A structural basis for the inhibitory effect of cholesterol on mitochondria outer membrane permeabilization by Bax

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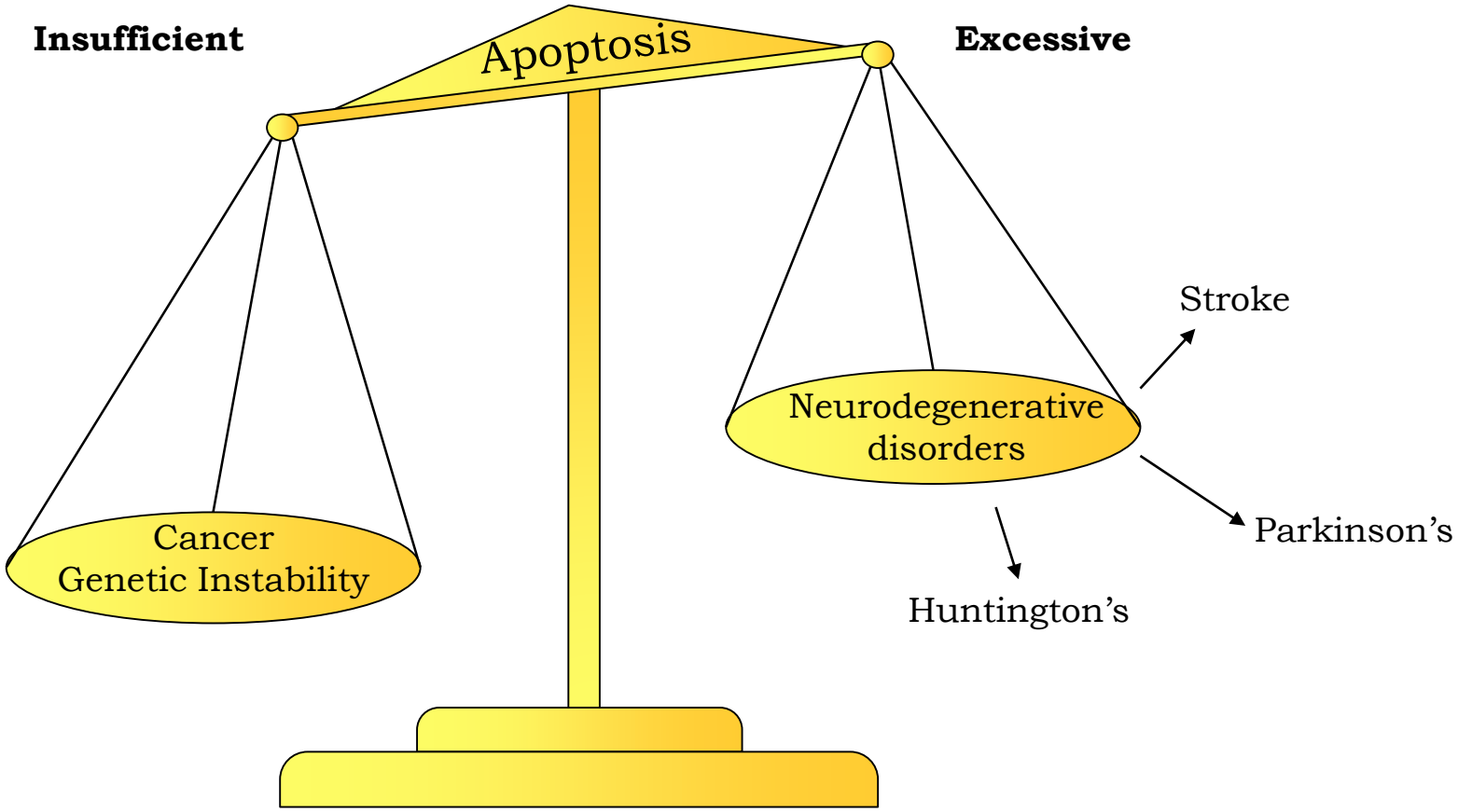
Goal of Research

- Study the structure and structural changes of a five component lipid membrane that models the mitochondria outer membrane with added cholesterol
- Use X-ray and Neutron Scattering

