Cell Membrane Training to Advance Industrial Processes

H2020-MSCA-COFUND-2018 Grant Agreement 847419

Guide for Applicants

Contact Details:


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Aston University
Birmingham

MemTrain Early Stage Researcher

Reference: R200087

Salary: £21,500 p.a.

Contract Type: Fixed term, 3 years

Basis: Full Time

Closing Date: 19th April 2020

Interview Date: May 2020

Start date: 1st October 2020

Applications must be submitted on line:
http://jobs.aston.ac.uk/

Aston University
Birmingham

1. Background

In May 2019, the Department of Biosciences at Aston University was awarded the EU Marie Skłodowska-Curie funded COFUND Doctoral Programme entitled MemTrain (Cell Membranes in Industrial Processes Training). The Programme is coordinated by Dr Alan Goddard who is a member of the Aston Membrane Protein and Lipid Research Group (AMPL). More information about AMPL can be found at:

https://www2.aston.ac.uk/lhs/research/centres-facilities/molecular-biomedical-research/ampl/Aston%20Membrane%20Proteins%20and%20Lipids%20(AMPL).

Over five years, MemTrain will train 12 Early Stage Researchers (ESRs; within 4 years of Masters qualification) undertaking a PhD in an area of research within the remit of AMPL, each of which is expected to have an intersectoral focus. Possible projects involve determining the roles of biological membranes in biotechnology or medicines discovery and the application of novel methodologies to investigate these membranes. Our 11 industrial partners will contribute to training of the researchers to generate a unique, integrated, learning environment in which graduate students benefit from industrial links, employability and entrepreneurial skills, and will leave as researchers who are highly competitive for jobs within academia and industry, as well as public policy, intellectual property law or as entrepreneurs.

The first cohort of ESRs were appointed in October 2019 and it is expected that 7 ESRs will be recruited in this call.

Further information about the MemTrain project and the team can be found on our website at:

https://www.memtrain.org.uk/

2. Research themes and projects available

In your application, please clearly indicate which project you are applying for based on the following descriptions. Candidates are strongly encouraged to have informal discussions with the relevant supervisor or MemTrain team over the suitability of their application before submission.

i. Development of therapeutics for dermal and ocular use based on plant bioactive compounds

The advancement of phytochemical interest and activity and a rising demand for plant bioactive compounds have led to elucidation of the chemical composition and biological properties of a number of plant species. Most of the bioactive compounds, including plant phenolics, tannins, terpenoids and proteins, are not readily able to cross the lipid barrier of the dermal surface. In consequence bioavailability and efficacy are relatively poor. The use of delivery complexes seems to cause least side effect in terms of tissue damage. Of this

family it is notable that improved drug absorption through the dermal layer is associated with lipid-based nanovesicles, such as liposomes, and ethosomes. Practical use of those vesicles is still limited, however, because of (e.g.) the lack of long-term stability and specific encapsulation requirements. There is, therefore, significant potential value in the design of synthetic polymers that can combine extraction and encapsulation ability whilst maintaining functionality of active compounds in the form of nanostructures. Such systems are equally applicable to the ocular environment.

The key to the success of this project will depend upon the design and development of an optimised hyper-associating styrene-maleic acid copolymer, or analogous structure, based on the expertise developed through existing collaborations between LHS (Rothnie, Goddard, Bill) and EAS (Tighe, Topham). A range of appropriate plant-based natural resources are available. The project will develop both synthetic and analytical skills.

The project will be supported by i+Med, a medical device research company specialising in controlled release and its biomedical applications

Project contacts:

Academic: Dr Alice Rothnie, Aston University: A.Rothnie@aston.ac.uk; Prof Brian Tighe, Aston University: b.j.tighe@aston.ac.uk

Industrial: Dr Virginia Saez: vsaez@imasmed.com

ii. Understanding age and diabetes-related changes in erythrocyte membranes.

There is evidence that both ageing and diabetes alter the fluidity of the red blood cell membrane leading to dysfunction, but the molecular basis for this is not well understood. Diabetes is a metabolic disorder closely linked to cardiovascular disease risk and inflammation, and is known to lead to the modification of proteins by the formation of advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs), an example of which is the widely used HbA1c diagnostic biomarker for chronic diabetes. Ageing has also been shown to result in an increase in lipid and protein oxidation.

