Photoconverters with organic semiconductors and photosynthetic bacteria: positioning the bacterial Reaction Center in nanostructures

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ABSTRACT

Photosynthetic Reaction Center (RC) is a transmembrane photoenzyme capable of converting absorbed photons into electron-hole pairs with almost unitary efficiency. The unique properties of this natural photoconverter attract considerable interest for its use as functional component in nanomaterials and bioelectronics devices. Implementation of RC into nanostructures or anchoring on devices' electrode surfaces require the development of suitable chemical manipulation. Here we report our methods to embed this protein in soft nanostructures or to covalently attach it on surfaces without denaturating it or altering its chemical properties.

1. INTRODUCTION

The Reaction Center (RC) is a specific transmembrane multi-subunit protein complex which represents the photochemical core of the photosynthetic processes in both plants and bacteria. Roderick Clayton reported, for the first time in 1968, its isolation from the purple Rhodobacter sphaeroides bacterium. The RCs of photosynthetic bacteria are simpler than those of the more evolved plant photosystems.[1]

RC is able to use solar energy to generate charge separated states with almost unitary conversion efficiency (close to 100%) of photons to electrons. The possibility of taking advantage of this unmatched photoconversion efficiency to create functional nanomaterials and bio-hybrid devices is very attractive.[2] In fact, using the complex functions of the RC in nanomaterials or optoelectronic devices would open the way to intriguing applications ranging from electronic biosensing to alternative approaches for transducing sunlight into electrical signals or chemical energy. More in general, the use of native or genetically-modified RCs from photosynthetic bacteria represents a paradigm of a new interesting concept that is worth investigating: the biotechnological production of optoelectronic materials as a green way to up-scaled low cost materials.

We have also recently demonstrated the possibility to increase the light harvesting capability of the natural photoenzyme by covalently affixing tailored molecular antennas, obtaining hybrid systems which outperform the native protein in light absorption and photogeneration.[3]

The fascinating possibility of RC integration in bioelectronics demands for proper straightforward and reliable approaches for implementing the biological photoconverter in either nanoconstructs and/ or bioelectronics devices. Effective methods for such implementation must fulfil two requirements: 1) maintain perfectly unaltered the functions of the RC photoenzyme; 2) favour proper interfacing between the biological structure and the nanostructured scaffold or the electrode surface. Efforts are continuously made to improve the "connectivity" between the enzymatic (the RCs), and

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the non-enzymatic components of the device, as witnessed by the growing number of publications on this topic in the last ten years, which have been also recently overviewed in various comprehensive review articles.[4]

So far, RCs extracted from Rhodobacter Sphearoides have been mainly applied in the fabrication of photoelectrochemical cells, devices which convert sunlight into electrical energy. In principle, a simple photoconverter architecture set-up consists of a layer of photoactive protein molecules which photogenerate electron-hole couples and inject charges into an electrode. The major issue of this simple configuration is represented by the conjugation of the biological structure with the electronic components. In fact, the extent of the photocurrent generated in the photoenzyme-based devices critically depends on the quality of their immobilization (orientation, which affects the efficiency of the charge extraction) and the number of proteins anchored to the electrode surface.

Various approaches can be adopted to interface RCs to electrodes, including immobilization by Layer-by-Layer [5] and Langmuir-Blodgett technique by entrapment in nanoporous materials [6] and sol-gel media,[7] by binding to nanostructured materials.[8] Moreover, the efficient physisorption-based immobilization of RC by laser printing technology has been recently reported.[9] A very effective approach to interface biological structures with artificial systems (electrodes surfaces, synthetic nanostructures) is based on the employment of suitable electrode/protein linkers and in highly selective chemical modification of the protein scaffold of the RC.[10]

Flexible spacers are well-suited to preserve the full functionality of the photoenzyme while maintaining the semiconducting properties of the underneath organic layer. We used a bifunctional flexible spacer, able to react at one end with lysines residues on the proteic scaffold, and at the other end with surface modified graphene, to bioconjugate the photosynthetic reaction center of Rb. sphaeroides R26 with reduced graphene oxide (rGO). [11] We preliminarly functionalized the protein with an aliphatic chain with terminal azide group and used click chemistry, *i. e.* by 1,3 dipolar addition reaction of the azide group pending on chemically modified RC molecules with the ethynylphenyl moieties present on the surface of a graphene sheet, to address this goal (Figure 1). The covalent approach was more profitable than simple physisorption, allowing to double the number of immobilized phooenzimatic units.

