Supporting information

Amphiphilic Janus Magnetoplasmonic Nanoparticles: pH-Triggered Self-Assembly and Fluorescence Modulation

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Materials

Inhibitor was removed from 2-(Diisopropylamino)ethyl methacrylate (DPA: Aldrich, 97%) and Poly(ethylene glycol) methyl ether methacrylate (Aldrich, average M_n 300) before use by passing through a basic alumina column. 2-Aminoethyl methacrylate hydrochloride (AMA: Aldrich, 90%) was recrystallized twice with isopropanol and ethyl acetate (3:7). Diethyl vinylphosphonate (Aldrich, 97%), Ammonia solution (Aldrich, 25%), Jones reagent (Aldrich), Poly(ethylene glycol) methyl ether (MeO-PEG-OH, average M_n 2000 and 5000, Aldrich), N,N′-dicyclohexylcarbodiimide (DCC, Aldrich, 99%), 4-(Dimethylamino)pyridine (Aldrich, ≥99%). Bromotrimethylsilane

(Aldrich, 97%), Triethylamine (TEA: Aldrich, ≥99%), 2,2′-Azobis(2-methyl prop ionitrile) (AIBN: Aldrich, 98%) was recrystallized with methanol. Tri-tert-butylphosphine (Aldrich, 98%), 2,2′-Bipyridyl (Bpy: Aldrich 99%), Manganese(II) acetate ($Mn(OAc)_2$), Iron(III) acetylacetonate ($Fe(acac)_3$), oleylamine (Aldrich, 70%) and (Alfa Aesar, 80-90%), Oleic acid (Aldrich, 90%), Dimethyl (2-hydroxyethyl)phosphonate (TCI, >92%) Hydrogen tetrachloro- aurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$: 99.99%, Alfa Aesar), Methacryloyl chloride (Alfa Aesar, 96%), Cyanine3 NHS ester (Lumiprobe Corporation, >95%).

Copper(I)chloride and 2, 2'-dithiobis [1-(2- bromo-2-methyl- propionyloxy)]s ethane (DTBE) were synthesized in our group.

The following solvents were used as received: dichloromethane (DCM: Labscan, AR grade), chloroform (CF: Labscan, AR grade), ethanol (EtOH: anhydrous, Lichrosolv, 99.9%, HPLC grade), n-Hexane (Labscan, AR grade), methanol (MeOH: anhydrous, Lichrosolv, 99.9%, HPLC grade), acetonitrile (CH_3CN, Lichrosolv, 99.9%, HPLC grade), N,N-dimethylformamide (DMF: Labscan, AR grade), tetrahydrofuran (THF: Lichrosolv, HPLC grade), isopropyl alcohol (IPA, anhydrous, Lichrosolv, 99.9%, HPLC grade).

Analytical Methodologies

Transmission Electron Microscopy (TEM) observations were conducted on a Jeol JEM 2010 electron microscope at an acceleration voltage of 300 kV and Jeol JEM 1410 electron microscope at an acceleration voltage of 100 kV. **Scanning electron**

microscopy (SEM) images were obtained on a FESEM (JSM-6700F, Japan). **UV-vis absorption** spectra were recorded by using a SHIMADZU UV-2501 spectrophotometer. **Thermogravimetric analysis** (TGA) was performed on a SDT Q600 thermogravimetric analyzer. Samples were placed in platinum sample pans and heated under a nitrogen atmosphere at a rate of 10 °C/min to 100 °C and held for 10 min to completely remove residual solvent. Samples were then heated to 800 °C at a rate of 10 °C/min. **Dynamic light scattering (DLS)** measurements were performed using a Malvern Zetasizer Nano Series running DTS software and operating a 4 mW He-Ne laser at 633 nm. Analysis was performed at an angle of 173° and a constant temperature of 25 °C. **Nuclear Magnetic Resonance (NMR)**. All NMR spectra were recorded on Bruker DRX 300 MHz spectrometer using an external lock ($CDCl_3$ or D_2O) at 298K. **Size Exclusion Chromatography (SEC)**. The molecular weight of homo- or co-polymers were determined by water phase SEC using a Waters Ultrahydrogel column with acidic buffer (0.5 M sodium acetate and 0.5 M acetic acid, with pH = 4.5) as eluent. **Fluorescence spectrophotometry.** Emission spectra of solutions were measured by fluorescence spectrophotometry with a Horiba Fluolog 3 spectrofluorometer at 25ºC. **Time-Resolved Photoluminesence Spectroscopy**. The excitation pulse (400 nm) was generated by frequency doubling the 800 nm pulse laser (100 fs, 80MHz). The repetition rate of the pulse was controlled by a pulse-picker to adjust the measurable range of decay time. The excitation was introduced to the solution sample through a 10 x long focus objective, and the back scattering photoluminescence signal was collected by the same objective. The photoluminescence signal was filtered by suitable cut-off

filters and detected by an avalanche photodiode connected to a single-photon counter (time-correlated single photon counting). **Static magnetic properties** were measured on a vibrating sample magnetometer (VSM, ADE Magnetics EV-9)

Computational Simulation

Theory:

The fluorescence rate γ_{em} of a molecule can be expressed as the product of excitation rate γ_{exc} and quantum yield q, defined as the fraction of radiative transitions from excited to ground state to the total decay rate. The fluorescence enhancement can then be expressed as

$$\frac{\gamma_{\text{em}}}{\gamma_{\text{em}}^{o}} = \frac{\gamma_{\text{exc}}}{\gamma_{exc}^{o}} \frac{q}{q^{o}} \qquad \text{(Equation S1)}$$

where the superscript 'o' indicates the corresponding free-space quantity. Below saturation, the excitation rate γ_{exc} is proportional to $|\mathbf{E}\cdot\mathbf{p}|^2$, with $\mathbf{E}$ being the local electric field and $\mathbf{p}$ the transition dipole moment. On the other hand, within the validity of Fermi's golden rule, the decay rate of the excited molecule in absence of the optical antenna $\gamma^o = \gamma_r^o + \gamma_{nr}^o$, where γ_r^o and γ_{nr}^o are the radiative and nonradiative decay rates of the excited molecule, respectively. The intrinsic quantum yield of the molecule is defined as $\gamma^o = \gamma_r^o/(\gamma_r^o + \gamma_{nr}^o)$. The presence of the nanoparticle introduces an additional nonradiative rate γ_{abs}, thereby modifying the quantum yield to $q = \gamma_r/(\gamma_r + \gamma_{nr} + \gamma_{abs})$. Using the definition of q^o, this can be rewriten as

$$q = \frac{\gamma_r/\gamma_r^o}{\gamma_r/\gamma_r^o + \gamma_{abs}/\gamma_r^o + (1-q^o)/q^o} \qquad \text{(Equation S2)}$$

Here γ_r is the radiative rate in presence of the optical antenna. We assume that the

nanoparticle does not influence the intrinsic nonradiative rate, i.e. $\gamma_{nr} = \gamma_{nr}^{o}$.

The energy transfer rate to the particle (γ_{abs}) can be calculated from the power P_{abs} that is radiated by the molecule and absorbed in the particle. Once P_{abs} is known, the normalized energy transfer rate is calculated as $\gamma_{abs}/\gamma_r^{o} = P_{abs}/P_o$, with $P_o = |\mathbf{p}|^2\gamma k^3/(12\pi\varepsilon_0)$ being the power radiated by a classical dipole with frequency ω and dipole moment **p**, and the associated wavevector being defined as $k = \omega/c$. Using $\gamma_r/\gamma_r^{o} = P_r/P_o$, the normalized power radiated by the system of molecule and particle is calculated.

Simulation method:

Numerical simulations were performed using COMSOL Multiphysics 5.0. The relative change in excitation rate was estimated by simulating the electric field intensity at the fluorophore position and averaged over the tangential and radial incident polarization. The relative change of the radiative and nonradiative decay rates were calculated by modeling the dye as a dipole current source with a determined orientation. The total power radiated into the far field and dissipated by the metallic nanoparticles is computed and normalized to the total power radiated into the far field in the absence of metallic nanoparticles. For these calculations, the intrinsic quantum yield of the Cy3-copolymer conjugate of 0.19 was considered. The excitation and emission wavelengths were set as 500 nm and 565 nm, respectively, consistent with the experiment setup.

Experimental Section

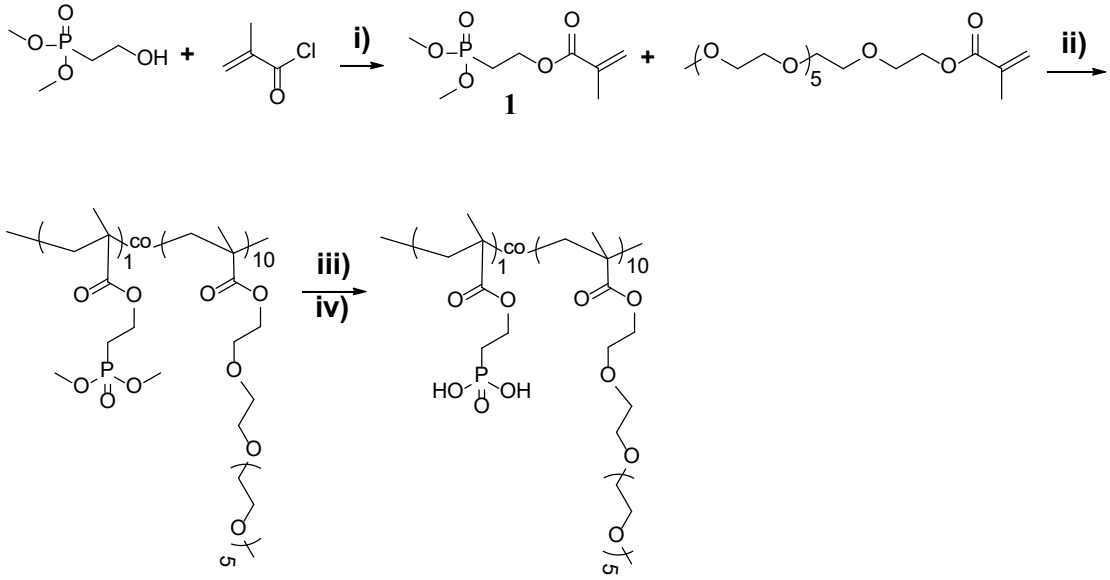
Synthesis of Au seed

To a mixture containing 1 mL of 1-octadecene, 4 mL of oleylamine (80-90%), and 0.4 mL of oleic acid, 1.6 mL of 50 mg/mL $HAuCl_4$ in CH_3CN was added. The mixture solution was heated up to 100 ºC to remove low boiling point compounds under reduced pressure. After that, the temperature of mixture was further increased to 135 ºC followed by applying nitrogen flow. The reaction was stirred at 135 ºC for 15 min, the mixture was cooled down room temperature. The red colored product was diluted by hexane and the AuNPs were obtained by the centrifugation at 3000 rpm for 5 min after the addition of ethanol. The AuNPs were dissolved in hexane and stored at 4 ºC for further use.

