

Visual pigment rhodopsin: a computer simulation of the molecular dynamics of 11-*cis*-retinal chromophore and amino-acid residues in the chromophore centre

Kholmirzo T. Kholmurodov,^a Tatyana B. Feldman^b and Mikhail A. Ostrovsky^{*b}

^a Joint Institute for Nuclear Research, 141980 Dubna, Moscow Region, Russian Federation.

Fax: +7 49621 65498; e-mail: mirzo@jinr.ru

^b N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 495 135 4101; e-mail: feldman@sky.chph.ras.ru

DOI: 10.1070/MC2006v016n01ABEH002255

Based on a computer molecular simulation, we have investigated the conformational dynamics of rhodopsin and its free opsin. A special emphasis was made on the behaviour of the chromophore group – 11-*cis*-retinal within the rhodopsin molecule in its dark-adapted state. The molecular dynamics trajectories were traced in the time range of 3000 ps. We have generated 3×10^6 discrete states of free opsin and rhodopsin in order to compare the rhodopsin and opsin structural conformation changes. Analysis of the 11-*cis*-retinal adjustment process in the chromophore site of opsin in correlation with the behaviour of the nearest surrounding amino acid residues has been carried out. The possible molecular mechanisms of the conformational adaptation of 11-*cis*-retinal in the protein binding pocket, which occur during the physiological regeneration of the visual pigment rhodopsin, are discussed.

Rhodopsin is a prototypical member of a large G-protein-coupled receptor (GPCR) family, which plays a key role in all regulatory processes of living organisms.¹

The signalling pathways regulated by these receptor proteins determine numerous crucial biological processes, including sensory reception, endocrine regulation and synaptic transmission. Approximately 5% of the human genome (above 600 genes) contains information about these proteins.² Over 40% of the currently used drugs aim at G-protein-coupled receptors as the main target.³

Any G-protein-coupled receptor is a heptahelical transmembrane protein where seven transmembrane α -helical parts ('helical bundles') are linked by six extramembrane fragments ('loops'). A general scheme of the function of G-protein cascade is universal.⁴

The visual pigment rhodopsin localised in the photoreceptor membrane of the photoreceptor cell rod outer segment is a perfect model for studies of the structure and functions of G-protein-coupled receptors.

Mikhail A. Ostrovsky is head of laboratory at N. M. Emanuel Institute of Biochemical Physics of the Russian Academy of Sciences (RAS) and professor at Moscow State University. He is a full member of the RAS. He graduated from Moscow State University; he received his Ph.D. in physiology from the Institute of Higher Nervous Activity and Neurophysiology of the RAS, and a degree of Doctor of Science in Biophysics from the Institute of Biological Physics of the RAS. Since 1994, he was a visiting professor or visiting researcher in the US at University of Maryland at College Park, at National Institute of Standard and Technology (NIST) and at National Institute of Health (NIH), as well as in the UK at Institute of Ophthalmology University College London, and in Finland at University of Helsinki.

His current research interests focus on biophysics and molecular physiology and pathology of vision, first of all, on photochemical reactions and conformation changes of the visual pigment rhodopsin. He and his co-workers have developed new ophthalmic technologies, including a new generation of coloured cut-off UV and partly blue light artificial lenses (intraocular lenses) protecting for sure the eye retina against hazard of light damage after cataract surgery. About one million of such lenses were implanted to patients since 1986.



Kholmirzo T. Kholmurodov graduated from the Tajik State University in 1981, fulfilled his diploma project at Joint Institute for Nuclear Research (JINR), Dubna, Moscow region, Russia. He received his Ph.D. (in 1984) and Doctor of Science (Physical and Mathematical, in 1997) degrees from JINR. Since 2003, he held a position of head of Computer Molecular Modeling Sector at the Laboratory of Radiation Biology, JINR. He was a visiting professor at Nagoya University (1997–1998), worked at Institute of Chemical and Physical Research, Japan (1998–2003). He is a member of Japanese Physical Society (JPS), Computational Collaborative Project 5 (CCP5) (UK), and International Association for Electrical and Electronics Engineers (IEEE).

His current research interests focus on the computer molecular simulations of the nano- and biological structures with machines of parallel-vector and special-purposes architectures.

Tatiana B. Feldman graduated from Moscow State University in 1985 with special interest in biophysics. She received her Ph.D. in biology from N. N. Semenov Institute of Chemical Physics of the Russian Academy of Sciences in 1990. Her Ph.D. thesis related to photo- and electro-induced processes in the visual pigment rhodopsin molecule.

Dr. Feldman is a senior researcher at N. M. Emanuel Institute of Biochemical Physics of the Russian Academy of Sciences, Moscow, and the Joint Institute for Nuclear Research, Dubna, Moscow region. Her current scientific interests focus the low-temperature and femtoseconds spectroscopy of rhodopsin. She is also involved in the photochemical studies of other eye pigments: carotenoids and lipofuscin.



Rhodopsin triggers the phototransduction process, which has been studied in sufficient detail. This process involves conversion, transmission and many-fold amplification of the primary light signal. The first event of phototransduction is photoisomerization of the rhodopsin chromophore group – 11-*cis*-retinal. This event leads to activation of the entire rhodopsin molecule and, as a result, its interaction with the G-protein.

In all other G-protein-coupled receptors, the first step of transduction mechanism involves binding a specific ligand, *e.g.*, an odour molecule, neurotransmitter or hormone.⁴ In phototransduction, light rather than a chemical substance is the physiological stimulus triggering a G-protein cascade.⁵

In this case, a unique feature of rhodopsin as a G-protein-coupled receptor is that its incorporated ligand, 11-*cis*-retinal, acts as a highly efficient antagonist that enables one to maintain rhodopsin in its dark state inactive.

As a result of photon absorption and 11-*cis* to all-*trans* retinal isomerization, the all-*trans*-retinal that is still covalently bound up with the protein part of molecule turns into a highly efficient agonist. In other words, in case of phototransduction all-*trans* retinal chromophore like any chemical substance acts as an agonist ligand that governs a universal scheme of the G-protein cascade operation.

A great progress in determination of the rhodopsin tertiary structure was made in the past five years. Followed by a number of unsuccessful attempts, the researchers succeeded in crystallising the membrane protein rhodopsin and determining its 3D structure by X-ray diffraction analysis, first with a resolution of 2.8 Å⁶ and then with a resolution of 2.2 Å.^{7,8}

This success stimulated theoretical studies that were aimed to describe the dark (unbleached) state of the rhodopsin molecule and intramolecular light-induced rhodopsin perturbation.^{9–12}

The computer simulation of the molecular dynamics of 11-*cis*-retinal chromophore and its nearest protein environment is of special importance in this context. Both the influence of the chromophore group on the nearest amino-acid residues and, *vice versa*, the effect of the protein environment on the chromophore conformation are the main target here. A sufficiently detailed description of the rhodopsin chromophore domain, namely, the spatial arrangement of 11-*cis*-retinal and its interaction with the nearest surrounding amino-acid residues, is currently available.^{5–8,13–15}

These data are necessary for understanding the intramolecular mechanisms of visual pigments spectral tuning (colour vision) and for explaining their unique photochemical properties.

