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Red Fluorescent Protein DsRed: Parametrization of Its Chromophore as an Amino Acid Residue for Computer Modeling in the OPLS-AA Force Field

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Abstract—Topology of the neutral form of the DsRed fluorescent protein chromophore as a residue of [(4-*cis*)-2-[(1-*cis*)-4-amino-4-oxobutanimidoyl]-4-(4-hydroxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]acetic acid was calculated with OPLS-AA force field. Use of this topology and molecular dynamics simulation allows calculating the parameters of proteins that contain such residue in their polypeptide chains. The chromophore parameters were obtained by *ab initio* (RHF/6-31G**) quantum chemical calculations applying density functional theory (B3LYP). Using this chromophore, we have calculated the molecular dynamics trajectory of tetrameric fluorescent protein DsRed in solution at 300 K (4 nsec). Correctness of the chromophore parametrization was revealed by comparison of quantitative characteristics of the chromophore structure obtained from the molecular dynamic simulations of DsRed protein with the quantitative characteristics of the chromophore based on the crystallographic X-ray data of fluorescent protein DsRed (PDB ID: 1ZGO, 1G7K, and 1GGX), and also with the quantitative characteristics of the chromophore obtained by quantum chemical calculations. Inclusion of the neutral form of DsRed protein chromophore topology into the OPLS-AA force field yielded the extended force field OPLS-AA/DsRed. This force field can be used for molecular dynamics calculations of proteins containing the DsRed chromophore. The parameter set presented in this study can be applied for similar extension in any other force fields.

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Fluorescent proteins are used in cell biology studies as potent noninvasive *in vivo* markers. They are employed for investigation of intracellular gene expression, localization of proteins and more complex structures in cells, and also for analysis of intra- and intercellular interactions [1-5]. Fluorescent proteins are also used as partners in fluorescence resonance energy transfer (FRET) with other fluorescent proteins or non-peptide compounds [6]; they represent a basis for development of sensors [7, 8], halide sensors [9], and timers [10].

Abbreviations: DsRed) *Discosoma striata* red fluorescent protein; GFP) *Aequorea victoria* green fluorescent protein; MD) molecular dynamics; OPLS-AA) the force field used in this study; QYG) DsRed chromophore formed from three amino acid residues, glutamine, tyrosine, and glycine, representing the residue of [(4-*cis*)-2-[(1-*cis*)-4-amino-4-oxobutanimidoyl]-4-(4-hydroxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]acetic acid; Phe-QYG) dipeptide formed by phenylalanine residues and QYG; $QYG^{MD,Average}$) QYG structure averaged by MD trajectory of DsRed protein; Phe-$QYG^{MD,Average}$) Phe-QYG structure averaged by MD trajectory of Phe-QYG dipeptide; QYG^{1G7K}, QYG^{1GGX}, and QYG^{1ZGO}) QYG structures in DsRed protein crystals (PDB ID: 1G7K, 1GGX, and 1ZGO, respectively); RMSD) root mean square deviation.

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The presence of the chromophore, a fragment of the polypeptide chain responsible for the major optical properties, is a characteristic feature of this protein family. A chromophore is formed during maturation of these proteins as a result of posttranslational, intramolecular, autocatalytic, chemical modification [11].

Green fluorescent protein (GFP) is a convenient small low-toxicity marker of gene expression [12]. Fluorescent proteins that emit in the red region of the spectrum become popular fluorescent probes. The most promising and intensively investigated red fluorescent proteins include tetrameric red fluorescent protein DsRed (drFP583) [13], monomeric red fluorescent protein mRFP1, and some others [14].

Fluorescence of proteins of this family depends on external factors: pH [15, 16], temperature [1], and ionic composition of the medium [6, 9]; however, the structure of the chromophore and its nearest environment are the factors responsible for fluorescence [1, 10]. Site-directed and random mutagenesis approaches are employed to obtain new mutant fluorescent proteins with improved fluorescent characteristics: quantum yield of the fluorescence, extinction coefficient, photo- and pH-stability, etc. [1, 17]. Studies also use theoretical, experimental, and computational methods for investigation of mechanisms underlying the fluorescence of these proteins [18-21].

Since optical properties of the fluorescent proteins are especially interesting for practical application, it is important to calculate them. The optical properties of a molecule are determined by the possibility of transitions between the ground and excited states of electrons and also oscillation and rotation energy levels, which may be calculated using quantum chemical methods [22-27]. However, the modern computer technologies cannot be used for complete quantum chemical calculation of protein molecules within reasonable time [28]. Calculations of these systems employ combined approaches in which some part of the information can be obtained using the method of molecular dynamics (MD) [29].

In the MD method, all atoms of a molecule are represented as the set of points, which move according to equations of classical mechanics. At any moment in time, the system state (the set of all coordinates and velocities of atoms of the analyzed system) may be shown as a representative point in multiparametric phase space. Movement of this point as a function of time describes the trajectory of this system. The potential energy of a molecule represents the sum of potentials (energy of bond stretching, energy of rotation around valence and dihedral angles, etc.); the set of potential parameters in the force field is based on experimental or calculated data [30-32].

The MD method is used for analysis of dynamic behavior of proteins, their conformational mobility, for analysis of changes in free energy of systems, which may exist in various states, and also for determination of local energy minima of molecules [20, 33]. Use of MD method for calculations of fluorescent proteins can give valuable information about the chromophore and its environment, which can be used in quantum chemical calculations.

MD calculation of protein trajectory in particular force field requires a set of potential parameters of atomic interactions in amino acid residues of a protein. In the case of fluorescent proteins, such parameters for atoms of the chromophore inserted into polypeptide chain as an amino acid residue are also required. The chromophore of green fluorescent protein GFP was parameterized in the CHARMM force field [20]. The chromophore of red fluorescent protein DsRed still has not been parameterized in any force field.

In this study using the quantum chemical approach *ab initio* we have parameterized potential parameters for interactions of atoms constituting the chromophore of DsRed, the red fluorescent protein, in OPLS-AA force field; the chromophore represents the residue of [(4-*cis*)-2-[(1-*cis*)-4-amino-4-oxobutanimidoyl]-4-(4-hydroxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]acetic acid (this name is given according to IUPAC nomenclature [34-36]).

Parameters for calculation of potentials of interatomic interactions, which constitute the chromophore topology of the DsRed protein, were used for modification of the initial OPLS-AA force field. The resulting extended OPLS-AA/DsRed[1] force field can be used for MD calculations for DsRed and other proteins containing a chromophore identical to that of DsRed.

MATERIALS AND METHODS

Method of molecular dynamics. *Force field and solvent type*. The initial OPLS-AA force field [37] additionally optimized for peptides [38] and the TIP4P model of water molecules [39] were used for MD calculations.

Potential energy equation. In the OPLS-AA force field as well as in our study, a set of constants of potential energy Eq. (1) determines the behavior of the whole system:

$$U = \sum_{r_{ij}} V_b(r_{ij}) + \sum_{\theta_{klm}} V_a(\theta_{klm}) + \sum_{\xi_{nopq}} V_{id}(\xi_{nopq}) + \\ + \sum_{\phi_{rstu}} V_{rb}(\phi_{rstu}) + \sum_{r_{vw}} V_{LJ}(r_{vw}) + \sum_{r_{xy}} V_C(r_{xy}) \quad , \qquad (1)$$

where $V_b(r_{ij})$ is harmonic potential of change of chemical bond length between two atoms i and j:

$$V_b(r_{ij}) = \frac{1}{2} k_{ij}^b (r_{ij} - b_{ij})^2, \qquad (2)$$

[1] Materials can be supplied in electronic version on request.

where k_{ij}^b is the rigidity of the bond formed by two atoms i and j (kJ/(mol·nm^2)), b_{ij} is equilibrium bond length (nm), and r_{ij} is current bond length (nm).

The expression $V_a(\theta_{klm})$ is harmonic potential of change of a valence angle formed by three atoms: k, l, and m:

$$V_a(\theta_{klm}) = \frac{1}{2} k_{klm}^{\theta} (\theta_{klm} - \theta_{klm}^0)^2 , \quad (3)$$

where k_{klm}^{θ} is rigidity of the valence angle (kJ/mol·rad^2) formed by the atoms, θ_{klm} is current value of this valence angle (degrees), θ_{klm}^0 is the equilibrium value of this angle (degrees).

The expression $V_{id}(\xi_{nopq})$ is harmonic potential of change of a dihedral angle formed by four atoms, n, o, p, and q:

$$V_{id}(\xi_{nopq}) = k_{nopq}^{\xi} (\xi_{nopq} - \xi_{nopq}^0)^2 , \quad (4)$$

where k_{nopq}^{ξ} is rigidity of this dihedral angle (kJ/mol·rad^2), ξ_{nopq} is current value of this dihedral angle (degrees), and ξ_{nopq}^0 is the equilibrium value of this dihedral angle (degrees).

The expression $V_{rb}(\phi_{rstu})$ is periodic potential of change of a dihedral angle formed by four atoms r, s, t, and u:

$$V_{rb}(\phi_{rstu}) = \sum_{n=0}^{5} C_{n+1} (\cos(\phi_{rstu} - 180^0))^n , \quad (5)$$

where ϕ_{rstu} is the current value of this dihedral angle (degrees) and C_n are potential constants (kJ/mol).

