Automated purification of dual-tagged proteins

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Automated purification of dual-tagged proteins

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Dual-tagged proteins

Recombinant proteins can be designed to contain N- or C-terminal affinity tags. If including two different tags in a construct, the target protein can be purified utilizing different affinity media, producing a purer protein with a straightforward purification scheme. If different tags are positioned on each end of the protein, a dual-tagged purification scheme ensures that a full length protein is the result. If cleavage sites are included inbetween the tag and the target protein, the affinity tag can be removed.

In this study two comparisons are made. First, the same protein is purified using two different affinity columns in two different set-ups. For another dual-tagged protein, final purity is compared when using only one affinity column, relative to using two different affinity columns.

ÄKTAxpress

ÄKTAxpress™ is a compact chromatography system supporting 28 different single- or multi-step protein purification protocols. Up to four different steps can be combined with or without on-column affinity tag cleavage.

Intelligent peak detection allows collection of peaks in capillary loops prior to injection onto the next column. Each sample is initially loaded on separate affinity columns (AC). Cleaning-in-place (CIP) is performed at the end of each run on all columns used. For higher throughput, up to twelve different ÄKTAxpress systems can be run in parallel and controlled from one computer.

For dual tagged proteins three different protocols are supported by ÄKTAxpress. Intermediate desalting (DS) is optional. The last step in the two four step protocols is either DS or gel filtration (GF). Up to three different samples can be processed automatically within a single run.

Fig 1. ÄKTAxpress™ is a compact chromatography system supporting 28 different single- or multi-step protein purification protocols

AC-(DS)-AC	3 samples in 7 hours
AC-(DS)-AC-DS	2 samples in 5.5 hours
AC-(DS)-AC-GF	2 samples in 12.5 hours

Conclusions

- By combining two different affinity tags a highly pure protein is achieved.
- Automation of purification of dual-tagged proteins on ÄKTAxpress is easy and convenient.
- The order in which affinity columns are used does not affect the end purity of the tested target proteins.

Reversing the purification order

To evaluate whether the order of affinity columns in a chosen purification method matters, proteins were purified using StrepTrap™ HP followed by HisTrap™ HP and subsequently in the reversed order. Fig 2 highlights one such comparison. The same amount (0.9 mg) of pure protein is achieved i both experiments, but an N-terminally truncated target protein (identified by MS analysis) passed through the HisTrap HP column more slowly than non-tagged proteins (hence the two peaks seen in the flow through; Fig 2A). According to SDS-PAGE analysis the end purity of the target protein was not affected by the order of the affinity columns used (SDS-PAGE images in Fig 2A vs Fig 2B). The smaller bands observed on the SDS-PAGE are a result of protein degradation upon SDS-treatment (data not shown).

Columns:	StrepTrap HP 1 ml HisTrap HP 1 ml
Sample:	$(Histidine)_6$-mCherry-Strep(II) in *E. coli* lysate
Chosen method:	AC-AC on ÄKTAxpress
Binding buffer (StrepTrap HP):	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0
Elution buffer (StrepTrap HP):	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8.0
Binding buffer (HisTrap HP):	20 mM phosphate, 500 mM NaCl, 5 mM Imidazole, pH 7.4
Elution buffer (HisTrap HP):	20 mM phosphate, 500 mM NaCl, 500 mM Imidazole, pH 7.4

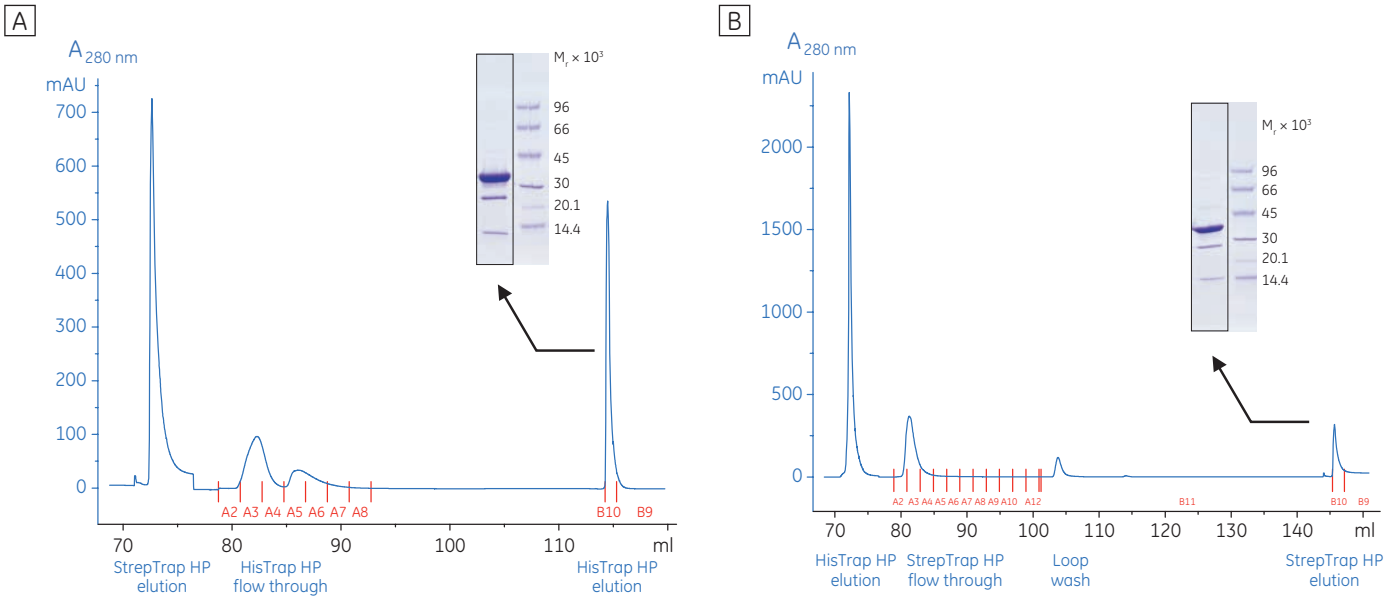

Fig 2. Chromatograms from purification of $(Histidine)_6$- and Strep(II)-tagged mCherry protein (M_r ~ 30 300) using the AC-AC method on ÄKTAxpress.

