Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 305 (2003) 116–121
BBRC
www.elsevier.com/locate/ybbrc

Fabrication and photoelectric response of poly(allylamine hydrochloride)/PM thin films by layer-by-layer deposition technique

Jinfang Chu, Xingchang Li, Jianping Zhang, and Ji'an Tang*

Key Laboratory of Colloid and Interface Science, Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

Received 8 April 2003

Abstract

Thin films of poly(allylamine hydrochloride) (PAH) and bacteriorhodopsin (bR) embedded in purple membrane (PM) have been prepared by layer-by-layer (LBL) self-assembly technique. The results obtained by UV–Vis spectroscopy and atomic force microscopy (AFM) analysis showed that the biological activity of bR was preserved and PM fragments could be well oriented onto the ITO substrate. A photo-electrochemical cell with the structure of ITO/$(PAH/PM)_n$/electrolyte (0.5 M KCl)/Pt was fabricated and studied. The photocurrent peaks of $(PAH/PM)_6$ corresponding to light-on and light-off were about 200 and 100 nA/cm^2, respectively, with the former enhanced 30% higher than that of the reference films made of $(PDAC/PM)_6$.

Keywords: Bacteriorhodopsin; Purple membrane; Layer-by-layer; Photocurrent; Films

In recent years, studies on the characterization, modification, and processing of biomaterials for device applications have been receiving technological importance. Among them, the study of bacteriorhodopsin (bR) protein and the elucidation of its function as a light driven proton pump represents one of the most interesting examples [1–3]. bR is a chromophore protein in the purple membrane (PM) isolated from *Halobacterium salinarium* and is a single-chain polypeptide with covalently bound chromophore retinal [4]. When this protein is activated by light, it undergoes a photocycle through which a proton is transported from the cytoplasmic side of the membrane to the extracellular side, and the resulting gradient of proton in the purple membrane generates electrochemical energy, which provides the electrical responses differently according to the illuminated light intensity change [5,6]. The photoelectric property can be utilized for photoelectric picture converters, such as motion sensors [7] and artificial retinas [8]. In order to obtain high response and excellent stability of bR films that are used in electric devices, the formation of organized molecular films of bR is required.

The Langmuir–Blodgett (LB) film technique and the electrophoretic sedimentation (EPS) technique have been commonly applied to the oriented film fabrication of bR [9,10]. Self-assembly (SA) technique was also used to oriented bR films for studying their photoelectric property [11]. Recently, He et al. [12,13] used a layer-by-layer (LBL) assembling method first to fabricate the oriented films of bR based on the self-adsorption of positively polycation and negatively charged purple membrane. LBL method [14,15] offers several advantages compared with other techniques mentioned above, such as it can be easily applied to water-soluble bR protein without mixing it into soya lecithin used in LB films; it does not require special instruments; it can be operated simply and be free of the limit of shape of substrate. He et al. have demonstrated that the layer-by-layer adsorption technique can keep bR from denaturation and should be an effective and facile method for the fabrication of a host of bioelectronic and

* Corresponding author. Fax: +86-10-8261-2484.
E-mail addresses: chujinfang@yahoo.com.cn (J. Chu), jatang@iccas.ac.cn (J. Tang).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00710-1

biooptical ultimately devices. In order to improve the performance of bR devices by the LBL technique, PAH was used as a polycation in the present paper. The characteristics of PAH/PM multilayer films were investigated by UV–Vis spectrophotometer and atomic force microscopy (AFM). The photocurrent responses were studied with the photoelectric measurement equipment (home-made) and the optimal conditions were obtained.

Materials and methods

Poly(allylamine hydrochloride) (PAH) and poly(dimethyldiallylammonium chloride) (PDAC) were purchased from Aldrich Chemical Co. and used without further purification. PAH was dissolved in doubly distilled water containing 0.5 M NaCl, with a concentration 2 mg/mL, and pH of the solution being 6.4. Solid supports used for depositing the PAH/bR composite films: quartz slides (10×40 mm) were used for UV–Vis absorption measurements; ITO glass slides (10×40 mm) were used for photoelectric property measurements and mica slices were applied for AFM analysis. In order to get a net negatively charged surface, all of the solid supports were treated according to that described in [12]. A halogen lamp (150 W) was used to investigate the photoelectric properties of the PAH/PM films and the stimulated light was white light (100 mW/cm^2). Other equipments were picoammeter (0.1 nA range) and a digital storage oscilloscope (20 MHz, Gold Star). The effective illumination area of PAH/PM films used in measurement was about 0.25 cm^2.

