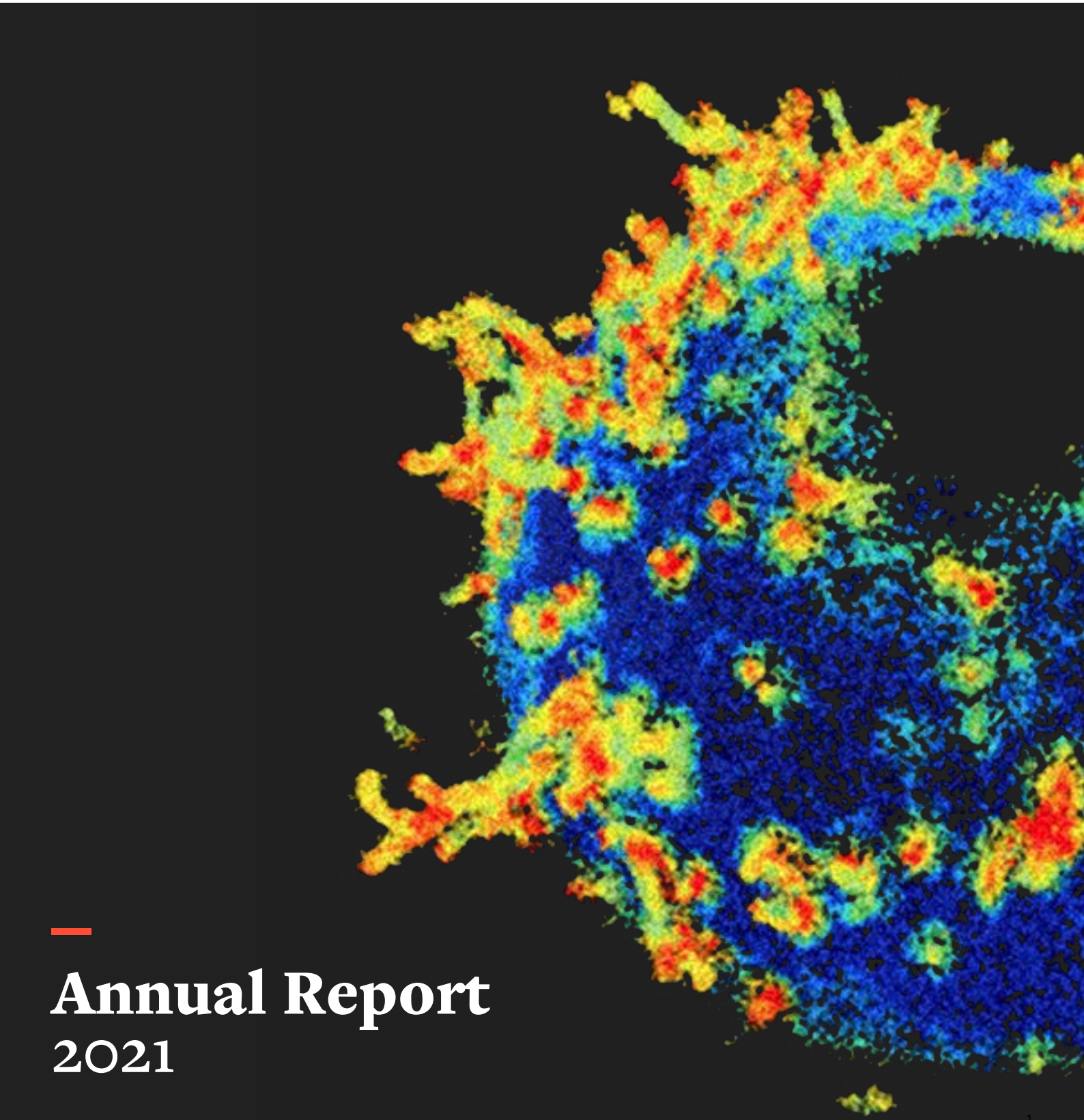
ASTBURY CENTRE

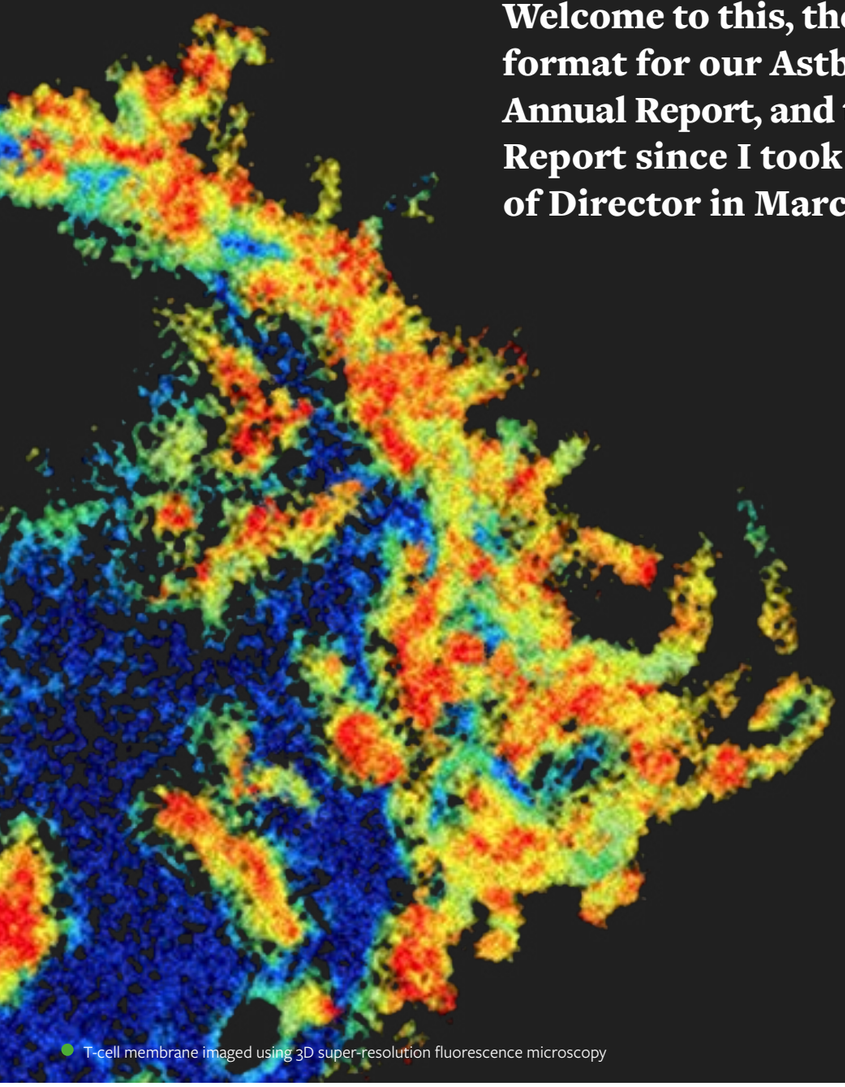
UNIVERSITY OF LEEDS



Annual Report
2021

Welcome from the Director

Professor Neil Ranson



T-cell membrane imaged using 3D super-resolution fluorescence microscopy

Welcome to this, the first of a new format for our Astbury Centre Annual Report, and the first Annual Report since I took up the post of Director in March 2021.

From left to right: Kate Langton (Astbury Research and Development Manager), Lucy Gray (Astbury Administrator), Adam Nelson (Astbury Deputy Director) and Neil Ranson (Astbury Director)

