Supporting Information

Compact Zwitterion-Coated Iron Oxide Nanoparticles for Biological Applications

*He Wei,† Numpon Insin,† Jungmin Lee, Hee-Sun Han, Jose M. Cordero, Wenhao Liu, and Moungi G. Bawendi**

Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, U. S. A.

E-mail: mgb@mit.edu

† These two authors contributed equally to this work.

Supporting Information Available. Experimental details of synthesis of iron oxide NPs, synthesis of dopamine sulfonate and zwitterionic dopamine sulfonate, ligand exchange of iron oxide NPs, dynamic light scattering measurements, serum binding test, synthesis of thiol-terminated catechol-derivative, dye and streptavidin conjugation for biotin plate tests.

Materials and analysis. All chemicals unless indicated were obtained from Sigma Aldrich and used as received. Air-sensitive materials were handled in an Omni-Lab VAC glove box under dry nitrogen atmosphere with oxygen levels <0.2 ppm. All solvents were spectrophotometric grade and purchased from EMD Biosciences. TEM images of the iron oxide NPs were obtained with a JEOL 200CX electron microscope operated at 200 kV. NMR spectra were recorded on a Bruker DRX 400 NMR spectrometer. UV-Vis absorbance spectra were taken using a HP 8453 diode array spectrophotometer.

Synthesis of iron oxide NPs. Maghemite (Fe_2O_3) magnetic NPs were prepared using a method modified from the literature.[1,2] As an example, for the synthesis of 8 nm NPs, 400 μL of $Fe(CO)_5$ was added to 0.75 mL of oleic acid in 20 mL of dioctyl ether at 100 °C. The temperature was increased at a rate of 2 °C/min to a final temperature of 290 °C, at which it was held constant for 1 h. After the mixture was cooled to room temperature, 0.32 g of $(CH_3)_3NO$ was added as an oxidizing agent.[3] The mixture was heated to 130 °C for 2 h and was then heated to 275 °C for 15 min. After cooling, the NPs were processed for storage by first adding ethanol to precipitate them. After centrifuging, the supernatant was discarded, and the NPs were then redispersed and stored in hexane. The typical concentration of iron oxide nanoparticles in hexane is ~2.8×10^{-5} mol (of NPs)/L (24 g/L).

Synthesis of Dopamine Sulfonate. Dopamine hydrochloride (1.1376 g, 6 mmol) was dissolved in 150 mL ethanol in a 500 mL round bottom flask. The flask was evacuated and back-filled with N_2, followed by slow addition of 28% ammonium hydroxide (416 μL, 3 mmol) and 1,3-propanesultone (799 mg, 6.5 mmol). The solution was heated to 50 °C and stirred for 18 hrs, resulting in the formation of white precipitates. The solvent mixture was filtered and the white precipitate was washed with ethanol three times. The residual white solid was dried under reduced pressure and characterized by NMR, which showed it to be pure (by NMR) dopamine sulfonate (DS). ^{1}H NMR (400 MHz, D_2O): δ (ppm) 2.08 (m, 2H), 2.88-2.90 (m, 2H), 2.94-2.99 (m, 2H), 3.14-3.17 (m, 2H), 3.26 (m, 2H), 6.71-6.74 (m, 1H), 6.82-6.88 (m, 2H). (Figure S1a) ^{13}C NMR (400 MHz, D_2O): δ (ppm) 21.11, 30.88, 46.11, 47.77, 48.57, 116.44, 121.06, 128.91, 143.01, 144.19. (Figure S1b)

