

## APPLICATIONS FUNDED THROUGH BRIC 2<sup>nd</sup> CALL JULY 2007

<b>Identifying and overcoming protein secretion bottlenecks in yeast and filamentous fungal cell factories</b>		
Professor David Archer	University of Nottingham	£362590
Professor Stephen Oliver	The University of Manchester	£422247
<p>The yeasts <i>Saccharomyces cerevisiae</i> and <i>Pichia pastoris</i> are the main experimental organisms in this study. Both species are used as cell factories (in the laboratory and commercially) for secreted protein production. <i>S. cerevisiae</i> is also an excellent model organism for investigations into the basic events involved in protein secretion and stress responses. <i>P. pastoris</i> is less amenable to basic studies but is an outstanding cell factory. This project will create some tools for basic studies in <i>P. pastoris</i> as well as using both organisms for comparative studies with each other and with the principal filamentous fungal cell factory, <i>Aspergillus niger</i>. We will examine the secreted expression of lysozyme and derived variant molecules, as well as scFv antibody proteins. Many of the necessary strains are already available although some will be constructed within the project. Controlled and reproducible cell culture is a necessary part of the studies proposed. The main technical objectives and main methods to be used in the study are: 1. Use transcriptomics and proteomics to examine the stress responses due to expression of variant lysozymes in <i>S. cerevisiae</i> and <i>P. pastoris</i>. 2. Examine the fates of selected lysozyme variants, including the folded states, using imaging, conformational antibody approaches and protein turnover studies. 3. Express and examine resulting secreted protein yields and stress responses from scFv proteins, measure thermal stability of purified scFvs, and compare with lysozymes. 4. Use comparative genomics methods to compare stress responses from <i>S. cerevisiae</i>, <i>P. pastoris</i> and <i>Aspergillus niger</i> to find commonality and differences. 5. Define and test a strategy for rational strain improvement for optimized secretion of scFvs based on stress response and protein fate studies.</p>		

<b>Combined /omics approaches to understand and control library enriched microbial cell factories</b>		
Professor Phillip Wright	University of Sheffield	£297941
<p>This project aims to apply a genome-wide, multiscale approach for functional genomics to improve the production of recombinant proteins in <i>Escherichia coli</i>, and to take this approach further to begin to understand how to improve the production of glycosylated proteins. We will integrate data obtained from DNA microarray inverse metabolic engineering tools such as SCALEs (multi-Scale Analysis of Library Enrichment), with that obtained from high throughput quantitative shotgun proteomics (building on 8-plex isobaric mass tag technology - iTRAQ) methods as an addition, as proteomics is a level closer to the functional understanding of a phenotype. We will analysis the data using a multivariate approach. We then will seek to move beyond simple statement of whether the transcriptomic and proteomic data are concordant or discordant, but rather how these then can be interpreted in the context of biological pathways. In particular those related to recombinant protein synthesis of the model glycoprotein. Implementation of /omic based tools and the resulting data is necessary to provide a systems level understanding of an organism so that a deeper functional understanding results in bioprocess engineers being able to take advantage of findings in the biosciences, and translate these to valuable processes and products for UK bioprocessing businesses. We seek to ultimately improve the production of glycosylated recombinant proteins such as the N-glycoprotein AcrA, in <i>E. coli</i> here as an exemplar project. This protein has been demonstrated as being possible to produce in <i>E. coli</i>, following the transfer of the N-glycosylation system from <i>Campylobacter jejuni</i> into <i>E.coli</i> cells.</p>		

<b>Pichia pastoris protein secretion: analysis of constraints, optimisation and methods development</b>		
Dr David Leak	Imperial College London	£724360
<p>The methylotrophic yeast <i>Pichia pastoris</i> is an established expression platform for secreted and membrane proteins and is being modified to "humanise" its glycosylation pathway. However, a number of secreted proteins do not express well in this host, partly because of inefficient trafficking through the ER, which leads to the induction of the unfolded protein response (UPR). Although the initial UPR expression of chaperones may be beneficial, it can ultimately result in reduced secretion, proteolysis and increased product heterogeneity. In this project we will undertake a global transcriptome and metabolic profile analysis of the UPR in <i>P. pastoris</i>, (initially on chemostat cultures then validated in a typical fed batch regime) and use the information gained to evaluate different potential reporters for the UPR including GFP, and metabolic fingerprinting. The optimum reporter system, based on factors such as responsiveness (correlated with the protein induction profile) and sensitivity (this may depend on the scale and type of culture) will be used to explore the potential for development of an on-line UPR monitoring and control system as well as for screening of constructs on a small scale. Applications of the reporter linked to moderate/ high throughput screening will also be investigated, with the aim of devising a strategy to screen large numbers of variants to select for those with improved secretion. Even when there is no evidence of induction of UPR, the specific productivity of secreted protein production is moderate, and nothing is known about what limits productivity. Therefore, we intend to explore the physiological status of highly secreting cells using combined transcriptomic, metabolomic and flux analysis of a construct with good secretion. This should indicate whether productivity limits are due to the secretion apparatus or biosynthetic capacity. In principle, a similar surrogate reporter approach may be used to indicate secretion saturation.</p>		

