Supporting information for

Amphipathic helical peptide-Nile Red probes for fluorescence probing of the lipid packing defects and their surrounding membranes on exosomes

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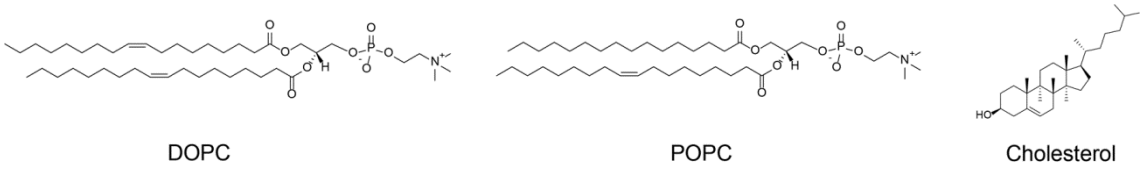

Figure S1. Chemical structures of lipids used for preparation of synthetic liposomes.

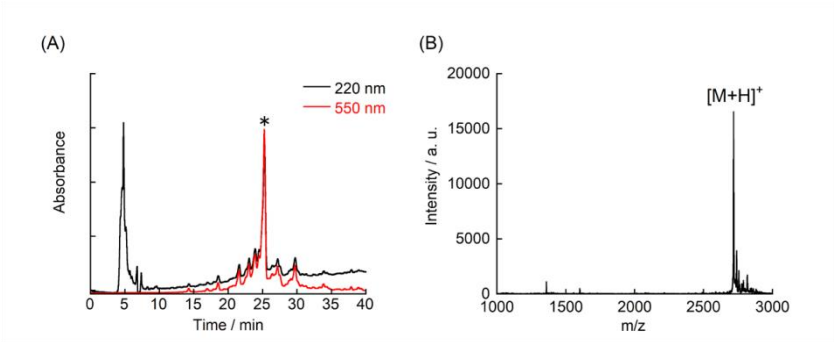

Figure S2. (A) HPLC profile for the purification of p2-23-NR. Gradient conditions: 40-60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) during 40 min. Absorbance at 220 nm and 550 nm were monitored for the peptide and NR unit in the probe, respectively. The peak (*) was collected and identified as the purified probe. (B) MALDI-TOF-MS spectrum of the purified probe.

Table S1. Probe characterization

	Observed mass (m/z)	Calculated mass $[M+H]^+$
ApoC-NR	2971.866	2971.586
ApoC-C1-NR	2915.742	2915.524
p2-23-NR	2717.248	2717.516
p2-23-C1-NR	2662.324	2662.461

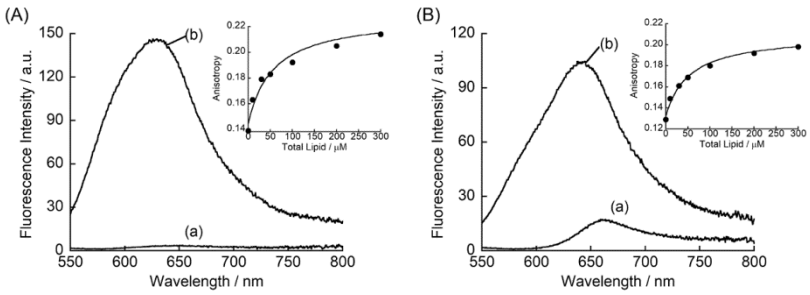

Figure S3. Fluorescence spectra of peptide-NR probe (2.0 μM: (A) ApoC-NR and (B) p2-23-NR) in the (a) absence and (b) presence of 50 μM DOPC liposome. Inset: Titration curve for the binding of the probe (2.0 μM) to DOPC liposomes, obtained by fluorescence anisotropy measurements. The obtained curve was analyzed with the fitting equation for determination of K_d value. Excitation, 552 nm. Analysis, 634 nm.

