**Project Title:**
Assessment of biological effects and molecular characterisation of novel analogues of natural product peptides for the treatment of type-2 diabetes

**Supervisor(s):** Dr Yasser Abdel-Wahab and Dr S McClean

**Contact Details:** y.abdel-wahab@ulster.ac.uk, Tel: (0)2870124354

**Level:** PhD

**Background to the project (200 words Max):**
As part of a programme of investigation targeted at isolation and characterization of novel insulin-releasing antihyperglycaemic agents, we have developed novel analogues of two previously described insulinotropic host defence peptides (PGLa-AM1 and hymenochirin-1b) isolated from *Xenopus amieti* and *Hymenochirus boettgeri* for the treatment of type 2 diabetes. Early results indicated that Ala\textsuperscript{14} → Lys substitution in PGLa-AM1 resulted in a significant augmentation of the insulinotropic effects of PGLa-AM1; producing enhanced *in vitro* insulinotropic effects as well as improved glucose tolerance and insulin release in obese insulin resistant mice. A similar result was obtained by substituting Asp\textsuperscript{9} in hymenochirin-1b with a D-Lysine isomer. This PhD proposal will focus primarily on the assessment of the longer term metabolic effects of these two peptide analogues in different animal models of type 2 diabetes. It will also seek to delineate the molecular mechanisms of action of these novel peptide analogues using a wide range of molecular biology techniques. Enhancement of insulinotropic effects and delineation of molecular mechanism of selected novel peptide analogues, using procedures consistent with sound research design, will provide a strong scientific basis for their clinical development as new therapeutic agents for type-2 diabetes.

**Objectives of the research project (400 words Max):**
The overall aim of this research is to examine the long-term metabolic effects and elucidate molecular mechanism of actions of novel peptide analogues of some recently discovered host defence peptides with potent insulin-releasing effects for potential development as novel type 2 diabetes drugs. We hypothesize that structural modification of the selected insulinotropic peptides will enhance their biological effects and delineation of the molecular mechanism of action will enhance the potential of the peptides for development as new antidiabetic agents. Therefore, the study will assess the effects of twice daily administration of the modified peptides, in mouse with environmental (diet-induced) and genetic aetiologies, on glycaemic control, glucose tolerance, insulin sensitivity, indirect calorimetry, fat deposition and beta cell function. We will investigate the interaction of the peptide with beta-cell membrane proteins as well as *in vivo* changes in the expression of key genes and proteins involved in glucose signalling via the KATP-dependent and the cAMP-dependent secondary messenger pathways resulting from the administration of the peptides to diabetic animals. This project will involve techniques such as HPLC, MALDI-TOF, tissue culture, small animal handling and experimentation, PCR, immunohistochemistry, flurometric assay, radioimmunoassay, etc. PCR and Western blotting will be utilised to explore both gene and protein expression of key elements involved in pathways of insulin secretion (such as *Ins1*, *Gck*, *Kcnj11*, *Abcc8*, *Cacna1c*), beta cell proliferation (such as *Pdx-1*) and insulin action (such as *Irs1*, *Pib1*, *Pi3kca*, *Pdk1*, *Akt1*, *Insr*, *Slc2a4*).
Methods to be used (400 words Max):
The proposed research will provide training in a wide range of techniques. In line with the objectives of this project, techniques involved are as follows:

Objective 1: Assessment of the longer term metabolic effects of selected peptide analogues in different animal models of type 2 diabetes

- Techniques: RP-HPLC, ESI, and MALDI-TOF mass spectrometry; CLAMS, DXA scanning, acute and long term studies in animal of obesity-diabetes, measurement of insulin secretion, radioimmunoassay, peptide iodination, ELISA

Objective 2: Delineation of molecular mechanism of actions of selected peptides

- Techniques: Tissue culture, signalling molecules (intracellular Ca^{2+} and other second messenger pathways, peptide fluorescence tagging, cell morphology, PCR, western blotting, immunohistochemistry

Established collaborations are in place for synthesis and large scale purification of synthetic peptides. This research will generate novel IP, high quality publications and potential exploitation through pharmaceutical development

Skills required of applicant (200 words Max):
The applicant should ideally have good practical laboratory, computer and skills and show enthusiasm and commitment to work diligently on all aspects the research project to completion under the leadership of his/her supervisors. A background in biomedical sciences, pharmacology, nutrition or a related subject would be desirable.