Growth, purification, and sample preparation of bacteriorhodopsin. The strain of *H. salinarium* was the ET1001 (also known as JW-1) strain. Growth, purification, and sample preparation of bacteriorhodopsin (bR) embedded in the purple membrane (PM) were implemented in our laboratory according to the standard method [16] with certain improved procedures. The main procedures were described as follows: the selected strain was inoculated into growth medium which has been autoclaved before inoculation and then the *Halobacterium* was cultured at 37 °C by ventilating with constant shocking. The cells were harvested after 7 days of incubation, then DNAse was added, and the PM suspension was dialyzed and washed. In order to get a good quality sample, sucrose density gradient was required. The sample was stored at 4 °C in doubly distilled water and the absorption ratio of $A_{280\ nm}/A_{570\ nm}$ was below 2.0, which suggested that it was a good quality sample. In this study, the PM fragments with a concentration of about 0.5 mg/mL bR were ultrasonically comminuted for about 200 s (power is 300 W), and pH of the PM suspension was adjusted to 9.4 using NaOH (0.1 M) solution.

Preparation of PAH/PM films by LBL method. ITO glass slide after negatively charged treatment was immersed into PAH aqueous solution (2 mg/mL, pH 6.4) for 5 min, rinsed with doubly distilled water (pH 6.4) for 1 min, and then dried with nitrogen flow, following which, the modified slide was immersed into PM suspension (about 0.5 mg/mL bR, pH 9.4) for 5 min, rinsed with doubly distilled water (pH 9.4) for 1 min, and then it was dried with nitrogen again. In this way, we obtained one bilayer of PAH/PM films that was marked as $(PAH/PM)_1$. This process was repeated until the required films were obtained. All adsorption steps were carried out at room temperature.

Photo-electrochemical cell. ITO (indium-tin-oxide) with PAH/PM films was used as working electrode and platinum wire as a counter electrode. The distance between the two electrodes in the photo-electrochemical cell was 0.8 cm and 0.5 M KCl aqueous solution was used as the supporting electrolyte, with a pH of 7.3.

Results and discussion

Studies of PAH/PM multilayers

It is well known that purple membrane (PM) fragments have the cytoplasmic and extracellular sides. In neutral and alkaline environments, the two sides are both negatively charged, so in this experiment, negatively charged PM fragments can be easily adsorbed onto the ITO glass surface with positive PAH layer. Fig. 1 shows the UV–Vis absorption spectra of the alternative PAH/PM multilayers when every bilayer was assembled onto the substrate. From this figure it can be seen that the bR embedded in the PM fragments is not denatured during the deposition process because the characteristic absorption at about 552 nm is still observed distinctly. There is about a 10 nm blue shift compared with the peak absorption of PM fragment suspension at 563 nm. The result of blue shift is consistent with bR LB films [17]. This phenomenon was explained as the dehydration effect on the Schiff base of the retinal chromophore in bR in the dried PAH/PM films [18], while in the situation with sufficient water, a red shift will be observed and the peak absorption can be recovered to that of PM solution [19]. This figure also shows that the characteristic absorbance at 552 nm is increased with the number of PAH/PM bilayers. The inset of the figure makes it clear that there is a linear relation between the number of PAH/PM bilayers and the UV–Vis absorbance. It suggests that each bilayer of PAH/PM multilayer films has equal thickness at each deposition step and the transfer amount of bR in PM fragments is equal. So we can conclude that the PAH/PM multilayer films are fabricated uniformly that can be applied to biological devices as a new kind of ultrathin films.

