Importance of Evaluating Dynamic Encapsulation Stability of Amphiphilic Assemblies in Serum
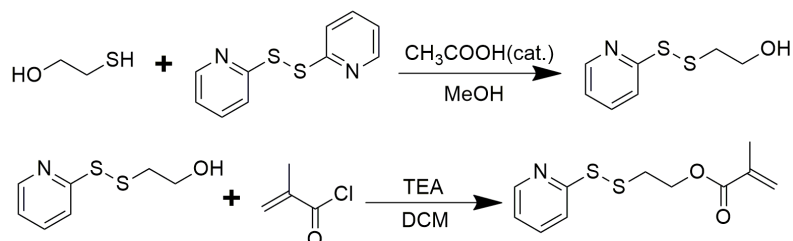
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Synthesis of the PDS monomer

Scheme S1. Synthesis of the PDS monomer

The PDS monomer was prepared by two steps according to our previous paper as shown in scheme S1. The NMR data of the monomers were shown in Fig. S1 and Fig. S2, which matched the literature results.[1]
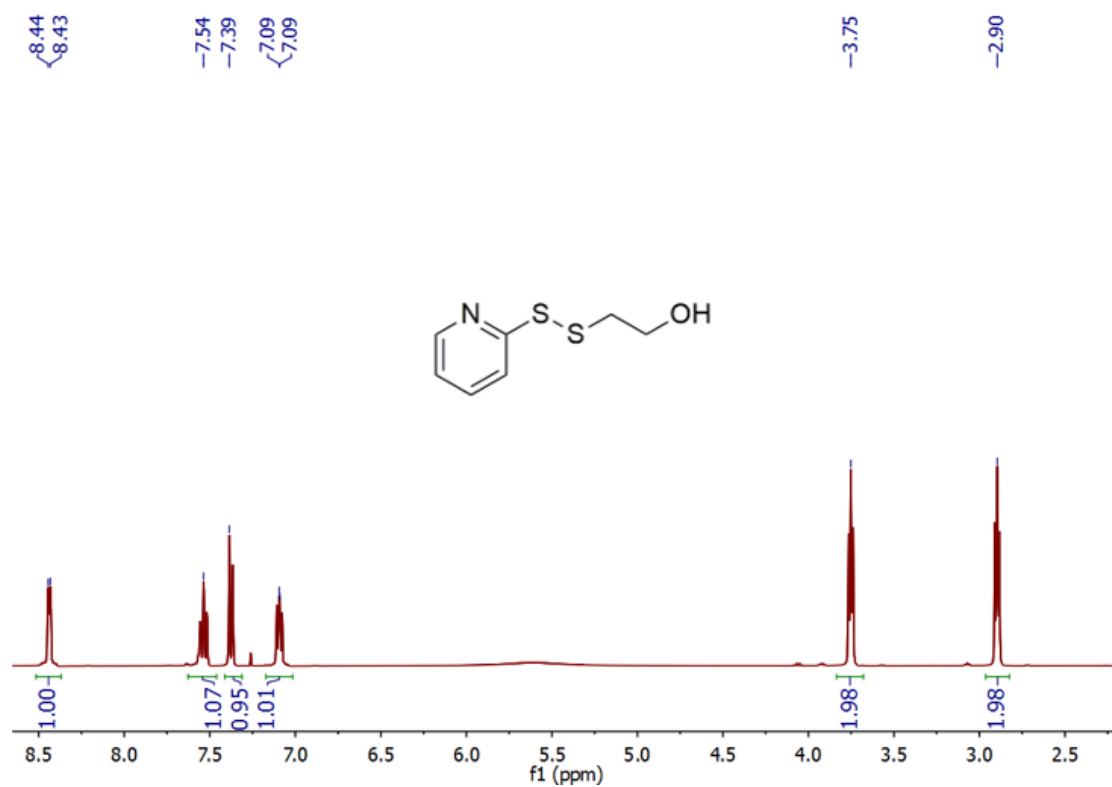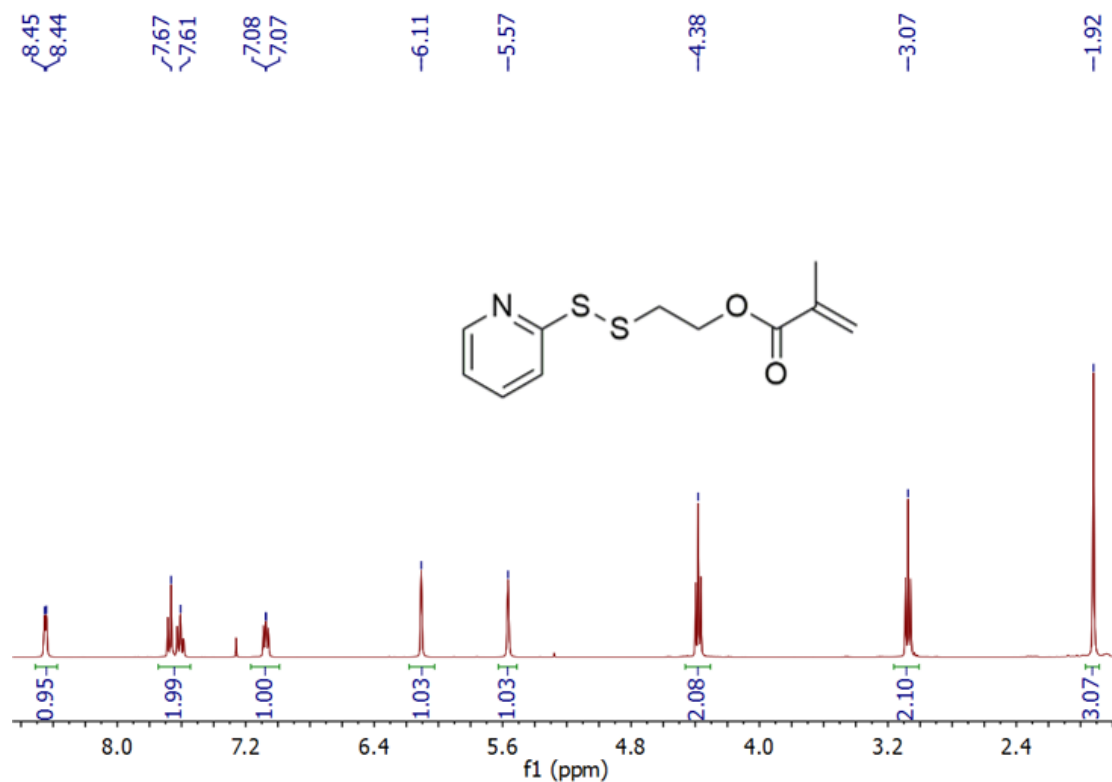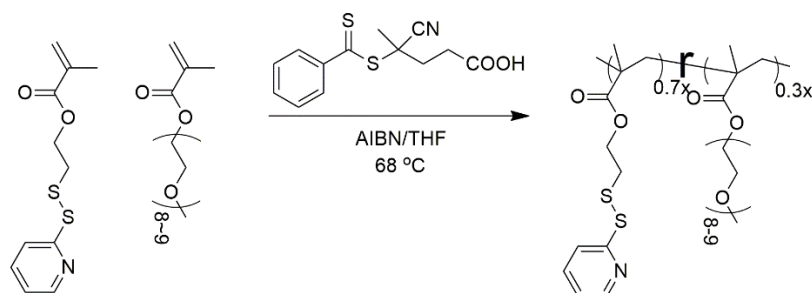
Fig. S1. NMR spectrum of PDS-OH.