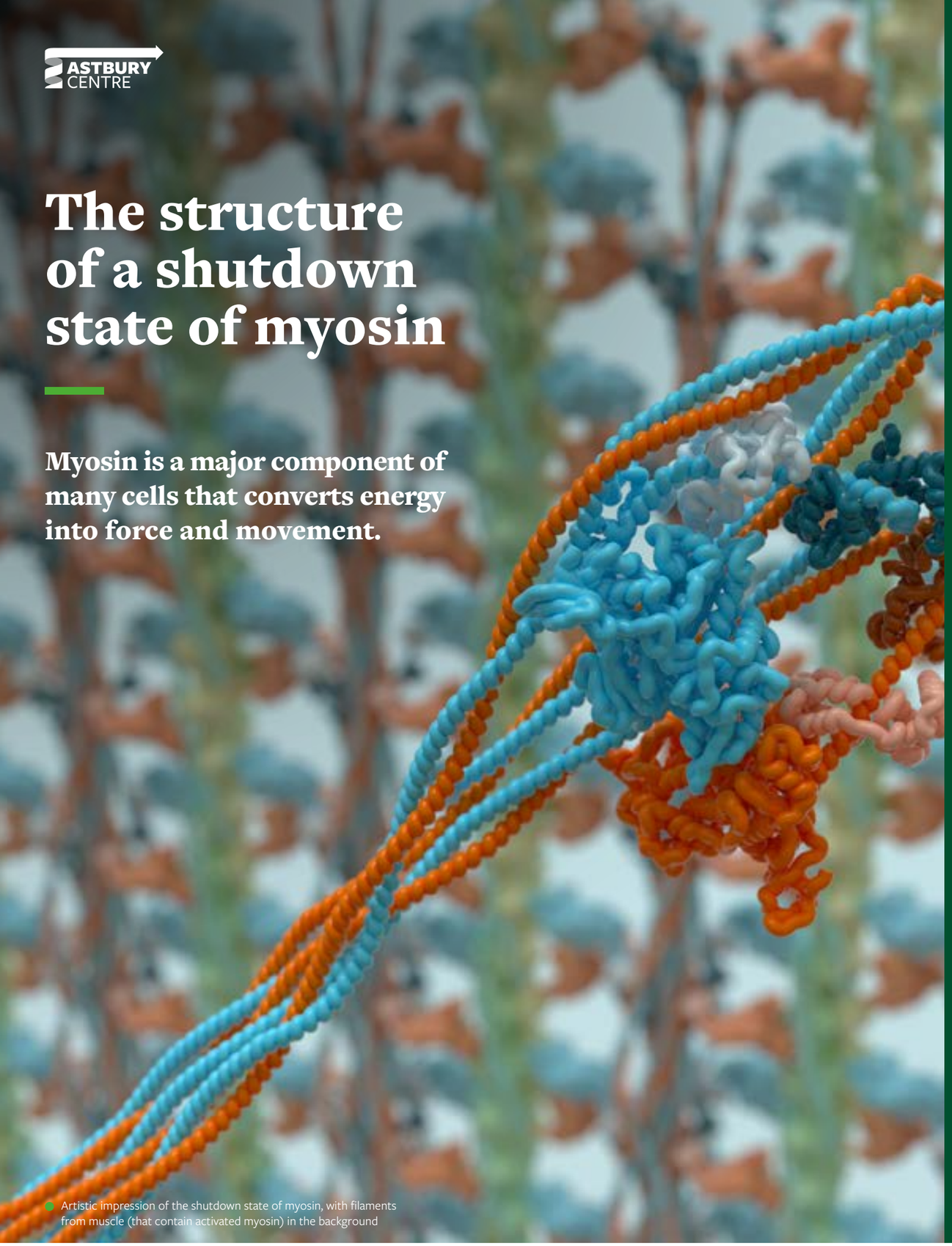
The Astbury Centre for Structural Molecular Biology is many different things. It is an interdisciplinary Centre that exists to promote research across disciplines, seeking to understand how molecules behave and how this behaviour impacts us in health and disease. The Centre brings together more than 400 researchers from biology, chemistry, medicine and physics, and we're all joined by a common passion to understand life in molecular detail. The Centre is also a vibrant, inclusive community where all members can tackle the toughest problems using interdisciplinary approaches 'without walls'.

