

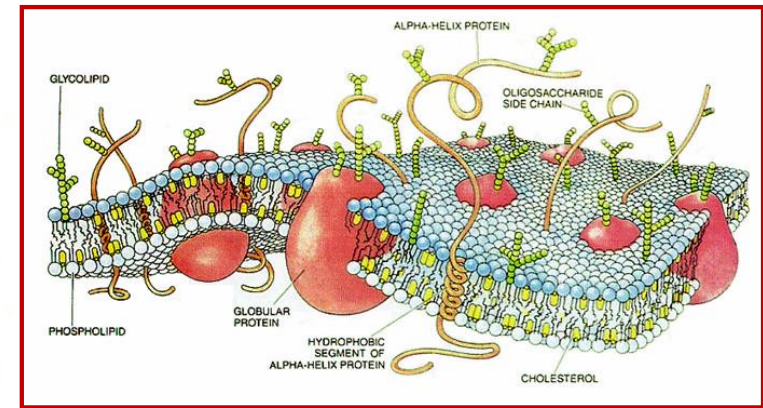
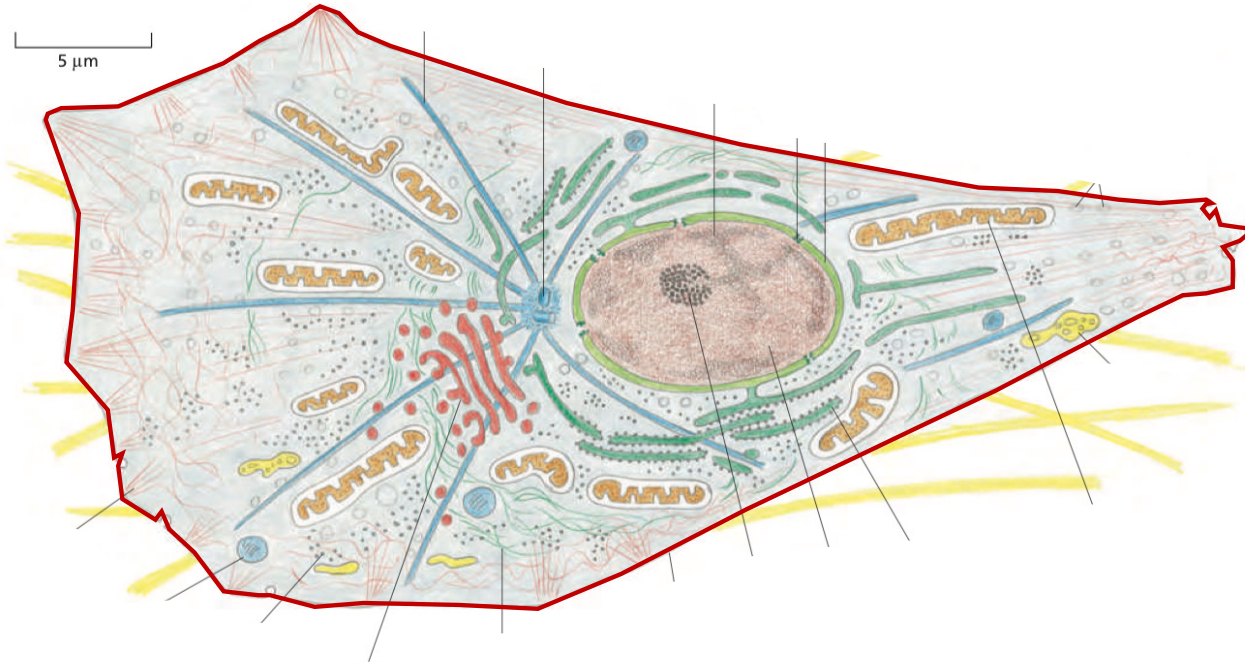
The dual-face nature of biological membranes

Milka Doktorova

Assistant Professor
Stockholm University

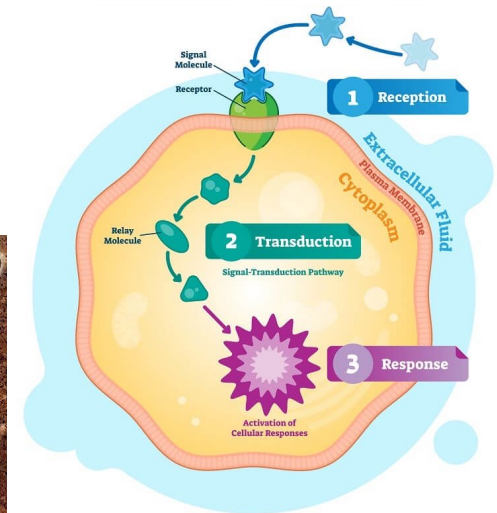
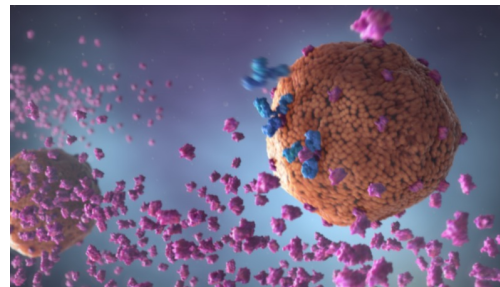
Phospholipid Research Center
August 31, 2026

Cell membranes: ubiquitous and multifunctional

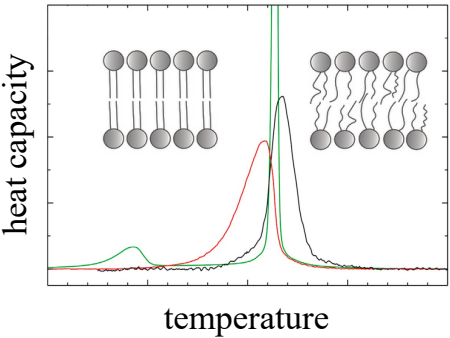
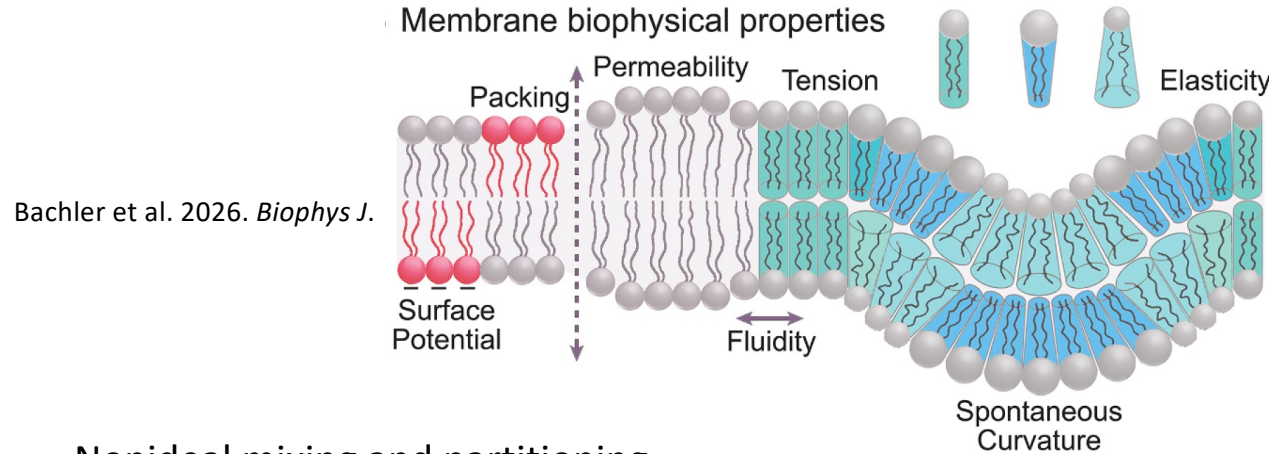
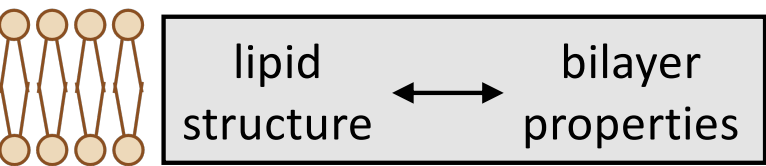


Alberts, Biology of the Cell

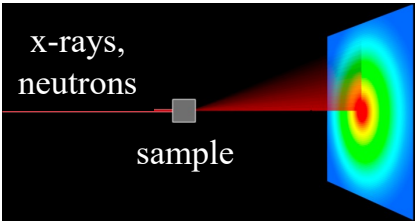
- Barrier
- Determinant of cell shape
- Major signaling hub