The expression $V_{LJ}(r_{vw})$ is combined potential of repulsion and dispersion interaction between two valence unbound atoms v and w (Lennard–Jones potential):

$$V_{LJ}(r_{vw}) = 4\varepsilon_{vw} \left(\left(\frac{\sigma_{vw}}{r_{vw}} \right)^{12} - \left(\frac{\sigma_{vw}}{r_{vw}} \right)^{6} \right) , \quad (6)$$

where r_{vw} is the distance between two unbound atoms v and w (nm), and σ_{vw} and ε_{vw} are set by formulas (7) and (8), respectively:

$$\sigma_{vw} = \frac{1}{2} (\sigma_{vv} + \sigma_{ww}) , \quad (7)$$

where σ_{vv} and σ_{ww} are expressed in nm;

$$\varepsilon_{vw} = \sqrt{\varepsilon_{vv} \varepsilon_{ww}} , \quad (8)$$

where ε_{vv} and ε_{ww} are expressed in kJ/mol.

Potential of electrostatic (Coulomb) interactions between atoms x and y is defined by:

$$V_C(r_{xy}) = \frac{1}{4\pi\varepsilon_0} \frac{q_x q_y}{\varepsilon_r r_{xy}} , \quad (9)$$

where r_{xy} is the distance between the atoms x and y (nm), q_x and q_y are effective point charges of the atoms (expressed in charge of the electron, $1.6 \cdot 10^{-19}$ Cl), ε_0 is absolute dielectric permeability (J·m^2/Cl^2), and ε_r is relative dielectric permeability.

Molecular dynamic calculations. *Program package for MD calculations.* The molecular dynamics simulation package GROMACS was used for MD calculations [33]. It supports OPLS-AA force field, TIP4P water model, and is characterized by high-performance simulation.

Initial DsRed protein structure for MD calculations. Initial tetrameric structure of DsRed used for MD calculations was obtained from the Protein Data Bank file (PDB ID: 1ZGO) (http://www.pdb.org) [40, 41]. This file contains information about coordinates of all atoms of the mature form of DsRed protein tetramer (except hydrogen atoms) obtained by X-ray analysis of the crystal of this protein [42].

Initial structure of the dipeptide Phe-QYG for MD calculations. The initial structure of the dipeptide Phe-QYG was set up using the initial structure of DsRed protein (employed for MD calculations); using the program spdbv [43] the "dipeptide" structure including chromophore with the preceding Phe amino acid residue was generated.

System setup for MD calculations. The system for MD calculation of DsRed protein contained DsRed tetramer, 12,510 water molecules, and four Na^+ ions in the periodic box of the following size: 63.28 × 76.89 × 71.43 Å. The system for MD calculations of Phe-QYG dipeptide contained Phe-QYG and 809 water molecules in the periodic box of the following size: 27.14 × 31.95 × 28.49 Å.

MD calculations. The energy of the system was minimized using the mdrun program [44, 45] and the method of steepest descent for finding of the energy minimum. Energy minimization options were: initial step-size Emstep = 0.01 nm, minimization was stopped when the maximum energy gradient was not more than Emtol = 2000 kJ/(mol·nm). Using the coordinate file obtained from the energy minimization, MD calculation with limitation of motion of polypeptide chain atoms (the position restraints procedure) was performed using the mdrun program. Parameters: integration step of 2 fsec, total integration period of 10 psec, Berendsen thermostat [45], temperature (ref_t) – 300°K, characteristic thermostat time (tau_t) – 0.1 psec. Using the coordinate file obtained during MD calculation with limitation of motion of polypeptide chain atoms, MD trajectory of the system at 300°K was calculated using the mdrun program.

Parameters: integration step of 2 fsec, Berendsen thermostat, temperature (ref_t) – 300°K, characteristic thermostat time (tau_t) – 0.1 psec, isotropic Parinello–Rahman thermostat, characteristic barostat time (tau_p) – 0.5 psec, pressure (ref_p) – 1.0 bar, compressibility – $4.5 \cdot 10^{-5}$.

Calculations gave MD trajectories for DsRed protein and Phe-QYG dipeptide containing information on values of coordinates and velocities for each atom, which were generated each 500 fsec.

Analysis of MD trajectories. For MD trajectories, root mean square deviations (RMSD) of atom positions from the positions of these atoms in the initial structure were calculated using the g_rms program. Using the method of least squares, a structure corresponding to some time t was superimposed onto the initial structure ($t = 0$) and RMSD was calculated using the following formula:

$$RMSD(t) = \left[\frac{1}{M} \sum_{i=1}^{N} m_i \left\| \vec{r}_i(0) - \vec{r}_i(t) \right\|^2 \right]^{\frac{1}{2}}, \qquad (10)$$

where $\vec{r}_i(t)$ is the radius vector of i-atom at time t, $\|\vec{r}_i(0) - \vec{r}_i(t)\|$ is the distance between the position of i-atom at t = 0 and at time t; m_i is the mass of i-atom; $M = \sum_{i=1}^{N} m_i$ (mass of the whole molecule), N is total number of atoms in the molecule.

The chromophore structure (denominated as $QYG^{MD,Average}$) averaged by MD trajectory of DsRed protein and by MD trajectory of Phe-QYG dipeptide structure (denominated as Phe-$QYG^{MD,Average}$) in aqueous solution were generated using g_covar program.

The valence and torsion angle value distributions in MD trajectories were generated using g_angle program.

Quantum chemical calculations. The quantum chemical calculations *ab initio* employed the GAMESS quantum chemistry program package [45].

Geometry optimization of molecular structure was carried out by quantum chemical calculation *ab initio* using the restricted Hartree–Fock (RHF) method. We selected the basis of wave functions 6-31G**. The calculation was carried out using the density functional theory (DFT) and Beck B3LYP parametric functional [45].

The R. E. D. program (RESP ESP charge Derive http://www.u-picardie.fr/labo/lbpd/RED) was used for determination of effective charge of chromophore forming atoms.

RESULTS

The QYG chromophore of mature DsRed protein is formed by glutamine (Q66), tyrosine (Y67), and glycine (G68) residues of the DsRed apoprotein; its formation involves cyclization followed by oxidation [46]. (Here and further numeration is given by the amino acid sequence of the DsRed apoprotein.) Figure 1 shows the structure of the neutral form of the QYG chromophore and its link to DsRed protein. The chromophore, representing an intrinsic unit of the polypeptide chain, may be therefore considered as a new amino acid residue.

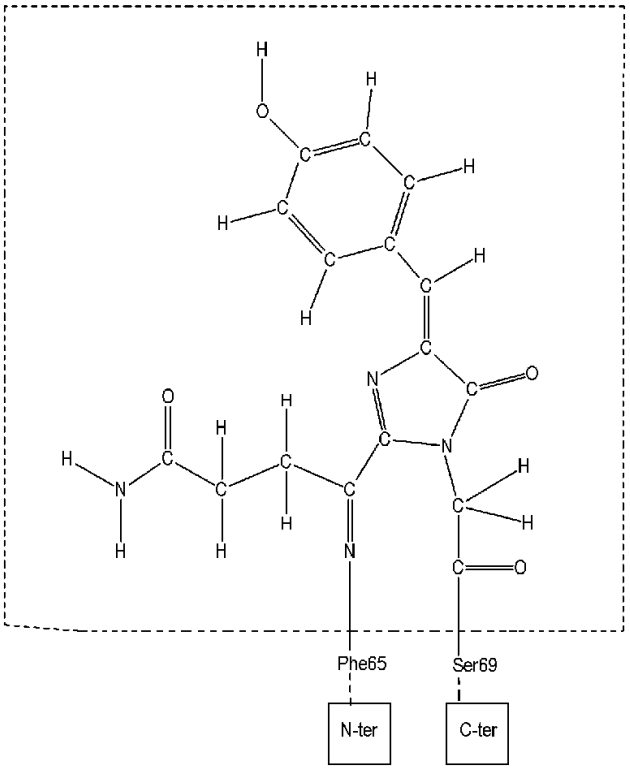

Fig. 1. Structure of the neutral form of the QYG chromophore in the DsRed polypeptide chain. The chromophore is framed by a dashed contour; N-ter and C-ter are the N- and C-terminal fragments of the polypeptide chain of the DsRed protein.

In the MD method the amino acid residue topology contains information about all atoms constituting this particular residue, their properties, type of interatomic interactions; it also includes quantitative description of interatomic interaction potentials for interactions of all these atoms and description of interaction potentials of atoms forming the peptide bond between particular amino acid residue and the previous one. Protein topology is thus a sum of topologies of amino acid residues forming the protein. In the present study we have generated topology of the neutral form of DsRed protein chromophore considered here as a new amino acid residue.

Each atom constituting this chromophore has been named, and Fig. 2 shows all names of atoms required for topology generation of the DsRed protein chromophore.

First approximation of the DsRed protein chromophore topology. The topology of the DsRed protein chromophore was generated in the first approximation

using types of atoms available in the OPLS-AA force field. The types of atoms were selected by analogy with compounds described in the OPLS-AA force field (Table 1).

The set of atom types determines the atomic parameters used in the OPLS-AA force field: atomic mass, effective atomic charge, Lennard–Jones potential parameters, and also the set of other force field quantitative parameters described by formulas (6)-(9). The resulting set of parameters obtained using chemical similarity approach (Table 1) is the first approximation of topology generation of the neutral form of the DsRed protein chromophore. The final version of topology has included the following parameters from the initial set: atomic parameters for calculation of Lennard–Jones potential, and rigidities of bonds, valence angles, and dihedral angles (excluding the angles -C_N1_CA1, -CA_-C_N1_CA1, and C_N1_CA1_C1; their rigidities were calculated separately) (Tables 1-5).

Model molecule for quantum chemical calculations. For specification of the set of parameters describing the chromophore, we have made quantum chemical calculations of effective atomic charges, equilibrium values of bond lengths, and valence and dihedral angles using specially constructed model molecule A (Fig. 3), whose structure is similar to that of the DsRed protein chromophore.

