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Facile Preparation of Multicolor Polymer Nanoparticle Bioconjugates with Specific Biorecognition

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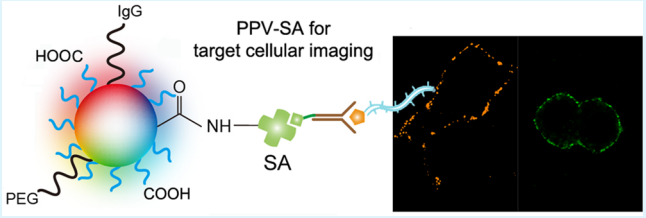
Supporting Information

ABSTRACT: A facile and efficient strategy to prepare multicolor and surface-functionalizable conjugated polymer nanoparticles (PPV_{seg}-COOH CPNs) was demonstrated. The CPNs with tunable photoluminescence colors and carboxylate groups were further covalently modified with a series of specific molecules such as streptavidin, IgG and poly(ethylene glycol) to show their generality for subsequent bioconjugation and biological applications. The streptavidin coating can significantly improve the photostability of the PPV_{seg}-SA CPNs, which indicates that specific biomolecules such as streptavidin functionalization of multicolor PPV_{seg}-COOH CPNs can be applied to achieve high optical stability of CPNs in various buffer solutions, metal ions for many biological applications. Furthermore, the resulted PPV_{seg}-SA CPNs also show efficient labeling ability in specific cellular imaging. The synthetic methods present the feasibility and versatility for further developing surface-functionalizable CPNs probes with full-color tunability for biological imaging and bioanalytical applications.



KEYWORDS: *conjugated polymer, nanoparticles, multicolor, bioconjugate, cellular imaging*

1. INTRODUCTION

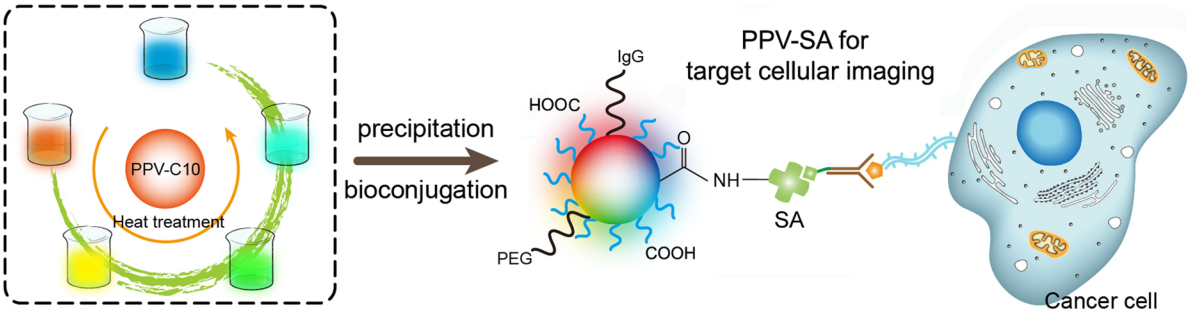
Convenient and cost-effective fluorescence techniques for cellular assays and in vivo imaging are highly desired in identification of cancer cells, medical diagnosis and therapy.[1,2] For the past two decades, considerable attention has been paid to small fluorescent molecules[3−5] and inorganic nanocrystals[6−8] for labeling biomedical entities such as bacteria, cells and tissues. However, conventional organic dyes are limited by their susceptibility to photobleaching.[9] The long-term toxicity of inorganic fluorescent nanocrystals such as CdSe and CdTe may cause serious damage to living biosubstrates and impede their final clinical application.[10−12] Another critical issue in the application of inorganic nanocrystals is these nanoparticles typically require surface modification, resulting in larger diameters, which may cause problems such as poor tissue penetration and nonspecific interaction.[13]

Recently, fluorescent conjugated polymer nanoparticles (CPNs) have emerged as a unique platform for nanoscale sensors and optical bioimaging due to their high fluorescence brightness, fast emission rates and lower toxicity.[14−16] Besides the applications as fluorescent probe for metal ion detection,[17,18] intracellular pH value[19] and temperature sensing,[20] CPNs have also been extensively used in cellular imaging and immunofluorescent labeling.[21] In general, multicolor CPNs are produced from organic conjugated polymers with different chemical structures through tedious synthetic modification. Furthermore, the surface functionalization and subsequent bioconjugation of CPNs is also essential for their effective utilization in biological applications. Using different strategies, CPNs with specific fluorescence have been functionalized with a variety of ligands.[22,23] However, these methods share the drawbacks such as lack of universality and need of sophisticated and respective functionalization for nanoparticles with different fluorescence colors, and the functional macromolecules are likely to dissociate from the formed CPNs due to the relatively weak noncovalent interactions.[24]

In this work, a simple strategy that allows the formation of multicolor CPNs bioconjugates with specific biorecognition was demonstrated. As shown in Scheme 1, multicolor CPNs formed from such a method would directly have functional groups available for subsequent bioconjugation, thus avoiding the respective and sophisticated surface modification of the nanoparticles and the dissociation of functional groups. The multicolor and surface-functionalizable PPV_{seg}-COOH was also successfully applied to prepare specific nanoparticle bioconjugates, thus indicating its general applicability and feasibility for

Received: February 6, 2014
Accepted: June 25, 2014
Published: June 25, 2014

Scheme 1. Schematic Illustration of the Preparation of Multicolor PPV_{seg} CPNs-Bioconjugates and PPV-SA for Target Cellular Imaging



biological imaging and bioanalytical applications. Compared with bare CPNs, the streptavidin-modified PPV_{seg}-COOH CPNs (PPV_{seg}-SA CPNs) exhibit dramatically improved photostability in buffer solutions and physiologically relevant environments. The multicolor PPV_{seg}-SA CPNs probes have also been applied for targeted cellular imaging, which demonstrates the ease of such functionalization can impart multicolor and desired properties such as specific recognition or biocompatibility simultaneously.

