OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

Please use the following link to submit this form online, https://forms.office.com/e/TryLU5d0tW
This Word copy is for preparation purposes only.

Supervisor(s): [1]David R. GREAVES, [1]Christoph TANG, [2]Angela J RUSSELL.

Named Day-to-Day Mentor (if different from above)[1]:

Department(s): [1]Sir William Dunn School of Pathology
[2]Departments of Chemistry and Pharmacology

E-mail: David.greaves@path.ox.ac.uk Tel: 01865 285519

Project Title: **Evolution of the GPR84 receptor - a conserved function in immune defence?**
.

Relevance of project to BBSRC themes (please underline all that apply):

Understanding the rules of life / Transformative technologies / Bioscience for sustainable agriculture and food / Bioscience for renewable resources and clean growth / Bioscience for an integrated understanding of health.

Relevance of project to DTP subject areas (please check all that apply):

- Animal Health
- Animal Welfare
- **X Cellular Mechanisms**
- Crop Science
- Developmental Biology
- **X Immunology**
- Industrial Biotechnology
- **X Microbiology**
- Neuroscience
- Plant Science
- Regenerative Biology
- Stem Cells
- Structural Biology
- Synthetic Biology
- Technology Development
- **X Other World Class Bioscience**

1 For laboratory-based projects this will normally be a postdoctoral researcher, senior technician or senior graduate student.

OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

Brief description of project (no more than 500 words): [Please provide a brief outline of the background and aims for the short project, including information on potential opportunities for extension to DPhil]

Aims (for the short project)

1) We will use genome engineering techniques to insert GPR84 orthologs into the hGPR84 gene locus to generate THP-1 cell clones that express variant GPR84 open reading frames expressed at the same level as hGPR84 in myeloid cells with physiologically relevant levels of signaling components.

2) GPR84 expression levels will be measured using RT-PCR, western blotting and flow cytometry. GPR84 function will be followed using cAMP and beta arrestin activation assays, cell impedance sensing, and measurement of live *E.coli* killing using techniques well established in the Greaves and Tang Laboratories.

3) We will use a range of GPR84 agonists to test macrophage phagocytosis of a range of targets; live and killed bacteria, yeast particles and cancer cells.

Background

GPR84 is a G Protein coupled receptor (GPCR) expressed by innate immune cells in mammals. While the receptor signals in response to medium chain fatty acids (MCFAs), the low potency (~10 mM) of these responses suggests that additional physiological or pathophysiological ligands exist and have yet to be identified, i.e. GPR84 is an orphan GPCR. It is intriguing that orthologs of this receptor are found in nearly all vertebrate genomes spanning over 500 million years of evolution (see Figure 1). The goal of this project is to extend our analysis of GPR84 to study the response of human and other vertebrate GPR84 orthologues to a wide range of bacterial lipids.

Introduction

We have shown that a range of low molecular weight chemical tool compounds can activate GPR84 signalling in mouse and human macrophages with high potency (5pM-10nM IC_{50}) and that these synthetic agonists can enhance inflammatory signalling as well as enhancing macrophage phagocytosis and killing of bacteria (Ref 1 & 3 and unpublished experiments).

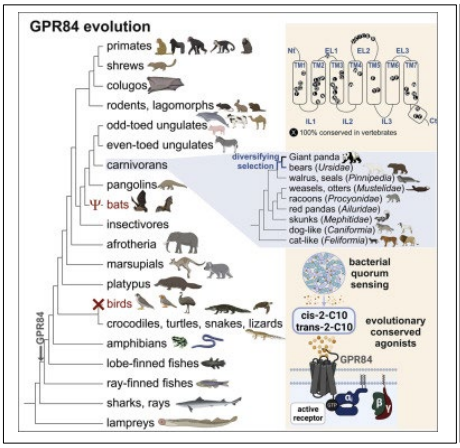

Figure 1. Evolution of the GPR84 gene in vertebrates.

This diagram (Schulze et al. 2022) shows the evolutionary relationships of GPR84 orthologues in selected vertebrates spanning over 500 million years of evolution.

The top right panel shows the conserved structure of the GPR84 receptor, Nt - NH2 terminus, TM1-7-transmembrane spanning domains, IL1-IL3 -intracellular loops, EL1-EL3 -extracellular loops including conserved amino acids across 101 mammalian orthologs and Ct-intracellular COOH terminus.

