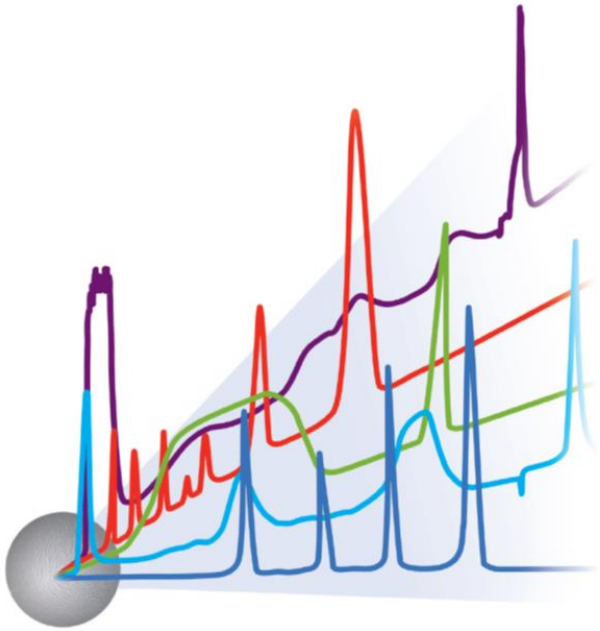
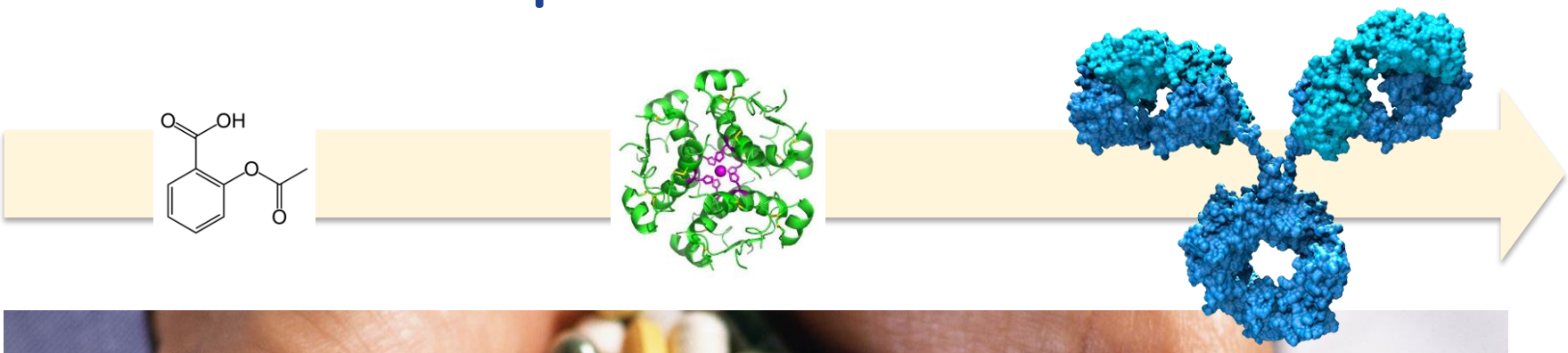


Introduction to purification strategy and purification optimization

Andy Chuang
Product Manager
andychuang@mail.level.com.tw

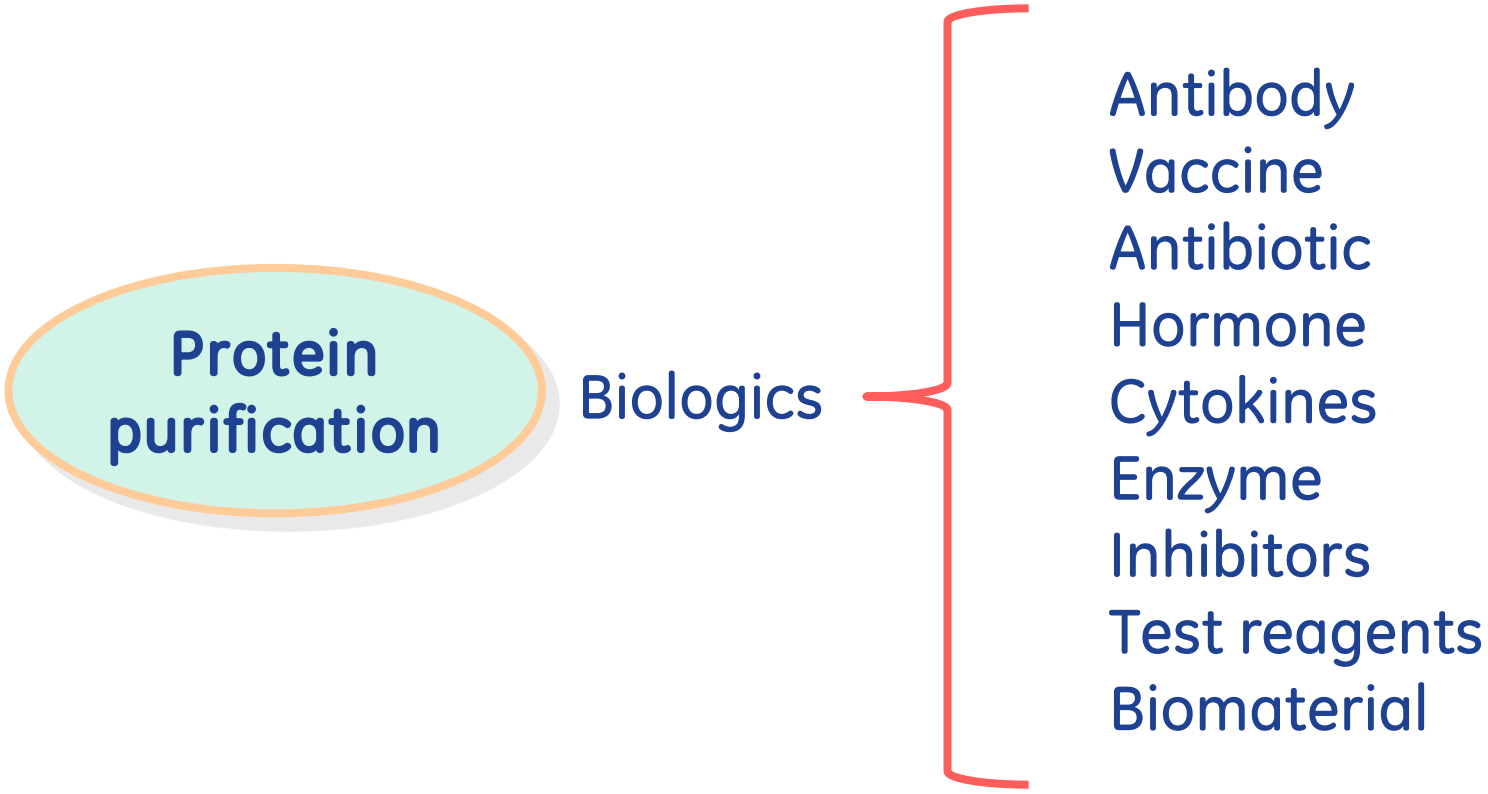
Level Biotechnology Inc.
進階生物科技股份有限公司
Discovering new boundaries

The trend of Biopharmaceutical?



In 2013, there were over 300 approved biopharmaceutical drugs on the market. A quarter of these drugs were related to monoclonal antibodies (MAbs).

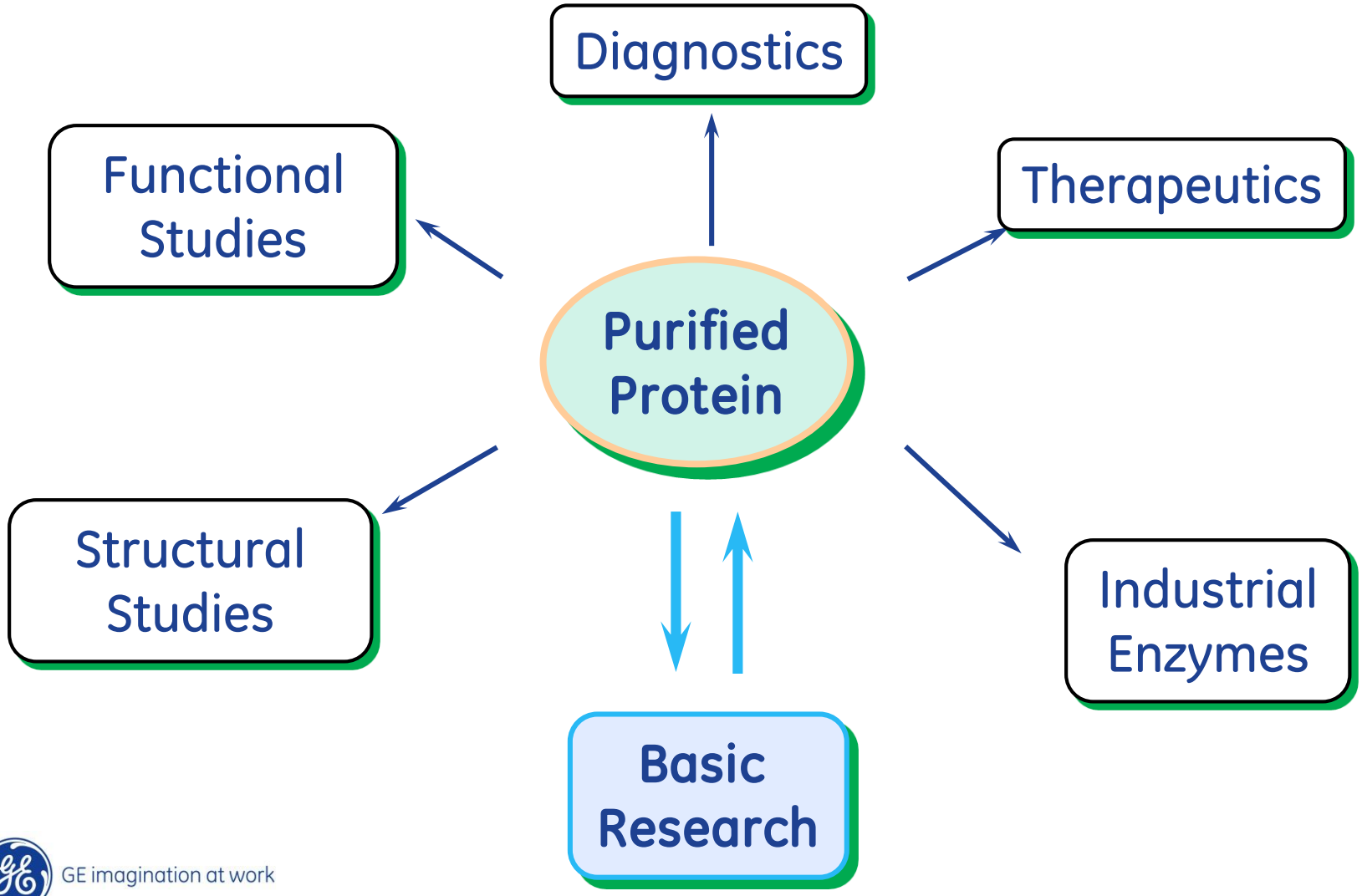
Why purify proteins?



Content

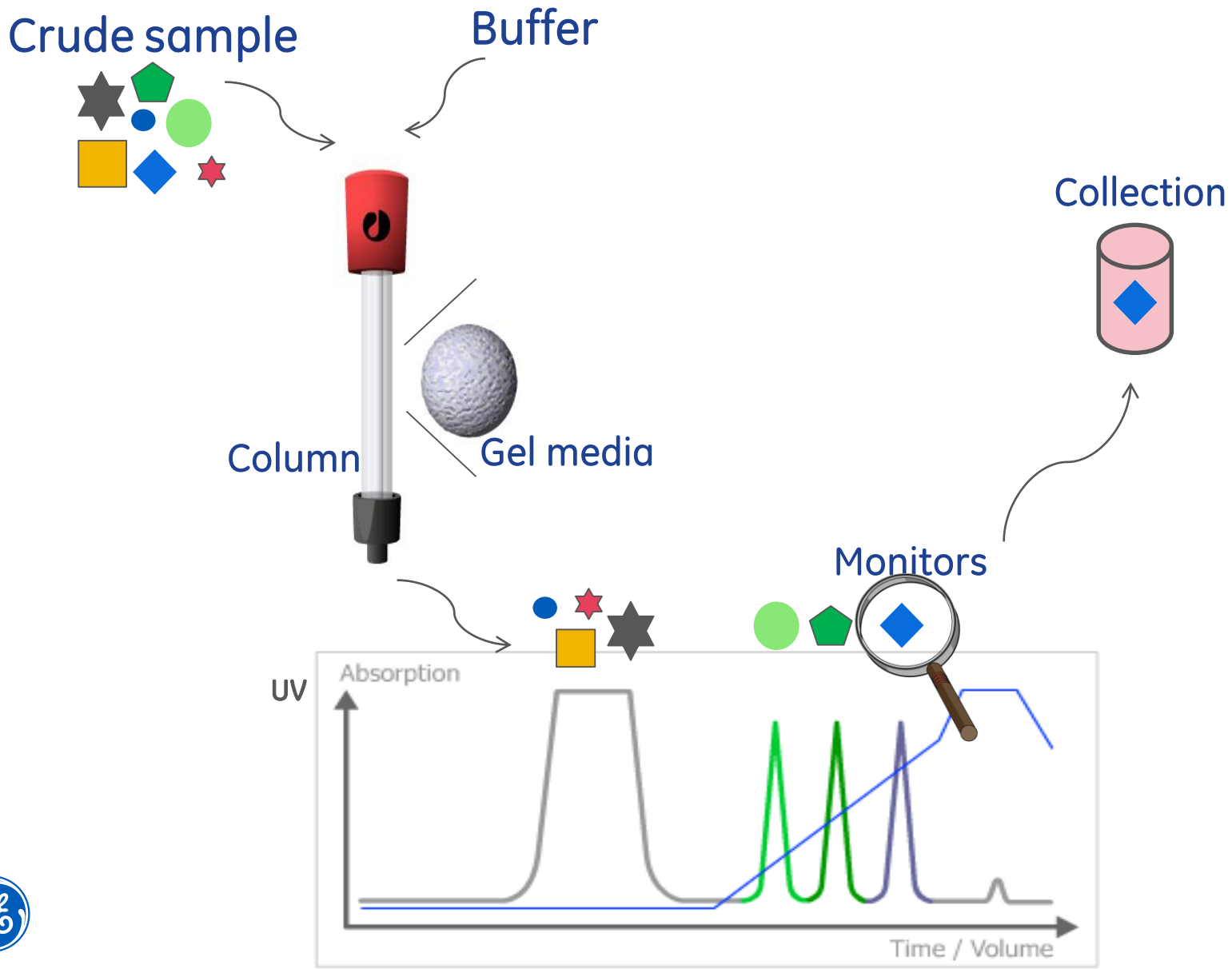
Introduction to protein purification

Why purify proteins?

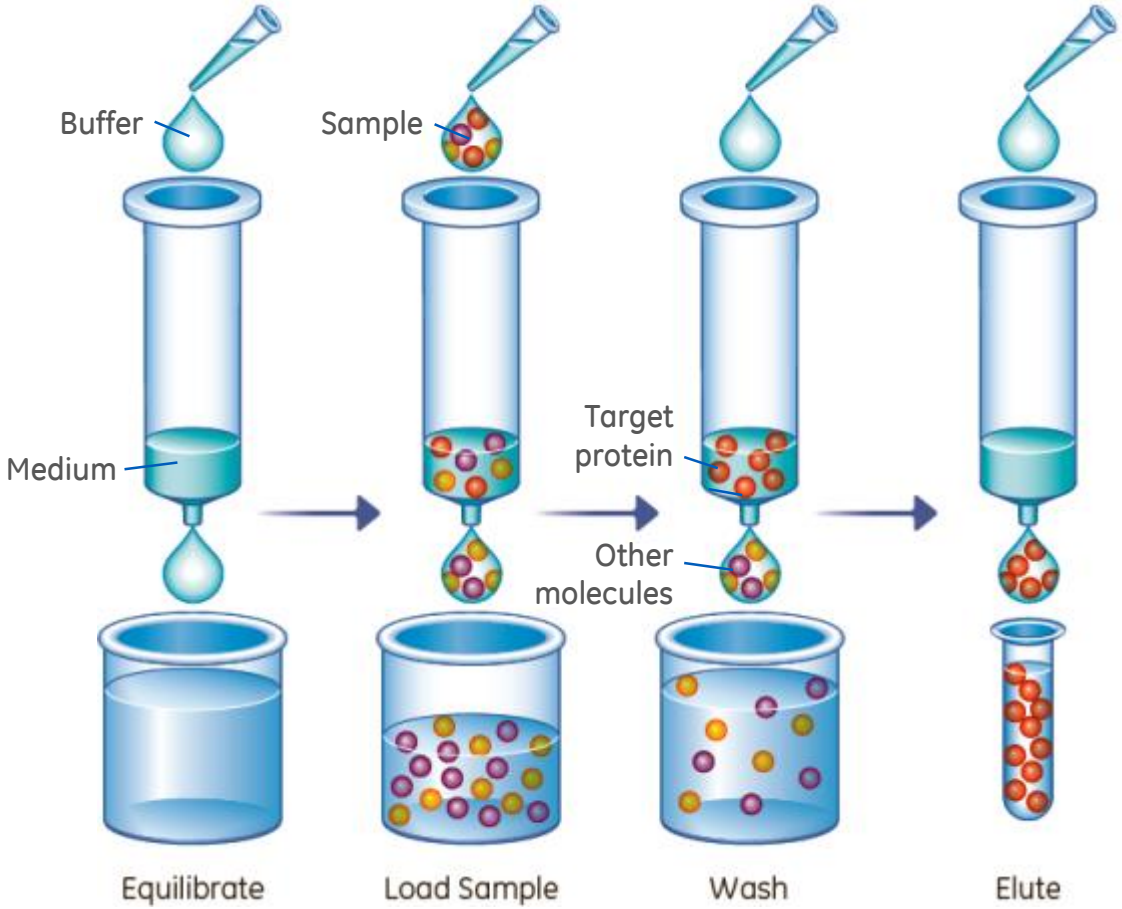


GE imagination at work

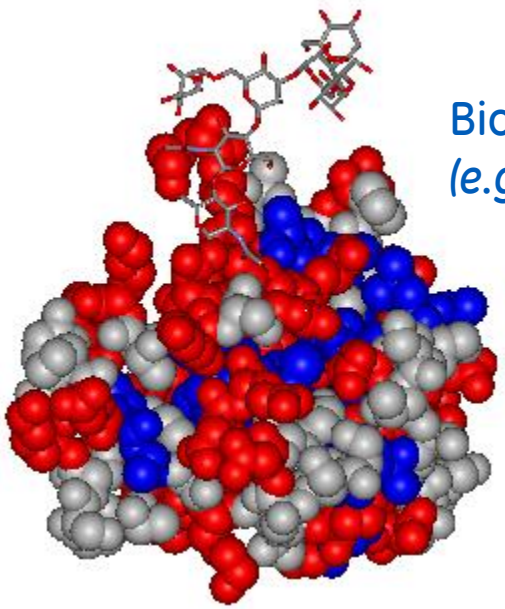
Chromatography



Chromatography principle



Protein properties that matter for protein purification



Biospecific affinity (*e.g.*, a tag) ---- Affinity chromatography (AC)

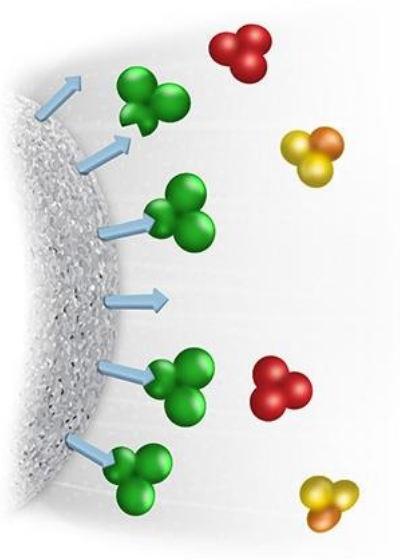
Net charge ------ Ion exchange chromatography (IEX)

Hydrophobicity --- Hydrophobic interaction chromatography (HIC)

Size ---------- Size exclusion chromatography (SEC) or gel filtration

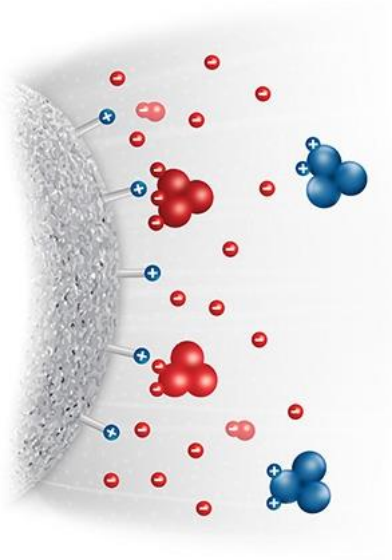
Schematics of common chromatography techniques

AC



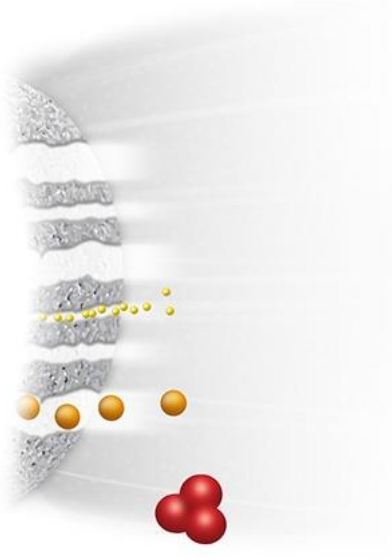
Watch AC principle video

IEX



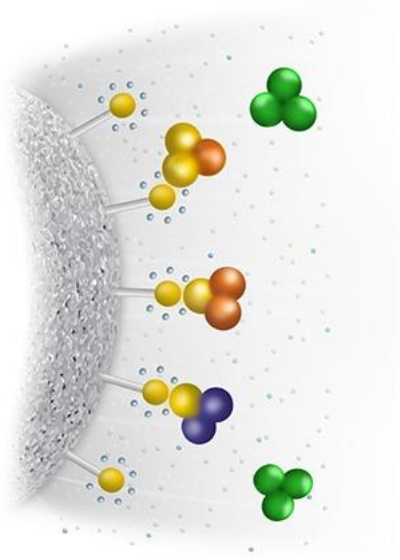
Watch IEX principle video

SEC



Watch SEC (or GF) principle video

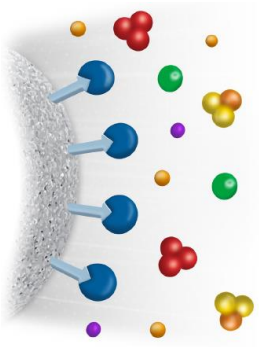
HIC



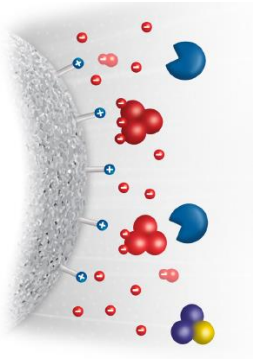
Watch HIC principle video

The principles of chromatography techniques

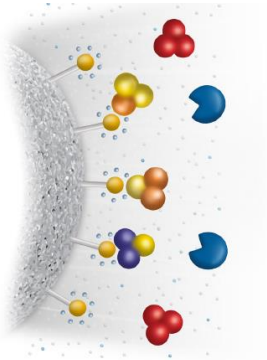
Affinity Chromatography (AC)



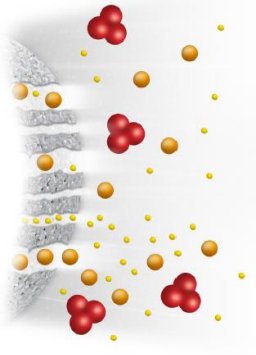
Ion exchange Chromatography (IEX)



Hydrophobic interaction Chromatography (HIC)



Size exclusion Chromatography (SEC)



- Bind – elute principle
- Requires specific elution conditions
- Concentrating effect

