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Supplemental Information

Membrane Phase-Dependent Occlusion of Intramolecular GLUT1 Cavities Demonstrated by Simulations

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Supporting Material

Membrane phase dependent occlusion of intramolecular GLUT1 cavities demonstrated by atomistic simulations

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Table S1. Average temperature values for the protein and membrane in the simulations considered.

Set Temperature	**323.15 K**	**308.15 K**
Protein	316.7 ± 2.1	303.3 ± 2.9
DPPC	325.1 ± 1.9	310.1 ± 2.5

Table S2. Average and maximum bottleneck radius values for the main inward and outward GLUT1 glucose translocation route. This pathway connects the central region of the transporter with the endofacial and exofacial sides of the membrane.

Phase (side of the membrane)	**Average Bottleneck Radius (Å)**	**Maximum Bottleneck Radius (Å)**
Gel (G_{in})	1.48 ± 0.32	2.58
Fluid (F_{in})	2.12 ± 0.31	3.00
Gel (G_{out})	0.96 ± 0.16	1.54
Fluid (F_{out})	1.31 ± 0.27	2.30
Gel - Warm Protein (IN)	1.2 ± 0.36	2.19
Gel - Warm Protein (OUT)	1.28 ± 0.28	2.25
Fluid - Cold Protein (IN)	2.02 ± 0.52	3.09
Fluid - Cold Protein (OUT)	1.95 ± 0.91	3.98

Table S3. Area per lipid measured using the whole 400 ns trajectories and the last 100 ns.

		Whole trajectory (400 ns) (Å^2/lipid)	Last 100 ns (Å^2/lipid)
Fluid Phase	external leaflet	72.8 ± 0.9	72.2 ± 0.9
	cytoplasmic leaflet	76.3 ± 1.1	75.8 ± 1.0
Gel Phase	external leaflet	66.3 ± 1.7	65.6 ± 0.8
	cytoplasmic leaflet	70.5 ± 1.2	70.0 ± 0.9

Figure S1. Temperature of the protein and membrane along the 400-ns trajectories calculated from the atom velocities. Temperature values averaged every 20 data points are shown in red and black for the membrane and protein respectively. Standard deviations every 20 points are shown in brown and orange for the membrane and protein, respectively. In the first case, the protein is at a higher temperature than the membrane, and the thermostat is able to maintain both systems at different temperatures. In the second system, when the protein is colder than the membrane, there is some energy transfer from the membrane to the protein that results in an increase of the temperature of the former. This simulation was repeated with a different thermostat (Berendsen instead of Parrinello-Raman) with similar outputs.

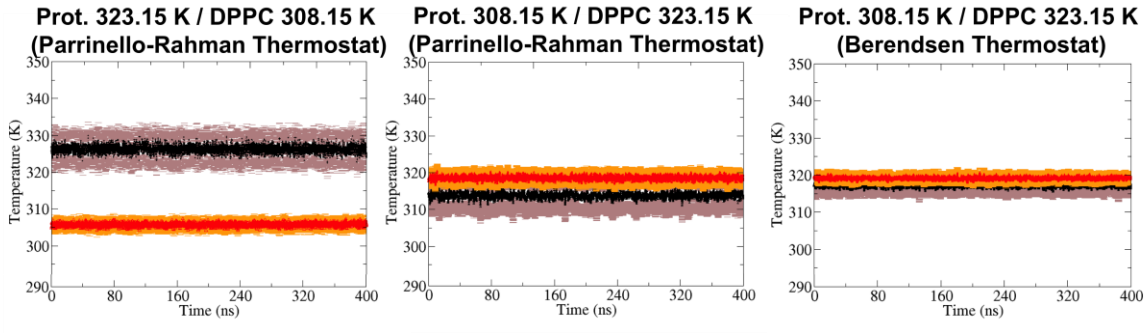


Figure S2. Evolution of the transmembrane (black) and IC domain (red) backbone RMSD values with respect to the original crystal structure as a function of time, when the protein is embedded in a gel or fluid membrane.

