The Astbury Centre for Structural Molecular Biology

UNIVERSITY OF LEEDS

ASTBURY CENTRE
UNDERSTANDING LIFE IN MOLECULAR DETAIL
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Welcome to the Astbury Centre for Structural Molecular Biology. Our Centre was formally constituted in 1999 and uses the latest tools available to understand life in molecular detail. We hope that you will find much of interest in this brochure that describes the full range of the Centre’s activities.

The Astbury Centre brings together around 60 academic staff and 300 researchers (including PhD students, post-doctoral scientists and research fellows) from physics, the biological and medical sciences and chemistry to facilitate interdisciplinary approaches to unravel life’s mechanisms.

The Centre has outstanding expertise and research infrastructure with all the major techniques required to carry out world-class structural molecular and cellular biology research. Our members address major questions associated with biological mechanisms in areas as diverse as chemical biology, structural biology, biophysics and molecular interactions in cells.

In the words of W.T Astbury in 1948:

"We need much more co-operation, much more fraternization, much more encouragement the one of the other, much more sharing of knowledge and much more asking of help."

Our centre today continues to embrace this mantra to the full, creating a lively, buoyant and invigorating research arena that allows new research discoveries to be made.

Professor Sheena Radford FMedSci, FRS
Director of the Astbury Centre
Chemical Biology is the application of chemical tools and approaches to address biological problems. Within the Astbury Centre, chemists are engaged in highly interdisciplinary and collaborative research programmes to address a wide range of biological, chemical and medical problems.

These include the design and synthesis of new drugs, the development and exploitation of small molecule tools to interrogate biological mechanisms, pioneering new approaches in synthetic biology and engineering new enzymes for use in synthetic chemistry.

We have superb facilities for Chemical Biology including integrated synthetic and biological laboratories, a computer cluster dedicated to ligand design, a facility for screening compound libraries for biological function and a suite dedicated to the high-throughput synthesis of small molecules.

We exploit these facilities, in conjunction with biophysical, structural and cellular approaches, to address a wide range of problems of direct relevance to society.

Activity Directed Synthesis

Activity Directed Synthesis is a new approach being developed at Leeds for the rapid discovery of novel bio-active molecules. Traditionally, chemists have discovered novel drug-leads and biological probes by separately preparing large numbers of molecules and screening these libraries of compounds for activity. One of the frustrations of this classical approach is that equal effort is applied to all compounds, even the inactive ones. The requirement to make a range of structural analogues also means that chemists tend to rely on a limited palette of reliable reactions to make their compound collections, limiting structural diversity.
PhD student George Karageorgis in the Astbury Centre has been working with Dr Stuart Warriner and Prof Adam Nelson to develop an alternative discovery approach which they have termed Activity Directed Synthesis. The method, published in *Nature Chemistry* in 2014, uses inherently unpredictable reactions which have numerous outcomes depending on the catalysts and reaction conditions used. Arrays of reactions are performed in 96 well plates and the reaction mixtures assessed for activity without purification. Reactions whose products generate the most active mixtures are then used as the starting points for future reaction arrays which are screened at progressively lower concentrations to identify the best combination of reagents, catalysts and conditions. Only at this point are the best reactions scaled up and the active products identified. By using this approach the team showed that Activity Directed Synthesis could be used to produce novel agonists of the androgen receptor with an unexpected β-lactam core. The ability to pan for desired compounds may revolutionise the search of chemical space for bio-active compounds.
STRUCTURAL BIOLOGY

The Astbury Centre has a strong history in structural studies from small molecules to large protein complexes. Bill Astbury’s definition of the alpha-helix and beta-sheet structures within proteins laid the foundations for the high resolution structural studies of bio-molecules that are carried out in the Centre today.

