Electronic supplementary information (ESI)

of

Micro-size Cell-like Vesicles Based on Gemini-like Amphiphilic Peptide

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Experimental Section

Materials

N-Fluorenyl-9-methoxycarbonyl (FMOC) protected L-amino acids (Fmoc-Orn (Boc)-OH, Fmoc-Cys (Trt)-OH) and 2-chloritrityl chloride resin (100-200 mesh, loading 1.18 mmol/g) were purchased from GL Biochem Ltd. (China) and used as received. Piperdine, trifluoroacetic (TFA), o-benzotriazole-N, N, N′, N′-tetramethyluroniumhexafluorophosphate (HBTU), and N-hydroxybenzotriazole (HOBt) were provided by Shanghai Regent Chemical Co. (China) and used directly. N, N-dimethylformamide (DMF), and diisoproylethylamine (DIEA) were obtained from Shanghai Reagent Chemical Co. (China) and distilled prior to use. Oleic acid were purchased from Shanghai Reagent Chemical Co. (China) and used after recrystallization from ethanol. Triisopropylsilane (TIS) and dimethylsulphoxide (DMSO) were purchased from ACROS (USA) and used without further purification. Methanol and dichloromethane (DCM) were obtained from Shanghai Chemical Co. (China) and used after distillation. Toluene was purchased from shanghai Chemical Co. (China) and dried by distillation over sodium. (3-Aminnopropyl) trimethoxysilane (APS) and (3-Mercaptopropyl) trimethoxysilane (MPS) were purchased from ACROS (USA) and used directly. Silica wafers were purchased from Huihong Electricity Co. Ltd. (China). All other reagents and solvents were of analytical grade and were used directly.

Synthesis of unsaturated peptide amphiphile by solid-phase peptide synthesis

Peptide amphiphiles were manually synthesized on the 2-chlorotrityl chloride resin employing a standard Fmoc solid phase peptide synthesis (SPPS) protocols. Before the synthesis, the resin was washed with dichloromethane (DCM) (three times) and DMF (three times) and then immersed in DMF for 30min. Thereafter, a DMF solution of FMOC protected amino acid (4 equiv relative to resin loading) and DIEA (6 equiv) was added to the resin and shaken for 2 h at room temperature. After the coupling, the FMOC group was removed by 20% (v/v) piperidine/DMF twice. After stirring for 30

min at room temperature, the reaction solution was drained off and the resin was washed with DMF (three times). The presence of free amino groups was indicated by a blue color in the Kaiser test. Then, a DMF solution of the mixture of FMOC protected amino acid (2 equiv), HBTU (3 equiv), HOBt (3 equiv) and DIEA (4 equiv) was added. After shaking for 1.5 h at room temperature, the reaction solution was drained off and the resin was washed with DMF (three times). The absence of free amino groups was indicated by a yellow color in the Kaiser test. After the repetition of deprotection and acylation reaction, oleic acid was conjugated to the resin with HBTU and HOBt as activating agents finally. After completion of the synthesis, the resin was washed with DMF and DCM three times, respectively. The solvent of the resin was removed under vacuum for 24 h (Scheme S1A).

Synthesis of unsaturated gemini-like amphiphilic peptides (u-GAPs)

Cleavage of the expected peptide amphiphiles and removal of side chain protected groups from the dried resin were performed using a cleavage cocktail comprised of TFA, TIS and deionized water in the ratio of 95:4:1. After shaking at room temperature for 2 h, the cleavage mixture and subsequently TFA washing were collected. Then the total 22.5% (v/v) DMSO was added into the combined solution in twice at 4 h intervals, directly oxidizing -SH to form disulfide bond, which connected two single peptide amphiphiles to form u-GAPs (Scheme S1B). After shaking for 24 h, the final mixture solution was concentrated to viscous solution by rotary evaporator. The remaining viscous u-GAPs solution was precipitated with cold ether. After washing with cold ether (five times), the resulting white product was collected and vacuum dried. Then, the product was dissolved in deionized water and was freeze-dried under vacuum for 3 days.

