

Supporting Materials for

Effect of Polycation Structure on Interaction with Lipid Membranes

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1. Polycations 1 and 2

Poly(allyltrimethylammonium chloride) (**1**) was synthesized according to the procedure reported previously.¹ Poly(allyltrimethylammonium chloride-*co*-allyl-*N,N*-dimethyl-*N*-dodecanylammonium chloride) (**2**) was prepared according to the modified procedure.²

2. Synthesis of polymers 3

Materials. Stearic acid (SA, 98.0%) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 98.0%) were purchased from Tokyo Chemical Industry. Triethylamine (TEA, 99.0%) and iodomethane (CH₃I, 99.5%) were purchased from Wako Pure Chemicals. Poly(allyltrimethylammonium chloride) (PHA, molecular weight: 15,000) was purchased from Aldrich. Dimethyl sulfoxide (DMSO) was dried over 4Å molecular sieves and purified by distillation under reduced pressure. Water was purified with a Millipore Milli-Q system. Other reagents were used as received.

Synthesis of poly(allyltrimethylammonium chloride-*co*-allyl-*N*-stearoylallylamine) (**3**) is shown in Figure S1.

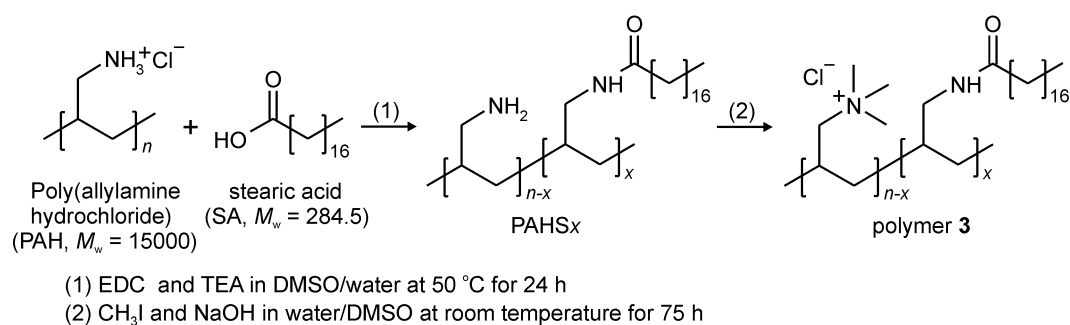


Figure S1. The synthesis route of poly(allyltrimethylammonium chloride-*co*-allyl-*N*-stearoylallylamine) (**3**).

Preparation of polymer 3a (PAH containing 1.5 mol% stearyl groups)

PAH (2.00 g, 21.4 mmol of amine group in PAH)) was dissolved in a mixture of DMSO and water (36 mL, 11/1 v/v). Then, SA (244 mg, 0.857 mmol), EDC (166 mg, 0.859 mmol), and TEA (84.9 mg, 0.839 mmol) were added to the solution. The solution was refluxed for 24 hours. After cooling, the mixture was dialyzed against methanol, water at pH 4, water at pH 10, and pure water continuously. The polymer (PAHS1.5) was collected by a freeze-drying technique (1.82 g, 90.6 %). The content of stearyl groups was calculated from the ^1H NMR (Figure 2b) integral intensity ratio of PAH (peak *c*) and stearyl groups (peak *g*). Thin layer chromatography (TLC) was performed using *n*-hexane/chloroform (4/1) as an eluent to confirm that there is not unreacted SA. The rate of flow (R_f) value of SA was 0.44. On the other hand, R_f value of PAHS1.5 was 0, which indicated that unreacted SA was not included in PAHS1.5.

Quaternization of amine groups in PAHS1.5

PAHS1.5 (0.679 g, 7.26 mmol of amine group in PAH) was dissolved in 1.33 M NaOH aqueous solution (20.4 mL) and DMSO (47.6 mL). Then, CH_3I (12.0 g, 84.8 mmol) was added to the solution, which was stirred at room temperature for 75 hours. The reacted solution was dialyzed against methanol, 0.1 M NaCl aqueous solution, and pure water continuously. The polymer **3a** was collected by freeze-drying technique (1.04 g, 100 %). From ^1H NMR spectrum for polymer **3a** in D_2O , the quaternized degree calculated from the ^1H NMR (Figure S2) integral intensity ratio of PAH (peak *g*) and trimethyl ammonium group (peak *i*) was 100 %.

