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A new regenerable biocomponent for biosensing?

Biosensor performance and specificity strongly depends on the successful functionalisation of the sensing surface with appropriate capture biocomponents. A variety of bio-functionalisation strategies have been described to date, ranging from simple and fast random physisorption to a variety of strategies for directed anchoring. The use of biotin-binding proteins is one of the preferred approaches when searching for biomolecule orientation and optimal performance.

Among others, avidin incorporation onto the sensing surface generates highly efficient and specific substrates; binding of the biotinylated biomolecule of choice is straight-forward, and a variety of biotinylation reagents and ready-to-use biotinylated molecules are commercially available. However, avidin Aquilles Heel is precisely its high affinity for biotin and thus the difficulty to regenerate and reuse the avidin-modified surfaces. In this way, sensor bio-functionalisation leads to disposable instead of reusable devices, increasing considerably the final production costs.

Captavidin is a biotin-binding protein derived from avidin, in which a tyrosine in the biotin-binding site has been nitrated. This modification permits dissociation of the captavidin–biotin complex at pH 10.0, a characteristic that has been exploited by the provider to develop regenerable reagents (such as captavidin conjugated to agarose and acrylamide, which are applicable to

affinity chromatography and isolation of biotinylated components). In this work, we use Surface Plasmon Resonance (SPR) to demonstarte that captavidin can be immobilised onto a sensing surface, which can then be successfully subjected to up to 9 serial capture and regeneration steps using a biotinylated antibody.

Alternatively, the surface could be used for the serial capture of biotinylated bacteria, applicable to the development of whole-cell microbial biosensors. Besides, a captavidin/biotin-Ab-modified sensor could be used to detect 3 consecutive protein-target captures. Our results indicate that captavidin is a promising regenerable molecular tool that could be used during biosensor optimisation and validation, and that captavidin-modified surfaces could be fine-tuned into truly reusable sensors.

In this work, captavidin, a recently described biotin-binding regenerable protein, is investigated as a new biocomponent of reusable biosensors using as a model SPR.

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References

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