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A new multifunctional peptide tag as an alternative to the well-established His tag in recombinant protein purification and immobilization

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Introduction

In biotechnology, the use of a L-histidine tag that is molecularly fused to a target protein and forms a selective coordinating bond with traditional IMAC, NTA, or IDA functionalized materials to analyze and purify proteins is well established. We want to introduce a new peptide tag made up of four L-arginine (R) and four L-histidine (H) placed in an alternating sequence. This $(HR)_4$ tag has no effect on the expression performance of recombinant proteins in E. coli and may be isolated with high purity and yield using IMAC affinity chromatography. Due to the tags polycationic nature, new materials may be used as affinity matrices, i.e. underivatized silica, magnetic nanoparticles. Protein purifications on underivatized silica have long been overlooked. The tag has a high affinity for deprotonated silanol groups because of the L-arginine groups interact with the surface via an ion pairing mechanism. The tag's affinity for the matrix resulted in protein purities (eGFP) of up to 93% after a single purification step, rivaling the polyhistidine tag used in nickel affinity chromatography.

Working Principle

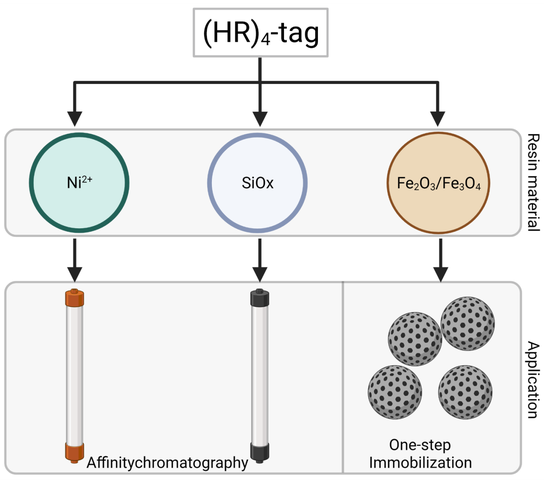

Figure 1: $(HR)_4$ applications schematic created with biorender

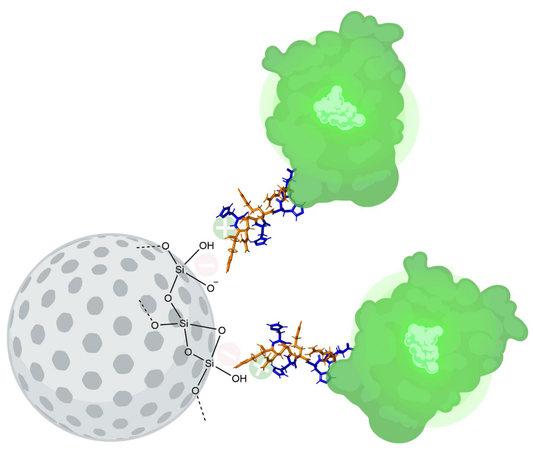

Figure 2: $(HR)_4$ tag interacting with the silica surface created with biorender

- The negatively charged silica surface is targeted by the polycationic $(HR)_4$ tag via an ion pairing mechanism
- The Ni^{2+} molecules are targeted by the imidazole ring of L-histidine in the $(HR)_4$ tag

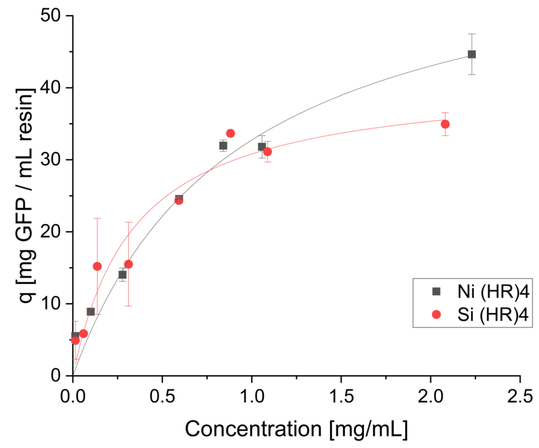

Figure 3: Equilibrium binding constant (24h) with non linear langmuir fit on respective resin material (1mg)

Process

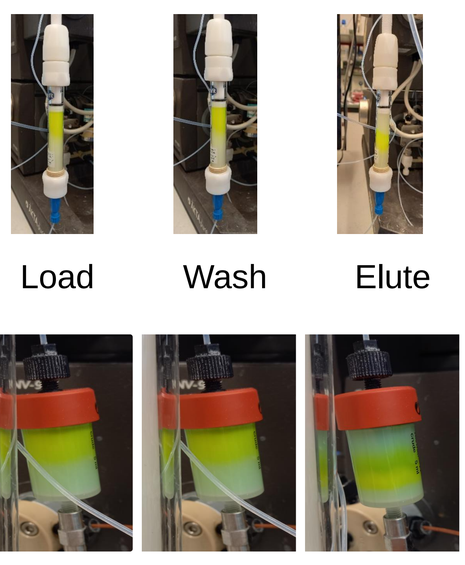

Figure 4: Silica and nickel columns during the different process steps

To determine the usability of the $(HR)_4$ tag, both resin materials were tested in stationary and dynamic experiments, under same process parameters (i.e. CV, flowrate)

