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Supporting information for:

Chemo –Photothermal Therapy of Cancer Cells Using Gold Nanorods -Cored Stimuli - Responsive Triblock Copolymer

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Experimental Section:

Materials

N-Isopropylacrylamide (NIPAAm, 97%, Sigma-Aldrich, USA) was purified by recrystallization from *n*-hexane/toluene mixture (90/10 v/v) before use. ε-Caprolactone monomer (Sigma-Aldrich) was dried by calcium hydride (CaH_2), vacuum-distilled, and then stored at -20 °C prior to use. Acrylic acid (Merck, Darmstadt, Germany) was distilled in vacuum, and then stored at -20 °C prior to use. The initiator 2,2´azobisisobutyronitrile (AIBN, Fluka, Switzerland) was recrystallized from ethanol at 50 °C before use. Toluene and tetrahydrofuran (THF) (Merck) were dried by refluxing over sodium wire and distilled under argon atmosphere prior to use. All other solvents with the highest purity grade were purchased from Merck, and were used as received. Dithiothreitol (DTT), sodium methoxide (CH_3ONa, 25 wt.% in methanol), mercaptoethanol, *N*,*N* dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), and tin octanoate [$Sn(Oct)_2$] were purchased from Sigma-Aldrich, and were used as received. All other reagents were purchased from Merck and purified according to the standard methods.

Synthesis of bis (2-hydroxyethyl) disulfide

A 50 mL round-bottom flask was charged with mercaptoethanol (0.07 mL, 1 mmol), ethyl acetate (5 mL), NaI (1.5 mg, 0.01 mmol), hydrogen peroxide (H_2O_2, 30%, 11 mL, 1 mmol). The mixture was stirred for about 30 minutes at room temperature. At the end of this time, a saturated solution of $Na_2S_2O_3$ (15 mL) was added to the flask, and the resulting mixture was extracted with ethyl acetate (3×30 mL). The combined organic phases was washed with brine (50 mL), and dried over anhydrous magnesium sulfate ($MgSO_4$). The solvent (ethyl acetate) was removed by a rotary evaporator, and the residue was purified by silica gel column chromatography using *n*-hexane:ethyl acetate (30:70 v/v) as the eluent to afford the pure product (Yield: 87%; colorless

oil). Figure S1 also reports ^{1}HNMR spectra of the synthesized bis(2-hydroxyethyl) disulfide agent

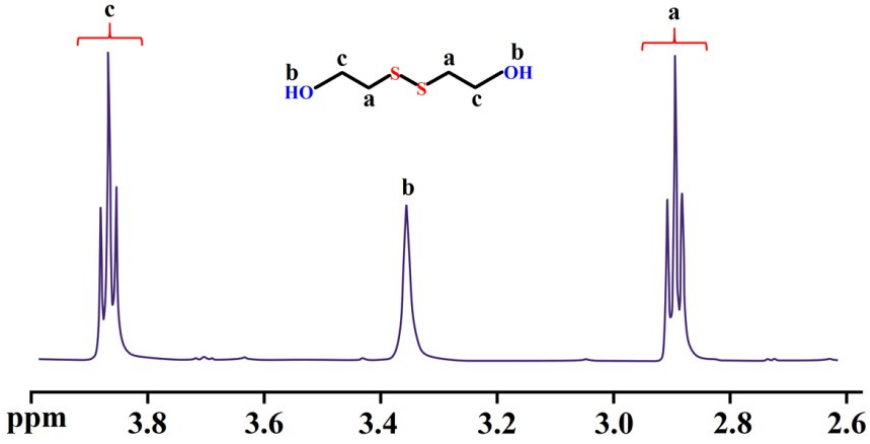

Figure S1. ^{1}H NMR spectrum of bis(2-hydroxyethyl) disulfide.