This project will investigate the correlation between membrane fluidity, (phospho)-lipid composition and the formation of AGEs and ALEs on erythrocyte membrane proteins, using erythrocytes that have been treated diabetes-related stress conditions or from aged volunteers. The methods with include fluorescent-based assays for membrane permeabilization and fluidity, western blotting for the formation of AGEs and ALEs, and mass spectrometry to monitor phospholipid changes. The pro-inflammatory effects of these model diabetic erythocytes will also be investigated. This will lead to an understanding of how diabetes and ageing lead to cellular dysfunction, and may provide insights that will lead to better management and treatment of disease and healthier ageing.

The project will be supported by our industrial partner SCIEX, who bring expertise in the analysis of biomolecules, especially using mass spectrometry.

Project contacts:

Joint Academic Supervisors: Prof Corinne Spickett. Aston University: c.m.spickett@aston.ac.uk; Dr Alan Goddard, Aston University: a.goddard@aston.ac.uk;

Associate Supervisor: Dr James Brown, Aston University: j.e.p.brown@aston.ac.uk

Industrial: Stephen Ayris, SCIEX: stephen.ayris@sciex.com

Industrial: To be advised

iii. The role of protein lipoxidation in cell membrane signalling.

During inflammation and metabolic imbalance reactive oxygen species are formed, which leads to oxidative stress resulting in oxidative damage to phospholipids in cell membranes, producing a variety of short and long-chain lipid oxidation products. The reaction of these oxidized lipid products with proteins (lipoxidation) can change the structure, activity and cellular effects of the protein. The formation of lipoxidation products has been demonstrated in a variety of metabolic, structural and signalling proteins across a range of cellular models relating to cardiovascular disease and cancer. Although the result is often loss of function, in some cases detrimental gain of function is observed, which may be related to a number of changes in the protein, particularly changes in cellular localization of the protein including targeting to membrane compartments

This project will investigate the effect of lipoxidation of the membrane-associated signalling proteins HRas, a small G-protein involved in MAPK signalling, and phosphatase and tensin homolog (PTEN), a regulator of the Akt pathway, both of which are central to key intracellular signalling pathways that are aberrantly activated in diseases such as cancer. Proteins will be treated with reactive lipid oxidation products in vitro and the protein isoforms will be characterized by novel chromatography approaches and ion mobility mass spectrometry. Cultured cell lines expressing wild type and mutant proteins will be treated with reactive lipid oxidation products and the effects on protein subcellular localization and activity will be monitored by fluorescence microscopy and western blotting respectively. This will lead to the identification of new mechanisms for disease development and progression and will enhance our understanding of the role of lipoxidation in disease.

The project will be supported by our industrial partners Waters, world leaders in ion mobility mass spectrometry and ThermoFisher for the development and application of advanced chromatography.

Project contacts:

Academic: Prof Corinne Spickett, Aston University: c.m.spickett@aston.ac.uk; Dr Cathy Slack, Aston University: c.slack@aston.ac.uk

Industrial: Dr James Langridge: James_Langridge@waters.com; Dr Ken Cook: ken.cook@thermofisher.com

iv. The effect of lipid oxidation on membrane protein activity.

During inflammation and metabolic imbalance that occur in diseases including cancer, cardiovascular disease and diabetes, reactive oxygen species are formed leading to oxidative

stress. This results in oxidative damage to phospholipids in cell membranes, producing a variety of reactive lipid oxidation products which form covalent adducts with proteins, altering their structure and activity and affecting their cellular function. However, we are only now starting to identify in sufficient detail the changes that occur to the proteins to link this to the effects, enabled by access to the latest technologies.

