Supporting Materials for "Preparation and Properties of Asymmetric Large Unilamellar Vesicles: Interleaflet Coupling in Asymmetric Vesicles is Dependent Upon Temperature but Not Curvature" by Hui-Ting Cheng and Erwin London

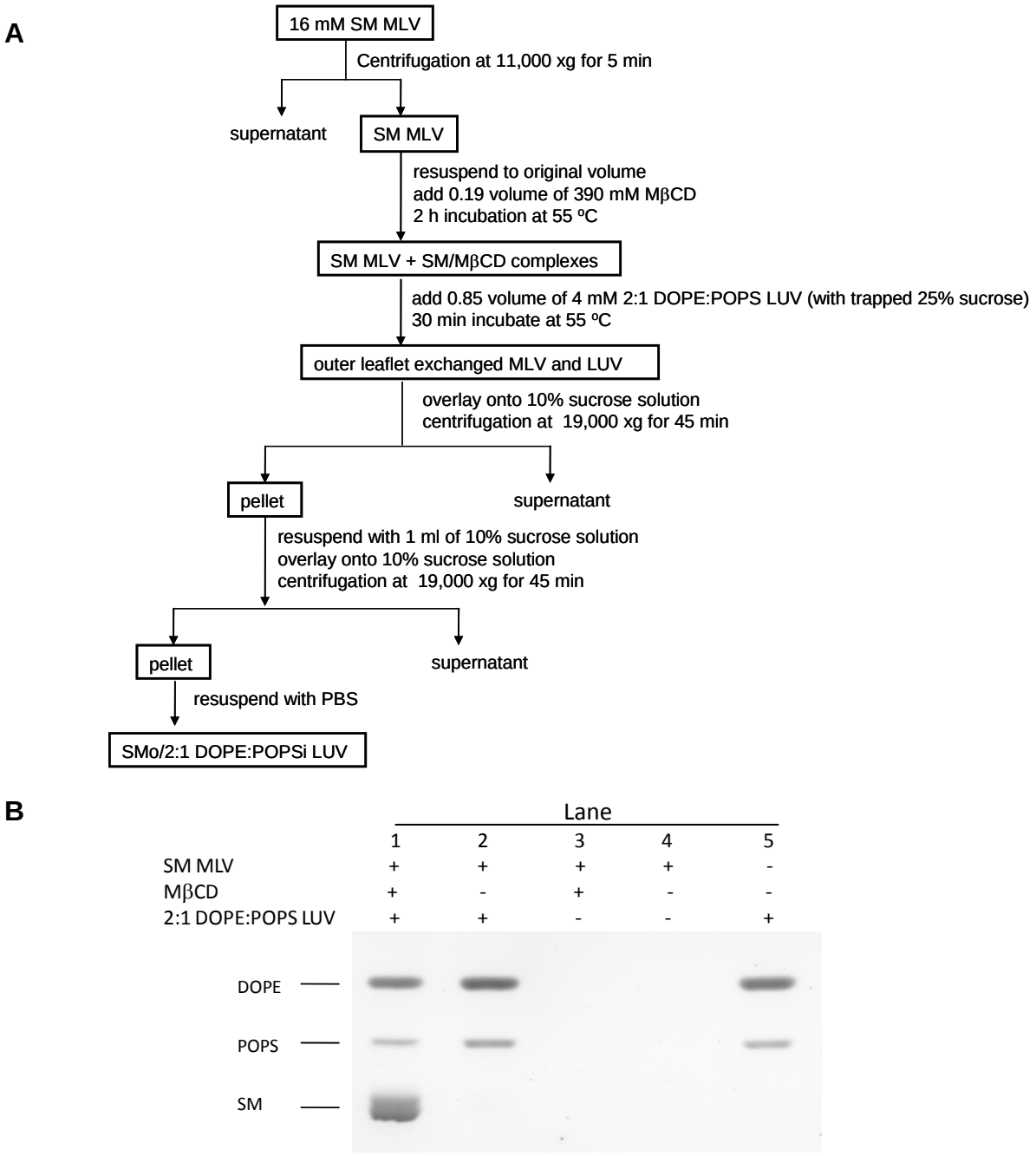

Figure S1 Preparation of asymmetric LUVs. (A) Flow chart of method for preparation of asymmetric SMo/2:1 DOPE:POPSi LUVs. (B) TLC analysis of final pellet from asymmetric LUV protocol. SM MLVs (lanes 1 to 4) or PBS (lane 5) were incubated with (lane 1 and 3) or without (lanes 2, 4, and 5) MβCD for 2h and 2:1 DOPE:POPS LUV (lanes 1, 2, and 5) or PBS (lanes 3 and 4) were then added, and incubated for 30 min, followed by centrifugation steps. HP-TLC analysis of resuspended pellets from each LUV preparation was performed as described in Materials and Methods.

