Color Recognition with Bacteriorhodopsin

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We have studied opto-electric properties of wild type bacteriorhodopsin and its two artificial variants. We have measured opto-electric responses with respect to wavelength for all three proteins and we describe the use of the proteins for color detection. Opto-electric responses of proteins to set of colored lights were measured and it has been shown that bacteriorhodopsin and its variants can be used to recognize color. A simple equation for estimating opto-electric response to arbitrary spectrum is given.

1 Introduction

Bacteriorhodopsin is the best understood protein of the class of rhodopsins - light sensitive pigments. Most known member of that family is rhodopsin, protein responsible for the light detection in human eye.

Bacteriorhodopsin has received increased attention from researchers during recent years, and number of different information processing applications which use bacteriorhodopsin (BR) has been reported [1 2 3 4 5 6]. Many groups research upon opto-electric activity of BR for constructing photosensors [7 8 9 10], and specially artificial retinas [7 9 10]. Yet, color sensitivity has not been an issue in such photosensors or it has been realized by using separate filters in front of the sensor as in e.g. CCD cameras.

By reconstituting bleached BR in vitro with synthetic retinal analogues, BR variants with different optical properties can be obtained [11]. In our earlier work [12] we have studied opto-electric properties of BR and its two variants: 4-keto and 3,4-dehydro. As it has been shown also by the others [13 14], both BR variants retained opto-electric activity. The measurements have also shown that the opto-electric response with respect to wavelength was different among the proteins. We have suggested to use the difference of responses for bacteriorhodopsin based color detection and consequently for adding color processing capability to BR based photosensors.

Our research is aimed towards BR based artificial retinas, especially towards color processing capability of such retinas. In this paper we will deal with color recognition ability of BR based photosensor. Firstly, we recall basic

properties of natural form of bacteriorhodopsin and we briefly review the results from our previous study [12]. Then we present color photosensor made of BR and its variants and we demonstrate its ability to recognize colors. Simple equation for estimating opto-electric response of proteins to light with certain spectrum is given too. A comparison of calculated and measured responses for set of colored lights is shown.

2 Bacteriorhodopsin

Bacteriorhodopsin is a photochromic protein found in membrane of microorganism *Halobacterium salinarium*. BR is composed of the protein part bound to the chromophore (retinal) with Schiff base linkage. Natural function of BR is to act as a light-driven proton pump, converting sunlight into chemical or electric energy.

BR has a photocycle with intermediates with different lifetimes and absorption spectra. The transition times vary from picoseconds to milliseconds. In the dark, BR is in a dark-adapted D-state. When illuminated, protein moves to B-state, that has a broad absorption band with maximum at 570 nm. Absorption of a photon causes, protein to move from the B-state through a number of intermediates into the M-state. The M-state has the absorption maximum at 412 nm and it has the longest lifetime of all the excited states. The transition from the B- to the M-state takes approximately 50 μs, during the transition a proton is released from the Schiff base. From the M-state protein thermally decays in approximately 10 ms back to the initial B-state, during the transition the Schiff base is reprotonated. The transition from the M- to the B-state can be also triggered directly by illumination with blue light.

The proton release and acceptance during switching to and from the M-state causes charge shifts within bacteria membrane or oriented protein film. This property is base for construction of opto-electric devices.

3 Materials and methods

BR in our study was used in a form of purple membrane isolated from *Halobacterium salinarium* wild type (S9), the membrane was isolated as described by Oesterhelt and Stoeckenius [15]. Two variants of wild type BR were prepared by reconstituting bleached BR with synthetic retinal analogues: 4-keto and 3,4-dehydro retinals. Opto-electric elements were produced from the three proteins as follows:

Polyvinylalcohol (PVA) films were prepared by mixing 750 μl of 15% PVA with 200 μl of BR solution and spread onto a conductive glass substrate. After

