TECH NOTE

Analysis of proteins containing Twin-Strep-Tags on Alto Digital SPR using capture immobilization strategies

Overview

The Twin-Strep-Tag® technology from IBA Lifesciences utilizes Strep-Tactin®XT to capture proteins labelled with a Twin-Strep-Tag directionally. Proteintech, a leading provider of antibody reagents, has also developed an anti-Strep VHH that may be used in place of Strep-Tactin XT to capture Twin-Strep-Tags. Both of these capture strategies offer an optimal orientation for analyte binding and enable users to capture ligands from crude samples or matrix compositions, which may be incompatible with direct coupling methods. Protocols for immobilizing Strep-Tactin XT and Strep VHH have been pre-optimized for Alto™, Nicoya's Digital surface plasmon resonance™ (SPR) platform, allowing users to reduce experiment design time by offering a pre-developed assay configuration.

Introduction

Streptavidin is a tetrameric protein best known for its exceptionally high binding affinity to biotin (also known as Vitamin H). The streptavidin-biotin interaction has an affinity on the order of 10^{-14} M, which is approximately 10^3 to 10^6 times stronger than a typical antibody-antigen interaction.[1] Due to this remarkable affinity, streptavidin is widely used in biotechnology applications.[2-5] However, the near-irreversibility of the interaction poses challenges for techniques such as surface plasmon resonance (SPR), where it is often necessary to regenerate the sensor surface to immobilize new ligands or perform multiple binding cycles.

The Twin-Strep-Tag and Strep-Tactin XT system from IBA Lifesciences

To overcome the limitations imposed by the strong biotin-streptavidin interaction, alternative systems have been developed that use engineered peptide tags to occupy

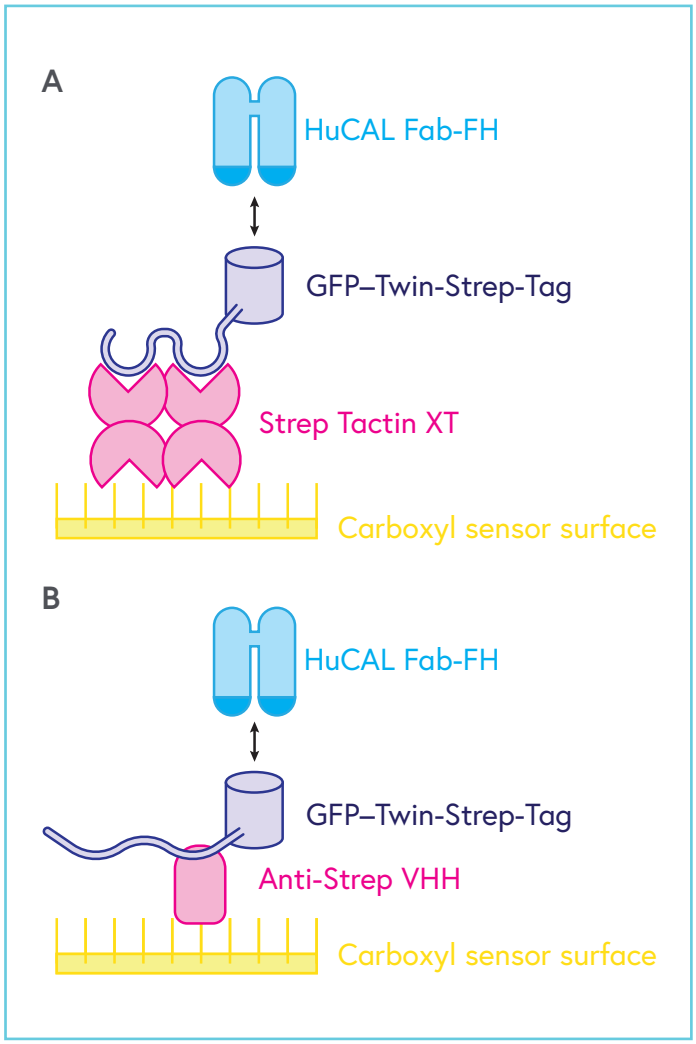

Figure 1: *Schematic representation of the capture systems used in this study to measure binding between GFP and a HuCAL Fab FH. A) Strep-Tactin XT, from IBA Lifesciences, is immobilized on the carboxyl sensor to capture GFP containing a Twin-Strep-tag. B) Anti-Strep VHH, from Proteintech, is immobilized on the carboxyl sensor to capture GFP containing a Twin-Strep-tag.*