Synthesis of Zwitterionic Dopamine Sulfonate. Dopamine sulfonate (0.3286 g, 1 mmol) was dissolved in 150 mL dimethylformamide (DMF) in a 500 mL round bottom flask. Anhydrous sodium carbonate (0.2544 g, 2.4 mmol) was added into the DMF solution, although the sodium carbonate did not completely dissolve initially. The flask was evacuated and back-filled with N_2 three times, followed by the addition of iodomethane (2.2 mL, 35 mmol). The solution was stirred for 5-10 hrs at 50 °C (the

sodium carbonate completely dissolved and the reaction mixture turned yellow upon completion of the methylation). The DMF was removed using a rotary evaporator at 40 °C and an oily mixture was obtained. 50 mL DMF/Ethyl acetate (1:10 v/v) was added to precipitate out a pale-yellow crude product. Following filtration, 50 mL DMF/Acetone (1:10 v/v) was added to the crude product and the mixture was refluxed at 55 °C for 2 hrs. The solution mixture was filtered again and the precipitate was collected. These reflux and filtration processes were repeated two more times and a white solid was obtained and characterized by NMR, which showed it to be pure (by NMR) zwitterionic dopamine sulfonate (ZDS). ^{1}H NMR (400 MHz, D_2O): δ (ppm) 2.21 (m, 2H), 2.92-2.95 (m, 4H), 3.13 (s, 6H), 3.47-3.51 (m, 4H), 6.74-6.76 (m, 1H), 6.83-6.88 (m, 2H). (Figure S1b) ^{13}C NMR (400 MHz, D_2O): δ (ppm) 18.12, 27.66, 47.10, 50.72, 62.02, 64.62, 116.49, 121.21, 128.23, 143.03, 144.18. (Figure S1d)