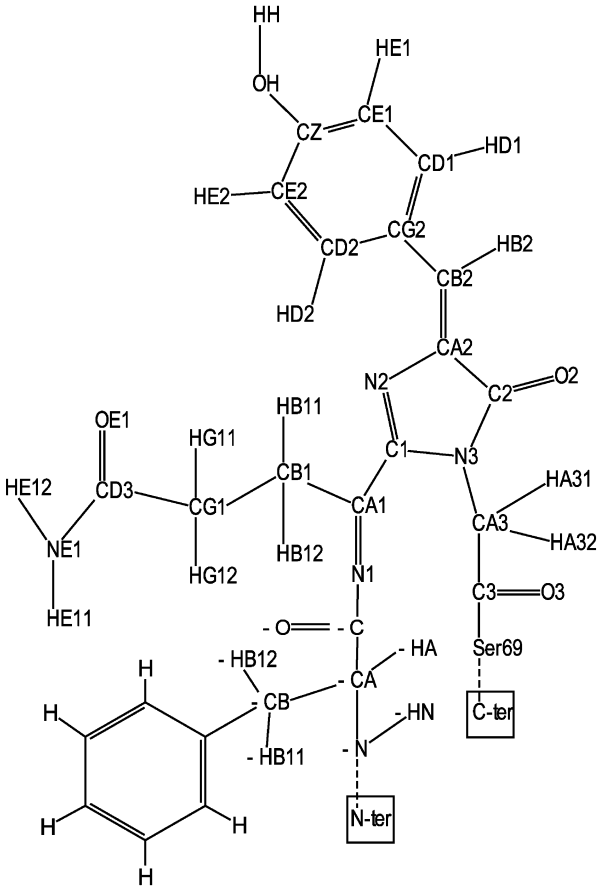

Fig. 2. Names of all atoms of DsRed polypeptide chain required for topology generation of the DsRed chromophore.

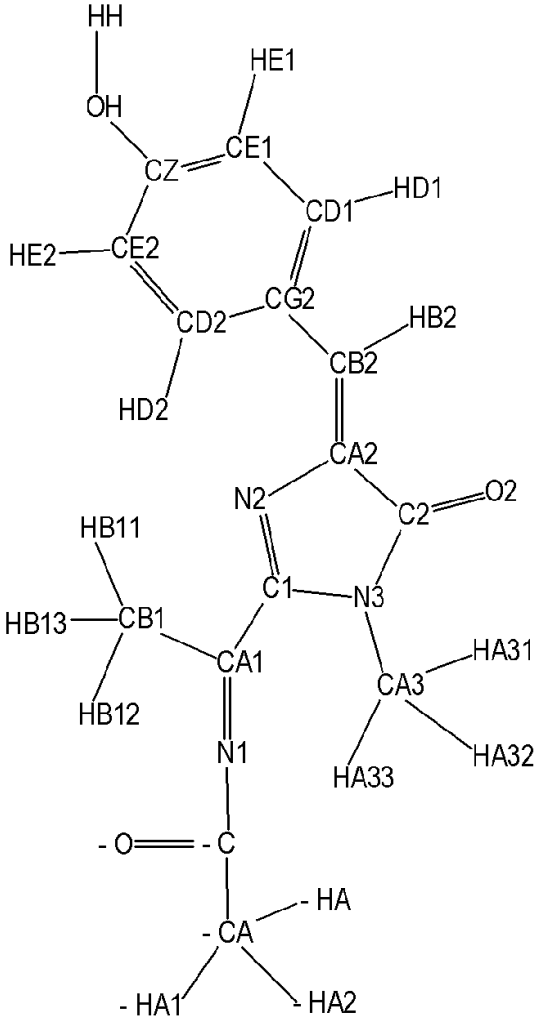

Fig. 3. Model molecule A.

In this study parameters of atoms and interatomic interactions of chromophore side radical corresponding to Q66 side-chain radical of immature DsRed protein have been set up by equal parameters of those for glutamine from OLPS-AA force field.

Quantum chemical calculation of equilibrium structure of model molecule A. For determination of effective atomic charges, equilibrium values of bond lengths, valence, and dihedral angles we have made quantum chemical optimization of geometry of the model molecule A. The structure of DsRed protein chromophore (PDB ID: 1GGX) [47] denominated as A^{1GGX} was used as the initial structure of model molecule A. Quantitative parameters of the A^{1GGX} structure are given in Tables 6-8.

Calculations gave the equilibrium structure of model molecule A, denominated as A^{eq}; it corresponds to the

Table 1. Atomic parameters of the DsRed protein chromophore

Atom name[2]	Atom type from OPLS-AA force field	Atom class from OPLS-AA force field	Effective partial atom charge q_i[3], set up by atom type	Effective partial atom charge q_i[3], obtained by quantum chemical calculations	Lennard–Jones potential parameters[4], set up by atom type		OPLS-AA force field atom ascribed to corresponding atom of the DsRed chromophore
					σ_{vv}, nm	ε_{vv}, kJ/mol	
1	2	3	4	5	6	7	8
N1	opls_335	NC	–0.54	–0.3426[1]	0.325[1]	0.711[1]	N3 atom of cytosine
CA1	opls_145	CA	–0.115	0.421[1]	0.355[1]	0.293[1]	C atom of benzene
CB1	opls_136	CT	–0.12	–0.12[1]	0.350[1]	0.276[1]	C atom aliphatic
HB11	opls_140	HC	0.06	0.04 (0.06[1, 6])	0.250[1]	0.126[1]	H atom aliphatic
HB12	opls_140	HC	0.06	0.04 (0.06[1, 6])	0.250[1]	0.126[1]	H atom aliphatic
CG1	opls_136	CT	–0.12[1]	n.d.[5]	0.350[1]	0.276[1]	C atom aliphatic
HG11	opls_140	HC	0.06[1]	n.d.[5]	0.250[1]	0.126[1]	H atom aliphatic
HG12	opls_140	HC	0.06[1]	n.d.[5]	0.250[1]	0.126[1]	H atom aliphatic
C1	opls_349	CB	0.38	0.0992[1]	0.350[1]	0.335[1]	C4 atom of adenine
N2	opls_352	NB	–0.49	–0.3415[1]	0.325[1]	0.711[1]	N7 atom of adenine
N3	opls_354	NA	–0.5	0.1093[1]	0.325[1]	0.711[1]	N9 atom of adenine
C2	opls_366	C	0.52	0.4[1]	0.375[1]	0.439[1]	C6 atom of guanine
O2	opls_370	O	–0.51	–0.5308[1]	0.296[1]	0.879[1]	O atom at C6 of guanine
CA2	opls_349	CB	0.38	0.0476[1]	0.350[1]	0.335[1]	C4 atom of adenine
CA3	opls_223B	CT_2	0.08	–0.12[1]	0.350[1]	0.276[1]	Cα atom of glycine
HA31	opls_140	HC	0.06	0.04 (0.06[1, 6])	0.250[1]	0.126[1]	H atom aliphatic
HA32	opls_140	HC	0.06	0.04 (0.06[1, 6])	0.250[1]	0.126[1]	H atom aliphatic
CB2	opls_145	CA	–0.115	–0.1164[1]	0.355[1]	0.293[1]	C atom of benzene
HB2	opls_146	HA	0.115	0.1439[1]	0.242[1]	0.126[1]	H atom of benzene
CG2	opls_145	CA	–0.115	0.0521[1]	0.355[1]	0.293[1]	C atom of benzene
CD1	opls_145	CA	–0.115	–0.1025[1]	0.355[1]	0.293[1]	C atom of benzene
HD1	opls_146	HA	0.115	0.1325[1]	0.242[1]	0.126[1]	H atom of benzene
CD2	opls_145	CA	–0.115	–0.1025[1]	0.355[1]	0.293[1]	C atom of benzene
HD2	opls_146	HA	0.115	0.1325[1]	0.242[1]	0.126[1]	H atom of benzene
CE1	opls_145	CA	–0.115	–0.2763[1]	0.355[1]	0.293[1]	C atom of benzene
HE1	opls_146	HA	0.115	0.1745[1]	0.242[1]	0.126[1]	H atom of benzene
CE2	opls_145	CA	–0.115	–0.2763[1]	0.355[1]	0.293[1]	C atom of benzene
HE2	opls_146	HA	0.115	0.1745[1]	0.242[1]	0.126	H atom of benzene

Table 1. (Contd.)

1	2	3	4	5	6	7	8
CZ	opls_166	CA	0.15	0.3365[1]	0.355[1]	0.293[1]	C atom of OH of phenol
OH	opls_167	OH	−0.585	−0.5158[1]	0.307[1]	0.711[1]	O atom of OH of phenol
HH	opls_168	HO	0.435	0.3811[1]	0.000[1]	0.000[1]	H atom of OH of phenol
OE1	opls_236	O	−0.5[1]	n.d.[5]	0.296[1]	0.879[1]	O atom of peptide bond carbonyl group
C	opls_235	C	0.5[1]	n.d.[5]	0.375[1]	0.439[1]	C atom of peptide bond carbonyl group
O	opls_236	O	−0.5[1]	n.d.[5]	0.296[1]	0.879[1]	O atom of peptide bond carbonyl group
CD3	opls_235	C	0.5[1]	n.d.[5]	0.375[1]	0.439[1]	C atom of peptide bond carbonyl group
NE1	opls_237	N	−0.76[1]	n.d.[5]	0.325[1]	0.711[1]	N atom of primary amide
HE11	opls_240	H	0.38[1]	n.d.[5]	0.000[1]	0.000[1]	H atom of primary amide
HE12	opls_240	H	0.38[1]	n.d.[5]	0.000[1]	0.000[1]	H atom of primary amide

[1] Parameters contributing to final version of chromophore topology of DsRed protein.
[2] Atom names given in this table correspond to those in Fig. 2.
[3] Values of effective partial atom charges are expressed in the charge of electron values (1 unit of charge of electron (q_{e^-}) is $1.6 \cdot 10^{-19}$ Cl).
[4] Lennard–Jones potential is described by formulas (6)-(8).
[5] This parameter was not determined (this atom is absent in model molecule A).
[6] Final version of DsRed protein chromophore topology included corrected values of effective partial charges of hydrogen atoms, which were calculated taking into consideration that chromophore topology contained just two pairs of such hydrogen atoms (HA31 and HA32) and (HB11 and HB12).

state of molecule A in its energy minimum. Tables 2-4 show parameters of the structure.

