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APPLICATION NOTE

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NiHC200M Biosensor Kinetic Assays on the Carterra® LSA®

This Application Note was developed in collaboration with Octapharma Biopharmaceuticals GmbH, Heidelberg, Germany.

Key Takeaways

- Perform kinetic screening using high-specificity capture of His-tagged proteins
- Benefit from low sample consumption to run experiments
- Characterize either crude or purified nanobody samples on the LSA

Overview

The polyhistidine-tag[1] is one of the most popular affinity tags for purification and detection of recombinant proteins. The histidine residues of the tag form coordination bonds with immobilized metal ions such as nickel, cobalt and zinc. This property is exploited for the stable capture of His-tagged protein. The NiHC200M biosensor is pre-derivatized with poly-Ni- NTA (nitrilotriacetic) groups for quick and easy immobilization of His-tagged molecules. In combination with the LSA instrument, the NiHC200M biosensor offers a fast and label-free approach for high-throughput screening and quantitation of His-tagged protein libraries.

In this application note, we describe a generic approach for the kinetic screening of His-tagged ligand using a NiHC200M biosensor. The presented protocol can be used as a starting point and can be adapted according to the properties of the sample.

Principle

The biosensor with pre-immobilized and nickel (Ni 2+) charged poly-NTA derivatized linear polycarboxylate hydrogel will specifically bind His-tagged ligand proteins. This approach can be adopted for the capture of purified or crude samples in quantitation and kinetic applications. Dissociation of the ligand and surface regeneration is achieved by the addition of a competitive ligand (imidazole) followed by the injection of a chelating agent (EDTA). Once the biosensor is regenerated, nitrilotriacetic groups are recharged with a solution of $NiCl_2$ prior to the capture of the next set of ligand proteins.

Experimental Conditions

Running buffer: HBST/PBST. Running buffer must not contain any chelating agent like EDTA/EGTA that would affect the stability of the His-tag/ Ni^{2+}/NTA complex.

Biosensor conditioning/regeneration: Sequential injection of imidazole 250 mM in ddH2O (2x2 minutes injection), 500 mM EDTA in ddH2O (2x2 minutes injection), 10 mM $NiCl_2$ in ddH2O (1x2 minutes injection).

Ligand capture: Ligands were prepared at a wide range of concentrations in running buffer **(Table 1)**, with a final volume 250 µL and captured on the surface for 15 minutes.

Antigen Binding Kinetics: Kinetic injections consisted of an eight-point three-fold analyte dilution series starting at 500 nM, with a 4 minute association and 10 minute dissociation. Fourteen injections were included (6 buffer injections + 8 antigen injections).

	Purified (Conc. ng/mL)						Periplasmic Protein Extracts (Dil. ratio)					
	1	2	3	4	5	6	7	8	9	10	11	12
A	NB_A (50ng/mL)	NB_A (33ng/mL)	NB_A (22ng/mL)	NB_A (15ng/mL)	NB_A (10ng/mL)	NB_A (6.5ng/mL)	NB_A (1:500)	NB_A (1:1000)	NB_A (1:2000)	NB_A (1:4000)	NB_A (1:8000)	NB_A (1:16000)
B	NB_B (50ng/mL)	NB_B (33ng/mL)	NB_B (22ng/mL)	NB_B (15ng/mL)	NB_B (10ng/mL)	NB_B (6.5ng/mL)	NB_B (1:500)	NB_B (1:1000)	NB_B (1:2000)	NB_B (1:4000)	NB_B (1:8000)	NB_B (1:16000)
C	NB_C (50ng/mL)	NB_C (33ng/mL)	NB_C (22ng/mL)	NB_C (15ng/mL)	NB_C (10ng/mL)	NB_C (6.5ng/mL)	NB_C (1:500)	NB_C (1:1000)	NB_C (1:2000)	NB_C (1:4000)	NB_C (1:8000)	NB_C (1:16000)
D	NB_D (50ng/mL)	NB_D (33ng/mL)	NB_D (22ng/mL)	NB_D (15ng/mL)	NB_D (10ng/mL)	NB_D (6.5ng/mL)	NB_D (1:500)	NB_D (1:1000)	NB_D (1:2000)	NB_D (1:4000)	NB_D (1:8000)	NB_D (1:16000)

Table 1: 96-well His-tagged nanobody ligand plate for kinetic assay

Results

The Carterra LSA performs high-throughput (HT) SPR analysis. Its one-on-many format enables screening of up to 384 interactions in parallel to generate high resolution binding kinetics results. The example below demonstrates the direct comparison of the capture of various concentrations of His-tagged nanobodies from both purified and crude samples from a periplasmic extract onto a NiHC200M biosensor. The results demonstrate capture of the ligands over a wide concentration range of sample.

