TEXAS INSTRUMENTS

APPLICATION BRIEF #001

Spreeta™

Immobilization of Ligand: Method 1

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Immobilization of Ligand: Method 1

ABSTRACT

In this method the ligand is linked to the gold surface via a cross-linked protein film using carbodiimide mediated amide bond formation.

Introduction

It is well documented that direct physisorption of protein to a gold surface induces denaturation of the adsorbed protein. Here we assemble a cross-linked protein film on the gold surface and then link the ligand of interest. Thus the ligand will not be in direct contact with the gold hence preserving its binding activity. Figures 1 is an illustration depicting the immobilization process while Figure 2 is an actual response curve for the immobilization of antibody using the method outlined below.

Materials and Methods

All reagents were obtained from Sigma-Aldrich unless otherwise stated. Note: Always use gloves when handling reagents and sensors. Ensure adequate safety precations are taken when handling all reagents espechially succinic anhydride, N-hydroxysuccinimide (NHS) or N-ethyl-N'(dimethylaminoporpyl)-carbodiimide (EDC). Refer to relevant MSDS for guidelines.

Preparation of Cross-linked Carboxymethylated Protein Film

Cleaning Gold

Use an ethanol saturated kimwip to gently wipe the gold surface thus removing contaminants. Be careful to ensure that no contact is made with the plastic surrounding the gold surface of Spreeta.

Cross-linked Film:

1. Prepare 5 mg/ml bovine serum albumin (BSA) in phosphate buffer saline (PBS), pH 7.4.
2. Prepare 10 mls of 0.4 M EDC and 10 mls 0.1 M NHS, aliquot each into 200 μl volumes and store at -20 °C.
3. Mix 200 μl of the EDC preparation with 200 μl of NHS preparation.
4. Add 0.5 mls of the BSA solution to the EDC/NHS mixture and incubate for 5 min at room temperature.
5. Place 10 μl the activated BSA solution onto the cleaned gold surface. Spread evenly over the surface. Ensure that the plane of the surface is horizontal and incubate for 15 min at room temperature.
6. Rinse with 20 mM HCl and then in water. Blow dry surface under dry air or nitrogen stream.
7. Add 0.2 g of succinic anhydride to 0.5 mls of DMSO and allow to dissolve (vortexing or sonication will help). Make up to a final volume of 10 mls with 50 mM disodium hydrogen phosphate. Add a few drops of 5M NaOH to adjust pH to around 6.5.
8. Incubate the BSA coated slide in the succinic anhydride preparation overnight at room temperature. Ensure that the gold surface is not in contact with any solid surface. Once coated any physical contact with the gold surface will destroy the film.

Ligand Coupling

Before activating the carboxylated BSA coated surface it is a good idea to test that the surface is properly formed by simply observing the charge preconcentration effect.

Preconcentration Test

1. Dock the sensor with fluidics, initialize, normalize and then equilibrate in water.
2. Dissolve ligand at 20 to 100 μg/ml in 10 mM sodium acetate buffer.
   (Note: when preparing the acetate buffer adjust the pH to 1 pH unit below the isoelectric point of the ligand (use 10% acetic acid to adjust pH)). The pH should not be set lower than 4.0 due to protonation of the surface carboxyl groups. Any acidic ligands with pI values less than 4.5 should be immobilized via avidin-biotin linkage (see Application Note 2).
3. Inject 50 μl of the ligand solution at 30 μl /min (ensure that the sensor and all lines are equilibrated in water). Should observe a rapid response due to attraction of the positively charged protein to the negatively charged surface.

4. Rinse with PBS. The high salt concentration in PBS negates the charge interaction and should remove all preconcentrated protein.

Ligand Coupling

1. Activate the surface by injecting a 1:1 mixture of 0.4 M EDC/0.1 M NHS. Allow a surface contact time of 2 - 7 min. Long contact times may convert all the carboxyl groups at the surface to NHS esters and this will destroy the preconcentration effect. It is vital that the majority of carboxyl groups are available to attract the positively charged ligand to the surface. Therefore some trial and error may be required to find the optimum concentration of reagents and appropriate contact time. In some cases EDC/NHS concentrations at 20% of the recommended concentrations proved appropriate.
2. Inject the ligand solution (prepared during the preconcentration test) for 10 min at room temperature. Should observe a response due to both preconcentration and coupling.
3. Rinse with water and then inject 1 M ethanolamine, pH 8.5, for 7 min, to cap residual NHS esters. It is preferable to use PBS, or HBS buffer, rather than water for continuous flow but one must ensure than dispersion within the system is low when using high salt buffer.
4. Check non-specific binding (NSB) using BSA and Ovalbumin at 1 mg/ml in PBS. This also doubles as a blocking step.

