Role of Lipids in Membrane Docking and Pore Formation of Pneumolysin

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Pneumolysin forms membrane pores

Streptococcous pneumoniae employs pneumolysin (PLY) to infect its human host. The specificity of PLY to cholesterol-rich membranes targets this virulence factor to mammalian cells.

PLY is released as a water-soluble monomer. The monomers dock to cholesterol-rich membranes and oligomerise into rings of ~400 Å diameter that insert into the lipid bilayer, forming large cytolytic pores.
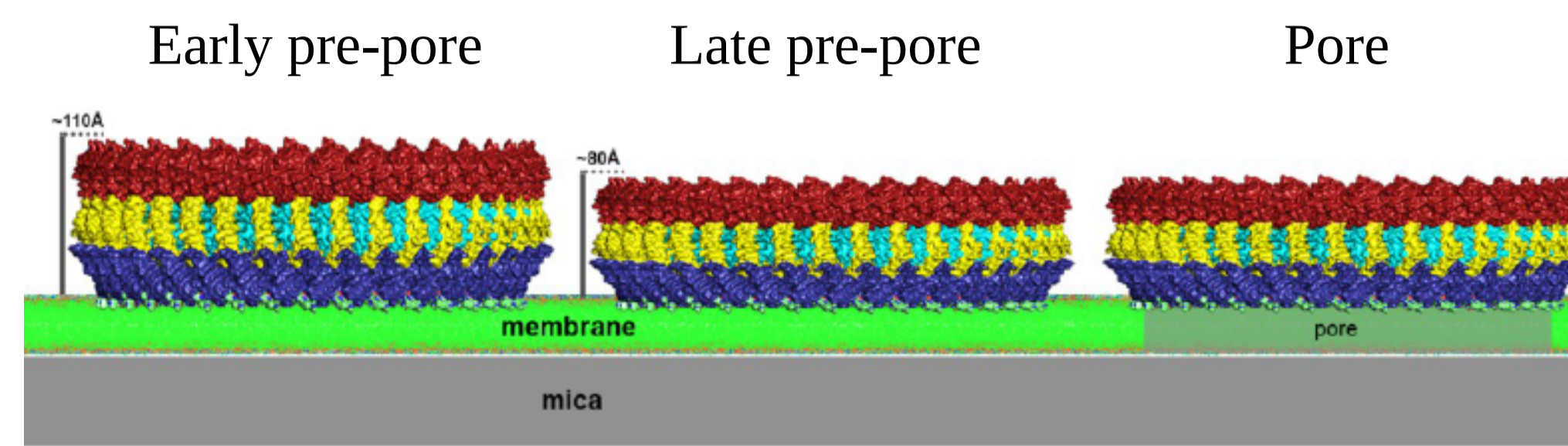
Early pre-pore   Late pre-pore   Pore

Figure 1: Early pre-pore, late pre-pore and pore structure of PLY on a membrane. [1]

Conformational States of PLY

X-ray structures of the soluble form [2,3] and cryoEM structures [2] as well as AFM studies [1] of the PLY pre-pore and pore have provided structures of three distinct stages of pore formation.
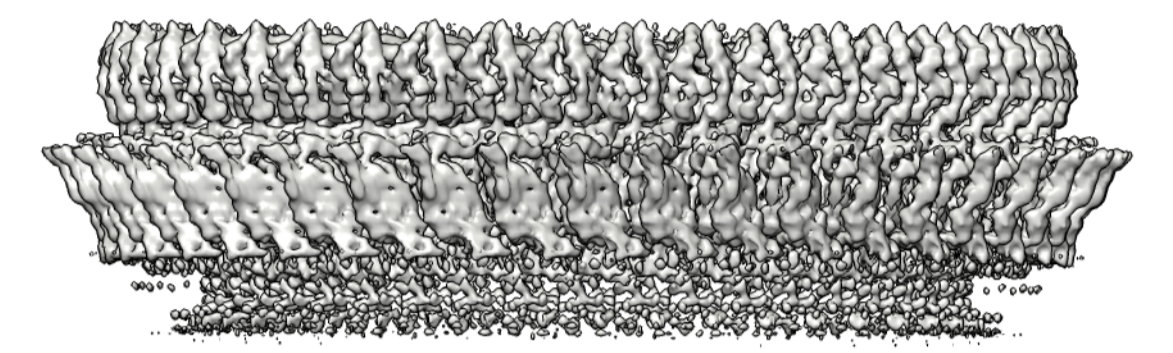

