Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Thermoresponsive Dual Emission Nanosensor Based on Quantum Dots and Dye Labeled Poly(N-isopropylacrylamide)

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Materials. CdO (99.998%, Puratronic®), Tri-n-octylphosphine oxide (98%, TOPO), tris(2-carboxyethyl)phosphine hydrochloride (98%, TCEP) and selenium (99.99% trace metals basis), were obtained from Alfa Aesar. n-Tetradecylphosphnic acid (97%, TDPA) was obtained from Strem Chemiclas. Trioctylphosphine (technical grade, 90%), hexadecylamine (HDA, technical grade, 90%), diethylzinc solution (1.0 M in heptane), hexamethyldisilathiane (synthesis grade), sodium borohydride ($NaBH_4$, granular, 99.99% trace metals basis), (±)-α-Lipoic acid (≥97%), Sephadex® G-25 (BioReagent, for molecular biology, DNA grade, Superfine), n-isopropylacrylamide (97%) and tetramethylammonium hydroxide pentahydrate (≥97%, TMAH) were purchased from Sigma-Aldrich. Texas red® C2 maleimide was obtained from Molecular Probes (Eugene, OR). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were obtained from Oakwood Products, Inc. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid were synthesized following published procedures.[1] Unless otherwise stated, all solvents used were reagent grade and all chemicals were used as received without further purification.

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded using Varian NMR spectrophotometer (300 MHz or 500 MHz). Number-average molecular weight (M_n), weight-average molecular weight (M_w), and molecular weight distribution ($Đ_M$) were determined by size exclusion chromatography (SEC), and molecular weight values were determined by light scattering. The SEC analysis were performed on a Waters 150-C Plus instrument equipped with three HR-Styragel columns and a triple detector system. THF was used as eluent at a flow rate of 1.0 mL/min at 35 °C. Absorption and photoluminescence spectra were obtained on Synergymx plate reader with temperature control (Bio-Tek Instrument, inc.). Matrix-assisted laser desorption/ionization-time-of-flight (MALDITOF) mass spectrum was obtained on a Bruker Ultraflex III TOF/TOF mass spectrometer (Bruker Daltonics, Inc., Billarica, MA). Transmission electron microscopy (TEM) images were obtained from a JEOL-1230 microscope with an

accelerating voltage of 120 kV. The hydrodynamic size of DHLA coated QDs and QDs-PNIPAM-TR at given temperature was determined by DLS measurements using a Malvern Zetasizer Nano ZS. The sample solutions were filtered through a 0.45 μm syringe membrane filter to remove any dust or aggregated particles before DLS measurements.

