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Effective Blocking of Streptavidin-coated BLI sensors by SophoMer F10

Application Note

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Introduction

Biolayer interferometry (BLI) is a gold-standard technique for measuring binding kinetics and dissociation constants between (bio)molecules. Its ability to directly resolve association and dissociation rates under well-controlled conditions makes BLI a trusted platform for generating accurate, quantitative affinity data across a broad range of biomolecular systems. However, non-specific binding of biomolecules, such as proteins or oligonucleotides, to glass-based BLI biosensors can significantly compromise data quality by increasing background signal and distorting measured kinetics. Effective surface blocking is therefore critical to ensure accurate affinity measurements and reliable interpretation of BLI results.

Experimental part

Surface blocking performance of SophoMer F10 polymer (Sophomer s.r.o., Prague, Czechia) was evaluated on streptavidin (SA)-coated BLI biosensors (*Sartorius, 18-5019*) and directly compared with Bovine Serum Albumin (BSA) under identical experimental conditions (Figure 1-left). Both blocking reagents were applied at the same (0.5%) concentration. Here, we present sensograms (BLI signals) from reference sensors, which are essential for correcting bulk refractive index changes, instrumental drifts, and mainly non-specific binding. We hydrated three SA-coated biosensors in parallel in 1X PBS, 1X PBS supplemented with 0.5% BSA, or 0.5% SophoMer F10 for 10 minutes. To present only signals coming from non-specific surface binding, no biotinylated ligand was loaded (immobilized) on the sensors. After baseline equilibration in the assay buffers, sensors were exposed to Human Insulin Receptor (HIR) protein at 50 nM concentration to measure association. After 500 seconds, the sensors were placed into the buffers to monitor dissociation for an additional 500 seconds. Figure 1-left shows an increase in BLI signal from the untreated SA sensor in the presence of the HIR protein. This reflects the level of non-specific protein binding to the SA sensor surface. When the sensor was exposed to HIR in the presence of BSA, a continuous increase in signal was observed with very limited surface-blocking effect. **When the reference sensor was exposed to HIR in the presence of SophoMer F10, there was no response observed, demonstrating full suppression of non-specific binding.**

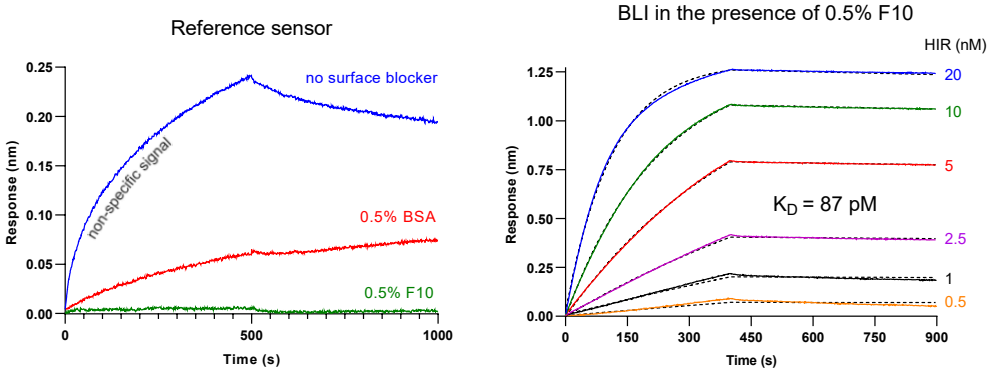

Figure 1. Surface-blocking effect of SophoMer F10. Left) Direct comparison with BSA in BLI measurement using SA-coated sensors. Sensor with no surface blocker represents the level of protein non-specific binding to the biosensor. Right) BLI kinetics (association and dissociation) between HIR protein and HIR-binding aptamer[1] in the presence of 0.5% SophoMer F10. Serial dilutions of HIR protein were used in the association step (20 to 0.5 nM). One biosensor without a loaded HIR-binding aptamer, treated with 50 nM HIR, was used as a reference sensor for background subtraction. Data was analyzed using GraphPad Prism 8.01 software.