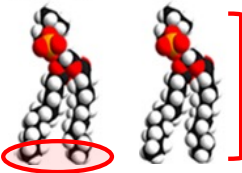
Lipid bilayers: rich biophysical profile



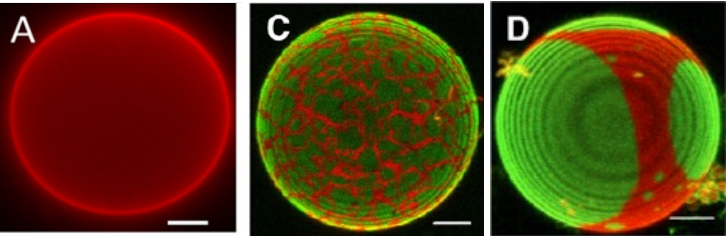
Transition temperatures



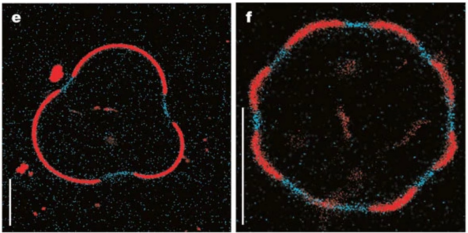
Area and thickness



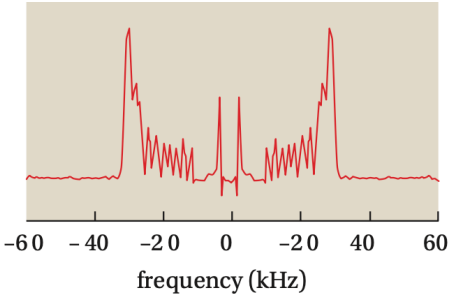
Nonideal mixing and partitioning



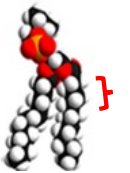
Zhao et al. 2007. *BBA*



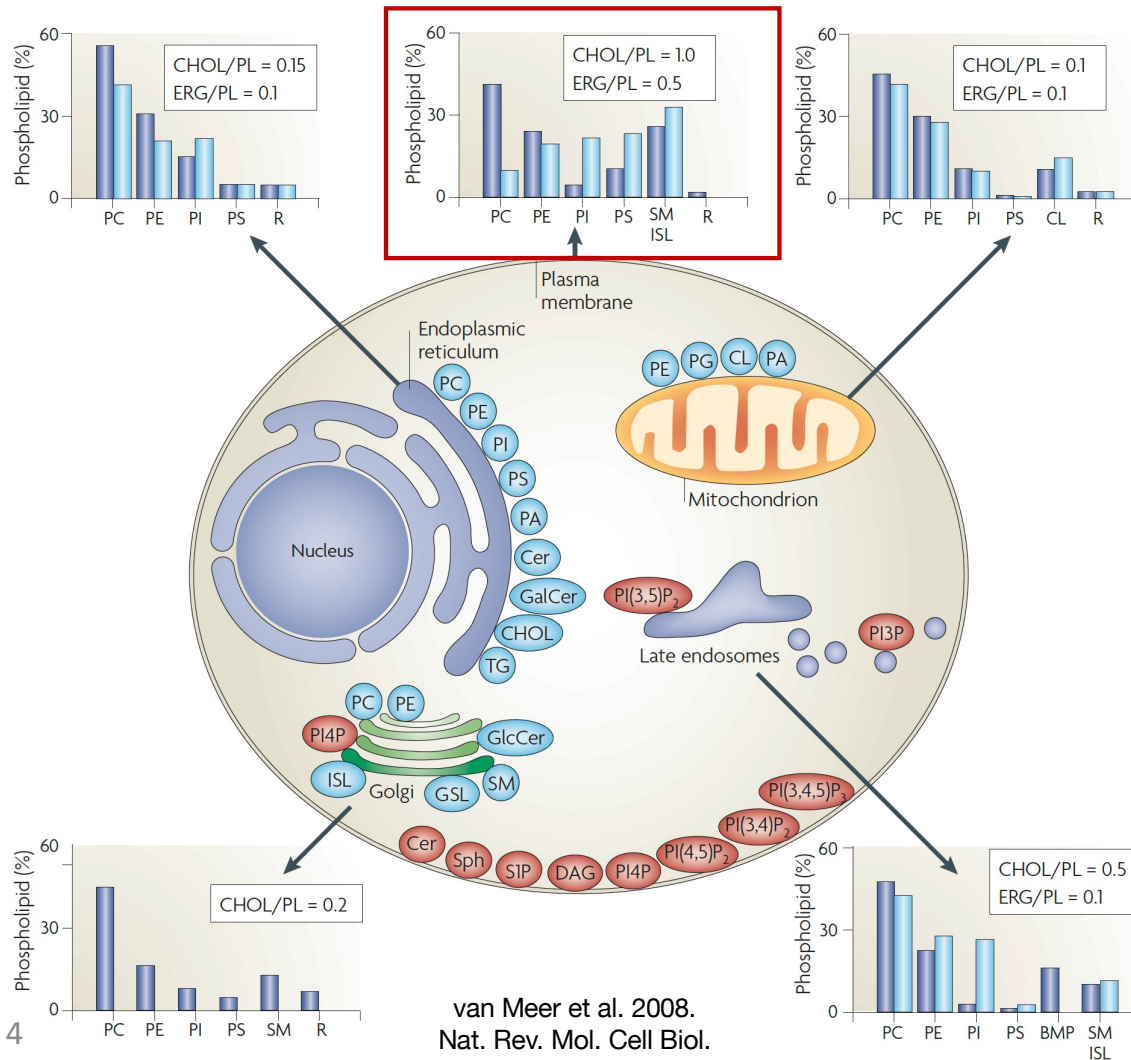
Baumgart et al. 2003. *Nature*



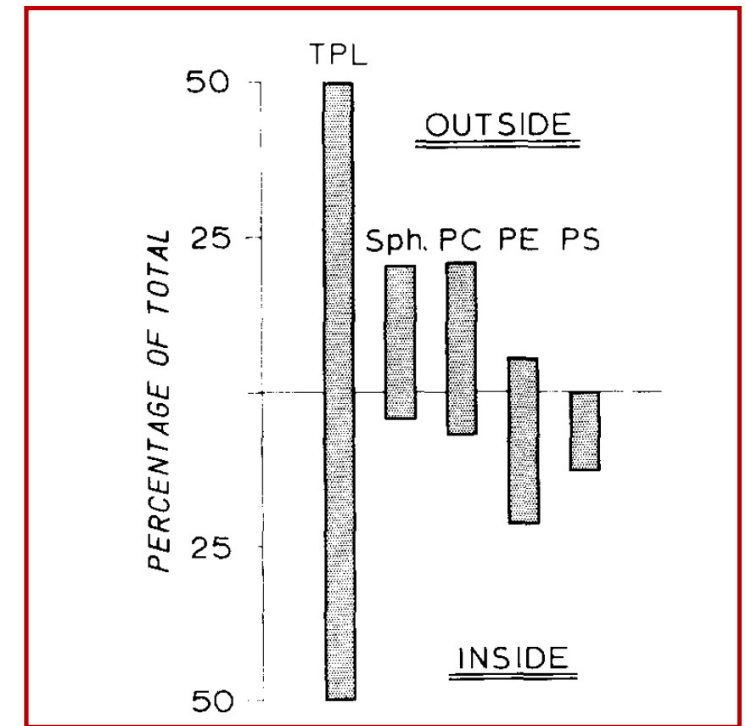
Chain dynamics



Inferences about biological membranes



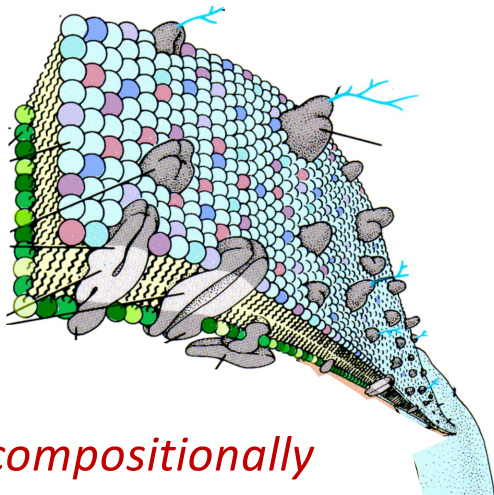
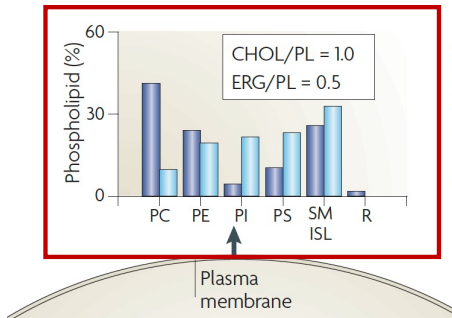
Plasma Membrane (PM)



