# Ultraviolet Protection Using Intensity-Dependent Spectral Shift in Bacteriorhodopsin

Yuhua Huang, Jie Fu, David J. Hagan, Shin-Tson Wu, *Fellow, IEEE*, Xiaohua Huang, and Mostafa A. El-Sayed

***Abstract*—The optical absorption properties of biological protein bacteriorhodopsin (bR) under the illumination of different intensity and pH environment were investigated. It is observed that the wild type bR film offers more than 30% effective UV protection. The UV protection effect can be further enhanced to 90% in some specific UV region by genetically or chemically modifying bR molecules or by the illumination of visible wavelengths ranging from green to red wavelengths. Preliminary results indicate that bR could be a promising material for UV protection.**

***Index Terms*—Bacteriorhodopsin (bR), optical absorption, ultraviolet exposure, ultraviolet protection.**

## I. Introduction

ULTRAVIOLET (UV) radiation, including UVA (320–400 nm) and UVB (280–320 nm), of solar light has been shown to be the major cause of skin damage such as skin aging, sunburn, immune suppression, and skin cancer [1], [2]. In addition, excessive exposure to UV radiation may also cause the formation of some eye disorders and cataracts [3]. Thus, it is very important to protect human subjects from UV exposure. The increased incidence of skin cancer and increased mortality rate in recent years has attracted great attention to UV protection.

Several measures, such as sun avoidance, sunglasses, sunscreens, and clothing, have been advised for UV protection. Among these methods, sun avoidance is obviously the most traditional and effective method [4]. However, it is not useful for those who cannot or will not avoid sun exposure. Sunglasses can only be used to protect the eyes from UV radiation. Sunscreens can provide the benefit of protecting against actinic keratoses, squamous cell, and basal cell carcinomas [5]. However, the use of sunscreen also has some disadvantages, including discomfort, the requirement for frequent reapplication, and potential hypersensitivity. In addition, most sunscreens on the market provide less protection for UVA than for UVB. The use of clothing for protection against UV radiation may be viewed as feasible addition or alternative to sunscreens. In fact, physicians have instructed the public to wear clothing as protection against UV exposure. So far, however, the present fabrics cannot effectively block UVA [6], [7].

Manuscript received December 15, 2004; revised October 17, 2005. This work was supported by the Defence Advanced Research Projects Agency Bio-Optic Synthetic Systems program under Contract DAAD 19-02-0208.

Y. Huang, J. Fu, D. J. Hagan, and S.-T. Wu are with the College of Optics and Photonics, University of Central Florida, Orlando, FL 32816 USA (e-mail: swu@mail.ucf.edu).

X. Huang and M. A. El-Sayed are with the Laser Dynamics Laboratory, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400 USA.

Digital Object Identifier 10.1109/JSTQE.2005.857696

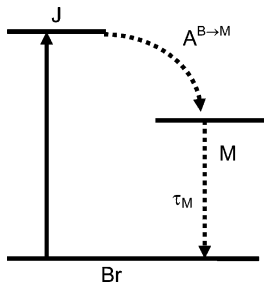



Fig. 1. Simplified energy level diagram of the bR molecule.

There is no doubt that UVB comprises the most energetic wavelengths and is more damaging. This does not mean that UVA is not hazardous. On the contrary, UVA has been found to produce skin damage and premature skin photoaging and wrinkling, as well as eye damage [8], [9]. Since UVA occupies the major portion ($\sim 94\%$) of the UV radiation reaching the surface of the earth [7], it is also very important to develop new materials and approaches for protection against UVA exposure.

In this paper, we studied the optical absorption properties in bacteriorhodopsin (bR) and found that it may be a promising material for protection against UV, especially UVA radiation. bR is a photochromic protein found in the purple membrane of the halophile bacteria *Halobacterium halobium*. Without illumination, bR is in its ground Br state which displays a broadband absorption with absorption peak at $\lambda$-570 nm. Upon illumination of green-red light, the molecules undergo several structural transformations corresponding to the intermediate states K, L, M, N, and O in a well-defined photocycle [10]. With the formation of each intermediate state, the absorption spectrum produces obvious shift, especially the absorption spectrum of the M intermediate state, which significantly shifts $\sim$160 nm toward blue with respect to the initial Br state spectrum. Since M is the most stable intermediate state, bR can be viewed as exhibiting a bistability between the Br and M states [11], as shown in Fig. 1. Various applications have been proposed based on this bistability [12]–[15], including optical limiting, optical switching, spatial light modulation, image processing, and optical logic gates. In this paper, we, for the first time, investigate its application for UV protection.

