Supporting Information

Supramolecular Biohybrid Construct for Photoconversion Based on a Bacterial Reaction Center Covalently Bound to Cytochrome *c* by an Organic Light Harvesting Bridge.

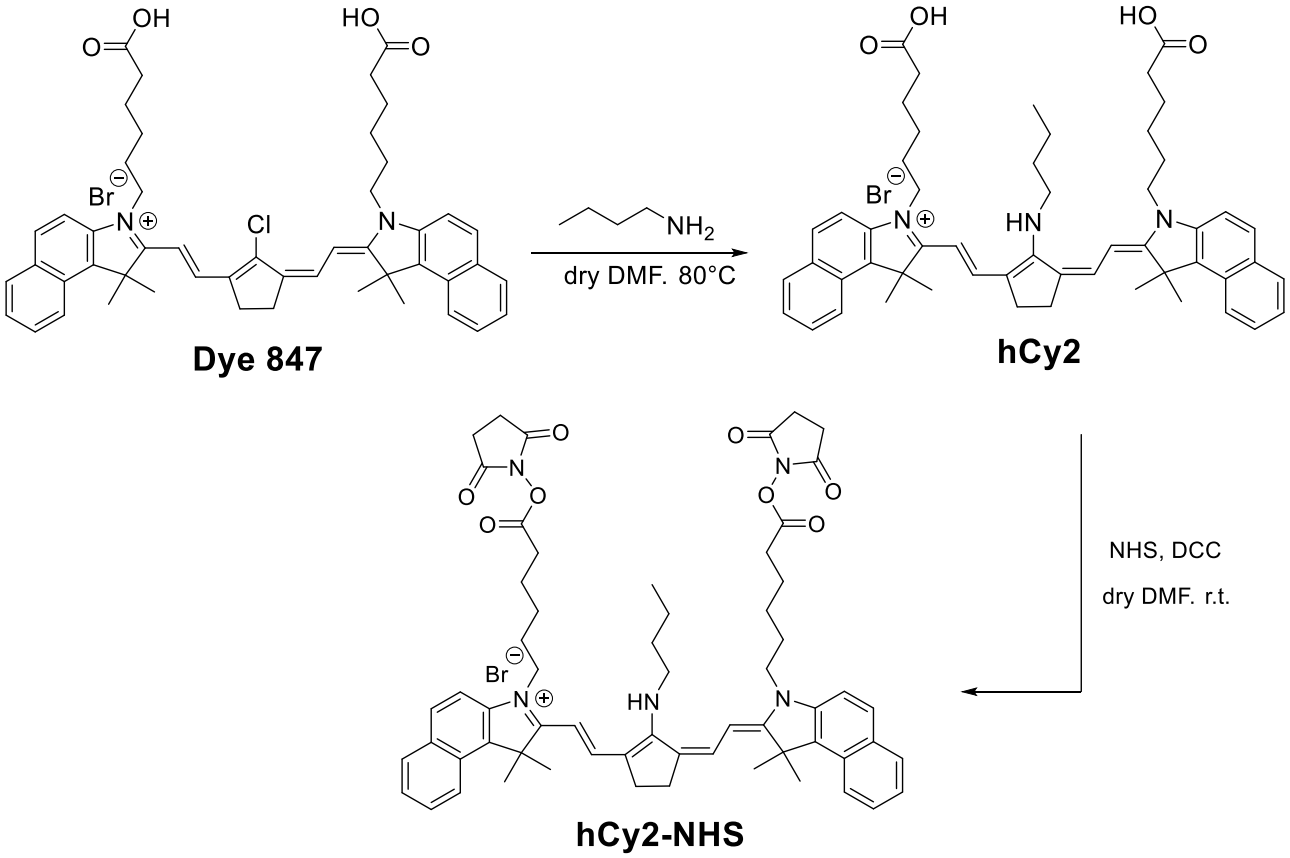
Gabriella Buscemi[†], Massimo Trotta[§], Danilo Vona[†], Gianluca M. Farinola[†], Francesco Milano[‡,*] and Roberta Ragni[†,*].

[†]Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Via Orabona, 4, 70126 Bari, Italy.

