

Electron transfer (ET) reactions between redox proteins generally occur over long distances. In these reactions, electrons are transferred through the protein matrix via mechanisms that are not yet fully understood. Specifically, it is still a matter of debate if the ET rate solely depends on the transfer distance, or if individual amino acids constitute specific ET pathways. Furthermore, little attention has been paid so far to the functional role of the protein binding domains to intermolecular ET processes. On the other hand, evidence has been provided that ET mechanisms and dynamics may be affected by electrostatic fields originating from local Coulombic interactions between oppositely charged amino acids in the protein binding domain as well as from transmembrane potential gradients [1]. In the present work, these topics are addressed by studying synthetic "de novo designed" heme proteins using a novel spectroscopic approach.

The *de novo* design of template-assembled four-helix-bundle heme proteins (Fig. 1) offers the possibility to vary, in a systematic manner, *individual* structural parameters that are assumed to be relevant to the intra- and intermolecular ET [2]: the axial ligands of the heme, the distance of the heme with respect to the electron exit/entry site of the protein (i. e. the interaction domain), individual amino acids along the putative ET pathway, and the polarity/hydrophobicity of the interaction domain. These proteins are immobilised on electrodes in order to study the heterogeneous ET process. Using Ag electrodes, surface-enhanced resonance Raman (SERR) spectroscopy can be employed that selectively probes the vibrational spectrum of the heme group solely of the adsorbed proteins. Furthermore, time-resolved SERR spectroscopy allows to monitor the dynamics of the interfacial ET reactions as well as possible coupled conformational changes of the redox sites so that this technique provides information about the ET mechanism not accessible by other methods [1,3,4].

The first series of synthetic proteins used in our study contain a bis-histidine ligated heme and a positively charged (lysine-rich) interaction site that serves as binding domain for bare Ag electrodes as well as for electrodes coated with self-assembled monolayers (SAM) of carboxyl-terminated alkanethiols. Such coatings do not only provide a biocompatible electrode surface. In addition, SAM-coated electrodes offer the opportunity to control the electric field strength at the protein binding site via variation of the alkyl chain length and thus allow to monitor electric field effects on the redox site structure and charge transfer dynamics [1,4].

In a second series of synthetic proteins the binding domain includes thiol functions instead of lysines allowing direct attachment to the electrode [5]. Such devices lack a charged protein/electrode interface and, hence, may provide complementary information about the electric field effects on the heterogeneous ET.

Potential-dependent SERR experiments have shown that, upon electrostatic immobilisation, strong electric fields as they exist at bare electrodes, destabilise the structure of the four-helix bundle proteins, eventually leading to the dissociation of (one of) the axial histidine ligands from the heme iron. This ligand dissociation causes the transition from the six-coordinated low-spin (6cLS) to the five-coordinated high-spin (5cHS) configuration, which, in turn, has a pronounced impact on the overall interfacial redox process. Conversely, no structural perturbations of the heme site were noted when the proteins were immobilised on SAM-coated electrodes. Under these conditions, the interfacial redox process can be described by a single ET reaction exclusively involving the 6cLS species. Currently, time-resolved SERR studies are under way to probe the dynamics of the interfacial ET processes.

#### References

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