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Protein Production & Assays

Application Note

THE STREP-TACTIN®XT TECHNOLOGY IN DOWNSTREAM APPLICATIONS

Benefits of using the Strep-Tactin®XT technology in studying receptor ligand affinities and kinetics by SPR and BLI

Introduction

In this application note is demonstrated how the Strep-Tactin®XT technology can be used in conjunction with biosensor platforms, such as surface plasmon resonance (SPR), and bio-layer interferometry (BLI) to generate useful affinity and kinetic measurements, or to determine the concentration of a protein of interest in cell culture supernatant.

The optical biosensor platforms BLI and SPR

Biomolecular interaction studies are vital for the understanding of diverse biological processes and play an essential role in biotherapeutic drug discovery and development. Out of various techniques for analyzing molecular interactions, optical biosensor applications such as SPR and BLI have emerged as powerful tools for direct, real-time, and label-free measurements. These devices share the principle of an optical measurement that detects binding of a biomolecule in solution (analyte) to a biomolecule that is immobilized on the surface of the biosensor (ligand). SPR detects changes in the refractive index that is proportional to the concentration of bound analyte at a gold-layered surface of a biosensor chip. In contrast, BLI measures changes in the interference patterns from light reflected by the surface of the biosensor, which lies at the tip of an optical fiber.

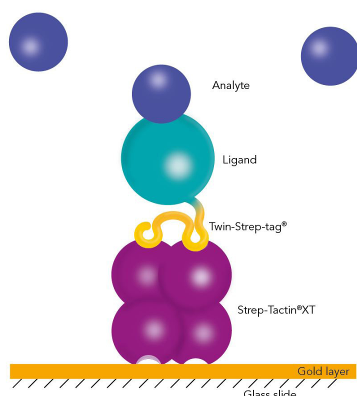


Fig. 1 Capturing Twin-Strep-tag® proteins with Biacore™.

Importance of protein immobilization

The full potential of biosensors is based upon a solid immobilization of the biomolecules serving as ligands on the sensor surface. However, the direct covalent immobilization of the ligand can change its biological activity or result in an undirected immobilization with reduced accessibility of the binding sites. The transient immobilization of tagged ligands via affinity capturing molecules overcomes these drawbacks **(Fig. 1)**. Nevertheless, tags used for such applications must bind efficiently to the capturing molecule with an affinity that is higher than that between analyte and ligand. His-tagged ligands that are immobilized on a NTA-surface with a KD in the nanomolar range can be used for measuring weaker interactions, but they fail in the measurement of high-affinity interactions with slow dissociation rates.

The Strep-Tactin®XT technology

The latest development of the Strep-tag® technology, the Strep-Tactin®XT, forms an exceptionally strong complex with the Twin-Strep-tag® (TST). An affinity in the low picomolar range allows measurements of long dissociation times and slow off-rates. Thus, the Strep-Tactin®XT technology enables a directed, high affinity immobilization that does not influence the ligands activity or requires any modifications. Due to the high affinity interaction and its high specificity, non-specific binding of host cell proteins is avoided, and ligands can be captured efficiently and directly from culture media. Moreover, Strep-Tactin®XT is compatible with many substances and the biosensors can be easily regenerated.

