

Supporting Information

Vesicular Self-assembly of Copolymer-grafted Nanoparticles with Anisotropic Shapes

1. Materials

Styrene (St), 2,2-Azobis(2-Methylpropionitrile) (AIBN), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPPA), *N,N'*-Dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), Hexadecyltrimethylammonium Chloride (CTAC, $\geq 98\%$), Gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, $\geq 99.9\%$ trace metals basis), Sodium bromide (NaBr, $\geq 99.0\%$), Ascorbic acid (AA, $\geq 99.0\%$), Sodium borohydride (NaBH_4 , 98%), tetrahydrofuran (THF), and *N,N*-dimethylformamide (DMF) were all obtained from Sigma-Aldrich. Styrene (St) were distilled under vacuum before use and stored in fridge at $-20\text{ }^\circ\text{C}$. 2,2-Azobis(2-Methylpropionitrile) (AIBN) was recrystallized twice from ethanol before use. The macro-(reversible addition fragmentation chain transfer) RAFT chain transfer agent (CTA), PEO_{45} -CTA, was synthesized by coupling poly(ethylene oxide) methyl ether (PEO, molecular weight of $2\text{ kg}\cdot\text{mol}^{-1}$) with 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPPA) through esterification reaction. Deionized water (Millipore Milli-Q grade) with resistivity of $18.0\text{ M}\Omega$ was used in all the experiments.

2. Synthesis of thiol-terminated *di*-block copolymers (BCPs)

PEO_{45} -*b*- PS_x -SH with different molecular weights were synthesized using reversible addition-fragmentation chain transfer (RAFT) polymerization reported previously (Figure S1)¹. Chain transfer agent (PEO-CTA) was synthesized by attaching CPPA to the poly(ethylene oxide) methyl ether through esterification. PEO_{45} -*b*- PS_x -CTA were prepared by RAFT polymerization. Briefly, styrene, PEO-CTA and AIBN were dissolved in dioxane. The solution was purged with argon for 30 min before the reaction was put into an $80\text{ }^\circ\text{C}$ oil bath. About 36 h later, the reaction was quenched by placing the solution in liquid nitrogen. The product was precipitated in hexane and dissolved in THF to remove unreacted monomers and impurities. This purification step was repeated for three times. By varying the amount of PEO-CTA, BCPs of PEO_{45} -*b*- PS_x -SH with different PS block lengths were synthesized. The PEO_{45} -*b*- PS_x -CTA was mixed with *n*-butylamine for 4 hours to convert the dithioester terminal into thiol groups. The product was purified by precipitating it in cold hexane for three times to remove excess *n*-butylamine and was subsequently dried under

vacuum at room temperature overnight. Molecular weights of polymers were characterized by gel permeation chromatography (GPC) and ^1H NMR.

3. Synthesis of Au nanocubes (Au NCs) and octahedrons

AuNCs and Au octahedrons were synthesized via a modified seed-mediated growth method reported previously.² The synthetic procedures are briefly described as follows.

3.1 Synthesis of Au nanocubes (Au NCs)

3.1.1 Synthesis of Au NCs seeds. In a 20 mL beaker, 0.32 g of CTAC was firstly dissolved in 5 mL water, and 5 mL of HAuCl_4 with a concentration of 0.5 mM was then added into the solution under magnetic stirring. Subsequently, 0.45 mL of freshly prepared ice-cold aqueous solution of NaBH_4 (0.02 M) was injected into the mixed solution. The seed solution turned brown immediately and was kept in 30 °C water bath for 1 h.

3.1.2 Synthesis of Au NCs with ~80 nm diameter. Growth solution A was prepared by adding 0.32 g CTAC in 9.5 mL of aqueous solution, followed by the addition of 0.25 mL of 10 mM HAuCl_4 solution, 0.1 mL of 1 mM NaBr solution, and 0.09 mL of 0.1 M ascorbic acid solution under magnetic stirring. Growth solution B was prepared by adding 1.6 g CTAC in 47.8 mL water, followed by the addition of 1.25 mL of 10 mM HAuCl_4 solution, 0.5 mL of 1 mM NaBr solution, and 0.45 mL of 0.1 M ascorbic acid solution. Both growth solution A and B were kept in a 30 °C water bath. About 10 min later, 0.025 mL of the seed solution was quickly added to the growth solution A and shook for ~10 s. Then 0.125 mL of solution A was added to the growth solution B with shaking ~15 s. The solution B was then left undisturbed for 20 min and collected by centrifugation.

