



## Photovoltage generation in enzymatic bio-hybrid architectures

Michele Di Lauro<sup>1</sup>, Gabriella Buscemi<sup>2,3</sup>, Michele Bianchi<sup>1</sup>, Anna De Salvo<sup>1,4</sup>, Marcello Berto<sup>5</sup>, Stefano Carli<sup>1</sup>, Gianluca Maria Farinola<sup>2</sup>, Luciano Fadiga<sup>1,4</sup>, Fabio Biscarini<sup>1,5</sup> and Massimo Trotta<sup>3\*</sup>

<sup>1</sup>Center for Translational Neurophysiology - Istituto Italiano di Tecnologia, Ferrara, Italy

<sup>2</sup>Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Bari, Italy

<sup>3</sup>CNR-IPCF Istituto per i Processi Chimico Fisici, Consiglio Nazionale delle Ricerche, Bari, Italy

<sup>4</sup>Section of Human Physiology University of Ferrara, Ferrara, Italy - <sup>5</sup>Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia, Modena, Italy

### Abstract

*Most of the photochemical activity of bacterial photosynthetic apparatuses occurs in the reaction center, a transmembrane protein complex which converts photons into charge-separated states across the membrane with a quantum yield close to unity, fuelling the metabolism of the organism. Integrating the reaction center from the bacterium Rhodospirillum rubrum onto electroactive surfaces, it is possible to technologically exploit the efficiency of this natural machinery to generate a photovoltage upon Near Infra-Red illumination, which can be used in electronic architectures working in the electrolytic environment such as electrolyte-gated organic transistors and bio-photonic power cells. Here, photovoltage generation in reaction center-based bio-hybrid architectures is investigated by means of chronopotentiometry, isolating the contribution of the functionalisation layers and defining novel surface functionalization strategies for photovoltage tuning.*

### INTRODUCTION:

Evolution has engineered multi-protein complexes [1] adapted to efficiently convert the solar radiation into chemical energy, sustaining all the energy needs of the life on planet Earth via the photosynthetic process. These multi-protein complexes act as photoenzyme, catalyzing the reduction of oxidized chemical species, performing an energetically "uphill conversion" by using visible light as the sole energy source. The enzymatic molecular machineries of photosynthetic organisms share peculiar common fundamental traits [2] that make higher and lower plants, algae, and some specialized bacteria the sole kind of organisms on the planet able to harvest extra-terrestrial energy

and store it, functioning as primary producers. Plants, algae, and cyanobacteria perform a complex set of reactions based on the harmonized function of two photosynthetic units, called Photosystems, which eventually oxidize water to molecular oxygen, while photosynthetic anoxygenic bacteria have developed simpler apparatuses based on a single functional unit. The photochemical cores of these photosynthetic apparatuses, called reaction centers (RCs), fuel the metabolism of the photosynthetic organisms by efficiently converting photons into charge-separated states across the membrane.

*Rhodobacter (R.) sphaeroides* is a deeply investigated purple non-sulphur bacterium often used as model organisms for the molecular mechanisms of photosynthesis. Much is known about the structure of the RCs from *R. sphaeroides* [3], here briefly summarized: the RC is three-subunit transmembrane protein sitting within the photosynthetic membrane. Eight cofactors within the protein scaffold are involved in the electron transfer reactions cascade that forms the hole-electron couple with quantum yields ranging from 0.98 to 1 [4]. In isolated RC, i.e. in absence of exogenous electron donors and acceptors, this state does not evolve further and has a lifetime that ranges from hundred milliseconds up to three seconds depending upon some operational conditions, such as the removal of one cofactor or the reconstitution of the protein in phospholipid bilayer [5].