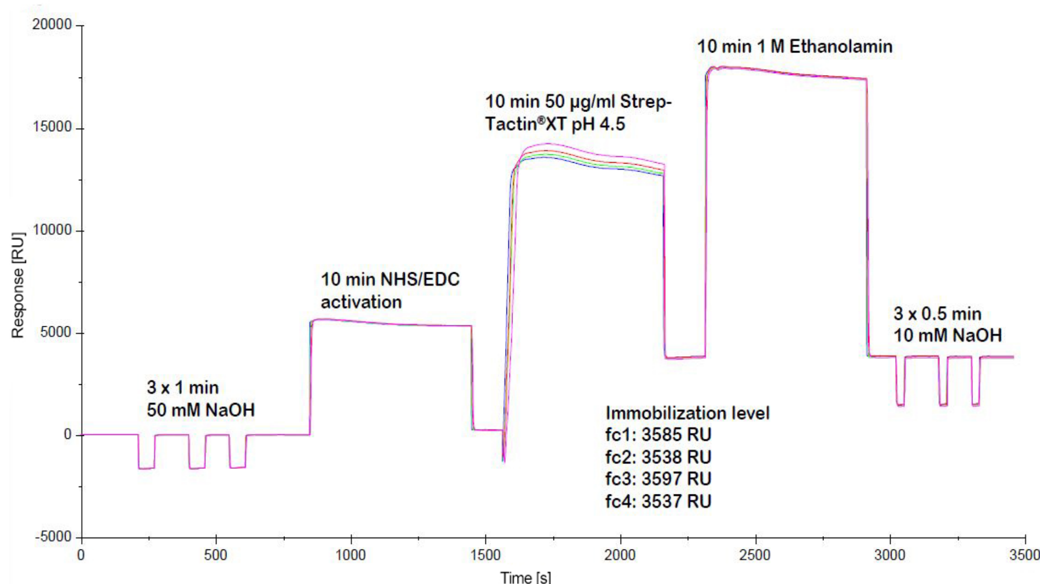


Fig. 2 Coupling of Strep-Tactin®XT on CM5 sensor chip is reproducible. The SPR sensogram shows the immobilization sequences of Strep-Tactin®XT with four flow cells simultaneously. The Biacore™ T200 (Cytiva) was used. Conditions and obtained response units (RU) were as indicated[1].

In this application note is shown that Strep-Tactin®XT in conjunction with Twin-Strep-tag® fusion proteins is a highly suited affinity capturing system for optical biosensor applications such as SPR and BLI.

Results and discussion

Application of Strep-Tactin®XT and Twin-Strep-tag® in SPR

To validate the capability of the Strep-Tactin®XT system for SPR assays, Strep-Tactin®XT was immobilized on CM5 sensor chips using the Twin-Strep-tag® Capture Kit (Cat. No. 2-4370-000) and the Amine Coupling Kit (Cytiva; BR-1000-50). Efficient and reproducible immobilization results were obtained using standard conditions as described in the Twin-Strep-tag® Capture Kit manual **(Fig. 2)**. The ability of Strep-Tactin®XT coated sensor chips to capture and release ligands that carry the Twin-Strep-tag® was tested with TST-fused ligands of different sizes and characteristics. TST-fused mCherry was reproducibly captured followed by regeneration on the Strep-Tactin®XT coated CMS sensor chip with 3 M GuHCl **(Fig. 3A)**. To test the usability of a different SPR sensor chip type, Strep-Tactin®XT was immobilized on a Xantec CMD200M chip. Likewise, capturing of anti-CD4-Fab-TST and regeneration of the chip were highly efficient **(Fig. 3B)**. Furthermore, capturing of the membrane protein CB2 (cannabinoid receptor type 2) on a Strep-Tactin®XT coated CM4 sensor chip

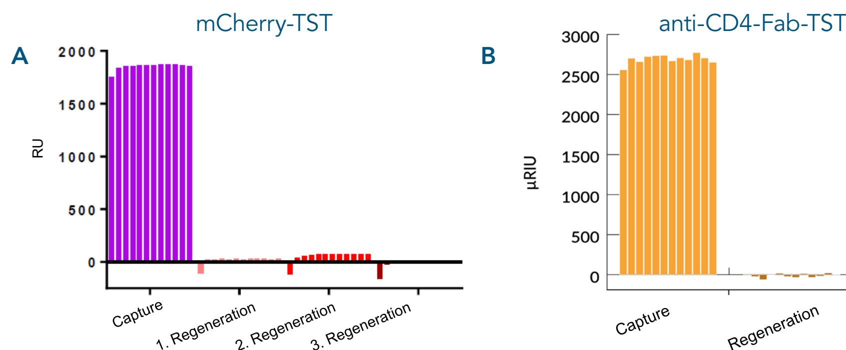


Fig. 3 Strep-Tactin®XT coated sensor chips can capture different TST fusion proteins and be efficiently regenerated. (A) TST-fused mCherry was sequentially captured on a Strep-Tactin®XT coated CM5 sensor chip by injections of TST-fusion protein (50 nM in 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% Tween 20). The chip was regenerated with 3 M GuHCl (3 x 0.5 min). Response units were 1858 ± 9 RU (CV:0.53%) and regeneration efficiency was 100%[1]. **(B)** Anti-CD4-Fab-TST was captured on a Strep-Tactin®XT coated Xantec CMD200M sensor chip at 25 °C with a flow rate of 100 µl/min. The working procedure was 2 min injection of anti-CD4-Fab-TST (500 nM in PBST) followed by 8 min injection of PBST. The chip was regenerated with 3 M GuHCl (3 x 0.5 min). Response units were 2691 ± 54 µRIU (CV: 2.00%), regeneration efficiency was 100%[2].

demonstrates the compatibility of Strep-Tactin®XT with various detergents and the usability in measuring interactions with high affinities **(Fig. 4)**.

