Supporting Information

Design polymer ligand for one-step preparation of highly stable fluorescent Ag_5 clusters for tissue labeling

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SI-1: Experiment Section

Quantum Yield (QY) Measurement. The QY of a compound is defined as the fraction of molecules that emit a photon after direct excitation by the source.[1] This quantity is not the same as the total number of emitted photons which escape a bulk sample divided by the total number of absorbed photons, although in many instances the two quantities are nearly equal. The measurement of QY was employed the compared method which is described below (Equation 1):

$$\Phi_{unk} = \frac{A_{std}}{A_{unk}} \times \frac{F_{unk}}{F_{std}} \times \frac{n_{unk}^2}{n_{std}^2} \times \Phi_{std} \qquad (1)$$

Φ = Quantum Yield; *unk* = Unknown Sample; *std* = Standard; *n* = Refractive index of solvent; *A* = Absorption at the selected excitation wavelength; *F* = Integrated fluorescence signal in the emission region. To calculate the QY of AgNCs, we measured a series of the samples and the standard Rhodamine 6G (Φ = 0.95 in ethanol). All the samples were diluted to ensure the optical densities less than 0.02 measured by Lambda 35 UV-visible spectrometer (Perkin-Elmer, USA) in order to reduce the error. The emission spectra were recorded on FP-6500 fluorescence spectrometer (Jasco, JPN) at 25 ^{o}C.

SI-2: The Characterization of Tridentate Polymer Ligands

Gel Permeation Chromatography. GPC was performed with an Agilent 1100 instrument using refractive index detector (RID) in order to obtain the molecular weights of polymer ligands. THF was used as the eluent at a flow rate of 1.0 mL/min at 23 ^{o}C. The polymer was first converted to the methyl ester using TMS-diazomethane reagent to render it soluble in THF.[2] The calculated molecular weights were based on a calibration curve for polystyrene standards (Polymer Laboratories).

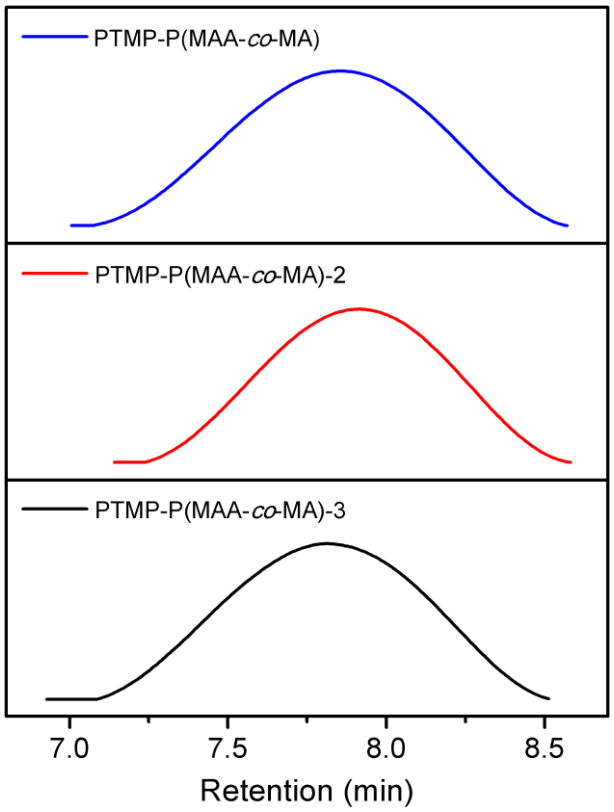

Fig. S1 GPC elution curves of polymer ligands PTMP-P(MAA-*co*-MA), PTMP-P(MAA-*co*-MA)-2, and PTMP-P(MAA-*co*-MA)-3 with MAA:MA monomer ratio of 6:1, 4:1, and 8:1, respectively.

Table S1. Molecular weight and poly dispersity of polymer ligands measured by GPC method.

