

# Real-Time Monitoring of Antibody Binding by Parallel MP-SPR, Potentiometry and Impedance Spectroscopy

*Multi-Parametric Surface Plasmon Resonance (MP-SPR) equipped with an electrochemistry (EC) flow-cell was used to run potentiometric and impedance measurements in parallel with the MP-SPR measurement. The binding reaction of anti-methotrexate to immobilized small molecule weight drug methotrexate (MTX) was followed by MP-SPR and by potentiometry and Electrochemical Impedance Spectroscopy (EIS). Methotrexate binding produced a relatively large impedance response and antibody molecules a small response, whereas potentiometry values stayed equal during interactions. The strong binding of antibody molecules was observed in the MP-SPR response.*

## Introduction

Surface Plasmon Resonance coupled to electrochemistry (EC-MP-SPR) can be used for a great variety of studies in the biochemical, biomedical, materials science and physics. Some basic examples are monitoring of electro-deposition and stripping of metals, e.g. to control the thickness, cleanliness and surface roughness of the metal surface, controlling the electro-deposition of conductive polymers or following their doping/de-doping processes. In the bio-nano-technological field EC-MP-SPR enables electrochemical control of self-assembly processes of organic monolayers (SAMs), as well as the study of potential or current-induced conformational changes of organic compounds, biomolecules or polymers.

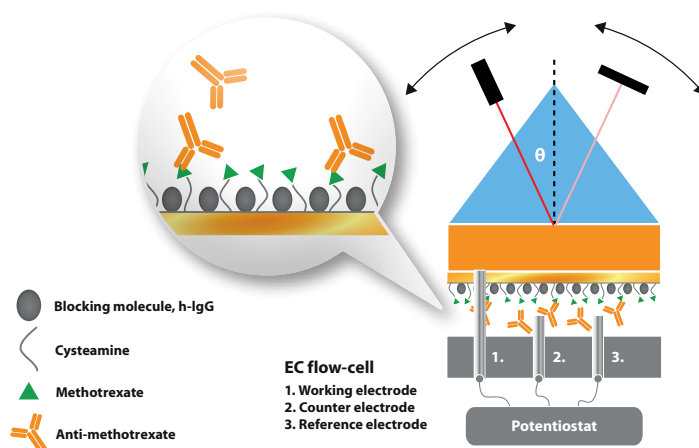
The unique MP-SPR instruments can perform measurements in a wide angular range (40-78 degrees) and at more than one wavelength, enabling layer characterization in terms of layer thickness (d), refractive index (n) and extinction coefficient (k). MP-SPR real-time interaction measurement with the unique PureKinetics™ feature provides affinity and kinetic constants of the interaction accurately. Even more complete characterization can be achieved when combined with electrochemical detection.

## Materials and methods

A MP-SPR-Navi™ 210A VASA instrument was used for MP-SPR measurements together with Palmsens3 potentiostat for potentiometric and impedance measurements. BioNavis electrochemistry flow-cell was equipped with a solid state reference electrode (SSRE), made of an Ag/AgCl wire covered with a chloride-containing PVC/PU membrane. These SSREs are available from BioNavis.

The binding anti-methotrexate (anti-MTX) to methotrexate was studied *in situ* by first immobilising MTX (molecular weight 454Da) onto a sensor slide which was first coated with a self-assembled layer of cysteamine and activated with glutar-aldehyde (GA) *ex situ*. Running buffer was PBS at a pH of 7.45. Injections were made of MTX (500 µg/mL), a non-specific protein for blocking (h-IgG, 100 µg/mL) and then increasing concentrations of anti-MTX (0.1-100 µg/mL). The MP-SPR shift in resonance angle was recorded at two wavelengths (670 nm and 785 nm) while the electrochemical measurements were run in parallel.

The EIS measurement cycle first determined the open circuit potential (Eoc), and then collected an impedance spectrum in the frequency range of 1 to 20 000 Hz at this potential. The cycle time for the potentiometry/EIS was 100 seconds. Experiments were run automatically overnight by use of the MP-SPR Navi™ 210A VASA instrument.



