Automated multistep purification of (histidine)$_6$– and GST-tagged proteins

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CY13484-15May20-PT

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Automated Multistep Purification of $(histidine)_6$– and GST-tagged Proteins

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Introduction

Automated multistep purifications of five affinity tagged proteins were performed using ÄKTAxpress™, a new chromatography system.

The following purification results are presented:

- One $(histidine)_6$-tagged protein using four different multistep protocols
- Four different $(histidine)_6$-tagged proteins using the same four-step protocol
- One $(histidine)_6$-tagged and one Glutathione S-transferase (GST)-tagged protein including automatic tag removal

ÄKTAxpress

- Automated multistep purification of affinity-tagged proteins
- Method wizard for easy creation of purification protocols
- Intelligent peak detection and collection in intermediate steps
- Optional on-column tag cleavage

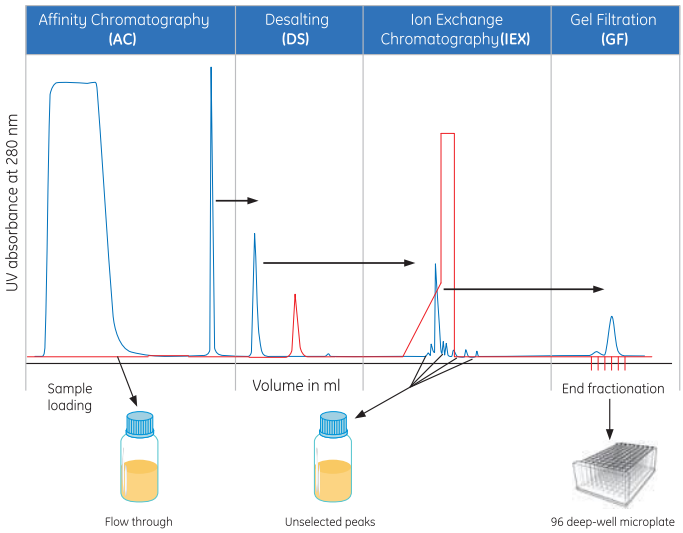
Conclusions

- Automated multistep protocols were used to produce proteins with high purity.
- Different peak shapes in intermediate purification steps are automatically handled by the system.
- Automatic tag removal was successfully performed.
- Yields of up to 50 mg protein were obtained.

ÄKTA™

Multistep purification using ÄKTAxpress

All protocols start with affinity chromatography followed by different combinations of desalting, ion exchange chromatography and gel filtration. The largest peak from each step is transferred to the next column.



Available multistep protocols	Effects from additional chromatographic steps
AC–DS	Buffer exchange
AC–GF	Separation from undesired aggregates and contaminants
AC–DS–IEX	Separation from other isoforms (e.g. heterogenously phosphorylated or glycosylated proteins)
AC–DS–IEX–DS	Separation from other isoforms on IEX and buffer exchange on DS
AC-DS-IEX-GF	Separation from other isoforms on IEX and removal of undesired aggregates and contaminants on GF

Material and methods

Samples

All used (histidine)$_6$- and GST-tagged proteins were expressed in *E. coli*. After harvest, cell lysis was performed by sonication. The samples were clarified by centrifugation prior sample loading.

Buffers

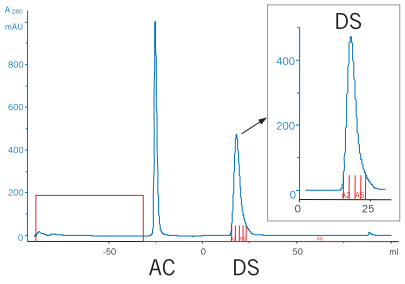
AC (His) binding buffer:	50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.5
AC (His) cleavage buffer:	50 mM Tris-HCl, 500 mM NaCl, 50 mM imidazole, pH 7.5
AC (His) elution buffer:	50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.5
AC (GST) binding and cleavage buffer:	50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5
AC (GST) elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
DS and IEX binding buffer:	50 mM Tris-HCl, pH 8.0
IEX elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 8.0
GF buffer:	50 mM Tris-HCl, 150 mM NaCl, pH 7.5

