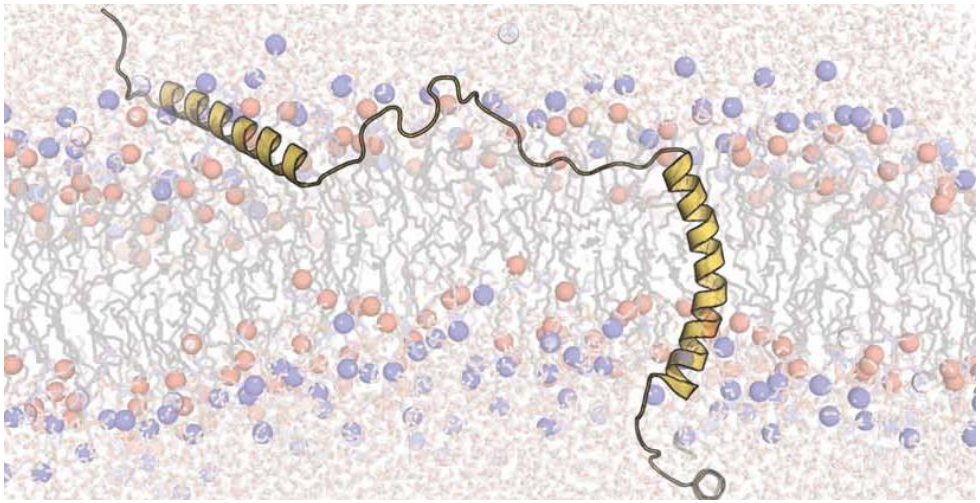
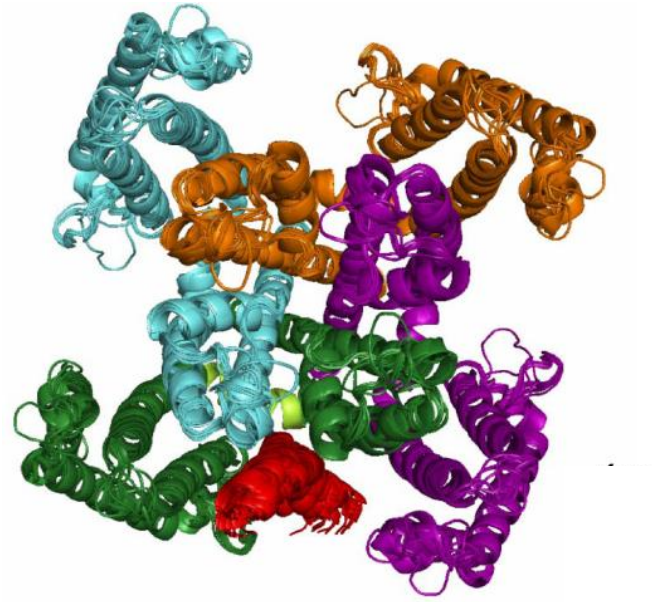
Studying Structural Dynamics of Human KCNE3 in Lipid Bilayers using EPR Spectroscopy





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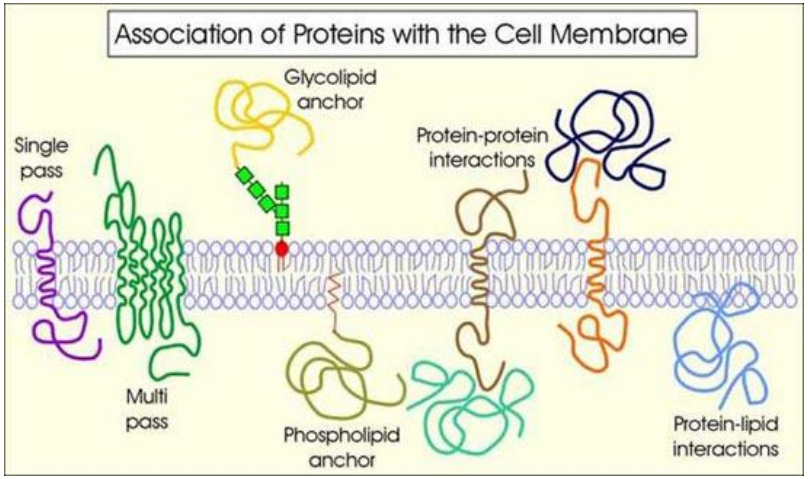
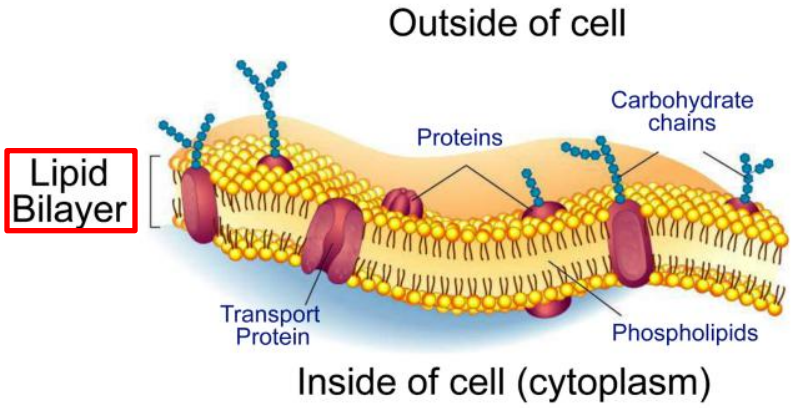
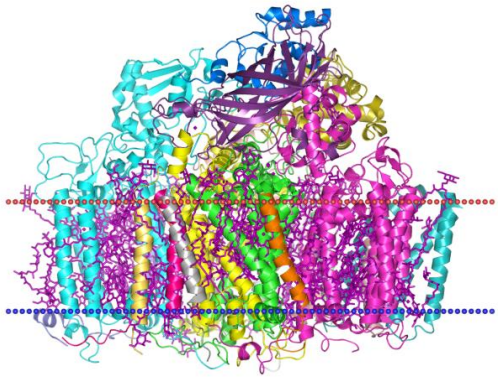
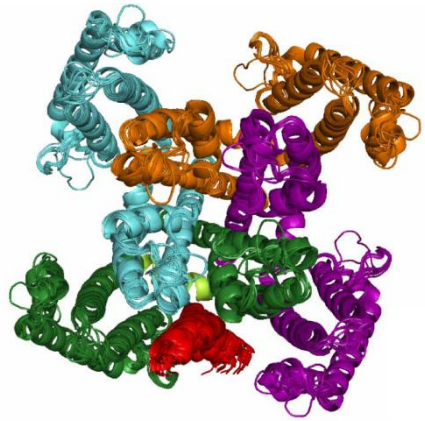
What are Membrane Proteins?

- Proteins that interact with biological membranes.
- Cellular transport, signaling, recognition and catalysis.
- The targets of more than half of the modern medicinal drugs.









