Hybrid semiconducting polymer nanoparticles as polarization-sensitive fluorescent probes

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Supporting Information

MATERIALS AND METHODS

Materials. Poly[(9,9-dioctylfluorenyl-2,7-diyl)-alt-co-(1,4-benzo-(2,1',3)-thiadiazole)] (PFBT, 10 kDa MW, polydispersity index (PDI) 2.3) was purchased from American Dye Source, Inc. (Quebec, Canada). Polystyrene-graft-poly(ethylene oxide) functionalized with carboxyl groups (PS-PEG-COOH, main chain MW 8,500, graft chain MW 1,200, total chain MW 21,700, PDI 1.25) was purchased from Polymer Source Inc (Quebec, Canada). Dimethyl sulfoxide (DMSO), casein, adenosine 5'-triphosphate magnesium salt (MgATP), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), (3-aminopropyl) triethoxysilane (APTES), poly(ethylene glycol) (PEG), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St. Louis, MO). 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) was purchased from Invitrogen (Carlsbad, CA). 30% Hydrogen peroxide was purchased from JT Baker (Mansfield, MA). Ultrapure water (Milli-Q) was produced by a Milli-Q water production unit; this production unit and ammonium hydroxide were purchased from EMD Millipore (Billerica, MA). Tubulin protein, biotinylated tubulin, fluorescent HiLyte 488 tubulin, paclitaxel ("Taxol"), guanosine-5'- triphosphate (GTP), and General Tubulin Buffer (GTB) were purchased from Cytoskeleton Inc. (Denver, CO). Full length kinesin motor proteins of two varieties, fruit fly kinesin and *Escherichia coli*, were kindly provided by the Wordeman lab at the University of Washington department of physiology and biophysics. All chemicals were used as-is unless stated otherwise.

P70 Polymer synthesis. The copolymer was synthesized by grafting hydrophobic dodecylamine onto the hydrophilic poly(isobutylene-alt-maleic anhydride) (Mw~6,000) backbone through spontaneous amide linkage, which converts one maleic anhydride into one corresponding amide and one free carboxylic acid. Typically, 0.5 g (0.083 mmol) of poly(isobutylene-alt-maleic anhydride) was dispersed in 20 mL of anhydrous THF in a 100-mL round flask. 0.43 g (2.32 mmol) dodecylamine dissolved in 40 mL of anhydrous THF was quickly injected to the polymer solution and kept at 60 °C with vigorous stirring. After 3 hours, the reaction mixture was concentrated to one third of the original volume under a reduced pressure. The concentrated solution was further refluxed overnight at 60 °C. The solvent was then slowly evaporated until the polymer was completely dry to obtain a pale yellow solid. The final product yield is ~0.88 g, 95%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.368-4.339 (br s, 2H), 3.74 (s, 2H), 2.516-2.483 (br s, 2H), 2.282-2.251 (br s, 2H), 1.848 (s, 2H), 1.264 (s, 22H), 0.879 (s, 9H).

Semiconducting Polymer Nanoparticle Formation. Polymer nanoparticles were formed by a nanoprecipitation technique. THF solutions with 20 μL of 10 mg/mL P70, 25 μL of 10 mg/mL PS-PEG-COOH, and 1 μL of 10 mg/mL PFBT were mixed with 5 mL of THF and the resulting solution was sonicated for 1 minute. Following sonication, the THF solution was quickly added to 10 mL of Milli-Q water under high sonication power. The polymer is insoluble in water and quickly formed polymer nanoparticles. The THF was then removed by heating the solution to 85 °C and bubbling nitrogen gas through the solution for 2 hours. Following removal of THF, the polymer dots were filtered through a 0.2-μm filter.

Polymer Dot Bioconjugation. Polymer nanoparticles were covalently bound to streptavidin in order to provide a convenient way to link nanoparticles with other biomolecules or structures of interest. 4 mL of nanoparticle solution with 0.1% PEG was mixed with 240 μL of streptavidin solution (1 mg/mL), and then 80 μL of freshly prepared EDC (5 mg/mL) was added to the solution. This was mixed for 4 hours by magnetic stirring at room temperature. After 4 hours, amine-terminated PEG was added along with an additional 80 μL of EDC in order to cap any remaining carboxylic acid groups on the surface of the polymer dots and to reduce nonspecific binding. The reaction was allowed to continue for another 2 hours and then quenched with 80 μL of a 10 wt% BSA solution. The polymer dots were then concentrated using a 100,000 MW cut-off centrifuge tube and purified on a size-exclusion column. The resulting streptavidin-and-PEG-functionalized polymer dots are stable for several months if refrigerated at 4 °C.