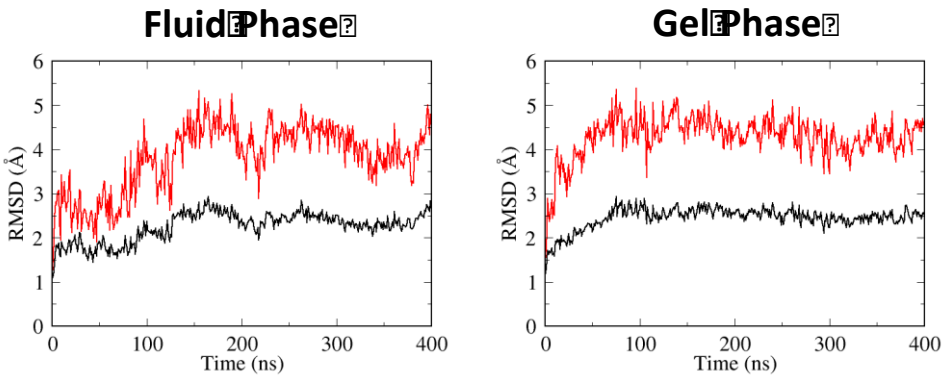


Figure S3. Structural alignment of the last snapshot of each 400-ns trajectory and the GLUT1 X-ray structure (pdb code: 4PYP). Carbon atoms from the MD trajectories and X-ray structure are shown in grey and cyan, respectively.

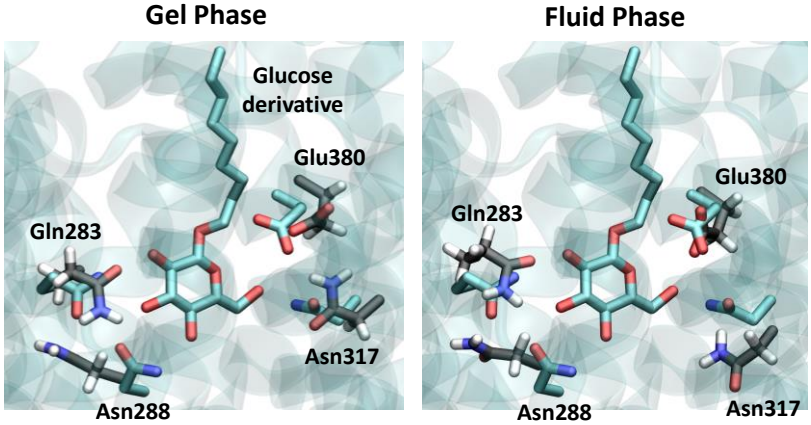


Figure S4. (A) Structural alignment of GLUT1 in a gel (cyan) and fluid (violet) membrane environment using the last frame of the 400-ns MD trajectories. (B) Orientation and interactions of the R153 and E243 residues in a gel (cyan) and fluid (black) membrane environment. Residues are shown in cyan and black for the gel and fluid membranes, respectively. IC2 and IC3 refer to the intracellular domain 2 and 3 of the GLUT1 transporter.

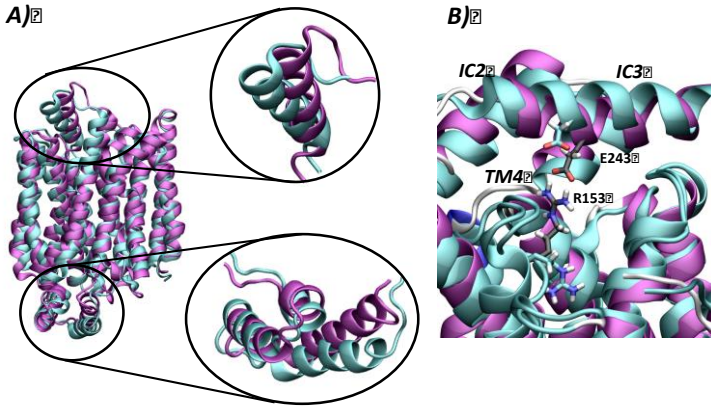


Figure S5. Docking poses of a glucose molecule in the GLUT1 transporter embedded in a gel or fluid membrane bilayer. G_{in}, G_{out}, F_{in} and F_{out} refer to the glucose translocation pathways studied.

