Priority Research Challenges

Biopharmaceuticals have come a long way since the first biological product, recombinant human insulin, was launched onto the market in 1982. By mid 2003 there were 148 biological products licensed in the USA and/or EU, and over one third of all products now in development are biopharmaceuticals.

This trend is set to continue and over the next five years the number of licensed biopharmaceuticals is expected to grow at around 20 % per year compared with 7 to 8% for the overall market. Estimated large-scale capacity demand for proteins was in excess of 2 metric tons in 2004, and is growing rapidly.

This growing demand for biopharmaceuticals stems from the demonstrable ability of biologicals to address unmet medical needs and there is consequently an increasing requirement to get new therapies to the clinic and market as quickly as possible. However this demand has also increased the need to address certain research challenges to ensure that new biopharmaceuticals do not languish in development and their therapeutic potential is realised. There are three principal drivers:

- Biological products are large and complex molecules which require equally complex manufacturing methods and a battery of analytical techniques. The development phase is therefore slow, expensive and complicated, frequently leading to a bottleneck in getting new products to the clinic. Since speed to clinic is vital, there is a need for new tools and methods which will contribute to accelerating development.
- The increasing demand for high volume products such as monoclonal antibodies is driving the need to improve cost efficiency. An improved understanding of the molecular and cellular processes which influence productivity is therefore increasingly important to improve bioprocessing efficiency.
- The complexity of biomolecules also presents a challenge in terms of understanding and controlling the effect of process conditions on product structure and heterogeneity.

The 2003 BIG-T report considered the following areas to be particularly important:

- Cell therapies and tissue engineering;
- Gene Therapy;
- Formulation and drug delivery;
- Novel manufacturing approaches for proteins and other biopharmaceuticals that allow them to be prepared in their bioactive state;
- High throughput bioprocess technologies, eg disposables and automation;
- Bioseparation technologies, including new development methods and novel separation techniques to improve efficiency.

The recommended research priorities described here address both the research drivers and BIG-T areas described above and have been identified following a close consultation with both academia and the bioprocessing industry.

The two priority research areas identified are:

- **Bioscience underpinning bioprocessing** – improving biological understanding to enhance bioprocessing. Understanding the cellular and

molecular processes which are predictive of process performance and which can inform strategies for process design and metabolic engineering
- **Improved Tools for Bioprocessing** – tools to accelerate bioprocess development including high throughput bioprocess research, process modelling, improved analytics and ultra-scale-down systems.

These research areas are relevant to bioprocesses based on microbial cell fermentation or mammalian cell culture in addition to emerging biological products based on stem cells and tissue engineering.

The overall output from the recommended research will be:

- a greater systems-based understanding of biology for improved bioprocessing;
- increased predictability of biological processes for bioprocessing, including improved scale-up and reproducibility;
- improved cost efficiency – both in manufacturing and development;
- increased flexibility to improve product characteristics and reduce product heterogeneity
- increased speed to clinic and market; and
- tools and methodologies for bioprocessing which may have potential for application in related fields

The research will have an impact on bioprocesses at all scales of operation, from the small amounts required for preclinical studies through to post-license bulk manufacture. The priority areas identified are potentially IP-rich and create opportunities for value creation. The advances delivered by the research will help to eliminate the bottleneck in the development of biotherapeutics and contribute to the development of a vibrant bioprocessing community, creating wealth for UK plc.

The priority research areas will now be described in detail. (Note that these research areas cover the whole of BRICs remits an it is likely that individual calls will be focused on aspects of theses areas.)

Research Area 1: Bioscience underpinning bioprocessing – improving biological understanding to enhance bioprocessing

Bioprocessing relies on the harnessing of cells and biological molecules to produce the desired product. However, current *a priori* understanding of the particular cellular mechanisms that control the phenotypic function or performance of biological systems *in vitro* is limited. Therefore one of the major research focuses is on understanding the cellular and molecular processes which limit or control phenotypic function relevant to bioprocessing.

Until we are able to understand such processes and their regulation we will struggle to make best use of cellular systems for our own purposes. In bioprocessing there are huge gaps in our knowledge of how cell biology and metabolism link to process performance. There is therefore a need for research on cellular systems to help process optimization and an understanding of the factors in cells that limit productivity and influence product characteristics.

Achieving this requires the identification of the molecular systems involved in each component of a process, elucidating the nature of any interactions and the molecular control mechanisms governing these interactions. Current state-of-the-art

technologies which investigate the molecular mechanisms at play at each stage throughout the cellular process (genomics, transcriptomics, proteomics, metabolomics) are allowing, researchers, for the first time, to understand biological systems at the molecular level and integrate information on whole systems. Metabolomics, for example, emphasises biosynthetic networks in their entirety, addressing questions of metabolic pathway reconstruction, thermodynamic feasibility, quantification of metabolites, their rate of conversion (flux), and control of this flux. We are only now in a position to begin using the information from such investigations to improve and redesign cell phenotypes and expression systems for a diverse range of purposes using a 'knowledge-based' strategy.

Such information provides effective strategies for cell engineering and bioprocess re-design and improvement based on:

- knowledge directly pertinent to the bioengineered system in question;
- utilisation of quantitative data streams derived from more than one level of cellular organisation, and
- generation of strategies derived from experimentally verifiable predictive models.

Important scientific challenges are:

- **Understanding, controlling and manipulating metabolism in microbial fermentation and mammalian cell culture** Developing an improvedunderstanding of cell physiology and cellular processes so as to improve the efficiency of bioprocesses. For instance, what factors limit the productivity of cells and how can we control and manipulate cellular processes so that bioprocess efficiency is improved both upstream and downstream and what are the underlying biological properties that define the process characteristics of cells? This includes expanding our ability to use microbial expression systems to effectively produce correctly folded and glycosylated complex proteins in manufacturing processes. Mammalian expression systems which minimize (host cell derived) damage to product integrity are also needed.
- **Growth of stem and tissue cells *in-vitro*** There is still an enormous deficit in understanding of basic biology of engineered stem and tissue cells which threatens to undermine their proper therapeutic use. For example, what properties of stem and tissue cells have an impact on their process properties and what factors influence scale-up and culture of these cells? An exploration of the cellular processes that permit adaptation of cells and tissues to function *in vitro,* and particularly for stem cell and tissue engineering therapies is vital before we can safely envisage their use *in vivo*. The outcome of this work will be the development of technology and protocols which will improve our ability to reproducibly grow and monitor stem and tissue cells *in vitro*.
- **Improved understanding of the properties of proteins** Controlling the higher order structure and self-association of therapeutic proteins is key to their successful application as biopharmaceutical products. Currently there are significant challenges in the ability to predict, early in development, protein pharmaceutical performance. This could be enhanced with tools facilitating collection of biophysical and biochemical data sets from the earliest stages of lead identification through the many stages of bioprocessing. Analysis of appropriately defined data sets would support the definition and control of parameters critical to biomanufacturing, particularly in downstream recovery and purification and in formulation, and help ensure

that safe, stable, economically produced biopharmaceuticals are successfully brought to market. This understanding may also contribute to the development of biopharmaceuticals in which manufacturability is built into the molecules during early discovery. The progress made in enhancing productivity of the mammalian cell culture operations used to produce biopharmaceuticals such as monoclonal antibodies threatens to outstrip the capacity of the downstream recovery and purification steps which follow on. New, high productivity technologies to process these culture supernatants while avoiding problems such as aggregation and isoform co-purification, requires new quantitative monitoring techniques and higher capacity molecular partition processes. This requires a better understanding of the biophysical properties of proteins and the materials and systems applied in their recovery.Similarly the formulation challenges posed by proteins which are to be given in high doses require further research into the factors affecting physico-chemical stability of the protein molecule. As the concentration increases the charged areas of the protein molecules are pushed together and this may result in agglomeration, precipitation, conformational changes, protein instability etc. As formulation is an inherent part of bio-processing, these factors must be better understood.

Research Area 2: Improved Tools for Bioprocessing

Bioprocess development involves the design and practical demonstration of scaleable, reproducible and regulatory-compliant processes. There are a large number of variables and decision points in this complex and time-consuming process. Accurately predicting the impact of decisions taken at an early stage in bioprocess development is currently difficult and may lead to unacceptable performance on scale-up. Processes must then be modified and re-tested, leading to delays in reaching a stable and acceptable manufacturing process and prolonging time to clinic and market. The ability to rapidly reach a stable, acceptable process which generates defined and required product characteristics is an important goal. Improved analytical techniques which determine, and improve our understanding of these product characteristics, including functionality, structure, stability and product heterogeneity are a vital element. Bioprocess research aimed at accelerating the development stage and improving design and predictability is therefore of great value and will have an immediate impact.

Research is therefore recommended which will lead to the development of tools which will help to improve bioprocessing efficiency. These include:

- Risk-based tools to give an early indication of whether a particular bioprocess development route is going to be problematical or not;
- Predictive tools to give a forecast output of a change to a bioprocess (for example to predict the impact of changing a construct on the output of a cell line).

Such predictive and risk-based tools are important in bioprocess development to assist in achieving a stable process as early on as possible. They may be used in bioprocess development at a number of scales including:

- Molecular – predicting the impact of molecular characteristics on processing decisions, performance and product properties;
- Cellular – the impact of cellular characteristics on processing efficiency

- Unit operation – predicting the behaviour of unit operations and sequences of operations at scale

Important scientific challenges are:

- **High-throughput process technologies** which will require automated ultra-scale-down techniques and predictive models. Such high-throughput technologies for cell line development and process optimization are applicable for both existing and emerging processes and products and can be used for the screening and optimisation of upstream and downstream conditions in addition to excipient compatibility and formulation screening. Novel high throughput technologies are also required, to enable the efficient 'scale out' production of personalised medicines. This is required so that these products can be produced at acceptable cost, even though normal economies of scale are not available. The development of appropriate analytical techniques for use in high throughput bioprocessing is also important, allowing real-time measurement of multiple parameters with negligible analyte consumption.
- **Effective modelling** of whole bioprocesses which allows the extrapolation of small-scale results to large scale prediction. Such improved models must be based on and demonstrated using industrially relevant bioprocesses.
- **Analytical Methodologies for Bioprocessing** The development of improved analytical methods and tools for the design, analysis and control of bioprocessing and bioprocess development through measurement of critical parameters. This in cludes technologies which will assist in the prediction and generation of defined product characteristics, including information on product functionality, structure, stability, heterogeneity and formulation. It could also lead to the development of technologies that can be applied more widely in the biological sciences. This includes the development of rapid or real time measurements as well as techniques for data based modeling and control for the bioprocessing industries.
- **Improved Downstream Processing** New approaches to the recovery, purification and formulation of products which match upstream improvements (in terms of both product titre and media formulations) are required to enhance process efficiency.The downstream processing of biopharmaceuticals employs a complex process where poor product yield remains an undesirable outcome. Furthermore future biotherapeutics (such as nanoscale viruses, plasmids and drug delivery vehicles) will have more challenging characteristics will add further complexity and require alternative approaches to separation technology. The development of novel approaches to improving downstream process efficiency is desirable. This will include a greater integration of upstream and downstream processes and the development of new separation technologies characterised by high capacity, selectivity and throughput in order to simplify processing or improve product yield and purity. One way of achieving this could be to develop tools and techniques for the rapid, cost effective design and development of new selective capture technologies for purification of new products, rather than adapting generic processes.

