Supporting Information for

Computational Investigation of Cholesterol Binding Sites on Mitochondrial VDAC

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note: Legends for Supporting Movies are provided at the end of this document

Figure S1. The location and identity of residues experimentally determined to bind cholesterol are shown in red sticks (reference (1) in the main text). N-terminal helix residues 2-25 are colored dark blue, and residues 26-283 are colored cyan.

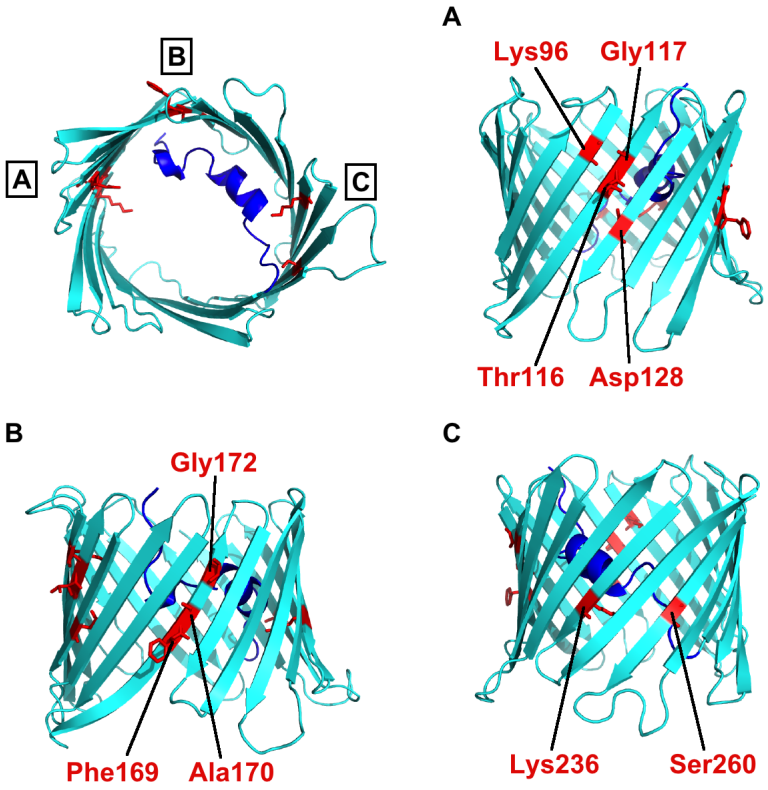


Figure S2. Shown in different colored sticks are the starting orientations for cholesterol during preliminary simulations that were used to build the final cholesterol-bound VDAC model. Each starting pose was a docking result that was returned from AutoDock. Residues that comprise each site are colored red.

