Supporting Information

Effect of Lipid Oxidation on the Channel Properties of Cx26 Hemichannels: a Molecular Dynamics Study

Maria C. Oliveira[a*], Rodrigo M. Cordeiro[b], Annemie Bogaerts[a]

[a]Plasma Lab for Applications in Sustainability and Medicine-Antwerp (PLASMANT), Department of Chemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium

[b]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Avenida dos Estados 5001, CEP 09210-580, Santo André, SP, Brazil

*Corresponding author

E-mail address: mariacecilia.oliveira@uantwerpen.be

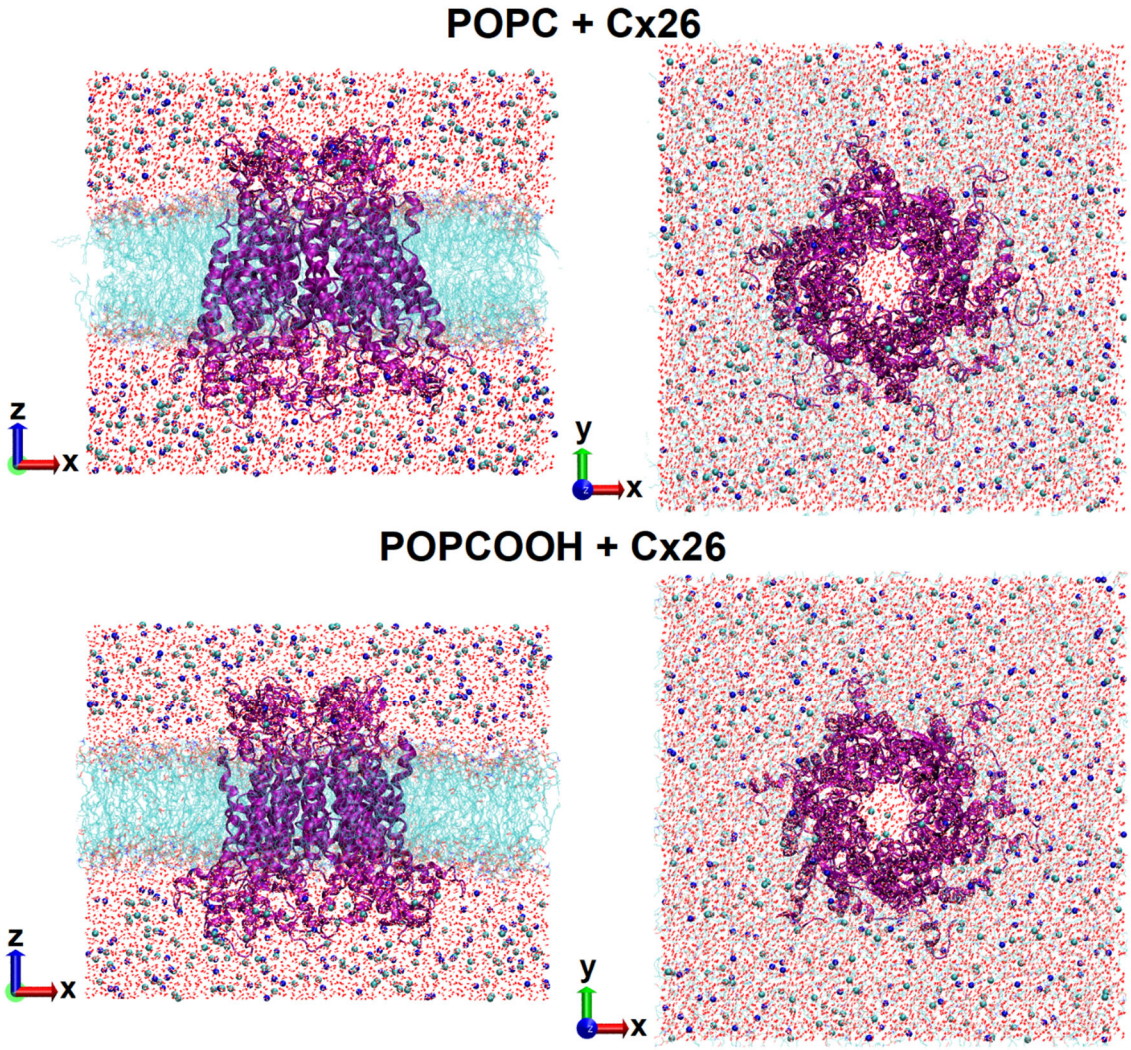

Figure S1. Representation of the initial simulated model systems, in side view (left) and top view (right). The Cx26 hemichannel is represented as purple ribbons, the lipids as lines of different colours, the water molecules as red points, and sodium and chlorine ions as van der Waals spheres in blue and cyan, respectively.

Table S1. Composition of each simulated model system.

