Supporting Information

Ohki et al.

SI Materials and Methods

Cloning, Expression and Purification. A codon-optimized synthetic gene (Eurofins Genomics) encoding OaPAC (accession number YP_007087096) was cloned into pCold I DNA (Takara Bio), resulting in the expression vector pCold-OaPAC, which encodes OaPAC protein carrying an N-terminal histidine tag, cleavable with TEV protease. This vector was transformed into *E.coli* ArcticExpress(DE3) (Agilent Technologies) and cultured in LB medium containing 50 μg/ml ampicillin and grown to $O.D._{600}$ between 0.5 to 0.6. The cells were cooled on ice for 20 min. and induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and cultured at 10°C for 5 days. These cells were harvested by centrifuge and stored at -80°C. The stored cells were resuspended in 40 ml lysis buffer (0.1 M Tris-HCl pH 8.0, 0.5 M NaCl and 1 mM PMSF) and disrupted by sonication. The crude sample was centrifuged at 38,000 *g* at 4°C for 30 min. The supernatant was passed through a 0.45 μm filter, and loaded into 5 ml TALON resin (GE Healthcare) equilibrated with wash buffer (50 mM Tris-HCl pH 8.0, 0.5 M NaCl, 10 mM imidazole) and agitated for 30 min. at 4°C. The resin was washed with 50 ml wash buffer and the protein eluted with 50 mM Tris-HCl pH 8.0, 0.5 M NaCl, 0.4 M imidazole. Suitable fractions were pooled and dialyzed against 50 mM Tris-HCl pH 8.5 and 100 mM NaCl. The dialyzed sample was applied onto a Q HP column (GE Healthcare) washed with 50 mM Tris-HCl pH 8.5, 50 mM NaCl. The protein was eluted with a linear gradient to 1 M NaCl. Suitable fractions were pooled and concentrated to 2 ml before loading onto a gel-filtration column (HiLoad 16/60 Superdex 200) washed with 10 mM HEPES pH 7.5 and 150 mM NaCl. Selected fractions were pooled and concentrated to 10 mg/ml for crystallization. TEV protease proved very ineffective at removing the affinity tag from this protein construct. Histidine-tag-free OaPAC protein was expressed directly using pCold. Bacterial culture was carried out as detailed above for the tagged protein. After purification using a Q HP column (with the same conditions as used above), ammonium sulfate was added to a final concentration of 1.2 M, and the protein was applied to a hydroxyapatite (BioRad) column (20 ml bed volume) and eluted with a linear gradient of potassium phosphate (10-800 mM potassium phosphate pH 6.8 with 0.1 M NaCl). The dialyzed sample was

re-applied onto the Q HP column (using the same conditions). The main peak fractions were pooled and concentrated to 10 mg/ml for crystallization.

Crystallization and structure determination. The closed-form OaPAC crystal was grown by vapor diffusion using the hanging drop method. Tagged protein (10 mM HEPES, pH 7.5, 150 mM NaCl) and reservoir solution (0.1 M sodium citrate pH 5.0, 10% PEG 20K), were mixed in a 1:1 ratio then equilibrated against 1 ml of reservoir solution at 20°C in the dark. Crystals grew in space-group $P2_12_12_1$, with a=85.4 Å, b=100.7 Å, c=120.7 Å and contained two molecules in the asymmetric unit. To make a heavy atom derivative, crystals were soaked in a solution containing crystallization buffer and 10 mM thimerosal (Hg) for 24 h before data collection. The open-form of OaPAC was crystallized by vapor diffusion using the hanging drop method. Tag-free protein (50 mM Tris/Cl, pH 8.5, 150 mM NaCl) and reservoir solution (0.1 M sodium citrate pH 5.0, 10% PEG 20K, 5 mM magnesium chloride, 5 mM ApCpp: $C_{11}H_{18}N_5O_{12}P_3$), were mixed in a 1:1 ratio then equilibrated against 1 ml of reservoir solution at 20°C in the dark. Crystals grew in space-group $P6_122$, with a= b=76.8 Å, c=204.8 Å and contained one monomer in the asymmetric unit. Diffraction data were collected using a crystal flash-frozen to -180°C in crystallization buffer containing 30% (v/v) glycerol. Diffraction data were collected on beam line BL-1A and BL17A stations at the Photon Factory, Tsukuba, Japan, using PILATUS-2M and PILATUS-6M detectors. All data were processed and scaled using HKL2000 and SCALEPACK (1).

The structure of closed-form OaPAC was initially solved to 3.0 Å resolution by the single wavelength anomalous dispersion (SAD) method. The mercury atom positions were determined using AutoSol within the PHENIX program suite (2), allowing much of the main-chain to be traced directly. Automatic model building using the AutoBuild module in PHENIX, manual refinement with COOT (3), and refinement with BUSTER (4) led to a final R-factor/Rfree of 27.1%/31.0% over the resolution range 50.0-2.9 Å. The open-form OaPAC structure was solved by molecular replacement using PHASER (5) with the previously solved closed-form structure as a starting model. One solution of open-form OaPAC were obtained with final TFZ (translation function Z-score) of 13.0. Refinement led to a final R-factor/Rfree of

21.2%/26.4% over the resolution range 50.0-1.8Å. Data collection and refinement statistics are shown in Table S1.

Mutagenesis. Site-specific mutagenesis was carried out by a simple PCR procedure using primer pairs (Table S3) to make 5' and 3' halves of the mutant sequence separately before a further reaction to create the full-length mutant sequence. The final PCR products were purified and treated with Nde I and Hind III (Thermoscientific), and cloned into pCold II vector treated with the same restriction enzymes.

In vitro adenylate cyclase assay. The adenylyl cyclase activity of OaPAC and its mutants were measured in an assay buffer (50 mM Tris-HCl, pH 7.5; 1 mM dithiothreitol; 1 mM $MnCl_2$; 0.1% bovine serum albumin; 100 μM ATP) in darkness or irradiated with blue light (70 μmol $m^{-2}s^{-2}$) from an LED illuminator (Sanyo QFC45-100B) for 10 min. The cAMP produced was assayed by an immune assay using cAMP Direct Biotrak EIA (GE Healthcare Life Sciences).

In vivo adenylyl cyclase assay using AC-deficient *E. coli.* The OaPAC gene was cloned into pGEM-5Zf(+) (Promega, Madison, WI, USA) of which *lac* promoter was removed, resulting in the complementation vector pGEMOaPAC3. Mutants of the vector were generated by site-directed mutagenesis using high-fidelity DNA polymerase (PrimeStar GXL, Takara, Otsu, Japan). An AC-deficient *E. coli* strain MK1010 (6) was transformed with the complementation vectors and was streaked on MacConkey agar plates (1% lactose; 100 μg/ml ampicillin; 25 μg/ml kanamycin). The plates were first incubated in darkness at 27°C for 7 h and then under white light (23 μmol m^{-2} s^{-2}) at 27°C for 22 h or kept in darkness.