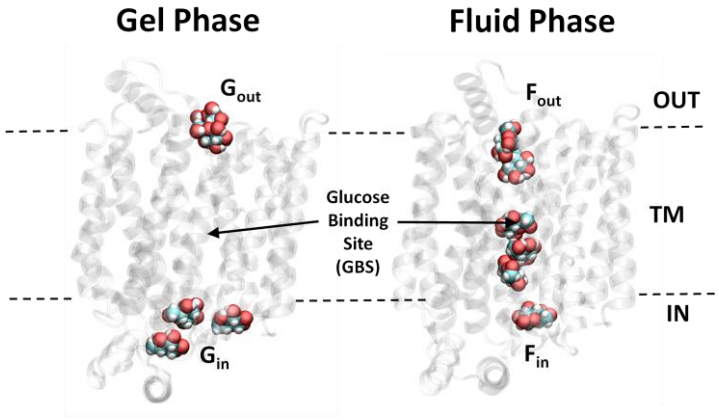


Figure S6. B-factors of GLUT1 residues from simulations where the protein is embedded in the fluid (black) and gel (red) phase bilayers.

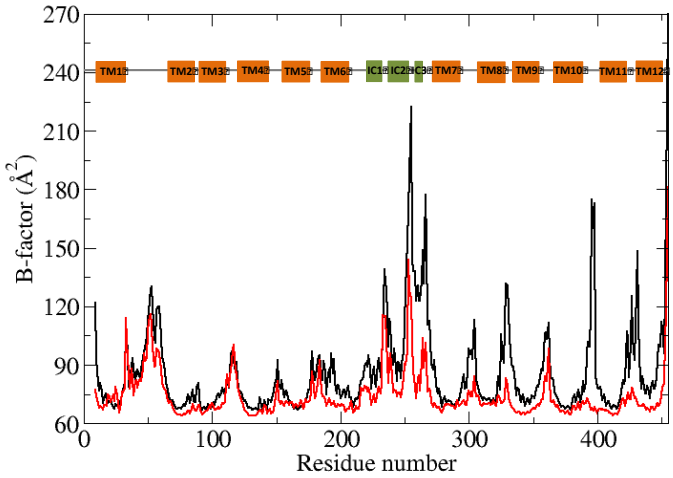
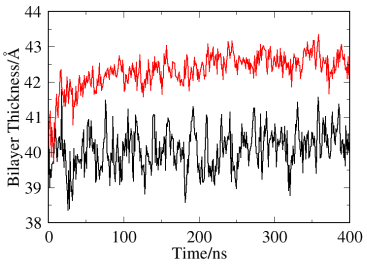
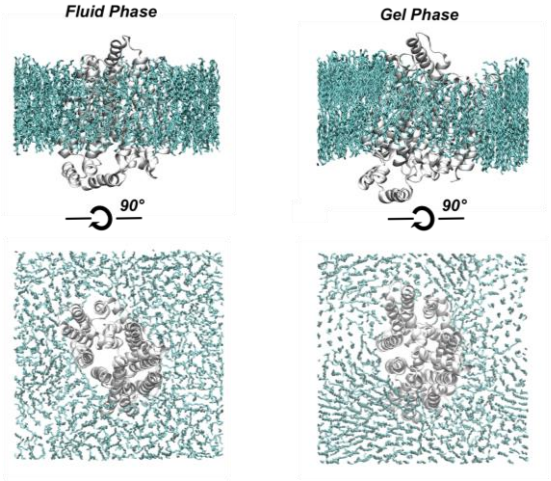


