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.