Building a Bilayer Overtone Analysis (BOA) Instrument

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NIST Center for Neutron Research

Neutron-Condensed Matter Science Group

SURF Colloquium August 2019

Physiological Homeostasis

Organization:

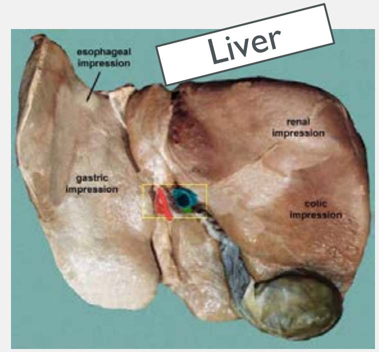
- Internal environment ≠ External environment

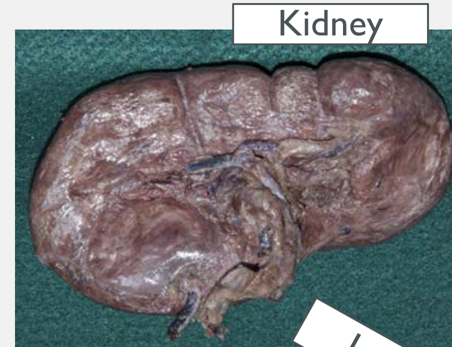
Compartmentalization:

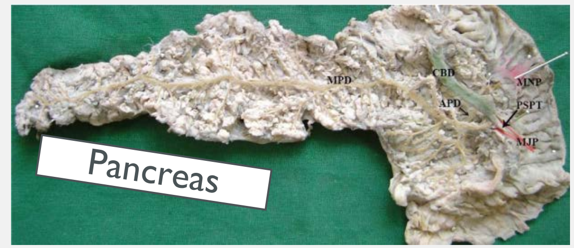
- Cells → Tissues → Organs → Organ systems
- No global variables!

Unsteady equilibrium:

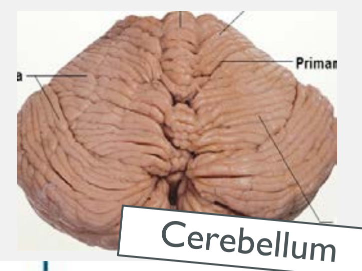
- Is complete stability a good thing?
- Newton's Third Law of Motion

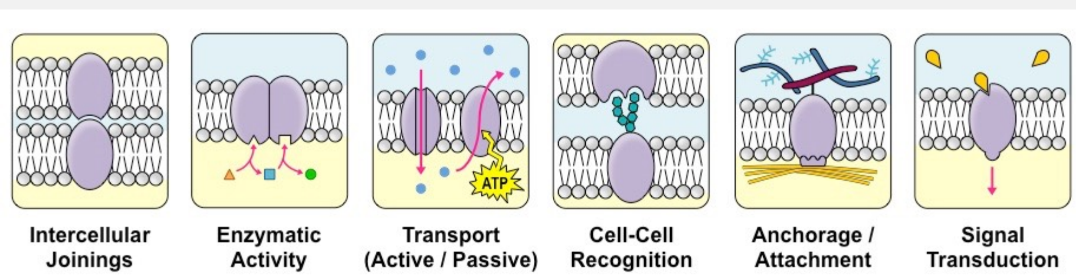






Maintaining **unsteady** equilibrium between constantly fluctuating internal and external environments





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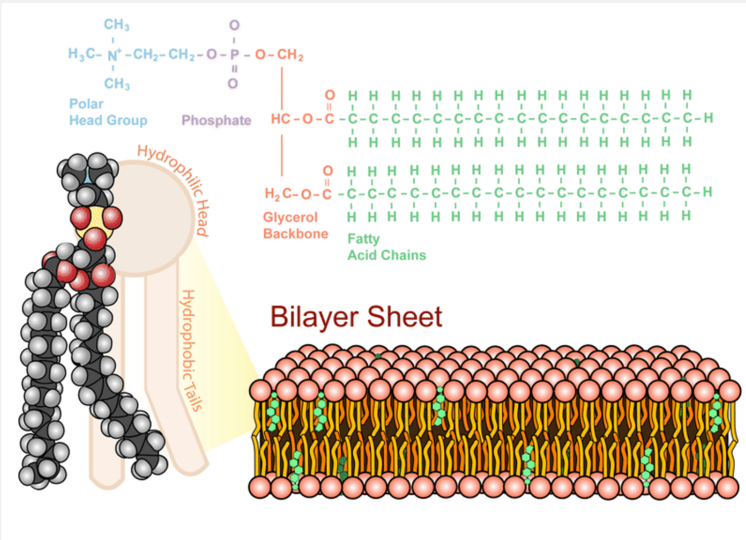
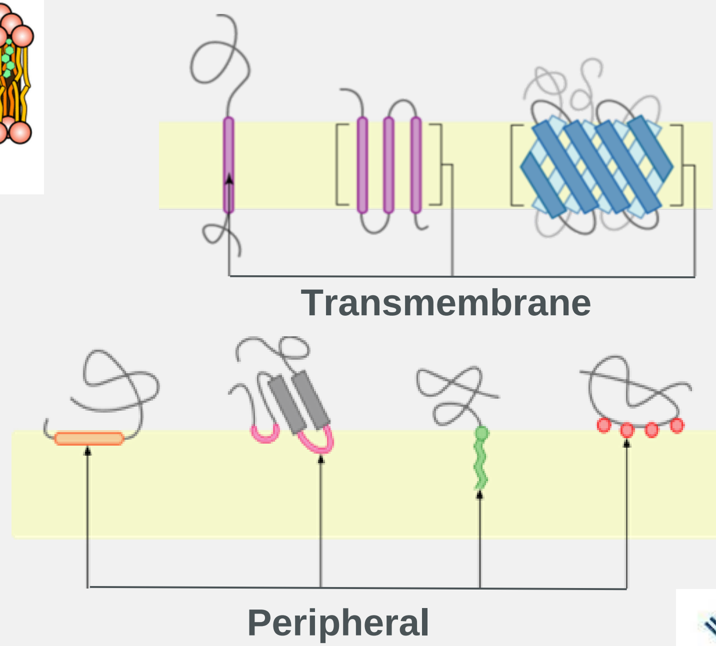
Almén MS, Nordström KJ, Fredriksson R, Schiöth HB (August 2009). "Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin". *BMC Biology*. **7**: 50. doi:10.1186/1741-7007-7-50. PMC 2739160. PMID 19678920.