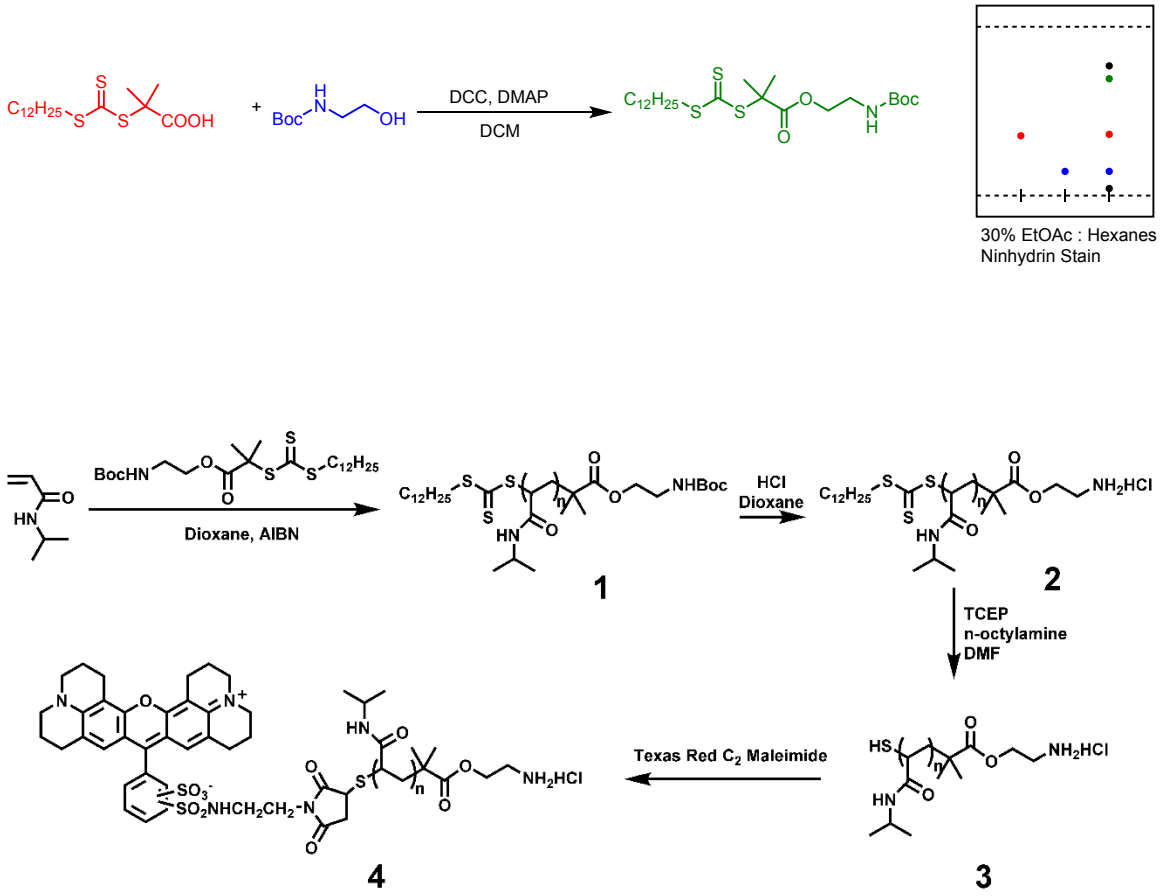
Synthesis of core CdSe nanocrystals: CdO (30.2 mg), TDPA (137 mg), TOPO (3.8 g) and HDA (3.8 g) were placed in a three necked round bottom flask and degassed at 80 °C for 2.5 h. The mixture was then heated to 320 °C under argon atmosphere. The color of solution turned from reddish to colorless at around 300 °C after the formation of Cd-TDPA complex. At this temperature, a TOPSe solution (97.2 mg elemental Se in 3 mL TOP prepare under argon) was injected quickly and the solution temperature dropped to 270 °C. The reaction was continued at 270 °C for 10 min. After cooling to room temperature, the solution was diluted with 2 mL of toluene. The core CdSe nanocrystals were precipitated by addition of methanol, separated by centrifugation. The nanocrystals were redispersed in hexane and precipitated with methanol again. Separated nanocrystals were redispersed in hexane.

Synthesis of core-shell CdSe/ZnS QDs: TOPO (3.2 g), HDA (1.6 g) and QDs in hexane were placed in a Schenk flask and freezed with liquid N_2. After the solvent was vacuumed away, the mixture was heated to 120 °C for 10 min, and then 170 °C. Required amount of hexamethyldisilathiane and diethyl zinc for 5 layers of ZnS shell were dissolve in TOP and loaded into a syringe. This mixture was added dropwise to QDs solution in 15 min. After addition, the temperature was lowered to 90 °C and the reaction continued for 2.5 h. After cooling to about 40 °C, 4 mL 1-butanol was added to the mixture. The solution was stored at -20 °C as a stock solution. The CdSe/ZnS QDs were purified by precipitation with methanol and redisperse in hexane for 3 times. After the final cycle, the QDs were dispersed in chloroform.

Preparation of (±)-dihydrolipoic acid (DHLA) coated QDs: (±)-α-Lipoic acid (107 mg) and $NaBH_4$ (39 mg) were mixed in $CHCl_3$/methanol mixed solvent (1 mL $CHCl_3$ + 2 mL methanol) and stirred vigorously under argon for 20 min. TOPO/HDA coated QDs in $CHCl_3$ (~1.95×10^{-5} mmol QDs) was added to the above mixture and stirred for 20 h under argon in the dark. 3 mL 1X PBS (phosphate buffered saline, pH = 7.4) was added and stirred for 30 min. The two phases were separated by centrifuge and aqueous layer containing QDs was collected. The QDs were further purified by running through a Sephadex G25 column using 1X PBS as eluent.

