16:45 – 17:20

Electronically Wired Proteins: Optimization of Structure-Function Relationships

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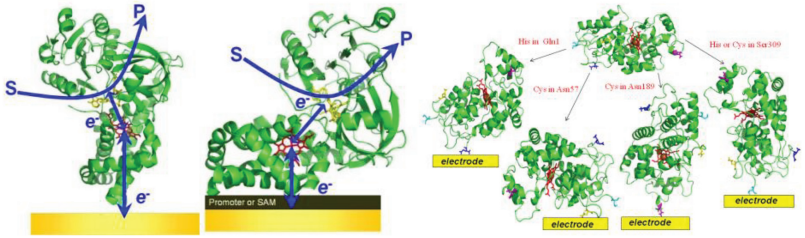
Electrocatalytic and biosensing properties of redox proteins depend on the efficiency of electron transfer (ET) between protein redox centres and electrodes, in particular on ET pathways preconditioned by protein assembly and orientation at electrodes [1]. Here, several strategies for controlled orientation of redox enzymes and proteins are discussed to gain knowledge of their directional ET reactions and ways they can be optimized. Heme- and flavo-containing redox proteins, such as horseradish peroxidase (HRP), containing surface His and Cys tags, bacterial truncated hemoglobin (trHb) and *E. coli* flavohemoglobin (HMP) were coupled to electrodes via genetically introduced surface tags [2], by reconstitution onto heme-terminated SAMs of alkanethiols [3], 3). Covalent attachment via preferentially charged surface aminoacid residues [4] and 3). Adsorbed onto promoter modified electrodes [4]

Attachment of HRP onto electrodes via tags provided its anisotropic orientations, with electrochemically estimated long-range ET rates between the electrode and heme of HRP consistent with theoretically predicted values. In contrast, reconstitution of HRP onto heme-terminated SAM resulted in a low-efficient ET and bioelectrocataysis. Covalent attachment of trHb to functionalized SAMs resulted in ET rates up to 2000 s^{-1} but only for preferential orientations via negatively charge surface domains. More complex



HMP, with a Hb-domain fused with an FAD-domain, required a promoter at the electrode surface to ensure site-specific immobilization of HMP via the heme domain enabling catalysis of reversible NADH/NAD^+ transformation. Comparison of several wiring strategies showed that the highest ET rates and bioelectrocatalysis might be achieved by protein site- and domain-specific immobilizations mimicking the natural environment of the protein and thus providing ET along the most favourable ET pathway within the protein matrix.

References

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