Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Supplementary Information

Enhancing the Photostability of Poly(phenylene ethynylene) for Single Particle Studies

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1. Purification of PPE-CO_2-49 coated NPs to remove unadsorbed polymer

The PPE-CO_2-49 coated NPs were prepared by adding aminosilanzed NPs ($SiO_2NH_3^+$) to an aqueous solution containing an amount of polymer in excess of what is required to passivate the surface. The PPE-CO_2-49 coated NPs were precipitated by centrifugation and 80% of the supernatant containing the excess, unadsorbed polymer was removed and replaced by an equal volume of water. Only 80% of the total volume was removed at a time to avoid disturbing the NP pellet. The UV-Vis absorption of the supernatants decreased after each centrifugation/washing step (Figure S1A). The expected absorbance of the supernatants was predicted based on a 5-fold dilution of the previous supernatant after each washing step and was compared to the actual absorbance measured (Figure S1B). The experimental values matched the expected values, indicating that the initially adsorbed polymer was not being removed from the NPs in subsequent washing cycles.

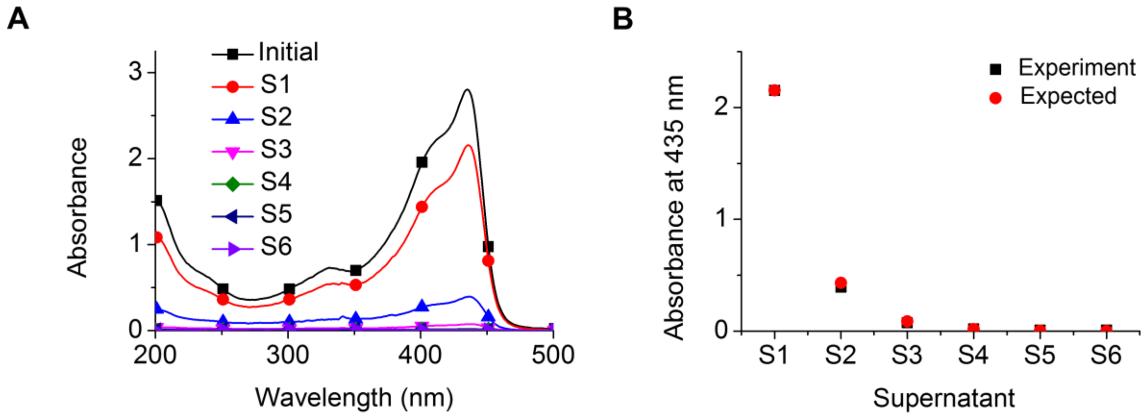

Figure S1. (A) UV-Vis absorption spectrum of the initial solution of PPE-CO_2-49 (50 µM) used to coat the NPs (black squares) and of the supernatants removed after centrifugation (S1-S6). The "Initial" and "S1" solutions were diluted to achieve an appropriate concentration for measurement and then these spectra were multiplied by the dilution factor to give the spectra plotted here. (B) The absorbance value of each supernatant at the PPE-CO_2-49 absorption maximum of 435 nm (black squares) is compared to the expected value based strictly on dilution of the remaining unadsorbed polymer after each washing step (red circles).

2. Calculation of the surface coverage of PPE-CO_2-49 on the NPs

To estimate the surface area of the NPs covered by PPE-CO_2-49, the number of polymer repeat units (PRUs) per NP was determined from UV-Vis absorption measurements. The polymer absorbance of the first supernatant was lower than that of the initial coating solution by a factor of 0.77, indicating that the missing 23% was adsorbed onto the surface of the NPs (Figure S1A). Given that the concentration of the initial coating solution was $5.0\text{x}10^{-5}$ M, this works out to a concentration of $5.0\text{x}10^{-5}$ M*0.23 = $1.15\text{x}10^{-5}$ M PPE-CO_2-49 PRUs adsorbed on the NPs. The surface area of

