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Research paper

# Potential of light-harvesting of bacteriorhodopsin co-sensitized with green fluorescence protein: A new insight into bioenergy application

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ABSTRACT

Herein we report for the first time on efficient and environmentally friendly bioenergy production from bacteriorhodopsin (bR) and green florescent protein (GFP) as co-sensitizers. bR as a transmembrane protein, acts like a light-driven proton pump in *Halobacterium salinarum*, converting light energy into a proton gradient. Employing GFP beside bR can enhance the photo-bioenergy production efficiency in two aspects: GFP can increase short circuit current by improvement in light absorption either by extending the sensitizingspectrumor making fluorescence in absorption region of bR. It can also enhance open circuit voltage more than 150 mV by improvement in photoelectrode converging and extending electron lifetime in photoelectrode. Maximum photovoltage of 680 mV and photocurrent of 1.2 mA $cm^{-2}$ have been achieved upon co-sensitization with bR/GFP. With the power conversion efficiency of 0.45%, the highest efficiency of photovoltaic cell based on bR has been reported in this research.



## 1. Introduction

Bio-sensitized solar cells (BSSCs) are a promising new kind of thin film solar cells that combine natural and technological components to make some kind of artificial leaves; they convert sunlight into electric energy based on the sensitization of wide band gap semiconductors by specific photo-active bio-molecules such as bacteriorhodopsin (bR) protein [1]and chlorophyll (Chl) [2].

BSSCs and dye-sensitized solar cells (DSSCs), third generation of photovoltaic devices, differ in the type of employed light-harvesting molecules. The advantages of bio-sensitizers over synthetic dyes include their abundant availability, renewability, low cost, andeasy extraction methods using cheap solvents which leads to production of environmentally friendly and fully biodegradable solar cells. In addition, like various types of natural leaves, they can operate at very low intensities of illumination and the power conversion efficiencies reaches saturation values at quite low light intensities [1–4].

However research in BSSCs is still in its infancy, but the progress in this field is really exciting.

Since the first report in 2009, several studies have been performed to improve the performance of bR based BSSCs. Applying various strategies, including using ZnO as a semiconductor [5], bR LB film formation [5], co-sensitization [1], control of dye-loading time [6],$TiCl_4$ treatment [6], and coating of a scattering layer [6] have led to improvement of the BSSCs. Photovoltaic characteristics of bR-based BSSCs reported in the literature are summarized in Table 1.

In our previous study, we reported the efficient bR based BSSC with optimization of the materials at the nano-bio interface and also morphology design [6]. This paper is a continuation of our study on BSSCs; we propose the use of green fluorescent protein (GFP) beside bR to extend light absorption spectra and enhance the efficiency of BSSC. As it is shown in Fig. 1a, employing GFP beside bR can enhance cell performance in two aspects, either by extending in light absorption spectrum or making fluorescence in the range of bR light absorption spectrum. Additionally as it has been shown in Fig. 1b, the lowest unoccupied molecular orbital (LUMO) of bR and GFP located at −3.8 eV and −3.43 eV respectively, which shows the favorable electron injection to the conduction band of $TiO_2$ located at −4.2 eV; on the other hand the highest occupied molecular orbital (HOMO) of bR and GFP located at −5.4 eV and −6.8 eV correspondingly which is suitable respect to redox potential of

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**Table 1**
Photovoltaic features of bR-based BSSCs.

| Sensitizer | Nanostructured semiconductor | Scattering layer | TiCl4 treatment | Jsc (mA $cm^{-2}$) | Voc (mV) | Efficiency (%) | Reference |
|---|---|---|---|---|---|---|---|
| 3Glu mutant | TiO2 NPs | no | no | 0.09 | 350 | – | [4] |
| wild-type bR | ZnO NPs | no | no | 0.39 | 500 | 0.1 | [5] |
| bR + bacterioruberin | TiO2 NPs | no | yes | 0.45 | 570 | 0.16 | [1] |
| wild-type bR | TiO2 NPs | yes | yes | 1 | 533 | 0.35 | [6] |