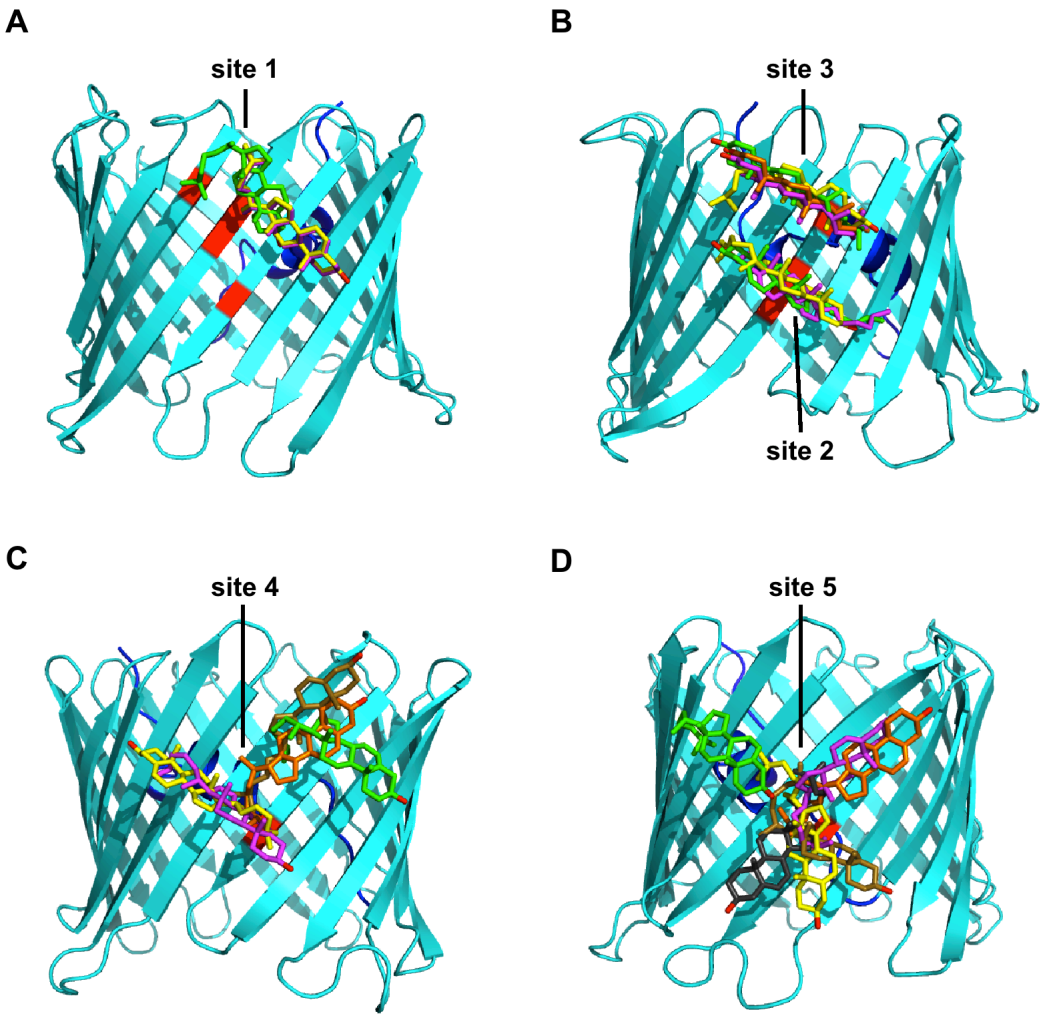


Figure S3. Minimum distance between cholesterol and a backbone amide atom (N or H) of the individual residues that were assigned to (A) site 1 and (B) site 2. A running average of 25 data points was used to reduce noise.

