Preparation and characterization of a new tethered bilayer lipid membrane (tBLM) model membrane system

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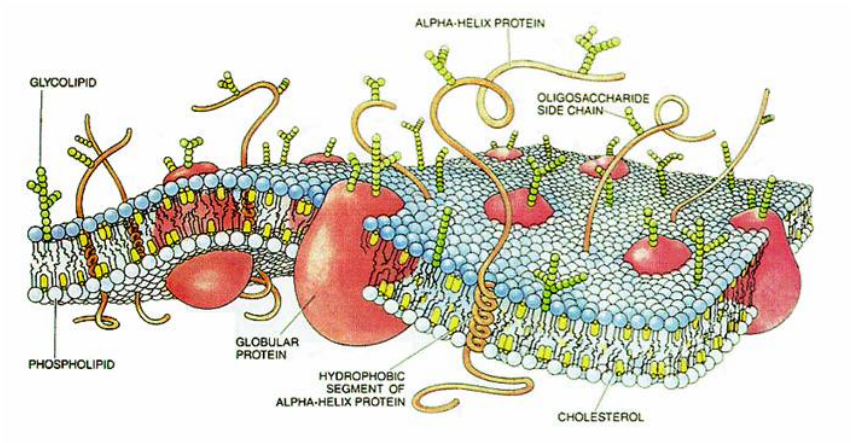
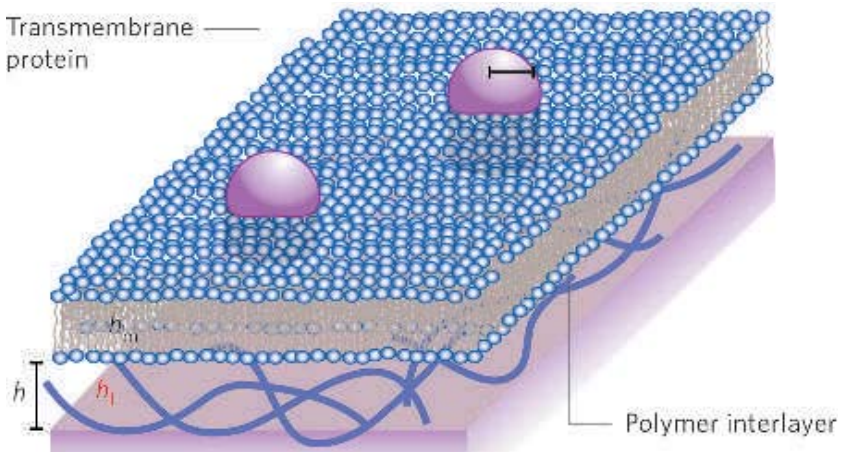
Biological membranes vs. model membranes