Capture Results Examining His-Tagged Nanobodies

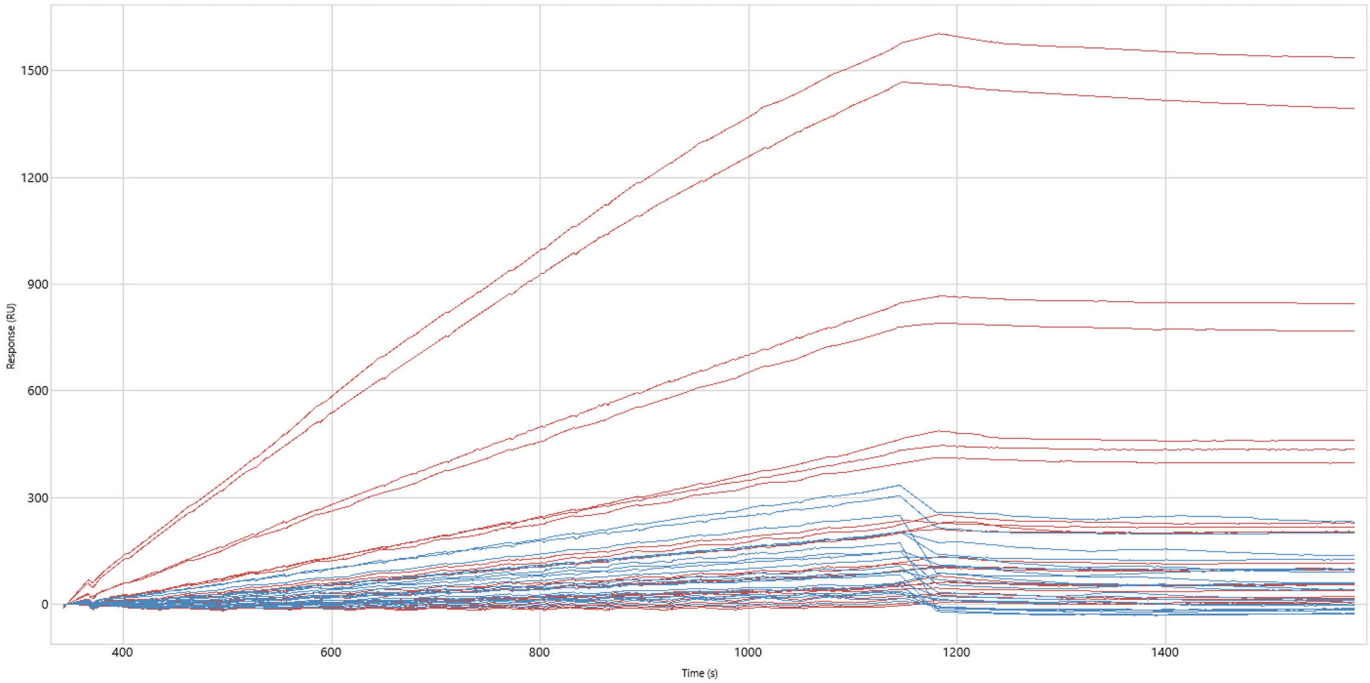

Figure 1: Capture of the purified and crude His-tagged nanobodies on NiHC200M surface in blue for the purified samples and red for the crude samples. Capture levels range from 0 to 1500 RU. Each ligand was captured at six different dilutions creating spots with different densities of each nanobody.

The use of multiple replicates at different densities across the array suggests that the apparent kinetic rate and affinity constants of each antigen/nanobody interaction could be reported with statistical confidence. Testing mass transport limitations and rebinding influences on generated binding kinetics can be examined by direct comparison of goodness of the fits. Also, the values of kinetic parameters determined for the different replicates and surface densities can be obtained by measuring replicate samples in the LSA.