Model system	Number of molecules				Box dimensions (nm)
	Lipids	Water	Na^+	Cl^-	
POPC + Cx26	500	38617	186	240	13.36, 13.36, 11.55
POPCOOH + Cx26	500	46455	211	265	14.26, 14.26, 11.50

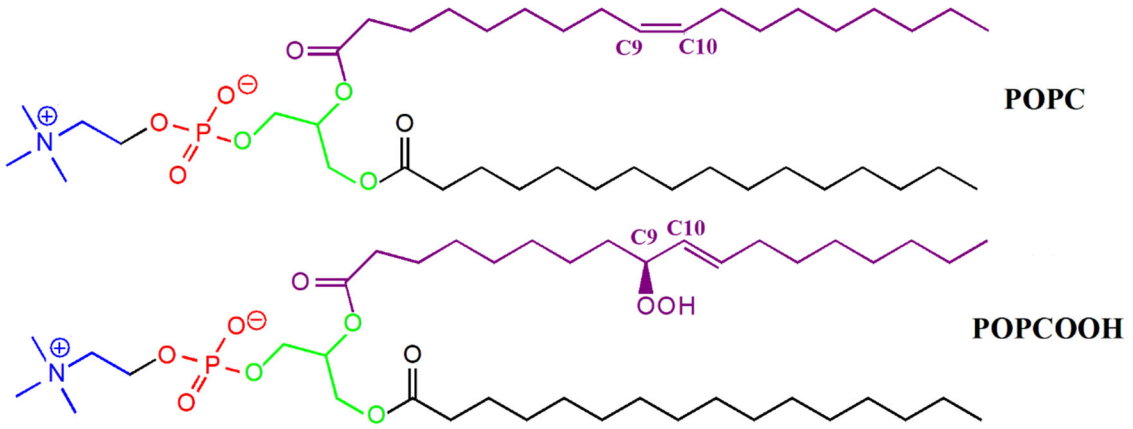

Figure S2. Structures of the POPC molecule and its oxidation product POPCOOH. The atoms in blue, red, and green represent choline, phosphate, and glycerol groups, respectively. The palmitoyl (*sn*-1) and oleoyl (*sn*-2) chains are represented by black and purple colours, respectively.