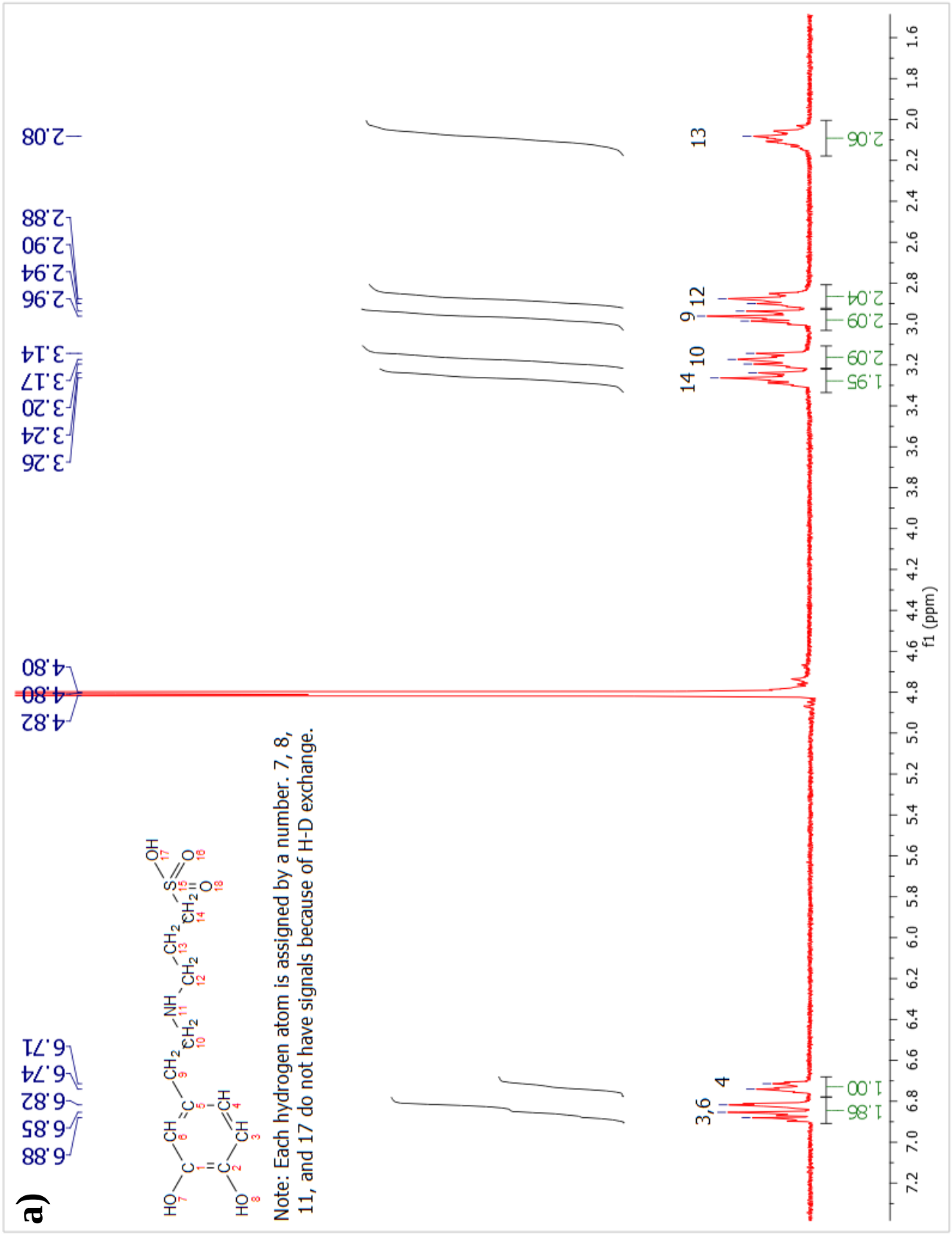


Figure S1a. ^{1}H NMR of DS (the peak at δ =4.80 is from D_2O solvents)

