

Application Note

High-affinity capturing of tagged proteins on the **switch**SENSE® biochip using Strep-Tactin®XT

Keywords: Strep-Tactin®XT | functionalization | sandwich assay | Strep-tag® | capture

Background and Aims

Reliable immobilization of biomolecules on sensor surfaces is a key approach for biomolecular kinetic studies. The unique sensor functionalization protocol of **switch**SENSE® technology allows to regenerate the sensor multiple times and supports a wide range of ligand immobilization methods, such as covalent coupling and His-tag-capturing.

Capturing proteins on a biosensor surface via tags is convenient, as many proteins are expressed with tags for purification. The most common tag is the polyhistidine tag, which can be captured by a tris-NTA modified surface with a K_D in the nanomolar range. This capture system works well for interactions featuring affinities which are weaker than the aforementioned K_D , but for the measurement of high-affinity interactions with slow dissociation rates below 10^{-3} s^{-1} , the $\text{His}_6\text{-NTA}_3$ complex is not stable enough. In contrast, the recently introduced Strep-Tactin®XT – Twin-Strep-tag® system by IBA GmbH forms an exceptionally strong complex with a K_D in the picomolar concentration range. Strep-Tactin®XT is a specifically engineered streptavidin variant with high affinity towards the synthetic Strep-tag®II peptide sequence (WSHPQFEK). Due to the avidity effect, even tighter binding can be achieved with the Twin-Strep-tag®, which consists of two Strep-tag®II motifs in series (WSHPQFEK-(GGGS)₂GG-SA-WSHPQFEK). In enabling the measurement of long dissociation times and the analysis of slow off-rates, this tag system is a promising means to stably immobilize Strep-tagged® proteins on biosensors.

Here, we measure the kinetic rates for the capturing of Twin-Strep-tag® and Strep-tag®II proteins by Strep-Tactin®XT. Subsequently, we analyze the well-studied interaction between protein A and adalimumab (Humira) using Twin-Strep-tag® capturing to show its applicability for the quantification of tight interactions with slow off-rates.

Methods

Measurements were performed with a dual-color DRX² instrument using the red fluorescence channel and analyzed with the kinetics module of the switchANALYSIS software (v 1.6, Dynamic Biosensors). For all measurements, a standard **switch**SENSE[®] chip (order no. MPC2-96-2-G1R1) was used and functionalized with Strep-Tactin[®]XT by conjugate hybridization using the Strep-Tactin[®]XT-kit (order no. CK-SXT-1-B96). Interaction kinetics were monitored in real-time by fluorescence. To measure the capturing kinetics of the Twin-Strep-tag[®] and of the Strep-tag[®]II by Strep-Tactin[®]XT, tagged GFP proteins (IBA GmbH, order no. 2-1006-005 and 2-1007-005) were used. Association measurements were performed at 6 different concentrations between 0 and 100 nM at a flow rate of 100 μ L/min. Dissociations were only observed for the highest GFP concentrations by pumping TE40 buffer (pH 7.4, order no. BU-TE-40-10) over the sensor surface at a fast flowrate of 1000 μ L/min to avoid rebinding artefacts. Before each association the sensor was regenerated by exposure to a high-pH regeneration solution (order no. SOL-REG-12-1). This procedure denatures the double stranded DNA by disrupting hydrogen bonds between the base pairs. The conjugate is washed away while the covalently attached single-stranded nanolevers remains on the surface and can be reused for a new functionalization step.