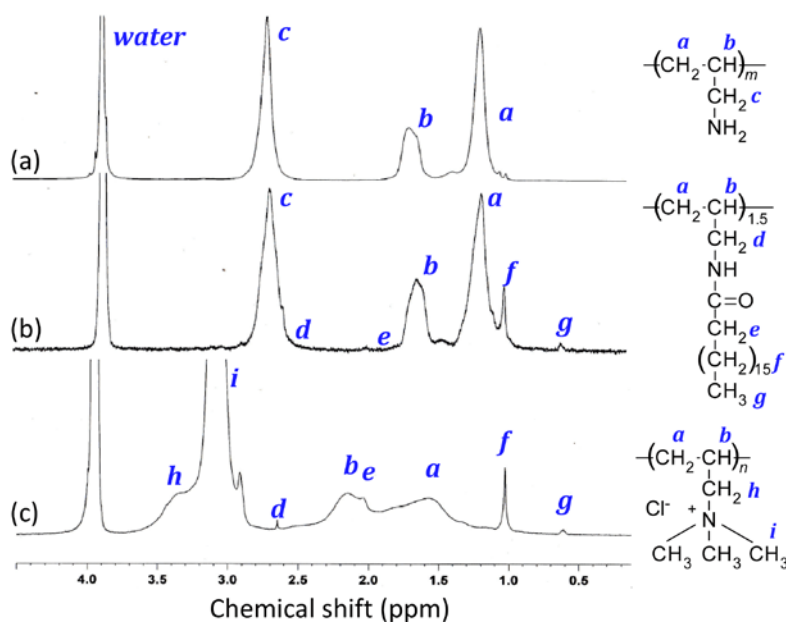


Figure S2. ^1H NMR spectra for (a) PAH, (b) PAHS1.5, and (c) polymer **3a** in D_2O at $80\text{ }^\circ\text{C}$.

Preparation of polymer **3b** (3.4 mol% stearyl group-containing poly(allylamine hydrochloride))

PAH (1.53 g, 16.4 mmol of amine group in PAH)) was dissolved in a mixture of DMSO and water (36 mL, 11/1 v/v). Then, SA (372 mg, 1.31 mmol), EDC (251 mg, 1.31 mmol), and TEA (133 mg, 1.31 mmol) were added to the solution and the solution was refluxed for 24 hours. The mixture was dialyzed against methanol, water at pH 4, water at pH 10, and pure water continuously. After the dialysis, the polymer (PAHS3.4) was collected by the freeze-drying technique (1.38 g, 90.2 %). The content of stearyl groups was calculated from the ^1H NMR (Figure 3a) integral intensity ratio of PAH (peak *c*) and stearyl groups (peak *g*). TLC was performed using *n*-hexane/chloroform (4/1) as an eluent to confirm that there is not unreacted SA. The R_f value of SA was 0.45. On the other hand, R_f value of PAHS3.4 was 0, which indicated that unreacted SA was not included in PAHS3.4.

Quaternization of amine groups in in PAHS3.4.

PAHS3.4 (0.648 g, 6.92 mmol of amine group in PAH) was dissolved in 1.33 M NaOH aqueous solution (20.4 mL) and DMSO (47.6 mL). Then, CH₃I (19.7 g, 141 mmol) was added and the solution was stirred at room temperature for 75 hours. The reacted solution was dialyzed against methanol, 0.1 M NaCl aqueous solution, and pure water continuously. The polymer **3b** was collected by freeze-drying technique (0.915 g, 99.4 %). From ¹H NMR spectrum for polymer **3b** in DMSO-*d*₆/D₂O (3/1, v/v), the peak *c* related to PAH was disappeared (Figure S3). Therefore, the quaternized degree of polymer **3b** was 100 %.