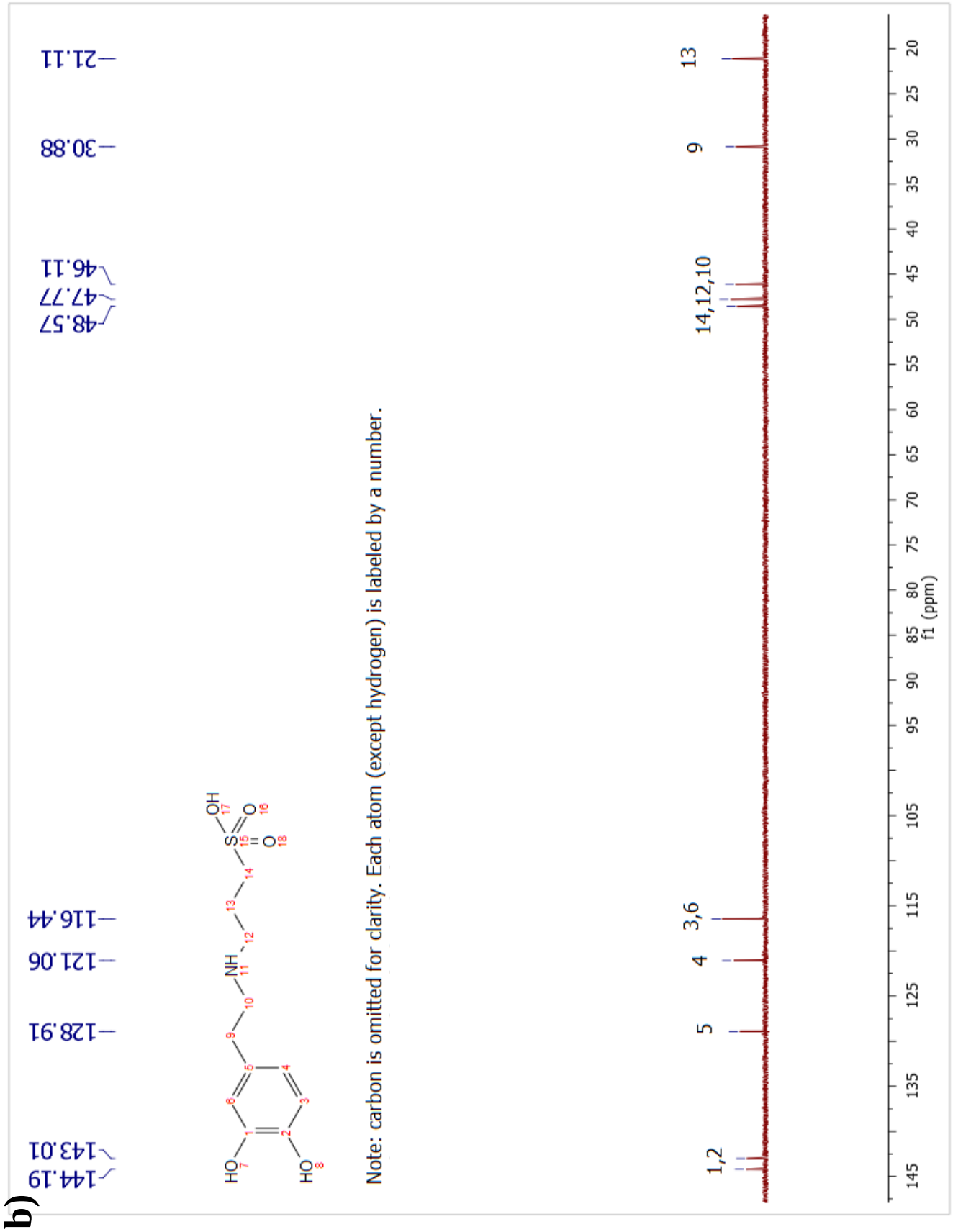


Figure S1b. ^{13}C NMR of DS

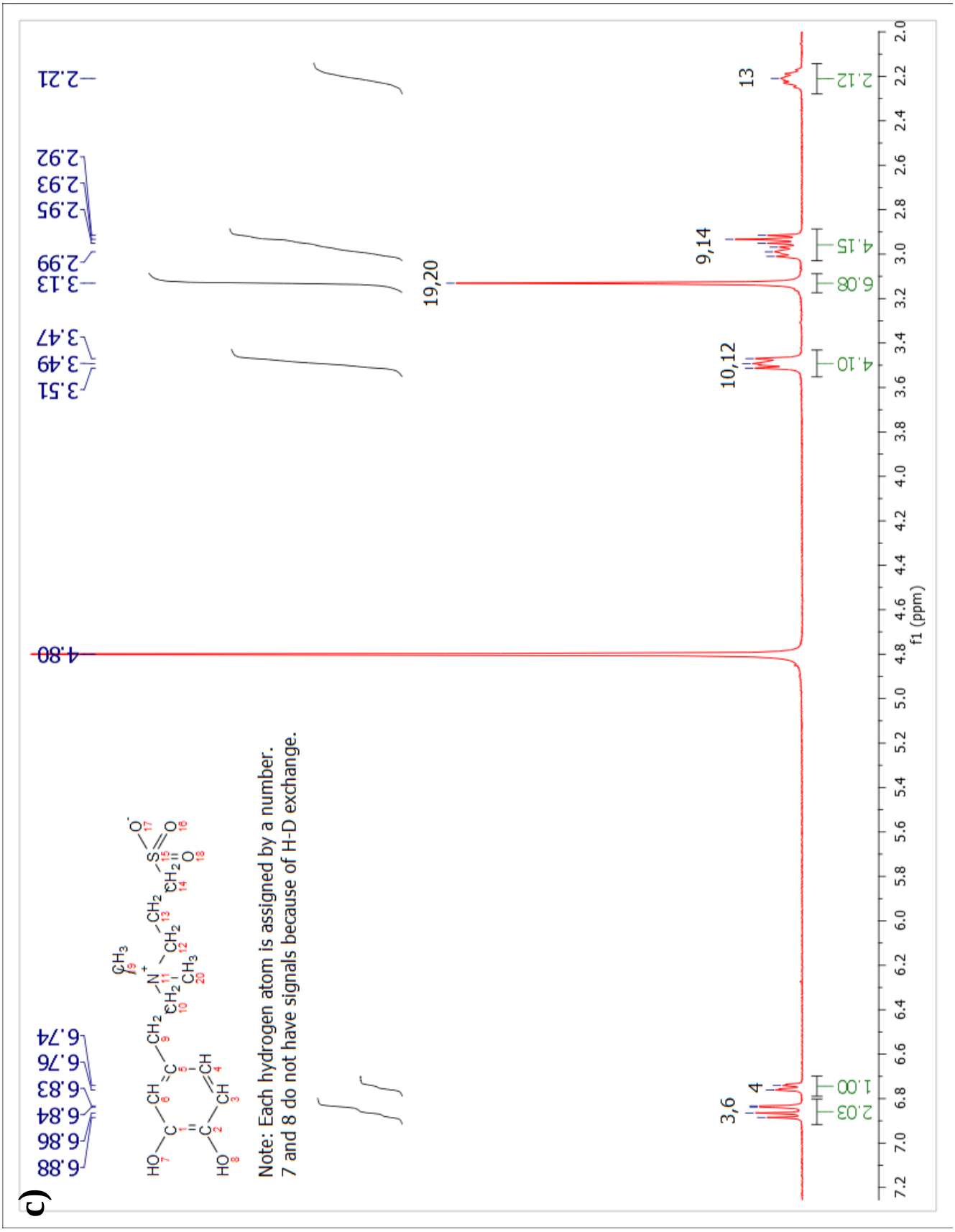


Figure S1c. ^{1}H NMR of ZDS (the peak at δ =4.80 is from D_2O solvents)