Synthesis of (PCL-S)$_2$

The (PCL-S)$_2$ was synthesized by ring-opening polymerization (ROP) of ε-caprolactone monomer using bis(2-hydroxyethyl)disulfide as an initiator. For this purpose, a 50 mL three-necked round-bottom flask equipped with a condenser, gas inlet/outlet and a magnetic stirrer, was charged with bis(2-hydroxyethyl) disulfide (212 mg, 1.375 mmol), Sn(Oct)$_2$ (96 mg, 0.237 mmol), ε-CL (12 g, 103 mmol), and toluene (20 mL). The reaction mixture was de-aerated by bubbling highly pure argon for 10 minutes, and then the flask was placed in an oil bath at 100 ± 3 ºC. The polymerization was allowed to proceed under stirring for 24 hours, and at the end of this time, the flask was cooled in ice/water bath, in order to quench the polymerization. The mixture was diluted with toluene (10 mL), and precipitated in cold acidic methanol (200 mL). The product was filtered, washed with methanol several times, and dried under vacuum at room temperature to afford white powder (Yield: 67%).

Synthesis of (CTA-PCL-S)$_2$ macro-RAFT agent

The (CTA-PCL-S)$_2$ macro-RAFT agent was synthesized through the Steglich esterification of (PCL-S)$_2$ with chain transfer agent (CTA; 4-cyano-4-[(phenylcarbothioyl)sulfanyl]pentanoic acid). For this, a 100 mL three-necked round-bottom flask equipped with condenser, gas inlet/outlet, and a magnetic stirrer, was charged with CTA (0.81 g, 3.0 mmol), DCC (1.24 g, 6 mmol), and dichloromethane (70 mL). The content of the flask was de-aerated by bubbling highly pure argon for 15 minutes, and stirred magnetically at room temperature for about 3 hours under argon protection. At the end of this period, the (PCL-S)$_2$ (4g, 0.48 mmol), and DMAP (0.12 g, 1 mmol) were added to the flask, and the reaction mixture was stirred for another 48 hours under an argon atmosphere at room temperature. The precipitated dicyclohexylurea salt (DCU) was filtered using filter paper (Whatman) to give a crude product. The product was precipitated into excess cold methanol twice to remove the unreacted reagents (CTA, DCC, and DMAP). The product was dried in vacuum at room temperature to afford yellow powder (Yield: 77%; monomer conversion ~ 90%). Figure S2 also reports ^{1}HNMR spectra of the synthesized (CTA-PCL-S)$_2$ macro-RAFT agent

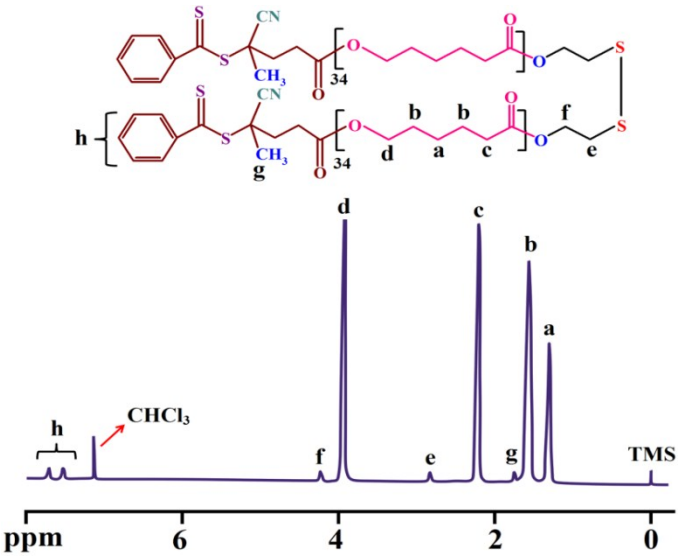

Figure S2. ^{1}H NMR spectrum of $(CTA\text{-}PCL\text{-}S)_2$ macro-RAFT agent.

Synthesis of (PNIPAAm-*b*-PCL-S)$_2$ triblock copolymer

In a typical experiment, a dry polymerization ampoule was charged with macro-RAFT agent [$(CTA\text{-}PCL\text{-}S)_2$; 1.0 g, 0.12 mmol], NIPAAm monomer (1.6 g, 15 mmol), AIBN (2.0 mg, 12 µmol), and dried 1,4-dioxane (8 mL). The ampoule was connected to the Schlenk line to degas *via* three freeze-evacuate-thaw cycles, and then immersed in a thermostated oil bath at 70±3 ºC for about 72 hours. At the end of this time, the ampoule was cooled in ice/water bath, in order to quench the reaction. The product was diluted with THF (10 mL), and then was dropped into a 200 mL of diethyl ether to afford the precipitate. This procedure was repeated three times to purify the product, and finally the obtained (PNIPAAm-*b*-PCL-S)$_2$ triblock copolymer was filtered and dried under reduced pressure at room temperature to afford light yellow powder (Yield: 70%; monomer conversion ~ 45%). Figure S3 also reports ^{1}HNMR spectra of the synthesized (PNIPAAm-*b*-PCL-S)$_2$ triblock copolymer