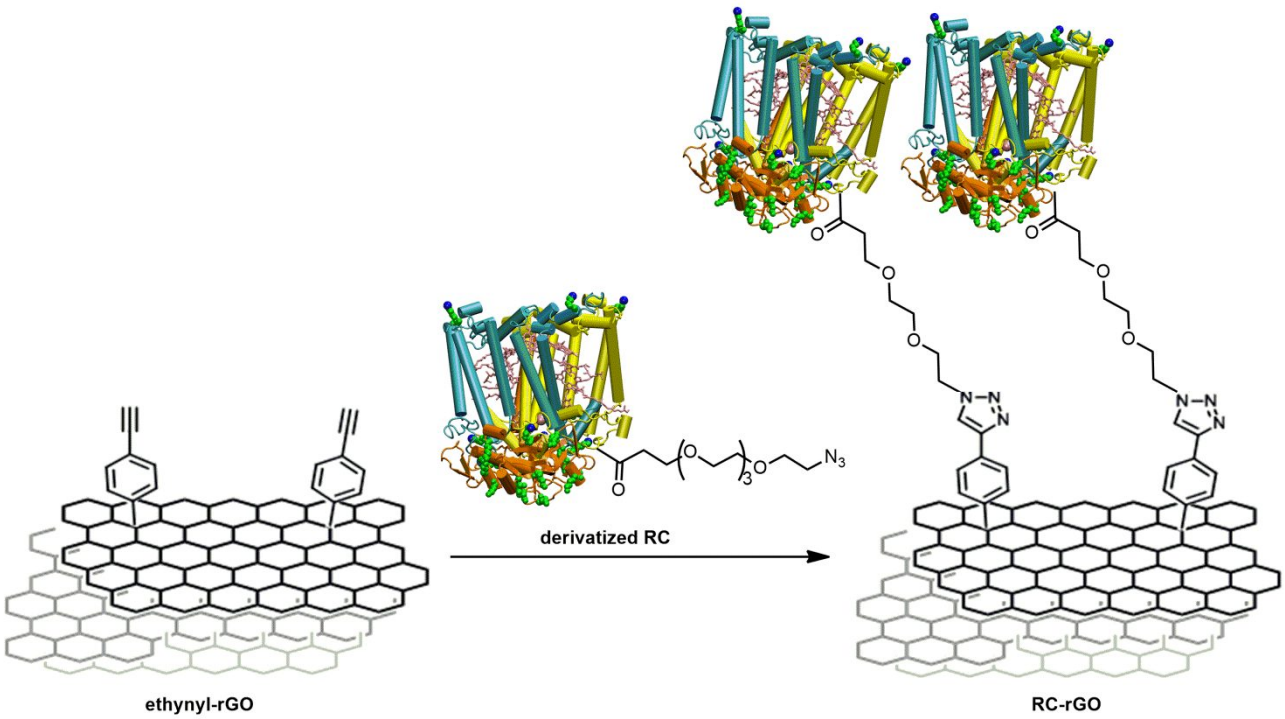


Figure 1

Following the same logic, direct bioconjugation with RCs in aqueous environment of hydrogen bonded pigments was achieved.[12] The representative semiconductors are epindolidione and quinacridone, materials used in optoelectronic

devices in the form of vacuum-evaporated polycrystalline films. These materials display low toxicity, good optoelectronic characteristics and thin film stability in a wide pH range.[13] In the first step of the bioconjugation process, shown in Figure 4 for epindolidione, an aqueous solution of disuccinimidylsuberate (SUB) is put in contact with the hydrogen-bonded pigment film. In the second step, the pigment-linker adduct reacts with RCs' lysines residues.