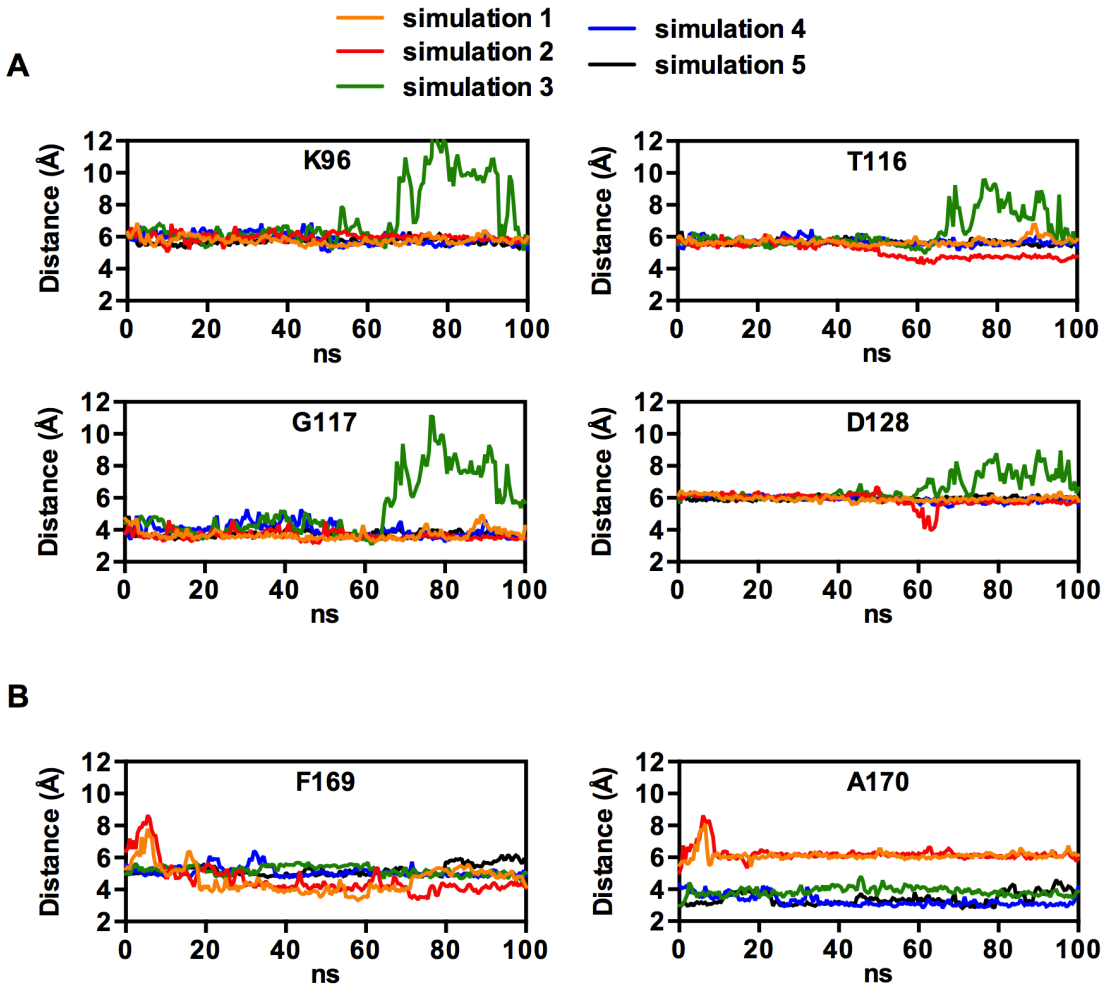


Figure S4. RMSD of cholesterol-bound and apo-VDAC backbones for each of ten simulations, showing the protein is both equilibrated and stable throughout the simulations. The trajectories were aligned to the backbone of the first frames (essentially, the humanized structure of PDB code 3EMN). These traces represent production runs following a short (0.725 ns) equilibration.

