Fluorescence-based sensing of active metabolites linked to the pathogenesis of neurodegenerative disorders

Highlights

Design of genetically-encoded biosensors, consisting of a molecular recognition element fused to one of more fluorescent proteins

Exploiting fluorescence imaging, resonance-energy transfer and fluorescence lifetime as readout methods for the biosensor

Investigating chemical mechanisms in yeast and mammalian cells and fruit flies using state-of-the-art laser-based biophysical techniques

Overview

Kynurenine is an intermediate in the pathway that converts the amino acid, tryptophan, to the redox enzyme, nicotinamide adenine dinucleotide (NAD+/NADH) - an essential molecule of life. The sequence of reactions is known as the kynurenine pathway, and their perturbation during inflammatory processes, leads to accumulation of neuroactive metabolites in the human central nervous system – a phenomenon implicated in Alzheimer’s, Parkinson’s and Huntington’s disease.

Recent research in Leicester has identified key regulatory enzymes in the pathway that could be potential drug targets for these disorders. Nonetheless, a precise understanding on how activation of the pathway is regulated in vivo is lacking, as well as the spatial and temporal changes in metabolism that occur during disease. This problem will be the focus of the project and will need to be addressed by a multidisciplinary approach encompassing biophotonics, chemical biology and genetics. While the successful applicant will be someone with demonstrable skills in biophotonics and chemical physics and based in the Department of Chemistry, they will need to work outside the confines of traditional departmental structures and collaborate with researchers in the Leicester Institute for Structural and Chemical Biology and the Department of Genetics and Genome Biology.
Yeast cells can be used to explore how particular genes can modify “symptoms” of neurodegenerative disease. The simplicity of the model system affords a great opportunity to develop new methodology to sense the real time production of kynurenine and its turnover into NAD+/NADH, which is upregulated in Huntington’s disease. We intend to design a fluorescence-based chemical sensor to detect kynurenine, and other metabolites, in the pathway. The changes in absolute levels of kynurenine, imaged in live cells and monitored in real time, will give a measure of activation of this pathway. Laser-based microscopy techniques will be employed in combination with the sensor, and various photophysical processes such as fluorescence resonance energy transfer and fluorescence lifetime will be exploited. We will begin by utilising yeast, but, in the longer term, the chemical sensor will be employed in both mammalian cells and fruit flies.

By making progress on understanding spatiotemporal accumulation of kynurenine within cells and the central nervous system of flies and the underlying regulatory processes, we will be making a transformative contribution to the study of neurodegenerative diseases.

Methodology, Critical Skills and Training and Development

Our proposed methodology is conceptually simple. We know the identity of a peptide sequence (mini-αβ-cystallin) that binds to kynurenine. We will use protein expression techniques to synthesis the peptide sandwiched between a pair of fluorescent proteins (see figure). We hypothesise that the binding of kynurenine to the peptide will result in a conformational change in the structure of the peptide leading to a photophysical phenomenon called Förster-energy transfer (or fluorescence resonance-energy transfer, FRET). We have the ability to detect this by microspectroscopy, and thus monitor in real time the progress of the kynurenine pathway. Once established, this sensor will be used to generate transgenic yeast. Conditions known to perturb the kynurenine pathway – such as neurodegenerative disease models – will be employed to assess the resolution of the nanosensor.

Further Reading

1. Bioimaging and spectroscopy
   d. ChemistrySelect 2, 3342–3346 (2017)
2. Neurodegeneration and the kynurenine pathway
   b. Current Biology 21, 961-966 (2011)

Additional Entry Requirements

- Interest in multidisciplinary approaches to research.
- Ability to work in a collaborative research team.
- Interest in spectroscopy, photophysics, microscopy and imaging.
• Willingness to broaden experience and engage in cell culture, protein expression and protein purification activities.

Funding
This research project is one of a number of projects in the College. It is in competition for funding with one or more of these projects. Usually the project which receives the best applicant will be awarded the funding.

Home/EU Applicants
This project is eligible for a fully funded EPSRC studentship which includes:

• A full UK/EU fee waiver for 3.5 years
• An annual tax free stipend of £14,777 (2018/19)
• Research Training Support Grant (RTSG)

Studentships are available to UK/EU applicants who meet the EPSRC Residency Criteria; if you have been ordinarily resident in the UK for three years you will normally be entitled to apply for a full studentship.

If you are an EU student and do not meet the residency criteria, please contact csepgr@le.ac.uk for more information on the funding options available.

International Applicants
• Unfortunately, there is no funding for international students on this project.

Application Instructions
The online application and supporting documents are due by Monday 21st January 2019.

Any applications submitted after the deadline will not be accepted for the studentship scheme.

References should arrive no later than Monday 28th January 2019.

Applicants are advised to apply well in advance of the deadline, so that we can let you know if anything is missing from your application.

Required Materials
1. Online application form
2. Two academic references
3. Transcripts
4. Degree certificate/s (if awarded)
5. Curriculum Vitae
6. EPSRC Studentship Form
7. English language qualification
Applications which are not complete by the deadline will not be considered for the studentship scheme. It is the responsibility of the applicant to ensure the application form and documents are received by the relevant deadlines.

All applications must be submitted online, along with the supporting documents as per the instructions on the website.

Please ensure that all email addresses, for yourself and your referees, are correct on the application form.

For more information, please visit our website at:
https://www2.le.ac.uk/colleges/scieng/research/postgraduate-opportunities/epsrc-2019/instructions