## MS13-P5 Structural basis for photoactivation of a light-regulated adenylate cyclase from the photosynthetic cyanobacterium *Oscillatoria acuminata*

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Cyclic-AMP is one of the most important second messengers, regulating many crucial cellular events in both prokaryotes and eukaryotes, and precise spatial and temporal control of cAMP levels by light shows great promise as a simple means of manipulating and studying numerous cell pathways and processes. The photoactivated adenylate cyclase (PAC) from the photosynthetic cyanobacterium *Oscillatoria acuminata* (OaPAC) is a small homodimer eminently suitable for this task, requiring only a simple flavin chromophore within a BLUF domain. These domains, one of the most studied type of biological photoreceptor, respond to blue light and either regulate the activity of an attached enzyme domain or change its affinity for a repressor protein. BLUF domains were discovered through studies of photo-induced movements of *Euglena gracilis*, a unicellular flagellate, and gene expression in the purple bacterium *Rhodobacter sphaeroides*, but the precise details of light activation remain unknown. Here we describe crystal structures and the light regulation mechanism of the previously undescribed OaPAC, showing a central coiled-coil transmits changes from the light-sensing domains to the active sites with minimal structural rearrangement. Site-directed mutants show residues essential for signal transduction over 30 Ångstroms across the protein. The use of the protein in living human cells is demonstrated with cAMP-dependent luciferase, showing a rapid and stable response to light over many hours and activation cycles. The structures determined in this study will assist future efforts to create artificial light-regulated control modules as part of a general optogenetic toolkit.

**Keywords:** cAMP, optogenetics, photoactivation, structural biology

## MS13-P6 Structural Basis of Selective Aromatic Pollutant Sensing by MopR, an NtrC Family Transcriptional Regulator

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Phenol and its derivatives are harmful to both terrestrial and aquatic life as they are carcinogenic, and embryotoxic in nature and their exposure, even in small quantities can be lethal. In recent years due to heavy industrial discharge these pollutants are released as major waste into the environment. Soil bacteria like *Pseudomonas sp.,* possess transcription regulators like XylR, DmpR and MopR that facilitate the natural catabolic degradation of these aromatic pollutants and hence have been used extensively over the years for design of effective biosensors that can detect these pollutants even at low levels. These proteins possess a modular architecture and are classified under the NtrC family of transcriptional regulators as they harbor a central AAA+ domain whose activity is regulated by the N-terminal sensor domain (1). Here, we have determined the crystal structure of the sensor domain of MopR ($MopR^{AB}$), from *Acinetobacter calcoaceticus*, in complex with phenol and its derivatives and investigated its binding profile using mutagenesis studies and various biophysical methods (2). The crystal structure of $MopR^{AB}$, which is the first structure from its family, possesses a unique fold and harbors a novel zinc motif that had remained elusive over the years. Based on the structure, we propose that the phenol binding pocket is dynamic in nature and is only formed when the ligand molecule occupies the binding site, triggered by the movement of the zinc-binding domain. The crystal structure is of great importance as it opens doors for selective and accurate design of broad-based/specific biosensors using rational approach. Over the past twenty years, plethora of efforts have been devoted to engineering of efficient aromatic biosensors based on these regulators. However, in the absence of any structure, most of the studies have failed to identify the exact sensor determinants. Our structure helps in not only identifying the correct pocket architecture but also aids in explaining the effects observed by other groups who have attempted to tweak the sensitivity of the sensor domain via either random mutagenesis or by creation of hybrid sensor domains. Moreover, based on the structure, we have been successful in undertaking preliminary studies, which serve as a stepping stone towards design of specific/broad based biosensors.

**References** (1) Shingler V. (2003) *Environ. Microbiol.* **5**, 1226-1241. (2) Ray S. et.al. *Manuscript under review in ACS Chemical Biology.*