Faculty of Biological Sciences
School of Molecular and Cellular Biology

Research Fellow in Structural Molecular Virology

Project Title: The roles of the pre-genomic RNA in Hepatitis B Virus nucleocapsid assembly

Full time, fixed term for 2 years

This project aims to determine the detailed molecular mechanism used by Hepatitis B Virus (HBV) in assembling its nucleocapsid using a combination of structure determination (especially electron microscopy), biophysics and biochemistry. HBV is estimated to have infected more than 2 billion people across the world, where chronically infected carriers can be >10% of the local population. Such carriers often progress to liver cancer or cirrhosis resulting in hundreds of thousands of deaths per annum. Current therapeutic options are very limited, so new drug targets such as those we will investigate during this project are urgently required.

This post offers an opportunity to join a highly productive, interdisciplinary team headed by Professor Peter Stockley (Leeds, where the post will be based) and Professor Reidun Twarock (York). Our team spans a wide range of expertises, including evolutionary biology and mathematical modelling (Twarock), structural virology (Neil Ranson, Joe Cockburn, Juan Fontana & David Rowlands), molecular genetics & biochemistry (Stockley), and biophysics (Roman Tuma). Our wider goal is to understand the molecular mechanisms used by ssRNA viruses to assemble their capsids during an infection cycle, and the constraints that these mechanisms place on their evolution. Such insights will enable the development of future anti-viral strategies. This class of viruses is ubiquitous in all kingdoms of life, causing devastating diseases in humans, animals and crop plants. They also constitute a significant fraction of newly emerging viral infections, so new therapeutic/defensive strategies are urgently required. HBV is part of this analysis because it initially assembles a nucleocapsid around a pre-genomic ssRNA version of its genome that is converted to DNA once encapsidated. Our hypothesis is that increased understanding of fundamental mechanisms will identify novel anti-viral targets.

We are looking for an ambitious, highly motivated structural biologist. You should have a PhD in structural biology or electron microscopy, although a motivated X-ray crystallographer with a desire to cross-train into EM will also be considered. Previous experience in one or more of the following areas is essential: microbiology, virology, biochemistry/chemistry, molecular genetics, or a closely allied discipline, including fluorescence spectroscopy, as will a strong drive to learn new techniques and tackle complex macromolecular systems. Your work will be at the interface between structural biology and Biochemistry/Biophysics. You will work on a day-to-day basis under the supervision of the grant PI, and actively collaborate with a large interdisciplinary team across the Stockley/Twarock research groups. In addition, you
will be expected to develop and implement new methods and participate in the supervision of graduate students. Stockley & Twarock are Wellcome Trust Joint Investigators, so extension of the post beyond the initial two-year period is a possibility.

University Grade 7 (£31,656 - £37,768 p.a.) Due to funding limitations, an appointment will not be made above £33,574

Informal enquiries may be made to Professor P. G. Stockley, email p.g.stockley@leeds.ac.uk or Dr. N. A. Ranson, email n.a.ranson@leeds.ac.uk

Closing Date: 1 June 2016

Ref: FBSMB1060

Click here for further information about working at the University of Leeds www.leeds.ac.uk/info/20025/university_jobs
Job Description

Responsible to: Head of School of Molecular and Cellular Biology
Reports to: Professor Peter Stockley

Background to the post

Single-stranded (ss)RNA viruses are major pathogens of humans, e.g. HIV & HCV; animals, e.g. Foot & Mouth Disease Virus; and plants, e.g. TBSV, causing deleterious impacts on human populations, farm animal health and crop yields. Despite the importance of this group of pathogens, there are still few anti-viral therapeutic options beyond vaccination. This is not always effective or practical. The assembly/disassembly pathways of ssRNA viruses are potential targets for novel anti-viral therapeutics, but they have mostly been ignored to date. In recent major contributions to understanding the assembly mechanisms of such viruses, we have shown that the lack of drugable targets in this area may be a consequence of neglecting the contributions of the viral RNA to these processes. We have shown that the genomes of ssRNA viruses play multiple roles during assembly, and probably during the initial stages of infection as capsids uncoat. These roles depend on multiple, degenerate packaging signals (PSs) that allow intrinsically asymmetric RNAs to be rapidly and faithfully encapsidated in highly symmetric protein shells in vivo in the presence of competing RNA substrates. Our studies have recently been described as a new paradigm for virus assembly “Prevelige PE Jr (2015) Follow the Yellow Brick Road: A Paradigm Shift in Virus Assembly, J Mol Biol. pii: S0022-2836(15)00700-7. doi: 10.1016/j.jmb.2015.12.009.”. Work pursued in this project will lay the foundations for the discovery of novel anti-viral ligands targeting PS-mediated features of viral lifecycles (see also: Stockley et al (2007) JMB 369, 541-552; Borodavka et al (2012) PNAS, 109, 15769-15774; Dent et al (2013) Structure 21, 1225-1234; Dykeman et al (2013) JMB 425, 3235-49; Dykeman et al (2014) PNAS, 111, 5361-6; Patel et al (2015) PNAS, 112, 2227-32 & Rolfsson et al (2015) JMB 428, 431-448).

