Supplementary Information for:

Composite Supramolecular Nanoassemblies with Independent Stimulus Sensitivities

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Experimental Section

Materials

2-(Diisopropylamino) ethyl methacrylate (DPA), 2-aminoethyl methacrylate hydrochloride (AMA), copper(I) bromide (CuBr), 2-propanol (IPA), 2, 2'-bipyridine (bpy),2,2′-dithiodipyridine, 2-mercaptoethanol, polyethylene glycol monomethyl ether methacrylate (MW 450), glycidyl methacrylate (GMA), D,L-dithiothreitol (DTT), 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), reduced glutathione (GSH), 2,2′-azobis(2-methylpropionitrile) (AIBN), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (chain transfer agent) and other conventional reagents were obtained from commercial sources and were used as received unless otherwise mentioned. Pyridyl disulfide ethyl methacrylate (PDSEMA) was prepared using a previously reported route.[1] ATRP initiator **1** was synthesized according to a reported procedure. [2]

Synthesis of $PDPA_{30}$-b-$PAMA_{15}$ block copolymer

$PDPA_{30}$-b-$PAMA_{15}$ was synthesized by one-pot ATRP. Catalyst CuBr (13.0 mg, 0.09 mmol), DPA (0.57 g, 2.7 mmol) and initiator **1** (30.0 mg, 0.09 mmol) were added into a 25 mL flask, which was sealed with a rubber septum. The mixture in the flask was degassed by performing three freeze-pump-thaw cycles. Then a solution of bpy (28 mg, 0.18 mmol) in 0.6 mL of IPA was degassed and injected into the flask under an argon environment. After 5 h at 50 °C, the monomer conversion was higher than 95%. A degassed solution of AMA (0.225 g, 1.35 mmol) in IPA-H_2O (0.36 mL-0.09 mL) was injected into the reaction mixture in argon atmosphere. After 24 h of the chain extension polymerization at 50 °C, the reaction mixture was diluted with deionized water and dialyzed against water (molecular weight cutoff 3500 g mol^{-1}) for three days to remove the catalyst and other small molecules. The block copolymer aqueous solution was freeze-dried to obtain the dry product.

Synthesis of random copolymer

Polyethylene glycol monomethyl ether methacrylate (1.8 g, 4.0 mmol), PDSEMA (0.76 g, 3.0 mmol), GMA (0.42 g, 3.0 mmol), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (28 mg, 0.1 mmol) and 2, 2′-azobis(2-methylpropionitrile) (5 mg, 0.03 mmol) were dissolved in 3 mL of tetrahydrofuran. The

mixture was poured into a 25 mL flask sealed with a rubber septum. Three freeze-pump-thaw cycles were performed to eliminate the oxygen in the mixture. After a 4 h polymerization at 70 °C in argon atmosphere, the resultant mixture was dissolved in dichloromethane (5 ml) and precipitated in hexane (200 ml) three times to yield purified copolymer.

Preparation of micelles

$PDPA_{30}$-b-$PAMA_{15}$ block copolymer was first dissolved in acetone to make solutions with 10 mg/mL concentration. Then the acetone solutions containing the block copolymers were injected into 10 mL of deionized water (with pH around 7.4). The obtained mixtures were left undisturbed at room temperature for 3 days to evaporate the acetone completely. To make micellar assemblies at different concentrations, 0.25, 0.5, 1.0 and 2.0 mL of copolymer acetone solutions were used.

Preparation of nanogels

For the preparation of 40 mol% crosslinked nanogel aqueous solution, 0.5 mL of random copolymer acetone solution (10 mg/ mL) solution was injected into 10 mL of deionized water (with pH around 7.4). The obtained mixtures were left undisturbed at room temperature for 3 days to evaporate the acetone completely. DTT (0.15 mg, 0.001 mmol, 20 mol% with respect to PDS groups) was added to crosslink the polymer to form the nanogel. Unreacted DTT and the byproductpyridothione were removed from the solution by ultrafiltration using a membrane with a molecular weight cutoff of 3,500 g mol^{-1}.

