iScience, Volume *23*

Supplemental Information

Light-Triggered Electron Transfer between a Conjugated Polymer and Cytochrome C for Optical Modulation of Redox Signaling

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Supporting Figures

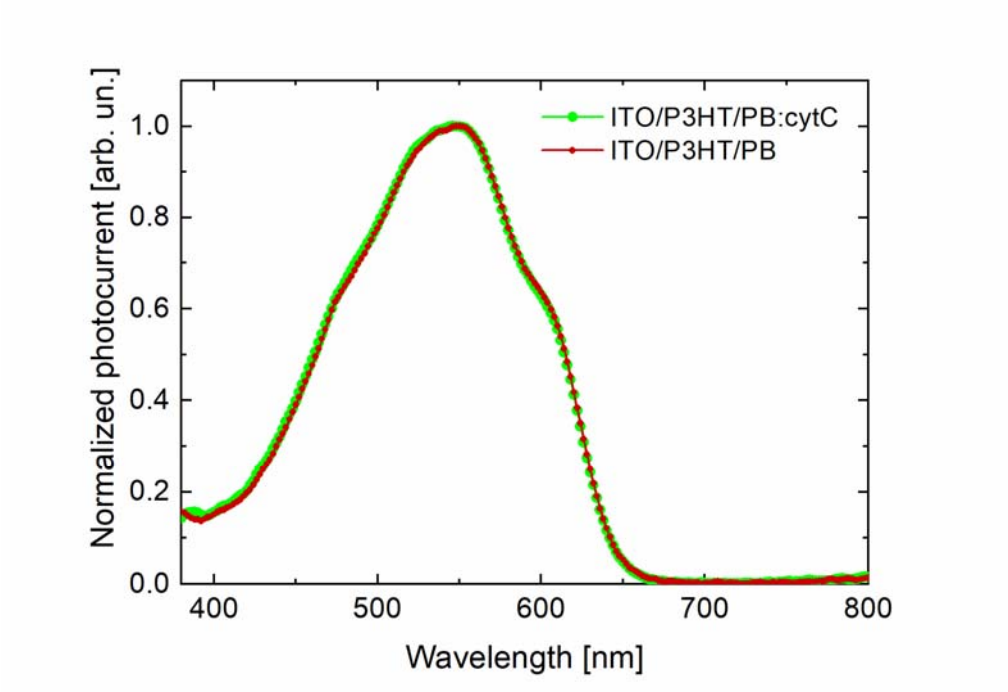

Figure S1. **Photocurrent action spectrum of ITO/P3HT/PB and ITO/P3HT/PB:CytC** (Related to Figure 1). The photocurrent action spectrum of ITO/P3HT in phosphate buffer (red dotted line) and in CytC-enriched PB solution (green dotted line) was recorded in a two-electrodes electrochemical cell coupled with a lamp, a monochromator and a photodiode. The signal was acquired by means of a lock in amplification system.