- Diffusion – no binding
- Any elution conditions
- Diluting effect

purification strategy

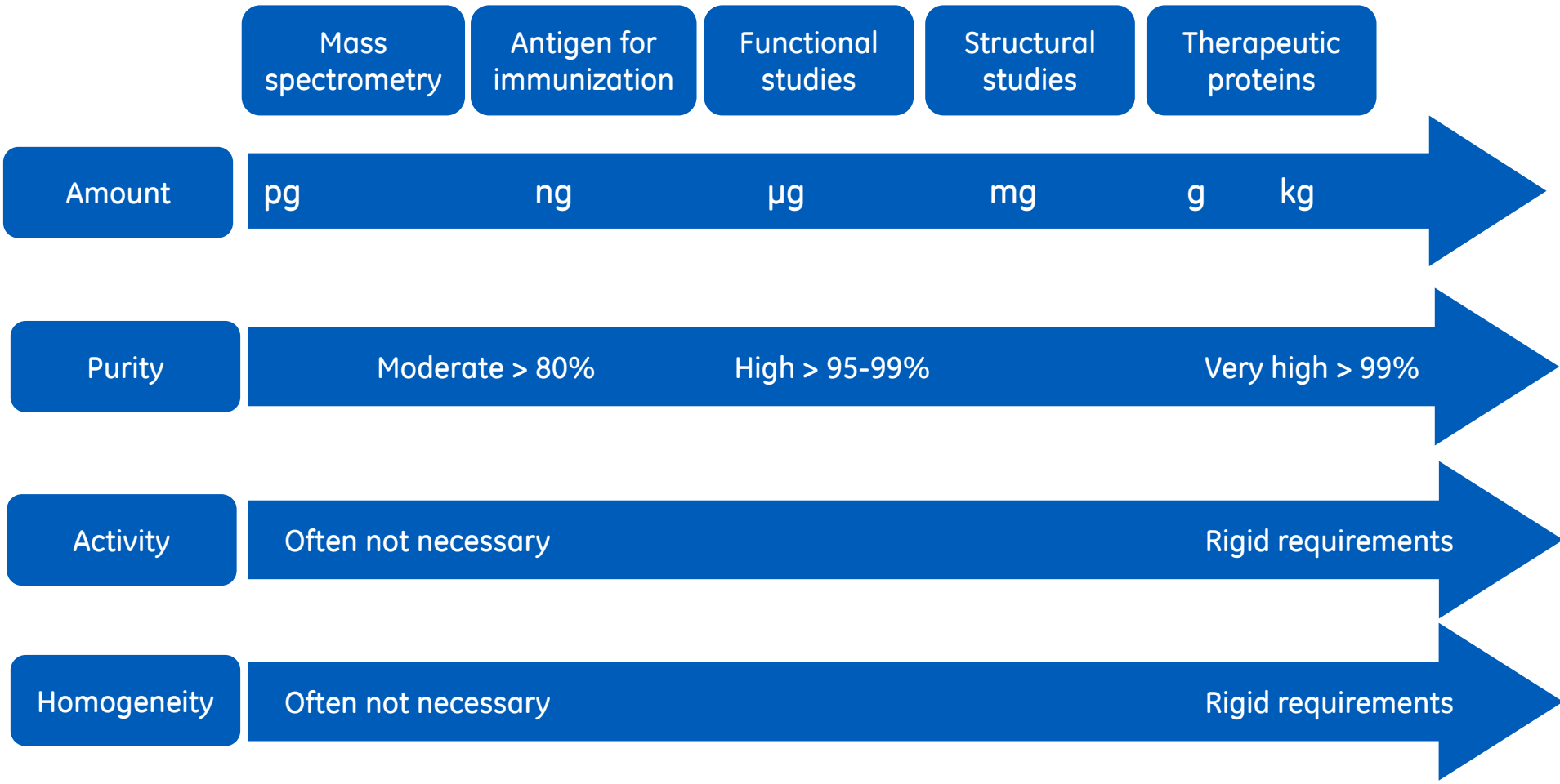
Purification strategy - key elements for success

1. Define the purification objectives
2. Build a purification scheme with different purification steps
   Capture, intermediate purification and polishing
3. Combine techniques
   Maximize separation power and minimize sample treatment
4. Use relevant analytical assays
   Protein ID, purity, quantity (activity, homogeneity)

Goals: successful protein purification

- Maintained biological activity
- Sufficient purity and quantity
- Good economy

Defining the purification objectives



Different Scales、Various equipments

BIOPROCESS SCALE

INCREASED THROUGHPUT
LAB SCALE

ÄKTAprocess™

ÄKTAxpress™

ÄKTApilot™

ÄKTAprime™ plus

ÄKTApure

ÄKTAstart™

LAB SCALE
SIMPLE PURIFICATION

LOW THROUGHPUT
ADVANCED PURIFICATION

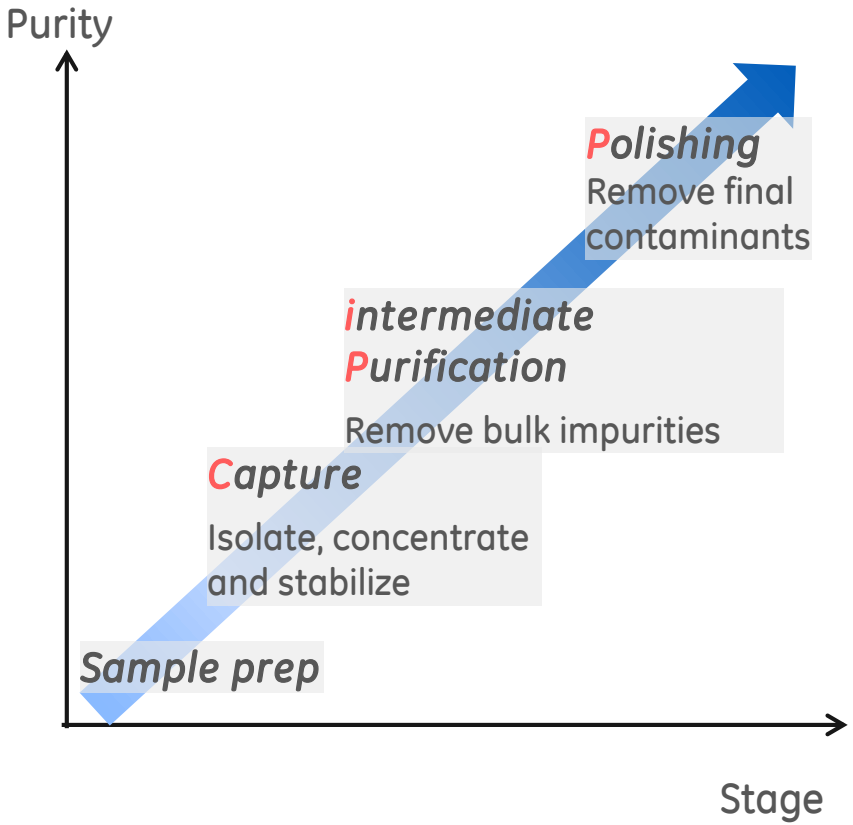
imagination at work

Purification strategy - key elements for success

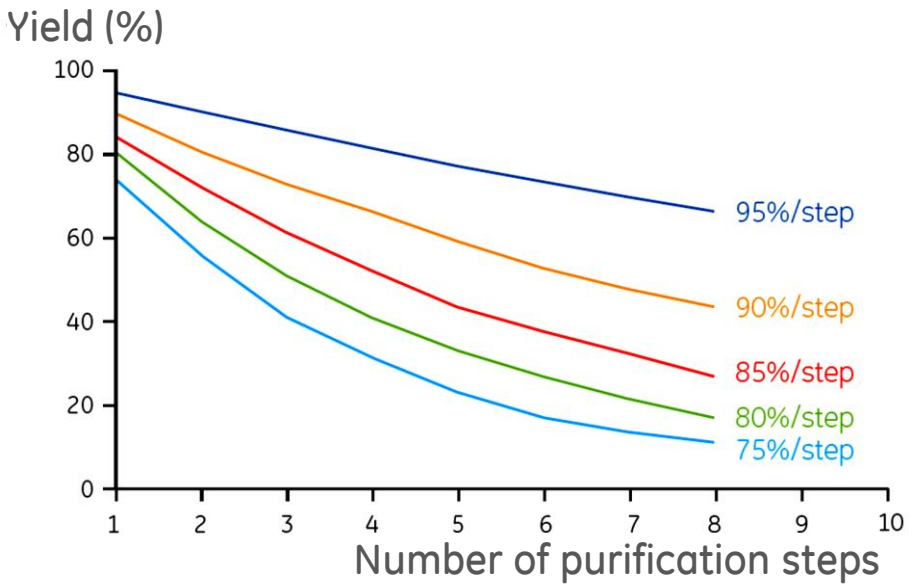
1. Define the purification objectives
2. Build a purification scheme with different purification steps
   Capture, intermediate purification and polishing
3. Combine techniques
   Maximize separation power and minimize sample treatment
4. Use relevant analytical assays
   Protein ID, purity, quantity (activity, homogeneity)

Introduction to CiPP purification strategy

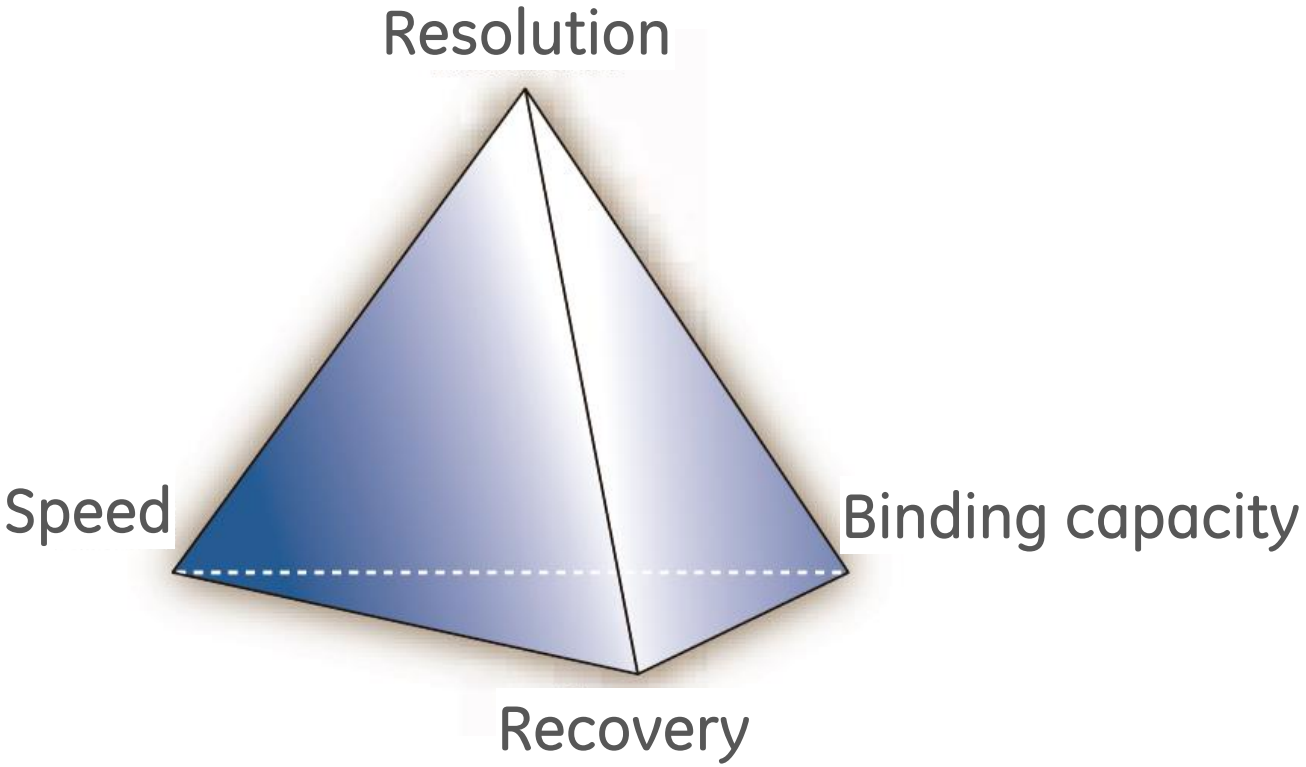
Purification strategy combining multiple steps



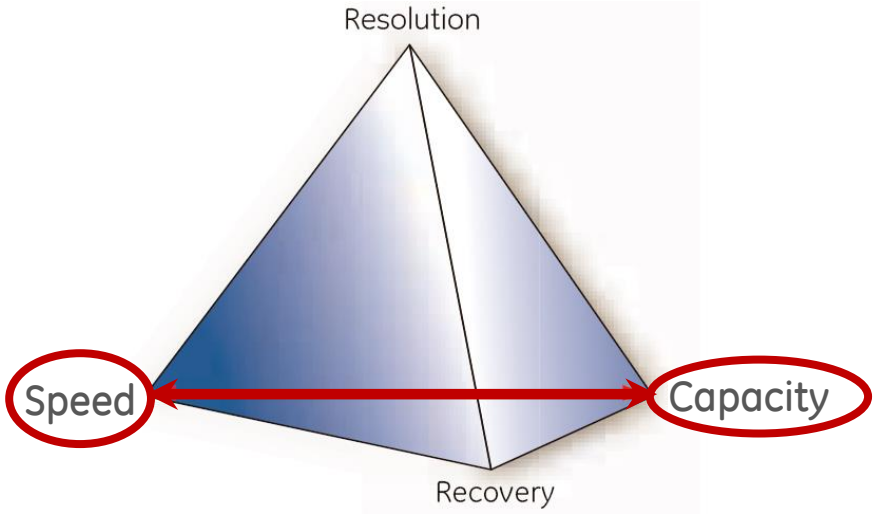
Protein recovery plotted against the number of purification steps

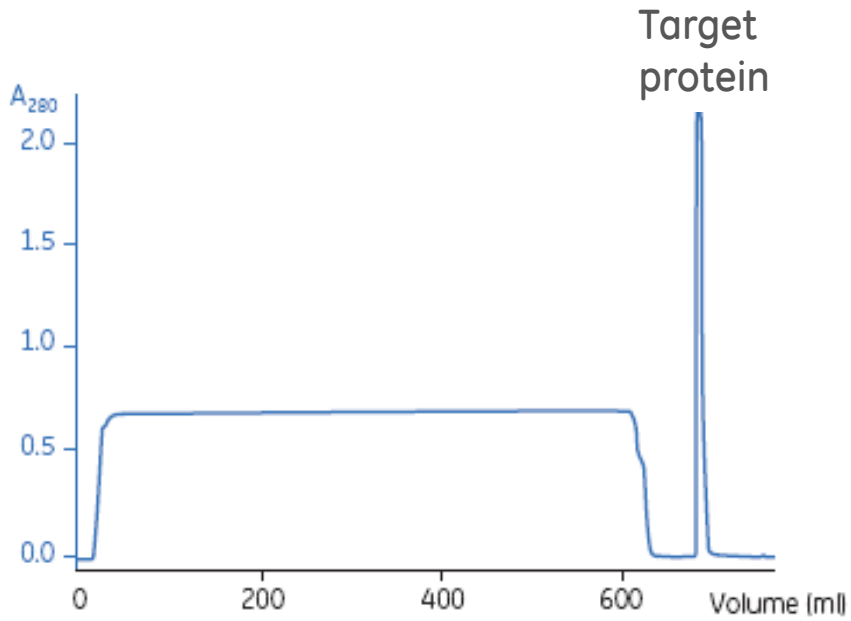


Important performance factors



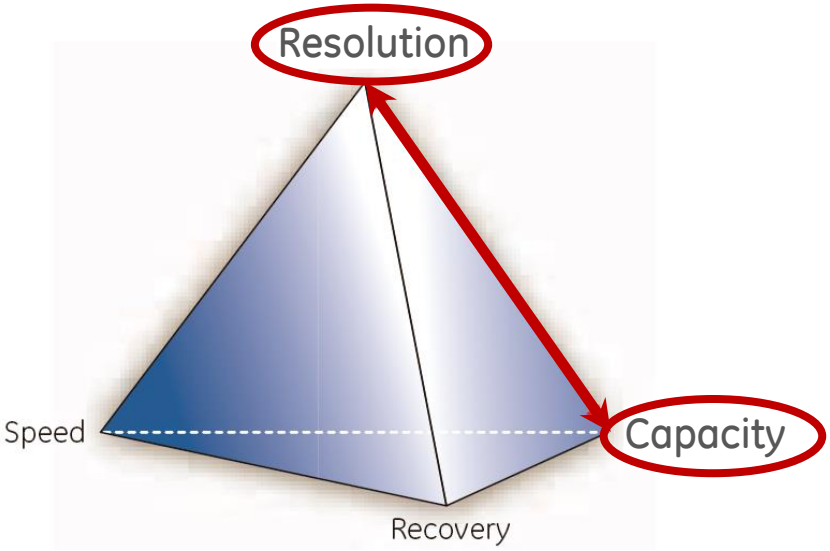
CiPP: Capture

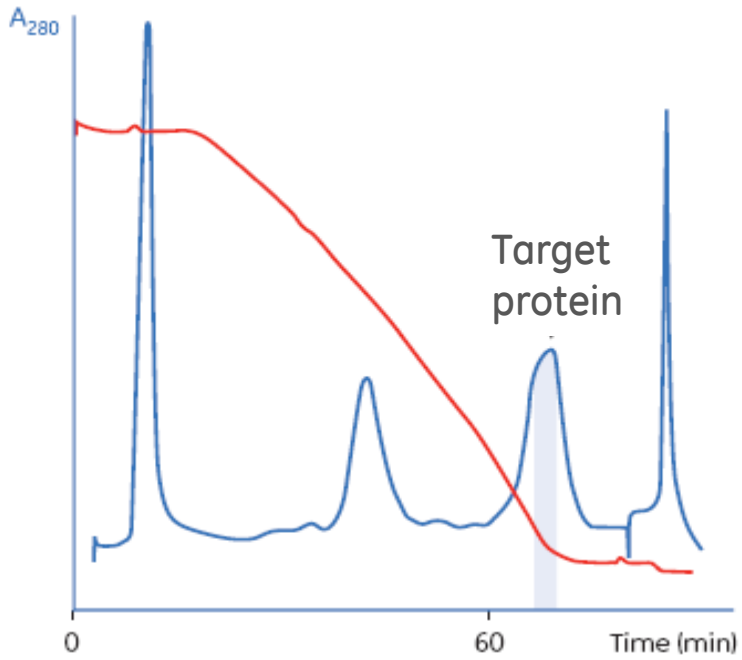




Technique: Affinity chromatography
Column: rProtein A Sepharose™ FF, XK16/20 (9.6 ml)
Sample: 88 mg of IgG2 in 600 ml clarified cell culture

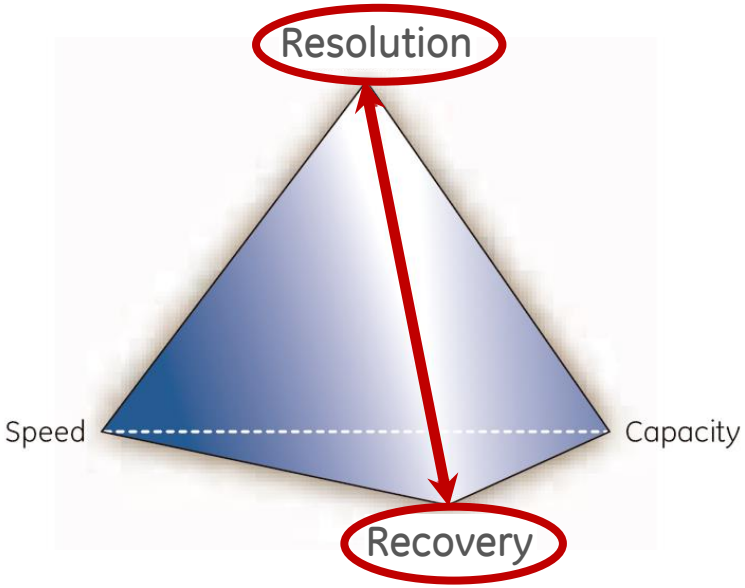
CiPP: Intermediate purification

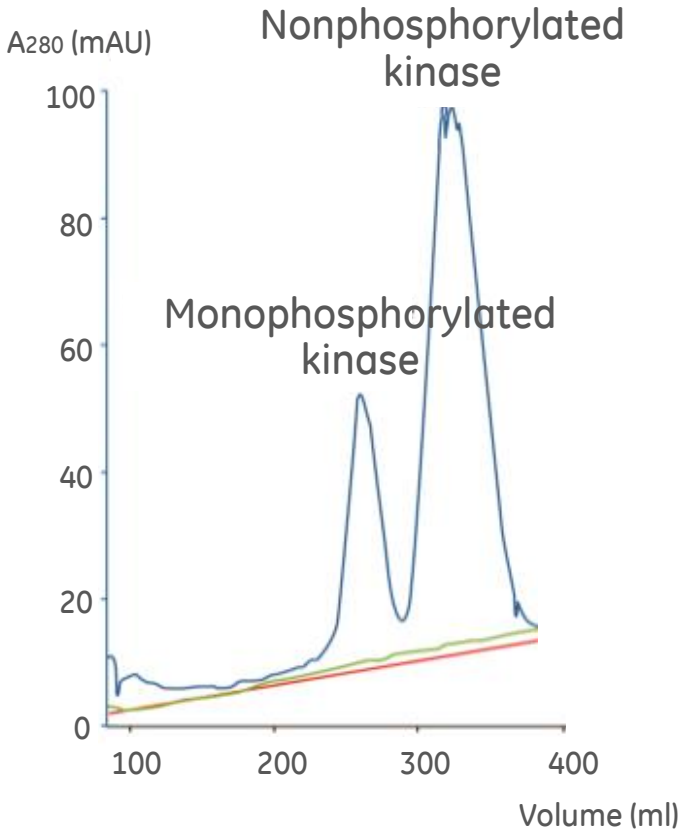




Technique: Hydrophobic interaction chromatography
Column: Butyl Sepharose™ 4 FF, XK16/20
Sample: 5 ml partially purified rec. annexin

CiPP: Polishing

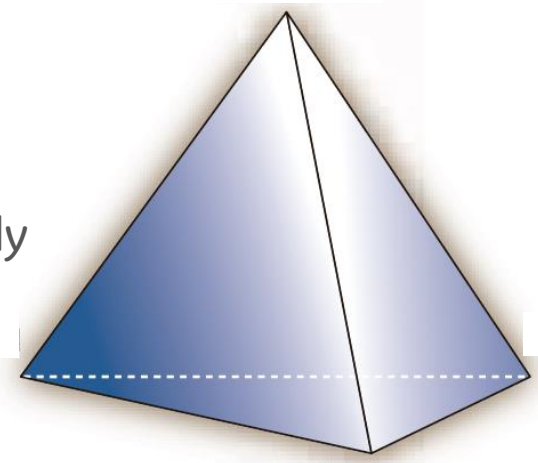




Technique: Ion exchange chromatography
Column: Mono S™ (8 ml column)
Sample: Zap-70 kinase

Which type of chromatography resin provides the desired performance?

Objective: High resolution
Small, uniformly sized beads (*e.g.*, 8-40 µm bead diameter).

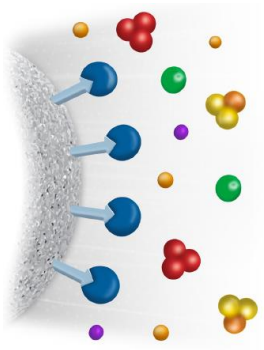
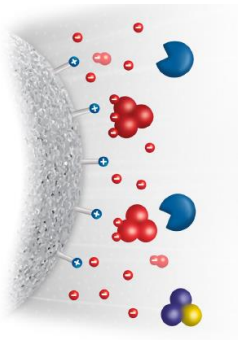
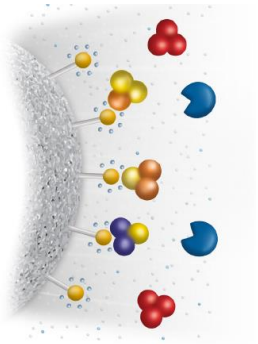
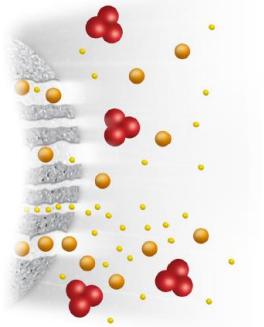


Objective: Speed
Large, rigid and uniformly sized beads provide the highest speed (*e.g.*, 50-100 µm, highly cross-linked agarose).

Objective: High binding capacity
Porous beads with high ligand density and directed ligand coupling.

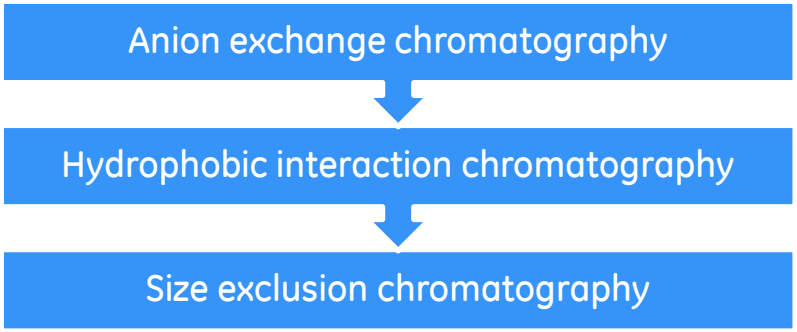
Objective: High recovery
Recovery is mostly dependent on buffer conditions and on how peaks are cut.

Recommendations: techniques in each CiPP step

	Affinity chromatography	Ion exchange chromatography	Hydrophobic interaction chromatography	Size exclusion chromatography
				
Capture	★★★	★★★		
Intermediate Purification		★★★	★★★	
Polishing		★★	★	★★★

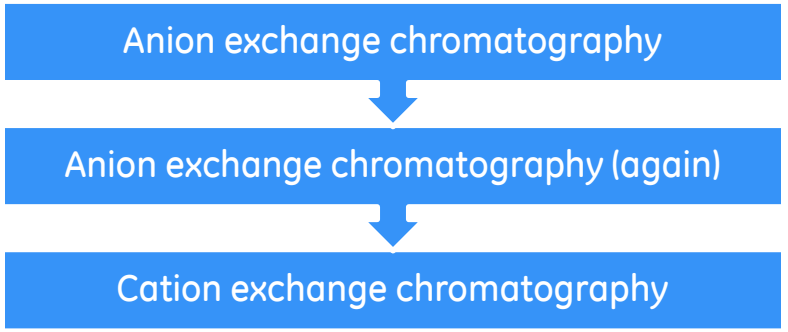
How to combine purification techniques

A good separation scheme



- Uses three different separation principles:
  charge, hydrophobicity and size.
- Requires little sample handling between steps.