Figure S7. (A) Time evolution of the membrane thickness for the simulations in the gel (red) and fluid (black) phases. (B) Representative snapshots of the simulation system with the membrane in the gel and fluid phases. Lipid molecules are shown in licorice representation. The tertiary structure of the protein is shown in white transparent representation. Polar head groups were omitted in the views shown from above for clarity. (C) Average volumetric maps of the membrane thickness for the last 100 ns of the trajectories in the gel and fluid phases. (D) Comparison of order parameters for lipids at greater or lower distances than 3 Å from the GLUT1 using the last 100 ns of the trajectories. Data associated with fluid phase is in black and red (<3 Å and >3 Å respectively), and with the gel phase is in green and blue (<3 Å and >3 Å respectively).

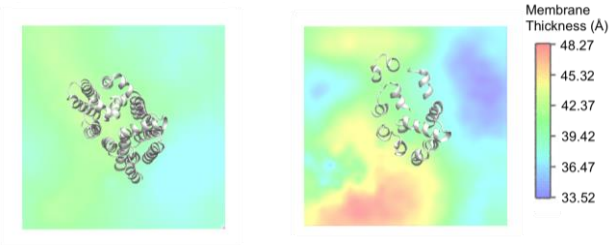
(A)



(B)



(C)



(D)

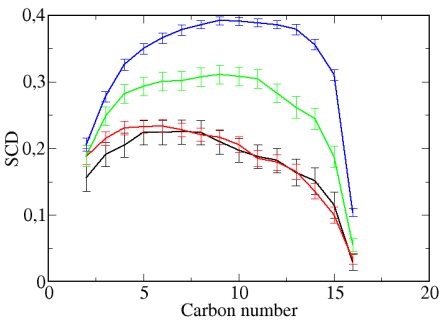


Figure S8. The thickness of DPPC bilayers were measured in aqueous multilamellar dispersions using synchrotron X-ray diffraction methods. Small-angle scattering intensities accumulated in 15 s were recorded for 5-orders of reflection and the phase angles obtained as described in Chen *et al* (2007) Biochim Biophys Acta 1768, 2873-2881.

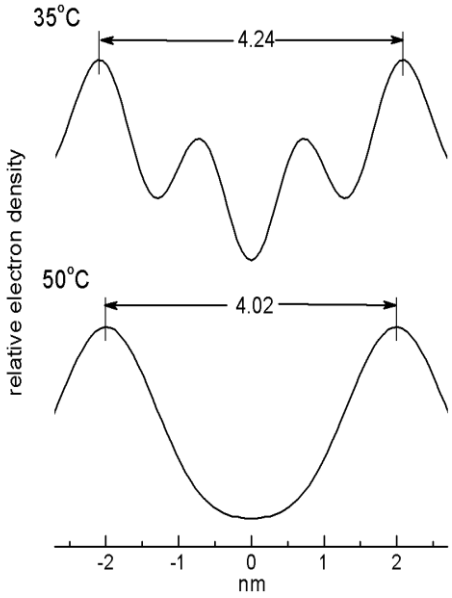


Figure S9. (A) Projection of the bottleneck radius (BR) onto the membrane area and membrane thickness for the G_{in} tunnel, obtained from MD simulations. (B) Cartoon representation of the effects of the gel phase bilayer membrane on the GLUT1 G_{in} conformation. An increase of membrane pressure (i.e. decrease of the membrane area) is translated into a closure of the C_{in} gate. An increase of membrane thickness causes the G_{in} gate to close probably by steric clashes between the lipid headgroups and the protein.

