein

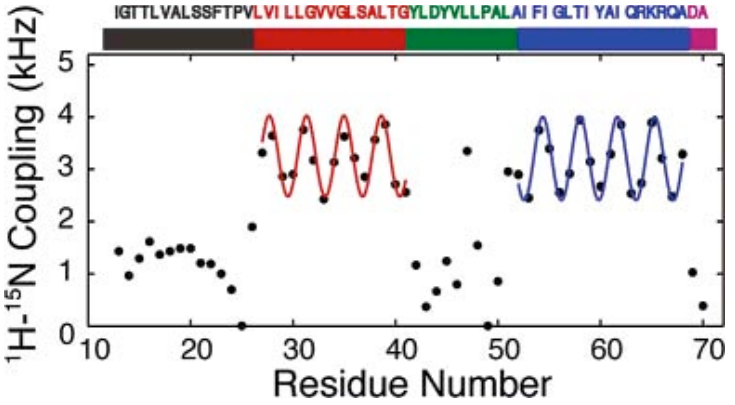
Tyr 42 - Leu 43

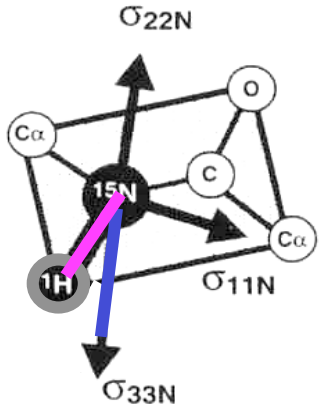


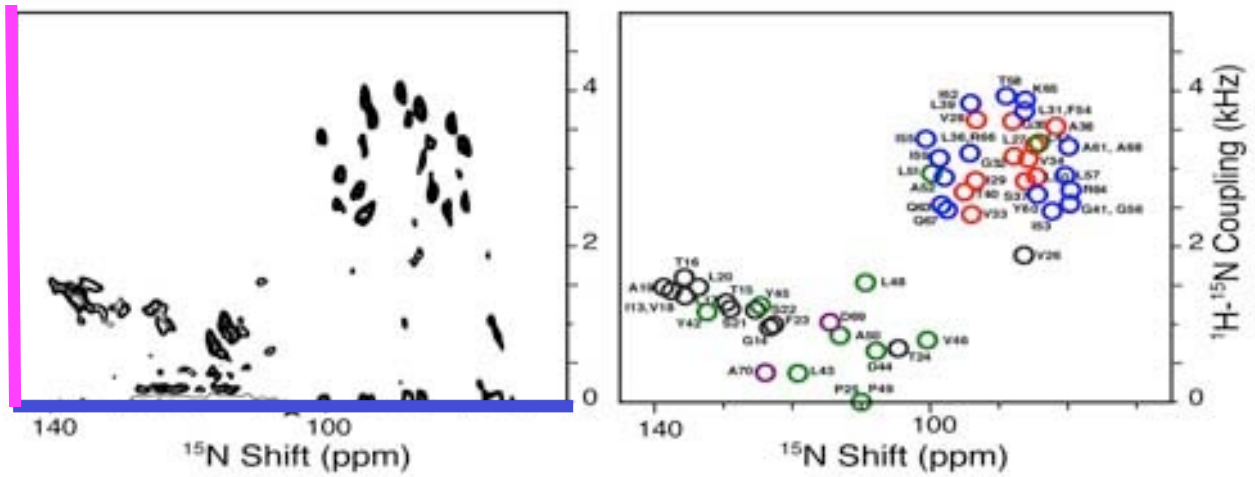
Resolution, measurement, and assignment of resonances.

MerFt (60 aa)

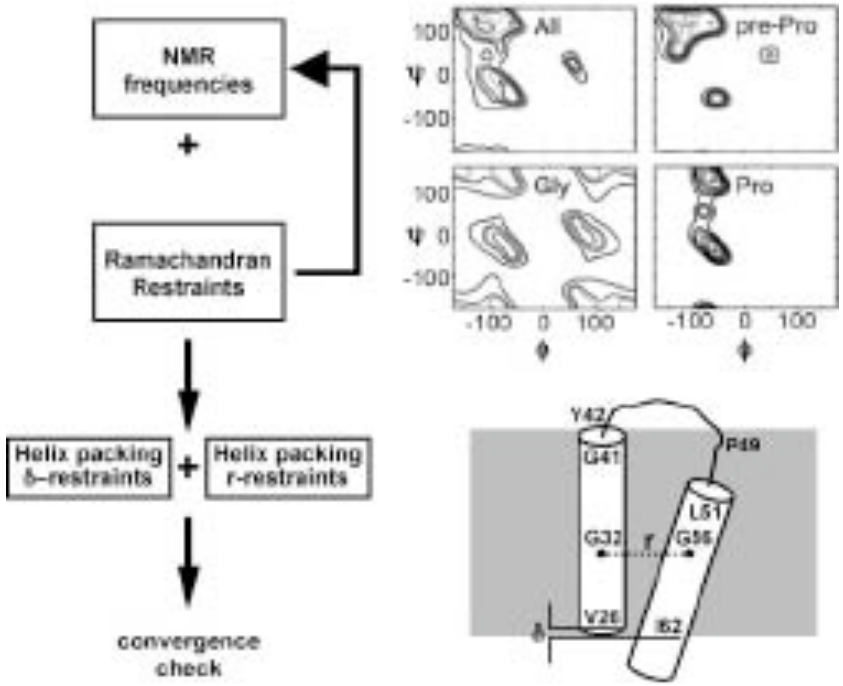




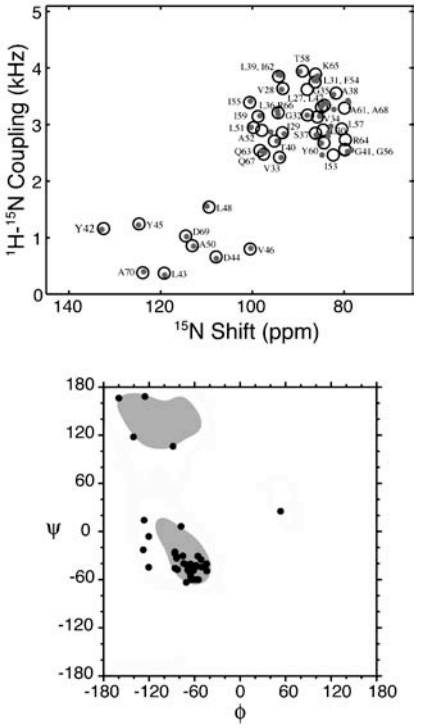




Two orientationally dependent frequencies for each residue enable calculation of three-dimensional protein structures.



