Supporting Information

Enveloped Artificial Viral Capsid Self-assembled from Anionic β-Annulus Peptide and Cationic Lipid Bilayer

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EXPERMENTAL SECTION

General. Reversed-phase HPLC was performed at ambient temperature using Shimadzu LC-6AD liquid chromatography system equipped with a UV/vis detector (220 nm, Shimadzu SPD-10AVvp) and Inertsil WP300 C18 (GL Science) column (250 × 4.6 mm and 250 × 20 mm). MALDI-TOF mass spectra were obtained using an Autoflex-T2 instrument (Bruker Daltonics) in linear/positive mode with α-cyano-4-hydroxy cinnamic acid (α-CHCA) as a matrix. Deionized water of high resistivity (>18 MΩ cm) was purified using a Millipore Purification System (Milli-Q water) and was used as a solvent for the present peptides. Reagents were obtained from commercial sources and used without further purification.

Synthesis of β-Annulus-EE Peptide. The peptide H-Ile-Asn(Trt)-His(Trt)-Val-Gly-Gly-Thr(tBu)-Gly-Gly-Ala-Ile-Met-Ala-Pro-Val-Ala-Val-Thr(tBu)-Arg(Pbf)-Gln(Trt)-Leu-Val-Gly-Ser(tBu)-Glu(OtBu)-Glu(OtBu)-Alco-PEG resin was synthesized on Fmoc-Glu(OtBu)-Alko-PEG resin (494 mg, 0.123 mmol/g; Watanabe Chemical Ind. Ltd.) using Fmoc-based coupling reactions (4 equiv of Fmoc amino acid). *N*-methylpyrrolidone (NMP) solution containing (1-cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) and diisopropylamine was used as the coupling reagent. Fmoc deprotection was achieved using 20% piperidine in *N*,*N*-dimethylformamide (DMF). Progression of the coupling reaction and Fmoc deprotection was confirmed by TNBS and chloranil test kit (Tokyo Chemical Industry Co., Ltd.). Peptidyl-resins were washed with NMP and were then dried under a vacuum. Peptides were deprotected and cleaved from the resin by treatment with a cocktail of trifluoroacetic acid (TFA)/1,2-ethanedithiol/triisopropylsilane/water = 3.76/0.1/0.04/0.1 (mL) at room temperature for 4 h. Reaction mixtures were filtered to remove resins, and filtrates were concentrated in vacuo. The peptide was precipitated by adding methyl *tert*-butyl ether (MTBE) to the residue and the supernatant was decanted. After three times of repetitive washing with MTBE, precipitated peptide was dried in vacuo. The crude product was purified by reverse-phase HPLC eluting with a linear gradient of CH_3CN/water containing 0.1% TFA (5/95 to 100/0 over 100 min). The fraction containing the desired peptide was lyophilized to give 33.5 mg of a flocculent solid (35% yield). MALDI-TOF MS (matrix: α-CHCA): *m/z* = 2563 $[M]^+$.

Synthesis of TAMRA-β-Annulus-EE Peptide. β-Annulus-EE peptide bearing Cys at the N-terminal (CINHVGGTGGAIMAPVAVTRQLVGSEE) was synthesized on Fmoc-Glu(OtBu)-Alko-PEG resin (400 mg, 0.1 mmol/g; Watanabe Chemical Ind. Ltd.) by almost the same procedure described above. The crude product was purified by reverse-phase HPLC eluting with a linear gradient of CH_3CN/water containing 0.1% TFA (5/95 to 100/0 over 100 min). The fraction containing the desired peptide was lyophilized to give 7.4 mg of a flocculent solid (29% yield). MALDI-TOF MS (matrix: α-CHCA): *m/z* = 2667 $[M]^+$. The obtained Cys-β-annulus-EE peptide powder was dissolved in 20 mM sodium phosphate buffer (926 μL, pH 7.0) in an Eppendorf tube. Then, 38 mM tris(2-carboxyethyl)phosphine

hydrochloride (TCEP-HCl, Wako Co., Ltd.) in Milli-Q water (26 μL) and 10.4 mM tetramethylrhodamine-5-maleimide (TAMRA-maleimide, Funakoshi Co., Ltd.) in dimethyl sulfoxide (48 μL) were added the solution, and then the mixture was incubated in the dark at 25 °C for 7 h (final concentration: 250 μM Cys-*β*-annulus-EE peptide, 1 mM TCEP-HCl, 500 μM TAMRA-maleimide). After dialysis (Spectra/por7, cutoff Mw 1,000, Spectrum Laboratories, Inc.) in water for 24 h, the sample was purified by reverse-phase HPLC eluting with a linear gradient of CH_3CN/water containing 0.1% TFA (5/95 to 100/0 over 100 min). The fraction containing the desired peptide was lyophilized and dissolved in water (100 μL) to give an aqueous solution of 129 μM TAMRA-*β*-annulus-EE (5.2% yield). MALDI-TOF MS (matrix: *α*-CHCA): *m/z* = 3148 $[M]^+$.

