

Covalent protein immobilization on Self assembled monolayer (SAM) for ligand binding



Material

- Affinité Instruments' Au sensors+16-MHA (SAM01-5) or sensors+AffiCoat (SAM02-5)
- Distilled water (DI)
- N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride, (EDC, Crystalline, Sigma-Aldrich E6383)
- N-Hydroxysuccinimide (NHS, 98%, Aldrich 130672)
- Running buffer: Phosphate Buffer Saline (PBS 1X, pH 7.4 Corning Cellgro 21-040-CV) or other
- Hydrochloric acid (HCl)
- Ethanolamine Hydrochloride ($\geq 99.0\%$, Sigma-Aldrich E6133)
- Sodium Hydroxide (NaOH)
- Sodium acetate
- Protein solution
- Ligand solutions
- Glycine regeneration solution

Protocol

Preparation of solution prior to experiment

- 1 M HCl
- 0.1 M NaOH
- Acetate solution pH 4.4: 10 mM sodium acetate in H₂O adjusted to pH 4.4 using 1M HCl. (can be of higher pH up to 5.5)
- Ethanolamine solution: 1M of ethanolamine in H₂O and adjusted to pH 8.5 with 0.1M NaOH.
- Protein solution: protein of 0.02 mg/mL in acetate solution (immobilization buffer).
- 400 mM EDC and 100 mM NHS mixed in 1 mL DDI. Make sure to prepare the solution immediately before the experiment to prevent degradation. Ensure that the concentrations are 400 mM EDC and 100 mM NHS after mixing in 1 mL.
- For ligand binding: solutions of different ligand concentration in running buffer (volume for each: 300 μ L)
- Glycine regeneration solution: 10 mM glycine in H₂O adjusted to pH 2.2 using 1M HCl.

*** Please make sure that the solutions are at room temperature before injection.

General notice

- Manual injection: inject at about 50 $\mu\text{L/s}$. Using 1 mL syringe, 1 drop is equivalent to 50 μL .
- Removing bubble: Inject at a higher flow rate of 200 $\mu\text{L/s}$ or perform pulsed injections to move smaller bubble away.
- Critical steps to avoid air bubble:
 - o EDC/NHS activation
 - o Immobilization
 - o Blocking

Always visually inspect the channels prior these steps.

- Minimum injection volume for each channel is 250 μL but usually 300 μL is recommended.

Setting up:

1. Insert the coated gold chip into the sample holder, lock the chip in place and inject 1 mL DI water in all channels.
2. Open software and allow 2 minutes for baseline stabilization. The baseline signal should not fluctuate more than 35 RU (0.1 nm).
3. Click on Start Recording to collect data.

EDC/NHS activation

4. Establish a stable baseline with DI water for 30 secs.
5. Inject 300 μL of fresh EDC (400 mM)/NHS (100 mM) into all channels for 2 minutes.
(Tip: prepare about a larger volume solution of each and aliquot the desired amount and freeze the aliquots in $-20\text{ }^{\circ}\text{C}$, once needed, aliquots can be thawed and mixed before use)
6. Wash both channels with 500 μL of acetate pH 4.4 (or other immobilization buffer) for 10 seconds.

Immobilization

7. Inject 300 μL of protein solution (e.g., antibody or peptide in immobilization buffer) in all channels and wait 20 minutes.
8. Wash all channels with 500 μL of acetate pH 4.4 (or other immobilization buffer) for 10 seconds.

Blocking

9. Inject 300 μL of 1M ethanolamine pH 8.5 in both channels and wait 10 minutes.
10. Wash both channels with 500 μL of running buffer (e.g., PBS, MOPS, etc.) for 10 seconds.
11. Make sure you have a stable signal over 15 min (900 seconds). Reinject buffer and wait until the surface is stabilized if needed.

Binding Assay (Example: protein-ligand binding)

12. Inject 300 μL of the least concentrated ligand solution in the sample channel(s) and inject 300 μL of control in the reference channel and wait 15 mins.

Note: regeneration does not need to be done after each ligand solution injection and can be done only at the end of the ligand solution series but it can be done after each ligand injection. See step 15.

