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Kinetics of picosecond laser pulse induced charge separation and proton transfer in bacteriorhodopsin

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Abstract. Bacteriorhodopsin (BR) films oriented by an electrophoretic method are deposited on a transparent conductive ITO glass. A counterelectrode of copper and gelose gel is used to compose a sandwich-type photodetector with the structure of ITO/BR film/gelose gel/Cu. A single 30-ps laser pulse and a mode-locked pulse train are respectively used to excite the BR photodetector. The ultrafast falling edge and the bipolar response signal are measured by the digital oscilloscope under seven different time ranges. Marquardt nonlinear least squares fitting is used to fit all the experimental data and a good fitting equation is found to describe the kinetic process of the photoelectric signal. Data fitting resolves six exponential components that can be assigned to a seven-step BR photocycle model: BR→K→KL→L→M→N→O→BR. Comparing tests of the BR photodetector with a 100-ps Si PIN photodiode demonstrates that this type of BR photodetector has at least 100-ps response time and can also serve as a fast photoelectric switch. *© 2003 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.1527626]

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1 Introduction

Bacteriorhodopsin (BR) is a photoactive retinal protein existing in the purple membrane isolated from *Halobacterium salinarium*. As a photoreceptor and photoenergy converter it functions as a light-driven proton pump in the living organism. BR consists of seven α-helix folded peptide chains and an inner retinal chromophore that is covalently bonded to Lys-216 through a protonated Schiff base. Upon absorption of photons, BR initiates a very fast photochemical isomerization of the retinal from all-trans to 13-cis on the femtosecond time scale and then thermally relaxes to its initial state within 10 ms, during which several different conformation intermediates (K, KL, L, M, N, O, etc.) are sequentially passed. Because BR possesses some photochromic and photoelectric properties,[1] it is attractive for many technical applications in optical data storage,[2] optical information processing,[3] artificial retinas,[4] biomolecular electronic devices,[5] etc. As a photoelectric material, BR has three important characteristics compared to other photoelectric materials, e.g., semiconductors. The first is the picosecond photoelectric responsibility, the second is the bipolar photoelectric response signal to short laser pulses, and the third is the differential photoelectric response to alternative light intensity. These characteristics originate from different carrier kinetics in the BR molecule. Here we only deal with the first two characteristics.

The ultrafast photoelectric response is directly caused by the primary photoisomerization of the chromophore, which generates the picosecond charge separation and then induces a displacement current in the circuit. By using dried oriented BR films deposited on transparent conductive glass and directly contacting the sampling head of sampling oscilloscopes, Groma,[6] Trissl,[7] and Simmeth[8] et al. respectively measured the charge separation time in the chromophore with a resolved time of 30, 20, and 5 ps. The theoretical limit should be 3 ps, the formation time of the first intermediate K. After the photoinduced charge separation of the chromophore, the protein conformation change is triggered to conduct the proton pump and the photocycle. In these thermal relaxation steps, protons move from one binding site to another site, which also induces a displacement current in the circuit, but mostly in the opposite direction to that of the photoinduced charge separation. This results in a bipolar photoelectric signal. This protein electric response signal (PERS) has been measured by many groups.[9–12]

We also prepared oriented BR films on the ITO conductive glass. But we did not use direct contact measurement. We constructed a photocell structure with the BR film and a gel buffer layer sandwiched between two electrodes. The most useful advantage of the gel-based BR photocell is its simple structure and ease of preparation compared to the direct contact structure used by other authors. The photoelectric signal is coupled into the oscilloscope through the two electrodes. We respectively used a single 30-ps laser pulse and a mode-locked pulse train to excite the BR photodetector and obtained the ultrafast falling edge and the bipolar response signal. We confirm that this type of BR photodetector has at least 100-ps response time and can also serve as a fast photoelectric switch.