Encapsulation of dyes in nanogels or micelles

For the preparation of nanogels encapsulated with dyes (such as DiI, pyrene and BDP-C12-I2), dye acetone solutions (10 mg/mL) were added when injecting block copolymer or random copolymer into deionized water. Other procedures were the same with the preparation of micelle or nanogel aqueous solutions. Excess insoluble dyes were removed by filtration. The content of dye used here were about 10 wt% of the polymer.

Core-shell nanostructures from the combination of micelles and nanogels

Core-shell nanostructures formed from micelles and nanogels was obtained by the surface reaction of amino and epoxy groups, as this reaction can take place in neutral aqueous solution. 0.1 mL of $PDPA_{30}$-b-$PAMA_{10}$ block copolymer micelle aqueous solution and 0.1 mL of crosslinked nanogel aqueous solution with the same concentration were mixed together. The mixture was left undisturbed overnight, to let the micelles combine with nanogels completely. Micelles and nanogels encapsulated with different dyes (such as DiI and BDP-C12-I2) were also used to make core-shell nanostructures loaded with guest molecules.

Characterization

^{1}H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer with 1000 scans at a relaxation time of 2 s. Molecular weights of the random copolymers were estimated by gel permeation chromatography (GPC) with a refractive index detector using THF as eluent (PMMA was used as

standard). Molecular weights of PDPA and $PDPA_{30}$-b-$PAMA_{10}$ block copolymer were measured by aqueous GPC at 35 °C using poly(2-vinyl pyridine) as standard. The eluent was a buffer solution containing 0.3M NaH_2PO_4 and 1.0M acetic acid (the pH is 3.3). Dynamic light scattering (DLS) and zeta potential measurements were performed using a Malvern Nanozetasizer. The fluorescence spectra were obtained from a JASCO FP-6500 spectrofluorimeter. UV/Vis spectra of the samples in aqueous solutions were measured on a Unico UV/Vis 2802PCS instrument. Transmission electron microscopy (TEM) images were taken from JEOL 100CX at 100 KV. To prepare the TEM samples, a small drop of the core-shell nanostructure aqueous solution was deposited onto a carbon-coated copper electron microscopy (EM) grid and then dried at room temperature. To distinguish the micelles from the nanogels, the heavy atom bearing dye molecule, BDP-C12-I2, was incorporated into the polymer micelle or the nanogel. Two types of incorporation were used: (1) BDP-C12-I2 was encapsulated in the nanogels, while keeping the micelles empty; (2) BDP-C12-I2 was encapsulated in the micelles, while leaving the nanogels empty.

Release of DiI or pyrene from core-shell nanostructures

Core-shell nanostructures with DiI encapsulated in nanogels were used to evaluate the release dye under GSH stimulus. The core-shell nanostructures loaded with DiI were first made in deionized water at pH around 7.4. Then HCl aqueous solution (0.01 mol/L) was used to adjust the solution pH the of the core-shell nanostructures to 6.5. Then, GSH was added to the mixture. The fluorescence spectra of the mixture were recorded at regular intervals to monitor the DiI release progress. Also, core-shell nanostructures with pyrene incorporated in the block copolymer micelles were adopted to test the release of dye in response to pH change. The core-shell nanostructures loaded with pyrene were first made in deionized water at pH around 7.4. Then HCl aqueous solution (0.01 mol/L) was used to adjust the solution pH the of the core-shell nanostructures to 6.5. The fluorescence spectra of the mixture were recorded at regular intervals to monitor the pyrene release progress.