Figure 2: CryoEM map of a 42-mer PLY ring with 4.5 Å resolution. [2]

Crystal Structure
(similar to pre-pore conformation)

Domain 1
Domain 2
Domain 3
Transforming helices
Domain 4
Loop L1
T459/L460/Y461
Trp-rich undecapeptide
W433/W435/W436

Figure 3: PDB 5CR6 [3]

Pneumolysin Pore in Coarse-Grained Computer Simulations

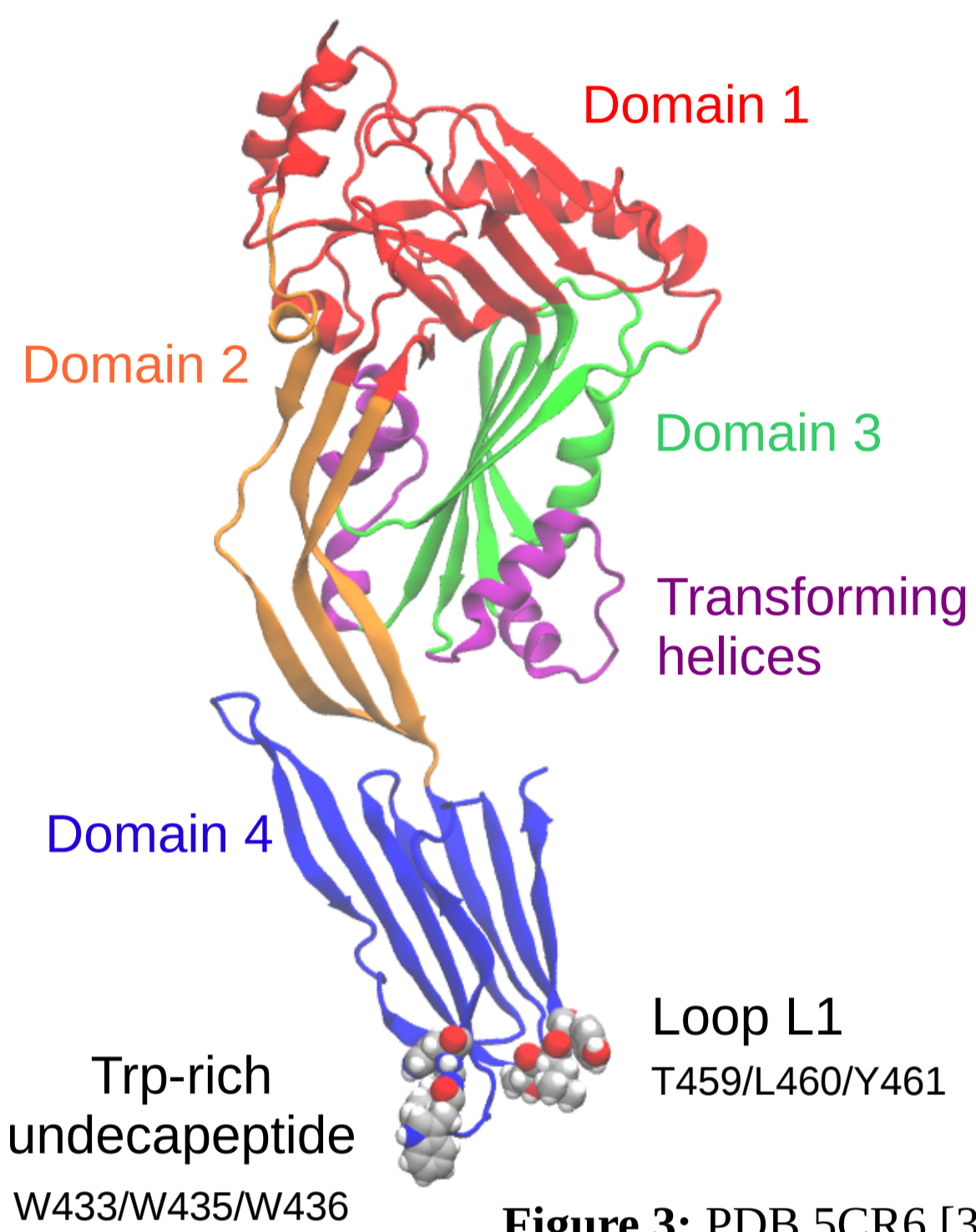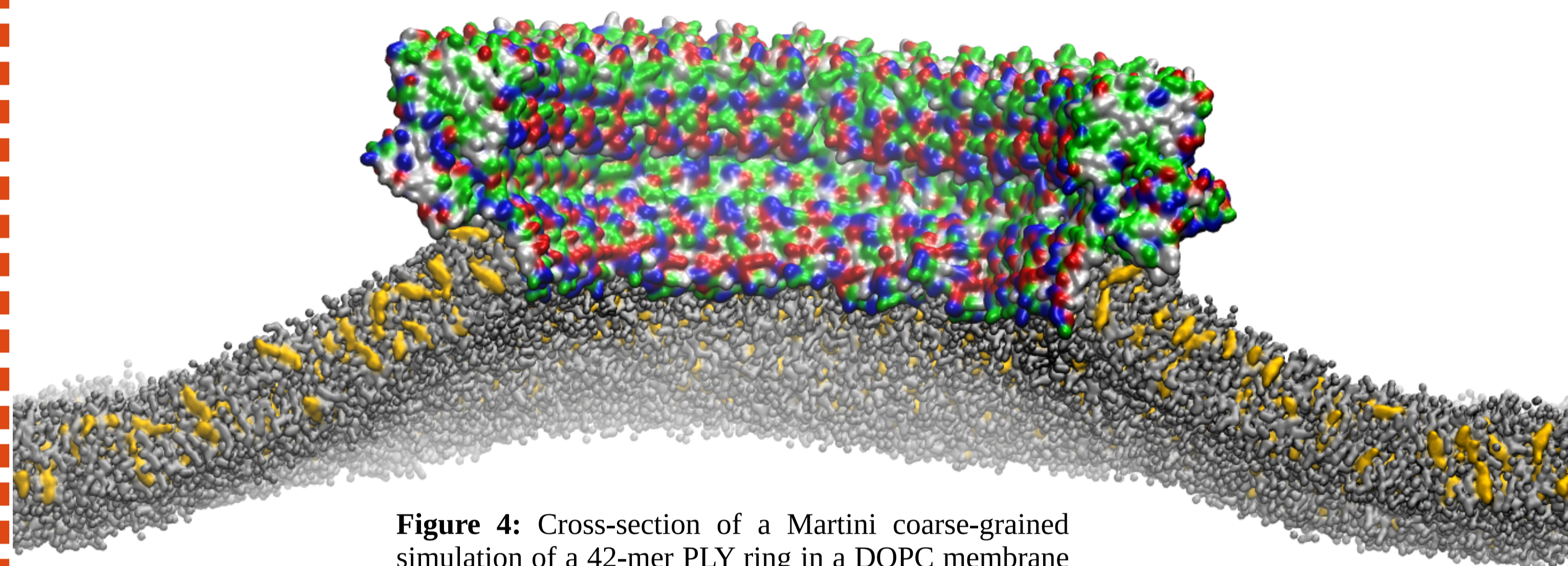**Figure 4:** Cross-section of a Martini coarse-grained simulation of a 42-mer PLY ring in a DOPC membrane with 30% cholesterol (yellow) in pore conformation.
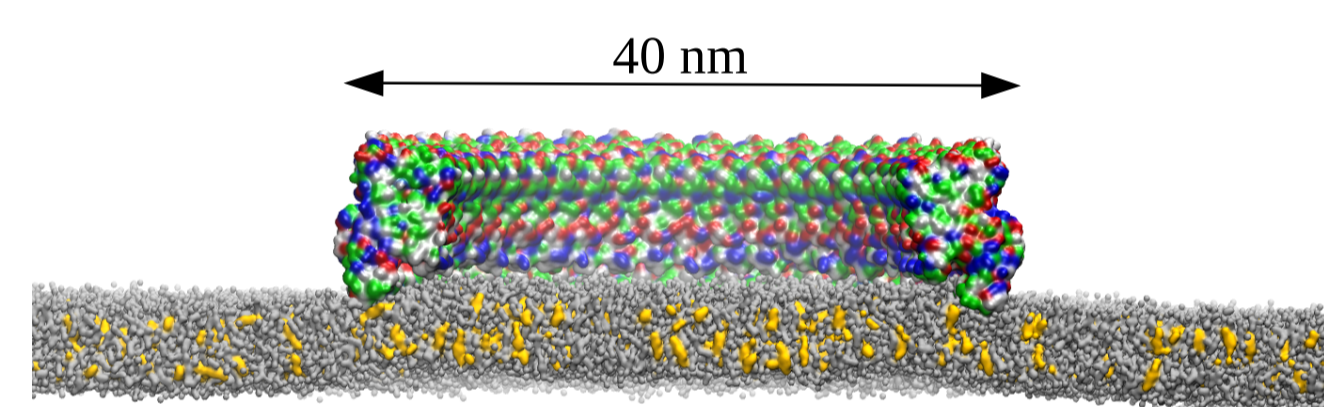
40 nm

