Determine biofunctional layer binding capacity with MP-SPR

MP-SPR is an effective reference tool for immunosensor development.

MP-SPR is utilized to determine affinity biosensor surface binding capacity of different parts of the immunosensor. Streptavidin binding capacity to the MBP-thiol SAM was 366 ± 2 ng/cm² and Bio-CRP antigen binding capacity to the MBP-thiol /streptavidin surface was 105 ng/cm². The MP-SPR also allowed for concentration optimization for the sensor system.
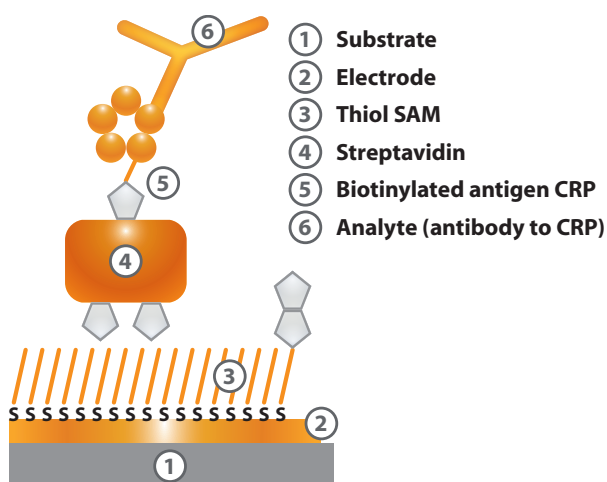
1	Substrate
2	Electrode
3	Thiol SAM
4	Streptavidin
5	Biotinylated antigen CRP
6	Analyte (antibody to CRP)

Figure 1. Schematic diagram of the structure (not to scale) of the affinity biosensor.

When developing new type of biosensors, it is usually of prime importance to evaluate the performance against an established method. Surface plasmon resonance has been utilized for biosensor studies for over two decades, and is an excellent reference method for this purpose.

Uniquely, BioNavis Multi-Parametric Surface Plasmon Resonance (MP-SPR) technology enables also for an easy *ex situ* preparation of custom sensors, including materials and methods that are not accessible with traditional SPR. MP-SPR is an excellent reference method for completely new types of biosensors.

- **ELISA assays**
  - Start with PC, PS or other typical laboratory plasticware material
  - Apply functionalization
  - Measure interactions in real-time and without labels

- **Point-of-care sensors**
  - Possibility to prepare own surfaces including CVD, ALD, sol-gel, LB methods
  - Surface materials including metals, metal oxides, ceramics
  - Optimize antibodies directly on final material of interest.

- **Traditional chemistry**
  - SiO2 surface as a model for glassware
  - *Ex-situ* modifications with dry chemistry followed by layer thickness in dry and wetted state, wetting process

Introduction

Clinical diagnostics is moving from centralized laboratories closer to the patient, into the doctor's office, pharmacies, homes. The requirements for such point-of-care (POC) devices differ greatly from centralized laboratory demands. POC devices should provide low-cost and easy to use analytical tool for rapid analysis of analytes with clinical relevance.

Many paper electronics opens up possibilities to do electronic platforms for cheap, disposable and recyclable applications that can be utilized in biosensing or medical diagnostic.

C-reactive protein (CRP) is a common inflammation marker in the body. Monitoring of CRP levels can be utilized to follow disease progress or treatment effectiveness.

Materials and methods

Standard gold sensor was coated with MuOH:Biotin-PEG-thiol (85:15 mol%) SAM (MBP-thiol SAM). Streptavidin interaction (2,5 – 300 nM) with MBP-thiol SAM sensor surfaces and bio-CRP antigen (1 – 20 µg/mL) interaction with MBP-thiol/streptavidin sensor surface was determined with SPR Navi™ 200 (Fig. 2A, Fig. 3A). For more details, please see the original publication [1]. The same protocol was also used for preparing the electrochemical printed sensors on paper.

Biofunctional layers binding capacity and mass areal density of analyte was determined with the Langmuir adsorption isotherm from the MP-SPR experiments.

Results and discussion

The MP-SPR results showed that streptavidin binding capacity to the MBP-thiol SAM was 366 ± 2 ng/cm² (Fig. 2B). High binding capacity confirmed that biofunctional layer is in the favourable orientation for streptavidin binding.