References (Maximum of ten references):


and islet function and decreases plasma lipids in high-fat fed mice. European Journal of pharmacology, 764:38–47.


Ethical Approval/Animal licence(s) Required - YES / NO (Delete as appropriate)
Animal licence(s) Required? YES/ NO (Delete as appropriate)

Use of Core Facilities including BBRU - YES / NO (Delete as appropriate)

If yes, please ensure that appropriate costings are obtained from Dr Le Roy Dowey and provided below
Animal project licences are already in place to facilitate the project. The successful candidate will complete the required training course.

Please identify how this project addresses/meets the research priorities of the Biomedical Sciences Research Institute/ Research Group:
This research project is consistent with the overall ethos of the Research Institute in that it focuses on nutritional aspects of chronic disease. The proposal fits fully within the priorities of the Diabetes Research Group which aims to increase knowledge at the forefront of diabetes research and improve the lives of people with diabetes.

Please provide a list of the titles you submitted for this year’s round of undergraduate student projects (or provide details of extenuating circumstances which prevented the submission of titles):

YAW
- Isolation, structural characterisation and mechanisms of action of novel insulin-releasing peptides from amphibian skin secretions (3 projects). Supervisor Y Abdel-Wahab
- Isolation and characterization of insulin-releasing and glucose-lowering agents from European and Indian plants used traditionally for the treatment of diabetes (3 projects). Supervisor Y Abdel-Wahab
- PRF
- Effects of bariatric surgery on small intestinal peptidomics in obese-diabetic rats (5 projects)

Please provide a list of the externally funded grants you have received within the last 3 years (print out from RO Required to be appended):

• Diabetica Ltd £29,952, Donation for Diabetes Research. (Prof PR Flatt, Prof NH McClenaghan, Prof FPM O’Harte; 2011-2016.)
• Irish Endocrine Society - Small Grant Scheme (Dr YHA Abdel-Wahab, Prof PR Flatt; 2012-2016): £8,292: Pharmacological studies of novel amphibian skin peptides for the treatment of type 2 diabetes.
• Sanofi-Aventis Gmbh (Prof PR Flatt; 2012-2017): £98,598 Pharmacological feasibility study of novel analogues at incretin receptors.
• EFSD (Prof PR Flatt; 2013-2016): £79,464 Production of GIP by islet alpha cells and role in beta cell compensatory response to pregnancy and cellular stress.
• Domain Therapeutics (Prof PR Flatt & Prof NH McClenaghan; 2012) £7,000 Evaluation agreement for human insulin secreting cell.
• ECACC (Prof PR Flatt & Prof NH McClenaghan; 2010-2012) £2,335 (10% total income which was £23,356 made up of 3 sums £13,632, £9,422 and £281): Sales of rodent and human insulin secreting cells.
• Invest Northern Ireland, Proof of Concept Programme (Prof PR Flatt & Prof FPM O’Harte; 2013-2014) £104,823: Novel dual agonists for diabetes therapy.
• Public Health England (Prof PR Flatt, Prof NH McClenaghan, 2013-2014) £2,073 (10% total income which was £20,733): Sales of rodent and human insulin secreting cells.
• Invest Northern Ireland, Proof of Concept Programme (Dr YHA Abdel-Wahab & Prof PR Flatt; 2013-2016) £105,919 Novel amphibian peptides for treatment of diabetes.
• Innovation Ulster, Proof of Principle Programme (Prof AM McKillop & Prof PR Flatt; 2014) £10,000: GPR84: A new therapeutic target for diabetes.
• Ono Pharmaceuticals (Prof PR Flatt & Prof NH McClenaghan; 2015): £21,850: Royalty payment for human 1.1E7 cells.
• Innovation Ulster, Proof of Principle Programme (Prof AM McKillop, Prof PR Flatt & Prof FPM O’Harte; 2016) £6,959 Novel marker of early beta cell dysfunction, pre-diabetes and diabetes.
• Innovation Ulster, Proof of Principle Programme (Dr N Irwin & Prof PR Flatt; 2016) £7,250 Novel bone specific GIP/xenin hybrids for bone fragility fractures.
• Diabetes UK (Prof JAM Shaw & Prof PR Flatt; 2015-2018): £92,460 Defining the role of dedifferentiation as a primary mechanism of beta cell dysfunction in type 2 diabetes.
• Invest Northern Ireland, Proof of Concept Programme (Prof FPM O’Harte & Prof PR Flatt; 2016-2017) £106,000 Validation of apelin peptide analogues for a new therapeutic approach to diabetes.
• Diabetes UK (Prof JAM Shaw, Dr H Marshall, Dr M Niven, Dr Y Porat & Prof PR Flatt; 2015-2018): £360,435 Development of novel donor human islet/recipient endothelial progenitor cell chimeric tissue transplant for type 1 diabetes.
• Lilly China R & D Centre (Prof PR Flatt & Prof NH McClenaghan; 2014) £25,000 Licensing of human insulin secreting cells.
• Public Health England (Prof PR Flatt & Prof NH McClenaghan; 2014-2015) £2,703 (10% total income which was £27,030) Sales of rodent and human insulin secreting cells.
• Public Health England (Prof PR Flatt & Prof NH McClenaghan; 2015-2016) £2,381 (10% total income which was £23,813) Sales of rodent and human insulin secreting cells.
- Zealand Pharmaceuticals (Prof PR Flatt; 2014-2015) Consultancy £16,530 Functional tests with human insulin secreting cells
- Innovation Ulster, Proof of Principle Programme (Prof FPM O’Harte & Prof PR Flatt; 2013) £7,500 Assessing the antidiabetic and antiobesity potential of Apelin-13 analogues
- Innovation Ulster, Proof of Principle Programme (Prof AM McKillop & Prof PR Flatt; 2014) £10,000 GPR84: A new therapeutic target for diabetes.

