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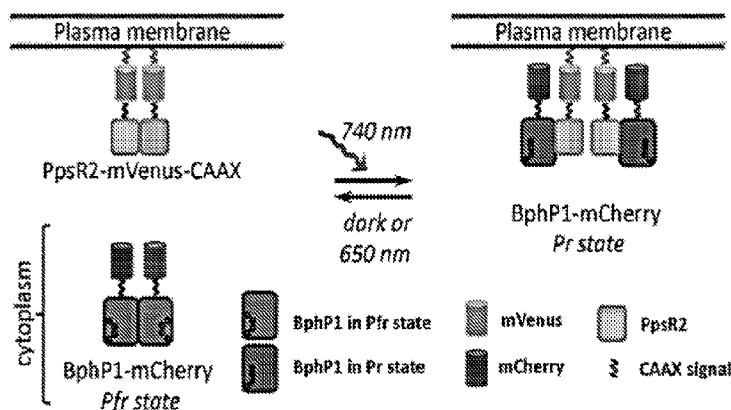


FIG. 2A

(57) Abstract: A novel optogenetic system, including constructs and methods, is provided based on the interaction of *Rhodospseudomonas palustris* BphP1 and *Rhodospseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.

AN OPTOGENETIC SYSTEM BASED ON BACTERIAL PHYTOCHROME
CONTROLLABLE WITH NEAR INFRA-RED LIGHT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/235,828, filed October 1, 2015, the contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Throughout this application various patents and other publications are referred to by number in parenthesis. Full citations for the references may be found at the end of the specification. The disclosures of these references and all patents, patent application publications and books referred to herein are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0003] This invention was made with government support under grant numbers GM073913, GM108579 and CA164468 awarded by the National Institutes of Health. The government has certain rights in the invention.

[0004] Precise spatiotemporal control of biochemical processes in live cells and animals will advance basic biology and biomedicine. A promising approach to provide such control is optogenetics, a field of biology focused on controlling desired cell functions utilizing light as an activation signal. Among several directions of optogenetics, the important one is a modulation of protein-protein interactions (PPIs) that regulate specific cellular pathways. The PPI optogenetic tools consist of fusions between the target proteins and the chromophore-containing protein domains capable of light-induced homo- or heterodimerization. Light-dependent genetically encoded systems exhibit several advantages over pharmacologically-based PPI constructs due to their non-invasiveness, high PPI activation rates, and absence of side effects caused by drugs.

[0005] Several genetically encoded PPI optogenetic systems have been engineered using different families of photoreceptors, such as LOV (light/oxygen/voltage) domains, BLUF (blue light utilizing FAD) domains (1), cryptochromes and subclass of phytochromes, such as plant phytochromes (1, 2, 3). Although use of these systems in

cultured mammalian cells has been shown, their application in living mammals could be complicated because of several factors.

[0006] First, a range of wavelengths where light exhibits maximum depth of tissue penetration due to low light-scattering and minimal absorbance of melanin, hemoglobin and water lies between 650 and 900 nm, and is called a near infra-red (NIR) tissue transparency window (4). The LOV, BLUF and cryptochrome-based optogenetic systems sense light in blue region of spectrum. Only phytochrome photoreceptors are activated with far-red or NIR light. Second, different phytochrome subfamilies incorporate different chromophores. Phytochromobilin and phycocyanobilin serve as chromophores in plant phytochromes, whereas bacterial phytochromes incorporate biliverdin IX α (BV) (4, 5, 6). These chromophores are products of heme degradation. A chromophore is autocatalytically and covalently attached via a thioether bond between the A-ring of tetrapyrrole and conserved cysteine residue of the phytochromes (6-8). Importantly, among these tetrapyrroles, BV exhibits the most NIR-shifted spectra of its absorption. Third, a use of the plant phytochrome based PPI optogenetic systems is limited by the requirement of exogenously supplied phycocyanobilin chromophore, which is unavailable in eukaryotic cells and organisms, including mammals (9). In contrast, the BV chromophore utilized by bacterial phytochromes is abundant in animal cells, and thus its exogenous supply is not required (10). This latter feature was used to engineer bacterial phytochromes into several types of NIR probes for mammalian tissues including permanently fluorescent proteins (11), photoactivatable fluorescent proteins (12) and PPI reporters (13).

[0007] Bacterial phytochromes are natural dimeric photoreceptors that control gene expression and second messenger signaling in bacteria in response to NIR light (14). They consist of a photosensory core module and an output effector module, which is represented by histidine kinase in canonical bacterial phytochromes, and by other domains in non-canonical ones. Spectral properties are defined by a photosensory core module where the protein-chromophore interaction occurs (5, 15, 16). Within a chromophore-binding pocket BV can adopt two conformational states, termed Pr and Pfr and absorbing far-red and NIR light, respectively. Isomerization of BV chromophore between Pr and Pfr states causes conformational changes in the protein backbone and activation of an effector module (17). Replacement of natural effector modules with phosphodiesterase and adenylyl cyclase allows for modulating levels of cyclic nucleotides (18-20). Most of bacterial phytochromes undergo photoconversion from a Pr state to the Pfr state with 660-700 nm light. However,

there is a small group, termed bathyphytochromes, which adopts Pfr as a ground state and undergoes Pfr→Pr photoconversion upon 740-780 nm light irradiation (21). Upon photoactivation, bacterial phytochromes can be converted back to the ground state either with light, which is absorbed by the activated state, or by means of thermal relaxation in darkness.

[0008] The present invention address the need for improved methods for controllably inducing protein-protein interactions or controllably inducing gene expression using convenient near infrared light.

SUMMARY OF THE INVENTION

[0009] An isolated nucleic acid is provided encoding (i) a protein having the sequence of *Rhodopseudomonas palustris* BphP1 and (ii) a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*. In an embodiment, the nucleic acid is a cDNA.

[0010] Also provided is an isolated nucleic acid encoding (i) a protein having the sequence of *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*. In an embodiment, the nucleic acid is a cDNA.

[0011] Also provided is an isolated nucleic acid comprising a first and second portion, the first portion encoding a protein having the sequence of *Rhodopseudomonas palustris* BphP1 and a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*, and the second portion encoding a protein having the sequence of *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*.

[0012] A cell is provided, wherein the cell is not in a human being, transformed with the nucleic acid of as described herein.

[0013] A fusion protein is provided comprising a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and a second protein or peptide. A fusion protein is provided comprising a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 and a second protein or peptide.

[0014] A method is provided for inducing interaction of a first protein with a second protein in a system, the method comprising providing a system comprising (a) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) the first protein, and (b) a fusion

protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) the second protein, and (c) an amount of biliverdin; and

irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof. In embodiments, the amount of biliverdin is present endogenously, added exogenously or produced enzymatically.

[0015] Also provided is a method for inducing translocation of a predetermined protein to a predetermined location in a cell comprising

providing a system comprising (a) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) a first protein, and (b) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) a second protein, and (c) an amount of biliverdin, wherein one of the first and second proteins is the predetermined protein and wherein the other of the first and second proteins is a protein that preferentially locates to the predetermined location in a cell; and

irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.

[0016] A kit is provided comprising any of the nucleic acid, fusion protein and/or system described herein and instructions for use.

[0017] The nucleic acids, fusion proteins and methods described herein are applicable *mutatis mutandis* to the non-canonical BphP and PpsR2 not present in *Rhodopseudomonas palustris*.

[0018] Additional objects of the invention will be apparent from the description which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Fig. 1a-1i: Spectral properties of BphP1 and characterizations of BphP1–PpsR2 interaction *in vitro*. (a) Absorbance spectra of BphP1 in the Pfr state (solid line), after photoconversion to the Pr state with 740/25 nm (dashed line) and after conversion back to the Pfr state with 636/20 nm (dotted line). (b) Action spectrum of the Pfr→Pr photoconversion measured as the relative decrease of Pfr absorbance detected at 780 nm upon irradiation with light of specific wavelength. (c) Dependence of the half-time of light-

induced Pfr→Pr photoconversion on the intensity of 740/25 nm light (n=3, error bars are s.e.m.). (d) Absorbance of BphP1 in the Pfr state during repeated illumination with 740/25 nm light followed by dark relaxation. (e) Dependence of the half-time of light-induced BphP1 Pr→Pfr photoconversion on the intensity of 636/20 nm light (n=3, error bars are s.e.m.). (f) Absorbance of BphP1 in the Pfr state during repeated illumination cycles with 740/25 nm light and then with 636/20 nm light. Absorbance in (b), (d) and (f) was measured at 780 nm. (g) Time-course of FRET changes during BphP1 photoswitching between the Pr and Pfr states either together with PpsR2-mRuby2 (solid line) or together with mRuby2 control (dashed line). Solid arrows correspond to 740/25 nm illumination. Dashed arrows correspond to 636/20 nm illumination. (h) Half-time of dark relaxation of BphP1 (Pr→Pfr transition) in the presence of various quantities of PpsR2-mRuby2 (n=3, error bars are s.e.m.). (i) Reversible dark relaxation cycles from the Pr to the Pfr state of a BphP1 and PpsR2 mixture with 8:1 ratio. Arrows correspond to 740/25 nm illumination.

[0020] Fig. 2a-2f: Light-induced re-localization of BphP1 to the plasma membrane. (a) A model for the light-induced interaction between cytoplasmic BphP1-mCherry and membrane-bound PpsR2-mVenus-CAAX. (b) Fluorescence images of a HeLa cell co-expressing PpsR2-mVenus-CAAX (green) and BphP1-mCherry (red) before illumination (left), after 3 min of 740/40 nm illumination with 0.9 mW cm^{-2} (middle), and after 20 min of dark relaxation (right). Bars, 10 μm . (c) Intensity profile of BphP1-mCherry fluorescence of the cell in (b) marked with a white line before (solid line) and after (dashed line) 3 min of 740/40 nm illumination. (d) Intensity profile of BphP1-mCherry fluorescence of the cell in (b) marked with a white line after 3 min of 740/40 nm illumination (dashed line) and after subsequent 24 min in darkness (dotted line). (e) Time-course of BphP1-mCherry fluorescence intensity in the cytoplasm during three cycles of 3 min of 740/40 nm irradiation with 0.2 mW cm^{-2} followed by 30 min in darkness (n=5; white lines represent mean $\pm$ s.e.m.). mCherry fluorescence was measured every 15 s during 740/40 nm light illumination and every 180 s during dark relaxation. (f) Time-course of the BphP1-mCherry fluorescence intensity in the cytoplasm during three cycles of 3 min of 740/40 nm irradiation with 0.2 mW cm^{-2} followed by 3 min of 650/10 nm irradiation with 0.35 mW cm^{-2} (n=5; white lines represent mean $\pm$ s.e.m.). mCherry fluorescence was measured every 15 s. All imaging was performed at 37°C using an epifluorescence microscope. Light intensities were measured at the back focal plane of a 60 $\times$ 1.35 NA objective lens.

[0021] Fig. 3a-3d: Light-induction of cellular cytoskeletal re-arrangements. (a) A model for the light-induced recruitment of cytoplasmic BphP1-mCherry-DHPH to membrane-bound PpsR2-mVenus-CAAX that results in cytoskeletal rearrangements of mammalian cells. (b, c) Fluorescence images of HeLa cells co-expressing either (b) BphP1-mCherry-DHPH (red) and PpsR2-mVenus-CAAX (green) or (c) BphP1-mCherry-DHPH (red) and mVenus-CAAX control (green) before (top) and after (bottom) 30 min irradiation with 740/40 nm light of 0.2 mW cm^{-2} (first 3 min continuous irradiation with 740/40 nm followed by 27 min of pulse illumination 15 s On 45 s Off). Bars, 10 μm . (d) Time dependent size changes of HeLa cells, which co-express either BphP1-mCherry-DHPH and PpsR2-mVenus-CAAX (n=5; error bars are s.e.m.), or BphP1-mCherry-DHPH and mVenus-CAAX control (n=5; error bars are s.e.m.), during irradiation with 740/40 nm light of 0.2 mW cm^{-2} (first 3 min continuous irradiation with 740/40 nm followed by 27 min of pulsed illumination 15 s On and 45 s Off). Fluorescent images were taken every 15 s during continuous and every 60 s during pulsed irradiation with 740/40 nm. All imaging was performed at 37°C using an epifluorescence microscope. The light power densities were measured at the back focal plane of a 60× 1.35 NA objective lens.

[0022] Fig. 4a-4d: Recruitment of BphP1 to the nucleus and light-induced transcription activation. (a) Fluorescence images of HeLa cells co-expressing NLS-PpsR2-mVenus and BphP1-mCherry incubated either in darkness or under irradiation with 740/25 nm pulsed light (30 s On and 180 s Off) of 0.2 mW cm^{-2} . Images were acquired at 37°C using an epifluorescence microscope. Bars, 10 μm . (b) Model of the light-inducible transcription activation system. NIR light converts BphP1 into the Pr state and induces heterodimerization with PpsR2. Nuclear localization signal (NLS) fused with PpsR2 facilitates translocation of the heterodimer to the nucleus where BphP1 fusions interact with *tetO* DNA repeats via fused TetR. VP16 fused with PpsR2 recruits the transcription initiation complex and triggers transcription of a reporter gene. (c) Kinetics of the light-to-dark ratio of SEAP signal detected in culture media of HeLa cells bearing BphP1-mCherry-TetR co-transfected with NLS-PpsR2-VP16 producing plasmid and pTRE-Tight-SEAP ($7\times\text{tetO}$) reporter plasmid after 48 h (n=3; error bars are s.e.m.). (d) Termination of SEAP transcription in HeLa cells with the same constructs as in (c) illuminated with 740/25 nm followed by 60 h of darkness or followed by 12 h of 636/25 nm illumination and then by 48 h of darkness. Data were normalized to SEAP signal of cells irradiated with 740/25 nm for 72 h; the signal from non-irradiated cells was subtracted (n=3; error bars are s.e.m.).

[0023] Fig. 5a-5h: Light-induced transcription activation in mice. (a) Rluc8 bioluminescence detected in mice with subcutaneously injected HeLa cells stably expressing BphP1-mCherry-TetR and co-transfected with the NLS-PpsR2-VP16 producing plasmid and pTRE-Tight-Rluc8 reporter plasmid kept either in darkness (top) or illuminated with 740/25 nm light of 1 mW cm^{-2} (bottom) for 48 h. (b) Rluc8 signals detected in dark-treated animals and in illuminated animals shown in (a) ($n=3$; error bars are s.e.m.). (c) Rluc8 signals in mice with subcutaneously injected HeLa cells co-transfected with pGAVPO plasmid encoding GAL4(65)-VVD-VP16 and pU5-Rluc8 reporter plasmid kept in darkness (top) or illuminated with 470/15 nm light of 1 mW cm^{-2} (bottom) for 48 h. (d) Rluc8 signals detected in the dark-treated and illuminated animals shown in (c) ($n=3$; error bars are s.e.m.). (e) Rluc8 signals detected in mice after hydrodynamic co-transfection with pKA-207I10 (encoding NLS-PpsR2-VP16-IRESv10-BphP1-mCherry-VP16) (50 μg) and pTRE-Tight-Rluc8 (5 μg) plasmids. Mice kept in darkness (top) or illuminated with 740/25 nm light of 5 mW cm^{-2} (bottom) for 48 h. (f) Kinetics of the Rluc8 expression in mice shown in (e) kept in darkness or illuminated for 72 h ($n=3$; error bars are s.e.m.). (g) Rluc8 signals detected in mice after hydrodynamic co-transfection with pGAVPO (50 μg) and pU5-Rluc8 (5 μg) plasmids. Mice kept in darkness (top) or illuminated with 470/15 nm light of 5 mW cm^{-2} (bottom) for 24 h. (h) Kinetics of Rluc8 expression in mice shown in (g) kept in darkness or illuminated for 72 h ($n=3$; error bars are s.e.m.).

[0024] Fig. 6(a) - 6(b): Kinetics of BphP1 photoconversion *in vitro*. (a) Dependence of the Pfr $\rightarrow$ Pr photoconversion on the 740/25 nm light intensity. The gradual increase in absorbance of the Pr state was observed for all tested light intensities. Kinetic curves can be fitted with monoexponential functions ($R^2 \geq 0.958$ for all curves). (b) Dependence of the Pr $\rightarrow$ Pfr photoconversion on the 636/20 nm light intensity. The achievable with 636 nm light 0.8 value of the Pfr ground state is likely represents an equilibrium between the Pfr and Pr states because 636/20 nm light is also absorbed by the Pfr state, thus causing its partial photoconversion back to the Pr state.

[0025] Fig. 7(a) - 7(c): FRET-based approach for characterization of BphP1-PpsR2 interaction. (a) Overlap of the emission spectrum of mRuby2 (dashed lines) and absorbance spectrum of BphP1 in the Pr state. (b) Changes of FRET (solid line) during Pfr $\rightarrow$ Pr photoconversion of BphP1-mRuby2 (dashed line). (c) Dependence of the FRET changes on power of 740/25 nm illumination of the PpsR2-mRuby2 and BphP1 mixture.

[0026] Fig. 8: Overlap of wavelengths used for mVenus and mCherry excitation with action spectrum of Pfr→Pr photoconversion. The lighter gray box outlines the wavelength range transmitted by 523/20 nm filter used for mVenus excitation, and the darker gray box outlines the wavelength range transmitted by 570/30 nm filter used for mCherry excitation. The action spectrum of the Pfr→Pr photoconversion was measured as the relative decrease of Pfr absorbance detected at 780 nm upon irradiation with light of specific wavelength (open squares).

[0027] Fig. 9: Initial recovery of the mCherry cytoplasmic intensity under 650 nm light and in darkness. The linear fitting of the mean recovery kinetics of mCherry fluorescence in cytoplasm of cells right after 740/40 nm illumination either in darkness (black line; n=5) or after turning on 650/10 nm light (red line; n=5). The HeLa cells were co-expressing BphP1-mCherry and PpsR2-mVenus-CAAX constructs. The tangent of the angles that correspond to the initial mCherry recovery rates, are 0.044 % s⁻¹ and 0.072 % s⁻¹, respectively.

[0028] Fig. 10: The relative decrease in mCherry cytoplasmic fluorescence and relative increase in cell area in 5 min periods from the beginning of cell illumination with 740/40 nm. The HeLa cells co-expressed the BphP1-mCherry-DHPH and PpsR2-mVenus-CAAX constructs (n=5, error bars are s.e.m.). The initial (during the first 5 min) relative decrease in the BphP1-mCherry-DHPH in cytoplasm was more than 2-fold larger than that of the increase in the cell area. No substantial difference between these two characteristics was observed in the later time periods.

[0029] Fig. 11: Analysis of light-induced recruitment of BphP1-mCherry to nucleus. mCherry nucleus-to-cytoplasm intensity ratio in HeLa cells stably expressing BphP1-mCherry transfected with pCMV-160 (dashed bars) or not transfected (solid bars). Cells were kept in darkness or irradiated with 740/25 nm light (30 s On, 180 s Off) of 1 mW cm⁻² for 24 h (n=20, error bars are s.e.m.). Cell imaging was performed using an epifluorescence microscope at 37°C.

