

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



Material

Overnight Immobilisation of SAM

- De-ionized Distilled water (DDI)
- Dimethylformamide (DMF) reagent grade
- Gold coated SPR sensor chip
- Petri-dish 60 mm (diameter) x 15 mm (height) (capacity of > 5 mL)
- 16-mercaptohexadecanoic acid (16-MHA)
- Parafilm
- Tweezer
- Kimwipe

Protein immobilization on SAM

- Prepared 16-MHA sensor chips or Affinité Instruments' Au sensors+16-MHA (SAM01-5)
- 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
- 1M Hydrochloric acid (HCl)
- Ethanolamine Hydrochloride ($\geq 99.0\%$, Sigma-Aldrich E6133)
- Sodium Hydroxide
- Sodium acetate
- Protein solution

Protocol

Overnight Immobilisation of SAM

1. Open a new pack of Au chips and put in a bottom glass petri dish.
2. Prepare a solution of 1 mg/mL of 16-MHA in DMF.
3. Transfer about 200 μ L of 16-MHA/DMF solution on the gold film. Make sure to cover the entire surface or use a larger volume of 5 mL of the 3-MPa solution and submerge the chips in the solution.
4. Put the top of the petri dish and let incubate in the dark for up to 16 hours (at least 12 hours) at room temperature.
5. Wash away thoroughly DMF with ethanol.
6. Dry with nitrogen and store the chips in Affinité's chip tray.

Protocol #1

Covalent immobilization of proteins



7. Store the coated chips dry in the dark for up to 6 months.
8. The surface of the sensor chip should not visually show any traces or residues. Make sure that the sensor chip's longer edges are dried and wiped gently with a kimwipe before placing into the P4SPR.

Protein immobilization on SAM

Preparation of solution prior to experiment

- 1 M HCl
- 0.1 M NaOH
- Acetate solution pH 4: acetate solution adjusted to pH 4 using 1M hydrochloric acid.
- Ethanolamine solution: 1M of ethanolamine in H₂O and adjusted to pH 8.5 with 0.1M sodium hydroxide.
- Protein solution: protein of 0.5 mg/mL in 1mL in running 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.**
- For subsequent assay: different concentration of ligands in running buffer

*** Please make sure that the solutions are at room temperature before injecting into the P4SPR system.

P4SPR experiment

General notice

- Manual injection flow rate: inject liquid at about 100 uL/s.
- Removing bubble: Inject at a higher flow rate of 300 uL/s or perform pulsed injections to move smaller bubble away.
- Critical steps to avoid air bubble:
 - EDC/NHS activation
 - Immobilization
 - Blocking

Always visually inspect the channels prior these steps.

Setting up the System:

1. Insert the 16-MHA coated gold chip into the chip cavity, close the latch and flow DI water through the fluidic in both channels.
2. Visually inspect to ensure there are no air bubbles in the channels.
3. The peak to peak variation of the signal should be 0.05 nm and no drift should be visible over 300 seconds.
4. Press Record. The graphs will reset and the backgrounds color will turn from gray to black indicating that the software is now recoding the data.
5. Do not activate the reference at prior the assay stage.

EDC/NHS activation

6. Establish a 2 min baseline in DI water.
7. Inject 300 uL of fresh EDC (400 mM)/NHS (100 mM) into both reference and sample channels for 2 minutes.
 - (Tip: prepare about a larger volume solution of each and then you can aliquot the desired amount and freeze them in -20 °C, once needed, aliquots can be thawed and used)
8. Wash both channels with 500 uL of acetate pH 4.0 (or other immobilization buffer) for 10 seconds.

Immobilization

Protocol #1

Covalent immobilization of proteins



9. Inject 300 uL of protein to immobilized (e.g., antibody or peptide in immobilization buffer) in both channels and wait 20 minutes.
10. Wash both channels with 500 uL of acetate pH 4.0 (or other immobilization buffer) for 10 seconds.

Blocking

11. Inject 300 uL of 1M ethanolamine pH 8.5 in both channels and wait 10 minutes.
12. Wash both channels with 1000 uL of running buffer (e.g., PBS, MOPS, etc.) for 10 seconds.
13. Make sure you have a stable signal (no drift) over 15 min (900 seconds). Reinject buffer and wait until the surface is stabilized.

Binding Assay (Example: aptamer-protein binding)

14. Activate the reference and deactivate the channel display of channel D.
15. Inject 300 uL of aptamer (5 μ M in MOPS) in the **SAMPLE** channel only and wait at least 15 mins to see a signal increase.
16. Press save shift to save the delta SPR signal of each sample channel.
17. To wash off the aptamer, urea (7 M urea) was injected with a short contact time of less than 5 seconds followed by MOPS buffer to investigate the disassociation of protein-aptamer. Concentration of urea may need to be adjusted in case the immobilized protein is denatured.
18. Subsequent aptamer injections can then be made by repeating steps 15-17.

Some Tips:

- Add feature in the software (right side in the bottom), will allow you to add multiple segments of the graph and you can have some comments about the segment saved
- Save shift, is a useful feature on the software that allow you to save a specific segment within the graph
 - For example, when the protein was injected, you can save the immobilization part by pressing save shift on the bottom right side of the software
- In case you forgot to save some segments, you can add a segment, then using red and yellow cursors you can select for the range of the segment you need and save the shift afterward
- We can also play with the time on the x-axis (i.e. zoom the graph, you can do this by pressing right click on the graph, then, autoscale x-axis
- Increasing the running window average in the parameter page, helps to decrease the noise in the graphs
- You can average your three channels (A, B, and C) by pressing average display (and check for the three channels) and a light blue (turquoise color curve will appear on the screen)
- You can remove the curves for the three channels (A, B, and C) by clicking on the channel that you would like to disappear on the right side of the software, and unclick the plot.