Synthesis of RAFT agent 2-((tert-butoxycarbonyl)amino)ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate:

2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoic acid (1.0 g, 2.74 mmol), dicyclohexylmethanediimine (DCC) (0.679 g, 3.29 mmol) and tert-butyl (2-hydroxyethyl)carbamate (0.531 g, 3.29 mmol) were dissolved in 15 mL anhydrous DCM and cooled to 0 °C. N,N-dimethylpyridin-4-amine (DMAP) (0.040 g, 0.329 mmol) was dissolved in (1 + 1) mL DCM and was added dropwise to the reaction mixture. The reaction was stirred at room temperature for 24 h. A white byproduct was filtered out. The filtrate was concentrated and purified by silica gel column chromatography using a gradient upto 30% EtOAc in Hexanes to obtain 2-((tert-butoxycarbonyl)amino)ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (1.00 g, 1.969 mmol, 71.8 % yield). ^{1}H NMR (500 MHz; $CDCl_3$): δ 4.18 (t, J = 4.2 Hz, 2H), 3.40 (d, J = 3.8 Hz, 2H), 3.29 (t, J = 7.5 Hz, 2H), 1.71 (s, 6H), 1.68 (t, J = 7.4 Hz, 2H), 1.45 (s, 9H), 1.40 (t, J = 7.3 Hz, 2H), 1.28 (d, J = 20.9 Hz, 16H), 0.89 (t, J = 6.8 Hz, 3H).



Synthesis of 1: In a typical polymerization, N-isopropylacrylamide (1 g, 8.84 mmol), RAFT agent (0.090 g, 0.177 mmol), AIBN (2.54 mg, 0.015 mmol) and anhydrous 1,4-dioxane (2.0 mL) were added to a microwave reaction vessel of volume 10 mL with an appropriate stir bar. The reaction vessel was degassed thrice by freeze pump thaw. Then the vessel was transferred to the microwave reactor and heated at 70 °C for 4 h under fast stirring conditions. The resulting polymer was purified by carrying out dialysis in MeOH while using a 1000 Da MWCO dialysis bag to yield 0.950 g of polymer (M_n = 4.7 kg/mol, PDI = 1.07).

Synthesis of 2: In a 20 mL scintillation vial equipped with a stir bar, 200 mg polymer **1** was added. 2 mL 4M HCl dioxane solution was added and stirred under N_2 for 2 h at room temperature. After removal of solvent, the obtained solid was dissolved in methanol and dialyzed

in methanol with a 2000 MWCO dialysis tube for 2 days. 160 mg light yellow solid was obtained after vacuum drying.

Synthesis of 3: 100 mg polymer **2** and 72 mg (0.25 mmol) TCEP were loaded into a 10 mL Schlenk flask equipped with a stir bar. The flask was vacuumed and backfilled with N_2 for 3 times. A solution of n-octylamine (65 mg, 0.5 mmol) in DMF (5 mL) was degassed with N_2 for 15 min and added to the flask via syringe. After stirring at room temperature for 20 h, DMF was removed *in vacuo*. The residue was dissolved in methanol and dialyzed in methanol with a 2000 MWCO dialysis tube for 3 days. After removal of methanol, the residue was dissolved in H_2O and freeze dried to give 50 mg white solid as product.

Synthesis of 4: 10 mg polymer 3 was dissolved in 1 mL degassed 1X PBS under argon in a 10 mL Schlenk flask, 5.7 mg TCEP was added and the mixture was stirred for 17 h. 1.5 mg Texas Red C_2 maleimide in 0.15 mL anhydrous DMSO was added to the above mixture and stirred for another 24 h. The reaction solution was dialyzed against H_2O with a 3000 MWCO dialysis tube for 3 days. 8 mg purple solid was obtained as product after lyophilization.

Preparation of QD-PNIPAM-TR hybrid: To a 200 μL PBS solution of DHLA coated QDs (2.05 μM) was added 4 mg EDC, the mixture was shaken under room temperature for 2 h. 400 μL 2 mg/mL TR-PNIPAM solution (in 1X PBS) was added and the reaction was continued at 4 °C for 48 h. The QD-PNIPAM-TR hybrid was obtained by size exclusion chromatography using Sephadex G25, PBS was used as eluent.

Calculate distance between QDs and dye:

Based on the ideal chain model, end to end distance of a polymer chain is

$$\sqrt{\langle \vec{R}^2 \rangle} = \sqrt{N} l$$

$\sqrt{\langle \vec{R}^2 \rangle}$: average end to end distance

l: length of each repeating unit (3.0 Å)

N: number of repeating unit

For a PNIPAM with molecular mass of 5 kD, the repeating unit is around 44, the end-to-end distance was calculated as:

$$\sqrt{\langle \vec{R}^2 \rangle} = \sqrt{44} \times 3.0\text{Å} \cong 20\text{Å}$$

Thus, for QDs donor with diameter of 35 Å, the center to center distance between QDs donor and dye acceptor is 37.5 Å.

