RESEARCH ARTICLE

Figures and figure supplements

Sublytic gasdermin-D pores captured in atomistic molecular simulations

Stefan L Schaefer and Gerhard Hummer.

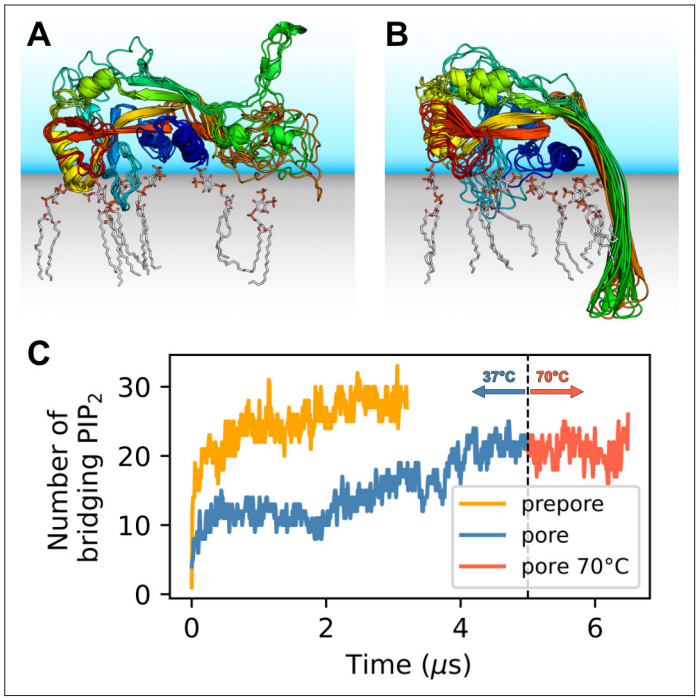

Figure 1. $GSDMD^{NT}$ interacts tightly with anionic lipids. Overlay of six representative $PI(4,5)P_2$ bound poses of the prepore monomer (**A**) and the 33-mer $GSDMD^{NT}$ ring in pore conformation (**B**). $GSDMD^{NT}$ is shown in cartoon representation and colored using a rainbow spectrum from blue (N-terminus) to red (C-terminus). The β1–β2 loop is colored in cyan, the α1 helix in dark blue, the α3 helix in yellow, and the C-terminus in red. $PI(4,5)P_2$ is shown in grey licorice representation with orange phosphorus and red oxygen atoms. Hydrogen atoms are not shown for clarity. The membrane and solvent are schematically shown with gray and blue shades, respectively. (**C**) Number of $PI(4,5)P_2$ molecules that interact simultaneously with two subunits of the prepore (orange) and pore (blue) 33-mer rings. After 5 μs at 37°C, the pore simulation was continued for 1.5 μs at 70 °C (red).