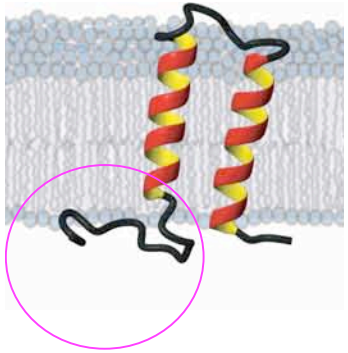
back-calculated spectrum

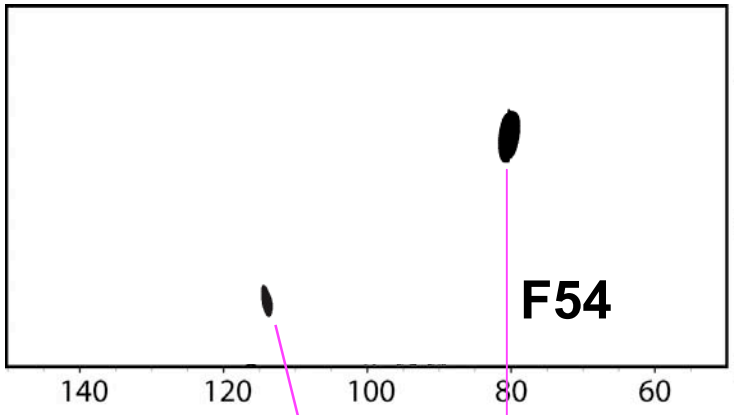


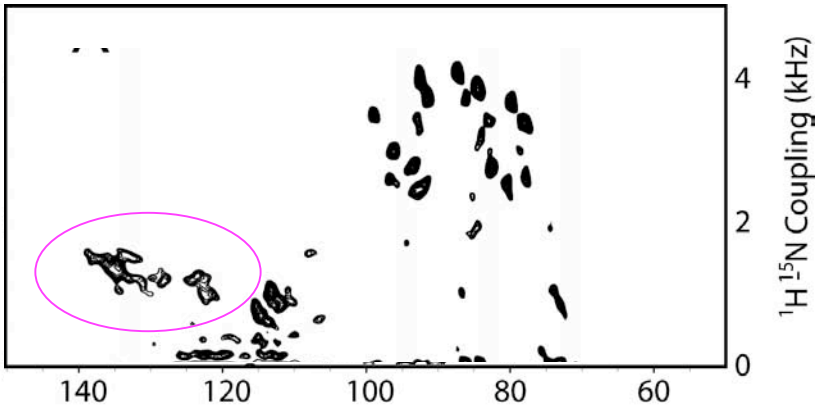
Structure and dynamics of terminal domains of MerF.

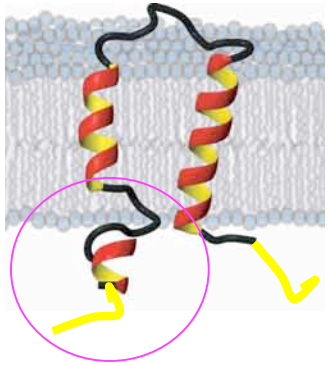
MerFt (60 aa)
MerFm (80aa)

