

# Electrostatic Force Microscopy Study of Open-Circuit Photovoltage from a bR/ITO Heterostructure

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## ABSTRACT

Non-contact electrostatic force microscopy techniques were used to study the open circuit photovoltage of purple membrane (PM) multilayers electrodeposited on indium tin oxide (ITO). A humidity dependant photovoltage response under illumination by a 635 nm photodiode was observed. Peak photovoltages in excess of 2V at  $\sim 36 \text{ W/m}^2$  were obtained for  $\sim 15\%$  relative humidity.

**Keywords:** bacteriorhodopsin, ITO, AFM, photovoltage.

## 1. INTRODUCTION

Bacteriorhodopsin (bR) is a light sensitive protein found in the cell membrane of the archaeobacterium *halobacterium salinarum*. bR arranges into a crystalline lattice of protein trimers, which are isolated in the form of purple membrane patches (PM). PM patches ( $\sim 300\text{nm}$  in diameter) have the potential to be used in future nanometer-size photodetectors. Such photodetectors would rely on many of the light sensitive properties inherent to the protein including a fast photoresponse, polarization sensitivity and the capacity to become well anchored and oriented onto a number of conductive substrates.[1-7]

While there have been a number of studies largely devoted to understanding the properties of bR in electrochemical environments[8], these results may not directly translate to bioelectronic heterostructures. A limited number of bR studies in non-aqueous environments have been previously reported [9-13] as well as studies of current flow through PM patches.[14,15]

Various PM deposition methods have been reported using Langmuir-Blodgett[16], electrostatic sedimentation[17], chemisorption[18], antigen-antibody interactions[19], and electrostatic layer-by-layer assembly.[20,21] Recently, Xu et al. have shown that PM multilayers deposited on GaAs substrates retain their light sensitive properties and produce a photocurrent in the nA range for a  $\sim 1 \text{ cm}^2$  contact area.[22]

To further investigate the compatibility of PM multilayers for advanced bio-electronic applications, we have measured the photoresponse of a PM/ITO heterostructure. Advanced applications will certainly employ PM patterned at the

nanoscale, so conventional measurement techniques of the photovoltage, which lack micron spatial resolution, are not of interest here.

It is worthwhile to briefly review what is known about the photocycle of bR. The observed photovoltage is caused by a complicated sequence of events initiated by an electric dipole moment that develops when a photon is absorbed by the retinal located at the center of the seven  $\alpha$ -helices (labeled A-G) that constitute the scaffold of the bR protein. The active photoresponse for PM has an absorption peak at 568 nm and half-width of roughly  $\pm 50 \text{ nm}$ [24]. Within picoseconds after photon absorption, the retinal undergoes a conformational change, inducing proton transfer toward the extracellular side of the protein. This triggers a complicated process resulting in the deformation of various helices as the retinal deprotonates and flexes. Crystallographic studies indicate lateral motions of  $\sim 0.35 \text{ nm}$  at the top of the F helix, evidently causing an opening of a channel to allow reprotonation of the retinal and return the protein to its resting state [25, 26].

In what follows, we take advantage of the bR photocycle and attempt to use it to effect charge separation in a non-aqueous environment.

## 2. EXPERIMENT

Wild-type PM patches in distilled water at pH  $\sim 6$  were electrodeposited onto an indium tin oxide (ITO) substrate (resistance  $\sim 30 \Omega/\text{square}$ , obtained from Delta Technologies Limited) by applying a potential difference of +5 V to the ITO substrate for 25 seconds.[17] The concentration of the PM solution was  $\sim 4.0 \text{ mg/ml}$ . After voltage application, a purple film appeared on the ITO. The thickness of the film was  $\sim 4 \text{ microns}$ . Given a PM height of  $\sim 5\text{nm}$ , we conclude that the film consisted of  $\sim 800$  layers.

Near neutral pH, bR prefers to anchor its extracellular face to the ITO surface.[22] Given that bR pumps protons towards the extracellular surface, we expect that, when activated, the bR/ITO interface will acquire a positive charge, rendering the bR/air interface negative.

Photovoltage response was measured using a custom-built air SPM. A schematic of which is shown in Figure 1. In this initial study, illumination of the PM multilayer was

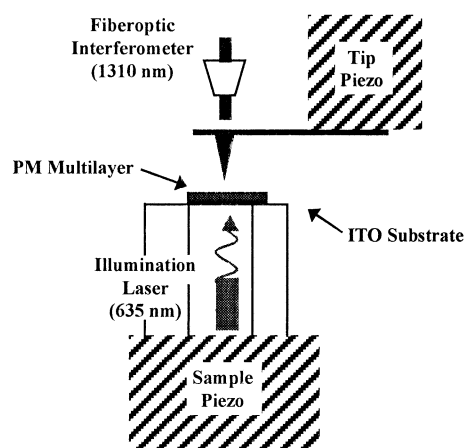
accomplished by a commercially available photodiode operating at 635 nm. Although this photodiode is not optimized for the bR absorption peak at 568 nm, it is sufficient for our purposes. A notable feature is the fiber-optic interferometer, operating at 1310 nm, used to monitor cantilever vertical deflection. This wavelength is far outside the region of active photoresponse for PM. Standard beam-bounce SPMs utilize red lasers to monitor cantilever position. This is well within the PM photoresponse region. In this situation, any light not blocked by the cantilever will confuse the interpretation of the photovoltage data. The SPM is housed in a clean stainless steel tube sealed by Conflat<sup>®</sup> flanges. This tube provides a light tight environment and also allows for controlled changes in the relative humidity (RH) surrounding the PM multilayer.