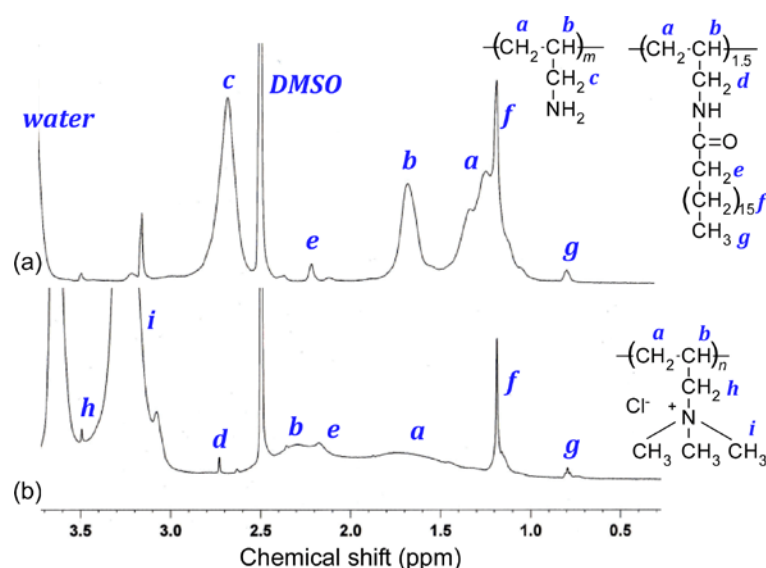


Figure S3. ¹H NMR spectra for (a) PAHS3.4 and (b) polymer **3b** in DMSO-*d*₆/D₂O (3/1, v/v) at 80 °C.

3. DLS measurements

Table S1. The values of the mean hydrodynamic diameter (d_z), dispersity (DI) and the zeta potential (ζ) of POPC SUVs ($c_{\text{lipid}} = 1.25 \text{ mg/mL}$) dispersed in a 1 mM NaCl solution at pH 7.4 and treated with polycations (values are the mean \pm standard deviation).

System	Polycation concentration [$\mu\text{g/mL}$]	Polycation content [wt%]	d_z [nm] ($n = 5$)	DI ($n = 5$)	ζ [mV] ($n = 5$)
POPC SUVs	0	0	116.2 ± 1.1	0.08 ± 0.02	-3.24 ± 0.97
POPC SUVs with polymer 1	40	3.2	145.4 ± 1.4	0.37 ± 0.01	2.18 ± 0.24
	50	4.0	159.3 ± 10.2	0.33 ± 0.02	3.46 ± 0.24
	60	4.8	168.6 ± 1.0	0.37 ± 0.01	3.71 ± 0.15
	70	5.6	147.1 ± 3.9	0.33 ± 0.02	3.51 ± 0.55
	80	6.4	152.1 ± 16.7	0.34 ± 0.04	4.24 ± 0.53
	90	7.2	151.8 ± 1.1	0.35 ± 0.02	4.62 ± 0.38
	100	8.0	155.0 ± 16.6	0.33 ± 0.04	4.52 ± 0.21
POPC SUVs with polymer 2a	40	3.2	118.6 ± 0.6	0.09 ± 0.01	12.2 ± 1.9
	60	4.8	118.9 ± 1.4	0.09 ± 0.02	13.0 ± 1.3
	80	6.4	125.4 ± 0.8	0.11 ± 0.01	23.2 ± 1.6
	100	8.0	123.8 ± 0.9	0.11 ± 0.01	21.3 ± 0.6
	120	9.6	124.7 ± 1.1	0.11 ± 0.03	25.2 ± 0.7
	140	11.2	125.7 ± 0.8	0.11 ± 0.02	27.6 ± 2.1
	180	14.4	130.4 ± 0.2	0.13 ± 0.01	33.2 ± 0.8
POPC SUVs with polymer 2b	50	4.0	131.6 ± 1.4	0.17 ± 0.02	21.1 ± 0.6
	60	4.8	130.5 ± 1.3	0.16 ± 0.01	25.6 ± 0.5
	70	5.6	135.0 ± 0.9	0.18 ± 0.01	35.2 ± 0.4
	80	6.4	136.7 ± 1.9	0.19 ± 0.02	37.2 ± 2.7
	90	7.2	138.7 ± 3.3	0.19 ± 0.02	38.4 ± 0.8
	100	8.0	143.5 ± 3.1	0.24 ± 0.02	38.6 ± 2.9
POPC SUVs with polymer 3a	40	3.2	132.8 ± 2.1	0.16 ± 0.01	17.7 ± 2.1
	50	4.0	133.6 ± 1.6	0.14 ± 0.01	24.2 ± 3.9
	60	4.8	135.8 ± 2.7	0.13 ± 0.01	28.8 ± 4.6
	70	5.6	135.9 ± 1.3	0.14 ± 0.02	31.8 ± 2.9
	80	6.4	139.4 ± 2.3	0.15 ± 0.01	33.8 ± 2.3
	90	7.2	138.3 ± 2.2	0.15 ± 0.01	38.5 ± 2.3
	100	8.0	139.4 ± 2.9	0.14 ± 0.02	41.0 ± 2.9
POPC SUVs with polymer 3b	20	1.6	127.5 ± 0.7	0.09 ± 0.02	22.9 ± 1.1
	30	2.4	129.0 ± 0.6	0.09 ± 0.02	33.7 ± 2.6
	40	3.2	130.2 ± 0.5	0.09 ± 0.02	36.0 ± 1.1
	50	4.0	131.1 ± 0.4	0.10 ± 0.02	39.2 ± 2.4

