

# Piscidin P1 uses soft spots in membranes as sites of action

Laura Lucas<sup>1</sup>, Myriam Cotten<sup>2</sup> and **Ella Mihailescu**<sup>3</sup>

<sup>1</sup>*Catholic University, Washington DC*

<sup>2</sup>*College of William and Mary, Williamsburg, VA*

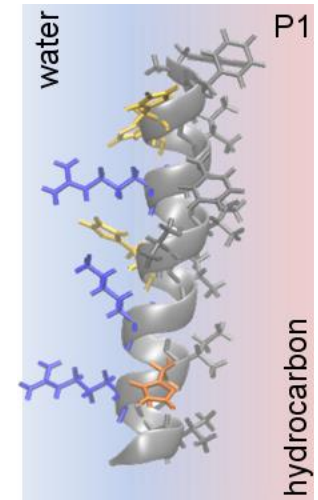
<sup>3</sup>*Institute for Bioscience and Biotechnology Research, Rockville MD*

*Biophysical Society Meeting, Feb-2018*

# Piscidins

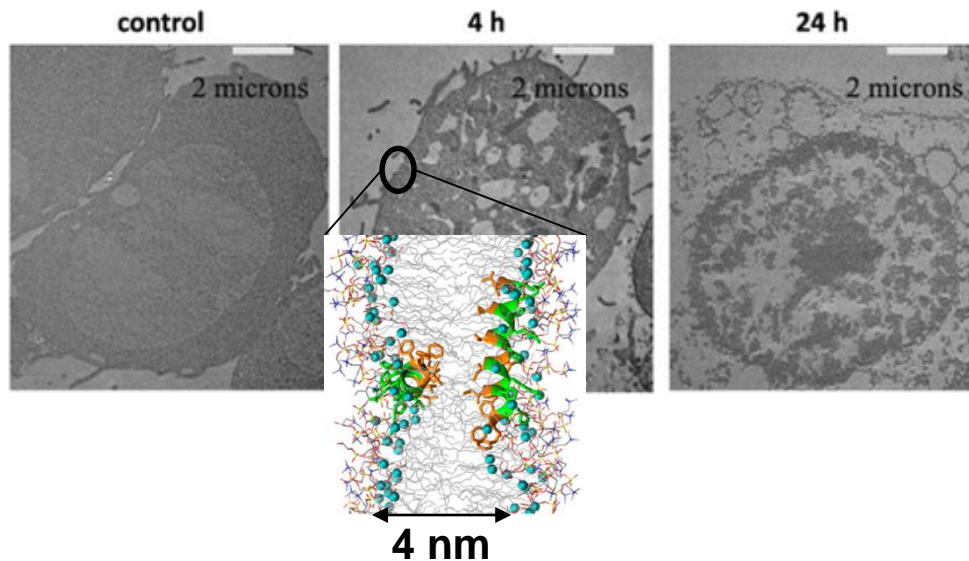
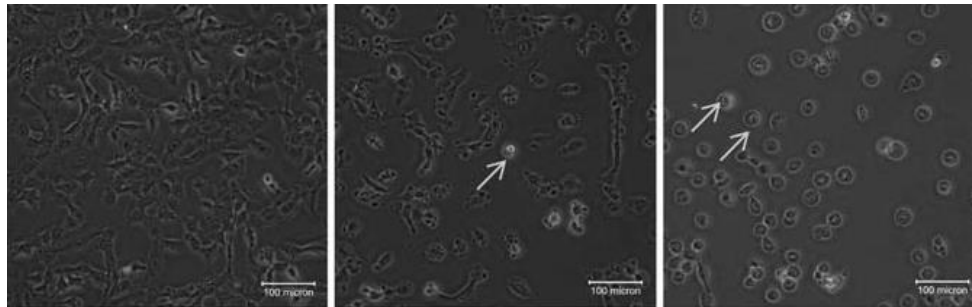
**P1** FFHHIFRGIVHVGKTIHRLVTG

- Hybrid striped bass (*Morone chrysops* x *Morone saxatilis*)
- First isolated from mast cells
- Broad spectrum activity against Gram-positive and –negative bacteria
- Highly conserved N-term sequence, but varied C-terminus
- Rich in HIS residues
- N-terminal metal binding ( $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ) motif (ATCUN)
- P1 minimum inhibitory concentrations (MIC) against:
  - *MRSA*  
< 2  $\mu\text{M}$
  - *E.Coli* :  
2-10  $\mu\text{M}$
  - *Bacillus Cereus*:  
< 2  $\mu\text{M}$
  - hemolytic activity (red blood cells):  
~50  $\mu\text{g/mL}$
- **P1 active against HIV-1, fungi and human carcinoma**



