Frontiers in Biotechnology 2013
Contents

25 February 2013

Manuel Carrondo
General Introduction to Animal Cell Technology

- Short introduction, historical perspective and future outcomes.
- Overview on criteria definition and concept driven bioprocess integration.

Paula Alves
Animal Cell Cultures in Biomedical Research

- Animal cells *in vitro* cultures (type, medium, culture conditions).
- Selection and design of bioreactors for animal cell culture (adherent versus suspension; operation modes).
- Case studies - Animal cells as tools for (i) research and for (ii) production of biopharmaceuticals.
- Stem cells (SCs) as cellular resources for cell therapy, tissue engineering, drug discovery and toxicology will also be addressed. Major challenges in the development of efficient systems for *in vitro* stem cell expansion and differentiation will be discussed.

Ana Sofia Coroadinha
Vectors design and cell line engineering

Overview on animal cell expression systems and its methodologies. Common Animal Cell Lines for protein expression. Stable vs. Transient expression systems. Expression Vector design and Genetic elements in expression cassettes. Selection Markers. Transfection Methods. Cell Engineering

25 February and 26 February 2013

Catarina Brito & Margarida Serra
Cell therapy

Tutorial
The aim of the "Cell Therapy" tutorial is to provide opportunities for the PhD students to earn knowledge on:
i) the clinical need for stem cell therapy and tissue engineering – stem cells as models for pre-clinical research and regenerative medicine applications;

ii) the basics of stem cell biology and cell therapy;
iii) bioprocessing stem cell for clinical application: quality control analysis and strategies for stem cell expansion, controlled differentiation and cryopreservation.

26 February 2013

Catarina Duarte
Drug delivery – challenges and strategies

- Introduction to Drug Delivery.
- Administration routes and the importance of adequate Drug Delivery Systems (DDS).
- Nanotechnology applied to Drug Delivery - Nanoparticles and nanoformulations
- Solid material processing and Preparation of DDS using SCFTechnology - particle formation methodologies and impregnation by adsoption
- Preparation of DDS - examples focused on Polymeric systems, Solid Lipid micro and nanoparticles, Hybrid structures and Microbubbles.

27 February 2013

Ana Luísa Simplício
Biological targets for prodrug strategy

Prodrugs are compounds with no pharmacological activity per se but which transform into active ingredients upon a metabolic transformation. They are prepared to circumvent solubility, drug delivery, bioavailability, targeting or side effect problems among others. The biotransformation may be accomplished by means of a chemical or enzymatic reaction. This module deals with the prodrug strategy for bioavailability enhancement and the biological targets usually used to release the therapeutic agent

Júlia Costa
Glycoproteomic technology for biomarker discovery

Definition and importance of biomarkers. Sample types for glycoprotein biomarker discovery. Glycan biomarkers. Glycoproteomic technology for biomarker identification. Use of lectins in glycoprotein detection and isolation. Glycan profiling. Structure analysis of protein glycosylation. Exoglycosidases. Glycan microarray technology.

Abel Oliva
Biosensors

Principles of biosensor function. Bioreceptors and bioaffinity. Transduction systems: electrochemical, optical, piezoelectric, calorimetrics. Imobilization of biomolecules.

Immobilization supports. Immunosensors. Enzymatic sensor. DNA sensors. MEMS. Lab-in-a-Chip. Biochip. Nanosensors. Rapid tests. Electronic nose.

Cláudia Santos
Potential of plant-derived compounds for preventing age-related neurodegeneration and cognitive decline

