Supporting Information of

Unraveling the Role of PEGylation in Anti-Aggregation Stability of Lipid Nanoparticles

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Contents

Initial structure construction

The initial configuration of the system was set up in the following sequential manner using Packmol package[1], as shown in Figure S1. The simulated membrane was built by a DOPC lipid bilayer containing a defined proportion of DOPC molecules on one leaflet replaced with DSPE-PEG molecules. The PEG chains are covalently conjugated to the DSPE lipid molecules (DSPE-PEG), forming an integral part of the membrane. This modified bilayer was then duplicated and oriented such that the PEG-grafted leaflets faced each other, creating a system for subsequent compression using steered MD simulations. The system was explicitly solvated with water molecules, and a certain amount of Na+ ions were added to maintain the charge neutrality. In our setup, the lipid bilayers were modeled as finite nanoscale patches rather than infinitely extended sheets spanning the entire cross-section of the box. This design avoids the unphysical trapping of water during compression and prevents the formation of artificial transmembrane voltages. As shown in Fig. S1, water molecules and Na^+ ions are free to diffuse throughout the simulation box.

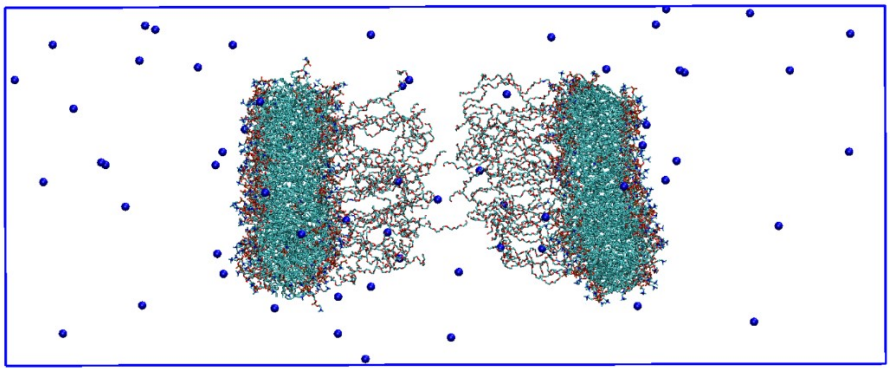

Fig. S1 The snapshot of initial structure. PEG chains are represented in gray, lipid headgroups in red, and lipid tails in dark cyan. Sodium ions are represented by blue beads, and water molecules were invisible in the figure.

Methods for regulating PEG polarity

The atomic partial charges of PEG were strategically modulated to evaluate the role of molecular polarity in interfacial energy by introducing a scaling factor α. The original charge of atom i is denoted as q_i. A scaling factor, α, was introduced to generate modified charges, q'_i, according to the following relation: $q_i^{'} = \alpha * q_i$. In this study, several distinct values for the scaling factor α were examined, including 0.8, 1.0 (representing the original unmodified charges), and 1.2. Since the ethylene glycol monomer unit is electrically neutral in the original force field (i.e., $\Sigma q_i = 0$ over all atoms i in the monomer), this scaling procedure ensures that each monomer unit, and thus the entire PEG chain, remains charge-neutral for all investigated values of α. A separate simulation system was prepared and simulated for each distinct value of α.

Potential of mean force calculation

The umbrella sampling method combined with the weighted histogram analysis method (WHAM)[2, 3] was used to calculate the potential of mean force (PMF) of the compression process of PEG chains. The separation distance between the two bilayers was defined as the reaction coordinate. To generate starting configurations for the umbrella sampling, a pulling simulation was carried out by slowly reducing the distance between two DOPC bilayers along the z-direction to compress PEG layers. At the beginning of the compression process, the PEG layer is in a slightly stretched configuration rather than in its equilibrium state. After compression, a selection of configurations was extracted from this trajectory and served as the input for the umbrella sampling windows. After that, a series of simulations were performed in which the two DOPC bilayers were restrained at a given distance using a harmonic

restraint force along the reaction coordinate *d*. The distance was decreased with 0.2 nm increments and the force constant was set as 1000.0 kJ·mol^{-1}nm^{-2}. Each umbrella sampling simulation was performed for 10 ns. The adequacy of sampling and overlap between neighboring windows for the WHAM analysis is shown in Fig. S2.