This project will use model liposome and cell systems to investigate how the G-protein coupled adenosine receptor A2aR is affected by oxidative changes in the surrounding membrane phospholipid environment. A2aR is present at high levels in basal ganglia, the vasculature and T-lymphocytes, has been shown to have a range of roles in the health of the heart and vasculature and in inflammation, and its activity has been linked to disease such as neurodegeneration and cancer. Changes in activity of the protein will be measured using binding assays, and mass spectrometry will be used to analyse phospholipid oxidation and covalent adducts with the protein. Styrene-maleic acid lipid particle technology (SMALPs, also known as nanodiscs) will be used to investigate the noncovalent interactions of oxidized and non-oxidised lipids with the protein, and the ability of A2aR in different lipid environments to be stimulated by agonists and downstream signalling will be measured and correlated with the membrane changes. This will answer fundamental questions on the role of protein-associated lipids and lipid oxidation products in the activity of a key class of membrane protein.

The project will be supported by the mass spectrometry team of our industrial partner Waters, a world-leading mass spectrometry and separations company.

Project contacts:

Academic: Prof Corinne Spickett, Aston University: c.m.spickett@aston.ac.uk; Dr John Simms, Aston University: j.simms3@aston.ac.uk

Industrial: Dr James Langridge: James_Langridge@waters.com

v. De Novo design of membrane protein channels using a multidisciplinary approach

Synthetic Biology has an enormous array of potential applications, ranging from the production of green biofuels to the use of programmable cells to treat cancer. One aspect of Synthetic Biology is the de novo design of protein sequences that result in novel biological building blocks with innovative functions. A significant bottleneck in the development of de novo design is an understanding of the rules that govern how proteins fold. Traditionally, biophysical methods such as FRET, CD and NMR are used to understand the folding of membrane proteins. However, computational methods are becoming key, not only in the analysis of data but also generating meaningful predictions of the structure of proteins intractable by other methods. Multidisciplinary approaches that combine computational prediction, as well as lab-based experiments, provide a deep insight into the structure of membrane proteins which can, in turn, be used to design novel sequences.

This is a challenging project and will address both the de novo design of novel sequences as well as the re-design of naturally occurring scaffolds to form membrane protein channels. This builds upon recent work in our lab which has successfully designed and expressed

stable helical bundles which autonomously fold and insert into a membrane environment. This multidisciplinary project will combine laboratory-based experiments (Molecular Biology, Protein Expression, Biophysics) with Computational Biology (Molecular Modelling and Membrane Protein Simulations) to generate a series of sequences which autonomously fold to act as ion channels. Furthermore, the experimental data discovered during the project will be used to further develop a novel, cutting edge computational structure prediction method with our industrial partner Syndial. Overall, this is a challenging, multidisciplinary project that will explore the exciting area of de novo protein design. In the process of this PhD, students will gain insight and technical expertise in both experimental-based lab work as well as computational approaches.

Project contacts:

Academic: Dr John Simms, Aston University: j.simms3@aston.ac.uk; Dr Alice Rothnie, Aston University: A.Rothnie@aston.ac.uk

Industrial: Professor David Lowe: david.lowe@syndial.net

vi. Identification of new inhibitors of aquaporin water channel function

Our consortium has access to the laboratory facilities, knowledge, data, technologies and networks needed to be able to progress a programme of medicines research and development.

With advisory input from our national and international partners, the student will work on the development of new drugs that target the function of aquaporin water channels (AQP). AQPs control the flow of water in all forms of life; in humans their dysfunction is associated with diverse diseases. The project will include the expression of AQPs in mammalian cells, their biochemical and biophysical assessment and compound screening. New aquaporin inhibitors will be benchmarked against existing molecules in novel functional assays.

The student will gain a broad overview of the drug discovery process. Furthermore, the student will gain specific skills such as tissue culture, protein biochemistry, high-throughput screening and biophysical characterization. Finally, the student will gain a unique perspective on a career in a not-for-profit, independent technology and innovation centre, including risk management and business development skills.

Project contacts:

Academic: Professor Roslyn Bill, Aston University: r.m.bill@aston.ac.uk

vii. Strain optimisation for production of high value chemicals.

