Electronic Supplementary Material (ESI) for Journal of Materials Chemistry C.

Supporting information

Bioconjugation of hydrogen-bonded organic semiconductors with functional proteins

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S1 FTIR spectroscopic characterization

Untreated QNC and EPI characterization

Figure S1.1 shows FTIR spectra of quinacridone and epindolidione films. These were acquired with a Perkin Elmer Spectrum One FTIR spectrometer equipped with the specular reflection device *AmplyfIR*. The spectral resolution used for all measurements was 4 cm^{-1}. The background spectrum was acquired using a neat ITO slide. Peak assignment is reported in table S1.1.

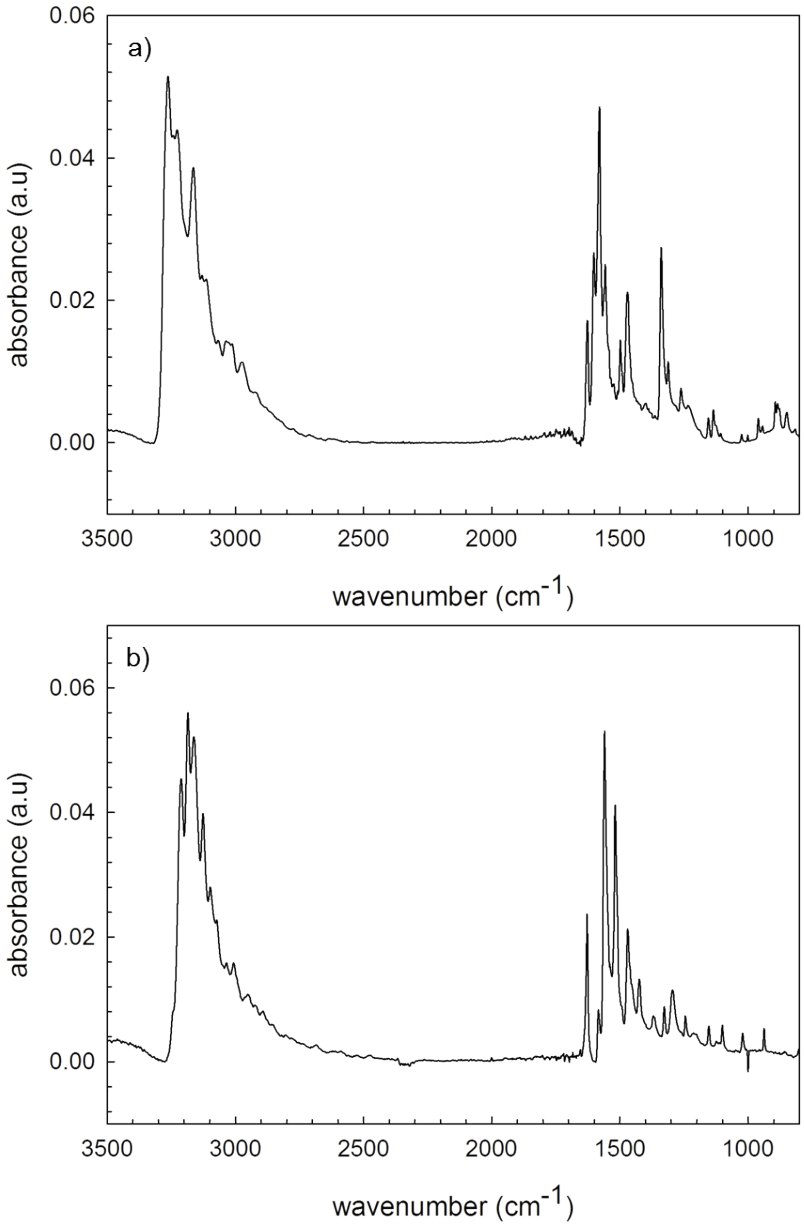

Fig. S1.1 FT-AmplifIR spectra of pristine quinacridone (a) and epindolidione (b) films.

Quinacridone wavenumber (cm^{-1})	Epindolidione wavenumber (cm^{-1})	Peak attribution
3267 (s)	3215 (s)	ν_s NH
3169 (s)	3164 (s)	ν_s NH
2923-3130 (b)	2930-3110 (b)	ν_s CH_{arom}
1625 (s)	1628 (s)	ν_{as} C=O
1603 (s)		ν C=C, δ NH
1582 (s)	1585 (m)	ν C=C, δ NH
1558 (s)	1560 (s)	δNH, ν C=C
1498 (m)	1519 (s)	ν C=C, δ CH
1470 (s)	1470 (m)	ν C=C, δ CH
	1428 (m)	ν C-H
1339 (b)	1329 (b)	ν C-C
1311 (b)	1300 (b)	δ NH, δ CH_{arom}
1262 (b)	1246 (b)	ν C-N, δ CH_{arom}
1135 (b)	1156 (b)	δ CH_{arom}
960 (b)	940 (b)	δ C-C-C_{arom}, δ CH
893 (b)		δ C-C-C_{arom}

ν stretching, δ deformation, s symmetric, as asymmetric; s strong, m medium, b broad

Table S1.1. IR peak attribution of quinacridone and epindolidione films by Spectrum v5.0.1 software (Perkin Elmer)

SUB-treated QNC and EPI characterization

Figure S1.2 shows a detail of SUB-treated epindolidione film FTIR spectrum after baseline correction. The broad peak at around 1725 cm^{-1} can be deconvoluted in two distinguished peaks: the one at 1729 cm^{-1} correspond to ν (C=O) ester vibration of the succinimidyl ring present at one end of SUB linker, while the band at 1700 cm^{-1} can be attributed to the amide binding between SUB linker ring and the amino groups of the organic semiconductors.