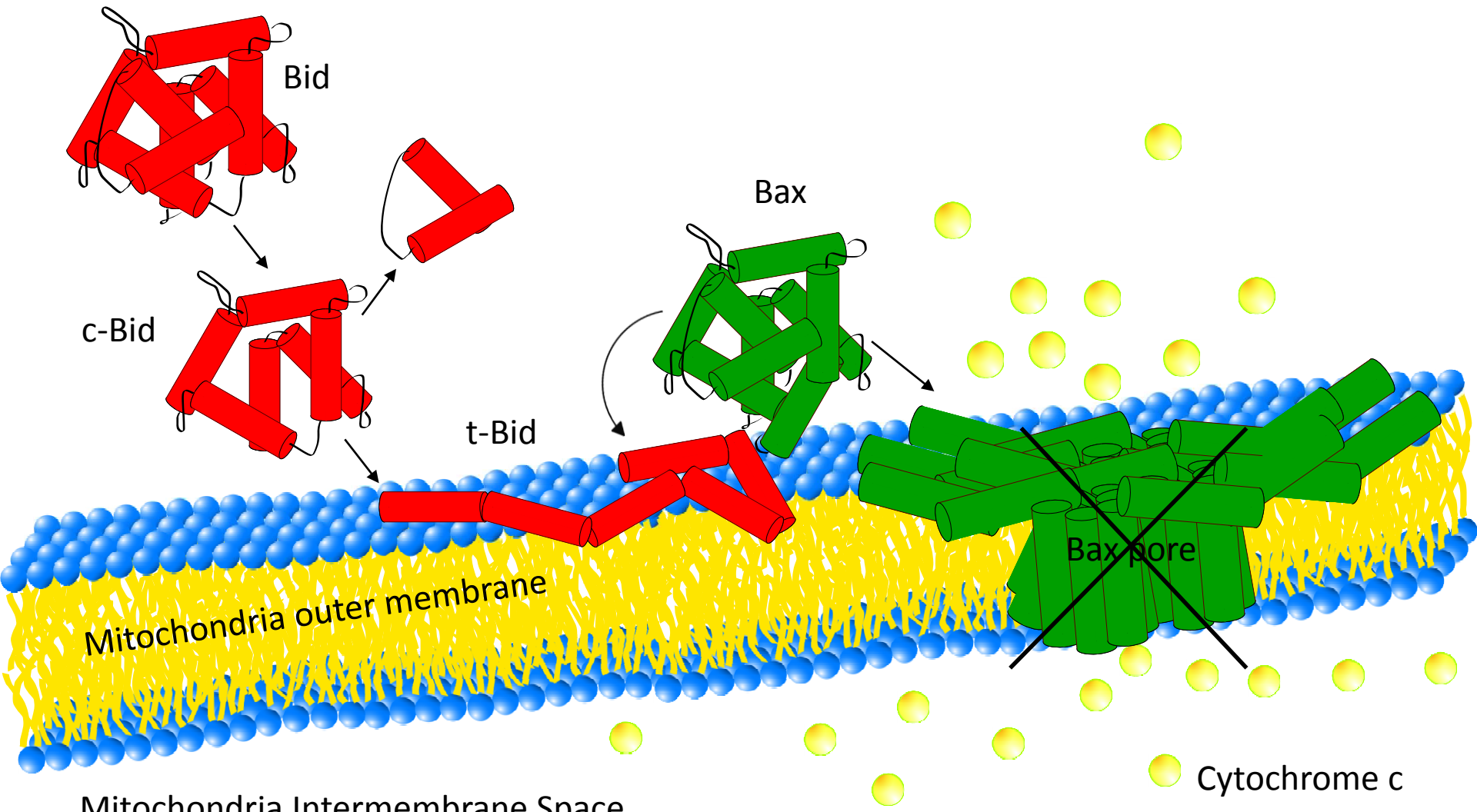
WHY ?

- The mitochondria outer membrane is involved in apoptosis
- Cholesterol inhibits a step in apoptosis involving the mitochondria outer membrane
- Our hypothesis is that the inhibition comes from a structural change in the membrane brought about by cholesterol.

Deregulation of Apoptosis Leads to Disease States



Bid and Bax Mediate Mitochondria Outer Membrane Permeabilization



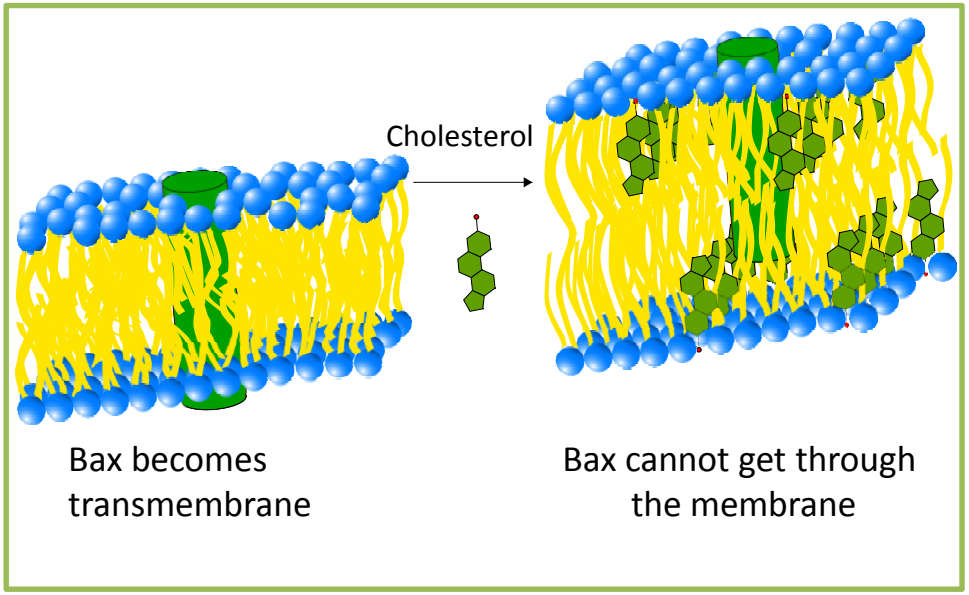
Cholesterol Inhibits Bax Insertion, Why?