one PRU was estimated to be 1.2 x 0.9 nm = 1.08 nm^2 using ChemBio3D Ultra version 12.0.2.1076 (CambridgeSoft). The total surface area of the polymer was then calculated to be $1.15\text{x}10^{-5}$ M*1.08 nm^2*N_A = $7.74\text{x}10^{18}$ nm^2/L. In turn, the concentration of NPs was $5.26\text{x}10^{-10}$ M and the average surface area of an NP was calculated from the formula for the surface area of a sphere, $4\pi r^2$, where the average value of r was estimated to be 55 nm based on dynamic light scattering data (Figure 1A). The total surface area of the NPs was then calculated to be $5.26\text{x}10^{-10}$ M*$3.80\text{x}10^{4}$ nm^2*N_A = $1.25\text{x}10^{19}$ nm^2/L. The percentage of the NP surface covered by PPE-CO_2-49 is equal to the surface area of the polymer divided by the surface area of the NPs: ($7.74\text{x}10^{18}$ nm^2/L/$1.25\text{x}10^{19}$ nm^2/L)*100 = 62%. We also note that this works out to an average of $1.15\text{x}10^{-5}$ M PPE-CO_2-49 PRUs/$5.26\text{x}10^{-10}$ M NPs = 21,900 PRUs per NP, or *ca.* 450 polymers where *n* = 49.