Synthesis of Au/$MnFe_2O_4$ (Au/MFO) Janus NPs

A mixture of 0.2 mmol of $Fe(acac)_3$, 0.1 mmol of $Mn(OAc)_2$, 4 ml of oleylamine (70%), and 2 mL of oleic acid was heated up to 80 ºC followed by adding 5 mL of AuNPs solution made in previous step., The water and hexane were removed in reduced pressure at 150 ºC. Then the mixture was slowly increased to 260 ºC under a N_2 flow. The reaction was stirred at this temperature for 20 min. After that, the mixture cooled down to 25 ºC, the products were precipitated by adding ethanol and separated with a magnet. The Au/MFO Janus NPs were dispersed in 10 mL of hexane for further modification.

Scheme S1: Synthetic route of multi-functional phosphonic acid POEGMA, 3



i) TEA, DCM, r.t., N_2, 12 h; ii) AIBN, THF, N_2, 12h; iii) TMSBr, N_2, DCM, r.t.; iv) MeOH, 24h, r.t..

Synthesis of phosphonate methacrylate monomer 1

To a 100 mL round bottom flask, Dimethyl (2-hydroxyethyl)phosphonate (3.4 g, 0.022 mol) and TEA (2.22 g, 0.022 mol) were dissolved in 15 mL dry THF. The flask was cooled down in ice bath followed by adding a mixture methacryloyl chloride (2.08 g, 0.2 mol) and 15 mL dry THF dropwise in 10 min. The reaction mixture was warmed up to room temperature and was stirred for 12 h. The solid salt was filtered out and the THF was removed by reduced pressure. The resultant content was again dissolved in 100 mL DCM, washed by saturated $NaHCO_3$ and brine sequentially. The organic phase was collected and dried over anhydrous $MgSO_4$, concentrated and purified by silica column chromatography with DCM/MeOH (15:1, v:v) as eluent. The fraction with R_f as 0.56 was collected and the collected fraction was concentrated and obtained as phosphonate methacrylate monomer **1** (2.79 g, yield 54%).

^{1}H NMR ($CDCl_3$, 298K, 300 MHz); δ 6.10 (br, 1H, C*H*H=C(CH_3)-), 5.54 (br, 1H, CH*H*=C(CH_3)-), 4.31-4.40 (m, 2H; -CH_2C***H***$_2$O-), 3.72,3.75(d, 6H; C*H*$_3$OP-, *J*=10.95 Hz), 2.13-2.25 (m, 2H; -PC***H***$_2$$CH_2$-), 1.91(s, 3H; methyl protons).

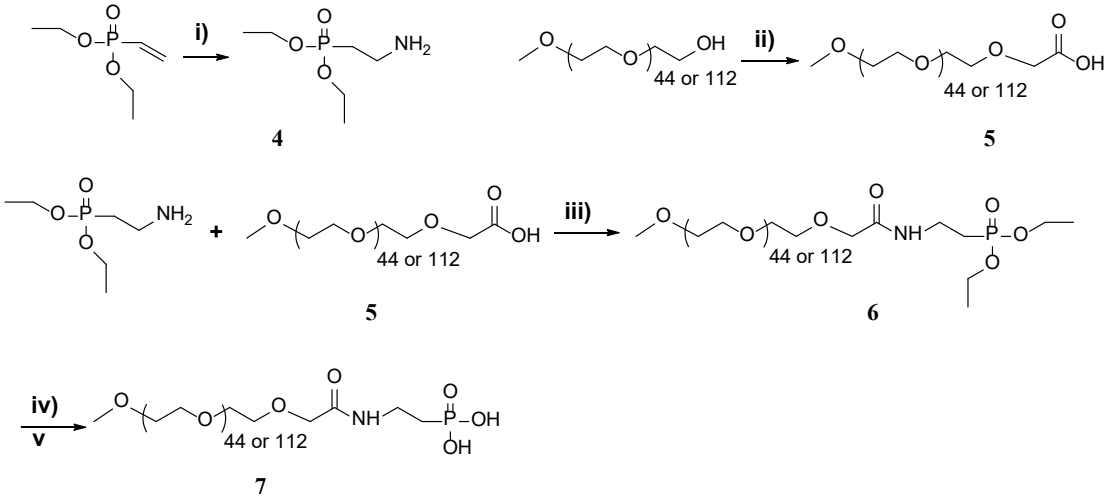
^{13}C NMR (CDCl3, 298K, 300 MHz); 165.98, 135.93, 126.01, 58.63, 52.54, 25.88, 24.01, 18.19. ^{31}P NMR (CDCl3, 298K, 300 MHz); 31.36

Synthesis of multi-functional phosphonic acid-functionalized POEGMA 3

To a 20 mL Schlenk Tube, POEGMA (1.53 g, average M_n=300, 5.1 x 10^{-3} mol), monomer **1** (0.106 g, 5.1 x 10^{-4} mol), and AIBN (17 mg, 1.02 x 10^{-4} mol) were dissolved in 6 mL THF. After 3 cycles of freeze-pump-thaw to remove the oxygen, the tube was placed in 70 °C temperature control oil bath. The reaction mixture was stirred for 12 h. The monomer conversion was reached to 96% (determined by ^{1}H NMR). The THF in the mixture was removed by reduced pressure, and further diluted by 20 mL DI H_2O. The resultant copolymer **2** was recovered by lyophilization.

The dry copolymer after lyophilization was dissolved in 9 mL dry DCM followed by adding TMSBr (0.38 g, 2.55 x 10^{-3} mol, 5 equivalents to monomer **10**). The mixture was stirred for 24 h, the DCM was removed by reduce pressure. Then 20 mL anhydrous MeOH was added and reacted for another 24 h. The resultant mixture was purified by dialysis against first with MeOH for one day, and further with DI H_2O for 2 days, followed by lyophilization and obtained sticky phosphonic acid-functionalized POEGMA **3**.

Scheme S2: Synthetic route of monofunctional phosphonic acid PEG



i)NH_3H_2O, r.t., 3 days; ii) CrO_3, H_2SO_4, H_2O, r.t., 24h; iii) DCC, DMAP, DCM, r.t., 24h; iv) TMSBr, N_2, DCM, r.t.; v) MeOH, 24h, r.t..

Synthesis of phosphonate amine 4

Diethyl vinylphosphonate (1.64 g, 0.01 mol) and 25 mL ammonium solution were added into 100 mL flask. The mixture was stirred for 3 days at room temperature. After that, 50 mL DI H_2O was added, followed by extracting with DCM (150 x 3 mL). The organic phase was collected and dried over anhydrous $MgSO_4$. The DCM was removed by reduced pressure and obtained as colourless liquid **4** and used for next step directly. ^{1}H NMR ($CDCl_3$, 298K, 300 MHz); δ 3.99-4.10 (m, 4H; $CH_3C\boldsymbol{H}_2O$-), 2.80-3.02 (m, 2H; -$CH_2C\boldsymbol{H}_2NH_2$-), 1.83-1.97 (m, 2H; -$PC\boldsymbol{H}_2CH_2$-), 1.26-1.31 (td, 6H; methyl protons, *J*= 7.05 Hz, *J*= 1.71 Hz).

^{13}C NMR (CDCl3, 298K, 300 MHz); 77.21, 67.91, 61.32, 16.45. ^{31}P NMR (CDCl3, 298K, 300 MHz); 30.24

Synthesis of PEG-COOH 5

To a 100 mL round bottom flask, PEG_{112}-OH (M_n=5000 Da, 5 g, 1 x 10^{-3} mol was dissolved in 25 mL H_2O. 6 mL 96 % H_2SO_4 was added dropwise into the solution above followed by adding 1.5 mL 2M Jones reagent dropwise into the mixture. The flask was then placed in ice bath and stirred for 30 min, then warmed up to room temperature. The reaction was stirred for another 24 h. After that, 50 mL DI H_2O was added to diluted the solution followed by the extraction with DCM (100 x 3 mL). The organic phase was collected and dried over anhydrous $MgSO_4$, concentrated, and finally precipitated in diethyl ether obtained as PEG_{112}-COOH **5a**.

The PEG_{44}-COOH (**5b**) with M_n =2000 Da was synthesized by the same procedure above.

Synthesis of monofunctional phosphonic acid-functionalized PEG 7

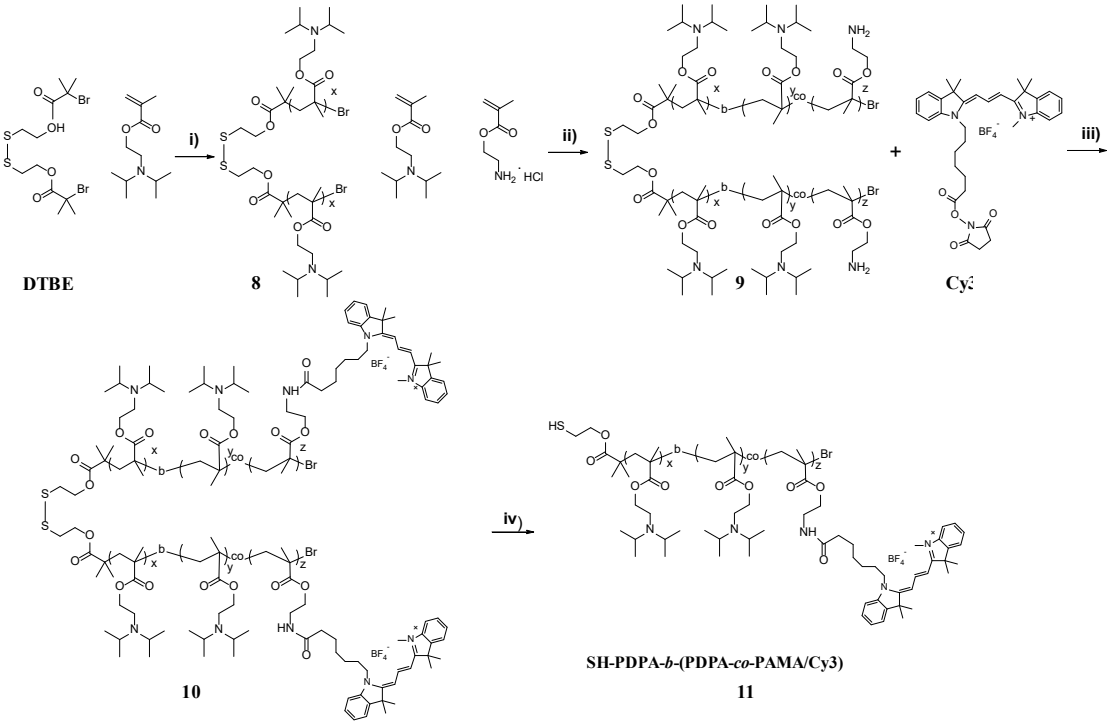
PEG-COOH **5a** (M_n=5000 Da, 0.3 g, 6.0 x 10^{-5} mol) and DMAP (7.3 mg, 6.0 x 10^{-5} mol) were dissolved in 5 mL dry DCM. A mixture of DCC (62 mg, 3.0 x 10^{-4} mol) and 3 mL dry DCM was added dropwise. The reaction mixture was stirred at room temperature for 48 h. The solid content was removed by filtration, the filtrate was concentrated and precipitated in diethyl ether obtained as phosphonate-PEG **6a**.