Cytotoxicity measurements

Cytotoxicity studies were performed on HeLa cells using Alamar Blue assay. HeLa cells were cultured in T75 cell culture flasks using Dulbecco's Modified Eagle Medium / nutrient mixture F-12 (DMEM/F12) with 10% fetal bovine serum supplement. Prior to the addition of test samples (micelles, nanogels or core-shell nanostructures) the cells were pre-incubated in a 96 well tissue culture plate with ~ 10,000 cells/well/200 µL at 37 ^oC and 5 % CO_2 for 24 hours. Separately different concentrations (1, 0.5, 0.25, 0.125, 0.062, and 0 mg/mL) of micelles, nanogels and core-shell nanostructures were prepared in 1:1 volume mixture of i) water with pH adjusted to 7.4, and ii)supplemented nutrient media, where 0 mg/mL corresponds to the control. In a typical experiment where the cells are pre-incubated, the nutrient media in the tissue culture plate were replaced with 200 µL of the test sample and incubated for an additional 24 hours at 37 ^oC and 5 % CO_2. After this, the solution was removed and replaced with 100 µL of fresh supplemented nutrient media containing 10% Alamar Blue, followed by 1.5 hours of incubation

in similar conditions. Cell viability was then calculated by comparing with controls by transferring 90 µL of this solution to a 96 well black flat bottom plate and measuring the emission intensity at 590 nm with excitation wavelength of 560 nm using fluorescence plate reader (Molecular Devices, SpectraMax M5). All experiments were done in triplicate and reported with standard deviations in the plots.

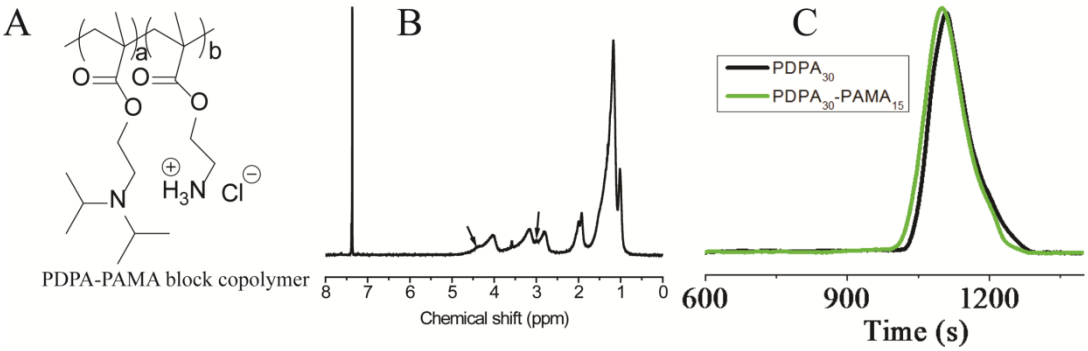

Fig. S1. Chemical structures of PDPA-b-PAMA block copolymer. (A), ^{1}H NMR spectrum of the $PDPA_{30}$-b-$PAMA_{15}$ block copolymer (peaks marked by the arrows represent the protons derived from the ethylgroup in AMA block) (B) and aqueous GPC curves of PDPA and $PDPA_{30}$-b-$PAMA_{15}$ block copolymer (C). We note that the chain lengths of both PDPA and PAMA calculated from the ^{1}H NMR spectra correlate well with that calculated from GPC curves.

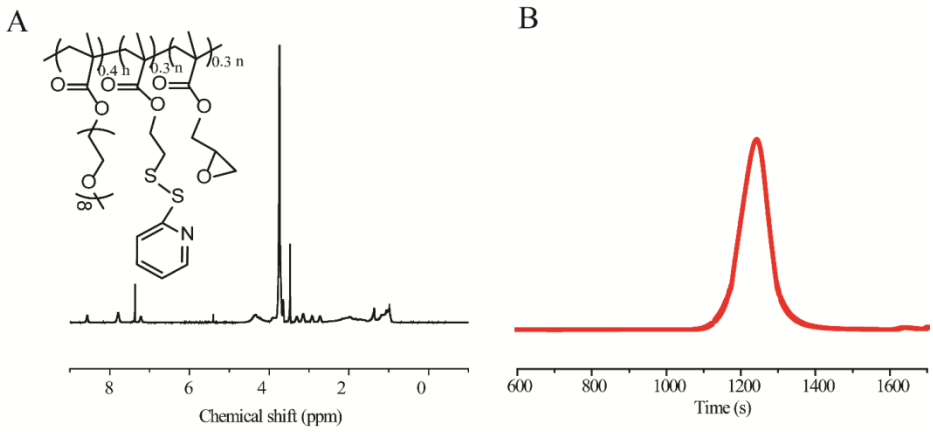

Fig. S2. Chemical structure of random copolymer and ^{1}H NMR spectrum of the randomcopolymer (A) and THF GPC curves of randomcopolymer (B).

