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Vectorial insertion of bacteriorhodopsin for directed orientation assays in various polymeric biomembranes

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Abstract

Here we demonstrate the rapid reconstitution and preserved functionality of vectorially-inserted bacteriorhodopsin from *Halobacterium Halobium* in ABA block copolymer thin films. We have utilized a triblock copolymeric membrane that simulates biological systems as a matrix to enable protein refolding to observe the activity of bacteriorhodopsin in purple membrane form as well as in individual molecules. In addition to observing protein–polymer compatibility properties using polymers of varying lengths (4 vs. 8.4 nm in height) and compositions (UV-cross-linkable methacrylate or acrylate ended), we have observed that vectorial insertion of the proteins, and hence, the directionality of proton pumping is dependent upon the pH of the medium to manipulate the asymmetric charge density of the polymer membrane during reconstitution.

Keywords: Protein; Block copolymer; Bacteriorhodopsin

1. Introduction

The harnessing of biological and chemical energetics to fabricate novel power sources has been the subject of much interest in the scientific community due to the high efficiencies and versatility of biomolecules. Purple membrane (PM) obtained from *Halobacterium halobium* is densely-packed with the light-dependent proton pump bacteriorhodopsin (BR). This membrane-bound protein serves as the mechanism for bacterial energy transduction to convert sunlight into a proton gradient, which is in turn coupled with ATP Synthase functionality to produce ATP from ADP and P_i [1]. PM has been the subject of much interest due to its unique photocycle intermediates that exist depending on the wavelength of light used to activate the retinal chromophore, which serves as the light-dependent element of the protein (500–650 nm, [2–5]). Studies have detailed its potential use in molecular electronics [6], optical devices [7], as well as bionanotechnology [8–10]. One of the more intriguing aspects of the BR protein lies in the fact that it is a 'vectorial catalyst', meaning that its processes of proton transport ensure that proton translocation is in a unidirectional manner [11]. This exists to ensure that gradients can in fact be developed due to light-activation of the protein.

While conventional studies of protein activity have relied on their interfacing with synthetic lipid systems, these methods usually require expanded lengths of time to prepare the vesicles as well as reconstitute proteins using dialysis. Furthermore, as the orientation of protein insertion is moderated by the pH of the external medium, lipid membranes retain impaired abilities to preserve protein activity in low pH solutions. This work details the preserved activity of BR based upon its reconstitution within block copolymeric membranes (Fig. 1). Block copolymers possess various advantages over lipid systems including block lengths/compositions that can be tailored for specific functionalities, as well as UV-cross-linking endgroups that can enhance membrane stability. In this work, PM activity was demonstrated while reconstituted within the ABA block copolymer (PMOXA–PDMS–PMOXA) [12–14]. Furthermore, it was observed that vectorial insertion of the BR, and the directionality of proton pumping could be controlled depending on the pH of the surrounding medium during protein reconstitution. The integration of biomolecules into robust, polymeric systems represents a promising approach to rapidly studying protein activity in various conditions.

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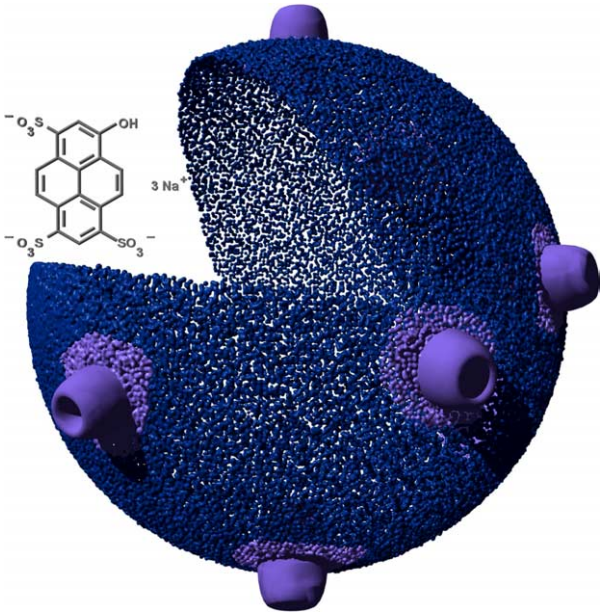

Fig. 1. Depiction of purple membrane/BR-functionalized copolymeric vesicle. A copolymeric vesicle is shown here that possesses embedded purple membrane structures that generate light-dependent proton gradients. These vesicles enclose a 8-hydroxyprene-1,3,6-trisulfonic acid (pyranine) dye, which optically monitors the pH changes occurring within the vesicle.