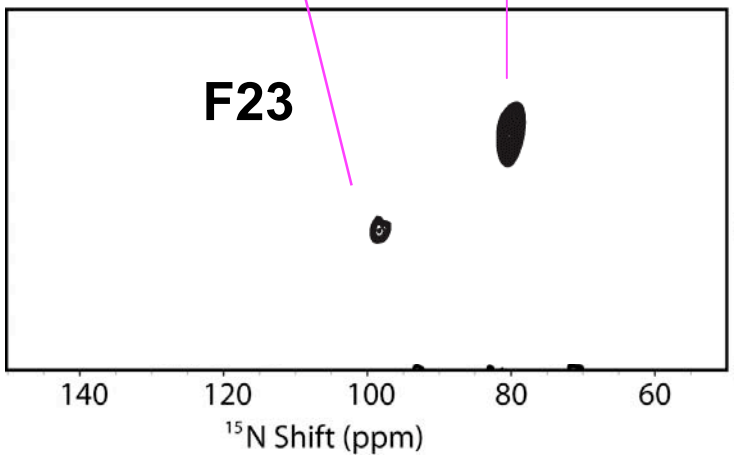
F23 **F54** **F76**

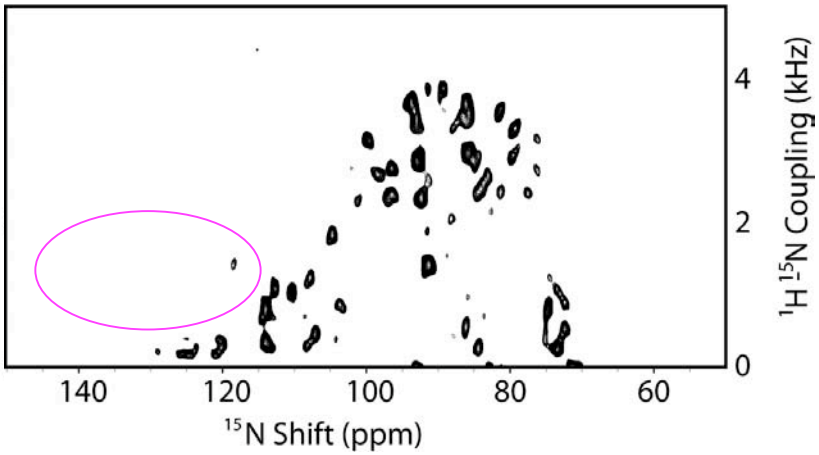








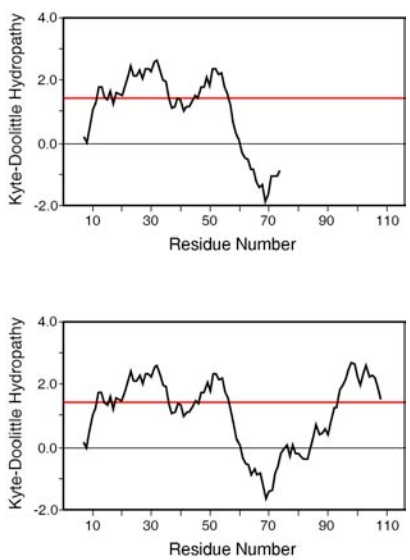


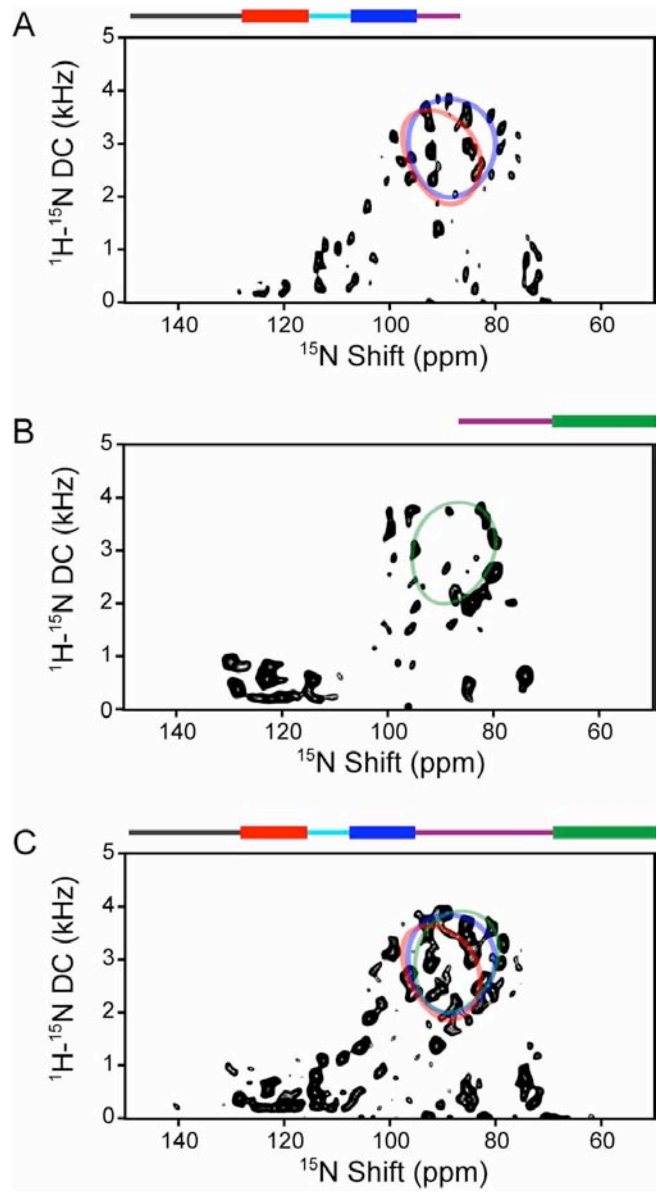


MerF (80 aa, 2 TM) vs. chimeric MerTf (114 aa, 3 TM).

```
MerFm  KDPKTLLRVSIIGTTLVALSSFTPVLVILLGVVGLSALTGYLDYVLLPALAIFIGLTIYAIQRKRQADASS---TPKFNGVKKZ-------------------------------
       |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||    |
MerTf  KDPKTLLRVSIIGTTLVALSSFTPVLVILLGVVGLSALTGYLDYVLLPALAIFIGLTIYAIQRKRQADASSAASKPGEVSAIPQVRATYKLIFWGVAVLVLVALGFPYVVPFFYZ
```

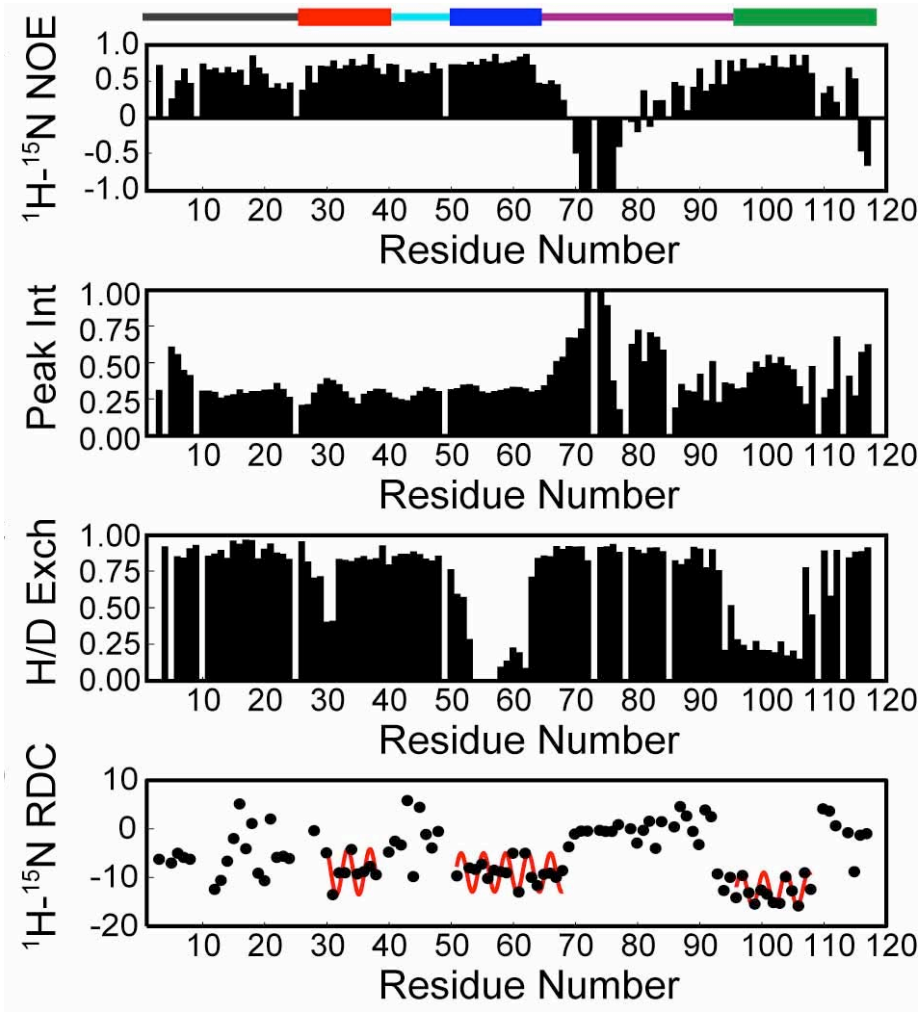
hydropathy plots



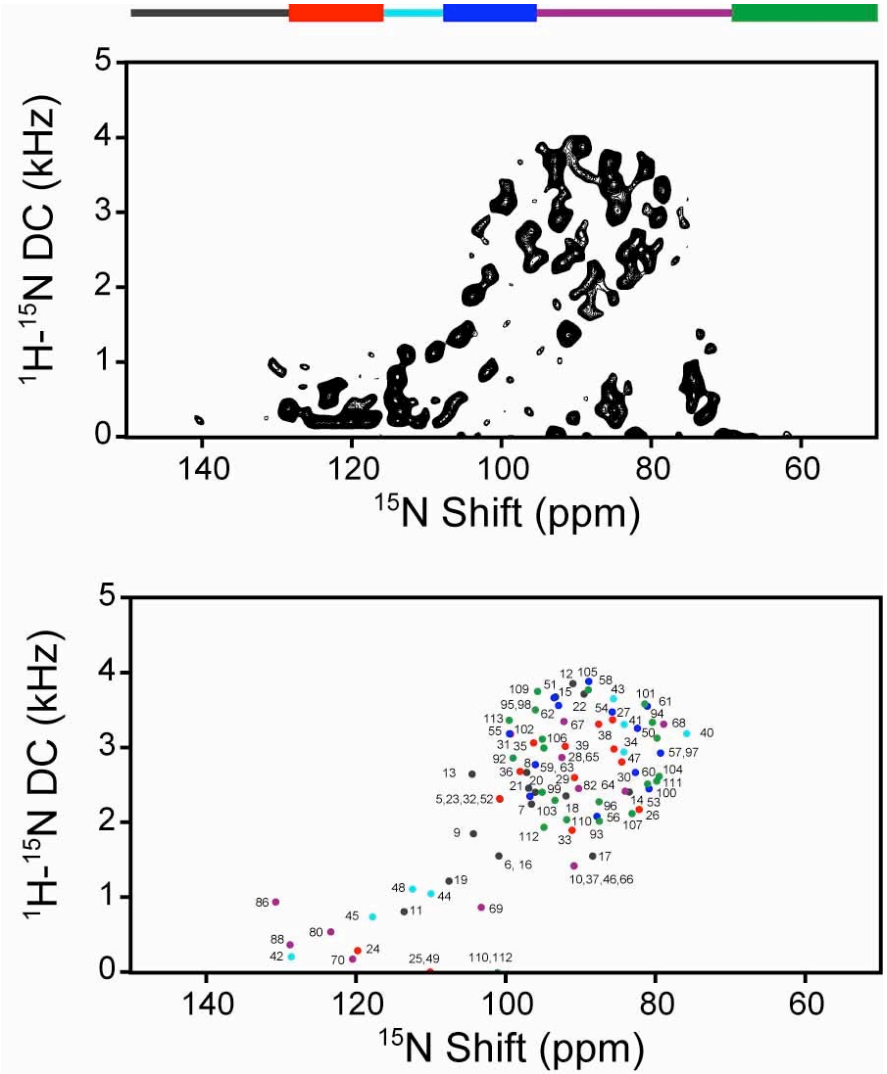


MerTf chimera in micelles vs. q=3.2 bicelles.