Label	Polymer Ligands	Monomer Mole ratio (MAA:MA)	GPC	
			Mn (g/mol)	*PDI*
A	PTMP-P(MAA-*co*-MA)	6:1	7400	1.40
B	PTMP-P(MAA-*co*-MA)-2	4:1	6900	1.47
C	PTMP-P(MAA-*co*-MA)-3	8:1	7850	1.42
D	PTMP-PMAA [†]	homopolymer	8400	1.31

[†]PTMP-PMAA was prepared by our previous work.[3]

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded in DMSO on a Bruker AV400 MHz spectrometer at room temperature using the δ scale and tetramethylsilane (TMS) as an internal standard. The ^{1}H NMR spectra were similar to our previous works.[4]

PTMP-P(MAA-*co*-MA) (DMSO-d_6) δ (ppm): 0.78-1.12 CH_3, 1.58-2.10 CH_2, 3.21-3.55 CH, 2.52-2.60 CH_2 (from PTMP), 2.62-2.74 CH_2 (from PTMP), 4.28-4.48 CH_2 (from PTMP).

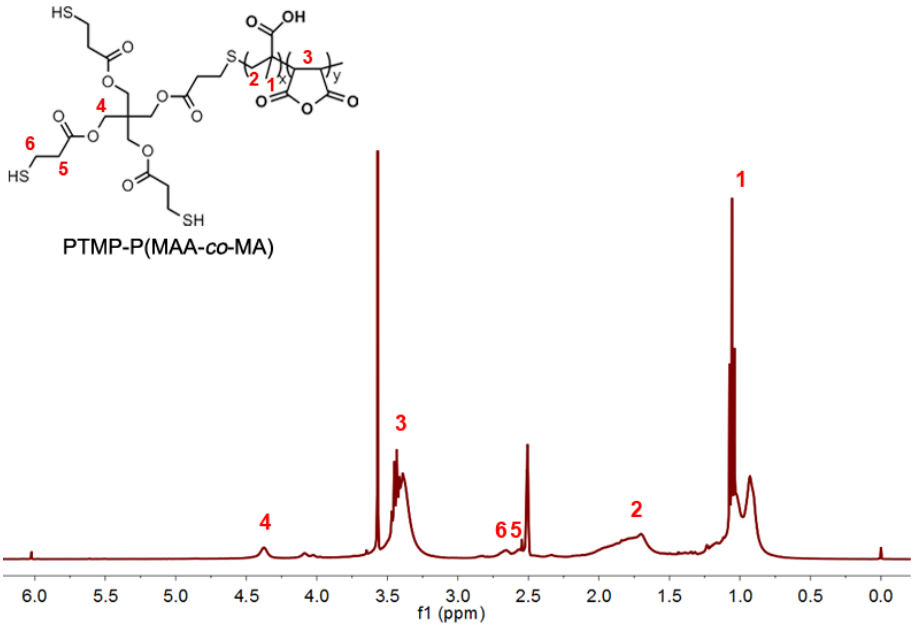

Fig. S2 ^{1}H NMR of thioether polymer ligands PTMP-P(MAA-*co*-MA) dissolved in DMSO-d_6.

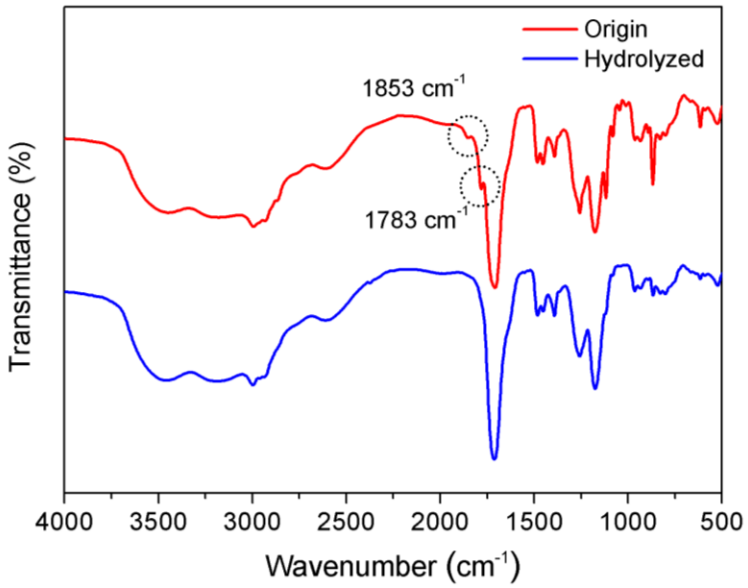

Fig. S3 FTIR spectra of PTMP-P(MAA-*co*-MA) before (red) and after (blue) hydorlyzed. The disappearance of anhydride groups at 1783 cm^{-1} and 1853 cm^{-1} indicates all the maleic anhydride groups had been hydrolyzed to maleic acid.