2. Materials and methods

2.1. Bacterial culture

Halobacterium salinarium was grown at 40 °C with rigorous shaking at 250 rpm for 48 h after the inoculation of the stock to the liquid medium. The *H. salinarium* stock was grown at the same condition as described, harvested in between the exponential and the pre-stationary growth phase ($ABS_{570\ nm} \leq 3.0$ a.u.) and stored at −80 °C with 15% glycerol. The composition of the liquid medium is as followed: for the final volume of 10 L, NaCl (2.5 kg), $MgSO_4$ (97.7 g, anhydrous), KCl (20.0 g), NH_4Cl (50.0 g), sodium citrate dehydrate ($C_6H_5O_7Na_3 \cdot 2H_2O$, 30.0 g), glycerol ($C_3H_8O_3$, 10.0 mL), KH_2PO_4 (1.0 g), and $CaCl_2$ (2.0 g, anhydrous).The sequence of these additions play a critical role in preventing precipitation in the mixture. Lastly, 100 (10 g/L) of Oxoid Bacteriological Peptone L-37® is added and pH is adjusted to 7.0 by NaOH pellets [15,16].

2.2. Isolation of purple membrane

Bacterial cells were lysed by osmotic force and successive centrifugations isolate purple membrane. First, cells were spun down by centrifuging the cell culture at 13,300*g* for 20 min. This pellet was resuspended in distilled water with 4.2 mM $MgSO_4$ and 0.1 mg/mL DNase I. After 24 h of stirring at room temperature, the solution of enzymatic digestion was centrifuged at 3000*g* for 20 min to spin down cell debris. The supernatant of suspended purple membrane was saved and concentrated by pelleting the purple membrane at 100,000*g* for 20 min. To remove contaminants such as carotenoid and red membrane, the pellets were resuspended in distilled water and the centrifugation at 100,000*g* for 30 min was repeated until the supernatant was clear. A minimum of eight runs were performed to obtain fairly pure pellets of purple membrane. The quality of the isolated purple membrane can be checked by measuring the absorbance at 265 and 568 nm. The ratio of ABS_{568}/ABS_{265} indicates the amount of bacteriorhodopsin to the total amount of protein, and should be greater than 45% for good bacteriorhodopsin-based device. Bacteriorhodopsin is the only membrane protein found in purple membrane. Also, the chance of contamination from other membranes containing halorhodopsin ($\lambda_{max} = 588$ nm) or sensory rhodopsins I and II ($\lambda_{max} = 587$ and 487 nm, respectively) is low, considering their low natural abundance [15,16].

2.3. Bacteriorhodopsin purification

To start the purification, 5 mg of purple membrane was resuspended in 4.5 mL of 25 mM phosphate buffer, pH 6.9 and 0.5 mL of 10% (v/v) Triton X-100 in 25 mM phosphate buffer, pH 6.9. After 30 s of sonication, it was kept in dark at room temperature for 24 h [17]. The undissolved materials were removed by centrifugation at 100,000*g* for 45 min. The sample was injected into Sephacryl-S100 16/60 column pre-equilibrated with 25 mM PBS with 1% (v/v) Triton X-100, pH 7.3. With a flow rate of 0.5 mL/min, bacteriorhodopsin was eluted, concentrated and stored at −80 °C. All the procedures were carefully performed in dark to minimize undesirable bacteriorhodopsin activity upon ambient light. The concentration of bacteriorhodopsin was estimated using a spectrophotometer with the extinction coefficient of $\Delta\varepsilon_{568}^{BR} = 63\ mM^{-1}\ cm^{-1}$.

2.4. Polymer structure and design

The triblock copolymer composed of polymethyloxazoline–polydimethylsiloxane–polymethyloxazoline (PMOXA–PDMS–PMOXA) was custom synthesized by Polymer Source, Inc., (Quebec, Canada). Based on that identified by Meier et al. [14], an initial conventional 8.4 nm thick polymer structural model terminated with UV-polymerizable methacrylate endgroups (8METH) was developed. Considering the thickness of natural bacterial membrane, we also have developed two different 4 nm thick (PMOXA–PDMS–PMOXA) ABA triblock copolymer species. One was terminated with the same methacrylate endgroup as found with the conventional thicker model (4METH), and the other polymer was terminated with an acrylate endgroup, which is also UV-polymerizable but much more susceptible to spontaneous, and hence, expedited cross-linking (4ACRYL). Block copolymer thicknesses were previously determined from measurements of capacitance of tethered membranes across hydrophobic septums using conventional 'black polymer membrane', or 'black lipid membrane techniques' [18].

