Autolab ESPRIT

Pre-treatment of the sensor disk for measurement of low molecular weight molecules

Low molecular weight molecules

Surface Plasmon Resonance is a surface sensitive technique used to measure a change of mass (change of refractive index) on a surface. The measurement is expressed in light incident angles (millidegrees). As the shift in angle is proportional with the increase in mass at the gold surface, there are limitations to the size of the molecule to be detected in a direct SPR measurement. Molecules smaller than 1000 Dalton are not big enough to change the refractive index on a surface with limited binding sites and, consequently, are difficult to detect with sufficient accuracy. This limitation can be overcome by increasing the ligand density on the disk.

Increasing ligand density

Increasing the density of immobilized ligand to provide more binding sites for the low molecular weight analyte can be accomplished by improving the efficiency of the immobilization process, or by increasing the surface of the monolayer (e.g. dextran modification).

In this application note we address the improvement of efficiency of the immobilization process. We show that three subsequent rounds of activation with EDC/NHS enhance the efficiency of immobilization of the ligand up to 150% as compared with a single round of activation.
Furthermore, the conditions of the coupling buffer are most important for a highly effective immobilization. Some considerations for the choice of coupling buffer are described.

Preparation of the surface

A first step towards an efficient immobilization is a clean and well stabilized modified gold surface. The Autolab ESPRIT instrument allows an automated protocol to stabilize and rehydrate the modified surface with one, two or three buffers. It is recommended to wash the surface with 0.1M NaOH and 0.1M HCl to get rid of any irregularities. Use the provided sequence: <*stabilize surface with stock 1 and stock 2 solutions.seq*>, in combination with HCl and NaOH in the stock solution 1 and 2 positions, respectively. Afterwards rinse and hydrate the surface with demineralised water using the provided sequence: <*stabilize surface with flask solution.seq*> in combination with demineralised water in the buffer flask.

Activation of the surface

The standard protocol of immobilization is to activate the carboxyl groups of a modified surface with a mixture of N-Hydroxysuccinimide (NHS) and 1-[3-(Dimethylamino)propyl]- 3-ethylcarbodiimide hydrochloride (EDC). It is known, however, that less than 15% of the carboxyl groups of the layer are activated in this way. Although, this is more than sufficient for ordinary measurements, it may be considered to improve this efficiency of activation in some special applications, to achieve a higher density of the immobilized ligand.

To see if multiple activations of the modified gold surface improve the efficiency of immobilization, we activated the carboxyl groups of a thiol monolayer with freshly mixed 0.4M EDC / 0.1M NHS (1:1) in water, for one single or for three successive periods of 5 minutes.

Immobilization

After the activation and a brief wash with demineralised water, a fixed concentration of 10µg/ml BSA in 5mM acetate buffer pH 4.5 (for choice of buffer see below) was applied to the surface, in one single time for 15 minutes, or in three times for 5 minutes. The immobilization curve was monitored for 15 minutes. Contact times longer than 30 minutes are ineffective due to the short half-life of the activated NHS-esters.

The effects of one (figure 1) and three (figure 2) rounds of activation with EDC/NHS, and the effect of one or three additions of ligand, on the efficiency of the immobilization are shown. The triple activation of the surface molecules in combination with three additions of ligand results a 150% higher efficiency of immobilization.
When the successive activation steps are interrupted with an immobilization step, this enhancement is not observed (not shown).

Choice of coupling buffer

The immobilization process is based upon the principle of electrostatic interaction of the ligand and

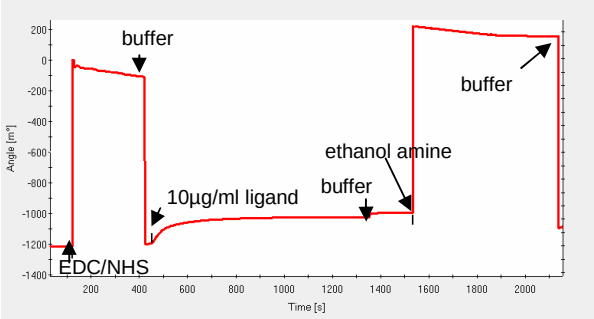


Figure 1 Immobilization of 10µg/ml BSA: 1 round of EDC/NHS, 1 addition of ligand.