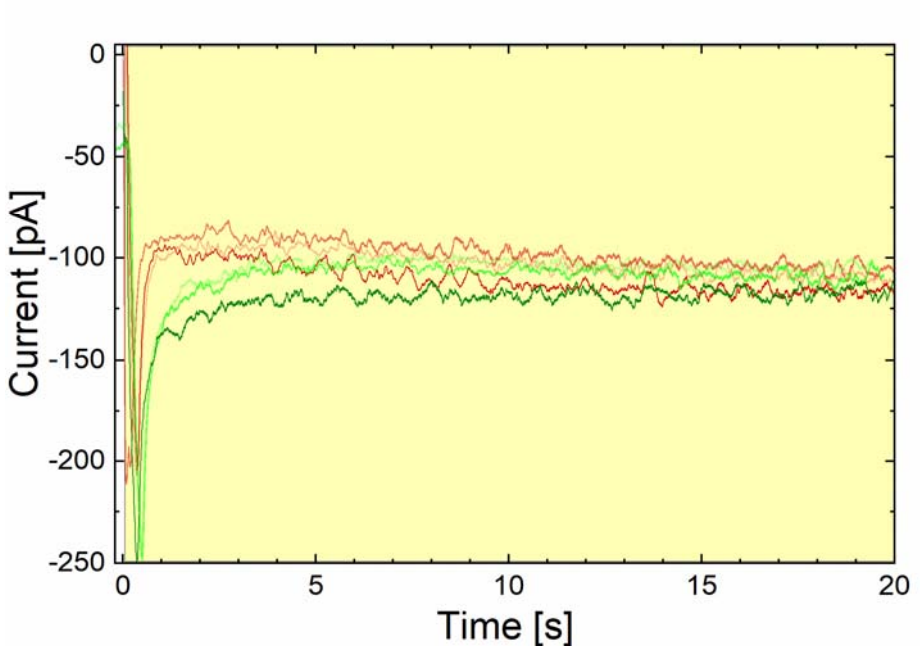

Figure S2. Chronoamperometry recording hydrogen peroxide production upon illumination (Related to Figure 1). Time course of hydrogen peroxide production upon polymer photoexcitation, in 4 mM phosphate buffer. Red, light red and orange have been recorded in absence of CytC; Dark green, light green and green traces are recorded in presence of 10 μM CytC. See Transparent methods for details on trace treatment.

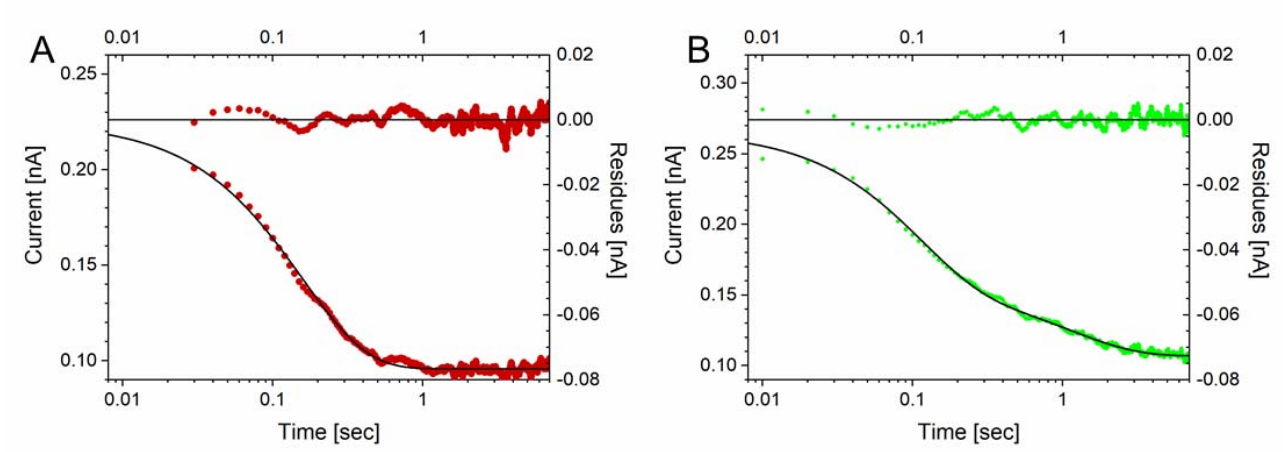

Figure S3. Fitting of chronoamperometry after the light onset (Related to Figure 1). Exponential fitting of the current observed in the time corse experiments, in absence (panel A) and in presence (panel B) of CytC. In both panels, the lower part report the experimental data as dotted lines and the exponential fitting curve as a black solid line, while in the upper part the residues are presented. The left axis refers to the experimental data, while the right axis to residues. In the ITO/P3HT/PB system a single exponential decay was used; for the system ITO/P3HT/PB:CytC a two-exponential fitting was necessary, based on the residues analysis.

