

# NiHC: High affinity poly-Ni-NTA sensor chips for fragment based drug discovery<sup>1</sup>

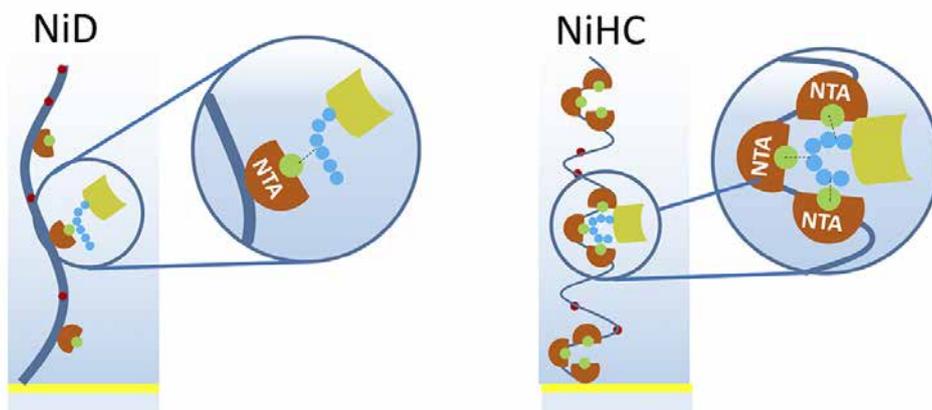
*Newsletter I 2019 (Update 05/2021)*

Fragment-based drug discovery (FBDD) has become a preferred alternative to high-throughput screening (HTS) to improve the discovery of small-molecule drug candidates. Screening of low-molecular-weight fragments can identify hit compounds with better efficiency and physiological profiles than HTS<sup>2</sup>.

SPR biosensor technology is one of the primary biophysical methods to screen fragment libraries<sup>3</sup> as newer instruments achieve sufficiently high signal-to-noise ratios to generate reliable data despite the low molecular weight and low affinity of many analytes.

In previous approaches to establish FBDD assays using SPR, the ligand was covalently immobilized on the sensor surface with high immobilization levels to ensure that the protein bound stably to the sensor chip surface. Alternatively, biotinylated proteins were immobilized on streptavidin coated sensor surfaces with the inherent drawback that the analyte could non-specifically interact with the streptavidin. Both immobilization methods lack the possibility to remove the bound ligand from the sensor surface which is critical, for example, when working with GPCRs or other sensitive proteins which often denature over long screening campaigns.

XanTec bioanalytics specializes in manufacturing high quality sensor chips compatible with all major surface plasmon resonance (SPR) instrument brands on the market. Through consistent research, XanTec has developed the widest portfolio of sensor chips available today, offering tailor-made solutions for almost any application.



A) Rigid carboxymethylated dextran hydrogel with single NTA groups with immobilized His<sub>6</sub>-tagged fusion protein.

B) Flexible HC hydrogel forming poly-NTA chelating "cages" for high affinity immobilization of His<sub>6</sub>-tagged fusion protein.

Figure 1. Comparison between NiD (dextran based) and NiHC (linear polycarboxylate based sensor chip coating for reversible immobilization of His<sub>6</sub>-tagged biomolecules.

To make this group of sensitive molecules accessible to FBDD, various attempts have been made to immobilize them reversibly via affinity based His<sub>6</sub>/nickel-nitrilotriacetic acid (NTA) coupling (Fig. 1). State-of-the-art NTA sensor chips are based on carboxymethyl dextran (CMD) hydrogel modified with NTA groups. Because of the relatively low affinity between the His<sub>6</sub>-tag and the Ni<sup>2+</sup>-NTA-complex, continuous dissociation of the ligand from the sensor chip surface leads to unwanted baseline drifts. Such drift effects can easily exceed the specific signal when screening small molecules and thus represent a major problem.