Membrane proteins are abundant and important.

http://evmestycor.blogspot.com/2011/05/cell-membrane-diagram.html

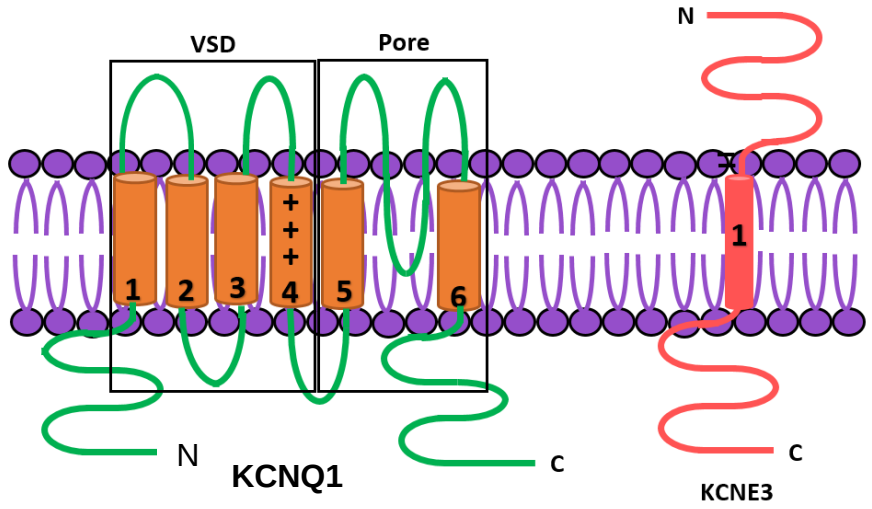
http://preib-biology.blogspot.com/2015/10/beginners-guide-to-cells.html

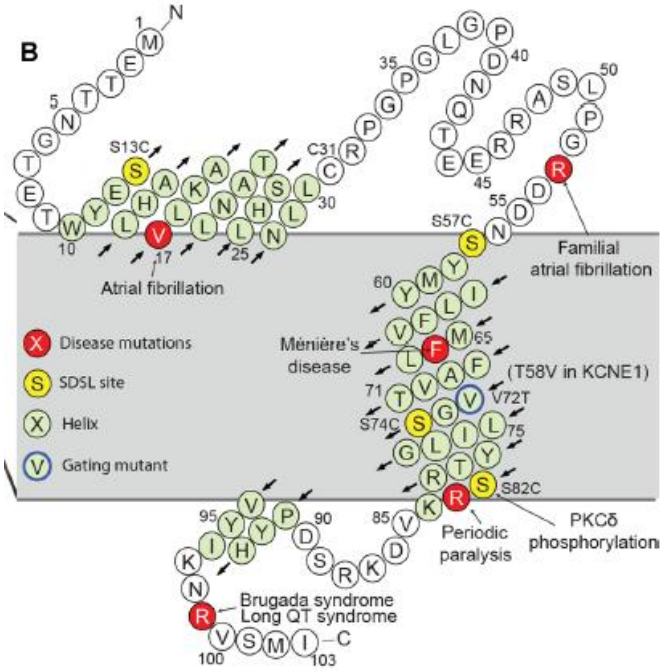
KCNE3: A Potassium Channel Accessory Protein

- A transmembrane protein
- 103 AA
- Molecular weight is 11.7 KDa

Function:

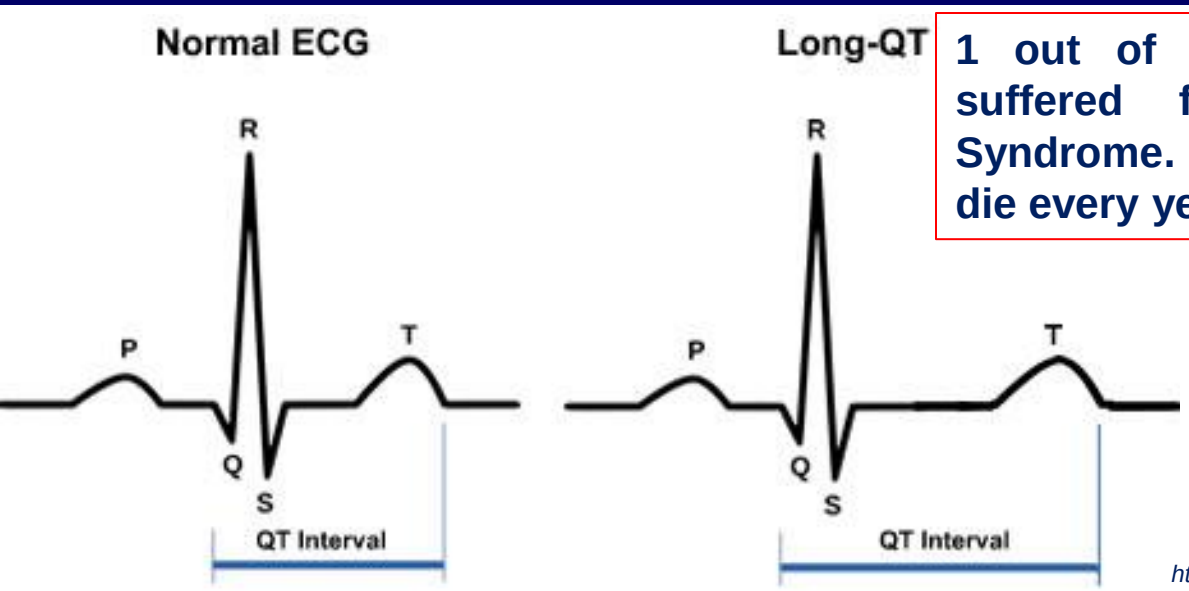
- KCNE3 holds open KCNQ1- a leaky channel
- Regulates ion homeostasis in the cell
- Malfunction of KCNE3 develops diseases: ***Long QT syndrome, Secretary diarrhea and CF (Cystic Fibrosis)***





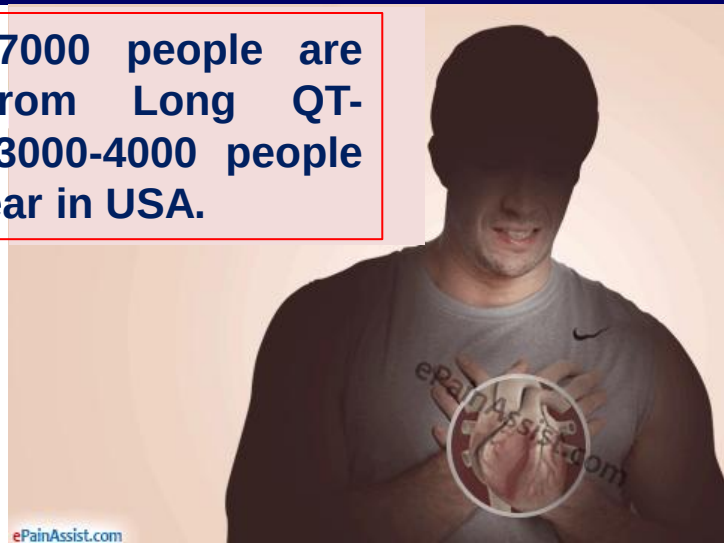
Topology of KCNE3

Long QT Syndrome



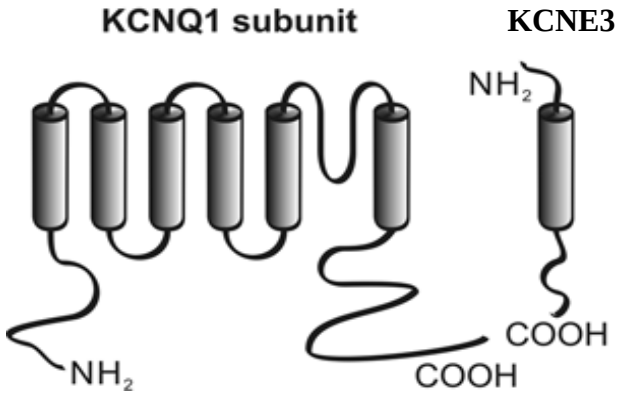
Schematic representation of ECG

1 out of 7000 people are suffered from Long QT-Syndrome. 3000-4000 people die every year in USA.



