

Underlying Mechanisms of Mitochondria-related Diseases through Cardiolipin-Cytochrome C Interactions

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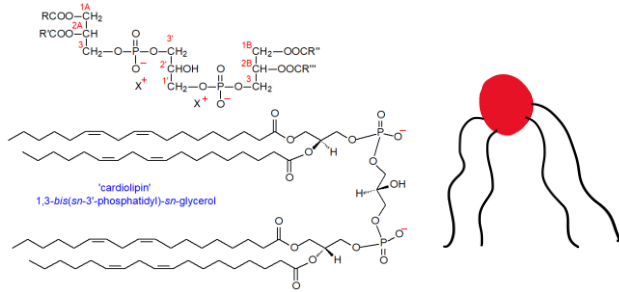
Host Lab: NIST Center for Neutron Research



FISCHELL DEPARTMENT OF
BIOENGINEERING

Key Components

Cardiolipin



Highly negative and unsaturated
Alterations to its structure and concentration lead to disease

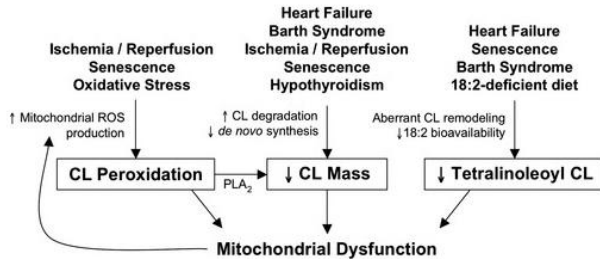
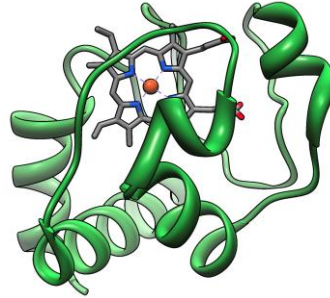


Figure 1: Chicco, Sparagna, *Am. J. Physiol. Cell. Physiol.* **2007**, 292, C33

Cytochrome



anchored to the outside of phospholipid membrane by CL

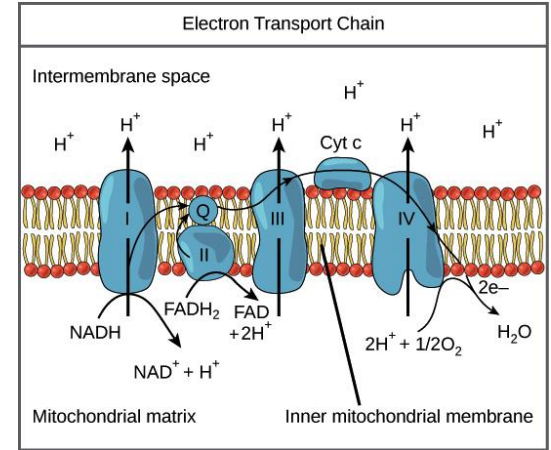
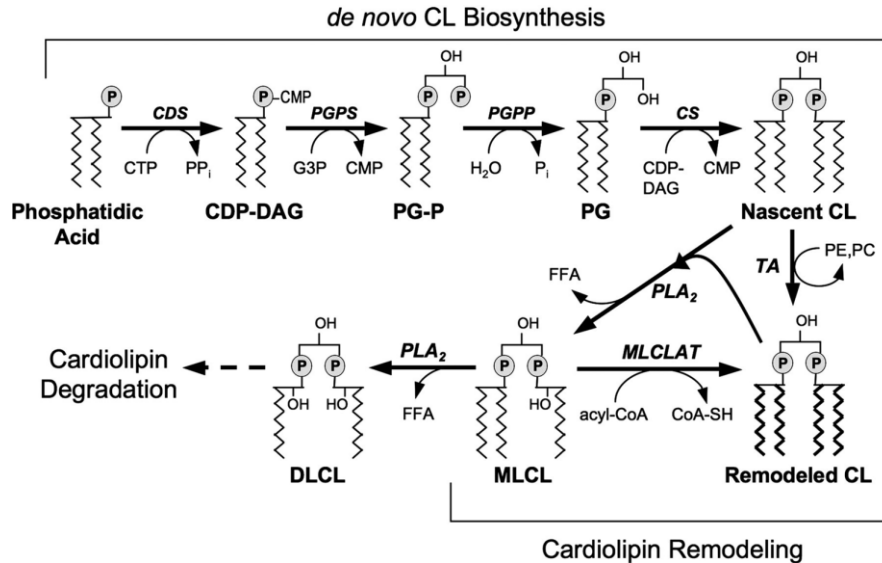