Understanding the structure of a protein or protein complex is fundamental to underpinning drug design and understanding the relationship between structure and function. The Astbury Centre has a wealth of expertise in X-ray crystallography, nuclear magnetic resonance spectroscopy (NMR), atomic force microscopy (AFM) and electron microscopy and this structural information is complemented by biophysical techniques such as analytical ultracentrifugation, isothermal titration calorimetry, circular dichroism spectroscopy (CD), surface plasmon resonance (SPR), and fluorescence. We take advantage of our excellent modern facilities, along with the many cross-disciplinary collaborations within the Centre, to investigate the structural biology of aspects of life sciences such as enzyme mechanisms, membrane proteins, molecular motors, virus biology, molecular dynamics, protein folding and more.
Towards a molecular understanding of amyloid diseases

The aggregation of normally monomeric globular proteins into ordered arrays of amyloid fibres is implicated in the aetiology of many of the debilitating conditions found in ageing populations such as Parkinson’s and Alzheimer's diseases.

However, despite the medical importance of these diseases, the molecular mechanisms by which functional soluble proteins re-configure into insoluble fibres is still largely unknown but is an area of intense international activity.

One method to treat amyloid diseases may be to prevent amyloid fibre formation by blocking the intermolecular interactions that initiate amyloid assembly, but to do this the binding interface must be characterised at the molecular level. A Wellcome Trust funded PhD student, Theo Karamanos together with Professor Sheena Radford achieved this for a protein called β₂m (the culprit protein in dialysis related amyloidosis) using a wealth of NMR techniques (chemical shift perturbation, paramagnetic relaxation enhancement and hydrogen exchange measurements) carried out on the Centre’s 750MHz NMR facility.

This work, published in Molecular Cell in 2014, reveals that the promotion of fibrillation involves the relatively weak association of monomers in a head-to-head orientation. This results in conformational changes in the initially non-fibrillogenic partner. This work opens the door to the design of molecules able to prevent amyloid formation which has major implications for this and other amyloid diseases.
BIOPHYSICS

The understanding of life requires a detailed knowledge of the structure of biological macromolecules and how they interact with each other and with other molecules in the living cell. Our researchers exploit the widest possible array of biophysical methods to determine the specificity, rates, and equilibrium free energies of protein-protein, protein-nucleic acid, protein-lipid and protein-ligand interactions.

Techniques such as SPR, isothermal titration calorimetry (ITC) and fluorescence based assays (intensity and anisotropy) are used to elucidate binding constants, whilst structural methods including CD, ion mobility mass spectrometry and NMR reveal information about conformational changes during binding.

Single molecule methods, including fluorescence resonance energy transfer (FRET) and force spectroscopy are also being used to reveal new information about protein stability and force-induced conformational changes.

Combined with insights from molecular dynamics simulations and mathematical modelling, our aim is to use state-of-the-art biophysical methods to develop new approaches able to elucidate how macromolecular recognition is controlled within cells to the exquisite sensitivity required for healthy life.

NANOSCALE MODEL MEMBRANES

Membrane proteins are key components of biological membranes and are responsible for regulating chemical and ionic gradients, metabolite and nutrient transfer, and signal transduction between the external environment and the cell interior. Approximately 30% of the genes in the human genome code for membrane proteins and, consequently, many licensed medicines target such proteins. Structural and functional investigations of membrane proteins are, however, challenging due to difficulties in their purification and handling outside of their membranous environment.

Methods that permit the study and manipulation of membrane components in situ are thus of great interest. PhD student George Heath working with Dr Simon Connell and Professor Stephen Evans developed a new route for the formation of model lipid membranes on surfaces, using the tip of an Atomic Force Microscope as a stylus to “write” nanoscale patterns of lipid bilayers. The method, published in Nano Letters in 2014, shows that bilayer patterns with dimensions less than 10 nm can be produced. Using the Centre’s fast-scan AFM imaging facility, the team were then able to observe single proteins diffusing within these nano-patterned membrane corals.
This method opens the way to study the role of confinement within membranes on protein and small molecule diffusion. Further, observation of membrane remodelling at the stripe edges could lead to new insights into lipid ordering near pores and be of significance for the development of electro- and sono-poration techniques for drug delivery.
MOLECULAR INTERACTIONS IN CELLS

To complement analyses using biophysical approaches *in vitro*, researchers in the Astbury Centre also study molecular interactions in the context of the whole cell.

Protein-nucleic acid, protein-protein and protein-lipid interactions within cells are investigated using biochemical approaches such as immunoprecipitation, purification via protein tags and the application of proteomic technologies.