RCs' high photoconversion efficiency, due to its finely tuned architecture, has spurred numerous attempts to mimic it, leading to the development of photoactive molecules called triads [6], passing through finely engineered molecular architectures [7], up to the very sophisticated design of the artificial leaf [8]. Biohybrids able to respond to light have been proposed basing their effect the light-response of non-photosynthetic artificial photoreceptors [9]. RC, conjugate to or interacting with organic moieties in bio-hybrid systems, has been demonstrated as effective transducer of solar radiation [10-12]. These bio-hybrids have also been demonstrated as materials for bio-optoelectronics [13,14], functionally integrated into devices [15-17] and exploited as active elements in bio-photonic power cells [18]. More recently, RC from *R. sphaeroides* has been deposited on the transparent gate electrode of electrolyte-gated organic transistors obtaining the Light-responsive Electrolyte-Gated Organic Transistor – LEGOT [19]. In LEGOTs, Near Infrared (NIR) illumination results in current amplification due to the generation of an additional DC potential, which may be as high as 60 mV, at the photosensitive gate electrode. This effect is observed only if the transparent Indium Tin Oxide (ITO) gate is covered with cytochrome c prior to RC deposition, as shown in Figure 1.

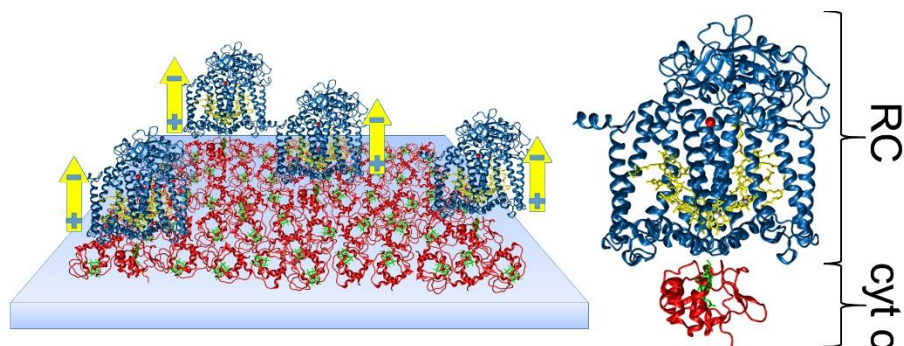


Figure 1. Schematic representation of the biohybrid architecture herein investigated. Oxidized cytochrome c (red-green) is deposited by drop-casting on the ITO surface; the transmembrane photosynthetic reaction center (blue-yellow) is successively deposited by drop-casting on top of the cyt c layer; the orientation of the photoinduced dipole is highlighted (yellow arrow)

The cyt c is a small redox protein analogue to the cyt  $c_2$  that, in its reduced form, acts as physiological electron donor to the positive charge of the hole-electron couple within the RC. For this electron transfer to occur it is necessary that the cyt c binds to a very specific docking site [20] in the periplasmic face of the RC. The docking site is formed independently on the redox state of the cyt [21] hence, in its oxidized form, cyt c can be used to preferentially orient the RC on a substrate via the docking site thereby avoiding any electron transfer. Preferential orientation is critical for the generation of photovoltage since random orientation of RC, and of the photogenerated dipoles, onto the bare ITO would result in net null potential variation [22,23].

In this work, photovoltage generation in such biohybrid electrodic structures is investigated by means of chronopotentiometry, focusing on the voltage contributions of each single layer and on photovoltage dependency on cyt c:RC ratio.

## **MATERIALS AND METHODS:**

### **Reaction center production/extraction:**

The RC was isolated and purified from cultured *Rhodobacter sphaeroides* and its integrity and activity were checked by UV-Vis-NIR spectroscopy and by flash induced absorbance change measurements performed on an instrument of local design [9,24,25].

### **Light-sensitive electrode fabrication:**

4 nmol of horse heart cytochrome c (Sigma-Aldrich, CAS: 9007-43-6) in phosphate 20 mM, Triton X-100 0.03%, EDTA 1 mM buffer (pH=8) were drop-cast onto Indium Tin Oxide. After drying, RC in the same buffer was cast on top of the cyt c layer, obtaining cyt c: RC ratios ranging from 55 to 1. The oxidation state of the cyt c stock solution was checked by Visible Absorption spectrophotometry.