Figure 2: Ofoedu, Chigozie, *Foods* **2021**, 10, 699

Internal Biosynthetic Pathway and Diseases



Heart Failure → decreased CL levels

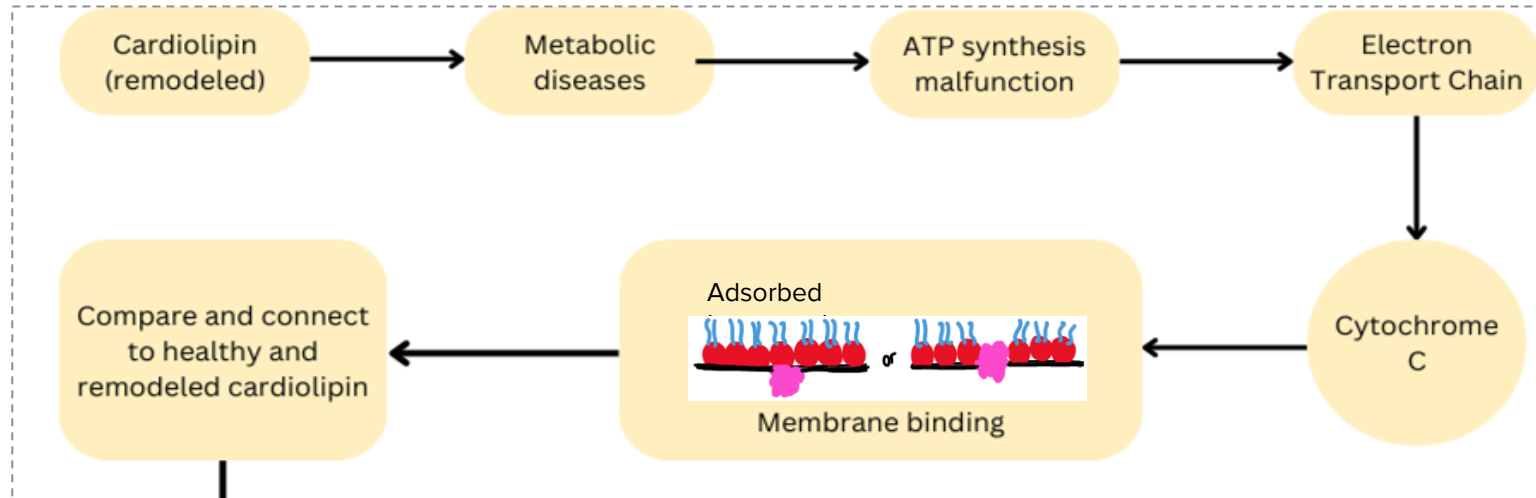
Ischemia and reperfusion → decreased CL levels
 Linoleic acid species diminish

Diabetic cardiomyopathy → decreased CL levels

Barth Syndrome → increase in **MLCL**
 (monolysocardiolipin)

Figure 3: Chicco, Sparagna, *Am. J. Physiol. Cell. Physiol.* **2007**, 292, C33

Motivation behind Project



I. Cardiolipin-Cytochrome c	
Materials (Lipids & Proteins)	
DPPC (16:0 PC)	
DPPE (16:0 PE)	
DMPC (14:0 PC)	
DMPE (14:0 PE)	
DMPG (14:0 PG)	
d-DMPC (14:0 PC-d54)	Control
d-DMPE (14:0 PE-d54)	
d-DMPG (14:0 PG-d54)	
(18:2)4CL (Heart, Bovine)	Healthy
Monolysocardiolipin (MLCL)	Diseased
(18:1)4CL	Diseased
(C22:6)4CL	
Cytochrome c	

Name	Mixed Lipid-CL Membranes
DPPC:DPPE:(18:2)4CL	(PC:PE)CL (4:3)0.15
DPPC:DPPE:(18:2)4CL	(PC:PE)CL (4:3)0.20
DPPC:DPPE:(18:2)4CL	(PC:PE)CL (4:3)0.40
DPPC:DPPE:MLCL	(PC:PE)MLCL (4:3)0.20
DPPC:DPPE:(18:1)4CL	(PC:PE)(18:1)4CL (4:3)0.20
DPPC:DPPE:DMPG	(PC:PE)(DMPG) (4:3)0.20
DMPC:DMPE:(18:2)4CL	(PC:PE)CL (4:3)0.15
DMPC:DMPE:(18:2)4CL	(PC:PE)CL (4:3)0.20
DMPC:DMPE:(18:2)4CL	(PC:PE)CL (4:3)0.40
DMPC:DMPE:MLCL	(PC:PE)MLCL (4:3)0.20
DMPC:DMPE:(18:1)4CL	(PC:PE)(18:1)4CL (4:3)0.20
DMPC:DMPE:DMPG	(PC:PE)(DMPG) (4:3)0.20

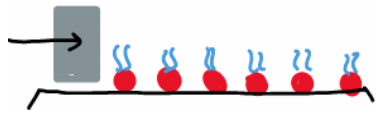
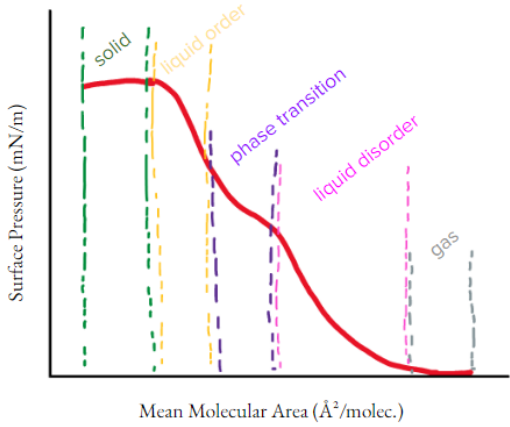
DP System (rows 1-6)
DM System (rows 7-12)

Techniques Employed

Langmuir isotherm:

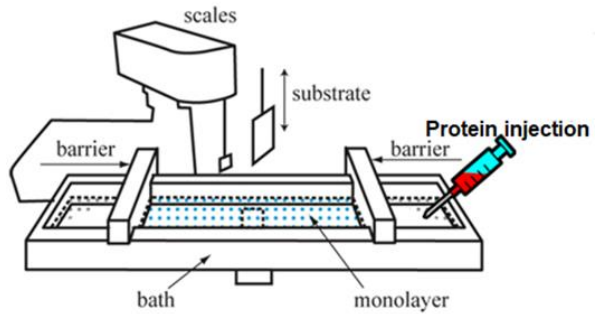
Fabricate the film that have the same packing density as the inner mitochondria (25mN/m)

Mix with different lipids to mimic the real



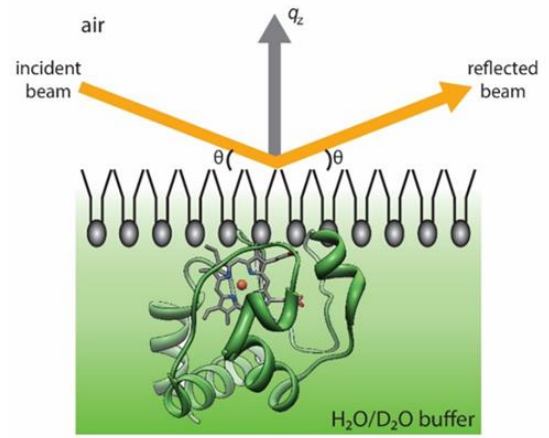
Adsorption Assay

Measure how much protein is absorbed by the interface and how it interacts with the membrane (how fast or slow)