3. Representative fluorescence intensity versus time trajectories of PPE-CO_2-49 coated NPs

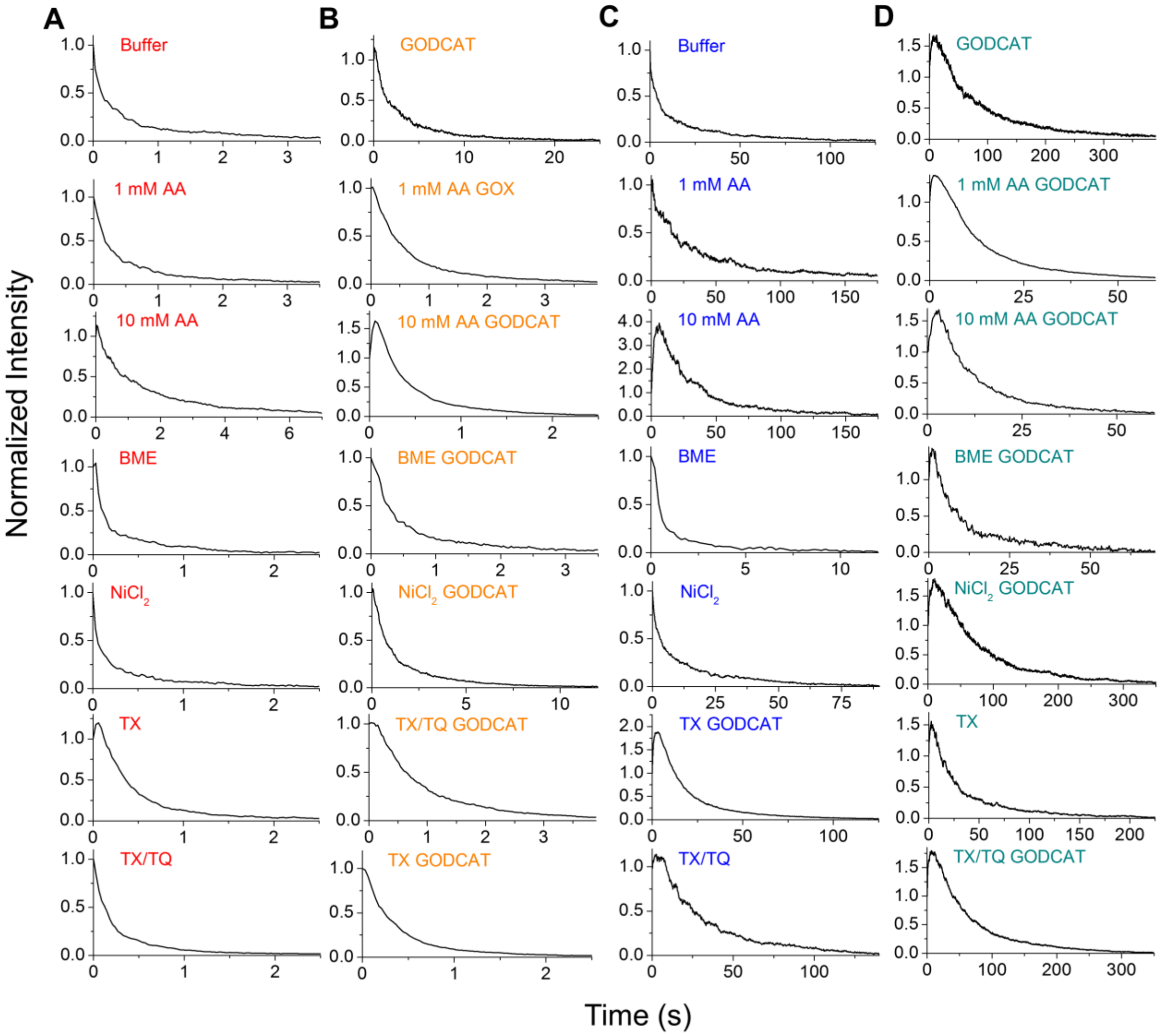

Figure S2. Fluorescence intensity versus time trajectories of representative NPs extracted from TIRFM movies acquired while flowing different additives in either buffer or a buffered solution containing GODCAT oxygen scavenger under an excitation power of either 4.4 or 0.1 mW. The trajectories are normalized to an initial intensity of 1. (A) Buffer, 4.4 mW. (B) GODCAT, 4.4 mW. (C) Buffer, 0.1 mW. (D) GODCAT, 4.4 mW.

