Application Note # 168

Regenerable avidin kit assays
Part II: Biomolecule affinity and concentration studies

This application note introduces the MP-SPR Navi™ Regenerable avidin kit, which allows to the use of same sensor for multiple different targets and ligands up to 100 regeneration cycles (Figure 1). The kit is based on avidin mutant protein, which enables to wash-off the ligand at relatively mild conditions and subsequently reload the surface with biotinylated probe molecules. The regeneration capacity of a single sensor is 100 cycles. The kit contains enough reagents for 40. For traditional bioassays, the carboxymethyldextran (CMD) sensor is used only for one ligand. After that the CMD sensor has to be changed and the whole pre-conditioning has to be executed again. Using regenerable avidin kit the whole process shortens significantly as same surface can be used for different ligands via regeneration. Please see the working principle of regenerable avidin kit in detail in Application Note #166.

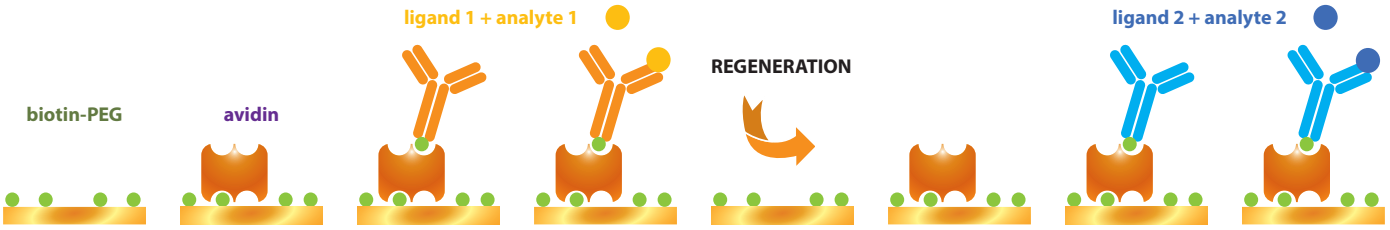

Figure 1. Schematic representation of the regenerable avidin kit working principle.

Introduction

Multi-Parametric Surface Plasmon Resonance (MP-SPR) is an excellent platform for affinity, kinetics and concentration analysis even in complex matrices, like serum. The proven SPR detection method is widely used in development of biosensors, antibody characterization, drug development and diagnostics. The robust fluidic setup and unique optical configuration of MP-SPR technology enable assays from crude samples such as serum and sea water.

Here we show two independent studies where Regenerable avidin kit was applied. Study I is a typical example of biomarker application where human IgG and Apolipoprotein-A1 (Apo-A1) can be detected in human serum. IgG level in serum provides key information of patient's humoral immune status (Gonzales-Guintela et al. 2008). Low Apo-A1 levels are associated with a higher risk risk of developing cardiovascular diseases (Florvall et al. 2006).

In study II, the affinity of intercellular signaling phosphoinositides (PIPs) to ORP2 protein was studied. ORP2 protein has an important role for instance in lipid and glucose metabolism or cell signaling pathways. PIPs are phospholipids attached to human cell membrane (molecular weight of around 700-800 Da) and contribute to cell regulation processes (Koponen et al., 2019).

Materials and methods

Study I

The measurements were carried out on MP-SPR Navi™ 220A NAALI at temperature of 22 °C with a flow rate of 30 µl/min. The running buffer was PBS + 0.05 % Tween20. Sensor functionalization with avidin was performed according to instructions of the kit.

Biotinylated anti-IgG (Jackson Immunoresearch, 109-065-003) and anti-Apo-A1 (Abcam, ab27630) antibodies were used as the ligand loaded onto avidin sensor at concentration of 25 µg/ml. The standard concentrations for IgG (Jackson Immunoresearch, 009-000-003) were between 0.25 – 66.7 nM. The standard concentrations for Apo-A1 (Sigma, A0722) were in the range of 1 – 283 nM. Before each new concentration, the analyte was regenerated from the antibody with short injection of 10 mM glycine at pH= 2.0. Analysis in serum was performed using human serum AB sample (Biowest, S4190-106, lot: S16095S4190) with dilution factor of 1/5000 - 1/2000 for detecting IgG and 1/300 – 1/13000 for apolipoprotein assays.

Study II

The measurements were performed using MP-SPR Navi™ 220A NAALI at temperature of 20 °C and 20 µl/min flow rate. The running buffer was 20 mM HEPES, 150 mM NaCl, pH 7.4.

pFOLD-Strep-ORP2 protein (recombinant fusion protein produced in *E.Coli*) was captured onto regenerable avidin sensor with 50 µg/ml solution until saturation was reached. The following PIPs molecules were tested: PI(4)P, PI(4,5)P_2 and PI(3,4,5)P_3. Analytes were sequentially loaded within the concentration range of 0.33 – 100 µM. The reference channel was functionalized with control pFOLD-Strept protein without ORP2 domains.

Data were analyzed with TraceDrawer™ v 1.8 in both studies.

AN_168.909.a

Excellence in Surface Plasmon Resonance
BioNavis

e-mail : info@bionavis.com
www.bionavis.com

Results and discussion

Study I

The kinetic analysis of sensograms obtained from IgG injections enabled to plot the calibration curve (Fig.2). IgG concentration in human male AB serum was determined to be 5.2 g/l.