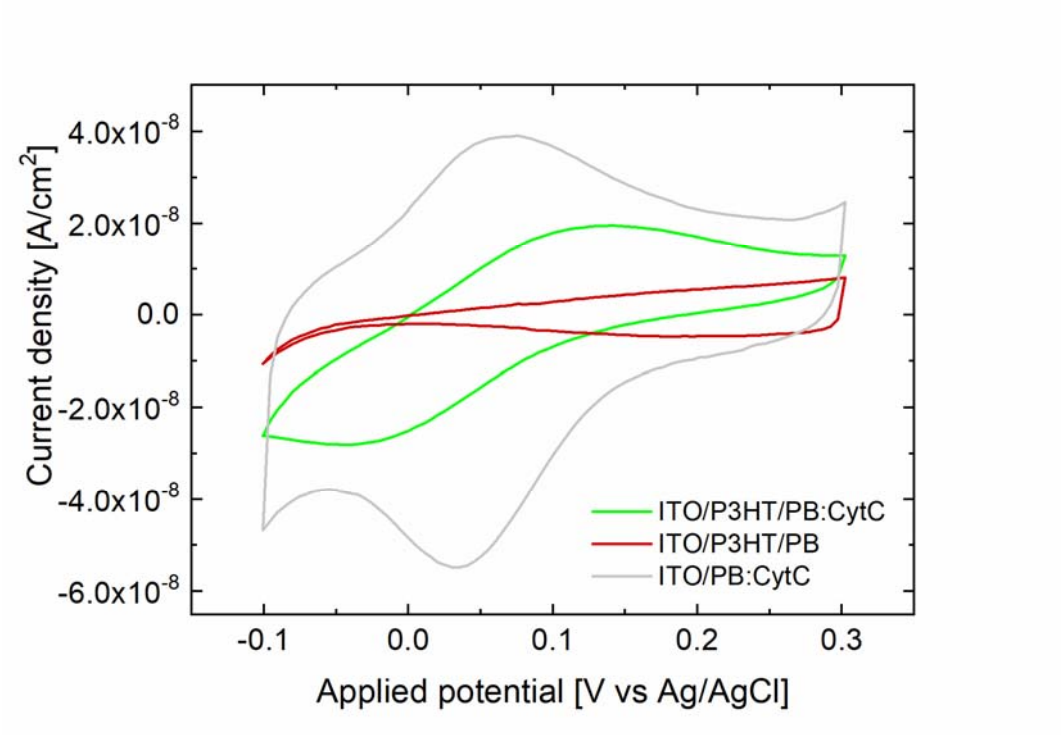

Figure S4. Cyclic Voltammetry of ITO/P3HT device in contact with CytC (Related to Figure 1). Cyclic voltammetry of ITO/P3HT working electrode in bare phosphate buffer (PB, red line) and in CytC-enriched PB (PB:CytC, 1 μM solution, green solid line). ITO working electrode in CytC-enriched PB (PB:CytC, 1 μM solution, grey solid line) is also shown for reference.