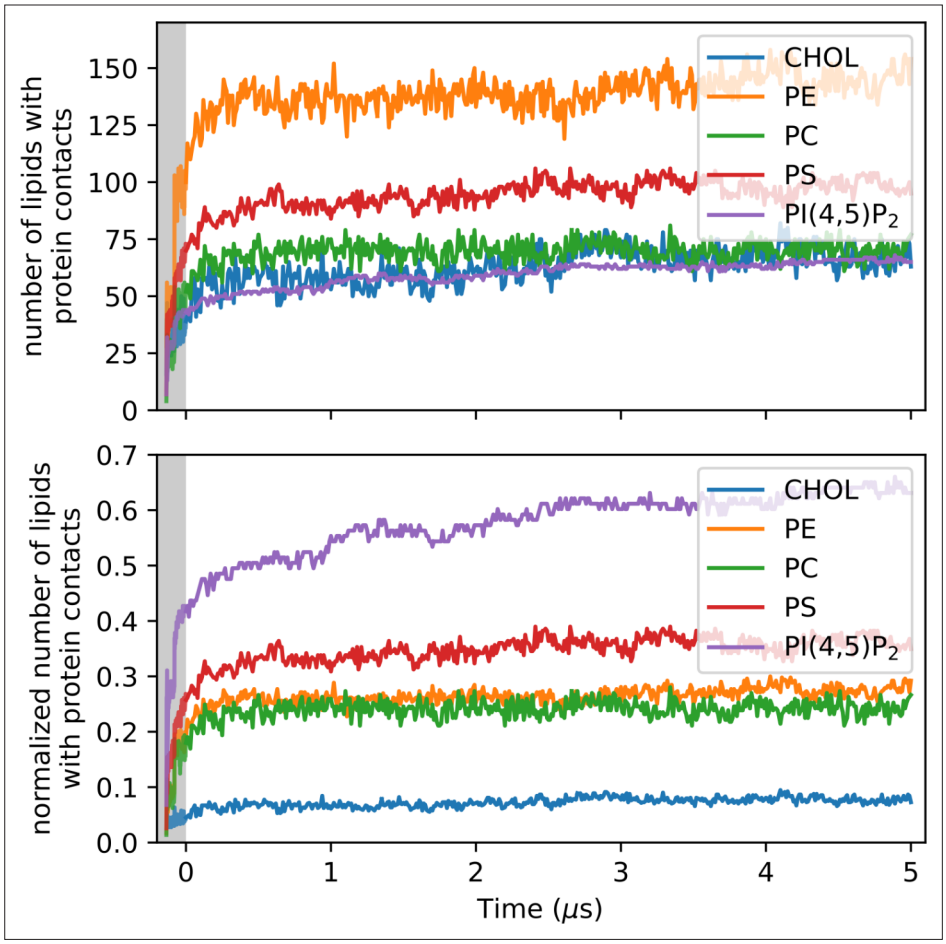

Figure 1—figure supplement 1. Change in the number of inner leaflet lipids, whose headgroups interact with at least one heavy atom of the 33-mer pore, in absolute counts (top) and normalized by the number of lipids of each lipid species in the inner leaflet (bottom). Lipids are grouped by their headgroup identity. The gray-shaded area represents the equilibration phase of the MD simulations.

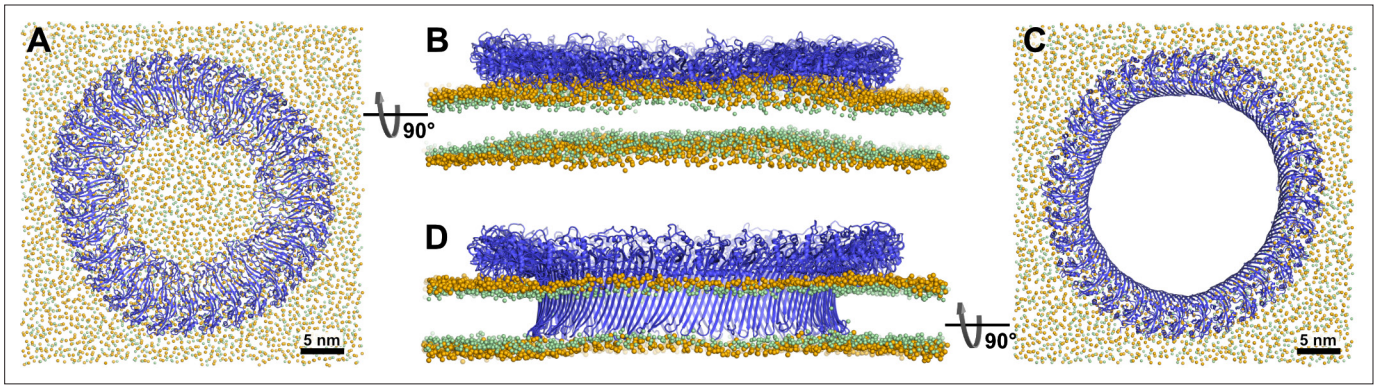

Figure 2. Atomistic MD simulations of GSDMD^{NT} 33-mer rings in prepore and pore conformation. Prepore (A,B; after 3.5 μs of MD) and pore rings (C,D; after 5 μs) are viewed from the top (**A,C**) and side (**B,D**). GSDMD^{NT} is shown in blue cartoon representation, lipid headgroup phosphates and glycerol oxygens are shown as orange and green spheres, respectively. Water, ions, and lipid tails are not shown for clarity. The membrane under the prepore ring (**A,B**) is continuous but visibly bent upwards into the ring (**B**). In the pore conformation (**C,D**), lipids are absent from the central pore, which is lined by a continuous, membrane spanning β-barrel.