The resultant phosphonate-PEG **6a** from previous step (M_n=5000 Da) was dissolved in 5 mL dry DCM followed by adding 40 µL TMSBr. The reaction mixture was stirred for 24 h. The DCM was removed by reduced pressure, then 5 mL dry MeOH was added into the resultant mixture and kept on stirring for another 24 h. The mixture was

concentrated and precipitated in diethyl ether. The precipitation was repeated three times and obtained as monofunctional phosphonoic acid functionalized PEG **7a** (M_n=5000 Da).

The monofunctional phosphonoic acid-PEG (**7b**) with M_n=2000 Da was synthesized by the same procedure above.

Scheme S3: Synthetic route of dye-conjugated pH responsive copolymer



i) CuCl, Bpy, IPA, 50 °C; ii) CuCl, Bpy, IPA, 50 °C; iii) DMF, r.t.; iv) P(*t*-Bu)$_3$, DMF, r.t..

Synthesis of copolymer 9 (-S-PDPA-*b*-(PDPA-*r*-PAMA))$_2$ in one-pot two step polymerization

DTBE was synthesized according to our previous report.[1] Freshly purified DPA (3.17 g, 1.49 x 10^{-2} mol), Bpy (83 mg, 5.32 x 10^{-4} mol), DTBE (60 mg, 1.33 x 10^{-4} mol), and IPA (7.1 mL) were added to a 20 mL Schlenk tube equipped with a magnetic stirrer and purged with argon for 30 min in ice bath. Cu(I)Cl (39 mg, 3.99 x 10^{-4} mol) was carefully added under positive argon flow, and the reaction mixture was purged with argon for another 5 min. The flask was placed in a temperature controlled oil bath at 50 °C for 16 h to obtain **8a**. The SEC sample was taken for measurement after 16 h. To the reaction mixture above, a degassed mixture containing DPA (1.13 g, 5.03 x 10^{-3} mol), AMA (0.13 g, 7.96 x 10^{-4} mol), Bpy (20 mg, 3.98 x 10^{-4} mol), IPA (3 mL) together with CuCl (13 mg, 1.33 x 10^{-4} mol) were added under positive argon flow. The mixture was kept on stirred in 50 ºC oil bath for another 3 h. The reaction was stopped by quenching in ice bath and exposure to air. The polymerization mixture was diluted with IPA. The diluted mixture was directly placed in a dialysis tube (cut off MWCO 3500) and dialyzed against with pH=4 aqueous solution for 2 days, the solvent was changed every 6 h. The copolymer **8a** was recovered by lyophilization.

8b, **9b**, **8c**, and **9c** were synthesized according to the same procedure as that of **8a** and **9a** but using different ratio of reactants/initiator and reaction time.

8b: DPA (3.72 g, 1.74 x 10^{-2} mol), Bpy (33 mg, 2.11 x 10^{-4} mol), DTBE (24 mg, 5.3 x 10^{-5} mol), CuCl (16 mg, 1.61 x 10^{-4} mol), and IPA (7.4 mL), 16 h.

9b: DPA (0.45 g, 2.12 x 10^{-3} mol), AMA (61 mg, 3.71 x 10^{-4} mol), Bpy (9.7 mg, 6.19 x 10^{-5} mol), IPA (3 mL) together with CuCl (6.1 mg, 5.3 x 10^{-5} mol), 6 h.

8c: DPA (3.53 g, 1.65 x 10^{-2} mol), Bpy (36.5 mg, 2.21 x 10^{-4} mol), DTBE (12.5 mg,

2.76 x 10^{-5} mol), and dry IPA (10 mL), 16 h.

9c: DPA (0.235 g, 1.10 x 10^{-3} mol), AMA (27 mg, 7.96 x 10^{-4} mol), Bpy (4.3 mg, 3.98 x 10^{-4} mol), IPA (1.5 mL) together with CuCl (2.73 mg, 1.33 x 10^{-4} mol), 9 h.

Synthesis of copolymer 10 (-S-PDPA-b-(PDPA-r-PAMA/Cy3))$_2$ and PDPACy3, 11, HS-PDPA-*b*-(PDPA-*r*-PAMA/Cy3)

For Cy3 conjugation, 100 mg copolymers **4** (**4a**, **4b**, and **4c**) were dissolved in 4 mL DMF in a 10 mL vial. Then Cy3-NHS ester (1.5 equivalents to molar amount of primary amine group) was added. The reaction mixture was stirred at room temperature for two days, the vial was wrapped by aluminum foil. After two days, Tri-tert-butylphosphine (4 equivalents to copolymer **10**) was added and continued to stir for 6 h. The resultant mixture was purified by dialysis against first with MeOH for 1 days and later with pH4 aqueous solution (with a few mg of TECP to avoid oxidation of copolymer) for 3 days in a container which wrapped by aluminum foil. The solvent was changed every 6 h. The copolymers **PDPACy3**,**11**, were recovered by lyophilization. Herein, **11a, 11b,** and **11c** with different molecular weight refer to **PDPACy3-I**, **PDPACy3-II**, and **PDPACy3-III**, respectively.

Synthesis of Au/MFO-POEGMA and Au/MFO-PEG Janus NPs *via* 'graft onto' method

To a 100 mL round bottom flask, 3 mL of Au/MFO solution (440 nM) was dissolved in 30 mL CF, followed by adding a mixture of 30 mg copolymer **3** and 20 mL CF. The

reaction was stirred for 12 h, followed by the centrifugation at 9000 rcf for 30 min. The centrifugation was repeated for 3 times. The liquid supernatant was removed and the resultant Au/MFO-POEGMA was dispersed in DMF for further use. The good dispersity of Au/MFO in DMF suggested that the successful conjugation of POEGMA brushes.

To a 100 mL round bottom flask, 3 mL of Au/MFO solution (440 nM) was dissolved in 30 mL CF, followed by adding the mixture of 15 mg phosphonic acid-PEG **7a** (M_n=5000 Da) and 20 mL CF. The reaction was stirred for 12 h, followed by the centrifugation at 9000 rcf 30 min. The centrifugation was repeated three times. The liquid supernatant was removed and the resultant Au/MFO-$PEG_{112}$was dispersed in DMF for further use. The Au/MFO-PEG_{44} was synthesized by the same procedure as above but used 6 mg phosphonic acid-PEG **7b** (M_n=2000 Da).

6 mg phosphonic acid-PEG (M_n=2000 Da).

Synthesis of (Cy3/PAMA-r-PDPA)-b-PDPA-Au/MFO-POEGMA or (Cy3/PAMA-r-PDPA)-b-PDPA-Au/MFO-PEG Janus NPs *via* 'graft onto' method

To 5 mL of Au/MFO-POEGMA DMF solution (220 nM), a mixture of **PDPACy3-I, 11a** (20 mg) and 15 mL DMF was added. The reaction was stirred for 24 h followed by centrifugation at 9000 rcf for 30 min. The centrifugation was repeated 3 times. The resultant amphiphilic **PDPACy3-I**-Au/MFO-POEGMA(**JNP1)** were dispersed in DMF for further self-assembly. To 5 mL of Au/MFO-PEG_{112} DMF solution (220 nM), a mixture of **PDPACy3-II, 11b** (30 mg) and 15 mL DMF was added into the solution

above. The reaction was stirred for 24 h followed by centrifugation at 9000 rcf for 50 min. The centrifugation was repeated three times. The resultant amphiphilic PDPACy3-II-Au/MFO-POEGMA (**JNP2**) were dispersed in DMF for further self-assembly.

To 5 mL of Au/MFO-PEG_{112} DMF solution (220 nM), a mixture of **PDPACy3-III, 11c** (40 mg) and 15 mL DMF was added into the solution above. The reaction was stirred for 24 h followed by centrifugation at 9000 rcf for 50 min. The centrifugation was repeated three times. The resultant amphiphilic PDPACy3-III-Au/MFO-PEG_{112} (**JNP3**) were dispersed in DMF for self-assembly.

To 5 mL of Au/MFO-PEG_{44} DMF solution (220 nM), a mixture of **PDPACy3-III, 11c** (40 mg) and 15 mL DMF was added into the solution above. The reaction was stirred for 24 h followed by centrifugation at 9000 rcf for 50 min. The centrifugation was repeated three times. The resultant amphiphilic PDPACy3-III-Au/MFO-PEG_{44} (**JNP4**) were dispersed in DMF for self-assembly.

Self-assembly of pH-responsive amphiphilic Janus NPs

Cluster:

1.6 mL pH 8.5 NaOH solution was added by feeding pump into of 0.4 mL DMF solution of **JNP1** in 100 min under gentle stirring, feeding rate 16 μL/min. The obtained solution was further dialyzed against pH 8.5 NaOH solution for 24 h. The solvent was changed every 6 h.

Lamellae and Vesicle:

1.6 mL pH 8.5 NaOH solution was added by feeding pump into of 1.6 mL DMF solution of **JNP2**, **JNP3** or **JNP4** (36 nM) in 100 min under gentle stirring, feeding rate 16 μL/min. The obtained solution was further dialyzed against pH 8.5 NaOH solution for 24 h. The solvent was changed every 6 h.