4. Histograms of the initial intensity of PPE-CO_2-49 coated NPs

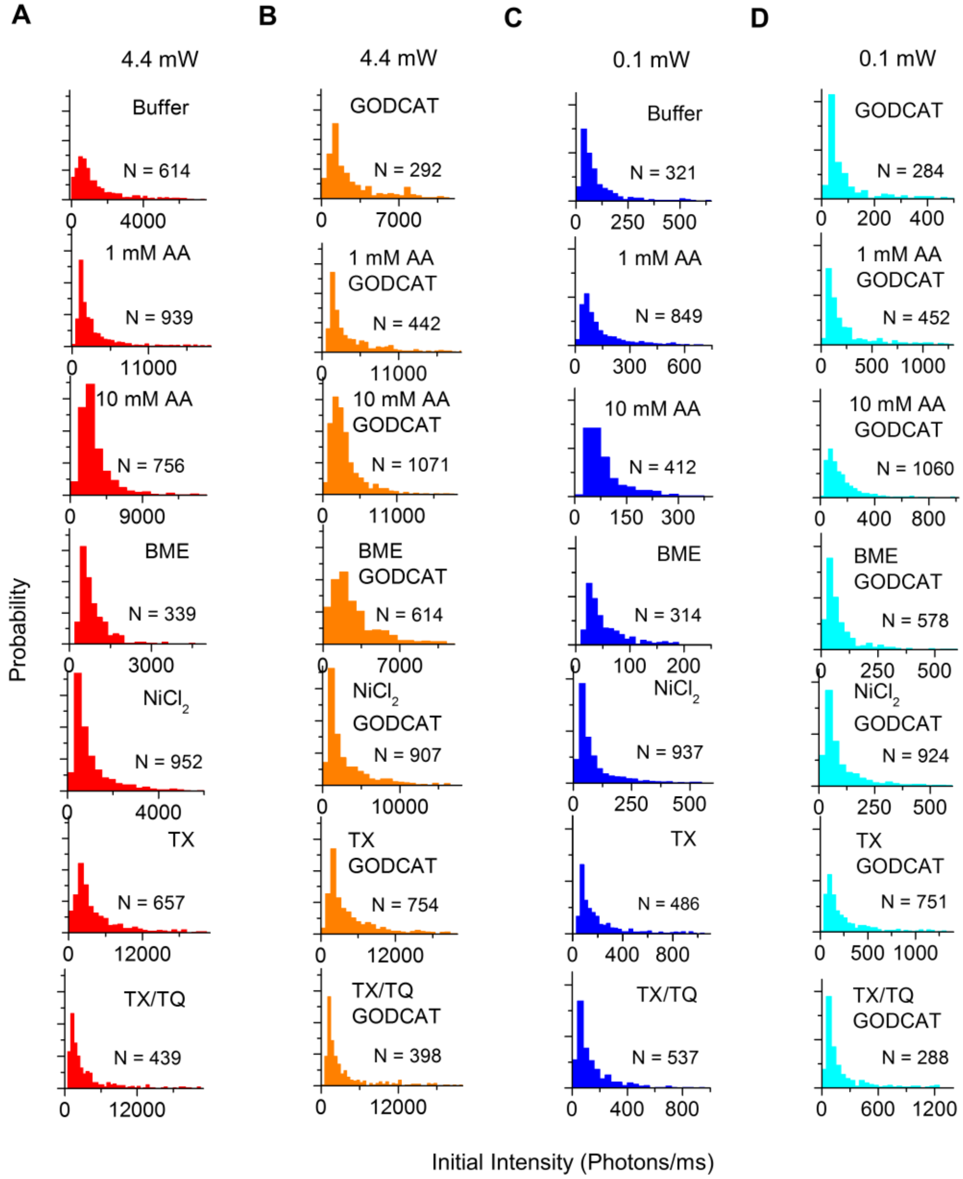

Figure S3. Histograms of the initial intensity of NPs in photons/ms extracted from TIRFM movies acquired while flowing different additives in either buffer or a buffered solution containing GODCAT oxygen scavenger under an excitation power of either 4.4 or 0.1 mW. Histograms are normalized to an area of 1. (A) Buffer, 4.4 mW. (B) GODCAT, 4.4 mW. (C) Buffer, 0.1 mW. (D) GODCAT, 4.4 mW.