Within the Astbury Centre these methods are complemented by a range of state-of-the-art bio-imaging equipment to investigate the biology of cells at different resolutions. Populations of cells are interrogated by flow cytometry and cell sorting.

At an individual cell level, conventional light microscopy (e.g. confocal and deconvolution) studies can be complemented by super-resolution approaches (PALM/STORM and SiM) and electron microscopy (transmission, scanning and tomography). Correlative light and electron microscopy (CLEM) allows the integration of datasets derived from different imaging modalities to provide unique insights into the localisation of biological molecules within cells.

Finally, a confocal microscope situated in the Category III containment facility provides the opportunity to study cells infected with pathogens.

**Investigating the cell biology of important human viral infections**

Viruses are intracellular parasites whose pathogenicity is dependent on the interaction with susceptible cells. To persist they must change host cell physiology to create an environment favourable for viral survival. By stopping viruses from manipulating the host cell, we can impede viral survival providing an avenue for development of anti-viral drugs.
Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic herpesvirus associated with multiple human AIDS-related malignancies. Like other herpesviruses, KSHV has a biphasic life cycle and both the lytic and latent phases are required for tumorigenesis.

Dr Brian Jackson, a Wellcome Trust Research Fellow, together with Prof Adrian Whitehouse demonstrated that lytically active KSHV infected cells induce a DNA damage response due to DNA strand breaks. The genomic instability observed, which may be a common feature in herpesvirus infection, is a consequence of R-loop formation – RNA:DNA hybrids that are known to drive genome instability in some cancers.

This study, published in *PLoS Pathogens*, highlights how KSHV and other herpesviruses manipulate key DNA damage responses during infection and describes for the first time a novel mechanism of genome instability that may lead to KSHV-associated tumours. The study increases our understanding of the oncogenic potential of herpesvirus infections which will aid in the development of much needed anti-KSHV therapeutics.
The discovery of novel safe and effective therapeutics remains a difficult and arduous task. For example, it has been estimated that bringing a new drug to market costs over £1 billion, including the costs of failed campaigns. Partnerships between academia and industry are an increasingly important mechanism to facilitate drug discovery.

The Astbury Centre is involved with the Leeds Pharmaceutical and Biopharmaceutical Innovation Hub which brings together expertise from across the University to pioneer innovative approaches to drug discovery. The Hub works with pharmaceutical and biotechnology companies, and allied supporting industries, to help discover safe new medicines faster. The Hub also supports Leeds researchers to commercialise their research. Reflecting areas of research strength at Leeds which align with industrial need, the Hub has established four research themes:

- predicting and preventing protein aggregation;
- modulating protein-protein interactions;
- targeted molecular delivery;
- ion channel research.

Alongside these themes, drug discovery projects and platform technologies to facilitate therapeutics discovery are also supported.

The Hub facilitates translational research through a range of activities including linkage to national initiatives, industry-focussed workshops and networking events, implementation of impact activities, funding of proof-of-concept projects and assisting academics to identify the best route to exploit their intellectual property.

To remain focussed on sector needs, the Hub is guided by an Industrial Advisory Board comprising representatives from large and small pharmaceutical/biotechnology companies and allied industries. The Hub also works closely with related Innovation Hubs at Leeds (including Stratified Medicine and High Value Chemical Manufacturing) to deliver coordinated support for (bio)pharmaceutical research at Leeds.

More information can be found at: [http://pharmahub.leeds.ac.uk/](http://pharmahub.leeds.ac.uk/)

"The Astbury Centre has a strong track record of industry engagement and their expertise has delivered value to our R&D."

Dr Mike Waring,
Principal Scientist - Medicinal Chemistry, AstraZeneca
Case study:
In addition to their ability to interact specifically with their target, potential drug molecules must have physico-chemical properties that allow their successful development into a therapeutic. Many drug discovery campaigns begin with a high-throughput screening step to identify lead molecules. Controlling the molecular properties of the compounds screened can increase the probability of successful translation to a clinical product. However, it is difficult to source diverse screening compounds with appropriate molecular properties:

“We recently evaluated 4.6 million commercially available compounds, and less than 1% of these molecules had optimal lead-like properties.”