A) StrepTrap HP followed by HisTrap HP. **B)** The reversed order is used. Images from the SDS–PAGE showing the end result are inserted next to each final elution peak. A low molecular weight marker is shown in the right lane.

Single versus two affinity steps

To compare purification using one tag or combining two different tags, a dual-tagged green fluorescent protein ((Histidine)$_6$-GFP-GST) was purified in three different ways (Fig 3A). The SDS-PAGE in Fig 3B Lane 2 shows the result from purification on HisTrap HP followed by desalting. The results show that the medium had affinity for other proteins as well. GSTrap™ 4B is much more specific in its binding (Lane 4) and the combination of the two tags (Lane 6) gave the highest purity.

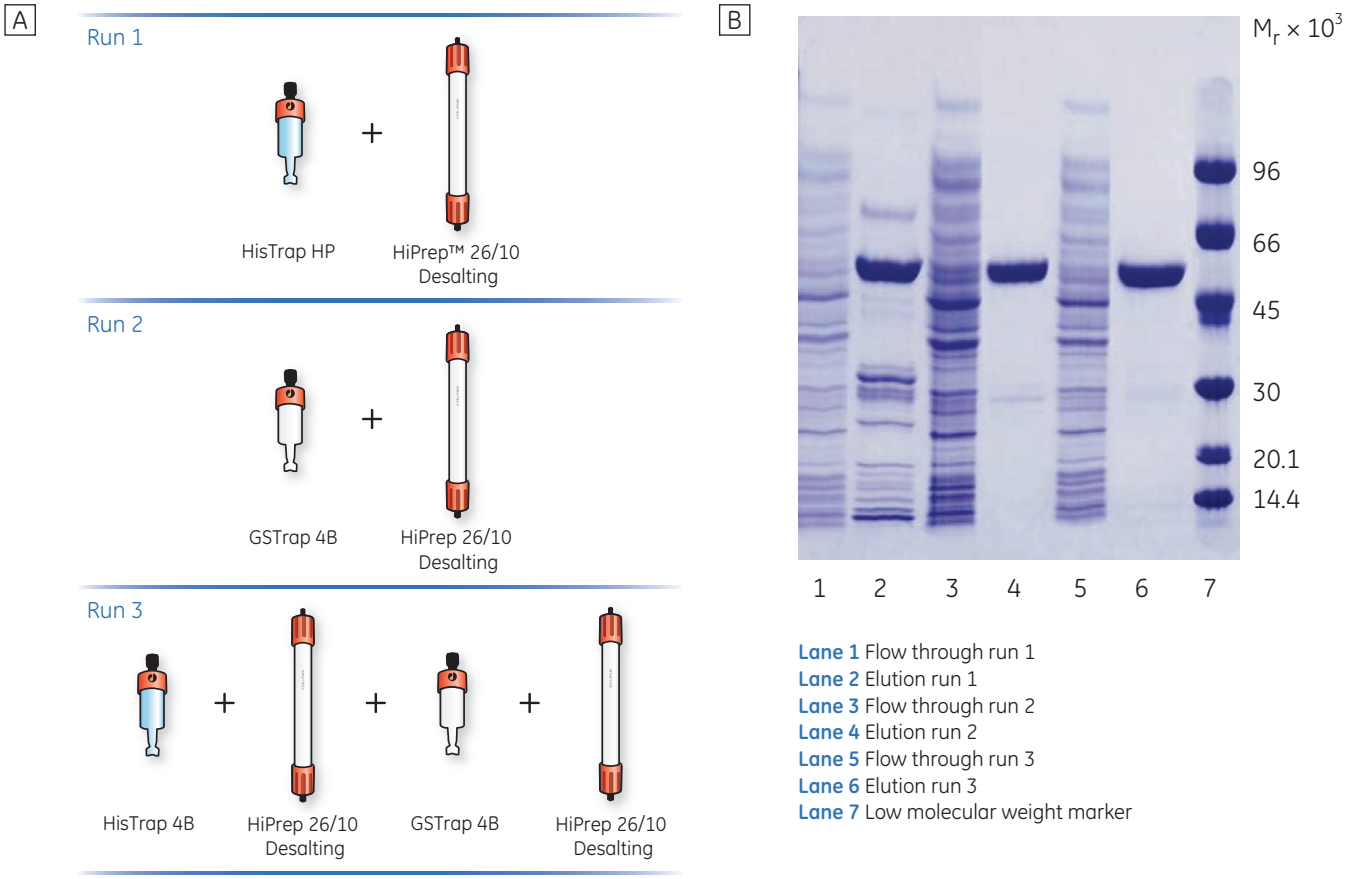

Fig 3. A) Summary of performed runs and columns used. **B)** SDS-PAGE analysis of fractions from purification of (Histidine)$_6$-GFP-GST.

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