Supporting Information for:

Understanding the Rod-to-Tube Transformation of Self-Assembled Ascorbyl Dipalmitate Lipid Nanoparticles Stabilized with PEGylated Lipids

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S1. Removal of PEGylated lipid micelles

The freshly prepared nanoparticle suspensions contained free PEGylated lipid micelles that were not incorporated into the nanoparticles.[1] Here, gel filtration chromatography was used to remove the PEGylated lipid micelles.[2] The nanoparticles and PEGylated lipid micelles in the suspensions were separated using Sepharose 4 Fast Flow (GE Healthcare Japan, Tokyo, Japan). Phosphate-buffered saline (PBS, pH = 7.4) was added as the eluent, and was collected every 20 drops as one fraction. Since the micelle solutions were transparent and colorless, Coomassie Brilliant Blue G-250 dye (Bio-Rad protein assay, USA) was used to detect the micelles.[3] The dye solution was added to each fraction solution after gel filtration. The absorbance of the solution fraction at 620 nm was measured using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). Independent micellar solutions of DSPE-PEG2000 and Chol-PEG1500 were prepared and measured as references.

Figure S1 shows the elution profiles obtained by gel filtration. Strong absorbance was observed in fractions 13–17 and 25–40, for the both nanoparticle suspensions of ASC-DP/DSPE-PEG2000 and ASC-DP/Chol-PEG1500. The solutions obtained for fractions 25–40 immediately after the gel filtration were transparent and colorless, whereas cloudy solutions were obtained for fractions 13–17. Independent micellar solutions of DSPE-PEG2000 and Chol-PEG1500 exhibited similar absorbance for fractions 25–40, indicating that the absorbance for fractions 25–40 was owing to the PEGylated lipid micelles. The cloudy solutions collected for fractions 13–17 exhibited mean particle sizes of 231 nm and 198 nm, for ASC-DP/DSPE-PEG2000 and ASC-DP/Chol-PEG1500, respectively (Figure 3d, e). Therefore, the absorbance for fractions 13–17 was owing to the nanoparticles. The solutions collected for fractions 13–17 were used as micelle-free nanoparticle suspensions.

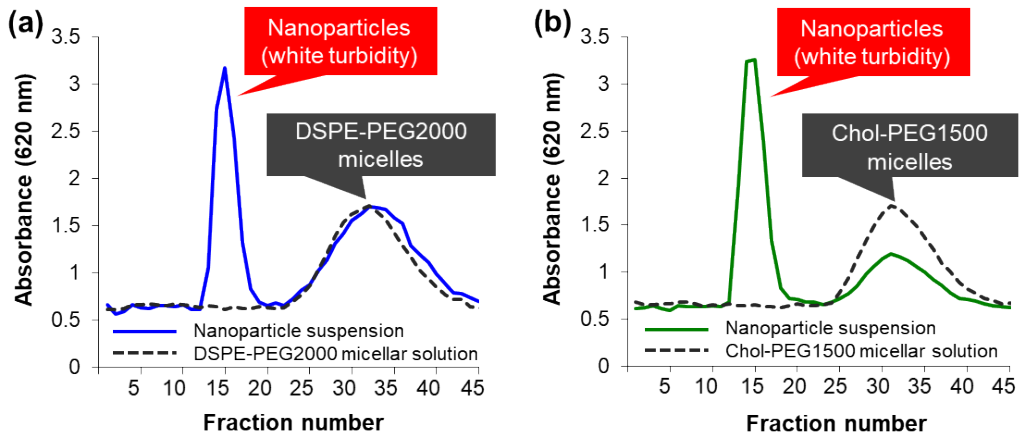


Figure S1. Elution profiles of gel filtration, for the (a) ASC-DP/DSPE-PEG2000 nanoparticle suspension and DSPE-PEG2000 micellar solution, and for the (b) ASC-DP/Chol-PEG1500 nanoparticle suspension and Chol-PEG1500 micellar solution.

S2. ^{1}H NMR spectra for determination of the nanoparticles' composition

The ^{1}H NMR spectra used for determining the nanoparticles' composition are shown in Figure S2. The alkyl methylene peak (1.25 ppm) and PEG methylene peak (3.63 ppm) were used to determine the concentrations of the ASC-DP and PEGylated lipids, respectively.

