

Lipid Corralling and Poloxamer Squeeze-out in Membranes

Ka Yee C. Lee

*Department of Chemistry, the Institute for Biophysical Dynamics & the James Franck Institute
The University of Chicago*

Workshop on Membrane Science
Argonne National Laboratory
Argonne, Illinois
August 18, 2004

LOSS OF MEMBRANE STRUCTURAL INTEGRITY

Electrically shocked cells suffer a loss of structural integrity



Electrically shocked cell

CF RELEASE FROM RAT SKELETAL MUSCLE CELLS

Shock

200 V/cm

Single 4 ms pulse at $t=5$ min

Results

Slope of dye loss approaches pre-shock state (slope ~ 0) *only for*

P188-treated cells

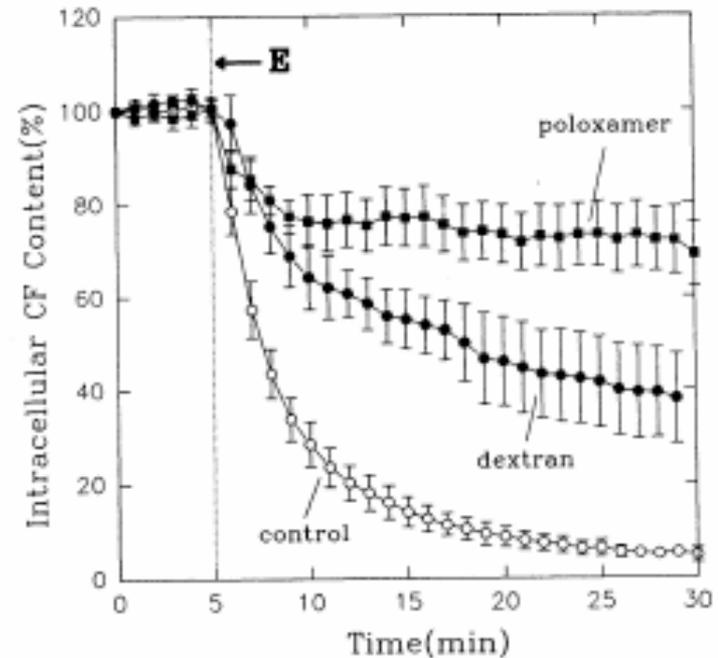


FIG. 2. CF release from isolated $1000 \times 30 \mu\text{m}$ (average length \times diameter) rat flexor digitorum brevis skeletal muscle cells ($n = 6$) after a single 200 V/cm, 4-ms-duration field pulse. The cells were preincubated in Ca^{2+} -free PBS with 1.5 mM MgCl_2 /25 mM HEPES buffer/20 μM CFDA for 2 hr. The cells were then incubated in the same solution without CFDA for 30 min, transferred to a custom microscope stage chamber (6) containing the buffered Mg^{2+} PBS, and then exposed to field pulse. The field pulse (E) was delivered at $t = 5$ min. The intracellular CF was excited at 480 nm and the emission at 520 nm was quantified by digital imaging processing methods as described (9). The procedure was repeated with PBS supplemented with either 8 mg of neutral dextran per ml (10.1 kDa; Sigma) or 8 mg of P188 per ml (BASF).

RESPONSE DEPENDS ON TIME OF P188 ADMINISTRATION

Shock

150 V/cm

Sixty 4 ms pulse every 10 s

Resistivity Results

Control

$49.9 \pm 3.6\%$

Saline control

$39.0 \pm 3.0\%$

Dextran control

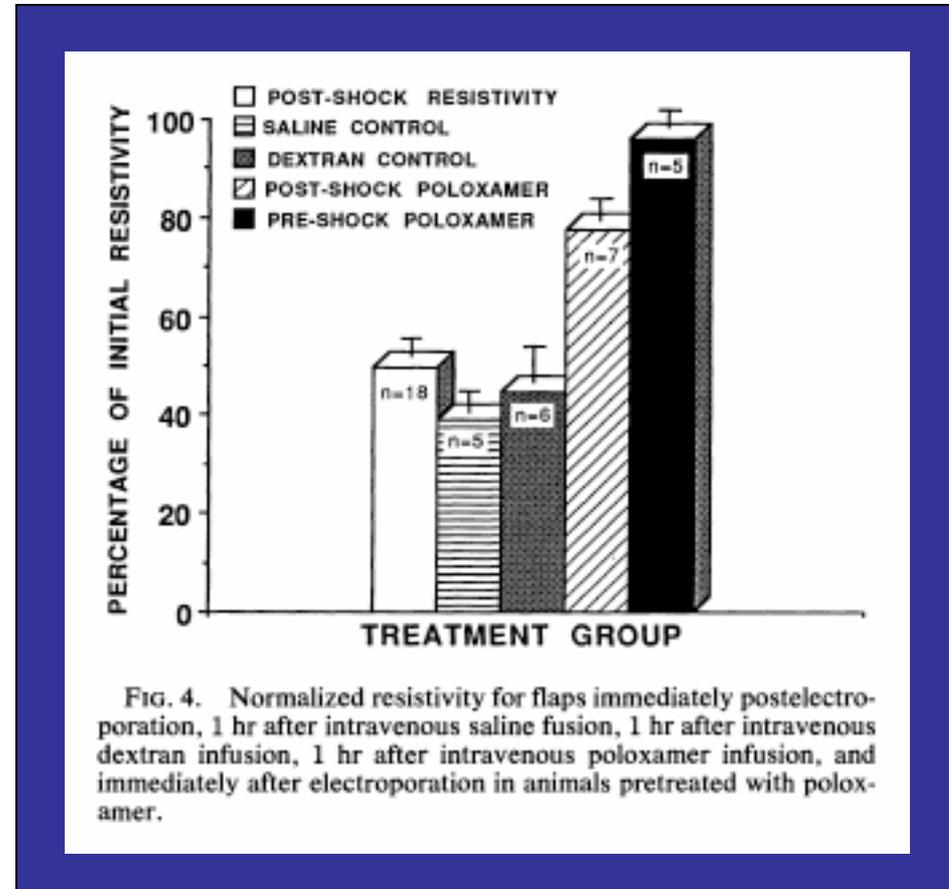
$44.8 \pm 8.3\%$

Post-shock P188

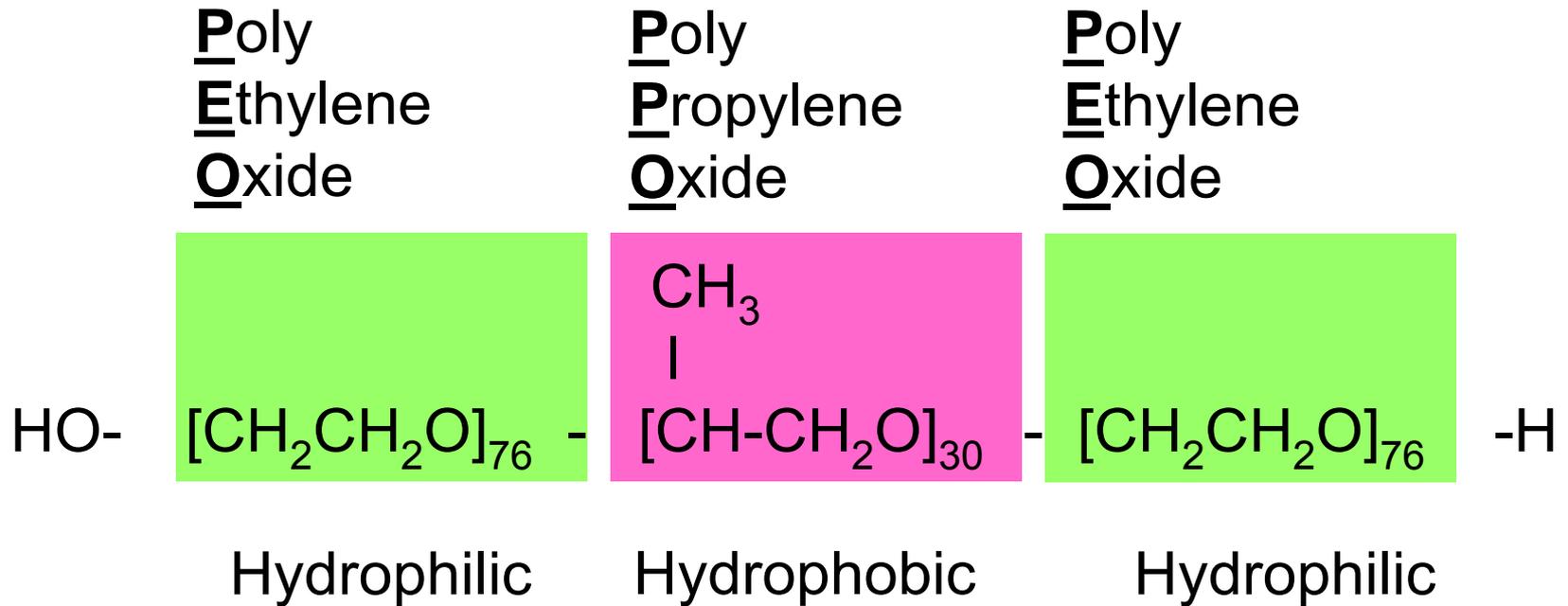
$77.4 \pm 4.8\%$

Pre-shock P188

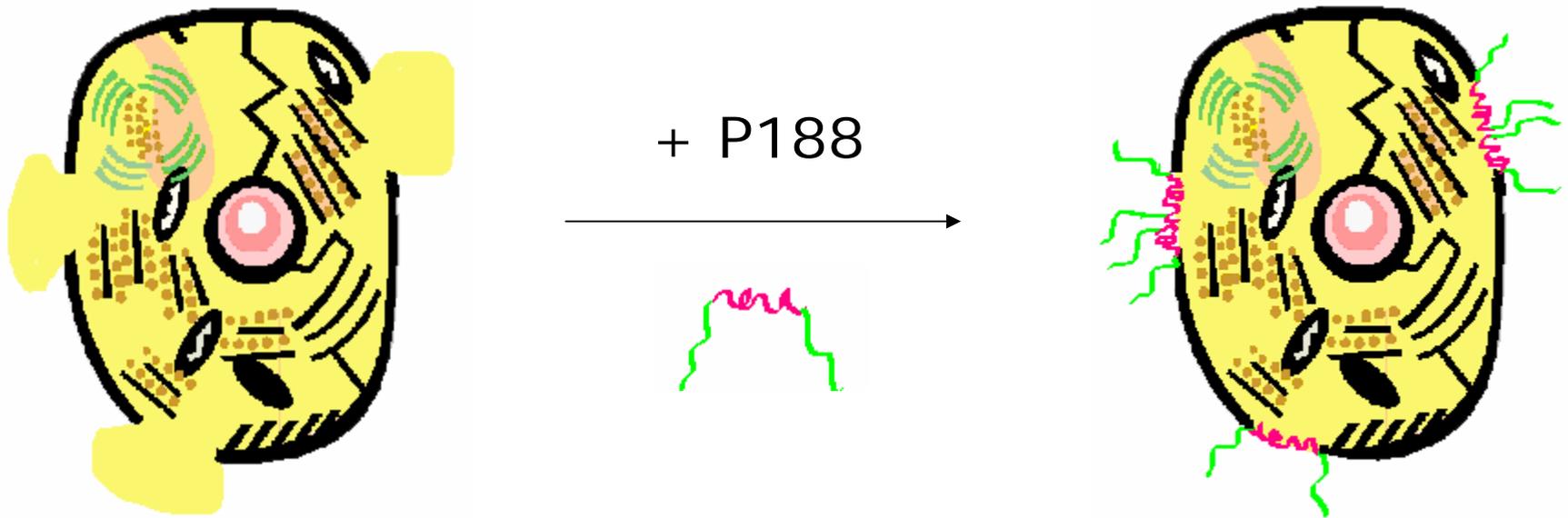
$96.3 \pm 1.8\%$



TRIBLOCK COPOLYMER P188



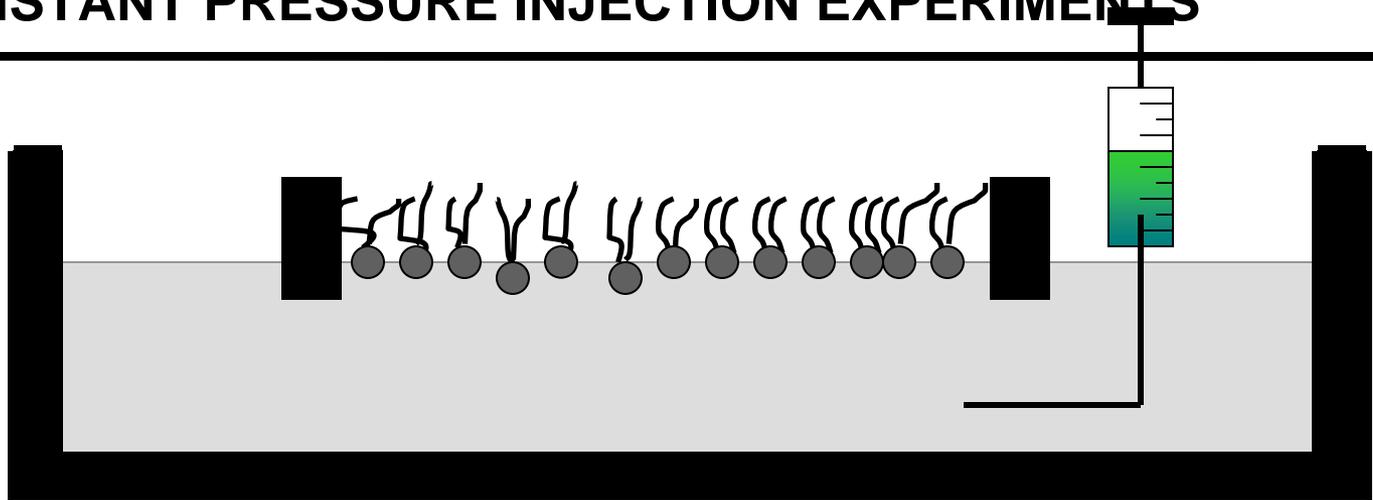
COPOLYMER SURFACTANT AS MEMBRANE SEALANT



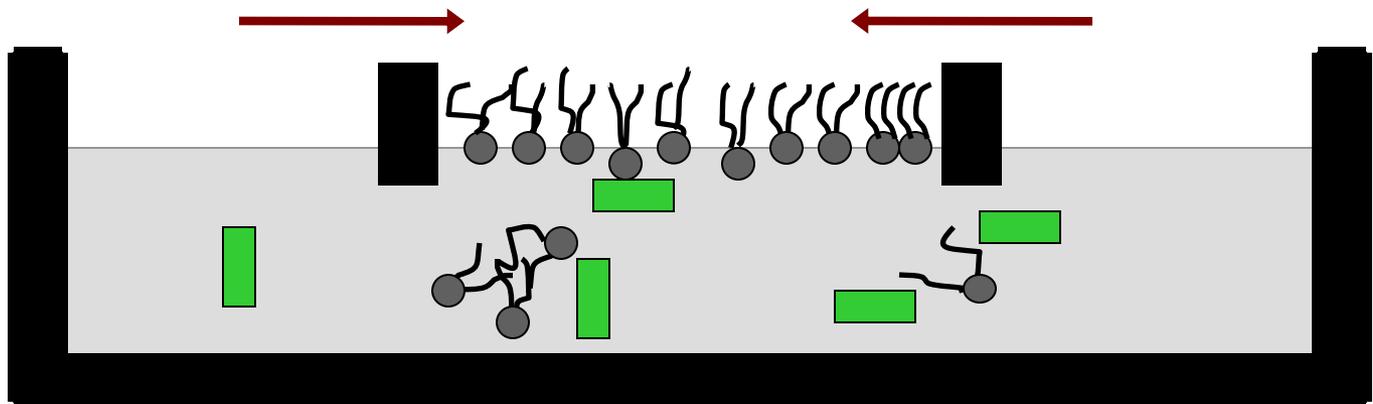
QUESTIONS TO BE ADDRESSED

- How does P188 interact with the cell membrane?
 - Does P188 insertion affect membrane lipid packing?
 - Is the sealing effect localized?
 - What is the fate of the polymer as the cell heals?
 - Effective treatment strategies? Designer polymers?
-
- How membrane packing regulates P188 insertion
 - How lipid electrostatics influence P188 insertion
 - Morphological changes using FM
 - High resolution imaging using AFM, TEM, Cryo EM
 - High resolution structural data by X-ray and neutron scattering

