Abstract

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Construction of an optimized Dye-Decolorizing Peroxidase based H2O2 biosensor following rational design

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Abstract

Dye-decolorizing peroxidases (DyP) are heme-containing enzymes that couple the oxidation of different organic substrates, with the reduction of H2O2 to water. Due to their broad substrate range, easy genetic manipulation and over-expression in E. coli, as well as their stability over wide ranges of pH and temperature, DyPs are considered to be attractive targets for development of biotechnological applications1. To that end, we explore DyPs from different bacterial organisms for construction of 3rd generation H2O2 biosensors. We use surface enhanced Resonance Raman (SERRS) spectro-electrochemistry to probe structure and function of immobilized DyPs. The enzyme is attached to Ag electrodes coated with alkanethiol self-assembled monolayers (SAM) to ensure biocompatibility. The choice of SAM takes in consideration the surface charge distribution of each DyP. The structural properties of the enzymes in solution and immobilized state are addressed by RR and SERRS, respectively, while electrocatalytic properties are simultaneously analysed by electrochemistry.We have previously shown that DyP from Pseudomonas putida can be used as biocatalyst in an efficient 3rd generation H2O2 biosensor, which reveals superior sensitivity in comparison to biosensors based on e.g. horseradish peroxidase2. Optimized 3rd generation DyP-based biosensors for H2O2 that show improved sensitivity, selectivity and stability are expected to be a valuable alternative to currently existing (commercial) devices that rely on other peroxidases. We therefore employ SERR spectro-electrochemistry to screen structural and redox properties of immobilized DyPs from different organisms in a fast manner, in the search for the best-behaved biocatalysts in terms of stability, sensitivity and selectivity.

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Biography

Catarina Barbosa is a PhD candidate at Raman BioSpectroscopy Lab - ITQB NOVA. She has a BSc. degree in Biochemistry from Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa (FCT/NOVA). In 2018 she obtained MSc. degree in Biochemistry for Health at ITQB NOVA. In 2019 she started research fellowship in Raman BioSpectroscopy Lab at ITQB NOVA. In 2021 she won a PhD Research Scholarship from Fundação para a Ciencia e Tecnologia. Her main research interest is the development of enzyme based electrochemical biosensors following insights obtained by vibrational spectroscopy

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