Complexation of β-Annulus-EE Peptide with DOTAP/DOPC Lipids. Stock solution of 10 mM 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP, Avanti Polar Lipids) in chloroform (3 μL), stock solution of 10 mM 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, Tokyo Chemical Industry Co., Ltd.) in chloroform (66 μL), methanol (33 μL) and chloroform (66 μL) were put in a glass tube and dried in vacuo for 6 h. The resulting lipid film was hydrated with solution of *β*-annulus-EE peptide solution (200 μL) in 10 mM Tris-HCl buffer (pH7.0) at 50 °C for 1 h, in which the cation / anion charge ratio of the complex was controlled to be 1: 1. Free liposomes were removed from the complex by ultracentrifugation using Optima MAX-TL Ultracentrifuge (25,000 rpm, 2 min, Beckman Coulter, Inc.). Figure S12 shows size distributions obtained from DLS and ζ-potential of enveloped artificial viral capsid immediately after complexing of *β*-annulus-EE peptide with DOTAP/DOPC (before equilibration for 1 h). The DLS showed multiple size distribution broader than that of the enveloped artificial viral capsid after incubation for 1 h (Figure 1C, 84 ± 23 nm). Therefore, incubation for 1 h after complexing plays an important role in equilibrating into stable and uniform enveloped artificial viral capsid.

Dynamic Light Scattering and ζ-Potential. Dynamic Light Scattering (DLS) of anionic artificial viral capsids, enveloped artificial viral capsids, and liposomes in 10 mM Tris-HCl buffer (pH 7.0) were measured at 25 °C using Zetasizer Nano ZS (MALVERN) with an incident He-Ne laser (633 nm) and ZEN2112-Low volume glass cuvette cell. During measurements, count rates (sample scattering intensities) were also provided. Correlation times of the scattered light intensities $G(\tau)$ were measured several times and there means were calculated for the diffusion coefficient. Hydrodynamic diameters of scattering particles were calculated using the Stokes-Einstein equation. ζ-Potentials of anionic artificial viral capsids, enveloped artificial viral capsids, and liposomes were measured at 25 °C using a Zetasizer Nano ZS with a DTS1070 clear disposable zeta cell.

Transmission Electron Microscopy. Aliquots (5 μL) of the DLS samples were applied to hydrophilized carbon-coated Cu-grids (C-SMART Hydrophilic TEM grids, ALLANCE Biosystems) for 1 min and then removed Subsequently, the TEM grids were instilled in the staining solution, 25% EM stainer aqueous solution (Nisshin EM Co., Ltd., 5 μL), for 15 min and then removed. After the

sample-loaded grids were dried in vacuo, they were observed by TEM (JEOL JEM 1400 Plus), using an accelerating voltage of 80 kV.

Imaging Flow Cytometry. Stock solution of 10 mM DOTAP (Avanti Polar Lipids) in chloroform (3 μL), stock solution of 10 mM DOPC (Tokyo Chemical Industry Co., Ltd.) in chloroform (28 μL), stock solution of 1 mM 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE, Avanti Polar Lipids) in chloroform (15 μL), methanol (23 μL) and chloroform (46 μL) were put in a glass tube and dried in vacuo for 6 h. The resulting lipid film was hydrated with solution (200 μL) of 49 μM *β*-annulus-EE + 1 μM TAMRA-*β*-annulus-EE peptide in 10 mM Tris-HCl buffer (pH7.0) at 50 °C for 1 h (final concentration: 49 μM *β*-annulus-EE, 1 μM TAMRA-*β*-annulus-EE, 150 μM DOTAP, 1425 μM DOPC, 75 μM NBD-PE), in which the cation / anion charge ratio of the complex controlled to be 1: 1. Free liposomes were removed from the complex by ultracentrifugation using Optima MAX-TL Ultracentrifuge (25,000 rpm, 2 min, Beckman Coulter, Inc.). The sample (200 μL) was observed by Imaging Flow Cytometer (Amnis FlowSight, Luminex Co., Ltd.) using an excitation laser (488 nm). The fluorescence intensity of 10,000 particles were analyzed by using software IDEAS (Luminex Co., Ltd.).

Fluorescence Resonance Energy Transfer (FRET). The enveloped artificial viral capsid labelled with NBD-PE (3.75 μM, 2.00 μM) as donor and TAMRA-*β*-annulus peptide (0−7.50 μM) as acceptor was constructed. The total concentration of unlabeled *β*-annulus-EE and TAMRA-*β*-annulus-EE were kept constant (50 μM). The fluorescence emission spectra (500-650 nm) exited at 460 nm of the samples was measured at 25°C by Jasco FP 8200 fluorescence spectrometer (JASCO Co., Ltd.) under temperature control using a Low Temp Bath BB301 (Yamato Scientific Co., Ltd.). The FRET efficiency *E* was calculated by using eq (1) [1]

$$E = 1 - \frac{I_{DA}}{I_D} \quad \cdot \cdot \cdot (1)$$

where I_D and I_{DA} are the fluorescence intensity of the donor in the absence and presence of the acceptor, respectively. The distance *R* between the donor and acceptor was calculated by using eq (2) [2]

$$R = R_0 \sqrt[6]{\frac{1-E}{E}} \quad \cdot \cdot \cdot (2)$$

where R_0 is Förster radius of the NBD/TAMRA-pair. It was set to R_0 = 5.1 nm according to the literature.[3]