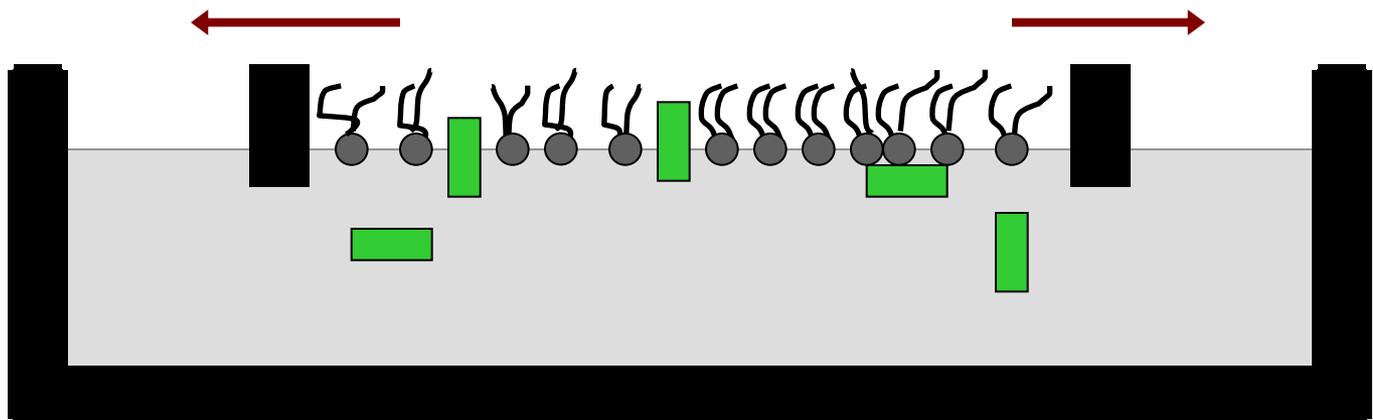
CONSTANT PRESSURE INJECTION EXPERIMENTS



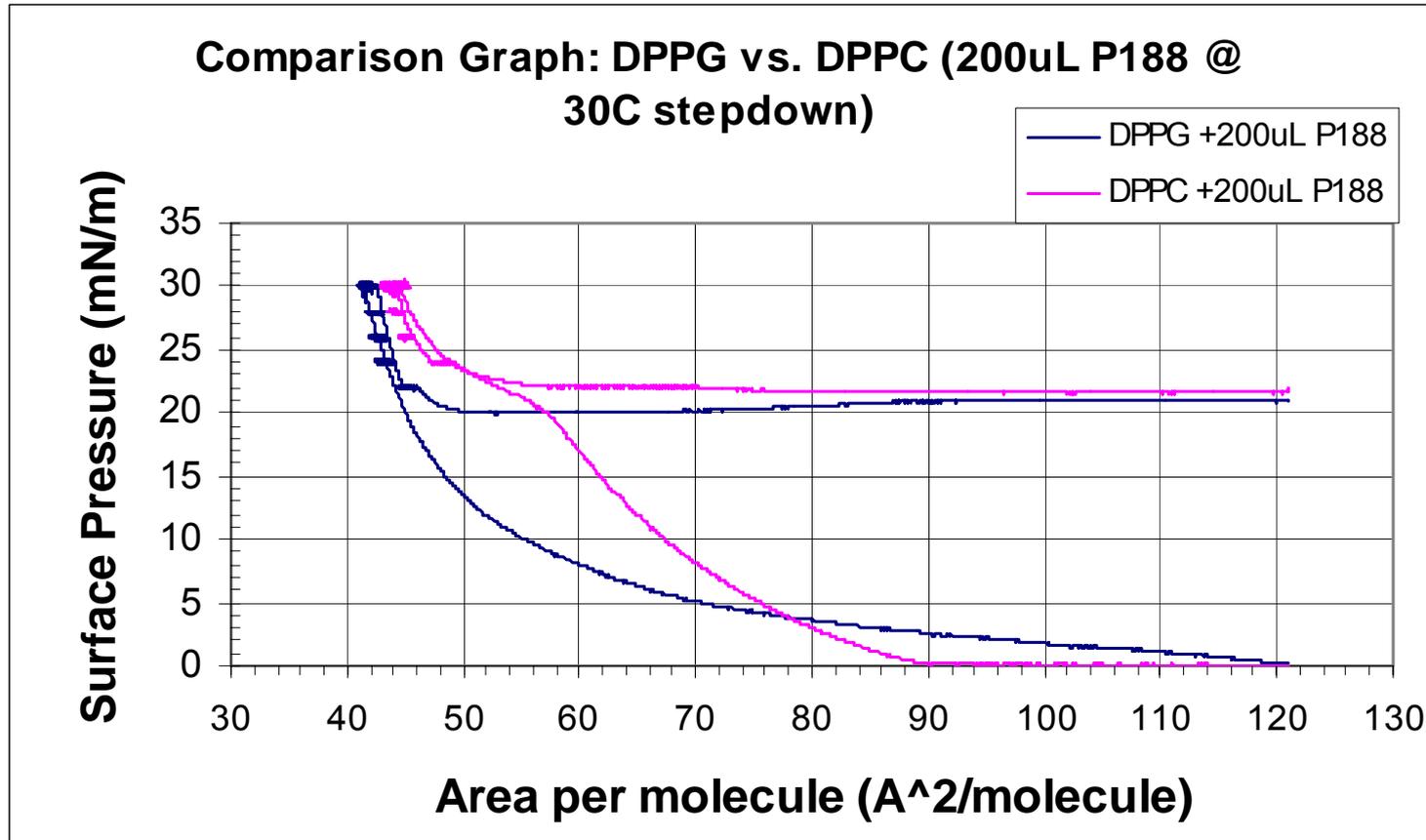
Desorption



Insertion

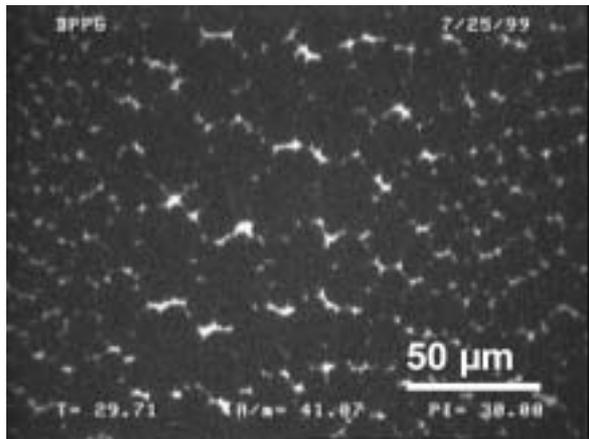


P188 INSERTION REGULATED BY LIPID PACKING DENSITY



DPPC + P188
DPPG + P188

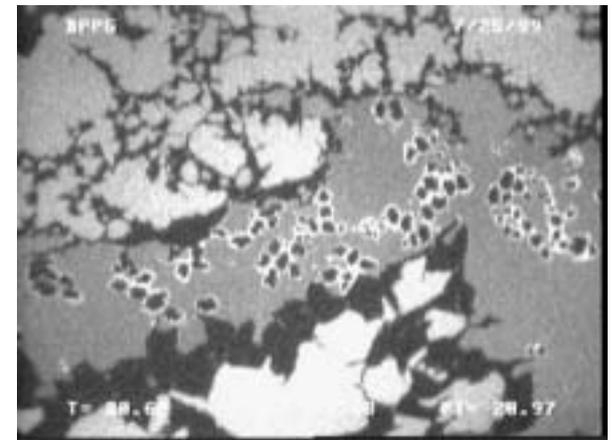
P188 INSERTION INTO DPPG MONOLAYERS



Pure **DPPG** at 30 mN/m
before P188 injection



DPPG + P188 at 20 mN/m
Area/ PG molecule = 78 \AA^2



DPPG + P188 at 20 mN/m
Area/ PG molecule = 120 \AA^2

CONSTANT PRESSURE EXPERIMENTS

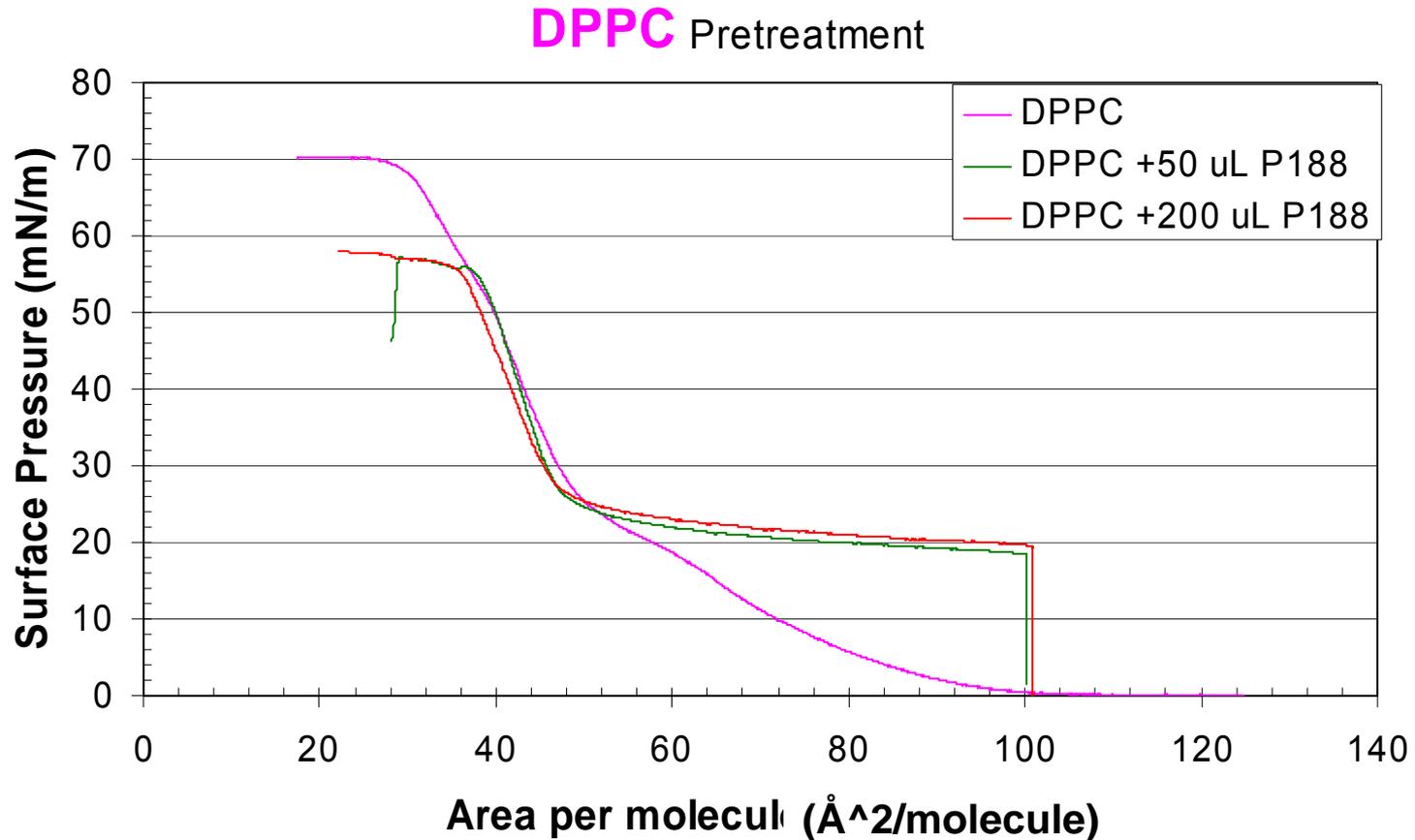
- P188 insertion insensitive to headgroup charge
- inserts into DPPC and DPPG monolayers at $\pi < 24$ mN/m
- only inserts into location where the lipid packing density has been lowered
- only adsorbs into damaged regions of cells
- does not non-specifically attach or interfere with healthy membranes
- insertion results in disordered structure of the membrane

What is the fate of the polymer as the cell heals?

PRETREATMENT EXPERIMENTS WITH DPPC MONOLAYERS

P188 is added before the lipid monolayer is compressed

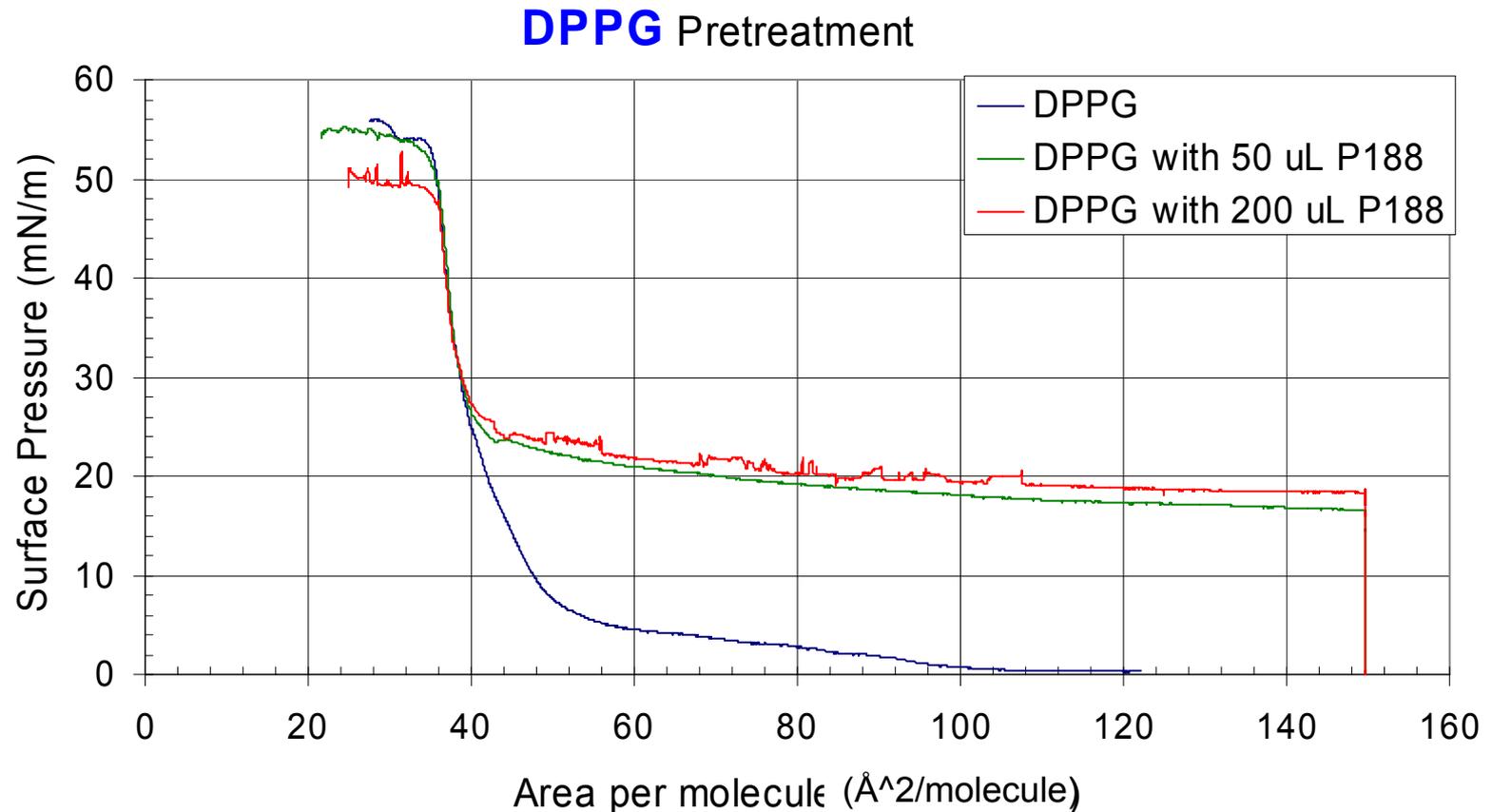
- initial lipid surface density is low
- mimics the condition of the existence of a pore