AFM analysis

The topographies of each bilayer of PAH/PM multilayers films were investigated by AFM in tapping mode. Because the mica has a hydrophilic surface with negative charge, polycation PAH (pH 6.4) can be easily adsorbed onto it by electrostatic attractive force, and then at appropriate conditions (concentration, pH, and ion intensity), electronegative PM fragments can also be spontaneously deposited onto positively charged PAH layer sequentially. It can be seen from Fig. 2A that polycation PAH is uniformly absorbed onto mica wafer and that the PAH layer surface is extremely smooth with almost no bulge observed from its three-dimensional image, which is consistent with that described in the literature [20]. Fig. 2B–D shows the topographical images of two, four, and eight bilayers of PAH/PM multilayer films (PM is the outer layer). In general, the multilayer films are fairly uniform and

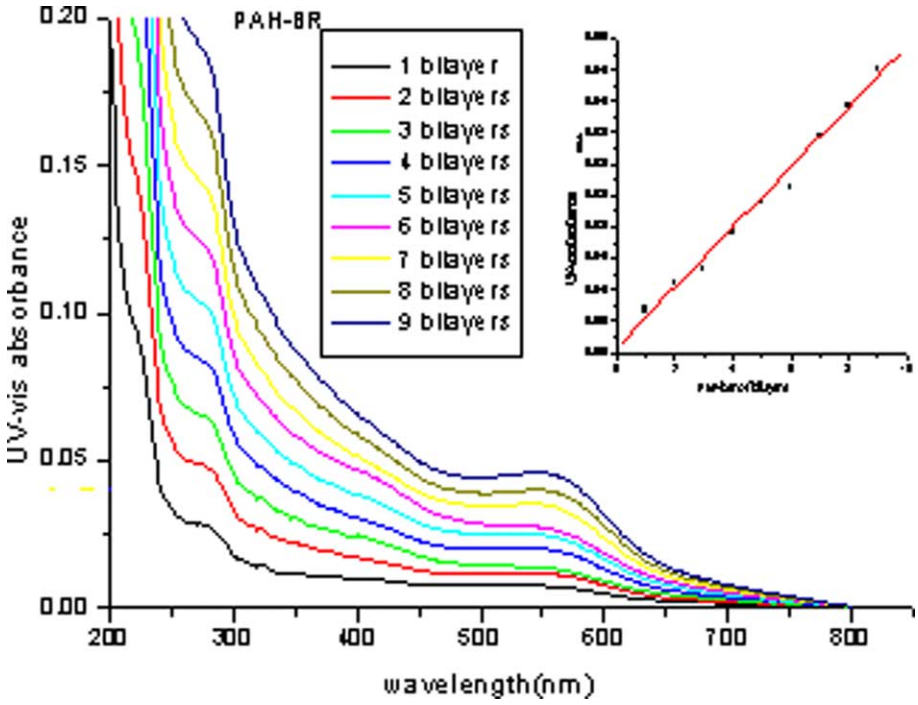

Fig. 1. UV–Vis absorption spectrum of PAH/PM multilayer films. The curves, from bottom to top, represent the adsorption of 1–9 bilayer of PAH/PM multilayers, respectively. The inset indicates the increase of absorbance of 552 nm with the number of bilayers.

smooth, while multitude of small domains can be observed, which are probably due to the different sizes of PM fragments and measly overlap of PM. Compared with the surface topography of bR film fabricated by LB and EPS technique, less aggregates of PM fragments are found by the LBL assembly and uniformity and the degree of bR orientation are much better [21,22].

Photoelectric properties

In alkaline environment, the cytoplasmic side is more negatively charged and is more strongly absorbed onto the PAH layer. So, for these fabricated PAH/PM films, the CP side is next to the PAH layer and this structure is in favor of transferring the proton. Fig. 3 shows a typical photoelectric response profile of the PAH/PM multilayer films. Positive and anodic responses are corresponding to light-on and light-off photocurrents that are caused by proton release and uptake, respectively [23]. It is observed that the signal shape is very similar to the typical photocurrent of the PM ultrathin films fabricated by LB and EPS technique [22,24,25]. The typical generated photocurrent indicates that the physiological activity of bR is preserved after electrostatic deposition. The observed photocurrent peaks of light-on and light-off are about 200 and 100 nA/cm^2, respectively, and the decay time is about 300 ms that is much faster than those of LB and EPS fabricated films, which can be explained for the better uniformity and orientation degree.

