**Proof of concept grants**

**Improved preservation of biologics by continuous intensified lyophilisation**

***Gary Montague, Teeside University; Lonza, Glythera, Accelyo***

This project proposes to perform experimental trials of model biologics using laboratory-scale continuous intensified lyophilisation; a technology that uses intensified controlled rate freezing followed by continuous sublimation. We will test two monoclonal antibodies and an antibody drug conjugate to investigate whether this improves activity, and provide data on throughput rates to quantify potential benefits.

**Investigating the effects of hydrodynamic force on the structure and biological integrity of a viral vector gene therapy product**

***David Brockwell, University of Leeds; Cobra Biologics***

We have developed and validated a device that generates a defined and controllable extensional hydrodynamic fluid flow field. This device can be used to identify ‘bioprocessible’ protein therapeutics and to optimize buffer conditions. Here we aim to find whether the device can also inform gene therapy viral vector development (flow parameters, buffer optimization, scaffold design) or as an analytical tool to differentiate between vectors with empty or full payloads.

**Top-down mass spectrometry methods for full characterisation of biopharmaceuticals**

***Perdita Barran, University of Manchester; Covance***

We aim to develop a new method for rapid analysis of intact mAbs. Our overarching aim is to characterize the primary sequence, to locate and analyse PTMs and to assess the three-dimensional structure and extent of aggregation of biopharmaceuticals as quickly as possible, with as little preparation. We will test a very new approach where direct mass analysis of native proteins is achieved directly from crude cell lysates, without any prior purification.

**Can archaeal minichromosome maintenance helicases enhance the performance of a
nanopore DNA sequencer?
*Michael Plevin, University of York, working with Oxford Nanopore Technologies***This project involves the engineering and production of novel protein nanopores. The collaborators will perform single molecule analyses of enzymes or proteins that transport chemicals or propagate signals across membranes to establish whether nanopore-based single molecule assays can be performed in parallel to generate more data more efficiently. This will provide an opportunity for new areas of research and development in protein biotechnology.

**Developing a tool kit for determining the manufacturability of new therapeutics in CHO cells
*Andrew Peden, University of Sheffield, working with UCB Pharma***Their project aims to identify bottlenecks in the production process of biologics by directly translating the understanding of basic biological processes into assays which will be useful for assessing the manufacturability of new drugs in mammalian cells. These novel and advanced assays will allow problems in the manufacturing process to be rapidly diagnosed and provide molecular clues to how the problems can be overcome.

**Analysis of host cell protein impurities using *in silico* approaches
*Lead applicant: Mark Wass, University of Kent*; *Centre for Process Innovation***There is no current resource that catalogues and records the occurrence and identification of host cell proteins and their interactions with process target proteins. Our work intends to develop a database of host cell protein data from several sources and investigate the use of bioinformatics methods to investigate the interactions of host cell proteins with biologics with the aim to understand these impurities and how to reduce them.

**Molecular imprinting for sustainable downstream processing of piopharmaceuticals
*Gyorgy Szekely, University of Manchester*; *FujiFilm Diosynth Biotechnologies***This project aims is to make paradigm shifts in the purification of biopharmaceuticals by replacing the tedious chromatographic steps currently employed with a single downstream operation unit. Molecularly imprinted membranes will be prepared with specific recognition sites for biopharmaceuticals. These sites will bind and protect certain parts of the crude biopharmaceutical whilst other parts are being transformedor removed.

**Hijacking intracellular storage bodies to create a novel mammalian cell-based expression system for the production of hard-to-express proteins.
*Marek Brzozowski, University of York***This project intends to develop a novel production system for difficult to express proteins. The key to our approach is to use an existing system of intracellular storage bodies, known as Weibel-Palade bodies that have a crucial role in the storage and controlled secretion of haemostatic proteins. Our intention is to show that we can hijack this already nature-tested packaging system and use it to form part of a novel protein expression system.