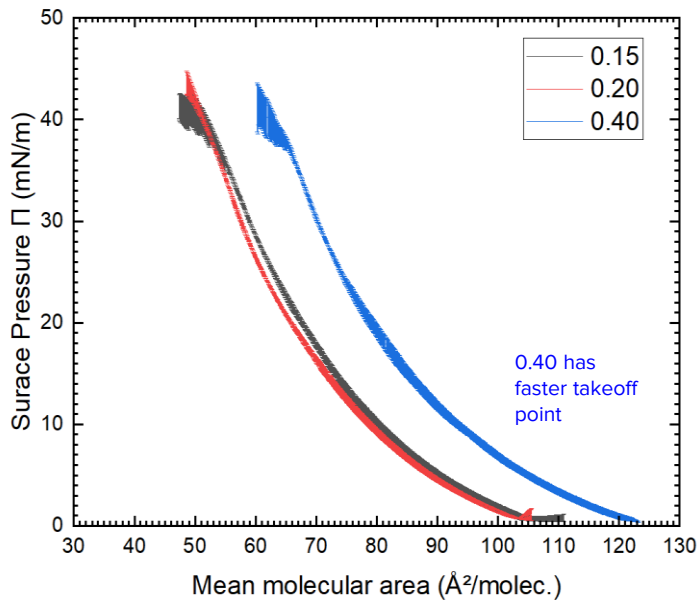
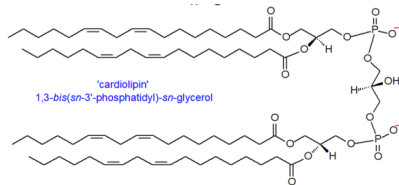
X-Ray Reflectometry

Allows us to get information about the thickness, density, roughness of membrane film

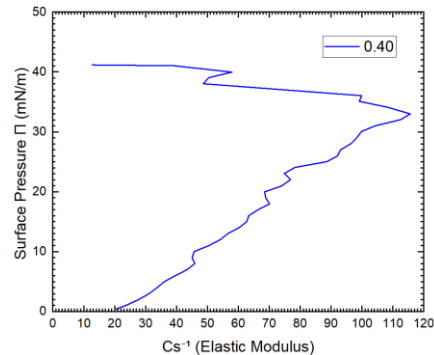
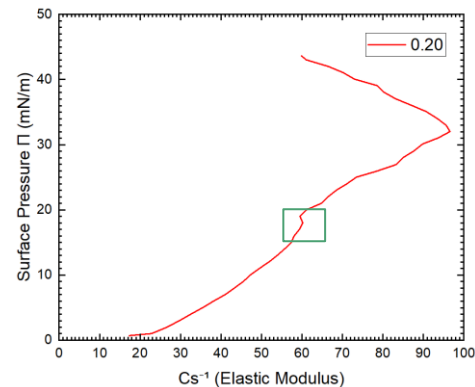
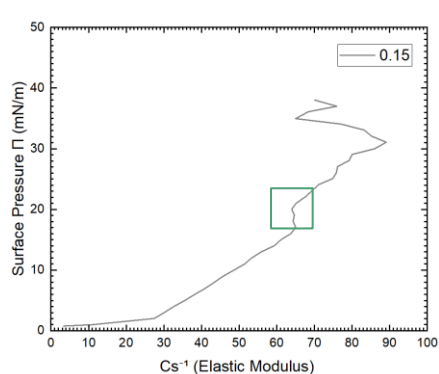


I. Concentration Effect

Isotherm Data: DMPC:DMPE w/ varied concentrations of healthy CL (18:2)4CL



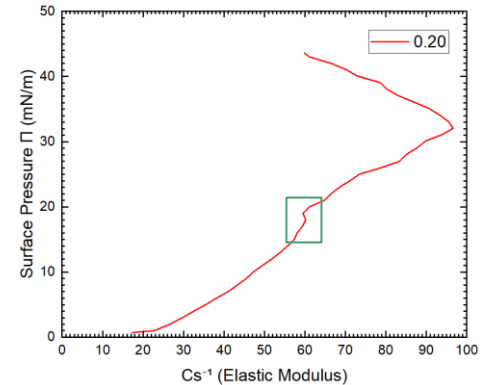
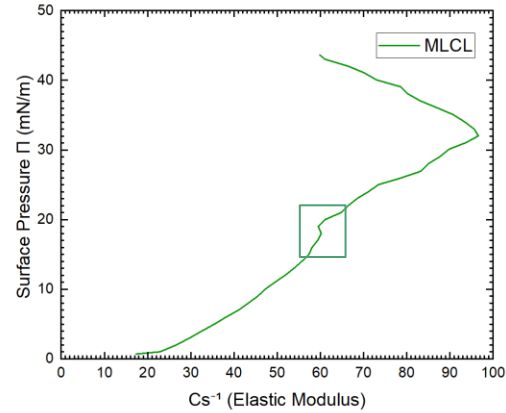
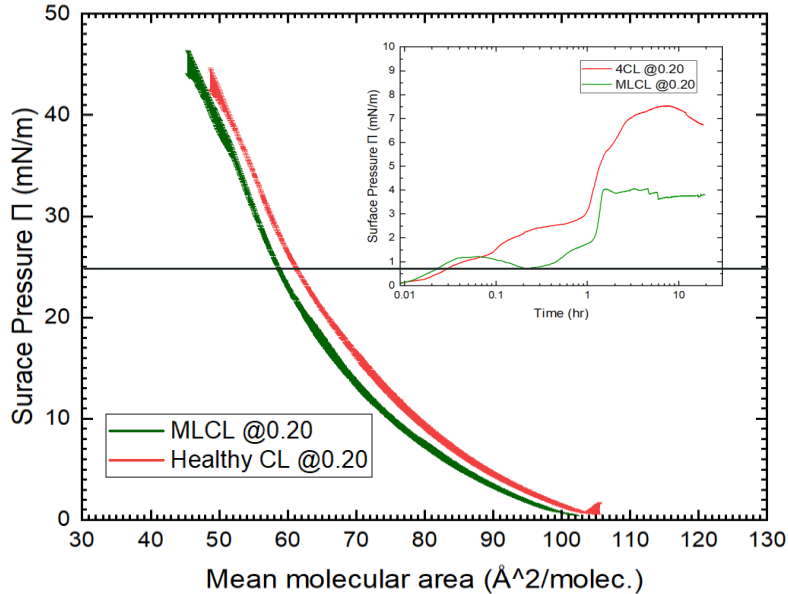
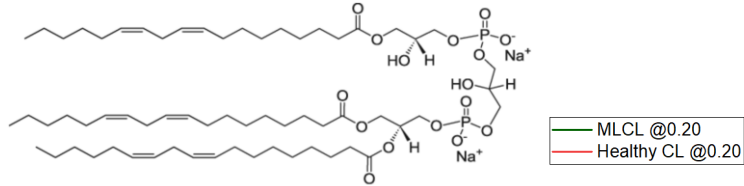
DMPC:DMPE:4CL @ 0.15, 0.20, 0.40 (mole percent)