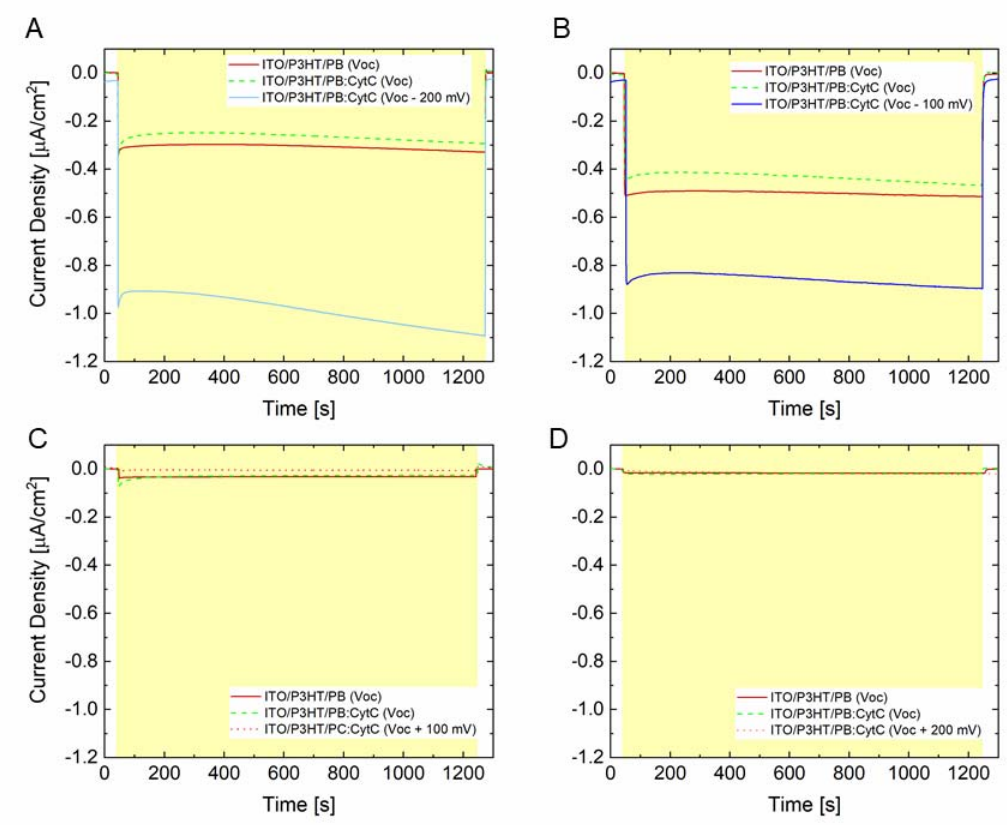

Figure S5. Photocatalytic activity of ITO/P3HT device in PB (Related to Figure 3). Chronoamperometry measurements upon externally applied positive and negative bias recorded during spectroelectrochemical measurements in a three electrodes electrochemical cell. In all panels, ITO/P3HT/PB photocurrent generated at open circuit potential vs Ag/AgCl in phosphate buffer is reported as a black solid line, ITO/P3HT/PB:CytC 10 μM at V_{OC} is reported as a dashed red line, ITO/P3HT/PB:CytC 10 μM at $V_{OC} \pm V_{app}$ is reported as a solid red line. The panels **A**, **B**, **C**, **D** are related to $V_{OC} - 0.2$ V, $V_{OC} - 0.1$ V, $V_{OC} + 0.1$ V, $V_{OC} + 0.2$ V vs Ag/AgCl, respectively. The yellow shaded area displays the illumination period, lasting for 20 minutes.

According to the photocathodic behaviour of the polymer/buffer interface, the bias negative respect to the V_{OC} value promotes electron transfer processes at the polymer surface, thus increasing the absolute value of the photocurrent density. Conversely, a bias more positive than the V_{OC} value hampers polaron dissociation and free electrons formation at the interface with the electrolyte, thus possibly reducing the overall oxygen reduction reaction efficiency and the photocurrent density absolute value. In this experimental configuration, the addition of CytC to the buffer does not lead to remarkable changes in the photocurrent dynamics.

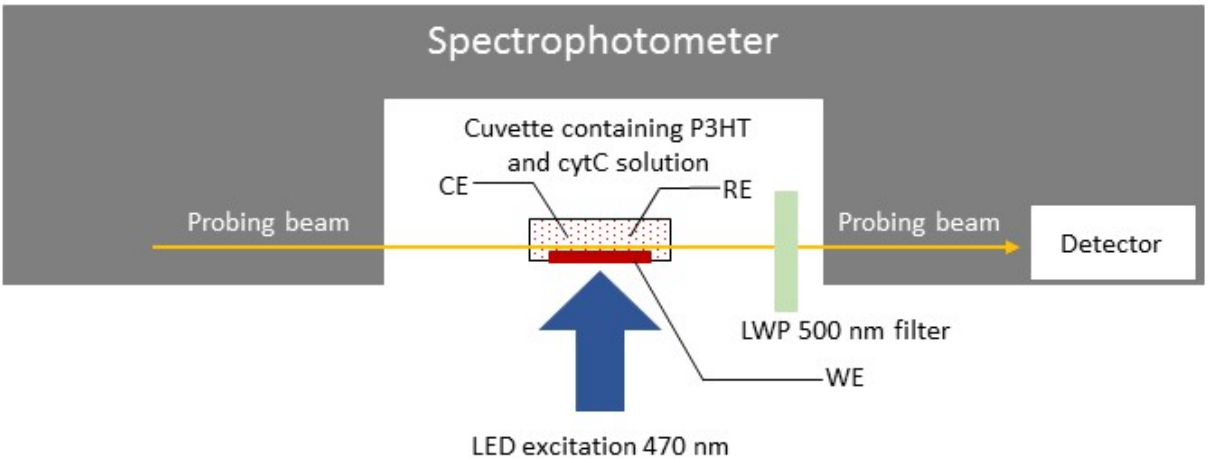

Figure S6. Spectroelectrochemical setup scheme (related to Figure 3 and Figure 4)**.** A cuvette containing ITO/P3HT thin film in contact with a solution containing CytC 10 μM dissolved in phosphate buffer (4 mM, pH 7.4) was positioned through the optical path of the probing beam of the spectrophotometer. The beam was carefully aligned with the cuvette, in order to avoid any interference with the polymer surface (i.e. with the working electrode, WE). The counter and the reference electrodes (CE and RE, respectively) were positioned far from the probing beam. A 470 nm LED was employed to photoexcite the polymer, the excitation optical path being orthogonal to the probing beam. A Long Wave Pass (LWP) filter at 500 nm was positioned at the entrance slit of the detector to avoid the cross talk between probing and excitation beam in the detection phase.

