Protein capture by streptavidin for ligand binding

Material

- Affinité Instruments' Au sensors+biotin
- Distilled water (DI)
- Streptavidin (5 mg Streptavidin for ELISA & Western Blotting) Millipore Sigma SA101
- Ethanol 95% (EtOH)
- Running buffer: Phosphate Buffer Saline (PBS 1X, pH 7.4 Corning Cellgro 21-040-CV) or other
- Protein solution
- Ligand solutions

Protocol

Preparation of solution prior to experiment

- Prepare 1mL of 0.025 mg/mL of streptavidin in DI.
- Protein solution: protein of 0.02 mg/mL in running buffer.
- For ligand binding: solutions of different ligand concentration in running buffer (volume for each: 300 µL)

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

General notice

- Manual injection: inject at about 50 µL/s. Using 1 mL syringe, 1 drop is equivalent to 50 µL.
- Removing bubble: Inject at a higher flow rate of 200 uL/s or perform pulsed injections to move smaller bubble away.
- 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.

Streptavidin capture

4. Establish a stable baseline with DI water for 30 secs.
5. Inject 250 uL of streptavidin solution (0.025 mg/mL) onto each channel and leave for 20 minutes.
6. Wash with DI for 5 minutes.
7. Inject running buffer and wait for 5 minutes or until the signal stabilizes.

Immobilization

8. Inject 300 µL of protein solution (e.g. antibody in running buffer) in all channels and wait 20 minutes.
9. Wash all channels with 500 µL of running buffer for 5 minutes or until the signal stabilizes. Reinject buffer and wait until the surface is stabilized if needed.

   Note: after immobilization the streptavidin chips should be immediately used and not stored over night.

Binding Assay (Example: protein-ligand binding)

10. 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: a wash step 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 needed, can also be done after each ligand injection.

11. Repeat step 10 with subsequent ligand solutions.
12. Click on Recording in Progress to stop collecting data.

System wash

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