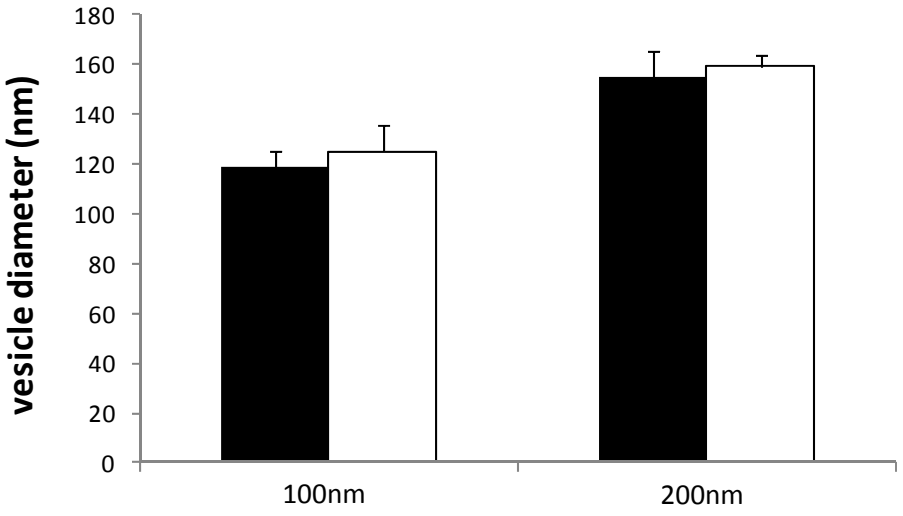

Figure S2. Comparison of vesicle sizes before and after MβCD-induced lipid exchange. 2:1 DOPE:POPS LUV were prepared by extrusion through either a 100 nm-pore size or 200 nm-pore size polycarbonate filter. LUV vesicle sizes before (black bar: 2:1 DOPE:POPS LUV) and after (white bar: SMo/2:1 DOPE:POPSi LUV) exchange were determined by dynamic light scattering. Average vesicle diameter and S.D. (represented by error bars) were obtained from 5 different preparations in samples prepared using a 100 nm-pore size filter (labeled as 100 nm in the figure) and from 3 different preparations in samples prepared using a 200 nm-pore size filter (labeled as 200 nm in the figure).