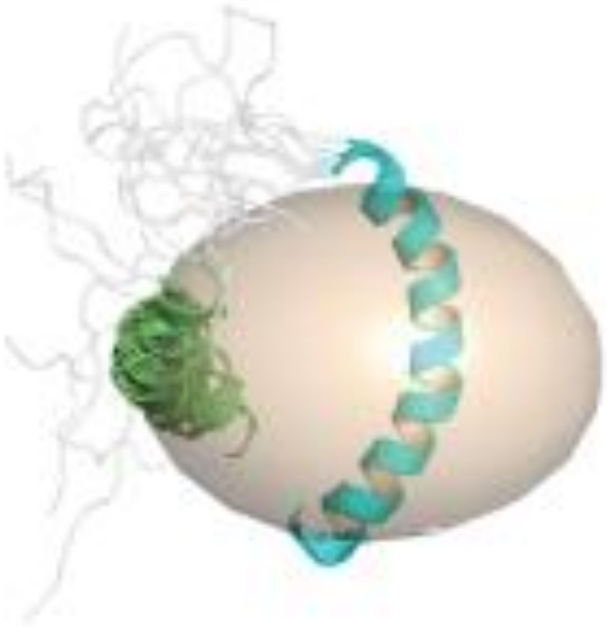
https://www.epainassist.com/chest-pain/heart/long-qt-syndrome'

Cardiac Long QT syndrome

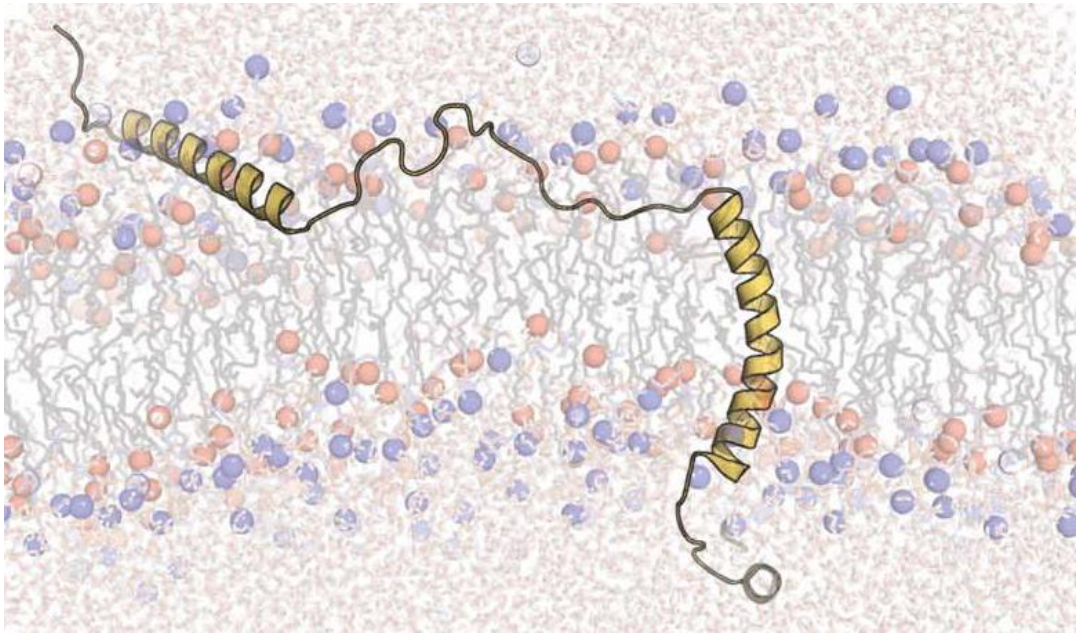


- A congenital heart condition with delayed excitation of the heart as observed on their ECG.
- Patients that suffer from long QT syndrome will go into ventricular fibrillation and sudden death.

3D-structure of KCNE3



Micelle structure



Isotropic bicelle structure

Micelle/Isotropic bicelle is not an ideal environment for proper function of membrane proteins.

Kroncke et al, Science Advances, *2016; 2 : e1501228*

Biophysical techniques for membrane proteins

Electron Paramagnetic Resonance (EPR) Technique:

- No protein size limitation
- Lipid bilayer environment
- High sensitivity

http://epr.miamioh.edu/instrumentation/bruker-emx-room-temperature-instrument; http://miamioh.edu/it-services/news/2018/11/hpc-redhawk.html

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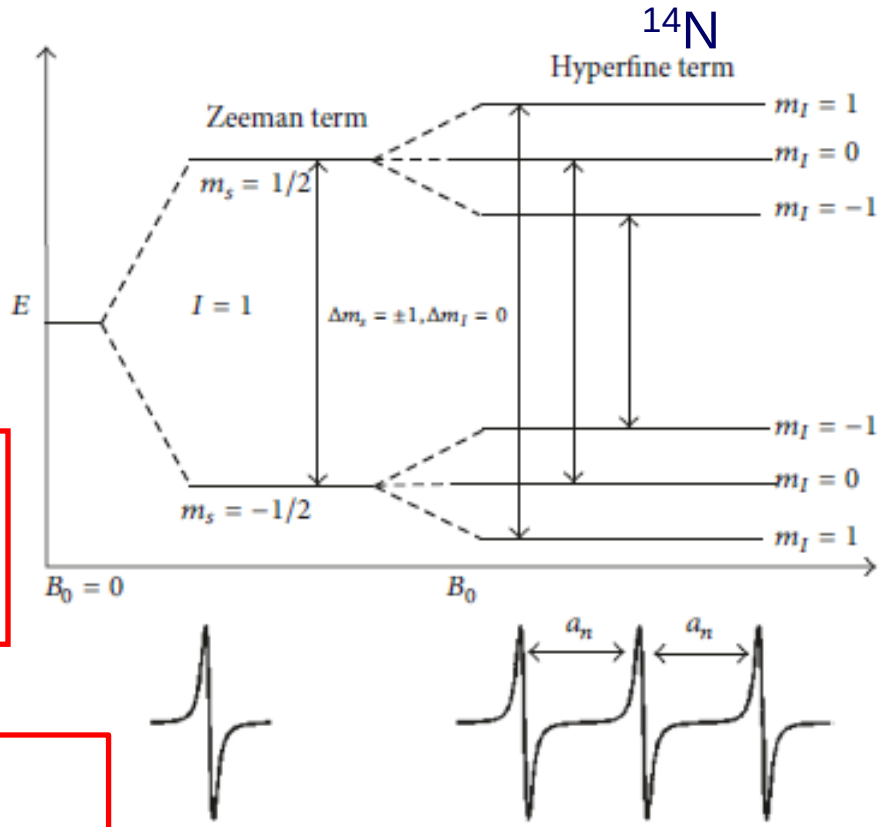
Electron Paramagnetic Resonance (EPR)

EPR measures the absorption of a microwave radiation by an unpaired electron in the presence of an external magnetic field.