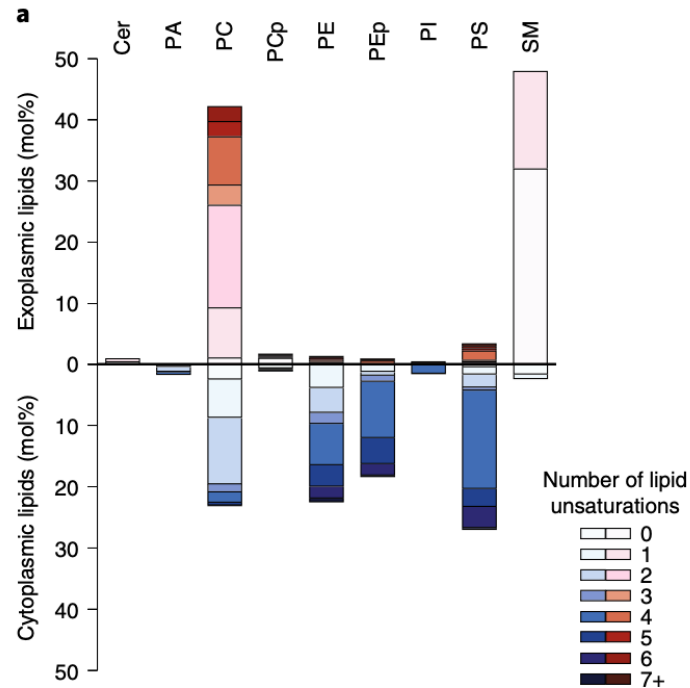
AJ Verkleij, ..., LLM Van Deenen. 1973. BBA

Biological membranes are asymmetric!

Inferences about biological membranes

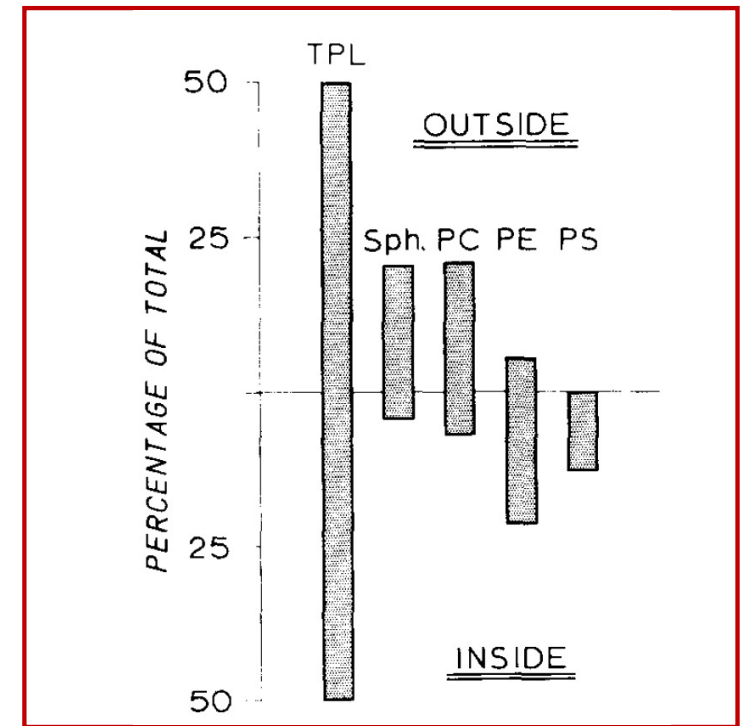


*compositionally
asymmetric!*



Lorent et al. 2020. *Nat. Chem. Biol.*

Plasma Membrane (PM)

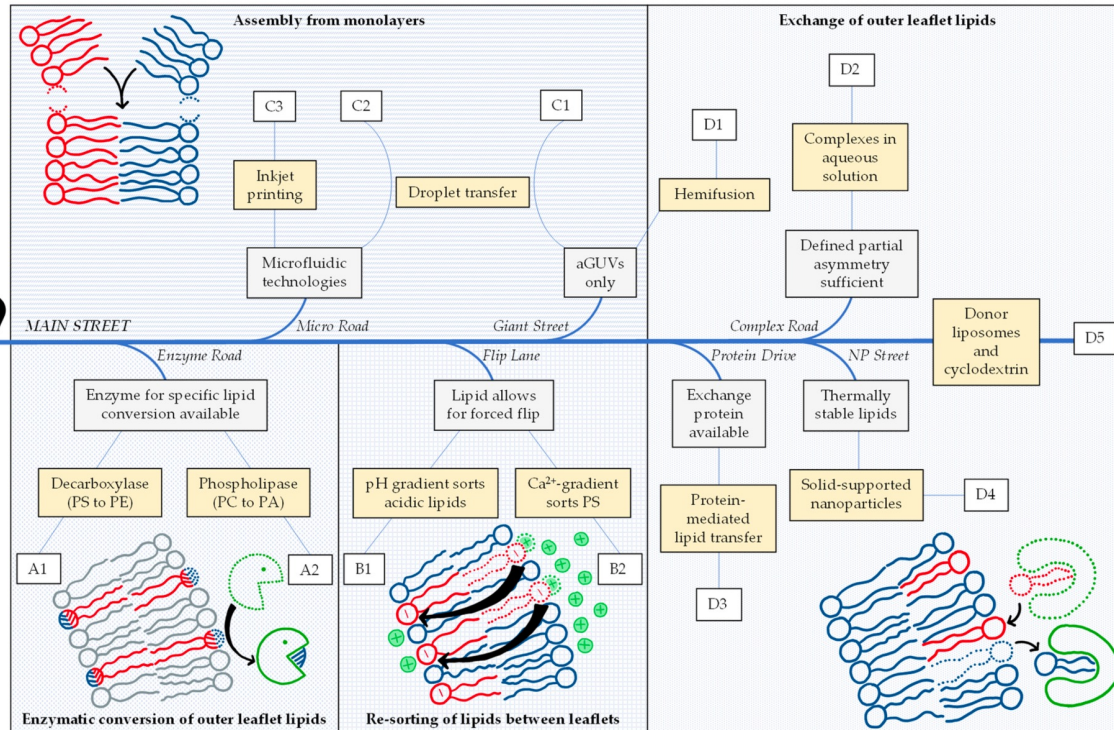


AJ Verkleij, ..., LLM Van Deenen. 1973. *BBA*

Symmetric bilayers with exo and cyto lipid compositions –
very different properties!

Model membranes in vitro show effects of asymmetry

Protocols for asymmetric model membranes



Krompers & Heerklotz. 2023. *Membranes*. 13:267

Leaflets in asymmetric bilayers have distinct biophysical properties relative to symmetric counterparts

