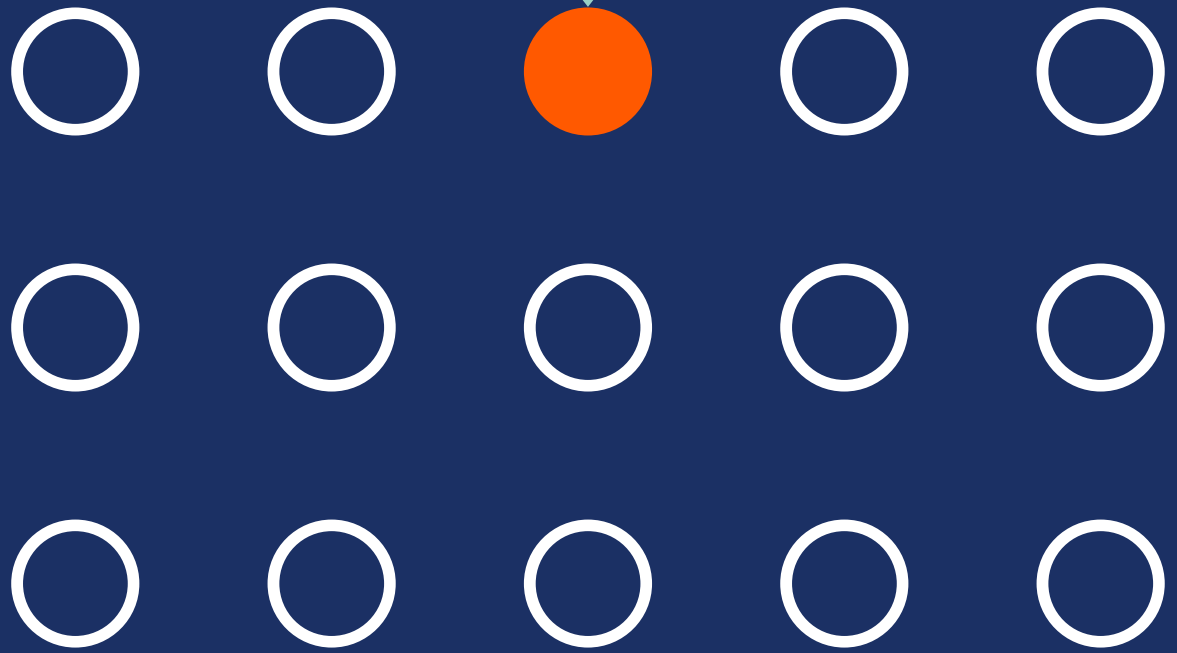
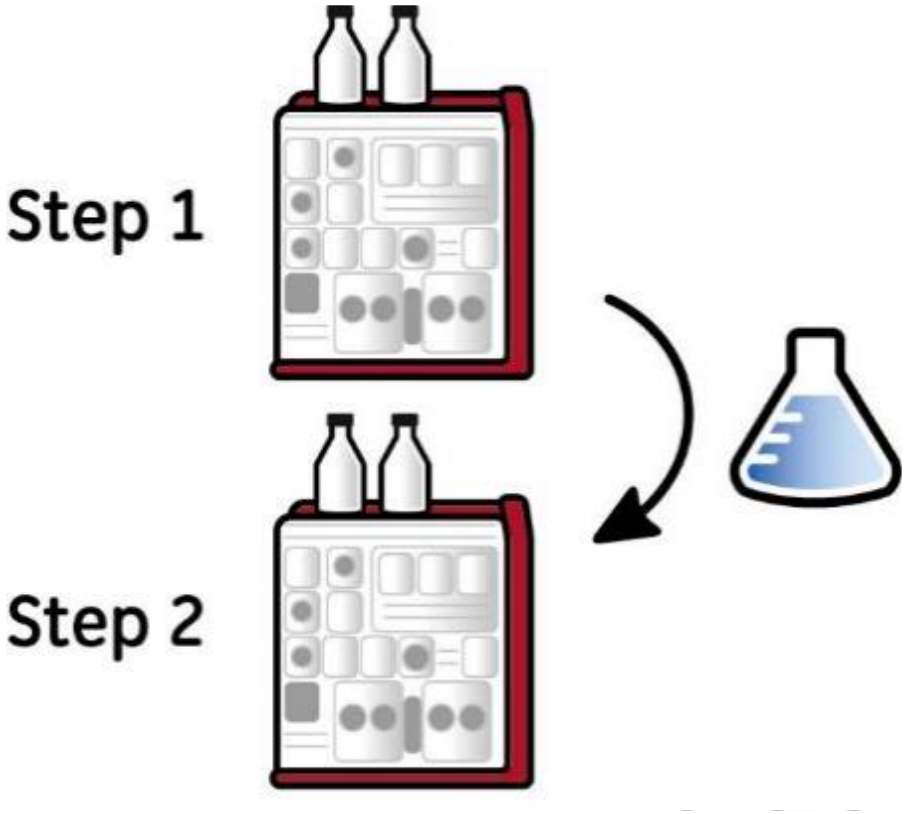
cytiva