Dr Ian Churcher,
GlaxoSmithKline screening collection enhancement group.

To address this unmet need, Professor Adam Nelson and his collaborators have been supported by the EPSRC to develop new methods for preparing diverse lead-like molecules. The Pharmaceutical & Biopharmaceutical Innovation Hub has since supported the realisation of a plan to bring such molecules to the market.
An abundance of instrumentation that includes both well-established and emerging techniques lies at the heart of the Astbury Centre’s research successes. Each is run by dedicated and highly experienced facility managers who offer training to all users. This infrastructure is maintained at the cutting-edge by funding from a diverse range of agencies including charities, private benefactors, the University of Leeds, BBSRC, EPSRC, MRC and the Wellcome Trust. Recent highlights include investments totalling over £5m in high resolution imaging methods, macromolecular NMR and X-ray crystallography, the latter including a new generator, robotics and screening methods for high-throughput membrane protein structure determination. This adds to a strong research base in chemical, biological and biophysical methods that include:

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<th>Chemical Biology</th>
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<td>automated NMR and mass spectrometric analysis</td>
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<td>high throughput robotics and screening</td>
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<td>multimode UV and fluorescence plate reader</td>
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<th>Structural Biology</th>
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<td>electron microscopy / single particle microscopy &amp; tomography</td>
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<td>X-ray crystallography</td>
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<td>electrospray ionisation mass spectrometry and ion mobility mass spectrometry</td>
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<td>biological computing (molecular dynamics and bio-informatics)</td>
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<th>Bio-molecular Interactions</th>
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<td>surface plasmon resonance</td>
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<th>circular dichroism spectroscopy</th>
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<td>fluorescence spectroscopy</td>
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<td>Optim: high throughput, microvolume protein analysis</td>
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<th>Cell Biology</th>
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<td>electron microscopy</td>
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<td>super resolution imaging (PALM, STORM and iSIM)</td>
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<td>X-ray photo-electron spectroscopy</td>
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<th>Single Molecule and other Methods</th>
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<td>single molecule fluorescence methods (FCS, TIRF, ALEX)</td>
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<td>single molecule force methods</td>
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<td>stopped-flow circular dichroism and fluorescence</td>
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For further details please see [www.astbury.leeds.ac.uk/research/facilities.php](http://www.astbury.leeds.ac.uk/research/facilities.php)
More than just a mass
In addition to accurate mass measurements of single proteins, mass spectrometry can also characterise intact bio-molecular complexes to yield information on mass, stoichiometry, size/shape and stability in a single experiment.

Using the Synapt HD-MS in the Centre’s Mass Spectrometry facility, Professor Ashcroft’s group has developed methods to study protein folding in relation to disease-associated protein self-aggregation and functional complex assembly. The state-of-the-art instrumentation couples ion mobility spectrometry to mass spectrometry, enabling amyloid assembly to be followed over time: measuring consumption of the protein monomer and the advent and depletion of protein oligomers as the final fibrils develop. Recently, Lydia Young, a PhD student sponsored by the BBSRC and Waters UK (manufacturers of mass spectrometers), and Professors Alison Ashcroft and Sheena Radford, devised a screening assay for potential small molecule inhibitors of protein self-assembly. Work published in J Am. Chem Soc. in 2014 describes the different modes of interaction of two amyloid inhibitors with amylin, a protein associated with type II diabetes.

Virus capsids, with their regular, geometrical shapes, are also products of protein assembly. Monitoring coat protein self-assembly via oligomers into the intact capsid and measurement of protein dynamics provides new details about the assembly process. These methods have been applied to Hepatitis B virus, in a study performed by Dale Shepherd, a PhD student sponsored by the EPSRC, with Professors Nicola Stonehouse and David Rowlands, and published in Biophys. J. in 2013. More recently mass spectrometry has been used to study the assembly pathway of bunyaviruses, a major threat to both humans and livestock, by Dale Shepherd and Antonio Ariza in collaboration with Astbury colleagues Dr John Barr, Dr Thomas Edwards and Professor Nicola Stonehouse, which was published in Rapid Commun. Mass Spectrom, 2014.
Overview

The Astbury Centre plays a major role in training highly-motivated researchers to become skilled in interdisciplinary life sciences. Funding for PhD training is provided from a variety of sources: The Centre holds a highly prestigious Wellcome Trust 4-year PhD programme and staff also play a major role in the White Rose BBSRC Doctoral Training Programme. Other sources of funding include the EPSRC and MRC and a growing number of PhD places are funded in collaboration with industry and through other bio-medical charities.