2.5. Polymer vesicle assembly

Polymer in ethanol of desired concentration (0.19 g/mL) was stirred at room temperature for 24 h until the mixture

showed no turbidity. The above polymer stock was placed in an amber vial with a rigorously stirring magnetic bar. Purple membrane was added slowly to the vial. Vigorous stirring was continued for 3 h in the dark to insure the homogeneous mixing. The polymer/protein mixture was then added to 0.1 M PBS buffer with pyranine at the rate of 10 μm every 5 min. To avoid the formation of multi-lamellar non-functional polymer vesicles, the mixture was syringe filtered through 0.22 μm membrane pores. Polymer vesicles were then UV radiated for 10 min to cross-link the acrylate or methacrylate endgroup of the functional polymer surface which stabilizes the vesicle structures. The final concentration of bacteriorhodopsin in the functional polymer vesicle solution was 8.6 mM. The excess pyranine which failed to be entrapped within the polymer vesicles is removed by successive dialysis overnight in the dark [19].

2.6. Monitoring activity of polymer vesicles

The proton pumping activity of purple membrane in polymer vesicle was monitored by the fluorescence probe, pyranine. The excitation scan by luminescence spectrometer (Perkin–Elmer) was performed in the range of 350–475 nm at emission 511 nm. External pyranine was quenched by the addition of *p*-xylenebispyridinium bromide (DPX) in the final concentration of 20 mM. The electrochemical gradient across the membrane was neutralized by 0.1 μM valinomycin. The conversion from the fluorescence to pH was performed as described in [20].

3. Results

3.1. Rapid reconstitution of protein in varying polymer constructs

Bacteriorhodopsin activity was observed in polymer membranes possessing varying lengths as well as compositions. One variation was 4 nm in length (thin) with acrylate endgroups that could be UV-cross-linked, while another variation was 8.4 nm in length (thick) with UC-reactive methacrylate endgroups. While rapid reconstitution and acquisition of protein functionality was observed for both polymers, these different constructs were utilized to observe effects of polymer block length as well as varying UV-reactivity of the endgroups on protein performance.

Bacteriorhodopsin is naturally incorporated into the purple membrane of its host to produce purple membrane sheets which in turn incorporate into the polymer membrane as patches. Polymeric vesicles functionalized with the protein enclosed the 8-hydroxyprene-1,3,6-trisulfonic acid (pyranine) dye, which optically monitors the pH changes occurring within the vesicle. Fig. 2 compares the average $|\Delta pH|$ values from the proton pumping activity of bacteriorhodopsin embedded in different polymers. The thick polymer membrane which is 8.4 nm in thickness preserved the enzyme activity, increasing $|\Delta pH|$ to be 0.1986 in 36 min. However, the acrylate-ended thin polymer of 4 nm thickness showed a dramatic change of 0.4856 in $|\Delta pH|$ in 30 min and further increased to 0.4958 at 1 h. It is believed that the long branches of the methacrylate endgroups on the hydrophilic surface of the thicker polymer