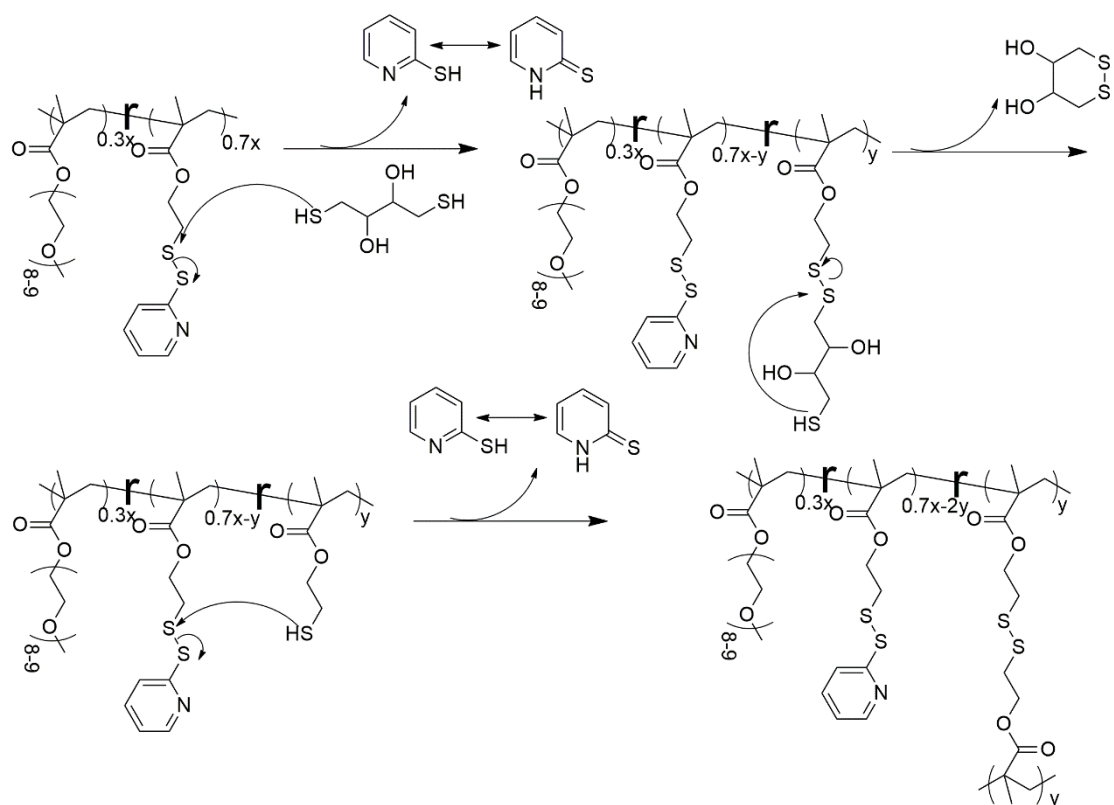**Fig. S2.** NMR spectrum of PDS monomer

Synthesis of PDS-co-PEG polymer

Scheme S2. Synthesis of PDS-PEG polymer

A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (24.3 mg, 0.087 mmol), PDSMA (776 mg, 3.04 mmol), polyethylene glycol monomethyl ether methacrylate (average MW: 500, 652 mg, 1.3 mmol) and AIBN (2.86 mg, 0.017 mmol) was dissolved in 3 mL THF and degassed with three freeze-pump-thaw cycles. Then the reaction was transferred to a pre-heated oil bath (68 °C) and stirred for 24 hours under argon atmosphere. The resultant polymer was precipitated in cold diethylether and washed with ether for several times to remove unreacted monomers. ^{1}H NMR spectrum of PDS-PEG was shown in Fig. S3. Based on the NMR spectrum, the ratio between PDS and PEG was calculated based on the NMR integration of peak at 8.45 ppm (PDS group) and 3.37 ppm (PEG group). The monomer ratio in polymer was calculated to be 7:3, which was the same as the feed ratio. The molecular weight of the polymer was determined by GPC and the results were shown in Fig. S4.

Fig. S3. NMR spectrum of PDS-PEG polymer

Mn (g/mol)	Mw (g/mol)	PDI
12121	14018	1.16

Fig. S4. GPC of PDS-PEG polymer

The crosslink mechanism of the nanogel

Scheme S3. The crosslink mechanism of the nanogel

Crosslink degree calibration and determination by UV

A

B

Equation	y = a + b*x		
Adj. R-Square	0.99997		
		Value	Standard Error
B	Intercept	-0.0055	0.00266
B	Slope	25.21023	0.05698