<b>Multifunctional Chromatography materials for improved downstream processing</b>		
Professor Owen Thomas	University of Birmingham	£595931
<p>The manufacture of many of today's biopharmaceuticals already stretches technical/economic acceptability to breaking point, and the move towards ever more sophisticated biologics and therapies is expected to compound these issues yet further. The explosion in new high-level expression systems for the production of recombinant proteins has reduced upstream processing costs to the point where concentration and purification operations, i.e. downstream processing (DSP), now dominates the overall manufacturing cost for many protein therapeutics. The success of future medicines, especially those characterized by very large physical size and referred to as nanoplexes, will to a great extent hang on our ability to introduce radical and prompt changes to current biomanufacturing thinking and practice. In light of the above, and given the dominant role that chromatography has played over the past forty years and is no doubt expected to play long into the future, shouldn't we now expect much more from 'next generation' chromatography matrices? The objective of this project proposal, which targets 'Improved Downstream Processing' of the BRIC initiative is to advance new 'multifunctional' chromatography materials that enable efficient separation of future nanoplex bioproducts from smaller, but chemically very similar 'problem' contaminants in a 'one column-one bead' process that combines size exclusion with ion exchange principles. The above responds expressly to the identified challenges of improved downstream processing, as well as to areas the BIG-T report considers vitally important, i.e. novel manufacturing and bioseparation technologies.</p>		

<b>Protein nucleation and crystallisation on novel 3-D templates</b>		
Dr Daryl Williams	Imperial College London	£384817
<p>The direct crystallisation of proteins from fermentation broths is an industrially attractive route for protein manufacture. This proposal describes an integrated and innovative research programme for the improved understanding of the effects of both surface chemistry and topography on heterogeneous protein nucleation and crystallisation via the use of novel templates. The main objectives of this study include: 1. The use of specific surface chemistry in combination with precise surface topographical features to allow novel surface templates to be created. 2. Use of these novel templates for protein crystallisation studies 3. An improved understanding of protein nucleation via novel detection methods. 4. Improved protein crystallisation fundamentals to enable the control and optimisation of bioprocessing. The methodologies to be employed include: 1. Sub 100 nm surface topographies will be templated onto surfaces by a PDMS stamping technique and via colloidal particle arrays. Other features will be fabricated via an anodisation approach. 2. A wide range of controllable surface chemistry's to be controlled via an established method; the self-assembled monolayers (SAMS). Surface characterisation of the templates will include wettability, FTIR, zeta potential, SEM, AFM, TEM. 3. A Quartz Crystal Microbalance capable of detecting depositions of nanogram levels protein onto the surfaces will monitor crystal nucleation, as well as measuring the viscoelastic properties of the protein layer. 4. The protein structure, morphology, habit and purity will be characterised for the crystals obtained. Our hypothesis is that these novel protein crystallisation templates will be superior to current nucleation media and methodologies. Coupled with an improved understanding of the fundamentals of protein nucleation and crystallization, these templates could directly, or indirectly, facilitate direct crystallisation in the reactor broth.</p>		

<b>Delta3D; Bench top assays for the rapid detection of protein 3D structural changes</b>		
Professor Jeremy Lakey	Newcastle University	£363525
<p>Proteins and complex biologicals (such as viral particles) are a significant growth area in pharmaceuticals and now account for 30% of the drug pipeline and 10% of sales. Biologics present an important extra variable compared to the small molecule therapeutics that once dominated the market and that is a complex non-covalent 3D structure. Changes to this are not revealed by normal analytical processes but can adversely affect solubility, stability and function. In recent years industry has adopted a series of biophysical techniques to measure the 3D structural integrity of proteins. These include fluorescence, circular dichroism spectroscopy, analytical ultracentrifugation, NMR, X-ray crystallography, light scattering and gel permeation chromatography. Whilst powerful, these methods are expensive, require specialist analytical knowledge and often require large amounts of protein. We are avid users of biophysical methods but also wish that non-specialists may be able to detect changes to the soft 3D structure of a known protein. The methods need not define the exact alteration as this can be done with the existing methods once the problem has been identified. Thus we hope to improve the early detection of structural changes or structural heterogeneity in samples. Furthermore, we hope to extend the analysis from the pure protein stage towards the fermentation and formulation stages. The methods which include spectroscopy of protein probe complexes, small scale hydrophobic interaction chromatography, cross-linking and limited proteolysis are not new but their design for robust generic analysis of protein structure by non-specialists has not been realised. We hope to develop the foundations for some commercialisable kits which will become commonly used in industry and academia.</p>		

<b>New approaches to high throughput protein, isolation, purification and concentration</b>		
Dr Barry Moore	University of Strathclyde	£392967
<p>This project will investigate non-chromatographic methods for purifying proteins based on selective coprecipitation of the target in the form of protein coated microcrystals (PCMC). The resultant precipitated PCMC particles consist of protein immobilised on the surface of a crystalline excipient carrier and are stable for long-term storage. The technique is expected to be particularly useful for isolation of complex protein assemblies not well suited to chromatography. We will investigate how coprecipitation compositions can be tuned to maximise selectivity and stability via changes to parameters such as excipient, solvent, pH and ionic strength. The scale-up potential of the process will be evaluated.</p>		

**Total £3,544,382**