The AppA BLUF (blue light sensing using FAD) domain from *Rhodobacter sphaeroides* serves as a blue light-sensing photoreceptor. The charge separation process between Tyr-21 and flavin plays an important role in the light signaling state by transforming the dark state conformation to the light state one. By solving the linearized Poisson-Boltzmann equation, I calculated $E_m$ for Tyr-21, flavin, and redox-active Trp-104 and revealed the electron transfer (ET) driving energy. Rotation of the Gln-63 side chain that converts protein conformation from the dark state to the light state is responsible for the decrease of 150 mV in $E_m$ for Tyr-21, leading to the significantly larger ET driving energy in the light state conformation. The $pK_a$ values of protonation for flavin anions are essentially the same in both dark and light state crystal structures. In contrast to the ET via Tyr-21, formation of the $W^+$ state results in generation of only the dark state conformation (even if the initial conformation is in the light state); this could explain why Trp-104-mediated ET deactivates the light-sensing yield and why the activity of W104A mutant is similar to that of the light-adapted native BLUF.

Sensing blue light is a prerequisite for organisms to maintain their functions. BLUF domains serve as a photoreceptor by regulating the activity of covalently attached effector domains (1). The photoactivation of the BLUF domain from *Rhodobacter sphaeroides* is mediated by a red shift intermediate state that mainly consists of FAD and Tyr-21 (2). The electronic excited FAD$^*$ state decays to the charge-separated $Y^-\cdot W^\cdot FAD^+$ state. Via proton transfer (PT) from $Y^+$ to FAD$^+$, the $Y^-\cdot W^\cdot FAD^+$ state transforms into the charge-neutral $Y^-\cdot W^-\cdot FADH^+$ state and finally decays to the ground state (3). Recent spectroscopic studies suggested that not only Tyr-21 but also Trp-104 act as electron donors in the charge separation process, where Tyr-21$^+$ is the functionally relevant charge state (i.e. the productive pathway in Ref. 4), whereas formation of Trp-104$^+$ (i.e. the nonproductive pathway in Ref. 4) does not contribute to light sensing of AppA BLUF (4). Note that although FAD is the cofactor in the native BLUF domain (5), the BLUF fragment contains a mixture of flavins (i.e. FMN (majority), FAD, and riboflavin) when expressed in *Escherichia coli* (6). In fact, FMN is identified in the BLUF crystal structures from *R. sphaeroides* (7, 8). Currently, there are several points that need to be clarified for understanding the BLUF domain as described below.

In the protein environment of the BLUF domain, the orientation of the key residue Gln-63 with respect to Tyr-21 is a matter of debate. From observations of the native BLUF crystal structure, Anderson *et al.* (7) proposed that the $–NH_2$ group of the Gln-63 side chain forms a hydrogen bond with Tyr-21 in the dark state and that the $–CO$ group of the Gln-63 side chain forms a hydrogen bond with Trp-21 in the light state (Fig. 1). In contrast, from observations of the C20S mutant crystal structure, Jung *et al.* (8) assigned “dark” and “light” states that were opposite to those assigned by Anderson *et al.* (7). The majority of spectroscopic and mutational studies (9–12) suggested the same assignment of the dark and light structures as that proposed by Anderson *et al.* (7). In the present study, I tentatively follow the definition of Anderson *et al.* (7) if not otherwise specified (see Fig. 1 for the definition used in the present study).

Another open question is the functionally relevant location of Trp-104 with respect to the FMN binding site. In the crystal structure of the native BLUF domain refined by Anderson *et al.* (7), the indole nitrogen atom of the Trp-104 side chain is at a hydrogen-bonding distance from the carbonyl oxygen atom of the Gln-63 side chain (i.e. $W_{in}$ conformation; see Fig. 2a). On the other hand, in the crystal structures of the C20S mutant by Jung *et al.* (8), Trp-104 is located on the protein surface and exposed to the bulk solvent (i.e. $W_{out}$ conformation; see Fig. 2b). The $W_{out}$ conformation is also identified in the BLUF domain from *Thermosynechococcus elongatus* (10). Jung *et al.* (8) argued that the $W_{in}$ conformation reported by Anderson *et al.* (7) may be induced by the presence of detergent molecules. However, the $W_{in}$ conformation can be seen in most NMR solution structural models of the BLUF domain (13). Recent spectroscopic studies suggested that Q63L and W104A mutants are insensitive to blue light, implying a functionally important role of a hydrogen bond between the two residues (that should exist only in the $W_{in}$ conformation) (14). It is known that photoactivation of the BLUF domain induces the rotation of the Gln-63 side chain (7). However, the driving force of the Gln-63 rotation is yet unclear. It was proposed that proton movements (namely
the PT from \( Y^+ \) to the N5 atom of FMN\(^7 \) underlie the rotation from the observed kinetic isotope effects (3).