Unable to define plateau

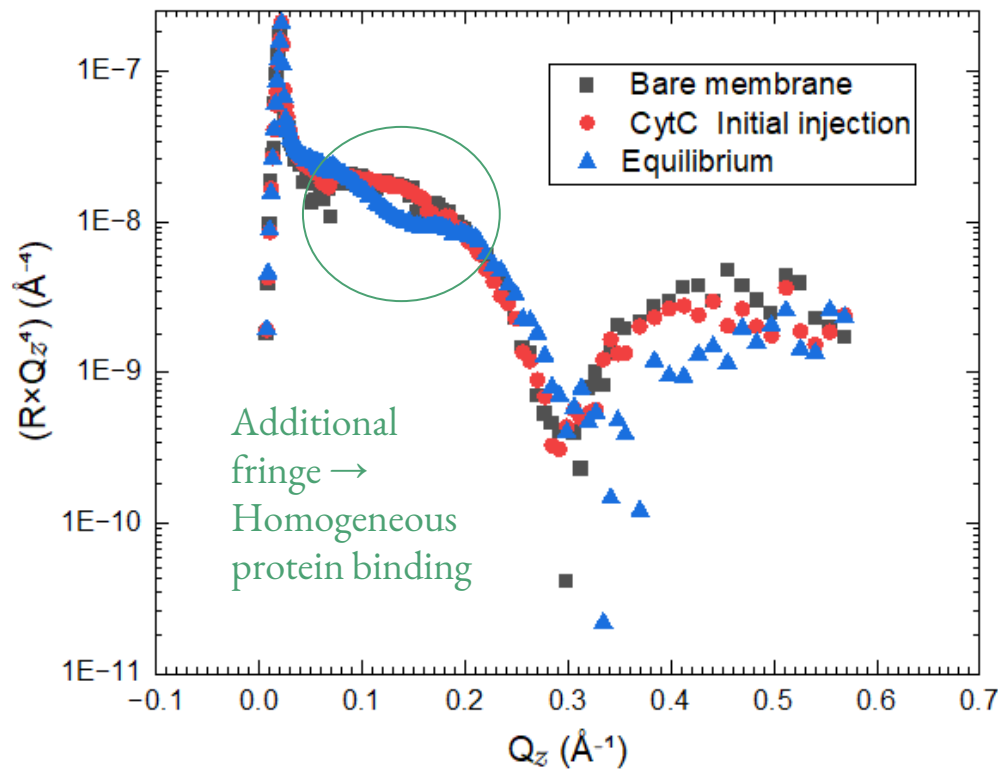
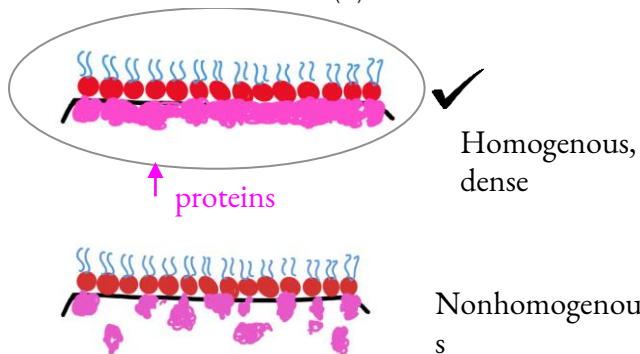
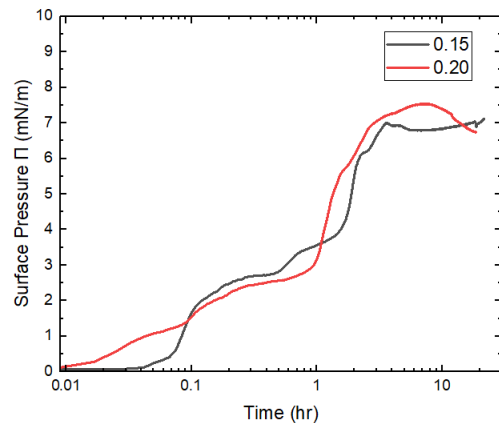
I. Effects of Structural Changes - Diseased CL