Key results:
- Selective binding to both resin materials
- Equilibrium load of silica is in a similar range to load on nickel
- Dynamic binding capacity (DBC_{10}) of silica was 2.4x lower than the capacity of the nickel column

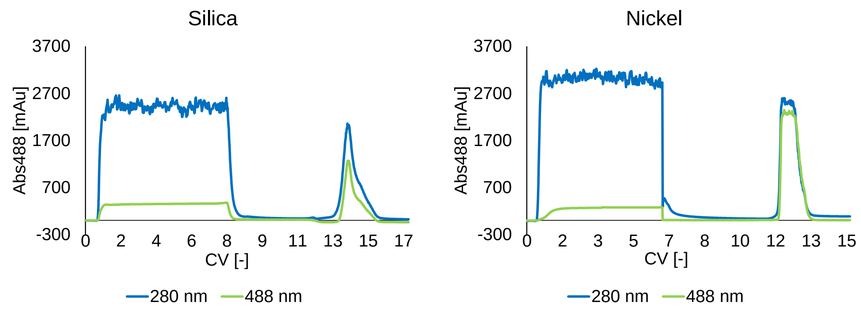

Figure 5: Chromatogramms of the purification process with $(HR)_4$ tagged eGFP

Table 2: Dynamic binding capacities of a 5 mL nickel and 5 mL silica resin and equilibrium binding capacities on 1 mg of the respective resin material

Process	DBC_{10} [mg/mL]	Retention time R_t [min]	Q_{max} [mg/mL]	Binding constant K_L [mM]
Ni-$(HR)_4$	3.25	5	62.73 ± 9.22	0.098
Si-$(HR)_4$	1.38	5	41.46 ± 4.32	0.037

Purities

In order to assess the functionality of each resin material the elution fractions were analyzed via SDS-PAGE (see figure 7).
The fractions of interest were then pooled and analyzed for the overall purity and effectiveness of each process.

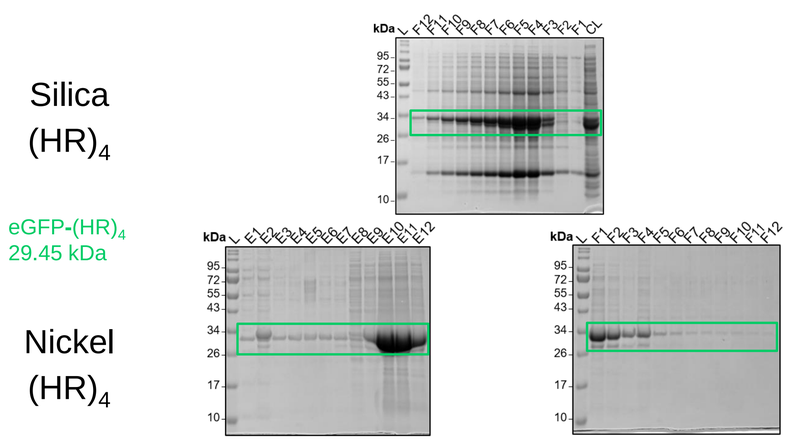

Figure 7: SDS-PAGE analysis of the elution fractions of both processes

The pooled fractions were analyzed via photometric, densitometric analysis.

Key results:
- Selective elution via L-lysine on silica and imidazole on nickel
- Nickel achieved an 8% higher initial purity

Table 3: Purity results of the densitometric SDS-PAGE analysis

GFP Proportion	Ni$(HR)_4$	Si$(HR)_4$
Lysate	~49%	~43%
Eluate	~93%	~85%

Summary and Outlook

The $(HR)_4$ tag binds to silica selectively. On a typical NiNTA column, the silica procedure yielded product purities of 85% with the $(HR)_4$-tag and 93% without the $(HR)_4$-tag. After a single step, the $(His)_6$-tagged GFP yielded purities of 95%.These findings demonstrate the $(HR)_4$ tag's superiority over the $(His)_6$-tag. The adaptability of the tag, on the other hand, allows for a variety of alternative purification and immobilization procedures without modifying the protein. Silica purification promises to be a low-cost method for purifying $(HR)_4$-tagged proteins in a single step. A 5 mL silica column requires 1.5 g of Davisil 643, which costs ~0.60€. In comparison, a 5 mL ready-to-use IMAC column costs 120€. The 200x cost reduction overcomes the decrease in dynamic binding capacity. Overall, $(HR)_4$-tag purification using bare silica is a promising technology due to the simple process parameters that result in high purities, which led to the patent application.

Forschungsnetzwerk Mittelstand

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