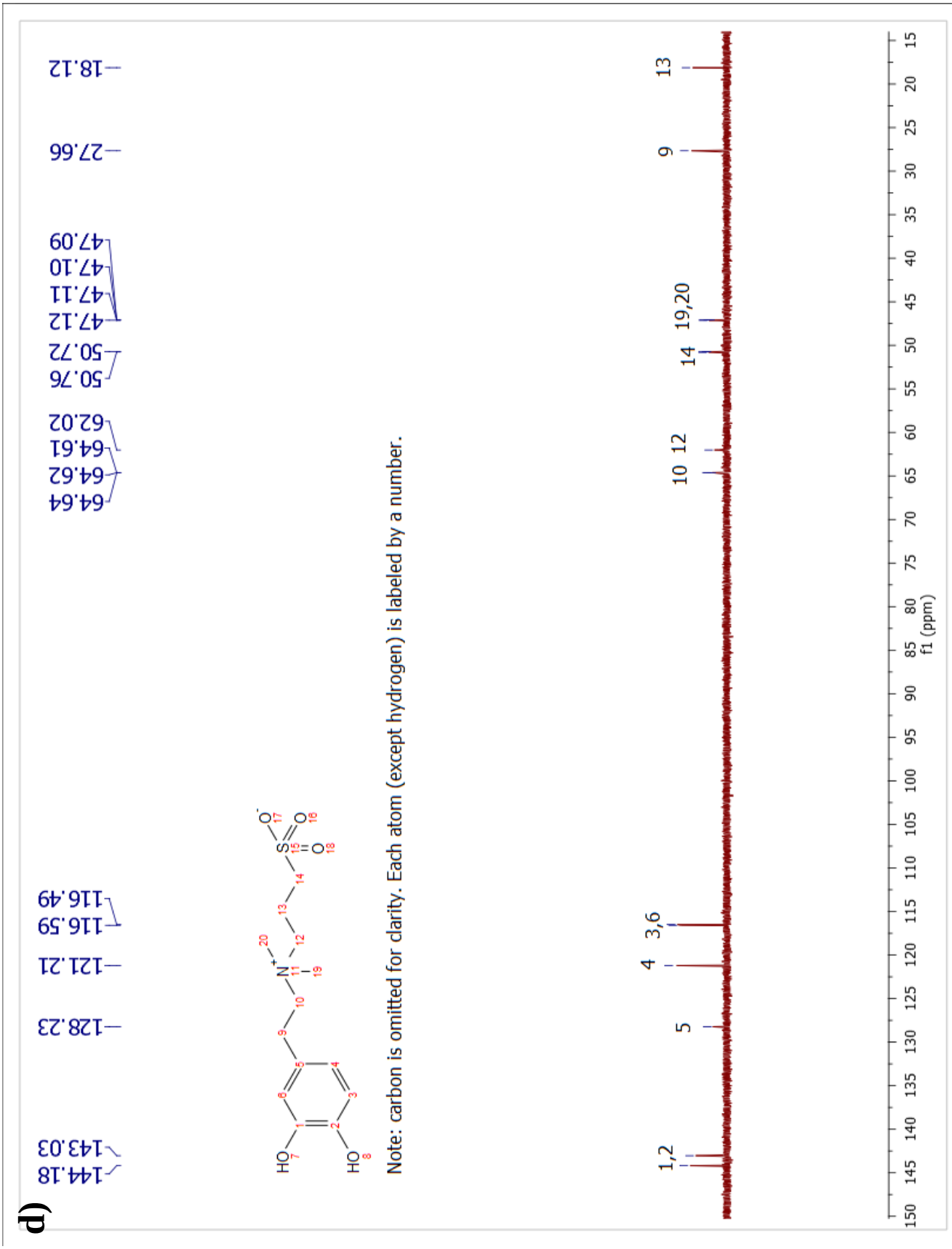


Figure S1a. ^{13}C NMR of ZDS

Ligand exchange of iron oxide NPs. Exchange of the native oleic acid surface ligands on NPs for the DS or ZDS ligand was carried out according to following procedures: To 25 μL of NPs in growth solution was added ethanol to the point of turbidity. Centrifugation and decantation yielded ~1 mg of dry pellet, to which 25 μL of neat 2-[2-(2-methoxyethoxy)ethoxy]acetic acid (MEAA) ligand and 75 μL of methanol were added. The mixture was stirred gently at 70 °C for 5 hrs and precipitated by adding 0.2 mL of acetone and 0.8 mL of hexane in succession. Centrifugation at 5800 RPM for 3 min yielded a clear supernatant, which was discarded. (Here the MEAA was used as an intermediate ligand to increase the solubility of NPs in 0.6 mL of DMF plus 0.3 mL of DI water, which was the solvent in the second ligand exchange process; this intermediate MEAA ligand is subsequently replaced by DS or ZDS later.) The pellet was then dispersed in 0.6 mL of DMF plus 0.3 mL of DI water, to which 50 mg of DS or ZDS ligand was added. Then the mixture was stirred again under N_2 at 70 °C for 12 hrs and precipitated by adding 5 mL of acetone. Centrifugation at 5800 RPM for 3 min yielded a clear supernatant, which was discarded. The pellet was then dispersed in 1 mL of phosphate buffer saline (PBS, pH=7.4: 1.5 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl) 1X and sonicated for 40 min. The sample was further purified using a dialysis filter (3 times) in order to thoroughly wash away excess ligand. DS or ZDS coated iron oxide nanoparticles could be dialyzed at least 10 times without noticeable aggregation, which is comparable to that of PEG-coupled nitrodopamine reported by Reimhult and co-workers.[4] The ZDS coated iron oxide NPs can reach a concentration of more than ~2×10^{-5} mol/L (17 g/L).