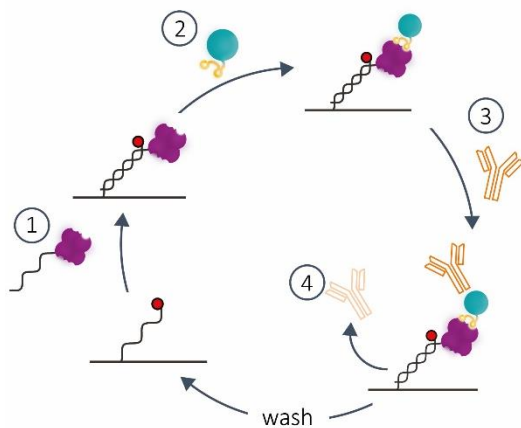


Figure 1 | Measurement workflow. ① Sensor functionalization with Strep-Tactin[®]XT: Covalently attached single-stranded DNAs on the measurement electrode are functionalized by hybridizing complementary DNA - Strep-Tactin[®]XT conjugates to the surface. ② Functionalization with the Strep-tagged protein. ③ Associations of the analyte. ④ Dissociation of the analyte. Regeneration: The DNA - Strep-Tactin[®]XT conjugate is removed from the surface by denaturing the DNA nanolevers at basic pH. The sensor is now ready for the next measurement cycle. To resolve kinetic rates, the cycle is typically repeated several times at increasing concentrations of analyte and the dissociation is only monitored for the highest concentration to reduce the measurement time.

Figure 1 shows the experimental workflow for the analysis of the protein A – adalimumab interaction. After hybridization of the Strep-Tactin[®]XT conjugate (①), the Twin-Strep-tagged protein A was captured at a concentration of 100 nM (②). Subsequently, adalimumab associations (③) were observed at 6 different concentrations ranging from 0 to 10 nM. The dissociation (④) was monitored only for the highest concentration. Before each measurement, the chip was freshly regenerated and functionalized with Strep-Tactin[®]XT and protein A.

Results

To validate stability of the two Strep-tag® based capturing systems, we first measure the kinetics of Strep-tagged and Twin-Strep-tagged GFP towards Strep-Tactin®XT. Figure 2 shows the measured kinetic curves for both interactions. In both cases, the fluorescence increases upon GFP binding due to changes in the physico-chemical environment of the dye. The associations and dissociations show single-exponential behavior, as expected for one-to-one interactions and can be described by the following equations with fit parameters a , k_{on} , k_{off} :