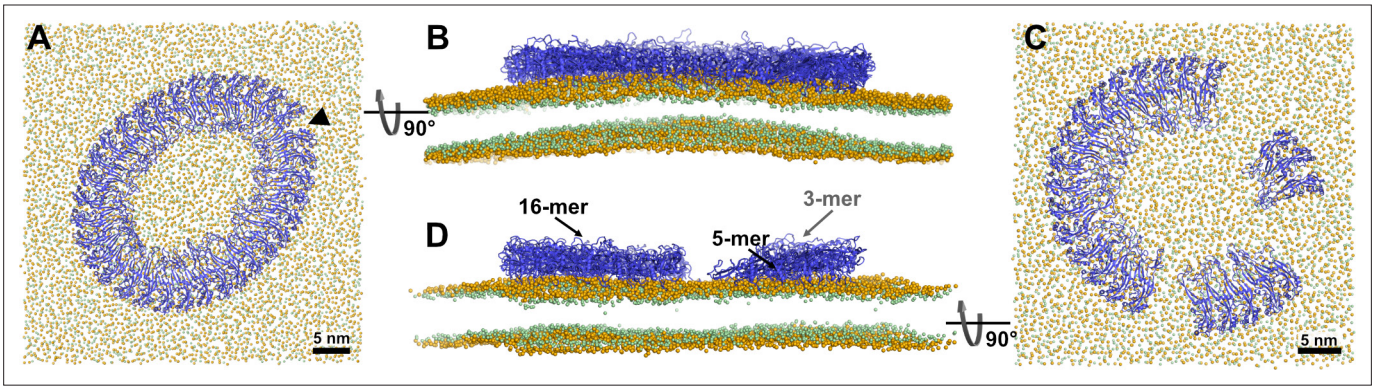

Figure 2—figure supplement 1. Snapshots of prepore 33-mer on a larger membrane patch and of prepore 3, 5, and 16-mer on one membrane patch. Snapshots of 33-mer GSDMD^{NT} prepore ring on 46×46 nm^2 membrane after 2.2 μs of MD simulation, viewed from the top (**A**) and side (**B**). Snapshots of the system with 3, 5, and 16-mer GSDMD^{NT} in prepore conformation after 1.5 μs of MD simulation, viewed from the top (**C**) and side (**D**). The GSDMD^{NT} backbones are shown in blue cartoon representation. Lipid headgroup phosphates and glycerol oxygen atoms are shown as orange and green spheres, respectively. Water, ions, and lipid tails are not shown for clarity. (**A**) The black triangle indicates where the contacts of the globular domains of two neighboring subunits broke up.