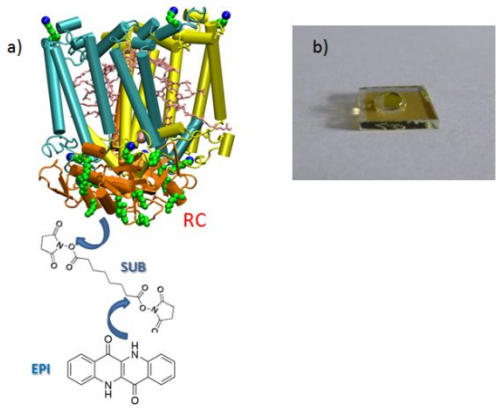


Figure 2. a) Bioconjugation procedure for the Rhodobacter sphaeroides photosynthetic reaction center (RC) to hydrogen-bonded pigment epindolidione using the disuccinimidyl suberate linker; b) an epindolidione 45 nm thick evaporated thin film on glass, with a drop of aqueous solution used to carry out the functionalization with SUB.

The experimental data collected by AFM suggested that treatment with the linker (panel b) does not disrupt pigments aggregation and morphology (Figure 3). Incubation with RC solution produces the surface with globular particles (panel c) that cover the underneath structure from the semiconductors. Washing the surface from physisorbed proteins reveals a nanostructure with relatively well-defined globules superimposed on the crystalline substrate (panel d).

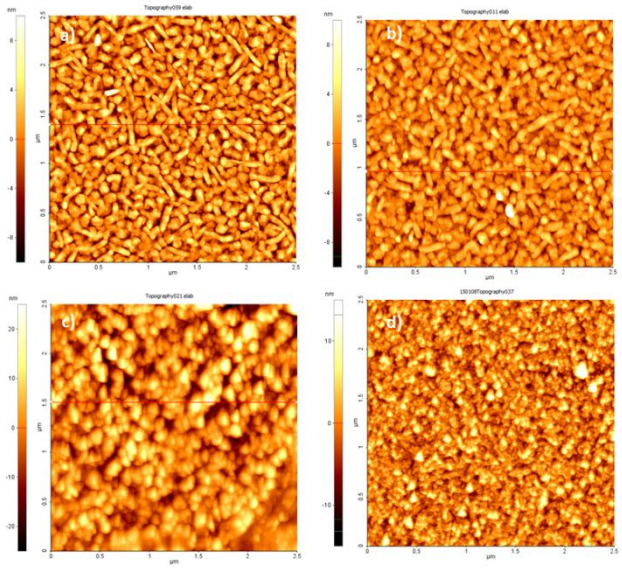


Figure 3. 2.5 µm x 2.5 µm topographies of a) pristine evaporated epindolidione film; b) SUB functionalized film; bacterial RC on SUB functionalized films before c) and after d) washing with buffer solution.

2. RESULTS

In this proceeding we would report our recent results in creating soft nanostructures able to incorporate the reaction center maintaining its full functional activity and possibly acting not only as supports but also as vehicle for the storage and transport of charges or redox-shuttle species able to interact with the RC photoenzymes.

