Supporting Information for

Imparting Nanoparticle Function with Size-Controlled Amphiphilic Polymers

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Overview:

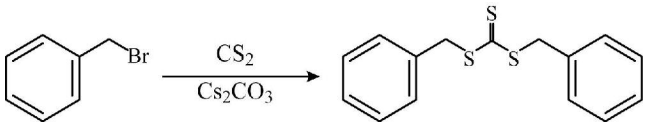
Reversible Addition Fragmentation chain Transfer (RAFT) is an effective way to control the size and chemical functionality of polymers.[1] This method employs a blocking RAFT agent that reversibly binds to the end of a growing radical polymer chain; the result is that all polymers grow in a nearly uniform manner. We generally vary the size of poly-acrylic acid (PAA) from 25, 50, and 100 monomers per polymer which are hydrophobically modified and then used to solubilize NPs. The thiol functionality formed on hydrolysis of the polymer is a convenient functional handle to further derivatize the NP materials in aqueous solution with maleimide functional dyes.

Experimental:

All reagents were purchased from either Fischer Scientific or Sigma-Aldrich Chemical Company, unless stated otherwise. Maleimide functional BODIPY 577/618 and fluorescein were purchased from Invitrogen (Carlsbad, Ca).

Synthesis of Benzyl Dithiobenzoate.

The procedure for this RAFT agent was followed directly from a recently reported procedure.[2] The as synthesized material was purified twice on a silica column using hexane as the eluent. We have found that the use of this RAFT initiator results in higher reaction yields compared to 4-cyanopentanoic acid dithiobenzoate and was used to make polymers for GPC and MALDI-TOF analysis. Most of the nanoparticle data was taken using 4-cyanopentanoic acid dithiobenzoate synthesized polymers, however in practice there is no difference between polymers synthesized using one RAFT agent or the other.



Schematic of synthesis of RAFT Benzyl Dithiobenzoate agent

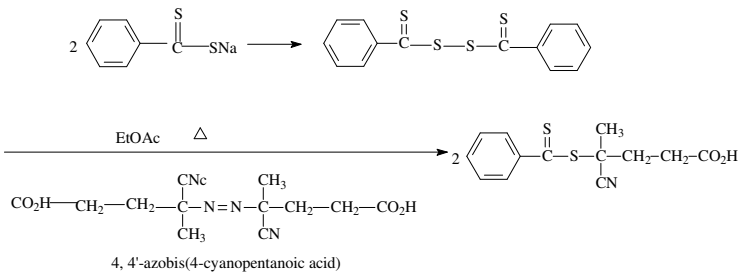
Synthesis of Dithiobenzoic Acid (DTBA).

In our initial studies we found that a Grignard reagent approach[1] towards the synthesis of DTBA produced materials of much higher purity compared to a common procedure involving sodium methoxide / elemental sulfur with benzyl chloride. First, 50 mL of tetrohydrofuran (THF) was added to a 200 mL flask with Mg turnings (0.41g, 17mmol) and a few iodine crystals (which acts as a catalyst). Next, 1.877 mL of bromobenzene (18mmol) dissolved in 8mL of THF was dripped slowly into the solution until the evolution of hydrogen was observed. Next, the solution was immersed in ice water and the remaining bromobenzene solution was added slowly. Next, the addition funnel was charged with 1 mL of carbon disulfide (17mmol) in 9 mL of THF which was slowly added to the solution while chilled. After addition of the CS_2, 50 mL water was added to the crude sodium dithiobenzoate solution which was transferred to a separatory funnel.

The solution was acidified with dilute HCl (3.0 mL of 12M HCl in 25 mL water). The product was then extracted into diethyl ether (3×20ml) from which the ionized product was re-extracted from using (3×125mL) NaOH basic water.

Synthesis of Di(thiobenzoyl) Disulfide.
The basic sodium dithiobenzoate solution was transferred immediately upon preparation to a 500 mL round-bottomed flask equipped with a magnetic stir bar. Potassium ferricyanide(III) (5.5g, 16.7mmol) was dissolved in deionized water and was added drop-wise via the addition funnel over 1h under vigorous stirring. The solution was washed several times with water; the red precipitate was subsequently isolated via centrifugation. The solid was dried under vacuum over night and was recrystallized from ethanol.

Synthesis of 4-Cyanopentanoic Acid Dithiobenzoate.
Dried di(thiobenzoyl) disulfide (2.0g, 6.59mmol) and 4,4'-azobis(4-cyanopentanoic acid) (2.75g 9.81mmol) was added to ethyl acetate (40mL) in a round-bottomed flask equipped with a magnetic stir bar. The reaction solution was heated to reflux (80 °C) for 18 hours. The ethyl acetate was removed under vacuum. The product was purified by silica gel column chromatography eluted with an ethyl acetate: hexane (2:3) mobile phase. The product was vacuum dried to finally yield purple powder which was then used as a RAFT polymerization initiator.