# Piscidin P1 induces apoptosis and necrosis of tumor cells

HT1080 cells (human fibrosarcoma) treated with 10 $\mu$ M P1  
Lin H -J. et al , Zool. Sci 29 (2012)



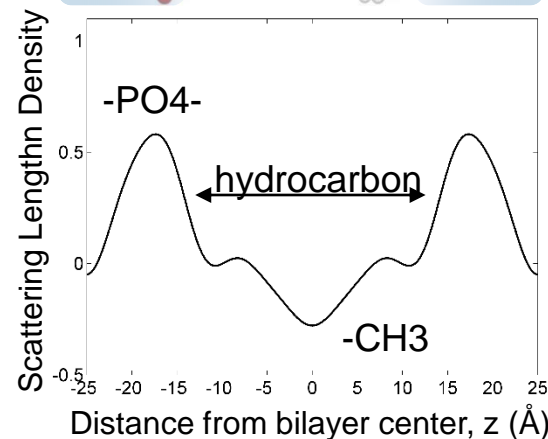
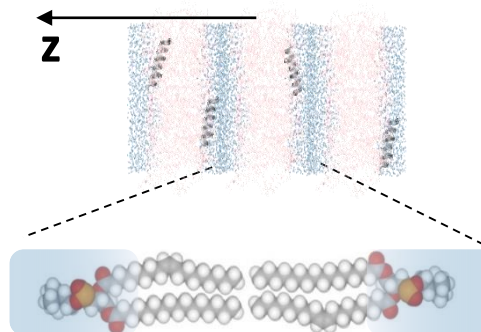
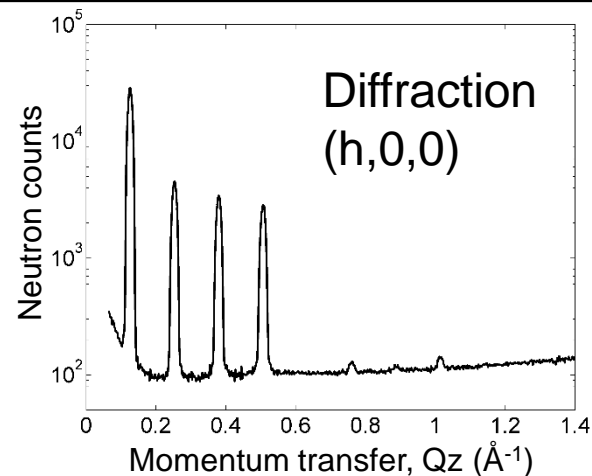
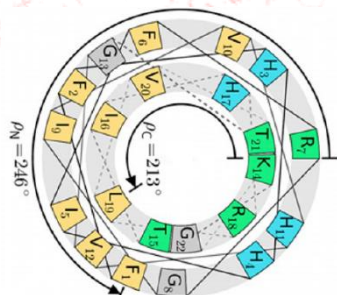
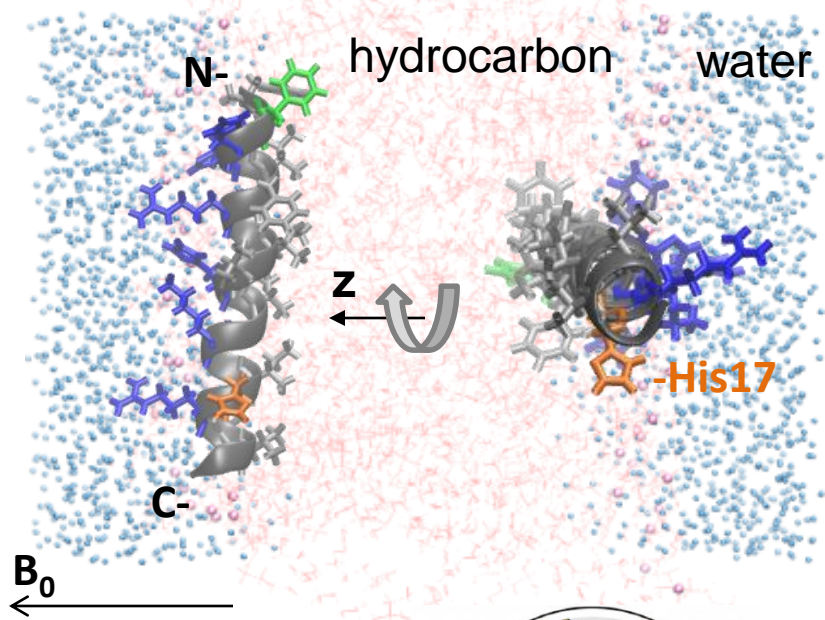
# Questions

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- How does P1 breach the lipid membrane barrier?
- Does the peptide show binding preference for specific lipid compartments in membranes?

