Rapid protein analysis and screening of membrane protein buffer conditions by gel filtration

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Rapid protein analysis and screening of membrane protein buffer conditions by gel filtration

Inger Salomonson[1], *Said Eshaghi*[2], *Fredrik Lundström*[1], *and Marianne Carlsson*[1]

[1] GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden
[2] Karolinska Institute, Stockholm, Sweden

Background

Screening of different conditions is often necessary to obtain protocols that result in pure and stable target proteins. Size-exclusion chromatography (SEC) is an excellent and gentle technique to study the state of aggregation of a protein as well as the purity of a target protein. SEC, however, is often time consuming and can also be hampered by the expense of sample and detergents. A new, small (3 ml) column format has been developed for rapid and reliable size-exclusion chromatography, employing Superdex™ 200, a powerful medium for size distribution analysis.

Column Characteristics

Column:	Tricorn™ glass column
Column dimensions:	5 mm diameter, 150 mm height
Bed volume:	3 ml
Medium:	Superdex 200
Separation range M_r:	10 000-600 000
Recommended flow rate:	0.15-0.6 ml/min
Maximum flow rate:	0.8 ml/min (245 cm/hr)
Recommended sample volume:	4-50 µl
Max. pressure over column:	15 bar (217 psi, 1.5 MPa)

Fig 1. Tricorn columns Superdex™ 75 and Superdex 200

Conclusions

- Protein-Protein interaction studies are easily carried out using Superdex 200 5/150 GL columns.
- Screening of buffer conditions for a membrane protein was performed within a few hours and with only 6 × 10 µl (68 µg) sample consumption.
- Rapid purity check of an IMAC purified protein gave results similar to SDS-PAGE analysis, but in significantly less time.

Protein-Protein Interaction

To evaluate the ability of Superdex 200 5/150 GL to monitor complex formation, Trypsin and Soy Trypsin Inhibitor (STI) were analyzed. First Trypsin and STI were run separately and then a 1:1 mixture was analyzed on the gel filtration column. Only one peak eluted from the mixture of Trypsin and STI with an elution volume shifted toward the void volume, indicating interaction between Trypsin and STI (Fig 2).

Column:	*Superdex 200 5/150 GL*
Samples:	*Trypsin 1 mg/ml; Soy Trypsin Inhibitor 1 mg/ml; Trypsin 1 mg/ml and Soy Trypsin Inhibitor 1 mg/ml*
Sample volume:	*12.5 µl*
Buffer	*PBS, pH 7.4*
Flow rate:	*0.3 ml/min*
System:	*ÄKTAexplorer™ 10*

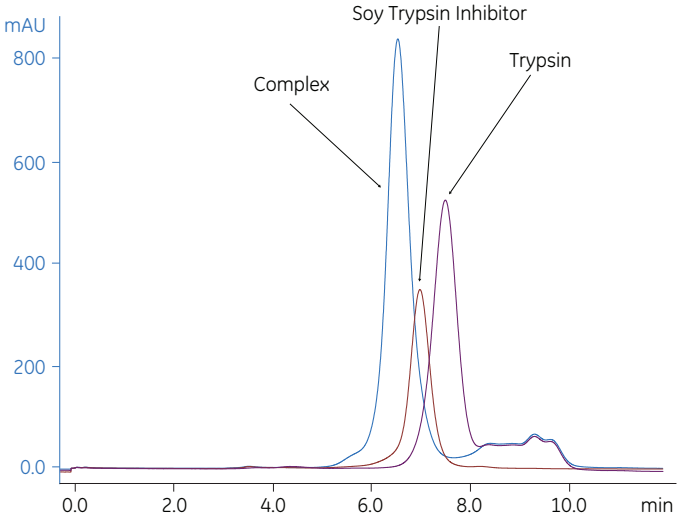

Fig 2. Analysis of complex formation of Trypsin and Soy Trypsin Inhibitor.

Screening of buffer conditions for a membrane protein

A 60 000 M_r integral membrane protein was purified for crystallization trials. The purified protein precipitated immediately during concentration at a neutral pH. Rapid gel filtration with Superdex 200 GL 5/150 was used to screen for a stable protein under various pH and salt conditions.

Column:	*Superdex 200 5/150 GL*
Sample:	*Integral membrane protein from E. coli*
Sample volume:	*10 µl*
Eluents (including 0.1 or 0.3 M NaCl):	*0.02 M sodium acetate, 0.03% dodecyl maltoside, 0.5 mM TCEP pH 5.2 0.02 M HEPES, 0.03% dodecyl maltoside, 0.5 mM TCEP pH 7.5 0.02 M CAPSO, 0.03% dodecyl maltoside 0.5 mM TCEP pH 9.5*
Flow rate:	*0.35 ml/min*
System:	*ÄKTAexplorer 10*