[0030] Fig. 12a-12c: Characterization of light-inducible activation of SEAP expression using the TetR-*tetO* system. (a) SEAP signal detected in culture media of HeLa cells bearing BphP1-mCherry-TetR co-transfected with pCMV-104 and pTRE-Tight-SEAP (7×*tetO*) reporter plasmid after 48 h in darkness or under 740/25 nm light (n=3; error bars are s.e.m.). (b) Light-to-dark ratio of the SEAP signal shown in (a). (c) The kinetics of SEAP accumulation in culture media of illuminated with 740/25 nm light (dark red) and

dark-treated (gray) HeLa cells stably expressing BphP1-mCherry-TetR, transiently co-transfected with pTRE-Tight-SEAP and pCMV-104 plasmids with a plasmids ratio of 1:5 (n=3, error bars are s.e.m.).

[0031] Fig. 13: Dependence of light-induced SEAP expression level on the power of 740 nm light. The light-induced expression of SEAP from the pTRE-Tight-SEAP reporter plasmid. SEAP signal was detected in HeLa cells with BphP1-mCherry-TetR, pTRE-Tight-SEAP and pCMV-104 plasmids. Cells were kept in darkness or under 740/25 nm light at different intensities (n=3, error bars are s.e.m.).

[0032] Fig. 14: Light-induced expression of EGFP from the pTRE-Tight-EGFP reporter plasmid. (a) Light-induced expression of EGFP from the pTRE-Tight-EGFP reporter plasmid. EGFP signal was detected in HeLa cells bearing BphP1-mCherry-TetR co-transfected with pCMV-104 and pTRE-Tight-EGFP plasmids. Cells were kept in darkness (gray) or under 740/25 nm light (dark red) for 48 h and analyzed using flow cytometry (n=5x10⁴) (n=3, error bars are s.e.m.). (b) Light-to-dark ratio of the EGFP signals shown in (a).

[0033] Fig. 15: Dose-response curves for light-induced SEAP expression for BphP1-PpsR2 and PhyB-PIF6 systems. SEAP signal was detected in HeLa cells with BphP1-mCherry-TetR, pTRE-Tight-SEAP and pCMV-104 plasmids (squares) and in HeLa cells with pTRE-Tight-SEAP plasmid and pKM022 plasmid (41), supplemented with 15 μ M of phycocyanobilin (circles). Cells were kept in darkness or illuminated with 740/25 nm light for the BphP1-PpsR2 system or illuminated with 660/20 nm light in a case of the PhyB-PIF6 system.

[0034] Fig. 16a-16d: Comparison of penetration depth of far-red and NIR light in different mammalian tissues. Dependences of the photon counts at 660 nm (dashed line) and 740 nm (solid line) wavelengths on the depth of penetration for (a) mammalian brain, (b) breast, (c) muscle and (d) bone tissues.

[0035] Fig. 17: Estimated efficiency of the SEAP light-induced expression for the BphP1-PpsR2 and PhyB-PIF6 systems at various depths in a muscle tissue. Dependence of the relative activation efficiency of the BphP1-PpsR2 and PhyB-PIF6 systems on the depth of a muscle tissue. The systems are activated with 740 nm (dark gray) and 660 nm (gray) light, respectively. Calculation of the relative efficiencies was based on the light-sensitivity measurements for both optogenetic systems (Fig. 15) and on the light-attenuation properties of a mammalian muscle tissue (Fig. 16c).

[0036] Fig. 18: Blue light-induced expression of Rluc8 in HeLa cells transfected with LightON system. The Rluc8 bioluminescence signal was detected in HeLa cells transiently transfected with the pGAVPO plasmid (42) and with the pU5-Rluc8 plasmid in a 1:1 ratio. 10 h after the transfection cells were continuously illuminated with 470/15 nm light (1 mW cm^{-2}) or remained in darkness for 48 h ($n=3$, error bars are s.e.m.).

[0037] Fig. 19: Spectral compatibility of LightON and BphP1–PpsR2 systems. The light-to-dark ratio of Rluc8 bioluminescence signal detected in HeLa cells co-transfected with the pGAVPO plasmid³ and with the pU5-Rluc8 plasmid in a 1:1 ratio (blue columns) and in HeLa cells expressing BphP1-mCherry-TetR co-transfected with the pTRE-Tight-Rluc8 and pCMV-104 plasmids with a 1:5 ratio (dark red columns). After the transfection, the cells were continuously illuminated for 48 h with either 470/15 nm (0.5 mW cm^{-2}) or 740/20 light (0.5 mW cm^{-2}) or remained in darkness ($n=3$, error bars are s.e.m.).

[0038] Fig. 20a-20c: Blue light-induced transcription activation in mice livers hydrodynamically transfected with high amounts of LightON plasmids. Rluc8 bioluminescence signals detected in mice after hydrodynamic co-transfection of the livers with the pGAVPO plasmid³ ($10 \text{ }\mu\text{g}$) and the pU5-Rluc8 reporter plasmid ($300 \text{ }\mu\text{g}$). Mice kept (a) in darkness or (b) illuminated with 470/15 nm light of 5 mW cm^{-2} for 24 h are shown. (c) The kinetics of the Rluc8 expression in mice kept in darkness or illuminated with 470/15 nm light at 5 mW cm^{-2} continuously for 72 h is quantified ($n=3$; error bars are s.e.m.). The color bar in (a) and (b) indicates the total bioluminescence radiance in [$\text{photons s}^{-1} \text{ cm}^{-2} \text{ steradian}^{-1}$].

DETAILED DESCRIPTION OF THE INVENTION

[0039] Light-mediated control of protein-protein interactions to regulate metabolic pathways and gene expression is an important approach of optogenetics. Herein is disclosed the first optogenetic system based on a reversible light-induced binding of a bacterial phytochrome *RpBphP1* and its natural partner *RpPpsR2* (its Cys439Ser mutant is denoted as PpsR2 herein) from *Rhodospseudomonas palustris* bacteria. The BphP1–PpsR2 interaction is herein characterized extensively both *in vitro* and in live mammalian cells, and then used to translocate target proteins to specific cellular compartments, such as plasma membrane and nucleus. Using this approach, a light-control of cell morphology was achieved resulting in the substantial increase of cell area. Next, a light-induced gene expression was

demonstrated with 40-fold contrast, and a Cre-mediated DNA recombination resulted in the 20-fold excess of positive cells. The unique characteristics of the BphP1–PpsR2 optogenetic system reported here are its sensitivity to 700-820 nm near-infrared light and its ability to utilize an endogenous biliverdin chromophore abundant in eukaryotes, including mammals and humans.

[0040] An isolated nucleic acid is provided encoding (i) a protein having the sequence of *Rhodopseudomonas palustris* BphP1 and (ii) a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*. In an embodiment, the nucleic acid is a cDNA.

[0041] Also provided is an isolated nucleic acid encoding (i) a protein having the sequence of *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*. In an embodiment, the nucleic acid is a cDNA.

[0042] Also provided is an isolated nucleic acid comprising a first and second portion, the first portion encoding a protein having the sequence of *Rhodopseudomonas palustris* BphP1 and a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*, and the second portion encoding a protein having the sequence of *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*.

[0043] In an embodiment, the first portion is contiguous with the second portion. In an embodiment, the first portion is contiguous with an oligonucleotide portion encoding a self-cleaving peptide, which oligonucleotide portion is contiguous with the second portion.

[0044] In an embodiment of the nucleic acids, the nucleic acid further encodes a detectable marker protein or detectable marker peptide. In an embodiment, the nucleic acid encodes a detectable marker protein which is a fluorescent protein.

[0045] A cell is provided, wherein the cell is not in a human being, transformed with the nucleic acid of as described herein.

[0046] A fusion protein is provided comprising a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and a second protein or peptide. A fusion protein is provided comprising a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and a second protein or peptide. In an embodiment, the second protein or peptide is heterologous relative to *Rhodopseudomonas palustris*. In an embodiment, the fusion protein further comprises a detectable marker

protein or detectable marker peptide. In an embodiment, the fusion protein comprises a detectable marker protein which is a fluorescent protein.

[0047] A method is provided for inducing interaction of a first protein with a second protein in a system, the method comprising providing a system comprising (a) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) the first protein, and (b) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) the second protein, and (c) an amount of biliverdin; and

irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof. In embodiments, the amount of biliverdin is present endogenously, added exogenously or produced enzymatically. An embodiment further comprises co-expression of heme oxygenase.

[0048] In an embodiment, the first or second protein is a DNA-binding protein. In an embodiment, the first or second protein comprises a transcriptional activator protein. In an embodiment, at least one fusion protein further comprises a detectable marker protein or detectable marker peptide. In an embodiment, the one fusion protein or both fusion proteins comprise different detectable marker proteins which are each fluorescent proteins. In an embodiment, the system is a eukaryotic cell. In an embodiment, the system comprises a plant, algae, fungi, yeast, insect, worm, avian, xenopus, fish or mammalian cell.

[0049] In an embodiment, the eukaryotic cell is *in vivo*. In an embodiment, the eukaryotic cell is *in vivo*.

[0050] In an embodiment, the method further comprises providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* BphP1.

[0051] In an embodiment, the method further comprises providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and the second protein.

[0052] Also provided is a method for inducing translocation of a predetermined protein to a predetermined location in a cell comprising

providing a system comprising (a) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) a first protein, and (b) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) a second protein, and (c) an amount of biliverdin, wherein one of the first and second proteins is the predetermined protein and wherein the other of the first and second proteins is a protein that preferentially locates to the predetermined location in a cell; and

irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.

[0053] In embodiments, the amount of biliverdin is present endogenously, added exogenously or produced enzymatically. An embodiment further comprises co-expression of heme oxygenase.

[0054] In an embodiment, the protein that preferentially locates to the predetermined location in a cell, preferentially locates to a plasma membrane of a cell.

[0055] In an embodiment, the protein that preferentially locates to the predetermined location in a cell, preferentially locates to an organelle of a cell.

[0056] In an embodiment, the protein that preferentially locates to the predetermined location in a cell, preferentially locates to a nucleus of a cell.

[0057] In an embodiment, the fusion protein comprising the first or second protein that preferentially locates to a nucleus, further comprises a DNA-binding protein.

[0058] In an embodiment, the fusion protein comprising the first or second protein that preferentially locates to a nucleus, further comprises a transcriptional activator protein.

[0059] In an embodiment, one fusion protein or both fusion proteins further comprise a detectable marker protein or detectable marker peptide.

[0060] In an embodiment, the one fusion protein or both fusion proteins comprises different detectable marker protein which are each fluorescent proteins.

[0061] In an embodiment of the methods, the cell is a eukaryotic cell. In an embodiment, the system comprises a plant, algae, fungi, yeast, insect, worm, avian, xenopus, fish or mammalian cell.

[0062] In an embodiment of the methods, the eukaryotic cell is *in vivo*. In an embodiment of the methods, the eukaryotic cell is *in vitro*.

[0063] In an embodiment, the method further comprises providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* BphP1.

[0064] In an embodiment, the method further comprises providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.

[0065] In an embodiment, the near infra-red light is 700 nm to 820 nm light. In an embodiment, the near infra-red light is activating infra-red light.

[0066] In an embodiment, the methods further comprise irradiating the system with far infrared light sufficient to induce dissociation of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof. In an embodiment, further comprising irradiating the system with far infrared light sufficient to induce dissociation, the method subsequently reduces the interaction of a first protein with a second protein in the system. In an embodiment, further comprising irradiating the system with far infrared light sufficient to induce dissociation, the method subsequently induces relocation of a predetermined protein away from the predetermined location in a cell.

[0067] A kit is provided comprising any of the nucleic acid, fusion protein and/or system described herein and instructions for use.

[0068] In an embodiment, this invention also encompasses isolated non-naturally occurring nucleic acids encoding a non-canonical BphP having an HOS domain and/or a PpsR2 interacting counterpart. In an embodiment, this invention also encompasses a fusion protein comprising a non-canonical BphP having an HOS domain and/or a PpsR2 interacting counterpart. In an embodiment, this invention also encompasses methods comprising use of a non-canonical BphP having an HOS domain and/or a PpsR2 interacting counterpart. Non-canonical BphP and PpsR2 are found not only in the *Rhodopseudomonas palustris* but in several other bacteria. For example, *Bradyrhizobium* in which there are BphP and PpsR2 which also can interact with each other when irradiated with near infra-red light. Use of and non-naturally occurring compositions comprising these isolated protein pairs from an bacteria with high homology between *Rp*BphP1 and *Br*BphP, as well as between *Rp*PpsR2 and *Br*PpsR2, are encompassed. *Br*BphP and *Br*PpsR2 proteins are cloned from *Bradyrhizobium* strain ORS278, and their nucleotide and peptide sequences are publically available in the NCBI/NIH databases. The nucleic acids, fusion proteins and

methods described herein are applicable mutatis mutandis to the non-canonical BphP and PpsR2 not present in *Rhodopseudomonas palustris*.

[0069] In an embodiment, the BphP1 has the sequence encoded by *Rhodopseudomonas palustris* gene *rpa1537*.

[0070] In an embodiment, the BphP1 has the sequence:

MVAGHASGSPAFGTADLSNCEREEIHLAGSIQPHGALLVVSEPDHRIIQASANA AEF
LNLGSVLGVPLAEIDGDLLIKILPHLDPTAEGMPVAVRCRIGNPSTEYDGLMHRPPE
GGLIILERAGPPIDLSGTLAPALERIRTAGSLRALCDDTALLFQQCTGYDRVMVYR
FDEQGHGEVFSERHVPGLSEYFGNRYPSDIPQMARRLYERQVRVRLVDVSYQPVP
LEPRLSPLTGRDLMSGCFLRSMSPHILQYLKNMGVRATLVVSLVVGKWLWGLVA
CHHYLPRFIHFELRAICELLAEAIATRITALESF AQSQSELFVQRLEQRMIEAITREGD
WRAAIFDTSQSILQPLHADGCALVYEDQIRTIGDVPSTQDVREIAGWLD RQPRAAV
TSTASLGLDVPELAHLTRMASGVVAAPISDHRGEFLMWFRPERVHTVTWGGDPKK
PFTMGDTPADLSPRRSFAKWHQVVEGTSDPWTAAADLAAARTIGQTVADIVLQFRA
VRTLIAREQYEQFSSQVHASMQPVLITDAEGRILLMND SFRDMLPAGSPSAVHLDD
LAGFFVESNDFLRNVAELIDHGRGWRGEVLLRGAGNRPLPLAVRADPVTRTEDQSL
GFVLIFSDATDRRTADAARTRFQEGILASARPGVRLDSKSDLLHEKLLSALVENAQL
AALEITYGVETGRIAEELLEGVRSMLRTAEVLGHLVQHAARTAGSDSSSNGSQNKK
(SEQ ID NO:1).

[0071] In an embodiment, RpBphP1 has the nucleotide sequence:

atgggtggcaggtcatgcctctggcagccccgcattcgggaccgccgatctttcgaattgcgaacgtgaagagatccacctcgccgg
ctcgatccagccgcatggcgcgcttctggctcgtcagcgagccggatcatcgcatcatccaggccagcgccaacgccgcggaattt
ctgaatctcgaagcgtgctcggcggtccgctcgccgagatcgacggcgatctgttgatcaagatcctgccgcatctcgatcccacc
gccgaaggcatgccggtcgcggtgcgctgccggatcggaatccctccacggagtagcagcggtctgatgcacggcctccggaa
ggcgggctgatcatcgagctcgaacgtgccggccccgccgatcgtctgtccggcacgctggcgccggcgctggagcggatccg
cacggcgggctcgctgcgcgcgctgtgcgatgacaccgcgctgctgtttcagcagtgacccggctacgacgggtgatggtgat
cgcttcgacgagcagggccacggcgaagtgttctccgagcgccacgtgccccgggctcgaatcctatttcggcaaccgctatccgtc
gtcggacattccgagatggcgggcggtgtacgagcggcagcgctccgctgctggtcgacgtcagctatcagccggtgcc
gctggagccgcggtgtcggctgaccggcgcgatctcgacatgtcgggctgcttctcgctcgatgtcggcatccatctgc
agtacctgaagaacatgggcgtgcgcgccaccctggtggtgtcgtggtggtcggcggaagctgtggggcctggttcctgtca
ccattatctgccgcttcatccatttcgagctcggggcgatctgcgaactgctcgggaagcgatcgcgacgcggatcaccgcgt
tgagagcttcgcgagagccagtcggagctgttcgtgcagcggctcgaacagcgcatgatcgaagcgatcaccctgaaggcga

ttggcgcgcagcgattttcgacaccagccaatcgatcctgcagccgctgcacgccgacgggtgcgcgctgggtgtacgaagaccag
 atcaggaccatcggtgacgtaccttcacgcaggatgttcgcgagatcgccgggtggctcgatcgccagccacgtgcggcggtga
 cctcgaccgcgtcgctcggtctcgacgtgccggagctcgcgcatctgacgcggatggcgagcggcgtgggtcgcggcgccgattt
 cggatcatcgccgcgagtttctgatgtgggtccgccccgagcgcgtccacaccgttacctggggcggcgatccgaagaagccgttc
 acgatgggcgatacaccggcggtatctgctgccggcgctccttcgccaatggcatcaggttgcgaaggcacgtccgatccgt
 ggacggccgcggtatcgcgcggctcgcaccatcggtcagaccgtcgccgacatcgctgcaattccgcgggtgcggacac
 tgatcgccgcgaacagtacgaacagtttctgctccaggtgcacgcttcgatgcagccgggtgctgatcaccgacgccgaaggccg
 catcctgctgatgaacgactcgttccgcgacatgttccggcggggtcgccatccgctccatctcgacgatctgcggggttcttc
 gtcgaatcgaacgatttctcgcaacgtcgccgaactgatcgatcacggccgcgggtggcgcgggcgaagtctgctgcgcggcg
 caggtaatcgcccgttgcgctggcagtcgcgcggatccggtagcgcgcagggaggaccagtcgctcggcttcgtgctgatttc
 agcgacgtaccgatcgtcgaccgcagatgccgcacgcacgcgtttccaggaaaggcatttctgccagcgacgtcccgcgctgc
 ggctcgactccaagtccgaccttctgcagagaagctgctgctccgcgtggctcgagaacgcgcagcttccgcatttgaaattactt
 acggcgctcgagaccggacgcacatcgccgagctgctgaaggcgttcgccagtcgatgctgcgcaccgccgaagtctcgcccatc
 ttggtgcagcacgcggcgcgacggccggcagcgacagctcgagcaatggctcgagaacaagaag (SEQ ID NO:9).

[0072] In an embodiment, the PpsR2 has the sequence encoded by the *Rhodopseudomonas palustris* gene *ppsR2*.