A poor separation scheme

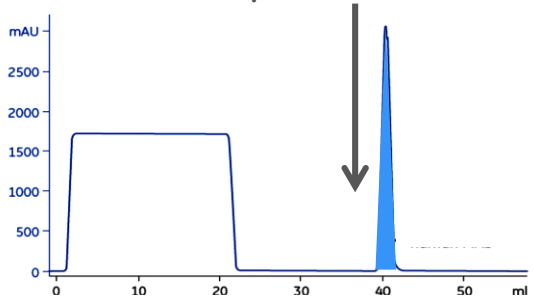


- Uses only one separation principle.
- Requires extensive sample handling between steps: dialysis or buffer exchange.

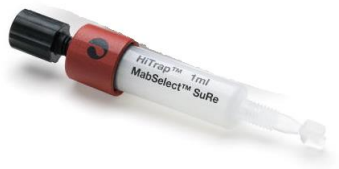
Application examples

A single capture step is sometimes sufficient

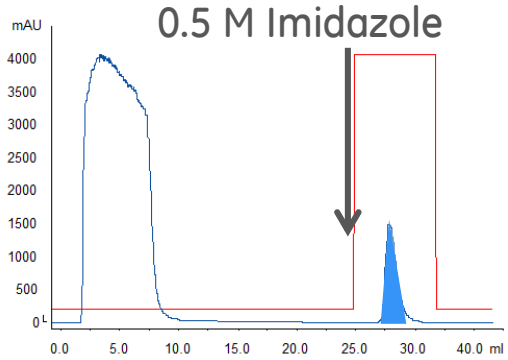
Monoclonal antibody purification



Column: HiTrap™ protein A 1 ml



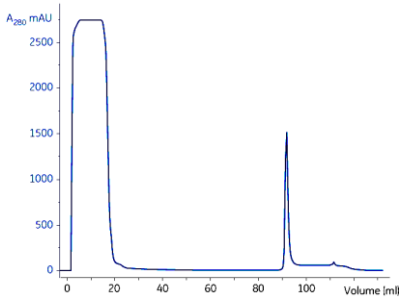
His-tagged protein purification



Column: HisTrap™ FF 1 ml

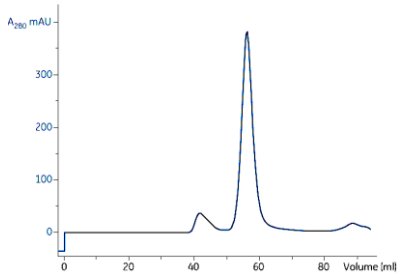
Example: Purification of a tagged protein*

1. Capture: Affinity chromatography

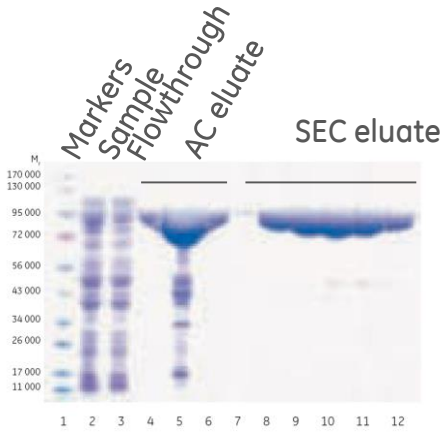


Column: MBPTrap™ HP 5 ml
Sample: 15 ml MBP-MCAD in *E. coli* lysate, M_r ~85 500

2. Polishing: Size exclusion chromatography



Column: HiLoad™ 16/60 Superdex 200 pg
Sample: 2 ml eluted fraction from AC

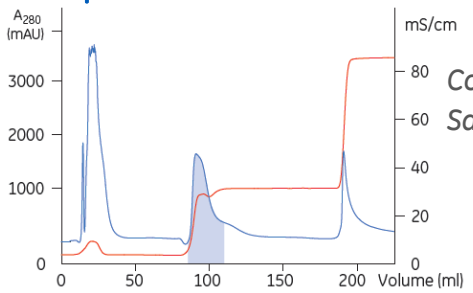


Purity check (SDS-PAGE)

* On all application examples shared in this presentation, different ÄKTA™ systems from GE Healthcare have been used for purification.

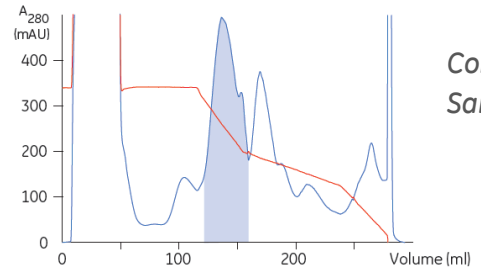
Example: Purification of an untagged protein

1. Capture: IEX



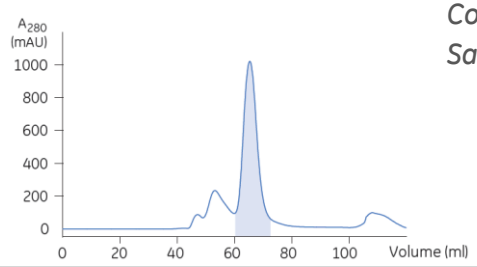
Column: *HiPrep™ Q XL 16/10*
Sample: *40 ml clarified E. coli extract with DAOCS*

2. Intermediate purification: HIC

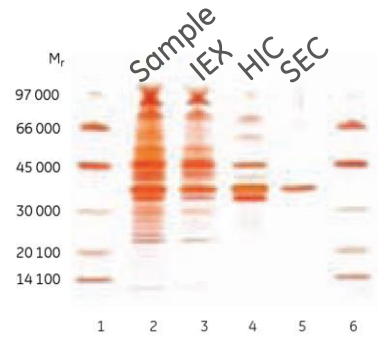


Column: *SOURCE™ 15ISO, packed in HR column 16/10*
Sample: *40 ml DAOCS pool from IEX*

3. Polishing: SEC

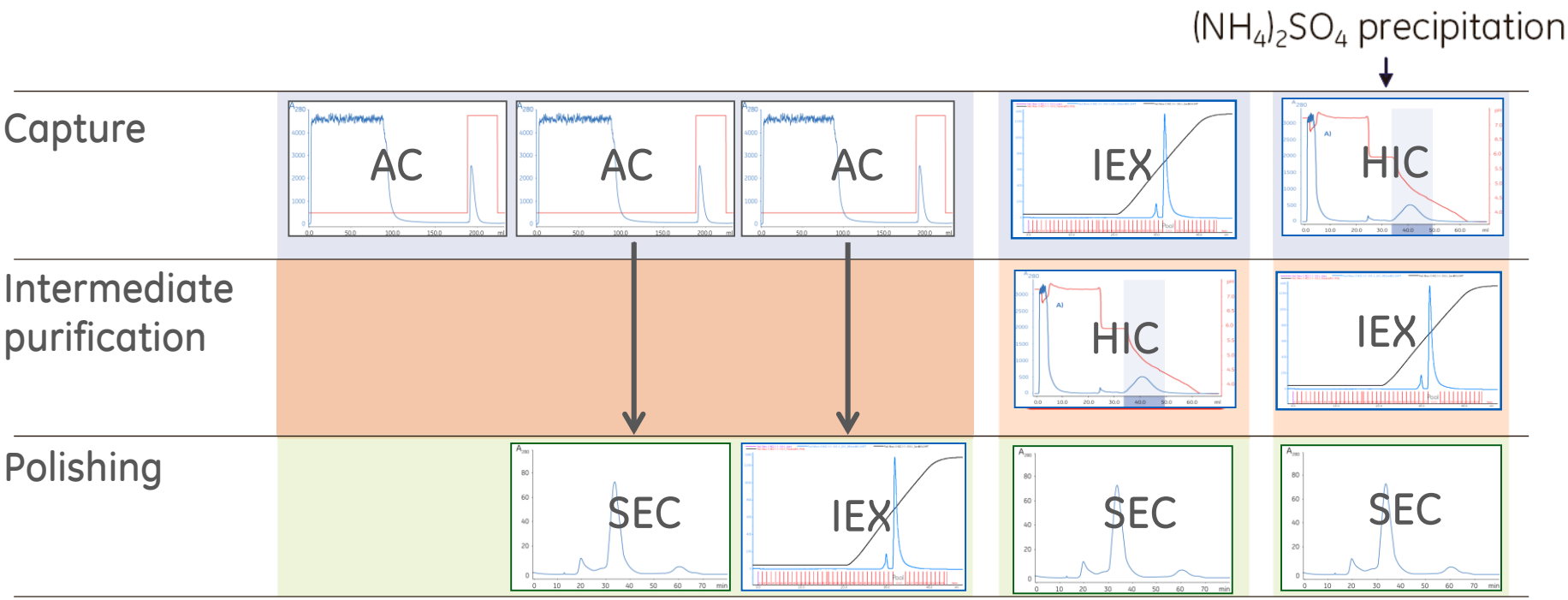


Column: *HiLoad™ 16/60 Superdex™ 75 pg*
Sample: *3 ml concentrated DAOCS pool from HIC*



Purity check (SDS-PAGE)

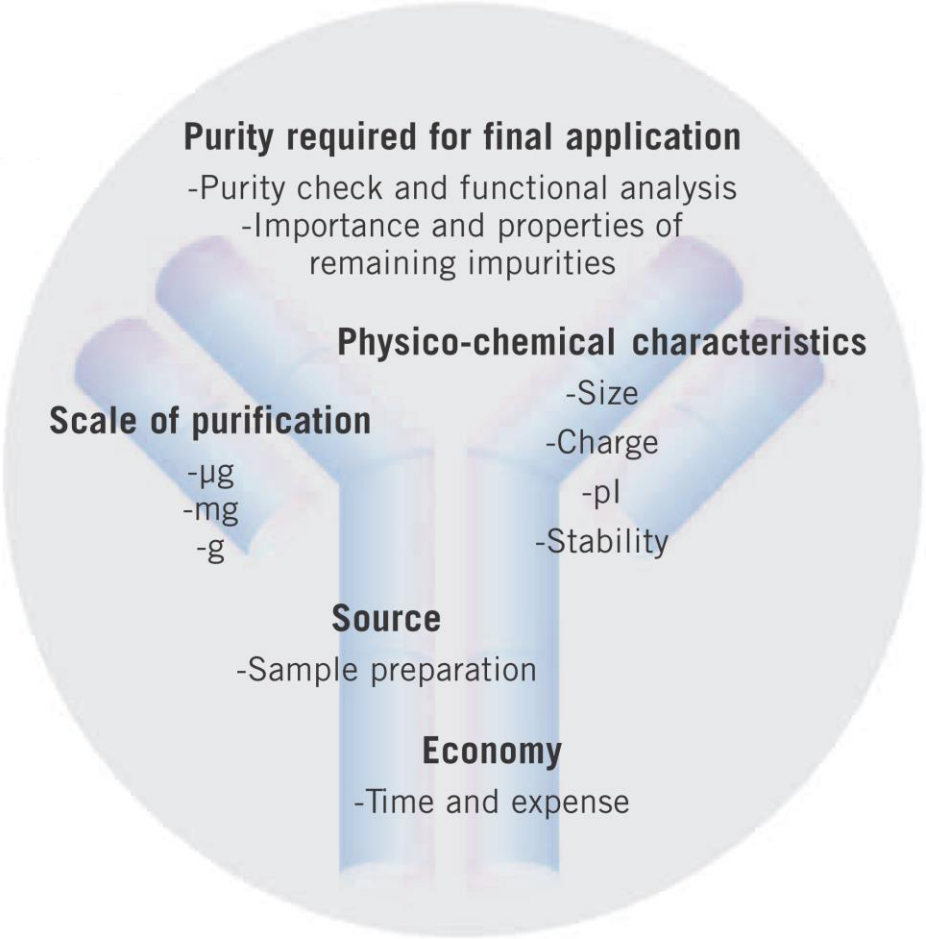
How to combine purification techniques



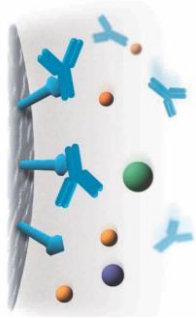
Use orthogonal techniques and minimize sample handling

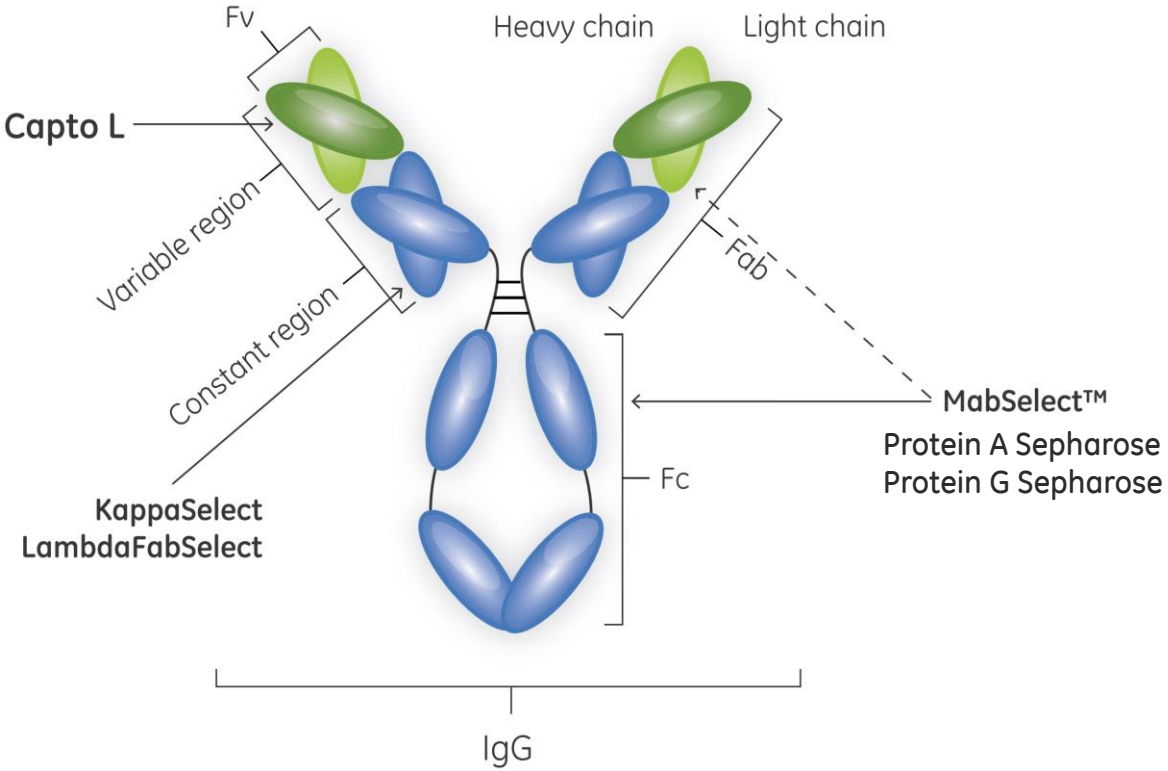
Application examples: Antibody purification

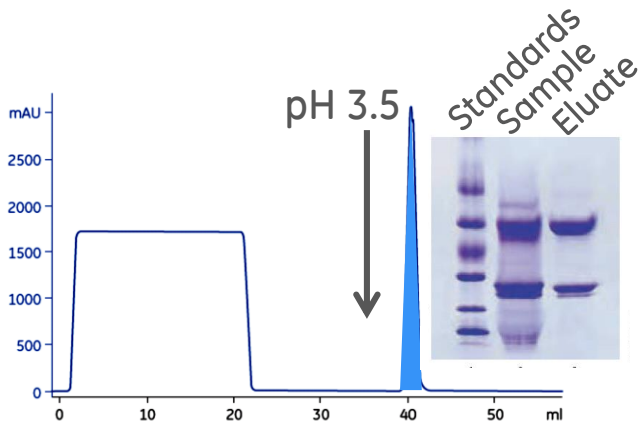
What is the goal of the purification?



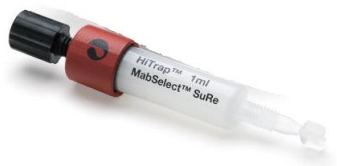
A platform approach for the purification of antibody or antibody fragments (Fabs)







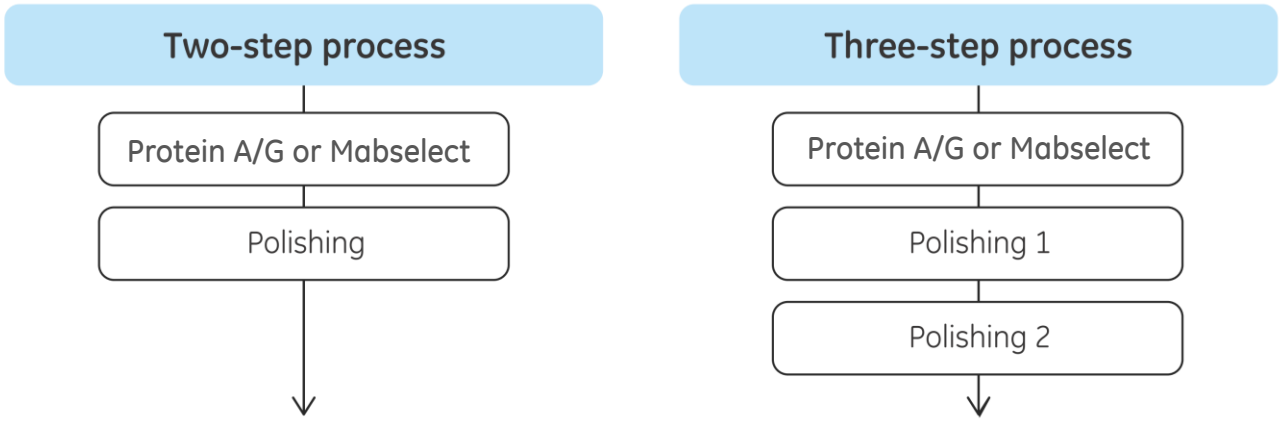
Column: HiTrap™ protein A 1 ml



Sample preparation
Sources and their associated contaminants

	Molecular types	Significant contaminants	Quantity
Source: native			
Human serum	Polyclonal IgG, IgM, IgA, IgD, IgE	albumin, transferrin, α_2-macroglobulin, other serum proteins	IgG 8–16 mg/ml IgM 0.5–2 mg/ml IgA 1–4 mg/ml IgE 10–400 ng/ml IgD up to 0.4 mg/ml
Hybridoma: cell culture supernatant with 10% foetal calf serum	Monoclonal	Phenol red, water, albumin, transferrin, bovine IgG, α_2-macroglobulin, other serum proteins, viruses	Up to 1 mg/ml
Hybridoma: cell culture supernatant serum free	Monoclonal	Albumin, transferrin (often added as supplements)	Up to 0.05 mg/ml
Ascites fluid	Monoclonal	Lipids, albumin, transferrin, lipoproteins, endogenous IgG, other host proteins	1–15 mg/ml
Egg yolk	IgY	Lipids, lipoproteins and vitellin	IgY 3–4 mg/ml
Source: recombinant			
Extracellular protein expressed into supernatant	Tagged antibodies, antibody fusion proteins, Fab or F(ab')$_2$ fragments	Proteins from the host, e.g. *E. coli*. General low level of contamination	Depends upon expression system
Intracellular protein expression		Proteins from the host, e.g. *E. coli*, phage	Depends upon expression system

What is your associated contaminants?



Common contaminants after initial purification are albumin, transferrin, DNA, immunoglobulins, antibody aggregates and leached protein A.

- Protein purity – defined by ***Resolution***
- Amount of target protein – defined by ***recovery***
- Ability to purify more protein if needed – defined by ***reproducibility***

Example: Aggregate removal using size exclusion

Mouse IgG_1 Purification

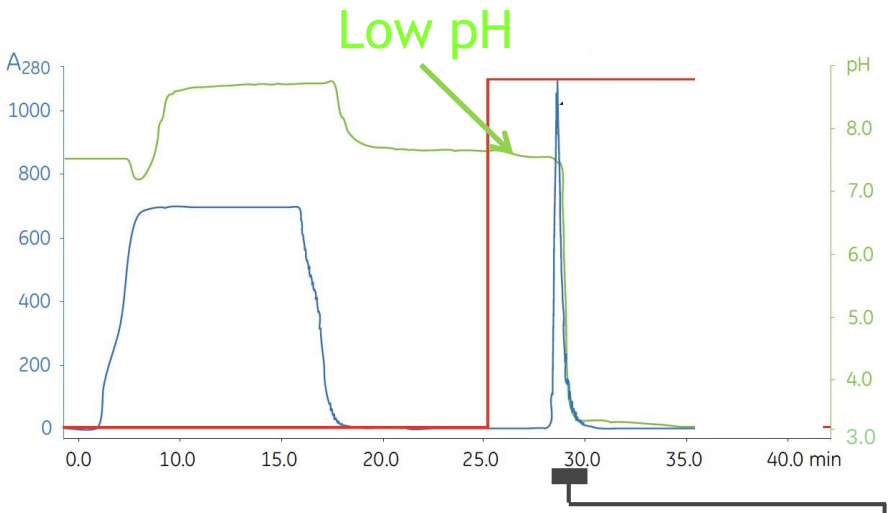
Affinity chromatography

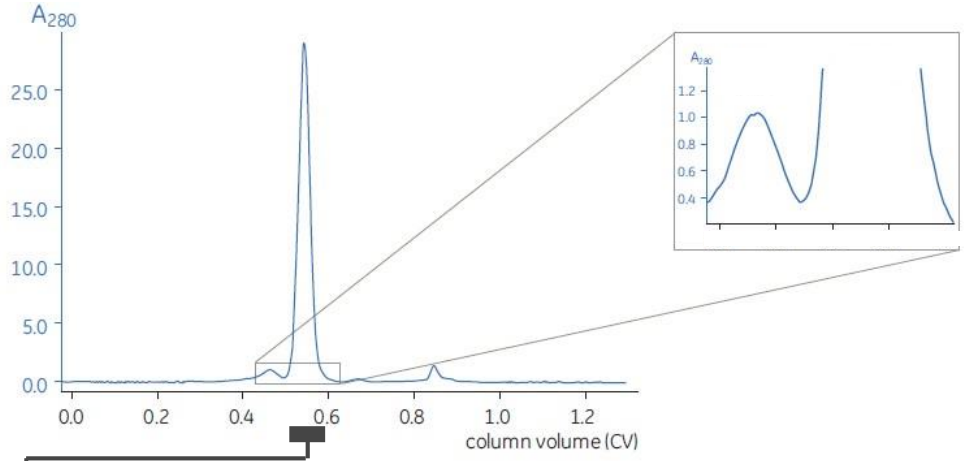
HiTrap™ Protein G HP 1 ml

Size exclusion chromatography

HiLoad™ 16/60 Superdex™ 200 pg

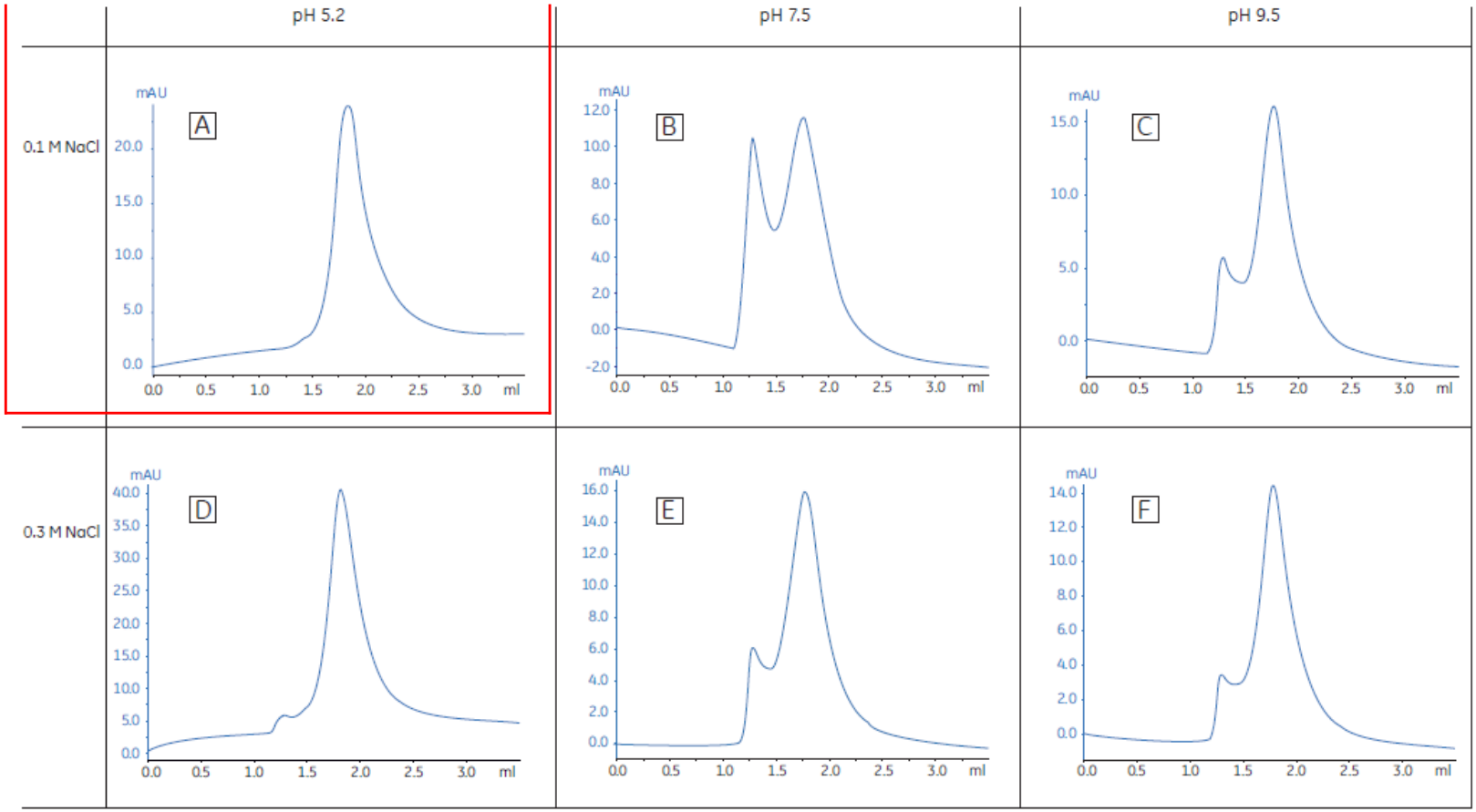
- Removal of dimers and aggregates
- Buffer exchange