C

D

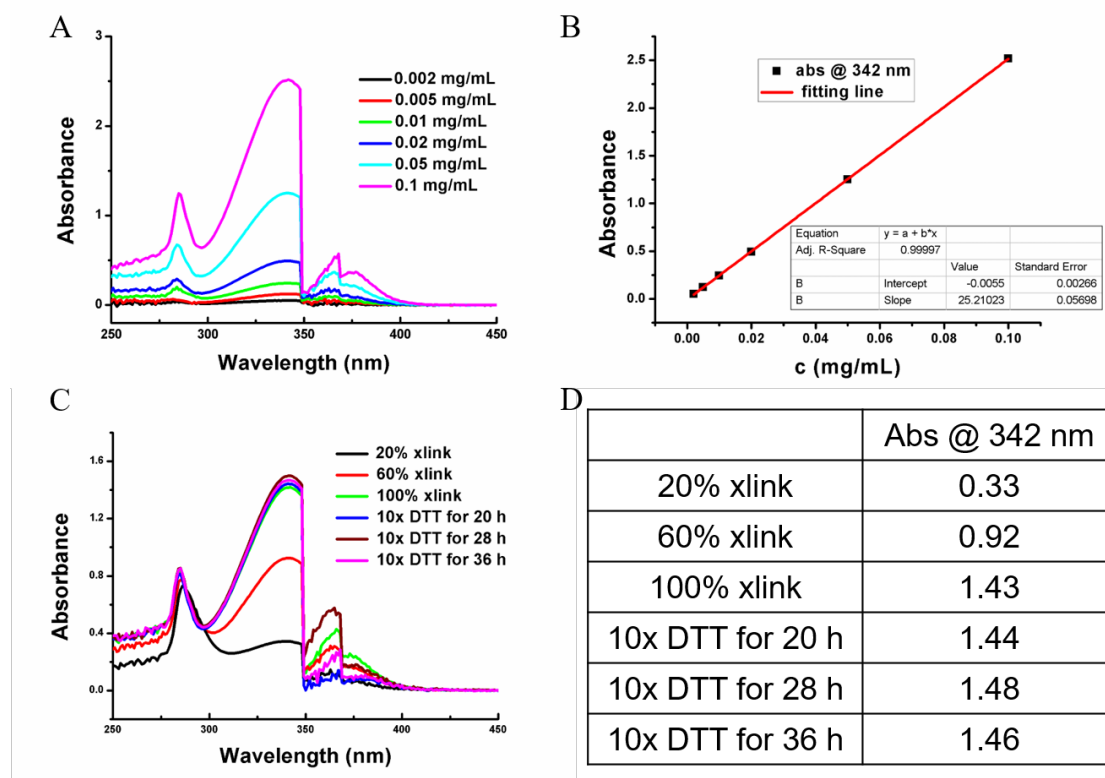|	Abs @ 342 nm
20% xlink	0.33
60% xlink	0.92
100% xlink	1.43
10x DTT for 20 h	1.44
10x DTT for 28 h	1.48
10x DTT for 36 h	1.46