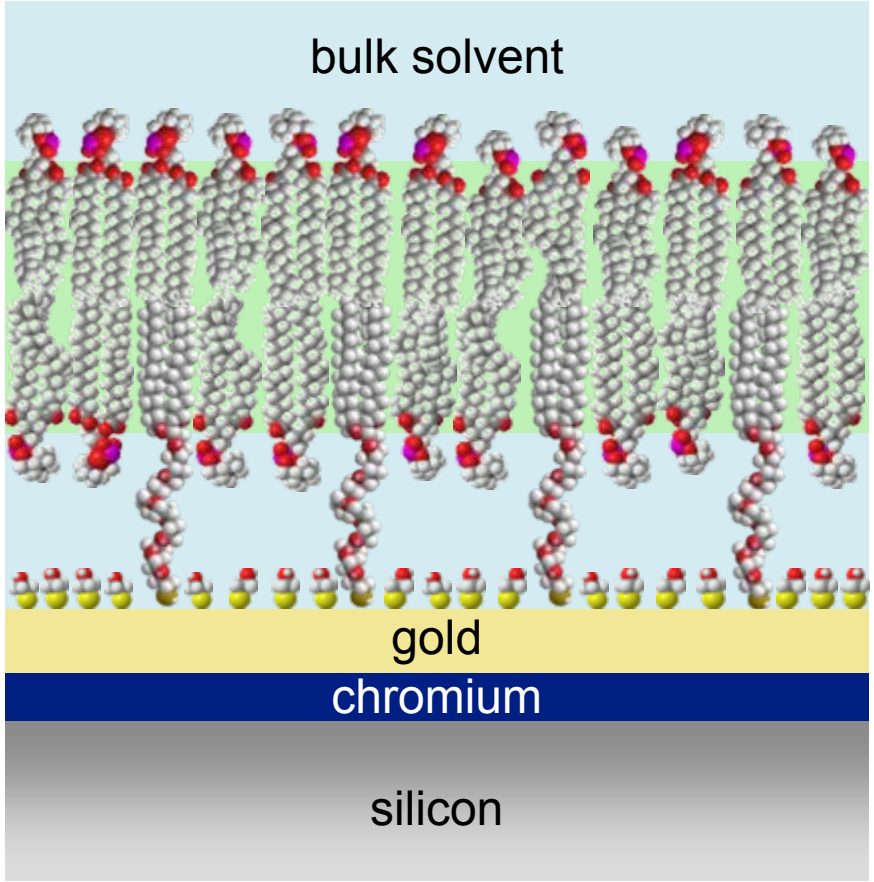


From Tanaka and Sackmann

- Semi-permeable phospholipid bilayer
- Hydrophobic and hydrophilic components
- Membrane proteins and other molecules in biomembranes
- Only lipids in model membranes

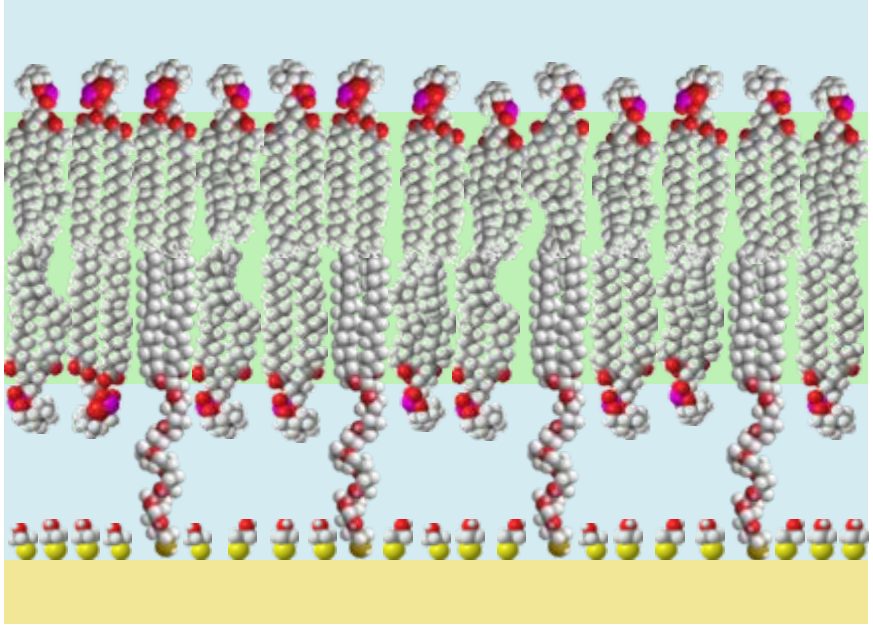
Tethered model membranes

- Tether molecule anchors bilayer to substrate
- Aqueous reservoir
- Membrane fluidity
- Protein incorporation
- Backfiller molecule β-mercaptoethanol (βME)



Objectives

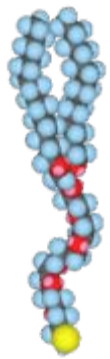
- Characterize and prepare model membranes on tethered systems
- Reduce surface density of tethers while maintaining bilayer structure
- Increase aqueous reservoir between membrane and substrate



Motivation

- Understand interaction between lipid bilayer and membrane proteins
- i.e. Alzheimer's, HIV Gag protein

New tether FC16: Better than WC14?



WC14

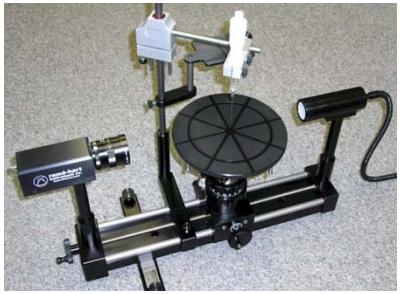
- 14-carbon alkyl chains
- 6 ethylene-oxide units

FC16

- 16-carbon alkyl chains
- 9 ethylene-oxide units

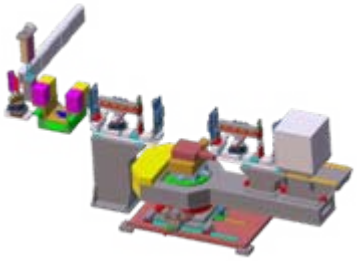
Will FC16 increase sub-membrane space, or fall over like a “drunken sailor”?