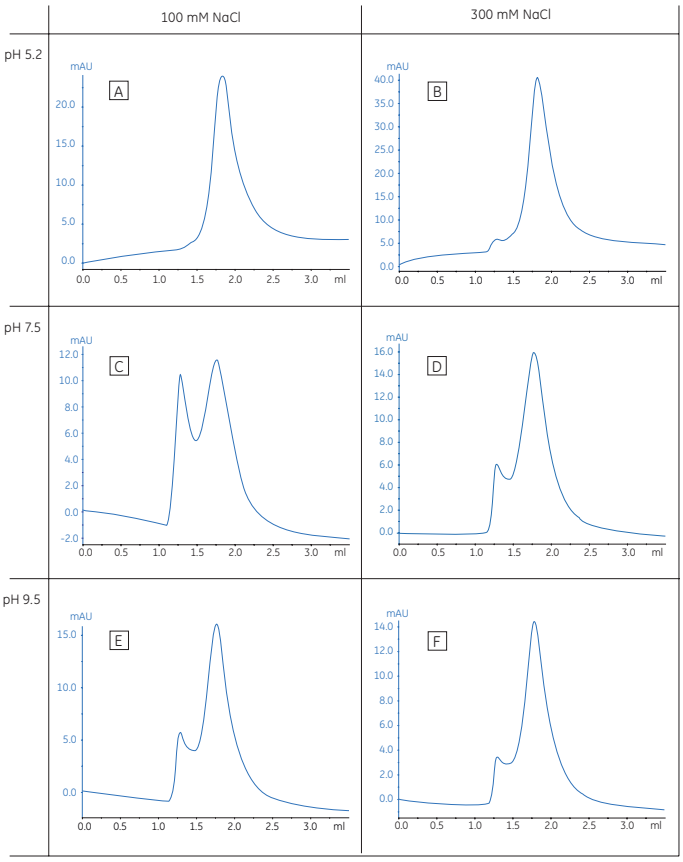

Fig 3. Screening of pH and ionic strength by gel filtration.

A symmetrical peak was observed when the separation was performed at pH 5.2 in 0.1 M NaCl (Fig 3A). This shows a homogenous size distribution of the protein under these conditions. At higher salt concentration (0.3 M NaCl, Fig 3B), a small peak appeared close to the void volume, indicating oligomerization or aggregation to a limited extent. Both at pH 7.5 and pH 9.5 (Figs 3C-F) large peaks were obtained close to the void volume, showing severe oligomerization or aggregation.

The complete screening procedure was performed in only a few hours, including the time for column equilibration. Sample consumption was 6 × 10 µl (68 µg protein) for the complete screen. Subsequently the protein was successfully concentrated in 0.1 M NaCl at pH 5.2 for crystallization trials.

Rapid Purity Check

To achieve high purity in IMAC purifications it is often necessary to optimize the imidazol concentration in the start buffer. An extract of histidine-tagged GFP was purified on a HisTrap™ HP 1 ml column in start buffers containing 0.005, 0.01, 0.02, 0.04, and 0.06 M imidazol. The bound protein was eluted stepwise with 0.5 M imidazol (data not shown).

Immediately after the first purification was finished, the eluted peak material was pooled and analyzed on Superdex 200 5/150 in Ettan™ LC. Each analytical run was finished within 15 min. The analysis of the fractions with gel filtration showed that the purity of the fractions increased with the imidazol concentration in the start buffer (Fig 4). In this case, 0.04 M imidazol in the start buffer was recommended for the final purification of GFPHis$_6$ and a similar conclusion could be drawn from the analysis of the fractions by SDS-PAGE electrophoresis (Fig 5).

Column:	Superdex 200 5/150 GL
Sample:	fractions of purified GFP-His$_6$
Sample volume:	50 µl
Buffer:	PBS, pH 7.4
Flow rate:	0.3 ml/min
System:	Ettan LC

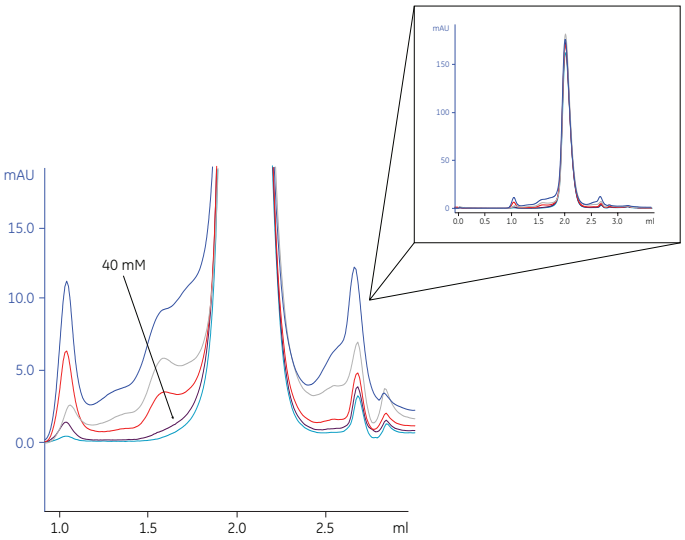

Fig 4. Gelfiltration analysis of fractions from purification of GFP-His$_6$.

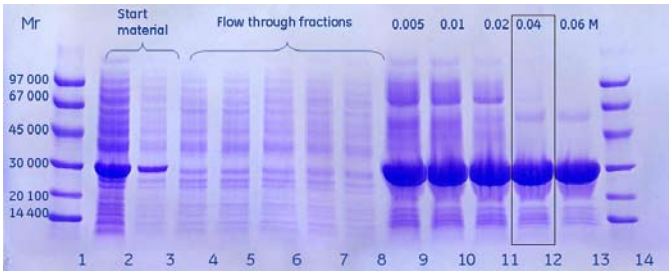

Fig 5. SDS-PAGE analysis of fractions from purification of GFP-His$_6$.

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

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