Conducting polymers as a versatile platform for protein nanoarrays technologies

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Abstract

Micro- and nano-arrays of biomolecules such as DNA, peptides and proteins offer exciting opportunities in both basic and applied research (*i.e.* diagnostics, drug screening and drug discovery) [1]. High-density miniaturized biochips can increase assays sensitivity and throughput while reducing sample consumption and processing time [1, 2]. While DNA micro-arrays are currently being realized and are showing all their potential in genomic applications, protein arrays are still in their infancy due to the delicate nature of proteins and their challenging interaction with solid substrates [1, 2]. Even though substantial advances in nano-patterning techniques have been achieved and protein nano-patterns have been realized using dip-pen lithography, electron beam lithography or nanografting, examples of proteins nano-arrays are still rare and without evidence of proteins activity and stability [1, 2].
Here we report a versatile platform for spatially and selective functionalization of electrically contacted gold micro- and nano-structures with biological molecules such as proteins. The method is based on the electrochemical functionalization of the gold surfaces with conducting polymers bearing biotin or metal ion units [3]. We first demonstrate that biotin-binding molecules such as streptavidin or histidine-tagged proteins can be selectively immobilized on the polymeric film [4, 5]. We then show that protein multiplexed nano-arrays can be successfully prepared by sequential polymerizations and biomolecular immobilizations. The platform can be further used to immobilize complex membrane proteins stabilized in amphipathic polymers (amphipols) [6]. In fact, by taking advantage of the high affinity between biotin and streptavidin, we immobilize distinct membrane proteins onto different electrodes via amphipols modified with a biotin tag (biotinylated amphipols, Figure 1) [7]. Antibody-recognition events indicate that the membrane proteins are stably anchored to the substrate and that the electropolymerization is compatible with their protein-binding activity. Finally we take advantage of the good conductivity properties of the conducting polymers and measure the direct electron transfer properties of a redox-active membrane protein bound to the substrate [8].
The platform described here is a first step for fabricating functional arrays of membrane proteins and we believe it will be a candidate of choice to produce electronically transduced nano-biosensors.

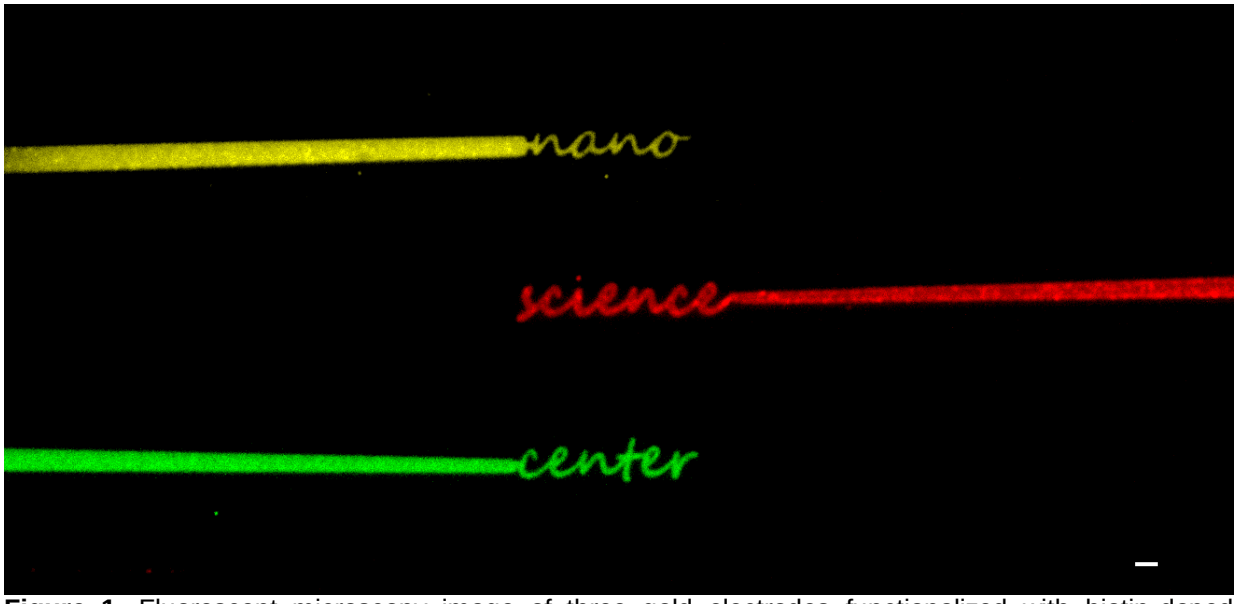

Figure 1. Fluorescent microscopy image of three gold electrodes functionalized with biotin-doped polypyrrole film and (top and central electrode) streptavidin and neutravidin Oregon Green (bottom electrode). By successive polymerization and protein incubation, the membrane protein tOmpA trapped in biotinylated amphipols and NBD fluorescent-labelled amphipols was immobilized on the top electrode and the membrane protein bacteriorhodopsin trapped in biotinylated amphipols and Alexa 647 fluorescent-labelled amphipols was immobilized on the central electrode. The image is obtained by overlaying fluorescence images obtained in three different channels. Scale bar is 5 µm.

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