13. Repeat step 12 with subsequent ligand solutions.

14. Click on Recording in Progress to stop collecting data.

Regeneration

15. After the ligand solution injection, inject 500 μL of Glycine solution pH 2.2 for 10 seconds.

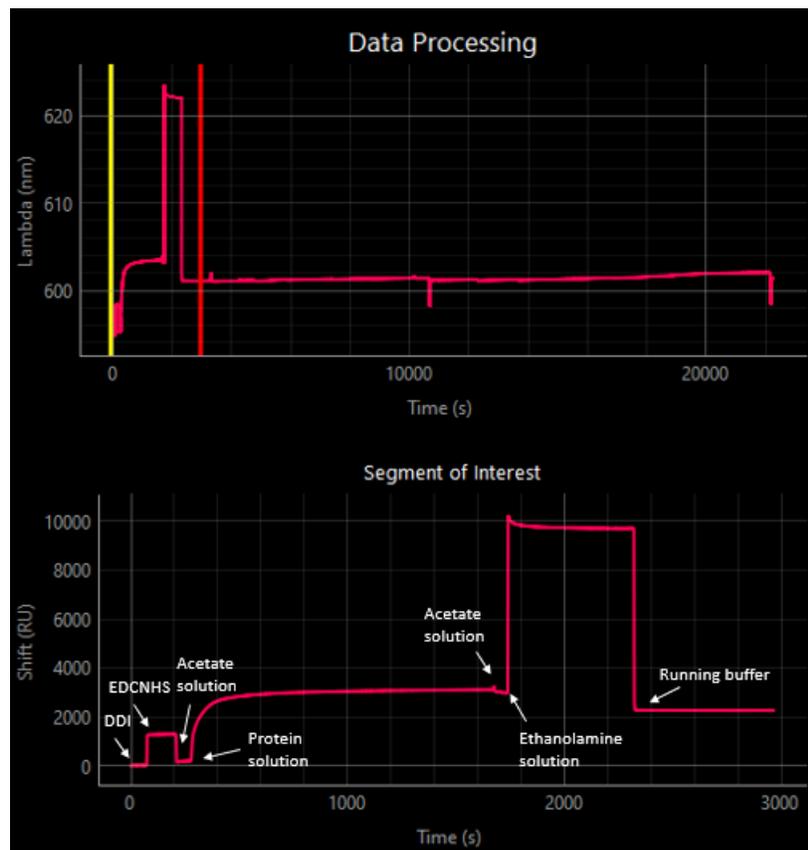
16. Re-inject running buffer and wait for 5 minutes or until the baseline stabilizes for the next ligand solution injection.

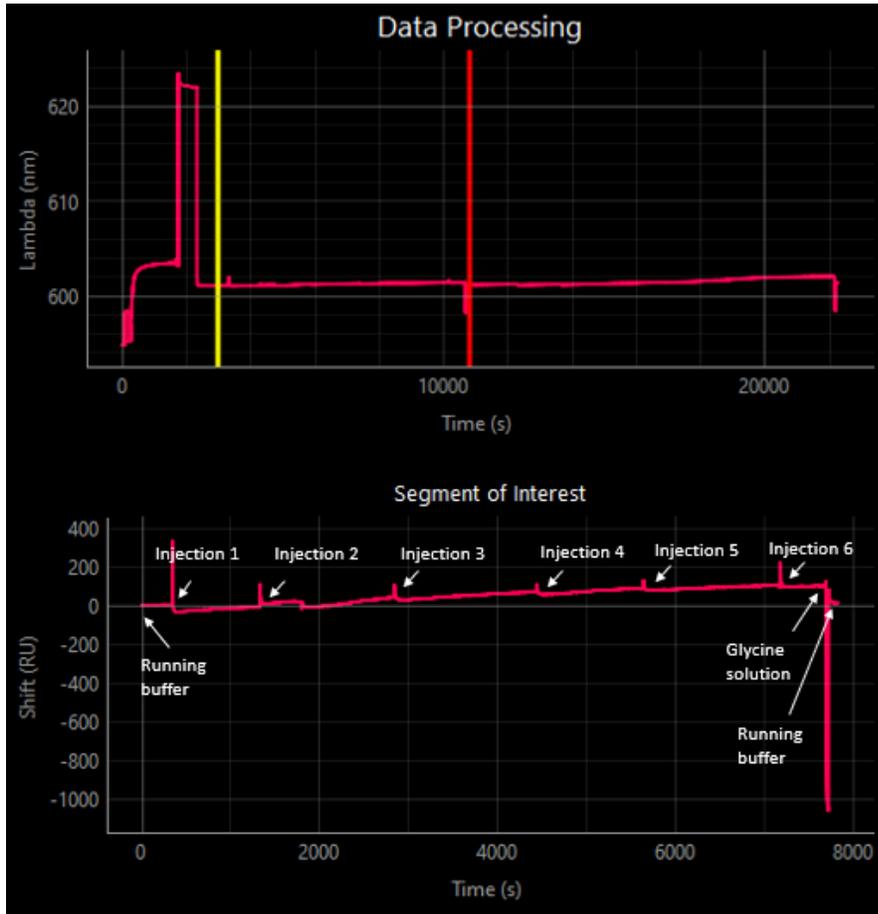
System wash

17. Inject 20 mL of DI water to rinse all channels then inject air to dry out the channels.

Example sensorgram:

Protein immobilization:





Regeneration

For regeneration to be considered acceptable:

$$\frac{\text{SPR shift (Running buffer 2 - Running buffer 1)}}{\text{SPR shift (Glycine solution - Running buffer 1)}} \times 100 \leq 10\%$$

$$\text{SPR shift (Glycine solution - Running buffer 1)}$$