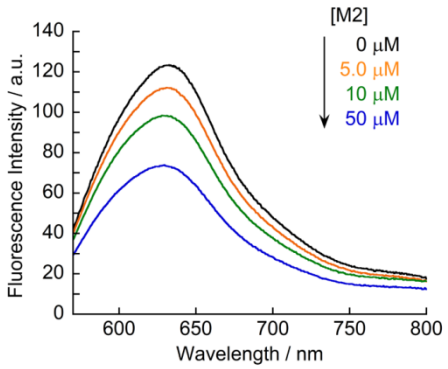

Figure S4. Fluorescence spectra of ApoC-NR in the presence of DOPC liposomes or M2/liposome complex. [ApoC-NR] = 2.0 μM, [DOPC liposome (total lipid)] = 500 μM, [M2 peptide] = 0-50 μM. Excitation, 552 nm.

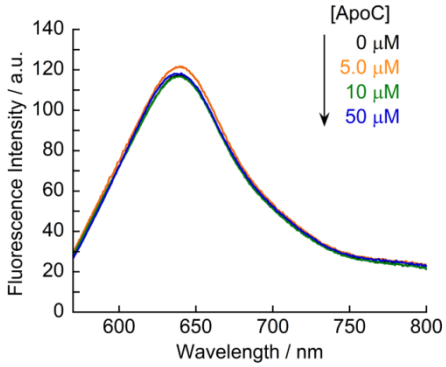

Figure S5. Fluorescence spectra of p2-23-NR in the presence of DOPC liposomes or ApoC/liposome complex. [p2-23-NR] = 2.0 μM, [DOPC liposome (total lipid)] = 500 μM, [ApoC peptide] = 0-50 μM. Excitation, 552 nm.

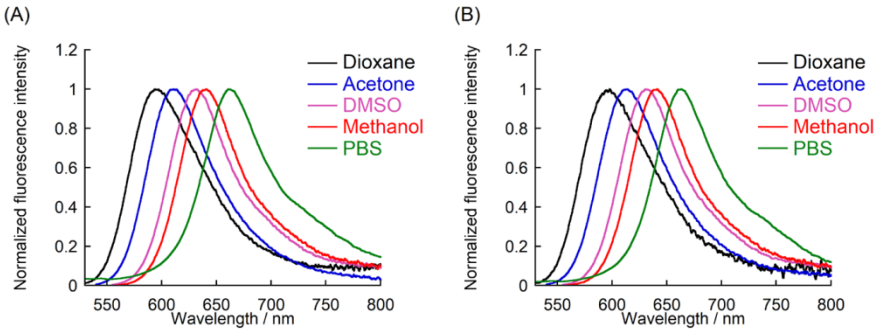

Figure S6. Normalized fluorescence spectra of the probe (2.0 μM: (A) ApoC-NR and (B) p2-23-NR) in various solvents. Excitation wavelength, 531 nm (Acetone), 532 nm (PBS), 523 nm (Dioxane), 550 nm (DMSO and methanol).

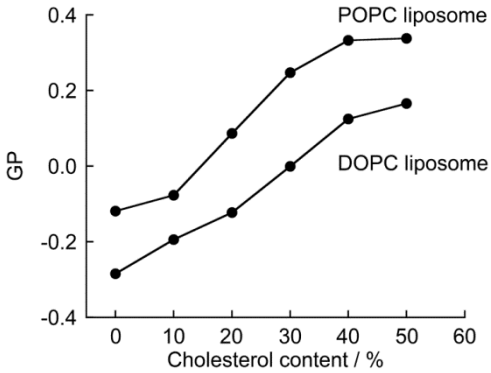

Figure S7. Evaluation of membrane polarity (GP) of DOPC or POPC liposomes with varying Chol contents (500 μM) based on the fluorescence response of Laurdan (0.5 μM).

