DNA Sequencing Using Biological Nanopores

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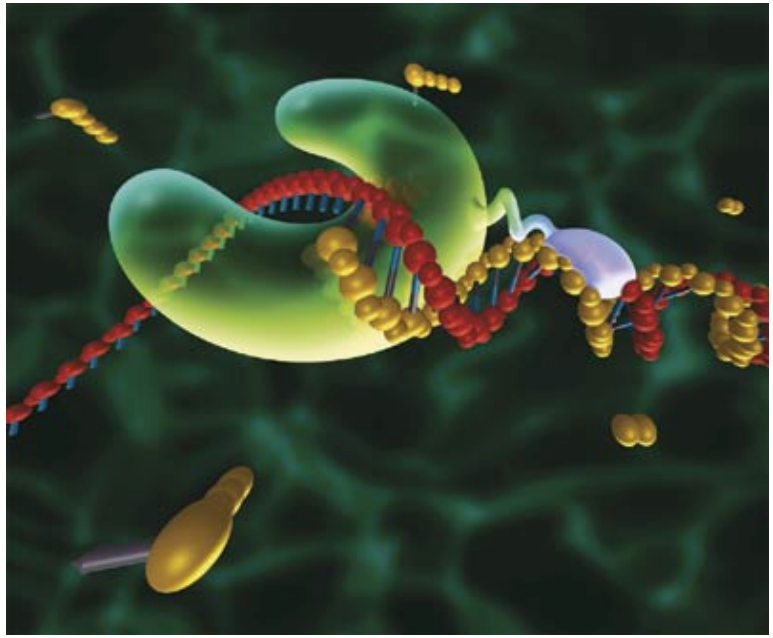
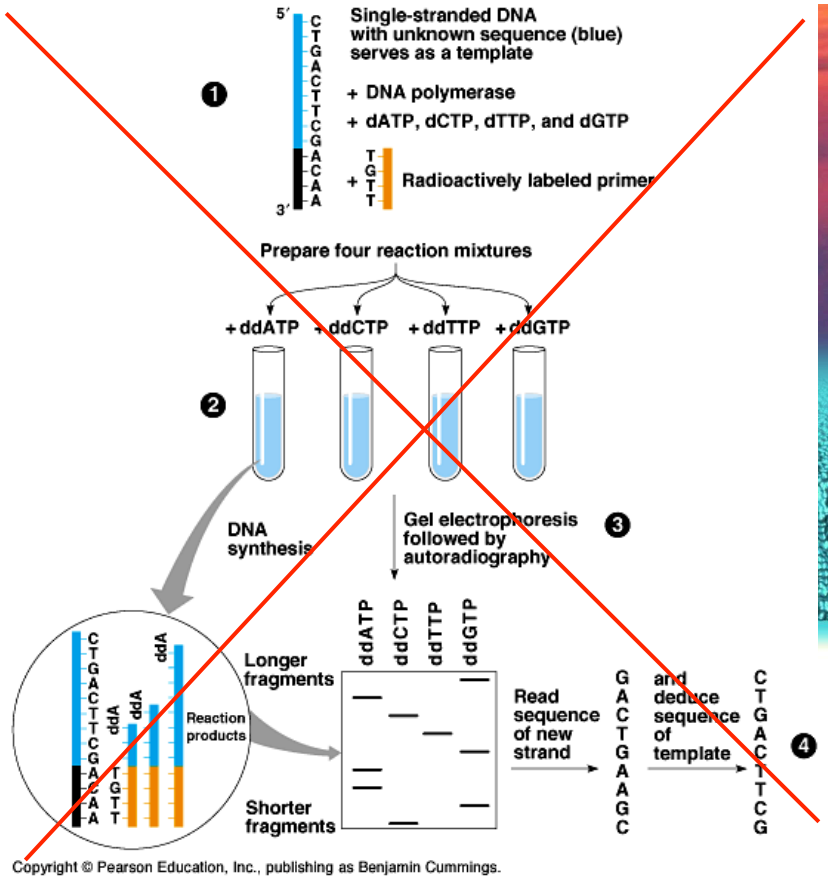
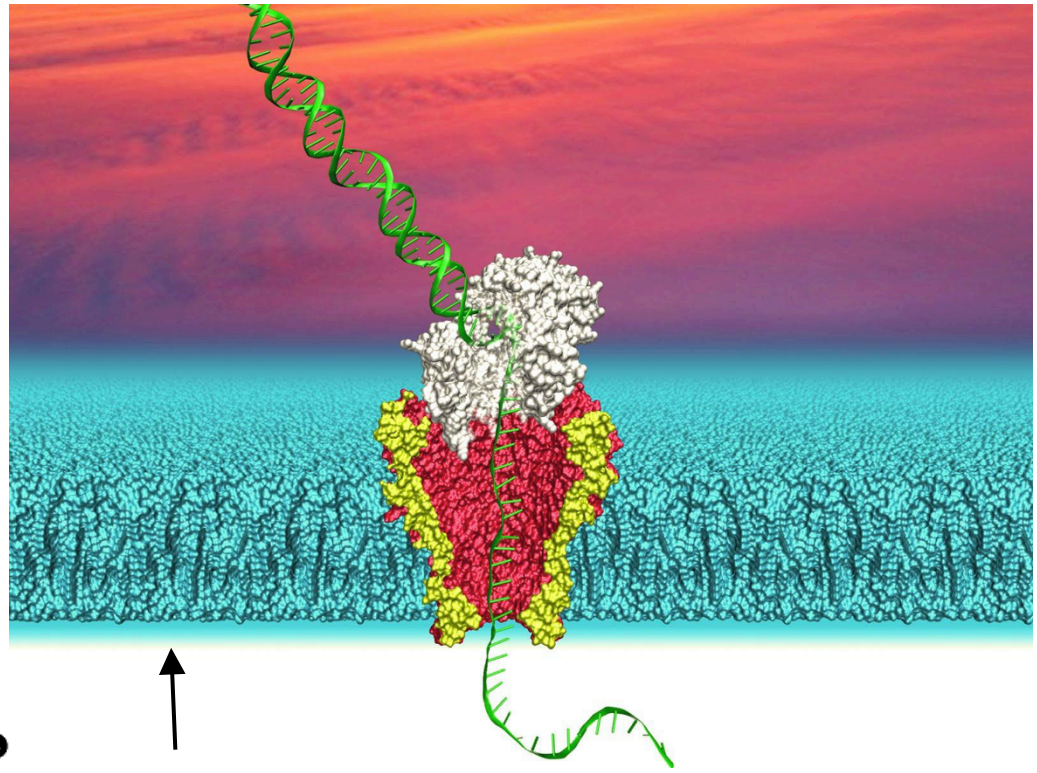
Conventional DNA sequencing