Fig. 4 shows the curves of the peak magnitude of photocurrent versus the number of bilayers in the PAH/PM assemblies. It is observed that the magnitude of the photocurrent is increased with the number of PAH/PM bilayers. Until to 4–6 bilayer, the photocurrent comes up to the maximum value. When the number of bilayers is over six, the magnitude of photocurrent is going to be decreasing with more bilayers absorbed. The probable reason is that the more bilayers are accumulated, the bigger the films have resistance. In this technique, $(PAH/PM)_6$ films are optimal for the measurement of photoelectric response.

Action spectrum of PAH/PM thin films was investigated in order to verify whether the generated photocurrent was the result of activity of bR or not. The multilayer PAH/PM films were generated by a series of monochromatic light with a series of interference filters that allow different wavelength-light to pass through. The monochromatic light is nearly constant from 400 to 660 nm. The action spectrum comparing with absorption spectrum is shown in Fig. 5. It is observed that the peak of the photocurrent of PAH/PM is consistent with the absorption spectrum peak in the visible light region. The optimal generation wavelength is the monochromatic light of 560 nm. This result shows that the photocurrent of PAH/PM films is due to the proton pumping activity of bR.

In order to compare the different influences between the cationic polymers, the films containing PDAC/PM have been prepared for comparison. $(PDAC/PM)_6$ films