Dye conjugation efficiency measurements

The Cy3 dye conjugation number in each copolymer chain and conjugating efficiency were determined by UV-vis absorbance of Cy3 dye and Cy3-copolymer conjugates. In brief, the Cy3 dye and Cy3-copolymer conjugates were dissolved in DMF. The absorbance of Cy3 standard and Cy3-copolymer solutions were determined at corresponding peak wavelength. The number of conjugated Cy3 was determined using the following equations:

$$N_{Cy3} = \frac{C_{Cy3}}{A_{Cy3}} \frac{A_{Cong}}{C_{Cong}} \frac{MW_{Cong}}{MW_{Cy3}} \qquad \text{(Equation S3)}$$

$$Efficiency\ (\%) = \frac{N_{Cy3}}{N_{NH2}} \times 100 \qquad \text{(Equation S4)}$$

Where N_{Cy3} and N_{NH2} are the conjugated dye number and the number of primary amine onto the copolymer **PDPACy3** backbones. A_{Cy3} and A_{Cong} are the absorbance of Cy3 dye reference and Cy3-copolymer conjugates. C_{Cy3} and C_{Cong} are the concentration of Cy3 dye reference and Cy3-copolymer conjugates. MW_{Cy3} and MW_{Cong} are the concentration of Cy3 dye reference and Cy3-copolymer conjugates, respectively.

Quantum yield measurements

The relative fluorescent quantum yield is determined by comparative method. The Rhodamine B was selected as reference dye, which the quantum yield was reported as 0.7 in ethanol.[2]

For quantum yield measurement, the Cy3-NHS and PDPA-Cy3 conjugates were dissolved in DMF and pH 4 and 6 aqueous solution and kept the absorbance of solution <0.05. The excitation wavelength 500 nm was used for all fluorescence spectra measurements. The integrated fluorescence intensities of two samples were calculated from the spectra. The quantum yield of Cy3-NHS and Cy3-PDPA conjugates were calculated using the following equation:

$$\Phi_{sample} = \Phi_{ref} \frac{I_{sample}}{I_{ref}} \frac{OD_{ref}}{OD_{sample}} \frac{n^2_{sample}}{n^2_{ref}} \qquad \text{(Equation S5)}$$

Where $\boldsymbol{\Phi}_{\mathbf{sample}}$ is the fluorescent quantum yield, $\boldsymbol{OD}_{\mathbf{sample}}$ is the absorbance of samples measured, $\boldsymbol{I}_{\mathbf{sample}}$ is the integrated emission intensity of samples measured, $\boldsymbol{n}_{\mathbf{sample}}$ is the refractive index of the solvent, and the subscript "***ref***" denotes the values for the reference sample.

The quantum yield of Cy3-PDPA (**PDPACy3-I** and **PDPACy3-III**) conjugated Au/MFO-POEGMA and Au/MFO-PEG_{112} were calculated using the following equation:

$$\Phi_{monomer} = \Phi_{Cy3-PDPA} \frac{I_{monomer}}{I_{Cy3-PDPA}} \frac{M_{cong}}{M_{Cy3-PDPA}} \qquad \text{(Equation S6)}$$

Where $\Phi_{monomer}$ is the quantum yield of Cy3-PDPA copolymer conjugated JNP1 or JNP3, $\Phi_{Cy3\text{-}PDPA}$ is the quantum yield of Cy3-PDPA copolymer, $I_{monomer}$ is the fluorescence intensity of JNP5 or JNP8 measured, $I_{Cy3\text{-}PDPA}$ is the fluorescence intensity of Cy3-PDPA copolymers measured. M_{cong} is the mass of Cy3-PDPA copolymer conjugated on Au/MFO JNPs, determined by TGA analysis (e.g., initial mass of JNP4 or JNP8 times wt% of hydrophobic grafts, Table S3). $M_{Cy3\text{-}PDPA}$ is the mass of Cy3-PDPA used for measurement. All measurements were carried out in DMF, 22°C. For JNP1, $\Phi_{monomer}/\Phi_{Cy3\text{-}PDPA}$ =0.051, for JNP3, $\Phi_{monomer}/\Phi_{Cy3\text{-}PDPA}$ =0.15.

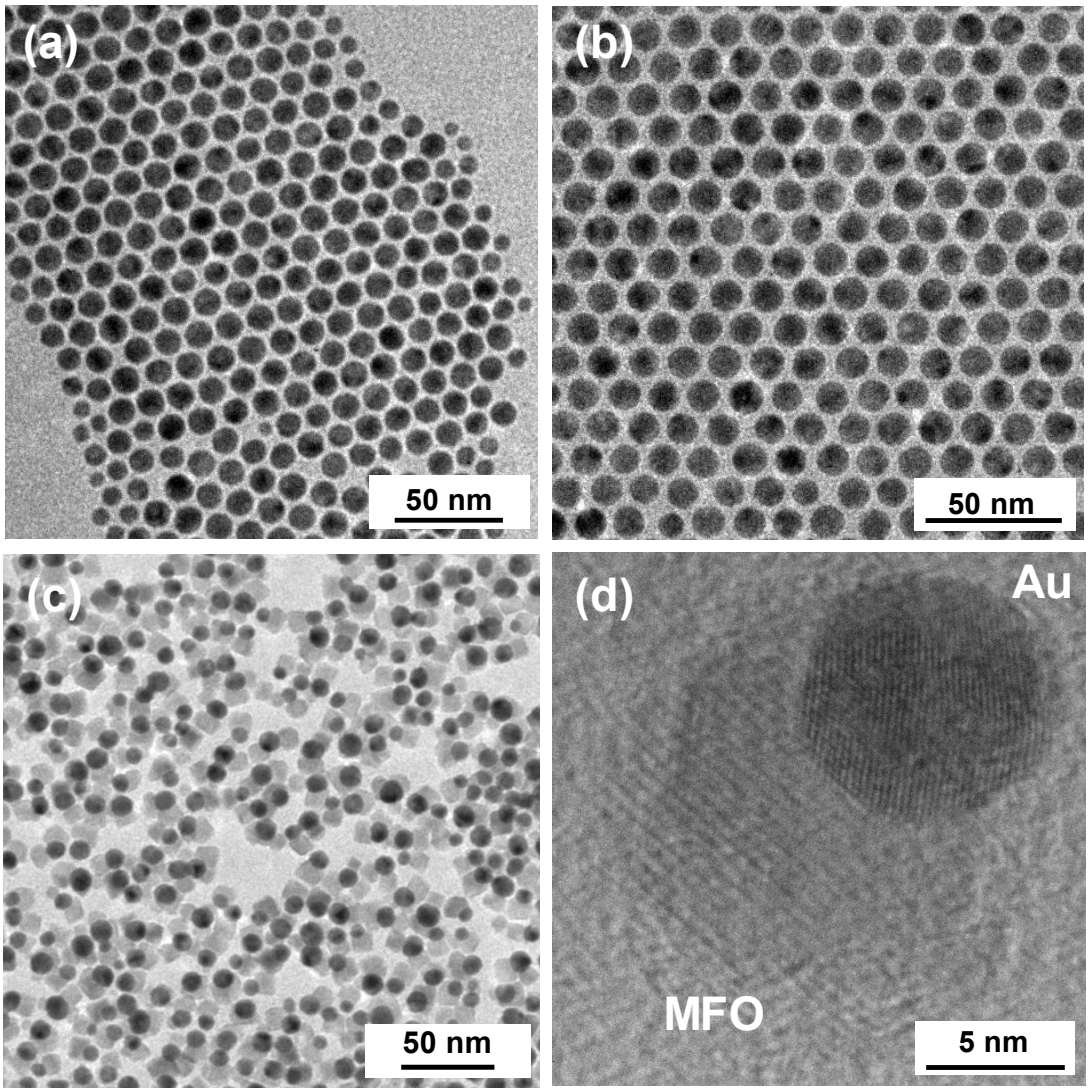


Figure S1: TEM images of Au seed (a, b) and Janus Au/MFO NPs (c, d). The average size of Au hemisphere ~9 nm, obtained by averaging the size of >50 Au/MFO JNPs).

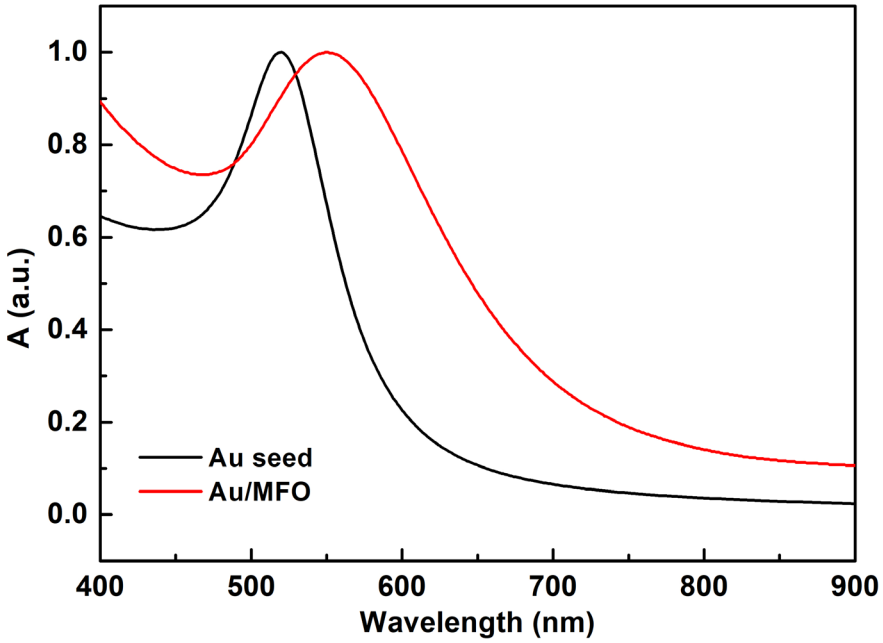


Figure S2: UV-Vis spectra of Au seed and Janus Au/MFO NPs in chloroform.