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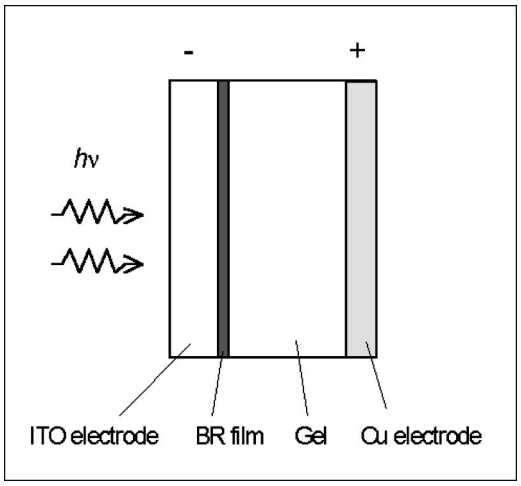

Fig. 1 Structural scheme of the oriented BR film photodetector.

2 Materials and Methods

Bacteriorhodopsin is embedded and crystallized in the purple membrane (PM), which is isolated from the R_1M_1 strain of *Halobacterium salinarium* according to the conventional method.[13] Each PM fragment is ~1 μm in diameter and contains ~10^5 BR molecules. PMs have a large permanent electric dipole moment in the direction of the membrane normal and a negative net charge due to the different negative surface charge densities of the cytoplasmic and the extracellular side, which allow them to be oriented in a moderate electric field. Here the electrophoretic sedimentation method is used. A drop of PM aqueous suspension is placed on a transparent conductive ITO glass and covered with a second electrode 1 mm away. Then a potential (~2 V) is applied between the electrodes. Under the electric field, the PM fragments partly orient, migrate, and finally deposit onto the anode (the ITO elec-