PRETREATMENT EXPERIMENTS WITH DPPG MONOLAYERS

P188 is added before the lipid monolayer is compressed

- initial lipid surface density is low
- mimics the condition of the existence of a pore

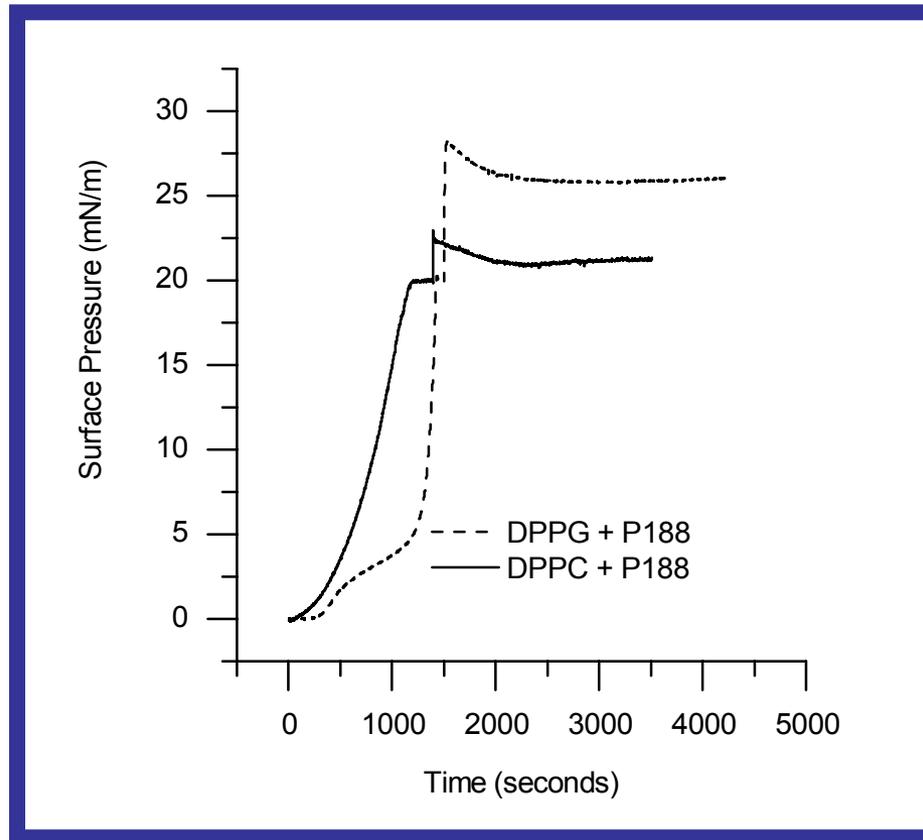


PRETREATMENT (PORE MIMICKING) EXPERIMENTS

- P188 is surface active and resides at the interface at low surface pressures (I.e. low surface lipid density)
- insertion pressure is similar to the equilibrium spreading pressure of P188
- polymer is “squeezed out” of the lipid layer when $\pi > 26$ mN/m
- a possible mechanism for it to leave the membrane surface when cell heals
- polymer will not interfere with the process of membrane healing
- no morphological information - black-out upon P188 adsorption to interface (will return to this later)

Does P188 insertion affect membrane lipid packing?

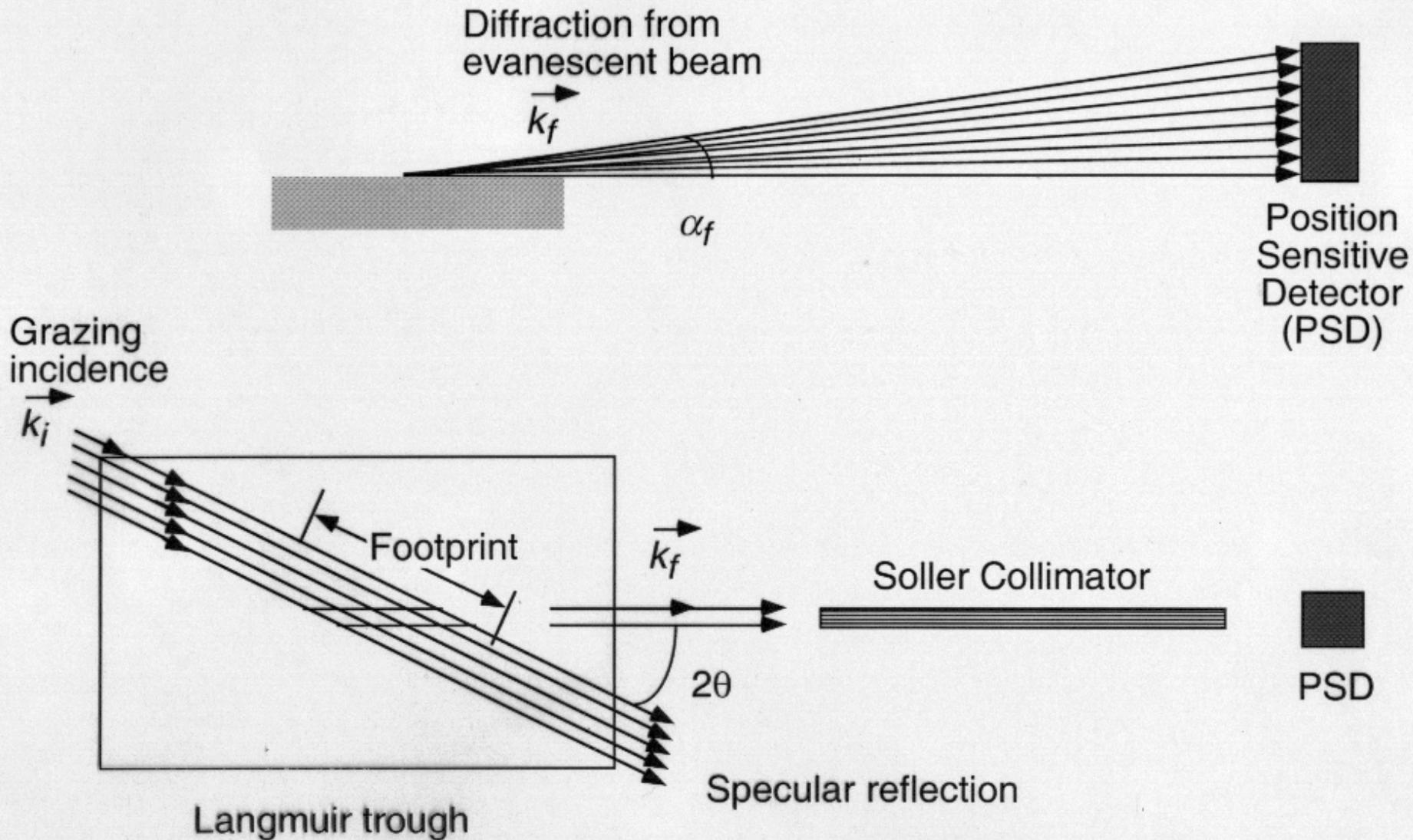
CONSTANT AREA EXPERIMENTS



- P188 enhances the packing density of lipid by inserting into the monolayer
- raises the surface pressure of the system

How does poloxamer insertion
lead to sealing?

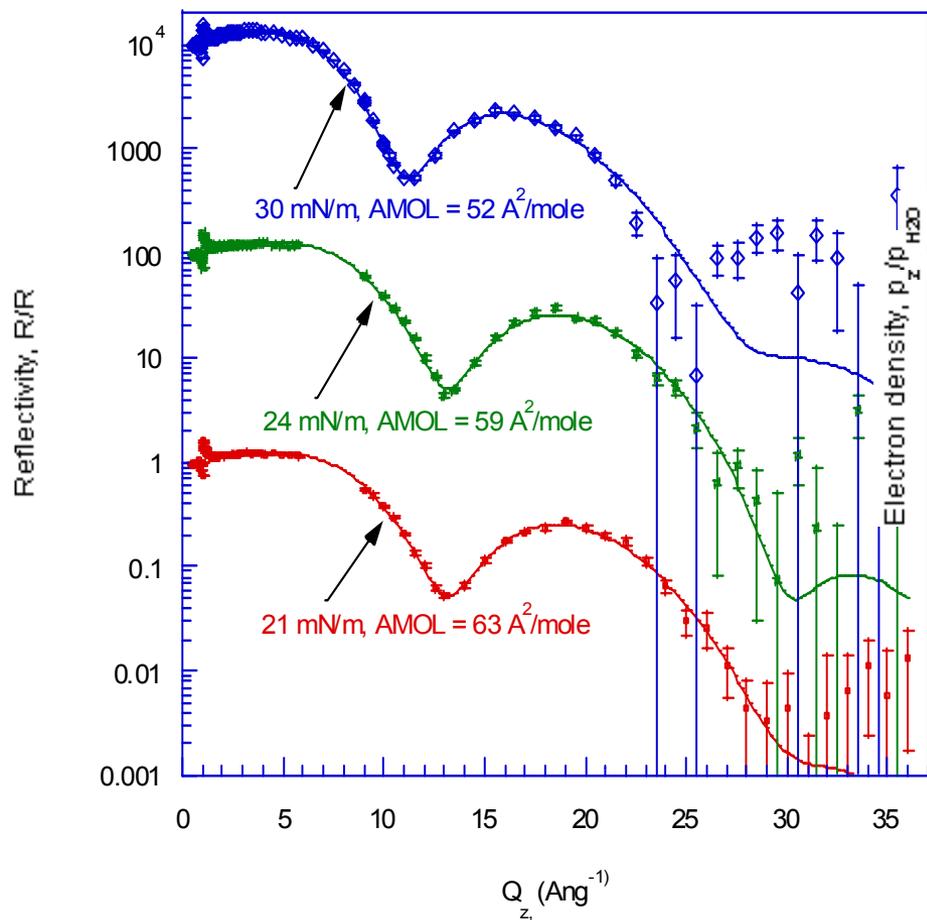
SCATTERING GEOMETRY



PURE DPPC MONOLAYER

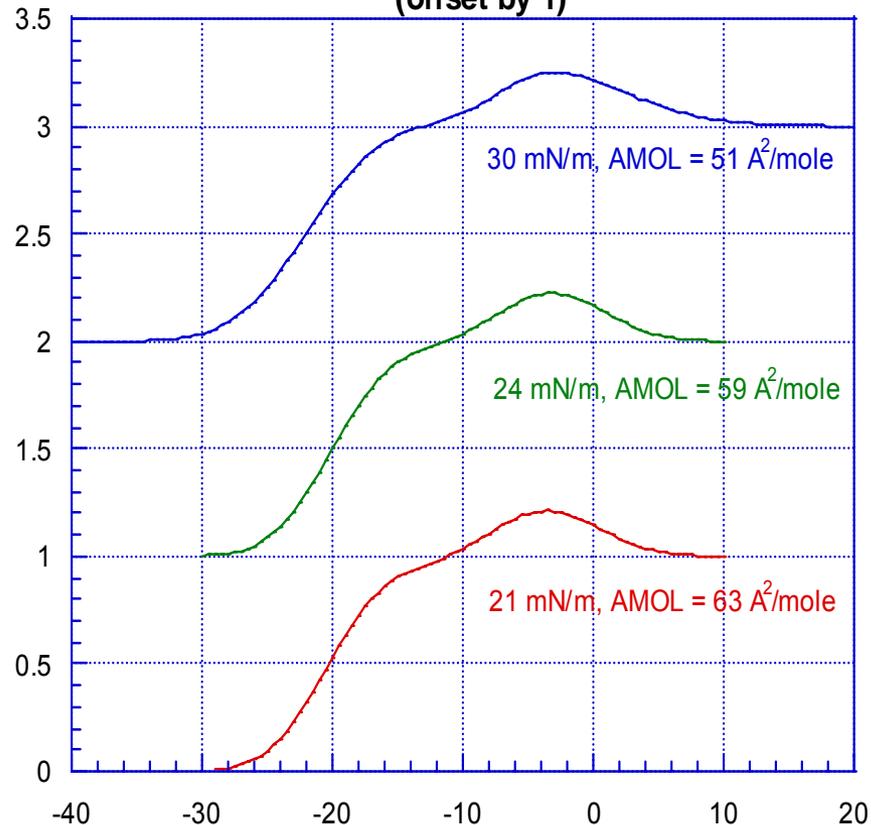
Reflectivity of DPPC

DPPC @ 30 °C on pure H₂O subphase
(offset by 100)

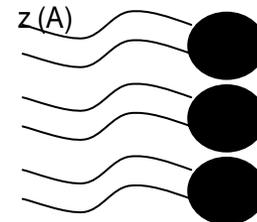


Electron density profile of DPPC

DPPC @ 30 °C on pure H₂O subphase
(offset by 1)

