Individual Proteins Recruited from Complex Mixtures

Summary

Ski Pro, a fluorescent-free biomolecular interaction analysis platform built around nanoporous optical interferometry (nPOI), is used to recruit selective proteins from very complex protein mixtures, such as plasma. Specifically, we demonstrate the efficient recruitment of a HyNic-tagged streptavidin (HyNic-SA) out of 10% rat plasma to a 4FB surface, and the recruitment of biotinylated BSA (bBSA) out of 10% rat plasma to a streptavidin coated surface.

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Introduction

Many research and clinical studies require the analysis of individual proteins that are part of a complex mixture of proteins, such as plasma. Key in these applications is to minimize non-specific interactions of random proteins with the surface, which could interfere with the specificity of binding and thus authenticity of results.

In the current study we demonstrate the utility of the Silicon Kinetics porous silicon (poSi) surface to selectively recruit individual proteins from a complex mixture of proteins. Specifically, we show that we can generate a streptavidin surface with hydrazone based coupling chemistry by recruiting HyNic-SA that was spiked into 10% diluted rat plasma, as well as that we can recruit biotinylated BSA (bBSA).

Flow cell data is taken using an automated configuration. We use a fully differential flow cell, with simultaneous sampling of sample and reference channels, using the SKi Pro instrument with the SKi AutoFlow fluidics modules and SKi AutoPrep autosampler. The differential flow cell, in this case, is used as a control for the presence of possible non-specific binding.

There are several options of SKi Sensor biochips which may be used with nPOI. Here, we use the 4FB chips. Rather than requiring an on chip activation, as with carboxyl chips, these require an off-line crosslinking of the receptor molecule.

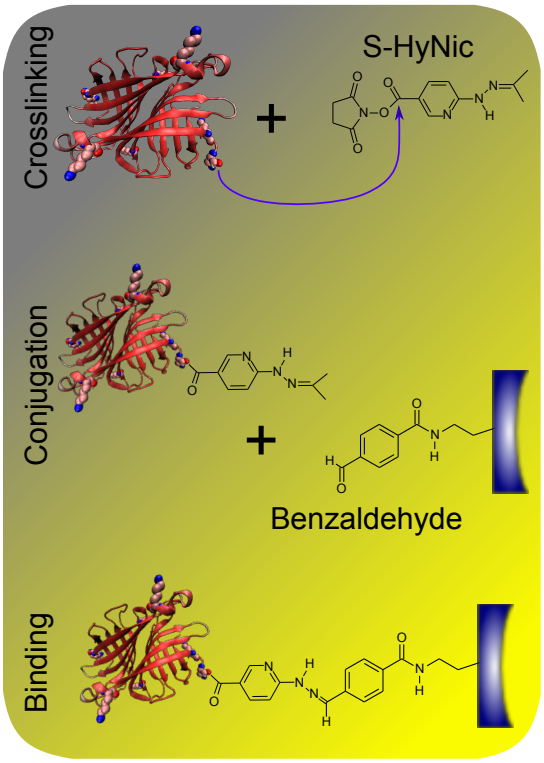

Figure 1: Immobilization chemistry used in this study. Streptavidin, shown with all of its lysines highlighted, is crosslinked externally with S-HyNic (Succinimidyl 6-hydrazinonicotinate acetone hydrazone). The succinimidyl group of S-HyNic leaves during the crosslinking step leaving the HyNic-SA complex. This is reacted with the 4FB (4-formylbenzoate) poSi biochips during the conjugation step. After this conjugation, the biochip surface is ready for binding.

Method

We use a hydrazone based chemistry, involving crosslinking a protein with S-HyNic off line as shown in Figure 1. This crosslinked complex is reacted with the 4FB-chips. Large batches of protein may be crosslinked offline and frozen as aliquots which may be used as needed.

To produce hydrazine-functionalized streptavidin, 5 mg of lyophilized streptavidin is dissolved in 0.5 mL of water. The dissolved streptavidin is equilibrated into PBS buffer, pH 7.2, using ZEBA columns. 1 mg S-HyNic is dissolved in 0.03 mL anhydrous DMF. After complete solubilization of the S-HyNic reagent, 15 μL of the reagent is added to the dissolved protein, followed by immediate rapid vortexing. After incubation of the labeling reaction at room temperature for 4 hours, unincorporated S-HyNic reagent is removed and buffer exchange into PBS, pH 6.0, performed using a ZEBA column. 20 μL of a 1 mg/mL HyNic-streptavidin solution in PBS, pH 6.0, is diluted ten-fold into 10% delipidated rat plasma. 50 μL of this solution is then applied to a 4FB poSi chip at a flow rate of 10 μL/min, followed by an exchange into PBS, pH 7.2 (sample).