To clarify the functional relevance of these possible four conformers (i.e. dark-\( W_{\text{out}} \) and light-\( W_{\text{out}} \) (Protein Data Bank 2IY1 and 2IYG, respectively) (Fig. 1) (8), dark-\( W_{\text{in}} \) (Protein Data Bank 1YRX) (7), and light-\( W_{\text{in}} \) (no corresponding Protein Data Bank entry; see Fig. 3)), the values of the ET driving energy for each protein conformer that are yet experimentally unavailable need to be clarified.

Recently, I calculated the redox (midpoint) potential \( (E_m) \) of flavin (15, 16) and redox-active tyrosine (17) precisely by considering the protonation states of all titratable sites in the proteins. In this study, I present the \( (E_m) \) of FMN for one-electron reduction \( (E_m(\text{FMN/FMN}^\cdot)) \), protonated tyrosine for one-electron oxidation \( (E_m(Y/Y^+)\) and, tryptophan for one-electron oxidation \( (E_m(W/W^+) \) in the BLUF domain, by solving the linearized Poisson-Boltzmann equation for all atoms in the crystal structures. From the calculated \( E_m \), I obtain the driving energy of the ET in the charge separation process (via Tyr-21 and Trp-104) of the known four BLUF conformers. Then I evaluate (i) functional relevance of the conformers assigned by Anderson et al. (7) and Jung et al. (8), (ii) difference of the ET energetics in the \( W_{\text{in}} \) and \( W_{\text{out}} \) structures, and (iii) why the charge separation process involving Trp-104 does not lead to the light signaling state.

Since the dark and light state structures are originally available only for the \( W_{\text{out}} \) structure (8) (i.e. without performing the modeling of protein atomic coordinates), the computational results of the \( W_{\text{out}} \) structure (8) are mainly presented if not otherwise specified. In particular, the results of \( W_{\text{out}} \) and \( W_{\text{in}} \) were essentially the same in the ET via Tyr-21 but not in the ET via Trp-104. In the present study, I used the same conditions sufficiently evaluated in previous studies on proteins that contain flavin (15, 16) and redox-active tyrosine (17).

**THEORY**

**Atomic Coordinates and Charges**—For performing computations of the BLUF domain, the crystal structures of the native BLUF domains by Anderson et al. (7) (Protein Data Bank code 1YRX) and the C20S mutant BLUF domains by Jung et al. (8) (Protein Data Bank codes 2IY1 and 2IYG) were used. For the \( W_{\text{out}} \) crystal structure by Jung et al. (8), I replaced the original \( S_b \) atom of the C20 side chain with an oxygen atom and used it as the native BLUF structure. In this study, the crystal structures with Protein Data Bank code 2IY1 and 2IYG were regarded as dark and light state structures (i.e. dark-\( W_{\text{out}} \) and light-\( W_{\text{out}} \)), respectively (Fig. 1). This definition of the dark and light structures is the same as that proposed Anderson et al. (7, 9–12).

For the \( W_{\text{in}} \) conformations, I used the crystal structure refined by Anderson et al. (7) as the dark state structure (i.e. dark-\( W_{\text{in}} \)). I modeled the light-\( W_{\text{in}} \) structure by rotating the side chain –NH\(_2\) and –CO groups of Gln-63 by 180° along the \( C_{\text{N}}-C_{\text{O}} \) axis (Fig. 3). The obtained atomic coordinates of the Gln-63 side chain for the light-\( W_{\text{in}} \) structure in the charge-neutral [Y-W-FMN] state are shown in Table S1.