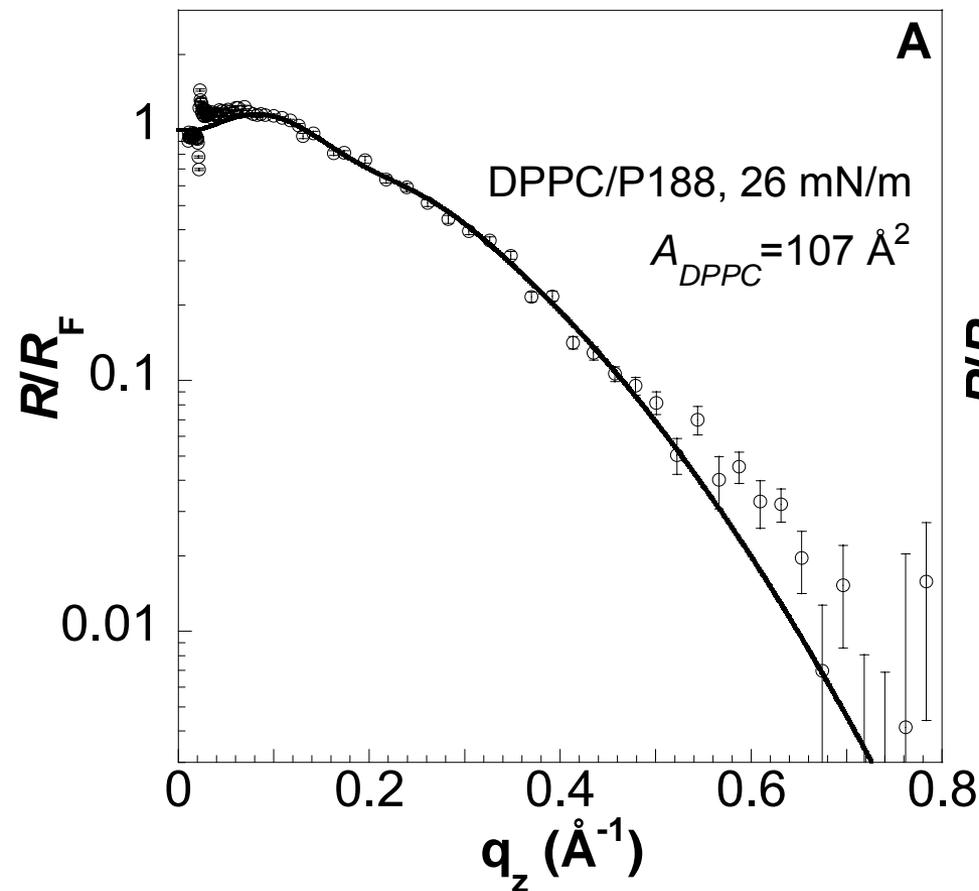


Surface pressure increases → molecular tilt decreases

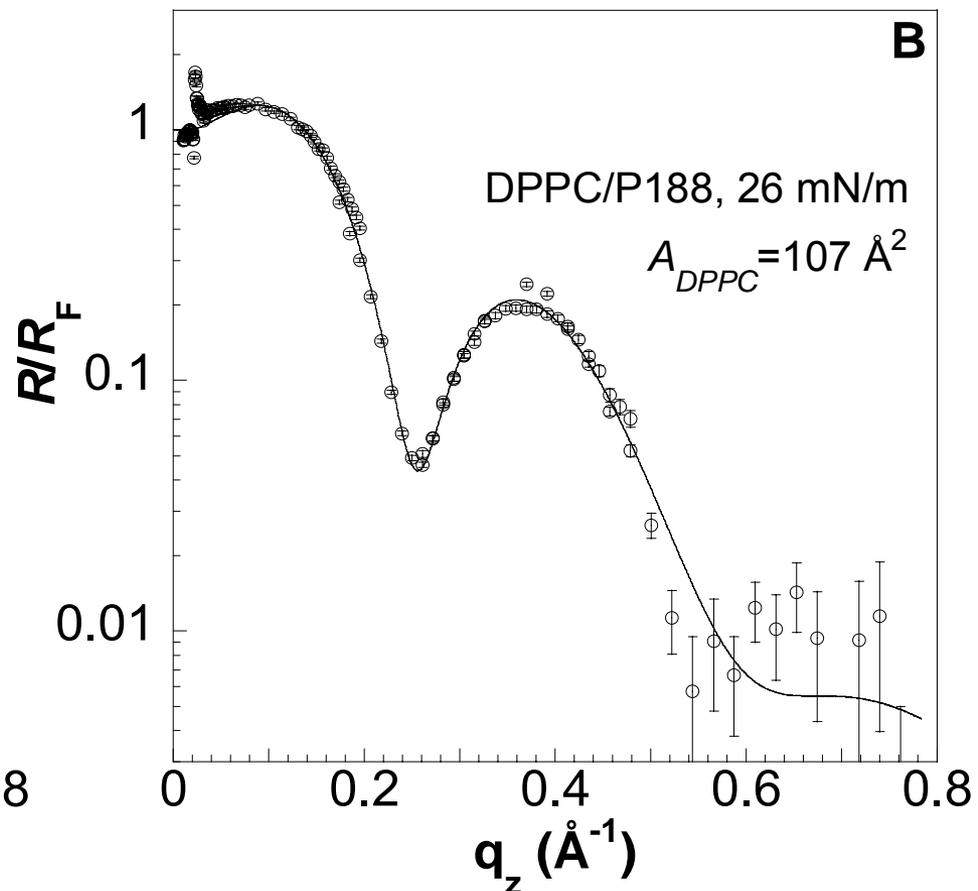


P188 Injection at High Surface Area - Surface Inhomogeneity Observed

P188-rich portion



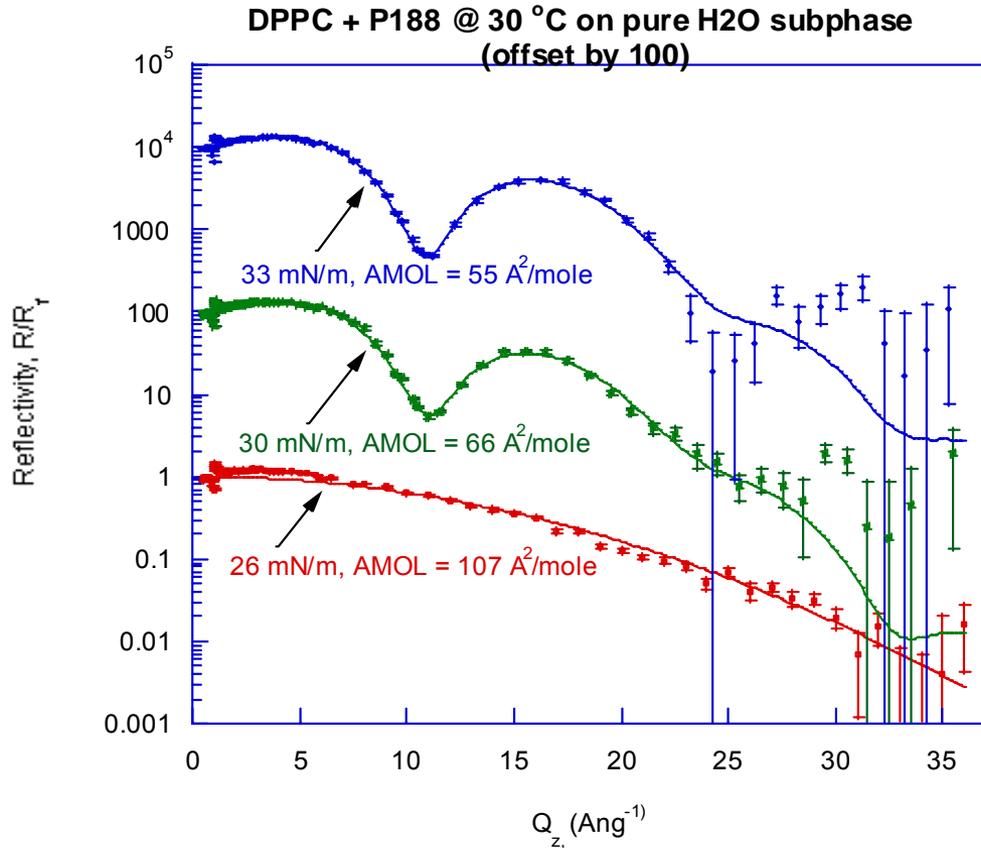
DPPC-rich portion



Surface inhomogeneity corroborates poloxamers' "corralling" effect showing that P188 forces DPPC to form a phase separated from the poloxamer

DPPC + P188 @ VARIOUS SURFACE PRESSURES (PRETREATMENT)

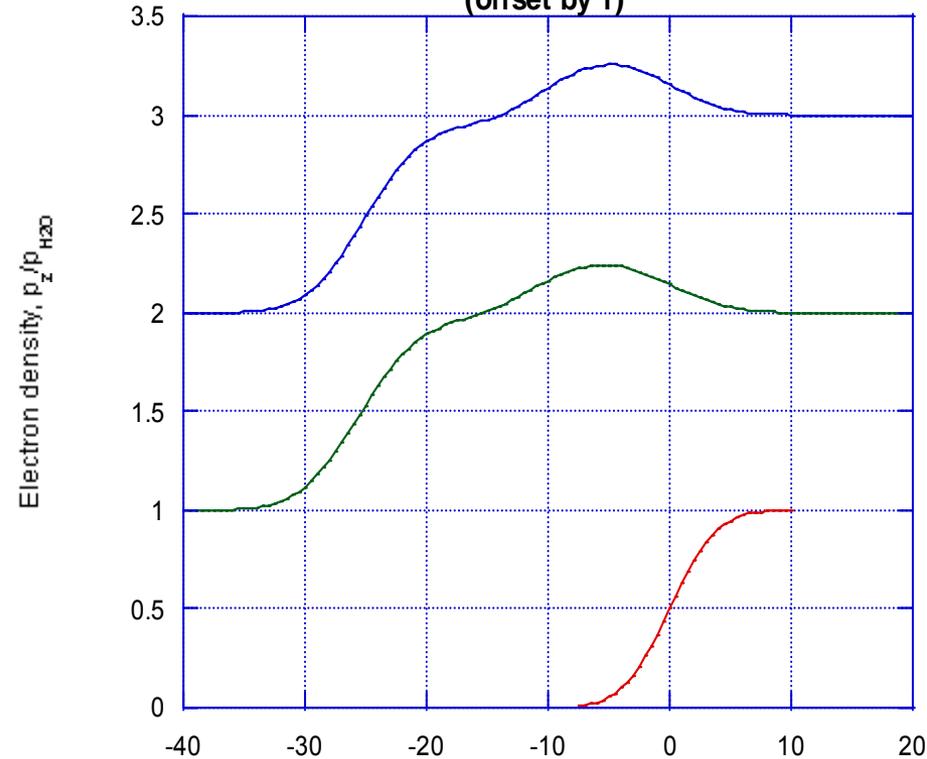
Reflectivity of DPPC+P188



Electron density profile

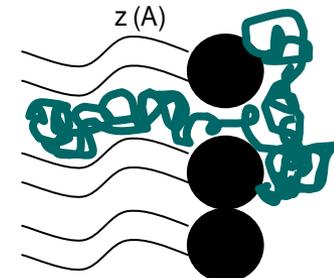
of DPPC+P188

DPPC+P188 @ 30 °C on pure H₂O subphase
(offset by 1)



High area/molecule → surface structure dominated by P188

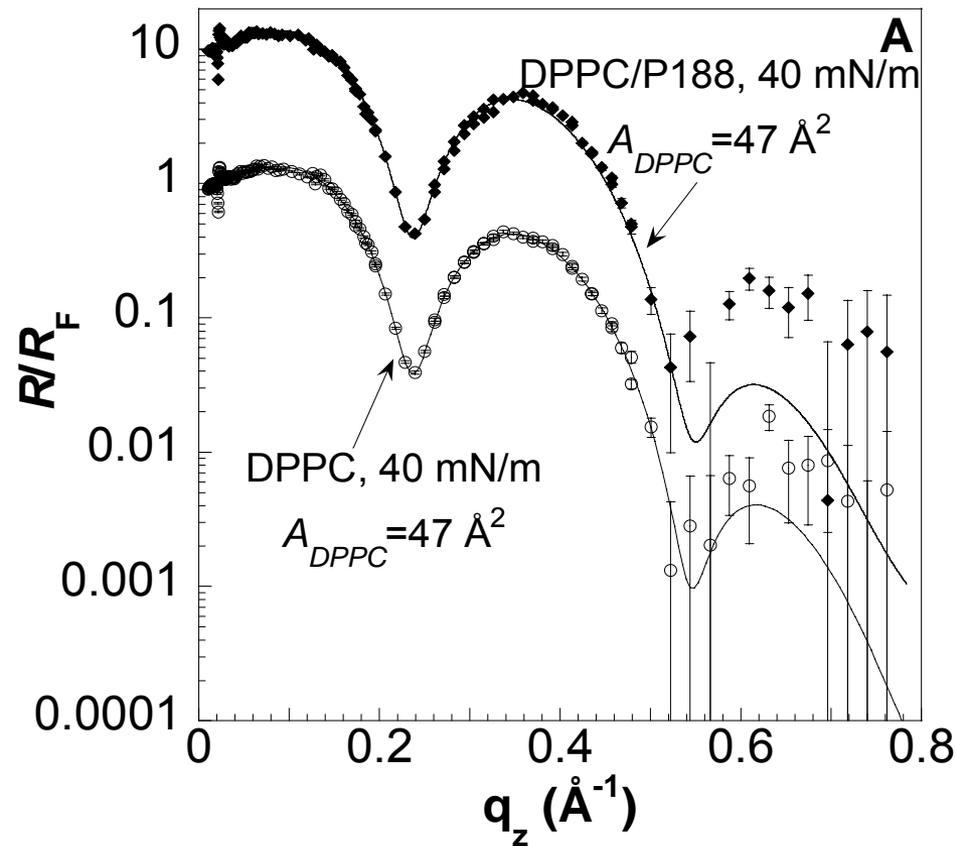
Low area/molecule → structure reverted to that of DPPC



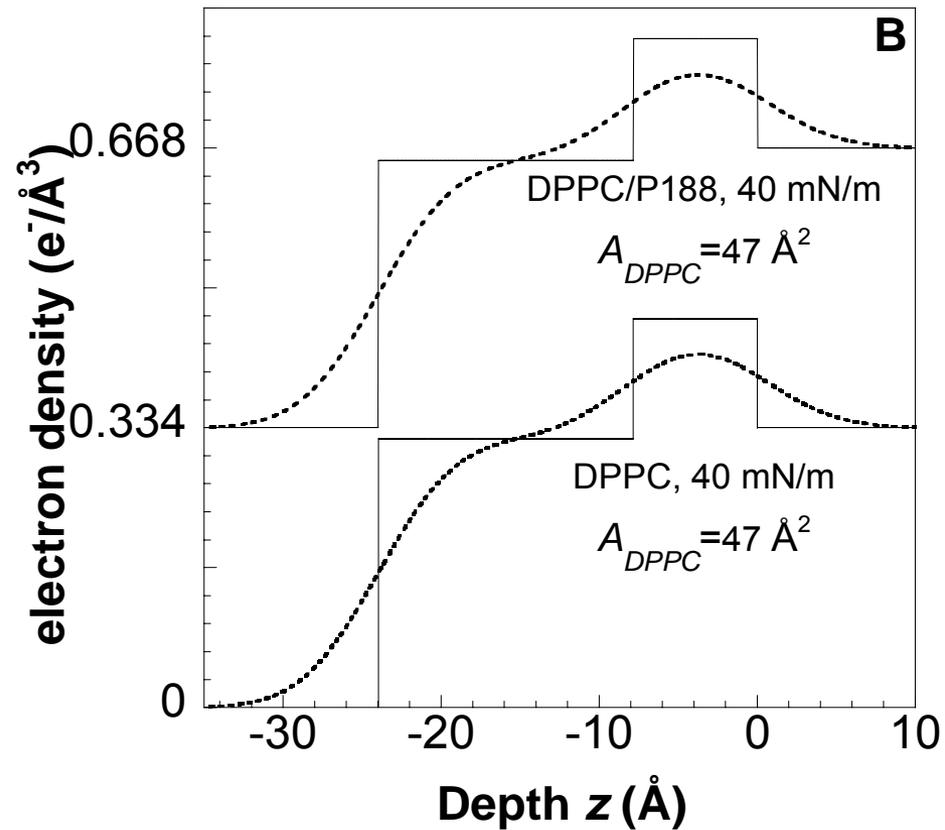
P188 is squeezed out beyond a given surface pressure!!

ELECTRON DENSITY PROFILE RESTORED AT HIGH PRESSURES

XR of DPPC & DPPC/P188

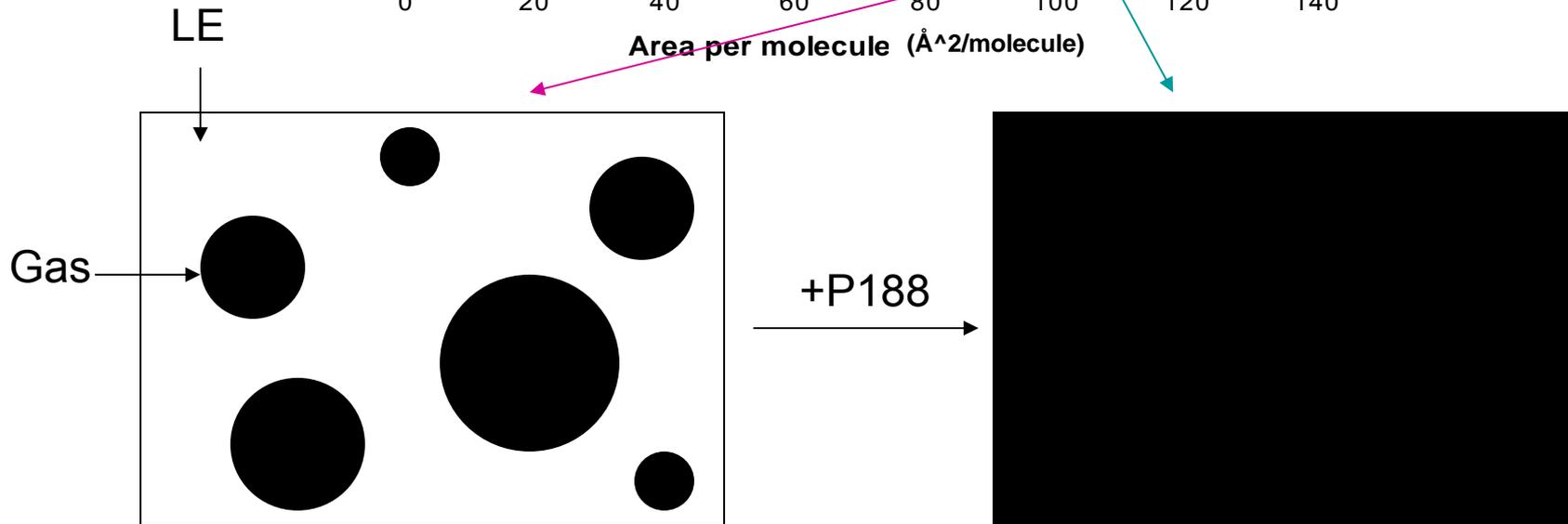
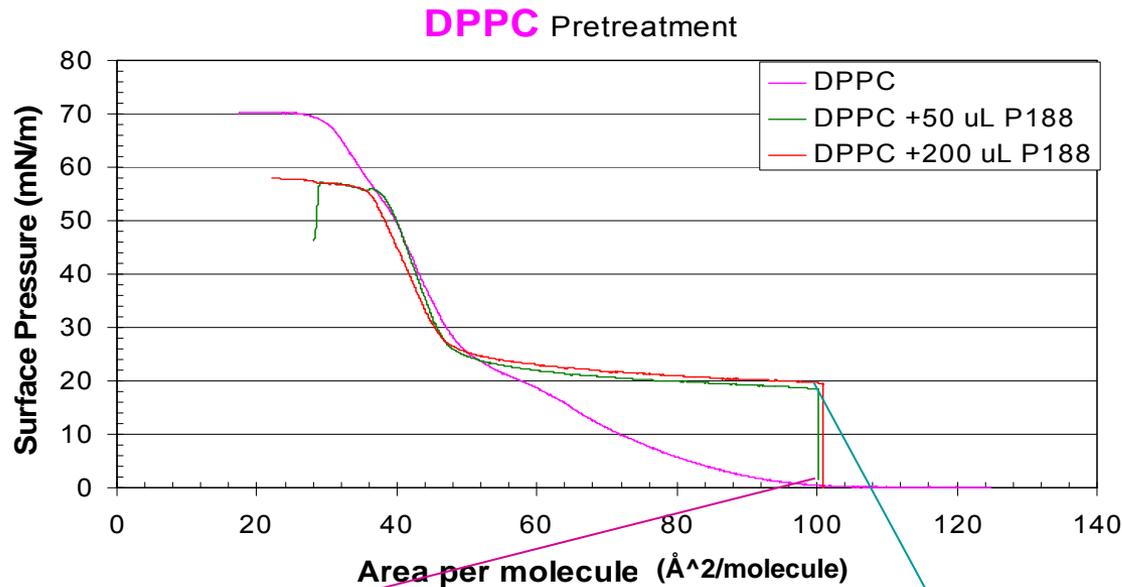