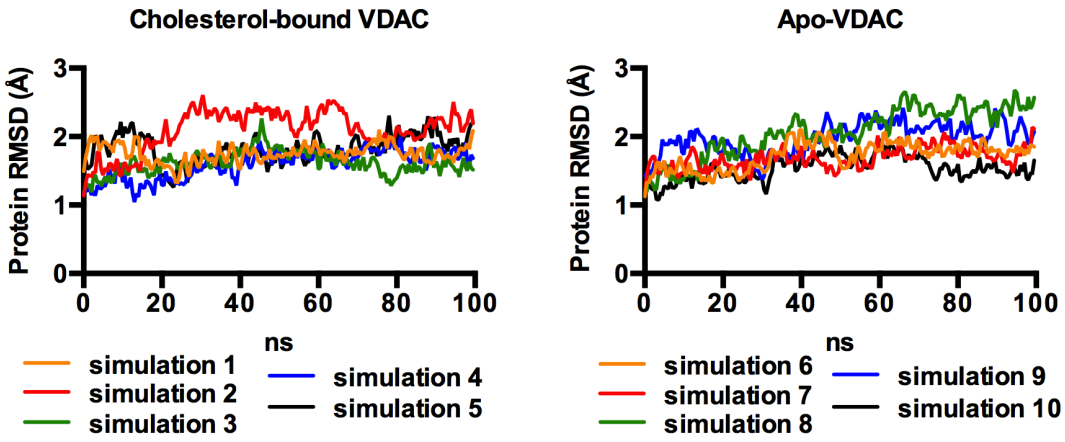
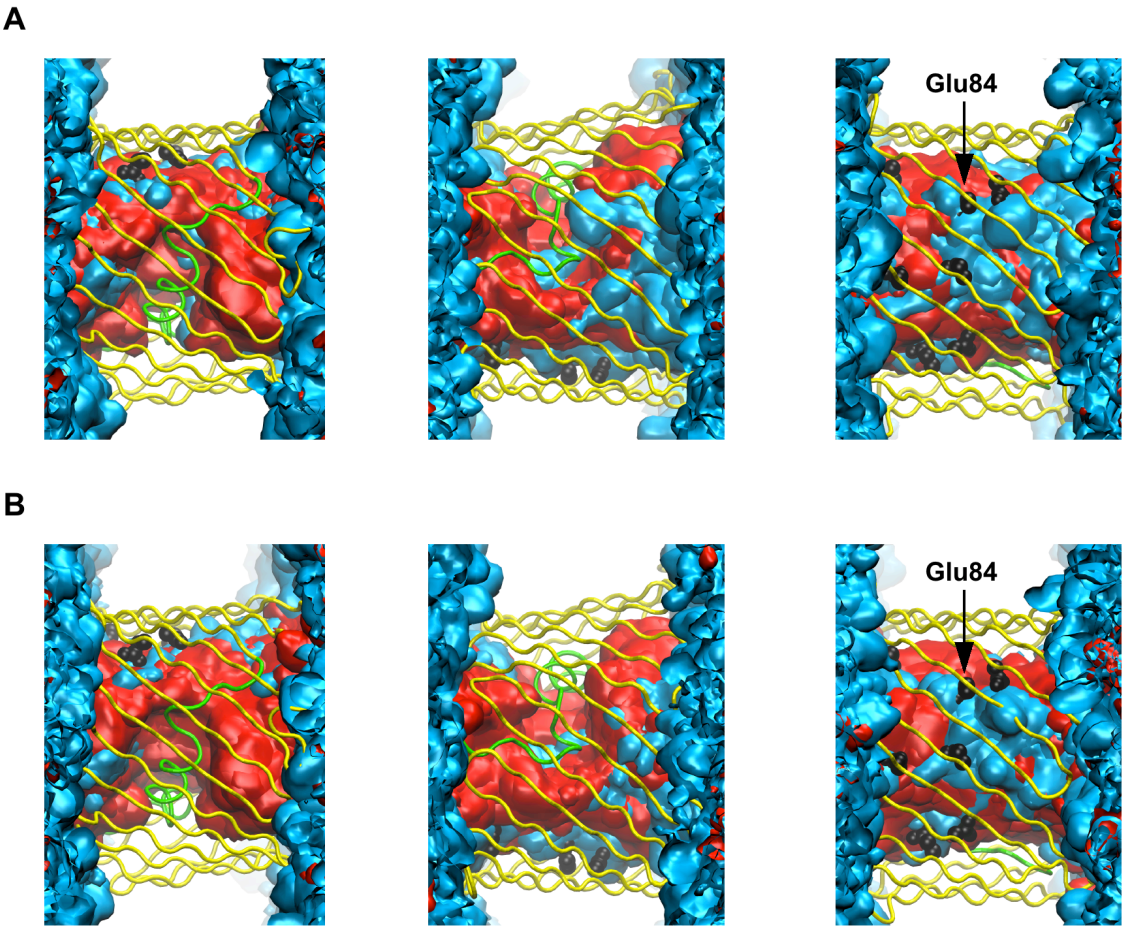


Table S1. Percent of simulation frames in which specific residue pairs hydrogen bond.[a]

Simulation	A2-H122	P4-N124	F18-K236	G25-T49	G25-L275	G23-S260	K20-K224	G23-L26	Y7-N168	V3-K119
1	56.3	49.8	44.8	52.5	7.1	30.4	0.0	9.1	0.0	0.0
2	53.1	43.6	43.4	7.5	29.4	27.2	51.5	5.8	0.0	0.0
3	52.9	50.3	38.8	15.9	3.8	20.4	59.9	11.2	0.0	0.0
4	43.6	50.7	44.9	44.2	42.6	29.9	3.3	9.5	0.0	0.0
5	57.5	46.8	46.8	42.5	37.8	29.2	3.0	7.3	0.0	0.0
6	52.1	50.3	43.4	41.1	37.7	23.7	47.9	8.9	0.1	0.0
7	50.1	46.8	50.2	20.6	46.8	34.7	0.4	7.0	0.0	0.0
8	37.1	52.1	34.9	67.8	19.2	23.5	30.9	9.0	0.1	0.0
9	54.7	50.5	46.2	40.3	40.4	32.5	2.3	6.1	11.8	0.0
10	52.9	41.1	48.9	33.0	35.2	35.7	34.7	8.7	0.6	0.0
Median	52.9	50.0	44.8	40.7	36.4	29.5	17.1	8.8	0.0	0.0