Solution spectroscopy. Spectral measurements were carried out with 2.0 and 1.9 mg/ml OaPAC solutions, respectively, without and with 1 mM ApCpp and 2 mM $MgCl_2$ in a 10 mM HEPES buffer, pH 7.5, containing 100 mM NaCl. The sample was kept at 20°C throughout the experiment using a thermostated cell-holder. Irradiation was performed for 5 s using a Hamamatsu L5662 spot light source equipped with a 200 W L6722 Hg-Xe lamp and optical fibers. An Edmund Lumilass-B glass was inserted between the light source and optical fibers to cut the UV light of wavelength less than 365 nm and

enhance the relative intensities of mercury emission lines at 405 nm (~0.7 mW/mm^2 at the sample position) and at 436 nm (~0.5 mW/mm^2 at the sample position). Transient absorption spectra during dark relaxation were measured using a Unisoku RSP-601 rapid scan spectrophotometer, equipped with a MOS-type highly sensitive photodiode array. To minimize actinic effects, the intensity of the measuring light (Hamamatsu 150W L2195 Xe lamp) was reduced with a mechanical iris and a mesh filter. The spectra between 353.0 and 560.5 nm during dark relaxation in a time frame of 0.1-50 s were globally fitted to a two-state (light to dark) model using the SPECFIT (Ver. 3.0.37, Spectrum Software Associates).

Single-crystal spectroscopy. A single crystal of OaPAC with 25 μl of its mother liquor was put onto a hole-slide glass, sealed with a coverslip, and observed under a Ziess UMSP 80 microspectrophotometer. The absorption spectrum of the crystal under the dark at 21-22°C was recorded between 350 and 600 nm with unpolarized, monochromatized light incident on (100) crystal face. Decay of the light-induced excited state in the crystal was monitored by absorption change at 492 nm after irradiation of the white light of the microspectrophotometer (Wotan 12V/100W halogen lamp) for 10 s. The occurrence of full excitation was confirmed by a linear relationship between the crystal thickness and the amplitude of the observed absorption change at 492 nm. The time course was fitted with a single exponential decay function.

Analytical ultracentrifugation. To investigate the oligomeric state of full-length OaPAC and OaPAC C-terminal domain in solution, sedimentation-velocity experiments were performed using an Optima XL-I analytical ultracentrifuge and an An-50 Ti rotor (Beckman Coulter) at 20°C. The concentrations of the loaded protein solutions were 0.25, 0.50 and 1.0 mg/ml in reference buffer (20 mM Tris, 150 mM NaCl, pH 8.0). Absorbance (280 nm) scans were collected during sedimentation at 50,000 rpm. Data analysis was performed with the programs SEDFIT (7).

OaPAC expression in human cells. A new OaPAC gene was chemically synthesized with codon usage optimized for human cells. This gene was inserted into the multiple cloning site of the mammalian expression vector, pEBmulti-Hygro (Wako, Osaka, Japan), which is distributed to daughter cells by episomal replication. Expression was

driven by the CAG promotor. Transfection was carried out by lipofection using FuGene HD (Promega KK, Tokyo), into a HEK293 cell line that stably expresses glosensor-22F from plasmid pGloSensor-22F (Promega KK, Tokyo). This luciferase-based cAMP reporter develops luminescence in response to intracellular cAMP. The original HEK293 cell (JCRB No.9068) was obtained from the National Institute of Biomedical Innovation. Three days after transfection with the OaPAC gene, the HEK293 cells were used for further experiments.

Constructs for exogenous expression in human cells. OaPAC and bPAC genes were chemically synthesized with codon usage optimized for mammalian cells. Bicistronic configurations such as [OaPAC-2A-RFP] and [bPAC-2A-RFP] were constructed where "2A" means 2A-peptides that ensure equal expression of the PAC and RFP (8). RFP is a variant species of red fluorescence protein (Rudolph-RFP, DNA2.0 Inc., Newark, California). Those bicistronic configurations were inserted in the multiple cloning site of the mammalian expression vector, pEBMulti-Hygro (Wako Pure Chemical Industries Ltd., Osaka, Japan), which is distributed to daughter cells by episomal replication. Expression was driven by the CAG promotor. A GloSensor^{TM}-22F cAMP gene (Promega Corporation, Fitchburg, Wisconsin), a luciferase-based cAMP reporter, was inserted in the multiple cloning site of the mammalian expression vector, pEBMulti-Neo (Wako Pure Chemical Industries Ltd., Osaka, Japan).

Building a cAMP-reporter expressing cell line. A pEBMulti-Neo-GloSensor^{TM}-22F cAMP plasmid was transfected by lipofection method using FuGENE^{TM} HD (Promega Corporation, Fitchburg, Wisconsin), into a HEK293 cells (obtained from DS Pharma Biomedical Co., Ltd., Osaka, Japan). The transfected cells were selected by addition of antibiotics (G418, Wako Pure Chemical Industries Ltd., Osaka, Japan) at 100-600 μg/ml to the culture medium and kept over 10 passages to establish a GloSensor^{TM} 22F cAMP stably expressing HEK293 cell line. When luciferase substrate (GloSensor^{TM} cAMP reagent, Promega Corporation, Fitchburg, Wisconsin) was added into the stable cells, luminescence was developed in response to the intracellular cAMP concentrations.

OaPAC and bPAC expression in human cells.

pEBMulti-Hygro-OaPAC-2A-RFP or pEBMulti-Hygro-bPAC-2A-RFP was transfected into the GloSensor^{TM}-cAMP stably expressed HEK293 cells via the lipofection method mentioned above. The transfected cells were selected by addition of an antibiotics (HygromycinB, Thermo Fisher Scientific Inc., Waltham, Massachusetts) at 600 μg/ml. Since expression level of PACs was equal to RFP in each cell due to action of the 2A-peptide, expression level of the PACs in each cell was monitored by fluorescence microscopy. Within the third passage after transfection, the cells showed various amount of expression, while after the forth the cells showed stable expression at high level. The cells within the third passage as well as after the forth passage were used for further experiments.

