

Reorganization of Cholesterol Containing Lipid Bilayers Through Membrane Active Peptides

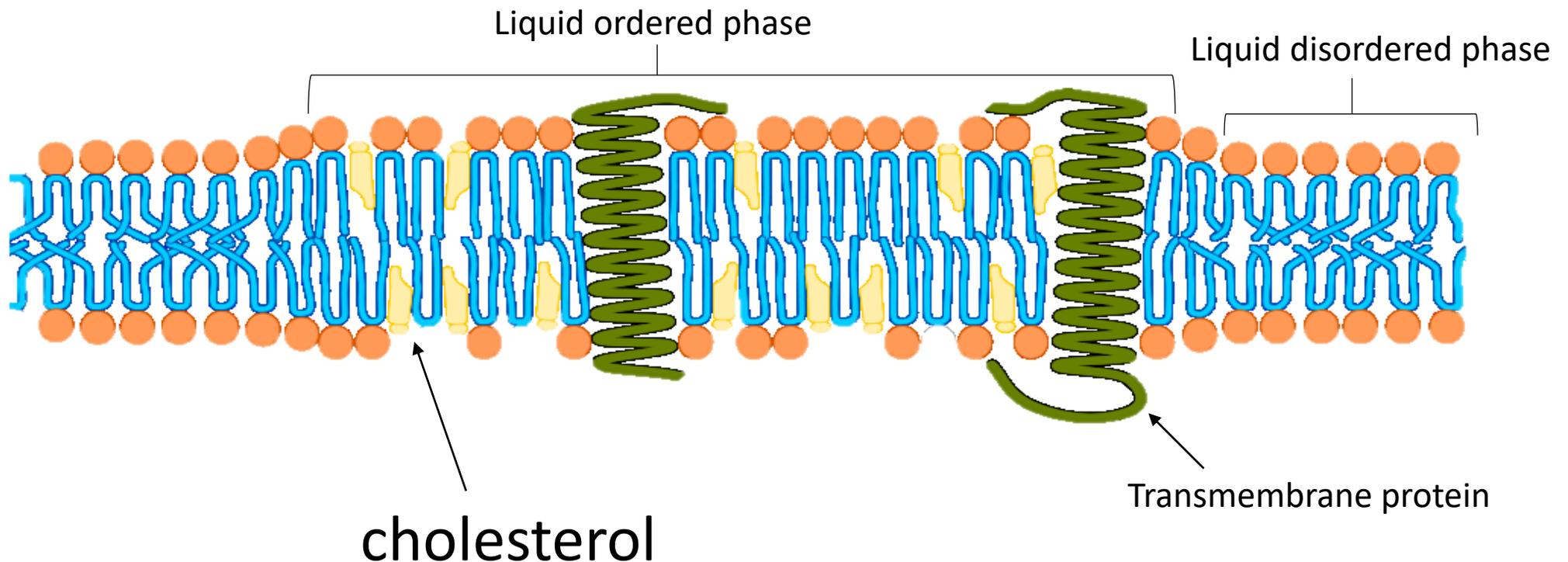
Laura Lucas

MML-Biomolecular Measurement Division-Biomolecular Structure and Function Group (IBBR)



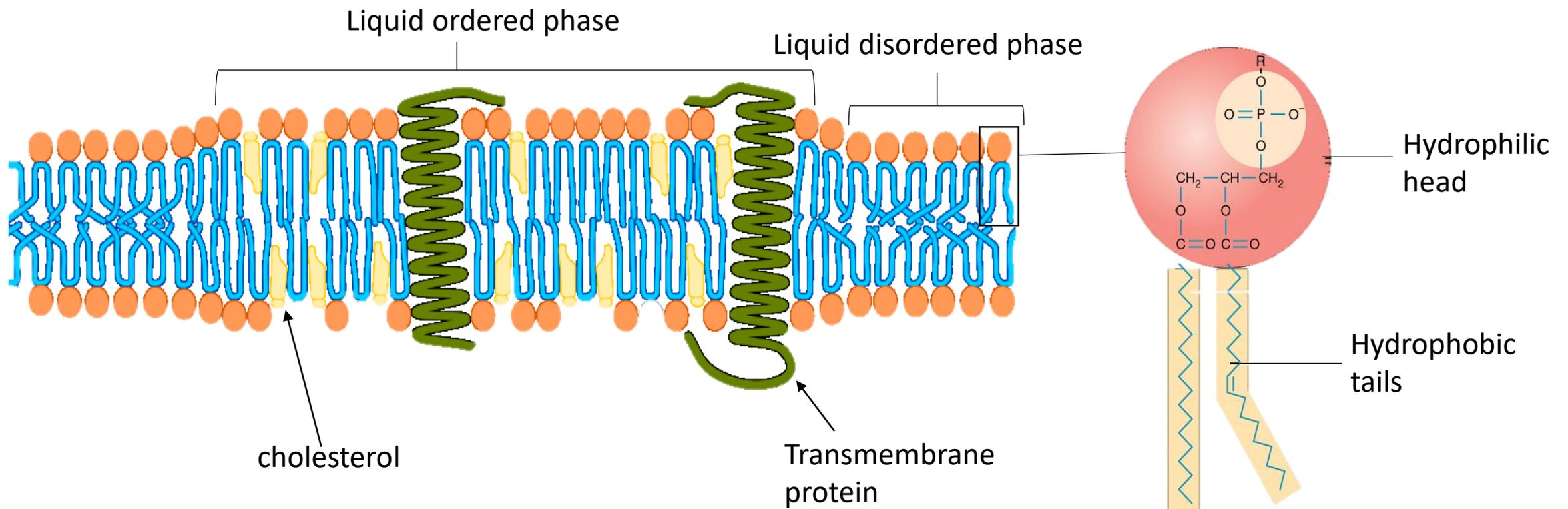
Complexity of the Plasma Membrane

- Pathogens use cholesterol to enter cells
- Cholesterol domains in plasma membrane (cell signaling)