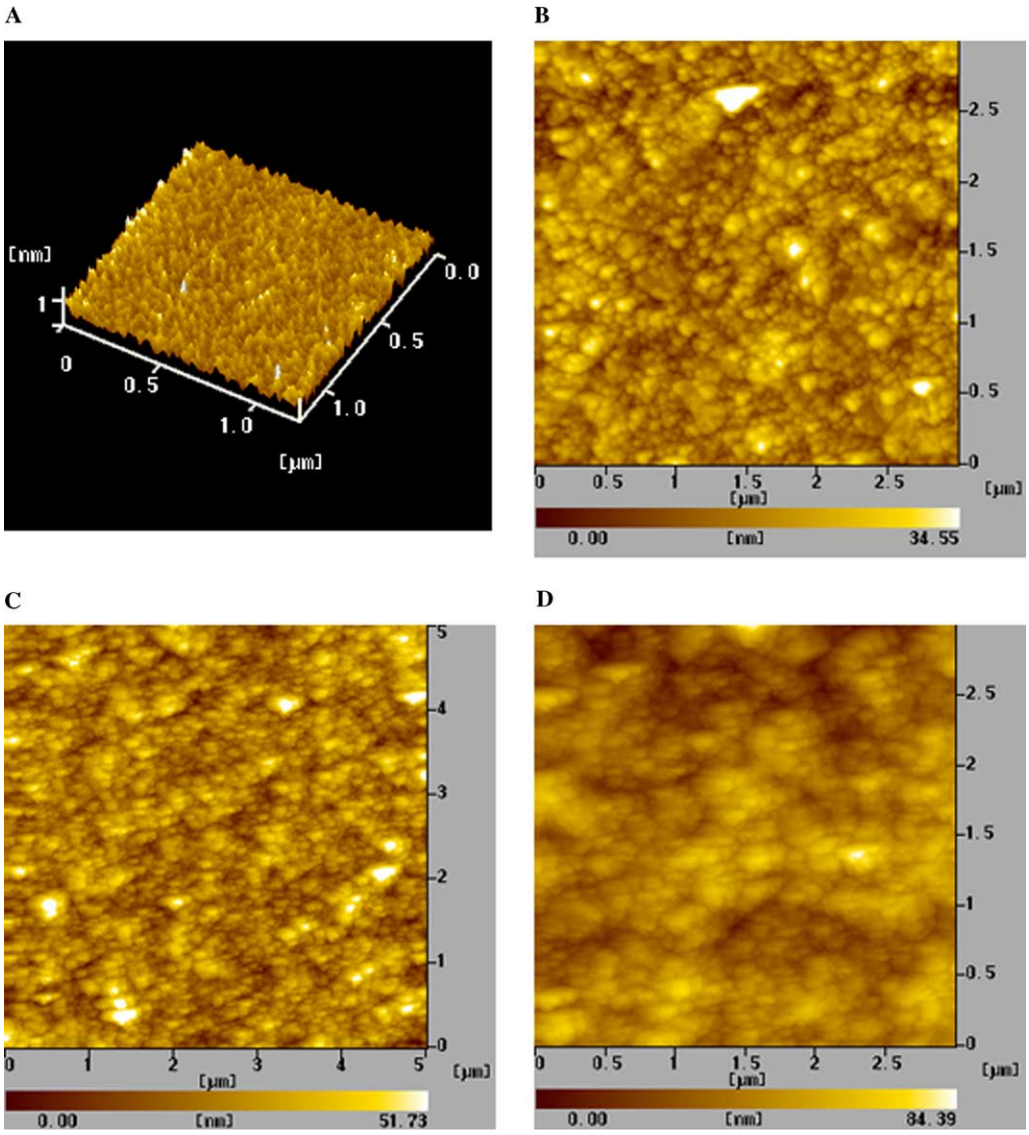

Fig. 2. AFM images of the $(PAH/PM)_n$ multilayer films. (A) PAH layer on a mica wafer surface, (B) two bilayers of PAH/PM films, (C) four bilayers of PAH/PM films, and (D) eight bilayers of PAH/PM films.

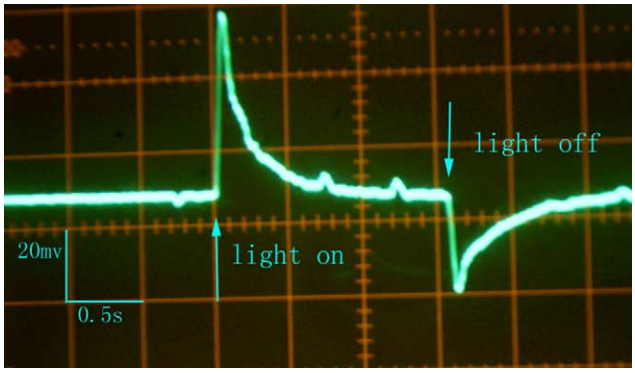

Fig. 3. A typical photoelectric response of the $(PAH/PM)_6$ multilayer films upon the light illumination.

were fabricated with laboratory-cultured material of bR according to that described in reference, and both of $(PAH/PM)_6$ and $(PDAC/PM)_6$ films were fabricated in the same concentration of bR. The photoelectric response of six bilayers of PDAC/PM $(PDAC/PM)_6$ is given in Fig. 6. Compared with Fig. 3, it is observed that the $(PAH/PM)_6$ and $(PDAC/PM)_6$ generate substantially different photocurrents which suggest that the physiological activity of the bR embedded in PM in both systems was preserved after electrostatic deposition. We can see that the light-on photocurrent of the $(PAH/PM)_6$ films is enhanced and increased up to 200 nA/cm^2, which is about 30% higher than that of the $(PDAC/PM)_6$ one under the same irradiation condition. At the same time, however, the light-off current is lower than that of the

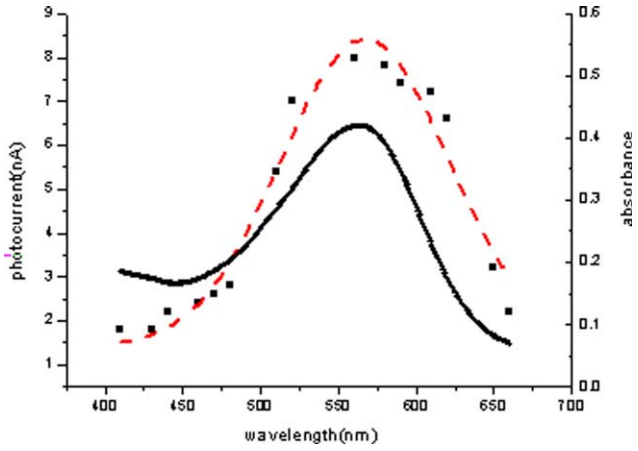

Fig. 4. Action spectrum of PAH/PM multilayer films. Dash curve: peak of photocurrent. Solid curve: absorption spectrum.