[0073] In an embodiment, the PpsR2 has the sequence:

gtggcgtaaagtccgttcacgacatcacccttctgctcgatggagggtgtgattcggaagccaccctgtctccgacgatgg
 cggccgagagcgtggacgggtgggtggggcgctgctggagcgacatcgccggcgccgaaggcggcgacaagggttcgccgat
 ggtcgaagacgcccgcgcagcggcatctcggttccgccagatcaatcagccttcccgagcggcgctcgaatcccgatcgaat
 tcaccacgatgctgctgggcgaccgcaccggcatgatcgggctggcaagaacatgcaggcggtcaccgagctgcattcccgcc
 tgatcgctgcgcagcaggcgatggagcgcgactattggcggttgcgtgaattggagactcgctaccgcctggtgttcgacgtgcc
 gccgatcgggtgatgatcgtctccggcgacatgcgcacgtcgaagccaaccggcgcggtgaatgcgatcagccgcgtc
 gagcgcggcaatgacgaccttgcggggcggtatttctcgccgaagtggcggtgctccgatcgcatgagggtgcgcgacatgctg
 gccaggtgcgtcagcgcggcaccgcactcagcgtcctcgttcacatctggccgttacaccgcgcctggatgctgcgcggttcgt
 gatgctcgcgagcgtcgtcagggtttctgctgcacttcacccgggtgaccacgactcccgcatcgacgacgtcgacgatgatgc
 cgtgctgcgcgggctgatcgatcgattcccagcgggttcgtcgactggattcggaaggcgtgcttcgtacgccaaccaggcgt
 ttctcgatctggtccagatcggtccaagcctgcggcggtcgagacatcgctggcgctctggatgggtcgtccggcgccgatctg
 tccagcttgcgtgacgtgctgcggcgctacaagacggtgcggctgttccaaacgacgatccgcggcgagctcggcaccgagactg
 aagtcgaggtctcgccgtcgacggcgaggacaccaatacatcgccgttctgatgcgaatgctgcgcgacgcctcgacgtgc
 ggacgaccacgatgccttgcgtcaggcgctcgccccgatcagcaagcagctcggcgatcctcgtcgcgaagctggtgaagaa
 cgccgtgagcattgtcgagcagcactacgtgaaggaaagcgtgttgcgatccaagggaatcgacggcaactgccgaactgctc

ggattgagccggcagagcctttatgcaaaactcaacagctacggcttcgacgacaaaggtgtcgttgcttctgctgccgacggtgca
gagggcgctcagacgacgagagat (SEQ ID NO:10).

[0074] In an embodiment, the *RpPpsR2* has the sequence:
MASKSVHADITLLLDMEGVIREATLSPTMAAESVDGWLGRRWSDIAGAEGGDKVR
RMVEDARRSGISAFRQINQPFPSGVEIPIEF TTMLLGDRTGMIAVGKNMQAVTELHS
RLIAAQQAMERDYWRLRELETRYRLVFDAAADAVMIVSAGDMRIVEANRAAVNA
ISRVERGNDDL AGRDFLA EVAAADRD A VRDMLAQVRQRGTALSVLVHLGRYDRA
WMLRGSLMSSERRQVFLHFTPVT TTPAIDDVDDD A VLRGLIDRIPDGFVALDSEG
VVRHANQAFLDLVQIGSKPAAVGRSLGVWMGRPGADLSSLLTLLRRYKTVRLFQT
TIRGELGTETEVEVSAVDGEDDQYIGVLMRNVARRLDAADDH DALRQALGPISKQ
LGRSSLRKL VKNASVIEQH YVKEALLRSKGNRTATAELLGLSRQSLYAKLNSYGF
DDKGVVASAADGAEGASDDAED (SEQ ID NO:2).

[0075] In an embodiment, the PpsR2 has the non-dimerizing variant sequence:
MASKSVHADITLLLDMEGVIREATLSPTMAAESVDGWLGRRWSDIAGAEGGDKVR
RMVEDARRSGISAFRQINQPFPSGVEIPIEF TTMLLGDRTGMIAVGKNMQAVTELHS
RLIAAQQAMERDYWRLRELETRYRLVFDAAADAVMIVSAGDMRIVEANRAAVNA
ISRVERGNDDL AGRDFLA EVAAADRD A VRDMLAQVRQRGTALSVLVHLGRYDRA
WMLRGSLMSSERRQVFLHFTPVT TTPAIDDVDDD A VLRGLIDRIPDGFVALDSEG
VVRHANQAFLDLVQIGSKPAAVGRSLGVWMGRPGADLSSLLTLLRRYKTVRLFQT
TIRGELGTETEVEVSAVDGEDDQYIGVLMRNVARRLDAADDH DALRQALGPISKQ
LGRSSLRKL VKNASVIEQH YVKEALLRSKGNRTATAELLGLSRQSLYAKLNCYGF
DDKGVVASAADGAEGASDDAED (SEQ ID NO:3).

[0076] Also provided is a method for artificially expressing a heterogenous gene *in vivo* in a subject comprising administering to the subject: (a) a nucleic acid encoding (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) a TetR tetracycline repressor protein (TetR) or a VP16 transcriptional activation domain (VP16); (b) a nucleic acid encoding (i) nuclear localization signal, a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (iii) a VP16 or a TetR; and (c) a nucleic acid encoding (i) a tetracycline response element (TRE) and the heterogenous gene;
wherein only one of (a) and (b) encodes a VP16 and the other of (a) or (b) encodes a TetR;
and

irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and thereby effect expression of the heterogenous gene.

[0077] In an embodiment, the method further comprises administering an amount of biliverdin to the subject.

[0078] In an embodiment, the method further comprises detecting the expression of the heterogenous gene in the subject.

[0079] In an embodiment, the method further comprises quantifying the expression of the heterogenous gene in the subject.

[0080] In an embodiment, the method further comprises administering to the subject a blue light-activated gene expression system for a second heterogeneous gene and irradiating the system with blue light sufficient to induce expression of the second heterogenous gene.

[0081] An exemplary blue light-activated gene expression system is that described in Wang et al., *Nature Methods*, 9(3):266-269, which is hereby incorporated by reference in its entirety. In an embodiment, the system is irradiated with blue light comprising 470 nm light.

[0082] In an embodiment, the TRE is comprises a pTRE plasmid.

[0083] Optionally, the activation domain of the methods and constructs described herein may be replaced with an alternative activation domain such as a p65 activation domain (GAVP) (See Wang et al., *Nature Methods*, 9(3):266-269).

[0084] In an embodiment of the methods, the method is performed on a eukaryotic cell. In an embodiment of the methods reciting a eukaryotic cell, the cell is in a human. In an embodiment of the methods reciting a eukaryotic cell, the cell is not in a human. In an embodiment of the methods reciting a eukaryotic cell, the cell is in a mammalian cell or mammalian-derived cell.

[0085] All combinations of the various elements described herein are within the scope of the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0086] This invention will be better understood from the Experimental Results, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

Experimental Results

Introduction

[0087] A non-canonical bathy bacterial phytochrome *RpBphP1* (BphP1) from purple photosynthetic bacteria *Rhodopseudomonas palustris* may perform its signaling function by interacting with a transcriptional repressor *RpPpsR2* when illuminated with NIR light (22). The effector module of BphP1 consists of two domains, PAS/PAC (PAS domain with additional C-terminal residues) and HOS (2-helix output sensor), with no detectable enzymatic activity. Recently, a BphP1–*RpPpsR2* heterodimerization was observed in the NIR-illuminated BphP1 and *RpPpsR2* protein mixture using gel filtration; however, no further characterization of this interaction was performed (23), and whether it is a practically usable interaction is unknown.

Examples

[0088] Properties of purified BphP1. In its ground Pfr state, BphP1 absorbs maximally at 412 nm (Soret band) and at 756 nm (Q band) (Fig. 1a). Upon NIR illumination, it photoconverts into the Pr state with absorbance at 390 nm and 678 nm for Soret and Q bands, respectively. A 4.3-fold decrease in absorbance at 756 nm upon 740/25 nm irradiation was observed (Table 1). The highest light sensitivity of the Pfr state was observed at ~740–780 nm, with notable BphP1 photoconversion at 800 nm (Fig. 1b). Kinetics of the Pfr→Pr photoconversion was monoexponential (Fig. 6a). The half-time of the Pfr→Pr transition was 28 s at 1 mW cm⁻² and decreased to 3.5 s at 27 mW cm⁻² (Fig. 1c). After the Pfr→Pr photoconversion, BphP1 returned to the ground state in darkness with a half-time of 170 s. BphP1 could undergo repeated cycles of Pfr→Pr photoconversion followed by dark relaxation back to the Pfr state (Fig. 1d). Irradiation with 636 nm light restored Pfr absorbance to ~80% (Fig. 1a). The remaining ~20% were restored during dark relaxation (Fig. 6b). The half-time of the Pfr→Pr conversion depended on the 636 nm light intensity, ranging from 21 s at 1 mW cm⁻² to 3.0 s at 45 mW cm⁻² (Fig. 1e). Multiple cycles of photoswitching did not lead to notable changes in absorbance (Fig. 1f).

[0089] BphP1–PpsR2 interaction *in vitro*. *RpPpsR2* has a single cysteine residue in position 439 and forms a noncovalent homodimer. To exclude the possibility of disulfide bond formation, cysteine 439 was substituted with serine and named this *RpPpsR2/C439S* variant PpsR2. mRuby2 was then fused to the C-terminus of PpsR2 and the BphP1–PpsR2

interaction characterized using FRET. mRuby2 emission and BphP1 absorbance in the Pr state have good spectral overlap (Fig. 7a), resulting in quenching of mRuby2 fluorescence (Fig. 7b). 740 nm light caused an increase in the Pr state, and up to 12% decrease in mRuby2 fluorescence resulted from the BphP1–PpsR2–mRuby2 binding. A 27-fold change in 740 nm light intensity did not cause changes in the kinetics of the mRuby2 fluorescence decrease (Fig. 7c). For all intensities tested, the fluorescence quenching exhibited a half-time of ~60 s, which was larger than observed for BphP1 alone (Fig. 1c). This suggested that PpsR2 did not bind BphP1 in the Pfr state, and that the BphP1–PpsR2 interaction was not limited by the rate of Pfr→Pr photoconversion but rather by BphP1 and/or PpsR2 structural changes involved in the interaction.

[0090] Using 740 nm for the Pfr→Pr and 636 nm for the Pr→Pfr photoconversion, several cycles of FRET changes (Fig. 1g) were monitored with half-times of ~60 s and ~30 s, respectively (Table 1). The observed incomplete Pr→Pfr switching with 636 nm light possibly was caused by overlap of the Pr and Pfr spectra at this wavelength (Fig. 1a). Dark relaxation in the BphP1–PpsR2 complex was slower than photoswitching with 636 nm and depended on the BphP1:PpsR2 ratio, with shorter half-times of Pr→Pfr dark relaxation (as detected by Pfr absorbance) corresponding to the larger BphP1 concentrations (Fig. 1h). The dark relaxation half-time was 900 s (Table 1), suggesting that the PpsR2 binding to BphP1 substantially slowed the Pr→Pfr relaxation and BphP1–PpsR2 dissociation in darkness. After complete dark relaxation the complexes can be formed again by 740 nm light illuminating (Fig. 1i).

[0091] Table 1 - Characteristics of BphP1 and its interaction with PpsR2 *in vitro*.

Proteins	Chromophore state	Absorbance maximum, nm	Extinction coefficient ¹ , M ⁻¹ cm ⁻¹	Absorbance change upon photoconversion, fold ²		Half-time (s) of ³	
				at 756 nm	at 678 nm	photoconversion	dark relaxation
BphP1	Pr	678	87,500	4.3 (7.0)	0.48 (0.45)	16 (Pr→Pfr, with 636/20 nm)	210
BphP1 and PpsR2						30 (Pr→Pfr, with 636/20 nm)	900
BphP1	Pfr	756	78,300			19 (Pfr→Pr, with 636/20 nm)	N.A.

						740/25 nm)	
BphP1 and PpsR2						60 (Pfr→Pr, with 740/25 nm)	

¹Calculated based on an extinction coefficient of BV, as described¹; ²Measured after irradiation either by a 740/25 nm LED array or by a 785/2 nm laser diode (in parenthesis); ³BphP1:PpsR2 molar ratio of 8:1 and photoconversion light intensity of 2 mW cm⁻² were used. N.A., not applicable.

[0092] BphP1–PpsR2 interaction in mammalian cells. To study the BphP1–PpsR2 interaction in mammalian cells a translocation assay was used (Fig. 2a). PpsR2 was fused with mVenus and a -CAAX plasma membrane localization signal, and BphP1 with mCherry for cytoplasmic expression. Excitation of these fluorescent proteins did not photoconvert BphP1 (Fig. 8). Illumination of HeLa cells with 740 nm light caused the translocation of BphP1-mCherry to the plasma membrane and a 25% decrease in mCherry cytoplasmic intensity after 3 min of 740 nm illumination detected with epifluorescence microscope (Fig. 2b,c). Subsequent dark incubation restored BphP1-mCherry fluorescence in the cytoplasm to the original level in ~24 min (Fig. 2b,d). The reversibility of the BphP1 translocation to the plasma membrane and back to the cytoplasm was demonstrated for 3 cycles of 3 min of 740 nm illumination followed by 30 min of dark relaxation (Fig. 2e), with the recovery of ~95% of the initial mCherry cytoplasmic intensity. Then effect of 650 nm light was studied on acceleration of the BphP1 dissociation from the plasma membrane and found a 1.6-fold higher initial rate of BphP1-mCherry cytoplasmic signal recovery as compared to that in darkness (Fig. 9). However, complete dissociation of the BphP1–PpsR2 complexes required darkness. Illumination with 740 nm and 650 nm light for 3 min each resulted in ~8% reversible changes of the BphP1-mCherry cytoplasmic signal after the initial 25% decrease (Fig. 2f), indicating that one third of the BphP1–PpsR2 complexes could be modulated with light.

[0093] Light-induced activation of a signaling pathway. The BphP1–PpsR2 interaction was next utilized for the recruitment of a DHPH domain of intersectin-1 (ref. 43) to the plasma membrane for activation of the small GTPase Cdc42 (ref. 44, 45). The DHPH domain was fused to the C-terminus of BphP1-mCherry and co-expressed the construct in HeLa cells with PpsR2-mVenus-CAAX (Fig. 3a). 740 nm illumination caused changes of cell morphology and a gradual increase of the cell area with a plateau after ~30 min (Fig.

3b). No notable changes were detected in cells co-transfected with BphP1-mCherry-DHPH and mVenus-CAAX (Fig. 3c). In the former experiment, the area increase reached 50% in some cells, with an average increase of ~25% (Fig. 3d). The initial relative decrease of BphP1-mCherry-DHPH in the cytoplasm was 2-fold higher than the cell area increase, indicating that BphP1-mCherry-DHPH translocated mainly during the first 5 min of 740 nm irradiation. Further changes were rather similar for mCherry fluorescence and cell area, suggesting that they were caused by an increase in the cell area (Fig. 10).

[0094] BphP1 recruitment to the cell nucleus. To examine whether optogenetically induced nuclear import could be achieved, HeLa cells that stably expressed BphP1-mCherry were transfected with PpsR2-mVenus containing a nuclear localization signal (NLS). In darkness NLS-PpsR2-mVenus localized to the nucleus, and BphP1-mCherry stayed in the cytoplasm (Fig. 4a), with a mCherry intensity ratio between the nucleus and cytoplasm of ~0.5 (Fig. 11). 740 nm illumination caused an increase of BphP1-mCherry fluorescence in the nucleus (Fig. 4a), resulting in a nucleus/cytoplasm ratio of 1.8. Cells expressing only BphP1-mCherry displayed nucleus/cytoplasm ratios below 0.5, both in darkness and after illumination (Fig. 11). Thus, the light-induced BphP1-PpsR2 interaction caused a ~3.5-fold increase of the BphP1-mCherry signal in nuclei of illuminated cells.

[0095] A light-inducible TetR-*tetO* transcription system. To develop a light-inducible transcription system, the light-induced recruitment of BphP1 to the nucleus was combined with a tetracycline repressor-based system (Fig. 4b). VP16 was fused to the C-terminus of NLS-PpsR2 and the tetracycline repressor (TetR) (ref. 46) to BphP1-mCherry. HeLa cells stably expressing BphP1-mCherry-TetR were co-transfected with a plasmid encoding NLS-PpsR2-VP16 and with a pTRE-Tight-SEAP plasmid containing 7×*tetO* upstream of the *SEAP* gene. 740 nm illumination increased SEAP levels ~40-fold over the levels in darkness after 48 h (Fig. 12a,b). The time course of SEAP production revealed a ~3-fold SEAP increase after 12 h of 740 nm illumination compared to dark-treated cells (Fig. 12c). SEAP accumulation exhibited a half-time of ~18 h and reached a plateau after 48 h (Fig. 4c, Fig. 12c). SEAP expression level depended on the power of activating light, with SEAP signal observed in cells irradiated with as low as 0.05 mW cm⁻² (Fig. 13). Similarly, the light-induced BphP1-PpsP2 interaction caused EGFP expression from a pTRE-Tight-EGFP reporter plasmid resulted in more than 27-fold higher EGFP signal in illuminated over dark-treated cells as detected by flow cytometry (Fig. 14).

[0096] Next studied was how fast the light-induced transcriptional activation could be terminated. Cells were illuminated with 740 nm light for 12 h and then kept in darkness. The SEAP reporter production increased ~2.6-fold during the first 12 h in darkness, likely due to pre-accumulation of SEAP mRNA, followed by its subsequent decrease with a half-time of ~8 h (Fig. 4d). It was tested whether 636 nm light would accelerate the termination of transcriptional activation and found that 12 h illumination with 636 nm after the 12 h-illumination period with 740 nm light decreased the SEAP production ~2.3-fold compared to the cells kept in darkness. Moreover, the SEAP decrease in the subsequent darkness after 636 nm illumination also was ~2-fold faster (Fig. 4d). Thus, similar to cell membrane re-localization (Fig. 2), the termination of gene transcription can be accelerated with 636 nm illumination, which causes dissociation of the BphP1-TetR and PpsR2-VP16 complexes in a nucleus.

[0097] Next compared were two similar TetR-*tetO* transcription activation systems, one based on the BphP1-PpsR2 pair and another on a PhyB-PIF6 pair (47), using the same reporter plasmid. The reporter expression was 2-fold higher for the BphP1-PpsR2 than for the PhyB-PIF6 interaction (Fig. 15). Comparison of light propagation for 660 nm and 740 nm wavelengths in various mammalian tissues revealed a substantially more effective penetration of NIR light (Fig. 16), resulting in higher reporter expression at larger tissue depth for the BphP1-PpsR2 system compared to the PhyB-PIF6 system (Fig. 17).

[0098] With regard to the results presented in Fig. 16, optical properties of mammalian tissues differ from type to type, and should be regarded as a complex function of different factors. A total absorption of a tissue depends on several parameters, such as an oxygenation degree of hemoglobin, a blood volume, a water content, a fat content, a concentration of endogenous chromophores (bilirubin, melanin, carotene, etc.), a Rayleigh light scattering and a Mie light scattering (48). The tissue optical properties are usually described in terms of three parameters: a scattering coefficient, μ_s , an absorption coefficient, μ_a , and a scattering anisotropy factor, g .

[0099] The absorption and scattering coefficients are defined as the probability of absorption or scattering, respectively, per a path-length unit. The third parameter is the scattering distribution in a turbid media, described by the anisotropy factor and obtained by computing the mean cosine of the scattering angle. The value of g , in a scale from -1 to 1, characterizes the direction of the scattering. In most biological tissue, the g value ranges from 0.70 to 0.99, indicating that photons are preferably scattered in the forward direction.

Using g factor one can compute a reduced scattering coefficient, which takes into account the anisotropy of scattering in a studied tissue:

$$\mu'_s = (1 - g)\mu_s \quad (1)$$

[00100] In a reality, when samples of a bulk tissue are analyzed, the above-mentioned parameters are difficult to evaluate, as these situations are characterized by the occurrence of multiple scattering and absorption events. As the consequence, to characterize optical properties of thick samples another parameter is utilized. An effective attenuation coefficient, μ_{eff} , is equal to an inverse of the attenuation length at which the incident spatial irradiance is attenuated by factor of $1/e$ (~37%). The relation between μ_{eff} , μ_a and μ'_s is defined as

$$[00101] \quad \mu_{eff} = \sqrt{3\mu_a(\mu_a + \mu'_s)} \quad (2)$$

[00102] The optical properties of different mammalian tissues have been studied by many research groups, and the μ_a and μ'_s coefficients were experimentally determined for many tissues (49-54). Thus, it is possible to calculate μ_{eff} using equation (2).

