Poster

DNA PROBE IMMOBILIZATION STRATEGIES FOR SENSITIVE SURFACE PLASMON RESONANCE IMAGING DETECTION OF DNA HYBRIDIZATION

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DNA microarrays based on multiplexed fluorescence measurements have revolutionized the practice of life sciences research, providing quantitative information on complex biological systems in a fraction of the time required by traditional methods. However, the application of such technology for quantitative measurement of biomolecules has been limited by laborious and time-consuming techniques associated with fluorescent labeling and detection. More recently, the technique of surface plasmon resonance imaging (SPRi) has attracted a lot of attention as a label-free, multichannel detection method well suited to the real-time monitoring of DNA hybridization. An essential requirement for SPR imaging of DNA binding interactions is the formation of robust, reproducible arrays of DNA sequences tethered to gold-coated surface. The method used for immobilization of the DNA probe onto the gold surface plays an important role in the overall performance of DNA biosensors, influencing the sensitivity and the limit of detection. The immobilization step should be carried out such that a well defined probe orientation, easily accessible by the target, is achieved.

In this paper two optimized DNA immobilization chemistries are used and compared. In particular, DNA immobilization using direct covalent attachment of thiol-modified DNA sequences is contrasted with the electro-polymerization method that uses poly-pyrrole DNA binding. Two different 24-mer oligonucleotide probe sequences (Zip9: GACCATCGTGCGGGTAGGTAGACC and Zip7: TGCGATCGCAGCGGTAACCTGACC) with T-10 spacers were immobilized on 50nm thick gold-coated SF-11 glass prisms. The optimized immobilization conditions for each method are as follows. For the first method, manual spotting of 1μM thiolated single-stranded ss-DNA probe in 1M K_2HPO_4 is performed in humid environment allowing probe self-assembly for 5 hours on the gold surface [1]. This is followed by 90 min passivation with mercaptohexanol, resulting in total preparation time of 7 hours (Fig.1a). The second method used automated spotting of a mix of 20mM poly-pyrrole and 1μM ss-DNA probe containing a pyrrole residue which polymerizes on the gold surface under a short electrochemical pulse, resulting in total preparation time of less than one hour (Fig.1b) [2].

Scanning-angle SPR imaging apparatus (Genoptics, France) at 800 nm wavelength is used to conduct the SPR kinetic analysis of DNA hybridization and assess the sensitivity, reproducibility and the limit of detection for each immobilization chemistry. It was found that the immobilization chemistry relying on thiolated DNA probe results in a signal that is approximately two times higher than that using poly-pyrrole electro-polymerization for both DNA sequences employed (Fig.2). In particular, the limit of detection for thiolated DNA probe was found to be an order of magnitude higher (1nM) compared to DNA-pyrrole probe (10nM). While both chemistries used were resistant to non-specific adsorption and gave reproducible results with no significant signal loss for repeated surface regeneration, substrates prepared using DNA-pyrrole chemistry have shown generally better thermal stability and long-term substrate integrity. These results are summarized in Table 1.

We have shown here a comparison of two different DNA immobilization chemistries well suited for high-throughput, label-free detection of DNA hybridization using SPR imaging. The choice of the chemistry employed will strongly depend on the application requirements including the sensitivity, thermal stability and the overall assay time.

References:

[1] A. W. Peterson, R. J. Heaton, and R. M. Georgiadis, Nucl. Acids Res. 29, 5163-5168 (2001).
[2] Fiche J.B., Fuchs J., Buhot A, Calemczuk R. and Livache T., Anal Chem, 80(4) 1049-57 (2008)

Figures and Tables:

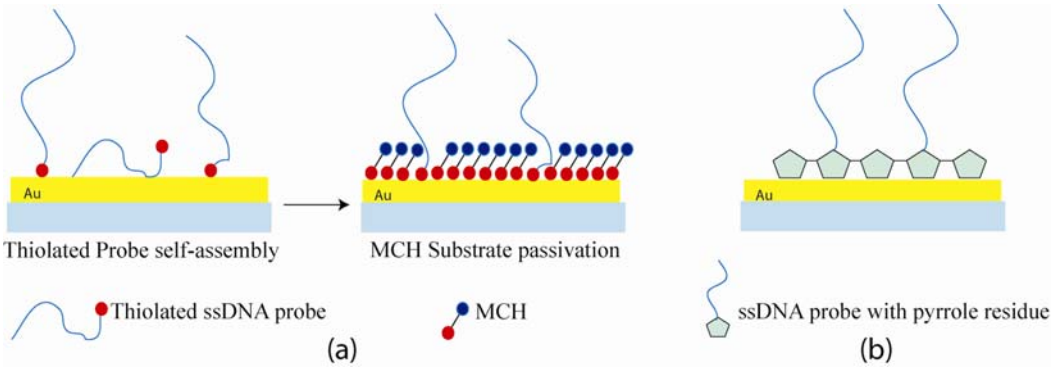

Figure 1. *DNA immobilization chemistry on gold-coated SF-11 glass prism (a) Self-assembly of the thiolated DNA probe followed by MCH surface passivation (b) Electropolymerization of DNA probe with pyrrole residue*

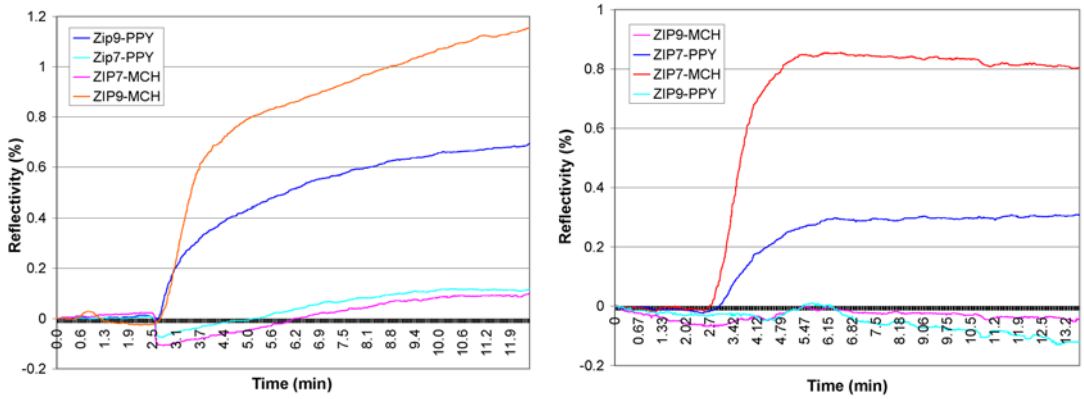

Figure 2. *Kinetic curves of DNA hybridization obtained using SPRi for (a) Zip 9 sequence (b) Zip 7 sequence*

Table 1. *Summary of results for the comparison of pyrrole and thiol based DNA immobilization chemistries*

Surface: **Property:**	**Poly-pyrrole**	**Thiols**
Preparation time	1h	7h
Thermal stability	Very good	Unstable
Long term stability	50% loss in ~3 months	65% loss in ~3 months
Reproducibility	Very good	Very good
Max SPR reflectivity change (Hybridization signal)	SPRi: ≤ 1.2%	SPRi: ≤ 2.5%
Detection limits	10nM	1nM