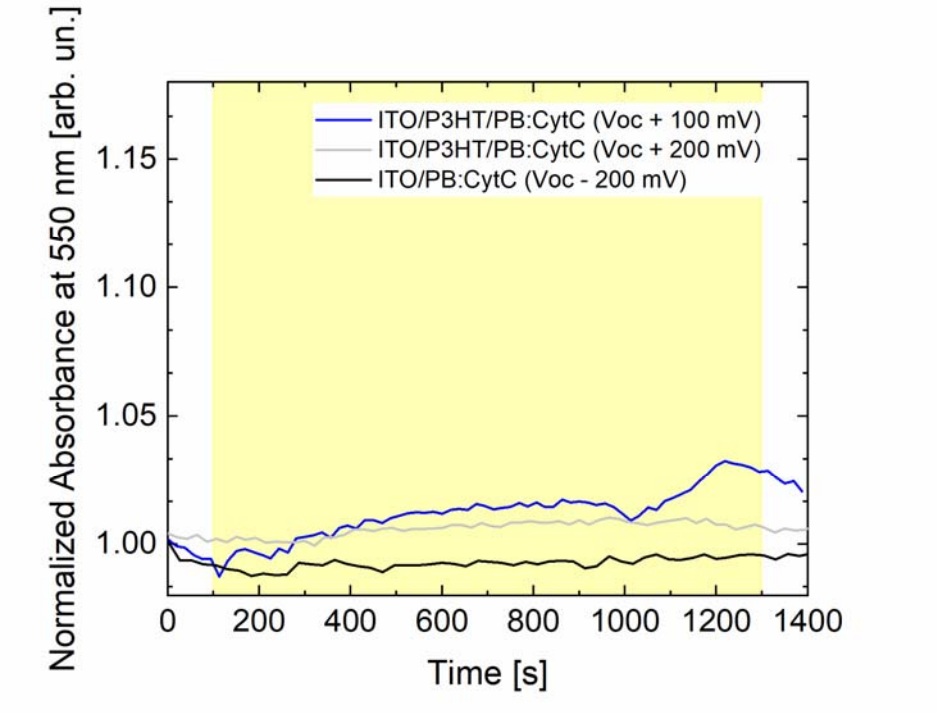

Figure S7. **550 nm peak variation of CytC upon positive biases** (Related to Figure 3). Relative temporal variation of the CytC absorbance peak at 550 nm peak, upon polarization and optical excitation of the ITO/P3HT working electrode, at positive (V_{OC} + 100 mV and V_{OC} + 200 mV, blue and grey lines respectively) and negative bias (V_{OC} – 200 mV). Bare ITO was used as a control substrate. The yellow shaded area shows the illumination period.