Figure 5: Cross-section of a coarse-grained simulation of a 42-mer PLY ring in pre-pore conformation.

Computer simulations using the coarse-grained Martini model [4,5] reproduce experimental findings: Lipids recede from the hydrophilic interior of the ring. The ring induces convex curvature of the membrane and stabilizes the pore.

Pore Conformation
(from CryoEM)

D1
D2
D3
D4
Docked to the membrane
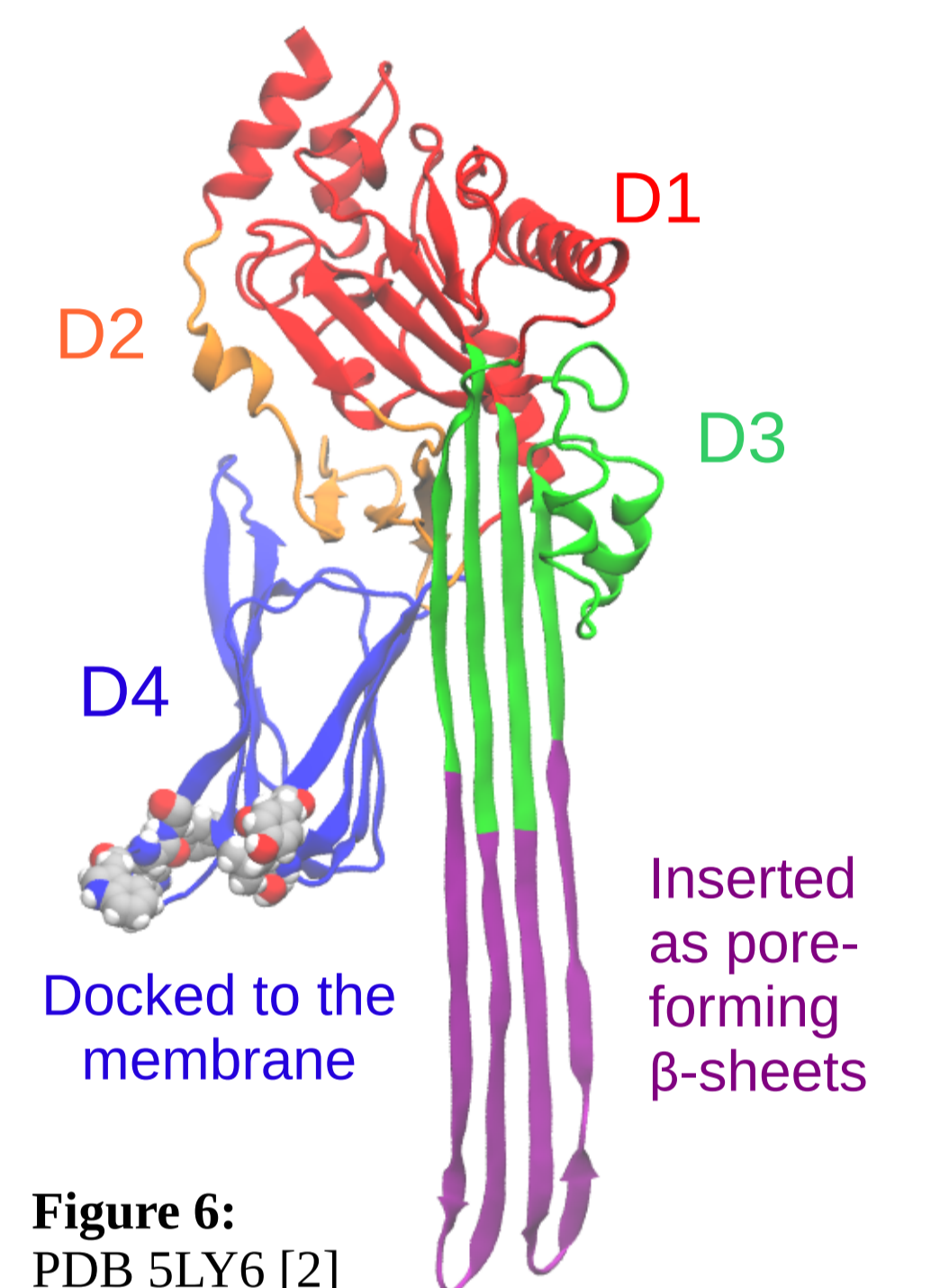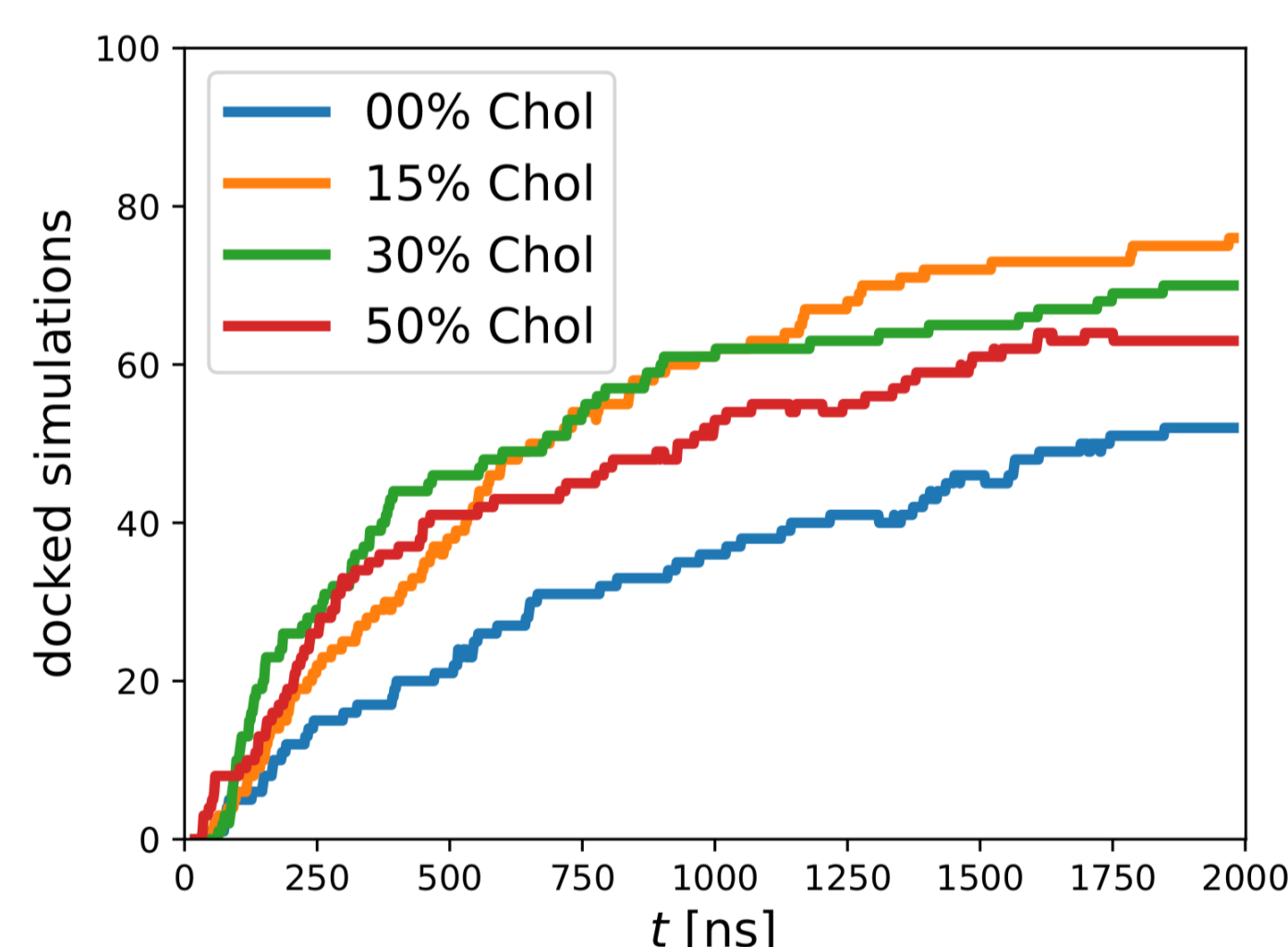Inserted as pore-forming β-sheets

Figure 6: PDB 5LY6 [2]

Membrane Docking

High-throughput simulations using the Martini model show:

- Cholesterol concentrations of 15-30% in the membrane increase docking events.
- Less docking for 50% Chol.

