Applications funded through BRIC 3rd call September 2008

Lyophilization of proteins - an in-situ study on structural changes and molecular interactions		
Professor Zhangfeng Cui	University of Oxford	£480559
The aim of the project is to identify and characterise protein secondary structure changes in each step of lyophilisation and link these to functional loss of proteins, which leads to guided approach to achieve better formulation. Experimental studies will be performed at well defined freezing and drying conditions using a commercial lyophilisation cryostage with exemplar proteins and chosen excipients. Focal Plane Array – Fourier Transform Infrared Spectroscopy (FPA-FTIR) and 3-dimensional spectral Multi-Photon Microscopy (MPM) are used in parallel for the in situ and in real time study on the structural changes of protein molecules and intermolecular interactions in protein formulations during each unit operation of the freeze drying process. FPA-FTIR characterises protein second structures and their changes in real time by analysis of the conformation sensitive Amid I band located between 1600 and 1700 1/cm. FPA technology permits the simultaneously measurements of 64x64 i.e. 4096 spectra in seconds. All 4096 spectra are spectroscopically evaluated, which means that the analysis can range from single-band intensity plots to mathematical approaches such as cluster analysis. MPM can reveal protein-protein interactions, protein aggregations, freeze concentration, the porous structure of the 'cake' and possible structural changes within three dimensional domain. Linking these spectral data at micro and nano scales to the outcome in an industrial freeze dryer will be made and validated. Attempts will be made to predict formulation outcome using heuristic and mechanistic modelling. The results will guide the selection of excipients and freeze drying protocols based on structural information of the proteins.		

An amphipathic reagent to extract, stabilize and purify proteins		
Dr Tim Dafforn	University of Birmingham	£416200
Biopharmaceutical products represent the largest growth area in the pharmaceutical sector. These products offer a high degree of efficacy and selectivity compared to many conventional small molecule drugs making their development a high priority. However, although biopharmaceutics offer many health benefits, their production is a significant technical challenge. This is the result of a number of factors, but perhaps two of the most important being the difficult of specifically releasing the product from the feedstock and the low stability of the product. This project aims to continue our pioneering development of a reagent that addresses both of these issues. The reagent, a substituted polystyrene (SPS) is a highly amphipathic entity that has a number of remarkable properties that make it an exceptionally suited to downstream processing of biopharmaceutics. The reagent disrupts lipid bilayers to form a nano-discoidal structure in which the lipid bilayer is stabilised by a "bracelet" of the SPS. The lipid-SPS assembly can also include membrane associated proteins allowing them to be stabilised in an entity that can be used in conventional chromatographic separations. The SPS can be easily removed from solution by a change is solution conditions leaving just the product solubilised in native membrane. In this project we will also demonstrate that SPS is multifunctional, being used to release products from the expression system and then acting as a stabilising agent for unstable products. The SPS has also been cleared by the FDA as a highly effective formulation and delivery system, allowing the potential to use a single reagent from cell disruption to administration. It therefore seems clear that, perhaps uniquely, SPSs can both improve product yield and purity of biopharmaceutic product as well as simplifying, and hence reducing the cost of the downstream process. This project aims to develop SPSs into a potent new entity for use in bioprocessing.		

Raman spectroscopy as a novel analytical bioprocessing tool for PAT		
Professor Royston Goodacre	The University of Manchester	£402612
This project will develop Raman spectroscopy with appropriate chemometric modelling as a novel analytical tool for high-throughput monitoring of biopharmaceutical processes. This will be employed throughout the whole production phase and in particular for in situ measurements in fermentation broths for assessing the levels of post-translational modifications to antibodies and non-antibody products (e.g., clotting factors), and to aid downstream product recovery by measuring the structure of purified proteins. During the course of the work the Raman spectroscopic approach will be benchmarked against purified antibodies		

and the same antibodies after modification (enzymatic deglycosylation and proteolytic degradation) as well as fermentation broths from mammalian cell lines producing antibodies. The later will also be performed using a tangential flow system to separate cells from fermentation broths thus allowing on-line analysis. Finally, we shall also develop Raman spectroscopy for the assessment of protein aggregation.