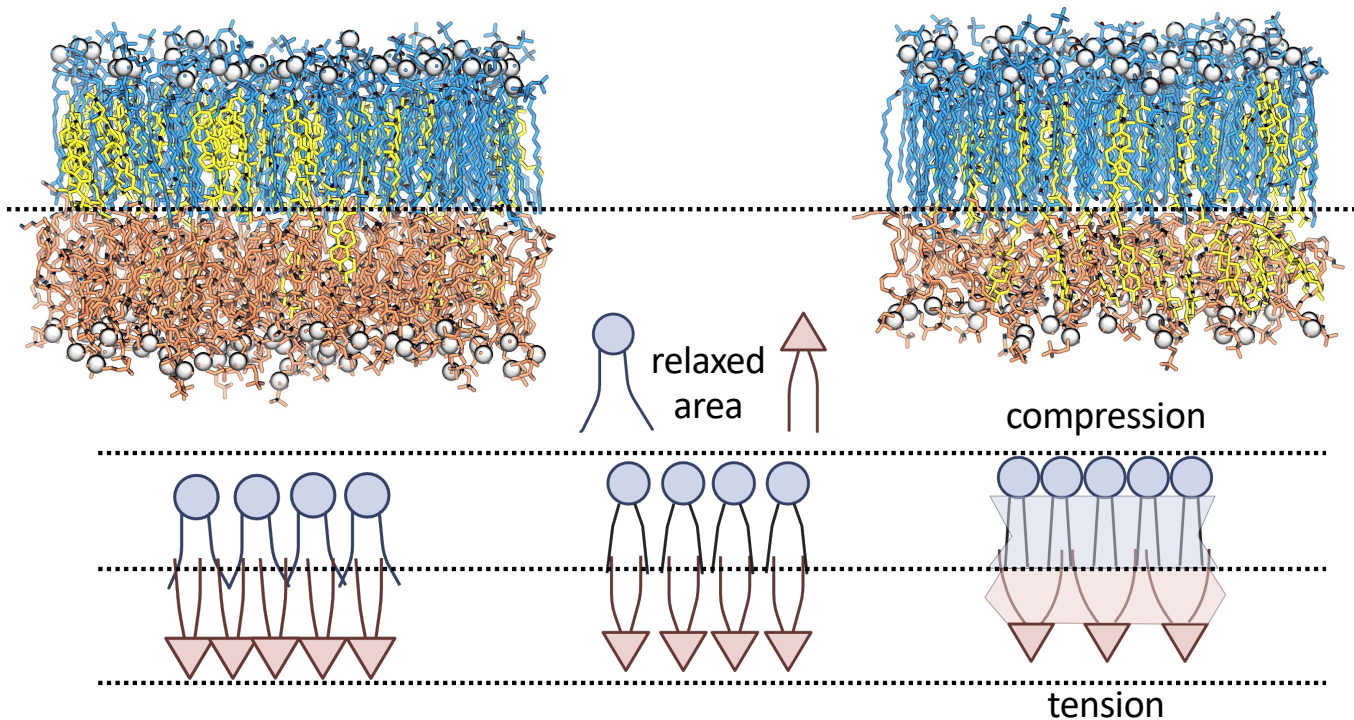
Relative to symmetric bilayers, compositionally asymmetric bilayers have different:

- Lipid order and packing
- Lipid diffusion
- Transition temperature
- Phase behavior
- Electrostatic potential
- Bending rigidity

Collins & Keller. 2008. PNAS
Chiantia & London. 2012. Biophys. J.
Elani et al. 2015. Chem. Commun.
Heberle et al. 2016. Langmuir.
Eicher et al. 2018. Biophys. J.
Markones et al. 2018. Langmuir.

Dreschler et al. 2018. Biophys. J.
Wang & London. 2018. Biophys. J.
Rickeard et al. 2020. Nanoscale
Enoki & Heberle. 2023. PNAS
Frewein et al. 2023. Biophys. J.
Reagle et al. 2024. Soft Matter

Studies in silico highlight effects of number asymmetry



Compositionally-identical asymmetric bilayers can have **VERY** different properties based on lipid number imbalance

Lipid number asymmetry & differential stress: **KEY** determinants of bilayer properties

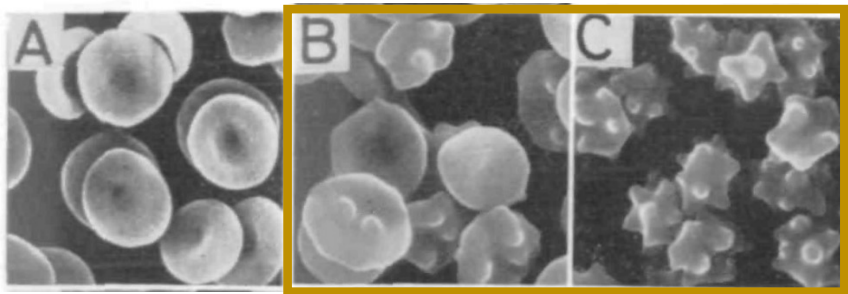
For a relatively large range of imbalances:

- Bilayer preserves its integrity
- The two leaflets develop contrasting properties

differential stress:
balancing *compression* and *tension* in the two leaflets

Esteban-Martín et al. 2009. JACS
Park et al. 2015. JCTC
Doktorova & Weinstein. 2018. Biophys. J.
Hosseini & Deserno. 2021. Biophys. J.
Pirhadi et al. 2022. JCTC
Heberle & Doktorova. 2025. Faraday Discuss.

Number asymmetry can be induced

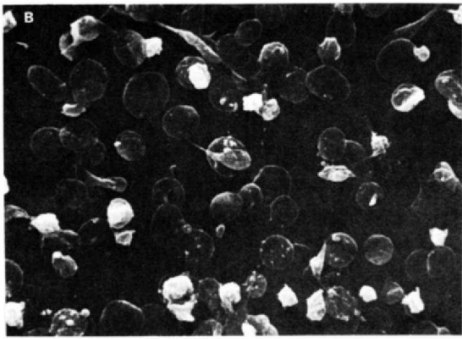
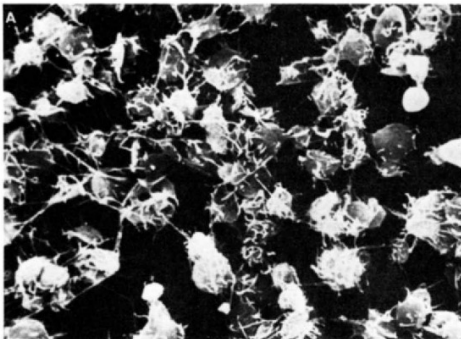


Tamura et al. 1982. *Biochemistry*

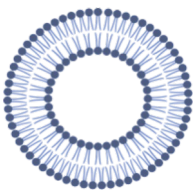
+ PC

+ PC

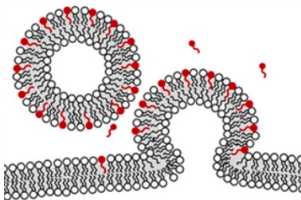
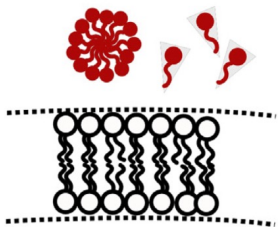
+ PS



8 Sune & Bienvenue. 1988. *Biochemistry*

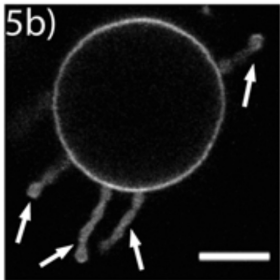


adding detergents

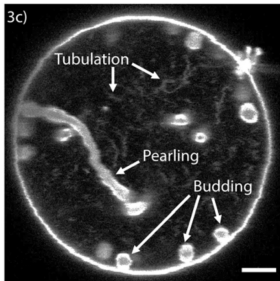


Hua et al. 2023. *Biophys. J.*
Hua et al. 2025. *J. Phys. Chem. B*

adding lipids

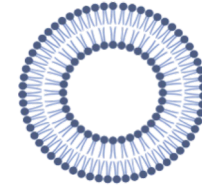


removing lipids
from outer leaflet



Reagle, ... Baumgart. 2024. *Soft Matter*

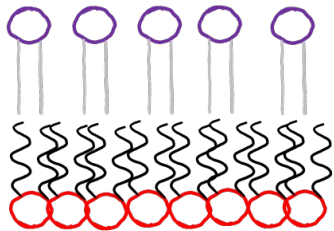
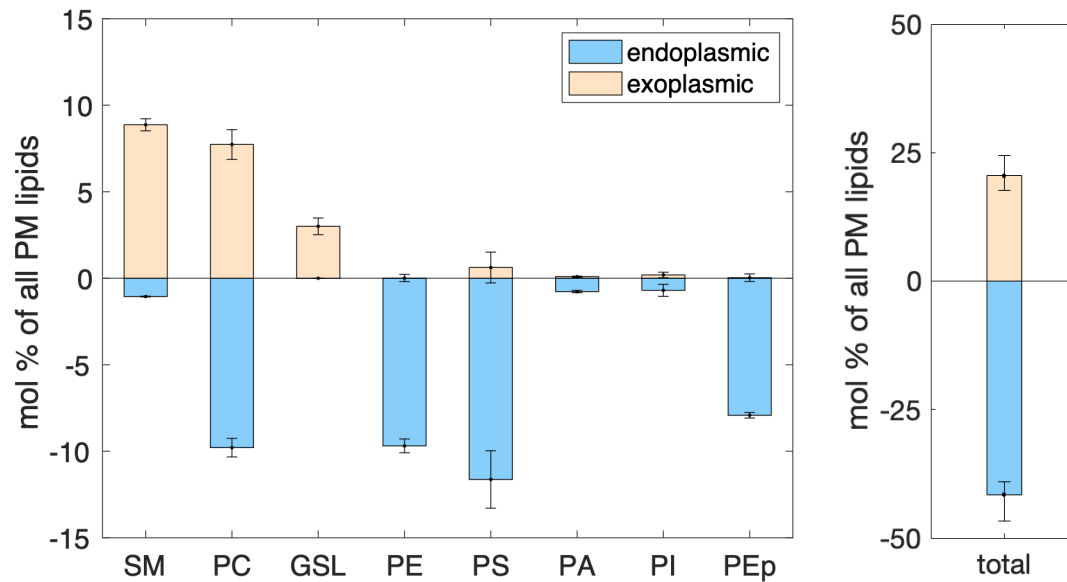
Number asymmetry can be hidden