solution NMR

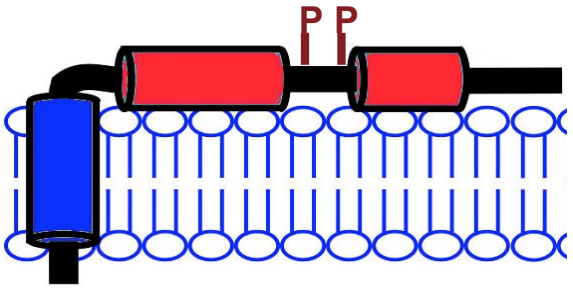


solid-state NMR

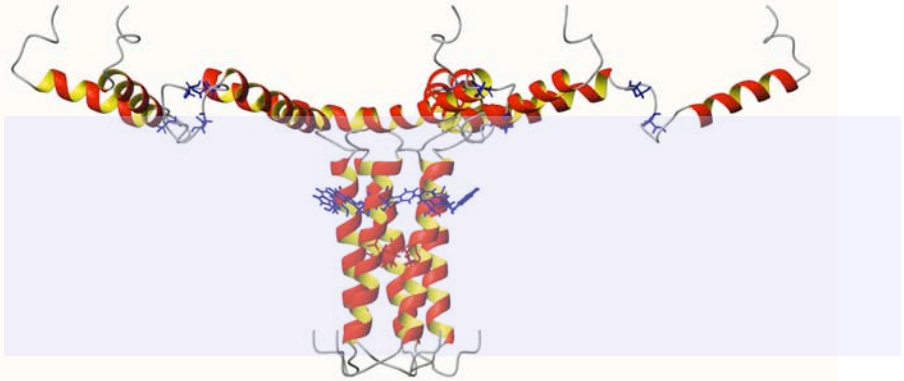


Vpu of HIV-1.

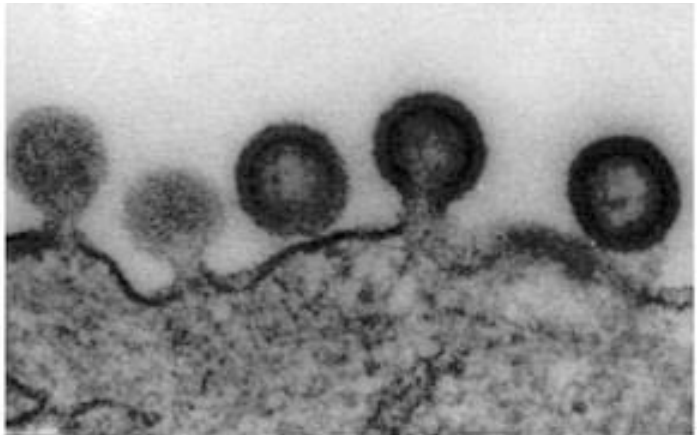
- 81 residue membrane protein.
- Enhances virus particle release from infected cells.
  - Associated with the trans-membrane domain.
  - Associated with ion channel activities.
  - Affected by “channel-blocking” drugs.
- Facilitates the degradation of CD4/gp160 complex.
  - Associated with the cytoplasmic domain.
  - Affected by phosphorylation of two conserved serines.