$h\nu = g_e \beta B_0$
Fundamental equation of EPR



Continuous Wave (CW) -EPR :
- EPR Lineshape Analysis
- Power saturation EPR

Pulse EPR
- Double electron electron resonance (DEER) technique : (20-60 Å)

Sahu et al. Bio Med Res.Int. 2018, 3248289

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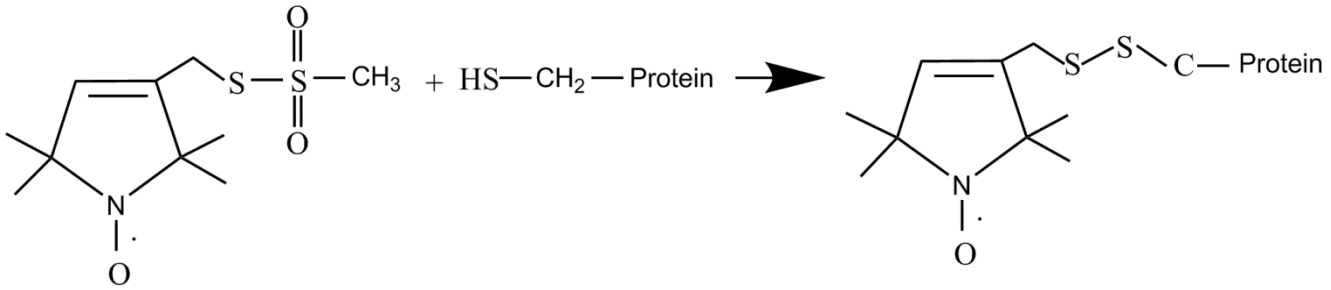
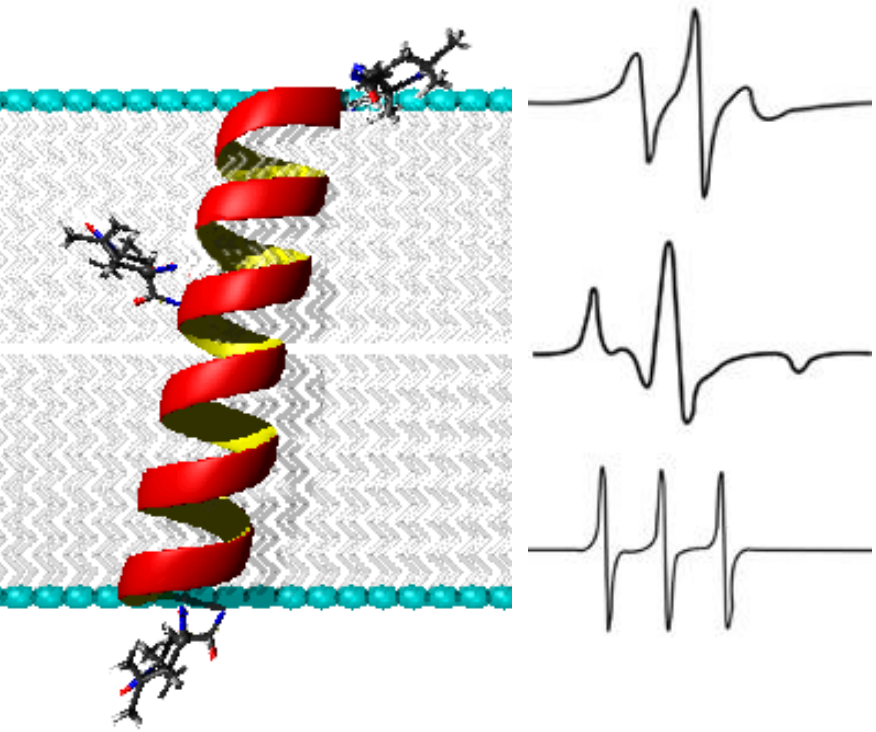
Site-directed spin labeling (SDSL) EPR



Methanesulfonothioate
Spin label (MTSL)

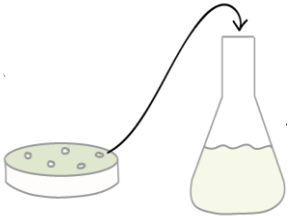


Application:

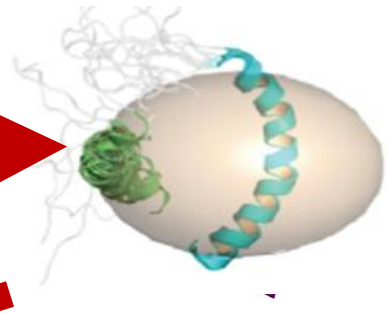
- Side-chain dynamics
- Backbone fluctuations
- Protein topology
- Conformational dynamics
- Distance restraints for protein structure
- Protein-protein interaction