Applications Funded through BRIC 1st Call June 2006

A new microfluidic tool for rapid analysis of protein stability and integrity in bioprocesses		
Dr Paul Dalby	University College London	£424203
Analysis of protein stability is currently too slow and requires too much of an exceedingly valuable biopharmaceutical to be useful in guiding bioprocess development or control. Introducing the first microfluidic method for protein stability testing will reduce sample use and cost of analysis by up to 108-fold over microwell-based analysis. Combined expertise from biochemical engineering and the London Centre for Nanotechnology will enable this analysis with parallelism for up to 1000 samples per day. The new generation of protein-based medicines has rapidly become a $30billion-a-year industry addressing previously untreatable diseases. They have the potential for much further growth but a principal constraint is the high cost of the manufacturing methods required to preserve the structural integrity of proteins with limited stability. The ability to perform rapid and parallel protein stability characterisation experiments, at the microfluidic scale, is essential to enable: a) the rapid optimisation of therapeutic protein formulations; and b) the real-time monitoring of protein product quality in process-, microwell- and microfluidic scale bioprocess development experiments. Our preliminary research has demonstrated protein stability determination using fluorescence measurements at the microwell scale (Aucamp et al., 2005). The aims of this proposal are to a) explore the fundamentals that impact on measurement accuracy and sensitivity at the microfluidic scale, so as to significantly decrease the sample volumes required for protein stability measurement; b) establish a microfluidic denaturation technique; c) overcome the challenges that will enable broad application to bioprocessing and formulation of biopharmaceutical protein products.		

Application of metabolomics profiling of recombinant mammalian cells to bioprocess design		
Professor Alan Dickson	The University of Manchester	£768109
The diagnosis and treatment of many clinical conditions is dependent on developments of new therapeutic drugs / complex protein molecules that require production by mammalian cells in culture. Advances in genetic engineering and understanding in gene expression has made it possible to turn mammalian cells in defined culture conditions into "factories" to permit harvest of the valuable therapeutic protein drugs. Hence it is possible to introduce into mammalian cells in culture a gene that will direct synthesise the desired drug and allow the mammalian cells to perform all the necessary reactions required to generate the therapeutic protein. Some of the drugs generated by these approaches are household names (eg insulin and clot-buster drugs) and others are not (eg antibodies), however all are critical and essential components in treatments for many life-threatening clinical conditions. A key problem is that the development and generation of sufficient amounts of therapeutic protein drugs is limited by the capacity to generate sufficient production from the cell cultures. Consequently, the investment of development and process time to harvest sufficient amounts of therapeutic proteins makes the process costly and these are expensive drugs. Despite significant effort by industrial and academic researchers we still do not fully understand the factors that limit production by the cell culture system and such information will be essential if we are to devise means to increase production. The unit cost for certain of these therapeutic protein drugs has implications for availability for treatment and for certain "difficult-to-make" drugs financial implications may prevent the drug being developed for the market. This proposal addresses this problem and seeks to use novel approaches to ask how we can improve the process of therapeutic protein formation by mammalian cells in culture. Our approach is to take a wide vision of the events within cells that limit formation of the desired drug. Too frequently experimental approaches to study the functions of cells focus on one specific aspect or examine functions in the cell for which there is no direct certainty of involvement in the processes under study. We are building complex models that will determine how the many factors in the cell interact to define how production of the therapeutic protein is controlled. We will then test the model in response to conditions that we impose in cells and, from this, determine if there is a means to identify those cells in a population with desirable characteristics that will give excellent production or if we can engineer these functions into cells. The research team in this programme have come together from distinctive areas of research, allowing a fusion of technologies to emerge from the interactions. The fusion of analytical scientists, cell biologists, chemical engineers and mathematical modellers generates a novel team-based approach to an industrially- (and, ultimately) clinically-relevant issue. The team will be part of a wider network of scientists (in academic and industrial groups) who are part of the Bioprocessing Research for Industry Club, the government/industrial initiative to maintain the UK research strength in this area.		

BRIC: Packaging cell lines for inherently manufacturable viral vectors

Professor Nigel Slater	University of Cambridge	£230652
Professor Farzin Farzaneh	Kings College London	£251318

Viruses dominate overwhelmingly the types of vectors currently being tested in clinical gene therapy trials and of these retro- and lentiviruses are the most numerous. Until recently two technological problems have hampered progress in gene therapy; production of high titre clinical grade virus and efficient tissue specific targeting. Research at Cambridge and King's College London has addressed the former and led to the development of a novel lentiviral vector packaging cell line in which manufacturability is built into the genome of the packaging cell and co-expressed on the surface of the viruses produced thereafter. We initially used simple retroviral vectors, and latterly the more complex lentiviral vectors based on a core of HIV-1, and have developed strategies for increasing the titre by several orders of magnitude. This is an active area of research amongst which our preliminary work with novel chromatographic techniques and paramagnetic particles set the foundation for a practical and efficient alternative technique to cumbersome ultracentrifugal concentration. For lentiviral vectors we engineered a new producer cell type that provides a biotin tag amenable to various lentiviral vectors produced from these cells using either VSV-G or MLV amphotropic envelopes. We have shown that these bio-lentiviral vectors are produced in the normal manner and only require the presence of biotin in the culture medium to manifest their affinity for streptavidin. Vectors can thus be retained on streptavidin Paramagnetic Magnespheres for infection, or eluted from streptavidin adsorbents. This cell line allows the capture of multiple envelope pseudotypes of lentiviral or MLV derived vectors, enabling production and concentration to titres that are several orders of magnitude higher. Using this scalable protocol we have concentrated lentivirus in excess of 4500-fold in only 3 h and have provided titers for both VSV-G and MLV amphotropic envelope pseudotypes of 1010 IU/ml. However, these viruses could not be easily eluted from adsorbents and required the addition of biotin to the growth medium of the packaging cells. This proposal aims to express the alternative desthiobiotin ligand on the surface of lentiviruses in such a way that elution from adsorbents may be more readily preformed to give higher process yields and the addition of an affinity ligand binding precursor to growth medium is avoided.

Characterization of post-transcriptional constraints that determine rP yield during bioprocessing in mammalian cells

Mark Smales	University of Kent	£987761

We all have an in-built defence mechanism to respond to infection when our body recognises a foreign 'invader'. A type of cell known as a B cell responds to infection by changing into an antibody-producing cell. Antibodies are proteins that work by attacking the foreign invader and destroying it, clearing infection by removing the foreign agent. Because antibodies are our body's natural defence against disease, many new antibody type drugs are being developed to help treat a number of human diseases such as cancer. These antibodies are usually produced by cells kept in a culturing solution under defined conditions. The problem is that these antibodies must be in a special shape; otherwise they do not work. The cells used to produce such antibodies have a very complex set of machinery to make the antibodies and put their components together into the right shape. This works very well when the cell is not expected to make much of the antibody in question. However, the cells we use to make antibodies are much less efficient at producing these drugs when we try and produce more of the product. As a result, we are not able to produce enough of these drugs and the cost and demand for them is therefore high e.g. the breast cancer drug Herceptin. The root of the problem is that when cells are asked to produce much more protein to meet our needs the machinery can no longer cope / the cells sometimes die or else don't produce antibodies of the right shape, of no clinical use whatsoever. It is largely agreed that this problem will become even serious as further antibody-based drugs are developed. The research proposed here will investigate how the cell machinery for making proteins works and examine whether, and in what ways, it can be manipulated to produce more antibody. We want to determine the different parts of this machinery that are limiting in terms of making the antibody, and then investigate how these parts work together to ultimately produce the antibody of interest. At present it is unknown if this is possible, and the process is poorly understood in the mammalian cells presently used to produce antibodies. We will employ a combination of new state-of-the-art technologies and approaches to take apart the antibody assembly line in mammalian cells in a step-wise manner, and then using the information gathered will determine the relationship between each step of the assembly process. Ultimately this should enable the manipulation of cells to change the balance of each step in the assembly line to produce more of the target antibody drug at reduced cost and higher quality. As stated above, this is extremely important as it is expected that with an increasing number of protein 'drugs' being developed we will lack the capability of producing large enough amounts to meet the required demand for these new drugs for the majority, as opposed to for those who can afford what must currently remain prohibitively expensive, but very effective, medicines.

A Novel Bioreactor System for Manufacturing in Stem Cell Therapy and Tissue Engineering

Professor Julian Chaudhuri	University of Bath	£186350
Professor Richard Oreffo	University of Southampton	£185475

The emergence of regenerative medicine offers the potential for new therapies and procedures for diseases and injuries that cannot currently be effectively treated. Regenerative medicine involves the use of living cells and other biological molecules to restore damaged structure and function in human organs and tissues. In addition, the recent excitement arising from the discovery and potential uses of stem cells makes it timely to investigate how stem cell research can be used to treat patients. One of the key issues is how to produce enough living cells (including the very rare stem cells) that have the correct function for these new therapies. The current laboratory cell culture procedures are not efficient, nor are they standardised and cannot meet the current clinical needs. This project aims to address this acute issue by the development of a new method of efficiently culturing stem cells and other human cells to give enough cells required to treat patients.

This system will be based on the idea of growing cells using a biomaterial derived from seaweed (alginate) that allows the cells to grow and develop normally. In this work, we will decide what effects the key features of the culture system will have on the growth and function of both human stem cells, and also specific human cell types (eg bone cells). As part of our experiments we will measure how the cells grow and behave in the different culture environments, and compare this to conventional methods.

Identification of novel signal transducers in the mammalian unfolded protein response

Dr Martin Schröder	Durham University	£677697

Human proteins produced in other organisms, i.e. bacteria or mammalian cell cultures are called recombinant or heterologous proteins. These proteins have many applications in industry and medicine. They are safer to administer in the clinic than their native counterparts purified from i.e. animals. Single point mutations in a protein, i.e. isolated from pigs, that deviate from the human sequence, can have serious side effects when the protein is administered to humans. Further, their posttranslational modifications, i.e. their glycosylation pattern, are similar to those found in humans. Again, minor deviations in the glycosylation pattern between an animal and a human protein can seriously affected the performance of the protein in the human body. Finally, the risk of contamination of the protein with infectious agents is much easier controlled in production processes for recombinant proteins compared to isolation of the protein from animals. Production processes for recombinant proteins for use in the clinic rely on mammalian cell lines. Growth conditions and sterility requirements for these production processes make them expensive. Further, the number of recombinant proteins for which there is a demand on a multikilogram per year scale is rapidly growing, requiring the development of large scale (several cubic metres) production processes. The cost efficiency of a production process often determines if a recombinant protein makes it to the market and to the clinic. Several strategies have been devised to improve product yield and in turn the cost effectiveness of these production processes. To date, the rate-limiting step for production of recombinant proteins is folding into their native, active, and non-immunogenic conformation. Protein folding is assisted by helper proteins that shield a folding protein from its environment, called chaperones, and that catalyse a subset of protein folding reactions, called foldases. Recent basic research has identified signal transduction pathways that sense the folding status of a protein, and that activate expression of these helper proteins to increase the efficiency of protein folding in response to this stress situation. Genetic engineering of these signalling pathways promises to alleviate the protein folding bottleneck in recombinant protein production. However, our current understanding of these signal transduction pathways is still limited. Based on our current knowledge the outcome of engineering of these pathways on recombinant protein production is unpredictable. To address this problem we propose to characterize these pathways on a genomic scale to define their complete substrate spectra by using recently developed genomic technologies to monitor the complete mRNA and protein population of a cell. In addition, we will exploit unique biochemical characteristics of these signalling pathways to focus our study on the pathways in question. In this way we will target three unique signal transduction mechanisms, non-spliceosomal splicing of mRNAs, preferentially translated mRNAs when translation of the majority of mRNAs is inhibited, and proteolytic activation of ER membrane proteins. We anticipate to identify new proteins involved in this response to protein unfolding. We will characterise the role of new genes involved in these signalling pathways identified in our study by characterising their role in chaperone and foldase expression, and survival of ER stress. Furthermore, through defining the complete substrate spectra of these pathways we will enable the engineering of these pathways with predictable outcomes on cell specific protein production and cell viability.