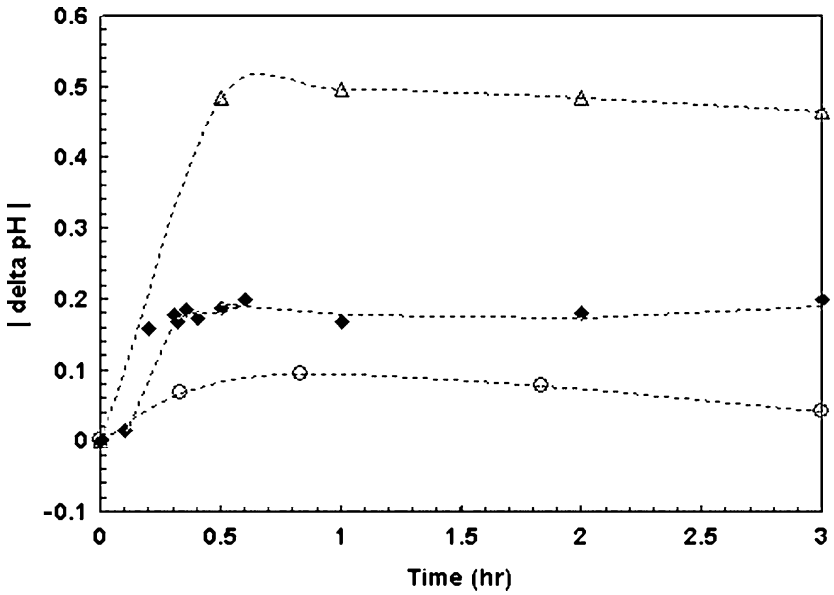

Fig. 2. Measurement of sustained protein functionality in varying polymer constructs. Proton pumping driven by bacteriorhodopsin was observed by reconstitution in polymeric vesicles enclosing 8-hydroxyprene-1,3,6-trisulfonic acid (pyranine) dye, which optically monitors the pH changes occurring within the vesicle. (–△–): Change in internal pH of the vesicle is observed for bacteriorhodopsin in UV cross-linked acrylate ended thin polymer which produced an average $|\Delta pH| = 0.4856$. (–■–): change in internal pH of the vesicle is observed for bacteriorhodopsin in UV cross-linked methacrylate ended long polymer which produced an average $|\Delta pH| = 0.19$. (–○–): Change in internal pH of the vesicle is observed for bacteriorhodopsin in non-UV cross-linked polymer matrix which produced an average $|\Delta pH| = 0.098$.

(8.4 nm) may have impeded the open face and hence the functionality of the membrane spanning proteins which are only 5 nm long in height as well as their conformational changes necessary to pump protons. Both the methacrylate-ended thick polymer and acrylate-ended thin polymer were UV-cross-linked to stabilize the polymersome structure and protein activity. Based upon the observations of pH change/preserved protein activity between the varying polymer constructs, it was believed that the short polymer created a more suitable environment for membrane proteins due to a membrane thickness which more closely resembles that found in biological systems. These samples that were examined did not contain oriented protein sheets. However, it was observed that the short polymer with the acrylate endgroup optimally supported and maintained protein activity. As such, experiments involving protein orientation were performed using acrylate or methacrylate ended 4 nm thickness polymer membranes.

The bacteriorhodopsin polymersome samples were exposed to continuous illumination of light (Fostec-150 W) for up to 1 h, and were then kept in the dark to test for membrane leakage against proton gradients over time. The peak $|\Delta pH|$ of 0.1986 at 36 min in the 8.4 nm-thick polymer was maintained for 2½ h in the dark, resulting in the $|\Delta pH|$ at 3 h being the same as 0.1986. The fluctuation of the $|\Delta pH|$ levels in the 4 nm-thick polymer was less than 5.84% for 2 h. It is believed that the methacrylate or acrylate endgroups formed a rigid but stable network on the membrane surface upon the UV radiation which maintained the pH gradient created across the membrane for over 10 h as it has been previously demonstrated that the mechanical stability of the membrane can be enhanced due to endgroup UV-reactivity (data not shown). Furthermore, the UV-induced free-radical polymerization process may have slightly altered the morphology of the hydrophilic polymer block by cross-linking of the polymethyloxazoline (PMOXA) 'A' blocks. This would have, in turn, resulted in a possible enhancement in resistance against transmembrane ion flux, as well as a reduction in shielding effects that would prevent the proteins from receiving light required to induce the all-trans to 13-cis conformational shift in the retinal chromophore and central mechanism of BR functionality that induces proton pumping due to the light scattering properties of the polymer [21]. Previous studies using dynamic light scattering have shown that polymer dynamics as well as properties such as the radius of gyration (R_g), and hydrodynamic radius (R_h), among others. In fact, it was shown that a steric contraction of the hydrophilic components during polymerization was observed as well [21]. Without cross-linking, the 4 nm-thick polymer produced a low activity of bacteriorhodopsin due to the instability of the membrane as well as an inability to prevent transmembrane ion flux [22]. The $|\Delta pH|$ commonly peaked at 30–50 min around to a level around 0.1 (as shown, $|\Delta pH| = 0.0953$ at 50 min) and exhibited a large decrease of 57.71% in 2 h. These results indicate that although membrane leakage to ions in non-cross-linked polymer membranes was observed, the polymer membrane stability was far improved over that of the lipid membranes considering the experiments were conducted at room temperature. In addition, the demonstration of protein functionality/reconstitution could be achieved in a matter of hours compared to several days for the lipid systems [10]. By stabilizing the interface between purple membrane and polymers, UV cross-linking not only enabled rapid and easily achievable detection of purple membrane activity, it also preserved the pH gradient across the membrane for over 10 h at room temperature.

3.2. Chemical orientation of proton pumps

To orient bacteriorhodopsin in the polymer membrane, the mixture of polymer molecules and the protein were exposed to a series of buffers in different pH conditions during the phase of protein incorporation into the polymer. Bacteriorhodopsin was mixed with polymer molecules as described. The buffer solution that was introduced during the polymersome formation phase possessed a pH range from 2.30 to 8.0 units. Since, bacteriorhodopsin is known to sustain its activity even at pH levels of 0 [23] and the exposure length of the proteins to the wide range of pH conditions was short in duration and only at the onset of polymersome formation, most of the proteins embedded in the purple membrane were considered to be preserved from the orientation procedure as measured by sustained activity. Extreme pH conditions lower than 2.35 were found to be effective in incorporating bacteriorhodopsin in the physiological orientation, characterized by an external (extracellular) proton pumping direction.

