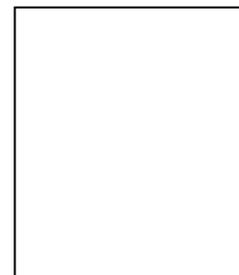


Mahidol - Liverpool Ph.D. Scholarships 2020
Application Form



First name: _____ Last name: _____

Name (in Thai): _____

Date of Birth: _____ Male/Female: _____

Please define your current status by checking / one of the following:

Graduate Staff / Faculty Member (Please specify your current Position):

Department/Faculty at Mahidol: _____

Graduation Year: _____ Cumulative GPA: _____

Address: _____

Home phone: _____ Mobile phone: _____

E-mail: _____

MU Staff member who can act as a reference:

Name _____

Position _____

E-mail _____ Mobile phone: _____

Have you taken any English Language examinations? Yes No

If Yes, give date of validation, overall score and individual subsets score¹:

IELTS overall: _____ Date: _____

TOEFL: _____ Date: _____

If No, you will be required to complete your language requirements by 30 September 2020.

¹ **English Requirements for UoL:** For overseas students, may require demonstration of your ability to speak and write English to an acceptable standard (IELTS 6.5 with minimum 5.5 in each component, or TOEFL Internet Based Test 88/89 with minimum scores in components as follows: Listening and Writing – 21, Reading – 22, Speaking – 23).

Eligibility:

- Thai citizenship.
- Awarded / expected 1st class degree with a GPA of 3.5 and above, from Mahidol University in related disciplines or currently a staff or faculty member at Mahidol University.
- English proficiency test with a minimum score that fulfills the requirements of the University of Liverpool. (IELTS 6.5 or TOEFL 570 (Paper Based), TOEFL 88-89 (Internet Based)).

Please submit completed application form with Transcript and one page CV to International Relations Division, Office of the President through your host faculty. (Deadline 30 September 2020).

Investigating the role of calmodulin in immunodeficiency and muscular hypotonia

<https://www.liverpool.ac.uk/translational-medicine/staff/nordine-helassa/>

Human genetic disorders such as “CRAC” (calcium release-activated calcium) channelopathies can cause severe immunodeficiency and muscular hypotonia. Recently, human gene mutations in the ion channel Orai1 (R91W, A88SfsX25) and its partner STIM1 (R426C, R429C) have been identified in patients with these conditions [1, 2].

The mutations are located in calmodulin-binding regions of the proteins, therefore we hypothesise that the calcium sensor calmodulin play an important role in the molecular mechanism of these conditions [3]. Calmodulin regulation in the context of CRAC channelopathies has not been investigated yet and remains unknown.

To get a better understanding of these diseases, we will investigate the role of calmodulin in the molecular mechanism of Orai1- and STIM1-associated human muscular hypotonia and immunodeficiency. We will determine the effect of Orai1-STIM1 mutations on:

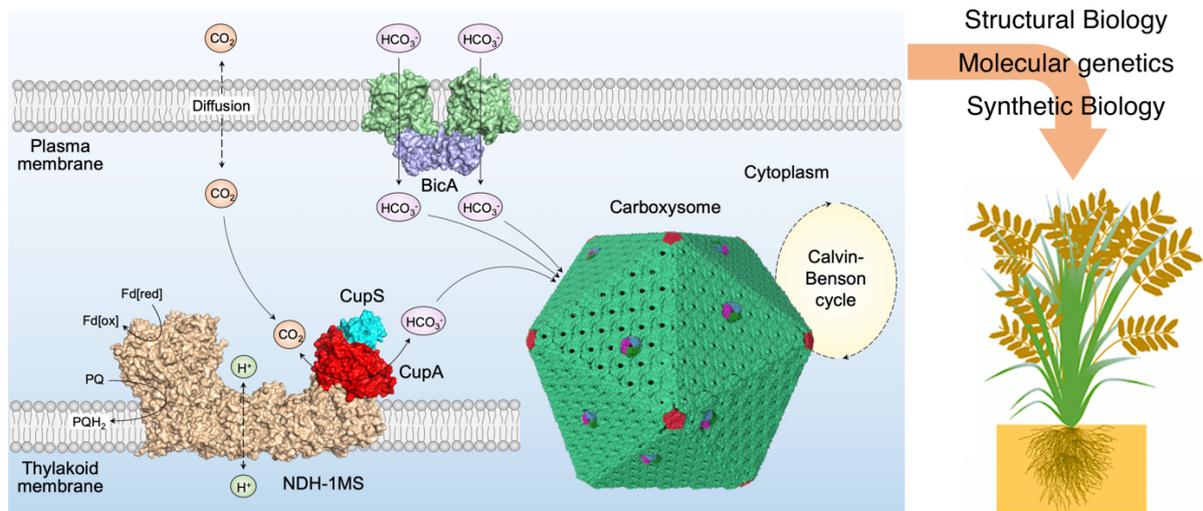
- (i) calmodulin binding to Orai1-STIM1
- (ii) calmodulin three-dimensional structure in complex with Orai1-STIM1
- (iii) the regulation of Orai1 channel activity by calmodulin

In this project, we will use a multidisciplinary approach to investigate the role of calmodulin in CRAC channelopathies. We will combine protein biophysics (ITC, fluorescence spectroscopy), structural biology (NMR and X-Ray crystallography), cell imaging (confocal microscopy) and electrophysiology (patch-clamp) to determine how calmodulin regulates Orai1-STIM1 function in human immunodeficiency and muscular hypotonia. Data obtained from this project will open opportunities for developing new therapeutic avenues of investigation.

The student will be trained and gain expertise in a palette of techniques including molecular biology, protein biochemistry, biophysics, structural biology and electrophysiology. The student will be hosted in the Department of Cardiovascular and Metabolic Medicine and benefit from the state-of-the-art facilities available at the Faculty of Health and Life Sciences at the University of Liverpool.

1. Zhang, S.L., et al. Proc Natl Acad Sci U S A, 2011. **108**(43): p. 17838-43.
2. Lacruz, R.S. and S. Feske. Ann N Y Acad Sci, 2015. **1356**: p. 45-79.
3. Li, X., et al. Nat Commun, 2017. **8**(1): p. 1042.

Characterisation and engineering of bacterial metabolic organelles for enhanced photosynthetic performance



With the rapid growth of the global population, the demand for food and energy is dramatically increasing. There is an urgent need to develop innovative strategies, by taking advantage of modern biotechnology, to enhance agricultural production. The single-cell photosynthetic microorganisms, cyanobacteria, account for over 25% of the global carbon fixation, thanks for their powerful CO₂-concentrating mechanisms. This unique system comprises proteins in cell membranes to pump CO₂ and bicarbonate through cell membranes and accumulate them in the cell, and the central CO₂-fixing organelle, named carboxysomes, to fix CO₂. The carboxysome has a virus-like protein-based structure, comprising an icosahedral shell made of thousands of protein peptides and the encapsulated cargo enzymes Rubisco and carbonic anhydrase. The carboxysome is one of the most important macromolecular complexes whose structures have not been solved.

This PhD project will unravel the structure and biogenesis pathway of carboxysomes in molecular details. We will study (1) the protein composition and stoichiometry of carboxysomes, (2) the three-dimensional structure of carboxysomes, and (3) the stepwise assembly process and regulation of carboxysomes, using multidisciplinary techniques including biochemistry, molecular biology, cryo-electron microscopy, and synthetic biology.

Advanced understanding of the molecular mechanisms that mediate carboxysome formation and regulation will offer great opportunities for the bioengineering of CO₂-fixing carboxysomes in other species to improve carbon fixation. For example, introducing functionally active carboxysomes into crop plants is considered as a promising strategy for boosting photosynthesis and crop yields. It will also inspire the development of new nanomaterials and protein scaffolds for biotechnological applications, e.g. underpinning cell metabolism, molecule delivery, and therapeutics.