Microbial physiology underpinning the production of difficult recombinant proteins

Professor Jeffrey Cole	University of Birmingham	£386309

Most people appreciate the need for pharmaceutical companies to develop new vaccines to prevent disease, or drugs to combat illness, not only for humans, but also for our domestic animals. In this context, biotechnology and genetic engineering are clearly tools to be used for the benefit of society. The development of new vaccines and drugs often depends upon the ability of bioprocessing companies to harness a cell factory to produce one or more target proteins. In many cases this will mean using simple but safe bacteria to generate the required product. Bacteria can be taught to synthesise almost any protein, once they have inherited the correct DNA coding sequence. However, under the conditions they are grown in the laboratory, they are often unable to assemble the protein correctly, so the product is useless. Sometimes it is better to make less product more slowly so that it is not toxic to the cell. In other cases, extra copies of helpful genes need to be transferred to the cell factory so it can assemble and modify the target protein after it has been generated from the genetic information provided. In this project, we will discover why some proteins are so difficult to make, and how to help bacteria make them more efficiently. We will start by making a protein that is located on the outer surface of the bacterium that causes the sexually transmitted disease, gonorrhoea. It is also found in bacteria that cause teenage meningitis (the so-called "kissing disease"). Vaccines are required for both gonorrhoea and type B meningitis. We will discover how to prevent the accumulation of useless product, and how to make authentic protein. The UK bioprocessing companies who have formed a club to support this type of research will then be invited to challenge us with one of their unsolved problems. This will allow us to test whether there are general rules that must be followed if other difficult proteins are to be generated for the benefit of human health.

Modelling cellular processes underpinning recombinant monoclonal antibody production by mammalian cells

Professor David James	University of Sheffield	£524266

This proposal is concerned with "bioprocessing". Bioprocessing collectively describes the range of manufacturing processes that enable the production of new biological medicines. You may be familiar with the one of the first biological medicines produced by recombinant DNA technology - a small protein called insulin. Insulin is now used very successfully to treat an increasingly common metabolic disease, diabetes. Before insulin, diabetics suffered a short life fraught with serious medical complications. This project is targeted at the production of other high-value therapeutic proteins by genetically engineered mammalian cells in culture, specifically monoclonal antibodies. In the body, natural antibodies present in the blood play an important role in our immune system: They target disease-causing microbes and foreign substances for removal. Recombinant monoclonal antibodies, being almost identical to natural antibodies, are specifically designed to target diseased cells. Unlike traditional small-molecule medicines such as penicillin and paracetamol, monoclonal antibody biopharmaceuticals are large, complex and relatively fragile proteins which have to be produced by living mammalian cells in culture, genetically engineered to produce the recombinant protein product. They are proving to be highly successful treatments for serious diseases such as rheumatoid arthritis and a range of cancers. It is anticipated that within the next five to ten years up to fifty percent of all drugs in development will be biopharmaceuticals; a very substantial proportion recombinant proteins produced by mammalian cells in culture. Since the first recombinant protein medicines produced by genetically engineered mammalian cells in culture were licensed as therapeutics over 25 years ago, we have learnt to substantially increase the productivity of biopharmaceutical manufacturing processes (bioprocesses). However, they are still complicated and expensive, and industry has to undertake time-consuming screening processes to find engineered cells making adequate amounts of recombinant protein. To date, the output of industrial bioprocesses has predominantly been increased by gradually improving the growth of producer cells in culture, and not by engineering each cell to make the product more efficiently. This is important, because if we knew how to instruct or programme the cell factory appropriately, we could substantially improve the productivity of manufacturing processes and decrease the time it takes to generate a productive cell culture. However this is not a simple problem. The cell utilises and coordinates a diverse range of its complex machinery to turn, for example, recombinant monoclonal antibody genes in its nucleus into a fully folded protein which can be secreted out of the cell. How can we understand this cellular "production line" well enough so that we can rationally implement strategies to improve flux from recombinant genes to protein product? In this project we will implement a novel, multidisciplinary combination of technical approaches to answer this question; mathematical modelling, gene expression, molecular cell biology, protein analysis and cell culture. We believe this is crucial - an integrated mathematical bioscience approach can massively increase the information content and utility of biological measurements and enable us to understand cellular processes from a systems control perspective. This project will, for the first time, provide a quantitative understanding of the cell factory on which to rationally build strategies to increase the productivity of therapeutic monoclonal antibody production systems. Without this knowledge, cell culture engineering will largely remain based on trial and error.

Strategy for the consistent preparation of sufficient non-viral large chromosomal vectors for biopharmaceutical applications

Dr Eli Keshavarz-Moore	University College London	£377945
Modern medicines and therapies are becoming increasingly complex and specific for a particular disease or group of patients. One very specific type of therapy is Gene Therapy. Gene therapy is the use of genes as medicines. These genes can be delivered to patients either by the use of genetically modified viruses to carry the genes or by using non-viral methods which employ circular DNA molecules called plasmids isolated from bacteria. Both techniques are still in their infancy but are already promising huge medical advances in vaccination, cancer therapy and the correction of genetic disorders. However, viruses suffer from several drawbacks including safety considerations and limited to carry large genetic information. On the other hand large plasmid DNA molecules called BACs (Bacterial Artificial Chromosomes) can be modified to incorporate a wide range of important control regions which allow expression of the gene in the correct tissue and at the correct time. This larger size poses a scientific and industrial challenge because the size limits the amount that can be made in a single bacterium, in addition, the size also calls for special processing considerations because BACs are fragile. The challenge is to be able to prepare the BAC molecules in the amounts needed to treat the numbers of people who could benefit from these medicines. We need to investigate then develop methods of making the DNA molecules at large scales in the biomanufacturing industry. The quality of the DNA molecules is also paramount. They need to be in the form that is most appropriate for delivering to humans (and animals) and free of contaminating material. The research we propose will enable industry to make DNA molecules that can be made at the large scales. The proposed research outcomes will allow others involved in gene therapy to prepare large DNA molecules for the treatment of cancers, for vaccines and to correct genetic disorders The science proposed will also allow other researchers in related disciplines to benefit from being able to make and manipulate large DNA constructs .We will also explore ways of making the DNA in the correct three dimensional form needed for efficient uptake into cells so that the DNA is an effective medicine.		

Total £5,068,335

APPLICATIONS FUNDED THROUGH BRIC 2nd CALL JULY 2007

Identifying and overcoming protein secretion bottlenecks in yeast and filamentous fungal cell factories		
Professor David Archer	University of Nottingham	£362590
Professor Stephen Oliver	The University of Manchester	£422247
The yeasts Saccharomyces cerevisiae and Pichia pastoris are the main experimental organisms in this study. Both species are used as cell factories (in the laboratory and commercially) for secreted protein production. S. cerevisiae is also an excellent model organism for investigations into the basic events involved in protein secretion and stress responses. P. pastoris is less amenable to basic studies but is an outstanding cell factory. This project will create some tools for basic studies in P. pastoris as well as using both organisms for comparative studies with each other and with the principal filamentous fungal cell factory, Aspergillus niger. 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 S. cerevisiae and P. pastoris. 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 S. cerevisiae, P. pastoris and Aspergillus niger 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.		

Combined /omics approaches to understand and control library enriched microbial cell factories		
Professor Phillip Wright	University of Sheffield	£297941
This project aims to apply a genome-wide, multiscale approach for functional genomics to improve the production of recombinant proteins in Escherichia coli, 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 E. coli here as an exemplar project. This protein has been demonstrated as being possible to produce in E. coli, following the transfer of the N-glycosylation system from Campylobacter jejuni into E.coli cells.		

Pichia pastoris protein secretion: analysis of constraints, optimisation and methods development		
Dr David Leak	Imperial College London	£724360
The methylotrophic yeast Pichia pastoris 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 P pastoris, (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.		

Multifunctional Chromatography materials for improved downstream processing		
Professor Owen Thomas	University of Birmingham	£595931
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.		

Protein nucleation and crystallisation on novel 3-D templates		
Dr Daryl Williams	Imperial College London	£384817
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.		

Delta3D; Bench top assays for the rapid detection of protein 3D structural changes		
Professor Jeremy Lakey	Newcastle University	£363525
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.		

New approaches to high throughput protein, isolation, purification and concentration		
Dr Barry Moore	University of Strathclyde	£392967
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 techniqe is expected to be particularly useful for isolaton of complex protein assemblies not well suited to chromotography. 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.		

Total £3,544,382

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

BRIC 2.1 PROJECT FURTHER DETAILS (6 projects)

Nigel Slater (University of Cambridge) & Dr Karen Coopman (Loughborough University) - Bioprocessing Research For Cellular Products

This project aims to develop novel preservation platform technologies required for the successful banking of human cells, an absolute prerequisite for their use as products. Many regenerative medicine products rely on the delivery of live cells to patients. At present this is exemplified by established therapeutic interventions such as bone marrow transplantation, blood transfusion and corneal grafting; future generations of products may include bio-artificial matrices that incorporate donor stem cells, for example bone replacement and repair devices, and artificial 'mini-organs' such as pancreas or liver. Current cryopreservation of stem cell based products results from historic work, using DMSO as a cryopreserving agent which is largely unsubstantiated with respect to final biological activity. DMSO can be toxic to cells, lead to low viabilities post thaw and both genetic and epigenetic instability (i.e. loss of pluripotency) over long term culture. Cryopreservation of blood cells has been attempted previously, with limited success due to loss of cell integrity, primarily due to the breakdown of the cell membrane and consequent loss of overall cell structure. A variety of techniques have been investigated for delivering trehalose, a membrane impermeable cryoprotectant, into mammalian cells, including microinjection, ion channel stimulation, pore formation using mutant bacterial toxins, fluid phase endocytosis, and internal trehalose synthesis via genetic engineering but intracellular trehalose concentrations achieved in erythrocytes has not exceeded 50 mM and is therefore below thresholds for cryoprotection

Biopolymer mediated cell loading achieves substantially increased intracellular trehalose concentrations of up to 251 mM and a concomitant improvement of erythrocyte cryosurvival of up to 20.4 % as compared with conventional methods of loading trehalose into cells. The technology utilizes novel amphiphilic biopolymers that interact with the external cell membrane to enable penetration and retention of cryoprotectant agents into the cells. Membrane permeabilisation by these Cell Permeating Polymers (CPPs) is rapid and completely reversible via washing with buffer. Cellular uptake of trehalose is dependent on polymer molecular structure, concentration, pH, external trehalose concentration, incubation temperature and time. Optimization of these parameters imparts cellular osmoprotection. Overall, a total cell recovery through a single freeze-thaw cycle at -80oC of 82.6 % has been achieved, which compares with a recovery of only 0.8 % for cells frozen in PBS.

This proposal aims to explore the CPP mediated loading of preservation agents into stem cells, to examine preservation by freezing and desiccation and to arrive at integrated processing routes for the preparation of optimally stable stem cells

Mark Cobbold (University of Birmingham) & Dr Eirini Theodosiou (Loughborough University) - Developing generic scalable and standardised selection methods for human therapeutic cells

This proposal aims to deliver practical, scientific and technologically innovative solutions to a real problem through partnership between neighbouring Universities: the University of Birmingham, Loughborough University and the University of Oxford. We aim to develop a device to allow blood cells to be selected directly from the blood. This device would allow new treatments to meet the bioselection challenges presented by advanced cellular therapies.