### **Chronopotentiometry:**

V vs t curves upon illumination were acquired using channel 1 of a Keysight B2912A Source-Measure Unit in current control ( $I = 0$  A) with a two electrode configuration, connecting the light-sensitive electrode and a Pt counter electrode ( $0.25$  cm<sup>2</sup>) to the high force and low force terminals of a Keysight N1294A-001 adapter, respectively. A 20 mM phosphate-buffered saline solution (pH = 8) has been used as electrolyte, while NIR excitation has been provided using an Osram LED (802 nm, 2.1 W).

## **EXPERIMENTS AND RESULTS:**

### **Photovoltage generation:**

Figure 2 shows chronopotentiometry of the photoactive electrode under three subsequent Near Infra-Red (NIR) photoexcitation cycles (duty cycle=0.5;  $\nu=10^{-2}$  Hz). This setup, featuring the highest RC/cyt c ratio herein investigated (i.e. RC : cyt c = 1 : 1), allows the photogeneration of a negative voltage contribution as high as  $-74$  ( $\pm 5$ ) mV.

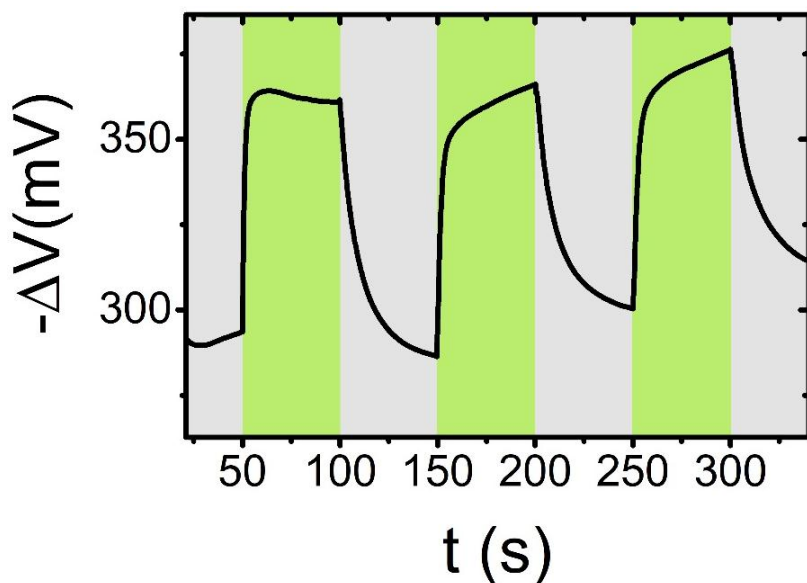


Figure 2. Chronopotentiometry of the photosensitive biohybrid architecture upon cyclic NIR illumination.

### Decoupling of the functionalization contributions:

In order to decouple the contributions of the various functionalization layers, the DC value of the potential difference between the biohybrid structure and the Pt counter electrode has been evaluated by chronopotentiometry in the dark, as reported in Figure 3.a. As expected, potential difference is influenced by surfaces functionalization and we can observe a DC offset while moving from the bare ITO ( $\Delta V \approx -10$  mV vs Pt) to the full biohybrid stack (i.e. ITO+cyt c+RC,  $\Delta V \approx -320$  mV vs Pt). It is important to notice how this offset is mainly contributed by the cyt c layer which, in this experiment, is in large stoichiometric excess with respect to RC (cyt c : RC = 5.5 : 1). These DC offset arises from differences between the Fermi levels of the electrode/electrolyte interfaces which, upon increasing functionalization complexity, deviate more and more from the bare ITO.

Upon NIR illumination, Figure 3.b, it is possible to observe photovoltage generation only in the full biohybrid stack, in which a uniform distribution of dipoles is induced at the surface, while in the presence of randomly oriented RC no net potential variation can be observed, confirming previous observations based on LEGOT behaviour [18]. Lacking chromophores, neither bare ITO nor ITO+cyt c exhibit photoactivity in the NIR.