the activated surface molecules, which results in a covalent binding. To ensure a positive net charge of the ligand required for an electrostatic interaction with the negatively charged surface molecules, the coupling buffer has to meet the correct pH value and ionic strength. As a general rule, the pH of the buffer should be at least 0.5 units below the iso-electric point (pI) of the ligand molecule. Furthermore, the lower the ionic strength, the more ligand will be preconcentrated and immobilized.

The best buffer conditions have to be determined empirically by preconcentration tests with one of the following buffers:
Sodium formate (2-20 mM) for a pH between 3.0 and 4.0, sodium acetate (2-20 mM) for a pH between 4.0 and 5.5 or sodium maleate (1-10 mM) for a pH between 5.5 and 7.0.

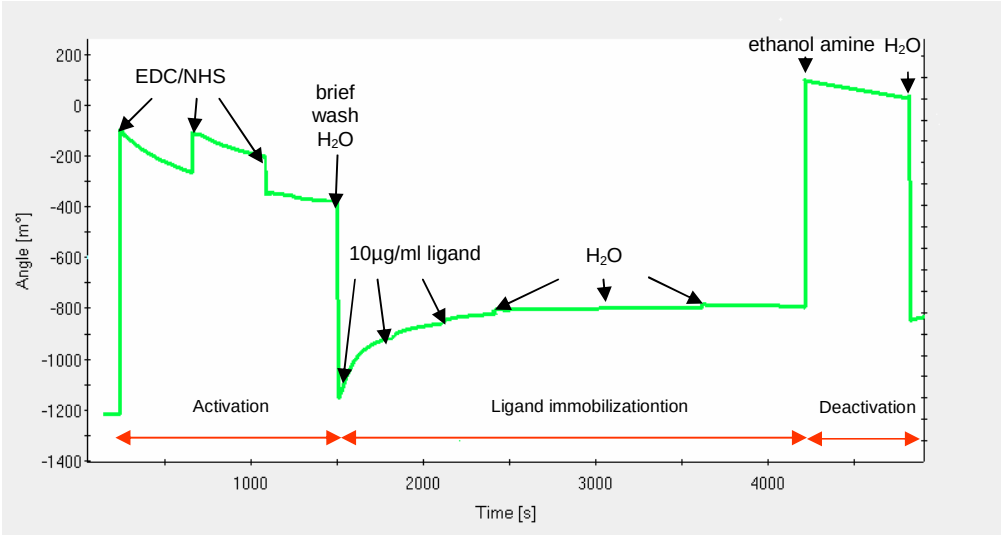
N.B. *Preconcentration experiments have to be carried out on the sensor surface without activation with EDC/NHS. By intermittent washes with NaOH and HCl, the test surface can be reused over and over again.*

By varying the concentration of the ligand, the optimal ligand concentration can be determined in these preconcentration tests as well.

Completion of the immobilization

In most cases the pH of the coupling buffer is below pH 7. However, at low pH, the covalent binding occurs at a relatively slow pace. Therefore, it is recommended to complete the immobilization process by incubation with demineralised water. Allow the immobilization to complete for 30 minutes, changing the demineralised water every 10 minutes.

Figure 2 Immobilization of 10µg/ml BSA: 3 rounds of EDC/NHS, 3 additions of ligand.



Deactivation of the surface

Even with a very efficient immobilization, not all surface molecules will be occupied by a ligand molecule. To prevent the low molecular weight analyte molecule from binding to the surface molecule rather than to the ligand, a deactivation step has to be preformed. For this, a 1M ethanol amine solution, pH 8, is commonly used.

Conclusion

To improve the sensitivity of the measurements of low molecular weight molecules, three subsequent rounds of activation with EDC/NHS is recommended for the activation of two-dimensional layers, such as a self assembled thiol monolayers, as well as three-dimensional dextran layered surfaces.