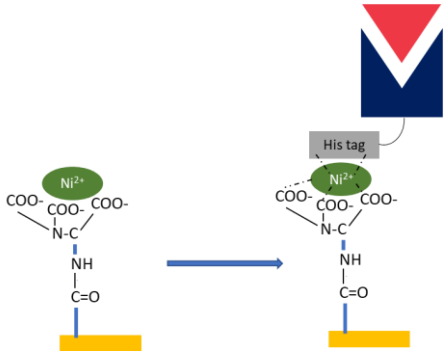
Protocol #2

Metal chelation of proteins

Histidine-tagged protein immobilization for antibody binding



Material

- Affinité Instruments' Au sensors+Ni-NTA (SAM03-5)
- Distilled water (DI)
- Running buffer: Phosphate Buffer Saline (PBS 1X, pH 7.4 Corning Cellgro 21-040-CV) or other
- Protein solution

Protocol

Preparation of solution prior to experiment

- Protein solution: protein of 0.1 mg/mL in 1mL of running buffer
- 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:
    - Immobilization

Always visually inspect the channels prior these steps.

Setting up the System:

1. Insert the Ni-NTA 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.

Immobilization

6. Inject 1 mL of PBS for 2 min to acquire a stable baseline.
7. Inject 0.4 mL of a solution of 0.1 mg/mL of His-tagged protein for 20 min to allow binding of the His-tag protein to the SPR chip.
8. Rinse with 1 mL of PBS for 2 min.

Binding Assay (Example: antibody-protein binding)

1. Activate the reference and deactivate the channel display of channel D.
2. Inject 300 uL of antibody in the **SAMPLE** channel only and wait at least 15 mins to see a signal increase.
3. Press save shift to save the delta SPR signal of each sample channel.
4. *Optional* Rinse with 1 mL of PBS for 2 min. Rinsing does not need to be performed between different ligand solutions but a final rinse with 1 mL of PBS should be done.
5. Repeat steps 2-4 for subsequent antibody solutions at different concentrations.

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.