Laboratory Practicals

S2005 Methods for characterization of biomolecular interactions

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During the exercise, we will be analyzing interaction between α-chymotrypsin (CHT, 29.3 kDa) and soybean trypsin inhibitor (STI, 20.1 kDa). Both stock solutions are prepared in PBS (20 mM Na-phosphate, 200 mM NaCl, pH 6.7).

MICROSCALE THERMOPHORESIS:

1) Initial check:

Prior to the affinity measurement, we need to choose the capillaries and check the suitability of the MST for the CHT-STI system. CHT was labeled in advance using NHS-Red labeling dye (Nanotemper) according to the product manual.

Sample preparation:

Prepare 500 ul of 10 nM labeled CHT working solution by diluting the stock solution in PBS. Use a black tube or protect the transparent tube from excessive light exposure.

Prepare 100 ul of 200 µM STI working solution by diluting the stock solution in PBS.

Measurement set-up:

Fill two standard capillaries and, using MO.control SW, perform a preliminary test to check the fluorescence signal and sticking of protein to the capillaries. If both parameters fulfill the criteria, continue with the next point. Otherwise, change the conditions (sample concentration, capillary type) and repeat the experiment.

Perform the binding test. In one tube, mix 30 µl of 10 nM CHT with 30 µl of PBS, and in the second tube mix 30 µl of 10 nM CHT with 30 µl of 200 µM STI solution. Fill four capillaries with each mixture and run the experiment.

Data analysis:

Using the MO.control SW, check the obtained data and automated evaluation. Discuss the results.

2) Affinity measurement:

Once the system's suitability is verified, we can measure the binding affinity.

Sample preparation:

Use the remaining working solutions from preliminary MST tests.

Prepare 16 tubes (use strips) of two-fold STI dilution with the highest concentration of 200 µM and 10 µl final volume in each tube. Add 10 µl of labeled CHT working solution (10 nM) into each tube.

Measurement set-up:

Fill in the information in the MO.control SW. Fill 16 capillaries with the prepared solutions, place them in the tray in the correct order and start the measurement.

Data analysis:

Using the MO.analysis SW, evaluate the experimental data. Try to calculate the affinity parameters using default and manual settings. If you have measured duplicate or triplicate, compare the results and perform a global analysis.

BIO-LAYER INTERFEROMETRY:

1) Protein immobilization:

The first step in BLI experiment is the immobilization of one binding partner. We will immobilize STI on AR2G biosensor using amine coupling approach.

Sample preparation:

Dilute the STI stock solution in 10 mM acetate, pH 4.0 to a working concentration of 50 µg/ml and a final volume 200 µl.

Measurement set-up:

- Take two unused AR2G biosensors and place them into desired positions of the sensor tray. Pipet 200 µl of MilliQ water into corresponding positions of hydration plate and insert the plate into tray.
- Prepare the experimental plate set-up using Data acquisition SW. Use following steps:

Step	Solution	Contact time [sec]
Baseline	PBS, pH 6.7	60
Activation	NHS/EDC mixture (mix immediately before run)	300
Binding	STI solution (active sensor) Acetate buffer pH 4.0 (blank)	600
Quenching	Ethanolamine pH 8	300
Wash	PBS, pH 6.7	120

- Pipet all solutions into corresponding wells (200 µl per well).
- Place sensor tray and experimental plate into BLI instrument and run the experiment at 20 °C.

Data analysis:

- Check the output sensorgrams. Compare the final response of active and blank channel.

2) Binding affinity assay:

The affinity towards CHT will be determined using direct-binding assay. The dissociation should be relatively fast and almost complete, therefore, no regeneration step will be introduced in between measurement cycles.

Sample preparation:

In 1.5 ml tubes, prepare six concentrations of two-fold dilution row of CHT into final volume of 400 µl per tube. The highest concentration shall be 150 µg/ml (app. 5 µM).

Measurement set-up:

- Take the AR2G biosensors prepared in the previous run and place them into new positions of the sensor tray. Pipet 200 µl of PBS buffer into corresponding positions of hydration plate and insert the plate into tray.
- Prepare the experimental plate set-up using Data acquisition SW. Use following steps for each of the CHT concentration:

Step	Solution	Contact time [sec]
Baseline	PBS, pH 6.7	60
Association	CHT solution of given concentration	180
Dissociation	PBS, pH 6.7 (same well as in Baseline step)	300

- Pipet all solutions into corresponding wells (200 µl per well).
- Place sensor tray and experimental plate into BLI instrument and run the experiment at 20 °C.

Data analysis:

- Using Data analysis SW, check the output sensorgrams.
- Perform blank sensorgram subtraction.
- Evaluate the curves using kinetic approach.
- Evaluate the curves using steady-state approach.
- Compare and discuss the results.