Analysis

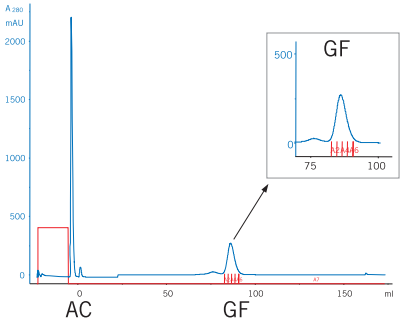
Purity of each sample was analyzed by Coomassie™-stained SDS polyacrylamide gels. The reduced samples were applied on 8–18 % gradient or 12.5 % homogenous ExcelGel™ SDS-PAGE gels. Approximately 7.5 µg of protein was loaded per lane.

One protein and four different protocols

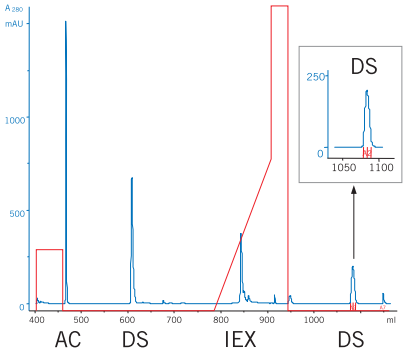
A (histidine)$_6$-tagged protein, APB7 (M_r 28x10^3, pI 6.0), was purified using four different multistep protocols. The pooled fractions from the final purification step were analyzed by SDS-PAGE.



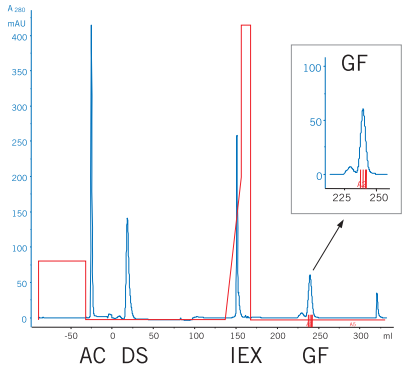
Columns:
HisTrap™ HP, 5 ml;
HiPrep™ 26/10 Desalting



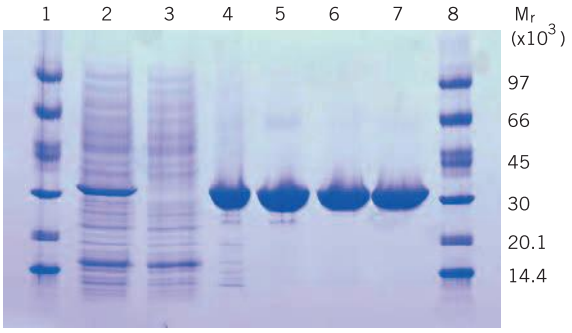
Columns:
HisTrap HP, 1 ml;
HiLoad™ 16/60 Superdex™ 75 pg



Columns:
HisTrap HP, 5 ml;
HiPrep 26/10 Desalting;
RESOURCE™ Q, 6 ml



Columns:
HisTrap HP, 5 ml;
HiPrep 26/10 Desalting;
RESOURCE Q, 1 ml;
HiLoad 16/60 Superdex 75 pg



SDS-PAGE analysis

The degree of purity as judged visually was in the following order:
AC–DS < AC–GF < AC–DS–IEX–DS < AC–DS–IEX–GF.

Lane 1. Low Molecular Weight marker
Lane 2. Start sample
Lane 3. Flow through
Lane 4. AC–DS
Lane 5. AC–GF
Lane 6. AC–DS–IEX–DS
Lane 7. AC–DS–IEX–GF
Lane 8. Low Molecular Weight marker

Four different proteins and one protocol

Four (histidine)$_6$-tagged proteins were purified using the same four-step protocol, AC–DS–IEX–GF. The ion exchange and gel filtration steps are shown below.