$$F_a(t) = a \exp(-(k_{on}c + k_{off}) t)$$

$$F_d(t) = a \exp(-k_{off} t)$$

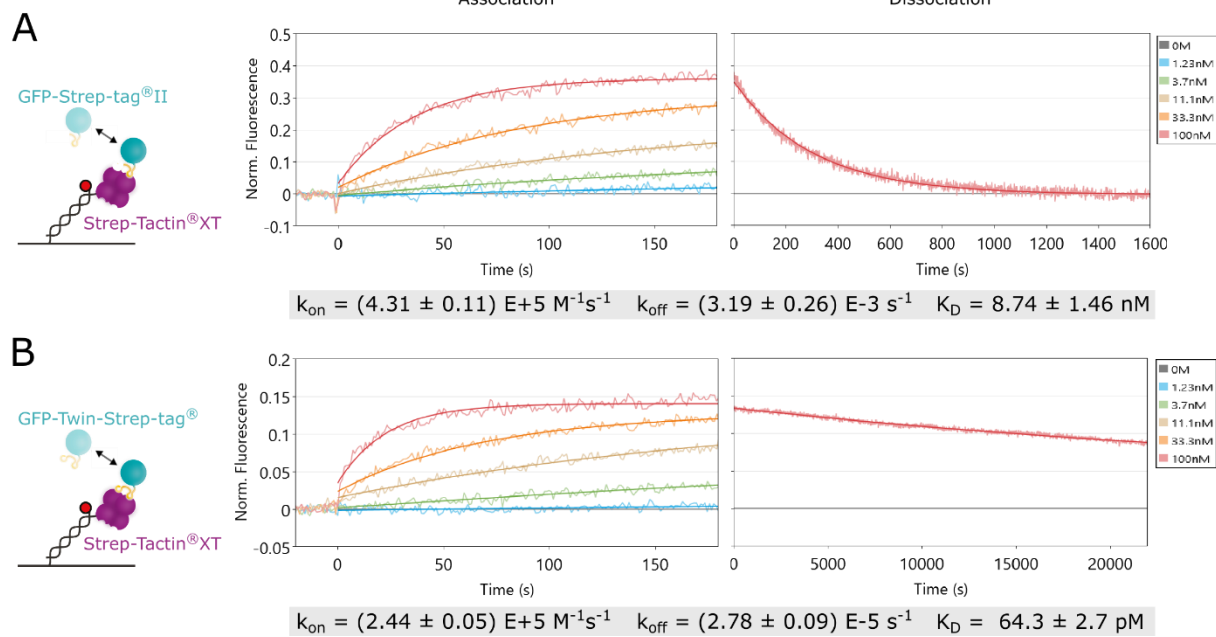


Figure 2 | Fluorescence signal and fits during association and dissociation phases of tagged GFP to/from immobilized Strep-Tactin®XT. Note the different x-axis scaling. **A:** Strep-tagged GFP. Global fitting of association and dissociation yields $k_{on} = (2.44 \pm 0.06) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $k_{off} = (3.19 \pm 0.26) \times 10^{-3} \text{ s}^{-1}$ and $K_D = 8.74 \pm 1.46 \text{ nM}$. **B:** Twin-Strep-tagged GFP. Global fitting of the association curves yields $k_{on} = (4.31 \pm 0.11) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $k_{off} = (2.78 \pm 0.09) \times 10^{-5} \text{ s}^{-1}$ and $K_D = 64.3 \pm 2.7 \text{ pM}$. Schematic figures courtesy of IBA GmbH.

For the Strep-tag®II interaction (Figure 2A), global fitting of all curves yielded $k_{on} = (2.44 \pm 0.06) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $k_{off} = (3.19 \pm 0.26) \times 10^{-3} \text{ s}^{-1}$ and $K_D = 8.74 \pm 1.46 \text{ nM}$. These parameters are comparable to the NTA₃-His₆-tag interaction ($k_{on} = 5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $k_{off} = 1 \times 10^{-3} \text{ s}^{-1}$, $K_D = 2 \text{ nM}$). Note that the numbers given here are typical values; the exact affinity of the His₆-NTA₃ complex depends on the nature of the tagged protein and the chemical environment and accessibility of its His₆-tag.¹ The Twin-Strep-tag® is expected to engage bivalently with the Strep-Tactin®XT on the surface and to be more stable bound than the monovalent Strep-tag®II. As shown in Figure 2B, the association of the bivalent Twin-Strep-tagged GFP, proceeds at a comparable on-rate, $k_{on} = (4.31 \pm 0.11) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$.

Remarkably, the dissociation measurement reveals the extraordinary strength of the new capturing method with a two orders of magnitude slower off-rate of $k_{off} = (2.78 \pm 0.09) \times 10^{-5} \text{ s}^{-1}$, corresponding to a K_D of $64.3 \pm 2.7 \text{ pM}$.

Table 1 | Kinetic parameters for the three tag capture systems: NTA₃ – His₆-Tag, Strep-Tactin®XT – Strep-tag®II, Strep-Tactin®XT – Twin-Strep-tag®

Tag capture system	$k_{on} (\text{M}^{-1}\text{s}^{-1})$	$k_{off} (\text{s}^{-1})$	K_D
NTA ₃ – His ₆ -tag	5×10^5	1×10^{-3}	2 nM
Strep-Tactin®XT – Strep-tag®II	2.4×10^5	3.2×10^{-3}	9 nM
Strep-Tactin®XT – Twin-Strep-tag®	4.3×10^5	2.8×10^{-5}	64 pM