**Expanding production time of mammalian cell cultures for biotechnological applications
*Martin Michaelis, University of Kent*; *MedImmune***The aim of this proposal is to develop improved manufacturing processes for the production of biologics in mammalian cells. The concept is based on a compelling observation that cells infected with the human parasite Cryptosporidium survive about five times longer in the presence of the same amount of nutrients than non-infected cell cultures. Within this proposal, we will investigate the mechanisms underlying this enhanced longevity of Cryptosporidium-infected cell cultures.

**Bioreactor design space identification with product quality constraints
*Lead Cleo Kontoravdi, Imperial College London: MedImmune***Current production methods yield drugs that exist in a non-homogeneous mix of glycoforms, and different glycoforms interact with the immune system in different ways. Our goal is to develop a cost-effective technology to determine the manufacturing conditions under which the desired glycoform profile can be produced. To do this we will use mathematical modelling to describe the interactions between the manufacturing conditions and the product glycoform.

**Towards a cell-free expression system based on *Pichia pastoris***

***Karen Polizzi, Imperial College London; FujiFilm Diosynth Biotechnologies***

This project aims to demonstrate the feasibility of using the yeast Pichia pastoris for cell-free synthesis of proteins. Cell-free synthesis is fast, meaning proteins can be produced more rapidly, and allows the production of proteins which are toxic to living cells. It can also be scaled to large volumes

**A suite of web tools to predict protein solubility for the biopharmaceutical and biotechnology sectors**

***Jim Warwicker, University of Manchester***

This project aims to develop web tools to predict protein solubility and/or aggregation, which could help separate well-behaving and poorly-behaving biologics through the design process. The key aim of this proposal is to encourage measurement of solubility and aggregation behavior, and the development of benchmark datasets for computational methods.

**Gene expression accuracy as a parameter in bioprocessing applications**

**Tobias von der Haar, University of Kent; MRC Technology, Cobra Biologics**

The project will conduct a small-scale pilot study that establishes for certain whether or not loss of optimization and reduced protein synthesis accuracy are real problems that warrant further exploration. It will evaluate new ways of determining protein synthesis accuracy in eukaryotic cells, and will then apply them to a selection of model proteins.

**Business interaction vouchers**

**Comparing the productivity of three cell-free extracts based on industrial cell lines**

***Karen Polizzi, Imperial College London; Lonza***

It is unclear how much variability there is between cell-free systems produced from different cell lines. This project aims to compare cell-free systems made from three industrial cell lines to understand how much their protein production capabilities vary.

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**Scale up of vaccine production in a microalgal host for animal trials**

***Saul Purton, University College London; MicrosynbiotiX***

In this project, the chloroplast of Chlamydomonas reinhardtii has been engineered to express a vaccine against the major fish pathogen. Working with MicrosymbiotiX, we will examine the pilot scale production, harvesting and recovery of C. reinhardtii biomass containing the vaccine. The goal of the work is to produce sufficient dried algal material for formulation into fish-feed and use in challenge trials.

**Assessing the production of human cysteine knot hormones in plant cell cultures
Jose Gutierrez-Marcos, University of Warwick, working with Mologic**
This project aims to implement a new methodology designed to streamline the rapid and cost-effective production of human chorionic gonadotropin in plants for its use in oncology diagnostics. To this aim we will assess the impact of culture media in protein production, purification and quality, which at present are the major limiting factors of protein production in plant cell cultures.

**A machine learning poly-omics classifier to improve protein production in CHO cells
Claudio Angione, Teesside University; Centre for Process Innovation**
Our objective is to combine novel machine learning techniques with polyomic analysis, building a computational method that: (i) accurately identifies whether target cells have optimal conditions for producing the target protein; (ii) if not, predicts genetic modifications that will likely increase protein production.

**Fermentation optimisation of biotherapeutic production by E. coli ‘TatExpress’ strains**C***olin Robinson, University of Kent; UCB-Celltech***
We have developed E. coli strains that export a range of biotherapeutics to the periplasm
via the Tat protein export pathway. This project will test and optimise growth under
fermentation conditions to determine their true abilities and capacities using high-throughput ‘ambr’ micro-bioreactor systems to systematically optimise fed-batch fermentation regimes.