4. MD Simulations

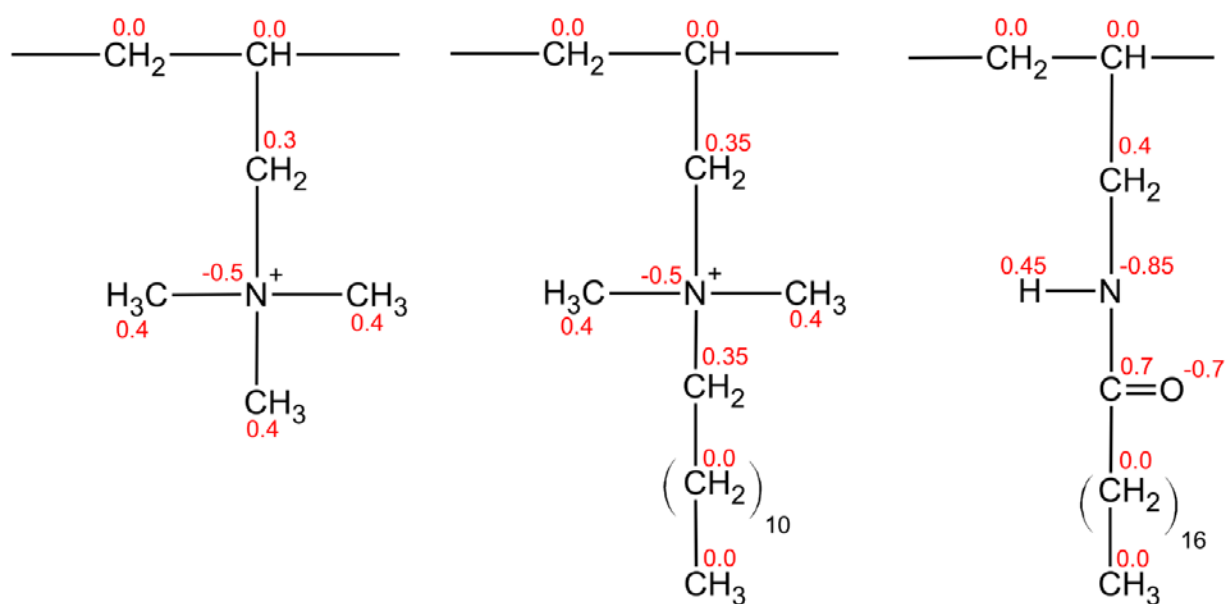


Figure S4. Atomic charges on the side groups of the **o1**, **o2** and **o3** oligomer models. The $-\text{CH}_n$ groups are treated as single sites, according to the united-atom model.