# Structures of Piscidins in bilayers

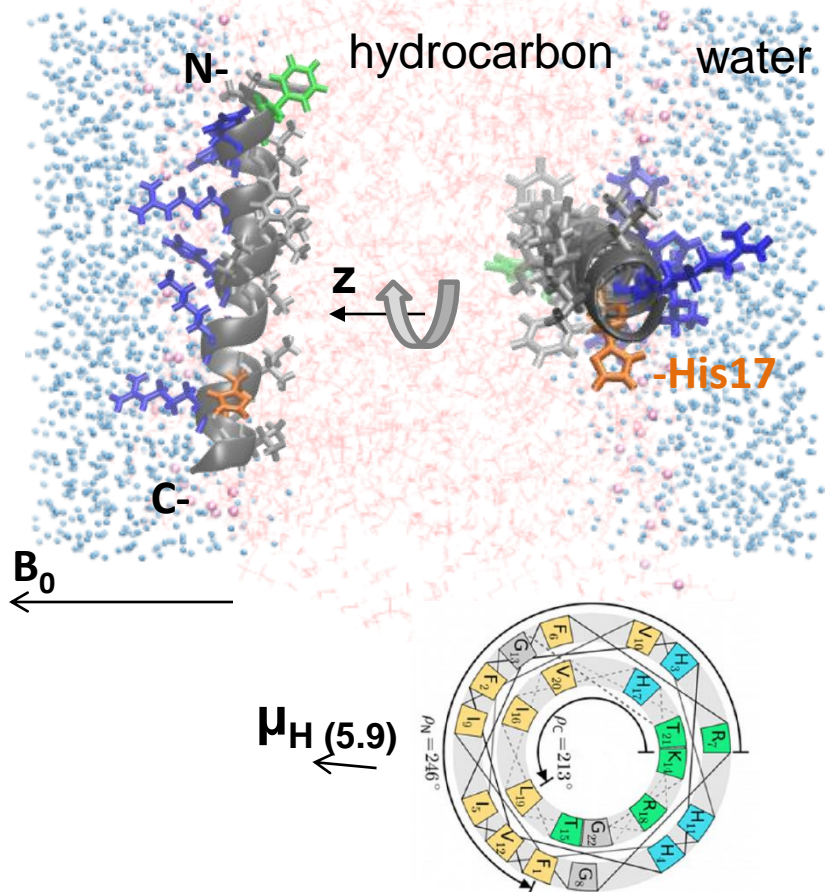
Solid state NMR structure of P1  
(in aligned multilayers DMPC/DMPG 3/1)



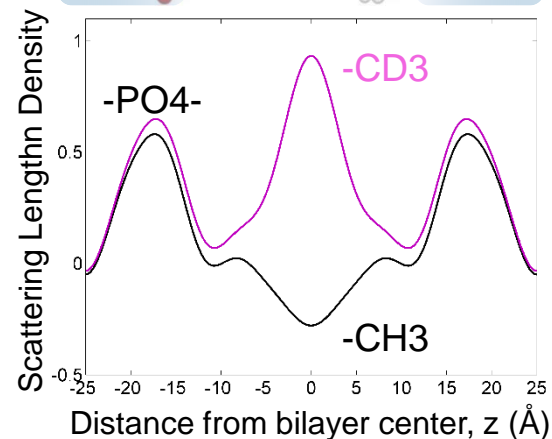
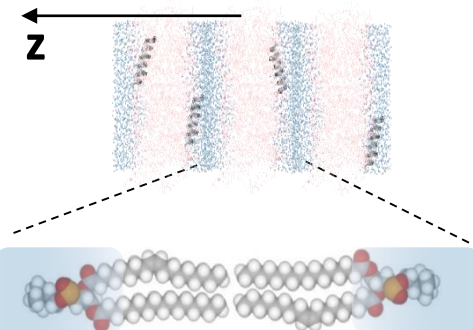
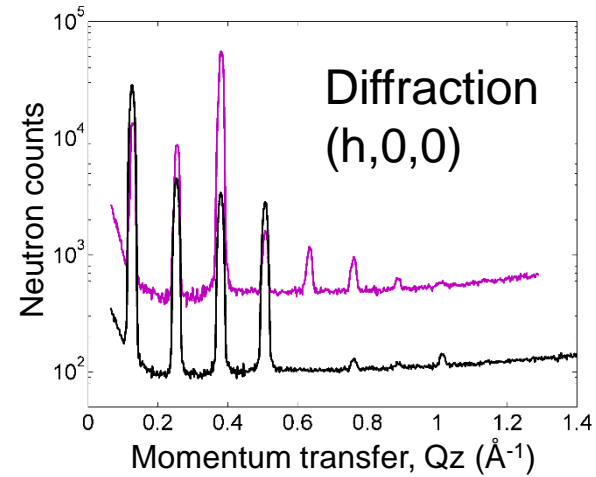
Fourier analysis