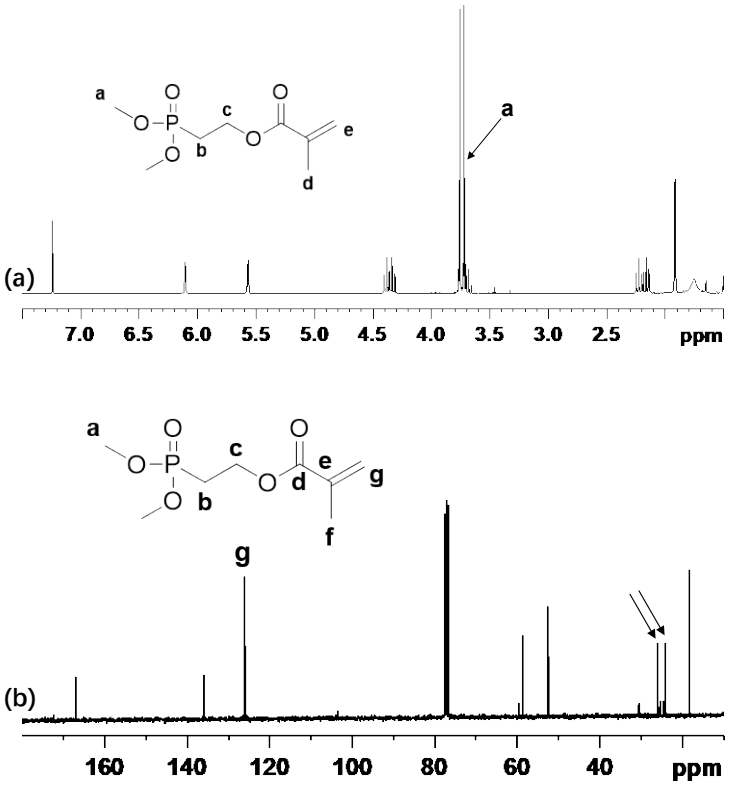


Figure S3: ^{1}H NMR (A) and ^{13}C NMR spectra of compound **1,** recorded in $CDCl_3$ at 298K, 300 MHz.

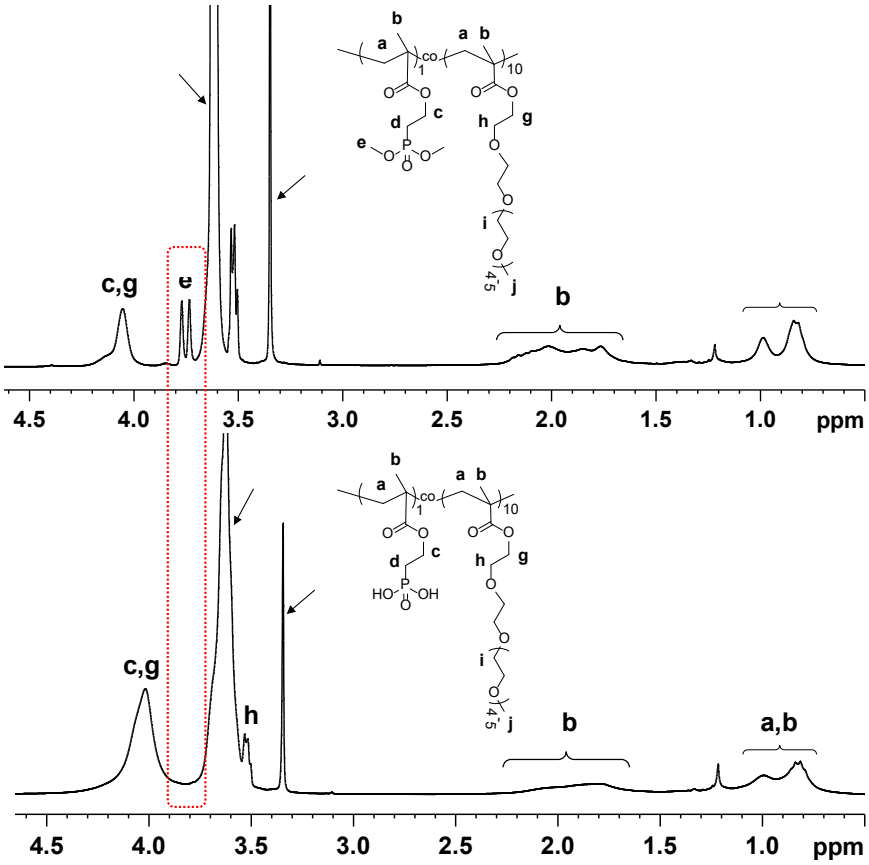


Figure S4: ^{1}H NMR spectra of Phosphonate-based, **2** (A) and Phosphonic acid-based **3** (B) POEGMA**,** recorded in $CDCl_3$ at 298K, 300 MHz, *=H_2O.

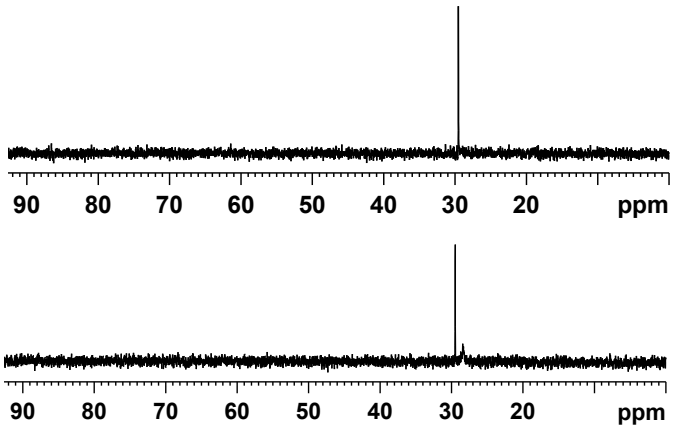


Figure S5: ^{31}P NMR spectra of Phosphonate-based, **2** (a) and Phosphonic acid-based **3** (b) POEGMA**,** recorded in $CDCl_3$ at 298K, 300 MHz.

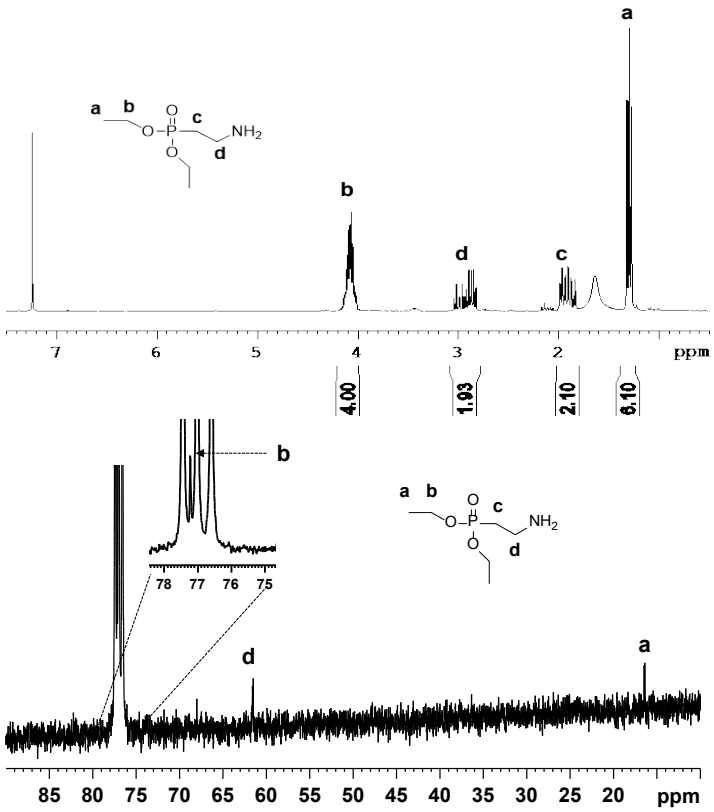


Figure S6: ^{1}H NMR (a) and ^{13}C NMR (b) spectra of compound **4,** recorded in $CDCl_3$ at 298K, 300 MHz.

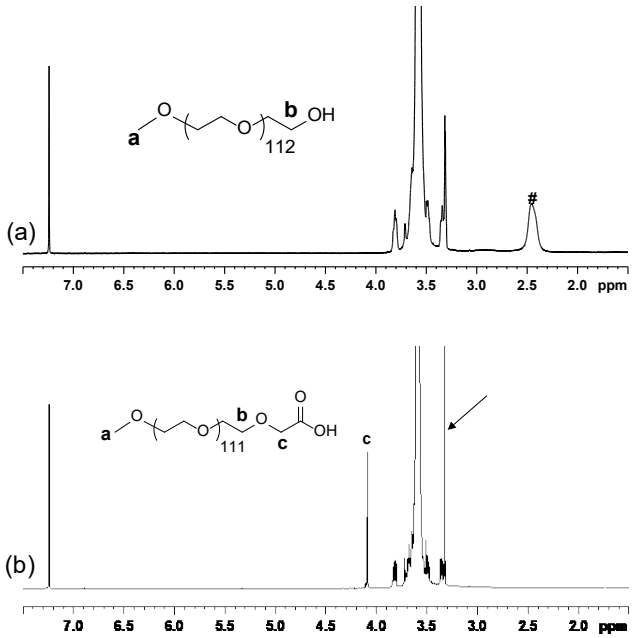


Figure S7: ^{1}H NMR spectra of MeO-PEG-OH (a) and MeO-PEG-COOH, **5** (b), recorded in $CDCl_3$ at 298K, 300 MHz, *=H_2O.

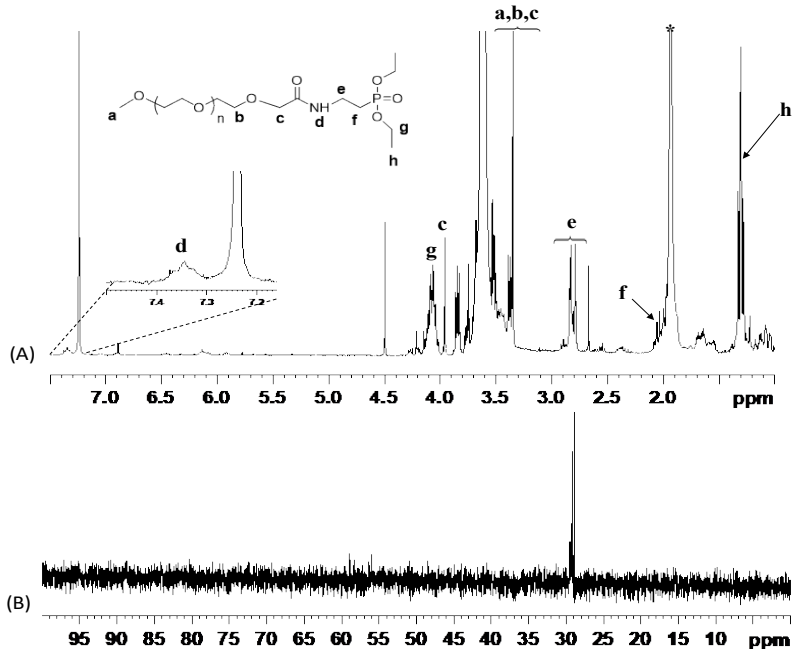


Figure S8: ^{1}H NMR (A) and ^{31}P NMR (B) spectra of Phosphonate-PEG **6**, recorded in $CDCl_3$ at 298K, 300 MHz, *=H_2O.

