Supporting Information: Sublytic gasdermin-D pores captured in atomistic molecular simulations

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Table S1: Asymmetric plasma membrane composition. Fatty acid tails abbreviated as FA.

Lipid	**FA**	**Full name**	**Charge**	**Inner leaflet [mol %]**	**Outer leaflet [mol %]**
CCHOL		Cholesterol	0	40.4	40.0
PSM	18:1/16:0	N-palmitoyl-D-erythro-sphingosylphosphorylcholine	0	1.0	12.0
NSM	18:1/24:1	N-nervonoyl-D-oleoyl-sphingosylphosphorylcholine	0	-	9.0
LSM	18:1/24:0	N-lignoceroyl-D-oleoyl-sphingosylphosphorylcholine	0	-	8.0
PLPC	16:0/18:2	1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine	0	8.1	15.0
SOPC	18:0/18:1	1-stearoyl-2-oleoylphosphocholine	0	-	7.0
PAPC	16:0/20:4	1-palmitoyl-2-arachidonoyl-glycero-3-phosphocholine	0	-	5.0
POPC	16:0/18:1	1-palmitoyl-2-oleoyl-glycero-3-phosphocholine	0	3.0	-
DPPC	16:0/16:0	1,2-dipalmitoyl-glycero-3-phosphocholine	0	2.0	-
PLA20(PE)	18:0/20:4	1-O-stearoyl-2-O-arachidonoyl-glycero-3-phosphoethanolamine	0	11.1	3.0
PDoPE	16:0/22:6	1-palmitoyl-2-docosahexaenoyl-glycero-3-phosphoethanolamine	0	8.1	-
SAPE	18:0/20:4	1-stearoyl-2-arachidonoyl-glycero-3-phosphoethanolamine	0	4.0	-
POPE	16:0/18:1	1-palmitoyl-2-oleoyl-glycero-3-phosphoethanolamine	0	3.0	-
PAPS	16:0/20:4	1-palmitoyl-2-arachidonoyl-glycero-3-phosphoserine	-1	13.1	-
SAPS	18:0/20:4	1-stearoyl-2-arachidonoyl-glycero-3-phosphoserine	-1	1.0	1.0
$PI(4,5)P_2$	16:0/18:2	1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoinositol-4,5-bisphosphate	-4	5.1	-

Table S2: Eisenberg hydrophobicity scores[S1] of the pore facing residues of human GSDMD and mouse GSDMA3 in kcal mol^{-1}

	human GSDMD		mouse GSDMA3	
Structural element	**Pore facing residues**	**$\sum$ Eisenberg hydrophobicity**	**Pore facing residues**	**$\sum$ Eisenberg hydrophobicity**
β3	ADQQSE	-2.73	MDQQLE	-1.93
β5	KAGASS	-0.96	TKKTGS	-2.66
β7	TKESRS	-4.18	TNNISP	-1.06
β8	QEQHSK	-3.76	LGQSNN	-1.54
$\sum$ full sheet		-11.63		-7.19

Table S3: Restraints used during energy minimization (EM) and stepwise equilibration (steps 1 to 6) of the asymmetric plasma membrane mimetic in kJ mol^{-1} nm^{-2}.

Step	Time [ns]	Timestep [fs]	Ensemble	Lipids	Dihedrals
EM				1000	1000
1	0.125	1	NVT	1000	1000
2	0.125	1	NVT	400	400
3	0.125	1	NPT	400	200
4	0.5	2	NPT	200	200
5	0.5	2	NPT	40	100
6	0.5	2	NPT	0	0

Table S4: Restraints used during energy minimization (EM) and stepwise equilibration (1 to 3) of the asymmetric plasma membrane mimetic in kJ mol^{-1} nm^{-2}.

Step	Time [ns]	Timestep [fs]	Backbone	Sidechains	Lipid headgroup (z)	Water (z)
EM			4000	2000	1000	1000
1	5*	2	2000	1000	10	50*
2	50	2	2000	1000	50	0
3	80	2	1000	500	0	0

* For the 27-mer system with an initially lipid filled pore this was extended to 10 ns with a force constant of 1000 kJ mol^{-1} nm^{-2} for restraining the z-position of water molecules.

Table S5: Atomistic GSDMD^{NT} simulations

Conformation	Subunits	Simulated time [μs]	Membrane size [nm^2]	No. of atoms
prepore	1	7.0	13.4×13.4	222740
prepore	33	3.5	38.9×38.9	1950552
prepore	33	2.2	45.8×45.8	3113594
pore	1	5.0	12.7×12.7	216375
pore	2	5.0	12.5×12.5	213665
pore	3	5.0	12.2×12.2	208656
pore	5	4.3	18.8×18.8	481242
pore	8	2.5	25.5×31.9	1079514
pore	10	3.0	25.4×31.7	1071088
pore	16	3.5	31.3×31.3	1329398
*pore	16	1.4	34.9×34.9	1497819
pore	27	4.1	37.2×37.2	2226909
pore	33	5.0	37.0×37.0	2119785
◊pore	33	1.5	37.0×37.0	2119785

