Impact Objectives

- Create a library of structurally diverse proteins, which can be studied from a microscopic and macroscopic viewpoint in different environments to obtain a comprehensive dataset
- Train PhD students in the biophysical and structural characterisation of proteins, thus creating a strong intersectoral network between academic and industrial sectors
- Ultimately improve healthcare due to more robust formulation development processes and at the same time improve the cost-effectiveness of biologics

Developing a library of proteins for drug research

The abundance of protein drugs on the market shows there is an increasing demand for knowledge within the formulation of biologics, as ***Professor Pernille Harris****, Principal Investigator and Coordinator of the* ***Protein-excipient Interactions and Protein-Protein Interactions in formulation (PIPPI)*** *project explains*

What are the key aims of the PIPPI project and what does it hope to achieve?

One intrinsic area of focus is training PhD students in the biophysical and structural characterisation of proteins in a pharmaceutical setting. They will create a strong intersectoral network between academic and industrial sectors. They will be key opinion leaders of the future. Scientifically, the aims are to create a representative library of structurally diverse proteins, and through a systematic study of their microscopic and macroscopic behaviour in different environments, we will obtain a comprehensive dataset. We will create a database enabling how to best predict protein stability on a minimum number of experiments and guide how to select the best experiments. We will bring biophysical understanding and characterisation tools for formulation of biologics to the next level.

Who will benefit from the research?

There will be widespread benefits across academia, industry and the wider public. Global academic research is likely to take a big step forward due to the unprecedented amount of coherent biophysical data that will be made available by the consortium. For industry, it will deliver the ability to recognise the potential values of biophysical techniques normally used only in academia. They will also gain an understanding of which of the currently used high throughput techniques add more value to the drug development process. For society, it will improve healthcare due to more robust formulation development processes, and at the same time, improve the cost-effectiveness of biologics. It will also allow the European Union (EU) to build on its strong pharmaceutical industry and take the lead in future drug development. Having the edge in knowledge on protein formulation will lower the risk of failure and increase the likelihood of better and more convenient dosage forms (for example, suitable for home treatment instead of hospitalisation). Overall, industry and academia in the EU will benefit from a large pool of highly skilled researchers and research groups.

Why do so few universities in Europe have formulation of biologics as a subject?

Except for a few examples, the use of biologics is a relatively new field within the medicinal industry. Traditionally, the big pharma industry is focused on small molecules, and teaching in academia, as well as public funding, are often driven by industrial opportunities. In society, it is recognised that protein drugs are more expensive and difficult to develop and less convenient for the patient, whereas it is not acknowledged that at the same time, biologics are often safer, with fewer side-effects and are much more precise in their mechanism of action and efficacious in treatment of diseases compared to small molecule-based drugs.

Who comprises the PIPPI consortium?

Partners in the PIPPI consortium have been selected to comprise a diverse set of scientific and industrial skills. Their selection is not based wholly on their strong CVs, but because of their high scientific level and innovative approach to scientific questions. This includes the industrial partners that are known to be excellent scientific collaborators with a serious commitment to training young researchers.

The academic partners include: Günther Peters and myself, Department of Chemistry, Technical University of Denmark (DTU); Robin Curtis, Alexander Golovanov, Alain Pluen, Jim Warwicker and Jeremy Derrick, University of Manchester, UK; Wolfgang Frieß and Gerhard Winter, Ludwig-Maximilians-Universität München, Germany; Mikael Lund, Lund University, Sweden; Åsmund Rinnan, University of Copenhagen, Denmark; and Marjolein Thunnissen, MAXIV Laboratory, Sweden. The industrial partners include: Shahid Uddin and Chris van der Walle, MedImmune; Werner Streicher and Allan Nørgaard, Novozymes; Dierk Roessner, Wyatt Technology Europe; Philipp Baaske, NanoTemper Technologies; Kishore Ravuri, F. Hoffmann-La Roche; and Jan Petersen, Danish National Metrology Institute.

