IITB-Monash Research Academy

An Indian-Australian research partnership

Indian Institute of Technology Bombay | MONASH University

Project Title:	**Structural characterization of a regulatory protein useful in developing biosensors for water purification system**	
Project Number	**IMURA0293**	
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Research Academy Themes:

Highlight which of the Academy's Theme(s) this project will address?

(Feel free to nominate more than one. For more information, see www.iitbmonash.org)

1. Advanced computational engineering, simulation and manufacture
2. Infrastructure Engineering
3. Clean Energy
4. **Water**
5. Nanotechnology
6. Biotechnology and Stem Cell Research

The research problem

Structural and biochemical studies of regulatory proteins of an operon which have significance in bioremediation and environmental medical diagnostic development with emphasis on biodegradation of phenol or benzene derivatives will enhance our ability to develop biosensors in order to detect these pollutants quickly and reliably. A whole-cell bacterial biosensor capable of detecting a wide range of pollutants can be created by placing a reporter gene under the control of an inducible promoter. Expression of the reporter gene provides a measurable response when the appropriate transcription activator protein interacts with a pollutant molecule to signal a particular environmental condition. We have targeted the regulatory proteins DmpR from Pseudomonas putida and other bacteria in order to understand their mechanism of action so that we can in the future design biosensors and develop strategies to remove these pollutants from the environment. In order to achieve this goal as a first step in this proposal we plan to clone, purify, crystallize and determine their 3D structures with the aim of

deciphering their functions. Subsequently we plan to develop high sensitivity bioassays and also engineer the protein of interest in order to design an enzyme-effector system, which can detect a host of pollutants efficiently and can be then implemented as a future diagnostic device.

Project aims

In this proposal our interest is two-fold and lies in understanding (1) the mode of binding of the aromatic alcohols (sensor molecule) and (2) in the downstream conformational change mediated by the binding of the sensor. Answering of both these queries is central in developing an efficient biosensor. Thus, the aim of the proposal is to; firstly, focus our initial effort on the N-terminal effector domain. This domain in the above class of proteins binds to aromatic alcohols. Although a body of work is available describing the various effects and the location of the individual domains, no structural information is available. Hence, as a first step we will concentrate are efforts on determining the structure of the effector domain. This will help in developing a thorough understanding of the active site and domain architecture of the effector domain. The structure will also provide insight into the specifics of individual residues involved in alcohol detection and from the structure we can develop strategies for engineering of the active site so that this domain can identify a broader range of aromatic alcohols. Secondly, we would also like to pursue the structure of the effector and ATPase domain in consort. This structure will be extremely valuable in understanding the conformation and the domain organization of both the effector domain and the ATPase domain with respect to each other. The structure of the apo form (no aromatic alcohol bound form) versus the structure with aromatic alcohol bound will help in mapping the conformational changes that occur upon ligand binding. This will help in further deciphering the mechanism of regulation of this class of effector-based sensor systems.

X-Ray crystallographic structure determination of the DmpR protein of *Pseudomas putida* provides a unique chance for the engineering of chemical sensing domain in order to respond large number of toxic aromatic compounds. In this context, DmpR from *Pseudomas putida* have been targeted for crystallization and structure determination.

The objectives are (i) cloning of these genes into several expression vectors to express the complete proteins and/or individual domains in soluble form, (ii) purification and characterization of these proteins/domains, (iii) crystallization, (iv) developing sensitive high-throughput biochemical assays to use effector molecules for screening, (v) diffraction data collection and phasing, (vi) model building and refinement and (vii) analysis of the structure. While the short-term goal is to determine the structure to understand the function, the long-term goal is to study interaction between regulatory proteins and effector molecules through high throughput screening thus leading to the development of biosensors.

Expected outcomes

In the short-term perspectives this project will be helping detailed understanding of the DmpR transcription factors. In addition this proposal aims to initiate the basic conceptualization behind development of a biosensor device for aromatic pollutants, which not only provide direct information that will result in changes in standards. In the long term perspective exploitation of the results developed through the project may result in changes in the way pollutants are analyzed, measured and controlled. The aim is to disseminate research information and results as early, fully and frequently as possible through a wide range of publications, mainly in high quality international scientific journals. The results from the project will be communicated by presenting results in international conferences within the field. The technology developed in this project will in the future be utilized by appropriate collaboration with the relevant expert team for creation of a medical diagnostic device.

How will the project address the Goals of the above Themes?

The number of toxic pollutants being discharged from industries has resulted in serious concern by pollution control authorities. There has been a significant increase in regulations that hold industrial entities accountable for the chemical pollution that results from their manufacturing activities. In order to comply with environmentally sensitive regulations, businesses must be able to identify contamination and monitor its remediation processes. The cost and technical complexity of chromatographic methods currently in use may act to limit characterization of contaminated sites. One way to lower the cost of detection is to use biosensors derived from genetic systems of bacteria that have evolved to use organic contaminants as growth media. The most basic whole cell bacterial biosensors can be created by placing a reporter gene under control of an inducible promoter. Expression of the reporter gene provides a measurable signal when the appropriate transcription activator protein interacts with an effector chemical. Operons encoding genes required for metabolism of phenol, toluene, benzene, and xylene. Transcription directed by these promoters occurs when the system's regulatory protein detects the presence of the substrate for the catabolic enzymes.

Capabilities and Degrees Required

The proposed projects represent a unique opportunity for a PhD student to acquire deep insights into molecular biology, biochemistry and structural biology using a variety of techniques. The duties/tasks and responsibilities of the PhD candidate are to lead the proposed project(s) under the supervision of his/her supervisors at IITB, and in close collaboration with colleagues at Monash University and Australian Synchrotron. By the end of the PhD study period, the candidate should have acquired a deep knowledge in molecular biology and biochemistry as well as a broad knowledge in structural biology. The PhD candidate should demonstrate excellent ability to design, perform, interpret, critically assess and contextualize generated data from experimental work. Furthermore, he/she should demonstrate aptitude to discuss and spread research results to both the national and international scientific community as well as to the non-scientific community.

The eligible candidate must hold a Master´s degree in Biotechnology, Biochemistry, biophysics, or equivalent. Previous experience in molecular biology and/or biochemistry as well as basic computational knowledge is necessary. Structural biology knowledge is desirable but not absolutely required. We are seeking a highly motivated, creative person with good technical skills. The successful applicant should be fluent in English, with excellent communication capacity combined with the ability to interact effectively and work productively in a team. Emphasis will be placed on personal suitability as well as genuine enthusiasm for the topic.