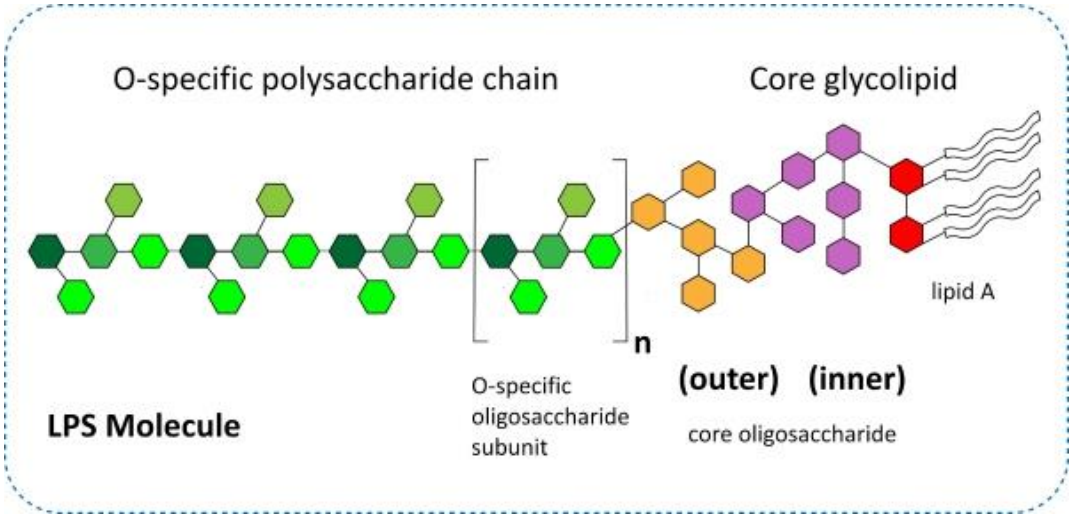
Remember: aggregates are invisible in reduced SDS-PAGE. The aggregates are found in the small peak that appears immediately before the main peak.

Example: Application: structure homogeneity



Screening of pH and ion strength conditions for optimal homogeneity and stability of a detergent-protein complex. Chromatogram A–F represent the results from the different screening conditions.

Example: Application: Endotoxins, DNA Cleaning

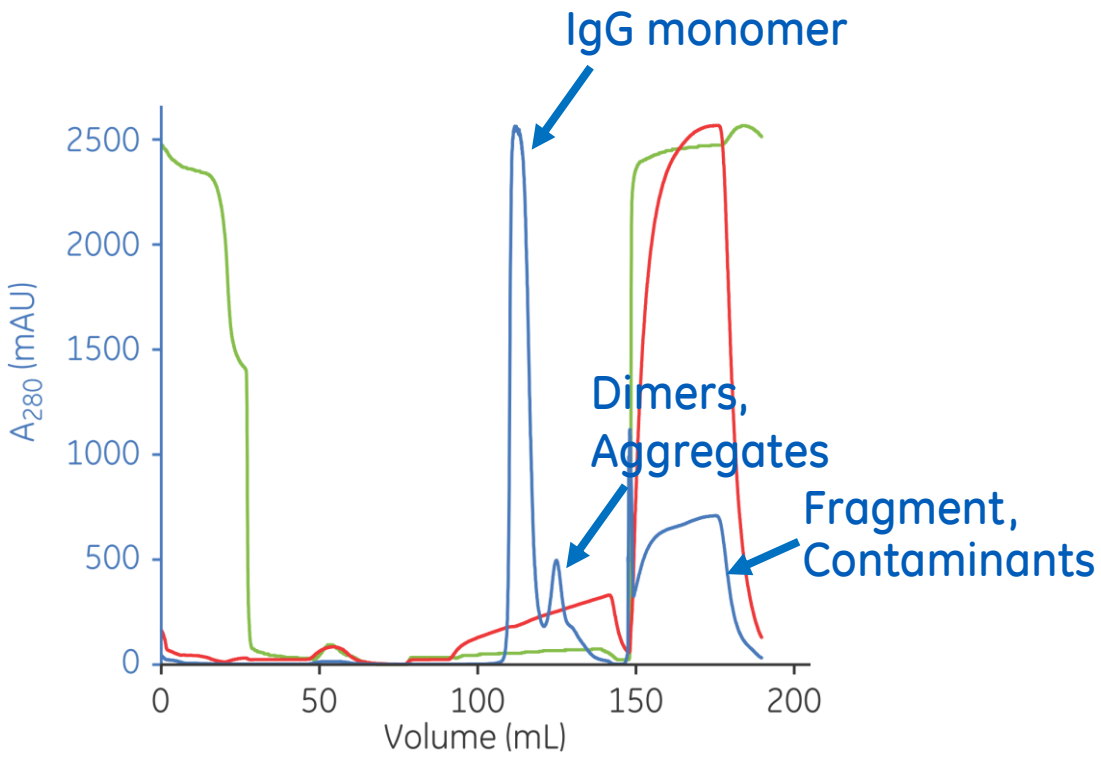


10~20 KD ~ 4 ×105 KD
< 0.25EU/ml

HiPrep DEAE FF 16/10

HiScreen™ Capto Q

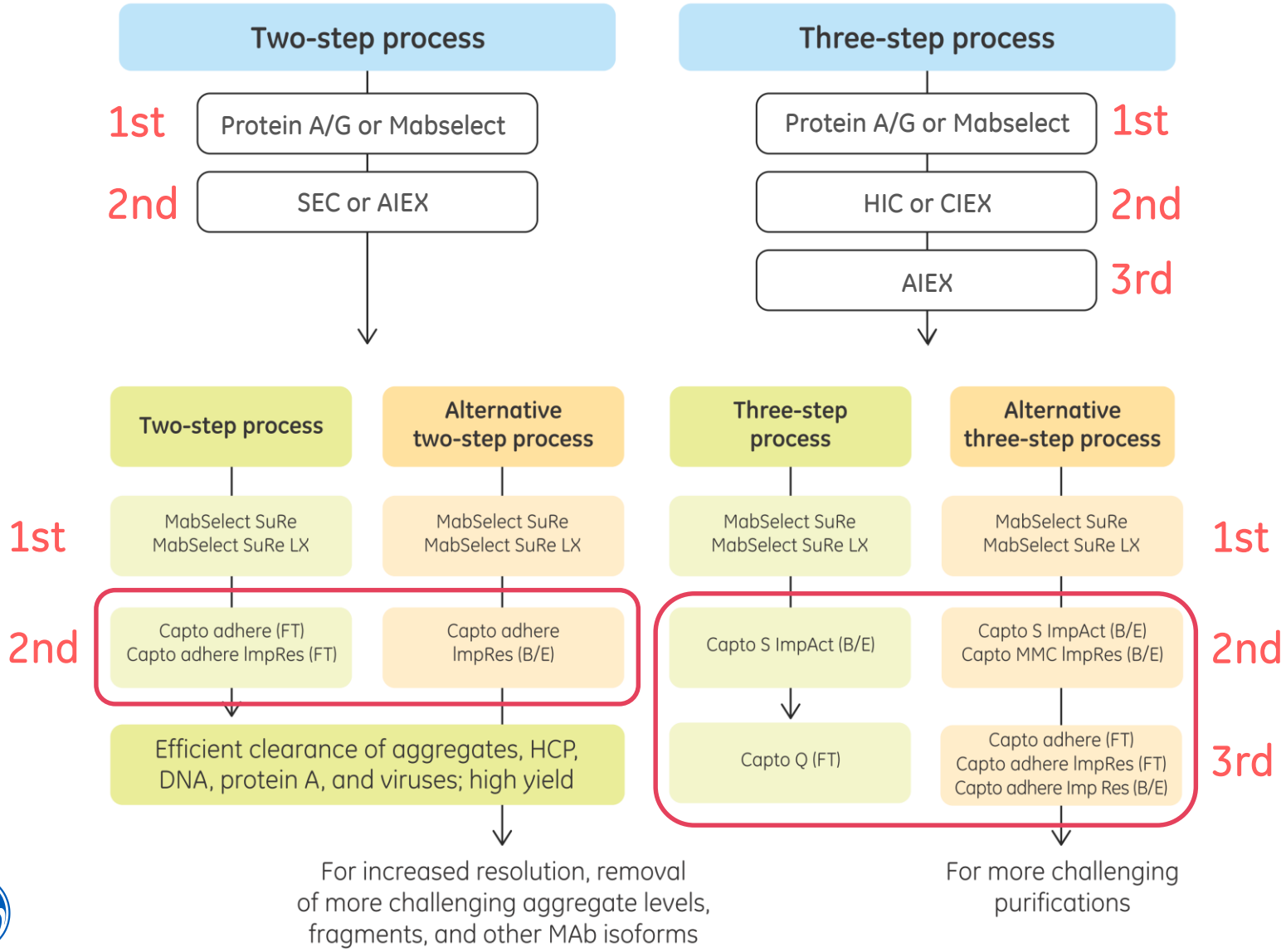
Example: Application: Aggregates and Truncated fragment



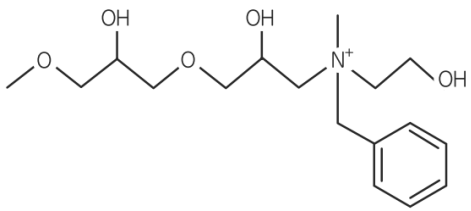
Column: Capto™ SP ImpRes

HiScreen™ Capto™ S
HiScreen™ Capto™ SP ImpRes
HiScreen™ family

Remove associated contaminants

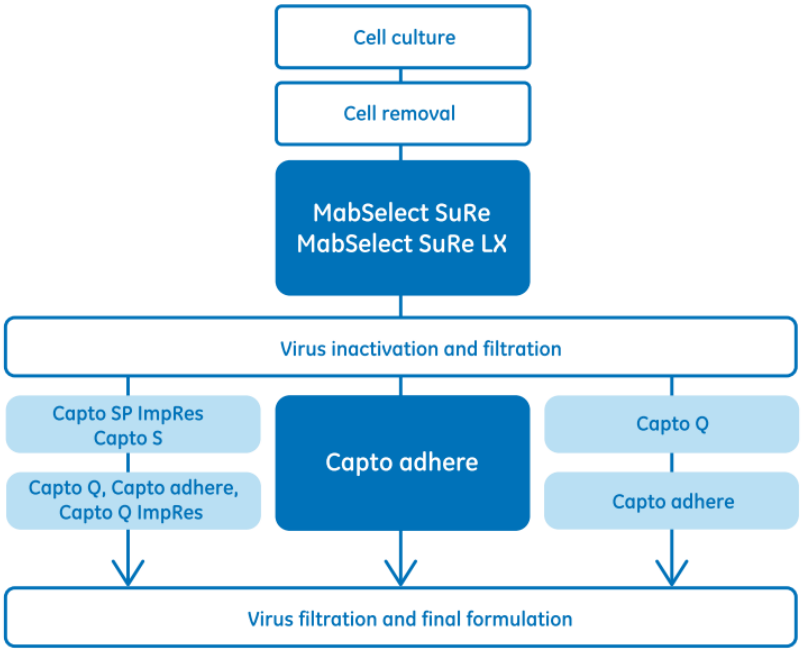


Example: Multimodal functionality gel

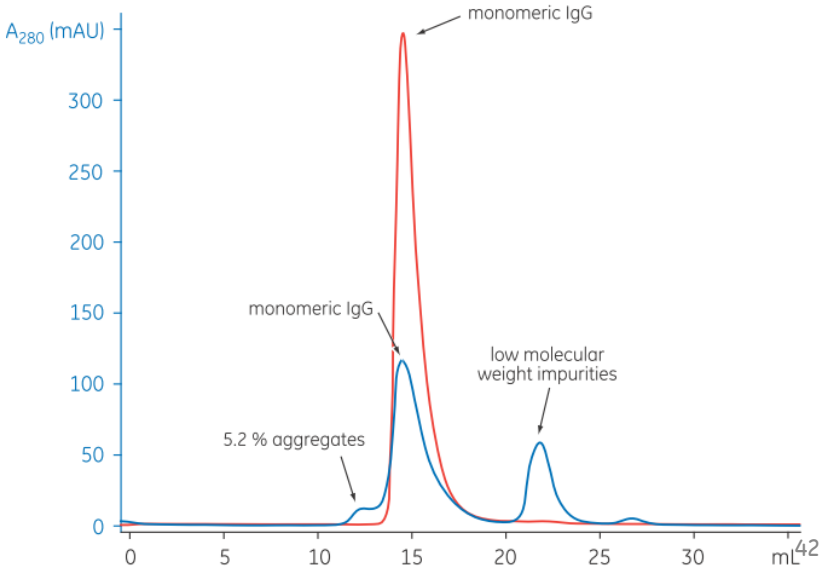


- aggregates
- DNA, viruses
- HCP
- endotoxin

Capto adhere

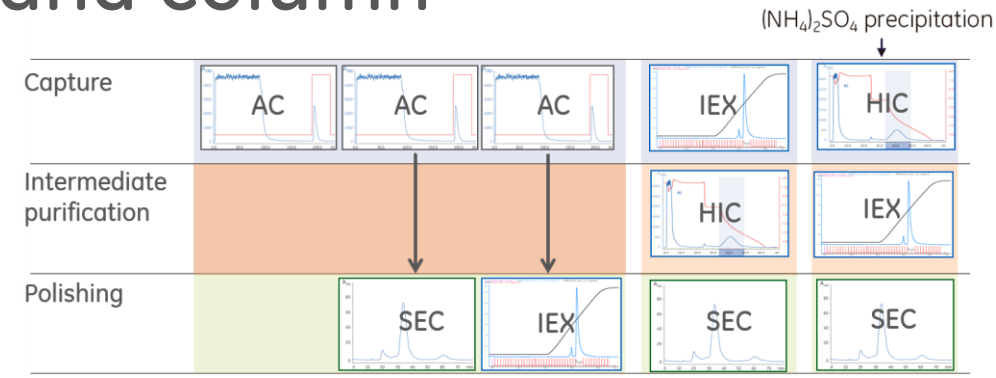


Column: Superdex 200 10/300
Sample: Flowthrough fraction (red) and eluate (blue) from the Capto adhere step
Sample load: 50 µL each
Loading buffer: 0.01 M sodium phosphate, 2.7 mM potassium phosphate, 137 mM sodium chloride, pH 7.4
Flow rate: 0.5 mL/min
System: ÄKTA chromatography system



How to choose media and column

How to choose media and column



To produce large quantities of the protein of interest

How to choose media and colum - AF

Advantages

- Simple
- Increase solubility/folding
- Increase yield (expression/stability)
- Easy to detection

Disadvantages

- Tag may interfere with structure/function

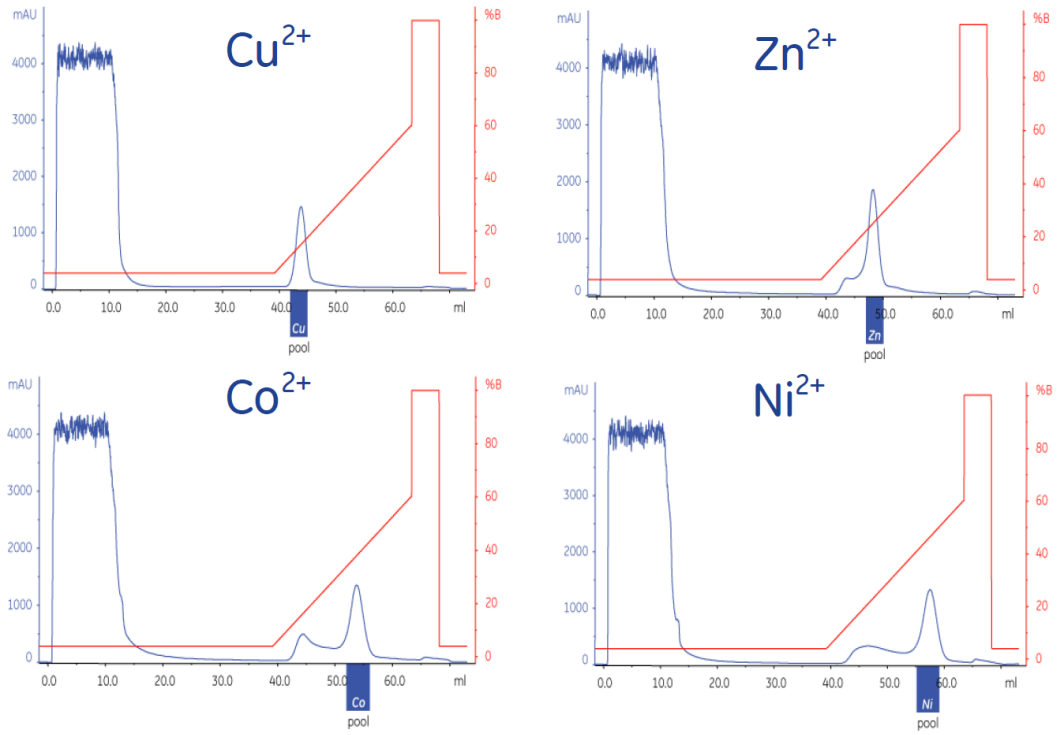
	GST	MBP	*Strep*-tag™ II	His	*Strep*-tag II-His
Size	26 kDa	40 kDa	8 aa	6 aa	14 aa
Capacity	30 mg/ml	10 mg/ml	6 mg/ml	40 mg/ml	NA
Purity	★★	★★	★★★	★	★★★★
Solubility	★★	★★	★	★	★

★ = Low

★★★★ = Highest

How to choose media and column - AF

Column: HiTrap™ IMAC FF
(prepacked with un-charged IMAC Sepharose™)



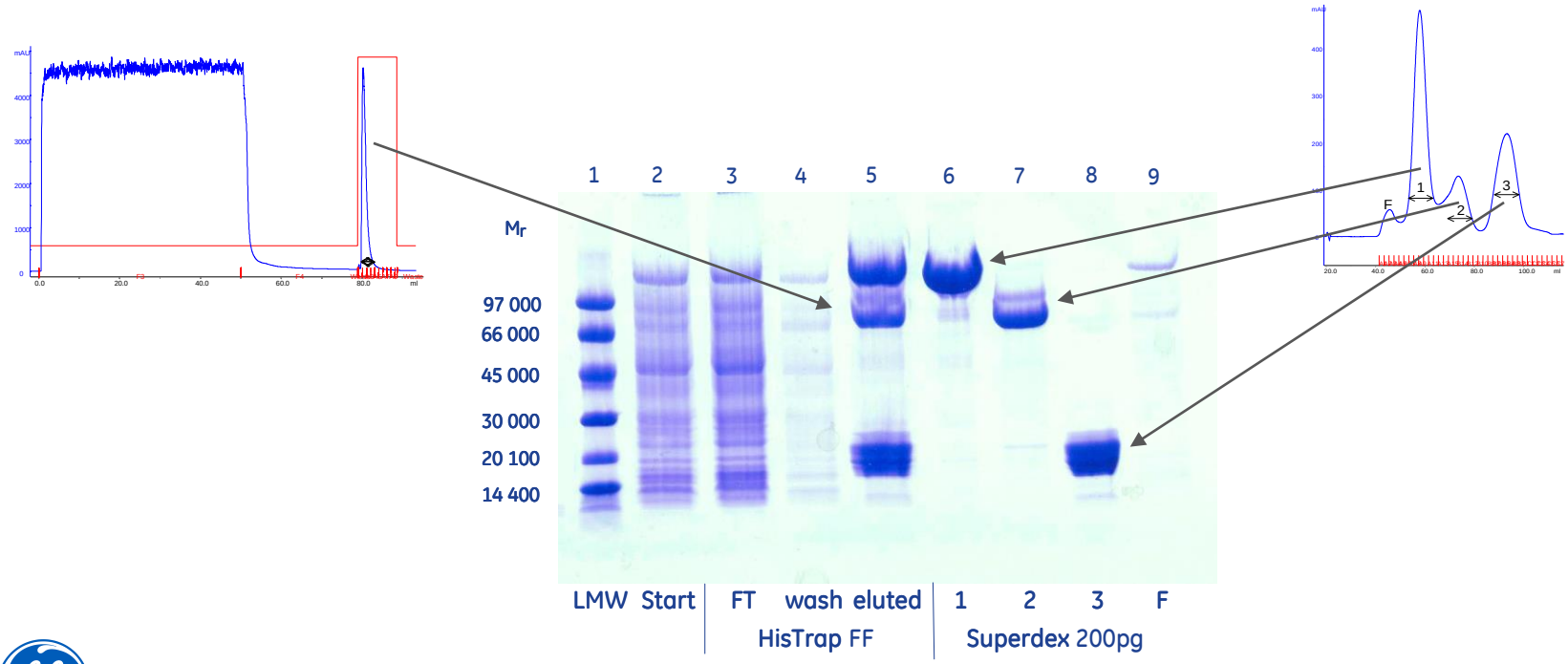
SEC: Two step purification

1. Affinity step

Column: HisTrap™ FF 1 ml
Sample: 50 ml $(His)_{10}$-Trx-P 450 in *E. coli* lysate
System: ÄKTAexplorer™

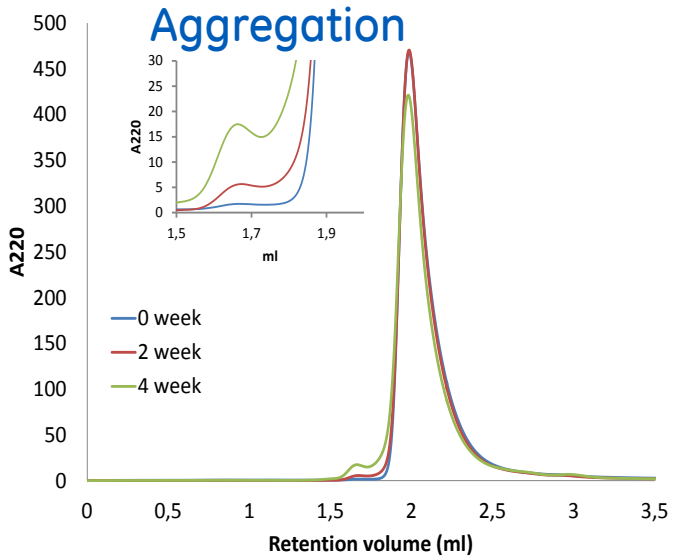
2. Gel filtration

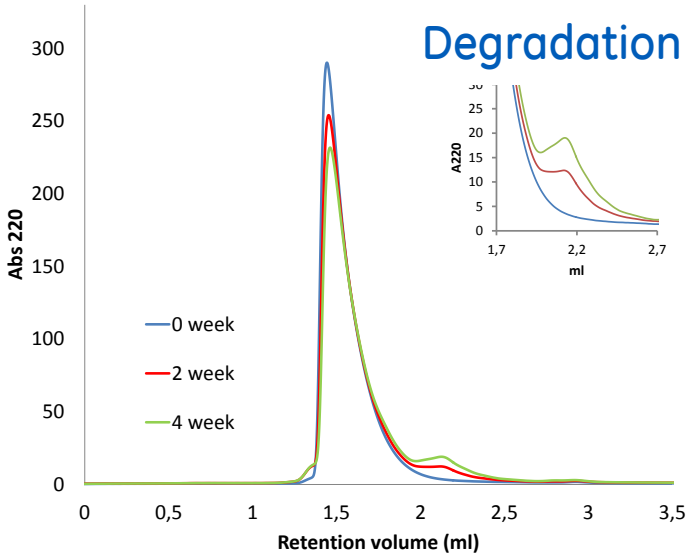
Column: HiLoad™ 16/60 Superdex™ 200 pg
Sample: 5.2 ml eluted pool from HisTrap
System: ÄKTAexplorer



