Regenerable avidin kit assays part I: Working principle

This Application Note introduces the working principle of MP-SPR Navi™ Regenerable avidin kit, which allows to use the same sensor for multiple capture of biotinylated ligands (Figure 1). The kit is based on a recombinant avidin, which enables to capture ligands in reversible manner. Indeed, biotinylated ligands can be washed from the sensor surface at relatively mild regeneration conditions and subsequently reloaded for multiple assays.

The regeneration capacity of a single sensor has been proven up to 100 cycles without affecting its ligand capture efficiency. The kit provided by BioNavis contains enough reagents for 40 complete cycles of regeneration. Planar biotin-PEG sensor surface is suitable for detection of analytes larger than 10 000 Da. Please see an assay example of regenerable avidin kit for affinity and concentration studies in Application Note #168.

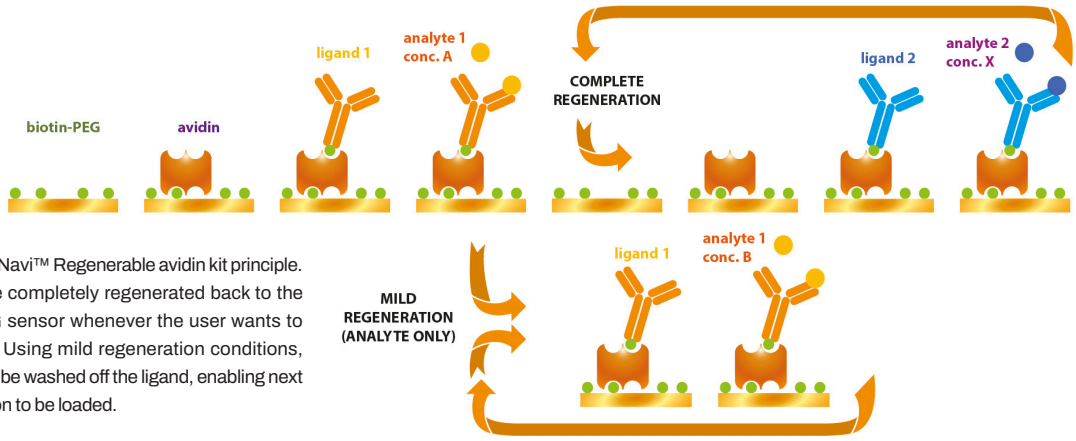

Figure 1. MP-SPR Navi™ Regenerable avidin kit principle. The surface can be completely regenerated back to the original biotin-PEG sensor whenever the user wants to change the ligand. Using mild regeneration conditions, only the analyte can be washed off the ligand, enabling next analyte concentration to be loaded.

Introduction

Surface Plasmon Resonance (SPR) is an optical phenomenon which is highly sensitive for detecting refractive index changes near the measurement surface which might in particular result from molecular binding or release. Based on the SPR phenomenon, Multi-Parametric Surface Plasmon Resonance (MP-SPR) technique has become a real-time and label free in vitro tool for affinity, kinetics and concentration analysis. The robust fluidic setup and wide angular range of MP-SPR technology enable these assays to be carried out even with crude samples such as 100 % serum, sea water and cell medium. The proven SPR detection method is widely used in development of drugs and biosensors, antibody characterization, diagnostics and even with uptake or adhesion studies with live cells.

This Application Note showcases the working principle and typical experimental protocol when working with Regenerable avidin kit for MP-SPR Navi™ instruments. In this case study, the concentration of IgG and Apolipoprotein-A1 was determined in human serum (see Application Note #168 for more information and detailed results).

In a traditional approach, affinity, kinetics and concentration studies are carried out with ligand being covalently immobilized onto the sensor surface. One typical strategy consists on activation of carboxymethyl dextran (CMD) based matrix followed by covalent attachment of ligand molecules (Figure 3). Immobilization of a new ligand (e.g. protein) requires assay development i.e. buffer optimization, regeneration scouting, optimization of ligand density and analyte concentration range or even selecting the most suitable CMD sensor type. Additionally, covalent immobilization procedures (e.g. EDC/NHS chemistry for amine coupling) may affect the ligand activity and its active concentration on the surface. Also, chemical immobilization of ligand onto CMD sensors provides a single-use solution with necessary exchange and functionalization of a new sensor when another ligand needs to be analyzed.

When using MP-SPR Navi™ Regenerable avidin kit, studies of biomolecular interactions or concentration analysis can be significantly shortened as the same sensor may be used for different ligands via regeneration. Through biotin-avidin bridge, any biotinylated ligand can be reversibly immobilized (captured) over the sensor surface without any particular immobilization buffers or chemical activation. In concentration analysis assay, the analyte introduced to the surface at a given concentration A can be mildly regenerated while keeping the same ligand for loading of further analyte concentrations (Figure 1 – mild regeneration). The sensor can be eventually regenerated to the original biotin-PEG layer where another ligand can be captured over the surface (Figure 1 – complete regeneration).

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Materials and methods

Concentration analysis were conducted using MP-SPR Navi™ 220A NAALI instrument and MP-SPR Navi™ Regenerable avidin kit. Entire measurement was carried out having PBS + 0.05 % Tween20 pH = 7.4 as running buffer. The flow rate was set to 30 µl/min and the measurement temperature was set to + 20°C.

