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BIOPHYSICS

LOV and BLUF Flavoproteins' Regulatory Photoreceptors of Microorganisms and Photosensory Actuators in Optogenetic Systems

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Abstract—In recent years, it has been shown that LOV (light, oxygen, voltage) and BLUF (Blue Light sensing Using FAD) photosensory proteins are functioning as photoreceptors of light-regulated processes not only in eukaryotes but also in numerous prokaryotes. In bacterial photoreceptors, LOV and BLUF domains with attached flavin chromophores are often associated with different effector domains, which possess enzymatic and other functions, constituting modular light-switchable systems. At present, some progress in uncovering the photoactivation mechanisms of such systems has been achieved. They are based on the chromophore photoreaction-induced changes in the photosensory domain structures and subsequent signal transduction to the effector domains. Understanding of the signal transduction principles in LOV and BLUF photosensors is important for designing, on their basis, photo-switchable enzymes and transcriptional systems applied in optogenetics—a new field in cell biology and biotechnology. The structural aspects of signal transduction by light-activated LOV and BLUF photoreceptors and their regulatory functions in bacteria, as well as some recent advances in using LOV and BLUF photosensors as activators in optogenetic systems for regulation of cellular processes are discussed.

Keywords: LOV and BLUF photoreceptors, signal transduction, bacteria, regulation, optogenetic systems, review.

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INTRODUCTION

Photobioregulatory processes are mediated by specialized photoreceptors that receive light signals and transform them into biochemical signaling pathways that cause physiological responses. Plants, fungi, and bacteria have been identified to have several types of regulatory photoreceptors, including sensitive to red phytochromes and sensitive to blue light cryptochromes, phototropins, and other proteins containing LOV and BLUF domains [1].

Photosensory BLUF and LOV domains consist of short (100–140 amino acid residues) α/β-modules that are capable of attaching flavins (FAD or FMN) as chromophores. LOV domain proteins constitute a large group of photoreceptors, originally identified in phototropins of plants, and later in fungi and many bacteria. Much less common, BLUF domain proteins are found so far only in some euglenids and bacteria. BLUF domains differ from LOV domains in terms of characteristics of the secondary structure and a specific type of primary phototransformations of their flavin chromophore. In BLUF domains, FAD in a photoexcited state initiates proton-coupled electron transport between the conserved tyrosine residue and chromophore followed by the reorganization of neighboring hydrogen bonds (Fig. 1). In LOV domains, FMN under the influence of blue light undergoes a photocycle comprising the formation of the thiol adduct between the isoalloxazine ring of flavin and the conservative cysteine residue of the protein. Structural changes arising in the photosensory domains induce modulation of the activity of effector domains or proteins interacting with photoreceptors [1, 2].

Recently, some progress in uncovering the mechanisms of receipt of light and signal transduction in BLUF and LOV photoreceptors has been achieved, and light-dependent regulatory processes mediated by them in bacteria have been identified and studied. There are also advances in the development of optogenetic systems based on LOV and BLUF photosensors and the aimed for light regulation of cellular processes