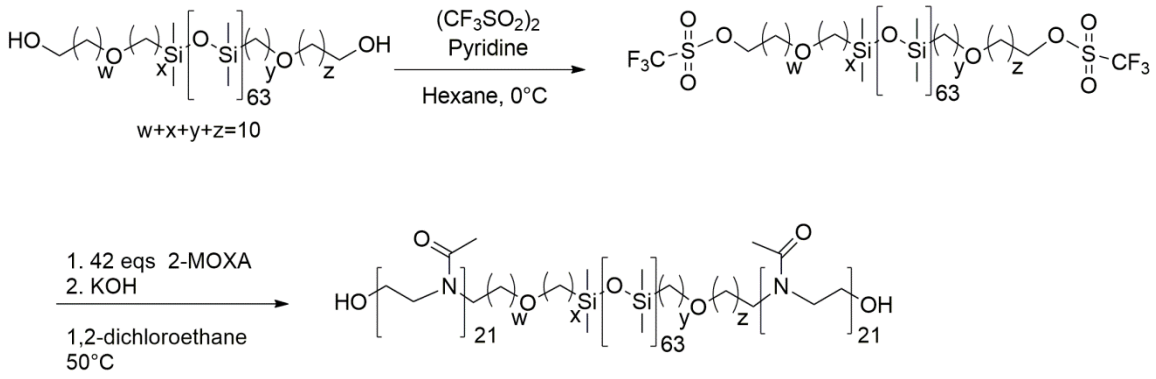
2.1 Introduction in nanostructures: polymersomes

In its physiological environment, RC sits in a phospholipidic bilayer, a membrane that provides the best protein orientation and allows to reach the maximum efficiency in charge separation and consequent activation of the photosynthetic cascade mechanism, fundamental in energy production. The photooxidised dimer in RC can be reduced back to its pristine form by interaction with cytochrome c; this last protein has a specific docking site on the reaction center: therefore proper protein orientation is crucial for maximum performances.

RC can be artificially reconstituted in liposomes[14] and polymersomes.[15] Also in this case, specific orientation of the protein is important to observe its full efficiency and functionality. Inclusion of RCs in artificial vesicles is very useful for successful solution manipulation of these photoenzimes without affecting their delicate structure.

Polymersomes can be prepared from amphiphilic block copolymers presenting alternating hydrophobic and hydrophilic portions of the chains.[16] A class of tri-block copolymers largely used for this purpose has two peripheral hydrophilic poly-(2-methyloxazoline) (PMOXA) blocks and a hydrophobic poly-(dimethylsiloxane) (PDMS) central core: $PMOXA_n$-$PDMS_m$-$PMOXA_n$, also shortened as $A_nB_mA_n$, with n and m being the number of monomeric units in the copolymer blocks.[17] We achieved reconstruction of the RC in polymersomes using micelle-to-vescicle transition (MVT) technique,[16] a protocol based on size exclusion chromathography, which is very mild if compared to other harsher procedures that are based on protein incorporation in preformed vesicles,[18] that would not guarantee preservation of the protein scaffold.

The synthesis of the $A_nB_mA_n$ block copolymer was performed according to the following synthetic sequence, reported in the Scheme 1.



Scheme 1. Synthetic sequence leading to $A_{21}B_{64}A_{21}$

$PMOXA_{22}$-$PDMS_{64}$-$PMOXA_{21}$ was synthesized starting from a commercial PDMS. The starting polymer possessed 64 repetition units and chain termination with hydroxyl groups appended to the main chain via aliphatic ether linkages. Trifluoromethanesulfonation of the terminal hydroxyl groups was achieved via reaction with triflic anhydride in dry hexane and pyridine at 0°C. The resulting material was used as initiator in the following polymerization; since the reaction is a cationic living polymerization, we assume that if 42 is the molar ratio between MOXA (2-methyloxazoline) and the polymeric initiator, two blocks of PMOXA of length 42/2 are grafted on both terminations of PDMS. The molar ratio between MOXA and PDMS monomers was confirmed by NMR integrals.