Ellipsometry



Contact angle

Impedance spectroscopy

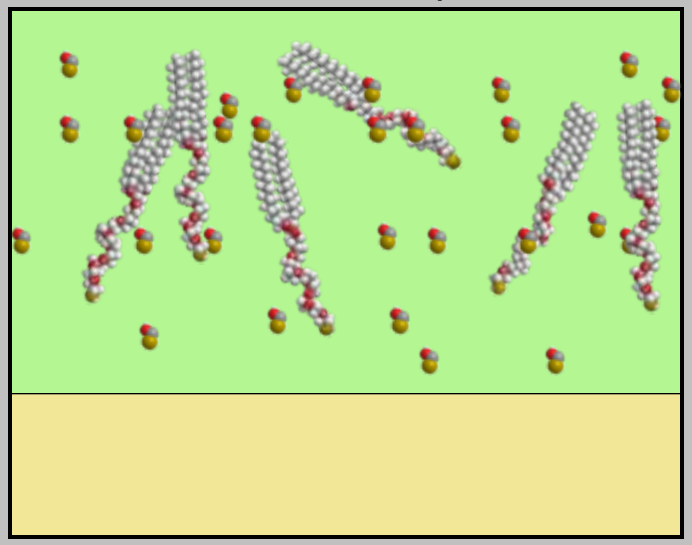


Neutron reflectometry

Sample Preparation

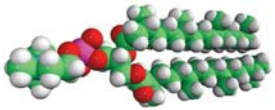
Self-Assembled Monolayers (SAMs)

Incubation with FC16/βME



Tethered Bilayer Lipid Membranes (tBLMs)

Rapid Solvent Exchange



DPhyPC lipid used for bilayer formation

Ellipsometry

PRINCIPLE

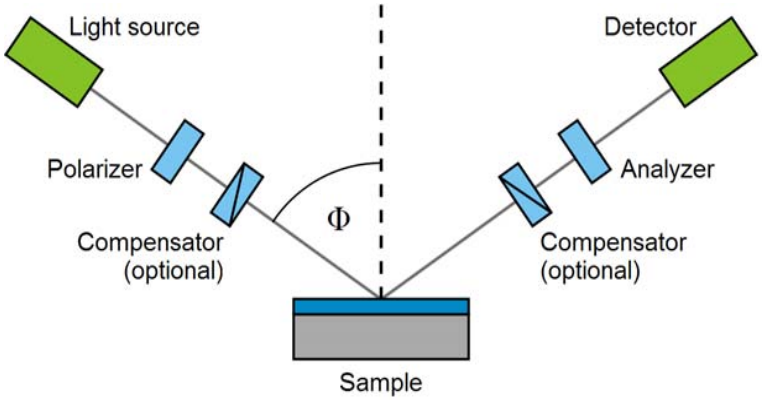
- Reflect polarized light
- Thickness of thin films