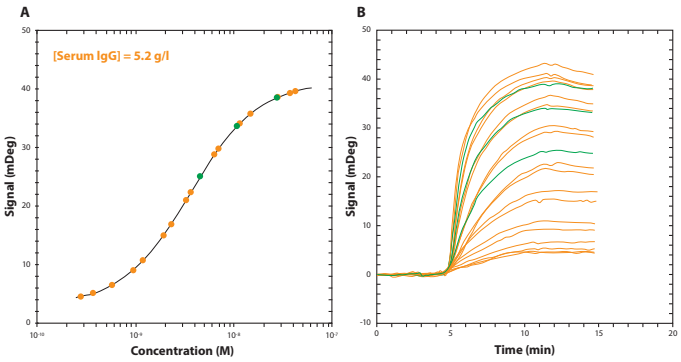

Figure 2. A. Calibration curve of IgG standards and serum samples. **B.** Sensograms of IgG standards and serum samples.

Concentration of apolipoprotein was calculated from calibration curves. Apo-A1 concentration in human male AB serum was determined to be 4.5 g/l (Fig 3.).

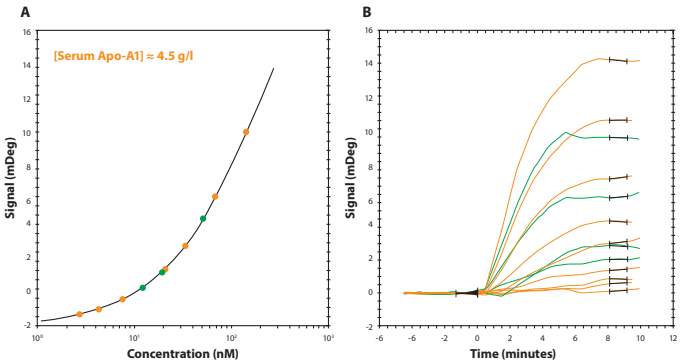

Figure 3. A. Calibration curve of Apo-A1 standards and serum samples. **B.** Sensograms of Apo-A1 standards and serum samples.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA, 220A NAALI, 400 KONTIO and 420A ILVES

Sensor surfaces: SPR102-AVI-2

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

Study II

The binding affinities (K_D) of PI(4)P, PI(4,5)P_2 and PI(3,4,5)P_3 molecules to ORP2 were successfully determined (Fig. 4). Kinetic analysis enabled to fit the experimental data to one-site binding model (TraceDrawer™).

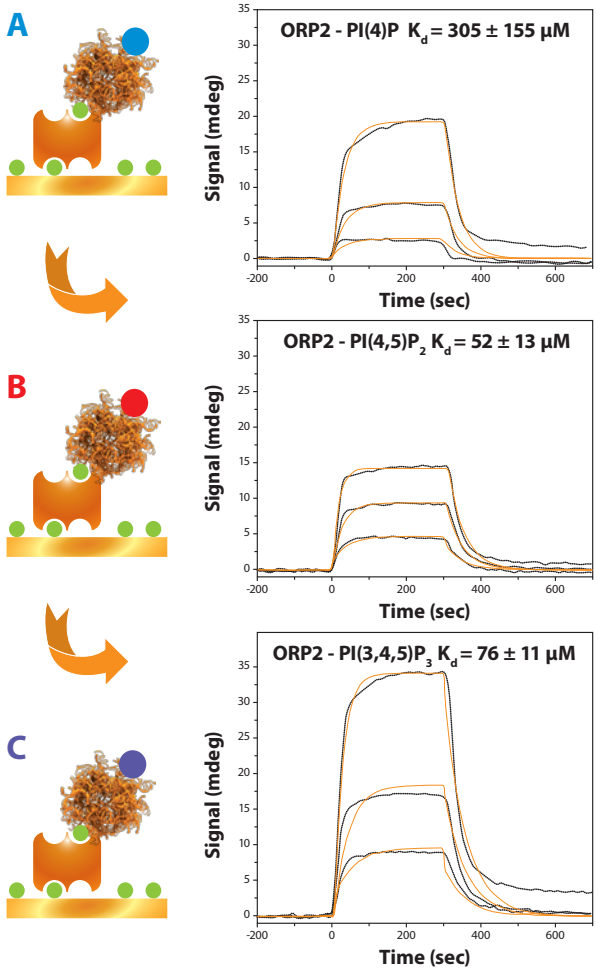

Figure 4. SPR binding curves of different PIPs binding to ORP2.

Conclusions

MP-SPR Navi™ Regenerable avidin kit is an excellent tool in development of MP-SPR based assays. Here, the specific binding affinity of lipid-based molecules towards sensor-captured protein was analyzed. Also, concentration analysis of diagnostically relevant biomarkers were performed in human serum samples.

Original article

Koponen A. et al. 2019, Biochimie Vol. 158 (90-101)

References:

Florvall et al. 2006, Jour. of Gerontology, Vol. 61 (12)

Gonzales-Guintela et al. 2008, Clin. Exp. Immunol. Vol. 151

AN_168.909.a

Excellence in Surface Plasmon Resonance
BioNavis

e-mail : info@bionavis.com
www.bionavis.com