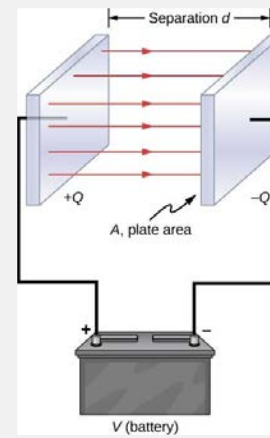
Lipid Bilayer



Structure: BI-LAYER (two leaflets)

- Separated by some distance
- Hydrophobic (non-polar) and hydrophilic (polar) organization
- Separation of **charge** on two surfaces across a **distance?**





Transport: proteins and ions!

- Diffusion vs. transport
- Many **classes** of membrane proteins
- Structure, function, membrane interaction
- Signaling, communication, etc.

Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 3.4, Membrane Proteins. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21570/

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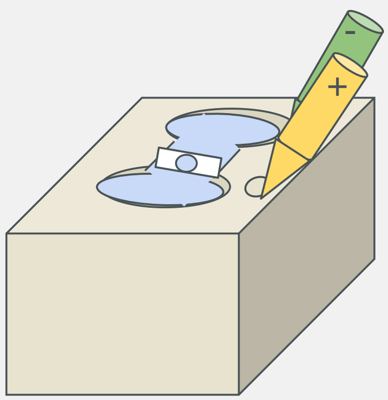
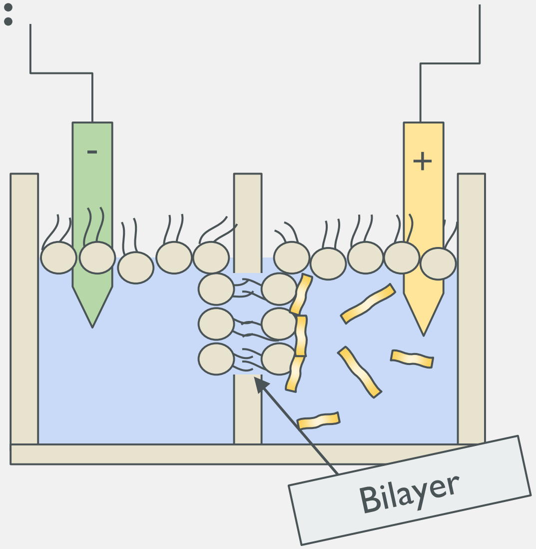
Lipid Bilayer: Functional Model

Basic principle:

- Asymmetry of the membrane results in an intrinsic transmembrane potential
  - Charges (ions) build up on each side of the membrane
- Membrane behaves like a **squishy capacitor**
  - Interactions with ions and proteins result in changes in the transmembrane pressure
  - Bilayer shrinks and expands! And we can model that!

Biophysical translation:

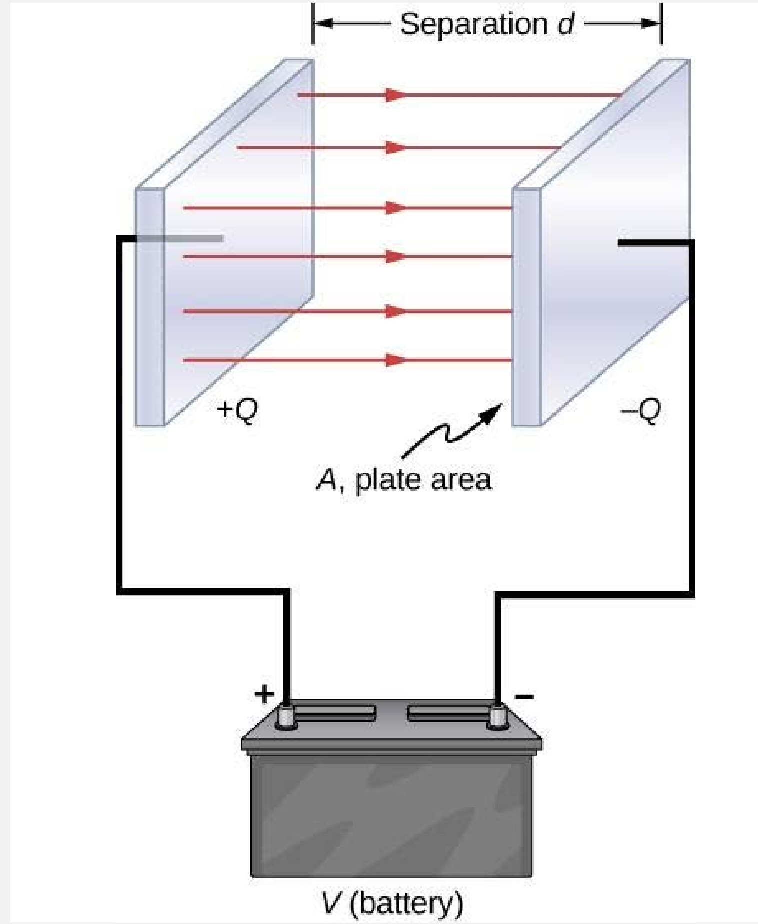




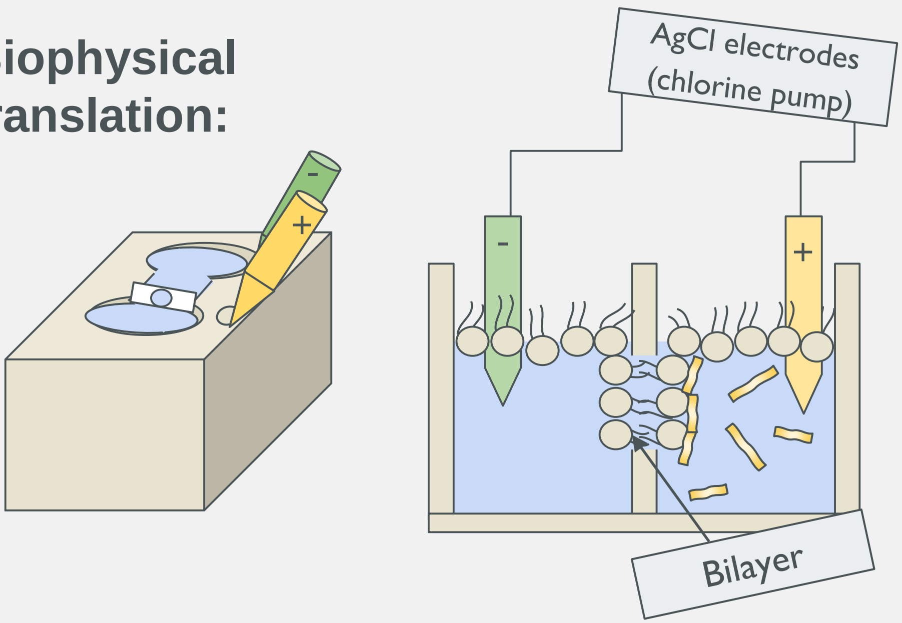
Lipid Bilayer: Functional Model

Capacitor equations:

$$Q = CV$$
$$i = \frac{dQ}{dt} = \frac{d}{dt}(CV)$$
$$V = f(t) = \varphi + V_{ac}\cos(2\pi f_0\, t) \text{ for AC signals, } \varphi = 0$$
$$C = f(V, t) = C_0(1 + \alpha V^2)$$
$$i_2 = 6\pi f_0\, \alpha C_0\, (\Psi + V_{dc})\, V_{ac}^{\;2}\, \sin(4\pi f_0 t)$$