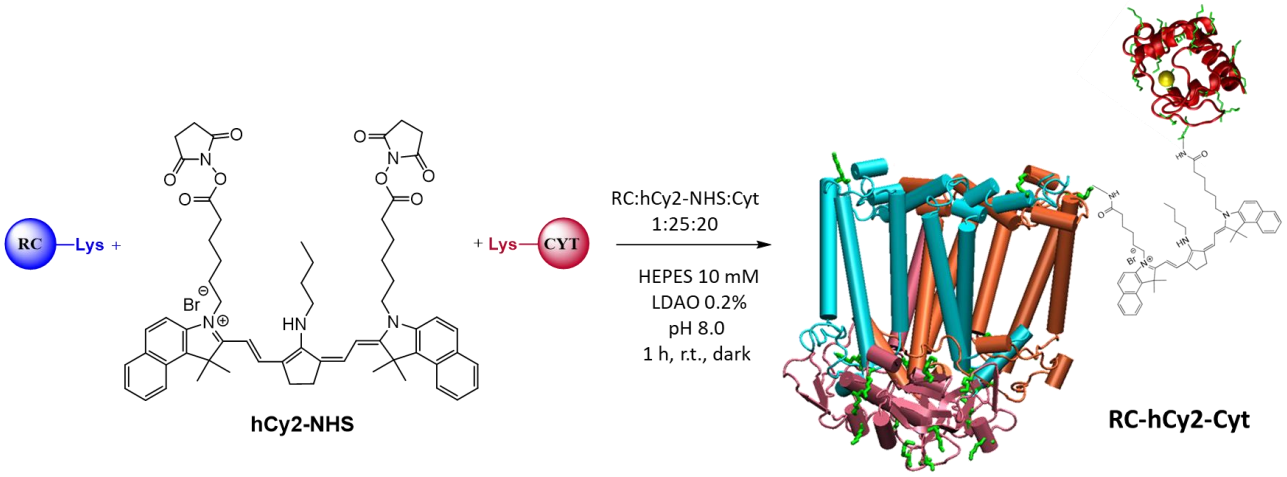
[§]Istituto per i Processi Chimico Fisici, Consiglio Nazionale delle Ricerche (CNR-IPCF), Via Orabona, 4, 70126 Bari, Italy.

[‡]Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche (CNR-ISPA), Via P.le Lecce-Monteroni, 73100 Lecce, Italy

Corresponding Authors: francesco.milano@cnr.it; roberta.ragni@uniba.it



Scheme S1: Synthesis of hCy2 and hCy2-NHS.



Scheme S2 Synthesis of RC-hCy2-Cyt c biohybrid.