In the developed world, the continuous increase in the population lifespan results in higher incidence of many age-related diseases. This results in higher health care costs, e.g. in 2008 for Alzheimer's disease and other forms of dementia recently estimated at €160 billion for the EU27 and €177 billion for the whole of Europe (Wimo et al., Int. J. Geriat. Psychiatry, 2011). Preventative, amelioration and/or, ideally, inhibition strategies to retard or reverse neuronal and behavioral deficits that occur in aging are under critical demand. However, delivery of (pharma) products and therapeutic strategies has been up to now limited.
One of the most promising research areas focuses on the potential of phytochemical-rich foods to prevent age-related neurodegeneration and cognitive decline. Plant-derived compounds have been observed to limit neurodegeneration and to prevent/reverse deteriorations in cognitive performance, though their precise mechanisms of action in the brain remain unresolved. Flavonoids have potential to protect neurons against neurotoxins, to suppress neuroinflammation and to promote memory, learning and cognitive function. The original idea that the antioxidant capacity determines their function is currently being revised, and accommodates now a complex view that involves other molecular mechanisms. These include interactions with protein kinase and lipid kinase signaling cascades, which regulate transcription factors and gene expression involved in both synaptic plasticity and cerebrovascular blood flow. The elucidation of molecular mechanisms underlying the neuroprotective effects of plant-derived compounds are only now gathering pace.

28 February 2013

Cristina Peixoto
Downstream Process of Biopharmaceuticals
New challenges in downstream processes research have emerged in the last years with the need to purify "advanced" biopharmaceuticals such as viral vectors and/or complex protein assemblies. Aiming at reducing the developing times and costs required for implementing novel strategies, companies developing biopharmaceuticals tend to use or adapt existing processes wherever possible.
Distinct case studies will be evaluated. The cases face unique challenges and show that there is no "general rule" in what concerns downstream. Many variables determine the strategy to purify the product: global production strategy (single cells in suspension *versus* use of microcarriers), medium composition (the presence of serum and phenol red), peculiar characteristics of the product, like high particle size, complex structure and intrinsic impurities (e.g. empty capsids, the non-well formed particle or aggregates).

Furthermore, the lack of specific analytical tools to monitor the quantity and the quality during downstream is also a bottleneck that needs to be overcome.

Tiago Bandeiras
Structure-based drug design - Protein domain design and stability studies for pre-clinical industrial projects

Protein domain design may overcome difficulties in expressing and purifying proteins, especially those who are multi-domain proteins connected by flexible aminoacid-stretches. Limited proteolyses is a powerful tool to identify new soluble domain borders, which can be used for optimized large-scale protein expression and purification approaches. Such process benefits from the production of stable, homogeneous and soluble proteins, which can be obtained by using the Thermo-Shift Assay. This method is used to quickly define optimal protein buffer and to identify potential physiological or non-physiological ligands which may stabilize or de-stabilize the protein by determining protein melting points.

António Cunha
Bioreaction scale up

The ability of biological systems for expressing a number of complex molecules lead to a fast development of the biotechnological industry. Different cultivations methodologies, and use of large scale bioreactors aiming the reduction of production costs, raised different problems that had to be addressed to have consistent product quality and productivity at different scales. Scaling up biological processes poses interesting challenges to the industry, being the main issues to be addressed the fulfilment of mass and heat transfer requirements at large scale, within the constraints of shear stress supported by the biological systems, and increased time constants of the process. The increased number of generations, which can influence the expression, namely due to possible genetic instability of the strains used for production is also a concern when scaling up biotechnological processes. These issues will be addressed, and the main variables to be taken into consideration will be discussed.

António Cunha and Tiago Bandeiras
Visit to the pilot plant

1 March 2013

Teresa Crespo
Different types of quality systems

A quality system refers to the activities a company/institution uses to promote quality within the organization. This system includes the plans, policies and procedures used in production of any type of good and in the production of services. Different quality

systems will be discussed, as well as the standards, legislation and guides that apply to each of them. As an example, the manufacturers of pharmaceutical and biopharmaceutical products must establish and follow a quality system to help ensure that their products consistently meet applicable requirements and specifications. The quality system that applies is known as current good manufacturing practices (cGMP's) and this is one of the topics that will included in the program of the class.

Adriano Henriques
Custom-made life, a new paradigm in Biotechnology?

Content: Metagenomics; the gastrointestinal tract microbiota; the human microbiome; commensals versus pathogens; uses of probiotics; mechanisms of probiosis; probiogenomics; in vivo expression technologies (IVET); use of spores as probiotics; new generation of probiotics. Synthetic biology. Custom-made life. Impact of synthetic biology on biotechnology and human health.

