Ligand Immobilization with Cysteamine-Glutaraldehyde for Surface Plasmon Resonance

Overview

Immobilizing ligands onto the OpenSPR sensor surface is a critical process for obtaining high quality results. One method for doing this is an amine coupling process using cysteamine-glutaraldehyde. Compared to the conventional EDC-NHS coupling method, this technique requires fewer steps and uses reagents that are more stable. Here we give a detailed description of how to immobilize an antibody for prostate specific antigen (PSA) onto an OpenSPR sensor chip using this process. Specific operating parameters have been optimized for the PSA system.

Materials & Equipment

- OpenSPR Instrument (SPR-01)
- TraceDrawer Kinetic Analysis software (TDS)
- Sensor Chip (SEN-AU-100)
- Deionized Water (DI water)
- 1x PBS Buffer pH 7.4, 0.05% Tween 20 (PBS-T)
- 10 mM Sodium Acetate Buffer pH 5.0 (IMB-5.0)
- Regeneration Solution - 5 mM HCl in DI water
- Cysteamine (1 mM in DI water)
- Glutaraldehyde (2.5% in PBS-T - must be used in a fume hood)
- Primary amine containing ligand (Anti-PSA 0.1 mg/mL in IMB-5.0)
- Target analyte (PSA – Prostate Specific Antigen in PBS-T)
- 1 M ethanolamine solution pH 8 in PBS-T
- Bovine serum albumin (3 mM in PBS-T)

Figure 1. Reaction mechanism of cysteamine and glutaraldehyde for immobilization of amine-containing ligand (R1). The thiol group of the cysteamine readily binds to the gold sensor surface.
Procedure

1. Follow all safety precautions outlined in material MSDS.
2. Following the typical startup procedure found in the OpenSPR manual, setup the OpenSPR instrument with a new sensor chip and flow cell. Refer to the “Tips and Tricks” document for important information on how to get the best results from your OpenSPR system.
3. Initiate running buffer of DI water at a flow rate of 200 µl/min and ensure the signal is flat and stable and the flow cell free of bubbles.
4. Reduce the flow rate to 30 µl/min.
5. Ensure the injection valve is in the “load” position. Inject 1 mM cysteamine in DI water into the 100 µl sample loop via the injection port, then turn the injection valve to the “inject” position. This process of filling the sample loop and injecting the solution remains the same for all injections and will be referred to as an “injection procedure”. As cysteamine enters the flow cell it will bind to the sensor surface and the signal will visibly increase and then stabilize.
6. Switch the running buffer to PBS-T, being careful not to introduce any bubbles. The signal will again increase and then stabilize.
7. Follow the injection procedure for 2.5% glutaraldehyde solution in PBS-T. The signal will increase due to the reaction of the glutaraldehyde with the cysteamine, and then it will stabilize.
8. Set the pump to the lowest speed setting (20 µl/min).
9. Dilute the ligand to be immobilized on the sensor surface into an appropriate immobilization buffer. In this case, anti-PSA is the ligand and the immobilization buffer is 10 mM acetate buffer at pH 5.0.
10. Follow the injection procedure for the 0.1 mg/mL anti-PSA solution. The signal will increase as the anti-PSA binds to the glutaraldehyde.
11. Once the ligand has exited the flow cell set the flow rate to 100 µl/min.
12. Follow the injection procedure using 5 mM HCl solution. This step removes excess ligand that is non-covalently bound to the sensor.
13. Set the flow rate to 30 µl/min. Follow the injection procedure using 1 M ethanolamine solution at pH 8. This step deactivates any remaining glutaraldehyde sites.
14. Set the flow rate to 100 µl/min, follow the injection procedure with 5 mM HCl. This removes any non-covalently bound ethanolamine.
15. Follow the injection procedure for 3 mM BSA in PBS-T. This ensures there is no non-specific binding and all non-specific sites are filled.
16. The sensor surface is now ready for analyte injection. In this example, 2400nM of PSA in PBS-T is injected.
Results
The sensorogram produced from the OpenSPR instrument throughout the surface functionalization process is shown in Figure 2. Antibody binding through the amine coupling reaction (d) is strong, and the stable baseline indicates that the antibody is not desorbing from the surface. The control injection of 3 mM BSA (h) as a non-specific protein does not produce any measureable response, indicating the surface is well blocked and that the response to PSA (i) is highly specific. Process parameters can be further optimized/adjusted to vary the density of antibody sites on the sensor surface to suit the desired application.

These results demonstrate a viable process for immobilizing a wide variety of antibody to the surface of the OpenSPR instrument via cysteamine glutaraldehyde functionalization.

Figure 2. Sensor response during functionalization process used to immobilize PSA antibody onto the OpenSPR sensor chip. Steps are as follows: (a) 1 mM cysteamine, (b) buffer switch to PBS-T, (c) 2.5% glutaraldehyde, (d) 0.1 mg/mL anti-PSA, (e) 5 mM HCl, (f) 1 M ethanolamine (g) 5 mM HCl, (h) 3 mM BSA, (i) 2400 nM PSA.