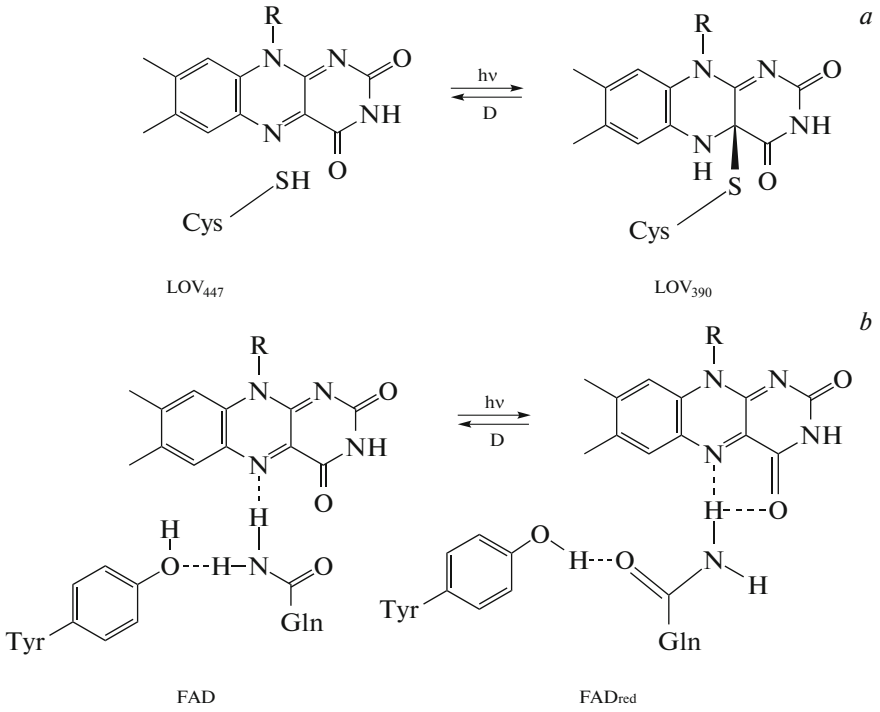

Fig. 1. (*a*) Light-induced (hν) generation in the LOV domain of the reversible in the dark (D) FMN-cysteinyl adduct detectable by the shift of the absorption maximum from 447 nm (LOV_{447}) to 390 nm (LOV_{390}); (*b*) light-induced (hν) reorganization of the network of hydrogen bonds between the FAD and tyrosine/glutamine residues causes generation in the BLUF domain of the reversible in the dark (D) FAD_{red} intermediate.

and functions. This review article focuses on the topical issues of functioning of LOV and BLUF photoreceptors.

Regulatory Functions of BLUF and LOV Photoreceptors

BLUF photosensory proteins. Many bacterial photoreceptors of this class consist only of the BLUF domain with two C-terminal α-helixes; they less often contain BLUF domain-related effector domains (see below), whose activity can be regulated by the photoexcited BLUF domain [3]. Short BLUF photosensors transmit signals and mediate physiological responses through the formation of complexes with signaling proteins, i.e., by light dependent protein–protein interaction [4].

As noted above, in BLUF domains, the FAD photocycle includes a proton-coupled electron transfer from the tyrosine residue to the photoexcited flavin with its transition into a neutral radical. It initiates a reorientation of hydrogen bonds near the flavin chromophore, thereby forming a reversible in the dark intermediate (FAD_{red}) with the absorption spectrum shifted by 10–15 nm to the red region. The hydrogen bond responsible for the formation of the intermediate is formed by conservative glutamine residues, which tautomerize under light excitation of the BLUF domain [5] (Fig. 1). Local changes in the orientation of hydrogen bonds induce conformational changes in the flavin-binding pocket, which, spreading through β5-sheet and C-terminal α-helixes of the BLUF domain, have a modulating effect on the activity of effector domains [6].

According to recent data, the photoexcited BLUF domain regulates the catalytic activity of enzyme effector domains involved in the synthesis and breakdown of second messengers. In the photosensory protein from bacteria *Klebsiella pneumonia*, BlrP1 (blue-light regulated phosphodiesterase) (Table 1), the BLUF domain modulates c-di-GMP-phosphodiesterase activity of its covalently attached EAL domain [7]. Crystallographic analysis of BlrP1 shows a dimeric nature of EAL domains with a conservative contact created by a composite coil formed of short helixes of each protomer. Photosensory BLUF domains are located close to the site of dimerization, and light absorption by the BLUF domain of one subunit of the antiparallel homodimer stimulates phosphodiesterase activity of the EAL domain of another subunit [7]. The regulation of the activity of the EAL domain by the BLUF sensor is carried out through a two-way allosteric communication between the photoinduced structural changes and the active center of EAL [8]. Light signals from both the BLUF domains are combined in a conservative dimerization area of

Table 1. Primary photoinduced processes and regulatory functions in individual bacterial BLUF photoreceptors