Vpu oligomer forms ion channels

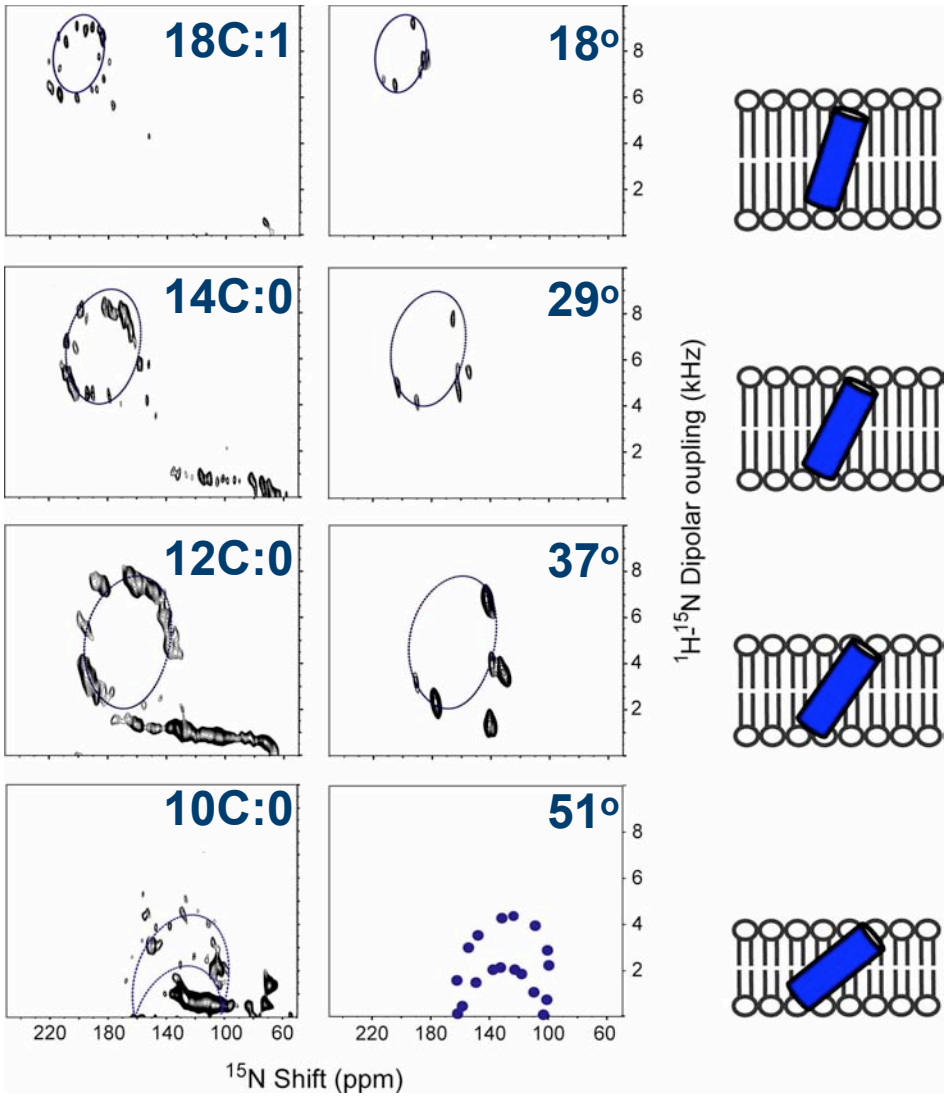


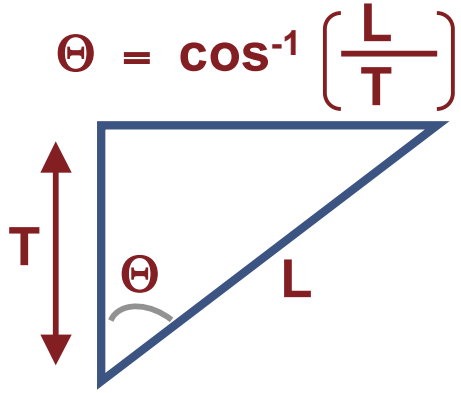
HIV budding from membrane

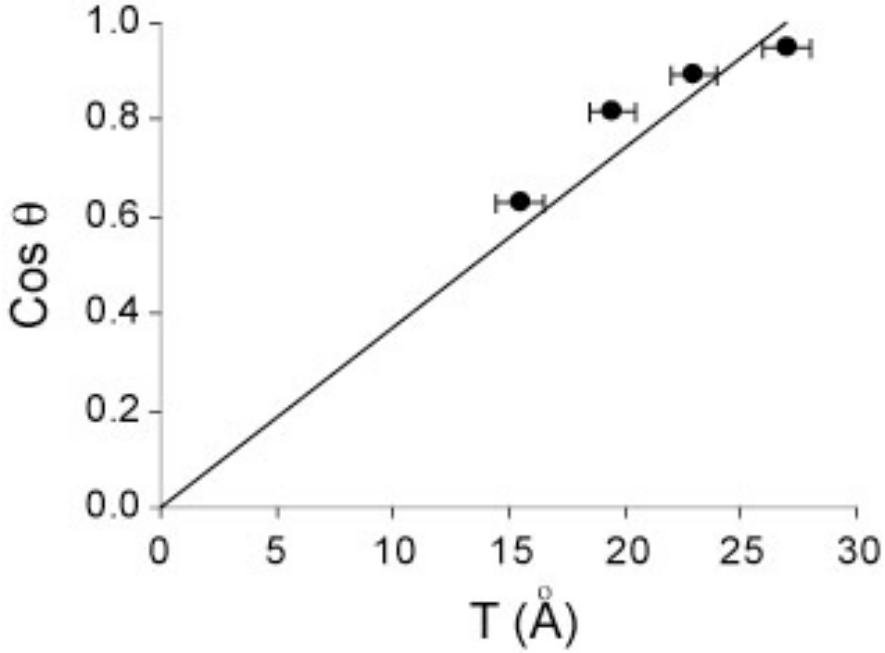


Ewart et al, Eur Biophys J 31, 26, 2002

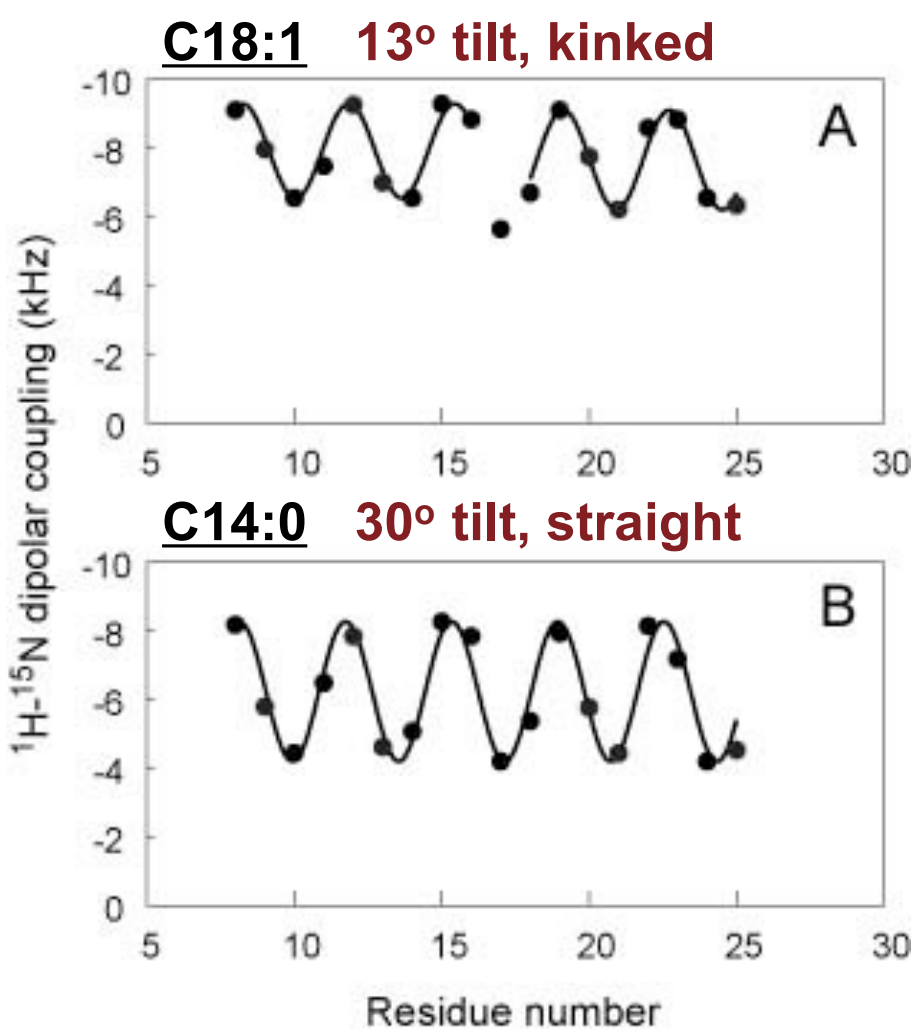
Tilt angle compensates for hydrophobic mismatch.
No change in rotation angle.

