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School of Life Sciences

Particle engineering for His-Tag protein immobilization and their shielding

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Bachelor thesis, Molecular nanotechnology

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ABSTRACT

Nanoparticles were engineered in order to collect and immobilize His_6-tag superfolder green fluorescent protein (GFP) from MES (50 mM pH 5.2) buffer and cell extract from *Escherichia* coli. The fluorescence of GFP was measured at 440nm/510nm.The immobilized GFP was afterwards directly shielded on the modified silica nano particles with a protective layer.

INTRODUCTION

Enzymes are natural biocatalyst which often display high chemo-and regio-selectivities and for this reason they are widely used in the pharma and chemical industry for the production of the desired products. During industrial processes enzymes get usually easily damaged. In order to decrease their fragility, immobilization represents an efficient solution. Between the several advantages, immobilized enzymes exhibit longer life-span and recycling and separation form the final product. Moreover, often immobilized enzyme are less prone to their environment.[1][2]

It has been recently shown that the presence of a organosilica layer, improves the stability of enzymes immobilized on silica nanoparticles (SNP). [3]

In this present work we aimed at producing engineered nanoparticles that allow to collect His-tag proteins directly from a cell lysate and to protect them with an organosilica layer. As model protein we have chosen a His_6-tag GFP.

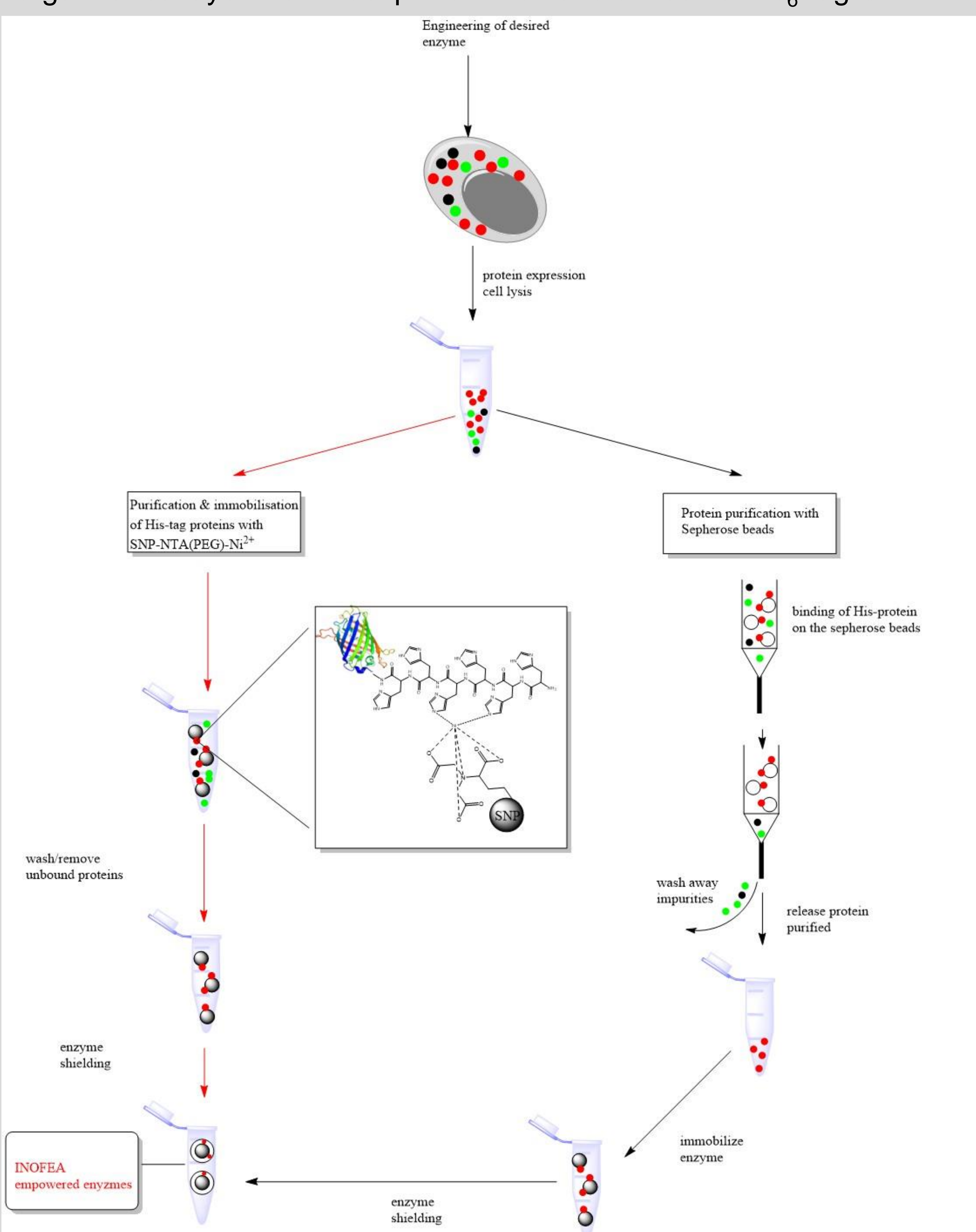

Fig. 1 Schematic representation of the two strategies to collect and shield proteins from a cell lysate. In a conventional procedure (black arrow), proteins must be first purified from a cell lysate and then immobilized on SNP through covalent crosslinking and shielded. Following our new strategy (red arrow), His_6-tag proteins are collected through the NTA modified SNP and directly shielded.

RESULT

The modified SNP were synthesized in order to collect protein through the NTA-Ni^{2+} His_6-tag complex, in a cell lysate. The GFP was measured by means of fluorescence.

Two different batches of modified SNP were synthesized and their capability to collect His_6-tag protein in a cell lysate was tested. One batch of SNP contains the NTA-Ni^{2+} functional group and the other was further modified with a PEG group to reduce unspecific binding.

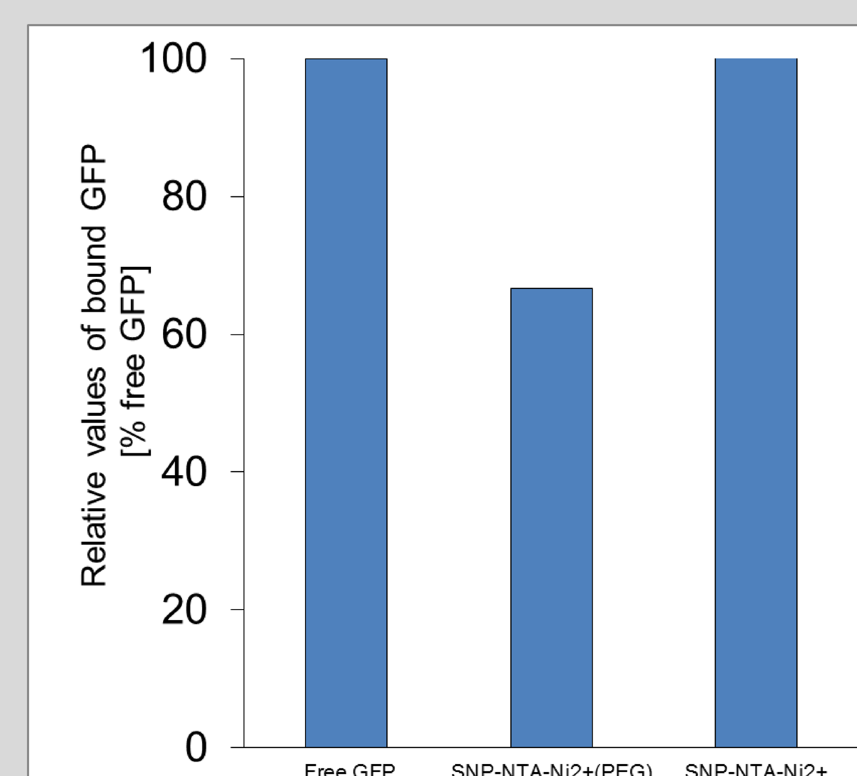

Fig. 2 Fluorescence of soluble His_6-tag GFP (left bar) and of His_6-tag GFP collected from a cell lysate through NTA-Ni^{2+}-(PEG) (middle bar) and NTA-Ni^{2+} (right bar) modified SNP.