The global economy has an unsustainable dependence on fossil raw material and concerns about environmental sustainability are becoming more acute. Biotechnological processes using microorganisms as cell factories to produce valuable compounds from renewable biomass are an attractive alternative, and an increasing number of platform and high-value chemicals are being produced at industrial scale using this strategy. However, many microbial processes are not implemented at industrial level because the product yield is

poorer and more expensive than achieved by chemical synthesis. It is well-established that microbes show stress responses during bioprocessing and one reason for poor product output from cell factories is production conditions that are ultimately toxic to the cells, often at the level of the cell membrane. Examples of stresses that are demonstrably membrane-centric are solvents, e.g. butanol production by Clostridia and ethanol production by yeast, and weak acids such a lactic acid produced by bacteria. This project will seek to alter the cell membrane of industrial microbes to increase tolerance to stresses during bioprocessing.

Biocleave have patented technology (CLEAVE™) for genome editing of Clostridial species. Clostridia have significant potential within bioproduction of both native compounds e.g. butanol, and to be engineered to make additional molecules. However, Clostridia have traditionally been difficult to manipulate on a genetic level. The introduction of CLEAVE technology, based on endogenous CRISPR, has the potential to be a game changer and provides an opportunity to greatly increase the utility of Clostridia in this field. This project will use a powerful combination of in vitro assays, microbial cell culture, and 'omics technologies to identify the molecular targets suitable for overcoming stresses in bioprocessing e.g. lipids and transporters. Following this, CLEAVE will be used to create new strains, followed by strain characterisation to determine if the desired membrane alterations have been achieved and if tolerance to a particular stress (or stresses) has been increased. Iterative design-build cycles will be undertaken as appropriate to further improve the strains.

Project contacts

Academic: Dr Alan Goddard, Aston University: a.goddard@aston.ac.uk

Industrial: Dr Liz Jenkinson: liz.jenkinson@biocleave.com

3. Eligibility criteria

The ideal applicants will have Masters degrees in biological science, biochemistry or a related discipline, and a strong drive to carry out cutting edge research for a doctoral degree. Each ESR will be expected to undertake research towards a PhD within one of the projects offered through MemTrain. Further information on the role and duties are provided in the Job Description. Further information about the specific projects on offer can be obtained by contacting the supervisors or Dr Alan Goddard (a.goddard@aston.ac.uk).

The prospective candidates must not have resided or carried out their main activity (e.g. work, studies) for **more than 12 months within the last three years in the UK** (prior to the closing date of this call). Furthermore, the candidates need to be in his/her first four years of his/her research career. The four years are counted from the date a degree was obtained which formally entitles the holder to embark on a doctorate either in the country in which the degree was obtained or in the country of employment. More details about these regulations can be found at

https://h2020.org.tr/sites/default/files/u390/cofund.gfa_.2018.pdf

In order to check your eligibility, please complete and upload the attached eligibility form to your application

Eligibility Form

You may apply to multiple projects but will only be interviewed by one panel. All applications are required to be completed in English and, to meet eligibility criteria, you will be required to have an IELTS score of 6.5[1] or above in all areas.

Applications will not be accepted after the published deadlines and will be rejected if they are incomplete. You do not need to wait until the application deadline to submit; you may submit your application at any time.

4. Evaluation process and timelines

There are three stages to the recruitment and evaluation process:

Stage 1. Applications will be checked for eligibility by the Programme Manager in conjunction with the Aston University admissions team. Eligibility criteria are outlined above and are in line with The European Charter for Researchers as well as internal regulations at Aston University. This panel will make an initial assessment of each application as:

- proceed – process application to Stage 2
- reject – application is incomplete or applicant does not meet minimum requirements
- further information required – an aspect of the application needs clarification e.g. provision of transcripts; such information must be supplied within **one working week** to avoid delay in the selection process. Unsuccessful candidates will be notified at this stage.

Stage 2. Candidates that are progressed to Stage 2 will have their applications anonymized by Aston University's Human Resources team and the MemTrain Programme Manager. Applications at this stage will be assessed by the external experts and Selection Committee in parallel.