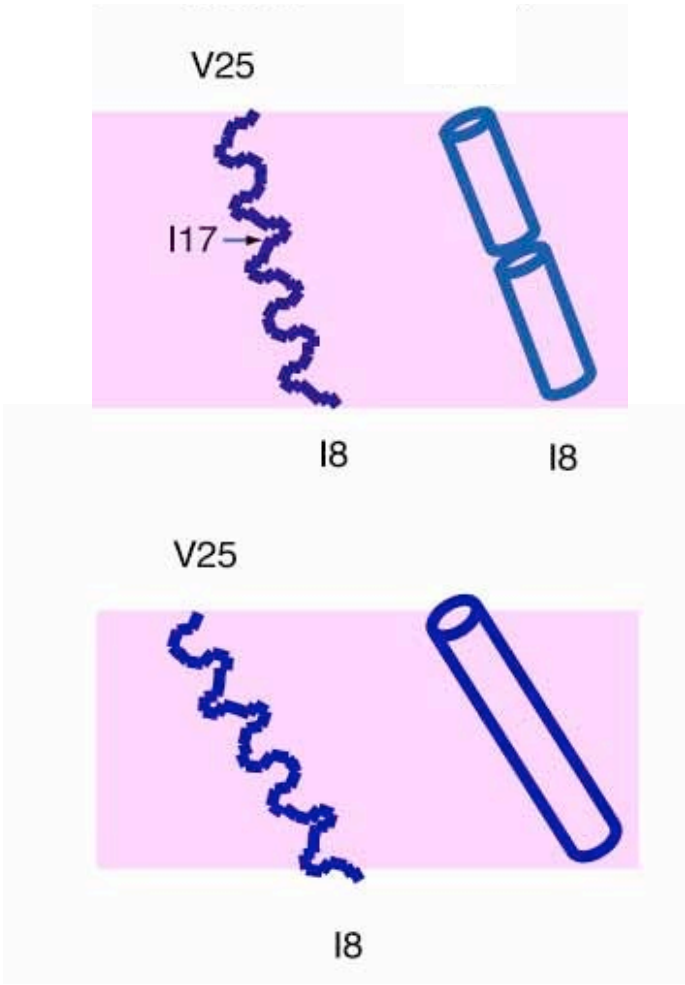




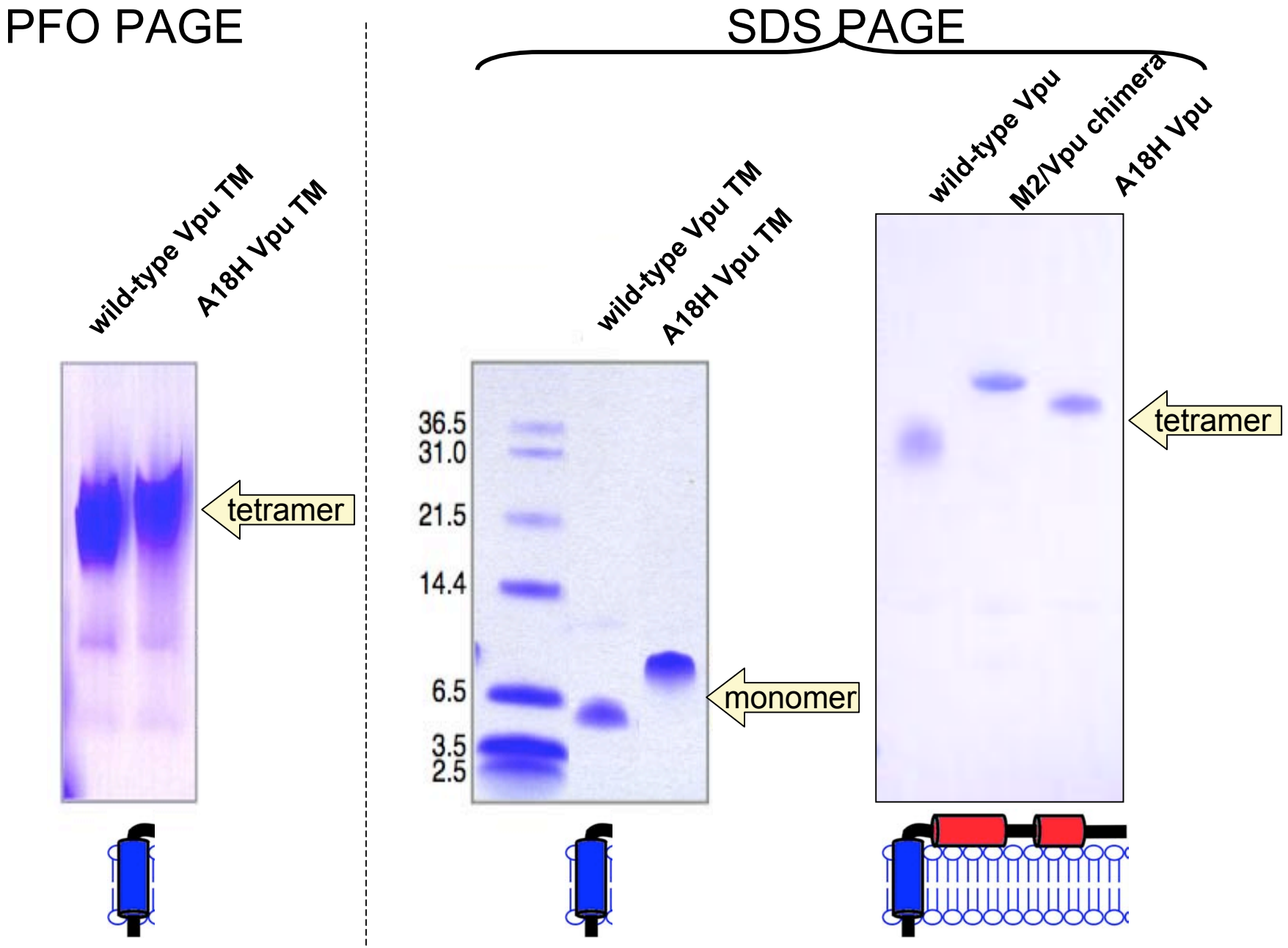


Lipids affect the structure of wild-type Vpu TM.

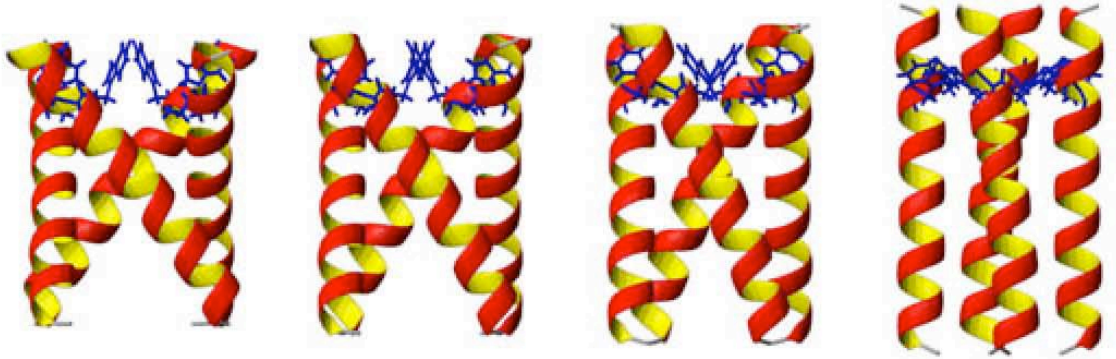




Gel electrophoresis suggests that Vpu forms a tetramer.



Structure varies with the type of biological membrane.

Type of biological membrane	Basolateral plasma membrane	Endoplasmic reticulum	Golgi	Apical plasma membrane
Hydrophobic thickness (A)	23.1 ± 0.6	25.0 ± 0.4	27.0 ± 0.4	30.0 ± 0.3
Predicted tilt angle (degrees)	40	34	26	4
Tetramer model				

A18H mutation makes Vpu of HIV-1 act like M2 of influenza.

```
M2 TM domain   -SSDPLVVAASIIGILHLILWILDRL-
Wild Vpu2-30+  QPIQIAIVALVVAIIIAIVVWSIVIIEGRGGKKKK
A18H Vpu2-30+  QPIQIAIVALVVAIIIHIVVWSIVIIEGRGGKKKK
```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Virology xx (2006) xxx–xxx

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A single amino acid substitution within the transmembrane domain of the human immunodeficiency virus type 1 Vpu protein renders simian–human immunodeficiency virus ($SHIV_{KU\text{-}1bMC33}$) susceptible to rimantadine