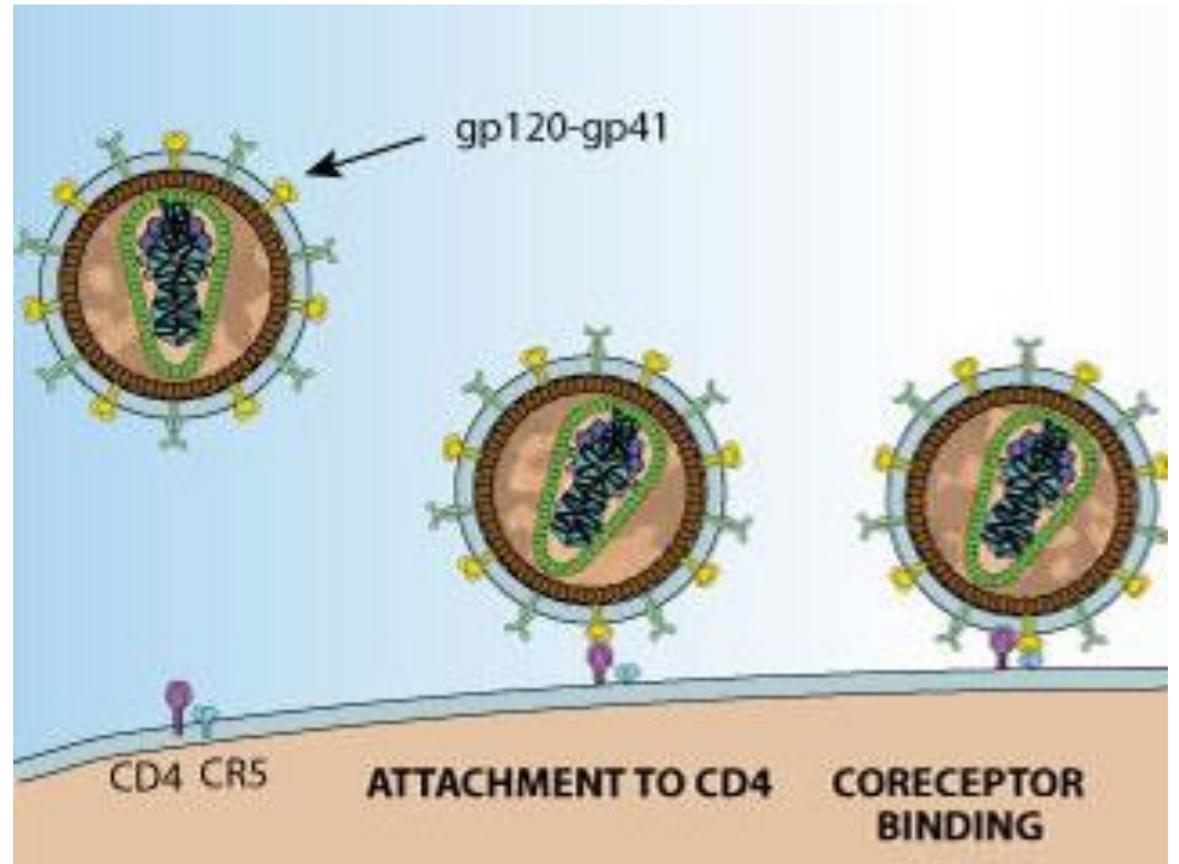
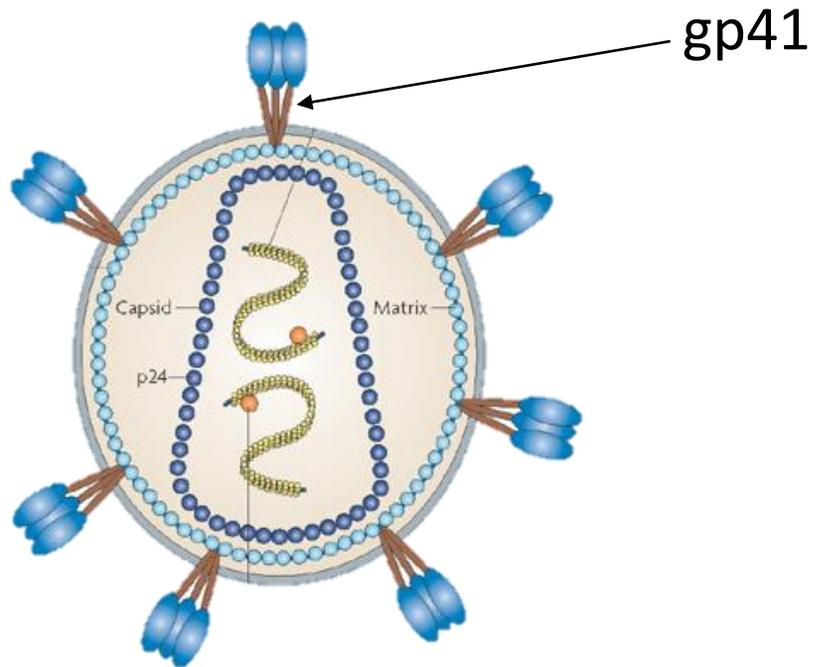
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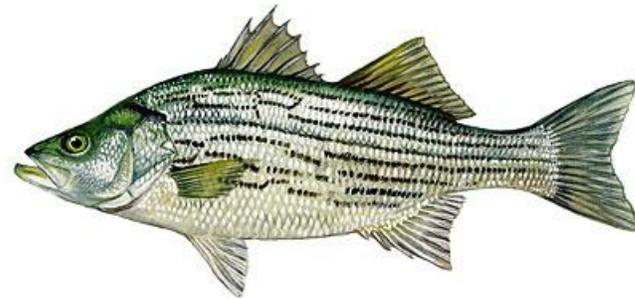
GP41

- 5 Amino Acid sequence LWYIK
- Part of the envelope of HIV
- Participates in fusion



Piscidin 1 (P1)

- Antimicrobial peptides
- 22 Amino Acid sequence
FFHHIFRGIVHVGKTIHRLVTG
- α -helix structure in membrane
- Anticancer properties

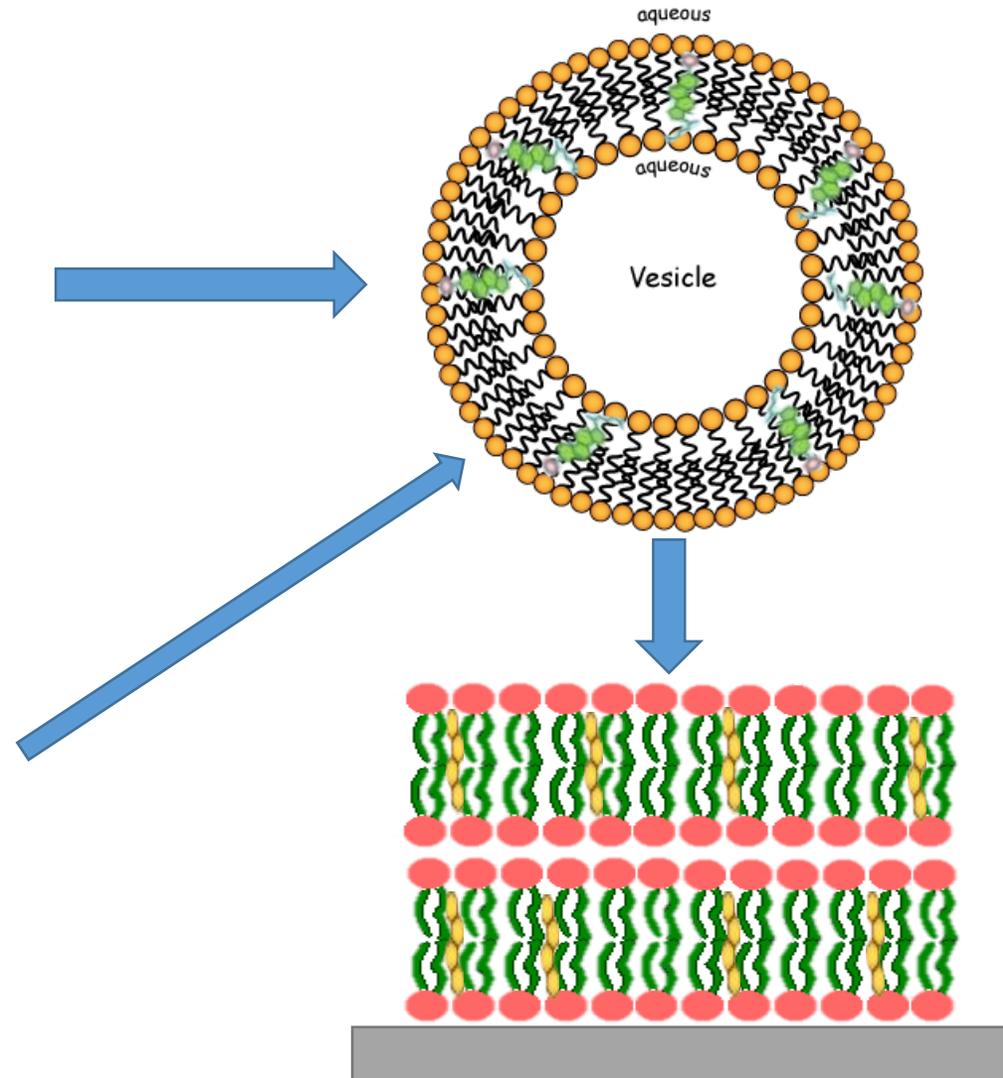
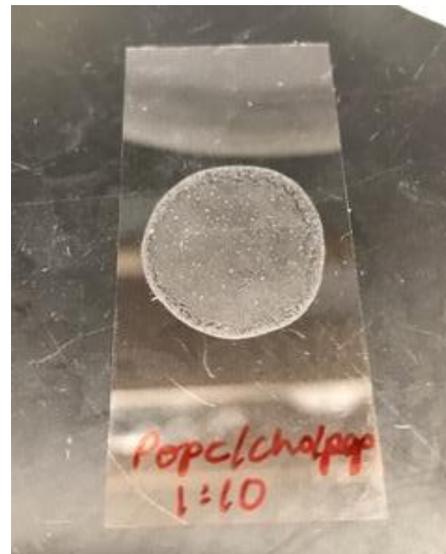