Type of photoreceptor	Primary photoinduced processes in BLUF photosensors	Structural bases of signal transduction	Regulation of cellular processes and functions
PixD (BLUF)	Proton-linked electron transfer from the Tyr residue to photoinduced flavin with its transition into a neutral radical; reorientation of hydrogen bonds near chromophore and the formation of intermediate FAD_{red}; conformational changes in C-terminal α-helixes of the BLUF domain [1, 2, 6]	Conformational change in PixD and breakdown of the oligomeric complex of PixD and regulatory protein PixE [11]	Control of phototaxis in *Synechocystis* sp. [11]
$BlrP_1$ (BLUF – EAL)		Allosteric bilateral association between structural changes in the BLUF domain and active site of EAL in dimeric $BlrP_1$ [8]	Stimulation of phosphodiesterase activity in the EAL domain in *K. pneumonia* [7]
YcgF (BLUF – EAL)		Dissociation of the complex of dimer YcgF and transcriptional repressor YcgE, followed by its release from the operon [13]	Formation of biofilm by *E. coli* cells [13]
AppA (BLUF – SCHIC)		Allosteric structural changes in the complex AppA–PpsR–DNA. Interaction of the complex AppA–PpsR with sites of PpsR repressor binding on the DNA prevents formation of the PpsR–DNA repressor complex [14]	Activation of expression of photosynthetic genes. Synthesis of components of the photosynthesis apparatus in *R. sphaeroides* [6, 14]
bPAC (BLUF – AC)		Not investigated	Increased activity of adenylyl cyclase (AC) and cAMP levels in *Beggiatoa* sp. [9]

EAL and are transmitted to the active center of the enzyme domain, causing its activation.

The photosensory protein identified in bacteria *Beggiatoa* sp. and containing the BLUF domain associated with adenylyl cyclase, bPAC (photoactivated adenylyl cyclase) [9] (or BlaC [10]), revealed to have a light-induced increase in the activity of this enzyme and cellular cAMP levels (Table 1). Of interest are the data on the conversion of BlaC to guanylate cyclase in terms of the constructed model of the nucleotide cyclase domain, in which several amino acid residues are replaced. The triple mutant, BlgC, has photoactivated guanylate cyclase in vitro. Blue light causes a significant increase in cGMP levels in mutant *Escherichia coli* expressing BlaC [10].

There were recent reports of photophysiological functions of BLUF photoreceptors that control the biological responses, such as phototaxis (Pix D in *Synechocystis* sp.), the formation of biofilms (YcgF in *E. coli*, PapB in *Rhodopseudomonas palustris*), virulence (BlsA in *Acinetobacter baumannii*), and synthesis of photosynthetic apparatus components (AppA in *R. sphaeroides*) [6]. The action of the marked photoreceptors is based on light dependent protein–protein interactions. In recent studies, new information about the molecular details of these light-controlled processes has been obtained.

The short BLUF photosensor PixD (Table 1), which participates in control of phototaxis in *Synechocystis* sp., reacts in the dark with a regulatory protein PixE, which induces the formation of an oligomeric complex composed of ten subunits of PixD and five subunits of PixE [11]. Light excitation of PixD causes conformational changes accompanied by breaking the complex into the dimers PixD and monomers PixE [4]. It is assumed that this light-induced process triggers a signaling cascade that controls phototaxis in bacteria [11].

In the other short BLUF protein, PapB from *R. palustris*, the photophysiological function is associated with negative regulation of the formation of biofilms. The photoresponse is based on the interaction of PapB with c-di-GMP-specific phosphodiesterase PapA, whose activity increases under light excitation of the photosensor. PapB, unlike PixD discussed above, which forms a complex with protein PixE only in the dark [11], reacts with PapA in the light. This reflects differences in the mechanisms of interaction between the two photosensors with the corresponding proteins in the signaling cascades that control various photobiological processes, such as phototaxis or the formation of biofilms [6, 12].

The BLUF protein of *E. coli* YcgF (Table 1) contains the EAL domain. However, unlike photoreceptor BlrP1 that is similar to it in terms of the domain organization (see above), YcgF does not bind to c-di-GMP and its EAL domain does not have the light-induced phosphodiesterase activity. It was established that YcgF functions as an antagonist of the transcriptional regulator YcgE [13]. The action of YcgE as a repressor is carried out through its binding to promoters in the operon encoding the proteins that can activate the biofilm matrix materials. Photoexcited YcgF temporarily forms homodimers, causing the dissociation of the complex YcgE–YcgF and releasing the repressor from

the operon. This indicates the photosensory function of YcgF in the modulation of the biofilm formation by *E. coli* cells [13].

The results of that study suggest that blue light in the BLUF protein with the degenerated EAL domain can activate a biological function that is different from the enzymatic due to the light dependent protein–protein interaction.

