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Batch Immobilization of a Biotinylated Ligand onto Streptavidin Biosensors and High Precision Streptavidin Biosensors (SAX, SAX2)

Technical Note

Scope
This technical note outlines a general batch mode procedure for immobilizing a biotinylated ligand onto Streptavidin Biosensors in the biosensor tray assembly on the benchtop. The goal is to develop a procedure that creates a binding surface having a maximum and reproducible response. This protocol outlines how to determine the optimal concentration and time for immobilization online prior to transferring these parameters to offline immobilization.

Abstract

Biotinylation of ligands is a critical technique in drug discovery because it allows for the specific and stable attachment of biotin to molecules of interest. This process plays an important role in target identification as biotinylation enables researchers to tag and isolate proteins, facilitating the identification of potential drug targets and identification of novel binding partners. High-throughput screening of samples, especially when determining kinetics and affinity of the interaction, can produce a bottleneck and therefore methods that streamline the process are highly desirable. Here, we show how multiple biosensors can be immobilized simultaneously for use on an Octet® Bio-Layer Interferometry (BLI) system, which helps remove the bottleneck and allow for the rapid and efficient testing of samples.

Overview

Octet® Streptavidin Biosensors enable the immobilization of a biotinylated ligand onto the biosensor surface. The immobilized molecule can then be used in subsequent kinetic or custom quantitation applications. The Streptavidin Biosensor surface has a biocompatible layer on which streptavidin has been immobilized. This surface allows for the quick and stable immobilization of biotinylated protein, peptide, dsDNA, or oligos. Best results are seen using a low molar coupling ratio of biotin to the molecule and using biotinylation reagents which incorporate a linker to allow for greater mobility once immobilized. Once the biotinylated ligand is immobilized onto the biosensor the resulting stable surface is suitable for most applications.

After immobilization parameters for a biotinylated ligand have been optimized online on the Octet® BLI system, batch immobilization offline (outside of the instrument) on the bench top is ideal for applications that require many biosensors with the same immobilized molecule. Batch processing in the biosensor tray provides a convenient way to immobilize protein onto many biosensors at once (Figure 1) and generates custom-coated biosensors suitable for long term storage.

Figure 1
Biosensor Tray Assembly.

Materials Required

- Octet® R8, RH16, or RH96 BLI system
- Streptavidin Biosensors, one of the following:
  - Octet® Streptavidin (SA) Biosensor for quantitation, screening, or kinetic applications (part no. 18-5019)
  - Octet® Super Streptavidin (SSA) Biosensors for small molecule kinetic analysis. (part no. 18-5057)
  - Octet® High Precision Streptavidin (SAX) Biosensors for high precision quantitation, screening, or kinetic applications (part no. 18-5117)
  - Octet® High Precision Streptavidin 2.0 (SAX2) Biosensors (part no. 18-5136)
- Biotinylated ligand to be immobilized. The ligand can be protein, DNA, or other biomolecules.
- (Optional) Analyte that binds the immobilized ligand. The analyte can be protein, DNA, or other biomolecules.
- 96 well, flat bottom polypropylene plates (Greiner Bio-one part no. 655209)
- Wash and immobilization buffer (typically HBS- or PBS-based)
- Sucrose (Sigma part no. S0389)

Reagent Preparation

- Immobilization and Wash Buffer. Typically, the same buffer is used for both the immobilization and wash, usually HBS- or PBS-based, at a neutral pH.

- Biotinylated Ligand Stock. It is important to remove any free biotin that may be present from the biotin-ligand stock. This can be accomplished via dialysis or desalting columns, following procedures outlined in the following Sartorius Technical Note: Biotinylation of Protein for Immobilization onto Streptavidin Biosensors.

Prepare a working stock of the biotinylated ligand in the immobilization buffer. Immobilization buffers are typically HBS- or PBS-based. The biotinylated ligand is typically used at a concentration of 10–25 µg/mL with 200 µL needed for each biosensor to be processed. Lower concentrations of the biotinylated ligand can be used, but the time of immobilization will need to be lengthened considerably.

- (Optional) Binding Molecule. The second molecule that binds to the immobilized biotinylated ligand (e.g. a known analyte) can be used to test the activity of the immobilized ligand. Prepare a stock solution at an appropriate concentration in the immobilization buffer. The concentration should be equivalent or greater than the expected affinity of the interaction to ensure a strong binding signal.

- Preservation Solution. Prepare a 15% *(w/v)* solution of sucrose in distilled, deionized water. Sterile filter through a 0.2 µm filter. This solution is used to preserve the biosensors once the ligand is immobilized.

Development Workflow

Successful assay development involves two phases:

1. Optimize the conditions for immobilizing the biotinylated ligand onto the Streptavidin Biosensor.
2. Transfer the optimized conditions for preparing ligand-coated biosensors to a batch-immobilization protocol. Preserve coated biosensors for storage. Assess the long-term stability of coated biosensors.