Approaches: Molecular Biology + EPR

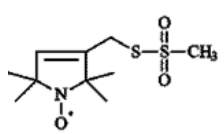
Side direct mutagenesis

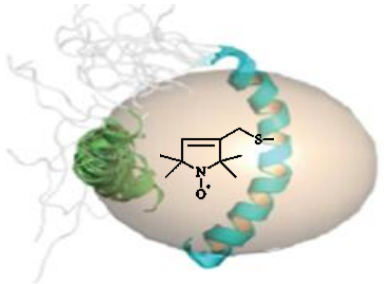


Protein expression

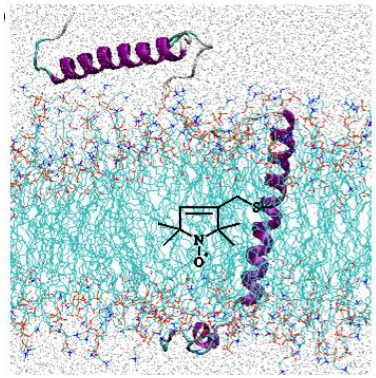


Spin labelling of protein

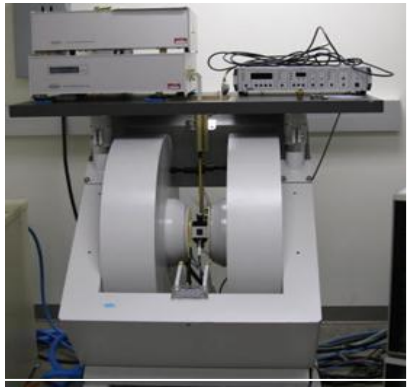




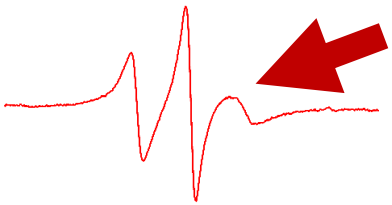
Purification in micelle



Vesicle incorporation

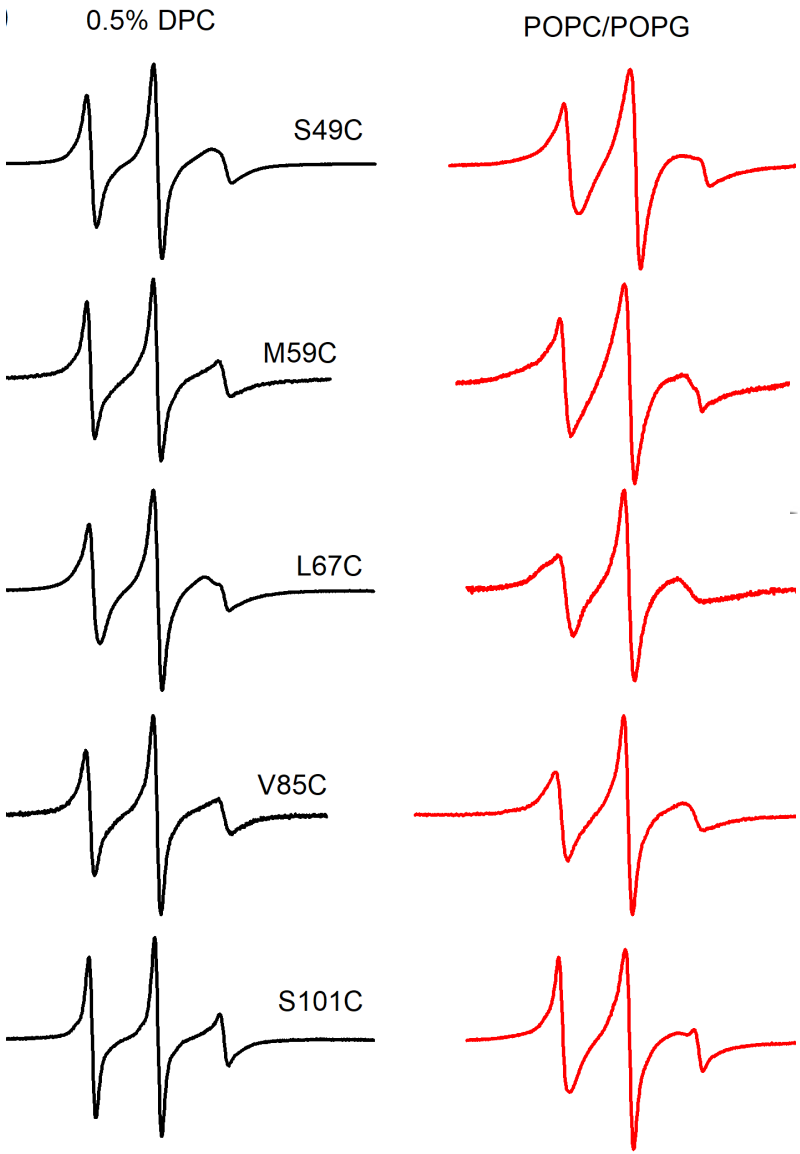


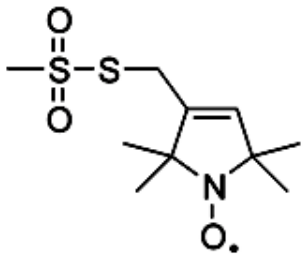
EPR analysis

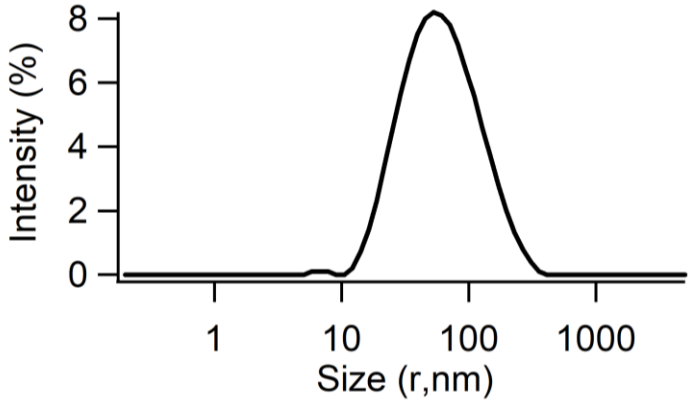


SDS-PAGE

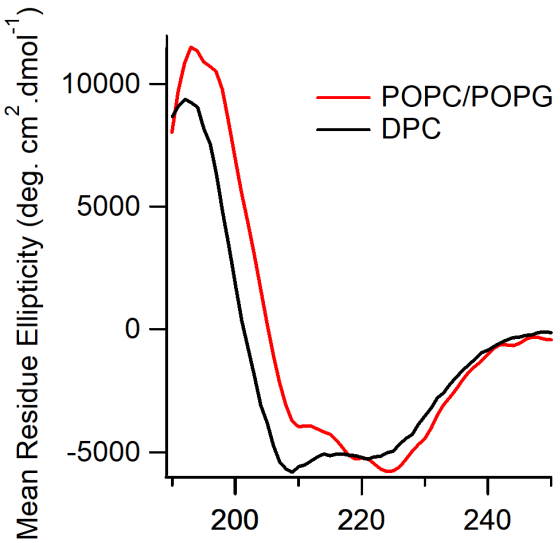
CW-EPR Spectroscopic measurements





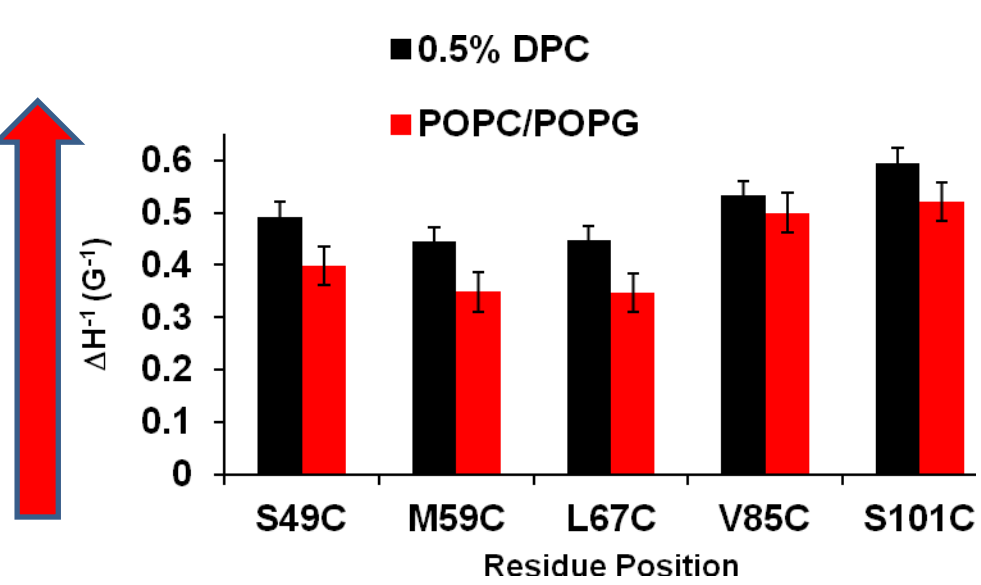


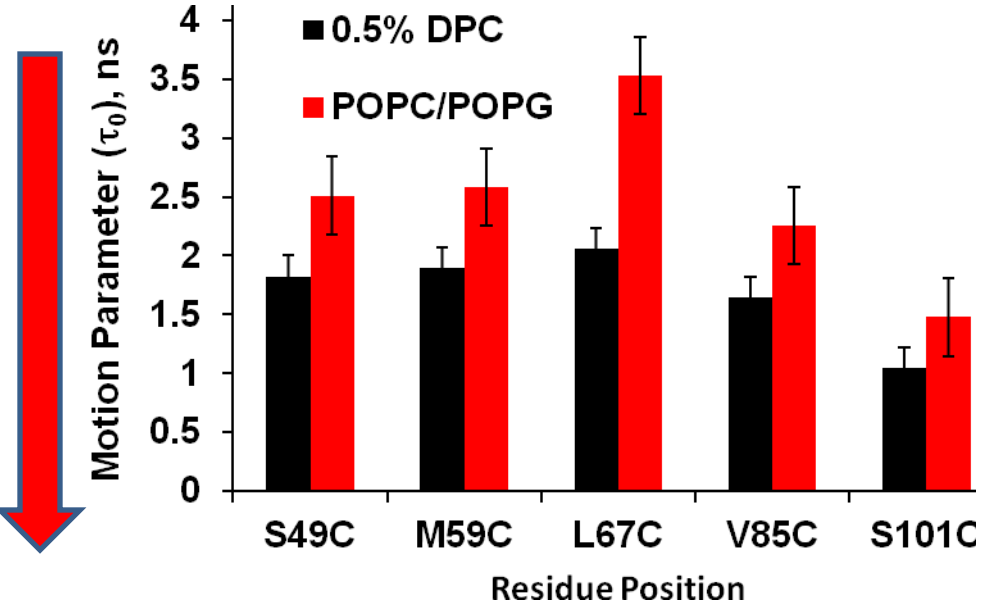
Dynamic Light Scattering (DLS) Size Measurement