**Project Costing:**

*Please identify the cost of undertaking the project and highlight current externally funded projects that align with this proposed project [Project Title, funding source, amount and effective dates].*

- POC 418 (Dr. YHA Abdel-Wahab and Prof. PR Flatt): £108,919,000 Novel Amphibian skin peptides for the treatment of diabetes (2014-2015)

<table>
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<th>Cost Categories &amp; (Brief Details)</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
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<td>Brief Consumables List</td>
<td>Peptides synthesis (£600) and in vitro screening in cultured cells (£300), Animal Licensing (£150)</td>
<td>In vitro studies using cultured cells and islets.</td>
<td>Further evaluation of lead compound in vitro and in vivo in mice</td>
<td>£9000</td>
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<td>Exceptional Items Details</td>
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<td>Sub Total</td>
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<td>£3000</td>
<td>£3000</td>
<td>£9000</td>
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</table>
Identify Source of Funding for the project and Confirmation that funds are available:
Substantial funds are available from active projects listed above. Monies will also be derived from studentship allocation and BMSRI membership.

The applicants and Research Group Leader can confirm that adequate funds are in place for this PhD project

Signed: [Signature]
(Lead Supervisor)

Anticipated Project Funding [please tick relevant box(es)]:

- DEL □ ✓
- VCRS □ ✓
- DARD □
- CAST □
- Self funding □ ✓

NOTE: Self funded students:
It is intended to advertise as many PhD projects on the web as possible (suitable for overseas self-funded students). Please note that all PhD projects for the 2016-2017 intake (including all projects to be offered to overseas self-funded students) should be included in this submission process.

Research Group Leader:
Research Group Leaders should sign to confirm that the project proposal aligns with the overall Research Group and RI strategy:

I confirm that this application meets the research strategy of the Research Group, can be conducted with available funds and has my full support:

Signature: [Signature]
(Appropriate Research Group Leader)

Date: ______________________ 10/11/16 ____________________________