Engineering of nanostructured electrodes and biocatalyst interfaces for efficient electron transfer

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The development of micro-biosensors and miniature biofuel cells is currently an important focus in bioelectronics, *e.g.*, for the development of miniaturised, self-sustaining biomedical devices working within tissues or organs for gathering spatially and temporally resolved chemical and physical information. Such self-contained, wireless biosensors have enormous scientific and practical importance in medicine as well as in the biotech industry. The key for miniaturised biosensors as well as for biofuel cells is the efficient electron transfer between the enzyme (bioelement) and the electrode, which guarantees a high sensitivity/current output even in miniaturised sensor/transmitter systems below 1 mm^3. In both cases maximum output voltage and current density are the *conditio sine qua non*. Currently, the discrepancy between the required power output and the necessary miniaturisation of electrode surfaces hampers practical realisation.

The project proposes two complementary approaches for achieving improved power output with miniaturised electrodes: (i) the application of conducting nanomaterials or nanostructures with large specific surface areas for high enzyme loading together with novel strategies for enzyme binding and orientation on the surface of these materials, and (ii) molecular engineering of enzymes for better electrode contact, orientation on the electrode and improved electron transfer. To this purpose, the blue multicopper oxidase laccase, which is capable of direct electron transport to an electrode, will be used for the optimisation of both, cathodic and anodic electrode reactions, based on the proposed strategies.

Laccase from the fungus *Botrytis aclada*, which will be in the focus of this project, was only recently discovered in our group at BOKU, and its 3D structure has been solved. It is an exceptionally chloride-tolerant laccase, and thus well suited to work under physiological conditions. It is the only fungal laccase to date that can be recombinantly expressed in very high yields using an expression host that can be easily manipulated. The enzyme will be engineered to (i) optimise the electron transfer from the electrode surface to the T1 copper center, (ii) modulate the redox potential of the T1 copper center, and (iii) improve enzyme binding, packing density and orientation by modifications of the enzyme surface. Purified laccase variants will be used to modify nanostructured electrodes or even micro- and nano-electrode arrays, based on *e.g.* carbon nanotubes, graphene materials, graphene-metal nanoparticle composites, graphene-carbon nanotube hybrid materials or nanostructured conductive polymer/metal oxide composites, and improve their performance by different adsorption and immobilisation techniques.

An applicant to this project should have profound knowledge in protein chemistry and molecular biology; in addition knowledge in electrochemistry/physical chemistry is an advantage.