It's impossible to talk about the last year without talking about COVID-19 and the huge impact it has had on every aspect of life in the Centre and beyond. We as a community have faced enormous challenges, from the fear and uncertainty many of us will have felt at times, through the rigours of home schooling, and sadly bereavement and loss. With this in mind, it's been a source of great pride (and huge pleasure!) to see the successes of Centre members in the face of such adversity this year. We have truly had a remarkable year with major successes in publishing and grant funding, and we hope you enjoy reading about some of these exciting stories in this report. These successes are a vindication of the Centre's culture and community that we've built together, and I'm confident that the Astbury Centre will go from strength to strength as we begin to return to the face-to-face networking and social activities that are a key feature of Astbury life.

I would like to personally thank everyone who has contributed to Astbury Centre life over the last couple of years. These thanks must start with our outgoing Director, Professor Sheena Radford, for her leadership of the Centre over the preceeding 9 years. Lucy Gray and Kate Langton help run the Centre and are at the heart of almost everything we do, working tirelessly to support our science. I'd also thank members of our Industrial Advisory Board, and the Executive Deans and Heads of School who support the Centre's work. Finally, I'd like to thank every Centre member at every career stage for making Astbury such a fantastic place to do our science.

The structure of a shutdown state of myosin

Myosin is a major component of many cells that converts energy into force and movement.



Artistic impression of the shutdown state of myosin, with filaments from muscle (that contain activated myosin) in the background

Team of Myosin researchers on their away day

Without myosin, muscles would not be able to contract, the heart would not beat, and cells would not be able to move.

In late 2020, researchers from the Astbury Centre solved the first 3-D structure of myosin that revealed the ingenious way in which myosin molecules shut themselves down to conserve energy when not involved in force generation.

Muscle myosin is found inside muscle fibres where it forms long filaments made up of hundreds of individual myosin molecules. When muscle activity ceases, these filaments are taken apart by the muscle cell, and individual myosin molecules are left in an inactive state.

Professor Michelle Peckham, who led the research said, *"We've known about the role of myosin in muscle contraction for decades, but how this 'switched off' state forms was unclear. The structure our team solved shows how the myosin folds up into a compact state that is inactive, and more easily transported through a crowded cell to where it's needed."*

Dr Charlie Scarff is now a British Heart Foundation Research Fellow in the Astbury Centre and said,

"I'm biased of course, but I think it's such a beautiful structure and shows so clearly how myosin activity is controlled. Myosin is just like a Brompton bicycle, kept in a folded state when not needed, and able to be quickly unfolded."

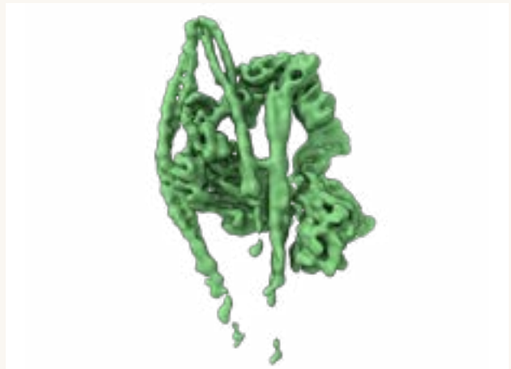
The structure of a shutdown state of myosin

The structure gives a direct insight into a number of human diseases. **Michelle** said,*"Mutations in muscle myosin cause many different muscle diseases. Our research helps explain how mutations or defects in the protein may be causing disease, and that opens the door to the possibility that we can develop new therapies that can help myosin to function normally."*

Charlie said,

"Solving the structure was a huge undertaking, and used nearly 100,000 individual views of the compact myosin molecule taken using a powerful electron microscope. I was lucky to work as part of a wonderful team, especially Glenn and David, but also with colleagues across the Astbury Centre and in the United States.

Everyone contributed something unique, based on their individual strengths. The work would not have been possible without them, and it is a great example of how we can achieve more together."



Three-dimensional reconstruction of shutdown myosin

Publications

The research was funded by the **Medical Research Council** and **Wellcome Trust,** and the work was published as **Scarff et al** (2020). Structure of the shutdown state of myosin-2. **Nature, 588, p515.**

Myosin is kept in folded, compact state – just like a Brompton bicycle – when not needed

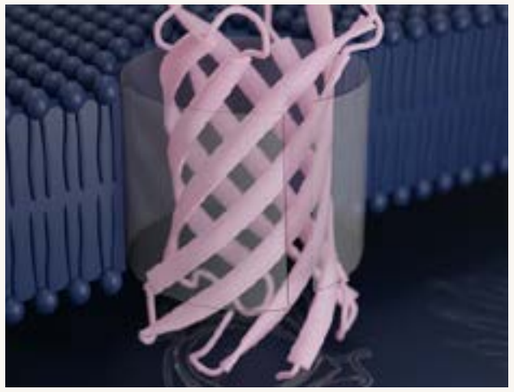
Designing new membrane proteins

Bacteria have thick, protective outer walls that are full of 'outer membrane proteins' or 'OMPs'. These act as gatekeepers in essential processes such as nutrient transport.

There is intense interest in designing artificial pores that can exploit the way nature moves "cargo" in and out of cells, for use in drug delivery or biotechnology. In 2021, scientists at Leeds were part of an international collaboration that designed such an artificial pore.