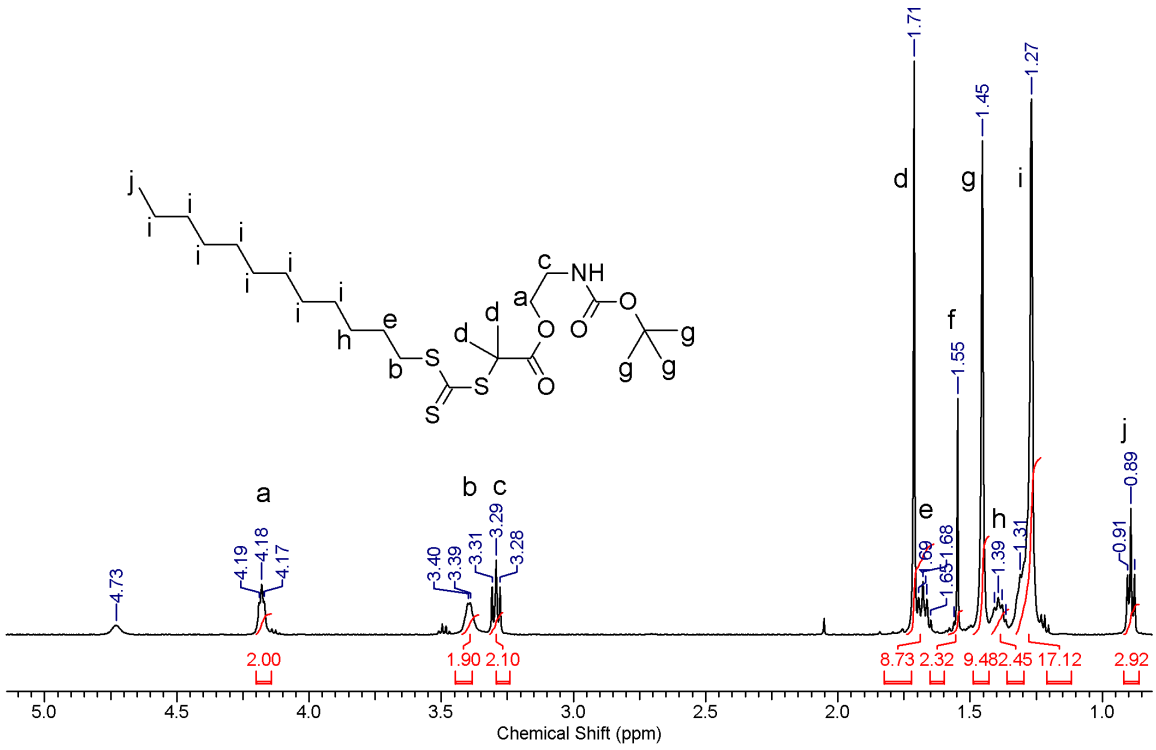


Fig. S1 ^{1}H NMR spectrum of RAFT agent 2-((tert-butoxycarbonyl)amino)ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate.

