MSc work proposal for 2016-2017, to be developed at the Abel Oliva laboratory, some of them in collaboration. More details proposals will be given after personalized request to -> oliva@itqb.unl.pt

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Title: **Metabolomic analysis of sweat, a contribution to the development of less-invasive diagnostic methods**

Field of work: NMR, Bioinformatics, Clinical Analysis

Summary:

There is a demand for the development of alternative non-invasive diagnostic methods due to the discomfort associated with needle picking for blood collection. Measurements in sweat collected at the skin surface, can represent a possible approach. Recently glucose in sweat was found to be correlated with blood glucose, an analysis with enormous interest for the diabetic population. Moreover it gave origin to the development of a patch to perform the measurements that was published in the highly renowned scientific magazine Nature Nanotechnology. Nevertheless the estimation of blood glucose concentration from sweat glucose measurements still presents an excessive variability with unknown origins. It is therefore necessary to understand more deeply the physiology of sweat production. The study of the correlation between sweat metabolite concentrations and the correspondent plasma metabolite levels can be a contribution to this field. ^{1}H-NMR metabolomic analysis is a powerful technique to address this question, as dozens of substances are measured simultaneously in a single spectrum. Furthermore it is particularly suited for polar metabolites, the main expected constituents of sweat.

In this project, the student will characterize simultaneously the range of metabolites found in sweat and plasma by ^{1}H-NMR metabolomic analysis. The correlation between the metabolites composition of both fluids will be determined. The results obtained in this project are expected to give origin to a scientific publication.

Collaboration: Biomolecular Diagnostic Lab. and Cell Physiology and NMR (ITQB/UNL Antonio Xavier)

Title: **Metabolomic characterization of different sweat samples collection methods towards clinical purposes**

Field of work: Sampling; NMR, Clinical Analysis

Summary:

There is a demand for the development of alternative non-invasive diagnostic methods due to the discomfort associated with needle picking for blood collection. Collection and measurements in sweat is an alternative approach, since concentration of substances in that fluid is related to the concentrations inside the body. Recently glucose in sweat was found to be correlated with blood glucose. In that study, the sweat response was induced by applying the cholinergic drug pilocarpine. Sweat can be explored as non-invasive human sample, which is possible to be obtained in a simple way. Therefore, the study of different methods of sweat collection towards diagnostic purposes is of highly interest. In this project, the student will obtain and analyse sweat of volunteers by different approaches: induced with pilocarpine; physical exercise and

natural sweat, and thereafter will identify all range of metabolites found in the samples by ^{1}H-NMR metabolomic analysis. The different methods of sweat collection will be compared and conclusion will be obtained after the interpretation of the results. A paper will be published at the end of this study.

Collaboration: Biomolecular Diagnostic Lab. and Cell Physiology and NMR (ITQB/UNL Antonio Xavier)

Title: **Production of hybrids from Medicago spp. protoplast by microfluidic chip**

Field of work: Single cell analysis, Microfluidics, Plant cells

Summary:

Plant breeders use protoplasts fusion as a tool to produce hybrid cultures, and thus achieve the improvement of their agronomical traits. This is of paramount importance when using sexually incompatible species, which could not be crossed by natural methods. Nevertheless, cell fusion is also a tool to mix genotypes and create new breeding lines which potentially present advantageous traits for farmers and consumers.

Although, this technique had been used on the last three decades, the frequency of viable hybrids produced is still reduced and the reasons unclear. Highlights on the factors which determine the viability of fused cells will boost this technique for one of the most used in plant breeding programs.

Knowing more about cell and molecular triggering of cell fusion will permit a more detailed evaluation of the process and contribute to better understand and improve it.

Therefore chips for this purpose has been designed and constructed towards the production of hybrid protoplast inside the microchambers. The microfluidic associated system will allow the introduction of cells and reagents and a real time observation of the phenomena as it occurs. Cell plants and enzymes meet in an area of incubation, where the cellular wall of the cells was degraded. Then the resultant protoplasts would be immobilized in the traps and treated with PEG to promote their fusion.

