

tained from Aldrich (USA). Chloroform, methanol, sodium chloride, Tris, and Triton X-100 were attained from Merck (Germany). All chemicals were utilized as received without further purification.

Preparation of Tween-grafted Lipid Vesicles Suspensions

Large multilamellar Tween-grafted lipid vesicles were prepared according to the method reported by Bangham et al.¹⁸ Briefly, the lipid mixture contained 0.59 mg/mL of α -tocopherol, 3.75 mg/mL egg PC, 9.6 mg/mL cholesterol, and various quantities of Tween 20 or 80 surfactants (from 0 to 8 wt%) dissolved in a chloroform/methanol mixture (2:1 v/v). The solvent was evaporated in a round-bottomed flask at 50 °C, and nitrogen was used to dry the lipid film for approximately 30 min in order to remove any traces of the organic solvents. The lipid bilayer film was then hydrated in 10 mL of buffer solution composed of 20 mM Tris, 100 mM NaCl, and 1 mM EDTA (pH adjusted to 7.4 with HCl) at 323 K.

Frozen and thawed (FT) lipid vesicles were prepared from the MLVs to reduce lamellarity.¹⁹ These lipid vesicles were frozen (in boiling nitrogen) and thawed (in a water bath at 323 K) 5 times, and were then sonicated for 30 min using a Bandelin HD-200 probe sonicator at a power setting of KE 76/D under nitrogen. The temperature was maintained at 323 K by circulating water in the round-bottomed flask. The final optically clear suspension was filtered through a 0.22- μ m Millipore filter for 3 cycles.

The same amount of α -tocopherol and cholesterol was added to the lipid vesicles. α -Tocopherol is believed to act as an antioxidant in lipid membranes to avoid oxidative damage caused by interactions between free radicals and to protect the PC against oxidative damages,²⁰ therefore, chemical instability of the experimental measurements was reduced.

PCS Determination of Particle Size Distribution of Lipid Vesicles

The lipid vesicle size distribution was determined by photon correlation spectroscopy (PCS) adopting a

zetasizer 3000 (Malvern Instruments, UK). Samples (lipid vesicle suspensions) were diluted in a buffer solution until concentrations were as low as possible to avoid hydrodynamic and electrostatic interactions between lipid vesicles but high enough to obtain the most-reliable auto-correction functions from the PCS measurements that could be obtained.²¹ All PCS measurements were performed at 298 K, and the viscosity of the sample solution was 1 cp. The lipid vesicle suspension was placed in a sample holder, and a laser light (helium-neon laser of wavelength 633 nm and 5 mW) was focused on the sample. Scattered light was detected at 90° to the incident beam using a photomultiplier tube. Five replicate measurements were made from each vesicle suspension, and the mean average particle diameter and polydispersity index were obtained by applying the cumulant analysis method with Malvern software.

ξ -Potential Measurement of Lipid Vesicles

The lipid vesicle suspension was prepared as previously described. The electrophoretic mobility and the zeta potential of the lipid vesicles suspensions were determined by laser Doppler electrophoretic mobility measurements via the zetasizer 3000 (Malvern Instruments).

Phase Transitions of Tween-grafted Lipid Vesicles by Differential Scanning Calorimetry

The concentration of the large multilamellar Tween-grafted lipid vesicles for the DSC experiment was 30 mM. DSC measurements were performed with a high-sensitivity adiabatic differential scanning calorimeter from Perkin-Elmer DSC-7 (USA) at a heating rate of 1 °C/min. The transition enthalpies were determined by planimetry of the peaks using the internal electrical power calibration signal as a reference.

Dilution Heat Determined by Isothermal Titration Calorimetry and the Interaction Potential of Tween-grafted Lipid Vesicles

Dilution heat of the Tween-grafted lipid vesicle