The bottom right panel shows intracellular signalling pathways activated by GPR84 balanced and biased ligands.

Schulze et al. hypothesise that evolutionary conservation of the GPR84 receptor is due to selection for defence against bacterial infection by signaling in response to components of bacterial cell walls or secreted bacterial quorum sensing molecules.

OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

Work leading up to the project
We have demonstrated the specificity of bacterial uptake and killing by our new synthetic GPR84 ligands by using the human monocytic leukaemia cell line THP-1 and wild-type and *Gpr84*$^{-/-}$ murine macrophages. We have used CRISPR-Cas9 to generate THP-1 clones in which the single copy hGPR84 gene has been deleted. GPR84 signalling and bacterial killing is completely lost in these THP-1 cell clones. We now want to reconstitute GPR84 signalling in these cell clones using naturally occurring amino acid variants of the human GPR84 receptor and GPR84 orthologs from a range of vertebrate species using genome engineering techniques.

Proposed methods

1) CRISPR Cas engineering of THP-1 cells and characterization by PCR, flow cytometry and western blotting
2) Phagocytosis assays using a range of different phagocytic meals
3) Live cell imaging microscopy

Opportunities for extension to a DPhil project

For students undertaking this project there will be opportunities to 1) synthesise new GPR84 agonists and study their effects on macrophages 2) to identify GPR84 signalling pathways that enhance macrophage phagocytosis and bacterial killing, *in vitro* and later *in vivo* 3) Working with local collaborators we could extend our in vivo studies into model organisms including *Drosophila* and *Caenorhabditis*.

[489 words]

References

1. Biased agonists of GPR84 and insights into biological control.
Luscombe VB, Wang P, **Russell AJ**, **Greaves DR** (2024) Br J Pharmacol. 181:1509-1523. doi: 10.1111/bph.16310.

2. Evolutionary analyses reveal immune cell receptor GPR84 as a conserved receptor for bacteria-derived molecules.
Schulze AS, et al. (2022) iScience. 6;25:105087. doi: 10.1016/j.isci.2022.105087.

3. Development of Highly Potent, G-Protein Pathway Biased, Selective, and Orally Bioavailable GPR84 Agonists.
Wang P, Raja A, Luscombe VB, Bataille CJR, Lucy D, Rogga VV, **Greaves DR, Russell AJ**. (2024) J Med Chem. 67:110-137. doi: 10.1021/acs.jmedchem.3c00951.

Supervision and training arrangements (no more than 300 words): [Please provide a brief description of the supervision and training arrangements for the project, including the role of all named supervisors and mentors, meetings and training available in specific techniques and opportunities for peer to peer interactions]

In the GPR84 project overall supervision will be provided by Professor Greaves. Training in key techniques (tissue culture, microscopy, phagocytosis assays) and hands on practical supervision will be provided by senior postgraduate students.

The project student will attend lab meetings in appropriate groups once a week for the 12 weeks of the project and the student will have scheduled 1-to-1 meetings with Prof Greaves, Prof Tang, Dr Rachel Exley in the

OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

Dunn School and Angela Russell in Chemistry. The project student will have the opportunity to take part and present in regular Journal Club meetings. The student will be encouraged to attend appropriate undergraduate lectures, seminars, and practical classes alongside regular departmental research seminars.

Graduate students on rotation in the Sir William Dunn School of Pathology are automatically enrolled in the Dunn School Graduate Students' Association (GSA) which organises regular careers seminars, Meet the PI sessions and social events - coffee and cake, film nights, Dunn drinks etc. Peer-to-peer interactions are strongly encouraged through multiple networking events across all three departments as well as through the Interdisciplinary Bioscience DTP.

Reasonable expected outcome of 12-week project: [Briefly explain what a student might realistically expect to achieve within 12 weeks]

In the GPR84 project the student will learn how to culture primary macrophages and macrophage reporter cell lines, how to perform macrophage phagocytosis experiments using live bacteria (the gentamycin protection assay) and /or how to synthesise and analyse key GPR84 agonists.

The student will learn how to acquire images by live cell imaging (using the EVOS 384 well microscope) and how to analyse resultant datasets (GraphPad Prism and ImageJ).