Collaboration: Biomolecular Diagnostic Lab. and Plant Cell Biotechnology (ITQB/UNL Antonio Xavier)

Title: **Single cell analysis of keratinocytes cells in microfluidic platform**

Field of work: Single Cell analysis; Imaging; Microfluidics. Animal cell culture

Summary:

Microfluidics, the study and control of the fluidic behavior in microstructures, has emerged as an important enabling tool for single-cell chemical analysis. The complex procedures for chemical cytometry experiments can be integrated into a single microfabricated device. The capability of handling a volume of liquid as small as picoliters can be utilized to manipulate cells, perform controlled chemical reactions, and efficiently minimize sample dilution after lysis. The microfluidic approach offers a rapid, accurate, and cost-effective tool for single-cell biology. In a way to explore the advantages of this microenvironment for analysis of few to single cells it is proposed to develop a microfluidic setup system based on a chip with cell trapping

arrangements, connected to different inlets/outlets that feeds specific reagents/growing media/activators/toxics etc. This will overcome the limitation of the static approach, also it will allow the experiment to be performed with very small quantities of reagents and cells, and will enable to get images (even fluorescent microscopy) in real time of the cells in each experimental step. The microfluidic chamber will be used for exploring the interaction of the keratinocytes cells with specific reagents and changes in the environmental surroundings conditions (temperature, gas mix, nutrients), allowing an individualized characterization of cell behavior.

Title: **Design and construction of microfluidic arrangement for downstream purification of proteins by ionic liquids.**

Field of work: Microfluidics; Monoclonal Antibodies; Ionic Liquids.

Summary:

This project envisages the development of microfabricated structures for manipulation of nature-inspired ionic fluids (NATIFs) and their assessment in the development of benign extraction processes using aqueous biphasic systems (ABS) for the purification of monoclonal antibodies (mAbs).

Monoclonal antibodies (mAbs) represent the fastest growing biopharmaceutical market segment, and their demand for the treatment of age and society related diseases (e.g. Alzheimer's, Parkinson's, cancer, or diabetes) continues to increase. 26 therapeutic mAbs are currently marketed in Europe, with sales of USD19.01 billion in 2011 and estimated to reach USD42.37 billion in 2018. The worldwide commercial pipeline includes ~350 mAbs in clinical trials. Downstream processing (dsp) of mAbs has however failed to keep up with the throughput of the upstream stage, resulting in a production bottleneck and in escalating costs.

The capability of handling a volume of liquid as small as picoliters in conjugation with the use of ionic liquids offer an attractive alternative to the standard procedures towards clarification of antibody solutions in a continuous way. The technique here proposed is a new approach that takes advantage of the many advantageous properties of ionic liquids (e.g. specific affinities, unique solubility characteristics, use of natural non-toxic reagents, etc.), that can be method with clear advantage related cost, efficiency and toxicity.

The fabrication of the microfluidic platform is an important aspect of this project, since the efficiency of the ionic liquids activity will be dependent of the suitability of the geometry and liquid transport inside the channels. The chips will be fabricated in PDMS, based on molds made of epoxy from an original SU8 cast constructed in the CENIMAT (FCT). The experiments with the chips will be performed at the Biomolecular Diagnostic Laboratory at ITQB/Oeiras. The work will be supported by a FCT project (PTDC/QEQ-FTT/3289/2014), started in 2016.

Collaboration: Biomolecular Diagnostic Lab.; Molecular Thermodynamics and CENIMAT at FCT/UNL

Title: **Sorting recombinant Escherichia coli cells inside droplets by a microfluidic arrangement**

Field of work: Single Cell analysis; Droplets; Microfluidics.

Summary:

Microfluidics, the study and control of the fluidic behavior in microstructures, has emerged as an important enabling tool for single-cell chemical analysis. The complex procedures for chemical cytometry experiments can be integrated into a single microfabricated device. Of the microfluidic techniques available for manipulating low volumes, as required for single-cell analysis, emulsion-based microfluidics shows greatest potential. This technology, also known as droplet microfluidics, has been successfully used to produce assays with volumes as low as several tens of femtoliters for a variety of chemical and biological applications. Droplet microfluidic systems form highly monodisperse emulsions drops, as in water droplets in oil, with diameters in the micrometer range. The development of microfluidic arrangements that allows the production of droplets and capturing of cells, surrounded by biocompatible oil, can provide an advantageous tool for isolating specific cells of interest and their protein expressions.

Directed evolution approaches have proven to be highly valuable for the study of the structure, function, and evolution of protein, as well as for obtaining improved proteins for biotechnological or industrial applications. By mimicking the principles of natural selection through iterative rounds of random mutagenesis and/or DNA recombination and screening, the epical time scale of evolution can be shortened to an experiment which can be conducted in the laboratory. Screening assays for the analysis of large mutant libraries (106-1012) are performed at a high cost of time and resources. These costs and the general need for high-throughput make further miniaturization of assay volumes attractive. Dye decolourising peroxidases (DyPs), the focus of the present proposal, constitute a new family of microbial heme-containing peroxidases that oxidize a remarkably wide range of substrates, from synthetic dyes to phenolic and nonphenolic lignin compound units, iron and manganese ions, showing an utmost importance for biomass degradation in the realm of Biorefinery and Industrial Biotechnology.

Collaboration: Biomolecular Diagnostic Lab. and Microbial and Enzyme Technology Labs/ ITQB NOVA