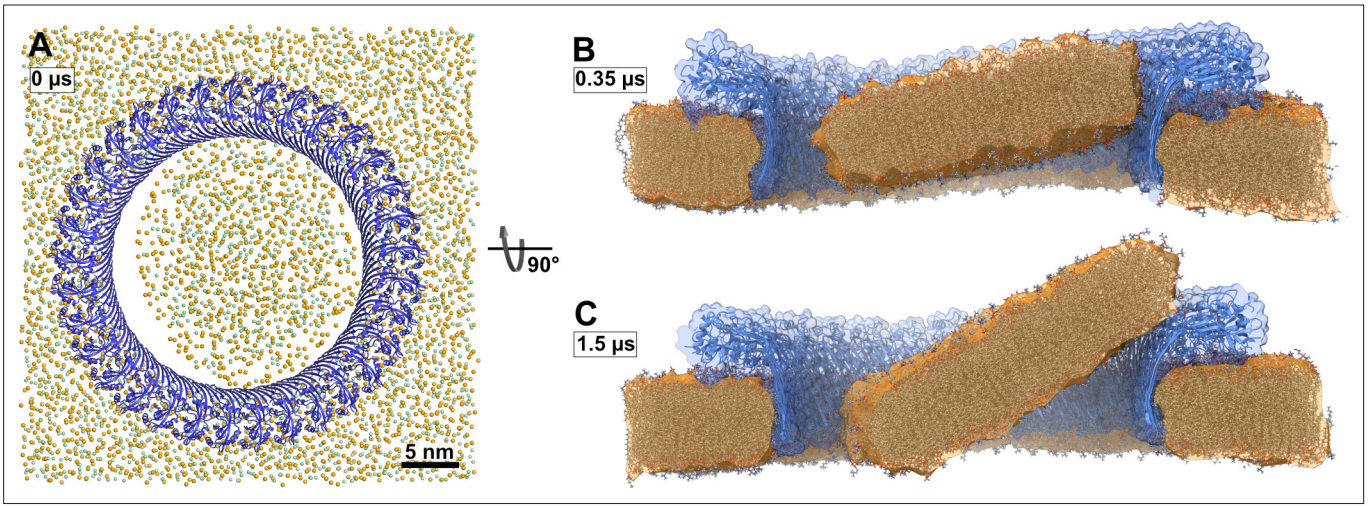

Figure 3. Atomistic MD simulations of GSDMD^{NT} 33-mer rings in pore conformation and filled initially with lipids. (**A**) Top view after equilibration. (**B,C**) Side views after 0.35 µs (**B**) and after 1.5 µs (**C**) of MD simulation shown as section through the pore center. The GSDMD^{NT} backbones are shown in blue cartoon representation. In (**A**), lipid headgroup phosphates and glycerol oxygen atoms are shown as orange and green spheres, respectively. Water, ions, and lipid tails are not shown for clarity. In the sections (**B,C**), lipid tails are shown in full licorice representation. The outlines of the protein and membrane are represented as transparent surfaces.

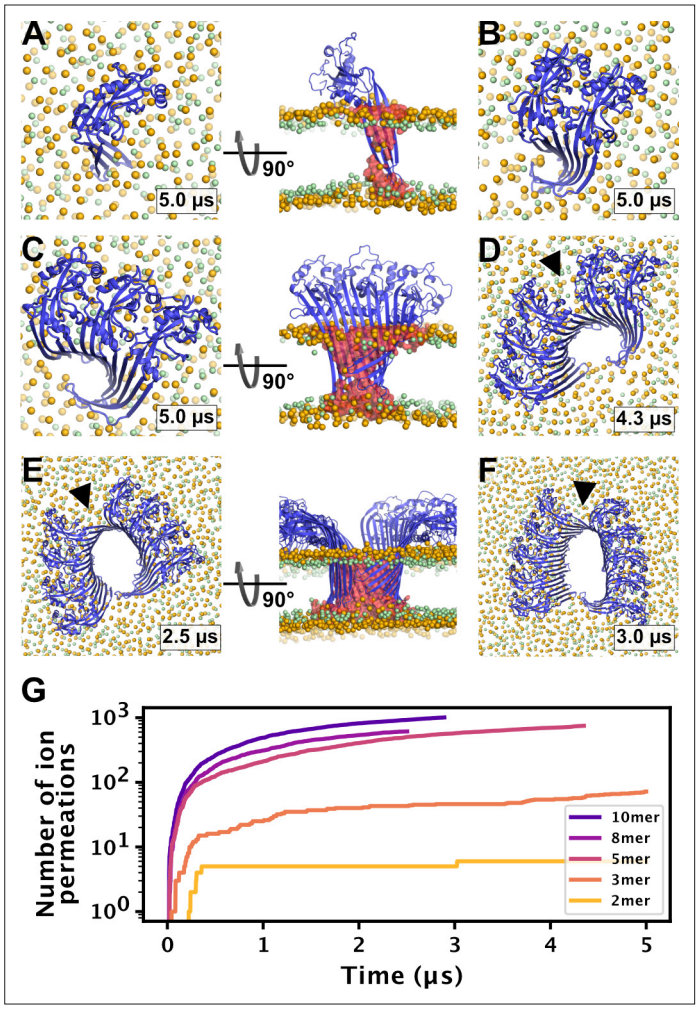

Figure 4. MD simulations of small GSDMD^{NT} oligomers. GSDMD^{NT} monomer (**A**), dimer (**B**), trimer (**C**), pentamer (**D**), octamer (**E**) and decamer (**F**) remain membrane inserted for the full duration of the respective MD simulations. The β-sheets of 2, 3, 5, 8, and 10-mers coil up into small membrane pores filled with water (water inside the pore shown as red volume in the right panels of A,C,E). The GSDMD^{NT} backbones are shown in blue cartoon representation. Lipid headgroup phosphates and glycerol oxygens are shown as orange and green spheres, respectively. Water, ions, and lipid tails are not shown for clarity except in the right panels of A,C,E. Black triangles indicate sites where the arc had cracked. (**G**) Cumulative sodium and chloride ion permeation events during the simulations. No ions permeated the membrane in the monomer simulation.