The positions of the hydrogen atoms were energetically optimized with CHARMM (18) by using the CHARMM22 force field. During this procedure, the positions of all nonhydrogen atoms were fixed, and the standard charge states of all of the titratable groups were maintained (i.e. basic and acidic groups were considered to be protonated and deprotonated, respectively). All of the other atoms whose coordinates were available in the crystal structure were not geometrically optimized. Note that the protein atomic coordinates obtained after the energy optimization process revealed that Tyr-21 is a hydrogen bond donor to the –NH\(_2\) and –CO groups of the Gln-63 side chain in the dark and light structures, respectively, in agreement with what was observed in recent density functional theory studies (19).

Atomic partial charges of the amino acids were adopted from the all atom CHARMM22 (18) parameter set. Atomic charges of the \( 5^-\)-phosphate group of FMN quinone (\( -\text{HPO}_3^- \)), \( -\text{H}_2\text{PO}_4^- \), and \( -\text{HPO}_4^{2-} \) were adopted from those of methYLphosphate. The charges of FMN, FMNH\(_2\), and FMN\(^7\) were from a previous report on flavodoxin (15). The atomic charges for the redox-active tyrosine were adopted from Ref. 20 (protonated tyrosine with neutral charge (Y) and protonated tyrosine radical with positive charge \( Y^+ \) in the redox pair \( Y/Y^+ \)). The atomic charges for the redox-active tetrypsolin were adopted from Ref. 20 (protonated tetrypsolin with neutral charge (W) and protonated tetrypsolin radical with positive charge \( W^+ \) in the redox pair \( W/W^+ \)).

**Protonation Pattern, Redox Potential, and \( pK_a \)**—The present computation is based on the electrostatic continuum model created by solving the linear Poisson-Boltzmann equation with the MEAD program (21). To facilitate a direct comparison with previous computational results, I uniformly used identical computational conditions and parameters, such as atomic partial charges and dielectric constants (e.g. see Refs. 15, 17, and 22). To obtain the absolute \( E_m \) values of the protein, we calculated the electrostatic energy difference between the two redox states in a reference model system using a known experimental \( E_m \) value. The difference in the \( E_m \) value of the protein relative to the reference system was added to the known \( E_m \) value (see below). All of the other titratable sites, including the \( 5^-\)-phosphate group, were fully equilibrated to the redox state of FMN during the titration. The ensemble of the protonation patterns was sampled by the Monte Carlo method with Karlsberg.\(^3\) The dielectric constants were set to \( \varepsilon_p = 4 \) inside the protein and \( \varepsilon_w = 80 \) for water. All computations were performed at 300 K, pH 7.0, and an ionic strength of 100 mM. The linear Poisson-Boltzmann equation was solved using a three-step grid-focusing procedure at resolutions of 2.5, 1.0, and 0.3 Å. The Monte Carlo sampling yielded the probabilities \( [A_{\text{ox}}] \) and \( [A_{\text{red}}] \) of the two redox states of molecule \( A \). \( E_m \) was evaluated using the Nernst equation. A bias potential was applied to obtain an equal amount of both redox states \( ([A_{\text{ox}}] = [A_{\text{red}}]) \), thereby yielding the redox midpoint potential \( E_m \), as the resulting bias potential.

From this analogy, using the Henderson-Hasselbalch equation, \( pK_a \) can be calculated as the pH at which the concentrations of the protonated and deprotonated residue species are equal (Henderson-Hasselbalch \( pK_a \)). For convenience, the computed

---

\(^3\) Rabenstein, B., Ullmann, G. M., and Knapp, E.-W. (1998) Eur. Bophys. J. 27, 626–637.
pKₐ and Eₘ Values in the Reference Model System—FMN⁺ forms FMNH₂ upon protonation at the N5 nitrogen in FMN quinone (Fig. 1). The value of 8.6 (23) was taken as the pKₐ(N5) value in the reference model system of FMN⁺/FMNH₂ equilibrium in aqueous solution. The value of 6.4 (24) was considered as the pKₐ value of the 5’-phosphate group of –H₂PO₃⁻/–HPO₃²⁻. Note that the 5’-phosphate group was permanently deprotonated in the –H₂PO₃⁻/–H₂PO₃₅⁻ equilibrium (pKₐ = 1.4 (24)) in all of the crystal structures that were investigated. Thus, in the present study, the –H₂PO₃⁻/–HPO₃²⁻ equilibrium (pKₐ = 6.4 (24)) was investigated, unless otherwise specified. As a reference model system, the following values for Eₘ versus the normal hydrogen electrode were used: Eₘ(Y/Y⁻+) = +1380 mV (25) and Eₘ(W/W⁺⁺) = +1070 mV (26) for one-electron oxidation in an aqueous solution. Eₘ(FMN/ FMN⁺) = −333 mV (15) was used as the reference model system of Eₘ(FMN/ FMN⁺) in an aqueous solution (see further discussion in the supplemental materials).