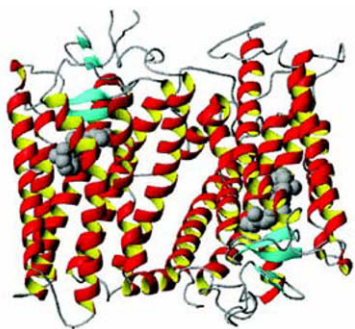


Figure 1 Computer model of a rhodopsin dimer (PDB file 1HZX, Palczewski *et al.*, 2000; Teller *et al.*, 2001). Chain A – left, chain B – right; 11-*cis*-retinal is represented as balls (side view).

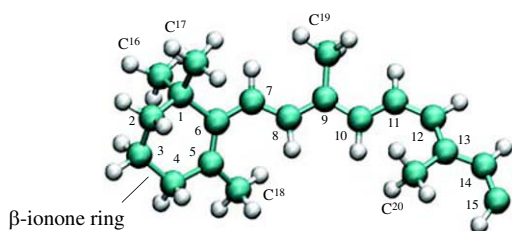


Figure 2 Molecule of 11-*cis*-retinal (6-*S-cis* configuration) with the numbering of all carbon atoms. C¹⁶–C²⁰ represent methyl groups.

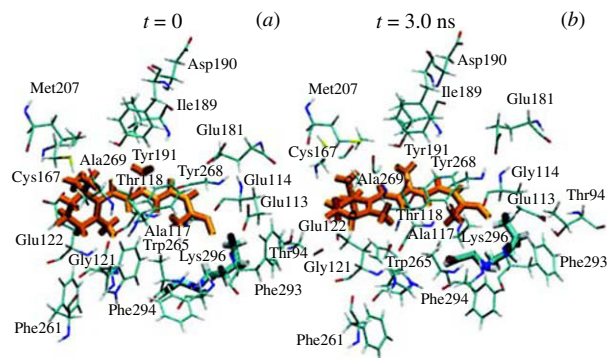


Figure 3 Molecular dynamics of 11-*cis*-retinal in the chromophore centre of the rhodopsin molecule at $T = 300$ K: (a) conformational state of the chromophore at start time; (b) conformational state of the chromophore after 3000 ps.

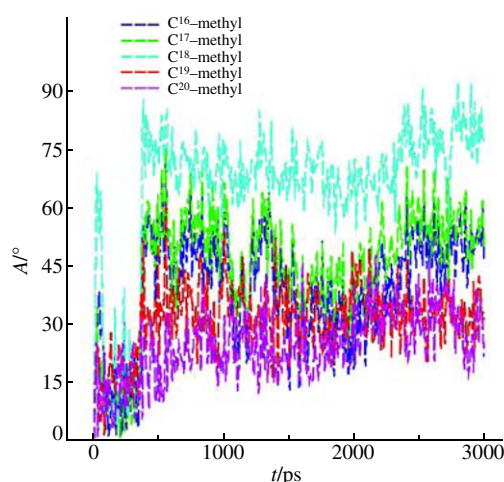


Figure 4 Torsional rotation angles of five methyl groups (C¹⁶–C²⁰) in 11-*cis*-retinal (Figure 3) during 3000 ps of rhodopsin molecular dynamics.

As to the latter, rhodopsin is characterised by an unusually high rate of chromophore isomerization (faster than 200 fs)^{16–18} and its high quantum yield (0.67).^{19,20}

The rate of 11-*cis*-retinal isomerization as a rhodopsin chromophore group is about two orders of magnitude higher than in solution.²¹ The crucial role of the protein environment is doubtless, but the underlying molecular mechanisms have not yet been studied in full.

The legitimacy and prognostic effectiveness of computer simulation for rhodopsin are supported by recent NMR data.^{13,22} The interaction of the 11-*cis*-retinal chromophore with amino-acid residues surrounding has been studied in detail. Many assumptions made previously on the basis of computer simulations^{9,12,23} have found experimental confirmation in these works.

In fact, it has been shown²² that one of the main events that turn rhodopsin after photon absorption into its physiologically active state involves conformational rearrangements in helical ‘bundles’ III and VI. Salgado *et al.*¹³ confirmed the experimental results and theoretical calculations regarding the β -ionone ring of 11-*cis*-retinal that has a twisted 6-*S-cis* conformation. It is no longer doubtful that 11-*cis*-retinal in rhodopsin exists in a twisted nonplanar state and the β -ionone ring is additionally turned by about 65° relative to the polyene chain.¹³ It is also believed that the twisting of the polyene chain plays an important role in retinal isomerization upon absorbing a photon.^{24,25}

However, many assumptions concerning the structural characteristics of the chromophore itself and its protein environment seem inconsistent at present. In particular, this concerns the conformation state of the β -ionone ring (6-*S-cis* or 6-*S-trans*) relative to the polyene chain of the retinal.^{14,26,27} Since conformational reorganization of this chromophore part is deemed to play an important role in the initiation of subsequent conformation reorganization in the protein part,^{15,28–30} studying the

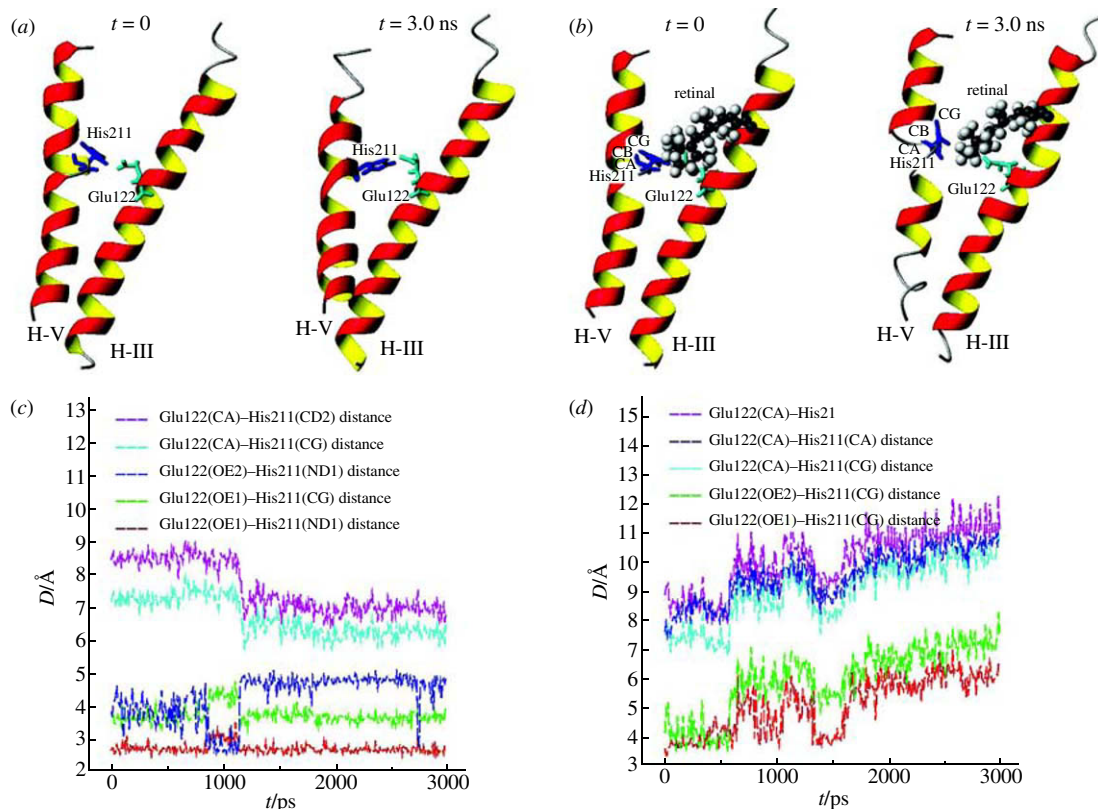


Figure 5 (Top) Molecular dynamics of the α -helices of H-III (Glu122) and H-V (His211): (a) in opsin (rhodopsin without the chromophore group); (b) in rhodopsin with the chromophore group, (11-*cis*-retinal) at simulation start time ($t = 0$) and end time ($t = 3000$ ps). 11-*cis*-retinal is shown as balls and amino acids are shown as 3D structures. (Bottom) Plots of interatomic distances between different atoms in Glu122 and His211 (the designations of amino-acid atoms according to the international nomenclature are shown in parentheses): (c) in opsin (rhodopsin without the chromophore group) and (d) in rhodopsin with the chromophore group (11-*cis*-retinal).

valid behaviour of the β -ionone ring itself and the nearest amino acid residues in the dark state and light-activated state remains an issue of current interest.

