UNIVERSITY OF LEEDS

# ADDITIONAL INFORMATION

## Background to the Programme and Composition of the Research Team

### Project Title: Protein-Protein Interactions in the Early Stages of Amyloid Formation

Increasing age and modern lifestyle place amyloidosis as 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 type II diabetes, Alzheimer's, prions, and Parkinson's); 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 have hope of controlling/curing amyloid disease. However, we currently lack the knowledge to enable us to achieve this goal: it is difficult to map the early stages of protein aggregation which represent the most tractable control points, and even more challenging to inhibit the transient interactions between non-native species which lead to aggregation and disease.

This project aims to provide this information. The programme will ultimately employ four PDRAs with complementary skills. Two PDRA have already been appointed (NMR/biophysics and biochemistry). The currently advertised post will bring the third person to the team. Exploiting the latest innovations in cell and structural biology methods, the post-doc employed to this position will use cell biology, imaging and structural methods to elucidate the molecular mechanisms by which protein aggregates of all sizes lead to cellular and organismal dysregulation, in human cell culture and *C. elegans* models of human disease. Focussing initially on $\beta_2$-microglobulin ($\beta_2$m) and amylin - proteins which are directly implicated in human disease – you will use super-resolution light microscopy, correlative light and electron microscopy, cryo-EM, cryo-ET and soft X-ray microscopy to understand which parts of cells and organisms are targeted, and how such disruption is manifested in three-dimensions at the maximum possible resolution. Other team members will use native mass spectrometry, NMR and other biochemical and biophysical methods to screen for, and further develop, small molecules or protein-based binding reagents (Affimers) that are able to control the rates of aggregation of $\beta_2$m or IAPP into amyloid. You will therefore have access to a growing library of potential anti-amyloid molecules, and be able to assess their effects in cells and *in vivo*. The overall vision of this project is to deliver new molecular understanding of the interactions that initiate amyloid formation and to discover new ways of controlling aggregation in vitro, in cells, in animal models and, ultimately, in human disease.

This post currently advertised will use structural biological methods, cell biology and bioimaging to:

(1) Generate human cell and *C. elegans* models of human amyloid disease
(2) Understand how different amyloids cause cytotoxicity in human cells using correlative light and electron microscopy methods and cryo-electron (and X-ray) tomography

(3) Understand the tissue tropism of different amyloid proteins at the level of the organism using *C. elegans* models of disease (e.g. using super-resolution light microscopy and cryo-focussed ion beam / scanning EM).
(4) Generate hypotheses for how cellular disruption proceeds and design experiments to test these ideas in cells and organisms
(5) Assess the protective effects of putative anti-amyloid compounds *in vivo.*
(6) Use all of the above approaches to develop new, fundamental molecular and mechanistic understanding of amyloid toxicity

Successful execution of this Wellcome Trust-funded 5 year Investigator Award will thus result in a molecular understanding of amyloid formation in unprecedented detail and open the door to new strategies to control aggregation in human disease.

**Aims and Key Research Questions**

There is mounting evidence that therapies for amyloid disease may be possible, despite the fact that the molecular precursors of aggregation are usually non-native, dynamic species which form weak and transient interactions. Only a single anti-amyloid drug is currently available in the clinic (tafamidis), a small molecule developed using structure-based design which prevents transthyretin amyloidosis by stabilising the native tetramer. Other inhibitors (still far from clinical use) include small molecules which prevent $\alpha$-synuclein, A$\beta_{42}$ or human islet-amyloid-polypeptide (hIAPP or amylin) aggregation. Thus, previously considered 'undruggable', disordered proteins can bind ligands with sufficient affinity to inhibit aggregation *in vitro* and *in vivo*. Other strategies use antibody domains/other scaffolds as preventative agents. The field urgently needs a toolkit of such reagents, so that well-defined ensembles of non-native species can be trapped, the structural mechanism of self-aggregation deduced, and the molecular culprits of cellular dysfunction defined.

Spurred by our recent successes (see references below), this project, which involves FOUR postdoctoral researchers in NMR/biophysics and biophysics/biochemistry (already appointed) Structural Cell Biology (advertised herein) medicinal chemistry (to be advertised later in 2017) aims to:

**(1)** use structural methods to map the protein-protein interactions that initiate amyloid formation of hIAPP and human $\beta_2$-microglobulin (h$\beta_2$m) *in vitro*;
**(2)** use existing structural knowledge, together with that from (**1**), to identify small molecules, and to design new proteins, able to control aggregation by targeting amyloid-initiating interfaces *in vitro;*
**(3)** discover whether the reagents developed in (**2**) affect aggregation in cells and in C. elegans models of $\beta_2$m/amylin amyloid disease,
**(4)** relate the structural/molecular information to insights at the cellular and organismal levels.

The overarching aim of the project thus is to derive generic principles about how these fundamentally different precursor types initiate aggregation, as well as details specific to each system.

**The FOUR PDRAs employed on this 5 year Wellcome Trust Investigator Award will thus work concurrently to answer a single key overarching question - "Can we inhibit amyloid formation by targeting early protein-protein interactions and hence derive new treatments for amyloid disease?"**

## Further Information

## Research Environment in Leeds

The Astbury Centre for Structural Molecular Biology (ACSMB) in Leeds provides an outstanding environment in which to do this research. ACSMB is an interdisciplinary research hub focused on understanding life in molecular detail. ACSMB has 68 academic members, a grant portfolio of >£90m, and expertise in physics, chemistry, medicine and biology. ACSMB hosts state-of-the-art facilities for MS, crystallography, NMR, force spectroscopy and fluorescence. ACSMB provides a vibrant environment for structural molecular biology, with experts in biophysics and structure, 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 new 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 includes **two Titan Krios EMs**, including one with an **energy-filtered Gatan K2, and Volta phase plate** – ideal for tomographic studies. We also operate Leica EM GP and FEI Vitrobot freezing devices, and all required ancillary equipment, and a **cryo-FIB/SEM** instrument is available for our use on the Leeds campus. With recent Wellcome Trust funding, are currently purchasing new equipment for cellular EM, including a **cryo-CLEM system**, **high-pressure freezer, freeze-substitution unit and cryo-ultramicrotome**.