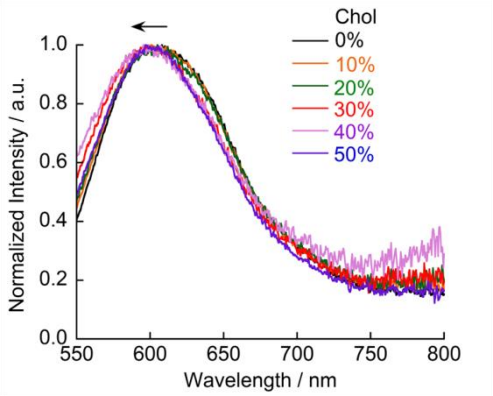

Figure S8. Normalized fluorescence spectra of ApoC- NR bound to the POPC liposomes with varing Chol content (0-50%). [ApoC-NR] = 2.0 μM, [Liposome] = 500 μM. Excitation, 470 nm.

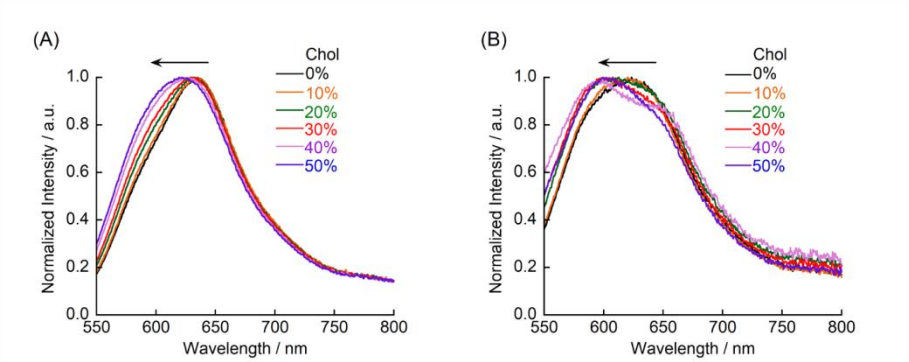

Figure S9. Normalized fluorescence spectra of p2-23-NR bound to the (A) DOPC or (B) POPC liposomes with varying Chol content (0-50%). [p2-23-NR] = 2.0 μM, [Liposome] = 500 μM. Excitation, 470 nm.