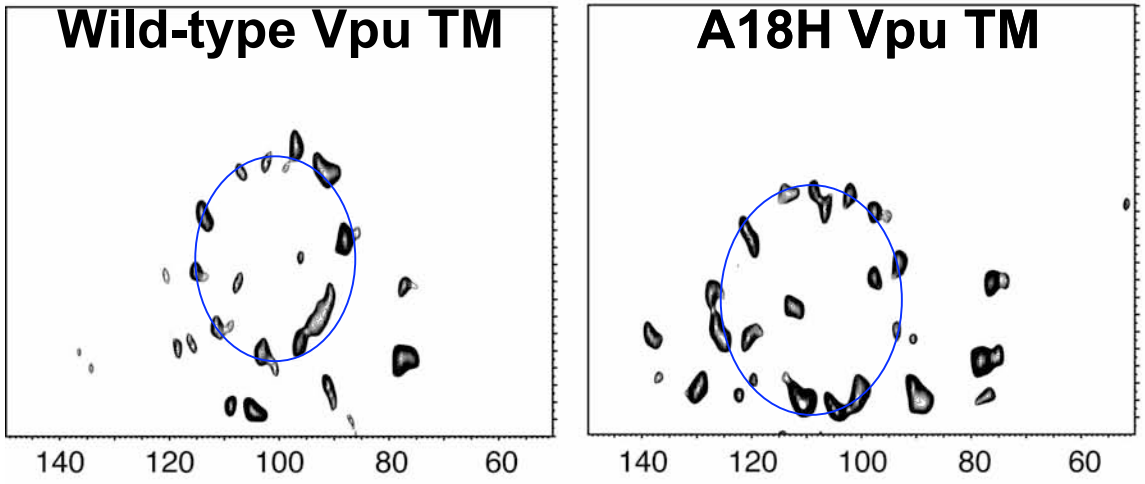
David R. Hout, Lisa M. Gomez, Erik Pacyniak, Jean-Marie Miller, M. Sarah Hill, Edward B. Stephens *

Department of Anatomy and Cell Biology, University of Kansas Medical Center 3901 Rainbow Blvd., Kansas City, KS 66160, USA

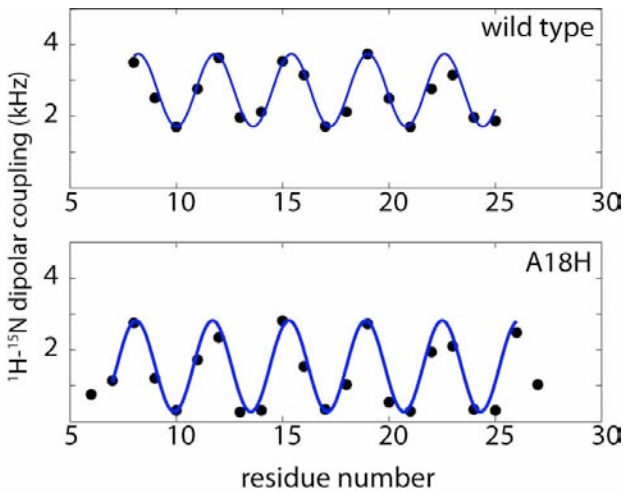
Received 7 September 2005; returned to author for revision 8 November 2005; accepted 9 December 2005

A18H mutation makes changes structure of Vpu.

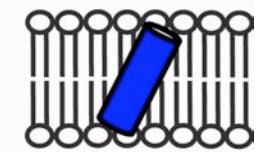
```
M2 TM domain   -SSDPLVVAASIIGILHLILWILDRL-
Wild Vpu2-30+  QPIQIAIVALVVAIIIAIVVWSIVIIEGRGGKKKK
A18H Vpu2-30+  QPIQIAIVALVVAIIIHIVVWSIVIIEGRGGKKKK
```



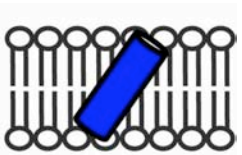
Mutation lengthens helix and increases tilt angle.



30°



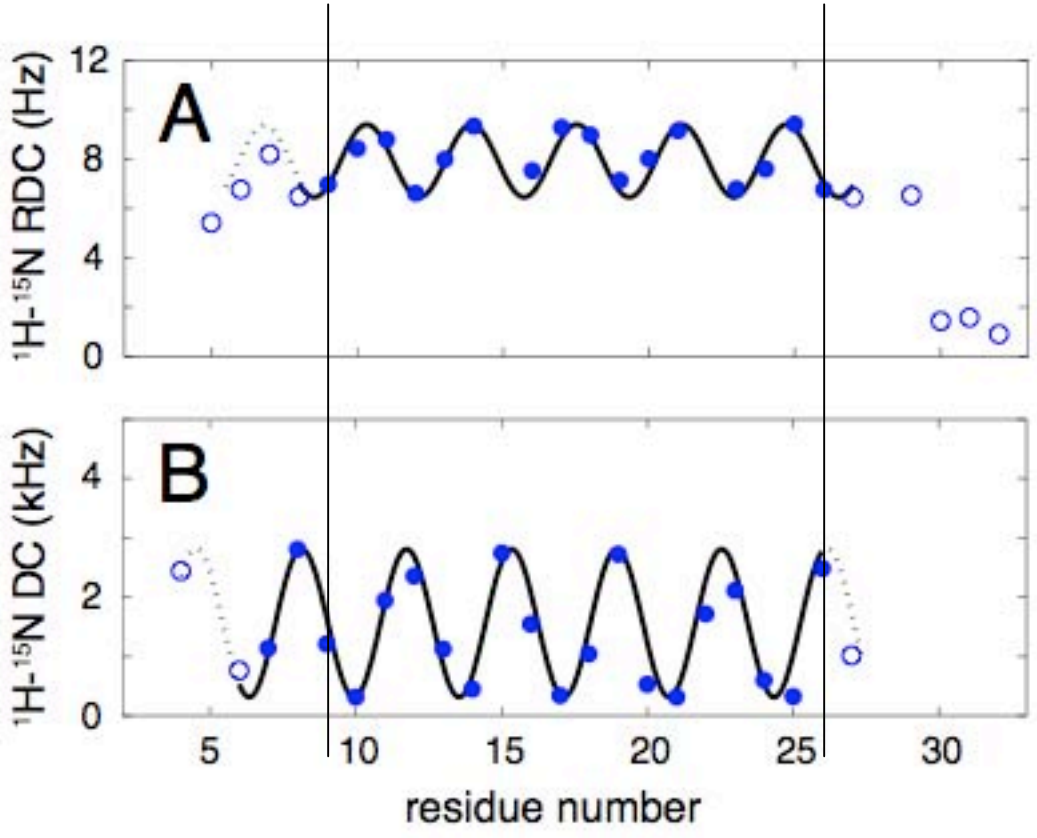
41°



Vpu mutation: Hout et al 2006

A18H Vpu TM domain: Micelles vs. Bicelles.