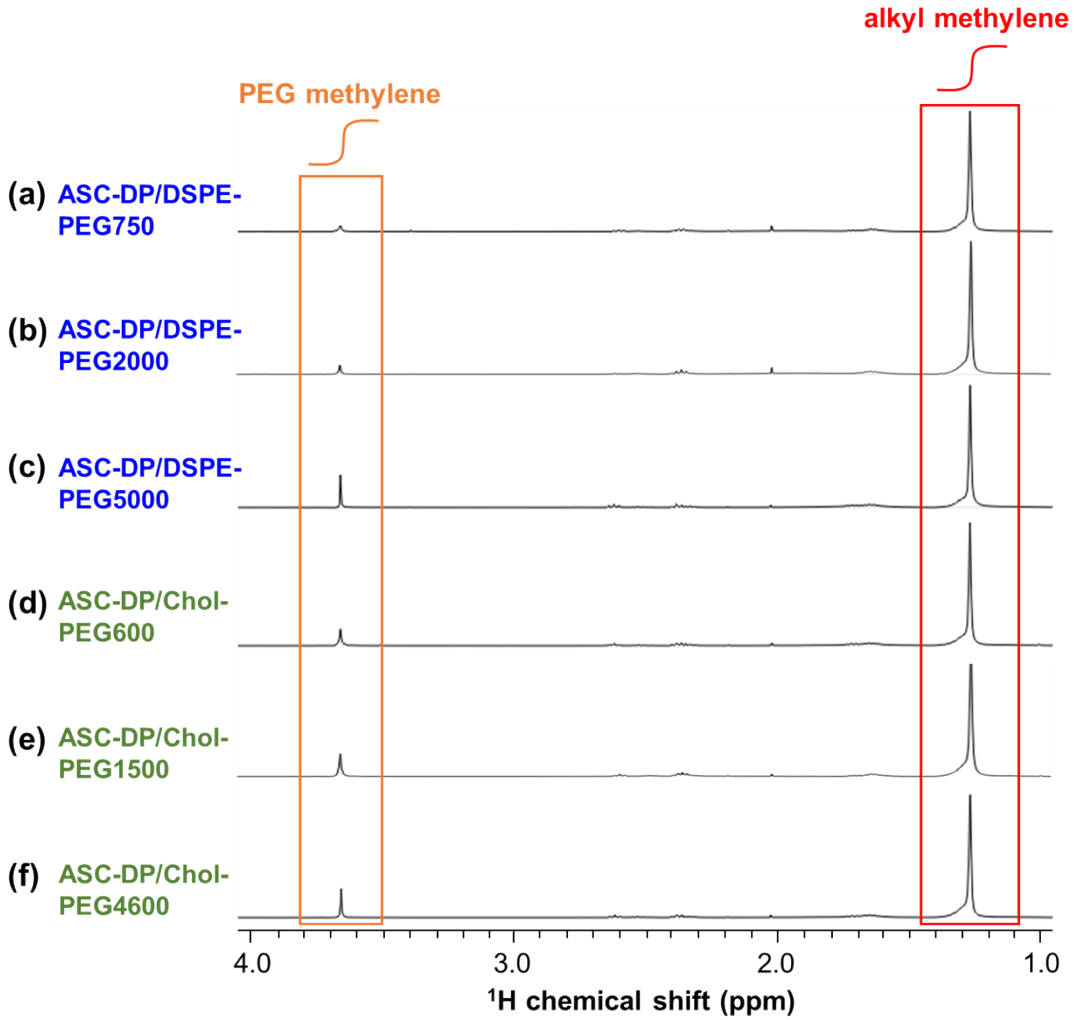


Figure S2. ^{1}H NMR spectra of the (a-c) ASC-DP/DSPE-PEG and (d-f) ASC-DP/Chol-PEG nanoparticles prepared at the molar ratio of 10:1 in $CDCl_3$. The PEG molecular weights of DSPE-PEG used to prepare the nanoparticles were (a) PEG750, (b) PEG2000, and (c) PEG5000, and those of Chol-PEG were (d) PEG600, (e) PEG1500, and (f) PEG4600, respectively.

