BSA Immobilization on sensor chip

BSA immobilization with amino coupling

Selected covalent coupling chemistry method depends on ligand reactive groups. Amine coupling is recommended as a first choice. Most macromolecules contain amine groups so they can be coupled with amine coupling method. Less suitable method is when amine is in the active site and binding can cause loss of activity or ligand is acidic.

Materials

MUA	Sigma Aldrich, 450561-5G
EDC, NHS	Merck, S6676607/S6676618
Glycine	Merck, k41484701
NaOH, NaCl	Merck, 130895598/K49437804743
PBS	Zist Mavad Pharmed, B No:Z96001-100

Equipment and supplies:

BioNavis, SPR Navi 210A

Nitrogen gas Cylinder

Eppendorf Samplers

Vortex Mixer

Microtubes

Distilled water

H2O2

Ammonia

Procedure Steps:

1. Sensor surface washing:

Materials: Have equal amounts of hydrogen peroxide, ammonia and distilled water**.**

Washing steps: For each chip, take 5 ml of ammonia, hydrogen peroxide and distilled water and put it at 90 degrees and boil the chips for 10 minutes. Rinse the chips with distilled water and 96% alcohol and dry with nitrogen gas.

2. Primary preparation of the chip with MUA:

First, take an initial scan of the empty gold chip with the SPR device and read the angle given by. Then, load 200 microliters of MUA on the metal surface of the chip and leave it at room temperature overnight so that it is not in the vicinity of the air. When using the MUA chip, wash the chip with ethanol and dry it.

3. Measurement procedure:

All operations are controlled through the Control window of the SPR**:**

•Prime system to get flow path filled with fresh buffer.

•Insert a sensor-slide-holder containing the type of sensor-slide that you wish to use for your experiment into the instrument and dock the slide.

•Set the target temperature from the SPR-Navi software. Wait at least 20 minutes for the instrument to reach thermal equilibrium before measurement.

•Check that amount of buffer(PBS) is enough (buffer filters needs to be totally under the liquid).

•Start the experiment by pressing Start at the “Initial scan” mode. Measurements for gas, liquid, full scan, or a custom angular range can be chosen.

•After the “Initial scan“ you should see a point of the steepest place on the slope of the measured SPR curve marked by a vertical line. The instrument is ready for the fixed angle measurement at this particular angle.

•On the Fixed Angle choose time of measurement, or choose the“ Continuous” option by clicking in the field next to it.

•Press Start, fill up the experiment data information the program asks for, and then press “Start measurement”. The Program will start measuring the baseline at the chosen angle.

•Allow the baseline to stabilize for approximately 10 minutes.

•After the measurement press “stop” and “end experiment” to stop measurement.

Remember to clean flow cell after measurement.

4. Measurement conditions:

- Selected 670 nm laser for both measurement channels.
- Temperature 25°C
- Running buffer PBS, pH 7.4
- Flow rate 30 ul/min
- Measurement in fixed Angle scan mode.

5. BSA(Bovine Serum Albumin) immobilization:

I. Cleaning: 7 minutes cleaning injection, NaCL 2M + NaOH 0.01M, to both channels.

II. Activation: 7 minutes activation injection, NHS 0.05M + EDC 0.2M, to both channels. Prepare EDC + NHS activation mixture just before use. Remember to degas the activation solution properly with syringe just before use!

III. Protein: 7 minutes BSA (0.2mg/ml) injection to one channel only. To the other channel only buffer is injected. (Autosampler: parallel injections)

IV. Deactivation: 5 minutes injection of 1M ethanolamine pH8 to the both channels.

V. Cleaning: 2x 1 minute NaOH 50mM injection to the both channels.

6.Interaction measurement:

1. Running Buffer:

Running buffer is important part of the experiment carrying analyte to the ligand surface. Running buffer can greatly affect to the interaction by its osmolality, composition and pH. e.g. PBS pH 7.4 or 10mM HEPES pH 7.4 are commonly used buffer in interaction measurements.

2. Analyte samples:

To calculate analyte affinity to the ligand, samples with different analyte concentration is measured. Minimum for the affinity calculation is five different concentrations. It´s recommended to measure also replicate from at least some sample concentrations to compare response.

3. Sample injections:

Inject analyte samples from lowest concentration to the higher. Injection time depends how fast steady state is forming. If kinetic is fast 4 minute injection is enough but if association is slowly

(e.g. with antibody) steady state forming takes more time and longer injection is needed to reach steady state. If dissociation is slowly after last sample (highest concentration) wait longer time to get enough slope for kinetic calculations, e.g. 20 minutes.

4. Regeneration:

If analyte is dissociating slowly from the ligand (e.g. antibody antigen interaction), analyte can be often removed injecting regeneration solution. Regeneration enables reuse of sensor slide surface e.g. another concentration or another molecule interaction to the same protein can be measured. Right regeneration solution depends physical forces responsible about binding so proper solution is not always the same. The most frequently used regeneration method is injecting low pH-buffer like 10 mM glycine pH 1.5 -2.5.

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