Two-photon fluorescence in red and violet conjugated polymer microspheres

Supporting Information

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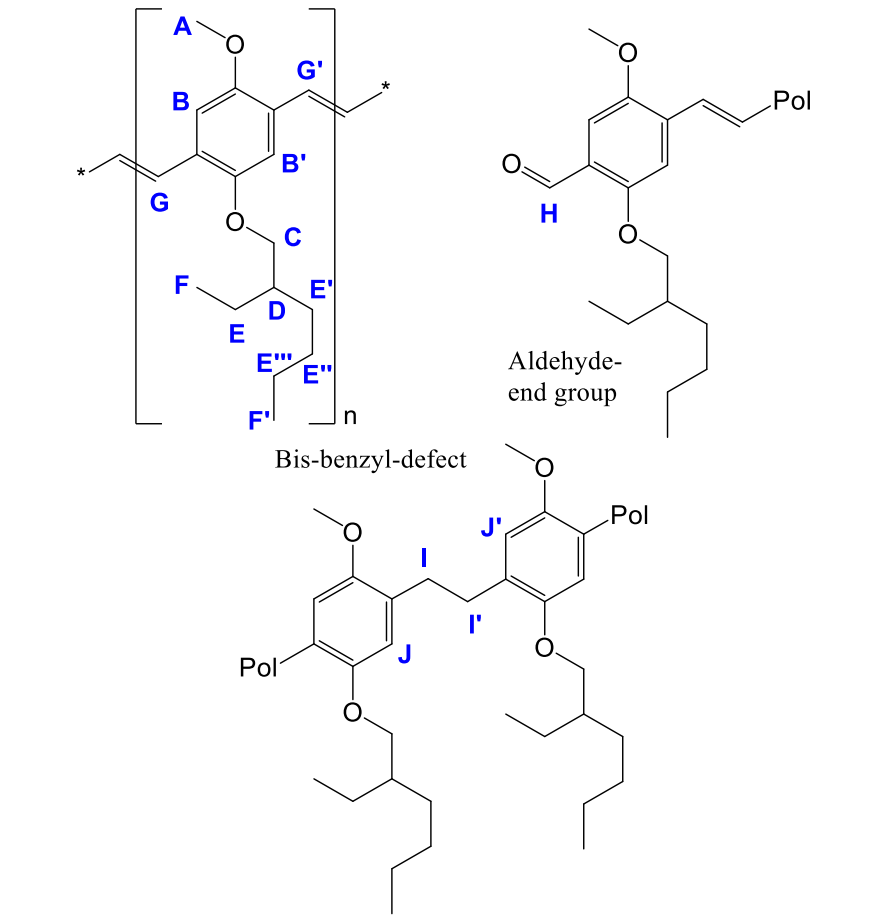


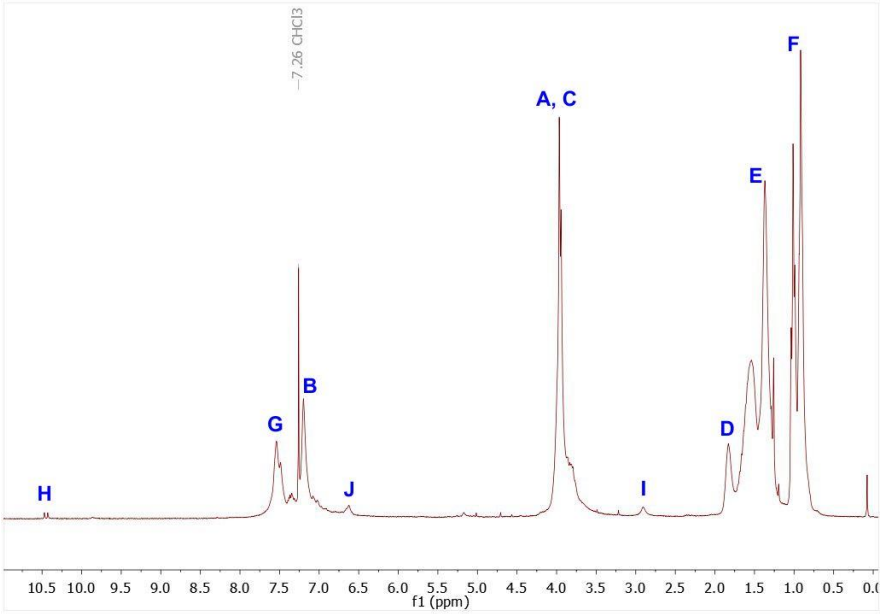


Fig. S1. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **MEH-PPV** and the signal assignment.

