

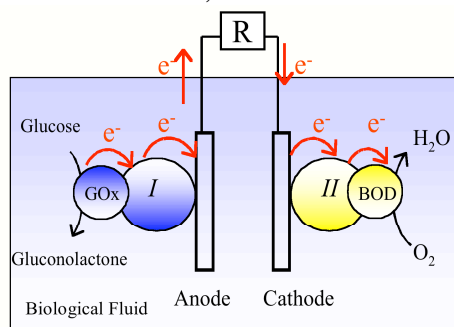
# Improving Glucose Oxidase for a miniaturized Biofuel Cell

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Enzymatic biofuel cells are promising power sources to drive miniaturized electronic devices and biosensors. Glucose oxidase is commonly used to provide electrons at the anode<sup>1</sup>. GOx is a soluble homodimer with a molecular weight of ~160 kDa, 16-25% (w/w) glycosylated, with each subunit containing one non-covalently bound flavin adenine dinucleotide (FAD) ~15 Å below the protein surface<sup>2</sup> and highly specific to glucose. Mechanistically, the oxidation of glucose proceeds concomitant to the reduction of enzyme-bound FAD to FADH<sub>2</sub>. In the natural environment, FADH<sub>2</sub> is oxidized by transferring two electrons and two protons to O<sub>2</sub>, the products being H<sub>2</sub>O<sub>2</sub> and FAD.

The purpose of present work is the design of modified glucose oxidases in order to improve the conversion of glucose chemical energy into electrical energy. Based on the biofuel cell described below, we focused on two complementary optimisations.



*Schematic diagram of the compartment-less biofuel cell<sup>3</sup>. The two electrodes, coated with different cross-linked electrostatic adducts of enzymes and redox polymers, reside in the same solution. At the anode, electrons are transferred from glucose to glucose oxidase (GOx), from GOx to Os (II) and from Os (II), to the electrode. At the cathode, electrons are transferred from the cathode to Os (III), from Os (II) to BOD, and from BOD to O<sub>2</sub>.*

I- Electron transfer from GOx is limited by the distance between the enzyme and the polymer redox centres. This can be improved by partial or complete removal of the carbohydrate chains<sup>4</sup>. Comparison between glycosylated and deglycosylated GOx showed no loss of activity in solution. Prior to electrochemical studies, the deglycosylated GOx was purified using anion exchange chromatography.

II- Electron transfer to the anode is also inhibited by molecular oxygen. As the O<sub>2</sub> partial pressure is increased, the anodic glucose electrooxidation current decreases because O<sub>2</sub> competes with the redox polymer for GOx-FADH<sub>2</sub> electrons. Decreasing the reactivity of GOx with O<sub>2</sub> will allow to accelerate electron transfer to the redox polymer. Several modifications are known to decrease electron transfer to molecular oxygen. Particular amino acids are known to be involved in electron transfer<sup>5</sup> from GOx to O<sub>2</sub>. By directed and random mutagenesis of the protein, we are trying to decrease electron transfer to O<sub>2</sub> and/or increase electron transfer to the redox polymer. Several studies also showed that modifications on FAD can decrease the rate of electron transfer to O<sub>2</sub>, without affecting half-reaction of glucose oxidation<sup>6</sup>. Variants of FAD have been synthesized and will be reintroduced into the protein. Activity of modified GOx will allow us to understand the balance between one-electron acceptor and O<sub>2</sub> pathways.

1. Mano N, Mao F, Heller A. *J Am Chem Soc.* 2002, **124**, 12962-3.
2. Wohlfahrt, G. Witt, S. Hendle, J. Schomburg, D. Kalisz, H. M. Hecht, H.-J., *Acta Cryst. Section D* 1999, **D55**, 969-977.
3. Mano N, Mao F, Heller A. *J Am Chem Soc.* 2003, **125**, 6588-94.
4. Fraser DM, Zakeeruddin SM, Grätzel M. *Biochim Biophys Acta.* 1992, **1099**, 91-101.
5. Roth JP, Klinman JP. *Proc Natl Acad Sci U S A.* 2003, **100**, 62-7.
6. Roth JP, Wincek R, Nodet G, Edmondson D, McIntire W, Klinman JP. *J Am Chem Soc.* 2004, **126**, 15120-31