Getting started with automated two-step protein purification



Benefits of automated two-step purification

Typically, manual intervention is needed



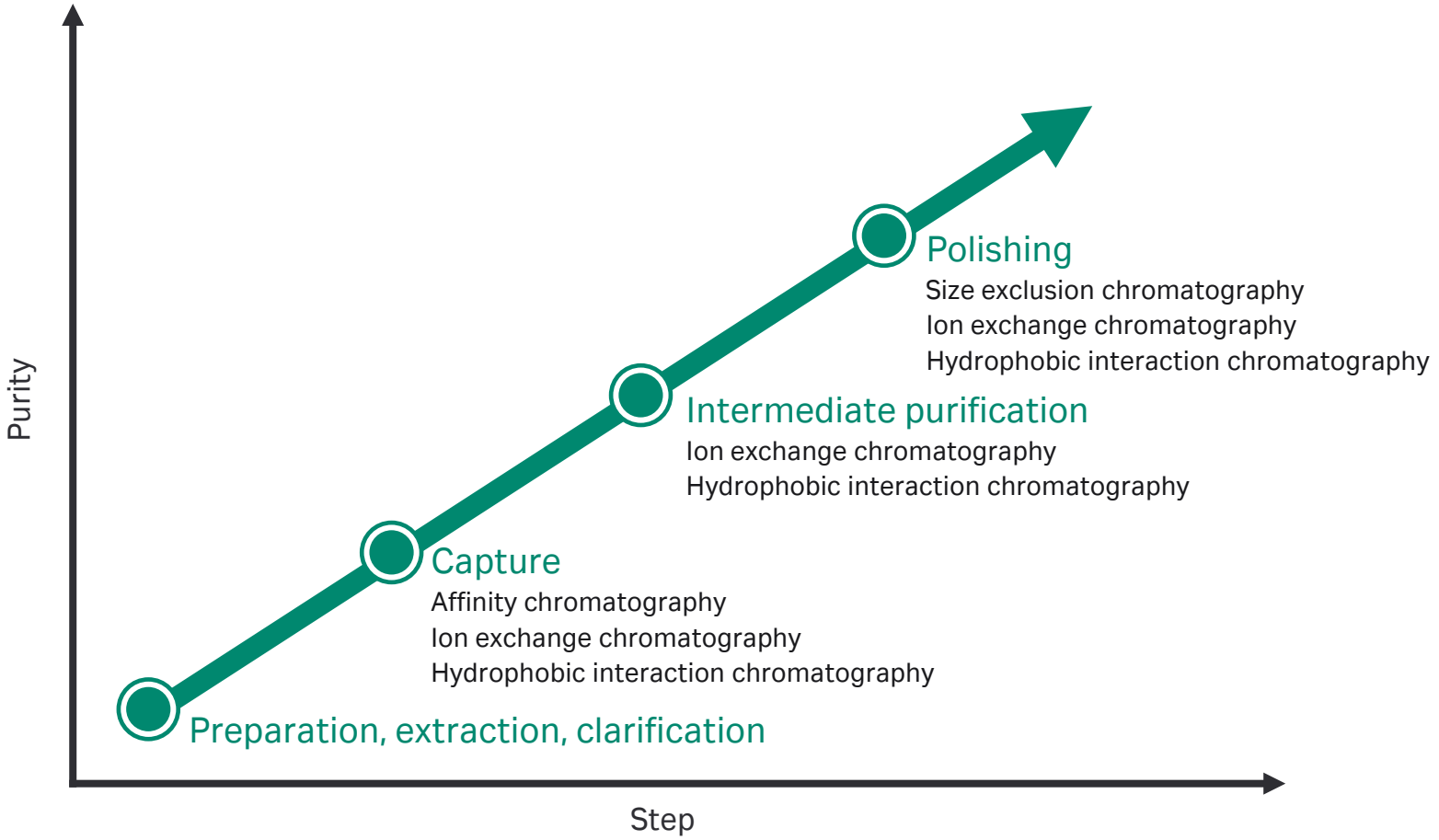
Reduce manual interactions

- Increase throughput
- Reduce labor
- Improve process consistency

Eliminate hold steps

- Ensure product stability
- Eliminate or reduce hold containers

Apply multistep purification on a wide range of purification schemes



Example application: Automated two-step purification using predefined methods

1. Capture

- Method Settings
- Equilibration
- Sample Application
- Column Wash
- Elution
- Column Wash
- Equilibration

Automatic transfer of target protein (peak) and start of second method

2. Polishing

- Method Settings
- Equilibration
- Sample Application
- Elution

Ways of working

1. Select purification protocol
2. Set up ÄKTA™ pure with components to support selected protocol
3. Set up capture method in UNICORN™ Method Editor
4. Set up polishing method in UNICORN Method Editor
5. Set up **Method queue**
6. Run the methods first with buffer and then with sample
7. Evaluate results

The following slides will demonstrate a workflow for a two-step purification with intermediate loop collection.

1. Select purification protocol

Select purification protocol

- Discuss with colleagues
- Web searches
- Reference literature
- Discuss with suppliers

Set up the protocol to combine a capture and a polishing step in one run.

2. Set up ÄKTA pure with components to support selected protocol using intermediate loop collection

The flexible system configuration allows for an optimized flow path

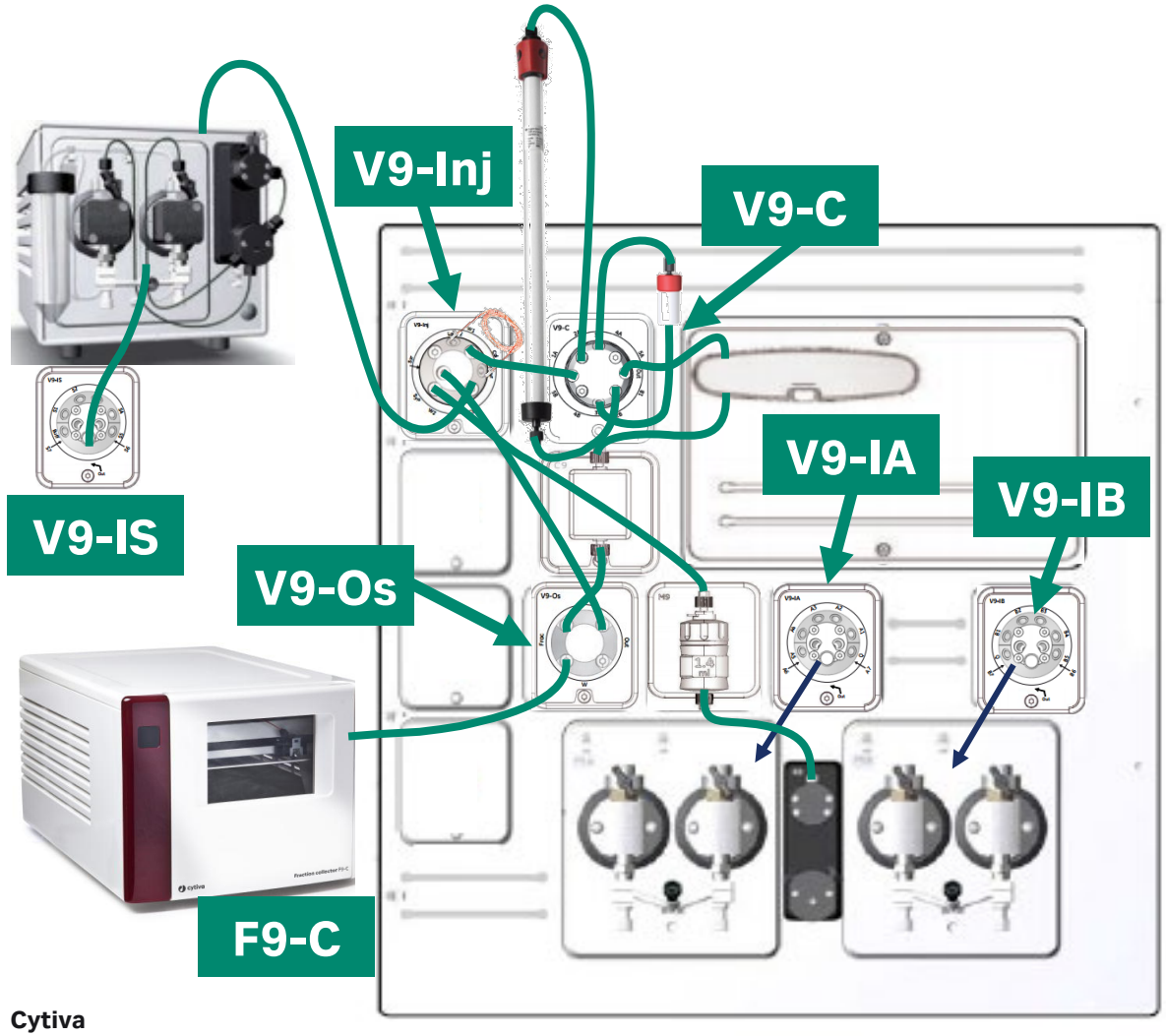
Components needed	Name
Sample inlet valve	V9-IS‡
Inlet valve	V9-IAB
Inlet valves*	V9-IA‡/ V9-IB‡
Injection valve	V9-Inj‡
Column valve	V9-C‡
UV monitor 1	U9-M‡/U9-L
Conductivity	C9n‡
Outlet valve	V9-O V9-Os‡
Versatile Valve†	V9-V
Fraction collector	F9-R‡/F9-C
Sample pump	P9S‡