Project students will be working directly with senior graduate students who are running experiments that will result in manuscripts to be submitted to peer-reviewed scientific journals. At the end of the 12-week projects students should have obtained pilot data that can quickly be turned into a full DPhil project.

Location: [Identify the department(s) or organisations in which work will be undertaken. Please state whether any overseas travel will be required]

The GPR84 short project will involve working in the Dunn School (~75%) and the Department of Chemistry (~25%).There is no requirement for overseas travel or fieldwork.

Timing: [Please state whether there are any restrictions on the timing of projects (e.g. sabbatical leave, access to facilities]. Projects will typically start in January and April

The GPR84 short project can start in either January or April.

Remote working: [Please indicate whether all or aspects of the project may be suitable for remote working]

To learn key techniques from lab members experienced in phagocytosis assays and chemical biology project students need to be present in the Dunn School or the Department of Chemistry full time. Project students will benefit greatly from in person training, weekly lab meetings and frequent 1-to-1 discussions with supervisors.

Any other specific points: [e.g. time required to learn software, suitable background and skills of student, animal handling license required etc]

Full training will be provided in microscopy, biological and chemical biology techniques as well as training in

1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

commonly used analysis software. An animal licence is not required for the short GPR84 project but would be useful if this became the main DPhil project.

Students with a background in biology, chemistry, pharmacology, mathematics, or engineering could all make important contributions to this established interdisciplinary research team.

OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

For administrative use only (the following pages will not be sent to students):

For University of Oxford based projects: What two letter code should be used to set up a consumables subtask on Oracle R12 for spending on this project? BV

Supervision arrangements

We confirm that the proposed supervisory team includes at least one academic member of staff at the University of Oxford or Oxford Brookes University who is eligible to supervise a DPhil/PhD student[2]: **YES**

We confirm that the current appointment of at least one academic member of the supervisory team extends for the duration of the proposed project/DPhil/PhD[3]:

Project: **YES**
DPhil/PhD: **YES**

Number of students supervised to completion of DPhil as main supervisor (please complete for all named supervisors in order initially listed)[4]:

Supervisor 1 (Greaves) 16
Supervisor 2 (Tang) 18
Supervisor 3 (Russell) >20

Number of students supervised as primary supervisor who will be enrolled at the University of Oxford or Oxford Brookes University in the academic years 2024/25 and 2025/26 (Please complete for all named supervisors in the order initially listed):

Supervisor 1 (Greaves) 2

2 Please see point 5 in the attached Guidance Notes. Please note that postdoctoral researchers cannot be named as the primary supervisor of a DTP short project and that postdoctoral researchers and senior graduate students cannot be asked to mentor more than one DTP or DTC student during a rotation period.
3 If you select NO this does not mean that a student cannot select the short project proposed. However, we will require that the supervisory team for any DPhil/PhD project arising from the short project includes an academic whose appointment extends for the duration of the DPhil/PhD (i.e. until September 2023). If the primary supervisor's current contract does not extend for the duration of the DPhil we will require confirmation from the relevant head of department that they are willing for the project to proceed to DPhil/PhD.
4 If the primary supervisor for a DPhil/PhD project has not supervised two students to completion of their DPhil/PhD as main supervisor, we will require that the DPhil/PhD supervisory team includes a senior academic who has supervised at least two students to completion of their DPhil/PhD. This restriction does not apply to short projects. However, supervisors who have not supervised two students to completion of their DPhil/PhD as main supervisor can only supervise one student for each rotation period.

OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

Supervisor 2 (Tang) 4
Supervisor 3 (Russell) 4

For administrative purposes, please indicate whether you currently hold, or have held BBSRC funding by ticking the relevant boxes below. If you have not held BBSRC funding this will not preclude you from offering a project.

☐Research Grant (current)

☐Research Grant (past)

☐Studentship (current)

☐Studentship (past)

☐Other (please specify)

Supervisor training
All supervisors are required to have completed the University of Oxford DPhil Supervision (Sciences) or an equivalent course prior to taking on a DPhil/PhD student.

We confirm that all members of the academic supervisory team have undertaken the following training sessions (all courses are available to University of Oxford staff with a single sign-on login via the Centre for Teaching and Learning and the Equality and Diversity Unit):

DPhil supervision at Oxford: online course https://www.ctl.ox.ac.uk/online-courses	**YES**
Implicit bias in the workplace: online course https://edu.admin.ox.ac.uk/training	**YES**
Equality and diversity briefing: online course https://edu.admin.ox.ac.uk/training	**YES**

If you are not based at the University of Oxford:
Please confirm what other training has been undertaken and confirm that you would be willing to undertake additional training if requested.

