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Humidity-dependent open-circuit photovoltage from a bacteriorhodopsin/indium tin oxide bioelectronic heterostructure

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Abstract

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

1. Introduction

Bacteriorhodopsin (bR) is a light-sensitive protein found in the cell membrane of the archae *Halobacterium salinarum*. The protein crystallizes within the cellular membrane in monoproteomic patches containing 75% bR and 25% lipid, called, for their colour, purple membrane (PM). PM patches ($\sim 300 \text{ nm}$ in diameter) have the potential to be used in future nanometre-size photodetectors, e.g. field effect transistors. bR-based 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 [1–8].

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 [9–14] as well as studies of current flow through PM patches [15, 16].

Various PM deposition methods have been reported using Langmuir–Blodgett [17], electrostatic sedimentation [18], chemisorption [19], antigen–antibody interactions [20], and electrostatic layer-by-layer assembly [21, 22]. Recently, Xu *et al* [23] have shown that PM multilayers deposited on GaAs substrates retain their light-sensitive properties and produce a photocurrent in the nanoampere range for a $\sim 1 \text{ cm}^2$

contact area. Combined with the recent measurements of the photoresponse of photosystem I proteins [24] these promising developments suggest that photoactive proteins may one day become integrated into modern semiconductor devices.

To further investigate the compatibility of PM multilayers for advanced bioelectronic applications, we have investigated the steady-state photoresponse of a PM/indium tin oxide (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. We employ scanning probe microscopy (SPM) techniques because of their ability to make measurements at size scales from tens of microns to nanometres, below the level of individual PM patches and below the level that one can easily fabricate complete semiconductor devices in which the bR can be integrated. Detailed topographic images of the surface morphology of the PM layers can be obtained using SPM, thereby allowing an optimization of the deposition conditions. In this way, SPM measurement techniques can be used to characterize both current multimicron-scale PM devices as well as future nanoscale devices built from one or a few individual bR proteins.

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 centre of the seven α -helices (labelled A–G) that constitute the scaffold of the bR protein [25]. The active photoresponse for PM has an absorption peak at 568 nm and a half-width of roughly ± 50 nm [26]. Within picoseconds after photon absorption, the retinal undergoes a conformational change, inducing proton transfer towards the extracellular side of the protein. This triggers a complicated process resulting in the deformation of various helices as the retinal deprotonates and flexes [25]. Crystallographic studies indicate lateral motions of ~ 0.35 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 [27]. It is well established that at low humidity, the bR photocycle does not operate normally, slowing down by orders of magnitude [14, 28, 29].

In what follows, we take advantage of the bR photocycle to form a useful bioelectronic heterostructure that functions under non-aqueous conditions typically encountered in semiconductor devices. In this way, we hope to produce a significant photovoltage that persists for long timescales.

2. Experiment

In this initial study wild-type PM patches in distilled water at pH ~ 6 were electrodeposited onto an ITO substrate by applying a potential difference of +5 V to the ITO substrate for 25 s [18]. The ITO areal resistance was $\sim 30 \Omega/\text{square}$ and was obtained from Delta Technologies Limited. The concentration of the PM solution was $\sim 4.0 \text{ mg ml}^{-1}$. After voltage application, a purple film appeared on the ITO. After drying, the thickness of the film at room temperature and an atmospheric relative humidity (RH) of $\sim 20\%$ was $\sim 4 \mu\text{m}$. Given a PM thickness of ~ 5 nm, we conclude that the film was ~ 800 layers thick.

It is known that near neutral pH, bR prefers to anchor its extracellular face to the ITO surface [23]. 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 diagram of this instrument 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 fibre-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® flanges. This tube provides a light-tight environment and also allows for controlled changes in the RH of the atmosphere surrounding the PM multilayer.

In order to make voltage measurements of the PM multilayer with micron spatial resolution, we used an electrostatic force technique. As is known [30, 31], by applying a dc voltage between the cantilever and sample,

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

(This figure is in colour only in the electronic version)

the electrostatic force acting on the cantilever can be nulled, a condition that is readily monitored by the fibre-optic interferometer. Calibration tests indicate that an electrostatic potential introduced between tip and substrate can be measured to an accuracy of better than ± 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 UltrasharpTM cantilevers commercially available from Veeco Inc. Electrical contact to the cantilevers was achieved by a separate wire. We used a versatile NanotecTM electronic control system to run the SPM and simultaneously acquire data on laser power and photovoltage from the illuminated PM multilayer.