[00103] Generally, a relation between absorbed light energy and pathway through which light travels is defined by Beer-Lambert law for thick layers:

$$[00104] \quad I(d) = I_0 e^{(-\mu_{eff} d)} \quad (3)$$

[00105] where I_0 is the initial light intensity, μ_{eff} is the effective attenuation coefficient of tissue at wavelength λ , and d is the path length of light through the sample.

[00106] Using equation (2) and (3) the dependence of relative photon counts for far-red (660 nm) and NIR (740 nm) light on the depth of penetration for 4 mammalian tissues was calculated and plotted, such as brain ($\mu_{eff}^{660} = 3.2$, $\mu_{eff}^{740} = 1.76$)⁹, breast ($\mu_{eff}^{660} = 5.5$, $\mu_{eff}^{740} = 3.13$)¹⁰, muscle ($\mu_{eff}^{660} = 3$, $\mu_{eff}^{740} = 1.55$)⁵ and bone ($\mu_{eff}^{660} = 2.24$, $\mu_{eff}^{740} = 1.77$)¹⁰. Substantial difference in penetration between 740 nm and 660 nm light at 1 cm depth is observed for the brain and muscle tissues where NIR light penetrates 4-fold more efficiently. The largest difference, ~11-fold, in penetration between 740 nm and 660 nm at 1 cm depth is observed for the breast tissue. This is because of the high level of fat and water content that resulted in the higher μ'_s for 660 nm light.

[00107] Overall, the considerations are consistent with the results published earlier (55). Importantly, in the NIR tissue transparency window the depth of light penetration is highly affected by light-scattering phenomenon: the absorption coefficients for many tissues within 650-900 nm range are ~10-fold lower than the light-scattering coefficients (54).

[00108] Light-activation of gene expression *in vivo*. For activation of gene expression *in vivo*, stably expressing BphP1-mCherry-TetR HeLa cells were co-transfected with NLS-PpsR2-VP16 and pTRE-Tight-Rluc8 plasmids and subcutaneously injected the cells into the interscapulum area of FVB mice 24 h after co-transfection. Then the animals were either illuminated with 740 nm light or kept in darkness. After 48 h a substantial increase of the Rluc8 signal was detected in the illuminated mice (Fig. 5a) as compared to those kept in darkness. The observed 32-fold activation contrast (Fig. 5b) was similar to that obtained in cell culture experiments (Fig. 12b).

[00109] Further compared was the BphP1-PpsR2 system with a blue light-activated LightON system (56). In cultured cells, LightON activation with 470 nm light resulted in 42-fold increase of the Rluc8 signal over the signal in dark treated cells (Fig. 18). However, experiments with subcutaneously injected cells in FVB mice led to only 15-fold Rluc8 activation contrast (Fig. 5c,d). This 2.8-fold drop in Rluc8 production in the LightON system was likely caused by the higher absorbance of 470 nm light than 740 nm light by ~1 mm thick tissue.

[00110] To determine whether both the LightOn and our system could potentially be used concurrently, optical crosstalk was tested for in cell culture. Activation of both systems in cultured cells with 470 nm and 740 nm light revealed their low cross-activation. 12-fold higher activation of the LightON system was detected with 470 nm light and 18-fold higher activation of the BphP1-PpsR2 system with 740 nm light (Fig. 19).

[00111] For deep tissues studies, the kinetics of light-induced Rluc8 expression in mice was assessed after hydrodynamic transfection (Fig. 5e-h, Fig. 20). After 24 h an increase of Rluc8 signal in livers of 740 nm illuminated mice in comparison with the dark treated animals (Fig. 5f) was detected. After 48 h the Rluc8 signal reached its maximum with a 5.7-fold light-to-dark signal ratio (Fig. 5e,f). Large differences of Rluc8 expression between the illuminated and dark-treated animals were observed up to 72 h after transfection (Fig. 5f). To test the LightON system mice were hydrodynamically transfected with the high amounts of the LightON and reporter plasmids used in the original paper (56) (Fig. 20) or with the amounts similar to those of the BphP1-PpsR2 system (Fig. 5g,h). In the former case, after 470 nm illumination of mice for 24 h, a 1.7-fold increase of the Rluc8 production was observed as compared to the dark treated animals. In the latter case, the signal increase was 2.8-fold (Fig. 5g,h), which was twice lower than that detected for the BphP1-PpsR2 system

(Fig. 5e,f). The Rluc8 'bell-shaped' kinetics of the LightON activation in liver observed in both conditions was similar to that reported (58).

Discussion

[00112] A novel optogenetic system is disclosed herein based on the light-inducible interaction of bacterial phytochrome BphP1 and its binding partner PpsR2 from *R. palustris*. This system takes advantage of a high-sensitivity of bacterial phytochromes to NIR light and a unique feature of bacterial phytochromes to incorporate heme-derived BV, which is abundant in mammalian cells and tissues (10), as a chromophore.

[00113] The induction of the BphP1–PpsR2 interaction occurs upon photoconversion of BphP1 from a ground Pfr state to the Pr state. Spectroscopic analysis revealed substantial difference in the absorption of Pfr band of BphP1 before and after the Pfr→Pr photoconversion. The photoconversion demonstrates high sensitivity to NIR 740-780 nm light and high rates of the Pfr→Pr transition. Characterization of BphP1–PpsR2 interaction *in vitro* and in live mammalian cells showed its reversibility in darkness. However, in experiments with light irradiation in Pr absorbing band the BphP1–PpsR2 dissociation was not complete. This might be caused by incomplete Pr→Pfr photoconversion (~80%) of BphP1 with far-red 636 nm light, which is absorbed by both Pr and Pfr states.

[00114] It was demonstrated that the BphP1–PpsR2 optogenetic system can be successfully used for activation of several types of cellular processes. First, a light-induced recruitment of one interacting partner to another partner residing in a specific cellular compartment was studied and it was found that using PpsR2-mVenus-CAAX as a partner, 25% of cytoplasmic BphP1 was recruited to plasma membrane. Excitation light for mVenus and mCherry had negligible overlap with the action spectrum of BphP1, allowing sampling of fluorescence images without altering of the BphP1 state. This approach was also utilized to activate Cdc42 signaling pathway by translocating the DHPH domain of intersectin-1 (24), which normally interacts with Cdc42 at plasma membrane. As a result an increase in cell area caused by lamellipodia formation was observed and changes in cell morphology (Fig. 4) consistent with the Cdc42 activity at lamellipodia (31).

[00115] The light-induced targeting approach was extended to the recruitment of BphP1 to a nucleus. To do this, nuclear-localized NLS-PpsR2-mVenus and cytoplasmic BphP1-mCherry constructs were co-expressed. Due to its molecular weight BphP1-mCherry could not undergo passive diffusion through nuclear pores and was localized to the cytoplasm in

darkness. NIR light caused formation of complexes between NLS-PpsR2-mVenus and BphP1-mCherry, resulting in substantially higher BphP1 concentration in the nucleus compared to cytoplasm.

[00116] The BphP1 nuclear recruitment was also used to activate transcription of several reporter genes downstream of the UAS or tetO binding sites for the light-induced PpsR2-VP16-BphP1-GAL4 and BphP1-tetR-PpsR2-VP16 complexes, respectively. This resulted in up to 40-fold increase in the reporter expression. The light-induced transcription was reversible in darkness. Furthermore, an irradiation with far-red light accelerated the termination of transcription by 2-fold, allowing its more precise control. Moreover, Cre recombinase expressed using this technique enabled substantial DNA recombination. In future studies, a combination of the Cre-mediated DNA recombination with the NIR light-control of Cre expression should provide a powerful non-invasive approach for genome editing in live animals (32).

[00117] These NIR light-inducible transcription approaches are easily transferable to model organisms genetically constructed to utilize the GAL4-UAS (28) and tetR-tetO (29) systems to enable protein expression in cells and tissues in spatiotemporal manner. Moreover, the light inducible tetR-based system will allow to avoid such drawbacks of consumption of tetracycline and its derivatives by experimental animals, as inhibition of proliferation, angiogenesis and cell migration, and induction of apoptosis in cancer models (33).

[00118] For *in vivo* applications, optogenetic systems sensing NIR light are required due to its deep tissue penetration and reduced phototoxicity compared to blue light utilizing LOV and cryptochrome systems. Majority of currently available far-red optogenetic systems are based on a PPI between plant phytochrome PhyB and its interacting partner PIF6 (3, 9, 34). These systems become active in response to far-red light peaked at 660 nm, whereas BphP1-PpsR2 interaction can be set off by NIR light with wavelengths of 740-780 nm. Moreover, a use of the PhyB-PIF6 systems is limited by the requirement to either supply exogenous phycocyanobilin chromophore (5, 6) or to produce it in cells by co-expressing several enzymes of bacterial origin (35), thus, affecting cellular metabolism.

[00119] Two similar TetR-*tetO* systems were compared, one based on the BphP1-PpsR2 described above and another system (9) based on the PhyB-PIF6 pair using the same pTRE-Tight-SEAP reporter plasmid. The BphP1-PpsR2 expressing cells were illuminated with 740 nm while the cells with the PhyB(1-650)-VP16-NLS and tetR-PIF6 constructs were

illuminated with 660 nm and also supplemented with 15 μ M of phycocyanobilin. Dependence of the efficiency of reporter activation on the light intensity was rather similar for the BphP1–PpsR2 and the PhyB–PIF6 systems and decreased with the attenuation of the light power. However, comparison of light propagation for 740 nm and 660 nm wavelengths in various mammalian tissues reveals substantially more effective penetration of NIR light. This results in much slower decrease of the level of reporter production with the tissue depth for the BphP1–PpsR2 system as compared to the PhyB–PIF6 system.

[00120] The *in vivo* comparison of transcription activation by the blue-light driven LightON system and NIR-light activated BphP1–PpsR2 system revealed a higher efficiency of the latter one at light intensities of ≤ 5 mW cm⁻², which are compliant with safety regulations. These light intensities caused a smaller increase of the Rluc8 signal in a deep-seated liver in the 470 nm illuminated animals as compared to the Rluc8 signal obtained with 90 mW cm⁻² illumination used in the original LightON paper (56).

[00121] In summary, the BphP1–PpsR2 light-controllable PPI was characterized both *in vitro* and in live mammalian cells and demonstration shown of the use of this light-sensitive pair as a novel optogenetic system for subcellular protein targeting, induction of intracellular enzymatic activity, activation of gene expression and control of DNA recombination. The BphP1–PpsR2 system is orthogonal to mammalian cells and minimally interferes with cellular metabolism.

Materials and Methods

[00122] Construction of bacterial and mammalian plasmids: A gene encoding *RpBphP1* from *R. palustris* was kindly provided by E. Giraud. A gene encoding *RpPpsR2* from *R. palustris* was kindly provided by M. Papiz. A DHPH domain of human intersectin-1 was PCR amplified from a pAL189 plasmid (Addgene #22278)(3). An mRuby2 gene was PCR amplified from a pcDNA3-mRuby2 plasmid (Addgene #40260)(36). A DNA binding domain of the transcription factor GAL4 from *S. cerevisiae* and a transactivation domain of transactivating tegument protein VP16 from *H. simplex* were PCR amplified from a pGV-2ER plasmid (Systasy). A reporter plasmid pG5-EGFP was purchased from Systasy. A SEAP gene was PCR amplified from a pKM611 plasmid kindly provided by W. Weber. A plasmid pKM022 encoding PhyB(1-650) was obtained by mutagenesis, using the pKM020 plasmid as a template (9). A Cre recombinase gene was PCR amplified from a pBS185-CMV-Cre (Addgene #11916) (37). A tetR gene (residues 1-207) that binds DNA in the

absence of tetracycline/doxycycline was PCR amplified from a pTet-Off vector (Clontech). The reporter plasmids pTRE-Tight-EGFP, pTRE-Tight-SEAP and pTRE-Tight-Cre were obtained by cloning of the EGFP, SEAP and Cre genes, respectively, into a pTRE-Tight2 vector (Addgene #19407). To develop stable preclonal cell mixtures of HeLa cells, plasmids encoding a transposase SB100X, pCMV(CAT)T7-SB100 (Addgene #34879)(38) and transposon bearing plasmids pT2/SVNeo and pT2/BH (Addgene #26553 and #26556)(39) were utilized. A reporter plasmid pCALNL-GFP was obtained from Addgene (#13770)(40).

[00123] For bacterial expression of the BphP1, BphP1-mRuby2 and PpsR2-mRuby2 proteins, a pBAD/His-D, a pBAD/His-B (Life Technologies-Invitrogen) and a pET22b (Novagen) vectors were used, respectively. In the pET22b vector an N-terminal pelB signal was replaced with a Strep-tag-II.

[00124] Mammalian expression plasmids were based either on a pEGFP-N1 vector (Clontech), having either a standard CMV promoter or a truncated to CMVd1 promoter, or on a pFC15K vector (Promega) with the truncated CMVd1 promoter. The flexible linkers of 10 (-DSAGSAGSAG-) (SEQ ID NO:1), 16 (-SAGGSAGGSAGGSAGG-) (SEQ ID NO:2), 20 (-SAGGSAGGSAGGSAGGSAGG-) (SEQ ID NO:3) or 24 (-SGGGSGGGSGGGSGGGSGGGSGGG-) (SEQ ID NO:4) amino acids, a C-terminal membrane-localization -CAAX signal from Kras4B (-KKKKKKSKTKCVIM) (SEQ ID NO:5), a nuclear localization signal of nuclear cap-binding protein subunit 1 (MSRRRHSYENDGGQPHKRRK-) (SEQ ID NO:6), a T2A peptide (-EGRGSLLTCDGVEENPGP-) (SEQ ID NO:7), and a Strep-tag-II (-WSHPQFEK-) (SEQ ID NO:8) were added by oligonucleotide annealing. The designed plasmids are summarized in Table 2.

[00125] Table 2. A list of the plasmids constructed in this study.

Plasmid	Figure	Vector backbone	Promoter	Insert
pKA-100	1, 6-8	pBAD/His-D	PBAD	<i>Bgl</i> II-BphP1- <i>Eco</i> RI
pKA-101	7	pBAD/His-B	PBAD	<i>Bgl</i> II-BphP1- <i>Eco</i> RI-10aaLinker- <i>Spe</i> I-mRuby2- <i>Hind</i> III
pKA-138.2	1, 7	pET-22b	T7	<i>Nde</i> I-Strep-tag II- <i>Nco</i> I-PpsR2- <i>Hind</i> III-20aaLinker- <i>Age</i> I-mRuby2- <i>Xho</i> I-His-tag
pKA-140	2	pEGFP-N1	CMV	<i>Kpn</i> I-PpsR2(C439S)- <i>Hind</i> III-20aaLinker- <i>Age</i> I-mVenus- <i>Nhe</i> I-Kras4BCT- <i>Not</i> I

pKA-141	2, 9	pFC15K	CMVd1	<i>NheI</i> -BphP1- <i>EcoRI</i> -10aaLinker- <i>SpeI</i> -mCherry- <i>XbaI</i>
pKA-142	2, 3, 9, 10	pFC15K	CMVd1	<i>AsiSI</i> -PpsR2(C439S)- <i>HindIII</i> -20aaLinker- <i>AgeI</i> -mVenus- <i>NheI</i> -Kras4BCT- <i>NotI</i>
pKA-144	3, 10	pFC15K	CMVd1	<i>NheI</i> -BphP1- <i>EcoRI</i> -10aaLinker- <i>SpeI</i> -mCherry- <i>BsrGI</i> -20aaLinker- <i>XbaI</i> -IntersectinDHPH- <i>NotI</i>
pKA-147	3	pFC15K	CMVd1	<i>AsiSI</i> -mVenus- <i>NheI</i> -Kras4BCT- <i>NotI</i>
pCMV-160	4, 11	pEGFP-N1	CMV	<i>AsiSI</i> -NLS- <i>AgeI</i> -PpsR2(C439S)- <i>HindIII</i> -20aaLinker- <i>AgeI</i> -mVenus- <i>XbaI</i>
pT2/SVNeo-103	4, 5, 11-15, 18	pT2/SVNeo	CMVd1	IRDR-CMVd1- <i>NheI</i> -BphP1- <i>EcoRI</i> -10aaLinker- <i>SpeI</i> -mCherry- <i>BsrGI</i> -24aaLinker- <i>XbaI</i> -TetR- <i>NotI</i> -NeoR-IRDR
pKA-207I10	5	pIRES-EGFP	CMV	<i>NheI</i> -NLS- <i>AgeI</i> -PpsR2- <i>HindIII</i> -20aaLinker- <i>NcoI</i> -VP16- <i>BglII</i> -IRESv10- <i>NcoI</i> -BphP1- <i>EcoRI</i> -10aaLinker-mCherry-TetR- <i>NotI</i>
pCMV-104	4, 5, 12-15, 18	pEGFP-N1	CMV	<i>AsiSI</i> -NLS- <i>AgeI</i> -PpsR2(C439S)- <i>HindIII</i> -20aaLinker- <i>AgeI</i> -VP16- <i>XbaI</i>
pTRE-Tight-SEAP	4, 12, 13 15	pTRE-Tight2	CMV _{min}	<i>XhoI</i> -7xtet-responsive element-P _{minCMV} - <i>BamHI</i> -SEAP- <i>NotI</i>
pTRE-Tight-EGFP	14	pTRE-Tight2	CMV _{min}	<i>XhoI</i> -7xtet-responsive element-P _{minCMV} - <i>BamHI</i> -EGFP- <i>NotI</i>
pTRE-Tight-Rluc8	5, 19	pTRE-Tight2	CMV _{min}	<i>XhoI</i> -7xtet-responsive element-P _{minCMV} - <i>BamHI</i> -Rluc8- <i>NotI</i>
pU5-Rluc8	5, 18-20	pU5-mCherry	min TATA-box promoter	5xGAL4-binding UAS-minimal promoter- <i>HindIII</i> -Rluc8- <i>BamHI</i>

[00126] Protein expression and purification: BphP1 and BphP1-mRuby2 proteins with polyhistidine tags on the N-terminus were expressed in LMG194 bacterial cells (Life Technologies-Invitrogen) containing a pWA23h plasmid encoding heme oxygenase for biliverdin synthesis in *E. coli* (12). The bacterial cells were grown in RM medium supplemented with ampicillin, kanamycin and 0.02% rhamnose for 6-8 h followed by an induction of the protein expression by adding of 0.002% arabinose. The proteins were purified using a Ni-NTA agarose (Qiagen). The PpsR2 and PpsR2-mVenus proteins with a Strep-tag-II at the N-terminus and a polyhistidine tag at the C-terminus was expressed in

BL21(DE3) bacterial cells grown in LB medium supplemented with ampicillin for 6 h, followed by an induction of a protein expression with 250 μ M IPTG. The proteins were first purified with a Ni-NTA agarose (Qiagen) followed by purification with a Strep-Tactin sepharose (IBA Lifesciences).