S3. FE-TEM images of the vertical positions of the nanoparticles

Figure S3 shows the FE-TEM images of some circle structures observed in the ASC-DP/DSPE-PEG2000 and ASC-DP/Chol-PEG1500 systems. These circle structures reflect the vertical positions of the cylindrical nanoparticles, further demonstrating the rod-like structure for the ASC-DP/DSPE-PEG2000 system and the tube-like structure for the ASC-DP/Chol-PEG1500 system.

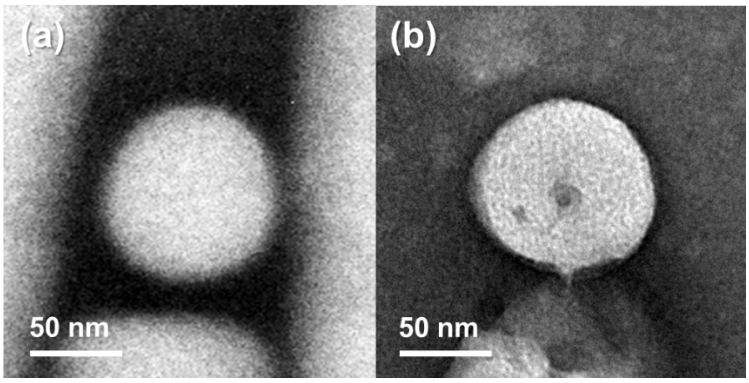


Figure S3. FE-TEM images of the vertical positions of the (a) rod-like ASC-DP/DSPE-PEG2000 and (b) tube-like ASC-DP/Chol-PEG1500 nanoparticles.

S4. Evaluation of the nanoparticles' formation at different preparation molar ratios for all systems

The mean particle sizes of ASC-DP/DSPE-PEG750, ASC-DP/DSPE-PEG2000, ASC-DP/DSPE-PEG5000, ASC-DP/Chol-PEG600, ASC-DP/Chol-PEG1500, and ASC-DP/Chol-PEG4600 nanoparticles prepared at various initial molar ratios, as determined by DLS, are shown in Figure S4-1. The particle-size distribution patterns at the molar ratio of 10:1 are shown in Figure S4-2.

