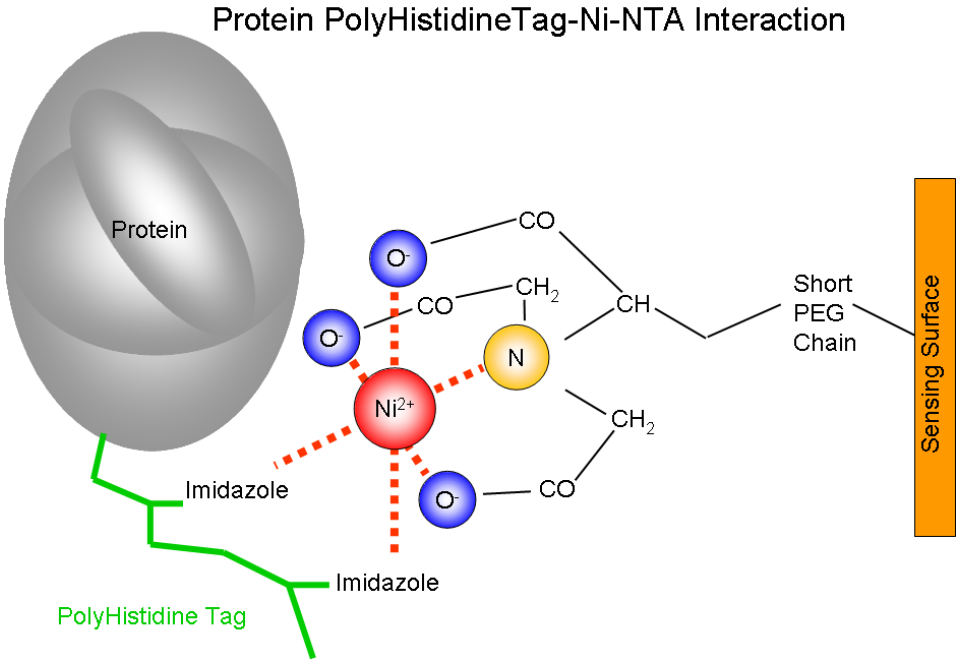
AFFINITY CAPTURE SURFACE FOR HISTIDINE-TAGGED RECOMBINANT PROTEINS (HisCap BIOSENSOR)

TECHNICAL NOTES

The SensíQ HisCap biosensor makes stable, reversible capture of polyhistidine-tagged proteins for surface plasmon resonance (SPR) experiments straightforward and swift. The baselines obtained with these immobilized proteins are stable enough for kinetic experiments, and the protein capture capacity is high enough for low molecular weight analytes such as fragments. The HisCap biosensor:

- Provides a convenient means of directed immobilization of his-tagged proteins [i], [ii].
- Is a suitable alternative for proteins that are not amenable to amine coupling.

The HisCap biosensor employs the long-established nitriloacetic acid (NTA) – nickel technique for protein attachment developed by Hoffman-LaRoche. In this technique, the imidazole side-chain of histidine in the protein of interest co-ordinate with surface-attached NTA-nickel complexes as shown in the diagram below. This technique is highly effective provided the protein has sufficient histidines – six in a typical histidine tag.



[i] Gershon PD, Khilko S. (1995) Stable chelating linkage for reversible immobilization of oligohistidine tagged proteins in the BIAcore surface plasmon resonance detector. J Immunol Methods 183:65-76

[ii] O'Shannessy DJ, O'Donnell KC, Martin J, Brigham-Burke M. (1995) Detection and quantitation of hexa-histidine-tagged recombinant proteins on western blots and by a surface plasmon resonance biosensor technique. Anal Biochem 229:119-24

HisCap Biosensor Advantages

- Protein production with His-tags is a long-established technology standard in labs that carry out recombinant protein work.

  → The HisCap biosensor is an obvious solution for immobilization of the abundant his-tagged proteins already available in today's research labs.

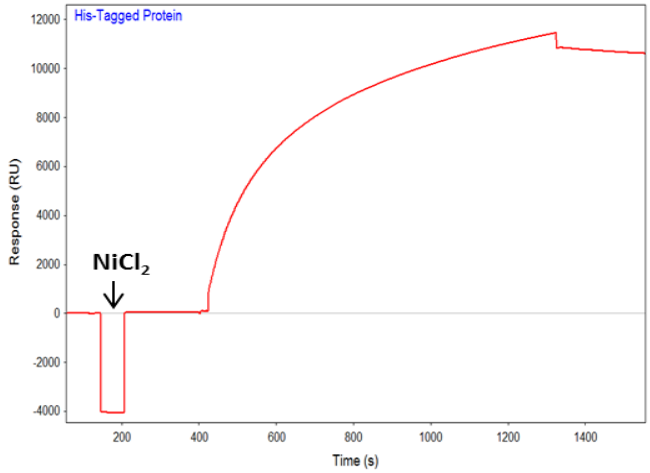
- Capture of his-tagged proteins using the HisCap biosensor provides a stable baseline

  → A stable baseline facilitates kinetic analysis of interactions; while drifting baselines can make kinetic analysis difficult if not impossible.

- The biosesnor can be regenerated using a variety of conditions, e.g. imidazole, SDS, or EDTA.

- The HisCap biosensor can be reused.

HisCap Biosensor Protein Capture Procedure



As shown in the above response curve, the user activates the biosensor by injecting nickel chloride: the nickel ions coordinate with the surface NTA residues. A his-tagged protein is subsequently injected for specific capture. EDTA or imidazole may be used to competitively reverse the interaction thereby restoring the original uncoated surface. The HisCap biosensor provides a platform for efficient, reproducible immobilization of his-tagged proteins. Most proteins captured establish a stable baseline suitable for kinetic analysis experiments.

Experimental Tip

We have found that non-specific binding to the electronegative NTA surface can be greatly reduced by blocking with a polyhistidine peptide or with a 6xHis tagged protein unrelated to the interaction of interest.

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