Application Note # 124

Selectively amplified SPR – New labeled method for enhancing biosensor performance

Real-time Analysis of DNA Hybridization with Enhanced Sensitivity and Specificity

Real-time analysis of DNA hybridization with SAMP-SPR yields high specificity, improved signal-to-noise ratio, and a significantly cleaner sensorgram.

Background

Surface plasmon resonance (SPR) is a well-established technique for the monitoring of biomolecular interactions. SPR has been frequently used for the real-time analysis of hybridization of DNA and RNA oligonucleotides. One challenge in this context is that SPR is a non-specific detection method, i.e. any substance that adsorbs onto the sensor surface is detected. For example, SPR analysis does not indicate which strands in a mixture of oligos hybridize with an immobilized DNA strand on the chip surface. This is in sharp contrast to the high specificity of fluorescence-based analysis, where only the labelled oligo is detected.

Multi-Parametric SPR (MP-SPR) is a novel method utilizing the same physical principles as SPR, where not only the SPR peak minimum shift, but also other parameters from the optical signal are measured as a function of time.

In this Application Note we demonstrate how real-time SAMP-SPR analysis of DNA hybridization with MP-SPR using oligos labelled with Episentec™ dyes results in a specificity comparable to that of fluorescence analysis. Also the sensitivity sensitivity improved and disturbing signals are reduced, resulting in a better signal to noise ratio.

Experimental

Hybridization of both unlabelled (native) and Episentec dye-labelled 25-mer DNA oligonucleotides (Episentec, www.episentec.com) were performed. Firstly, biotin-BSA conjugate was spontaneously adsorbed onto a clean gold sensor chip followed by binding of avidin. A 25-mer DNA oligo with a spacer coupled to a biotin entity was then bound to the avidin. Subsequently, a number of samples containing either native DNA, or DNA labelled with Episentec dye B10, were injected and hybridized. Denaturation was performed with 25 mM sodium hydroxide. All experiments were performed using BioNavis Multi-Parametric SPR Navi™ 200. Enhanced sensorgrams were calculated in accordance with methods implemented in the EpiGrammer™ software.

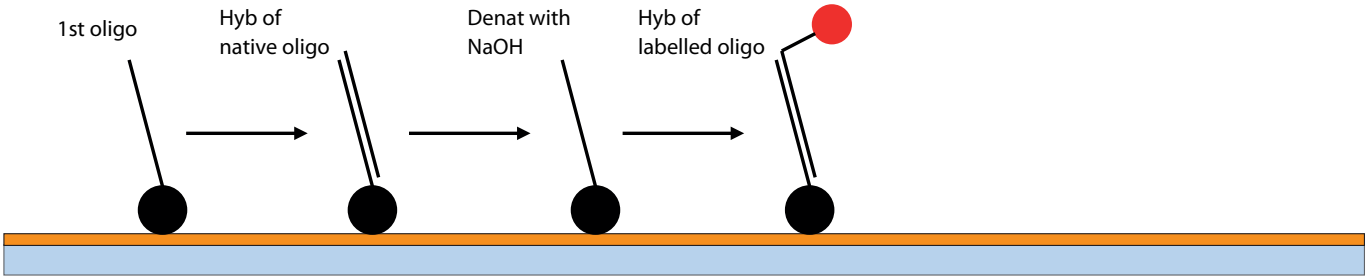

Figure 1. The principle of surface competition. The analyte competes with the labelled analyte analogue for binding to the ligand on the surface.

Excellence in Surface Plasmon Resonance
BioNavis

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 44 5872001
e-mail : info@bionavis.com
www.bionavis.com

