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Photoinduced Electron Transfer Facilitates Tautomerization of the Conserved Signaling Glutamine Side Chain in BLUF Protein Light Sensors

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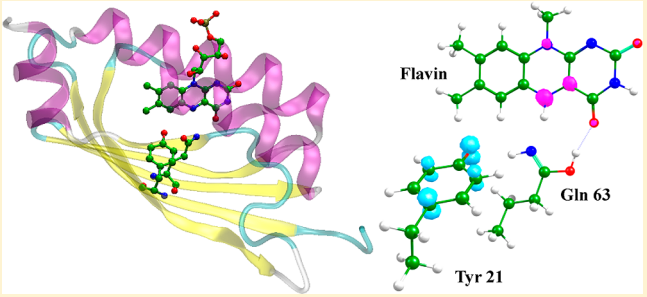
Supporting Information

ABSTRACT: The BLUF domain (sensor of blue light using flavin adenine dinucleotide) from a bacterial photoreceptor protein AppA undergoes a cascade of chemical transformations, including hydrogen bond rearrangements around the flavin adenine dinucleotide (FAD) chromophore, in response to light illumination. These transformations are initiated by photoinduced electron and proton transfer from a tyrosine residue to the photoexcited flavin which is assisted by a glutamine residue. According to the recent studies, the proton-coupled electron transfer leads to formation of a radical-pair intermediate Tyr●···FADH● and a tautomeric EE form of glutamine in the ground electronic state. This intermediate is a precursor of the light-induced state of the BLUF photoreceptor implicated in biological signaling. In order to describe evolution of the radical pair, we computed reaction pathways on the ground state potential energy surface employing quantum-chemical calculations in the DFT PBE0/cc-pVDZ approximation for a molecular cluster mimicking the chromophore containing pocket of the AppA BLUF protein. We found a minimum-energy pathway comprised of the following consecutive reaction steps: (1) rotation of the imidic group of the EE glutamine side chain around the Cγ−Cδ bond; (2) flip of the OεH group and formation of the ZE form of the glutamine side chain; and (3) biradical recombination via coupled proton and electron transfer, leading to the ZZ form of the glutamine side chain. The potential-energy barriers for stages 1−3 do not exceed 9 kcal/mol. Energy barrier 3 describing the ZE to ZZ glutamine tautomerization is significantly smaller in the BLUF model than in isolated glutamine, since tautomerization in BLUF is facilitated by electron transfer and radical recombination. Thus, our study shows that tautomerization of the conserved glutamine is coupled to the light-induced electron transfer process in BLUF and, thus, is a viable candidate for the photoactivation mechanism which at present is very much debated.



INTRODUCTION

Flavin containing photoreceptor proteins with a BLUF (blue light using flavin adenine dinucleotide (FAD)) domain mediate physiological responses to blue light in bacteria and euglenas. A BLUF photoreceptor domain was first found in the purple bacterial photoreceptor AppA involved in the light-dependent regulation of photosynthesis gene expression.[1,2] Since then, many other BLUF-containing proteins were identified, and their mechanisms of light response were intensively studied (for recent reviews, see refs 3 and 4). In the BLUF domains, blue light activates the flavin chromophore. After flavin light absorption, a putative signaling state is formed that is characterized by an ∼10 nm red-shifted flavin absorption with respect to the initial dark state. The small spectral change indicates that the flavin chromophore is present in the oxidized state in both the dark-adapted and light-induced states but its immediate environment undergoes changes. The IR and Raman spectra of AppA BLUF indicate that a strong hydrogen bond between flavin and protein is formed in the 10 nm red-shifted light state.[5−12] Structural, mutagenesis, and further spectroscopy studies identified a conserved glutamine and tyrosine residues being responsible for these specific interactions.[13−21] However, the structural interpretation of the light-induced structural change is still a matter of intense studies including computational papers[22−26] addressing the relationship between the spectroscopic signatures and structure of BLUF.

Our standpoint[27−29] is that the dark state of the AppA BLUF domain is represented by the protein structure proposed by Jung et al. (PDB code 2IYG)[30] on the basis of their X-ray diffraction crystallography study. In this structure, the side chain of the critical residue Gln63 forms a hydrogen bond with the conserved Tyr21 as a proton acceptor and another

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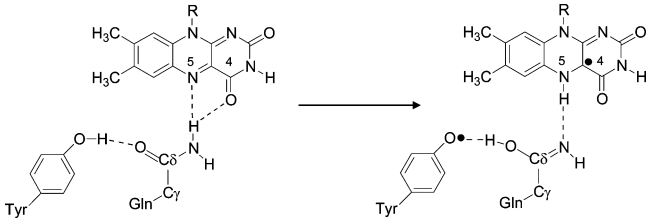
hydrogen bond with the N5 of the isoalloxazine ring as a proton donor, as shown in the left side of Scheme 1

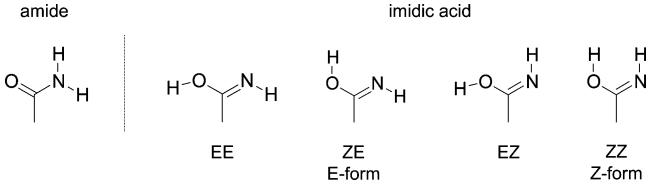
Scheme 1. Initial Stage of Photoreaction Involving the Flavin Chromophore, Glutamine, and Tyrosine in the BLUF Domains[5,7]



(numbering of all atoms in the isoalloxazine ring of the chromophore is illustrated in Figure S1 of the Supporting Information to this paper). Other structural[31−33] and also computational studies[23,34] indicated that the hydrogen-bonding network involving flavin is rather dynamic in the BLUF dark state. In particular, multiple orientations of the Gln63 side chain can be proposed on the basis of the solution NMR data.[31] The distribution of these orientations might depend on the fold of the BLUF C-terminus, the so-called Trp-in[33] and Met-in[30] BLUF conformations. In our previous studies,[28,29] we considered the hydrogen-bond dynamics of the AppA BLUF in the Met-in conformation and found that this structure corresponded to a low-energy minimum on the protein potential energy hypersurface.

Experimentally, it is established that the light activation is triggered by the photoinduced proton coupled electron transfer (PCET) involving Tyr and flavin,[35−41] resulting in formation of a flavin−tyrosine radical pair. In the AppA BLUF protein, the radical pair was not detected because of a short lifetime,[40,41] but the respective absorbance transients were observed for another BLUF protein PixD[37] structurally very similar to the AppA BLUF. The mechanism of formation and recombination of the radical pair was considered in computational chemistry studies.[42,43] In particular, by using multiconfigurational quantum chemical approaches, Udvarhelyi and Domratcheva[43] showed that PCET mediated the decay of the photoexcited flavin to the electronic ground state, leading to the radical-pair species Tyr• + FADH• and the tautomeric form of the Gln63 side chain (right side in Scheme 1). Thus, the radical pair containing the EE tautomeric glutamine was proposed as a primary photoproduct of the BLUF photoreaction.[42,43] Throughout this paper, we use the nomenclature for the glutamine side chain isomers, as illustrated in Scheme 2. Radical recombination via a back electron transfer to flavin yields the red-shifted light-induced state of BLUF. Formation of the red-shifted state from the radical-pair photointermediate is a primary subject of the present study.