RESULTS AND DISCUSSION

Driving Energy in the ET Involving Tyr-21—By calculating Eₘ(FMN/FMN⁺) and Eₘ(Y/Y⁻+), I obtained the driving energy (ΔG) of the ET between FMN and Y to be −156 and −317 meV for the dark and light state structures in the charge-separated [Y⁺⁻ W-FMN⁺] state, respectively (Table 1). I found that the calculated ΔG in the light state structure (−317 meV) is greater by 160 meV than that in the dark state structure (−156 meV; Table 1). This tendency essentially holds true for the charge-neutral [Y-W-FMN] state. Since the light state structure is energetically much favorable for the ET than the dark state structure in the present study, the charge separation process via Tyr-21 ([Y-W-FMN] → Y⁺⁻ W-FMN⁺) should complete with the light state structure (Fig. 1).

The revealed larger ET driving energy (i.e. energetically more exergonic) in the light state structure in the present study supports the validity of the assignment of the dark and light state conformations by Anderson et al. (7) and those suggested in spectroscopic and mutational studies (9–12).

Influence of Gln-63 on Eₘ(FMN/FMN⁺) and Eₘ(Y/Y⁻+)—I investigated the influence of the BLUE protein environment on Eₘ(FMN/FMN⁺) and Eₘ(Y/Y⁻+). The different orientations of the –NH₂ and –CO groups of Gln-63 in the dark and light structures alter Eₘ(Y/Y⁻+) by 230 mV (Table 2). Note that this influence is partially compensated by associated changes in the protonation states of titratable residues and the hydrogen atom coordinates of some residues, resulting in the net Eₘ shift of 154 mV (see Table 1). The lower Eₘ(Y/Y⁻+) in the light state structure implies that obviously, the proximity of the oxygen atom of the –CO group can stabilize the Y⁺ state effectively. In the dark state structure, the –CO group, in turn, destabilizes the Y⁺ state by 67 mV because of the proximity of its polar carbon atom to Tyr-21. On the other hand, the side chain orientation of Gln-63 does not essentially affect Eₘ(FMN/FMN⁺) (Tables 1 and 3). Thus, the significant change in ΔG by switching from the dark state to the light state structure can be attributed predominantly to the change in Eₘ(Y/Y⁻+) and not that in Eₘ(FMN/FMN⁺).

Driving Force of Side Chain Rotation of Gln-63—Regardless of the difference in the hydrogen bond pattern with regard to the Gln-63 and FMN pair, the calculated pKₐ(N5) values of FMN are essentially the same in both dark and light state structures (pKₐ(N5) = 12.2 and 12.3 in the [Y-W-FMN] state and 14.0 and 13.9 in the [Y⁺⁻ W-FMN⁺] state, respectively). In other words, ΔG for the PT process from Y⁺ to FMN⁺ is essentially the same in the dark and light state structures. On the other
Influence of the protein environment on Influence of the protein environment on In analogy, the light-induced charge of the \([Y^-\cdot \cdot \cdot \cdot FMN^-]\) state (rather than protonation process) may be the driving force for the rotation of the Gln-63 side chain; the Gln-63 reorientation is probably synchronized with the completion of the charge-separation process \([Y^-\cdot \cdot \cdot \cdot FMN^- \rightarrow Y^-\cdot \cdot \cdot \cdot FMN^-]\).

\[\Delta G \text{ for the ET } [Y^-\cdot \cdot \cdot \cdot FMN^- \rightarrow Y^-\cdot \cdot \cdot \cdot FMN^-] \text{ is } 160 \text{ meV greater in the light state structure than that in the dark state structure (}[Y^-\cdot \cdot \cdot \cdot FMN^-]\) state; Table 1]. It is unlikely that the BLUF must maintain the dark state conformation during the charge separation process at the expense of this available 160-meV energy of the ET. Instead, it appears that the driving force of the Gln-63 side chain rotation is a result of the light-induced charge \([Y^-\cdot \cdot \cdot \cdot FMN^-]\) (rather than the different \(\Delta G\) for the PT from \(Y^-\) to the N5 atom of FMN).