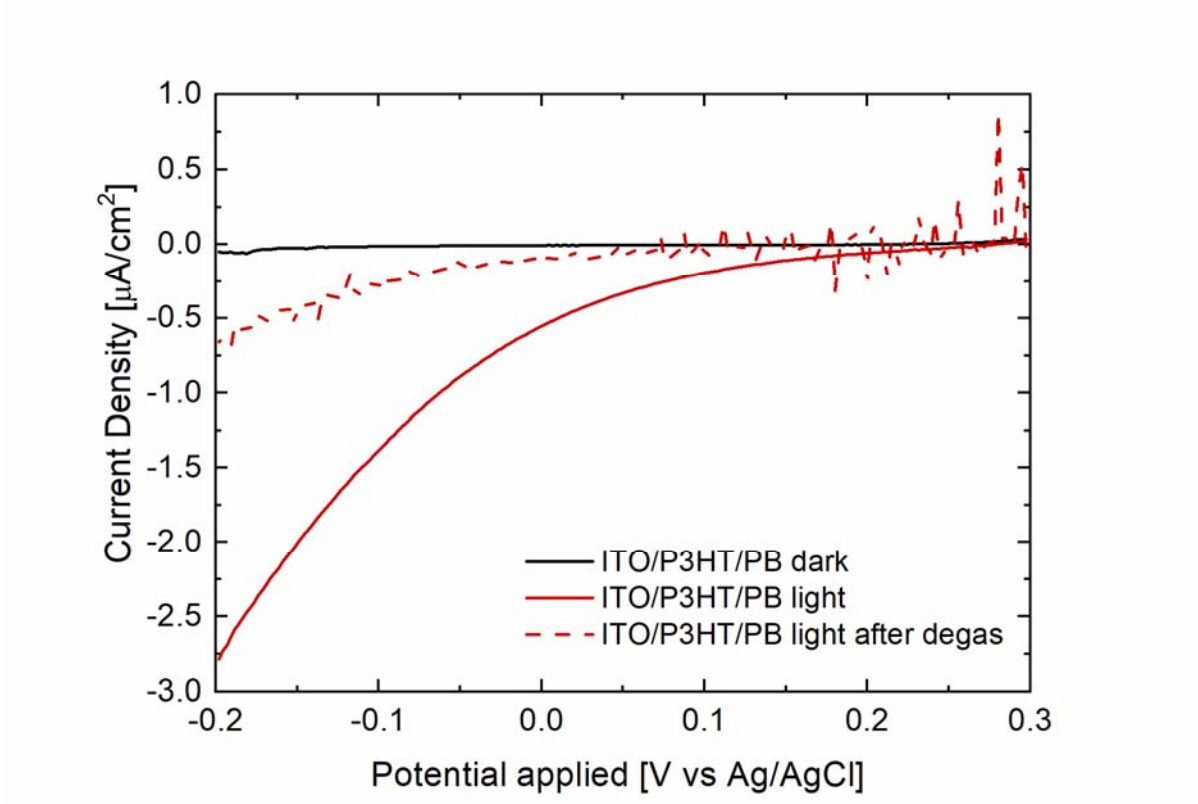

Figure S8. Linear Sweep Voltammetry of ITO/P3HT device upon nitrogen purging (Related to Figure 4). Linear sweep voltammetry (LSV) of ITO/P3HT working electrode recorded in a three electrodes electrochemical cell. LSVs were recorded in phosphate buffer in dark condition (black solid line), upon optical excitation of the ITO/P3HT electrode (red solid line) and upon removal of oxygen from the solution (red dashed line).

Transparent Methods

1 Sample preparation.

Poly-3-Hexyl-Thiophene (P3HT) polymer (15000-45000 MW, Sigma Aldrich) was dissolved in Chlorobenzene (Sigma Aldrich) up to a final concentration of 20 mg/mL. The solution was stirred at 65 °C for 6 hours. Indium-Tin-Oxide (ITO)/glass substrates (XynYan Technology, 15 nm thickness, sheet resistance 15 ohm/sq) were subsequently sonicated in water, acetone, isopropanol (10 minutes each). P3HT solution was spin coated (1500 rpm for 1 minute) on the ITO slabs, obtaining a final thickness of 130 nm and an optical density of 0.6 (at the main absorption peak).

Electrochemical measurements.

Chronoamperometry (CA), cyclic voltammetry (CV) and linear sweep voltammetry (LSV) measurements were carried out with a potentiostat (PGSTAT 302N, AutoLab), in a two-compartments, three electrodes photoelectrochemical cell. The counter and the reference electrodes (platinum wire and saturated KCl Ag/AgCl electrode, respectively) were separated from the working electrode (ITO/P3HT electrode) by a saline bridge. Both CAs and CVs were carried out in Phosphate Buffer (PB), pH = 7.4, 4 mM concentration. CytC from equine heart (Sigma Aldrich) was diluted in phosphate buffer (PB) up to a final concentration of 10 μM. The CA measurements were carried out both in dark and upon polymer photoexcitation. For optical excitation a continuous light source (Thorlabs LED M470L3-C5, 470 nm central emission wavelength) was employed, with a power density of 2.7 mW/mm^2. Light was impinging on the ITO side of the working electrode. CA measurements were carried out first at the open circuit potential (V_{oc}), in absence of external bias, and then by applying a potential difference in the range [V_{oc} - 200 mV, V_{oc} + 200 mV], with 100 mV steps. LSV were acquired in similar conditions, at 5 mV/s scan rate and upon external bias in the range [-200 mV, +300 mV]. CA, CVs and LSVs were also carried out in controlled oxygen concentration, by fluxing nitrogen until the dissolved oxygen concentration was reduced by 80% (evaluated at – 200 mV). Data were recorded using NOVA 1.11 software and analysed by OriginPro 8.5.

