Surface functionalization strategies for optimal DNA biosensor performance

P. Robin[1], A. Kavand[1], P. Skigin[1], L. Mayoraz[1], S. Marocco[2], J. Pagnoncelli [2], D. Staedler[3], I. Stefanini[2]*, S. Gerber-Lemaire[1]*

[1]Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, [2] Department of Innovative Technologies, University of Applied Sciences of Southern Switzerland, [3] TIBIO Services, Scitec Research SA

The emergence of the COVID-19 pandemia highlighted the need for rapid, accurate and massive virus detection techniques to control the circulation of infectious diseases. Surface DNA-biosensors are interesting candidates for this purpose as they provide a high sensitivity detection, are affordable and can be implemented in microfluidic systems for automated detection.[1]

The design of such surfaces must address a number of parameters, such as the density and orientation of immobilized single-strand DNA (ssDNA) probes, which have been identified as key factors to control the performance of the biosensing device.[1,2] The available binding sites should be maximized to provide a large number of anchoring sites for the targeted gene, in order to increase the sensitivity of the sensor.

We suggest a variety of strategies to immobilize ssDNA probes on glass surfaces. The resulting sensing surfaces displayed different DNA probe densities, geometrical configurations and behaviours. A first simple system was obtained by conjugating ssDNA probes to amino-coated surfaces via a short succinic linker. Later, branched-peptides were developed to reach a higher immobilization density on borosilicate surfaces.[3] Then, the use of spacers of different lengths were developed to study the effect of the DNA/surface spacing on the hybridization density. Finally, ssDNA probes were conjugated through a stimuli-responsive polymer to obtain DNA biosensors with antifouling properties.

Overall, diverse surface functionalization strategies were developed for the conjugation of ssDNA probes on glass surfaces to obtain DNA biosensors with tailored configurations and behaviours. The resulting sensing surfaces were further implemented in a microfluidic screening platform for the detection of unamplified viral RNA from saliva samples. As a proof-of concept, with a simple functionalization pattern, a detection limit of 10 aM was reached for the identification of SARS-CoV-2 RNA by fluorescence readout, in 10 minutes from human saliva with a high specificity.[4] The detection efficiency of the newly developed sensing surfaces is under study for several viral RNAs.

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