3.1.3 Synthesis of Au NCs with ~52 nm diameter. Growth solution A was prepared by adding 0.32 g CTAC in 9.5 mL water, followed by the addition of 0.25 mL of 10 mM HAuCl_4 solution, 0.1 mL of 1 mM NaBr solution, and 0.09 mL of 0.1 M ascorbic acid solution under magnetic stirring. Growth solution B was prepared by adding 1.6 g CTAC in 47.6 mL water, followed by the addition of 1.25 mL of 10 mM HAuCl_4 solution, 0.5 mL of 1 mM NaBr solution, and 0.45 mL of 0.1 M ascorbic acid solution. Both growth solution A and B were kept in a 30 °C water bath. About 10 min later, 0.045 mL of seed solution was quickly added to the growth solution A with shaking ~10 s. Then 0.225 mL of solution A was added to the growth solution B and shook for ~15 s. The solution B was then left undisturbed for 20 min and collected by centrifugation.

3.2 Synthesis of Au octahedrons with ~50 nm edge length. Growth solution A was prepared by adding 0.32 g CTAC in 9.5 mL water, followed by the addition of 0.25 mL of 10 mM HAuCl₄ solution, 0.1 mL of 1 mM NaBr solution, and 0.15 mL of 0.1 M ascorbic acid solution with strong magnetic stirring. Growth solution B was prepared by adding 1.6 g CTAC in 47.6 mL water, followed by the addition of 1.25 mL of 10 mM HAuCl₄ solution, 0.5 mL of 1 mM NaBr solution, and 0.75 mL of 0.1 M ascorbic acid solution. Both growth solution A and B were kept in a 30 °C water bath. About 10 min later, 0.045 mL of seed solution was quickly added to the growth solution A and shook for ~10 s. Then 0.225 mL of solution A was added to the growth solution B and shook for ~15 s. The solution B was then left undisturbed for 20 min and collected by centrifugation.

4. Surface modification and Self-assembly of Au NCs

AuNCs were modified by thiol-terminated BCPs using an interfacial ligand exchange method.³ In brief, 2.5 mg of PS-*b*-PEO-SH was dissolved in 10 mL of DMF. 5 mg Au NCs was concentrated into 0.3 mL aqueous solution by centrifugation. The concentrated Au NCs solution was slowly added into the BCPs solution under ultrasonic. The mixture was then sonicated for 1 h and kept undisturbed for 8 h. The solution was centrifuged 6 times in THF to remove free polymers in solution and finally re-dispersed in 4 mL THF as the stock solutions. Self-assembly of Au NCs was carried out by dialyzing the stock solution against deionized water under slight stirring for 24 h.

5. Characterization

¹H NMR spectra were recorded using Bruker AV-400 MHz high resolution NMR spectrometer. All copolymers were dried under vacuum at 80 °C overnight to remove trace amount of solvent. The NMR samples were prepared by dissolving polymers in deuterated reagents.

GPC measurements were performed on an Agilent GPC 1260 equipped with a refractive index detector. PS standards were used for molecular weight and molecular weight distribution calibration, and THF was used as the elution solvent with a flow rate of 0.8 mL/min.

UV-vis Absorption Spectroscopy was measured in a range of 400-1100 nm using a Perkin-Elmer Lambda 750 system.

Thermogravimetric Analysis (TGA) was used to characterize the copolymers grafting density on the surface of Au NCs by METTLER TOLEDO TGA1. The experiments were carried under nitrogen flow, with a scan rate of 25 °C·min⁻¹ from room temperature to 600 °C. During the scan process, the temperature was maintained at 100 °C for 10 min to further remove moisture.

The morphologies of assembled vesicles were imaged using a Gemini Ultra55 (Zeiss) SEM and a HT7800 (Hitachi) TEM. Samples for SEM observations were prepared by dropping 10 μL of sample solution onto silicon wafers and dried at room temperature. The TEM samples were prepared by casting $\sim 1.0\text{-}1.5$ μL of sample solution onto copper grids covered with carbon film, and drying them under a stream of dry air at room temperature.

6. Polymer grafting density calculation

Based on the measured weight loss f by TGA, the grafting density (σ , chains $\cdot\text{nm}^{-2}$) of copolymers on AuNCs was estimated by the equation:⁴

$$\sigma = \frac{f}{1-f} \cdot \frac{N_A \rho D}{6M_n}$$

where f is the weight fraction of the polymeric ligands determined by TGA, N_A is the Avogadro constant, ρ is the bulk density of Au core material ($19.32 \text{ g}\cdot\text{cm}^{-3}$), D is the edge length of NCs, and M_n is the number-average molecular weight of the copolymer ligands the BCP, determined by ^1H NMR. In the above estimation, we assumed that the density of the inorganic NP core is identical to the density of its bulk counterpart and no free polymer is present in the system.