Application of Strep-Tactin®XT and Twin-Strep-tag® in BLI

To analyze the capability of the Strep-Tactin®XT for BLI approaches, Strep-Tactin®XT was immobilized on Amine Reactive (AR2G) biosensors. After hydration in water, BLI sensors were activated in EDC/NHS for 10 min. Coupling reactions were performed with 45 µg/ml Strep-Tactin®XT in 10 mM sodium acetate (pH 5) for 10 min followed by blocking in 1 M ethanolamine (pH 8.5) for 10 min. After treatment in 50 mM NaOH for 100 s, sensors were equilibrated in 1x Buffer W (Strep-Tactin®/XT Wash Buffer) and then deployed in BLI measurements. A first test of the Strep-Tactin®XT coated biosensors resulted in reproducible capturing of TST-SEAP on freshly prepared biosensors as well as on the regenerated biosensors **(Fig. 5A)**.

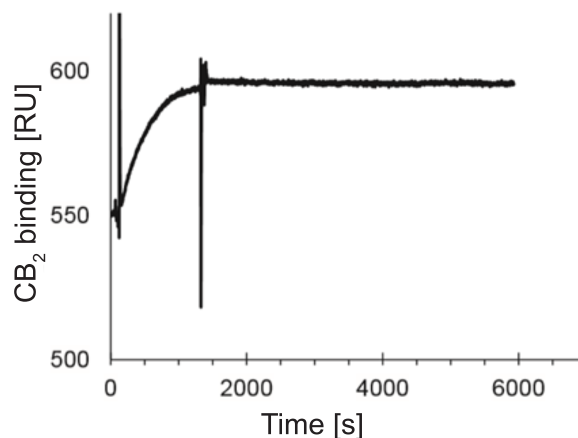


Fig. 4 Strep-Tactin®XT coated CM4 sensor chip can be captured with a TST-fused membrane protein. TST-fused CB2 was captured on a Strep-Tactin®XT coated CM4 sensor chip at 10 °C with a flow rate of 5 µl/min. 100 nM TST-CB2 in running buffer (50 mM Tris pH 7.5, 100 mM NaCl, 10% glycerol, 0.1% DDM, 0.5% CHAPS, 0.1% CHS) were injected for 20 min followed by injection of running buffer for more than 1 h[3].