Nobel Prize in Chemistry, 1980.
Sanger Method: As DNA is synthesized, nucleotides are added onto the growing chain by DNA polymerase.







Ahh, much better!

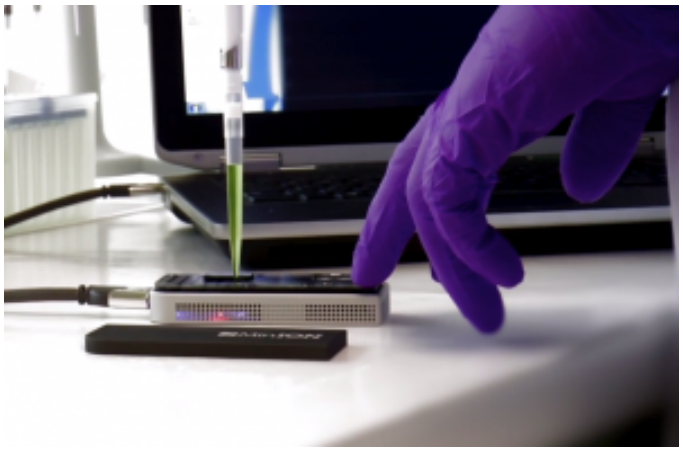
Image: Venkatesan *et al*, Nature Nano

Nanopores for DNA sequencing

- Fast and cheap sequencing
- Can reveal predispositions to a variety of illnesses
- Technology may go from: lab → industry → clinic → household → ??
- Multidisciplinary research

