

Immobilization of a ligand on gold chip surface:

(Protocol adapted from the BIACORE Sensor Surface Handbook.)

Required chip:

CM-5

Supplied solutions for amine coupling (in kit):

EDC : 0.4M of 1-ethyl-3-(3-dimethylpropyl)-carboimide in water

NHS : 0.1M N-hydroxysuccinimide in water.

Ethanolamine : 1 M ethanolamine-HCl pH 8.5

Required solutions:

Ligand: Immobilization requires about 200 μ l of 50 μ g/ml.
Ideally ligand is dissolved in water and is at a high concentration (1 mg/ml)

Analyte : About 100 μ l of 1 mg/ml Should be in identical buffer as running buffer.

General Immobilization procedure for CM5 chip (as later performed by Wizard):

Activate the surface	EDC/NHS	Flow rate: 5 μ l/min Contact time: 10 minutes
Immobilize ligand	ligand	Flow rate: 5 μ l/min Contact time: 10 minutes
Deactivate excess active groups:	ethanolamine	Flow rate: 5 μ l/min Contact time: 7 minutes

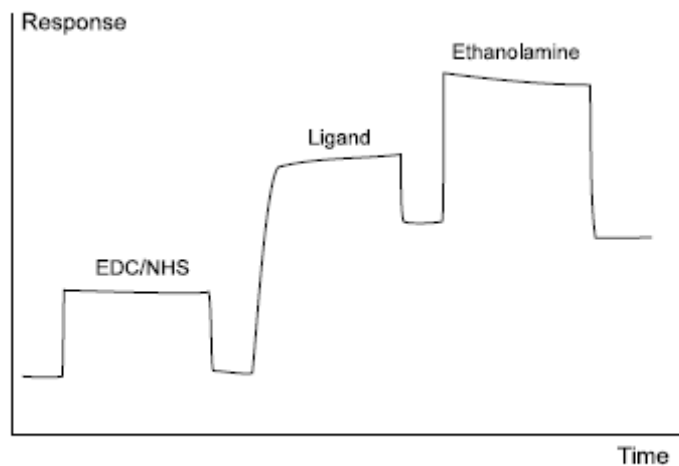


Figure 4-6. Schematic sensorgram showing the results of amine coupling.

General outline of an experiment:

- A. pH scout for pre-concentration
- B. Immobilization of ligand on a chip
- C. Interaction analysis

Start up procedures:

1. Make sure all buffers are filtered and all tubing is inserted properly.
2. Open Biacore T100 control software.
3. Undock old chip/maintenance chip using the command on the menu bar.
4. When the compartment opens, remove old chip and insert new chip then manually close the compartment.
5. Dock new chip from the menu bar.
6. Choose **tools: prime** to prime the system.
7. Eject sample rack and insert the required samples, noting their placement on the grid.
8. Normalize the signal response.
 - Go to tools: More Tools.
 - Select normalize in the maintenance tools folder and click on start.
 - Click on next in the subsequent dialogue box.
 - Fill a 7mm vial with 120uL of BIA normalizing solution 70% (in kit) and place in the correct position on the reagent rack.

A. pH-scouting for pre-concentration:

(note: pH of the immobilization buffer should be higher than 3.5 and lower than the isoelectric point of the ligand)

Purpose: In preparation of the immobilization of the ligand onto the chip, the pH at which the ligand is most attracted to the negatively charged dextran surface must be found.

Buffer to be used: 10mM sodium acetate

Total ligand needed: 5µg of ligand

Dilute ligand to a final concentration of 50 µg/ml (~100 µl final volume) in 10mM sodium acetate at the following pH values:

pH 4

pH 4.5

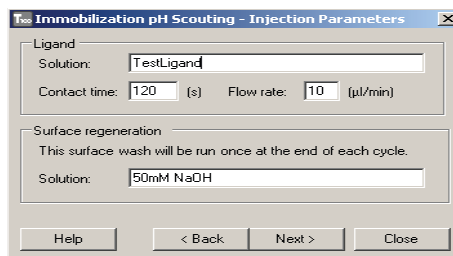
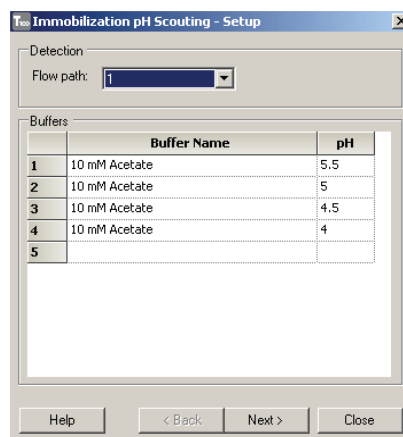
pH 5

pH 5.5

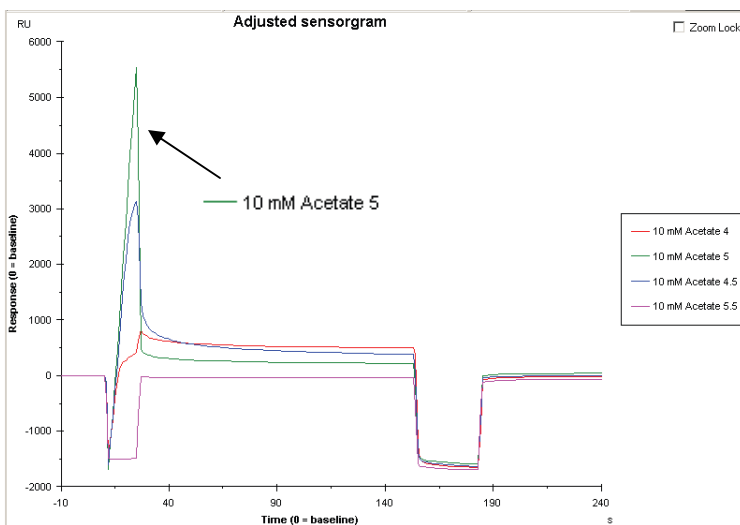
Pre-concentration pH scouting:

Procedure:

1. From the run menu, choose **Wizard**. Select **Immobilization pH Scouting** in the Surface Preparation folder.
2. Set flow path. As no immobilization will be performed during the pH scouting, any flow path may be chosen.
3. Set injection parameters. Increase/decrease flow rate and/or contact time if needed.
4. Run Wizard.