→ **Figure 7:** Number of simulations (out of 100) in which pneumolysin docked to the membrane as a function of simulation time.


All-atom MD simulations show:

- Only Trp433 of the undecapeptide is completely buried within the membrane.
- Loop 1 is more buried and can interact with cholesterol via Leu460 and Thr459.

→ **Figure 8:** Snapshot of Domain 4 from a simulation of PLY docked to a DOPC membrane with 30% cholesterol. Important residues are shown as spheres.

PLY D4
Trp436
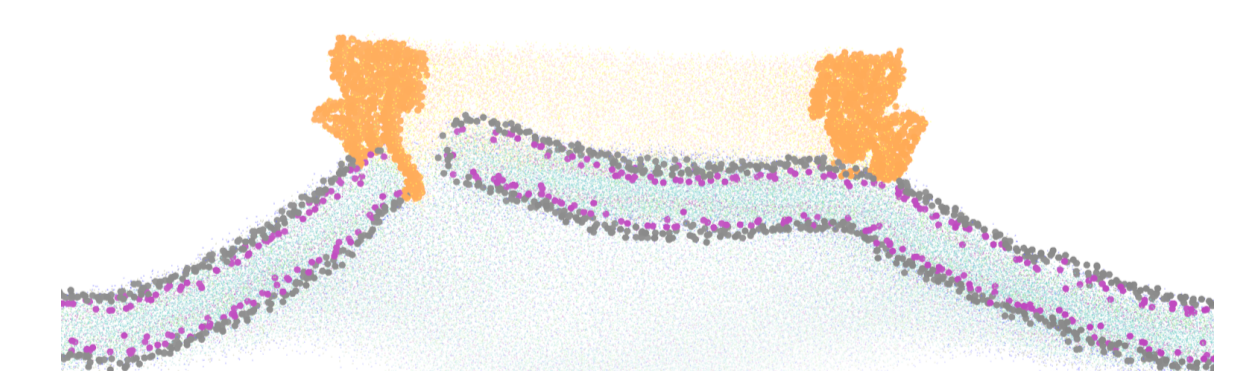Trp435
Tyr461
Thr459
Trp433
Leu460
Cholesterol
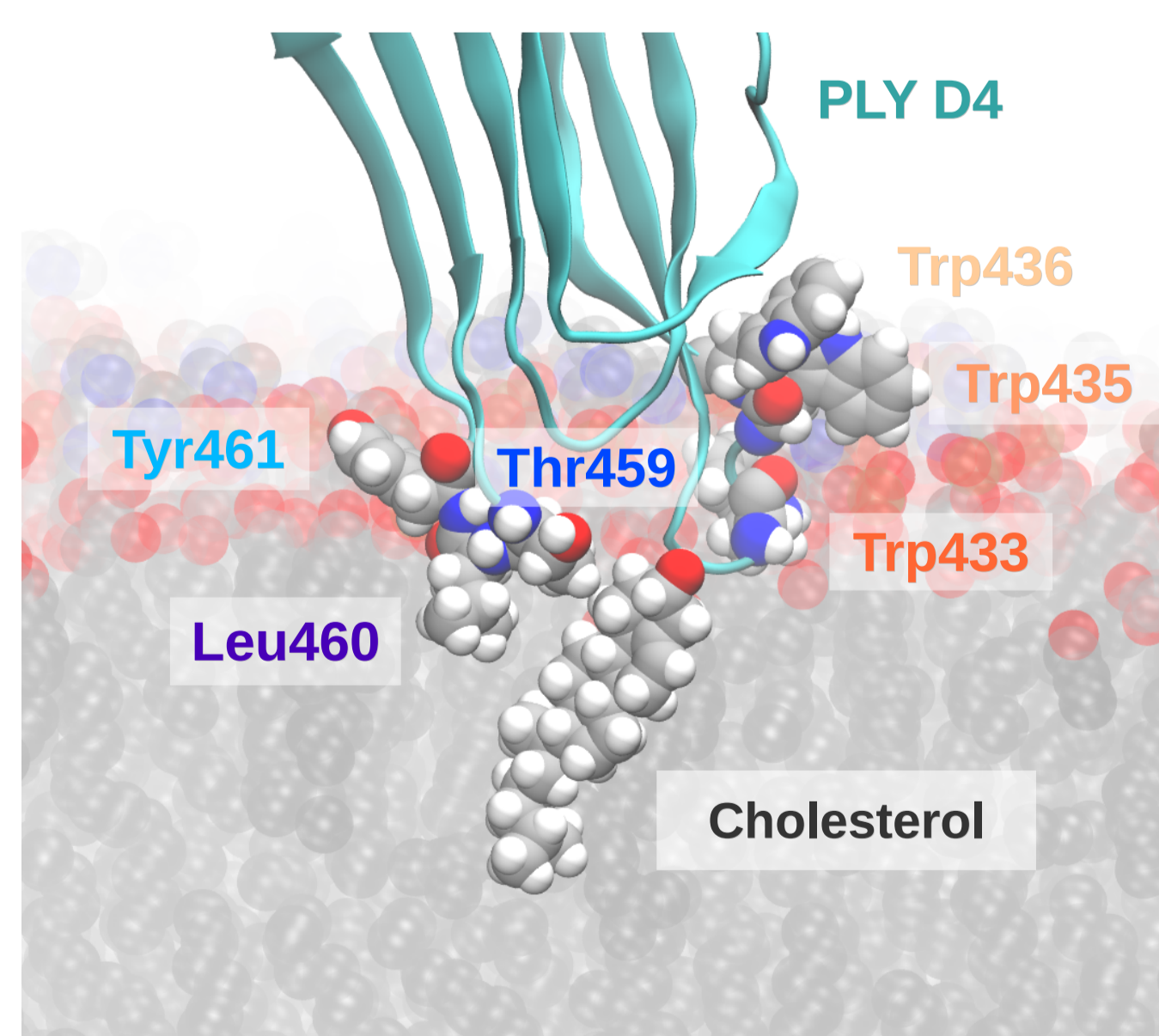
Pore Formation