In order to make voltage measurements of the PM multilayer, we used an electrostatic force technique. As is known[27,28], by applying a DC voltage between the cantilever and sample, the electrostatic force acting on the cantilever can be nulled, a condition that is readily monitored by the fiber-optic interferometer. Calibration tests indicate that an electrostatic potential introduced between tip and substrate can be measured to an accuracy of better than  $\pm 20$  mV with a time constant for the feedback system of about 100 ms.

To improve the performance of the interferometer, we used Au coated cantilevers that were prepared by evaporating 5 nm Ti followed by 80 nm Au on standard Ultrasharp<sup>™</sup> cantilevers commercially available from Veeco Inc. Electrical contact to the cantilevers was achieved by a separate wire. We used a versatile Nanotec<sup>™</sup> electronic control system to run the SPM and simultaneously acquire data on laser power and photovoltage from the illuminated PM multilayer.

RH was controlled by adjusting the flow rate of dry nitrogen bubbled through deionized Millipore<sup>™</sup> water. RH was sensed electronically using a commercially available, calibrated humidity sensor. Repeated experiments indicate that it is easy to maintain the humidity constant to within  $\pm 1\%$  for the duration of a measurement. At this time, we are unable to quantify the actual hydration level of the PM multilayer as a function of chamber RH although long-time separated measurements show no difference in photoresponse between samples brought to a stable RH for one and ~45 minutes.

The 635 nm, 2 mW photodiode is mounted below the sample to avoid any shadowing by the cantilever. This also dictated our choice of substrate since ITO is a transparent conductor. Optical power output of the photodiode as a function of applied voltage was calibrated to an accuracy of about  $\pm 5\%$ . This indicates incident intensity on the PM multilayer was about 3% of the solar level.[29]



**Figure 1.** Schematic of the SPM head. Tip deflection monitored with 1310nm laser via fiber optic cable, sample illuminated with 635nm. Crosshatched areas are tip and sample XYZ piezos. Fiber attachment to piezo, mechanical head support, and the surrounding stainless steel chamber are not shown.

We conducted numerous tests on bare ITO substrates to confirm that the sample/tip voltage was independent of illumination. Heating of the photodiode caused rapid thermal drifts in XYZ, preventing us from imaging the spatial dependence of the photovoltage and requiring a large tip-sample distance to avoid contacting the surface. Standard feedback was capable of keeping the tip sample distance fixed, but we were unable to maintain a constant XY tip position once the laser was turned on.

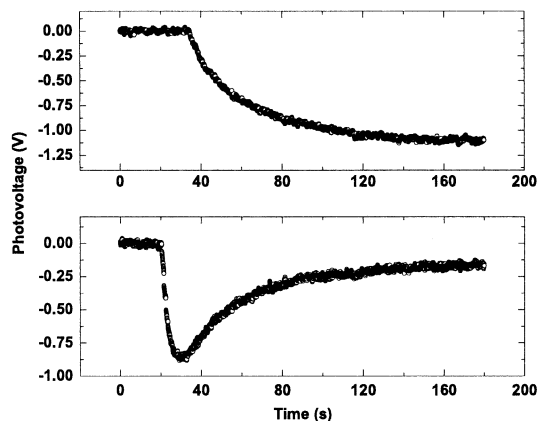
Any voltages measured using an electrostatic SPM will have a finite spatial resolution governed by the tip-sample separation. Heat induced drift caused us to set the tip-sample separation to about 1 micron, rendering the tip itself essentially irrelevant to the voltage measured. In this experiment, only the main cantilever beam contributes significantly to the electrostatic force and, hence, measured photovoltage.[27,28] We estimate that the measured photovoltage represents an average value over a few hundred square micron area.

### 3. RESULTS AND DISCUSSION

The photovoltage as a function of time produced by the illuminated PM film is plotted at two different humidities in Figure 2. Four interesting features are evident from this data:

- As expected, the PM/air interface goes negative when the light is on;
- The overall time evolution of the photovoltage depends strongly on RH;

- The time constant for photovoltage turn-on as well as the maximum photovoltage also depend strongly on RH;
- The time constants characterizing the turn-on of the photovoltage are different for constant RH.



**Figure 2.** Photoresponse of PM multilayer at 3% RH (top) and 36% RH (bottom).

Given the long time constants observed for charging and discharging the PM multilayer (orders of magnitude greater than the feedback response time constant) and the observed polarity of the photovoltage, we conclude that the dominant charging species must be ionic, as opposed to electronic, in nature. Presumably, the impeded mobility of  $H_3O^+$  through the multilayer accounts for the time constants observed. This observation is consistent with the observed difference in the time constants for turn-on and turn-off. One would expect that bR driven diffusion during illumination would proceed faster than the drift diffusion that occurs when the light is turned off. Further study of the observed photovoltage response in conjunction with known properties of PM may yield information on lateral and vertical diffusion within the multilayer.[30, 31]

#### 4. CONCLUSIONS

We have shown that SPM techniques can successfully measure the open circuit photovoltage of PM multilayers with micron-scale spatial resolution. We find  $\sim 4$  micron thick PM multilayers are capable of generating photovoltages in excess of 2V when illuminated by a 635 nm photodiode. Over long illumination timescales the photovoltage displays complex humidity dependant behavior.

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