# Structures of Piscidins in bilayers

Solid state NMR structure of P1  
(in aligned multilayers DMPC/DMPG 3/1)



(Perrin et al, JACS 2013)

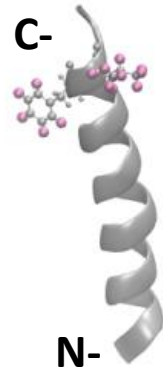


Fourier analysis

# Determining peptide conformation with specific deuteration

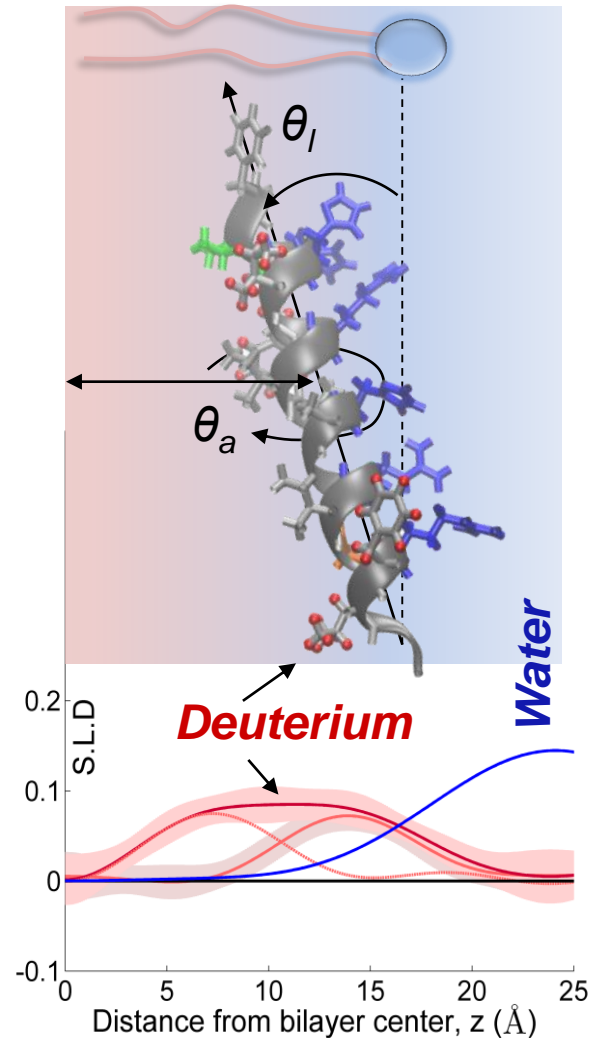
dP1 FFHHIFRGIVHVGKTIHRLVTG (d18)