BLI kinetic analysis for HIR protein and HIR-binding aptamer[1] was performed to elucidate whether SophoMer F10 does not affect either the process of immobilization of biotinylated aptamer to SA-coated biosensor or protein-aptamer interaction itself (Figure 1-right). The dissociation constant (K_D) was consistent with the published[1] value, which was experimentally measured using AR2G biosensors (*Sartorius, 18-5092*) optimized for covalent immobilization using EDC/NHS chemistry. This consistency indicates that **SophoMer F10 does not interfere with any of the key biomolecular processes during kinetic analyses.**

Different concentrations of SophoMer F10 were tested to show its effectiveness in surface blocking (Figure 2-left). As the concentration of F10 decreased, some instrumental drifts were repeatedly observed. To elucidate whether these drifts are affecting immobilization of the aptamer or protein-aptamer intermolecular interactions, BLI kinetic analysis was repeated using 0.1% of SophoMer F10 (Figure 2-right). An almost identical K_D value suggests that **even a 0.1% SophoMer F10 blocks the sensor surface effectively enough, with no negative effect on the BLI kinetic measurement and outcome data.** However, to ensure optimal BLI performance, a 0.5% F10 concentration is recommended.

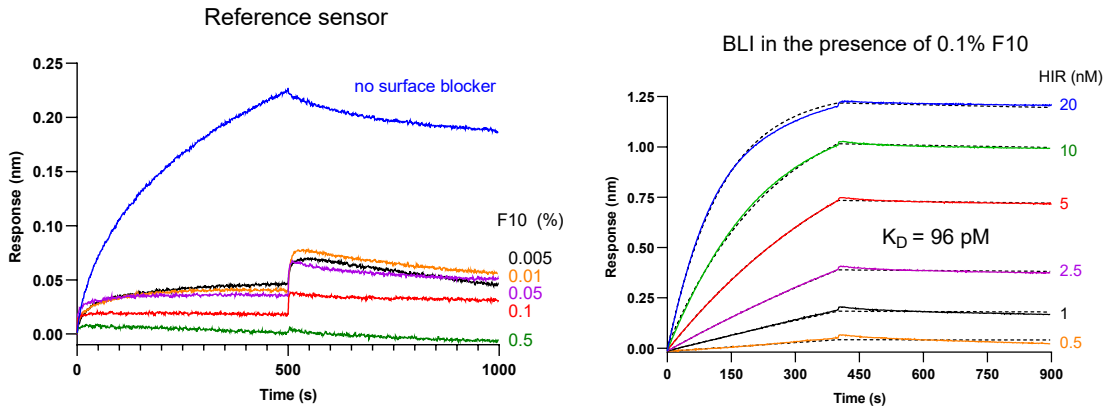

Figure 2. Left) Surface-blocking performance of SophoMer F10 under decreasing concentrations. Right) BLI kinetics between HIR protein and HIR-binding aptamer[1] in the presence of 0.1% SophoMer F10. Serial dilutions of HIR protein were used in the association step (20 to 0.5 nM). One biosensor without a loaded HIR-binding aptamer, treated with 50 nM HIR, was used as a reference sensor for background subtraction. Data was analyzed using GraphPad Prism 8.01 software.

Performance Summary

The data presented in this application list provides strong evidence for SophoMer F10 and its role in biosensing and analytical assays, particularly in applications where traditional protein-based blockers fail. At identical concentrations and under the same assay conditions, SophoMer F10 effectively passivated the glass-based BLI sensor surface, resulting in complete suppression of non-specific protein binding. The BLI kinetic assay yielded a consistent dissociation constant as previously published under a different BLI setup, providing proof that SophoMer F10 does not interfere with any of the key biomolecular processes of the assay. In contrast, frequently used BSA showed limited blocking activity and failed to prevent non-specific interactions with the sensor surface. Although lower F10 concentrations yield identical results, 0.5% concentration is preferable. Nevertheless, SophoMer F10 showed an effective surface blocking effect, ensuring accurate affinity measurements and reliable interpretation of BLI data.

References

1. Franco-Urquijo, P.; Ondruš, M.; Kurfürst, J.; Škerlová, J.; Selicharová, I.; Mužíková Čechová, L.; Šváchová, H.; Semerádtová, A.; Filimoněnko, A.; Fejfarová, A.; Homola, J.; Kouba, T. and Hocek, M. Over-Represented DNA Libraries Containing Two Hydrophobic (Het)aryl-Linked Nucleotides for Expedient Single-Round Selection of a Specific Aptamer Targeting Human Insulin Receptor. **2026**, Under review – already available as pre-print:,https://doi.org/10.21203/rs.3.rs-7946390/v1.

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