ESI-MS (LCQ Advantage, Finigan, USA, Fig. S1). $(C_{18}\text{-C-}O_3)_2$: Calcd. 1457; Found 729.7 ($[M+2H]^{2+}$) (Fig. S2A); $(C_{18:1}\text{-C-}O_3)_2$: Calcd. 1453; Found 727.8 ($[M+2H]^{2+}$), 1456.9 ($[M+H]^{+}$) (Fig. S2B); $(C_{18:2}\text{-C-}O_3)_2$: Calcd. 1449; Found 725.3 ($[M+2H]^{2+}$), 1448.6 ($[M+H]^{+}$) (Fig. S2C)

Purity analysis. The purity of these obtained peptides was determined by high-pressure liquid chromatography (HPLC, Prominence LC-20A, Shimadzu, Japan) with a C18 reversed-phase column

by using a linear gradient from 100% to 5% H2O/acetonitrile containing 0.1% TFA in 1 ml/min for 18 min. The data was shown in Fig. S3.

Self-assembly of u-GAP building blocks in the bulk solution

These lyophilized GAPs were dispersed rapidly in distill water with 0.5 mg/ml at 25 °C to form optically clear dispersions and these dispersions were cultivated for various times to determine the dynamic changes of these assemblies. Additionally, the experiments at basic medium by were also carried out to study the effect of pH on the self-assembly of GAPs.

Critical micelle concentration (CMC) determination

Fluorescence spectra were recorded on a LS55 luminescence spectrometer (PerkinElmer). Pyrene was used as a hydrophobic fluorescent probe. Aliquots of pyrene solution (1.2×10^{-7} mol ml^{-1} in acetone, 50 μL) were added to containers, and the acetone was allowed to evaporate. One milliliter aqueous solution of GAP with a particular concentration was added to the container, which contained the pyrene residue. The aqueous sample solutions containing pyrenet residue at the same concentration of 6×10^{-6} M were kept at room temperature for 24 h to reach the solubilization equilibrium of pyrene in the aqueous phase. Emission wavelength was carried out at 393 nm, and excitation spectra were recorded ranging from 300 to 360 nm. Both excitation and emission slit widths were 10 nm. From the pyrene excitation spectra, the intensity ratio I_{338}/I_{335} was analyzed as a function of logarithm of the GAP concentration. A CMC value was determined from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentration[1].

Transmission electron microscopy (TEM)

5~10 μl aliquot of the peptide solution was placed on a copper grid. After several min, excess fluid was removed and a 1~2 μl aliquot of phosphotungstic acid negative stain solutions was dropped on the copper grid. After two min, excess negative stain solutions were removed and then the copper grid was dried in air. Samples were observed by using a TEM (FEI Tecnai G2 20 TWIN) with an acceleration voltage of 200 kV.

Differential scanning calorimeter (DSC)

Before the measurement, the peptide solution was rapid-freezing by the liquid nitrogen and was freeze-dried in a Freeze Drier (Labconco) under vacuum. The thermograms were recorded on a DSC (Pyris 1, Perkin-Elmer) under dry nitrogen and the scan speed for the heating cycle was 10 °C/min.

Circular dichroism spectrum (CD)