5. Histograms of the total photon output of PPE-CO2-49 coated NPs

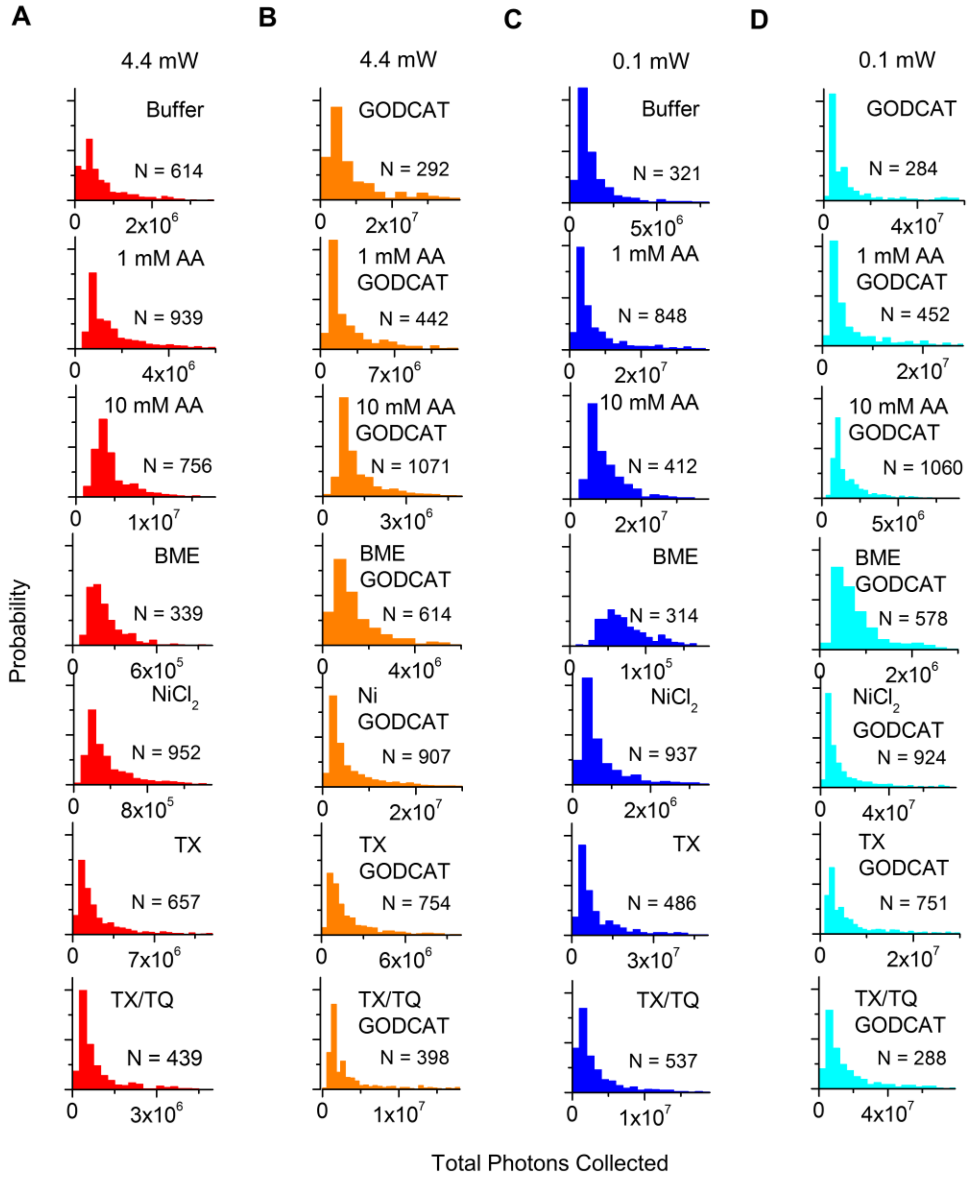

Figure S4. Histograms of the total photon output of NPs extracted from TIRFM movies acquired while flowing different additives in either buffer or a buffered solution containing GODCAT oxygen scavenger under an excitation power of either 4.4 or 0.1 mW. Histograms are normalized to an area of 1. (A) Buffer, 4.4 mW. (B) GODCAT, 4.4 mW. (C) Buffer, 0.1 mW. (D) GODCAT, 4.4 mW.