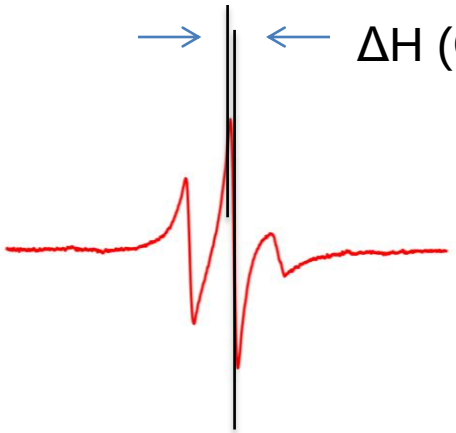


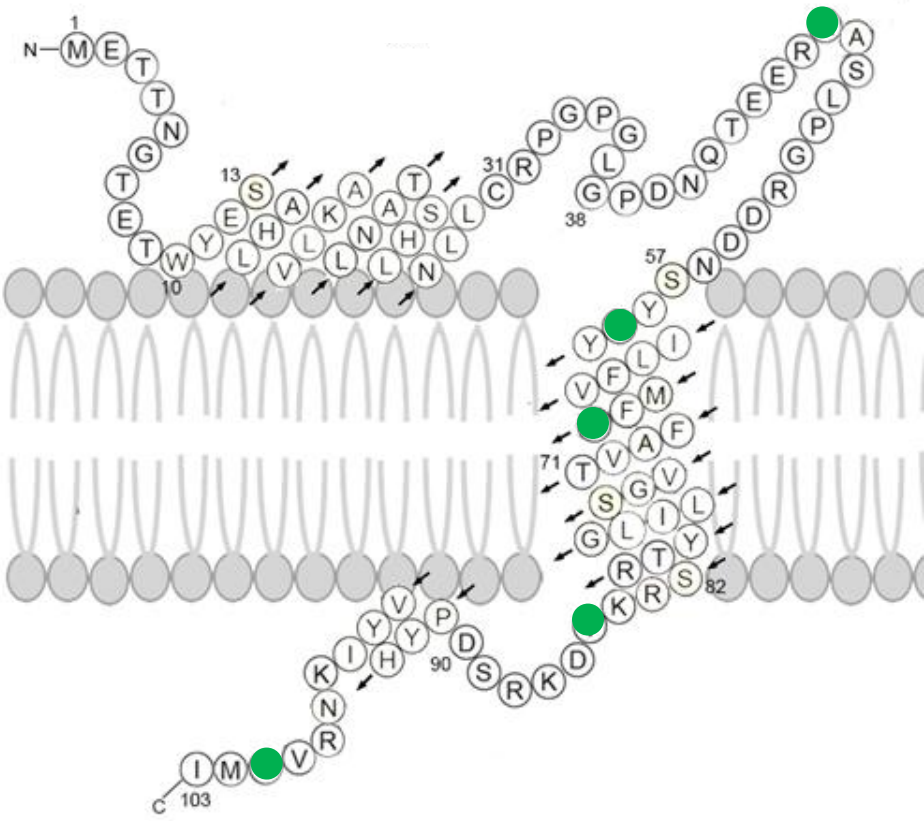
Circular Dichroism (CD) spectra

CW-EPR Spectroscopic measurements

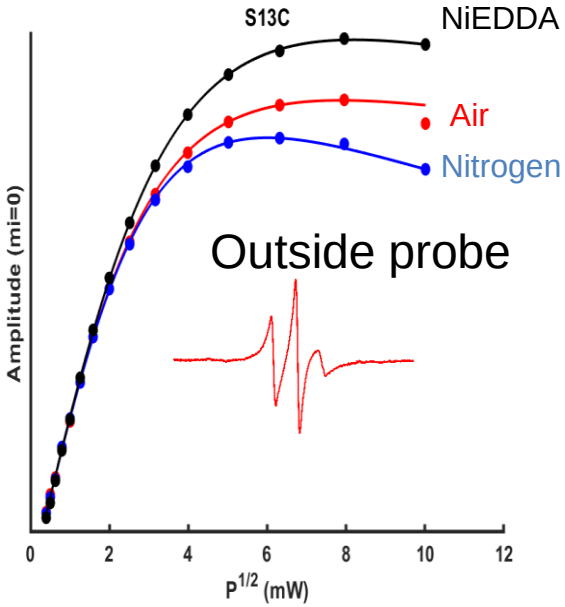


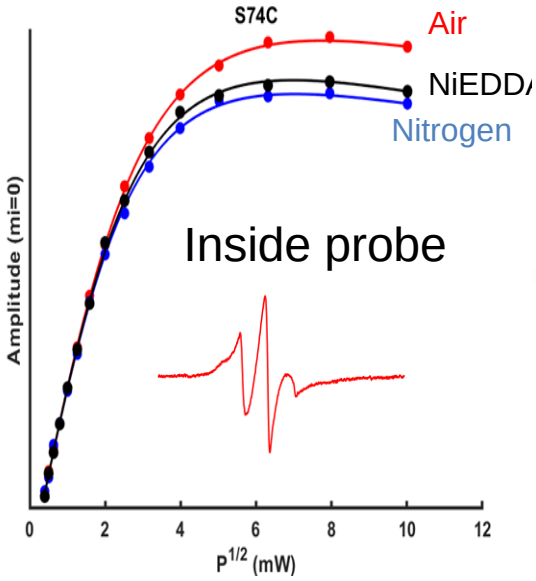


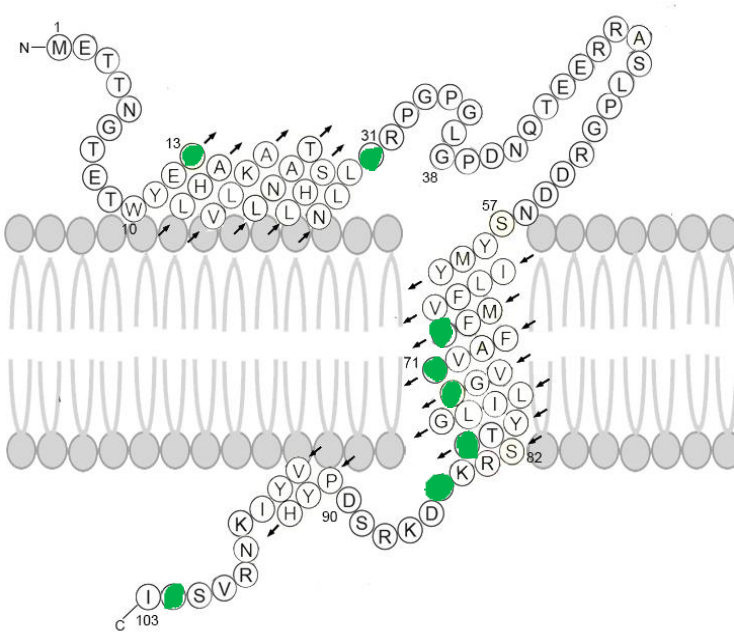




EPR Power Saturation Data: Incorporation of KCNE3 into POPC/POPG Lipid Bilayered Vesicle







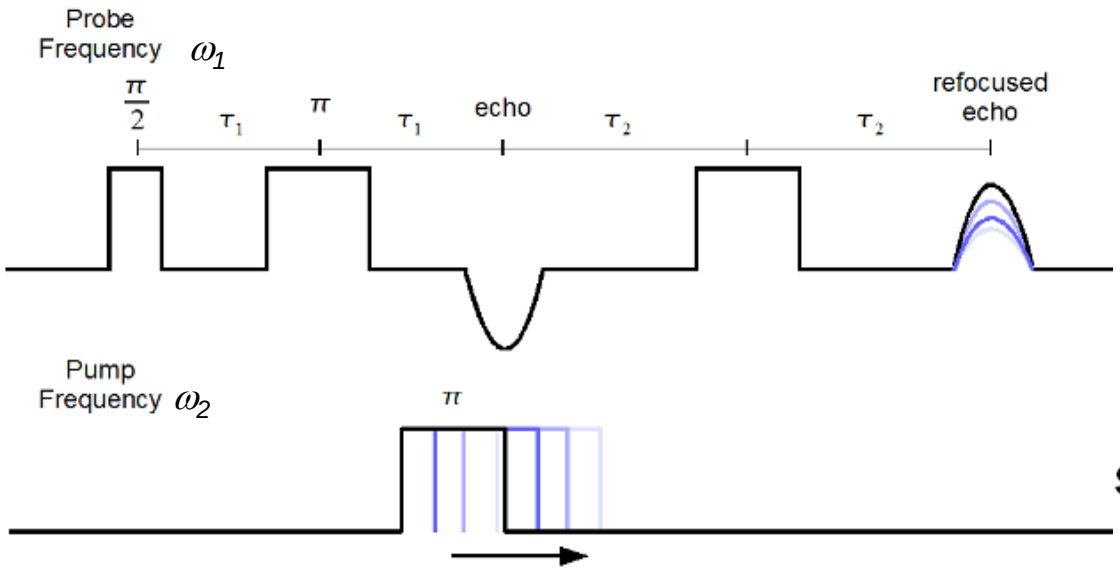
Residue positions	Depth Parameter (Φ)
S13C	-1.3
C31 (wt)	-0.9
L67C (inside)	1.36
T71C (inside)	1.49
S74C (inside)	1.3
R81C	-0.8
V85C	-1.7
M102C	-1.09