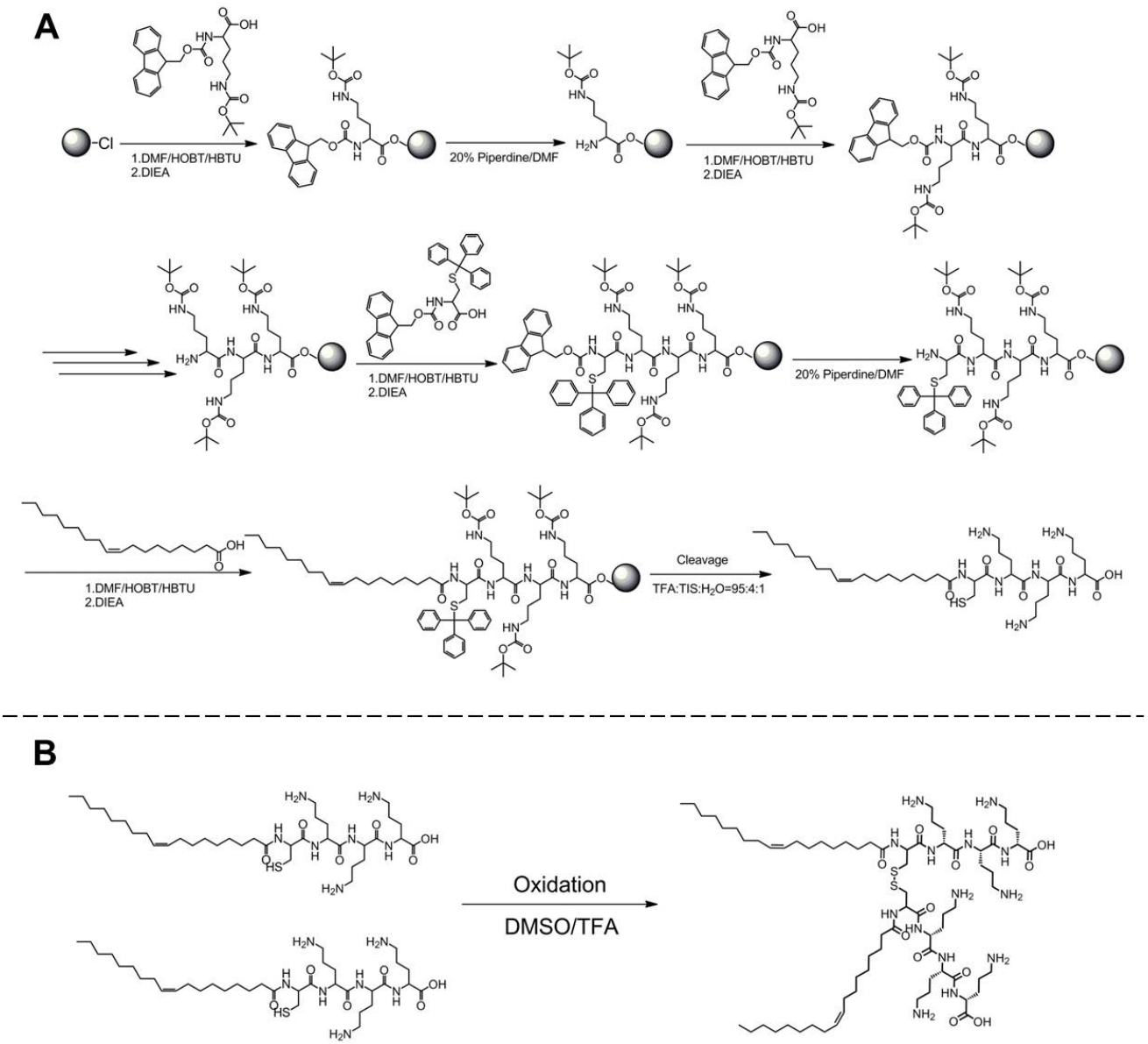
The peptide solution was fixed in a 0.5 mm quartz cell and CD was recorded on a Jasco J-810 spectropolarimeter (Jasco, Japan).

Fourier transform infrared spectroscopy (FT-IR)

The peptide solution was rapid-freezing by the liquid nitrogen and was freeze-dried in a Freeze Drier (Labconco) under vacuum. Then the sample was pressed into potassium bromide (KBr) pellets and FT-IR measurement was performed on a FT-IR spectrophotometer (Perkin-Elmer Spectrum One).

2. References

1. H. Wei, C. Cheng, C. Cong, W. Q. Chen, S. X. Cheng, X. Z. Zhang, R. X. Zhuo, Langmuir 2008, 24, 4564-4570.



Scheme S1 The synthesis of u-GAPs. A) The corresponding unsaturated single amphiphile, $C_{18:1}$-C-O_3, was synthesized by the solid phase peptide synthesis (SPPS) technique. B) U-GAP, $(C_{18:1}\text{-C-}O_3)_2$, was synthesized through the formation of disulfide bonds between $C_{18:1}$-C-O_3S in the cleavage mixeture by using the method of DMSO-TFA oxidation.

