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Structure guided design of aromatic biosensors for water quality monitoring

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Phenol, benzene and their derivatives are harmful to both terrestrial and aquatic life as they are carcinogenic, mutagenic 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 [1]. It is extremely challenging to employ suitable chemical strategies to develop specific sensors for these pollutants. However, 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 [2]. These proteins possess a modular architecture and are classified under the NtrC family of transcriptional regulators as they harbor a central AAA+ ATPase domain whose activity is regulated by the N-terminal pollutant sensing domain [2]. Here, we have determined the first crystal structure of the sensor domain of one of these proteins, MopR, from Acinetobacter calcoaceticus, in complex with phenol and its derivatives and investigated its binding profile using mutagenesis studies and various biophysical methods [3]. The crystal structure is of great importance as it opens doors for selective and accurate design of tunable biosensors using rational approach. Over the past twenty years, plethora of efforts had been devoted to engineering of efficient aromatic biosensors based on these NtrC regulators. However, in the absence of any structure, most of the studies 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 [3]. Hence, we used the X-ray structure of the sensor domain of MopR as a scaffold to perform accurate site-specific mutagenesis within the binding pocket in order to design a series of logic-based in vitro broad-based/selective biosensors based on the ATPase activity of MopR for detection of a spectrum of toxic aromatic pollutants. Moreover, to increase the efficiency of the biosensors, we translated the targeted biosensor designs into a whole cell setup, which could detect the said pollutants with a much higher sensitivity. Based on our structure guided biosensor designs, future efforts can be undertaken to establish a sensing system having engineered protein coated sensor chips, which are portable and can be directly used for variety of pollutant monitoring in real time environmental sites like river water, lake water etc. This would be a great stepping stone towards efficient bioremediation of target aromatic pollutants.

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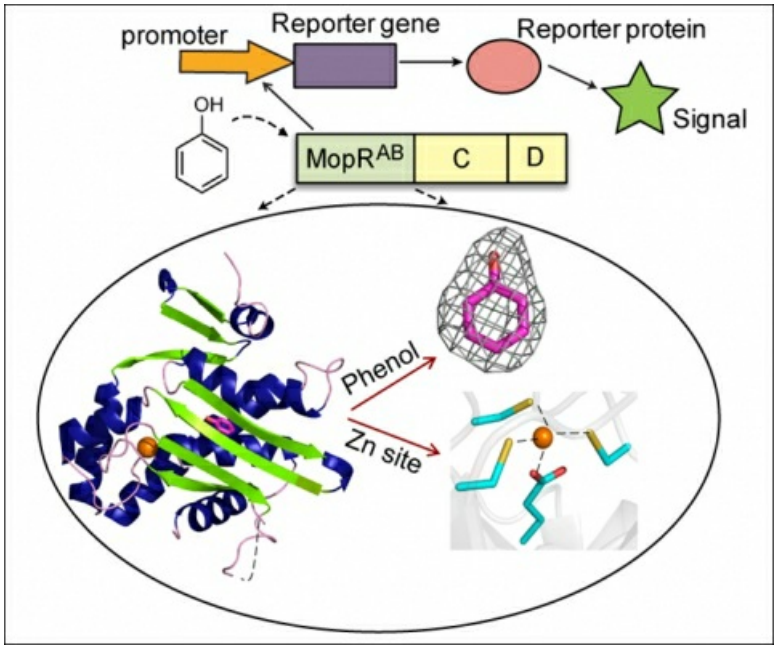

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