Scheme 2. Notation of the Isomers of the Glutamine Side Chain



Scheme 3 illustrates two possible reaction pathways (designated as A and B) leading to the red-shifted light-induced state in the ground electronic state studied in the present work. The difference between these pathways is in the ordering of two events, namely, transformation of the Gln63 side chain and radical-pair recombination. We characterize possible intermediates along pathways A and B and estimate potential energy barriers. Recombination of the BLUF radical pair in the BLUF-BlrB protein following pathway B was previously considered in the study of Sadeghian et al.[42] They found a low-energy pathway leading from the initial radical pair containing the EE form of Gln to the closed-shell structure (i.e., containing oxidized flavin and reduced tyrosine), with the ZE form of glutamine (Scheme 3, bottom). The ZE glutamine tautomer, however, cannot form a strong hydrogen bond with flavin, and thus, its formation does not explain the red-shifted absorption of the light-induced state.[42] In contrast, the ZZ form is capable of forming two hydrogen bonds (Scheme 3, the right side); however, upon transition from ZE to ZZ, the Nε—H group flip with respect to the Cδ=Nε double bond has a very high energy barrier.[42] In this study, we show that formation of the ZZ form of glutamine in BLUF is achieved by coupling this reaction to electron transfer from flavin to tyrosine and radical recombination.

MODELS AND COMPUTATIONAL PROTOCOL

The molecular cluster model used for quantum chemistry calculations was constructed as follows. Initial coordinates of heavy atoms were taken from the crystal structure with the PDB code 2IYG[30] attributed to the dark-adapted state of AppA BLUF in the so-called Met106-in protein conformation. The geometry coordinates of all atoms were optimized in the quantum mechanics/molecular mechanics (QM/MM) approximation as described previously.[28,29] The molecular cluster selected for further quantum-chemical calculations includes the lumiflavin molecule representing the izoalloxazin part of the flavin chromophore and the side chains of Tyr21, Asn45, Leu54, Gln63, Leu65, Ile79, and Met106 amino acid residues. The hydrophobic side chains Leu54, Leu65, and Ile79 were included to model the immediate environment of flavin and Gln63. Figure 1 provides a view of the entire model cluster with the amidic form of Gln63. Since our molecular model is rather large, to help better visualization of the computed reaction pathways in energy diagrams, we show only a small fraction of the molecular system (a selected group of atoms at their respective geometry arrangements), as illustrated in Figure S2 of the Supporting Information to this paper.

To construct the ground state energy profiles for the reaction stages shown in Scheme 3, we performed the relaxed scans of the potential energy surface. During geometry optimization, the coordinate-locking scheme[44] was employed by fixing certain atomic coordinates of terminal carbon atoms of each molecular fragment to the values obtained in the QM/MM model.[28,29] To scan the potential energy surface, the following geometry parameters were chosen as the reaction coordinates: (1) the Cβ—Cγ—Cδ—Nε dihedral angle of Gln63; (2) the Cγ—Cδ—Oε—H dihedral angle of Gln63; (3) the —Cδ=Nε—H angle of Gln63. When radical-pair recombination was considered, the distance between flavin's N5 and H atoms served as a reaction coordinate.

Stationary points referring to the radical-pair electronic structure were calculated in the UDFT approximation[45] with the PBE0 functional[46] and the cc-pVDZ basis set.[47] We paid

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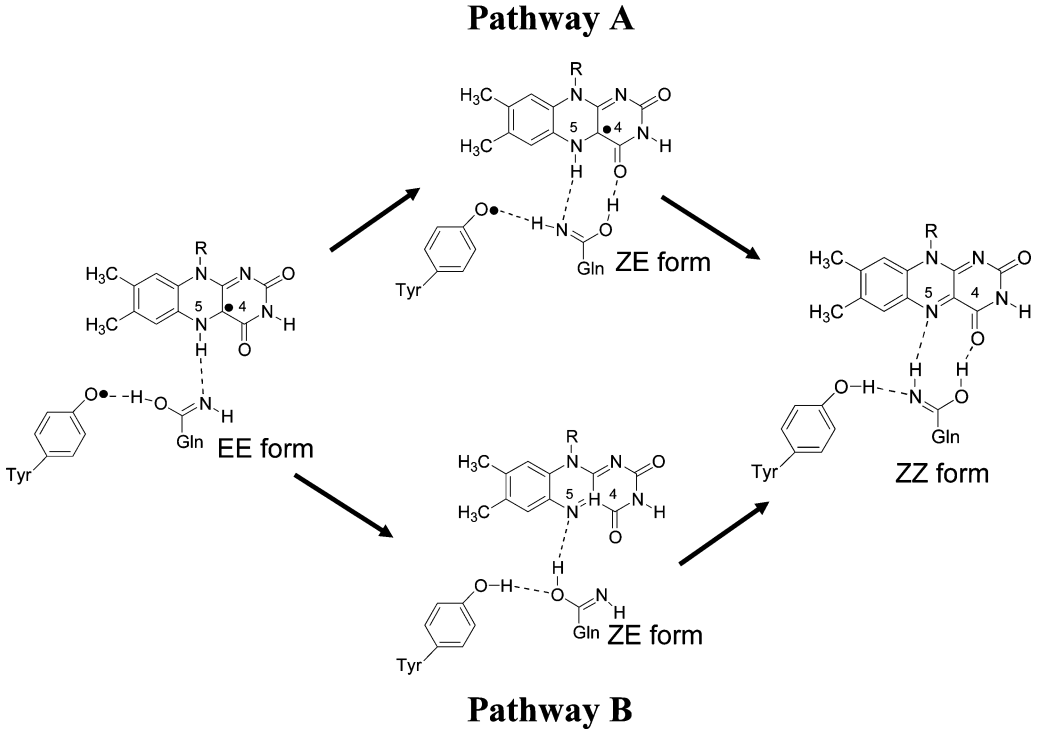
Scheme 3. Two Alternative Pathways Connecting the Primary Radical-Pair Intermediate with the Light-Induced State in BLUF



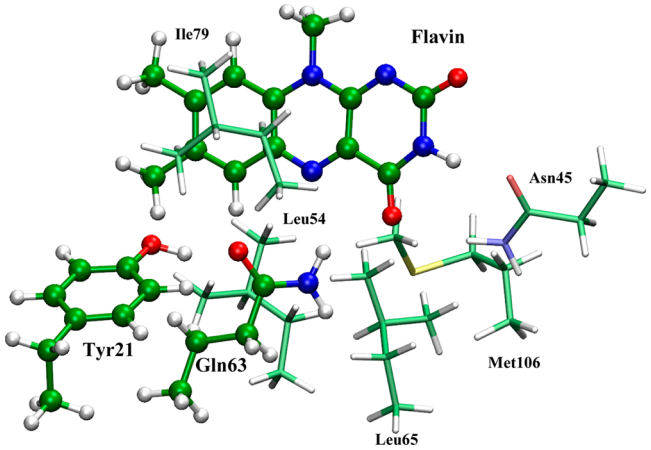


Figure 1. Molecular cluster employed in calculations of the energy profiles. Here and in all figures below, carbon atoms are colored in green, nitrogen in blue, and oxygen in red.

special attention to describe correctly the electronic structure of the model system with two spatially separated singly occupied molecular orbitals located on flavin and Tyr21. Additional verification of the electronic structure was performed by using the CASSCF(4/3)/6-31G** method with the subsequent perturbation theory (MRMP2) energy correction.[48] The closed-shell structures referring to pathway B were computed by using the conventional closed-shell DFT PBE0/cc-pVDZ approach. Quantum chemistry computations for all model systems were performed with the Firefly quantum-chemistry package,[49] partially based on the GAMESS (US)[50] source code.