Professor Sheena Radford FRS FMedSci co-supervised the research that was conducted in the Astbury Centre. She said:

"These pores are fascinating molecular machines that sustain life, but re-engineering them to perform new functions is really challenging. There's been a lot of progress in designing bespoke proteins, but up until now it's not been possible to design a protein that would form the kind of β-barrel structure found in bacterial OMPs, so what's been achieved in this study is a major advance."

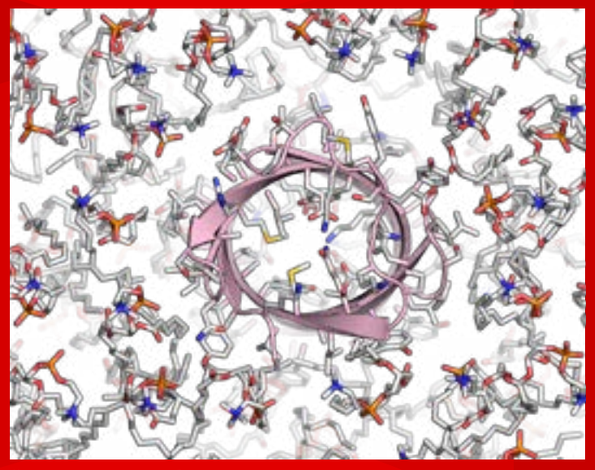


Artist's impression of an β-barrel protein embedded in a membrane

Professor Sheena Radford and Professor David Brockwell

The first author of the study was Dr Anastassia Vorobieva, from Professor David Baker's team at the Institute for Protein Design at the University of Washington School of Medicine, USA. They used a computer algorithm called 'Rosetta' to design new β-barrels comprising eight ribbon-like strands from scratch. The Astbury Centre's role in the research was to evaluate the designed protein and show that it did in fact fold into a barrel and embed itself into a membrane. The analysis showed the protein could fold up efficiently without the help of any accessory proteins. This is in marked contrast to how natural transmembrane β- barrels fold.

Designing new membrane proteins



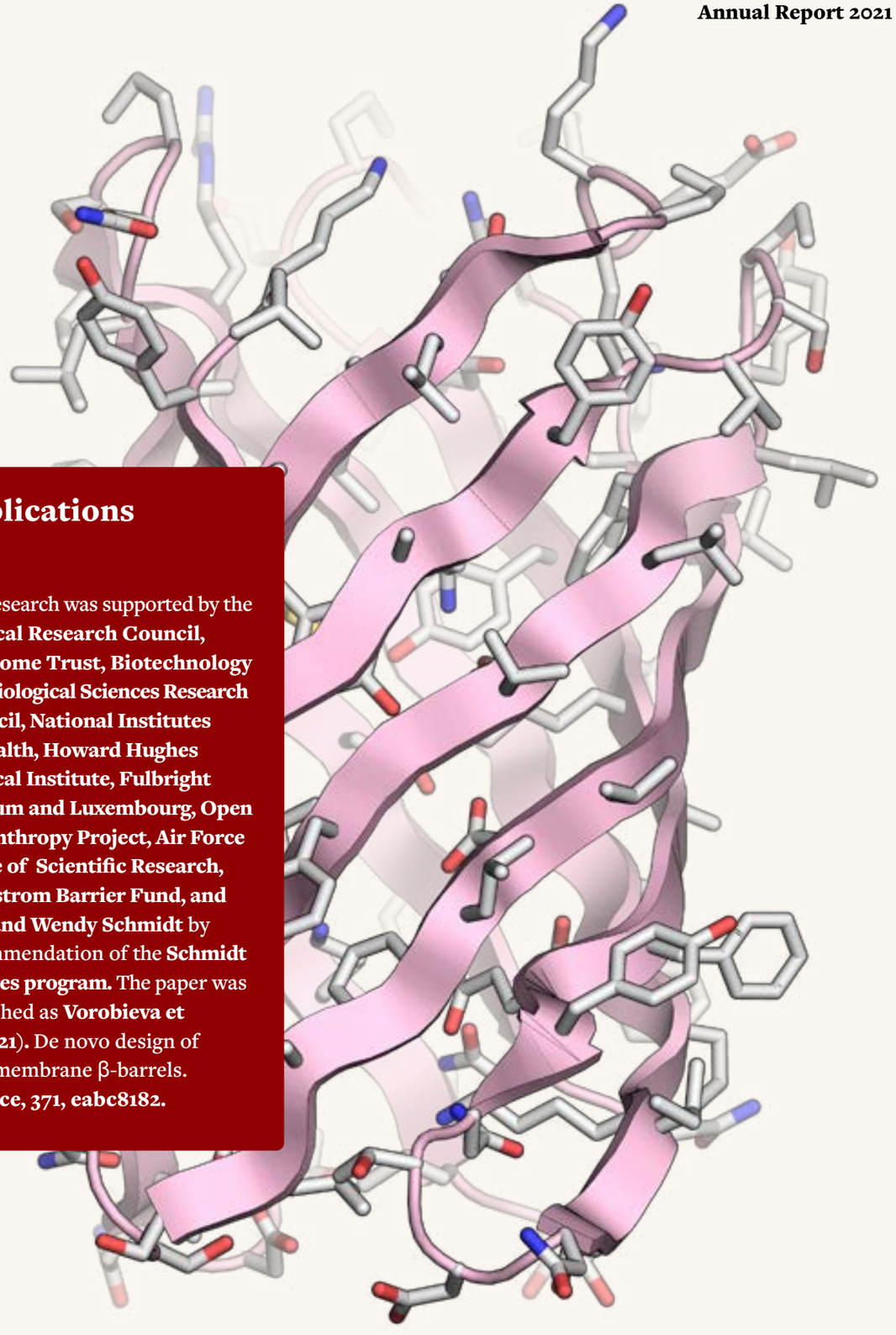
Model of a designed β-barrel protein embedded in a membrane

Professor David Brockwell was the other co-supervisor of the research in the Astbury Centre. David says,

"These designed proteins are fascinating because they have no evolutionary history. By studying them, we can discover some of the essential features that allow natural β-barrel proteins to fold into a membrane. If we can better understand how that happens we might be able to discover new ways to target bacterial infections."

