

Chemically induced enhancement of the opto-electronic response of *Halobacterium* purple membrane monolayer

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Acetylation of purple membranes (PM) significantly enhances the surface photovoltage that they exhibit, if adsorbed as a monolayer on a solid surface; we suggest that this increase is due to the improved orientation of the PM on the surface.

Bacteriorhodopsin (bR) is a protein–chromophore complex that serves as a light-driven proton pump in the purple membrane (PM) of *Halobacterium salinarum*.¹ Its remarkable photovoltaic and photochromic properties, extraordinary stability against thermal, chemical and photochemical degradation, together with an ability to maintain its biological activity if immobilized on solid support,² has made bR a promising material for bioelectronics. In order to achieve bR-based photoelectric devices, it is essential that the bR films are oriented so as to optimize their photoresponse.³ Several interesting approaches, such as specific antigen–antibody or biotin–streptavidin molecular recognition⁴ and vesicle fusion⁵, have been developed to obtain highly oriented PM films. However, for fundamental studies, simpler but efficient techniques to prepare oriented PM films (especially monolayers) are needed.

PM monolayers are difficult to prepare due to aggregation and stacking of PM patches (even at sub-monolayer coverage). Apart from Langmuir–Blodgett deposition,^{6a,b} electrostatic assembly^{6c} has been used as a simple and efficient method to prepare PM (sub-)monolayers. However, bR orientation is still poor because both PM surfaces are negatively-charged⁷ and are practically electrostatically equivalent if viewed from a distance of a few Debye lengths, despite their asymmetric charge distribution. Therefore, the internal electrostatic asymmetry of bR is not very effective in orienting PM patches upon spreading them on a substrate.^{6b} We show here that acetylation of PM significantly increases its permanent dipole, which is likely to improve the orientation of electrostatically adsorbed PM. Clear evidence for this is derived from the stronger surface photovoltage responses of acetylated PM monolayers compared to non-acetylated samples.

Acetylation processing of bR is especially useful, since it efficiently derivatizes the lysine residues,⁸ and neutralizes its positive charges. Each bR molecule comprises seven lysine residues,⁹ of which five are located in the cytoplasmic (CP) and one on the extracellular (EC) side. One lysine is bound to a retinal chromophore, which prevents its acetylation. As the PM lipids are also devoid of amino groups, it is expected that following acetylation, the CP surface potential will be much more net negative than the EC side, compared to the unacetylated native

membrane.⁷ The increased permanent dipole of PM as a result of acetylation should assist in orienting electrostatically-assembled bR monolayers on the positively-charged substrate surface.

PM patch fragments were prepared by a standard method¹⁰ and acetylation was carried out as previously described.^{8,9c} Acetylation does not change the optical absorption spectrum of bR ($\lambda_{\text{max}} = 564 \text{ nm}$).^{9c} PM and acetylated PM (A-PM) were electrostatically self-assembled onto an Al substrate ($\sim 50 \text{ nm}$ thick film of Al, evaporated on quartz, with a few-atom-thick layer of natural aluminum oxide on its surface, represented here as AlOx), which was silanized with (3-aminopropyl)trimethoxysilane (APTMS)¹¹, followed by treatment with 0.1 M HCl to obtain a positively-charged surface. The Al substrate was then transferred into freshly prepared PM or A-PM suspensions ($\sim 0.34 \text{ OD}$ in 20 mM tris buffer, pH 9.2) for 5 min, rinsed with tris buffer (pH 9.2) for 1 min and dried in a flow of N₂ gas.

Atomic force microscopy (AFM) images were acquired using the tapping mode, in ambient air, on a Nanoscope IIIa (DI, now VEECO) instrument, utilizing standard silicon tips (ULTRASHARP, NSC/12/50). Fig. 1 shows representative AFM images of the (sub-)monolayers of PM and A-PM. The typical PM patch size is $\sim 1 \mu\text{m}$. It is difficult to obtain full monolayer coverage of PM patches *via* electrostatic deposition due to the (relatively) large patch size and their strong tendency to aggregate in-plane, as shown by AFM. PM and A-PM differed markedly in their photovoltaic responses, as evidenced by contact potential difference (CPD) measurements, although there was no pronounced difference in their patches' morphology surface coverage ($\sim 70 \pm 10\%$). The CPD measurements were performed, both in the dark and under illumination, using a commercial (Besocke) Kelvin probe, as part of a home-built set-up inside a N₂-filled glove-box (relative humidity $\leq 20\%$). Time-dependent CPDs between an Au reference and the sample surface, before and after illumination, are shown in Fig. 2. CPD measurements were started

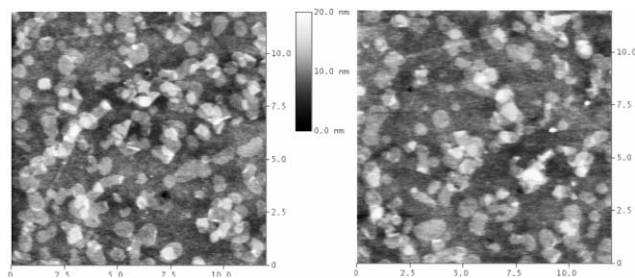


Fig. 1 Representative $12 \times 12 \mu\text{m}$ AFM images of (sub-)monolayers of PM (left) and A-PM (right) prepared by 5 min adsorption on an Al/AlOx substrate derivatized with APTMS. Scale bar: 20 nm.