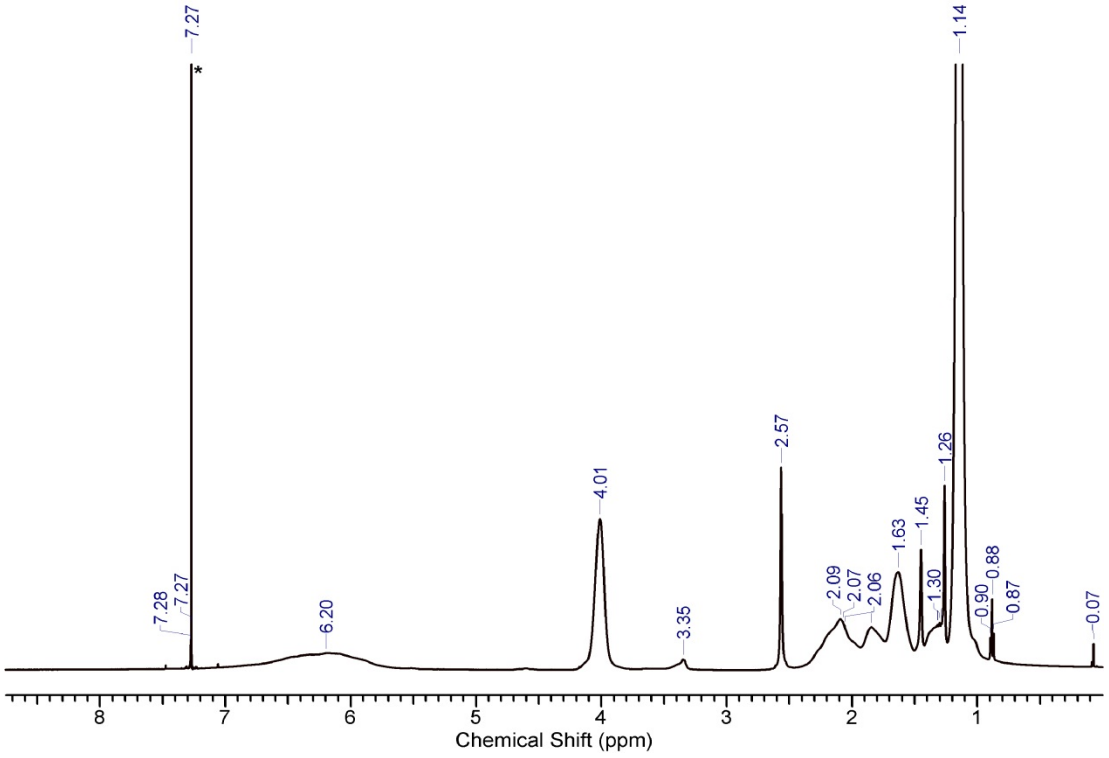


Fig. S2 ^{1}H NMR spectrum of polymer **1**. * $CHCl_3$.

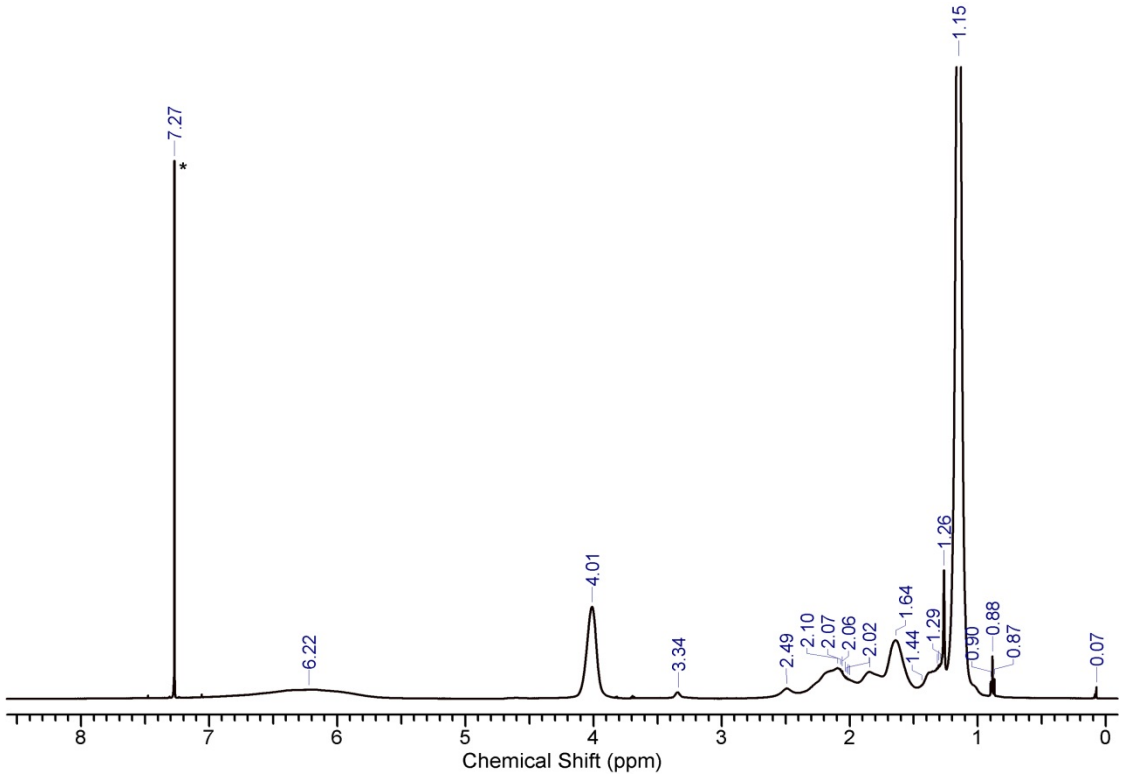


Fig. S3 ^{1}H NMR spectrum of polymer **2**. * $CHCl_3$.