Manipulation of surface charges of the hydrophilic ends of the ABA polymer (PMOXA) found with polymer vesicles was a key factor in determining the orientation of purple membrane patches in the vesicles. This phenomenon occurred in a similar manner as previously observed with the interaction between the surface charge of lipid vesicles and the lipids of the purple membrane [24–28]. Fig. 3 shows that the change in pH was equal to the difference between the initial inner pH of the vesicle (cytoplasmic side) and the final outer pH of the vesicle. A positive ΔpH meant that bacteriorhodopsin was pumping protons towards the luminal side, lowering the internal pH of the vesicles. Activity plots of polymersomes prepared in a buffer of pH 7.3 showed the unidirectional pumping of purple membranes from the external side to the luminal side of the vesicles, arriving at $+0.69$ units in 6 min (Fig. 2(A)). This rate of change in ΔpH corresponded to the reported value of turnover rate of bacteriorhodopsin (100 protons per second) [29]. However, in sample B (prepared at pH 3.0), the partial orientation of purple membrane resulted in a delayed, as well as lower change in ΔpH of $+0.559$ units at 9.5 min. In this study, the critical point of orienting purple membrane in the polymer matrix in the physiological orientation occurred between pH 2.5 and 2.35. Previous studies have reported the directional change of the proton pumping from artificial bacteriorhodopsin assemblies prepared in similar manner in the range of pH 2.75–3.52 [24–28]. With variable rates in the change of ΔpH and fluctuations, it was not feasible to define the trend in the orientation in between these pH conditions. Sample D (prepared at pH 2.35) showed the activity of the

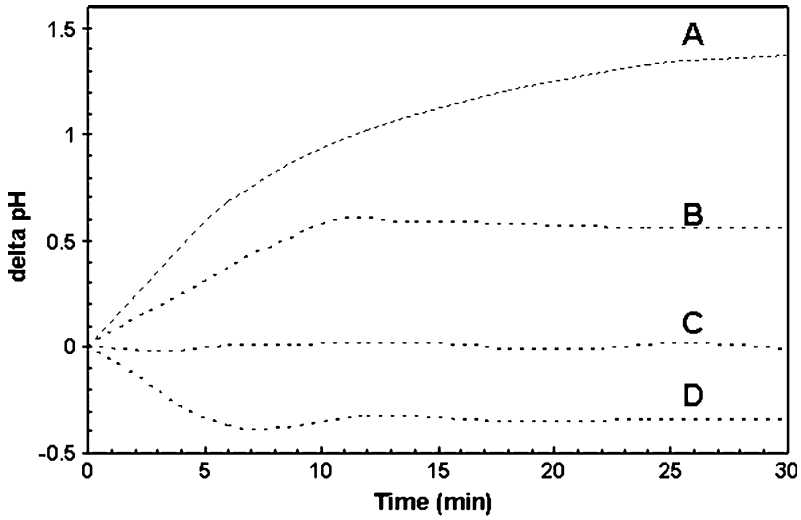

Fig. 3. Chemical orientation of purple membrane. Variation of the external pH conditions surrounding polymeric vesicles resulted in the ability to manipulate the proton pumping directionality. As the UV cross-linked thin polymers produced optimal protein activity, they were used for the orientation trials. (A) Using a buffer with pH 7.3, a maximum delta pH was observed to be +1.37 pumping in the intracellular (non-physiological) direction. (B) Using a buffer with pH 3.0, a maximum delta pH was observed to be +0.59 pumping in the intracellular (non-physiological) direction. (C) This sample served as a control for D with proteopolymersomes measured without illumination with an identical preparation for sample D. (D) Using a buffer with pH 2.35, a maximum delta pH was observed to be −0.38, which indicates the net proton efflux to the external side of the vesicles (physiological orientation).