Photoactivation of PACs and monitoring cAMP-generation. The exogenous genes expressing HEK293 cells were inoculated in 35 mm cell culture dish (BioCoat Collagen I, Corning Incorporated, NY) with 2 ml of usual culture medium (DMEM high glucose with 10% FBS and 4 mM L-glutamine, Thermo Fisher Scientific Inc., Waltham, Massachusetts). Several hours before experiment, CO_2-independent medium (Thermo Fisher Scientific Inc., Waltham, Massachusetts) was substituted for the usual medium and luciferase substrate (final concentration 1.2 mg/ml) was added. Photoactivation experiments were performed in a dark room where the temperature and the humidity were kept at 27°C and 30%, respectively. An EM-CCD digital camera (ImagEM C9100-13, Hamamatsu Photonics K.K., Hamamatsu, Japan) and a blue LED module (maximum emission wavelength of 447 nm, Luxeon Rebel LED, Philips Lumileds Lighting Company, CA) were attached to an inverted microscope (Eclipse TE-300, Nikon Corp., Tokyo) mounted with an objective lens (S Fluor 20x, NA of 0.75, Nikon Corp., Tokyo). The EM-CCD camera and the blue-LED were computer-controlled through a serial communication bus. Blue-light irradiation, with a photon flux of 1010 μmol m^{-2} s^{-1}, was applied to the cells for 0.6 s, 6 s and 60 s, respectively. Immediately after irradiation, consecutive luminescence imaging was begun (at 60 s per frame) with the EM-CCD camera in photon-counting mode (EM-gain of 240, binning of 1x1, exposure time of 60 s) until the cAMP-dependent luminescence disappeared. In the same field of view, fluorescence imaging for RFP also carried out under green light excitation (Texas Red filter set: Ex;540-580 nm, DM; 595 nm, BA; 600-660 nm, an

additional long-pass filter (LP570) was inserted in the excitation optics). From those obtained images, intensities for luminescence as well as fluorescence in each cells were quantified using ImageJ software (http://imagej.nih.gov/ij).

Primary culture of dentate granule cells. All experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo and according to the University of Tokyo's guidelines for the care and use of laboratory animals. Cultures of dissociated granule cells were prepared from postnatal three-to-four-day-old (P3-4) SD rats as previously described (9, 10). Briefly, the dentate gyri were dissociated in ice-cold Gey's balanced salt solution (GBSS) enriched with D-glucose (6.50 g/L) and subsequently treated with 0.25% trypsin and 0.01% DNase I at 37°C for 30 min. After trypsinization was stopped via the addition of horse serum, cells were centrifuged at 1200 rpm for 5 min. The supernatants were removed, and the remaining cells were dispersed in 2 ml of culture medium with arabinofuranosyl cytidine and serum at 37°C. For primary cultures, the dissociated granule cells were plated onto poly-D-lysine coated 12-mm cover slips (H-12-PDL; neuVitro) at a cell density of 5.0×10^3 cells/cm^2 in culture medium in 24-well plates and incubated at 37°C in a humidified 5% CO_2 and 95% air atmosphere. Medium was changed at DIV 1 and every 3 days after. The culture plates were wrapped with black tape to avoid exposing the PAC-transfected cells to light. For real-time imaging, cells were plated on glass-bottomed 35 mm dishes instead of 24-well plates.

DNA and siRNA transfection. For granule cells, DNA constructs and siRNAs were transfected using Lipofectamine 2000 according to the manufacturer's instructions. For one well of a 24-well plate, 0.4 μg of DNA or 20 pmol of siRNA was first dissolved in 50 μl OptiMEM I Reduced-Serum Medium as solution A, and 0.75 μl Lipofectamine 2000 was added to 50 μl OptiMEM as solution B. Solutions A and B were mixed, added to 400 μl of transfection culture medium (95% Neurobasal medium, 2 mM L-glutamine, and 1 mM sodium pyruvate at 37°C in a humidified 5% CO_2 and 95% air atmosphere) and used to replace the culture medium. The mixed solution was replaced with culture medium after 1 h. DNA constructs for oaPAC, PACα and membrane-GFP were used. Membrane-GFP and membrane-tdTomato constructs were from Clontech. mCherry-2A-PACα was expressed under the control of the EF1α promoter.

Blue-light stimulation. For all cultured cells, blue-light stimulation was applied using 20000-mcd LED lamps. Four LED lamps were connected in a parallel circuit with a 3-V DC power source. The intensity of the light stimulus was approximately 200 μmol/m^2/s. The duration of the light pulse was set to 2 s, and the interval was set to 3 s. Each LED lamp was fixed to four corners of the lid of a 24-well plate 3 mm above the surface of the culture medium.

Immunocytochemistry. Cultured neurons were fixed with 4% paraformaldehyde for 30 min. at 4°C. Fixed samples were washed three times with phosphate-buffered saline (PBS). The samples were then incubated with blocking solution (5% goat serum and 0.1% Triton-X 100 in PBS) for 1 h at room temperature. The samples were subsequently incubated with primary antibodies overnight at 4°C. The samples were washed with PBS and incubated with secondary antibodies with or without the presence of rhodamine-conjugated phalloidin (1:50) for 6 h at room temperature. The primary antibodies were as follows: rabbit anti-mCherry (1:500; Abcam); mouse anti-tau-1 (1:1000; Chemicon); chicken anti-GFP (1:1000; Abcam); The secondary antibodies were as follows: Alexa 405-labeled anti-mouse IgG (1:500; Invitrogen); Alexa 594-labeled anti-rabbit IgG (1:500; Invitrogen); and Alexa 488-labeled anti-chicken IgY (1:500; Abcam).

Morphology analysis and fluorescence quantification. Axon lengths were measured using ImageJ (NIH). The longest axon from each cultured neuron was defined as the primary axon, and the axon segments emerging from other axon segments were defined as axonal branches. Axonal branches shorter than 5 μm were excluded from the analysis.

References

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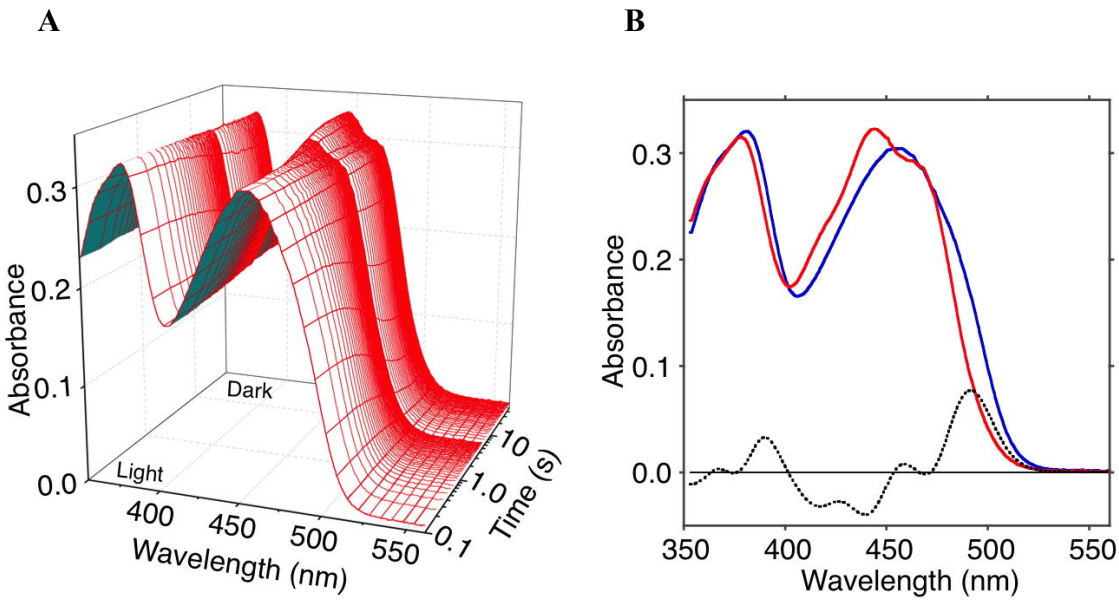