6. Correlation between initial intensity and total photon output of PPE-CO$_2$-49 coated NPs

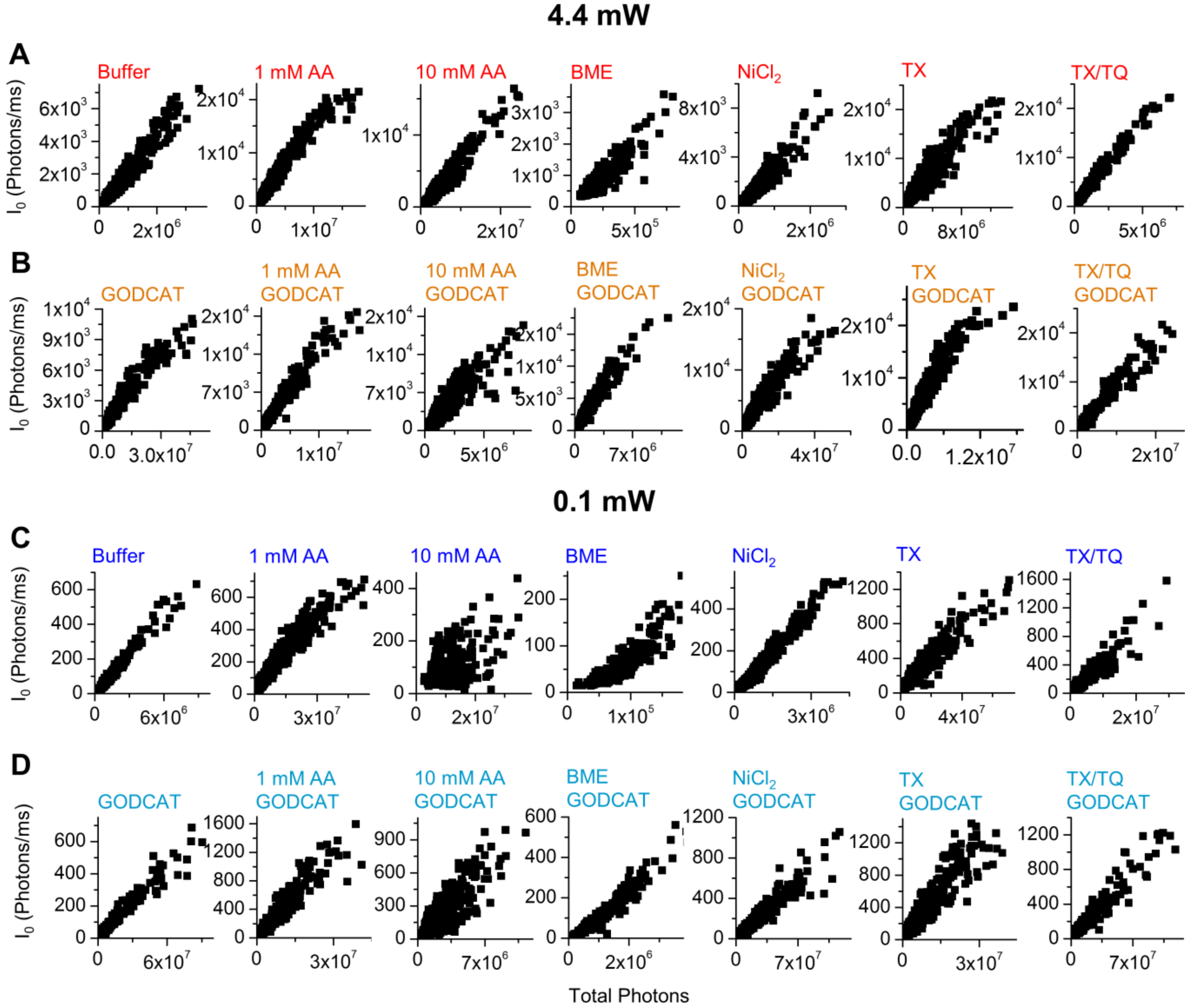

Figure S5. Correlation plots of the initial intensity versus the total photon output of the PPE-CO$_2$-coated NPs. The plotted values were obtained from individual intensity *versus* time trajectories extracted from TIRFM movies acquired while flowing different additives in either buffer or a buffered solution containing GODCAT oxygen scavenger under an excitation power of either 4.4 or 0.1 mW. (A) Buffer, 4.4 mW. (B) GODCAT, 4.4 mW. (C) Buffer, 0.1 mW. (D) GODCAT, 0.1 mW.

7. Effect of the additives on the ensemble UV-Vis absorption and emission spectra of PPE-CO_2-49

The UV-Vis absorption spectrum of 1.6 μM PPE-CO_2-49 in buffer was acquired and compared to the spectrum in GODCAT (Figure S6A). The spectrum in GODCAT was noticeably different in both shape and absorbance. The absorbance spectrum of GODCAT solution was also acquired (absorbance in the UV region was anticipated due to the presence of amino acids in the enzymes glucose oxidase and catalase) (Figure S6A). The curve obtained by summation of the spectrum of GODCAT and the spectrum of PPE-CO_2-49 in buffer coincided with the spectrum of PPE-CO_2-49 in GODCAT, indicating that this spectrum is a convolution of its components, not that the components of GODCAT scavenger were influencing the absorptive properties of the polymer. Similarly, the other additives were not found to affect the absorptivity of PPE-CO_2-49 in either buffer or in GODCAT (Figure S6B and S6C, respectively).