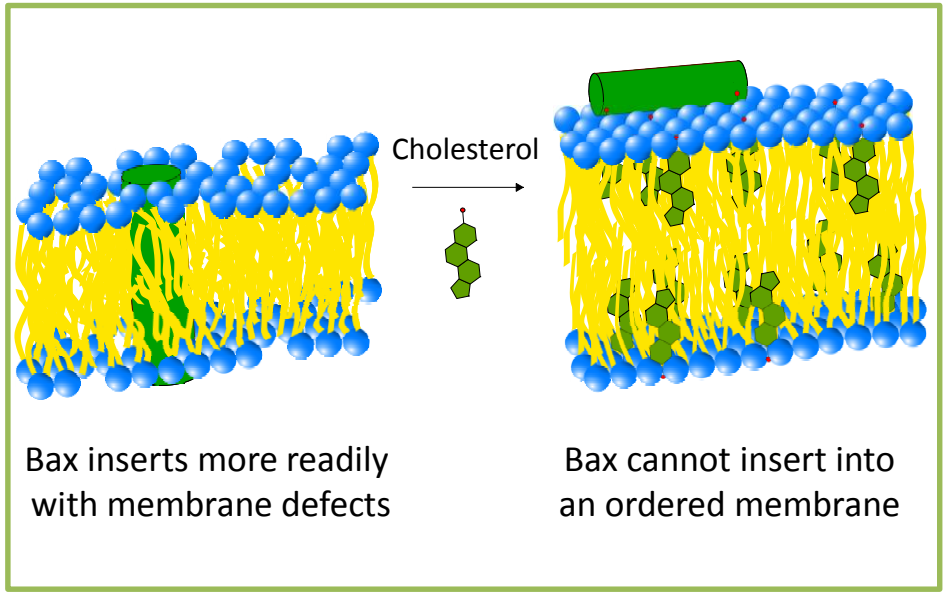
- Enantiomer of cholesterol also has this inhibitory effect ruling out a biochemical interaction

Structural Basis for Inhibition

Thickness changes

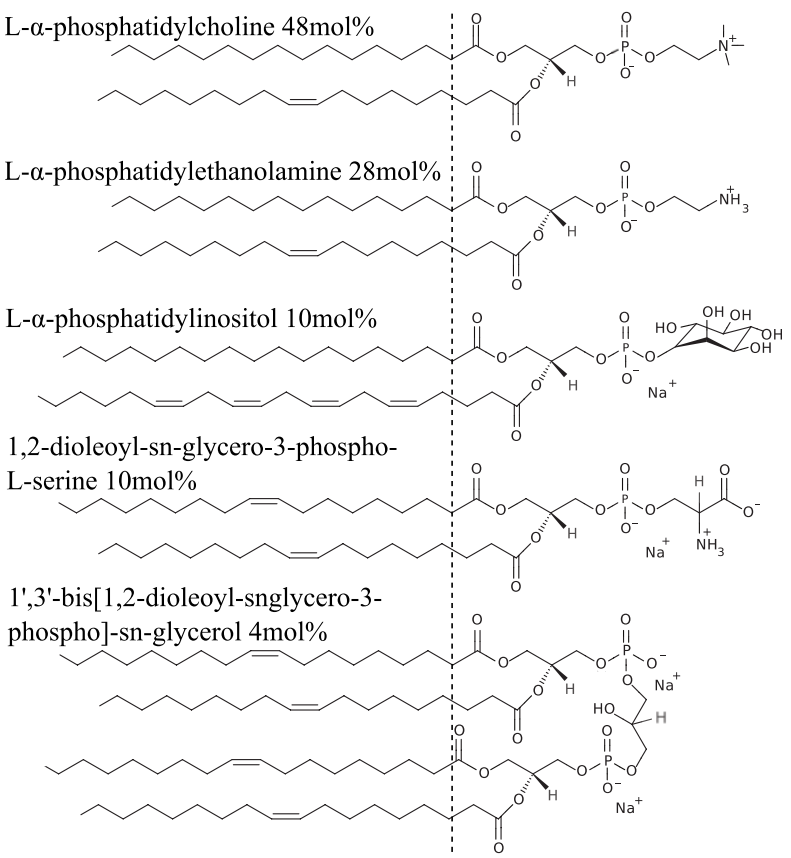
Condensation effect and membrane fluidity





