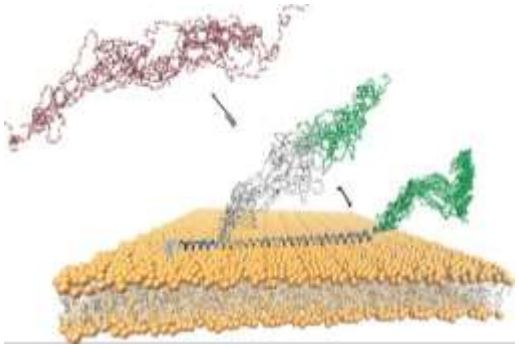
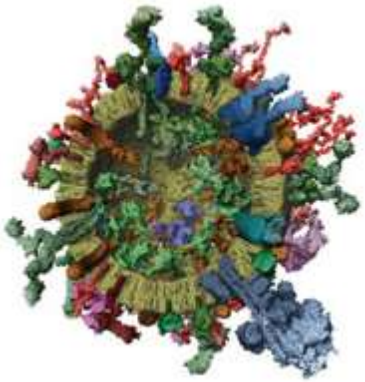
2017 Soft Matter Summer School on Membranes

Protein-Membrane Interaction Studies using NMR Spectroscopy





Jung Ho Lee

Department of Chemistry
Seoul National University, Korea

Motivation

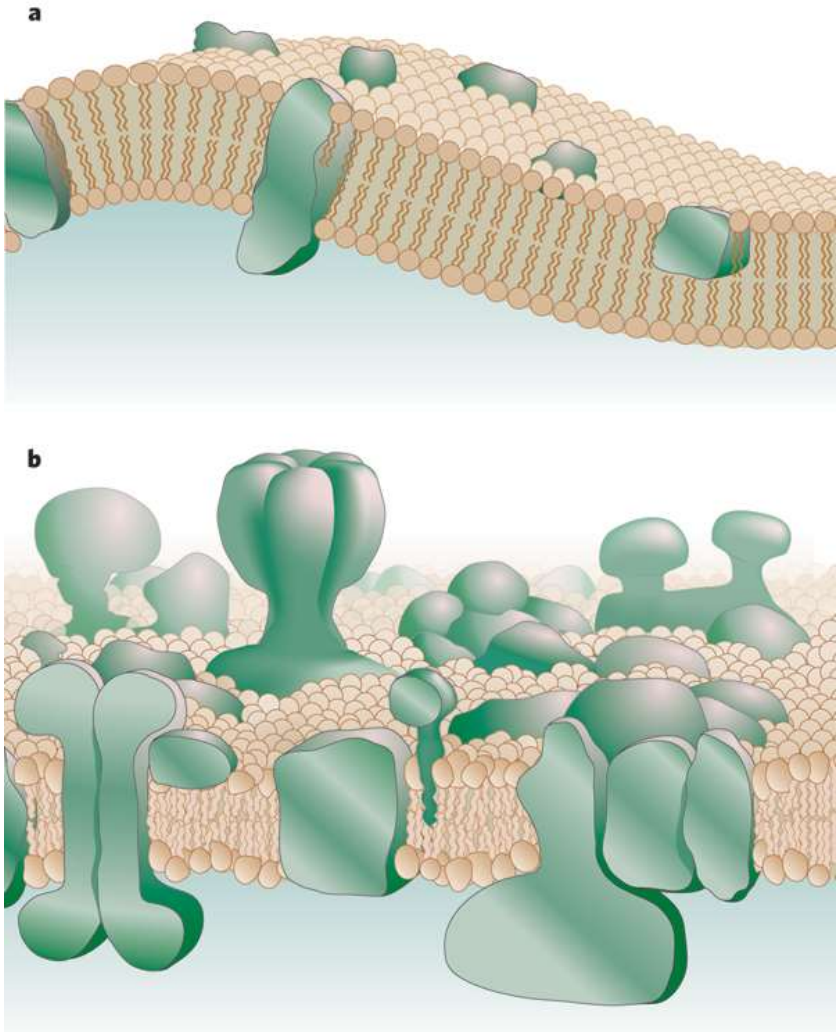


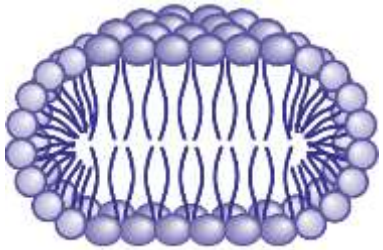
Image: Nature 438, 578-580 (2005)

- Compartmentalization by membranes is essential for life.
- There must be specific ways to overcome the boundaries of the compartment (membrane).
- Macromolecules exist to pass materials and information between a cell and its environment.
- **a**: Fluid Mosaic Model (Science 175, 720-731, 1972)
- **b**: High protein occupancy, variable patchiness, and thickness.

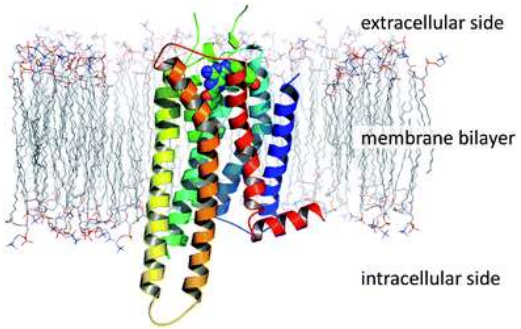
Course Outline

1. NMR spectroscopy

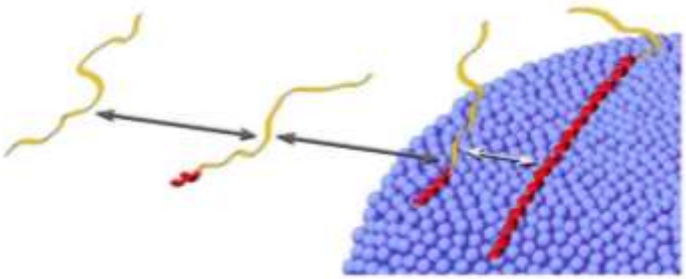
2. Membrane mimetics for NMR studies



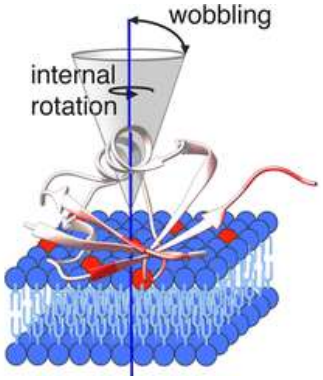
3. Membrane proteins



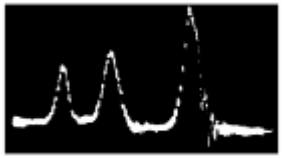
4. Induction of Protein Structure upon Lipid Binding



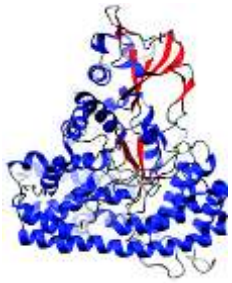
5. Protein Motions on the Membrane



1. Nuclear Magnetic Resonance (NMR)

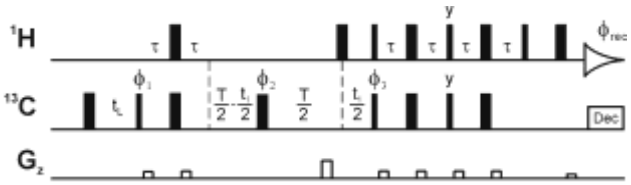


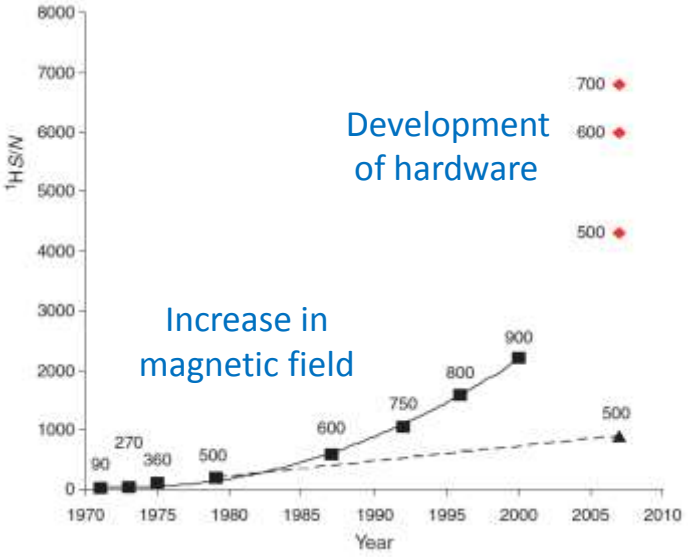
ethyl alcohol (1951)



(21st century)

Development of pulse sequence

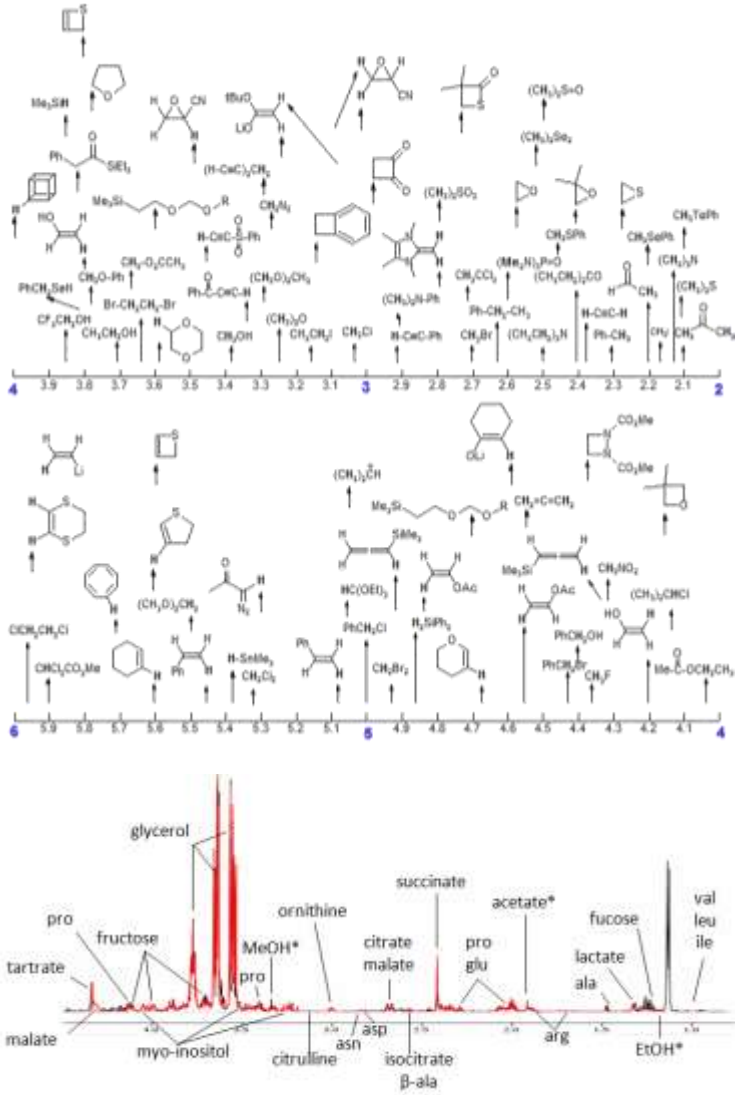




- 1952 *Physics* Bloch, Purcell NMR discovery
- 1991 *Chemistry* Ernst High-resolution FT NMR
- 2002 *Chemistry* Wüthrich 3D structure of biological macromolecules by NMR
- 2002 *Medicine* Mansfield, Lauterbur MRI

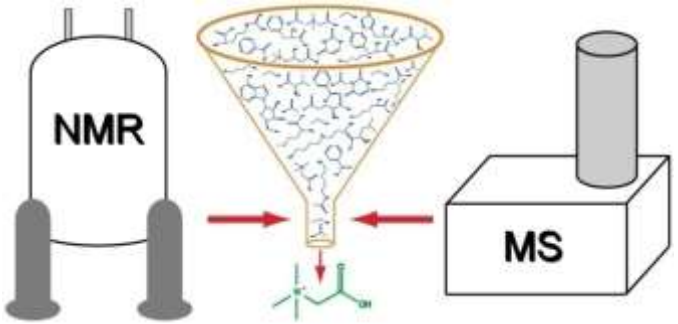
Applications of NMR

Structure of small molecules

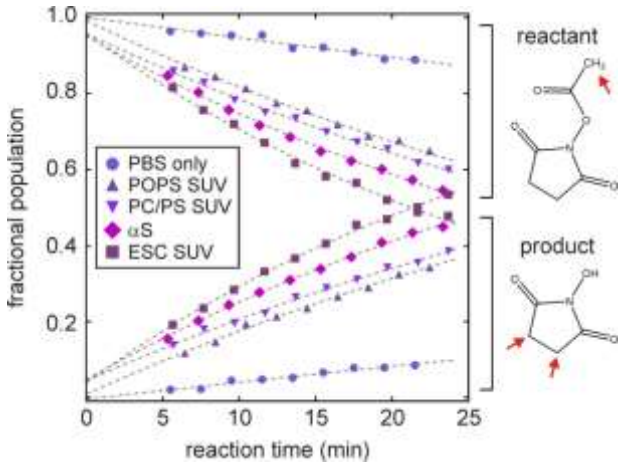


Metabolomics

Identification and quantitative measurement of many metabolites in biological samples.



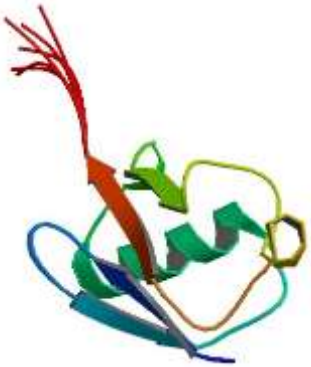
Reaction Kinetics



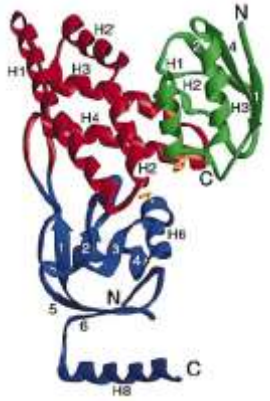
Lee J.H., et al. *Biochemistry* **2016**