Current Technology ↑

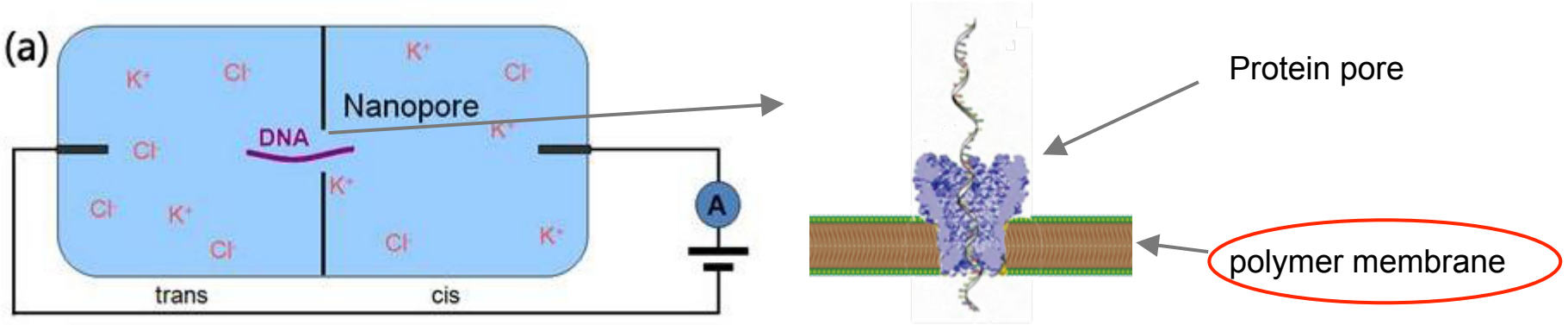
vs.



← Nanopore Technology (potentially)

Image: Oxford Technologies

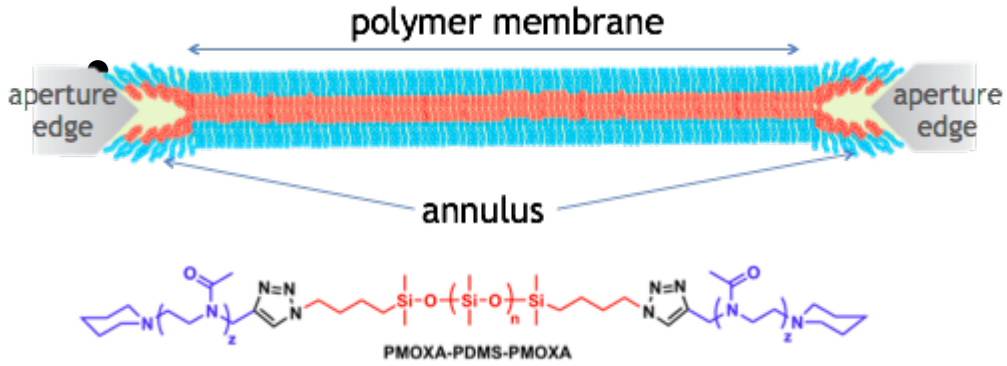
So how does it work?



- DNA passes through and creates a blockade of current

Polymer Membranes as the Platform

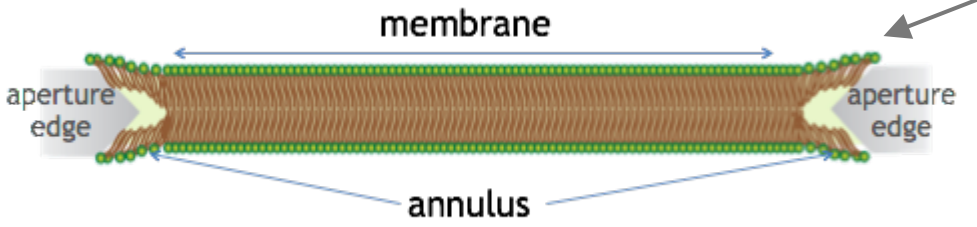
Goal #1: Form polymer membranes



- Highly Stable
- Long-Lasting

instead of...

lipid membrane



- Very Fragile!
- Limited lifetime

Image: Courtesy of Dr. Luke Theogarajan

Proteins behave differently in polymer membranes

Certain bilayer properties can give rise to certain protein conformations

Different protein shape = different protein function.