Biophysical translation:

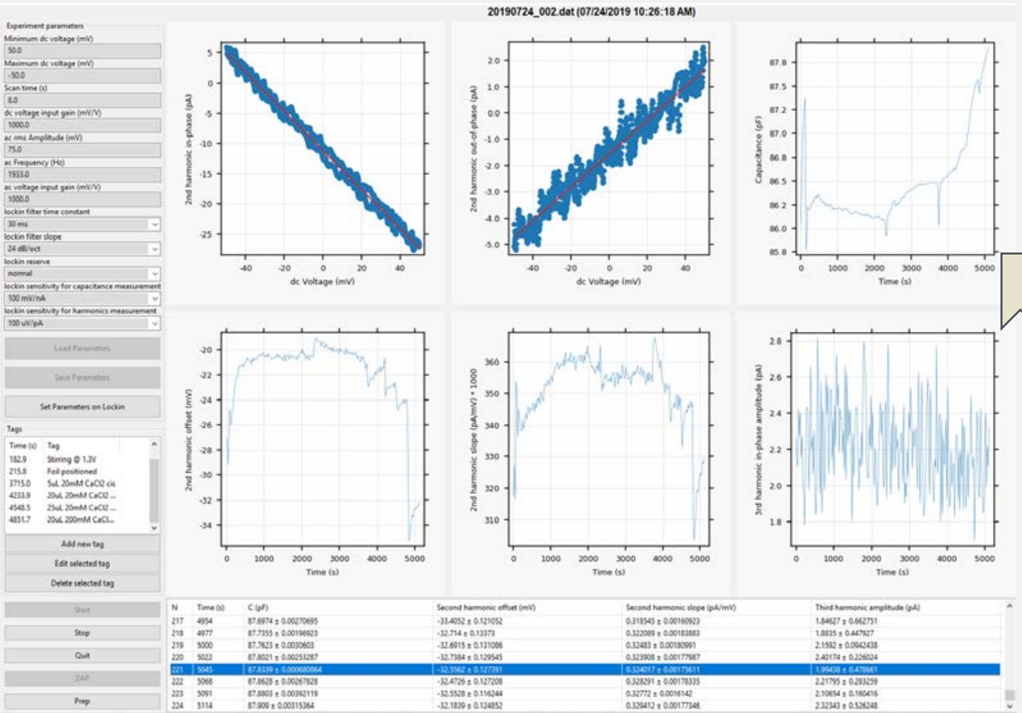


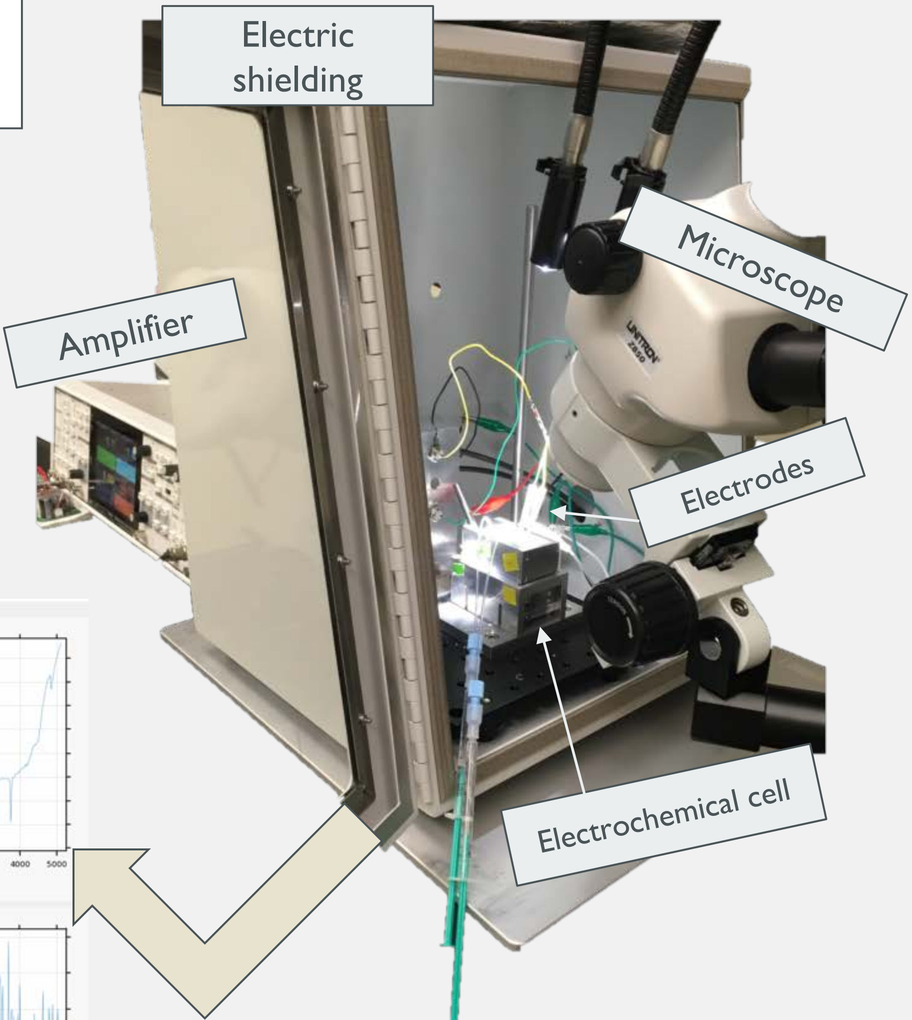
Katz D.M. Physics for Scientists and Engineers: Foundations and Connections. Cengage Learning; Boston, MA, USA: 2016

What is a BOA?

Component pieces:

- Lock-in amplifier- SR860
- Control software and GUI- for DAQ!
- Physical cell, teflon partition, aluminum chamber
- Silver chloride electrodes
- Electromagnetic shielding, vibration isolation stage





Biophysical tool: semi-bio, semi-physical!