Applications of NMR

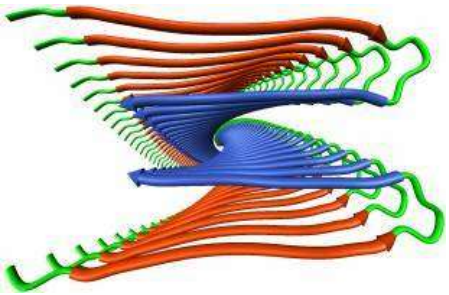
Structure of large biomolecules



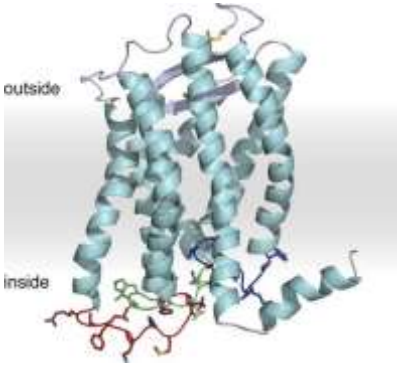
small proteins



biomolecular complexes

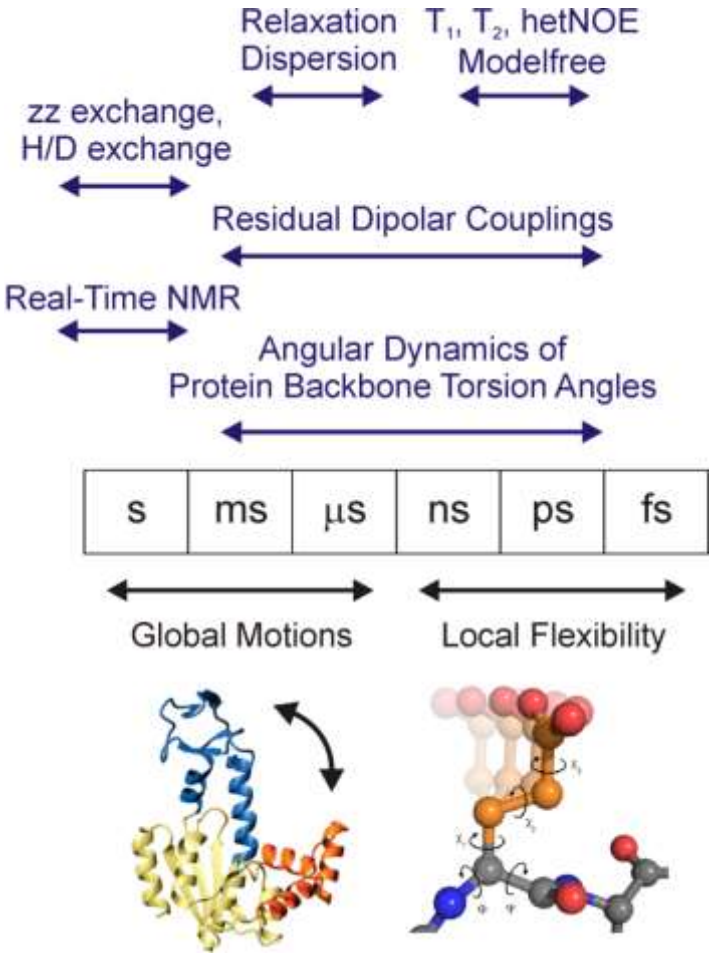


amyloid fibrils



membrane proteins

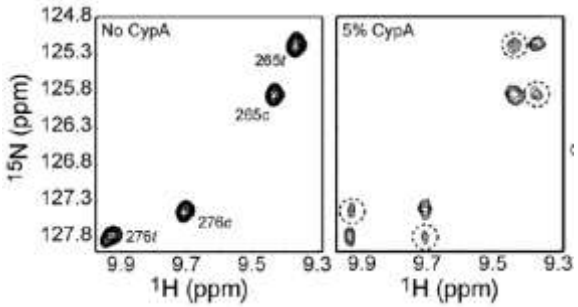
Dynamics of large biomolecules



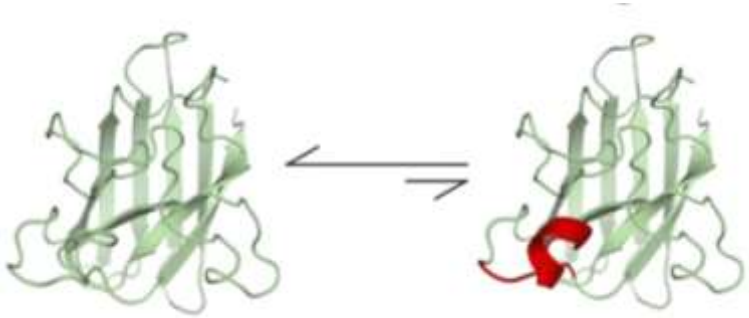
Applications of NMR

Dynamic Equilibrium

Slow exchange process

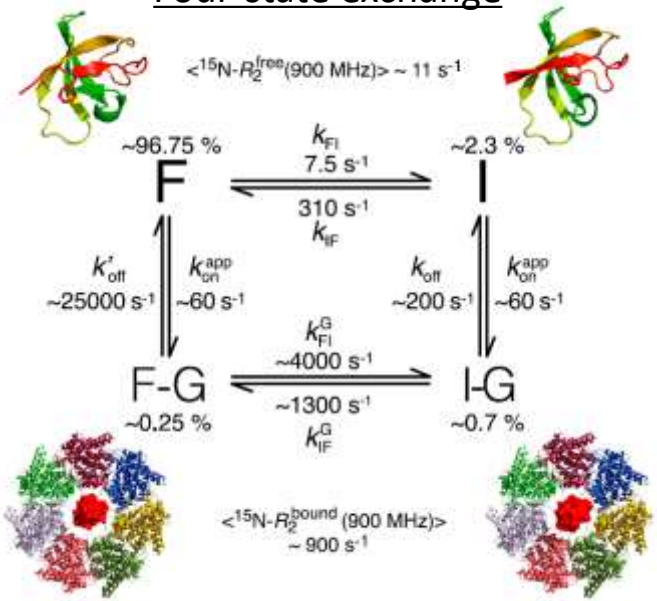


Spectroscopically invisible states

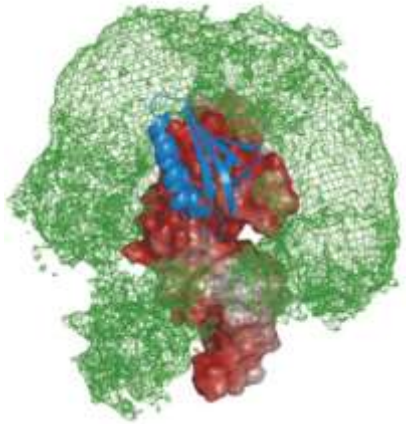


Sekhar A. et al. *eLife* **2015**, *4*, e07296

Four-state exchange



Libich D.S., et al. *Proc. Natl. Acad. Sci. U. S. A*. **2015**, *112*, 8817



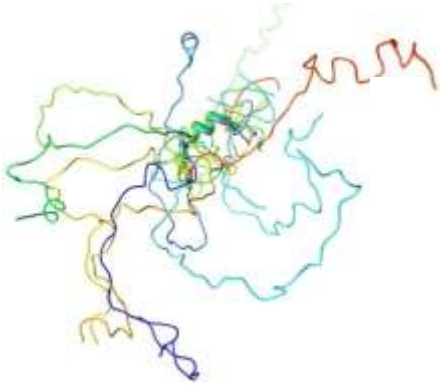
Ensemble of encounter complexes

Tang C., et al. *Nature* **2006**, *444*, 383.

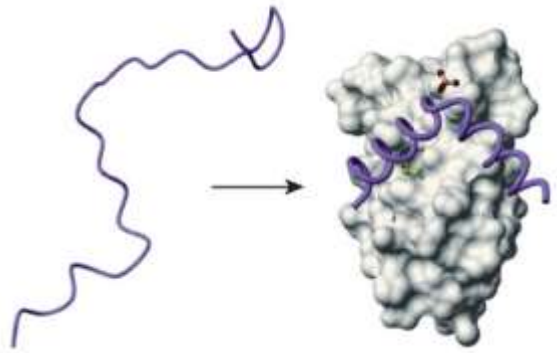
Applications of NMR

Flexible Proteins and Regions

Conformation

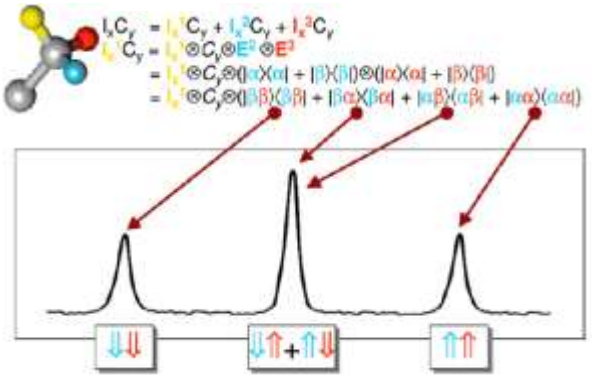


Binding

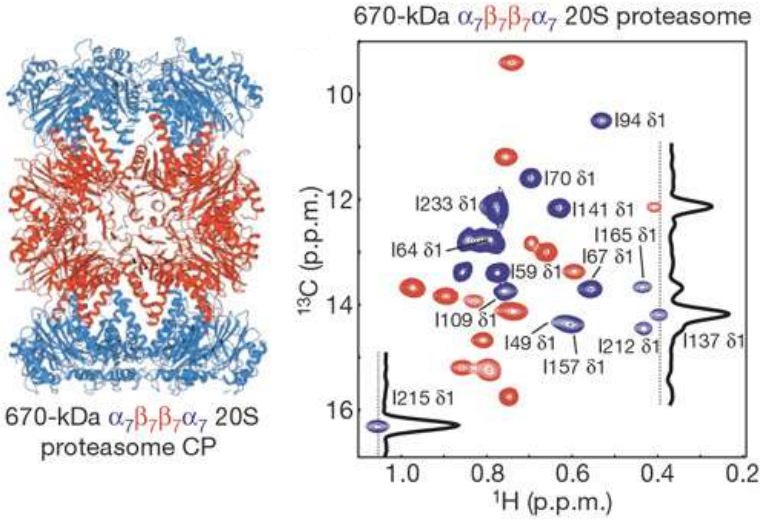


Sugase K. et al. *Nature* **2007**, *447*, 1021.

~ MDa Proteins



Kay L.E. *J. Magn. Reson*. **2005**, *173*, 193.

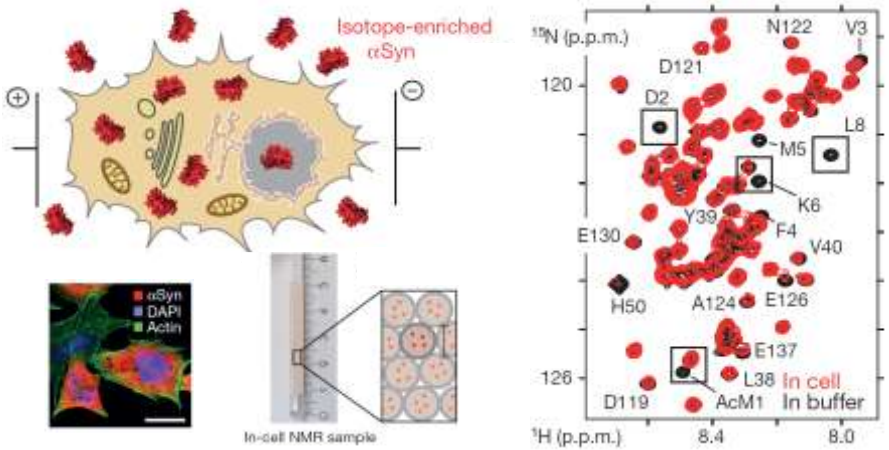


Sprangers R., Kay L.E. *Nature* **2007**, *445*, 618.

Applications of NMR

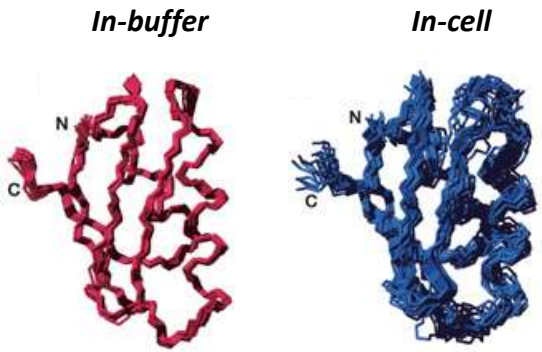
In-Cell NMR

Protein conformation in live *neuron* cells



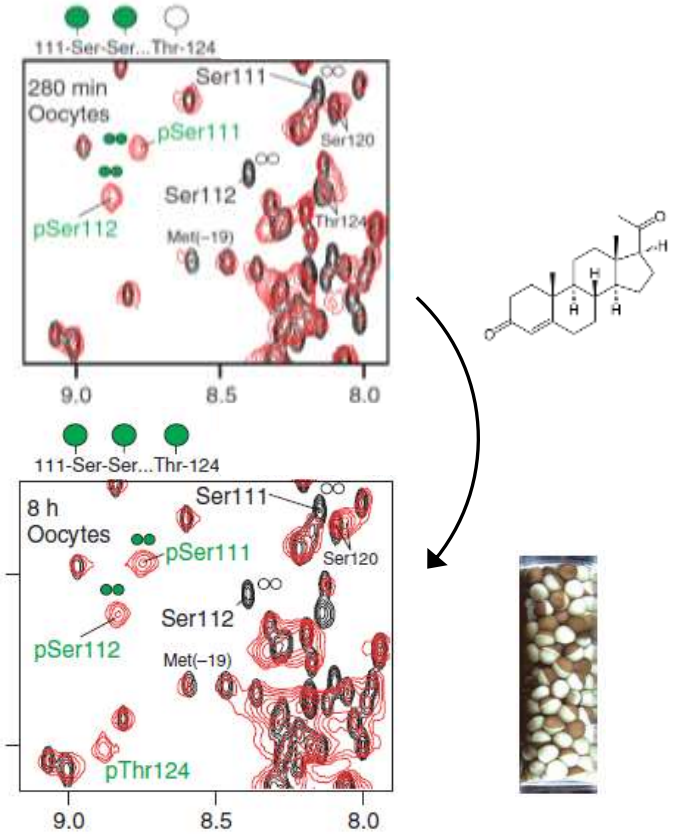
Selenko P. et al. *Nature* **2016**, *530*, 45.

Protein structure in live *E. coli* cells



Sakakibara D. et al. *Nature* **2009**, 458, 102-105.

Protein phosphorylation in live *oocyte* cells

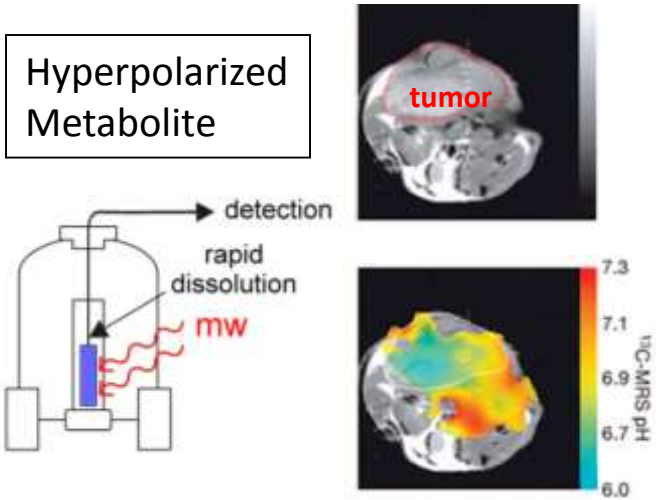


Selenko P. et al. *Nat. Struct. Mol. Biol.* **2008**, *15*, 321-329.

Applications of NMR

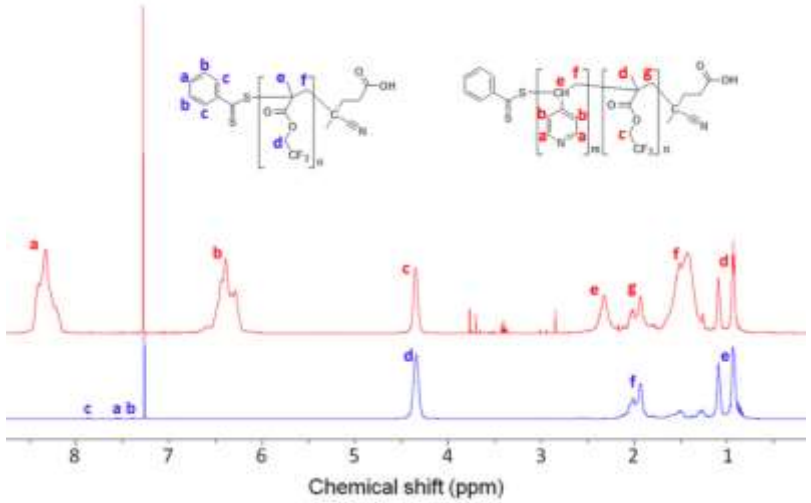
MRI

Hyperpolarized Metabolite

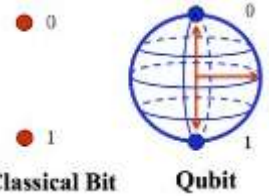


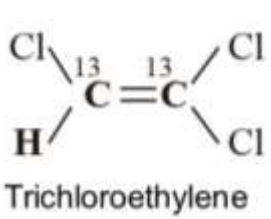
Gallagher, F. A., et al. *Nature* **2008**, *453*, 940.

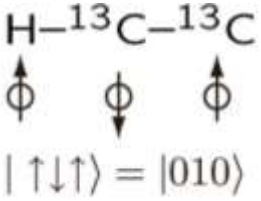
Polymers



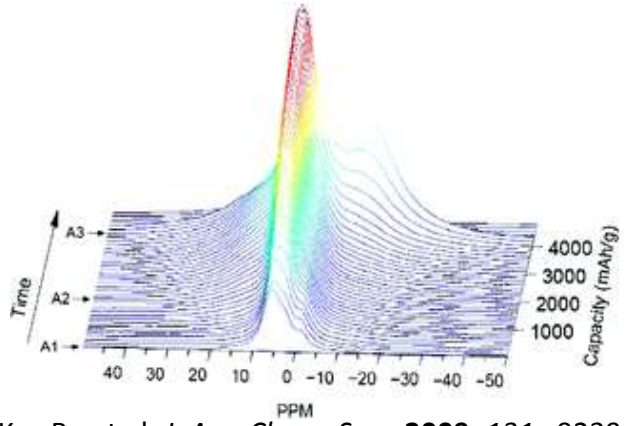
Quantum Computation







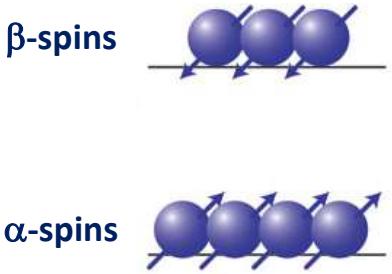
Lithium-ion batteries

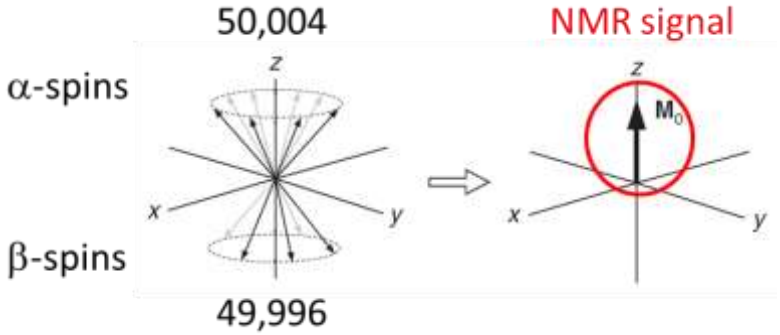


Key B., et al. *J. Am. Chem. Soc.*, **2009**, *131* , 9239.

NMR Signal

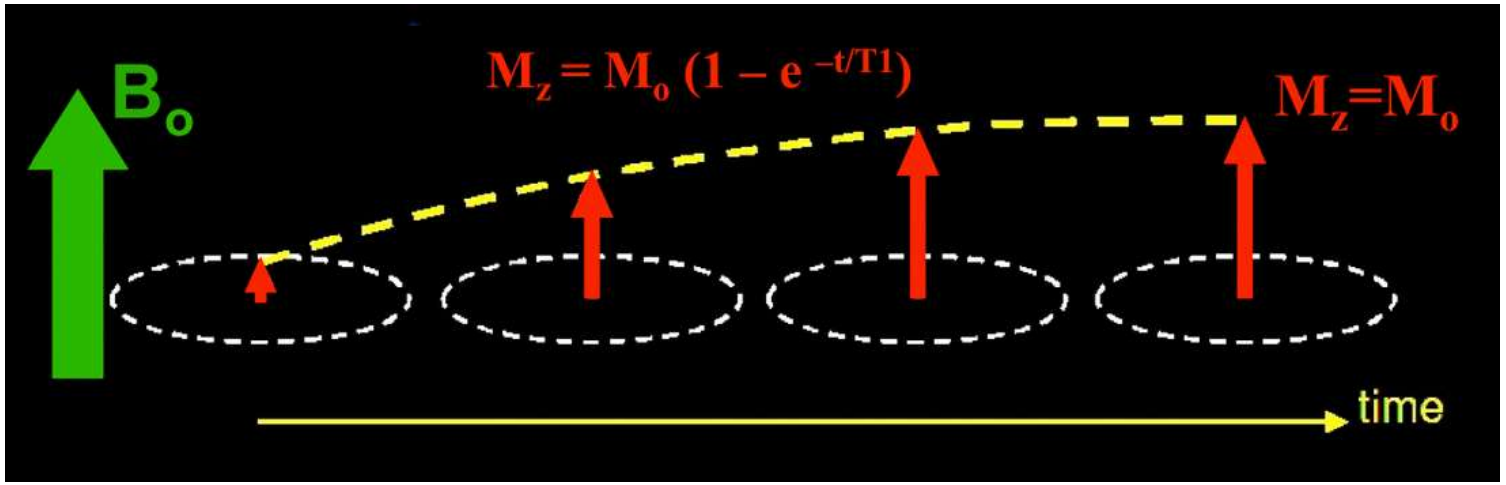
Boltzmann Distribution of Nuclear Spins

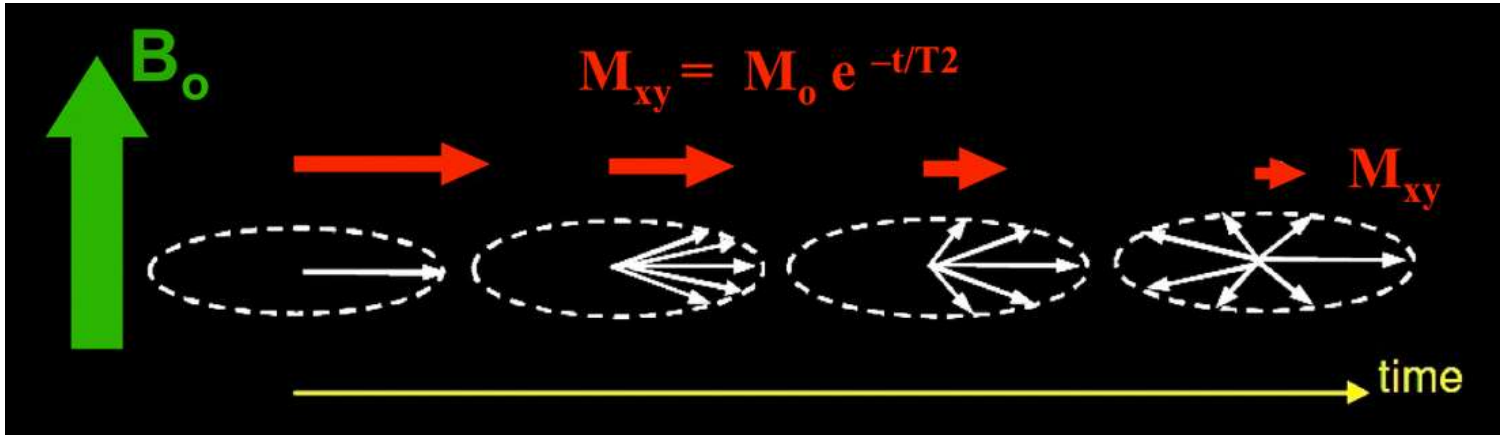




http://mutuslab.cs.uwindsor.ca/

T_1 and T_2 Relaxation

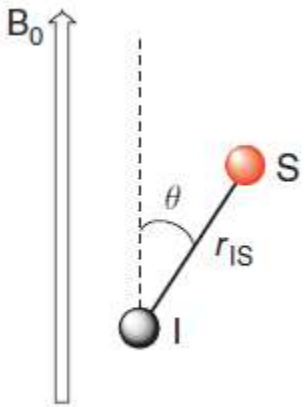


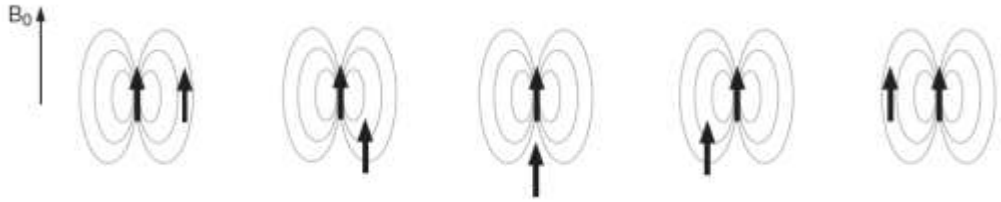


http://mri-q.com/

Rate of relaxation: $R_1=1/T_1$ and $R_2=1/T_2$

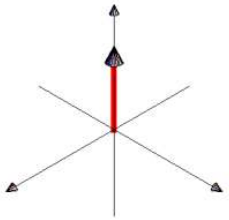
Dipolar Interactions between Nuclear Spins