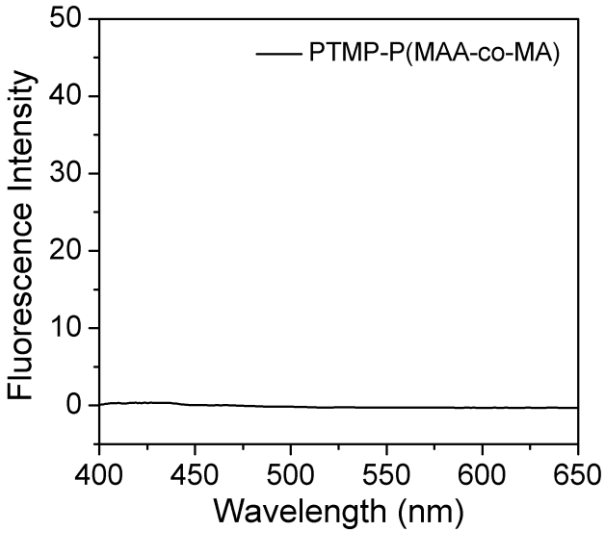

Fig. S4 Fluorescence emission spectra of PTMP-P(MAA-*co*-MA) ligand.

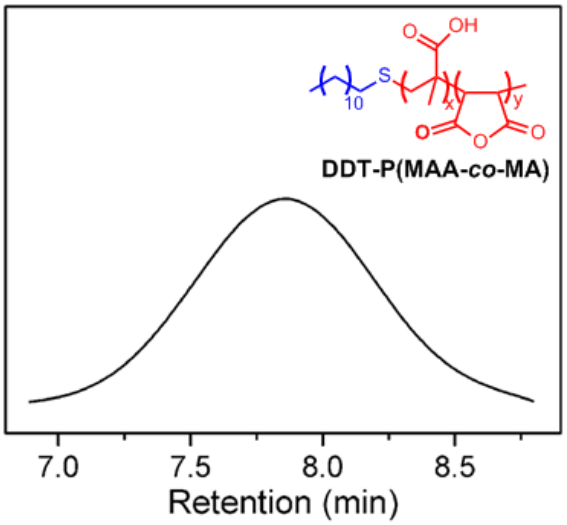

Fig. S5 GPC elution curves of polymer ligands DDT-P(MAA-*co*-MA) (*Mn* = 7750, PDI = 1.51).

SI-3: The Characterization of AgNCs

Fig. S6 Photographs of AgNCs@PTMP-P(MAA-*co*-MA) in aqueous solution (left) and a solid powder (right) under room light.

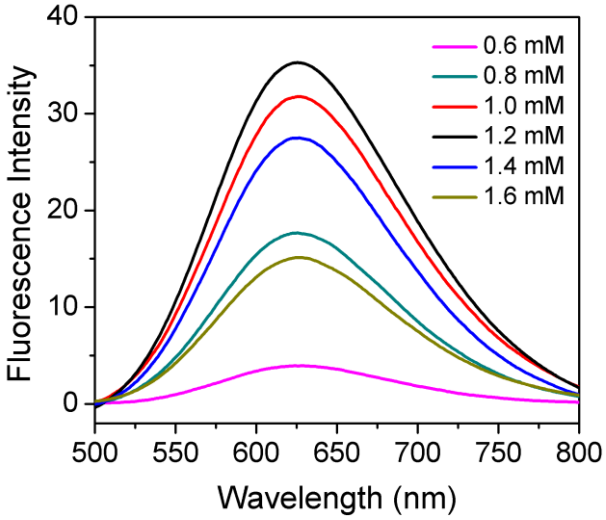

Fig. S7 Fluorescence emission spectra of AgNCs@PTMP-P(MAA-*co*-MA) prepared with different ligands concentrations.

