NiHC: High affinity poly-Ni-NTA sensor chips for fragment based drug discovery[1]

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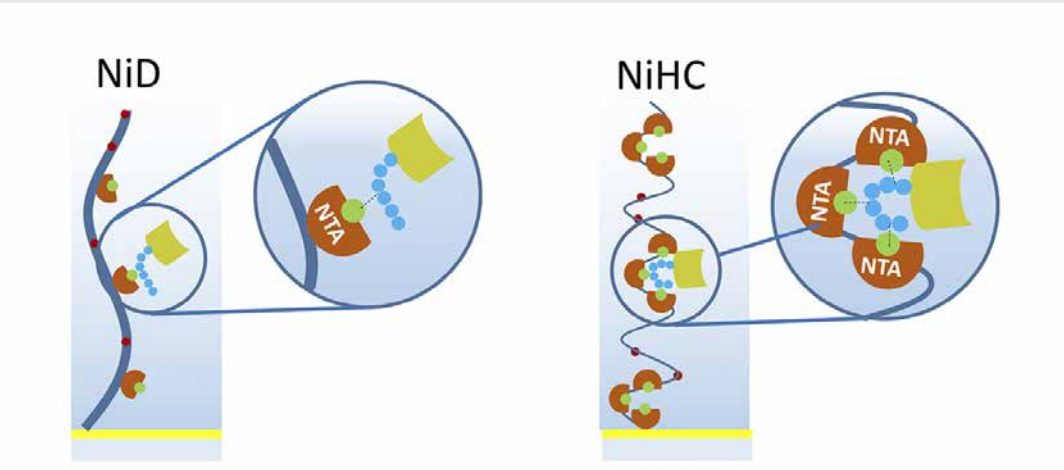
Fragment-based drug discovery (FBDD) has become a preferred alternative to high-throughput screening (HTS) to improve the discovery of small-molecule drug candidates. Screening of low-molecular-weight fragments can identify hit compounds with better efficiency and physiological profiles than HTS[2].

SPR biosensor technology is one of the primary biophysical methods to screen fragment libraries[3] 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.

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.

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.



A) Rigid carboxymethyled dextran hydrogel with single NTA groups with immobilized His_6-tagged fusion protein.

B) Flexible HC hydrogel forming poly-NTA chelating "cages" for high affinity immobilization of His_6-tagged fusion protein.

Figure 1. Comparison between NiD (dextran based) and NiHC (linear polycarboxylate based sensor chip coating for reversible immobilization of His_6-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_6/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_6-tag and the Ni^{2+}-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.

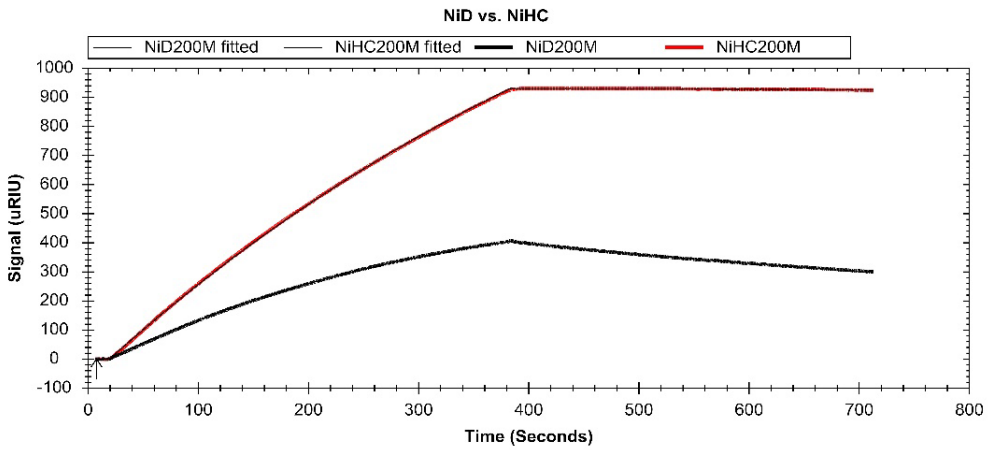


Figure 2. Overlay plot of two sensorgrams comparing immobilization capacity and stability of His_6-tagged fusion protein (59.7 kDa; AbCam #ab52213) on NiD and NiHC sensor chips. Both interactions were fitted based on a diffusion corrected 1:1 binding model. The immobilization on NiHC shows an approx. 200-fold higher stability compared to the immobilization on a NiD sensor chip.