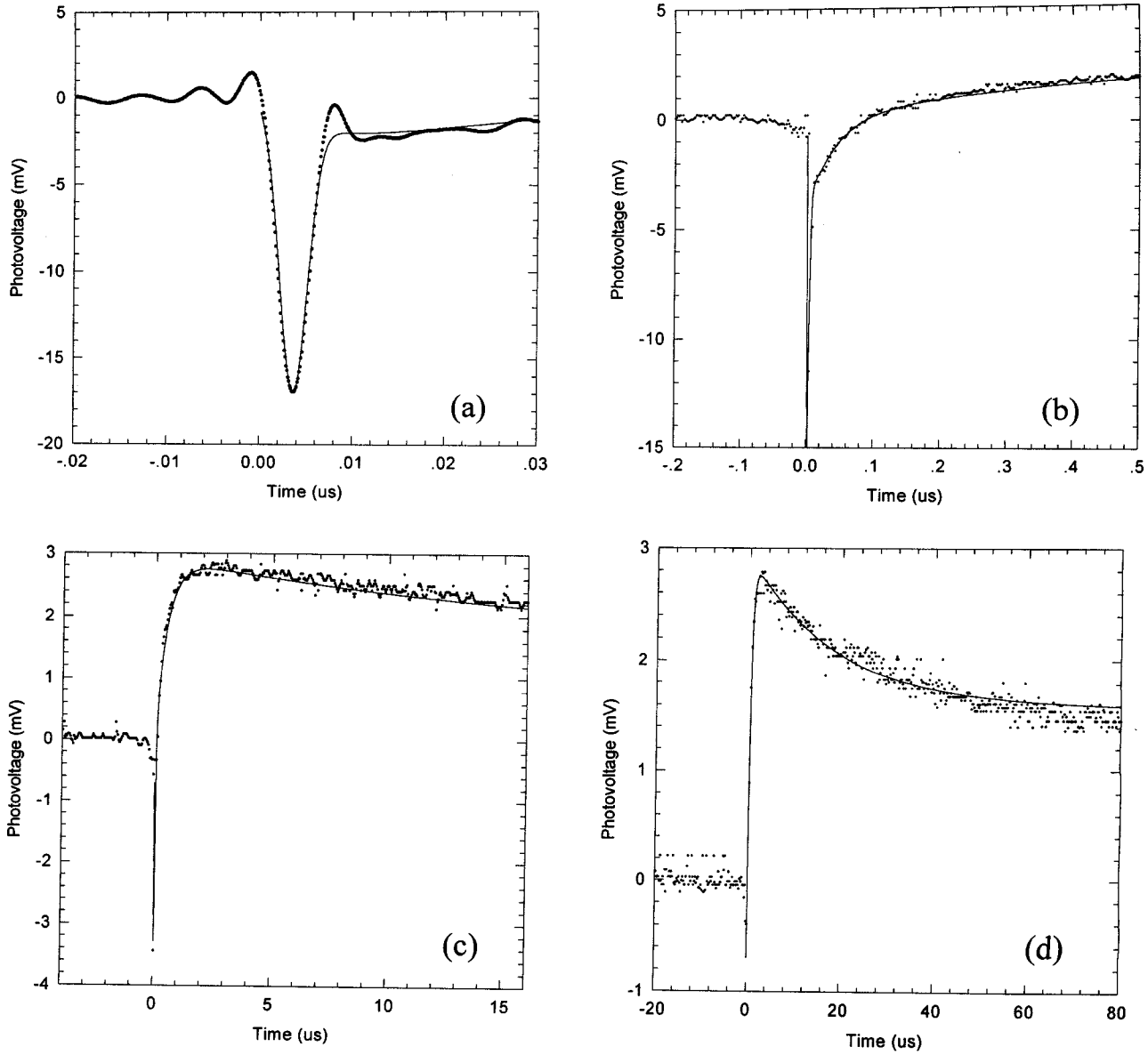

Fig. 2 Photoelectric response signal of the BR photodetector (ITO/BR film/gel/Cu) to a 30-ps laser pulse, measured under seven different time scales [(a) to (g)]. Each curve consists of 500 dots. Dots: experimental data; lines: calculating results by Eqs. (2) and (3) with the parameters in Table 1.

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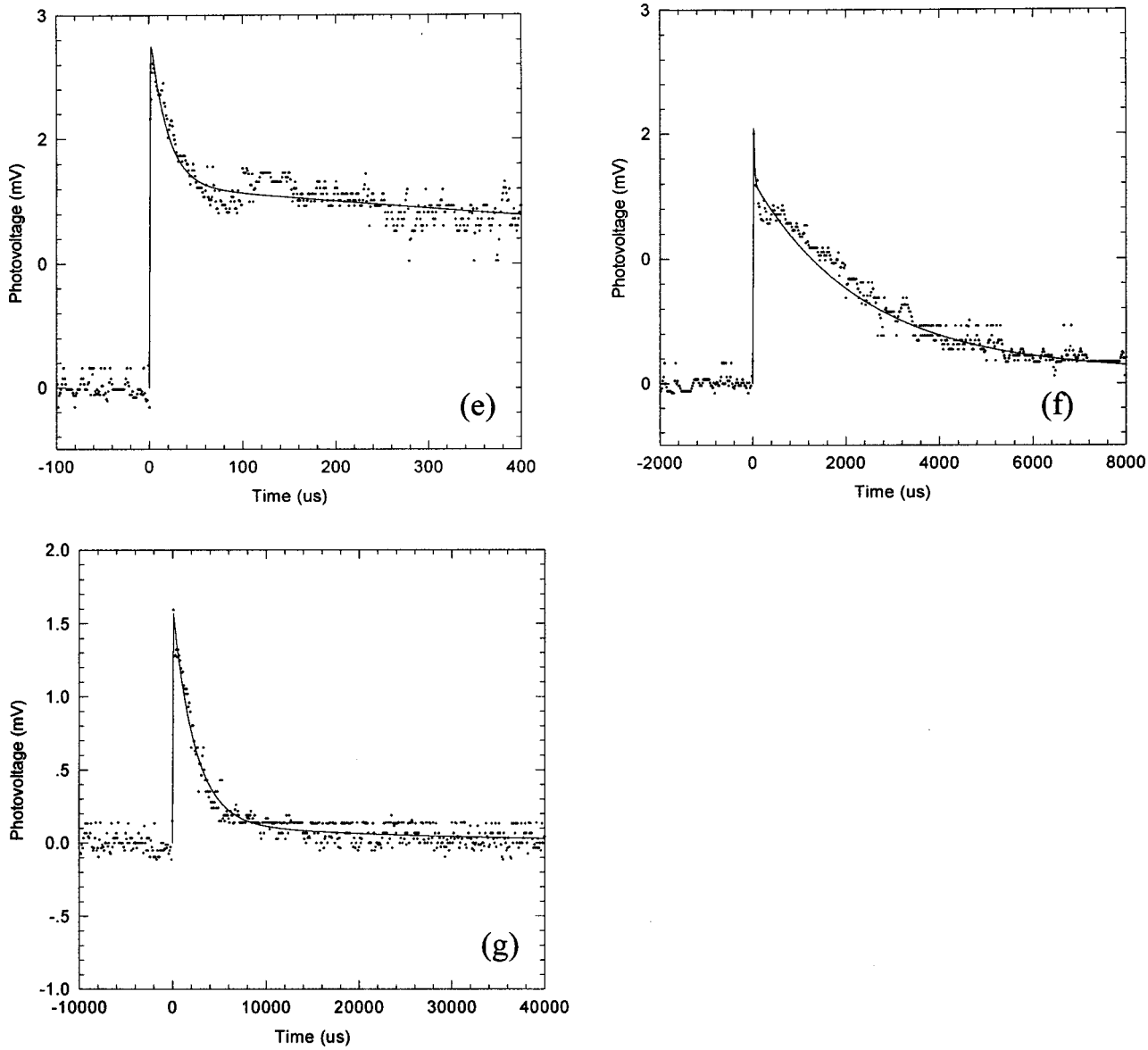