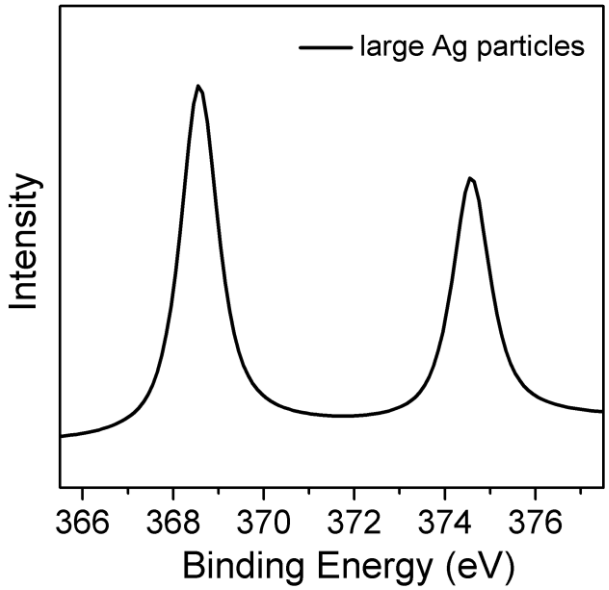

Fig. S8 XPS spectra of large Ag particles prepared by directly reduction of $AgNO_3$ by $NaBH_4$ without ligand. The binding energy (BE) of Ag $3d_{5/2}$ and Ag $3d_{3/2}$ of large Ag particles were 368.5 eV and 374.5 eV, respectively.

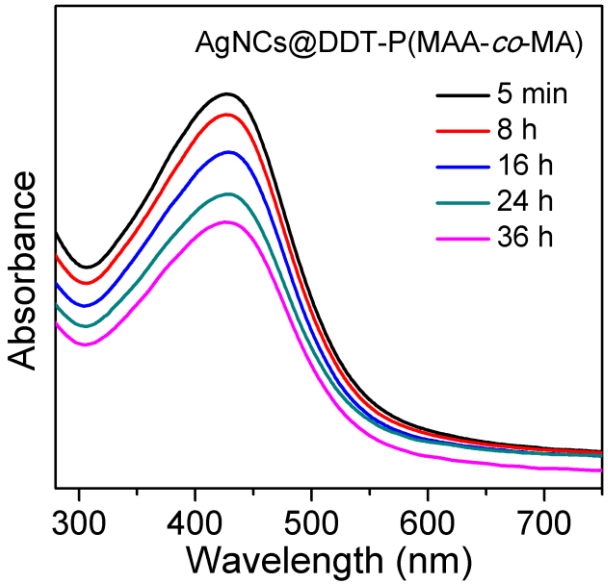

Fig. S9 Time dependent evolution of UV-Vis absorption spectra of aqueous AgNCs@DDT-P(MAA-*co*-MA), ligand concentration was 1.2 mM.

