nature | **methods**

Enabling IMAC purification of low abundance recombinant proteins from *E. coli* lysates

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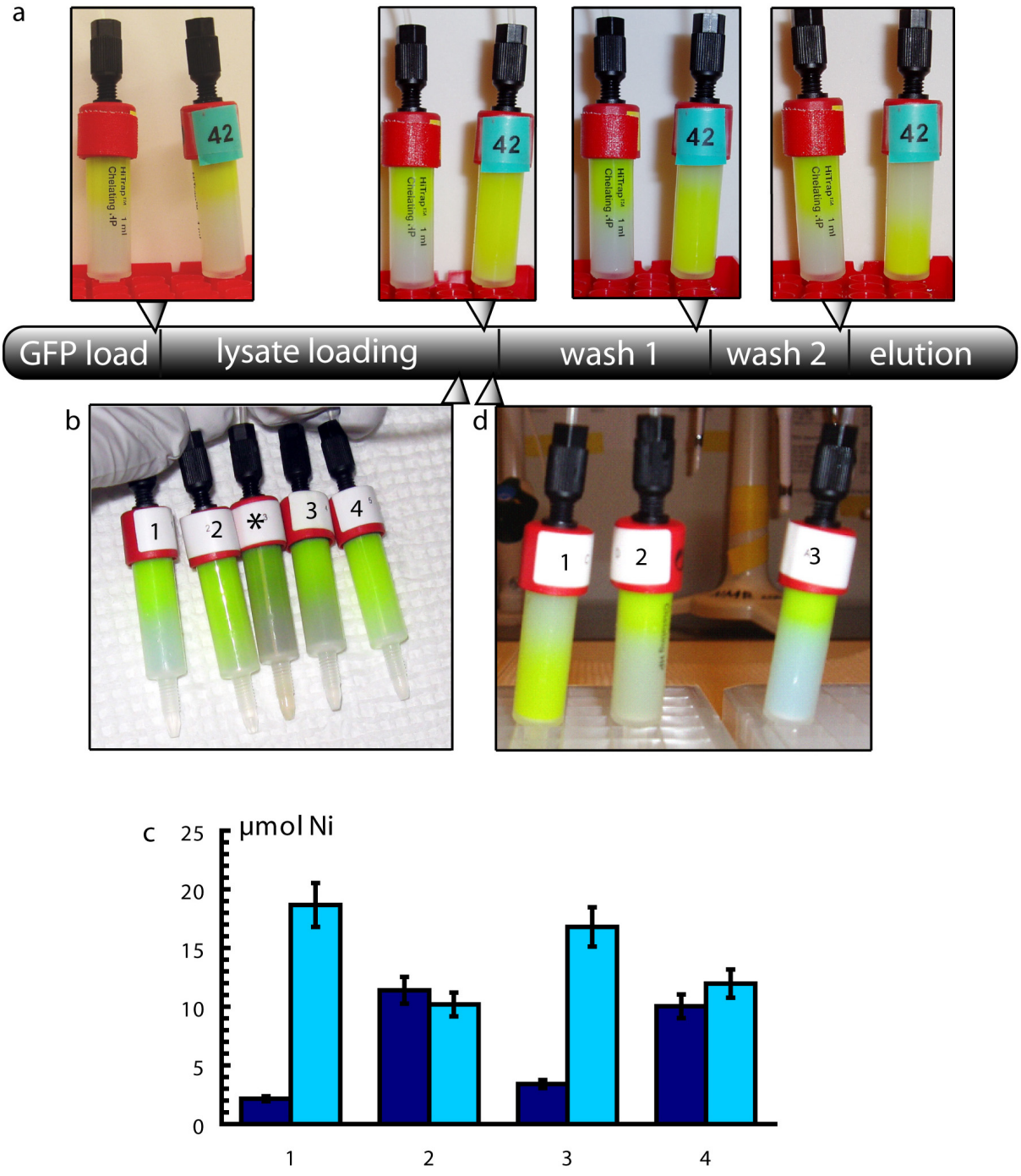
Supplementary Methods	

Supplementary Figure 1.