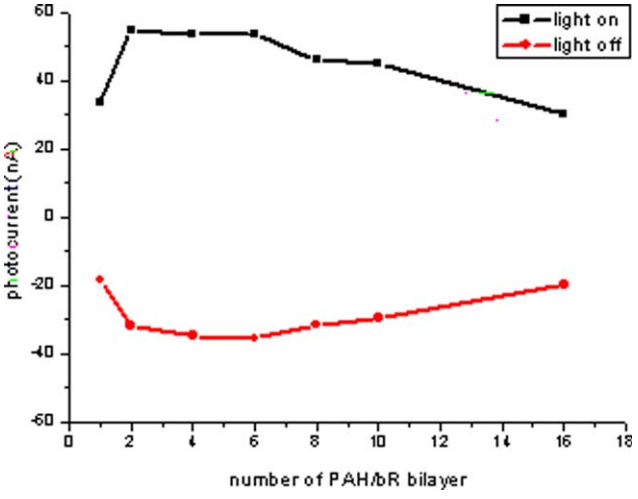

Fig. 5. The curves of photocurrent versus the number of PAH/PM bilayers.

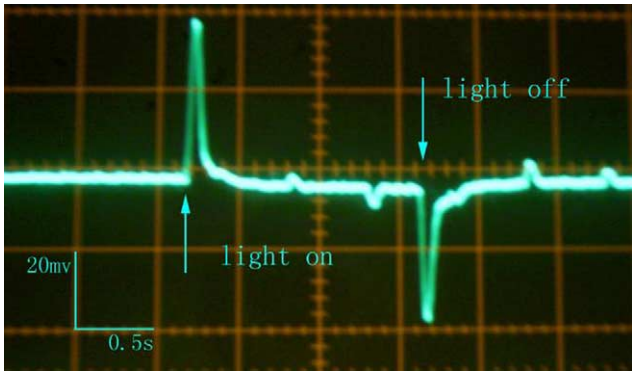

Fig. 6. Photoelectric response of $(PDAC/PM)_6$ multilayer films.

$(PDAC/PM)_6$ films. From the higher light-on photoelectric response, we can know that the photoelectric sensitivity of $(PAH/PM)_6$ films is higher than that of the $(PDAC/PM)_6$ one. As for the lower light-off photoelectric response, we can not give the exact explanation now, but we know that the light-off photocurrent of bR corresponds to the net proton uptake which is determined by the decay of the M intermediate. Because the surface charge densities, steric hindrance of both polycations, and the electrostatic forces between PM and polycations are quite different, this may lead to varying degrees of inhomogeneity in the orientation of both types of multilayers during the electrostatic layer-by-layer deposition process. The influences of different polycations on the performance of bR devices are in progress.

Conclusion

In this work, PAH and PM can be used easily to fabricate well-oriented thin films with the LBL self-assembly technique. The absorption spectrum and photocurrent response of PAH/PM multilayer films indicate that the biological activity of bR is preserved well after process. The light-on photocurrent is enhanced by about 30% as compared with the PDAC/PM one, showing that PAH/PM films are a promising combination for the fabrication of bR molecular devices.

Acknowledgments

This work is supported by Nanometer Devices and Their Fundaments, Key Program of Knowledge Innovation Projection of Chinese Academy of Sciences (KJCX1-SW-07).

References

[1] D. Oesterhelt, C. Brauchle, N. Hampp, Bacteriorhodopsin: a biological material for information processing, Quart. Rev. Biophys. 24 (4) (1991) 425–478.
[2] R.R. Birge, Photophysics and molecular electronic applications of the rhodopsins, Ann. Rev. Phys. Chem. 41 (1990) 683–733.
[3] J.K. Lanyi, Proton translocation mechanism and energetics in the light-driven pump bacteriorhodopsin, Biochim. Biophys. Acta Bioenerget. Rev. 1183 (1993) 241–261.
[4] D. Oesterhelt, W. Stoeckenius, Rhodopsin like protein from purple membrane of *Halobacterium halobium*, Nat. New Biol. 233 (1971) 149–152.
[5] T. Miyasaka, K. Koyama, I. Itoh, Quantum conversion and image detection by a bacteriorhodopsin-based artificial photoreceptor, Science 225 (1992) 342–344.
[6] H. Takei, A. Lewis, Z. Chen, I. Nebenzahl, Implementing receptive fields with excitatory and inhibitory optoelectrical responses of bacteriorhodopsin film, Appl. Opt. 30 (1991) 500–507.
[7] T. Miyasaka, K. Koyama, Image sensing and processing by a bacteriorhodopsin-based artificial photoreceptor, Appl. Opt. 32 (1993) 6371–6379.
[8] L. Lensu, M. Frydrych, C. Aschi, J. Parkkinen, S. Parkkinen, T. Jaaskelainen, Towards color sensitivity of protein based artificial retina, Comput. Nanosci. 1 (2001) 17–20.