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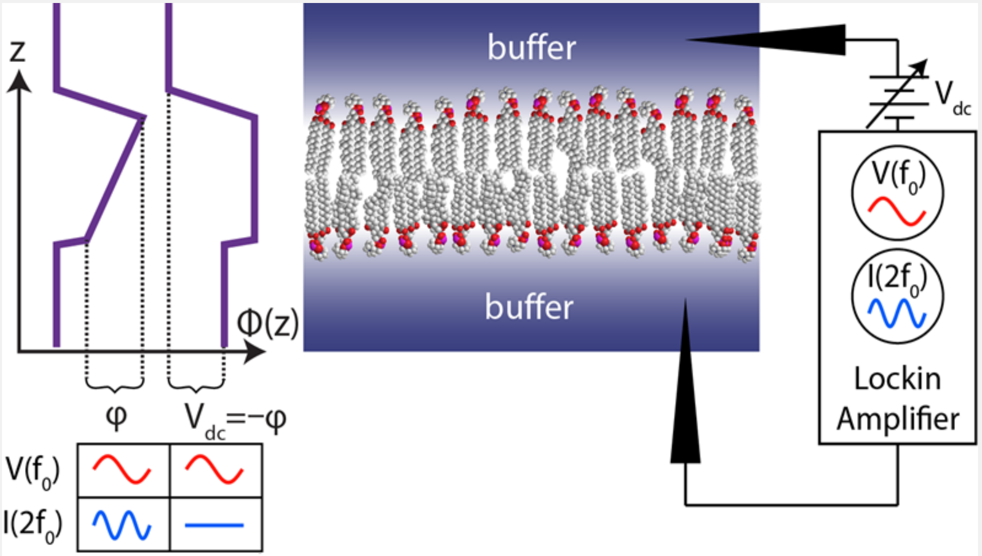
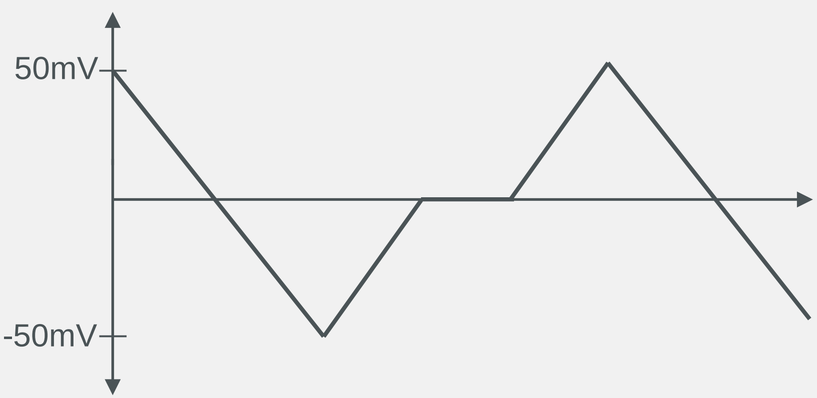
Electrical Signal & Measurement



$$i = -6\pi f_0 \, \alpha C_0 \, (\Psi + V_{dc}) \, V_{ac}^2 \, sin(4\pi f_0 t)$$

SR860 Lockin amplifier:

- $f_0 \approx$ 2kHz, measure current at $2f_0$
- Measure capacitance (first harmonic), V_{ac} = 75mV
- Scan V_{dc} from -50 - 50 mV over 8s
  - Wait at 0V for transients
  - Measure second harmonic amplitude (slope) and phase (offset)



X – in-phase component
Y – out-of-phase component
DC - voltage scan
θ – degree offset

Tubulin on biomimetic mitochondrial membranes, David P. Hoogerheide, Sergei Y. Noskov, Daniel Jacobs, Lucie Bergdoll, Vitalii Silin, David L. Worcester, Jeff Abramson, Hirsh Nanda, Tatiana K.Rostovtseva, Sergey M. Bezrukov, Proceedings of the National Academy of Sciences May 2017, 114 (18) E3622-E3631; DOI:10.1073/pnas.1619806114

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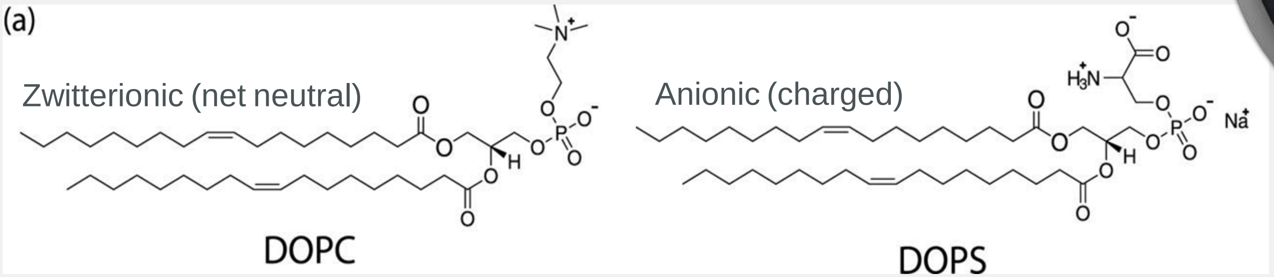
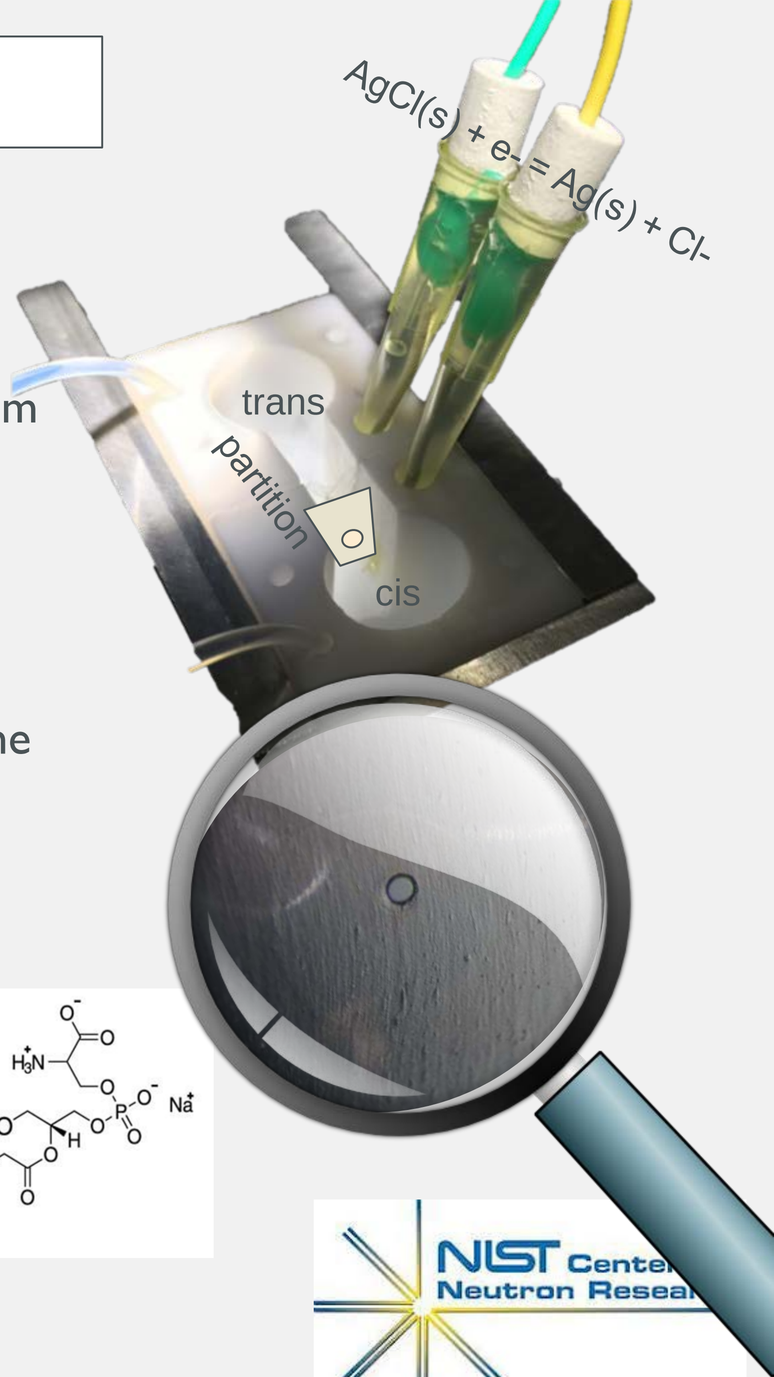
Experimental Methods