Effective cellular therapies are not new and date back to 1665 when the first successful blood transfusion took place. Today, 81 million units of blood are taken each year, saving hundreds of thousands of lives worldwide. In addition, at least 50,000 patients receive stem

cell transplants (SCT; also known as Bone Marrow Transplants) globally every year (one of the few therapies able to cure individuals with cancer) with each costing around £100,000.
The blood stem cells account for only 0.2% of all white blood cells in the blood. Unfortunately it is not possible to isolate just the stem cells from blood, but instead, a large number of white cells are removed from the donor's blood via a process termed leukapheresis. The donor quickly replenishes their white cells over the next few weeks. One side effect for the donor is that their bone marrow is stimulated to grow which frequently leads to bone pain.
These stem cells potentially offer the gift of life to the recipient who typically will have leukaemia or lymphoma where these cells migrate to the bone marrow and start producing blood for the patient.
However some of the white blood cells from the donor have to potential to cause harm, these immune cells recognise the recipient as foreign and try to attack them. To prevent this, the stem cells are purified from the white cells and infused in a purer form.
Scientists are understanding more about stem cells than ever before and can now expand these rare cells in the laboratory and even create new organs from them (in mice at least). The challenge now is to bring these new techniques and knowledge to the forefront of clinical medicine. Before this can be done, new technologies are required to manipulate these cells in a manner that will not introduce infection and ensures the cells are of sufficiently high quality to be effective for the patient.
The aim of this proposal is to develop a new device which would allow the enrichment of cells. The device will be engineered to be very versatile and would allow the enrichment of any cell type and thus will be of broad interest to many companies seeking to develop cellular therapies. We propose two steps, first a 'capture' device which could even be used to directly isolate cells from the circulating blood of a donor/patient. This could reduce side effects of stem cell isolation such as the bone pain by only picking out the cells of interest. The next step would be to wash these captured cells and pass them over smaller purification columns, which would remove unwanted cell types and capture the cells of interest. This is important as currently there are no ways to select subpopulations of cells. Finally the cells will be released and analysed for their properties. This work has been principally developed by a clinician familiar with the problems delivering new therapeutics to the clinical coalface. We have a working prototype able to capture and release cells from whole blood and are asking for funding to develop this further. We have designed the device with the NHS in mind, to be safe, effective and importantly affordable.

Karen Polizzi (Imperial College London) - A platform for the optimisation of metabolic pathways for glycosylation to achieve a narrow and targeted glycoform distribution

Recently, the development of treatments for new disease has shifted away from traditional chemical compounds and towards protein therapeutics (biopharmaceuticals) like antibodies for the treatment of cancer and hormones for chronic diseases. Nearly 70% of these protein therapeutics have sugar molecules attached to them naturally which affect their function and how long they remain in the body. Because the sugars are so important for the drug function, one of the biggest problems in their manufacture is how to control what sugars are added (glycoform) and to ensure that all the proteins produced have the same sugars on them (homogeneous glycoform profile). Current production methods yield a non-homogeneous mix of glycoforms. Also, different glycoforms interact with the immune system in different ways, so it would be of benefit to be able to produce certain glycoforms over others depending on what the drug is and how it is meant to function.
Our goal is to develop technology to rapidly determine the effects of different production methods on which glycoforms are produced and how homogeneous the glycoform profile is. To do this we will develop proteins which are produced inside the cells that are also producing the biopharmaceutical that report the concentrations of nutrients that are already

known to influence glycoforms. Alongside, we will develop a computer model of the metabolism of the cells which can predict which glycoforms are produced. Using these two together, we should be able to design new media for the cells to use that result in a more homogeneous glycoform profile which we can change based on what the cells are fed with. We can also suggest genetic changes to the cells that would further help us produce a single, designed glycoform. This could lead to the production of drugs that are safer and require lower doses because they have a single glycoform attached which is the most appropriate for the function of that drug.

Paul Dalby (University College London) - Elucidating aggregation mechanisms in antibody fragment-based therapeutics to improve their manufacturability

Recently, the development of treatments for new disease has shifted away from traditional chemical compounds and towards protein therapeutics (biopharmaceuticals) like antibodies for the treatment of cancer and hormones for chronic diseases. Nearly 70% of these protein therapeutics have sugar molecules attached to them naturally which affect their function and how long they remain in the body. Because the sugars are so important for the drug function, one of the biggest problems in their manufacture is how to control what sugars are added (glycoform) and to ensure that all the proteins produced have the same sugars on them (homogeneous glycoform profile). Current production methods yield a non-homogeneous mix of glycoforms. Also, different glycoforms interact with the immune system in different ways, so it would be of benefit to be able to produce certain glycoforms over others depending on what the drug is and how it is meant to function.
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Robin Curtis (University of Manchester) - Understanding and predicting aggregation in biopharmaceuticals

Currently, one of the bottle necks to developing cheaper protein therapeutics is the cost of the downstream bioprocessing and formulation steps. A key problem is the loss of active protein therapeutic to irreversible aggregation throughout the bioprocess. Other problems can arise during chromatography or filtration when encountering high protein concentrations which could lead to high viscosites or even precipitation. The focus of this work is to develop predictive methods for identifying problematic conditions early on in the bioprocess. These could then be used for identifying changes to the protein to minimize the problems. Alternatively, the method could be used for optimizing the solvent properties (pH, buffer type and concentration) or finding other small molecule additives to be used in order to avoid aggregation or increase protein solubility. We benchmark our approach by studying antibodies and antibody fragments due to their growing importance as human therapeutics.

Hans Westerhoff (University of Manchester) - Predictable Protein Production

Recently, the development of treatments for new disease has shifted away from traditional chemical compounds and towards protein therapeutics (biopharmaceuticals) like antibodies for the treatment of cancer and hormones for chronic diseases. Nearly 70% of these protein therapeutics have sugar molecules attached to them naturally which affect their function and how long they remain in the body. Because the sugars are so important for the drug function, one of the biggest problems in their manufacture is how to control what sugars are added (glycoform) and to ensure that all the proteins produced have the same sugars on them (homogeneous glycoform profile). Current production methods yield a non-homogeneous mix of glycoforms. Also, different glycoforms interact with the immune system in different ways, so it would be of benefit to be able to produce certain glycoforms over others depending on what the drug is and how it is meant to function.
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BRIC 2.2 PROJECT FURTHER DETAILS (12 projects)

Bernadette Byrne (Imperial College London) - Application of ATR-FTIR to industrial scale production of therapeutic antibodies

Summary
Biopharmaceuticals, proteins used as drugs, are an emerging area in the treatment of a range of diseases. The large scale production of these molecules requires a number of discrete steps including recombinant expression, isolation by a technique called column chromatography and development of an optimal final formulation. The isolation of the biopharmaceuticals from all other contaminating material usually involves a number of steps. The column chromatography used exploits a specific interaction between the target protein and another molecule immobilized on a support, the resin.
Ideally all the contaminating material is washed from the column and the conditions are changed in order to release the protein from the resin. Optimal binding, washing and release steps often require significant changes in the properties of the surrounding solution, for example pH and ionic strength. In addition, it is possible to bind very high concentrations of the protein onto these resins. The concentrations reached are likely to be higher than those achieved at any other point in the production process. It is not known exactly what effects the different solution conditions and protein concentrations have on the quality of the protein. Indeed they may result in highly undesirable effects such as non-specific aggregation. Another key issue that scientists isolating biopharmaceuticals encounter is the binding of unwanted contaminant materials which reduces the ability of the column to isolate the target protein. Regular replacement of the resin significantly increases the cost of the isolation process and thus of biopharmaceutical production. Here we aim to investigate these issues with a view to improving isolation protocols using a specialized technique called Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. This technique

allows us to chemically image (take chemical photographs of) the target protein molecules under different conditions both in static droplets and in dynamic systems similar to those used for large scale isolation. We can also directly image used resin with a view to understanding what contaminating molecules reduce the useful lifetime of the material. We anticipate that the results of these experiments will inform optimization of isolation protocols to both minimize target protein losses on-column and increase resin lifetime.

Che Connon, (University of Reading) - Investigation of optimal gel conditions for stem cell preservation at room temperature and scaling up of selected methodology

Summary
For numerous reasons (financial, resource, scientific etc) the centres of excellence responsible for the preparation/manufacture (cell banking, growth, culture, purity and QA etc) of the Living Biologic (LB) in any Regenerative Therapeutic (RT) are geographically separated to that of the point of use. Therefore, a major challenge is to deliver to the clinic the LB in a validated, quality, 'fit for purpose' form. Across the global market place there is a pressing need to retain the key LB characteristic for at least several days to one week. Although suitable for longer term banking, Cryofreezing for the final product is not an option due to the acknowledged negative effects on the LB. As important, the system needs to be simple, rapid and easy to use at the point of care in order to ease uptake by the clinician. Our recent BRIC enabling grant has shown that an alginate gel cell encapsulation system meets many of these needs. We have a growing body of evidence that demonstrates it is effective at preserving a number of cell types (including stem cells) over extended periods in room temperature conditions. However, further research is needed to fully understand how it works and demonstrate that the technology can be implemented in an industrial setting (i.e. scalable).

Paul Dalby (University College London) - Multi-modal fluorescence spectroscopy for online analysis of proteins in bioprocesses

Summary
Biopharmaceutical proteins are typically purified from clarified fermentation broths using multiple chromatographic steps where each separates the proteins based on one or more physico-chemical feature such as net charge, size, hydrophobicity and biological affinity. The elution peak containing the native protein product at each step is often contaminated by host cell proteins, or with slightly modified versions of the product which can be almost indistinguishable from the native form and therefore very challenging to remove. The precise product profile at each step is therefore very sensitive to small changes in upstream variability, buffer composition and pH, and the gradual fouling of chromatographic resins that affects performance through repeated re-use. It is therefore imperative to be able to monitor the product profile, preferably in-line or on-line, to be able to adjust the process parameters in real time, or to make a responsive decision as to when to start and stop collecting the product fraction within the elution peak. Real time, low cost and low volume analysis of proteins and protein mixtures suitable for online monitoring of chromatography, is generally limited to simple absorbance, refractive index and conductivity measurements which only provide basic peak detection and no detailed characterisation of the protein product profile. Multi-angle laser light scattering (MALLS) has some potential for online characterisation of approximate molecular masses, whereas accurate online mass-spectrometry is too expensive and technically demanding. We will take advantage of our recent advances in both the biophysical analysis and rapid laser-induced temperature perturbations of unlabelled proteins in microcapillary flow, as well as state-of-the-art optical components, to

establish a low volume flow-detector for use in chromatography, that can evaluate the heterogeneity of the protein product profile in real time. A single set of optics for the intrinsic fluorescence of proteins will be set up to measure fluorescence intensity (FLI), time-resolved fluorescence (TRF) and fluorescence correlation spectroscopy (FCS) and so simultaneously characterise orthogonal features of the protein product profile. These will measure protein quantity (peak detection), and detect underlying variability in solution conformation, oligomeric state, and particle sizes, including the soluble aggregates. FCS is more sensitive and quantitative for relative particle concentrations than DLS or MALLS which are disproportionately sensitive to larger particles. To further resolve product heterogeneity, the flowing sample (continuously split from the main elution stream) will also be subjected to a rapid (12 ms) temperature jump of up to 70degC using our recently demonstrated microfluidic IR-induced heating technology. This will induce partial structural unfolding of protein domains, and the dissociation of soluble oligomers, with kinetics and amplitudes that are characteristic to each different protein species in the sample, and thus provide further online resolution of the sample complexity relative to known reference standards or previous elution profiles. As an additional bonus, the detector will also be suitable for standalone sample analysis, such as for the profiling of dosage formulations and their viscosities. The benefits of each new spectroscopic mode will be demonstrated for a wide range of relevant proteins from our lab and from other BRIC members, including IgG, Fab, GCSF. To test the range of heterogeneity that can be detected, these will be syringe-pumped into the detector and analysed in partially and fully purified forms, and also after deliberate modification by partial proteolysis, partial unfolding and aggregation at low pH, oxidation, and shear damage. Online application to chromatography will then be demonstrated using a flow splitter to give a continuous flow into the detector in parallel to fraction collection.