From the calculated σ , the average distance (d) between anchoring points of adjacent ligands can be estimated by using equation⁴

$$d = \frac{2}{\sqrt{\pi\sigma}}$$

where σ is estimated from TGA. The footprint of a single copolymer ligand on NP surface is considered as a circular area with a diameter equal to d .

The root-mean-square end-to-end distance of PS block (R_0) in corresponding copolymer can be calculated by the following equation⁴

$$R_0 = bN^{0.5}; N = M_n(\text{PS})/M_0$$

where b is the Kuhn length ($b = 1.8 \text{ nm}$ for PS), N is the number of Kuhn segments calculated from the molar mass of Kuhn monomer M_0 ($M_0 = 720 \text{ g/mol}$ for PS), and $M_n(\text{PS})$ is the number-average molecular weight of PS block.

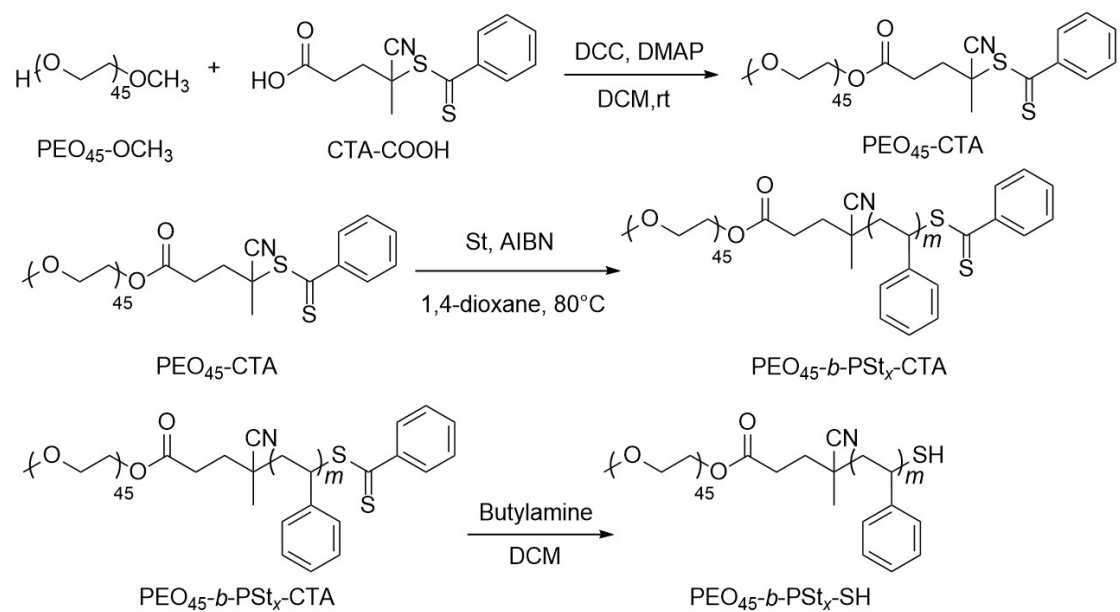


Figure S1. The synthetic route of thiol-terminated PEO₄₅-*b*-PS_{*x*}-SH.

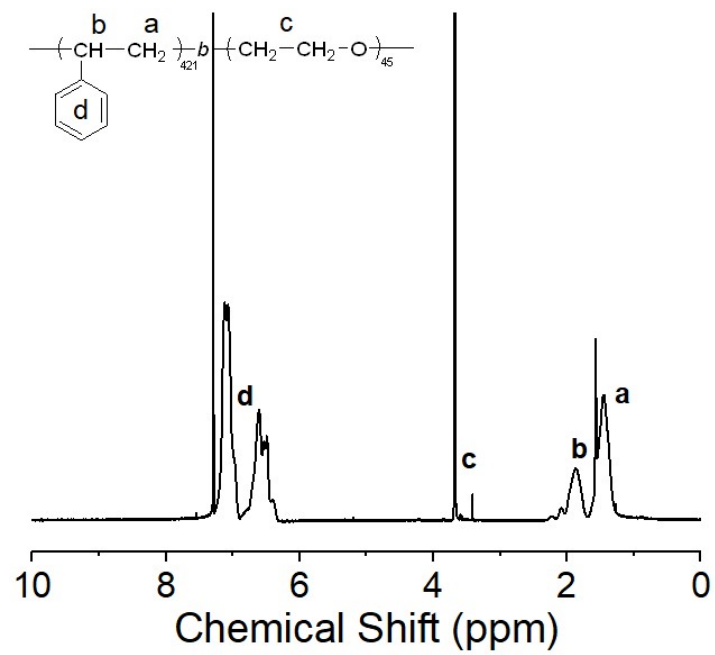


Figure S2. ^1H NMR spectrum of $\text{PEO}_{45}\text{-}b\text{-PS}_{421}\text{-SH}$ in CDCl_3 .

