

Supplementary Material

# Efficient Purification of Polyhistidine-Tagged Recombinant Proteins Using Functionalized Corundum Particles

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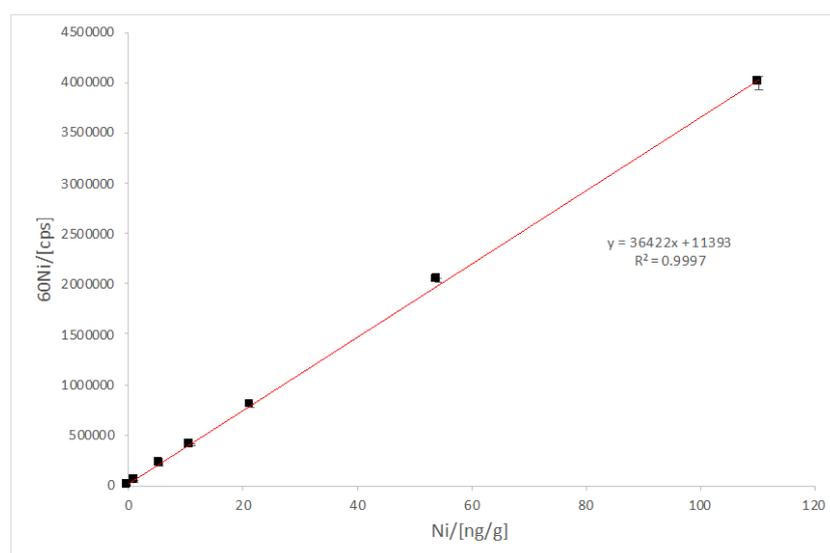
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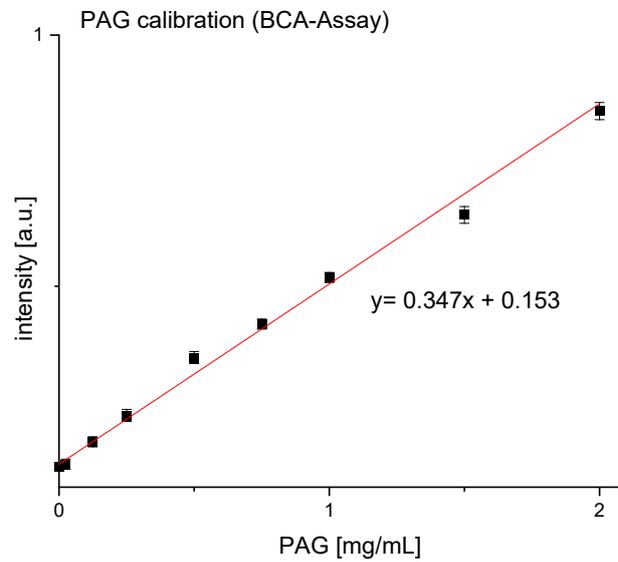
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## 1. ICP-MS – Nickel calibration



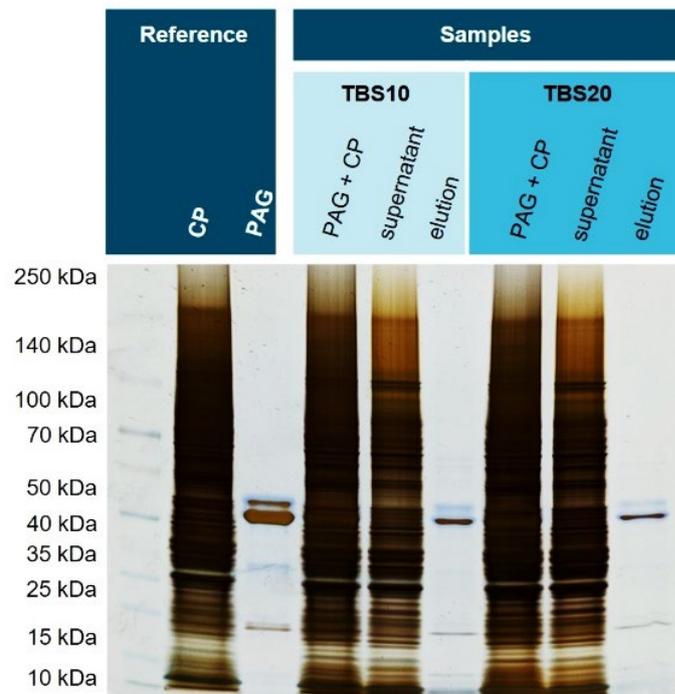
**Figure S1.** External Ni-calibration for the Ni-determination on functionalized corundum via ICP-MS using a Thermo iCAP Q and the multi-element standard for external calibration, and yttrium as internal standard.