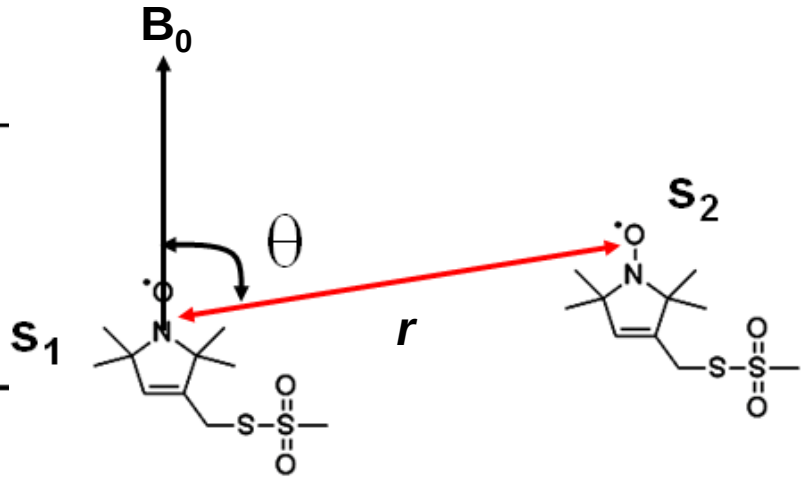
$$A = I\sqrt{P}\,[\,1+(2^{1/\varepsilon}-1)\,P/P_{1/2}]^{-\varepsilon}$$

$$\Phi = \ln\left(\frac{\Delta P_{1/2}(O_2)}{\Delta P_{1/2}(\text{NiEDDA})}\right)$$

Double Electron Electron Resonance (DEER) Spectroscopy

Four Pulse DEER Sequence

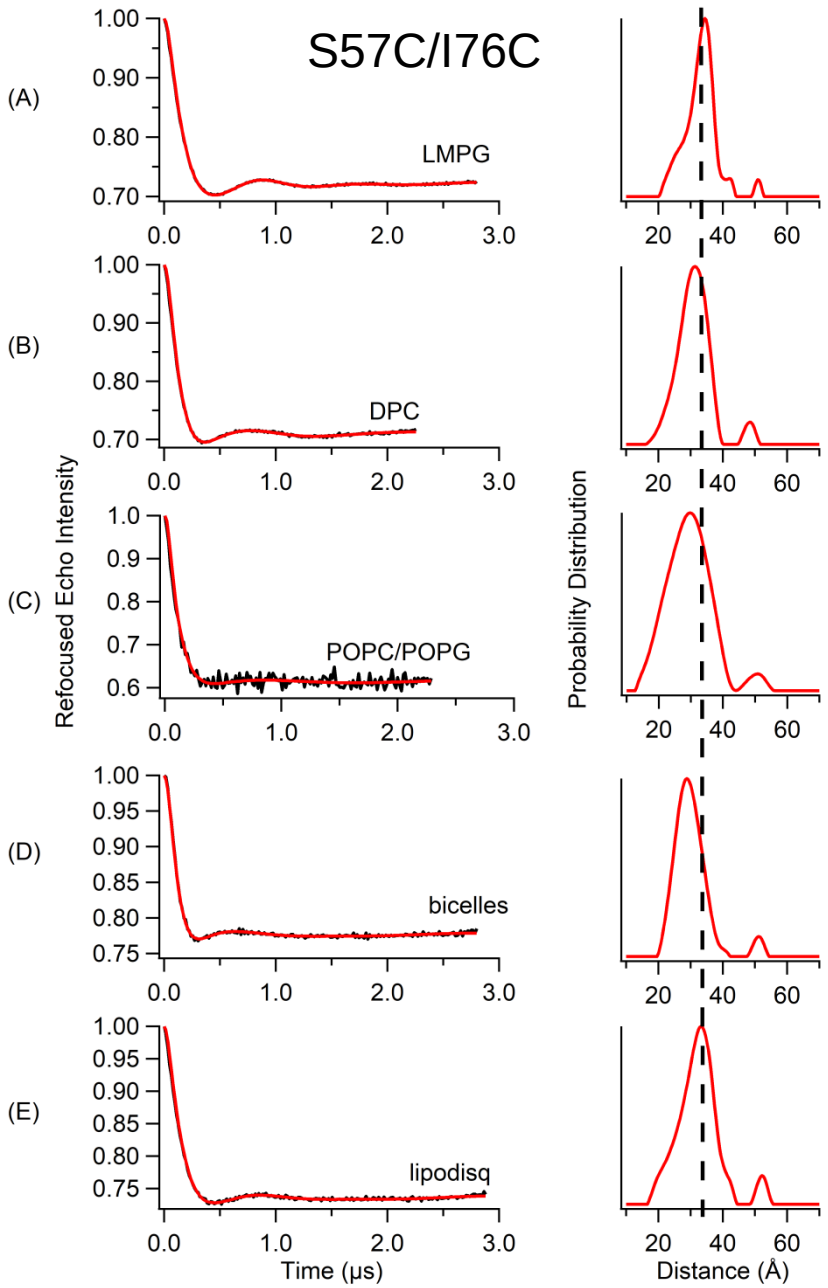


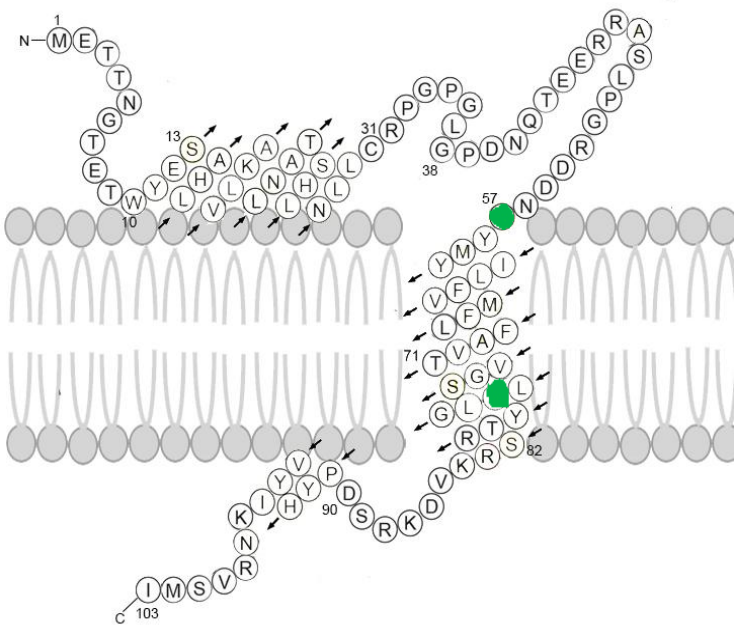


Dipolar coupling frequency is given by,

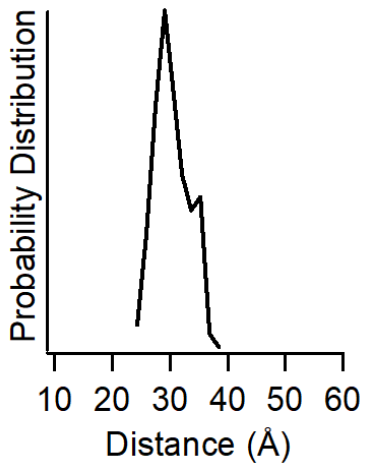
$$\omega_{ee} = \frac{\mu_0 g_1 g_2 \beta_e^2}{4\pi\hbar} \frac{1}{r^3} (3cos^2\theta - 1)$$