This ambitious project builds on the world-class expertise of Professor Luning Liu (University of Liverpool, www.luningliu.org) in photosynthesis, carboxysome assembly, molecular genetics, microscopy, and synthetic biology, and Professor Peijun Zhang (University of Oxford) in state-of-the-art cryo-electron microscopy. It will provide excellent opportunities for the PhD student to learn a wide spectrum of experimental techniques and produce high-quality publications.

Key publications of the research groups:

- (1) Zhao et al., and Liu LN* (2020) *Nature Plants*, accepted.
- (2) Yang et al., and Liu LN* (2020) *Nature Communications*, DOI: 10.1038/s41467-020-15888-4.
- (3) Wang et al., and Liu LN, Zhang P* (2019) *Nature Plants*, 5:1184-1193.
- (4) Sun et al., and Liu LN* (2019) *Plant Cell*, 31:1648-1664.
- (5) Zhang P* (2019) *Curr Opin Struct Biol*, 58:249-258.
- (6) Himes BA, Zhang P* (2018) *Nature Methods*, 15:955-961.

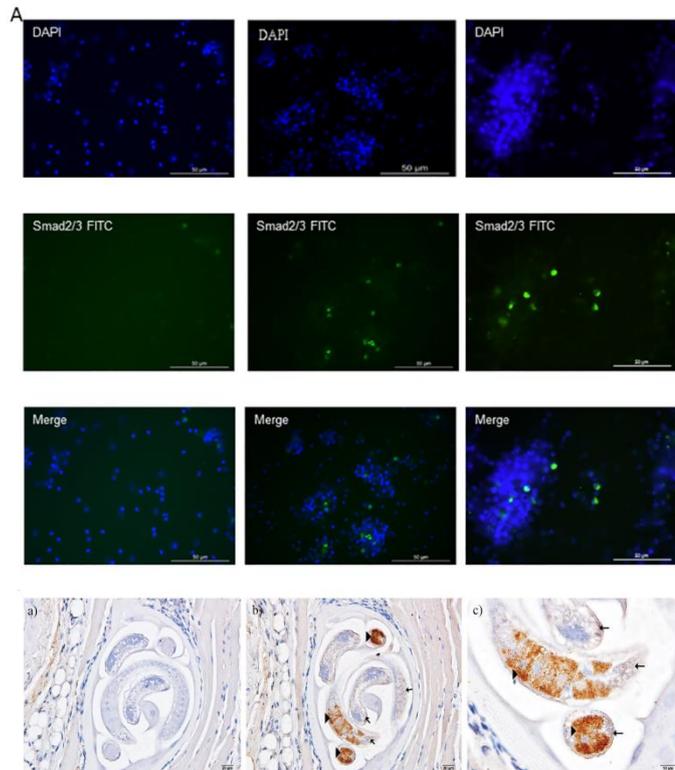
Mechanisms of interspecies signalling in nematodes

Dr Robin J Flynn University of Liverpool & Dr Poom Adisakwattana University of Mahidol

Parasitic worms provoke a strong type-2 (Th2) inflammatory response during the course of infection; however, parasites have developed complex lifecycles to evade host immune responses and avoid expulsion from the host. These often involve phases of migration within the host or release of proteins that can modulate the host immune response. One parasite which combines both of these tactics is the zoonotic nematode *Trichinella spiralis*.

Adult stages of *T. spiralis* are expelled from the host gut by type-2 immunity; however, new-born L1 larvae (NBL) persist in tissues in particular the diaphragm and muscles of legs. Residing within muscle cells, that the parasite converts to nurse cells, NBL evade immunity and reprogram host metabolism sustaining infection. Published data shows that the host immune response and metabolic pathways converge here to benefit the parasite.

Recently, our groups have identified a number of parasite proteins which interact specific host immune signalling pathways or host cytokine receptors. We now propose to characterise the proteins secreted from *T. spiralis* NBL that signal through cytokine receptors or immune signalling pathways to manipulate the host response for a survival advantage.



