Project: Novel biosensors for medical diagnosis

Areas: Nanobiotechnology/Biochemistry

Supervisor: Lígia O. Martins **Co-supervisor: Vânia Brissos**

Modern biosensors can be miniaturized, mass produced and easily transported. They can measure analytes in real-time, which is extremely useful for monitoring rapid changes in biological fluids.

Enzyme paper-based sensors have become a promising platform for *lab-on-a-chip* devices, offering high selectivity and sensitivity for application areas such as, health diagnostic, food quality control and environmental monitoring.(1,2) This is mainly related to their simplicity, portability and low-cost associated with the high selectivity of enzymes that enable the determination of single analyte species in complex mixtures. In order to operate, enzymes must be optimized to catalyze a specific biochemical reaction and be stable under the normal operating conditions. Therefore, designing enzyme-based biosensors requires consideration of both the target enzyme and the complexity of the matrix in which the analyte will be measured.

Pyranose 2-oxidases (P2Oxs) are flavoproteins that catalyze the oxidation of glucose and other aldopyranoses with concomitant reduction of O_2 to H_2O_2. P2Oxs can be employed for the determination of 1,5-anhydro-D-glucitol (1,5-AG), a natural analogue of D-glucose present in human cerebrospinal fluid and in serum (3). It is known that a decrease of this compound in serum is related with hyperglycemia or renal dysfunction and P2Oxs can be used as a biosensor of glycemic control in diabetes mellitus, with improved sensitivity as compared with current devices.

In this proposal protein engineering approaches will be followed to improve the efficiency and stability of the enzyme AsP2Ox from *Arthrobacter siccitolerans* towards their successful application in cost-effective portable biosensor devices This enzyme, the first from bacterial origin, was recently characterized in our lab and showed many advantages as compared with known glucose oxidases. (4) Previous work was performed in the optimization and validation of mutagenesis protocols of AsP2Ox, cell growth in 96 well plates and robotic high-throughput assays and after one round of evolution a hit enzyme was found with improved catalytic efficiency for glucose. (5) In this proposal new rounds of mutagenesis and screening will be accomplished to improve the properties of the enzyme of interest. Paper-based immobilization will be performed to improve its stability and allow for its recyclability toward the construction of a sensing device for glucose detection.

1. Costa, M. N., Veigas, B., Jacob, J. M., Santos, D. S., Gomes, J., Baptista, P. V., Martins, R., Inacio, J., and Fortunato, E. (2014) A low cost, safe, disposable, rapid and self-sustainable paper-based platform for diagnostic testing: lab-on-paper. *Nanotechnology* **25**, 094006
2. Rocchitta, G., Spanu, A., Babudieri, S., Latte, G., Madeddu, G., Galleri, G., Nuvoli, S., Bagella, P., Demartis, M. I., Fiore, V., Manetti, R., and Serra, P. A. (2016) Enzyme Biosensors for Biomedical Applications: Strategies for Safeguarding Analytical Performances in Biological Fluids. *Sensors* **16**
3. Adamson, T. L., Cook, C. B., and LaBelle, J. T. (2014) Detection of 1,5-Anhydroglucitol by Electrochemical Impedance Spectroscopy. *Journal of diabetes science and technology* **8**, 350-355
4. Mendes, S., Banha, C., Madeira, J., Santos, D., Miranda, V., Manzanera, M., Ventura, M. R., van Berkel, W. J. H., and Martins, L. O. (2016) Characterization of a bacterial pyranose 2-oxidase from Arthrobacter siccitolerans. *J. Mol. Cat. B: Enzymatic*, 10.1016/j.molcatb.2016.1011.1005
5. Santos, D. (2017) *Improving a bacterial pyranose 2-oxidase from Arthrobacter siccitolerans through directed evolution.* Master Degree, Universidade Nova de Lisboa

Contact:

Lígia O. Martins

Microbial and Enzyme Technology Lab

ITQB NOVA, Oeiras

Tel: 214469534,

E-mail: lmartins@itqb.unl.pt

http://met.itqb.unl.pt/