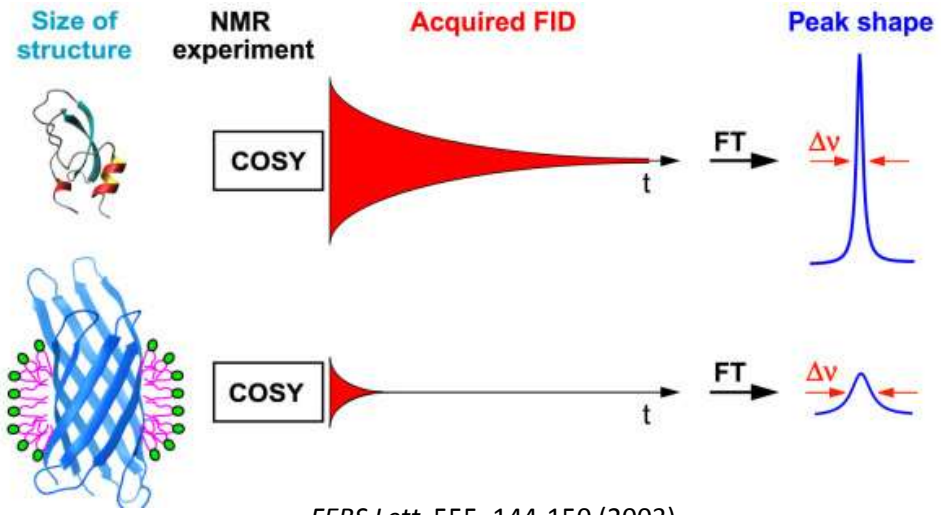




Claridge TDW. High-Resolution NMR Techniques in Organic Chemistry 3rd Ed. Elsevier Science

$$H_D = \frac{\hbar \gamma_I \gamma_S}{4\pi r_{IS}^3}[1 - 3\cos^2\theta](3I_zS_z - I \cdot S)$$





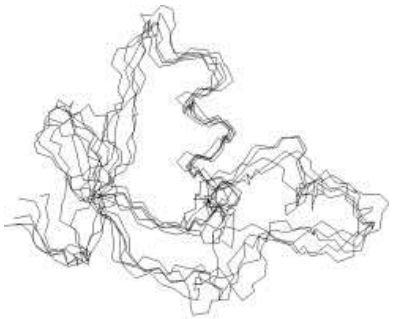
FEBS Lett. 555, 144-150 (2003)

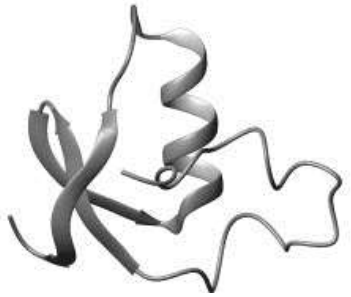
Nuclear Overhauser Effect (NOE)

Saturation of S spin

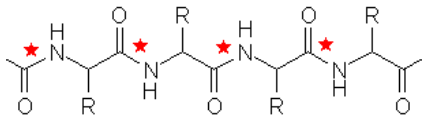
I-S dipolar interaction mediated NOE

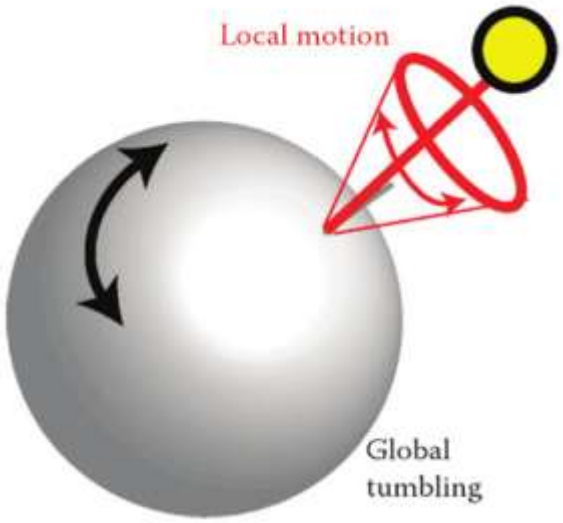
The first protein structure determined by NMR





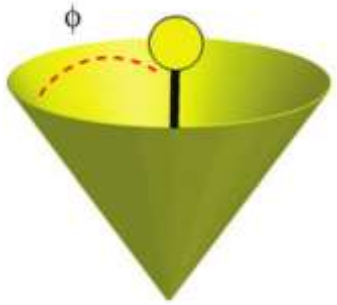
Dipolar Relaxation through a Covalent Bond



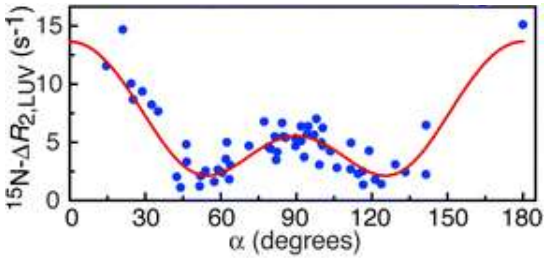


- Ratio of global to local motion (order parameter) → local dynamics
- Timescale of local and global motions

- Local motions are often described as a bond wobbling in a cone

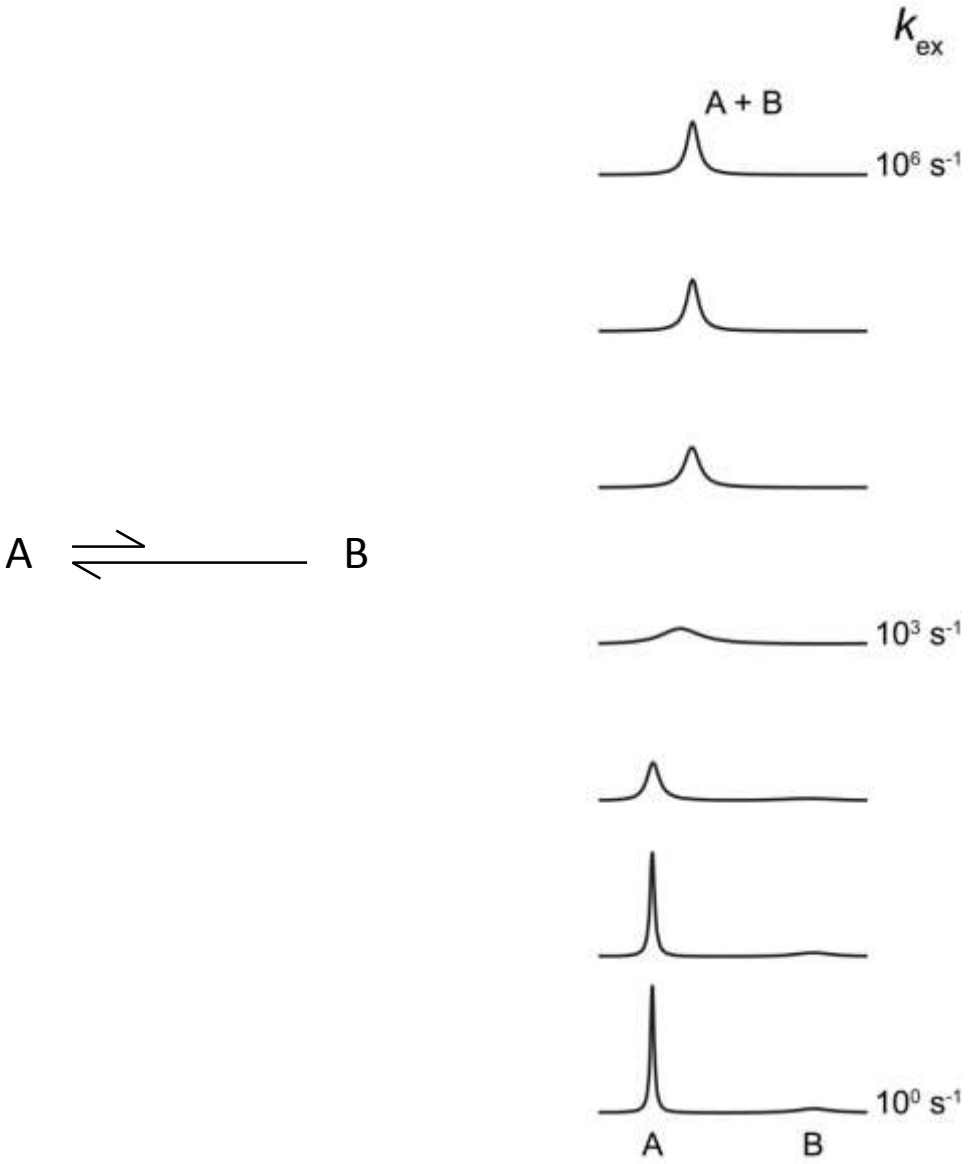


- Special motions lead to certain relaxation patterns.



J. Am. Chem. Soc. 104, 4546–4559 (1982)
Image: Introduction to Fluorescence. CRC Press (2014)

Line Broadening by Chemical Exchange



2. Lipid Membrane Mimetics for NMR studies

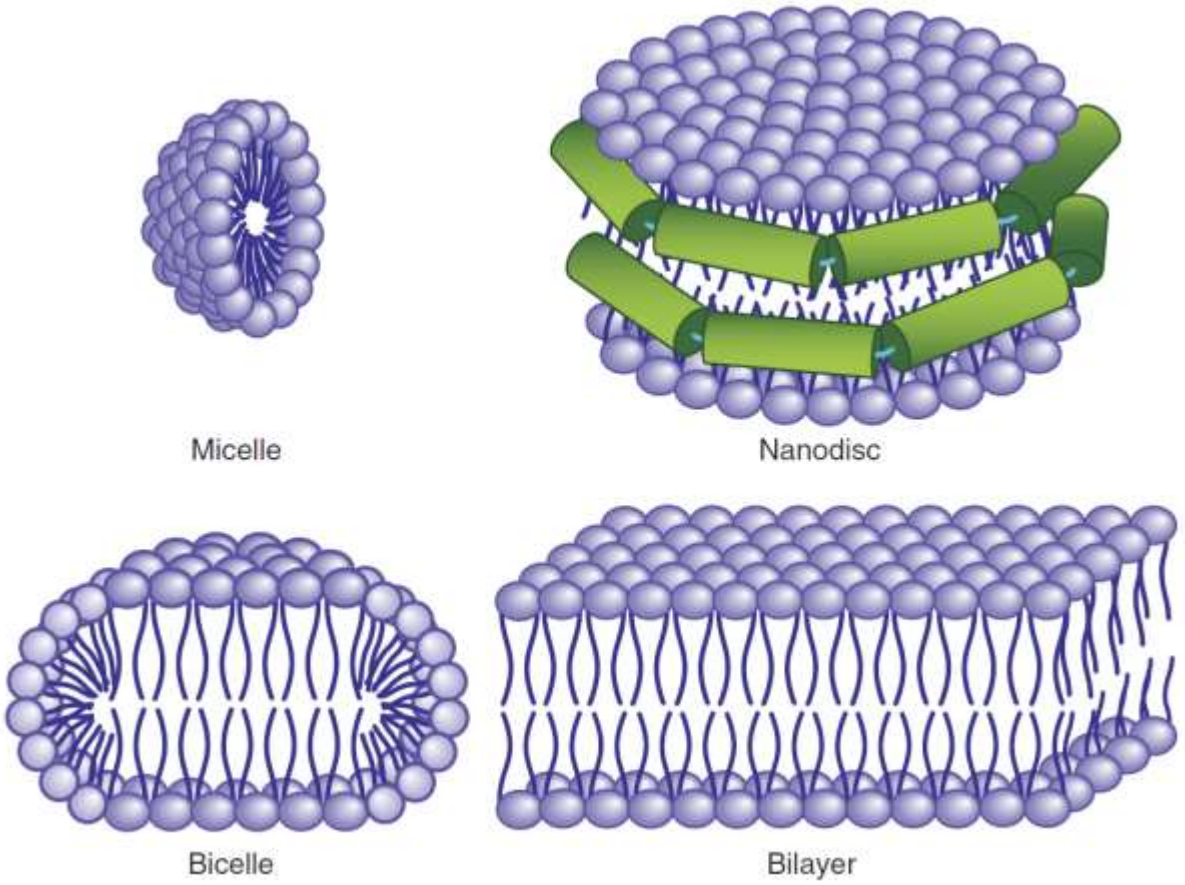
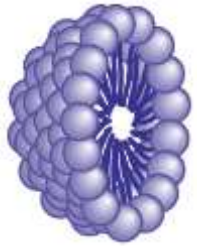


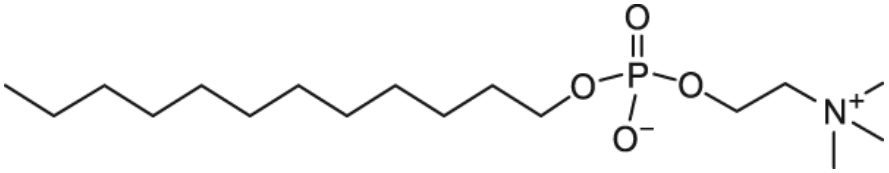
Image: Nat. Struct. Mol. Biol. 23, 468–474 (2016)

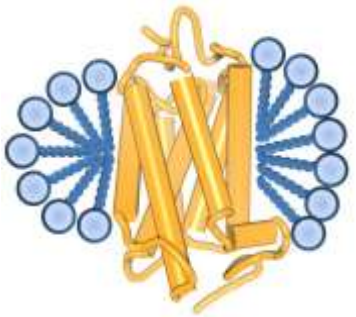
Micelle



- The most popular and simplest way to prepare membrane proteins for NMR studies is to disperse them in lipid micelles.
- Denatured (e.g. by urea) membrane protein is added dropwise to a detergent solution to refold the protein → Micelle solution is added dropwise to the refolded protein while stirring → Buffer is exchanged to the micelle solution.

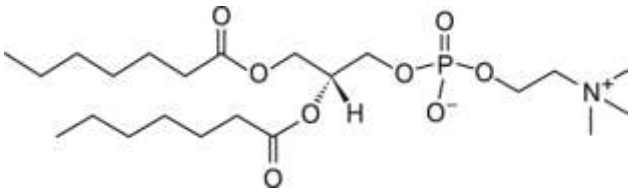
dodecylphosphorylcholine (DPC)

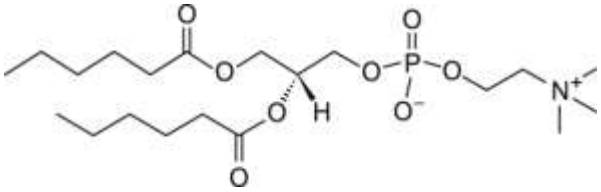




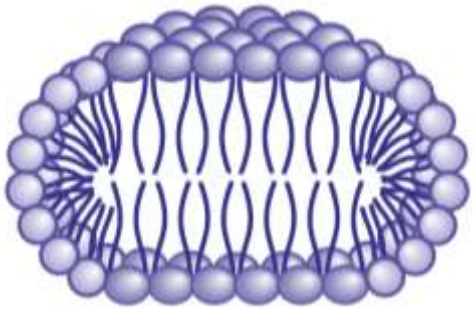
Front. Pharmacol. 6, 1-24 (2015)

diheptanoyl / dihexanoyl phosphocholine (DHPC)



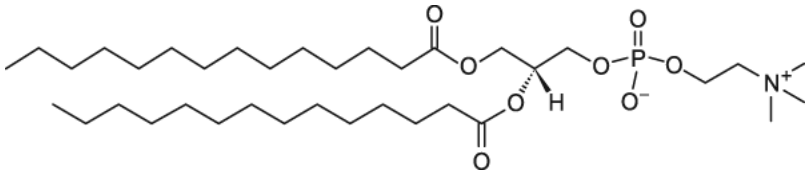


Bicelle

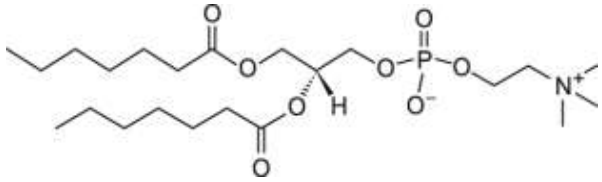


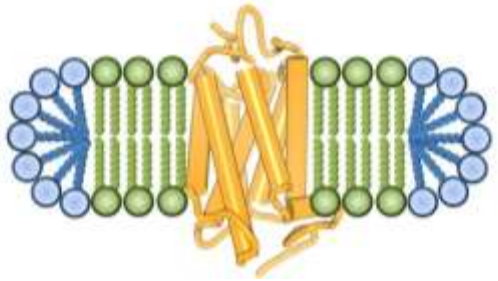
- Bicelles are mixture of bilayer-forming phospholipids (e.g. DMPC) and non-bilayer-forming phospholipids (e.g. DHPC).
- The ratio DMPC(long-chain)/DHPC(short-chain) determines the size of the bicelle, with higher ratio leading to larger and flatter bicelle.