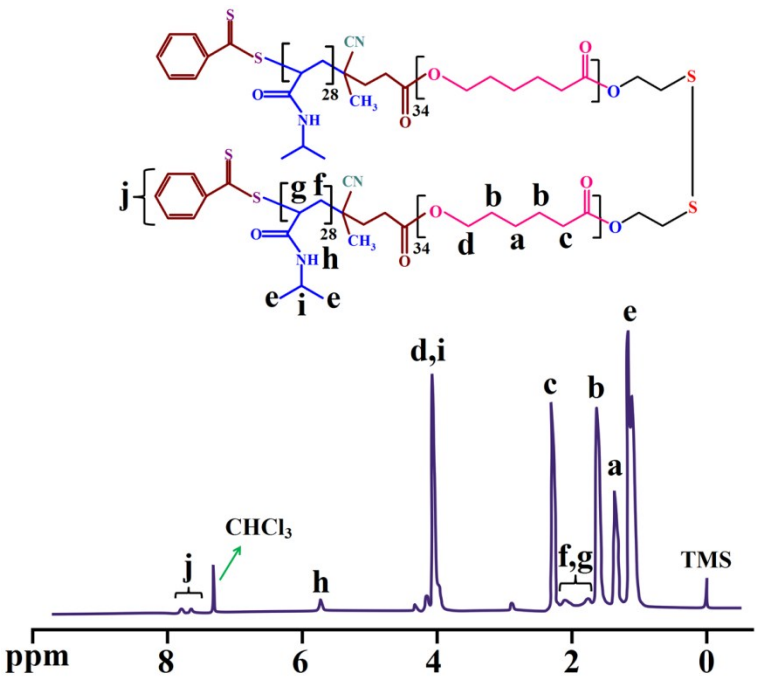

Figure S3. ^{1}H NMR spectrum of the (PNIPAAm-*b*-PCL-S)$_2$ triblock copolymer

Synthesis of (PAA-*b*-PNIPAAm-*b*-PCL-S)$_2$ pentablock copolymer

The (PAA-*b*-PNIPAAm-*b*-PCL-S)$_2$ pentablock copolymer was synthesized through the RAFT technique by copolymerization of AA monomer using (PNIPAAm-*b*-PCL-S)$_2$ as the macroinitiator. For this purpose, a dry polymerization ampoule was charged with macro-RAFT agent [(PNIPAAm-*b*-PCL-S)$_2$; 1.0 g, 0.068 mmol], AA monomer (1.5 g, 20 mmol), AIBN (2.0 mg, 12 μmol), and dried 1,4-dioxane (8.0 mL). The polymerization ampoule was degassed with several freeze–pump–thaw cycles, sealed off under vacuum, and placed in an oil bath at 70±3 °C for about 24 hours. At the end of this time, the reaction mixture was diluted with 1,4-dioxane (10 mL), and then precipitated in cold diethyl ether (200 mL). The product was filtrated, and dried in vacuum at room temperature (Yield: 80%; white yellow powder; monomer conversion ~ 68%).

Synthesis of PAA-*b*-PNIPAAm-*b*-PCL-SH triblock copolymer

To a stirred THF solution of (PAA-*b*-PNIPAAm-*b*-PCL-S)$_2$ (1.0 g, 0.035 mmol), and DTT (0.15 g, 1.0 mmol) a catalytic amount of CH_3ONa (2.4 mg, 0.044 mmol) was added under argon protection. The reaction was allowed to proceed for 30 hours at room temperature. The resulting polymer was precipitated in degassed cold diethyl ether (using argon gas to prevent disulfide formation), filtrated, and dried in vacuum at room temperature (Yield: 76%; white yellow powder).