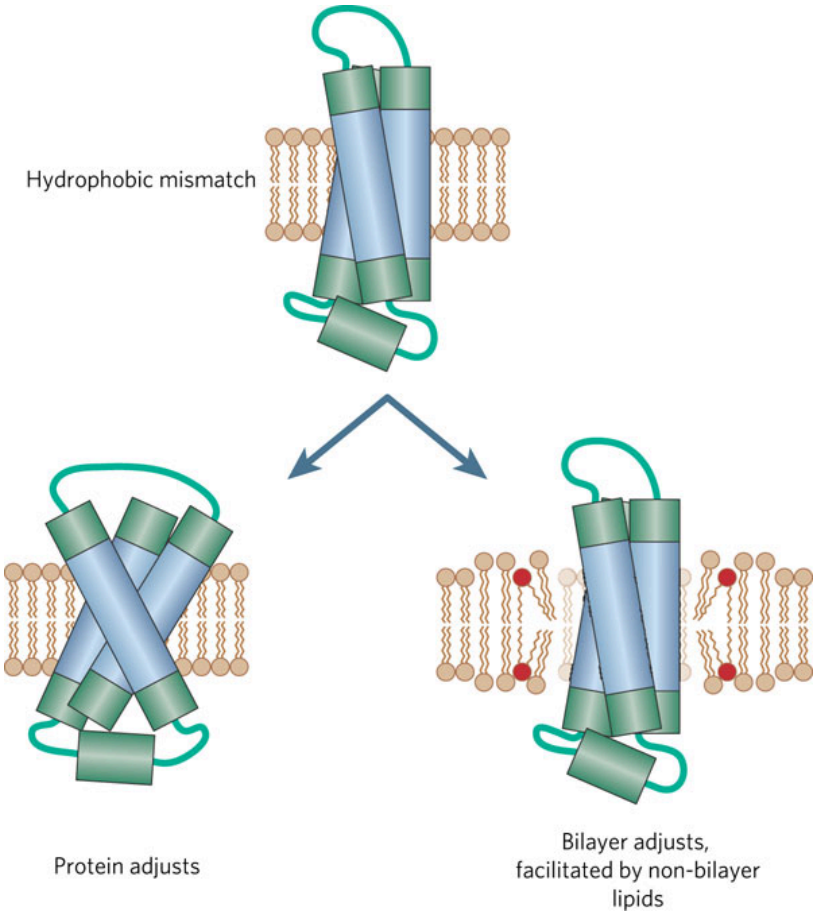
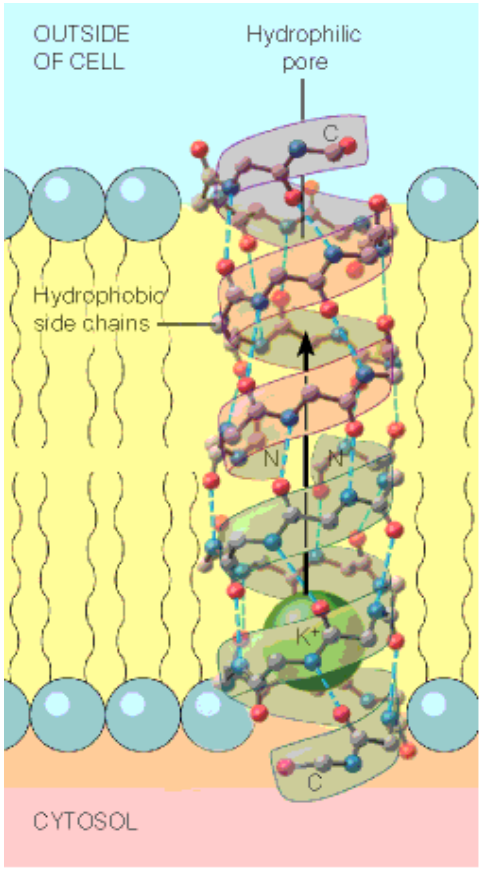


Image: Bowie, J.U., "Solving the memrane protein folding problem," *Nature* 438, 581-589.

Gramicidin A as a Molecular Force Probe



Goal #2: Use Gramicidin A, a peptide, to probe membrane properties and understand membrane energetics

