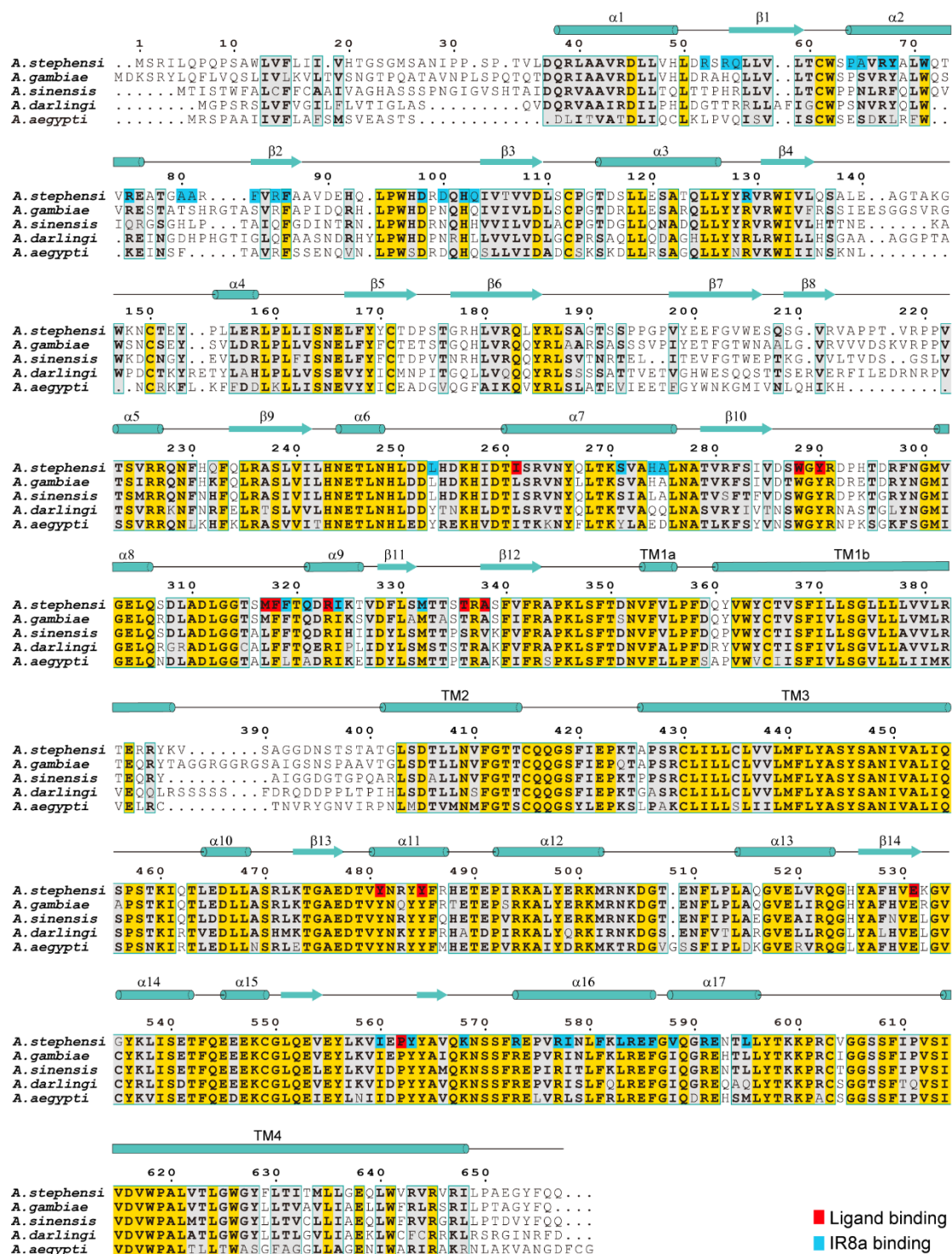
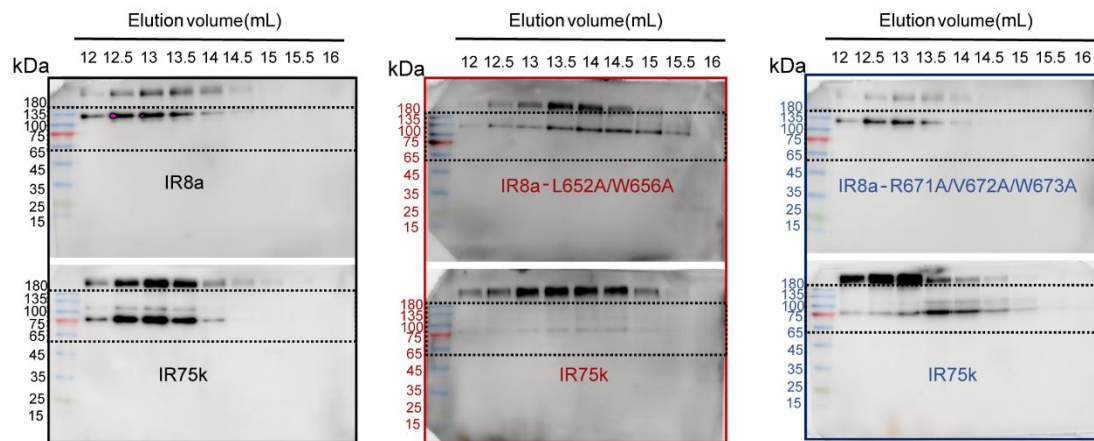




