ANTIBODY FUNCTIONALIZATION OF QCM GOLD ELECTRODES BY PHOTONIC INDUCED TECHNIQUE

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The interest in the development of measurement tools for rapid, low cost and real time analysis is a continuous motivation for the search of biosensors capable to recognize analytes at very low concentrations. One of the main issue is the difficulty in finding biological molecules able to bind the desired compound while being apt to tether the particular transducers. In this context we applied a new technique named Photonic Immobilization Technique (PIT) to anchor large amount of antibodies onto gold surfaces with their variable part side-up [1]. PIT exploits the advantage of the high stability in the gold-sulfur interactions, the sulfur generally exposed in the form of free thiol group by biomolecules. While the thiol groups are usually added by chemical synthesis, we use UV light at 258 nm to excite the tryptophan residues that release the energy to the next disulfide bridges entailing their opening [2] (the triad Try-Cys-Cys is relatively common in antibodies). Although the absorption mechanism is essentially of the first order (one-photon absorption) the high UV power required to render the PIT effective poses the question about the most appropriate laser source for the UV radiation. Thus, we compared femtosecond and nanosecond pulsed laser sources not only in their final effect on the biosensor but also in their effectiveness in the disulfide bridges opening by means of the so-called Ellman's assay [3]. Moreover, the occurrence of conformational changes of the bio-molecules when irradiated has been evidenced by measuring the steady state fluorescence [4], whereas AFM analysis of the gold electrode in several irradiation conditions has also been performed. All these analysis reveal a significant difference between the two laser sources for PIT: in particular, the irradiation by nanosecond laser pulses seems to lead to a more rapid "photo-bleaching", as a consequence of the high fluence conveyed by these pulses, making femtosecond rather than nanosecond laser sources more suitable for PIT.

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