Model System: Mitochondria-like Membrane and Cholesterol

5 component Mitochondria-like membrane



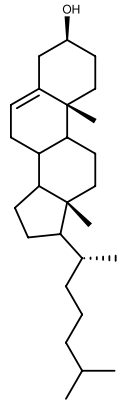
PC 16:0, 18:1

PE 16:0, 18:1

PI 18:0, 20:4

DOPS 18:1

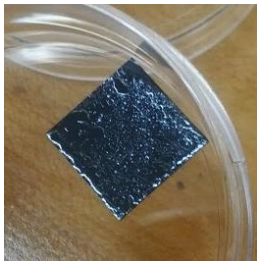
TOCL 18:1

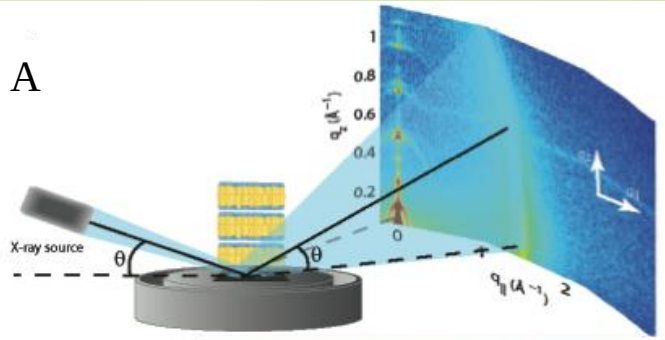


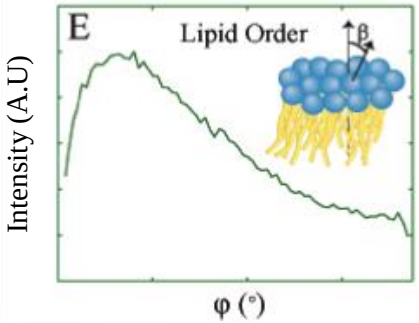
Cholesterol

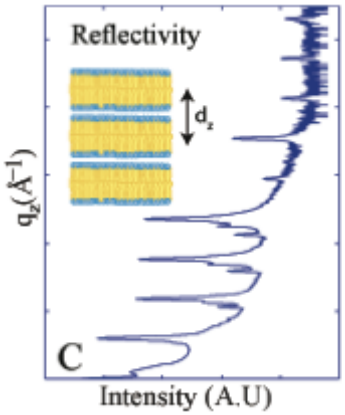
Experimental Setup & Results

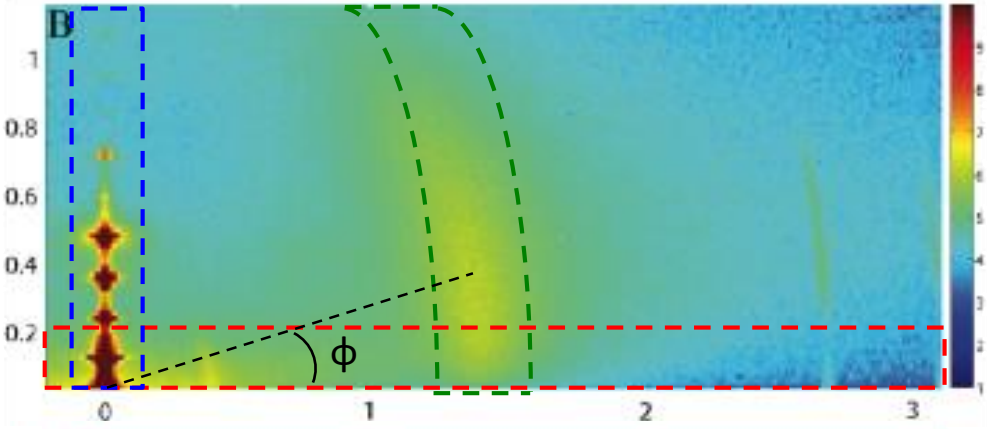
X-ray: Information for Different Scans

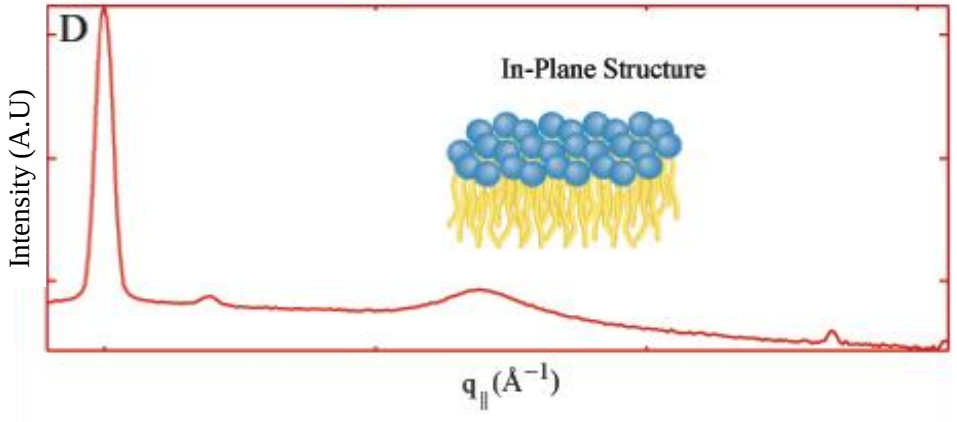








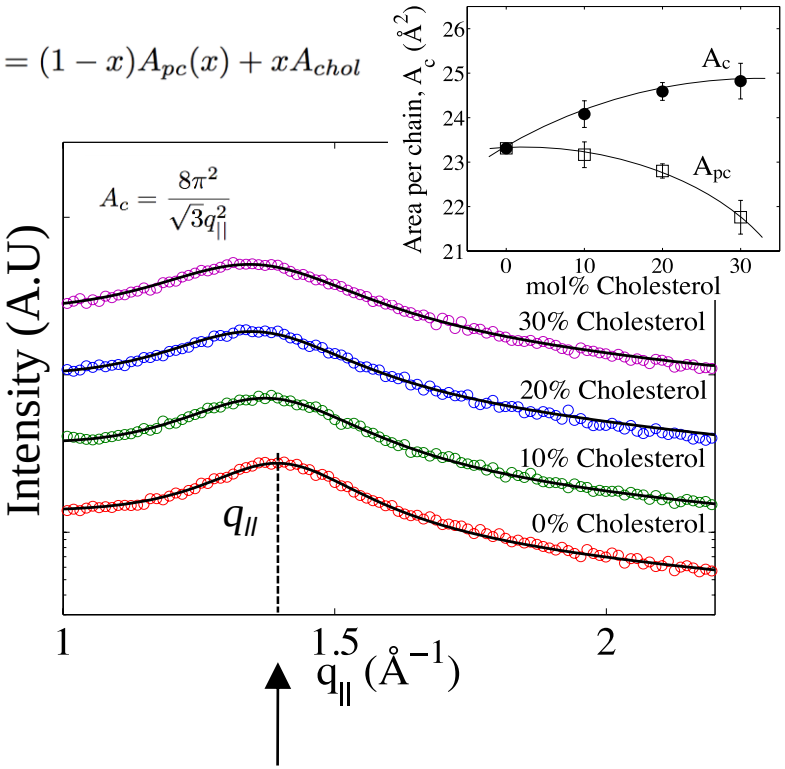