physiologically-oriented proteins, reaching a maximum ΔpH of −0.38 units at 6 min. The acidic medium of very low ionic strength was believed to be effective in orienting bacteriorhodopsin as in living cells. Normally the preferred orientation of bacteriorhodopsin in in vitro conditions is the opposite of the naturally occurring direction. Previous studies reported the success in orienting bacteriorhodopsin in the naturally occurring direction by lowering the pH of the medium at the phase of the protein insertion to the lipid membranes depends on lowering the negative charge of phosphate head group of lipid [24,26,27]. Similar orientation of bacteriorhodopsin results were reported when polymeric spacer was used to modify the surface charge of the carrier beads before the deposition of lipid and the protein [25]. By chemically lowering the negative charge density of the polymer membranes, the 'right-side-out', or physiologically-oriented incorporation of bacteriorhodopsin in purple membrane form was achieved. Note that the unidirectional pumping of sample A exhibited a logarithmic increase in ΔpH while samples B and D exhibited a linear increase until a saturation/equilibrium point was reached, characterized by the counter-directional pumping of protons to prevent any further pH change. The control (sample C) which had been prepared in the same manner as sample D showed negligible change in ΔpH ($\leq$0.02) in the absence of light which indicated impermeability of the polymer membrane to protons.

In addition to purple membrane/polymer hybridization, it was also shown that individual bacteriorhodopsin molecules, or monomers, extracted out of the native purple membrane of *H. halobium* could be successfully reconstituted into the polymer matrix for functionality assays. The proteins were extracted from purple membrane as monomers using further processing steps, and the exposure time of the proteins to polymer/ethanol mixture was shortened to 2 h since the membrane protein monomers were suspected to be less robust without the chemical and mechanical support of the surrounding membranes as in purple membrane. As shown in Fig. 4, varying pH change rates and levels were observed according to the properties of the polymer in which the protein was inserted. It was observed that bacteriorhodopsin monomers exhibited a slower change in pH of 0.153 in 7 min while residing in the thicker polymer. This could have been attributed to several reasons, including decreased efficiencies in passively orienting the BR monomers compared with the ability to passively orient larger sheets of BR present in purple membrane by taking

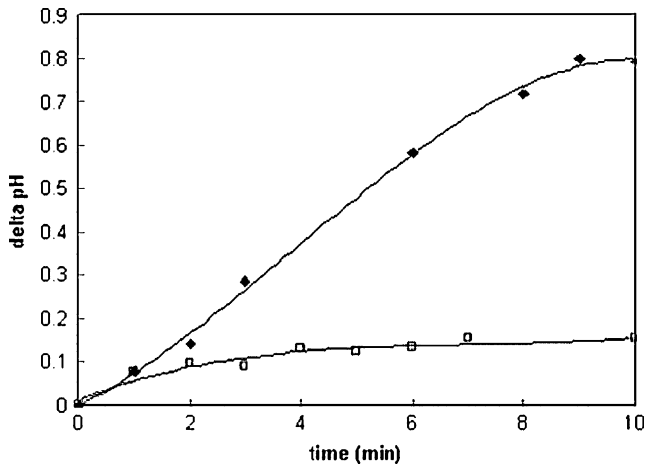

Fig. 4. Proton pumping activity of bacteriorhodopsin in varying polymer structures. With the potential light shielding effect of the thick polymer matrix, bacteriorhodopsin had a slower change in pH of 0.153 in 7 min (–□–). However, when there was a high number of bacteriorhodopsin reconstituted in the short polymer matrix (77.46% was estimated to be functionally incorporated), the change in ΔpH was as high as 0.802 in 9 min (–■–). The controls without proteins had proton leakage fluctuations within $\leq$0.01 in both types of polymers.

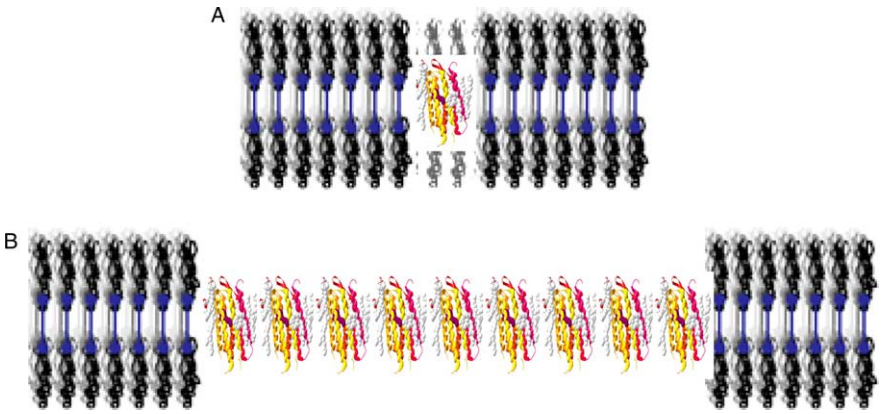

Fig. 5. Depiction of BR monomer and purple membrane embedded within copolymer membrane. (A) Insertion, or burying of BR monomers amongst copolymer molecules may result in a higher likelihood of polymeric shielding when compared with the reconstitution of purple membrane patches (B) within the copolymer where densely packed arrays of BR that reside within their native cell membranes are not buried within the copolymer molecules.