Ambient RH of the chamber was controlled by adjusting the flow rate of dry nitrogen bubbled through deionized MilliporeTM water. Chamber RH was sensed electronically using a commercially available, calibrated humidity sensor located at the bottom of the steel tube. Repeated experiments indicate that it is easy to maintain the atmospheric 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. We have performed simple studies on samples to look for hydration time constant effects. As an example, one sample was quickly brought from a zero RH level to a stable RH value. No difference in the photoresponse was measured after the stable RH was maintained for either 1 or 45 min, suggesting that the multilayer hydration reaches a stable value in times short compared to a minute. All measurements were made at room temperature, $\sim 22^\circ\text{C}$.

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 that the incident intensity on the PM multilayer was about 3% of the solar level [32]. Data was acquired at a rate of 1 kHz.

Figure 2. (a) Photoresponse of PM multilayer at 3% RH. (b) Photoresponse of PM multilayer at 20% RH. (c) Photoresponse of PM multilayer at 33% RH. (d) Maximum photovoltage as a function of RH.

We conducted numerous tests on bare ITO substrates to confirm that the tip-sample 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 $1\ \mu\text{m}$, 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 [30, 31]. We estimate that the measured photovoltage represents an average value over an area of a few hundred square microns.

3. Results and discussion

The photovoltage as a function of time produced by the illuminated PM film is plotted as a function of humidity in figures 2(a)–(c). The typical noise in a photovoltage measurement was found to give a standard deviation of $\sim 14\ \text{mV}$, a value small compared to the magnitude of the peak photovoltages observed.

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

Figure 2(d) plots the maximum photovoltage measured by our apparatus as a function of RH. It can be seen that the maximum photovoltage occurs around 15% RH. When the RH approaches 40%, the photovoltage essentially disappears.

From figure 2, the time constants after laser turn-on (or turn-off) clearly decrease with increasing RH. This seems reasonable if we assume that the hydration of the PM increases with atmospheric RH as this would provide more H_2O to hydrate the membrane. From neutron studies, a stiffening of the surrounding lipid bilayer is a consequence of dehydration. This stiffening is assumed to impede the bending of the F-chain, and increases the lifetime of one of the intermediate states (M) of the bR photocycle [28]. Dehydration of the bR heterostructure would lead both to increased time constants as the photocycle slows down [14] and to the observed decrease in the peak photovoltage below 15% RH as more of the bR in the sample effectively ceases to function [28].

It is instructive to estimate the size of the charge density that develops in the PM multilayer as a function of time. From previous studies, 15% RH is far too low to allow normal photocycle kinetics [28, 29]. The bR photocycle effectively pauses halfway through, leaving it with a net dipolar charge. A simple model is that the PM multilayer forms a polarized slab of material. There is then a net charge on surfaces making a finite angle with the polarization vector. The size of the surface polarization charge can be estimated by considering

the electrostatic potential that develops from two charged discs of radius R , each carrying a charge density $|\sigma|$ and separated by a distance d in free space. Assume the negatively charged disc lies in the xy plane at $z = 0$ and the positively charged disc lies a distance d below it. Then the voltage V as a function of distance z from the negatively charged plate is

$$V(z) = -\frac{\sigma d}{2K\epsilon_0} \left(1 - \frac{2z+d}{2R} + \dots \right) \quad (1)$$

where, $K\epsilon_0$ is the dielectric permittivity of the region between the charged discs. When the potential is measured close to the disc (i.e. when $z \ll R$), the potential becomes

$$V \cong -\frac{\sigma d}{2K\epsilon_0}. \quad (2)$$

We can now estimate the charge density that develops at the top and bottom surfaces of the PM multilayer. From figure 2, choosing the data at RH of 15%, the maximum photovoltage is found to be about 2.5 V. Using this in equation (2) and assuming a dielectric constant $K = 2$, we find a maximum charge density $\sigma \approx 20 \mu\text{C m}^{-2}$ for a measured d of $\sim 4 \mu\text{m}$. This model is useful for estimating the voltage that develops as a function of multilayer thickness.

One can also estimate the resistivity of the PM multilayer by measuring the relaxation to charge neutrality when the light is turned off. We identify the measured time constant (τ) obtained from figure 2 with the dielectric relaxation time of the PM multilayer [33]. Since $\tau = \rho\epsilon$ with $\epsilon \approx 2\epsilon_0$, we estimate $\rho \approx 10^{11} - 10^{12} \Omega \text{ m}$, depending on humidity.

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 $\sim 4 \mu\text{m}$ thick PM multilayers are capable of generating open-circuit photovoltages in excess of 2 V when illuminated by a 635 nm photodiode. Over long illumination timescales the photovoltage displays a humidity-dependent behaviour attributed to changes in the bR photocycle.

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