Electron density profile



XR of DPPC & DPPC/P188 films at 40 mN/m are identical, confirming P188 removal
Surface pressure $> \pi_{SO}$, P188 is squeezed out of the lipid film

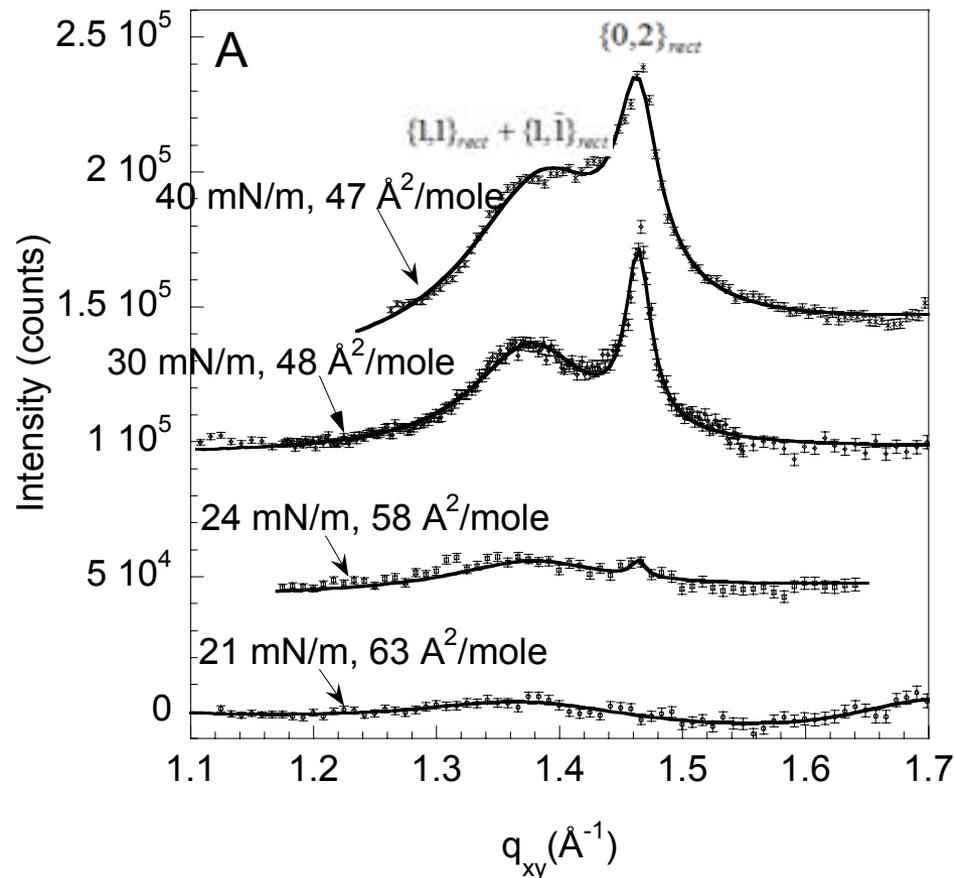
BLACK-OUT IN PRETREATMENT EXPERIMENT (REVISTED)



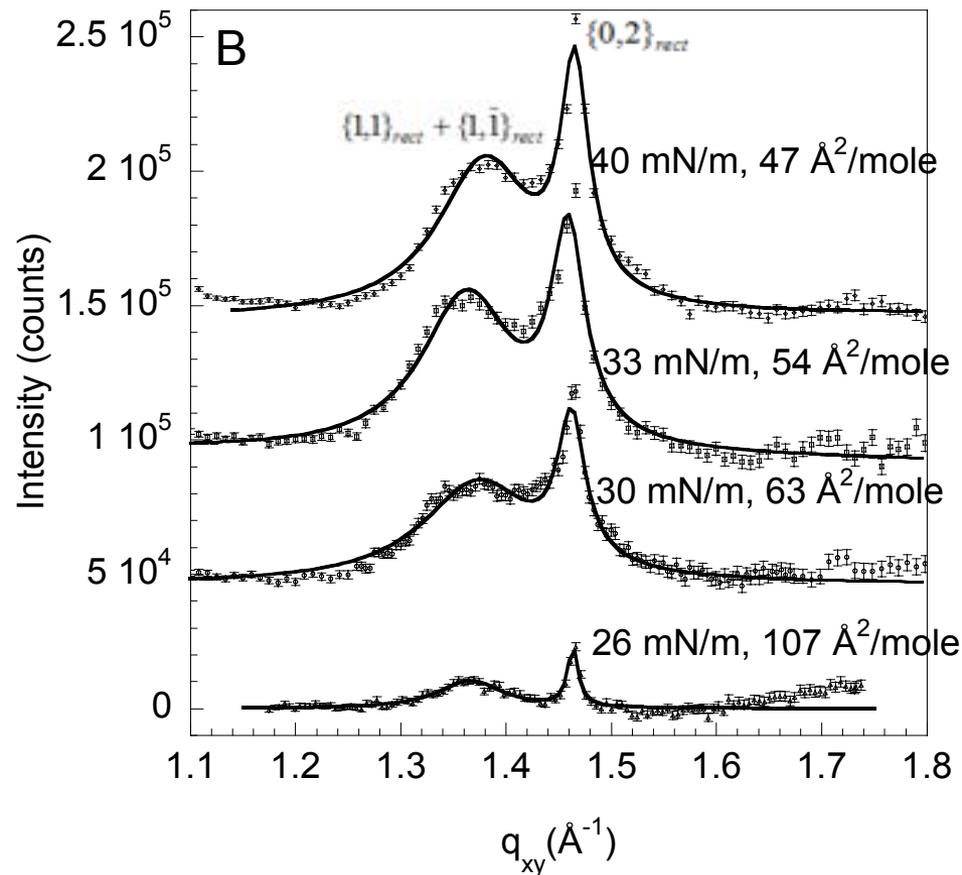
What is the fate of the lipid molecules upon the addition of P188?
Phase separated? Homogeneously mixed?

CORRALLING OF LIPID MOLECULES BY P188

Bragg peaks of DPPC



Bragg peaks of DPPC/P188



P188 corrals the lipid molecules together to form packed domains even at low surface lipid density!!

X-RAY – CONCLUSIONS

- Incorporation of P188 under pore-mimicking conditions results in surface inhomogeneity
- When surface pressure is increased beyond a certain point, XR results show P188 is squeezed out of tail region
- Even at high area per molecule (disordered phase for pure lipids), the presence of P188 corrals lipids to pack to form ordered 2-D crystallites
- GIXD results show that P188 inserts into the disordered phase of lipid monolayer

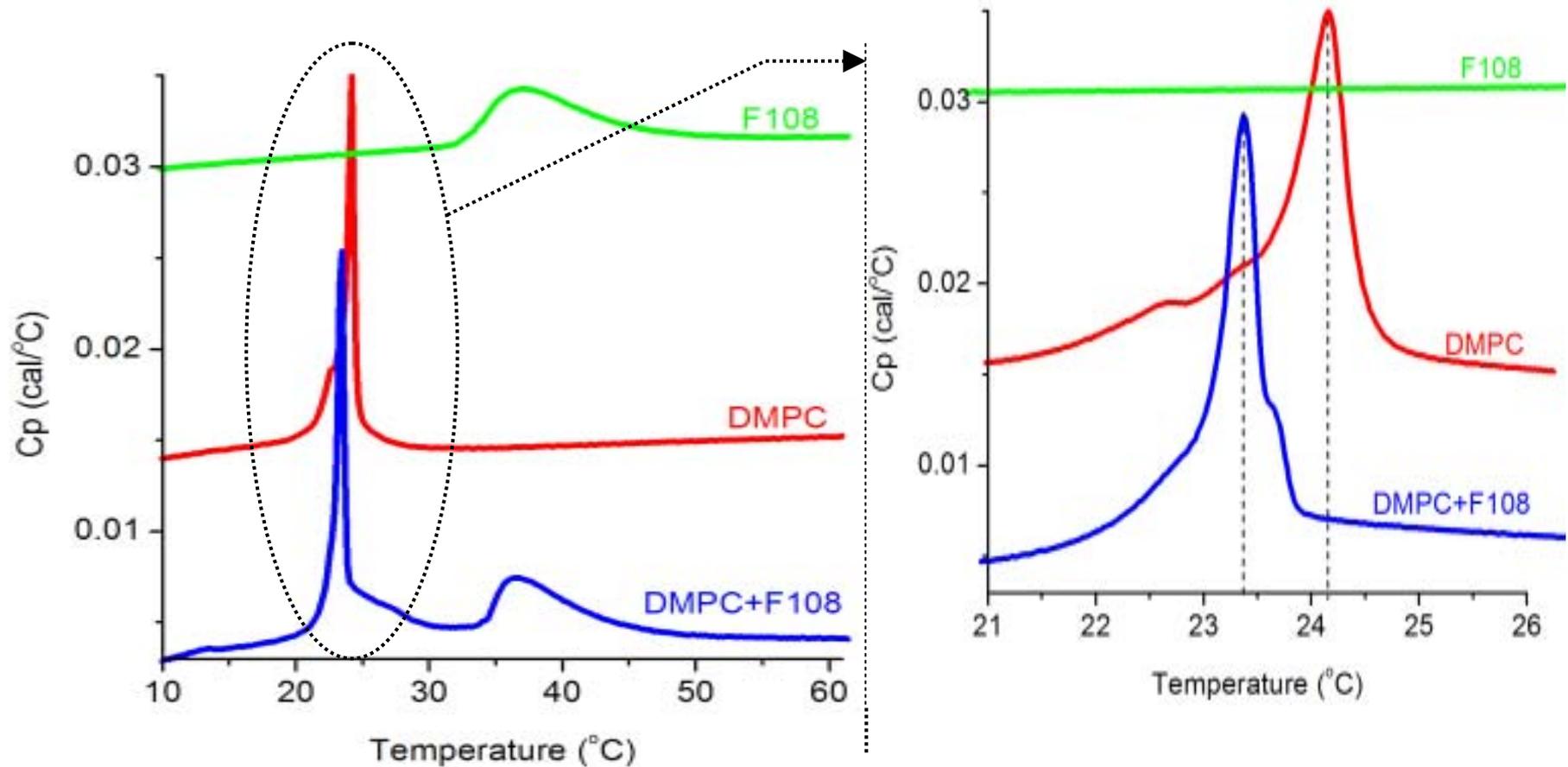
Wu, Majewski, Ege, Weygand, Kjaer & Lee, *PRL* 93 (2004) 028101

SIMULATION SHOW CORRALING AND SQUEEZE-OUT

QuickTime™ and a YUV420 codec decompressor are needed to see this picture.

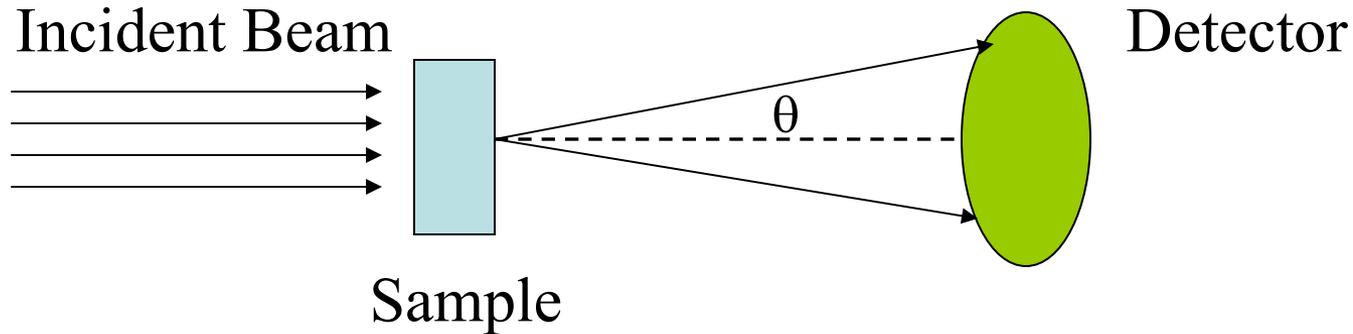
Courtesy of Igal Szleifer (Purdue University)

DIFFERENTIAL SCANNING CALORIMETRY ON POLOXAMER AND LIPID UNILAMELLAR VESICLES



- Poloxamer slightly decreases the gel-liquid main phase transition of vesicles.
- Poloxamer starts to change from free chains to micelle at ~35 °C at this conc.
- Two species of poloxamer exist:(1) interacting with DMPC vesicles;
(2) free

SMALL ANGLE NEUTRON SCATTERING



$$I(q) = n \Delta \rho^2 V^2 F(q) S(q), \quad q = 4\pi \sin \theta / \lambda$$

n : the number density of the particles

$\Delta \rho$: contrast between the particle and surrounding medium

V : volume of the particles

$F(q)$: particle form factor

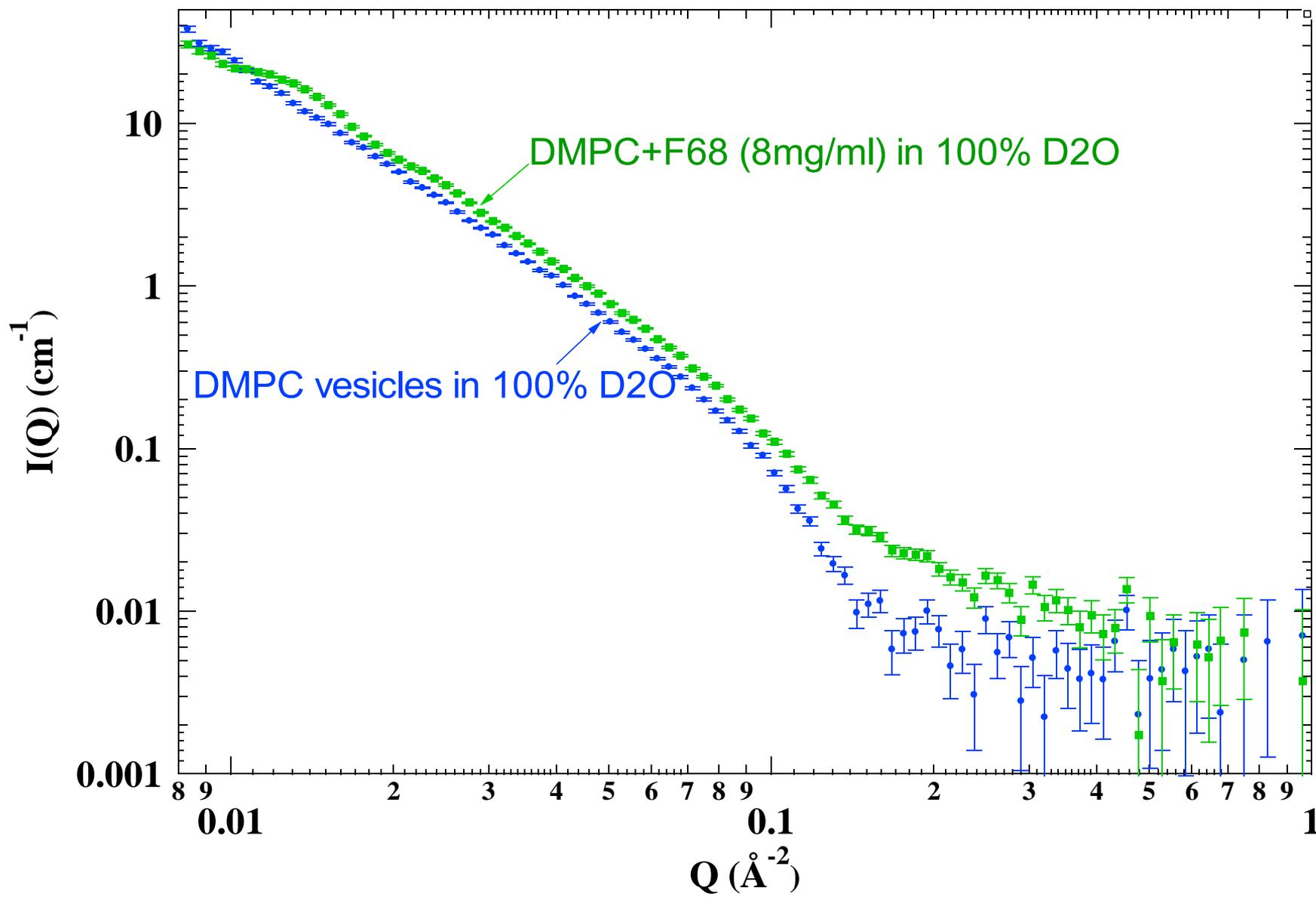
$S(q)$: the structure factor.