## 2. His6-PAG calibration by BCA protein assay



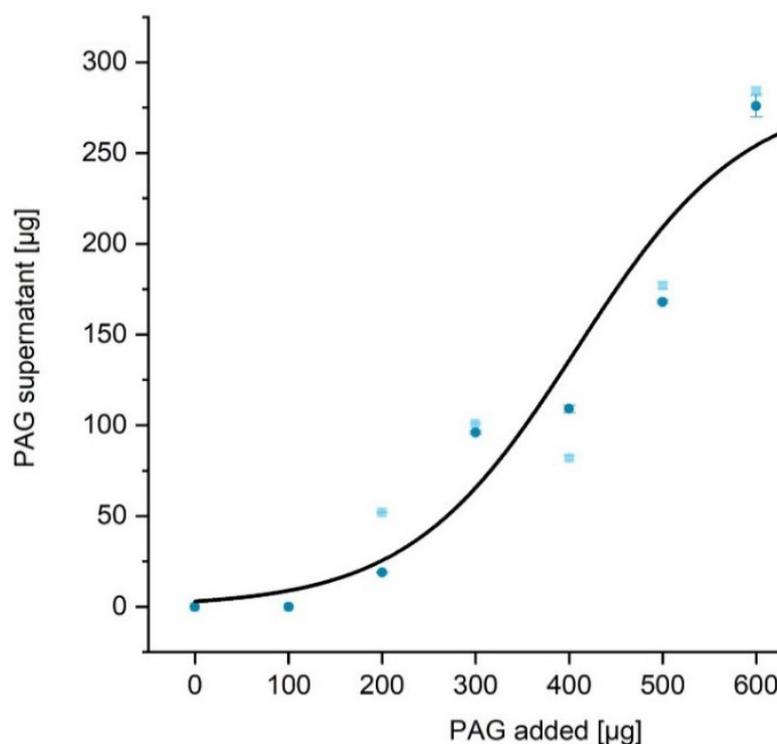
**Figure S2.** His6-PAG calibration by BCA protein assay. His6-PAG was diluted in different concentrations for external calibration. The absorbance was measured at 562 nm.

## 3. Isolation of His6-PAG spiked into *E. coli* NiCo21(DE3) cell lysate – Silver-stained gel



**Figure S3.** Silver-stained gel showing the isolation of His6-PAG spiked into *E. coli* NiCo21(DE3) cell lysate. For both binding buffers TBS 10 and TBS20, there seems to be no difference in purity. This confirms that for a cytoplasm optimized for IMAC, the use of 20 mM imidazole as a dilution buffer does not compromise selectivity.

#### 4. BCA assay for the determination of protein binding capacity in TBS20 and TBS40 in the supernatant



**Figure S4.** Determination of the protein binding capacity (binding buffer TBS40 dark blue, TBS20 light blue). A maximum of around 300 mg His6-PAG in the supernatant was determined when incubating 100 mg functionalized corundum with 600 µg His6-PAG.

#### 5. Ni-NTA Agarose Protocol Jena BioScience (AC-501-10)

50 µL of agarose beads in 0,5 mL native lysis buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 10 mM imidazole, pH 8.0) were equilibrated and washed. The supernatant was discarded after a centrifugation step (6000 rpm, 5 min). Then 1 mL cytoplasm containing 1 mg spiked 6His-PAG and 0,5 mL native lysis buffer were incubated for 1 h at room temperature. The supernatant was removed and collected for the SDS-PAGE. The particles were washed 3 times with 0.5 mL washing buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 20 mM imidazole, pH 8.0). Elution took place for 10 min with 50 µL elution buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 250 mM imidazole pH 8.0). Particles were centrifuged and the eluate was taken for SDS-PAGE analysis.