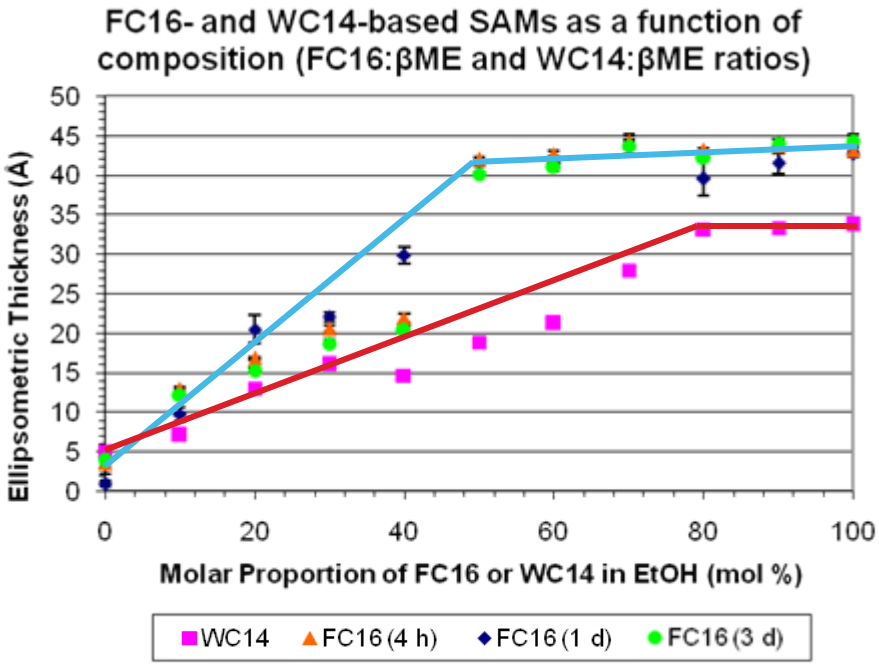
EXPERIMENT

- Different proportions of FC16:βME SAMs

RESULTS

- FC16 SAM 10 Å thicker
- Time independence of SAM formation





Contact Angle

PRINCIPLE

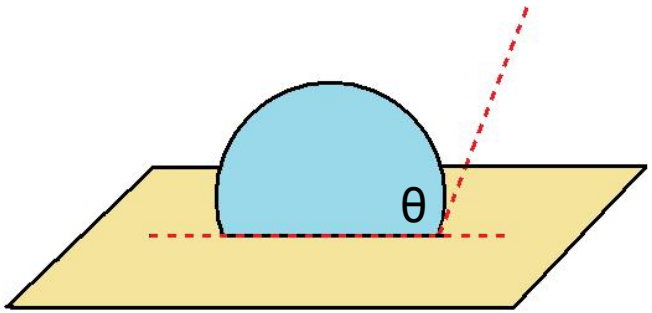
- Angle of air/liquid interface at surface
- Hydrophobicity of SAM