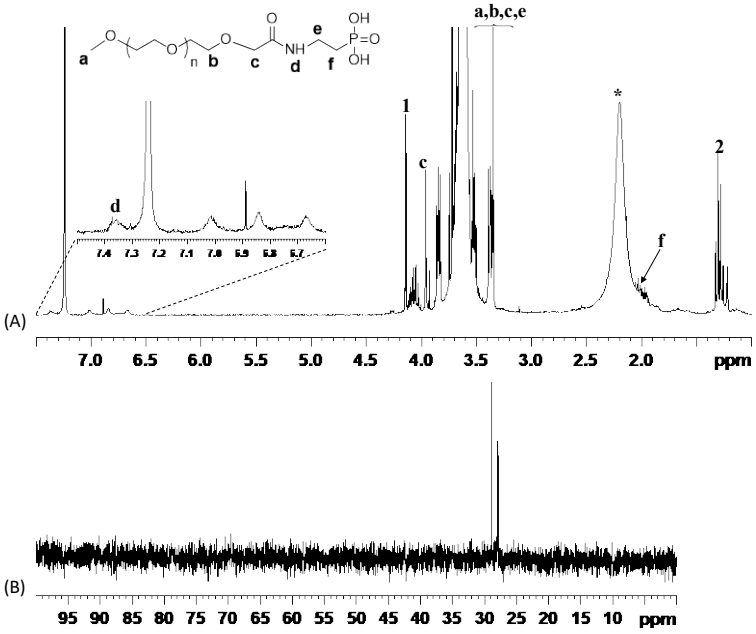


Figure S9: ^{1}H NMR (A) and ^{31}P NMR (B) spectra of Phosphonic acid-PEG **7**, recorded in $CDCl_3$ at 298K, 300 MHz, *=H_2O.

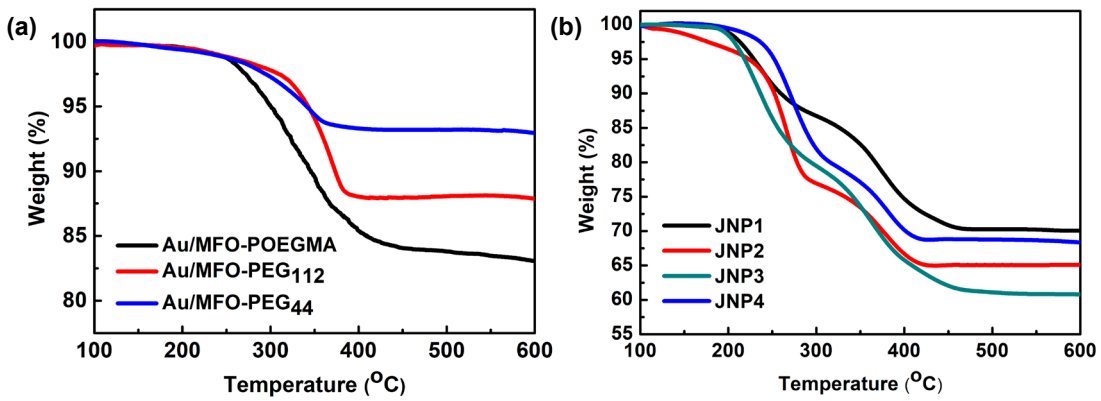


Figure S10: TGA curves of (a) POEGMA or PEG modified JNPs and (b) amphiphilic JNPs (from JNP1 to JNP4).

Table S1. TGA analysis results of polymer-conjugated Au/MFO.

Entry	Total Weight-Loss (%)[a]	Hydrophobic wt%[b]	Hydrophilic wt%[c]	Amphiphilic Ratio[d]
Au/MFO-POEGMA	17	/	17	/
Au/MFO-PEG_{112}	13	/	13	/
Au/MFO-PEG_{44}	8	/	8	/
JNP1	30	13	17	0.76
JNP2	35	22	13	1.69
JNP3	40	27	13	2.08
JNP4	33	25	8	3.12

a Determined by TGA (Figure S14), for Au/MFO-POEGMA, Au/MFO-PEG_{112}, Au/MFO-PEG_{44}, JNP1-4.
b Weight percentage of hydrophobic polymers content grafted on Au/MFO, obtained by the total weight loss subtract the weight loss of hydrophilic polymers of Au/MFO-POEGMA, Au/MFO-PEG_{112}, and Au/MFO-PEG_{44}.
c Weight percentage of hydrophilic polymers content grafted on Au/MFO, it is the same as their precursors Au/MFO-POEGMA, Au/MFO-PEG_{112}, and Au/MFO-PEG_{44}.
d Amphiphilic Ratio=Hydrophobic wt%/ Hydrophilic wt%

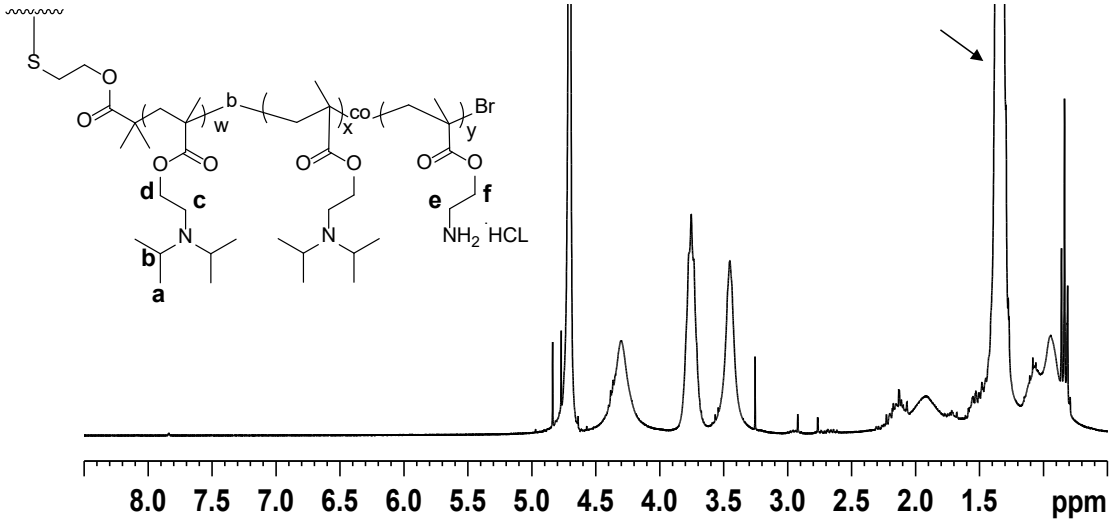


Figure S11: ^{1}H NMR spectrum of copolymer **9**, recorded in $CDCl_3$ at 298K, 300 MHz.

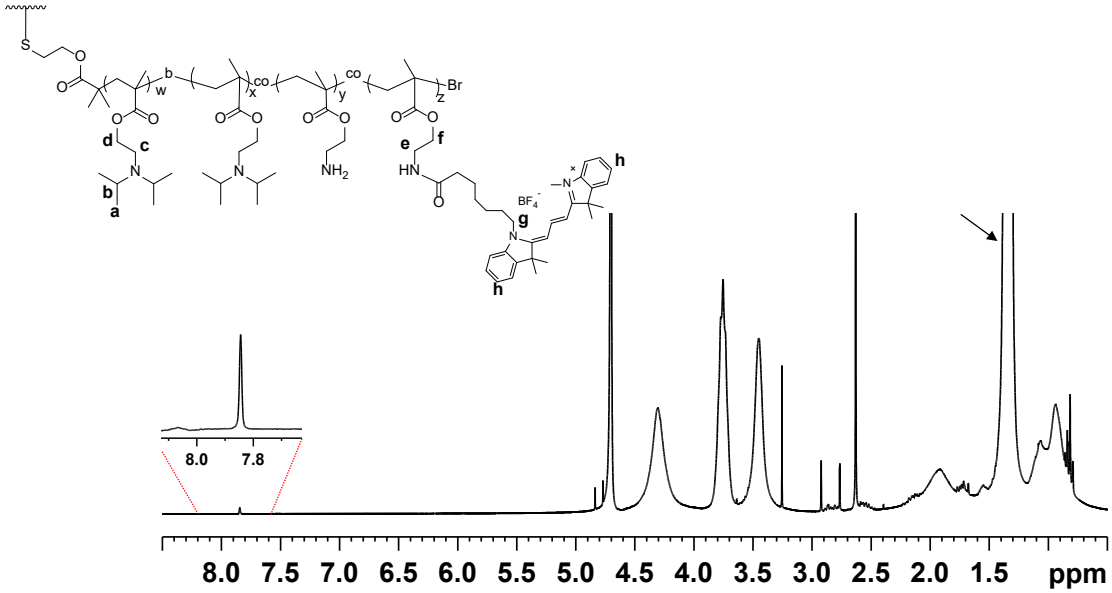


Figure S12: ^{1}H NMR spectrum of copolymer **10**, recorded in $CDCl_3$ at 298K, 300 MHz.

