*Int. J. Bio-Inorg. Hybr. Nanomater., Vol. 3, No. 4 (2014), 207-213*

*ISSN Print: 2251-8533*
*ISSN Online: 2322-4142*

**International Journal of Bio-Inorganic Hybrid Nanomaterials**

# Nano-bio Hybrid Material Based on Bacteriorhodopsin and ZnO for Bioelectronics Applications

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Received: 20 July 2014; Accepted: 23 September 2014

**ABSTRACT**

Bioelectronics has attracted increasing interest in recent years because of their applications in various disciplines, such as biomedical. Development of efficient bio-nano hybrid materials is a new move towards revolution of nano-bioelectronics. A novel nano-bio hybrid electrode based on ZnO–protein for bioelectronics applications was prepared and characterized. The electrode was made by covalent attachment of bacterorhodopsin (bR) on to the ZnO substrate. The protein was attached to the ZnO nanoporous film coated on FTO glass with and without linker. In the immobilization method by adsorption without linker, protein was bonded to ZnO via electrostatic interactions and in the immobilization method protein was attached covalently. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were employed to investigate the surface features of the ZnO thin film and attached protein. ATR-FTIR was used to confirm the protein attachment.

**Keyword:** Bioelectronics; ZnO Nanoparticle; Bacteriorhodopsin; Immobilization; AFM; Covalently Attachment.

## 1. INTRODUCTION

Immobilization of proteins on various substrates has gained a lot of interest in biology and biotechnology. With the rise of nanotechnology and bioelectronics, protein immobilization on nano-structures has been explored to develop novel nano-bio hybrid systems [1, 2]. Using nano-structured solid substrates can improve the performance of these hybrid systems. Especially different nanoparticles can be assisted to design different bioelectrical devices [3-6].

Recently, zinc oxide (ZnO) nanoparticles have attracted a lot of attention as a promising alternative material for bio-application as an example of bio-imaging, cancer detection [7], biosensors, and biomolecule-sensitized solar cells. Compared to other semiconductors, ZnO has unique properties such as, wide band gap of 3.37 eV [8], higher electron mobility and higher exction binding energy of 60 meV [9, 10]. ZnO also has been explored and designed for some devices such as

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ultraviolent laser diodes [11], chemical sensor [12], photo-catalyst [13], solar cells [14], piezoelectric tranducers [15], plasma-flashover cathode emitters [16, 17] and nanoresonators [18]. Other advantages of ZnO nanoparticle are that it can be processed in an easy method by wet chemical etching and has great stability under high energy radiation [19, 20] and has a good biocompatibility and chemical stability and is non-toxic [21, 26].

However, ZnO nanoporous films with various nanostructures prepared by different fabrication techniques have been widely used for enzyme and protein immobilization, but all the previous studies have focused on electrostatic immobilization of proteins. Covalently immobilization of proteins on the substrates is an effective way to improve the stability of them. Therefore, devices with covalently attached proteins can be more effective and stable than non-covalently attached proteins.

In previous study, we reported the design of an efficient bio-sensitized solar cell (BSSC) using electrostatically adsorption of a photoactive protein, bacteriorhodopsin (bR), on the surface of ZnO NPs [22]. Bacteriorhodopsin is a membrane protein found in the purple membrane (PM) of Halobacterium salinarium. Because of the unique photochrornic, electrochromic, and photoelectric properties, bR has attracted much attention in bioelectronics [23-27].

In the present study, for the first time, we attached bR, to the ZnO nanoporous film by covalent bonds. Covalently attachment of bR to ZnO nanoparticle can result in improvement of the efficiency and stability of bR for the various bioelectronics applications.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

Purple membrane and toluene were purchased from Sigma Aldrich. ZnO nanoparticle paste (20-60 nm) was acquired from Sharif-Solar, Tehran, Iran. Fluorine-doped tin oxide (FTO) glass was obtained from Solaronix. 3-glycidoxypropyltrimethoxysilane (GPS), Acetone and Ethanol were purchased from Merk.