In this project we will examine how PS-mediated assembly applies to a DNA virus, namely HBV, which assembles a nucleocapsid, principally with \( T=4 \) quasi-symmetry, around a pre-genomic RNA. We have identified PS sites within the HBV genome, shown that they are strongly conserved across strain variants, and that oligonucleotides encompassing individual PS sites trigger highly efficient virus-like particle (VLP) assembly at low nanomolar concentrations in a sequence-specific fashion. Preliminary cryo-EM reconstructions of these VLPs at intermediate resolution (5-12 Å), both with and without symmetry averaging, suggesting that assembly can be triggered by a unique initiation complex involving contacts between several PS sites in the pre-genomic RNA and the C-terminal arginine-rich domain of the HBV core protein. These structures also hint at the molecular basis of quasi-conformer specification, as HBV is known to assemble into both \( T=3 \) and \( T=4 \) structures.
Summary of the research programme

The successful candidate will prepare HBV VLPs assembled around pre-genome RNA fragments guided by single molecule fluorescence assays and existing protocols. They will then determine cryo-EM structures to the highest possible resolution, with the aim of achieving a detailed molecular understanding of the mechanism of assembly initiation. This will include asymmetric EM reconstructions of particles with multiple copies of individual PS sites, as well as longer pre-genome fragments that are candidates for the natural initiation site in vivo. Site-directed mutagenesis of the HBV core protein and or the presence of additional virally encoded components such as the polymerase on the assembly process will also be examined. If suitable samples can be obtained crystallisation and X-ray structure determination may also be an option. Note, all the work will be with recombinantly-produced proteins and synthetic oligonucleotide materials and none of it will pose any potential health hazard.

Research environment

The Astbury Centre for Structural Molecular Biology (ACSMB) was established in 1999 as an interdisciplinary research centre of the University of Leeds. Prof. Stockley was its Director from 2001 until 2008. It was founded to carry out international quality research in all aspects of structural molecular biology. ACSMB brings together over sixty academics from across the University of Leeds who share a common interest in understanding life in molecular detail.

The ACSMB is an ideal environment in which to pursue this research, which is heavily dependent on cryo-EM. The University of Leeds has recently committed >£17M to fund the Astbury BioStructure Laboratory, to be equipped with state-of-the-art EM & NMR instruments. The cryo-EM facility, led by Neil Ranson, will shortly (May/June 2016) install TWO FEI Titan Krios EMs, each equipped with direct detectors. One will also be equipped with a Volta phase-plate and energy-filtered Gatan K2 detector. For NMR, we have just installed a Bruker Aeon 950 MHz spectrometer). These instruments, together with dedicated technical support staff, will make Leeds amongst the best-equipped places for cryo-EM in the world, allowing near-atomic resolution structures of viruses and virus-like particles to be determined in a matter of weeks. ACSMB is a renowned interdisciplinary centre, with multiple, large-scale funding from both the Research Councils and The Wellcome Trust (e.g. recent grants of £0.75M & £1M from the Wellcome Trust to support developments in NMR and EM, respectively). Our single molecule facility provides access to total internal reflection fluorescence microscopy (TIRFM), as well as alternating excitation (ALEX) and fluorescence correlation spectroscopy (FCS) confocal instruments equipped with multiple colour detectors. In addition, several fluorimeters are available for both routine spectral, as well as advanced fluorescence lifetime and anisotropy measurements.
Main duties and responsibilities

- To study the molecular mechanism of pre-genome packaging specificity in HBV, especially by structure determination of VLPs assembled around genomic RNA fragments encompassing PSs
- To generate and pursue independent and original research ideas as appropriate in the subject area
- To keep up-to-date with the scientific literature on virus structure, assembly and anti-viral chemotherapy
- To pro-actively build and maintain interactions with collaborators both at Leeds and elsewhere
- To attend Stockley/Twarock team meetings and ACSMB seminars
- To design and conduct a programme of investigation in consultation with the PI and collaborating groups, as appropriate
- To evaluate methods and techniques used and results obtained by other researchers and to relate such evaluations appropriately to their own work
- To communicate or present research results through publication or other recognised forms of output
- To attend scientific meetings and seminars locally, nationally and internationally
- To take part in knowledge-transfer activities, where appropriate and feasible
- To contribute to the supervision of junior researchers, as appropriate
- To maintain their own continuing professional development and act as a mentor to less experienced colleagues, as appropriate
- To maintain a safe work environment, including ensuring compliance with legislation and the undertaking of risk assessments
- To undertake any other duties commensurate with the post as requested by the Director of Institute 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.
Person Specification