Table S2: Molecular data measured by aqueous SEC

Polymer	Molecular weight[a]			Hydrodynamic diameter (nm)		
	$M_{n,RI}$	$M_{w,RI}$	PDI	$D_{h,DMF}$[b]	$D_{h,HCl}$[c]	$D_{h,SEC}$[d]
8a	20440	25550	1.25	/	/	/
8b	32380	40730	1.21	/	/	/
8c	45160	56410	1.22	/	/	/
9a	24830	30790	1.20	6.2	7.6	10.8
9b	38000	47880	1.26	9.2	10.3	14.0
9c	52200	63680	1.22	12.5	14.0	16.6

a Determined by water phase SEC using a Waters Ultrahydrogel column with acidic buffer (0.5 M sodium acetate and 0.5 M acetic acid, with pH 4.5) as eluent.
b Determined by DLS in DMF.
c Determined by DLS in pH 4 HCl solution.
d Determined by Mark-Houwink equation[3, 4]:

$$R_h^3 = \frac{3KM^{a+1}}{10\pi N_A} \qquad \text{(Equation S7)}$$

Where R_h is hydrodynamic radius, N_A is Avogadro's number, a = 0.7 (in a good solvent for PDPA), K = 0.0537 $cm^3\ g^{-1}$
and hydrodynamic diameter:

$$D_h = 2R_h$$

Table S3 Composition of copolymer 11 and theoretical gap size

Entry	Number of units [a]			M_n [c]	D_h (nm)[d]	Max gap size(nm)[e]	Min gap size(nm)[f]	Conjugating Efficiency (%)[g]
	x	y	z[b]					
11a	48	18	1.2	12410	5.4	10.8	4.7	69.0
11b	76	21	1.4	19000	7.0	14.0	6.1	61.0
11c	106	29	1.7	26100	8.3	16.6	7.2	54.0

a Determined by molecular weight data from SEC, ^{1}H NMR, and UV-vis spectra.
b Determined by the UV-vis absorbance of Cy3 dye and Cy3-PDPA conjugates using equation S3.
c Half of M_n of 9a, 9b, and 9c.
d Determined by equation 7, Table S1, based on M_n of 11a (PDPACy3-I), 11b (PDPACy3-II), and 11c (PDPACy3-III).
e Theoretical maximum gap size of assemblies, equal to 2-fold of corresponding D_h of 11a, 11b, and 11c.
f Theoretical minimum gap size of assemblies, calculated by maximum gap size divided by shirking ratio 2.3. For example, minimum gap size of cluster using 11a as spacer is equal to 10.8/2.3, gave result of 4.7.

g Cy3 conjugating efficiency on copolymer, determined by equation S4.

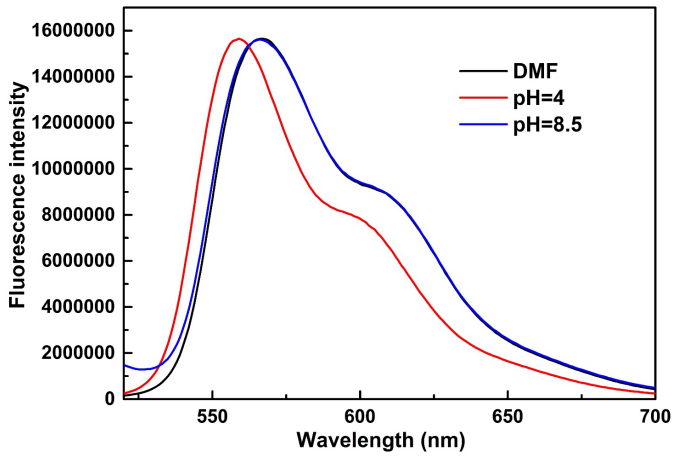


Figure S13: Fluorescence emission intensity spectra of copolymer **PDPACy3** in DMF, pH 4 and 8.5, excited at 500 nm. The QY of Cy3-PDPA copolymer **PDPACy3** are the same in three different solvent (0.19), calculated by Equation S5.

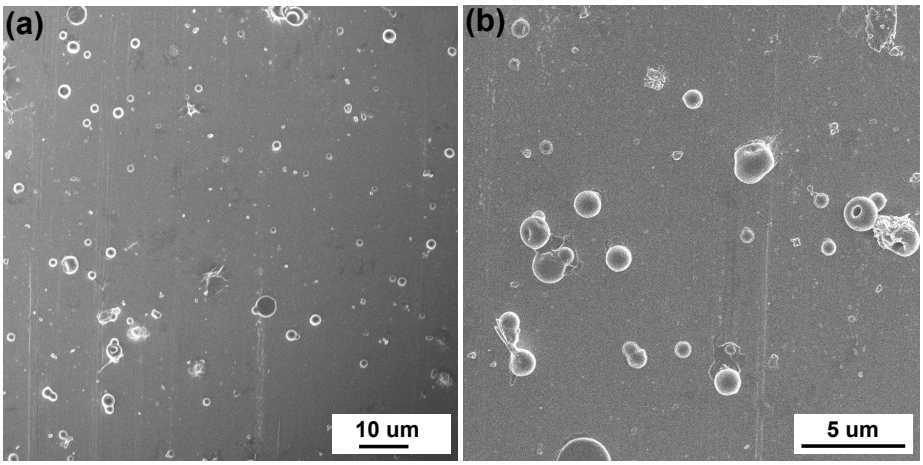


Figure S14: SEM images of vesicle assemblies. Full (a) and expanded (b).

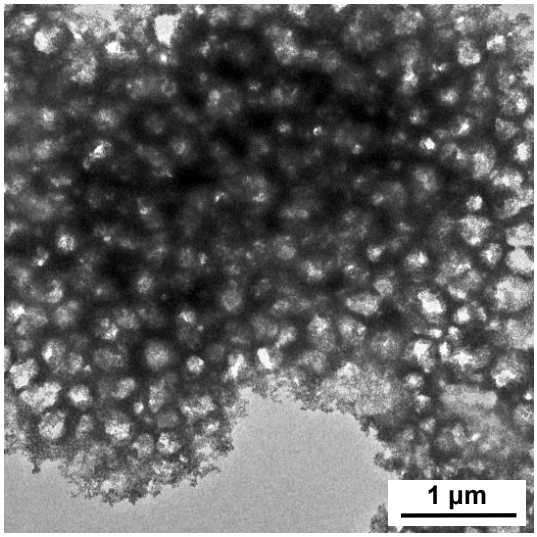


Figure S15: TEM images of aggregates derived from the self-assembly of JNP4 in pH 8.5 aqueous solution.

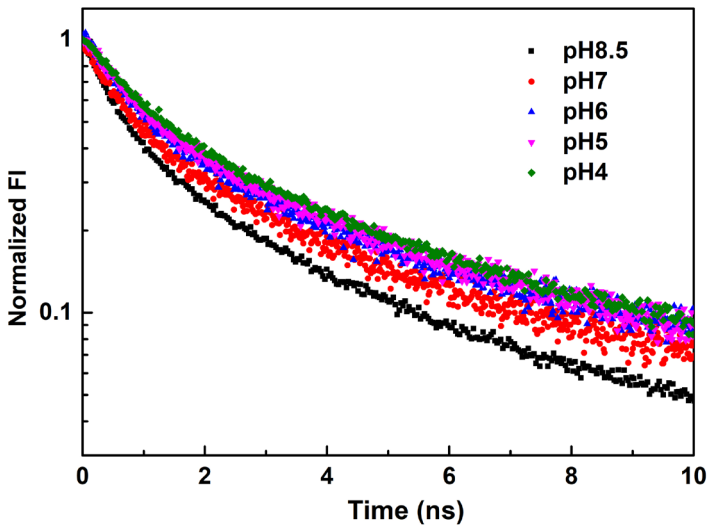


Figure S16: Time-resolved fluorescence intensity (FI) decays of clusters in aqueous solution as different pH, the curves were fitted with biexponential function.

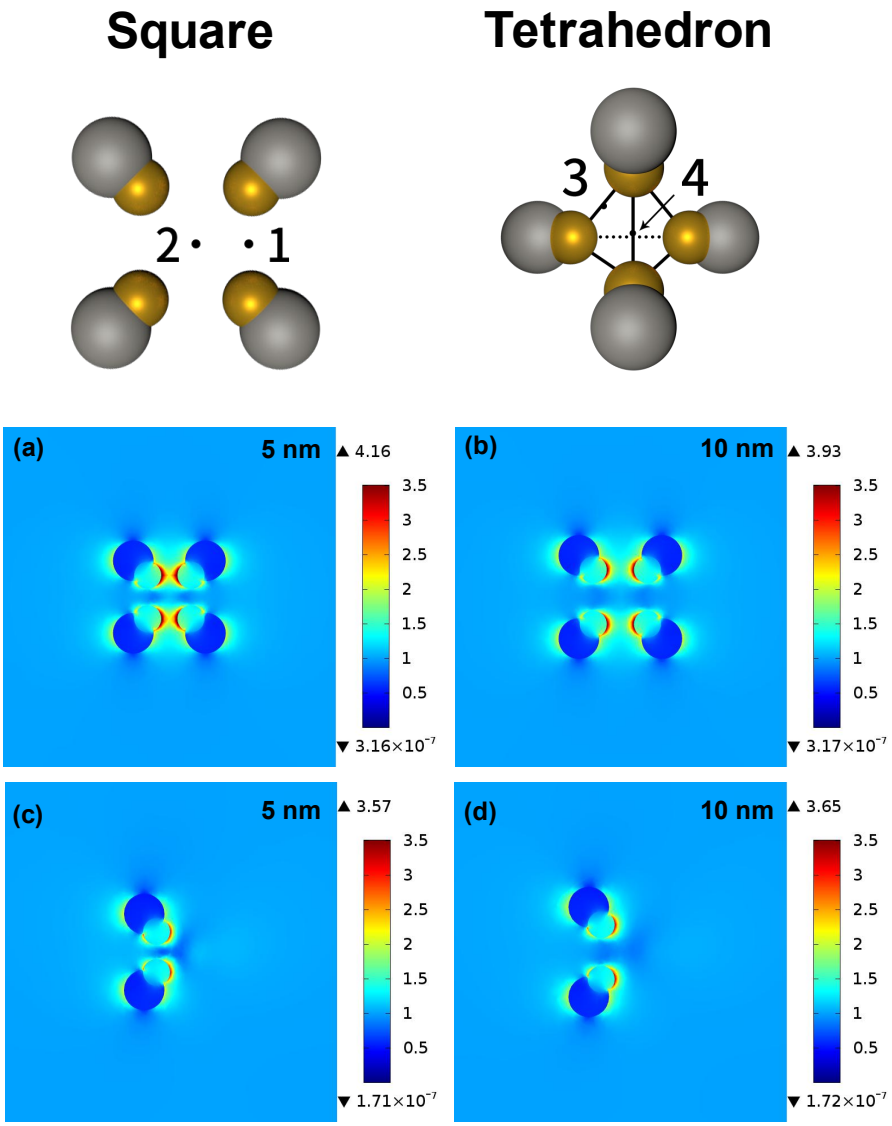


Figure S17: Cartoon illustrations of possible spatial structures of clusters, 1, 2, 3, and 4 refer to the four positions of Cy3 in the cluster for simulation; (a-d) Electric field profiles of the cluster. Refer to the square structure with gap size in 5 nm (a) and 10 nm (b); tetrahedron structure with gap size in 5 nm (c) and 10 nm (d).