Publications

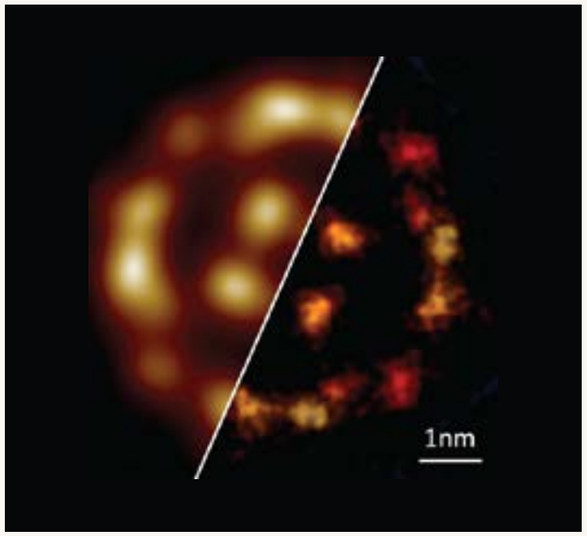
The research was supported by the **Medical Research Council, Wellcome Trust, Biotechnology and Biological Sciences Research Council, National Institutes of Health, Howard Hughes Medical Institute, Fulbright Belgium and Luxembourg, Open Philanthropy Project, Air Force Office of Scientific Research, Nordstrom Barrier Fund, and Eric and Wendy Schmidt** by recommendation of the **Schmidt Futures program.** The paper was published as **Vorobieva et al (2021).** De novo design of transmembrane β-barrels. **Science, 371, eabc8182.**



A designed OMP, with the β strands that make up the barrel in pink

High-speed microscopy in the Wolfson Imaging Facility

A collaboration between the Astbury Centre and the Bragg Centre for Materials Research is transforming our capabilities in high-speed imaging, with the establishment of the Wolfson Imaging Facility.



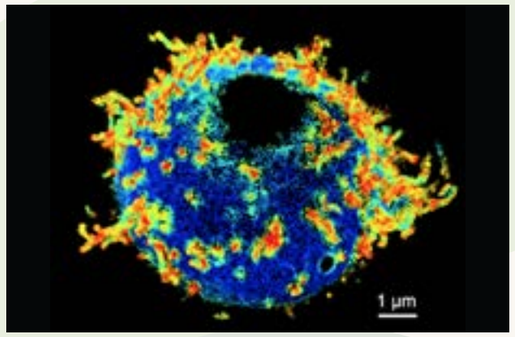
Surface structure of the membrane protein Aquarporin Z observed by Atomic Force Microscopy (left) and Localization Atomic Force Microscopy (right)

Dr George Heath and Dr Aleks Ponjavic

The facility is focused on the latest developments in high-resolution high-speed microscopy, and is housed in a new state-of-the-art lab in the University's new £100m Sir William Henry Bragg Building. The Wolfson facility is built around high-speed fluorescence and high-speed atomic force microscopy instrumentation being developed by the research teams of Dr George Heath and Dr Aleks Ponjavic, and will enable us to see complex and dynamic molecules performing functions and interacting at unprecedented time resolution.

Led by former Astbury Director Professor Sheena Radford, and Director of the Bragg Centre Professor Edmund Linfield, the facility will solve biological and medical challenges through the engineering and development of new microscopy methods. The ability to see how a particular molecule behaves in a cell, interacting with neighbouring structures, will give important indicators of whether a cell is healthy or a disease process is starting. Researchers will be able to watch how viruses attack cells, how immune cells get ready to attack their targets and how proteins carry out their functions. They will also be able see how these mechanisms go awry in diseases and how potential new drugs interact with their targets.

> ***"Methods to study molecules in atomic detail typically involve a static snapshot and averages of many molecules, but a new generation of high-speed atomic force microscopes can image individual molecules in action and in response to different triggers."***
>
> **Dr George Heath**
> School of Physics and Astronomy



T-cell membrane imaged using 3D super-resolution fluorescence microscopy

Our investment

Approximately **£2.6 million** of the investment was made by the **University of Leeds**, and **£750,000** by the **Wolfson Foundation** – an independent charity. A further donation was made by a former student, **Dr Chris Pointon** (Chemistry and Earth Sciences 1970).

Protein unfolding controls the structural and mechanical properties of protein networks

Hierarchical assemblies are essential to all living systems, demonstrating extraordinary mechanical strength and resilience, whilst also being able to change and adapt to environment changes.

Proteins are the building blocks of these assemblies, performing their function through structural and mechanical changes. Globular proteins have a specific and well-defined folded structure that responds to mechanical force. To study how protein unfolding translates to the structural and mechanical properties of a hierarchical assembly of proteins, Astbury researchers in Physics and Biology studied 3D networks of chemically cross-linked bovine serum albumin (BSA). The team used chemical "nanostaples" to provide molecular reinforcement of individual proteins, preventing the BSA protein from unfolding when experiencing force. Upon removal of these nanostaples, the BSA proteins could more easily be unfolded. Using a combination of structural techniques, including small angle neutron scattering and x-ray at the ISIS neutron and muon facility and Diamond Light Source, rheological studies at Leeds and computer modelling using an in house modelling platform called BioNet, the researchers were able to determine the impact of in situ protein unfolding in network formation.