Microtubule Preparation. Microtubules were polymerized from tubulin by mixing 10 μL GTB with 9 μL of 10 mg/mL tubulin, 1 μL 10 mg/mL biotinylated tubulin, 2 μL anhydrous DMSO, and 0.5 μL GTP. The solution was placed in a 37 °C water bath and taxol was added to a final concentration of 2 μM, 20 μM, and 200 μM at 0, 10, and 20 minutes, respectively. Polymerization was continued for 60 minutes and the microtubules were shortened by pipetting the microtubule repeatedly before use. Fluorescently labeled microtubules, used as a control, were prepared by replacing the biotinylated tubulin with HiLyte 488 tubulin and polymerizing the tubulin as described above.

Microtubule Gliding Assay. All glass coverslips were cleaned by sonication for 30 minutes in a 2% Micro-90 solution, followed by thorough rinsing in Milli-Q waterand sonication for 30 minutes in Milli-Q water. The glass was then boiled in a 3:2:1 solution of Milli-Q:NH_4OH:H_2O_2 for 60 minutes, followed by thorough rinsing with Milli-Q water before use. A channel with the approximate dimensions of 2.5 cm × 1 cm × 1 μm, was created using a clean glass slide attached to a 1" coverslip using double-sided sticky tape. Gliding microtubules with attached polymer dots were visualized using a slightly modified technique previously described by Wang et al.[25] A

series of five solutions, 10 μL each, were introduced to the channel and allowed to sit for 5 minutes after their introduction. Solution 1 contained GTB with 0.5 mg/mL casein. Solution 2 contained GTB with 0.2 mg/mL casein, 0.3 mM MgATP, and kinesin. Solution 3 contained GTB with 0.5 mg/mL casein, 0.3 mM MgATP, 10 μM Taxol, and 0.05 μL of the microtubule solution. Solution 4 contained GTB with 0.5 mg/mL casein, 0.3 mM MgATP, 10 μM Taxol, and 50 pM functionalized polymer dots. Solution 5 contained GTB with 0.5 mg/mL casein, 1.5 mM MgATP, 10 μM Taxol, and an oxygen scavenging system (50 μg/mL glucose oxidase, 4μg/mL catalase, 1% glucose, and 0.1% β-mercaptoethanol). The final solution was introduced three times to flush out the free polymer nanoparticles.

Optical Setup. Polarized wide field illumination was accomplished using best form spherical singlet lenses, 488 nm λ/2 waveplates, and polarizers (Thorlabs, Newton, NJ). Through careful placement of polarization optics, we achieved an $I_{\parallel}$:$I_{\perp}$ ratio of 100:1.The 488-nm dichroic mirror we used was chosen for its insensitivity to polarization for reflection and transmission. Fluorescence excitation came from a Sapphire 488-nm laser (Coherent, Santa Clara, CA). Excitation light was filtered out using a 500-nm longpass filter. Imaging was carried out using a TE-2000 microscope (Nikon, Melville, NY) and a 100× 1.3 numerical aperture objective. A home-built setup described previously (Zeigler et al)[19] was used to separate the orthogonally polarized fluorescence components, and the resulting pair of images were captured on two halves of the same Cascade 512b EMCCD camera (Photometrics, Tucson, AZ).

Observation of Individual Surface Bound Polymer Nanoparticles. Individual polymer nanoparticles were observed by applying very dilute (1 pM) solutions of polymer nanoparticles to (3-aminopropyl)triethoxysilane treated number 1 glass coverslips (Bellco, Vineland NJ). The coverslips were photoetched to assist in positioning the coverslip under bright-field illumination. After five minutes, the coverslips were rinsed with Milli-Q water, dried, and made in a channel as described previously. Polymer nanoparticles polarization sensitivity was demonstrated by filling the channel with water and inserting the channel into a rotating stage mounted on the microscope. The polymer nanoparticles were illuminated and stage was rotated manually to visualize the selectively polarized emission of individual polymer nanoparticles.