RESULTS

Pathway A. As shown in Scheme 3, our calculations of the reaction-energy profile start from a radical-pair complex, containing Gln63 in the EE form designated as the **Reag** structure. Pathway A assumes an initial evolution of the system in the electronic configuration of a radical-pair state located at Tyr21 and flavin. The respective α and β spin densities are illustrated in Figure S3 of the Supporting Information to this paper. When performing energy scans at all stages along pathway A, the electron density distributions were carefully monitored. The closed-shell electronic configuration corresponding to the back electron transfer from flavin to Tyr21 was recovered only at the last step (3) of the reaction pathway A which, thus, corresponded to radical recombination.

The first step (1) on pathway A is the rotation of the imidic group of the EE glutamine side chain around the Cγ−Cδ bond. The dihedral angle Cβ−Cγ−Cδ−Nε of Gln63 was chosen as a reaction coordinate, and two energy profiles were computed for the clockwise and counterclockwise internal rotations of the Gln63 functional head. The results of these calculations are illustrated in Figure 2 containing relative energies computed with respect to the energy of the **Reag** complex. Gln rotation results in a rupture of the hydrogen bond involving the −OεH group of Gln63. After rotation, the position of the OεH group is taken up by the NεH group. The energy of the rearranged radical-pair intermediate, designated as **A-I(1)** in Figure 2, is slightly higher (by 2.2 kcal/mol) than that of the initial structure **Reag**. The energy barriers at the transition state structures, **A-TS(1)**$_1$ and **A-TS(1)**$_2$, in Figure 2, are about 8 kcal/mol.

To clarify the origin of the energy barriers, we considered similar transformations of the isolated acetamide molecule. The results of our quantum chemical calculations of the acetamide molecule are presented in the Supporting Information to this paper. In particular, Figure S4 (Supporting Information) shows the computed energy profile for the internal rotation in the EE form which has an energy barrier smaller than 2 kcal/mol. Thus, we found that the intrinsic rotation energy barrier is significantly smaller than the barrier shown in Figure 2, which

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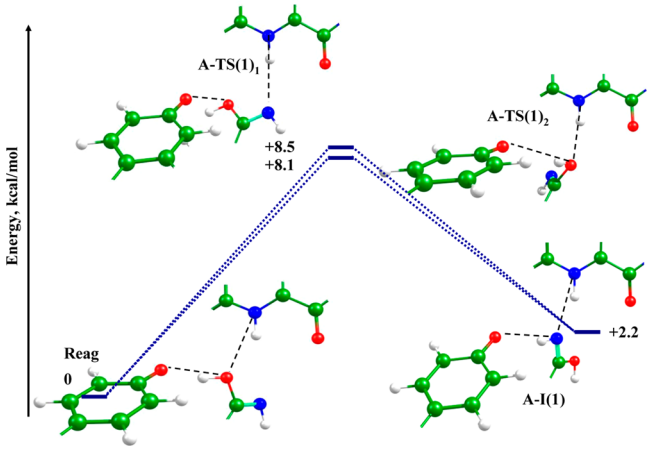

Figure 2. Computed energy profile and geometry changes corresponding to the rotation of the EE imidic group of Gln63 around the Cγ−Cδ bond.

allowed us to conclude that the much higher energy barriers in the BLUF cluster model were caused by the disruption of the hydrogen bonds. In addition, along the reaction pathway, Oε-Gln approaches either Leu65 or Leu54 (see Figure 1) when rotating counterclockwise or clockwise, respectively, which may contribute to the energy barriers in the form of a steric hindrance energy.

The next step (2) along pathway A is the isomerization of the Gln63 side chain from the EE form to the ZE form. The dihedral angle Cγ−Cδ−Oε−H of Gln63 was selected as a coordinate in the relaxed energy scan, and two possible directions of rotation were considered. The results are illustrated in Figure 3. The radical-pair reaction intermediate

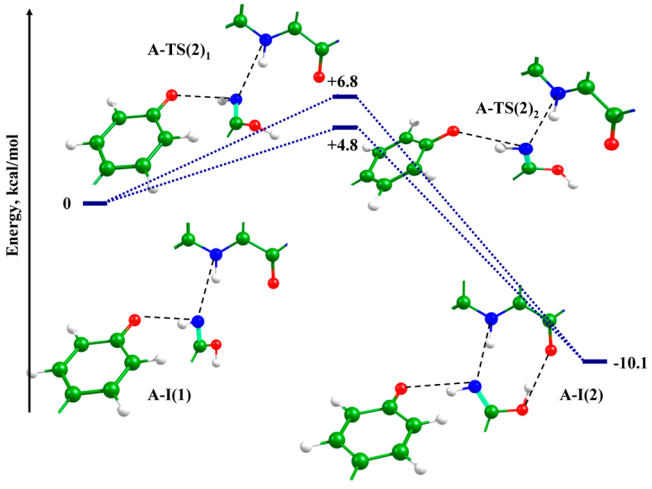

Figure 3. Computed energy profile and geometry changes corresponding to the rotation of the OεH group of Gln63 around the Oε−Cδ bond.

A-I(2) containing the ZE form of Gln63 in the right-hand side of Figure 3 is characterized by the energy lower than that of the intermediate **A-I(1)** by about 10 kcal/mol. The energy decrease is consistent with the properties of the imidic tautomeric forms: in vacuo, the ZE tautomer is the most stable isomer; the ZE form of acetimide is about 8 kcal/mol lower in energy than the EE form, as shown in Figure S5 in the Supporting Information. In addition, formation of an additional hydrogen bond Gln63(Oε)−H···(O4)flavin in the BLUF cluster model further decreases the energy. Two transition-state structures, **A-TS(2)**$_1$ and **A-TS(2)**$_2$, were found with the energies 6.8 and 4.8 kcal/mol (relative to the energy of the **A-I(1)** structure) for the counterclockwise or clockwise rotation, respectively. The intrinsic energy barrier of the EE to EZ isomerization is about 5 kcal/mol (see Figures S5 and S6 of the Supporting Information) which is in a good correspondence with the lowest-energy barrier calculated for the cluster model.

