Supplementary Information for

Direct translocation of a negatively charged nanoparticle across a negatively charged model cell membrane

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A. Supplementary Text

A.1 Molecular composition of the simulation system

Table S1 shows the molecular composition of the simulation system without ionic charge imbalance. The concentration of NaCl in both the outer and inner solvent compartments was 154 mM, which is equivalent to the concentration of isotonic saline solution. The identical NaCl concentration ensured that there was no osmotic pressure across the lipid bilayer. To compensate for the negative charges of 1,2-dipalmitoyl-phosphatidylglycerol (DPPG) and the nanoparticle, sodium ions were added to each solvent compartment, thereby maintaining their electroneutrality. The amount of DPPG in the lipid bilayer was 16 mol%, mimicking the lipid composition of human red blood cell membranes.[1]

Table S2 shows the molecular composition of the simulation system with ionic charge imbalance. The number of sodium ions in the inner compartment was set to be higher than that in the outer compartment. Hence, the total net charges in the outer and inner compartments were –65 e and +65 e, respectively. We preliminarily confirmed that the $\pm 65\ e$ charge imbalance induced a membrane potential of 40 mV. The details can be found in Section A.2 and Fig. S2.

A.2 Relationship between the net charge difference and the resulting transmembrane electric potential

Figure S2 shows the relationship between the net charge difference between the inner and outer compartments (ΔQ) and the resulting transmembrane electric potential ($\Delta\psi_{\mathrm{imb}}$). ΔQ was defined as $\Delta Q = q_{\mathrm{inner}} - q_{\mathrm{outer}}$, where q_{inner} and q_{outer} are the total charges in the inner and outer compartments, respectively. q_{inner} and q_{outer} were set to be positive and negative charges, respectively, so that the electric potential in the inner compartment was higher than that in the outer compartment. The total charge of the entire simulation system was set to zero (i.e., $q_{\mathrm{inner}} + q_{\mathrm{outer}} = 0\ e$) to maintain electroneutrality. As shown in Fig. S2, $\Delta\psi_{\mathrm{imb}}$ increased with an increase in ΔQ. From the plot, the ΔQ that yields $\Delta\psi_{\mathrm{imb}} = 40$ mV, which was the target intensity for a complementary transmembrane potential in this study, was determined to be +130 e.

B. Supplementary Tables and Figures

Table S1 Molecular composition of the simulated system without ionic charge imbalance.[a]

Total number of lipid molecules	2304
Number of DPPC molecules	1936
Number of DPPG molecules	368
Number of CG-water sites	123466
Number of CG-sodium ion sites in outer compartment	1014
Number of CG-sodium ion sites in inner compartment	894
Number of CG-chloride ion sites in outer compartment	710
Number of CG-chloride ion sites in inner compartment	710
Number of nanoparticles (with surface charge of –120 *e*)	1
Total net charge in outer compartment	0 *e*
Total net charge in inner compartment	0 *e*

[a] DPPC: 1,2-dipalmitoylphosphatidylcholine; DPPG: 1,2-dipalmitoyl-phosphatidylglycerol; CG: coarse-grained

Table S2 Molecular composition of the simulated system with ionic charge imbalance. The net charge difference corresponded to a membrane potential of 40 mV. [a]

Total number of lipid molecules	2304
Number of DPPC molecules	1936
Number of DPPG molecules	368
Number of CG-water sites	123466
Number of CG-sodium ion sites in outer compartment	949
Number of CG-sodium ion sites in inner compartment	959
Number of CG-chloride ion sites in outer compartment	710
Number of CG-chloride ion sites in inner compartment	710
Number of nanoparticles (with surface charge of –120*e*)	1
Total net charge in outer compartment	–65 *e*
Total net charge in inner compartment	+65 *e*

[a] DPPC: 1,2-dipalmitoylphosphatidylcholine; DPPG: 1,2-dipalmitoyl-phosphatidylglycerol; CG: coarse-grained