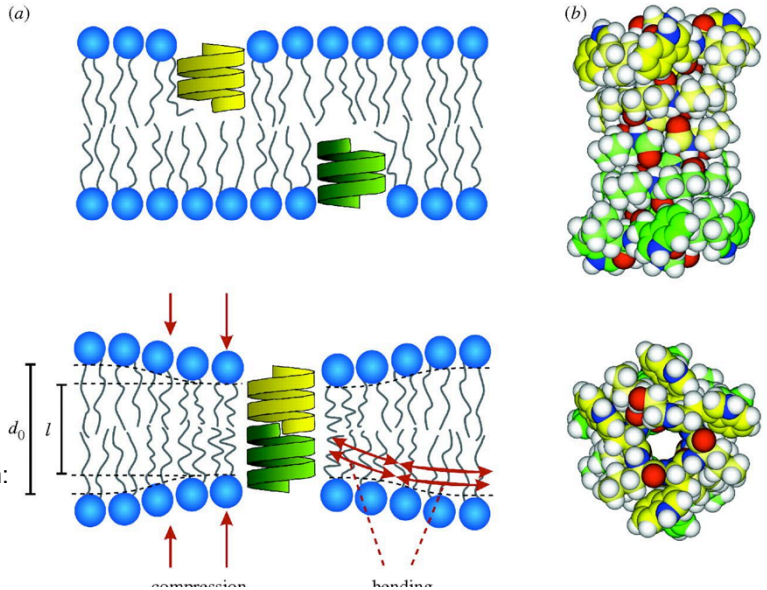


Image: Andersen O. S., Koeppe R. E. II
2007 Bilayer thickness and membrane protein function: an energetic perspective. *Annu. Rev. Biophys. Biomol. Struct.* 36, 107–130.

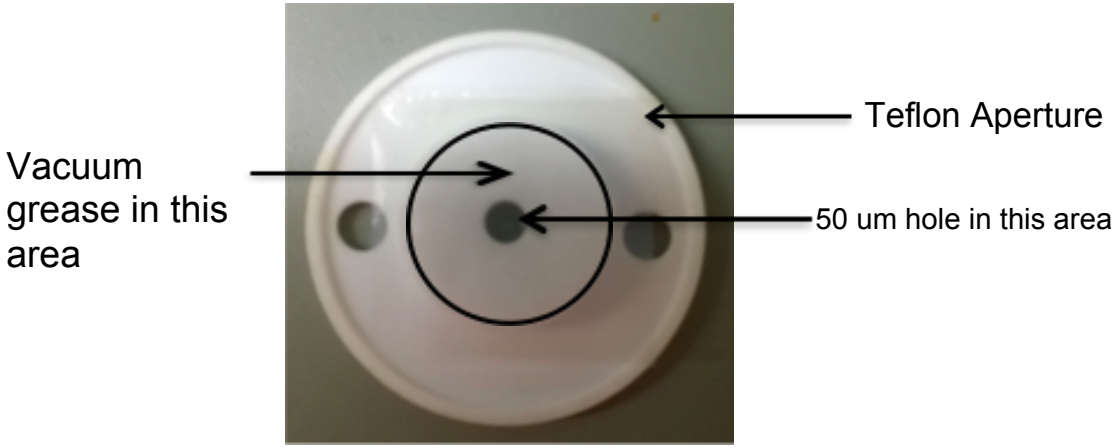
Methods

1. Making membranes

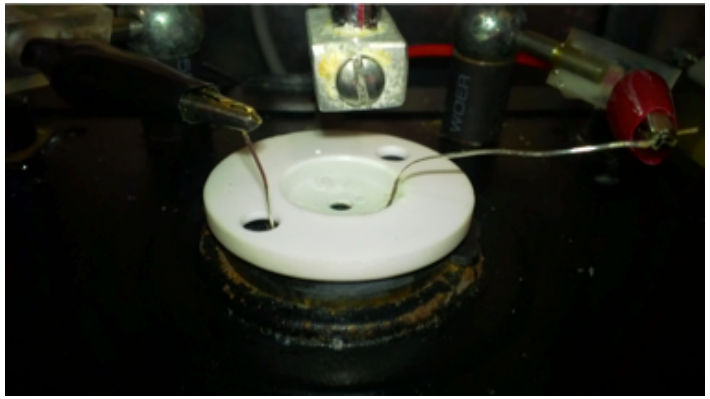
2. Inserting Gramicidin A

Forming Membranes

The setup



Apply a voltage to form the membrane



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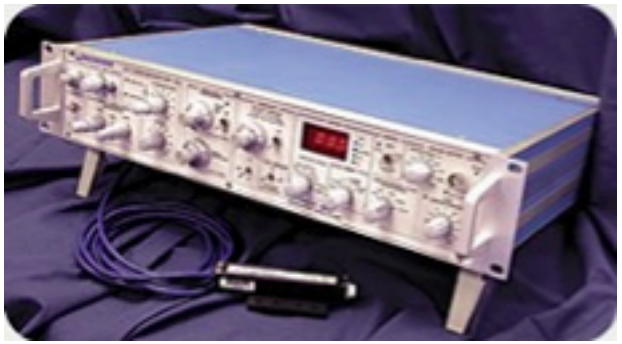
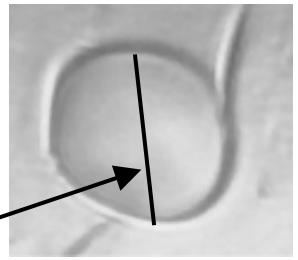