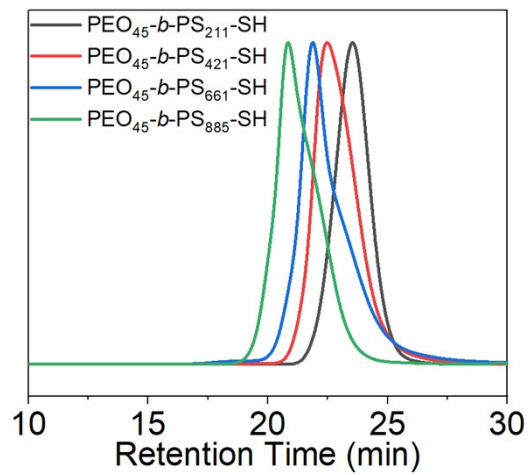


Figure S3. The GPC elution curves of representative PEO₄₅-*b*-PS_{*x*}-SH BCPs.

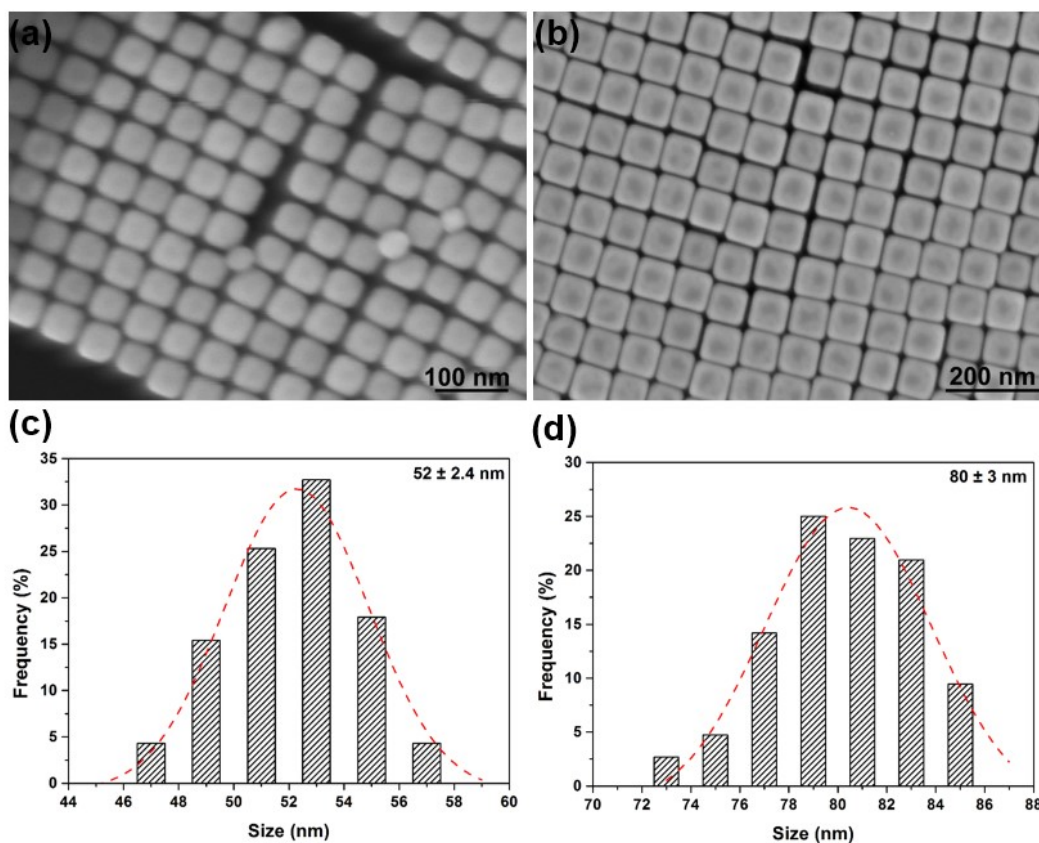


Figure S4. Representative SEM images of Au nanocubes with different edge length (D) and corresponding size distribution histogram: $D = 52$ nm (a, c), $D = 80$ nm (b, d). Red dashed lines show the Gaussian fit to the size distribution.