The stability of the Twin-Strep-tag® interaction allows to study tight interactions with slow of rates. To show this, we captured Twin-Strep-tagged protein A on a Strep-Tactin®XT functionalized chip and measured its interaction with the adalimumab antibody. The antibody association and dissociation curves were fitted globally assuming a biexponential dissociation to account for the slow dissociation of the Twin-Strep-tag®. The fitting yields $k_{on} = (2.11 \pm 0.05) \times 10^7 \text{ M}^{-1}\text{s}^{-1}$, $k_{off} = (6.20 \pm 0.98) \times 10^{-4} \text{ s}^{-1}$ and $K_D = 29.4 \pm 4.7 \text{ pM}$, which is in good agreement with literature.² The superposed affinity-tag off-rate is one order of magnitude slower and was fitted as $k_{off}^{tag} = (7.13 \pm 3.23) \times 10^{-5} \text{ s}^{-1}$, which agrees well with the rate determined above for the Twin-Strep-tagged GFP.

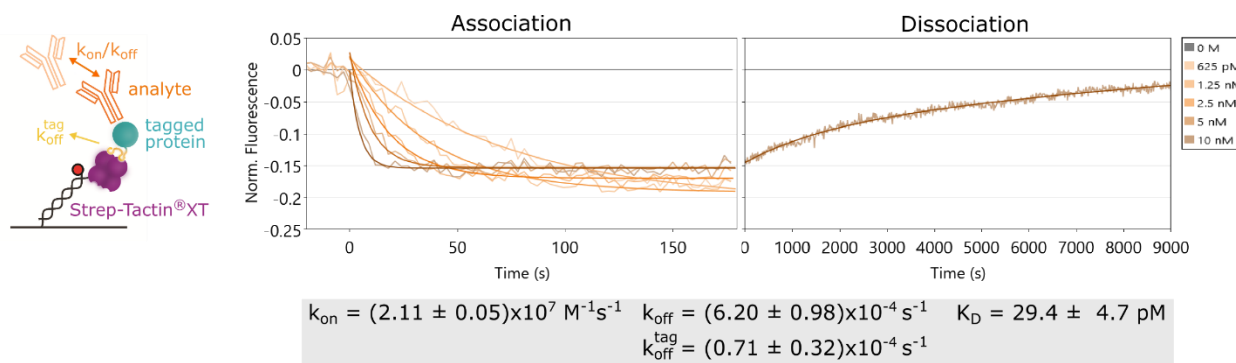


Figure 3 | Fluorescence signal and fits during association and dissociation phases of adalimumab to/from captured Twin-Strep-tagged protein A with adalimumab concentrations ranging from 0 to 10 nM. The curves were fitted globally assuming a biexponential dissociation to account for the slow dissociation of the tag. The fit yields $k_{on} = (2.11 \pm 0.05) \times 10^7 \text{ M}^{-1}\text{s}^{-1}$, $k_{off} = (6.20 \pm 0.98) \times 10^{-4} \text{ s}^{-1}$ and $K_D = 29.4 \pm 4.7 \text{ pM}$, and $k_{off}^{tag} = (7.13 \pm 3.23) \times 10^{-5} \text{ s}^{-1}$. The rate agrees well with literature² and the previously determined off-rate of the Twin-Strep-tag®-GFP. Schematic figures courtesy of IBA GmbH.

Conclusions

In conclusion, Strep-Tactin®XT functionalized **switch**SENSE® biosensor surfaces are very well suited to capture proteins tagged with any of the two Strep-tags: Strep-tag®II and Twin-Strep-tag®. While Strep-tag®II shows a moderate stability and should be used to investigate relatively fast kinetics and weaker-than-nM affinities only, the bivalent Twin-Strep-tag® system proved to be exceptionally stable. The Twin-Strep-tag® features a pM dissociation constant and overcomes up-to-date limitations of capture techniques. For the first time, the Twin-Strep-tag®/Strep-Tactin®XT system enables the kinetic analysis of strong binders with long dissociation times without the need for covalent coupling.

References

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