DATE: Day 14 Month 05 Year 2018

SUMMARY of
2017 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Investigation of Transcriptional Regulators as Potential Drug Targets**
Applicant	**Name**	**Dr. Inoka C. Perera**
	Affiliation	**University of Colombo**
	Present Title	**Senior Lecturer**
Research Collaborator (Host PI)		**Prof. Genji Kurisu**

Summary

DNA-binding transcriptional regulators, are fundamental key operators involves in gene expression by influencing RNA polymerase activity in a gene specific manner. Therefore identification and characterization of transcriptional regulators is real beneficiary in drug discovery. This project aims on characterizing two transcriptional regulators Rv0792 from *Mycobacterium tuberculosis* and PA1526 from *Pseudomonas aeruginosa.*

As the last year work summary, the protein Rv0792 which is a possible oxidative stress responsive protein in *M. tuberculosis* was expressed in *E. coli* Bl21(DE3)pLyS and eluted to near homogeneity with Ni-NTA chromatography. Thrombin cleavage optimization was carried out for His tag Rv0792, since precipitation occurred during concentrating the protein which leads to loss of protein yield rapidly. N-terminal analysis was carried out to investigate the His tag cleavage of protein with thrombin which revealed self- cleaved thrombin and non-specific cleavage of the protein. CD spectrum was carried out identify the secondary structural compositions of protein with and without His tag. Thermal stability of the protein was determined using DSC, which given a value of 57.2 ± 0.6°C for protein without His tag. DSC graphs revealed the folding of Rv0792 protein occurs through stable intermediates and dimeric nature of the protein. Pure fractions of size exclusion chromatogram was pooled and further concentrated up to 1.2 mg/ml. This was used for crystallization screening using several kits, including Index I and II, Crystal screen I and II, PEG/ Ion I and II, Wizard I, II, III and IV under different temperature conditions for few days. Only few salt crystals were observed in screening, this was mainly due to low concentration of protein used and three protein expression attempts were carried out in order to get concentrated protein samples, therefore further concentration and optimization is needed for the crystallization process of Rv0792.

Transcriptional regulator PA1526 in *Pseudomonas aeruginosa* is proposed as a potent drug target to battle infections of *P. aeruginosa* as it regulates an important pathway responsible for the ubiquitous existence of the organism. Three dimensional (3D) structure of the target protein needs to be determined for structure based drug discovery.

Protein structural studies were performed to determine the model of PA 1526. Recombinant PA1526 was purified to near homogeneity. Gel filtration chromatography revealed PA1526 is a dimeric protein. Circular dichorism (CD) spectroscopy revealed PA 1526 has high number of α helixes. PA 1526 crystal formation

screened with PEG Rx I and II, Index I and II, Crystal screen I and II, PEG/ Ion I and II, Wizard I and II, Wizard III and IV crystal screening kits was not successful due to poor yield of PA 1526 (1.25 mg/ml).
Optimization of the protein purification conditions will aids successful PA 1526 crystal development and structure determination experiments.

***Deadline: May 18, 2018**
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