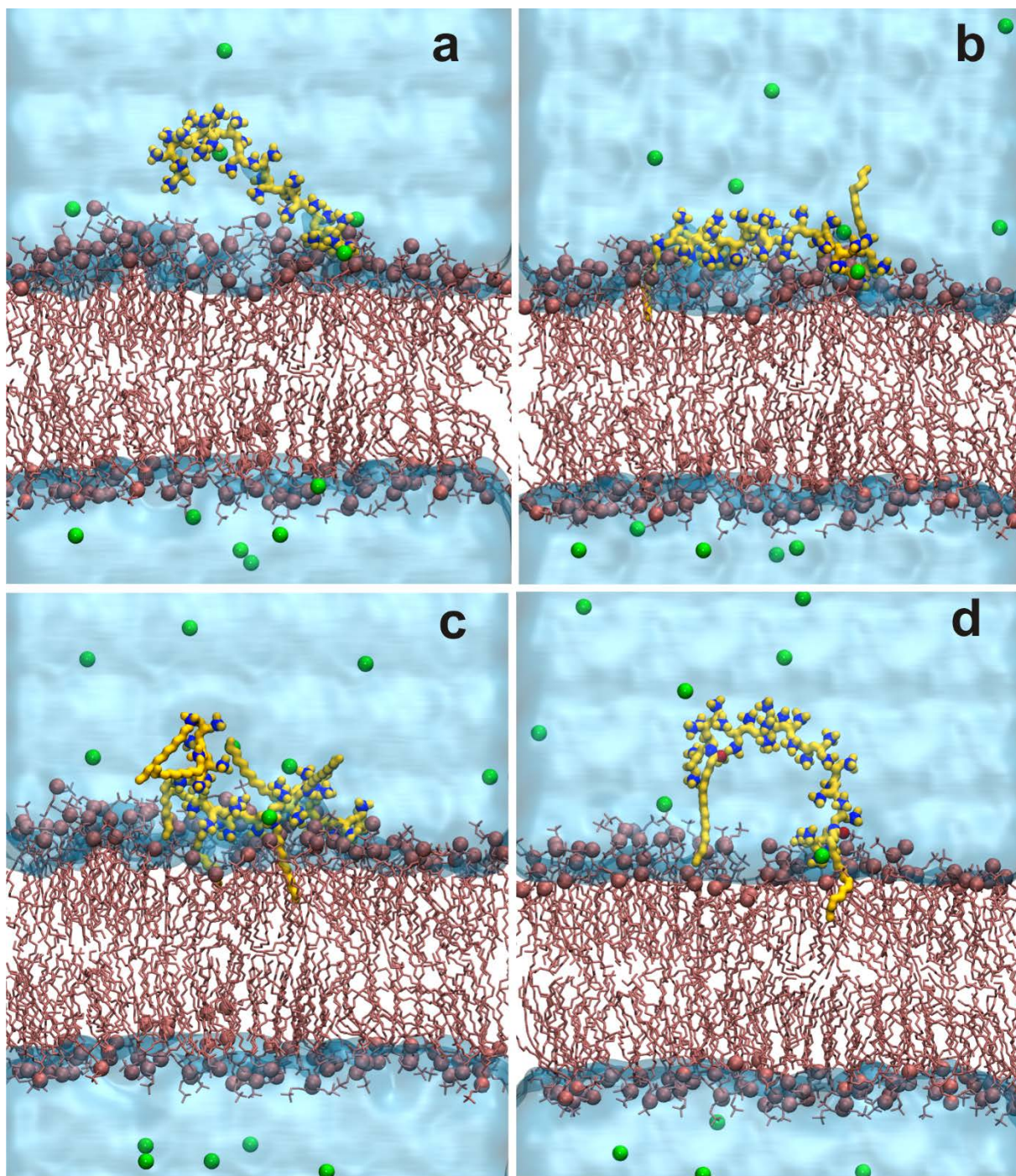


Figure S5. Initial configurations of the oligomer-POPC systems: S-o1 (a), S-o2a (b), S-o2b (c), S-o3b (d). The oligomer molecules are shown in yellow with the N atoms marked in blue. Lipids are shown as maroon sticks and their phosphate groups are shown as maroon spheres. Cl^- anions are represented by green spheres.