Materials and Methods

Differential
Scanning
Calorimetry

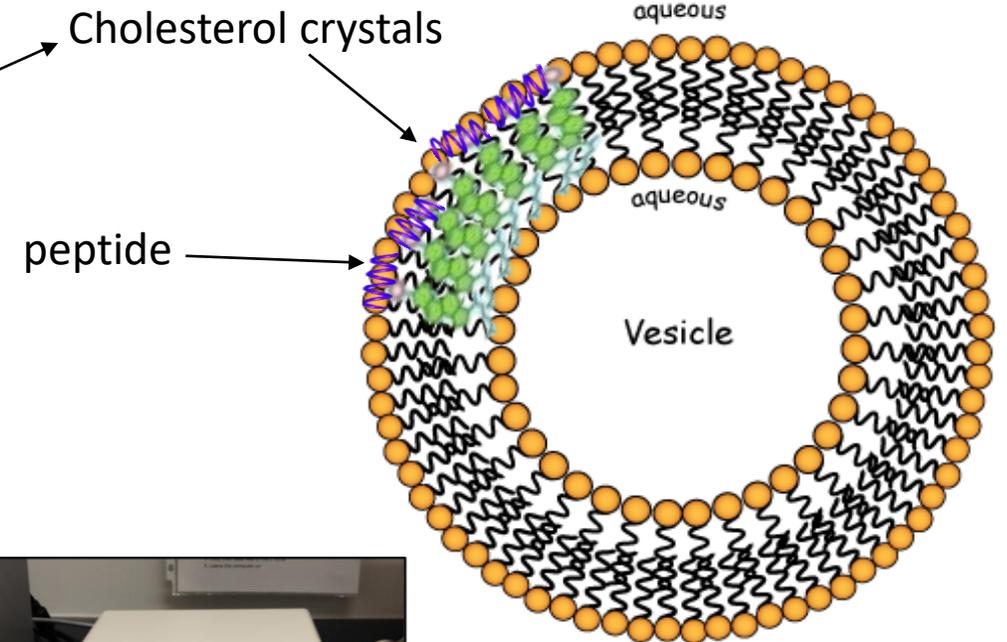
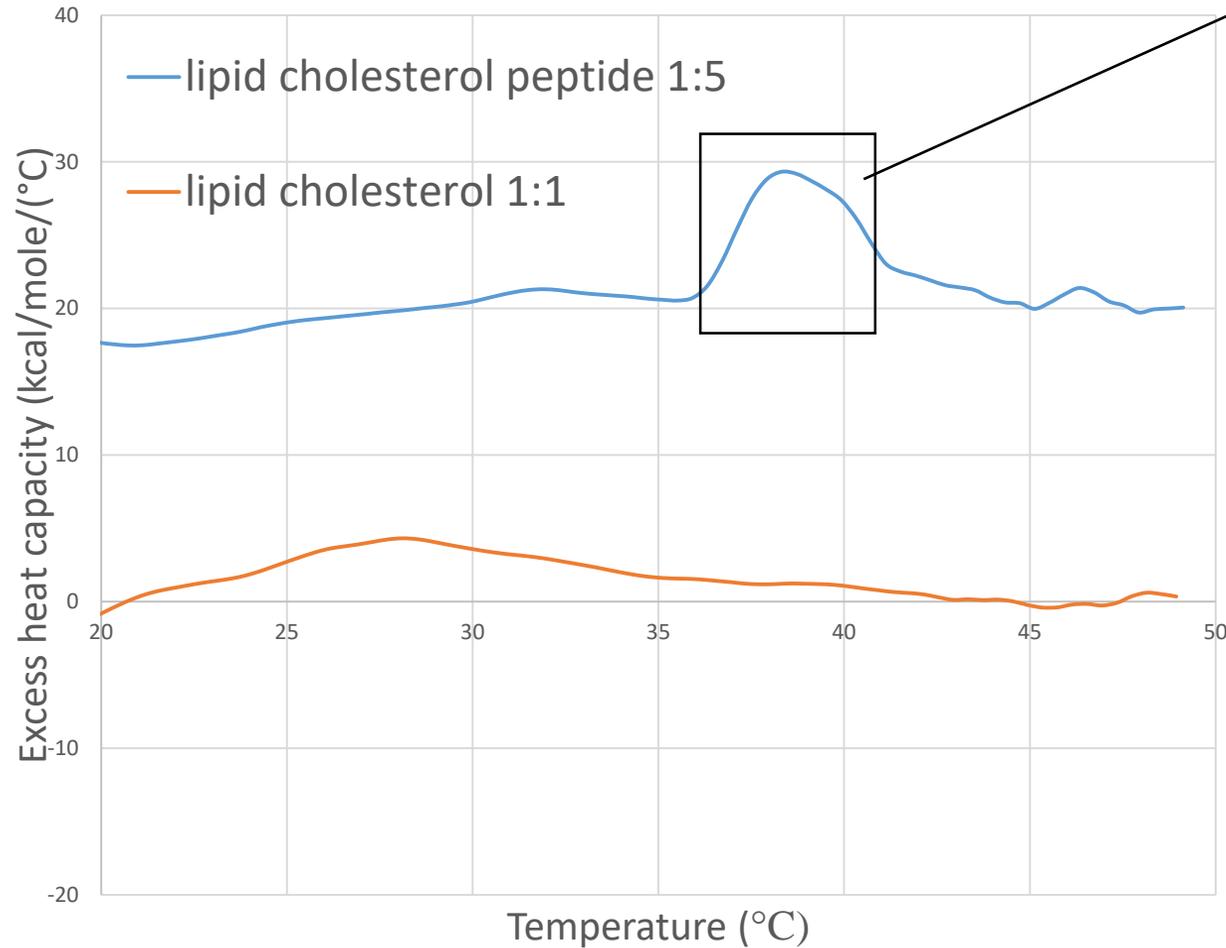


X-ray
Diffraction



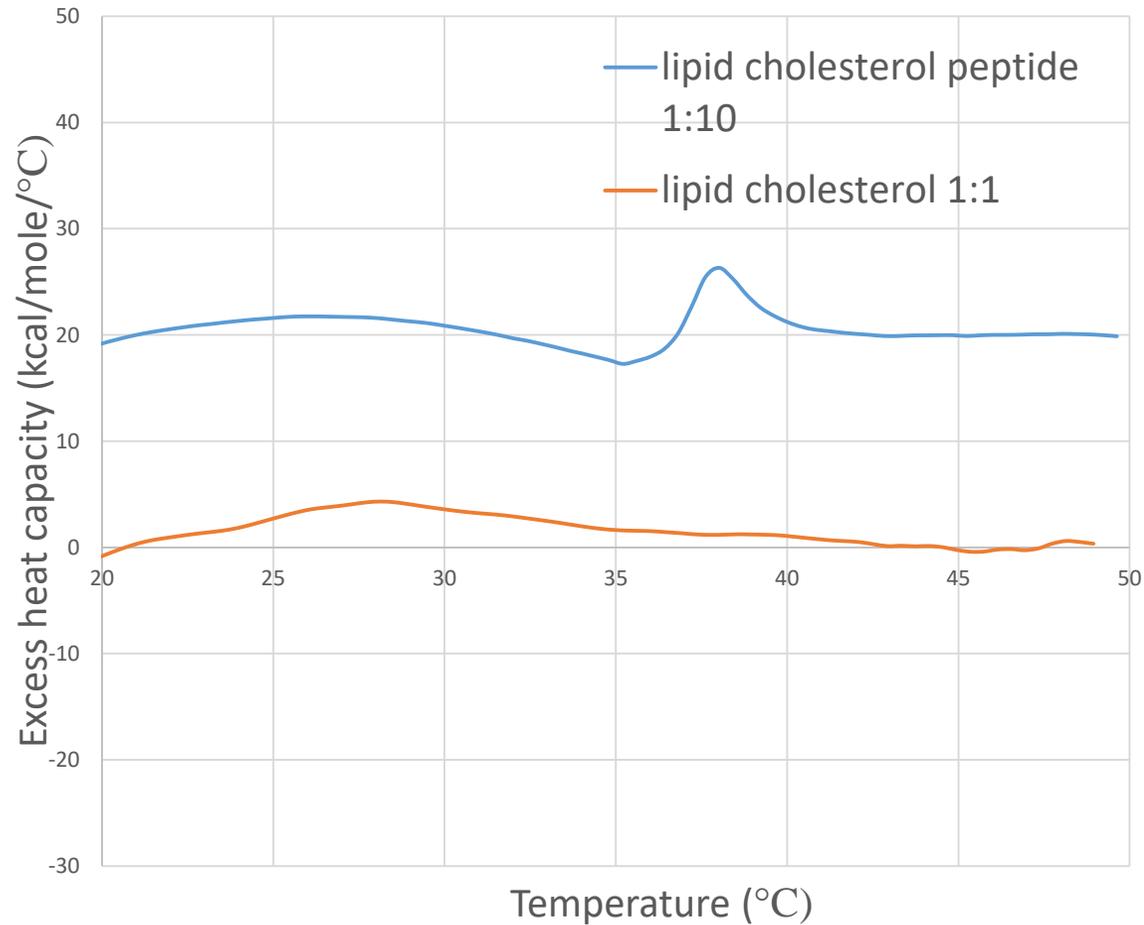
Differential Calorimetry Results with GP41 Peptide