DMPC

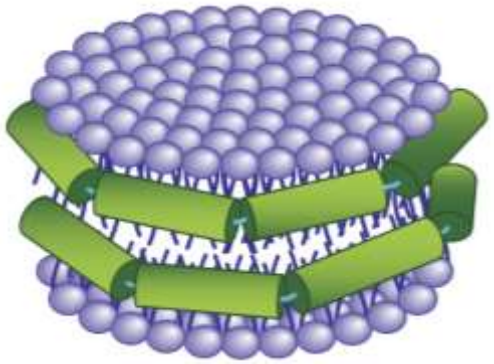


DHPC

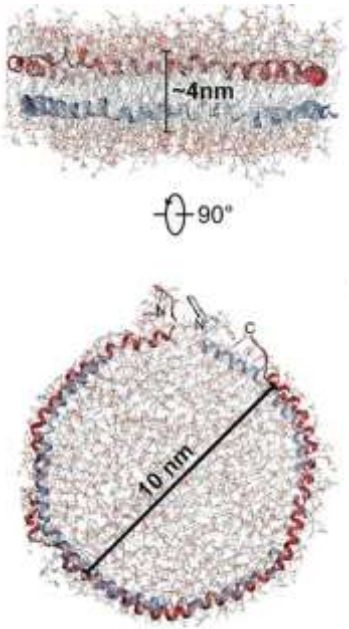


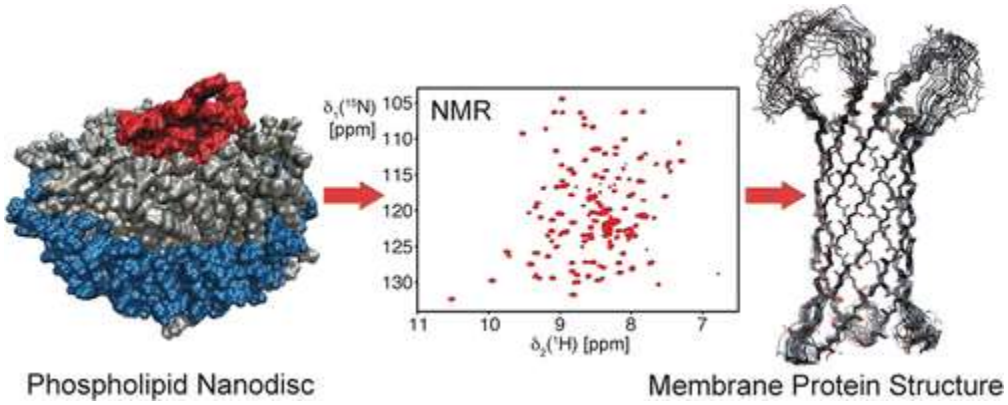


Nanodisc



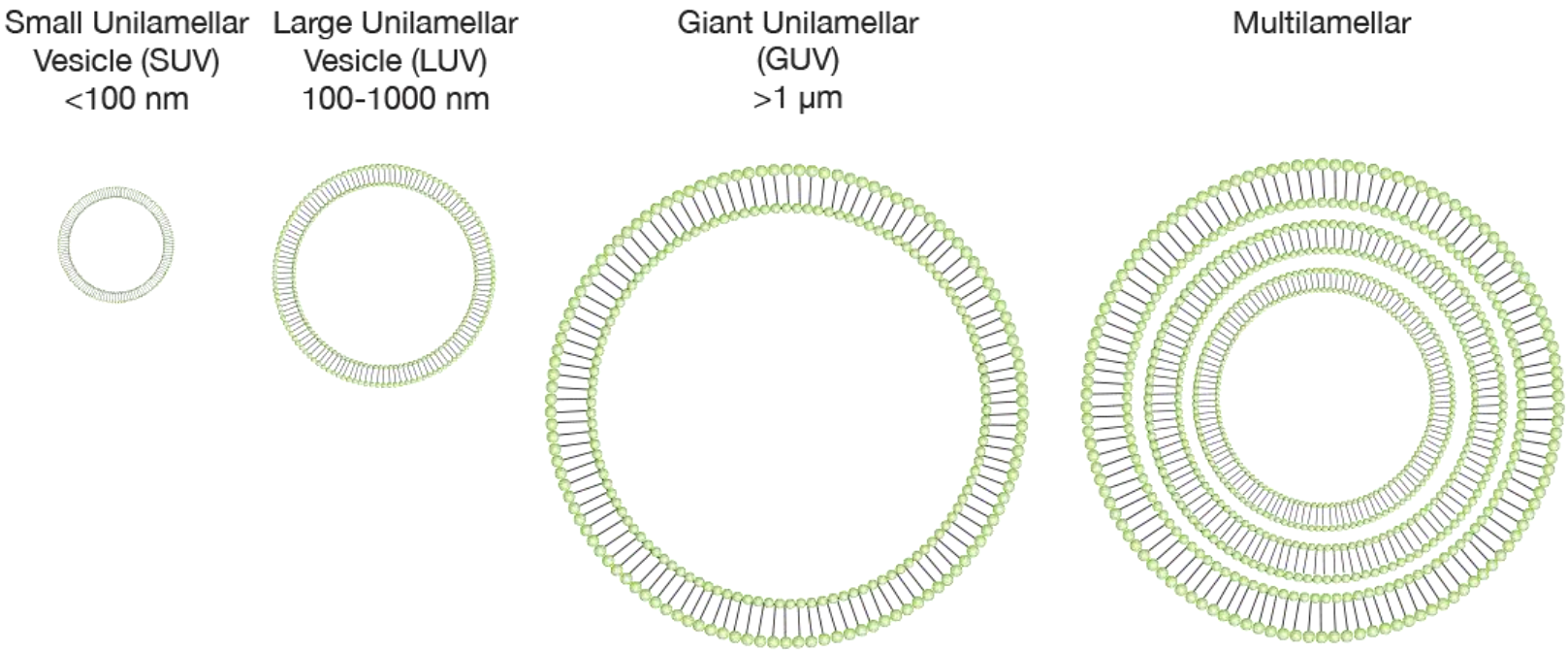
- Nanodiscs are small patches of lipid bilayer surrounded by segments of amphipathic helical proteins that stabilize the patches.
- Usually two copies of the membrane scaffold protein (MSP) are at their perimeter. MSPs are derived from apolipoprotein A-1.
- Nanodiscs are assembled by adding MSPs to cholate-solubilized phospholipids and detergent-solubilized membrane proteins. Detergents are removed afterwards.





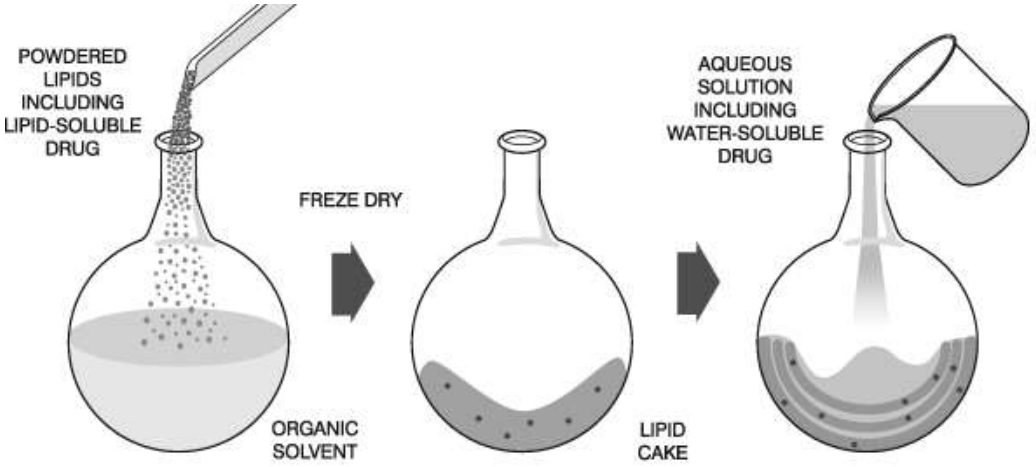
Nano Letters 2, 853–856 (2002)
J. Am. Chem. Soc. 135, 1919–1925 (2013)

Liposomes for Functional Studies

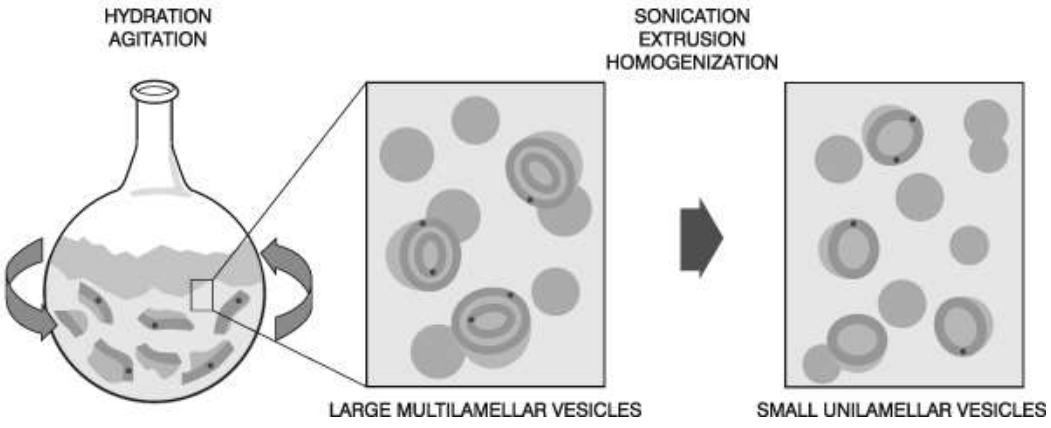


https://www.mirusbio.com/

Making Liposomes



Sonication → SUVs



Extrusion → LUVs

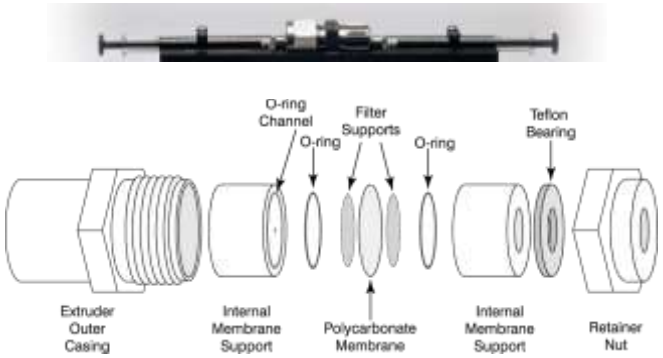
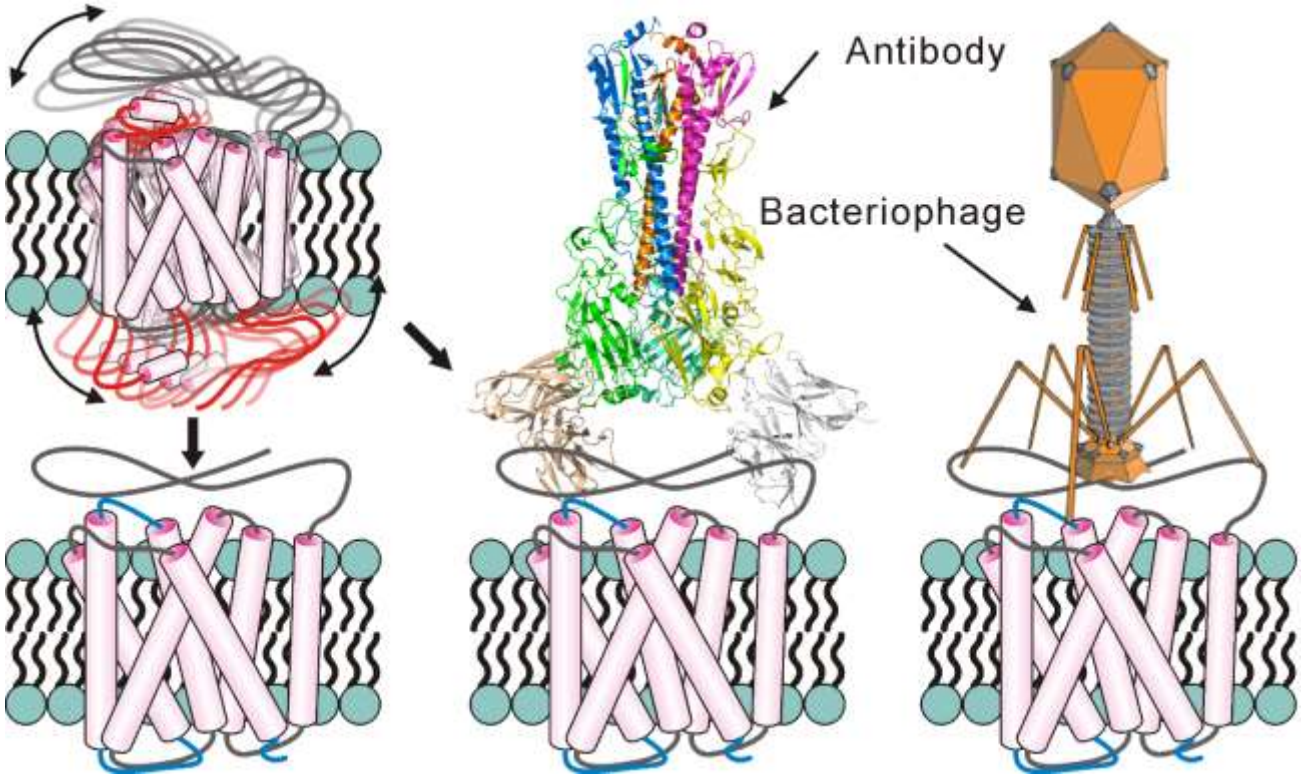
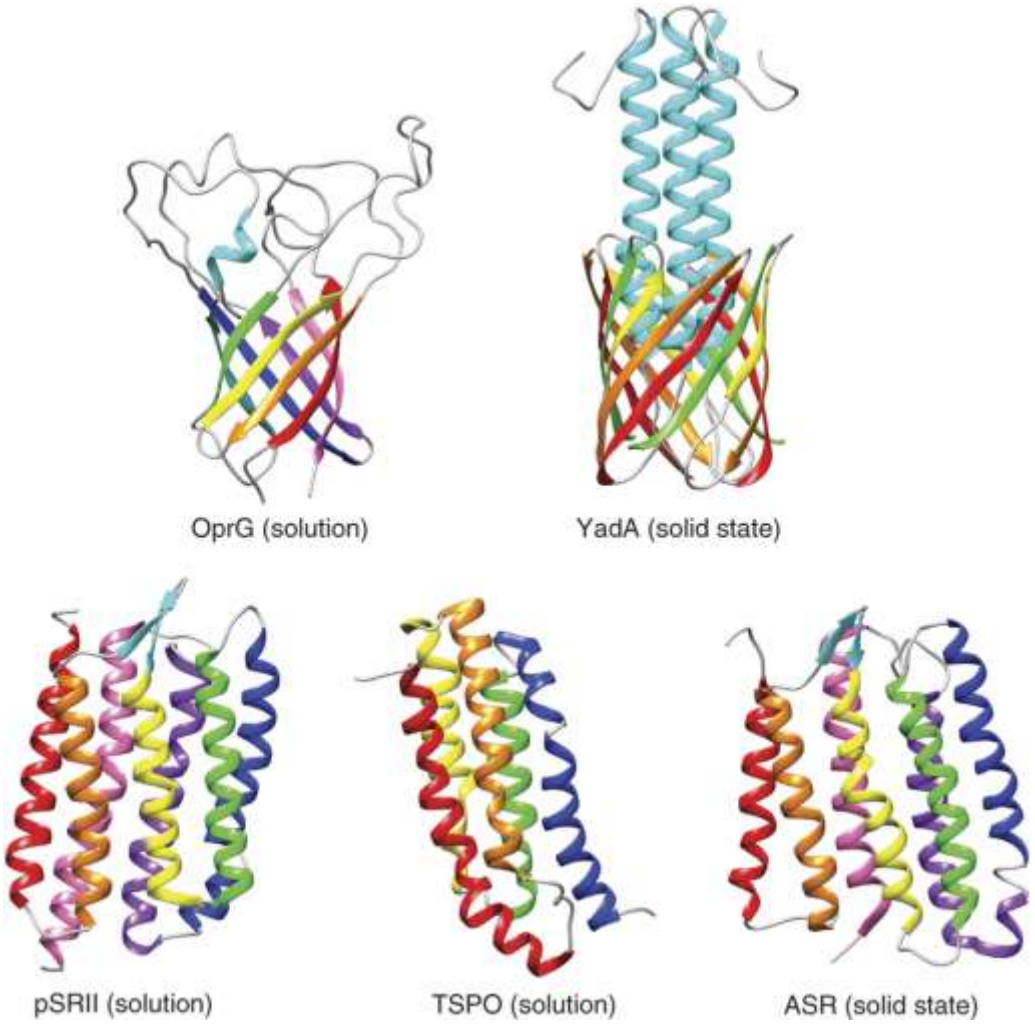


Image: Avanti Lipids

Motivation for Studying Membrane Proteins by NMR



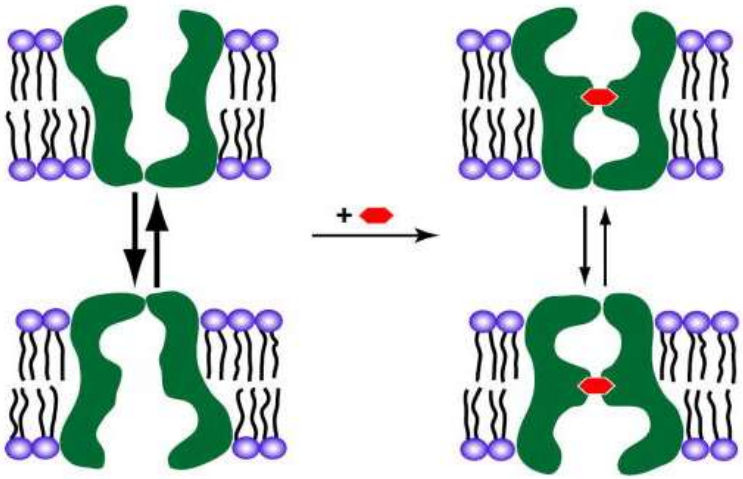
3. Characterization of Membrane Proteins by NMR



Nat. Struct. Mol. Biol. 23, 468–474 (2016)