*C-term  
deuteration*



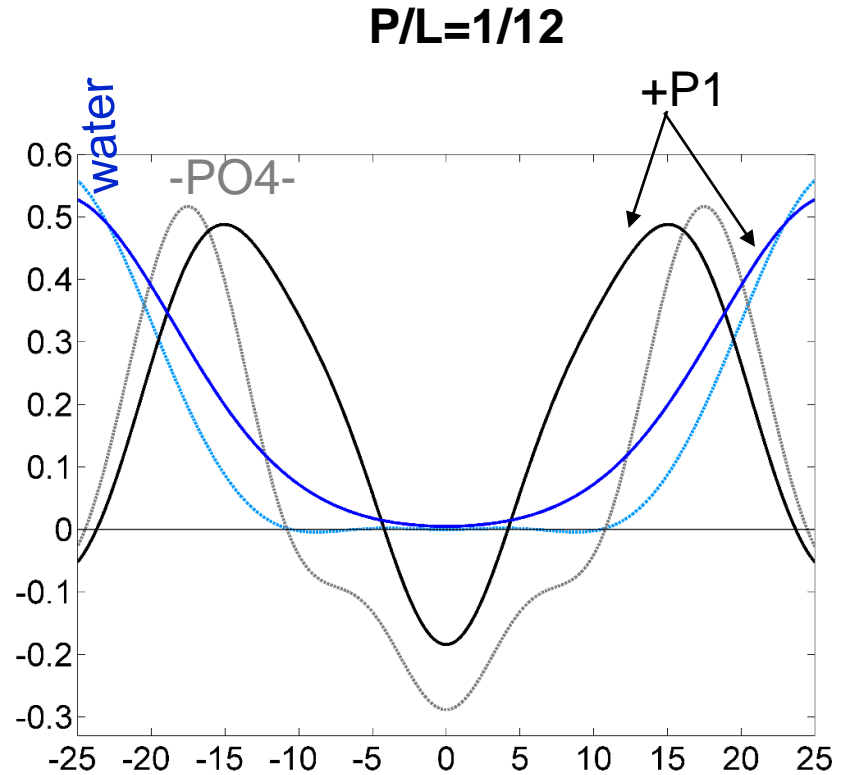
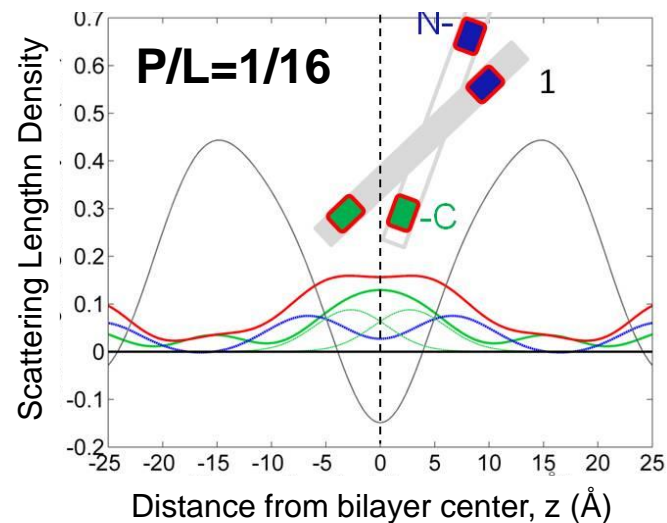
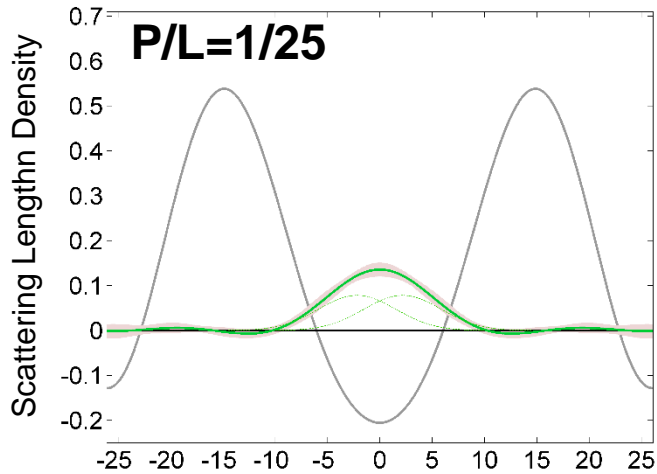
ddP1 FFHHIFRGIVHVGKTIHRLVTG (d33)