Isotherm Data: DMPC:DMPEMLCL



I. Concentration Effects - Healthy Membrane Model

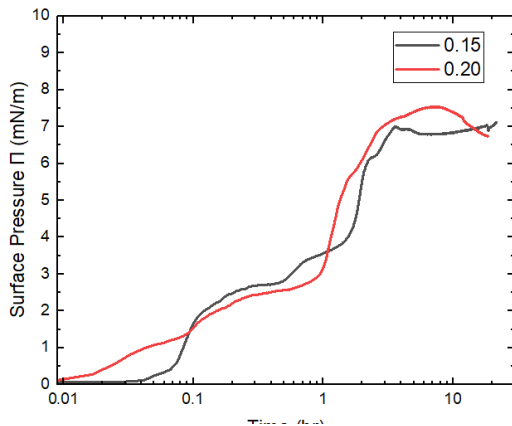
XRR Data DMPC:DMPE:4CL@0.20



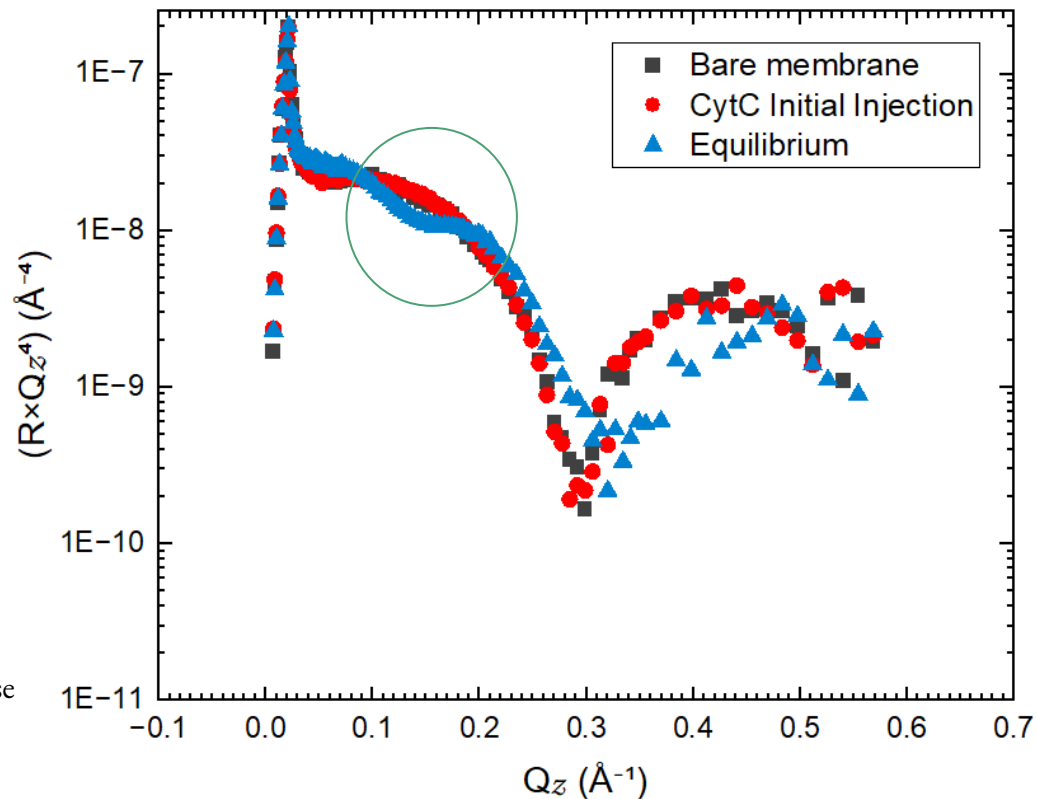
I. Concentration Effects - Diseased Membrane Model

XRR Data DMPC:DMPE:4CL @0.15

Homogeneous protein binding, but less than 0.20



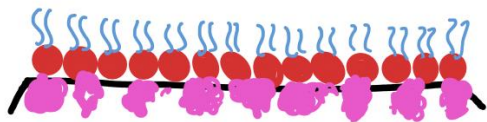
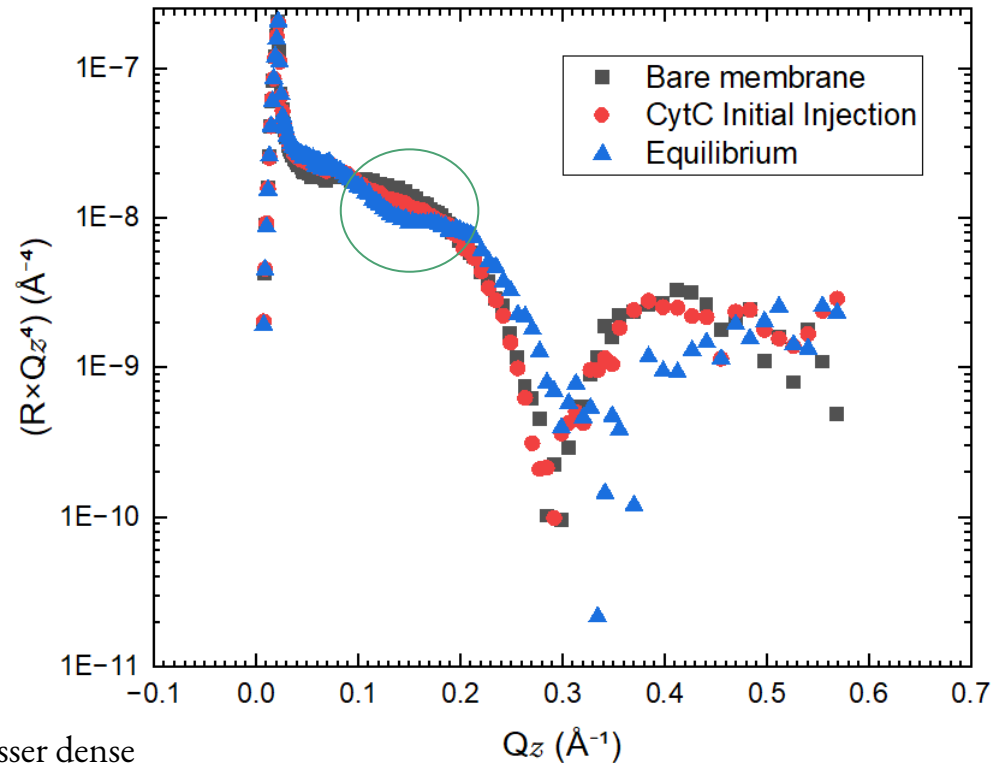
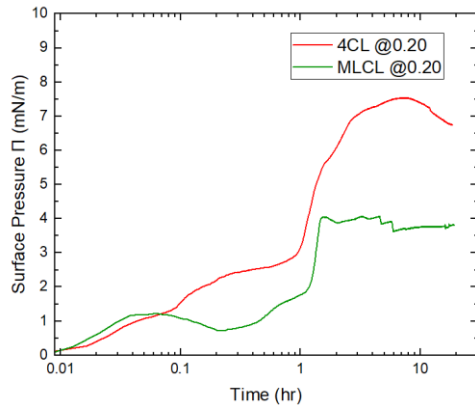
↑ proteins