## II. Experiment

Three types of bR samples, including wild type bR aqueous solution, wild type bR film, and the bR mutant D96N film, were used in our experiment. bR molecules were obtained from Halobacterium halobium cells by the standard procedures described in [16]. The concentration of the bR molecules in the

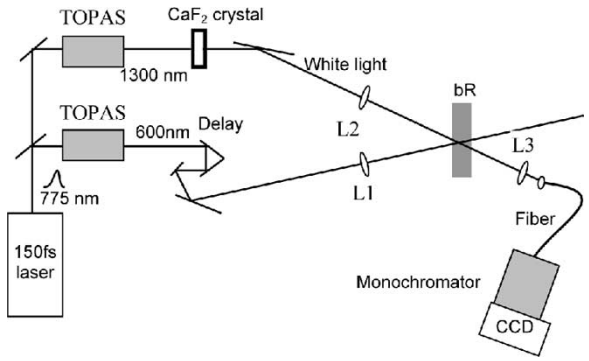



Fig. 2. Experimental apparatus for studying the absorption spectrum of bR samples.

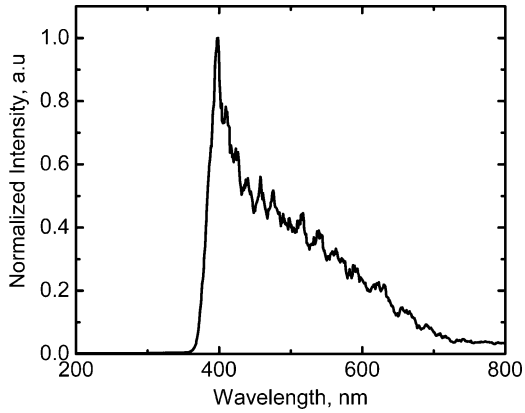



Fig. 3. The spectrum of the white light source produced by the femtosecond pulsed laser.

aqueous solution was $\sim 1.72 \times 10^{-7}$ mol/ml. HC1 and NaOH were added to the solution to change the pH value of the bR aqueous solution within the 0–12 pH range. The bR aqueous solution was filled in a 1 mm thick cuvette for the measurement. The wild type and D96N bR films with thickness of $\sim 80$ $\mu$m, optical density of 3 and 2 at $\lambda = 570$ nm, and thermal relaxation time of $\sim 0.5$ s and 60 s, respectively, were purchased from Munich Innovative Biomaterials GmbH.

Fig. 2 depicts the experimental apparatus. In our experiment, the pulsed laser beam at $\lambda = 775$ nm with 150 fs of full-width half maximum (FWHM) and 1 kHz repetition rate output from a Ti:Sapphire femtosecond pulsed laser (CPA2001 from Clark-MXR company) was split into two beams. One was used to pump a travel optical parametric amplifier system (TOPAS 4/800) to produce $\lambda = 600$ nm output, which is used as the pump beam. The other one was used to pump another TOP AS 4/800, from which the 1300 nm laser output was chosen to pump a piece of calcium fluoride ($CaF_2$) crystal to obtain a broadband white light for probing bR's absorption spectrum. The spectrum of the white light is shown in Fig. 3. The pulse energy of the white light was set at $\sim 1$ nJ to avoid unnecessary excitation of the sample. The white probe beams were focused by a lens. The red pump beam (diameter $\sim 3$ mm) and the foci of the white beam (diameter $\sim 100$ $\mu$m) were overlapped on the bR sample. A translation stage was used to adjust the optical path of the white probe beam to make sure that the pump and probe beams arrive at the sample at the same time. A silicon diode array-based spectrometer was placed behind the bR sample to detect the transmission spectrum of the white probe beam.