Supporting figures

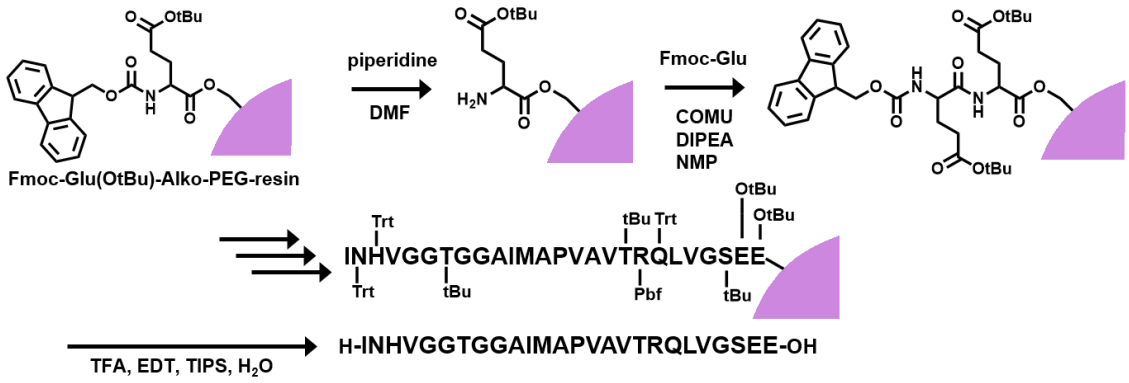

Figure S1. Synthesis of β-annulus-EE peptide by solid-phase Fmoc-chemistry.

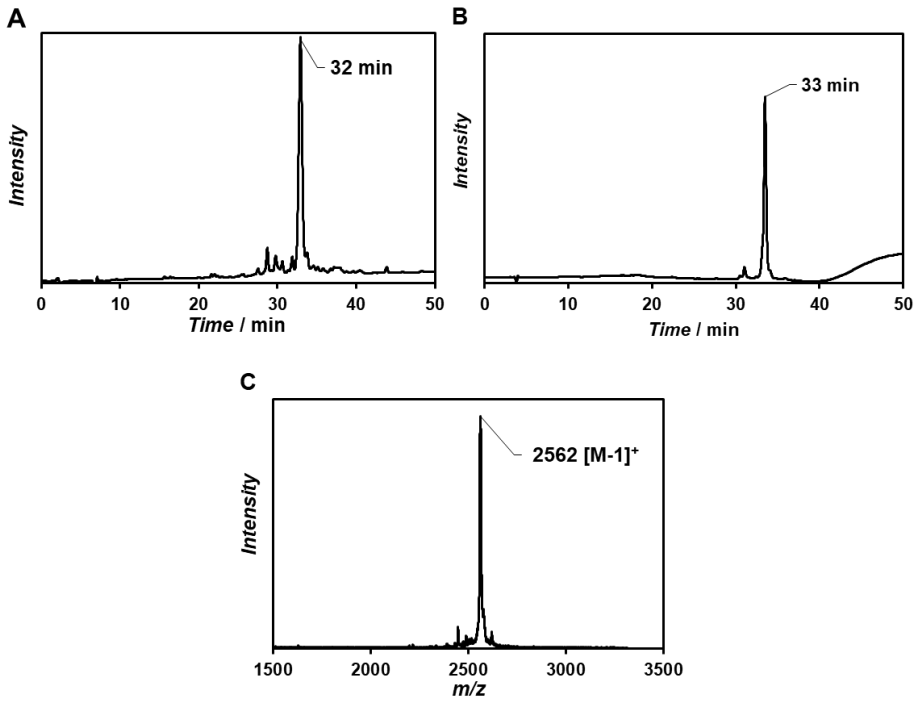

Figure S2. Reversed-phase HPLC chart of crude (A) and purified (B) β-annulus-EE peptide eluted with a linear gradient of CH_3CN/water containing 0.1% TFA (5/95 to 100/0 over 100 min), and MALDI-TOF-MS (C).

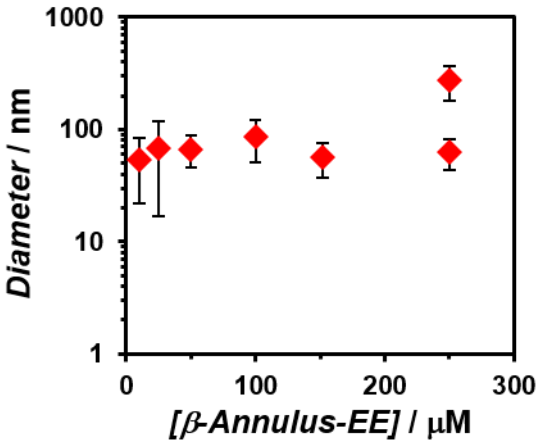

Figure S3. Concentration dependence of the size distribution obtained from DLS of *β*-annulus-EE peptide in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