**Industry biotechnology for the production of hyaluronic acid from Streptococcus equi: understanding hyaluronic acid expression**
**Garry Blakely, University of Edinburgh; Hyaltech**
We wish to understand how the production of hyaluronic acid in Streptococcus equi changes during large-scale fermentation. To achieve this, we will study the genetic mechanisms that regulate expression in order to yield quantitative improvements in production.

**Exploring the feasibility of nonlinear acoustic detection technique for online bioprocess monitoring**
**Sourav Ghosh, Loughborough University; Centre for process innovation**
This project proposes to evaluate whether a nonlinear acoustic detection technique could offer a sensitive yet fast and reliable alternative to current industrial standard analytical methods (such as immunoassay, HPLC/UPLC).

**Supercritical fluid processing to improve the stability and delivery of low dose biopharmaceuticals**
**Helen Philippou, University of Leeds; Crystec**
Crystec has innovative supercritical fluid technology that can be used can process larger molecules to form room-temperature stable particles. This project will be used to evaluate whether supercritical fluid technology can be used on a micro-scale to successfully process low-dose biomolecules.

**A collaboration to engineer a novel protein nanopore for single molecule DNA sequencing applications**
**Michael Plevin, University of York; Oxford Nanopore Technologies**
Oxford Nanopore Technologies produce phone-sized DNA sequencer, in which DNA is sequenced as it passes through a motor protein-controlled nonopaore. We will determine whether recmoninant proteins native to heat-loving microbes can be engineered to improve the DNA reading capabilities of the sequencer.

**Developing a novel fluorescence-based biopharmaceutical quality control technology**
**Christopher Pudney, University of Bath; Bath ASU**
We will develop a novel technology to accurately perform quality control on biopharmaceuticals. The technology is based on the quantification of the fluorescence edge shift phenomenon giving a library of spectroscopic fingerprints for different biopharmaceuticals, which accurately quantifies subtle changes to protein structure.

**Predictive tools for folding-supportive sequence design spaces
Tobias von der Haar, University of Kent; UCB Celltech**
This project aims to test if it is possible to combine high yield and high activity during the production of cell-derived, protein-based pharmaceuticals. We will test whether tools to boost yield can be adapted to improve production of novel-format antibodies.

**Collaboration on a bio-process to optimize the yields and to characterize and test unique exopolysaccharides from two microalgal strains
Kevin Flynn, Swansea University; John Dodd, Algaecytes**
The aim of the project is to grow two microalgae strains in large volumes and concentrate the exopolysaccharides by the use of a novel reverse osmosis technique (a new bio-process). Samples will then be chemically characterized using FTIR/EDAX techniques. The exopolysaccharides will be screened empirically for bioactivity to seek commercial application of a biologic product.

**Enhancing cell growth to allow selection of biopharmaceutical-producer cell lines with favourable properties**
**Lisa Swanton,, University of Manchester; Alasdair Robertson, SAL Scientific**
We will test whether the addition of novel supplements that enhance cell growth/survival during cell line selection of stable cell lines (CHO S cells, transfected to express ‘difficult-to-express’ variants of erythropoietin) enhances the recovery of productive clones in greater numbers and with greater specific productivity of recombinant protein.

**Monitoring of host cell proteome expression during bioprocessing of CHO cells expressing recombinant proteins**
**Martin Michaelis, University of Kent; Michael Hutchins, TotalLab**
Here we will use 2D-PAGE to analyse the HCP proteome during culture of CHO cells, and potentially during early purification steps, and then apply new software developed by TotalLab to determine those HCPs present and their relative abundance. These data will provide the basis for risk-based assessment of the presence and relative concentration of specific HCPs to provide better control of HCP amounts and improved assurance of recombinant protein product quality.