Table S1. Summary ofM_n, M_w and Đ of the PDPA, PDPA-b-PAMA block copolymer and randomcopolymer.

	M_n	**M_w**	**Đ**
$PDPA_{30}$	6458	8085	1.52
$PDPA_{30}$-b-$PAMA_{15}$	9036	11926	1.68
Copolymer 1	21044	26691	1.26

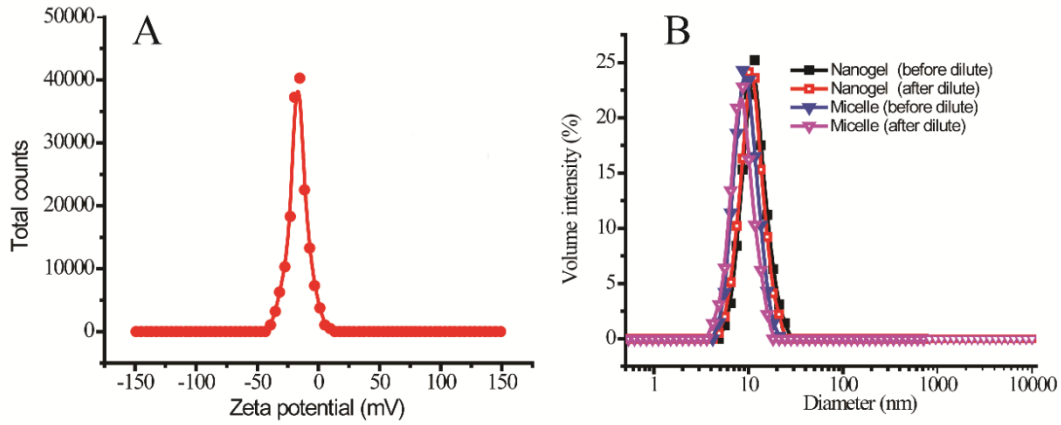

Fig. S3. Zeta potential of nanogels with 0.5 mg/mL concentration (A); diameters of the nanogels and micelles with 0.5 mg/mL concentration before and after diluting to double volume (B). The nanogels used in these testing were 40% crosslinked.

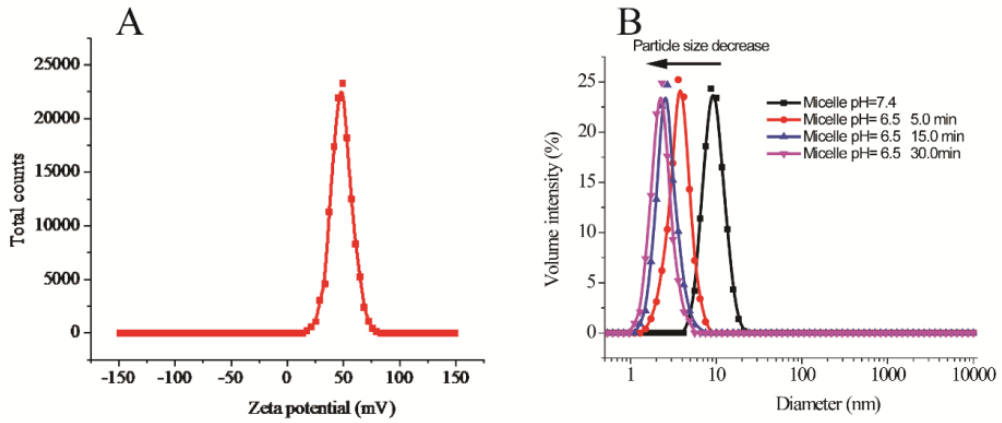

Fig. S4. Zeta potential of the micelles with 0.5 mg/mL concentration (A); pH sensitivity of the micelles (with concentration 0.5 mg/mL).

