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Bacteriorhodopsin and its mutants for light-induced anisotropy and dynamic holography recording

D.Stepanchikov, N.Burykin[1], *T.Dyukova*[2], *T.G.Ebrey*[3], *S.P.Balashov*[4], *E.Korchemskaya*[5]

Zhytomir State University, Zhytomir 10008, Ukraine
[1]Institute of Applied Optics, National Academy of Sciences, Kyiv 01054, Ukraine
[2]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow region 142290, Russia
[3]University of Washington, Seattle WA 98195, USA
[4]University of California, Irvine CA 92697, USA
[5]Institute of Physics, National Academy of Sciences of Ukraine, 46 Nauki Ave., Kyiv 03028, Ukraine

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We present our results on the optimization of light-induced anisotropy characteristics and holography recording on the films of genetically and chemically modified bacteriorhodopsin (BR), the photochromic retinal protein. Gelatin films with chemically modified D96N and D96E BR mutants might be promising for both optical data storage and spatial light modulators. We were the first to show that E204Q BR films (wherein the proton release complex in the protein is affected), exhibit a considerable increase in the diffraction efficiency and initial peak sharpness in the holography recording kinetics as compared to wild type BR and D96N BR. The E204Q BR is the only known holographic reversible material where such sharpness of the initial transient process is observed in dynamic recording by low-intensity red light from a cw He–Ne laser.

Приведены результаты по оптимизации характеристик фотоиндуцированной анизотропии и голографической записи на пленках бактериородопсина (БР), фотохромного ретиналь-содержащего белка, посредством его генетической и химической модификации. Желатиновые пленки с химически модифицированными D96N и D96E мутантами БР могут являться перспективными как для хранения оптических данных, так и для пространственных световых модуляторов. Впервые было показано, что в пленках с мутантом БР E204Q (в котором изменен комплекс выброса протона в молекуле белка) наблюдается существенное увеличение дифракционной эффективности и остроты начального пика в кинетике голографической записи в сравнении с немодифицированным БР и D96N БР. Пленки E204Q БР являются единственными из известных реверсивных голографических материалов, в которых такая острота начального кратковременного процесса получена в динамической записи красным светом низкой интенсивности He–Ne лазера.

One of the main problems for optical data processing is to find real-time photomaterials with the most effective combination of optical properties. The key element in many optical applications is a thin film of a photosensitive material. Biological optical materials have attracted a lot of attention in recent years. The retinal-protein bacteriorhodopsin (BR), similar to visual human pigment rhodopsin, a biological photoreceptor and photoactive proton pump in the purple membrane (PM) of the microor-

ganism *Halobacterium salinarum*, is one of the bright examples [1]. Evolution has optimized this protein for high photochemical efficiency, highly directional nature, thermal and photochemical stability and rapid cycling [2]. The proton pumping mechanism is realized via a series of conformational changes of the chromophore and the protein during the photochemical cycle (Fig. 1). All photocycle intermediates are photoactive [3] and can be spectroscopically distinguished by the shift of absorption maximum. The initial state of BR, bR570, has an absorption peak at 570 nm, and the longest-lived short-wavelength absorbing intermediate M412 (or M-state) has one at 412 nm. The optical and functional properties of BR molecules are preserved in isolated PMs and partially even in dry films. This makes it possible to obtain samples with PM fragments to be used in real-time optical information processing [4].

Gelatin films were prepared using casting procedure wherein the photosensitive mixture of a BR protein (in a form of aqueous PM suspension), 8 % (w/v) gelatin solution and chemical additives (arcaine sulfate, *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine and 1,2-diaminopropane or triethanolamine) is introduced between two glass supports separated with 500 to 1000 μm thick spacers and is allowed to gel at 10°C. After 1 h, the upper (hydrophobic) support is removed, and the layer is dried at 10°C and 10–15 % r.h. [4]. The resulting dry film thickness can be varied between 30 to 70 μm (depending on spacers). All films described here had the same BR concentration in the gelatin matrix, their thickness was nearly 50 μm and optical density, measured spectroscopically at 570 nm, is about 2.0. The gelatin-BR films show very high photosensitivity (several mW/cm^2 of cw visible laser irradiation), their spatial resolution is typically at least 5000 lines/mm; they require no external processing and may be cycled through the write/erase process over millions of times without degradation. The photosensitivity and cyclicity of BR films are far beyond than those of synthetic materials [2, 5].