2. EXPERIMENTAL SECTION

Materials and Characterization. All reagents for polymer preparation were used without further purification as purchased from Sigma-Aldrich Chemical Co. and Aladdin reagent (Shanghai, China). Distilled tetrahydrofuran (THF) was used for the preparation of multicolor segmented PPV conjugated polymers to ensure anhydrous conditions. The following materials and chemicals for bioconjugation and cell study were used as received. Amine-terminated poly(ethylene glycol) (PEG) (MW: 5000) was purchased from Jenkem Technology (Beijing, China). Goat antimouse IgG was purchased from Solarbio Science & Technology (Beijing, China). 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Aladdin reagent for bioconjugation. Streptavidin and biotinylated primary anti-EpCAM antibody were purchased from Sigma-Aldrich Chemical Co. and Abcam Inc., respectively. The human breast adenocarcinoma cell line MCF-7 cells, 90% MEM Eagle for cell culture experiment and PBS buffer (pH = 7.0) were purchased from KeyGEN Biotec (Nanjin, China). The color changes in PL spectra were measured using a Shimadzu RF-5301PC spectrophotometer. The NMR spectrum was recorded on a Bruker AV 400 MHz NMR spectrometer. The gel permeation chromatography (GPC) analysis was conducted at room temperature on a Shim-pack GPC-80X column using polystyrene as a standard and tetrahydrofuran (THF) as the eluant. The size and ζ-potential data of CPNs were obtained from a Brukehaven ZetaPALS with a He−Ne laser (633 nm). Confocal laser scanning microscope images of multicolor PPV_{seg}-SA CPNs were taken on a Olympus Fluo-view 1000. The PL quantum yields of PPV_{seg}-COOH in THF and PPV_{seg}-COOH CPNs in water were determined against 9,10-diphenylanthracene (cyclohexane, QY = 0.90),[25] coumarin-6 (ethanol, QY = 0.76)[26] and rhodamine 110 (ethanol, QY = 0.92)[27] depending on fluorophore. PL spectra of PPV_{seg}-COOH, PPV_{seg}-COOH CPNs and dyes were taken under identical spectrometer conditions. The absorbance was kept lower than 0.08 for all QY measurements to avoid the self-quenching. The integrated intensities of the emission spectra were used to calculate the quantum yields.

General Procedure for Synthesis of PPV-COOH. The PPV-COOH were synthesized by copolymerization of monomers 1,4-bis(bromomethyl)-2-(2-ethylhexyloxy)-5-methoxybenzene (**M1**) and ethyl 4-(2,5-bis(bromomethyl)-4-methoxyphenoxy)butanoate (**M2**) by Gilch coupling according to the procedure described in literature.[28] The molar fraction of side-chain carboxylic acid groups in the polymers could be tuned by changing the feed ratios of monomers in the synthesis step. Poly(*p*-phenylenevinylene) synthesized from 10% **M2** (abbreviated as PPV-C10) was used as an example: to a solution of monomers **M1** (0.45 g, 1.06 mmol) and **M2** (0.05 g, 0.12 mmol) in 40 mL anhydrous THF under nitrogen was added 4 equiv of KO^{t}Bu(0.45 g, 4 mmol) dissolved in anhydrous THF (60 mL). The reaction mixture was allowed to stir at room temperature for 24 h. The obtained solution was poured into methanol. The formed solid was collected and dried in a vacuum.

The obtained polymer solid (250 mg) was dissolved in THF (25 mL) and methanol (2 mL) and treated with NaOH (1 g, 25 mmol). The mixture was then stirred at 70 °C for 12 h and acidied with hydrogen chloride. The obtained solution was poured into ethanol and collected to give PPV-C10 as a bright red solid.

Spectroscopic data and chemical structure of the obtained polymer PPV-C10 is as shown in Figure S1 (Supporting Information).

Preparation of Multicolor Segmented Conjugated Polymer Nanoparticles (PPV_{seg}-COOH CPNs). Multicolor PPV_{seg}-COOH CPNs in aqueous solution were prepared by a reprecipitation method. In a typical preparation, PPV-C10 was first dissolved in anhydrous tetrahydrofuran (THF) to make a 0.4 mg/mL stock solution. After heat treatment at 50 °C for various times, the obtained multicolor PPV_{seg}-COOH solutions were further diluted in THF. A 2 mL quantity of THF solution of PPV_{seg}-COOH was quickly added to 8 mL of Milli-Q water in a vigorous bath sonicator. The THF was removed by partial vacuum evaporation, followed by filtration through a 0.22 μm filter. The PPV_{seg}-COOH CPNs were stable and could be stored at 4 °C for more than 3 months without signs of aggregation.

Bioconjugation of PPV_{seg}-COOH CPNs. All reagents were immediately handled and used before bioconjugation. Bioconjugation of PPV_{seg}-COOH CPNs with different molecules was conducted by standard carbodiimde chemistry. The synthesis of PPV_{seg}-SA CPNs was used as an example. To a solution of PPV_{seg}-COOH CPNs and streptavidin in HEPES buffer (0.67 mM, pH = 7.3) solution containing 5 wt % polyethylene glycol (PEG, MW: 3350), was added directly EDC and the reaction mixture was stirred for 3 h in a dark room at room temperature. Unbound streptavidin from the PPV_{seg}-SA CPNs solution were removed by centrifugal washing with Milli-Q water several times using a microcentrifuge tube (MWCO = 100 000).