Visualisation of the mesoscale structure of a cross-linked BSA protein network

Dr Matt Hughes and Professor Lorna Dougan

Matt Hughes was first author and winner of the VC Jordan/PR Radford prize for the best PhD in the Astbury Centre 2021, and he studied the mechanics of the networks, expecting the networks to get softer when the proteins could unfold. *"Actually we saw the opposite!"* he explains; *"the unfolded proteins form additional connections between clusters of folded proteins, highlighting the importance of the unfolded protein in defining the network mechanics."*

Professor **Lorna Dougan,** who led the EPSRC funded project said,

"Our vision is to uncover the rich complexity of protein unfolding across length scales. This will provide powerful new opportunities to design responsive biomaterials which exploit both the mechanical properties of proteins and their inherent biological functionality. This vision can only be realised with interdisciplinary collaboration and a multi scale experimental and modelling approach."

Publications

This work was funded by the EPSRC, and published in **ACS Nano,** as **Hughes et al (2021).** Control of Nanoscale In Situ Protein Unfolding Defines Network Architecture and Mechanics of Protein Hydrogels, **15, 11296.**

Targeting protein-protein interactions

Protein-protein interactions (PPIs) are involved in all cellular processes. Therefore, changes to these interactions can lead to the development of diseases such as cancer and neurodegenerative conditions (e.g. Alzheimer's).

Modulating PPIs therefore holds potential for treatment but identifying small-molecule inhibitors has been challenging. Researchers from Chemistry and Biology in the Astbury Centre, collaborating with AstraZeneca have established a new method to overcome this problem and enable targeting of PPIs.

Initially 'hot spot' residues (those amino acids that are most important for the interaction) from one of the proteins at the interface were computationally identified. Then a 'query' was generated which has the shape of one of the proteins found at the interface and bearing the hot-residues.

The query was then used to virtually screen a library of potential inhibitors to determine those with similar shape and recognition features to the query. Once candidates were identified, they were tested using a variety of biophysical techniques to show they interacted with the target protein and inhibited the PPI. Further rationale design was used to improve the potency of the initial candidates, demonstrating the approach to be viable for identification of starting points for drug discovery.

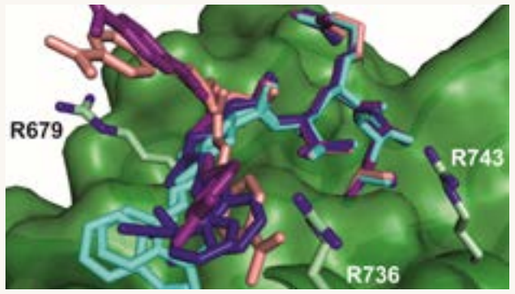
As examples, the team used the p53/hDM2 interaction, which is involved in the development and progression of some cancers, and the GKAP/SHANK1-PDZ interaction, which has a key role at the synaptic junction and is relevant to neurological disorders. Using the new method, the researchers were able to find inhibitors for both interactions, including the more difficult GKAP/SHANK1-PDZ interaction that is mediated by a β-strand.

Lead investigator **Prof Andy Wilson** stated

"This exciting proof-of-concept establishes an approach for the identification and prioritisation of small molecules that mimic diverse secondary structures to inhibit PPIs; it will help broaden the range of accessible drug discovery targets and could ultimately lead to new therapies for unmet medical need."

Publications

This work was funded by the **EPSRC** as part of the **PoPPI consortium** and was published as **Celis et al (2021)** Query-guided protein-protein interaction inhibitor discovery **Chemical Science 12, 4753-4762.**



Overlaid crystal structures of different peptide-fragment hybrids (tubes) bound to the SHANK1-PDZ domain (green)

The Astbury Community

The Astbury Centre places its members at the heart of everything we do, and we strive to develop a sense of community from which collaboration flows.

Astbury Centre members at the research retreat in 2019

To support research, we run an international seminar series, PI members' meetings, and an early career researcher forum. Crucially we run an annual research retreat where Centre members can focus on their science in an informal, fun atmosphere. The flagship of our scientific programme is the biennial Astbury Conversation – an international 2-day research symposium and public engagement event that is one of the highlights of the University year. This year Richard Henderson, winner of the Nobel prize for Chemistry in 2017 led a virtual Conversation as we recovered from Covid-19, but we plan to come back bigger and better than ever in 2024.

Beyond research, the Centre maintains a diverse social calendar led by the Astbury Society that fosters networking, innovation and participation, whilst giving back to the local community by fundraising for local charities.

The Astbury summer ball is the centrepiece of our social calendar, with its eighth iteration planned for Summer 2022 after a three-year hiatus when mass gatherings have been difficult. Previous years have seen resounding success, and this year we will be looking to build upon the £981.23 and £1223.91 raised for Leeds Children's charity at the 2018 and 2019 events respectively.

Astbury Christmas Quiz

Our famous Christmas quiz night endured the medium of Zoom in 2020, but made its in-person return in 2021 with a refreshed Astbury Society committee. For many, this event provided the first face-to-face socialisation on campus for 18 months, and gives us a fantastic platform to reintroduce in-person events to our calendar for 2022 and beyond.