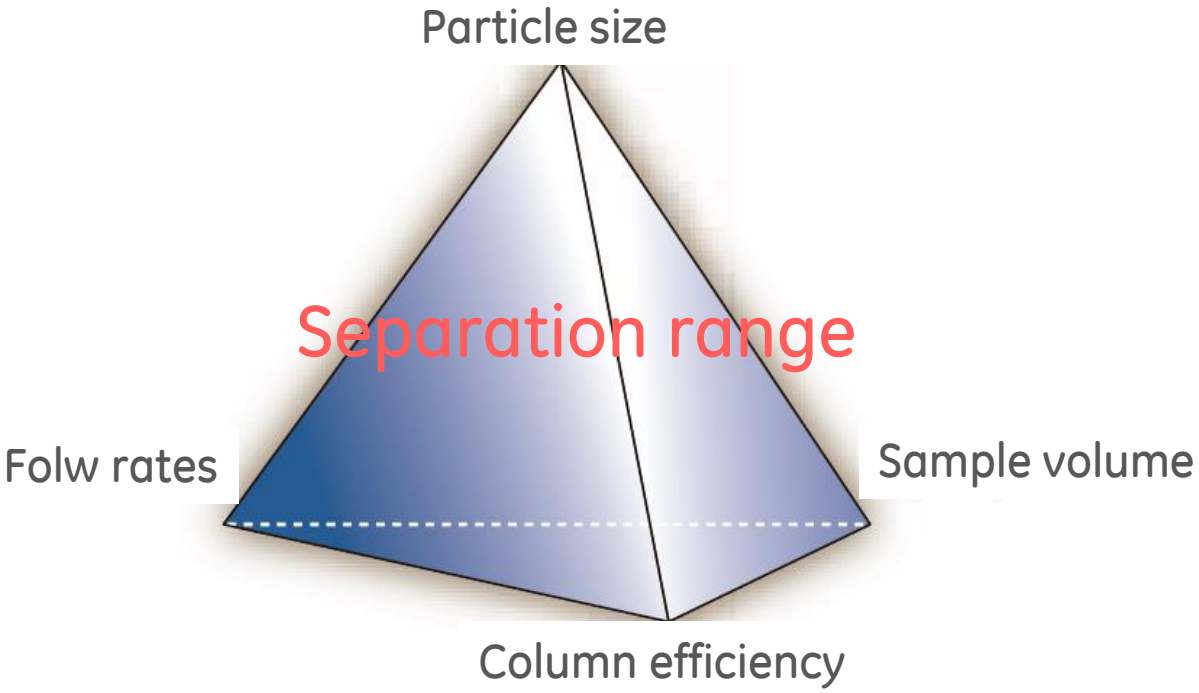
SEC: Product-related impurities

Column: Superdex™ 75 Increase 5/150
Sample volume: 10 µL, *Flow rate:* 0.5 mL/min
System: Agilent 1100

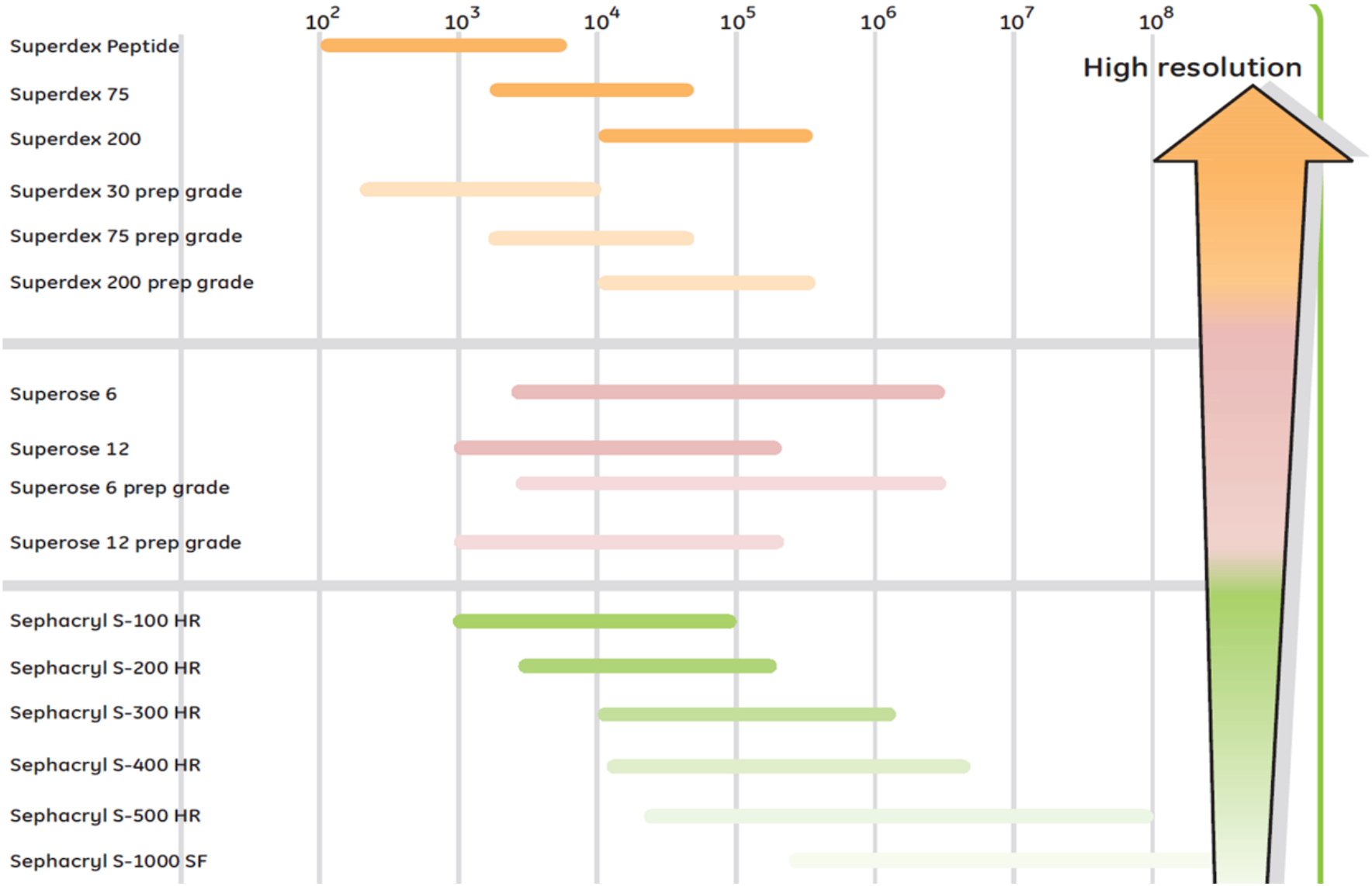




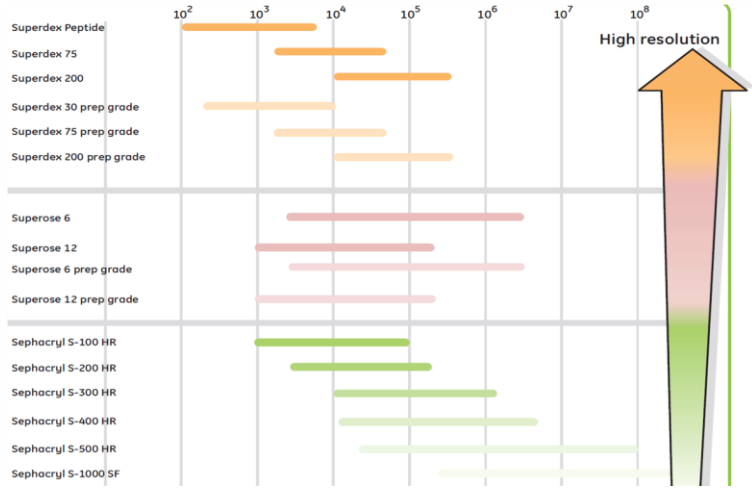
SEC: Important performance factors



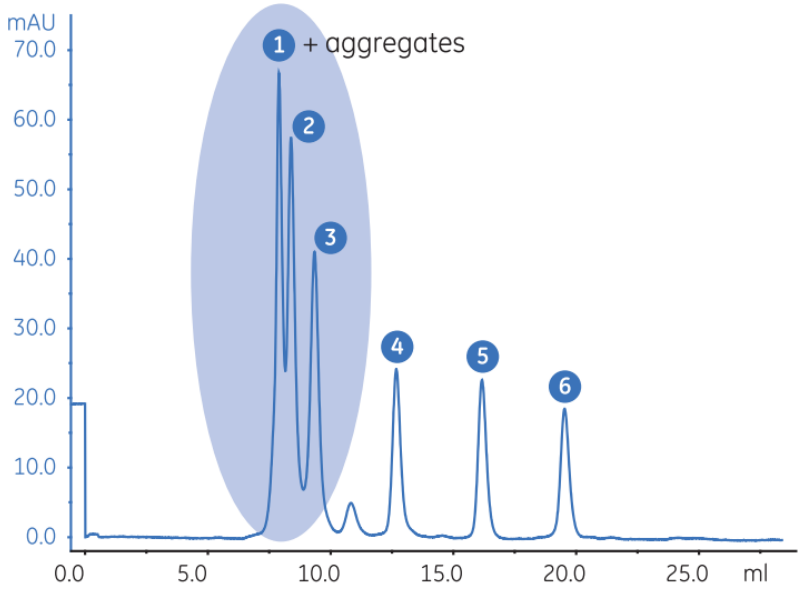
How to choose media and column - GF



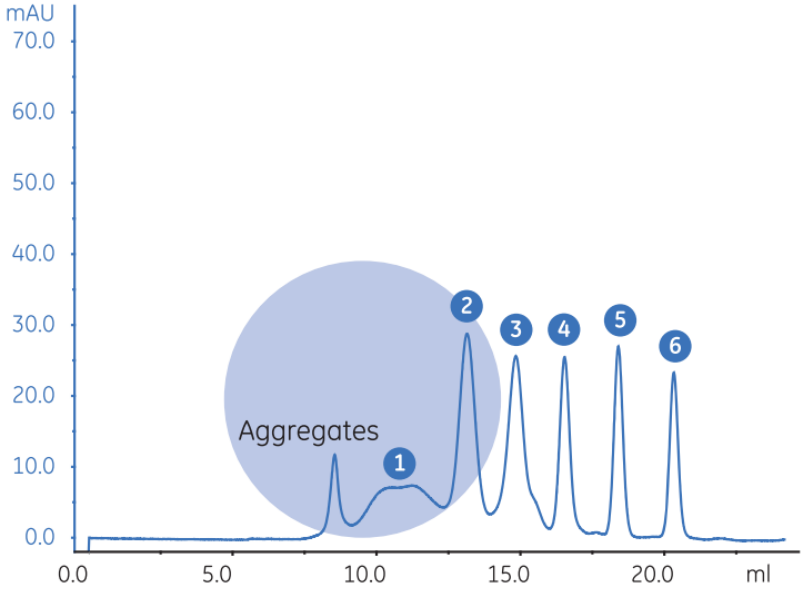
Sample:	M_r
1 IgM	970,000
2 Thyroglobulin	669,000
3 Ferritin	440,000
4 BSA	66,000
5 Myoglobin	17,600
6 Vitamin B	1,300



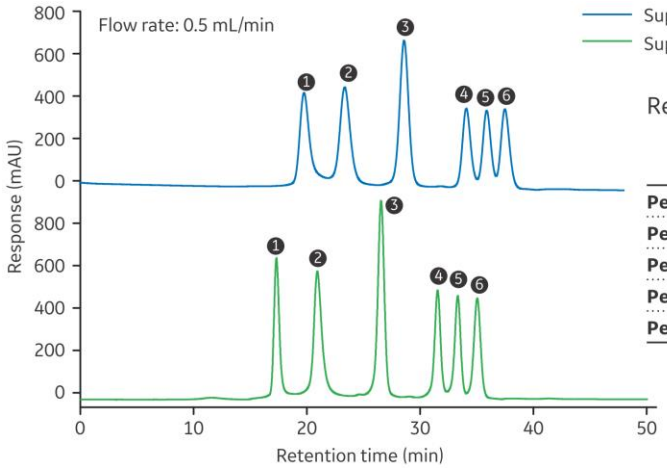
Superdex 200 Increase 10/300 GL



Superose 6 Increase 10/300 GL

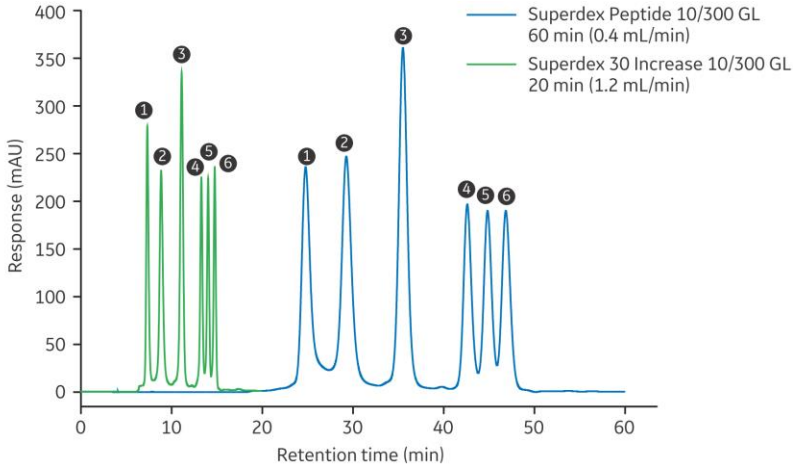


New generation Size Exclusion Chromatography (SEC) columns - Increase



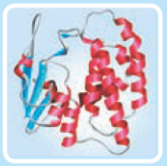
Resolution (Rs) data

	Superdex Peptide	Superdex 30 Increase
Peak 1-2	2.19	3.41
Peak 2-3	3.24	4.84
Peak 3-4	3.88	4.94
Peak 4-5	1.33	1.82
Peak 5-6	1.16	1.64



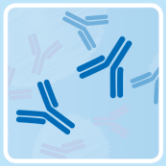
Superdex 30 Increase

Peptides and other small biomolecules
Fractionation range M_r ~ 100 to 7000



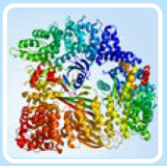
Superdex 75 Increase

Recombinant tagged proteins
Fractionation range M_r ~ 3000 to 70 000



Superdex 200 Increase

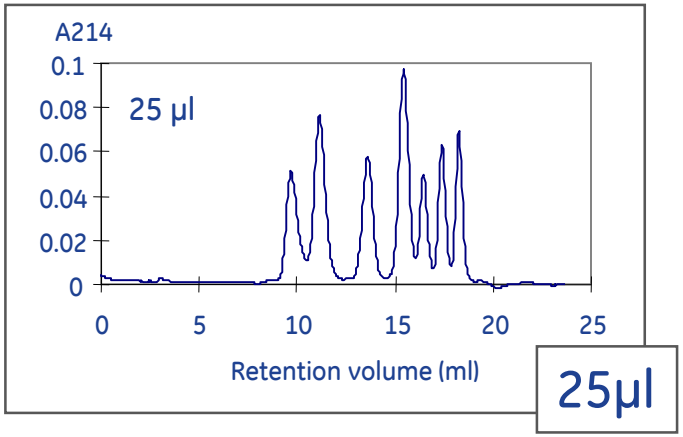
mAb and other antibodies
Fractionation range M_r ~ 10 000 to 600 000



Superose™ 6 Increase

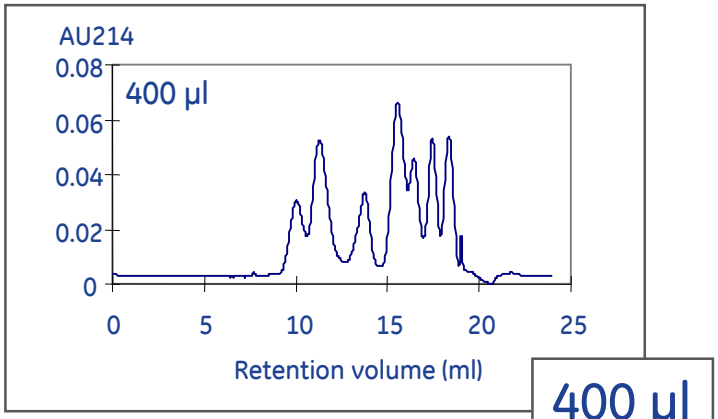
Larger proteins and protein complexes†
Fractionation range M_r ~ 5000 to 5 000 000

How to choose media and column - GF



25µl

0.1% Vt



400 µl

<2% Vt

Superdex™ Peptide column: 10/30_24ml

Desalting and buff exchange 25% CV

PD-10 (gravity) loading capacity 2.5 ml
HiTrap™ 5 ml loading capacity 1.5 ml
HiPrep™ 53 ml loading capacity 15 ml

Polishing–Fractionation 0.5% ~ 5% CV

µl – analytical column 1% CV
- 10/300 loading capacity 250 µl
- 5/150 loading capacity 50 µl

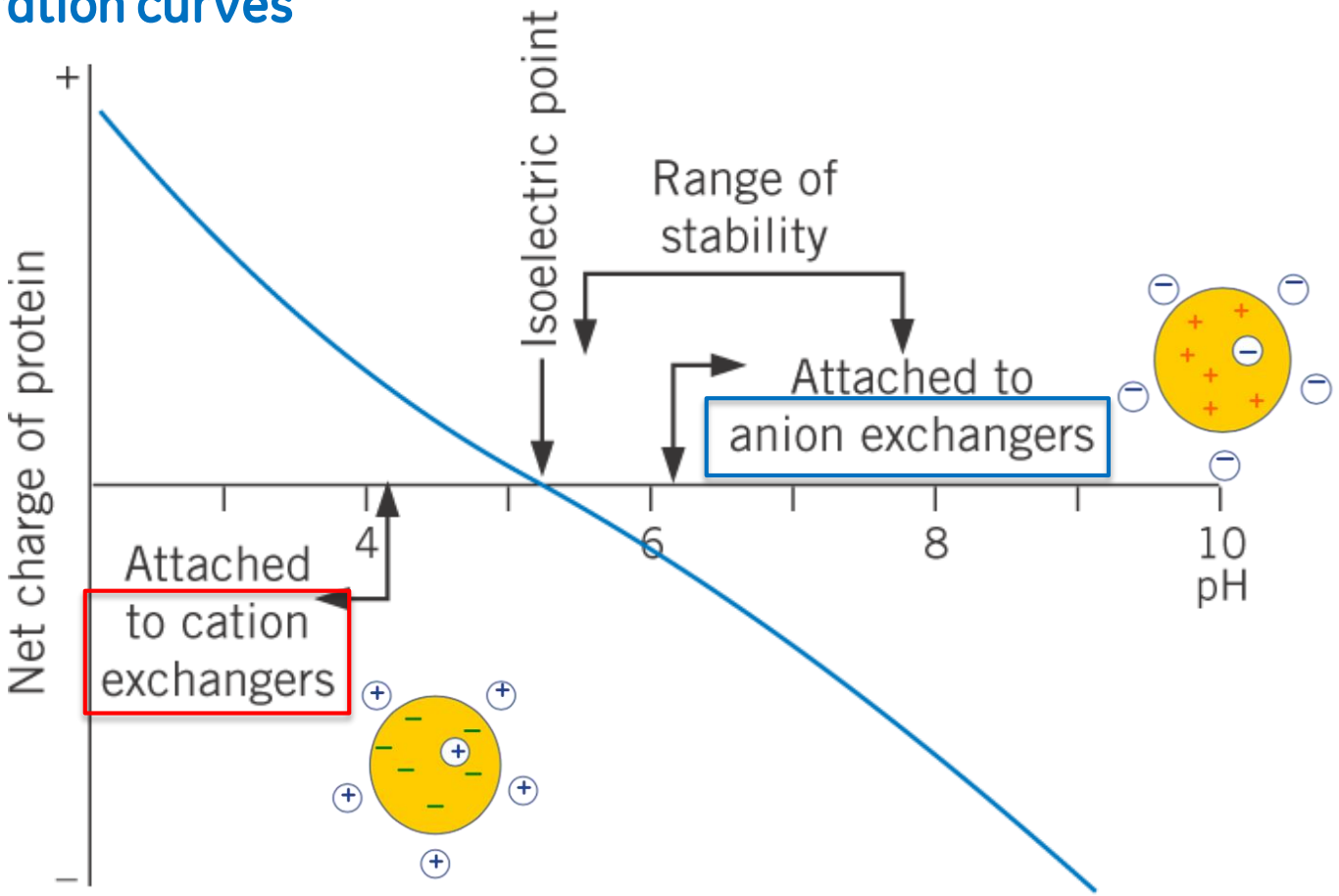
ml – preparative column 5% CV
- 16/60 loading capacity 5 ml
- 26/60 loading capacity 13 ml

How to choose media and column - column

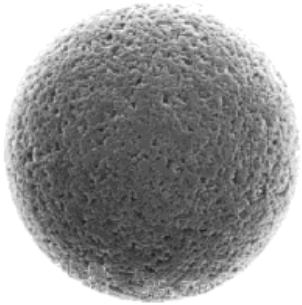
Description	Fractionation Range (kD)	Bed volume (ml)	Particle size	Sample volume
Superdex Peptide 10/300 GL	0.1~7	24	13	25~250 ul
Superdex 75 10/300 GL	3~70		13	25~250 ul
Superdex 200 10/300 GL	10~600		13	25~250 ul
HiLoad 16/60 Superdex 30 pg	up to 10	120	34	≤ 5 ml
HiLoad 16/60 Superdex 75 pg	3~70		34	≤ 5 ml
HiLoad 16/60 Superdex 200 pg	10~600		34	≤ 5 ml
HiLoad 26/60 Superdex 30 pg	up to 10	240	34	≤ 13 ml
HiLoad 26/60 Superdex 75 pg	3~70		34	≤ 13 ml
HiLoad 26/60 Superdex 200 pg	10~600		34	≤ 13 ml
HiPrep 16/60 Sephacryl 100	1~100	120	50	≤ 5 ml
HiPrep 16/60 Sephacryl 200	5~250		50	≤ 5 ml
HiPrep 16/60 Sephacryl 300	10~1500		50	≤ 5 ml
HiPrep 26/60 Sephacryl 100	1~100	240	50	≤ 13 ml
HiPrep 26/60 Sephacryl 200	5~250		50	≤ 13 ml
HiPrep 26/60 Sephacryl 300	10~1500		50	≤ 13 ml

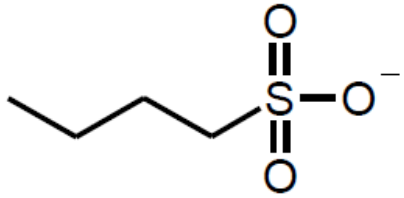
How to choose media and column - IEX

Titration curves



How to choose media and column - IEX





Resolution increases

Matrix (particle size)
MiniBeads (3)
MonoBeads (10)
Source 15 (15)
Source 30 (30)
Sepharose High Performance (34)
Capto™ ImpRes (40)
Capto™ ImpAct (40)
Sepharose Fast Flow (90)
Capto (90)

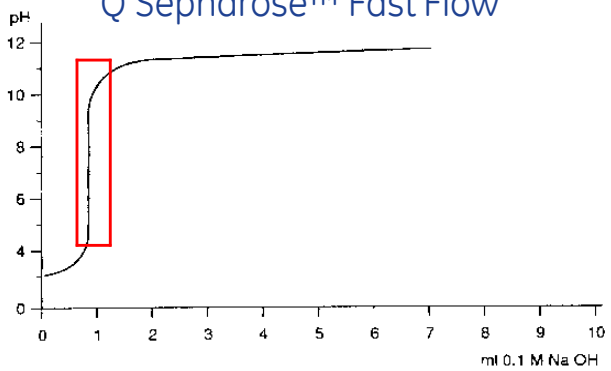
Anion exchanger
Q (strong)
ANX (weak)
DEAE (weak)

Cation exchanger
SP (strong)
CM (weak)