### TABLE 2

| Redox potential | \(E_{\text{FMN}}\) | \(E_{\text{FMN}}\) | \(\Delta E_{\text{FMN}}\) |
|----------------|----------------|----------------|----------------|
| Dark^-Wout \((2IYG)\) | -654 | -333 | -231 |
| Light^-Wout \((2IYG)\) | -209 | -333 | -254 |

### TABLE 3

| Redox potential | \(E_{\text{FMN/FMN}}\) | \(E_{\text{FMN/FMN}}\) | \(\Delta E_{\text{FMN/FMN}}\) |
|----------------|----------------|----------------|----------------|
| Dark^-Wout \((2IYG)\) | -654 | -333 | -231 |
| Light^-Wout \((2IYG)\) | -209 | -333 | -254 |

Indeed, the functional relationship between the rotation of a \(-CO\)-containing group and the light-induced charge can be seen in other photosassociated systems. In bacterial photosynthetic reaction centers from \(R. \text{sphaeroides}\), light-induced charge separation leads to electron transfer from a monomeric bacteriochlorophyll to a bacteriopheophytin. It has been reported from spectroscopic studies that there exist two distinct conformations of the bacteriopheophytin in the reduced state with regard to the orientation of the acetyl group (27). The rotation of the acetyl group of the electron acceptor bacteriopheophytin takes place in coupling with the charge separation process after the excitation of chromophores (completed in 4 ps) (27). As a consequence, the rotation of the acetyl group contributes to a stabilization of the reduced state of the bacteriopheophytin by upshifting its \(E_m\) (28). Notably, this “light-induced acetyl conformational switch” can be activated by induced anionic charges on the bacteriopheophytin upon its photoconversion, leading to the stabilization of the charge-separated state in the photosynthetic reaction centers (27).

In analogy, the light-induced charge of the \([Y^-\cdot \cdot \cdot \cdot FMN^-]\) state (rather than protonation process) may be the driving force for the rotation of the Gln-63 side chain; the Gln-63 reorientation is probably synchronized with the completion of the charge-separation process \([Y^-\cdot \cdot \cdot \cdot FMN^- \rightarrow Y^-\cdot \cdot \cdot \cdot FMN^-]\).

\[\Delta G \text{ for the ET } [Y^-\cdot \cdot \cdot \cdot FMN^- \rightarrow Y^-\cdot \cdot \cdot \cdot FMN^-] \text{ is } 160 \text{ meV greater in the light state structure than that in the dark state structure (}[Y^-\cdot \cdot \cdot \cdot FMN^-]\) state; Table 1]. It is unlikely that the BLUF must maintain the dark state conformation during the charge separation process at the expense of this available 160-meV energy of the ET. Instead, it appears that the driving force of the Gln-63 side chain rotation is a result of the light-induced charge \([Y^-\cdot \cdot \cdot \cdot FMN^-]\) (rather than the different \(\Delta G\) for the PT from \(Y^-\) to the N5 atom of FMN).
Recent studies on Y21F and W104F mutants suggested that ET via Tyr-21 and Trp-104 compete in the charge separation process (4). Hence, if the dark-W out structure is the only functionally relevant conformation, ET via Trp-104 may not be able to compete with ET via Tyr-21 due to its very low ET coupling. Thus, the Win structure is probably necessary to explain the competition of the two ET processes (see below for further discussion).

Transformation of the Light-W in Conformation to the Dark-W in Conformation—In the W in crystal structure, only the atomic coordinates of the dark state conformation (where the –NH2 group of Gln-63 forms a hydrogen bond with the Tyr-21) are available (7). Therefore, to investigate the light-W in conformation, I modeled the atomic coordinates by rotating the side chain –NH2 and –CO groups of the Gln-63 side chain by 180° along the Cγ-Cα axis and energetically optimizing the atomic coordinates of the Gln-63 side chain. In the [Y-W-FMN] state, J(W/W ) of the dark-W in structure was lower by ~120 mV than that of the modeled light-W in structure (Table 1), indicating that W+ can be stabilized effectively in the dark state. Note that the significantly destabilized W+ state in the modeled light-W in structure is a result of the loss of the hydrogen bond between the indole nitrogen atom of Trp-104 with the –CO group of Gln-63 (Table 4).