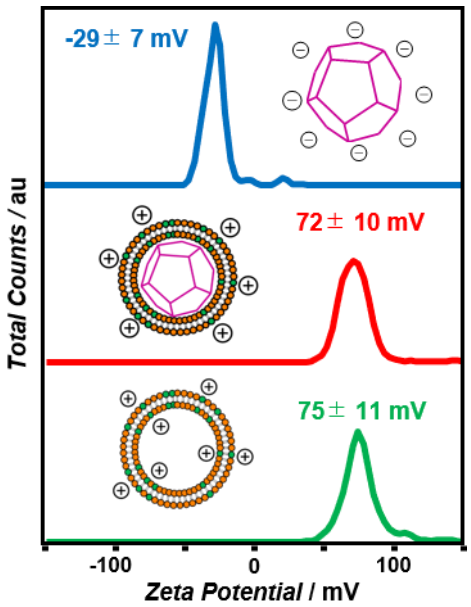

Figure S4. ζ-Potential of the artificial viral capsid bearing anionic surface (50 μM *β*-annulus-EE peptide, blue), enveloped viral capsid (50 μM *β*-annulus-EE peptide, 150 μM DOTAP and 1500 μM DOPC, red), liposome (150 μM DOTAP and 1500 μM DOPC, green) in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

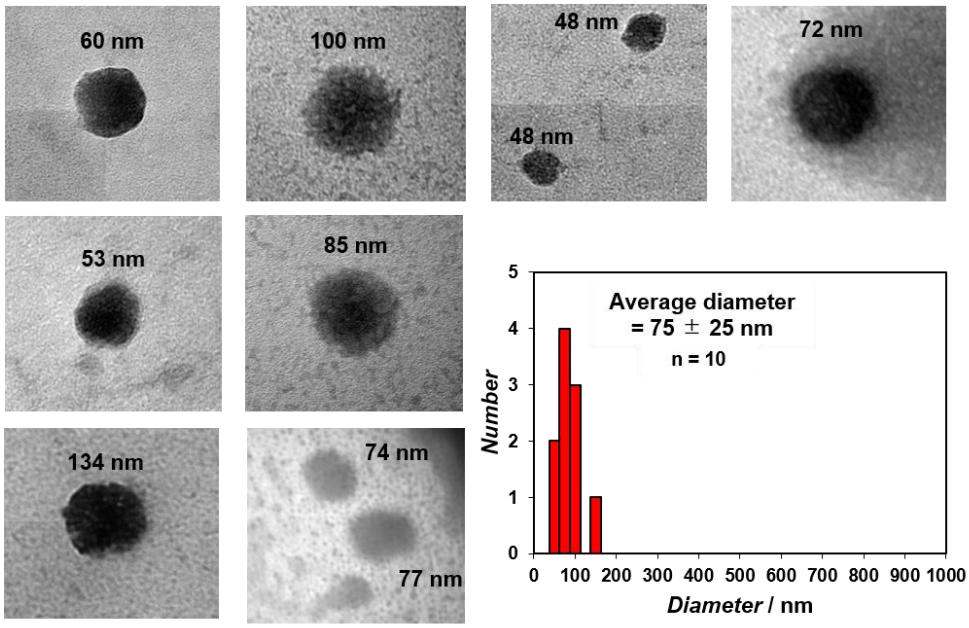

Figure S5. TEM images of the artificial viral capsid bearing anionic surface (50 μM *β*-annulus-EE peptide) and the size distribution.

