Application Note # 122

Selectively amplified SPR – New labeled method for enhancing biosensor performance

Enhanced Sensitivity and Specificity of a Small Molecule Model System

The use of Selectively Amplified Surface Plasmon Resonance (SAMP-SPR) greatly enhances the analysis of low molecular weight compounds with SPR. Improvements include:

- *Enhanced sensitivity: Typically 100-fold or better increase in signal-to-noise ratio.*
- *Enhanced specificity: Only the dye label is detected, minimizing non-specific interference.*

Background

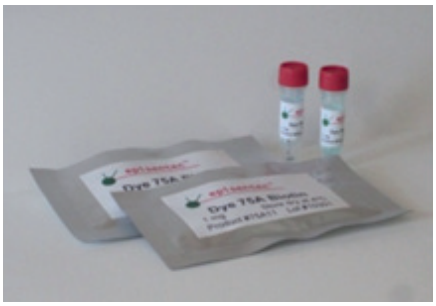
Surface plasmon resonance (SPR) is a well established technique for monitoring biomolecular interactions and determining the concentration of different molecular species. However, since SPR instruments are designed to measure the mass of the interacting molecules, their sensitivity is often limited when working with small molecules. Traditional SPR detection is non-specific in the sense that anything that adsorbs onto the sensor surface generates a signal. Non-specific binding of even small amounts of large molecules, e.g. proteins, interfere with the quantitative analysis of low molecular weight compounds.

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 combining novel MP-SPR technology from BioNavis together with labelling of small molecules with Episentec™ dyes results in enhances both the sensitivity and the specificity of SPR mediated detection.

Experimental

Binding of the vitamin biotin (244 Da) was used as a small molecule model system. Avidin in SSC (saline sodium citrate) buffer was allowed to spontaneously adsorb onto two clean SPR gold sensor chips in the BioNavis MP-SPR instrument. On the first chip, 400 µM of native biotin – enough to completely saturate the avidin binding sites – in SSC/DMSO 75/25 was injected and the binding reaction was monitored. Then 5 µM and 25 µM of biotin labelled with Episentec dye B12 (Episentec, www.episentec.com) was injected in order to study possible non-specific binding effects of the labelled biotin. On the second chip, 5 µM and 25 µM of dye-labelled biotin was injected without the preceding injection of native biotin. Enhanced sensorgrams were calculated in accordance with methods implemented in the EpiGrammer™ software.



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Results and discussion

Figure 1 shows the binding of two avidin pulses. The upper trace is the standard sensorgram. The lower trace is the enhanced sensorgram that only monitors the binding of dye-labelled molecules. The signal is essentially zero: the specificity is very high and the binding of non-labelled molecules is not monitored.

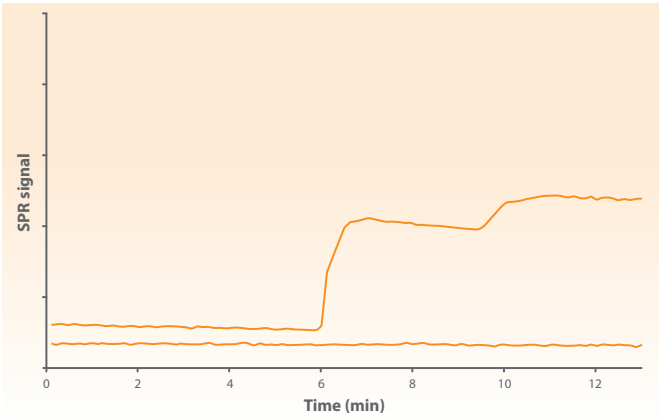

Figure 1. Upper trace: Standard sensorgram of two avidin pulses. Lower trace: Enhanced sensorgram of two avidin pulses

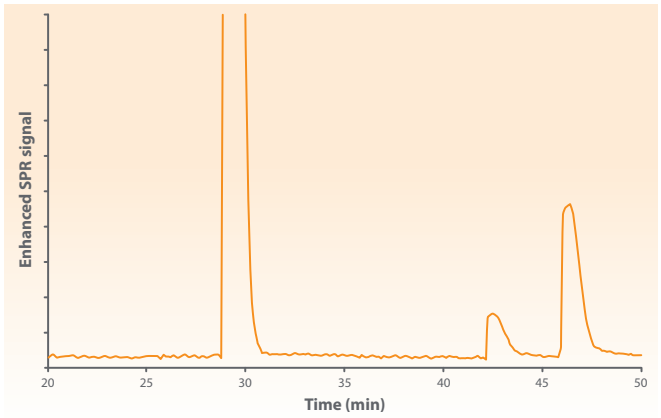

Figure 2. Enhanced sensorgram of biotin at 29 minutes and labelled biotin at 42 and 46 minutes.

Figure 2 shows the binding of native biotin followed by two pulses of labelled biotin on the first avidin chip after signal enhancement. After linear baseline correction there is no detectable non-specific binding of labelled biotin neither to the saturated avidin nor to possible naked gold spots on the surface.

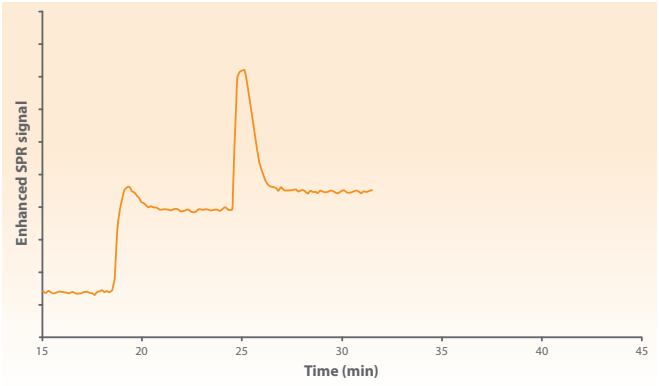

Figure 3. Enhanced sensorgram of labelled biotin at 19 and 25 minutes. Same scale as Fig 2.

Figure 3 shows the binding of two pulses of dye-labelled biotin onto the second chip after signal enhancement. Both binding steps are clearly detected. The signal-to-noise ratio for the binding of labelled biotin is 100. Since native biotin was not detected (the signal-to-noise ratio by definition being <1), the sensitivity enhancement using dye-labelled biotin is greater than a factor of 100.

Conclusions

The unique combination of BioNavis MP-SPR and Episentec amplification dyes allow utilization of the new SAMP-SPR technology. In the analysis of low molecular weight compounds, the method allows unique benefits compared to existing technology:

- Enhanced sensitivity: The signal-to-noise ratio may be increased at least 100-fold.
- Enhanced specificity: Only the dye label is detected while the non-specific binding of any colourless material is totally cancelled.

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