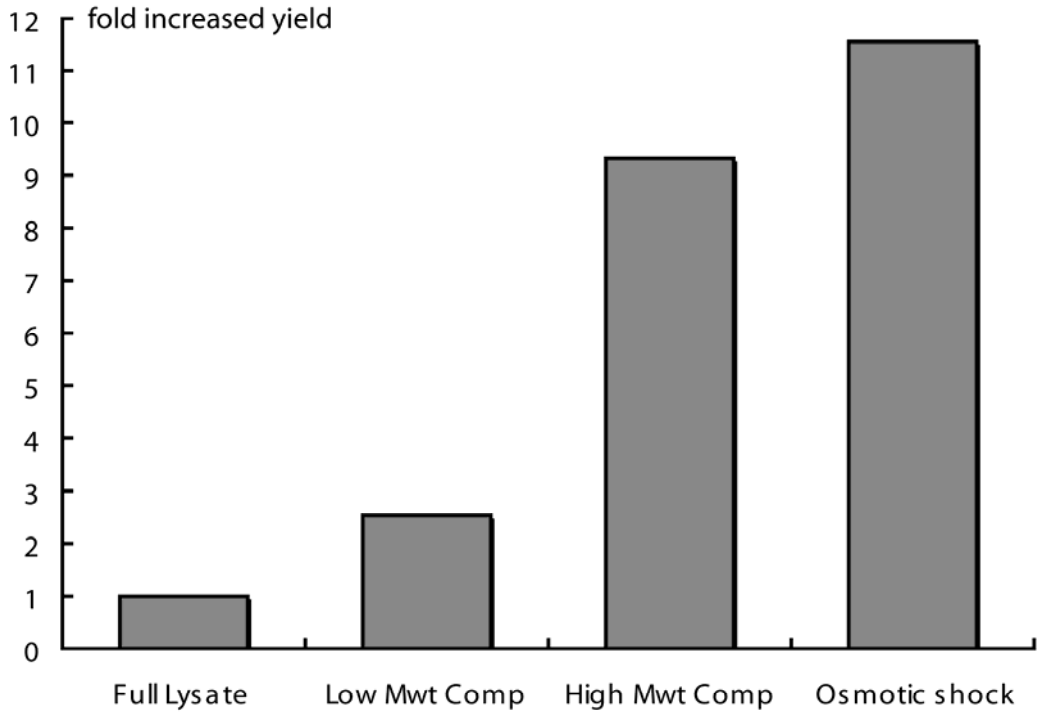


Supplementary Figure 1: *E.coli* lysate causes migration of His tagged target proteins on IMAC columns

(**a**) Migration of pre-bound His tagged GFP during treatment of columns with *E. coli* lysate (right) and wash1 buffer (left) followed by two wash steps and elution.
(**b**) Photograph taken of IMAC columns pre-loaded with His tagged GFP after treatment with 1:wash1 buffer, 2: *E. coli* lysate, 3: *E. coli* lysate devoid of low molecular weight components, 4: the low molecular weight fraction, and *: *E. coli* lysate supplemented with 2 mM $FeSO_4$. (**c**) Analysis of the nickel content after treatment of IMAC columns with 1: wash1 buffer, 2: *E. coli* lysate, 3: *E. coli* lysate devoid of low molecular weight components,

and 4: the low molecular weight components of *E. coli* lysate. Dark blue bars represent the amount of nickel extracted during treatment, light blue bars represent nickel remaining on column after sample load. (**d**) Photograph taken of IMAC columns pre-loaded with His-tagged GFP after treatment with 1: *E. coli* lysate 2: lysate of *E. coli cells* devoid of periplasmic material 3: wash1 buffer.