Specific Cellular Imaging. For specific cellular imaging study, the human breast adenocarcinoma cell line MCF-7 cells with overexpressed EpCAM receptors was chosen as an example. The MCF-7 cancer cells were cultured at 37 °C, 5% CO_2 in 90% MEM Eagle supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were precultured prior to experiments until confluence was reached. The cells were harvested from the culture flask by briefly rinsing with culture media followed by incubation with 1 mL of trypsin−EDTA solution (0.25 w/v % trypsin, 0.02% w/v EDTA) at 37 °C for 3 min. After complete detachment, the cells were rinsed, centrifuged and resuspended in 90% MEM Eagle. Ten thousand MCF-7 cells were plated on a 15 mm-diameter glass-bottomed culture dish and cultured until the density

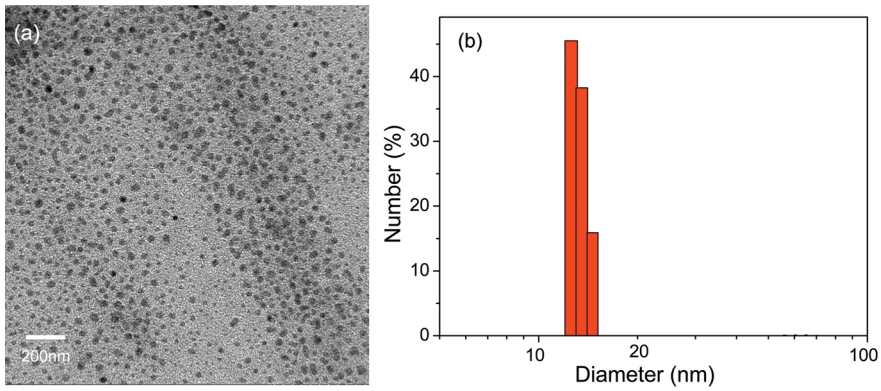


Figure 1. (a) Transmission electron microscopy for PPV_{Cya}-COOH CPNs. (b) Dynamic light scattering measurements of PPV_{Cya}-COOH CPNs.

reached confluence for PPV_{seg}-SA CPNs labeling and fluorescence imaging.

MCF-7 cells were blocked with BlockAid blocking buffer for 30 min and then were incubated sequentially with biotinylated primary anti-EpCAM antibody and PPV_{seg}-SA CPNs for 30 min. After incubation, the cells were washed twice with 1× PBS buffer solution and imaged by confocal laser scanning microscopy (CLSM). The MCF-7 cells incubated with PPV_{seg}-SA CPNs at 37 °C without biotinylated primary anti-EpCAM antibody pretreatment, and MCF-7 breast cancer cells treated with biotinylated primary antibody and free PPV_{seg}-COOH CPNs in the absence of streptavidin were used as the control.

3. RESULTS AND DISCUSSION

Synthesis and Characterization of PPV_{seg}-COOH CPNs.

After elaborately investigating the bioconjugation efficiency of PPV_{seg}-COOH CPNs to different molecules, it was found that the effectiveness of bioconjugation depended on the carboxylic acid ratio of the polymers. And considering that a higher density of carboxylic acid groups may lead to nonspecific binding in cellular labeling,[24] PPV-C10 was utilized for the preparation of multicolor PPV_{seg}-COOH in this work. The multispectral PPV_{seg}-COOH was first prepared by heat treatment of PPV-C10 (the chemical structure of PPV-C10 is shown in Figure S1 (Supporting Information)) in anhydrous THF at 50 °C as previously reported.[29] The PL spectra of PPV-C10 are shown in Figure S2(Supporting Information). It is apparent that there is a continuous blue shift in the emission maxima with decreases in the extent of conjugation during heat treatment of PPV-C10, which is similar to that for previously reported MEH-PPV.[29,30] The fluorescence color tuning of PPV-C10 solution from orange to blue under UV illumination is shown in Figure S3 (Supporting Information) and this phenomena could be ascribed to thermo/photo-oxidation of PPV species.[31−33] Furthermore, the color changes of PPV-C10 could also be controlled upon addition of H_2O, and the fluorescence color changes of PPV_{Gre}-COOH after heat treatment at 50 °C with and without 50 μL H_2O is shown in Figure S4 (Supporting Information). The carboxylate-functionalized and multicolor segmented conjugated polymers (PPV_{Yel}-COOH, PPV_{Gre}-COOH, PPV_{Cya}-COOH and PPV_{Blu}-COOH) in the presence of H_2O are stable and can be stored at 4 °C for more than 3 months. The PL quantum yields of pristine PPV-C10, PPV_{Yel}-COOH, PPV_{Gre}-COOH, PPV_{Cya}-COOH and PPV_{Blu}-COOH in THF were determined to be 13.7%, 15.9%, 33.7%, 26.8% and 16.0%, respectively. The results demonstrate that the fluorescence efficiency of the polymers could be obviously improved by the heat treatment of PPV-C10, which is probably due to the intrachain energy transfer in the presence of statistical distribution of conjugation lengths or the exciton confinement in the "quantum well" of these segmented conjugated polymers.[29,30] The effect of the heat treatment on molecular weight of PPV-C10 was monitored with gel permeation chromatography (GPC) and is summarized in Table S1 (Supporting Information). The M_n of the polymers decreased with increased heating time, which is consistent with improved solubility of PPV_{seg}-COOH and suggests that heat treatment causes partially polymer backbone cleavage.