I. Effects of Structural Changes - Diseased CL

XRR Data DMPC:DMPE:MLCL

MLCL associates less tightly with proteins than CL does → Less homogenous binding



Homogenous, lesser dense

Conclusion

At pressures before 25 mN/m,

- As CL concentration increases in the fabricated membrane, the phase transition is less prominent
- The MLCL- and healthy CL-containing membranes have almost identical phase behaviors

In membranes w/ lower concentration of CL mass (0.15), the binding of CytC is less homogenous than the binding of the membrane w/ 0.20 CL

- When MLCL is introduced, CytC is much less absorbed in the membrane.

How CytC binding affects the membrane structure still requires further analysis of X-ray (is the membrane in tact or disrupted?)

The decrease in CytC binding leads to the progression of metabolic diseases

Acknowledgements

Dr. Minh Phan - Mentor

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- Leland Harriger

NIST SURF - Cara O'Malley

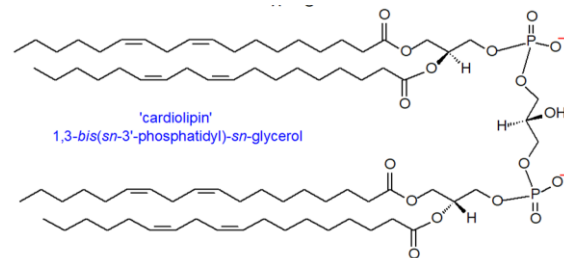
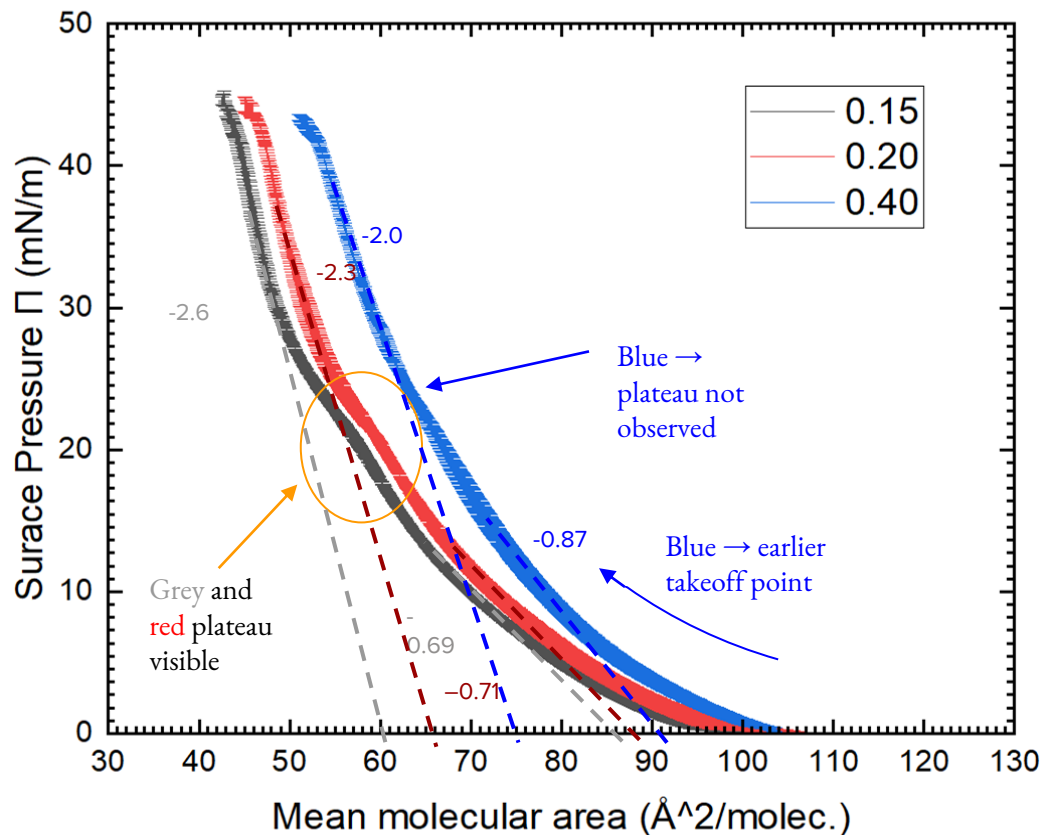


Backup Slides

I. Concentration Effect

Isotherm Data: **DP- System** w/ varied concentrations of healthy CL (18:2)₄CL

DPPC:DPPE:4CL @ 0.15, 0.20, 0.40 (mole percent)



- CL is rigid \rightarrow very dynamic and able to communicate w/ other molecules easily
- CL favors disorder \rightarrow diminishes the plateau