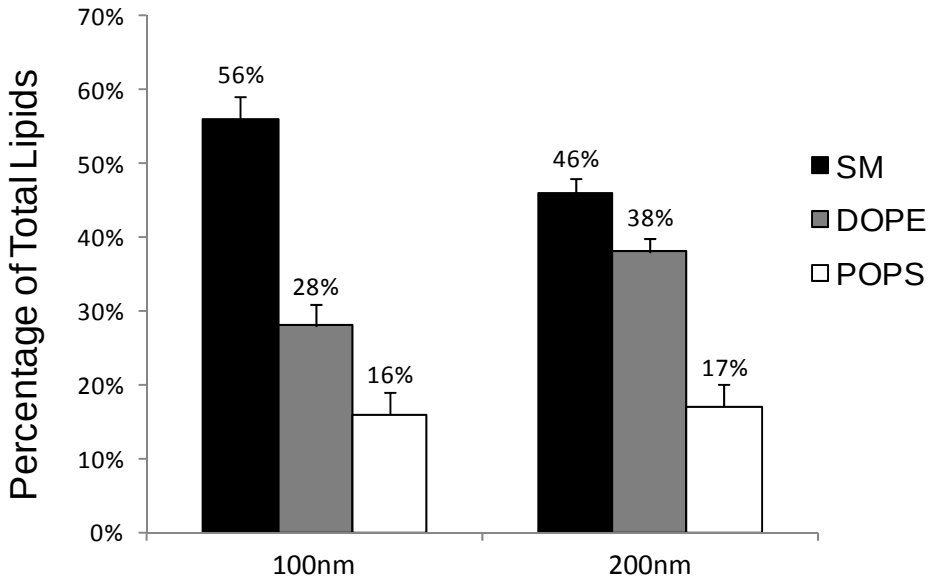

Figure S3. Lipid composition of asymmetric SMo/2:1 DOPE:POPSi LUVs. Lipid composition was analyzed by HP-TLC. Average values and S.D. from 8 different preparations made using 100 nm-pore size filters (labeled as 100 nm in the figure) and from 3 different preparations made using 200 nm-pore size filters (labeled as 200 nm in the figure) are shown.