* *Enables additional inlet buffers*

† *Use a versatile valve in front of injection valve if syringe position is needed for manual loop filling*

‡ *Used in the example described in the following slides*

2. Set up ÄKTA pure with components to support selected protocol (cont.)



Optimized flow path with intermediate loop collection used in the application example

A sample pump facilitates sample loading in capture step.

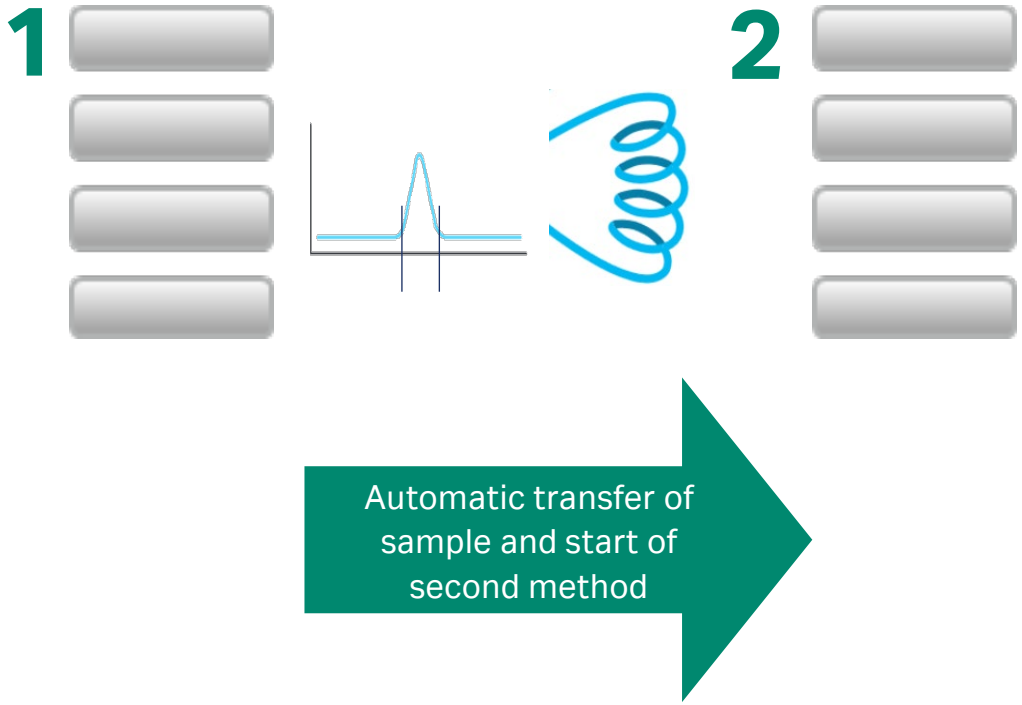
Connect outlet position from Outlet valve to Syringe port of injection valve.

When using V9-Os a fraction collector must be used.

Note: As an alternative, the sample can be loaded using the system pump and a mixer by-pass valve.

3. Method setup: Automated two-step purification with intermediate loop collection

Include first and second method in a *Method queue*



1. Set up your methods for the two steps as individual methods using the predefined protocols
   - **Capture step**
     Use peak fractionation (via the outlet valve) to direct the eluted peak from the capture step to the loop
   - **Polishing step**
     Fractionate normally to Fraction collector or V9-O
2. Create **Method queue** and start the run in System Control

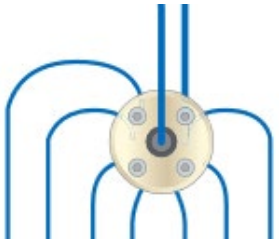
3. Method setup

Method 1: Capture step

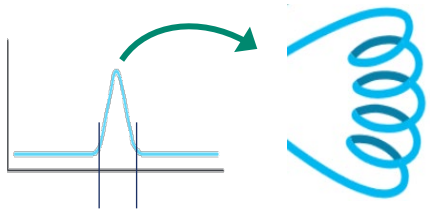
Method 2: Polishing step

Load sample for the capture step

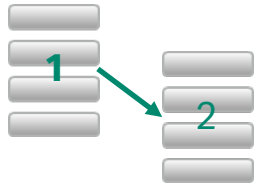
Eluted peak is automatically directed from outlet valve via syringe port to injection valve loop



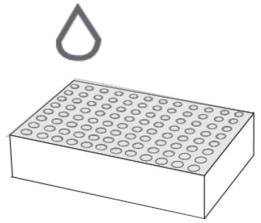
Sample (peak) from capture is stored in loop between first and second method