Fig. S5. A) the absorbance spectrum of 2-pyridinethione at different concentrations in water. B) the linear fitting curve of the 2-pyridinethione based on the absorbance at 342 nm. C) and D) the absorbance for different crosslink degree. The absorbance of nanogel for different crosslink degrees are very similar to the designed crosslink degree. Further, the 10-fold excess of DTT induced the similar absorbance as that of 100% crosslink degree. All these results demonstrated that the crosslink degree can be well controlled by the feed amount of the DTT.
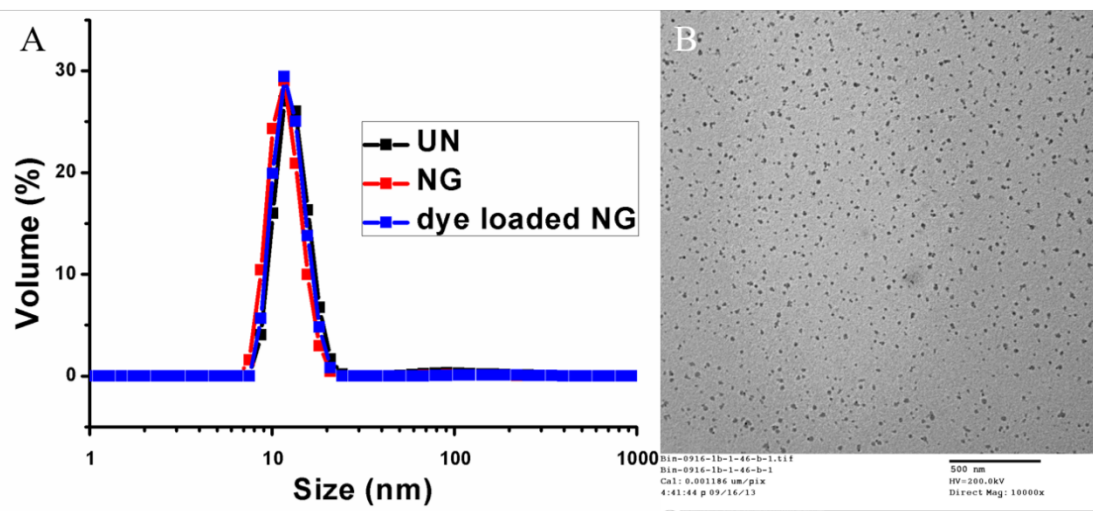
Fig. S6. A) DLS of the uncrosslinked nanoassembly (UN), nanogel (NG) and dye loaded nanogel, B) TEM image of dye loaded nanogel.
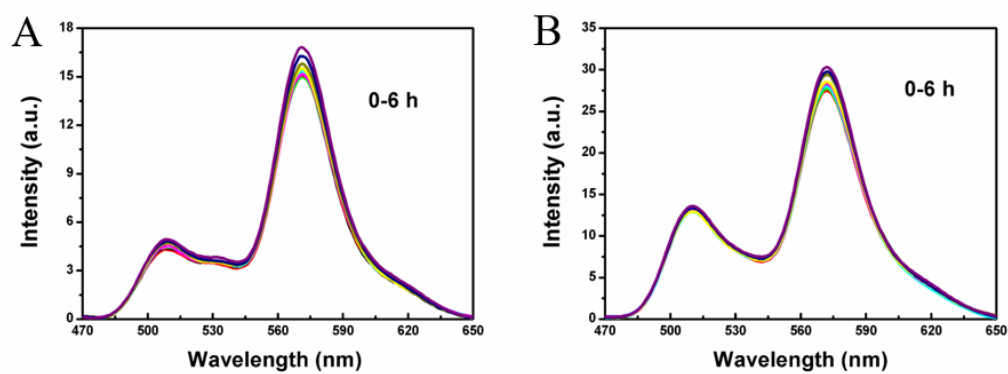

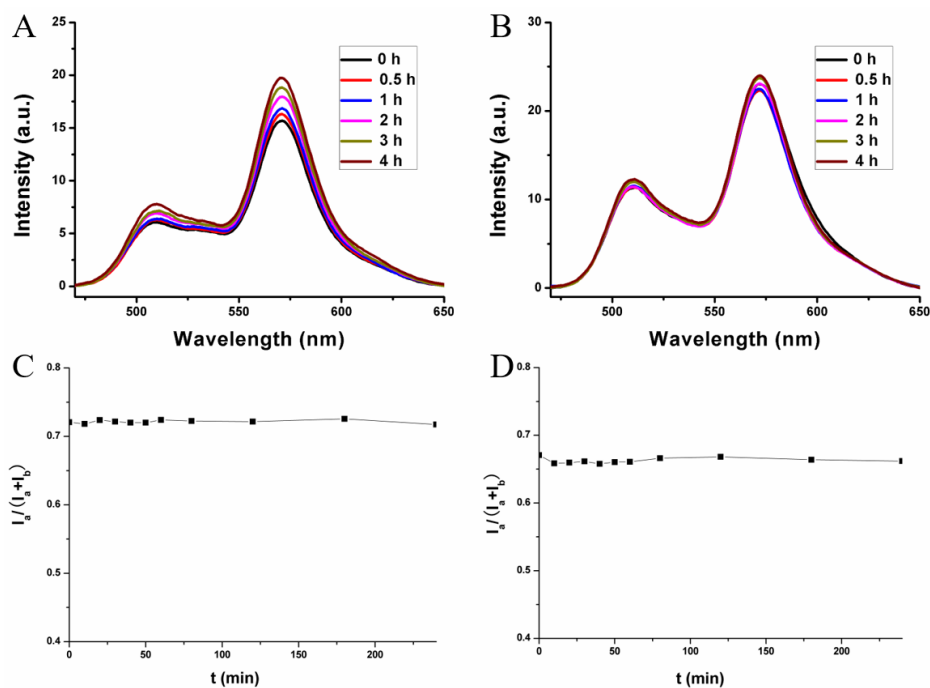**Fig. S7.** The FRET evolution in water: A) uncrosslinked nanoassembly, b) nanogel.

