# **Queen’s Doctoral Training Programme - Multi-dimensional approaches to understanding microbe/host interactions in the context of disease, therapeutics and community resilience**

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| **Title of studentship** | **Mechanistic and structural studies of two novel antimalarial drug targets** |
| **Project summary (max 250 words – this will be used to advertise the project if selected).** | ***The Problem:*** Malaria is a serious infectious disease caused by protozoan parasites of the *Plasmodium* genus that results in over 660,000 deaths per year worldwide, predominantly in children and pregnant women. Effective treatment of malaria is under threat due to the rise of drug-resistant parasites. Consequently, there is a pressing need to identify and validate novel antimalarial targets. ***Opportunity:***We have identified two proteins in *Plasmodium* as novel drug targets: (i) an iron transporter; and (ii) aminopeptidase. The Law laboratory has extensive experience in the biophysical and biochemical characterization of the iron transporters, whereas our international collaborator is a leader in the biochemistry of aminopeptidase. Dysfunction of these malarial proteins interferes with iron homeostasis and supply of amino acids, respectively, during the intraerythrocytic stage of the parasite life cycle. Therefore, inhibition of these proteins offers an attractive strategy for development of novel anti-malarial therapies. The Tikhonova laboratory possesses expertise in molecular modelling for the study of protein dynamic behaviour and using the gained insights, to predict novel pharmaceutical interventions. Recent advances in structural studies of both proteins provide a unique opportunity for our teams to explore the molecular basis of protein function. ***Strategy:***This project aims to provide the structural context of the substrate-protein interaction of each target and to validate computer predictions through mutagenesis and functional assays. This project will provide a gateway for development of anti-malarial drugs. This work has direct relevance to pharmaceutical industry and sustainability in the developing world. The project facilitates skills development applicable in academia and industry. |
| **Supervisor(s)** | Dr. Irina Tikhonova and Dr. Christopher Law |
| **What types of new collaborative relationships would this studentship support (e.g. development of national and international collaborations or industrial involvement/financial support) (100 words max)**  | The studentship will help to develop new internal collaboration between Tikhonova and Law and external relationships with Evotec, a leading biotechnology company. In addition, the studentship will develop new relationships with Prof. John Dalton from the University of Galway, RoI and Dr Petra Rohrbach of McGill University, Canada. In addition, it is envisaged that the data generated will lead to significant funding income from joint SFI and RCUK programs.  |
| **Research centre / School** | School of Pharmacy and School of Biological Sciences |
| **Subject area** | Biochemistry, parasitology, computational biology, drug design |
| **Candidate requirements / Key skills required for the post.** Please note for the QUB-DTP awards applicants must have a 1st or 2.1 Honours degree (or equivalent) in a relevant subject. | Applicants should have a 1st or 2.1 honours degree (or equivalent) in a relevant subject. Relevant subjects include Pharmacy, Molecular Biology, Pharmaceutical Sciences, Biochemistry, Biological/Biomedical Sciences, Chemistry, Engineering, or a closely related discipline. Students who have a 2.2 honours degree and a Master’s degree may also be considered, but the School reserves the right to shortlist for interview only those applicants who have demonstrated high academic attainment to date |
| **Relevant links for project advertisement/ more information**  | <http://www.qub.ac.uk/schools/SchoolofPharmacy/Research/PostgraduatePositions/><http://www.qub.ac.uk/schools/SchoolofPharmacy/Research/> |
| **Keywords for search filters** | Malaria, drug design, molecular modelling, mutagenesis, parasitology, computational biology |
| **Training provided through the research project** | This research will be conducted at the School of Pharmacy Queen's University Belfast which in 2018 was ranked 2nd in the UK for Pharmacy and Pharmacology according to the Guardian University Guide 2018, underpinning the school's investment in and commitment to world-class facilities and staff, with internationally leading research programmes. The School of Pharmacy Queen’s University Belfast was particularly outstanding ranking as first amongst Pharmacy submissions in REF 2014. The School of Pharmacy is a leading UK centre for pharmaceutical research and has been supported by philanthropic donations of more than £7 million for strategic research developments. The School's research strategy has focused on developing high profile projects, including in cancer and dermatological therapies that ultimately have the potential to meet identified clinical needs and, consequently, also have high priority status with the pharmaceutical industry. As a member of the Russell Group, Queen's University Belfast which is consistently recognised as one of the leading universities for knowledge exchange in the UK, thus ensuring research is creating jobs, wealth, skills and innovation. The Postgraduate Research Committee (PGRC) advises and supports all PGR students, ensures appropriate training is provided, considers all matters relating to recruitment, admission, progress and examination for postgraduate degrees, monitors and reviews supervision, appoints external examiners, reviews complaints, refers student appeals to the University Postgraduate Appeals Committee and also submits an annual report to the University Postgraduate Office. The School of Pharmacy expects monthly meetings with students where electronic records must be kept. Students must also complete a three-month initial review and annual progress review to proceed to years two and three. The annual progress review involves written work, presentation and/or mini viva. These are the standard management and monitoring arrangements that must be adhered to by the academic partners. As such the School of Pharmacy has the best PhD completion rates within Queen's University Belfast. Each PhD student must also complete the centrally organised Queen's University Belfast researcher development framework program consisting of 30 days of training. These have been created by Vitae, and endorsed by the QAA and RCUK. Dr. Tikhonova will supervise the computational work of the project. Dr Law and our international collaborator will provide advice and supervision on mutagenesis and functional studies. The PhD will have an opportunity of industrial supervision and guidance from Dr. Hefetz, Evotec. The supervisors will form a scientific advisory board to evaluate the execution of the project and help the PhD student in meeting all the objectives. The training areas include four domains that encompass: (A) knowledge and intellectual abilities, (B) personal effectiveness, (C) research governance and organisation and (D) engagement influence and impact. For this studentship the student will be trained in the following generic skills; developing writing skills, developing presentation skills, power point for academic presentations and posters, communication skills, introduction to research design, academic plagiarism, basic and advanced statistics, networking and negotiating, lab demonstrating and introduction to ref works. Students are also encouraged to use the Personal Development Planning (PDP) process to build a portfolio on learning, performance, and achievement. PDP encourages the students to adopt a good work practice and supports the timely submission of thesis. The student will receive formal training in the following specialist skills necessary for this project- molecular modelling and dynamics, atomistic trajectory analyses, date mining, biochemical and biophysical methods. The combination of these skills is highly transferable and should give the student a distinct advantage either in academia or industry. |
| **Expected impact activities** | There is critical need for novel drugs to target malaria. Information on the *Plasmodium* transporter and aminopeptidases will be of value to the UK pharmaceutical sector in that it could be exploited to drive the design, development, and manufacture of novel antimalarials. The applicants have close ties with the pharmaceutical sector, and these will be exploited for commercially useful outputs that arise from the research. It is envisaged that the data generated will lead to significant funding income from RCUK. Several publications to high impact journals will be generated. The results of work will be presented at the national and international conferences. Furthermore, the project facilitates skills development in therapeutics research, which is applicable in academia and industry.  |