Although BR films are now used widely for optical processing, the wild-type (WT), i.e. genetically unmodified BR is not necessarily successful [6]. For various systems of optical information processing, there is a strong demand for generation of the films with maximum bleaching and diffraction efficiency and considerably prolonged M-state

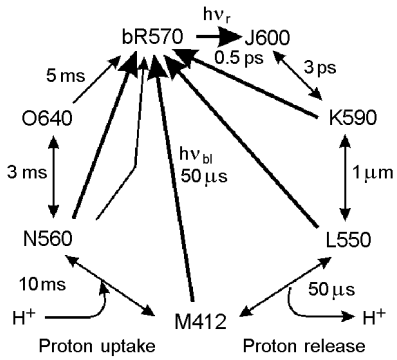

Fig. 1. Main reactions of the BR photocycle. Intermediates are designated by letters J through O accompanied by position of absorption maximum in nm.

lifetimes. For example, the time scale of 10 ms (limited by the natural thermal relaxation time of the initial bR570 state) is not enough for cache-memory applications using the BR films. Alternatively, when photoinduced population distribution is used to store information recorded by light, of critical importance is the maximum occupancy of M, i.e. the total number of BR molecules converted into the M-state upon illumination. It is known that there exist several possible approaches to improve the properties of the BR films for information processing without appreciable loss of photosensitivity and cyclicity. The BR molecular properties can be modified by several means: changing physical/chemical conditions of the medium, retinal replacements, genetic modification of the BR primary sequence [7], chemical modification of the BR-polymer environment by adding chemicals [4], etc. Here, we discuss the combination of two approaches to improve the characteristics of BR films for information storage and processing: the use of BR genetic mutants and chemical modification of the protein environment. The genetic engineering of BR has become a high-effective tool for designing novel photochromic materials. The replacements of D96 and E204 residues (which are functionally important in the proton transfer pathway) can offer the desired performance characteristics in the BR genetic mutants D96N, D96E, and E204Q BR by changing their photocycle kinetics.

One of the areas where modification of BR and the resulting prolonged M lifetimes appeared to be quite effective is photoanisotropic response in BR films. The photoinduced anisotropy occurs in BR film upon illumination with linearly polarized light [8]. The BR films are unique materials for

dynamic holography recording [9]. The light-induced anisotropy and dynamic holography recording in BR films have recently drawn a great attention in the field of optical image processing [10–12]. Here, we present our results on the optimization of light-induced anisotropy characteristics and holography recording by means of genetic and chemical modifications.

The BR molecules both in the initial state bR570 and longest-lived intermediate M412 possess anisotropic absorption. We designate the photoinduced anisotropy method based only on anisotropic properties of bR570 as "B type anisotropy", that based only on anisotropic properties of M412 as "M type anisotropy", and the method based on anisotropic properties of both bR570 and M412 as "B-M type anisotropy" [13]. An experimental setup to detect photoanisotropic responses of the B, M and B-M types is shown in Fig. 2. To induce the B type anisotropy, a linearly polarized beam of a He–Ne laser, $\lambda = 633$ nm initiating solely bR570→M412 transition is sufficient. To induce the M and B-M types anisotropy, it is necessary to initiate bR570→M412 transition and run simultaneous anisotropic photoselection of M412 molecules with linearly polarized blue light (Fig. 1). We concurrently employ a He–Cd laser, $\lambda = 442$ nm, that activates predominantly the long-lived short-wavelength absorbing photocycle intermediate M412, and He–Ne laser, $\lambda = 633$ nm, to induce the M and B-M types anisotropy. The linear polarization of He–Cd laser beam and circular polarization of He–Ne laser beam induce the M type anisotropy. The orthogonally linearly polarized beams of the He–Cd and He–Ne lasers induce the B-M type anisotropy. The intensities of the exciting beams are varied over a wide range by two Glan prisms arranged in series and measured by photodiodes PD2 and PD3, respectively. The He–Ne exciting beam polarization is varied by rotating the $\lambda/4$ plate. The BR film is mounted between a crossed polarizer P and analyzer A; the optical axis induced by the exciting beams is inclined at 45° to the polarizer axis. Testing is also performed by the He–Ne laser beam. With high precision, the photoanisotropic response value can be determined at a high accuracy by measuring the transmittance TP-BR-A of the system P-BR-A using the photodiode PD1. We have shown that the mixed B-M type anisotropy produces the highest photoanisotropic response: it is

