CHARGE TRANSFER CHARACTERISTICS OF REDOX PROTEINS ON MACROSCOPIC AND NANOSIZED ELECTRODES USING NOVEL IMMOBILIZATION STRATEGIES

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Within the last decade protein electrochemistry at miniaturized electrodes has gained in importance not only for the study of the charge transfer properties of redox proteins but also for developing sensitive biosensor and bioelectronic devices [1-4]. One of the major challenges within this field of research is the directed coupling between electronic and biological compounds without losing the biofunctionality [5].

Proteins from natural biological electron transfer systems like respiratory chain or photosynthesis are preferably used in the field of protein bioelectronics. They typically have redox active centers accessible to the outer surface and are thus able to efficiently communicate with electrodes [6]. The challenging task for useful biosensor/bioelectronic application is to remain both, good communication between electrode and immobilized protein as well as their functional integrity.

In this work we report on electrochemical investigations of the charge transfer behavior of redox proteins depending on both, electrode dimension and type of the immobilization strategy. Therefore we basically focus on the charge transfer behavior of cytochrome *c* (cyt *c*) while immobilized on macroscopic and nanosized electrodes. We used reported immobilization strategies like attachment via electrostatic interaction and moreover developed novel strategies for a directed protein immobilization. Our interest lies in the optimized orientation of the protein on the electrode allowing a fast and reversible electron transfer between electrode and protein as well as the conservation of their biofunctionality. Therefore we developed new strategies for directed immobilization of horse heart cyt *c* on gold electrode surfaces using bioaffinity tags at specified positions within the amino acid sequence. We used an existing expression system for cyt *c* in *E.coli* [7] and modified the protein sequence genetically by incorporation of his- and/or cys-tags at defined positions. We performed comparative immobilization studies of different mutants and native cyt *c* by means of SPR and AFM. Biofunctionality was proven by UV-Vis spectroscopy and cyclovoltammetric studies. We aim to derive a protein with optimized tag position which facilitates a strong electronic coupling between protein and electrode and at the same time still remains an accessible active site that allows binding to other enzymes and which preserves its catalytic activity.

Finally the combination of different immobilization strategies e.g. his-tag and electrostatic immobilization leads to completely new possibilities for bivalent immobilization of proteins, meaning that they can be immobilized on two sites at the same time. This might be of special interest in particular for molecular bioelectronic and biosensing applications where the proteins are either immobilized between two crossing electrodes or bridging a nano gap within single molecule approaches.

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