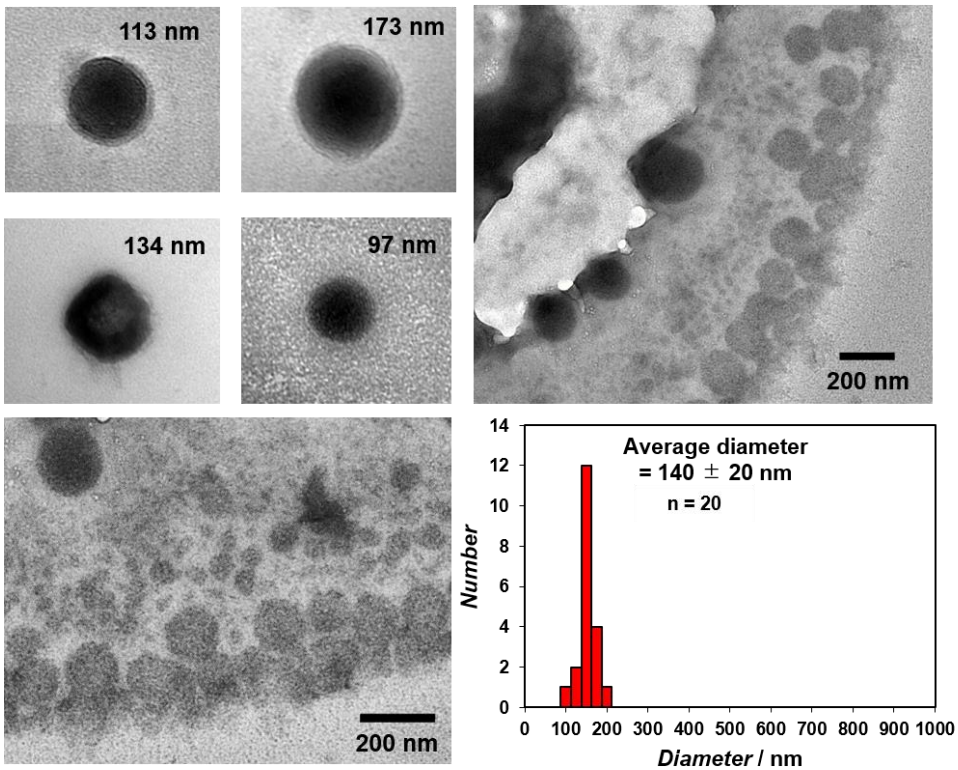

Figure S6. TEM images of enveloped artificial viral capsid (50 μM *β*-annulus-EE peptide, 150 μM DOTAP and 1500 μM DOPC) and the size distribution.

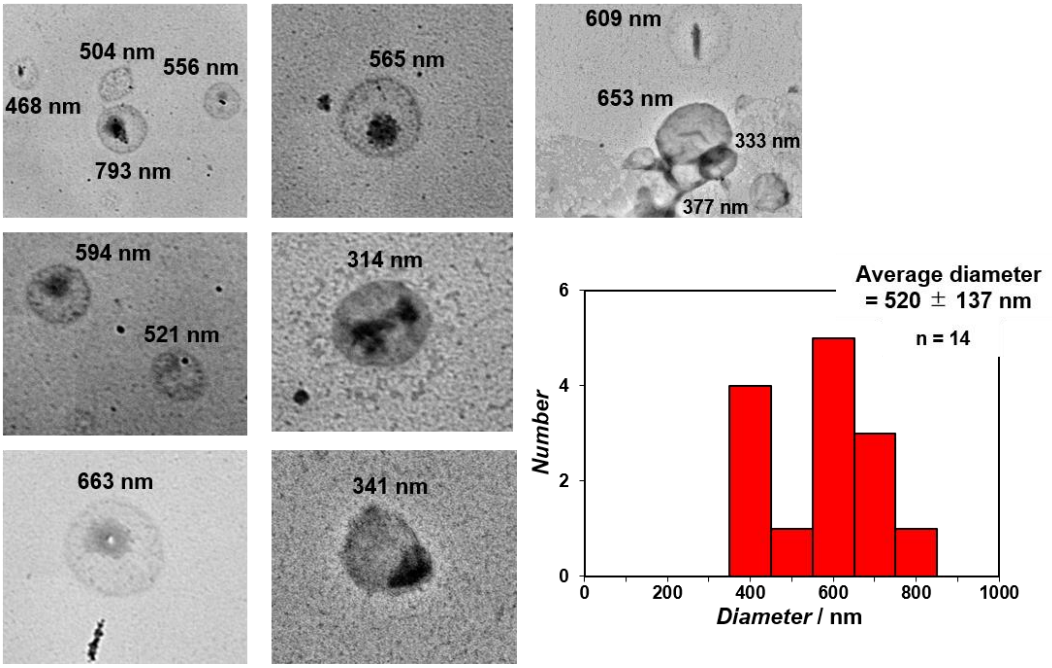

Figure S7. TEM images of liposome (150 μM DOTAP and 1500 μM DOPC) and the size distribution.

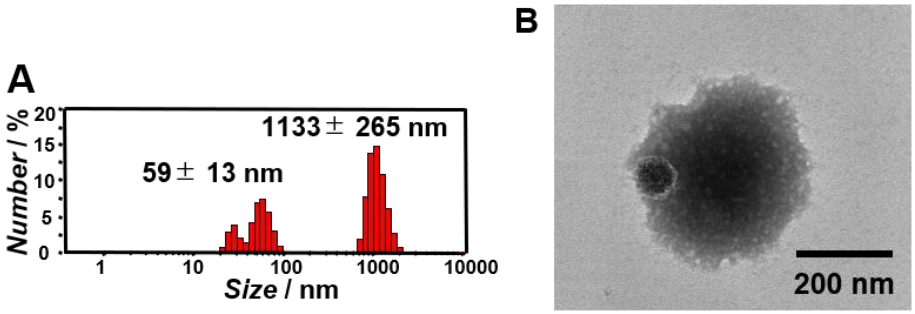

Figure S8. Size distribution obtained from DLS (A) and TEM image (B) of complex of anionic artificial viral capsid (50 μM *β*-annulus-EE peptide) with 1650 μM DOPC in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC. TEM sample was stained with sodium phosphotungstate.

