Electronic Supplementary Information

for

A Controllable Approach to Development of Multi-spectral Conjugated polymer Nanoparticles with Increased Emission for Cell Imaging

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Contents in electronic supplementary information:

1. Experimental Section

Reagents and Chemicals. Poly[2-methoxy-5-(2'-ethyl-hexyloxy)-1,4-phenylenevinylene] (MEH-PPV, Mn 40,000-70,000) was purchased from sigma-Aldrich. Distilled THF was used for the preparation of multicolor segmented PPV conjugated polymers to ensure anhydrous conditions. All other reagents and solvents were obtained from commercial sources and used as received unless otherwise statement.

Characterization. The color changes in PL spectra were measured using a Shimadzu RF-5301PC

spectrophotometer. UV-vis spectra were acquired on a Shimadzu UV-3600PC UV-visible scanning spectrophotometer at room temperature. The infrared spectra were collected on a Shimadzu IRPrestige-21 Fourier transform infrared spectrophotometer (using KBr pellets) in the range 500 - 4000 cm^{-1}. The NMR spectrum was recorded on a Bruker AV 400 MHz NMR spectrometer. The gel permeation chromatography (GPC) analysis were conducted at room temperature on a Shim-pack GPC-80X column using polystyrene as a standard and tetrahydrofuran (THF) as the eluant. Transmission electron microscopy (TEM) was performed on a Hitachi HT7700 operating at 100 kV accelerating voltage. Laser confocal scanning microscope images of PPV_{seg} CPNs were taken on a Olympus Fluo-view 1000. The PL quantum yields of PPV_{seg} in THF and PPV_{seg} CPNs in water were determined against 9,10-diphenylanthracene (cyclohexane, QY = 0..90)[1], coumarin-6 (ethanol, QY = 0.76)[2], and rhodamine 110 (ethanol, QY = 0.92)[3] depending on fluorophore. PL spectra of PPV_{seg}, PPV_{seg} 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 Gilch Reactions. The PPV-COOH was synthesized by Gilch coupling according to the procedure described in literature.[4] Under an atmosphere of nitrogen, monomers 1,4-bis(bromomethyl)-2-(2-ethylhexyloxy)-5-methoxybenzene, ethyl 4-(2,5-bis(bromomethyl)-4-methoxyphenoxy)butanoate are dissolved in dry and degassed THF. At 0 °C, 4 equiv of KO^{t}Bu, dissolved in dry THF (3.3 mol/L) is added. The reaction mixture is allowed to warm up to room temperature and to stir for 24h. The obtained solution is poured into methanol. The formed solid is collected and dried in vacuum. Spectroscopic data and chemical structure of the obtained polymers PPV-COOH are as shown in Figure S3.

Preparation of Multi-color segmented PPV conjugated polymer nanoparticles (PPV_{seg} CPNs). PPV_{seg} CPNs in aqueous solution were prepared by a modified reprecipitation method. In a typical preparation, MEH-PPV was first dissolved in anhydrous tetrahydrofuran (THF) to make a 0.1 mg/mL stock solution. After heat treatment at 50℃ for various times, the obtained multicolor polymer solutions were further diluted in THF. 8 mL MilliQ water was quickly added to a 2-mL quantity of THF solution of segmented PPV conjugated polymers 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} CPNs were stable and could be stored for months without signs of aggregation.

Cell culture. The human hepatocellular liver carcinoma cell line HepG2 was ordered from Roswell Park Memorial institute. Cells were cultured at 37℃, 5% CO_2 in RPMI 1640 supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were pre-cultured 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℃ for 3 min. After complete detachment, the cells were rinsed, centrifuged, and resuspended in RPMI 1640. Ten thousands of HepG2 cells were plated on a 15-mm-diameter glass-bottomed culture dish, and cultured until the density reached confluence for PPV_{seg} CPNs labeling and fluorescence imaging.