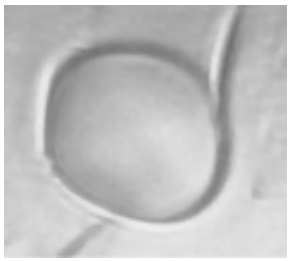
and the result...



Amplifier sensitive to very small current levels (picoamp range)

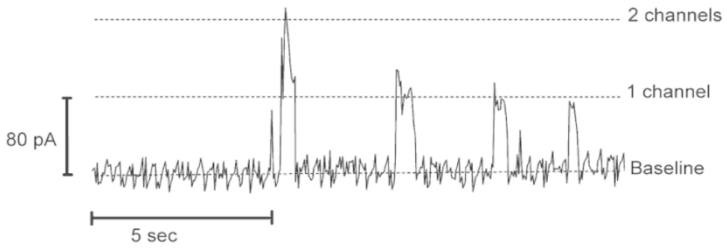
Inserting Gramicidin A

Form membrane (make sure it's stable!) under applied voltage



Pipet Gramicidin A near aperture

Look for characteristic current traces

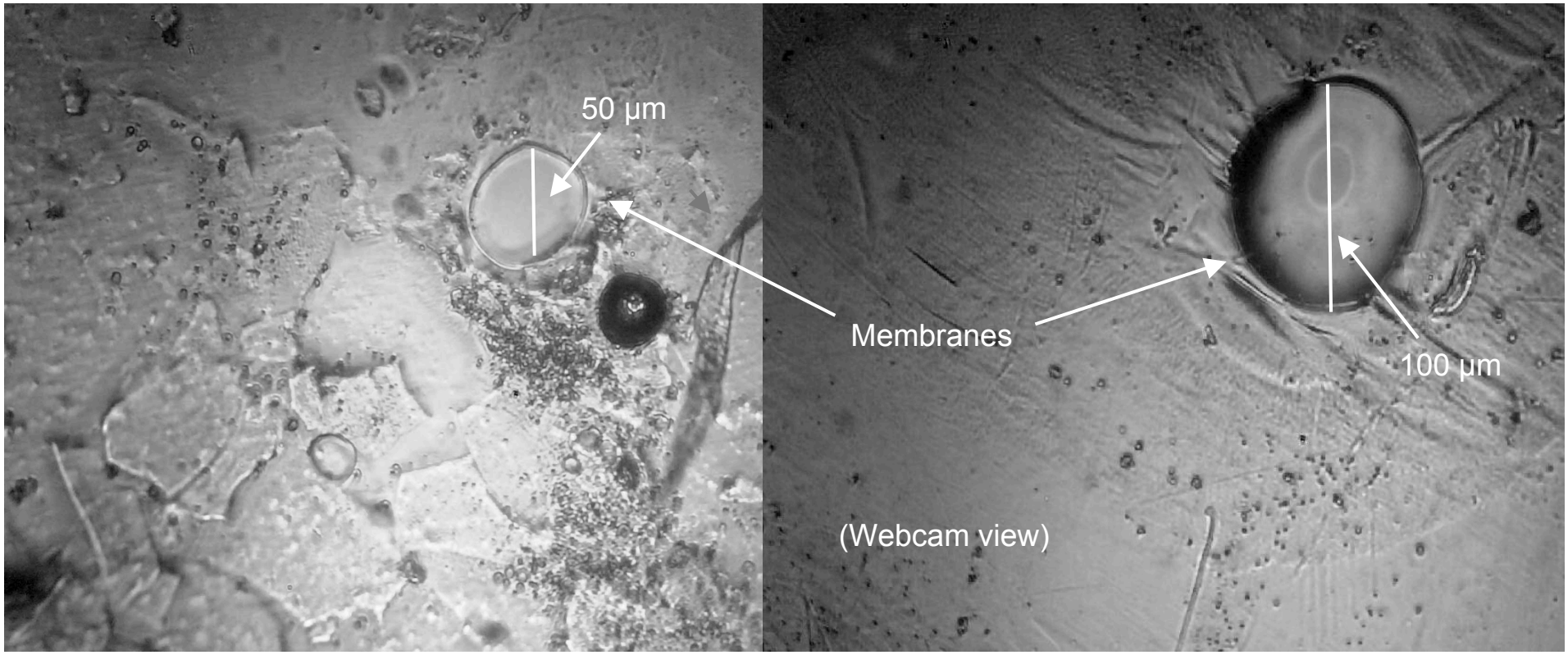


Results

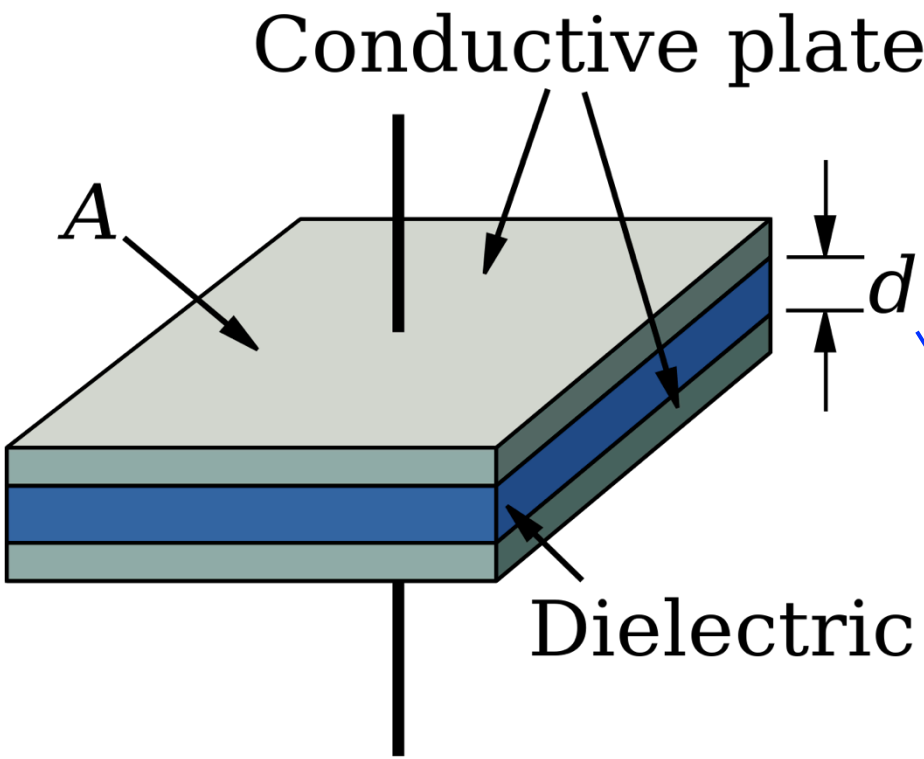
1. Stable membranes

2. Protein insertion

Results: Formed Membranes



Model membrane as a parallel plate capacitor