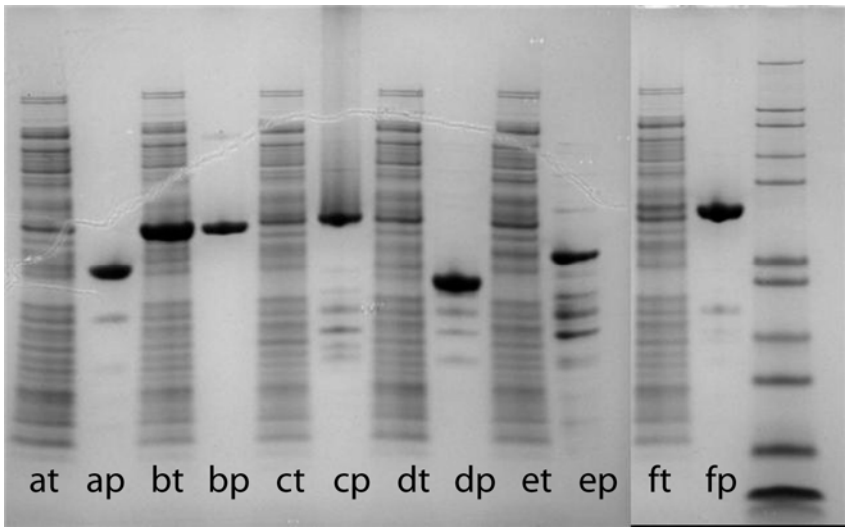
Supplementary Figure 2:



Supplementary Figure 2: Periplasmic low molecular weight components of lysate reduce the binding capacity of IMAC columns

The relative gain of removing the high molecular weight compounds, low molecular weight compounds, or the periplasmic material before IMAC purification compared with cells that were lysed directly according to standard procedure. *E. coli* cells containing His-tagged GFP were mixed with cells lacking any recombinant protein in order to simulate the conditions of a low abundance His-tagged target protein. All the samples were purified using IMAC and the yield of His-tagged GFP was determined by measuring the fluorescence of the pooled elution fractions.

Supplementary Figure 3:



Supplementary Figure 3: Removing periplasmic material from lysate before applying it on IMAC columns improves yield for a wide range of target protein abundance.

SDS PAGE showing the input and the resulting batch from a triple sized culture treated with osmotic shock. t: soluble total protein, p: purified. The target proteins were a: glutaminase domain of GLS, b: ATPase domain of HSPA6, c: KH and DEAD domains of DDX53, d: glutaminase domain of CTPS2, e: C-terminal domain of GLE1, and f: ACOT1. Not surprisingly, a strong correlation between the abundance of the target protein in the lysate and the homogeneity of the purified target protein is observed. For example, the highest abundance protein (b) in this study is very pure after the two-step automated purification while the lowest abundance protein (e) is rather impure.

Supplementary Methods:

No comparisons were made between different experiments so small discrepancies in cell weights, buffer volumes or composition, incomplete lysis efficiency, etc. will not effect the conclusions drawn. Buffer compositions can be found at the end.

E. coli strains, plasmids and media:
BL21(DE3)gold pRARE2 harboring expression plasmid pTH27:GFP (1) were grown in TB medium supplemented with glycerol (8 g/l), ampicillin (100 µg/ml), and chloramphenicol (34 µg/ml). Cell pellets of *E. coli* containing GFP expression plasmids were bright green. BL21(DE3)gold pRARE2 harboring pUC18 or BL21(DE3) pLysS were grown in TB medium supplemented with glycerol (8 g/l), chloramphenicol (34 µg/ml), as well as ampicillin (100 µg/ml) when pUC18 was included.

Cultivation:
Typically, starting cultures were inoculated with cells from glycerol stocks and grown over night at 30 °C in shaking incubators. The following day the starting culture was diluted 1:50 and cultured in shaking incubators at 37 °C until OD_{600} reached ~1.5. The temperature was lowered to 18 °C and protein expression was induced by addition of IPTG (0.5 mM) when appropriate**.** The following day cells were harvested by centrifugation at 4,500 x g for 20 minutes at 10 °C. The cell pellets were weighted and resuspended in 1.5-2.2 ml/g_{pellet} lysis buffer using a shaking table at 8 °C and finally frozen in -80 °C unless destined for removal of periplasmic material.