Paving the way for protein-based drugs

There is a constant pressure to develop safer and more effective medicines, which has resulted in a shift in interest by the pharmaceutical industry towards protein-based drugs. To improve our understanding of these drugs, the ***PIPPI*** *research project will develop a representative library of structurally diverse proteins*

There is currently a great deal of interest in the pharmaceutical community around proteinaceous drugs. These are drugs where the active ingredient is a protein. Proteins are generally large complex molecules and when used as drugs, they are characterised by a higher specificity with fewer side-effects compared to drugs based on small molecules. A classic example of this is found in the treatment of Type 2 diabetes, where the small molecule drug metformin may be compared to the protein drug, insulin. Side-effects from the use of metformin outnumber and are much more severe than the side-effects from the use of insulin. Insulin also gives a substantial improvement in glycemic control, with a significantly improved benefit risk profile as a result. Protein drugs are already utilised for so-called replacement theory, where the drug is replacing a malfunctioning or missing protein in the body, such as insulin for the treatment of diabetes, human growth hormone for the treatment of poor growth, and blood clotting factors VII, VIII and IX for the treatment of haemophilia. Proteins such as monoclonal antibodies, interferons and interleukins are used for the treatment of autoimmune diseases and cancer immuno therapy, where the drug is up- or down-regulating biological processes, such as the immune system to assist the body in fighting the disease.

A LIVING SYSTEM

So how do proteinaceous drugs, or biologics, differ from conventional drugs? Primarily, a biologic is manufactured in a living system, such as a micro-organism or plant or animal cells. Most biologics are very large, complex molecules or mixtures of molecules. Many biologics are produced using recombinant DNA technology. In contrast, a small molecule drug is typically manufactured through chemical synthesis, which means it is made by combining specific chemical ingredients in an ordered process.

Small molecule drugs generally have well-defined chemical structures, and a product can usually be analysed to determine all its various components. By contrast, it is difficult, and sometimes impossible, to characterise a complex biologic by testing methods available in the laboratory, and some of the components of a finished biologic may be unknown. Therefore for biologics, the product is the process. Because the finished product cannot be fully characterised in the laboratory, manufacturers must ensure product consistency, quality and purity by ensuring the manufacturing process remains substantially the same over time. By contrast, a small molecule drug manufacturer can change the manufacturing process extensively and analyse the finished product to establish that it is the same as before the manufacturing change. The revolution of biotechnology has led to the creation of various types of therapeutic biologics with the potential to provide treatment for many chronic and malignant diseases. The potential advantages of biologics lay in their high specificity and potency combined with few side-effects. Unfortunately, their formulation remains a large challenge to pharmaceutical scientists.

CHARACTERISING BIOLOGICS

To overcome this difficulty in characterising biologics, scientists in the field of structural biology, biophysics, protein formulation and stability have formed the Protein-excipient Interactions and Protein-Protein Interactions in formulation (PIPPI) project, to use an interdisciplinary approach to systematically map physicochemical properties of biologics, formulation conditions and protein stability. The main objective of this consortium is to provide a new generation of innovative and entrepreneurial researchers who will develop methodologies, tools and databases to guide the robust formulation of biologics. 'Understanding the molecular mechanisms behind protein stability and solubility is difficult and complex, but it is not recognised as such,' Pernille Harris, Associate Professor in the Department of Chemistry at the Technical University of Denmark (DTU), and Principal Investigator of PIPPI explains. 'Therefore, little training and general understanding is available in the community. The combined efforts in the consortium will provide the necessary molecular understanding to guide the development of future biologics.

> *'In the PIPPI project we are investigating many proteins under many conditions using a diverse set of techniques'*

'Also, technologies in many non-pharmaceutical industries are based on protein science, and for a sustainable future, the world requires these up-coming technologies in fields such as bioethanol, biocatalysis, biopolymers and bioagriculture.' Harris explains that if the knowledge of how the formulation affects the protein structure and behaviour increases, then this fundamental understanding will guide the choice of formulations that are more likely to be correct on the first try. This will decrease the time it takes for the protein to reach the market and hence the patients. 'In the PIPPI project we are investigating many proteins under many conditions using a diverse set of techniques,' she continues. 'Therefore, our main challenge is to utilise all results coherently. We are getting interesting results and those results give rise to new interesting questions that cannot all be investigated in-depth within the time limit of the current grant.'