First, 50 µg/ml of avidin was injected over sensor slide for 7 min with 5 min post-wait time. 25 µg/ml of biotinylated anti-human IgG (Jackson Immunoresearch, 109-065-003) was subsequently injected for 7 min and captured over the sensor surface (5 min post-wait time). One channel was left as reference where no biot-antibody was introduced. After the ligand capture, the sensor was measured against 0.25 – 66.7 nM of human IgG standard samples (Jackson Immunoresearch, 009-000-003) and human serum AB samples (Biowest, S4190-106, lot: S16095S4190) with dilution factor of 1/5000 - 1/2000. The analytes were loaded within 7 min injections followed by 5 min post-wait time. 10 mM Glycine pH = 2 was used as a mild regeneration solution with 2 min injection between each analyte concentration. After measuring all IgG standards and serum samples, complete regeneration was done with REG2 and REG1 solutions provided with the kit (2 min injection of each followed by 5 min post-wait time).

The very same sensor slide was subsequently functionalized with 25 µg/ml of biotinylated anti-Apo-A1 (Jackson Immunoresearch, 009-000-003) and similar experimental protocol was carried out for concentration analysis. Apolipoprotein-A1 standard samples (Sigma, A0722) were measured with a concentration range of 1 – 283 nM. Dilution factor 1/300 – 1/13000 was used for Apo-A1 detection in serum. After the measurement and complete regeneration, the sensor slide was rinsed with MilliQ water, dried and stored in fridge for next set of experiments.

Results and discussion

One single regenerable avidin kit sensor slide was successfully used to measure both IgG and Apo-A1 concentration in human serum. Injections of analyte standards enabled to establish a calibration curve from which the analyzed serum samples were assessed using TraceDrawer™ for MP-SPR Navi™. The human serum samples resulted in 5.2 g/l of IgG and 4.5 g/l of Apo-A1 which is expected for physiological values. Please see AN#168 for detailed results with standard curves and stacked sensograms.

Figure 2 represents the experiment workflow based on measured sensograms. Fig. 2A shows consequent injections of sample concentrations over two ligands: anti-IgG and anti-Apo-A1. Fig. 2B is a close-up of functionalizing the sensor for IgG measurement and loading of the first two serum concentrations. In 1 hour since the beginning of the run, the MP-SPR Navi™ Regenerable avidin kit enables already to inject second analyte concentration while it would be the minimum time needed for ligand immobilization if CMD sensor was used. Additionally, the preparation of all different immobilization reagents is more time consuming as compared to direct preparation of sample dilutions into running buffer in case of the Regenerable avidin kit.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA, 400 KONTIO, 410A KAURIS and 420A ILVES

Sensors surfaces: MP-SPR Navi™ Regenerable avidin kit

Software: MP-SPR Navi™ Control, DataViewer and TraceDrawer™ for MP-SPR Navi™

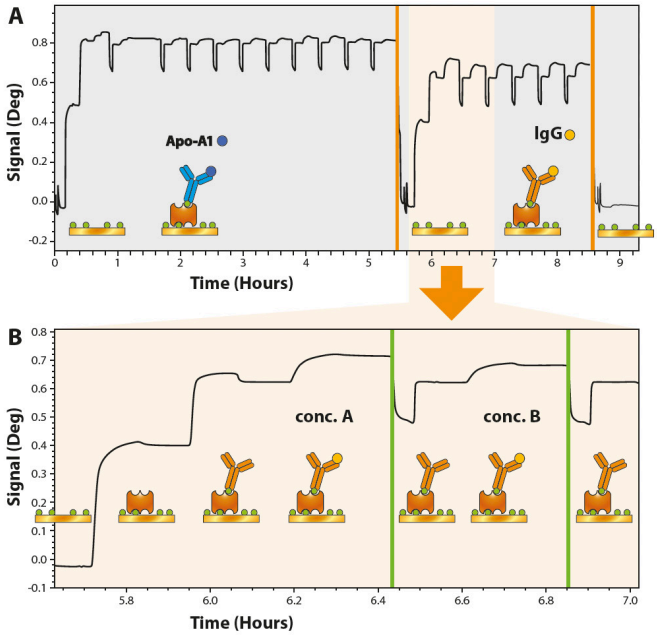

Figure 2. Measurement sensogram representing raw data of IgG and Apo-A1 concentration studies with MP-SPR Navi™ Regenerable avidin kit. A. Full measurement sensogram. Orange lines represent the time points of complete regeneration B. Close-up represents anti-IgG immobilization with biotin-avidin bridge and measuring the first two analyte concentrations. Green lines represents the time points of mild regeneration.

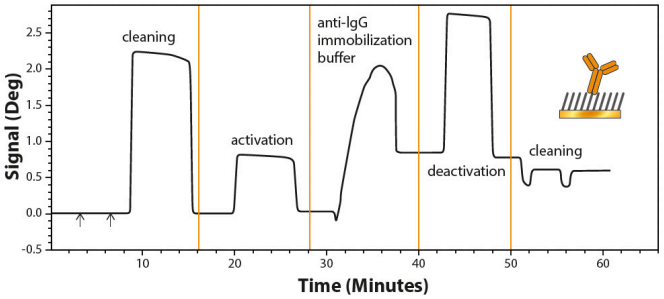

Figure 3. Anti-IgG immobilization over CMD sensor slide. The sensor slide needs to be cleaned, activated, deactivated and cleaned again besides injecting anti-IgG in correct immobilization buffer.

Conclusions

MP-SPR Navi™ Regenerable avidin kit is an excellent tool in development of MP-SPR based assays. The kit allows the user to conduct precise and highquality measurements in shorter time and convenient experimental set-up. Besides having the ligand available with biotin-conjugation, one does not need to optimize buffers and immobilization conditions. Finally, the costeffectiveness of SPR measurements improves since the same sensor slide can be completely regenerated up to 100 times.

For further reading, please see Application Note #138 for interaction analysis with CMD sensor and Application Note #168 for MP-SPR Navi™ Regenerable avidin kit for detailed kinetics and concentration assay examples.

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