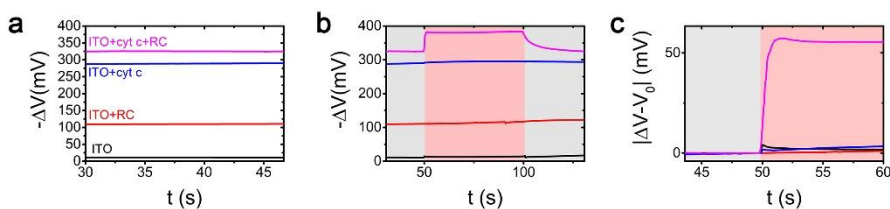


Figure 3: a) Chronopotentiometry in the dark of bare ITO (black), ITO+RC without cyt c (red), ITO+cyt c without RC (blue) and full ITO+cyt c+RC stack (pink); b) photovoltage is generated only with the full biohybrid architecture under NIR illumination ( $\lambda = 802$  nm); c) Absolute values of the photoinduced potential variation after baseline subtraction.

## Effect of the cyt c/RC ratio:

To corroborate the hypothesis that photogenerated voltage arises as a consequence of dipole formation in the RC and orientation of dipoles at the electrode/electrolyte interface we performed photoexcitation experiments, as described in Figure 2, on biohybrid stacks with area equal to  $0.25 \text{ cm}^2$ , varying systematically the amount of RC between 0.073 nmol and 3.65 while keeping cyt c amount constant to 4 nmol. Results are shown in Figure 4.

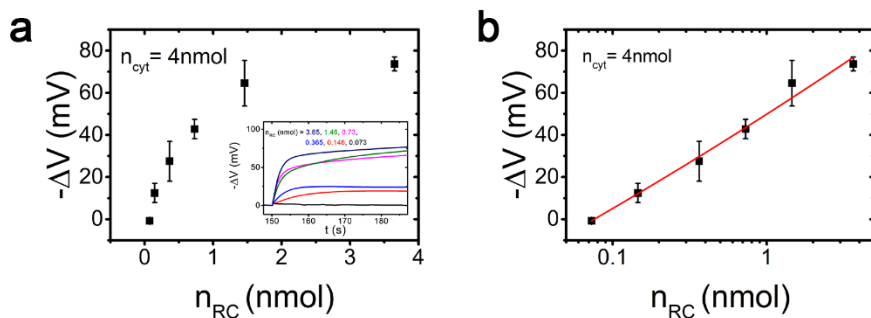


Figure 4: Dependence of the generated photovoltage on the ITO in the full biohybrid architecture under NIR illumination ( $\lambda = 802 \text{ nm}$ ) represented a) in a lin-lin plot and b) in a lin-log plot vs the nmoles of RC added to a fixed amount of cyt c. Inset in a) shows the actual chronopotentiometries recorded for different RC amounts; red solid line in b) is a Boltzmann fit of the experimental data [26].

The dependence of the photovoltage generated by light upon the amount of RC used in the stack, shown in Figure 4, clearly indicates the increase in the coverage of the electrode with photoactive enzymes. The plateau in the  $\Delta V$  value registered when the ratio between cyt c and RC approaches unity should not be claimed as a full coverage of the electrode. It should be, rather, interpreted as the functional reconstitution of the sole correctly oriented cytochrome proteins distributed on the ITO surface that allow the formation of the photoactive stack, which can be roughly estimated to be between 1-5 % of the overall cytochromes.

## CONCLUSIONS

The generation of a photovoltage by a biohybrid architecture developed for LEGOT, Light-responsive Electrolyte-Gated Organic Transistor, was investigated by means of chronopotentiometry on ITO surface. It was confirmed, in full analogy to the LEGOT, the need of a functional stack including the photoenzyme and the oxidised form of the redox protein cytochrome c, needed for photoenzyme docking on the ITO surface. Furthermore, it was showed that the photogenerated voltage increases with the amount of RCs added to the stack up to a plateau value which indicates the full coverage of the correctly oriented cytochromes.

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