The resulting values of the lengths of the bonds and angles of the A^{eq} structure were then used as corresponding values of equilibrium lengths of the bonds b_{ij} (Table 2, column 2), valence angles θ^0_{klm} (Table 3, column 2), and dihedral angles ξ^0_{nopq} (Table 4, column 2) in the final description of the DsRed protein chromophore topology in OPLS-AA force field.

Specified values of effective atomic point charges of DsRed protein chromophore. The values of effective atomic point charges of the A^{eq} structure were obtained by quantum chemical calculation. The resulting charge values (Table 1, column 5) were then used as the specified chromophore atomic charges and were included in the final version of the topology (Table 1, column 4).

Specified parameters of chemical bonds of DsRed protein chromophore. Equilibrium values of lengths of the bonds b_{ij} and bond rigidity constants k^b_{ij} present in formula (2) are determined in the OPLS-AA force field by the types of atoms forming the bond (Table 2, columns 3 and 4). In this study, the equilibrium values of the lengths of the bonds from the first approximation of chromophore topology were substituted by the values of the lengths of the bonds from the A^{eq} structure (Table 2, column 2).

Bond rigidities included in the final version of the chromophore topology remained the same as they were set in the OPLS-AA force field on the basis of the types of atoms forming the bond (Table 2, column 4).

Specified parameters of valence angles of DsRed protein chromophore. Equilibrium values of valence angles θ^0_{ijk} and valence angle rigidity constants k^θ_{ijk} present in formula (3) are determined in the OPLS-AA force field by the types of atoms forming valence angle (Table 3, columns 3 and 4). In this study, the equilibrium values of the valence angles from the first approximation of the DsRed protein chromophore topology were substituted

Table 2. Parameters of chemical bond for description of the DsRed chromophore topology

Pair of atoms forming bond[2]		Equilibrium bond length $b_{ij}^{(3)}$, nm		Bond rigidity $k_{ij}^{b(3)}$, set up by atom types, kJ/mol·nm^2
		obtained by quantum chemical calculations	set up by atom types	
1		2	3	4
N1	CA1	0.128[1]	0.134	404174.4[1]
CA1	CB1	0.151[1]	0.151	265265.6[1]
CB1	HB11	n.d.[4]	0.109[1]	284512[1]
CB1	HB12	n.d.[4]	0.109[1]	284512[1]
CB1	CG1	n.d.[4]	0.153[1]	224262.4[1]
CG1	HG11	n.d.[4]	0.109[1]	284512[1]
CG1	HG12	n.d.[4]	0.109[1]	284512[1]
CG1	CD3	n.d.[4]	0.152[1]	265265.6[1]
CD3	OE1	n.d.[4]	0.123[1]	476976[1]
CD3	NE1	n.d.[4]	0.134[1]	410032[1]
NE1	HE11	n.d.[4]	0.101[1]	363171.2[1]
NE1	HE12	n.d.[4]	0.101[1]	363171.2[1]
CA1	C1	0.148[1]	0.140	392459.2[1]
C1	N2	0.131[1]	0.139	346435.2[1]
N2	CA2	0.139[1]	0.139	346435.2[1]
CA2	CB2	0.136[1]	0.140	392459.2[1]
CB2	HB2	0.109[1]	0.108	307105.6[1]
CB2	CG2	0.145[1]	0.140	392459.2[1]
CG2	CD1	0.142[1]	0.140	392459.2[1]
CD1	HD1	0.109[1]	0.108	307105.6[1]
CG2	CD2	0.142[1]	0.140	392459.2[1]
CD2	HD2	0.108[1]	0.108	307105.6[1]
CD1	CE1	0.138[1]	0.140	392459.2[1]
CE1	HE1	0.109[1]	0.108	307105.6[1]
CE1	CZ	0.140[1]	0.140	392459.2[1]
CD2	CE2	0.138[1]	0.140	392459.2[1]
CE2	HE2	0.108[1]	0.108	307105.6[1]
CE2	CZ	0.140[1]	0.140	392459.2[1]
CZ	OH	0.136[1]	0.136	376560[1]
OH	HH	0.097[1]	0.095	462750.4[1]
CA2	C2	0.149[1]	0.142	374049.6[1]
C2	O2	0.122[1]	0.123	476976[1]
C2	N3	0.141[1]	0.139	349782.4[1]
N3	C1	0.139[1]	0.137	364844.8[1]
N3	CA3	0.148[1]	0.147	282001.6[1]
CA3	HA31	0.109[1]	0.109	284512[1]
CA3	HA32	0.109[1]	0.109	284512[1]
CA3	C3	n.d.[4]	0.152[1]	265265.6[1]
C3	O3	n.d.[4]	0.123[1]	476976[1]
-C	N1	0.141[1]	0.136	382417.6[1]

[1] Parameters included in final version of DsRed chromophore topology.
[2] Atom names given in this table correspond to those of Fig. 2.
[3] Parameters for calculation of bond energy by formula (2).
[4] This parameter was not determined (this bond is absent in model molecule A).

Table 3. Parameters of valence angles of the DsRed protein chromophore

Triplets of atoms forming valence angle[2]			Equilibrium value of valence angle θ^{0}_{ijk}[3], degrees		Rigidity of valence angle k^{θ}_{ijk}[3], kJ/mol·rad^2, set up by atom types
			obtained by quantum chemical calculations	set up by atom types	
1			2	3	4
-CA	-C	N1	113.6[1]	116.6	585.8[1]
-O	-C	N1	122.4[1]	122.5	669.44[1]
-C	N1	CA1	123.8[1]	121.9	418.4 (279.3[1, 4])
N1	CA1	CB1	125.7[1]	116.0	585.8[1]
CB1	CA1	C1	115.8[1]	128.6	585.8[1]
CA1	C1	N3	121.5[1]	121.5	585.8[1]
C1	CA1	N1	118.4[1]	121.5	585.8[1]
CA1	CB1	HB11	109.5[1]	109.5	292.9[1]
CA1	CB1	HB12	109.5[1]	109.5	292.9[1]
CB1	CG1	HG11	n.d.[5]	110.7	313.8
CB1	CG1	HG12	n.d.[5]	110.7	313.8
CA1	CB1	CG1	n.d.[5]	112.7	488.273
CB1	CG1	CD3	n.d.[5]	111.1	527.184
CG1	CD3	OE1	n.d.[5]	120.4	669.44
CG1	CD3	NE1	n.d.[5]	116.6	585.76
OE1	CD3	NE1	n.d.[5]	122.9	669.44
CD3	NE1	HE11	n.d.[5]	119.8	292.88
CD3	NE1	HE12	n.d.[5]	119.8	292.88
CA1	C1	N2	120.8[1]	123.3	585.8[1]
CA1	C1	N3	125.0[1]	123.3	585.8[1]
C1	N2	CA2	106.1[1]	110.0	585.8[1]
C2	CA2	N2	109.3[1]	106.2	585.8[1]
CA2	C2	N3	103.1[1]	111.3	585.8[1]
C1	N3	C2	107.3[1]	109.8	585.8[1]
N3	C1	N2	114.2[1]	113.9	585.8[1]
CB2	CA2	C2	112.2[1]	123.3	585.8[1]
CB2	CA2	N2	128.5[1]	123.3	585.8[1]
CA2	CB2	CG2	130.1[1]	128.6	585.8[1]
CA2	C2	O2	131.1[1]	128.8	669.4[1]
N3	C2	O2	125.8[1]	120.6	669.4[1]
C1	N3	CA3	130.8[1]	123.3	585.8[1]
C2	N3	CA3	121.3[1]	123.3	585.8[1]
C3	CA3	N3	110.1[1]	110.1	527.2[1]
HA31	CA3	N3	109.5[1]	109.5	292.9[1]
HA32	CA3	N3	109.5[1]	109.5	292.9[1]
CD1	CG2	CD2	117.7[1]	120.0	527.2[1]
CE1	CZ	CE2	119.9[1]	114.1	585.8[1]
CA2	CB2	HB2	113.8[1]	120.0	292.9[1]
HB2	CB2	CG2	116.1[1]	120.0	292.9[1]

Table 3. (Contd.)

1			2	3	4
CB2	CG2	CD1	121.2[1]	120.0	527.2[1]
CB2	CG2	CD2	121.2[1]	120.0	527.2[1]
CG2	CD1	HD1	119.0[1]	120.0	292.9[1]
CG2	CD2	HD2	119.0[1]	120.0	292.9[1]
CG2	CD1	CE1	121.3[1]	120.0	527.2[1]
CG2	CD2	CE2	121.3[1]	120.0	527.2[1]
HD1	CD1	CE1	119.6[1]	120.0	292.9[1]
HD2	CD2	CE2	119.6[1]	120.0	292.9[1]
CD1	CE1	HE1	120.7[1]	120.0	292.9[1]
CD2	CE2	HE2	120.7[1]	120.0	292.9[1]
CD1	CE1	CZ	119.9[1]	120.0	527.2[1]
CD2	CE2	CZ	119.9[1]	120.0	527.2[1]
HE1	CE1	CZ	119.5[1]	120.0	292.9[1]
HE2	CE2	CZ	119.5[1]	120.0	292.9[1]
CE1	CZ	OH	120.0[1]	120.0	585.8[1]
CE2	CZ	OH	120.0[1]	120.0	585.8[1]

[1] Parameters included in final version of the DsRed chromophore topology.
[2] Atom names given in this table correspond to those of Fig. 2.
[3] Parameters for calculation of valence angle bond energy by formula (3).
[4] Rigidity value was corrected using quantum chemical calculations.
[5] This parameter was not determined (this angle is absent in model molecule A).

by the values obtained during quantum chemical calculations of the A^{eq} structure (Table 3, column 2).