Schematic of synthesis of the 4-Cyanopentanoic Acid Dithiobenzoate RAFT agent

Polymerizations.
A 50 mL three-necked round-bottomed flask equipped with a magnetic stir bar and a rubber septum was charged with anhydrous ethanol (9mL), followed by addition of freshly distilled acrylic acid (3.0mL, 43.7mmol), RAFT agent (benzyl dithiobenzoate: 0.244g, 0.876mmol, or 4-cyanopentanoic acid dithiobenzoate: 0.254g, 0.876mmol) and 4, 4'-azobis(4-cyanopentanoic acid) (0.0245g, 0.0875mmol). The reaction solution was purged with N_2 and then heated at reflux ~1 hour at 95 °C. The reaction was stopped by adding 2,6-di-tert-butyl-4-methylphenol (0.21g, 0.96mmol) in benchtop ethanol. The polymerization yield is much higher (~100%) when using benzyl dithiobenzoate. Some material was saved for GPC analysis while the remainder was used for hydrophobic modification.

GPC Characterization.
After polymerization of the poly-acrylic acid (PAA) backbone, some material was hydrolyzed in basic water overnight which was analyzed by gel permeation chromatography (GPC). We used a low pressure system (Ätka Prime, GE Biosciences) which required a large amount of experimentation to determine the optimal conditions to analyze PAA samples. We found that a Superdex Peptide 10/300 GL column from GE Biosciences using pH 5 phosphate buffer solution was optimal towards analyzing the

molecular weight of the PAA polymers, which were calibrated against poly-acrylic acid standards from American Polymer Standards. We found that calibration against any other polymer or protein results in significant error when applied towards analyzing poly-acrylic acid. Very low concentrations are also necessary to properly analyze the poly-acrylic materials. Shown in Fig. S1 is a GPC molecular weight distribution of our benzyl dithiobenzoate synthesized PAA from which we calculated the number average (Mn) and the weight average (Mw) molecular weight. We have found that our Benzyl Dithiobenzoate synthesized PAA polymer has weights of Mn=1,810 and Mw=2,300. The number average molecular weight is on target for our synthetic strategy; the polydispersity (PDI) factor of 1.27 is typical for RAFT synthesized polymers while we found that a commercial source of PAA has a PDI of 2.3, again typical for uncontrolled polymerizations. The yield of RAFT PAA synthesized using 4-cyanopentanoic acid dithiobenzoate was smaller and thus the size could not be determined on our column. It should also be noted that not all polymers likely contain a thiol functional group as other hydrolysis products from the RAFT initiators can be formed.[3]

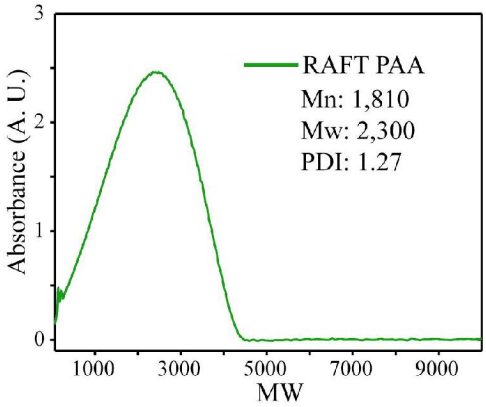


Figure S1. GPC data of benzyl dithiobenzoate synthesized poly-acrylic acid.

MALDI-TOF Characterization.

We also characterized benzyl dithiobenzoate synthesized PAA using MALDI-TOF, the spectrum of which is shown in Figure S2. These results suggest that the RAFT agent was the dominant initiator, however we note that these data proved very difficult to reproduce. We have discussed this issue with several experts in the field which confirmed that MALDI-TOF of PAA tends to be very difficult to take; this data set represents our best results. We feel that GPC characterization is easier to run and analyze and was much more reproducible than mass spectrum data.

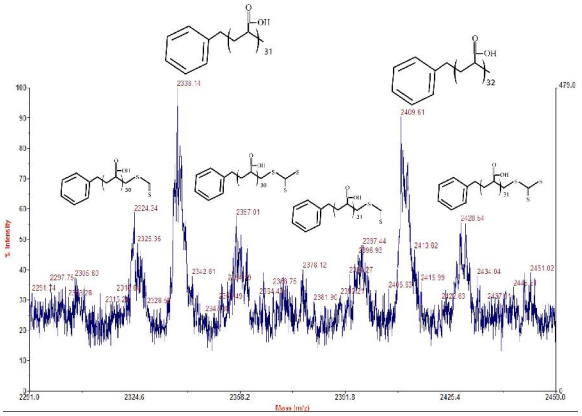


Figure S2. MALDI-TOF spectrum of RAFT PAA synthesized in our laboratories.