Bio-CRP antigen binding capacity to the MBP-thiol /streptavidin surface was 105 ng/cm² (Fig. 3B). MP-SPR measurement proved that Bio-CRP antigen adsorbed on the streptavidin surface well. Based on Bio-CRP antigen size, the surface coverage was determined to be approximately 67%.

As the SPR Navi™ standard Au sensors could be treated with same protocols as the printed electrode sensors, the MP-SPR experiments were an excellent reference method in developing affinity biosensor. The MP-SPR also allowed for concentration optimization for the sensor development of an optimal biotin/streptavidin/biotinylated-CRP-antigen/anti-CRP antibody – sensor system (Fig. 1). The system was also evaluated by impedance measurements on printed gold electrodes on disposable paper substrate.

Conclusions

MP-SPR is an excellent reference method in developing affinity biosensors, because:

- Many materials, including polymers, ceramics, metals, metal oxides, nanolaminates can be used as substrates

- Sensor slide surfaces can be treated with same methods as final POC product

- The biomolecular interaction measurement is label-free and real-time

References

[1] Ihalainen et al. Biosensors 2013, 3,1-17

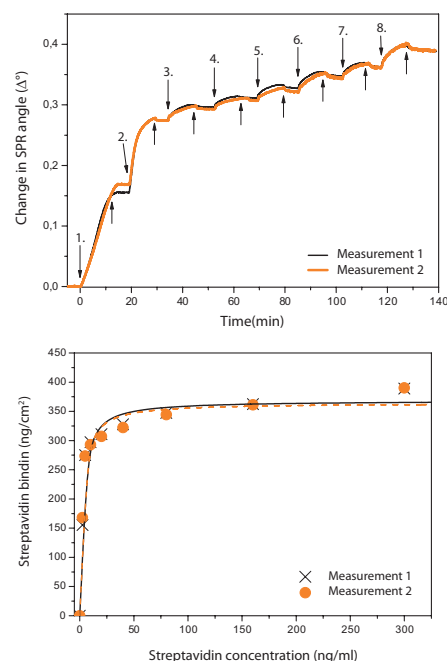| Recommended instrumentation for reference assay experiments |
| --- |
| SPR Navi™ 210A or SPR Navi™ 200 |
| Immobilizer – can be used for immobilization to save instrument time |
| Sensor surfaces: Au, other metal or inorganic coating |
| Software: SPR Navi™ Control, DataViewer, optionally TraceDrawer |

Figure 2. (A) MP-SPR signal response after injecting 1.25–300 nM streptavidin over MuOH:Biotin-PEG-thiol (85:15 mol%) SAM sensor surface. Down arrows represents the time of streptavidin injections: 1. = 2.5, 2. = 5, 3. = 10, 4. = 20, 5. = 40, 6. = 80, 7. = 160 and 8. = 300 nM. Up arrows represent the injection of buffer without streptavidin. (B) Mass areal density curve for streptavidin showing the Langmuir fit over the data points.
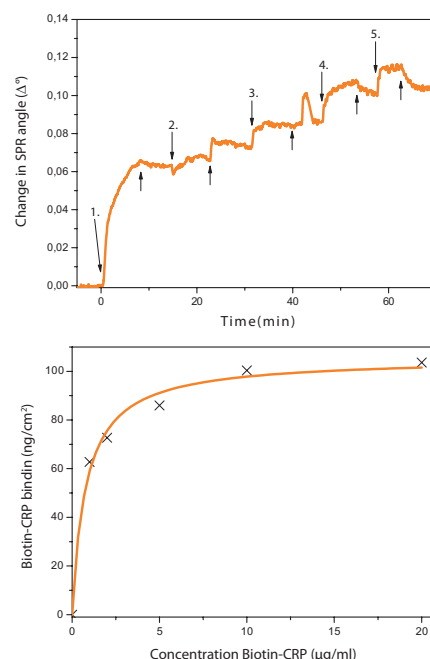

Figure 3. (A) MP-SPR signal response after injecting 1–20 µg/mL bio-CRP antigen over MuOH:Biotin-PEG-thiol (85:15 mol%)/streptavidin sensor surface. Down arrows represents the time of bio-CRP antigen injections: 1. = 1, 2. = 2, 3. = 5, 4. = 10 and 5. = 20 µg/mL. Up arrows represents the injection of buffer without bio-CRP antigen. (B) Mass areal density of bio-CRP antigen showing the Langmuir fit over the data points.