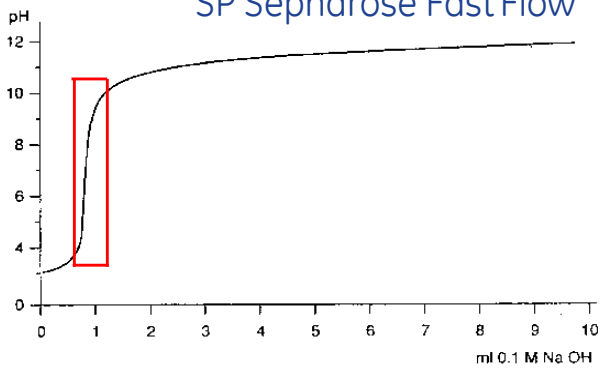
How to choose media and column - IEX

strong ion exchangers: capacity is constant over a wide range of pH

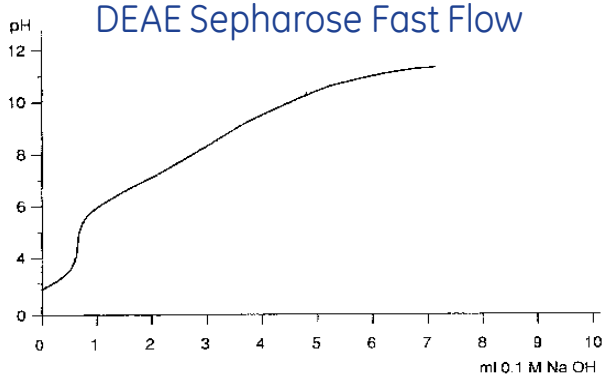
Q Sepharose™ Fast Flow



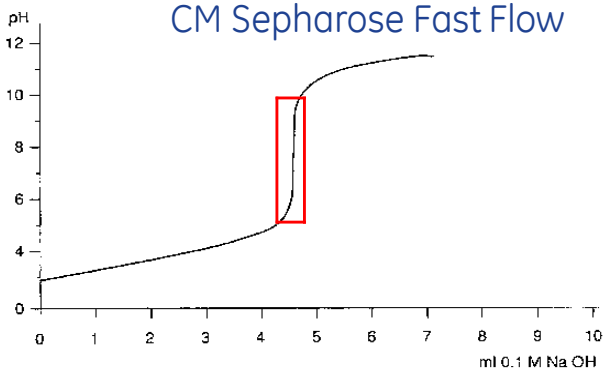
SP Sepharose Fast Flow



DEAE Sepharose Fast Flow

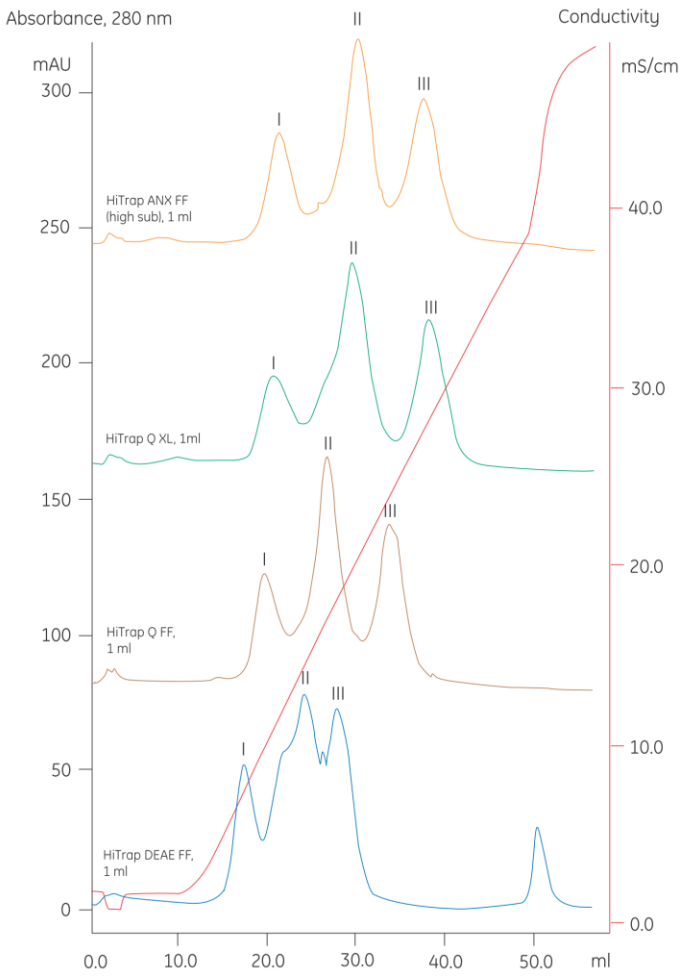


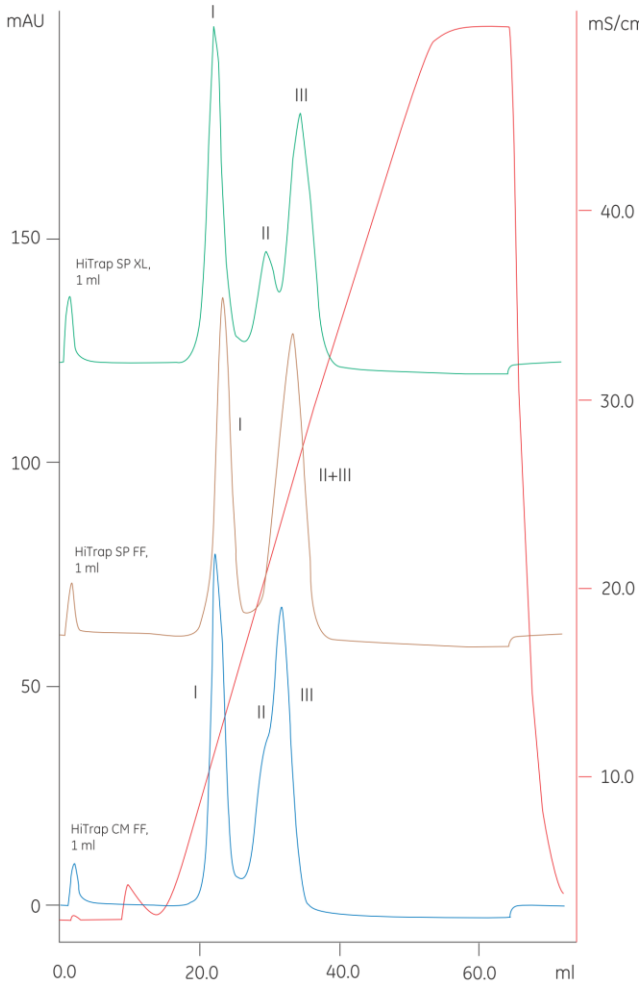
CM Sepharose Fast Flow



weak ion exchangers: capacity varies with pH

Use HiTrap selection kit to Quick screening





HiTrap IEX selection kit 7 x 1 ml

HiTrap Capto IEX selection kit 5 x 1 ml

HiTrap HIC selection kit 7 x 1 ml

Purification tools
ÄKTA™ System

Purification tools

Manual purification



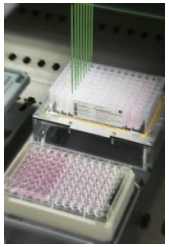


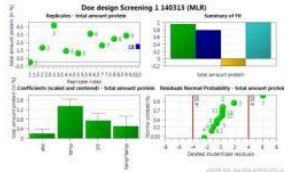
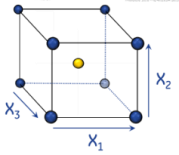



Automated purification





Comparison: manual vs LC system purification

Manual purification

Little training or start-up time required.

Easy to do parallel runs for increased throughput.

LC system purification
e.g. ÄKTA™ instruments

Automation – convenience

Gradient elution – high resolution

Reproducibility

Documentation

Select chromatography system based on the research application that matters to you

Standardization

Automation

Documentation

ÄKTA pure

Free up time

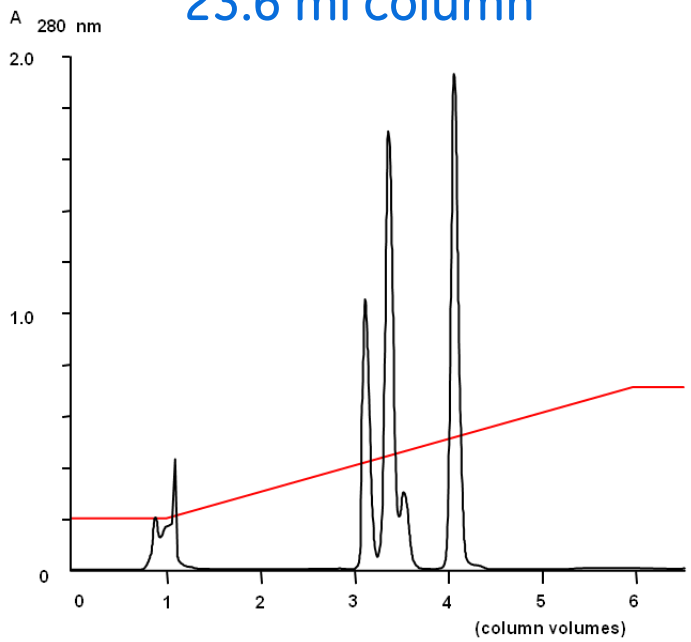
Purification efficiency

Minimize human error

And more.....

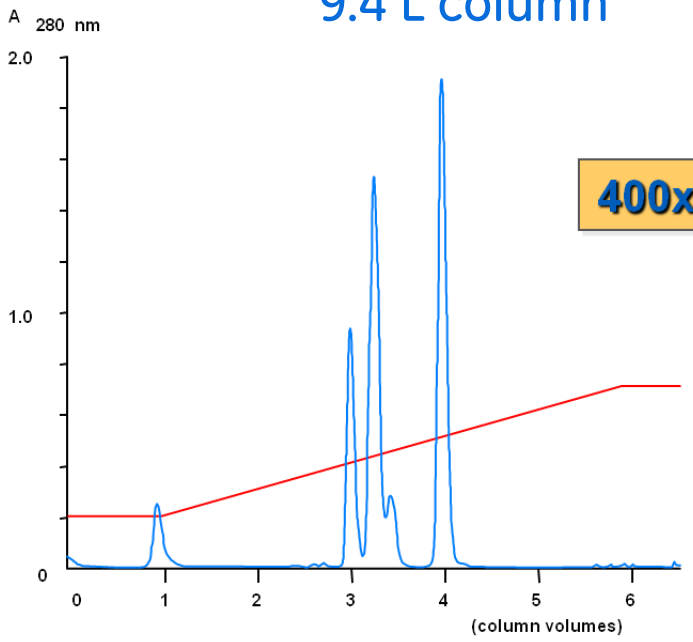
ÄKTA system purification

ÄKTA™ AKTApure 25
23.6 ml column



10 mm column diameter

ÄKTA™ process
9.4 L column

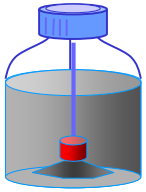


400x scale up

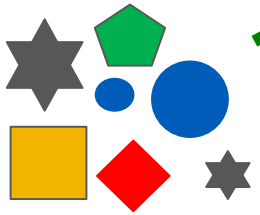
200 mm column diameter

Linear scale-up in the same AKTA platform

Basic Liquid Chromatography System



Buffer



sample

Pump

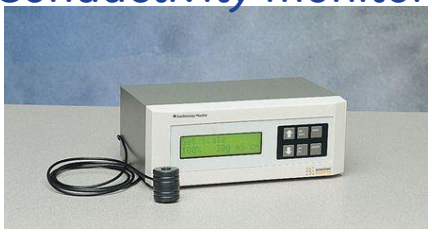
HiLoad pump P-50

Peristaltic Pump P-1

COLUMNS

Monitor UVis-920

Conductivity monitor



Frac-920 fraction collector

Proven technology

Continues ÄKTA™ tradition

- Incorporates over 50 years of experience in protein research
- Backed by 30 years of experience in developing protein purification systems
- ÄKTA systems are used by 100,000 researchers globally

2012—ÄKTA pure: protein purification your way

1959—Sephadex™, the world's first gel filtration medium

1982—FPLC™ system released, predecessor to ÄKTA systems

1997 - ÄKTApurifier™ · single platform for all chromatography techniques

New Generation of ÄKTA pure

New System

ÄKTA™ pure

Functions & Price

Classic System

ÄKTAprime™ plus

ÄKTApurifier™ UPC

ÄKTAFPLC™

ÄKTApurifier™

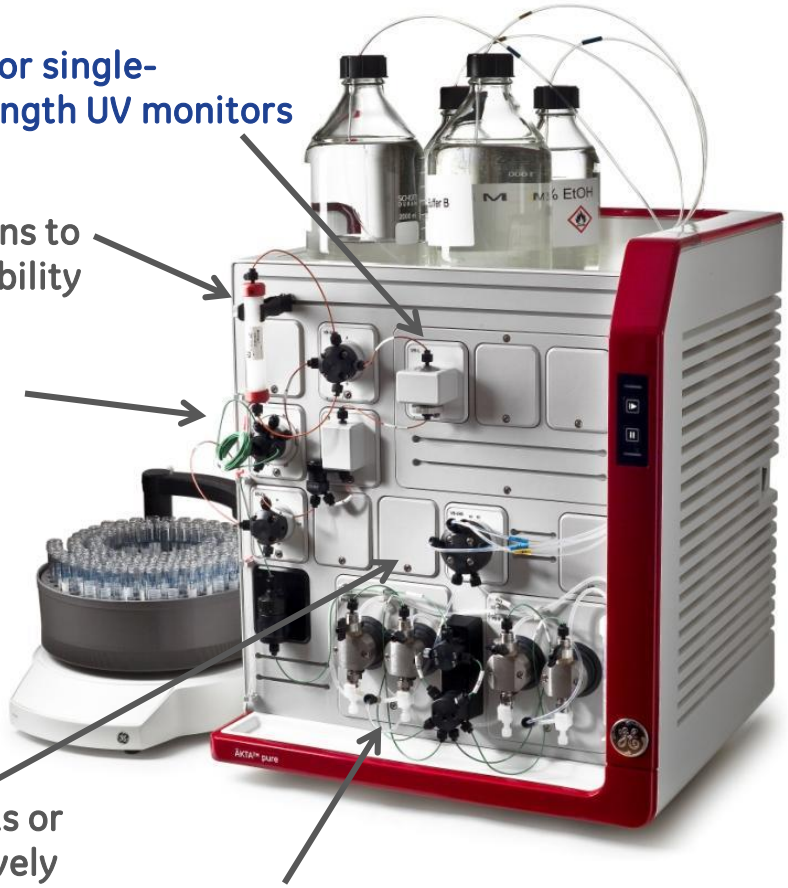
ÄKTAexplorer™

New Generation of ÄKTA pure

Flexible

Intuitive

Reliable



Triple- or single-wavelength UV monitors

•High-quality, prepacked columns to ensure reproducibility

Modular design

Intuitive viewing and new interactive process picture

Valve options to simplify basic tasks or automate extensively

Choose your scale: High precision pumps that go to 25 ml/min or 150 ml/min

Protein purification your way

Two versions for different needs

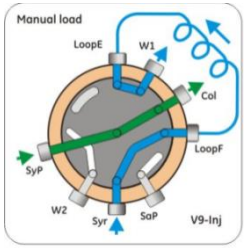
	ÄKTA pure 25	ÄKTA pure 150
Flow Rate	0.001~25ml/min	0.01~150ml/min
column packing flow	50ml/min Max.	300ml/min Max.
Operating Pressure Max.	20Mpa	5Mpa
Flow rate of sample pump	0.001~50ml/min	0.01~150ml/min
Operating Pressure [Sample Pump] Max.	10Mpa	5Mpa
Column	26~50mm	70~100mm
Productivity	ug~g of protein	10g of protein

Core modules

System pumps P9 A, P9 B

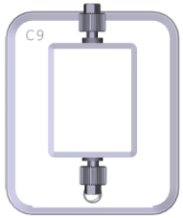
- 0.1-25 ml/min, 0-20 MPa
- Automatic pressure control

System pressure monitor R9



Injection valve V9-Inj

- No need to re-plumb when changing sample application technique



Conductivity monitor C9

- Up to 999.99 mS/cm
- Flow cell volume 22µl
- Mixer size stored in result file

Mixer M9

- Chambers 0.6, 1.4, 5 ml
- Integrated in-line filter
- Mixer size stored in result file
- Gradient composition accuracy: ± 0.6%

Instrument control panel B9

- Status lamps (power, run, pause, alarm/error)
- Pause / continue buttons

Module options

Core modules

- System pump P9 A
- System pump P9 B
- System pressure monitor R9
- Mixer M9
- Injection valve V9-Inj
- (ICU I9n – internal module)
- (ICP B9 – internal module)

Core modules = mandatory

Optional modules

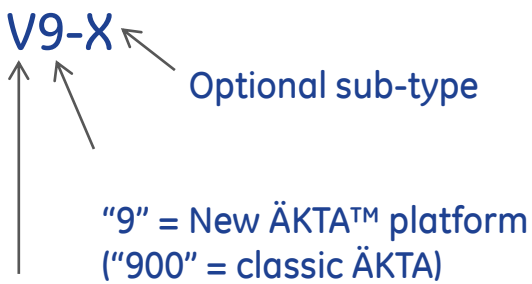
- Inlet valve V9-IAB
- Inlet valve V9-IA
- Inlet valve V9-IB
- Inlet valve X1
- Inlet valve X2
- Mixer valve V9-M
- Column valve V9-Cs
- Column valve V9-C
- pH valve V9-pH
- Outlet valve V9-Os
- Outlet valve V9-O
- Versatile valve V9-V
- Versatile valve V9-V, 2nd
- UV monitor U9-M
- UV monitor U9-L
- UV monitor U9-L, 2nd
- Cond. monitor C9n

Improved vs ÄKTApurifier™

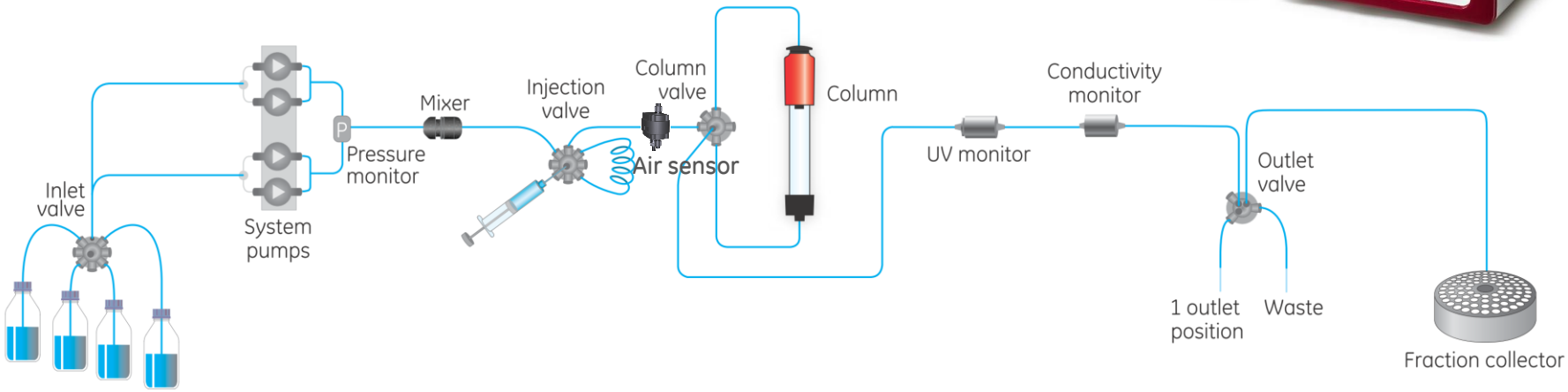
Optional modules (external)

- Air sensor L9, 1
- Air sensor L9, 2
- Air sensor L9, 3
- Air sensor L9, 4
- I/O-box E9
- I/O-box E9, 2nd
- Fraction collector F9-R
- Fraction collector F9-R, 2nd

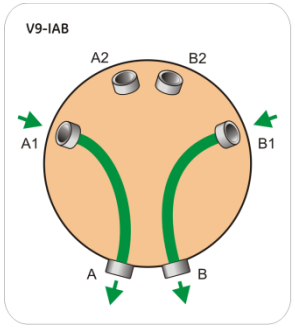
Module naming



Flexibility to match your applications



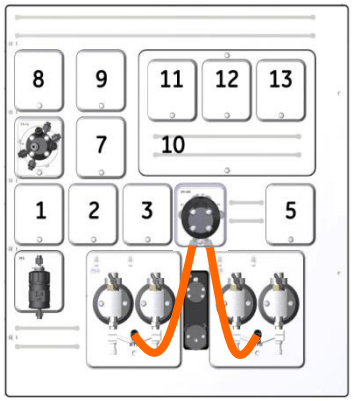
Buffer Inlet valve



Inlet valve V9-IAB

- - One valve for both A and B inlets

- 4 inlet positions (2A and 2B)



Benefits:

- Buffer scouting
- Large sample volume loading
- CIP solution and storage solution operation (NaOH & 20% EtOH)

System pump P9 A/P9 B

ÄKTA pure25:

Flow rate: 0.001-25 ml/min

Max. flow rate: 50ml/min

Pressure: 0-20 MPa

ÄKTA pure150:

Flow rate: 0.01-150 ml/min

Max. flow rate: 300ml/min

Pressure: 0-5 MPa

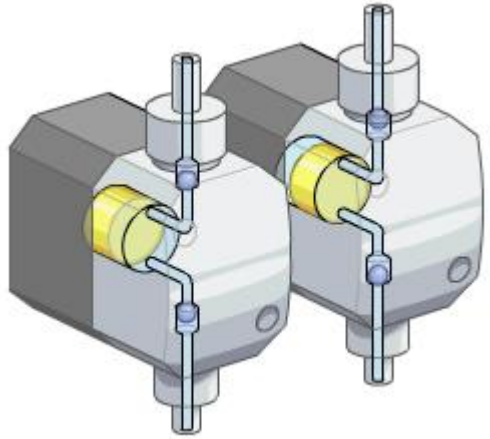
--Fulfill any purification method。

--Compatible for any bio-molecules

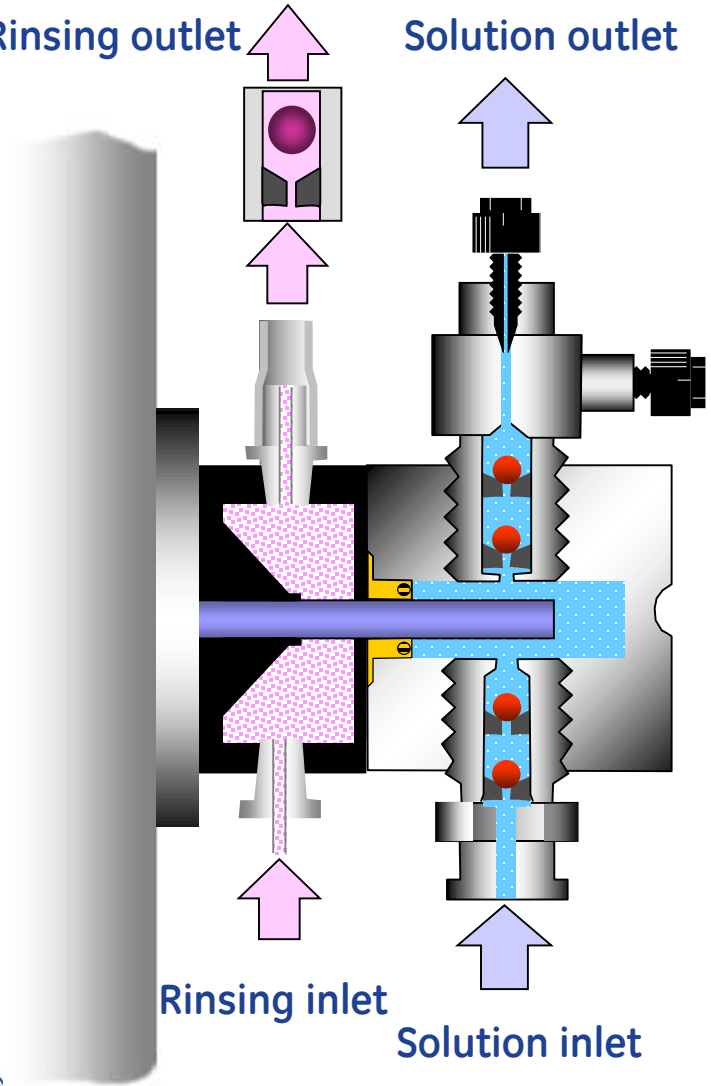
--Generate isocratic or gradient elution

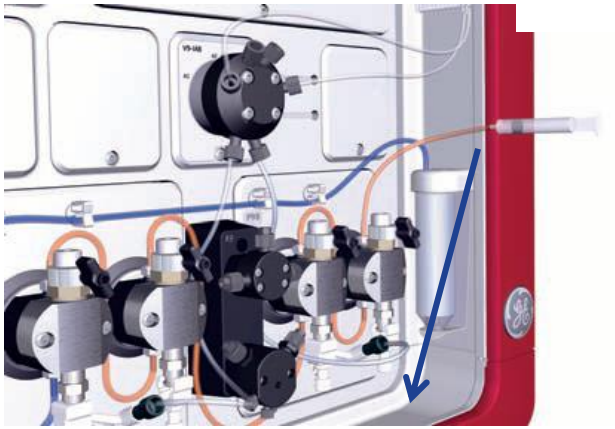
--Two pump heads that work alternately to give a continuous, low pulsation, liquid delivery.