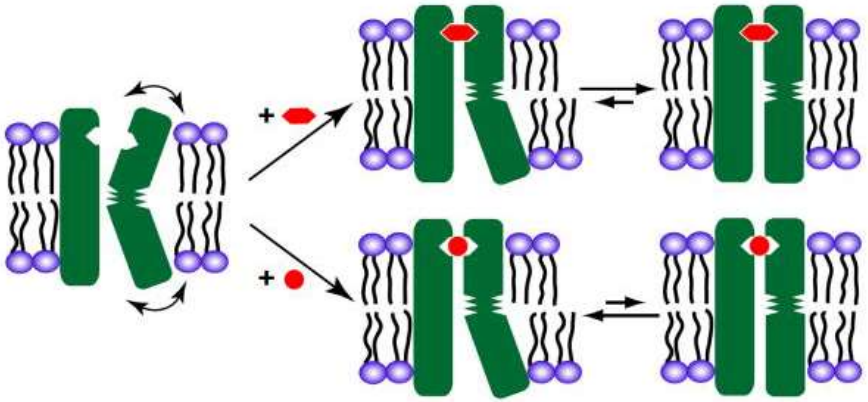
Membrane-Protein Function and Dynamics

Faster Dynamics in the ligand-free apo form



J. Am. Chem. Soc. 136, 8072-8080 (2014)

Ligand-induced conformational selection

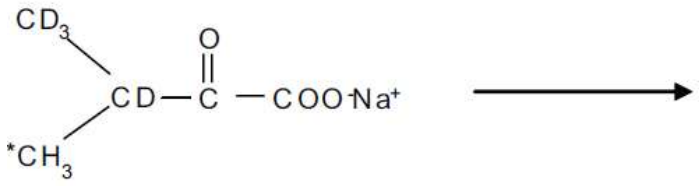


Science 355, 1106-1110 (2012)

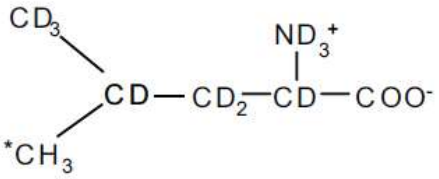
Val, Leu, Ile methyl-protonated and ^{15}N-, ^{13}C-, ^{2}H-labeled membrane protein

^{15}N ammonium chloride, U-^{13}C/^{2}H-labeled glucose, $^{2}H_2O$

+

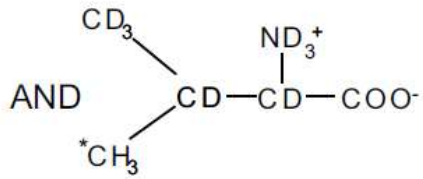


CDLM-7317 α-Ketoisovaleric Acid, Sodium Salt
(3-Methyl-^{13}C, 99%; 3,4,4,4-D_4, 98%)

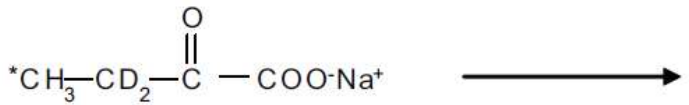


(^{1}H-δ-Methyl) -Leucine

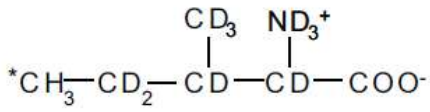
AND



(^{1}H-γ-Methyl) -Valine



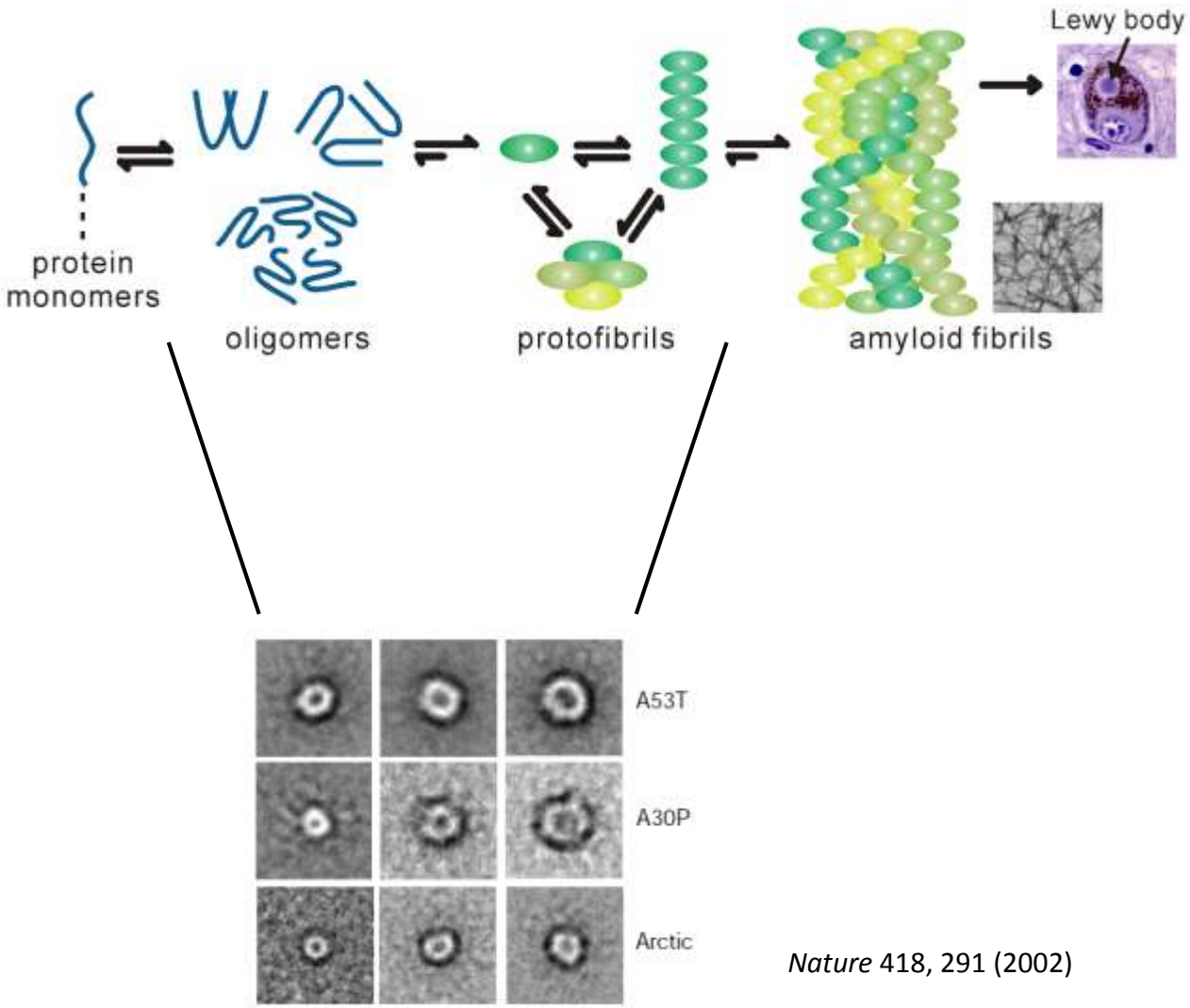
CDLM-7318 α-Ketobutyric Acid, Sodium Salt
(Methyl-^{13}C, 99%; 3,3-D_2, 98%)



(^{1}H-δ Methyl)-Isoleucine

J. Biomol. NMR. 13, 369–374 (1999)

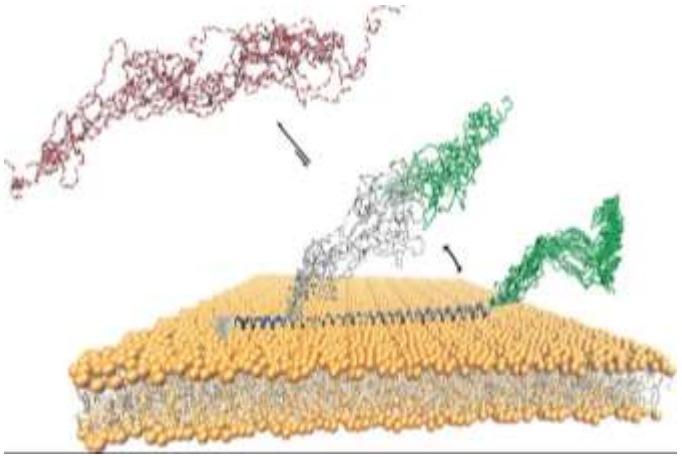
Proteins Affect Membranes in Many Ways



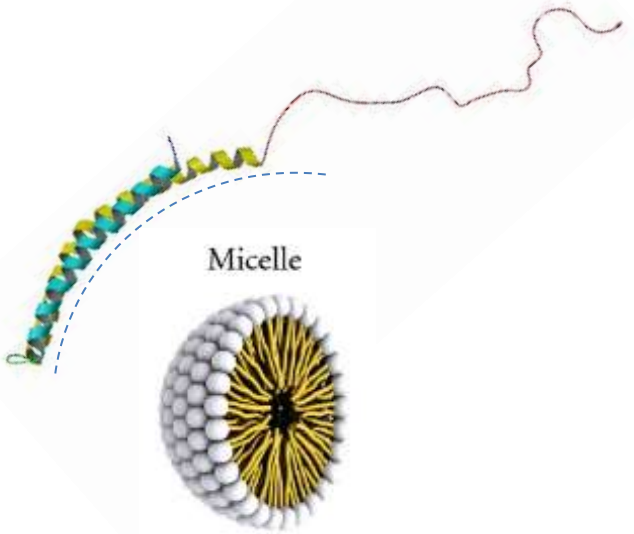
Nature 418, 291 (2002)

4. Structure Induction upon Membrane Binding

Alpha-Synuclein (αS) and Lipid-Membrane Interaction



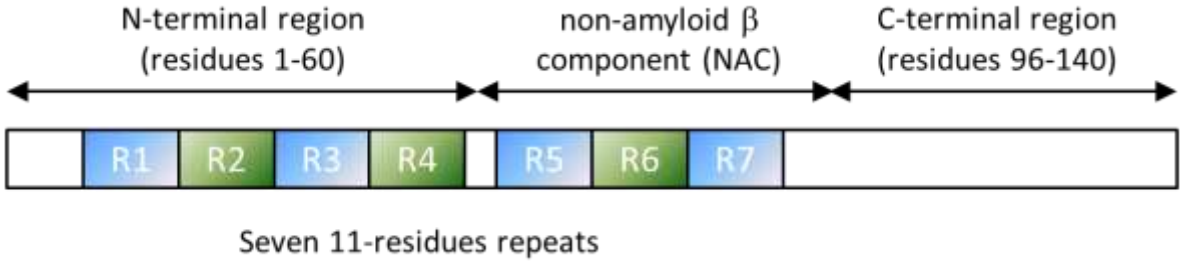
Nat. Commun. 5, 1-8 (2014)



J. Biol. Chem. 280, 9595-9603 (2005)

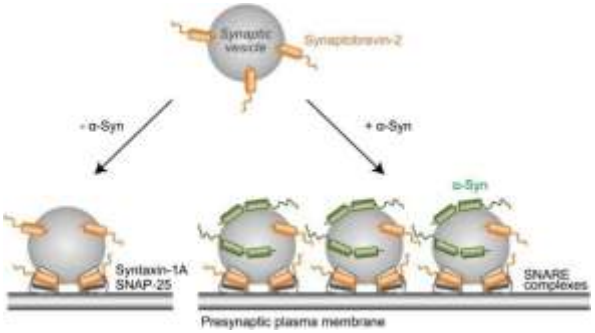
Alpha-Synuclein (αS) Protein

αS Primary Structure

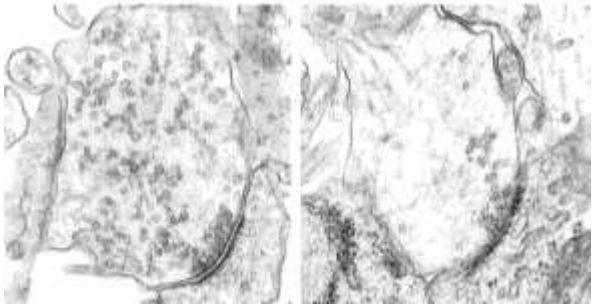


αS Function

Helps SNARE-Complex Assembly



Maintain the size of synaptic vesicle pools



Control **Deplete αS**

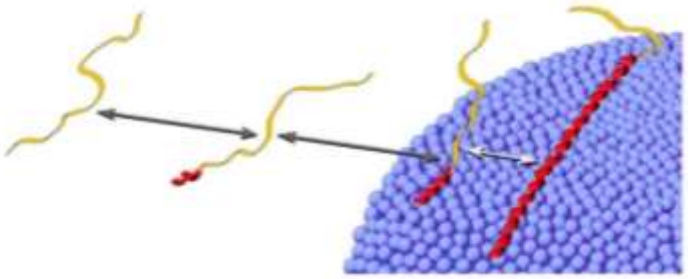
Impaired learning and memory in αS knockout mice

αS and Parkinson's Disease (PD)

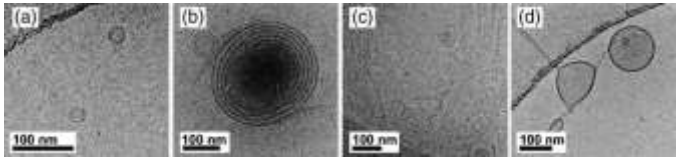
- αS gene triplication and mutations are found in familial PD.
- αS is the main component of Lewy bodies, which are aggregates of proteins in PD patient's brain.

Challenges in Studying αS-Membrane Interaction

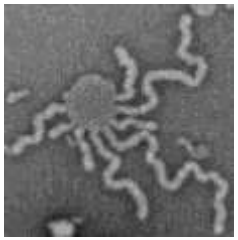
Dynamic Equilibrium



Heterogeneous Population

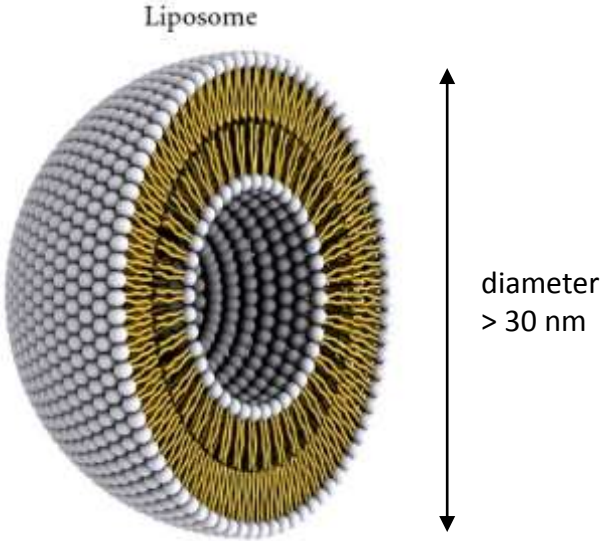


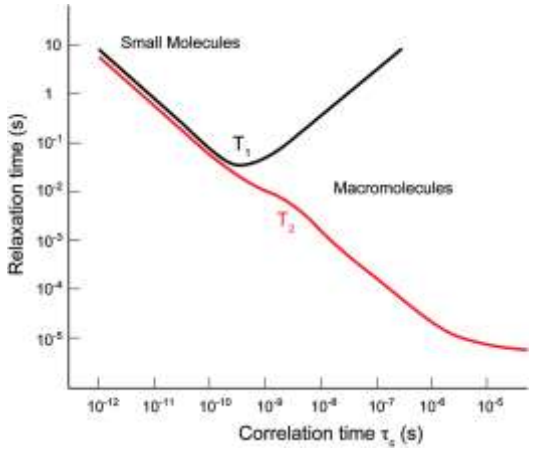
Bodner et al. *J. Mol. Biol.* (2009)



Jiang et al. *J. Am. Chem. Soc.* (2013)

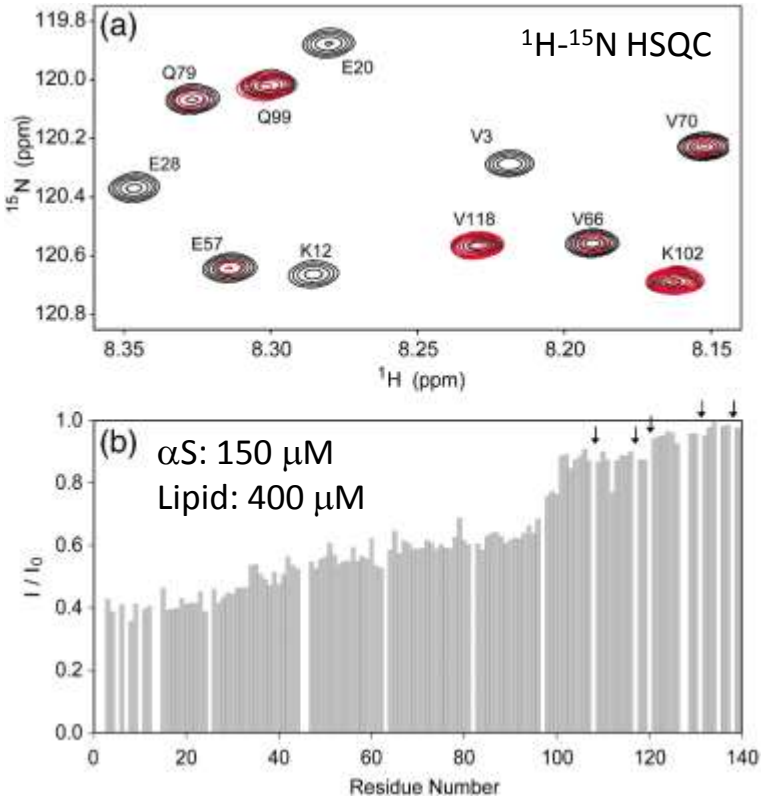
αS-Membrane Complex is too large for NMR