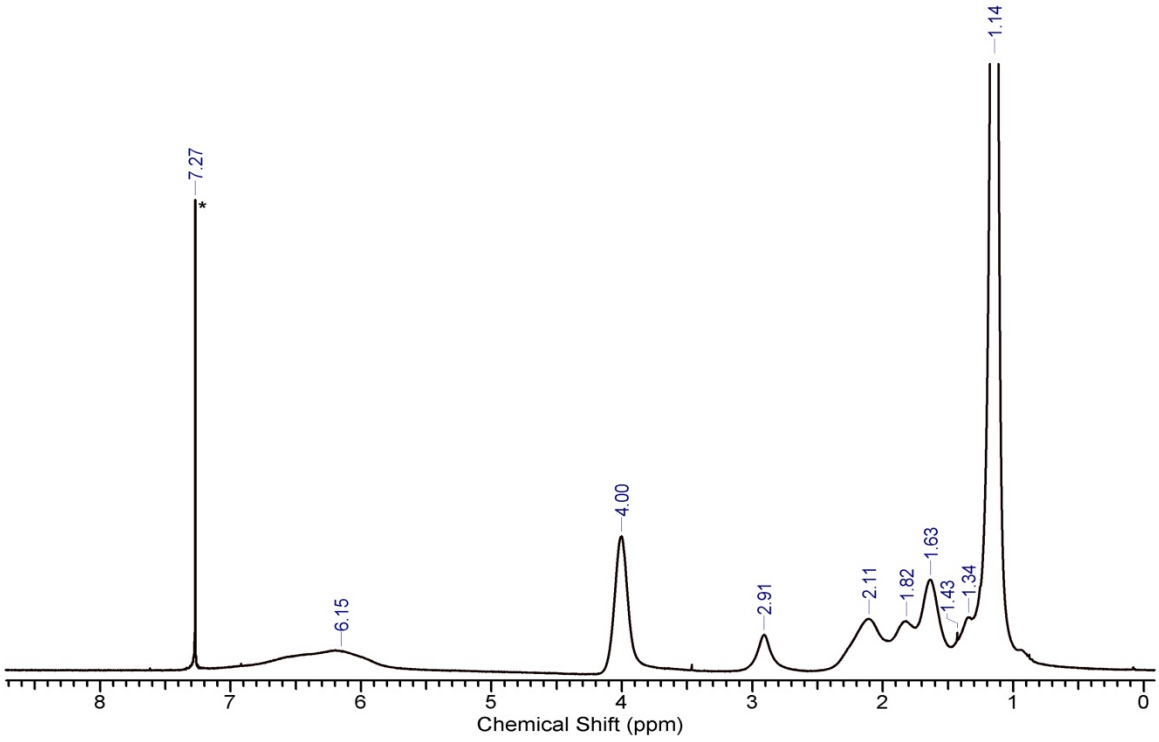


Fig. S4 ^{1}H NMR spectrum of polymer **3**. * $CHCl_3$.