[00127] *In vitro* characterizations of BphP1 properties: For absorbance measurements, a Hitachi U-2000 spectrophotometer was used. For fluorescence measurements of mRuby2 fusions, a FluoroMax-3 spectrofluorometer (Horiba-Jobin Yvon) equipped with a 600 nm shortpass filter before detector was used. A photoconversion of BphP1 containing proteins was performed with 740/25 nm and 636/20 nm custom assemble LED sources in quartz microcuvettes (Starna Cells). A determination of action spectrum was performed by measurement of changing in absorbance of Pfr state BphP1 at 780 nm upon illumination with photoconversion light. As a source of light the FluoroMax-3 spectrofluorometer was used. The illumination time was normalized to total amount of irradiated light energy, measured with a PM100 optical powermeter equipped with a S130A sensor (ThorLabs) at the respective wavelength. A half-time of Pr→Pfr transition (or dark relaxation) was measured by registering of absorbance at 780 nm after 5 min illumination of samples with 740/25 nm. Samples containing fixed quantity of BphP1 (5 μ M) and various quantities of PpsR2 (0-5 μ M) were pre-incubated in darkness for 30 min. Reversible dark relaxation cycles were obtained by registration 780 nm absorbance of protein mixture at BphP1:PpsR2 molar ratio of 8:1. To study changes in mRuby2 fluorescence due to FRET between the PpsR2-mRuby2 fusion and the Pr state of BphP1, the proteins (2.5 μ M each) were pre-incubated in darkness for 30 min and then transferred to a microcuvette. An mRuby2 fluorescence intensity was registered each 30 s with excitation 545/2 nm and emission 585/10 nm. All spectroscopic measurements were performed at a room temperature in PBS.

[00128] Mammalian cell culture: HeLa cells were grown in DMEM medium supplemented with 10% FBS, penicillin-streptomycin mixture and 2 mM glutamine (all from Life Technologies-Invitrogen) at 37°C. For experiments 10⁵ cells were plated on a pre-coated with ECL mixture (EMD-Millipore) 35 mm glass-bottom culture dishes (MatTek). Transient cell transfections were performed using an Effectene reagent (Qiagen).

[00129] Preclonal mixtures of HeLa cells were obtained using the plasmid-based Sleeping Beauty transposon system. For this, the desired for integration into genome sequences were cloned into the transposon bearing plasmids pT2/BH or pT2/SVNeo and co-transfected with a plasmid encoding a hyperactive transposase SB100X. Cells were further

selected with 700 µg/ml of G418 antibiotic for two weeks and enriched using a FACSARIA sorter (BD Biosciences), resulting in the preclonal HeLa cell mixtures stably expressing NLS-VP16-PpsR2-T2A-BphP1-mCherry-GAL4 or BphP1-mCherry-tetR.

[00130] For light-induced re-localization to plasma membrane, HeLa cells were transiently co-transfected with pKA-140 or pKA-142 and pKA-141 plasmids in 1:1 ratio. For light-induced cytoskeletal rearrangement, cells were co-transfected with pKA-142 (or pKA-147) and pKA-144 plasmids in 1:2 ratio. The cell light-activation and imaging were typically performed 48 h after the transfection. For light-induced nuclear re-localization, HeLa cells stably expressing BphP1-mCherry-tetR were transiently transfected with pCMV-160. To study transcription activation, HeLa cells stably expressing NLS-VP16-PpsR2-T2ABphP1-mCherry-GAL4 were transfected with a pG5-EGFP for light-induced EGFP expression or with a pG5-SEAP for kinetic studies. To study light-induced transcription activation in tetR-based system HeLa cells stably expressing BphP1-mCherry-tetR were co-transfected with pCMV-104 and either pTRE-Tight-EGFP or pTRE-Tight-SEAP plasmids in 5:1 ratio. To study Cre-driven expression of EGFP HeLa cells stably expressing BphP-mCherry-tetR were co-transfected with pTRE-Tight-Cre, pCALNL-GFP and pAS-104 plasmids in 0.6:1:2 ratio. To compare the PhyB-PIF6 system with the BphP1-PpsR2 system, HeLa cells were transfected with pKM022 and pTRE-Tight-SEAP plasmids in 2:1 ratio. All procedures after the transfection were performed as described in the respective PhyB-PIF6 paper (9).

[00131] Cell light-activation and imaging: Imaging was performed using an Olympus IX81 inverted epifluorescence microscope equipped with a 200 W metal halide arc lamp (Lumen 220PRO, Prior) and a 60x 1.35 NA oil immersion objective lens (UPlanSApo, Olympus). During imaging HeLa cells were incubated in a cell imaging medium (Life Technologies-Invitrogen) and kept at 37°C. Yellow (523/20 nm exciter and 565/40 nm emitter) and red (570/30 nm exciter and 615/30 nm emitter) channel filter sets (Chroma) were used for detection of mVenus and mCherry fluorescence, respectively. The Pfr→Pr photoconversion of BphP1 was done by illuminating with 740/40 nm filter (Chroma).

[00132] For Pfr→Pr re-localization assay, HeLa cells were exposed to 740/40 nm for 3 min while imaged every 15 s. An intensity of activation light was 0.9 mW/cm². To quantify mCherry fluorescence in cell cytoplasm during BphP1 dark relaxation, HeLa cells were imaged every 3 min during 24 min, starting immediately after the Pfr→Pr photoconversion. Intensity profile of mCherry fluorescence through cell was determined using an ImageJ

v.1.46f software. Intensity of mCherry fluorescence in cytoplasm and nucleus was measured using a SlideBook v.4.2.09.

[00133] To study light-induced cytoskeletal rearrangements, the Pfr→Pr photoconversion was done by illuminating cells using 740/40 nm filter for 3 min and imaged every 15 s, followed by further maintaining of BphP1 in the Pr state by illuminating with 740/40 nm for 15 s every minute and imaged every 60 s. An intensity of activation light was 0.2 mW/cm². The total time of cells imaging was 30 min. The light power densities were measured at a back focal plane of a 60x 1.35 NA objective lens. Membrane-localized mVenus was used for determination of cell area. The data were analyzed using an ImageJ v.1.46f software.

[00134] Unless otherwise indicated, a light-induced transcription activation HeLa cells was performed with 740/25 nm LED source at 1.0 mW cm⁻² using the 30 s On and 180 s Off cycle in CO₂ incubator at 37°C. Time of illumination varied in different experiments. For nuclear localization and EGFP or SEAP transcription activation experiments, cells were illuminated for 24 and 48 h, respectively. For kinetic studies of SEAP accumulation in culture media, 3 different illumination regimes were used: 72 h of 740/25 nm; 12 h of 740/25 nm followed by 60 h of darkness; and 12 h of 740/25 nm followed by 12 h of 636/20 nm followed by 38 h of darkness. To measure light sensitivity of activation of SEAP expression, HeLa cells were irradiated with either 740/25 (with BphP1–PpsR2) or 660/20 nm (with PhyB–PIF6) light of various power densities (5-1000 μW cm⁻²). Light power densities and durations of illumination were converted to photons counts.

[00135] Light-induced LightON transcription activation of *Rluc8* in HeLa cells was performed as described in the original paper (56). In brief, HeLa cells were transfected with pGAVPO and pU5-*Rluc8* plasmids in 1:1 ratio. Then cells were kept in darkness for 10 h. After change of culture medium, the cells were continuously illuminated with a 470/15 nm LED array (LuxeonStar) of 1 mW cm⁻² or remained in a darkness for 48 h before analysis. For an analysis, the cells were resuspended in PBS and disrupted by freezing-thawing. An *Rluc8* bioluminescence signal was measured in supernatants after adding of 5 μM h-coelenterazine (NanoLight Technology). A signal was detected using an IVIS Spectrum instrument (PerkinElmer/Caliper Life Sciences) and analyzed using Living Image 3.0 software (PerkinElmer/Caliper Life Sciences).

[00136] Flow cytometry analysis: Flow cytometry analysis of a light-induced EGFP expression and a Cre mediated EGFP expression was performed 48 h after the HeLa cell

transfection using a LSRII flow cytometer (BD Biosciences) equipped with a 488 nm laser and a 530/40 nm emission filter and with a 561 nm laser and 610/20 nm emission filter. To calculate an efficiency of the light-induced EGFP expression, a mean fluorescent intensity of EGFP positive cells was multiplied by a number of the positive cells, resulting in the total amount of synthesized proteins. In contrast, for a Cre mediated EGFP expression, the EGFP positive cells were counted only. Gates for counting of EGFP positive cells were set using non-transfected cells as a negative control. Typically, the cell samples were triplicated. Minimally, 5×10^4 cells were analyzed per a sample. The data were analyzed using a FACSDiva v. 6.1.3 and a FlowJo v. 7.6.2 software.

[00137] Secreted alkaline phosphatase assay: For SEAP detection in culture media, a Great EscApe fluorescent SEAP Assay kit (Clontech) was used. 25 μ l aliquots of cell culture media from wells of a 6-well plate or a 12-well plate were collected at each time point and stored at -20°C. For kinetics studies, a culture medium was changed with a fresh medium at each time point. A fluorescence intensity of the SEAP reaction product was measured using the SpectraMax-M2 plate reader.

[00138] Light-activation and imaging in mice. The FVB 2-3 month old female mice (National Cancer Institute, NIH) of 20-25 g body weight were used for *in vivo* comparison of the LightON and BphP1-PpsR2 systems. To compare an efficiency of the light-induced transcription activation, HeLa cells bearing either the LightON system or the BphP1-PpsR2 system were injected subcutaneously in the interscapular area of FVB mice. For better illumination the fur on the back was removed using a depilatory cream. For the LightON system, HeLa cells were co-transfected with the pGAVPO and pU5-Rluc8 plasmids in a 1:1 ratio, and for the BphP1-PpsR2 system, HeLa cells stably expressing BphP1-mCherry-TetR were co-transfected with the plasmid encoding NLS-PpsR2-VP16 and the pTRE-Tight-Rluc8 reporter plasmid in a 5:1 ratio. For both systems, the 3×10^6 HeLa cells in 100 μ l of RPMI-1640 media supplemented with 2 mM L-glutamine were injected subcutaneously 24 h after the transfection. All manipulations with cells before and during the injection were performed under a 640 nm safelight for the LightON system and under a 530 nm safelight for the BphP1-PpsR2 system. After cells injection, mice were placed in transparent cages with the top illumination of either the 470/15 nm LED array or the 740/25 nm LED array, respectively. Intensities of 470/15 nm light and 740/25 nm light were the same and equal to 1 mW cm⁻². Control mice after injection of the cells were kept in darkness in conventional

cages. Animals were continuously illuminated or kept in darkness for 48 h, and every 12 h were released and fed for 30 min. Each experimental group contained 3 mice.

[00139] For hydrodynamic transfection into liver (57), the Swiss Webster 2-3 month old female mice (National Cancer Institute, NIH) with body weight of 22-25 g were used. For the BphP1-PpsR2 system, 50 µg of the pKA-207I10 plasmid and 5 µg of the pTRE-Tight-Rluc8 reporter plasmid in 2.5 ml of PBS were intravenously injected through a tail vein. For hydrodynamic transfection by the LightON system, the plasmid amounts either indicated in the original paper (56) were used (10 µg of the pGAVPO plasmid and 300 µg of the pU5-Rluc8 plasmid) or similar to those used for the BphP1-PpsR2 system (50 µg of the pGAVPO plasmid and 5 µg of the pU5-Rluc8 plasmid). The mice were placed in transparent cages and illuminated from the bottom with the 470/15 nm LED array and the 740/25 nm LED array, respectively. Intensity of the both activation light was the same and equal to 5 mW cm⁻². For better illumination and imaging the belly fur was removed using a depilatory cream. Control animals were kept in conventional cages in complete darkness. Animals were continuously illuminated or kept in darkness for 72 h, and every 12 h were released and fed for 30 min. Each experimental group contained 3 mice.

[00140] For bioluminescence detection, 48 h after the HeLa cells injection or every 24 h after the hydrodynamic transfection of livers the animals were imaged using an IVIS Spectrum instrument (Perkin Elmer/Caliper Life Sciences) in luminescence mode with an open emission filter. Throughout the imaging, animals were maintained under anesthesia with 1.5% vaporized isoflurane. Prior to imaging, 80 µg of Inject-A-Lume CTZ native (NanoLight Technology) were intravenously injected through a retro-orbital vein. Data were analyzed using Living Image 3.0 software (Perkin Elmer/Caliper Life Sciences).

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What is claimed is:

1. An isolated nucleic acid encoding (i) a protein having the sequence of *Rhodopseudomonas palustris* BphP1 and (ii) a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*.
2. An isolated nucleic acid encoding (i) a protein having the sequence of *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*.
3. An isolated nucleic acid comprising a first and second portion, the first portion encoding a protein having the sequence of *Rhodopseudomonas palustris* BphP1 and a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*, and the second portion encoding a protein having the sequence of *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and a protein or peptide which is heterologous relative to *Rhodopseudomonas palustris*.
4. The nucleic acid of Claim 3, wherein the first portion is contiguous with the second portion.
5. The nucleic acid of Claim 3, wherein the first portion is contiguous with an oligonucleotide portion encoding a self-cleaving peptide, which oligonucleotide portion is contiguous with the second portion.
6. The nucleic acid of any of Claims 1-5 further encoding a detectable marker protein or detectable marker peptide.
7. The nucleic acid of Claim 6, wherein the nucleic acid encodes a detectable marker protein which is a fluorescent protein.
8. A cell, wherein the cell is not in a human being, transformed with the nucleic acid of any of Claims 1 to 7.

9. A fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and a second protein or peptide.
10. A fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 a non-dimerizing variant thereof and a second protein or peptide.
11. The fusion protein of Claim 9 or 10, wherein the second protein or peptide is heterologous relative to *Rhodopseudomonas palustris*.
12. The fusion protein of any of Claims 9-11 further comprising a detectable marker protein or detectable marker peptide.
13. The fusion protein of Claim 12, wherein the fusion protein comprises a detectable marker protein which is a fluorescent protein.
14. A method for inducing interaction of a first protein with a second protein in a system, the method comprising
providing a system comprising (a) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) the first protein, and (b) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) the second protein, and (c) an amount of biliverdin; and
irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.
15. The method of Claim 14, wherein the first or second protein is a DNA-binding protein.
16. The method of Claim 14 or 15, wherein the first or second protein comprises a transcriptional activator protein.

17. The method of any of Claims 14-16, wherein at least one fusion protein further comprises a detectable marker protein or detectable marker peptide.
18. The method of Claim 17, wherein the one fusion protein or both fusion proteins comprise different detectable marker proteins which are each fluorescent proteins.
19. The method of any of Claims 14-18, wherein the system is a eukaryotic cell.
20. The method of 19, wherein the eukaryotic cell is *in vivo*.
21. The method of 19, wherein the eukaryotic cell is *in vivo*.
22. The method of any of Claims 19-21, further comprising providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* BphP1.
23. The method of any of Claims 19-22, further comprising providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and the second protein.
24. A method for inducing translocation of a predetermined protein to a predetermined location in a cell comprising
providing a system comprising (a) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) a first protein, and (b) a fusion protein comprising (i) a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (ii) a second protein, and (c) an amount of biliverdin, wherein one of the first and second proteins is the predetermined protein and wherein the other of the first and second proteins is a protein that preferentially locates to the predetermined location in a cell; and
irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.

25. The method of Claim 24, wherein the protein that preferentially locates to the predetermined location in a cell, preferentially locates to a plasma membrane of a cell.
26. The method of Claim 24, wherein the protein that preferentially locates to the predetermined location in a cell, preferentially locates to an organelle of a cell.
27. The method of Claim 24, wherein the protein that preferentially locates to the predetermined location in a cell, preferentially locates to a nucleus of a cell.
28. The method of Claim 27, wherein the fusion protein comprising the first or second protein that preferentially locates to a nucleus, further comprises a DNA-binding protein.
29. The method of Claim 27, wherein the fusion protein comprising the first or second protein that preferentially locates to a nucleus, further comprises a transcriptional activator protein.
30. The method of any of Claims 24-29, wherein one fusion protein or both fusion proteins further comprise a detectable marker protein or detectable marker peptide.
31. The method of Claim 30, wherein the one fusion protein or both fusion proteins comprises different detectable marker protein which are each fluorescent proteins.
32. The method of any of Claims 24-31, wherein the cell is a eukaryotic cell.
33. The method of Claim 32, wherein the eukaryotic cell is *in vivo*.
34. The method of Claim 32, wherein the eukaryotic cell is *in vitro*.
35. The method of any of Claims 24-34, further comprising providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* BphP1.

36. The method of any of Claims 24-35, further comprising providing the system by transfecting the cell with a nucleic acid encoding the fusion protein comprising a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.

37. The method of any of Claims 14-36, wherein the near infra red light is 740 nm to 780 nm light.

38. The method of any of Claims 14-37, further comprising irradiating the system with far infrared light sufficient to induce dissociation of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof.

39. The method of Claim 38, wherein the method subsequently reduces the interaction of a first protein with a second protein in the system.

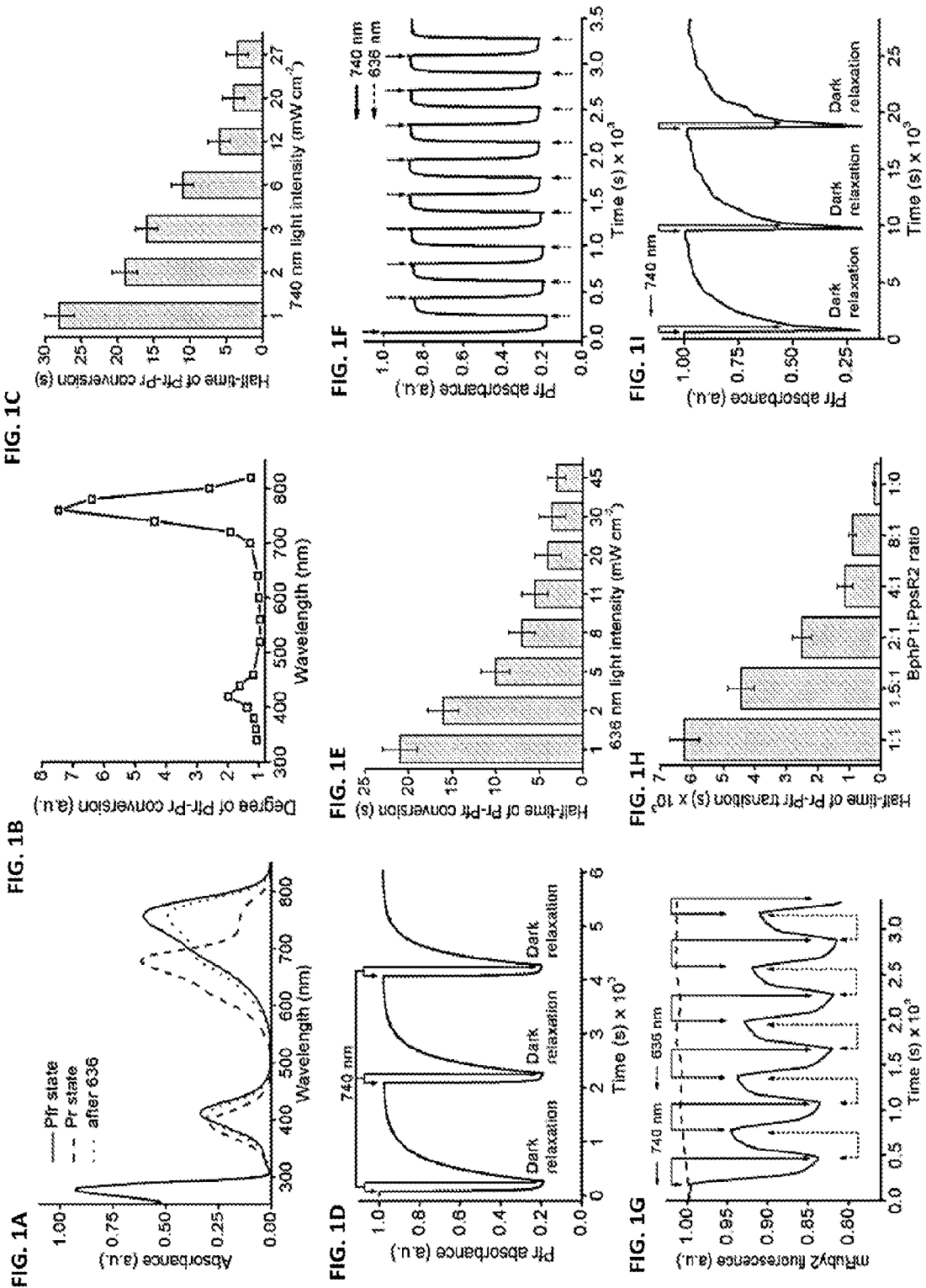
40. The method of Claim 38, wherein the method subsequently induces relocation of a predetermined protein away from the predetermined location in a cell.