In vitro cell imaging. For Confocal fluorescence imaging study, HepG2 cells in the glass-bottomed culture dish was incubated with PPV_{seg} CPNs for 30 min, followed by two washing steps after incubation. The PPV_{seg} CPNs-tagged cells were then imaged on the fluorescence confocal microscope.

2. Supplemental Figures:

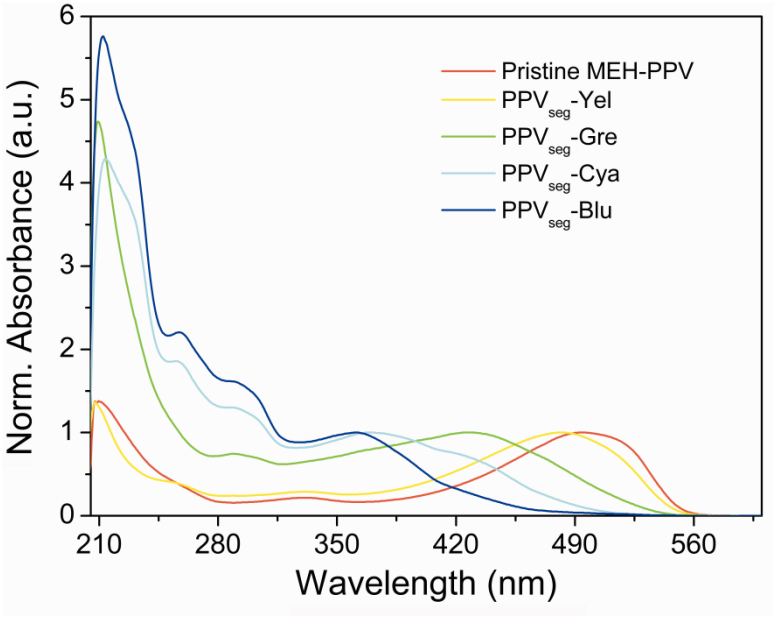
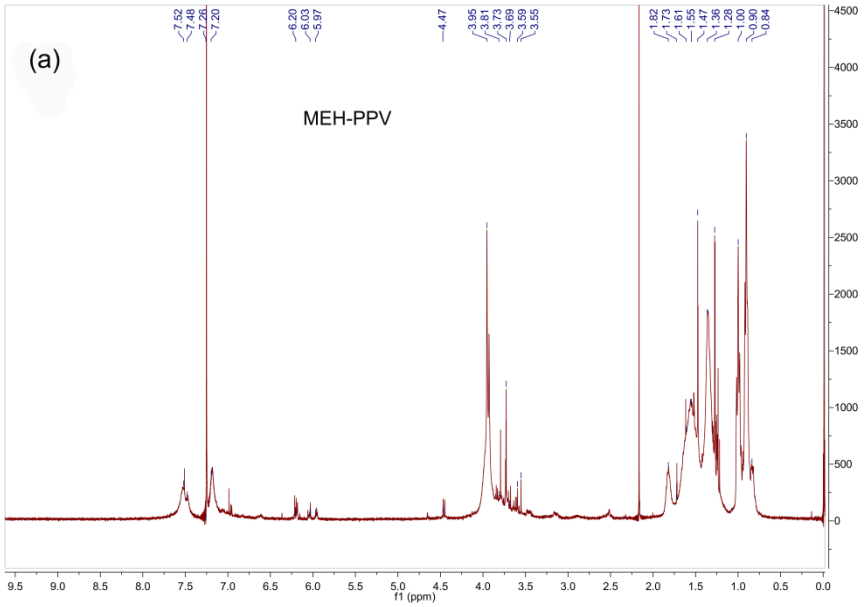
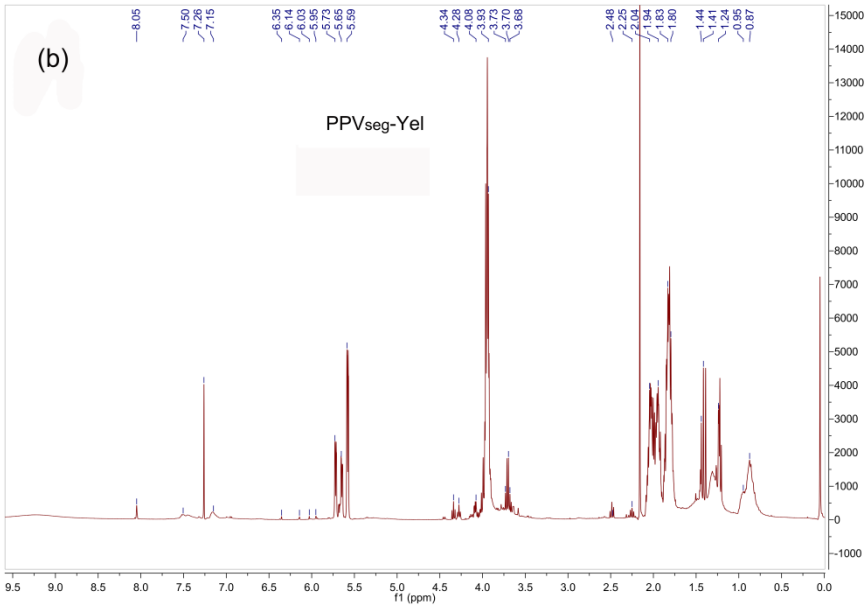