*C-term + N-term  
deuteration*



# P1 distribution and bilayer distortions

Neutron diffraction: P1 in POPC/POPG 3/1, RH=93%, T=23°C



- P1 recruits the lipid headgroups and water into the bilayer interior

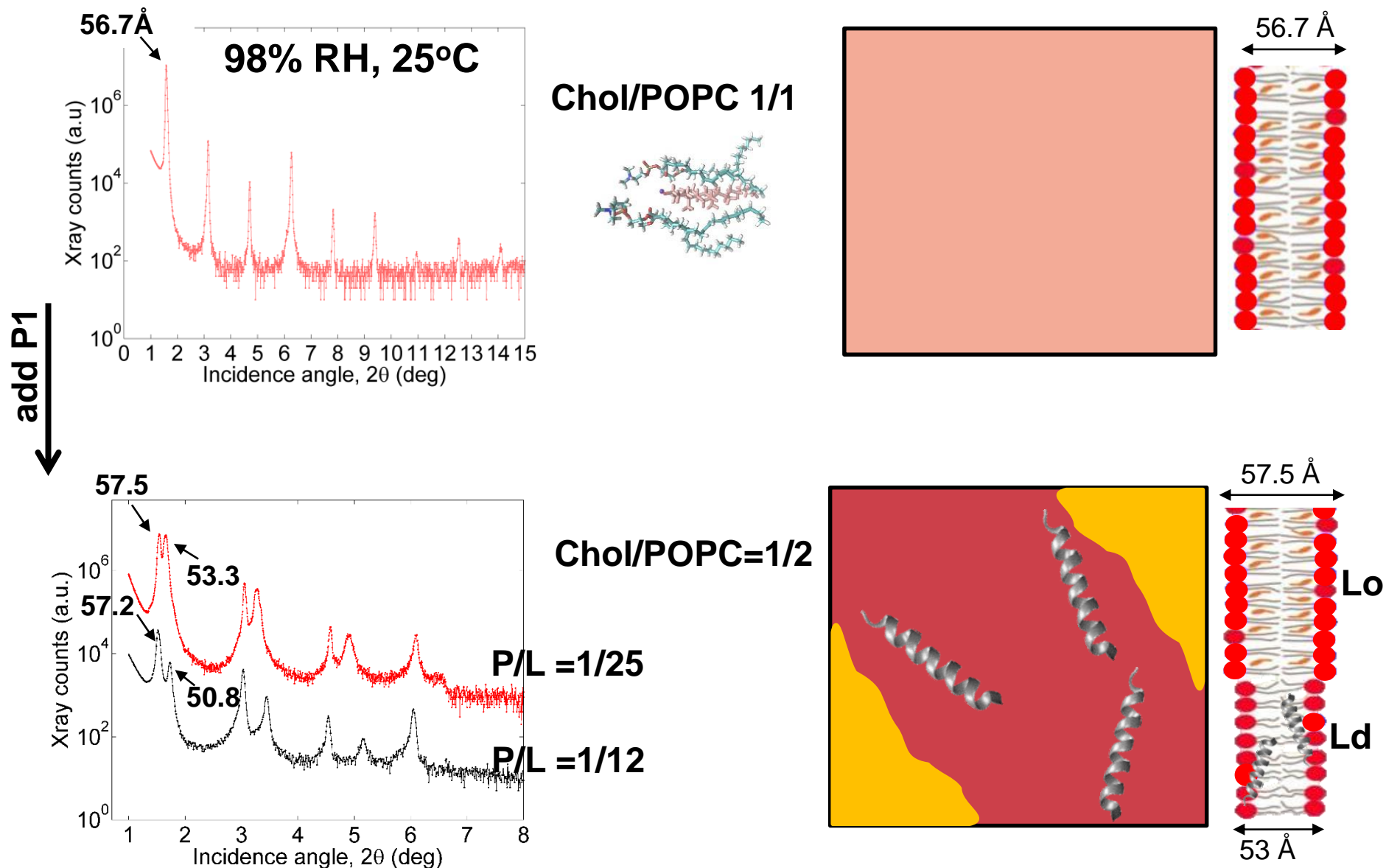


# Questions

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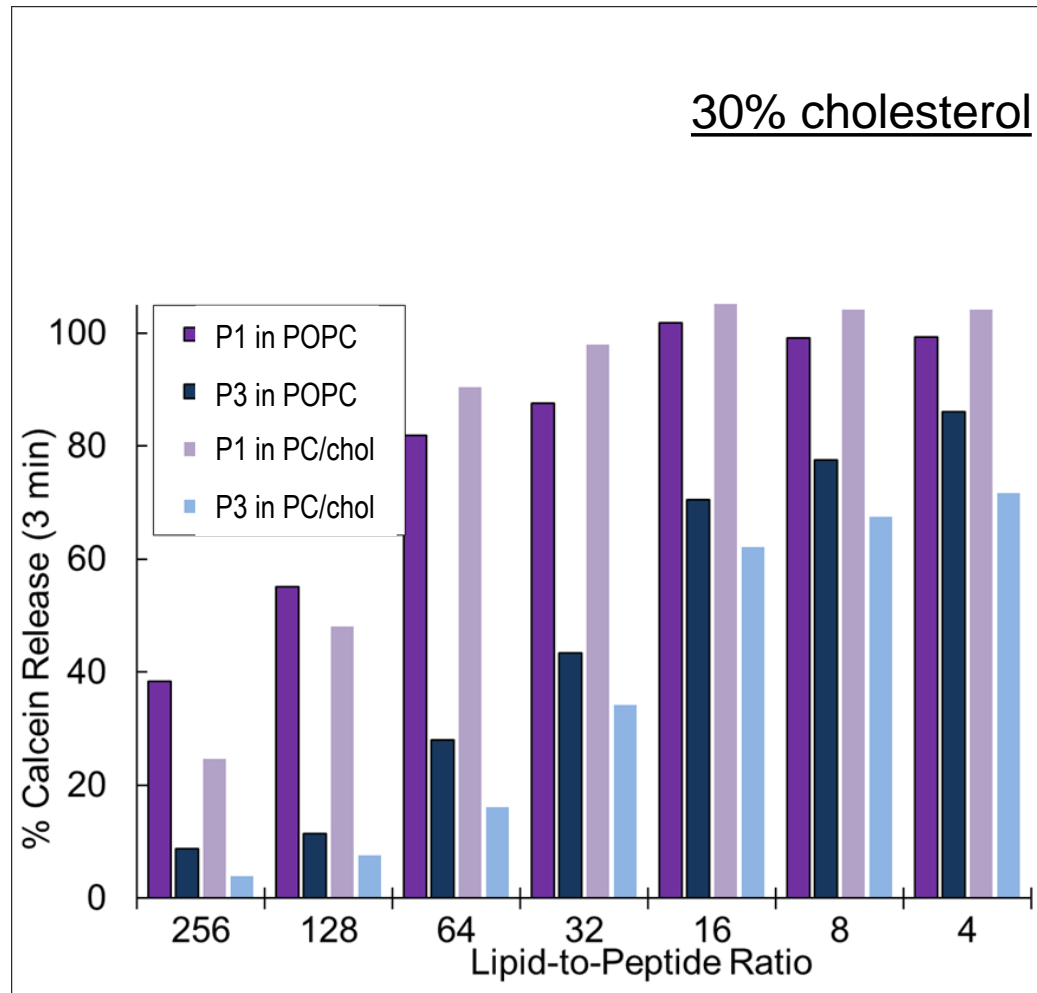
- How does P1 breach the lipid membrane barrier?
- Does the peptide show binding preference for specific lipid compartments in membranes?