Fig. S1. The transient absorption spectrum of OaPAC. *(A)* The spectral change of OaPAC in solution, with 1 mM ApCpp and 2 mM $MgCl_2$, during dark relaxation after light activation. The reversion to the dark state is shown on the logarithmic time axis, from 0.1 – 50 s, with the light-stimulated state shown in front and the dark-adapted state shown behind. *(B)* The spectra of the fully activated (blue) and deactivated (red) states. The difference between the two is shown as a dotted black line.

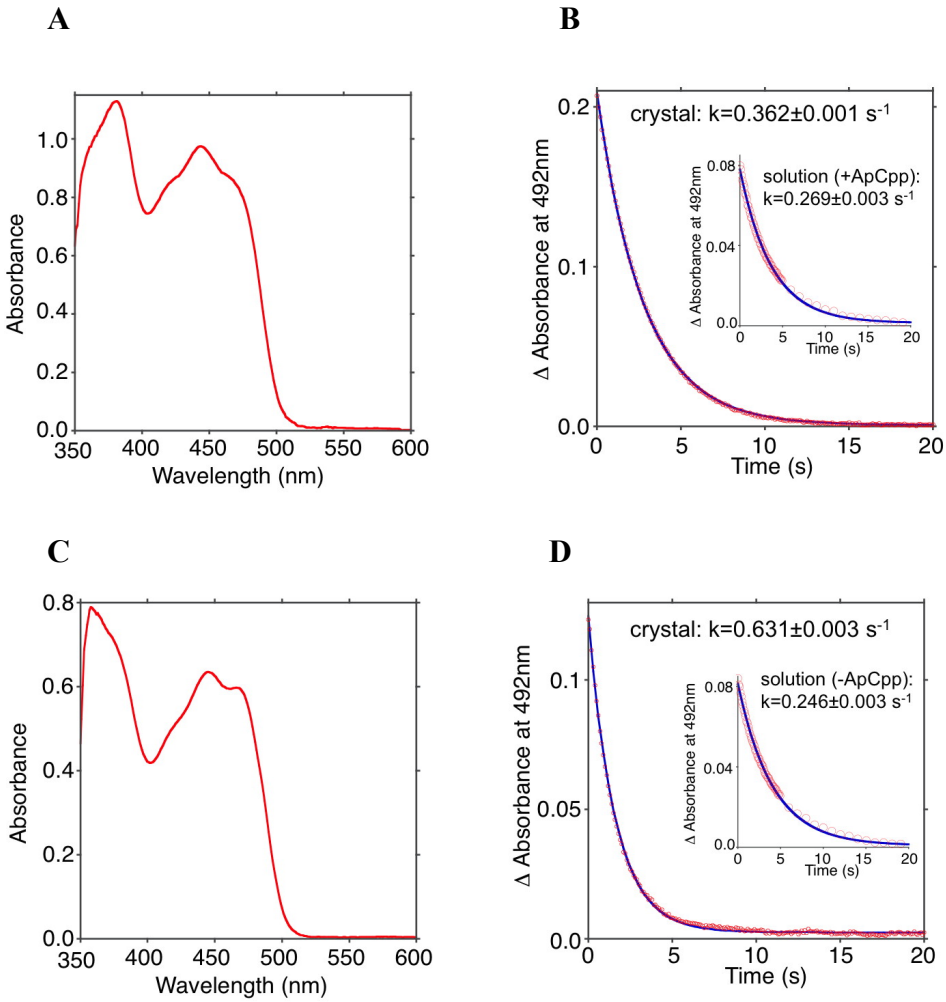


Fig. S2. The absorption spectra of the OaPAC crystal forms. *(A)* The spectrum of the dark-adapted state with ApCpp present. *(B)* The decay of the light-induced excited state in the hexagonal crystal form, monitored by change in absorption at 492 nm after removing illumination. The change was fitted to a first-order rate constant. The inset shows the equivalent results for the change in solution. In the presence of ApCpp, the rate-constant is comparable in solution and in the crystal. *(C)* The spectrum of the dark-adapted state with no ATP homologue present. *(D)* The decay of the light-induced excited state in the orthorhombic crystal form, monitored at 492 nm after removing illumination. The inset shows the fitting to a first-order rate constant in solution. The crystal shows a reversion rate twice that of the protein in solution, indicating that crystal contacts favour the dark-adapted state.