Spectro-electrochemistry.

Spectro-electrochemical characterization was carried out by combining potentiostat recordings with optical absorbance spectra measurements (Perkin-Elmer Lambda 1050). Upon photoexcitation of the ITO/P3HT

working electrode, both CytC absorption spectrum and CA measurements were simultaneously monitored, either at the equilibrium potential or upon external bias. In this measurement, a single compartment cell was employed. CytC absorption spectrum was probed in the range 520 nm - 560 nm, 2 nm steps. The ITO/P3HT working electrode was optically excited as previously described. P3HT optical excitation and spectrophotometer beam were in orthogonal configuration (see Figure S5 for the configuration sketch). Any interference between the spectrophotometer beam and the electrodes was carefully avoided. The cell was black-shielded and a high-pass filter (500 nm cut frequency, Thorlabs) was employed at the entrance of the detector. The absorbance variations have been calculated as the difference between the CytC absorbance before and after the illumination protocol, normalized to the absorbance before the stimulus ($\Delta\alpha = (\alpha_{post} - \alpha_{pre})/ \alpha_{pre}$). Spectroelectrochemical measurements were carried out also upon controlled oxygen atmosphere, by fluxing nitrogen until the dissolved oxygen concentration was reduced by 80%. Electrochemical data were recorded with NOVA 1.11, optical data with UV-VIS WinLab Perkin Elmer software, and then merged together with OriginPro 8.5.

Photocurrent action spectrum.

Photocurrent action spectrum was recorded by employing a single compartment, two electrodes electrochemical cell, in which the ITO/P3HT slab acted as the working electrode and a platinum wire was used as the counter electrode. A tungsten light source, filtered by a double grating monochromator and focused on the sample by a spherical mirror, was used for polymer photoexcitation. Photocurrent spectrum was detected by a lock-in technique, at a modulation frequency of 570 Hz. System response (light source emission spectrum, gratings responsivity, optical components) was measured by using a calibrated Si photodiode and was taken into account to properly normalize the spectra. Data were analysed with OriginPro 8.5 software.

Scanning electrochemical microscopy.

Scanning electrochemical microscopy (SECM) studies were carried out both on ITO/P3HT and ITO electrodes. Substrates were placed at the bottom of a Petri dish using Gel-Film (WF-35-X8-A, Gel-Pak). 4 mM phosphate-buffer was employed, with or without 10 μM CytC. The polymer was photostimulated through a mercury lamp of a Nikon Eclipse Ti inverted microscope, filtered with a Nikon Texas Red HYQ cubic filter (excitation wavelength range, 532-587 nm, emission wavelength range, 608-683 nm). The power density of

the photoexcitation source, measured at 550 nm with an optometer at its focal plane, is 70 mW/mm^2. All SECM measurements were carried out with a CHI910B SECM bipotentiostat from CH Instruments Inc. (Austin, Texas), employing an Ag/AgCl (KCl 3 M) as reference electrode and a platinum wire as counter electrode. Platinum microelectrodes (CHI Pt 10 μm diameter, RG 10) were employed as working electrodes; ultramicroelectrodes were modified with nanoporous black platinum by electrodeposition, as previously described, in order to increase the sensitivity towards hydrogen peroxide. The approach curves were carried out by applying a bias of 0.6 V or -0.7 V (for the positioning of the ultramicroelectrode at 25 μm from the substrate) vs Ag/AgCl (KCl 3 M) at the microelectrode. The current signals reported in the SECM approach curves were normalized to the i_{inf} value, namely the bulk current estimated applying 0.6 V vs Ag/AgCl (KCl 3 M) at a distance higher or equal to 350 μm. Scan lines were carried out in constant height mode. Fitting of decay kinetics of Figure 1D were performed by least-squares minimization routines based on grid search algorithms, using a *in house* developed software. Figure presentation: an offset at 25 s after switching on the illumination has been applied to time coarse of hydrogen peroxide production (Figure 1C), to compare kinetics in presence or absence of CytC, thus eliminating contributions due to the fouling of the black platinum microelectrode.