By using the same procedure, I tried to generate the modeled light-W in structure for the charge-separated [Y-W+FMN+] state. Unexpectedly, I could obtain only the dark state conformation for the [Y-W+FMN+] state and not the light state conformation (Fig. 3). In other words, when I apply the [Y-W+FMN+] charge state to the light state structure and then perform energy optimization of the atomic coordinates, the Gln-63 side chain undergoes a 180° rotation along the Cγ-Cα axis, resulting in the atomic coordinates of the dark state structure during the energy optimization process (Fig. 3).

Functional Relevance of the W in Conformations—The present study demonstrates that the light-W in conformation of the [Y-W+FMN+] state is unusually unstable due to its energetically unfavorable hydrogen bond pattern. Thus, it is likely that the involvement of Trp-104 in the W in charge separation process would lead to the elimination of the light-W in conformation and, as a consequence, a decrease in the yield of the signaling state of the BLUF domain. Indeed, spectroscopic studies on the W104F mutant indicate its 1.5-fold increase in the quantum yield of signaling state formation with respect to the native BLUF (32), implying that Trp-104 in the native BLUF is likely to lower the quantum yield of signaling state formation. This is in agreement with the present result.

In addition, recent mutational studies have suggested that there exist two competing light-induced ET pathways in the BLUF domain: one ET pathway via Tyr-21 that can form the signaling state and another ET pathway via Trp-104 that leads to the deactivation of signaling state formation (4). From the
present result, only the $W_{in}$ conformation is able to yield the dark state structure upon the formation of the [Y-W$^+$-FMN$^\ast$] state (Fig. 3). In the $W_{out}$ structure, there does not exist such a strict constraint on the orientation of the Gln-63 side chain because of the isolation of Trp-104 from Gln-63 (14.5 Å). Thus, to explain competitions of ET via Tyr-21 and Trp-104 (4, 32), the existence of the $W_{in}$ conformation (7) is functionally required, as implied in former studies (7, 12–14).

Remarkably, spectroscopic studies by Masuda et al. (14) demonstrated that (i) the W104A mutant is insensitive to blue light and (ii) its activity is similar to that of the light-adapted native BLUF. These two facts are indicative that the loss of Trp-104 in the native $W_{in}$ structure does not result in the transformation to the dark state structure and that the BLUF domain is always adapted to the light state structure in the absence of Trp-104. Formation of $W^\ast$ in the $W_{in}$ structure could reset the Gln-63 orientation to the dark (i.e. ground) state so that the BLUF domain can again perform photosensing effectively.

CONCLUSION

The driving energy of the ET via Tyr-21 in the light state structure is greater by 160 meV than that in the dark state structure (Table 1 and Fig. 1); this suggests that assignments of the BLUF dark/light conformers by Anderson et al. (7) are more reasonable than those by Jung et al. (8) to explain the energetics of the photoinduced ET event.

The calculated $pK_a$ values indicate that the driving energy for the PT from Y$^\ast$ to FMN$^\ast$ is essentially the same in the dark and light state structures.

The driving energy of the ET via Trp-104 is larger than that via Tyr-21.

I observed that $W_{in}^\ast$ transforms the light state to the dark state (Fig. 3). This is in contrast to the case where Y$^\ast$ transforms the dark state to the light state. Hence, $W_{in}^\ast$ may facilitate to reset the Gln-63 orientation to the dark (i.e. ground) state so that the BLUF domain can again perform photosensing effectively. These are probably the reasons why the W104A mutant is insensitive to blue light and its activity is similar to that of the light adapted native BLUF in spectroscopic studies (14).

Acknowledgments—I am grateful to Dr. Arieh Warshel and Dr. Ernst-Walter Knapp for useful discussions.