83%RH
28 ˚C
BLADE

X-ray: In-Plane Diffraction Gives Lateral Structure

$$A_c(x) = (1-x)A_{pc}(x) + xA_{chol}$$



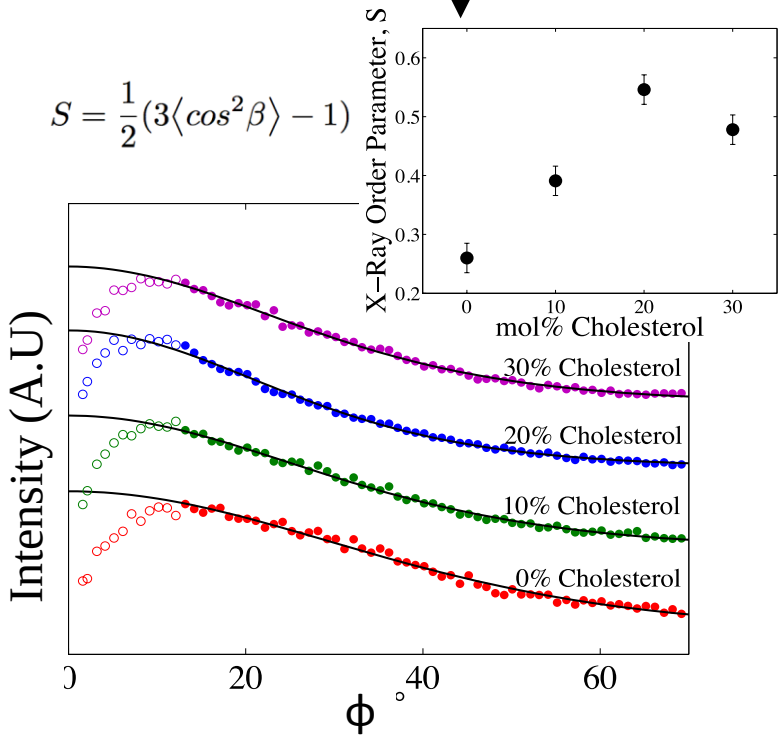
Cholesterol Condensation Effect

In plane X-ray diffraction shows cholesterol increases the area per total chain molecule while decreasing the area per phospholipid chain

Cholesterol Increases Lateral Order

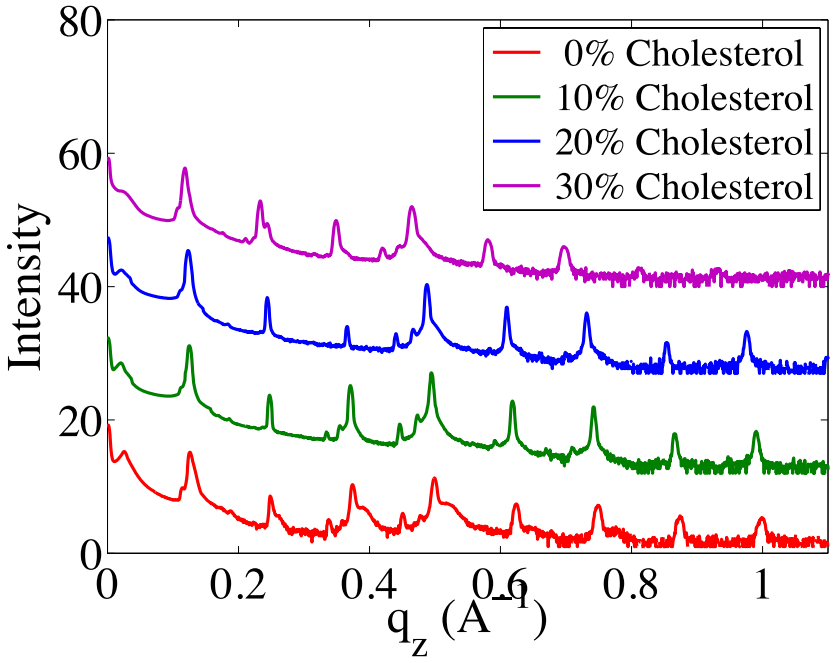
Integrating along the in plane correlation peak shows cholesterol increases the x-ray order parameter, thus decreasing the fluidity of the membrane