The multicolor PPV-C10 and PPV_{seg}-COOH CPNs were then formed by a reprecipitation procedure in which the polymers in THF solution were injected quickly into water under ultrasonication. After vacuum removal of the THF, a stable aqueous suspension of PPV-C10 and PPV_{seg}-COOH CPNs was obtained. To investigate their particle size and morphologies, the as-prepared PPV-C10 and PPV_{seg}-COOH CPNs were characterized by both TEM and dynamic light scattering (DLS). Figure 1 shows representative TEM data and size distribution of PPV_{Cya}-COOH CPNs. The particle size obtained from DLS indicated that the majority of PPV_{Cya}-COOH CPNs possessed hydrodynamic diameters in the range of ∼13 nm (Figure 1b). Under the same preparation conditions, the bare PPV_{seg}-Cya CPNs without carboxylic acid functionalization showed a hydrodynamic diameter about 5 nm. The hydrodynamic size difference between the two CPNs species may be caused by differences in the hydrophilic functional groups of the polymers and the molecular weight of the original polymer precursors.[34]

The changes in PL spectra upon particle formation are shown in Figure 2. The PL spectra of PPV-C10 and PPV_{seg}-COOH CPNs are red-shifted as compared to those of the corresponding polymers in THF solutions, which can be attributed to the increased interchain interactions and multiple energy transfer in nanoparticles as has often been observed in thin films.[35,36] For PPV-C10 and PPV_{seg}-COOH CPNs, the fluorescence spectra are devoid of any fine structure, which is due to individual segment emission, even in the case of PPV_{Blu}. This was attributed to the more rapidly energy transfer between flexible segmented chromophores in nanoparticles as in thin films when compared to dilute solutions.[29] Under UV irradiation (365 nm), PPV-C10 and PPV_{seg}-COOH CPNs show multicolor fluorescence spanning from red to cyan, as shown in Figure 2b. The PL quantum yield of PPV-C10 CPNs was determined to be 1.8%, which was similar to a previous report.[14] And the PPV_{Yel}-COOH, PPV_{Gre}-COOH, PPV_{Cya}-COOH and PPV_{Blu}-COOH CPNs all exhibit bright fluorescence with PL quantum yields of 2.4%, 14.5%, 10.0% and

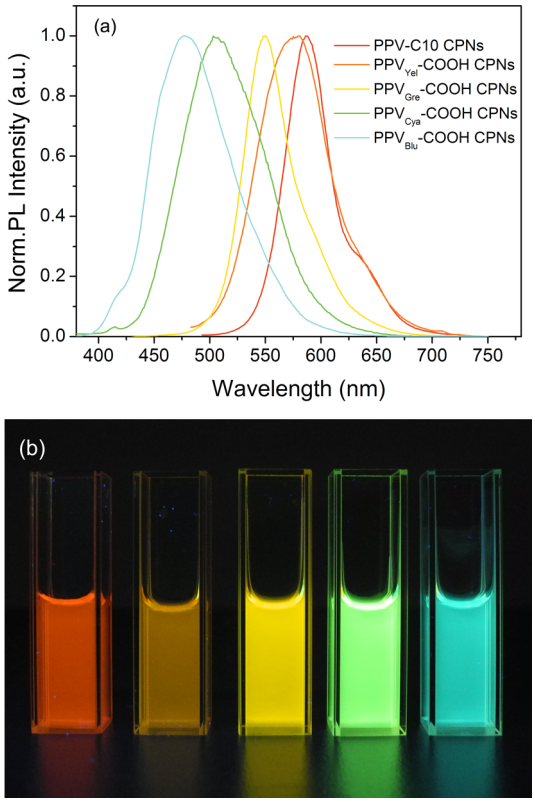


Figure 2. (a) Normalized PL spectra of pristine PPV-C10 and PPV$_{seg}$-COOH CPNs. (b) Full emission colors of PPV-C10 and PPV$_{seg}$-COOH CPNs under UV illumination.

3.0% respectively, which represents a general increase in emission. The emission efficiency of CPNs is critically important for many fluorescence-based imaging applications, which make these multicolor CPNs promising for both future bioimaging and bioanalysis.

Bioconjugation of PPV$_{seg}$-COOH CPNs and Specific Cellular Imaging. To show this strategy is simple and versatile for the generation of multicolor and surface-functionalizable CPNs, subsequent bioconjugation of PPV$_{seg}$-COOH CPNs to different molecules (such as streptavidin, IgG and aminated PEG) have also been conducted via conventional bioconjugation chemistry using 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC) as a catalyst. Representative electrophoresis images of streptavidin conjugated PPV-C10 CPNs (PPV-SA CPNs) are shown in Figure 3a and additional electrophoresis data for multicolor PPV$_{seg}$-SA CPNs are listed in Figure 3b–e. In Figure 3a, lane 1 has only PPV-C10 CPNs before streptavidin bioconjugation and shows the corresponding red band. In lane 4, PPV-SA CPNs exhibit apparent slower migration mobility compared to bare PPV-C10 CPNs. PPV-C10 CPNs treated with free streptavidin and PPV-C10 CPNs treated directly with EDC in the absence of streptavidin were used as control as shown in lane 2 and lane 3. Aminated poly(ethylene glycol) (PEG) was also selected as a ligand because it has been widely used as effective biocompatible building blocks to suppress nonspecific adsorption of biological substances. Bioconjugation of PEG and antibody IgG to the PPV$_{Yel}$-COOH CPNs surface was also performed and confirmed by agarose gel electrophoresis (0.5%), which is shown in Figure 4a. After conjugation to IgG and PEG, gel electrophoresis showed shifted migration bands of the CPNs