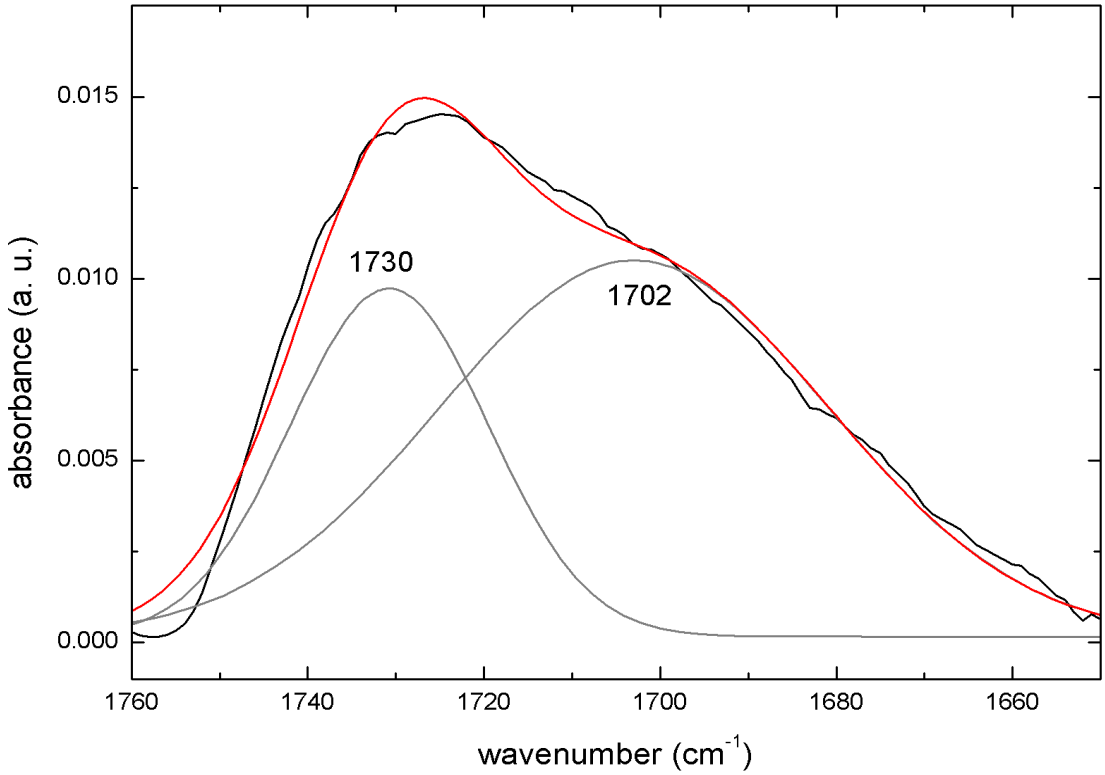

Figure S1.2. Deconvolution of carbonyl IR peaks of suberate after linking to semiconductor surfaces (epindolidione case is shown in this figure). Deconvolution was performed with Origin software after baseline correction.

S2 Cytochrome assay on washing solutions of RC-treated EPI and QNC

Experimental procedure

RC-modified (bioconjugated or physisorbed) semiconductor samples on ITO slides were immersed in a 1.2 mL cuvette filled with 0.8 mL of PBS with LDAO 0.025 mL for 1 minute and then removed. Reduced horse heart cytochrome c (5 µM) and decylubiquinone (50 µM) were subsequently added to this washing solution. The rate of photoinduced cytochrome oxidation was determined following the absorbance change at 551 nm upon illumination for 3 minutes with a red-filtered light. The measured rate was correlated to the RC concentration by a previously built calibration curve using known amounts of RC. This procedure was repeated three times for evaluating the protein release in subsequent washing steps. This cytochrome assay was found to quantify RC up to a detection limit of 5 nM, about 100-fold more sensible of a direct RC absorbance quantification.

Discussion

The cytochrome oxidation kinetic in RC photocycle was employed to detect the removal of the protein from the incubated substrates after rinsing in phosphate-buffered saline. In figure S2.1 the kinetic traces recorded after each washing step for RC-physisorbed (upper panels) and bioconjugated (bottom panels) samples are shown, the first ones used as control test.