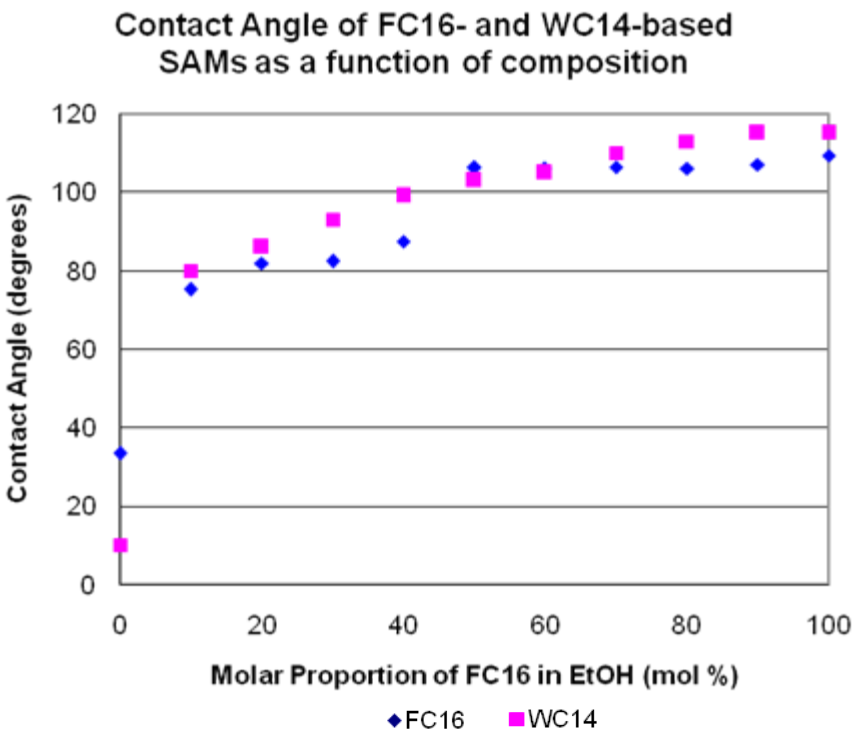
EXPERIMENT

- Different proportions of FC16:βME SAMs

RESULTS

- FC16 hydrophobic at low proportions

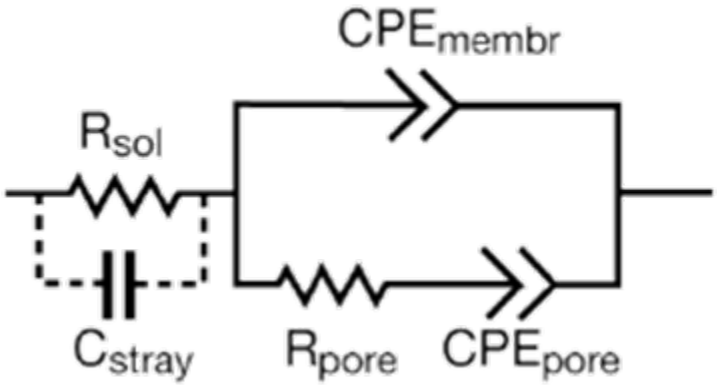




Electrochemical Impedance Spectroscopy

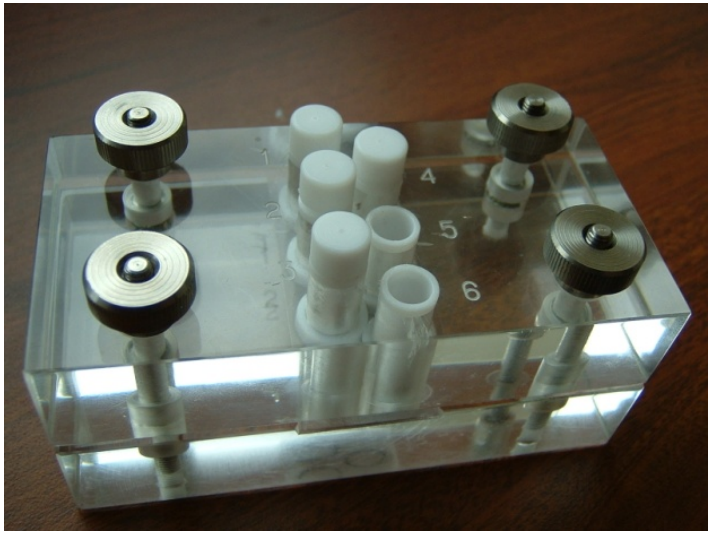
PRINCIPLE

- Bilayer acts as a capacitor
- Defects in bilayer lower resistance



EXPERIMENT

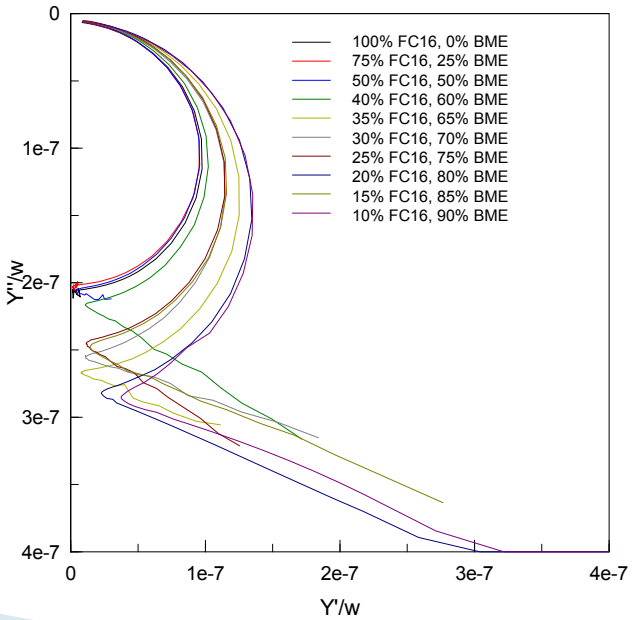
- Different proportions of FC16: βME SAMs and bilayers (focus on low FC16: βME ratios)