Developing scalable and standardised manufacturing methods for human pluripotent stem cells

Professor Chris Hewitt	Loughborough University	£376648
Professor Lorraine Young	University of Nottingham	£366643

Pluripotent hESCs are a major emerging platform for a wide range of therapeutic cell based products and pharmaceutical assays, however there are major barriers to their commercial-scale production. Our multidisciplinary collaboration will improve the understanding and reliability of cell expansion for pluripotent human embryonic stem (hESC) and induced pluripotency (hIPSC) cells by: 1) investigating properties of pluripotent cells that influence their processing and scale-up using our experience of multiple cell lines and culture conditions to scope generic process conditions 2) optimising and validating automated bioprocess protocols to enable robust and reproducible manufacture of hESC-based products at commercial scales. To maximise the range of manufacturing scales that are likely to be required for e.g. pharmaceutical screening processes or regenerative medicine applications, we will develop medium scale (entirely automated 90 X T175 flask T-flask culture in the CompacT SelecT) and larger scale (for potentially up to 1000L bioreactor) systems in parallel, using the same source of highly characterised cells. The processes that we deliver will have improved cost-effectiveness over current systems and will allow standardised culture protocols to be applied to multiple human pluripotent cell lines. Statistically-designed factorial experiments, underpinned by systematic process improvement, will identify the variables in manual culture methods that affect the practicality of scaled hESC manufacture. Factorial experimentation & quality optimisation (biological function, variation & cost) of the bioprocessed cell product will be achieved through gaining an understanding of all relevant variables through a unique collaboration between stem cell biologists and bioprocess/biomanufacturing engineers.

Non-invasive biophotonics tool for phenotypic identification of pluripotent stem cells and their progeny

Dr Ioan Notingher	University of Nottingham	£543484

While embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos, induced pluripotent stem cells are generated by viral transduction of somatic cells with four key transcription factors. For both pluripotent stem cell types, a large number of cell types can be derived following differentiation, including cardiomyocytes, osteoblasts, neurons, beta-cells, and haematopoietic cells.

However, the conditions to derive specific cell types remain suboptimal, reflecting the limited understanding of cell differentiation. Therefore current techniques generally produce only low yields of the desired differentiated lineages within a highly heterogeneous population of mainly unwanted cell types, which are not suitable for clinical applications.

In this project, we will develop a non-invasive tool based on Raman micro-spectroscopy for phenotypic discrimination of individual differentiated cells derived from pluripotent stem cells. Since the discrimination will be based on the intrinsic biochemical composition of the cells, the technique has the potential to be used for simultaneously identification of a large number of cell types without affecting their viability. Multivariate methods will be used for the analysis of the spectral markers to establish lineage-specific spectral markers. The quantification of these markers will allow time-course measurements on individual cells to follow the biochemical changes during their differentiation and response to various physiochemical stimuli.

The technology will provide on-line information regarding differentiation of pluripotent stem cells and assess their phenotypic characteristics. This will have a huge beneficial impact on refinement and standardisation of differentiation protocols and could help overcoming the current bottlenecks in the manufacturing and quality assessment of cell products, which are key factors for the future advancement and widespread clinical use of regenerative medicine therapies.

Exploitation of the Tat export machinery for protein production by bacteria

Professor Colin Robinson	University of Warwick	£346970
Dr Eli Keshavarz -Moore	University College London	£334424

The proposed research will generate a new platform for the production of recombinant biopharmaceuticals in bacteria. Many recombinant proteins are currently produced in bacteria, especially Escherichia coli, and a favoured approach is to target ('export') the protein of interest into the periplasmic space by the secretory (Sec) pathway. We propose to develop an entirely novel platform that exploits the unique abilities of the more recently-discovered Tat export pathway. This system exports fully folded proteins to the periplasm, thereby bypassing major technical problems associated with the Sec system's need to transport unfolded proteins. The project has been designed as follows:

Phase 1 will involve a detailed assessment of E. coli strains that already export proteins at moderate rates via the Tat system, together with an initial step-wise enhancement of export rates. The aims are to understand the physiological consequences of tat gene overexpression, and to enhance the export capacity of the cells through a combination of efficient Tat signal peptide and overexpression of tat genes and substrates.
Phase 2 will further improve export capacities through a systematic overexpression of cytoplasmic chaperones (essential for substrate folding), reduction of periplasmic proteases and manipulation of redox levels to support export of disulfide-bonded proteins. In parallel, each stepwise improvement will be assessed under industrial fermentation conditions to provide continual feedback of the effects on key physiological / process parameters.
Phase 3 will generate super-secreting strains using a combination of engineered characteristics identified in Phases 1 and 2, with the ultimate aim of achieving an optimised balance of high export flux vs minimised downstream processing complications. In this Phase we will also carry out a feasibility study in which we explore the potential of Tat-dependent export in the Gram-positive organism Bacillus subtilis.