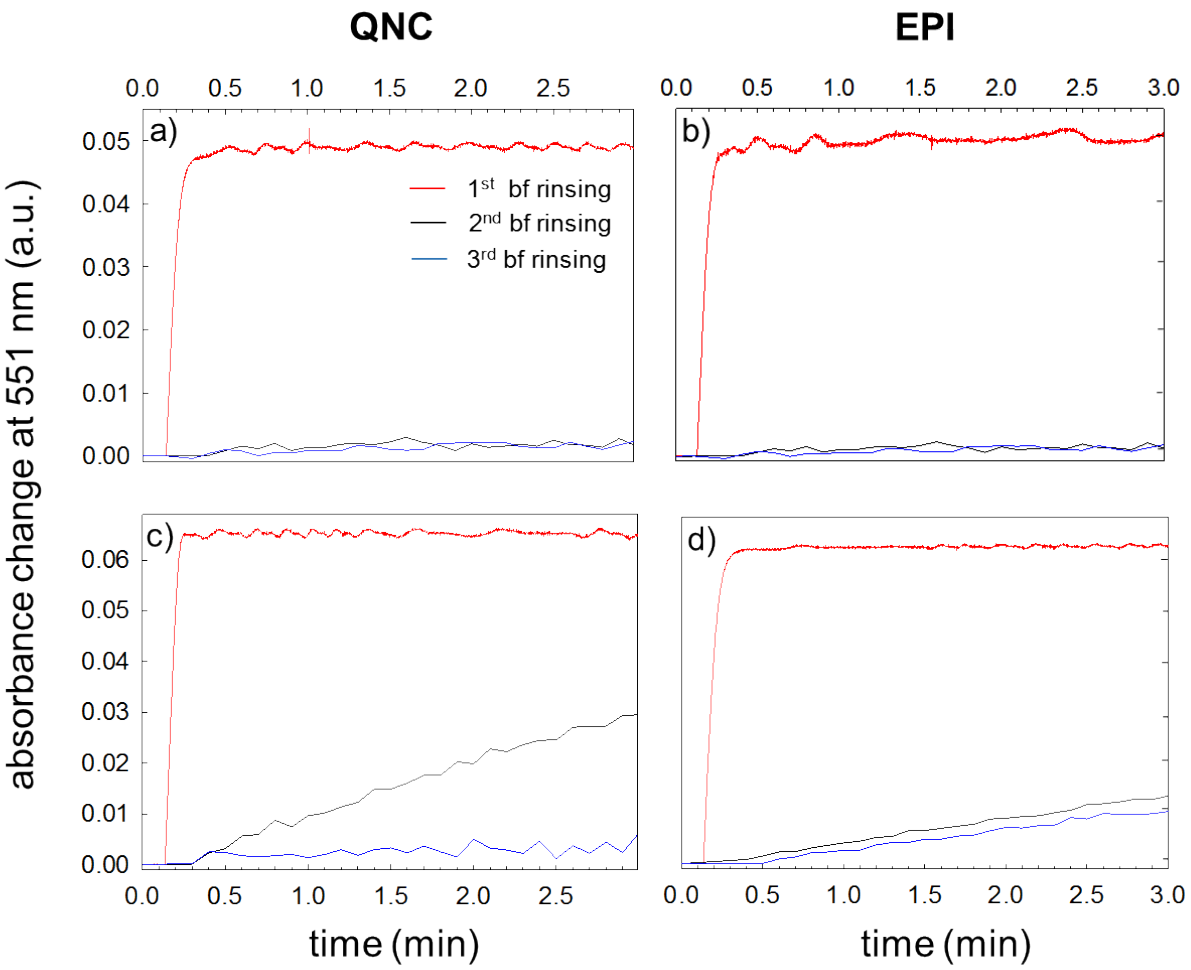

Fig. S2.1 Time courses of cytochrome oxidation evaluated as absorbance change at 551 nm after each rinsing step with PBS (bf.) for physisorbed RC-quinacridone (a), physisorbed RC-epindolidione (b), bioconjugated RC-quinacridone (c) and bioconjugated RC-epindolidione (d). Experimental: each slide immersed in 1 mL of 5 µM cytochrome c^{2+}, 50 µM decylubiquinone in PBS; excitation by a red-filtered light for 3 minutes.

According to both bioconjugation and physisorption protocols, the total RC amount cast onto each slide was 4×10^{-10} mol: since 1 mL of the phosphate buffer was used for the washing procedure, a concentration of 400 nM is expected in the case of complete protein removal. Table S2.1 indeed shows that for physisorbed samples the first washing step removes all the RC from the substrates, since the protein amount contained into the second washing solution is below the detection limit (1 nM) for the used assay. This rules out any adventitious RC interaction with the organic semiconductors. Conversely for bioconjugated samples (table S2.2) a more gradual removal of unbound RC from semiconductor surfaces, especially for quinacridone, can be observed. These findings can be explained by hypothesizing the generation of a covalently linked protein layer which favors hydrophobic aspecific protein-protein interaction with unbound RC, making harsher the removal of unbioconjugated excess. In the optimized bioconjugation protocol, RC-modified semiconductor substrates are rinsed four times with buffer and DI water to ensure the unreacted protein excess removal.