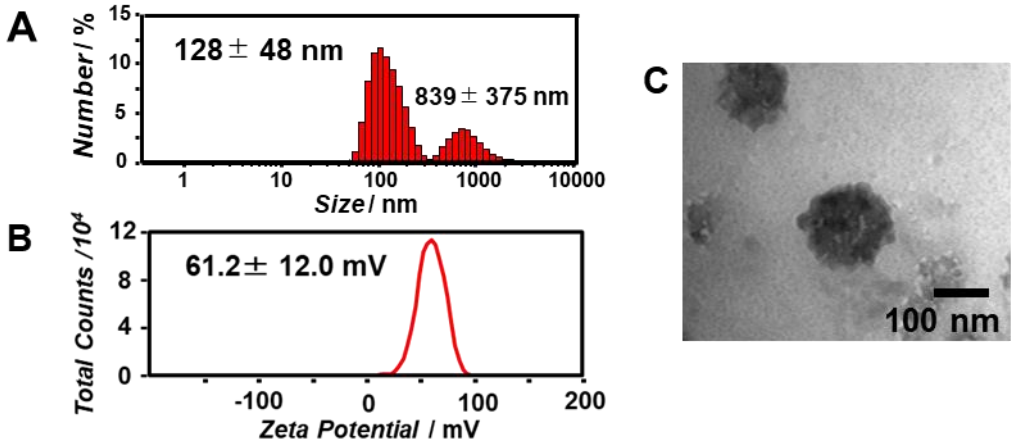

Figure S9. Size distributions obtained from DLS, ζ-potential and TEM image of enveloped artificial viral capsid complexing of 100 μM *β*-annulus-EE peptide with 300 μM DOTAP in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

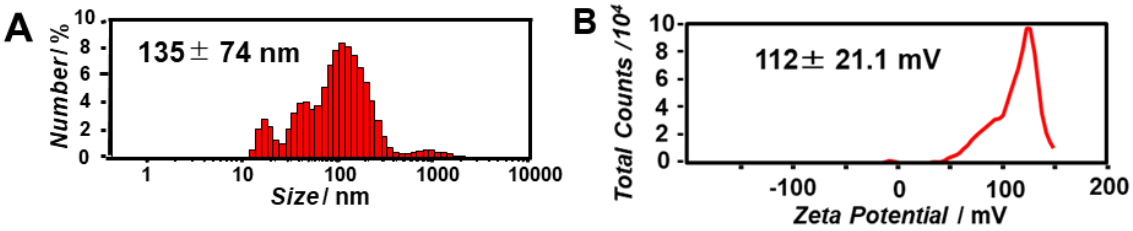

Figure S10. Size distribution obtained from DLS (A) and ζ-potential (B) of liposome consisting of 300 μM DOTAP in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

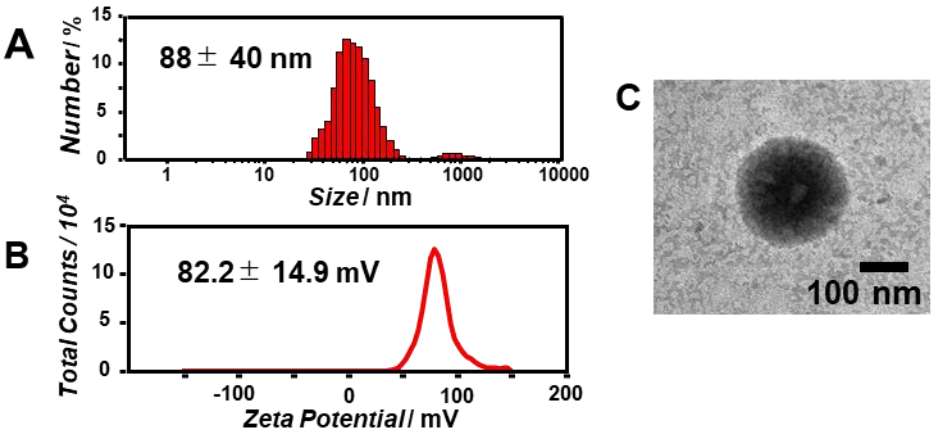

Figure S11. Size distribution obtained from DLS(A), ζ-potential (B) and TEM image (C) of enveloped artificial viral capsid consisting of 50 μM *β*-annulus-EE peptide, 150 μM DOTAP and 750 μM DOPC in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

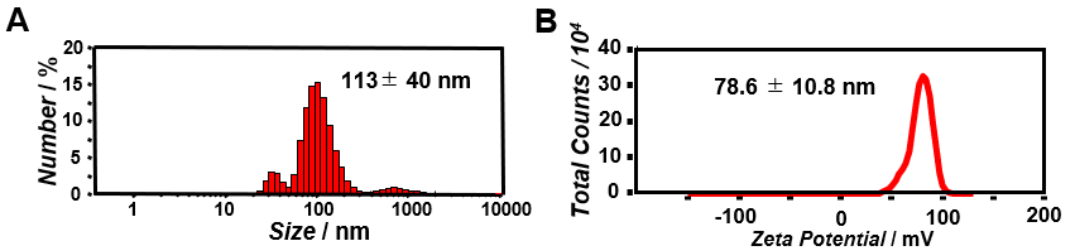

Figure S12. Size distributions obtained from DLS (A) and ζ-potential (B) of enveloped artificial viral capsid immediately after complexing of 50 μM *β*-annulus-EE peptide with 150 μM DOTAP and 1500 μM DOPC (before equilibration) in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