SDS-PAGE

Lane 1. Low Molecular Weight marker
Lane 2. Start sample
Lane 3. Flow through
Lane 4. Pooled protein after AC–DS–IEX–GF

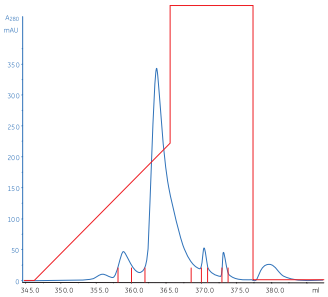
Ion Exchange Chromatography

Gel Filtration

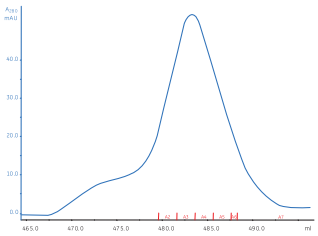
SDS-PAGE

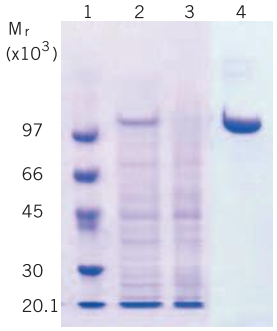
Sample:
P-450, M_r 121 x10^3, pI 5.3

Column: HiLoad 16/60 Superdex 200 pg



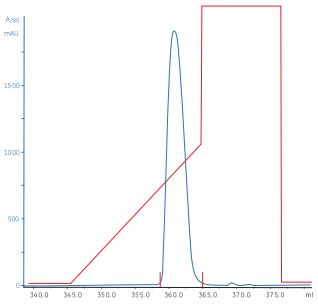
Column: RESOURCE Q, 1ml



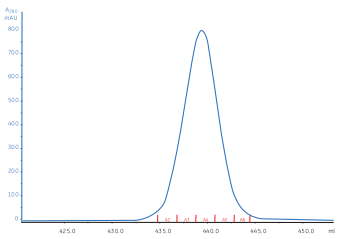


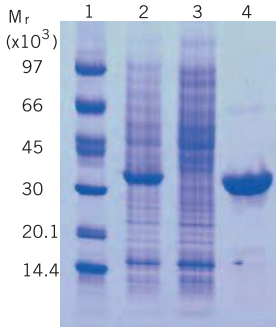
Sample:
APC234, M_r 32.5 x10^3, pI 5.8

Column: RESOURCE Q, 1ml



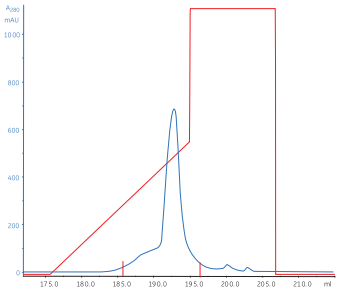
Column: HiLoad 16/60 Superdex 75 pg



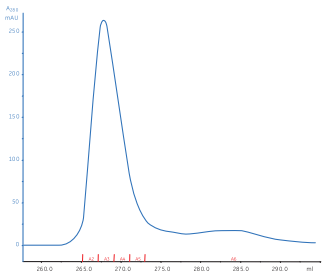


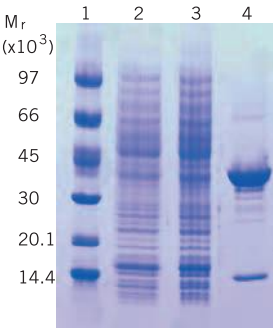
Sample:
APC1040, M_r 38.9 x10^3, pI 5.7

Column: RESOURCE Q, 1ml



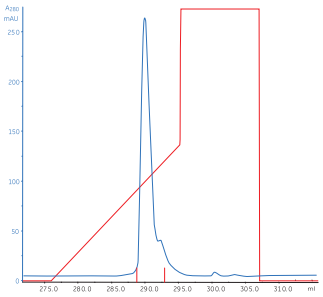
Column: HiLoad 16/60 Superdex 75 pg



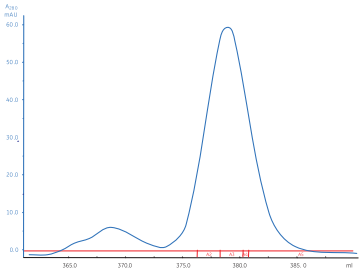


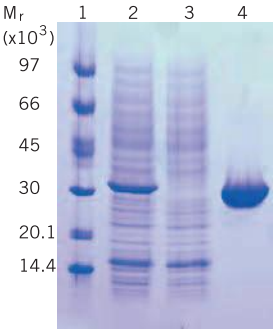
Sample: APB7,
M_r 28 x10^3, pI 6.0

Column: RESOURCE Q, 1ml



Column: HiLoad 16/60 Superdex 75 pg





Ion Exchange Chromatography

The software and hardware were able to detect and handle peaks with different shapes. The largest peak was injected on to the gel filtration column.