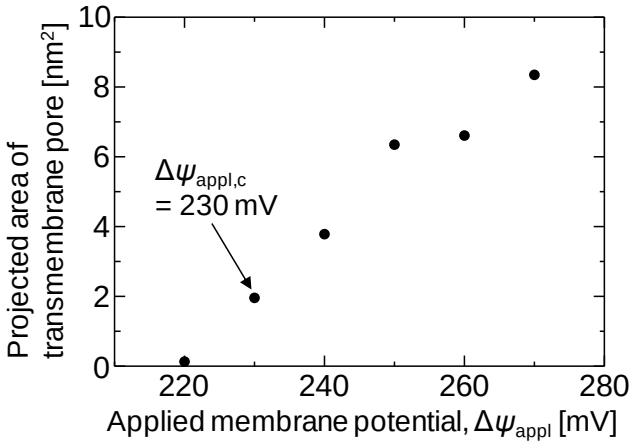

Fig. S1 Projected area of transmembrane pore as a function of applied membrane potential $\Delta\psi_{appl}$. To determine the critical applied potential for membrane breakdown ($\Delta\psi_{appl,c}$), molecular dynamics simulations without the nanoparticle and without ionic charge imbalance ($\Delta\psi_{imb} = 0$ mV) were performed under different $\Delta\psi_{appl}$ values. From the results, $\Delta\psi_{appl,c}$ was determined to be 230 mV.

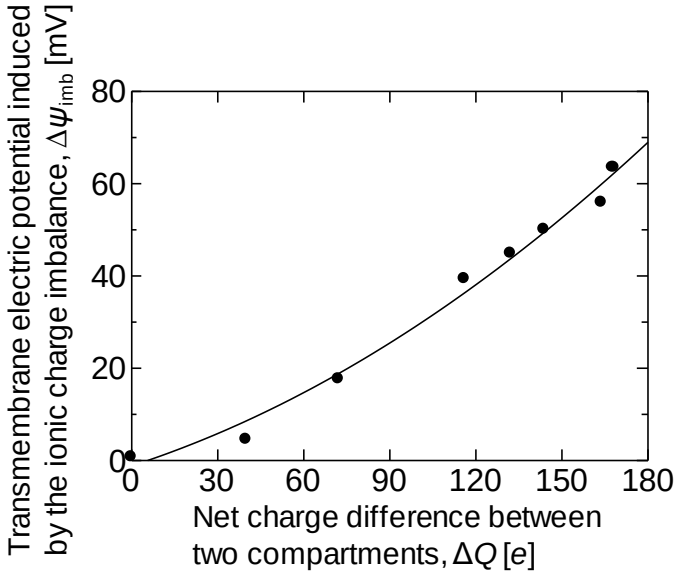

Fig. S2 Relationship between the net charge difference ΔQ and the transmembrane electric potential induced by the ionic charge imbalance, $\Delta\psi_{imb}$. ΔQ was defined as $\Delta Q = q_{inner} - q_{outer}$, where the q_{inner} and q_{outer} were the total net charges in the inner and outer compartments, respectively. The data was obtained at an applied potential $\Delta\psi_{appl}$ of 0.0 mV.