[9] T. Miyasaka, Y. Maekawa, K. Koyama, A novel photoreactive amphiphile of nitrophenylazide for immobilization of bioactive proteins, Thin Solid Films 180 (1989) 73–83.
[10] A.A. Kononenko, E.P. Lukashev, A.V. Maximychev, Oriented purple membrane films as a probe for studies of the mechanism of bacteriorhodopsin functioning. The vectorial character of the external electric-field effect on the dark state and the photocycle of bacteriorhodopsin, Biochim. Biophys. Acta 850 (1986) 162–169.
[11] H.G. Choi, J. Min, K.K. Han, Photoelectric conversion of bacteriorhodopsin films fabricated by self-assembly technique, Synt. Met. 117 (2001) 141–143.
[12] J.A He, L. Samuelson, L. Li, J. Kumar, S.K. Tripathy, Oriented bacteriorhodopsin/polycation multilayer by electrostatic layer-by-layer assembly, Langmuir 14 (1998) 1674–1679.
[13] J.A. He, L. Samuelson, L. Li, J. Kumar, S.K. Tripathy, Photoelectric properties of oriented bacteriorhodopsin/polycation multilayer by electrostatic layer-by-layer assembly, J. Phys. Chem. B102 (1998) 7067–7072.
[14] G. Decher, Fuzzy nanoassemblies: towards layered polymeric multicomposites, Science 277 (1997) 1232–1237.
[15] J.H. Fendler, F.C. Meldrum, The colloid chemical approach to nanostructured materials, Adv. Mater. 7 (1995) 607–632.
[16] D. Osterhelt, W. Stoeelcenius, Isolation of cell membRane of *Halobacterium halobium* and its fractionation into red and purple membrane, Methods Enzymol. 31 (1974) 667–678.
[17] S.-B. Hwang, J.I. Korenbrot, W.J. Stoeckenius, Structural and spectroscopic characteristics of bacteriorhodopsin in air–water interface films, Membr. Biol. 36 (1977) 115–135.
[18] M. Ikonen, J. Peltonen, E. Vuorimaa, H. Lemmetyinen, Study of photocycle and spectral properties of bacteriorhodopsin in Langmuir–Blodgett films, Thin Solid Films 213 (1992) 277–284.
[19] P. Hildebrandt, M. Stockburger, Role of water in bacteriorhodopsin's chromophore resonance Raman study, Biochemistry 23 (1984) 5539–5548.
[20] G. Kurth, D. Volkmer, M. Ruttorf, B. Richter, A. Müller, Ultrathin composite films incorporating the nanoporous isopolyoxymolybdate "Keplerate" $(NH_4)_{42}[MO_{132}O_{372}\text{-}(CH_3COO)_{30}(H_2O)_{72}]$, Chem. Mater. 12 (2000) 2829–2831.
[21] J. Min, H.G. Choi, J.W. Choi, W.H. Lee, U.R. Kim, Photocurrent of bacteriorhodopsin films deposited by electrophoretic method, Thin Solid Films 327/329 (1998) 698–702.
[22] J. Min, H.G. Choi, J.W. Choi, W.H. Lee, U.R. Kim, Optimal fabrication condition of bacteriorhodopsin films by electrophoretic sedimentation technique, Supramol. Sci. 5 (1998) 687–690.
[23] K. Koyama, T. Miyasaka, R. Needleman, J.K. Layni, Photoelectrochemical verification of the proton-releasing groups in bacteriorhodopsin, Photochem. Photobiol. 68 (1998) 400–406.
[24] J.P. Wang, J.R. Li, P.D. Tao, X.C. Li, L. Jiang,Photoswitch, based on bacteriorhodopsin Langmuir–Blodgett films, Adv. Mater. Opt. Electron. 4 (1994) 219–224.
[25] H.H. Weetall, L.A. Samuelson, Optical and electrical properties of bacteriorhodopsin Langmuir–Blodgett films, Thin solid Films 312 (1998) 306–312.