In this work, we used computer simulations to analyse the molecular dynamics of 11-*cis*-retinal and its proper conformational ‘adaptation’ in the rhodopsin chromophore centre.

Computer simulation methods. The dynamics of conformational changes in rhodopsin was computed for rhodopsin without chromophore group, *i.e.*, free opsin, and rhodopsin with the 11-*cis*-retinal chromophore.

The initial configuration of the visual pigment was chosen by analogy with other reported computer simulation studies.^{9,23} The rhodopsin molecule from the rhodopsin dimer model was used (PDB file 1HZX, chain A) (Figure 1).^{6,14}

Since the initial configuration of the molecule was incomplete (some parts of the PDB file were empty), preliminary cal-

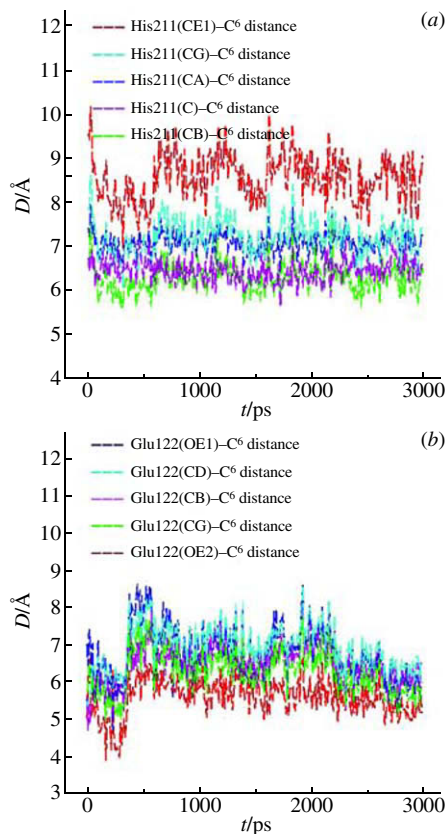


Figure 6 Interatomic distances between C⁶ of the β -ionone ring in 11-*cis*-retinal and different atoms of (a) His211 and (b) Glu122.

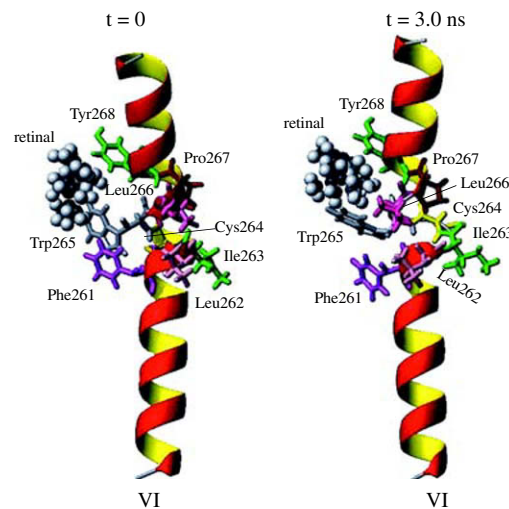


Figure 7 Molecular dynamics of the H-VI α -helix and its amino acid residues located near 11-*cis*-retinal at simulation start time ($t = 0$) and end time ($t = 3000$ ps). 11-*cis*-retinal is shown as balls and amino acid residues are shown as 3D structures.

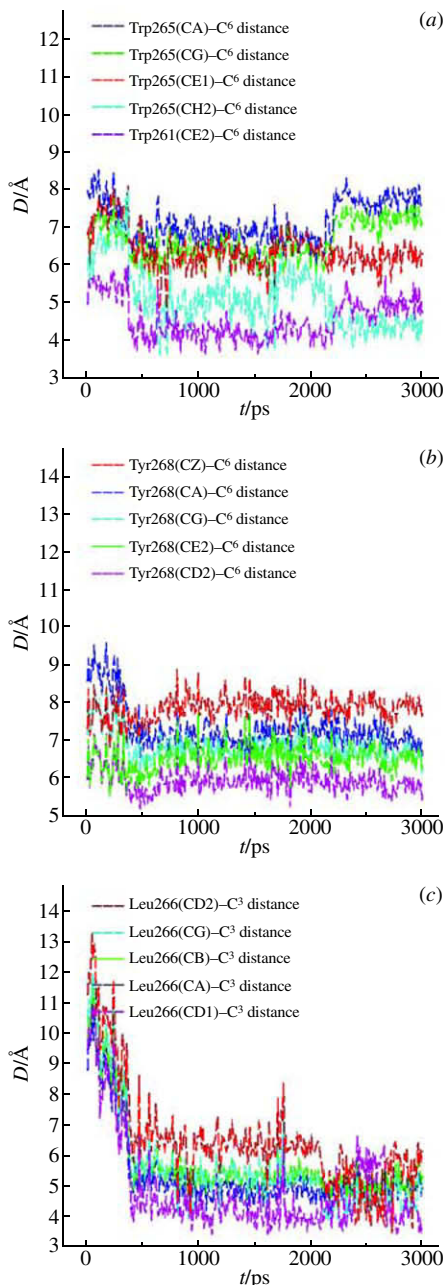


Figure 8 Interatomic distances between C⁶ of the β -ionone ring in 11-*cis*-retinal and different atoms of (a) Trp265 and (b) Tyr268, as well as (c) between C³ of the β -ionone ring in 11-*cis*-retinal and different atoms of Leu266.

culations were carried out in order to determine the complete tertiary structure of the rhodopsin molecule before the simulations. Based on the primary rhodopsin structure,³¹ we found and minimised the missing fragments of amino-acid sequences 236–240 and 331–333, which were embedded in the initial structure (PDB file 1HZX, chain A) using the MOE software

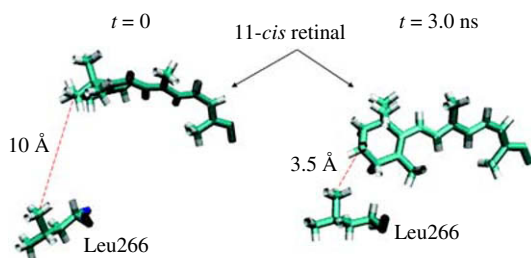


Figure 9 Molecular dynamics of 11-*cis*-retinal and Leu266 in a rhodopsin molecule at start time and after 3000 ps. View from the side of the molecule cytoplasmic part.

