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-- Supporting Information --

A Compact and Highly Fluorescent Orange-Emitting Polymer Dot for Specific Subcellular Imaging

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Materials and Methods

Functionalization of semiconducting polymer dots. The fluorescent semiconducting polymer Poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-(1-cyanovinylene-1,4-phenylene)] (CN-PPV, MW 15,000, polydispersity 5.9) was purchased from ADS Dyes, Inc. (Quebec, Canada). The copolymer poly(styrene-co-maleic anhydride) (PSMA, cumene terminated, average Mn ~1,700, styrene content 68%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents for nanoparticle preparation were purchased from Sigma-Aldrich (St. Louis, MO, USA), and all experiments were performed at room temperature unless indicated otherwise. Functionalized Pdots in

aqueous solution were prepared by using a modified nanoprecipitation method. In a typical preparation, the fluorescent semiconducting polymer CN-PPV was first dissolved in tetrahydrofuran (THF) to make a 1 mg/mL stock solution. The copolymer PSMA was also dissolved in THF and mixed with a diluted solution of CN-PPV to produce a solution mixture with a CN-PPV concentration of 50 μg/mL and a PSMA concentration of 10 μg/mL. The mixture was sonicated to form a homogeneous solution. A 5-mL quantity of the solution mixture was quickly added to 10 mL of MilliQ water in a bath sonicator. The THF was removed by nitrogen stripping, and the solution concentrated to 5 mL on a 90 ºC hotplate followed by filtration through a 0.2 micron filter. During nanoparticle formation, the maleic anhydride units of PSMA molecules were hydrolyzed in the aqueous environment, generating carboxyl groups on Pdots. The Pdot dispersions were clear and stable for months without signs of aggregation.

Biomolecular conjugation to functionalized Pdots. Streptavidin was purchased from Invitrogen (Eugene, OR, USA). In this paper, we performed bionconjugation by utilizing the EDC-catalyzed reaction between carboxyl groups on Pdots and amine groups on streptatvidin. In a typical bioconjugation reaction, 100 μL of polyethylene glycol (5% w/v PEG, MW 3350) and 100 μL of concentrated HEPES buffer (1 M) were added to 5 mL of functionalized Pdot solution (50 μg/mL in MilliQ water), resulting in a Pdot solution in 20 mM HEPES buffer with a pH of 7.3. Then, 200 μL of streptavidin (1 mg/mL) was added to the solution and mixed well on a vortex. Finally, 100 μL of freshly-prepared EDC solution (5 mg/mL in MilliQ water) was added to the solution. The mixture was left on a rotary shaker for 4 hours at room temperature. The resulting Pdot-

streptavidin bioconjugates were separated from free streptavidin by gel filtration using Sephacryl HR-300 gel media.

Characterization of functionalized Pdots. The particle size of Pdots in bulk solution was characterized by dynamic light scattering (Malvern Zetasizer NanoS). For the TEM measurements, one drop of the Pdot dispersion was placed on a carbon-coated copper grid. After evaporation of the water, the nanoparticles were imaged with a transmission electron microscope (FEI Tecnai F20). UV-Vis absorption spectra were recorded from the Pdots in 1-cm quartz cuvettes with a DU 720 scanning spectrophotometer (Beckman Coulter, Inc., CA USA). Fluorescence spectra were obtained using a commercial Fluorolog-3 fluorometer (HORIBA Jobin Yvon, NJ USA). Agarose gel electrophoresis of the functionalized Pdots was carried out using a Mupid®-exU submarine electrophoresis system. Pdots (in 30% glycerol) were loaded onto a 0.7% agarose gel containing 0.1% PEG. The Pdot-loaded gel was run for 20 min at 135 V in tris-borate-EDTA (TBE) buffer, and then imaged on Kodak image station 440CF system.

Cellular and subcellular labeling. The MCF-7 cells were labeled for flow cytometry with primary anti-EpCAM antibody (0.5 mg/mL, Biolegend) and biotin secondary anti-mouse IgG (0.5 mg/mL, Biolegend). Other reagents were the labeling buffer (1× PBS containing 1% BSA and 2 mM EDTA) and BlockAid^{TM} blocking buffer (Invitrogen). A million MCF-7 cells were dispersed in 100 µL of labeling buffer in a 5-mL round-bottom tube. 1 µL of primary anti-EpCAM antibody (0.5 mg/mL) was added to the cell suspension. The cell suspension was left on a rotary shaker for 30 minutes. Then 4 mL of labeling buffer was added to the tube. The cells were centrifuged down to the tube bottom to wash off free primary antibody. The cells were dispersed in100 µL of

labeling buffer. An aliquot of1 μL of biotin secondary anti-mouse IgG (0.5 mg/mL, Biolegend) was added, and the cell suspension was left on a rotary shaker for 30 minutes. Next, 5 μL of Pdot-steptavidin conjugates (~200nM) was well-dispersed into 200-μL BlockAid™ blocking buffer (Invitrogen). The final Pdot concentration for cell labeling was ~5 nM. An aliquot of 4 mL of labeling buffer was added to the cell tube after 30 minutes of secondary antibody incubation. The cells were pelleted by centrifugation and then resuspended with 200 μL of Pdot-steptavidin in BlockAid™ buffer. The cell suspension was left on rotary shaker for 30 minutes. An aliquot of 4 mL of labeling buffer was added to the cell tube and the cells were pelleted by centrifugation to wash off free Pdot-strepavdidin conjugates. The step was repeated twice, and the resultant cells were run through the flow cytometer.

For subcellular microtubule-labeling experiment, BlockAid™ blocking buffer was purchased from Invitrogen (Eugene, OR, USA). Ten thousands of HeLa cells were plated on a 22×22-mm glass coverslip and cultured until the density reach 60-70% confluence. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton-X 100 in PBS for 15 minutes, and blocked in BlockAid™ blocking buffer for 30 minutes. The fixed and blocked HeLa cells were incubated sequentially with 5 μg/mL biotinylated monoclonal anti-α-tubulin antibody (Biolegend, San Diego, CA, USA) for 60 minutes, and 5-nM CN-PPV-streptavidin conjugates for 30 minutes. The Pdot-tagged cells were then counterstained with Hoechst 34580 and imaged immediately on a fluorescence confocal microscope (Zeiss LSM 510).

Subcellular Imaging. The fluorescence images of HeLa cells were acquired with a fluorescence confocal microscope (Zeiss LSM 510). Blue channel, green channel

emission and bright field image were simultaneously obtained. The blue channel was excited by 405-nm diode laser, while the green channel was excited by 488-nm diode laser. A Carl Zeiss 63X ("C-Apochromat" 63X/1.2 oil Corr) and a Carl Zeiss 100X ("C-Apochromat" 100X/1.45 oil Corr) objective lens were utilized for imaging and spectral data acquisition. The laser was focused to a spot size of ~5 μm, and the laser power density in the sample stage was about ~9.0×10^3 W/cm^{-2} for 488-nm diode laser.