For the inclusion of RC in ABA micelles, a stock solution of $A_{22}B_{61}A_{22}$ was used to prepare a thin film deposited on the walls of an Eppendorf tube. Then, a cholate solution in pH 7 buffer was used to dissolve the film. The stock solution of RC was added to the suspension and the detergent was separated from the micelles by size exclusion chromatography. Dinamic light scattering analysis on polymersomes with and without RC showed an average hydrodynamic diameter of 130±30 nm. The indipendence of vesicles diameter from RCs inclusion induced the hypothesis that RCs are retained in the PMOXA palisade and hence they are more available to interact with the hosting solution. To demonstrate this hypothesis, charge recombination kinetics were recorded on RCs embedded in $A_{21}B_{64}A_{21}$ polymersomes and in POPC liposomes (giant phospholipid vesicles). The RC reconstitution in ABA-polymersomes was done with excess dQ (an exogeneous quinone) and charge recombination kinetic was measured. The k_S value obtained for RC-ABA-polymersome was 1.88±0.05s^{-1}. In the absence of exogenous quinone a value of 1.70±0.05 s^{-1} was recorded. These k_S values are remarkably higher than 0.98±0.05 s^{-1}, obtained under the same conditions (i.e. dQ as exogenous quinone) in RC-POPC liposomes, where the protein is firmly packed in the hydrophobic portion of the phospholipid bilayer. In the artificial aggregates the Q_B^- appears to be less stable than in the hydrophobic portion of liposome.

All these observations support the intriguing hypothesis that the RC may accumulate in the PMOXA portion of the polymersome. However, to further support this hypothesis, a new charge recombination experiments was performed adding a suitable excess of the reduced form of cytochrome c (cyt c^{2+}) to the preformed vesicle. The recombination kinetic curves are shown in the figure 6. Cyt c^{2+} will only be able to reduce the photooxidized dimers facing toward the exterior of the vesicle, while the portion facing the interior of the vesicle will be unaffected by cyt c^{2+}. As shown in figure 4 the addition of cyt c^{2+} only halves the amplitude of the signal in RC in POPC liposomes. Conversely, in RC-polymersomes the interaction with cyt c^{2+} fully reduces the oxidized dimer and, accordingly the charge recombination signal disappears.

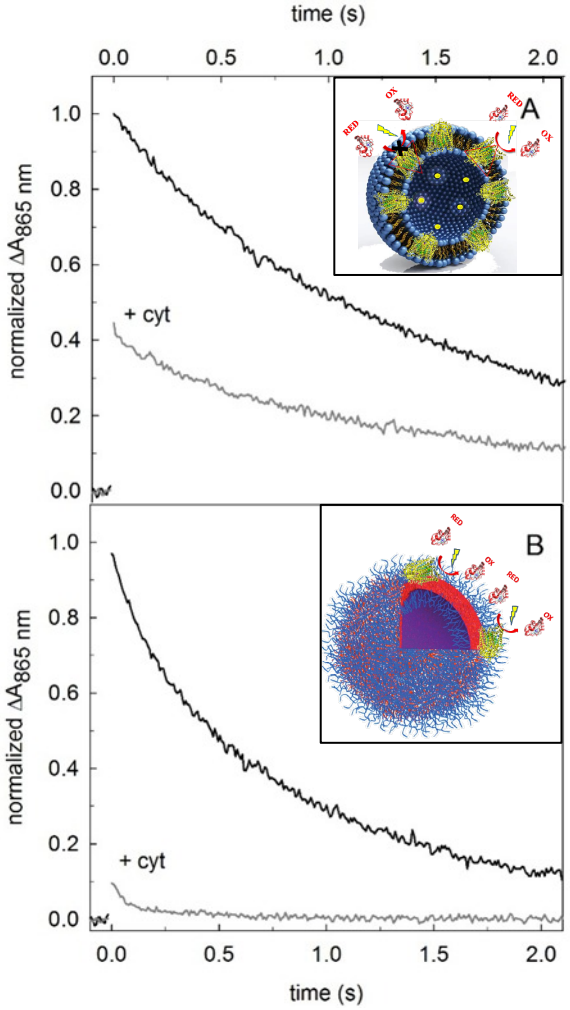
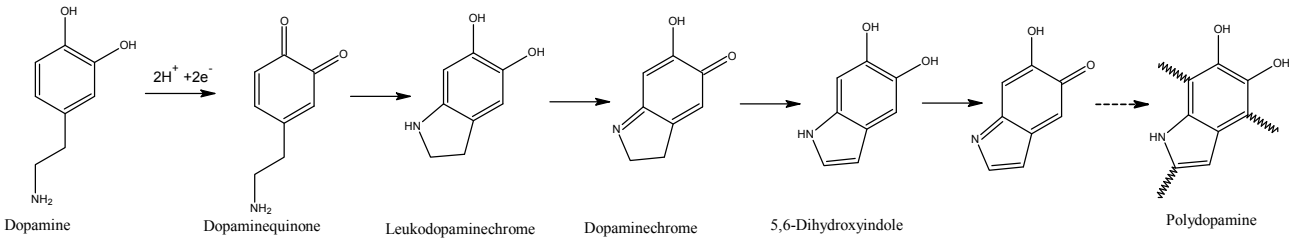