Characterization of Polymer Nanoparticles. All fluorescence spectra were taken in 20 mM HEPES buffer, pH 7.4, supplemented with 0.1% PEG. Quantum yield measurements were taken using a Hamamatsu absolute PL quantum yield measurement system (Hamamatsu, Shizuoka, Japan), excited at 450 nm with a xenon lamp. Fluorescence lifetime and time-resolved fluorescence anisotropy measurements were taken using a Pico Quant Fluo time 100 system and a 470 nm picosecond laser as per manufacturer's recommendation and analyzed using commercial Fluo Fit software (Picoquant GmbH, Berlin, Germany). Nanoparticle size and zeta potential were characterized using a Zetasizer Nano ZS (Malvern, Philadelphia, PA). Fluorescence spectra were taken using the Fluorolog-3 fluorospectrometer (HORIBA, JobinYvon, NJ). Fluorescence spectra were taken in 20 mM HEPES buffer, pH 7.4, with 0.1% PEG.

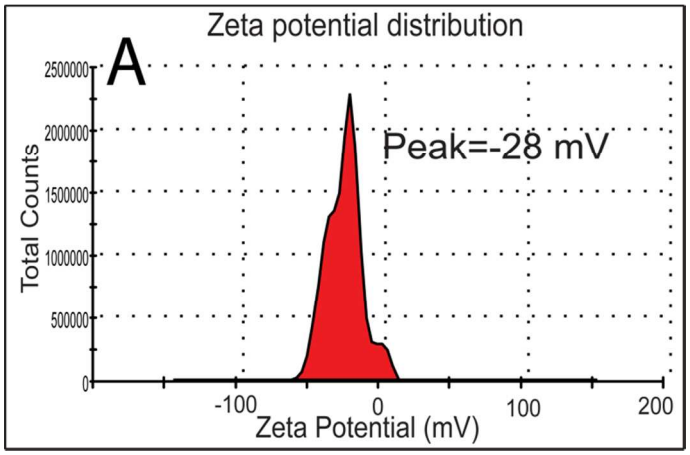
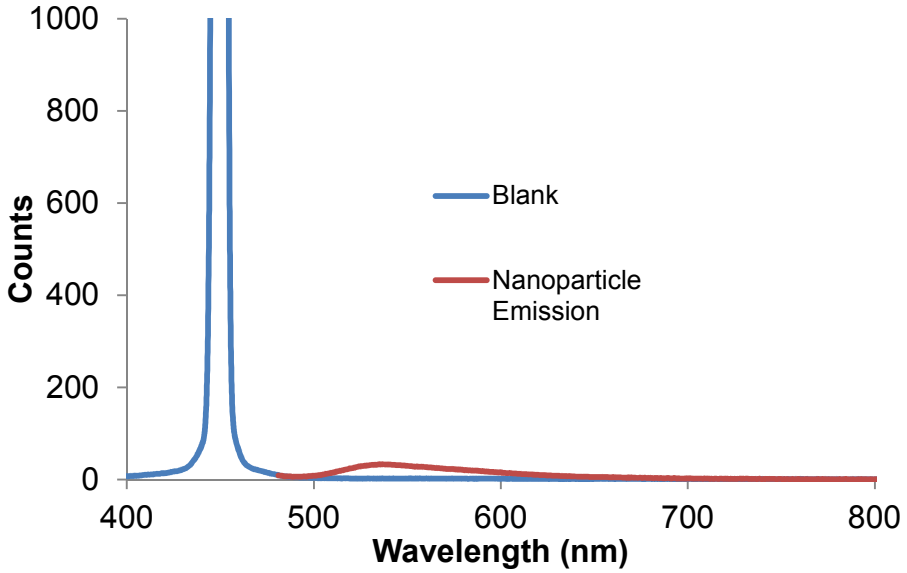


Figure S1. The zeta potential of bioconjugated polymer nanoparticles. Despite the low zeta potential, polymer nanoparticle sizes remained stable for months and aggregation was not observed.



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No.	Counts 439 - 460(nm)	Counts 483 - 868(nm)	QuantumYield
Blank	2.81×10^{7}	---	---
PFBT Nanoparticles	2.61×10^{7}	1.52×10^{6}	0.75

Figure S2. Quantum yield of polymer nanoparticles.