A surprising result



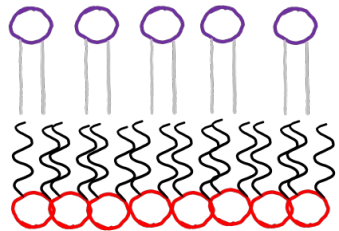
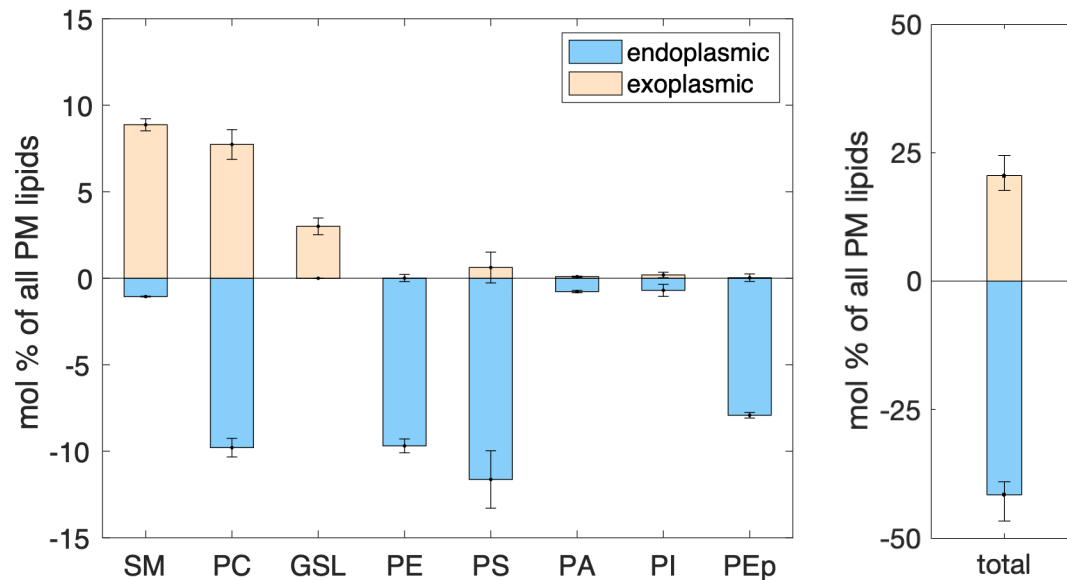
What is the relative total abundance of lipids in the two leaflets?



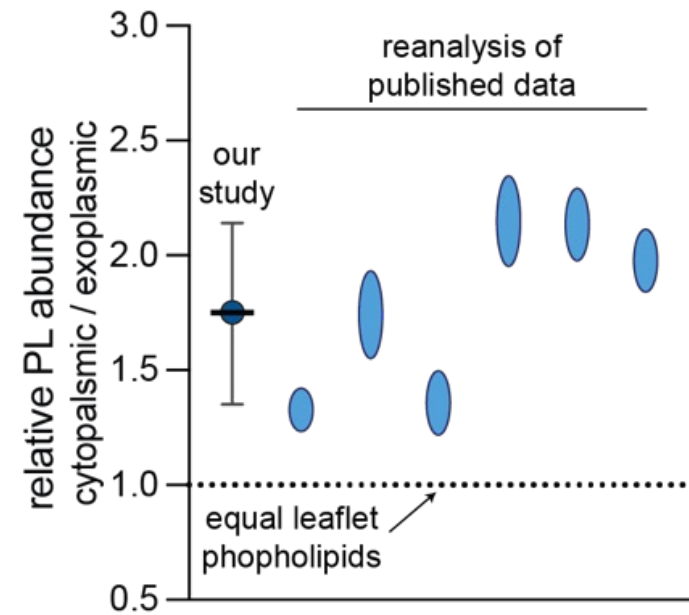
exoplasmic (SM-rich) leaflet
much FEWER phospholipids than
cytosolic (PUFA-rich) leaflet!

A (not so) surprising result

What is the relative total abundance of lipids in the two leaflet?



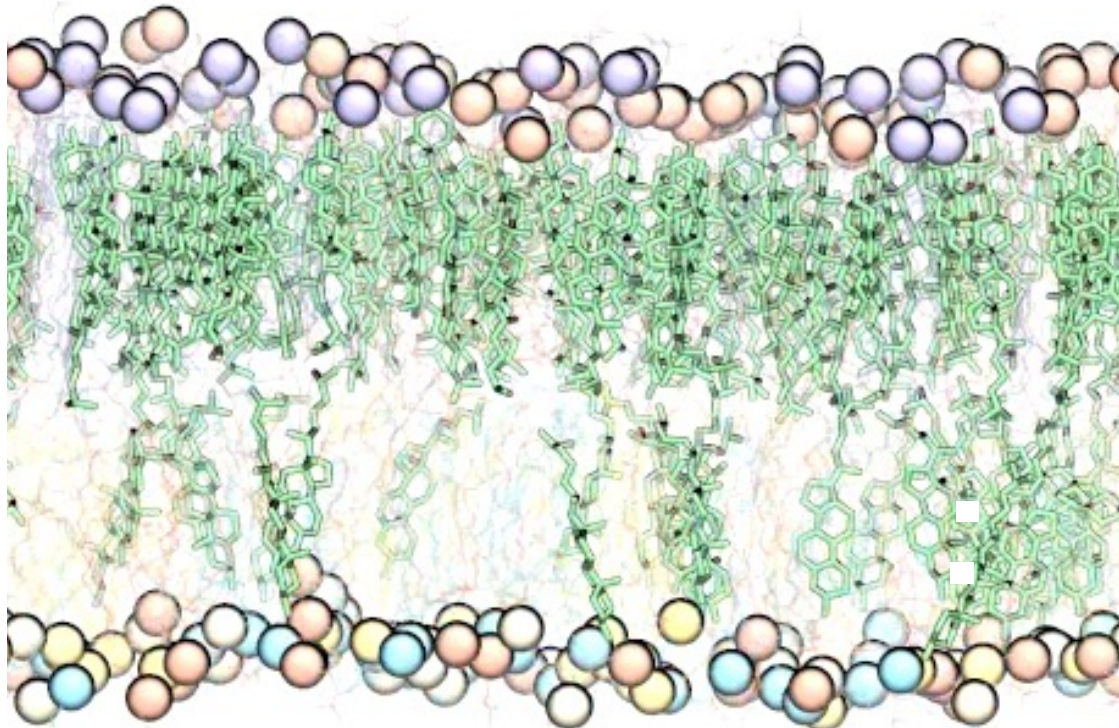
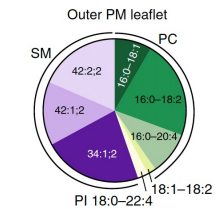
exoplasmic (SM-rich) leaflet
much FEWER phospholipids than
cytosolic (PUFA-rich) leaflet!



Phospholipid (PL) abundance
imbalance is evidenced in
published data!

How is that possible?