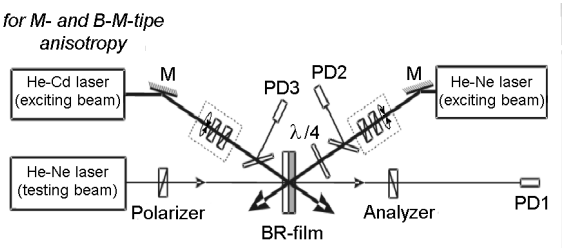

Fig. 2. Experimental setup for the detection of the photoanisotropic response of the B, M and B-M types in BR films.

nearly three times greater than that for the M type and twice as large as that for the B type [13].

The obtained results are explained in terms of a model of the reversible anisotropic photoselection of BR molecules under linearly polarized light. The distribution of molecular populations between the initial state bR570 (N^B) and intermediate M412 (N^M) under excitation with He–Ne laser and He–Cd laser beams is described by the following balance equation:

$$\frac{dN^B}{dt} = \left(\sigma_{bl}^M(\varphi)A_{bl}^{M\to B}\frac{I_{bl}\lambda_{bl}}{hc} + \tau^{-1}\right)N^M - \left(\sigma_r^B(\varphi)A_r^{B\to M}\frac{I_r\lambda_r}{hc} + \sigma_{bl}^B A_{bl}^{B\to M}\frac{I_{bl}\lambda_{bl}}{hc}\right)N^B,$$

where σ^B and σ^M are absorption cross-sections for the B-state and M-state at exciting wavelength, respectively; $A^{B\to M}$ and $A^{M\to B}$ are quantum yields of $bR570\to M412$ and $M412\to bR570$ transitions, respectively; τ is an average lifetime of the M-state, I_r and I_{bl} are He–Ne laser and He–Cd laser intensities (indices r and bl correspond to wavelengths $\lambda = 633$ nm and $\lambda_{bl} = 442$ nm, respectively, see Fig. 1). It is assumed that in all cases $N^B + N^M = N_0$, where N_0 is the total density of BR molecules.

In general, an anisotropically absorbing molecule can be approximated by mutually orthogonal linear oscillators, so that the absorption cross-section dependence on angle φ between a long absorption axis of a molecule and electric field vector of light takes the form

$$\sigma_{bl,r}^{B,m}(\varphi) = \sigma_{\|bl,r}^{B,M}\cos^2\varphi_{bl,r} + \sigma_{\perp bl,r}^{B,M}\sin^2\varphi_{bl,r},$$

where $\sigma_{\|bl,r}^{B,M}$ and $\sigma_{\perp bl,r}^{B,M}$ are absorption cross-sections for the light polarized parallel and perpendicular to a long absorption axis of a molecule, respectively. The absorption

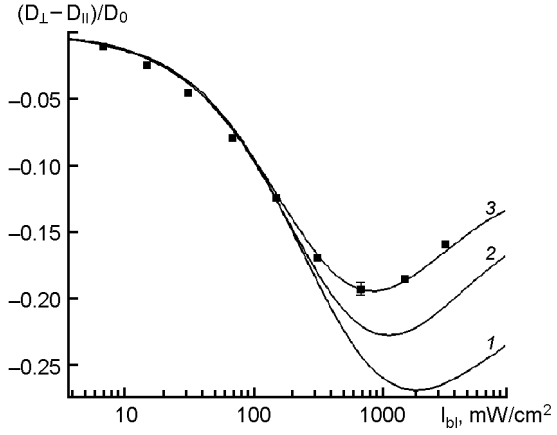

Fig. 3. Experimental dependences of photoinduced dichroism in WT BR film on the intensity of linearly polarized He–Cd laser exciting beam $I_{bl}(\lambda_{bl} = 442$ nm) under concurrent excitation by circularly polarized He–Ne laser beam at the intensity $I_r = 500$ mW/cm^2 and calculated dependences at different values of molecular dichroism k^B and k^M. (*1*) — k_B= 0.2, k_M=0; (2) — k_B= 0.4, k_M=0.05; (3) — k_B= 0.6, k_M=0.11.

cross-section for the circularly polarized light takes the form $\sigma_r^B = 0.5(\sigma_{\|r}^B + \sigma_{\perp r}^B)$. The anisotropic absorption of molecules is described by the molecular dichroism, k: $k = \sigma_\perp / \sigma_\|$. As a result, the heterogeneous distribution of angular concentration of molecules occurs. The photostationary specific angular distribution of molecules in bR570 state is written as Eq.(1).