DEVELOPING PROTEIN LIBRARIES

During the project, researchers will select a protein library, which will be characterised systematically using current state-of-the-art excipients. On the microscopic level, the molecular interactions will be examined using advanced experimental and *in-silico* techniques. On the macroscopic level, the critical formulation properties such as shelf-life stability and phase behaviour will be assessed. 'All the results will be collected in a database that will contain comprehensive data of protein structure, self-interactions and interactions with state-of-the-art excipients, protein stability and behaviour,' Harris adds. 'The database will enable the consortium to use modern data treatment of large datasets to link the results from the different studies and provide novel and in-depth insight into important protein properties that govern protein stability. The consortium is highly-skilled and covers the required expertise and innovation power to catalyse this scientific field and set it in motion.'

The programme will collect a large, comprehensive, coherent dataset on several proteins, where all data will be publicly available in a database. 'We will treat the complete dataset using a Big Data approach with state-of-the-art multivariate analysis,' Harris continues. 'I am confident that we will make the first small, but crucial step towards a scientific revolution within this scientific field with an approach similar to the one seen with the large human genome projects.'

PARTNER PARTICIPATION

In addition, PIPPI will generate 15 highly skilled young researchers with a comprehensive knowledge of research in academia and in industry. They will have obtained training in protein formulation. Alongside DTU, there are five other partners – University of Manchester, UK; Ludwig-Maximilians-Universität München, Germany; Lund University, Sweden; University of Copenhagen, Denmark; and MAXIV Laboratory, Sweden – that form a strong academic partnership.

Alexander Golovanov of the University of Manchester explains that he was keen to participate in PIPPI because the consortium gives unprecedented access to a wide range of biopharmaceutically-relevant protein models, and brings together a wide range of techniques to look at the behaviour of these from different perspectives, collating all the information together and making sense of it all. 'If we recall an ancient parable of the blind men and an elephant, when several men were touching and exploring different parts of the same elephant, which they have never seen before, and later disagreeing with each other what the elephant is – in a way we are in the same position, looking at the same thing from different perspectives,' Golovanov explains. 'What, however, differentiates us from these old men is there are more of us exploring the same elephant, and more importantly, we do it systematically and talk to each other.'

Project Insights

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COLLABORATORS

Academic Partners

Günther Peters and Pernille Harris, Department of Chemistry, Technical University of Denmark (DTU) • Robin Curtis, Alexander Golovanov, Alain Pluen, Jim Warwicker and Jeremy Derrick, University of Manchester, UK • Wolfgang Frieß and Gerhard Winter, Ludwig-Maximilians-Universität München, Germany • Mikael Lund, Lund University, Sweden • Åsmund Rinnan, University of Copenhagen, Denmark • Marjolein Thunnissen, MAXIV Laboratory, Sweden

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CONTACT

Professor Pernille Harris
Principal Investigator

T: +45 45252024
E: ph@kemi.dtu.dk
W: www.pippi-itn.eu

PRINCIPAL INVESTIGATOR BIO

Associate Professor Pernille Harris has an interest in the fundamental relationships between protein structures and physical/chemical properties with an offset in X-ray techniques. In PIPPI her focus area is the study of solution structures of protein-protein complexes and investigations of protein-protein interactions, including studies of high-concentration samples where long-acting intermolecular interactions may be described. Apart from her role as Principal Investigator in PIPPI, Harris also possesses significant organisational skills, which are instrumental in successfully running a large international research network. At DTU Chemistry, Harris is part of the department management team, head of the study board and she is member of the Board of Danscatt (Danish Large Facility users).