Upper panel from PLOS Path 2016,
lower panel PLOS NTD 2020

Training & Techniques:

This project will offer an opportunity for a candidate to receive training in molecular cloning of parasite genes and preparation of recombinant proteins. Downstream there will be opportunities to develop skills in reporter assays and functional assays using *in vitro* systems. Importantly, the candidate will have an opportunity to develop parasite knockdowns using RNAi technologies. These knockdown parasites will be phenotyped in terms of their *in vivo* immune responses in a murine model of infection, providing training in ex vivo immunological analysis.

Recent References:

Kobpornchai P, Flynn RJ, Reamtong O, Rittisoonthorn N, Kosoltanapiwat N, Boonnak K, Boonyuen U, Ampawong S, Jiratanh M, Tattiyapong M, Adisakwattana P. A novel cystatin derived from *Trichinella spiralis* suppresses macrophage-mediated inflammatory responses. PLoS Negl Trop Dis. 2020 Apr 1;14(4):e0008192. doi: 10.1371/journal.pntd.0008192. PMID: 32236093.

Sulaiman AA, Zolnierczyk K, Japa O, Owen JP, Maddison BC, Emes RD, Hodgkinson JE, Gough KC, Flynn RJ. A Trematode Parasite Derived Growth Factor Binds and Exerts Influences on Host Immune Functions via Host Cytokine Receptor Complexes. PLoS Pathog. 2016 Nov 2;12(11):e1005991. doi: 10.1371/journal.ppat.1005991. PMID: 27806135; PMCID: PMC5091765.

The role of a novel point mutation in the small calcium binding protein, CaBP4, in a rare form of inherited human epilepsy.

Dr. Lee Haynes <https://www.liverpool.ac.uk/translational-medicine/staff/lee-haynes/>

Epilepsy is a relatively common (~1 in 100) neurological disorder affecting all ages. It is characterised by sudden unusual bursts of excessive or synchronous neuronal activity that can lead to characteristic seizures. A significant percentage of epilepsy sufferers have a refractory form of the disease and do not respond to medication. Unfortunately, most of these individuals are also unsuitable for surgery and therefore their symptoms can be extremely difficult to treat. There is a pressing need to identify new molecular pathways involved in epilepsy which could reveal new therapeutic targets for the disease.

Calcium controls or influences almost all aspects of mammalian cell behaviour and is essential for normal neurotransmission. Calcium signals in the mammalian central nervous system (CNS) are detected by families of specific calcium sensing proteins [1]. These proteins then interact with downstream effectors to drive processes as diverse as neurotransmitter release, alterations in neuronal gene expression, changes in neuronal excitability and regulation of neuronal architecture and connectivity. As such, they are involved in high-level functions including learning, problem solving and memory acquisition. Dysregulated CNS calcium signalling has been linked to epilepsy in humans and rodents [2]. A recent genotyping study of a family having a rare inherited form of the disease identified a single point mutation in the calcium sensor, Calcium Binding Protein 4 (CaBP4^{G155D}) as a possible underlying cause [3].

Although CaBP4 has important functions in the regulation of voltage gated calcium channels essential for neurotransmission in the mammalian auditory system, no CNS localised functions for CaBP4 have been reported. The G155D mutation suggests that CaBP4 may have a yet undiscovered CNS specific function that is linked to epilepsy.