Method queue **function automatically starts second method**

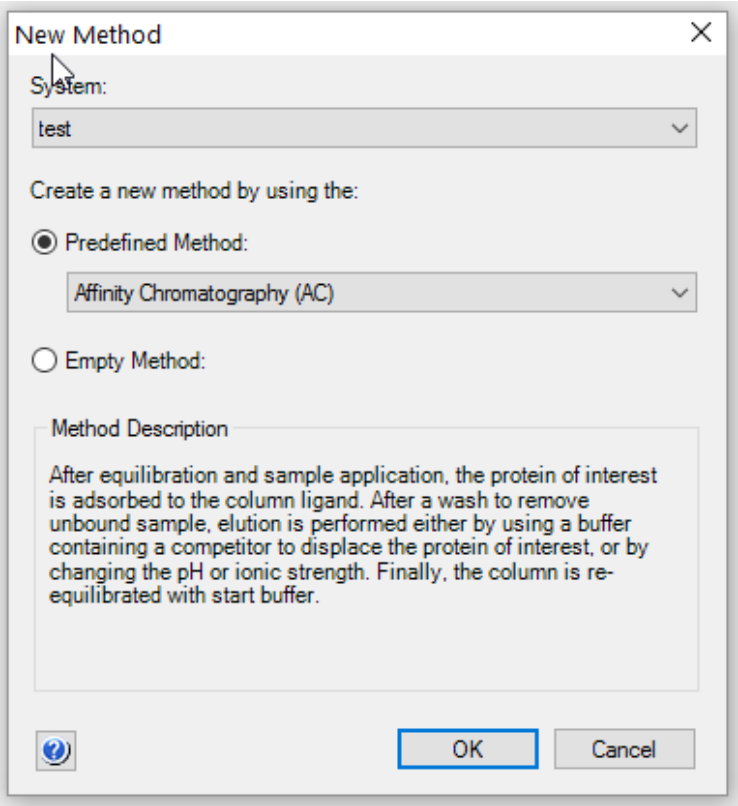


Target molecule is fractionated



3. Method setup: Capture
Select adsorption technique

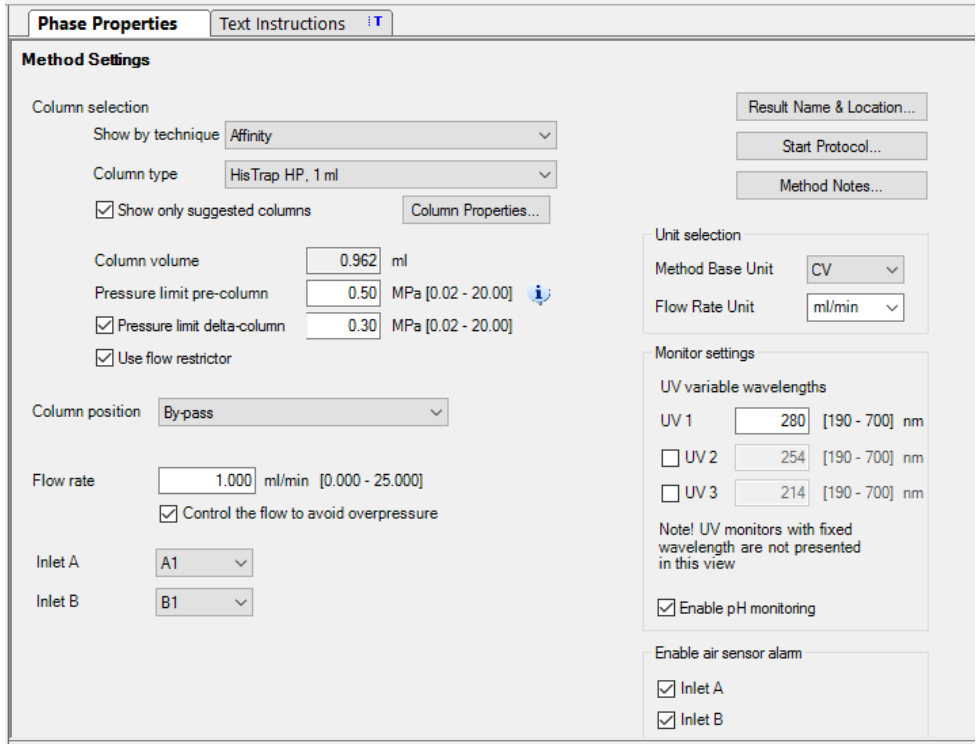
Select predefined method in *Method editor*:



Different phases (steps) will be displayed in *Method outline*

3. Method setup: Capture
Adjust **Phase properties**

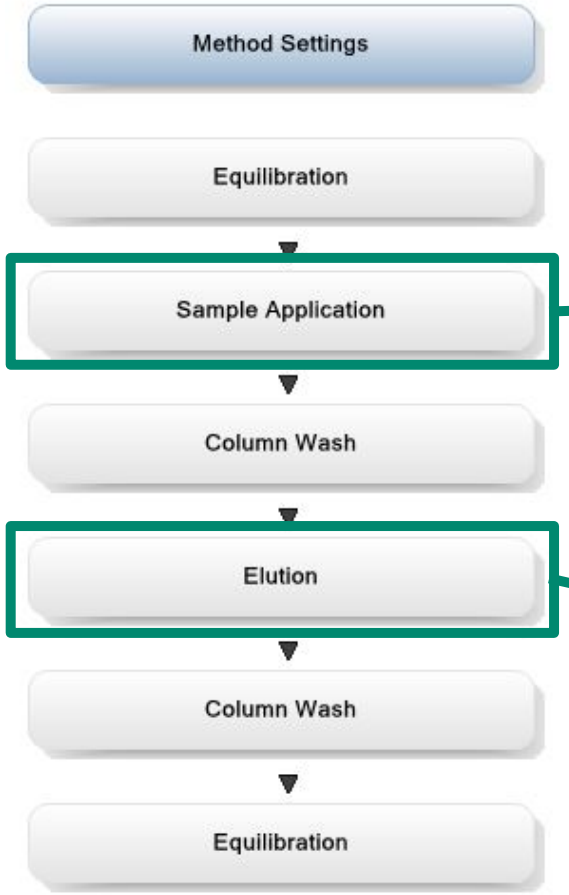
Define parameters for each phase in *Phase properties* tab