the biotin-binding site of native or modified streptavidin. The first such system was the Strep-Tag®, which binds streptavidin with an affinity of approximately 37 µM.[6] Continued engineering of both binding partners by IBA Lifesciences led to the development of the Twin-Strep-Tag and Strep-Tactin XT, a modified form of streptavidin which includes a second, engineered biotin-binding site. The Twin-Strep-Tag was created by fusing two Strep-Tag-like motifs together with a flexible linker. This design allows the Twin-Strep-Tag to simultaneously bind both the native biotin pocket and the engineered binding site of Strep-Tactin XT, resulting in a dissociation constant (K_D) of approximately 10 pM.[7-9] The high affinity of this interaction is ideal for SPR ligand capture. Sensors can be functionalized with Strep-Tactin XT to capture Twin-Strep-tagged ligands and measure analyte interactions with slow off-rate and long dissociation times. Unlike the irreversible streptavidin-biotin interaction, the Twin-Strep-tagged ligand can be efficiently removed with glycine-HCl, pH 1.5, preserving the Strep-Tactin XT surface for subsequent binding cycles.

Proteintech Strep VHH

As an alternative to Strep-Tactin XT, Proteintech offers a nanobody that binds with high affinity to both the Strep-Tag and related sequences, including the Twin-Strep-Tag. Nanobodies, or VHH fragments, are small (12–15 kDa) antibody derivatives consisting of a single variable domain, originally based on the unique heavy-chain–only antibodies found in camelids.[10] Their compact size and monomeric structure make them well-suited for use as SPR capture reagents, often providing improved stability, orientation, and surface density compared to conventional IgG antibodies. In addition to these performance benefits, Strep VHH is a cost-effective alternative to Strep-Tactin XT, making it an attractive option for routine or high-throughput applications.

In collaboration with Proteintech, this technical note demonstrates the use of Proteintech's Strep VHH alongside IBA Lifesciences' Strep-Tactin XT and as capture surfaces on Alto for characterizing interactions involving Twin-Strep-tagged ligands. To illustrate compatibility and performance, we measured the binding kinetics of a Twin-Strep-tagged GFP (GFP-TST) interacting with an anti-GFP Human Combinatorial Antibody Library Fab (HuCAL Fab-FH) as the analyte.

Materials & Equipment

- Alto 16-Channel Instrument with Nicosystem Pro Software (ALTO16)
- Alto 16-Channel Carboxyl Cartridge (KC-CBX-CMD-16)
- Running Buffer: PBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-PBST)
- Alto Carboxyl Surfacing Kit: Cleaning, normalization, activation solutions (ALTO-R-CBX-SURF)
- Regeneration Buffer: Glycine-HCl, pH 1.5 (ALTO-R-GLYHCl-1.5)
- Capture molecule: ChromoTek Strep VHH, recombinant binding protein: Proteintech cat# qt
- Analyte: BioRad HuCAL Fab-FH Negative Control: CAT# HCA045
- IBA Lifesciences Twin-Strep-Tag Capture Kit: CAT# 2-4370-000
  - Capture molecule: Strep-Tactin XT
  - Ligand: GFP-Twin-Strep-Tag (GFP-TST)

Assay optimization tips

- Optimizing buffer conditions to capture the ligand is not necessary; it is recommended (but not required) that the ligand be in the running buffer.
- For best performance, it is recommended (but not required) that samples are purified. However, crude matrices are compatible with Alto.
- For most applications, the user should choose the lowest ligand density that still provides an analyte binding signal to prevent multi-phasic behavior and other artefacts from over saturating the sensor surface. It is recommended that the user choose a ligand density that gives a maximum analyte response (R_{max}) between 50-150 RU to give a sufficient signal-to-noise ratio to resolve kinetics while avoiding steric hindrance or mass transfer effects.
- Both Strep-Tactin XT and Strep VHH are compatible with a wide range of running buffers and buffer additives. Recommended buffers:
  - PBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-PBST)
  - HBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-HBST)
  - TBS-T* (0.1% Tween 20), pH 7.4 (ALTO-R-TBST)