This project is designed to investigate this possibility by examining the structure and function of the protein to explain the epilepsy phenotype. The student will employ a diverse range of experimental techniques, routine in our laboratory [4], that will provide excellent training in modern protein biochemistry and cell biological approaches. They will determine the structure of CaBP4 wild-type (CaBP4^{WT}) and CaBP4^{G155D} through protein crystallisation (collaboration with Dr Andrew Lovering, University of Birmingham, UK). The effect of the mutation on the chemical stability, calcium and target peptide binding properties of CaBP4 will be examined using a combination of biochemical and biophysical approaches including: Protease sensitivity, intrinsic fluorescence, circular dichroism spectroscopy and isothermal titration calorimetry. The project will then address the functional differences imparted by the G155D mutation. HEK cells expressing CaBP4^{WT} or CaBP4^{G155D} and transfected with known CaBP4 regulated voltage gated calcium channels will be loaded with calcium dye and depolarisation induced calcium fluxes monitored by confocal microscopy. These combined approaches will allow us to link structure to function and will provide insights into the molecular basis of a unique form of human epilepsy. This in turn could reveal novel potential therapeutic targets for future investigation.

References

1. Burgoyne RD et al. Cold Spring Harb Perspect Biol. 2019 May 1;11(5). pii: a035154.
2. Zamponi GW et al. Pflugers Arch. 2010 Jul;460(2):395-403.
3. Chen ZH et al. Oncotarget. 2017 Sep 5;8(45):78940-78947.
4. Helassa N et al. Hum Mol Genet. 2017 Jul 1;26(13):2426-2435.

Dissecting human INPP5K: cell biology, structural determination by X-ray crystallography and enzymology of a key protein implicated in muscular dystrophy, insulin signalling, spastic paraplegias and Parkinsonism.

Primary Supervisor Dr Laura Swan

We are offering an exciting interdisciplinary project combining structural biology/biophysics, cell biology and molecular neuroscience which will give PhD student possibility to work in 3 laboratories (the Swan lab (<http://pcwww.liv.ac.uk/~lauras/>), Molecular-Biophysics group <http://www.biophysics.liv.ac.uk/> and <http://pcwww.liv.ac.uk/~maxstagi/>) based in newly established Institute of Systems, Molecular and Integrative Biology (Faculty of Health and Life Sciences) and learn complementary techniques.

The lipid metabolising enzyme called **INPP5K** (<https://pubmed.ncbi.nlm.nih.gov/28190456/>) has many very intriguing roles in axonal regeneration, in insulin signalling and in cancer biology. Its mutants are seen in patients with inherited diseases of the brain, eyes and muscles. But details of its involvement are not very clear yet, which makes this project very interesting.

INPP5K and several other critically important proteins in the cell (such as the immune system protein and mitophagy regulator NDP52) contain a protein domain called a SKICH domain, which is essential to recruit proteins to cell membranes. The Swan lab has found a new group of small molecules that bind the SKICH domain, which you will characterise by X-ray crystallography (with Dr Svetlana Antonyuk) and in neuronal cell culture (with Dr Massimiliano Stagi) as part of your PhD project.

You will be based in the Swan lab working on INPP5K biology (mouse models, live imaging and enzymology), with collaborators in Germany, UK and USA.

During 4 years of PhD you will learn key lab techniques in cell biology, protein biology, biophysics and structural biology which are can be applied widely in your future academic career.

You are welcome to contact Dr Swan directly if you have any questions:

laura.swan@liverpool.ac.uk

A comparative functional genomics approach towards unravelling the molecular mechanisms underlying floral organ photosynthesis

(Subject area: functional genomics / photosynthesis / plant development / comparative genomics / gene regulatory networks)

Primary Supervisor: Dr Diarmuid Ó Maoiléidigh, Ph.D. (Institute of Integrative Biology, University of Liverpool)

Increased crop productivity is required to provide enough food for an ever-growing population, which is expected to double by 2050. Photosynthesis is a key process that controls plant growth and improvement of photosynthetic efficiency is an important strategy towards fortifying global food security. Photosynthesis is often viewed as a leaf-centric process, where photosynthates generated in the leaf are transported to other parts of the plants, such as the developing fruit and seeds, to support their growth. Although this process is important during plant development, a large body of research indicates that photosynthesis occurring in the fruits and seeds themselves is a vital source of energy. Fruits and seeds of a large variety of plants are photosynthetically active, however, it is unclear how this process is controlled on a transcriptional level. By understanding the composition of the gene regulatory networks underlying floral organ photosynthesis, strategies to improve the photosynthetic capacity of fruits and seeds can be implemented.