Results and discussion

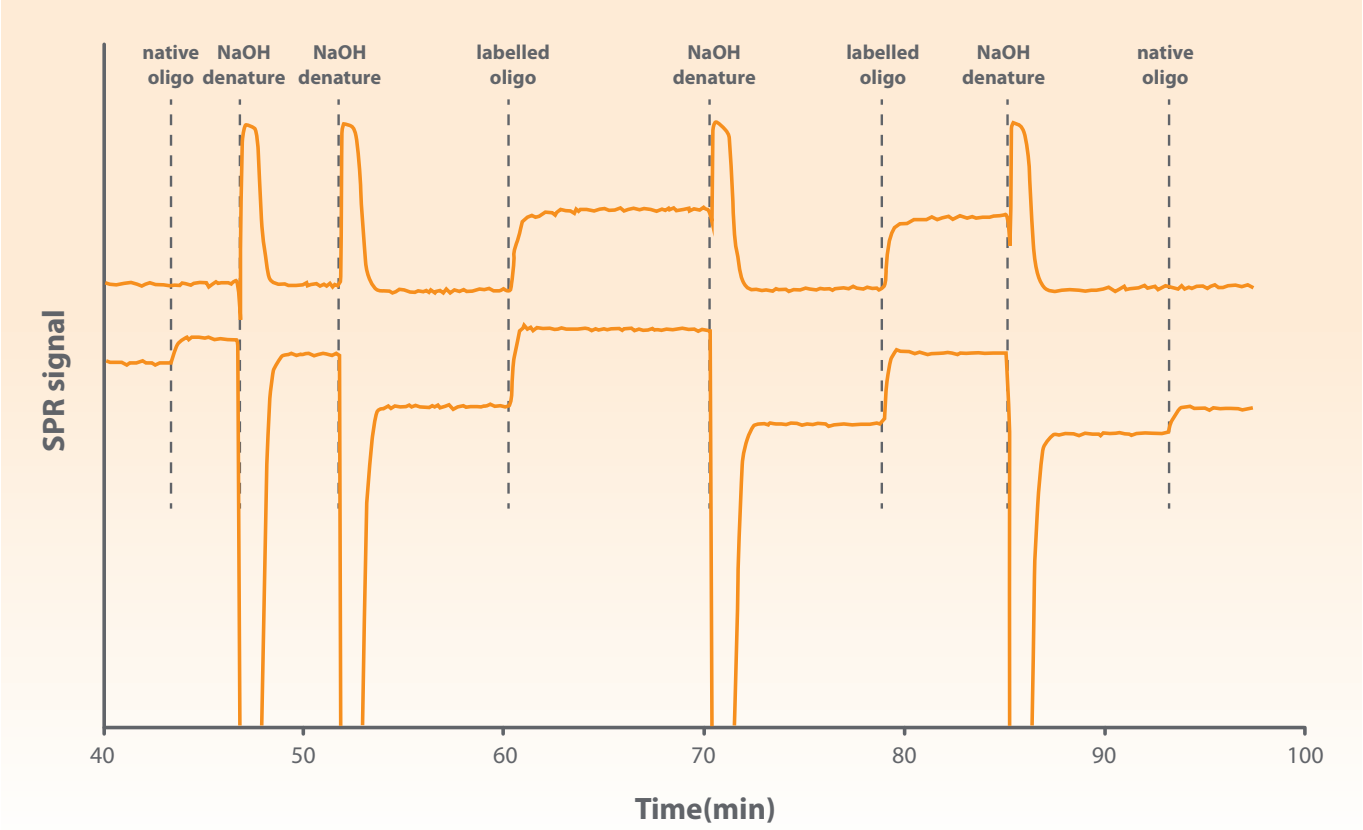

Figure 2. Sensorgrams for four DNA oligo hybridizations and denaturation with sodium hydroxide. Upper trace: enhanced sensorgram. Lower trace: traditional SPR sensorgram (SPR peak minimum).

Figure 2 is a plot of the resulting sensorgrams - the standard sensorgram and the sensorgram enhanced by the EpiGrammer software - for four DNA hybridization steps and subsequent denaturation steps with sodium hydroxide (NaOH). The injections are shown in the upper part of the plot. The two sensorgrams are scaled to the same height for the labelled DNA injections. Three features are obvious from a comparison of the sensorgrams:

Specificity: The upper, enhanced sensorgram shows only signals from hybridization of dye-labelled DNA, with no signal from hybridization of native DNA.

Sensitivity: The signal-to-noise ratio of hybridization of dye-labelled DNA in the enhanced sensorgram is significantly higher than that of native DNA in the standard sensorgram.

Sensorgram clean-up: The sodium hydroxide peaks represent the contribution from a change of the bulk liquid composition – the heights of these peaks are reduced by almost 90% in the enhanced sensorgram. Also, the sodium hydroxide injections cause a pronounced baseline shift due to desorption of bound material (i.e. loosely bound BSA and SA) from the surface in the standard sensorgram – this shift is absent in the enhanced sensorgram.

Conclusions

Real-time analysis of DNA hybridization with using SAMP-SPR method yields high specificity and improved signal-to-noise ratio compared to traditional SPR detection.

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 44 5872001
e-mail : info@bionavis.com
www.bionavis.com