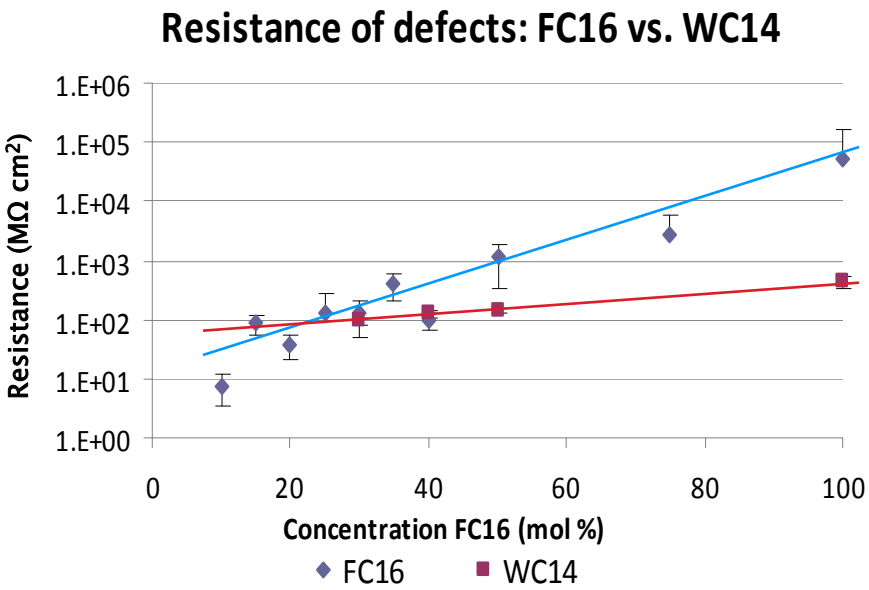


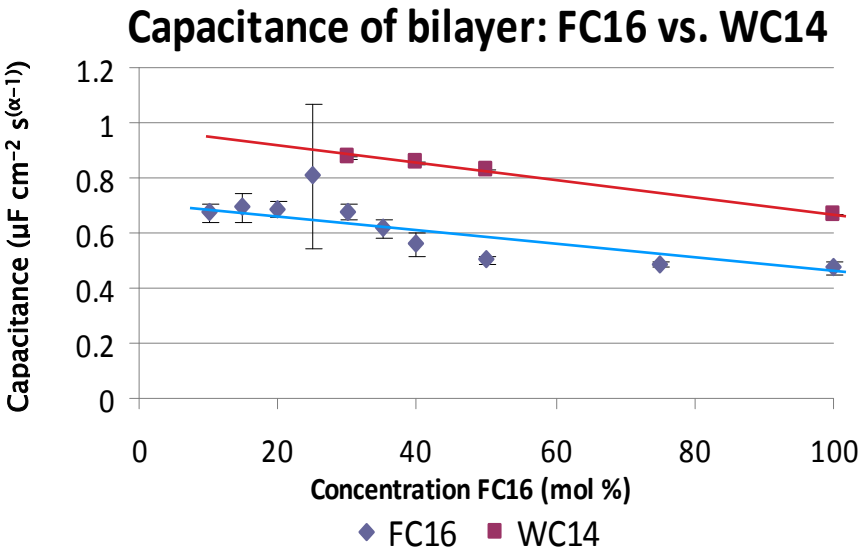
Electrochemical Impedance Spectroscopy

RESULTS

- FC16 has lower capacitance, higher resistance than WC14







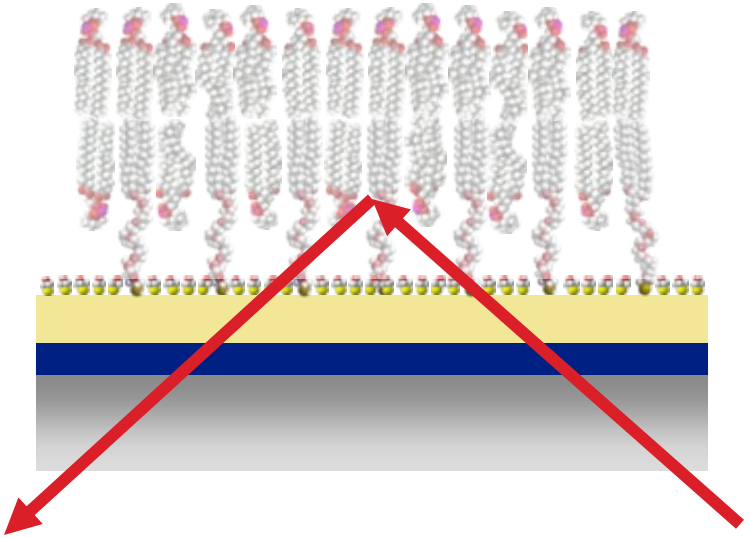
Neutron Reflectometry

PRINCIPLE + OBJECTIVE

- Intensity and angle of reflected neutron beam give neutron scattering length densities
- Solvent contrasts
- Characterize structure, determine hydration

EXPERIMENT

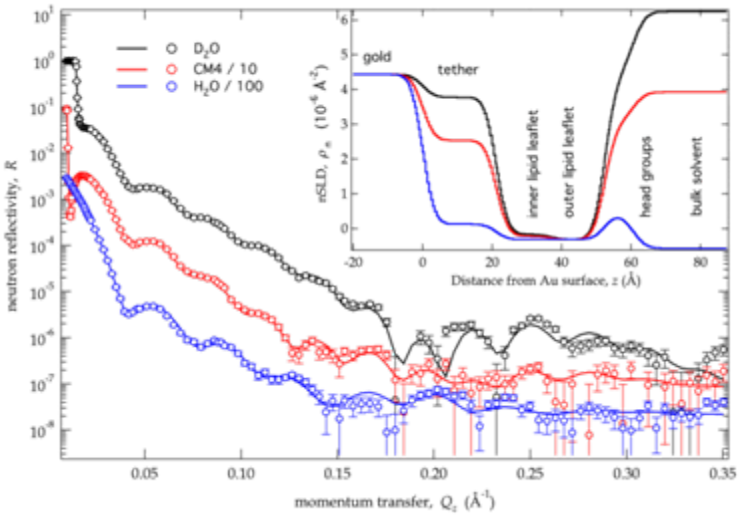
- 30 mol % and 15 mol % FC16, DPhyPC bilayer