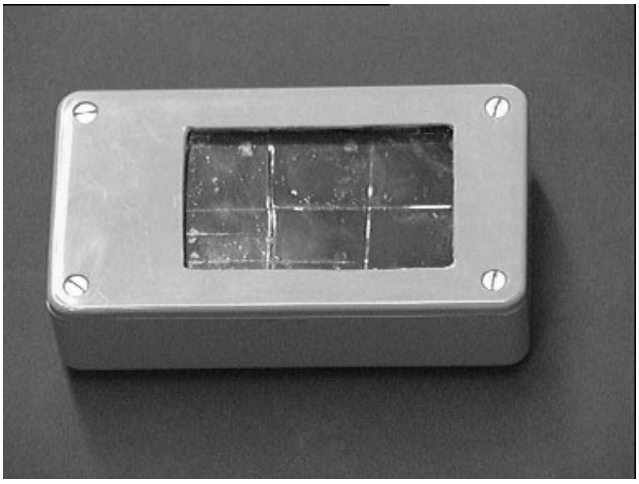

Figure 1: BR element

drying 24 hours, the gold layer of about 40 nm was sputtered on the PVA film to form counter electrode for the conductive glass. Thin wire was connected to the corner of gold layer by silver paint to form an electric connection from the gold layer. The element system (figure 1) containing altogether six such elements was made. There are three pairs of elements in the sensors, each pair containing one of the three proteins. All elements have the same size, about 18×17 mm.

Absorption spectra for all three proteins were measured using standard spectrophotometer, measurements were done in 1 nm steps form 189 to 900 nm.

To measure the wavelength response curves, a Canon speedlite 188 camera flash was used together with the Oriel narrow band interference filters. The filters were every 20 nm from 400 to 700 nm, the half width of the filters was about 10 nm. Since the only relative responses for comparison of elements with different proteins are needed, the flash was sufficient. Responses for changes both from the B- to the M-state and from the M- to the B-state were measured. When measuring change from the B- to the M-state, the molecules was enforced into the B-state using 420 nm light pulses, in order to assure that the protein is initially in the B-state. When measuring change from the M- to the B-state, the element was kept in the M-state by continuous yellow light. The electric signal was measured as potential change between electrodes using standard 100 MHz oscilloscope.

3.1 Color recognition

The differences in opto-electric responses among BR variants allow for color recognition with BR. To see how well BR variants are able to distinguish colors in practice, we have measured the electric responses of our simple element system to set of 84 colored lights. The colored lights were produced by filtering a flash light through different colored plastic filters. The flash was the same as in the previous measurements. A triplet of responses (one for each of the three proteins) was recorded for each colored light.

For each filter in the set we have also measured transmittance spectrum in 1nm step from 379 to 780nm. The same spectrophotometer as for measuring BR absorption spectra was used.

3.2 Calculation of opto-electric response

The optical properties of wild type BR have been extensively studied in the literature and several two-level approximate models has been applied to describe the properties. Less attention has been paid to study and model optical properties of BR variants, only recently [16], steps towards modeling optical properties of 4-keto BR has been made. However, there is not any model for opto-electric properties of BR.

Having a model for opto-electric properties is particularly important if one needs to compute electric response of sensor elements to arbitrary colored light. Model of only optical properties is not sufficient for that. Once the opto-electric properties are modelled, it is possible to simulate experiments with various configurations of proteins in a sensor for finding proper layout of actual sensor.

For our purpose it is sufficient to model the electric response of the protein to a single light pulse only. The response in such a case depends on following factors - spectral distribution of the light, length of the pulse, distribution of the states in protein at the beginning of the pulse, the thickness and area of the protein patch, and RC constant of the circuit used to measure the response. If we assume that all factors except the spectrum of the light are constant, as it is in case of our element system and measuring equipment, we can simplify the model considerably.

The response of proteins to colored light can be calculated as:

$$Phe_1 = k_1 * \int S(\lambda)\, A(\lambda)\, d\lambda, \tag{1}$$

where $S(\lambda)$ is the spectrum of input light, $A(\lambda)$ is absorption spectrum of the protein, k_1 is a constant, and λ is wavelength. The equation expresses an ideal

case - the amount of molecules making transition from the B- to the M-state depends linearly on spectral power of absorbed light. In practice it is not so, with increasing spectral power of the light the amount of molecules being able to make the transition increases only up to a certain level, and then saturates.