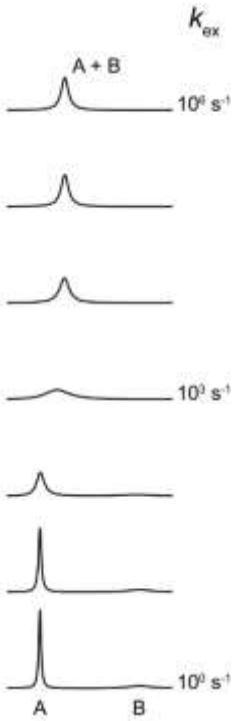


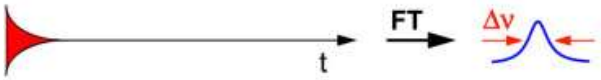


Liposome-αS Interaction

αS: 150 μM
Lipid: 400(black), 800(red) μM



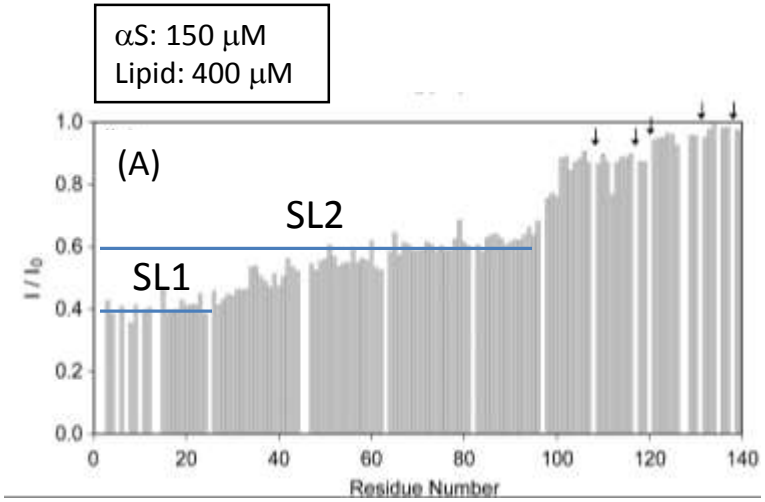


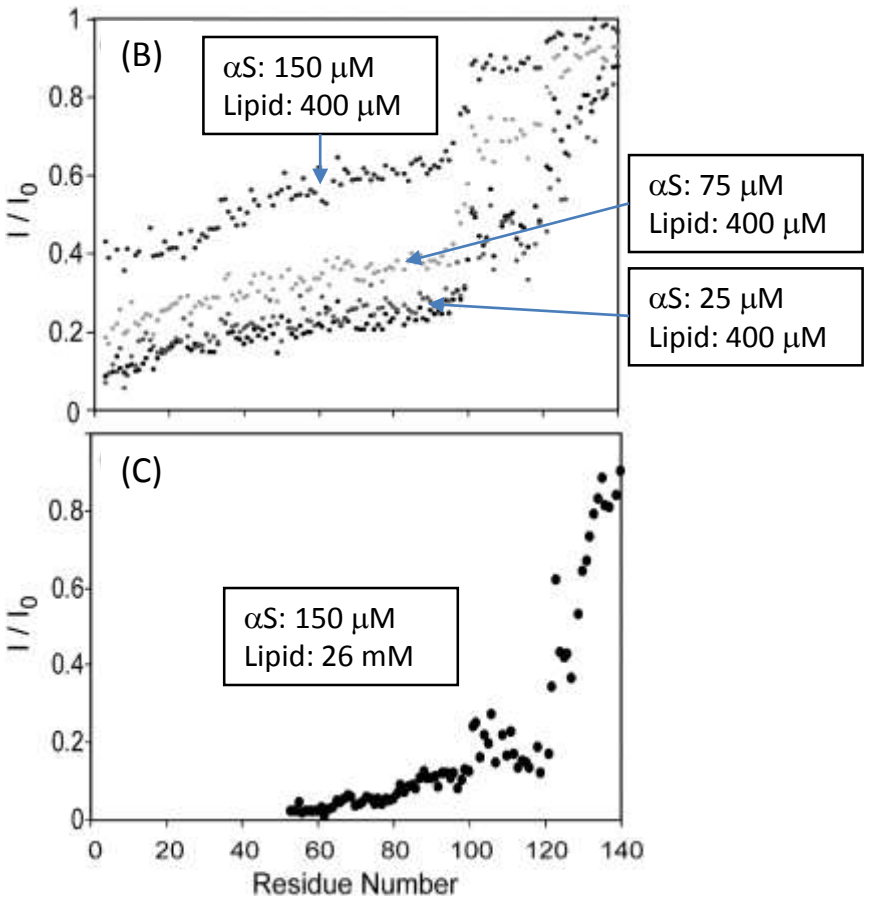


- Signals from the N-terminal residues are significantly more attenuated than signals from the C-terminal residues upon addition of SUVs.
- No new or shifted resonance positions are observed.
- Minimal line-broadening is observed.
- Exchange is slow on the NMR timescale (<10 s^{-1}).

J. Mol. Biol. 390, 775-790 (2009)

Multiple Competing-Membrane-Binding-Modes of αS



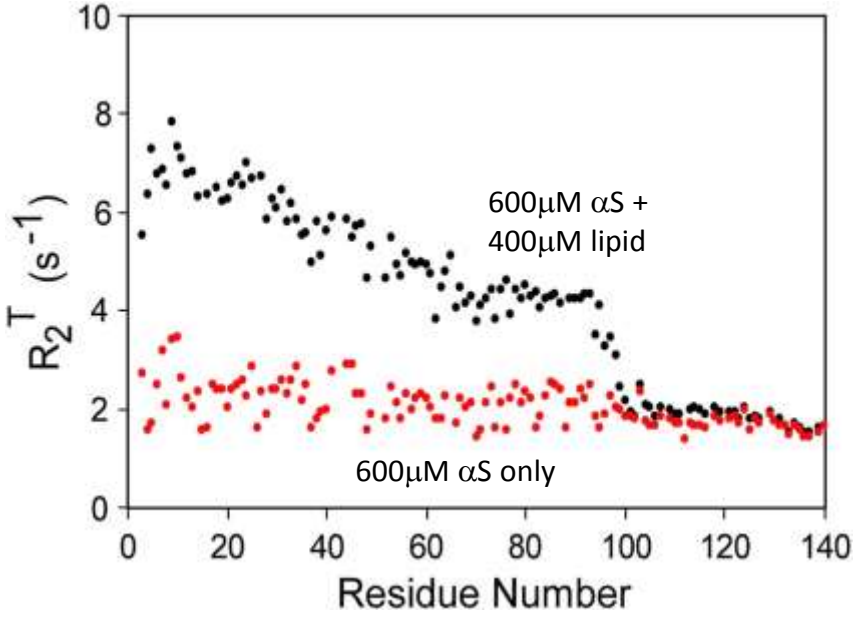


- At low lipid/protein ratios (A), there exists a pool of protein, where only the first ~25 N-terminal residues are bound (SL1), and the second pool (SL2), where residues 1-97 are bound and invisible.
- Residues that are not directly bound to the lipids are dynamically disordered, even when other parts of the same protein molecule are immobilized.
- The relative degree of attenuation changes when the protein concentration is lowered (B), indicating competition between different binding modes. For example, SL1 is 20% and SL2 is 40% in (A).
- NMR data recorded at high lipid/aS ratio (C), suggests that more than two distinct states exist.

J. Mol. Biol. 390, 775-790 (2009)

Kinetics of Binding

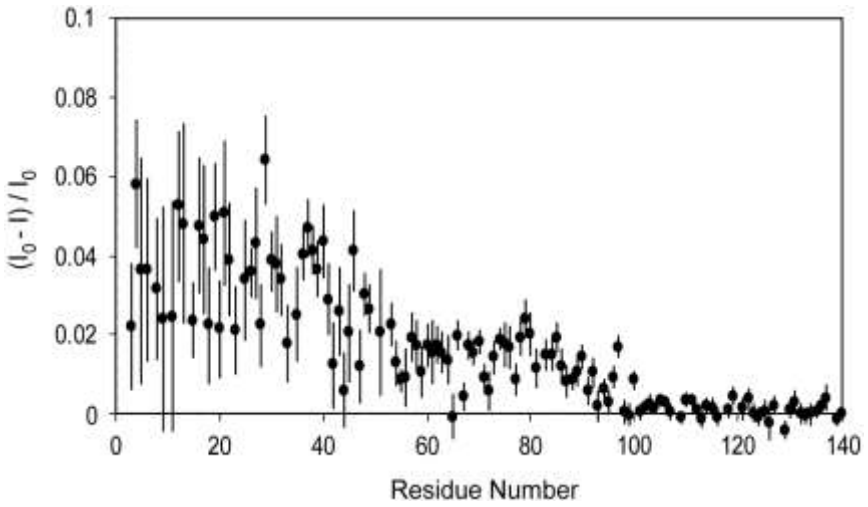
Transverse relaxation rate



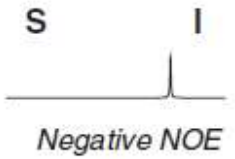
- The observed R_2^T equals the sum of the R_2^T of the highly mobile $R_2^T{}_{random\text{-}coil}$ and the forward rate of the free-to-bound transition k_{on}.
- k_{on} = 3-5 s^{-1}

Truncated signal

Saturation transfer NMR



- Selective saturation of the magnetization of phospholipid methylene resonances at 1.16 ppm.
- The fact that magnetization can be transferred from the lipid-bound state to the free state indicates that the timescale of bound-to-free transition is on the order of longitudinal relaxation rate of aS amide protons. k_{off}= ~1s^{-1}.

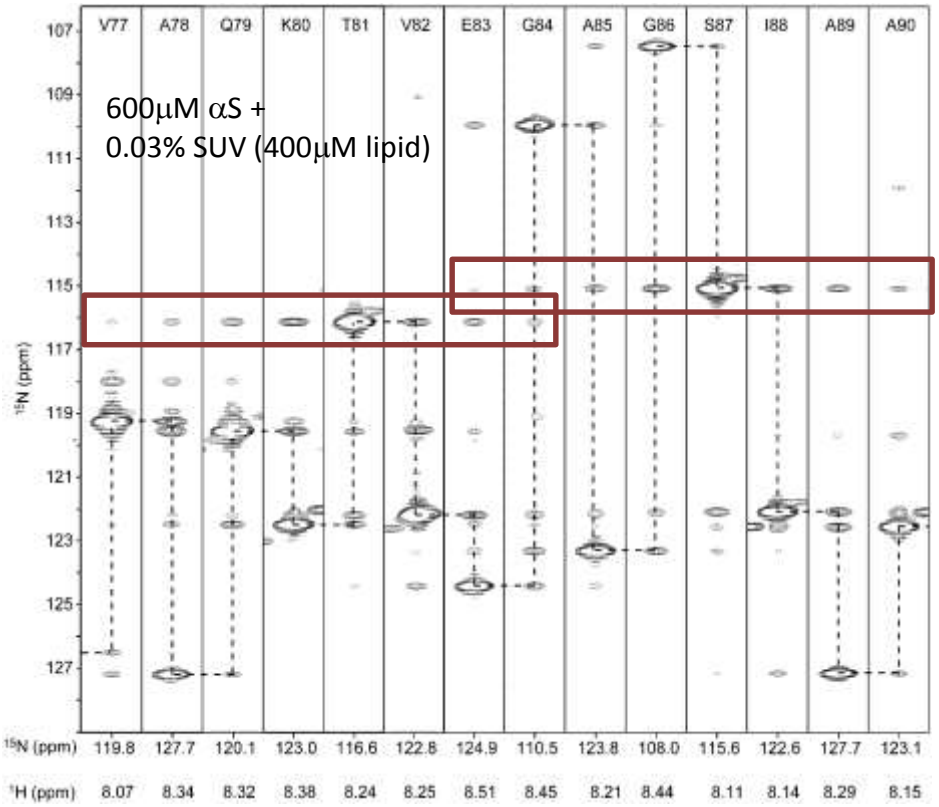


Negative NOE

J. Mol. Biol. 390, 775-790 (2009)

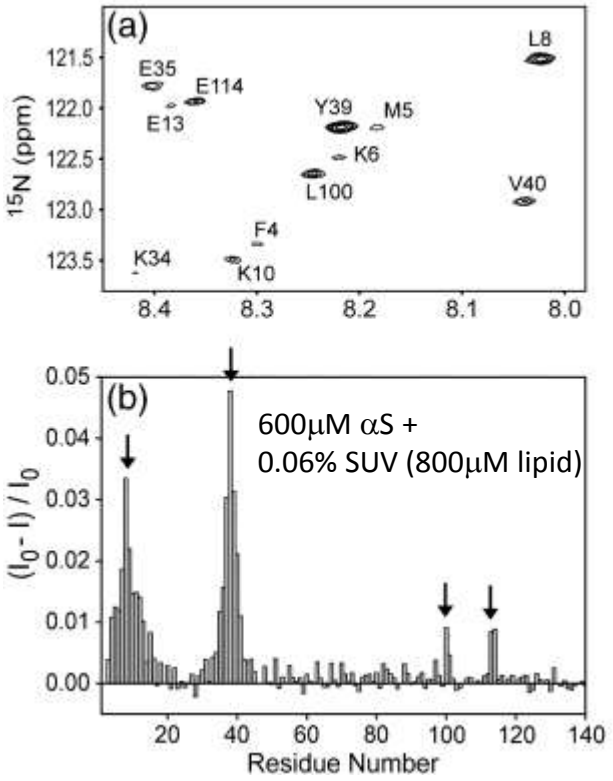
Probing the Invisible Bound State of αS

Transferred NOE between H^N and H^N



- (Free state → Bound state → Free state) during the NOE mixing time. Fast NOE transfer indicates that the bound complex is huge and αS is helical.
- Both SL1 and SL2 regions show H^N-H^N connectivities to eight or more adjacent amide protons.

Transferred NOE between Leu-CH_3 and H^N



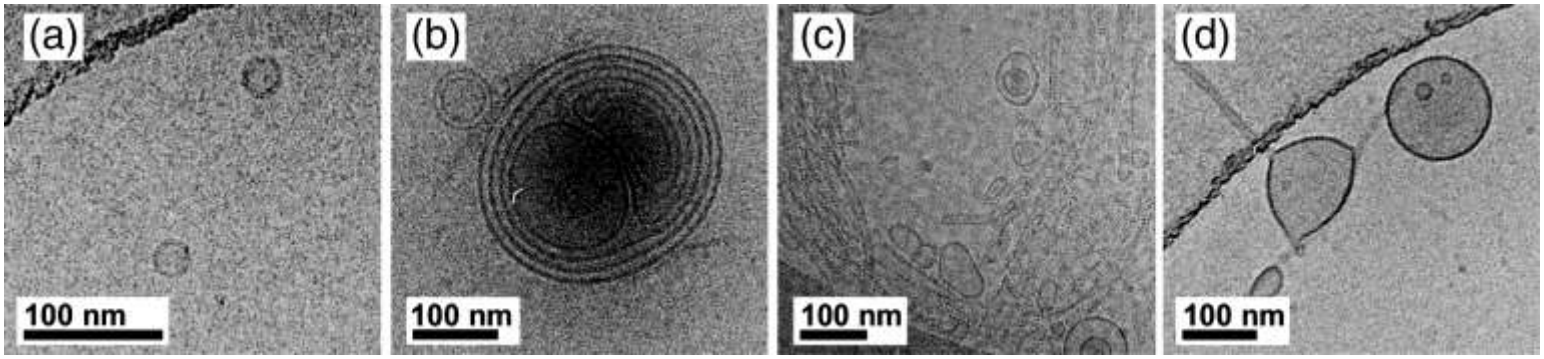
- NOE difference effect is large for Leu8 and Leu38 and extends over a significant number of residues.

J. Mol. Biol. 390, 775-790 (2009)

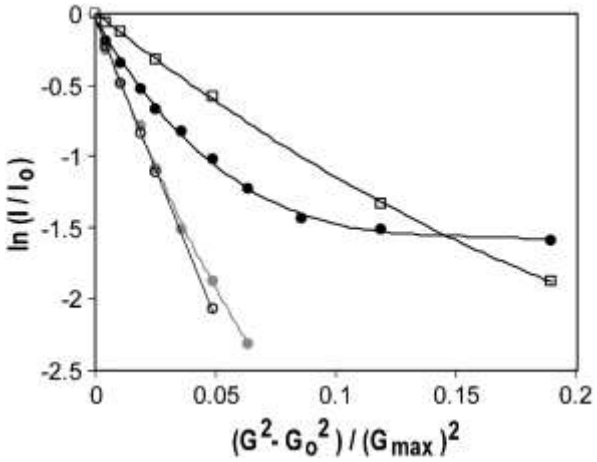
Size of the αS-Lipid Complex

Cryo-EM Images

600μM αS +
0.03% SUV (400μM lipid)



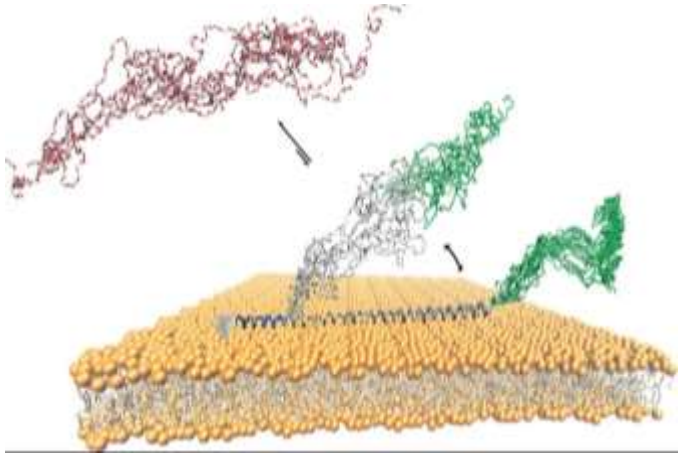
NMR diffusion experiment

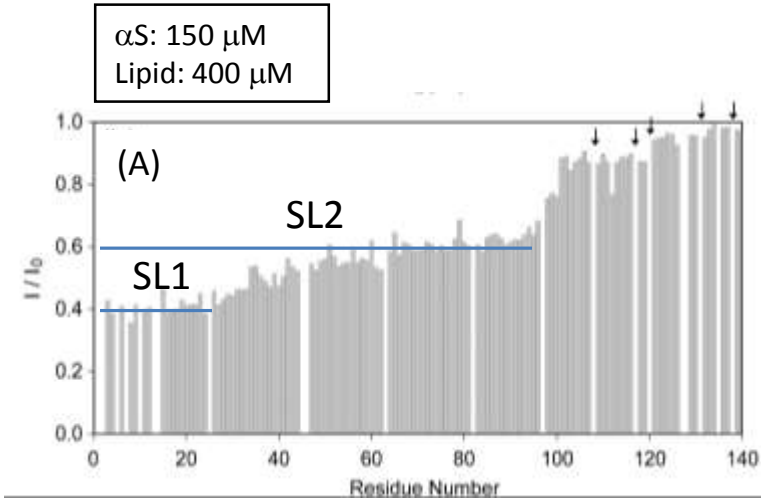


Sample	D_s (× 10^{-11} m^2 s^{-1})	R_h (Å)[a]
150 μM αS[b]	5.77 ± 0.12	26.6 ± 0.5
150 μM αS[b] + 0.03% SUV	4.1 ± 0.2	37 ± 2
150 μM αS[b] + 2.0% SUV	0.15 ± 0.01	990 ± 30
2.0% SUV[c]	0.99 ± 03	152 ± 5

J. Mol. Biol. 390, 775-790 (2009)

Open Questions



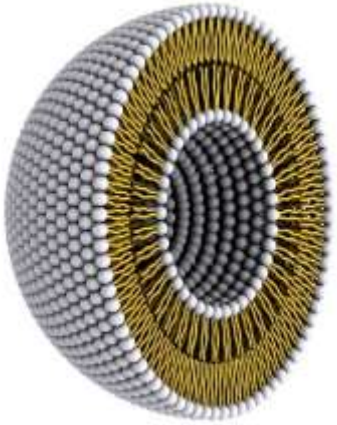


J. Mol. Biol. 390, 775-790 (2009)

- If αS binds to the surface of SUVs with its 100 N-terminal residues in a contiguous α-helical conformation, it would occupy a minimum of 1400 Å^2, approximately to the surface area of 28 phospholipid headgroups. (surface area of a single lipid headgroup in a bilayer is 50 Å^2).
- With two leaflets per bilayer, the minimal stoichiometry for such a binding mode requires at least 56 lipids per αS molecule, assuming that the surface of an SUV to be 100% covered by αS.
- Even with αS : lipid = 1 : 2.6, 40% of αS is bound in SL2 mode.
- The N-terminally acetylated form of αS binds to the lipid membranes even more tightly worsening the dilemma.
- Does a special stable, oligomeric, lipid-bound species of αS exist (e.g. bundle of aS with a modest number of phospholipids at its core)?

