UNIVERSITY OF LEEDS

ADDITIONAL INFORMATION

Background to the Project and Composition of the Research Team

Project Title: The structure and function of the beta-barrel assembly machinery: an Achilles heel of Gram-negative pathogens

OMP biogenesis – a fascinating and vital bacterial assembly process. Despite its name, the outer membrane (OM) of Gram-negative bacteria is contains relatively little lipid, but instead is packed with hundreds of thousands of β-barrel outer membrane proteins (OMPs), with >100 different sequences known in *E. coli*. The density of proteins in the OM reflects its essential roles: the OM is vital for nutrient uptake, for the assembly of host invasion apparatus, and mechano-chemical signalling. The biogenesis of OM proteins (OMPs) is a complex process: OMPs are translated on cytoplasmic ribosomes, and pass through the inner membrane (via the SecYEG translocon). Thereafter, these highly aggregation-prone proteins must traverse the aqueous environment of the periplasm to reach the inner leaflet of the OM, and then insert into a crowded environment and fold to a functional structure. Given the importance of the OM for bacterial survival, it is unsurprising that an obligate protein complex, the β-barrel assembly machinery (BAM), is essential for OMP folding and assembly *in vivo*. In addition, periplasmic chaperones such as SurA and Skp, are also vital for transport of different OMPs to BAM.

Determining how BAM functions is of both fundamental and strategic importance. The BAM-assisted folding of OMPs must be fundamentally different from the well-characterised folding of cytosolic proteins by ATP-dependent cytosolic chaperones, because there is no source of ATP in the periplasm. Furthermore, BAM is an essential protein complex in Gram-negative bacteria, and given that BAM is surface-located and conserved, it is an exciting potential target for new antibiotics against major pathogens. Furthermore, the fundamental mechanisms of OMP folding are much less well understood than those of water-soluble proteins. This is due to the lack of structural information about the BAM complex itself, a dearth of tractable model OMPs (OMPs are hydrophobic and aggregation-prone), and a lack of biophysical methods with which to determine their folding mechanisms in mechanistic detail. Excitingly, this situation is changing: structures of the whole BAM complex have now been solved by both X-ray crystallography and here in Leeds by cryo-EM. In addition, we and others have recently developed methods for studying the kinetics of OMP folding. We now want to exploit these achievements to understand the molecular details of how OMPs of different sequence are recognized, inserted and folded into the OM by BAM

This research spans the Radford and Ranson laboratories, you will join a large well-resourced team funded by MRC and BBSRC grants to understand OMP and OM biogenesis, consisting of PhD and Post-doctoral researchers. You will work in a vibrant, interdisciplinary environment alongside a range of outstanding researchers working on many aspects of protein folding and aggregation, membrane protein structure, and structural virology. For a full list of publications from the Radford and Ranson laboratories, please see our Google Scholar pages:

https://scholar.google.com/citations?user=8nEAkSoAAAAJ&hl=en&oi=sra
https://scholar.google.com/citations?user=djqT8TIAAAAJ&hl=en

This post, which is primarily focused on cryoEM structure determination and will be based in the Ranson laboratory, will be funded by the remaining 2.5 years of a 5-year MRC Programme grant which started in 2017. The programme seeks to address a single key overarching question - "How does the intact BAM complex fold OMPs?"

Main duties and responsibilities.
Specific Duties

- Optimise the purification of BAM and it's reconstitution into a variety of bilayer mimetics such as detergent, nanodiscs etc.
- Devise and create BAM complexes with OMPs stalled at different stages of BAM-catalysed folding
- Determine the structure of BAM and the complexes it makes, including stalled substrate complexes, using cryoEM and single particle analysis.
- Produce and site-specifically label outer membrane proteins (OMP) for analysis by cryo-EM and biophysical methods
- To reconstitute BAM into proteoliposomes, use cryoET and sub-tomogram averaging to determine its structure
- Determine the structure of BAM and the complexes it makes in outer membrane vesicles (OMVs) and in native outer membranes;
- To keep informed of recent advances in the fields of OMP/BAM folding

Additionally you will also:

- Generate and pursue independent and original research ideas in Biochemistry/Biophysics
- Design and conduct a programme of investigation in consultation with the principal investigators, as appropriate
- Evaluate methods and techniques used and results obtained by other researchers and to relate such evaluations appropriately to their own work
- Communicate or present research results through publication or other recognised forms of output
- Understand broader issues relating to the management of research
- Take part in knowledge-transfer activities, where appropriate and feasible
- Contribute to the supervision of junior researchers, as appropriate
- Maintain own continuing professional development and act as a mentor to less experienced colleagues, as appropriate
- Maintain a safe work environment, including ensuring compliance with legislation and the undertaking of risk assessments

- Collaborate closely with all members of the research team and integrate their own results with those of others
- Prepare written summaries of their work and meet with the full team to discuss these reports on a regular basis
- Attend group meetings and present their work to others
- Undertake any other duties commensurate with the post as requested by the Head of School or nominee

Career Expectations

The University of Leeds is committed to developing its staff. All staff participate in the Staff Review and Development scheme and we continue to work with individuals, supporting them to maximise their potential.

Progression to a higher grade is dependent on an individual taking on an increased level of responsibility. Vacancies that arise within the area or across the wider University are advertised on the HR website - http://jobs.leeds.ac.uk - to allow staff to apply for wider career development opportunities.