This gives rise to a photoinduced anisotropy: birefringence, and macroscopic dichroism. The specific angular distribution of molecules in bR570 state for the B-M type anisotropy is more anisotropic than those observed for the anisotropy of B and M types.

Comparing the experimental dependences of nonlinear photoinduced macroscopic dichroism on the laser intensity with similar calculated dependences, it is possible to determine the molecular dichroism of BR for the initial state of the photocycle bR570, k^B, and the longest-lived intermediate M412, k^M [14]. The photoinduced macroscopic dichroism $D_\perp - D_\|$ is written as:

$$D_\perp - D_\| = \frac{2D_0}{N_0}\,\frac{1 - k^B_{test}}{1 + k^B_{test}} \int_0^{2\pi} \frac{\partial N^B}{\partial \varphi}(\sin^2\varphi - \cos^2\varphi)d\varphi,$$

where $D_\perp$ and $D_\|$ are optical densities of a sample in the testing beam polarized perpendicular and parallel to an induced optical axis, respectively. Here, $D_0 = N_0\sigma^B_{\|test}\,(1 + k^B_{test})d_0/\ln 10$ is the initial optical density of photocycling molecules (d_0 is the sample thickness, $\sigma_{\|test}$ is the absorption cross-section at λ_{test}). For example, the experimental and calculated dependences of the M type macroscopic photoinduced dichroism on the exciting He–Cd laser intensity for different molecular dichroism values are shown in Fig. 3. It is seen that the experimental and theoretical curves agree very well at $k^M = 0.11 \pm 0.01$ with $\lambda = 442$ nm. An additional information for the estimation of k^B and k^M was obtained using excitation solely with the He–Cd laser and testing using the collimated lamp beam with $\lambda_{test} = 570$ nm [14].

The photoinduced anisotropy was studied in polymer films with the WT BR, genetic mutants D96N (Asp96→non-ionizable Asn), D96E BR (Asp96→ionizable Glu) and in respective chemically modified BR films. Both Asp96 mutants show a slowed photochemistry, since the replacement of Asp96, an internal proton donor in the proton pathway, can provide a mutant protein with a slowed later photocycle part. The lack of the internal proton donor in the protein slows down sharply the proton uptake, therefore, the lifetime of M intermediate is also increased (Fig. 1). Besides, the chemical modification of BR typically produces 8-to-10 times increase in the M state lifetime as well, resulting in its higher population. We believe that the chemicals affect the pKa of the functionally important residues involved in the cytoplasmic part of the proton transfer

$$\frac{\partial N^B}{\partial \varphi} = \frac{N_0}{2\pi}\,\frac{\sigma^M_{bl}(\varphi)A^{M\to B}_{bl}\,\dfrac{I_{bl}\lambda_{bl}}{hc} + \tau^{-1}}{\left(\sigma^M_{bl}(\varphi)A^{M\to B}_{bl} + \sigma^B_{bl}(\varphi)A^{B\to M}_{bl}\right)\dfrac{I_{bl}\lambda_{bl}}{hc} + \sigma^B_r(\varphi)A^{B\to M}_r\,\dfrac{I_r\lambda_r}{hc} + \tau^{-1}}. \qquad \text{(Eq.1)}$$

pathway, thus hindering conformation changes of the later photocycle part and slowing down the initial state recovery.