Settings available in *Phase properties*

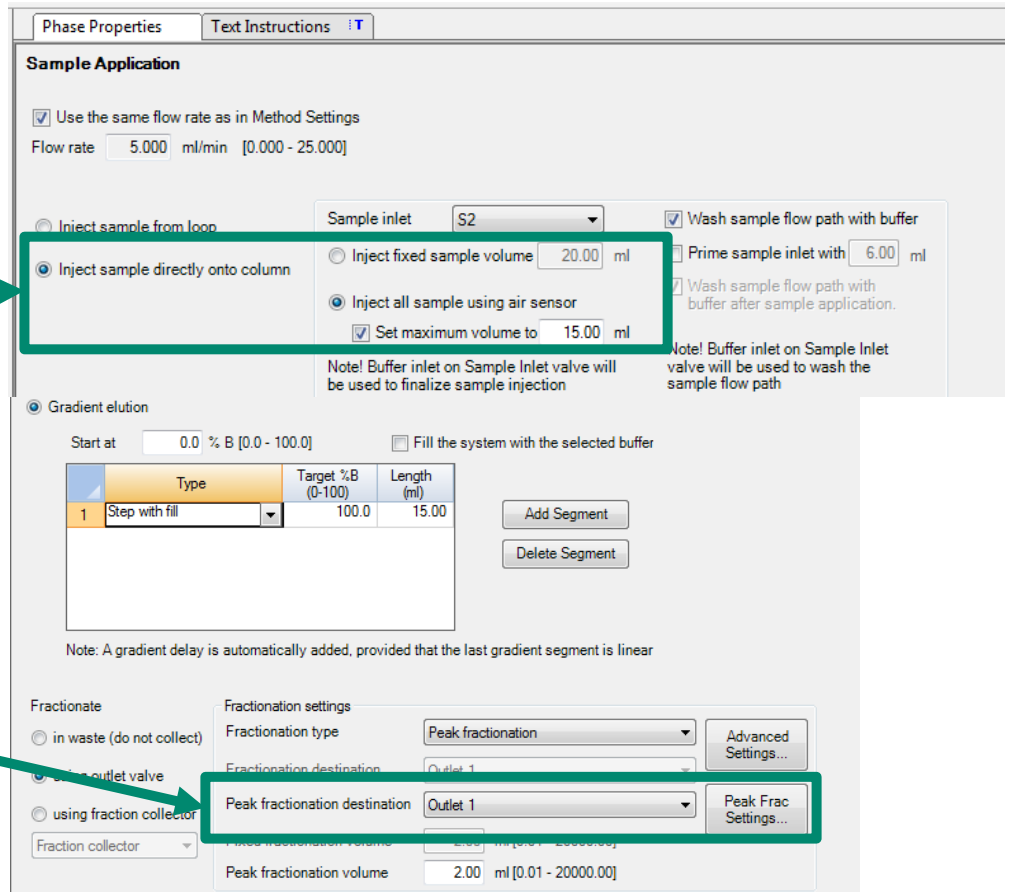
- Flow rate and pressure limits for selected column
- Column position
- Inlets to be used
- Base unit
- Monitor settings

3. Method setup: Capture
Sample application and **Elution** properties



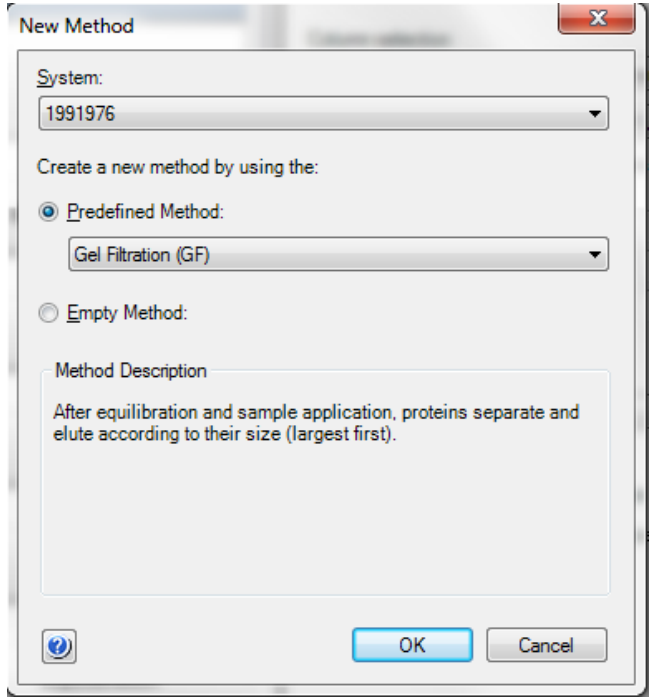
Sample application
Load sample using a sample pump.

Elution
Use peak fraction to collect the eluting peak in a loop.