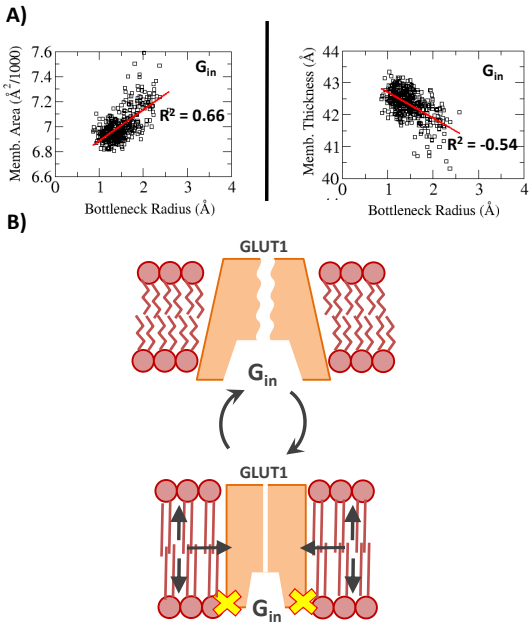


Figure S10. (A) Total number of water molecules in the protein, and (B) average number of water molecules in the protein (with standard deviation) in the fluid (black) and gel (red) phases over time. (C) Fraction of water molecules in the protein inserted in a lipid bilayer in fluid and gel phases during the simulation.

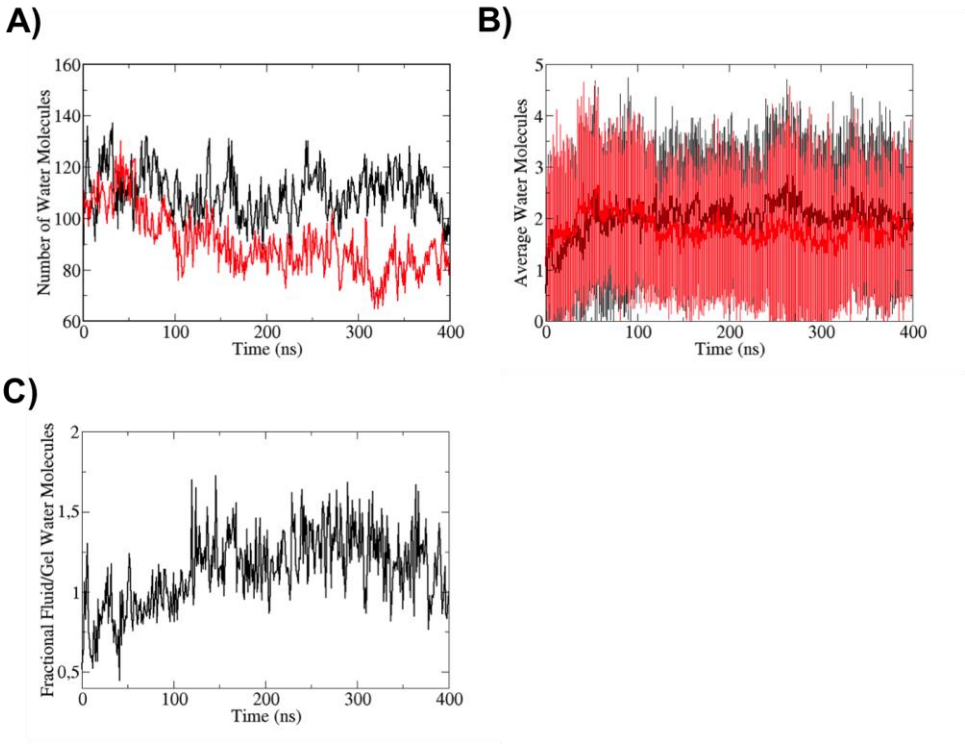


Figure 11. (A) Spatial correlations of water molecules in the central channel inserted in a lipid bilayer in gel phase. (B) Spatial correlations of water molecules in the central channel inserted in a lipid bilayer in fluid phase