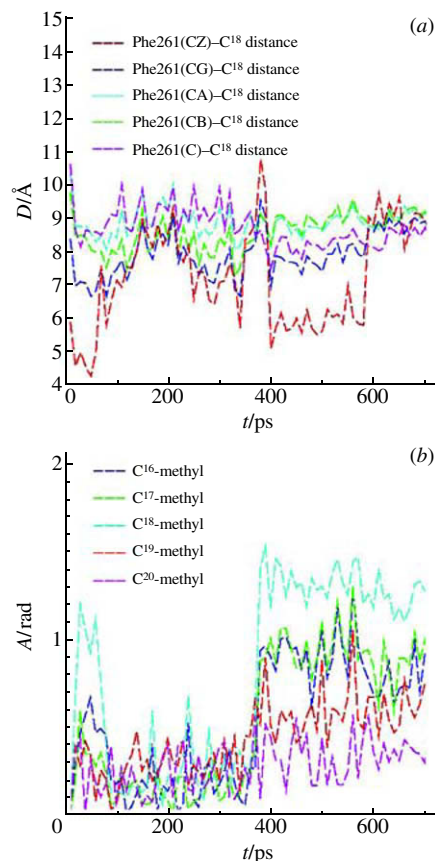


Figure 10 (a) Interatomic distances between C¹⁸ of the β -ionone ring in 11-*cis*-retinal and different atoms of Phe261. (b) Torsional rotation angles of five methyl groups (C¹⁶–C²⁰) in 11-*cis*-retinal.

package for computer simulations of biomolecules.³² After that, the entire 1HZX molecule was minimised with missing fragments already embedded.

The next simulation stage involved the molecular-mechanical calculation of the molecule energy minimum and heating of the system from the crystalline state at $T = 0$ K to a physiological temperature of 300 K. The molecular structure that would correspond to the minimum energy at $T = 300$ K was searched for with very slow heating in about 20-degree steps in order to avoid strong system ‘oscillations’ during the heating. After the system was brought to $T = 300$ K, it took a few thousand ‘steps’ (50–70 ps) for it to reach an equilibrium state with a minimum energy at this constant temperature. As a matter of fact, it is in this equilibrium state with a minimum invariable energy of the system as a whole where the simulation process started. This time point of simulation start was considered as zero time.

The system temperature was kept constant (300 K) for three million steps using the Berendsen algorithm with a thermostat relaxation time of 0.2 ps.³³ An integration step of 1 fs was chosen for Newtonian motion equations; thus, the total simulation time amounted to 3000 ps.

The simulation of rhodopsin was carried out in an aqueous solution by means of the AMBER 5.0 (Parm94) software package^{34–36} and its modified version AMBER 7.0 (Parm96) for the MDGRAPE-2 special-purpose computer.^{37,38}

The solvation of the system was carried out using the procedure of solvation by the TIP3P water model in a specified spherical space.³⁹

The lengths of the bonds involving only hydrogen atoms were calculated using the standard SHAKE method.⁴⁰

All atomic interactions and trajectories were calculated and the structure of the entire rhodopsin molecule was found. Simulations employed the Cornell atomic-power field method.⁴¹ The energy state of the system or the total interaction potential corresponded to the equilibrium state of the system where attraction forces were equilibrated by repulsion forces. Different types of

interaction that contribute to the stabilization of the biomacromolecule structure were taken into account:

$$\begin{aligned}
 U(r) = & \sum K_r(r - r_{eq})^2 + && \text{potential of intramolecular} \\
 & + \sum K_\theta(\theta - \theta_{eq})^2 + && \text{potential of angular bonds} \\
 & + \sum 1/2 K_\phi[1 + \cos(n\phi - \gamma)] + && \text{potential of dihedral (torsion)} \\
 & + \sum (A_{ij}/r_{ij}^{12} - B_{ij}/r_{ij}^6) + && \text{potential of nonvalence} \\
 & + \sum q_i q_j / \epsilon r_{ij} && \text{electrostatic potential.}
 \end{aligned}$$

Computation of the following values is presented in this work.

(i) Interatomic distances d with coordinates of individual atoms i and j :

$$\begin{aligned}
 D_1(x_1, y_1, z_1) = D_1(r_1) \text{ and } D_2(x_2, y_2, z_2) = D_2(r_2), \\
 d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2} = |r_2 - r_1|,
 \end{aligned}$$

at selected time t . In other words, r_1 and r_2 are the spatial position vectors of atoms i and j , respectively, at the given time point t (in the time range from $t = 0$ to $t = 3000$ ps).

(ii) Torsional rotation angles for individual segments of the chromophore, viz., 11-*cis*-retinal (Figure 2). Angle A between straight lines D_1D_2 and D_3D_4 , the directions of which are determined by vectors D_1D_2 and D_3D_4 , was calculated by the following formula:

$$\begin{aligned}
 \cos A = \frac{d_{12}d_{34}}{|d_{12}||d_{34}|}, \text{ where} \\
 |d_{12}| = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}, \\
 |d_{34}| = \sqrt{(x_4 - x_3)^2 + (y_4 - y_3)^2 + (z_4 - z_3)^2}.
 \end{aligned}$$

That is,

$$\cos A = \frac{(x_2 - x_1)(x_4 - x_3) + (y_2 - y_1)(y_4 - y_3) + (z_2 - z_1)(z_4 - z_3)}{|d_{12}||d_{34}|},$$

where d_{12} is the segment length vector at starting time point $t = 0$, d_{34} is the length vector of the same segment at time point $t > 0$ (in the time range up to 3 ns).

For methyl groups C¹⁶, C¹⁷, C¹⁸, C¹⁹ and C²⁰ in retinal, $|d_{12}|$ is the length of segment (C¹–C¹⁶) for C¹⁶; length of segment (C¹–C¹⁷) for C¹⁷; length of segment (C⁵–C¹⁸) for C¹⁸; length of segment (C⁹–C¹⁹) for C¹⁹; and length of segment (C¹³–C²⁰) for C²⁰, respectively.

Results and Discussion. It is well known that 11-*cis*-retinal in dark-state rhodopsin has a nonplanar, distorted and twisted conformation.^{13,14} This differs its conformational state from that of the retinal protonated Schiff base in solution.⁴⁶ The role of the protein environment in the formation of this configuration of 11-*cis*-retinal in rhodopsin is doubtless. However, the molecular mechanisms that ‘force’ the chromophore to assume such an energetically ‘unfavourable’ configuration still remain unclear. This was a key starting point for present simulations, and we first build a real-time model for the adaptation of 11-*cis*-retinal in opsin. Based on animated pictures and over 3000 ps calculations of interatomic distances, torsional rotation angles, etc., we describe below the model of dark ‘adaptation’ of retinal incorporated into opsin. The model assumes to explain why 11-*cis*-retinal exists in the configuration that makes it stable but ready, at the same time, for efficient ultrafast photoisomerization.

(A) *Molecular dynamics of the chromophore group – 11-cis-retinal.*

The simulation results have shown that the β -ionone ring of 11-*cis*-retinal in rhodopsin has a 6-*S-cis* configuration with respect to the polyene chain (Figure 2). This is in good agreement with experimental data of X-ray diffraction^{6,8,14} and NMR spectroscopy.^{13,47}

When considering the chromophore molecular dynamics over 3000 ps of simulation, it is easy to notice obvious changes in the spatial configuration of 11-*cis*-retinal.