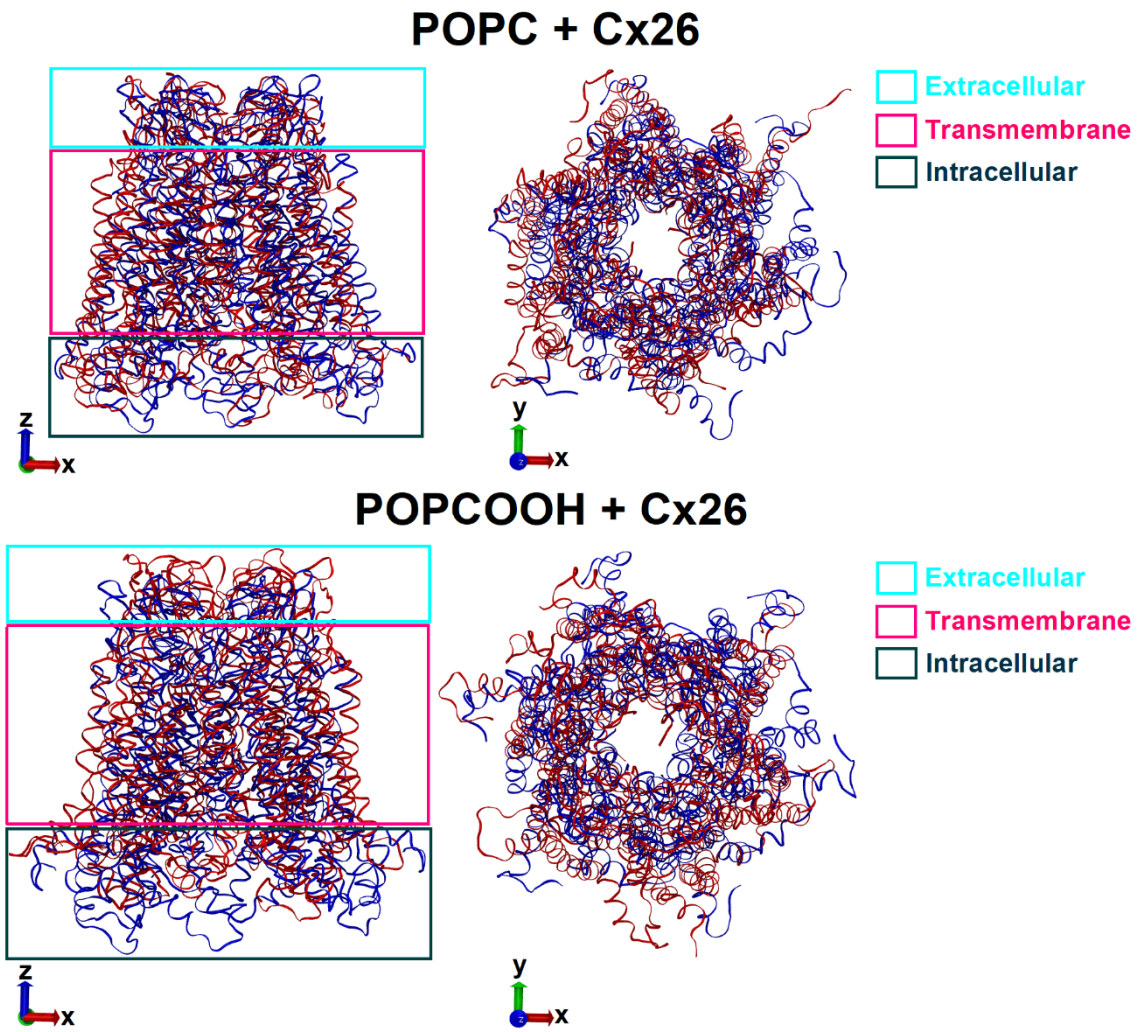

Figure S3. Comparison between the initial configuration of the Cx26 hemichannel (in blue) and the equilibrated hemichannel at 500 ns of simulation (in red). We can note a higher deviation in the intracellular side in the presence of oxidized lipids.

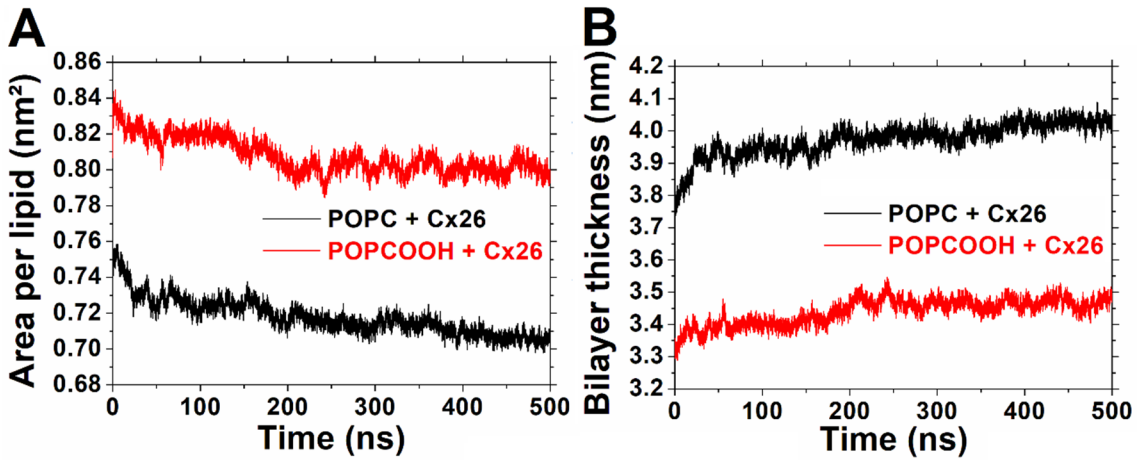

Figure S4. Temporal evolution of the structural properties of POPC and POPCOOH membranes: (**A**) area per lipid and (**B**) bilayer thickness.