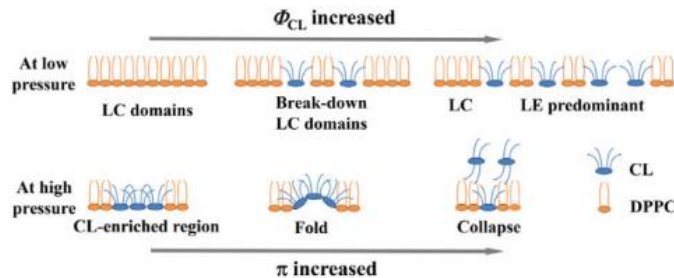
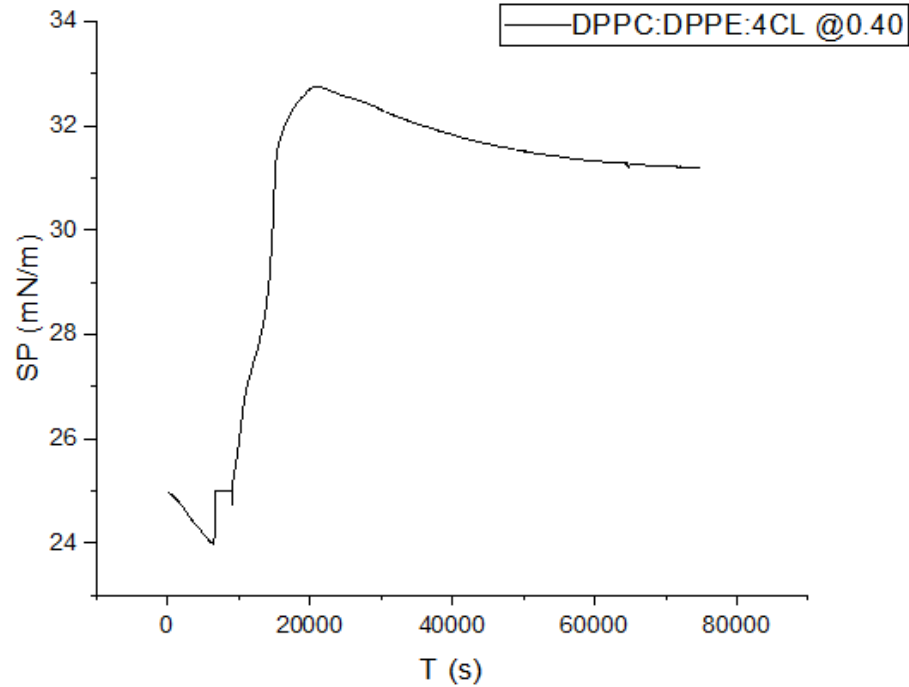


Figure 4: Phan *et al.*, *Biophys. J.* 2015, 108, 1977

Adsorption Assay: DPPC:DPPE:4CL (0.40 concentration)

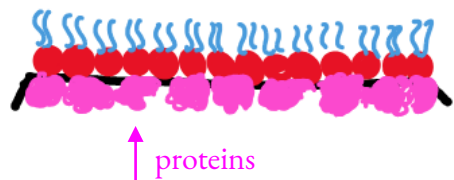


XRR Data DPPE:DPPE:4CL@0.20

$$Q \sim 2\pi/d$$

Left or right shift of Kiessig fringe shows us change in thickness of membrane

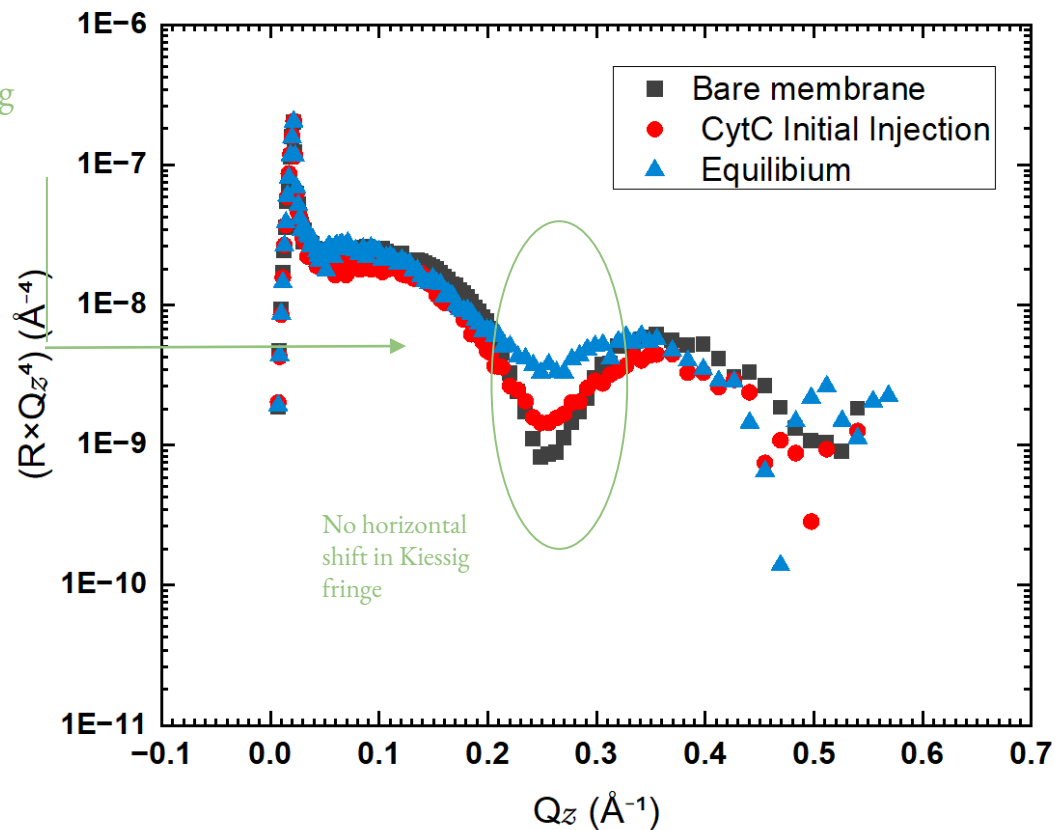
The less pronounced the Kiessig fringe is at equilibrium, the more rough our membrane is
→ CytC bounded less homogenous