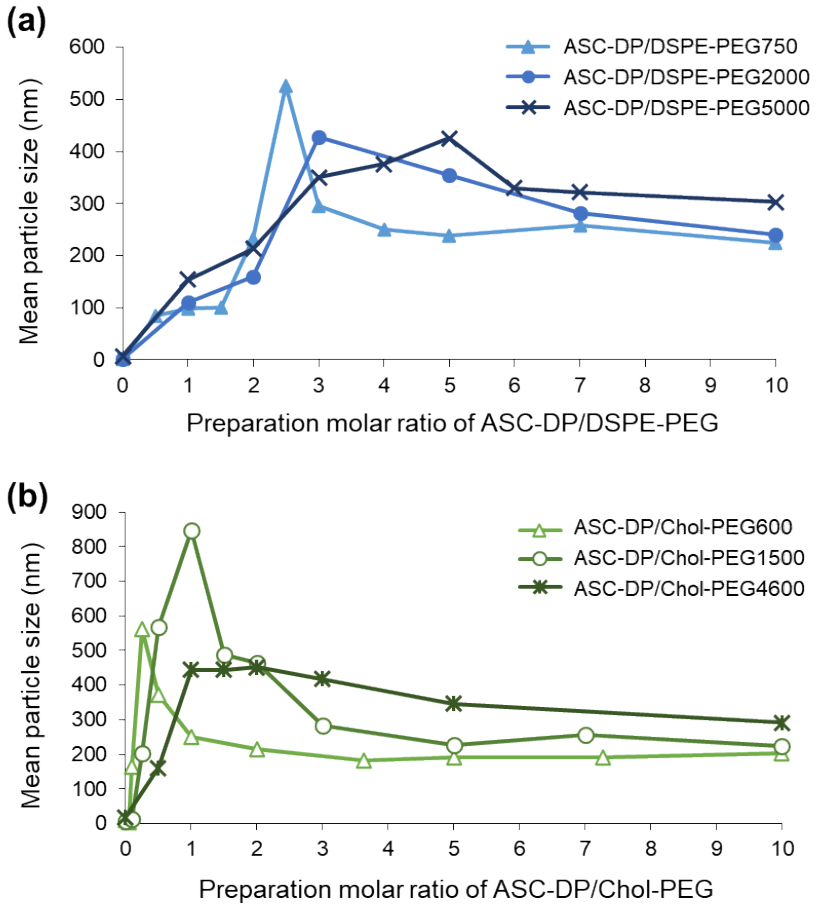
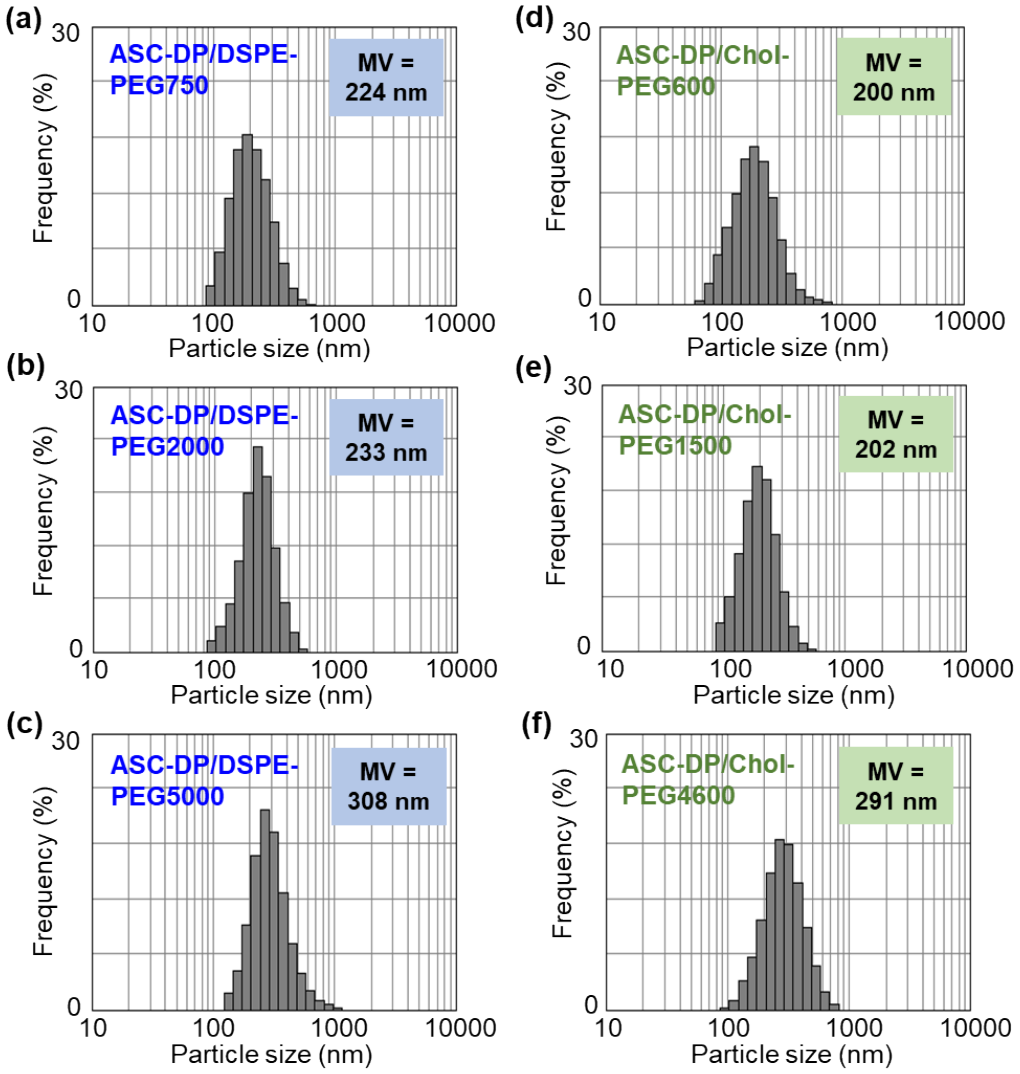


Figure S4-1. Mean particle sizes, for the (a) ASC-DP/DSPE-PEG and (b) ASC-DP/Chol-PEG nanoparticles prepared at various initial molar ratios.