### *2.2. Preparation of ZnO thin films*

FTO substrates were first cleansed thoroughly by sonication sequentially in detergent, distilled water, acetone, and ethanol solution. The ZnO electrodes were prepared by squeezing the ZnO paste onto the FTO glass (1.5 cm × 2.0 cm, 15 Ω $sq^{-1}$) by doctor blade method [28]. Subsequently, the as-prepared electrodes

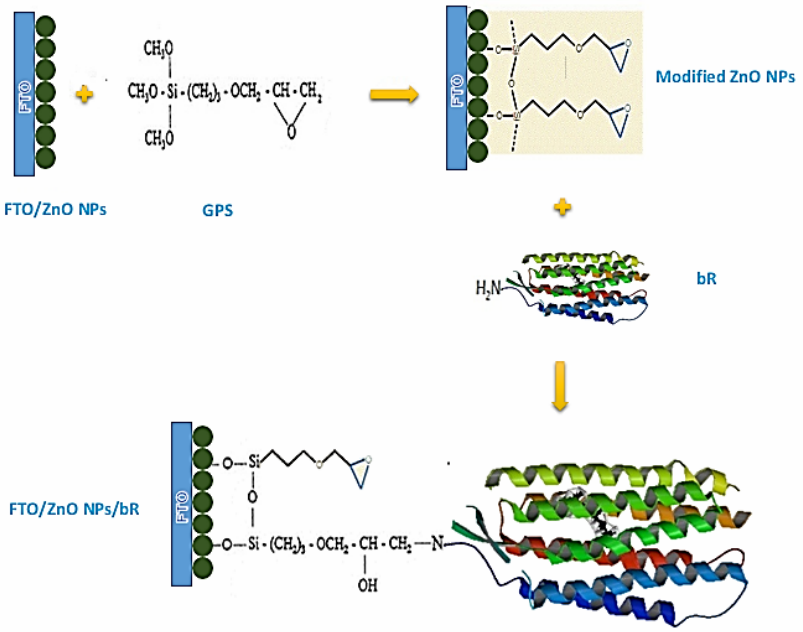


***Figure 1:*** *Schematic of covalently immobilization of bacteriorhodopsin protein on FTO/ZnO electrode.*

were annealed at 450ºC for 2 h and cooled naturally.

### *2.3. Protein immobilization*

Two methods were used to adsorb bR on ZnO surfaces. In the first, Immobilization of bR onto the ZnO film was accomplished by attaching of bR to the ZnO surface via electrostatic interactions. Then, 50 mL of 1 mg $mL^{-1}$ bacteriorhodopsin was dropped on the ZnO electrode and dried for 12 h at room temperature. Finally, the ZnO electrode was rinsed in water carefully to remove non-immobilized proteins. In the other method, silanization was done.

Initially, the silane solution was providing with 2% 3-lycidoxypropyltrimethoxysilane (GPS) solution in toluene (V/V). The ZnO electrodes were kept in silane solution at 60˚C for 4-5 h. Then all modified samples were washed with toluene to remove non-modified samples and dried thoroughly in the vacuum system (Concentrator 5301). All the prepared samples were placed in oven at 100˚C for 30 min. After dropping 50 µL of 1 mg $mL^{-1}$ bacteriorhodopsin on the modified ZnO electrode, FTO/ZnO/GPS/bR electrode incubated at room temperature in overnight and were rinsed in water carefully to remove non-attached protein molecules. Figure 1 represents a schematic illustration of the immobilization of bR onto FTO/ZnO/GPS electrode.

### *2.4. Measurements*

Morphological studies of bare ZnO nanoporous surface and bR attached ZnO electrods were performed by using atomic force microscopy (AFM) (model TN2582), scanning electron microscopy (SEM), and ATR–FTIR (Thermo Niocolet; model SMART-MULTI-BOUNCEHATR). Structural studies of bR and bR immobilized on surface of ZnO thin film were investigated by a Unicam UV-300 spectroscopy.