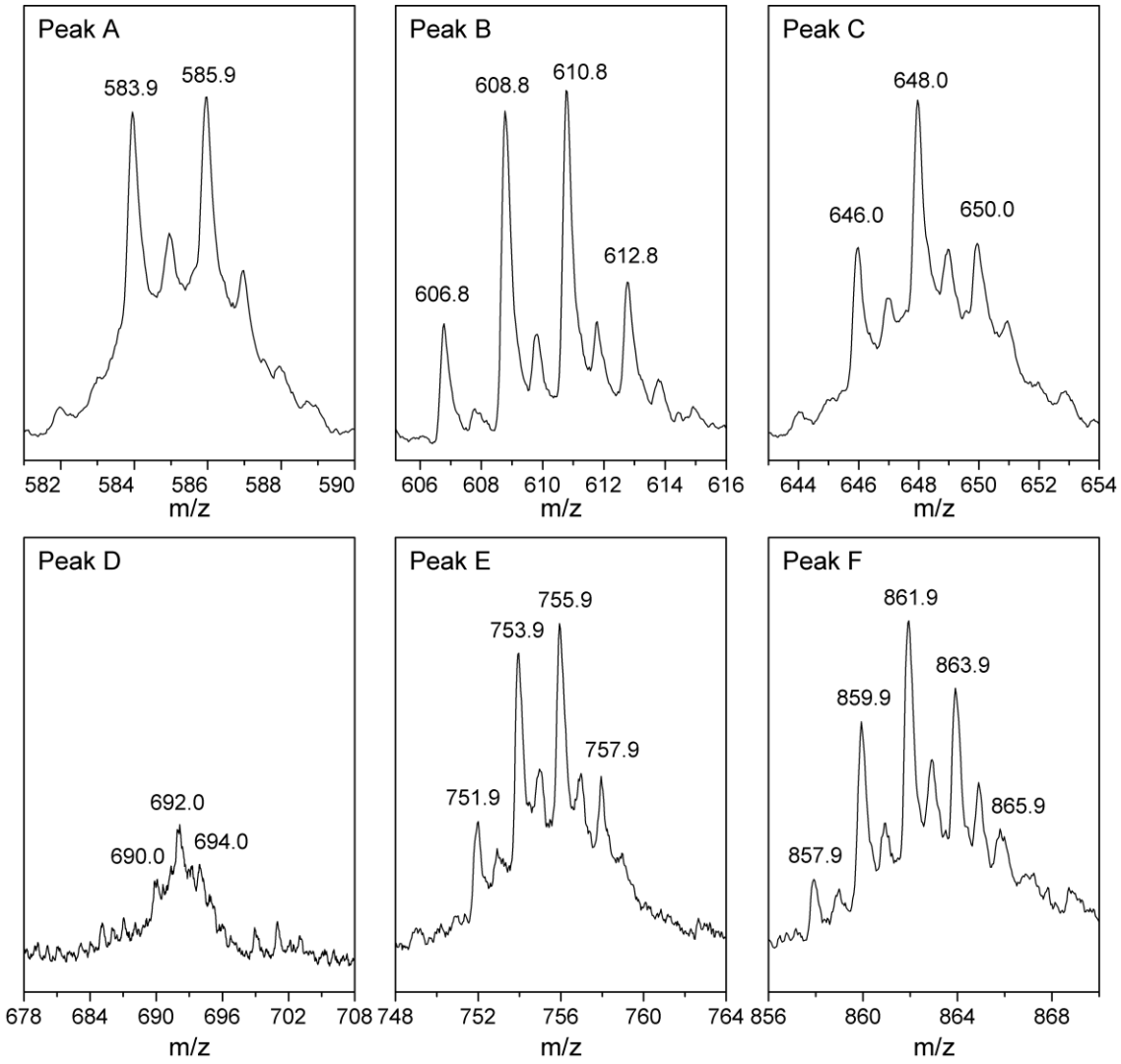

Fig. S10 MALDI-TOF results of isotopic patterns for species A-F (see labels in Fig. 2a). It is a little diffiicult to

determine the number of Ag atoms in AgNCs because the isotopic distributions of silver clusters are complicated significantly by the two abundant naturally occurring silver isotopes, 107 Ag and 109 Ag.

Table S2. List of isotopic patterns for species A-F determined by MALDI-TOF.

Peak	m/z	Composition
A	585.9	Ag_4S_2 - 4H + 4Na
B	608.8	Ag_4S_2 - 5H + 5Na
C	648.0	Ag_5S_2 - 2H + 2Na
D	692.0	Ag_5S_2 - 4H + 4Na
E	755.9	Ag_6S_2 - 2H + 2Na
F	862.0	Ag_7S_2 - H + Na

