# NOVEL ELECTROCHEMICAL BIOSENSOR SURFACE MODIFICATION METHOD BASED ON PHOTOBLEACHING

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## ABSTRACT

Methods for fast and reliable biochemical surface functionalization are required for all microfluidic assays involving the selective interaction of biochemically relevant ligands such as, e.g., antibodies, proteins or peptides with technical surfaces. In this work we describe a very fast and reliable methods for the modification of gold electrodes for electrochemical assays which allow the creation of dense, highly specifically binding surfaces with very low electrochemical impedance. The method uses photobleaching as coupling technique which has, as war as the authors are aware, never been used for electrode surface modification.

**KEYWORDS:** Electrochemical Sensor, Microfluidics, Photobleaching, Surface Modification, Biosensor

## INTRODUCTION

There are a number of surface modification techniques for label-free affinity assays available. The most commonly used methods reported in literature include physical adsorption, self-assembled monolayers and conductive polymers [1, 2]. In this work we propose a novel surface modification technique based on photobleaching. Photobleaching is a photochemical process which leads to the permanent damage of fluorophores after excessive irradiation. The overexposure of the fluorophores induces their destruction and the formation of short-lived radicals which can be used to couple ligands to BSA [3] and unsaturated compounds [4] coated surfaces.

## EXPERIMENTAL

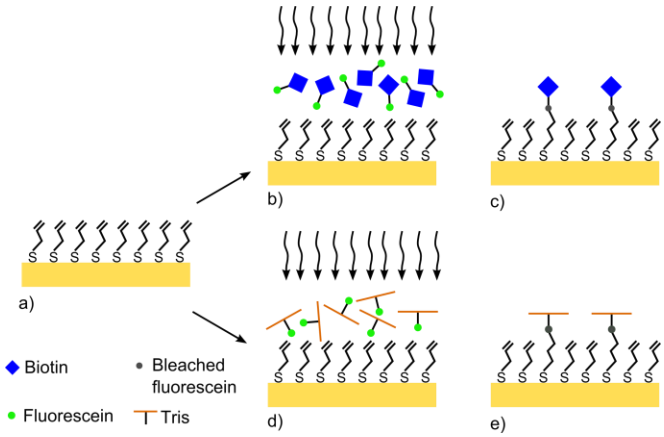


*Figure 1: Schematic diagram of the photobleaching-based immobilization technique. a) Self-assembled monolayer of ally mercaptan formed in the gas phase. b) Fluorescein-biotin is photobleached. c) Immobilization of biotin to the non-saturated surface. d) Fluorescein-tris is photobleached. e) Immobilization of tris to the non-saturated surface.*

A gold surface was coated with allyl mercaptan in gas phase, creating a self-assembled monolayer surface suitable for immobilization via photobleaching. Fluorescein was the fluorophore chosen because it is inexpensive, biologically compatible (absorption in visible light spectrum) and commercially

available as a separate dye as well as conjugated to a variety of proteins and antibodies. Two different functionalizations were performed and investigated using fluorescence microscopy (see Figure 1). Firstly, biotin coupled to fluorescein was applied to the gold surface, covered with a glass coverslip and exposed to fluorescein's adsorption wavelength for 10 minutes at a power intensity of about 4 mW/cm². The same procedure was repeated by substituting biotin-fluorescein with Tris(hydroxymethyl)aminomethane-(tris)-fluorescein. The first surface was functionalized with biotin and the second with a hydrophilic molecule which hinders nonspecific adsorption. In order to make the immobilization visible the substrate was probed with streptavidin-Cy3 (see Figure 2). After thoroughly washing the substrate in phosphate buffer saline (PBS) it was dried under nitrogen and scanned in the fluorescence scanner (Figure 2).