Fig. 2 (***Continued.***)

trode) with the cytoplasmic side toward the electrode. The PM fragments on the anode retain their orientation when the electrodes are separated and the sample is dried. The thickness of the film is a few micrometers. The BR film photodetector is constructed as a sandwich-type electrochemical cell comprising junctions of ITO/BR film/aqueous electrolyte gel (1 mm thick)/counterelectrode (copper) as shown in Figure 1. The viscous gel is composed of 10% carboxymethyl chitin and 1 M potassium chloride (pH6), which are very common conditions used by other authors. The gel mostly influences the resistance and capacitance of the BR photocell depending on the concentration, humidity, and pH value. It affects the measured time constant and the output amplitude. The larger the resistance and capacitance of the gel, the larger the measured time constant and the smaller the output amplitude.

Ultrashort laser pulses are generated by a Nd:YAG CPM oscillator. The mode-locked pulse train is selected by a Pockels cell to output a single pulse, then amplified by Nd:YAG and frequency-doubled by KTP. The output laser pulse is 532 nm, 30 ps, and 10 mJ. The photoelectric response signal of the BR film photodetector is directly coupled into a digitizing oscilloscope (HP54505B, real-time bandwidth 125 MHz). Through an IEEE488 GP-IB interface card, a PC microcomputer running on our specially developed software controls the oscilloscope to acquire data, store waveforms, and process the experimental results. The experiment is performed at room temperature.

3 Results and Discussion

The photoelectric response signal of the BR film photodetector to a 30-ps laser pulse is shown in Figure 2. To observe the whole process and the detail of each time domain, the oscilloscope is set to capture data on different timebases. It can be seen that the signal has two peaks, one negative and one positive, i.e., a bipolar signal. The fast negative peak [Figure 2(a)]

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reflects the ultrafast charge separation of the chromophore. The measured output signal is determined by the convolution of the input and the weighting function of each subsystem,[6] that is,

$$V_{fast}(t) = I_{ex}(t) \otimes \exp(-t/\tau_i) \otimes \exp(-t/RC) \otimes O(t), \quad (1)$$

where $I_{ex}(t)$ is the time distribution of the exciting laser pulse described by a Gaussian function with halfwidth of 30 ps, τ_i is the decay time constant of the PERS signal in a certain transition step of the BR photocycle, RC is the time constant of the measuring circuit, and $O(t)$ is the weighting function of the oscilloscope described by a Gaussian function with halfwidth of ~2.8 ns. Because the bandwidth of the oscilloscope is low, the negative pulse is widely broadened and mainly determined by the RC and the weighting function of the oscilloscope. We cannot resolve the picosecond charge separation time of the chromophore. By using Marquardt nonlinear least squares fitting, we find the best-fitting equation for the negative pulse is

$$V_{fast} = -15.6 \exp[-2.77(t-3.6)^2/3.5^2] + 3.36 \exp(-t/5) - 4.08 \exp(-t/41) + 0.72, \quad (2)$$

where the unit of time is nanoseconds. It is not a pure Gaussian function and contains two exponential components. This indicates that in this time domain (0 to 30 ns) there are two other transition steps going on. According to the values of the time constants, we suppose that 5 ns corresponds to the transition K→KL and that 41 ns corresponds to the transition KL→L.