Hydrophobic Modification.

RAFT synthesized poly-acrylic acid (3.0g, 36.7mmol) in anhydrous DMF solvent (8ml) was added to a dried 50 mL, round-bottomed flask equipped with a magnetic stir bar. Next, EDC (2.81g, 14.6mmol) was dissolved in a small amount of DMF and then injected into the poly-acrylic acid solution. The mixture was stirred for 0.5h following by addition of octylamine (2.43ml, 14.7mmol). The reaction solution was stirred at room temperature

overnight followed by solvent removal under vacuum ~5h to reduce the volume. Precipitation of the polymer was accomplished via the addition of distilled water which was isolated by centrifuge. Sodium hydroxide (100ml, 0.1M) was added and the solution was stirred for 24h to hydrolyze the polymer. The crude modified PAA solution was transferred to a 1 L separatory funnel and was washed with ethyl acetate (3×20mL). A dilute solution of HCl was added to the aqueous layer until the pH was below 5 to precipitate the polymer. The material was then neutralized and lyophilized for storage and subsequent use.

Water solubilized NPs with RAFT amphiphilic polymer.

Nanoparticles in growth solution were precipitated with a few drops of isopropyl alcohol and excess methanol. The flocculate was centrifuged for 4 min at 3.0K RPM followed by removal of the supernatant. The precipitation was performed again by dissolving the NPs in hexane, transferring to a pre-weighed vial and re-precipitating as before. After vacuum drying generally ~10mg of bare NPs are isolated. Amphiphilic RAFT synthesized polymers (50mg) were added to the NPs followed by chloroform (5mL) and ~two drops of methanol which was sonicated until the polymer solubilizes. The addition of a very small amount of methanol was found to be crucial to make the solubilization procedure reproducible with high yield. The solution was dried under vacuum at room temperature for 1h; basic water was then added resulting in clear, highly fluorescent aqueous solution of NPs. Consequently, there does not appear to be any interaction of unreacted RAFT agents or by-products which cause quenching or precipitation of the nanoparticles. Magnetic (and non-fluorescent) Fe_2O_3 NPs do not solubilize as well and required filtering with 100nm syringe filters. Dialysis was performed using 50K MW cutoff filters from Millipore usually 6-7 times or until no more free polymer is observed in the filtrate (as confirmed by acidification of the filtrate, no excess polymer precipitation is observed when the material has been purified). Some samples required purification by GPC. The solutions are dialyzed against pH 6 phosphate buffer solution which is a good pH for conjugation of maleimide functional dyes with the NPs.

Conjugation of maleimide functional dyes and RAFT polymer encapsulated NPs.

A small amount of a DMF solution of BODIPY 577/618 maleimide dye was added to polymer coated 580 nm emitting CdSe/ZnS NPs in pH 6 buffer. The concentrations dye and NP in solution was determined to be 3.9775×10^{-5} M and 1.1067×10^{-6} M, respectively (thus a dye/NP ratio of ~3.6) The absorption/emission was monitored every 10 to 15 minutes at first while the solutions are stirred; the data are shown in Figure 2 of the main text. To analyze the kinetic data, we first assume that the

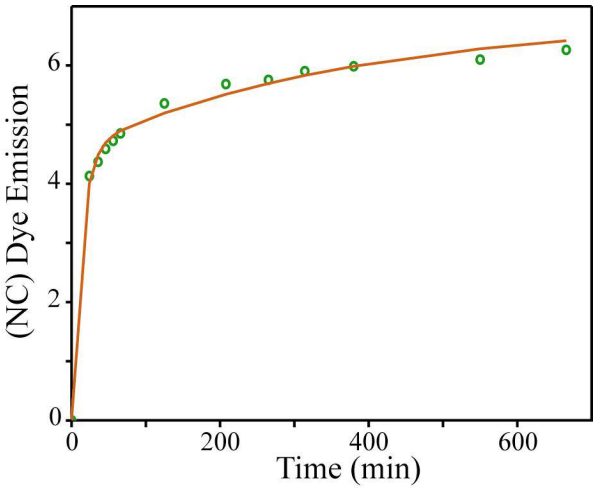


Figure S3. Kinetic trace of the formation of the CdSe/ZnS NP / BODIPY 577/618 dye coupled product over time.

dye component of the emission is entirely from the coupled construct given that the NP / dye system is excited where the dye has very little absoprtion. By decoupling the dye from NP emission using Matlab scripts and plotting the relative dye emission intensity over time allows us to calculate the rate of the reaction. Next, we note that the CdSe/ZnS NPs have a static concentration profile as a single NP can react with more than one dye molecule. We thus analyzed the kinetics shown in Fig. S3 under pseudo first order conditions where we find two processes are active with rate constants of 1.18×10^{3} $M^{-1}s^{-1}$ and 40.4 $M^{-1}s^{-1}$ (the per molar unit is with respect to the NP concentration). We do not know the origin of the biphasic kinetics however we assume that capture of the hydrophobic dye within the hydrophobic interior region of the polymer matrix and subsequent reaction with the thiol are two separate possible dynamical channels.