The immobilized GFP was further shielded with a protective organosilica layer (Figure 3) of 52 ± 7 nm.

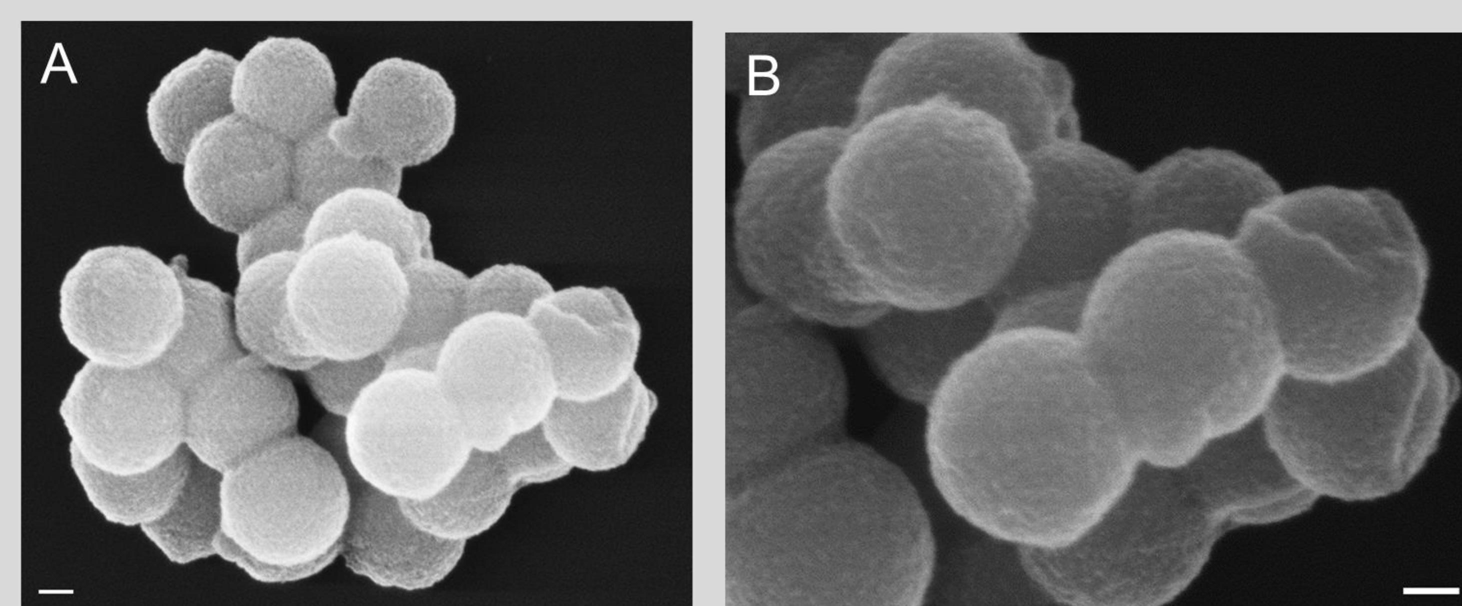

Fig. 3 A: SEM micrograph of shielded NTA-Ni^{2+}-(PEG) SNP. B: Detail showing a damaged shield. (Scale bars are 100 nm)

The fluorescents of the GFP could be measured through the shielded SNP, therefore the three dimensional structure was not damaged during the organosilica layer growth.

CONCLUSION

In the present work, we show a new strategy to produce modified SNP allowing to collect His_6-tag proteins from a cell lysate. Furthermore the bound proteins were successfully shielded with a protective layer preserving its three dimensional conformation.

REFERENCES

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