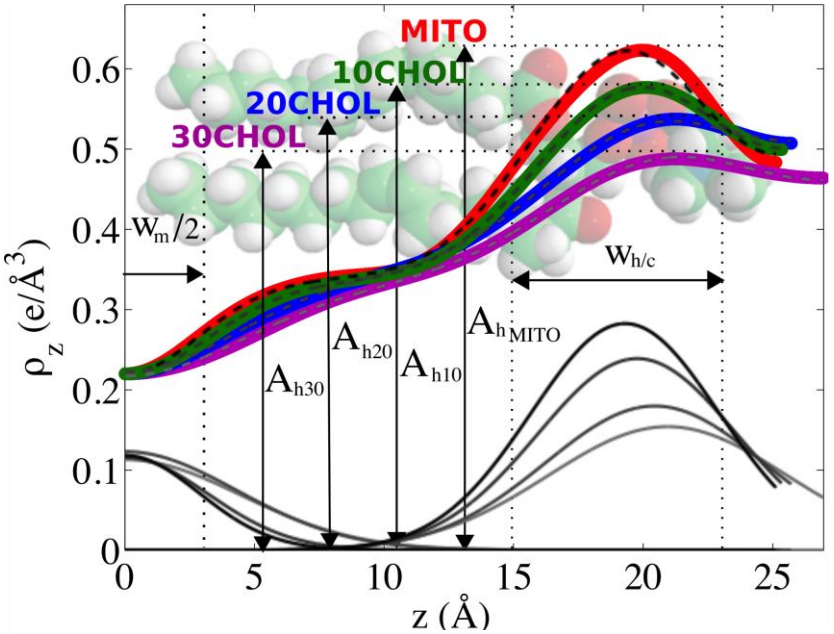
$$S = \frac{1}{2}(3\langle cos^2\beta\rangle - 1)$$



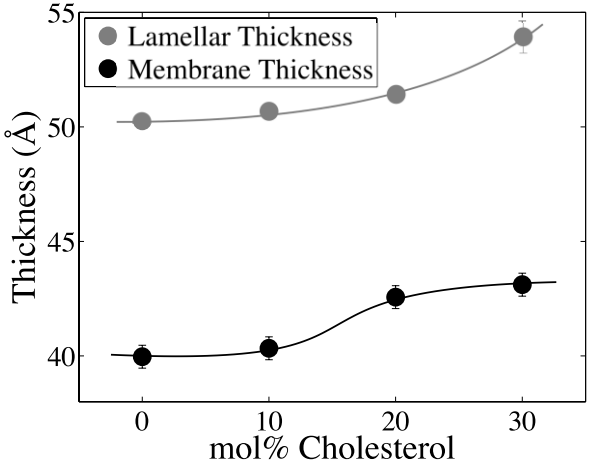
Paper in preparation: Cholesterol Increases Order in Membranes with Complex Mitochondria-Like Lipid Composition

X-ray: Out-of-Plane Diffraction Gives Vertical Structure





Reflectivity shows up to 8 orders of Bragg peaks for samples with varying amounts of cholesterol

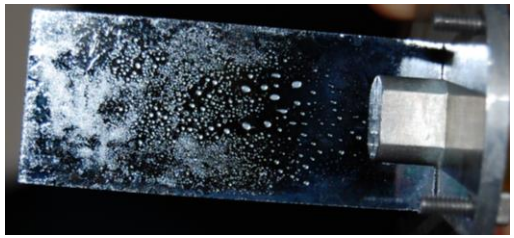


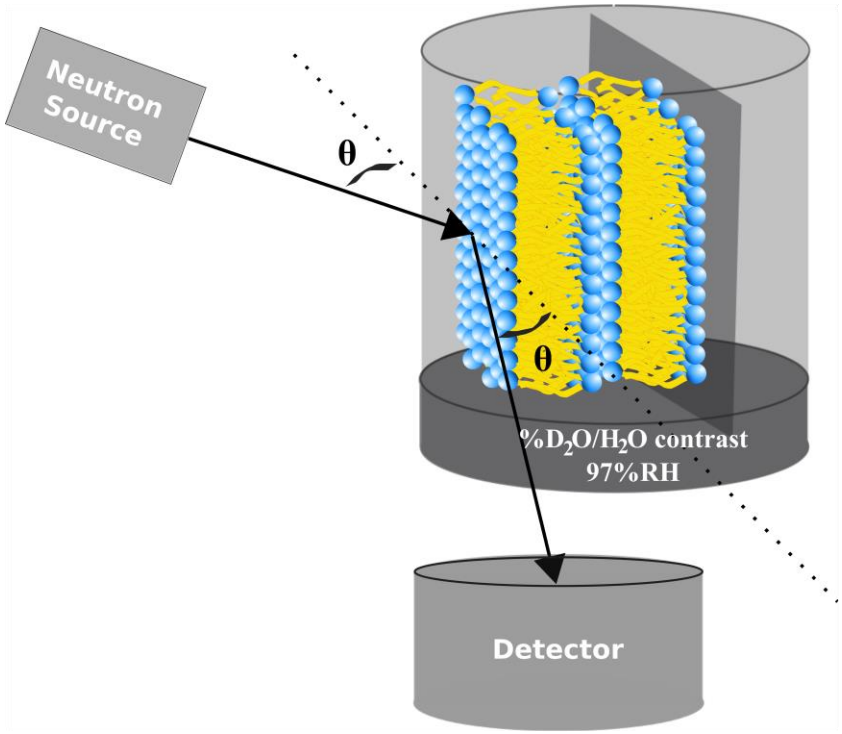
Cholesterol Thickening Effect

Electron density profiles indicate cholesterol increases lamellar and membrane thickness

