Protein and Electrode Engineering for the Covalent Immobilization of P450 BMP on Gold

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Site-directed mutagenesis and functionalization of gold surfaces have been combined to obtain a stable immobilization of the heme domain of cytochrome P450 BM3 from Bacillus megaterium. Immobilization experiments were carried out using the wild type protein bearing the surface C62 and C156 and the site-directed mutants C62S, the C156S, and the double mutant C62S/C156S (no exposed cysteines). The gold surface was functionalized using two different spacers: cystamine-N-succinimidyl3-maleimidopropionate and dithio-bismaleimidoethane,both leading to the formation of maleimide terminated monolayers capable of covalent linkage to cysteine. Tapping mode atomic force microscopy experiments carried out on cystamine-N-succinimidyl 3-maleimidopropionate
derivatized gold led to good images with expected molecular heights (5.5-6.0 nm) for the wild type and the C156S mutant. These samples also gave measurable electrochemical signals with midpoint potentials of -48 and -58 mV for the wild type and C156S, respectively. On the other hand, the dithio-bismaleimidoethane spacer led to variability on the molecular heights measured by tapping mode atomic force microscopy and the electrochemical response. This is interpreted in terms of lack of homogeneous dithio-bismaleimidoethane monolayer on gold. Furthermore, results from tapping mode atomic force microscopy show that the double mutant and the C62S did not lead to stably immobilized P450 protein, confirming the necessity of the solvent exposed C62.