Fig. S8. The FRET evolution of the nanogels: A) and B) Fluorescence spectra, C) and D) FRET ratio. Condition for A and C: 0.02 mL of the dye (DiI+DiO) loaded NG (2 mg/mL), 0.08 mL of the blank uncrosslinked nanoassembly (2 mg/mL) and 0.9 mL H$_2$O. Condition for B and D: 0.02 mL of the dye (DiI+DiO) loaded uncrosslinked nanoassembly (2 mg/mL), 0.08 mL of the blank NG (2 mg/mL) and 0.9 mL H$_2$O.

Synthesis of the amphiphilic random copolymer
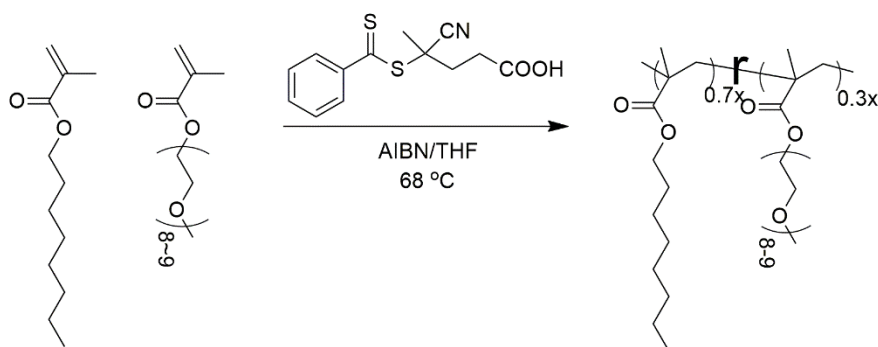
Scheme S4. Synthesis of the amphiphilic random copolymer