Source: [https://www.jenabioscience.com/files/jenabioscience/datasheet\\_extern/AC-501.pdf](https://www.jenabioscience.com/files/jenabioscience/datasheet_extern/AC-501.pdf) (Accessed 03.04.2023)

#### 6. Production of His-6-tagged MBP-mSA2 and blanks in different E. coli strains

BL21(DE3) pLysS and NiCo21(DE3) competent *E. coli* cells were transformed with vector pET-MBP-mSA2 and selected on LB plates supplemented with 30 µg/mL kanamycin and 2 % glucose. Inoculated liquid cultures were grown in 4 mL of LB medium containing already mentioned additives overnight shaking at 200 rpm and 37 °C. Prewarmed TB medium (12 g/L tryptone, 24 g/L yeast extract, 4 mL/L glycerol, 17 mM potassium hydrogen phosphate, 72 mM dipotassium hydrogen phosphate) was inoculated 1:100 with overnight culture and grown under stated conditions until the optical density at 600 nm (OD<sub>600</sub>) reached 0.6. Protein expression was induced with 1 mM isopropyl β-D-1-

thiogalactopyranoside (IPTG, final conc.) and allowed to proceed overnight shaking at 200 rpm and 16 °C. The bacterial cells were harvested by centrifugation (10,000 × g, 4 °C, 20 min) and frozen at -20 °C. For cell disruption, the pellet was resuspended in lysis buffer (300 mM NaCl, 46.6 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.9 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM imidazole, pH 8) with additional 1 mg/mL lysozyme as well as protease inhibitors and lysed via sonication. Insoluble cell debris was collected through centrifugation (10,000 × g, 4 °C, 20 min) and subsequently, a soluble fraction of the cell lysate was taken off and filtered through a 0.22 μm filter before recombinant protein purification.

For the production of blanks, single colonies of both competent cell lines were generated by dilution-plating on LB plates with 2% glucose. The further procedure was analogous to the expression conditions of pET-MBP-mSA2 without adding kanamycin during cultivation. In 1 mL blanks, 1 mg of His6-PAG was spiked and then diluted with 1 mL of TBS binding buffer containing different amounts of imidazole.

### 7. Production of SARS-CoV-2-S-RBD-His8 in human Expi293F cell line

Expi293F-cells (Thermo Fisher Cat#A14527) were cultivated in serum-free and ready-to-use Expi293 expression media (Thermo Fisher Cat#A1435101). Cell growth occurred in sterile, ventilated 125 mL Erlenmeyer flasks (Fisher Scientific Cat#BBV12-5) shaking at 37°C, 125 rpm with 80% humidity and 8% CO<sub>2</sub>. The transient transfection of the cells took place with the plasmid pcDNA3-SARS-CoV-2-S-RBD-8his (Addgene #145145) [21,22] and the Gibco™ ExpiFectamine™ 293 Transfections Kit (Thermo Fisher Cat#A14524) according to the manufacturer. The cell density was adjusted to 3 × 10<sup>6</sup> cells/mL in a final volume of 25 mL Expi293-expression media in 125 mL Erlenmeyer flasks. For the transfection, 25 μg plasmid and 80 μL of ExpiFectamine293 were diluted in 1.5 mL Opti-MEM-Medium. Later the reagents of both tubes were homogenized, incubated for 15 min at room temperature, and added to the cell suspension dropwise. After 20 hours, 150 μL of ExpiFectamine™ 293 Transfection Enhancer 1 and 1,5 mL of ExpiFectamine™ 293 Transfection Enhancer 2 were added to the cell suspension and incubated further whilst shaking. Cells were counted each day after transfection and checked on their viability (%) (automatic cell counter EVE, VWR # 734-2675). Protein harvest was performed 4 days after transfection; cells were centrifuged for 30 min at 4000 × g and the decanted supernatant was stored at 4°C until protein purification.