Figure S1. UV-visible absorption spectra of pristine MEH-PPV and PPV_{seg} in anhydrous

THF solution after heat treatment at 50 °C. The UV-visible absorption spectra of MEH-PPV and PPV_{seg} also show the expected blue shift in the absorption maxima, which reflects a gradual increase in the population of shorter conjugated segments.





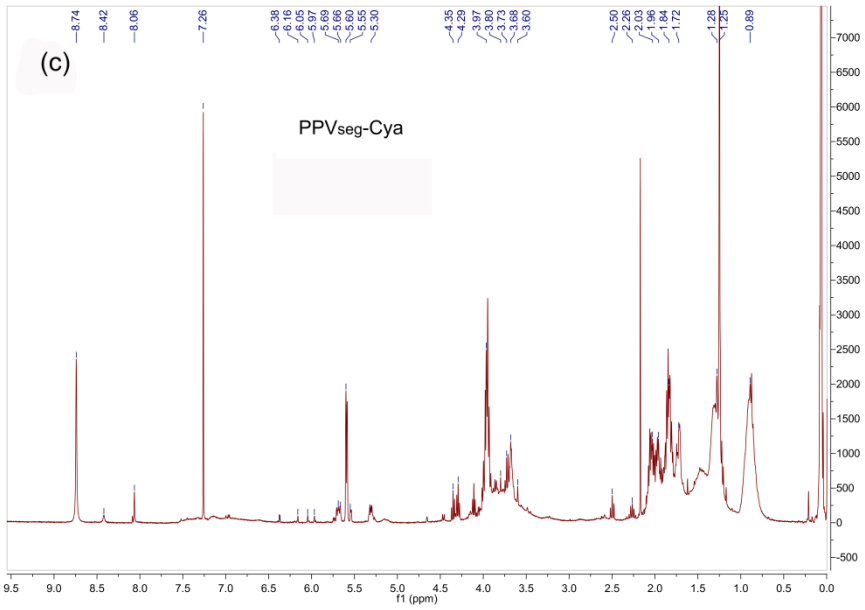


Figure S2. ^{1}H NMR spectra changes of (a) pristine MEH-PPV, (b) PPV_{seg}-Yel, (c) PPV_{seg}-Cya in $CDCl_3$ after heat treatment at 50 °C. The results shows that the ^{1}H NMR spectrum of pristine MEH-PPV are match with previous literatures [5,6] and signals related to structural defects from the polymer synthesis are shown at low proportions. [7] After heat treatment, new signals are observed in different regions of the spectrum with low intensity (2.3-3.0, 5.0-5.8, and 8.0-9.0 ppm). The initial structural defects are still present with small alterations. The spectral changes give us a strong indication of structural changes in the polymer chain and the signals at 8.06 and 8.74 ppm are typical of MEH-PPV oxidation after heat treatment.