Antigen Binding Kinetics

The capture kinetic experiment was performed on a nanobody panel as shown in **Figure 2**. These results highlight the efficiency of the His-capture approach in combination with the flow printing technology to extract ligands from low concentration sample solutions and to retain them tightly on the surface, which is necessary for the characterization of binding kinetics. His-tag proteins should be evaluated for the stability of the NiNTA capture, as His-tag exposure can vary from construct to construct and ligand drift can make the fit of kinetic data tedious or impossible and lead to distorted results.

Additionally, the possibility to analyze up to 384 clones in parallel enables low analyte consumption and is very time efficient. Each antigen concentration only requires a single 270 µL volume to be utilized to collect kinetic data for the entire array of ligands.

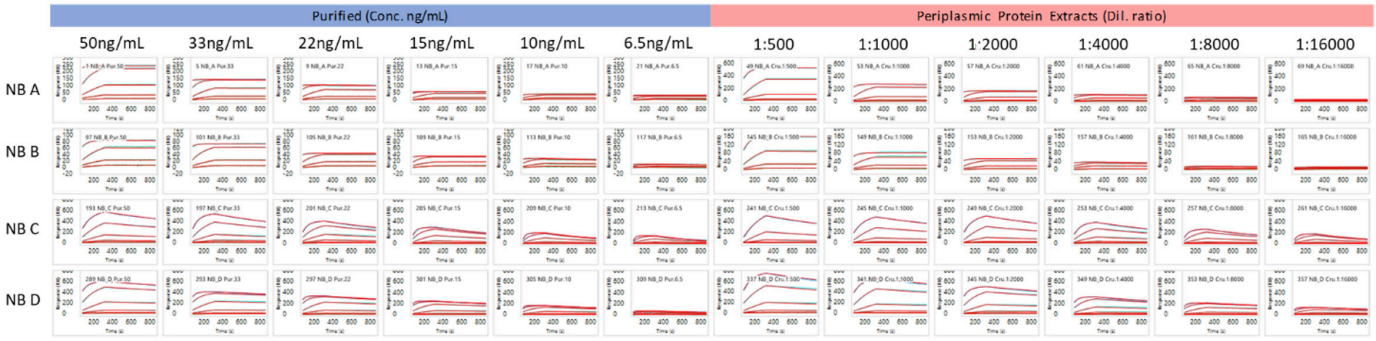

Figure 2: Ligand array view of kinetic data. Capture kinetics was performed on 24 ligands simultaneously using the LSA. The array comprised 4 unique clones provided as purified and crude sample, each captured onto 12 individual locations of an NiHC200M chip. The specific monomeric antigen was injected as analyte from 228pM to 500nM over the entire ligand set.

The binding kinetics obtained using the purified and crude nanobody samples are in good agreement **(Figure 3)**. This demonstrates the reliability and accuracy of HT-SPR on the LSA to produce high quality kinetics data not only from purified protein but also from crude samples. Note that a k_d value of $1x10^{-5}$ s^{-1} was used as a limit in the analysis for clones showing barely any detectable dissociation within the allowed dissociation phase (of 10 minutes). For the accurate estimate of slower dissociation rates much longer dissociation phases are needed to obtain a sufficient signal decay for calculation. Two of the characterized clones (clones NB A and NB B) display very slow dissociation with their k_d reaching the limit of $1x10^{-5}$ s^{-1} demonstrating that the His-capture exhibits a sufficient binding stability to allow the characterization of high affinity clones. Noteworthy, His capture efficiency and stability can vary between different constructs and is known to be influenced, e.g. by the length of the His-tag[2,3].