Lipids: DOPC, DOPS, DOPC/PS

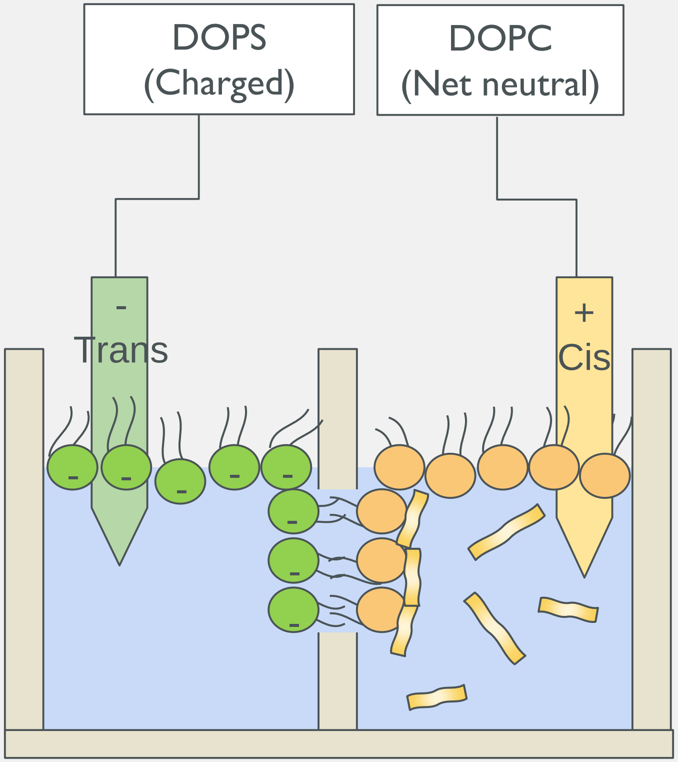
- ~75ug lipid suspended *on top of* 1M KCl 7.0pH buffer solution (~1mL total cell volume)
- 1% hexadecane- to make things “sticky” at ~13um
- ~30-60min equilibration period
  - Foil shielding, stirring, voltage scan
- Divalent ion series: 100uM-100mM in 5-25uL increments
  - 2x concentration KCl buffer of equal volume added to opposite side as control
  - Contents weighed for more accurate concentration



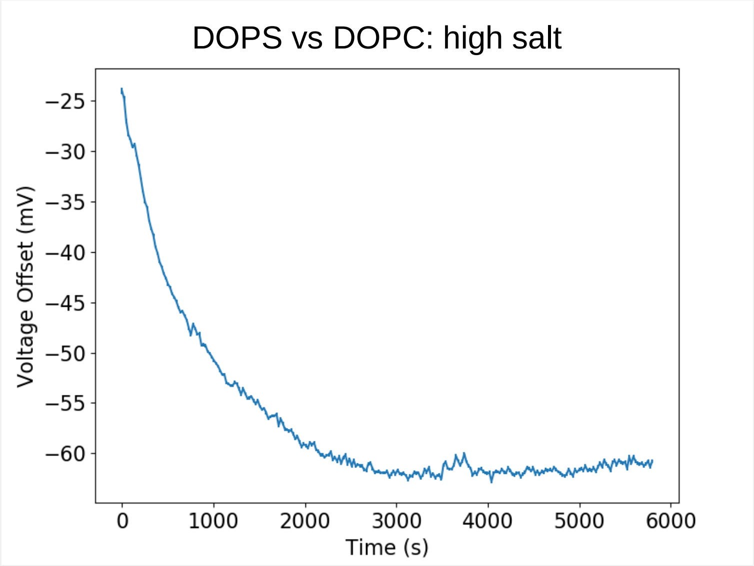
Redondo-Morata, L.; Giannotti, M.I.; Sanz, F. Structural impact of cations on lipid bilayer models: Nanomechanical properties by AFM-force spectroscopy. *Molecular Membrane Biology* **2014**, 31, 17.



Experimental Results: Asymmetric membrane



Charge density (cis):
$$-62mV \approx 1e^{-}/0.66nm^2$$



Asymmetric membrane: DOPC (cis), DOPS (trans)