Essential

- A first degree in a biological or physical science, and a PhD in structural biology or electron microscopy, although a motivated X-ray crystallographer with a desire to cross-train into EM will be considered
- Familiarity with relevant software systems such as: e.g. for EM (RELION, EMAN2, XMIPP etc; e.g. for X-ray (PyMol, CCP4, Coot etc)
- Previous experience in one or more of the following areas: microbiology, virology, biochemistry/chemistry, molecular genetics, or a closely allied discipline, including fluorescence spectroscopy
- Excellent data management, analytical and computer skills
- A strong drive to learn new techniques and tackle complex macromolecular systems
- A proven ability to work effectively and responsibly without close supervision, designing, executing and writing up a programme of experimental work independently
- Good organisational and time management skills
- Good written and verbal communication skills
- Good presentational skills
- An ability to work to deadlines
- Have the ability to deal with and prioritise varied tasks
- Professional ambition and enthusiasm

Desirable

- A detailed understanding of virus structure, molecular evolution and nucleic acid biochemistry
- Previous experience of inter-disciplinary biosciences
- In-depth theoretical and practical experience of a range of complementary structural methods, e.g. SAXS, light-scattering, model building
- Peer-reviewed publications in the relevant field
**Additional Information**

The University offers generous terms and conditions of employment, a wide range of benefits, services, facilities and family friendly policies. Full details are available on the Human Resources web pages accessible at [www.leeds.ac.uk/hr](http://www.leeds.ac.uk/hr).

**The Partnership**

The Partnership has been developed by students and staff and describes the mutual expectations of us all as members of the University of Leeds community. More information about the Partnership is available at [http://partnership.leeds.ac.uk](http://partnership.leeds.ac.uk).

**Disclosure and Barring Service checks**

A Disclosure and Barring Service (DBS) Check is not required for this position. However, applicants who have unspent convictions, cautions, reprimands and warnings, including any pending criminal proceedings must indicate this in the ‘other personal details’ section of the application form and send details to the Recruitment Officer at disclosure@leeds.ac.uk.

**Disabled Applicants**

The post is located in the Faculty of Biological Sciences. Disabled applicants wishing to review access to the building are invited to contact the department direct. Additional information may be sought from the Recruitment Officer, email disclosure@leeds.ac.uk or tel + 44 (0)113 343 1723.

Disabled applicants are not obliged to inform employers of their disability but will still be covered by the Equality Act once their disability becomes known.

Further information for applicants with disabilities, impairments or health conditions is available in the applicant guidance.
School of Molecular and Cellular Biology

The School, comprising some 40 principal investigators, together with our sister Schools of School of Biomedical Sciences and School of Biology, was formed in September 2005. The aim of the school is to provide a stimulating environment for the prosecution of world-class research. We have a strong emphasis on interdisciplinary activity, with the aim of developing the boundaries between traditional disciplines. To this end, collaborations between members of SMCB and our sister schools within FBS are strongly encouraged. Moreover, the Astbury Centre for Structural Molecular Biology is a cross-faculty centre that includes staff from the Faculty of Mathematics and Physical Sciences and the Faculty of Medicine and Health.

Biological Sciences

The Faculty of Biological Sciences is one of the leading groups of life-science researchers within the UK, offering superb facilities, providing a high quality research training environment and delivering an exceptional student education.

Our position amongst the UK elite for bioscience research was confirmed in the results of the recent Research Excellence Framework (REF) where we were ranked as 6th in the country for research impact. The assessment also identified that over 80% of biological science research at Leeds has a top quality rating of either “world leading” or “internationally excellent”.

In addition to 110 academic staff, the Faculty has over 400 postdoctoral fellows and postgraduate students supported by a current active research grant portfolio of around £50m derived from a range of sources including charities, research councils, the European Union and industry.

With around 2000 undergraduate students and 150 taught postgraduate students, we are one of the largest life sciences faculties in the UK. Our programmes cover the breadth of the biological sciences with undergraduate programmes in the areas of biology, biochemistry, microbiology, sport and exercise sciences and medical sciences including physiology and neuroscience.

Significant investments in our infrastructure contribute to our dynamic and vibrant research environment, offering excellent opportunities for leading edge research focused around key areas, including neuroscience, sports and exercise science, membrane biology, and structural molecular biology.

The Faculty has 3 Schools:

- School of Biomedical Sciences
- School of Molecular and Cellular Biology
- School of Biology

Find out more about the Faculty [here](#).