University Values

All staff are expected to operate in line with the university's values and standards, which work as an integral part of our strategy and set out the principles of how we work together. More information about the university's strategy and values is available at http://www.leeds.ac.uk/comms/strategy/

The University of Leeds' commitment to women in science has been recognised with a national accolade. The University has received the Athena SWAN Bronze Award and the Faculty of Biological Science holds the Athena SWAN Bronze Award in recognition of our success in recruiting, retaining and developing/promoting women in Science, Engineering and Technology (SET). We are proud of our commitment to equality and inclusiveness.

Protected characteristics are under-represented in the Faculty in posts in this area. We would therefore particularly welcome applications from members of such groups, however, any appointment will be made entirely on merit.

Research Environment in the Astbury Centre for Structural Molecular Biology, University of Leeds

The Astbury Centre for Structural Molecular Biology (ACSMB) in Leeds provides an outstanding environment in which to perform this research. ACSMB is an interdisciplinary research hub focused on understanding life in molecular detail. ACSMB has >70 academic members, a grant portfolio of >£110m, and expertise in physics, chemistry, medicine and biology. ACSMB hosts state-of-the-art facilities for MS, EM, crystallography, NMR (600-950MHz), force spectroscopy and biophysics. ACSMB provides a vibrant environment for structural molecular and cellular biology, with experts in biophysics and structural biology, chemical biology, membrane biology, virology, enzymology and bioninformatics. Of particular importance for this project, we will exploit the state-of-the-art facilities for cryo-EM in the Astbury Biostructure Laboratory, established in 2016 with a £17m strategic investment from the University of Leeds, with ~£2m from the Wellcome Trust. The EM facility is **superbly equipped** for this project,

and includes **two Titan Krios EMs**, including one with an **energy-filtered Gatan K2, and Volta phase plate**, and **one with a Falcon3-EC.** We also operate Leica EM GP and FEI Vitrobot freezing devices, and all required ancillary equipment. With more recent Wellcome Trust funding, we also have equipment for cellular EM, including a **cryo-CLEM system**, **high-pressure freezer, freeze-substitution unit and cryo-ultramicrotome**. The faculty of Biological sciences also has excellent facilities for cell imaging including two **Zeiss LSM880 with Airyscan microscopes**, which have 1.7x the resolution of standard confocals. One of LSM880 microscopes is inverted enabling imaging of live cells. The confocal microscopes are complemented with super resolution microscopes with capabilities for **STORM** and **PALM** for fixed samples and **iSIM** for imaging fixed and live cells. In addition, a **Multiphoton/ TCSPC** system enables optical sectioning of thick sections and FLIM. Facilities also exist for **single molecule fluorescence** and FRET experiments, including **FCS** and **FCCS** and **FRET** using multicolour and alternating laser excitation protocols. **TIRF** enables single molecule analysisof immobilised samples, including membranes/membrane proteins, individual macromolecular complexes and cells.

Recent Relevant Publications

Madej, M., White, J., Nowakowska, Z., Rawson, S.D., Pothula, K., Scavenius, C., Enghild, J., Kleinekathoefer, U., **Ranson, N.A.,** Potempa, J. & van den Berg, B. (2019). Dynamic peptide acquisition by the RagAB TonB-dependent transporter from *Porphyromonas gingivalis.* ***BioRxiv*** DOI:10.1101/755678

Humes, J.R., Schiffrin, B., Calabrese, A.N., Higgins, A.J., Westhead, D.R., Brockwell, D.J. & **Radford, S.E.** (2019). The Role of SurA PPIase Domains in Preventing Aggregation of the Outer-Membrane Proteins tOmpA and OmpT. *J. Mol. Biol.*, DOI: 10.1016/j.jmb.2019.01.032.

Horne, J.E., Walko, M., Calabrese, A.N., Levenstein, M.A., Brockwell, D.J., Kapur, N., Wilson, A.J. & **Radford, S.E.** (2018). Rapid Mapping of Protein Interactions Using Tag-Transfer Photocrosslinkers. *Angew. Chem. Int. Ed. Engl*., DOI:10.1002/anie. 201809149

Schiffrin, B., Brockwell, D.J. & **Radford, S.E.** (2017). Outer membrane protein folding from an energy landscape perspective. *BMC Biol.,* **15:**123. DOI:10.1186/s12915-017-0464-5.

Iadanza, M.G*., Higgins, A.J.*, Schiffrin, R., Calabrese, A., Brockwell, D.J., Ashcroft, A.E. **Radford, S.E.‡ & Ranson, N.A.‡** (2016). Lateral opening of the intact β-barrel assembly machinery captured by cryo-EM. *Nature Comms.* 10.1038/ncomms12865

Schiffrin, B., Calabrese, A. N., Devine, P. W. A., Harris, S. A., Ashcroft, A. E., Brockwell, D. J., & **Radford, S. E.** (2016). Skp is a multivalent chaperone of outer-membrane proteins. *Nature Structural & Molecular Biology*, ***23***(9), 786–793.

Allen, W. J., Corey, R. A., Oatley, P., Sessions, R. B., Baldwin, S. A., **Radford, S. E.,** et al. (2016). Two-way communication between SecY and SecA suggests a Brownian ratchet mechanism for protein translocation. *eLife*, *5*, 6545.

A full list of recent publications from Ranson, Radford and their collaborators and more information about the Astbury Centre for Structural Molecular Biology can be found at: http://www.astbury.leeds.ac.uk