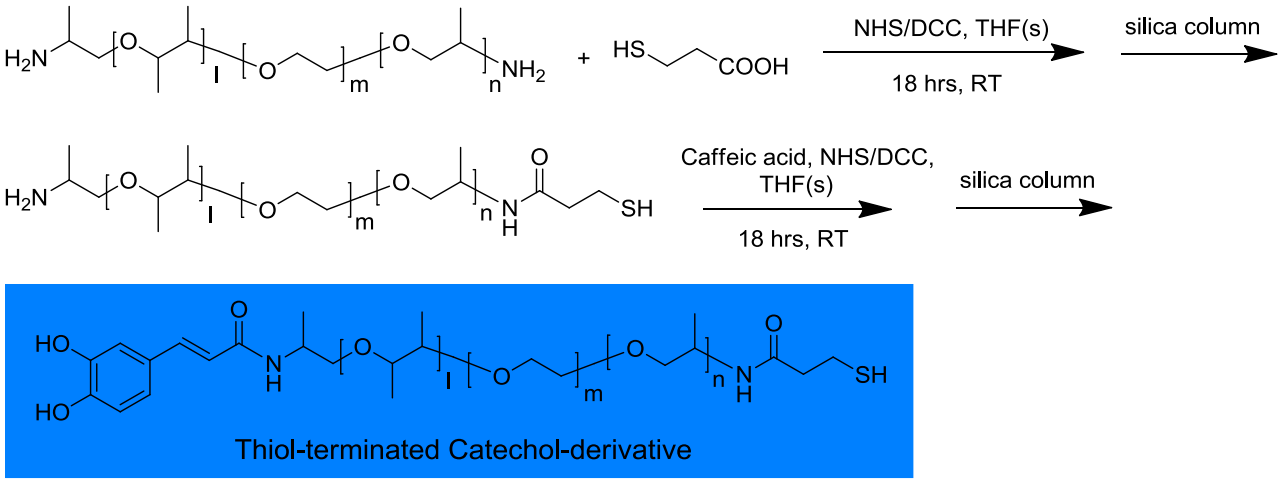
Dynamic Light Scattering and pH Stability Measurements. Light-scattering analysis was performed using a Malvern Instruments Nano-ZS90. While pHs varied from 6.0 to 8.5 with 0.5 spacing, all NP samples had the same concentration. Each autocorrelation function (ACF) was acquired for ~5 s, and averaged for ~10 min per measurement. Hydrodynamic diameters were obtained from a volume-

weighted size distribution data analysis and reported as the average of triplicate measurements (error bars in Figure 1b were the standard deviations of the values given by three parallel measurements).

Serum Binding Test. ZDS coated iron oxide NPs (ZDS-NPs) and DS coated iron oxide NPs (DS-NPs) were washed by phosphate buffer containing Mg^{2+} (pH=7: 1.5 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 137 mM NaCl, 10 mM $MgCl_2$, 2.7 mM KCl) and plain PBS 1X in succession, followed by the filtration using 0.2 μm HT Tuffryn® membranes. The resulted ZDS-NPs and DS-NPs were incubated with 10% and 20% Fetal Bovine Serum (FBS) solution (in PBS 1X) as well as plain PBS 1X,[5] respectively. Kept at room temperature for four hours, the six samples (ZDS-NPs with PBS, ZDS-NPs with 10% FBS, ZDS-NPs with 20% FBS, DS-NPs with PBS, DS-NPs with 10% FBS, DS-NPs with 20% FBS) were filtered by 0.2 μm HT Tuffryn® membranes before they were separately injected into a Superose™ 6 (GE Healthcare, 10/300 GL) size-exclusion column via a high-performance liquid chromatography machine manufactured by Agilent Technologies. With a flow rate of 0.5 mL PBS 1X per min, the iron oxide NPs were eluted and the absorption was monitored at 400 nm referencing to 600 nm and the resulted chromatograms were normalized by area.

Synthesis of Thiol-terminated Catechol-derivative and Its Ligand Exchange. The synthesis scheme is described in Scheme S1. 3-Mercaptopropionic acid (0.73 g, 6.9 mmol) was dissolved in 40 mL tetrahydrofuran (THF) in a 200 mL flask, followed by adding N-hydroxysuccinic anhydride (NHS, 1.03 g, 9.0 mmol) and N,N'-Dicyclohexylcarbodiimide (DCC, 1.84 g, 9.0 mmol). The solution was stirred at room temperature (RT) for 18 hrs, filtered, and the solvent removed using a rotary evaporator, yielding 3-Mercaptopropionic NHS ester. 8.26 g (13.7 mmol) O,O′-Bis(2-aminopropyl) polypropylene glycol-block-polyethylene glycol-block-polypropylene glycol (Jeffamine® ED-600) was dissolved in 40 mL THF in a 200 mL flask, and the 3-Mercaptopropionic NHS ester and triethyl amine (TEA, 0.77g, 7.6