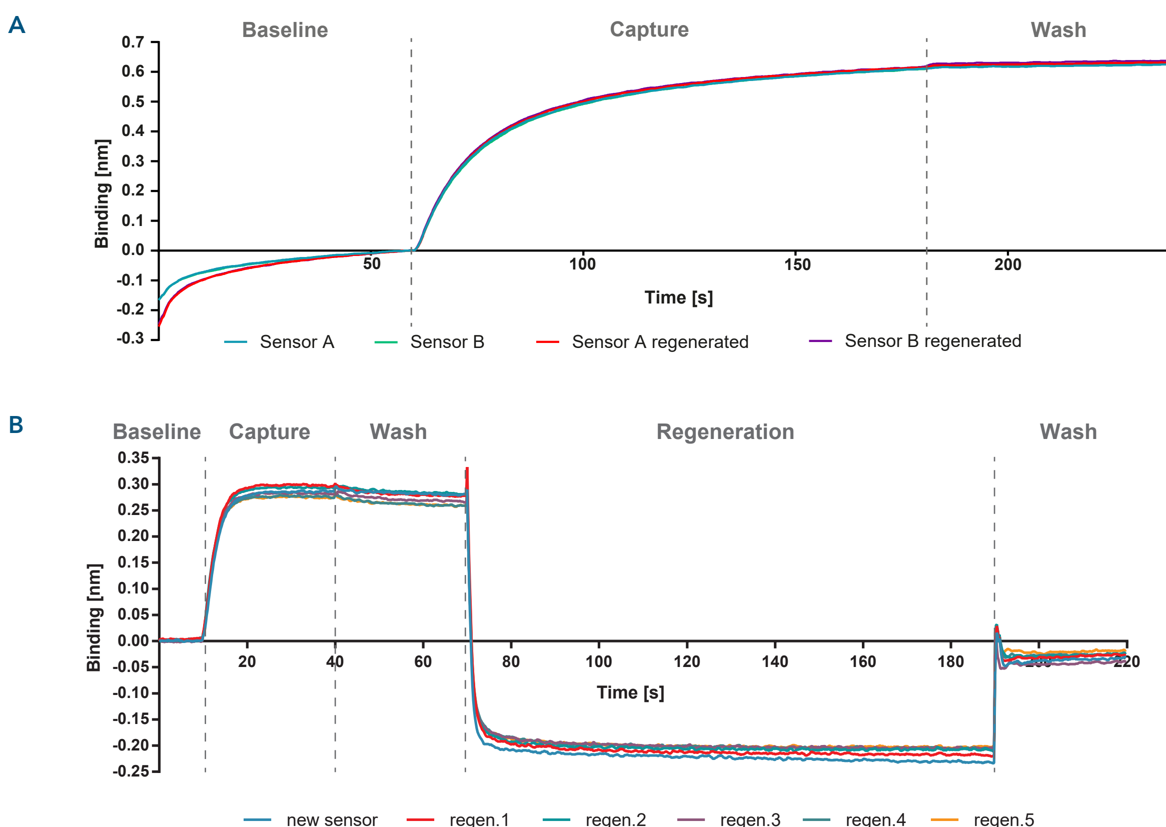


Fig. 5 Strep-Tactin®XT coated biosensors provide reproducible results (A) and can be regenerated several times (B). (A) TST-SEAP was in a first step independently captured on two freshly prepared Strep-Tactin®XT biosensors. The following working procedure was performed in Buffer W using the BLItz® System (Pall Fortebio Corp.): baseline for 60 s, association in 586 nM TST-SEAP for 120 s, dissociation for 60 s. Biosensors were regenerated in 3 M GuHCl for 2 min followed by 60 s in Buffer W (not shown) and by a second capturing step of TST-SEAP on the regenerated biosensors. **(B)** TST-GFP was captured on a freshly prepared Strep-Tactin®XT biosensor. The following working procedure was performed in Buffer W using the BLItz® System (Pall Fortebio Corp.): baseline for 10 s, association in 1100 nM TST-GFP for 30 s, and dissociation for 30 s. The biosensor was regenerated in 3 M GuHCl for 2 min followed by 30 s in Buffer W and reused for 5 times.

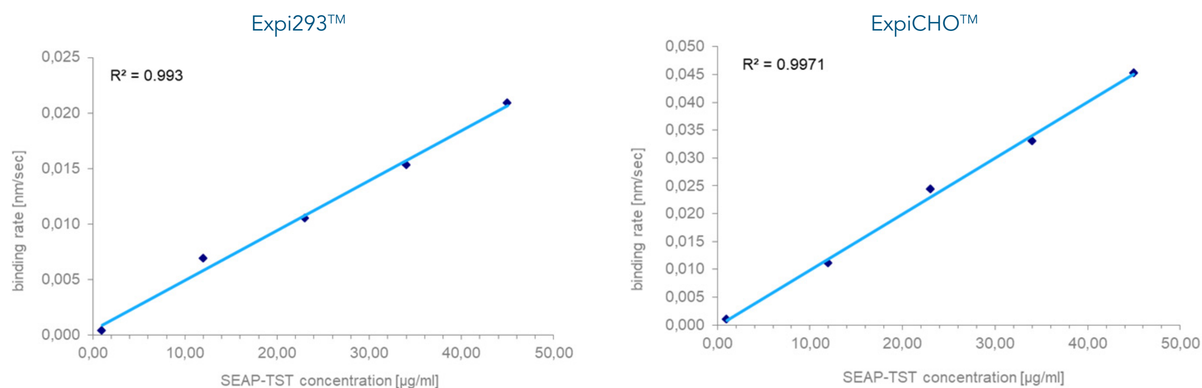
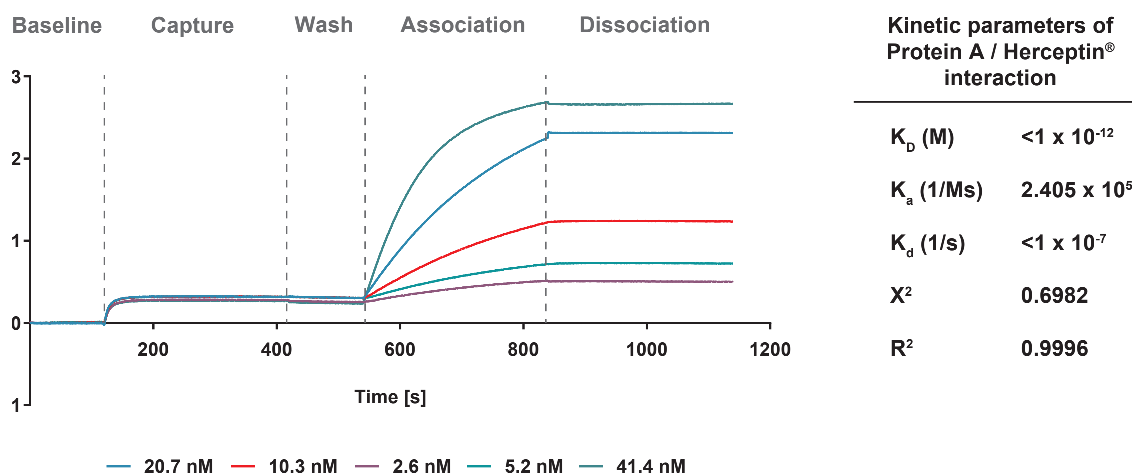