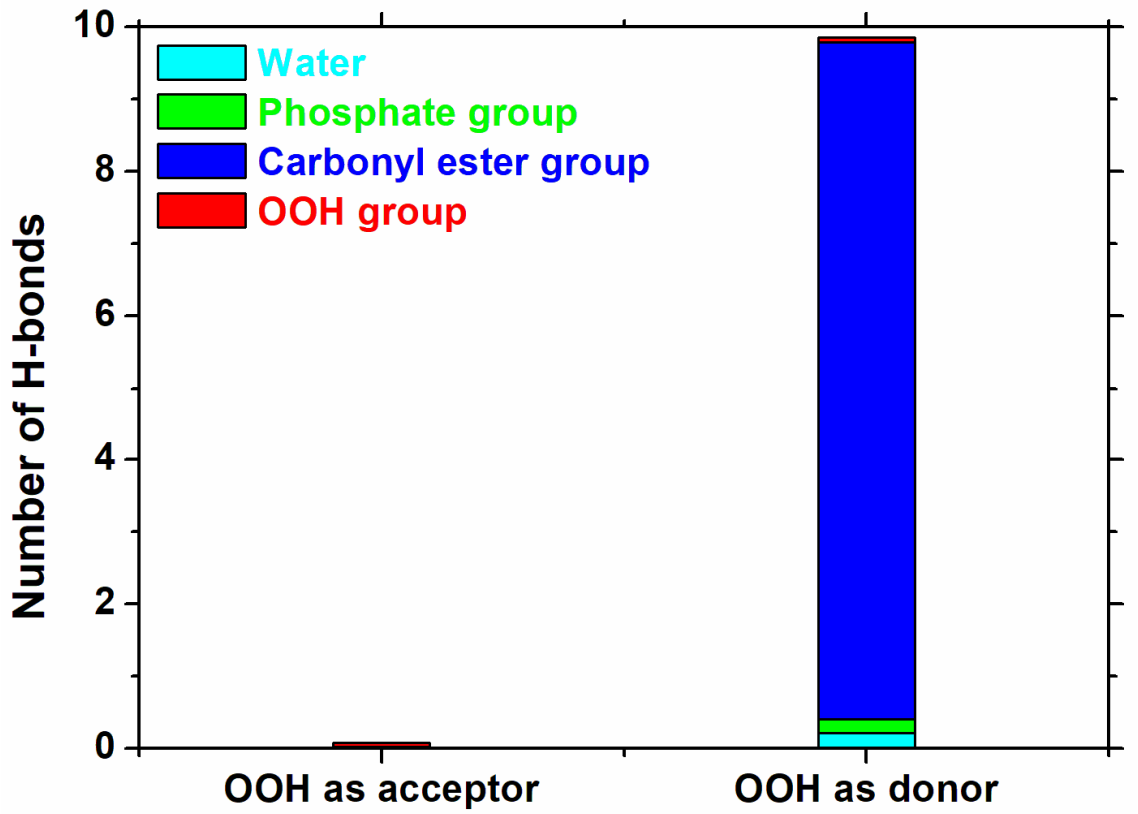

Figure S5. Average number of hydrogen bonds (H-bonds) established between the —OOH group of POPCOOH lipids, with water and other groups of POPCOOH lipids. The number of bonds were defined at a maximum distance of 0.25 nm for the acceptors and averaged over the last 200 ns of the simulation.

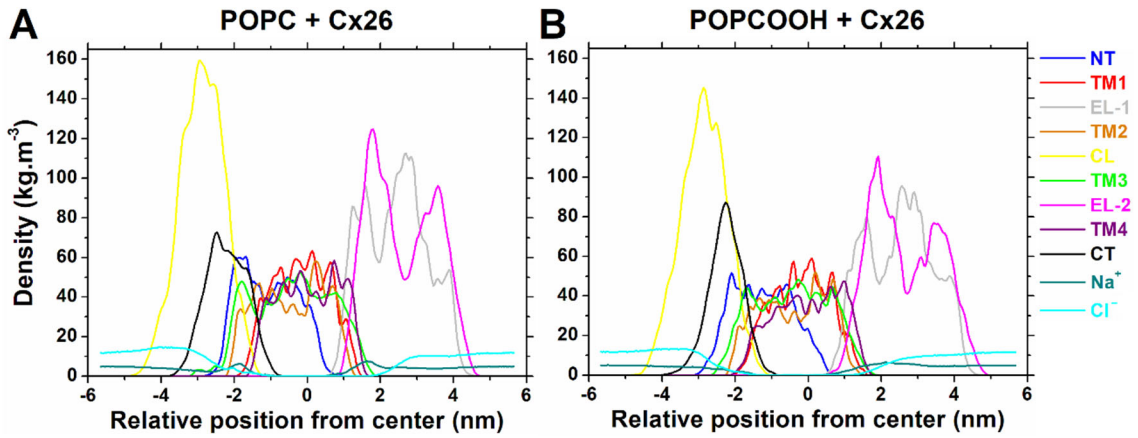

Figure S6. Density profiles of the Na^+ and Cl^- ions, and intracellular and extracellular regions of the Cx26 hemichannel embedded into the (**A**) POPC membrane and (**B**) POPCOOH membrane.

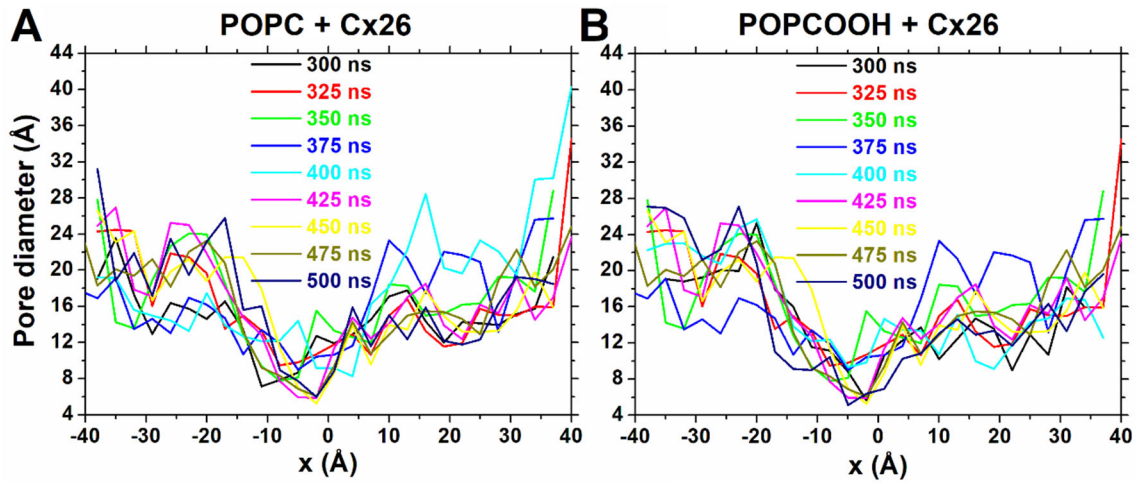

Figure S7. Pore diameter profiles of the Cx26 hemichannel embedded into the (**A**) POPC membrane and (**B**) POPCOOH membrane, for different simulation times.