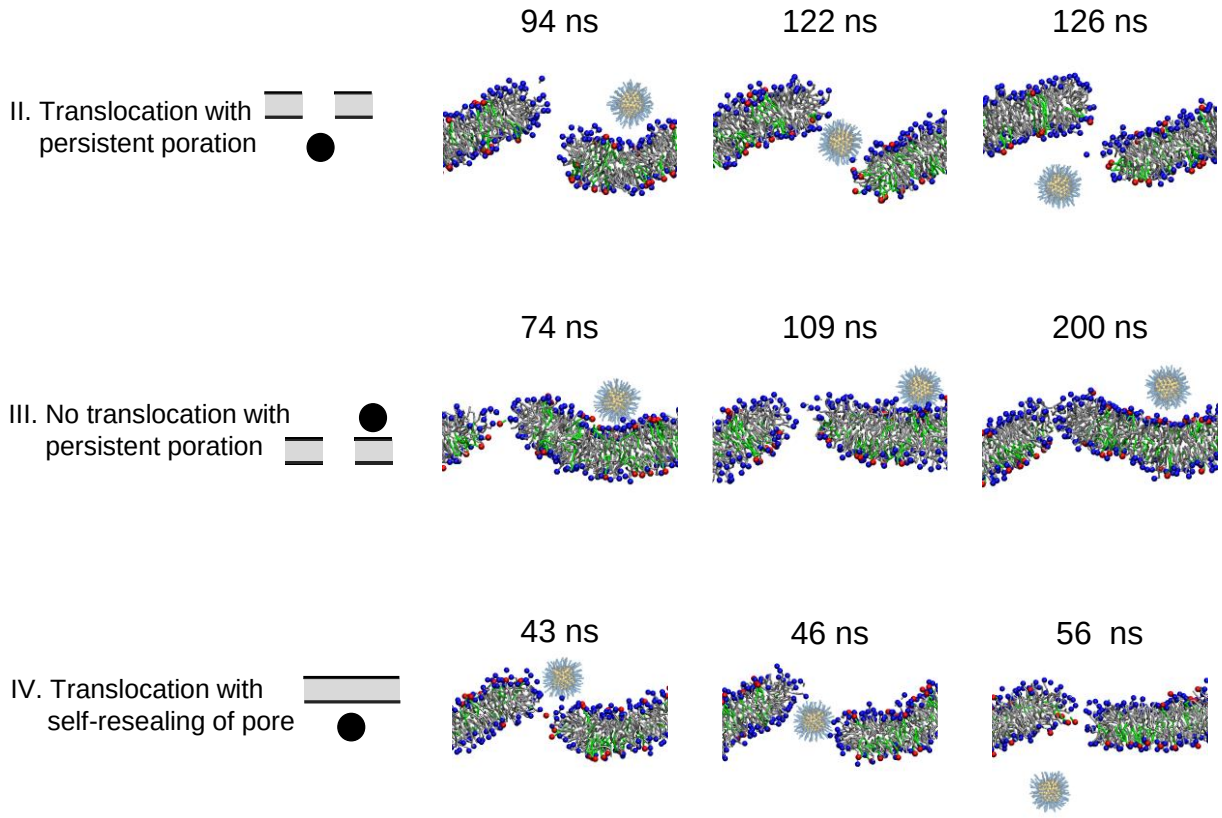

Fig. S3. Cross-sectional side views of transmembrane pores observed in the mode II, III, and IV. The blue and red spheres correspond to the hydrophilic heads of DPPC and DPPG, respectively. Waters and ions were not shown for clarity.

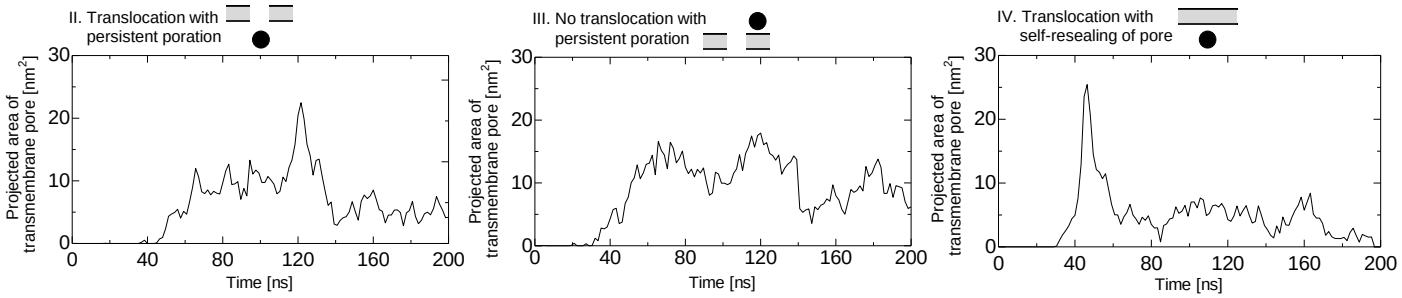

Fig. S4. Temporal changes in the projected area of the transmembrane pores in the mode II, III, and IV.