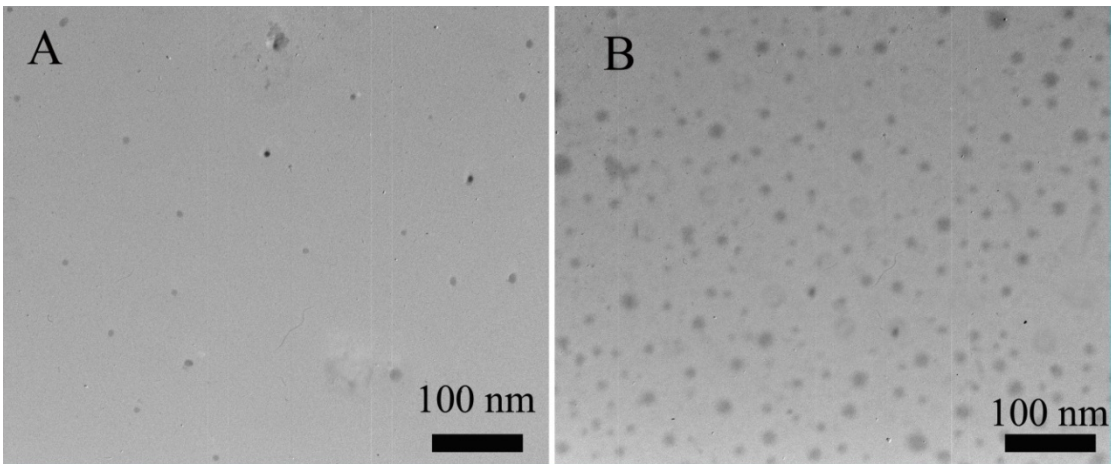

Fig. S5. TEM images of micelles formed from 0.5 mg/mL (A) and 1.0 mg/mL (B) of $PDPA_{30}$-b-$PAMA_{15}$ block copolymer.

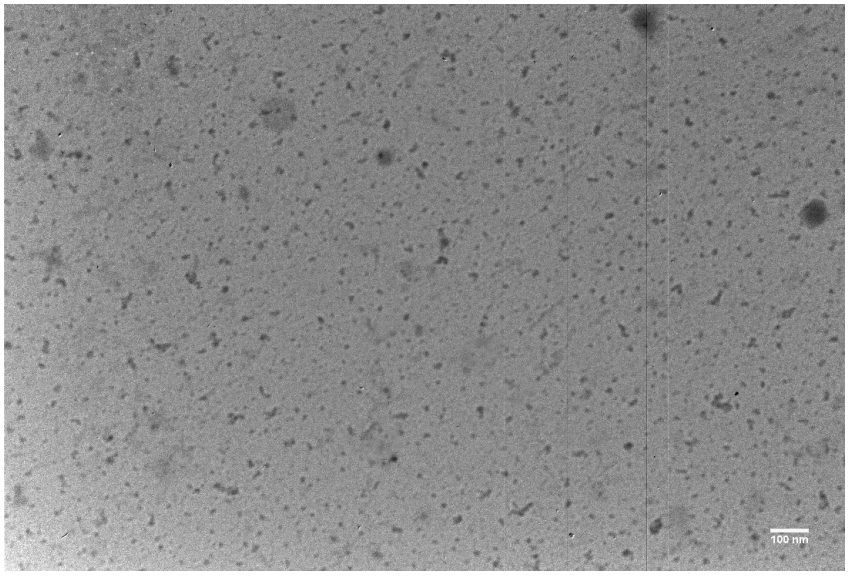

Fig. S6. TEM image of the core-shell nanostructure after the disassembly of micelle core at pH=6.5.