Table S2.1 Unbound-RC amounts in the three washing solutions of RC-physisorbed QNC and EPI samples corresponding to figure S2.1 a) and b) respectively. Each value was determined from experimental oxidation rates, by a previously built calibration curve using known amounts of RC.

	a) RC-physisorbed QNC (nM)	b) RC-physisorbed EPI (nM)
1st bf rinsing	410 ± 20	390 ± 20

2nd bf rinsing	n.d.	n.d.
3rd bf rinsing	n.d.	n.d.

n.d. values are below the determined limit of detection (LOD)

Table S2.2 Unbound-RC amounts in the three washing solutions of RC-bioconjugated QNC and EPI samples corresponding to figure S2.1 c) and d) respectively. Each value was determined from experimental oxidation rates, by a previously built calibration curve using known amounts of RC.

	c) RC-bioconjugated QNC (nM)	d) RC-bioconjugated EPI (nM)
1st bf rinsing	400 ± 20	370 ± 20
2nd bf rinsing	7.2 ± 1.3	2.5 ± 1.3
3rd bf rinsing	n.d.	2.1 ± 1.3

n.d values are below the determined limit of detection (LOD)

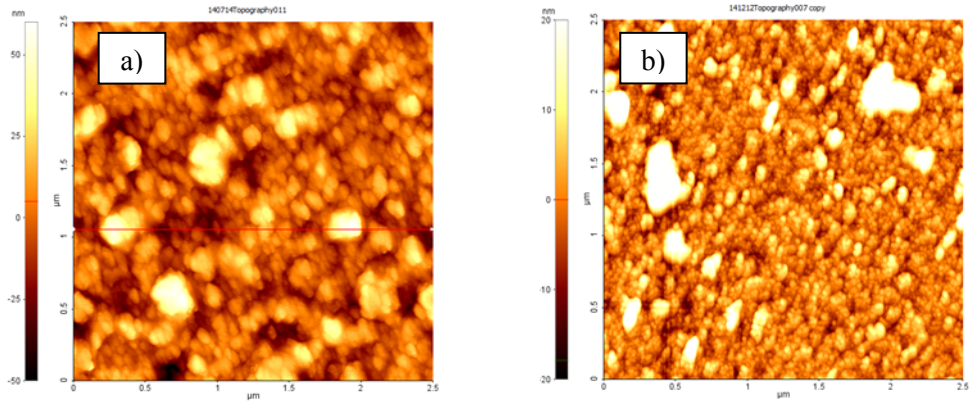

Fig. S3 a) Quinacridone after treatment with SUB solution, followed by washing and 4 hours dipping in RCs solution; b) same treatment on epindolidione.

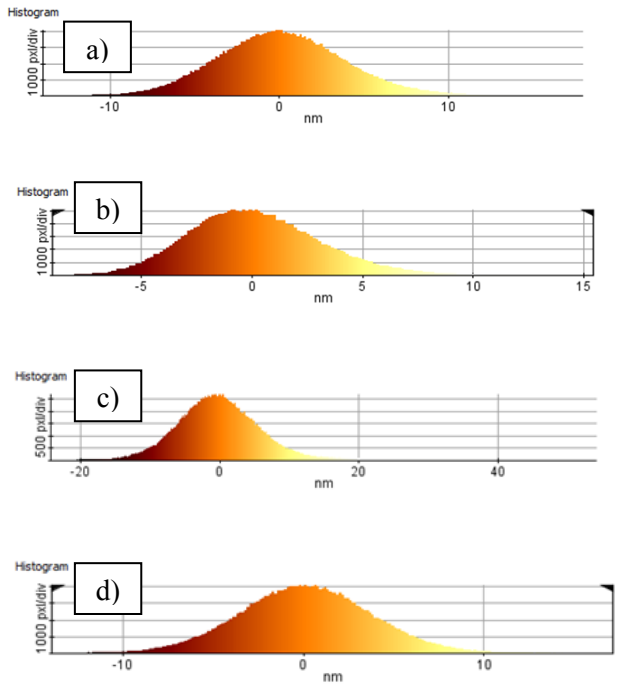

Fig. S4 Height Distribution histograms for QNC films: a) as evaporated; b) after SUB functionalization; c) after functionalization with SUB and bacterial reaction center; d) after contact with bacterial reaction center solution and washing away physisorbed material.