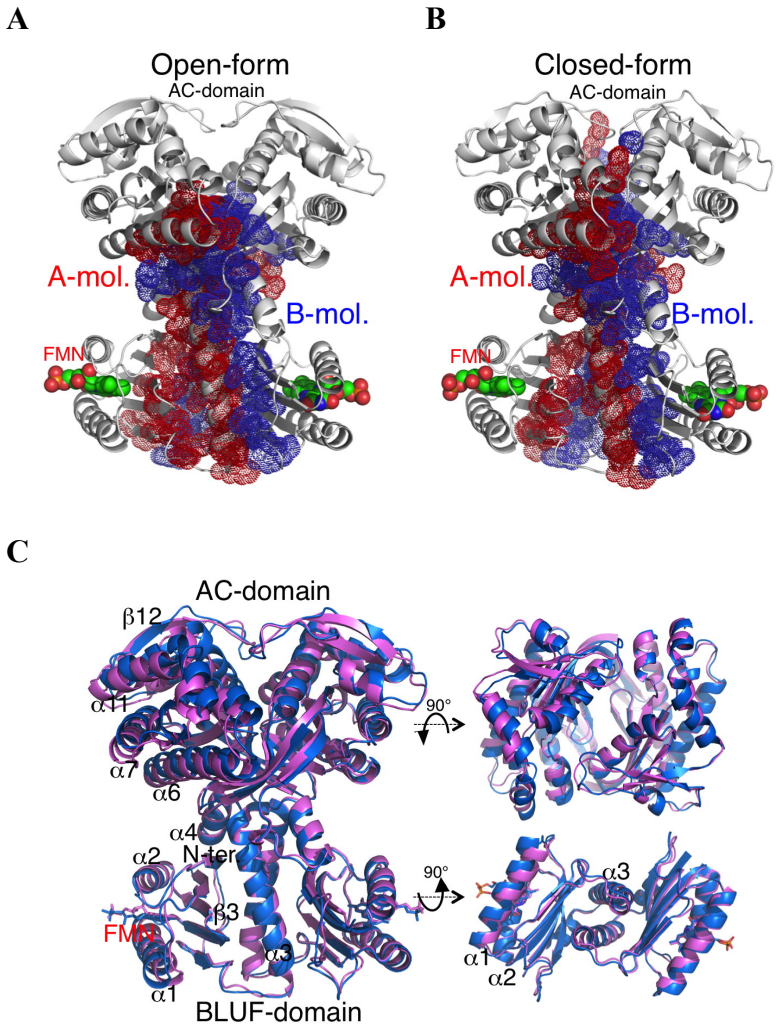


Fig. S3. Open and closed-form structure of OaPAC. *(A)* The open (hexagonal) form of OaPAC, with contact residues of monomers A and B shown as red and blue van der Waal surfaces. The FMN molecules are shown as CPK models. The buried surface area is approximately 3660 Å^2. *(B)* The closed (orthorhombic) form of OaPAC, with contact residues shown as in (A). The buried surface area is approximately 4020 Å^2, slightly larger than in the open form due to the closer approach of the AC domains. *(C)* A superposition of the open (pink) and closed (blue) forms, viewing the BLUF and AC domains along the dyad axis.

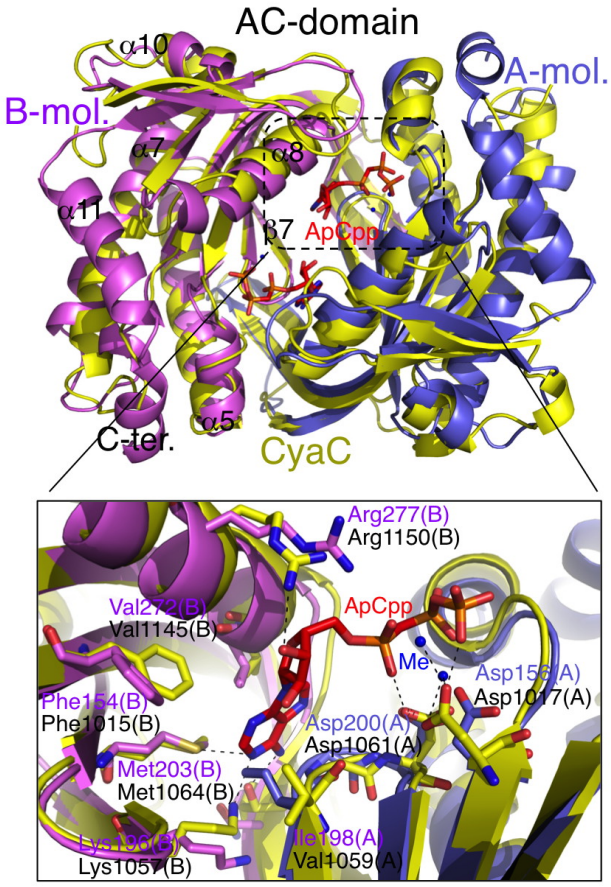


Fig. S4. Superposition of OaPAC AC domains. Superposition of the Cα traces of OaPAC open form (pink and purple) with CyaC with bound non-hydrolysable ApCpp in each active site (PDB entry 1WC5 (8)). The inset shows a close-up view of one active site, showing the conserved side-chains around the substrate molecule. Residue labels of CyaC are in black, and magnesium ions are shown as spheres. Side-chains from each monomer contribute to each active site.

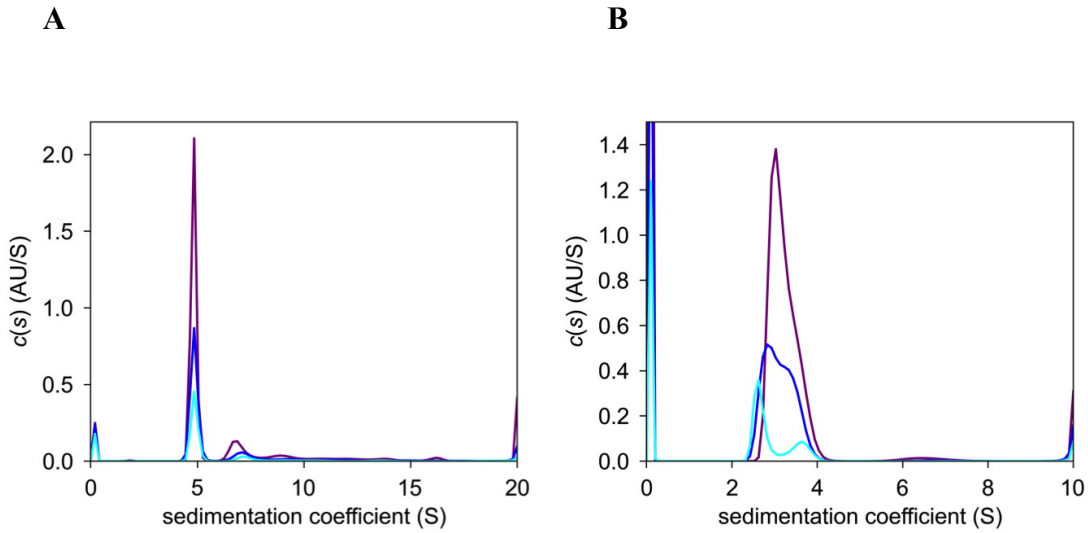


Fig. S5. Analysis of the oligomeric state by analytical ultracentrifugation. *(A)* The c(s) distribution of full-length PAC showed the presence of one principal species in solution with a sedimentation coefficient (s) of 4.8 S, corresponding to a PAC dimer. The c(s) distribution showed no significant change with protein concentration, implying the protein exists as a stable dimer. *(B)* The c(s) distribution of the PAC C-terminal AC domain showed protein concentration dependence. At low protein concentration the c(s) distribution showed two peaks. At higher protein concentration, the weight average increased, but did not exceed the dimer mass. These results are consistent with a relatively rapid reversible equilibrium between the monomer and dimer species.

