IITB-Monash Research Academy

Indian Institute of Technology Bombay | MONASH University

An Indian-Australian research partnership

Project Title:	**The novel nuclear transport molecule importing from *Plasmodium falciparum*; potential drug target**	
Project Number	**IMURA0716**	
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IITB Department:	Biosciences & Bioengineering	

Research Clusters:

Highlight which of the Academy's CLUSTERS this project will address? *(Please nominate JUST **one.** For more information, see www.iitbmonash.org)*	
1	*Material Science/Engineering (including Nano, Metallurgy)*
2	*Energy, Green Chem, Chemistry, Catalysis, Reaction Eng*
3	*Math, CFD, Modelling, Manufacturing*
4	*CSE, IT, Optimisation, Data, Sensors, Systems, Signal Processing, Control*
5	*Earth Sciences and Civil Engineering (Geo, Water, Climate)*
6	*Bio, Stem Cells, Bio Chem, Pharma, Food*
7	*Semi-Conductors, Optics, Photonics, Networks, Telecomm, Power Eng*
8	*HSS, Design, Management*

Research 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*
7	*Humanities and social sciences*

The research problem

Define the problem

Importins (Imps) are the central transporters of molecules between the cytoplasm and the nucleus, with multiple forms of α and β types known in higher eukaryotes. Using *in vitro* binding assays, Prof. Patankar's lab. has shown that the nuclear transport molecule importin α from *P. falciparum* (PfImpα) can bind a nuclear localisation signal (NLS) from the *P. falciparum* trimethyl guanosine synthase (PfTGS1) protein (Bawankar et al, Mol Biol Rep, 2010, Babar et al, Mol Biochem Parasitol, 2016). These assays involved immobilising PfImpα onto Ni-NTA beads and assaying for binding with the PfTGS1 NLS fused to GFP. PfImpα was found to bind to NLS-GFP specifically, in contrast to control molecules.

Interestingly, the *P. falciparum* genome has a single gene for PfImpα, unlike the genome of the human host, which encodes 7 distinct genes for Impα, implying a lack of redundancy in the role of PfImpα in *P. falciparum*. In all eukaryotes thus far analysed including yeast, Impα only binds NLSs with high affinity in the presence of Impβ1, which it binds via its N-terminal Impβ binding (IBB) domain; truncation of this N-terminal IBB can also allow high affinity binding through relieving auto-inhibition conferred by the IBB on NLS binding by Impα. Intriguingly, full-length PfImpα can bind to NLSs in the absence of Impβ1 even when its IBB is present.

SPR assays have been developed in the Patankar lab. and used to determine the binding constants of full-length PfImpα to NLS-GFP (Kd in the low nM range). In contrast, the Kd of NLS-binding of human Impα1 to NLS is in the low μM range. It seems reasonable to predict that small molecules that target the PfImpα protein by mimicking the NLS are likely to bind to PfImpα with much higher affinity than human Impα.

The Jans lab. has developed an ALPHAscreen based binding assay to assess Imp-NLS binding, and used this to screen small molecule inhibitors (Wagstaff et al, J Biomol Screen, 2011), identifying ivermectin and mifepristone as molecules able to inhibit viral infection (HIV-1, Dengue, Venezualan Encephalitis VIrus; Wagstaff et al, Biochem J, 2012; Tay et al, Antiviral Res, 2013; Lundberg et al, Antiviral Res, 2013). A subsequent screen found a potent specific inhibitor (4HPR) of Dengue virus (Fraser et al, J Infect Dis, 2014). Screening a small molecule library for inhibitors of PfImpα–NLS binding is an exciting possibility, likely to reveal potential inhibitors of *P. falciparum* itself.

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Kaur, G., Delluc-Clavieres, A., Poon, I.K.H., Forwood, J.K., Glover, D.J. & Jans, D.A. (2010) "Calmodulin-dependent nuclear import of HMG-box family nuclear factors; importance for the role of SRY in sex reversal" **Biochem. J.** 340, 39-48

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Tay, M.Y.F., Fraser, J.E., Chan, K.K., Moreland, N.J., Rathore, A.P., Wang, C., Vasudevan, S.G. & Jans, D.A. (2013) “Nuclear localization of Dengue Virus (DENV) 1-4 Non-Structural Protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin” **Antiviral Res.** 99, 301-306

Wagstaff, K.M., Rawlinson, S.M., Hearps, A.C. & Jans, D.A. (2011) “An ALPHAscreen based assay for high-throughput screening for specific inhibitors of nuclear import” **J. Biomol. Screening** 16, 192-200