The effect of the additives on the fluorescence quantum yield of PPE-CO_2-49 was also investigated by comparing its emission profile and intensity in each solution condition. GODCAT alone showed a weak fluorescence signal, but even after considering its contribution, the emission intensity of PPE-CO_2-49 was still enhanced by a factor of 1.2-fold in GODCAT compared to buffer (Figure S6D). As for the other additives, TX and 1 mM AA did not affect the fluorescence quantum yield, BME caused a slight enhancement, and $NiCl_2$ and 10 mM AA caused fluorescence quenching. These results were consistent in both buffer and in GODCAT (Figure S6E and S6F, respectively).

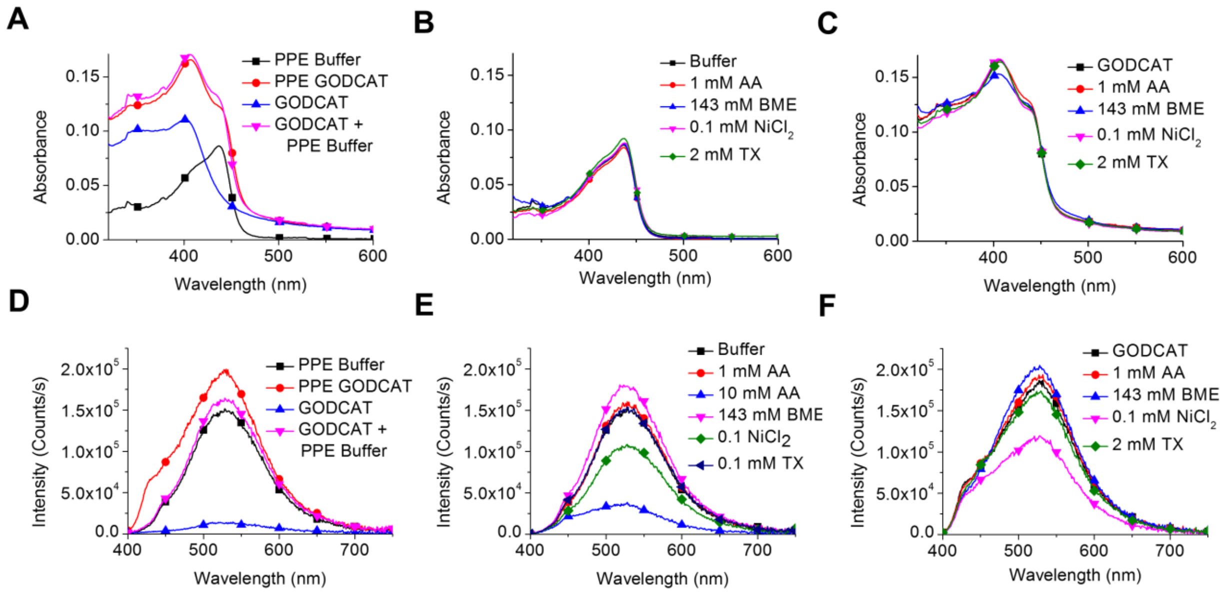

Figure S6. (A) UV-Vis absorption spectra of 1.6 µM PPE-CO$_2$-49 in buffer or GODCAT (black squares and red circles, respectively), GODCAT (blue triangles), and the spectrum obtained by summation of the spectrum of 1.6 µM PPE-CO$_2$-49 in buffer and the spectrum of GODCAT (purple inverted triangles). (B) and (C) UV-Vis absorption spectra of 1.6 µM PPE-CO$_2$-49 in a buffered solution or a buffered solution containing GODCAT oxygen scavenger, respectively (black squares) as well as containing either 1 mM AA (red circles), 143 mM BME (blue triangles), 0.1 mM NiCl$_2$ (purple inverted triangles) or 2 mM TX (green diamonds). (D), (E), and (F) Emission spectrum of the samples whose UV-Vis absorption spectra is shown in (A), (B) and (C), respectively. The excitation wavelength was 390 nm. Spectra are corrected for background and photomultiplier tube sensitivity.