Rigidity of the valence angle included in the final version of the DsRed protein chromophore topology was corrected for the angle -C_N1_CA1 (Fig. 2). For all other valence angles of the chromophore rigidity values included in the final version of topology remained unchanged as they were set in the OPLS-AA force field on the basis of the types ascribed to atoms that form the corresponding angle (Table 3, column 4).

Specified parameters of dihedral angles of DsRed chromophore. In this study, dihedral angle energy has been described by harmonic potential in accordance with formula (4) (Table 4) or by periodic potential according to formula (5) (Table 5).

Equilibrium values of dihedral angles of the DsRed chromophore characterized by energy described by harmonic potential obtained corresponding values from quantum chemical calculations of the A^{eq} structure (Table 4, column 2). Rigidities of these angles remained unchanged (as they were set in the OPLS-AA force field on the basis of the types of atoms forming the angle) (Table 4, column 4).

Energy of the dihedral angles -CA_-C_N1_CA1 and C_N1_CA1_C1 were set in this study by periodic potential using formula (5). Parameters of these angles included in the final version of the DsRed chromophore topology have been calculated separately, as described below.

Parameters of other dihedral angles with energy set by periodic potential remained unchanged as they were set on the basis of the types of atoms forming the angle (Table 5).

Parameters of valence angle -C_N1_CA1. For calculation of parameters of the valence angle -C_N1_CA1 the A^{eq} structure was used as the initial one. In the A^{eq} structure we varied the value of the valence angle -C_N1_CA1 (θ) from the equilibrium θ° value of 123.8° (Table 3) by increasing or decreasing it within $\Delta\theta$ in the range from -5° to $+5^\circ$ (with step of 1°); the values of other angles and bonds remained unchanged. These manipulations yielded the set of 10 non-equilibrium structures defined as $B^*(\Delta\theta)$.

For each structure, the quantum chemical geometry optimization was carried out at fixed values of the valence angle θ. This resulted in generation of 10 new quasi-equilibrium structures with fixed value of valence angle θ, which were defined as $B^{qeq}(\Delta\theta)$.

All these structures (as well as the A^{eq} structure) have a given deviation $\Delta\theta$ of the valence angle θ from its equilibrium value ranging from -5° to $+5^\circ$ including 0° (equilibrium value for the A^{eq} structure) and geometrically

Table 4. Parameters of dihedral angles of the DsRed protein chromophore set by harmonic potential

Quartet of atoms forming dihedral angle[2]				Equilibrium value of dihedral angle ξ^0_{nopq}[3], degrees		Angle rigidity k^{ξ}_{nopq}[3], kJ/mol·rad^2, set up by atom types
				obtained by quantum chemical calculations	set up by atom types	
1				2	3	4
CG1	NE1	CD3	OE1	n.d.[4]	180[1]	43.9[1]
CD3	HE11	NE1	HE12	n.d.[4]	180[1]	4.2[1]
CG2	CE2	CD2	HD2	179.97[1]	180	4.6[1]
CD2	CZ	CE2	HE2	−179.95[1]	180	4.6[1]
CD1	CZ	CE1	HE1	179.97[1]	180	4.6[1]
CG2	CE1	CD1	HD1	179.84[1]	180	4.6[1]
CD1	CD2	CG2	CB2	179.80[1]	180	4.6[1]
CE1	CE2	CZ	OH	−179.90[1]	180	4.6[1]
N1	CB1	CA1	C1	−179.17[1]	180	4.6[1]
CA1	N2	C1	N3	−179.80[1]	180	4.6[1]
N3	CA2	C2	O2	179.83[1]	180	43.9[1]
C1	C2	N3	CA3	177.33[1]	180	4.2[1]
N2	CB2	CA2	C2	179.36[1]	180	4.6[1]
CA2	CG2	CB2	HB2	179.67[1]	180	4.6[1]

[1] Parameters included in final version of DsRed chromophore topology.
[2] Atom names given in this table correspond to those of Fig. 2.
[3] Parameters for calculation of dihedral angle energy by formula (4).
[4] This parameter was not determined (this bond is absent in model molecule A).

optimized structure of all other parts of the molecule. The A^{eq} structure can be considered jointly with the set of the resulting structure assuming that $B^{qeq}(0) \equiv A^{eq}$.

For all 11 resulting structures, energy values were obtained by quantum chemical calculations. Figure 4 shows the dependence of energy of resulting structures on the value of deviation angle Δθ forming these structures.

The resulting set of points was approximated using formula (3), in which θ° = 123.8°. The equilibrium value of the valence angle θ° and obtained rigidity value k^{θ} of the angle -C_N1_CA1 were included into the final version of the DsRed chromophore topology (Table 3, columns 2 and 4).

Parameters of dihedral angles -CA_-C_N1_CA1 and C_N1_CA1_C1. For calculation of parameters of the dihedral angles -CA_-C_N1_CA1 and -C_N1_CA1_C1 we employed a procedure similar to used for calculation of rigidity of the valence angle -C_N1_CA1.

For determination of potential parameters of the dihedral angle -CA_-C_N1_CA1 (**ϕ**), the equilibrium A^{eq} structure was used as the initial one. Changes in the angle -CA_-C_N1_CA1 (**ϕ**) in the A^{eq} structure resulted in generation of a set of non-equilibrium structures C*(**ϕ**), in which the angle had the following values: $\phi = -180 + 20k$, where $k = 0, 1, ..., 18$.

For each structure, the quantum chemical geometry optimization was carried out at fixed values of angle ϕ. This generated a new set of quasi-equilibrium structure with fixed value of the valence angle ϕ, which were

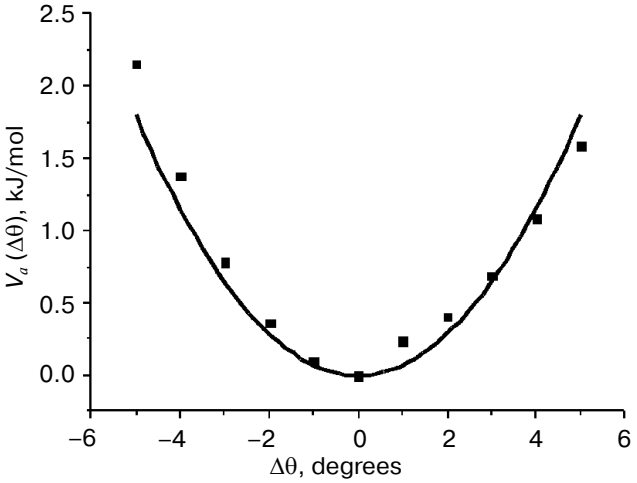

Fig. 4. Dependence of $V_a(\Delta\theta)$ energy values of B^{qeq} structures on deviation value (Δθ) of valence angle -C_N1_CA1 from its equilibrium value (see explanation in the text for model molecule A). Quadrants show energy values obtained by quantum chemical calculations, solid line shows approximation made by formula (3) at θ° = 123.8°.

Table 5. Parameters of dihedral angles of the DsRed protein chromophore set by periodic potential

Quartet of atoms forming dihedral angle[2]				Parameters of periodic potential set by types of atoms[3]					
				C_1	C_2	C_3	C_4	C_5	C_6
1				2	3	4	5	6	7
N1	CA1	CB1	CG1	1.92464[1]	2.61081[1]	2.20079[1]	−6.73624[1]	0[1]	0[1]
CG1	CB1	CA1	C1	−2.13384[1]	5.63166[1]	−3.49782[1]	0[1]	0[1]	0[1]
CB1	CG1	CD3	NE1	6.64001[1]	−10.5521[1]	−10.9663[1]	14.8783[1]	0[1]	0[1]
N1	-C	-CA	-N	10.36376[1]	−6.60654[1]	−10.4935[1]	6.73624[1]	0[1]	0[1]
-HA	-CA	-C	N1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-HA1	-CA	-C	N1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-HA2	-CA	-C	N1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-CB	-CA	-C	N1	5.00825[1]	−1.6987[1]	−0.37238[1]	−2.93716[1]	0[1]	0[1]
HB11	CB1	CA1	N1	0.97069[1]	2.91206[1]	0[1]	−3.88275[1]	0[1]	0[1]
HB12	CB1	CA1	N1	0.97069[1]	2.91206[1]	0[1]	−3.88275[1]	0[1]	0[1]
C1	N3	C2	O2	30.334[1]	0[1]	−30.334[1]	0[1]	0[1]	0[1]
C1	N3	C2	CA2	30.334[1]	0[1]	−30.334[1]	0[1]	0[1]	0[1]
CA3	N3	C2	O2	30.334[1]	0[1]	−30.334[1]	0[1]	0[1]	0[1]
CA3	N3	C2	CA2	30.334[1]	0[1]	−30.334[1]	0[1]	0[1]	0[1]
C3	CA3	N3	C1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
C3	CA3	N3	C2	4.73838[1]	−1.52507[1]	1.30541[1]	−4.51872[1]	0[1]	0[1]
HA31	CA3	N3	C2	0.87864[1]	2.63592[1]	0[1]	−3.51456[1]	0[1]	0[1]
HA32	CA3	N3	C2	0.87864[1]	2.63592[1]	0[1]	−3.51456[1]	0[1]	0[1]
-C	N1	CA1	CB1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
				n.d.[4]	n.d.[4]	n.d.[4]	n.d.[4]	n.d.[4]	n.d.[4]
-C	N1	CA1	C1	(24.65[1, 5])	(1.15[1, 5])	(−17.6[1, 5])	(−8.2[1, 5])	0[1]	0[1]
-N	-CA	-C	CA1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-HA	-CA	-C	CA1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-CB	-CA	-C	CA1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-CA	-C	N1	CA1	n.d.[4]	n.d.[4]	n.d.[4]	n.d.[4]	n.d.[4]	n.d.[4]
				(−4.08[1, 5])	(6.62[1, 5])	(12.18[1, 5])	(−1.48[1, 5])	0[1]	0[1]
-O	-C	N1	CA1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-CA	-C	CA1	N1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-CA	-C	CA1	CB1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-CA	-C	CA1	C1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-O	-C	CA1	N1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-O	-C	CA1	CB1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-O	-C	CA1	C1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
N1	-C	CA1	CB1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
N1	-C	CA1	C1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-C	CA1	CB1	HB11	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-C	CA1	CB1	HB12	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
-C	CA1	CB1	CG1	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
C1	N3	CA3	HA31	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
C1	N3	CA3	HA32	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]
N3	CA3	C3	O3	0[1]	0[1]	0[1]	0[1]	0[1]	0[1]