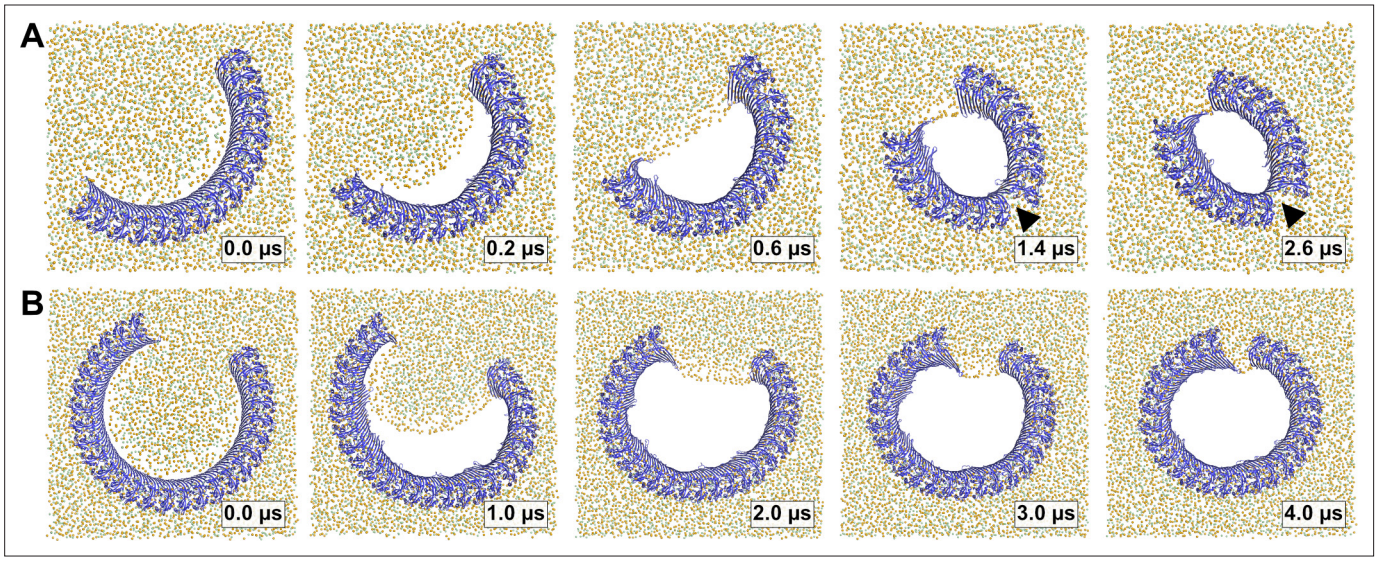

Figure 5. Arc-shaped GSDMD^{NT} oligomers transition into slit or ring-shaped membrane pores. Top views of GSDMD^{NT} arcs comprising 16 (**A**) and 27 (**B**) subunits in pore conformation along MD simulation trajectories (time points indicated) show phospholipid headgroups (orange spheres) and cholesterol oxygens (green spheres) receding from the inserted β-sheet, before the open protein edges approach each other and close into slit-shaped (**A**) or ring-shaped (**B**) pores. Water, ions, and lipid tails are not shown for clarity.