--Automatic pressure control



System Pump Design





Pump head rinsing system

Function：

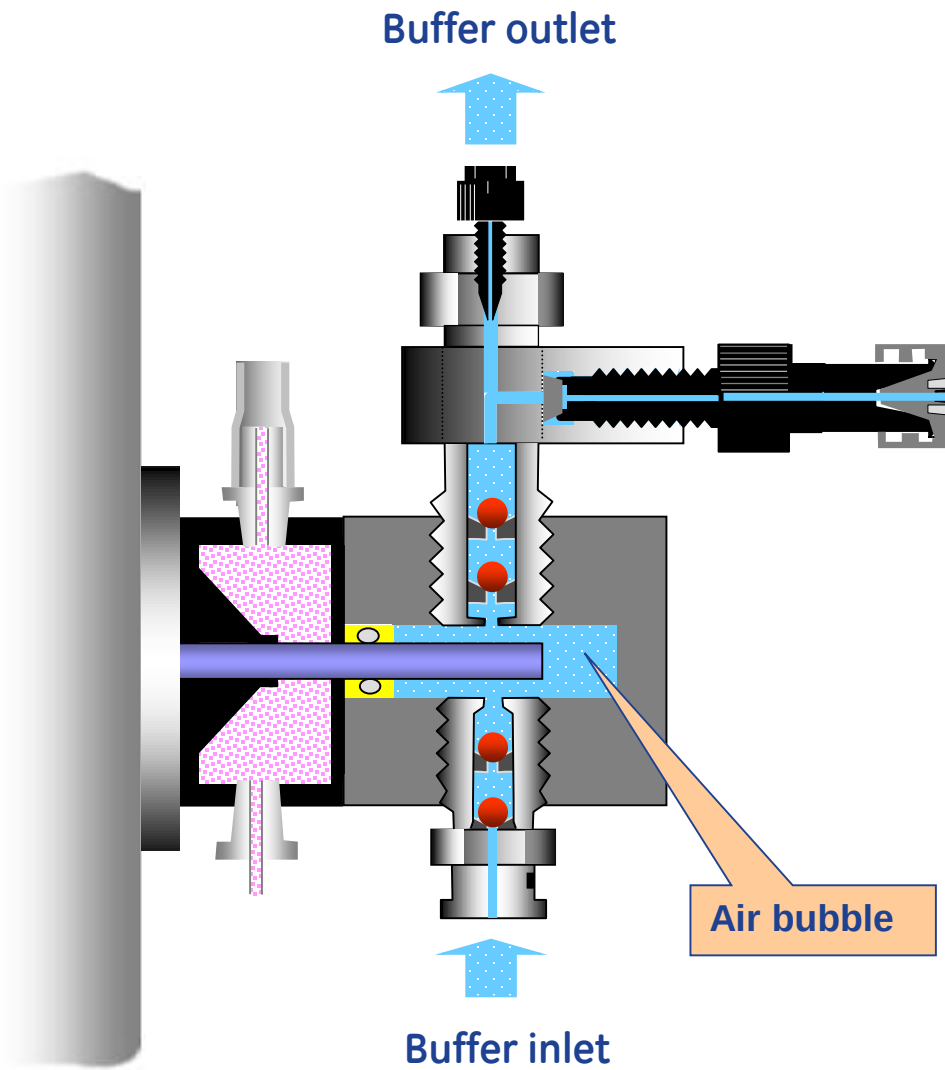
--Rinse piston；

--Rinse out buffer salt；

Maintenance： 20% EtOH

（change rinsing solution every week）

Air in Pump



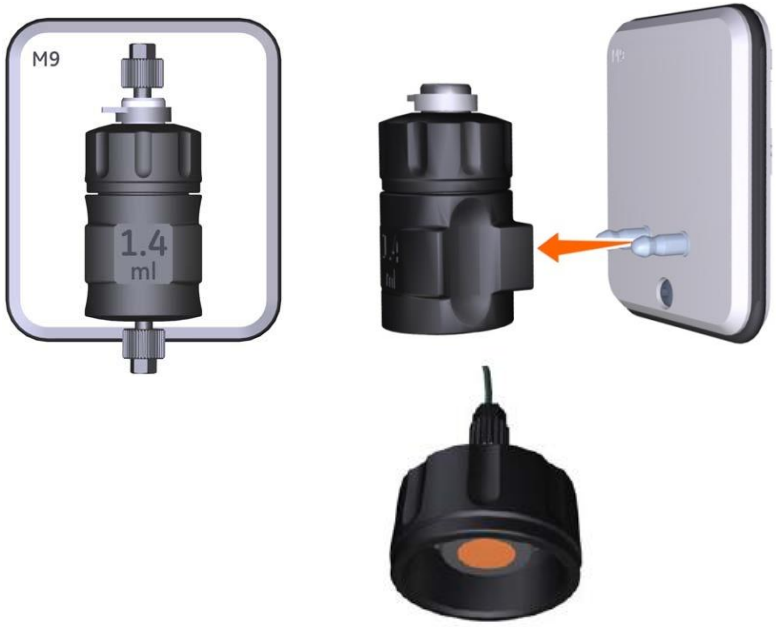
- If air gets stuck in the pump, manual purge might be necessary
- For lower amount of air, use pump wash

Mixer M9

dynamic mixer for high-performance gradients

- Mixed to give a homogenous buffer composition
- Integrated in-line filter (pore size 10 µm)
- Four mixer chamber size

Mixer chamber size	Flow rate two pump gradient
0.6 ml	0.1 - 5 ml/min
1.4 ml	0.5 – 10 ml/min
5 ml	5 – 25 ml/min
15ml	25~150ml/min



In-line filter

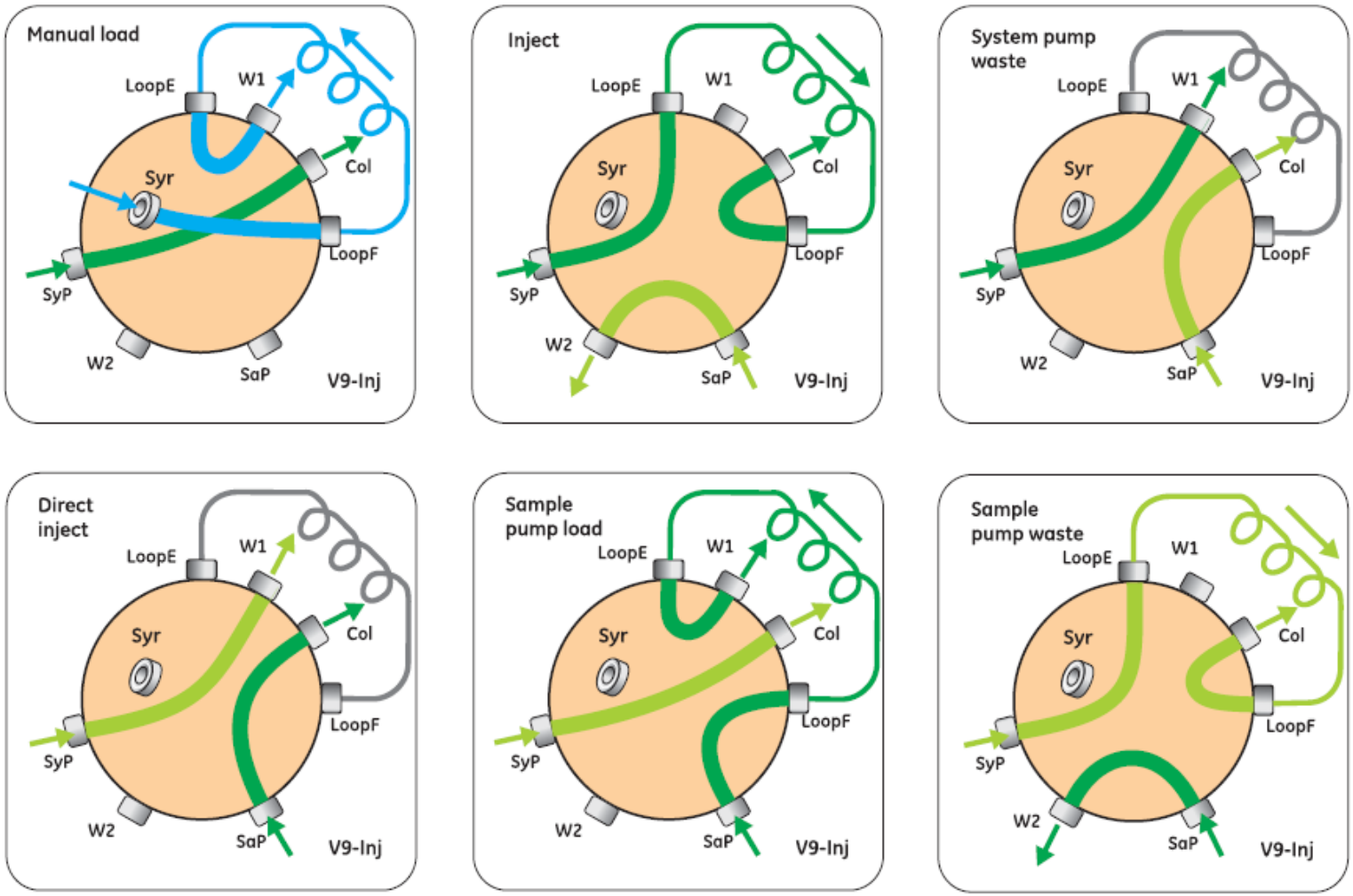
Injection Valve V9-Inj

enables usage of a number of different sample application techniques.

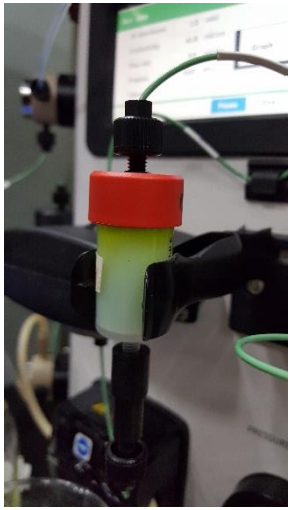
sample application techniques：

- Fill sample loop manually using a syringe
- Fill superloop (10ml, 50ml, 150ml) automatically using a syringe
- Sample injected directly onto the column using a system pump
- Work with mixer valve V9-M for by-pass of mixer/in-line filter

Ports and flow paths of the Injection valve

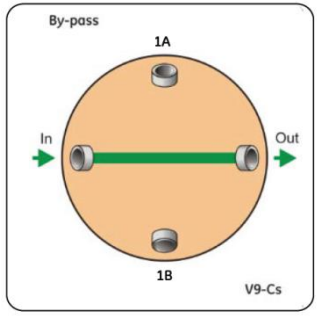


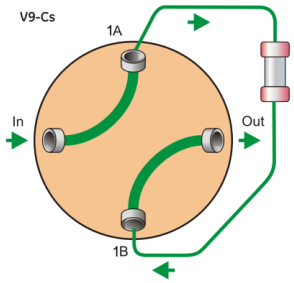
Column valve V9-Cs, V9-C



No column valve

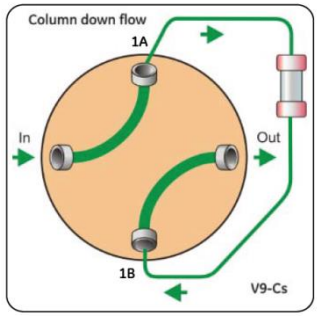
- One column position, no by-pass

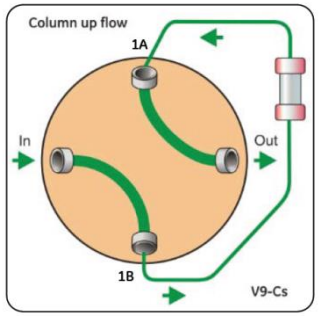


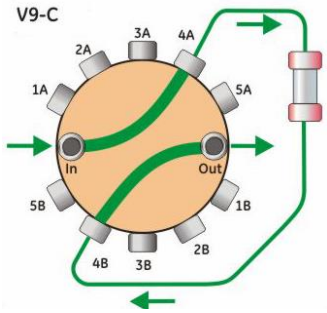


Column valve V9-Cs

- One column position
- Built-in by-pass position
- Reversed flow







Column valve V9-C

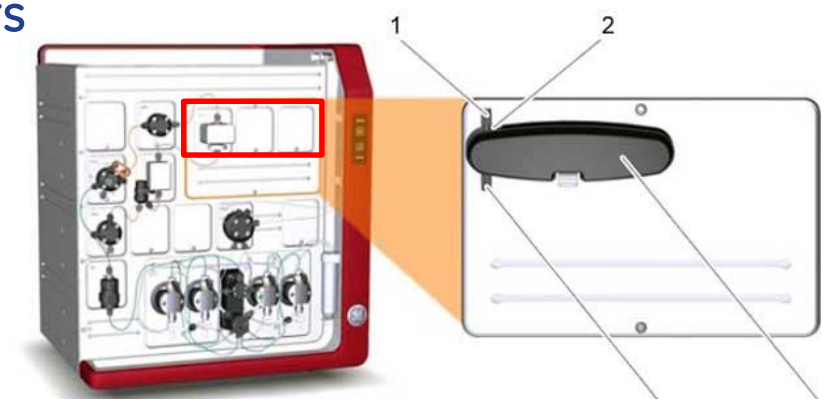
- 5 column positions
- Integrated pressure sensors
- Built-in by-pass position
- Reversed flow

Benefits：

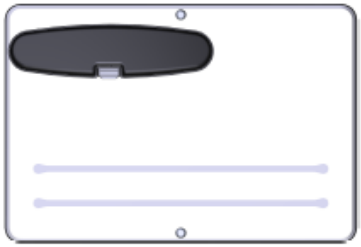
- By-pass position：enables system wash only
- Downflow or upflow enables column operation and cleaning

Basic configuration - UV monitor options

Triple- or single-wavelength UV monitors

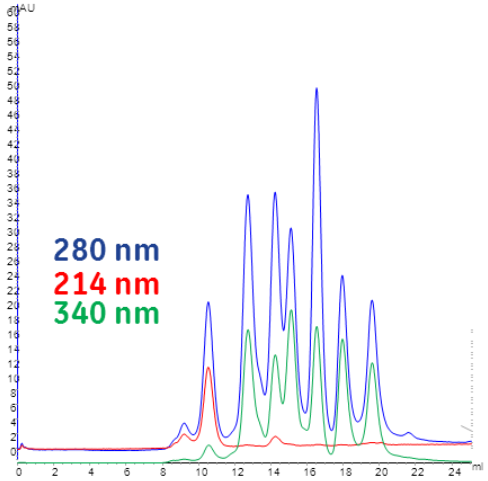


190-700 nm



Flash lamp and fibre optics

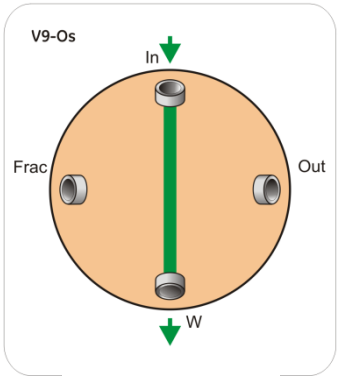
- no warm up time
- no sample warming
- long life time



Outlet Valve V9-Os, V9-O

Outlet valve V9-Os

- Waste
- Frac
- 1 outlet position **(Support for the second fraction collectors)**



imagination at work

Fraction collector F9-R

Automatic collection of sample fraction

- Fixed volume fractionation
- Peak fractionation
- Combined fixed volume fractionation and peak fractionation
- 3, 5, 8, 15, 50 ml tubes

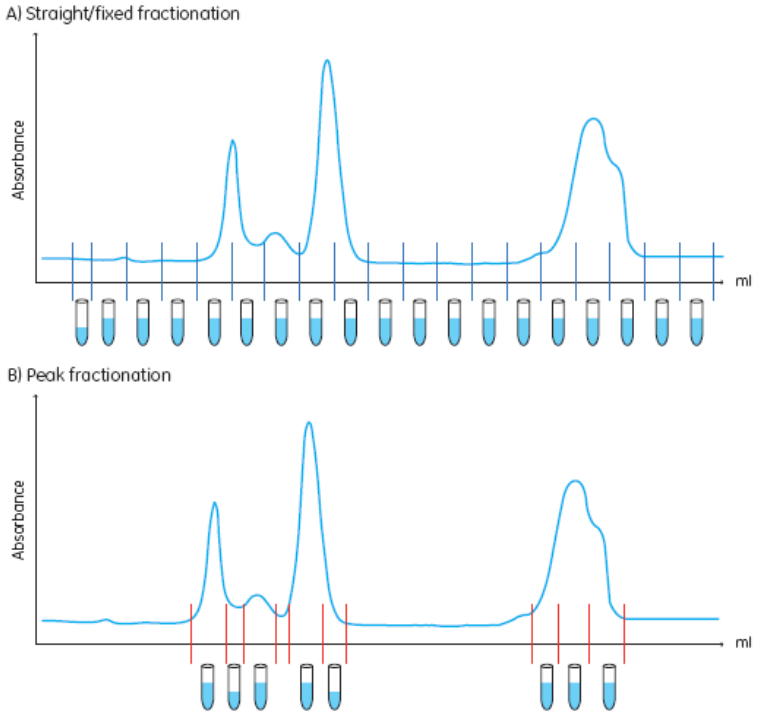


Fig 9.1. Straight fractionation (A), Peak fractionation (B).