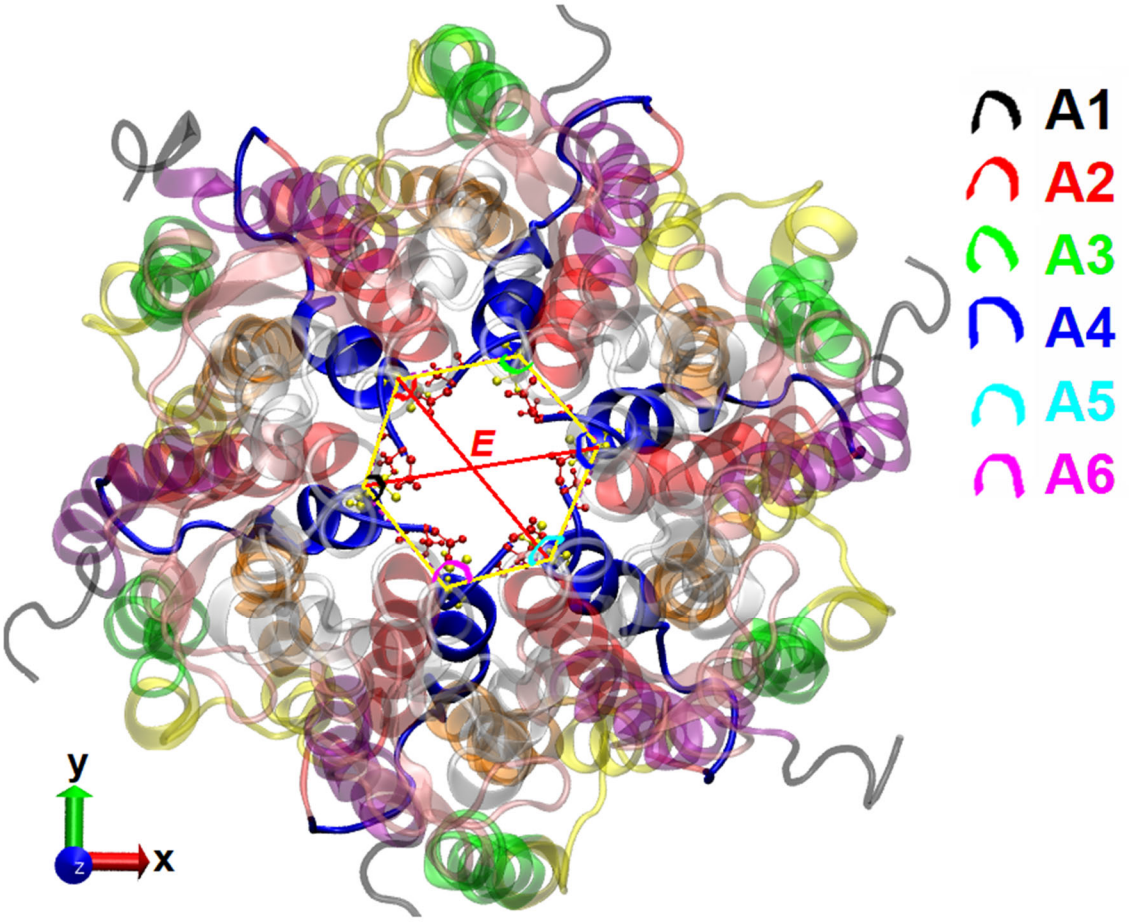

Figure S8. Schematic representation of how the eccentricity coefficient (*E*) and angles (*A*) were calculated for the Cx26 hemichannel. The maximum and minimum diameters are represented as red lines, and the hexagon to calculate the angles as yellow lines. The maximum and minimum diameters were calculated by the average distance between the Cα of the six asparagine residues within the NT domains (represented as red balls and sticks), calculated from the last 200 ns of simulation. The angles were calculated as the angle between the Cα of the six threonine residues within the NT domains (represented as yellow balls and sticks), calculated from the last 200 ns of simulation. Each angle is represented as a half circle of different colour lines: black, red, green, blue, cyan, and pink. Each domain of each Cx26 protein is represented as ribbons rendered with different colours: TM1 (red), TM2 (orange), TM3 (green), TM4 (purple), EL-1 (white), EL-2 (pink), CL (yellow), NT (blue), and CT (black).