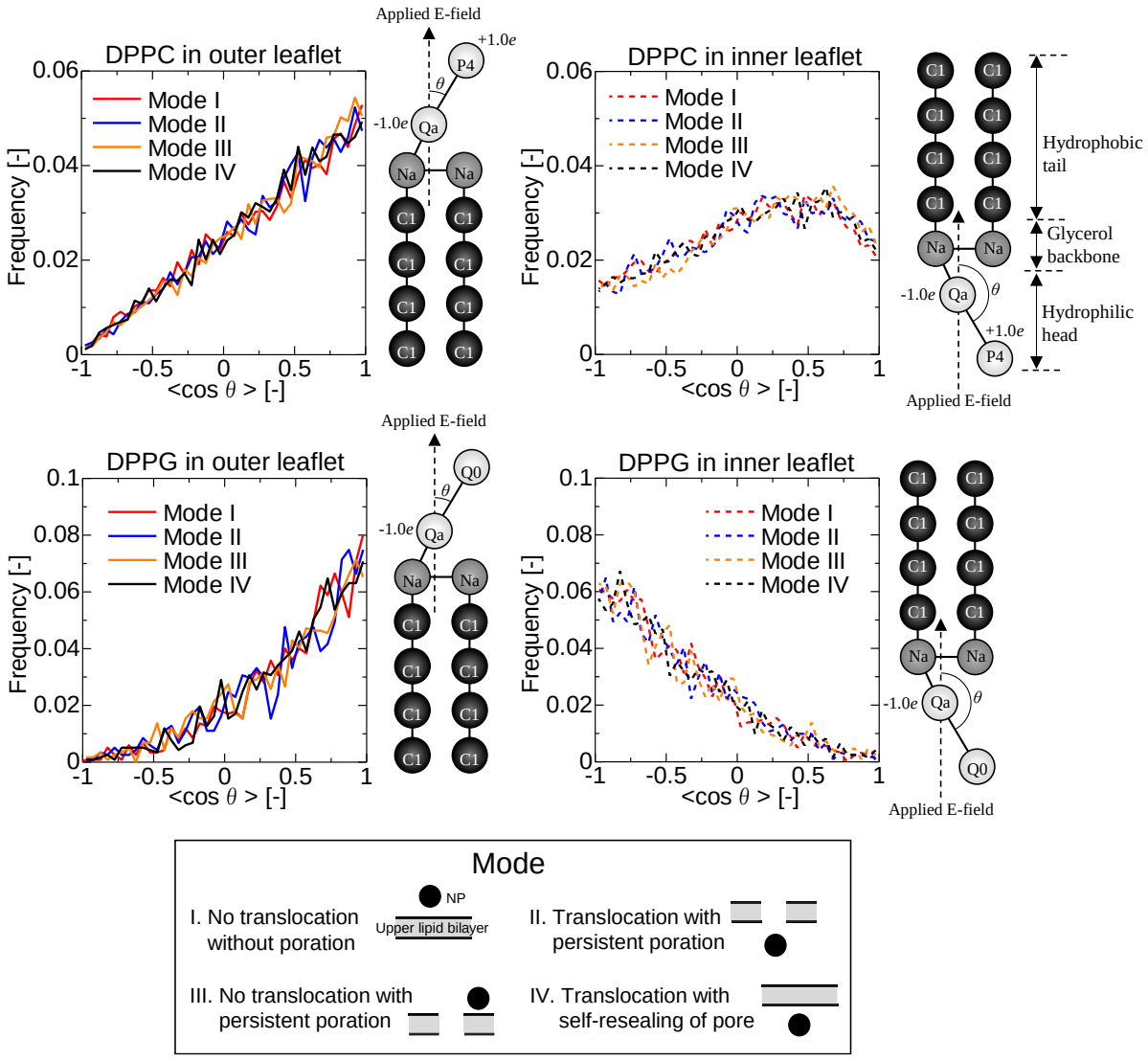

Fig. S5. Distributions of lipid head orientations with respect to the applied external electric field (E-field) in each mode. The lipid molecules existing in the inner and outer leaflets of the upper lipid bilayer were analyzed. θ corresponds to the orientation angle with respect to the applied E-field. P4, Qa, Q0, Na, and C1 correspond to types of the coarse-grained sites defined by the MARTINI force field.

C. Supplementary Reference

1 A. Zachowski, *Biochem. J.*, 1993, **294**, 1–14.