Application Note # 123

Selectively Amplified SPR (SAMP-SPR) – A novel labelled method for enhancing optical biosensor performance

Competitive Analysis of a Small Molecule Model System

Analysis of small molecules using SAMP-SPR in a competitive assay format increases the sensitivity of SPR to levels previously unattainable in the absence of labelling with large molecules.

Competitive analysis with small dye labels is beneficial for:

- *Determining equilibrium constants and affinity ranking*
- *Performing competitive kinetic analysis.*

Background

Surface plasmon resonance (SPR) is a well established technique for monitoring biomolecular interactions. However, the sensitivity of SPR is often limited when working with small molecules. In order to increase the sensitivity to small molecules, the analysis is often performed in a surface competition format [1]. In this format (Figure 1), the binding of the analyte molecule competes with the binding of an analyte analogue labelled with a heavier, more detectable molecule, e.g. an antibody. A drawback is that labelling with large, bulky molecules induces significant changes in the physicochemical properties of the small analyte, complicat¬ing the study of both equilibrium and kinetic properties.

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. This utility is taken advantage of in the current application.

In this Application Note we demonstrate a novel competitive analysis technology known as SAMP-SPR, that is at least ten-fold more sensitive than conventional competitive analysis achievable via conventional SPRusing BioNavis MP-SPR technology in conjunction with competing small Episentec™ dye molecules. The use of small dye labels facilitates the study of both equilibrium and kinetics.

Experimental

Binding of the vitamin biotin (244 Da) was used as a small molecule model system. First, the biotin-binding protein avidin in SSC (saline sodium citrate) buffer was allowed to spontaneously adsorb onto clean gold sensor chips in the MP-SPR instrument. Then, a number of samples containing 50 µM biotin labelled with Episentec dye B12 (Episentec, www.episentec.com) mixed with native biotin in the concentration range 0-50 µM in SSC/DMSO 75/25 (v/v) were injected. All experiments were performed using the BioNavis SPR Navi™ 200 MP-SPR instrument. Enhanced sensorgrams were calculated in accordance with methods implemented in the EpiGrammer™ software.

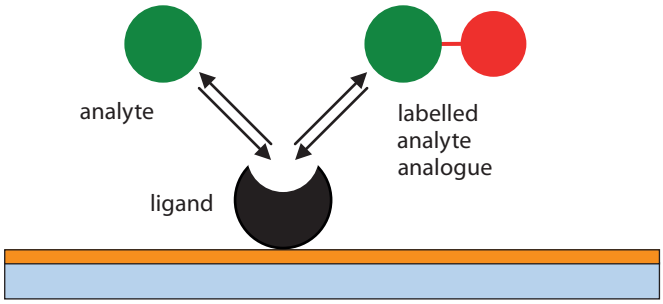

Figure 1. The principle of surface competition. The analyte competes with the labelled analyte analogue for binding to the ligand on the surface.

Excellence in Surface Plasmon Resonance
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Results and discussion

Figure 2 is a graph where the inverse of the measured signal (1/signal) is plotted against the ratio of native biotin to labelled biotin. As expected, from the theory of competitive analysis [2], this yields a straight line graph. The fit to the straight line is excellent with R2 being >0.99.

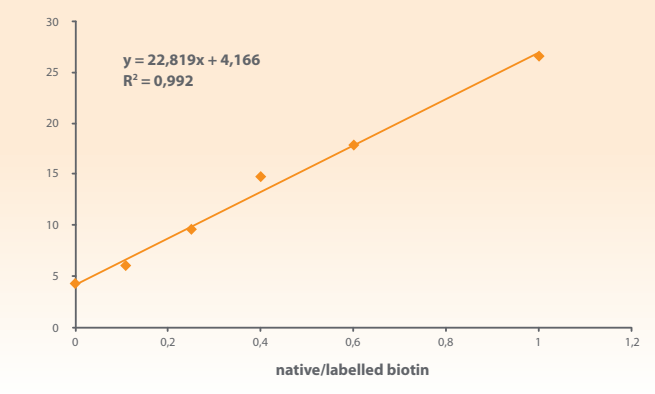

Figure 2. Resulting plot of inverse signal versus relative concentration of native biotin.

The relative equilibrium binding constant can be calculated by dividing the slope of the straight line with the intercept. In this case, the equilibrium constant for native biotin is 5.5 times higher than that of dye-labelled biotin – a relatively small difference when considered that affinity is an exponential property.

By using the methods of competitive kinetics [2], it is also possible to evaluate the binding kinetics. For accurate results, the mass transfer rate and the kinetic constants for the analyte and the labelled analyte analogue should be in the same range. This condition is fulfilled for labelling with small dye molecules, but not for labelling with large, bulky molecules.

Conclusions

Analysis of small molecules within a competitive assay using SAMP-SPR increases the sensitivity to levels previously unattainable in the absence of labelling with large molecules. Competitive analysis with small dye labels is beneficial for:

- Determining equilibrium constants and affinity ranking
- Performing competitive kinetic analysis.

[1] R.B.M. Schasfoort, A.J. Tudos (Eds.) "Handbook of Surface Plasmon Resonance" RSC Publishing, Cambridge, 2008, Chaps. 7 & 11
[2] R. Karlsson, Anal. Biochem. 1994, 221, 142-151.

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