Hydrogen $b = -0.374 \times 10^{-4} \text{\AA}^{-2}$

Deuterium $b = 0.665 \times 10^{-4} \text{\AA}^{-2}$

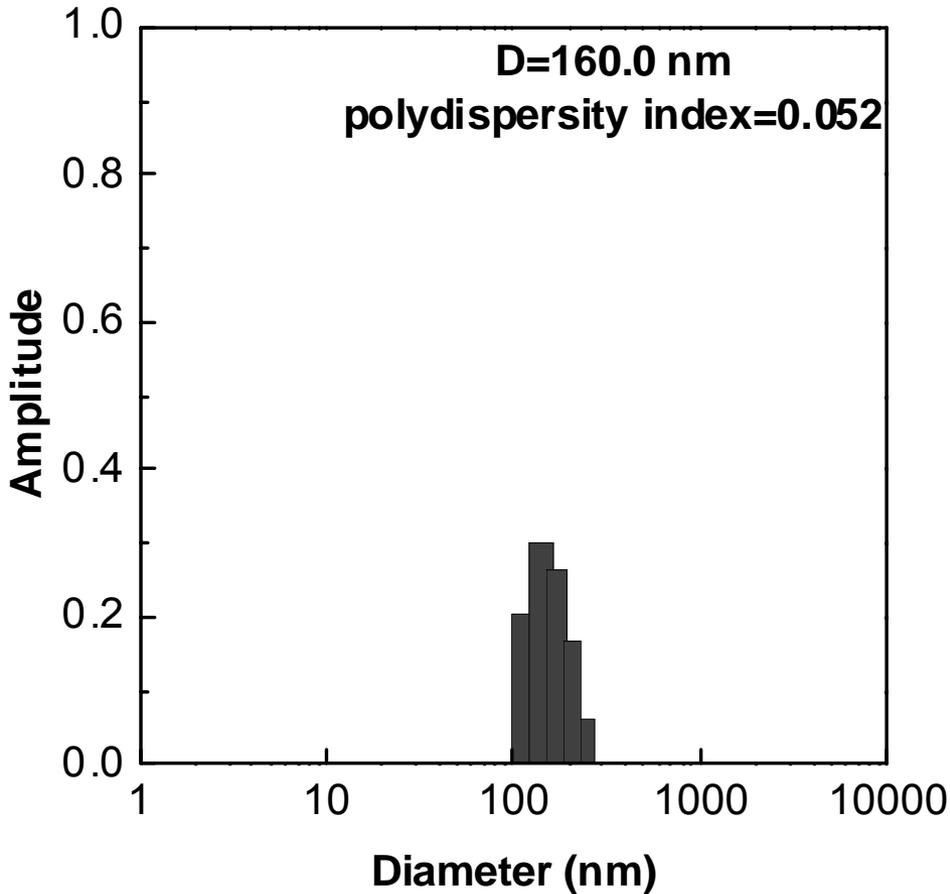
Small Angle Neutron Scattering

The oscillation indicates the particles are more mono-disperse

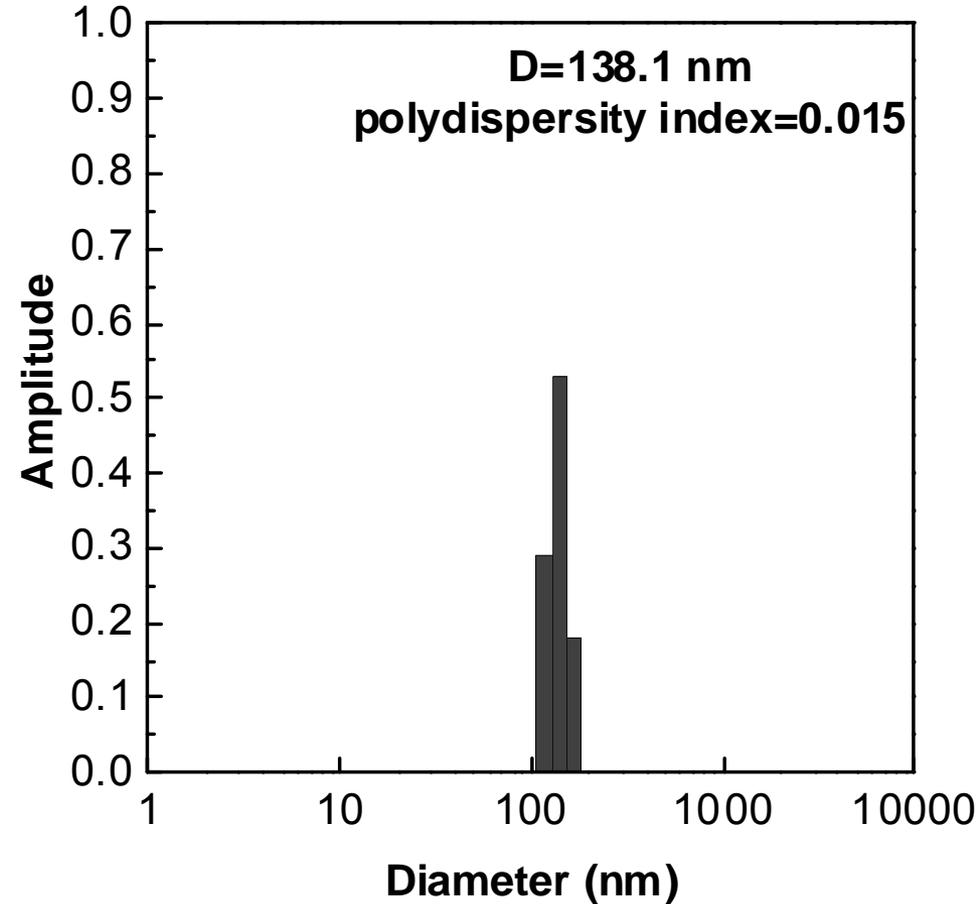


DYNAMIC LIGHT SCATTERING

POPC vesicle



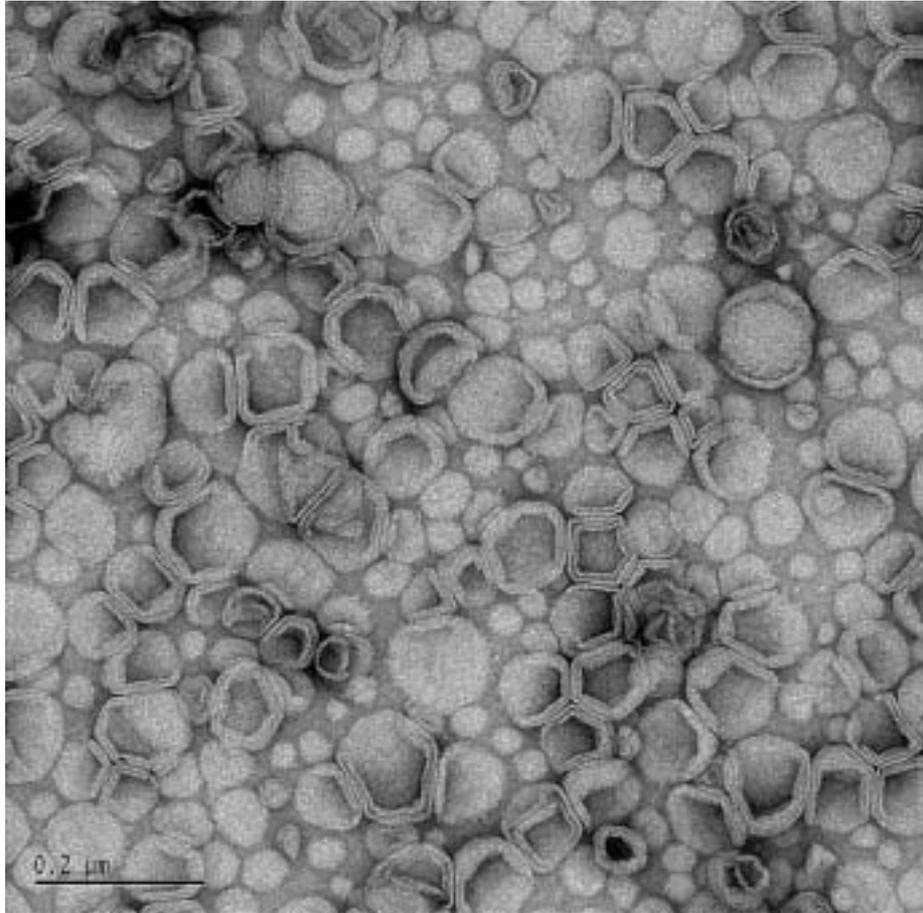
POPC/P188 vesicle



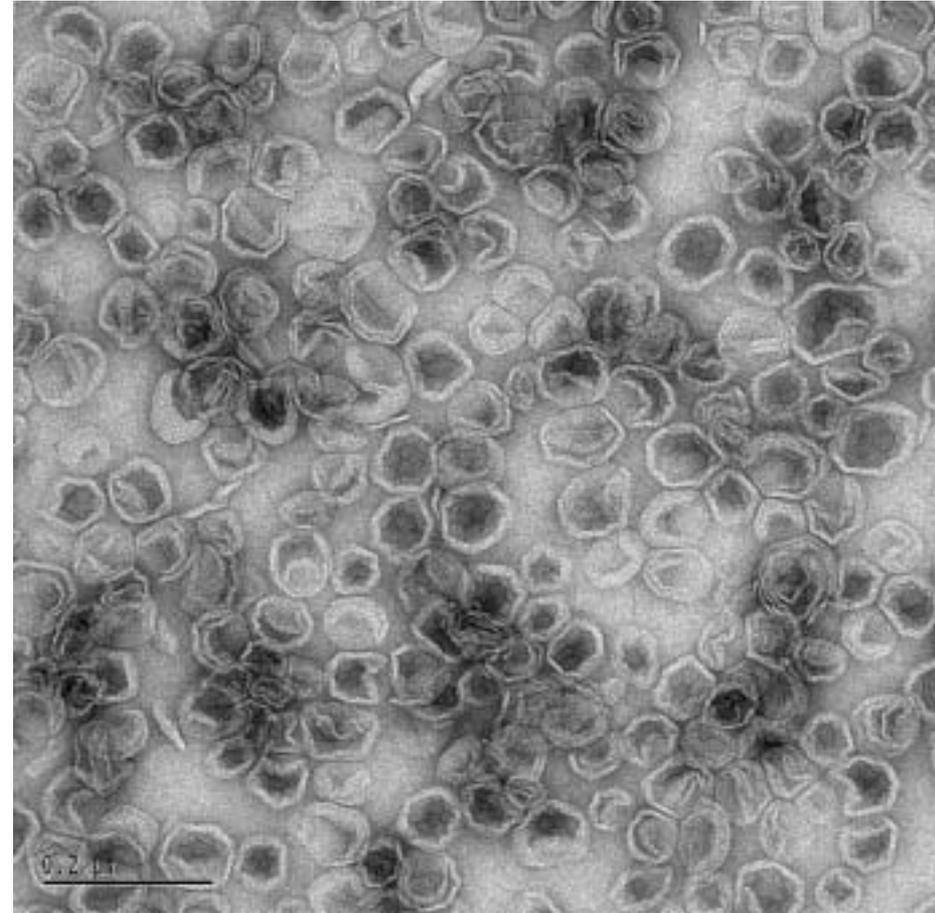
- Lipid vesicle size distribution gets more mono-disperse with presence of Poloxamer.