DEER Data on KCNE3 in Lipodisq





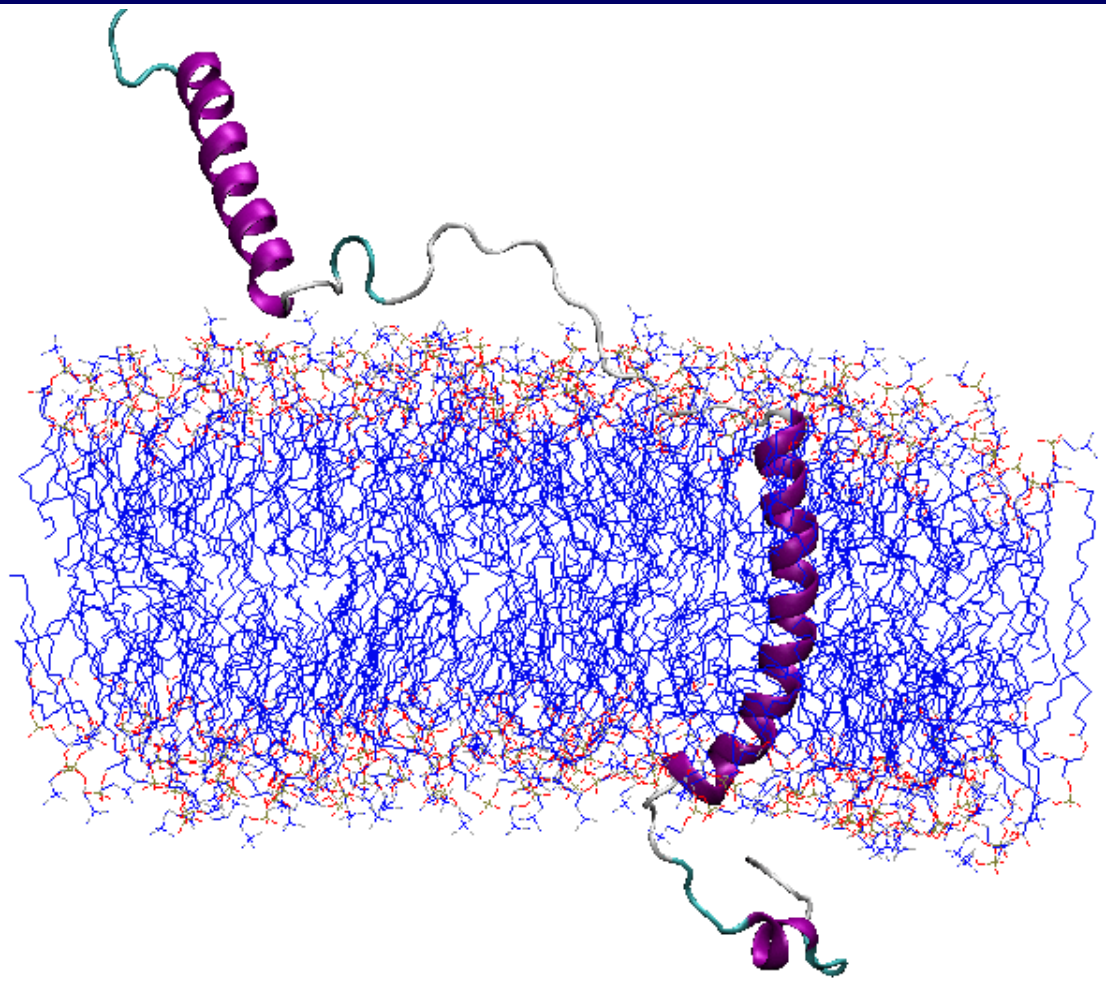
Membrane Mimetic	KCNE3 Double Mutant (Ser57/Ile76)	
	Distance (Å)	FWHM (Å)
LMPG Micelles	34	~9
DPC Micelles	31	~11
POPC/POPG	30	~17
Bicelels	29	~11
Lipodisq	34	~11



Lipodisq nanoparticles provide improvement in S/N of the time domain data.

How does KCNE3 interact with Lipid Bilayers?

Molecular dynamics simulations on KCNE3 membrane protein



Summary

- CW-EPR Spectroscopic lineshape analysis data provide sidechain dynamic properties of KCNE3 in lipid bilayered membrane.
- EPR power saturation data provide topology of KCNE3 in lipid bilayer membrane.
- The DEER distance distribution data indicated that the lipidisq nanoparticle provide better membrane mimic when compared to that of the liposomes for Biophysical measurement.
- Developing several biophysical techniques for studying structural and conformational dynamics of KCNE3.

Acknowledgements

Current Lab Members:

1. Mathew Scheyer
2. William David Carbo
3. Conner Campbell
4. Afsana Begam
5. Mustakim Hussain
6. Patrick Logan Williams
7. Aliyah Taylor
8. Isaac Asare
9. Sebastian Escobar Fonseca
10. Andres Bastidas Garcia
11. Supriya Maharjan Sapkota
12. Umesh Adhikari
13. Peyton Dabney
14. Nima Patel
15. Alberto Perez
16. Samuel Haralu

Collaborators:

Prof. Gary A. Lorigan — Miami University

Prof. Shudha Chakrapani — Case Western Reserve University

Prof. Charle R. Sanders — Vanderbilt University

NSF MSB-2040917

Open for new research collaboration

Selected Publications

- Indra D Sahu, Gunjan Dixit, Warren D Reynolds, Ryan Kaplevatsky, Benjamin D Harding, Colleen K Jaycox, Robert M McCarrick, Gary A Lorigan, **Characterization of Human KCNQ1 Voltage Sensing Domain(VSD) in Lipodisq Nanaoparticles for EPR Spectroscopic Studies of Membrane Proteins, *J. Phys. Chem. B*** 2020, 124, 2331-2342

- Brett M. Kroncke, Wade D. Van Horn, Jarrod Smith, CongBao Kang, Richard C. Welch, Yuanli Song, David P. Nannemann, Keenan C. Taylor, Nicholas J. Sisco, Alfred L. George Jr., Jens Meiler, Carlos G. Vanoye, Charles R. Sanders**, Structural basis for KCNE3 modulation of potassium recycling in epithelia, *Sci. Adv. 2016; 2 : e1501228***

- Indra D. Sahu, Robert M. McCarrick, Gary A. Lorigan, **Use of Electron Paramagnetic Resonance to solve Biochemical Problems**, ***Biochemistry***, 2013, *52* (35), 5967–5984.

- **Indra D. Sahu**, Rongfu Zhang, Megan M. Dunagan, Andrew Craig, and Gary A. Lorigan, **Characterization of Lipodisq nanoparticles for EPR Spectroscopic study of Membrane Protein, *J. Phys. Chem. B*,** 121, 5312–5321, 2017

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