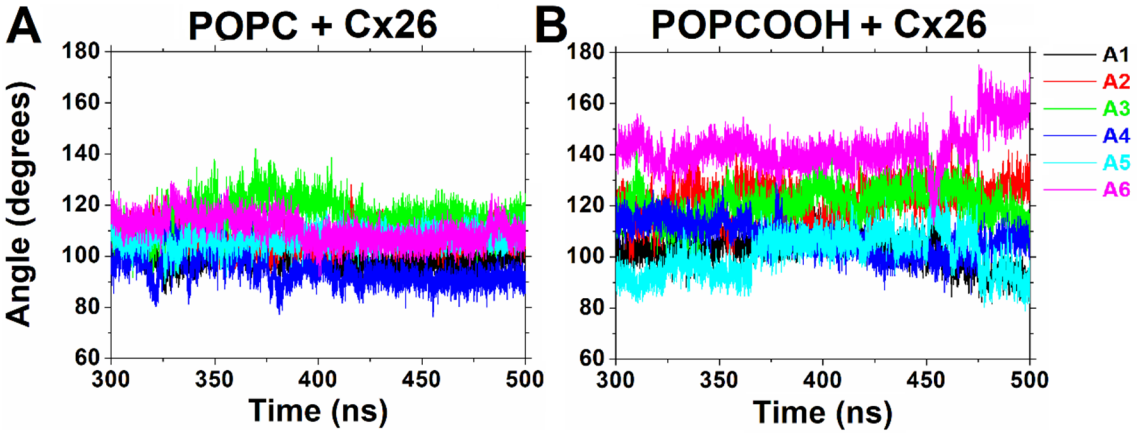

Figure S9. Temporal evolution of the angles in the Cx26 hemichannel embedded into (**A**) the POPC membrane and (**B**) the POPCOOH membrane.

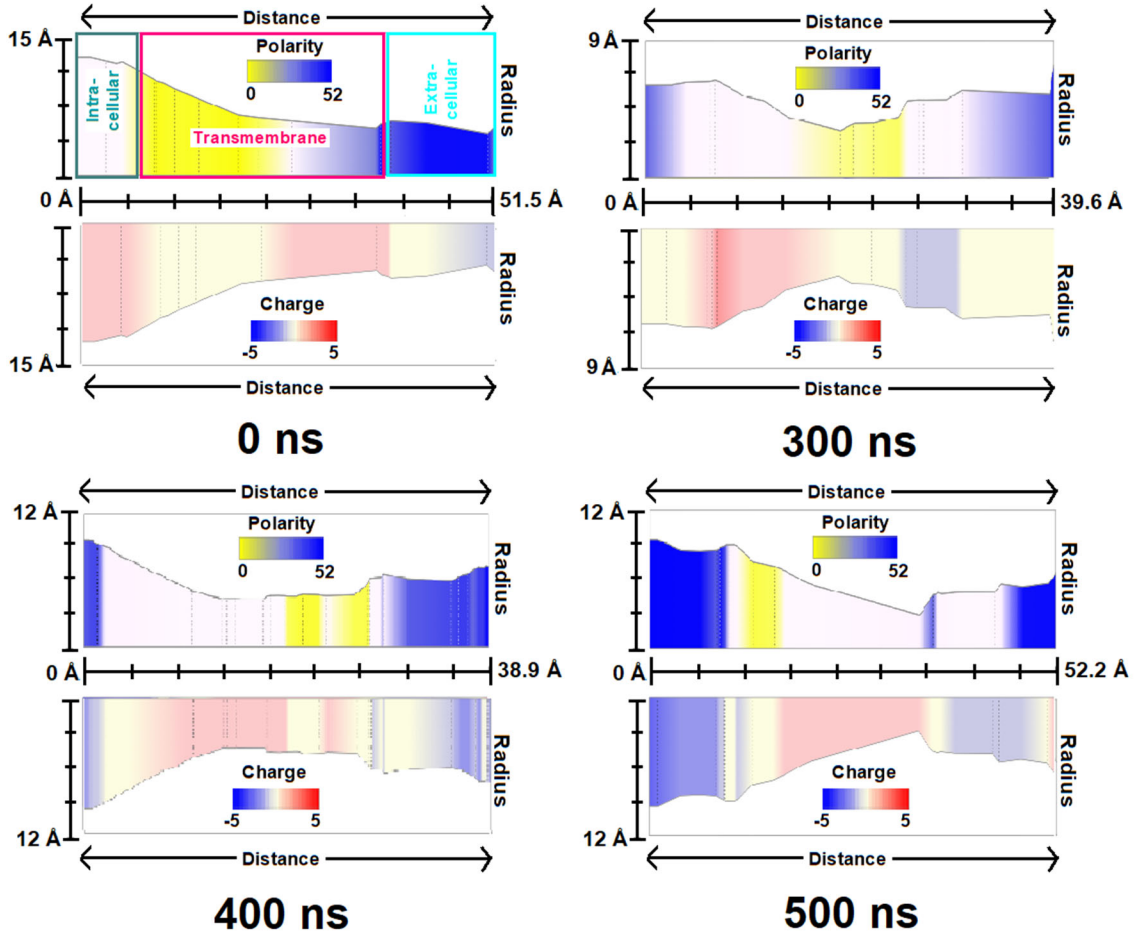

Figure S10. Characterization of the Cx26 hemichannel pore embedded into the POPC membrane, calculated with MOLEonline. The probe radius is 13 Å and the interior threshold is 0.8 Å.