advantage of asymmetric purple membrane charge properties [28]. As such, this would have possibly resulted in commonly observed 50/50 monomer orientations to decrease unidirectional pumping capabilities. In addition, polymer shielding effects could have also played a role in light scattering while not allowing the maximum amount of light to reach and activate the protein [21,22]. The shielding effects seemed to be far greater with bacteriorhodopsin monomers compared with polymers containing reconstituted purple membrane. This could be exhibited by the maximum pH change of 0.802 exhibited by the 4 nm thick PMOXA–PDMS–PMOXA vesicles containing BR monomers, uncross-linked pH changes of less than 0.1, as well as 1.37 exhibited by samples containing purple membrane reconstituted within copolymers comprised of the 4 nm thick triblock structure. This was an expected outcome in both the long and short polymers as the embedded monomers were typically buried within and intercalated between the copolymer molecules while purple membrane contains a major portion of proteins that reside within their native membrane as patches, and are not surrounded by and in direct with copolymer molecules thus reducing shielding capabilities. More specifically, and structurally speaking, BR monomers inserted within the polymer can be thought of as individual molecules that are buried amongst the copolymer molecules, and are bordered by polymer molecules on all sides, increasing the likelihood of shielding effects, or transmembrane ion leak at these borders. However, purple membrane inserted into the polymer are actually large sheets of BR that are already oriented, and with exception to the edges of the purple membrane, none of the proteins are buried within the polymer network, but in fact, are residing in their native environment and are not shielded from light. In addition, they are not subject to the morphological and textural properties unique to the artificial polymeric membranes that may also contribute to transmembrane ion flux at the protein–polymer junction (Fig. 5). Overall, the perceived benefit of embedding the purple membrane into the polymer network represents the ability to functionalize the abiotic polymer with biological activity which still possesses enhanced robustness over a vesicle that is completely made from lipids due to the cross-linking capabilities of the copolymer material. In addition, we have recently shown that the ability to insert proteins into polymeric matrices may also be dependent upon the thickness of the polymer membrane as there is a potential correlation between membrane thickness and fluidity properties which regulate biomolecule insertion efficiencies. For example, using Langmuir isotherm studies, subphase additions of protein to the 8.4 nm-thick polymer resulted in a surface pressure change of 6.62 mN/m [30]. However, when there was a high number of bacteriorhodopsin reconstituted in the short polymer matrix, surface pressures in excess of 7.33 mN/m were observed which indicate a substantial increase in proteins that could be incorporated. In addition, the change in ΔpH was as high as 0.802 in 9 min in the 4 nm-thick membranes (77.46% was estimated to be functionally incorporated). This observation was also additionally believed to be due to the decreased shielding of light from the bacteriorhodopsin due to the much thinner membrane structure (4 nm) which improved the conditions for light activation of the protein. The controls without proteins had proton leakage fluctuations within ≤ 0.01 in both types of polymers.

4. Conclusions

This work has demonstrated the rapid reconstitution of the bacteriorhodopsin protein into block copolymeric membranes that simulate natural biomembranes. Though conventional bacteriorhodopsin refolding into lipid vesicles for optical measurement of proton pumping requires multiple dialysis steps that can take as many as 4 days to complete, bacteriorhodopsin incorporation into polymer membranes as well as functionality recovery have been accomplished in as rapidly as 2 h. In addition, we have shown that the orientation of the reconstituted protein can be controlled based upon the pH of the surrounding medium during insertion due to chemical orientation of the asymmetric membrane charge density. Measurement and dependence of protein functionality on membrane properties such as block composition as well as block lengths were also observed. Methodologies for rapid protein activity assays will be increasingly important as the

emerging field of bionanotechnology has begun to evaluate the feasibility of using membrane proteins as core technologies for practical devices. Polymer–protein hybridization techniques such as those discussed in this work provide a promising approach towards meeting the demands required of these novel methodologies.