**Design consultation and testing of a membrane photobiorector suitable for advanced biologic production from micro algae**
**Mike Allen, Plymouth Marine Laboratory, Joe McDonald, Varicon Aqua Solutions**
This project will develop a prototype membrane photobioreactor for high value biologic production from microalgae — bench top size; about 5l volume — and will assess biologic production capability using a reference algal system: a genetically modified strain of *Phaeodactylum tricornutum* expressing the venus yellow fluorescent protein.

**Evaluating the use of Raman Spectroscopy to determine topological isoforms of plasmid DNA**
**Lorna Ashton,, Lancaster University; Cobra biologics**
We will test if Raman spectroscopy can identify different plasmid DNA topologies, and if the methodology is able to provide quantitative data, compared with standard orthogonal analytical approaches, on the various plasmid isoform levels. A better knowledge of DNA topology will aid the formulation and in-process development of biological molecules.

**Production of therapeutic and industrial proteins in microalgae
Anil Day, University of Manchester; Protein Technologies**Modern manufacture of therapeutic and industrial proteins for specific applications requires a well characterized, reliable and safe host cell to manufacture proteins. In this project we will use microalgae, which provide an attractive new system, which is low-cost, safe and sustainable, to make proteins with applications in healthcare.

**Exploiting advanced electron microscopy to optimise protein and biologic expression platforms
Corinne Smith, University of Warwick; Jeol UK**We propose to work with Jeol U.K. to exploit advanced electron microscopy technology to image organisms such as E. coli in fine detail using zero-loss cryo-electron tomography and direct electron detection. This will provide ultra-high-resolution data on the effects of high-level recombinant protein production in terms of: (i) Distribution of any insoluble protein aggregates (ii) Effects on levels and distribution of protein export systems, (iii) Generalised effects on membrane structures.

**Development of a crossflow filtration dynamic flux control system to reduce cell harvest time
Yuhong Zhou, University College London; BioProControl Tech**Crossflow filtration is one of the key operations for cell harvest, particularly when cells are
shear sensitive such as infectious cells and cell therapy products. A dynamic control system that balances the fluxand the fouling over the entire operation has the potential to shorten the operating time. A dynamic control system combining model-based control and feedback control strategies is proposed. The feasibility will be tested in E. coli cell harvest.

**Rapid processing to recover high value microbial by-products
Paul Clegg and Joe Tavacoli, University of Edinburgh; Recyclatech**
The project aims to develop a rapid recovery system, using extensive expertise available at the University of Edinburgh, for these commodities by forming droplets that hold the bacteria and cell-associated biosurfactants. The bio-material can then be facilely removed by a skimming process. Better and/or more cost-effective ways to recover spent medium will benefit many bioprocessing applications.

**A pilot study to improve the expression of a Clostridium difficile toxin-based fragment in E.coliTarit Mukhopadhyay, University College London; Public Health England**
Clostridium difficile is a major cause of hospital and community-acquired infections. Public Health England is developing an antibody-based therapy that will neutralize these toxins and halt infection symptoms. To do this, toxin-derived antigens are required to produce ovine antibodies and in this project we seek to express these antigens in harmless strains of E. coli. This project will investigate antigen expression and manufacturing protocols for this new treatment.

**Evaluating enhancement of Secretion for Recombinant Proteins in CHO cells via overexpression of 7SL RNA
Bob White, University of York; Cobra Biologics**We will test if the overexpression of 7SL RNA can increase the amount of a therapeutically-relevant monoclonal antibody secreted by a stably-transfected cell line. We will also examine the intracellular trafficking of the antibody to better understand the secretion bottleneck.

**Initial development of novel product concepts with unique pharmacokinetic characteristics Randall Mrsny, University of Bath and Arecor**Arecor has developed technologies for stabilising therapeutic proteins. University of Bath has developed an in vitro technique for modelling interactions between a biopharmaceutical and hypodermis components that mimics SC injection events. This project will combine respective strengths of Arecor and Bath to generate proof-of concept data on selected therapeutic proteins, demonstrating the ability to produce a desirable pharmacokinetic profile.