# P1 causes segregation of cholesterol in membranes

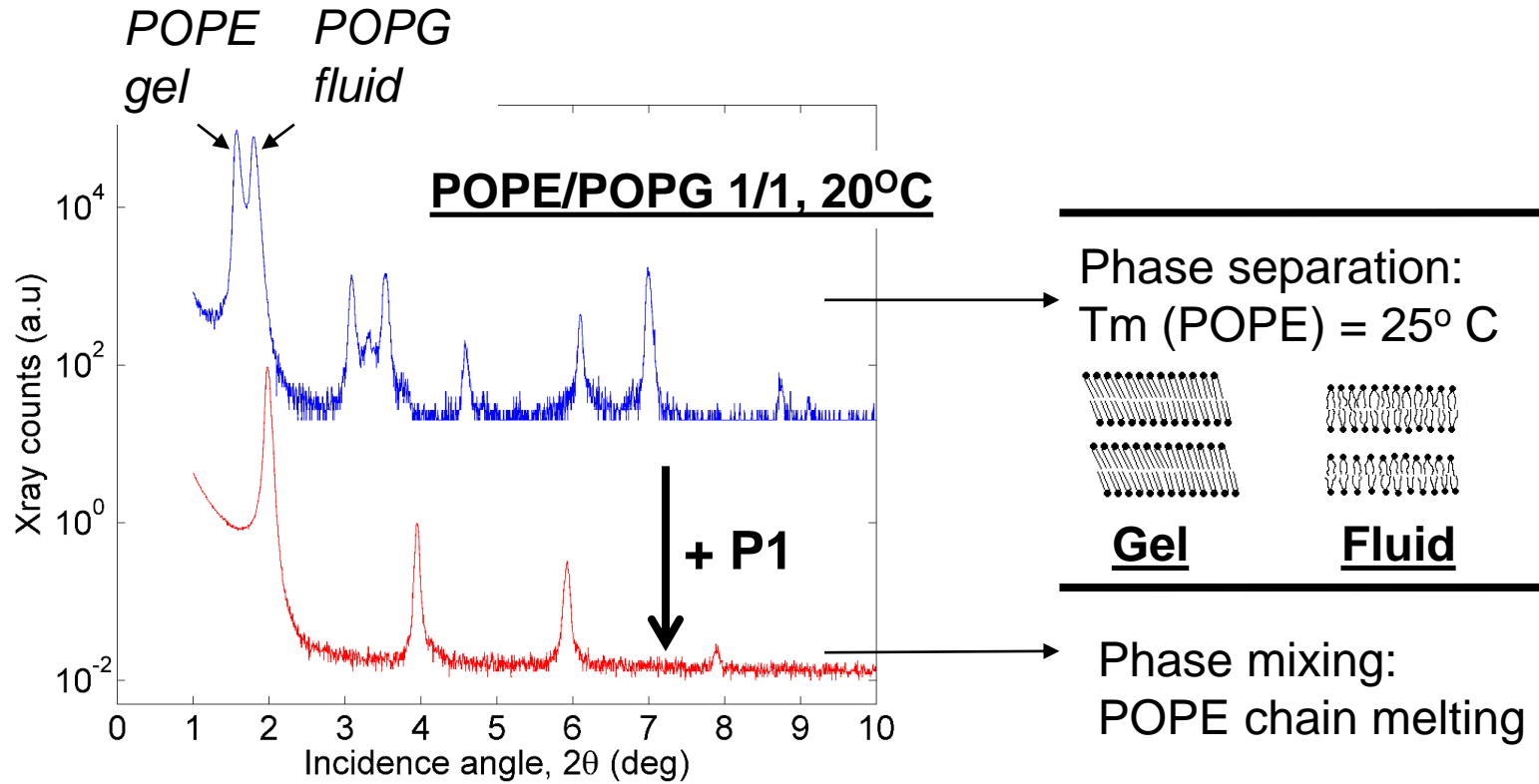


# P1 is not inhibited by cholesterol in membranes

Dye leakage measurements in liposome

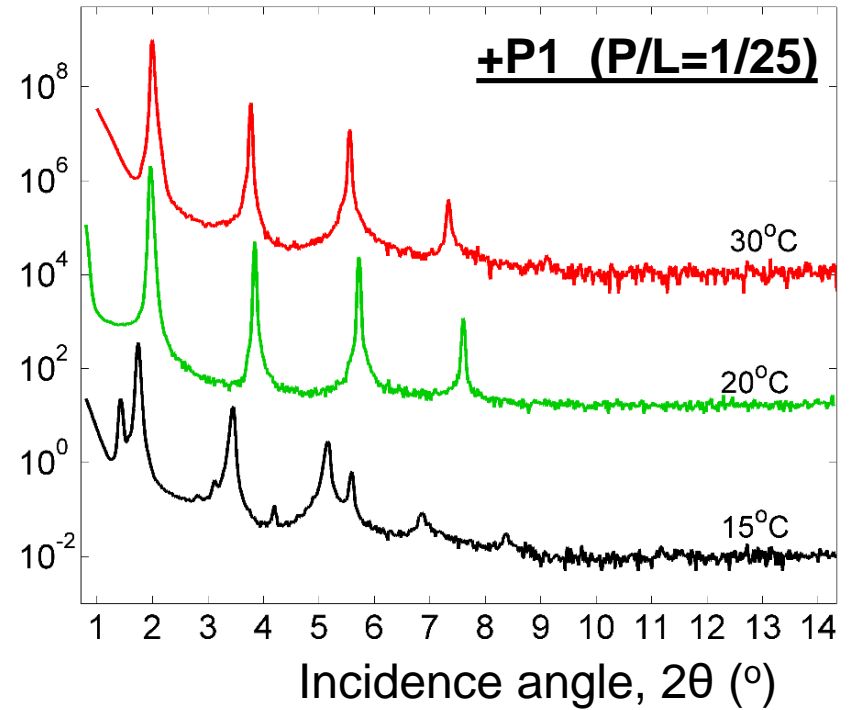
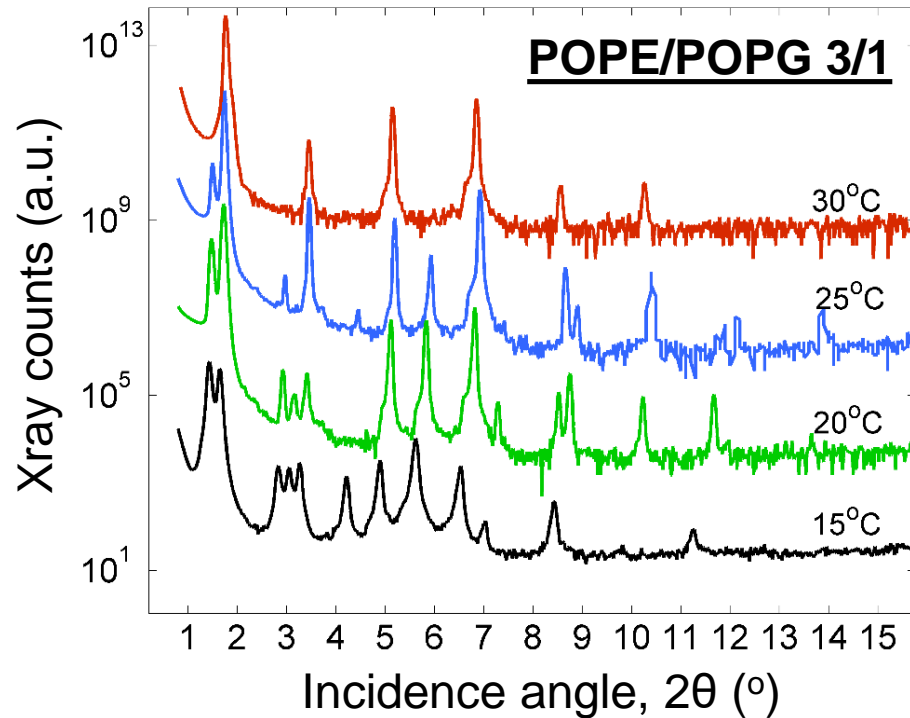


# P1 causes chain melting



# P1 causes chain melting

98% RH, 25°C



- P1 inhibits the gel state of POPE at low temperatures

# Summary

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- **Neutron diffraction with specific D-labeling is used to assess the structural interactions of membrane-active peptides with bilayers on a molecular level**
- **Piscidin P1 inserts the C-terminus and breaches the hydrophobic barrier by recruiting lipid headgroups and water into the membrane interior**
- **No indication that stable pores are forming (defects are predominant)**
- **P1 exploits soft spots in the membrane for entry (regions with high curvature strain and higher fluidity)**

# Acknowledgements

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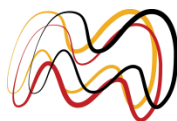
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