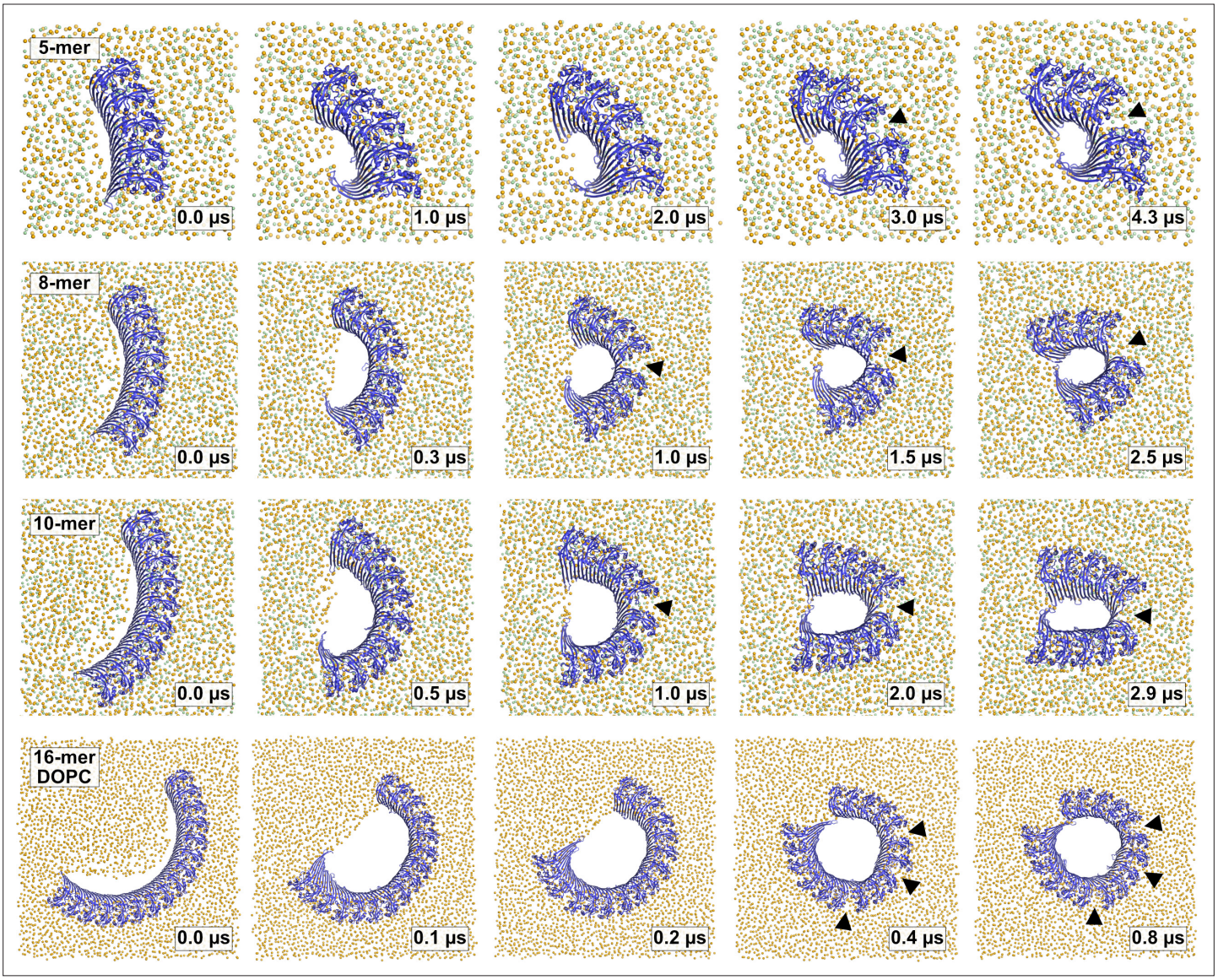

Figure 5—figure supplement 1. Time lapse images of pore formation from 5–10-meric arcs in the plasma membrane and a 16-meric arc in a pure DOPC membrane. Top view snapshots of $GSDMD^{NT}$ arcs comprising 5–16 subunits in pore conformation along MD simulation trajectories show lipids (orange spheres) and cholesterol (green spheres) receding from the inserted β-sheet, before the open protein edges come closer to each other. In the case of the 16-mer in a fluid DOPC membrane, the arc closes and forms a ring shaped pore. In all cases, the interfaces between globular domains broke in one to three positions, as indicated with black triangles. Water, ions, and lipid tails are not shown for clarity.