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References

[1] Racker E, Stoeckenius W. Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphate formation. J Biol Chem 1974;249:662–3.
[2] Dioumaev AK, Brown LS, Shih J, Spudich EN, Spudich JL, Lanyi JK. Proton transfers in the photochemical reaction cycle of proteorhodopsin. Biochemistry 2002;41:5348–58.
[3] Brown LS, Chamorovsky SK. Light adaptation blockage in dehydrated purple membrane does not result from retinal isomerization inhibition. J Photochem Photobiol B 1993;18:123–6.
[4] Oesterhelt D. The structure and mechanism of the family of retinal proteins from halophilic archaea. Curr Opin Struct Biol 1998;8:489–500.
[5] Baudry J, Tajkhorshid E, Schulten K. Complementarities and convergence of results in bacteriorhodopsin trimer simulations. Biophys J 2004; 87:1394–5.
[6] Birge RR. Photophysics and molecular electronic applications of the rhodopsins. Annu Rev Phys Chem 1990;41:683–733.
[7] Downie JD, Smithey DT. Measurements of holographic properties of bacteriorhodopsin films. Appl Opt 1996;35:5780–9.
[8] Okamoto T, Yamaguchi I, Boothroyd S, Chrostwiski J. Novelty filter that uses a bacteriorhodopsin film. Appl Opt 1997;508–11.
[9] Ho D, Chu B, Lee H, Montemagno CD. Nanoscale hybrid protein/polymer functionalized materials. Proc Int Soc Opt Eng 2004;5389:192.
[10] Ho D, Chu B, Lee H, Kuo K, Montemagno CD. Block copolymer-based biomembranes functionalized with energy transduction proteins. Proc Mat Res Soc 2004;823:W11.8.1.
[11] Stoeckenius W. A model for the function of bacteriorhodopsin. Biochem Biophys Acta 1979;505:215.
[12] Graff A, Sauer M, Van Gelder P, Meier W. Virus-assisted loading of polymer nanocontainer. Proc Natl Acad Sci USA 2002;99:5064.
[13] Nardin C, Widmer J, Winterhalter M, Meier W. Amphiphilic block copolymer nanocontainers as bioreactiors. Eur Phys J E 2001;4:403.
[14] Meier W, Nardin C, Winterhalter M. Reconstitution of channel proteins in (polymerized) ABA triblock copolymer membranes. Angew Chem Int Ed 2000;39:4599.
[15] Oesterhelt D, Stoeckenius W. Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. Nat New Biol 1971;233:149–52.
[16] Oesterhelt D, Stoeckenius W. Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. Methods Enzymol 1974;31:667–78.
[17] Dencher NA, Heyn MP. Preparation and properties of monomeric bacteriorhodopsin. Methods enzymol 1982;88:5–10.
[18] Ho D, Chu B, Schmidt JJ, Brooks EK, Montemagno CD. Hybrid protein–polymer biomimetic membranes. IEEE Trans Nanotechnol 2004;3(2): 256–63.
[19] Ho D, Chu B, Lee H, Montemagno C. Protein-driven energy transduction across polymeric biomembranes. Nanotechnology 2004;15:1084–94.
[20] Hazard A, Montemagno C. Improved purification for thermophilic F_1F_0ATP synthase using *n*-dodecyl β-D-maltoside. Arch Biochem Biophys 2002;407:117–24.
[21] Nardin C, Hirt T, Leukel J, Meier W. Polymerized ABA-triblock copolymer vesicles. Langmuir 2000;16:1035.
[22] Ho D, Chu B, Lee H, Brooks EK, Kuo K, Montemagno CD. Fabrication of biomolecule-copolymer hybrid nanovesicles as energy conversion systems. Nanotechnology 2005;16:3120–32.
[23] Hong FT. Interfacial photochemistry of retinal proteins. Prog Surf Sci 1999;62:188.
[24] Happe M, Teathera RM, Overath P, Knobling A, Oesterhelt D. Direction of proton tanslocation in proteoliposomes formed from purple membrane and acidic lipids depends on the pH during reconstitution. Biochim Biophys Acta 1977;465:415–20.
[25] Rothe U, Aurich H, Engelhardt H, Oesterhelt D. Oriented incorporation of bacteriorhodopsin into the lipid shell of phospholipids-coated polymer particles. FEBS 08356 1990;2:308–12.
[26] Ihara K, Mukohata Y. Proteoliposomes with right-side-out oriented purple membrane/bacteriorhodopsin require cations inside for proton pumping. FEB 06502 1988;1 and 2:148–52.
[27] Hellingwerf K, Scholte B, Van Dam K. Bacteriorhodopsin vesicles: an outline of the requirements for light-dependent H^+ pumping. Biochim et Biophys Acta 1978;153:66–77.
[28] Ovchinnikov YA. Rhodopsin and bacteriorhodopsin: structure-function relationships. FEBS Lett 1982;148:179–91.
[29] Vsevolodov N. Biomolecular electronics: an introduction via photosensitive proteins.: Birkhauser Boston; 1998 p. 73–74.
[30] Ho D, Chang S, Montemagno CD. Nanomedicine: Nanotechnology, Biology and Medicine, in press.