A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (24.3 mg, 0.087 mmol), octyl methacrylate (OMA, 603 mg, 3.04 mmol), polyethyleneglycol monomethylether methacrylate (average Mw: 500, 652 mg, 1.3 mmol) and AIBN (2.86 mg, 0.017 mmol) were dissolved in 3 mL THF and degassed with three freeze-pump-thaw cycles. Then the reaction mixture was transferred to a pre-heated oil bath (68 °C) and stirred for 24 hours under argon atmosphere. The resultant polymer was precipitated in cold diethylether and washed with ether for several times to remove unreacted monomers. ^{1}H NMR spectrum of POMA-PEG was shown in Fig. S6. Based on the NMR spectrum, the ratio between POMA and PEG was calculated based on the NMR integration of peak at 4.08 ppm (POMA group) and 3.38 ppm (PEG group). The monomer ratio in polymer was calculated to be 7:3, which was the same as the feed ratio. The molecular weight of the polymer was determined by GPC and the results were shown in Fig. S8.
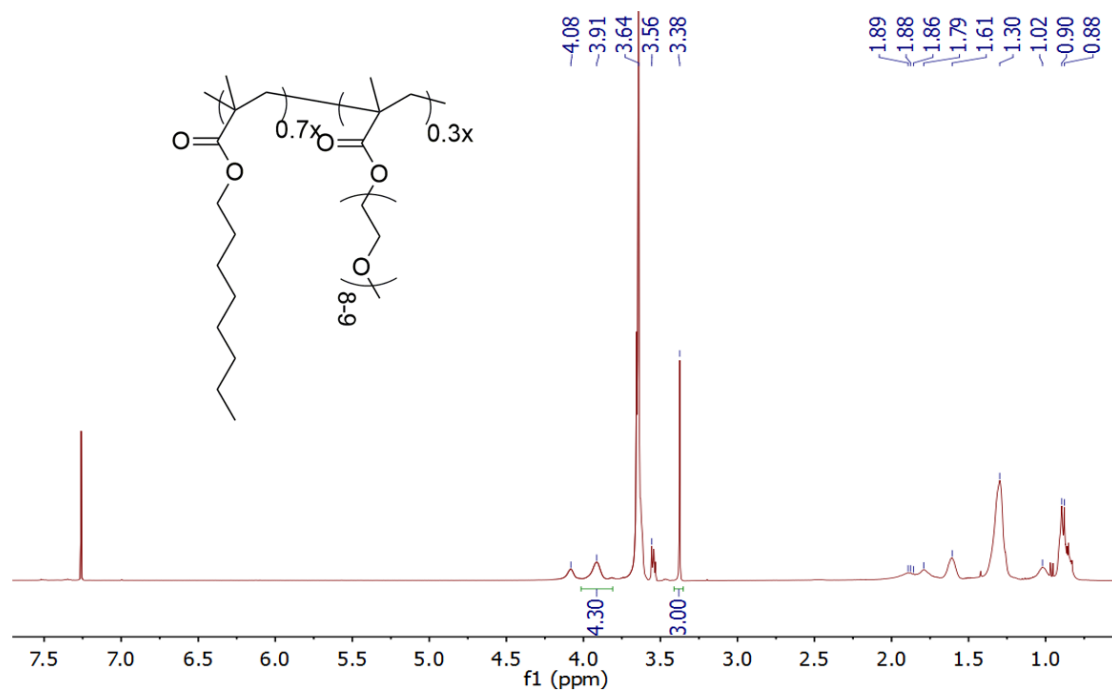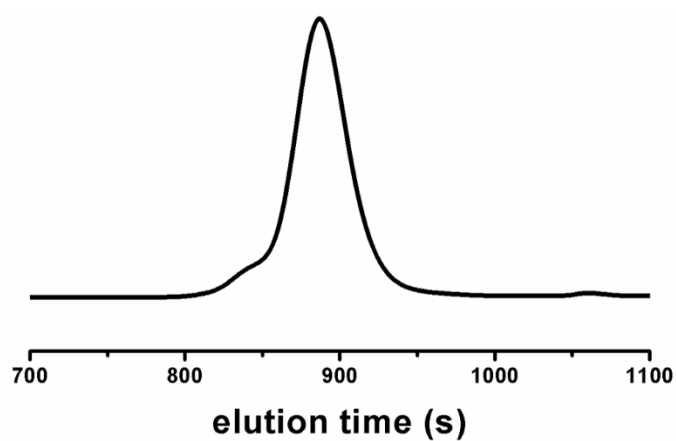
Fig. S9. NMR spectrum of amphiphilic random copolymer

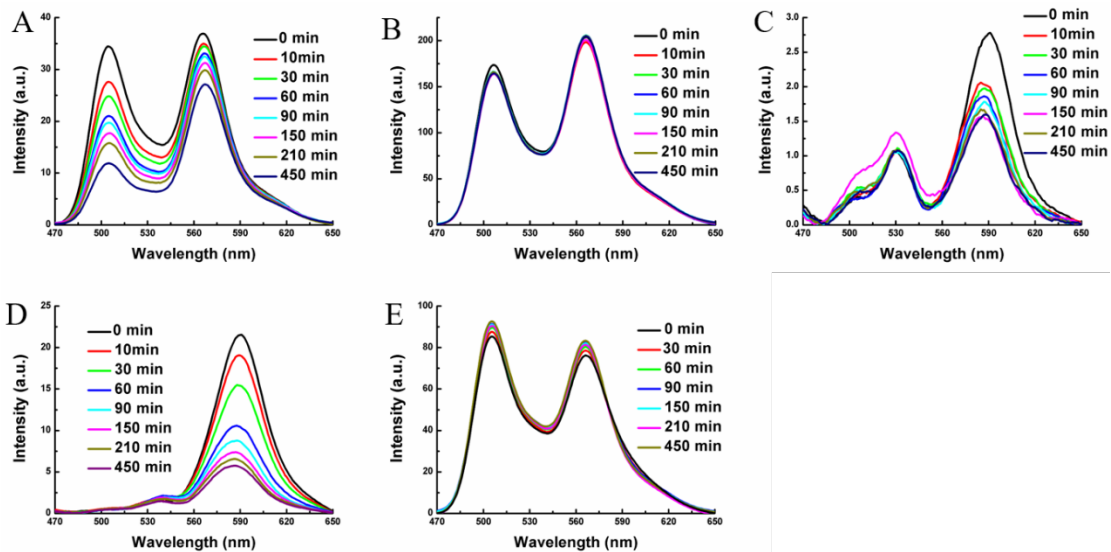| Mn (g/mol)	Mw (g/mol)	PDI
11701	13162	1.12