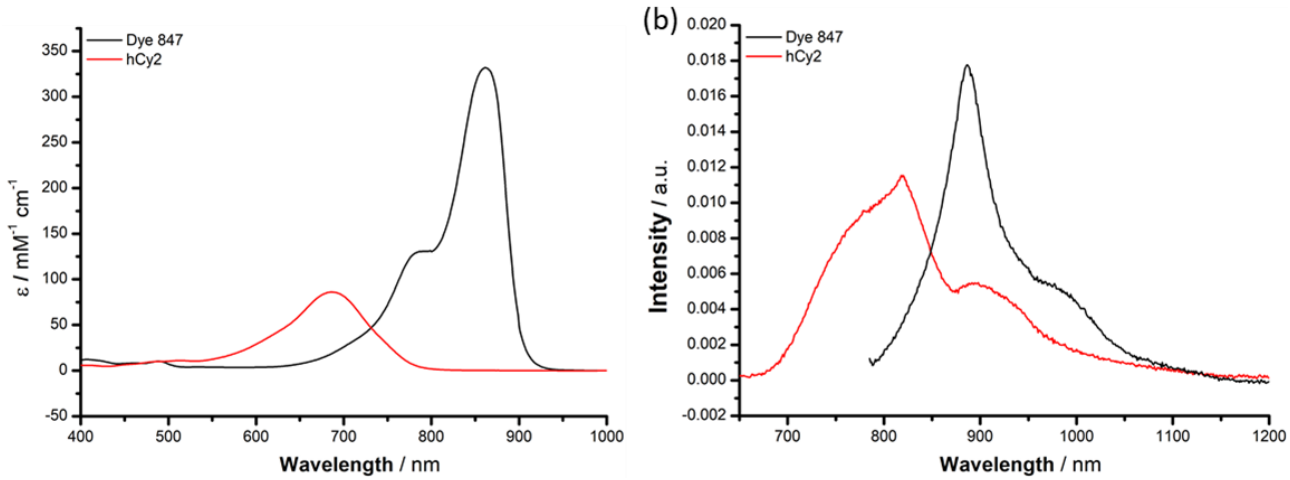


Figure S1 (a) Absorption spectra in $H_{10}L_{0.2}$ of Dye847 (black) and hCy2 (red); (b) Emission spectra in $H_{10}L_{0.2}$ of Dye847 (black, λ_{exc} = 775), hCy2 (red , λ_{exc} = 680).

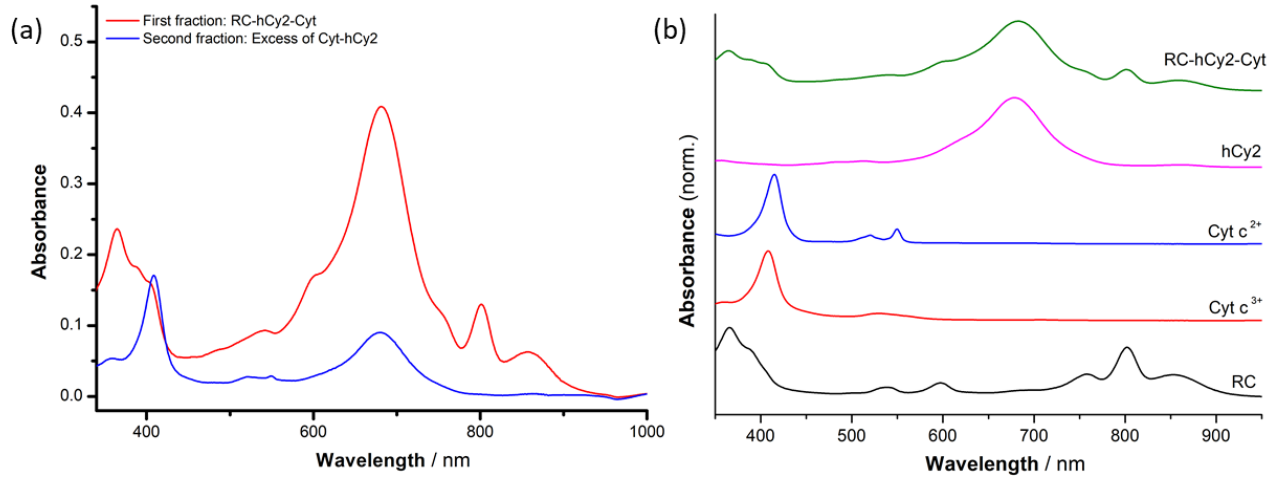


Figure S2: (a) Absorption spectra of the first (red) and second (blue) fractions eluted from the size exclusion chromatographic column; (b) Absorption (arbitrary scale) spectra of RC (black), Cyt c^{3+} (red) Cyt c^{2+} (blue), hCy2 (magenta) and the RC-hCy2-Cyt c biohybrid (green).