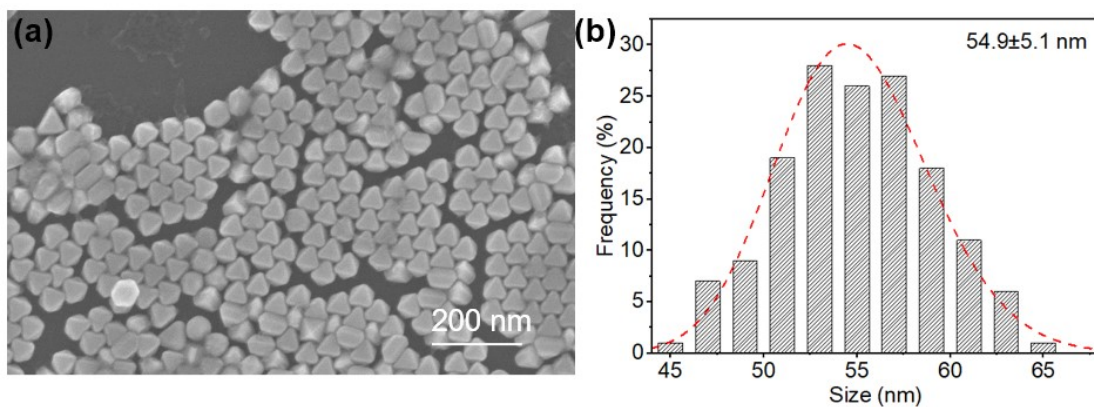


Figure S5. Representative SEM images (a) and size distribution histogram (b) of Au octahedrons. Red dashed lines show the Gaussian fit to the size distribution.

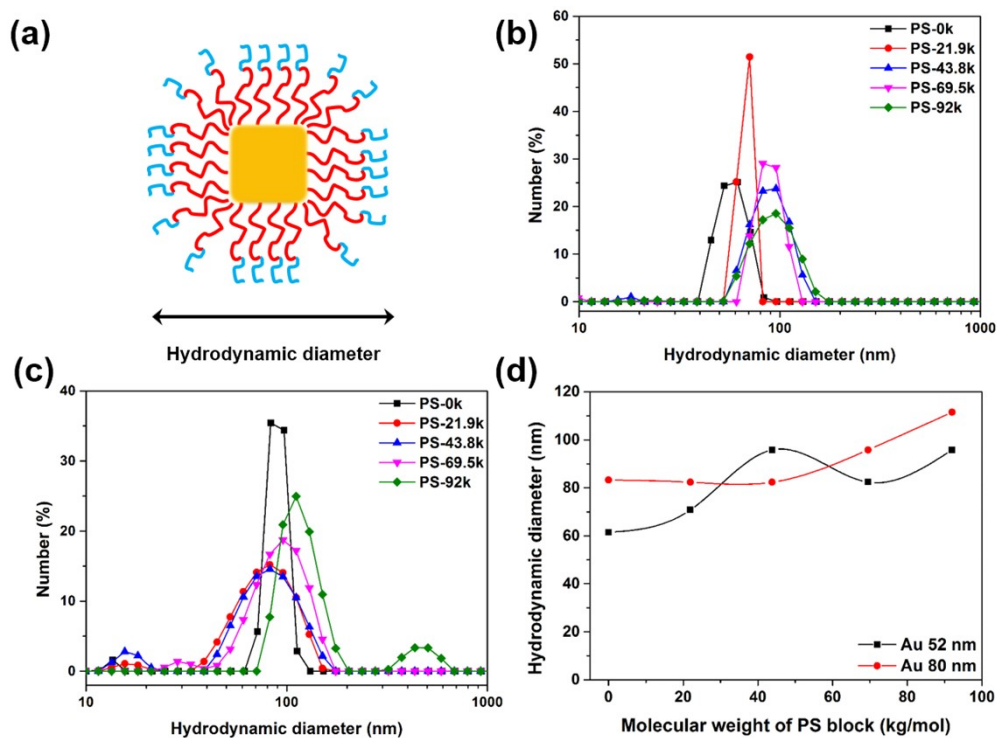


Figure S6. (a) Schematic illustration of $\text{PEO}_{45}\text{-}b\text{-PS}_x\text{-SH}$ BCPs-grafted Au NCs. (b) The hydrodynamic diameter of BCPs-grafted ~ 52 nm Au NCs with different PS lengths (PS-21.9k ($x=211$), PS-43.8k ($x=421$), PS-69.5k ($x=668$), and PS-92k ($x=885$)) (c) The hydrodynamic diameter of BCPs-grafted ~ 80 nm Au NCs with different PS lengths (PS-21.9k ($x=211$), PS-43.8k ($x=421$), PS-69.5k ($x=668$), and PS-92k ($x=885$)). (d) The dependency of hydrodynamic diameter of BCPs-grafted Au NCs on the M_n of PS block.