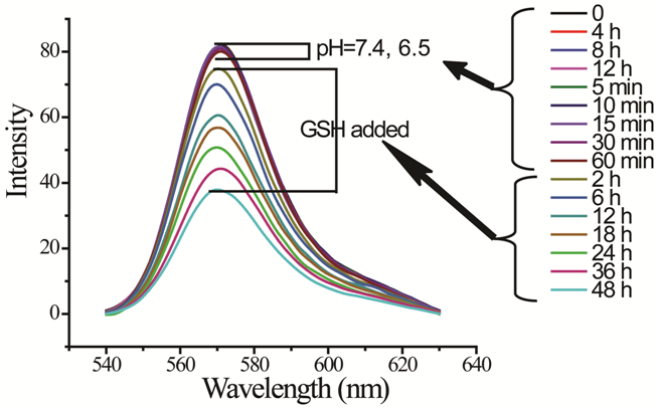

Fig. S7.Fluorescence spectra trace the release of DiI from the core-shell nanostructures in response to 5.0 mM of GSH (DiI was incorporated in thenanogels).

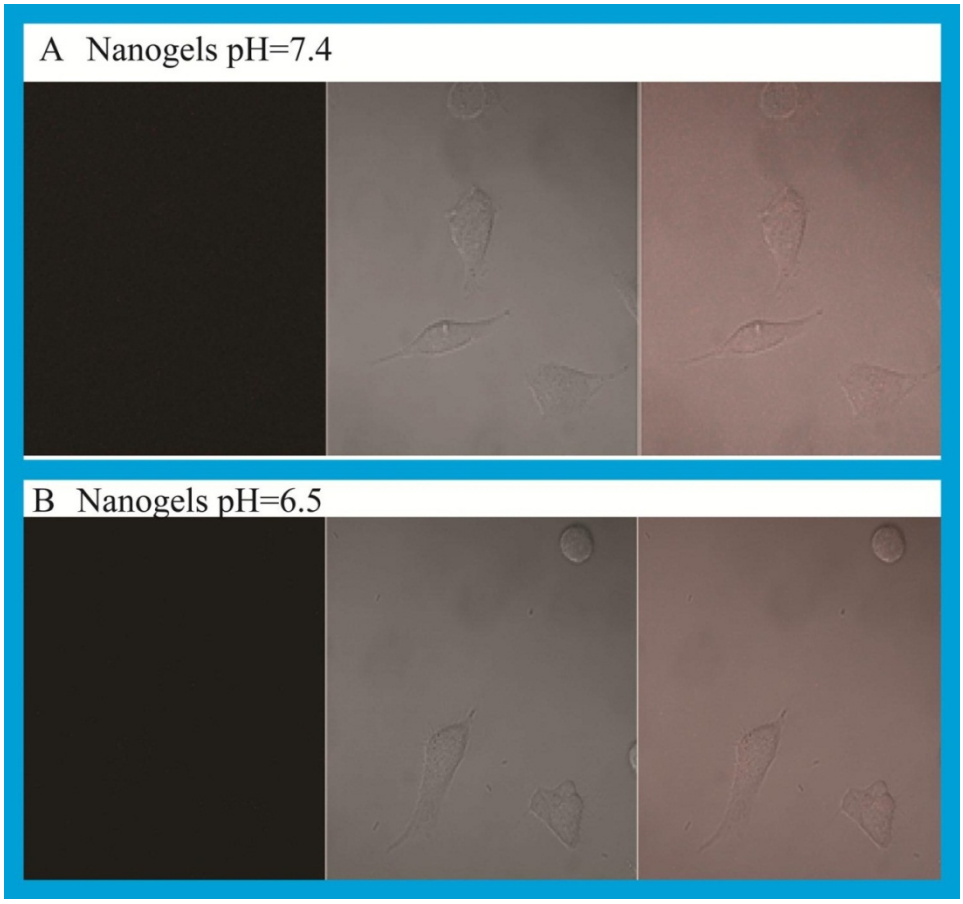

Figure S8. Cell uptake of nanogels at pH 7.4 and 6.5 after incubation with cells for 30 min..

References:

1 S.Ghosh, S. Basu and S. Thayumanavan, *Macromolecules,*2006, **39**, 5595-5597.

2 D. Bontempo, K. L. Heredia, B. A. Fish and H. D. Maynard, *J. Am. Chem. Soc.,*2004,**126**, 15372-15373.