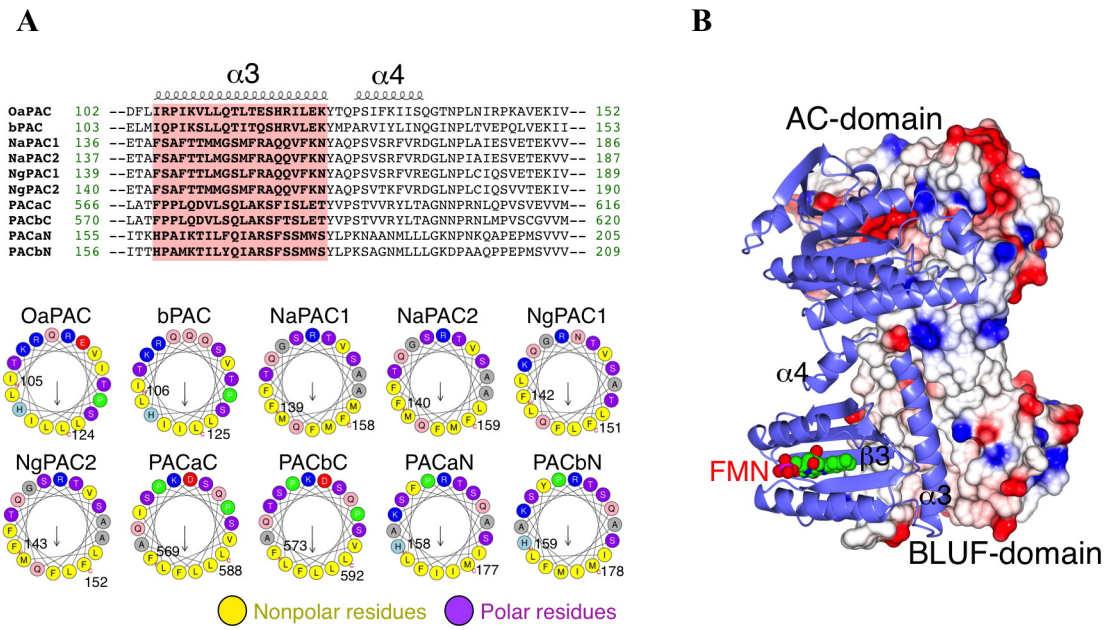


Fig. S6. Sequence alignment of helix α3 in the BLUF domain family. *(A)* The sequences of 13 BLUF domain proteins (bPAC: *Beggiatoa sp.* (11); NaPAC: C-terminal of *Naegleria australiensis* (12); NgPAC: C-terminal of *Naegleria gruberi* (13); PACaC: C-terminal region of the α-chain of *Euglena gracilis* (14); PACbC: C-terminal region of the β-chain of *Euglena gracilis* (14); PACαN: N-terminal region of the α-chain of *Euglena gracilis* (14); PACβN: N-terminal region of the β-chain of *Euglena gracilis* (14)), were aligned and the region (from Asp 102 to Val 152) including the helices α3 and α4 is shown. The sequences are also shown as helical wheels, indicating the conserved hydrophobic interface around the dimer interface side of the α3 helix. *(B)* The open form of the OaPAC dimer, with one monomer shown as a surface representation coloured by electrostatic potential (red negative, blue positive), and the other as a simple Cα trace.