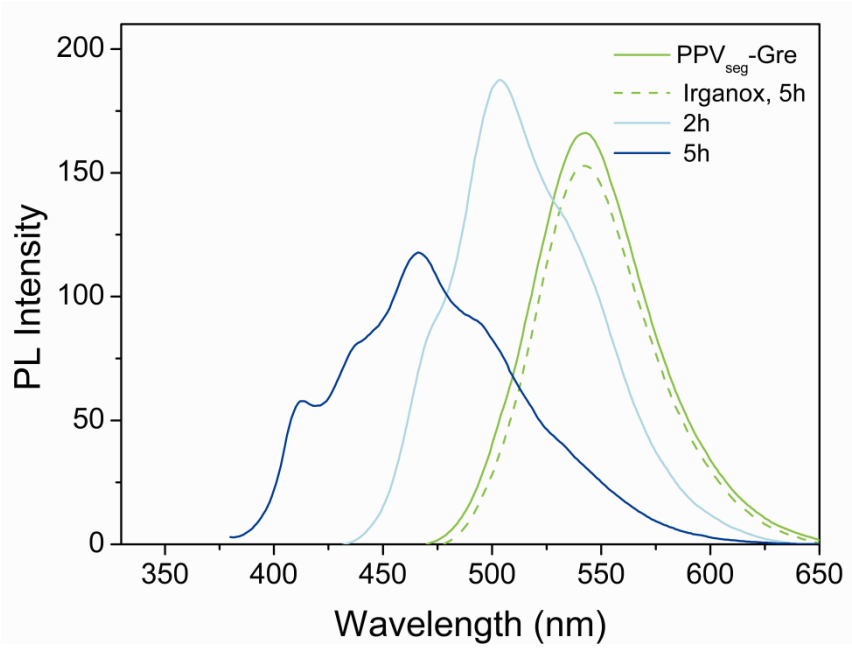


Figure S3. Color tuning of the PL spectra for PPV_{seg}-Gre and PPV_{seg}-Gre: Irganox 1010 (1/4 ratio in mass) during heat treatment at 50 °C.

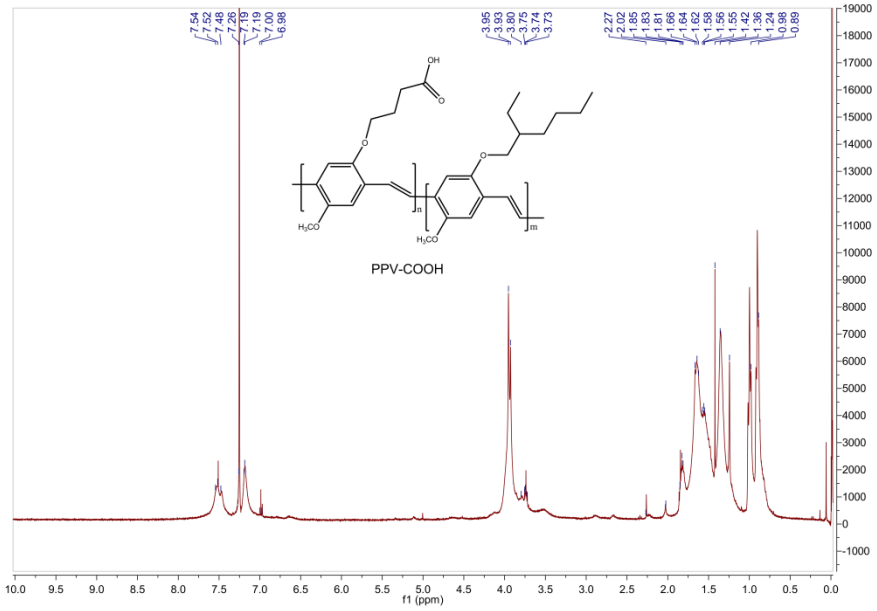


Figure S4. ^{1}H NMR spectrum of PPV-COOH in $CDCl_3$.