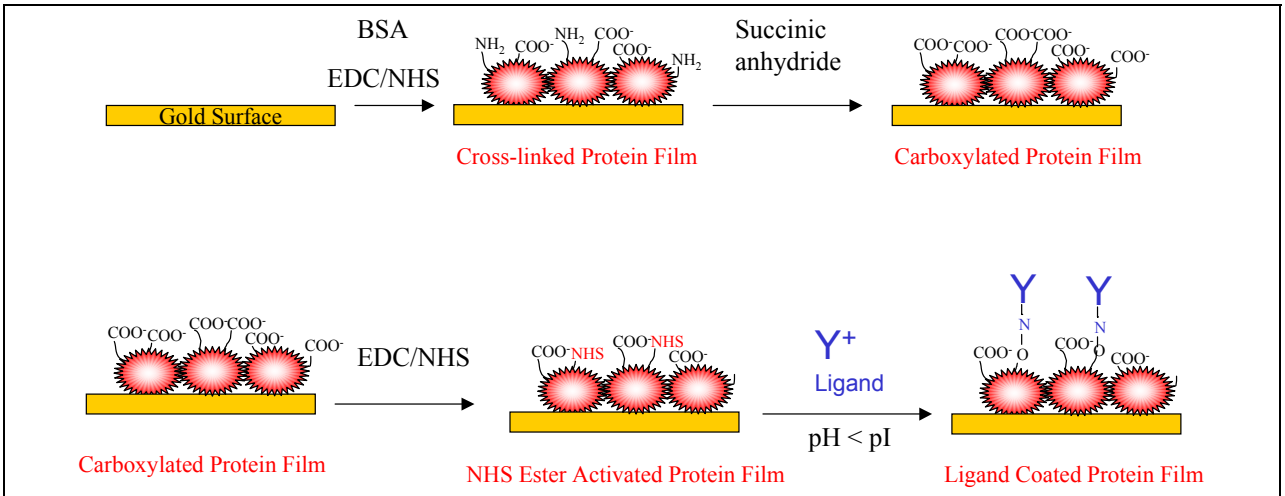

Figure 1. Preparation of cross-linked protein film and immobilization of Ligand

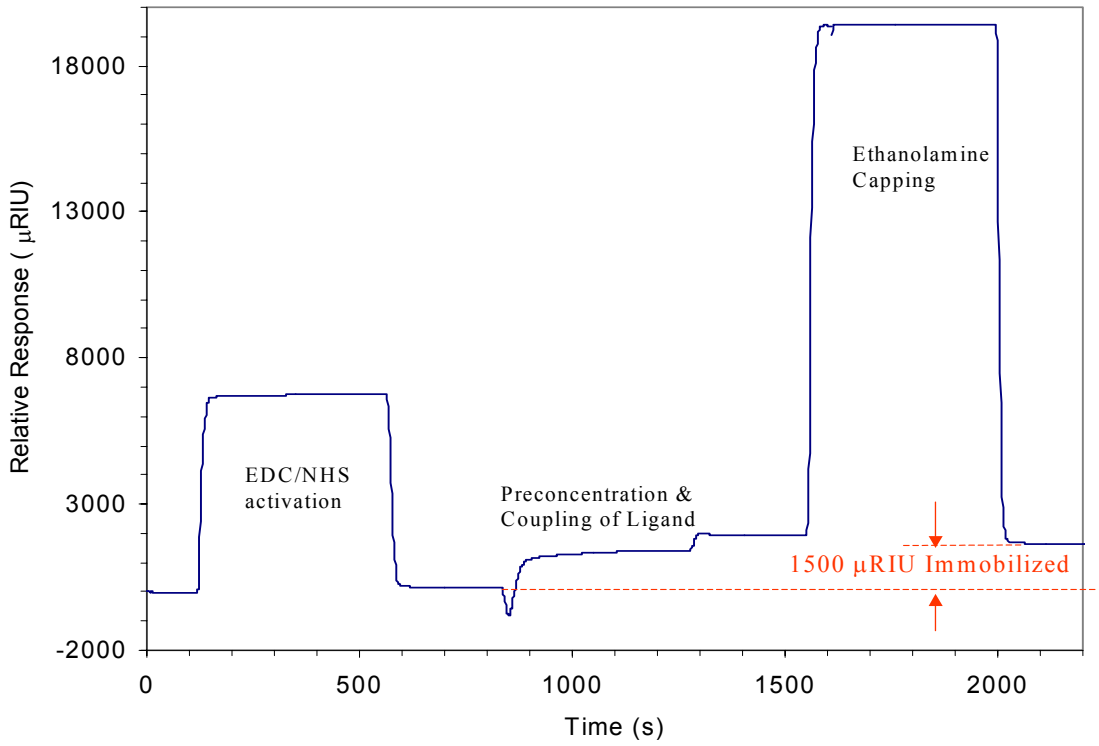

Figure 2. Response curve for immobilization of Goat IgG onto carboxylated protein film by amine coupling. PBS was employed as running buffer.