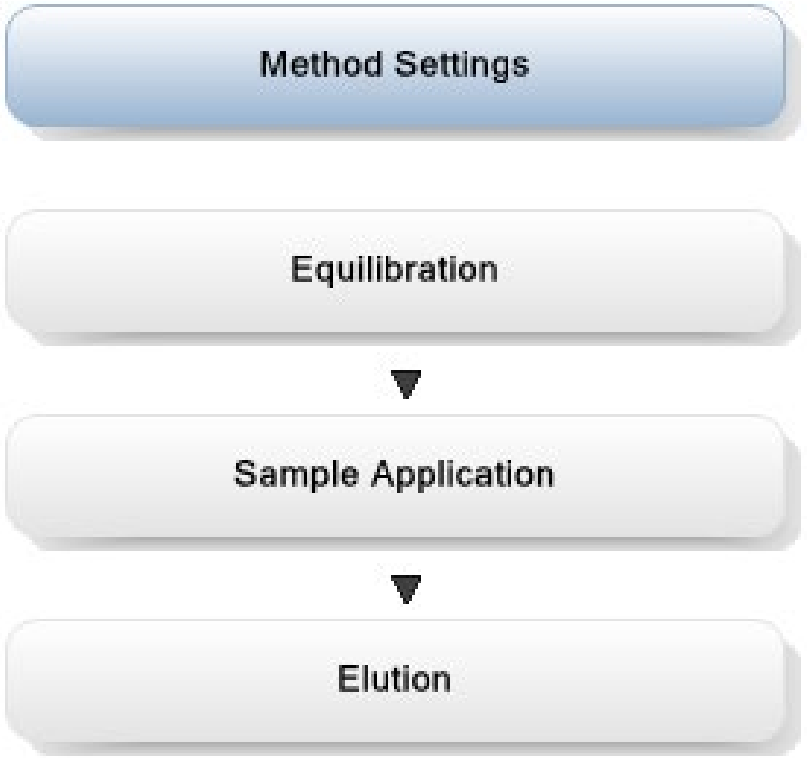


4. Method setup: Polishing
Select **SEC*/Desalting** in **Method editor**

Select predefined method: *SEC*/Desalting* in *Method editor*



Method will be displayed in *Method outline*

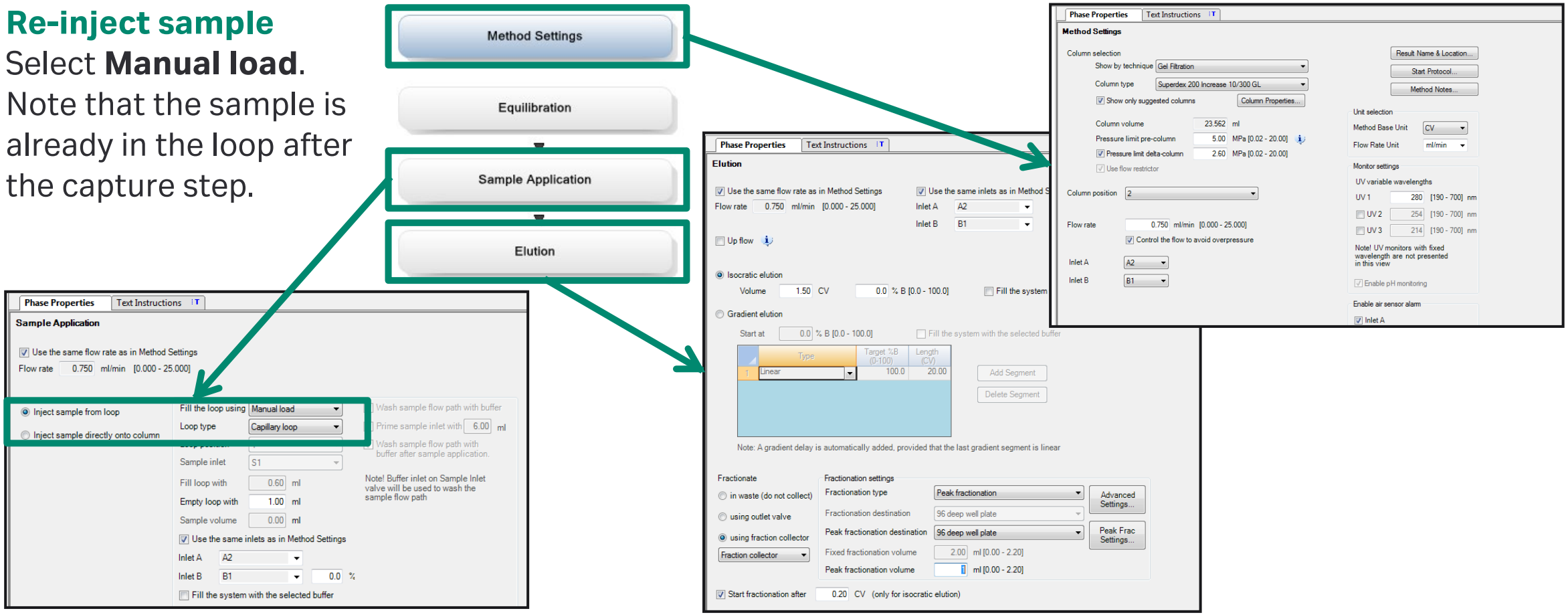


*Size exclusion chromatography, also called gel filtration (GF)

4. Method setup: Polishing
Method settings, **Sample application**, and **Elution** properties

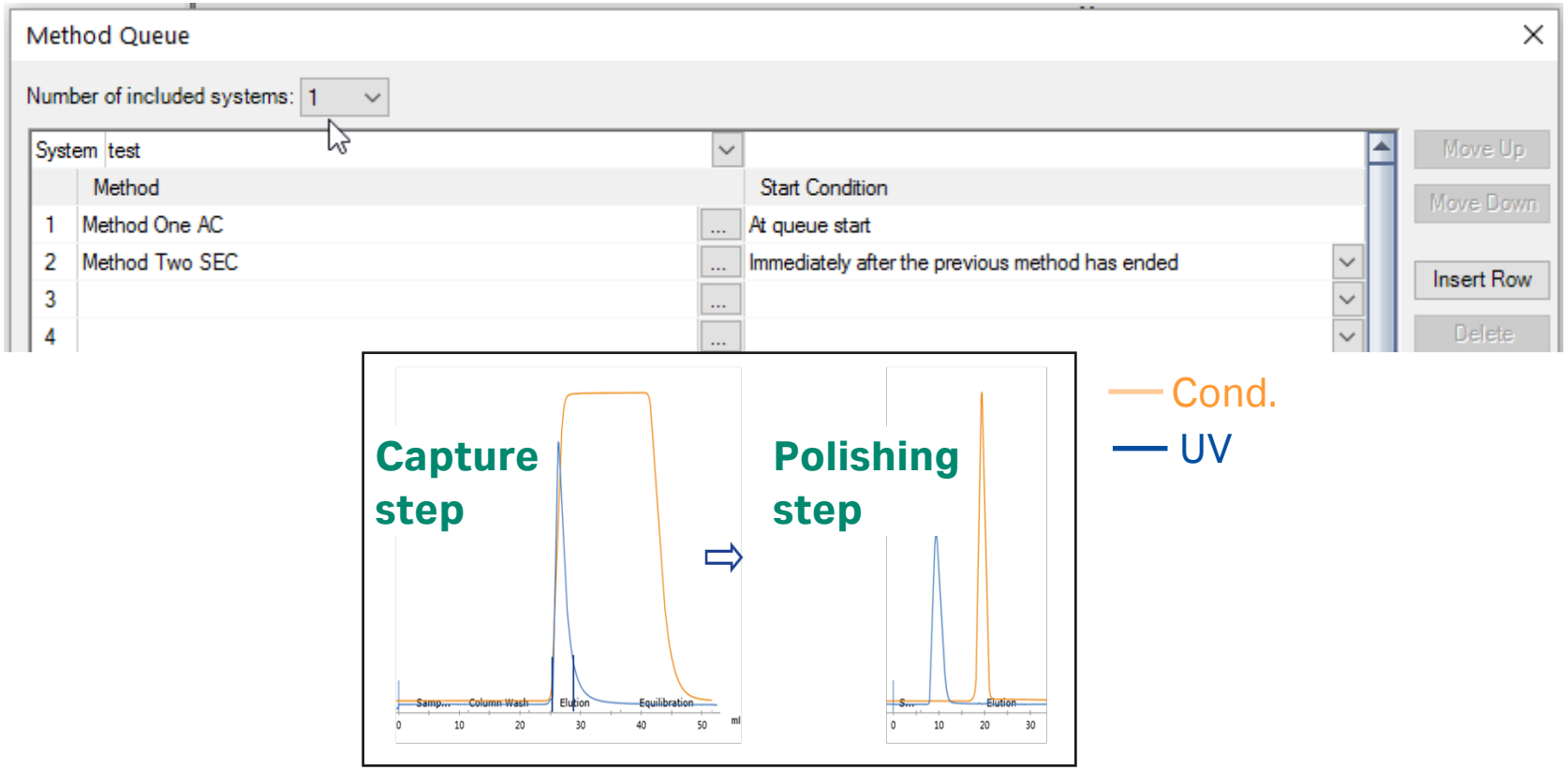
Re-inject sample

Select **Manual load**.
Note that the sample is already in the loop after the capture step.