The last step (3) along pathway A refers to biradical recombination occurring via coupled proton and electron transfer. Starting from the **A-(I)2** intermediate, the relaxed energy scan was performed for the gradually increased N5−H distance of the flavin radical. The results are presented in Figure 4. The highest-energy structure **A-TS(3)** with the energy 5

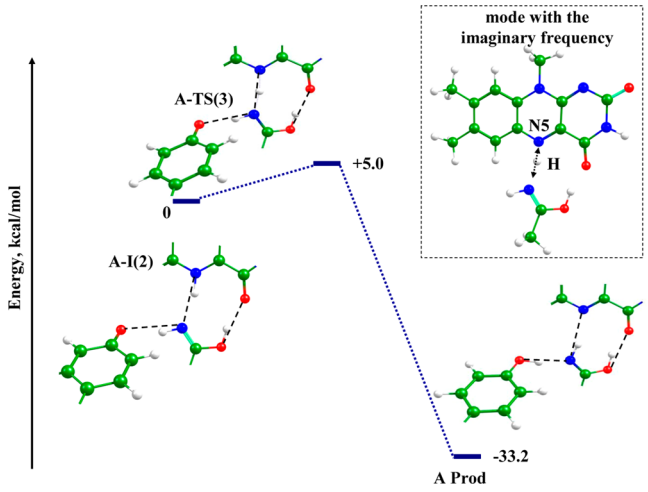

Figure 4. Computed energy profile and geometry changes corresponding to the radical-pair recombination and formation of the ZZ form of Gln63 along pathway A.

kcal/mol above the radical pair **A-I(2)** is described by a geometry configuration with a proton equally distant (1.25 Å) from the flavin N5 atom and the Gln Nε atom. The computed **A-TS(3)** structure is characterized by a single imaginary frequency 1347i cm^{-1} corresponding to the stretching normal mode involved in the proton transfer from flavin to Gln63. Upon this proton transfer, the electronic structure changes from radical pair to closed shell which is consistent with electron transfer from flavin● to Tyr●. The transition to the closed-shell electronic configuration is clearly indicated by the gradual decrease of the S^2 value along the computed reaction coordinate. Thus, the transition state **A-TS(3)** corresponds to the proton-coupled electron transfer. In both UDFT PBE0/cc-pVDZ and CASSCF(4/3)/6-31G** calculations, we observed that the radical-pair character is retained until the transition state **A-TS(3)**. After **A-TS(3)**, the model system is described by the closed-shell electronic configuration. Thus, it contains neutral oxidized flavin, cationic protonated Gln63, and anionic deprotonated reduced Tyr21. After radical recombination, another proton, initially assigned to the −Nε−H group of Gln63, is transferred to the oxygen atom of Tyr21 without an energy barrier. Radical recombination leading to the ZZ form of the Gln63 side chain lowers the energy of the system by more than 30 kcal/mol. In an isolated acetimide molecule, the ZE to ZZ tautomerization has a very high energy barrier, as shown in Figure S5 in the Supporting Information. In BLUF, this energy

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is lowered by coupling of the NεH flip and radical recombination.

Pathway B. The first step (1) of the pathway B (Scheme 3 and Figure 5) is a radical recombination accompanied by the

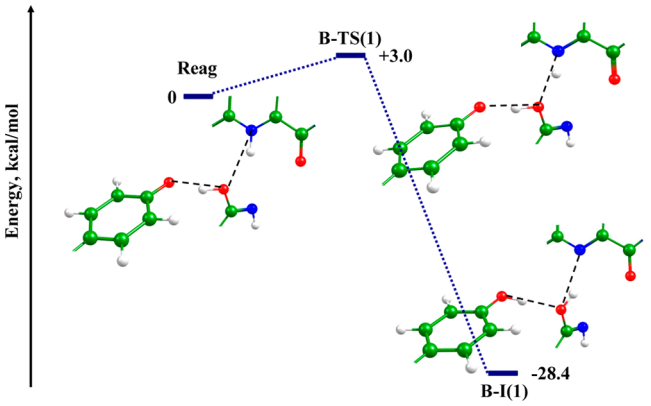

Figure 5. Computed energy profile and geometry changes corresponding to the radical-pair recombination and formation of the ZE form of Gln63 along pathway B.

glutamine EE to ZE isomerization. Similar to step (3) of pathway A, radical recombination is a proton-coupled electron transfer reaction. However, on pathway B, the conformation of the OεH group instead of the NεH group changes from E to Z in intermediate **B−I(1)** (Figure 5). According to our estimates, the energy of the **B-TS(1)** transition state is 3.0 kcal/mol. At the transition state structure, the proton is still bound to the N5 atom: the N5−H and Oε−H distances are 1.06 and 1.75 Å, respectively. A similar energy barrier was previously computed by Sadeghian et al.[42] After radical recombination, intermediate **B−I(1)** gains the closed-shell electronic structure and its energy is significantly lowered compared to the energy of the initial radical-pair state. **B−I(1)** consists of the oxidized flavin, the reduced tyrosine, and the ZE isomer of glutamine which accepts a hydrogen bond from tyrosine and donates a hydrogen bond to flavin. Further glutamine transformations are necessary to enhance glutamine−flavin hydrogen bonding interactions and to reach the red-shifted spectroscopic state of BLUF.

To study rearrangement of the glutamine tautomer after radical recombination, we performed the relaxed energy scan from the closed-shell intermediate **B−I(1)** along the reaction coordinate defined by the changes of the Cβ−Cγ−Cδ−Nε dihedral angle of Gln63. Two directions of the rotation (clockwise and counterclockwise) were considered. Figure 6 shows the computed energy diagram. The energies of the starting structure **B−I(1)** and of the product structure **B−I(2)** differ only by 0.5 kcal/mol. Depending on the direction of rotation, we found two geometry configurations, **B-TS(2)**$_1$ and **B-TS(2)**$_2$, corresponding to the energy barriers of 6.5 and 5.1 kcal/mol, respectively. The energy barrier of the ZE imidic group intrinsic rotation is about 1 kcal/mol (Figure S4 in the Supporting Information). Thus, in our BLUF model, the higher energy barriers compared to the acetamide molecule are coursed by intermolecular interactions: hydrogen bonding and steric hindrances.

To complete the transformations along pathway B, in the last step (3), the ZE form is transferred to the ZZ form of Gln63 via a proton flip in the Cδ═Nε—H group (Figure 7). We chose the —Cδ—Nε—H angle in Gln63 as a reaction coordinate to perform the relaxed energy scan. The geometry configurations

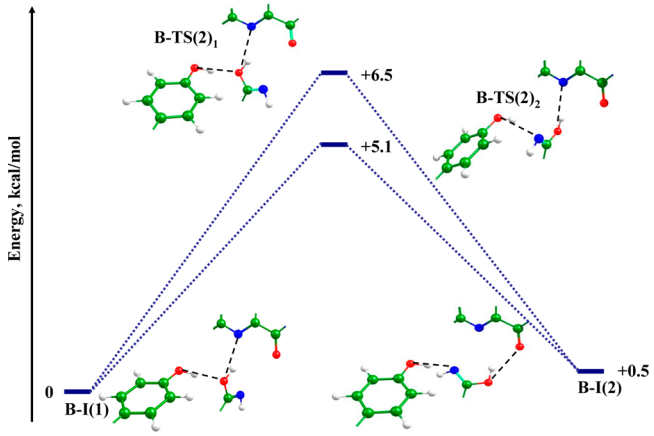

Figure 6. Computed energy profile and geometry changes corresponding to the rotation the ZE imidic group of Gln63 around the Cγ−Cδ bond.