Wagstaff, K.M., Sivakumaran, H., Heaton, S., Harrich, D. & Jans, D.A. (2012) “Ivermectin is a specific inhibitor of importin ???-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus” **Biochem. J.** 443, 851-856

Project aims

Define the aims of the project

im 1. To test inhibitors of nuclear trafficking for the ability to inhibit PfImpα–NLS binding and *P. falciparum* infection, to confirm PfImpα–NLS binding as a viable target for high throughput screening (IITB/Monash Uni.). Ivermectin, mifepristone, 4-HPR as well as a range of small molecules identified by the Jans lab. will be tested in SPR (Patankar lab.) and ALPHAscreen (Jans lab.) binding assays for the ability to inhibit PfImpα–NLS binding. In parallel, all small molecules will be assayed for the ability to inhibit *P. falciparum* parasites in culture (routine in the Patankar lab.). *These experiments will validate PfImpα–NLS binding as a target for future drug screening.* Time-permitting, this may prove possible during the 1.5 years of the project at Monash.

Aim 2. To characterise the IBB domain of PfImpα by site-directed mutagenesis to determine the basis of lack of auto-inhibition (see above) (IITB/Monash Uni.). Mutagenesis will be performed of the IBB domain of PfImpα and human Impα proteins (Patankar lab.) to determine the key differences conferring relief from auto-inhibition. Binding of the mutant derivatives to Impβ1 and NLSs in the absence or presence of Impβ1 will be carried out using SPR (Patankar lab.) and/or ALPHAscreen (Jans lab.). *This will define key aspects of PfImpα function.*

Aim 3. To develop a nuclear transport assay for *P. falciparum* (Monash Uni./IITB). Transfection of *P. falciparum* in culture shows efficiencies of approximately one parasite in a million and does not lead to rapid generation of stable lines, so assays for nucleocytoplasmic transport become difficult. We propose to establish semi-intact cell assays, such as the mechanical perforation (wet cleavage) method, established in the Jans lab. (eg. Kaur G et al, Biochem J, 2010; Kaur and Jans, FASEB J, 2011; Misheva et al, BBA Mol Cell Res, 2014), and the digitonin permeabilization assay (Fineberg et al, Biochemistry, 2003) that is commonly used in many labs. These assays would be used to study nuclear transport of NLS-GFP and control molecules in *P. falciparum* infected cells. The assay systems will be learnt at Monash Uni., and the technology then transferred back to IITB to develop similar assays with parasite cells. The assay will then be used to analyse nuclear transport kinetics of NLS-GFP and other controls in *P. falciparum* infected cells

Expected outcomes

Highlight the expected outcomes of the project

1. Validation of PfImpα as a drug target – will lead to establishing a high throughput screening platform for drugs to inhibit PfImpα. These drugs could also potentially affect parasite viability in culture and be lead compounds for further development.
2. Delineation of the novel mechanism of NLS binding/lack of autoinhibition of PfImpα – will lead to X-ray crystallographic studies to both confirm findings, and give further insight into PfImpα as a drug target.

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

Describe how the project will address the goals of one or more of the 6 Themes listed above.

The project addresses an early stage of drug discovery for a globally prevalent infectious disease, malaria. The results from the project may be of interest to Indian pharmaceutical companies.

Capabilities and Degrees Required

List the ideal set of capabilities that a student should have for this project. Feel free to be as specific or as general as you like. These capabilities will be input into the online application form and students who opt for this project will be required to show that they can demonstrate these capabilities.

i. M.Tech. or equivalent degree in Biotechnology. i. M.Tech. or equivalent degree in Biotechnology.

ii. M.Sc. or equivalent degree in subjects related to Life Sciences OR B.Tech in Biotechnology with one of the following:
   a) a valid GATE score
   b) a valid CSIR/UGC/DBT JRF or a valid ICMR JRF not linked to ICMR project
   c) Minimum of 2 years of professional experience (acquired after obtaining the qualifying degree and completed before the starting of the semester in which admission is sought)

Experience with recombinant DNA technology, protein expression and purification is desirable.

Potential Collaborators

Please visit the IITB website www.iitb.ac.in OR Monash Website www.monash.edu to highlight some potential collaborators that would be best suited for the area of research you are intending to float.

Select up to **(4)** keywords from the Academy's approved keyword list **(available at www.iitbmonash.org)** relating to this project to make it easier for the students to apply.

Bioengineering and bio science