REFERENCES

1. Han, Y., Brautsch, S., Osterloh, L., and Klug, G. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 12306–12311
2. Kraft, B. J., Masuda, S., Kikuchi, J., Dragnea, V., Tollin, G., Zaleski, J. M., and Bauer, C. E. (2003) Biochemistry 42, 6726–6734
3. Gauden, M., van Stokkum, I. H. M., Key, J. M., Luhrs, D. C., van Grondelle, R., Hegemann, P., and Kennis, J. T. M. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 10895–10900
4. Gauden, M., Grinstein, J. S., Laan, W., van Stokkum, I. H. M., Avila-Perez, M., Toh, K. C., Boelens, R., Kaptein, R., van Grondelle, R., Hellingwerf, K. J., and Kennis, J. T. M. (2007) Biochemistry 46, 7405–7415
5. Masuda, S. and Bauer, C. E. (2002) Cell 110, 613–623
6. Laan, W., van der Horst, M. A., van Stokkum, I. H. M., and Hellingwerf, K. J. (2003) Photochem. Photobiol. 78, 290–297
7. Anderson, S., Dragnea, V., Masuda, S., Ybe, J., Moffat, K., and Bauer, C. (2005) Biochemistry 44, 7998–8005
8. Jung, A., Reinstein, J., Domratcheva, T., Shoeman, R. L., and Schlichting, I. (2006) J. Mol. Biol. 362, 717–732
9. Masuda, S., Hasegawa, K., and Ono, T.-A. (2005) Biochemistry 44, 1215–1224
10. Kita, A., Okajima, K., Morimoto, Y., Ikeuchi, M., and Miki, K. (2005) J. Mol. Biol. 349, 1–9
11. Unno, M., Masuda, S., Ono, T.-A., and Yamauchi, S. (2006) J. Am. Chem. Soc. 128, 5638–5639
12. Grinstein, J. S., Avila-Perez, M., Hellingwerf, K. J., Boelens, R., and Kaptein, R. (2006) J. Am. Chem. Soc. 128, 15066–15067
13. Grinstein, J. S., Hsu, S.-T., Laan, W., Bonvin, A. M., Hellingwerf, K. J., Boelens, R., and Kaptein, R. (2006) ChemBioChem 7, 187–193
14. Masuda, S., Tomida, Y., Ohta, H., and Takamiki, K. (2007) J. Mol. Biol. 368, 1223–1230
15. Ishikita, H. (2007) J. Biol. Chem. 282, 25240–25246
16. Ishikita, H. (2008) Biochemistry 47, 4394–4402
17. Ishikita, H., and Knapp, E. W. (2006) Biophys. J. 90, 3886–3896
18. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., and Karplus, M. (1983) J. Comput. Chem. 4, 187–217
19. Takahashi, R., Okajima, K., Suzuki, H., Nakamura, H., Ikeuchi, M., and Noguchi, T. (2007) Biochemistry 46, 6459–6467
20. Popovic, D. M., Zniric, A., Zaric, S. D., and Knapp, E.-W. (2002) J. Am. Chem. Soc. 124, 3775–3782
21. Bashford, D., and Karplus, M. (1990) Biochemistry 29, 10219–10225
22. Ishikita, H., Saenger, W., Biesiada, J., Loll, B., and Knapp, E.-W. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 9855–9860
23. Draper, R. D., and Ingraham, L. L. (1968) Arch. Biochem. Biophys. 125, 802–808
24. Kumler, W. D., and Eiler, J. J. (1943) J. Am. Chem. Soc. 65, 2355–2361
25. Tommos, C., and Babcock, G. T. (2000) Biochim. Biophys. Acta 1458, 199–219
26. Tommos, C., Skalicky, J. I., Pilloud, D. L., Wand, A. J., and Dutton, P. L. (1999) Biochemistry 38, 9495–9507
27. Müh, F., Williams, J. C., Allen, J. P., and Lubitz, W. (1998) Biochemistry 37, 13066–13074
28. Ishikita, H., Loll, B., Biesiada, J., Galstyan, A., Saenger, W., and Knapp, E.-W. (2005) FEBS Lett. 579, 712–716
29. Harriman, A. (1987) J. Phys. Chem. 91, 6102–6104
30. Moser, C. C., Keske, J. M., Warncke, F., Farid, R. S., and Dutton, P. L. (1992) Nature 355, 796–802
31. Page, C. C., Moser, C. C., Chen, X., and Dutton, P. L. (1999) Nature 402, 47–52
32. Laan, W., Gauden, M., Yeremenko, S., van Grondelle, R., Kennis, J. T., and Hellingwerf, K. J. (2006) Biochemistry 45, 51–60
33. Ishikita, H., Soudackov, A. V., and Hammes-Schiffer, S. (2007) J. Am. Chem. Soc. 129, 11146–11152

Redox Potentials of the BLUF Domain Cofactors

VOlUMe 283 • NUMBER 45 • NOVEMBER 7, 2008 30623