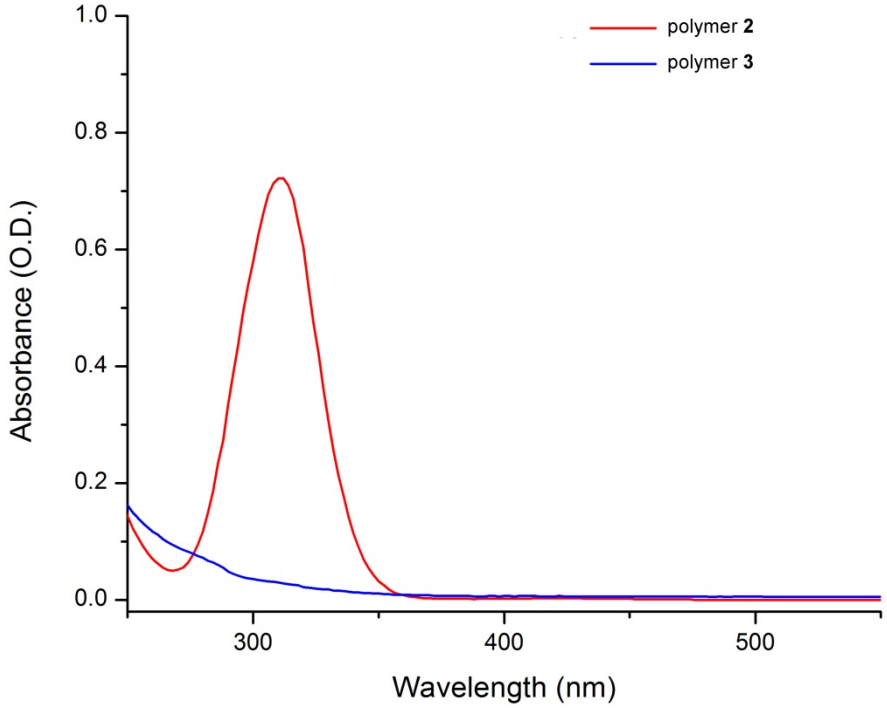


Fig. S5 UV-vis spectra of polymer **2** and polymer **3** in $CHCl_3$.

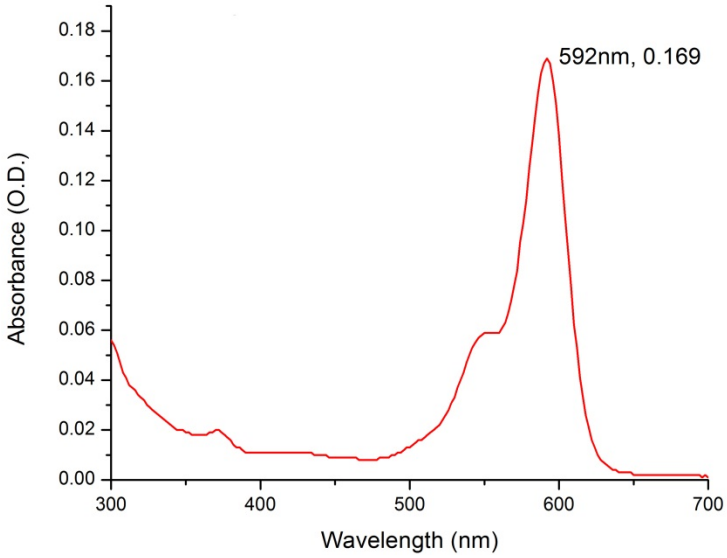


Fig. S6 UV-vis spectrum of TR-PNIPAM (polymer **4**) in H_2O.