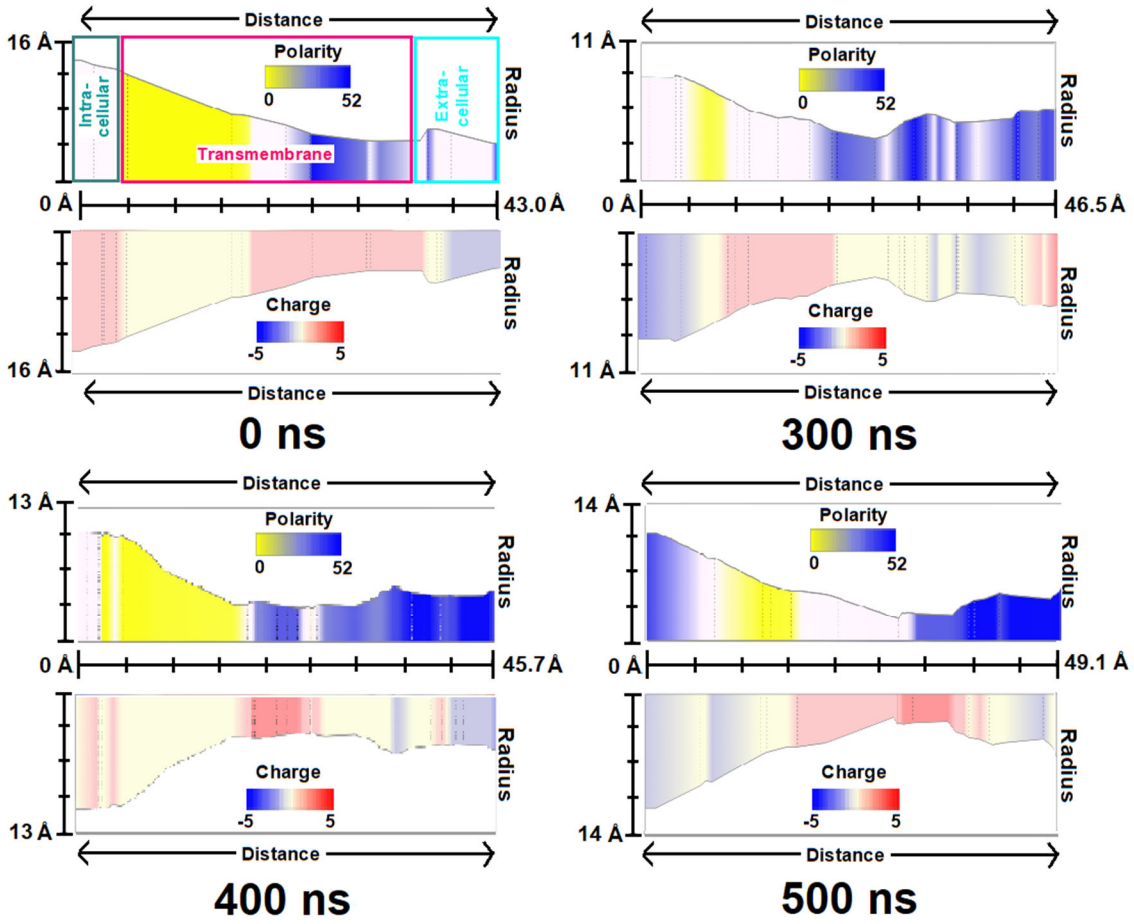

Figure S11. Characterization of the Cx26 hemichannel pore embedded into the POPCOOH membrane, calculated with MOLEonline. The probe radius is 13 Å and the interior threshold is 0.8 Å.

Table S2. Minimum distances between each amino acid residue of the NT domains and the center of mass of $HO_2^{\bullet}$ radicals, for the Cx26 hemichannel embedded into the POPCOOH membrane. The values in red represent the shorter average distances between them. The values were calculated from the last 50 ns of simulation. *Abbreviations*: Met (methionine); Asp (asparagine); Trp (tryptophan); Gly (glycine); Thr (threonine); Leu (leucine); Gln (glutamine); Ile (isoleucine); Val (valine); Asn (asparagine); Lys (lysine); Hie (protonated histidine); and Ser (serine).

	Distance (nm)						
Residue	**Chain 1**	**Chain 2**	**Chain 3**	**Chain 4**	**Chain 5**	**Chain 6**	**Average**
Met_1	0.194	0.194	0.189	0.177	0.160	0.205	0.186
Asp_2	0.154	0.160	0.152	0.150	0.145	0.144	0.151
Trp_3	0.152	0.191	0.180	0.181	0.139	0.147	0.165
Gly_4	0.254	0.146	0.200	0.175	0.148	0.188	0.185
Thr_5	0.225	0.166	0.175	0.182	0.145	0.217	0.185
Leu_6	0.274	0.317	0.175	0.182	0.152	0.273	0.229
Gln_7	0.168	0.140	0.147	0.139	0.147	0.186	0.154
Thr_8	0.306	0.160	0.259	0.372	0.262	0.144	0.250
Ile_9	0.205	0.173	0.163	0.265	0.152	0.273	0.205
Leu_{10}	0.183	0.152	0.134	0.222	0.286	0.163	0.190
Gly_{11}	0.383	0.162	0.184	0.236	0.163	0.157	0.214
Gly_{12}	0.231	0.168	0.184	0.290	0.270	0.222	0.227
Val_{13}	0.297	0.192	0.214	0.418	0.282	0.225	0.271
Asn_{14}	0.164	0.164	0.187	0.218	0.170	0.183	0.181
Lys_{15}	0.186	0.171	0.153	0.182	0.167	0.200	0.176
Hie_{16}	0.170	0.149	0.161	0.150	0.248	0.177	0.176
Ser_{17}	0.175	0.182	0.153	0.156	0.149	0.149	0.161
Thr_{18}	0.182	0.162	0.149	0.206	0.149	0.157	0.167
Ser_{19}	0.482	0.370	0.174	0.143	0.152	0.142	0.244
Ile_{20}	0.490	0.571	0.292	0.188	0.198	0.260	0.333