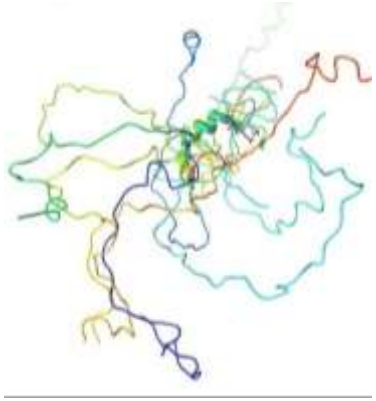
Monitoring Acetylation Reactivity of Lysine Side Chains

Small Unilamellar Vesicles (SUV)
DOPE:DOPS:DOPC = 5:3:2

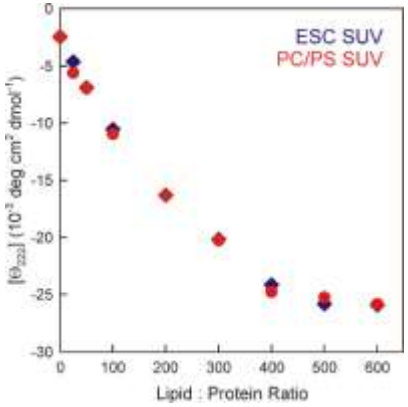


+

α-Synuclein

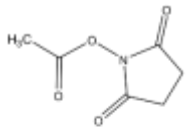


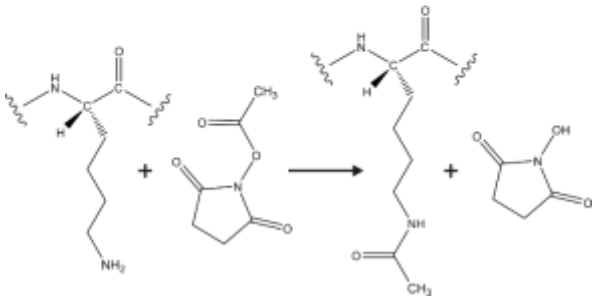
Helical Structure of α-Synuclein



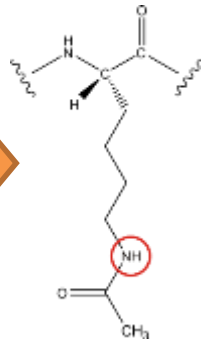
Add

N-Succinimidyl-Acetate

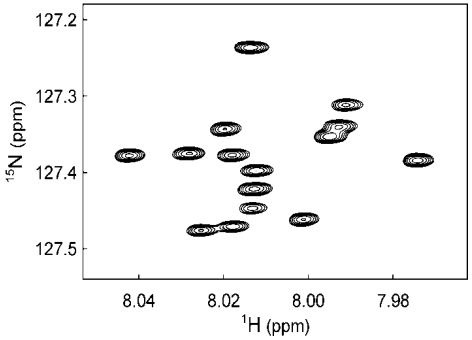




Lipid removal by methanol precipitation



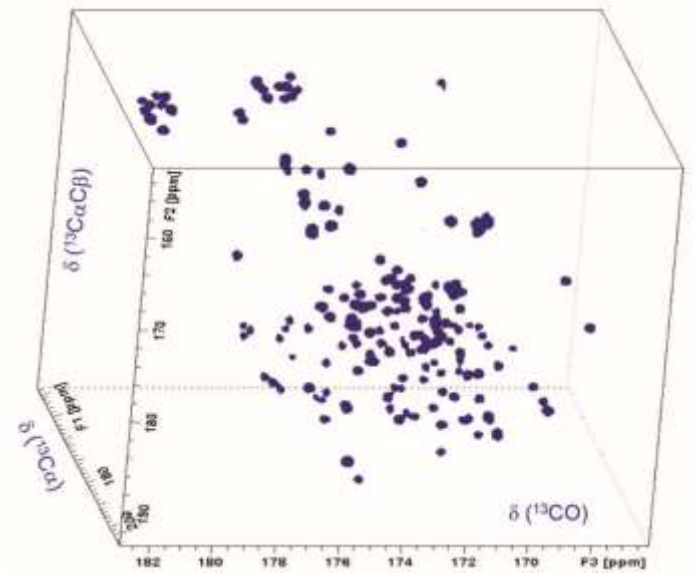
High resolution NMR of acetylated lysine side chains

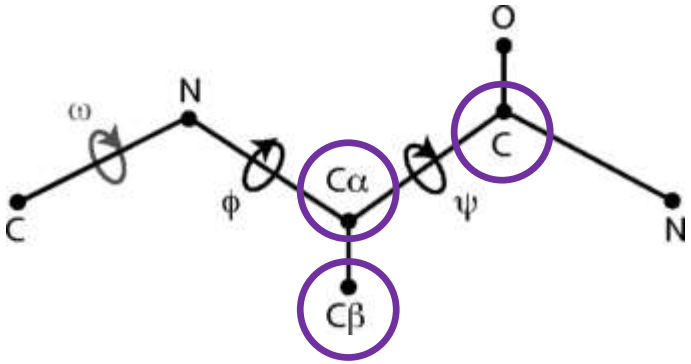


Lee JH et al. *Biochemistry.* 55, 4949 (2016)

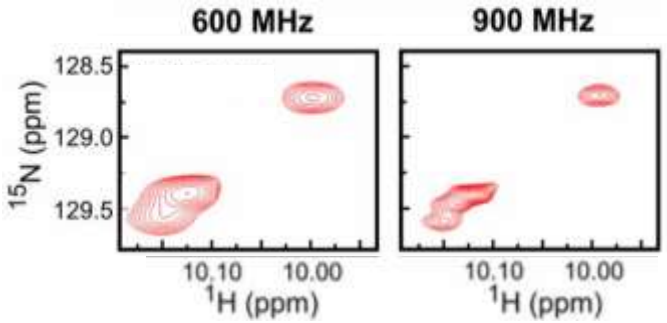
NMR is a High Resolution Technique

Multidimensional NMR



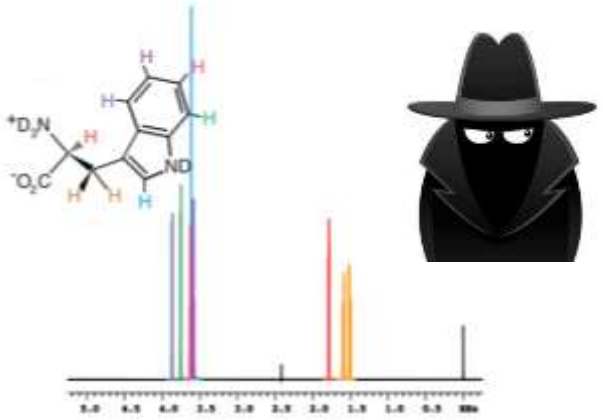


High Magnetic Field



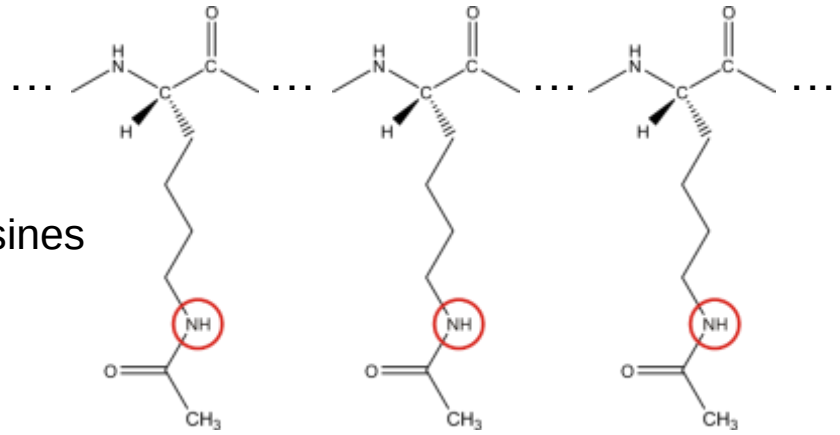
Lee, J.H., et al. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112,* E4206

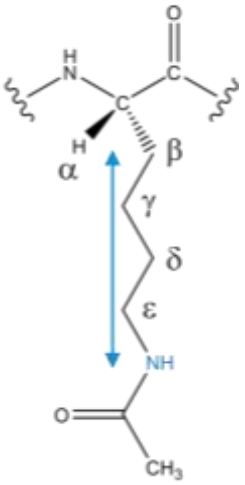
A Non-Destructive Technique

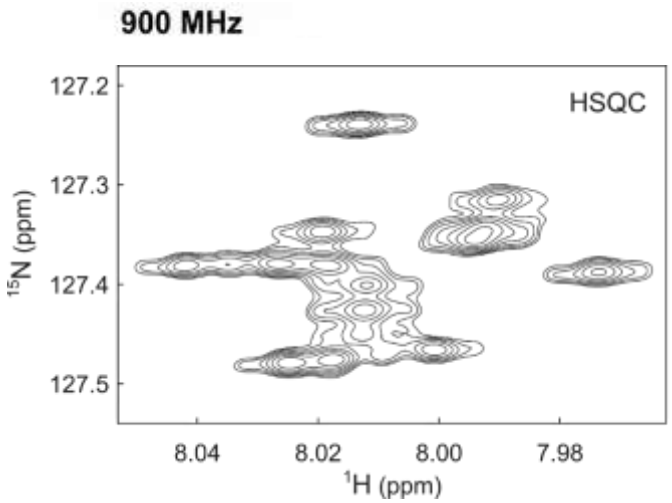


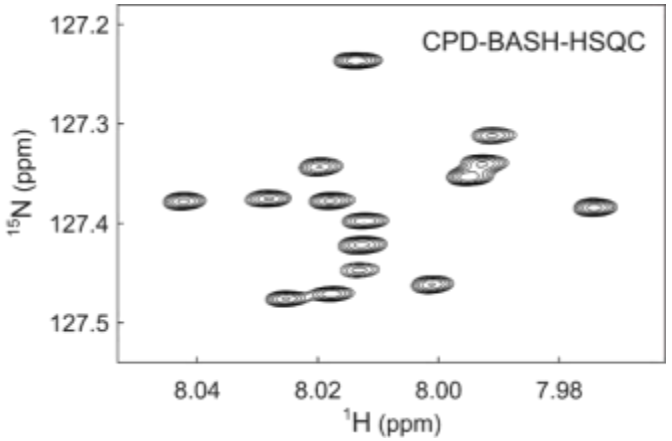
Assignment of Acetylated αS Lysine Side Chains