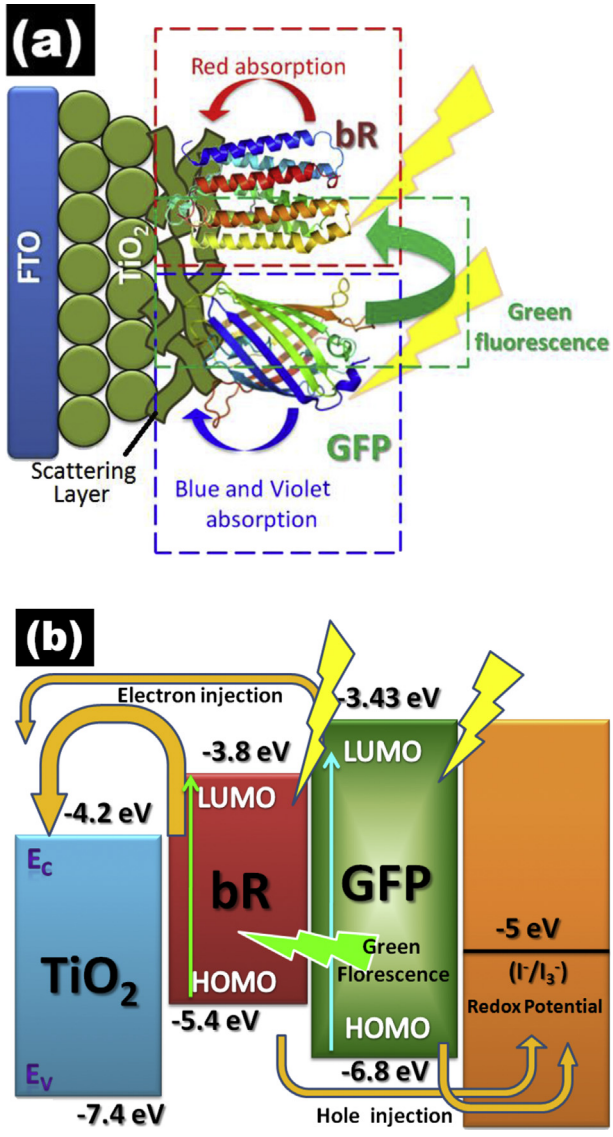


**Fig. 1.** (a) Schematics of co-sensitization of photoelectrode with bR and GFP. (b) Energy level diagram of BSSC respect to vacuum energy based on $TiO_2$photoelectrode co-sensitized with bR and GFP.

iodine based electrolyte at −5 eV [7,4]. On the other hand the florescence spectrum of GFP can absorb by bR which can help the light absorption efficiency.

Green fluorescent protein isolated from jellyfish *Aequorea victoria* has attracted a lot of attention since Shimomura discovered the first GFP in 1962 [8], and more so after it was cloned in 1992 [9]. This 27-kDa protein, contains a highly fluorescent fluorophore that is formed auto-catalytically by internal cyclization and oxidation of the Ser-Tyr-Gly sequence at positions 65–67 within the 238 amino acid [10,11]. Because of its unique properties, GFP has considered in recent years as a unique and promising material for biosensors and bioelectronics [12–15]. Here we present potential application of this protein in solar energy conversion.

## 2. Experimental

### 2.1. Gene cloning, protein expression and purification

Synthetic sequence was designed from the wild type sequence of GFP mRNA, with the insertion of NdeI (5/end) and XhoI (3/end) sites, subsequently. This sequence was cloned into EcoRV site of pBluescriptIISK(−) (Bio S&T company, Toronto, Canada). In this synthetic sequence, nucleotide "t" in position 231 was replaced with nucleotide "c" to remove the restriction site of NdeI for its cloning between NdeI and XhoI site in pET21(a). Also six histidine codon, (cac) 6, was designed after start codon (atg) and restriction site of NdeI for one-step purification by immobilized metal-ion chromatography.

GFP was produced in *Escherichia coli* cells, strain BL21 (DE3) using pET expression system, and purified with chelating Sepharose fast flow column (Roche Company, Germany) that was charged with 0.2 M $NiSO_4$. The purity of GFP was confirmed by SDS-PAGE. The protein concentration was measured by Bradford method. The purified recombinant GFP was mixed in phosphate buffer saline (PBS, 20 mM $Na_2HPO_4$, 0.15 M NaCl, pH 7.4) with or without 30 mM sorbitol as stabilizer and stored at −80 °C.

Purple membrane (PM) of *Halobacterium salinarum* containing bacteriorhodopsin was isolated by the well-known method of Oesterhelt and Stoeckenius [16].

### 2.2. Fabrication of BSSCs

A nanostructured photoanode was prepared as described previously [6]utilizing 10 μm transparent $TiO_2$ layer (20 nm) as the active medium (Fig. 2a) for sensitization and micron-length nanofibers synthesized thorough electro-spinning method employed as scattering layer (Fig. 2b). The parameters obtained from our previous study on bR based BSSC were applied to improve the interface of protein and nanostructures [4]. Prepared photo-anodes were immersed in 2 $cm^3$ of 1 kg $m^{-3}$ bR and 324 g $m^{-3}$ GFP for 1 h at 70 °C separately. Also, both bR and GFP were adsorbed on a $TiO_2$ electrode to harvest the broader range of wavelengths over the solar spectrum. Therefore, the electrode was first immersed in 1 kg $m^{-3}$bRfor 1 h at 80 °C, and then immersed in 324 g $m^{-3}$ GFP for 1 h at 22 °C.The Platinum coated FTO sheets were used as counter electrode. The working and counter electrodes, were assembled into sealed sandwich solar cell with hot-melt Surlyn film (30 μm thickness) as spacer between the electrodes. Redox electrolyte was introduced through small holes drilled in the counter electrode that were sealed afterward. The composition of the electrolyte was as follows: 0.1 M $I_2$, 0.1 M LiI, 0.6 M tetrabutylammonium iodide, 0.5 M 4-tertbutylpyridine in acetonitrile.