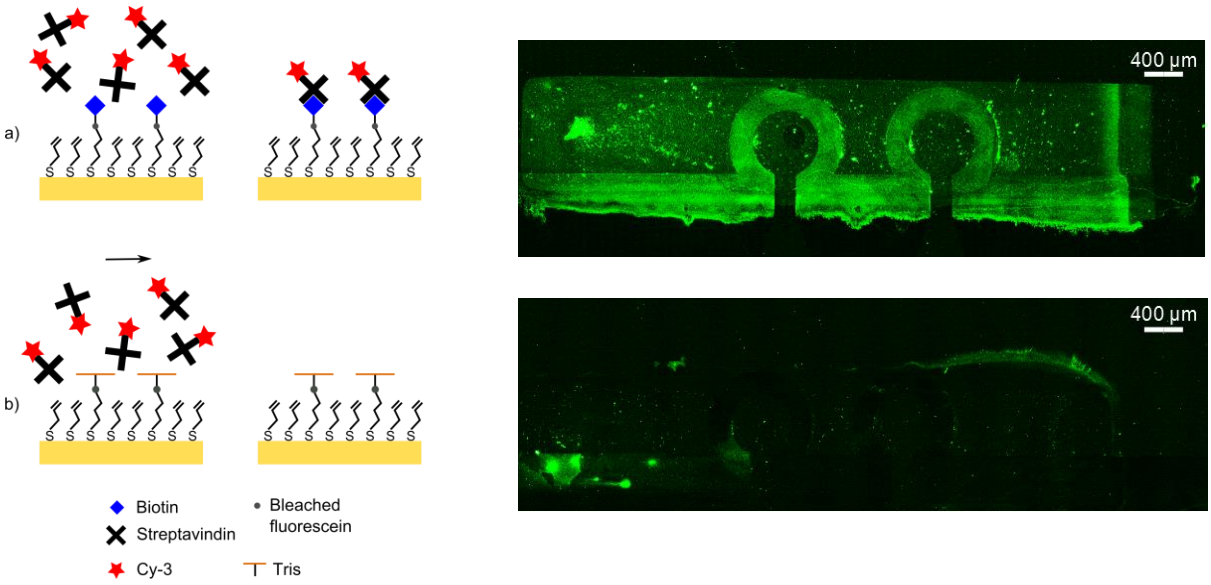


*Figure 2: Schematic diagram of previously prepared surfaces staining with streptavidin-Cy3. a) The biotin coated surface enables the binding with streptavidin-Cy3, thus creating bright areas in the fluorescence microscopy images. b) The tris coated surface blocks the unspecific binding of streptavidin-Cy3 showing as dark areas in the fluorescence microscopy images.*

## RESULTS AND DISCUSSION

As expected, the biotin functionalized surface is bright and the surface blocked with tris remains dark. The evaluation by means of EIS (see Figure 3) required some minor changes during the surface preparation. The two different functionalizations were carried subsequently out on the same electrode, thus immobilizing biotin and, at the same time, suppressing nonspecific adsorption. The staining step for fluorescence microscopy was carried out in a silicone microchannel over the electrodes. In order to assess the nonspecific adsorption the electrode was probed twice with a 1 mg/ml BSA solution. Finally, solutions containing 1 and 10 ng/ml biotin were probed across the electrodes resulting in clear signal increases.

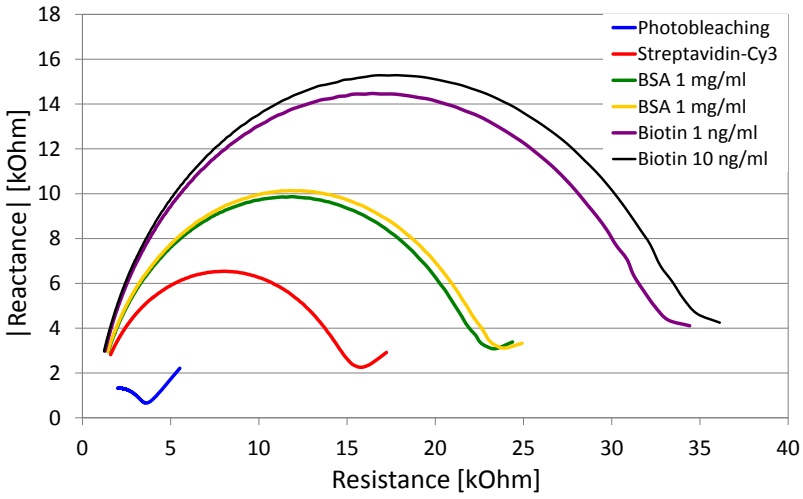


*Figure 3: Nyquist diagram of electrodes modified by photobleaching. Blue curve represents the electrode impedance after the coupling of biotin and tris to the surface. The red curve was measured after the incubation of streptavidin-Cy3. The green and yellow curves were obtained after probing the electrode with 1 mg/ml of BSA. Purple and black curves show the electrode impedance after probing with 1 and 10 ng/ml of biotin, respectively.*

## CONCLUSION

Our novel surface modification based on photobleaching provides a simple, fast and flexible method to immobilize ligands. Measurements based on fluorescence microscopy and EIS have confirmed its efficiency and demonstrated detection down to as little as 1 ng/ml for biotin. This method provides an additional advantage for EIS as it generates low-impedance layers which enable the fabrication of high sensitivity biosensors. This process can be easily adapted to work with other fluorescein labelled ligands (e.g., IgG and enzymes).

## REFERENCES

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