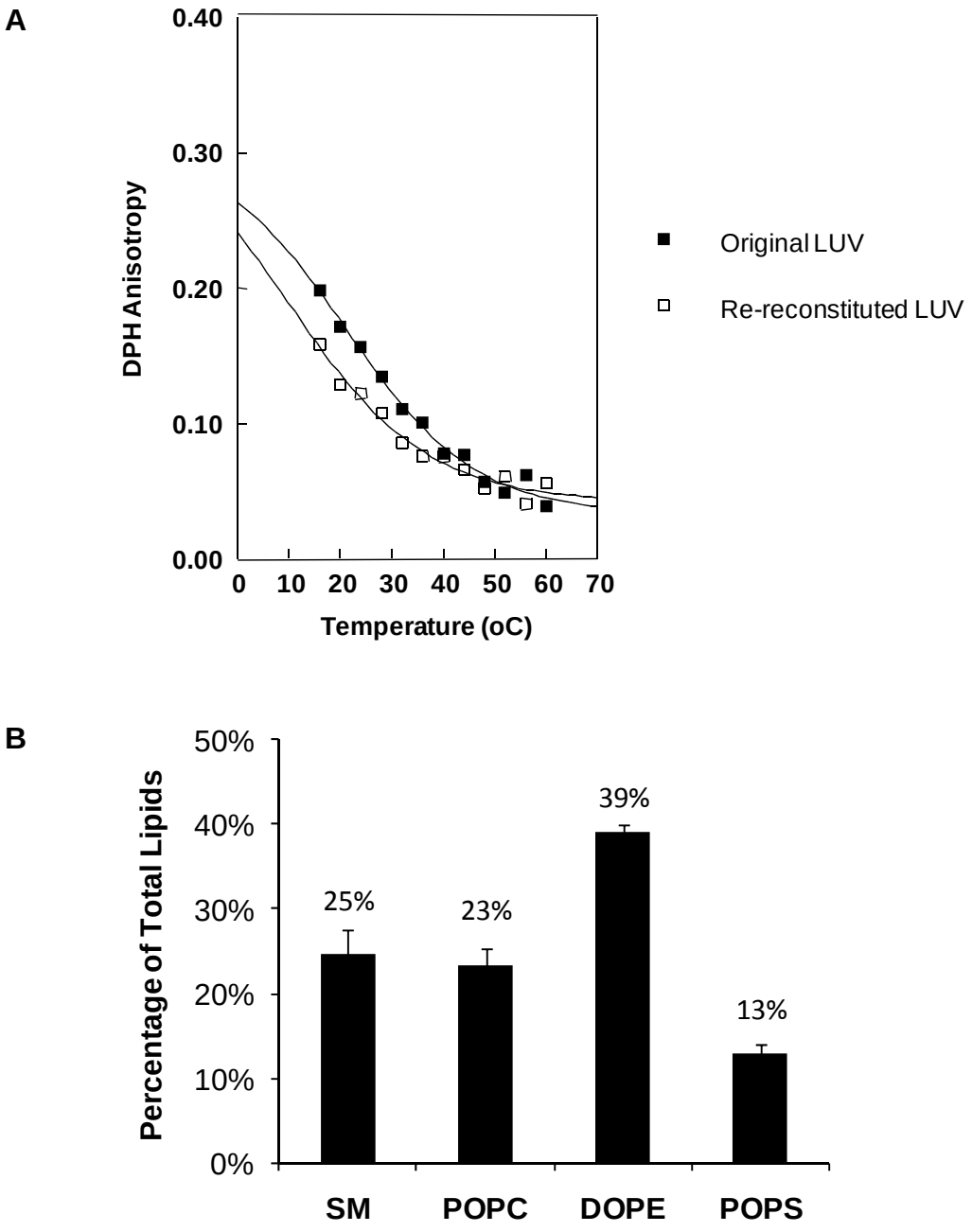

Figure S4. Preparation of asymmetric 1:1 SM:POPCo/2:1 DOPE:POPSi LUVs. (A) Temperature dependence of DPH anisotropy in original (asymmetric) and re-reconstituted LUVs. Rereconstituted LUVs were made by dissolving the asymmetric vesicles in solvent and reforming them into ordinary (symmetric vesicles). See methods for details. Symbols: ■: original (asymmetric) LUVs, □: re-reconstituted LUVs. (B) Lipid composition of asymmetric 1:1 SM:POPCo/2:1 DOPE:POPSi LUVs. Lipid compositions were analyzed by HP-TLC. Average values and S.D. from 3 different preparations in samples made using 100 nm-pore size filters are shown.