Alan Dickson (The University of Manchester) - Application of single cell metabolite profiling to optimisation of stem cell bioprocessing

Summary
The potential of stem cells as treatments for disease appears in the public arena with increasing frequency. Fundamentally, these are cells that may have the ability to be switched to take on the function of cells within critical roles in the body. Consequently, when the normal cells are damaged, as in heart attacks or paracetamol-induced liver damage, the stem cells should have the ability, as a transplant, to take up the function of the heart or liver cells. The scope exists for stem cell treatments to cure/improve the well-being of patients suffering from life-threatening/-changing conditions. Tremendous advances have been made but a number of key hurdles must be overcome before there is a more complete realisation of the undisputed potential. Chief amongst these is the fact that stem cells do have the potential to take on the functions of many different cell types and the control of this process is not fully understood. Placing this in to the context of our present proposal, the loss of insulin-production by cells of the pancreas generates diabetes. This can be treated by insulin injection, with all the consequences for monitoring and life-style, but the possibility of transplantation of insulin-producing cells to patients offers a vision of cure, rather than therapy. Hence, the key challenge is to understand the processes that control the change from stem cell to a pancreatic cell. This is against a background of stem cells undergoing changes towards many cell types - nerve, liver, muscle, kidney, heart - and generating a mixture of cell types simultaneously. Transplantation of a mixture of cells, rather than the insulin-secreting cell would not be effective. This aspect remains a major limitation for the potential of stem cell therapy to become a reality and it is the focus for this proposal. Our proposal will apply novel approaches to look at stem cells as individual cells using our pancreatic cell lines as a powerful model system. The reason this work is important is that it aims to take "fingerprints" of the metabolism of each cell as an individual entity, enabling us to relate a "fingerprint" with the controlling events that determine if a cell becomes nerve,

liver, pancreas etc. The information will aid us to identify processes for maintenance of cells in whatever functional state is desired and to select for cells with a particular functional state. This type of processes can be applied to large-scale generation of cells with defined function - taking the production of cells to the commercial level that will move us towards manufacture of cells in sufficient quantity, as well as quality, to use as real-life medicines. Our approach is one that is unique due to the collaborative positioning of research groups with disruptive new technologies and model cell lines for effective development of a more general technology

Nikolaj Gadegaard (University of Glasgow) - Development of nanopatterned substrates for the delivery of high quality stem cells

Summary
The use of stem cells in regenerative medicine holds great potential and with an increasingly aging population, we need to look for new opportunities. Their potential use span from orthopaedic applications such as arthritis and osteoporosis to neurodegenerative disorders such as Parkinson's and Alzheimer's, to name a few. The body has a constant source of stem cells located in niches within the body. From a scientific and clinical point of view, one of the most exploited sources for adult stem cells is the bone marrow. The bone marrow is relatively easy to access and stem cells can be easily isolated from the extracted cell population. However, until recently, a major hurdle is that the stem cells cannot be cultured for extended periods in culture and maintain their regenerative potential (multipotent). The very potential of stem cells, that they can change into many different cell types and so help repair damage on demand, means that their profile (phenotype) is unstable in culture. Hence, as we culture stem cells they soon lose the very potential and potency we want to exploit. We have recently (2011) demonstrated that by culturing the cells on a uniquely nanopatterned surface (nanopits, 100 nm in diameter and 100 nm deep, arranged in a square lattice) it is indeed possible to keep the cells in the multipotent state in prolonged culture as well as expand the number of cells. These nanopatterned surfaces resemble the tracks on a Blu-Ray disc and indeed our technology is very similar to the production of optical media where nanopatterns can be injection moulded into polymer discs in high volumes and at a very low cost. We can change the arrangement of the nanopatterns, thereby tuning the stem cell response to growth without profile (phenotype) drift and to target desired changes to tissues we want (known as differentiation). This is has important implications on the design of implants (like a hip replacement implants) where a specific cell changes (differentiation) is desirable. An example of this is again, orthopaedic implants where differentiation of cells to bone is desirable, or an area with perhaps even more potential is the growth of large numbers of stem cells. Thus, a key research goal is to take a patient's stem cells, grow them in the laboratory to useful numbers, and then place them back into the patient to spark regeneration. Scale up of our technology will allow this.
The technology we have used so far has only allowed us to explore the stem cell interaction to a very limited number of different geometries (<10). In this proposal we will develop a new platform where a single sample will contain 1000 different patterns thereby allowing us to investigate a much larger library of nanopatterns and their ability to influence the fate of the stem cells. From these libraries new patterns will be identified and we will investigate them in more detail using mass spectrometry to identify small molecules influencing the cell fate. Importantly, to see real benefit of these discoveries, it is vital that we are able to scale the materials used to large areas to sustain the growth and expansion of stem cells used for regenerative medicine or pharma. As described above, our technology is very similar to the production of DVDs and Blu-Rays, which means that it lends itself to a cost effective mass production of the nanopatterned surfaces. To demonstrate this potential, we will expand extracted bone marrow stem cells to 5 million cells, the number of cells used for the fully tissue engineered trachea demonstrated in 2008.

Chris Hewitt (Loughborough University) - Expansion of human mesenchymal stem cells in aqueous / aqueous two phase systems

Summary
In order that people can live longer and more active lives there is a need to develop new affordable and effective medicines. In some cases cells that we have within our own bodies can be used to repair damaged tissues. However, in adults, this repair mechanism is very limited and often inefficient. Stem cells produced by the body are those that can go on to make all of the different types of cells in the human body and these so called 'stem cells' when harvested have the potential to repair many types of diseased tissue in adults. Although stem cells can now be grown in laboratories, one highly trained person can only grow a few million cells in a week. Since it takes 5 billion heart cells to repair the heart muscle of a heart-attack patient, growing these cells at laboratory scale is useful for research but not for treating multiple patients in practise. This project aims to combine the expertise of both biologists and engineers, to create scalable system for the "manufacture" of large numbers of stem cells so the potential of stem cell therapies can be realised. Once enough stem cells can be routinely grown and harvested, it is more likely that they can begin to treat a variety of diseases.

David James (University of Sheffield) - Linking recombinant gene sequence to protein product manufacturability using CHO cell genomic resources

Summary

Biopharmaceutical companies producing the new generation of recombinant DNA derived therapeutic proteins (e.g. cancer medicines such as Herceptin and Avastin) often use mammalian cells grown in culture to make the protein product. All production processes are based, fundamentally, upon the ability of the host mammalian cell factory to use a synthetic DNA genetic "code" to manufacture the complex protein product. This is a cornerstone of modern biotechnology. However, because protein synthesis is so complex, involving many cellular resources and machines, it is extremely difficult for genetic engineers to design a DNA code that will best enable the mammalian cell factory to operate most efficiently. Moreover, as individual mammalian cell factories can be very variable, they may differ substantially in their relative ability to make the product. As a consequence, a lot of time and money has to be spent by companies on the initial phases of the biopharmaceutical development process conducting intensive screening operations to find the best cell factory (out of a large population) able to use the genetic code it has been given. For a different protein product it is necessary to start the whole development process again. In this project we will utilise recently available high information content molecular analysis technologies and computational tools to "de-convolute" the complexity of protein synthesis in mammalian cell factories. Effectively, we know that the mammalian cell factory uses its own genetic code to make thousands of its own proteins (machines) that together perform a variety of functions that enable the cell to grow and divide. The rate at which these proteins are made varies hugely, over 1000-fold, so that the cell can make each bit of protein machinery in the right quantity to do its job. We will measure how efficiently each cellular protein is made then using advanced biological information analysis (bioinformatics) and mathematics we will determine how the cell uses pieces of information embedded in each of its genes to vary the rate at which a specific protein is made. This will enable us to create, for the first time, a usable set of "design rules" (computer programmes) that genetic engineers and cell factory developers can employ to (i) reliably design the best genetic code for any given protein

product and (ii) accurately predict how much of the protein product the mammalian cell factory can make. This is important as it means that biopharmaceutical companies can design a predictable production system from scratch, enabling a more rapid transition through lengthy cell factory development processes towards (pre-)clinical trials

Stephen Oliver (University of Cambridge) - Development of an integrated continuous process for recombinant protein production using *Pichia pastoris*

Summary
This project will build on the successes of our two projects in phase 1 of the Bioprocessing Research Industry Club (BRIC1) to develop a system for the growth-associated production of commercially important protein products in a commerciallyimportant production organism - the yeast Pichia pastoris. Proteins, particularly antibodies, are now playing a major role in the treatment of human disease. They are usually produced using animal cells in culture, a procedure that is both costly and time-consuming. An attractive alternative is to engineer a yeast cell to produce human proteins of therapeutic value. Pichia pastoris is often used for this purpose since it may be grown at high cell densities and has an efficient secretion system to release the protein product from the cells. Current processes induce protein production by repeatedly adding methanol to the yeast cultures. Our studies in BRIC1 demonstrated that this is exactly the wrong way to go about this since the cells are repeatedly stressed and produced badly folded proteins that have low biological activity and are not secreted out of the cells. What is required is a continuous process for protein production and recovery and this is what our BRIC2 project aims to achieve. The product proteins to be studied will be guided by our contacts within the BRIC member companies and include a number of proteins used in human therapies. Growth-associated production will enable continuous processes and thus increase commercial productivity. Computer models developed in BRIC1 will be improved, extended, and used to control the novel process. Moreover, the fast protein separation methods that we also developed in BRIC1, will permit the continuous retrieval of product, thus obviating the major barrier to the commercial adoption.

Colin Robinson (University of Kent) - Development of new-generation bacterial secretion process platforms

Summary
Many important therapeutic products are proteins - often termed biopharmaceuticals - that have to be produced in a living organism and then purified. Over 30% of the currently licensed therapeutic proteins are made in the bacterium Escherichia coli, which can be quickly grown in large amounts. Some of these proteins are synthesised in the cell interior (cytoplasm) but a favoured strategy is to 'export' the protein product to the periplasmic space between the two cell membranes. The reasons are two-fold. First, the contents of the periplasm can be extracted relatively easily, by selectively rupturing the outer membrane. Secondly, the periplasm is an oxidising environment, and is thus the only place where disulphide bonds form naturally. These bonds are essential structural features of some proteins. Industrial applications almost always use the bacterial 'secretory' (Sec) pathway to export the protein product to the periplasm. This system transports the protein through the inner membrane in an unfolded state, after which the protein refolds in the periplasm. This often works very well but the system has serious limitations: some proteins fold too quickly for the Sec system to handle, and others may not fold correctly in the periplasm, which lacks the natural 'chaperone' molecules that normally help most proteins to fold in the cytoplasm.

This application aims to exploit a second bacterial protein export pathway, known as the Tat pathway. This can also export foreign proteins, but the major difference is that it transports proteins in a folded state. Importantly, it appears only to transport proteins in a correctly-folded state, and it therefore offers potential for (i) exporting proteins that the Sec pathway cannot handle, and (ii) producing products of particularly high quality, since they should be correctly folded and hance active. In a previous project, we showed that E. coli strains over-expressing Tat could export a test protein at very high rates - easily sufficient for industrial applications. This project aims to develop two important variants of these strains, each with unique properties. The project will involve collaboration between Warwick and UCL. The partnership is important: the
Warwick group are experienced in Tat studies while the UCL partner is able to rigorously test the quality of strains and their readiness for use by industry.
The first part of the project will create strains that can export prefolded proteins that are disulphide-bonded. Disulphide bonds normally only form in the periplasm, but a Finnish group has developed new E. coli strains which express a thiol oxidase that enables efficient disulphide bond formation in the cytoplasm. Recent collaborative studies have shown that three disulphide-bonded test proteins are efficiently exported by Tat if a signal peptide is attached. These strains offer a new means of producing disulphide-bonded proteins in high quantities, with the potential of generating a product of exceptional folding fidelity.
The second part of the project aims to exploit a surprising recent finding by the applicants' groups. The E. coli Tat pathway normally exports proteins to the periplasm, and the outer membrane almost invariably remains intact during fermentation processes. We have replaced the native E. coli Tat system with a Tat system from Bacillus subtilis (TatAdCd; patent application filed) and have shown that the system also exports proteins to the periplasm with high efficiency. However, during fermentation the outer membrane becomes selectively leaky, and releases periplasmic proteins into the extracellular medium ('broth'). The net result is that even in simple batch fermentations, the broth contains high levels of the protein product and this means that the product can be harvested directly from this broth without the need for extraction of the periplasm. This may be a very cost-effective new means of producing therapeutic proteins

Ivan Wall (University College London) - Commercial scale manufacture of adult allogeneic cell therapy for regenerative medicine