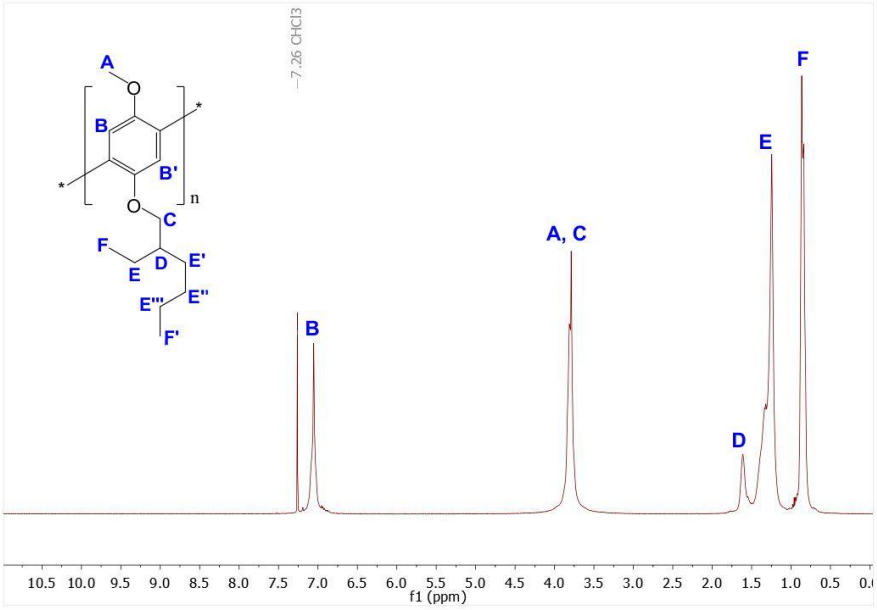


Fig. S2. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **MEH-PPP** and the assignment of the signals.

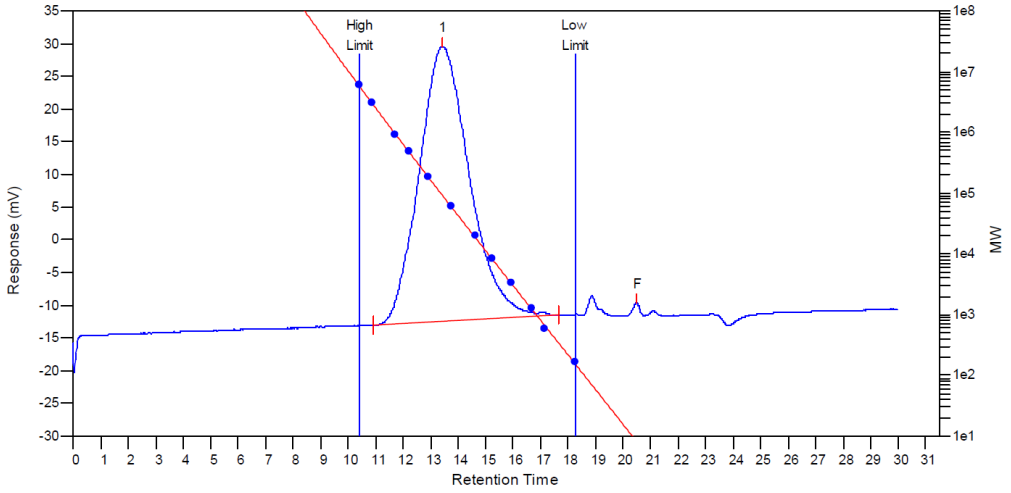
The following conditions for GPC-measurements were applied:

1 ml/min flow rate, approx. 1mg/ml sample concentration, two PLgel Mixed-C columns from Agilent (7.5 mm diameter and 600 mm total length), 40°C (THF) or 25°C (chloroform). The separation range of the columns is from 200 Da to 2 MDa according to the manufacturer.

The GPC columns were calibrated using 11 to 12 narrow polystyrene standards (blue points in the GPC-elugrams) purchased by Agilent, and the calibration curves shown as red lines were fitted as polynomial (either first or third order, depending on where the best fit was achieved). The upper and lower limits of the calibration are indicated with the vertical blue lines.