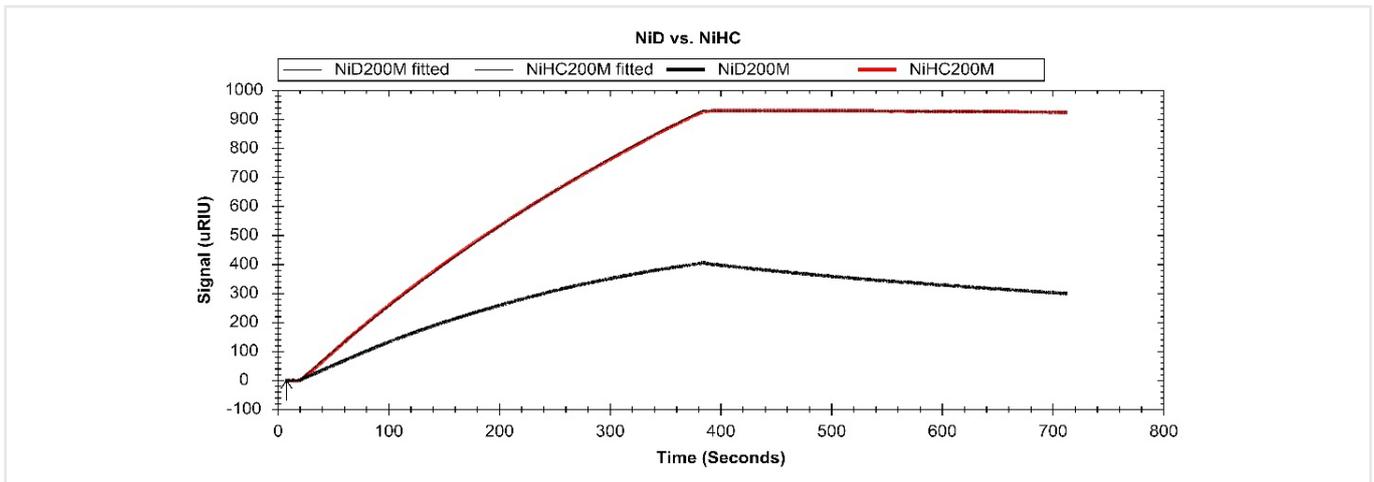


Figure 2. Overlay plot of two sensorgrams comparing immobilization capacity and stability of His<sub>6</sub>-tagged fusion protein (59.7 kDa; AbCam #ab52213) on NiD and NiHC sensor chips. Both interactions were fitted based on a diffusion corrected 1:1 binding model. The immobilization on NiHC shows an approx. 200-fold higher stability compared to the immobilization on a NiD sensor chip.

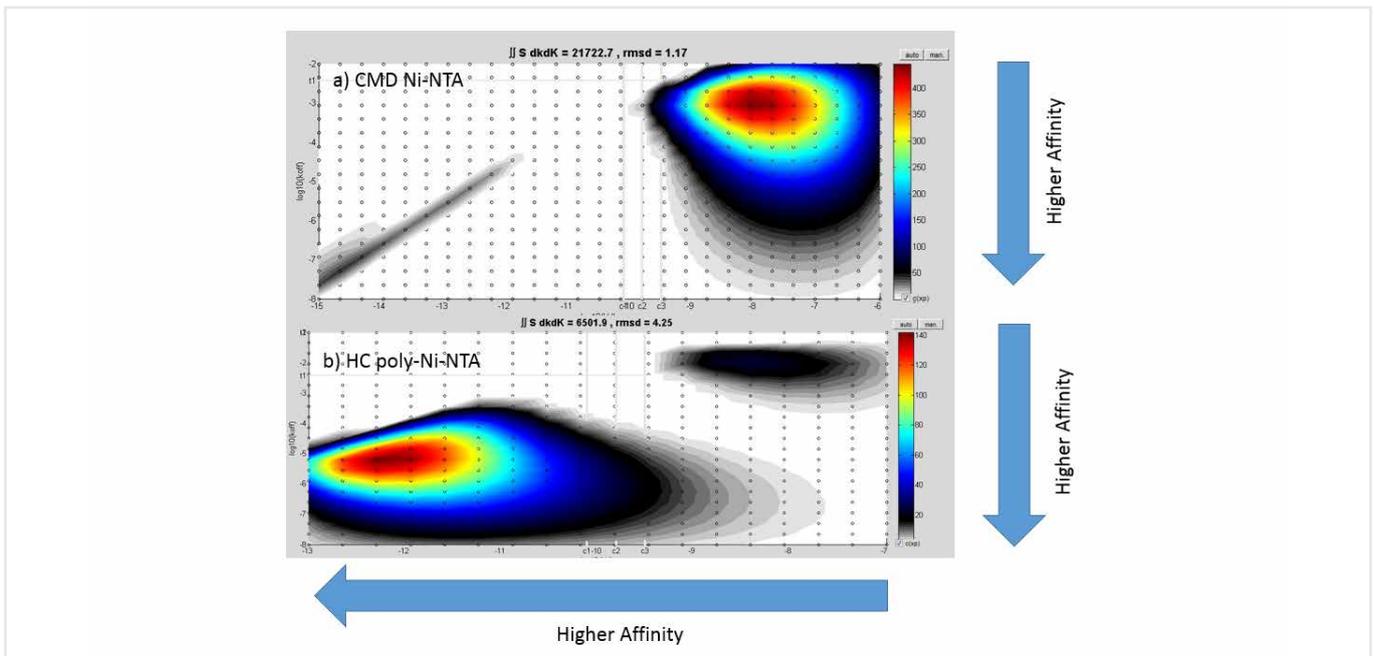


Figure 3. Interaction/affinity map of His<sub>6</sub>-tagged protein A/G fusion protein with mono-NTA derivatized carboxymethyl dextran (top) and XanTec's poly-NTA sensor chip (bottom). Increasing affinity from the upper right corner to the lower left corner. The affinity map was calculated by association/dissociation rate constants using EvilFit<sup>5</sup>.