## III. Results and Discussions

First, we investigated the intensity-dependent optical absorption spectrum of the wild type bR film, as shown in Fig. 4(a). Without the red pump beam, the bR molecule is on its initial Br state, which has strong absorption in the green–yellow region and weak absorption in UV region ranging from 200–400 nm. The excitation of the red beam makes bR's absorption shift from green–yellow toward the blue and UV region. With the increase of the red pump beam intensity, the absorption of green-yellow wavelengths decreases, and that of the blue and UV region increases. The intensity-dependent absorption at 570

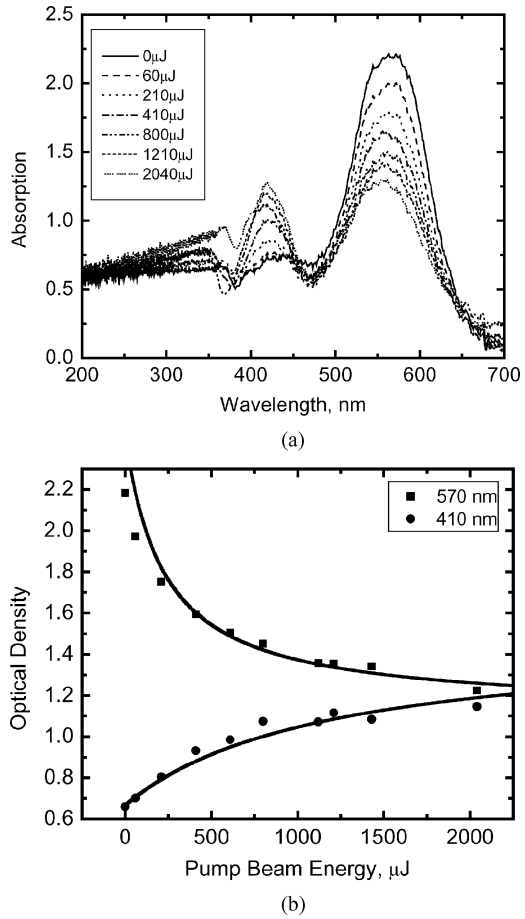



Fig. 4. (a) Light intensity dependent absorption spectrum of the wild type bR. (b) Experiment and simulation results at $\lambda = 570$ and 410 nm.

and 410 nm extracted from Fig. 4(a) is plotted in Fig. 4(b). This is easy to understand. Before the illumination, bR molecules are in the initial state. Therefore, it exhibits a strong absorption in the green-yellow region and small absorption in the UV region. After the red light illumination, a portion of bR molecules are excited to the M state. Since the M state has a strong absorption peak at $\lambda = 410$ nm, the absorption ranging from blue to UV wavelengths around 410 nm increases, and that of the

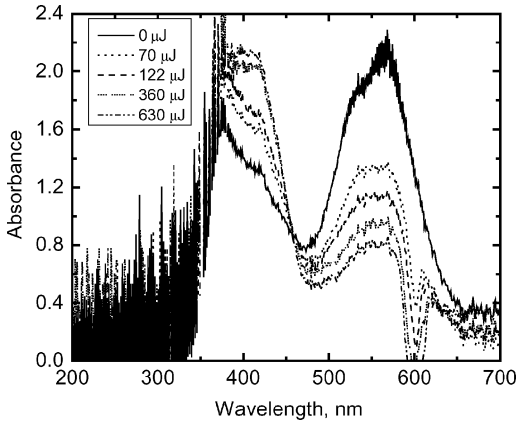



Fig. 5. Light intensity dependent absorption spectrum of mutant D96N bR.

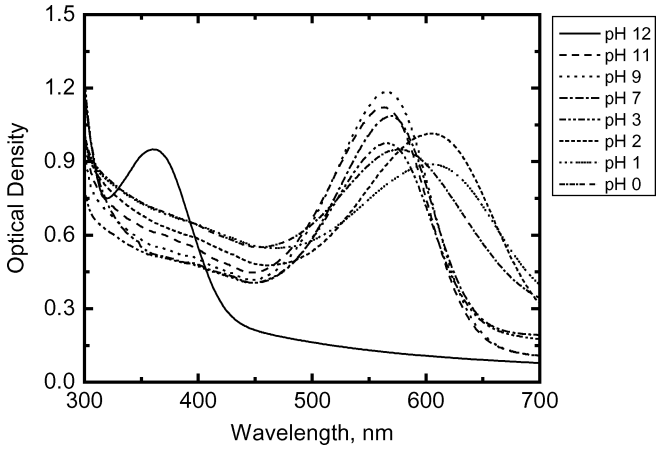



Fig. 6. pH value dependent absorption spectrum of wild type bR aqueous solution.