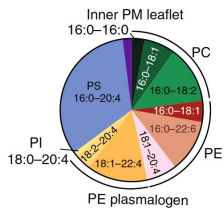
All-atom simulation of PM model



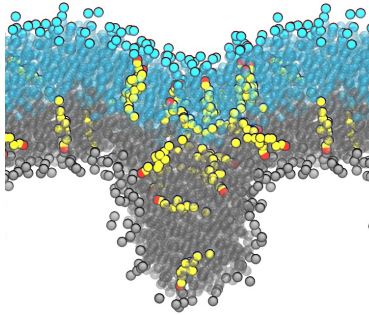
Cholesterol



Phospholipid (PL) abundance imbalance is tolerated via **cholesterol asymmetry**



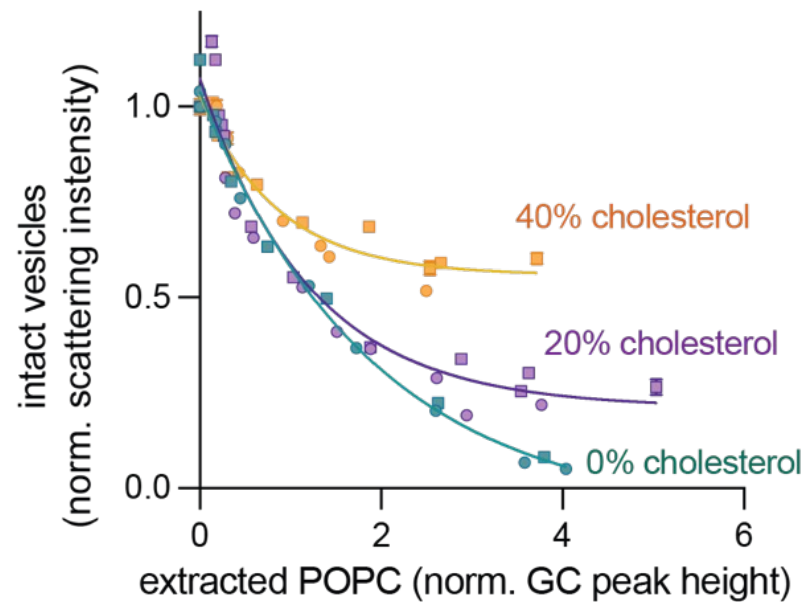
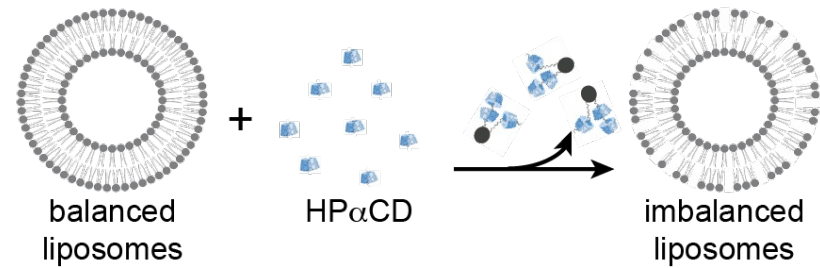
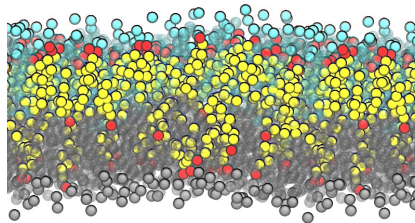
Chol increases tolerance for PL imbalance



↑ + little Chol (10–20%)

fixed PL imbalance

↓ + more Chol (40–50%)



High Chol mol%



more phospholipids
extracted from outer
leaflet before vesicle
dissolves

Chol is enriched in the outer leaflet

The exoplasmic PM leaflet is both

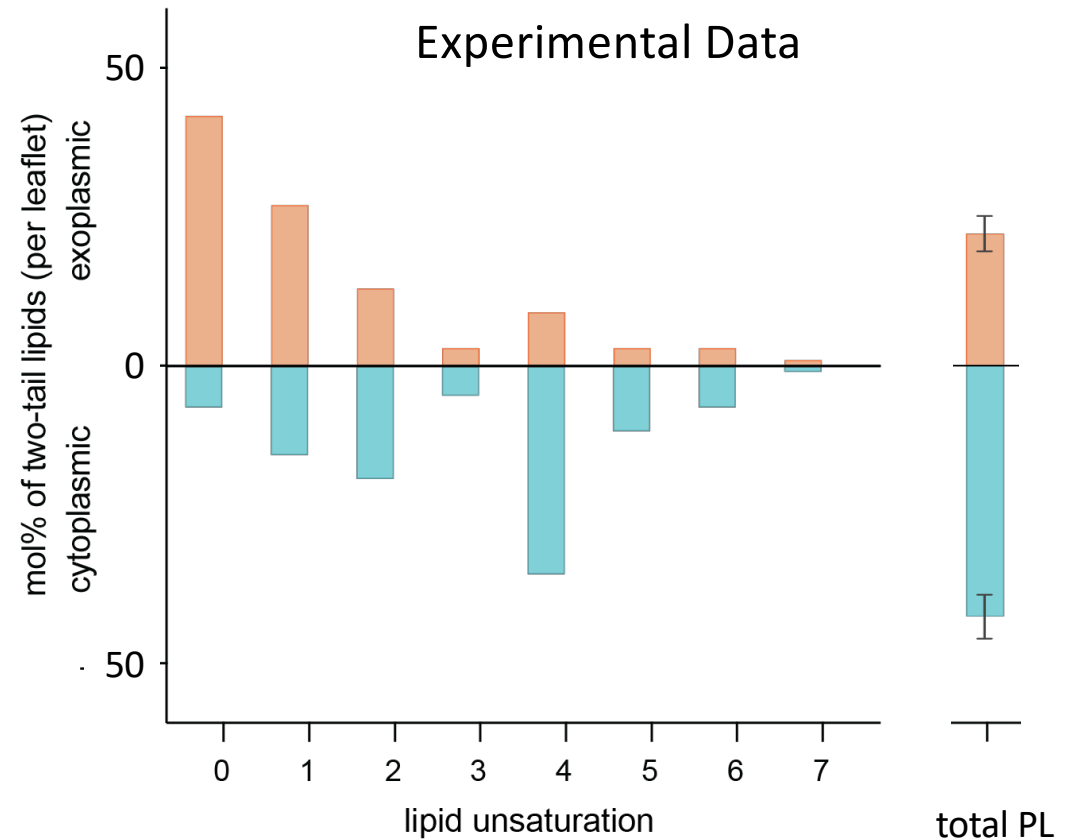
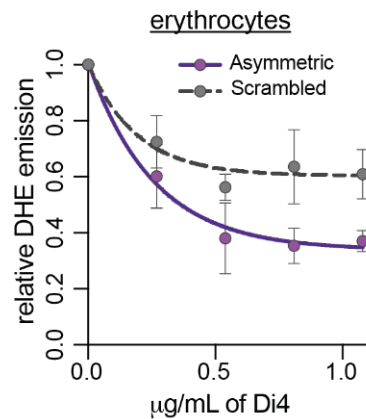
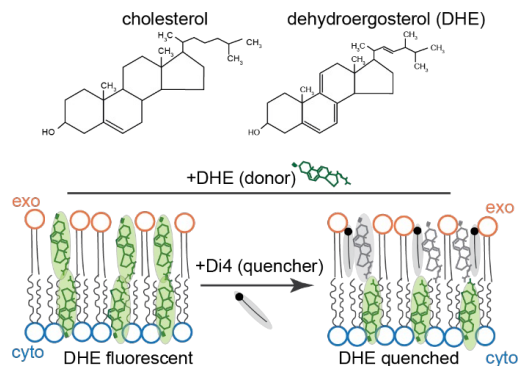
more saturated

AND

underpopulated!



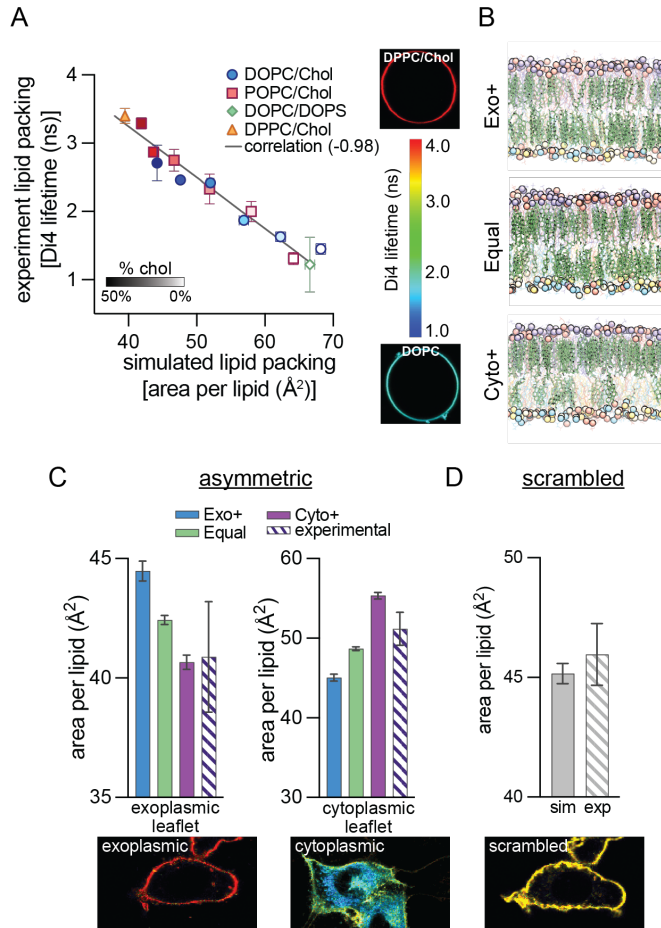
Chol must be enriched there



~2/3 of all Chol is in the exoplasmic leaflet in RBCs.