The molecular dichroism of bR570 and the M412 intermediate in these BR films has been estimated (Table). The molecular dichroism of bR570 in D96E BR, D96N BR and chemically modified BR films is slightly increased as compared to the unmodified WT BR film. This is an indication that site-specific mutations in D96E and D96N BR as well as the addition of chemicals into gelatin matrix result in insignificant weakening of chromophore/protein interaction as against the chemically untreated WT BR [14]. For B type anisotropy, the transmittance $T_{P\text{-}BR\text{-}A}$ in chemically untreated WT, D96E and D96N BR films and in the respective chemically modified films is plotted in Fig. 4 as a function of exciting He–Ne laser beam intensity I_r. The chemically modified BR films exhibit almost fourfold increase of the photoanisotropic response and a 100-fold increase in photosensitivity. It should be noted that, the BR concentration being the same in all films, the photoinduced anisotropy was considerably higher in chemically modified BR films due to a significant increased fraction of BR molecules converted into M [13]. The photoinduced anisotropy model in the BR films [14] implies that photoanisotropic response grows as the total number of bleached BR molecules converted into the M state increases, since the effective optical density (the difference between the initial and residual optical densities upon saturation intensity irradiation) is proportional to the amount of bleached BR molecules. The M decay time constants τ_1 and τ_2 (s) for chemically modified WT BR, D96E and D96N BR in ambient conditions are 21.9/221.1; 20.6/414.3; 41.9/567.8, respectively, as compared with 2.8/28.5 for chemically untreated WT BR [15]. Thus, the B type anisotropy in these BR films seems to be of interest for optical data storage [10]. For spatial light modulators, the M and B-M type anisotropy of these BR films are more promising [12]. In the latter cases, the cycle process time is about 100 μsec (Fig. 1).

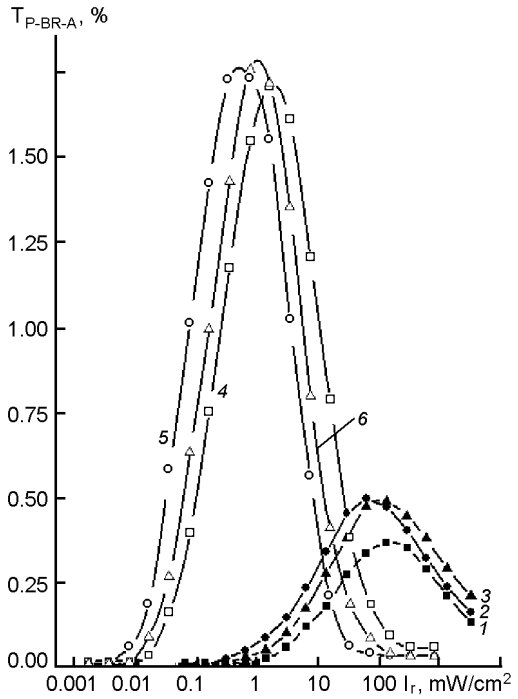

Fig. 4. Transmittance $T_{P\text{-}BR\text{-}A}$ for the chemically untreated WT, D96E and D96N BR films and the respective films with chemical additives as a function of the linearly polarized He–Ne laser exciting beam intensity I_r (λ_r = 633 nm). *1* — WTBR; *2* — D96E BR; *3* — D96N BR; *4* — WT BR with add.; *5* — D96E BR with add.; *6* — D96N BR with add.

It is seen that the BR films are low-saturated dynamic recording materials (Fig. 4). The diffraction efficiency kinetics in BR

Table. Molecular dichroism of the initial state bR570, k^B and the longest-lived M412 intermediate, k^M for the WT, D96E and D96N BR in chemically untreated gelatin films and similar films with chemical additives

Intermediate of photocycle	BR films	Wavelength (nm)	Molecular dichroism k
Initial state, bR570	WT, D96E, D96N, WT + additives, D96E + additives, D96N + additives	633, 510, 488, 633, 510, 488	0.04±0.005, 0.06±0.005
Longest-lived intermediate M412	WT, D96E, D96N, WT + additives D96E + additives D96N + additives	442	0.11±0.01

films consists of two stages: an initial peak followed by a decrease to a steady state [16, 17]. The high amplitude of the transient process in BR films (the initial peak sharpness) of holography recording kinetics in Raman-Nath thin grating is a useful feature which can be utilized in novelty filter and detection of rapid object movement. Recently, we have proposed to use the initial overshoot in the first-order self-diffraction beam kinetics in thin holography grating to detect small periodic vibrations [17, 18]. In this method, the vibration detection is based on the holographic grating recording by the shifted interference pattern in a novel place of a BR film. Hence, the efficiency and time resolution of vibration detection do not depend on the vibration shape. They depend only on the amplitude and sharpness of the initial peak in the first-order self-diffraction beam recording kinetics.