green–yellow region decreases. Higher pumping intensity leads more bR molecules to the M state. Therefore, the absorption around 410 nm increases, while that of the green–yellow region decreases with the increased pumping intensity. Since the time interval (1 ms) of the femtosecond laser pulses is much shorter than the thermal relaxation time of the bR sample, the illumination of the femtosecond pulsed laser is equivalent to a CW laser. Therefore, the relationship between the population distribution and the pump beam intensity can be simply written as follows:

$$\frac{dN_{\rm Br}}{dt} = -\frac{\sigma_{\rm Br} A^{{\rm Br}\to M} I}{hv} N_{\rm Br} + \frac{N_M}{\tau_M} \tag{1}$$

$$N_M = N - N_{\rm Br} \tag{2}$$

where $N$ is the total population density in the bR sample. $N_{\rm Br}$ and $N_M$ are the population density of the Br and M states, respectively; $h$ is Planck's constant; $v = C/\lambda$, is the illuminating light frequency; $A^{B\to M} = 0.64$ is the quantum efficiency for the transition from Br state to the excited state [6]; $\sigma_{\rm Br}$ is the absorption cross section of bR's Br state at the excitation wavelength. Using the above equations, we can simulate the experimental results, as shown by the solid line in Fig. 4(b). In our simulations, we used $\sigma_{\rm Br} = 2.4 \times 10^{-16}$ and $\sigma_{\rm Br} = 0.65 \times 10^{-16}$ cm$^2$ at 570 and 410 nm [12], respectively, and $\tau = 0.5$ s.

From Fig. 4(a), we can see that although the bR sample in the initial Br state displays smaller absorption in the UV region than in the green-yellow region, it can still block more than 30% of UV light in the whole UV region. The most interesting feature is that the additional illumination of the green–red wavelengths can further enhance the protection efficiency up to 70% for the UVA wavelengths. Since the major part of the sunlight is in the visible wavelengths ranging from 400–700 nm, the illumination of sunlight will increase bR's UV protection effect. This indicates that bR film can be an effective UV protector.

The UV protection effect of bR in some specific region can be further enhanced by modifying bR molecules genetically or chemically. Here, we just take the mutant bR D96N and the wild type bR under different pH environments as examples to show the UV protection capability. Fig. 5 shows the absorption spectrum of a mutant bR D96N under the illumination of different red beam intensities. The downward pulse around 600 nm is caused by the scattering of D96N bR sample to the red pump beam. Similar to the wild type bR, the absorption of D96N in the wavelengths around 410 nm increases with the increase of the red pump beam intensity. It is noticed that D96N bR exhibits much stronger absorption in the UVA region than wild type bR. Overall, the D96N sample can block more than 30% of UVA wavelengths. In the 355–400 nm spectral range, more than 90% of light is blocked.

These results indicate that the wild type bR and mutant D96N bR films can effectively block UV radiation. In addition, they also block more than 90% visible light. It does not matter if they are used as clothing or sunscreen for protecting against UV exposure. However, the visible transmittance is an important consideration if we would use bR film as UV protection sunglasses. Our study of the pH value effect on the absorption spectrum shown in Fig. 6 illustrates that this can be solved by choosing a suitable pH environment during the fabrication processes. From Fig. 6, the absorption spectrum barely changes between $\rm pH = 3$–$8$. However, in the environment with $\rm pH < 3$, the absorption produces a noticeable red shift due to the protonation of counterion Asp 85 [17]. The absorption in the UV region also increases correspondingly. In the high pH region, especially when $\rm pH > 12$, the absorption spectrum shifts toward UV region because of the deprotonation of the counterion Asp 96 [17]. In this case, the bR film is transparent in the visible region while its UV blocking efficiency is significantly enhanced. UV protection sunglasses using bR films therefore become feasible.

