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# From monomeric to multimeric His-tag proteins conjugation to magnetic nanoparticles through NTA-$Me^{2+}$: shape and size effects

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Protein immobilization is a technique that offers many advantages, but also poses some challenges, among them the conservation of the original 3D structure and the protein orientation that guarantees a good activity of the immobilized variant. One the most widely used strategy to control enzyme orientation and avoid structural changes is the use of His-tag recombinant variants: a 6 histidine residues tail is added at N or C-terminus and the imidazole side chain could be coordinated by divalent transition metal ions on the surface of magnetic nanoparticles thanks to a chelating agent, the nitriloacetic acid, NTA. [1]

Herein, we explore the immobilization of His-tagged modified proteins, with different quaternary structure, to magnetic nanoparticles: monomeric fragment of E-Cadherin, monomeric Green Fluorescent Protein (GFP), dimeric Alcohol Dehydrogenase (ADH) from *T. thermophilus* and tetrameric Alcohol Dehydrogenase from *B. stearothermophilus.* [2] The conjugation is studied using different amounts of $Co^{+2}$ and $Ni^{+2}$, that present different affinity and specificity for the histidine imidazole side chain. Besides, we evaluate how the use of one transition metal, along with proteins with different quaternary structure affect, first the immobilization yield and the binding specificity and, later, the activity of the enzymes bound to the MNPs.

Our preliminary results suggest that proteins shape and size, in association with the amount of available metal site, play a central role in the binding yield, specificity of the binding and particles colloidal stability.

## References

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