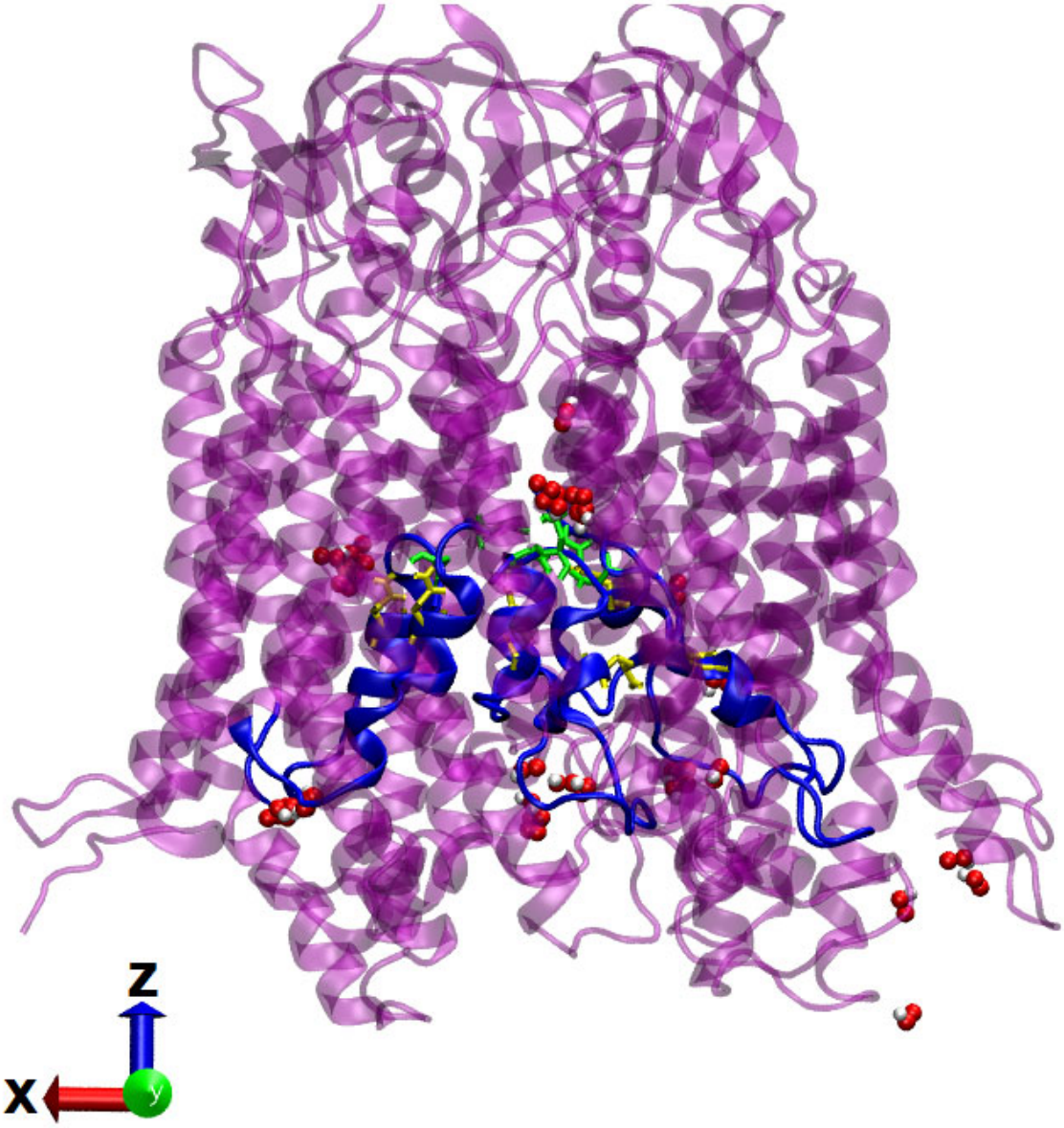

Figure S12. Snapshot of the trajectory of $HO_2^{\bullet}$ radicals inside the Cx26 hemichannel embedded into the POPCOOH membrane. All frames of the last 10 ns are overlaid in order to highlight the interaction between $HO_2^{\bullet}$ radicals with the NT domains of the hemichannel. Only $HO_2^{\bullet}$ radicals within 0.3 nm of the NT domains are represented. The membrane was removed for the sake of clarity. The NT domains are represented as blue ribbons and its asparagine Asp_2 and glutamine Gln_7 amino acid residues are shown in green and yellow bonds, respectively. The $HO_2^{\bullet}$ radicals are shown as red and white van der Waals spheres. We can see that these $HO_2^{\bullet}$ radicals interact more with the asparagine Asp_2 and glutamine Gln_7 amino acid residues of the NT domains.