Summary
Commercial growth within the cell therapy industry heavily relies on development of new platform technologies that offer rapid, cost-effective bioprocessing solutions to produce large quantities of high-quality cell product. In this proposal we aim to achieve that by translating a regulator approved cell immortalization method pioneered by British company ReNeuron (Guildford, Surrey) to a new cell type to produce a cell therapy to cure spinal cord injury. Patients who suffer acute neurologic injury (such as stroke and spinal cord injury) require rapid treatment in order to prevent loss of function. In the
UK, 2,000 mostly young people become paralyzed each year due to spinal trauma. Currently there is no cure and overall 50,000 British people suffer from spinal cord injury ranging from paraplegia to complete below the neck paralysis. Such disability has major consequences for patients and also for their families, carers and the whole healthcare system. It significantly impacts on society and the UK economy due to costs associated with unemployment and social care. The average cost (direct healthcare and indirect social costs) per patient per year is in the order of £100,000. Cell therapy offers a real possibility to cure spinal cord injury if treated within a few days of the injury. As a general rule, the central nervous system does not regrow after damage. One exception is in the olfactory system where regeneration of nerves (that are destroyed for example during a common cold when

we lose our sense of smell) happens throughout life. This regeneration is possible thanks to a unique cell population called olfactory ensheathing cells (OECs) that are found in the nose and promote new neuronal connections by producing guidance channels. Over the last 20 years Prof. Raisman's group have identified and tested the ability of OECs in combination with co-located fibroblasts to regenerate the spinal cord after injury. Repeated animal studies have demonstrated safety and efficacy of this dual-cell approach and appropriate UK regulator (MHRA) and ethical approvals for a clinical trial are in place. However, there is a significant hurdle. The present method uses the patient's own (autologous) cells but treatment needs to occur in less than 14 days. This is not adequate time to take a tissue biopsy, process the cells to a therapeutic dose range, characterize and implant into the damaged spinal cord.
We will therefore make a step change to universal (allogeneic) OEC cell lines and explore parallel single cell cultures and direct co-culture options. We will use the ReNeuron approach, with advice from their Chief Scientific Office, Dr. John Sinden, to make a series of universal OEC cell lines that are easily grown in the lab and then reliably silenced upon transplantation into the patient, making it a safe option. OEC-fibroblast preparations will be produced at different ratios and we will identify key characterization and monitoring issues associated with co-culture. Preparations will be tested for potency and regenerative potential using robust high-throughput in vitro screening assays developed in our lab. GMPcompliant manufacture of potent cell line will then be initiated. This project will benefit those affected by acute spinal cord injury. Significantly, the bioprocess industry will gain a pioneering generic platform technology that has been tested in multiple cell types for several indications and gain understanding of manufacturing considerations for co-culturing of cells for therapy. Successful cell based products of the future are likely to comprise two different cell types to produce the therapeutic effect. Therefore it is crucial to derive robust co-culturing methodologies applicable to scalable GMP manufacture. This project will accelerate a cure for spinal cord injury and expand the UK's bioprocess knowledge pool and skills-base in the area of co-culture to increase the UK's commercial competitive advantage.

Phillip Wright (University of Sheffield) - Improving biopharmaceutical production in microbial systems: Engineering GlycoPEGylation in *E.coli*

Summary

We aim to produce an example therapeutic protein (medicine) in the bacterium Escherichia coli (E. coli) that can be purified and then efficiently modified to improve its biological and physical characteristics and thus overall effectiveness. Although ca. 30% of the genuinely new biopharmaceuticals (protein-based medicines) approved between 2006-10 employed E. coli, there is an opportunity to improve this host system. For example, smaller proteins or protein fragments such as antibody fragments can be made more efficiently in E. coli compared to mammalian or plant cell systems due to relatively inexpensive growth requirements, high cell densities and high protein yields. Although effective as medicines, the smaller size of these proteins means they have a higher clearance rate in humans (ie the drug is removed by the kidneys), reducing overall efficacy of the dose. This project builds on the concept of post-production modification to increase the circulatory half-life of these type of proteins (the drug lasts longer in the body). An inert, synthetic polymer, polyethylene glycol (PEG) is commonly used in industry and will be employed here - its attachment to the drug is known as PEGylation.

The target protein IFN-a2b (a member of the interferon family of medicine known as cytokines) will serve as the exemplar 'drug' for this project as it is a well understood and widely manufactured therapeutic agent. In addition, it has been PEGylated previously and it's selection has been supported by BRIC industrial partners (Lonza and Fuji Diosynth). Optimising the process of PEGylation has received a lot of attention as the efficiency directly

translates into manufacturing costs (high efficiency means reduced manufacturing costs). Traditional methods have led to random PEGylation that means many different protein forms are made, reducing productivity (and increasing costs). Several site-directed methods were subsequently proposed including one where the protein is purified from E. coli and then two enzymes (biological catalysts) are used in a separate process outside E .coli after the protein has been made (in vitro) to add a sugar (enzyme1) and then sugar-PEG (enzyme 2). The process is referred to as glycoPEGylation. This project builds on this concept and exploits the newly discovered ability of E. coli to glycosylate proteins (add sugar groups to the protein in the cell) using enzymatic machinery from another microorganism (BRIC1 - funded). By designing a sugar (glycosylation) attachment site into the protein target, we have shown that the sugar-adding (glycosylation) machinery in E. coli can recognise and add a specific sugar to the site (with IFN-a2b and other proteins such as GFP). We propose that this modified protein can be purified and then used in a one-step reaction outside the cell where PEG is added. This requires a specific enzyme that will be designed and optimised.
We will use a combination of cutting edge biological engineering techniques, now considered part of an emerging field known as synthetic biology, to manipulate E. coli to produce the modified protein target IFN-a2b. We will employ in-house metabolic engineering strategies (forward and backward/inverse) to improve yields. To improve PEGylation efficiency, the sugar acceptance site in IFN-a2b will be varied to optimise enzyme recognition of the added sugar. For rapid translation to industry the optimised cell system and protein will be tested in bioreactors which we have already shown increases antibody fragment production yields in E. coli. We wish to gain insight into how easy this product would be to manufacture (manufacturability) and we will design fermentation with E. coli and discuss this with BRIC members. For quality control, the modified IFN-a2b will be tested for biophysical stability throughout using a combination of tools. Also, cost comparisons to the existing site-directed glycoPEGylation methodology, will be performed throughout.

Xue-Feng Yuan (The University of Manchester) - Bioprocessing of high concentration protein solutions: quality by digital design approach

Summary
There is a need for underpinning research to support industrial development of novel protein therapeutics for more convenient delivery by subcutaneous injection (SC). This is an increasing priority for biopharmaceutical companies such that patients can administer the medicines at home, rather than having to visit hospital for a lengthy infusion. The challenge for bioprocessing research is to dissolve the dose of protein required in a small volume, usually 1 ml, that can be self-injected. The protein therefore must be soluble up to 300 mg/ml, and it is desirable that the liquid can be stored at 2-8 C for 2 years or more without precipitation, aggregation or other instability. In addition, the liquid must not be too viscous,otherwise the injection will require too high a pressure or may take too long to administer. There is also a need to prevent damage to the protein during the process of forcing the liquid through a narrow needle, into the tissue under the skin. The proposed research will develop methods for use by industry to screen protein formulations for viscosity and other flow properties, using small quantities of protein. This will enable methods for viscosity reduction to be developed. It is known that similar proteins differ widely (by a factor of two or more) in their viscosity at similar concentrations, and that alterations in co-solvent can reduce the viscosity of a formulation. To achieve this, we propose to apply comprehensive rheological characterisation, RheoChip rheometry, and advanced modelling as a platform, which can be used by industry to select the protein and formulation for development of the final dosage form, at an earlier stage than it is possible today. This should save time and cost in development of many new protein medicines. The research will build on existing methods, which are already well-established for rheological characterisation of water soluble polymers and BSA solutions, and adapt and apply them to the bioprocessing and injectability of high concentration protein biopharmaceutical solutions.

Comprehensive rheological characterisation of protein solutions has not yet been published. In addition, there is the potential for this new knowledge to be applied in industry to improve the production of biopharmaceutical proteins, as high concentrations may be reached during bioprocessing, e.g. freeze drying, tangential flow filtration (TFF) etc. and there can be difficulties in processing viscous solutions, e.g. nanofiltration for virus removal may be impractical. The deliverables of the project will be the form of instrumentation, rheological characterisation methods more relevant than current viscosity measurement, and computational tools.

The project has five work packages (WPs). WP1 and WP2 will focus on development of new enabling technologies. The output of WP1 will be the first high throughput characterisation platform for screening protein formulations under the flow conditions encountered in bioprocessing while requiring minimal sample. WP2 will construct a computational platform for predictive modelling of concentrated protein fluid flows. WP3 and WP4 will critically validate these enabling technologies using both model and industrially relevant protein solutions under the complex flows, including TFF and SC injection. The output will be an integrated approach for design and optimisation of (nonlinear) scale-up protein production, based on high throughput rheological data obtained from Rheo-chip and predictive modelling of protein flows and protein stability during processing. WP5 will correlate the rheological properties and flow behaviour of concentrated protein solutions with the effects of excipients and/or formulation conditions on the conformational stability and self-association in dilute solution. This will help to establish the molecular determinants of high viscosities and flow induced protein aggregation leading to rational design of high throughput screens.

BRIC 2.2 PROJECT FURTHER DETAILS (12 projects)

Bernadette Byrne (Imperial College London) - Application of ATR-FTIR to industrial scale production of therapeutic antibodies

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Biopharmaceuticals, proteins used as drugs, are an emerging area in the treatment of a range of diseases. The large scale production of these molecules requires a number of discrete steps including recombinant expression, isolation by a technique called column chromatography and development of an optimal final formulation. The isolation of the biopharmaceuticals from all other contaminating material usually involves a number of steps. The column chromatography used exploits a specific interaction between the target protein and another molecule immobilized on a support, the resin.
Ideally all the contaminating material is washed from the column and the conditions are changed in order to release the protein from the resin. Optimal binding, washing and release steps often require significant changes in the properties of the surrounding solution, for example pH and ionic strength. In addition, it is possible to bind very high concentrations of the protein onto these resins. The concentrations reached are likely to be higher than those achieved at any other point in the production process. It is not known exactly what effects the different solution conditions and protein concentrations have on the quality of the protein. Indeed they may result in highly undesirable effects such as non-specific aggregation. Another key issue that scientists isolating biopharmaceuticals encounter is the binding of unwanted contaminant materials which reduces the ability of the column to isolate the target protein. Regular replacement of the resin significantly increases the cost of the isolation process and thus of biopharmaceutical production. Here we aim to investigate these issues with a view to improving isolation protocols using a specialized technique called Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. This technique allows us to chemically image (take chemical photographs of) the target protein molecules under different conditions both in static droplets and in dynamic systems similar to those used for large scale isolation. We can also directly image used resin with a view to understanding what contaminating molecules reduce the useful lifetime of the material. We anticipate that the results of these experiments will inform optimization of isolation protocols to both minimize target protein losses on-column and increase resin lifetime.

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works and demonstrate that the technology can be implemented in an industrial setting (i.e. scalable).