The response can also be calculated as:

$$Phe_2 = k_2 * \int S(\lambda)\, p(\lambda)\, d\lambda, \tag{2}$$

where $S(\lambda)$ is as above, $p(\lambda)$ is the opto-electric response of the protein, and k_2 is a constant. Unfortunately we do not know the response function $p(\lambda)$, but only its rough approximation using narrow-band responses. If we further assume the spectrum $S(\lambda)$ is "smooth" enough, we can write:

$$Phe_3 = k_3 * \sum_b S_b p_b, \tag{3}$$

where S_b is the relative spectral power over the band b, p_b is the opto-electric response for the band b, k_3 is the constant. Again, in equations 2 and 3 the saturation is not taken into account.

4 Results

Figure 2 shows relative absorbances of the three BR variants from 400 to 700 nm. Absorption maxima were: for BR wild type at 564 nm, for 4-keto at 508 nm and for 3,4-dehydro at 590 nm. Slight difference of absorbtion maxima from values reported in literature (570 nm for BR-wt and 503 nm for BR-4-keto) are due to uneven intensity distribution of the flash, especially it has low intensity in blue part of its spectrum. Relative intensity distribution of the flash filtered through the interference filters is in figure 6.

The wavelength dependency curves of the opto-electric response from bacteriorhodopsin PVA films are drawn in figure 4; the curve of 4-keto BR is inverted, since the signal had opposite polarity compared to other proteins. The response curves are shown for both transitions; from the B- to the M-state and from the M- to the B-state. The voltages have opposite signs for different state changes. The strongest signal for B- to M-state transition was measured for wild type BR being several volts for unfiltered flash. Signals for 4-keto-BR and 3,4-dehydro-BR were half and 1/10th of that amplitude, respectively. Examples of recordings of pulses for BR wild type and 4-keto BR are in figure 3.

Figure 5 shows the triplets of responses to each color. It can be seen from the figure, that the BR based sensor could distinguish colors of colored plastic

filters. The responses of wild type BR to the set of filters were compared to values calculated by equation 4 and 2, respectively. The constants k_1 and k_2 were estimated so that the error of calculated responses was minimized in least square sense.

Measured and calculated responses are in figure 7. One can recognize, that, for some colors, the models give rather good estimates of the responses. On the other hand, the experimental maxima are smaller than calculated for certain colors. This is possibly because only the transition from the B- to the M-state is included into the model. However the RC constant of the measuring system introduces a delay which may be long enough for the transition from the M- to the B-state to have significant influence for the response. The charge shift of that transition has opposite direction, and thus decreases the response. Saturation at the sensor plays a role too. Why in some points experimental values clearly exceed calculated ones. is not yet known.

It can also be seen from the figure 7, that the differences between values calculated by equation 1 and 3 are relatively small. We expect to get better estimate of responses by applying proper model which will consider also the saturation and temporal properties of the system.

5 Conclusion

In this paper we have described basic opto-electric properties of three photoactive proteins. Those proteins have been extensively studied by chemists and biochemists, there are also efforts towards their use for information processing. The applications cover broad area of technologies including holographic memories, spatial light modulators and artificial retina. In our present study we have concentrated on color processing.

We have shown that these proteins can be used as light sensitive elements in an image detector. Furthermore, it was possible to produce proteins with different wavelength characteristics. This forms a basis for a color sensitive sensor. The color sensitivity expands also possibilities in constructing artificial retinas based on bacteriorhodopsin molecules. So far only gray-level processing was considered.

We have built a simple color sensitive detector using these proteins. We have demonstrated its ability to recognize colors, despite the visible color space have not been covered efficiently by the elements. All three proteins are little sensitive to blue part of spectrum, however we expect to expand the coverage by producing new variants of BR. The number of different proteins in the system is, of course, not limited to three. One can build a sensor containing four or more proteins, the actual number of proteins will depend on application

and availability of particular proteins.