41. A method for artificially expressing a heterogenous gene *in vivo* in a subject comprising administering to the subject: (a) a nucleic acid encoding (i) a protein having the sequence of a *Rhodopseudomonas palustris* BphP1 and (ii) a TetR tetracycline repressor protein (TetR) or a VP16 transcriptional activation domain (VP16); (b) a nucleic acid encoding (i) nuclear localization signal, a protein having the sequence of a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and (iii) a VP16 or a TetR; and (c) a nucleic acid encoding (i) a tetracycline response element (TRE) and the heterogenous gene;

wherein only one of (a) and (b) encodes a VP16 and the other of (a) or (b) encodes a TetR; and

irradiating the system with near infrared light sufficient to induce interaction of a *Rhodopseudomonas palustris* BphP1 with a *Rhodopseudomonas palustris* PpsR2 or a non-dimerizing variant thereof and thereby effect expression of the heterogenous gene.

42. The method of Claim 41, further comprising administering an amount of biliverdin to the subject.
43. The method of Claim 41 or 42, further comprising detecting the expression of the heterogenous gene in the subject.
44. The method of Claim 41, 42 or 43, further comprising quantifying the expression of the heterogenous gene in the subject.
45. The method of any of Claims 41-44, further comprising administering to the subject a blue light-activated gene expression system for a second heterogeneous gene and irradiating the system with blue light sufficient to induce expression of the second heterogenous gene.
46. The method of Claim 45, wherein the system is irradiated with blue light comprising 470 nm light.
47. The method of any of Claims 41-45, wherein the TRE is comprises a pTRE plasmid.
48. A kit comprising any of the nucleic acids, fusion proteins and/or systems described herein and instructions for use.



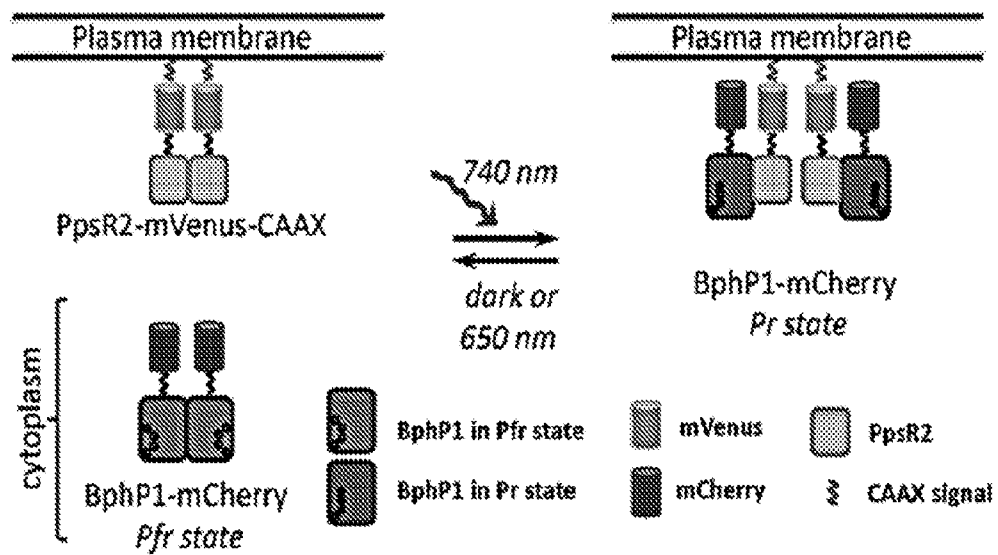


FIG. 2A

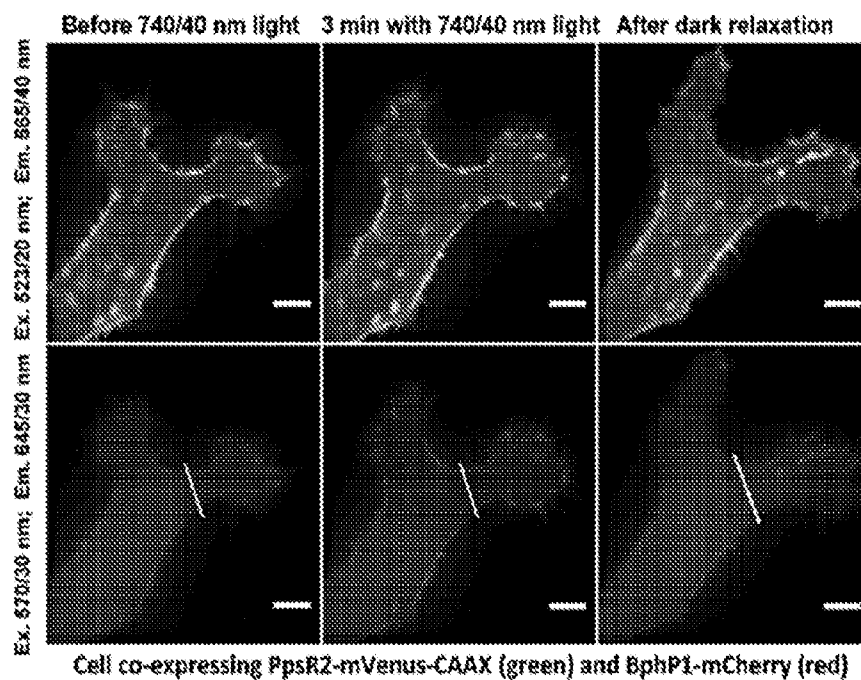


FIG. 2B

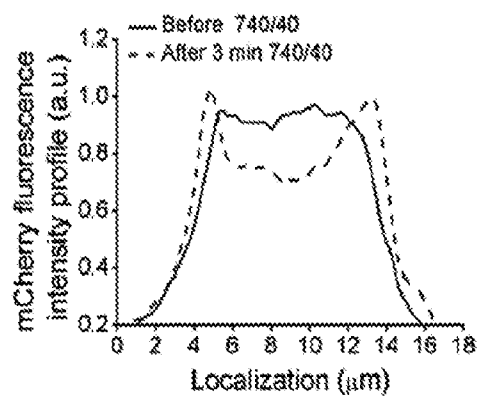


FIG. 2C

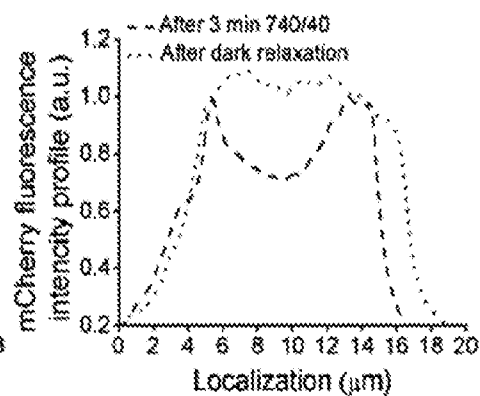


FIG. 2D

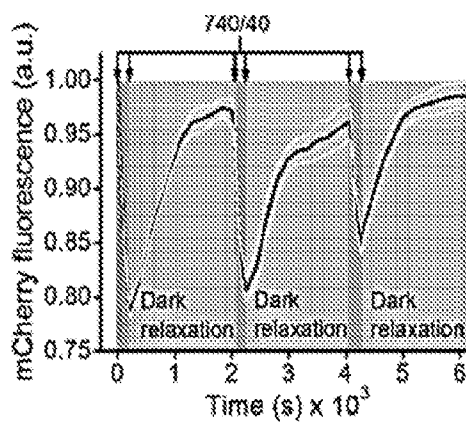


FIG. 2E

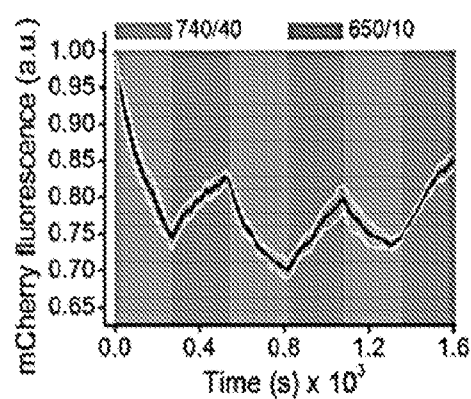
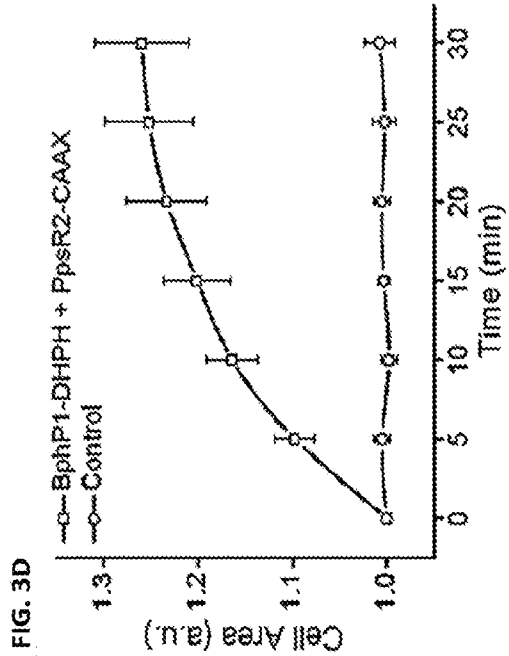
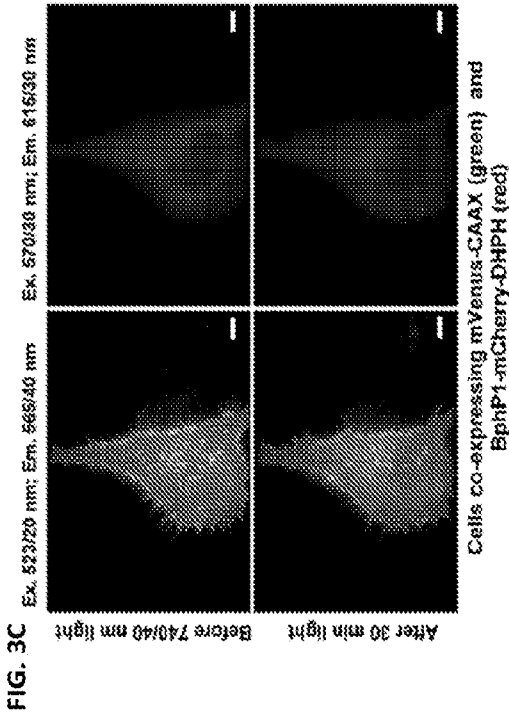
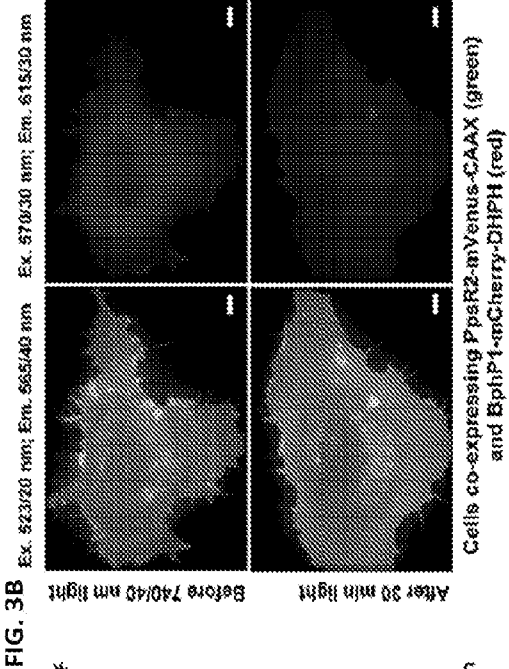
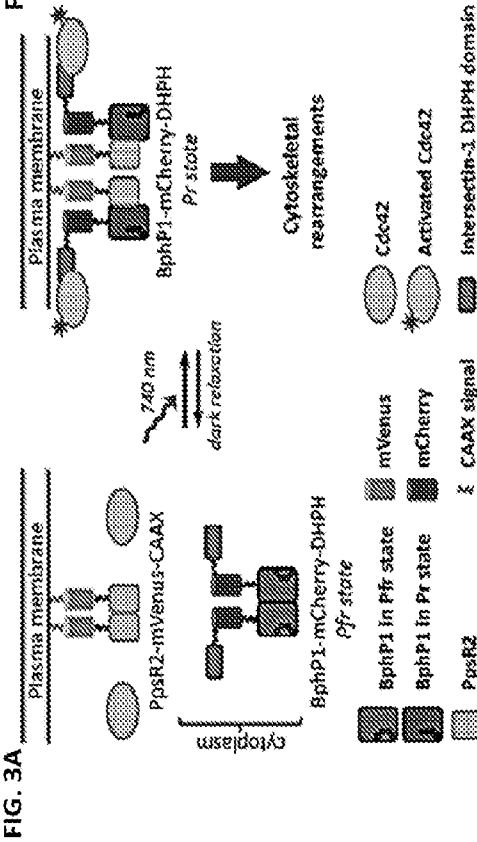
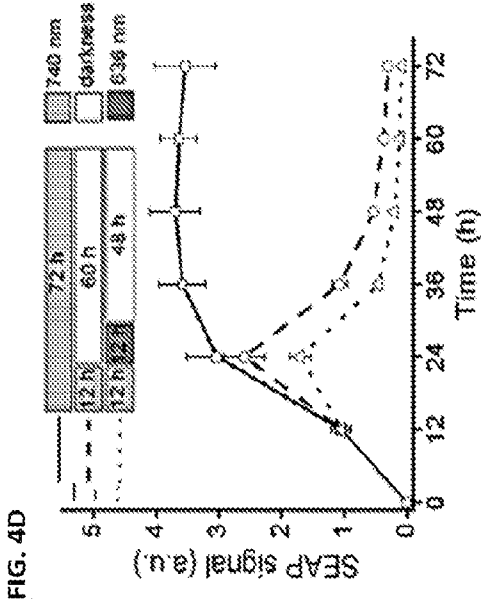
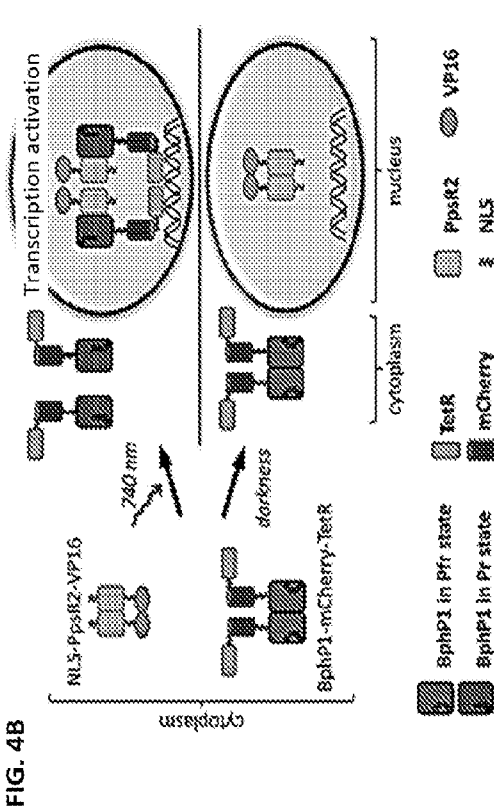
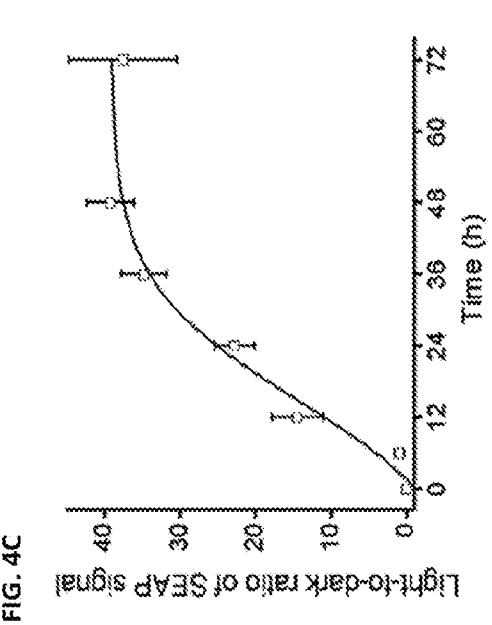
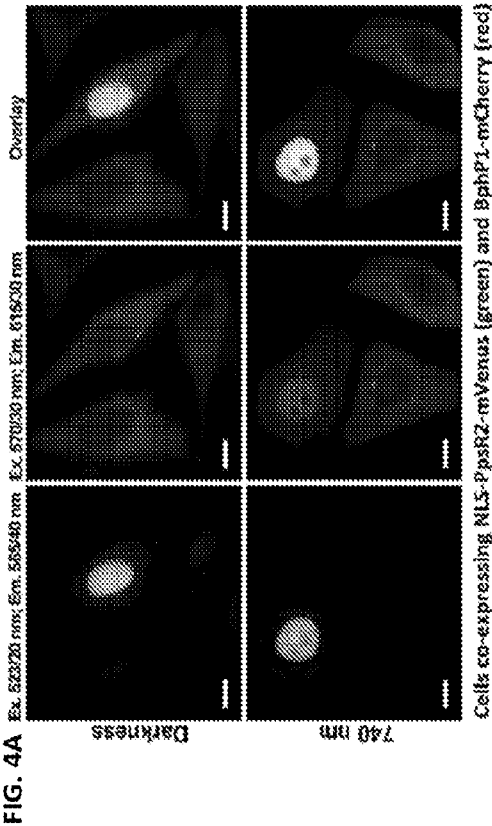