Membrane asymmetry in cells

Model validation



Membrane properties

Permeability

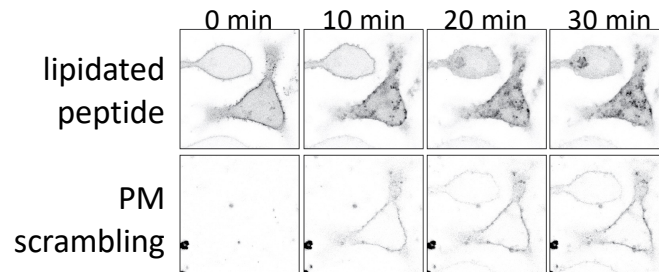
- Less permeable than scrambled PM.
- **Exo** leaflet **bottleneck** of permeability

Differential stress

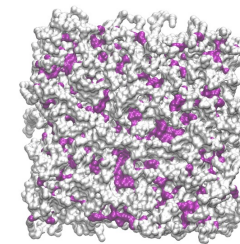
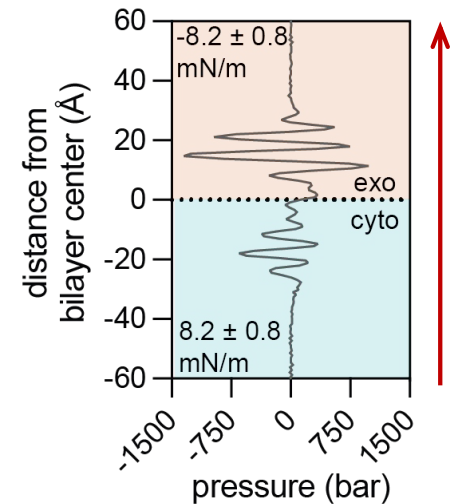
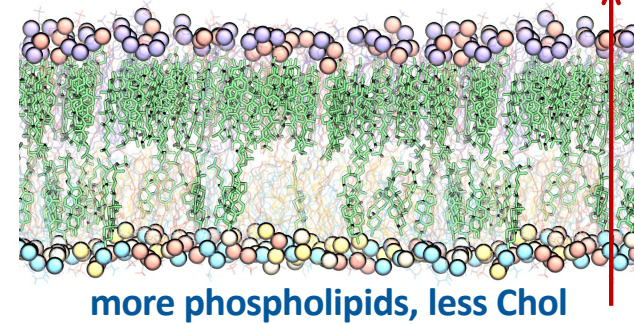
- Exo leaflet compressed
- Cyto leaflet has tension

Protein-membrane interactions

- Hydrophobic **defects** modulate binding of lipidated proteins to cyto leaflet

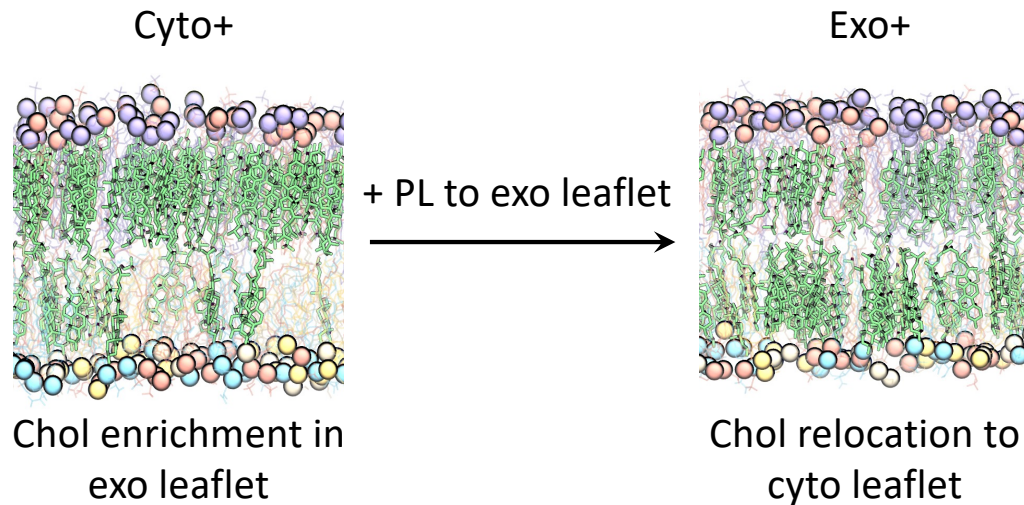


less phospholipids, more Chol

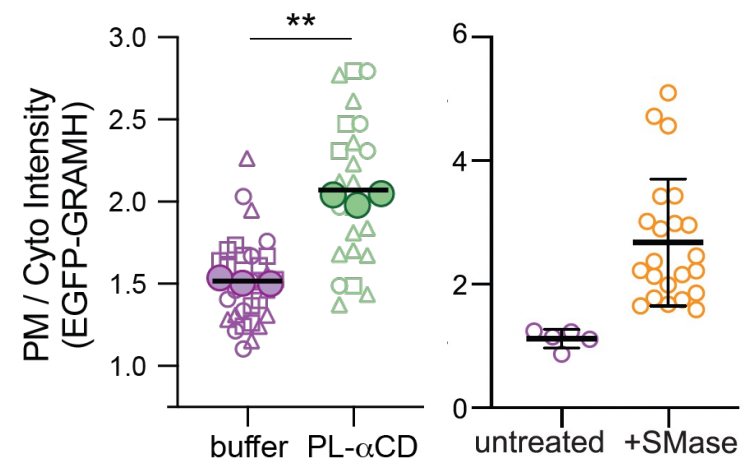
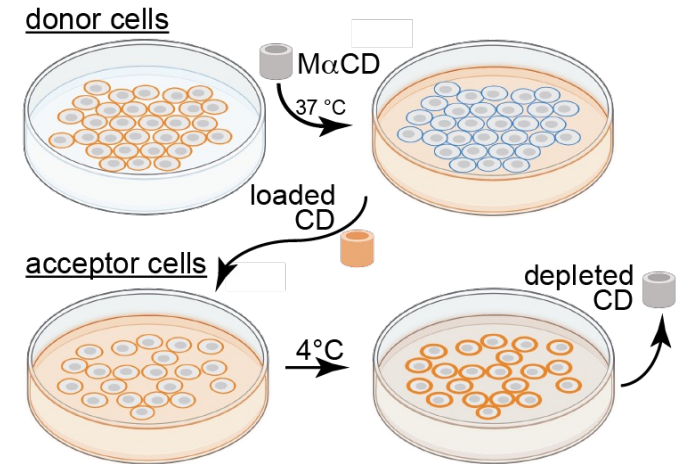
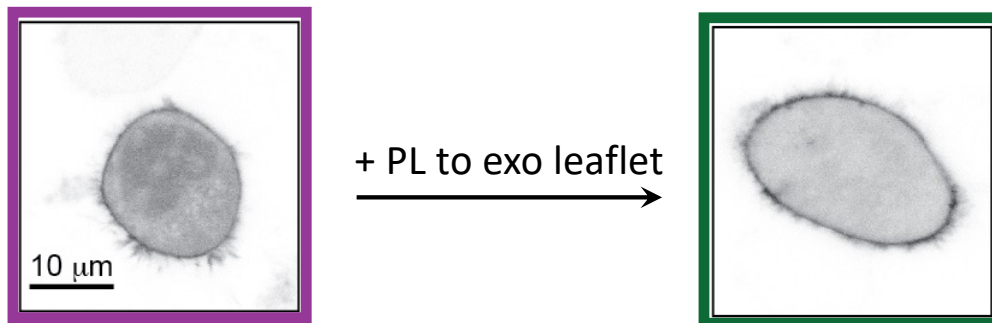