Figure S5. dynamic-light-scattering measurements of PPV_{seg}-Cya CPNs. The PPV_{seg}-Cya CPNs exhibit a diameter of ~5.3 nm based on DLS measurements, which are larger than those measured by TEM. DLS size measurements are expected to be somewhat higher than those measured by TEM, since the measured sizes from DLS reflect the hydrodynamic diameter and can

be inflated by the presence of even small amounts of aggregate.

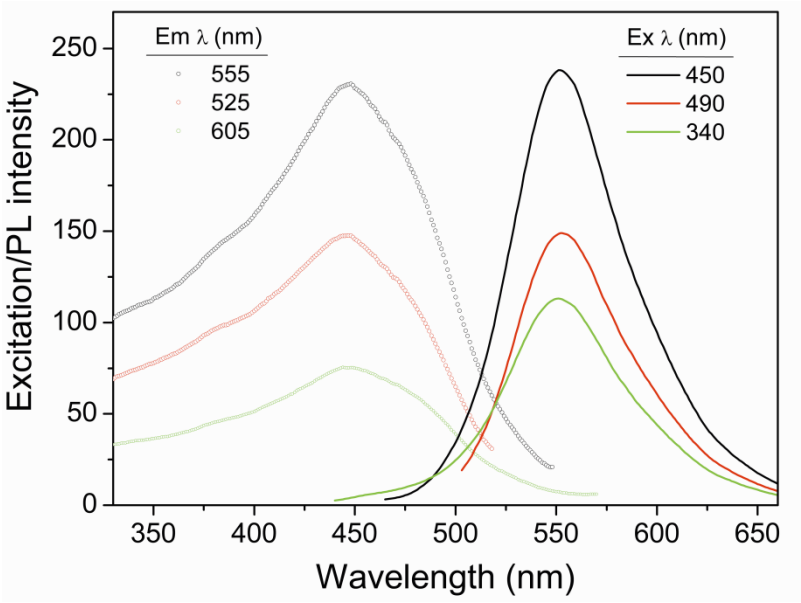


Figure S6. Excitation (○)and PL spectra (solid line) of PPV_{seg}-Gre.

Table S1 GPC data of the polymers

Polymers	Pristine MEH-PPV	PPV_{seg}-Yel	PPV_{seg}-Gre	PPV_{seg}-Cyan	PPV_{seg}-Blu
Mn [a] (10^4)	5.15 [c]	2.58 [c]	1.63 [c]	0.57 [c]	0.41[c]
Mw [a] (10^4)	19.37	5.75	4.17	0.93	0.55
PDI [b]	3.76	2.22	2.55	1.62	1.33

[a] Determined with GPC (THF) against polystyrene standards. [b] Mw/Mn. [c] Degree of polymerization (DP) based on polymer repeat unit is 177 for pristine MEH-PPV, 89 for PPVseg-Yel, 56 for PPVseg-Gre, 20 for PPVseg-Cya and 14 for PPVseg-Blu. The M_n and polydispersity (PDI) of the polymers both decrease with increased heating time, which is consistent with improved solubility of PPV_{seg} and suggests that heat treatment causes partially polymer backbone cleavage.

3. Refenrences:

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[7] T. Schwahn, J. Wiesecke, S. Immel, M. Rehahn, Macromol. Rapid Commun. 2009, 30, 1295-1322.