Figure 4: Normalized charge recombination kinetics of 1 µM RC in POPC liposome (fig. A) and ABA polymersomes (fig. B) in the absence (black line) or in the presence (gray line) of 10 µM reduced cytochrome c in phosphate buffer, KCl 10 mM at pH 7. Insets: pictorial representations of positioning of RCs and their interaction with cyt c^{2+}.

2.2 Embedding the RC in polydopamine nanoparticles

Following our previous investigations on the optoelectronic properties of melanin-like materials [19], we also recently carried out a preliminary study to demonstrate that the RC can be stably embedded into polydopamine nanoparticles. Polydopamine (PDA) represents the most common type of synthetic melanin and is extensively used in various fields, including biology, energy science and sensing.[20] PDA can be easily prepared by a polymerization reaction of dopamine, a biomolecule that contains catechol and amine functional groups, found also in high concentration in mussel adhesive proteins.[21] The physicochemical properties of polydopamines are similar to those ones of natural melanins, including thermoregulation, biocompatibility, antibiotic and free radical quenching function, photoprotection of humans and animals from ultraviolet injury, high light absorbivity in the visible and also in the near infrared (NIR) regions.[22] Polydopamine, as a synthetic melanin, represents a promise as photoprotector,[23] antioxidant,[24] semiconductor[25] and as biomedical material.[26].

Although the mechanism of self-polymerization has not yet been clearly elucidated, it occurs by the oxidation of the catechol unit of dopamine to quinone unit, which further reacts inter and intramolecularly with amine groups and other catechols/quinones. (Scheme 2)



Scheme 2. Mechanism of self-polymerization of dopamine

In general, dopamine polymerization can be easily carried out in water, in the presence of oxygen which favours the oxidation of catechol and at alkaline pHs to allow the addition of nucleophilic amino groups to the quinone rings.[27] In particular, we have carried out the synthesis of PDA nanoparticles with embedded RC molecules by suspending both the reaction center and dopamine hydrochloride in aqueous solution of 250 mM tris-HCl buffer kept at pH=8. The buffer was used to preserve the reaction center, avoiding its denaturation. The concentrations of RC and dopamine in the reaction mixture were 1 μM and 20 mM, respectively. The RC is expected to concurr to the polymerization reaction by the nucleophilic amino groups of lysine residues that may react with the quinone rings of dopamine. After stirring in air the reaction mixture for 24 hours, the resulting nanoparticles were isolated after various cycles of centrifugation and washing in tris-HCl and finally suspended in the same buffer.

As confirmed by microscopy images, the resulting nanoparticles have an average diameter of 100 nm (Figure 5).