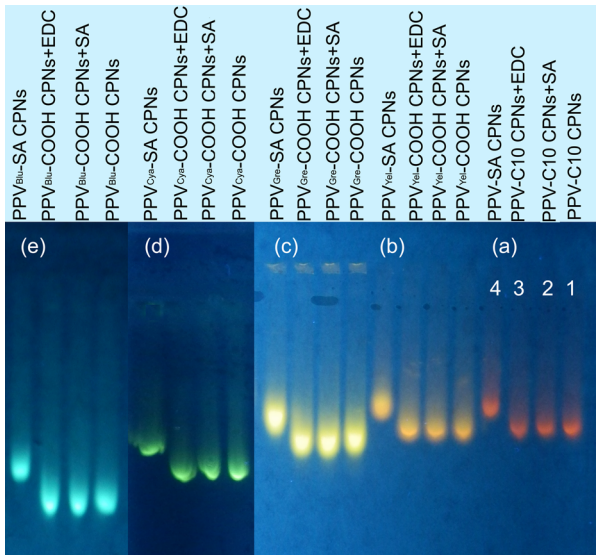


Figure 3. Gel electrophoresis of (a) PPV-SA and (b,c,d and e) multicolor PPV$_{seg}$-SA CPNs. Photographs were taken under UV light (excited at 365 nm) to visualize multicolor PPV$_{seg}$-COOH CPNs and PPV$_{seg}$-SA CPNs at the same time.

conjugates, which was caused by the changes in surface charge and different molecular weight of the molecules. The broad feature of the band is likely to be the result of the polydispersity of the polymers. These results clearly indicate successful carboxylate functionalization of the CPNs as well as the subsequent surface bioconjugation of multicolor CPNs with specific molecules, which indicate the generality of this method for preparing multicolor conjugated polymer nanoparticle bioconjugates.

To demonstrate the nanoparticle stability of multicolor PPV$_{seg}$-COOH CPNs, we measured the ξ-potential of bare PPV$_{seg}$-Yel CPNs without carboxylic acid functionalization, PPV$_{Yel}$-COOH CPNs and PPV$_{Yel}$-SA CPNs to be −22.76, −43.50 and −25.85 mV, respectively, which indicates that the surface of PPV$_{Yel}$-COOH CPNs is more negative than that prepared from bare PPV$_{seg}$-Yel CPNs. No obvious aggregation was observed for PPV$_{Yel}$-COOH CPNs in various buffers such as PBS, Tris–HCl and in blood plasma. To further confirm the improved colloidal stability of bioconjugated PPV$_{seg}$-COOH CPNs, the intensity of fluorescence emission from bare PPV$_{seg}$-Yel CPNs and PPV$_{Yel}$-SA CPNs were measured as an example. Figure 4b shows the fluorescence intensities of bare PPV$_{seg}$-Yel CPNs and PPV$_{Yel}$-SA CPNs dispersed in PBS, Tris–HCl buffer solution and in physiological conditions (e.g., 20 vol % serum). The fluorescence intensity of bare PPV$_{seg}$-Yel CPNs showed up to 74% fluorescence quenching in PBS solution and nearly a 53% reduction in Tris–HCl and 20 vol % serum. On the contrary, PPV$_{Yel}$-SA CPNs displayed a significant improvement of optical stability and do not show obvious fluorescence change in PBS and Tris–HCl. Furthermore, PPV$_{Yel}$-SA CPNs display slightly enhanced fluorescence in the case of 20 vol % serum and it may be due to the nonspecific interactions of PPV$_{Yel}$-SA CPNs with biological macromolecules.[37,38] For evaluating the effect of different ions on the optical stability of PPV$_{Yel}$-SA CPNs, PPV$_{Yel}$-SA CPNs solution was also titrated with different metal ions such as 0.4 mM Fe(II) solution, 0.5 mM Cu(II) solution, 0.5 mM Ca(II) solution and 0.5 mM Mg(II) solution. As shown in Figure 4b, the addition of Fe(II) and Cu(II) both lead to effective quenching of the bare PPV$_{seg}$-

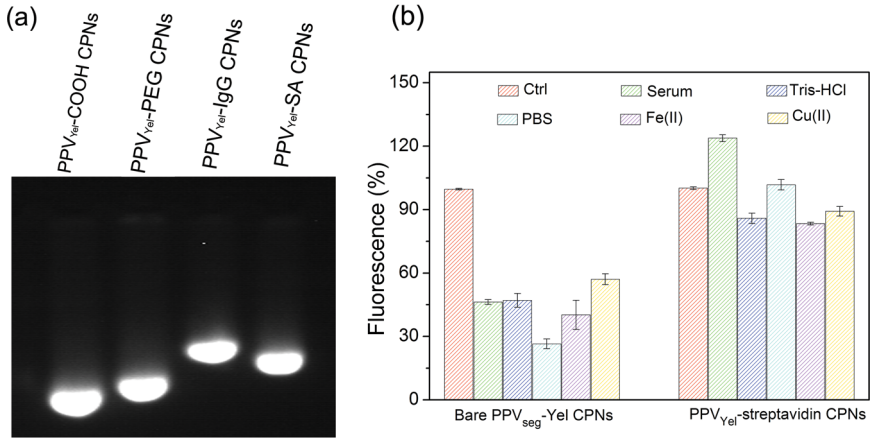


Figure 4. (a) Gel electrophoresis of PPV$_{Yel}$-COOH CPNs and specific bioconjugated PPV$_{Yel}$-COOH CPNs. (b) Changes in fluorescence intensity of bare PPV$_{seg}$-Yel CPNs and PPV$_{Yel}$-SA CPNs in Tris−HCl, PBS, 20 vol % serum, Cu(II) and Fe(II). Control samples were dispersed in deionized water.