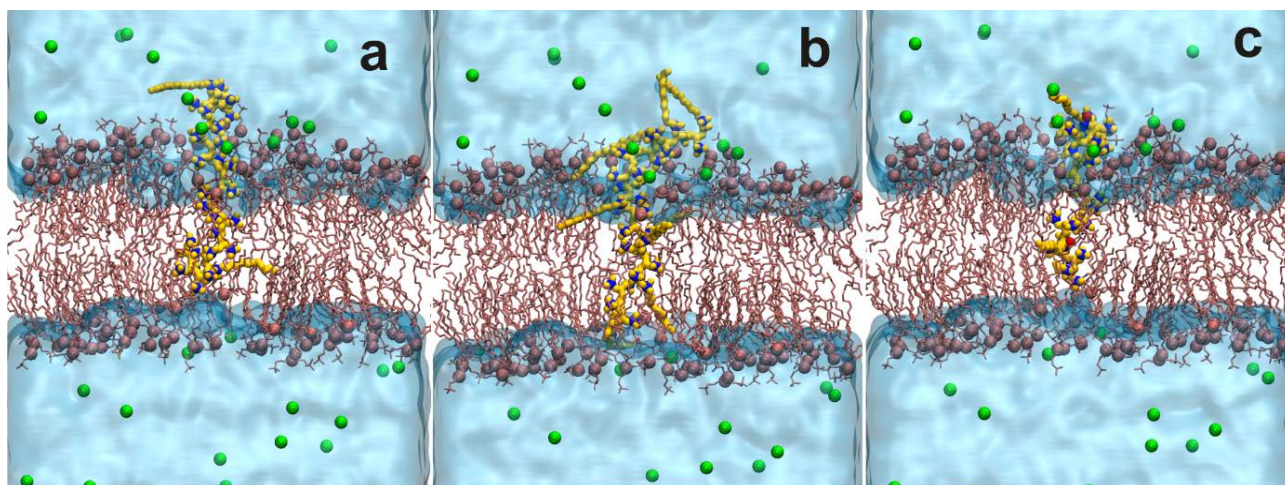


Figure S6. Initial configurations of the oligomer-POPC systems: E-o2a (a), E-o2b (b), E-o3b (c). The oligomer molecules are drawn in yellow with the N atoms marked in blue. Lipids are shown as maroon sticks and their phosphate groups are shown as maroon spheres. Cl^- anions are represented by green spheres.