**TBS-T should not be used in auxiliary buffer well for capture molecule immobilization.*

Method

Experimental setup

The experimental setup was completed remotely on Alto's Nicosystem™ user portal, followed by run initiation on the instrument:

1. From a laptop, the experiment was designed and saved in the Nicosystem.
2. On the Alto, the designed method was selected to launch Alto's on-screen setup guide.
3. An Alto 16-Channel Carboxyl Cartridge was placed in the instrument, and samples were loaded into the cartridge following the experiment setup guide.
4. The experiment was initiated on the Alto device by selecting "Run Method".

Alto assay protocol

The following steps were completed automatically by Alto with no operator supervision:

1. Carboxyl sensors were normalized with normalization solutions.
2. Carboxyl sensors were cleaned with 10 mM HCl for 60 s.
3. Carboxyl sensors were activated with 200 mM EDC/NHS for 600 s.
4. 5 µg/mL Strep-Tactin XT diluted in 10 mM sodium acetate, pH 4.5 or 5 µg/mL Strep VHH diluted in 10 mM MES, pH 6.0 was immobilized onto all sensors for 600 s.
5. All sensors were blocked with the 1 M ethanolamine for 300 s to quench any remaining active carboxyl groups.
6. All sensors were conditioned for 60 s with 10 mM glycine-HCl, pH 1.5.
7. GFP-TST in the running buffer (PBS-T) was introduced to each even-numbered active sensor for 300 s. GFP-TST was loaded at 20 nM when used on a Strep-Tactin XT surface and 10 nM when used on the Strep VHH surface.
8. Alto executed five automated HuCAL Fab-FH serial dilutions on the cartridge. Each sample was diluted from 100 nM stock, producing 0.411 nM, 1.23 nM, 3.74 nM, 11.1 nM, and 33.3 nM solutions in the running buffer.
9. The lowest HuCAL Fab-FH concentration was exposed to each sensor for 300 s, followed by dissociation in the running buffer for 600 s, and a 60 s regeneration step with 10 mM glycine-HCl, pH 1.5.
10. Steps 7 and 9 were repeated for the remaining four HuCAL Fab-FH analyte concentrations, which constitutes a full multi-cycle kinetics (MCK) round.

Alto data analysis

1. The test was opened under the analysis tab in the Nicosystem user portal.
2. The "Build Capture Surface" tab was opened to assess immobilization levels of the capture molecule across 4 lanes of the cartridge to ensure sufficient and/or optimal levels.
3. The "Raw data" tab was opened to ensure that the ligand capture levels and regeneration are sufficient and/or optimal.
4. The "Capture Kinetics" tab was opened and a 1:1 Langmuir binding model was automatically applied to the data.
5. Processing tools were used as required.
6. Final images and .CSV files were downloaded.

Results & Discussion

The capability of IBA Lifesciences' Strep-Tactin XT and Proteintech's Strep VHH to serve as capture surfaces in SPR assays on the Alto platform was evaluated. Two cartridges were used for this study, one per capture molecule, with the capture molecule immobilized on both the active and reference sensors. Figure 2 shows the immobilization of Strep-Tactin XT for one cartridge used in this study, with an average immobilization level of 1596 RU. Figure 3 shows the immobilization of the Strep VHH on the other cartridge used as part of this study, with an average immobilization level of 3578 RU. Table 1 summarizes the average binding response and standard deviations for both capture molecule immobilization and subsequent ligand capture. The ligand capture by Strep-Tactin XT of 20 nM GFP-TST averaged 132.7 RU and the ligand capture by the Strep VHH of 10 nM GFP-TST averaged 87.9 RU.