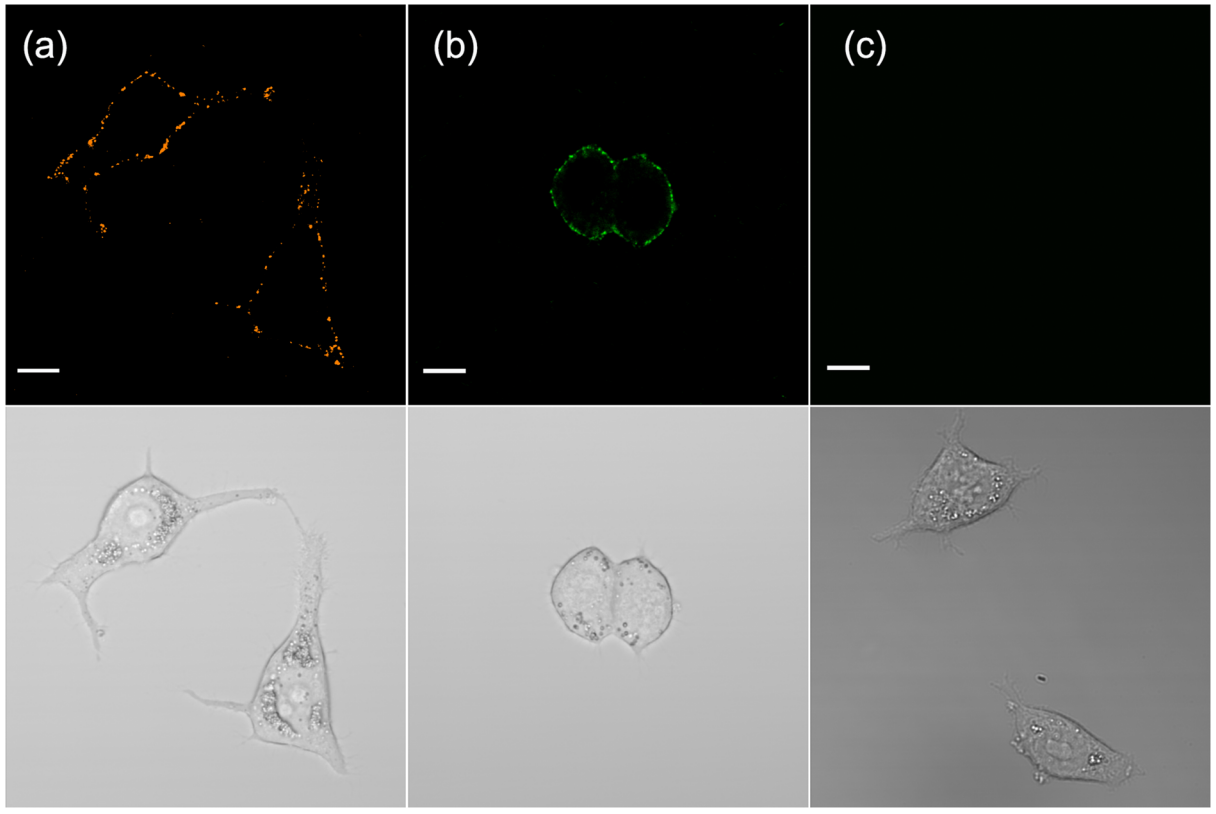


Figure 5. Confocal images of live MCF-7 cells (a) positively labeled with PPV$_{Yel}$-SA CPNs, (b) positively labeled with PPV$_{Cya}$-SA CPNs and (c) negatively labeled with PPV$_{Cya}$-SA CPNs in the absence of biotinylated primary antibody. Scale bar: 10 μm.

Yel CPNs. The mechanisms of Cu(II) and Fe(II)-induced fluorescence quenching of bare PPV$_{seg}$-Yel CPNs may be due to the screened charge repulsion of nanoparticles by the increase in ionic strength and the specific interactions between CPNs and the biovalent metal ions.[17,22] Fluorescence measurements also shows no obvious change of fluorescence intensity for PPV$_{Yel}$-SA CPNs in the presence of Cu(II) and Fe(II) solutions, which may be due to the combination of charge and steric stabilization by streptavidin coating. These results demonstrate that specific biomolecules such as streptavidin functionalization of multicolor PPV$_{seg}$-COOH CPNs can be applied to achieve high optical stability of CPNs in various buffer solutions, metal ions for many biological applications.

To further demonstrate the applicability of multicolor bioconjugated PPV$_{seg}$-COOH CPNs in biological analysis and bioimaging, we use PPV$_{seg}$-SA CPNs for targeted imaging of MCF-7 breast cancer cells. The detection of live MCF-7 breast cancer cells was demonstrated by sequentially staining EpCAM receptors with biotinylated primary anti-EpCAM antibody and PPV$_{seg}$-SA CPNs, and the corresponding fluorescence images are shown in Figure 5. It is obvious that PPV$_{seg}$-SA CPNs are more concentrated along the cell periphery than in the cytoplasm of MCF-7 breast cancer cells, which indicates the

effective labeling of MCF-7 breast cancer cells by PPV_{seg}-SA CPNs. In the control experiments without biotinylated primary antibody, no fluorescence on the cell surface was detected (Figure 5c), confirming the specific interaction between PPV_{seg}-SA CPNs and EpCAM receptors. Furthermore, MCF-7 breast cancer cells treated with biotinylated primary antibody and free PPV_{seg}-COOH CPNs in the absence of streptavidin were also used as a control for specific cellular labeling studies of MCF-7 cancer cells (data not presented). The CLSM image also reveals that no fluorescence was observed on the cell surface, confirming that the bioconjugation of streptavidin to PPV_{seg}-COOH CPNs was covalent and the conjugation of streptavidin onto the cell surface was specific through biotin−streptavidin interaction rather than the nonspecific interaction between nanoparticles and cell surfaces.