Fig. 6 Strep-Tactin®XT biosensors can be used for quantification of SEAP-TST from Expi293™ and ExpiCHO™ cell culture supernatants. Strep-Tactin®XT biosensors were hydrated in Buffer W for 10 min. Sensors were incubated for 4 min in Expi293™ and ExpiCHO™ medium (+ 1/10 10x Buffer W and BioLock). TST-SEAP association values were measured in different concentrated cell culture supernatants from either medium for 2 min using one individual biosensor per measurement. Biosensors were regenerated in 3 M GuHCl for 2 min followed by 4 min incubation in Expi293™ or ExpiCHO™ medium. The BLItz® System (Pall Fortebio Corp.) and the program "Create Standard Curve" of the BLItz Pro™ Data Analysis software were used for measurements.

A simple regeneration procedure is one of the key benefits of Strep-Tactin®XT. To validate the regeneration capability of Strep-Tactin®XT coated biosensors, TST-GFP was captured followed by regeneration in 3 M GuHCl for 5 cycles. The data for all 5 capture and regeneration cycles closely align **(Fig. 5B, previous page)**, demonstrating that Strep-Tactin®XT coated biosensors can be regenerated several times. The possibility to capture ligands directly from complex samples would dispense the need for the ligand purification step and provide time- and cost-efficient analyses. Thus, the direct capture of SEAP-TST was tested from different concentrated Expi293™ and ExpiCHO™ cell supernatants. A linear correlation between binding rate and SEAP-TST concentration could be obtained with low variances **(Fig. 6)**. To test, if the Strep-Tactin®XT enables the kinetic analyses of strong binding analytes, TST-Protein A was captured on a Strep-Tactin®XT coated biosensor and its interaction with the Trastuzumab (Herceptin®) antibody was analyzed. The antibody association and dissociation curves were fitted globally assuming a 1:1 binding model **(Fig. 7).** The resulting kinetic parameters of Protein A/IgG are in good agreement with the literature[4], demonstrating the suitability of Strep-Tactin®XT for kinetic analyses.



Kinetic parameters of Protein A / Herceptin® interaction

K_D (M)	<1 x 10^{-12}
K_a (1/Ms)	2.405 x 10^{5}
K_d (1/s)	<1 x 10^{-7}
X^2	0.6982
R^2	0.9996

Fig. 7 High binding affinities of Strep-Tactin®XT biosensors allow kinetic measurements. A sandwich-based (2-step) assay was used to quantitate protein A/IgG binding kinetics. TST-Protein A was captured on a Strep-Tactin®XT biosensor followed by association of the Trastuzumab (Herceptin®) IgG1 at indicated concentrations. The following working procedure was performed using the BLItz® System (Pall Fortebio Corp): baseline for 120 s, association of TST-Protein A for 300 s, second baseline for 120 s, association of Herceptin® for 300 s, dissociation for 300 s. The program "Advanced Kinetics" of the BLItz Pro™ Data Analysis software was used for measurements. The table shows the kinetic parameters of the Twin-Strep-tag® Protein A/IgG interaction after step correction of the TST-Protein A immobilization and global fit to a 1:1 binding model.

Conclusion

In conclusion, Strep-Tactin®XT can be efficiently immobilized on SPR and BLI sensors and enables capturing of TST-fused ligands with exceptionally high affinity. The system allows kinetic analyses of strong binding analytes with long dissociation times and thus overcomes the current limitations of other affinity tag-based capture systems, such as the His-tag. The possibility to capture diverse ligands directly from culture media and a simple regeneration procedure of the biosensors add major value to the application of Strep-Tactin®XT in optical biosensor assays.

References

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