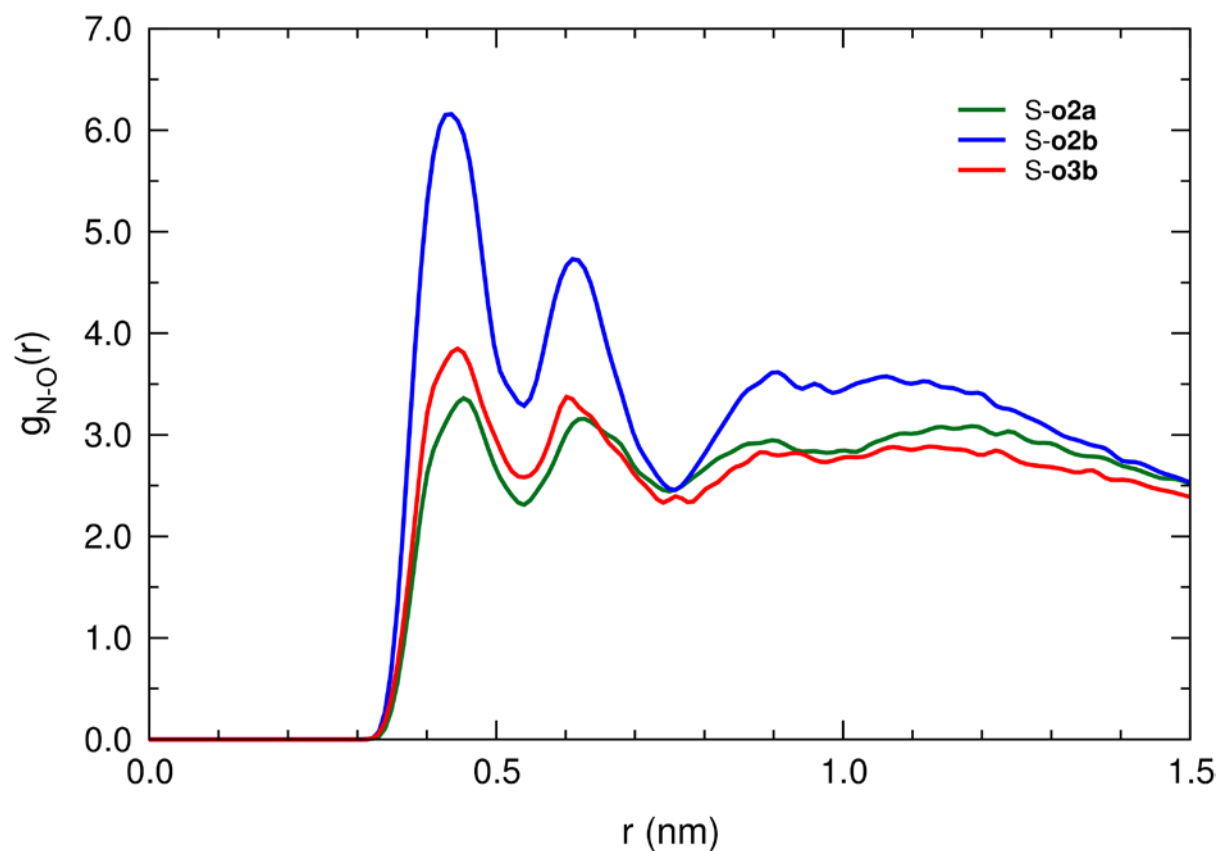


Figure S7. Radial distribution function (rdf) for the N-O(P) atomic pair in the S systems.