While we could not determine the absolute reaction yield of direct BODIPY 577/618 maleimide to thiol coupling given the hydrophobic nature of the BODIPY dye, we were able to measure a reaction efficiency of hydrophilic fluorescein maleimide dye conjugated to blue emitting CdS/ZnS NPs at 30 ± 10% in buffered pH 6 water. This was calculated as follows- after several days of stirring, the NP / dye was dialyzed using 50,000 molecular weight (MW) cutoff centrifuge filters from Millipore. Approximately half the sample is dialyzed. We then compare the relative percent dye concentration in the NP:dye + unbound dye in the high molecular weight portion to the amount of unbound dye in the filtrate. The percent yield is then 100% - 100×[dye in filtrate]/[dye in high MW portion]%. The samples were purified by GPC to assure that the coupling occurs only to dye and NP bound polymers vs. dye conjugation to free polymers.

The kinetic rate constants for CdS/ZnS and fluorscein maleimide coupling could not be determined nor the dye to NP ratio as the molar absoptivity of CdS/ZnS NPs is unknown. The quantum yield of the aqueous blank CdS/ZnS NPs was measured to be 12.4% which lowers to 6-8% upon conjugation to fluorescein maleimide (quantum yields are all relative to Coumarin 104 in methanol). This is likely due to the highly self-absorptive nature of NP / dye constructs. We also determined the FRET efficiency as a function of solution pH via quenching of the donor to be: pH 5 = 59%, pH 6 = 67%, pH 7 = 72%, and pH 8 = 74%. The efficiency is modulated by the increased absoprtion of the fluorescein acceptor (as shown in Figure S4) as the solution turns more basic which results in a naturally ratiometric fluorescent response to pH.

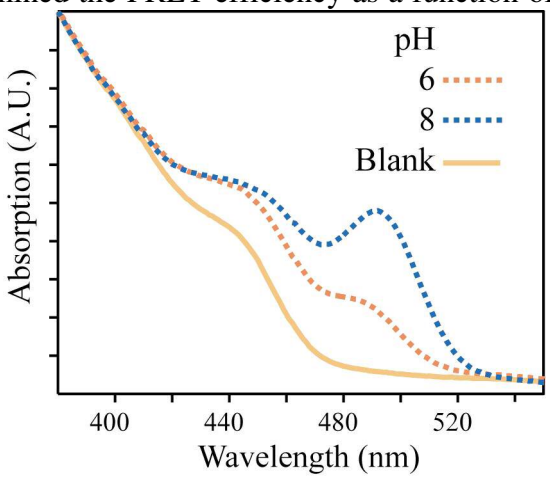


Figure S4. The increase of the absorption of the fluorescein dye with base is discernible in the absoprtion spectra of our CdS/ZnS : fluorescein dye product.

For the Fe_2O_3 NP / fluorescein system we were again unable to determine the dye / NP ratio, however we were able to deconvolve the fluorescein absorption from the iron oxide to show that the NP bound dye is quenched by 43% relative to unconjugated fluorescein. If the total absoprtion of the

material is considered, the NP / dye construct is 15% as bright as the free dye.

Many reported NP functionalization strategies involve conjugating water soluble, antibody functionalized NPs attached to dye labeled antigens. Using a biological coupling strategy is costly and requires disparate knowledge of both inorganic synthesis and biological labeling. Further, the coupled chromophore distance is very difficult to manipulate which can result in poor energy transfer efficiency.[3] Using commercially available materials we have coupled NPs to dyes via thioether links which represents a biologically stable bonding motif. Since the reaction conditions are very mild, have high conjugation efficiencies and involve no other coupling reagent (such as EDC, which tends to cause PAA coated NP's to precipitate) we may synthesize these functional materials on a very large scale.

Reference:

1. Hans de Brouwer "RAFT memorabilia: living radical polymerization in homogeneous and heterogeneous media." Technische Universiteit Eindhoven, 2001.
2. Aoyagi, N.; Ochiai, B.; Mori, H.; Endo, T. *Synlett.* **2006**, *4*, 636-638.
3. Hohng, S.; Ha, T. *Chem. Phys. Chem.* **2005**, *6*, 956.