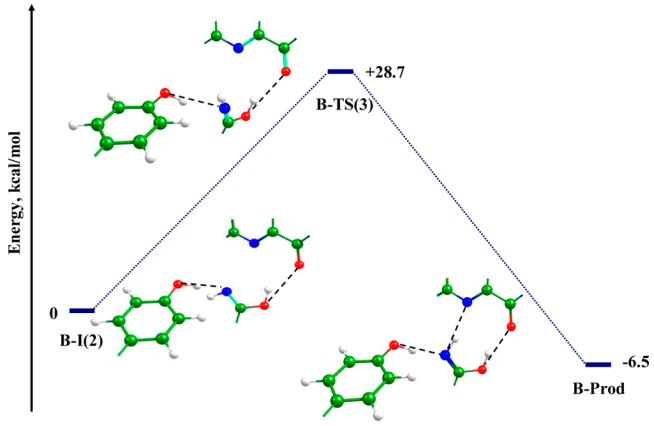

Figure 7. Computed energy profile and geometry changes corresponding to the flip of the NH group and formation of the ZZ glutamine side chain.

of the found transition state **B-TS(3)** are characterized by a linear arrangement of the atoms of the Cδ—Nε—H fragment. The corresponding energy barrier is 31 kcal/mol, which is the highest barrier found and characterized in this work. The high energy barrier separating the EZ and ZZ forms is explained by "rotation" around the Cδ═Nε double bond. A similar high energy barrier more than 25 kcal/mol was found in our calculations of the isolated acetimidic acid (Supporting Information, Figures S5 and S7) and also in the previous study of BLUF-BlrB.[42] Apparently, this step is a bottleneck for the entire pathway B: after "early" radical recombination, the high energy barrier prohibits further isomerization around the Cδ═Nε double bond. We also note that an equally high energy barrier is expected for transformation of the ZE form to the lowest-energy amide form (Figure S5, Supporting Information), which in the case of the BLUF active site corresponds to the recovery of the dark state. The energy of the BLUF dark state is about 14 kcal/mol lower than the energy of the light-induced ZZ form, as estimated in our previous study.[29]

DISCUSSION AND CONCLUSION

A photoreaction of the BLUF photoreceptor proteins occurring in response to blue-light illumination results in a hydrogen bond rearrangement around the flavin chromophore. This photoreaction, unique to the BLUF domain, is triggered by a

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photoinduced electron transfer from a conserved tyrosine residue to the flavin chromophore, leading to a radical-pair intermediate containing Tyr• and FADH•. A conserved glutamine residue mediates interactions between the tyrosine and flavin in BLUF and plays a key role in the photoreaction. To explain the BLUF photoreaction, several molecular mechanisms were proposed that either implicate Gln rotation[8,11,17,23,25,26,33,34,37−39] or Gln tautomerization.[10,18,19,27−29,42,43] The first group of mechanisms excludes photochemical rearrangement of covalent bonds in the light-induced state. Instead, a rearrangement of hydrogen bonds is considered which is achieved by rotation of the conserved glutamine side chain around the Cβ−Cγ bond (a dihedral angle change). In contrary, the mechanisms involving glutamine tautomerization consider a photoinduced chemical modification of the glutamine side chain from the low-energy amide to the high-energy imidic ZZ form. The Z conformations of both the NεH and OεH groups are capable of forming two hydrogen bonds with flavin and at the same time may accept two hydrogen bonds from the neighboring amino acids.[27−29] Thus, the distinct hydrogen-bonding abilities of the glutamine ZZ form allows for a considerably different hydrogen bonding network around flavin which triggers signal transduction in BLUF proteins. In our previous works,[27−29] we have demonstrated that the hydrogen bonding network accommodating the glutamine ZZ form induces a red-shifted flavin absorbance spectrum in the visible and IR regions.

Notably, the light-induced glutamine tautomerization was proposed on the basis of computational quantum-chemical studies.[27−29,42,43] A low-activation-energy path was computed that connects the photoexcited flavin and a radical-pair intermediate containing the EE tautomeric form of glutamine shown in Scheme 1.[42,43] Along this pathway, photoinduced electron transfer first results in a charged radical pair FAD•− anion and Tyr(H)•−cation. The radical pair is further stabilized via proton transfer neutralizing the charge of the system. The conserved glutamine mediates this proton transfer, resulting in glutamine tautomerization. At the end of this process, the system decays to the ground electronic state, however, retaining the radical-pair electronic structure. The radical pair is still a high-energy species which further evolves through radical-pair recombination. In this study, we consider the recombination leading to the formation of the ZZ form of glutamine (Scheme 3).

We applied quantum chemical calculations to characterize the reaction pathway leading from the radical-pair intermediate to the red-shifted photoinduced state. We selected a fairly large molecular model (Figure 1) that included polar side chains forming hydrogen bonds with the flavin•−Tyr21• radical pair as well as hydrophobic side chains creating steric hindrances. We treated the radical pair as well as its decay to the closed-shell state by using the unrestricted-spin DFT calculations, an approach similar to that used in the earlier study of the BLUF radical pair.[42] We compare in Figure 8 the computed energy diagram for both considered pathways. Pathway A includes (1) rotation of the imidic group of the EE form around the Cγ—Cδ bond, (2) a flip of the OεH group followed by (3) radical recombination. Along this pathway, the highest energy barrier is found in step (1) for the EE form rotation around the C—C bond breaking hydrogen bonds. Pathway B, in contrast, starts from (1) radical recombination, which is followed by (2) rotation and (3) tautomerization of the glutamine side chain. Along this pathway, we found a high energy barrier in step (3),

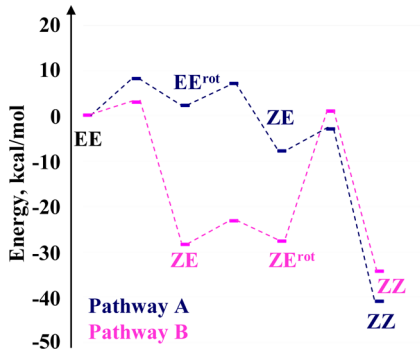

Figure 8. Comparison of the computed glutamine tautomerization pathways A and B in the BLUF active site.

corresponding to the formation of the Z conformation of the NεH group via a proton flip with respect to the Nε═Cδ double bond. The ZZ photoproduct from pathway A is somewhat lower in energy compared to its counterpart from pathway B. The difference in energy is explained by slight variations in conformations of the Met106, Asn45, Leu65, and Leu54 side chains. These differences characterize the conformational flexibility of the BLUF active site.