mmol) were slowly added into this solution. The resulting solution was also stirred at RT for 18 hrs, filtered, and the solvent removed using a rotary evaporator. The oily product was purified via a silica column with 20% MeOH/Dichloromethane (v/v) as the eluting solvent, yielding the thiol-terminated Jeffamine. Separately, caffeic acid (1.25 g, 6.9 mmol) was dissolved in 80 mL THF in a 200 mL flask, followed by the addition of N-hydroxysuccinic anhydride (NHS, 1.03 g, 9.0 mmol) and N,N'-Dicyclohexylcarbodiimide (DCC, 1.84 g, 9.0 mmol). The solution was stirred at RT for 18 hrs, filtered, and the solvent removed using a rotary evaporator, yielding the caffeic NHS ester. Finally, the thiol-terminated Jeffamine was dissolved in 80 mL THF in a 200 mL flask, followed by adding the caffeic NHS ester and TEA (0.77g, 7.6 mmol). This solution was stirred at RT for 18 hrs, filtered, and the solvent removed using a rotary evaporator. The crude product was again purified via a silica column using 20% MeOH/Dichloromethane (v/v) as the eluting solvent, yielding the thiol-terminated catechol-derivative. The presence of free thiol groups was confirmed by Ellman's test. The ligand exchange of TD/ZDS was done by using the same procedure in section "Ligand exchange of iron oxide NPs", except that 15% TD and 85% ZDS ligands (in molar ratio) were used yielding TD/ZDS coated iron oxide NPs (TD/ZDS-NPs).



Scheme S1. Synthesis Route of Thiol-terminated Catechol-derivative

Dye and Streptavidin Conjugation for Biotin Plate Test. TD/ZDS-NPs were first treated with tris(2-carboxyethyl)phosphine to reduce any potential disulfide bonds, which may form during the ligand

exchange, to free thiol groups. 0.1 mg Alexa Fluor 488 maleimide dye (purchased from Invitrogen) and 1 mg streptavidin-maleimide (SA) were reacted with 200 μL TD/ZDS-NPs at 37 °C for 4 hrs. When the reaction was finished, the excess dye and streptavidin were immediately removed by dialysis (3 times), yielding Dye-SA-TD/ZDS-NPs with a hydrodynamic diameter of ~17 nm. Then three samples were prepared: the first one was 100 μL plain PBS 1X, which was used as a blank; the second one was 100 μL Dye-SA-TD/ZDS-NPs in PBS 1X incubated with 50 μL biotin solution (80 mM in dimethyl sulfoxide) for 20 min, where SA should be saturated by biotin; the third one was 100 μL Dye-SA-TD/ZDS-NPs in PBS 1X. The three samples were then incubated in biotin-coated wells on a strip plate (purchased from Thermo Scientific, Reacti-Bind™ Biotin coated clear strip plate) for 20 min. The sample solutions were then removed and the biotin-coated wells were rinsed with PBS 1X three times and 100 μL PBS 1X was added to each well. The fluorescence profiles of these three wells were measured by Plate Reader Machine (BioTek, SYNERGY 4) with emission wavelength from 520 nm to 750 nm. Both the results of the second sample (pre-biotin-saturated Dye-SA-TD/ZDS-NPs) and the third sample (Dye-SA-TD/ZDS-NPs) were blanked with the first sample (PBS 1X only).

REFERENCES:

1. Insin, N.; Tracy, J. B.; Lee, H.; Zimmer, J. P.; Westervelt, R. M.; Bawendi, M. G. *ACS Nano* **2008,** *2*, 197.
2. Woo, K.; Hong, J.; Choi, S.; Lee, H. W.; Ahn, J. P.; Kim, C. S.; Lee, S. W. *Chem. Mater.* **2004,** *16*, 2814.
3. Hyeon, T.; Lee, S. S.; Park, J.; Chung, Y.; Bin Na, H. *J Am Chem Soc* **2001,** *123*, 12798.
4. Amstad, E.; Gillich, T.; Bilecka, I.; Textor, M.; Reimhult, E. *Nano Lett.* **2009,** *9*, 4042.
5. Zheng, M.; Davidson, F.; Huang, X. *J. Am. Chem. Soc.* **2003,** *125*, 7790.