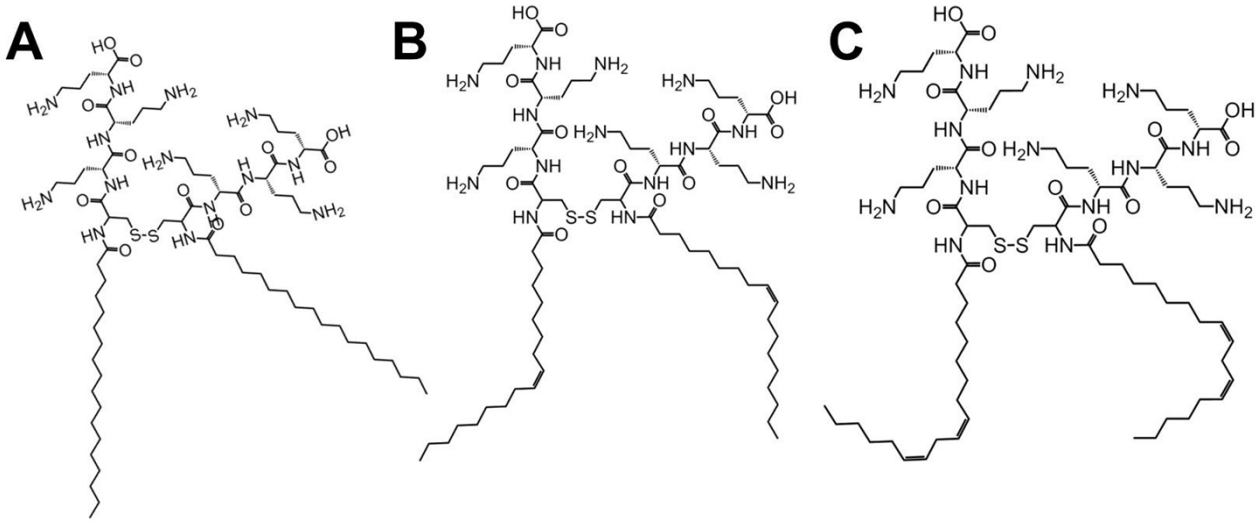


Figure S1. Molecular structures of GAPs. A) s-GAP; B) o-GAP; C) l-GAP.

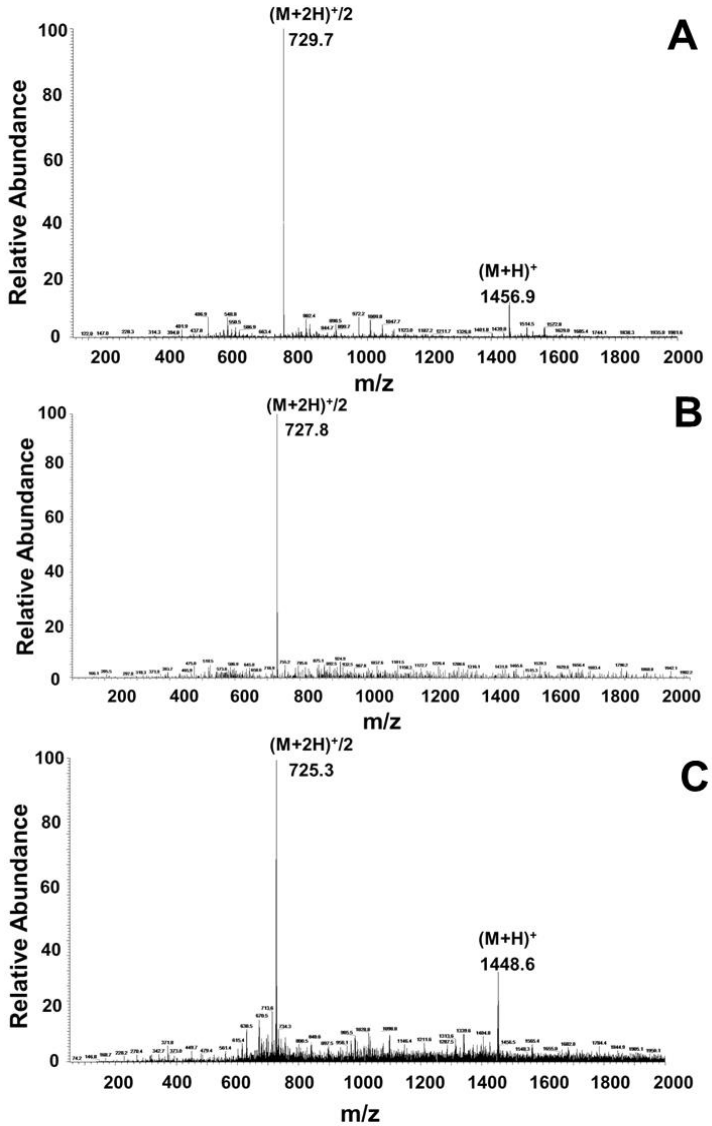


Figure S2. ESI-MS profiles of GAPs: A) s-GAP; B) o-GAP; C) l-GAP.

