UNIVERSITY OF LEEDS

ADDITIONAL INFORMATION

Background to the Project and Composition of the Research Team

Project Title: The structural biology of amyloid aggregation

Increasing age and modern lifestyle means that amyloidosis is a major threat to human health, in both the developed and developing worlds. Despite the increasing prevalence of amyloid disorders, which include >50 human diseases (most famously Alzheimer's and Parkinson's diseases, type II diabetes); few successful routes exist for their prevention, treatment or cure. A detailed molecular understanding of the mechanisms of protein misfolding and aggregation is needed if we are to control and/or cure amyloid disease. Importantly, despite recent advances in our ability to determine the structures of amyloid fibrils, driven in large part by advances in cryoEM, the diversity of possible amyloid structures remains unclear and how fibril structure relates to disease type remains mysterious. In particular, the effects of disease-relevant mutations and post-translation modifications on amyloid fibril structure has not been mapped and, crucially, how the structures of fibrils formed *in vitro* for different amyloid precursors relate to those that form in patients remain unclear.

This project, which will be based in the Laboratories of Professors Neil Ranson and Sheena Radford, aims to answer these key questions. The project will include two PDRAs with complementary skills. One in structural biology (advertised here) and one in biochemical analysis of protein conformation and post-translational modifications (advertised separately).

Exploiting the latest innovations in structural biology, especially in cryo-EM and image processing, the successful applicant will investigate the structure of a range of amyloid fibrils purified ex vivo from patient samples, alongside structures of fibrils formed in vitro from recombinant proteins or synthesized peptides. This work will be carried out alongside a Wellcome Trust-funded programme (Investigator Award to Radford) which is investigating the molecular mechanisms by which protein aggregates of all sizes lead to cellular and organismal dysregulation, using both human cells and *C. elegans* models of disease. Initially you will focus on β_2-microglobulin (β_2m) and α-synuclein, protein aggregates isolated from human tissue, and which are directly implicated in human amyloid disease. You will use cryo-EM to determine the structure of amyloid fibrils extracted from amyloid plaques in 3D at the maximum possible resolution, and with the second PDRA use mass spectrometry to identify mutations and/or post-translational modifications in the aggregated proteins.

The person appointed to this position will have extensive experience in the use of structural methods to understand protein structure and function. Ideally this would be cryo-EM or cryo-ET, but candidates with a strong track record in X-ray crystallography or super-resolution light microscopy should also apply, as training in EM methods can be provided for outstanding candidates without EM experience. Experience working with human tissue samples would be an advantage but is not required.

The wider amyloid team in the Astbury Centre for Structural Molecular Biology at Leeds includes members who are using cryoEM, native mass spectrometry, NMR, chemical biology, and other biochemical and biophysical methods to characterise all aspects of amyloid aggregation pathways and cytotoxicity, and to screen for, and further develop, small molecules or protein-based binding reagents (Affimers) that are able to control the rates of aggregation. You will therefore join a dynamic interdisciplinary team of research scientists working in the amyloid field (see http://www.astbury.leeds.ac.uk/bmbsgi10/sermembers.php). The overall vision of this project is to (a) relate the structure of ex vivo amyloid fibrils to those grown in vitro; and (b) to deliver new molecular understanding on the effects of mutations and post-translational modifications to the primary sequence of precursor proteins on the structure of amyloid fibrils

This post will:

(1) Use cryo-EM to determine the structure of in vitro created and ex vivo amyloid fibrils of α-synuclein, β_2m, and sequence variants thereof,
(2) Purify amyloid fibrils from ex vivo patient samples;
(3) Prepare proteins/peptides for amyloid fibril growth in vitro;
(4) Use the above approaches to develop new, fundamental molecular and mechanistic understanding of amyloid structure;

Successful execution of this MRC funded project will thus result in a molecular understanding of amyloid structure in patients, and of the relevance of in vitro models of amyloid fibrils for studies in human disease.

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 analysis of immobilised samples, including membranes/membrane proteins, individual macromolecular complexes and cells.

Recent Relevant Publications

Recent publications from the Radford/Ranson groups on amyloid formation including cryo-EM structures of amyloid and other macromolecular complexes include:

1. Gallardo, R., **Ranson, N.A.** & **Radford, S.E**. (2020) Amyloid structures: Much more than just a cross-β fold *Curr. Op. Struct. Biol,* 60, 7-16 DOI:10.1016/j.sbi.2019.09.001
2. Iadanza, M.G., Silvers, R., Boardman, J., Smith, H., Karamanos, T., Griffin, R.G., **Ranson, N.A.** and Radford, S.E. (2018). The cryo-EM structure of a β_2-microglobulin fibril shows the molecular basis of a common amyloid architecture. *Nature Communications***,** DOI:10.1038/s41467-018-06761-6
3. Iadanza, M.G., Jackson, M.P., Hewitt, E.W., **Ranson, N.A.** & Radford, S.E. (2018). Seeing Amyloid Structures: A New Era for Understanding Amyloid Disease***.*** *Nat. Rev. Mol. Cell Biol.,* DOI:10.1038/s41580-018-0060-8
4. Structural mapping of oligomeric intermediates in an amyloid assembly pathway. Karamanos, T.K., Jackson, M.P., Calabrese, A.N., Goodchild, S.C., Cawood, E.E., Thompson, G.S., Kalverda, A.P., Hewitt, E.W., & **Radford, S.E.** (2019) *eLife,* **8**, e46574. DOI: 10.7554/eLife.46574.
5. Dynamic peptide acquisition by the RagAB TonB-dependent transporter from *Porphyromonas gingivalis.* 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). ***BioRxiv,*** DOI: 10.1101/755678
6. Cryo-EM structure of the spinach cytochrome b_6f complex at 3.6 Å resolution. Malone, L.A., Qian, P., Mayneord, G.E., Hitchcock, A., Farmer, D.A., Thompson, R.F., Swainsbury, D.J.K., **Ranson, N.A.,** Hunter, C.N. & Johnson, M.P. (2019). *Nature*, **575**, 535-39. DOI:0.1038/s41586-019-1746-6
7. The 3.3 Å structure of a plant geminivirus using cryo-EM. Hesketh, E.L, Saunders, K., Fisher, C., Potze, J., Stanley, J., Lomonossoff, G.P. & **Ranson, N.A.** (2018). *Nature Communications,* 9, **2369.** DOI:10.1038/s41467-018-04793-6
8. The HBV RNA pregenome encodes specific interactions with the viral core protein that can promote nucleocapsid assembly.Patel, N., White, S.J., Thompson, R.F., Weiß, E.U., Bingham, R., Zlotnick, A., Dykeman, E., Twarock, R., **Ranson, N.A.** & Stockley, P.G. (2017). *Nature Microbiology,* **DOI:10.1038/nmicrobiol.2017.98**

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