Both MEH-PPV and MEH-PPP samples elute completely within the calibration limits. A flow marker signal (indicated as F in the elugrams) was used for correction of the retention times of the standards and unknown sample in chloroform, and the calibration curve was used to calculate the average molecular weights according to usual algorithms. The recording of elugrams and all calculations were performed using a Cirrus GPC Software package.

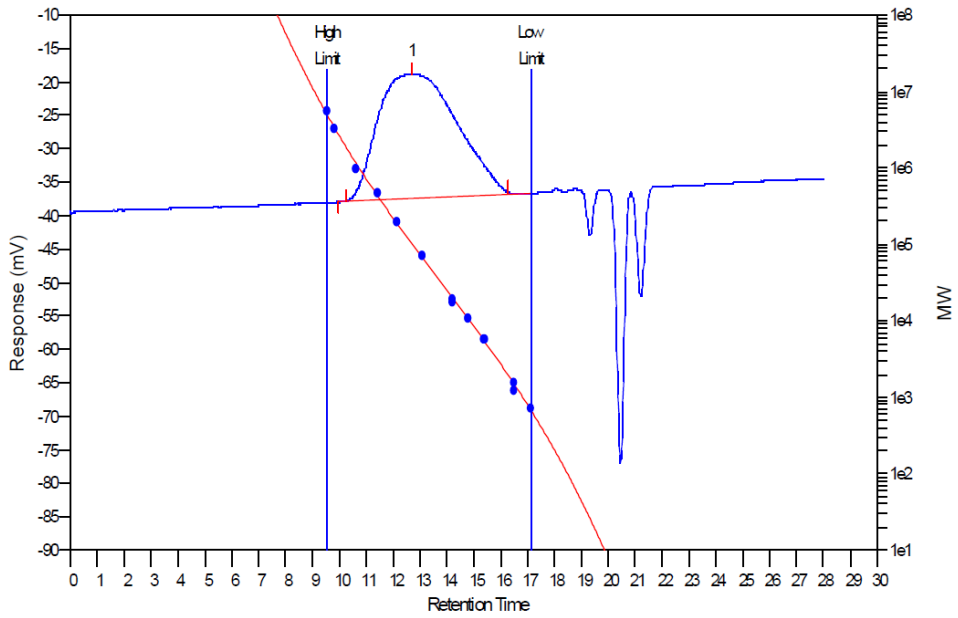
The represented GPC-curves are recorded with a differential refraction index detector, and due to the linear GPC-columns they reflect roughly the polymer molecular weight distribution curves. The latter would be plotted in the axes <d(m)/d(log(M)) vs. log(M)>, where the highest molecular weight corresponds to the shortest retention time in the GPC curve. Both MEH-PPV and MEH-PPP samples have relatively high polydipersity (4.5 and 5, respectively).



MW Averages

Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	93226	30968	139531	358156	644934	117904	4.50565
2	0	0	0	0	0	0	0

Fig. S3. GPC-trace (differential refractive index detector) of **MEH-PPV** in chloroform with the average molecular weights calculated relative to polystyrene.



MW Averages

Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	108966	30226	149677	390619	631577	123561	4.95193

Fig. S4. GPC-trace (differential refractive index detector) of **MEH-PPP** in THF with the average molecular weights calculated relative to polystyrene.

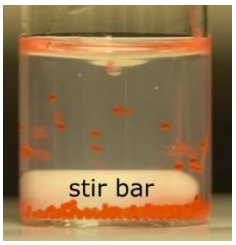


Fig. S5. Photograph of MEH-PPV-in-chloroform (20 mg/mL) droplets being injected into the stirred water bath. The chloroform evaporates during the stirring process until only solid polymer microspheres remain in the beaker.

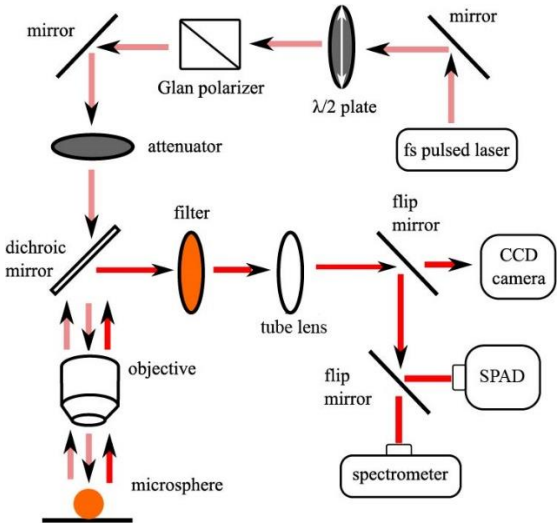


Fig. S6. Experimental setup for PL spectra and lifetime measurements of single microspheres. The light paths drawn in pink and red denote the pump laser light and the fluorescence, respectively.