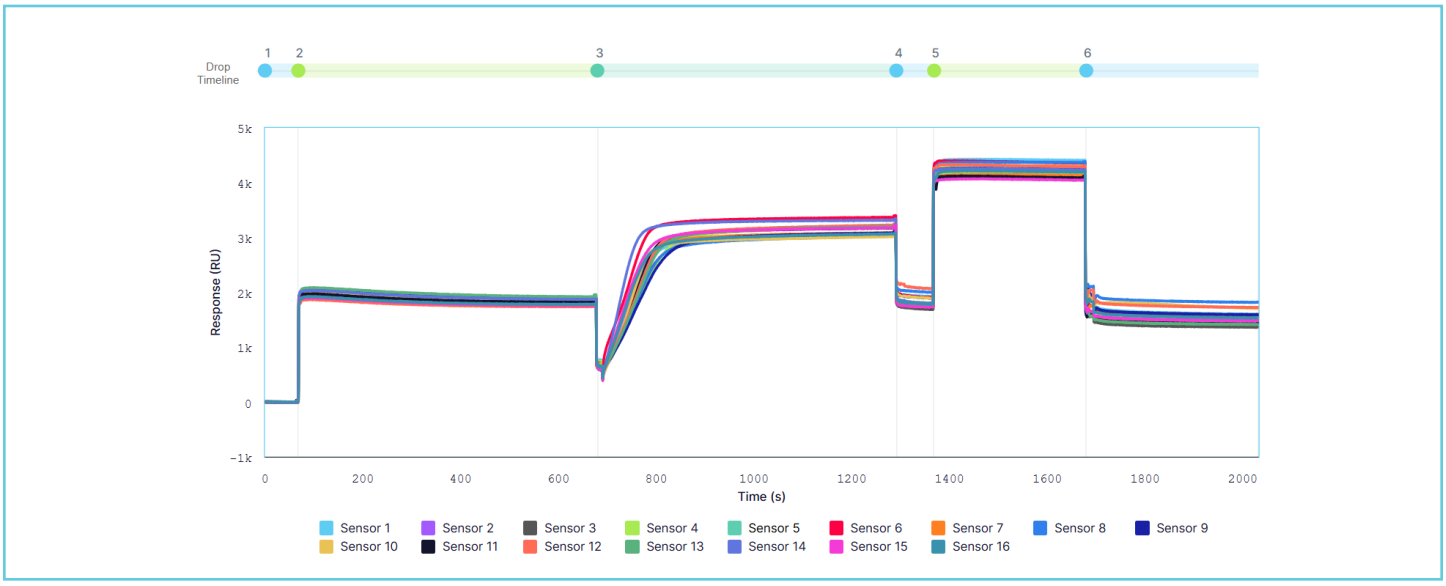

Figure 2: *Immobilization of Strep-Tactin XT on the Alto carboxyl cartridge. The capture surface building activity includes activation of all 16 sensors with 200 mM EDC and 200 mM NHS from Nicoya's Surfacing Kit for 600 s, followed by immobilization of 5 µg/mL Strep-Tactin XT in sodium acetate pH 4.5 for 600 s, and blocking of sensors with 1 M ethanolamine for 300 s.*