Lígia O. Martins
Integrating Protein Science and Technology: The Laccase-Study.

In view of increasing environmental and economic pressures to use renewable sources of energy and chemical feedstocks in industry, biocatalysts look like potentially attractive technological tools. Biocatalysts based processes require less energy and minimize wastes load and at the same time could improve the quality and functional specifications of products. Efforts to search natural biodiversity for useful activities have been done but natural diversity cannot address all practical problems. In addition, some fields of chemical industry or those of bioremediation require enzymes which can recognize especial materials, sometimes nonexistent in nature, as their substrates. Many problems are best attacked by engineering the catalysts itself, whether it is a single enzyme, multiple enzymes or even a whole cell. Recombinant DNA technology provides a means to redesign nature's catalysts at the molecular level, to adapt their functions for applied ends. Either existing biocatalysts can be fine-tuned by rational redesign, or combinatorial techniques can be used to search for useful functionality in libraries generated at random and improved by suitable selection methods. In this lecture, we will focus on the development of strategies targeted at the improvement of bacterial laccases as biocatalysts: (i) **selection** (screening genomes, enzymes or cells), (ii) **overproduction and purification**, (iii) **characterization** (catalytic properties, reaction conditions, structural information), (iv) **engineering** (process or enzyme engineering), (vi) **application processes**.

4 March 2013

José Canongia Lopes
Ionic liquids: theory and fundamentals

The development and application of atomistic forcefields to the study of ionic liquids using quantum and statistical mechanics methods has led to the discovery and analysis of the unique nature of their liquid phases, i. e. the notion that ionic liquids are nano-segregated fluids with structural and dynamic heterogeneities at the nanoscopic scale. This successful contribution of theoretical chemistry to the field of ionic liquids will serve as a guide throughout the ensuing discussion that will highlight the fundamental characteristics of ionic liquids as a novel and complex class of compounds and the use of theoretical and computational chemistry tools as ways to probe the inner structural features of these (or other) chemical entities.
In this lecture we will review the processes that led to the development of one of the most widely used force fields in the area of ionic liquids modeling, analyze its subsequent expansions and alternative models, and consider future routes of improvement to overcome present limitations. This includes the description and discussion of i) the rationale behind the generic and systematic character of the CL&P force-field, namely its built-in specifications of internal consistency, transferability and compatibility; ii) the families of ionic liquids that have been (and continue to be) parameterized over the years and those that are the most challenging both in theoretical and applied terms; iii) the steps that lead to a correct parameterization of each type of ion and its homologous family, with special emphasis on the correct modeling of their flexibility and charge distribution; iv) the validation processes of the CL&P and other force-fields; and finally v) the compromises that have to be attained when choosing between generic or specific force-fields, coarse-grain or atomistic models, and polarizable or non-polarizable methods.

Isabel Marrucho
Ionic Liquids: Green Solvents for separations

Green chemistry is a matter of chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances. As a chemical philosophy, green chemistry applies to organic chemistry, inorganic chemistry, biochemistry, analytical chemistry and even physical chemistry. Its focus is on minimizing the hazard and maximizing the efficiency of any chemical choice.
The practical implementations of environmentally green solvents in separation processes are critical in preventing pollution. Along with supercritical carbon dioxide, which is an important commercial and industrial solvent exhibiting low toxicity and little environmental impact, ionic liquids have virtually no vapor pressure and can also be classified as green solvents. A wide range of ionic liquids separation applications are being studied and have gained great attention, in the areas of extraction, absorption, and adsorption with immobilized IL. The success of using ionic liquids for separations is due to their unique combination of properties and tunability which can be tailored to meet a specific purpose. Several examples already implemented in industry will be discussed.