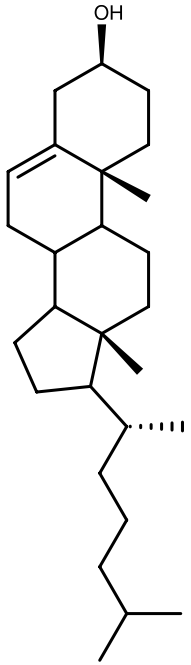
Paper in preparation: Cholesterol Increases Order in Membranes with Complex Mitochondria-Like Lipid Composition

Neutron: Geometry

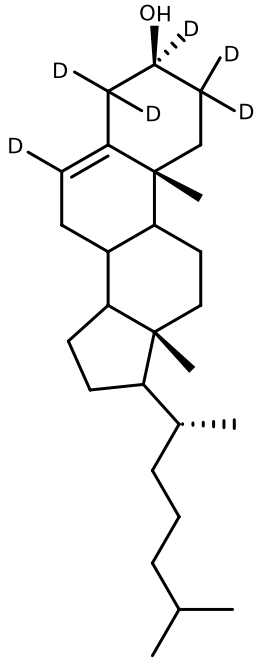




Cholesterol

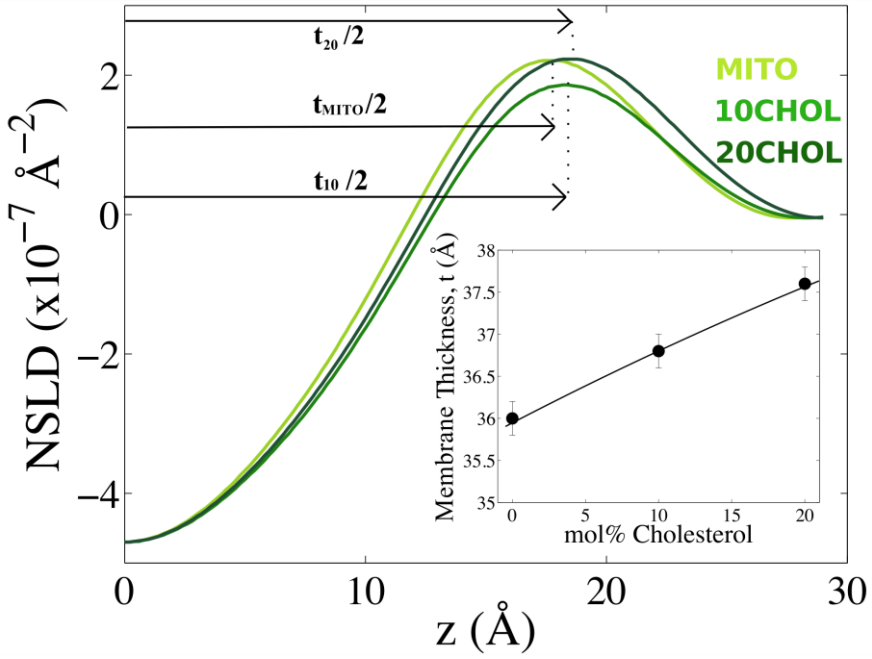


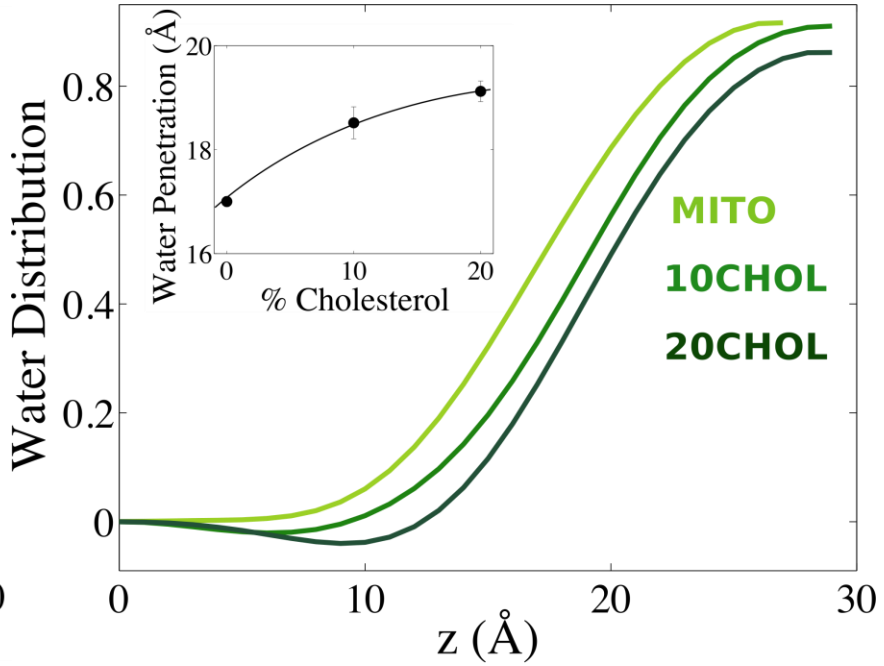
Cholesterol-2,2,3,4,4,6-d_6



N5 Beamline at the Canadian Neutron Beam Center

Neutron: Water Penetration and Thickness





Membrane thickness is increased with Cholesterol

Water penetration is increased with Cholesterol

Conclusions and Interpretations

Conclusions and Interpretations

- Both X-ray and Neutron scattering revealed an increase in membrane thickness, comparable to increases seen in simple membranes in similar conditions.[1]
- X-ray scattering demonstrated area condensation and an increase in chain ordering, again comparable to the increase seen in simple membranes.[2]
- Due to volume conservation, neutron data suggests area condensation
- Neutron scattering showed a decrease in water penetration of the same magnitude as the increase in membrane thickness; they are the same effect.
