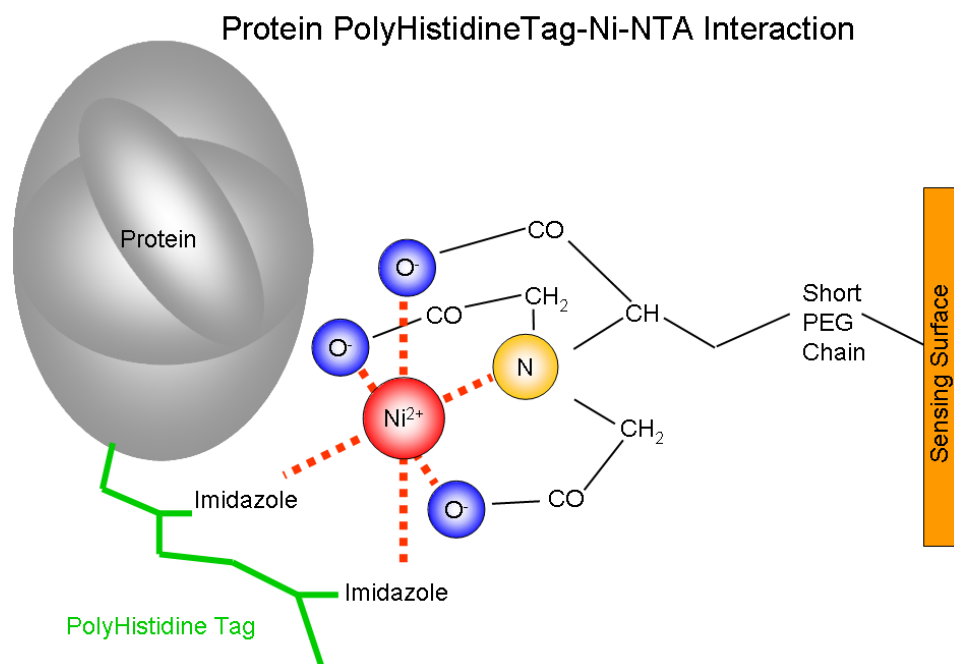


AFFINITY CAPTURE SURFACE FOR HISTIDINE-TAGGED RECOMBINANT PROTEINS (HisCap and HisHicap CHIPS)

The ICx Nomadics HisCap chip makes stable, reversible immobilization of polyhistidine-tagged proteins for surface plasmon resonance (SPR) experiments straightforward. The baselines obtained with these immobilized proteins are stable enough for kinetic experiments. The HisCap chip:

- Provides a convenient means of directed immobilization of his-tagged proteins ^{i, ii}.
- Also works reasonably well for any protein with sufficient numbers of histidine residues.

The HisCap chip employs the long-established nitriloacetic acid (NTA) – nickel technique for protein attachment developed by Hoffman-LaRoche ⁱⁱⁱ. 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 above diagram. This technique is highly effective provided the protein has sufficient histidines – six in a typical histidine tag but as few as three may be sufficient.



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

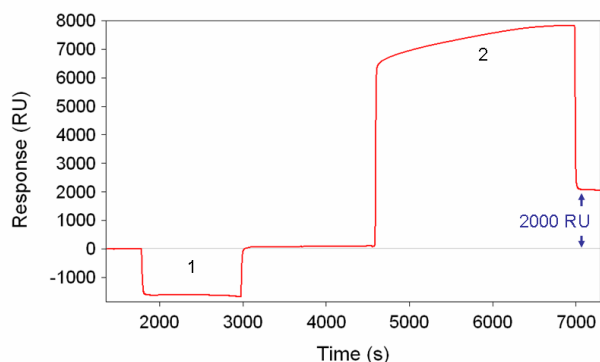
ⁱⁱⁱ Hoffman-LaRoche's patent expired in 2005

HisCap CHIP ADVANTAGES

- His-tagging is a long-established technology, standard in labs that carry out recombinant protein work.
 - The HisCap chip 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 chip provides a stable baseline
 - A stable baseline facilitates kinetic analysis of interactions; drifting baselines make kinetic analysis extremely difficult if not impossible.
- The chip can be regenerated using mild conditions, e.g. imidazole or EDTA.
- The HisCap chip can be reused.

HisCap CHIP IMMOBILIZATION

The ICx Nomadics HisCap chip is prepared by immobilizing an amine derivative of the NTA ligand onto a planar PEG surface.



As shown in the response curve, the user activates the chip by injecting nickel chloride (1): the nickel ions coordinate with the surface NTA residues. A His-tagged protein is subsequently injected (2) resulting in affinity capture of a mass equivalent to 2000RU. EDTA, or alternatively imidazole may be used to competitively reverse the interaction thereby restoring the original uncoated surface. The HisCap chip provides a platform for efficient,

reproducible immobilization of his-tagged proteins. The protein does not leach from the surface resulting in a stable baseline suitable for kinetic analysis experiments.

EXPERIMENTAL TIP

Occasionally we experience non-specific binding of electropositive analyte to the electronegative NTA-coated surface. We have found that non-specific binding can be greatly reduced by blocking these excess sites using a polyhistidine peptide or with a 6xHis tagged protein unrelated to the interaction of interest.

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