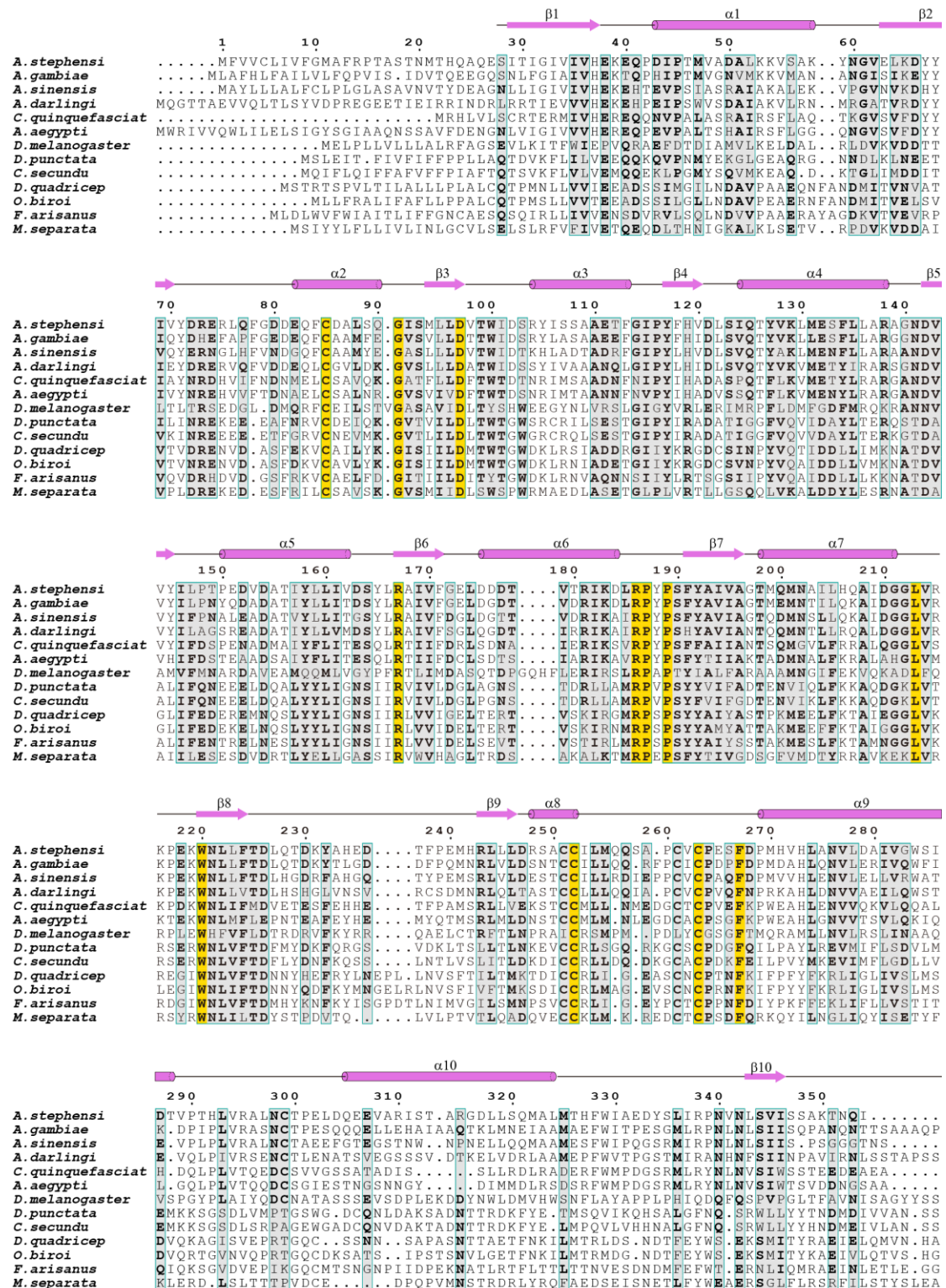
Supplementary Fig. 1 | Sequence alignment of IR75k among mosquitoes.