$$C = \frac{\varepsilon A}{d}$$

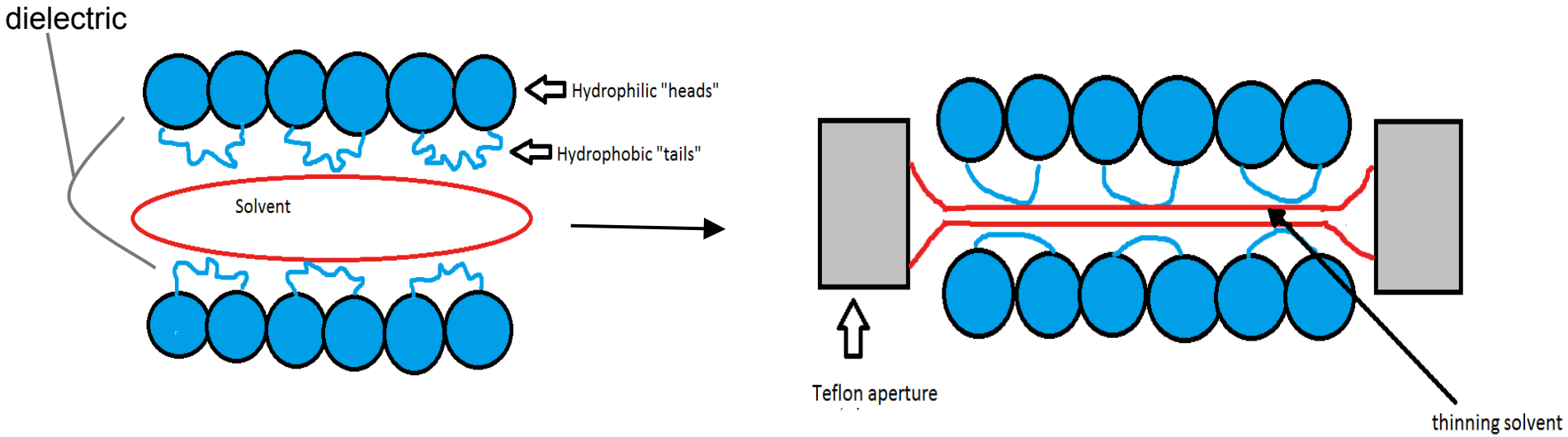
Where,

- C = Capacitance in Farads
- ε = Permittivity of dielectric (absolute, not relative)
- A = Area of plate overlap in square meters
- d = Distance between plates in meters

Bilayer thickness

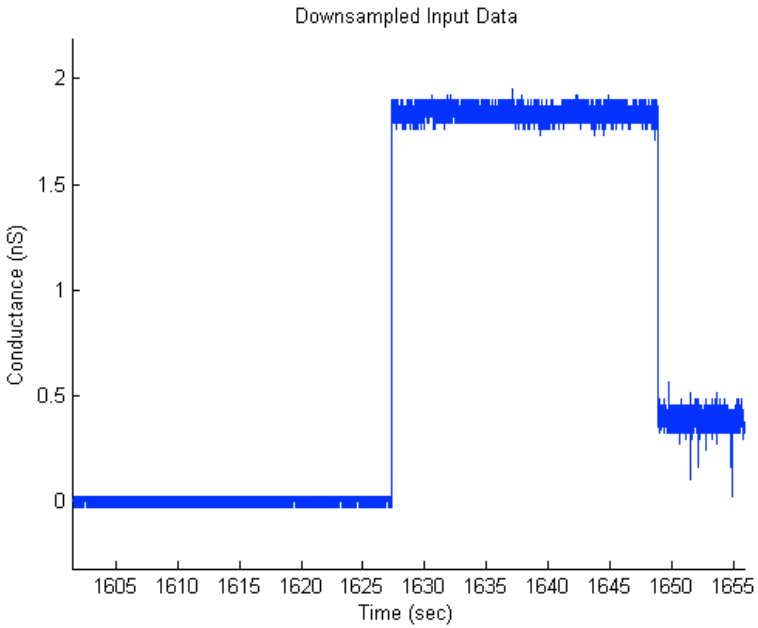
Membrane hydrophobic region

Membrane Thinning



- As membrane thins, capacitance values increase = indicative of stability

Results: Protein Insertion



Protein insertion at 180mV

Stepwise conductance increase characteristic of insertion

We predict gramicidin will have a similar transition, but with lower values

Summary of Progress

➔ Refining protocol for gramicidin assay

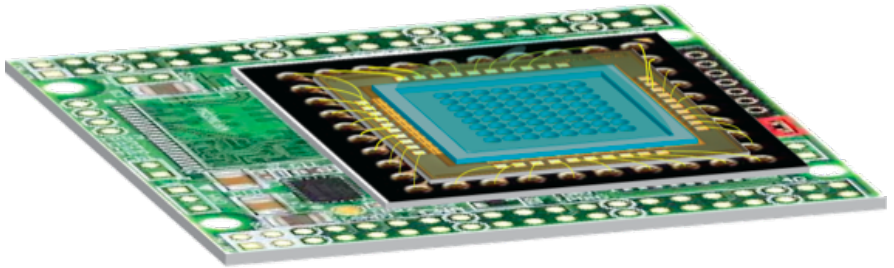
➔ Learned prep work for membrane formation

➔ Learned how to form membranes under applied voltage

Future of Research

Continue with gramicidin study to optimize polymer/protein interaction

Long term: Nanopore array- allows high throughput sequencing



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