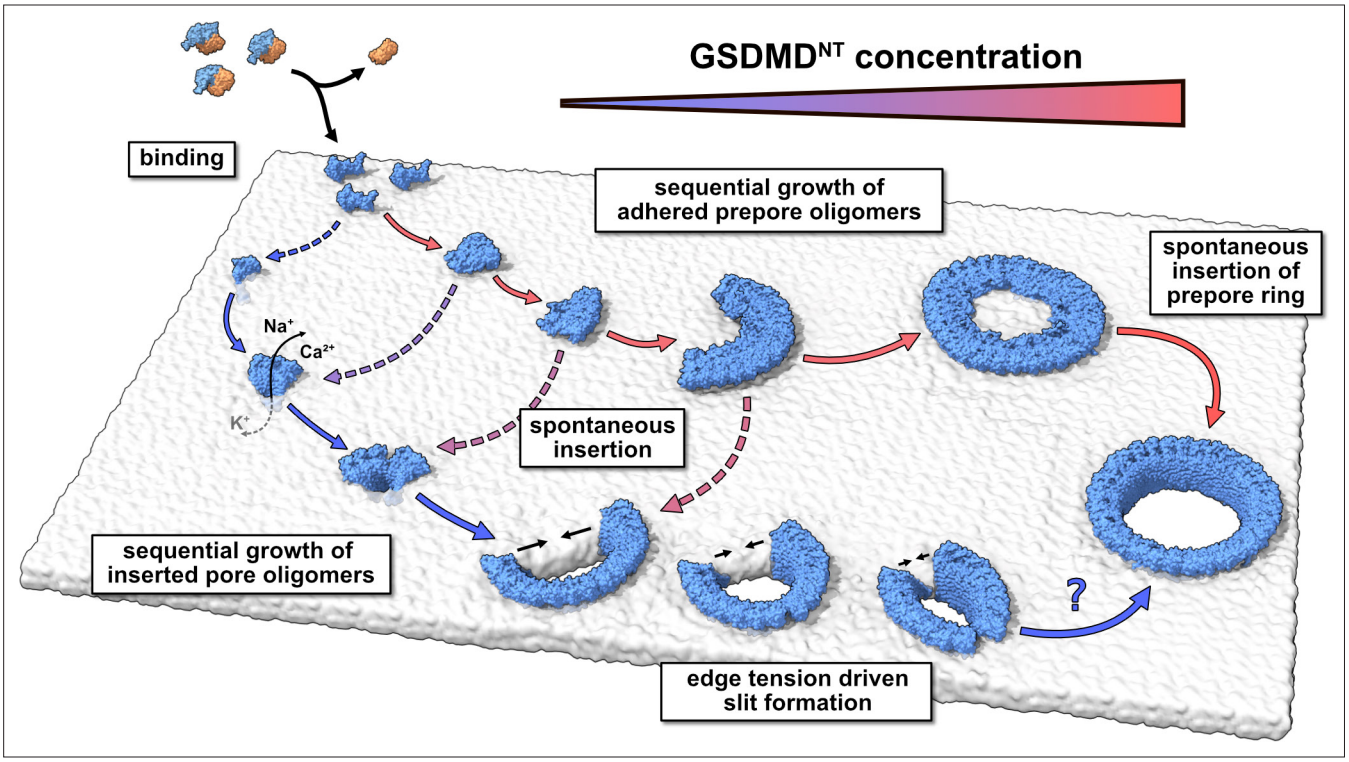

Figure 6. Model of membrane pore formation by GSDMD^{NT}. After proteolytic cleavage, GSDMD^{NT} monomers bind the inner leaflet of the plasma membrane. Aided by specific lipid interactions they multimerize and, at a critical size, spontaneously insert into the membrane. Depending on the concentration of membrane adhered GSDMD^{NT}, the insertion may proceed either from a fully formed prepore ring or from small oligomeric assemblies. Dotted arrows indicate that the mechanism of β-sheet insertion so far remains unresolved. Pores formed by small oligomers cause early nonspecific ion flux and can combine with one another or grow sequentially by the attachment of uninserted monomers. Depending on the edge tension in the cellular milieu, arcs would continue to grow or crack to form slit-shaped pores (bottom). Whether slit-shaped pores can grow to circular pores by subsequent mono- or oligomer attachment is unclear.