Cristina Silva Pereira
Ionic liquids: whole cell catalysis

Biocatalysis underpins some of the oldest chemical transformations known to humans, the Sumerians, for instance, produced at least 19 different types of beer. Biocatalysis

carry out the chemistry of life, the controlled chemical transformations in primary metabolism and the generation of natural- product diversity in secondary metabolism of plants and microbes. The diversity of potentially useful enzymes at the chemist's disposal is now vast, being deployment in applications ranging from chiral resolution to bioremediation of pollution and offering solutions to synthetic problems that seem intractable to artificial catalysts.
Traditionally, enzymes have been regarded as catalysts designed to work in water, but some can develop altered selectivities and enhanced thermal stability in nonaqueous solvents. However, the volatility and toxicity of many conventional organic solvents are obvious drawbacks of this approach. To substitute these organic solvents, the ionic liquids, a class of alternative green solvents, have recently gained growing attention in biocatalysis, mainly due to their extremely low vapor pressures and designable physical/chemical properties. Fungal biocatalysis using ionic liquids favors white-biotechnology development, optimising nature toolset exploitation to industrial production and enhancing considerably the processes green record.

5 March 2013

Cristina Silva Pereira, Isabel Marrucho and Jóse Esperança
A technological mix of Microbiology and Chemistry

Tutorial
Lecture Structure:
The class will be previously divided in 6 Groups (~4 students each).
The groups will be assigned to identify either the benefits (groups 1,2,3) or the drawbacks (groups 4, 5 and 6) of biocatalysis in ionic liquids.

The groups should select a single paper (not revision paper and not older than 2005). The lecture will start with each group presenting in 5'(± 2') the basic principles of the corresponding ionic liquid based technology and its advantages or disadvantages, relatively to traditional approaches. After the presentations the groups (1, 2 and 3) and (4,5 and 6) are suppose to identify rapidly (10') the arguments for a animated debate.

6 March 2013

Margarida Oliveira
Main strategies and achievements in Plant Genetic Engineering

- The innovation of genetic engineering in plant breeding
- The basis of plant genetic engineering - Agrobacterium vectors
- Other methods to achieve transgene expression in plant cells
- Some examples of targets for plant genetic engineering (fundamental studies, plant quality, industrial characteristics, molecular farming, biotic and abiotic stresses...)
- Safety and public concern

Nelson Saibo
Biotechnology for crop improvement: responding to environmental and population changes

Crop production is threatened by global climate change, and recent demands for crops to produce bio-fuels have started to affect the worldwide supply of some of the most important foods. In addition, it is predicted that by 2050 we will need to produce at least twice as much food as we do today to meet the demands of the growing world population. Therefore, an understanding of the history and future of the plant biotechnology is particularly relevant as we look ahead to the challenges brought by increasing population, degrading soils, disappearing water reserves, escalating energy prices and climate change. In this lecture, we will talk about plant domestication, green revolution, the modern plant breeding programs, and the use of biotechnology to accelerate the progress in solving the challenges of food security, energy, climate change, health and well-being, sustainability and environmental protection. Given that development of crops that are better adapted to abiotic stresses is extremely important for food production in many parts of the world today, a particular emphasis will be given to this topic. The future of biotechnology approaches for crop improvement will also be discussed.
By the end of this lecture the student should be able to answer why biotechnology is necessary, to describe Key agricultural biotechnology approaches, and to identify the constraints and opportunities for the use of plant biotechnology.

6 and 7 March 2013

Célia Miguel and Nelson Saibo
Plant Biotechnology and Functional Analysis Tools

Tutorial
1. Presentation of some examples of tools and techniques used in Plant Functional Genomics and Biotechnology (room 2.13)
Visit to the Forest Biotech and Genomics of Plant Stress Labs
2. Presentation of scientific articles by the students and group discussion

7 March 2013

Rita Abranches
Molecular Pharming

- Molecular Pharming: historical perspective on the use of plants as alternative systems for the production of recombinant proteins
- Plant-based productions systems: advantages and disadvantages
- Transient systems vs. stable transformation; whole-plant systems vs. plant cell cultures

- Plant specific post-translational modifications; implications for the product quality
- Factors affecting the intracellular fate of a recombinant protein
- Optimization procedures: protein stabilization, novel production schemes, modeling, scale up
- Downstream processing and regulatory considerations

Do we need plant genetic engineering?- Group discussion
Margarida Oliveira & Ana Sanchez