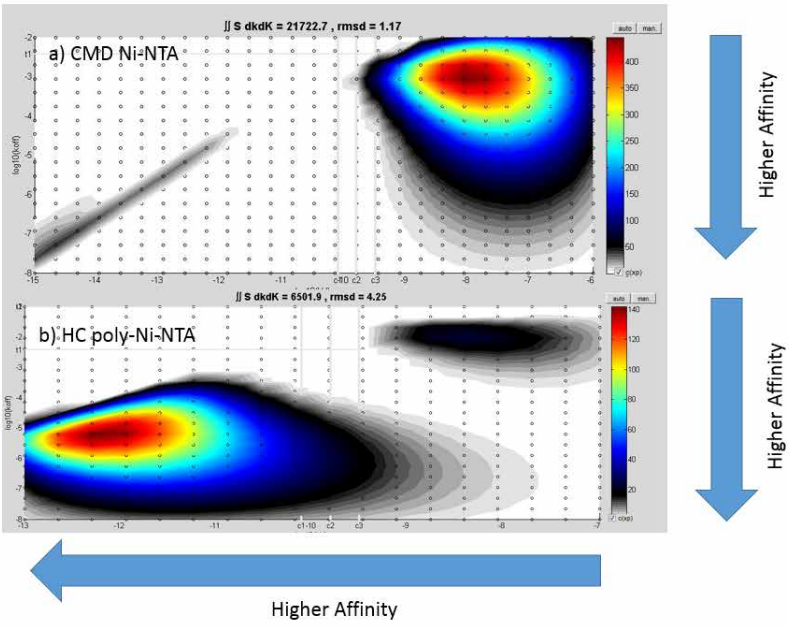


Figure 3. Interaction/affinity map of His_6-tagged protein A/G fusion protein with mono-NTA derivatized carboxymethyl dextran (top) and **XanTec's** poly-NTA sensor chip (bottom). Increasing affinity from the upper right corner to the lower left corner. The affinity map was calculated by association/dissociation rate constants using EvilFit[5].

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_6-tagged ligands by 2-3 magnitudes, matching the high affinity of the recently-developed Tris-NTA[4].

Figures 2 and 3 are showing the much higher stability of a captured His_6-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.

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

1. *1. Faoro, C., Wilkinson-White, L., Kwan, A. H., & Ataide, S. F. (2018). Discovery of fragments that target key interactions in the signal recognition particle (SRP) as potential leads for a new class of antibiotics. PloS One, 13(7), e0200387.*
2. *2. Retra, K., Irth, H., & van Muijlwijk-Koezen, J. E. (2010). Surface Plasmon Resonance biosensor analysis as a useful tool in FBDD. Drug Discovery Today: Technologies, 7(3), e181-e187.*
3. *3. Shepherd, C. A., Hopkins, A. L., & Navratilova, I. (2014). Fragment screening by SPR and advanced application to GPCRs. Progress in Biophysics and Molecular Biology, 116(2-3), 113-123.*
4. *4. Lata, S., Reichel, A., Brock, R., Tampé, R., & Piehler, J. (2005). High-affinity adaptors for switchable recognition of histidine-tagged proteins. Journal of the American Chemical Society, 127(29), 10205-10215.*
5. *5. Gorshkova, I. I., Svitel, J., Razjouyan, F., & Schuck, P. (2008). Bayesian analysis of heterogeneity in the distribution of binding properties of immobilized surface sites. Langmuir, 24(20), 11577-11586.*

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