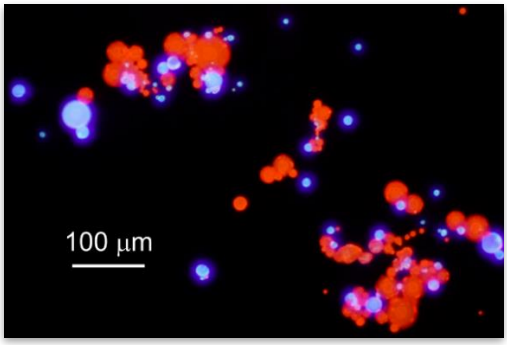


Fig. S7. Fluorescence image of a sample of CP microspheres from violet and red polymer droplets (dissolved in chloroform) injected simultaneously into the water/PVA solution from two syringe pumps. The two types of microspheres appear not to have mixed.

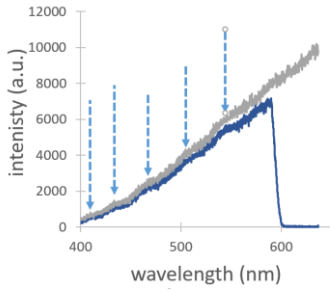


Fig. S8. The "bumps" in the TPF of the blue particles likely arise from the optical system. When passing a calibration lamp through the system, we observed small features in the transmission spectra that occurred at almost the same wavelengths as the observed features.

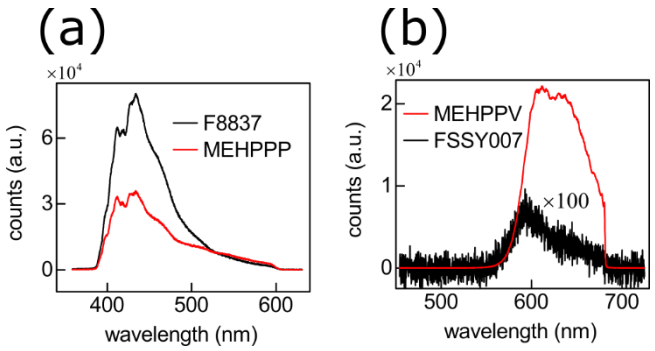


Fig. S9. A comparison of the TPF fluorescence intensity of (a) the violet CP microsphere with a blue-violet commercial dye-doped polystyrene sample (F8837 from Thermo-Fisher), and (b) a red CP microsphere with a commercial dye-doped polystyrene sample ("Suncoast Yellow" from Bangs Labs).

Laser power density estimation

The spot size of the laser is ~1.3 μm at 800 nm, and ~1.1 μm at 700 nm. The power density was estimated according to:

$$D = \frac{P}{\pi\left(\frac{d}{2}\right)^2} \qquad \text{(Eq. S1)}$$

where D is the power density, P is the laser power, d is the diameter of the spot size. Taking the spot size as 1.3 μm and the laser power as 2 mW, the power density is thus estimated as 150 kW/cm^2.

Fluorescence comparison of MEH-PPV and MEH-PPP microspheres

When collecting the two-photon fluorescence spectra in Fig. 5 and Fig. 7, the spectrometer was not always set the same. Because the two-photon PL of the MEH-PPP microsphere is much weaker than that of the MEH-PPV microsphere, two actions were made to ensure a reasonable signal-to-noise-ratio (SNR): i) the entrance slit width of the spectrometer was increased, ii) the "gain" function of the spectrometer was turned on. Therefore, the fluorescence counts cannot be used to directly compare the fluorescence intensity of the MEH-PPV and MEH-PPP microspheres; however, for comparing them to their commercial analogues, care was made to keep the conditions the same.

