Center for Bioprocess Innovation

A Center for Bioprocess Innovation headquartered at the University of Maryland provides a unique research base for bioprocessing research. Bioprocess engineers at the University of Maryland College Park (UMCP) and other Maryland research universities have the expertise in miniaturization, biochemical engineering, and bioprocess development to overcome the resource-draining roadblocks that hinder the development of new drugs.

BACKGROUND

Bioprocessing, also known as bioprocess engineering, is the sub-discipline of biotechnology involved in the production of biological products. Bioprocess engineers work with live cells extracted from mammals, insects, bacteria, plants and fungi to manufacture molecules that we cannot produce by synthetic chemical processes. For more than two decades, cell-based production methods have been the manufacturing foundation of the biotechnology industry.

Basic production methods are currently used to produce antibodies, enzymes, and other proteins, but it typically takes more than ten years and $1 billion to bring a new therapeutic to market. Innovative bioprocessing solutions can overcome the bottlenecks that increase the time and the costs of drug development; the research universities of Maryland support researchers with the expertise and the facilities to develop and test such creative bioprocessing innovations.

Several investigators in the Maryland area are eager to contribute to a Center for Bioprocess Innovation: Dr. William Bentley- University of Maryland College Park (UMCP)/University of Maryland Biotechnology Institute(UMBI), Dr. Michael Betenbaugh- John Hopkins University (JHU), Dr. Mark Marten- University of Maryland, Baltimore County (UMBC), Dr. Greg Payne (UMBI), and Dr. Govind Rao (UMBC). These researchers have access to state-of-the-art facilities. The Bioprocess Scaleup Facility (BSF) at UMCP is one of few facilities in the U.S. to offer complete pilot-scale bioprocessing. Further, the Current Good Manufacturing Practices (cGMP) facility of UMBI is available for both training and manufacturing. The bioprocessing capabilities at the nearby National Cancer Institute (NCI) enable full-scale production of new products.

Issues to be Addressed:

1. Unit operations - difficult to express products; separation and selection of closely related proteins; post-translational modifications

The products of biomanufacturing are often structural or functional proteins. Some proteins are difficult to express, either because they are normally expressed in very low quantities, or because of conditions in the bioreactor which are inimical to production. Approaches to resolve this issue include traditional **biochemical engineering (**Rao, Marten**)** – manipulating conditions in the bioreactor – and genetic and **metabolic engineering** (Bentley, Betenbaugh) – altering the actual biochemical pathways of the organism. Proteins are currently separated based on charge and size using many techniques. Proteins which are very similar may therefore be difficult to separate, and multi-dimensional protein separation techniques are needed (Payne). Minor alterations to a protein such as glycosylation (adding sugars) can have an enormous effect on the function of a protein, and manufacturing processes must account for this.

Increasingly greater demands have been placed on a cell's protein synthesis machinery that have come from enhanced analytical techniques that define regions of the protein that confer function. That is, the exact glycosylation pattern for enabling function can be defined and now its synthesis must be controlled. **Cellular engineering** methods (Bentley, Betenbaugh) have emerged to build and control biocatalytic functions that are tailored for the specific protein of interest. Importantly, there is increased realization that the product's host cell might also be a product of the process development program. Thus, a tailored cell line is created for a specific protein product. The assembled team is expert in designing cell lines and engineering cell systems for optimized synthesis of drugs with native biological activity.

2. High Content Methods for Identifying Best Drug Candidates

How does this become generalized for any protein of interest? How do we "discover" the best unit operations processes (fermentation, cell culture, lysis, separation, purification, etc.) for a specific protein of interest? A solution we propose is to create tools and devices that efficiently scale down all these processes so that they can be interrogated in parallel. This includes microbioreactors, microseparators, micropurifiers, that are all on-chip and built to enable parallel testing of candidate drugs, host cell systems, bioprocess unit operations, and bioprocessing conditions, all at the same time. Importantly, scaling parameters must be developed that enable the translation of processing information at the microscale to predict full-scale production methods.

We are faced with a rapidly approaching untenable situation in human health. Blockbuster drugs, which cure major diseases afflicting huge populations, are being pulled from the shelves (e.g.,

Vioxx). They are being replaced by drugs that have smaller market potential and more localized impact (e.g., FluMist). The current cost of developing a drug and getting it to market exceeds $1 billion and the process takes over ten years. These competing forces cannot be resolved without truly transformational changes in the way drugs are discovered, developed and approved.

The promise of the human genome project is in developing drugs that are tailored for individuals – matching their own genetic makeup and behavioral lifestyle. Even personalized regenerative medicine is rapidly becoming realized.

The Center for Bioprocess Innovation (CBpI) program will transform the way we develop drugs. This is accomplished by bringing biological systems, synthetic biology, and microfabricated devices into the mainstream for accelerating clinical testing and bioprocess development. We will reduce the process to practice in specific ways and develop methodologies that will be broadly applicable. Drug companies have already developed high throughput techniques for identifying drug targets and lead candidates; they have not, however, developed high throughput methods for testing efficacy and process development.

Our approach combines microfabrication and systems biology for creating experimental platforms that will be used to characterize component biological systems. By creating component structures, we will move this industry closer to an in silico process – which has widely been cited as necessary.

Microfabrication also has much to offer. It is capable of generating devices by localizing components of ever-decreasing size, and these capabilities are being extended from two dimensions (printed circuit boards) to three dimensions (microfluidic networks). Microfabricated devices can be constructed for near-instantaneous signal processing – to impose stimuli, to detect response, and to transmit this information. Microfabricated systems can be constructed of immense complexity – where optical, electrical, pneumatic and fluidic systems are all integrated "on-chip" to enable high throughput, massively-parallel operation.

SPECIFIC CBpI PROJECTS

1. Quorum sensing.

The ability of a population of cells to communicate with each other is a function of their aggregate metabolic activity and ability to sense the environment, and is referred to as quorum sensing. Quorum sensing, which enables single-celled organisms to behave as a multicellular unit, has enormous effects on both cell growth and protein production because one grows the host cells in large-scale high density systems and their control is of paramount importance. This project would identify the control factors inherent in quorum sensing and permit the manipulation of metabolic pathways, hence the production process to be automated by the cells.

2. Flexible biomanufacturing of high value products.

Cells can be programmed to produce any of a large number of protein products on demand. A combination of microfluidics and metabolic engineering will permit the on-demand production of a range of high-value biological products in theatre.

3. Hybrid bioreactor production and separation.

Product proteins are tailored so that they can be separated and purified directly from cell extracts using novel enzymatic, on-chip, signal-directed unit operations. Reducing the footprint of the production process while maintaining efficiency and flexibility would be the focus of this effort.

4. Multi-dimensional protein separation.

Using physico-chemical dimensions beyond charge and size, this project will focus on recovery of closely related and/or hard-to-separate protein products, allowing for the production of protein which were heretofore impossible to obtain. Miniaturizing the process will allow selections to be made within hours rather than weeks, speeding the manufacturing process and reducing costs.

6. Translation to full scale processes and GMP manufacturing.

Processing parameters are non-dimensionalized and run at small scale using the same processing parameters as large scale. That is, large-scale systems have optimal and suboptimal operations; these can all be scaled down so that the same optimal and suboptimal conditions are reproduced at the micro-scale. Tests can then be performed at the micro-scale which will then be scaled back up to the pilot scale at the BSF and at the GMP manufacturing facilities.

The entire scenario combines state-of-the-art research on cellular and metabolic engineering, MEMS and microfluidics, bioprocessing, with high quality full-scale bioprocessing efforts common to many large pharmaceutical houses and biotechnology companies.

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