Figure S4, S5, table S1, figure S6 also reports ^{1}HNMR spectra, GPC, Molecular weight analysis data and image of synthesized materials respectively.

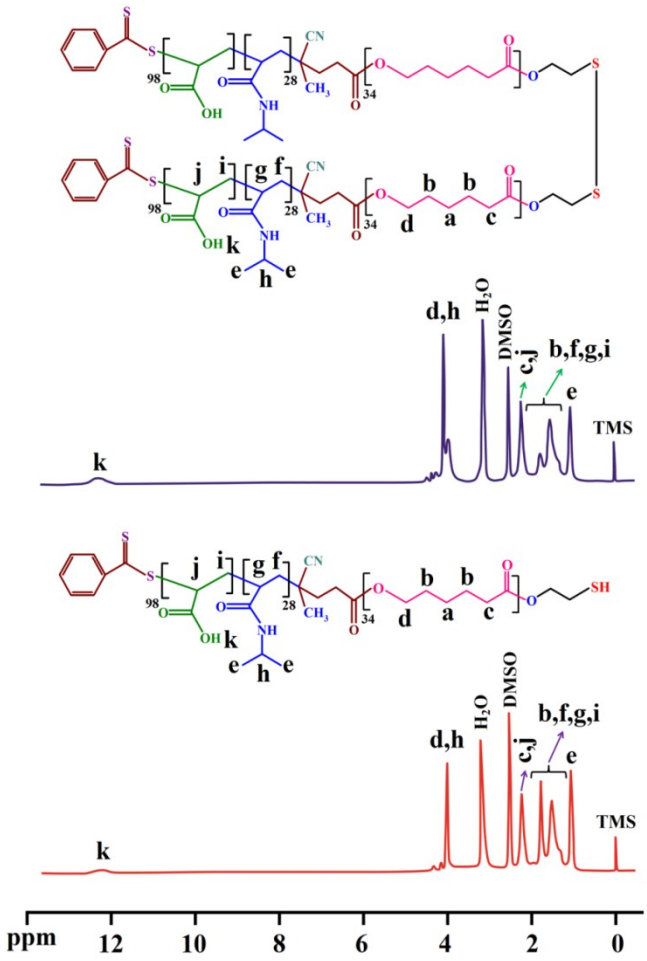

Figure S4. ^{1}H NMR spectra of the (PAA-*b*-PNIPAAm-*b*-PCL-S)$_2$ pentablock copolymer, and PAA-*b*-PNIPAAm-*b*-PCL-SH triblock copolymer

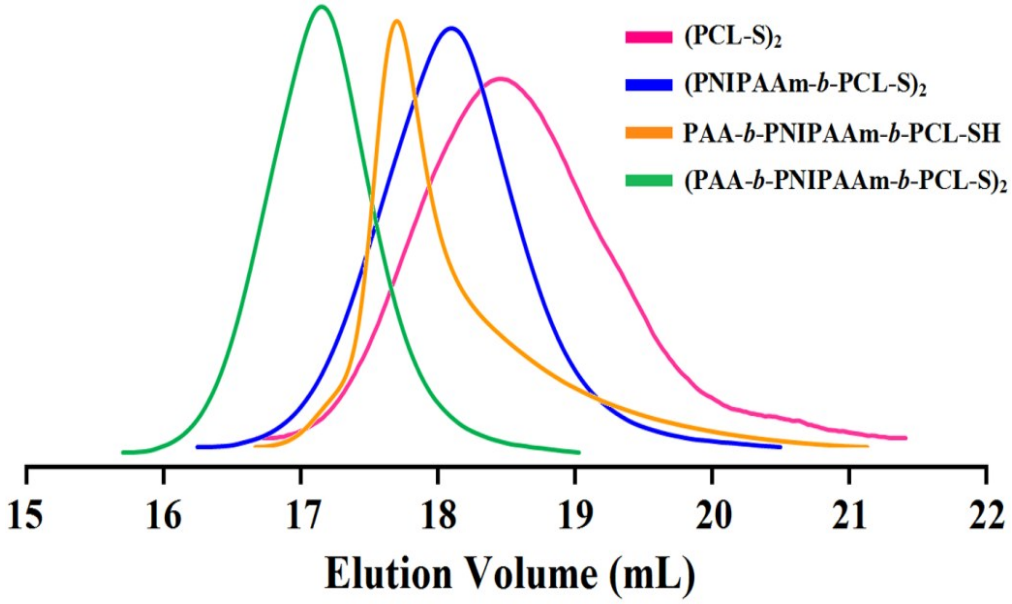

Figure S5. GPC chromatograms of (PCL-S)$_2$, (PNIPAAm-*b*-PCL-S)$_2$, (PAA-*b*-PNIPAAm-*b*-PCL-S)$_2$, and PAA-*b*-PNIPAAm-*b*-PCL-SH samples.