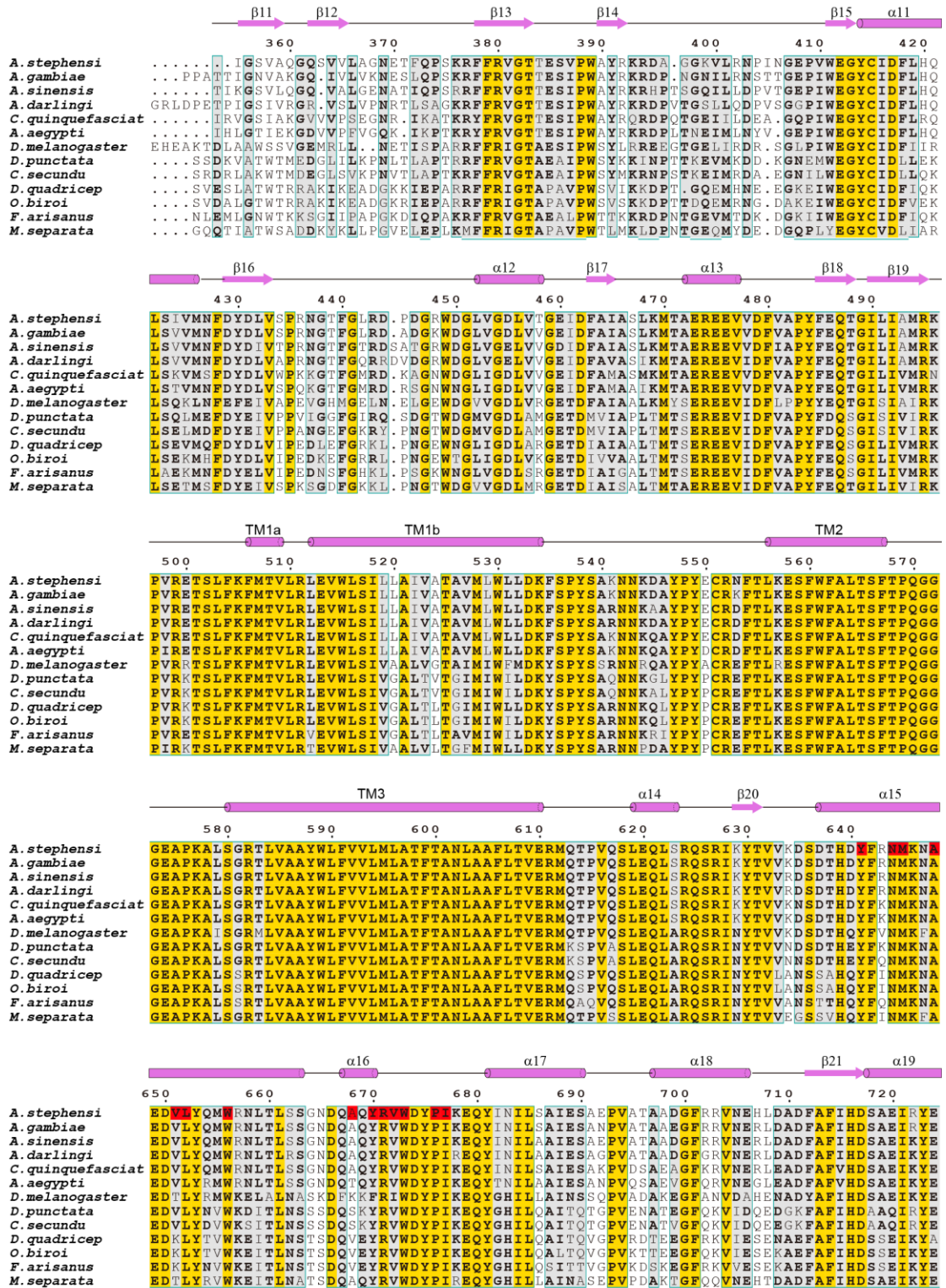
The sequences were aligned using Clustal Omega¹. Secondary structural elements of *Anopheles stephensi* IR75k are indicated above the sequences. Invariant residues are