Optimize Immobilization Conditions

The optimization goal is to develop an immobilization procedure that creates an analyte-binding surface with a maximized and reproducible response. While, ultimately, the ligand will be batch-immobilized onto the biosensors offline, the protocol should be optimized online for easier visualization. Table 1 shows a suggested assay method on the Octet® BLI system for optimizing the immobilization conditions.

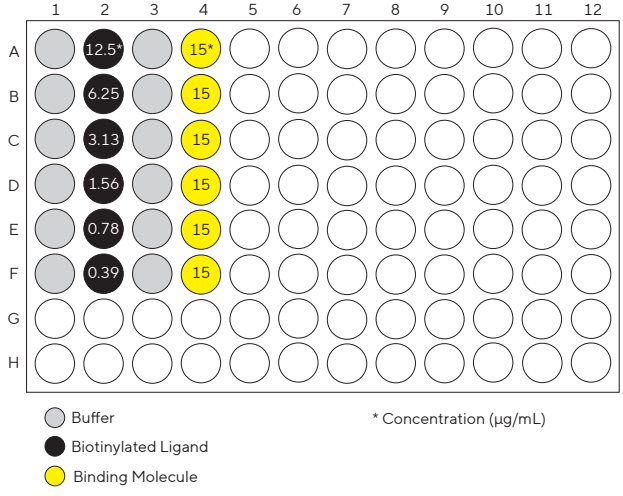
Table 1
Assay Method to Optimize Immobilization of a Biotinylated Ligand onto Streptavidin Biosensors.

Step	Data Name	Assay Time (sec)	Shake Speed (RPM)	Step Type
1	Buffer	120	0	Baseline
2	Biotinylated ligand dilutions	600	0	Loading
3	Buffer	120	1000	Baseline
4	Binding molecule	300	1000	Association

Assay Setup

1. Prepare several dilutions of the biotinylated ligand in immobilization buffer (>225 µL per biosensor). For example, Figure 2 shows an assay including six concentrations of biotinylated ligand from 0.39 - 12.5 µg/mL.
2. (Optional) Prepare a solution of the binding molecule in immobilization buffer (>225 µL per biosensor) at a concentration above the expected K_D. For example, Figure 2 specifies a 15 µg/mL solution of binding molecule.
3. Prepare a sample plate according to the plate map in Figure 2 (200 µL per well).
4. In the Kinetics mode of the Octet® Software, create the assay method shown in Table 1. The biotinylated ligand is immobilized at a shake speed of zero to more closely approximate the conditions of offline loading. Immobilization can be performed at the standard running temperature of 30°C.
5. Hydrate the Streptavidin Biosensors in immobilization buffer.
6. Place the sample plate and the hydrated biosensors in the Octet® BLI system and start the assay, setting a delay of 600 seconds to allow the samples to equilibrate to temperature.

Figure 2
Sample Plate Layout of a Kinetic Assay to Screen Biotinylated Ligand Concentrations for Immobilization onto Streptavidin Biosensors.



Results and Data Analysis

1. Examine the data to determine the best immobilization conditions. For example, in Figure 3, biotinylated ligand in the range 12.5 - 3.13 µg/mL with a loading time of 300 seconds provide sufficient ligand for maximum analyte binding (Figure 4). Note that if the amount of biotin-ligand is limiting, a low concentration can be used with an extended immobilization period (*e.g.* overnight at 4°C).
2. After immobilization, the biotinylated ligand should produce a stable binding surface. During optimization it is important to make certain that the post-immobilization baseline is flat and that the immobilized ligand binds the analyte of interest.

Transfer Immobilization Protocol to Batch (Offline) Mode and Validate

This section describes the basic procedure for immobilizing the biotinylated ligand offline (*i.e.*, in a batch). After developing a batch-immobilization protocol, it is highly recommended to perform an experiment confirming the activity of the biosensors immobilized offline against that of biosensors immobilized online in the Octet® BLI system.

If biosensors are to be stored longer than 24 hours before use, it may be useful to preserve and dry them. The protocol below outlines a preservation and storage procedure that produces stable biosensors for most proteins. Stability can be determined by testing preserved biosensors against a control sample at different storage times. Correctly stabilized biosensors will not show significant deterioration in binding during storage.

Figure 3

Example Binding Chart of a Screening Assay to Optimize Immobilization of a Biotinylated Ligand to Streptavidin Biosensors.