[a]These pairs are specific to the N-terminal helix (residues 2-25) hydrogen bonding with the remainder of the protein (residues 26-283), and include polar atoms from side chains and the protein backbone. These have >10% occupancy in at least one simulation. Red indicates >10% difference from the median for that specific pair, and blue indicates >25% difference. The crystal structure from PDB code 3EMN contains hydrogen bonds between P4-N124, G25-T49, G25-L275, G23-S260, and V3-K119.

Figure S5. K^+ ions concentrate around negatively charged residues inside the channel on the opposite side of the barrel from the N-terminal helix. The cyan and red correspond to the aggregate volumetric densities of K^+ and Cl^- ions, respectively, at $3*10^{-5}$ amu/Å^3. (A) Apo-VDAC and (B) cholesterol-bound VDAC are shown, with the *trans* pore entrance to the left, and the panels representing a 360˚ rotation around the channel viewed from the within the membrane. Shown in black spheres are the sidechain atoms of Asp30, Glu40, Glu59, Glu84, Glu88, Asp100, and Glu280, all of which have decreased average RMSF in cholesterol-bound VDAC. Note the confluence of K^+ density across the entire channel (from left to right) and surrounding the negatively charged residues, particularly adjacent Glu84, in cholesterol-bound VDAC. In contrast, Cl^- density is concentrated around the N-terminal helix (colored green); this is increasingly evident in cholesterol-bound VDAC compared to apo-VDAC.



Supporting Movie Legends

Movie S1. 100 ns trajectory of docked cholesterol occupying site 1. The view is aligned along the x axis, with the N- and C-termini ($z < 0$, or the *trans* side) at the top of the screen. The protein without hydrogen is represented in QuickSurf, with amino acids colored by residue type (white, hydrophobic; green, polar; blue, positively charged; red, negatively charged), except for targeted site 1 residues (Lys96, Thr116, Gly117, and Asp128), which are colored magenta. Cholesterol is shown in sticks without hydrogen.

Movie S2. 100 ns trajectory of docked cholesterols occupying sites 2 and 3. The view is aligned along the x axis, with the N- and C-termini ($z < 0$, or *trans*) at the top of the screen. The protein without hydrogen is represented in QuickSurf, with amino acids colored by residue type (white, hydrophobic; green, polar; blue, positively charged; red, negatively charged), except for targeted site 2 residues (Phe169 and Ala170) colored magenta and the site 3 residue (Gly172) colored orange. Cholesterol is shown in sticks without hydrogen.

Movie S3. 100 ns trajectory of docked cholesterols occupying sites 4 and 5. The view is aligned along the x axis, with the N- and C-termini ($z < 0$, or *trans*) at the top of the screen. The protein without hydrogen is represented in QuickSurf, with amino acids colored by residue type (white, hydrophobic; green, polar; blue, positively charged; red, negatively charged), except for targeted site 4 residue (Lys236) colored magenta and the site 5 residue (Ser260) colored orange. Cholesterol is shown in sticks without hydrogen.

Movie S4. 100 ns trajectory of cholesterols occupying sites 4 and 5, with a random membrane cholesterol replacing the originally docked cholesterol in site 4. The view is aligned along the x axis, with the N- and C-termini ($z < 0$, or *trans*) at the top of the screen. The protein without hydrogen is represented in QuickSurf, with amino acids colored by residue type (white, hydrophobic; green, polar; blue, positively charged; red, negatively charged), except for targeted site 4 residue (Lys236) colored magenta. Cholesterol is shown in sticks without hydrogen.