[1] Parameters included in final version of DsRed chromophore topology.
[2] Atom names given in this table correspond to those of Fig. 2.
[3] Parameters for calculation of dihedral angle energy by formula (5).
[4] Dihedral angle parameters were not determined in OPLS-AA force field.
[5] Parameters obtained in this study by means of quantum chemical calculations.

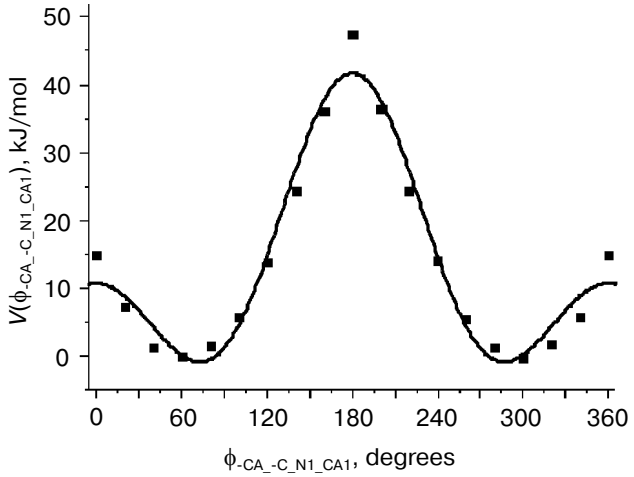

Fig. 5. Dependence of $V(\phi_{-CA\_-C\_N1\_CA1})$ energy values of C^{qeq} structures on value of dihedral angle -CA_-C_N1_CA1 ($\phi_{-CA\_-C\_N1\_CA1}$). Quadrants show energy values obtained by quantum chemical calculations; solid line shows approximation made by formula (5).

defined as $C^{qeq}(\phi)$. For these structures, the energy value was obtained by quantum chemical calculations. Figure 5 shows the dependence of energy of these structures on the dihedral angle ϕ value.

Similar procedures were employed for elucidation of parameters of the dihedral angle -C_N1_CA1_C1 (ψ), for which a set of quasi-equilibrium structures defined as $D^{qeq}(\psi)$, with fixed values of the angle ψ was obtained. For these structures, the energy value was obtained by quantum chemical calculations. Figure 6 shows the dependence of energy of these structures on the dihedral angle ψ value.

The resulting dependences (Figs. 5 and 6) were approximated by functions describing the periodic potential of change in dihedral angle according to formula (5). The potential parameters C_n (where n = 1, 2, ..., 6) for angles -CA_-C_N1_CA1 and -C_N1_CA1_C1 were included in the final version of the DsRed chromophore topology (Table 5, columns 2-7).

Final version of DsRed protein topology and extension of OPLS-AA force field to OPLS-AA/DsRed field. Tables 1-5 show potential parameters of interatomic interactions constituting the DsRed chromophore topology.

These parameters have been included in initial OPLS-AA force field; such extension required editing of corresponding files containing topology parameters of amino acid residues, rules for hydrogen atom addition to amino acid residues, parameters of atoms, and interactions. This resulted in the extended version of the OPLS-AA force field denominated as OPLS-AA/DsRed.

MD trajectory of DsRed protein in force field OPLS-AA/DsRed. Using the OPLS-AA/DsRed force field, the MD calculation was made for the DsRed protein tetramer in aqueous solution during 4.185 nsec. Protein structure analysis covered the last 3.185 nsec, because during the first nsec the system underwent relaxation; this period was further defined as the productive trajectory (MD trajectory) (Fig. 7).

Comparison of DsRed chromophore structures. Chromophore structures of fluorescent proteins were denominated by the QYG chromophore "carrying" index, indicating source (or in some cases method) used for generation of corresponding chromophore structure.

Using MD trajectories of DsRed protein the chromophore structure averaged by trajectory was obtained; this structure was denominated $QYG^{MD,Average}$.

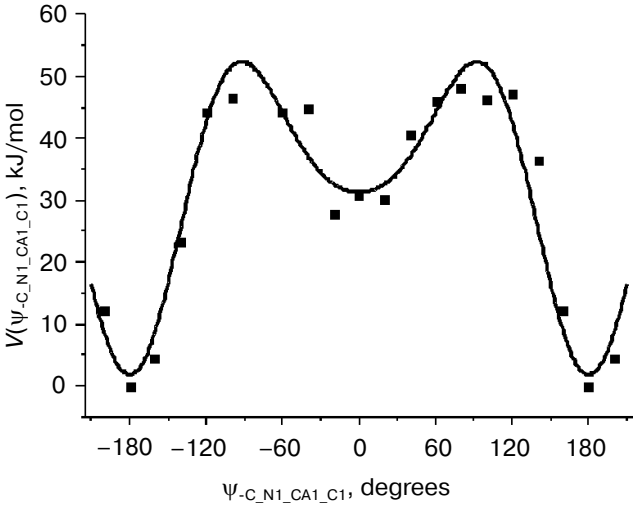

Fig. 6. Dependence of $V(\psi_{-C\_N1\_CA1\_C1})$ energy values of the $D^{qeq}(\psi)$ structures on value of dihedral angle -C_N1_CA1_C1 ($\psi_{-C\_N1\_CA1\_C1}$). Quadrants show data obtained by quantum chemical calculations; solid line shows approximation made by formula (5).

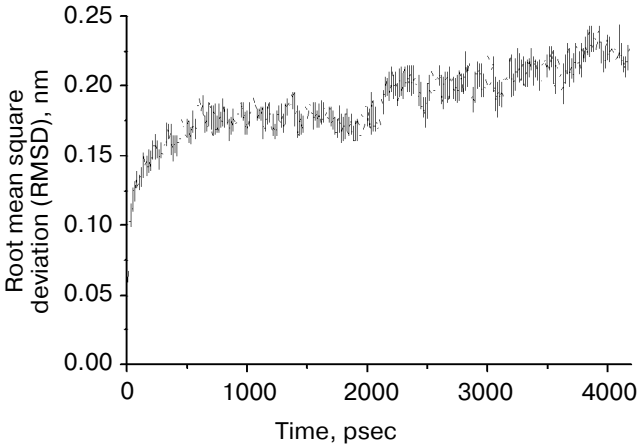

Fig. 7. Root mean square deviation (RMSD) of atomic coordinates of DsRed protein chain A (excluding hydrogen atoms) in aqueous solution as a function of time according to MD calculation.

Table 6. Lengths of bonds r_{ij} in the chromophore (Å) based on X-ray analysis and quantum chemical and MD calculations

Atoms forming bond[1]		Length of bond in structure QYG^{1G7K}	Length of bond in structure QYG^{1GGX}	Length of bond in structure QYG^{1ZGO}	Length of bond in structure A^{eq}	Length of bond in structure $QYG^{MD,Average}$
1		2	3	4	5	6
CA1	N1	1.31	1.29	1.29	1.28	1.28
-C	N1	1.33	1.39	1.31	1.41	1.40
CB1	CA1	1.58	1.53	1.55	1.51	1.51
C1	CA1	1.25	1.44	1.34	1.48	1.47
N2	C1	1.34	1.34	1.35	1.31	1.31
N3	C1	1.45	1.42	1.35	1.39	1.39
C2	N3	1.43	1.42	1.36	1.41	1.40
O2	C2	1.26	1.25	1.21	1.22	1.21
C2	CA2	1.49	1.48	1.40	1.49	1.48
CA2	N2	1.49	1.37	1.37	1.39	1.39
CA3	N3	1.42	1.52	1.45	1.46	1.47
CB2	CA2	1.36	1.42	1.34	1.36	1.36
CG2	CB2	1.38	1.42	1.38	1.44	1.44
CD1	CG2	1.42	1.48	1.40	1.41	1.40
CD2	CG2	1.44	1.47	1.41	1.42	1.40
CE1	CD1	1.37	1.40	1.41	1.39	1.37
CE2	CD2	1.37	1.39	1.41	1.38	1.37
CZ	CE1	1.48	1.48	1.40	1.40	1.39
CZ	CE2	1.46	1.47	1.40	1.40	1.39
OH	CZ	1.27	1.25	1.24	1.36	1.35
CB1	CG1	1.56	1.54	1.54	n.d.[2]	1.50
CG1	CD3	1.52	1.52	1.51	n.d.[2]	1.50
CD3	OE1	1.24	1.23	1.21	n.d.[2]	1.21
CD3	NE1	1.32	1.36	1.34	n.d.[2]	1.31
CA3	C3	1.50	1.57	1.49	n.d.[2]	1.50
C3	O3	1.24	1.23	1.39	n.d.[2]	1.11

[1] Atom names given in this table correspond to those of Fig. 2.
[2] This parameter was not determined (this bond is absent in model molecule A).