Table S1. Molecular weight analysis data of (PCL-S)$_2$, (PNIPAAm-*b*-PCL-S)$_2$, (PAA-*b*-PNIPAAm-*b*-PCL-S)$_2$, and PAA-*b*-PNIPAAm-*b*-PCL-SH samples by GPC.

Sample	M_n **(gmol^{-1})**	$Đ^a$
(PCL-S)$_2$	8400	1.65
(PNIPAAm-*b*-PCL-S)$_2$	14700	1.38
(PAA-*b*-PNIPAAm-*b*-PCL-S)$_2$	28700	1.24
PAA-*b*- PNIPAAm-*b*-PCL-SH	15100	1.29

Figure S6. The image of synthesized materials

Micelle Formation of PCL-b-PNIPAAm-b-PAA-SH

Thiol-end capped triblock copolymer (PCL-*b*-PNIPAAm-*b*-PAA-SH) micelle suspension in aqueous media was prepared *via* a membrane-dialysis approach. Briefly, the synthesized triblock copolymer (50 mg) was dissolved in 10 mL DMSO to obtain an initial concentration of 5000 mgL^{-1}. Then the solution was put into a dialysis tube (molecular weight cut-off: 2000 $gmol^{-1}$) and subjected to dialysis against 1000 mL deionized water for about 72 hours with vigorous stirring. The water was replaced overnight, especially changed once per hour in first 3 hours. The solution changed from transparent to translucent during the dialysis, which confirms the formation of micelle. The micellar solution was diluted with deionized water to the desired concentration for further measurements.

AFM observation

AFM image (Figure S7) show GNRs had a relatively smooth surface with low valleys (dark regions) and hills, and good structural integrity. After graft onto GNRs backbone, the surfaces of the samples demonstrate relatively rough (brightest regions) with some protuberances, due to the grafting process. Also, it can be seen that the GNRs@polymer films highly covered compared with GNRs surface and height of the line scan of GNRs@polymer is higher compared with GNRs surface due to the grafting process.