## IV. Conclusion

In conclusion, the absorption spectrum of bR under different pump beam intensities and pH values was studied. It is observed that the wild type bR can block a portion ($>30\%$) of UV wavelengths. The illumination of an additional beam ranging from green to red wavelengths will enhance bR's UV protection efficiency. By genetically mutating or chemically modifying the bR molecules, the UV protection efficiency can be further enhanced in some specific region. The results show that bR is a promising candidate material for UV protection.

## REFERENCES


[1] D. L. Bissett, D. P. Hannon, and T. V. Orr, "Wavelength dependence of histological, physical, and visible changes in chronically UV—irradiated hairless mouse skin," *Photochem. Photobiol.*, vol. 50, pp. 763–769, 1989.

[2] N. Lowe, D. Meyers, and I. Scott, "Low dose of repetitive ultraviolet a induce morphologic changes in human skin," *J. Invest. Dermatol.*, vol. 105, pp. 739–743, 1995.

[3] J. Longstreth, F. De Grujil, M. Kripke, Y. Takizawa, and J. van der Leun, "Effects of increased solar ultraviolet radiation on human health," *Ambio*, vol. 24, pp. 153–165, 1995.

[4] H. W. Lim and W. Bergfeld, "Prevention strategies," *J. Amer Acad. Dermatol.*, vol. 41, pp. 95–96, 1999.

[5] B. A. Gilchrest, "Sunscreen: A public health opportunity," *N. Engl. J. Med.*, vol. 329, pp. 1193–1194, 1999.

[6] K. Hoffman, K. Kaspar, T. Gambichler, and P. Altmeyer, "*In vitro* and *in vivo* determination of the UV protection factor for lightweight cotton and viscose summer fabrics: A preliminary study," *J. Amer. Acad. Dermatol.*, vol. 43, pp. 1009–1016, 2000.

[7] S. Q. Wang, A. W. Kopf, J. Marx, A. Bogdan, D. Pilsky, and R. S. Bart, "Reduction of ultraviolet transmission through cotton T-shirt fabrics with low ultraviolet protection by various laundering methods and dyeing: clinical implications," *J. Amer. Acad. Dermatol.*, vol. 44, pp. 767–774, 2001.

[8] A. R. Kaidbey, "The soectral dependence for UVA-induced cumulative damage in human skin," *J. Invest. Dermatol.*, vol. 108, pp. 17–21, 1997.

[9] I. Sayer, J. Canno, M. Picot, J. Meynadier, and L. Meunier, "Immunosuppression induced by acute solar simulated ultraviolet exposure in humans: prevention by a sunscreen with a sun protection factor of 15 and high UVA protection," *J. Amer. Acad. Dermatol.*, vol. 37, pp. 187–194, 1997.

[10] R. R. Birge, "Photophysics of light transduction in rhodopsin and bacteriorhodopsin," *Annu. Rev. Biophys. Bioeng.*, vol. 10, pp. 315–354, 1981.

[11] T. H. Zhang, C. P. Zhang, G. H. Fu, Y. D. Li, L. Q. Gu, G. Y. Zhang, Q. W. Song, B. Parsons, and R. R. Birge, "All-optical gates using bacteriorhodopsin films," *Opt. Eng.*, vol. 39, pp. 527–534, 2000.

[12] Y. Huang, G. Siganakis, M. G. Moharam, and S. T. Wu, "Broadband optical limiter based on nonlinear photoinduced anisotropy in bacteriorhodopsin film," *Appl. Phys. Lett.*, vol. 85, pp. 5445–5447, 2004.

[13] P. Ormos, L. Fábián, L. Oroszi, E. K. Wolff, J. J. Ramsden, and A. Deŕ, "Protein-based integrated optical switching and modulation," *Appl. Phys. Lett.*, vol. 80, pp. 4060–4062, 2002.

[14] J. Hoseph, F. J. Aranda, D. V. L. N. Rao, B. S. DeCristano, B. R. Kimball, and M. Nakashina, "Optical implementation of the wavelet transform by using a bacteriorhodops in film as an optically addressed spatial light modulator," *Appl. Phys. Lett.*, vol. 73, pp. 1484–1486, 1998.