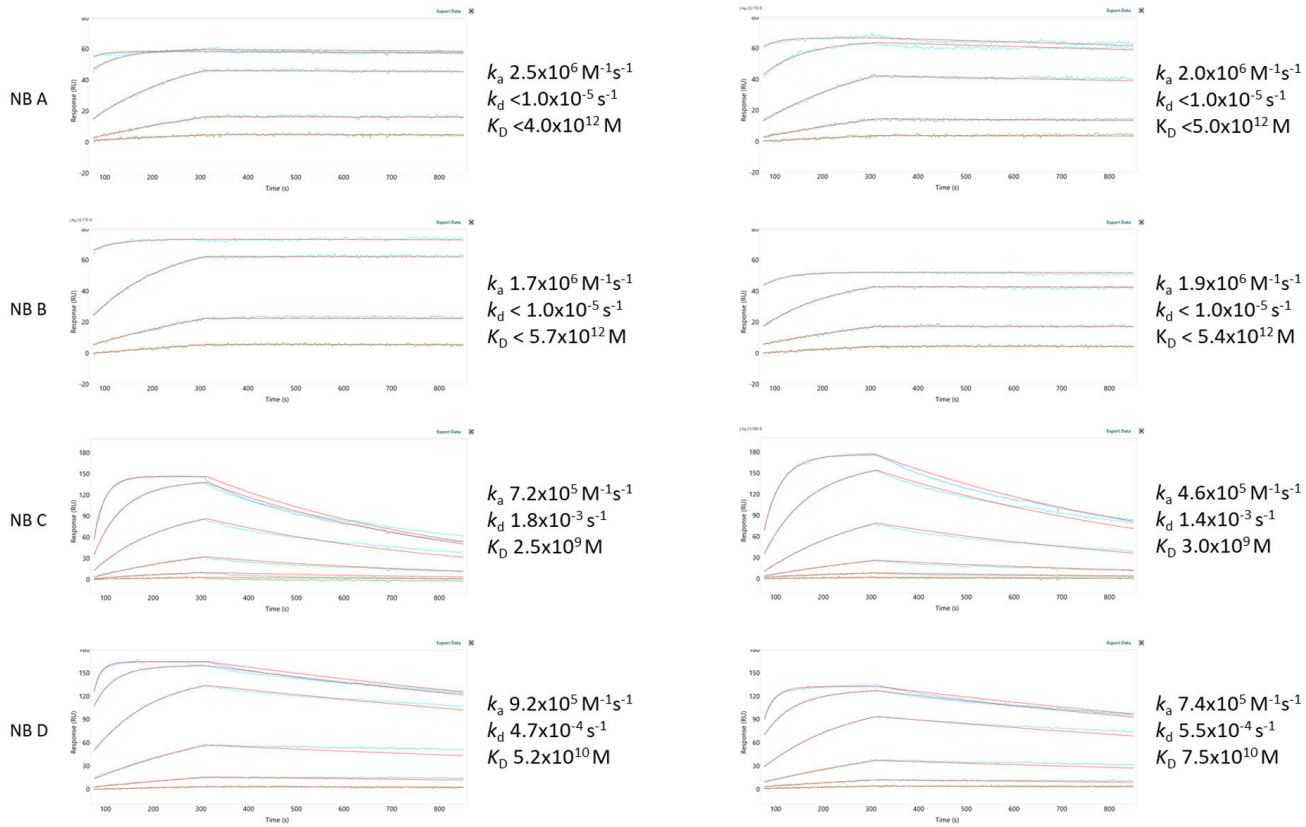

Figure 3: Representative antigen binding profiles of the captured nanobodies. 1:1 kinetic model fit (red) overlays the sensorgrams. The purified nanobodies are shown on the left side while binding curves for the crude nanobodies are displayed on the right side. The overlaid red lines represent the kinetic fit of the binding response signals at different analyte concentrations to a 1:1 interaction model. The best fit value of the rate constant of association (k_a) and dissociation (k_d) as well as the equilibrium dissociation constant (K_D) are reported at the right side of each sensorgram set.

Summary

The NiHC200M chip is an optimal solution for the capture of His-tagged proteins. The stability of the capture as well as the possibility to fully regenerate the ligand make this biosensor an ideal choice for the reliable and accurate characterization of binding kinetics for His-tagged ligands. In combination with the high-throughput flow printing technology of the LSA that uses bi-directional sample delivery across spots on a chip surface to efficiently capture ligands even from low titer samples, it constitutes an ideal tool for the kinetic screening of His-tagged ligand libraries. The possibility to capture from crude production medium and the low sample consumption of the LSA perfectly comply with the requirements for early lead discovery projects. Additionally, the analysis of these large data sets is quick and easy using Carterra's powerful and intuitive Kinetics software, with a typical analysis taking only a few minutes and providing sophisticated tools for visualization and data summary plots.

References

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2. Hemdan ES1, Zhao YJ, Sulkowski E, Porath J. Surface topography of histidine residues: a facile probe by immobilized metal ion affinity chromatography. *Proc Natl Acad Sci USA*. 1989;86(6):1811-5.
3. Terpe, K. (2003) Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems, *Appl. Microbiol. Biotechnol.*, 60, 523533.

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