Paul Dalby (University College London) - Multi-modal fluorescence spectroscopy for online analysis of proteins in bioprocesses

Summary
Biopharmaceutical proteins are typically purified from clarified fermentation broths using multiple chromatographic steps where each separates the proteins based on one or more physico-chemical feature such as net charge, size, hydrophobicity and biological affinity. The elution peak containing the native protein product at each step is often contaminated by host cell proteins, or with slightly modified versions of the product which can be almost indistinguishable from the native form and therefore very challenging to remove. The precise product profile at each step is therefore very sensitive to small changes in upstream variability, buffer composition and pH, and the gradual fouling of chromatographic resins that affects performance through repeated re-use. It is therefore imperative to be able to monitor the product profile, preferably in-line or on-line, to be able to adjust the process parameters in real time, or to make a responsive decision as to when to start and stop collecting the product fraction within the elution peak. Real time, low cost and low volume analysis of proteins and protein mixtures suitable for online monitoring of chromatography, is generally limited to simple absorbance, refractive index and conductivity measurements which only provide basic peak detection and no detailed characterisation of the protein product profile. Multi-angle laser light scattering (MALLS) has some potential for online characterisation of approximate molecular masses, whereas accurate online mass-spectrometry is too expensive and technically demanding. We will take advantage of our recent advances in both the biophysical analysis and rapid laser-induced temperature perturbations of unlabelled proteins in microcapillary flow, as well as state-of-the-art optical components, to establish a low volume flow-detector for use in chromatography, that can evaluate the heterogeneity of the protein product profile in real time. A single set of optics for the intrinsic fluorescence of proteins will be set up to measure fluorescence intensity (FLI), time-resolved fluorescence (TRF) and fluorescence correlation spectroscopy (FCS) and so simultaneously characterise orthogonal features of the protein product profile. These will measure protein quantity (peak detection), and detect underlying variability in solution conformation, oligomeric state, and particle sizes, including the soluble aggregates. FCS is more sensitive and quantitative for relative particle concentrations than DLS or MALLS which are disproportionately sensitive to larger particles. To further resolve product heterogeneity, the flowing sample (continuously split from the main elution stream) will also be subjected to a rapid (12 ms) temperature jump of up to 70degC using our recently demonstrated microfluidic IR-induced heating technology. This will induce partial structural unfolding of protein domains, and the dissociation of soluble oligomers, with kinetics and amplitudes that are characteristic to each different protein species in the sample, and thus provide further online resolution of the sample complexity relative to known reference standards or previous elution profiles. As an additional bonus, the detector will also be suitable for standalone sample analysis, such as for the profiling of dosage formulations and their viscosities. The benefits of each new spectroscopic mode will be demonstrated for a wide range of relevant proteins from our lab and from other BRIC members, including IgG, Fab, GCSF. To test the range of heterogeneity that can be detected, these will be syringe-pumped into the detector and analysed in partially and fully purified forms, and also after deliberate modification by partial proteolysis, partial unfolding and aggregation at low pH, oxidation, and shear damage. Online application to chromatography will then be demonstrated using a flow splitter to give a continuous flow into the detector in parallel to fraction collection.

Alan Dickson (The University of Manchester) - Application of single cell metabolite profiling to optimisation of stem cell bioprocessing

Summary
The potential of stem cells as treatments for disease appears in the public arena with increasing frequency. Fundamentally, these are cells that may have the ability to be switched to take on the function of cells within critical roles in the body. Consequently, when the normal cells are damaged, as in heart attacks or paracetamol-induced liver damage, the stem cells should have the ability, as a transplant, to take up the function of the heart or liver cells. The scope exists for stem cell treatments to cure/improve the well-being of patients suffering from life-threatening/-changing conditions. Tremendous advances have been made but a number of key hurdles must be overcome before there is a more complete realisation of the undisputed potential. Chief amongst these is the fact that stem cells do have the potential to take on the functions of many different cell types and the control of this process is not fully understood. Placing this in to the context of our present proposal, the loss of insulin-production by cells of the pancreas generates diabetes. This can be treated by insulin injection, with all the consequences for monitoring and life-style, but the possibility of transplantation of insulin-producing cells to patients offers a vision of cure, rather than therapy. Hence, the key challenge is to understand the processes that control the change from stem cell to a pancreatic cell. This is against a background of stem cells undergoing changes towards many cell types - nerve, liver, muscle, kidney, heart - and generating a mixture of cell types simultaneously. Transplantation of a mixture of cells, rather than the insulin-secreting cell would not be effective. This aspect remains a major limitation for the potential of stem cell therapy to become a reality and it is the focus for this proposal. Our proposal will apply novel approaches to look at stem cells as individual cells using our pancreatic cell lines as a powerful model system. The reason this work is important is that it aims to take "fingerprints" of the metabolism of each cell as an individual entity, enabling us to relate a "fingerprint" with the controlling events that determine if a cell becomes nerve, liver, pancreas etc. The information will aid us to identify processes for maintenance of cells in whatever functional state is desired and to select for cells with a particular functional state. This type of processes can be applied to large-scale generation of cells with defined function - taking the production of cells to the commercial level that will move us towards manufacture of cells in sufficient quantity, as well as quality, to use as real-life medicines. Our approach is one that is unique due to the collaborative positioning of research groups with disruptive new technologies and model cell lines for effective development of a more general technology

Nikolaj Gadegaard (University of Glasgow) - Development of nanopatterned substrates for the delivery of high quality stem cells

Summary
The use of stem cells in regenerative medicine holds great potential and with an increasingly aging population, we need to look for new opportunities. Their potential use span from orthopaedic applications such as arthritis and osteoporosis to neurodegenerative disorders such as Parkinson's and Alzheimer's, to name a few. The body has a constant source of stem cells located in niches within the body. From a scientific and clinical point of view, one of the most exploited sources for adult stem cells is the bone marrow. The bone marrow is relatively easy to access and stem cells can be easily isolated from the extracted cell population. However, until recently, a major hurdle is that the stem cells cannot be cultured for extended periods in culture and maintain their regenerative potential (multipotent). The very potential of stem cells, that they can change into many different cell types and so help repair damage on demand, means that their profile (phenotype) is unstable in culture.

Hence, as we culture stem cells they soon lose the very potential and potency we want to exploit. We have recently (2011) demonstrated that by culturing the cells on a uniquely nanopatterned surface (nanopits, 100 nm in diameter and 100 nm deep, arranged in a square lattice) it is indeed possible to keep the cells in the multipotent state in prolonged culture as well as expand the number of cells. These nanopatterned surfaces resemble the tracks on a Blu-Ray disc and indeed our technology is very similar to the production of optical media where nanopatterns can be injection moulded into polymer discs in high volumes and at a very low cost. We can change the arrangement of the nanopatterns, thereby tuning the stem cell response to growth without profile (phenotype) drift and to target desired changes to tissues we want (known as differentiation). This is has important implications on the design of implants (like a hip replacement implants) where a specific cell changes (differentiation) is desirable. An example of this is again, orthopaedic implants where differentiation of cells to bone is desirable, or an area with perhaps even more potential is the growth of large numbers of stem cells. Thus, a key research goal is to take a patient's stem cells, grow them in the laboratory to useful numbers, and then place them back into the patient to spark regeneration. Scale up of our technology will allow this.
The technology we have used so far has only allowed us to explore the stem cell interaction to a very limited number of different geometries (<10). In this proposal we will develop a new platform where a single sample will contain 1000 different patterns thereby allowing us to investigate a much larger library of nanopatterns and their ability to influence the fate of the stem cells. From these libraries new patterns will be identified and we will investigate them in more detail using mass spectrometry to identify small molecules influencing the cell fate. Importantly, to see real benefit of these discoveries, it is vital that we are able to scale the materials used to large areas to sustain the growth and expansion of stem cells used for regenerative medicine or pharma. As described above, our technology is very similar to the production of DVDs and Blu-Rays, which means that it lends itself to a cost effective mass production of the nanopatterned surfaces. To demonstrate this potential, we will expand extracted bone marrow stem cells to 5 million cells, the number of cells used for the fully tissue engineered trachea demonstrated in 2008.

Chris Hewitt (Loughborough University) - Expansion of human mesenchymal stem cells in aqueous / aqueous two phase systems

Summary

In order that people can live longer and more active lives there is a need to develop new affordable and effective medicines. In some cases cells that we have within our own bodies can be used to repair damaged tissues. However, in adults, this repair mechanism is very limited and often inefficient. Stem cells produced by the body are those that can go on to make all of the different types of cells in the human body and these so called 'stem cells' when harvested have the potential to repair many types of diseased tissue in adults. Although stem cells can now be grown in laboratories, one highly trained person can only grow a few million cells in a week. Since it takes 5 billion heart cells to repair the heart muscle of a heart-attack patient, growing these cells at laboratory scale is useful for research but not for treating multiple patients in practise. This project aims to combine the expertise of both biologists and engineers, to create scalable system for the "manufacture" of large numbers of stem cells so the potential of stem cell therapies can be realised. Once enough stem cells can be routinely grown and harvested, it is more likely that they can begin to treat a variety of diseases.

David James (University of Sheffield) - Linking recombinant gene sequence to protein product manufacturability using CHO cell genomic resources

Summary

Biopharmaceutical companies producing the new generation of recombinant DNA derived therapeutic proteins (e.g. cancer medicines such as Herceptin and Avastin) often use mammalian cells grown in culture to make the protein product. All production processes are based, fundamentally, upon the ability of the host mammalian cell factory to use a synthetic DNA genetic "code" to manufacture the complex protein product. This is a cornerstone of modern biotechnology. However, because protein synthesis is so complex, involving many cellular resources and machines, it is extremely difficult for genetic engineers to design a DNA code that will best enable the mammalian cell factory to operate most efficiently. Moreover, as individual mammalian cell factories can be very variable, they may differ substantially in their relative ability to make the product. As a consequence, a lot of time and money has to be spent by companies on the initial phases of the biopharmaceutical development process conducting intensive screening operations to find the best cell factory (out of a large population) able to use the genetic code it has been given. For a different protein product it is necessary to start the whole development process again. In this project we will utilise recently available high information content molecular analysis technologies and computational tools to "de-convolute" the complexity of protein synthesis in mammalian cell factories. Effectively, we know that the mammalian cell factory uses its own genetic code to make thousands of its own proteins (machines) that together perform a variety of functions that enable the cell to grow and divide. The rate at which these proteins are made varies hugely, over 1000-fold, so that the cell can make each bit of protein machinery in the right quantity to do its job. We will measure how efficiently each cellular protein is made then using advanced biological information analysis (bioinformatics) and mathematics we will determine how the cell uses pieces of information embedded in each of its genes to vary the rate at which a specific protein is made. This will enable us to create, for the first time, a usable set of "design rules" (computer programmes) that genetic engineers and cell factory developers can employ to (i) reliably design the best genetic code for any given protein product and (ii) accurately predict how much of the protein product the mammalian cell factory can make. This is important as it means that biopharmaceutical companies can design a predictable production system from scratch, enabling a more rapid transition through lengthy cell factory development processes towards (pre-)clinical trials

Stephen Oliver (University of Cambridge) - Development of an integrated continuous process for recombinant protein production using *Pichia pastoris*

Summary

This project will build on the successes of our two projects in phase 1 of the Bioprocessing Research Industry Club (BRIC1) to develop a system for the growth-associated production of commercially important protein products in a commerciallyimportant production organism - the yeast Pichia pastoris. Proteins, particularly antibodies, are now playing a major role in the treatment of human disease. They are usually produced using animal cells in culture, a procedure that is both costly and time-consuming. An attractive alternative is to engineer a yeast cell to produce human proteins of therapeutic value. Pichia pastoris is often used for this purpose since it may be grown at high cell densities and has an efficient secretion system to release the protein product from the cells. Current processes induce protein production by repeatedly adding methanol to the yeast cultures. Our studies in BRIC1 demonstrated that this is exactly the wrong way to go about this since the cells are repeatedly stressed and produced badly folded proteins that have low biological activity and are not secreted out of the cells. What is required is a continuous process for protein production and recovery and this is what our BRIC2 project aims to achieve. The product proteins to be studied will be guided by our contacts within the BRIC member companies and include a number of proteins used in human therapies. Growth-associated production will enable continuous processes and thus increase commercial productivity. Computer models developed in BRIC1 will be improved, extended, and used to control the novel process. Moreover, the fast protein separation methods that we also developed in BRIC1, will permit the continuous retrieval of product, thus obviating the major barrier to the commercial adoption.