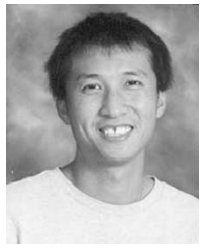
[15] Y. Huang, S. T. Wu, and Y. Zhao, "All-optical switching characteristics in bacteriorhodopsin and its applications in integrated optics," *Opt. Express*, vol. 12, pp. 895–906, 2004.

[16] D. Oesterhelt and W. Stoeckenius, "Isolation of the cell membrane of Halobacterium halobium and its fractionation into red and purple membrane," *Methods Enzymol.*, vol. 31, pp. 667–678, 1974.

[17] H. Otto, T. Marti, M. Holz, T. Mogi, L. J. Stern, F. Engel, H. G. Khorana, and M. P. Heyn, "Substitutions of amino acids Asp-85, Asp-212, and Arg-82 in bacteriorhodopsin affect the proton release phase of the pump and the pK of the Schiff base," *Proc. Nat., Acad. Sci.*, vol. 87, pp. 1018–1022, 1990.


**Yuhua Huang** received the B.A. degree in electronics from Hunan Air Force Aviation Technology College in 1994, the master degree in optics from Shanxi University in 1997, and the Ph.D. degree in optics from Fudan University in 2000, respectively.

She was an Assistant Professor at East China Normal University from July 2000 to June 2002. Then she joined the College of Optics and Photonics, University of Central Florida (UCF), Orlando, as a Research Scientist.



**J. Fu** was born in Beijing, China. He received the B.S. and M.S. degrees from Tsinghua University, Beijing, China, in 1997 and 2000, respectively. He is currently working toward the Ph.D. degree in optics at the College of Optics and Photonics: CREOL & FPCE, University of Central Florida, Orlando, where his research work is focused on developing and using novel nonlinear spectroscopy to study structure/nonlinear optical properties relations of materials.

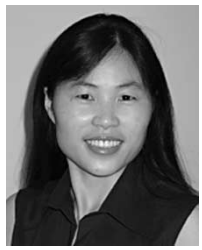
His research work in Tsinghua University was studying frequency splitting phenomenon in He-Ne laser and using it to develop novel displacement sensor.

**D. J. Hagan**, photograph and biography not available at time of publication.

**Shin-Tson Wu** (M'98–SM'99–F'04) received the B.S. degree in physics from National Taiwan University, Taipei, and the Ph.D. degree from the University of Southern California, Los Angeles.

He is a PREP professor at the Collage of Optics and Photonics, University of Central Florida (UCF). Prior to joining UCF in 2001, he was with Hughes Research Laboratories, Malibu, CA, for 18 years. His studies at UCF concentrate in foveated imaging, bio-photonics, laser beam steering, liquid crystal displays, and liquid crystal materials. Prof. Wu has Co-Authored two books: "*Reflective Liquid Crystal Display*" (New York: Wiley, 2001) and "*Optics and Nonlinear Optics of Liquid Crystals*" (Singapure: World Scientific, 1993).

Dr. Wu is a Fellow of the Society for Information Display and the Optical Society of America.



**Xiaohua Huang** received the B.S. degree in chemistry from Jilin University, China, in 1996, and the master degree in chemistry from Peking University, China, in 2001. She has been a Ph.D. student in chemistry at Georgia Institute of Technology, Atlanta, since 2002.

**Mostafa A. El-Sayed** received the B.Sc. degree from Ain Shams University, Cairo, Egypt and the Ph.D. degree from Florida State University, Tallahassee.

He has held Research Associate positions at Harvard, Yale and California Institute of Technology. He taught at UCLA until 1994 when he moved to Georgia Institute of Technology. He is Julius Brown Chair and Regents Professor in the School of Chemistry and Biochemistry at the Georgia Institute of Technology, where he is also Director of the Laser Dynamics Laboratory. Using different laser spectroscopic techniques, his group examined the dynamical processes and energy conversion in molecules, in gaseous clusters, in organic and inorganic solids as well as in photobiological systems, such as bacteriorhodopsin. More recently, his group began active research on the ultrafast electron and hole dynamics in semiconductor nanoparticles and the shape dependent physical (optical and nonradiative) of Nobel metals and the chemical (nanocatalysis) of transition metal nanoparticles.