85 **Figure S4-2**. Particle size distributions, for the (a-c) ASC-DP/DSPE-PEG and (d-f) ASC-DP/Chol-PEG nanoparticle suspensions prepared at the molar ratio of 10:1.

S5. XRD patterns of ASC-DP/PEGylated lipid nanoparticles prepared at the molar ratio of 10:1 for all systems

Figure S5 shows the XRD patterns of the nanoparticles prepared with the ASC-DP/PEGylated lipid molar ratio of 10:1; the patterns are shown for the ASC-DP/DSPE-PEG750, ASC-DP/DSPE-PEG2000, ASC-DP/DSPE-PEG5000, ASC-DP/Chol-PEG600, ASC-DP/Chol-PEG1500, and ASC-DP/Chol-PEG4600 systems. All the systems exhibited similar patterns, with diffraction peaks at 2θ = 2.3, 4.6, and 6.8º, and broad peaks for 2θ in the 19–24º range.

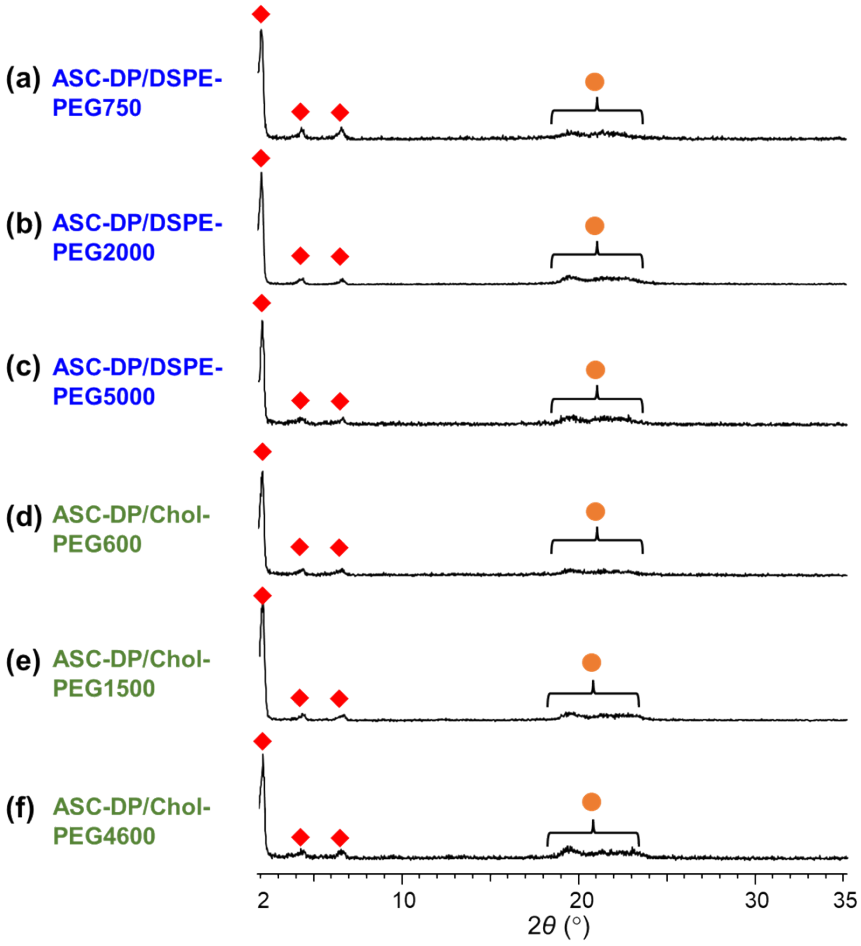


Figure S5. XRD patterns of the (a-c) ASC-DP/DSPE-PEG and (d-f) ASC-DP/Chol-PEG nanoparticles prepared at the molar ratio of 10:1.

References

1. M. Johnsson, P. Hansson and K. Edwards, *J. Phys. Chem. B*, 2001, **105**, 8420-8430.
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3. K. S. Rosenthal and F. Koussaie, *Anal. Chem.*, 1983, **55**, 1115-1117.