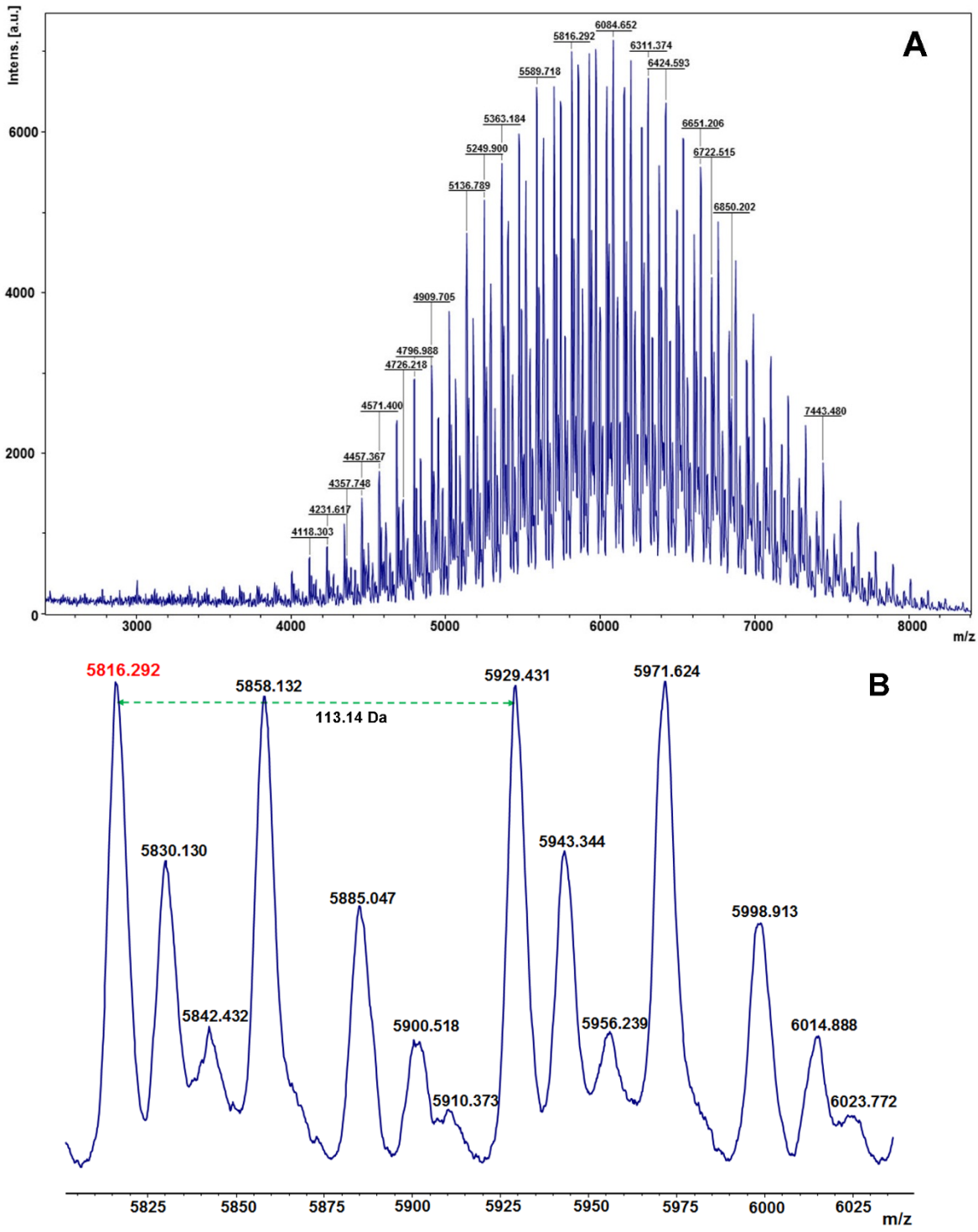


Fig. S7 (A) MALDI-TOF spectrum of TR-PNIPAM (polymer **4**). (B) Zoomed in of spectrum A showing DP = 43 (DP: degree of polymerization). Emprical formula: $[C_{301}H_{524}N_{49}O_{52}S_3Cl + Na]^+$. Calculated avg. mass: 5816.854, measured avg. mass: 5816.292

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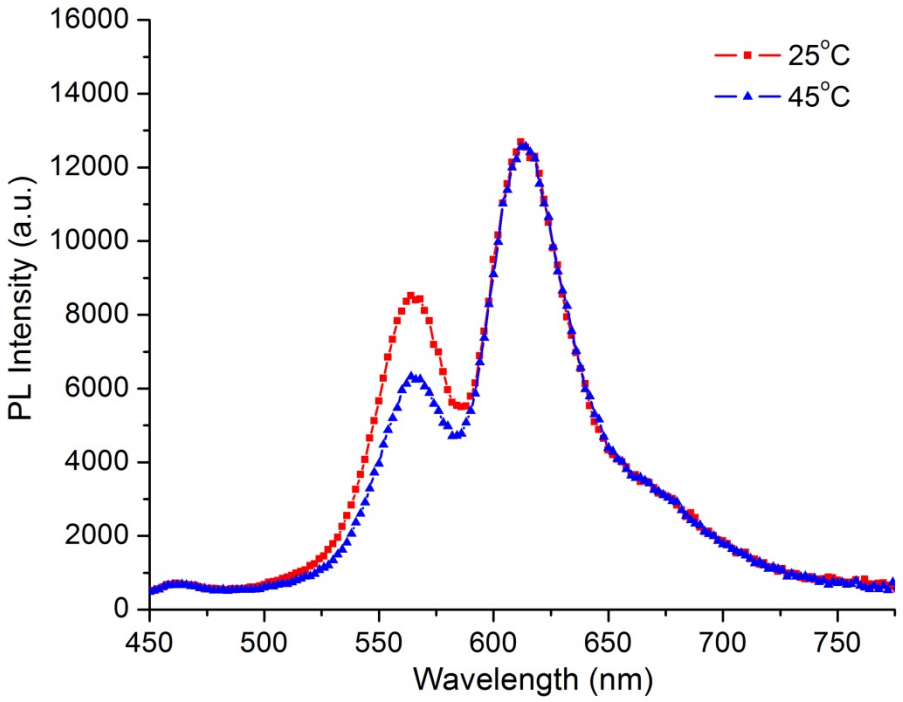

Fig. S8 PL spectra of the mixture of DHLA coated QDs and TR-PNIPAM under 25 ℃ and 45 ℃.

1. Lai, J. T.; Filla, D.; Shea, R., *Macromolecules* **2002,** *35*, 6754-6756.