Dipolar Waves

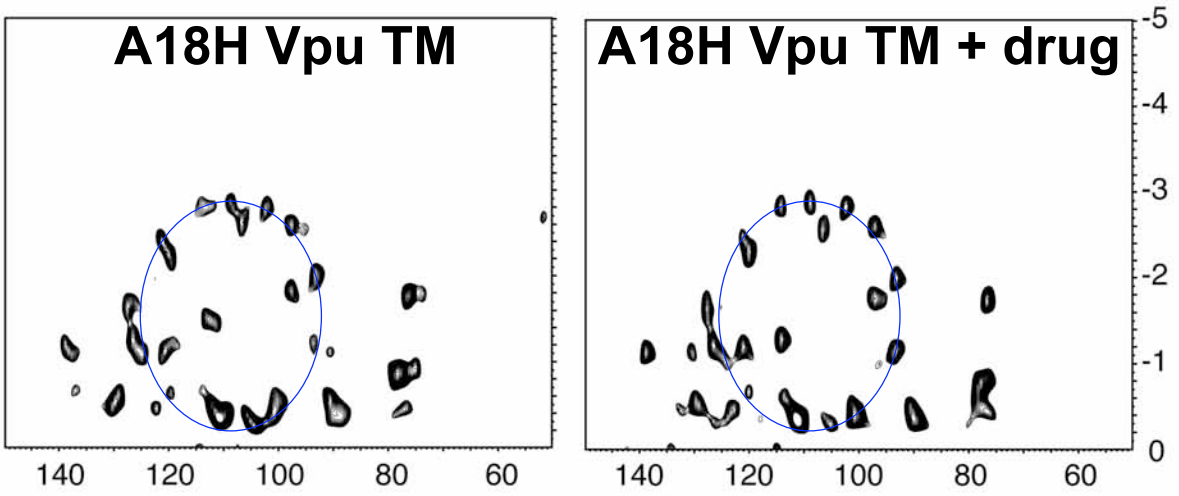


DHPC micelles
q=0

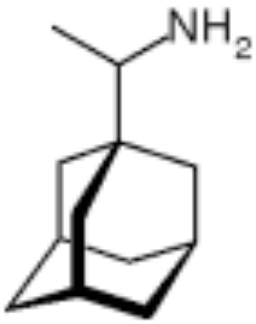
DMPC:DHPC bicelles
q=3.2

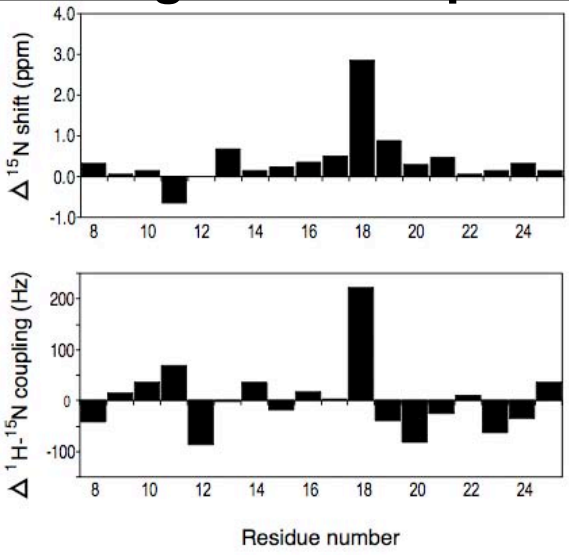
A18H mutation makes HIV-1 infection sensitive to rimantidine.

```
M2 TM domain   -SSDPLVVAASIIGILHLILWILDRL-
Wild Vpu2-30+  QPIQIAIVALVVAIIIAIVVWSIVIIEGRGGKKKK
A18H Vpu2-30+  QPIQIAIVALVVAIIIHIVVWSIVIIEGRGGKKKK
```



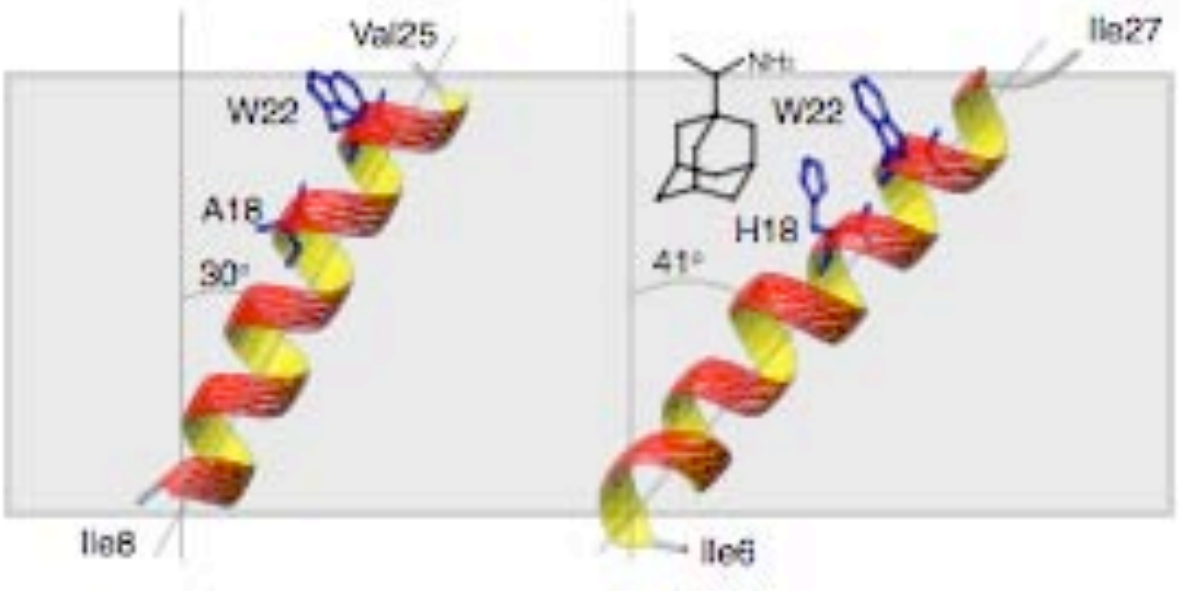
Drug binding induces spectral changes



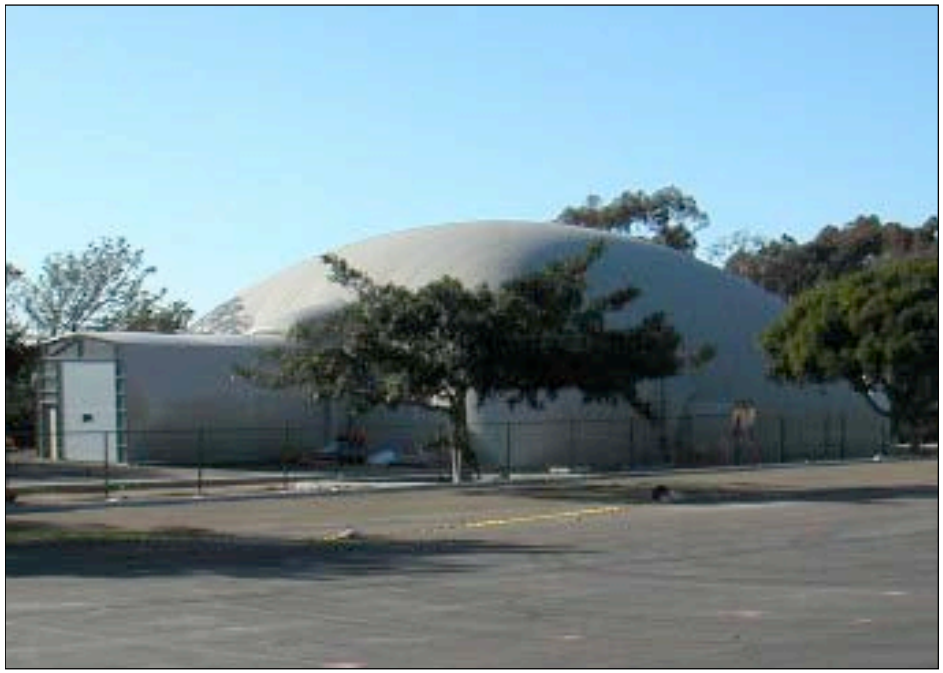


Structures of Vpu TM: willd-type vs. A18H mutant.

Wild type Vpu TM **A18H Vpu TM + drug**



Biotechnology Resource for NMR Molecular Imaging of Proteins at UCSD.



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