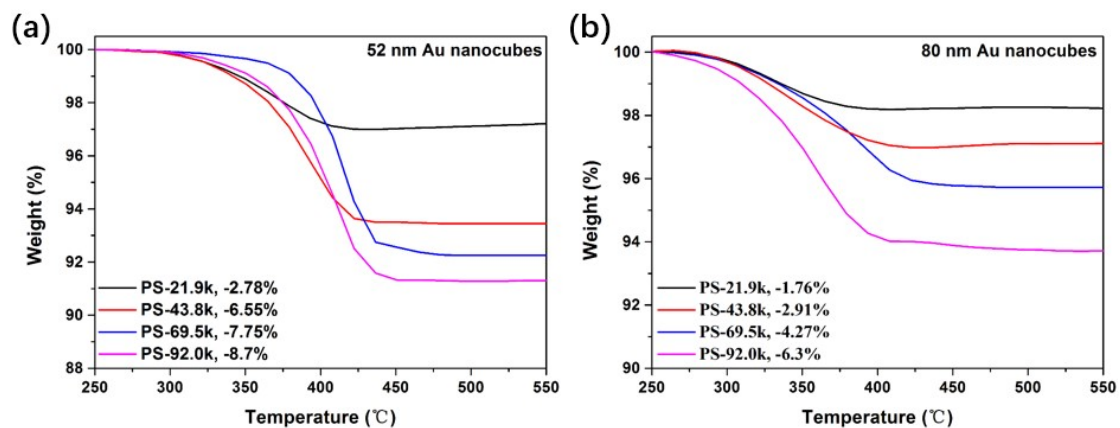


Figure S7. TGA curves of (a) ~52 nm and (b) ~80 nm Au NCs grafted with PEO₄₅-*b*-PS_{*x*}-SH BCPs of different PS lengths (PS-21.9k (*x*=211), PS-43.8k (*x*=421), PS-69.5k (*x*=668), and PS-92.0k (*x*=885)).

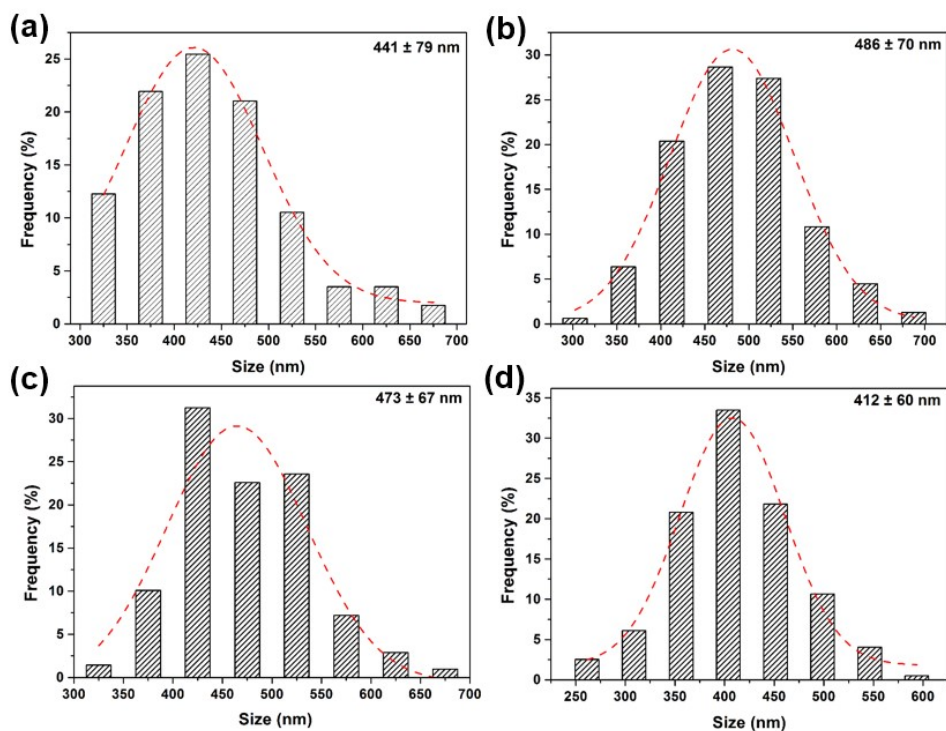


Figure S8. Size distributions of vesicles assembled from ~80 nm Au NCs grafted with PEO₄₅-b-PS_x-SH of different PS block lengths: (a) PS-21.9k ($x=211$), (b) PS-43.8k ($x=421$), (c) PS-69.5k ($x=668$), and (d) PS-92k ($x=885$). Red dashed lines show the Gaussian fit to the size distribution.