FIG. 2F





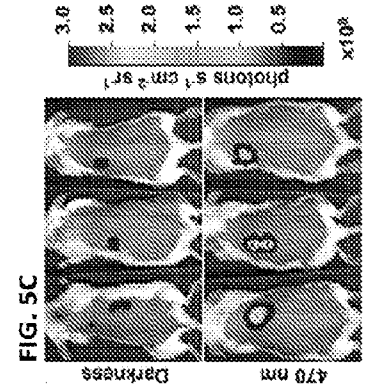
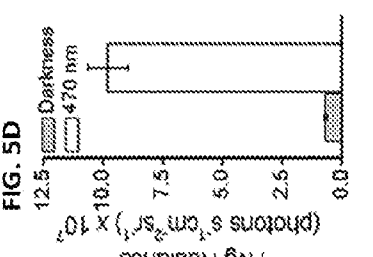
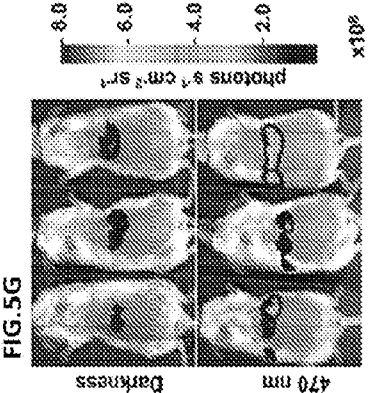
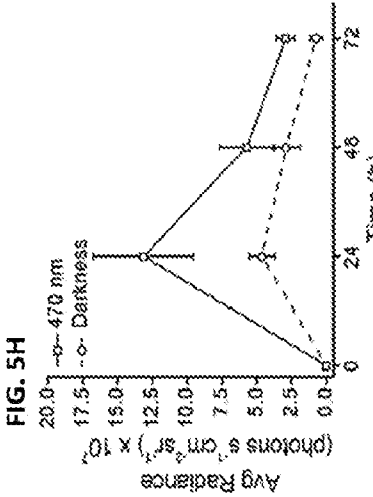
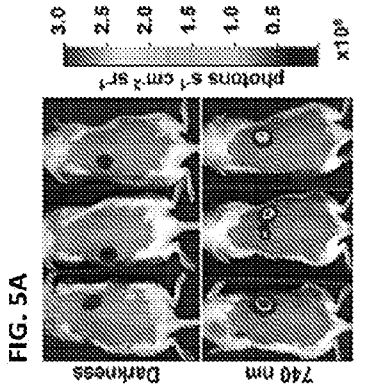
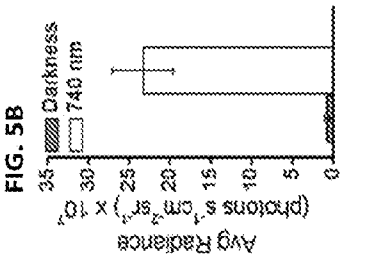
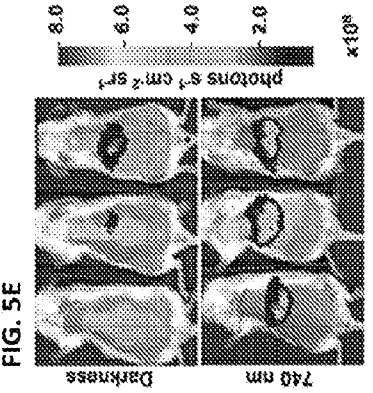
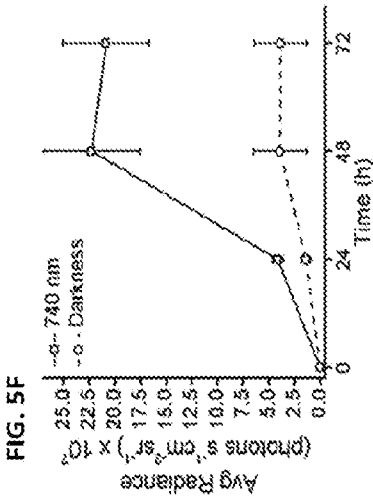


FIG. 6B

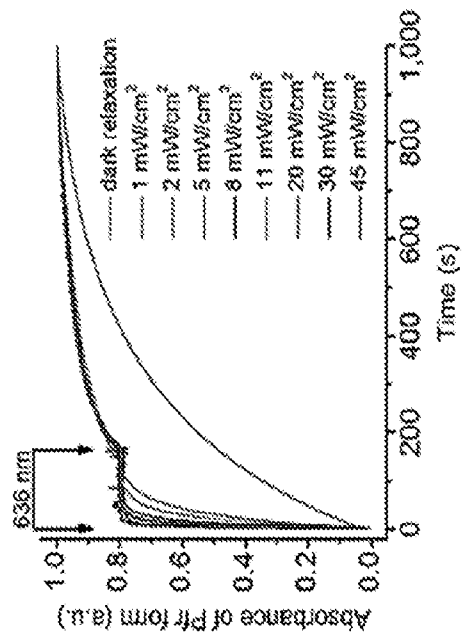


FIG. 6A

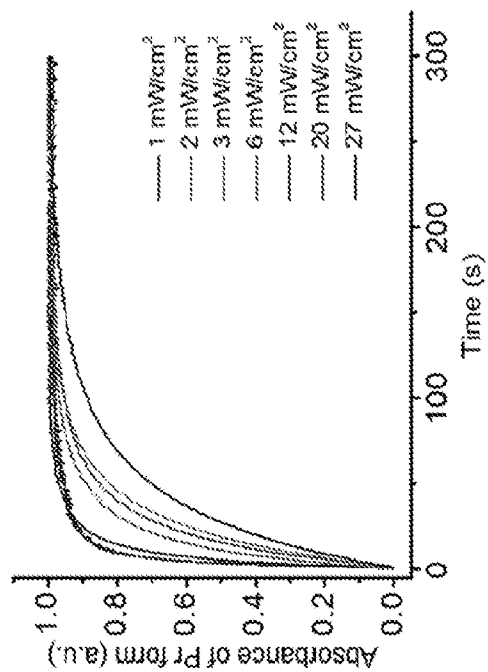


FIG. 7A

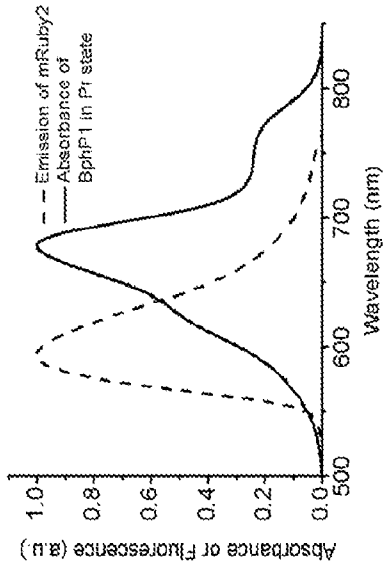


FIG. 7B

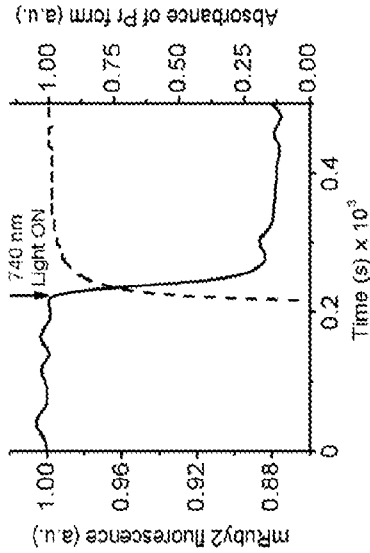
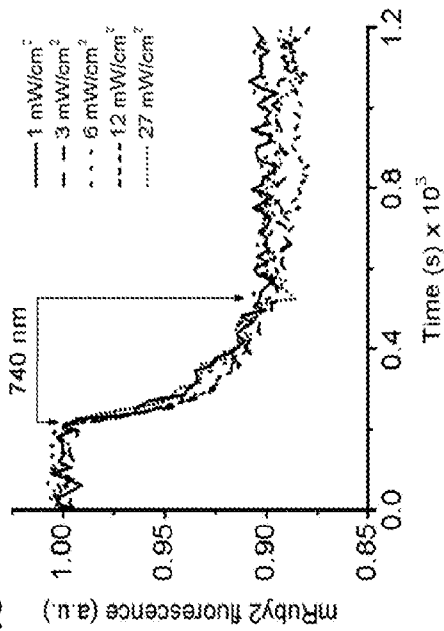