4. CONCLUSIONS

In summary, we demonstrate the smart and versatile preparation of covalently functionalized PPV_{seg}-COOH CPNs with tunable fluorescence simultaneously by just heat treatment of PPV-C10 solution and further reprecipitation procedure. We show that this strategy is simple and general enough to be applied to prepare multicolor and surface-functionalizable CPNs simultaneously. These multicolor PPV_{seg}-COOH CPNs can then be conjugated to different biologically relevant molecules such as streptavidin, IgG and PEG for specific biological applications. Furthermore, the streptavidin functionalization dramatically improves the photostability of the CPNs, which indicates that specific biomolecules functionalization of multicolor PPV_{seg}-COOH CPNs can be applied to achieve high optical stability of CPNs in buffer solutions and other harsh conditions. To show the applicability of multicolor PPV_{seg} CPNs bioconjugates for specific cellular imaging and bioanalysis, the PPV_{seg}-SA CPNs were then successfully used for the targeting imaging of live MCF-7 cancer cells. As the fluorescent properties of PPV_{seg}-COOH can be fine-tuned through addition of H_2O and the recognition ability of PPV_{seg}-COOH CPNs can be desirably modified through conjugation with diverse biorecognition moieties, this study provides a novel molecular design concept to further develop multicolor and surface-functionalizable CPNs probes simultaneously for complicated biological imaging and detection.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H NMR spectrum of PPV-C10 in $CDCl_3$, PL spectra and emission color of the polymers, color tuning of PPV_{Gre}-COOH and GPC data of the polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge support of this work by the National Basic Research Program of China (2012CB933301), the National Natural Science Foundation of China (21204038, 81273409, 51173081), the Ministry of Education of China (IRT1148, 20123223110007), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and the Natural Science Foundation of Jiangsu Province (BK20130862).

■ REFERENCES

(1) Lichtman, J. W.; Conchello, J. A. Fluorescence Microscopy. *Nat. Methods* **2005**, *2*, 910−919.

(2) Weissleder, R. Molecular Imaging in Cancer. *Science* **2006**, *312*, 1168−1171.

(3) Yin, J.; Kwon, H.; Kim, D.; Lee, D.; Kim, G.; Hu, Y.; Ryu, J. H.; Yoon, J. Cyanine-based Fluorescent Probe for Highly Selective Detection of Glutathione in Cell Cultures and Live Mouse Tissues. *J. Am. Chem. Soc.* **2014**, *136*, 5351−5358.

(4) Vendrell, M.; Zhai, D. T.; Er, J. C.; Chang, Y. T. Combinatorial Strategies in Fluorescent Probe Development. *Chem. Rev.* **2012**, *112*, 4391−4420.

(5) Li, J.; Zhang, C. F.; Yang, S. H.; Yang, W. C.; Yang, G. F. A Coumarin-based Fluorescent Probe for Selective and Sensitive Detection of Thiophenols and Its Application. *Anal. Chem.* **2014**, *86*, 3037−3042.

(6) Biju, V. Chemical Modifications and Bioconjugate Reactions of Nanomaterials for Sensing, Imaging, Drug Delivery and Therapy. *Chem. Soc. Rev.* **2014**, *43*, 744−764.

(7) Dirin, D. N.; Dreyfuss, S.; Bodnarchuk, M. I.; Nedelcu, G.; Papagiorgis, P.; Itskos, G.; Kovalenko, M. V. Lead Halide Perovskites and Other Metal Halide Complexes as Inorganic Capping Ligands for Colloidal Nanocrystals. *J. Am. Chem. Soc.* **2014**, *136*, 6550−6553.

(8) Kim, J. Y.; Voznyy, O.; Zhitomirsky, D.; Sargent, E. H. Colloidal Quantum Dot Materials and Devices: A Quarter-Century of Advances. *Adv. Mater.* **2013**, *25*, 4986−5010.

(9) Resch-Genger, U.; Grabolle, M.; Cavaliere-Jaricot, S.; Nitschke, R.; Nann, T. Quantum Dots versus Organic Dyes as Fluorescent Labels. *Nat. Methods* **2008**, *5*, 763−775.

(10) Yong, K. T.; Law, W. C.; Hu, R.; Ye, L.; Liu, L. W.; Swiharte, M. T.; Prasad, P. N. Nanotoxicity Assessment of Quantum Dots: From Cellular to Primate Studies. *Chem. Soc. Rev.* **2014**, *42*, 1236−1250.

(11) Zhang, W. J.; Lou, Q.; Ji, W. Y.; Zhao, J. L.; Zhong, X. H. Color-Tunable Highly Bright Photoluminescence of Cadmium-Free Cu-Doped Zn-In-S Nanocrystals and Electroluminescence. *Chem. Mater.* **2014**, *26*, 1204−1212.

(12) Bottrilla, M.; Greena, M. Some Aspects of Quantum Dot Toxicity. *Chem. Commun.* **2011**, *47*, 7039−7050.

(13) Erathodiyil, N.; Ying, J. Y. Functionalization of Inorganic Nanoparticles for Bioimaging Applications. *Acc. Chem. Res.* **2011**, *44*, 925−935.

(14) Wu, C. F.; Chiu, D. T. Highly Fluorescent Semiconducting Polymer Dots for Biology and Medicine. *Angew. Chem., Int. Ed.* **2013**, *52*, 3086−3109.

(15) Feng, L.; Zhu, C.; Yuan, H.; Liu, L.; Lv, F.; Wang, S. Conjugated Polymer Nanoparticles: Preparation, Properties, Functionalization and Biological Applications. *Chem. Soc. Rev.* **2013**, *42*, 6620−6633.

(16) Pecher, J.; Mecking, S. Nanoparticles of Conjugated Polymers. *Chem. Rev.* **2010**, *110*, 6260−6279.

(17) Chan, Y. H.; Jin, Y. H.; Wu, C. F.; Chiu, D. T. Copper(II) and Iron(II) Ion Sensing with Semiconducting Polymer Dots. *Chem. Commun.* **2011**, *47*, 2820−2822.

(18) Childress, E. S.; Roberts, C. A.; Sherwood, D. Y.; LeGuyader, C. L. M.; Harbron, E. J. Ratiometric Fluorescence Detection of Mercury Ions in Water by Conjugated Polymer Nanoparticles. *Anal. Chem.* **2012**, *84*, 1235−1239.