The area of the electrods in the sensor elements was about 3 cm^2. The signal from elements was measured without amplification and was up to several volts. This leads us to believe that the elements size in actual sensor could be small enough for practical applications. Efforts to construct high resolution BR sensors have been reported also by the others [10], although they are at the initial stage too.

Acknowledgements

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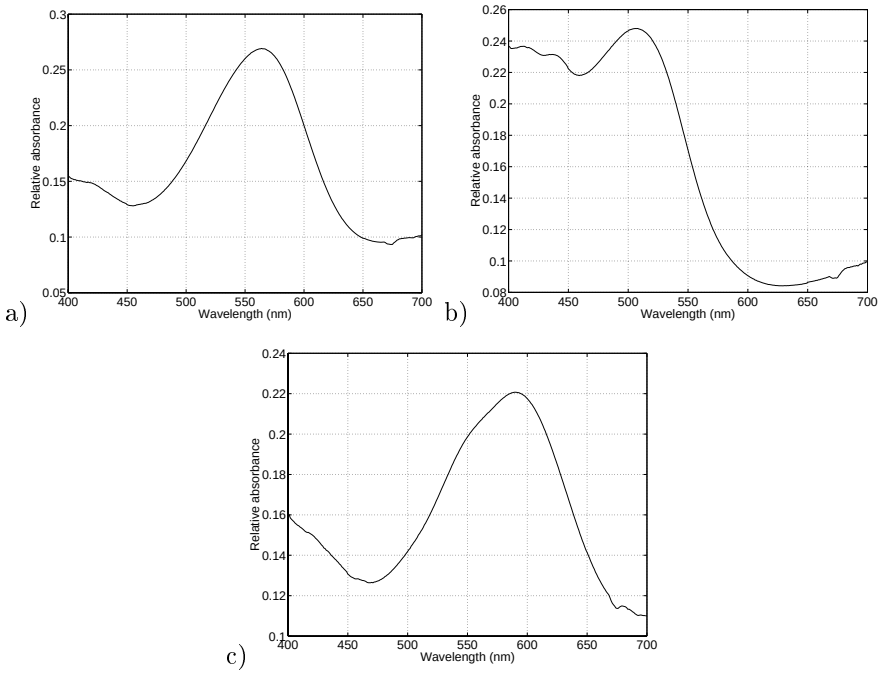


Figure 2: Absorption spectra. BR-wt (a), BR-4-keto (b) and BR-3,4-dehydro (c).

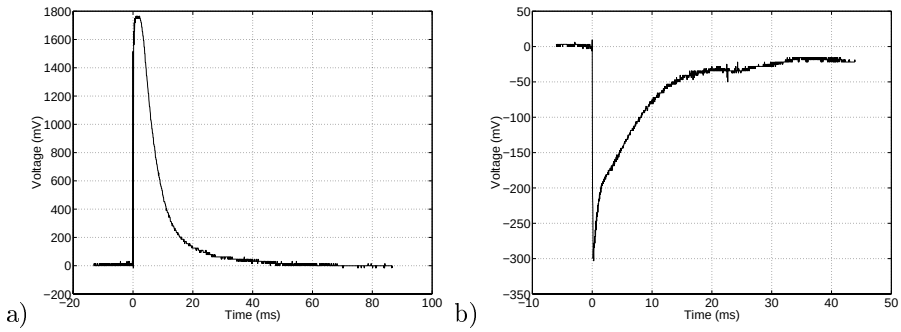
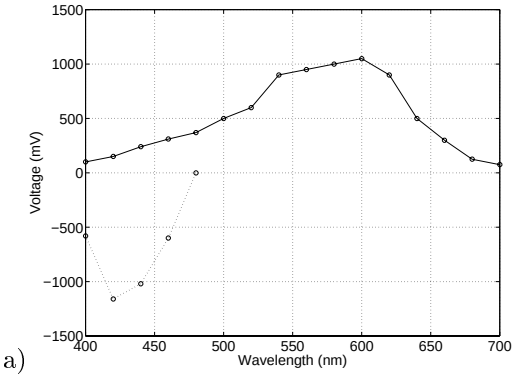
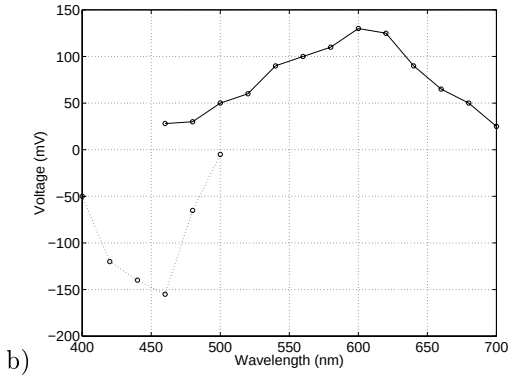


