Investigating non-specific protein binding and streptavidin binding using QCM-D sensing

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Abstract

This protocol describes how to check the quality of modified QCM-D sensors and how to study protein interactions using QCM-D sensing.

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Before start

All QCM-D measurements should be performed with an E4 Q-Sense instrument. All protein interaction experiments should be performed in duplicates, under a continuous flow of 50-100 µl/min and with a negative control for which PBS is flown instead of the other reagents.

Materials

- HEPES View by P212121
- Sodium chloride View by P212121
- BSA-Molecular Biology Grade - 12 mg B9000S by New England Biolabs
- Streptavidin - 1.0 mg N7021S by New England Biolabs
- 1X PBS (Phosphate-buffered saline) by Contributed by users
- Distilled Water by Contributed by users
- Fetal Bovine Serum by Contributed by users
- biotin-BSA by Contributed by users
- biotin-protein-A by Contributed by users
- anti-BSA by Contributed by users
- NaOH by Contributed by users

Protocol

Step 1.

Prepare a Hepes Buffered Saline (HBS) solution containing 150 mM NaCl and 10 mM Hepes. Then prepare a solution of 25 µg/ml streptavidin in HBS.

Step 2.
Using QCM-D sensing, make a baseline of the HBS solution.

Step 3.
Expose the modified sensors to non-diluted Fetal Bovine Serum (FBS) for 30-60 min under static conditions in QCM-D and measure the amount of adsorbed FBS relative to the HBS baseline obtained in the previous step.

Step 4.
Rinse the sensors with the HBS solution.

Step 5.
Flow the streptavidin solution over the modified sensors (100-500 µl/min), until they are saturated.

Step 6.
Rinse the sensors with the HBS solution.