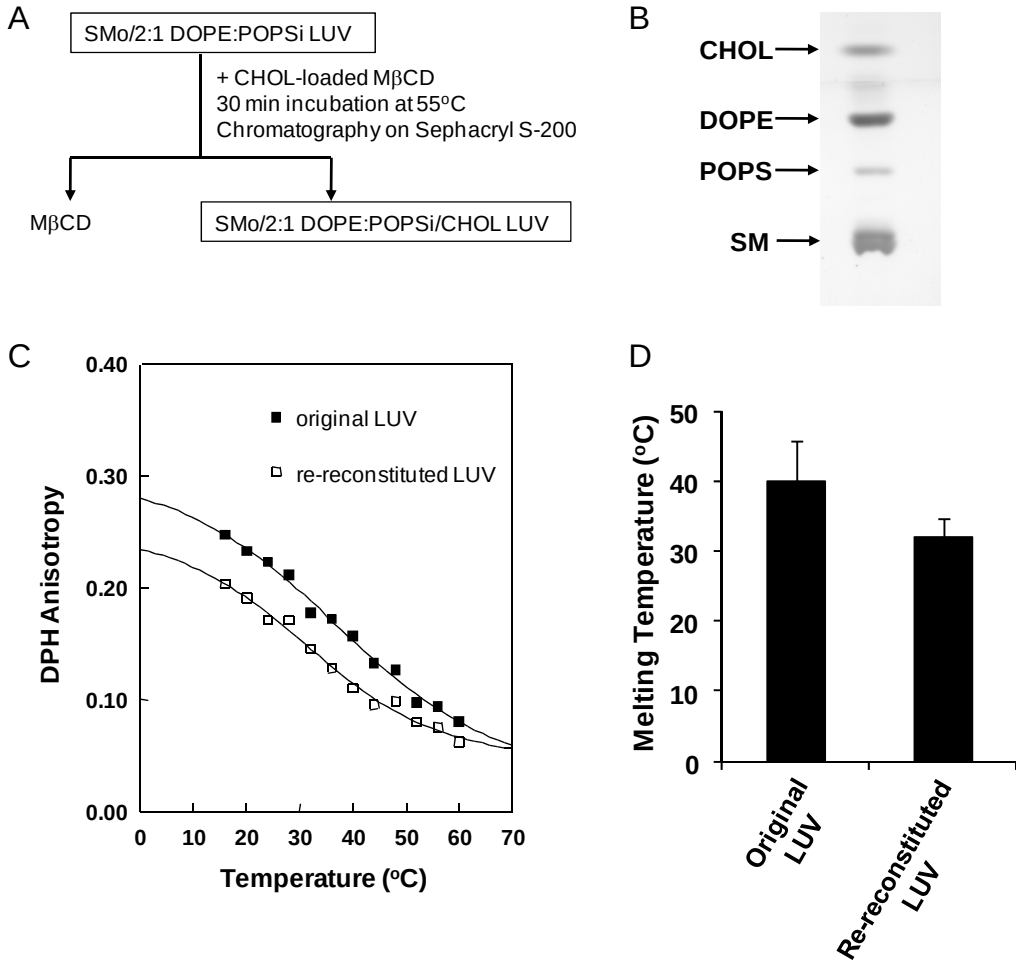

Figure S5. Preparation of SMo/2:1 DOPE:POPSi/CHOL LUVs. (A) Flow chart of method for preparation of asymmetric SMo/2:1 DOPE:POPSi/CHOL LUVs from SMo/2:1 DOPE:POPSi LUVs. (B) HP-TLC analysis of combined LUV-containing fractions. In this preparation, total lipid concentration was 282μM in a 2 ml volume. The molar ratio of SM:DOPE:POPS:CHOL was 32:34:15:19. (C) Temperature dependence of DPH anisotropy in original and re-reconstituted LUVs. Symbols: ■: original (asymmetric) LUVs, □: re-reconstituted LUVs. (D) Melting temperature of original (asymmetric) and re-reconstituted LUVs. Rereconstituted LUVs were made by dissolving the asymmetric vesicles in solvent and reforming them into ordinary (symmetric vesicles). See methods for details.

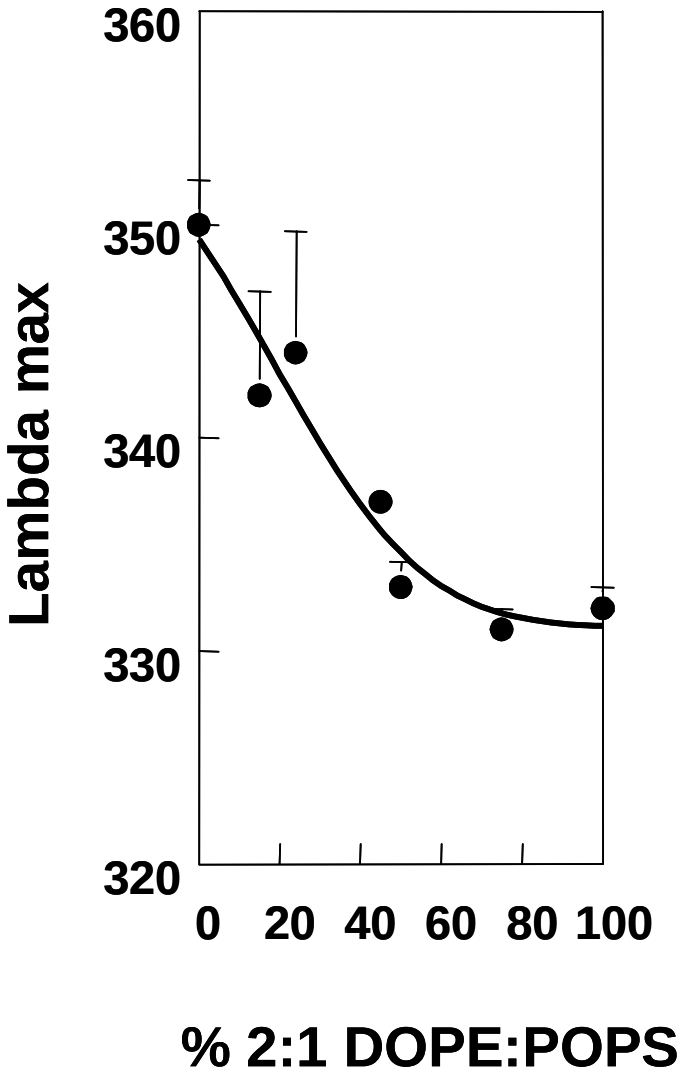

Figure S6. Dependence of fluorescence emission λmax of pL4A18 peptide upon the fraction of 2:1 DOPE:POPS in ordinary (symmetric) vesicles composed of SM, DOPE and POPS and with a 2:1 DOPE:POPS ratio. % 2:1 DOPE:POPS equals sum of % DOPE + % POPS. The % SM in the vesicles is 100% - (% DOPE + %POPS). Average values and standard deviation from three samples are shown. (Point at 45% without error bar is from a single sample.)