Removal of periplasmic fraction:
Osmotic shock: Newly harvested cells were re-suspended in 5 ml/g_{pellet} sucrose buffer (50 mM HEPES, 20% sucrose, 1 mM EDTA pH 7.9) before re-pelleting by centrifugation at 7,000 x g for 30 min at 4 °C. The supernatant was discarded and the pellet re-suspended in 5 ml/g_{pellet} of 5 mM $MgSO_4$ and incubated on ice for 10 min. Cells were finally pelleted by centrifugation at 4,500 x g for 20 min, the supernatant was discarded and the pellet was re-suspended in 1.5 ml/g_{pellet} lysis buffer and frozen in –80 °C until lysis.

EDTA+lysozyme treatment: Newly harvested cells were re-suspended in 5 ml/g_{pellet} lysis buffer without any reducing agent. EDTA (1 mM) and lysozyme (0.5 mg/ml) was added and the suspension was left shaking at 20 °C for one hour. Cells were pelleted by centrifugation at 4,500 x g for 20 min, the supernatant was discarded and the pellet was resuspended in 1.5 ml/g_{pellet} lysis buffer and frozen in -80 °C until lysis.

Lysis:
Cell suspensions were thawed and lysis was achieved by sonication for 3 min in 4 s pulses. The lysates were centrifuged at 50,000 x g for 15 min, the supernatants were decanted and filtered through a 0.45 µm filter. All steps were performed on ice or at 4°C.

Separation of low and high molecular weight components:
Four HiPrep desalting 26/10 columns (GE Healthcare) were coupled in serial on an ÄKTAxpress system (GE Healthcare), equilibrated with IMAC wash1 buffer and loaded with

60 ml lysate. The lysate components were eluted with IMAC wash1 buffer and all fractions were collected. Fractions corresponding to a strong peak at the 280 nm absorbance spectrum that eluted between 40-145 ml were pooled and referred to as the high molecular weight components of the lysate. All fractions after this peak were pooled and referred to as the low molecular weight components of the lysate. The flow-rate was set to 4 ml/min.

General GFP migration experimental setup:
1 ml HiTrap Chelating HP columns (GE Healthcare) were prepared and charged with nickel according to the manufacturer's instructions. Prior to use the columns were washed with water and equilibrated with 5 CV IMAC wash1 buffer. A multiple channel peristaltic pump (Watson Marlow) was used to first load His-tagged GFP and then to expose the columns to lysate, buffer, or manipulated lysate versions. The flow-rate was set to 1 ml/min in all setups. After sample load, the columns were washed with 10 CV IMAC wash1 buffer, 6 CV IMAC wash2 buffer, before eluting bound protein with 6 CV IMAC elution buffer. During the whole process 2-ml fractions were collected for fluorescence measurements. The experiments were performed at 8 °C or at room temperature. An *E. coli* cell pellet weight of 25 g was used for each column in these experiments. A similar experiment was carried out using HiTrap IMAC HP column (GE Healthcare). Also on this column extensive migration of His-GFP was observed even though it was less profound.

Fluorescence measurements:
The fluorescence of GFP was measured in 300 µl aliquots 96 well black plates on a Synergy HT plate reader (BioTek, USA) using λ_{ex}= 485 nm and λ_{em}= 528 nm. If the read out exceeded 500 the sample was diluted due to the non-linearity at higher fluorescence values.