Peptide:lipid 1:5

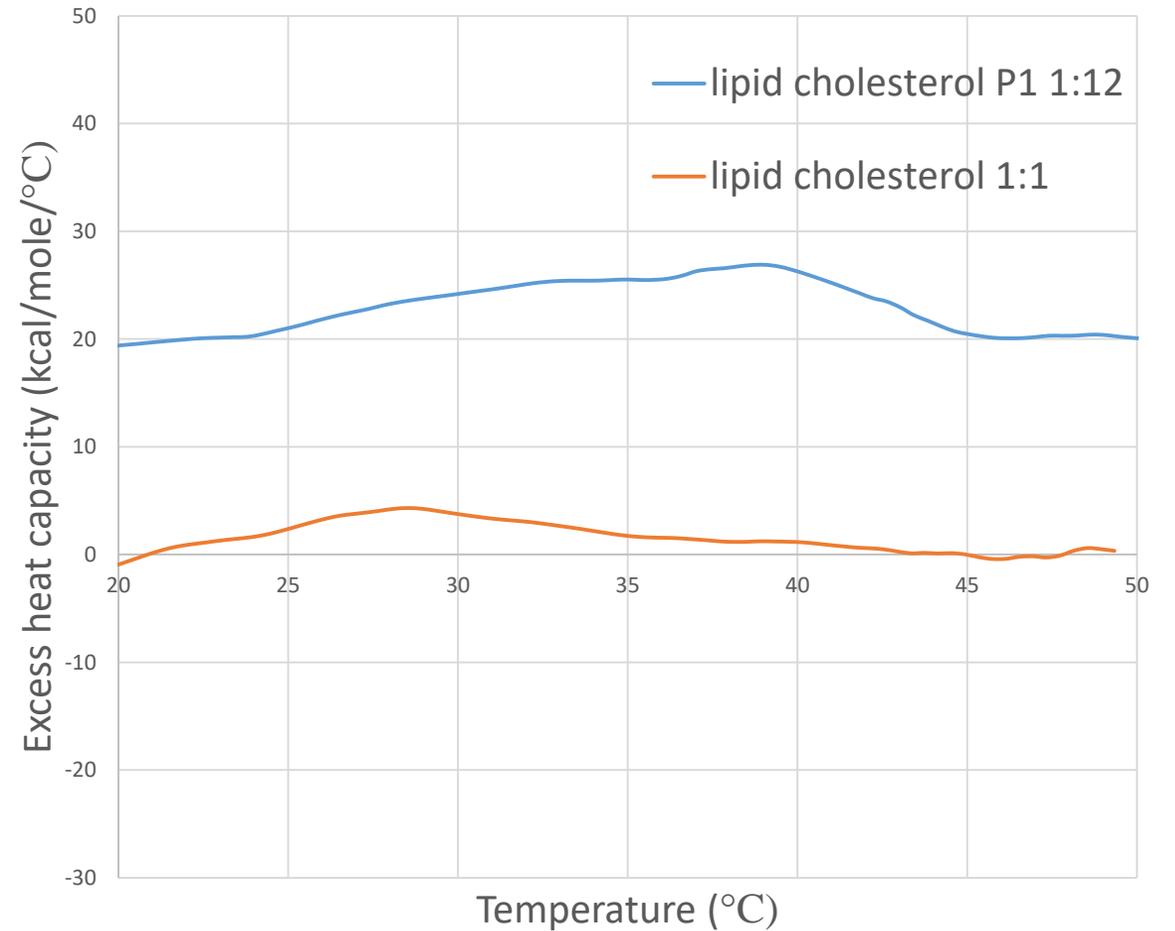


Differential Calorimetry Results with P1

GP41 Peptide:lipid 1:10



P1:lipid 1:12



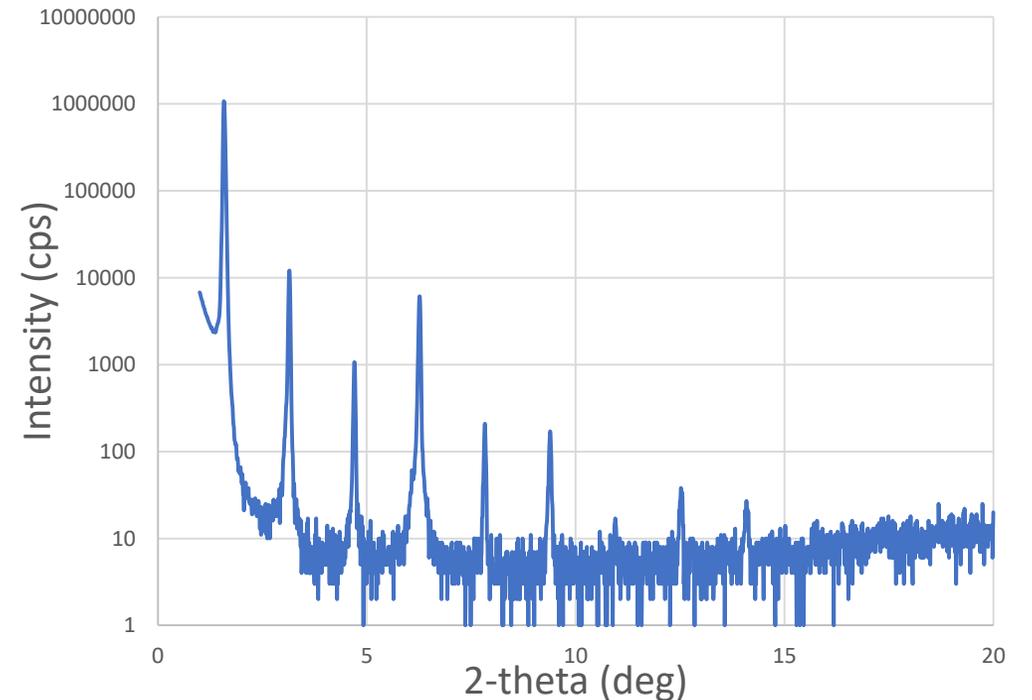
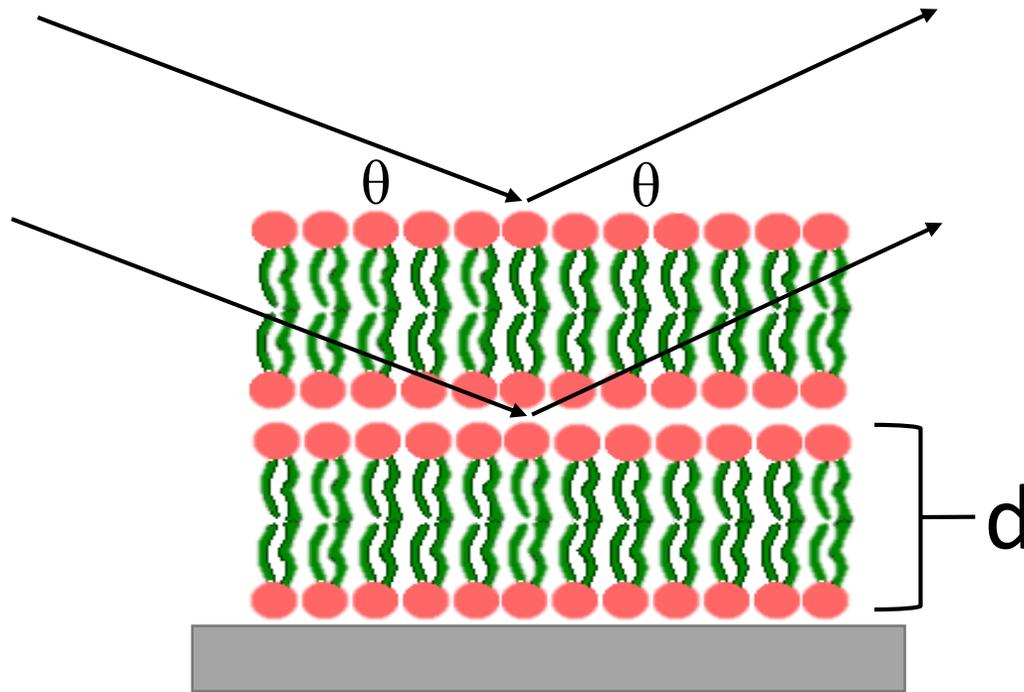
X-ray Diffraction and Bragg's Law