## Recent Relevant Publications

Recent publications from the Radford/Ranson groups and their collaborators relevant to the project advertised include:


**1.** A population shift between sparsely populated folding intermediate determines amyloidogenicity. Karamanos, T.K., Pashley, C.L.,Kalverda, A.P., Thompson, G.S., Mayzel, M., Orekhov, V.Y. & **Radford, S.E**. (2016) *J. Am. Chem. Soc.* **138**, 6271-6280

*In the manuscript we show that subtle modulation of the folding energy landscape protects from aggregation. NMR, stopped-flow-fluorescence and HX revealed that murine $\beta_2$m (90% homologous to human $\beta_2$m) is not amyloidogenic because the critical aggregation intermediate ($I_{trans}$) is destabilised relative to a more unstructured non-amyloidogenic intermediate.*

2. pH-induced molecular shedding drives the formation of amyloid fibril-derived oligomers. Tipping, K.W., Karamanos, T.K., Jakhria, T., Iadanza, M.G., Goodchild, S.C., Tuma, R., **Ranson, N.A., Hewitt, E.W. & Radford, S.E.** (2015) *PNAS,* **112**, 5691-5696

*In this paper we reveal how fibril fragmentation/dynamics may contribute to disease. We showed that nanoscale length fibrils may contribute to disease by their unique ability to enter cells and release cytotoxic oligomers within acidic lysosomes.*

3. Screening and classifying small molecule inhibitors of amyloid formation using ion mobility spectrometry-mass spectrometry. Young, L.M., Saunders, J.C., Mahood, R.A., Revill, C.H., Foster R.J., Tu, L.-H., Raleigh, D.P., **Radford, S.E.** & Ashcroft, A.E. (2015) *Nature Chemistry,* **1**, 73-81

*Here we describe a new screen for ligands able to inhibit aggregation of intrinsically disordered precursors using ESI-IMS-MS and discovered a new inhibitor of amylin aggregation.*

4. An *in vivo* platform for identifying inhibitors of protein aggregation. Saunders, J.C., Young, L.M., Mahood, R.A., Revill, C.H., Foster, R.J., Jackson, M.P., Smith, D.A.M., Ashcroft, A.E., Brockwell, D.J. & **Radford, S.E**. (2016) *Nature Chem. Biol.,* **12**, 94-101

*Here we devise a new approach able to screen for aggregation inhibitors in vivo. Exploiting the accessibility of the E.coli periplasm to small molecules and a □-lactamase sensor we discovered another inhibitor of amylin aggregation and demonstrated the utility of the system for biopharma.*

5. Visualization of transient protein-protein interactions that promote or inhibit amyloid assembly. Karamanos, T.K., Kalverda, A.P., Thompson, G.S & **Radford S.E***.* (2014) *Molecular Cell,* **55**, 214-226

*In this manuscript we use PRE and other dynamic NMR methods to determine how the □2m protein known as ΔN6 assembles into amyloid fibrils and is inhibited by the non-amyloidogenic murine variant.*

6. 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). The HBV RNA pregenome encodes specific interactions with the viral core protein that can promote nucleocapsid assembly**.** *Nature Microbiology,* **DOI:10.1038/nmicrobiol.2017.98**

*Here we use single particle EM to examine asymmetric assembly intermediates of an important human pathogen*

7. 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.* **DOI:10.1038/ncomms12865***.*

*A joint study between the Radford/Ranson groups showing the first EM structure of the BAM complex, an important protein complex that assists the correct folding of bacterial outer membrane proteins.*

8. Hesketh, E.L., Meshcheriakova, Y., Dent, K.C., Saxena, P., Thompson, R.F., Cockburn, J.J.B, Lomonossoff, G.P. & **Ranson, N.A.** (2015). Mechanisms of assembly and genome packaging in an RNA virus revealed by high-resolution cryo-EM. *Nature Comms.,* **DOI:10.1038/ncomms10113**

*The first high resolution EM structures from the Ranson lab, at 3.0Å resolution, giving important clues about virus self assembly, and scaffolding/chaperoning of the process by viral proteins.*

9. Dent, K.C., Thompson, R., Barker, A.M., Barr, J.N., Hiscox, J.A., Stockley, P.G. & **Ranson, N.A**. (2013). The asymmetric structure of an icosahedral virus bound to its receptor suggests a mechanism for genome release. *Structure*, **21**, p1225.

*This study uses cryo-ET and sub-tomographic averaging to reveal the first asymmetric structure of a virus:receptor complex, and the fact that the RNA genome is packaged in a unique conformation inside the capsid.*

**10. O'Brien D, van Oosten-Hawle P. (**2016). Regulation of cell-non-autonomous proteostasis in metazoans. Essays in Biochemistry, **60**,~~:~~ 133-142.

**11. van Oosten-Hawle P**, Porter RS, Morimoto RI (2013). Regulation of organismal proteostasis by transcellular chaperone signaling. Cell, **153**,1366-78.

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