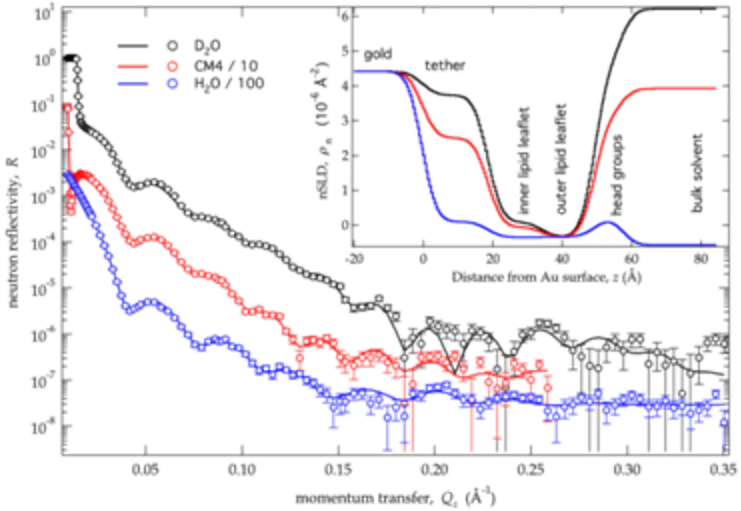
Neutron Reflectometry

RESULTS

- Complete bilayer, only minimal hydration of inner leaflet
- 30 mol % FC16: 21 Å aqueous reservoir
- At 15 mol %, 19 Å thick
- Tether region >50% hydration