Hydrophobic defects

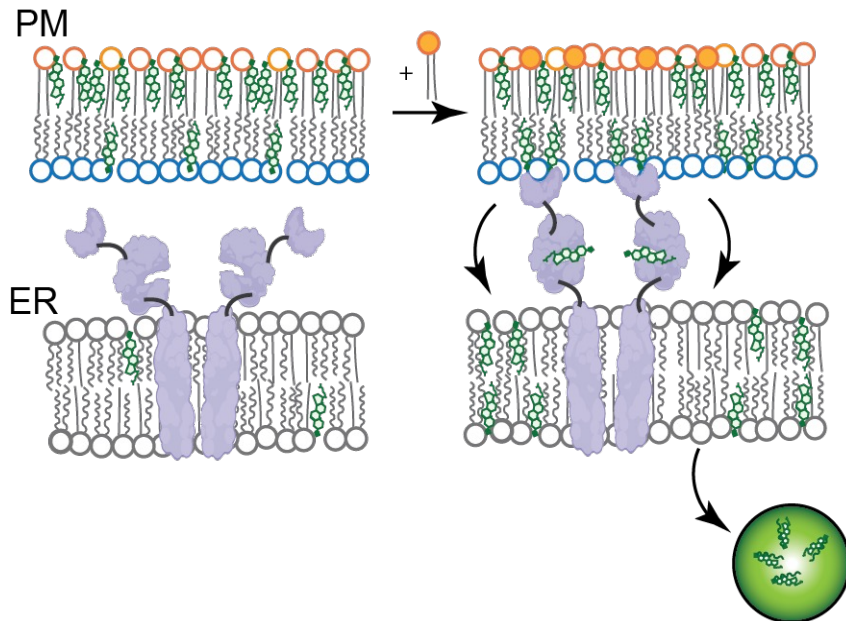
Physiological implications of PM lipid imbalances



**Chol-binding protein
EGFP-GRAM-H**

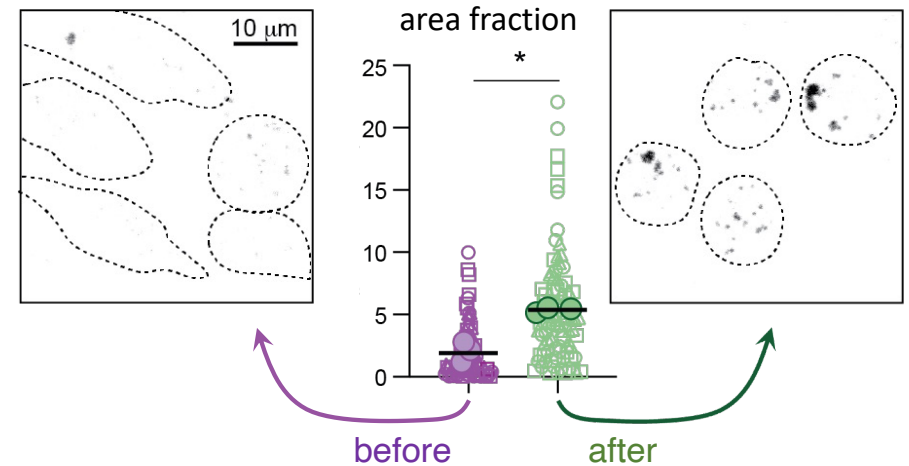


Physiological implications of PM lipid imbalances



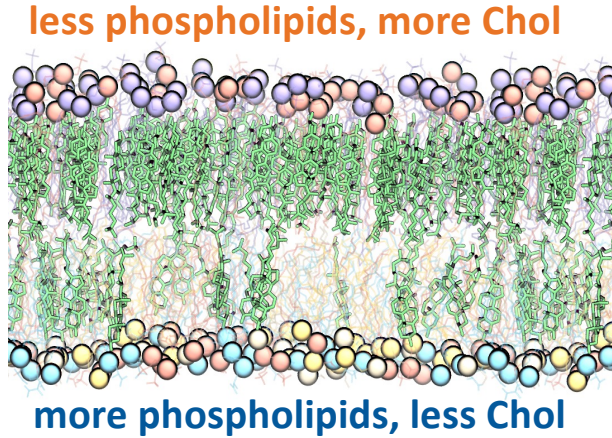
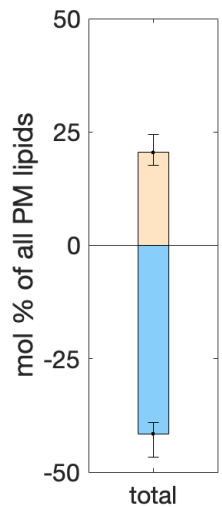
- Same total Chol amount but **more in cytoplasmic leaflet**
- Higher Chol **in cyto leaflet** perceived as excess Chol in PM
- Triggered response to remove and discard

Lipid droplet marker



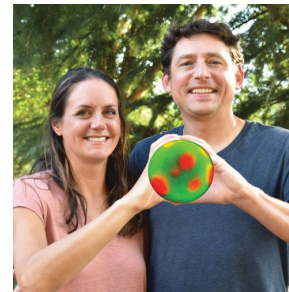
Lipid imbalances between leaflets
affect cellular cholesterol
homeostasis!

Number asymmetry can be hidden



In mammalian plasma membranes:
exists naturally and is functional

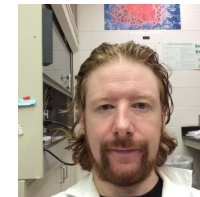
Doktorova, ... KR Levental, I Levental. 2025. *Cell*. 188:2586



Ilya & Kandice Levental
Jessica Symons
Xiaoxuan Zhang
Hong-Yin Wang



Ed Lyman
U Delaware



Joseph Lorent
UCLouvain



Fred Heberle
UT Knoxville
Stockholm U



Jan Schlegel
Erđinć Sezgin
SciLifeLab

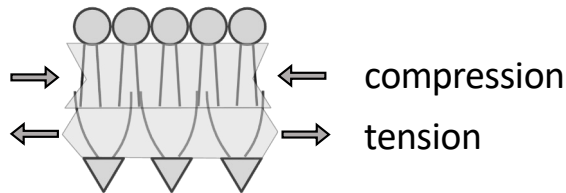


XSEDE

Extreme Science and Engineering
Discovery Environment

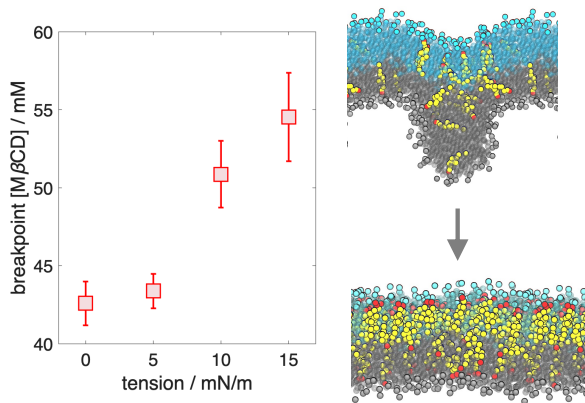
NIH National Institutes of Health

Summary

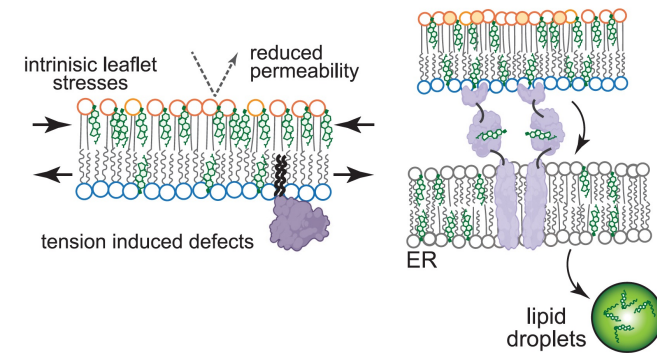


- Number asymmetry: relatively unexplored dimension of membrane structure

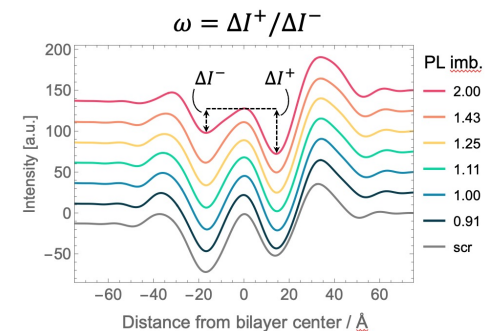
- Phospholipid imbalance in biological membranes can exist naturally and be functional



- Net tension and cholesterol can increase membrane tolerance for number asymmetry



- Cryo-EM emerging as a promising tool for analyzing relative leaflet packing in liposomes with and without Chol



The CMBL team

Aymane Laabadi
Emines, Morocco