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**Project details including expertise in area and workplan (max 1200 words)**

**PROJECT DETAILS:**

**Project Titles: Mechanistic and structural studies of two novel antimalarial drug targets**

**Background study:**

Malaria is a serious parasitic disease, caused by unicellular protozoa of the genus *Plasmodium*, which is responsible for high morbidity and mortality rates among those infected. In 2013 alone, the most virulent human malaria parasite, *P. falciparum*, was responsible for an estimated 198 million clinical cases of infection and more than 0.5 million deaths[1](#_ENREF_1). Although currently confined to tropical and subtropical regions of Africa, Asia, Latin America and the Indian subcontinent, the change in climate suitability for transmission of malaria is associated with a predicted net increase in the global population at risk. Due to lack of a vaccination programme (even though at least one vaccine is currently undergoing trials), and emerging resistance against the drugs commonly used to control infection, there is a clearly critical need for a more intimate understanding of aspects of the biochemistry and physiology of the malaria parasite to inform future strategies of control; one such aspect that requires more investigation is the function and mechanism of *Plasmodium* membrane transporter proteins.

The malaria parasite undergoes a complex life cycle. Once the parasite is inside the host it proceeds to the intra-erythrocytic stages of development. This stage coincides with the time when the parasite is most metabolically active and when most clinical symptoms are manifested. During this stage, the degradation of host cell haemoglobin, and uptake and efflux of nutrients and ions are necessary to support protein synthesis and metabolism. Peptide degradation is supported by parasite M1 alanyl aminopeptidase (**PfM1-AAP)** but the transmembrane movement of solutes is facilitated by the ion transporter **(PfVIT).** The inhibition of these malarial proteins prevents either the supply of amino acids or iron metabolism during the intra-erythrocytic stage of the parasite life cycle and, therefore, is an attractive strategy for the development of novel combination anti-malarial therapies.