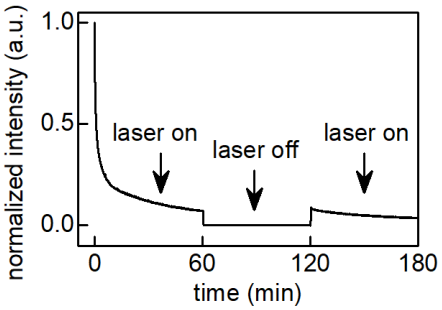


Fig. S10. Photobleaching graph for an MEH-PPV microsphere TPF (2 mW excitation power). During the first hour and the third hour, the laser was on. During the second hour, the laser was shut off.

In order to compare the fluorescence intensity of the MEH-PPV and MEH-PPP microspheres with similar diameters under the same irradiance power, the TPF counts of those two types of single microspheres were collected using an avalanche photo-diode (APD). The ratio of the TPF counts is shown in Fig. S7, indicating that the MEH-PPV (red) microsphere was always much brighter than the MEH-PPP (violet) microsphere.

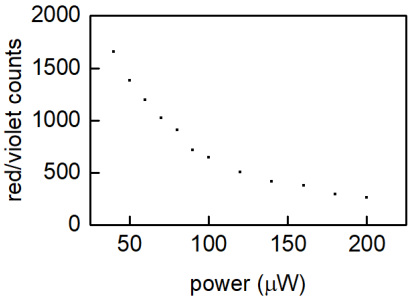


Fig. S11. The ratio of the two-photon fluorescence intensity (counts) from MEH-PPV to those from MEH-PPP microspheres as a function of irradiance power.

TRPL

The TRPL dynamics were modeled using Eq. 1 in the main text. The method used was least-absolute residuals. Manual trials had to be extensively performed to narrow down the parameters prior to automated curve fitting. The result of the deconvolution yielded a single exponential time constant comparable or shorter value than the IRF temporal width (~250 ps); thus, these exact values should be taken cautiously. Most notable is the consistent enhancement of the lognormal part (the small B parameter) in the TPF cases. Note that the negative time constants for the lognormal part (τ_1) do not imply real negative lifetimes in the lognormal distribution (which is entirely positive, as seen in Fig. 6).

Table S1. TRPL fitting parameters (Eq. 1) for MEH-PPP and MEH-PPV microspheres, where τ_0 refers to the single exponential component, and τ_1 and $\Delta\tau_1$ are for the lognormal component (hence, τ_1 can be negative without implying negative decay times). The weighted mean lifetime is given by τ_{avg}.

parameter	B	τ_0 (ns)	τ_1 (ns)	$\Delta\tau_1$ (ns)	t_{avg} (ns)
OPF-PPP	0.96	0.14	0.42	0.57	0.21
OPF-PPV	0.45	0.22	-3.23	1.35	0.23
TPF-PPP	0.06	2.23	-0.50	0.68	1.04
TPF-PPV	0.13	0.60	-2.11	0.95	0.34

Photobleaching

The spectral response of the photomultiplier tube (PMT) is fairly constant over the MEH-PPV emission range, so the effects of the responsivity function on the TRPL data should be relatively small. The photobleaching dynamics did not follow a single-exponential fitting, and were instead fit using a double-exponential function, which is commonly used when studying two-photon fluorescence lifetime [1]:

$$I = a_1 \cdot e^{-t/\tau_1} + a_2 \cdot e^{-t/\tau_2} + dc \qquad \text{(Eq. S2)}$$

where I is the intensity (or counts) at time t, a_1 and a_2 are scaling factors, τ_1 and τ_2 are corresponding time constants, and dc is an offset constant. The mean weighted decay times are calculated as

$$\tau = \frac{a_1}{a_1 + a_2} \cdot \tau_1 + \frac{a_2}{a_1 + a_2} \cdot \tau_2 \qquad \text{(Eq. S3)}$$

The two-photon florescence of a MEH-PPV microsphere was excited under IR irradiance with a power of 2 mW. The laser was firstly on, and bleaching was observed, as shown in Fig. S8. The laser was then shut off for an hour. The TPF did not recover appreciably.

[1] A. S. Kristoffersen, S. R. Erga, B. Hamre, and Ø. Frette, "Testing Fluorescence Lifetime Standards using Two-Photon Excitation and Time-Domain Instrumentation: Rhodamine B, Coumarin 6 and Lucifer Yellow," **Journal of Fluorescence** 24, 1015-1024 (2014).