- Measures diffraction intensity at different angles
- From angle of diffraction, we can find the repeat spacing

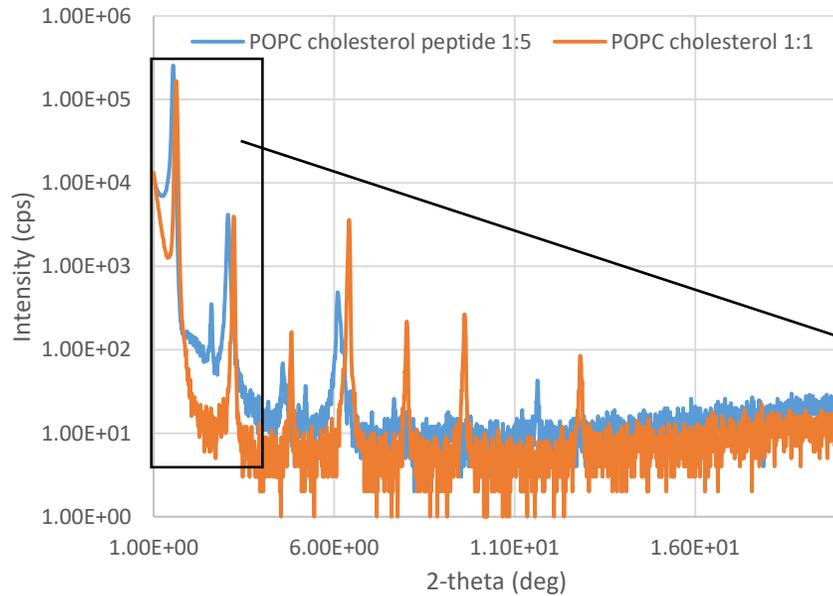
$$n \lambda = 2 * d * \sin \theta$$

$$Q = (4\pi/\lambda) * \sin \theta$$

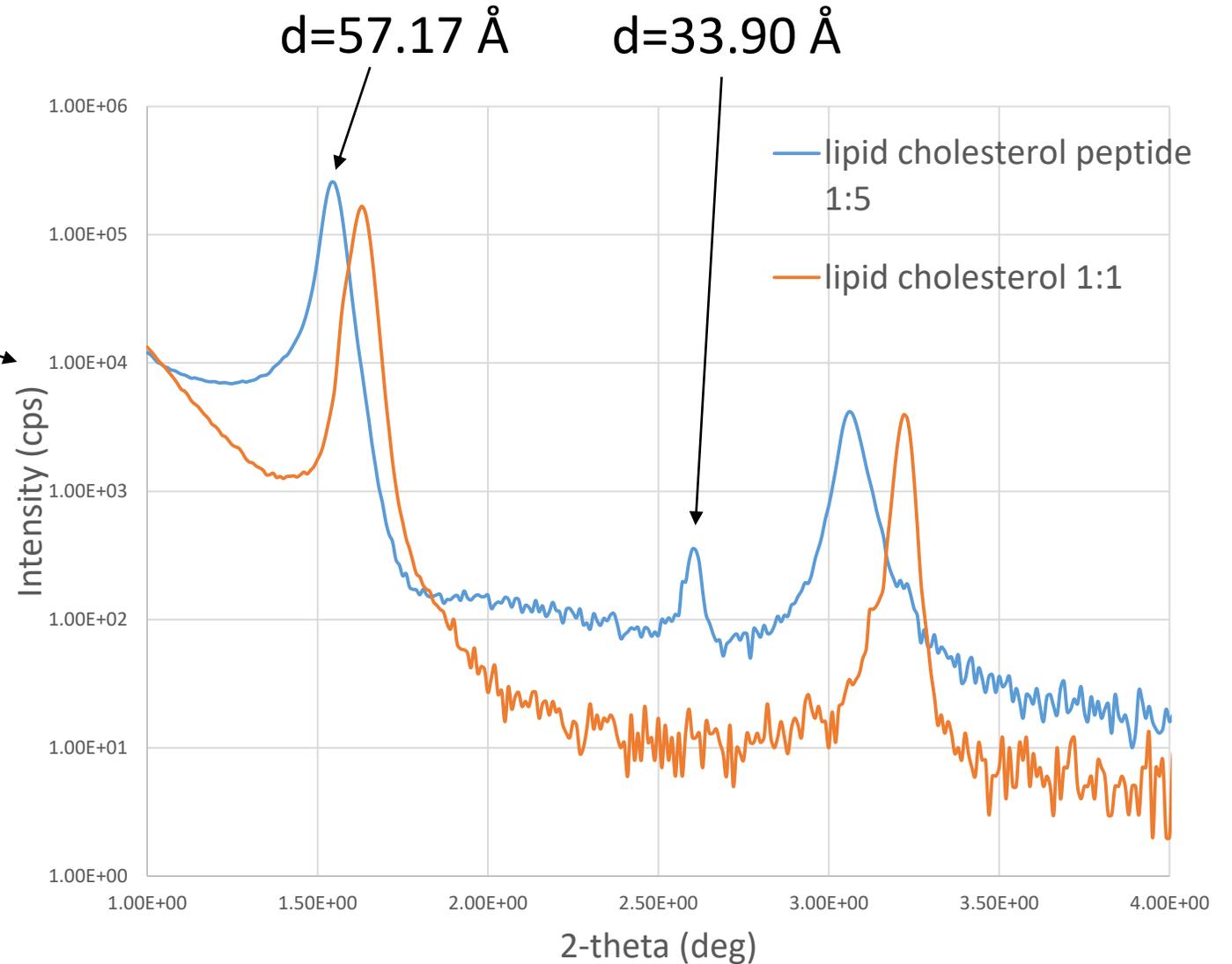
$$d = \lambda / 2 * \sin \theta$$



Diffraction Results with GP41 Peptide



- Additional intermediate peaks from cholesterol