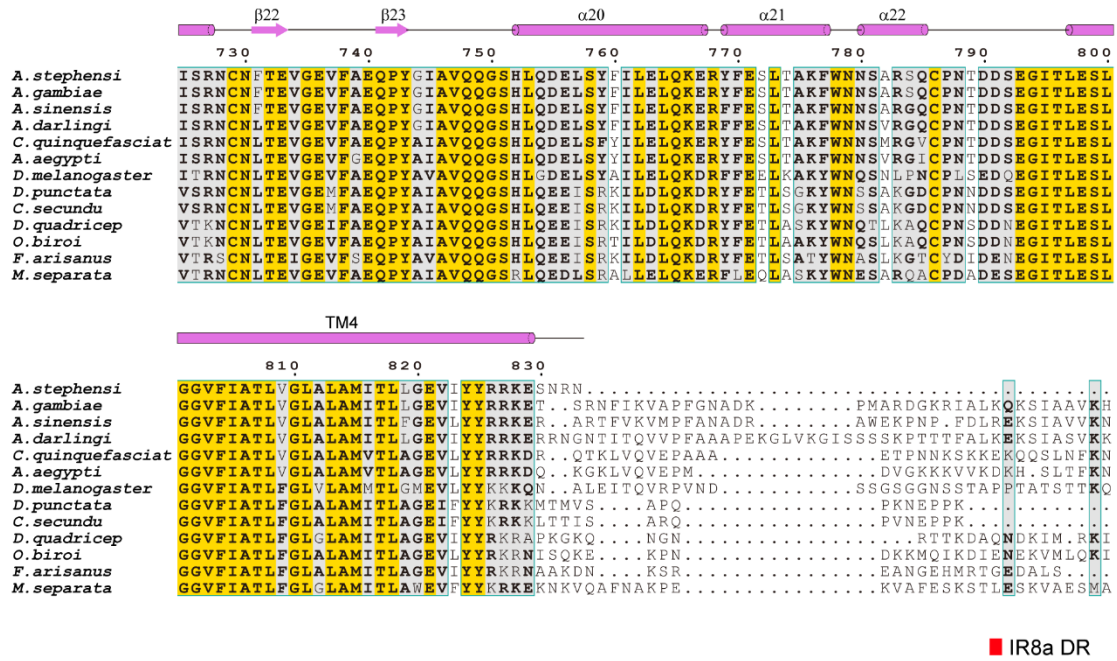
shaded yellow, and the conserved ones are shaded gray. The residues involved in ligands binding are shaded red, while the residues participated in IR8a extracellular domains' binding are shaded light blue. The sequence IDs or Uniprot IDs for the aligned sequences are: *Anopheles stephensi*: XP 035916906.1; *Anopheles gambiae*: Q7PT48; *Anopheles sinensis*: A0A084WNB9; *Anopheles darlingi*: W5JST1; *Aedes aegypti*: A0A6I8TFY9.



Supplementary Fig. 2 | Uncropped western blot of IR8a-IR75k variants for Extended Data Fig. 8h. Western blot of corresponding SEC fractions confirms complex integrity: Wildtype IR8a-IR75k (black), IR8a-L652A/W656A-IR75k (red) and IR8a-R671A/V672A/W673A-IR75k (blue). The cropped areas for use in the Extended Data Fig. 8h are indicated by a black dashed box.







Supplementary Fig. 3 | Sequence alignment of IR8a from different mosquito species.

The sequences were aligned using Clustal Omega¹. Secondary structural elements of *Anopheles stephensi* IR8a are indicated above the sequences. Invariant residues are shaded yellow, and the conserved ones are shaded gray. The residues involved in dimerization region are shaded red. The sequence IDs or Uniprot IDs for the aligned sequences are: *Anopheles stephensi*: XP 035894330.1; *Anopheles gambiae*: UPI0020FF7C0F; *Anopheles sinensis*: A0A084VTS8; *Anopheles darlingi*: W5JU59; *Culex quinquefasciatus*: B0XL62; *Aedes aegypti*: Q17GP1, *Drosophila melanogaster*: Q9W365; *Diptera punctata*: A0AAD8ELN2; *Cryptotermes secundu*: A0A2J7RGC7; *Dinoponera quadricep*: A0A6P3Y1C0; *Ooceraea biro*: A0A026WMY8; *Fopius arisanus*: A0A9R1STA8; *Mythimna separata*: A0AAD7Y5U0. DR: dimerization region.