Colin Robinson (University of Kent) - Development of new-generation bacterial secretion process platforms

Summary
Many important therapeutic products are proteins - often termed biopharmaceuticals - that have to be produced in a living organism and then purified. Over 30% of the currently licensed therapeutic proteins are made in the bacterium Escherichia coli, which can be quickly grown in large amounts. Some of these proteins are synthesised in the cell interior (cytoplasm) but a favoured strategy is to 'export' the protein product to the periplasmic space between the two cell membranes. The reasons are two-fold. First, the contents of the periplasm can be extracted relatively easily, by selectively rupturing the outer membrane. Secondly, the periplasm is an oxidising environment, and is thus the only place where disulphide bonds form naturally. These bonds are essential structural features of some proteins. Industrial applications almost always use the bacterial 'secretory' (Sec) pathway to export the protein product to the periplasm. This system transports the protein through the inner membrane in an unfolded state, after which the protein refolds in the periplasm. This often works very well but the system has serious limitations: some proteins fold too quickly for the Sec system to handle, and others may not fold correctly in the periplasm, which lacks the natural 'chaperone' molecules that normally help most proteins to fold in the cytoplasm.
This application aims to exploit a second bacterial protein export pathway, known as the Tat pathway. This can also export foreign proteins, but the major difference is that it transports proteins in a folded state. Importantly, it appears only to transport proteins in a correctly-folded state, and it therefore offers potential for (i) exporting proteins that the Sec pathway cannot handle, and (ii) producing products of particularly high quality, since they should be correctly folded and hance active. In a previous project, we showed that E. coli strains over-expressing Tat could export a test protein at very high rates - easily sufficient for industrial applications. This project aims to develop two important variants of these strains, each with unique properties. The project will involve collaboration between Warwick and UCL. The partnership is important: the
Warwick group are experienced in Tat studies while the UCL partner is able to rigorously test the quality of strains and their readiness for use by industry.
The first part of the project will create strains that can export prefolded proteins that are disulphide-bonded. Disulphide bonds normally only form in the periplasm, but a Finnish group has developed new E. coli strains which express a thiol oxidase that enables efficient disulphide bond formation in the cytoplasm. Recent collaborative studies have shown that three disulphide-bonded test proteins are efficiently exported by Tat if a signal peptide is attached. These strains offer a new means of producing disulphide-bonded proteins in high quantities, with the potential of generating a product of exceptional folding fidelity.
The second part of the project aims to exploit a surprising recent finding by the applicants' groups. The E. coli Tat pathway normally exports proteins to the periplasm, and the outer membrane almost invariably remains intact during fermentation processes. We have replaced the native E. coli Tat system with a Tat system from Bacillus subtilis (TatAdCd; patent application filed) and have shown that the system also exports proteins to the periplasm with high efficiency. However, during fermentation the outer membrane becomes selectively leaky, and releases periplasmic proteins into the extracellular medium ('broth'). The net result is that even in simple batch fermentations, the broth contains high levels of the protein product and this means that the product can be harvested directly from this broth without the need for extraction of the periplasm. This may be a very cost-effective new means of producing therapeutic proteins

Ivan Wall (University College London) - Commercial scale manufacture of adult allogeneic cell therapy for regenerative medicine

Summary
Commercial growth within the cell therapy industry heavily relies on development of new platform technologies that offer rapid, cost-effective bioprocessing solutions to produce large quantities of high-quality cell product. In this proposal we aim to achieve that by translating a regulator approved cell immortalization method pioneered by British company ReNeuron (Guildford, Surrey) to a new cell type to produce a cell therapy to cure spinal cord injury. Patients who suffer acute neurologic injury (such as stroke and spinal cord injury) require rapid treatment in order to prevent loss of function. In the
UK, 2,000 mostly young people become paralyzed each year due to spinal trauma. Currently there is no cure and overall 50,000 British people suffer from spinal cord injury ranging from paraplegia to complete below the neck paralysis. Such disability has major consequences for patients and also for their families, carers and the whole healthcare system. It significantly impacts on society and the UK economy due to costs associated with unemployment and social care. The average cost (direct healthcare and indirect social costs) per patient per year is in the order of £100,000. Cell therapy offers a real possibility to cure spinal cord injury if treated within a few days of the injury. As a general rule, the central nervous system does not regrow after damage. One exception is in the olfactory system where regeneration of nerves (that are destroyed for example during a common cold when we lose our sense of smell) happens throughout life. This regeneration is possible thanks to a unique cell population called olfactory ensheathing cells (OECs) that are found in the nose and promote new neuronal connections by producing guidance channels. Over the last 20 years Prof. Raisman's group have identified and tested the ability of OECs in combination with co-located fibroblasts to regenerate the spinal cord after injury. Repeated animal studies have demonstrated safety and efficacy of this dual-cell approach and appropriate UK regulator (MHRA) and ethical approvals for a clinical trial are in place. However, there is a significant hurdle. The present method uses the patient's own (autologous) cells but treatment needs to occur in less than 14 days. This is not adequate time to take a tissue biopsy, process the cells to a therapeutic dose range, characterize and implant into the damaged spinal cord.
We will therefore make a step change to universal (allogeneic) OEC cell lines and explore parallel single cell cultures and direct co-culture options. We will use the ReNeuron approach, with advice from their Chief Scientific Office, Dr. John Sinden, to make a series of universal OEC cell lines that are easily grown in the lab and then reliably silenced upon transplantation into the patient, making it a safe option. OEC-fibroblast preparations will be produced at different ratios and we will identify key characterization and monitoring issues associated with co-culture. Preparations will be tested for potency and regenerative potential using robust high-throughput in vitro screening assays developed in our lab. GMPcompliant manufacture of potent cell line will then be initiated. This project will benefit those affected by acute spinal cord injury. Significantly, the bioprocess industry will gain a pioneering generic platform technology that has been tested in multiple cell types for several indications and gain understanding of manufacturing considerations for co-culturing of cells for therapy. Successful cell based products of the future are likely to comprise two different cell types to produce the therapeutic effect. Therefore it is crucial to derive robust co-culturing methodologies applicable to scalable GMP manufacture. This project will accelerate a cure for spinal cord injury and expand the UK's bioprocess knowledge pool and skills-base in the area of co-culture to increase the UK's commercial competitive advantage.

Phillip Wright (University of Sheffield) - Improving biopharmaceutical production in microbial systems: Engineering GlycoPEGylation in *E.coli*

Summary

We aim to produce an example therapeutic protein (medicine) in the bacterium Escherichia coli (E. coli) that can be purified and then efficiently modified to improve its biological and physical characteristics and thus overall effectiveness. Although ca. 30% of the genuinely new biopharmaceuticals (protein-based medicines) approved between 2006-10 employed E. coli, there is an opportunity to improve this host system. For example, smaller proteins or protein fragments such as antibody fragments can be made more efficiently in E. coli compared to mammalian or plant cell systems due to relatively inexpensive growth requirements, high cell densities and high protein yields. Although effective as medicines, the smaller size of these proteins means they have a higher clearance rate in humans (ie the drug is removed by the kidneys), reducing overall efficacy of the dose. This project builds on the concept of post-production modification to increase the circulatory half-life of these type of proteins (the drug lasts longer in the body). An inert, synthetic polymer, polyethylene glycol (PEG) is commonly used in industry and will be employed here - its attachment to the drug is known as PEGylation.

The target protein IFN-a2b (a member of the interferon family of medicine known as cytokines) will serve as the exemplar 'drug' for this project as it is a well understood and widely manufactured therapeutic agent. In addition, it has been PEGylated previously and it's selection has been supported by BRIC industrial partners (Lonza and Fuji Diosynth). Optimising the process of PEGylation has received a lot of attention as the efficiency directly translates into manufacturing costs (high efficiency means reduced manufacturing costs). Traditional methods have led to random PEGylation that means many different protein forms are made, reducing productivity (and increasing costs). Several site-directed methods were subsequently proposed including one where the protein is purified from E. coli and then two enzymes (biological catalysts) are used in a separate process outside E .coli after the protein has been made (in vitro) to add a sugar (enzyme1) and then sugar-PEG (enzyme 2). The process is referred to as glycoPEGylation. This project builds on this concept and exploits the newly discovered ability of E. coli to glycosylate proteins (add sugar groups to the protein in the cell) using enzymatic machinery from another microorganism (BRIC1 - funded). By designing a sugar (glycosylation) attachment site into the protein target, we have shown that the sugar-adding (glycosylation) machinery in E. coli can recognise and add a specific sugar to the site (with IFN-a2b and other proteins such as GFP). We propose that this modified protein can be purified and then used in a one-step reaction outside the cell where PEG is added. This requires a specific enzyme that will be designed and optimised.
We will use a combination of cutting edge biological engineering techniques, now considered part of an emerging field known as synthetic biology, to manipulate E. coli to produce the modified protein target IFN-a2b. We will employ in-house metabolic engineering strategies (forward and backward/inverse) to improve yields. To improve PEGylation efficiency, the sugar acceptance site in IFN-a2b will be varied to optimise enzyme recognition of the added sugar. For rapid translation to industry the optimised cell system and protein will be tested in bioreactors which we have already shown increases antibody fragment production yields in E. coli. We wish to gain insight into how easy this product would be to manufacture (manufacturability) and we will design fermentation with E. coli and discuss this with BRIC members. For quality control, the modified IFN-a2b will be tested for biophysical stability throughout using a combination of tools. Also, cost comparisons to the existing site-directed glycoPEGylation methodology, will be performed throughout.

Xue-Feng Yuan (The University of Manchester) - Bioprocessing of high concentration protein solutions: quality by digital design approach

Summary

There is a need for underpinning research to support industrial development of novel protein therapeutics for more convenient delivery by subcutaneous injection (SC). This is an increasing priority for biopharmaceutical companies such that patients can administer the medicines at home, rather than having to visit hospital for a lengthy infusion. The challenge for bioprocessing research is to dissolve the dose of protein required in a small volume, usually 1 ml, that can be self-injected. The protein therefore must be soluble up to 300 mg/ml, and it is desirable that the liquid can be stored at 2-8 C for 2 years or more without precipitation, aggregation or other instability. In addition, the liquid must not be too viscous,otherwise the injection will require too high a pressure or may take too long to administer. There is also a need to prevent damage to the protein during the process of forcing the liquid through a narrow needle, into the tissue under the skin. The proposed research will develop methods for use by industry to screen protein formulations for viscosity and other flow properties, using small quantities of protein. This will enable methods for viscosity reduction to be developed. It is known that similar proteins differ widely (by a factor of two or more) in their viscosity at similar concentrations, and that alterations in co-solvent can reduce the viscosity of a formulation. To achieve this, we propose to apply comprehensive rheological characterisation, RheoChip rheometry, and advanced modelling as a platform, which can be used by industry to select the protein and formulation for development of the final dosage form, at an earlier stage than it is possible today. This should save time and cost in development of many new protein medicines. The research will build on existing methods, which are already well-established for rheological characterisation of water soluble polymers and BSA solutions, and adapt and apply them to the bioprocessing and injectability of high concentration protein biopharmaceutical solutions. Comprehensive rheological characterisation of protein solutions has not yet been published. In addition, there is the potential for this new knowledge to be applied in industry to improve the production of biopharmaceutical proteins, as high concentrations may be reached during bioprocessing, e.g. freeze drying, tangential flow filtration (TFF) etc. and there can be difficulties in processing viscous solutions, e.g. nanofiltration for virus removal may be impractical. The deliverables of the project will be the form of instrumentation, rheological characterisation methods more relevant than current viscosity measurement, and computational tools.

The project has five work packages (WPs). WP1 and WP2 will focus on development of new enabling technologies. The output of WP1 will be the first high throughput characterisation platform for screening protein formulations under the flow conditions encountered in bioprocessing while requiring minimal sample. WP2 will construct a computational platform for predictive modelling of concentrated protein fluid flows. WP3 and WP4 will critically validate these enabling technologies using both model and industrially relevant protein solutions under the complex flows, including TFF and SC injection. The output will be an integrated approach for design and optimisation of (nonlinear) scale-up protein production, based on high throughput rheological data obtained from Rheo-chip and predictive modelling of protein flows and protein stability during processing. WP5 will correlate the rheological properties and flow behaviour of concentrated protein solutions with the effects of excipients and/or formulation conditions on the conformational stability and self-association in dilute solution. This will help to establish the molecular determinants of high viscosities and flow induced protein aggregation leading to rational design of high throughput screens.