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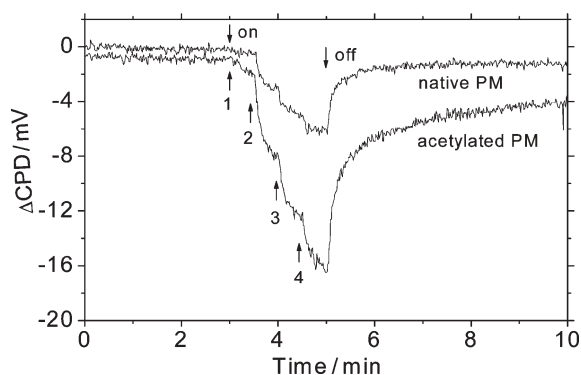


Fig. 2 Surface photovoltage (SPV) responses of (sub-)monolayers of PM and A-PM, respectively, on an APTMS-modified Al/AIO_x substrate, following illumination (after 3 min in the dark) with a green light. The light intensity was 1, 4, 18 and 28 mW cm⁻² (1–4, respectively) with 30 s intervals.

in the dark and followed by sample illumination. Illumination with green light (>495 nm, obtained with a tungsten-halogen light source in combination of a cut-off filter (495 nm)) caused a decrease in both samples' surface photovoltage (SPV: the change in CPD upon illumination), indicating that green light increased the surface dipole. This observation is in keeping with PM fragments being attached to the substrate surface mainly *via* their CP side,¹² since the proton is ejected towards the EC side upon illumination with ~560 nm light.¹³ The SPV increased as the light intensity was increased, and reached saturation at 6 and 16 mV for PM and A-PM films, respectively. Control substrates without PM did not show any pronounced photoresponse (<2 mV at 28 mW cm⁻²). Because the surface coverage was similar for both A-PM and PM (see Fig. 1), the ~2.5-fold signal enhancement of A-PM can be ascribed to the increased permanent dipole of the PM. Most likely, this will improve PM orientation and thus its photoresponse, although the possibility that the increased permanent dipole somehow increases the photoresponse per PM cannot be excluded.

The photoactivities of the two samples were examined by irradiating them with green and blue lights. In the case of native PM patches in solution, blue light accelerates the decay of the M intermediate that is formed upon green light irradiation to the bR ground state. We interpret our results (*cf.* Fig. 2 and Fig. 3) in a similar fashion. Except for the ~2.5-fold signal enhancement of A-PM, similar CPD signal oscillations were observed for both samples if green and blue illumination were alternated (Fig. 3). We also examined the effect of simultaneous illumination using both lights on the CPD responses of A-PM films. If blue light was added to green light, a small decrease in CPD (which became ~4 mV less negative) was found (Fig. 4). This change in CPD strongly indicates that the effect of adding blue to green light is to decrease a green light-induced dipole. Such a sequence is consistent with green light-induced formation of the M intermediate and its blue light-induced decay.

In summary, we have shown that acetylation of PM increases the permanent dipole of PM, which appears to improve their orientation upon electrostatic self-assembly on a solid substrate, as evidenced by an enhancement of the surface photovoltage response of the acetylated PM monolayer. This finding is important for the

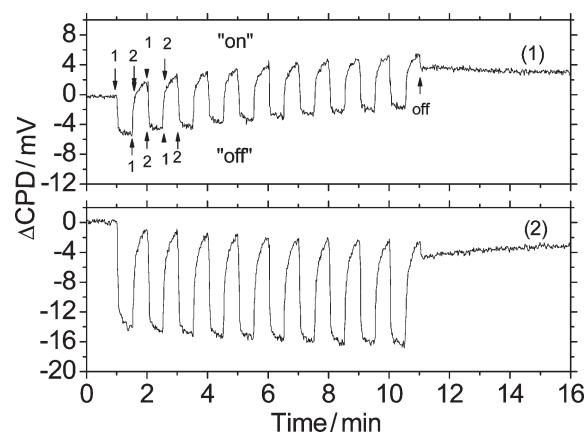


Fig. 3 CPD responses of (1) PM and (2) A-PM (sub-)monolayer on an Al/AIO_x/APTMS surface with the sample first in the dark for 1 min, then illuminating it for 30 s with green light (>495 nm, 28 mW cm⁻²), followed by turning off the green light and immediately illuminating with blue light (405 nm laser, 5.5 mW cm⁻²) for 30 s. The Figure shows the results for 10 consecutive repetitions of this illumination protocol, which, in the top part, are indicated as follows: 1 - green light; 2 - blue light.

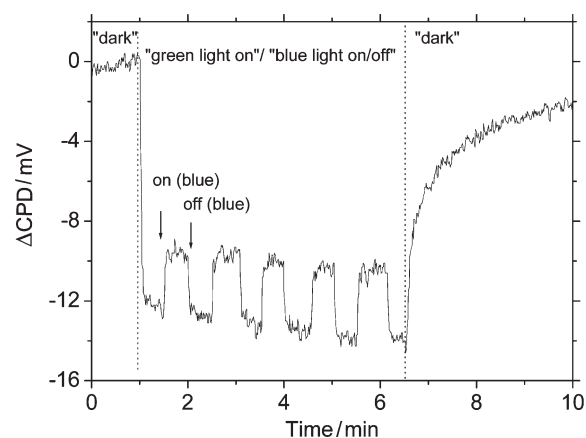


Fig. 4 CPD responses of A-PM monolayer on an Al/AIO_x/APTMS surface upon illumination with green light ("on"), to which blue light is intermittently added ("on/off"). Illumination intervals are 30 s. The Figure shows the results for 5 consecutive repetitions of this illumination protocol.

future use of the monolayers of PM patches for electrical studies and devices.

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