Supplementary Video Legends

Supplementary Video 1 | Molecular dynamics simulation of the NA-bound IR8a-IR75k complex under an external electric field.

The simulation was initiated from our experimental determined cryo-EM structure of the NA-bound IR8a-IR75k complex. The complex was embedded in a lipid bilayer and subjected to an external electric field of 0.015 V/nm along the membrane. Sodium ion (green sphere) is shown permeating the channel in a partially dehydrated state during the simulation.

Supplementary Video 2 | Molecular dynamics simulation of the apo state IR8a-IR75k complex under an external electric field.

The simulation was initiated from our experimentally determined cryo-EM structure of the apo state IR8a-IR75k complex under the same simulation conditions as Supplementary Movie 1. No sodium ion permeation through the channel was observed during the simulation, supporting the conclusion that ligand binding is required to trigger the conformational cascade necessary for channel gating and sodium ion permeation.

Supplementary Table 1 | Reliability and reproducibility checklist for molecular dynamics simulations.

*All boxes must be marked YES by acceptance unless an N/A option is available		Yes	N/A	Response (Please state where this information can be found in the text)
1. Convergence of simulations and analysis				
1a. Is an evaluation presented in the text to show that the property being measured has equilibrated in the simulations (e.g. time-course analysis)?		☒		Time-course root-mean-square deviation analyses demonstrating equilibration are provided in Methods.
1b. Then, is it described in the text how simulations are split into equilibration and production runs and how much data were analyzed from production runs?		☒		This information is described in Methods.
1c. Are there at least 3 simulations per simulation condition with statistical analysis?		☒		Each system was subjected to three independent simulations with different initial random seeds, as detailed in Methods.
1d. Is evidence provided in the text that the simulation results presented are independent of initial configuration?		☒		All replicate trajectories were initiated from independent starting configurations with different random seeds, as described in Methods.
2. Connection to experiments				
2a. Are calculations provided that can connect to experiments (e.g. loss or gain in function from mutagenesis, binding assays, NMR chemical shifts, J-couplings, SAXS curves, interaction distances or FRET distances, structure factors, diffusion coefficients, bulk modulus and other mechanical properties, etc.)?		☒		Electrophysiological experiments and structural analysis supporting the MD simulations results, as shown in Fig.2a, b and Extended Data Fig. 1b,c.
3. Method choice				
3a. Do simulations contain membranes, membrane proteins, intrinsically disordered proteins, glycans, nucleic acids, polymers, or cryptic ligand binding?		☒		The simulations include lipid bilayers, embedded membrane proteins, and ligands, as described in Methods and shown in Movies S1 and S2.
3b. Is it described in the text what force field and water model are used and why?		☒		This information is described in Methods.
3c. Is the timescale of the event(s) under investigation beyond the brute-force MD simulation timescale in this study that enhanced sampling methods are needed?			☒	Enhanced sampling methods are unnecessary for our systems.
	If YES, are the parameters and convergence criteria for the enhanced sampling method clearly stated?			N/A

	If NO , is the evidence provided in the text?		☒	
4. Code and reproducibility				
4a. Is a table provided describing the system setup, such as simulation box dimensions, total number of atoms, total number of water molecules, salt concentration, lipid composition (number of molecules and type)?		☒		All system parameters are described in Methods.
4b. Are other parameters for the system setup described in the text, such as protonation state, type of structural restraints if applied, nonbonded cutoff, thermostat and barostat, etc.?	☒			This information is described in Methods.
4c. Is it described in the text what simulation and analysis software and which versions are used?	☒			All simulation and analysis software, along with their corresponding versions, were described in Methods.
4d. Are initial coordinate and simulation input files and a coordinate file of the final output provided as supplementary files or in a public repository?	☒			All MD coordinate and simulation input files are available at https://doi.org/10.5281/zenodo.23033096 .
4e. Is there custom code or custom force field parameters?		☒		No custom code or force-field parameters were used.
	If YES , are they provided as supplementary files or in a public repository?			N/A

Reference:

- 1 Larkin, M. A. *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-2948, doi:10.1093/bioinformatics/btm404 (2007).