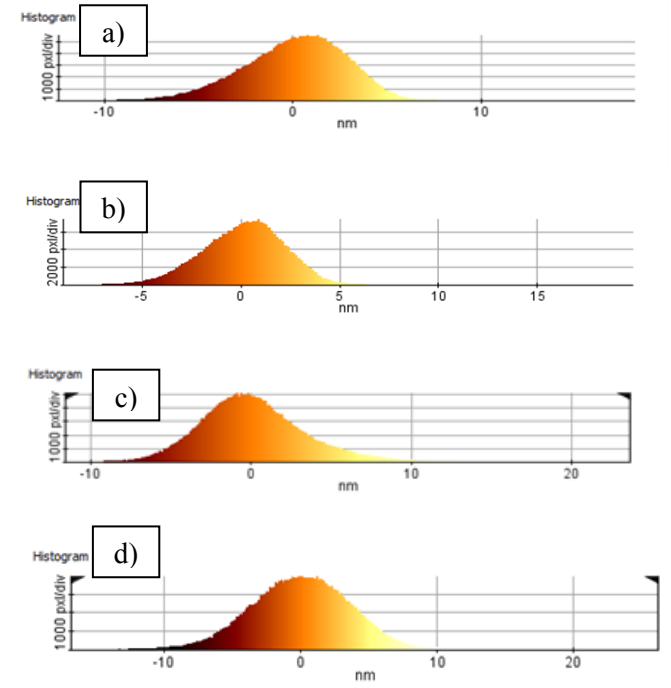

Fig. S5 Height Distribution histograms for EPI films: a) as evaporated; b) after SUB functionalization; c) after functionalization with SUB and bacterial reaction center; d) after contact with bacterial reaction center solution and washing away physisorbed material.

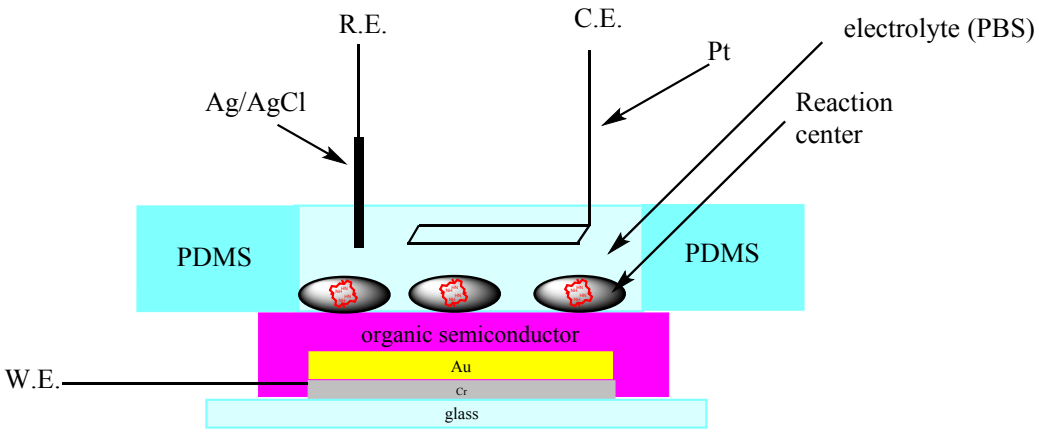

Fig. S6 Set-up for (photo)electrochemistry of the RC on the HBP films. Ag/AgCl was used as the quasi-reference electrode, a Platinum counter electrode was used, while the semiconductor-covered gold film functioned as the working electrode. The gold film was prepared by evaporating a 2-mm wide stripe of 70 nm Au on glass, using 2 nm chromium as a sticking layer. The working electrode area was 0.04 cm^2, and was defined by a poly(dimethylsiloxane) block with a molded hole in it. This PDMS block served to confine a given electrolyte volume (~50 µl) over the working electrode. Contacting the working electrode, and reference and counter electrodes were manipulation was carried out using a modified microscope probe station.

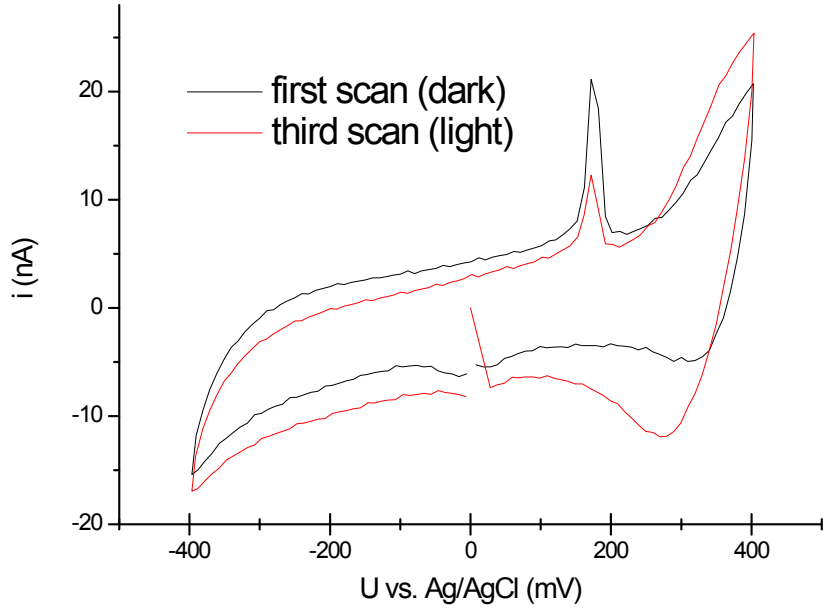

Figure S7. CV measured on the setup shown in S6, in the dark and under illumination, λ > 600 nm. Scan rate = 50 mV/s.

