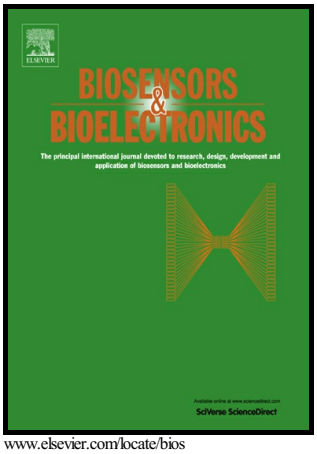

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Regenerative biosensor for use with biotinylated bait molecules

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ABSTRACT:

Label-free biosensors are ideally suited for the quantitative analysis of specific interactions among biomolecules or of biomolecules with drugs, as well as for quantitation of diagnostic markers in biofluids. In contrast to the label-dependent methods, a new assay for a particular prey molecule can be set up within few minutes by immobilizing the corresponding bait molecule on the sensor surface, using one of the common immobilization procedures. Unfortunately, the extensive application of label-free biosensors is still hampered by the fact that the immobilization of the bait molecule is usually irreversible; for that reason, a new chip (which is expensive) is required for every successful or futile attempt. Here, we present a general method for the switchable immobilization of biotinylated bait molecules on a new desthiobiotin surface, using wild-type streptavidin as a robust bridge between the chip and the biotinylated bait. The immobilization of the bait is very stable, so that many cycles of prey injection and subsequent prey removal can be carried out. For the latter, common reagents like HCl, Na_2CO_3, glycine buffer, or SDS are employed. When desired, however, streptavidin plus the biotinylated bait can be completely removed by 3 min injections of biotin, guanidinium thiocyanate, pepsin, and SDS, which makes it possible to immobilize new biotinylated bait. The number of *in situ* regeneration cycles is unlimited during the lifetime of the chip (2-3 weeks). One chip can easily be shared by many users with unrelated tasks (as is typical in academics), or used for the fully automated screening of many different interactions (for example in pharmaceutical research). In comparison to other regenerative chips, the new chip surface has much wider applicability and all of its structural and functional parameters have been disclosed.

Keywords: Regenerative biosensor, Desthiobiotin surface, Wild-type streptavidin, Reversible immobilization, Surface plasmon resonance