## 3. RRSULTS AND DISCUSSION

### *3.1. Morphological studies of ZnO film surface and protein immobilization*

SEM and AMF were used to investigate the ZnO nanoporous film and immobilized protein morphologically. Figure 2 shows three-dimensional AMF images

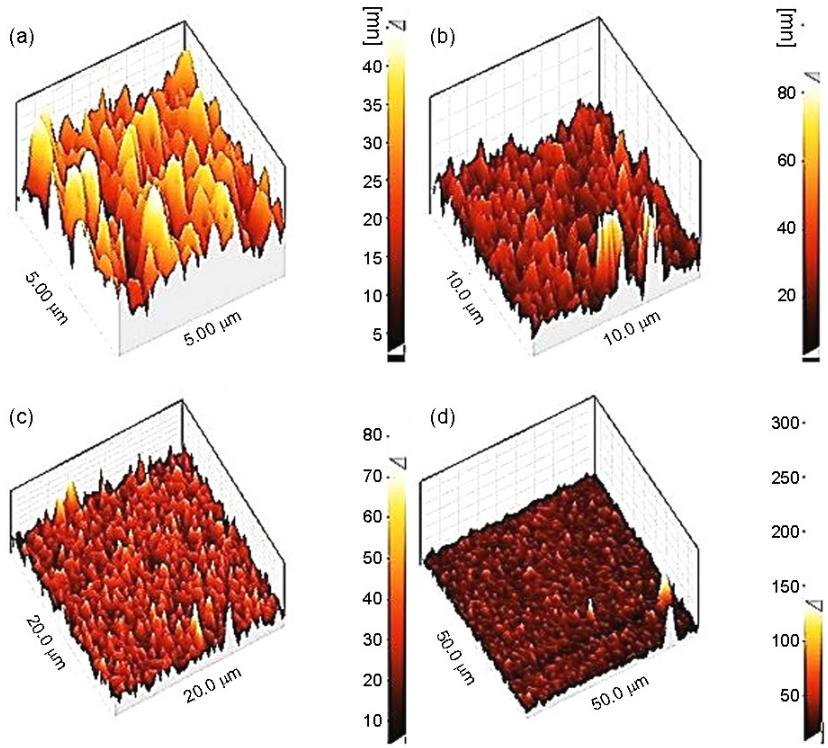


***Figure 2:*** *AFM images of ZnO nanoparticles films at different magnification (a) 5 µm × 5 µm, (b) 10 µm × 10 µm, (c) 20 µm × 20 µm, and (d) 50 µm × 50 µm.*

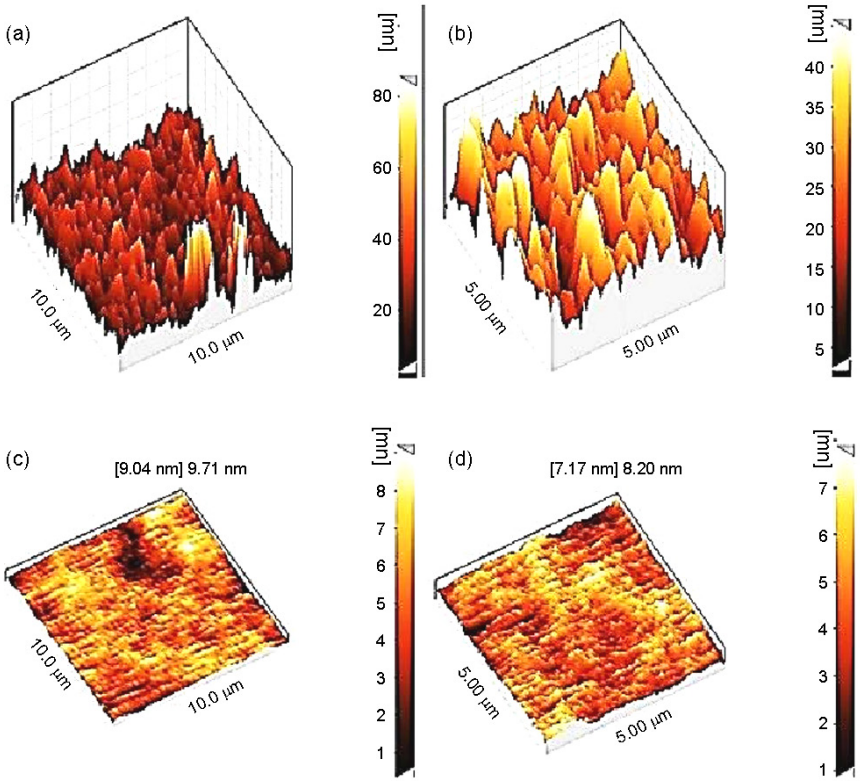


***Figure 3:*** *The morphological changes of (a,b) ZnO, (c,d) ZnO/bR electrods at the magnification of 5 µm × 5 µm, 10 µm × 10 µm.*