- Cholesterol=16-17 Å

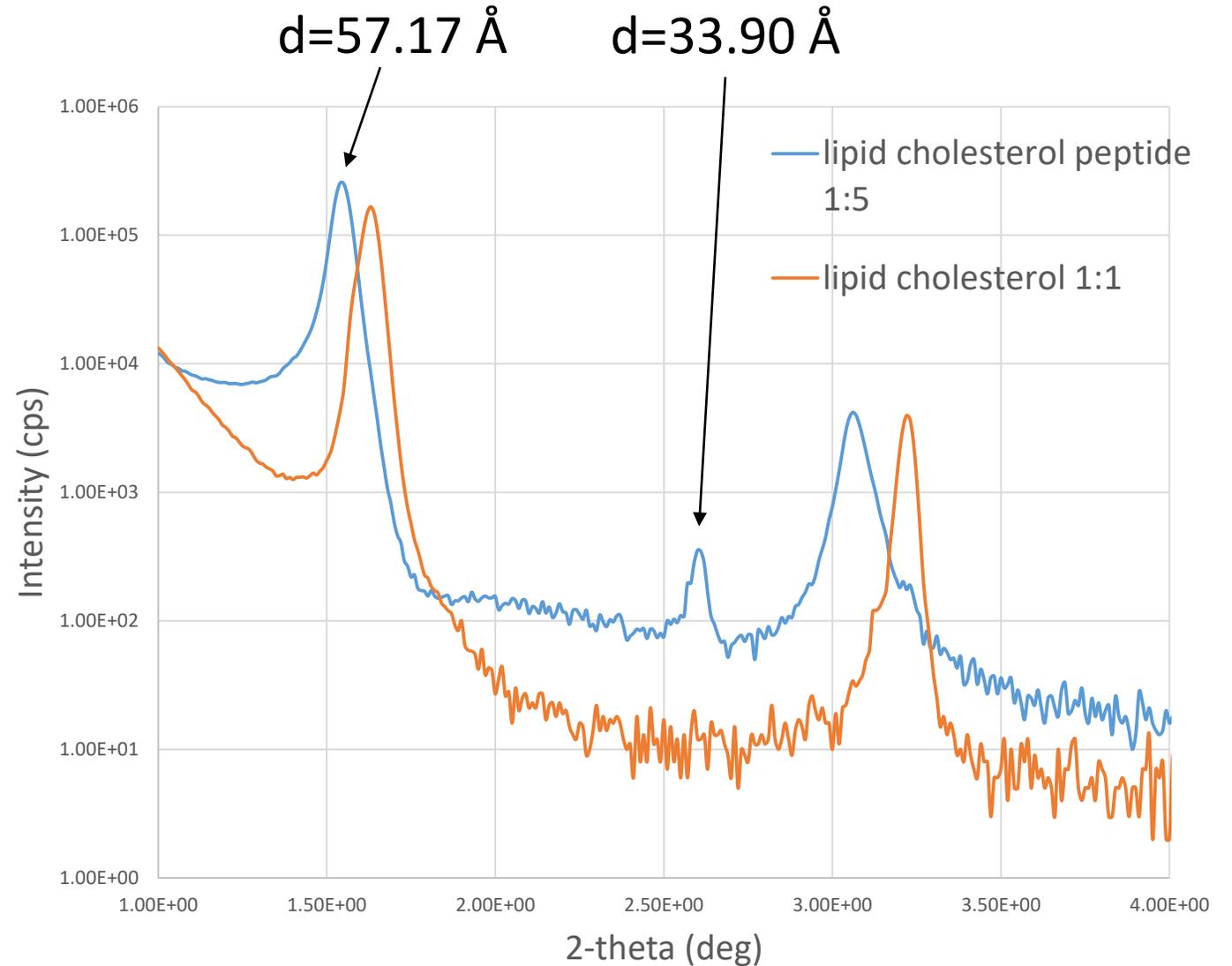
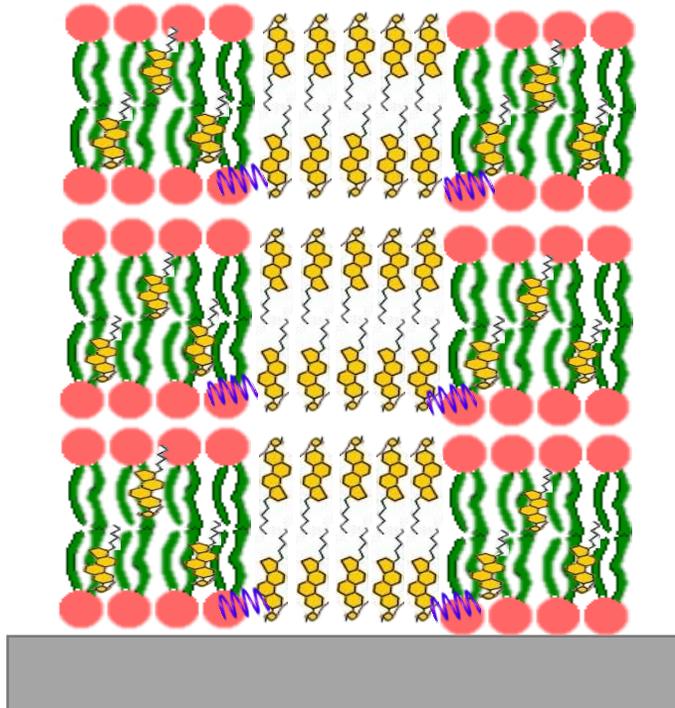


Shieh, H. S., et al. Crystal Structure of Anhydrous Cholesterol. *Nature* (1977): 287-289.

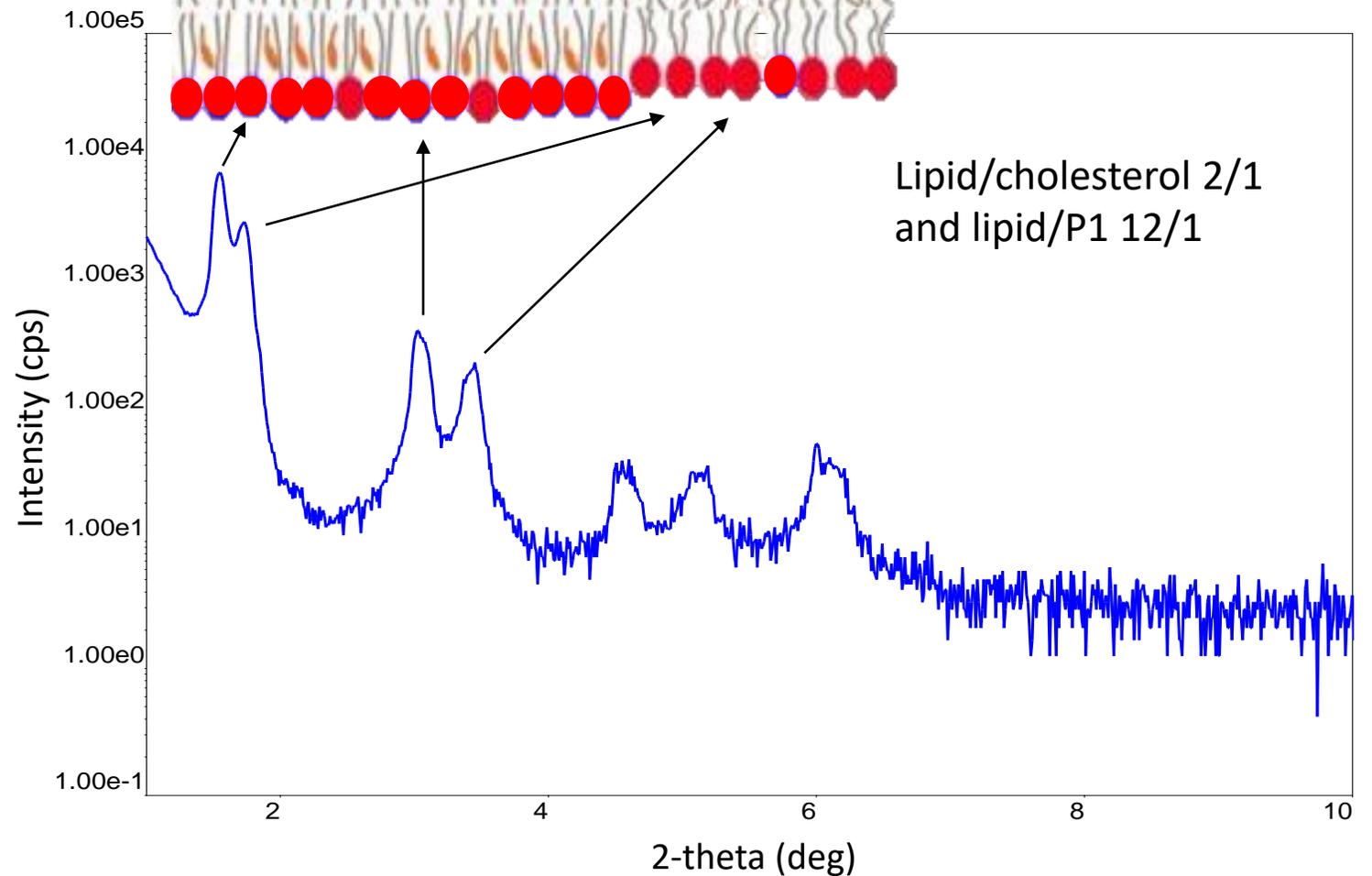
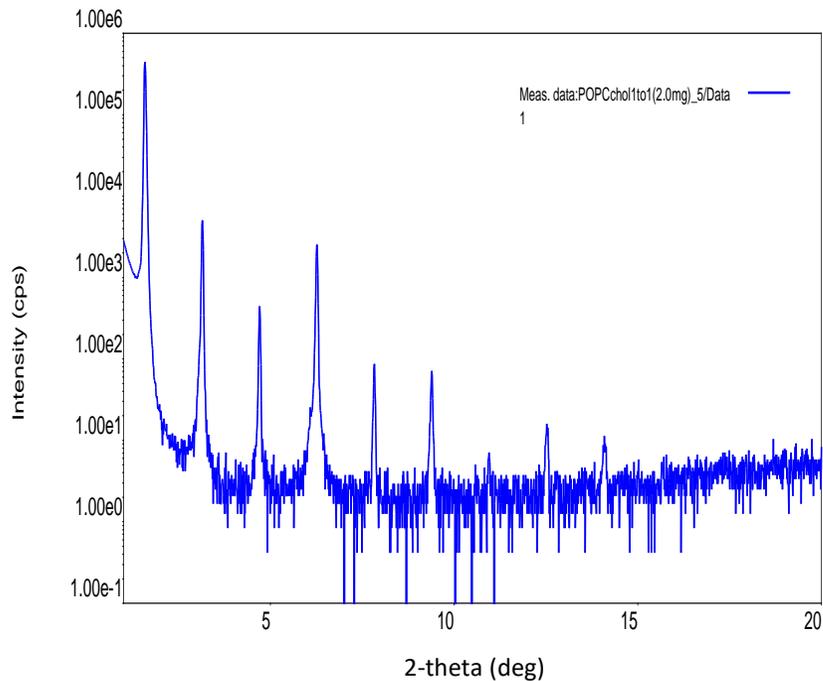
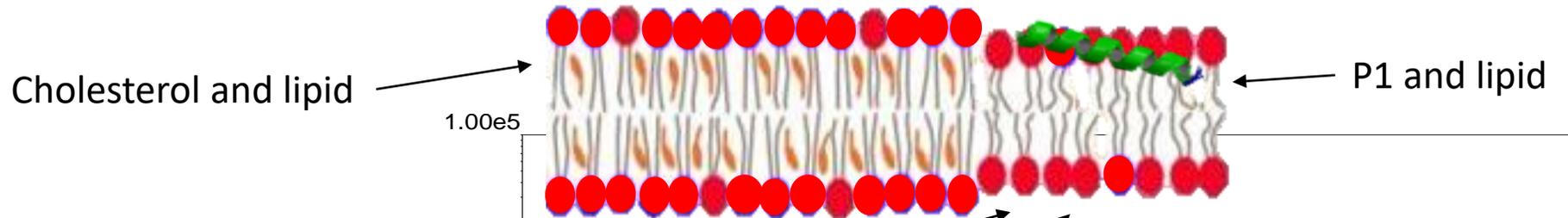
Diffraction Results with GP41 Peptide