Analysis of the results shows that the facilitated ZE to ZZ tautomerization along pathway A is achieved by the coupling of the NεH group proton flip to radical recombination. Because of the ZE to ZZ energy barrier, only pathway A leads to the ZZ form, whereas the EZ form is stable along pathway B. The EZ form is characterized by distinct hydrogen bonding as compared to the amide glutamine; however, this isomer is unlikely to form an enhanced hydrogen bond with flavin. In contrast, along pathway A, the radical recombination directly leads to the light-induced red-shifted state containing the ZZ form. The BLUF photodynamics studies[8,37,39] provide evidence that the red-shifted state is directly formed from the radical pair, in total agreement with the mechanism assuming pathway A. The coupling of glutamine tautomerization and radical recombination is achieved through specific hydrogen bonding in the radical-pair intermediate **A-I(2)** shown in Figure 4, i.e., by placing the glutamine E-NεH group in between the electron donor flavin• and the electron acceptor Tyr21•. Thus, glutamine rotation is closely linked to the formation of the ZZ form in BLUF and only rotated ZZ tautomer is formed in photoreaction.

Previously,[28] we found that, in the BLUF active site containing the rotated ZZ glutamine, the flavin visible band and the flavin CO stretching band undergo downshifts consistent with experimental observations. A recent spectroscopic study[21] found an unusually downshifted Tyr21 OH stretching frequency in the light state. Here we extend our previous study[28] by considering the OH stretching frequency of tyrosine. The computed harmonic frequencies of BLUF models are collected in Table 1. We assign the unusual downshift of the Tyr21 OH stretching to its hydrogen bond with the Z conformation of the imidic C═NH group. Thus, the IR spectrum of ref 21 demonstrates photochemical changes in BLUF that are consistent with the computed pathway A.

Along pathway A, we find two sources of energy barriers: hydrogen bonds together with steric constraints and PCET. These barriers control the lifetime of the BLUF radical intermediates. Comparison of the BLUF pathways with the respective acetamide pathways (Figure S8 of the Supporting

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Table 1. Computed Harmonic Frequencies of the BLUF Models

structure of Gln63 in BLUF models	frequencies (cm^{-1}) of the harmonic vibrations of the Tyr21 OH stretching contributions
amide	3373, 3406, 3410
rotated ZZ isomer (Scheme 3)	3091, 3097, 3100
ZZ isomer without rotation (the photoproduct proposed in ref 42)	3576

Information to this paper) reveals that hydrogen bonds and steric hindrances complicate rotation of the glutamine functional group. The highest energy barrier 8.1 kcal/mol is explained by cleavage of hydrogen bonds in step A(1). This estimate provides an upper bound because it is computed for a system without contributions of the thermal energy. We also assume that all possible stabilizing hydrogen bonds are formed in all considered intermediates which may not be the case in the dynamic protein environment. In our previous molecular dynamics and QM/MM study of AppA BLUF,[29] we found that the dynamics of the binding pocket may promote glutamine rotation. We also note that glutamine may rotate in the excited state in the course of the first PCET reaction.[43]

According to the computational model presented here, the short lifetime of the radical pair in AppA BLUF[40,41] may be ascribed to the mobile hydrogen-bonding network that promotes glutamine rotation. Evidence of such dynamics was observed in low-temperature experiments.[20,51,52] At low temperatures, the paramagnetic photointermediates are characterized by μs lifetimes,[51] but at temperatures higher than 160 K, the intermediates are not detectable by using the EPR method.[52]

The energy barrier of the PCET reaction A(3) is related to the experimentally observed H/D effect on the radical-pair lifetime.[37−39] According to our calculations, this energy barrier is rather low. However, presently we cannot definitely state if the U-DFT approach and the limiting size of our molecular model results in underestimating the radical recombination energy. In contrast to the previously discussed rotation barriers, the thermal motion and the hydrogen bond dynamics should not have a drastic effect on radical recombination. It is noteworthy that the radical recombination activation energy is related to the redox potential of the flavin−tyrosine redox pair in a particular BLUF protein. A link between redox properties and photoactivation of the BLUF sensor was established for the first time in a very recent spectroscopy study.[39] The intriguing redox aspect of the BLUF photochemistry calls for further computational studies. In particular, computations of radical recombination by various quantum-chemical methods, including multiconfigurational approaches, are needed to provide reliable energy benchmarks.

To conclude, our current BLUF photoactivation model explains the hydrogen bond switch around the flavin chromophore by the light-induced formation of the ZZ form of the conserved glutamine. The ZZ glutamine is capable of forming a maximum number of hydrogen bonds in the BLUF binding pocket. Photoactivation involves two PCET reactions: photoinduced formation of the neutral radical pair and radical-pair recombination. The first light-induced PCET reaction yields a radical pair containing the EE form of glutamine. The radical pair then undergoes further chemical evolution including the rotation of the functional headgroup and formation of the Z conformation of the Oε—H group. The second PCET and radical recombination catalyzes the NεH flip with respect to the Cδ═Nε double bond, leading to the ZZ form of glutamine. The interactions of the ZZ form with flavin and neighboring amino-acid side chains are enhanced as compared to the dark-state amide form, which is observed as characteristic red-shifts of the flavin spectral signatures. More importantly, the enhanced interactions provide a pathway to alter the BLUF protein dynamics triggering the light response.

■ ASSOCIATED CONTENT

Supporting Information

Complete ref 19, additional figures, and table showing the coordinates of the structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ REFERENCES

(1) Gomelsky, M.; Kaplan, S. AppA, a Novel Gene Encoding a Trans-Acting Factor Involved in the Regulation of Photosynthesis Gene Expression in Rhodobacter Sphaeroides 2.4.1. *J. Bacteriol.* **1995**, *177*, 4609−4618.

(2) Masuda, S.; Bauer, C. E. AppA Is a Blue Light Photoreceptor That Antirepresses Photosynthesis Gene Expression in Rhodobacter sphaeroides. *Cell* **2002**, *110*, 613−623.

(3) Zoltowski, B. D.; Gardner, K. H. Tripping the Light Fantastic: Blue-Light Photoreceptors as Examples of Environmentally Modulated Protein−Protein Interactions. *Biochemistry* **2011**, *50*, 4−16.

(4) Losi, A.; Gärtner, W. Old Chromophores, New Photoactivation Paradigms, Trendy Applications: Flavins in Blue Light-Sensing Photoreceptors. *Photochem. Photobiol.* **2011**, *87*, 491−510.

(5) Masuda, S.; Hasegawa, K.; Ishii, A.; Ono, T. Light-Induced Structural Changes in a Putative Blue-Light Receptor with a Novel FAD Binding Fold Sensor of Blue-Light Using FAD (BLUF); Slr1694 of Synechocystis sp. PCC6803. *Biochemistry* **2004**, *43*, 5304−5313.

(6) Masuda, S.; Hasegawa, K.; Ono, T. Light-Induced Structural Changes of Apoprotein and Chromophore in the Sensor of Blue Light Using FAD (BLUF) Domain of AppA for a Signaling State. *Biochemistry* **2005**, *44*, 1215−1224.

(7) Hasegawa, K.; Masuda, S.; Ono, T. Light Induced Structural Changes of a Full-Length Protein and Its BLUF Domain in YcgF(Blrp), a Blue-Light Sensing Protein That Uses FAD (BLUF). *Biochemistry* **2006**, *45*, 3785−3793.