Offset voltage (mV)	Standard deviation
-62.148	0.050

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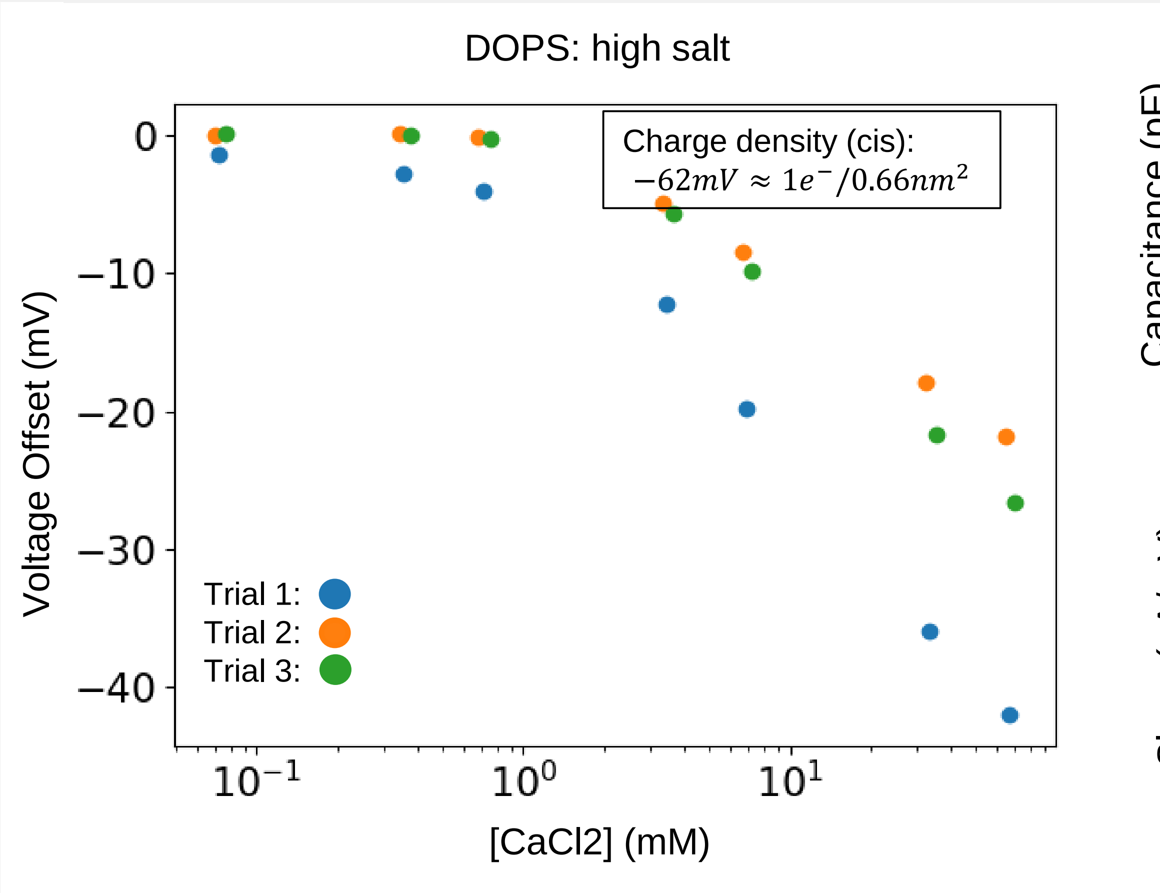
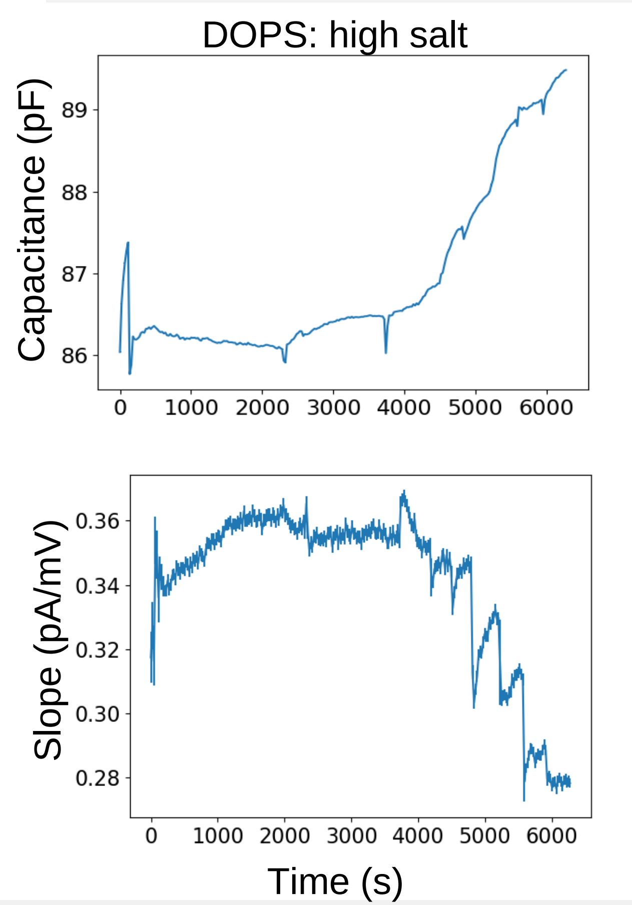
Experimental Results





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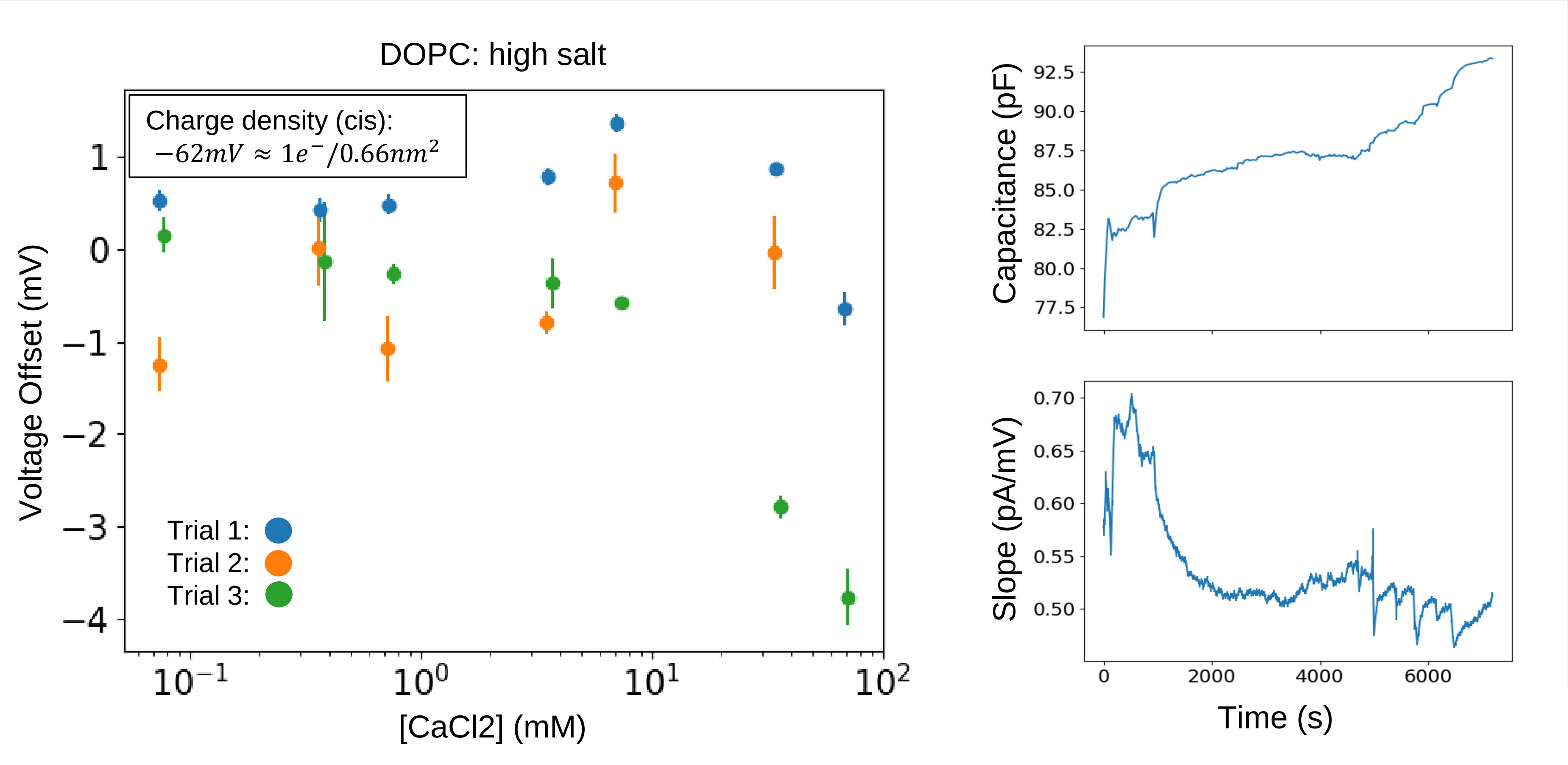