*simulated in pure DOPC membrane

◊ 70°C continuation of the 37°C simulation after 5μs

Supporting Figures

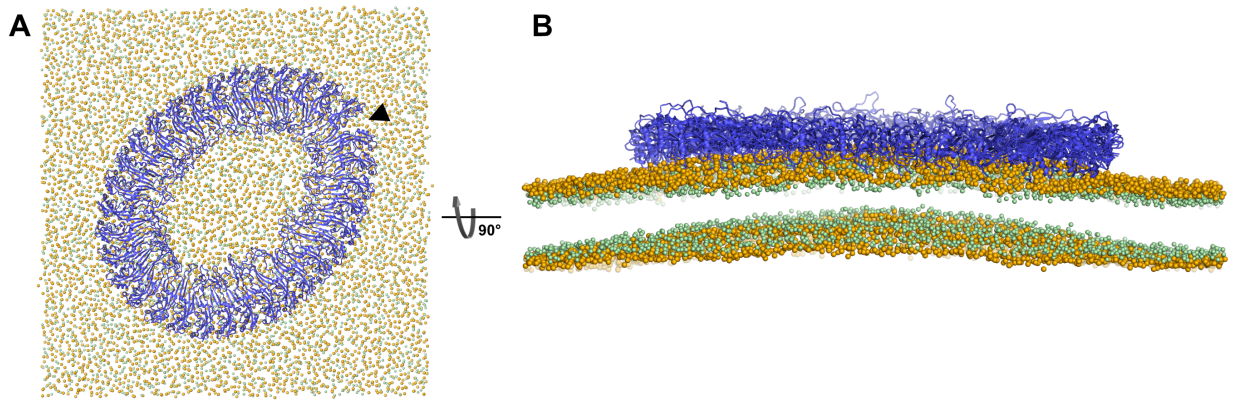

Figure S1: **Snapshot of 33-mer GSDMD^{NT} prepore ring on 46 × 46 nm^2 membrane after 2.2 μs of MD simulation, viewed from the top (A) and side (B).** The GSDMD^{NT} backbones are shown in blue cartoon representation. Lipid headgroup phosphates and glycerol oxygen atoms are shown as orange and green spheres, respectively. Water, ions, and lipid tails are not shown for clarity. (A) The arrow indicates where the contacts of the globular domains of two neighboring subunits broke up.

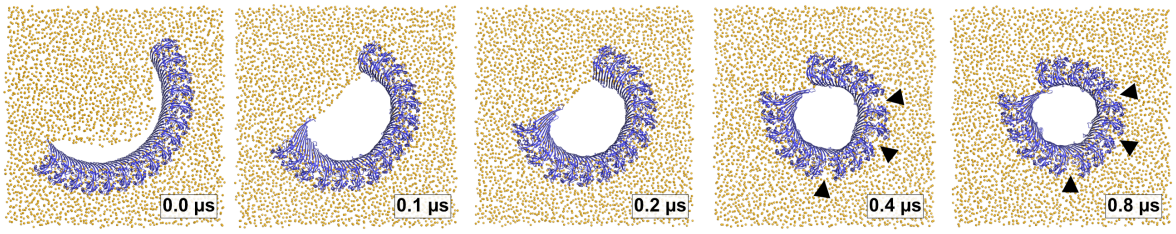

Figure S2: **Time lapse images of pore formation from a 16-meric arc in DOPC membrane.** Top view snapshots of GSDMD^{NT} arcs comprising 16 subunits in pore conformation along MD simulation trajectories show lipids (orange spheres) receding from the inserted β-sheet, before the open protein edges come closer to each other and close a ring shaped pore. The interfaces between globular domains broke in three positions, as indicated with black triangles. Water, ions, and lipid tails are not shown for clarity.

Supporting Movie Legends

Supporting Movie S1

Formation of a slit-like pore from a membrane inserted 16-meric arc. Trajectory from 2.0 μs of simulation, showing the plasma membrane edge receding from the protein and then shortening. This draws the ends of the multimer together, causes breakage of the contacts of two neighboring globular domains and ultimately the slit-shaped pore of Figure 4A (2.6 μs) develops. Lipid headgroup phosphates are shown as yellow spheres, cholesterol oxygen atoms as light green spheres, and the protein is shown in blue cartoon representation. Water and ions are not shown for clarity.

Supporting Movie S2

Formation of a ring-like pore from a membrane inserted 27-meric arc. Trajectory from 4.0 μs of simulation, showing the plasma membrane edge receding from the protein. Lipid efflux first increases the distance between the arc ends, before the open membrane edge then draws the ends together to form the ring-shaped pore of Figure 4B (4.0 μs). Lipid headgroup phosphates are shown as yellow spheres, cholesterol oxygen atoms as light green spheres, and the protein is shown in blue cartoon representation. Water and ions are not shown for clarity.

References

(S1) Eisenberg, D.; Weiss, R. M.; Terwilliger, T. C.; Wilcox, W. Hydrophobic moments and protein structure. *Faraday Symp. Chem Soc.* **1982**, *17*, 109–120.