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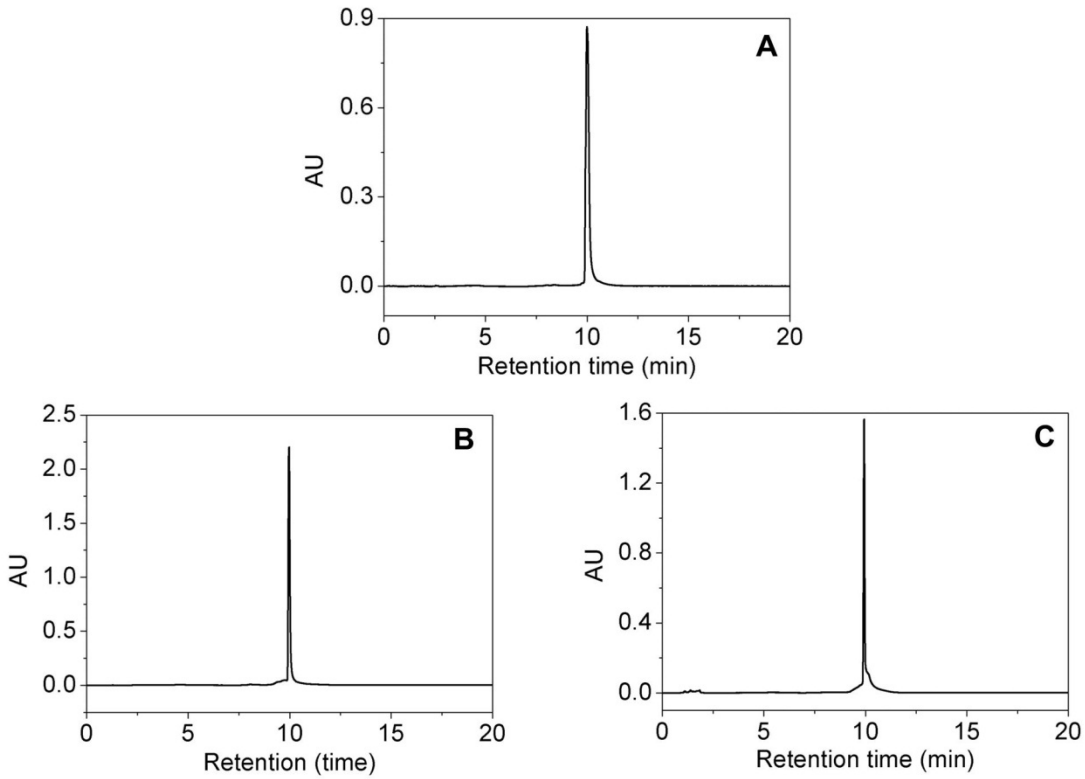

Figure S3. HP-LC analysis of GAPs: A) s-GAP; B) o-GAP; C) l-GAP.