The following evaluation criteria will be used during Stage 2:

- Quality of CV including relevant past experience and personal motivation (0-30 points, threshold 18);
- Academic track record including both undergraduate and postgraduate education in terms of metricised achievement and relevance of the subjects to the project of interest (0-30 points, threshold 18);
- Experience and skills including industrial / academic placements and practical research experience (scored out of 20, threshold 12);

[1] Successful candidates will be required to provide documentary evidence of their IELTS score before enrolling onto the PhD programme

Selection will use a standardised scoring sheet along with free text feedback to produce an Evaluation Report for each candidate. Two external experts will be used for each applicant, determined by the theme of the project within MemTrain. External experts and members of the Selection Committee will return completed Evaluation Reports to the Programme Manager who will collate the scores (each assessor carries equal weighting) and produce a ranked list of candidates for each project. No fewer than 3 applicants per project will be shortlisted but more may be shortlisted in event of a tie of scores.

It is anticipated that shortlisted candidates will be notified within 2 working weeks of the closing date.

Stage 3. Shortlisted applicants will be invited to attend an interview. You will have 3 options:

- **Attend interview in person**. Interviews will be held at Aston University, Birmingham, B4 7ET, UK. We are able to provide limited financial support for travel.
- **Attend interview by Skype**. If you are unable to travel to Birmingham for the interview then we would be happy to interview you by Skype. If you do not attend the interview in person then, if successful, you will have the opportunity to visit Aston at a mutually-convenient time before making your final decision.
- **Inform us that you no longer wish to be considered for this position.**

As part of your interview you will be required to make a 10 minute presentation detailing your past research experience (or a selection thereof) and, importantly, how this relates to the MemTrain project you have applied for.

Each candidate will be interviewed by appropriate selected members of the Selection Committee and any other additional supervisors on the project including an industry representative.

These will be scored as outlined below:

- Presentation including timekeeping, quality of oral and visual aspects of the presentation, clarity of explanation of research and ability to satisfactorily answer questions (scored out of 40, threshold 25);
- Interview responses, including the ability to articulate thoughts, communication of motivations and desire to undertake the selected project, knowledge of the scientific and industrial background of the project and mobility, including ability to work across national and discipline / sectoral boundaries (scored out of 40, threshold 25);
- Practical abilities including accuracy, precision, dexterity and knowledge of the underlying principles of relevant techniques (scored out of 20, cut-off 14);

Following the interview, the Programme Manager will collate interview scores and produce a ranked list of candidates for each project. The Selection Committee will meet to review these scores and to make a final decision on which candidates will be offered each project. In addition, if candidates are suitable, a reserve list will be created. All applicants will be notified of the decision of the Selection Committee and successful applicants will be given

two working weeks to accept or decline the offer. Once this period has passed the applicants who are on the reserve list will be notified of their final outcome.

Candidates will be notified of the outcome of their interview no later than 2 working weeks.

5. Appeals process

An applicant may appeal on one or more of the following grounds:

- Procedural error where the process leading to the decision being appealed against was not conducted in accordance with Aston University's procedure, such that there is reasonable doubt as to whether the outcome might have been different had the error not occurred;
- Exceptional circumstances, illness, or other relevant factors that had, for good reason, not been made known at the time of application or had not been taken into account properly. 'Good reason' requires an applicant to demonstrate circumstances outside his/her control that prevented the relevant factors being disclosed at the time of application. Personal embarrassment or an unwillingness to disclose personal circumstances do not count as 'good reason' for the purposes of this policy. If claiming the grounds for appeal as detailed above, additional information not known at the time of application will be requested.

Appeals will be considered when made by the individual applicant but not third-party representatives, such as parents, representatives, or other third-parties, unless the applicant's situation is such that they require third-party representation. All appeals will be considered on their merit, however appeals that are based exclusively on one or more of the grounds below will be rejected automatically; in such cases, the decision will be final.

- Appeals against the academic judgement of selectors;
- Appeals where the application was received after the published deadline;
- The retrospective reporting of extenuating circumstances that might have been reasonably made known at the time of application;
- Failure by the applicant to attain the entry qualifications specified as conditions of the offer.

Appeals must be lodged with the Programme Manager within one week of the candidate receiving their decision. This information will be passed on to Aston University HR department who will review the case within two weeks. Candidates will be informed of the decision of the appeal within a further one-week period.