According to our observations, within a short period of time, that is, from about 300 to 400 ps from the start of simulation, the β -ionone ring turns around the C⁶–C⁷ bond by about 60° with respect to the starting configuration of 11-*cis*-retinal (PDB file 1HZX, chain A^{6,14}) (Figure 3). This agrees with the theoretical and experimental data^{9,13} according to which the β -ionone ring in dark and non-irradiated rhodopsin is turned by about 50–65° with respect to the plane of the planar structure of free 11-*cis*-retinal, which creates its strained twisted conformation. At the same time, it is worth noting that the spatial model of the rhodopsin molecule obtained by X-ray diffraction analysis does not provide an accurate description of the spatial configuration of 11-*cis*-retinal in its chromophore section.^{46,48} Analysis of the crystal structure of rhodopsin and the comparison with the animated molecular-dynamics images clearly show that at the initial stage of simulation the plane of the β -ionone ring in 11-*cis*-retinal is almost perpendicular to the membrane plane, while the chromophore structure is nearly planar as a whole. The simulation results meanwhile show also that, after the β -ionone ring has turned, its plane becomes nearly parallel to the membrane plane.

It seems that the amino acid residues that surround the chromophore ‘help’ or ‘force’ 11-*cis*-retinal to ‘adapt’ a proper spatial configuration, necessary for a rhodopsin molecule to be a highly efficient trigger of phototransduction (this is possibly the simplest and, at the same time, might be the proper molecular mechanism of physiological regeneration of the visual pigment).

Furthermore, it has been reported that the central part, C¹⁰–C¹³, of the retinal polyene chain has a twisted strained configuration as well.^{49,50} In order to assess a ‘twisting’ degree of different retinal segments, we computed the torsional angles of all methyl groups. First, we calculated the torsional angles of methyl groups C¹⁶, C¹⁷ and C¹⁸ with respect to their positions at the start of simulation in order to evaluate the rotation of the β -ionone ring plane. Next, we calculated the torsional angles of C¹⁹ and C²⁰ in order to determine the conformational changes in the polyene chain central part. Figure 4 shows the diagrams of the rotational angles of the five methyl groups specified above. It is evident that the turning of these methyl groups occurs abruptly, that is, in a very short time interval (approximately, in the range 370–390 ps) and rather simultaneously. The mean rotational angles of methyl groups in this time interval are 75° for C¹⁸, 60° for C¹⁶ and C¹⁷, 40° for C¹⁹ and 30° for C²⁰. Though the fluctuation of these groups have a high amplitude (on average, about 20°), it is still evident that the more distant a methyl group from the β -ionone ring, the smaller its deviation from the initial state. It seems that the twisted distorted conformation of 11-*cis*-retinal in rhodopsin dark state is a result of a turn of the β -ionone ring that entails the ‘twisting’ of the retinal polyene ring.

Note that the methyl group C¹⁸ has the largest rotational angle. The rotational oscillations of this group at the start of ‘adaptation’ of the chromophore to the protein have the largest amplitude. Hence, this group probably plays a role of a key in 11-*cis*-retinal twisting.

Crozier *et al.*¹² have calculated the possible spatial coordinates for all heavy atoms in retinal within which all the chromophore atoms oscillate. It has been shown that C¹⁸ has the most significant oscillation dynamics among the five most mobile methyl groups including C¹⁶, C¹⁷, C¹⁹ and C²⁰ (here, the methyls are arranged in the order of decreasing mobility). These data are in good correlation with our calculations and demonstrate a motion dynamics of methyl groups in retinal.

The rotational angles of methyl groups C¹⁹ and C²⁰ are in a good agreement with experimental NMR-spectroscopic data,⁵¹ where the deviation angles of the C⁹–C¹⁹ and C¹³–C²⁰ vectors from the axis perpendicular to the photoreceptor membrane are approximately 42° and 30°, respectively.

Thus, our simulation results on the chromophore dynamics correlate well with both experimental^{5,6,8,13,14,47} and theoretical data.^{9,12,52} This clearly demonstrates that retinal in dark rhodopsin

has a twisted distorted configuration. However, note that the rotation of the β -ionone ring in these studies is incomparable with changes in the conformation of 11-*cis*-retinal polyene chain, and more specifically, its central part C¹⁰–C¹³. The two chromophore segments are considered in these papers independently.

Our simulation results suggest that all processes related to 11-*cis*-retinal dark adaptation in the chromophore centre have probably a common starting point. That is a large rotation of C¹⁸ methyl group. Presumably, fluctuations of this methyl in comparison with the others cause the twisting of the entire polyene chain in 11-*cis*-retinal.

(B) Molecular dynamics of amino acid residues in the chromophore region.

In order to determine the role of the nearest amino acid residues surrounding retinal and governing its conformational transformations over a period of 3000 ps, we calculated changes in the interatomic distances between the β -ionone ring and the adjacent amino acid residues Glu122, His211, Phe261, Trp265, Tyr268 and Leu266. For the distance between any heavy atom of the amino-acid residue and the β -ionone ring, we used the C⁶ atom of the β -ionone ring since it had the smallest mobility.

(1) *Glu122, His211 and the β -ionone ring.* Amino-acid residues Glu122 and His211 attract much attention due to their proximity to the β -ionone ring. It is believed that the interaction of Glu122 and His211 determines the mobility of the H-III and H-V α -helices, which, in turn, participate in the rhodopsin activation.⁵³ According to X-ray diffraction data,⁶ there is a hydrogen bond between these amino acid residues. In other words, the distance between them should be about 3 Å. In fact, the simulation of the rhodopsin dynamics in the absence of retinal confirmed this fact [Figure 5(a)]. One can see in the figure that the smallest distance between these amino acid residues is 3 Å throughout the entire simulation. It suggests that a stable hydrogen bond actually exists between Glu122 and His211. However, we obtained unexpected results in the case of dynamics simulation for the rhodopsin molecule with 11-*cis*-retinal. During the adaptation of retinal over 3000 ps after the start of the simulation, the distance between Glu122 and His211 increases [Figure 5(b)]. The shortest distance between Glu122(OE1) and His211(ND1) increases from 3 to 6–7 Å.

In other words, it appears that the hydrogen bond between Glu122 and His211 breaks, and that undoubtedly 11-*cis*-retinal initiates the process. It should be noted that the changes in the distance occur stepwise rather than smoothly. Approximately 580 ps after the start of the simulation run, the distance between various atoms of these amino acid residues begun to increase. Patel *et al.* claim¹¹ that the bond between H-III and H-V is cleaved when rhodopsin is activated, assuming that a hydrogen bond between Glu122 and His211 exists in the dark state of rhodopsin. According to our results, this bond is already cleaved in the protein inactive dark state. We may just assume that this event increases the instabilities in the system as a whole, and that it is a premise for the ‘alert’ of 11-*cis*-retinal for ultrafast efficient photoisomerization.

To understand the role of 11-*cis*-retinal in this process, we calculated the distances between these amino acid residues and the C⁶ atom of the β -ionone ring. Analysis showed that His211 does not manifest any noticeable regular dynamics with respect to the β -ionone ring [Figure 6(a)]. On the other hand, the behaviour of Glu122 looks unusual [Figure 6(b)]. First, in the range from 0 to 350 ps, it slightly approximates the β -ionone ring, then Glu122 abruptly becomes farther from 11-*cis*-retinal. The interatomic distances increase by 2 Å on average. This result might assume that the interaction between Glu122 and the β -ionone ring (350 ps) is the reason for the cleavage of the hydrogen bond between Glu122 and His211 (580 ps).