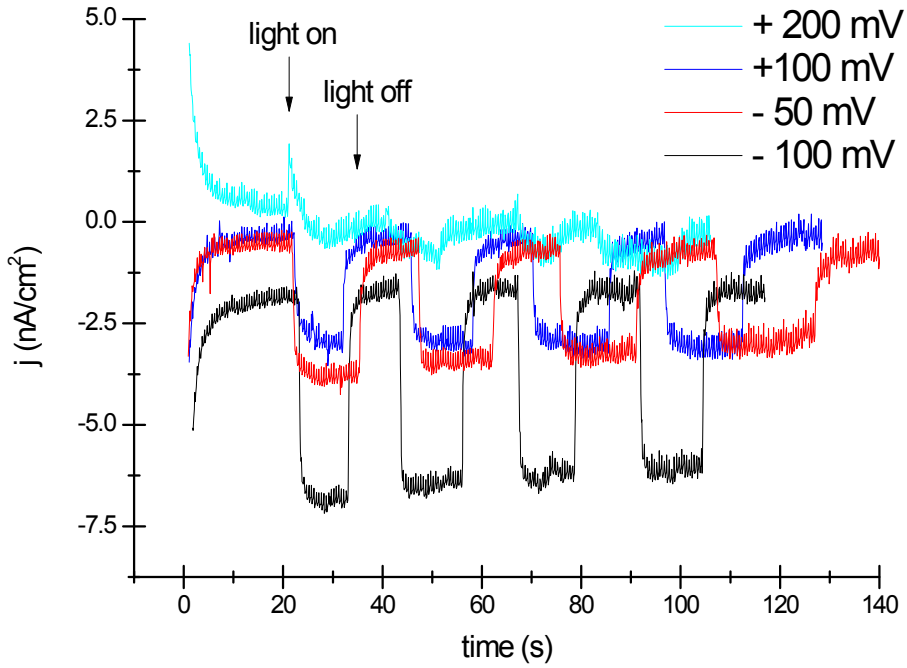

Fig. S8. Chronoamperometry at different working electrode potentials for immobilized RC on EPI electrodes, measured as shown in Fig. S6. Light with λ> 600 nm was turned on for 10-second periods. Scans for different working electrode potentials are shown. Up to +200 mV, a photocathodic current is observed upon illumination.

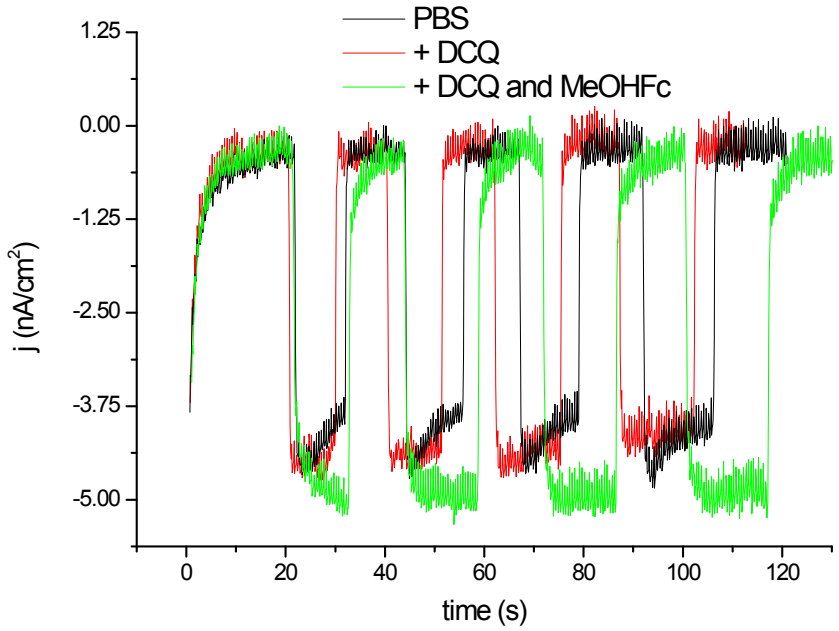

Fig. S9. Dark/light current recorded at 0V vs. Ag/AgCl for EPI with immobilized RC as a function of presence of sacrificial electron donor (Ferrocenemethanol) and electron acceptor (decylubiquinone). Light was turned on for ≈10s. Addition of both electron donor and acceptor to the electrolyte reliably led to larger photocurrents, but compared with plain PBS solution, the enhancement was very small.