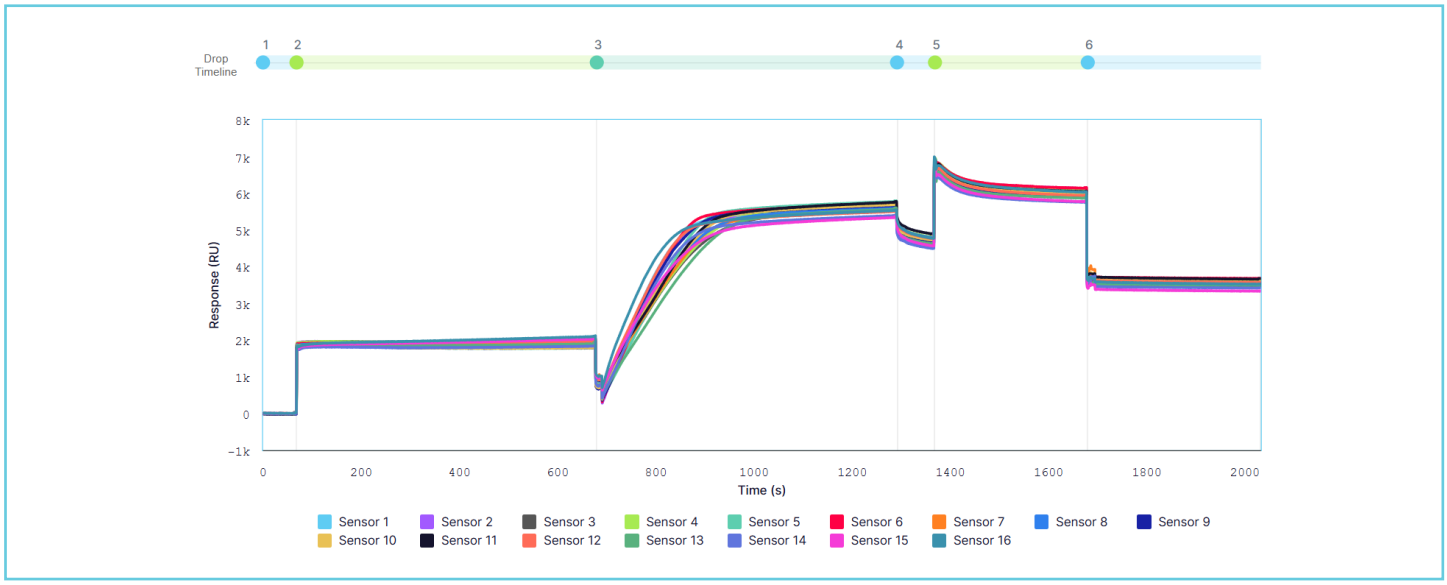

Figure 3: *Immobilization of Strep-tag VHH on the Alto carboxyl cartridge. The capture surface building activity includes activation of all 16 sensors with 200 mM EDC and 200 mM NHS from Nicoya's Surfacing Kit for 600 s, followed by immobilization of 5 µg/mL Strep-tag VHH in MES pH 6.0 for 600 s, and blocking of sensors with 1 M ethanolamine for 300 s.*

Capture molecule	Strep-Tactin XT Immobilization	GFP-TST capture by Strep-Tactin XT	Strep VHH Immobilization	GFP-TST capture by Strep VHH
Average (RU)	1596	132.7	3578	87.9
Standard deviation (RU)	133	18.5	91	8.7
% CV (n=16)	3.7	13	2.5	9.9

Table 1: *Average immobilization and ligand capture levels for Strep-Tactin XT and Strep VHH.*

To evaluate the performance of Strep-Tactin XT and Strep VHH in kinetic experiments, MCK assays were performed to measure the kinetics of a HuCAL recombinant antibody binding to captured GFP-TST. The recapture of the ligand was consistent across all five analyte concentrations and regenerations as shown in Figure 4A for Strep-Tactin XT and in Figure 4B for Strep VHH in Figure 4B. These figures also demonstrate the low susceptibility of non-specific binding (NSB) to the capture surfaces as evidenced by the minimal response in the reference channels for the HuCAL analyte. Both capture surfaces were fully regenerated with 10 mM glycine-HCl, pH 1.5, demonstrating their reusability for multiple binding cycles. The ligand, GFP-TST, shows minimal dissociation following binding to both Strep-Tactin XT and Strep VHH, highlighting the rigidity of both capture surfaces.