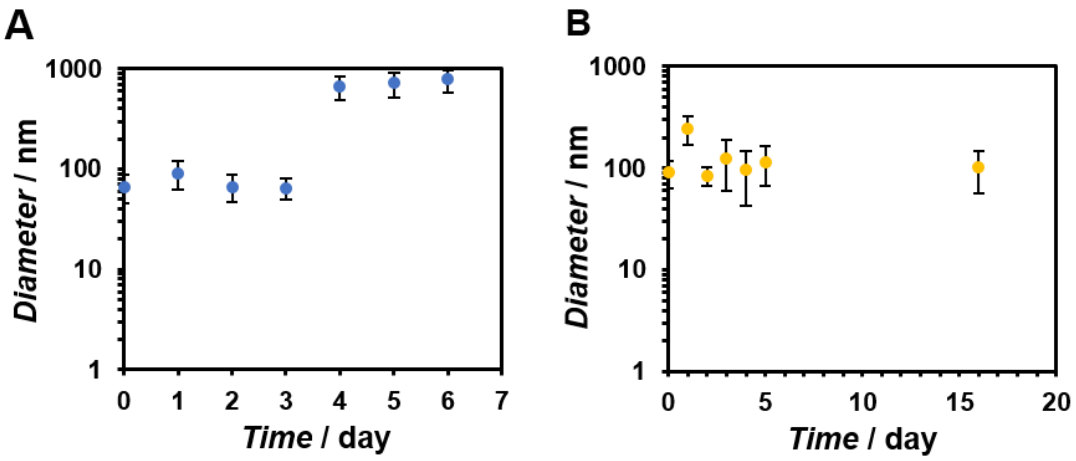

Figure S13. Time course of the size distribution of (A) 50 μM *β*-annulus-EE peptide and (B) enveloped artificial viral capsid ([*β*-annulus-EE] = 50 μM, [DOTAP] = 150 μM and [DOPC] = 1500 μM) in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

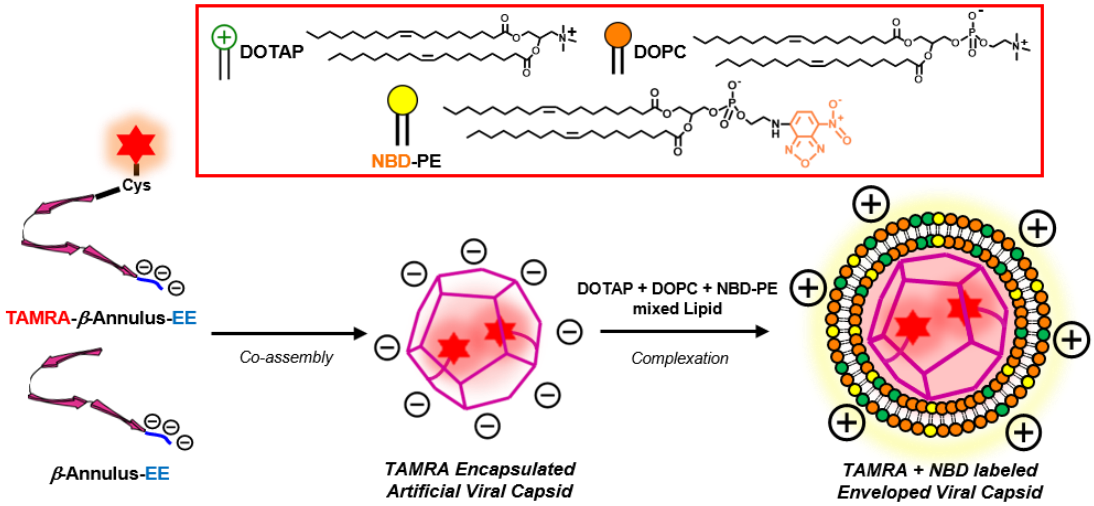

Figure S14. Schematic illustration of the construction of TAMRA/NBD-labelled enveloped artificial viral capsid.

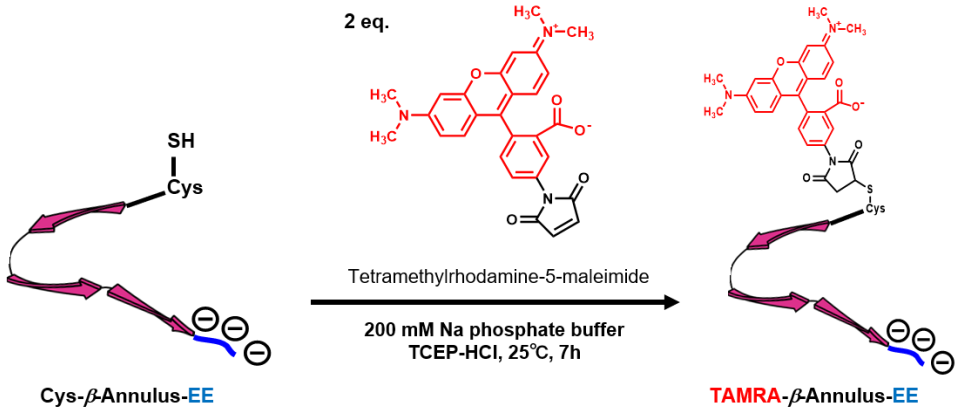

Figure S15. Synthesis of TAMRA-*β*-annulus-EE peptide.

