S011 Bioenergetics at the gold surface: SEIRAS probes photosynthetic and respiratory reactions at the monolayer level

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A novel concept is introduced for the oriented incorporation of membrane proteins into solid supported lipid bilayers. Recombinant cytochrome c oxidase solubilized in detergent was immobilized on a chemically modified gold surface via the affinity of its histidine (His)-tag to a nickel chelating nitrilo-triacetic acid (NTA) surface. The oriented protein monolayer was reconstituted into the lipid environment by detergent removal. The individual steps of the surface modification, were followed *in-situ* by means of SEIRA, SERRS, and by STM and AFM. The high surface sensitivity of SEIRAS allows for the identification of each chemical reaction process within the monolayer at the molecular level. Finally, full functionality of the surface tethered cytochrome c oxidase was demonstrated by cyclo-voltammetry after binding of the natural electron donor cytochrome c. As an additional benefit of the methodology, the His-tag can be attached to virtually any recombinant protein making it an universal approach. Moreover, the position of the His-tag is easily changed reverting the orientation of the protein with respect to the electrode. Recently, we succeeded to tether photosystem II to the gold surface which provides the basis for setting up a biomimetic system for H_2 production. Finally, the influence of the membrane potential on the functional mechanism of a membrane protein was elucidated by applying a transmembrane voltage across sensory rhodopsin II. Our results demonstrate that IR difference spectroscopy can be applied to processes that depend on the membrane potential as in voltage-gated ion channels, transporters, as well as in receptors.