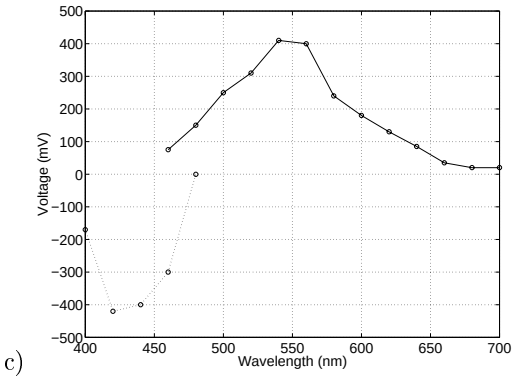
Figure 3: Examples of pulses. BR-wt (a) and BR-4-keto (b).



a)

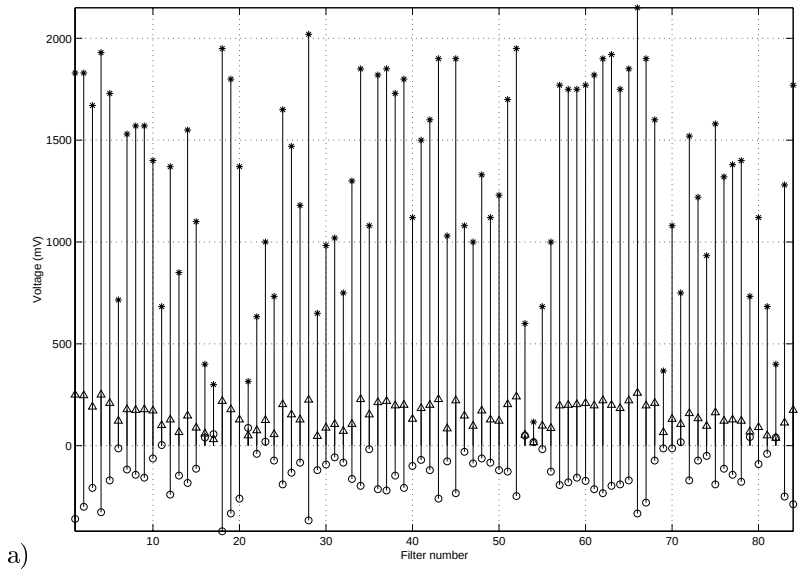


b)