of ZnO nanoporous films at different magnification. The AFM photographs of ZnO nanocrystalline films show a uniform distribution on to the FTO substrate. The AFM photographs demonstrate the increase at the size of ZnO NPs from 20-60 nm to 200-600 nm. Uniform structure and distribution of ZnO nanoporuos film demonstrate that it has appropriate spread for immobilization of protein.

The AFM micrographs of ZnO, and protein coated ZnO are shown in Figure 3. Considerable morphologi-

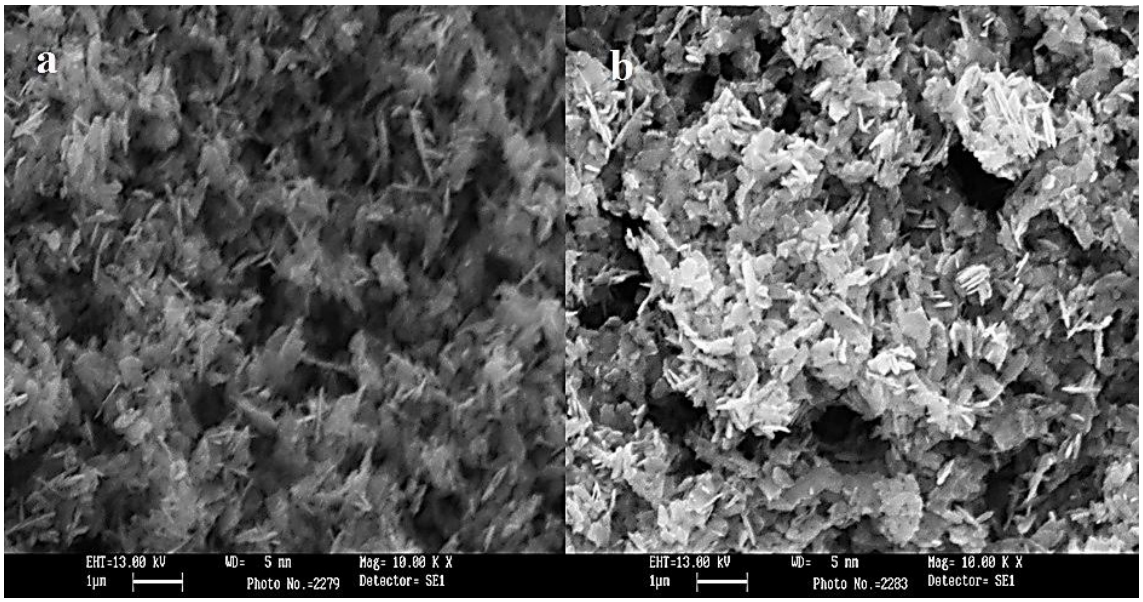


***Figure 4:*** *SEM images of (a) ZnO electrode and (b) immobilized protein on ZnO electrode.*

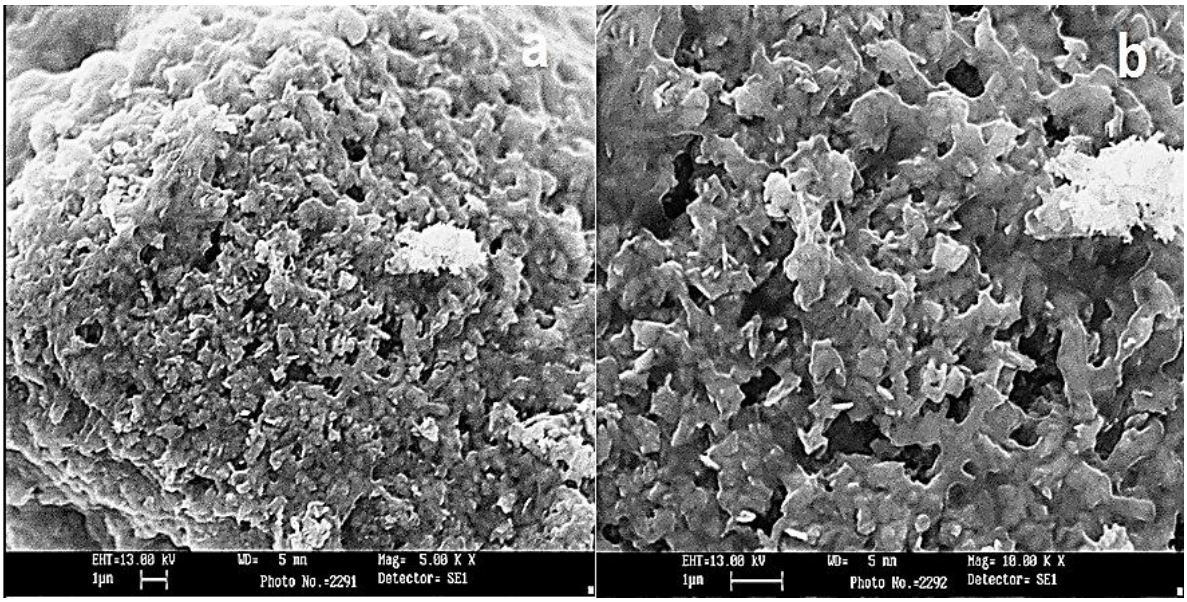


***Figure 5:*** *SEM images of non-washed immobilized protein on ZnO nanoporous surface at (a) 5x, and (b) 10x magnification.*

cal changes of ZnO film after protein immobilization indicates the protein have been deposited on the surface.

Morphological studies of ZnO nanoparticles surface and protein immobilization was also investigated by the Scanning electron microscopy (SEM). Figure 4 shows SEM images of ZnO nanoporous film on the FTO substrate. The SEM photographs show a uniform distribution for bare ZnO nanoporous film over the substrate which this uniform structure is destroyed and agglomerated after the attachment of protein.

Figure 5 presents SEM images of non-washed immobilized protein on ZnO nanoporous surface at magnification of 5x (Figure 5-a), and 10x (Figure 5-b). Non-washed immobilized protein on ZnO results in an aggregation of protein on the surface of ZnO nanoporous film which decreases performance of bioelectrical devices.

### *3.2. Structural studies of protein*

Structural studies of bacteriorhodopsin were investigated by UV-Vis spectrophotometer (Unicum 300). Figure 6 shows the absorption spectra of bR suspension and bR immobilized on ZnO. The standard bacteriorhodopsin protein has two sharp peaks at 280 nm and 568 nm which, 280 nm corresponds to aromatic amino acids (Try, Phe, Thr) and 568 nm attribute to a retinal chromophore. After immobilization of bacteriorhodopsin on ZnO nanoporous film, strong adsorptions at about 568 nm were observed.

### *3.3. Studies of covalently immobilized protein on ZnO*

ATR-FTIR was used to evaluate the attachment of protein to the ZnO nanoporous film. FTIR spectrum of protein, ZnO NPs modified by silane, and immobilized protein on ZnO is depicted in Figure 7. In the case of protein a broad N-H stretching band at 3334.81