The photoreceptor of bacteria *R. sphaeroides* AppA (Table 1), a light and redox regulator of photosynthetic gene expression, consists of the BLUF domain and redox sensor domain SCHIC (Sensor Containing Heme Instead of Cobalamin) [6]. Light and oxygen are received by means of the AppA–PpsR-regulatory system, where PpsR is a repressor of photosynthetic genes containing the (HTH)-motif (helix-turn-helix-motif) for binding to the DNA, and AppA is an antirepressor capable of forming a noncovalent AppA–$PpsR_2$-complex through the SCHIC-domain.

A recent study based on the analysis of crystal structures of both the proteins and their complex showed that light activation of AppA alters the effector region of PpsR within the complex. In addition, it demonstrated the formation of a photosensitive ternary complex AppA–PpsR–DNA, through which the signal can be transmitted through allosteric structural changes. According to the proposed mechanism, the photomodified complex AppA–$PpsR_2$ interacts with sites of PpsR binding on the DNA, thus preventing the formation of the PpsR–DNA-repressor complex, leading to activation of photosynthetic gene expression [14].

LOV photosensory proteins. The photosensory function of the LOV domain was first revealed in the identification of phototropin containing two FMN-binding LOV domains. Of these, LOV2 is the main in the regulation of photoreceptor activity; it is coupled by the Jα-helix with the effector serine-threonine kinase domain. The photocycle of FMN with a maximum absorbance at 447 nm (LOV_{447}) includes formation of the FMN-cysteinyl adduct (LOV_{390}) reversible in the dark (Fig. 1). This form is a signal state of the photoreceptor, which is associated with destructurization of the Jα-helix induced by the adduct formation and with a consequent increase in the kinase activity [1, 2].

It is important to note that the same principle of light activation of LOV proteins (except for some parts of the structural mechanisms of signal transduction) remained preserved among distant phylogenetic groups of organisms, including fungi and bacteria [1, 2, 15, 16]. All the LOV photoreceptors of fungi and bacteria contain one LOV domain, and it is closely related to various effector domains in many prokaryotes, forming the modular systems whose activity can be regulated by light.

In fungi, the LOV base photoreceptor system is basically represented by two FAD-containing LOV domain proteins: WC-1 (White Collar) and VVD, mediating the light regulation of launching and the circadian rhythm phase in *Neurospora crassa* [17]. The photosensory protein WC-1 is a transcription factor, which forms a heterodimeric complex WCC together with another transcription factor WC-2. Photoactivation of WC-1 in this complex causes homodimerization of WCC [18]. The WC-complex activated by light induces transcription of many light-inducible genes, including the gene encoding the VVD photosensor, which is necessary for the proper regulation of circadian rhythms [17, 19]. Induced by the photoadduct formation, the conformational changes in LOV/VVD are accompanied by homodimerization of VVD and its interaction with WCC [20], determining the formation of negative feedback to WCC in the control of circadian gene expression.

The modern model of the antagonistic action of VVD with respect to the WC-complex is based on the direct interaction and competitive heterodimerization between VVD–VVD/LOV and WC–1/LOV [18, 19]. The formation of the heterodimer VVD : WC-1 makes the organism adaptable to light by blocking the originally initiated wave of gene expression under continuous lighting, thus preventing the overexpression of WCC-transcribed genes [18, 19, 21].

In recent years, bacterial LOV photoreceptors containing various effector domains associated with the photosensory domain were revealed to have light-dependent activity; some of them also perform photobiological functions [2, 15]. LOV histidine(H)-kinases contained in bacteria *Caulobacter crescentus* (LovK), *Brucella abortus* (LOV HK) (Table 2), and *Pseudomonas syringae* show photocycles of flavin chromophore typical for LOV domains, which are accompanied by changes in the tertiary structure and autophosphorylation of kinases in vitro, as well as the transfer of the phosphate group to the corresponding proteins—response regulators (RRs) (Table 2). Thus, in LOV kinases, RRs constitute a two-component system of light signal transduction [3]. Photoactivation of LovK and LOV HK causes physiological responses in bacteria: *B. abortus* were observed with a 10-fold increase in cell proliferation in macrophages and *C. crescentus* with a sharp increase in cell adhesion [15].

In cyanobacteria *Synechococcus elongates*, the LOV domain associated with GGDEF-EAL domains mediates photoinduced regulation of the phosphodiesterase activity of EAL in vitro [22] (Table 2). Based on this fact, it is assumed that the blue light activated LOV domain can control the level of cellular c-di-GMP, which is involved as a second messenger in the regulation of several physiological functions (motility, virulence, etc.).