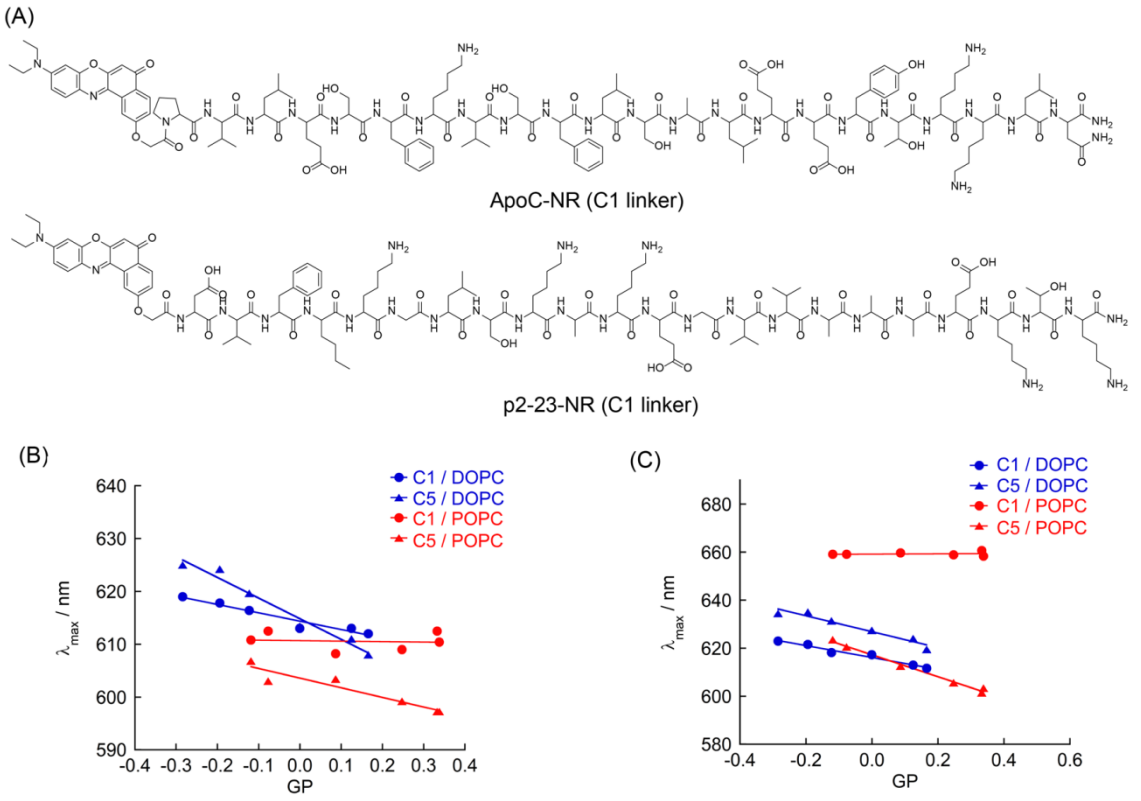

Figure S10. (A) Chemical structures of AH peptide-NR probes carrying a C1 linker. (B, C) Effect of linker length between AH peptide and NR units on changes of maximum emission wavelength of the probes (2.0 μM: (B) ApoC-NR and (C) p2-23-NR) in 500 μM liposomes with different membrane polarity (GP).

In both probes, the maximum emission wavelength of C5 linker-carrying probes showed higher correlation with the membrane polarity determined by Laurdan assay (ΔGP) of the liposomes compared to C1 linker-carrying ones. These results strongly suggest that C5 linker in the probes is favorable for assessment of the membrane lipid packing order.

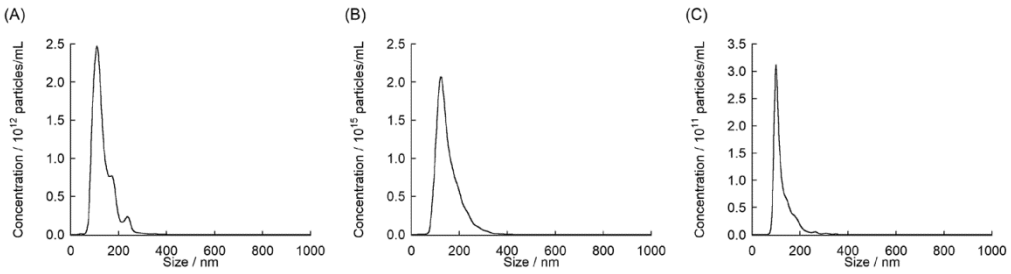

Figure S11. Size distribution profiles of (A) ExoA549, (B) ExoHela, and (C) ExoMCF7, obtained by nanotracking analysis.

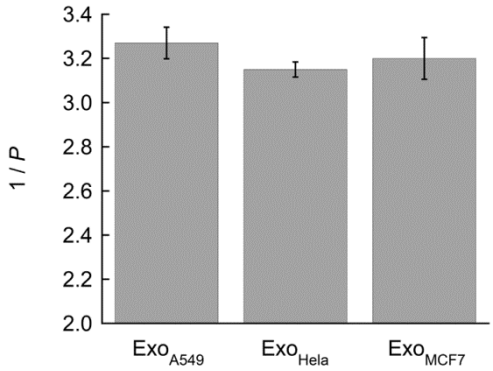

Figure S12. Estimation of membrane fluidity of exosomes using DPH. [Exosome] = 3.9×10^7 particles/μL, [DPH] = 0.5 μM. Excitation, 360 nm.

Membrane fluidity of exosomes was determined based on the change of fluorescence polarization of DPH according to the literature (ref. 7c in the main text). The polarization (*P*) of DPH at 430 nm in the presence of exosomes was measured. The 1/*P*, reciprocal of polarization (*P*) was calculated as the membrane fluidity.