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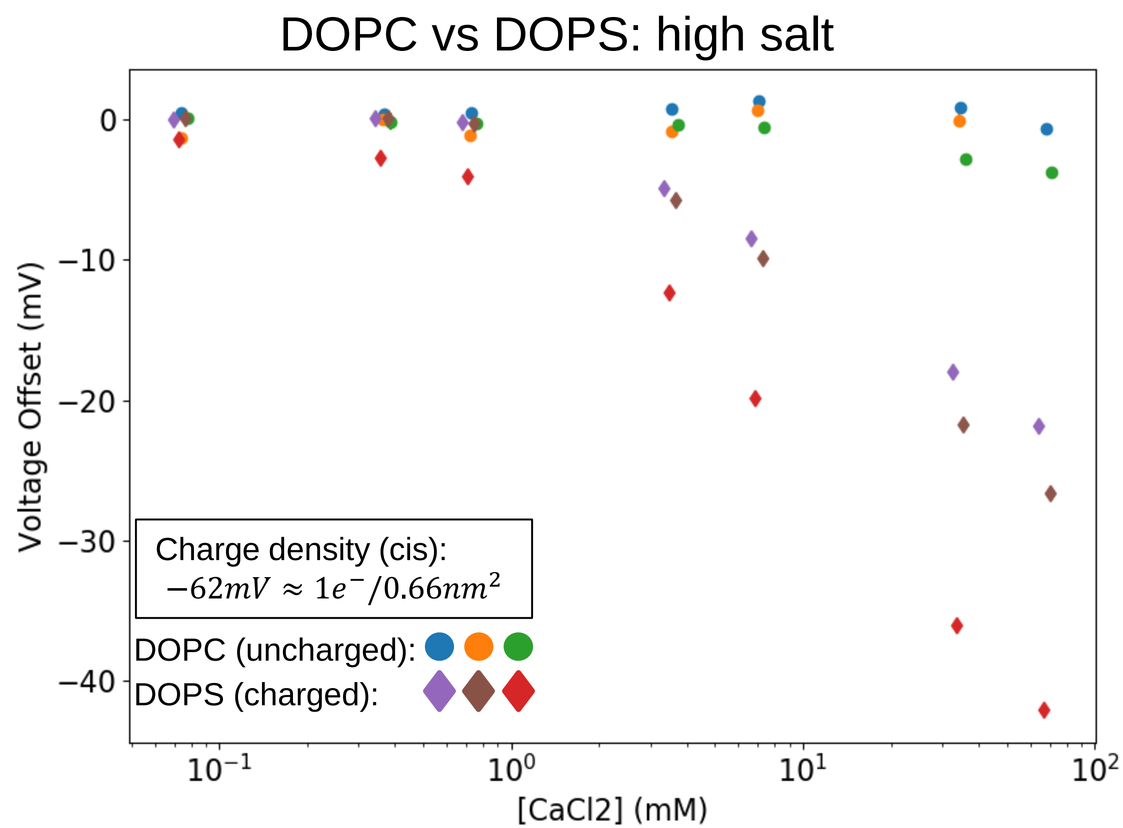
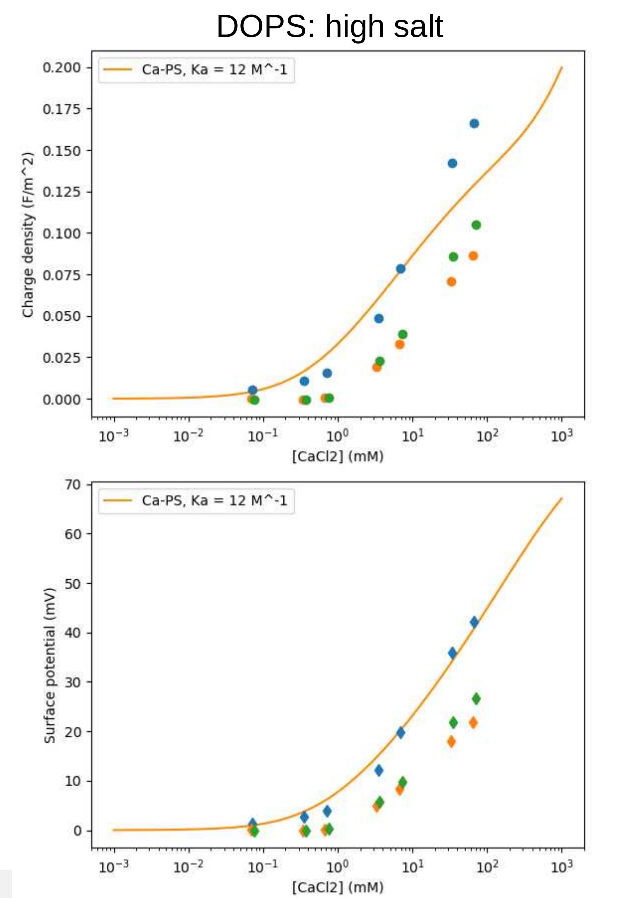
Experimental Conclusions





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Discussion & Future Experiments

Pros of this set-up:

- We can 'see' everything!