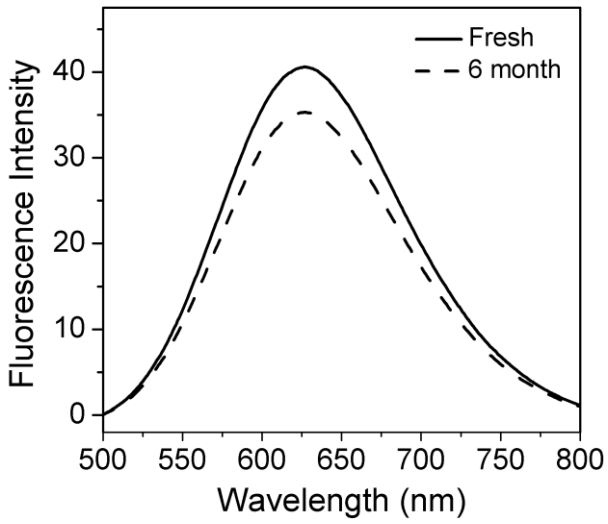

Fig. S11 Fluorescence emission spectra of fresh AgNCs@PTMP-P(MAA-*co*-MA) solution (solid line) and after 6 month storage (dash line).

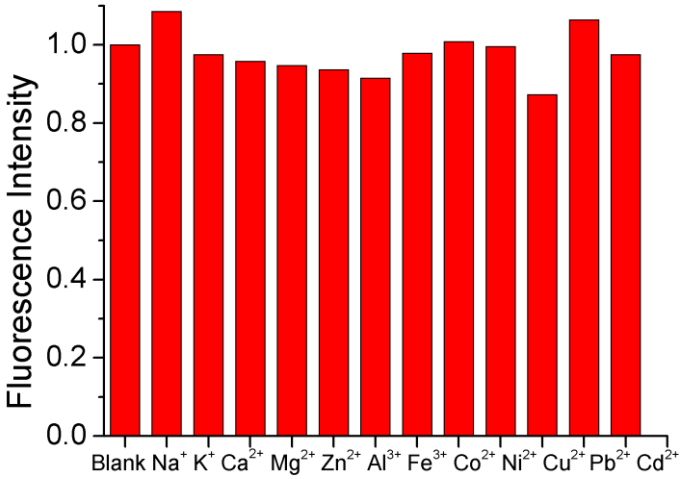

Fig. S12 The histogram of relative fluorescence intensity of AgNCs@PTMP-P(MAA-*co*-MA) in different metal ion solutions. The concentration of metal ions nitrates solution (0.2 M) were prepraed firstly, then AgNCs were added in to each solution and the final concentration of AgNCs was 0.25 mM.

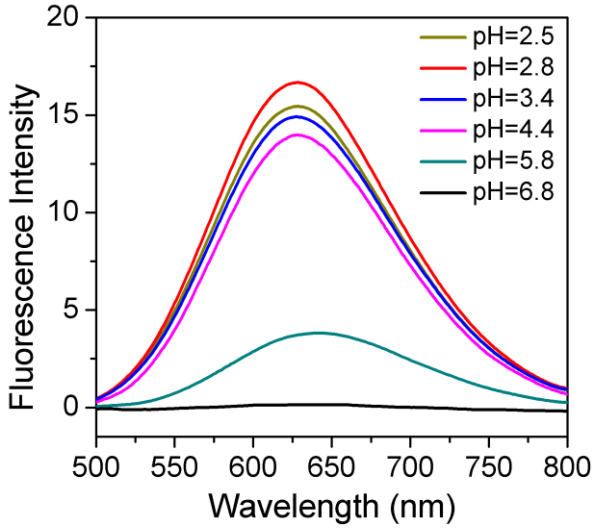

Fig. S13 Fluorescence emission spectra of AgNCs@PTMP-P(MAA-co-MA) dissoved in buffer solutions with different pH value, respectively.

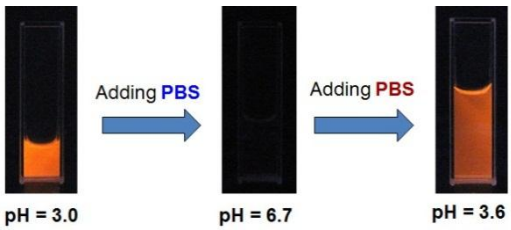

Fig. S14 Photographs of AgNCs@PTMP-P(MAA-co-MA) solution under UV light adjusted to different pH by PBS. The fluorescence of AgNCs disappeared when the pH was adjusted to neutral and returned back when the solution pH was changed back to acid.