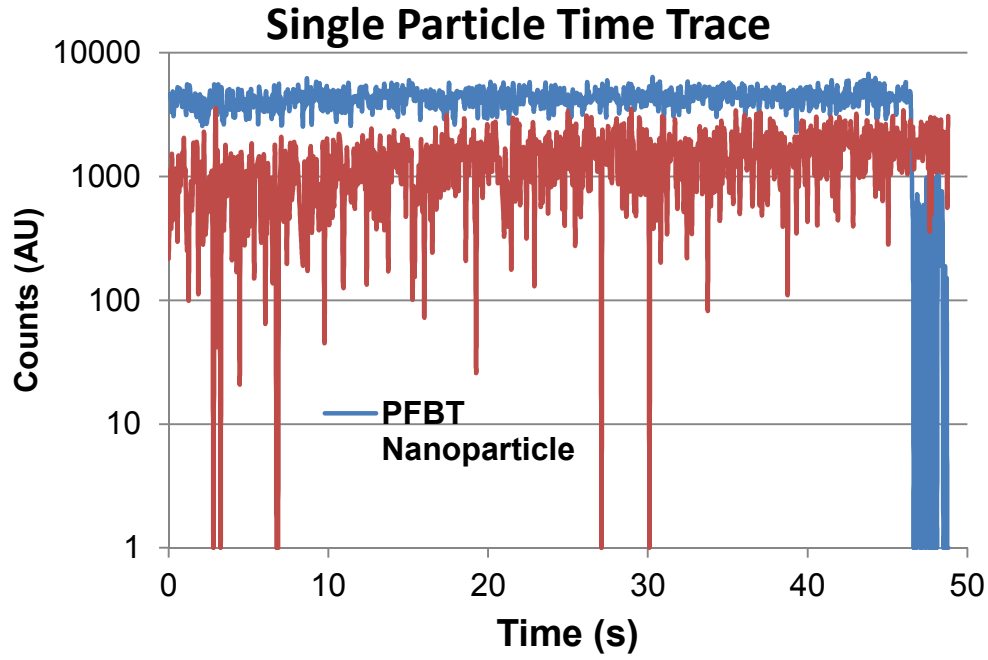

Figure S3. Intensity time trace of a single polymer nanoparticle and a single quantum dot. This PFBT nanoparticle was bound to a stationary microtubule and shows little blinking and a single photobleaching event. The nanoparticles used did show intermittent blinking when passively adsorbed to a glass coverslip, but this blinking was almost entirely extinguished when bioconjugated nanoparticles were bound to microtubules. Although the brightness of this

PFBT nanoparticle was higher than the quantum dot, single-particle brightness measurements show that they have comparable average brightness.

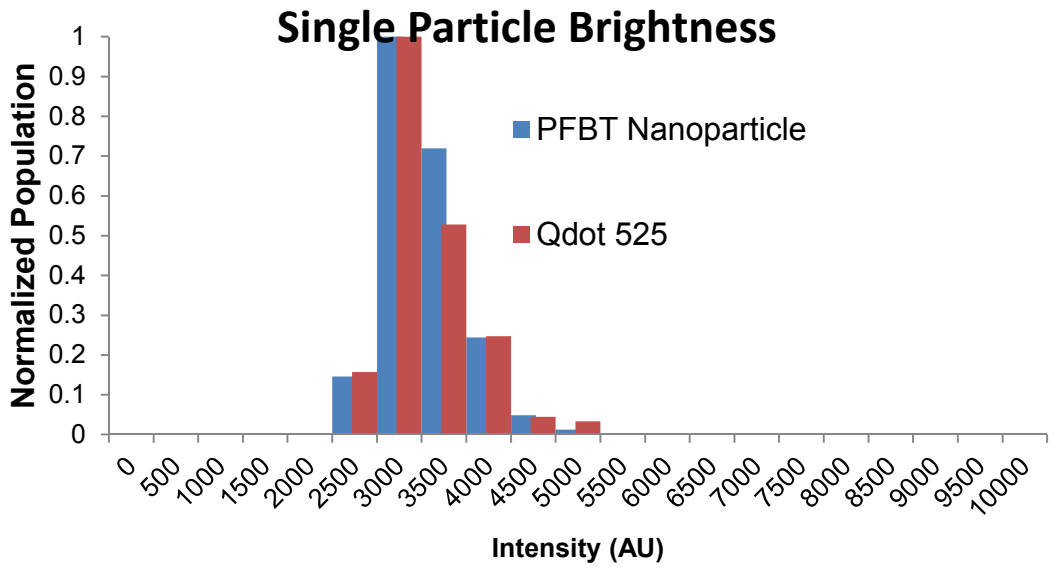

Figure S4. Single particle brightness histogram comparing polymer nanoparticles and Qdot 525. Mean brightness was 3035 ± 424 (n=178) and 3020 ± 492 (n=179) for polymer nanoparticles and 525 nm emission quantum dots, respectively.