Immobilization of proteins on NPS Epoxy sensors

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Introduction

Nanoplasmonic Sensing (NPS) is a powerful technology that allows you to explore surface phenomena with sensitivity while being free of bulk events. The key to making NPS sensors specific is the modification of the surface with molecules that interact exclusively with your target analyte. Insplorion now introduces a NPS sensor surface with active epoxy groups that allow easy modification of the sensor surface.

The NPS Epoxy sensors are covered with an Ultra-thin (1-2 nm) layer with a rigid structure presenting an epoxy group. NPS Epoxy sensors provide a new option tailored for those looking for an easier covalent immobilization of (bio)molecules on the surface. The epoxy group reacts with nucleophilic groups such as amines, thiols, hydroxyl groups and carboxyl groups, under forming of a covalent bond. The remainder of the surface is uncharged, providing intrinsic low fouling properties.

Instruments and Materials

You will require the following (in brackets what was used in the presented example):

- Insplorion S2 or Insplorion XNano
- NPS Epoxy sensors
- Compatible biomolecule for conjugation on the surface (Biotinylated-BSA)
- Target analyte (Streptavidin)
- Blocking solution (BSA)
- Running Buffer (PBS, pH8)
- MiliQ water
- Humid chamber (a closed box with soaked wipes and a sensor holder)

Figure 1: *The Insplorion S2 was used to perform the experiments.*

Sensor modification procedure

Conjugating your desired molecules to the NPS Epoxy sensor is a simple process. In this example we immobilized biotinylated-BSA. A volume of 20 µL of a 0.5mg/mL solution of Biotinylated-BSA in PBS was spotted on top of the NPS Epoxy sensors. The sensor was then placed in a humid chamber at 4°C overnight (successful modification was observed with spotting times as short as 4h). The excess of liquid was removed after immobilization.

Protein-protein interaction

The modified NPS Epoxy sensors were placed on the Insplorion S2. PBS buffer was flowed through the system until a stable baseline was achieved. BSA (2.5 mg/mL in PBS) was used to block unreacted epoxy groups, after which PBS was flowed. A solution of 0.5mg/mL of Streptavidin was then injected into the system after which, buffer was flowed to remove unbound protein.

Tip! The NPS Epoxy sensors are the perfect complement for the Insplorion S2

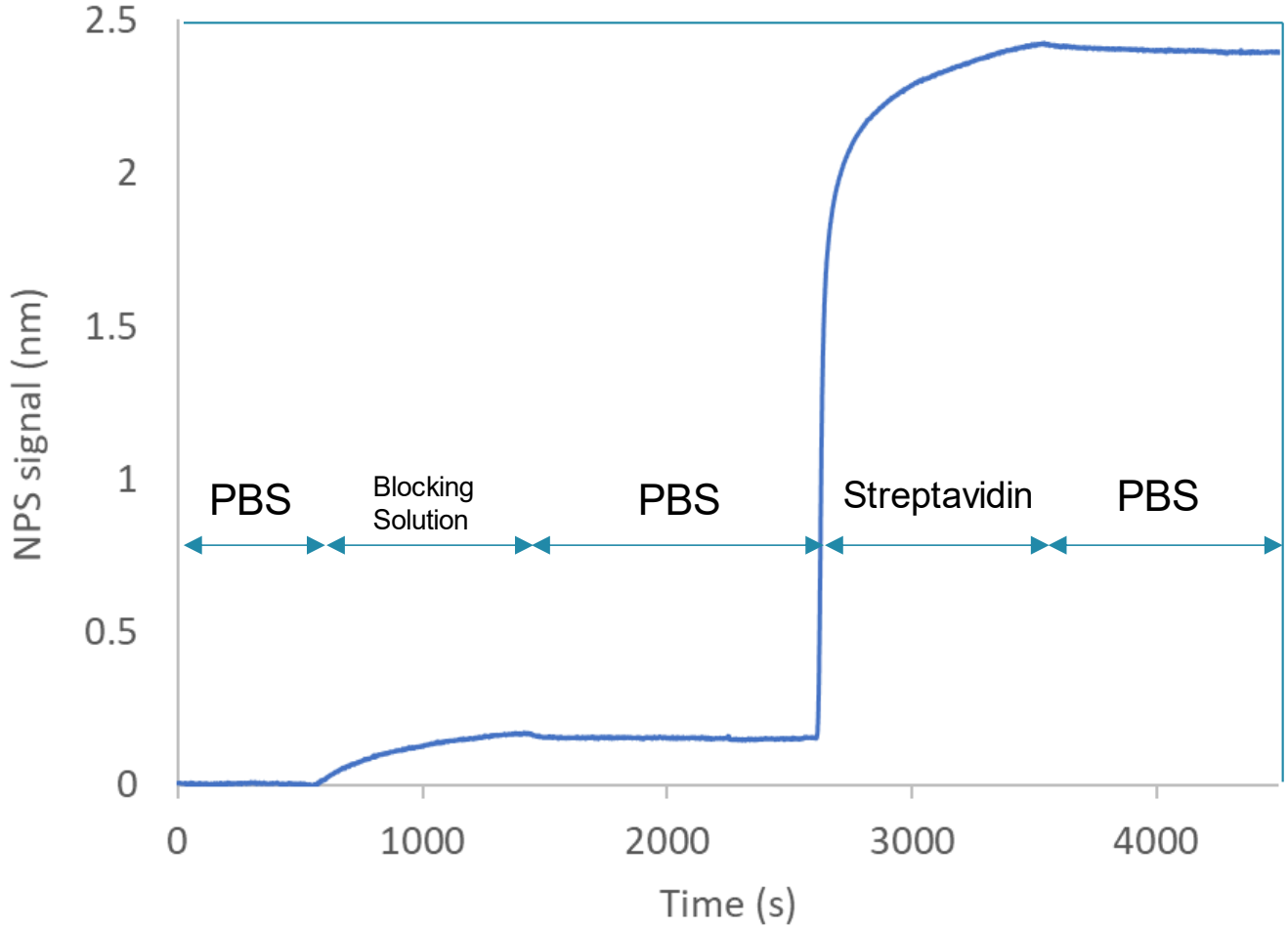

Figure 2: *Spectrogram of Streptavidin binding to NPS Epoxy sensors modified with Biotinylated-BSA.*

Results and Conclusions

Figure 2 shows the interaction of the Biotinylated-BSA-modified NPS Epoxy sensors with the solutions used as described in the previous section.

The interaction with the blocking solution shows that not all the surface area was covered during the activation as there is mass uptake, reflected by the increase in NPS signal. The stability of the signal in the following PBS step shows that the blocking agent promoted an interaction with the surface that was sufficiently strong to keep the uptaken mass in place, highly likely due to a reaction with the leftover available epoxy groups.

When injecting streptavidin, there is a clear binding event between the biotin in the biotinylated-BSA and the injected streptavidin. Using the model available in Technical Note 1, one can estimate the amount of protein binding to the surface based on the experimentally acquired NPS signal (2.25nm), the thickness of the material (5nm) the dn/dc of the material (0.181), the sensitivity of the sensor (100 nm/RIU) and the decay length of the enhanced field (30 nm). Using this model, one can estimate a binding of 2.2 nm/mm^2 to the surface.

Potential applications

The NPS Epoxy sensors make the modification of the surface sensors a simple process. The epoxy chemical groups react with amines, carboxyl groups, thiols and hydroxyl groups, making them perfect for conjugation with biomolecules or other molecules that can react with epoxy groups. Modification of NPS sensors has never been easier.

This study was performed at Insplorion's facility using the Insplorion S2 and the NPS Epoxy sensors.