Note that the interaction between Glu122 and the β -ionone ring precedes the rotation of the β -ionone ring itself (370 ps). Thus, we suppose that Glu122 participates actively in conformational transformations of the retinal itself and its nearest environment.

The results on the molecular dynamics of Glu122 and His211 correlate well with the results of a theoretical study¹² where the

interaction energies of amino acid residues in the chromophore segment with 11-*cis*-retinal were calculated. The mean interaction energy of His211 with the β -ionone ring is -7.6 kcal mol⁻¹ (*i.e.*, these groups are attracted strongly to each other) and that for Glu122 is $+9.1$ kcal mol⁻¹ (*i.e.*, strong repulsion forces exist between the β -ionone ring and this amino acid residue).

(2) *Trp265, Tyr268, Leu266, Phe261 and the β -ionone ring.* The β -ionone rings of 11-*cis*-retinal in rhodopsin are mainly surrounded by aromatic amino acid residues.^{6,8,14} It is believed that the aromatic cluster plays an important role in maintaining the twisted strained configuration of 11-*cis*-retinal in the dark inactive state of the pigment.^{5,9,22,54} In this case, the main attention is given to Trp265 located at a distance of 3.8 Å from retinal, which is quite sufficient for strong electrostatic interactions with the chromophore.^{5,6}

It is believed that, on the one hand, the β -ionone ring blocks the movement of Trp265, thus stabilizing H-VI and preventing dark rhodopsin from passing to the active state, and, on the other hand, Trp265 stabilises the energetically unfavourable twisted configuration of the β -ionone ring. Many authors believe that Phe261 and Tyr268 also play an important role in stabilising the β -ionone ring, in determining the spectral sensitivity of the eye pigments, and in processes of rhodopsin activation.^{5,55}

In this work, we have analysed the dynamic behaviour of aromatic amino acid residues Phe261, Trp265, Tyr268 and Leu266 with aliphatic lateral chain and computed changes in the interatomic distances between these amino acid residues and the β -ionone ring of 11-*cis*-retinal (Figures 7 and 8).

The presence of Leu266 in this group is not coincidental. When analysing animated files, we have noted an unusual behaviour of this amino acid residue. The functional properties of Leu266 and its role in both maintaining the dark inactive state of rhodopsin and rhodopsin activation have almost not been discussed in the literature. It was reported that Leu266, like Trp265, is located very close to the β -ionone ring.⁵⁶ Simulation results show that during the adaptation of 11-*cis*-retinal in the chromophore region, Trp265 becomes closer to the β -ionone ring by about 2 Å [Figure 8(a)]. The smallest distance between these atomic groups is about 3.8 Å, which correlates well with published data. The dynamics of the approaching is stepwise in the region of 380 ps. Tyr268 also becomes closer by approximately 1–2 Å to the β -ionone ring [Figure 8(b)]. The approach is gradual in comparison with Trp265 and the process is completed by about 400 ps. However, note that the fluctuation amplitude on the curves becomes smaller as Tyr268 is approximating the β -ionone ring. It can be assumed that electrostatic interaction forces between Tyr268 and the β -ionone ring decrease the mobility of both group atoms. One can clearly see in Figure 7 that, in comparison with the initial state ($t = 0$), Trp265 and Tyr268 more completely ‘enfold’ 11-*cis*-retinal on two sides by $t = 3000$ ps of the simulation, whereas their aromatic rings become almost parallel to each other. These results are in good correlation with the concept of the role of these aromatic amino acids in stabilising and retaining the chromophore in a twisted distorted configuration.

Figure 7 also distinctly demonstrates an abrupt change in the position of Leu266. Analysis of the dynamics of changes in the interatomic distances between Leu266 and the β -ionone ring also distinctly shows the unusual behaviour of this amino acid residue [Figure 8(c)]. It is remarkable that the distance between Leu266 and the β -ionone ring decreases, on the average, by 6 Å in 400 ps, while the smallest distance between these groups is about 3.5 Å.

In other words, a strong electrostatic bond is established between these groups. It should be noted that the approximation of Leu266 and the β -ionone ring is observed starting from almost the zero time of simulation and ends by 400 ps, and that the mutual arrangement of these atomic groups remains unchanged during the subsequent simulation.

It is easy to notice while analysing the animation files that the distance mainly changes due to the rotation of the β -ionone ring.

The motive force of this process remains unknown. However, one can discuss the functional role of this conformational transformation. For example, it is assumed that Leu47 is involved in

the orientation of the side chain of Lys296 along the long axis of the rhodopsin molecule.⁵ Leu266 can also participate in the reorientation of the β -ionone ring. It is evident from Figure 9 that the plane of the β -ionone ring after it has turned (*i.e.*, after 400 ps) is arranged in the same plane with the two symmetric methyl groups of Leu266. However, no symmetry is observed in the arrangement of these groups at the start time of the simulation ($t = 0$).

Presumably, Trp265 and Tyr268 act as ‘clamps’ in this conformational transformation. If one compares the molecular dynamics time parameters for these amino acid residues, an impression appears that the interaction of Leu266 with the β -ionone ring (360–400 ps) occur faster than the conformational transformations of Trp265 (380 ps) and Tyr268 (up to 500 ps).

In other words, it can be assumed that approximation of the β -ionone ring with Leu266 occurs first, followed by its ‘clamping’ by Trp265 and Tyr268.

The molecular dynamics of Phe261 is also unusual. Two abrupt approximations of this residue to the β -ionone ring are clearly seen in Figure 10.

They occur within the time ranges from 0 to 50 ps (first) and from 370 to 600 ps (second). Comparison of this diagram with the curves reflecting the rotation angles of the methyl groups in the β -ionone ring on the same time scale (3000 ps) clearly shows a time correlation between the changes in the interatomic distances between Phe261 and the β -ionone ring, on the one hand, and the torsional oscillations of the C¹⁸ methyl group, on the other hand. An impression arises that, as Phe261 approximates the β -ionone ring, it attracts C¹⁸. It can be assumed that it is Phe261 that ‘swings’ C¹⁸, thus initiating the rotation of the entire β -ionone ring. However, it remains unclear what initiates this dynamics of Phe261 behaviour and why it moves again away from the β -ionone ring after 600 ps. It cannot be excluded that the fluctuations of Phe261 force Leu266 to move towards the β -ionone ring.

Therefore, it can be presumed that Phe261 fluctuations swing the C¹⁸ methyl group and simultaneously initiate the movement of Leu266 towards the β -ionone ring. In the instant when Phe261 approximates the C¹⁸ methyl group and Leu266, in turn, approximates the β -ionone ring to a distance sufficient for electrostatic interactions to appear between them, the β -ionone ring undergoes turns. Strong electrostatic interactions are established between Leu266 and the β -ionone ring; it is these interactions that define the rotation angle of the β -ionone ring and fix it in the new position. After that, Trp265 and Tyr268 additionally strengthen this bond. The appearance of uncharged (neutral) Leu266 in the environment of the β -ionone ring causes repulsion forces between the β -ionone ring and protonated (neutral) Glu122, which in turn results in the cleavage of the hydrogen bond between Glu122 and His211, and eventually, the H-III and H-V α -helices move apart.