Nickel leakage analysis:
54 g cells of *E. coli* without recombinant protein were lysed and the soluble fraction was divided in two parts of 60 ml. The first part was passed over four serially coupled HiPrep desalting 26/10 columns (GE Healthcare) to separate high and low molecular components. The low molecular components eluted in a volume of 160 ml, why the high molecular weight components also were diluted to 160 ml with IMAC wash1 buffer, as was the second part of the lysate. The three lysate variants and an additional control consisting of 160 ml IMAC wash1 buffer were applied to four 1 ml HiTrap Chelating HP columns and the flow-trough was collected. After completed sample load the metal ions remaining on the columns were eluted with 20 CV of EDTA solution (50 mM). The amount of nickel in input buffers, lysate variants, flow-through, and EDTA elutions was analyzed by MeAna-konsult, Uppsala Sweden. The amount of nickel in all input buffers and lysates was below detection limits.

Simulation of a low abundant His-tagged target by mixing His-tagged GFP and E. coli lysate devoid of recombinant protein:
For each column approximately 100 g of *E. coli* cells without any recombinant protein was mixed with approximately 10 g of GFP containing cells and were either separated into high and low molecular components, exposed to osmotic shock, or treated as usual before lysis. The samples were loaded on 1 ml HiTrap Chelating HP columns, washed and eluted using an ÄKTAxpress system basically as described above. The yield of His-tagged GFP was determined by measuring the fluorescence of the eluted fractions. For the sample devoid of

high molecular weight components, an aliquot of 10 g of GFP containing cells was added prior to IMAC load.

Purification of human His-tagged target proteins:
Cells were grown in TB medium supplemented with glycerol (8 g/l), antibiotics, and Antifoam 204 (Sigma) in a LEX system (Harbinger Biotechnology) at 37 °C until OD_{600} reached approximately 2. The cultures were cooled to 18 °C and protein expression was induced by addition of IPTG (0.5 mM) and allowed to continue over night at 18 °C. Cells were harvested by centrifugation at 4,500 x g for 10 min at 4 °C and divided into single and triple sized cell- pellets. The single sized cultures corresponded to 1.5 litres and resulted in cell pellet wet weights ranging from 19 to 35 g, with an average of 27 g.
A set of the triple sized cultures were subjected to osmotic shock or treated with the EDTA+lysozyme method as described above. The remaining cells, corresponding to 4.5 and 1.5 l culture, respectively, were directly re-suspended in 1.5 ml/g_{pellet} lysis buffer and stored at -80 °C. The frozen cell suspensions were thawed and the cells were lysed by sonication. After centrifugation at 49,000 x g for 20 min at 4°C the soluble fraction was decanted and filtered through 0.45 µm filters.
Purification was performed in a two step procedure on an ÄKTAxpress system using 1 ml HiTrap Chelating HP and HiLoad 16/60 Superdex 75 or 200 Prep Grade columns (GE Healthcare). Prior to purification the columns were equilibrated with IMAC wash1 and SEC buffer, respectively. The protein sample was loaded on the IMAC column, washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC columns with IMAC elution buffer and automatically loaded on the gel filtration column.

Buffer composition:
All solutions were filtered through 0.22 µM filter. TCEP was always added right before use and once TCEP was added, the buffer was not used for more than one day.

Lysis buffer: 100 mM HEPES, 500 mM NaCl, 10% glycerol, 10 mM imidazole, 0.5 mM TCEP, supplemented with one tablet of Complete EDTA-free protease inhibitor and 2,000 U Benzonase per 100 ml buffer, pH 8.0
IMAC wash1 buffer: 20 mM HEPES, 500 mM NaCl, 10% glycerol, 10 mM imidazole, 0.5 mM TCEP, pH 7.5
IMAC wash2 buffer: 20 mM HEPES, 500 mM NaCl, 10% glycerol, 25 mM imidazole, 0.5 mM TCEP, pH 7.5
IMAC elution buffer: 20 mM HEPES, 500 mM NaCl, 10% glycerol, 500 mM imidazole, 0.5 mM TCEP, pH 7.5
SEC buffer: 20 mM HEPES, 300 mM NaCl, 10% glycerol, 0.5 mM TCEP, pH 7.5