BRIC 2008: Bioprocess Intensification by MicroCapillary Separations Systems

Professor Nigel Slater	University of Cambridge	£327494

Our plan is to implement high definition micro-channel separations within an extruded flat film, modular format using a novel microstructured material, a MicroCapillary Film (MCF), which has been invented in this laboratory by the co-applicant. An MCF typically contains 19 parallel capillaries, each of 410 micron diameter, within a flat polymer film (20 mm by 2 mm). Any number of capillaries can be incorporated into a film by simply changing the extruder die. MCFs can be made reproducibly with capillary diameters down to 100 microns and they can be fabricated from a range of low-cost polymer resins (PMMA, MBS, polystyrene blends) using a high-throughput extrusion process. They can be manufactured precisely, cheaply and in bulk.

MCFs can be fabricated into MicroFlow Devices (MFDs) that from established theory are expected to display high dynamic binding capacities at high flow rates. Residence time distributions for flows in the MFD are remarkably narrow, even at high throughput; the 8 cm spiral MFD displays a similar number of theoretical plates to a conventional 20 cm Sepharose packed column when operated with a flow velocity of 160 m/h, compared to 100 cm/h for the column. This provides the ideal plug-flow behaviour that is needed for sharp chromatography column breakthrough at high liquid flow rates. As a result of these desirable capacity and flow properties MFDs represent a clear alternative to polymer monolith and membrane chromatography systems for bio-separations.

This project will demonstrate the advantages of MFDs over conventional chromatography technologies. We will fabricate a cation-exchange functionalised MCF and use this for the purification of monoclonal antibodies.

Integrating upstream host cell line selection and development with improved downstream bioprocessing

Dr Mark Smales	University of Kent	£323538
Dr Daniel Bracewell	University College London	£365107

Over the last 20 years recombinant protein yields from in vitro cultured mammalian cells have at times exceeded 5 g/L, however there have been fewer major advances in the downstream bioprocessing (DSP) of proteins produced in this manner. In DSP, removal of host cell protein (HCP) is a major goal, however there have been few public reports focussed upon identifying the HCP complement from industrially relevant cells. This is surprising as the rational application/manipulation of DSP approaches would be enhanced by knowledge of the principal contaminants, whether these change/accumulate during fermentation/recovery, which HCPs specific steps remove

and, if specific types of product influence the HCP profile. Such insights would aid the design of novel/improved DSP approaches and inform upstream strategies for improved DSP. Here we will test the hypothesis that identification/characterization of the major CHO HCPs will allow the design of more efficient, or alternative, purification strategies and the rational selection and/or engineering of hosts to limit HCP levels. We will characterise the links between fermentation length and HCP accumulation in null and producer cell lines. Any interactions between HCPs and the target protein and the influence this has on product yield/ease of DSP, the fate of HCPs throughout DSP, and the effect removal of specific HCPs by RNAi has on DSP will be assessed. The direct outcomes will be (1) a CHO HCP profile for a model antibody and an understanding of how this changes/accumulates during fermentation, (2) knowledge as to whether the target protein changes the HCP profile and the ease with which these contaminants are removed, (3) an understanding of the HCPs removed throughout the template DSP, (4) methods of monitoring/measuring HCPs, (5) determination of the effects of eliminating specific HCPs on cell phenotype and subsequent DSP, and (6) the design of alternative processes to remove HCPs via either up- or down-stream approaches.

A novel characterisation and separation technique for pluripotent human embryonic and hematopoeitic stem cells

Dr Nicholas Willoughby	Heriot-Watt University	£310945
Dr Paul De Sousa	University of Edinburgh	£104711

This proposal intends to develop a scalable separation/purification technique for human stem cells capable of non-invasive and reversible processing of cells that will supercede current methods based on flow cytometry, centrifugation or magnetic separation. By using Atomic Force Microscopy with charged tips to generate charge maps the research will identify surface electrical charge differences associated with specific human stem cell populations, namely embryonic and adult haematopoietic (CD34+) stem cells. This information will be used to computationally model complimentary interactive substrates providing reversible affinity. Small-scale experimental work will be used to evaluate substrate prototypes for their capacity to enrich for stem cell populations without altering their viability or pluripotency, assessed using a range of standardised in vitro assays for cell molecular marker expression, and differentiation potential. By focusing on both charge and topography of surfaces the work will produce an adsorption-based separation technology more specifically suited for cell purification than current chromatographic techniques. This novel technology should be scalable to processing larger quantities of cells (10 E9-10) than current methods, thus making it a commercially useful solid-phase separation technique.

Total £4,699,342