FIG. 7C



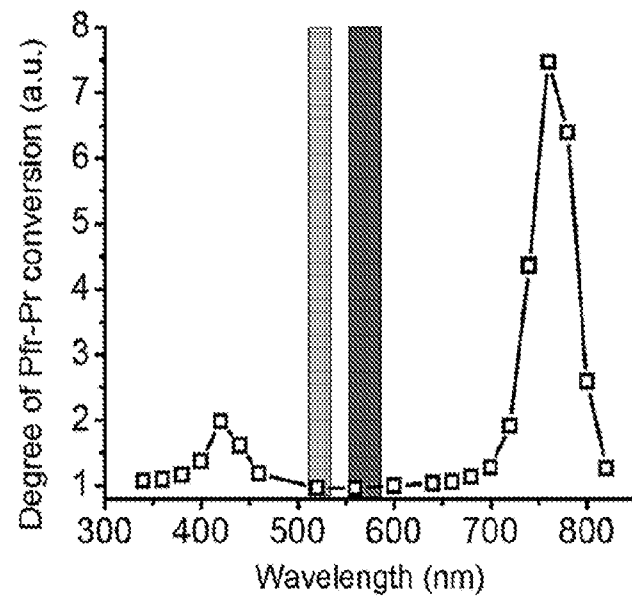


FIG. 8

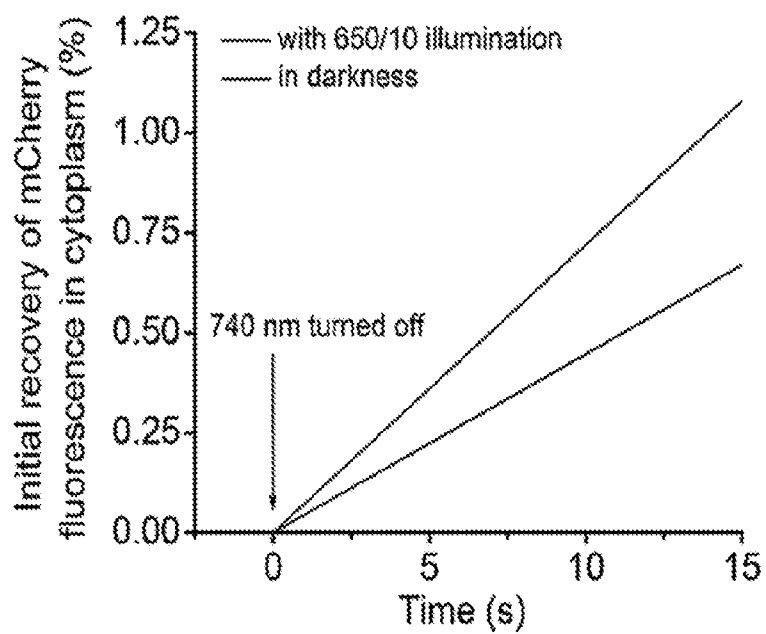


FIG. 9

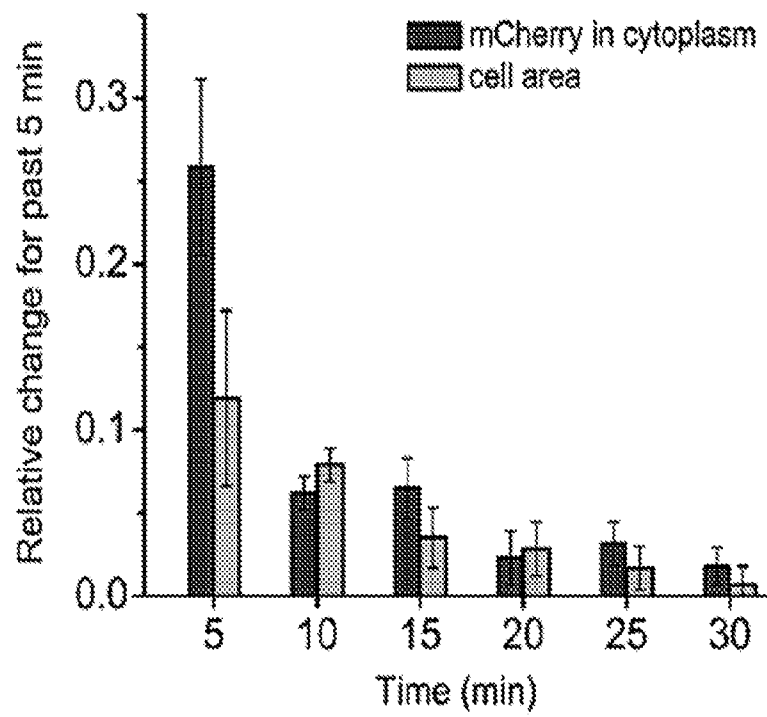


FIG. 10

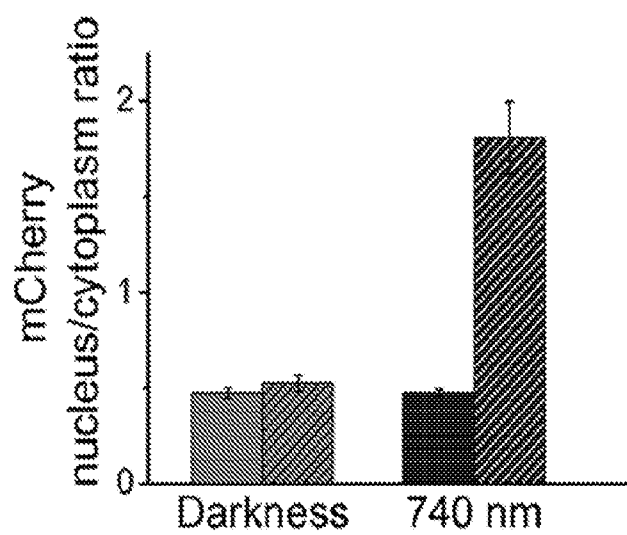


FIG. 11

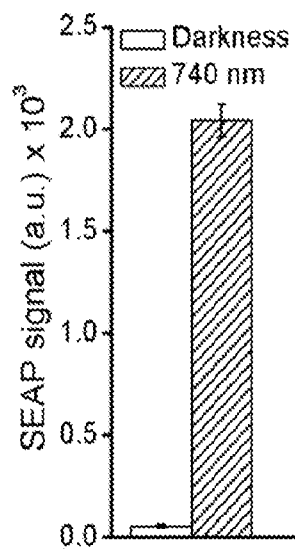


FIG. 12A

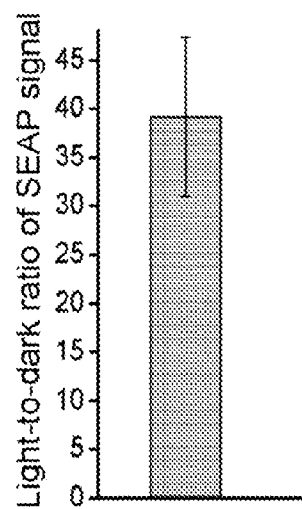


FIG. 12B

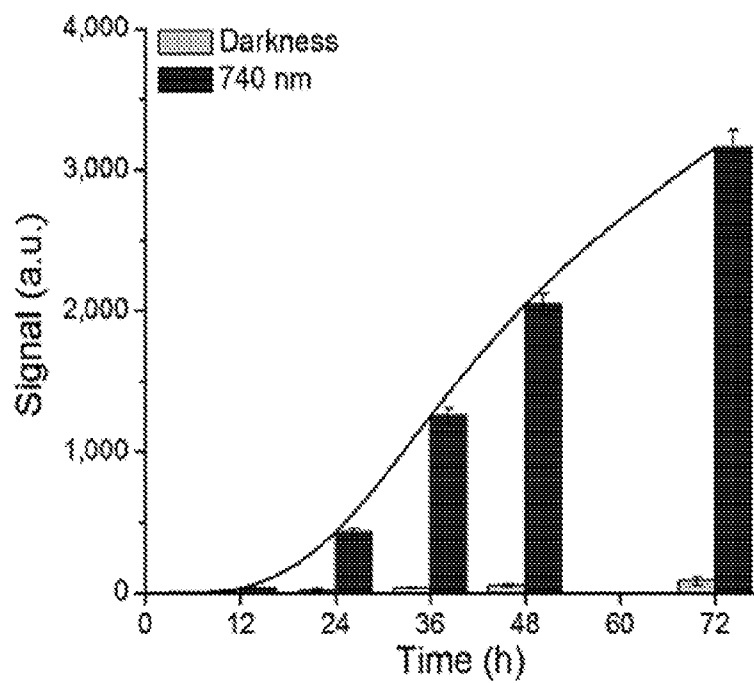


FIG. 12C

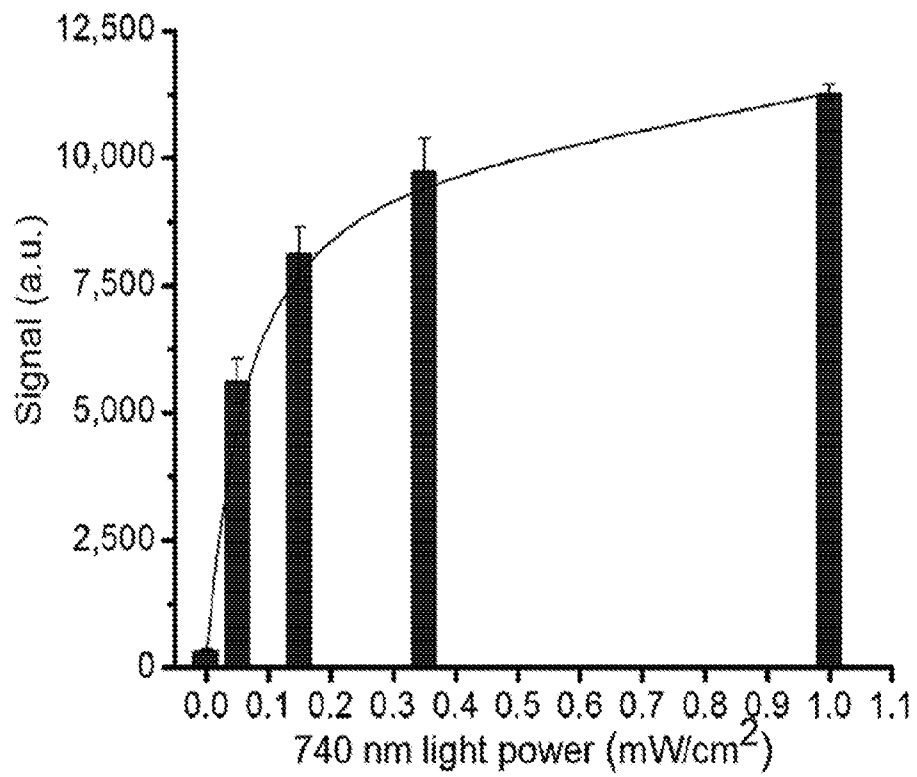


FIG. 13

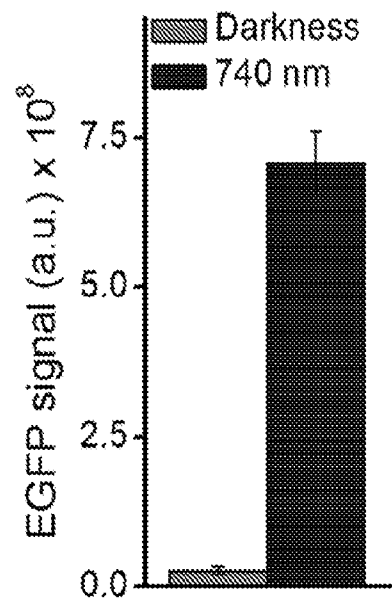


FIG. 14A

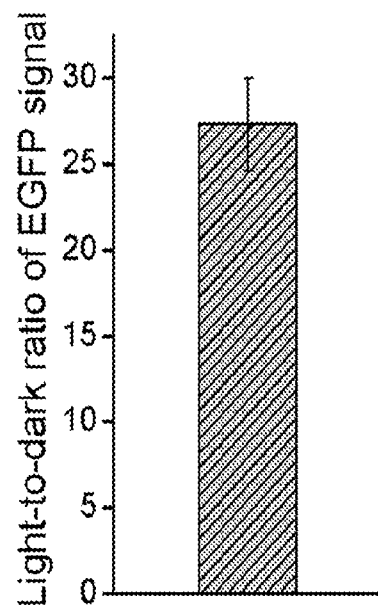


FIG. 14B

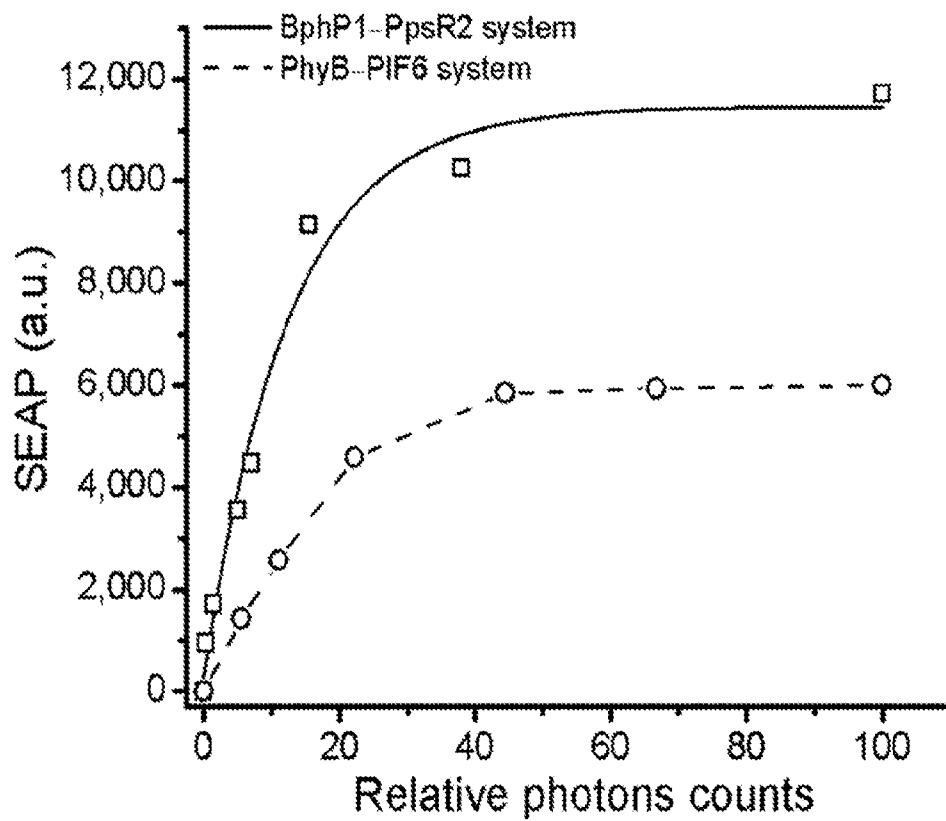


FIG. 15

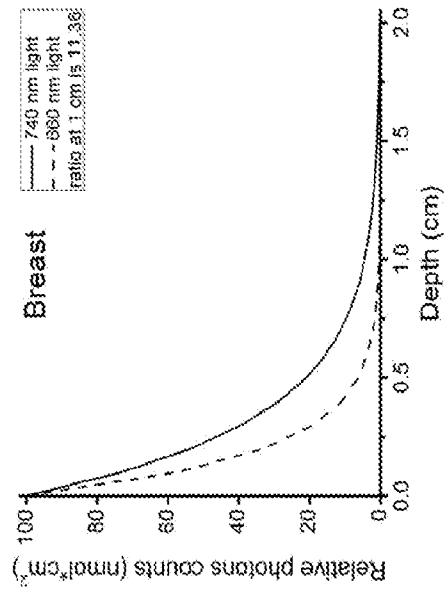


FIG. 16B

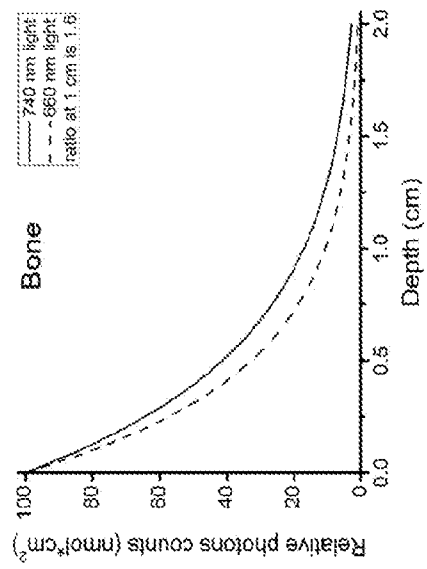


FIG. 16D

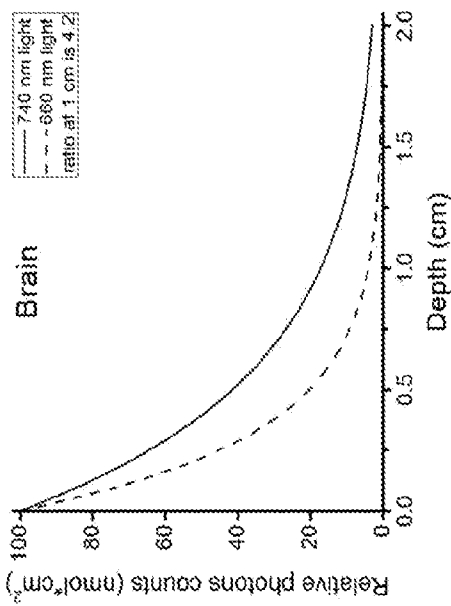


FIG. 16A

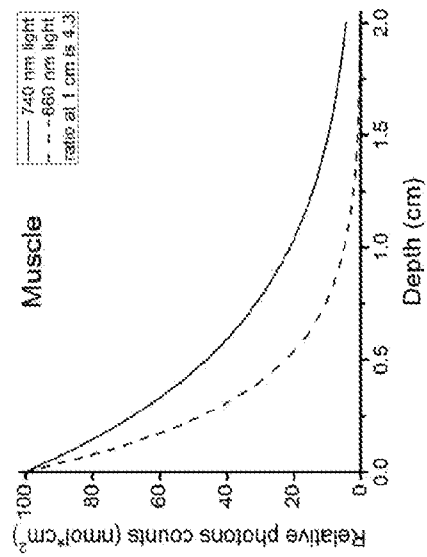


FIG. 16C

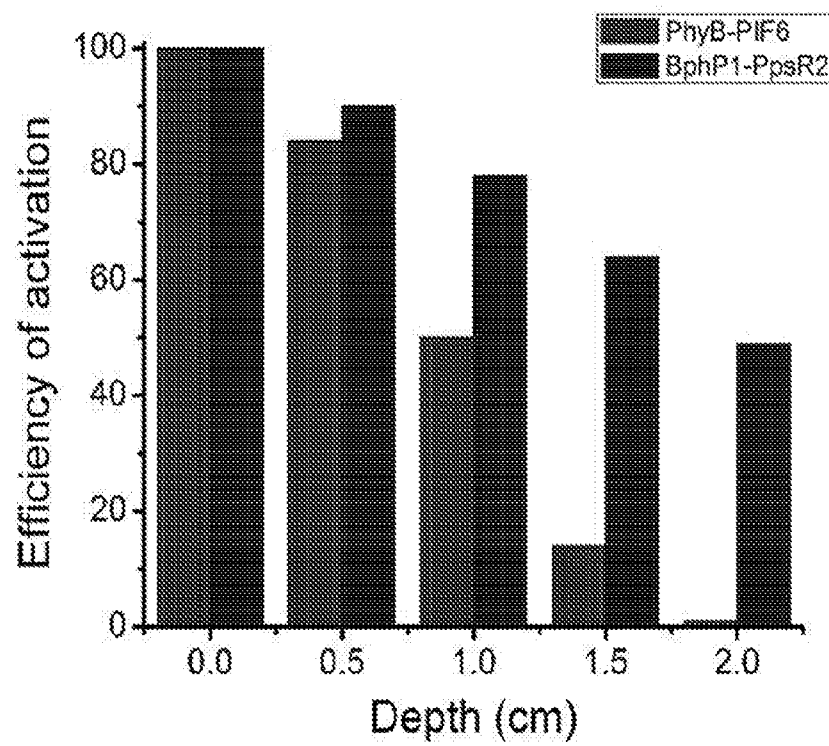


FIG. 17

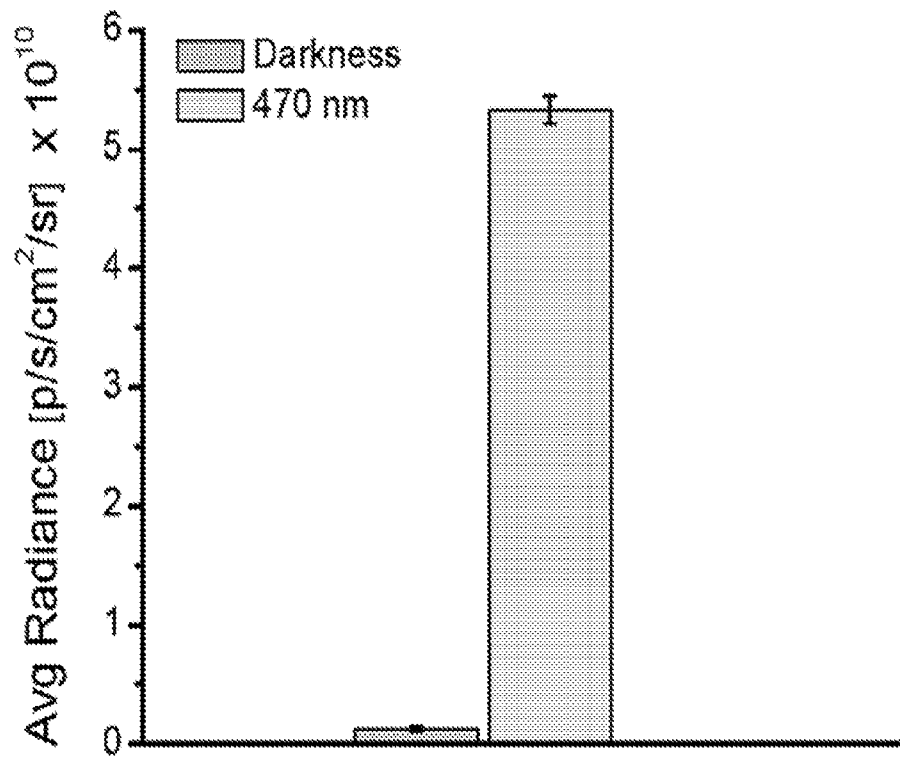


FIG. 18

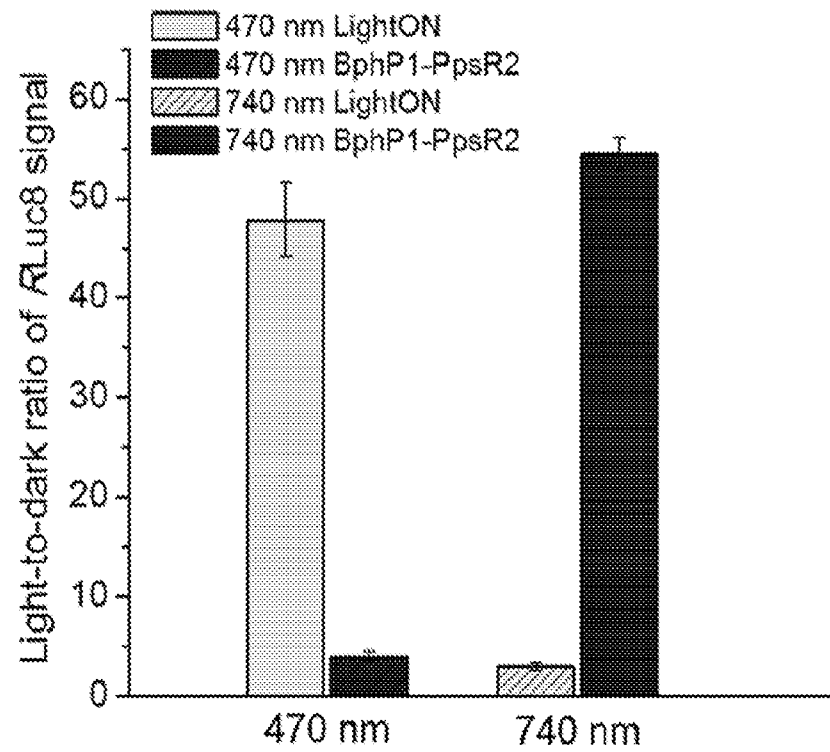


FIG. 19

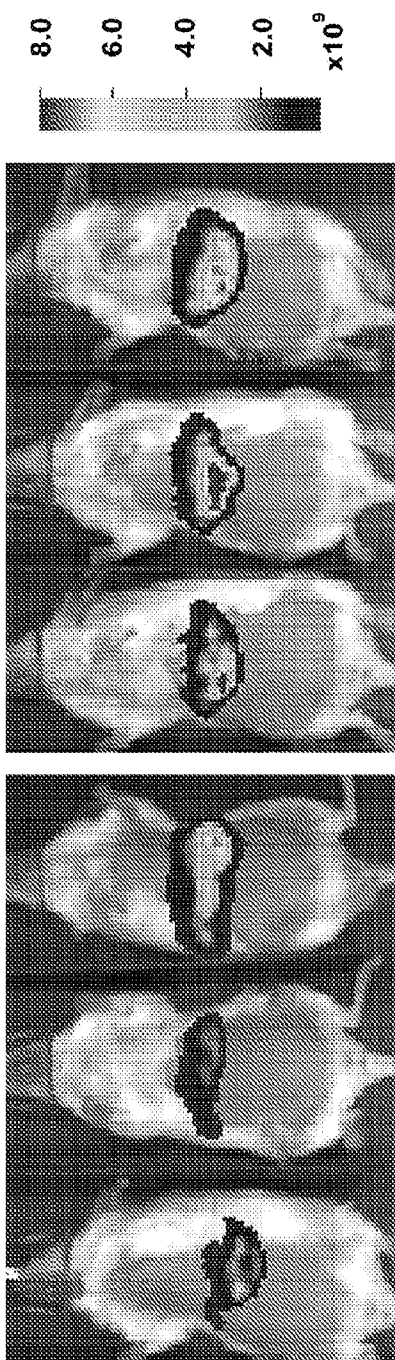


FIG. 20B

FIG. 20A

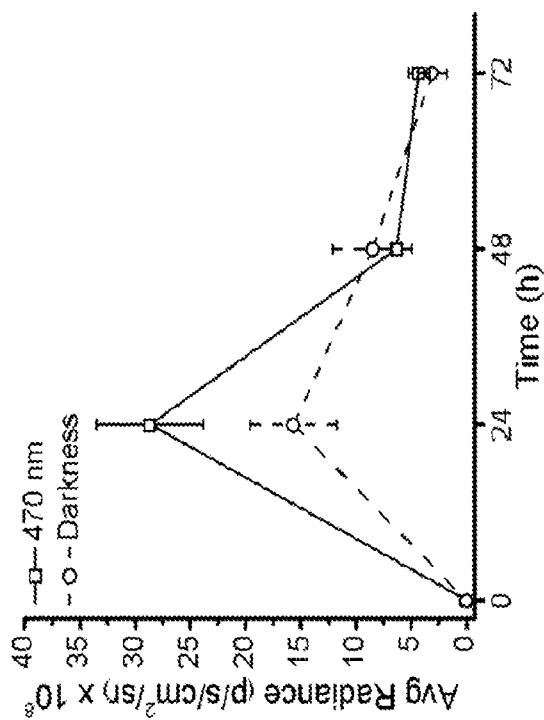


FIG. 20C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/54322

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 8, 12-13, 17-23, 32-40, 44-48
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/54322

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/195; C12N 15/62, 15/09; A61K 35/74, 47/42 (2016.01)

CPC - C07K 14/195; A61K 49/0045, 35/74, 47/42; C12N 15/625, 15/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C07K 14/195; C12N 15/62, 15/09, 15/63, 5/00; A61K 35/74, 47/42, 49/00; G01N 33/52, 21/64; C12Q 1/02, 1/68 (2016.01)

CPC: C07K 14/195; A61K 49/0045, 35/74, 47/42, 49/0002; C12N 15/625, 15/09, 15/85; G01N33/52, 33/64, 33/542, 33/582

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; Pubmed; EBSCO

Keywords: bphp1, ppsr2, Rhodospseudomonas palustris, fusion, optogenetic, infrared, biliverdin, protein, phytochrome, bacteria*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	(BELLINI, D et al.) Structure of a Bacteriophytochrome and Light-Stimulated Protomer Swapping with a Gene Repressor. Structure. 12 July 2012; Vol. 20, No.8; pages 1436-1446; page 1441, column 1, paragraph 2; page 1441, column 2, paragraph 2; DOI: 10.1016/j.str.2012.06.002	1-5, 6/1-5, 7/6/1-5, 9-10, 11/9-10, 14-15, 16/14-15, 24-29, 30/24-29, 31/30/24-29, 41-42, 43/41-42
A	(BRAATSCH, S et al.) The O2-Responsive Repressor PpsR2 but not PpsR1 Transduces a Light Signal Sensed by the BphP1 Phytochromein Rhodospseudomonas palustris CGA009. FEMS Microbiology Letters. 24 April 2007; Vol. 272, No. 1; pages 60-64; page 61, column 2, paragraph 3.	1-5, 6/1-5, 7/6/1-5, 9-10, 11/9-10, 14-15, 16/14-15; 24-29, 30/24-29, 31/30/24-29, 41-42, 43/41-42
A	US 2011/0099646 A1 (LACOSTE, A et al.) 28 April 2011; paragraph [0006]	1-5, 6/1-5, 7/6/1-5, 9-10, 11/9-10
A	(PIATKEVICH, KD et al.) Engineering of Bacterial Phytochromes for Near-Infrared Imaging, Sensing, and Light-Control in Mammals. Chemical Society Reviews. 29 January 2013; Vol.42, No.8; pages 1-18; figure 3	1, 6/1, 7/6/1, 9, 11/9
A	(BRAATSCH, S et al.) Rhodospseudomonas palustris CGA009 Has Two Functional ppsR Genes, Each of Which Encodes a Repressor of Photosynthesis Gene Expression. Biochemistry. 05 December 2006; Vol. 45, No. 48; pages 14441-14451; page 14444, column 1, paragraph 5; DOI: 10.1021/bi061074b	2-5, 6/2-5, 7/6/2-5, 10, 11/10, 14-15, 16/14-15, 24-29, 30/24-29, 31/30/24-29, 41-42, 43/41-42

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

29 December 2016 (29.12.2016)

Date of mailing of the international search report

23 JAN 2017

Name and mailing address of the ISA/US

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Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/54322

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	(MULLER, K et al.) A Red/Far-Red Light-Responsive Bi-Stable Toggle Switch to Control Gene Expression in Mammalian Cells. Nucleic Acids Research. 25 January 2013; Vol. 41, No. 7; pages 1-11; page 5, column 2, paragraph 2; DOI: 10.1093/nar/gkt002	41-42, 43/41-42
P, X	(KABERNIUK, AA et al.) An Optogenetic System Based on Bacterial Phytochrome Controllable with Near-Infrared Light. Nature Methods. 09 May 2016; Vol. 13, No. 7; pages 1-20; whole document; DOI: 10.1038/nmeth.3864.	1-5, 6/1-5, 7/6/1-5, 9-10, 11/9-10, 14-15, 16/14-15, 24-29, 30/24-29, 31/30/24-29, 41-42, 43/41-42