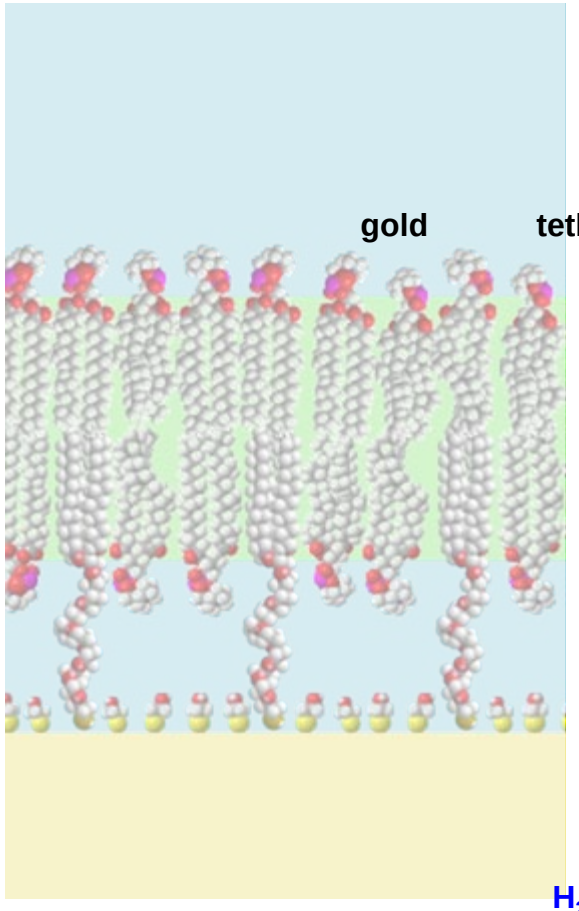


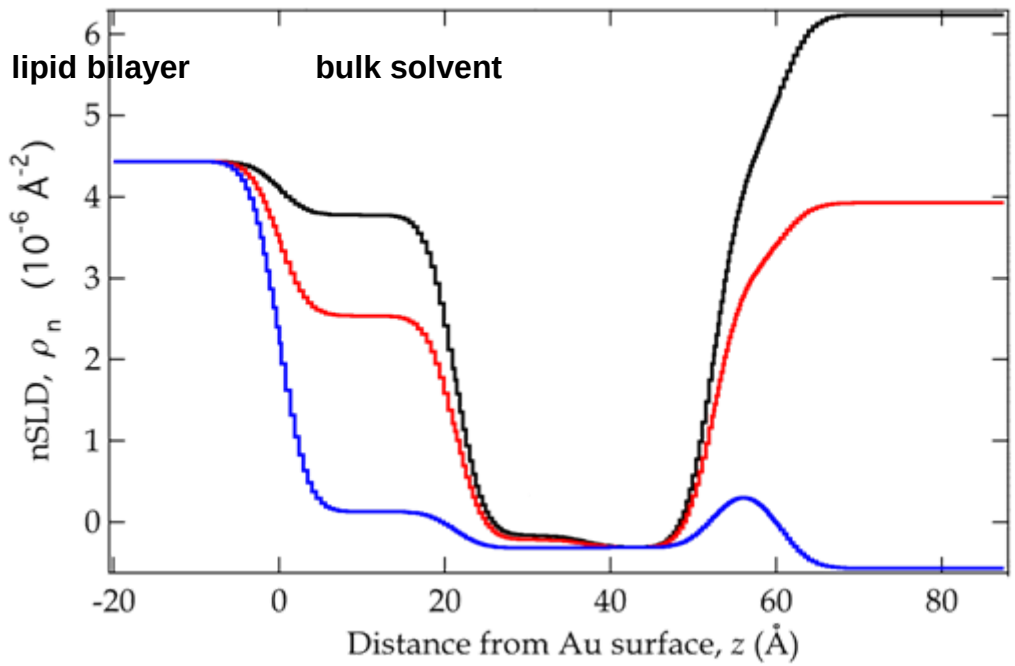
Reflectivity curves, fit and nSLD profile of the 30:70 FC16:βME sample completed with DPhyPC.



Reflectivity curves, fit and nSLD profile of the 15:85 FC16:βME sample completed with DPhyPC.

Neutron Reflectometry





H_2O D_2O CM4

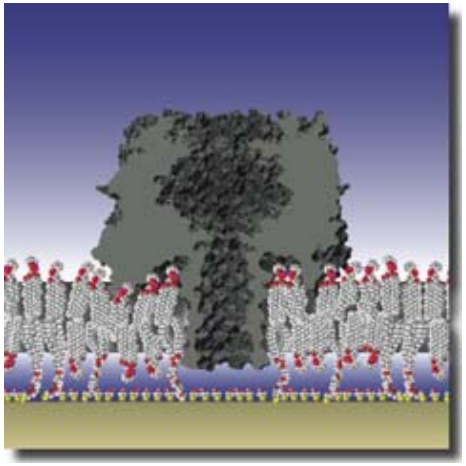
The final showdown: FC16 vs. WC14

FC16		WC14
43	Ellipsometric thickness of 100% tethered SAM (Å)	33
0.67	Capacitance of bilayer at 30% tether (μF cm^{-2} $s^{(\alpha-1)}$)	0.87
132	Resistance of defects in bilayer at 30% tether (kΩ cm^2)	93
21	Submembrane space thickness at 30% tether with bilayer (Å)	13
54	Hydration of submembrane space at 30% tether (vol %)	64
100	Bilayer completeness (outer leaflet) at 30% tether (%)	98

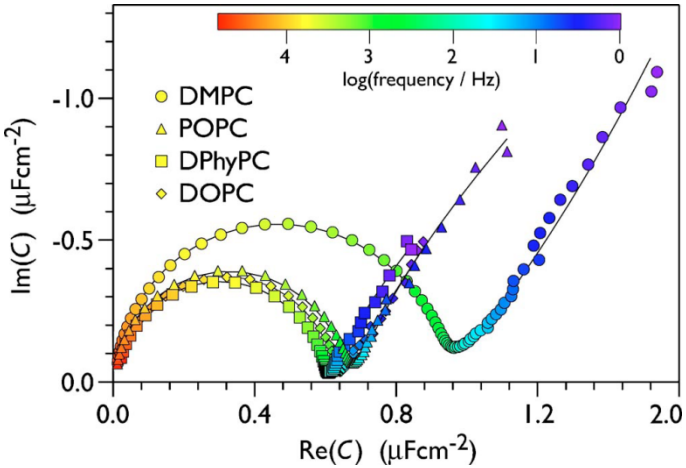
Compared to WC14, FC16 increases submembrane space and is able to support a more completed bilayer

Future Work

- Use of FC16 for studies on structure and function of biomembranes



- Further characterization of FC16 tBLMs with other lipids, esp. charged lipids



Acknowledgements

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Questions?