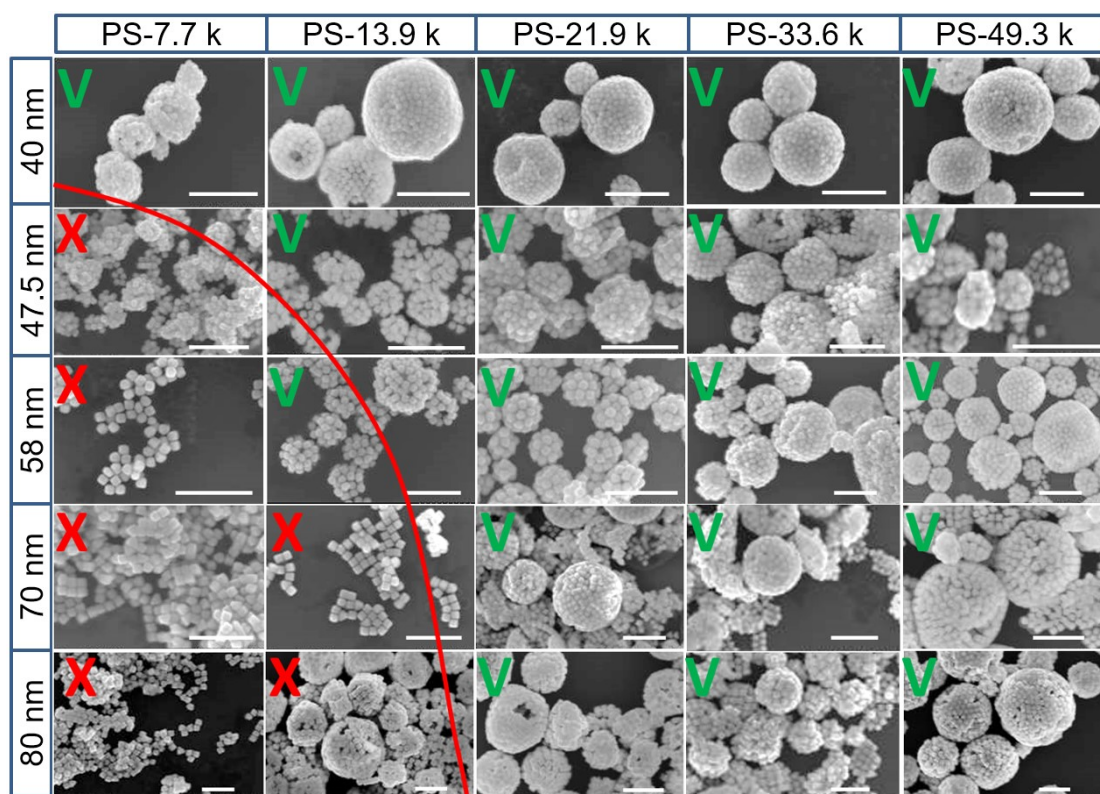


Figure S9. Vesicles or clusters assembly from Au NCs with different sizes (40 nm, 47.5 nm, 58 nm, 70 nm and 80 nm) modified by PEO₄₅-*b*-PS_{*x*}-SH BCPs with different PS block lengths (PS-7.7k (*x*=74), PS-13.9k (*x*=133), PS-21.9k (*x*=211), PS-33.6k (*x*=323), and PS-49.3k (*x*=473)). Red X means no vesicle formed while green V means vesicles formed in the condition. Scale bars in all images are 500 nm.

Table S1. Characterization of amphiphilic BCPs PEO₄₅-*b*-PS_{*x*}-SH.