**Figure 1.** MP-SPR measurement combined with potentiostat with BioNavis electrochemistry flow-cell.

## Results and discussion

Parallel MP-SPR, potentiometry, and EIS measurements were performed successfully utilizing the BioNavis electrochemistry flow-cell. Figure 2. summarizes the MP-SPR and potentiometry response to injections of MTX, h-IgG and anti-MTX in a broad concentration range, while Figure 3. shows the results for the MP-SPR in parallel with the modulus of the impedance. The results show that a longer equilibration time before the first injection would provide better results. However, the effect of the initial drift, that is likely due to the glutaraldehyde layer, was minor, except in potentiometry where the effect of drift was large. The injection of the MTX produced a relatively large impedance response, while the reaction of antibody molecules were more strongly observed in the MP-SPR response. The potential of the electrode did not markedly change upon binding of MTX, IgG and anti-MTX.

Potentiometry, MP-SPR and EIS thus provided valuable real-time information on the binding of a drug molecule and subsequent biomolecules, which are in concordance with the expected penetration depth of the different techniques; potentiometry only responds to reactions happening very close to the surface (a few ångströms), while the EIS and MP-SPR reach out further from the surface.

## Conclusions

MP-SPR instruments can be used in parallel with a variety of electrochemical techniques for following in real-time not only optical features, but also the potentiometric, amperometric or impedance parameters. MP-SPR equipped with an electrochemistry flow-cell (EC-MP-SPR) is an excellent tool for obtaining parallel MP-SPR and electrochemistry measurements. Simultaneous measurement of different surface properties is valuable for obtaining a more complete analysis of binding reactions, such as that between an antibody and an antigen.

If you are interested to learn how to get thickness and refractive index from the MP-SPR measurement, see Application Note #128.

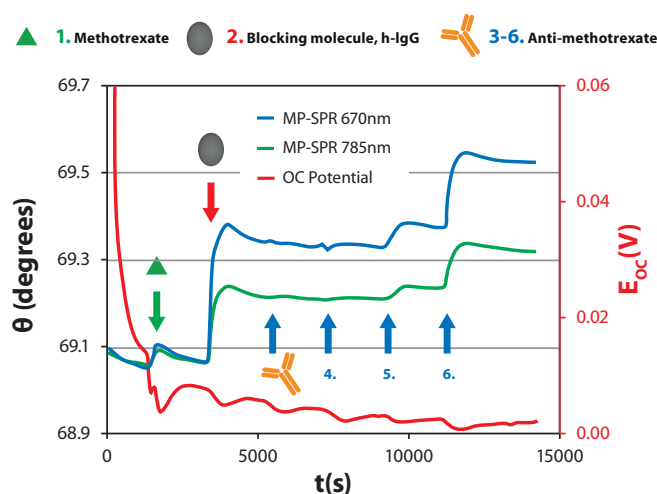
### Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO or 210A VASA equipped with electrochemistry flow-cell SPR321-EC

Sensor surfaces: SPR102-Au or other metal

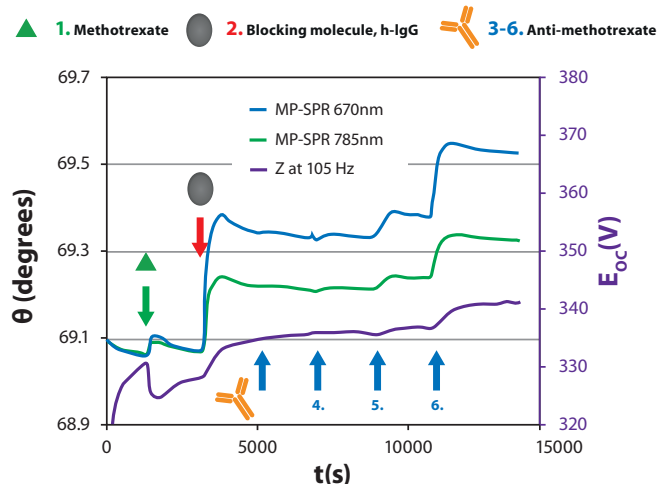
Software: MP-SPR Navi™ Control, DataViewer and TraceDrawer™ for MP-SPR Navi™.

### MP-SPR and Potentiometry



**Figure 2.** Binding of anti-MTX to MTX monitored by MP-SPR and potentiometry. 3. Anti-MTX 0.1 µg/mL, 4. 1 µg/mL, 5. 10 µg/mL and, 6. 100 µg/mL.

### MP-SPR and Impedance Spectroscopy



**Figure 3.** Binding of anti-MTX to MTX monitored by MP-SPR and Impedance Spectroscopy. 3. Anti-MTX 0.1 µg/mL, 4. 1 µg/mL, 5. 10 µg/mL and, 6. 100 µg/mL.