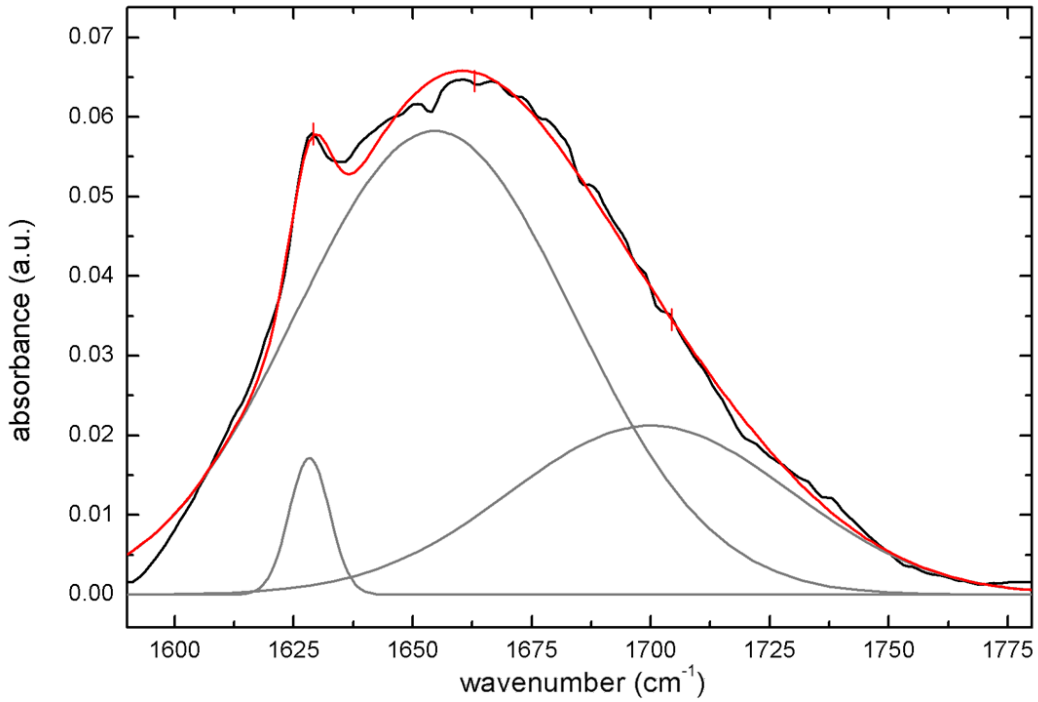

Figure S10. Deconvolution of ureoid carbonyl stretching IR peak of B7 after linking to semiconductor surfaces. Deconvolution was performed with Origin software after baseline correction.

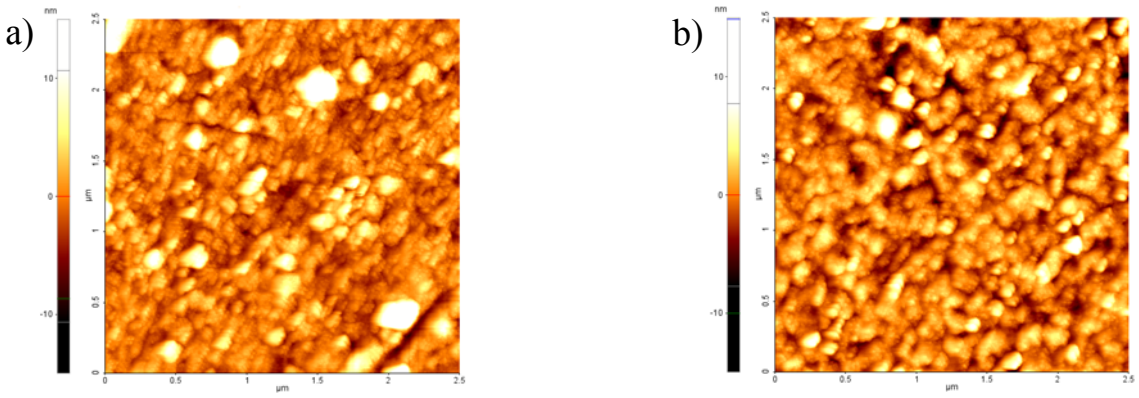

Fig. S11 a) Quinacridone after treatment with B7 solution, followed by washing and 20 minutes dipping in SA solution; b) same treatment on epindolidione.

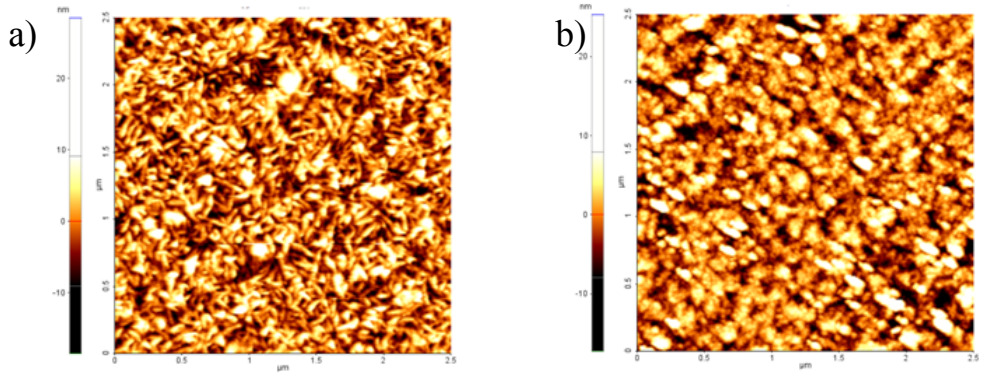

Fig. S12 a) Quinacridone after 20 minutes dipping in SA solution and washing away physisorbed protein. RMS 4.3±0.5 nm; b) same treatment on epindolidione. RMS 4.6±0.6 nm.