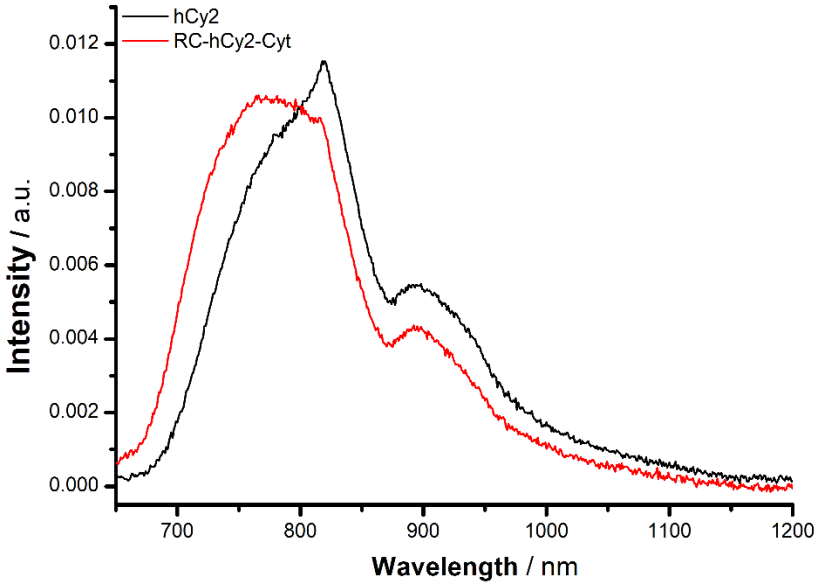


Figure S3: Emission spectra of hCy2 (black) and RC-hCy2-Cyt c (red) in $H_{10}L_{0.2}$ using λ_{exc} = 680.

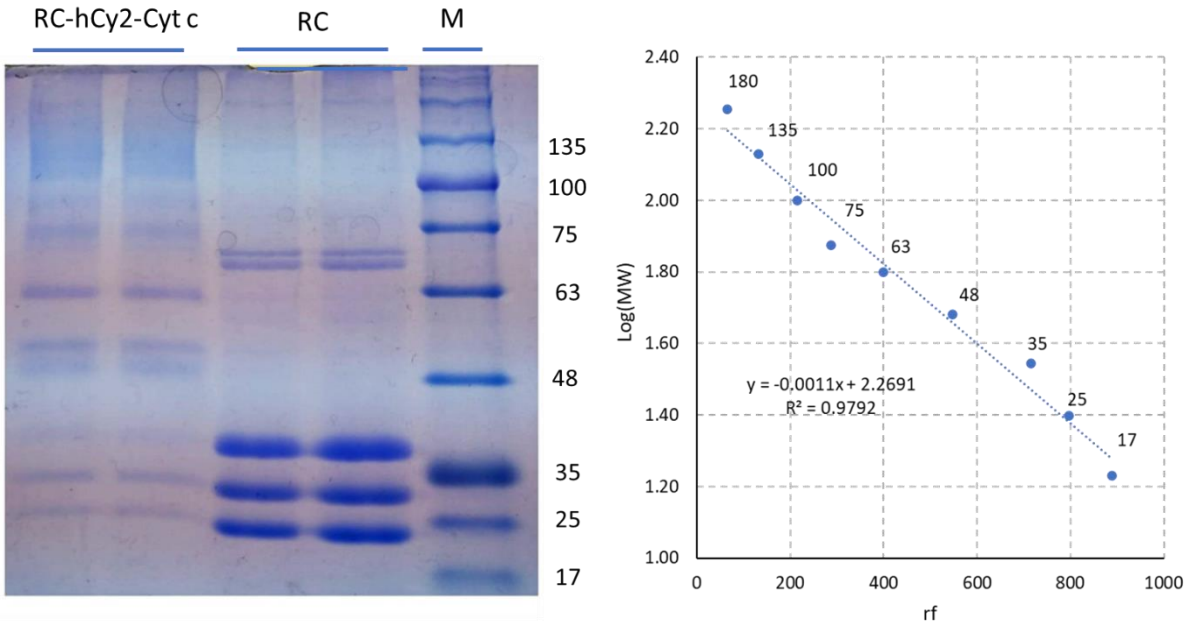


Figure S4: Left panel, SDS-PAGE gel electrophoresis of RC-hCy2-Cyt c, RC and MW markers: 20 µg were loaded for both RC and biohybrid. Right panel: calibration curve reporting the logarithm of molecular weight of markers bands versus the relative migration distance R_f.