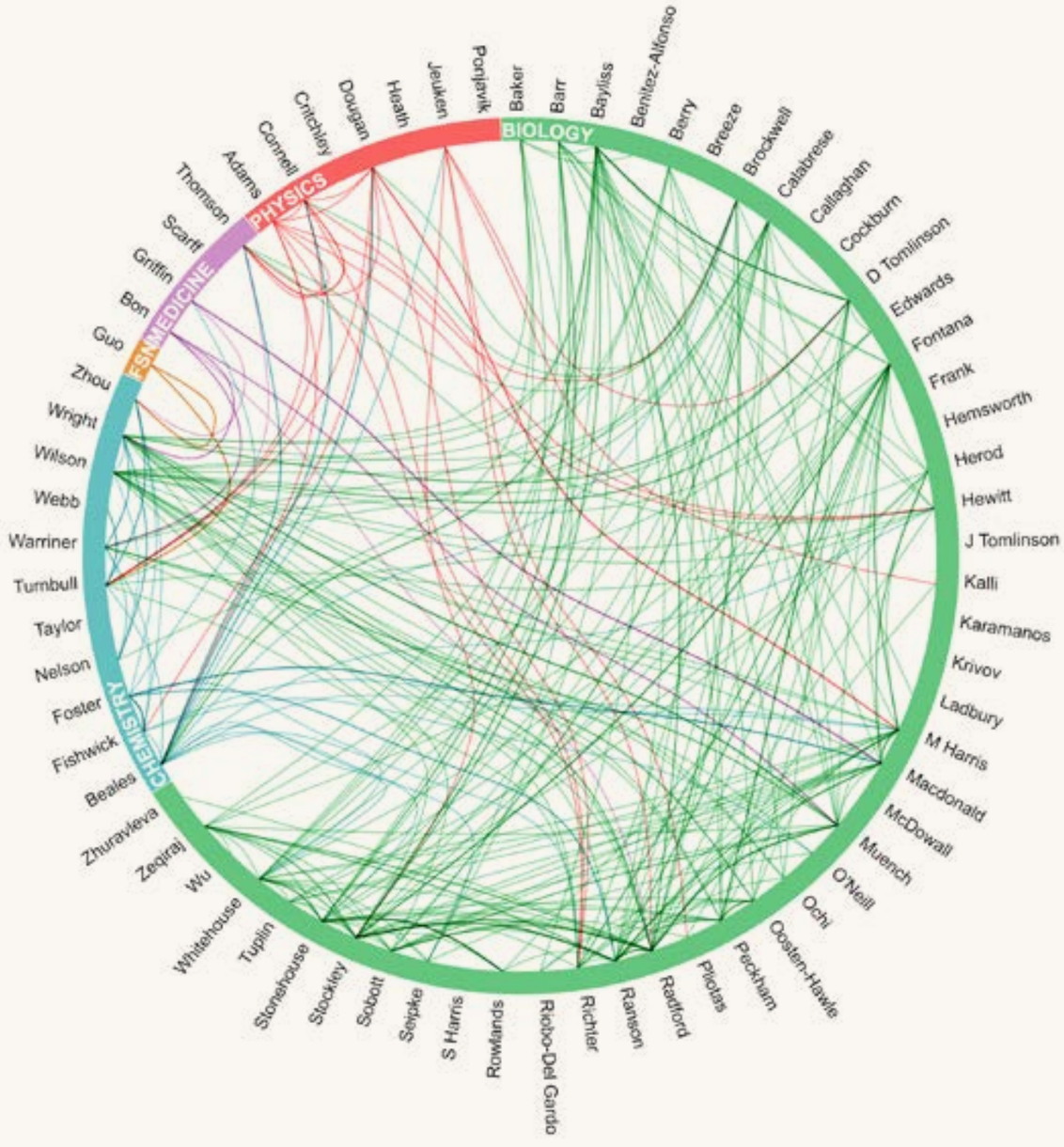
Other calendar highlights include our annual cheese and wine night, a summer barbeque and our sports day. Combined with the funds raised from the Astbury summer balls, the Society has to date raised a total of £6,600.00 for Leeds Children's charity.

Our funders

We rely on the generous support of a wide range of funders, who underpin every aspect of the Centre's research. Members of the Astbury Centre have £80.5M in live grant funding (with a total value of £125.4M to the University of Leeds).

Figure 1 captures just how interdisciplinary our science is, showing how Centre members secure funding together.

The illustration shows the aggregation of a human peptide hormone into an amyloid fibril. Image created by Phospho Animation

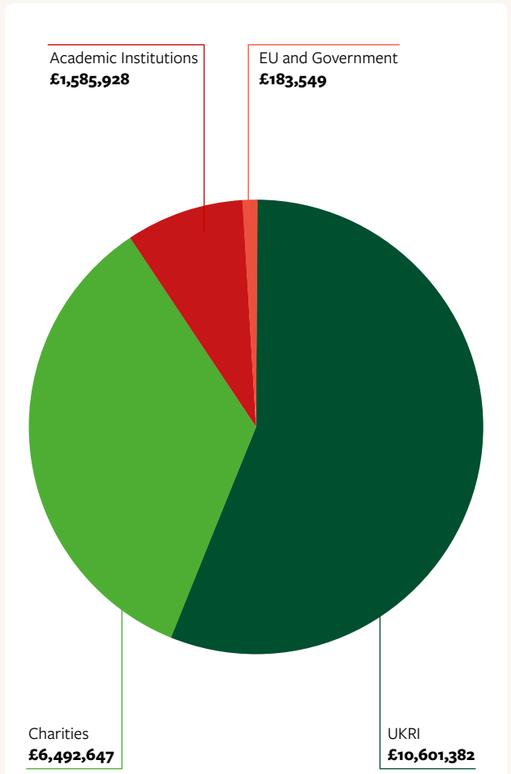


Network diagram showing the collaborative research funding held by Astbury Centre members (2017-2021)

Our funders

Grant successes in 2021.