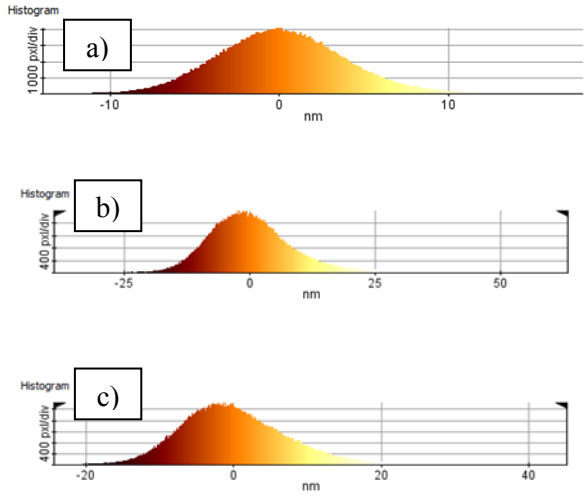

Fig. S13 Height Distribution histograms for QNC films: a) as evaporated; b) after B7 functionalization; c) after contact with streptavidin solution and washing away physisorbed material.

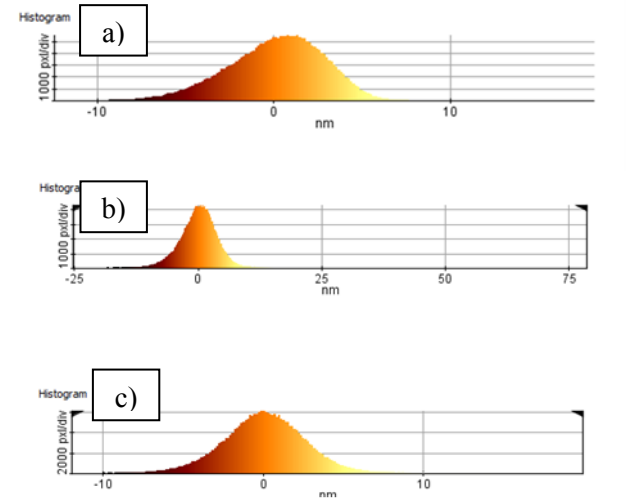

Fig. S14 Height Distribution histograms for EPI films: a) as evaporated; b) after B7 functionalization; c) after contact with streptavidin solution and washing away physisorbed material.

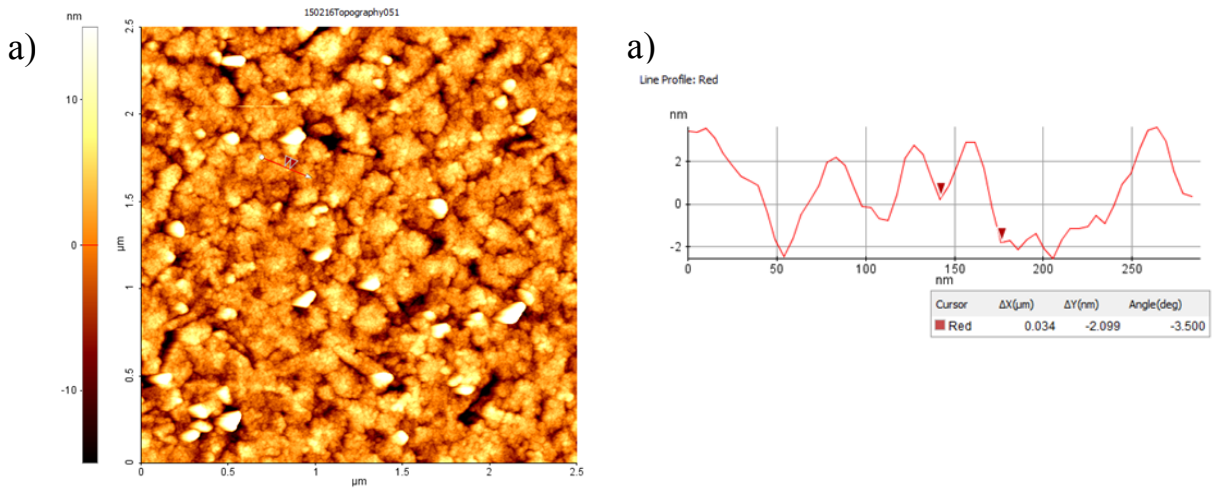

Fig. S15 a) SA bioconjugate on EPI and b) a segment of height profile evidencing the presence of small aggregates on the surface of EPI.