Homogenous



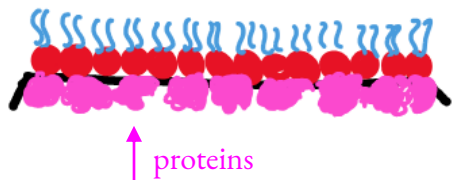
Less homogenous



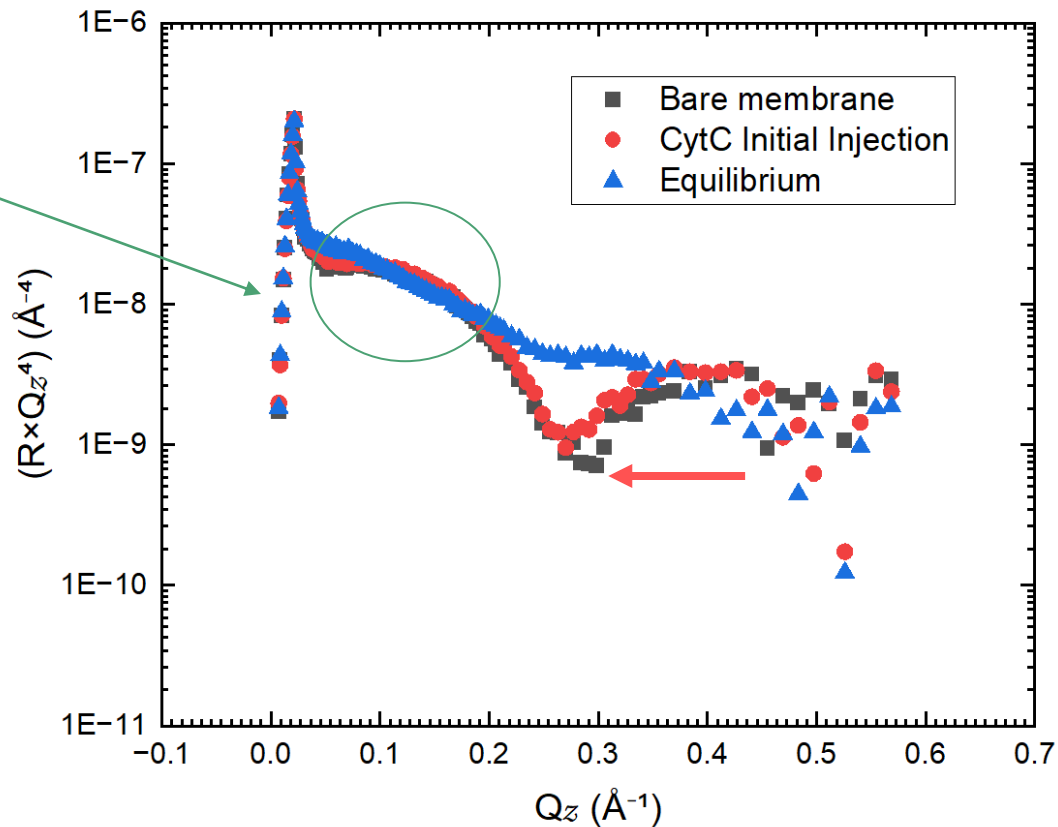
XRR Data DPPE:DPPE:4CL@0.40

Additional oscillation/fringe present \rightarrow CytC formed homogenous layer under membrane,

Red shifted left \rightarrow thickness of membrane increased
- More thick, better binding of protein

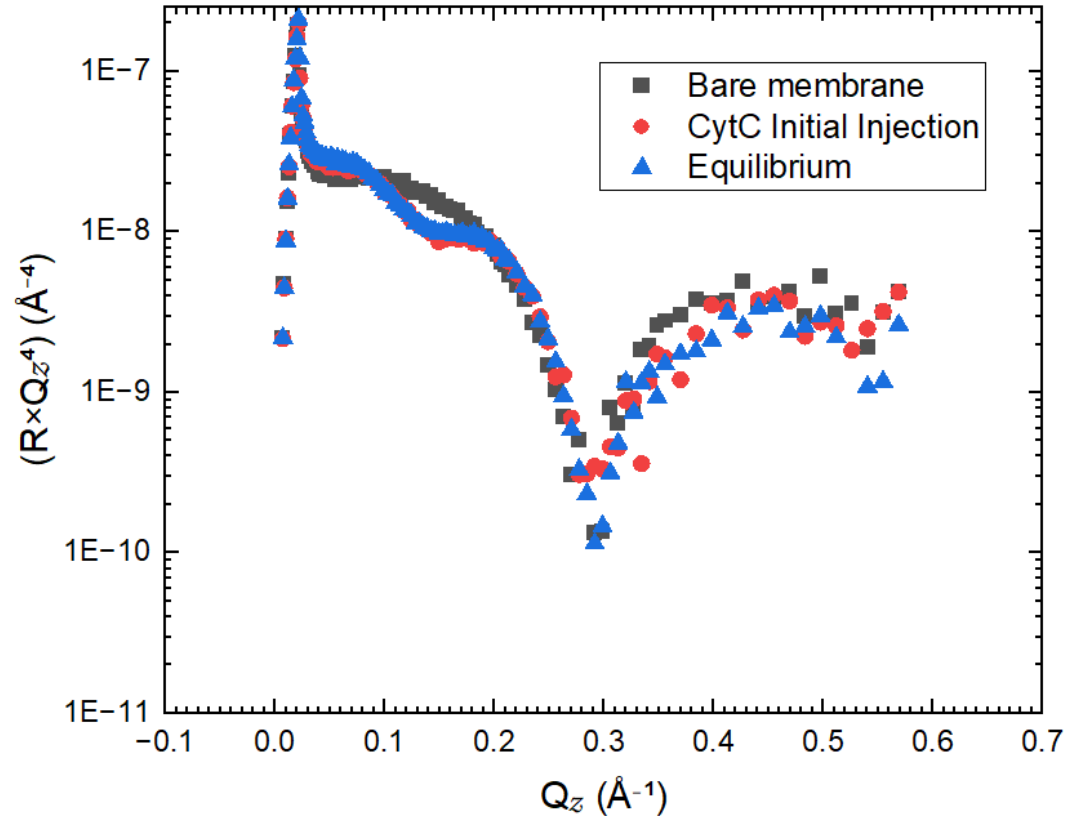


Homogenous



XRR Data DMPC:DMPE:DMPG

Homogenous protein binding in
in initial injection stage and
equilibrium



Isotherm Data: **DM System** w/ control (DMPG)

DMPC:DMPE:DMPG