Sample	Compositions	M_n (kg/mol)		PS length (kg/mol)	D_m	R_0^c (nm)
		GPC ^a	NMR ^b			
BCP-1	PEO ₄₅ - <i>b</i> -PS ₂₁₁ -SH	16.0	23.9	21.9	1.15	9.93
BCP-2	PEO ₄₅ - <i>b</i> -PS ₄₂₁ -SH	68.7	45.8	43.8	1.08	14.04
BCP-3	PEO ₄₅ - <i>b</i> -PS ₆₆₈ -SH	62	71.5	69.5	1.09	17.68
BCP-4	PEO ₄₅ - <i>b</i> -PS ₈₈₅ -SH	71	94.0	92.0	1.08	20.35

^a Number-average molecular weight determined by GPC using polystyrene standards for calibration.

^b Molecular weight calculated from ¹H NMR measurements.

^c Root-mean-square end-to-end distance of PS blocks, calculated from $R_0 = bN^{0.5}$; $N = M_n(\text{PS})/M_0$, where b is the Kuhn length ($b=1.8$ nm for PS), N is the number of Kuhn segments calculated from the molar mass of Kuhn monomer M_0 ($M_0=720$ g/mol for PS), and $M_n(\text{PS})$ is the number-average molecular weight of PS block.

Table S2. Parameter of Au NCs and BCPs in Figure 2 and Figure 3.

Au NCs & BCP	σ (chains \cdot nm ⁻²)	d (nm)	R_0' (nm)	R_0'/d
Au-80 & PEO ₄₅ - <i>b</i> -PS ₂₁₁ -SH	0.12	3.31	10.78	3.26
Au-80 & PEO ₄₅ - <i>b</i> -PS ₄₂₁ -SH	0.10	3.54	14.66	4.14
Au-80 & PEO ₄₅ - <i>b</i> -PS ₆₆₈ -SH	0.10	3.63	18.17	5.01
Au-80 & PEO ₄₅ - <i>b</i> -PS ₈₈₅ -SH	0.11	3.39	20.78	6.13
Au-52 & PEO ₄₅ - <i>b</i> -PS ₂₁₁ -SH	0.19	2.62	10.78	4.11
Au-52 & PEO ₄₅ - <i>b</i> -PS ₄₂₁ -SH	0.24	2.32	14.66	6.33
Au-52 & PEO ₄₅ - <i>b</i> -PS ₆₆₈ -SH	0.18	2.64	18.17	6.87
Au-52 & PEO ₄₅ - <i>b</i> -PS ₈₈₅ -SH	0.16	2.85	20.78	7.30

The root-mean-square end-to-end distance of BCP (R_0') can be calculated by the following equation⁴

$$R_0' = [(Nb^2)_{\text{PS}} + (Nb^2)_{\text{PEO}}]^{0.5}$$

where b is the Kuhn length ($b=1.8$ nm for PS, $b=1.1$ nm for PEO) and N is the number of Kuhn segments calculated from the molar mass of Kuhn monomer M_0 ($M_0=720$ g/mol for PS and $M_0=137$ g/mol for PEO).

Table S3. D of Au NCs and R_0 of BCPs in Figure 2 and Figure 3 and corresponding yield of hexagonally packed vesicles.

Au NCs & BCP	D (nm)	R_0 (nm)	R_0/D	hexagon yield
Au-80 & PEO ₄₅ - <i>b</i> -PS ₂₁₁ -SH	80	9.93	0.12	20%
Au-80 & PEO ₄₅ - <i>b</i> -PS ₄₂₁ -SH	80	14.04	0.18	46%
Au-80 & PEO ₄₅ - <i>b</i> -PS ₆₆₈ -SH	80	17.68	0.22	61%
Au-80 & PEO ₄₅ - <i>b</i> -PS ₈₈₅ -SH	80	20.35	0.25	85%
Au-52 & PEO ₄₅ - <i>b</i> -PS ₂₁₁ -SH	52	9.93	0.19	80%
Au-52 & PEO ₄₅ - <i>b</i> -PS ₄₂₁ -SH	52	14.04	0.27	91%
Au-52 & PEO ₄₅ - <i>b</i> -PS ₆₆₈ -SH	52	17.68	0.34	96%
Au-52 & PEO ₄₅ - <i>b</i> -PS ₈₈₅ -SH	52	20.35	0.39	99%

References

1. J. He, Y. Liu, T. Babu, Z. Wei and Z. Nie, *J Am Chem Soc*, 2012, **134**, 11342-11345.
2. H.-L. Wu, C.-H. Kuo and M. H. Huang, *Langmuir*, 2010, **26**, 12307-12313.
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4. X. Lin, S. Ye, C. Kong, K. Webb, C. Yi, S. Zhang, Q. Zhang, J. T. Fourkas and Z. Nie, *J Am Chem Soc*, 2020, **142**, 17282-17286.