- Neutron scattering indicates that cholesterol's polar head is located at 13.6Å from the membrane center with cholesterol residing in the canonical position. This is consistent with its position in saturated membranes.[3]
- These results suggest that both hypotheses for why Bax insertion is inhibited by cholesterol are possible. Need to distinguish between these effects by changing one without changing the other.

[1] Kucerka et al (2009) *What determines the thickness of a biological membrane*. Gen. Physiol. Biohys. **28**: 117-125

[2] Mills et al (2008) *Order parameters and areas in fluid-phase oriented lipid membranes using wide angle X-ray scattering*. Biophys. **95**: 669-681

[3] Haldar et al (2012) *Differential effect of cholesterol and its biosynthetic recurs on membrane dipole potential.* Biophys. 102:1561-1569

Thank you!

Dr. Cecile Fradin
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Dr. Norbert Kucerka

Fradin Lab

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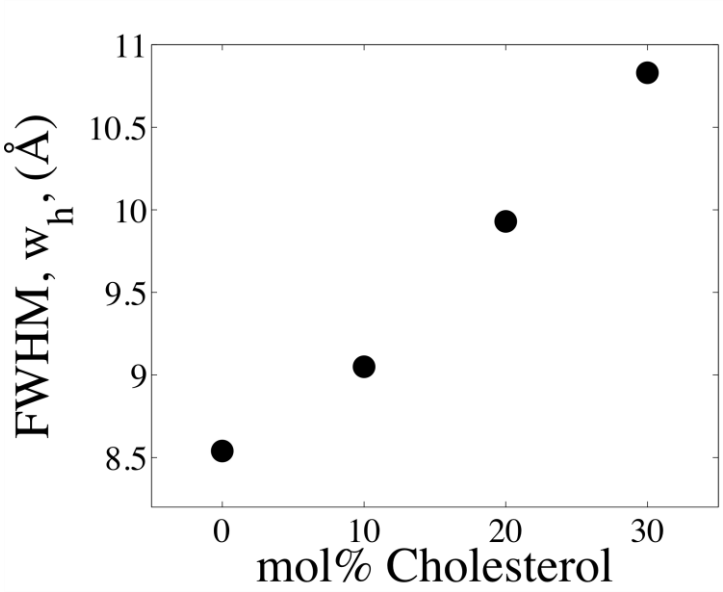
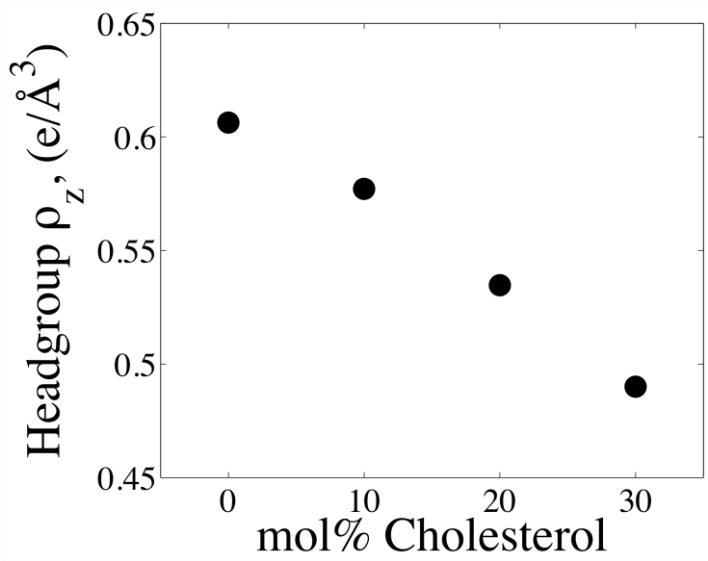
- Aisha Shamas-Din
- Annie Cheung

NSERC
CRSNG

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

Extra: X-ray Analysis





Extra: Neutron Analysis