The exact masses of the H, M and L subunits are 28, 34 and 31 KDa, respectively.[1] However, literature reports that the hydrophobic M and L subunits undergo an increase of the electrophoretic mobilities, reaching apparent MW values of 23 and 21 KDa respectively.[2] In our gel, the apparent MW of H, M and L are slightly higher both in the native RC and in the biohybrid as shown in Table S1. These discrepancies are due to the well-known difficulty of denaturing membrane-bound proteins and to the different amount of SDS molecules that proteins can bind.[3] Moreover, two bands at 76 and 89 kDa are observed for RC, likely related to residual non-denatured RC. At least four additional weak bands at 48, 53, 67, 88 kDa can be distinguished in the biohybrid lanes and, although their attribution to specific biohybrid structures would be questionable, their presence supports that bioconjugation occurred involving all three RC subunits.

Table S1. R_f values and calculated molecular weights of bands detected in the SDS-PAGE of native RC and RC-hCy2-Cyt c.

R_f for RC	**Calculated MW**	**R_f for RC-hCy2-Cyt c**	**Calculated MW**
810	23 (L)	777	25 (L)
754	27 (M)	718	29 (M)
671	33 (H)	670	33 (H)
350	76	524	48
328	80	488	53
		398	67
		290	88

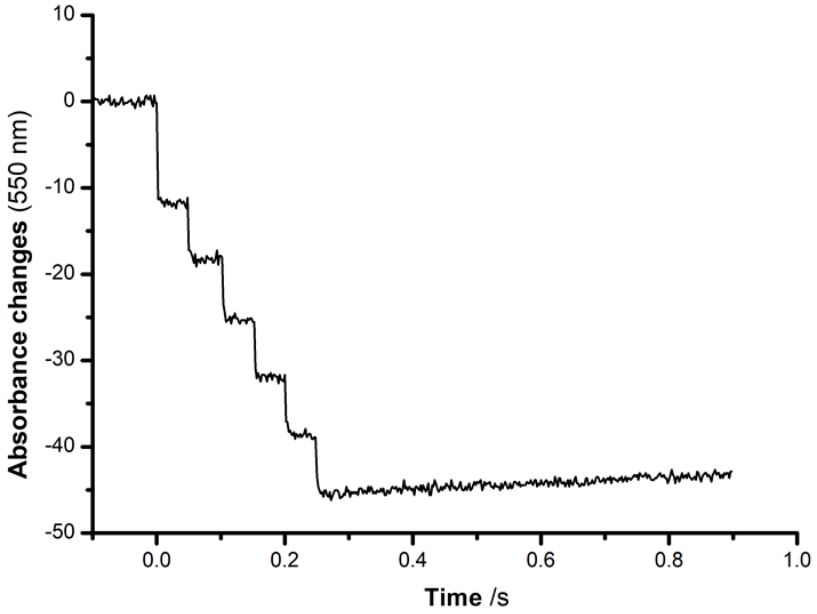


Figure S5: Absorbance changes at 550 nm for 0.8 µM RC in $H_{10}L_{0.2}$ mixed with Cyt c^{2+} (1:20) and dQ 50 µM The sample was flashed 6 times every 50 ms.