MD simulations were initiated from different possible intermediate steps along pore formation.
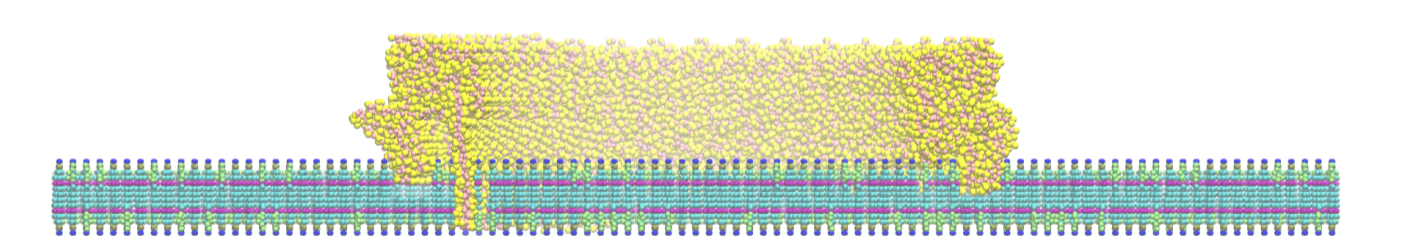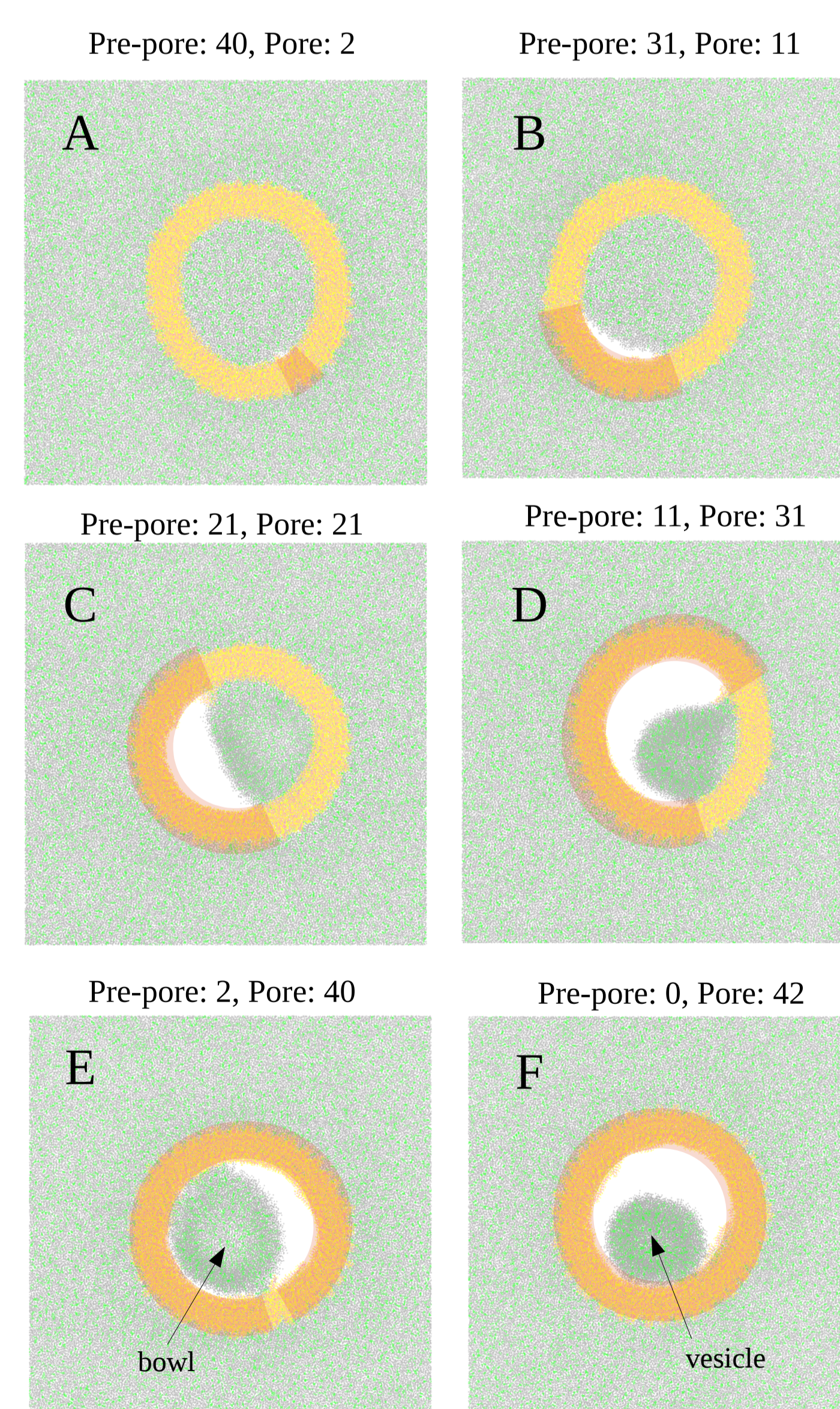

