

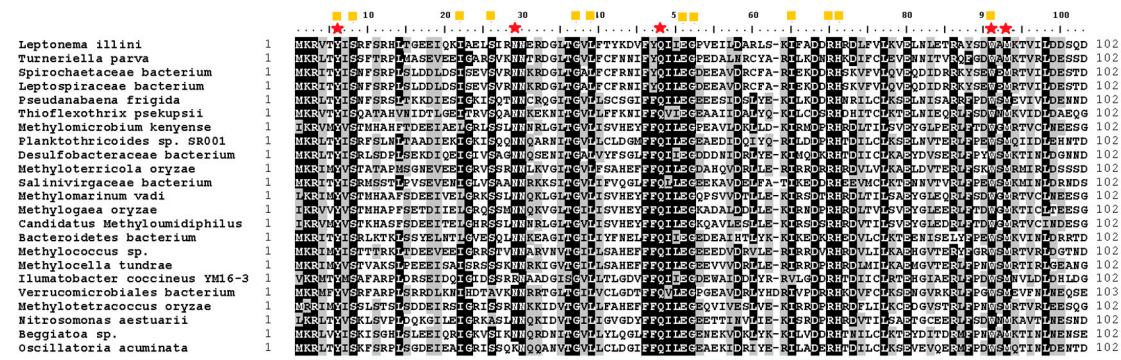
# Establishment of Optogenetic Modulation of cAMP for Analyzing Growth, Biofilm Formation, and Virulence Pathways of Bacteria Using a Light-Gated Cyclase

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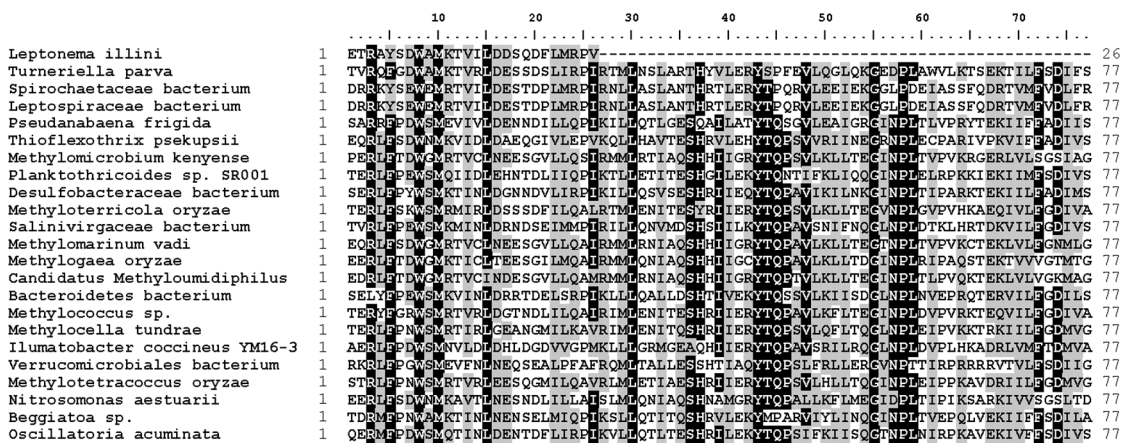
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## Supplementary Materials:



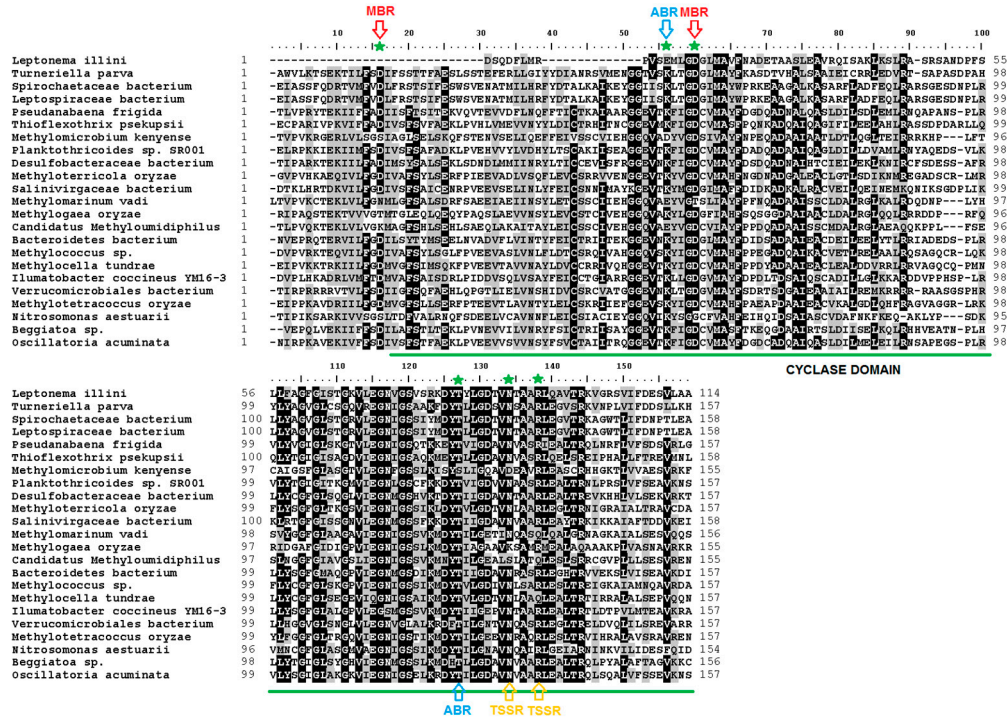
BLUF DOMAIN

### A



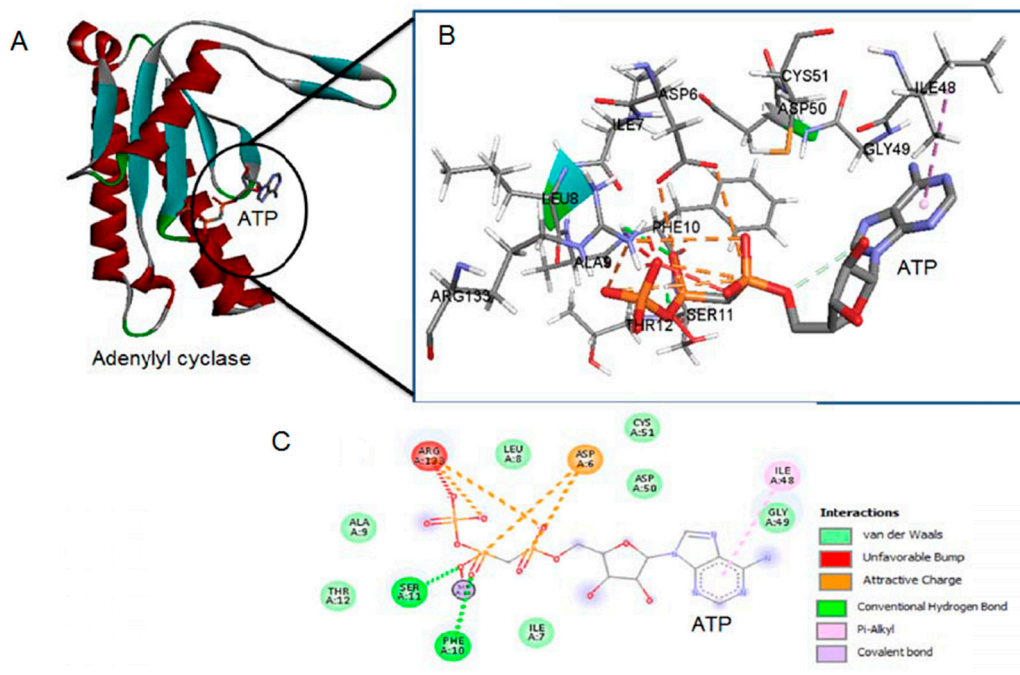
LINKER REGION

### B

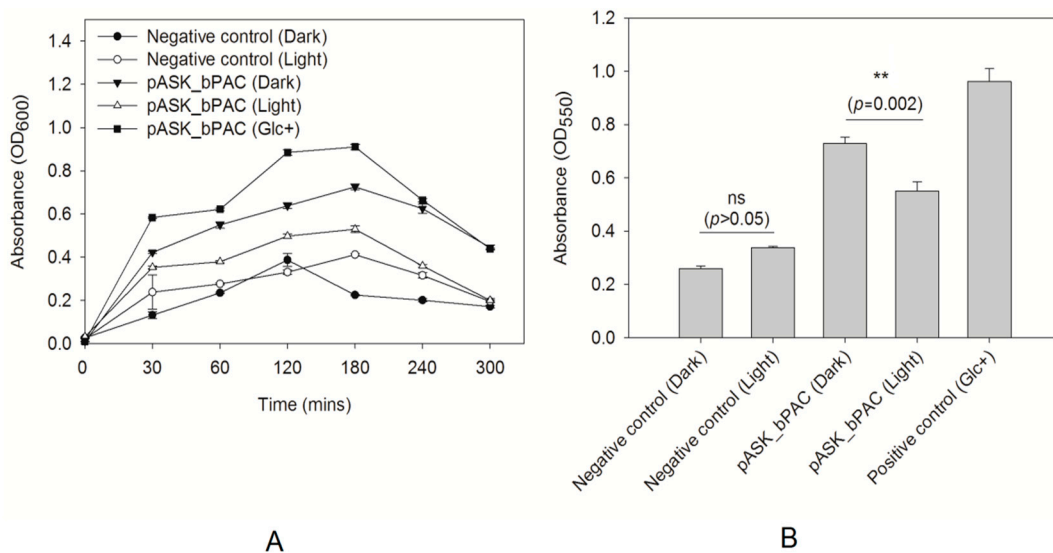


C

**Figure S1.** Multiple sequence alignment of the BLUF (blue light using flavin) domain (A), linker region (B), and cyclase domain (C) from different organisms with that of the well-characterized BLUF-Cyclase proteins from *Oscillatoria acuminata* depicting the functionally important amino acids in the BLUF domain (red star represent the amino acids important for photocycle and photodynamics of the BLUF while the orange square denotes the residues essential for the flavin binding pocket), in the cyclase domain [green stars represent important amino acids required for ATP binding, red arrows depict metal-binding residues (MBR), blue arrows correspond to the essential adenine-binding residues (ABR), and orange arrows denote transition state-stabilizing residues (TSSR)], respectively. The length of the linker region is crucial for light-gated cyclase activity of all the BLUF; cyclases were shown to be similar except in *Leptonema illini*, where the length of the linker region was surprisingly very short.



**Figure S2.** LgAC–ATP interaction analysis. **(A)** A 3-D model of the cyclase domain of LgAC docked with the ATP as the ligand (encircled). **(B)** Interaction between ATP and amino acid residues forming the ATP binding pocket within the cyclase domain (Inset). **(C)** 2-D interaction image depicting the amino acid residues and the nature of interaction involved in the binding of ATP. The green, yellow, and violet dashed lines represent conventional hydrogen bonding, attractive charge-based bonding, and pi-alkyl bonding, respectively.



**Figure S3.** **(A)** Growth curve of *E. coli* expressing recombinant LgAC in the dark and BL. Growth was estimated at 600 nm **(B)** Biofilm formation by *E. coli* carrying LgAC under dark and BL. The biofilm formation of *E. coli* was estimated at 550 nm. Data are presented as mean  $\pm$  SD ( $n = 3$ ). Statistical comparisons were performed using ANOVA using SPSS version 16.0. Asterisks (\*) represents the level of significance difference as compared to control: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  and “ns”, not significant. Positive control was *E. coli* cells supplemented with 0.1% glucose and negative control was untransformed *E. coli* cells only.