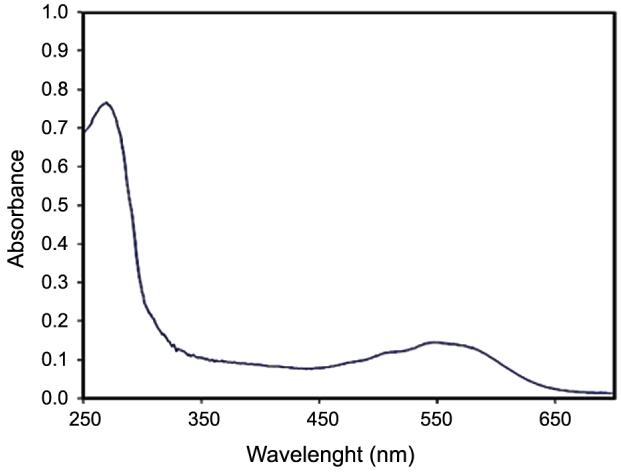


***Figure 6:*** *The optical absorption spectra of bacteriorhodopsin in solution. Inset shows absorption spectra of bR onto ZnO transparent anode.*

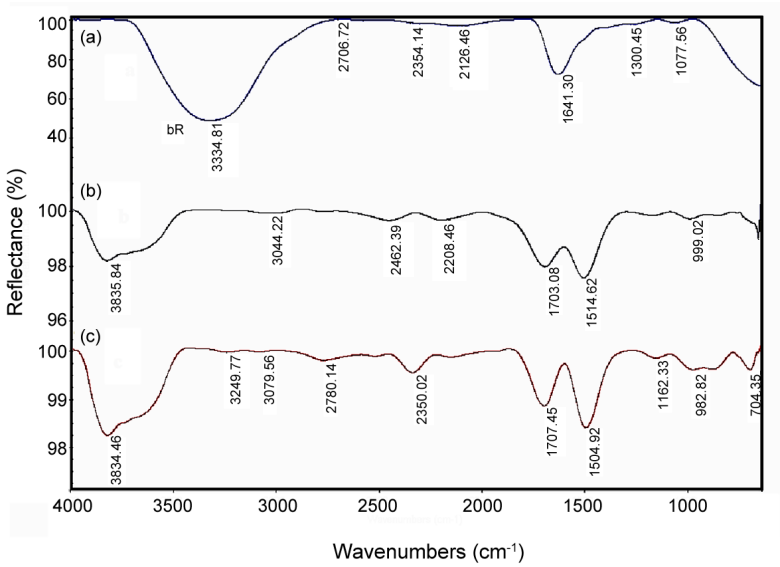


***Figure 7:*** *ATR-FTIR spectrum of (a) protein, (b) ZnO modified by silane, and (c) immobilized protein on ZnO.*

$cm^{-1}$ was observed. A weak band at 2706.72 $cm^{-1}$ corresponds to the C-H stretching band. A strong peak at 1641.30 $cm^{-1}$ was observed due to C=N stretch of protonated Schiff base. The peaks at 1300.45 and 1077.56 $cm^{-1}$ correspond to the C-C and N-C groups.

In the case of ZnO modified by silane, the N-H and C-H broad peaks were observed at 3835.84 and 3044.22 $cm^{-1}$. A weak band at 2208.46 $cm^{-1}$ is due to CN group. A strong peak at 1730.08 $cm^{-1}$ correspond to a C=O stretching band. A Si-O bending band was observed at. Signals at 999.02 $cm^{-1}$ arise due to both epoxy groups and Si-OH or Si-O groups. The covalently immobilized protein has a broad band at 3834.46 $cm^{-1}$ related to N-H stretching band. An O-H stretching group was observed at 3249.77 $cm^{-1}$. A weak band at 3079.56 $cm^{-1}$ related to a C-H stretching band. The strong bands at 2350.02 and 1707.45 $cm^{-1}$ are due to Si-H and C=O strong bands. A fairly strong band at 1504.92 $cm^{-1}$ correspond to a C=N group. A Si-O-Si bending band was observed at 1162.33 $cm^{-1}$. A weak band at 2780.14 $cm^{-1}$ is due to Aldehyde C–H stretching band.

## 4. CONCLUSIONS

In this study, a novel method has been investigated for covalently attachment of bacteriorhodopsin to the nanoporous ZnO film. The AFM and SEM images confirm the immobilization of bacteriorhodopsin on ZnO nanoprous surface as well as ATR-FTIR. Covalently attached proteins have high stability compared to that of immobilized via electrostatic interaction. This ZnO-bR hybrid electrode possesses versatile bioelectronics applications such as biosensors and biosolar cells.

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