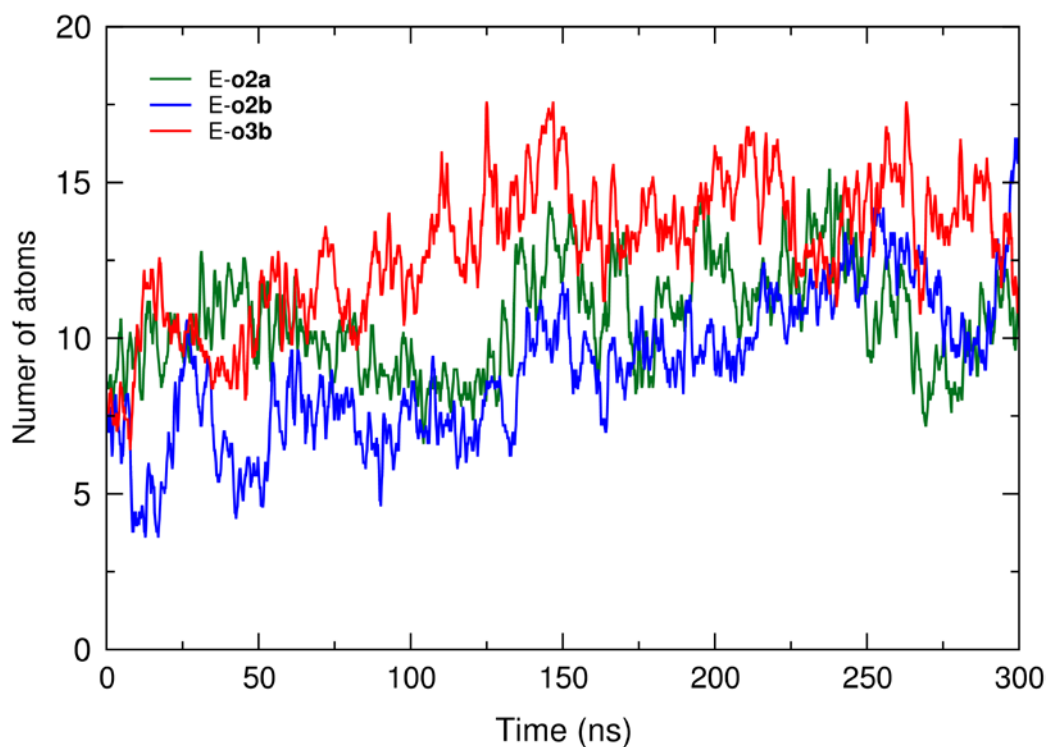


Figure S8. Number of lipid phosphates inside the pore in the systems: E-**o2a** (green), E-**o2b** (blue) and E-**o3b** (red).

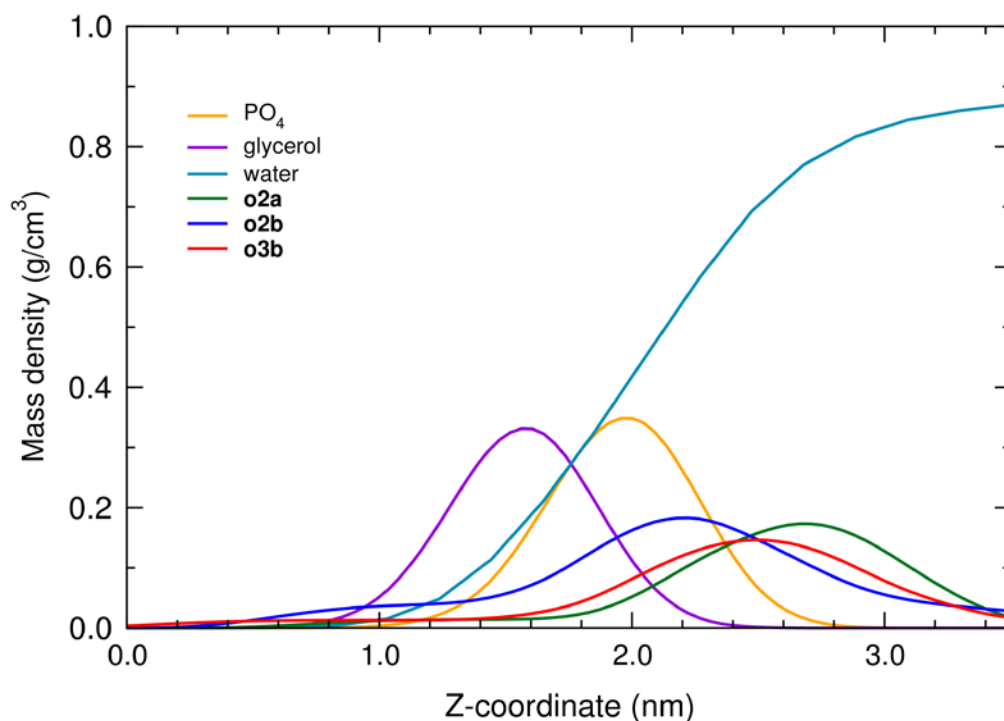


Figure S9. Mass density profiles of the oligomers and the selected POPC groups along the bilayer normal in the systems: S-**o2a**, S-**o2b** and S-**o3b**. Profiles are computed over the final 150 ns of trajectories. The profiles of the oligomers were multiplied by 3 for better comparison.

References

- (1) Wytrwal, M.; Koczurkiewicz, P.; Wójcik, K.; Michalik, M.; Kozik, B.; Zylewski, M.; Nowakowska, M.; Kepczynski, M. Synthesis of Strong Polycations with Improved Biological Properties. *J. Biomed. Mater. Res. A* **2014**, *102A*, 721–731.
- (2) Kepczynski, M.; Jamróz, D.; Wytrwal, M.; Bednar, J.; Rząd, E.; Nowakowska, M. Interactions of a Hydrophobically Modified Polycation with Zwitterionic Lipid Membranes. *Langmuir* **2012**, *28*, 676–688.