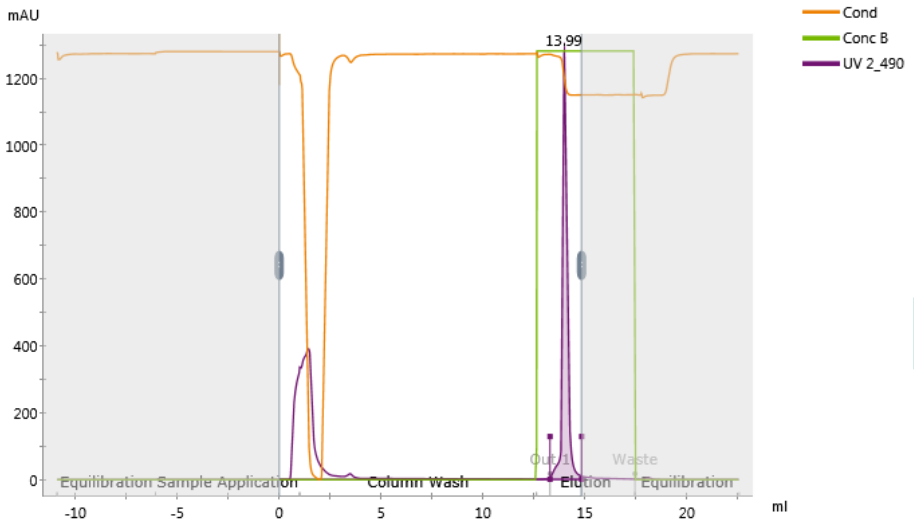
5. Method setup: Create **Method queue**
Include the capture and polishing step in **Method queue**

Method queue* is set in UNICORN™ *Method editor

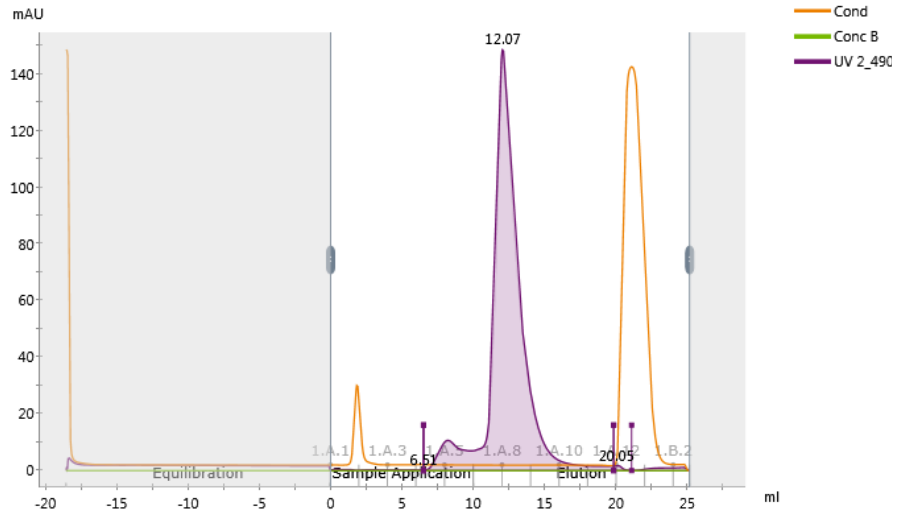


6. Running the methods
Automated two-step purification, example 1

Capture: Affinity chromatography with HisTrap™ HP 1 mL



Polishing: SEC* Superdex™ 75 Increase



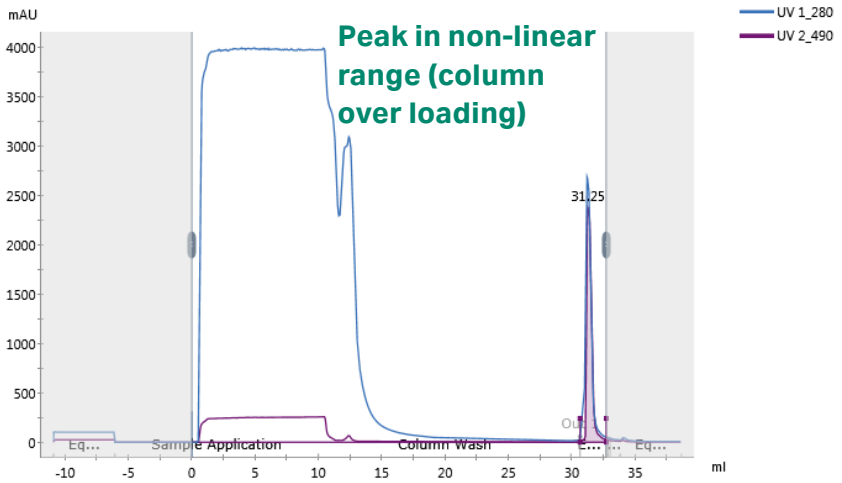
Method	Peak	Retention (mL)	Area (mL*mAU)	Volume (mL)
AC	Peak A	13.990	313.2	1.549
SEC	Peak A	12.070	295.4	13.329

Purple peaks are similar in area indicating minimal sample loss.

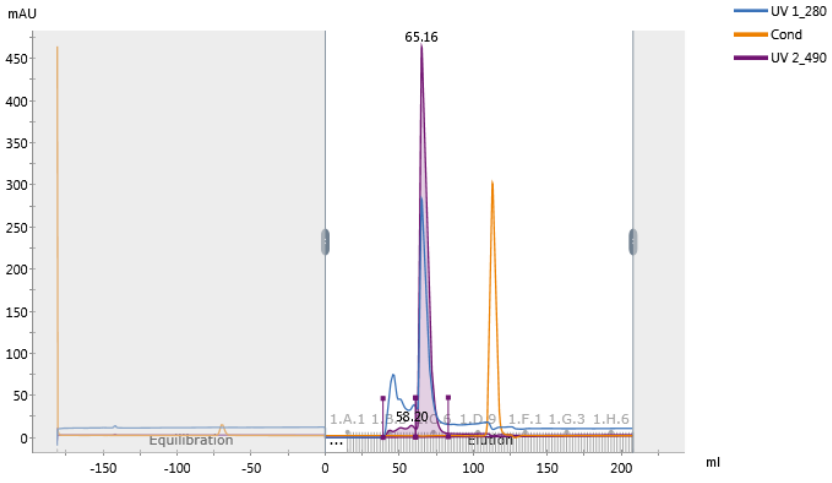
Sample: GFP-His (clarified homogenate, 5.3 mg GFP-His/g *E. coli* cell pellet)
*Size exclusion chromatography, also called gel filtration (GF)