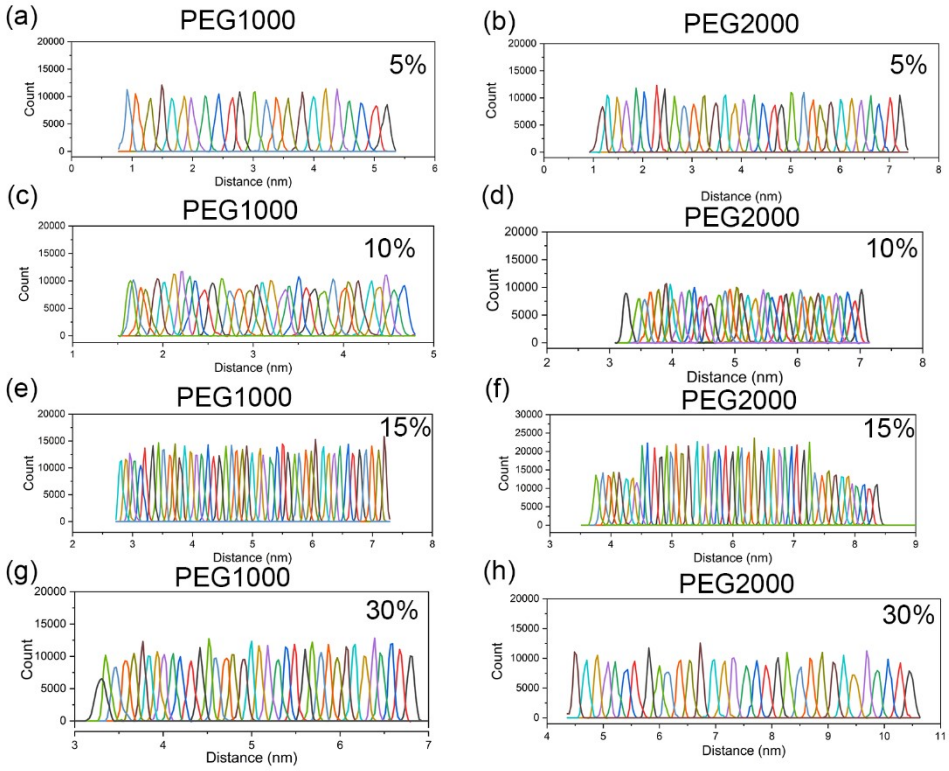

Fig. S2 The corresponding umbrella histograms of PMF of all systems.

Experimental reagents

Microfluidic polydimethylsiloxane (PDMS) chips (channel depth: 100 μm, width: 200 μm) were obtained from Jianmi Zhikong Technology Co., Ltd. (Wuhan, China). mPEG-DSPE was purchased from Carbosynth Ltd. (Guangzhou, China). DOPC was sourced from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous ethanol, sodium hydroxide, and hydrochloric acid were acquired from Sinopharm Chemical Reagent

Co., Ltd. (Shanghai, China). Dialysis membranes with a 3.5 kDa molecular weight cutoff (MWCO) were procured from Yuanye Biotechnology Co., Ltd. (Shanghai, China).

Preparation of PEGylated liposomes via microfluidic method

PEGylated liposomes with varying PEG concentrations and molecular weights (including a non-PEGylated DOPC control and PEG-modified liposomes containing 5%, 15%, or 30% of either 1k or 2k mPEG-DSPE) were prepared using a hydrodynamic flow-focusing microfluidic device. Briefly, DOPC and mPEG-DSPE (1k or 2k Da) were dissolved in anhydrous ethanol at specific ratios to achieve PEG contents of 5%, 15%, or 30% relative to total lipid mass, with a final total lipid concentration of 10 mg/mL. The lipid-ethanol solution (organic phase) and ultrapure water (aqueous phase) were co-injected into a PDMS microfluidic chip at a total flow rate of 1,000 μL/min and a flow rate ratio (aqueous-to-organic phase) of 4:1. The resulting liposome suspensions were dialyzed against ultrapure water for 24 h using 3.5 kDa molecular weight cutoff (MWCO) dialysis membranes to remove residual ethanol and unincorporated lipids.

Supporting figures

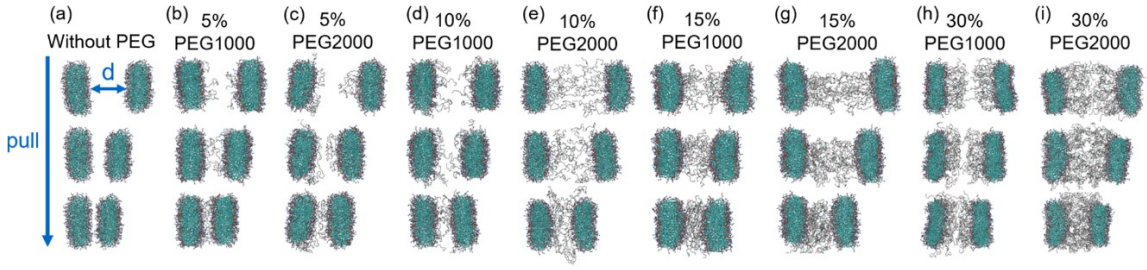

Fig. S3 Snapshots of PEGylated lipid bilayers during compression with varying PEG molecular weights (m_W = 1000 Da, 2000 Da) and grafting densities (ρ_g = 0%, 5%, 10%, 15%, and 30%). PEG chains are represented in gray, lipid headgroups in red, and lipid tails in dark cyan. The separation distance d is defined as the distance between two PEG-grafted lipid surfaces.

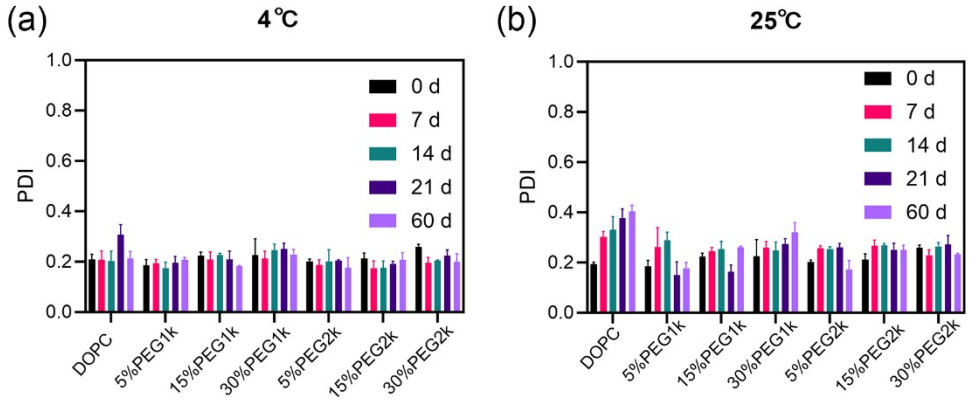

Fig. S4 Polydispersity index (PDI) of DOPC liposomes modified with varying molar percentages (5%, 15% and 30%) of PEG1000 or PEG2000, stored at (a) 4 °C and (b) 25 °C for up to 60 days. Unmodified DOPC liposomes serve as a control.

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References

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