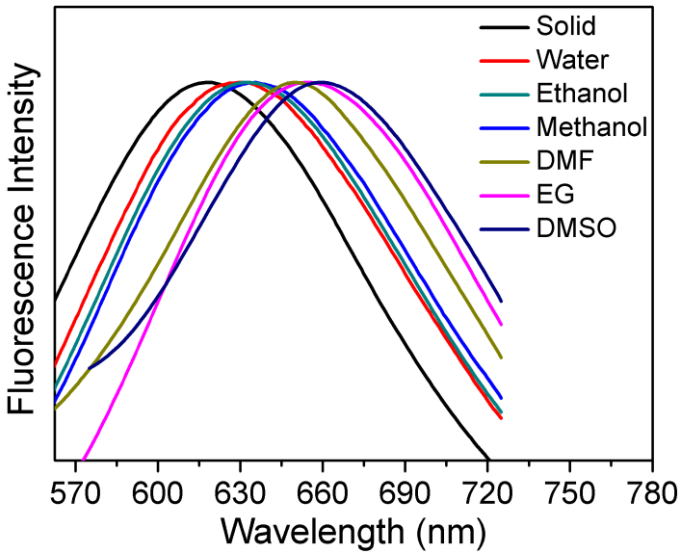

Fig. S15 Normalized fluorescence emission spectra of AgNCs@PTMP-P(MAA-*co*-MA) redissolved in different solvent including water.

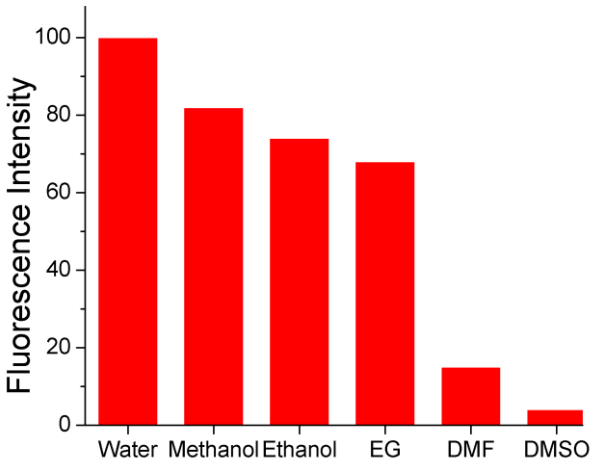

Fig. S16 Relative fluorescence intensity of AgNCs@PTMP-P(MAA-*co*-MA) dissolved in different solvent. Comparing to protic solvents, the aprotic solvents DMF and DMSO brought about a dramatically fluorescence quenching. This may be due to the protic solvents could formed strong hydrogen bond with carboxyl groups of the ligand to formed compact structure of polymer chain and resist solvent quenching.

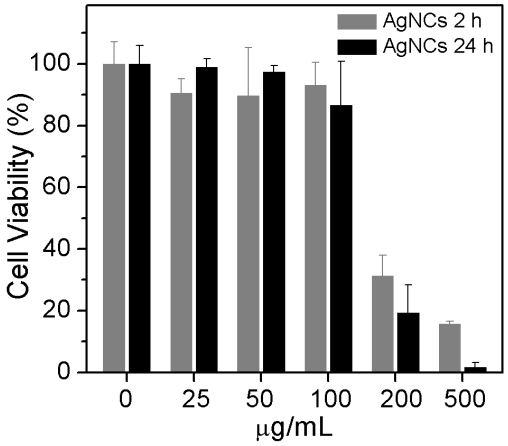

Fig. S17 Cytotoxic responses to AgNCs in Human Umbilical Vein Endothelial Cells (HUVEC) measured by the MTT assay.

SI-3: References and Notes

1. G. A. Crosby and J. N. Demas, *J. Phys. Chem.* , 1971, **75**, 991-1024.
2. L. Couvreur, C. Lefay, J. Belleney, B. Charleux, O. Guerret and S. Magnet, *Macromolecules*, 2003, **36**, 8260-8267.
3. H. Zhang, X. Huang, L. Li, G. Zhang, I. Hussain, Z. Li and B. Tan, *Chem. Commun.* , 2012, **48**, 567-567.
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