(8) Bonetti, C.; Mathes, T.; van Stokkum, I. H. M.; Mullen, K. M.; Groot, M.-L.; van Grondelle, R.; Hegemann, P.; Kennis, J. T. M. Hydrogen Bond Switching Among Flavin and Amino Acid Side Chains in the BLUF Photoreceptor Observed by Ultrafast Infrared Spectroscopy. *Biophys. J.* **2008**, *95*, 4790−4802.

(9) Majerus, T.; Kottke, T.; Laan, W.; Hellingwerf, K.; Heberle, J. Time-Resolved FT-IR Spectroscopy Traces Signal Relay within the Blue-Light Receptor AppA. *ChemPhysChem* **2007**, *8*, 1787−1789.
(10) Stelling, A. L.; Ronayne, K. L.; Nappa, J.; Tonge, P. J.; Meech, S. R. Ultrafast Structural Dynamics in BLUF Domains: Transient Infrared Spectroscopy of AppA and Its Mutants. *J. Am. Chem. Soc.* **2007**, *129*, 15556−15564.
(11) Unno, M.; Masuda, S.; Ono, T.; Yamauchi, S. Orientation of a Key Glutamine Residue in the BLUF Domain from AppA Revealed by Mutagenesis, Spectroscopy, and Quantum Chemical Calculations. *J. Am. Chem. Soc.* **2006**, *128*, 5638−5639.
(12) Takahashi, R.; Okajima, K.; Suzuki, H.; Nakamura, H.; Ikeuchi, M.; Noguchi, T. FTIR Study on the Hydrogen Bond Structure of a Key Tyrosine Residue in the Flavin-Binding Blue Light Sensor TePixD from Thermosynechococcus elongatus. *Biochemistry* **2007**, *46*, 6459−6467.
(13) Laan, W.; Gauden, M.; Yeremenko, S.; van Grondelle, R.; Kennis, J. T. M.; Hellingwerf, K. J. On the Mechanism of Activation of the BLUF Domain of AppA. *Biochemistry* **2006**, *45*, 51−60.
(14) Dragnea, V.; Arunkumar, A. I.; Yuan, H.; Giedroc, D. P.; Bauer, C. E. Spectroscopic Studies of the AppA BLUF Domain from Rhodobacter sphaeroides: Addressing Movement of Tryptophan 104 in the Signaling State. *Biochemistry* **2009**, *48*, 9969−9979.
(15) Dragnea, V.; Arunkumar, A. I.; Lee, C. W.; Giedroc, D. P.; Bauer, C. E. A Q63E Rhodobacter sphaeroides AppA BLUF Domain Mutant Is Locked in a Pseudo-Light-Excited Signaling State. *Biochemistry* **2010**, *49*, 10682−10690.
(16) Okajima, K.; Fukushima, Y.; Suzuki, H.; Kita, A.; Ochiai, Y.; Katayama, M.; Shibata, Y.; Miki, K.; Itoh, S.; Ikeuchi, M. Fate Determination of the Flavin Photoreceptions in the Cyanobacterial Blue Light Receptor TePixD (Tll0078). *J. Mol. Biol.* **2006**, *363*, 10−18.
(17) Bonetti, C.; Stierl, M.; Mathes, T.; van Stokkum, I. H. M.; Mullen, K. M.; Cohen-Stuart, T. A.; van Grondelle, R.; Hegemann, P.; Kennis, J. T. M. The Role of Key Amino Acids in the Photoactivation Pathway of the Synechocystis Slr1694 BLUF Domain. *Biochemistry* **2009**, *48*, 11458−11469.
(18) Lukacs, A.; Haigney, A.; Brust, R.; Zhao, R.-K.; Stelling, A. L.; Clark, I. P.; Towrie, M.; Greetham, G. M.; Meech, S. R.; Tonge, P. J. Photoexcitation of the Blue Light Using FAD Photoreceptor AppA Results in Ultrafast Changes to the Protein Matrix. *J. Am. Chem. Soc.* **2011**, *133*, 16893−16900.
(19) Haigney, A.; Lukacs, A.; Zhao, R.-K.; Stelling, A. L.; Brust, R.; Kim, R.-R.; Kondo, M.; Clark, I.; Towrie, M.; Greetham, G. M.; et al. Ultrafast Infrared Spectroscopy of an Isotope-Labeled Photoactivatable Flavoprotein. *Biochemistry* **2011**, *50*, 1321−1328.
(20) Fukushima, Y.; Murai, Y.; Okajima, K.; Ikeuchi, M.; Itoh, S. Photoreactions of Tyr8- and Gln50-Mutated BLUF Domains of the PixD Protein of Thermosynechococcus elongatus BP-1: Photoconversion at Low Temperature without Tyr8. *Biochemistry* **2008**, *47*, 660−669.
(21) Iwata, T.; Watanabe, A.; Iseki, M.; Watanabe, M.; Kandori, H. Strong Donation of the Hydrogen Bond of Tyrosine during Photoactivation of the BLUF Domain. *J. Phys. Chem. Lett.* **2011**, *2*, 1015−1019.
(22) Obanayama, K.; Kobayashi, H.; Fukushima, K.; Sakurai, M. Structures of the Chromophore Binding Sites in BLUF Domains as Studied by Molecular Dynamics and Quantum Chemical Calculations. *Photochem. Photobiol.* **2008**, *84*, 1003−1010.
(23) Götze, J.; Saalfrank, P. Serine in BLUF Domains Displays Spectral Importance in Computational Models. *J. Photochem. Photobiol., B* **2009**, *94*, 87−95.
(24) Götze, J. P.; Greco, C.; Mitrić, R.; Bonačić-Koutecký, V.; Saalfrank, P. BLUF Hydrogen Network Dynamics and UV/Vis Spectra: A Combined Molecular Dynamics and Quantum Chemical Study. *J. Comput. Chem.* **2012**, *33*, 2233−2242.
(25) Rieff, B.; Bauer, S.; Mathias, G.; Tavan, P. DFT/MM Description of Flavin IR Spectra in BLUF Domains. *J. Phys. Chem. B* **2011**, *115*, 11239−11253.
(26) Hsiao, Y.-W.; Götze, J. P.; Thiel, W. The Central Role of Gln63 for the Hydrogen Bonding Network and UV−Visible Spectrum of the AppA BLUF Domain. *J. Phys. Chem. B* **2012**, *116*, 8064−8073.
(27) Domratcheva, T.; Grigorenko, B. L.; Schlichting, I.; Nemukhin, A. V. Molecular Models Predict Light-Induced Glutamine Tautomerization in BLUF Photoreceptors. *Biophys. J.* **2008**, *94*, 3872−3879.
(28) Khrenova, M. G.; Nemukhin, A. V.; Grigorenko, B. L.; Krylov, A. I.; Domratcheva, T. M. Quantum Chemistry Calculations Provide Support to the Mechanism of the Light-Induced Structural Changes in the Flavin-Binding Photoreceptor Proteins. *J. Chem. Theory Comput.* **2010**, *6*, 2293−2302.
(29) Khrenova, M. G.; Domratcheva, T.; Schlichting, I.; Grigorenko, B. L.; Nemukhin, A. V. Computational Characterization of Reaction Intermediates in the Photocycle of the Sensory Domain of the AppA Blue Light Photoreceptor. *Photochem. Photobiol.* **2011**, *87*, 564−573.
(30) Jung, A.; Reinstein, J.; Domratcheva, T.; Shoeman, R. L.; Schlichting, I. Crystal Structures of the AppA BLUF Domain Photoreceptor Provide Insights into Blue Light-Mediated Signal Transduction. *J. Mol. Biol.* **2006**, *362*, 717−732.
(31) Grinstead, J. S.; Hsu, S.-Te D.; Laan, W.; Bonvin, A. M. J. J.; Hellingwerf, K. J.; Boelens, R.; Kaptein, R. The Solution Structure of the AppA BLUF Domain: Insight into the Mechanism of Light-Induced Signaling. *ChemBioChem* **2006**, *7*, 187−193.
(32) Wu, Q.; Gardner, K. Structure and Insight into Blue Light-Induced Changes in the BlrP1 BLUF Domain. *Biochemistry* **2009**, *48*, 2620−2629.
(33) Anderson, S.; Dragnea, V.; Masuda, S.; Ybe, J.; Moffat, K.; Bauer, C. Structure of a Novel Photoreceptor, the BLUF Domain of AppA from Rhodobacter sphaeroides. *Biochemistry* **2005**, *44*, 7998−8005.
(34) Meier, K.; Thiel, W.; van Gunsteren, F. On the Effect of a Variation of the Force Field, Spatial Boundary Condition and Size of the QM Region in QM/MM MD Simulations. *J. Comput. Chem.* **2012**, *33*, 363−378.
(35) Dragnea, V.; Waegele, M.; Balascuta, S.; Bauer, C.; Dragnea, B. Time-Resolved Spectroscopic Studies of the AppA Blue-Light Receptor BLUF Domain from Rhodobacter sphaeroides. *Biochemistry* **2005**, *44*, 15978−15985.
(36) Shibata, Y.; Murai, Y.; Satoh, Y.; Fukushima, Y.; Okajima, K.; Ikeuchi, M.; Itoh, S. Acceleration of Electron-Transfer-Induced Fluorescence Quenching upon Conversion to the Signaling State in the Blue-Light Receptor, TePixD, from Thermosynechococcus elongatus. *J. Phys. Chem. B* **2009**, *113*, 8192−8198.
(37) Gauden, M.; van Stokkum, I. H. M.; Key, J. M.; Luhrs, D. C.; van Grondelle, R.; Hegemann, P.; Kennis, J. T. M. Hydrogen-Bond Switching through a Radical Pair Mechanism in a Flavin-Binding Photoreceptor. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10895−10900.
(38) Mathes, T.; Zhu, J.; van Stokkum, I. H. M.; Groot, M. L.; Hegemann, P.; Kennis, J. T. M. Hydrogen Bond Switching among Flavin and Amino Acids Determines the Nature of Proton-Coupled Electron Transfer in BLUF Photoreceptors. *J. Phys. Chem. Lett.* **2012**, *3*, 203−208.
(39) Mathes, T.; van Stokkum, I. H. M.; Stierl, M.; Kennis, J. T. M. Redox Modulation of Flavin and Tyrosine Determines Photoinduced Proton-coupled Electron Transfer and Photoactivation of BLUF Photoreceptors. *J. Biol. Chem.* **2012**, *287*, 31725−31738.
(40) Gauden, M.; Yeremenko, S.; Laan, W.; van Stokkum, I. H. M.; Ihalainen, J. A.; van Grondelle, R.; Hellingwerf, K. J.; Kennis, J. T. M. Photocycle of the Flavin-Binding Photoreceptor AppA, a Bacterial Transcriptional Antirepressor of Photosynthesis Genes. *Biochemistry* **2005**, *44*, 3653−3662.
(41) Toh, K. C.; van Stokkum, I. H. M.; Hendriks, J.; Alexandre, M. T.; Arents, J. C.; Perez, M. A.; van Grondelle, R.; Hellingwerf, K. J.; Kennis, J. T. M. On the Signaling Mechanism and the Absence of Photoreversibility in the AppA BLUF Domain. *Biophys. J.* **2008**, *95*, 312−321.
(42) Sadeghian, K.; Bocola, M.; Schütz, M. A Conclusive Mechanism of the Photoinduced Reaction Cascade in Blue Light Using Flavin Photoreceptors. *J. Am. Chem. Soc.* **2008**, *130*, 12501−12513.