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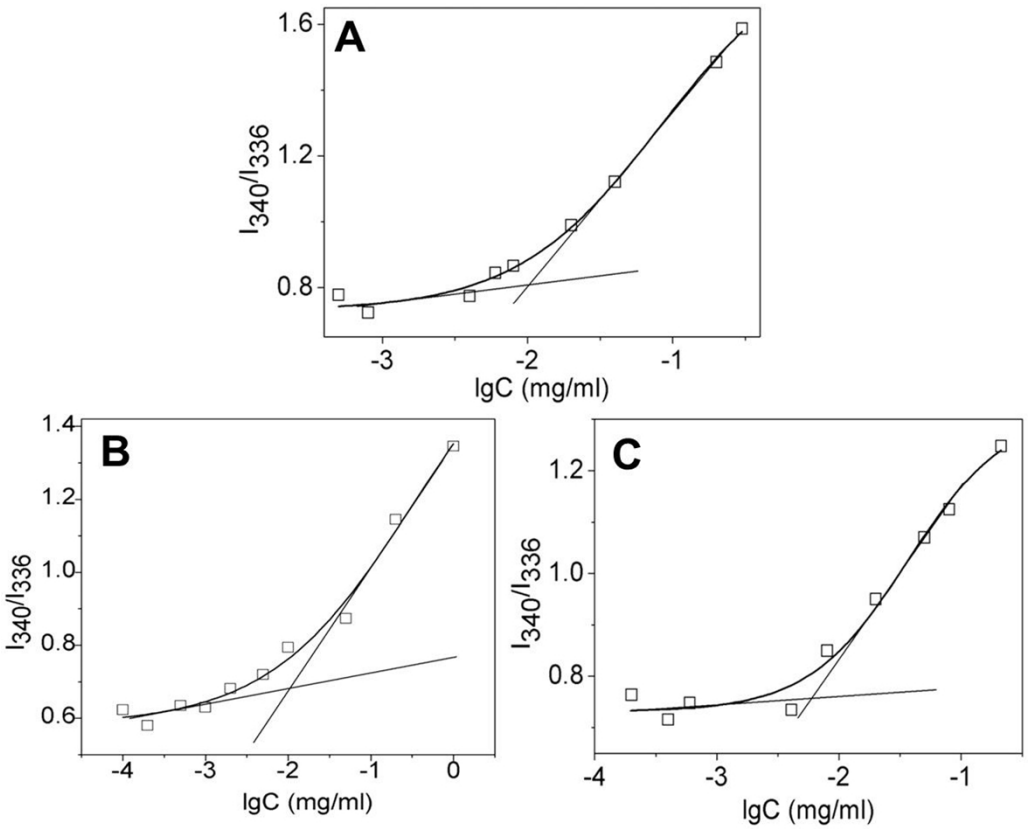

Figure S4. The intensity ratio I_{338}/I_{335} in the excitation spectrum as a function of logarithm of GAPs concentration. A) s-GAP; B) o-GAP; C) l-GAP.