Transmission Electron Microscope

DMPC

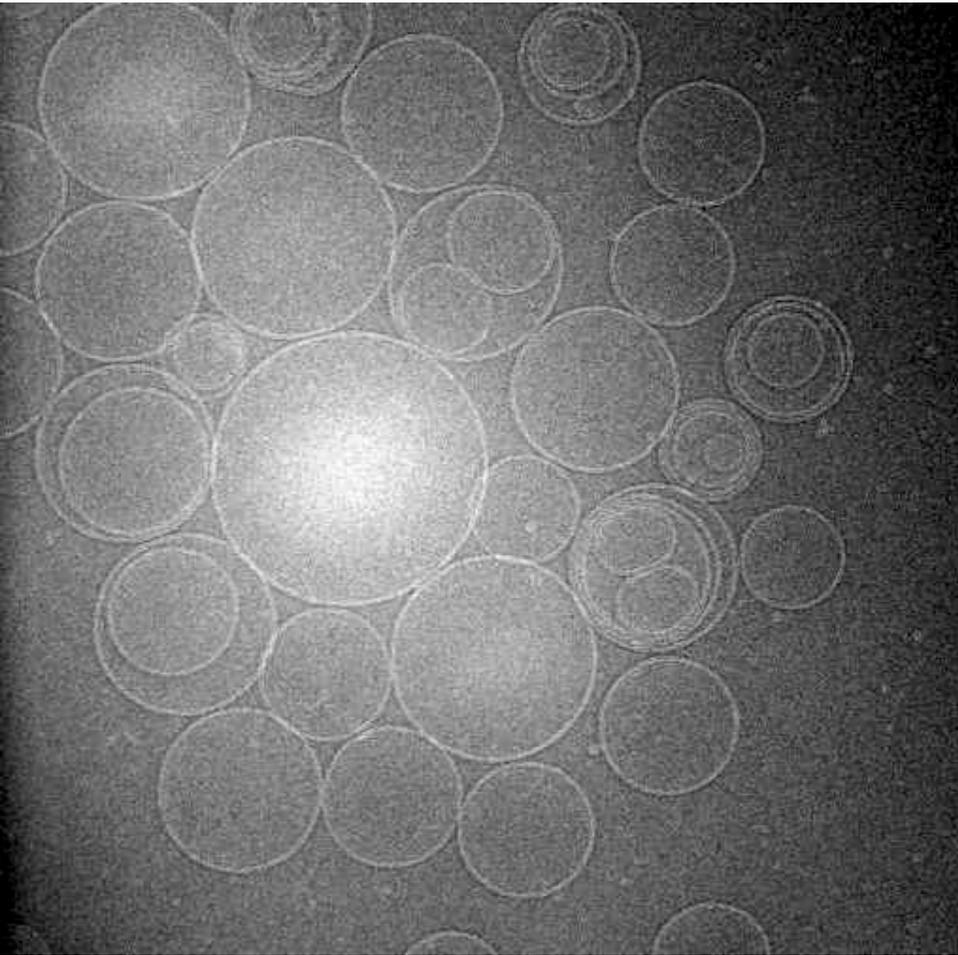


DMPC+F108

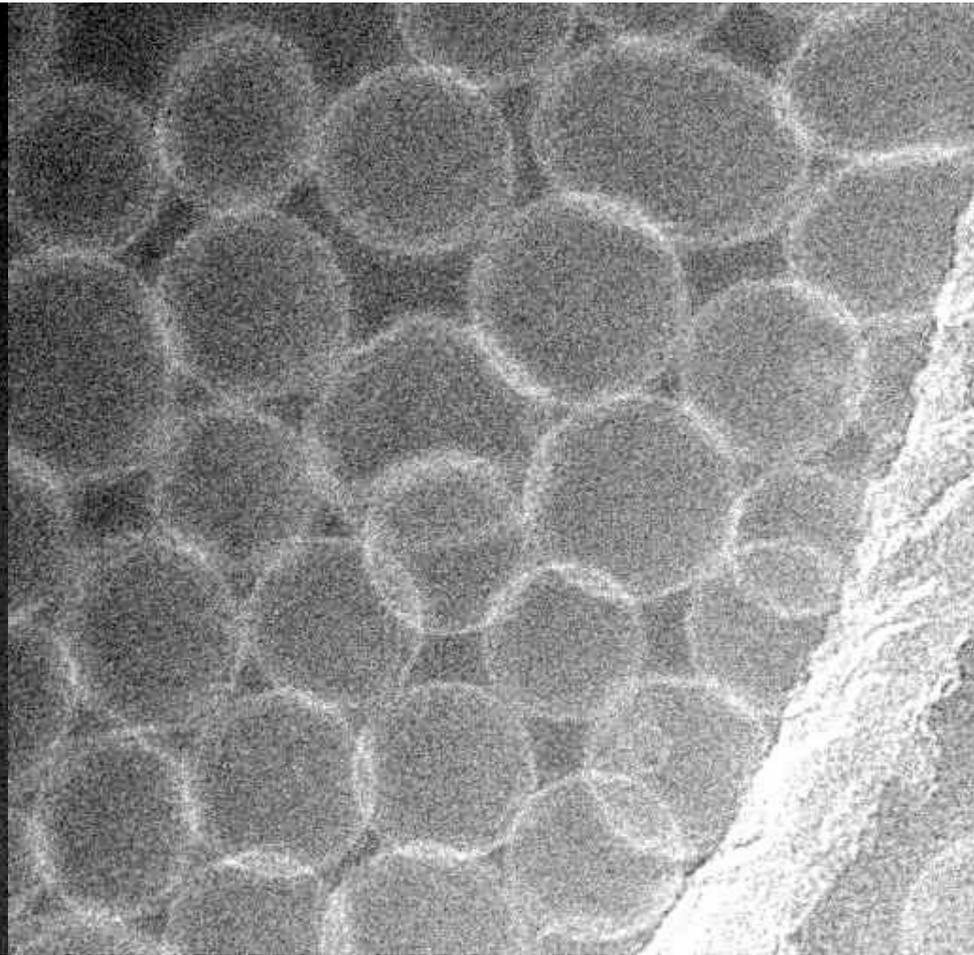


Cryo - EM

POPC (10 mg/ml)

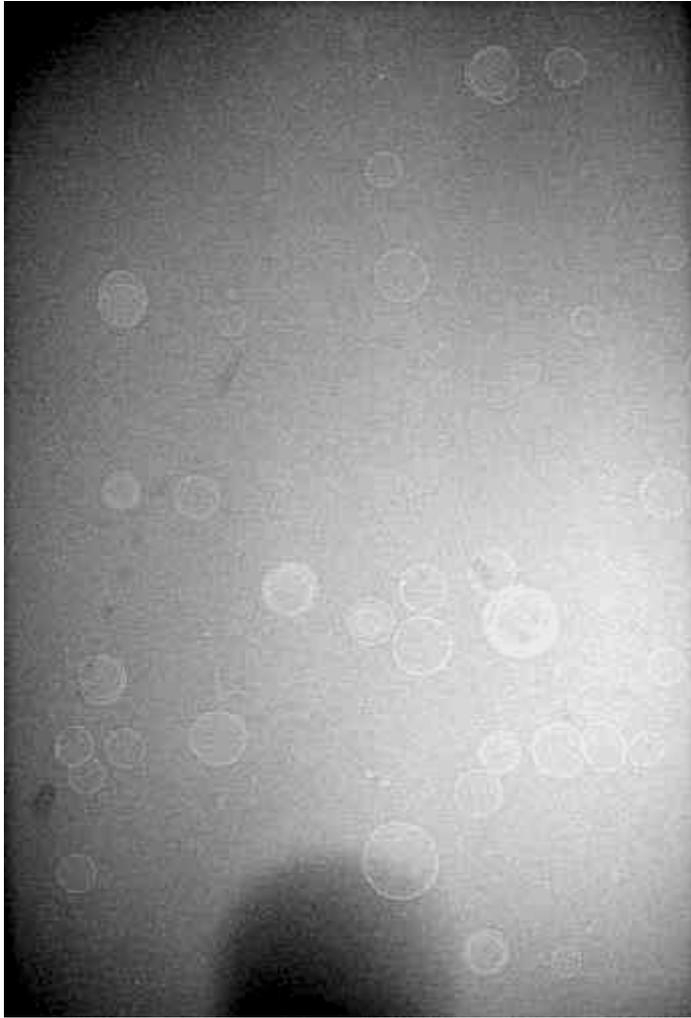


POPC-F108 (10-10 mg/ml)