The slow process of the signal [Figures 2(b) to 2(g)] corresponds to the proton transfer in the BR and is relatively simple to analyze. Because for these transitions the measuring circuit and the oscilloscope are fast enough to respond, according to Eq. (1) the output signal directly reflects the exponential decay processes of the thermal relaxation steps of the BR photocycle. So this slow signal should be a multiexponential process described by the following equation:

Table 1 Decay time constants and amplitudes of the exponential components, fitted from the photoelectric response signal of the BR photodetector.

i	1 (K→KL)	2 (KL→L)	3 (L→M)	4 (M→N)	5 (N→O)	6 (O→BR)
τ_i	5 ns	41 ns	0.48 μs	18 μs	2.35 ms	26.7 ms
u_i (mV)	3.36	−4.08	−2.91	1.30	1.49	0.137

$$V_{slow}(t) = \sum_{i=1}^{6} u_i \exp(-t/\tau_i). \quad (3)$$

To obtain the parameters τ_i and u_i, we still use Marquardt nonlinear least squares fitting to fit the above experimental curves. But it should be noted that it is impossible to use the six-exponential equation in one curve fitting. Because there are too many parameters to be determined, for different time range curves the determined parameters will be very different. Here we adopt multistep correlated regional fitting to determine the parameters of Eq. (3). This is based on the fact that in certain time ranges some relatively faster components can be ignored and some relatively slower components can be treated as constants, therefore the fitting equation can be simplified into two- or three-exponential functions in this time range. Using this method we fit all the experimental data and obtain the parameters listed in Table 1. In Figures 2(b) to 2(g) the solid lines calculate results by Eq. (3) with the parameters in Table 1. It gives a very good fitting to every time range curve. From these data we suppose the six exponential components correspond to the six intermediates of the BR photocycle, which are also listed in Table 1. These time constants may be different from the values obtained by optical measurements in some literature. Because the mechanism of the BR photoelectrical signal is very complicated, we think this difference is mostly from the differences of the measuring and analyzing methods as well as the differences of the sample conditions.

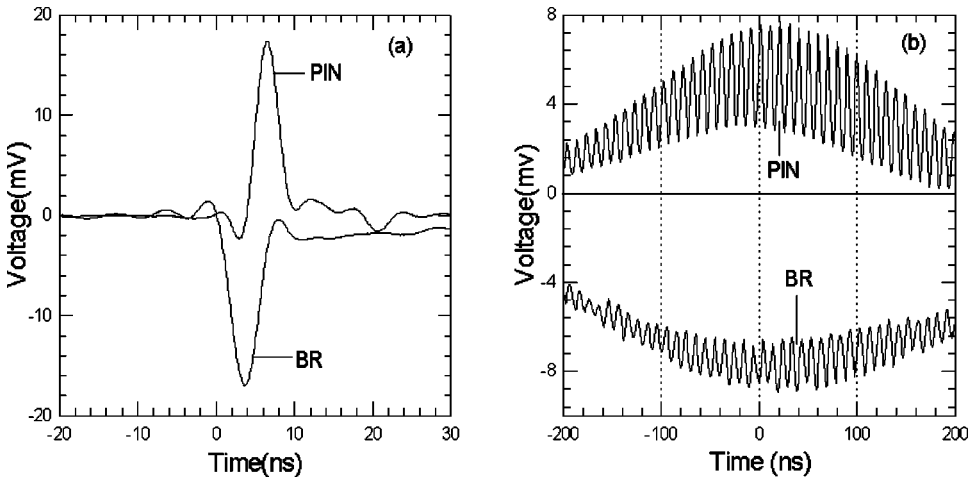

Fig. 3 Comparing measurement between the BR photodetector and a 100-ps Si PIN photodiode to the same 30-ps single laser pulse (a) and the mode-locked pulse train (b).

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To test how fast a BR photodetector with this kind of structure can respond, we make a comparison measurement. We respectively use the BR photodetector and a 100-ps Si PIN photodiode to measure the same single 30-ps laser pulse and the mode-locked pulse train. The results are shown in Figure 3. It clearly shows that the BR photodetector and the PIN photodiode give the same measuring results, which indicates that this BR photodetector has at least 100-ps response time.