Gel Filtration and SDS-PAGE analysis

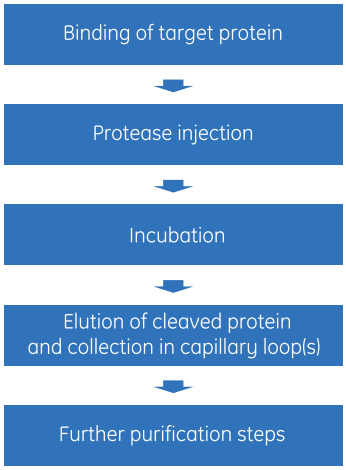
In the final step, peak fractionation was performed and all peaks were collected in the integrated fraction collector.

For all four proteins high purity was achieved using the same pre-optimized protocol.

Automated tag removal

All multistep purification protocols in ÄKTAxpress can be combined with automated on-column tag cleavage. Tag cleavage is always performed on the affinity column prior further purification steps. When the cleaved protein has been eluted the affinity column is regenerated and affinity tag, tagged protease and remaining uncleaved protein is collected in separate outlet.

Procedure

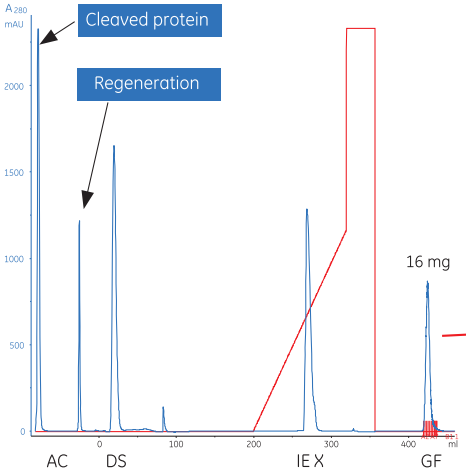


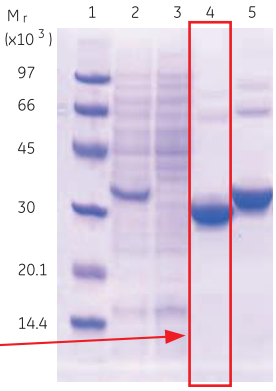
Four-step protocol: (histidine)$_6$-tagged protein cleaved with AcTEV protease

Sample: APC234, M_r 32.5 x10^3 (Cleaved product: M_r 30.1 x10^3)

Columns: HisTrap HP, 5 ml; HiPrep 26/10 Desalting; RESOURCE Q, 6 ml; HiLoad 16/60 Superdex 75 pg

Cleavage conditions: 200 units of AcTEV™ Protease (Invitrogen)/mg protein, 8 hours incubation time at room temperature.





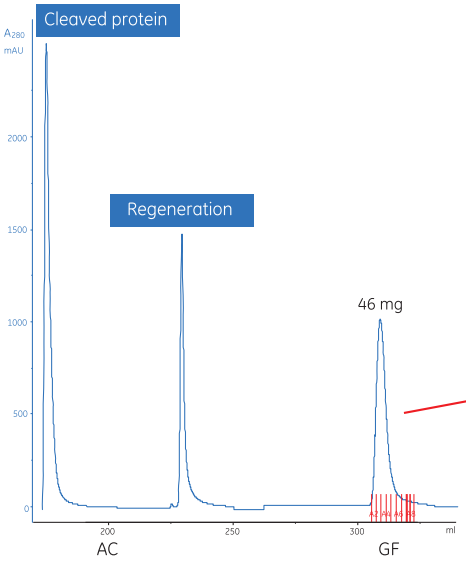
Lane 1. Low Molecular Weight marker
Lane 2. Start sample
Lane 3. Flow through
Lane 4. Purified cleaved APC234
Lane 5. Reference: uncleaved APC234