Please state whether you have undertaken any DPhil/PhD supervisor training and provide details:

Please state whether you have undertaken Unconscious Bias Training and Equality and Diversity Training and provide details:

OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

DPhil/PhD supervision/EDI/Unconscious Bias Training is not available through my/our organisation.

Best practice in supervision*

We will require all supervisors who agree to supervise a DTP student as primary supervisor for their DPhil project to attend a doctoral supervision workshop in the year that students commence their DPhil/PhD research. This invitation will be optionally extended to co-supervisors and to all current supervisors in subsequent years. Please note that attendance at this workshop is required of both experienced and inexperienced supervisors, as experienced supervisors will make an important contribution by sharing their experiences and insights, as well as gaining additional insight into effective practices and challenges in supervision. If the primary supervisor is based at a non-university partner institution, the university co-supervisor will also be strongly encouraged to attend the first year workshop.

Additionally, we may require supervisors to undertake additional training or to provide information on their supervisory practices as required by UKRI-BBSRC or agreed by the DTP directorate. Please complete the following statements.

We confirm that we will undertake any training required by the DTP or provide information on request if a student chooses to undertake a rotation project or doctoral project in my research group:

*Please note that if you are currently the primary supervisor for a DTP student who started their studies in 2021 and their substantive DPhil project in 2022 your project proposal will not be circulated to students until you have attended a DTC doctoral supervision workshop. We will be offering additional workshops, for those who have not yet attended a workshop, in Aug-Sept 2023. Potential supervisors who have not yet attended a workshop, but who are interested in offering a project are also welcome to attend.

Commented [GP1]: Link needs to be added

Research environment

We confirm that the student will have access to the facilities needed to carry out the proposed research, including appropriate office/laboratory bench space and access to computer facilities: **YES**

We confirm that all relevant licences and approvals to undertake the proposed work are in place[5]: **YES**

5 If you select NO we will seek confirmation that all licences are in place before the project can commence

OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership
1-4 Keble Road, Oxford OX1 3NP
Email: dtpenquiries@biodtp.ox.ac.uk
Tel: +44 (0)1865 610656

Interdisciplinary Bioscience DTP Project Proposal Form 2024-2025

Contribution to the DTP

The success of the DTP depends on the ongoing contributions of DTP supervisors not only to supervision, but also to other programme activities, such as the assessment of DTP project write-ups and project proposals, contributions to training and admissions processes, and participation in DTP events. The DTP Directorate reserves the right to exclude supervisors who routinely decline reasonable requests for assistance, or fail to complete required tasks such as reporting on student progress[6].

Please confirm that in submitting this proposal you agree to take on the following responsibilities.

1. To advise, read and comment on the project report and project proposals written by any students you supervise in a timely manner.
2. To report on the progress of the students you supervise at the request of the DTP and on a quarterly basis via GSR for students who proceed to DPhil study (GSR registered supervisors only).
3. To read and provide written comments on project reports submitted by DTP students who are supervised by other supervisors on request and in a timely manner.
4. To participate in DTP project proposal assessments. These involve two supervisors with relevant expertise reading a student's project proposal and discussing the proposal with them in a meeting that typically takes 45 minutes to 1 hour. Following the meeting the assessors are required to provide a joint written report to the DTP, which is shared with the student and their supervisors.

Additionally, please confirm that you are willing to take on at least one of the following responsibilities if requested:

1. To participate in DTP admissions panels
2. To contribute to DTP skills training and mentoring (please note any areas of training where you are willing to provide specific expertise, including computational and quantitative skills and advanced methods, bioscience for physical scientists, career development, equality, equity, diversity and inclusion (EEDI), professional skills and contributing to the organisation of challenge-led study groups involving non-academic organisations)
3. To offer summer internship projects on UKRI-BBSRC relevant research topics for undergraduate students from disadvantaged and under-represented backgrounds through the University of Oxford's UNIQ+ programme

6 The DTP will endeavour to ensure that any requests made are reasonable, and will take into account specific circumstances such as sick leave, sabbatical leave or conflicts of interest where relevant.