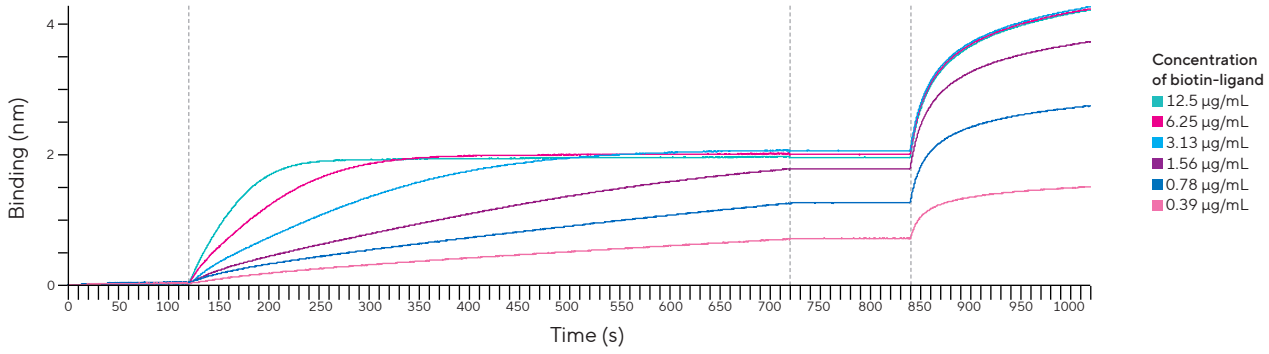
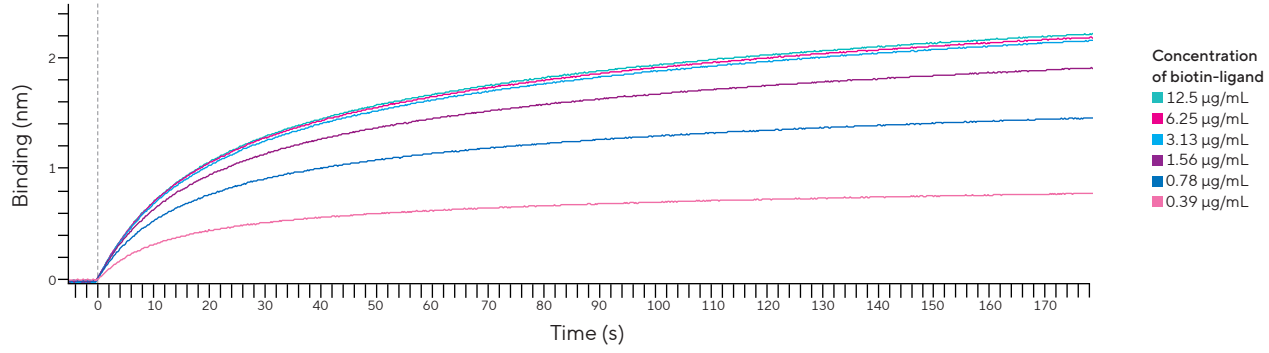


Figure 4

Normalized Analyte Binding Response Curves From Corresponding Immobilized Ligands As Shown in Figure 3.



Batch-Immobilize Biotin-Ligand onto Streptavidin Biosensors

During batch immobilization, the hydration, immobilization, washing and optional preservation steps are performed offline in the biosensor tray assembly (Figure 1).

Note: Fully equilibrate reagents and samples to room temperature before beginning batch immobilization.

1. To "translate" the online immobilization conditions to an offline protocol at room temperature, multiply the online incubation time at 30°C by 2 to achieve more reproducible ligand immobilization. For example, in the data shown in Figure 3, for a 12.5 µg/mL solution of biotin-ligand, the online immobilization time is 300 seconds, so for batch immobilization at room temperature the time will be doubled to 600 seconds (Table 2).
2. Determine the number of biosensors to be prepared in the batch. If you are going to process only part of a biosensor tray, carefully transfer the biosensors to an empty biosensor tray without touching the active tips of the biosensors. It is recommended to use the Octet® AT biosensor transfer tool (part no. 18-5159) when transferring biosensors.
3. Prepare a sufficient volume of the biotinylated ligand (200 µL/well) at the optimal concentration determined by the screening experiment above.
4. Prepare a separate microplate for each incubation step of the batch-immobilization protocol detailed in Table 2, filling the wells that match the location of the biosensors. Exchange the plate in the bottom tray holder for each incubation step.

Note: Take care not to let the biosensors sit out of liquid for too long to prevent them from drying out.

5. After completing the batch immobilization protocol, remove the biosensors from the sucrose solution and allow to dry. This can be done at room temperature for 5 minutes or in an incubator at 37°C for 1–2 minutes.
6. Store the preserved biosensors in the original foil pouch with desiccant at room temperature.

Note: Preserved biosensors must be hydrated in buffer immediately prior to use.

Table 2

Streptavidin Biosensor Batch Immobilization Protocol (At Room Temperature on the Benchtop).

Plate	Step	Well Contents	Incubation Time
1	Hydrate biosensors	Immobilization buffer	5 minutes
2	Immobilize biotinylated ligand*	Biotinylated ligand	2X online immobilization time
3	Wash 1	Immobilization buffer	5 minutes
4	Wash 2	Immobilization buffer	5 minutes
5	Preserve for storage	15% sucrose in PBS	1–2 minutes

* Use the optimum concentration of the biotinylated ligand and optimum incubation time determined from the screening assay. Alternatively, step 2 can be performed using a low concentration of biotinylated ligand (*e.g.,* 1 µg/mL) and incubated overnight at 4°C.

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