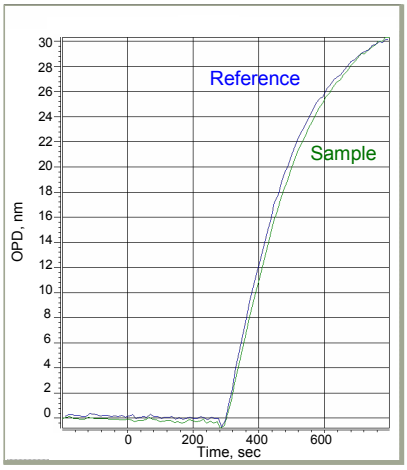

Figure 2: HyNic-SA is immobilized onto the 4FB poSi chip surface in both the sample and reference channels. A ΔOPD of 30 nm indicates the immobilization.

Alternatively, 50 μL of a 1 mg/mL HyNic-streptavidin solution in PBS, pH 6.0, is applied as a comparison (reference). In a separate experiment 50 μL of a 200 μg/mL biotinylated BSA solution in 10% delipidated rat plasma (sample), or in PBS buffer, pH 7.2 (reference), is applied at a flow rate of 10 μL/min, followed by a buffer step to test for dissociation.

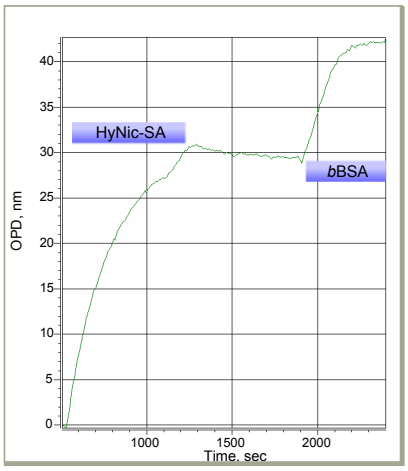

Figure 3: Recruiting HyNic-SA from Rat Plasma. The 50-fold excess of protein in the rat plasma does not interfere with the covalent hydrazone chemistry used to link the HyNic-SA to the surface. Here, ca. 30 nm of ΔOPD shift is seen as before. The shift due to bBSA non-covalently binding, likewise recruited from 10% rat plasma, shows a ΔOPD of 13 nm.

Results

We first ensure that the sample and reference flow cells are identical. As shown in Figure 2 sample and reference cells are similar in terms of being able to immobilize equivalent amounts of HyNic-SA to a 4FB surface. Using the same flow cell with a new chip, we further demonstrate that there is no appreciable difference in either rate or absolute quantity of HyNic-SA binding to the surface, suggesting that the aromatic hydrazone/benzaldehyde reaction is not affected by the presence of other proteins (Fig. 3).

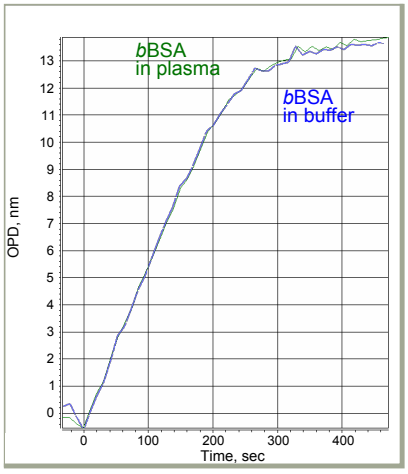

Figure 4: Recruiting bBSA from Rat Plasma and from buffer with a HyNic-SA surface. Both case show ca. 13 nm of ΔOPD.

These surfaces are equally competent in being able to bind biotinylated substrates (not shown). We then exposed a streptavidin surface to either bBSA dissolved in 10% delipidated rat plasma (sample), or dissolved in PBS, pH 7.2 (reference). As shown in Figure 4, there is no significant difference in either rate or quantity of bBSA binding when the protein is recruited from buffer, or from a complex protein mixture of 50 fold excess protein mass.

Discussion

A comparison of specific protein recruitment from buffer or from a complex mixture of proteins demonstrates that the porous silicon chips are able to specifically recruit proteins to either a chemical or to a protein affinity surface. It is now possible to either use hydrazone-functionalized proteins, or biotinylated proteins, or similar affinity reagents to recruit proteins and protein complexes from complex protein mixtures.

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