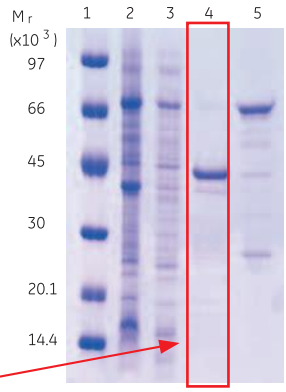
Two-step protocol: GST-tagged protein cleaved with PreScission protease

Sample: GST-purα, M_r 61.6 x10^3 (Cleaved product: M_r 35.2 x10^3)

Columns: GSTrap™ HP, 5 ml; HiLoad 16/60 Superdex 75 pg

Cleavage conditions: 20 units of PreScission™ protease/mg protein, 8 hours incubation time in cold room.

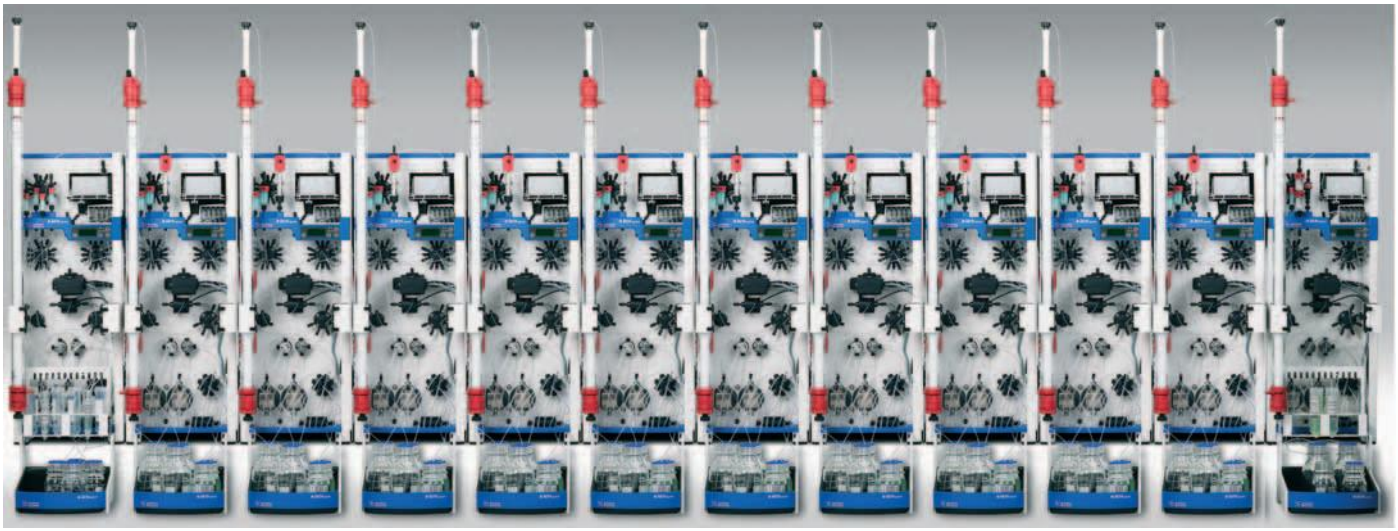




Lane 1. Low Molecular Weight marker
Lane 2. Start sample
Lane 3. Flow through
Lane 4. Purified cleaved GST-purα
Lane 5. Reference: uncleaved GST-purα

High-throughput protein purification

- Up to 12 modules in parallel can be controlled from one computer
- Up to 4 samples can be purified per module
- Up to 48 proteins can be purified within 14 hours using a two-step protocol
- Up to 24 proteins can be purified within 11 hours using a four-step protocol



Acknowledgements

We are thankful to Dr. Darcy Birse at the Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden and TGN Biotech Inc., Québec, Canada, for providing us with GST-purα protein. We are also grateful to Argonne National Laboratory, US, for providing us with APC234 and APC1040, two soluble protein targets of the Midwest Center for Structural Genomics (www.mcsg.anl.gov).

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