lipid bilayer
with cholesterol

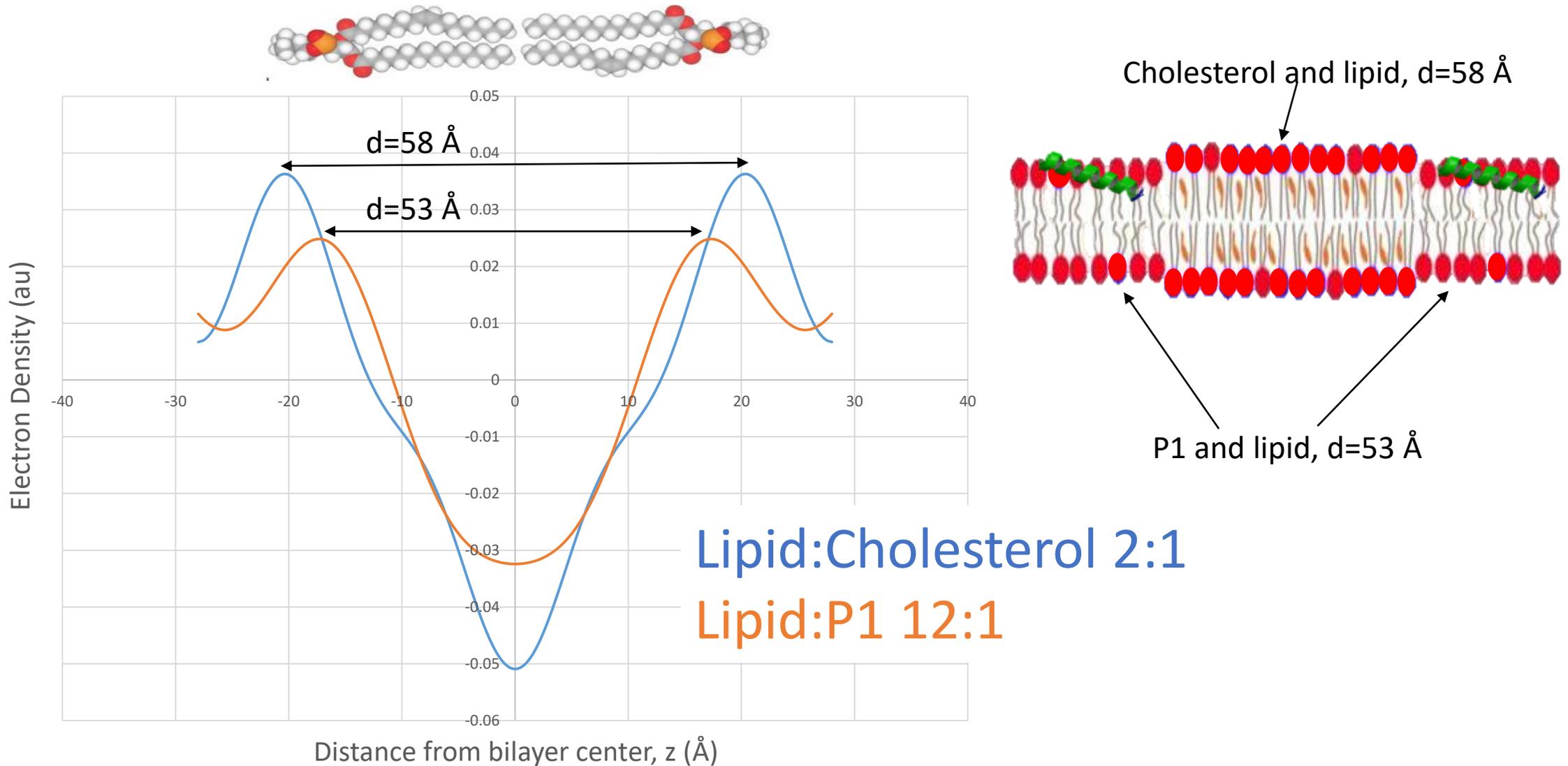
Cholesterol
crystals



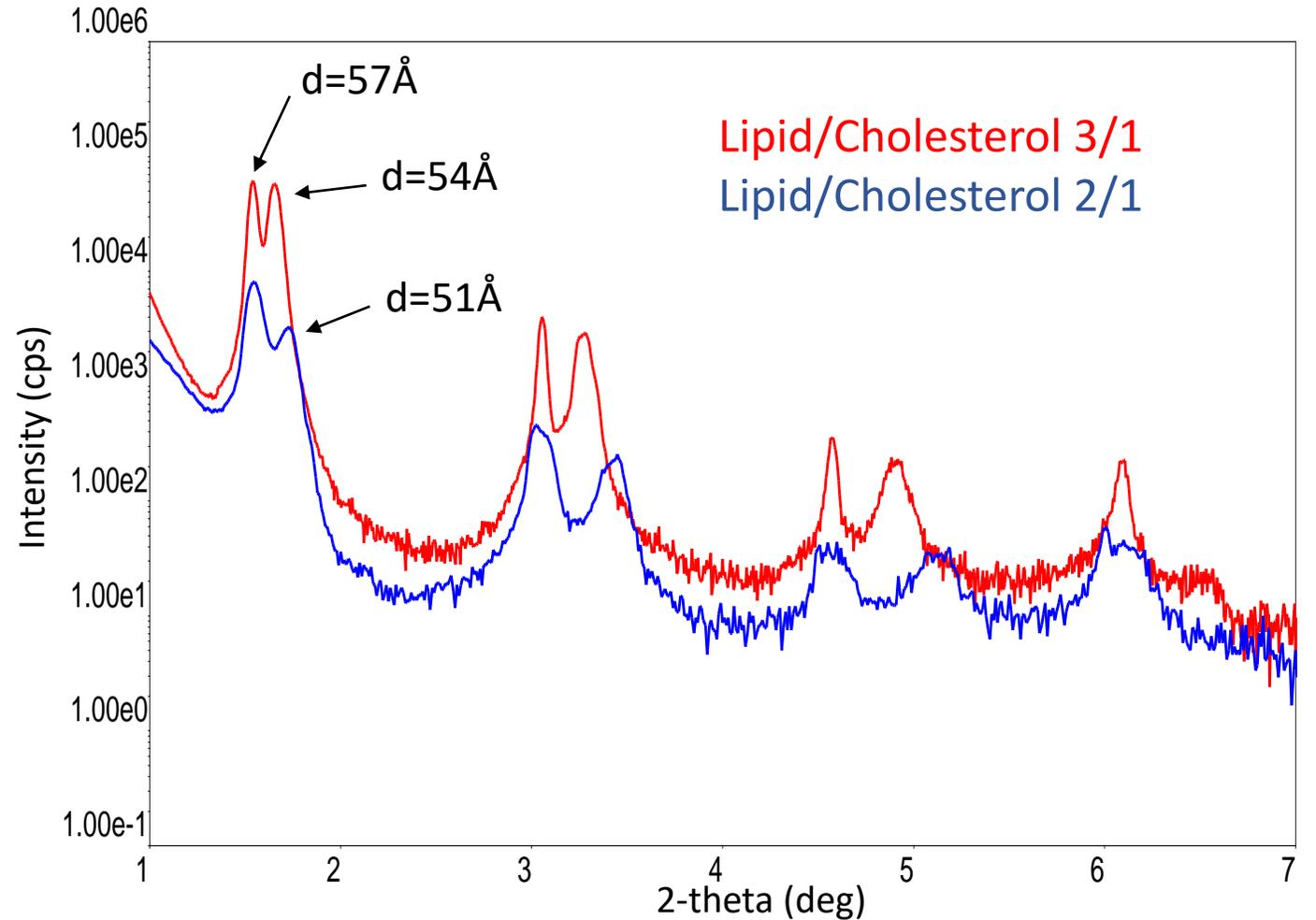
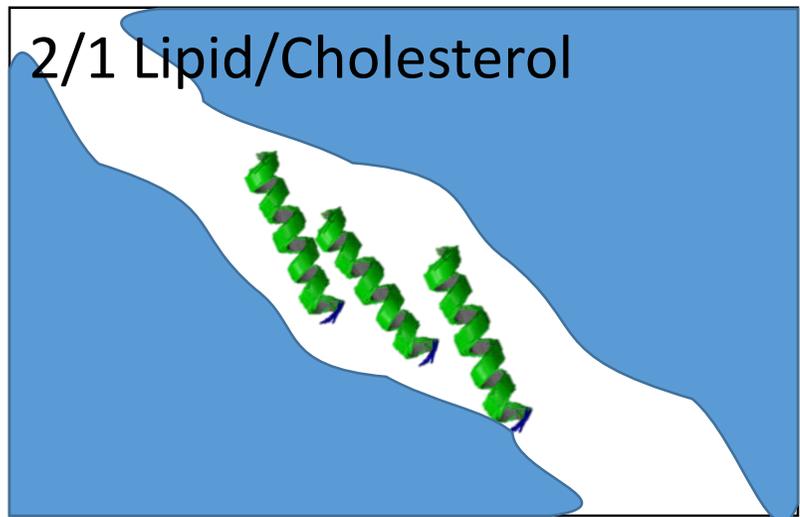
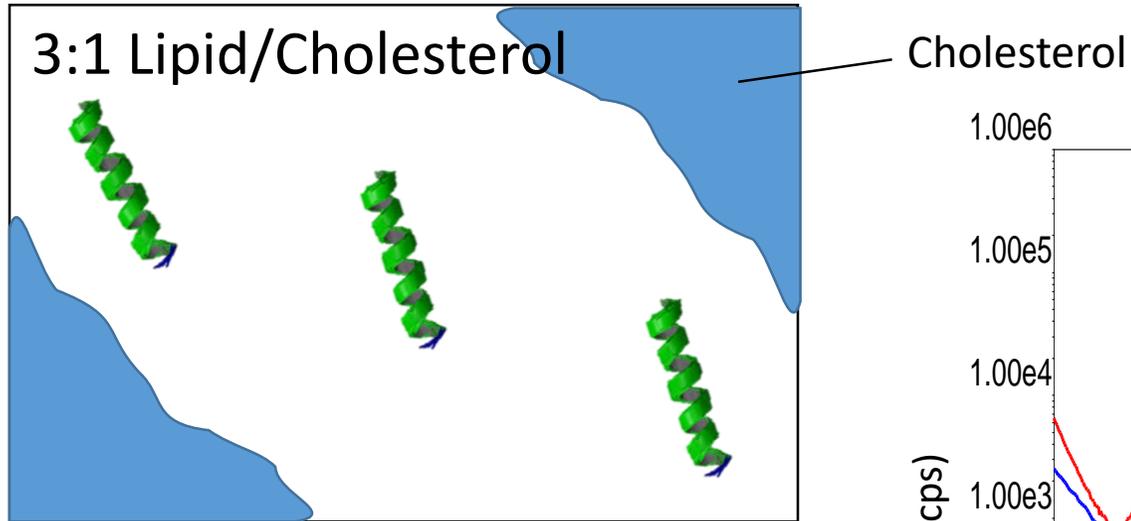
Diffraction Results with P1 Peptide



Effect of P1 on Bilayer Structure and Organization

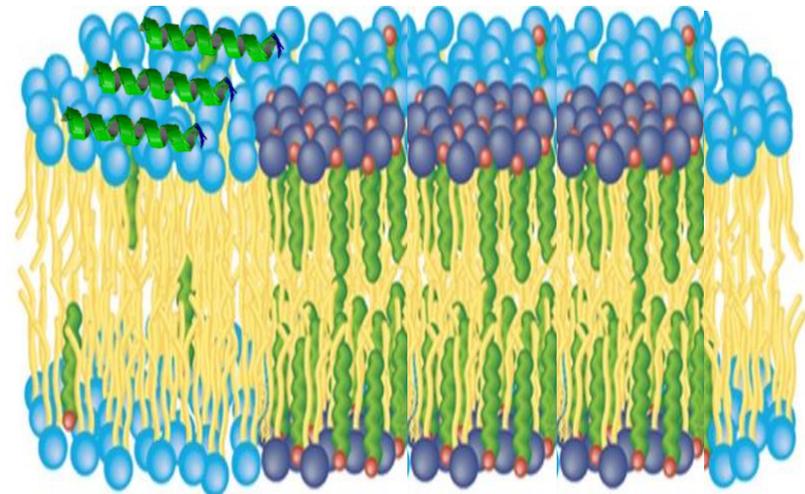


Diffraction Results with P1 Peptide



Conclusions

- Both peptides reorganize cholesterol, but by different mechanisms
 - GP41 peptide interacts directly with cholesterol and promotes the formation of cholesterol crystals
 - Piscidin avoids cholesterol and destroys the integrity of the membrane
- Membrane proteins and pathogenic peptides create their own microenvironments for their function



Acknowledgements

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 - David Vanderah