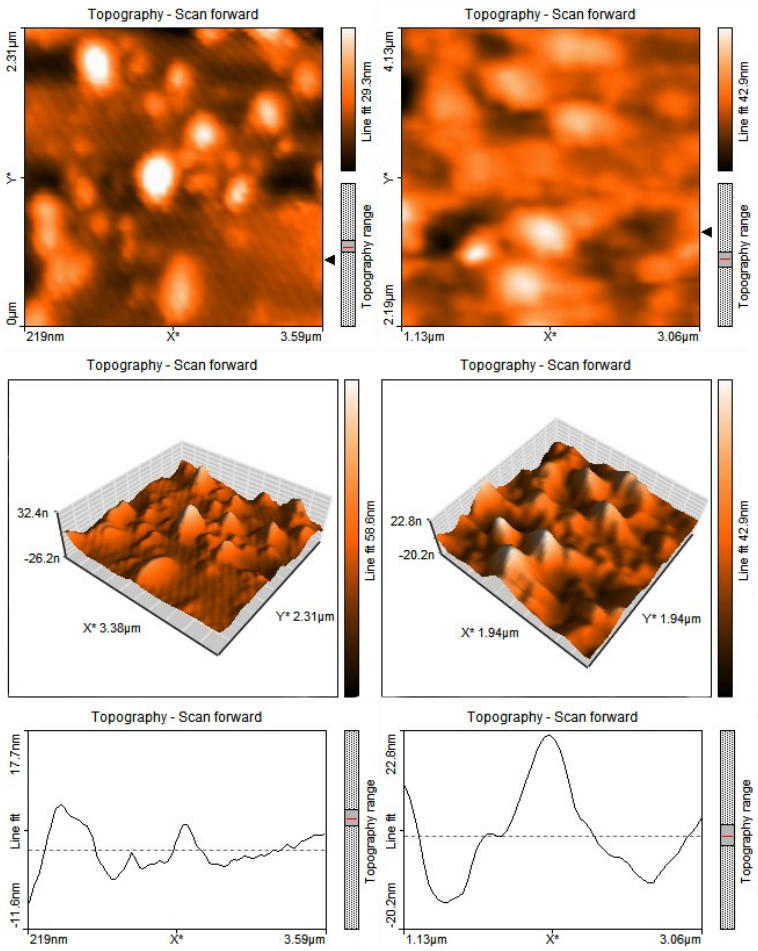

Figure S7. AFM of GNRs (left side) and GNRs@polymer (right side) with three dimensional and cross section images

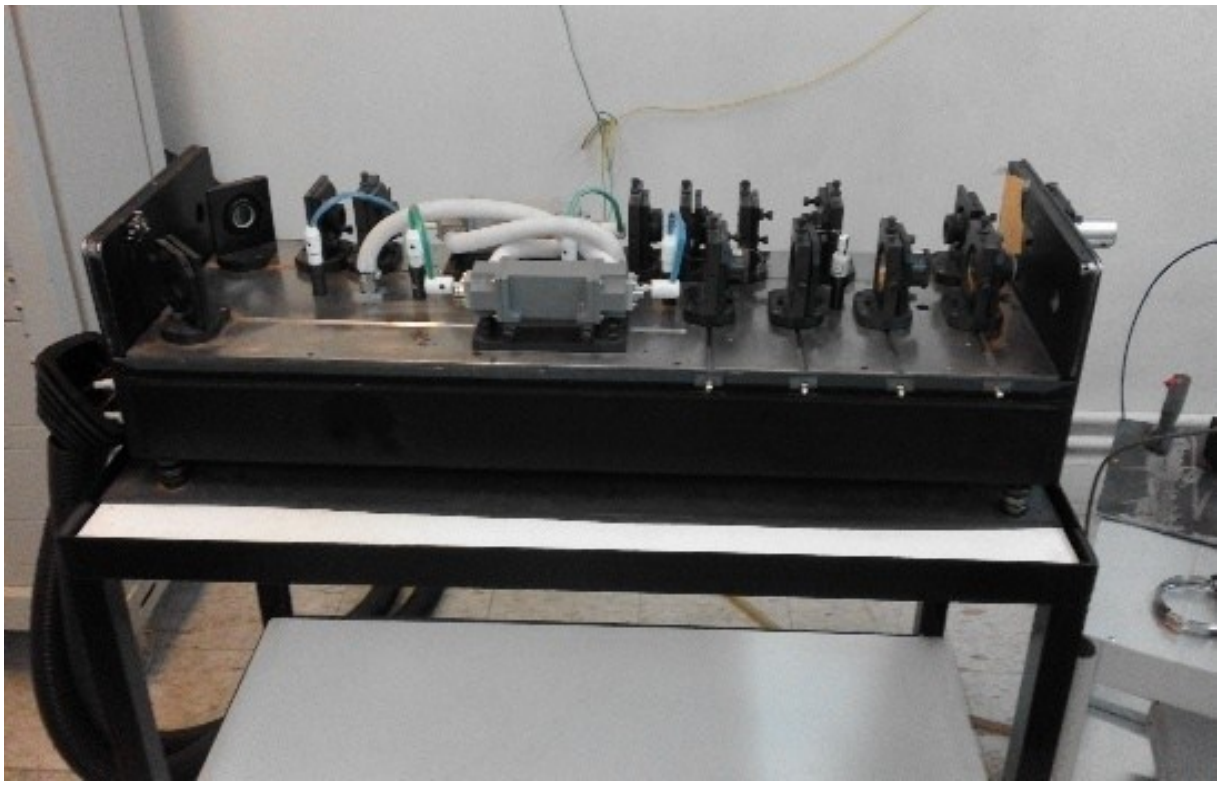

Figure S8: The image of pulsed Q-switched Nd:YAG laser