In bacteria *Bacillus subtilis*, the LOV STAS (sulfate transporter/antisigma-factor antagonist)-protein YtvA (Table 2) functions as a photoreceptor in vitro and in vivo. The STAS-domain determines the blue light modulated ability of YtvA to bind GTP in vitro,

Table 2. Primary photoinduced processes and regulatory functions in some selected bacterial LOV photoreceptors

Type of photoreceptor	Primary photoinduced processes in LOV domains	Structural bases of signal transduction	Regulation of cellular processes and functions
LovK (LOV -H-kinase)	Photoinduced FMN undergoes a photocycle that involves formation of a thiol adduct between the isoalloxazin ring of chromophore and Cys residue of the protein; conformational changes in the LOV domain initiated by reorientation of the glutamine residue and a change in hydrogen bonds involving peripheral amino acid specific signal transmitters [1, 2, 15]	Changes in the tertiary structure of the H-kinase domain [2, 15]	Phosphorylation of H-protein kinase and transphosphorylation of the response regulator (RR) protein. Increased cell adhesion in *C. crescentus* cells [3, 15]
LOV -HK (LOV -H-kinase)		Changes in the tertiary structure of the H-kinase domain [2, 15]	Phosphorylation of H-protein kinase and transphosphorylation of the response regulator (RR) protein. Increased cell proliferation in *B. abortus* [3, 15]
YtrA (LOV – STAS)		Conformational changes in the LOV dimer cause rotation of two monomers by 4–5° relative to each other [26]. The impact of these structural changes on the conformation of the STAS-domain has not been investigated	GTP binding by the STAS-domain. Upregulation of the transcription factor of the general stress of σB in *B. subtilis* [23]
LOV – GGDEF-EAL		Not investigated	Regulation of phosphodiesterase activity of EAL in LOV photoreceptor from *S. elongates* in vitro [22]

which is a second messenger in response to stress in *B. subtilis*. In addition, the photoactivated YtvA in vivo acts as a positive regulator of the transcription factor of the general stress of σB [23]. Since the mutations that violate the binding of GTP in the STAS-domain or cross-domain distribution of the light-induced signal in the photoreceptor inhibit the blue light activated σB-dependent transcription in vivo, it is believed that binding of GTP is necessary for the manifestation of functional activity of YtvA [23–25].

Studies of the structural changes occurring in the LOV domain of YtvA, which is connected to the effector STAS-domain through the Jα-helix, showed that, unlike those in phototropins, they do not affect the destructurization of the Jα-helix [24, 26]. This is due to differences in the quaternary structure and orientation of Jα, whose domain forms a coiled-coil configuration in the YtvA-LOV [23]. Isolated YtvA-LOV domains are constitutive dimers whose monomers upon photoactivation rotate relative to each other by 4–5° [26]. In the propagation of light-induced structural rearrangements in YtvA-LOV, as well as in LOV domains of phototropin and VVD, the primary role is played by the glutamine residue undergoing reorientation in its side chain.

Analysis of the data obtained in the study of the structural aspects of signal transmission in LOV photoreceptors indicates that in bacterial, just as in eukaryotic, LOV proteins, the light signal transduction in the initial stages occurs in terms of the common mechanism. It is based on the conformational changes of the β-sheet of the LOV core, which are induced by the photoadduct formation. An important role in their initiation is played by the conserved glutamine residue, which directly interacts with photoactivated flavin chromophore. Restructuring of glutamine induces changes in hydrogen bonds involving several peripheral amino acid residues that vary in different LOV proteins and act as specific signal transmitters (Table 2). Structural arrangements of the subsequent steps of signal transmission, which allow the LOV domains to regulate the activity of effector domains, also vary [15].

Application of LOV and BLUF Photosensors in Optogenetic Systems

Optogenetics is a new field of cell biology that combines optical and genetic approaches to regulate cellular processes by light using photosensory proteins. In recent years, optogenetics has become a key biotechnology as genetically encoded photosensory activators may be functionally introduced into cells of any type, where, after light activation occurring with high spatiotemporal precision, they are capable of inducing the gene expression regulation, enzymatic activity, and other biological functions [27].