6. Running the methods
Automated two-step purification, example 2

Capture: Affinity chromatography with HisTrap™ HP 1 mL



Polishing: SEC* HiLoad™ 16/600 Superdex™ 75 pg

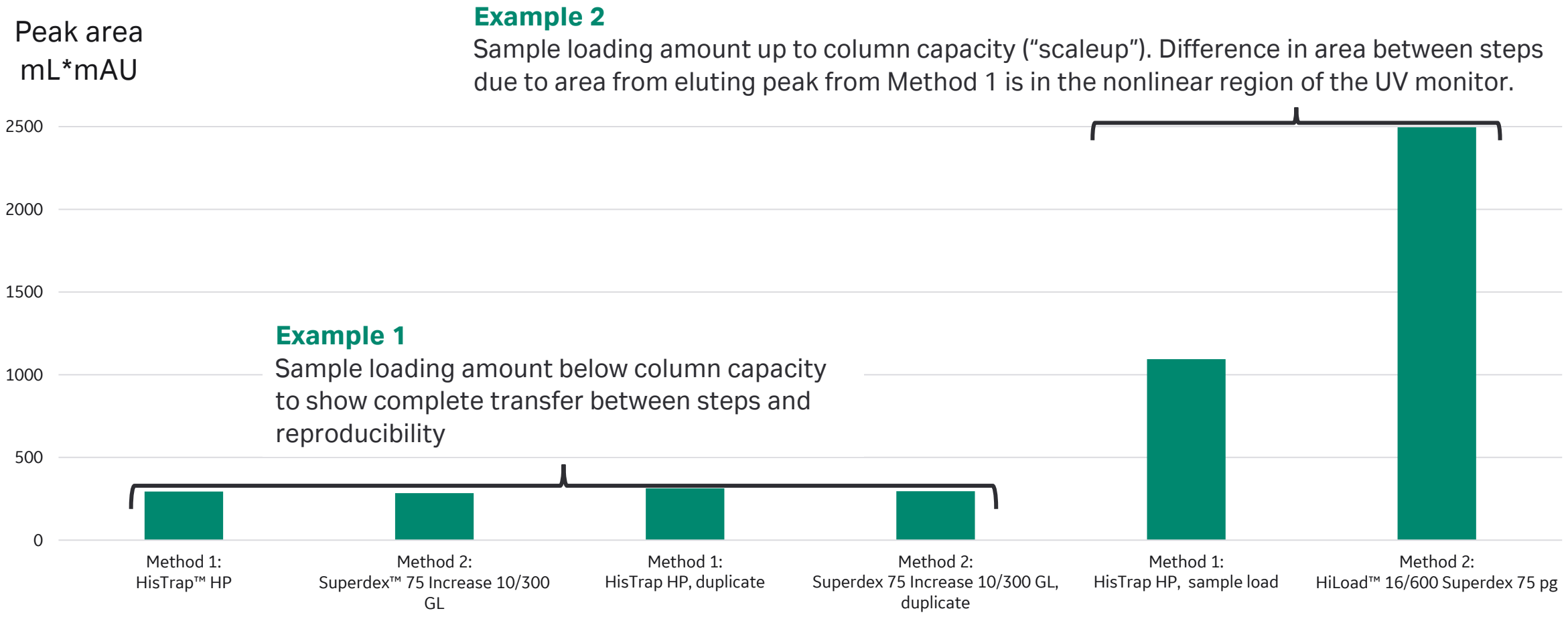


Method	Peak	Retention (mL)	Area (mL*mAU)	Volume (mL)
AC	Peak A	31.246	1094	2.076
SEC	Peak B	65.158	2496	22.000

In the capture step UV is measured outside of the linear range of the monitor, therefore peak areas cannot be compared.

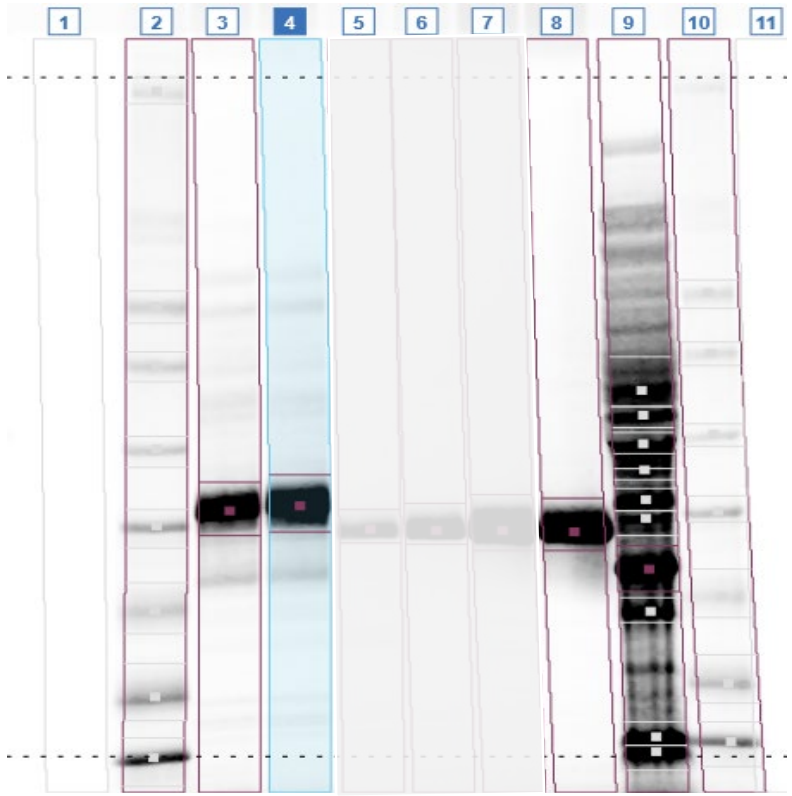
Sample: GFP-His (clarified homogenate, 5.3 mg GFP-His/g *E. coli* cell pellet)
*Size exclusion chromatography, also called gel filtration (GF)

7. Evaluation
Integration results from example 1 and 2



7. Verification
Reference analysis with electrophoresis

Results form electrophoresis



Reference analysis to verify identified peaks

Sample ID:

1. Blank
2. Amersham™ WB molecular weight markers
3. Two-step purification protocol AC-GF HisTrap™ 1 mL + Superdex™ 75 Increase 10/300 GL
4. Two-step purification protocol AC-GF HisTrap 1 mL + Superdex 75 Increase 10/300 GL, duplicate

5.–7. Blanks

8. Two-step purification protocol AC-GF HisTrap 1 mL + HiLoad™ 16/60 Superdex 75 pg, scaleup
9. Start material His-GFP, *E. coli*
10. Amersham WB molecular weight markers

Utilize standard hardware and predefined UNICORN methods for automated two-step purification

ÄKTA™ systems in automated multistep purification

Combine different chromatography steps in automated multistep purification to save time and add consistency.

Set up automated, two-step purification using predefined methods and **Method queue** function to quickly get started.

Store peak from step one in loop before automatically continuing to second step.

ÄKTA pure was used in the example application shown. ÄKTA avant can also be used in the same way.

Thank you

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