↑ **Figure 9:** Starting structure of a simulation with mixed pore and pre-pore conformations.

Lipids recede from the inner rim of the PLY ring. At the later stages, the inner lipids form a vesicle.

↑ **Figure 10:** Side view of system B.

← **Figure 11:** Top views of simulations with different numbers of monomers in pore (orange) and pre-pore (yellow) conformation.

A — Pre-pore: 40, Pore: 2
B — Pre-pore: 31, Pore: 11
C — Pre-pore: 21, Pore: 21
D — Pre-pore: 11, Pore: 31
E — Pre-pore: 2, Pore: 40
F — Pre-pore: 0, Pore: 42
bowl
vesicle

Conclusions

The combination of results from atomistic and coarse-grained simulations refines the current picture of the docked structure of PLY and of its cholesterol dependent membrane binding. Simulations of intermediates of a possible step-by-step pore formation show the behavior of the receding lipids.

Due to the high similarity of pore-forming toxins, these results give general insight into the function of other proteins in this class.

References

[1] van Pee et al., *Nano Letters*, 2016

[2] van Pee et al., *eLife* 2017

[3] Marshall et al., *Sci. Rep.* 2015

[4] Marrink et al., *J. Phys. Chem. B*, 2007

[5] Monticelli et al., *J. Chem. Theory and Comput.*, 2008,

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