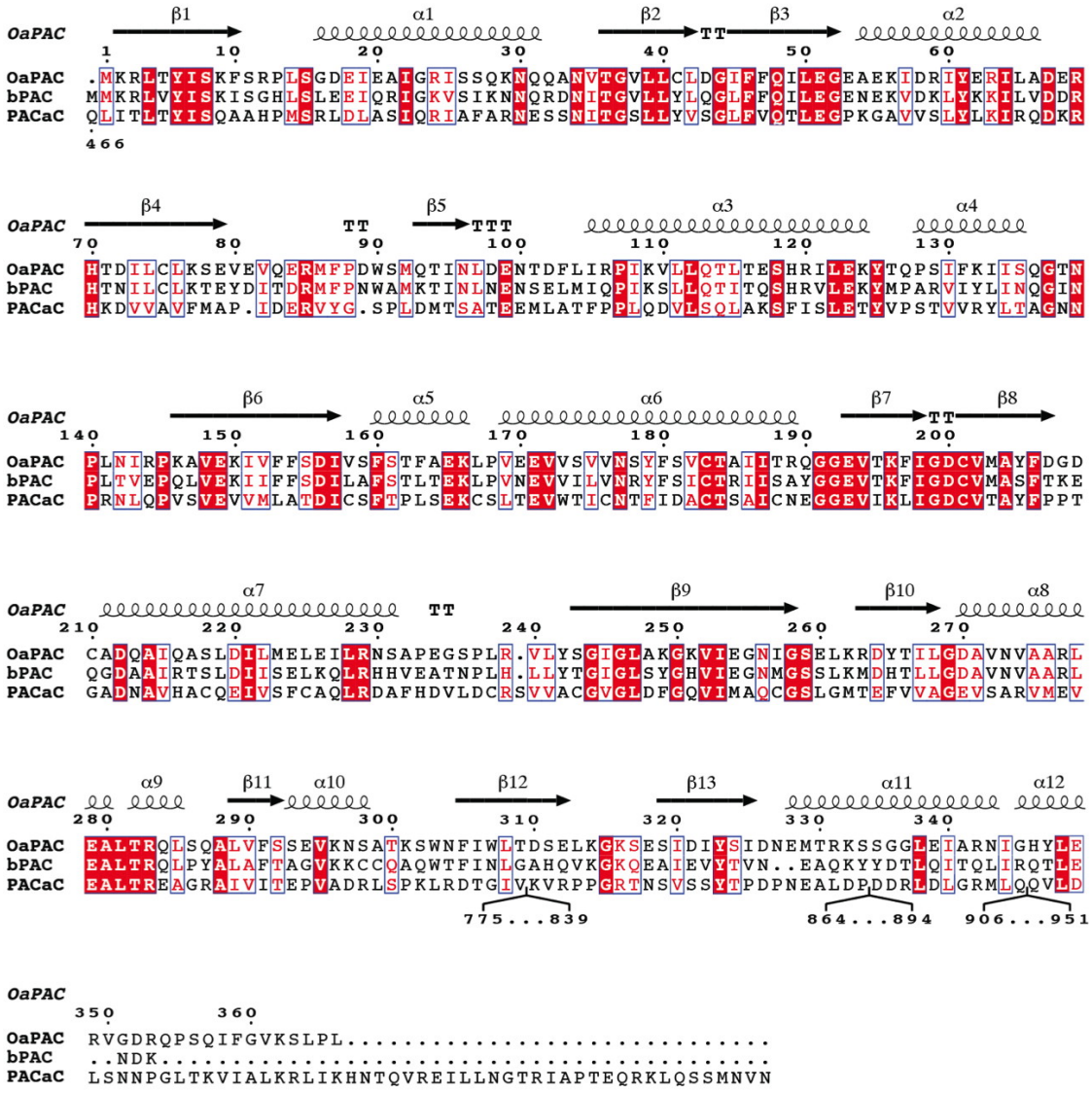
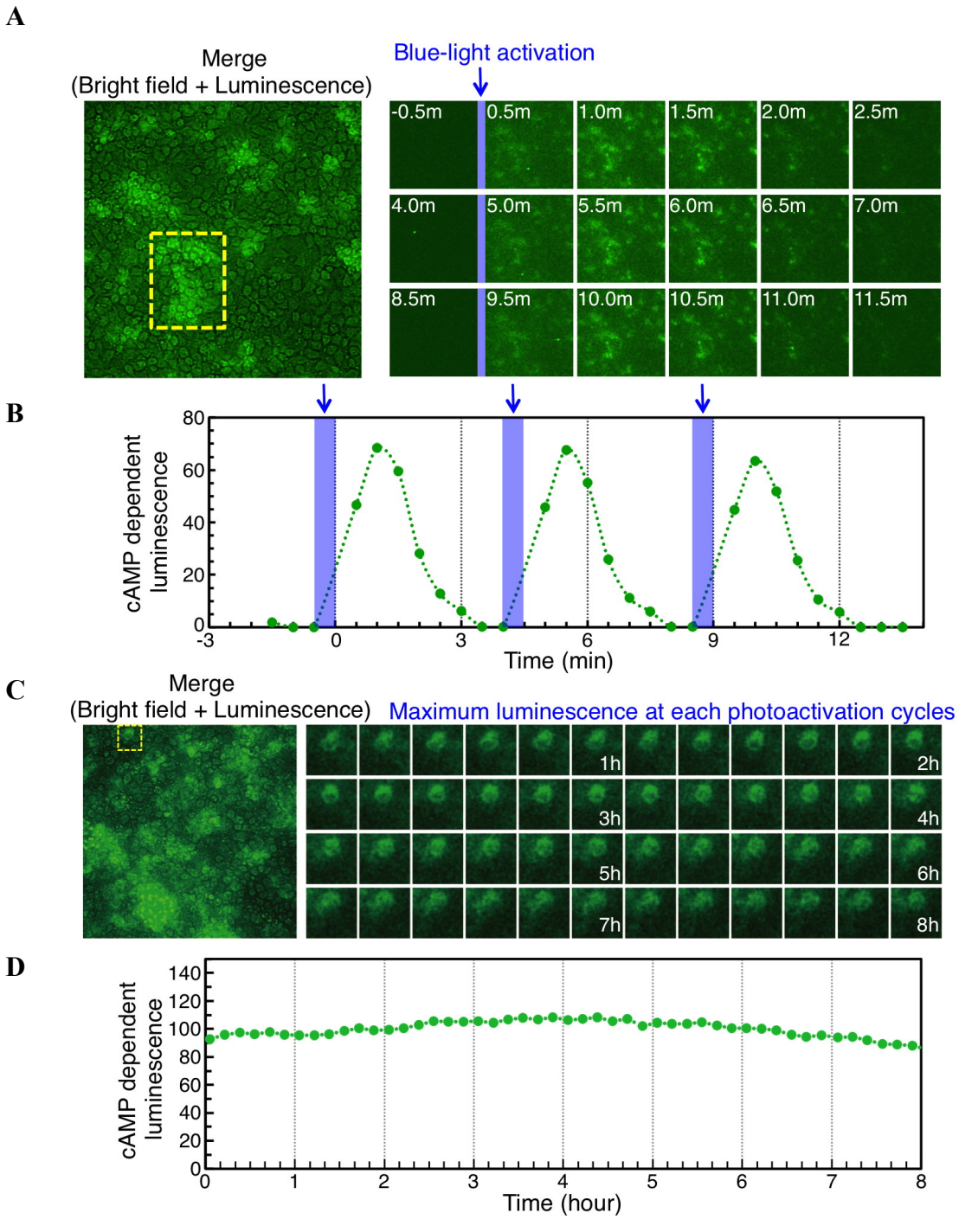


Fig. S7. The secondary structure of OaPAC shown with the sequence alignment with bPAC from *Beggiatoa sp.* (11) and the C-terminal region of PAC α-chain from *Euglena gracilis* (14). Disordered residues are shown as dotted lines. Conserved residues are shown in white on red. OaPAC and bPAC share very similar FMN binding sites, but Trp 90 and Met 92 are not conserved in PACαC.


A
Merge
(Bright field + Luminescence)
Blue-light activation
-0.5m
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8.5m
9.5m
10.0m
10.5m
11.0m
11.5m
B
cAMP dependent luminescence
Time (min)
C
Merge
(Bright field + Luminescence)
Maximum luminescence at each photoactivation cycles
1h
2h
3h
4h
5h
6h
7h
8h
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cAMP dependent luminescence
Time (hour)

Fig. S8. Use of OaPAC to generate cAMP in cultured human HEK293 cells.

OaPAC expressed in HEK293 (human embryonic kidney) cells was activated by blue-light, and the rise in intracellular cAMP was monitored by luminescence imaging of cAMP-dependent luciferase.

(A) A merged micrograph and time sequential images of cAMP-dependent luminescence and bright-field image of the cells. Cellular luminescence is shown in green. The dotted square indicates the area selected for quantification of luminescence.

(B) Initial time-course of intracellular cAMP concentration showing reproducible cycles with repeated OaPAC activation every 270 s.

(C) A merged bright-field micrograph (left) and time sequential images (right) of maximum cAMP-dependent luminescence at each activation cycle (measured 2 min. after a 1 min. light pulse). The dotted square indicates the area selected for quantification of luminescence in single cell. The cycle was repeated 6 times per hour for a total period of 8 h from first photo-stimulation.

(D) Maximum luminescence (in arbitrary units) observed in the micrographs shown in *(C)*, plotted over time. Each point indicates the value for one activation cycle, showing that the response is essentially unchanged over 8 h.