To account for this well-known disadvantage, surface chemists at XanTec developed a poly-NTA sensor chip hydrogel coating based on a strongly hydrated and very flexible polycarboxylate polymer backbone. Compared to the standard CMD-NTA chemistry, these new coatings, which are available in 30, 200, 1000 and 1500 nm thickness, can improve the stability of captured His<sub>6</sub>-tagged ligands by 2-3 magnitudes, matching the high affinity of the recently-developed Tris-NTA<sup>4</sup>.

Figures 2 and 3 are showing the much higher stability of a captured His<sub>6</sub>-tagged ligand (a protein A/G fusion protein) on XanTec's poly-NTA surface NiHC1000M than on NTA-derivatized CMD hydrogel (mono-NTA). Despite the high affinity of this surface, the regeneration conditions (EDTA) are mild, and identical to those of the standard mono-NTA sensor chips.

The effective use of polydentate high-affinity Ni-NTA surfaces in SPR measurements of small molecules is vividly demonstrated in a publication by Byun et al.<sup>6</sup>. A direct binding assay was used to investigate the kinetics of cGMP (345 Da) and a cGMP analog (8-NBD-cGMP, 605 Da) binding to the catalytic domain of *Plasmodium falciparum* (PfPKG), the malaria-associated parasite. Even at high immobilization capacities, the XanTec bioanalytics high-affinity NiHC NTA sensor chip used showed no leaching effects, so R<sub>max</sub> values well below 10 RU could be evaluated (Fig. 4).

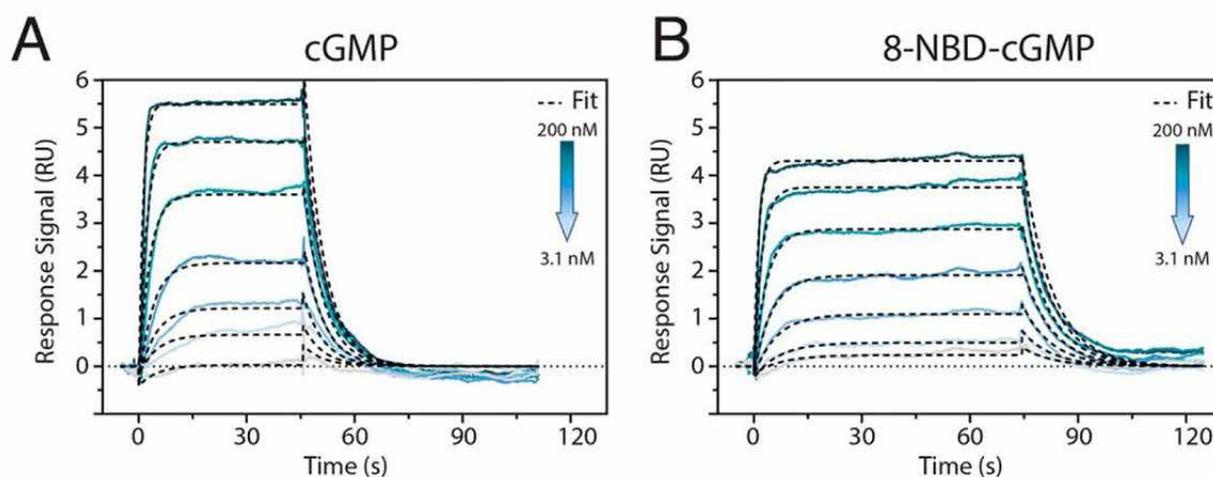


Figure 4. Surface plasmon resonance analysis of binding of cGMP and 8-NBD-cGMP to the catalytic domain of PfPKG (residues 401–853) demonstrates similar binding kinetics. Direct binding assays were performed on a Biacore™ by capturing polyhistidine-tagged PfPKG 401–853 on a XanTec bioanalytics high-affinity NiHC NTA sensor chip and injecting a dilution series of cGMP (345 Da) for 45 s (A) or 8-NBD-cGMP (605 Da) for 70 s (B). A Langmuir 1:1 model was applied for data fitting.

## Conclusion

With XanTec's unique poly-NTA sensor chips (NiHC group) it is possible to establish higher immobilization levels compared to NTA-derivatized carboxymethyl dextran with the additional benefit of drastically reduced leaching, resulting in a practically drift-free baseline. This allows repeated immobilization of sensitive ligands during extended FBDD campaigns, as NiHC chip surfaces are fully regenerable over many interaction cycles.

## References

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