Fig. S10. GPC of amphiphilic random copolymer

Fig. S11. The FRET evolution of different nanoassemblies in water: A) CTAB, B) Tween 20, C) P85, D) F127, E) amphiphilic polymer.
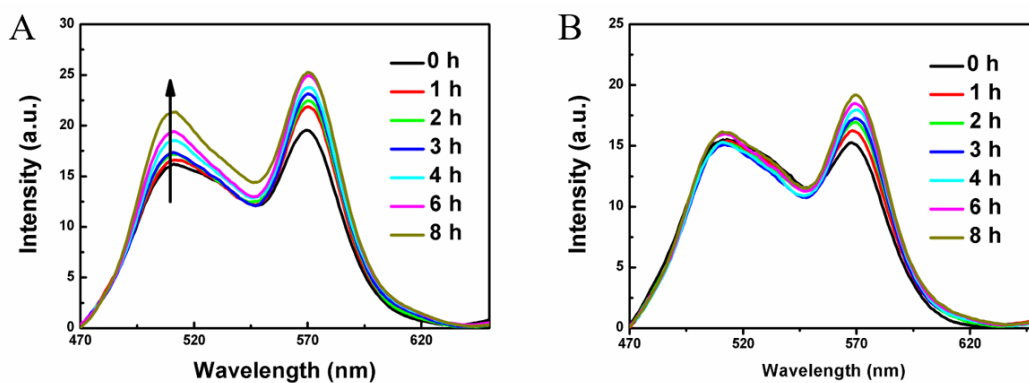

Fig. S12. FRET evolution of nanogels in albumin solution: A) 20% crosslink degree, B) 60% crosslink degree.
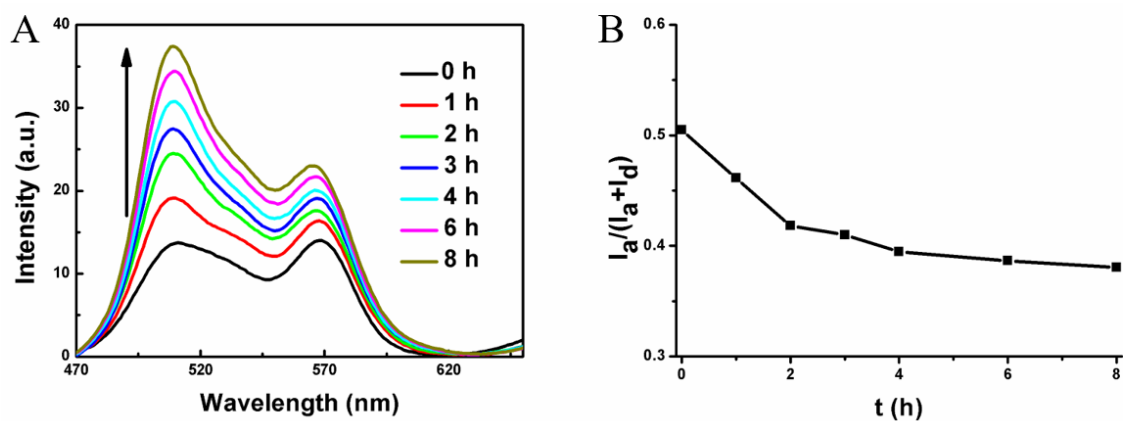

Fig. S13. De-crosslink process inside the albumin solution: A) FRET evolution over time, B) FRET ratio change.

Reference

1. Ghosh, S.; Basu, S.; Thayumanavan, S. Stimultaneous and Reversible Functionalization of Copolymers for Biological Applications. *Macromolecules* **2006**, 39, 5595-5597.