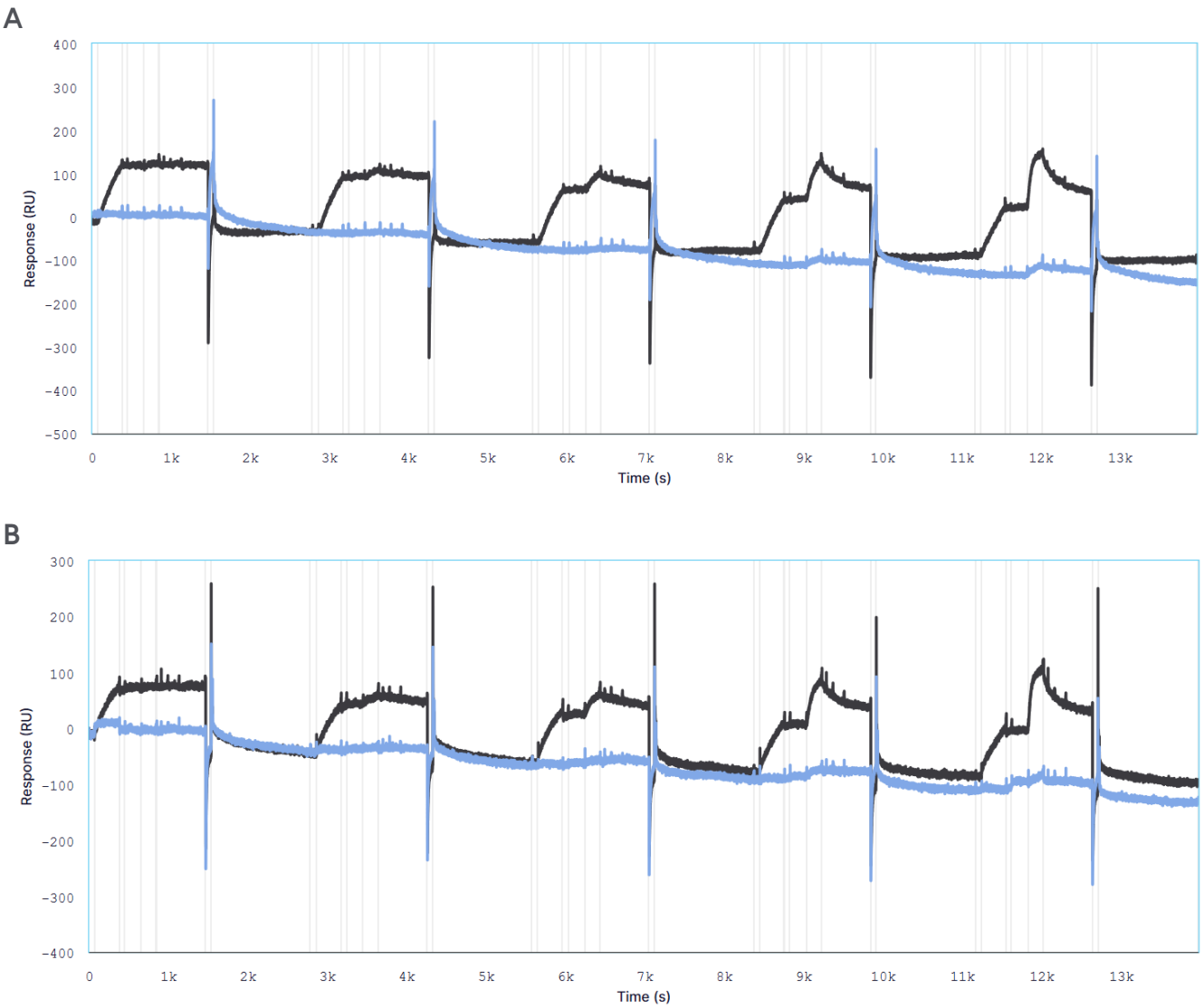

Figure 4: *Raw sensorgrams from a multi-cycle kinetic (MCK) experiment showing HuCAL Fab-FH binding to GFP-TST captured by A) Strep-Tactin XT or B) Strep VHH. In each of the five cycles, GFP-TST was captured on the sensor, followed by a single HuCAL Fab FH concentration (412 pM, 1.23 nM, 3.74 nM, 11.1 nM, and 33.3 nM, in order of increasing concentration). Each cycle included: GFP-TST capture (900 s), buffer baseline (150 s), analyte association (180 s), dissociation (600 s), and regeneration with glycine-HCl, pH 1.5 (60 s) to remove both ligand and analyte. Sensorgrams were used to generate corrected data for analysis using the Nicosystem software.*

Kinetic values were calculated based on the sensorgrams obtained on one cartridge with Alto, tested across 2 rounds (n=16). Both assays demonstrated excellent reproducibility across all replicates. A representative example of a sensorgram is shown in Figure 5 for Strep-Tactin XT and Figure 6 for the Strep VHH. The data were fit to a Langmuir 1:1 binding model analyzed in the Nicosystem software and kinetic parameters are reported in Table 2 for both capture surfaces. From the kinetic analysis, when Strep-Tactin XT was used as the capture molecule, association and dissociation rate constants (k_a and k_d) were determined to be 6.29 x 10^5 $M^{-1}s^{-1}$ and 4.21 x 10^{-3} s^{-1}, respectively, resulting in a K_D of 6.93 nM. When the Strep VHH was used as the capture molecule, association and dissociation rate constants (k_a and k_d) were determined to be 3.85 x 10^5 $M^{-1}s^{-1}$ and 2.37 x 10^{-3} s^{-1}, respectively, resulting in a K_D of 6.78 nM.