LOV and BLUF photosensors have key properties ideal for use in optogenetics. They have a small size and, as chromophores, use photochemically active flavin cofactors present in all cell types. Furthermore, the ability of LOV and BLUF photosensors to form functional modular structures with effector domains

provides a basis for designing their combinations with other proteins/enzymes whose activity is subject to light-induced allosteric control [28–31].

A number of studies for the allosteric control of protein functions used the LOV2-domain of vegetative phototropin, which undergoes significant structural changes in photoexcitation. LOV2-Jα joins the target protein so that conformational changes in LOV2 induce conformational changes in the protein. By this mechanism, LOV2-Jα causes light dependent regulation of the activity of several enzymes, including the expression in animal cells [32–34].

Of particular interest are the data on light regulation of GTPase Rac1 in fibroblasts expressing hybrid LOV2-Jα-Rac1-protein [33]. It is shown that, in the dark, LOV2 sterically inhibits the Rac1 activity by blocking its binding to effector proteins. The action of focused laser light causes destructurization of the Jα-helix, removing steric inhibition and, thereby, providing the interaction of Rac1 with effector molecules. Since Rac1 is a key protein that regulates cytoskeleton dynamics, the obtained results show that light exposure can be used to remotely control the mobility of cells expressing the photoactivatable Rac1 [33].

The photoregulatory action of LOV2-Jα is also shown for the DNA-binding activity of the fusion protein enabling a bacterial repressor of tryptophan TrpR [35]. Light-induced binding is based on disconnecting the Jα-spiral from the LOV core followed by a conformational change and activation of the effector domain (TrpR).

Note that, in addition to using phototropin LOV2 as a light-dependent regulator of biological processes, this domain was used as a basis for the creation of fluorescent reporter molecules [2, 27, 30]. They have been successfully used to control populations of bacteria in anaerobic conditions and viral infections of plants. In these cases, the LOV domain reporter with sufficiently intense green fluorescence of flavin chromophore exceeds GFP (green fluorescent protein), which requires the presence of oxygen for the formation of fluorescent chromophore. Although the fluorescence of flavin chromophore disappears during the photochemical cysteinyl adduct formation, this process can be prevented by replacing the cysteine residue for alanine and serine, which provides a constantly fluorescent molecule.

Along with LOV2-Jα for optogenetic applications, bacterial LOV photosensors have also been studied. Based on the property of bacterial LOV domain photoreceptors to incorporate H-regulated kinase as an effector domain, synthetic light dependent H-kinase has been designed in which the nonphotosensory PAS-domain from the oxygen sensor FixL from bacteria *Bradyrhizobium japonicum* is replaced for the LOV domain of the photoreceptor YtvA from *B. subtilis* [36]. Further application of this fusion protein is associated with engineering of plasmids for light dependent induction (pDawn) or repression (pDusk) of bacterial genes [37].

Recently, with the help of genetic engineering, other light dependent transcription systems have been created. Very promising is the design consisting of the LOV photosensory VVD protein from *N. crassa* and the DNA-binding and activation domains; it is used to control gene expression in animal cells [38]. Also of great interest is the study of the LOV domains recently identified in the genomes of bacteria isolated from different habitats, including those extreme [39]. The combination of these photosensory modules with different effector domains can provide a wide range of designs for optogenetic systems.

Research of BLUF photosensors for use in optogenetics gave rise to works with PAC from *Euglena gracilis*, a photoreceptor containing cAMP-cyclase. Expressed in neurons of animals, the BLUF photoreceptor causes light dependent activation of the cyclase followed by a rapid increase in cAMP levels, which, by means of a cascade of phosphorylation, regulates gene expression and a number of biological processes [40].

The above-discussed BLUF adenylyl cyclase (bPAC/BlaC) of bacteria *Beggiatoa* sp. is promising for future applications in optogenetics. Indeed, light activated bPAC is highly efficient in cAMP-regulated processes, including behavioral responses, in integration with neurons of some animals [9]. In addition, BlaC can be converted by mutation into photoactivatable cGMP-cyclase (BlgC), extending its scope [10]. As is known, the products of adenylyl and guanylyl cyclases, cAMP and cGMP, are versatile second messengers that regulate many processes in organisms. Therefore, the ability of bacterial PAC to control, under light exposure, the cAMP/cGMP synthesis coupled with their small size and high degree of light activation opens prospects for using these BLUF cyclase photoreceptors in optogenetics.

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