The diversity of our science and the Centre itself is also reflected in the wide range of academic disciplines and nationalities of the students that are studying with us. Our PhD students have a small supervisory team to look after them and to help promote our ethos of multidisciplinary training. Students form an integrated cohort within the Centre through training days and other activities such as the Annual Research Retreat which all students are invited to attend.

Wellcome Trust 4-year PhD Programme

This programme is focussed on investigating “the molecular basis of biological mechanisms” and trains students in the wide range of techniques in cell biology, biological chemistry and biophysics that are needed to push back the frontiers in topical areas of medical relevance. An outstanding feature of this programme is the foundation year where students undertake three laboratory research rotations together with taught sessions and workshops. Together these provide excellent training in practical skills, allowing students to develop a deep understanding of modern techniques, their principles and applications. Students then undertake a full research project in years 2-4, selecting from the wide range of topics offered by the programme’s supervisors, and are able to tailor-make their project of choice.

BBSRC Doctoral Training Programme

The White Rose BBSRC PhD programme “mechanistic biology and its strategic application” is focussed on important biological questions within the BBSRC’s strategic themes of world-class bioscience, food security and bioenergy and industrial biotechnology.

Students undertake a four-year research project and are provided with bespoke cohort-wide training in core skills such as new technologies, mathematics, data-analysis and modelling. These generic skills are supplemented with practical training and skills modules in techniques specifically related to their PhD project. During their PhD, students undertake a 3 month ‘Professional Internship for PhD Students’ (PIPS) gaining experience of work in a professional environment and transferable skills, beneficial for their future careers.

Application deadlines vary, please see www.astbury.leeds.ac.uk/join/phd.php for more details.
LIFE IN THE
ASTBURY CENTRE

The Astbury Society
The Astbury Society, comprised of PhD students and postdoctoral members, plays a key role in encouraging cross-departmental collaborations by organising social events including an Annual Sports Day, the May Ball, team working events and seminars with guest speakers. The events of the Astbury Society are enjoyed by postgraduate students, postdoctoral researchers and academic staff alike.

“Meeting various people from different academic departments in a social environment allows for time to discuss aspects of my PhD and to get a range of ideas that might have not been brought to my attention otherwise.”

Paul Devine, PhD student

“Being part of the Astbury Society has certainly had a positive impact on my PhD, predominantly due to the interdisciplinary nature of the Centre. There are always people, from a range of scientific backgrounds, who will happily let you bounce ideas off them to help you along with your PhD.”

Claire Windle, PhD student
INDUSTRY FOCUS

www.astbury.leeds.ac.uk

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The staff list is up to date as of day of printing but is subject to change. Please check the Astbury Centre website for up to date information www.astbury.leeds.ac.uk/people/people.php
The Astbury Centre for Structural Molecular Biology was formally constituted as a University Interdisciplinary Research Centre in 1999. The Centre builds on the visions and achievements of the pioneers of modern biophysics and is named after W.T. (Bill) Astbury FRS, a biophysicist who laid many of the foundations of the field during a long research career at the University of Leeds (1928-1961). Astbury originally identified the two major recurring patterns of protein structure (alpha and beta), took the first X-ray fibre diffraction pictures of DNA (in 1938) and is widely credited with the definition of the field of molecular biology. Astbury’s work, in turn, followed on from the pioneering work of Sir William Bragg who was the Cavendish Professor of Physics in Leeds from 1908-1915.

We are at the dawn of a new era, the era of ‘molecular biology’ as I like to call it, and there is an urgency about the need for more intensive application of physics and chemistry, and specially of structure analysis, that is still not sufficiently appreciated.

Bill Astbury 1947

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The research undertaken by Astbury Centre investigators is described in greater detail in our annual reports. These accounts, first published in 2000, are available online: www.astbury.leeds.ac.uk/research/reports.php