Thus, we summarise that the process of 11-*cis*-retinal ‘accommodation’ (adaptation) in opsin chromophore centre is important for maintaining the dark inactive state of the molecule (remember that the chromophore acts as a powerful antagonist ligand) and for the readiness of 11-*cis*-retinal to undergo ultrafast efficient photoisomerization.

It is so far unknown how and in what spatial configuration in the course of physiological regeneration process the 11-*cis*-retinal is delivered to the rhodopsin chromophore site. Probably, the initial configuration of 11-*cis*-retinal entering the chromophore region differs from that of its stable dark-adapted state. It should be noted that the chromophore domain of the dark-adapted rhodopsin is not a specific binding site only for 11-*cis*-retinal. It is rather spacious and may also adopt other retinal isomers (*e.g.*, 9-*cis*-retinal), as well as their modifications.^{48,57} This may serve as an indication that, when 11-*cis*-retinal enters the chromophore site, it adopts an energetically unfavourable configuration. This is likely due to interactions with the amino acid residues that surround it rather than due to a steric hindrance. It might be true that the primary configuration of 11-*cis*-retinal is nearly

planar when it enters the chromophore segment, but in subsequence, owing to its interactions with the surrounding amino acid residues, the β -ionone ring turns by 60° and the chromophore polyene chain is twisted.

Though the topography of the rhodopsin molecule in the photoreceptor membrane has been described in considerable detail, X-ray diffraction analysis yet does not provide a unique answer regarding the true configuration of 11-*cis*-retinal in dark-adapted rhodopsin.

Using X-ray diffraction data,⁶ in the simulations we next allowed a crystal structure to find by itself the true and optimum arrangement, interacting thereby with all rhodopsin segments at physiological temperature (300 K). It is worth noting once more that the structure of the molecule in the initial point of the simulation was completely identical to that derived from X-ray diffraction data.

Though our model is a protein molecule in an aqueous environment (rhodopsin is a membrane protein), the rhodopsin dynamics described is nevertheless in good correlation with many experimental and theoretical studies, including those with models in lipid environments.^{9,12,46} This allows one to extrapolate the simulation results to the *in vivo* dark physiological regeneration of rhodopsin.

Most likely, the adaptation time of 11-*cis*-retinal into opsin and the initial configuration in our model system do not ideally match the parameters *in vivo*. However, the interaction processes for the chromophore with the surrounding amino acid residues we believe are the same. The situation might look, of course, more complex; it might be not like a key (retinal chromophore) and a lock (opsin protein) matching the key. Not only do the chromophore and opsin structurally match each other, but the protein forces 11-*cis*-retinal to adopt a proper spatial configuration that would match, on the other side, its function as an antagonist ligand and efficient trigger of phototransduction.

Thus, only during the physiological regeneration process we have to expect that the mutual structural adaptation and adjustment of the chromophore with its protein environment occur.

In this study, we have only analysed the region of the β -ionone ring of 11-*cis*-retinal with the surrounding amino acid residues. At the next step, we assume to analyse the intramolecular interaction mechanisms in the Schiff base region. This might shed light on how 11-*cis*-retinal, upon interaction with opsin, becomes a highly efficient phototransduction trigger (its photoisomerization rate is less than 200 fs, while the quantum yield of the reaction is 0.67), unlike free 11-*cis*-retinal in solution (the photoisomerization rate is about 10 ps, while the quantum yield of the reaction does not exceed 0.20) and an antagonist ligand, *etc.*

It is worth noting that the issue of the chromophore function as an antagonist ligand, we have considered previously⁵⁸ with regard to the dynamics of behaviour of hydrophilic ‘loops’ of the rhodopsin molecule on the cytoplasmic surface of the photoreceptor membrane.

This study was aided by computer hardware at the Computational Astrophysics Laboratory (CAL) of the Institute of Physical and Chemical Research (RIKEN), Japan. We are grateful to the Laboratory for the time provided for work on a supercomputer and a MDGRAPE-2 special-purpose computer for computing the molecular dynamics of protein molecules. We are grateful to the Director of the Laboratory, Professor Toshikazu Ebisuzaki for providing licensed software and MDGRAPE-2 computers with a total processing power of 25 teraflops.

This work was supported by the Program for fundamental studies of the Presidium of the Russian Academy of Sciences ‘Theoretical and experimental investigation of the nature of chemical bond and mechanisms of major chemical reactions and processes’ (Program no. 1 of Division for Chemistry and Materials Science of the RAS), the Russian Foundation for Basic Research (grant no. 05-04-49945), and the Program for fundamental studies of the Presidium of the Russian Academy of Sciences ‘Integrative regulation mechanisms of organism functions’.