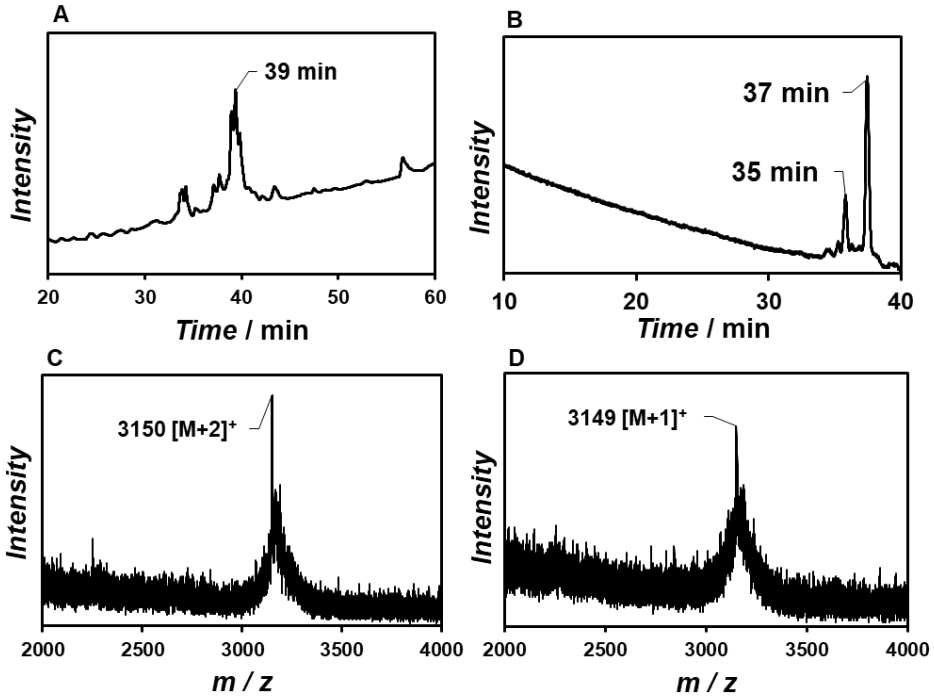

Figure S16. Reversed-phase HPLC chart of crude (A) and purified (B) TAMRA-*β*-annulus-EE peptide eluted with a linear gradient of CH_3CN/water containing 0.1% TFA (5/95 to 100/0 over 100 min), and MALDI-TOF-MS of peaks at 35 min (C) and 37 min (D). The two peaks at 35 and 37 min in chart (B) might be caused by equilibrium of opened-closed from of spirolactone ring of TAMRA.

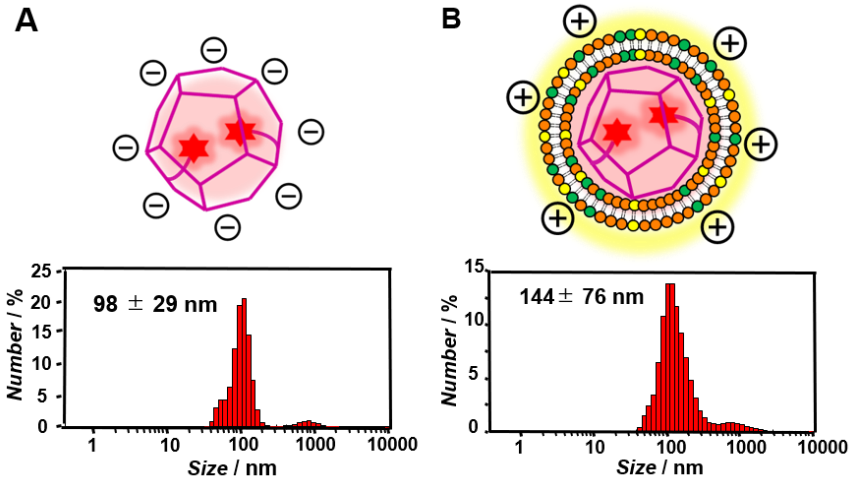

Figure S17. Size distributions obtained from DLS for (A) TAMRA-labelled artificial viral capsid (1 μM TAMRA-*β*-annulus-EE, 49 μM *β*-annulus-EE peptide), (B) TAMRA/NBD-labelled enveloped artificial viral capsid (1 μM TAMRA-*β*-annulus-EE, 49 μM *β*-annulus-EE peptide, 150 μM DOTAP, 1425 μM DOPC, 75 μM NBD-PE) in 10 mM Tris-HCl buffer (pH 7.0) at 25 ºC.

References

(1) *Principles of Fluorescence Spectroscopy*, J. R. Lakowicz, 3rd Ed. Springer, 2006.

(2) *Molecular Fluorescence: Principles and Applications*, B. Valeur, 2nd Ed. Wiley-VCH, 2012.

(3) P. E. Schneggenburger, S. Mullar, B. Worbs, C. Steinem, U. Diederrichsen, *J. Am. Chem. Soc*., 2010, **132**, 8020.