To verify the experimental reliability, we constructed another BR photocell with the ITO electrode substituted by a stainless steel slide. This BR photocell has a similar structure to the former, i.e., stainless steel slide/BR film/aqueous electrolyte gel (1 mm thick)/counterelectrode (copper). The photoelectric response signals of this BR photocell to the 30-ps laser pulse and the mode-locked pulse train are similar to the above results. So we believe this fast photoelectrical signal must come from the photoinduced charge separation of the chromophore.

4 Conclusion

We have constructed a sandwich-type BR photocell with a gel buffer layer between two electrodes. The experiment proves this type of BR photodetector has at least 100-ps response time. So it can serve as a fast photoelectric switch. Theoretical analysis and mathematical fitting to the data suggest the kinetics of the photoelectric signals relate to the photoinduced charge separation of the chromophore and the proton transfer in BR. According to the fitting results we suppose this photoelectric process may correspond to the BR photocycle model of BR→K→KL→L→M→N→O→BR.

Acknowledgments

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References

1. N. Vsevolodov, *Biomolecular Electronics: An Introduction Via Photosensitive Proteins*, Birkhauser, Boston (1998).
2. R. R. Birge, N. B. Gillespie, E. W. Izaguirre, A. Kusnetzow, A. F. Lawrence, D. Singh, Q. W. Song, E. Schmidt, J. A. Stuart, S. Seetharaman, and K. J. Wise, ''Biomolecular electronics: protein-based associative processors and volumetric memories,'' *J. Phys. Chem. B* **103**, 10746–10766 (1999).
3. N. Hampp, ''Bacteriorhodopsin as a photochromic retinal protein for optical memories,'' *Chem. Rev.* **100**, 1755–1775 (2000).
4. T. Miyasaka and K. Koyama, ''Image sensing and processing by a bacteriorhodopsin-based artificial photoreceptor,'' *Appl. Opt.* **32**, 6371–6379 (1993).
5. F. T. Hong, ''Interfacial photochemistry of retinal proteins,'' *Prog. Surf. Sci.* **62**, 1–237 (1999).
6. G. I. Groma, G. Szabo, and G. Varo, ''Direct measurement of picosecond charge separation in bacteriorhodopsin,'' *Nature (London)* **308**, 557–558 (1984).
7. H. W. Trissl, G. Gartner, and W. Leibl, ''Reversed picosecond charge displacement from the photoproduct K of bacteriorhodopsin demonstrated photoelectrically,'' *Chem. Phys. Lett.* **158**, 515–518 (1989).
8. R. Simmeth and G. W. Rayfield, ''Evidence that the photoelectric response of bacteriorhodopsin occurs in less than 5 picoseconds,'' *Biophys. J.* **57**, 1099–1101 (1990).
9. L. Keszthelyi and P. Ormos, ''Electric signals associated with the photocycle of bacteriorhodopsin,'' *FEBS Lett.* **109**, 189–193 (1980).
10. A. A. Kononenko, E. P. Lukashev, S. K. Chamorovsky, A. V. Maximychev, S. F. Timashev, L. N. Chekulaeva, A. B. Rubin, and V. Z. Paschenko, ''Oriented purple membrane films as a probe for studies of the mechanism of bacteriorhodopsin functioning II. photoelectric processes,'' *Biochim. Biophys. Acta* **892**, 56–67 (1987).
11. H. W. Trissl, ''Photoelectric measurements of purple membranes,'' *Photochem. Photobiol.* **51**, 793–818 (1990).
12. Y. Huang, Y. Zhao, Z. Zhang, X. Ou-Yang, Q. Gong, L. Chen, F. Li, J. Liu, J. Ding, and Q. Li, ''Light-induced charge separation and transfer in bacteriorhodopsin,'' *Chem. Phys. Lett.* **17**, 301–303 (2000).
13. D. Oesterhelt and W. Stoeckenius, ''Isolation of the cell membrane of Halobacterium halobium and its fraction into red and purple membrane,'' *Methods Enzymol. Biomembr.* **31**, 667–678 (1974).

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