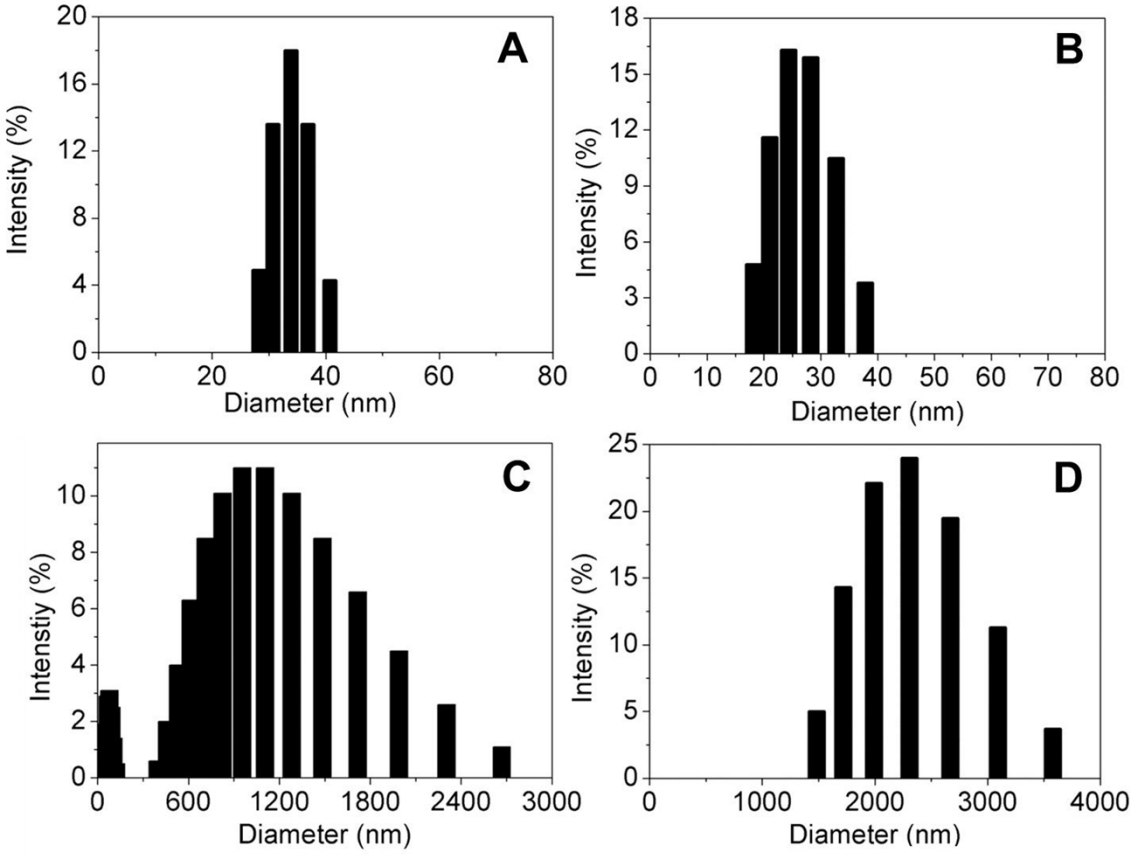

Figure S5. Size distribution of assembled vesicular structures. A) formed by s-GAPs; B~D) formed by o-GAPs: B) at begin; C) after 2 h; D) after 4 h.

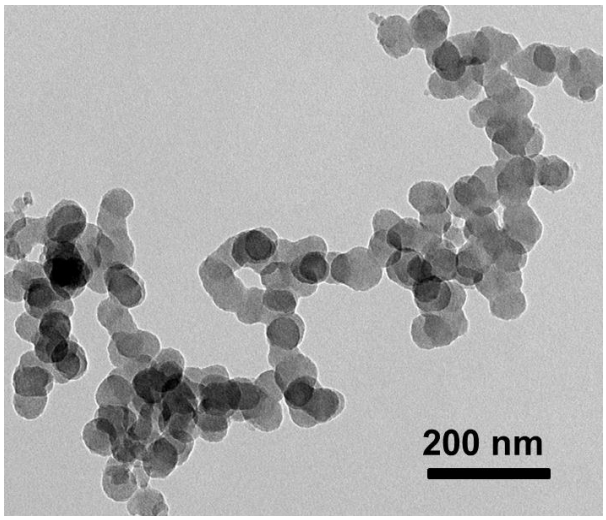

Figure S6. TEM image of adhesive and fused small nanovesicles formed by o-GAPs.

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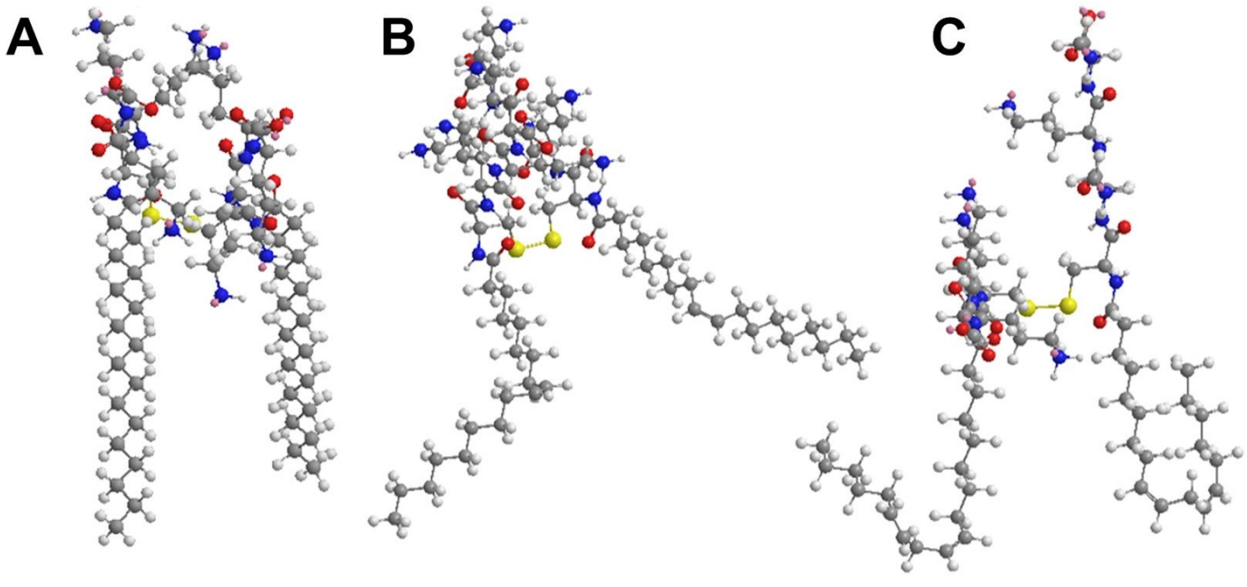

Figure S7. Molecular modeling structures of A) s-GAP; B) o-GAP; C) l-GAP.