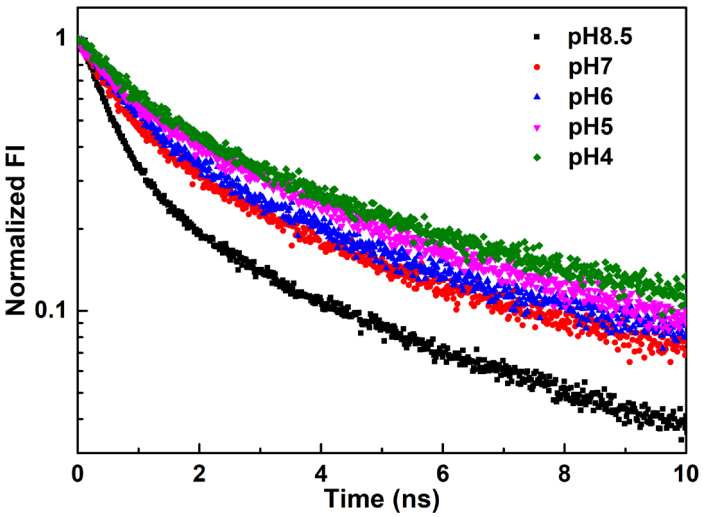


Figure S18: Time-resolved fluorescence intensity (FI) decays of vesicle in aqueous solution as different pH, the curves were fitted with biexponential function.

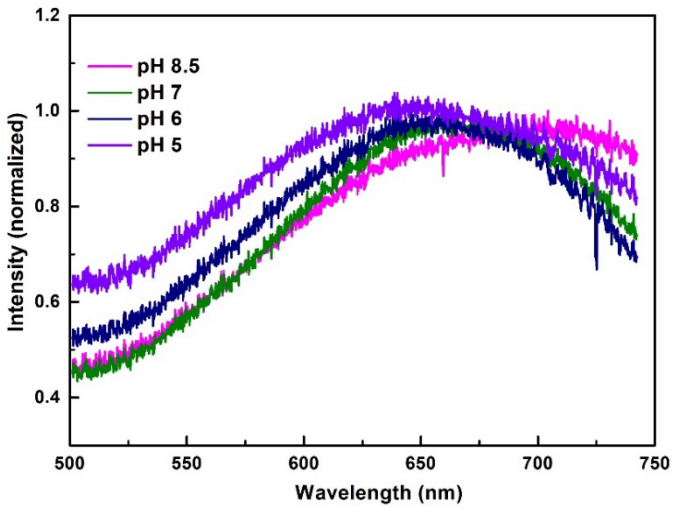


Figure S19: Scattering spectra of vesicles at different pH value. The vesicles assembled from JNP3. The scattering spectrum at pH 4 it too weak to be detected.

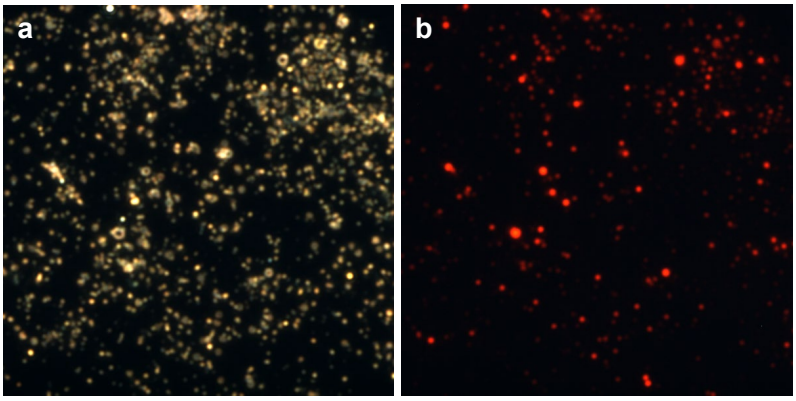


Figure S20: Dark-field (a) and fluorescence (b) images of vesicle ensembles at pH 6 aqueous solution.

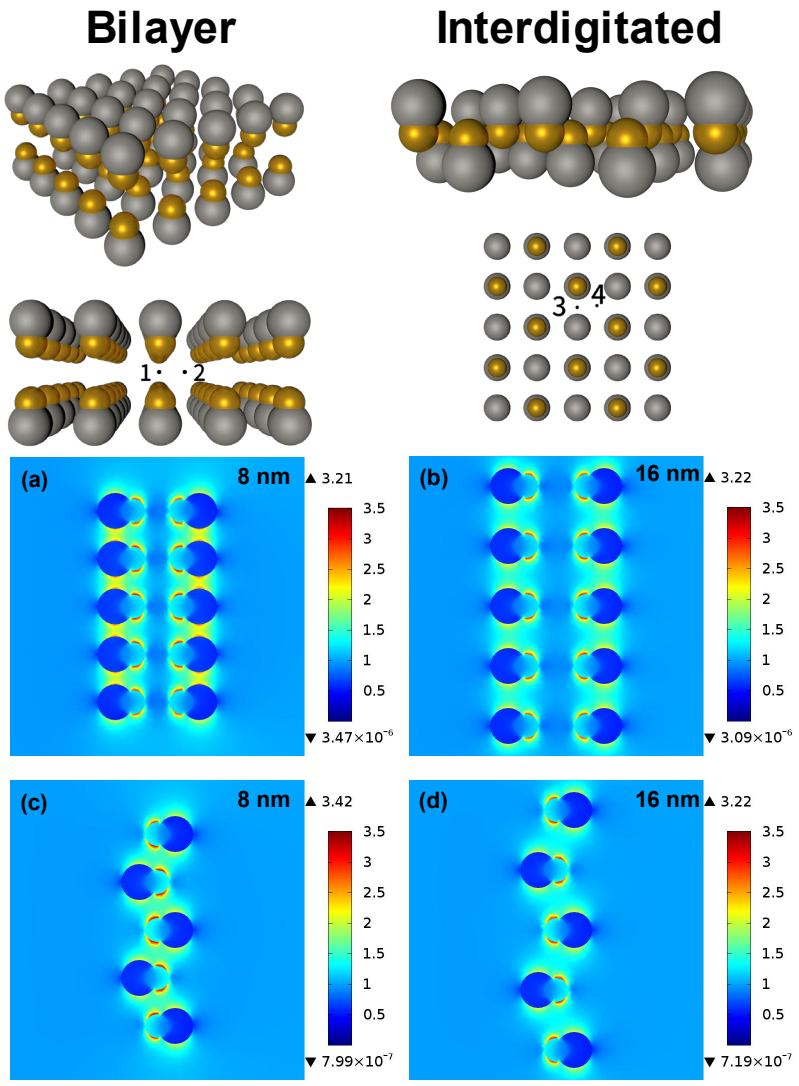


Figure S21: Cartoon illustrations of possible spatial structures of vesicles. 1, 2, 3, and

4 refer to the four positions of Cy3 in the vesicle for simulation; (a-d) Electric field profiles of the cluster and vesicle assemblies. Refer to bilayer structure with gap size in 8 nm (a) and 16 nm (b); interdigitated structure with gap size in 8 nm (c) and 16 nm (d).

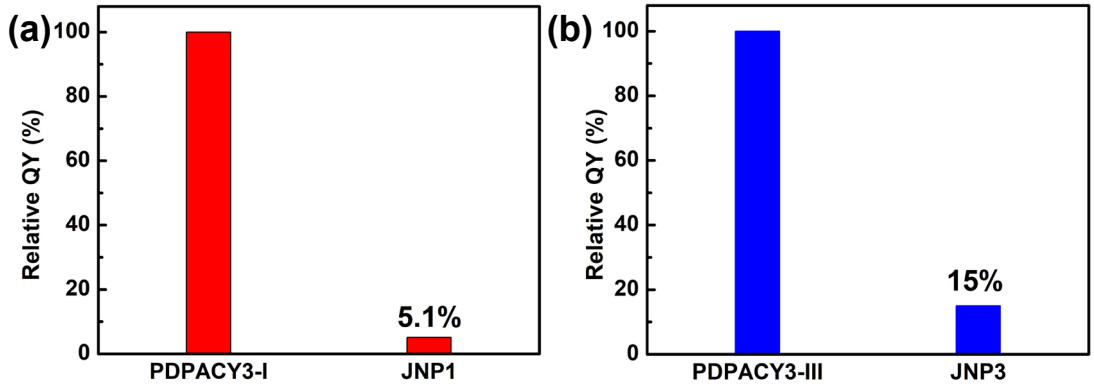


Figure S22: Relative QY of monomer JNP1 (a) and JNP3 (b) compared to corresponding PDPACy3 copolymers. Calculated by Equation S6.

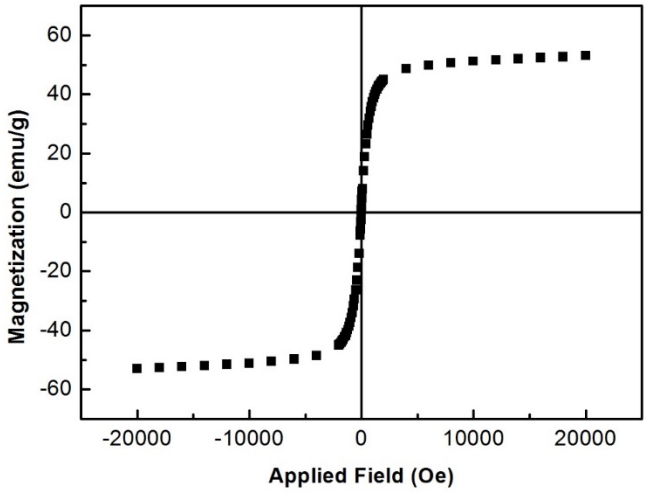


Figure S23: Hysteresis loop of Au/$MnFe_2O_4$ Janus nanoparticles at room temperature.

References:

1. Song, J. B.; Cheng, L.; Liu, A. P.; Yin, J.; Kuang, M.; Duan, H. W. *J Am Chem Soc* **2011,** 133, (28), 10760-10763.

2. Brouwer, A. M. *Pure Appl Chem* **2011,** 83, (12), 2213-2228.

3. Gois, J. R.; Rocha, N.; Popov, A. V.; Guliashvili, T.; Matyjaszewski, K.; Serra, A. C.; Coelho, J. F. J. *Polym Chem-Uk* **2014,** 5, (12), 3919-3928.

4. Lu, D. R.; Hossain, M. D.; Jia, Z. F.; Monteiro, M. J. *Macromolecules* **2015,** 48, (6), 1688-1702.