References

- 1 T. Mirzadegan, G. Benko, S. Filipek and K. Palczewski, *Biochemistry*, 2003, **42**, 2759.
- 2 J. C. Venter, M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith, M. Yandell, C. A. Evans, R. A. Holt *et al.*, *Science*, 2001, **291**, 1304.
- 3 D. R. Flower, *Biochim. Biophys. Acta*, 1999, **1422**, 207.
- 4 U. Gether and B. K. Kobilka, *J. Biol. Chem.*, 1998, **273**, 17979.
- 5 S. T. Menon, M. Han and T. P. Sakmar, *Physiol. Rev.*, 2001, **81**, 1659.
- 6 K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto and M. Miyano, *Science*, 2000, **289**, 739.
- 7 Y. Liang, D. Fotiadis, S. Filipek, D. A. Saperstein, K. Palczewski and A. Engel, *J. Biol. Chem.*, 2003, **278**, 21655.
- 8 T. Okada, M. Sugihara, A.-N. Bondar, M. Elstner, P. Entel and V. Buss, *J. Mol. Biol.*, 2004, **342**, 571.
- 9 J. Saam, E. Tajkhorshid, S. Hayashi and K. Schulten, *Biophys. J.*, 2002, **83**, 3097.
- 10 R. S. H. Liu and L. U. Colmenares, *PNAS*, 2003, **100**, 14639.
- 11 A. B. Patel, E. Crocker, M. Eilers, A. Hirshfeld, M. Sheves and S. O. Smith, *PNAS*, 2004, **101**, 10048.
- 12 P. S. Crozier, M. J. Stevens, L. R. Forrest and T. B. Woolf, *J. Mol. Biol.*, 2003, **333**, 493.
- 13 G. F. J. Salgado, A. V. Struts, K. Tanaka, N. Fujioka, K. Nakanishi and M. F. Brown, *Biochemistry*, 2004, **43**, 12819.
- 14 D. C. Teller, T. Okada, C. A. Behnke, K. Palczewski and R. E. Stenkamp, *Biochemistry*, 2001, **40**, 7761.
- 15 W. L. Hubbell, C. Altenbach, C. M. Hubbell and H. G. Khorana, *Adv. Protein Chem.*, 2003, **63**, 243.
- 16 L. A. Peteanu, R. W. Schoenlein, Q. Wang, R. A. Mathies and C. V. Shank, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 11762.
- 17 G. Haran, E. A. Morfino, J. Matthes, R. H. Callender and R. M. Hochstrasser, *J. Phys. Chem. A*, 1999, **103**, 2202.
- 18 H. Kandori, Y. Furutani, S. Nishimura, Y. Shichida, H. Chosrowjan, Y. Shibata and N. Mataga, *Chem. Phys. Lett.*, 2001, **334**, 271.
- 19 H. Dartnall, *Vision Res.*, 1968, **8**, 339.
- 20 J. E. Kim, M. J. Tauber and R. A. Mathies, *Biophys. J.*, 2003, **84**, 2491.
- 21 H. Kandori, Y. Katsuda, M. Ito and H. Sasabe, *J. Am. Chem. Soc.*, 1995, **117**, 2669.
- 22 P. J. R. Spooner, J. M. Sharples, S. C. Goodall, P. H. M. Bovee-Geurts, M. A. Verhoeven, J. Lugtenburg, A. M. A. Pistorius, W. J. DeGrip and A. Watts, *J. Mol. Biol.*, 2004, **343**, 719.
- 23 U. F. Röhrig, L. Guidoni and U. Rothlisberger, *Biochemistry*, 2002, **41**, 10799.
- 24 G. G. Kchendoerfer, P. J. E. Verdegem, I. van der Hoef, J. Lugtemburg and R. A. Mathies, *Biochemistry*, 1996, **35**, 16230.
- 25 F. DeLange, P. H. M. Bovee-Geurts, J. Van Oostrum, M. D. Portier, P. J. E. Verdegem, J. Lugtemburg and W. J. DeGrip, *Biochemistry*, 1998, **37**, 1411.
- 26 P. J. R. Spooner, J. M. Sharples, M. A. Verhoeven, J. Lugtenburg, C. Glaubitz and A. Watts, *Biochemistry*, 2002, **41**, 7549.
- 27 G. Gröbner, I. J. Burnett, C. Glaubitz, G. Choi, A. J. Mason and A. Watts, *Nature*, 2000, **405**, 810.
- 28 T. Okada, O. P. Ernst, K. Palczewski and K. P. Hofmann, *Trends Biochem. Sci.*, 2001, **26**, 318.
- 29 B. Borhan, M. L. Souto, H. Imai, Y. Shichida and K. Nakanishi, *Science*, 2000, **288**, 2209.
- 30 J. E. Kim, D. Pan and R. A. Mathies, *Biochemistry*, 2003, **42**, 5169.
- 31 Yu. A. Ovchinnikov, N. G. Abdulaev, N. Yu. Feigina, I. D. Artamonov, A. S. Zolotarev, M. B. Kostina, A. S. Bogachuk, A. I. Miroshnikov, V. I. Martynov and A. B. Kudelin, *Bioorg. Khim.*, 1982, **8**, 1011 (in Russian).
- 32 MOE/MOE (Molecular Operating Environment) (<http://www.chemcomp.com>; used within 2002–2003, by license of CAL RIKEN).
- 33 H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. DiNola and J. R. Haak, *J. Chem. Phys.*, 1984, **81**, 3684.
- 34 D. A. Case, D. A. Pearlman, J. W. Caldwell, T. E. Cheatham, W. S. Ross, C. L. Simmerling, T. A. Darden, K. M. Merz, R. V. Stanton, A. L. Cheng, J. J. Vincent, M. Crowley, D. M. Ferguson, R. J. Radmer, G. L. Seibel, U. C. Singh, P. K. Weiner and P. A. Kollman, AMBER 5, 1997, University of California.
- 35 D. A. Pearlman, D. A. Case, J. W. Caldwell, W. R. Ross, T. E. Cheatham, S. DeBolt, D. Ferguson, G. Seibel and P. Kollman, *Comp. Phys. Commun.*, 1995, **91**, 1.
- 36 J. W. Ponder and D. A. Case, *Adv. Prot. Chem.*, 2003, **66**, 27.
- 37 T. Narumi, R. Susukita, T. Ebisuzaki, G. McNiven and B. Elmegreen, *Molecular Simulation*, 1999, **21**, 401.
- 38 T. Narumi, R. Susukita, H. Furusawa and T. Ebisuzaki, *Proc. 5th Int. Conf. on Signal Processing*, 2000, Beijing, pp. 575–582.
- 39 W. L. Jorgensen, J. Chandrasekhar and J. D. Madura, *J. Chem. Phys.*, 1983, **79**, 926.
- 40 J. P. Ryckaert, G. Ciccotti and H. J. C. Berendsen, *J. Comput. Phys.*, 1997, **23**, 327.
- 41 W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, Jr., K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell and P. A. Kollman, *J. Am. Chem. Soc.*, 1995, **117**, 5179.
- 42 K. T. Kholmurodov, M. V. Altaisky, I. V. Puzynin, T. Darden and F. P. Filatov, *PEPAN (Physics of Elementary Particles and Atomic Nuclei)*, JINR, Dubna, Moscow, 2003, vol. 34, part 2, pp. 474–510.
- 43 K. Kholmurodov and T. Ebisuzaki, *ICMS-CSW2004*, Tsukuba, 2004, vol. C4, pp. 9–11, <http://www.jinr.ru/publish/Pepan/v-34-2.html>.
- 44 R. A. Sayle and E. J. Milner-White, *Trends Biochem. Sci.*, 1995, **20**, 374.
- 45 R. Koradi, M. Billeter and K. Wüthrich, *J. Mol. Graphics*, 1996, **4**, 51.
- 46 M. Sugihara, V. Buss, P. Entel, M. Elstner and T. Frauenheim, *Biochemistry*, 2002, **41**, 15259.
- 47 M. Han and S. O. Smith, *Biochemistry*, 1995, **34**, 1425.
- 48 D. Singh, B. S. Hudson, C. Middleton and R. R. Birge, *Biochemistry*, 2001, **40**, 4201.
- 49 P. J. Verdegem, P. H. Bovee-Geurts, W. J. de Grip, J. Lugtenburg and H. J. de Groot, *Biochemistry*, 1999, **38**, 11316.
- 50 S. W. Lin, M. Groesbeek, I. van der Hoef, P. Verdegem, J. Lugtenburg and R. A. Mathies, *J. Phys. Chem.*, 1998, **102**, 2787.
- 51 G. Grobner, G. Choi, I. J. Burnett, C. Glaubitz, P. J. Verdegem, J. Lugtenburg and A. Watts, *FEBS Lett.*, 1998, **422**, 201.
- 52 A. Yamada, T. Yamato, T. Kakitani and S. Yamamoto, *Photochem. Photobiol.*, 2004, **79**, 476.
- 53 M. Beck, T. P. Sakmar and F. Siebert, *Biochemistry*, 1998, **37**, 7630.
- 54 A. F. Creemers, S. Kiihne, P. H. Bovee-Geurts, W. J. DeGrip, J. Lugtenburg and H. J. de Groot, *PNAS*, 2002, **99**, 9101.
- 55 S. Jager, M. Han, J. W. Lewis, I. Szundi, T. P. Sakmar and D. S. Kliger, *Biochemistry*, 1997, **36**, 11804.
- 56 K. Nakanishi, H. Zhang, K. A. Lerro, S. Takekuma, T. Yamamoto, T. H. Lien, L. Sastry, D. J. Baek, C. Moquin-Pathey, M. F. Boehm, F. Derguini and M. A. Gawinowicz, *Biophys. Chem.*, 1995, **56**, 13.
- 57 Y. Imamoto, M. Sakai, Y. Katsuta, A. Wada, M. Ito and Y. Shichida, *Biochemistry*, 1996, **35**, 6257.
- 58 Kh. T. Kholmurodov, T. B. Feldman and M. A. Ostrovsky, *Rossiiskii Fiziologicheskii Zhurnal im. I. M. Sechenova*, 2005, **91**, 1377 (in Russian).

Received: 20th October 2005; Com. 05/2596