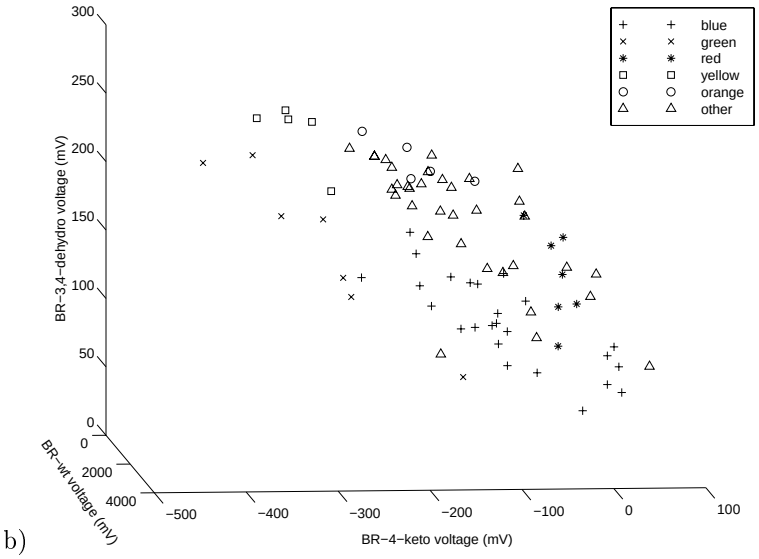


c)

Figure 4: Responses of transition from the B- to the M-state (full line) and from the M-to the B-state (dotted line). BR-wt (a), BR-3,4-dehydro (b) and BR-4-keto (c).



a)



b)

Figure 5: a) The opto-electric responses of BR-wt (stars), BR-4-keto (circles) and BR-3,4-dehydro (triangles). b) Triplets of opto-electric responses drawn in 3D space.

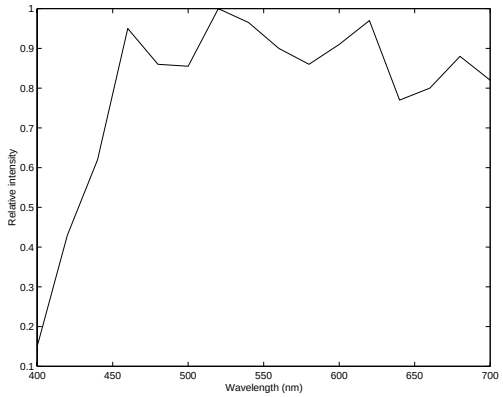


Figure 6: Relative intensity of filtered flash.

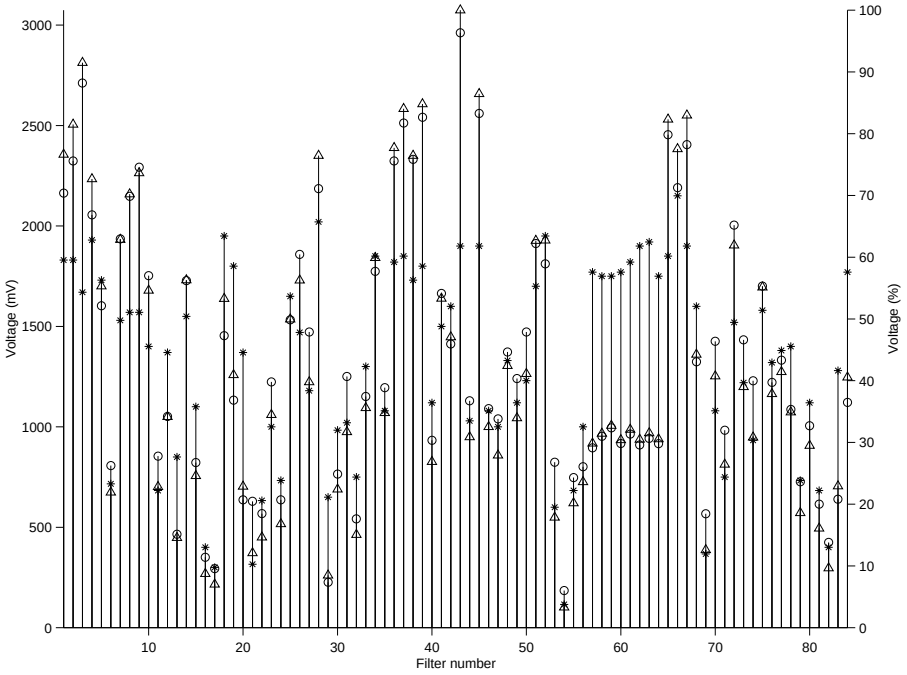


Figure 7: Responses of wild type BR to colored plastic filters (stars) and calculated responses; using equation 1 (circles), and equation 3 (triangles).