This project will focus on two evolutionarily conserved transcription factor paralogs that control photosynthetic capacity in both vegetative and reproductive structures. State-of-the-art techniques will be implemented during this project, which will be performed in at least two model plant species. This work will improve our understanding of how whole plant photosynthesis is coordinated to drive plant growth.

References

1. Brazel, A.J. and Ó'Maoiléidigh, D.S. (2019) Photosynthetic activity of reproductive organs. *Journal of Experimental Botany* 70(6): 1737–1754
2. Aschan G, Pfanz H. 2003. Non-foliar photosynthesis—a strategy of additional carbon acquisition. *Flora* 198: 81–97

Developing cross-linked glycan for potential colonic drug delivery

Primary supervisor: Dr Alan Cartmell

<https://www.liverpool.ac.uk/integrative-biology/staff/alan-cartmell/>

Background to the project

The development of small molecules to treat human disease and enhance quality of life, is one of the most important areas in biotechnology. There are several key aspects of small molecules which need to be considered, including; (i) the efficacy against their target, (ii) establishing what the off target effects are, and (iii) determining how the molecule should best be delivered. Both efficacy and off-target effects can be optimised through effective, targeted, drug delivery. For instance, the ability to target a drug to a specific, localised area, will increase its effective concentration and limit its interactions to molecules in that localised area, thus reducing off-target effects.

Complex glycans (GAGs) already make it to the colon intact and the bacteria in the colon have the required enzymatic apparatus to metabolise them once there. This makes GAGs an ideal potential mechanism for colonic drug delivery. Two key parameters are needed to utilise GAGs for drug delivery: knowledge of the glycan's composition and structure and detailed molecular insights into the proteins that metabolise them. It is with these key parameters in mind that the focus of this proposal will be to develop cross-linked glycans for potential drug delivery. Furthermore, GAGs, as dietary components themselves they are safe and unaffected by the presence of other components in the diet. This is ideal for a drug delivery system as the GAG vehicle will be resistant to sudden dietary changes.

Preliminary data

We have recently characterised the genetic locus PUL_{CS/DS/HA} from the colonic organism *bacteroides thetaiotaomicron* (*B.theta*). PUL_{CS/DS/HA} confers the ability on *B.theta* to metabolise GAGs¹. **PUL_{CS/DS/HA} is conserved in significant number of species.** We have determined many of the biochemical activities of the proteins encoded by PUL_{CS/DS/HA} and solved several structures with large GAG substrates and products. We have extensive experience in the chemical modification and characterisation of GAGs, including modifications to their sulfation patterns the removal of N-acetyl groups and the generation of cross-linked gels by several different methods, including cross-linking of the amine groups between GAGs.

Project description

The project provides a truly multidisciplinary experience with the student developing skills in carbohydrate chemistry, carbohydrate active protein biochemistry and structural biology. Training in carbohydrate chemistry is needed to be able to effectively crosslink and fluorescently label the GAGs to be utilised in this study. Training in recombinant protein expression, enzymology and structural biology will also be provided by Dr Cartmell. This will enable further characterisation of the surface GAG degradative apparatus, building on our recently published work of the GAG degradative apparatus¹. This training is invaluable in being able to rationally design the GAGs for drug delivery. These training aspects will be carried in Liverpool and training provided by the Dr Cartmell and Dr Yates who share a laboratory space and already work closely together. The skill acquired from this project will enable the applicant to operate as an effective glycobiologist of the highest level.

Reference:

1. Metabolism of multiple glycosaminoglycans is orchestrated by a versatile core genetic locus.
Ndeh D, Baslé A, Strahl H, Yates EA, McClurg UL, Henrissat B, Terrapon N, Cartmell A.
Nat Commun. 2020 Jan 31;11(1):646. doi: 10.1038/s41467-020-14509-4.