For comparison of the resulting structure with X-ray data the chromophore structures QYG^{1G7K}, QYG^{1GGX}, and QYG^{1ZGO} were obtained from PDB files: 1GGX [47], 1G7K [48], and 1ZGO [42], respectively. Tables 6-8 compare chromophore structure parameters obtained by X-ray data, averaged by MD trajectory, and also obtained by quantum chemical calculations.

Distribution of angles -C_N1_CA1, -C_N1_CA1_C1, and -CA_-C_N1_CA1 in MD trajectory of DsRed protein. Distribution of angle values in the DsRed protein in MD trajectory was obtained for the valence angle -C_N1_CA1 and dihedral angles -C_N1_CA1_C1 and -CA_-C_N1_CA1, whose parameters were earlier determined by quantum chemical calculations (Fig. 8).

Averaged values and half-width of distribution of chromophore dihedral angle values in structures $QYG^{MD,Average}$ and Phe-$QYG^{MD,Average}$. Distributions of chromophore dihedral angle values were obtained using MD trajectory of the DsRed protein; they were then used for calculations of the averaged values of chromophore dihedral angles and half-width of distribution of their values. In similar manner, the averaged values of the dihedral angles of the Phe-QYG dipeptide were obtained from its MD trajectory (Table 9).

Table 7. Chromophore valence angle values θ_{klm} (degrees) based on X-ray analysis and quantum chemical and MD calculations

Atoms forming valence angle[1]			Value of angle in structure QYG^{1G7K}	Value of angle in structure QYG^{1GGX}	Value of angle in structure QYG^{1ZGO}	Value of angle in structure A^{eq}	Value of angle in structure $QYG^{MD,Average}$
1			2	3	4	5	6
N1	CA1	CB1	105.8	121.0	113.05	126.44	116.7
N1	CA1	C1	136.7	119.7	132.9	118.14	116.7
C1	CA1	CB1	117.4	119.3	114.0	115.42	118.9
CA1	CB1	CG1	100.0	114.5	109.6	n.d.[2]	114.3
CB1	CG1	CD3	117.1	120.4	120.85	n.d.[2]	116.0
CG1	CD3	OE1	120.4	118.1	120.2	n.d.[2]	121.6
CG1	CD3	NE1	117.2	119.5	117.4	n.d.[2]	117.4
NE1	CD3	OE1	122.4	122.4	122.4	n.d.[2]	121.0
-C	N1	CA1	163.1	142.95	110.95	123.77	131.5
CA1	C1	N2	123.75	120.7	131.85	120.75	119.5
CA1	C1	N3	123.52	127.84	123.7	125.25	127.3
N2	C1	N3	112.7	111.4	104.3	114.00	113.1
C1	CA1	N1	136.68	119.71	132.9	118.14	116.65
C1	N2	CA2	107.6	107.8	113.05	106.34	107.4
C1	N3	C2	105.4	107.3	111.7	107.28	107.4
N3	C2	CA2	104.8	103.5	106.9	103.11	103.8
C1	N3	CA3	129.6	128.1	123.9	131.27	133.3
C2	N3	CA3	124.15	124.6	124.4	121.39	119.3
N2	CA2	C2	109.3	110.0	104.1	109.27	108.0
N3	C2	O2	124.3	126.05	124.7	125.74	126.3
CA2	C2	O2	130.9	130.4	128.4	131.15	130.0
C2	CA2	CB2	119.8	122.2	125.3	122.42	121.85
N2	CA2	CB2	130.8	127.9	130.6	128.31	130.1
CA2	CB2	CG2	133.7	132.2	137.6	130.35	133.6
C3	CA3	N3	116.29	126.59	116.69	n.d.[2]	113.1
CB2	CG2	CD1	119.4	125.9	123.7	123.52	122.6
CB2	CG2	CD2	124.1	117.7	119.2	118.48	120.2
CD1	CG2	CD2	116.45	116.4	117.1	118.00	117.1
CG2	CD2	CE2	122.8	122.1	121.0	120.93	121.85
CG2	CD1	CE1	122.1	121.7	122.3	121.37	121.3
CD2	CE2	CZ	121.6	122.05	121.25	120.05	119.6
CD1	CE1	CZ	122.1	121.65	119.8	119.57	120.1
CE2	CZ	CE1	114.6	116.2	118.5	120.09	119.9
CE1	CZ	OH	122.65	122.2	121.5	122.97	120.0
CE2	CZ	OH	122.7	121.6	120.0	116.94	120.0
-CA	-C	N1	118.17	136.62	149.90	n.d.[2]	118.4
-O	-C	N1	122.11	102.91	104.83	n.d.[2]	120.7

[1] Atom names given in this table correspond to those of Fig. 2.
[2] This parameter was not determined (this angle is absent in model molecule A).

Table 8. Chromophore dihedral angle values (degrees) based on X-ray analysis and quantum chemical and MD calculations

Atoms forming dihedral angle[1]				Value of angle in structure QYG^{1G7K}	Value of angle in structure QYG^{1GGX}	Value of angle in structure QYG^{1ZGO}	Value of angle in structure A^{eq}	Value of angle in structure $QYG^{MD,Average}$
1				2	3	4	5	6
N1	CA1	CB1	CG1	−45.6	−36.8	−42.2	n.d.[2]	−81.8
CG1	NE1	CD3	OE1	180.69	181.5	180.8	n.d.[2]	178.55
CD1	CD2	CG2	CB2	181.69	180.36	180.17	180.57	177.5
CE1	CE2	CZ	OH	181.34	180	180.67	180	182.5
N1	CB1	CA1	C1	183.8	178.67	177.1	181.47	178.4
CA1	N2	C1	N3	178.37	180.1	175.7	181.33	182.5
N3	CA2	C2	O2	177.96	180.26	180.0	180.0	179.8
C1	C2	N3	CA3	189.42	181.42	181.74	177.0	178.1
N2	CB2	CA2	C2	178.68	179.64	181.12	179.0	177.0
CB1	N1	CA1	C1	175.1	181.3	183.6	179.15	181.4
N1	CA1	C1	N2	183.5	191.5	166	183.72	186
N1	CA1	C1	N3	5.3	11.4	−9	3.41	3.2
CA1	C1	N3	C2	182.3	180	175.5	179.93	187.6
C1	N3	C2	O2	179.6	180	180.2	180.21	179.6
C1	N3	C2	CA2	−2.28	0.21	0.17	0.7	−0.65
CA1	C1	N2	CA2	177.7	179.7	185.1	180	170.4
CA1	C1	N3	CA3	−7.8	−1.4	−7.2	3.10	10
CA3	N3	C2	O2	9.01	1.39	1.92	2.3	−2.3
CA3	N3	C2	CA2	187.15	181.64	181.92	177.7	177.5
C1	N2	CA2	CB2	181.1	180	180.2	179.19	189.1
N2	CA2	CB2	CG2	0	2	0.9	−0.12	−6.6
CA2	CB2	CG2	CD1	3.5	2.4	3.3	−0.03	−6.6
CA2	CB2	CG2	CD2	181.8	182	183.5	179.84	176.2
CB2	CG2	CD1	CE1	182.7	180	181.4	180.15	185.1
CB2	CG2	CD2	CE2	181.5	180	179.2	179.86	176.2
CG2	CD2	CE2	CZ	−1.1	0.1	−1.6	0.02	−1.3
CD2	CE2	CZ	OH	180.5	180.3	181.8	180	185.4
CA1	CB1	CG1	O	195.1	163.4	185.4	n.d.[2]	163.8
C1	N3	CA3	+C	81.2	48.1	74.7	n.d.[2]	81
N3	CA3	+C	O	181.2	148.7	180.5	n.d.[2]	161
C3	CA3	N3	C1	81.15	48.12	74.73	n.d.[2]	80.85
-C	N1	CA1	CB1	−83.8	−66.83	−75.01	4.5	−22.24
-C	N1	CA1	C1	91.26	114.51	108.63	183.0	159.33
C3	CA3	N3	C2	−110.67	−133.60	−107.22	n.d.[2]	−96.7
CB1	CG1	CD3	NE1	−15.33	−217.21	−180.0	n.d.[2]	−175.0
CG1	CB1	CA1	C1	138.17	141.91	134.85	n.d.[2]	96.8
N1	-C	-CA	-N	−24.12	−17.63	−21.45	n.d.[2]	7.3

[1] Atom names given in this table correspond to those of Fig. 2.
[2] This parameter was not determined (this angle is absent in model molecule A).