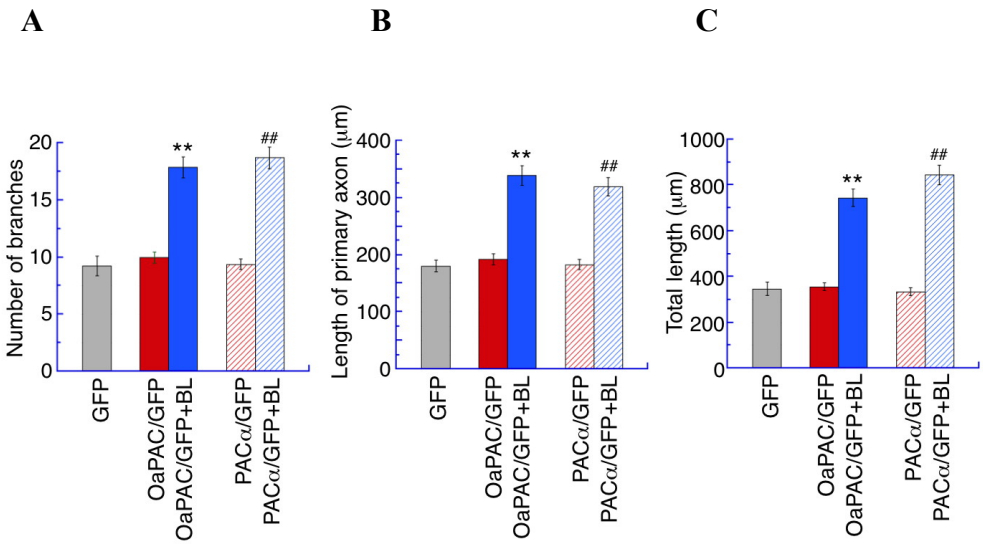

Fig. S9. The activation of OaPAC promotes axonal growth in neurons. Primary cultures of hippocampal neurons were prepared from P3-4 rat pups. Membrane-GFP (mGFP), mGFP/OaPAC or mGFP/PACα were transfected on 1DIV. The blue light stimulation (BL) was applied for 30 min. on 4DIV. Cells were fixed on 7DIV, and subsequently immunostained for GFP, mCherry, and the neurite marker tau-1. (*A*) Bar graphs indicating the number of branches. Activation of either OaPAC or PACα with blue light increased the number of axonal branches and branch length. $^{**}p < 0.01$ vs. OaPAC w/o light, and $^{\#\#}p < 0.01$ vs. PACα w/o light; Steel-Dwass test after Kruskal-Wallis test, n = 30 cells for each group. (*B*) Bar graphs indicating the length of primary axons. Activation of OaPAC as well as PACα with a blue light stimulation increased the length of primary axon. $^{**}p < 0.01$ vs. OaPAC w/o light, and $^{\#\#}p < 0.01$ vs. PACα w/o light; Tukey's test after one-way ANOVA, n = 30 cells for each group. (*C*) Bar graph indicating the total axonal length. Activation of OaPAC as well as PACα with a blue light stimulation increased the total axon length. $^{**}p < 0.01$ vs. OaPAC w/o light, and $^{\#\#}p < 0.01$ vs. PACα w/o light; Tukey's test after one-way ANOVA, n = 30 cells for each group.

Table S1. Data collection, phasing and refinement statistics.

Data Set	PAC (Open-form)	PAC (Closed-form)	Hg (derivative)
Resolution range (Å)	50.0 – 1.8	50.0 – 2.9	50.0 – 3.0
Space group	$P6_122$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions (Å)	a= b=76.84, c=204.78	a=85.35, b=100.70, c=120.67	a=85.73, b=100.05 c=122.64
	α=90°, β=90°, γ=120°	α=90°, β=90°, γ=90°	α=90°, β=90°, γ=90°
Reflections (Measured/Unique)	309,162 / 33,448	103,207 / 22,797	90,114 / 19,753
Completeness (%)	97.7 / 85.5	96.1 / 92.8	91.3 / 61.6
Mean $<I>$ / $<\sigma(I)>$	8.7 / 0.8	14.9 / 3.0	7.2 / 1.2
Multiplicity	9.3 / 2.5	4.6 / 2.9	4.7 / 2.5
*R*merge (%)	9.4 / 67.1	6.7 / 37.5	8.8 / 35.8
B factor from Wilson plot (Å^2)	28.1	66.0	46.2
Figure of merit (after AutoSol in PHENIX)			0.340 / 0.821
Refinement Statistics			
Resolution range (Å)	50.0 – 1.8	20.0 – 2.9	
R-factor / free *R*-factor (%)	21.2 / 26.4	27.1 / 31.0	
Rms deviations from ideals			
bond lengths (Å) / bond angles (°)	0.008 / 1.178	0.009 / 1.12	
No. of atoms (protein/FMN/water)	2758 / 31/ 152	5536 / 62 / 0	
B-values (all atoms)			
Average B-factor (protein/FMN/water, Å^2)	34.5 / 35.7 / 48.8	99.8 / 120.3 / -	
Ramachandran plot			
residues in most favorable regions (%)	97.7	94.6	
residues in additional allowed regions (%)	2.3	5.0	
residues in generously allowed regions (%)	0	0.4	

Values in outer shell are for the highest shell with a resolution of 3.05-3.00 Å, 2.95-2.90 and, 1.83-1.80.

Table S2. The superpositions of separate domains of the open and closed forms in OaPAC.

Data Set	A(open) vs. A(closed)	A(open) vs. B(closed)
Oa-PAC	1.75 / 3.61 / 2.34 / 1.06	2.14 / 3.75 / 2.62 / 0.73
BLUF	0.88 / 2.03 / 1.34 / 0.69	1.12 / 1.96 / 1.43 / 0.51
AC	2.63 / 5.02 / 3.32 / 0.66	2.91 / 4.97 / 3.47 / 0.66

The open form has a single copy in the asymmetric unit (chain A), and the closed form has a complete dimer (chains A and B).

The rmsd values (Å) for superpositions of main-chain atoms, side-chain atoms, all protein atoms, Cα atoms.

BLUF-domain: residues 1-136; AC-domain: residues 146-350.

Table S3. Sequences of OaPAC mutagenesis PCR primers.

Mutant	Sequence
L11A/L115A	5’-CGCCCGATCAAAGTAGCGCTGCAGACTGCGACCGAAAGCCACCG-3’
	5’-CGGTGGCTTTCGGTCGCAGTCTGCAGCGCTACTTTGATCGGGCG-3’
Y125A	5’-GCCACCGTATTCTGGAGAAAGCCACACAACCG-3’
	5’-CGGTTGTGTGGCTTTCTCCAGAATACGGTGGC-3’
F197S	5’-GTGACCAAAAGCATTGGAGACTGTGTTATGGCC-3’
	5’-GGCCATAACACAGTCTCCAATGCTTTTGGTCAC-3’
N256A	5’-GGCAAAGTAATCGAAGGGGCCATTGGCTCAGAACTGAAACGCG-3’
	5’-CGCGTTTCAGTTCTGAGCCAATGGCCCCTTCGATTACTTTGCC-3’
D200N	5’-CCAAATTCATTGGAAACTGTGTTATGGCC-3’
	5’-GGCCATAACACAGTTTCCAATGAATTTGG-3’