In 2021, Centre members secured £18.9m of new awards:



Funding sources for new grants in 2021

Some major award highlights include:

Yoselin Benitez-Alfonso – UKRI Future Leaders Fellowship – PDWallMech: Harnessing PlasmoDesma Wall Mechanics for plant biotech and biomaterials – £1.55m

Anton Calabrese – Wellcome/Royal Society Sir Henry Dale Fellowship – Molecular Basis of Phase Separation in Viral Replication and Neurodegenerative Disease – £1.2m

Andy Wilson (PI), Richard Bayliss, Takashi Ochi, Sheena Radford, Darren Tomlinson, Roman Tuma, Megan Wright, and colleagues in Cambridge – BBSRC sLOLA – Deciphering the function of intrinsically disordered protein regions in a cellular context – £4.55m

René Frank – UKRI Future Leaders Fellowship – In situ architecture of Alzheimer's disease-associated pathology – £1.55m

Theo Karamanos – Wellcome/Royal Society Sir Henry Dale Fellowship – unpicking the specificity of the protein quality control network in health and disease – £ 1.382m

Michelle Peckham – Wellcome Investigator Award – Regulatory mechanisms in myosins - £1.3m

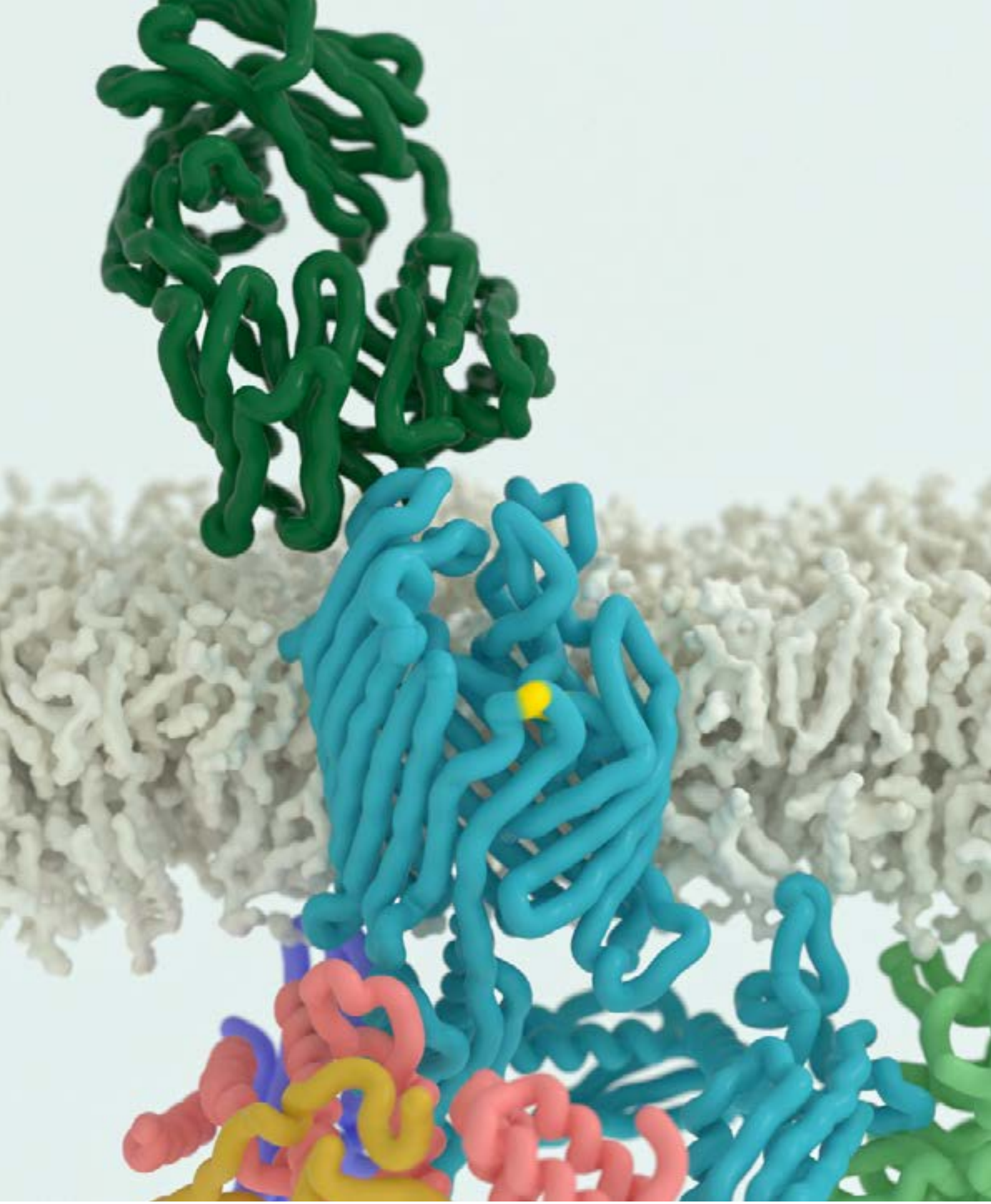
Elton Zeqiraj – Wellcome Senior Fellowship – Regulation of immune signalling via ubiquitin-mediated receptor degradation – £2.55m

Astbury members at a PI Away Day

Publications

The centre published **212** peer-reviewed publications in **2021** – with some of our most popular destinations including:

- ***Biophys J.*** 9
- ***Nature Communications*** 8
- ***Scientific Reports*** 7
- ***PNAS*** 6
- ***Chemical Science*** 5
- ***Communications Biology*** 5
- ***eLIFE*** 3
- ***Journal of General Virology*** 3
- ***Journal of the American Chemical Society*** 3
- ***Small*** 3
- ***Nature*** 2
- ***Science*** 2



The Astbury Centre for Structural Molecular Biology

Annual Report 2021

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