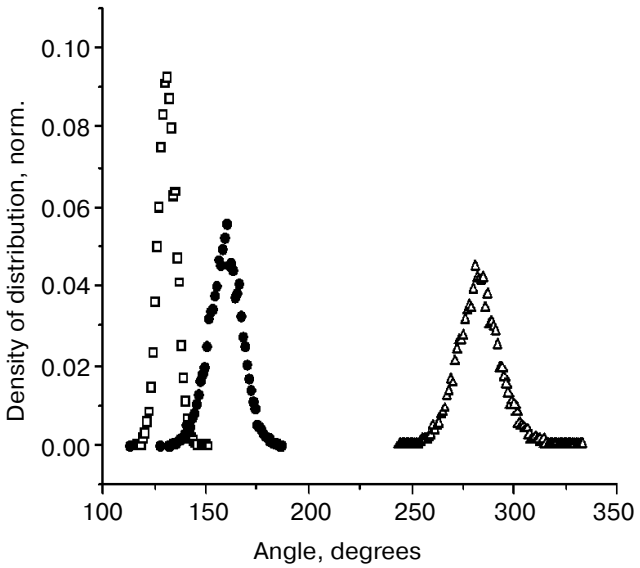

Fig. 8. Distribution of angle values of valence angle -C_N1_CA1 (squares) and dihedral angles -C_N1_CA1_C1 (circles) and -CA_-C_N1_CA1 (triangles) in MD trajectory of DsRed protein in aqueous solution.

DISCUSSION

For MD calculation of DsRed fluorescent protein and its analogs, we have parameterized the DsRed chromophore in the OPLS-AA force field as an amino acid residue of the polypeptide chain. The first approximation of the chromophore topology was obtained by assigning parameters to atoms forming the chromophore, which are related similar groups of atoms from compounds described in the OPLS-AA force field (Table 1). The resulting set of parameters was specified by quantum chemical calculations of model molecule A, which shares structural similarity with the chromophore (Fig. 3). The values calculated for model molecule A were then assigned to similar parameters of the DsRed chromophore: the effective partial charges of chromophore atoms (Table 1) and values of equilibrium lengths of the bonds and valence and dihedral angles (Tables 2-4). Parametrization of the valence angle -C_N1_CA1 (Fig. 4, Table 3) and the dihedral angles -CA_-C_N1_CA1 (Fig. 5, Table 5) and -C_N1_CA1_C1 (Fig. 6, Table 5) related to the chromophore fragment including atoms N1, CA1, C1, -C, and -CA (Fig. 2) was carried out separately by means of quantum chemical calculations because the OPLS-AA force field lacked compounds containing similar groups of atoms. Additionally, the double bond between atoms N1 and CA1 is essential for shifting the fluorescence to the red region [46]. After inclusion of parameters of the DsRed chromophore topology into the OPLS-AA force field, the resulting extended force field denominated as the OPLS-AA/DsRed became applicable for MD calculation of the DsRed protein and also other proteins containing chromophore identical to that of the DsRed protein.

Adequacy of the chromophore parametrization was evaluated by MD calculation of the DsRed protein using the OPLS-AA/DsRed force field, and the MD trajectory of the DsRed protein in an aqueous solution was obtained. This calculation employed the DsRed protein tetramer because in aqueous solution [16] and in three known crystal structures of this protein (PDB ID: 1ZGO, 1GGX, 1G7K) [42, 47, 48] it exists as a tetramer.

RMSD analysis made for atoms of the chain A of the DsRed protein (excluding hydrogen atoms) in MD trajectory revealed lack of conformational transitions. So in subsequent analysis of the DsRed chromophore structure we have used the averaged chromophore structure calculated by MD trajectory of the DsRed protein ($QYG^{MD,Average}$) (Tables 6-8). Quantitative characteristics of the $QYG^{MD,Average}$ structure (Tables 6-8) were compared with the quantitative characteristics of the equilibrium A^{eq} structure obtained by quantum chemical calculations and also with quantitative characteristics of three structures of the DsRed protein chromophore obtained from X-ray data of its crystals: QYG^{1GGX}, QYG^{1G7K}, and QYG^{1ZGO} (Tables 6-8). For evaluation of significance of differences between values of lengths of the bonds and angles, we have employed corresponding criteria. An average energy per degree of freedom was 1/2 RT (1.25 kJ/mol at 300°K). The average deviations of parameters from their equilibrium values, which correspond to such values, have been calculated. These are 0.02 Å for lengths of the bonds, 4° for valence angles, and 15° for dihedral angles.

Using these criteria we analyzed differences between the structure of the $QYG^{MD,Average}$ chromophore and the equilibrium A^{eq} structure of the model molecule A (Tables 6-8, columns 5 and 6). It should be noted that in these two structures the values for all bonds and dihedral angles can be considered as similar ones. Values of valence angles can be considered as similar in these two structures with the exception of angles -C_N1_CA1 and N1_CA1_CB1; the latter can be attributed to the influence of amino acid environment on the chromophore in the DsRed protein structure.

Based on the distribution of values for valence angle -C_N1_CA1 and dihedral angles -C_N1_CA1_C1 and -CA_-C_N1_CA1, the average values (131°, 159°, and 283°, respectively) and half-widths of value distributions (9°, 16°, and 19°, respectively) were obtained by MD calculations (Fig. 8). The values of the same angle in the equilibrium A^{eq} structure are 124°, 183°, and 234°. Differences in the corresponding values of these angles can be attributed to the influence of amino acid environment on the chromophore in the DsRed protein structure.

For comparison of structure parameters of the isolated chromophore of the DsRed protein with the parameters of chromophore structure in its protein environment,

Table 9. Averaged values and half-widths of distribution of dihedral angles forming the system of coupled π-bonds of the chromophore and theoretical values of angles in the case of planar chromophore structure

Atoms forming dihedral angle[(1)]	Mean value of angle by MD trajectory of DsRed protein, degrees	Half-width of distribution of angle value by MD trajectory of DsRed protein, degrees	Mean value of angle by MD trajectory of Phe-QYG dipeptide, degrees	Postulated angle value in case of planar chromophore structure, degrees
1	2	3	4	5
-C_N1_CA1_C1	159	15	172.54	
N1_CA1_C1_N2	186	14	178.22	180
N1_CA1_C1_N3	3	16	−1.91	0
CA1_C1_N2_CA2	171	12	180.96	180
CA1_C1_N3_CA3	10	15	−0.73	0
CA1_C1_N3_C2	187	12	178.87	180
C1_N2_CA2_CB2	187	14	179.5	180
C1_N2_CA2_C2	5	12	−0.48	0
C1_N3_C2_O2	179	12	180.88	180
C1_N3_C2_CA2	−1	10	0.86	0
N2_CA2_CB2_CG2	−6	16	0.07	0
N2_CA2_C2_O2	177	12	179.72	180
N3_C2_CA2_CB2	175	13	179.76	180
N3_C2_CA2_N2	−2	11	−0.26	0
C2_CA2_CB2_CG2	243	12	180.06	180
CA2_CB2_CG2_CD1	−6	16	−0.4	0
CA2_CB2_CG2_CD2	176	16	179.6	180
O2_C2_CA2_CB2	−5	14	−0.26	0
O2_C2_N3_CA3	−3	13	0.54	0
CB2_CG2_CD1_CE1	184	13	179.82	180
CB2_CG2_CD2_CE2	176	13	179.9	180
CG2_CD1_CE1_CZ	0	13	0.14	0
CG2_CD2_CE2_CZ	−1	13	0.00	0
CD1_CG2_CD2_CE2	−1	12	0.11	0
CD1_CE1_CZ_CE2	−2	13	−0.02	0
CD2_CE2_CZ_CE1	3	13	−0.06	0
CD2_CG2_CD1_CE1	2	12	−0.19	0
OH_CZ_CE1_CD1	175	14	180	180
OH_CZ_CE2_CD2	185	14	179.93	180
-CA_-C_N1_CA1	283	19	279.7	

[(1)] Atom names given in this table correspond to those of Fig. 2.

we have made MD calculations of Phe-OYG dipeptide. The resulting chromophore structure averaged by the MD trajectory Phe-QYG^{MD,Average} was compared with the QYG^{MD,Average} structure. This comparison revealed that the values of all these parameters (except values of dihedral angle C2_CA2_CB2_CG2 (Fig. 2)) coincide. In these structures, difference between the values of the dihedral angle C2_CA2_CB2_CG2 exceeds the value characterizing motion (Table 9). This results in extrusion of the C2 atom from the chromophore plane. In the case of isolated chromophore (Phe-QYG dipeptide), all chromophore atoms are within one plane (Table 9). This is consistent with notion that the chromophore represents a planar aromatic system and thus suggests correct parametriza-

tion of the dihedral atoms. Difference in the value of the dihedral angle C2_CA2_CB2_CG2 found in the structures Phe-QYG^{MD,Average} and QYG^{MD,Average} can be attributed to the influence of amino acid environment on the chromophore in the DsRed protein structure (e.g. by forming of hydrogen bonds between the chromophore and amino acid residues R95, E148, and Y181).

Comparison of the QYG^{MD,Average} chromophore structure with the chromophore structure in crystals QYG^{1GGX}, QYG^{1G7K}, and QYG^{1ZGO} shows that most bonds and valence and dihedral angles of the chromophore satisfy the selected criteria.

For bonds N2_C1 and OH_CZ the values of corresponding lengths in the three crystal structures are almost the same, but differ from the length of this bond in the QYG^{MD,Average} structure; this may be explained by differences in protein structure in crystal and aqueous solution [20].

The structure of the DsRed protein obtained by MD calculations using topology of the neutral form of the DsRed protein chromophore must not significantly depend on charge of the chromophore. However, it should be noted that topologies for all other possible forms of the chromophore [1, 15, 16] should by calculated separately.

Thus, in the present study we have generated topology for the neutral form of the DsRed protein chromophore and demonstrated the adequacy of its application for molecular dynamics due to similarity of the QYG^{MD,Average} structure with QYG^{1GGX}, QYG^{1G7K}, QYG^{1ZGO}, and A^{eq} structures. Using the topology obtained we have modified the initial OPLS-AA force field. The extended OPLS-AA/DsRed force field gives the possibility for MD calculations for the DsRed proteins and other proteins that contain a chromophore identical to the DsRed protein chromophore.

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