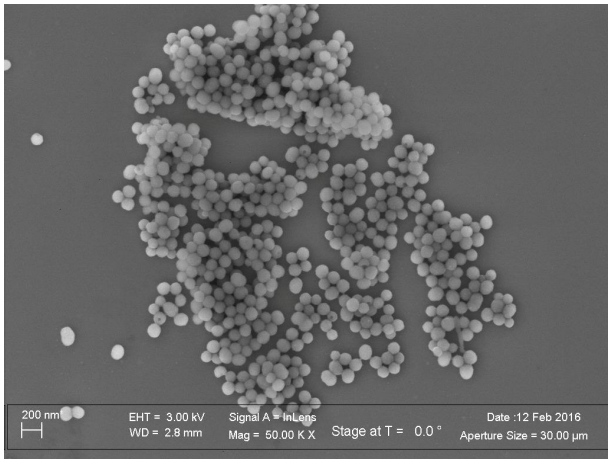


Figure 5. AFM image of polydopamine nanoparticles

Moreover, the presence of the embedded RC into the PDA nanoparticles is confirmed by the exhistence of the maximum absorption peak at 800 nm in the hybrid system (Figure 6). The absorption spectrum profile of the hybrid resembles indeed to the superimposition of the absorption spectra of pristine RC and PPDA nanoparticles.

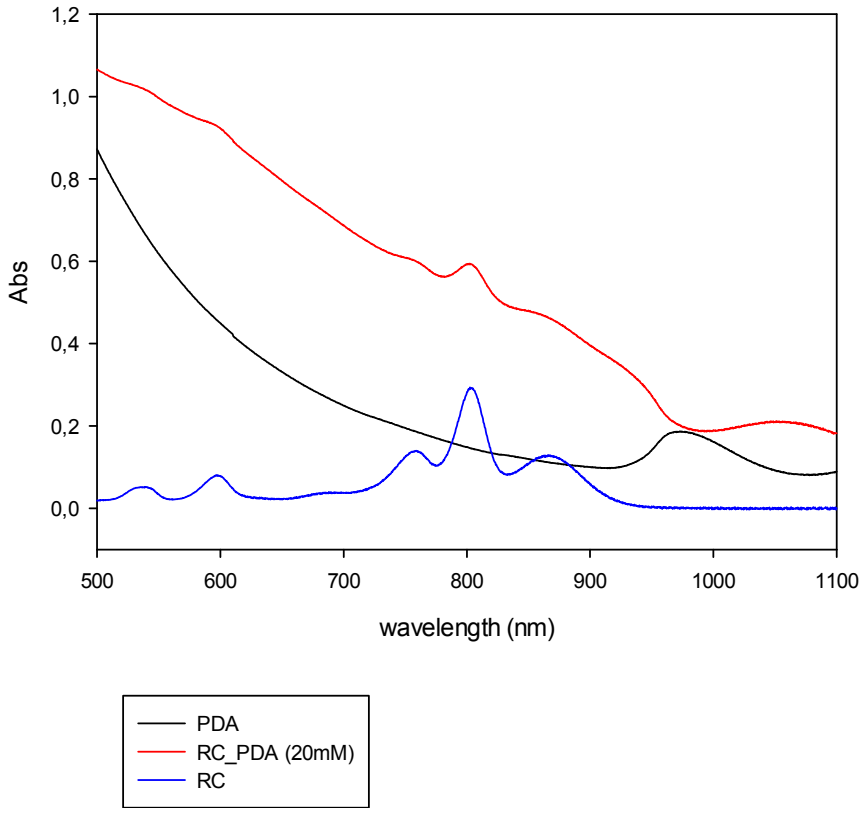


Figure 6. Absorption spectra of the PDA nanoparticles, pristine RC and RC_PDA.

3. CONCLUSIONS

Achievement of fully functional electronic devices including as active component the photosynthetic reaction center represents a perfect case of study of the emerging field of organic bioelectronics. Enormous potentialities arise from the combination of highly specialized and efficient protein natural structures with organic electronic devices. Especially, it is possible to envisage the exciting perspective of combining biotechnological production of active materials with the electronic technologies. On the other hand, the main issues are related to the manipulation of the complex and delicate biological macromolecules for implementation in electronic devices. Highly selective chemical functionalization to selectively address these systems at the interfaces with electronic devices may be a successful approach to accomplish bio-hybrid devices. We consider that the chemical tools of organic synthesis and self-assembly will play a key role in developing organic bioelectronics, bridging the gap between biotechnological production of the materials and engineering of the devices.

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