Recently, structural information for both proteins has become available. Our international collaborator has crystallised several structures of the PfM1-AAP. The crystal structure of the *E. grandis* VIT1 transporter that is homologues to PfVIT has been published, which allow building a reliable 3D model of PfVIT. New structural information has opened new opportunities to initiate an understanding of the mechanisms of substrate binding and recognition in these two proteins and used new knowledge for structure-based computer-aided compound design.

**Recent discoveries that motivate the project:**

The Tikhonova lab has started computer simulations of PfM1-AAP and showed that the migration of substrates through the C-terminal channel is predominantly controlled via long-range electrostatic interactions (**1**). This atomistic description provided a possible clue to the failure of random screenings and suggested opportunities to design a novel class of antimalarial agents in a rational way. The Tikhonova lab has also built the preliminary homology models of PfVIT and suggested residues for mutagenesis. The Law lab has obtained overexpressed and purified PfVIT and developed substrate binding and transport assays (**2**), which allow investigation of PfVIT mechanism of regulation and compound screening.

***References:***

[***Steered molecular dynamics simulations reveal critical residues for (un)binding of substrates, inhibitors and a product to the malarial M1 aminopeptidase.***](https://pubmed.ncbi.nlm.nih.gov/30379805/)Moore DS, Brines C, Jewhurst H, **Dalton JP**,**Tikhonova IG.** PLoS Comput Biol. 2018 Oct 31;14(10):e1006525.

***Recombinant vacuolar iron transporter family homologue PfVIT from human malaria-causing Plasmodium falciparum is a Fe2+/H+exchanger.***  Labarbuta P, Duckett K, Botting CH, Chahrour O, Malone J, **Dalton JP**, **Law CJ**. Sci Rep. 2017 Feb 15;7:42850.

**Specific aims:**

1. **Structure-function characterization of the ion transporter (PfVIT)**

We will focus on the identification and characterization of the PfVIT transport mechanism and a role for activity-dependent regulation of PfVIT that could be exploited for therapeutic intervention. We will identify key amino acids in PfVIT involved in substrate binding and/or transport activity. We will model the structure of PfVIT in the realistic membrane environment and apply enhanced sampling molecular dynamics techniques to study the ion transport and substrate binding. The results of *in silico* work will be validated in the transport assay and substrate binding assays developed in the Law lab.

**2. Structure-function characterization of M1 alanyl aminopeptidase (PfM1-AAP)**

We will focus on the mechanism of substrate and inhibitor binding and identify key amino acid residues in the substrate and inhibitor recognition. We will also compare the binding site of M1-AAP with apicomplexan parasite homologs isolated in the Dalton lab. All computer predictions will be validated using mutagenesis and various enzymatic assays established in the Dalton laboratory.

**3. *In silico* and *in vitro* compound library screening against PfVIT and PfM1-AAP**

The gained above structural insight in substrate-protein binding will be used for virtual compound screening and subsequent experimental validation against both targets by Law and Dalton labs. Prof. Dalton in collaboration with NIH has performed high throughput screening of NIH compound libraries against several malarial proteins. This data will be incorporated in computational screening efforts with a goal to identify pan-inhibitors.

**Expertise**

This interdisciplinary project is a collaborative effort bringing together skillsets to ensure that structural modelling of novel targets can be linked with basic biology and parasitology and translated towards drug design.

Tikhonova (School of Pharmacy) brings expertise in molecular modelling and simulations of soluble and membrane proteins, chemoinformatics, drug-target interaction.

Law (School of Biological Sciences/IGFS) provides expertise in the production, purification, characterisation (using a variety of biophysical and biochemical methods) and crystallisation of integral membrane proteins.

Dalton (University of Galway, RoI) provides expertise in anti-malaria drug design, vaccine development against helminth parasites of animals and humans, and discovery of novel helminth parasite-derived immunotherapeutics.

Heifetz (Evotec, Oxford England) provides expertise in industrial drug discovery and development.