(19) Chan, Y. H.; Wu, C. F.; Ye, F. M.; Jin, Y. H.; Smith, P. B.; Chiu, D. T. Development of Ultrabright Semiconducting Polymer Dots for Ratiometric pH Sensing. *Anal. Chem.* **2011**, *83*, 1448−1455.

(20) Ye, F. M.; Wu, C. F.; Jin, Y. H.; Chan, Y. H.; Zhang, X. J.; Chiu, D. T. Ratiometric Temperature Sensing with Semiconducting Polymer Dots. *J. Am. Chem. Soc.* **2011**, *133*, 8146−8149.
(21) Geng, J. L.; Liu, J.; Liang, J.; Shi, H. B.; Liu, B. A General Approach to Prepare Conjugated Polymer Dot Embedded Silica Nanoparticles with a SiO_2@CP@SiO_2 Structure for Targeted HER2-Positive Cellular Imaging. *Nanoscale* **2013**, *5*, 8593−8601.
(22) Jin, Y. H.; Ye, F. M.; Wu, C. F.; Chan, Y. H.; Chiu, D. T. Generation of Functionalized and Robust Semiconducting Polymer Dots with Polyelectrolytes. *Chem. Commun.* **2012**, *48*, 3161−3163.
(23) Howes, P.; Green, M.; Levitt, J.; Suhling, K.; Hughes, M. Phospholipid Encapsulated Semiconducting Polymer Nanoparticles: Their Use in Cell Imaging and Protein Attachment. *J. Am. Chem. Soc.* **2010**, *132*, 3989−3996.
(24) Zhang, X. J.; Yu, J. B.; Wu, C. F.; Jin, Y. H.; Rong, Y.; Ye, F. M.; Chiu, D. T. Importance of Having Low-Density Functional Groups for Generating High-Performance Semiconducting Polymer Dots. *ACS Nano* **2012**, *6*, 5429−39.
(25) Eaton, D. F. Reference Materials for Fluorescence Measurement. *Pure Appl. Chem.* **1988**, *60*, 1107−1114.
(26) Reynolds, G. A.; Drexhage, K. H. New Coumarin Dyes with Rigidized Structure for Flashlamp-Pumped Dye Lasers. *Opt. Commun.* **1975**, *13*, 222−225.
(27) Kubin, R. F.; Fletcher, A. N. Fluorescence Quantum Yields of Some Rhodamine Dyes. *J. Lumin.* **1982**, *27*, 455−462.
(28) Schwalm, T.; Rehahn, M. Efficient Oxygen-Induced Molar-Mass Control of Poly(p-phenylene vinylenes) Synthesized via the Gilch Route. *Macromol. Rapid Commun.* **2008**, *29*, 207−213.
(29) Bao, B. Q.; Tao, N. J.; Yang, D. L.; Yuwen, L. H.; Weng, L. X.; Fan, Q. L.; Huang, W.; Wang, L. H. A Controllable Approach to Development of Multi-Spectral Conjugated Polymer Nanoparticles with Increased Emission for Cell Imaging. *Chem. Commun.* **2013**, *49*, 10623−10625.
(30) Padmanaban, G.; Ramakrishnan, S. Conjugation Length Control in Soluble Poly[2-methoxy-5-((2′-ethylhexyl)oxy)-1,4-phenylenevinylene] (MEHPPV): Synthesis, Optical Properties, and Energy Transfer. *J. Am. Chem. Soc.* **2000**, *122*, 2244−2251.
(31) Scurlock, R. D.; Wang, B. J.; Ogilby, P. R.; Sheats, J. R.; Clough, R. L. Singlet Oxygen as a Reactive Intermediate in the Photodegradation of an Electroluminescent Polymer. *J. Am. Chem. Soc.* **1995**, *117*, 10194−10202.
(32) Chambon, S.; Rivaton, A.; Gardette, J.; Firon, M. Reactive Intermediates in the Initiation Step of the Photo-Oxidation of MDMO-PPV. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 6044−6052.
(33) Chambon, S.; Rivaton, A.; Gardette, J.; Firon, M. Photo- and Thermo-Oxidation of Poly(p-phenylene-vinylene) and Phenylene-vinylene Oligomer. *Polym. Degrad. Stab.* **2011**, *96*, 1149−1158.
(34) Yu, J. B.; Wu, C. F.; Zhang, X. J.; Ye, F. M.; Gallina, M. E.; Rong, Y.; Wu, I. C.; Sun, W.; Chan, Y. H.; Chiu, D. T. Stable Functionalization of Small Semiconducting Polymer Dots via Covalent Cross-Linking and Their Application for Specific Cellular Imaging. *Adv. Mater.* **2012**, *24*, 3498−504.
(35) Li, K.; Liu, B. Polymer Encapsulated Conjugated Polymer Nanoparticles for Fluorescence Bioimaging. *J. Mater. Chem.* **2012**, *22*, 1257−1264.
(36) Nguyen, T. Q.; Martini, B.; Liu, J.; Schwartz, B. J. Controlling Interchain Interactions in Conjugated Polymers: The Effects of Chain Morphology on Exciton-Exciton Annihilation and Aggregation in MEH-PPV Films. *J. Phys. Chem. B* **2000**, *104*, 237−255.
(37) Zheng, J.; Swager, T. M. Biotinylated Poly(p-phenylene ethynylene): Unexpected Energy Transfer Results in the Detection of Biological Analytes. *Chem. Commun.* **2004**, 2798−2799.
(38) Thomas, S. W.; Joly, G. D.; Swager, T. M. Chemical Sensors Based on Amplifying Fluorescent Conjugated Polymers. *Chem. Rev.* **2007**, *107*, 1339−1386.