Cons of this set-up:

- We can see ***everything***

Lingering questions:

- What are the markers of a stable membrane at high salt?
  - At low salt we can look at the slope
- What are the effects of hexadecane?
  - At the torus and bilayer interior

Future experiments:

- Phospholipases and mutant forms
  - Study integral and peripheral membrane proteins
- Try more concentrated solutions of divalent ions
- Optimize membrane formation at a lower salt concentration
  - Study less concentrated solutions of divalent ions

Complementary technique:

Integrate with other biophysical tools to get the complete picture!

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ACKNOWLEDGEMENTS

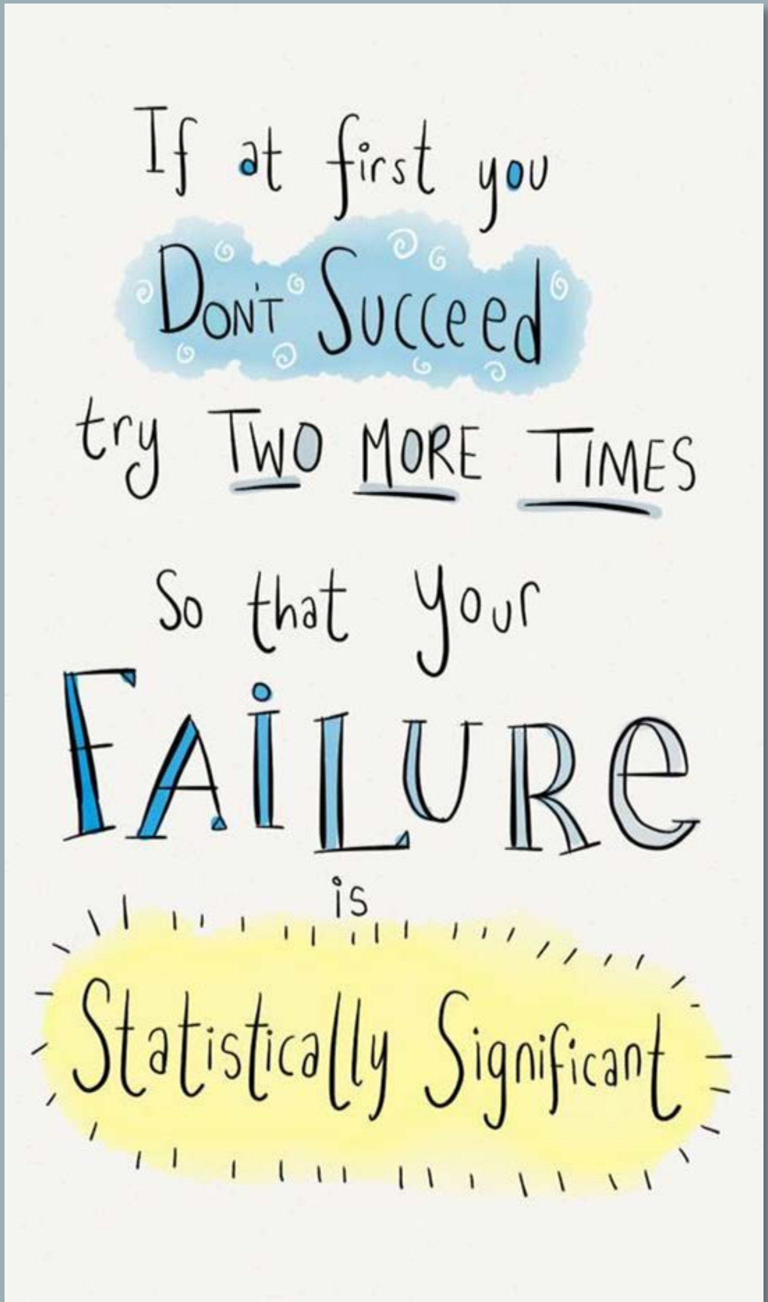
Dr. David Hoogerheide
- Mentor, NCNR Project Leader

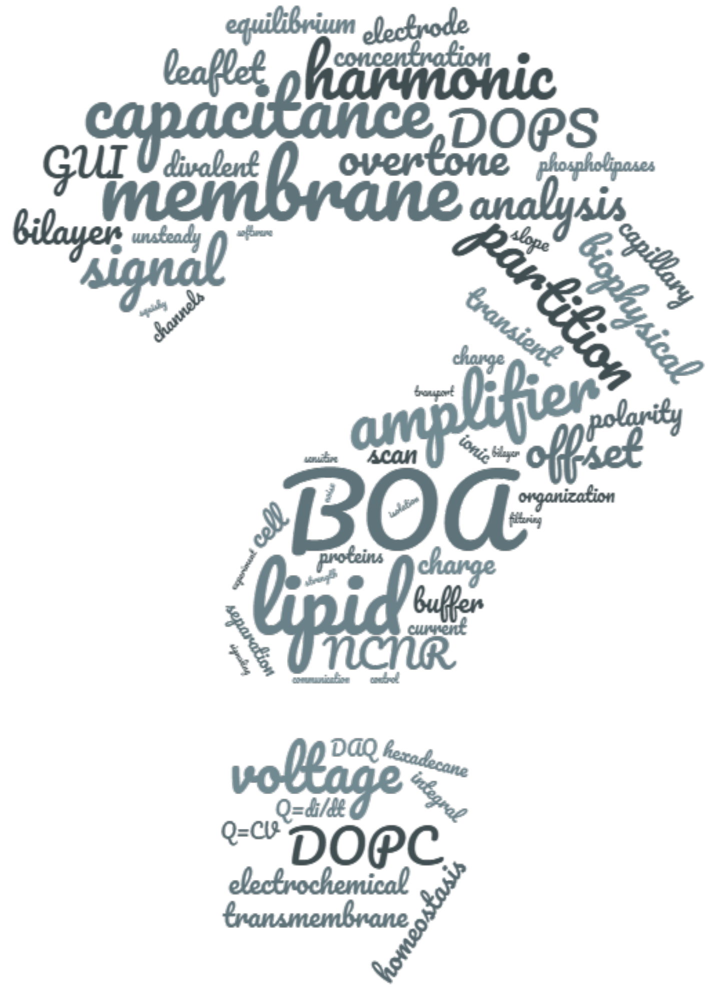
Dr. Joseph Dura & Dr. Julie Borchers
- NCNR SURF Directors

Dr. Brandi Toliver
- SURF Program Director

Lisa Fronczek
- EL SURF Director, Office of Advanced Manufacturing

Fellow NCNR SURFers!
- Shark Tank, Submarine, and Fish Bowl





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