5. After run is done. Examine the results. For the immobilization, choose the buffer with the pH that gives the most response. (e.g. 10 mM acetate pH5.0 on left)



B. Immobilization:

Required solutions:

EDC

NHS

Ethanalmine

140 µl of 50 µg/ml of ligand solution.

Procedure:

1. From the run menu, choose **Wizard**. Select immobilization in the Surface Preparation folder.

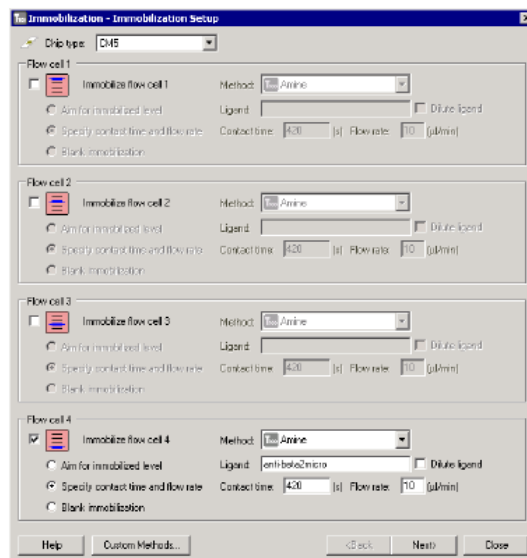
Select chip type: CM-5

Immobilize flow cell 4

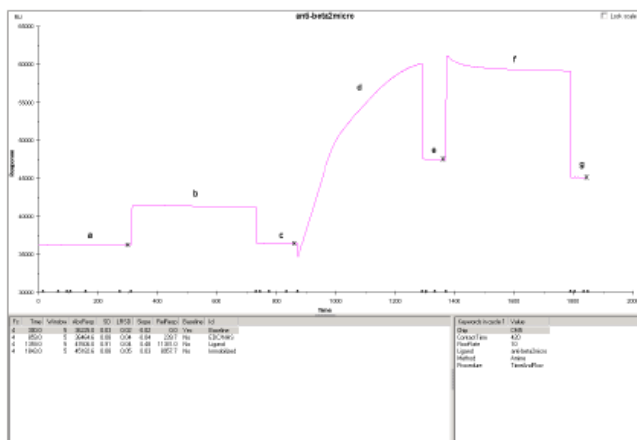
Contact time: 420 sec (7 min)

Flow rate: 5 µl/ml

2. Click on **next**.
3. System Prep box pops up. If pre conc. has just been performed, uncheck: **Prime before run** and **Normalize detector**. If not, leave them checked.
4. Click on **next**.
5. Choose solution/rack positions and click on next and the prepare run protocol box should pop up.
6. Run method.



Typical immobilization result:



An immobilization level of about 8000 RU is good.

Calculate the Rmax:

$$R_{max} = \text{immobilized RU} \times \text{stoic ratio} \times \frac{m_{w, \text{analyte}}}{m_{w, \text{ligand}}}$$

Stoic ratio: if each analyte molecule can bind 5 ligand molecules, then the ratio is 1/5.

Immobilized RU: difference between A and G on the sensorgram

- a) Baseline.
- b) EDC/NHS activation of the dextran matrix.
- c) Baseline after activation.
- d) Attraction and covalent coupling of the ligand to the dextran matrix
- e) Buffer washes away loosely associated ligand. The response level at this point gives a first indication of the immobilized amount.
- f) Deactivation and further washing away of loosely associated ligand.
- g) The difference in response between points a and g reflects the amount immobilized.

C. Interaction analysis:

Analyte: About 100µl of 0.1 - 1 mg/ml to start
(*protein concentration might have to be changed*)
Regeneration buffer: Depends on application (see manual). Regeneration
scouting wizard may need to be run.

1. Choose run: wizard. Select binding analysis in the assay folder and click on new.
2. In the injection sequence box: Use default settings Sample: 1 and Regeneration:1 then click next.
3. In the setup box: Choose detection in Flow path: 4-3. (Remember: we immobilized the ligand on flow path 4, flow path 3 will give the nonspecific binding). Click on next.
4. In the injection parameters box:
In sample box enter:
 Contact time: 180 sec
 Flow rate: 10 µl/min
 Dissociation time: 0 sec
In regeneration box:
 Contact time: 30 sec
 Flow rate: 10 µl/min
 Stabilization period: 5 sec
Click on next.
5. Enter sample names in sample dialog box. Click on next.
6. In the system preparations dialog box, uncheck prime before run and normalize detector (already been done during immobilization. Click on next.
7. Prepare samples and enter assign rack positions as indicated on the screen. Click on next.

Alternatively, from the Run Menu, one can run other Assay Wizards including: Binding analysis, Concentration analysis, Kinetics/Affinity and Thermodynamics.