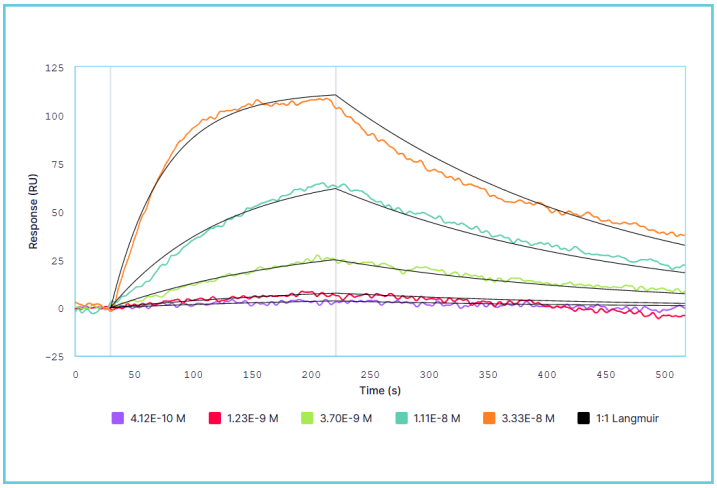

Figure 5: *Representative sensorgram showing the binding of HuCAL to GFP-TST that is captured by Strep-Tactin XT in an MCK format. Image was generated by Nicosystem analysis software.*

Regeneration

Surface regeneration in SPR involves the removal of non-covalently bound reagents from the sensor and restoring it for subsequent binding events. The choice of regeneration solution must be optimized for each specific interaction.

An ideal regeneration solution is strong enough to fully remove the ligand and analyte but not harsh enough to damage the capture surface. As shown in Figure 4, each regeneration step results in a sharp change in signal that returns it to the same baseline position as before the ligand capture step. This is indicative of a successful regeneration. Glycine-HCl, pH 1.5 (ALTO-R-GLYHCl-1.5) is the recommended regeneration solution for Strep-Tactin XT and Strep-tag VHH capture of Twin-Strep-tagged molecules.

Several regeneration solutions are available in Nicoya's Regeneration Optimization Kit (ALTO-R-REGEN-OPT), for those who wish to find the best reagent that suits their binding interaction.

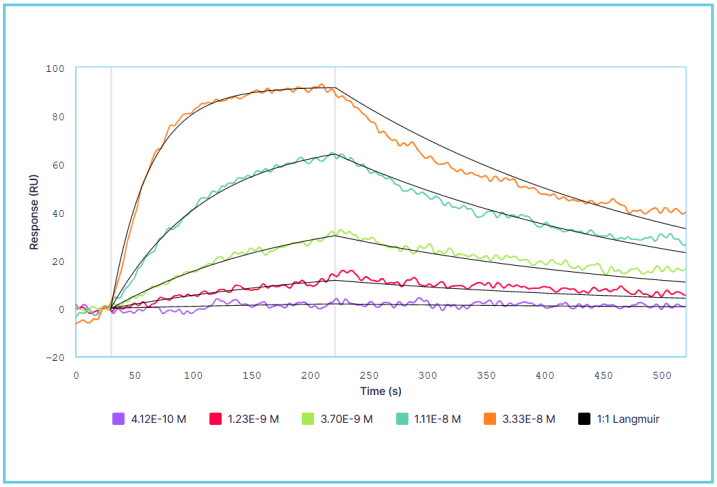

Figure 6: *Representative sensorgram showing the binding of HuCAL to GFP-TST that is captured by the Strep-tag VHH in an MCK format. Image was generated by Nicosystem analysis software.*

Capture molecule	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
Strep-Tactin XT (n=16)	6.29 x 10^5 ± 1.23 x 10^4	4.21 x 10^{-3} ± 3.59 x 10^{-4}	6.93 ± 1.34
Strep VHH (n=16)	3.85 x 10^5 ± 1.19 x 10^4	2.37 x 10^{-3} ± 3.74 x 10^{-4}	6.78 ± 2.62

Table 2: *Kinetic parameters measured for HuCAL Fab-FH using each capture molecule.*

References

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