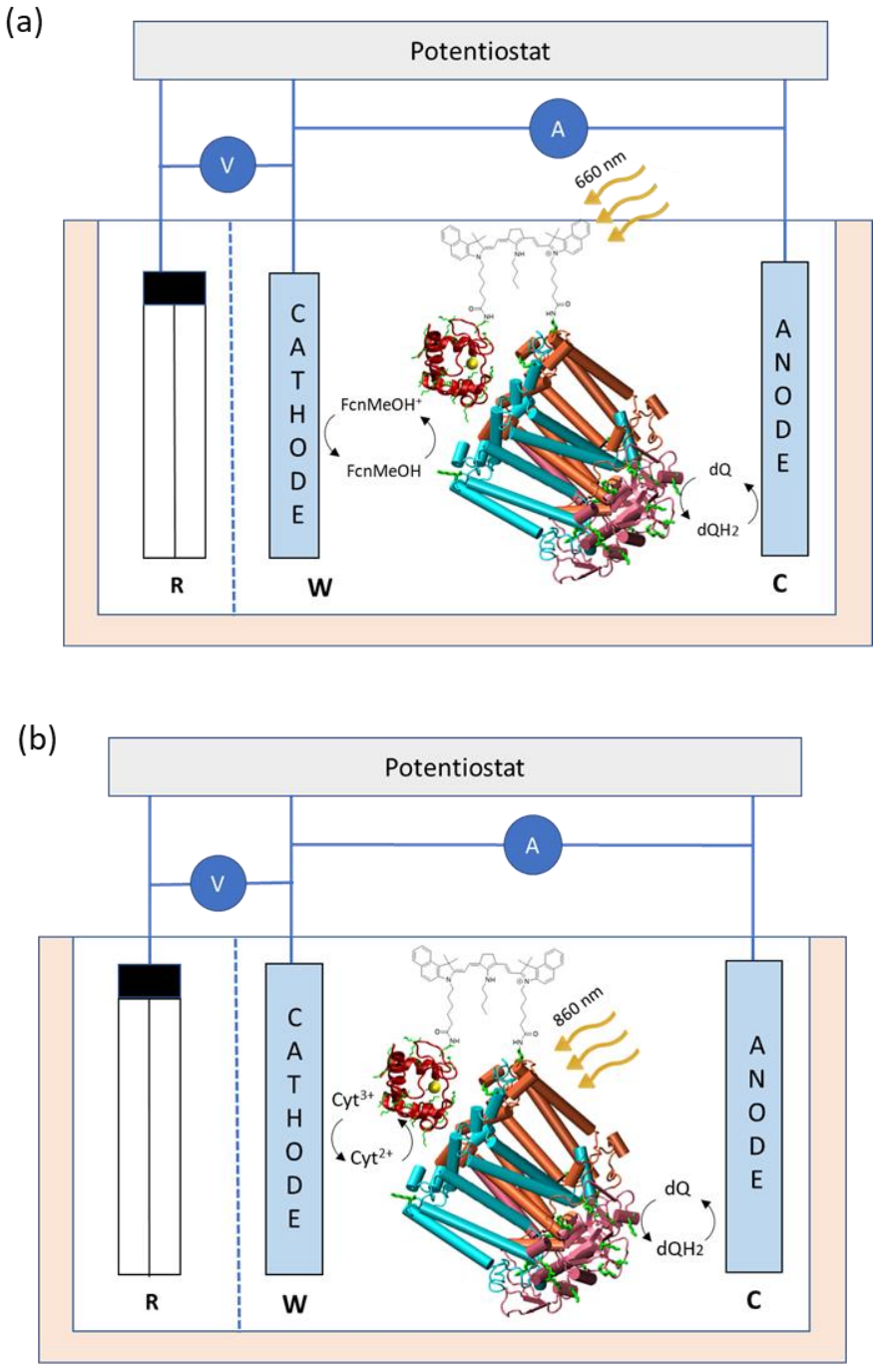


Figure S5: Electrochemical setups used to evaluate (a) the hCy2 antenna effect in RC-hCy2-Cyt c^{3+} and (b) the photocurrent density of RC-hCy2-Cyt c^{2+} or a mixture of RC and Cyt c where cytochrome is used as the electron donor.

Table S2: Kinetic parameters of the charge recombination reaction for RC, a mixture of RC and ascorbate, and RC-hCy2-Cyt c^{3+}.

Parameter	RC	RC+ asc	RC-hCy2-Cyt c^{3+}
ΔA_{tot}	52±1	56±3	52.5±0.1
A_s	22.8±0.9	27±1.0	3.6±0.1
K_s	4.4±0.1	4.7±0.1	0.8±0.1
k_f	10.0	10.0	10.0

References

(1) Williams, J. C.; Steiner, L. A.; Feher, G., Primary structure of the reaction center from Rhodopseudomonas sphaeroides. *Proteins* **1986,** *1* (4), 312-25.
(2) Feher, G., Some chemical and physical properties of a bacterial reaction center particle and its primary photochemical reactants. *Photochem. Photobiol.* **1971,** *14* (3), 373-387.
(3) Rath, A.; Glibowicka, M.; Nadeau, V. G.; Chen, G.; Deber, C. M., Detergent binding explains anomalous SDS-PAGE migration of membrane proteins. *Proceedings of the National Academy of Sciences* **2009,** *106* (6), 1760-1765.