(43) Udvarhelyi, A.; Domratcheva, T. Photoreaction in BLUF Receptors: Proton-coupled Electron Transfer in the Flavin-Gln-Tyr System. *Photochem. Photobiol.* **2011**, *87*, 554−563.
(44) Siegbahn, P. E. M.; Himo, F. The Quantum Chemical Cluster Approach for Modeling Enzyme Reactions. *Wiley Interdiscip. Rev.: Comput. Mol. Sci.* **2011**, *1*, 323−336.
(45) Gräfenstein, J.; Kraka, El.; Filatov, M.; Cremer, D. Can Unrestricted Density-Functional Theory Describe Open Shell Singlet Biradicals? *Int. J. Mol. Sci.* **2002**, *3*, 360−394.
(46) Adamo, C.; Barone, V. Toward Reliable Density Functional Methods Without Adjustable Parameters: The PBE0 Model. *J. Chem. Phys.* **1999**, *110*, 6158−6170.
(47) Dunning, T. H. Gaussian Basis Sets for Use in Correlated Molecular Calculations. I. The Atoms Boron Through Neon and Hydrogen. *J. Chem. Phys.* **1989**, *90*, 1007−1023.
(48) Nakano, H.; Uchiyama, R.; Hirao, K. Quasi-Degenerate Perturbation Theory with General Multiconfiguration Self-Consistent Field Reference Functions. *J. Comput. Chem.* **2002**, *23*, 1166−1175.
(49) Granovsky, A. A. *Firefly*, version 7.1.G; URL http://classic.chem.msu.su/gran/firefly/index.html.
(50) Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; et al. General Atomic and Molecular Electronic Structure System. *J. Comput. Chem.* **1993**, *14*, 1347−1363.
(51) Nagai, H.; Fukushima, Y.; Okajima, K.; Ikeuchi, M.; Mino, H. Formation of Interacting Spins on Flavosemiquinone and Tyrosine Radical in Photoreaction of a Blue Light Sensor BLUF Protein TePixD. *Biochemistry* **2008**, *47*, 12574−12582.
(52) Weber, S.; Schroeder, C.; Kacprzak, S.; Mathes, T.; Kowalczyk, R. M.; Essen, L.-O.; Hegemann, P.; Schleicher, E.; Bittl, R. Light-Generated Paramagnetic Intermediates in BLUF Domains. *Photochem. Photobiol.* **2011**, *87*, 574−583.
(53) Voevodin, Vl. V.; Zhumatiy, S. A.; Sobolev, S. I.; Antonov, A. S.; Bryzgalov, P. A.; Nikitenko, D. A.; Stefanov, K. S.; Voevodin, V. V. Practice of "Lomonosov" Supercomputer. *Open Systems J.* **2012**, *7* (in Russian).