It was assumed before that a substantial gain in the bleaching efficiency and hence in diffraction efficiency of the BR film could be reached with just Asp96 genetic mutants lacking the internal proton donor in the molecule (the proton uptake pathway is affected), e.g. D96N BR. We were the first to show that E204Q BR (Glu204→non-ionizable Gln) wherein the proton release complex in the protein is affected (this mutant exhibits a strongly delayed light-induced proton release) provides a considerable gain in diffraction efficiency as compared to WT and D96N BR (Fig. 5a). Unlike the transmittance kinetics in the WT and D96N BR for He–Ne laser, $\lambda = 633$ nm, the transmittance kinetics in E204Q BR film (Fig. 5b) demonstrates two stages: an initial peak followed by a decrease to a steady state. This could result from the formation of a considerable amount of O-intermediate [19] even in thin 50 μm E204Q BR film (Fig. 1). The E204Q BR film has a larger fraction of bleached molecules at the initial photoexcitation stage than the D96N and WT BR ones (Fig. 5b). The existence of two stages in the transmittance kinetic results mainly in the increase of the initial peak sharpness of holography recording kinetics in E204Q BR film (Fig. 5a). The ratio between the peak and steady-state values is 5.8, 6.9 and 9.6 for the WT, D96N and E204Q BR, respectively.

To conclude, the gelatin films with chemically modified Asp96 mutants might

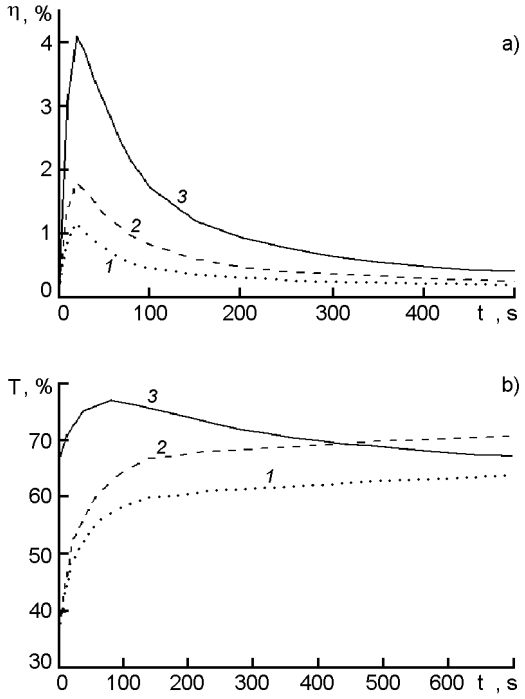

Fig. 5. The first-order self-diffraction kinetics in Raman-Nath thin grating (a) and transmittance kinetics (b) for the He–Ne laser recording in WT (*1*), D96N (*2*) and E204Q (*3*) BR gelatin films at 95 % relative humidity.

be of promise for both optical data storage (using B type anisotropy) and spatial light modulators (using M and B-M type anisotropy). The film with genetic mutant E204Q BR (where the proton release complex is affected) has been shown to exhibit an increased diffraction efficiency. A considerable population of red-light absorbing O-state arises in the E204Q film in the later part of the BR photocycle. This results in a significant gain in the initial peak sharpness. The E204Q BR film is the only known holographic reversible material where such sharpness of the initial transient process is observed in dynamic recording by low-intensity red light from a cw He–Ne laser. This could be extremely useful for the novelty filter and detection of object rapid movement.

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Бактеріородопсин та його мутанти для фотоіндукованої анізотропії та динамічної голографії

Д.Степанчиков, М.Бурикін, Т.Дюкова, Т.Д.Ібрі, С.П.Балашов, О.Корчемська

Подано результати з оптимізації характеристик фотоіндукованої анізотропії та голографічного запису на плівках бактеріородопсину (БР), фотохромного ретинального протеїну, за допомогою генетичної та хімічної модифікацій БР. Желатинові плівки з хімічно модифікованими D96N та D96E мутантами БР можуть бути перспективними як для оптичного зберігання даних, так і просторових світових модуляторів. Вперше показано, що у плівці з мутантом БР E204Q, в якому комплекс викидання протону у молекулі протеїну змінено, спостерігається значне підвищення дифракційної ефективності та гостроти початкового піку у кінетиці голографічного запису у порівнянні з природним немодифікованим БР та D96N БР. Плівка з E204Q БР є єдиним оборотним голографічним матеріалом, де така гострота початкового короткочасного процесу має місце у динамічному запису під дією красного світла низької інтенсивності з He–Ne лазера.