15 Lysines

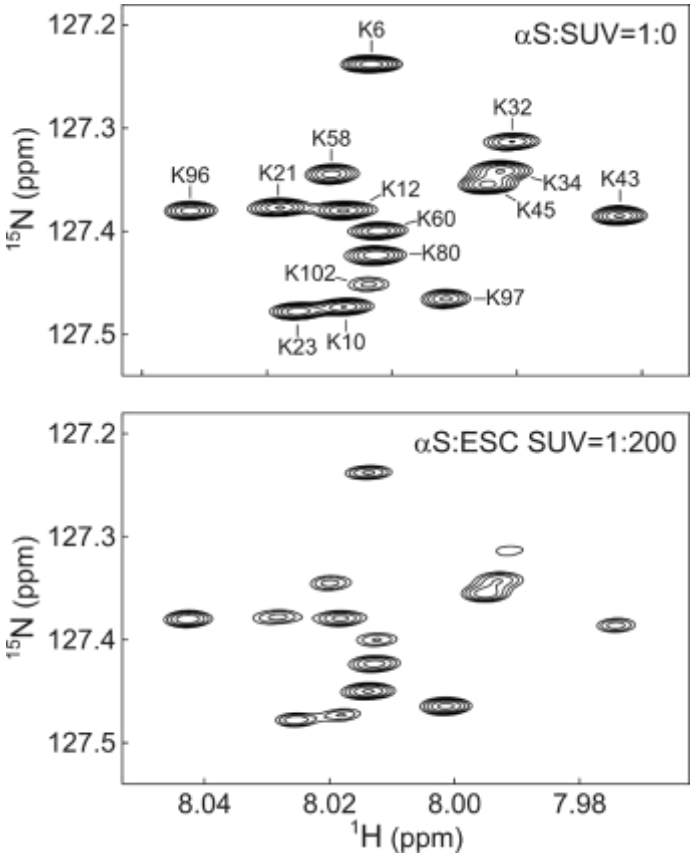


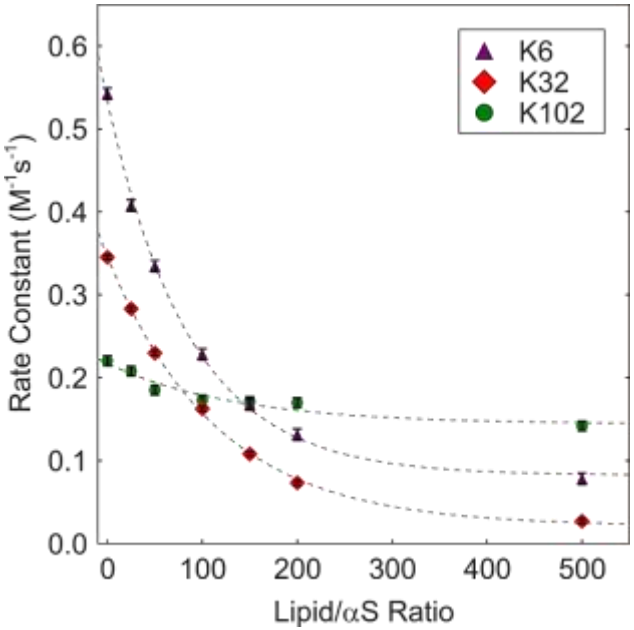


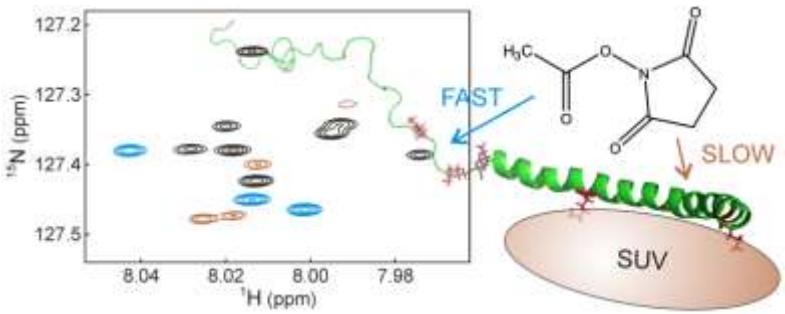




Lipid-Induced Protection of αS Acetylation

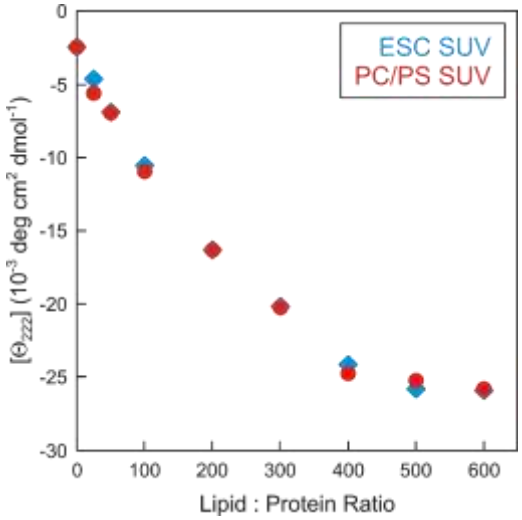




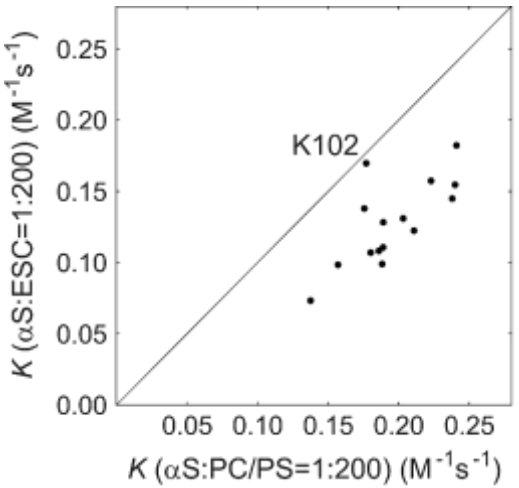


Effect of SUV Chemical Composition on αS Binding

Same Helicity

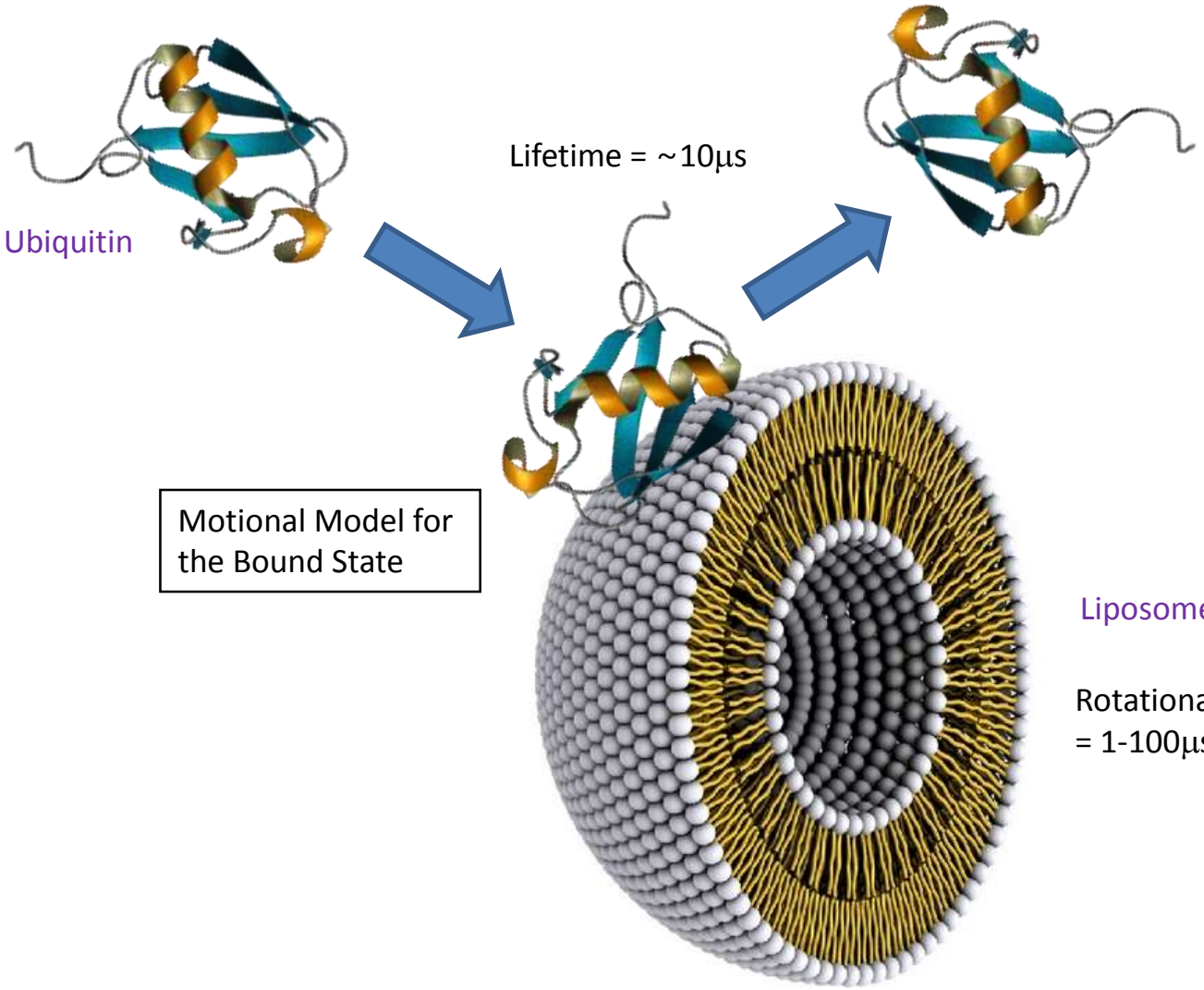


Different Protection



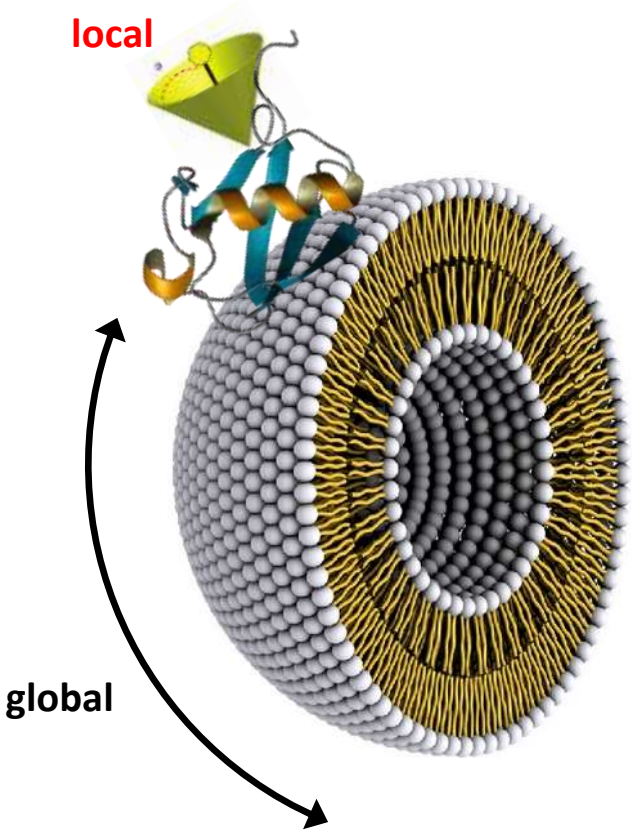
αS may preferably bind to regions of lipid disorder and annealing defects OR lipid rafts

5. Transient Membrane-Protein Interactions

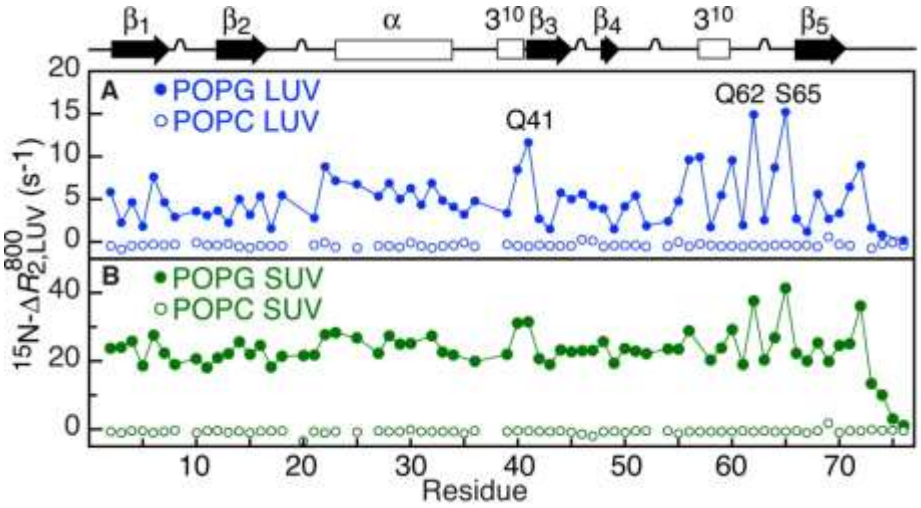


Sequence Variation of ^{15}N-ΔR_2

Local and global motions of the NH bond

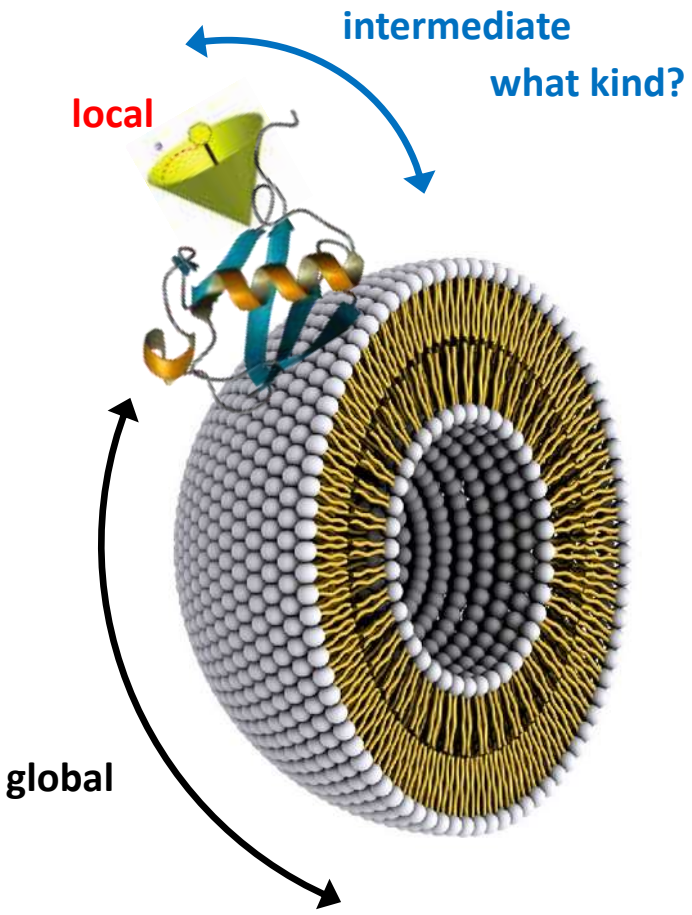


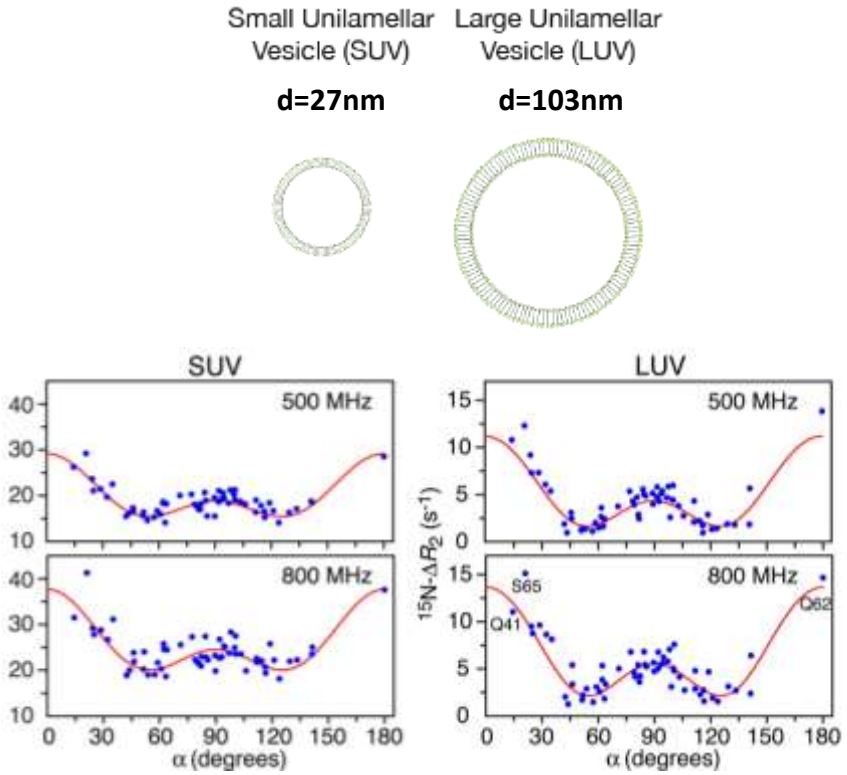
^{15}N Line-broadening



J. Am. Chem. Soc. 138, 5789-5792 (2016)

Rotation of Ubiquitin on the Surface of Liposome

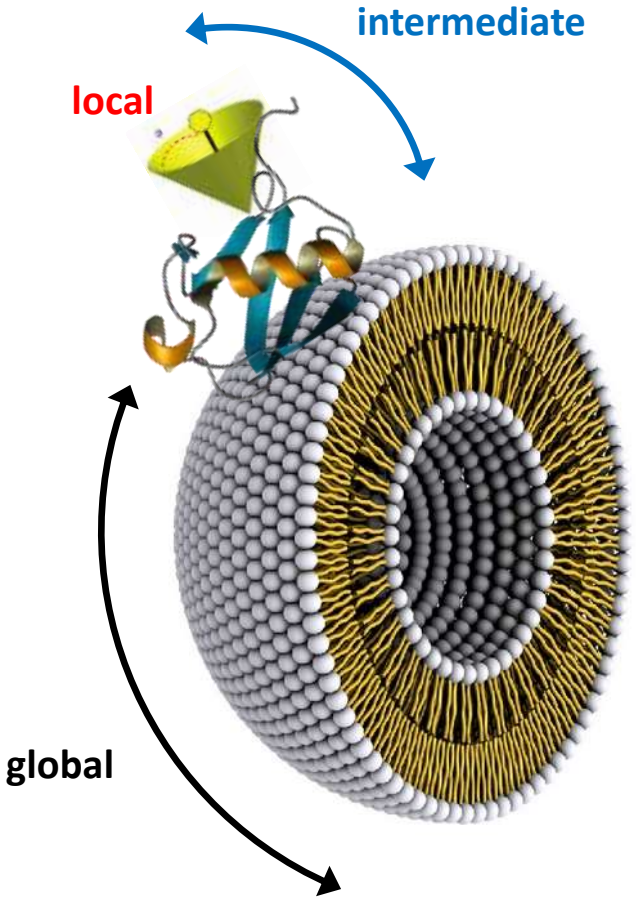




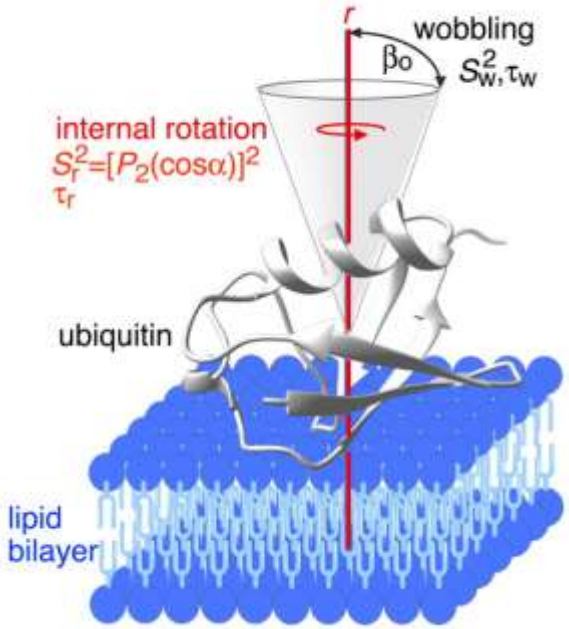
α (degrees): the angle between N-H bond vector and the axis of rotation

J. Am. Chem. Soc. 138, 5789-5792 (2016)

Rotation of Ubiquitin on the Surface of Liposome



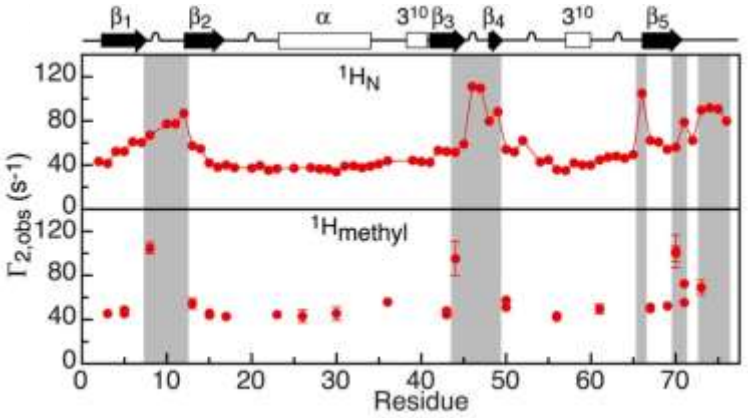
Ubiquitin rotates and wobbles on the surface of a liposome, upon encounter



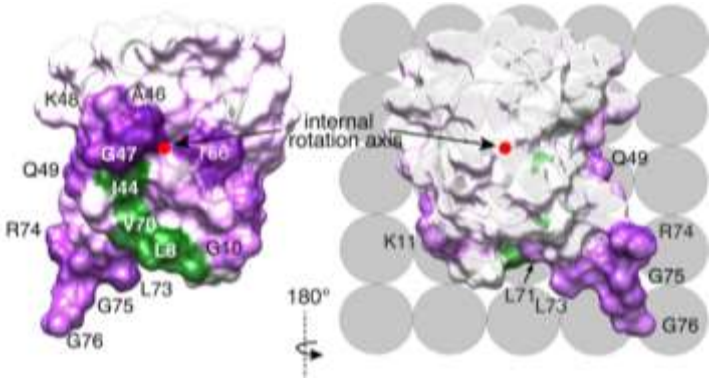
Probing the Surface of Interaction

Paramagnetic Relaxation Enhancement (PRE)

Ubiquitin in the presence of Gd^{3+}-tagged POPG LUVs

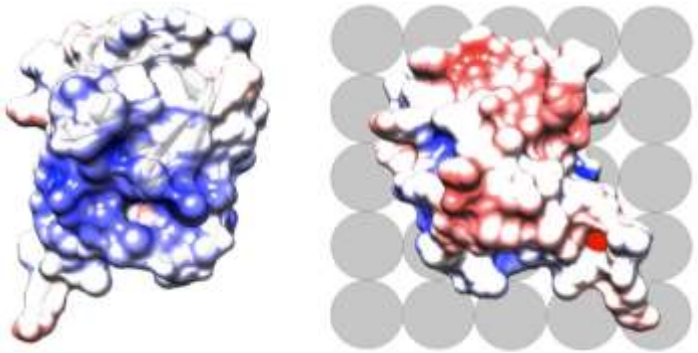


PRE mapping at the interface



purple: high $^1H^N$ PRE
green: high methyl PRE

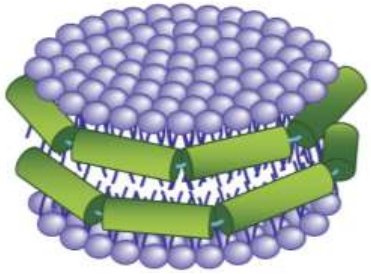
Electrostatic potential



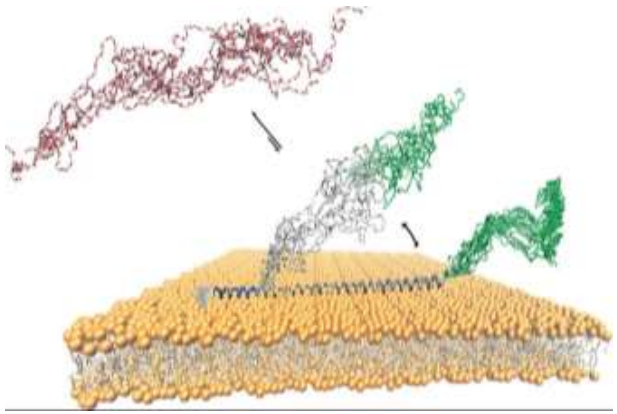
blue: positive, white: neutral, red: negative

Summary

Membrane Mimetics and Study of Membrane Proteins

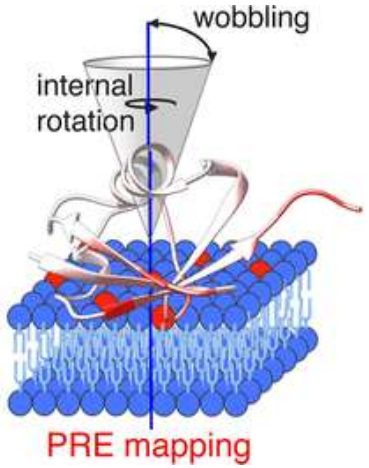


αS-Membrane Interaction



NMR Spectroscopy

Ubiquitin-Membrane Interaction



Further Reading

1) Cavanagh et al. Protein NMR spectroscopy: Principles and Practice. Academic Press, 2nd edition (2006)

2) Engelman D.M. "Membranes are more mosaic than fluid", *Nature*. 438, 578-580 (2005)

3) Liang B. and Tamm L.K. "NMR as a tool to investigate the structure, dynamics and function of membrane proteins", *Nat. Struct. Mol. Biol.* 23, 468–474 (2016)

4) Bodner C.R., Dobson C.M., Bax A. "Multiple tight phospholipid-binding modes of alpha-synuclein revealed by solution NMR spectroscopy", *J. Mol. Biol.* 390, 775-790 (2009)

5) Ceccon A., Tugarinov V., Bax A., Clore G.M. "Global dynamics and exchange kinetics of a protein on the surface of nanoparticles revealed by relaxation-based solution NMR spectroscopy", *J. Am. Chem. Soc.* 138, 5789-5792 (2016)