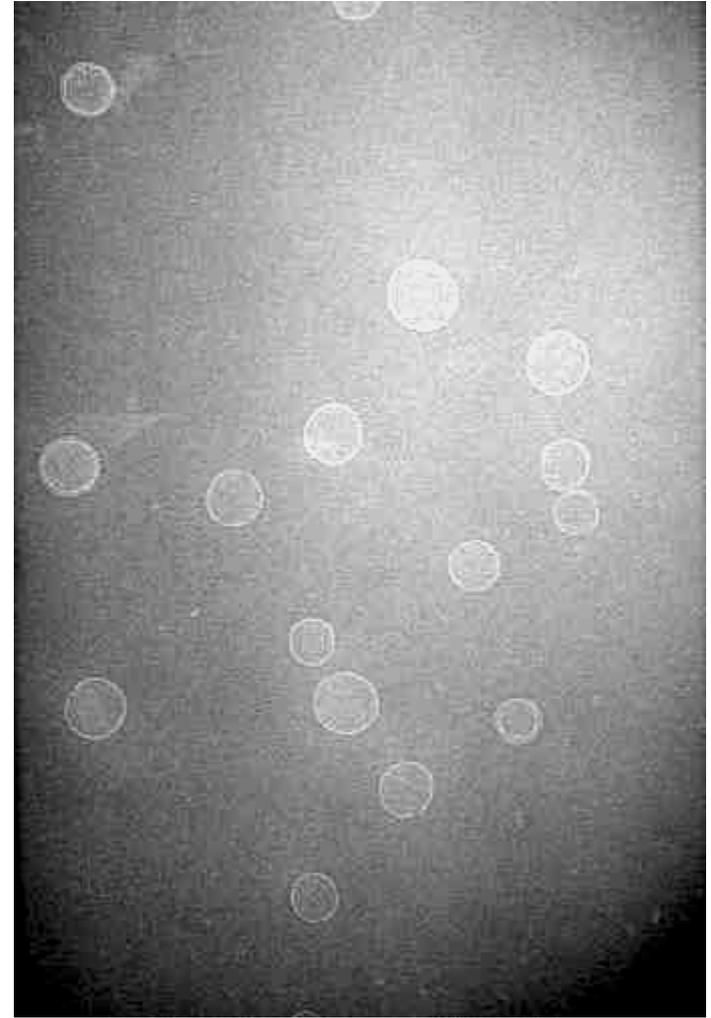


Cryo - EM

DLPC vesicle

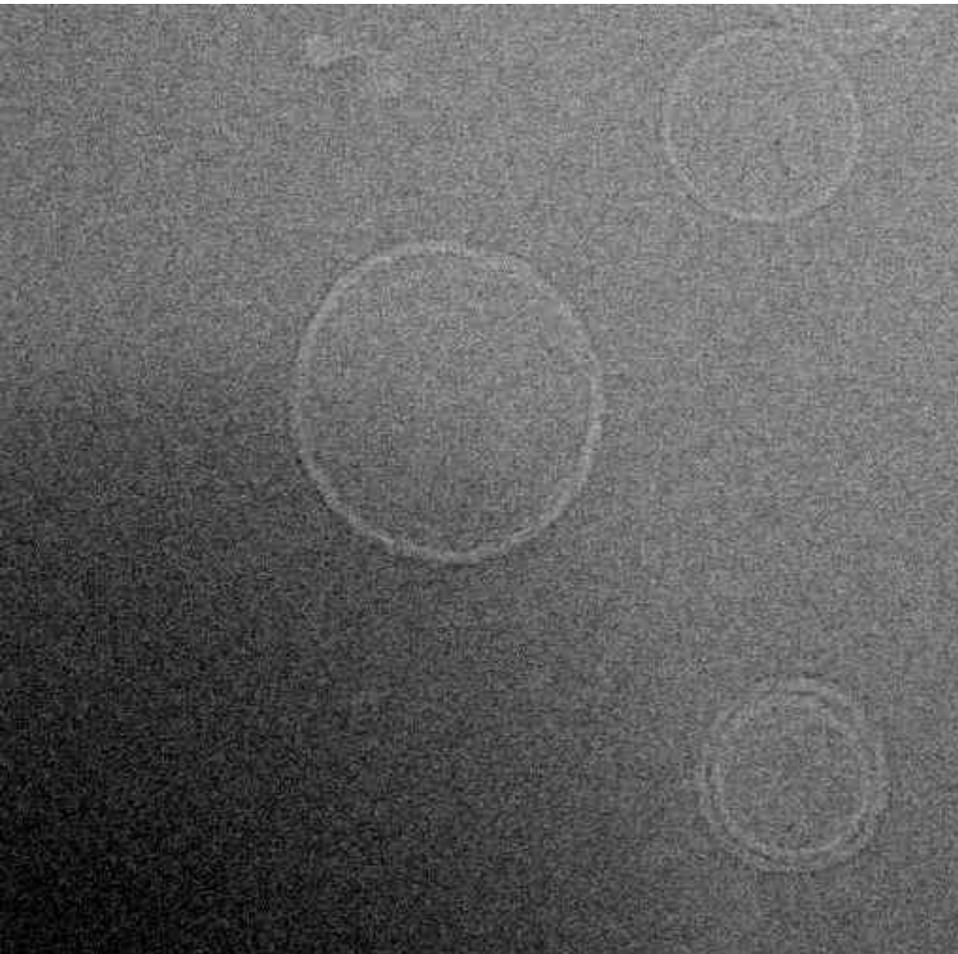


DLPC/P188 vesicle

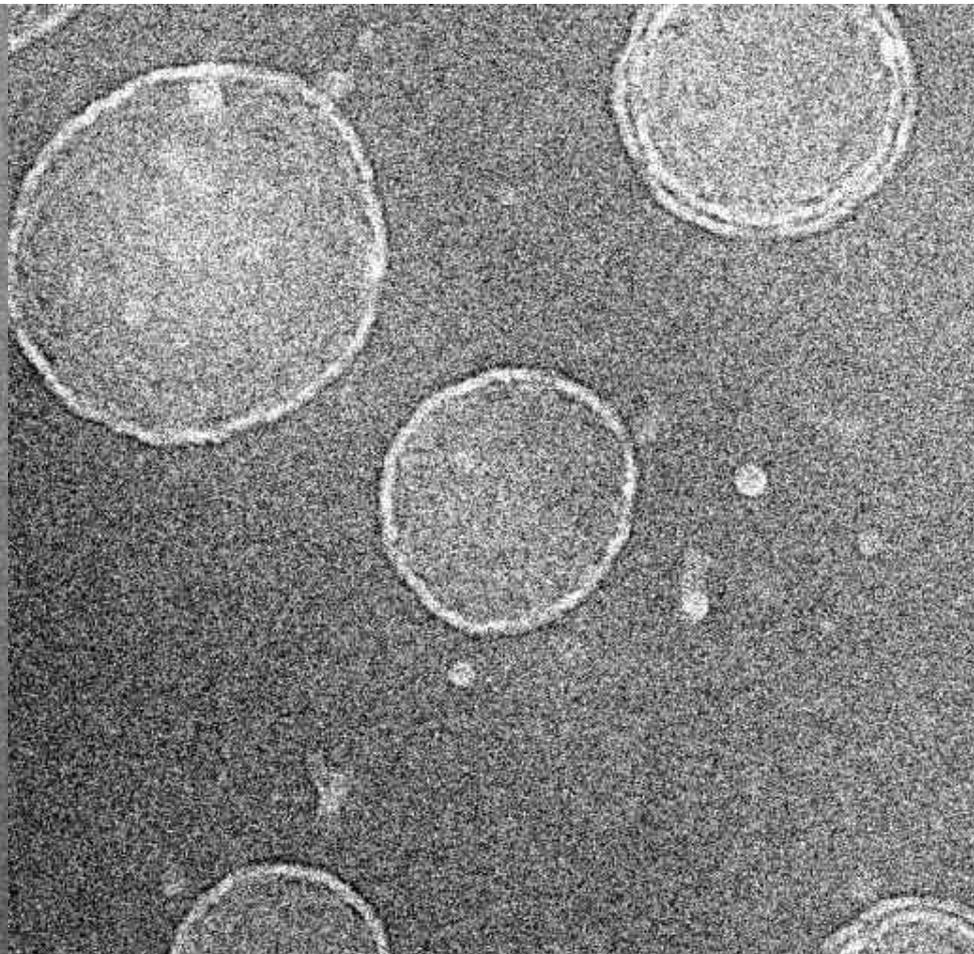


Cryo - Electron Microscope

DLPC vesicle

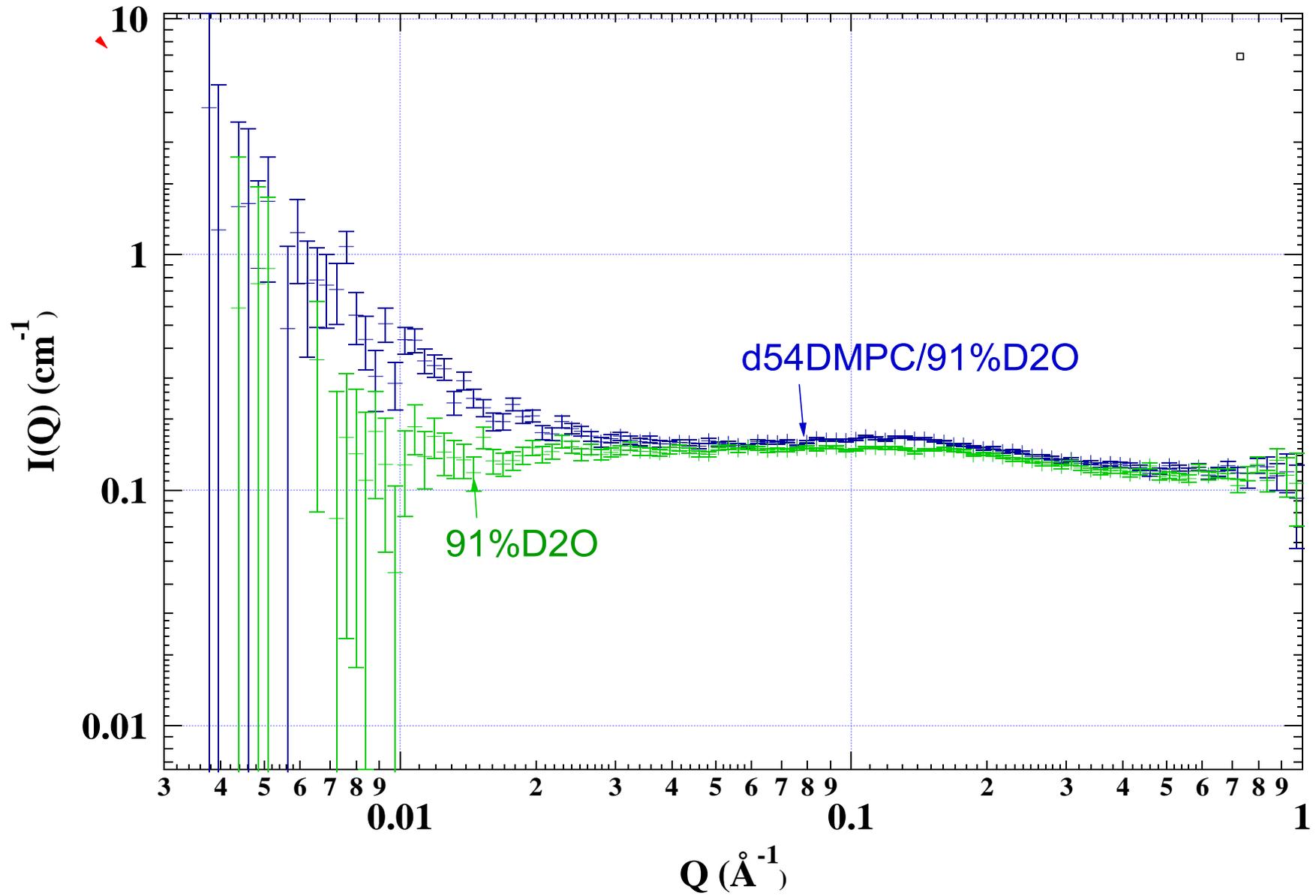


DLPC/P188 vesicle

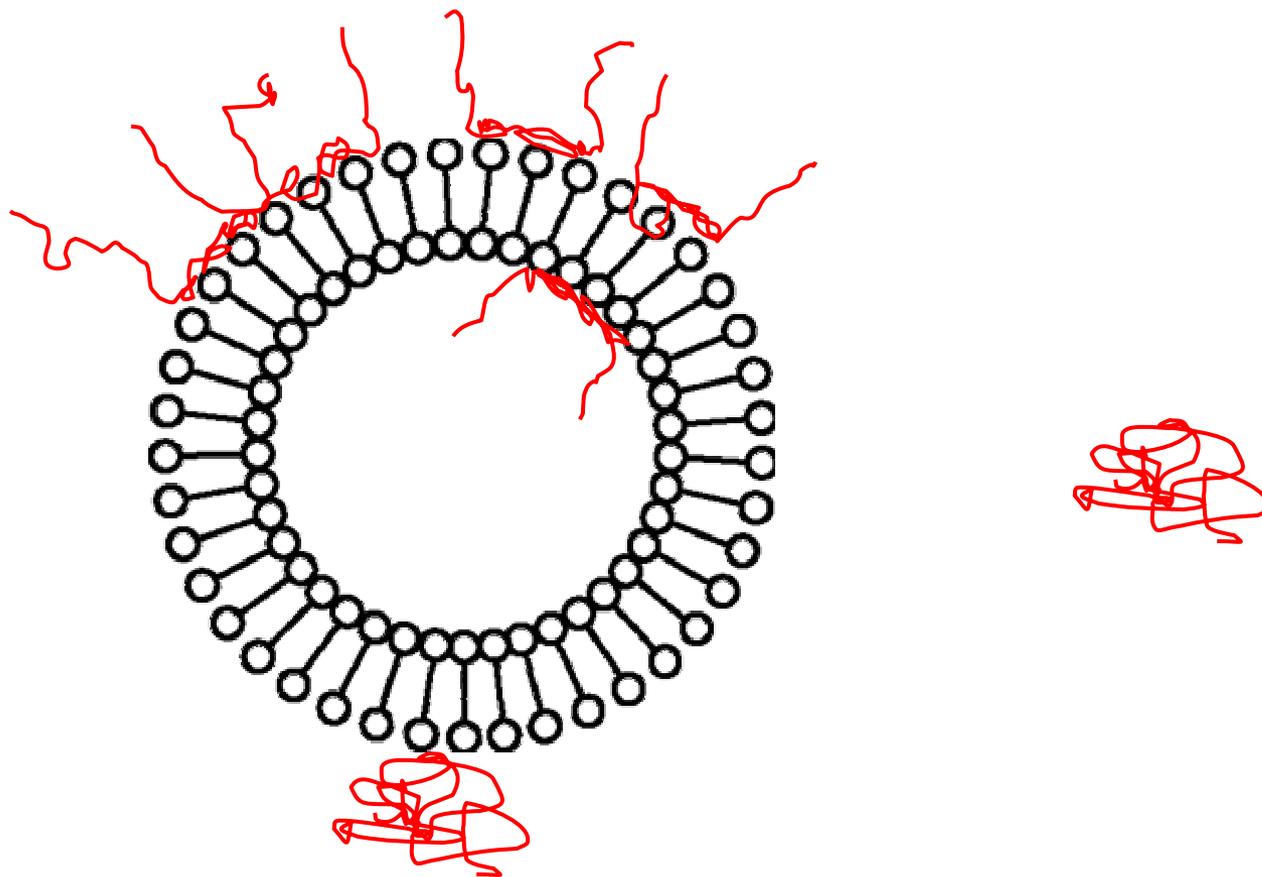


INDEX MATCHING EXPERIMENT

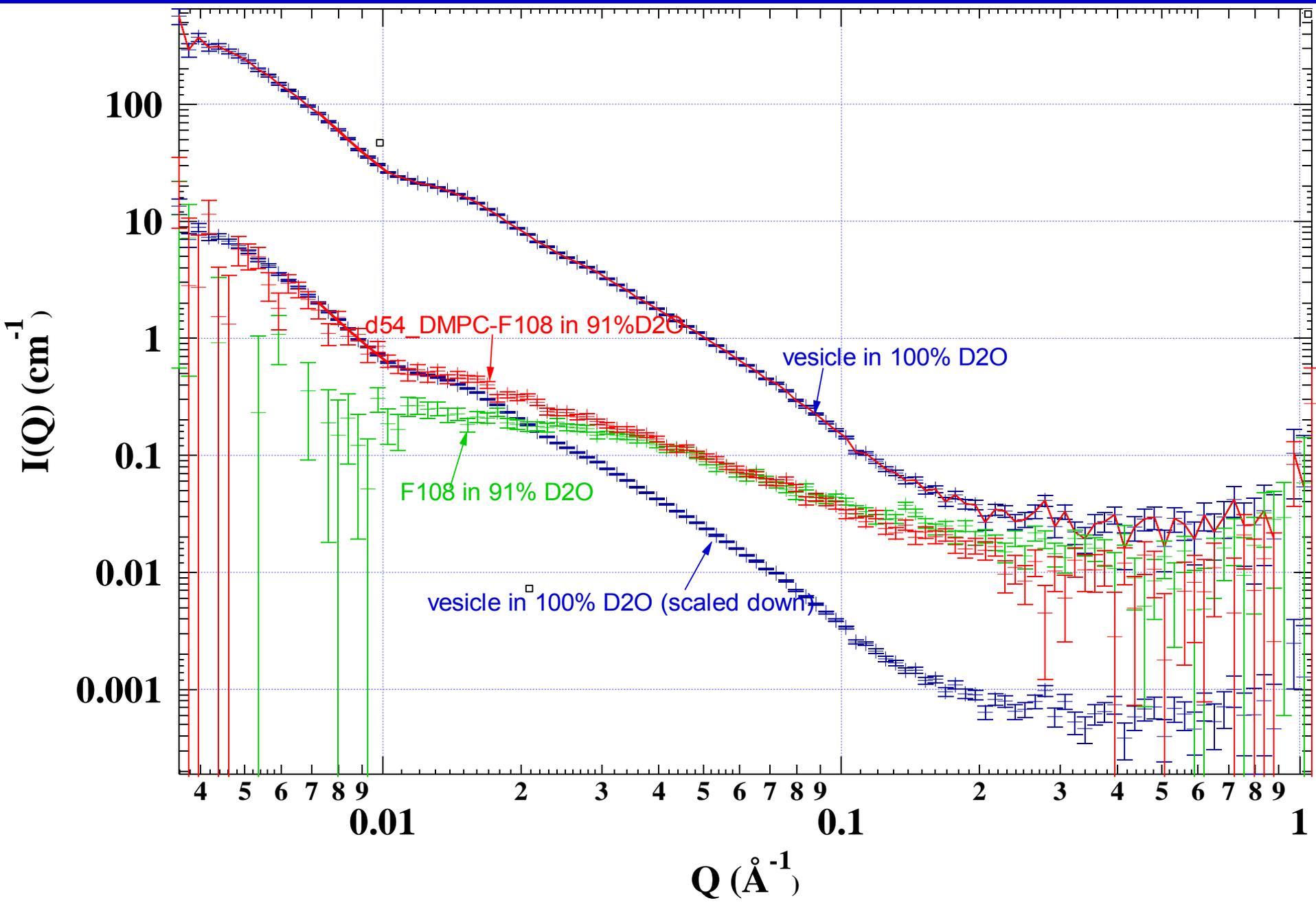
D54-DMPC IN 91% D2O



WE ONLY SEE POLYMERS (VESICLES TRANSPARENT FOR NEUTRON)



BOTH ADSORBED AND FREE POLYMER EXIST



DMPC/F108 VESICLES

Turbidity

Vesicles stored at 31 °C -> turbid

Brought to room temperature -> solution becomes clear

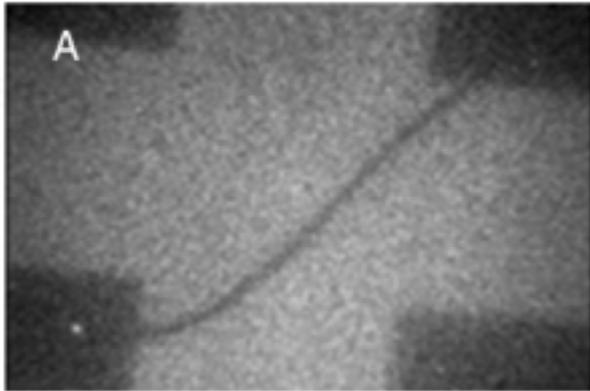
Stay in room temperature for a short time

- warm up in palm
 - shake
- > turbid

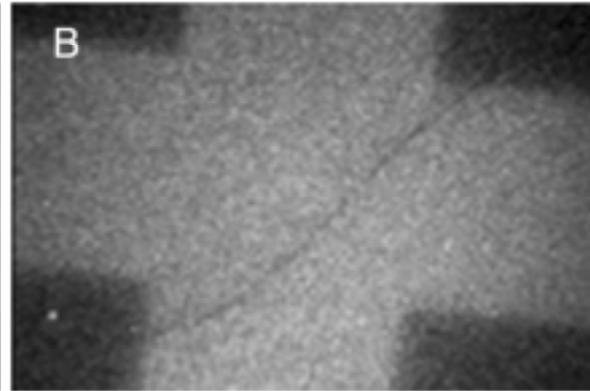
Stay in room temperature for a long time

-> turbidity returns only after incubating in oven for an extended period

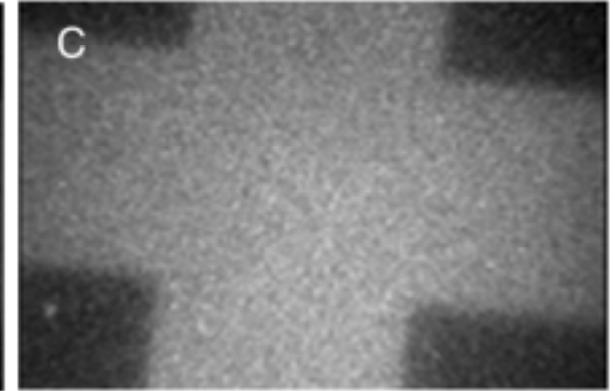
HEALING ON SUPPORTED BILAYERS



Supported bilayers
with a scratch



Poloxamer insertion
Reduces the scratch

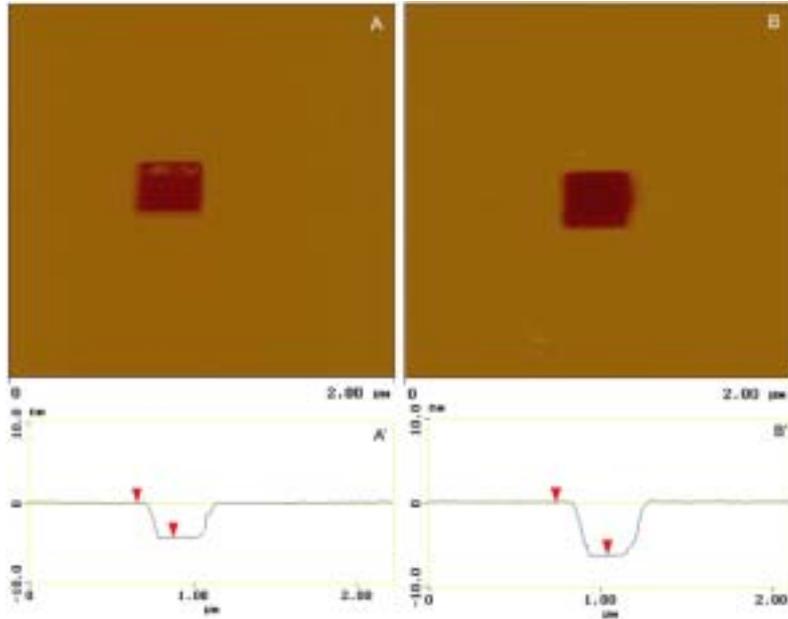


Complete elimination
of the scratch

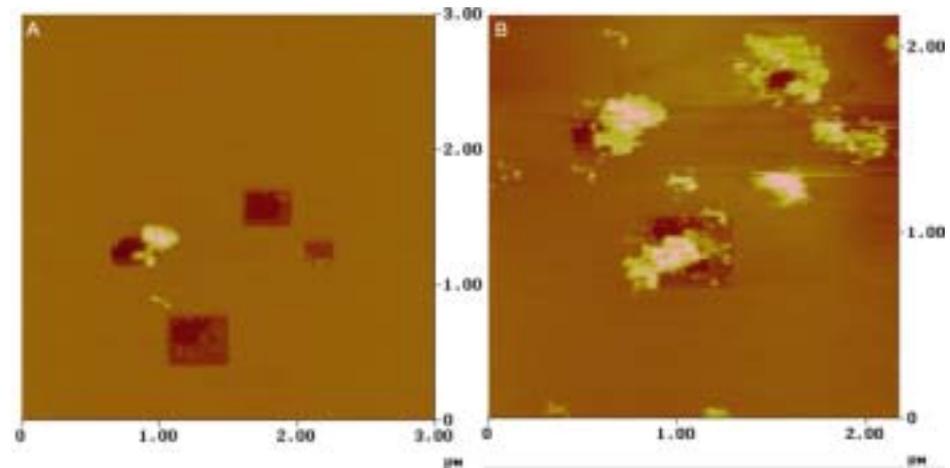
HIGH RESOLUTION INSERTION PROFILE BY AFM

Without P188

With P188



Preferential P188 adsorption
to defect edges



CONCLUSIONS

Hydrophobic/hydrophilic ratio dictates insertion characteristics

Squeeze-out of poloxamer occurs at high packing density

Phase separation into polymer-rich and polymer-poor phases

Inserted polymers force lipids to pack more tightly
→ help seal membrane

Polymer incorporation results in lower polydispersity

Both free polymer and polymer incorporated into vesicles exist

Polymer insertion eradicates physical damages
in supported bilayers

ACKNOWLEDGMENTS

Poloxamer

Raphael C. Lee (Surgery, U of C)
Luping Yu (Chemistry, U of C)
Heinrich Jaeger (Physics, U of C)
Karl Freed (Chemistry, U of C)
Igal Szleifer (Chemistry, Purdue University)

Scattering

Jaroslav Majewski (LANL)
Kristian Kjaer (Risø National Lab, Denmark)
Jyotsana Lal (ANL)
BW1, HASYLAB, DESY, Hamburg, Germany
IPNS, ANL

\$\$\$\$

**Alfred P. Sloan Foundation, Alzheimer's Association, American Health Assistance Foundation
American Lung Association, Camille and Henry Dreyfus Foundation
David and Lucile Packard Foundation, March of Dimes, NSF
NSF-MRSEC, Searle Scholars Program/The Chicago Community Trust**

THIS TALK IS BROUGHT TO YOU BY ...

Guohui Wu

Kinlok Lam



Stacey Maskarinec (now at Caltech with Tirrell)