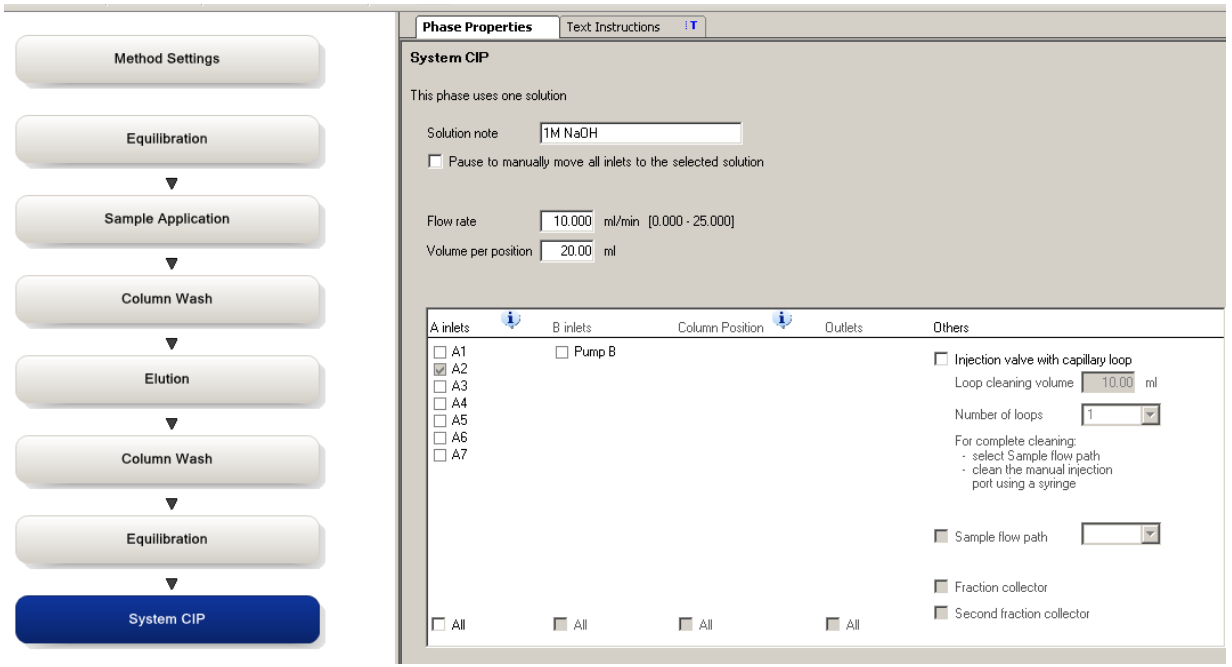
imagination at work

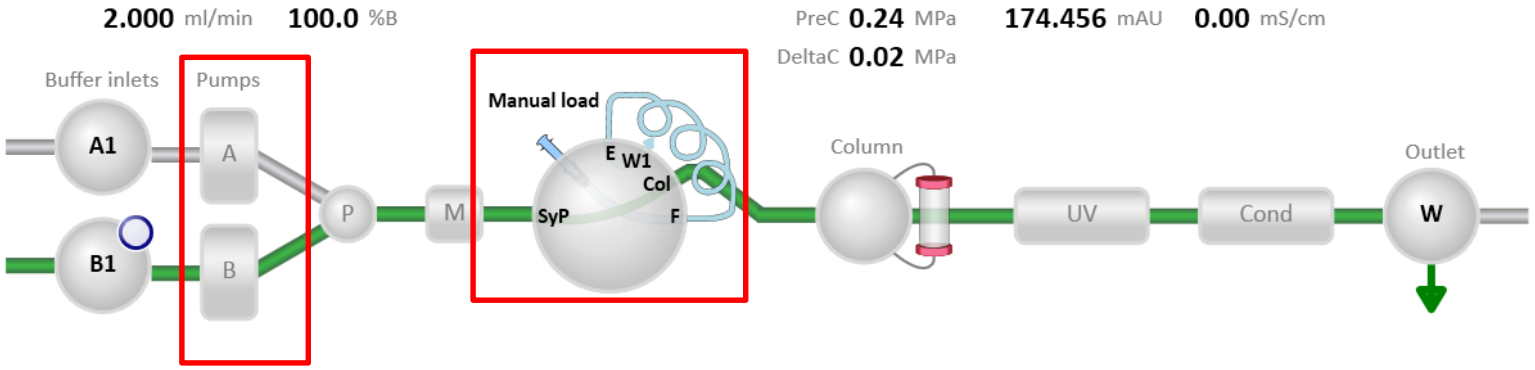
Introduction to UNICORN™ 7 software

Fast method setup

Predefined methods

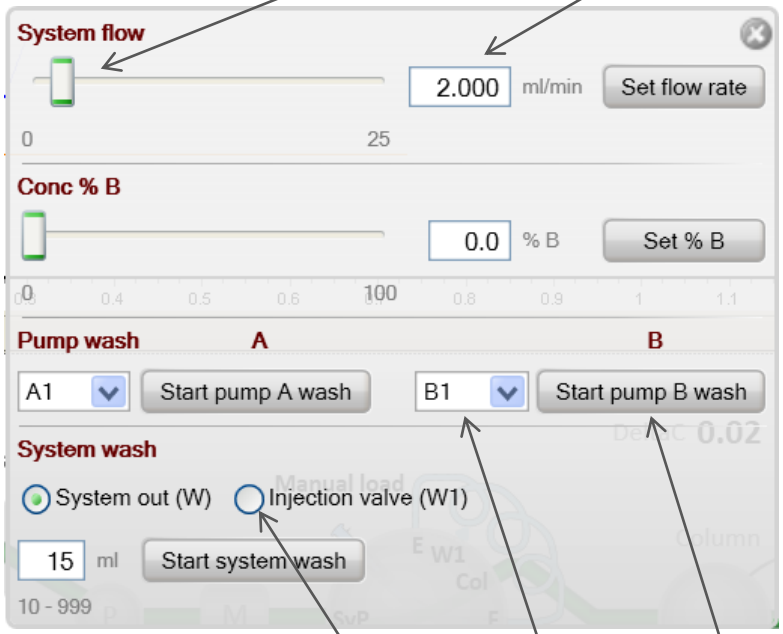
✓ Drag-and-drop programming

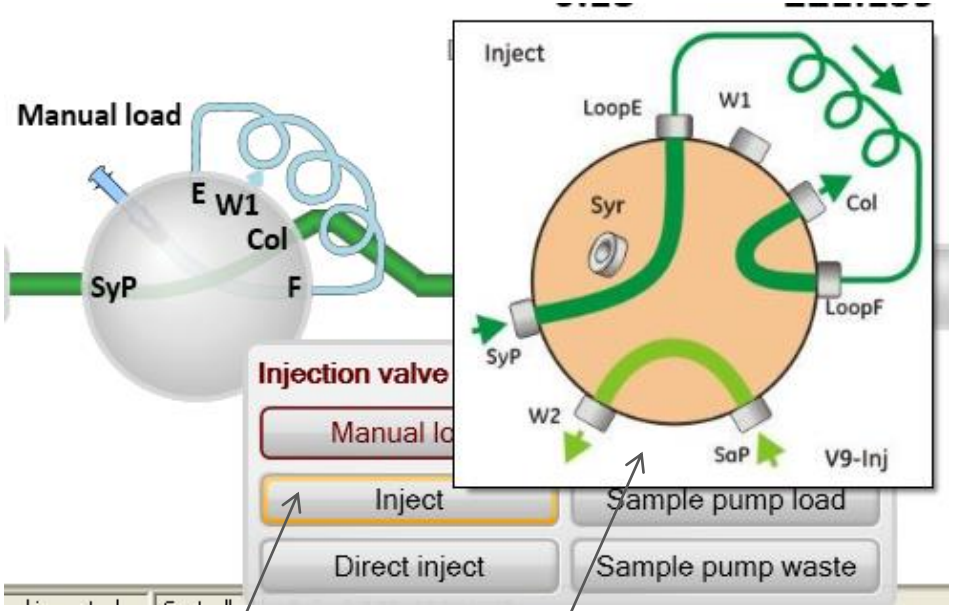




Sliders

Numerical input





Pop-up window

Execute buttons

Detailed valve pictures

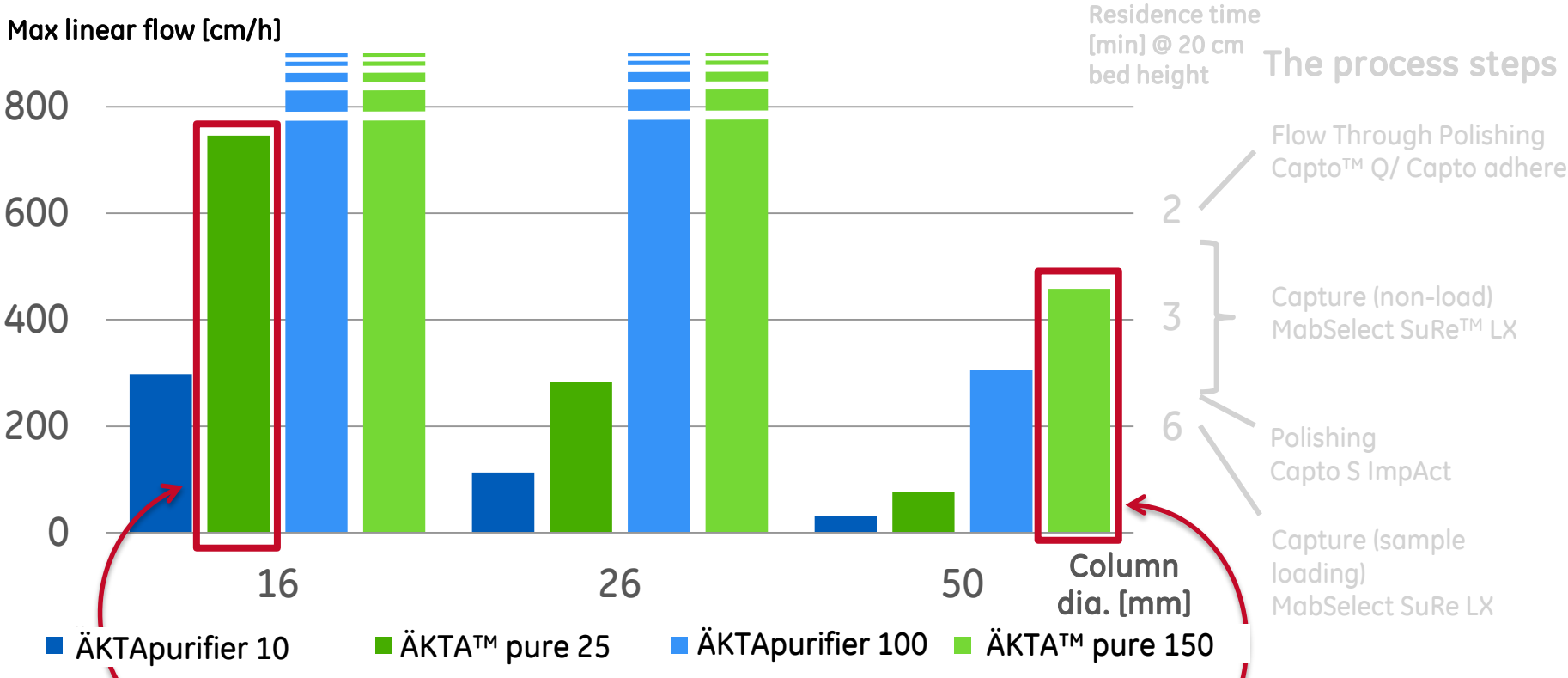
Drop-down lists

Direct selection buttons

Radio buttons

Purification optimization for ÄKTA™ System

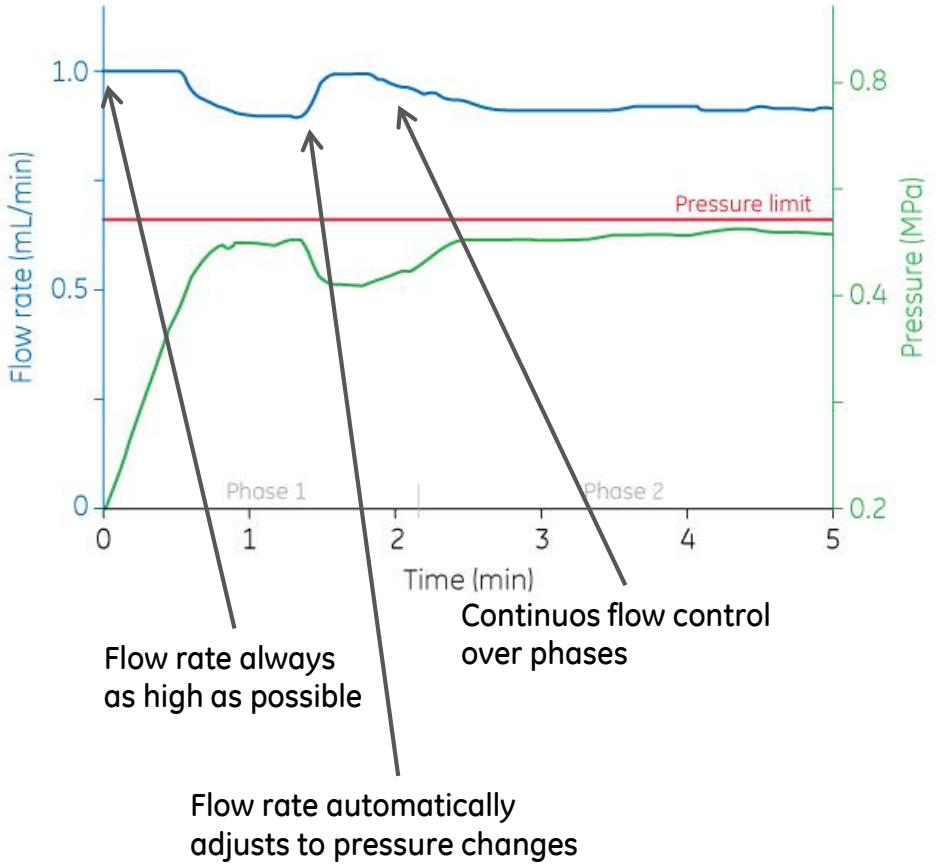
MAb purification example



All process steps supported in 16 mm columns on ÄKTA pure 25

Capture, polishing and capture non-load steps supported in 50 mm columns on ÄKTA pure 150

Maximize flow rates and protect your columns



Save time on your runs

Pressure sensors within the column valve

Flow control based on precolumn and delta pressure

Advanced continuous control algorithm

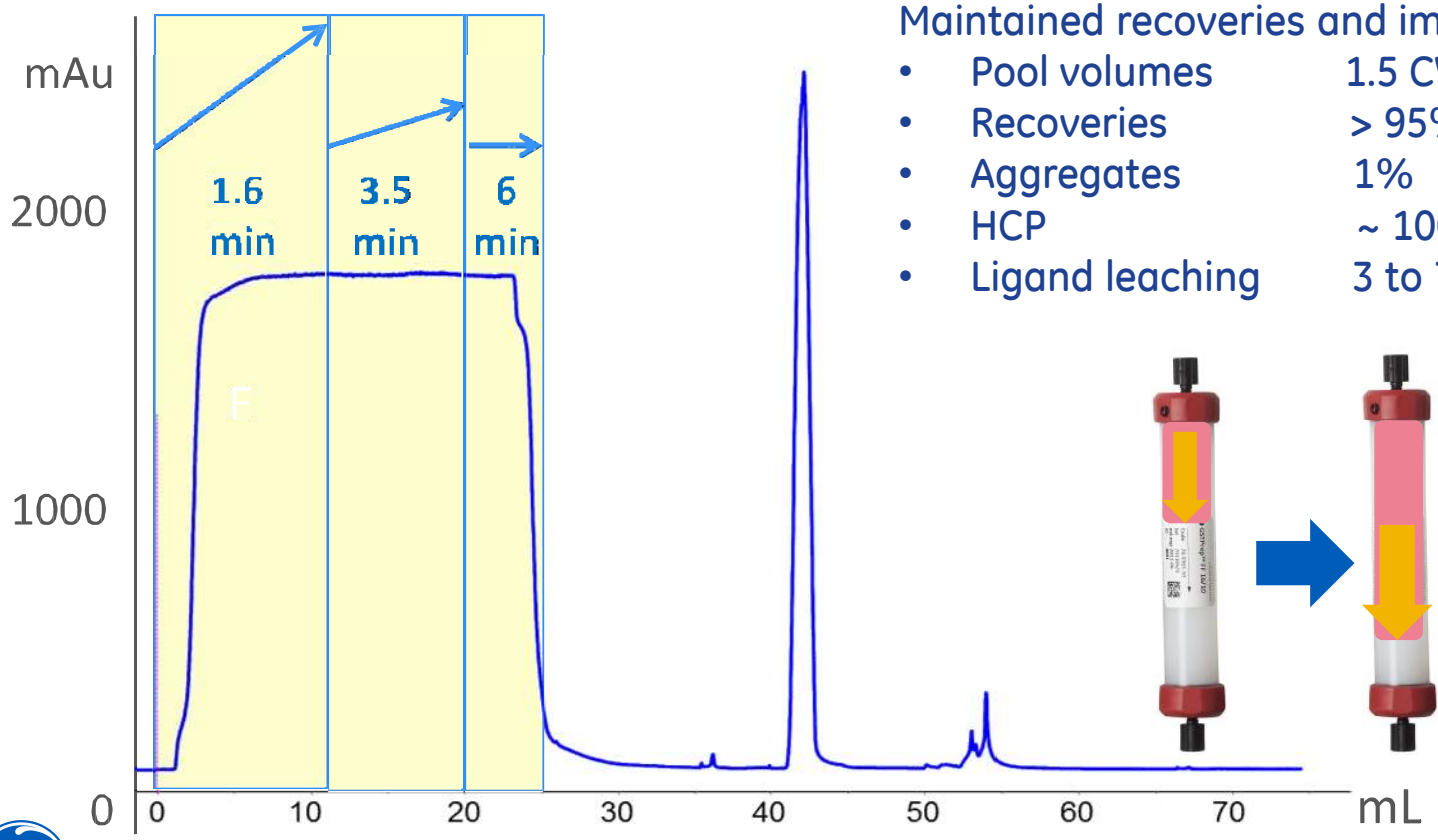
Connect up to 5* columns

* Installing optional extra column valves enables simultaneous use of up to 10 columns.

PID control

Cycle time variable loading - Improving productivity

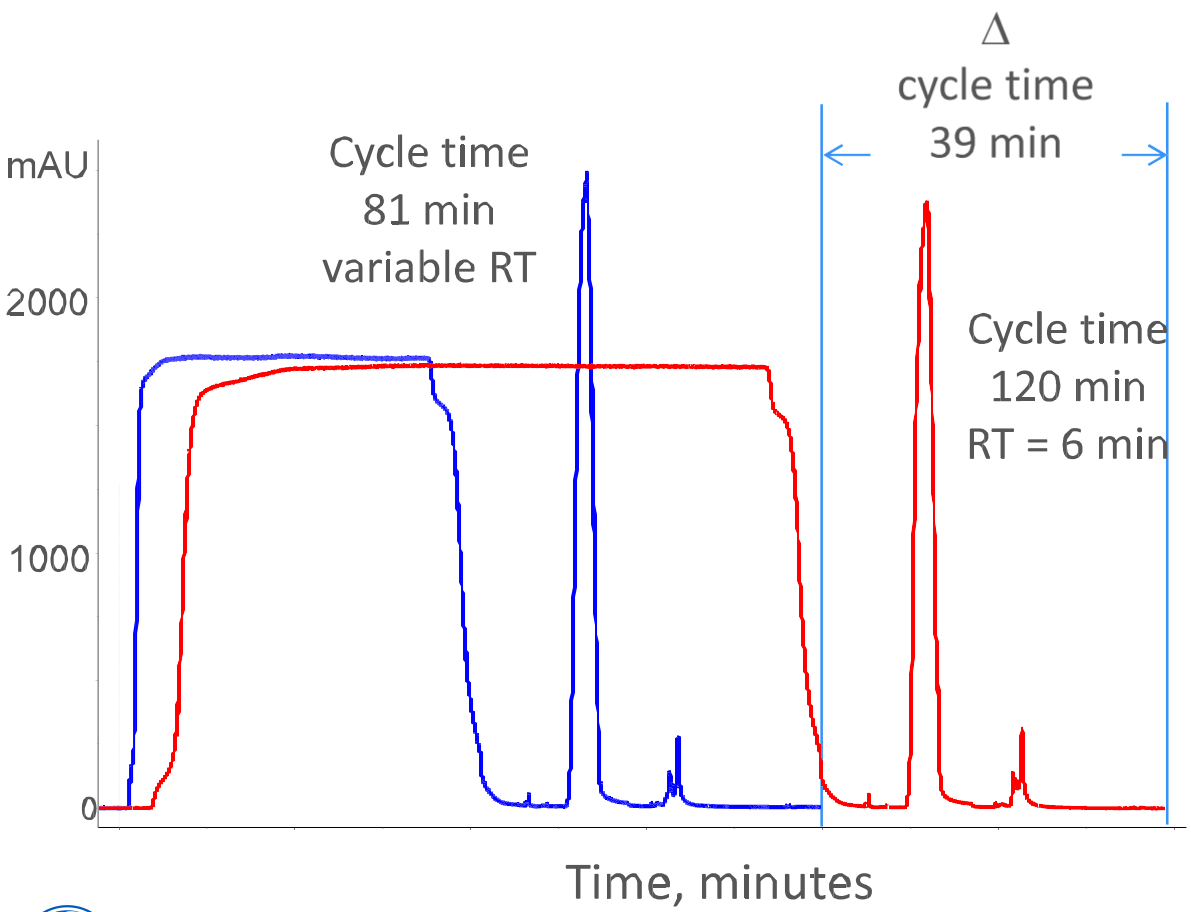
80% of QB10 was loaded for each residence time on MabSelect SuRe™ LX
DoE study peformed to optimize each residence time



Maintained recoveries and impurity profile

- Pool volumes 1.5 CV
- Recoveries > 95%
- Aggregates 1%
- HCP ~ 1000ppm
- Ligand leaching 3 to 7 ppm

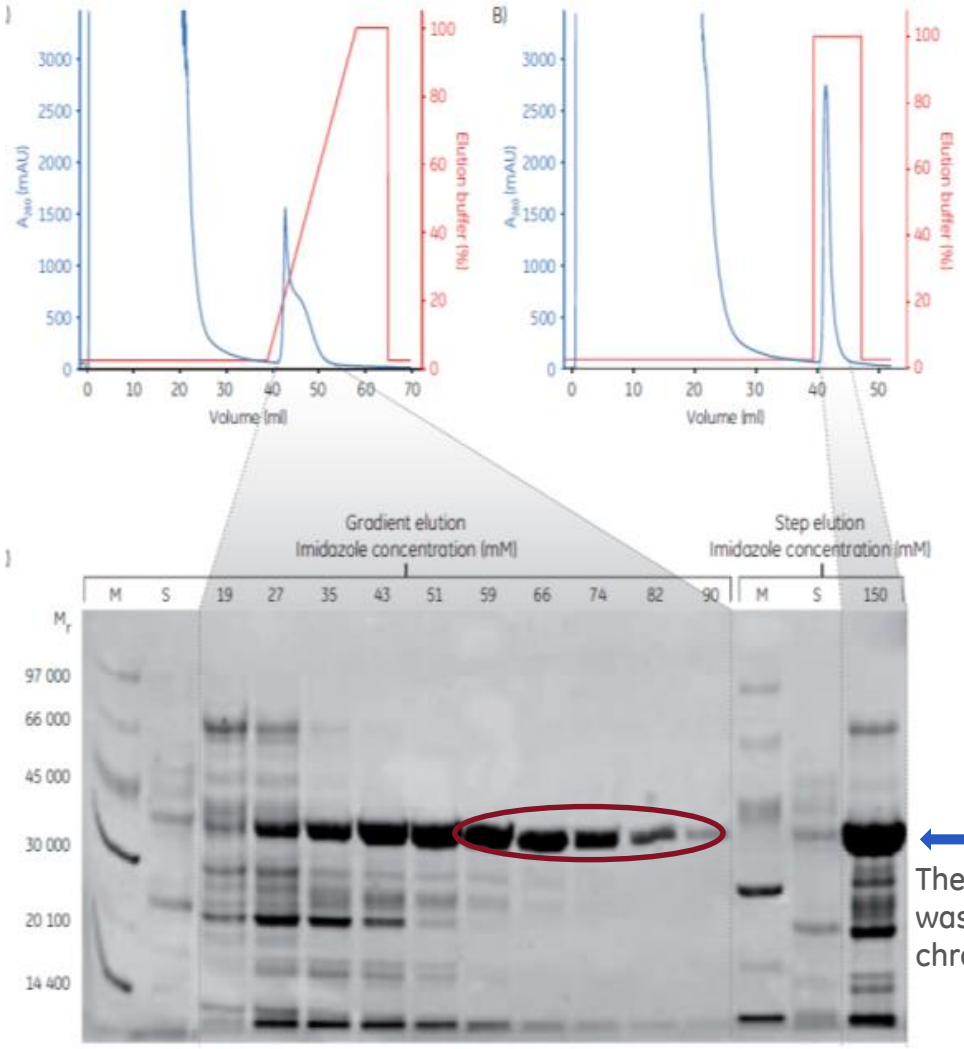
Cycle time normal loading Vs variable loading strategy



40% productivity increase. Maintained capacity and performance.

ÄKTA system: Linear gradient elution is an efficient approach for obtaining high purity

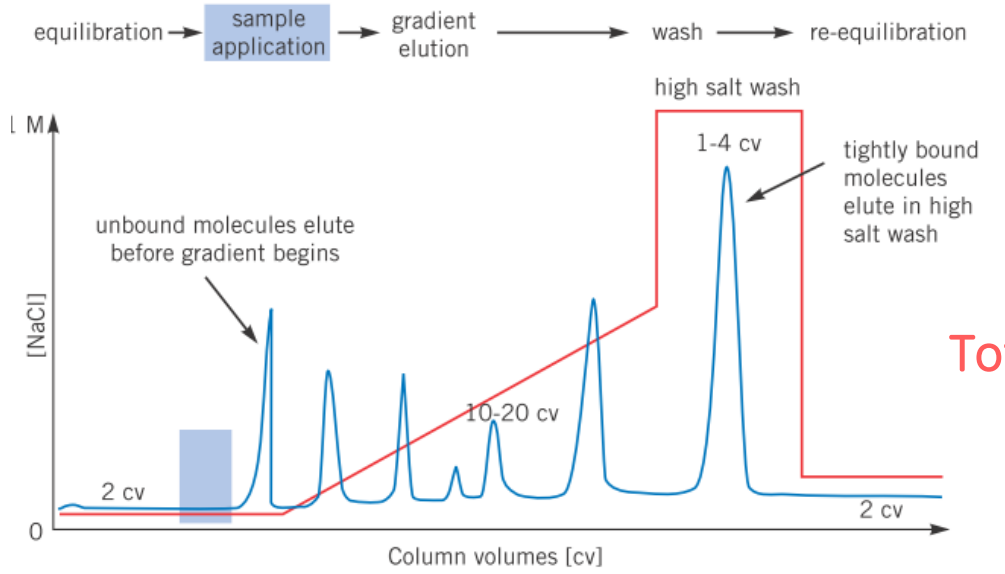
Gradient elution



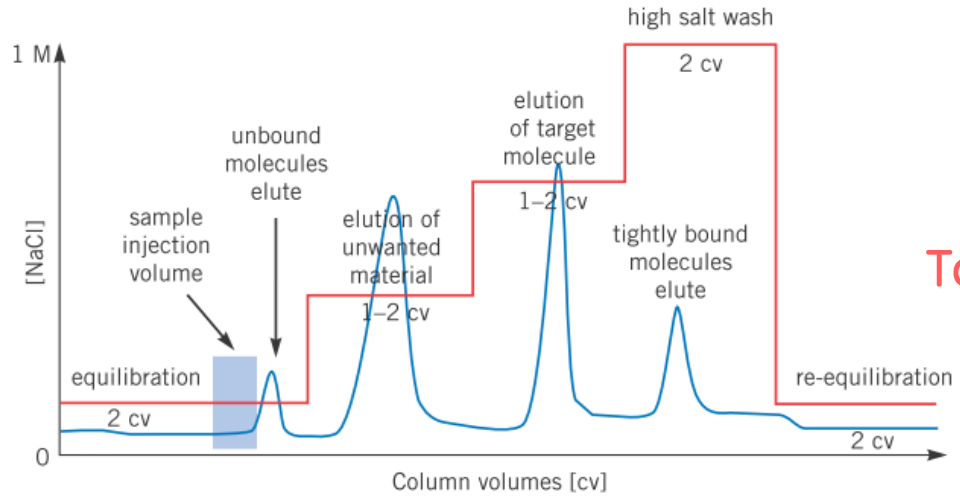
Step elution

The linear gradient in this example was easily created by using an ÄKTA™ chromatography system

ÄKTA system: Different elution conditions can be used

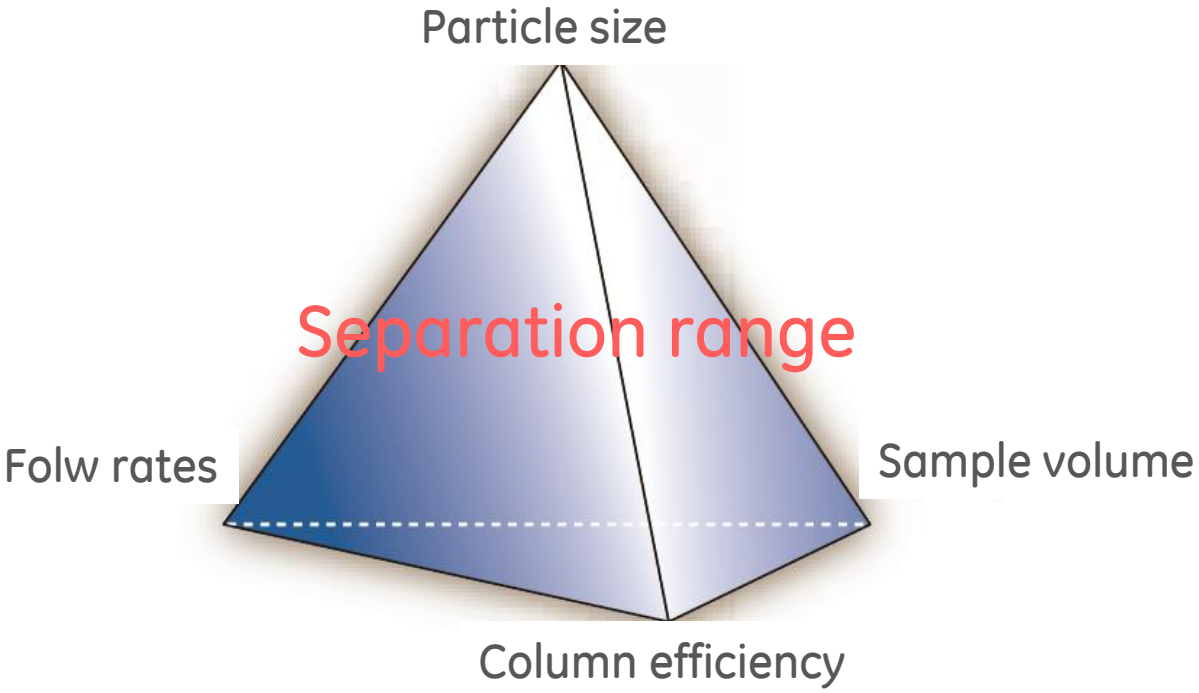


Total 26 CV



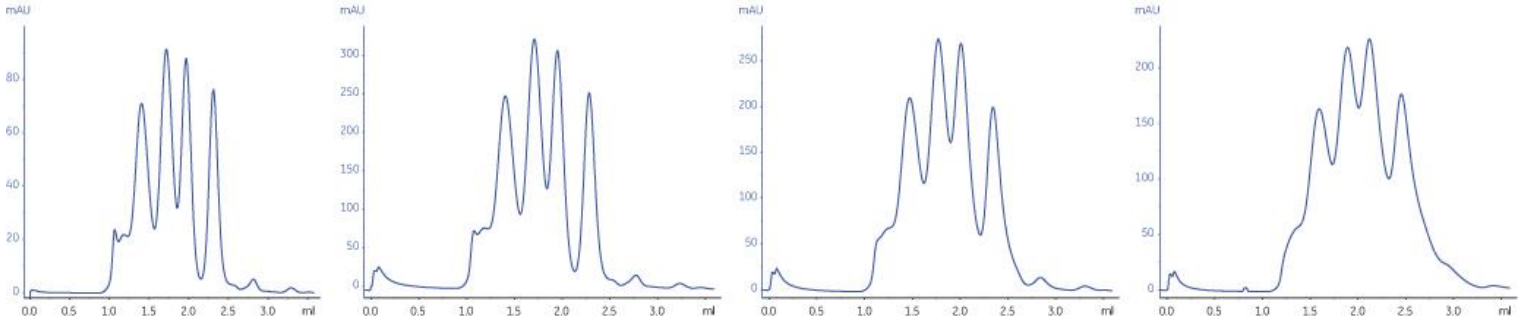
Total 10 CV

SEC: Important performance factors



SEC: Narrow system tubing and slow flow rate improves resolution

Tubing diameter 0.15 mm 0.25 mm 0.50 mm 0.75 mm

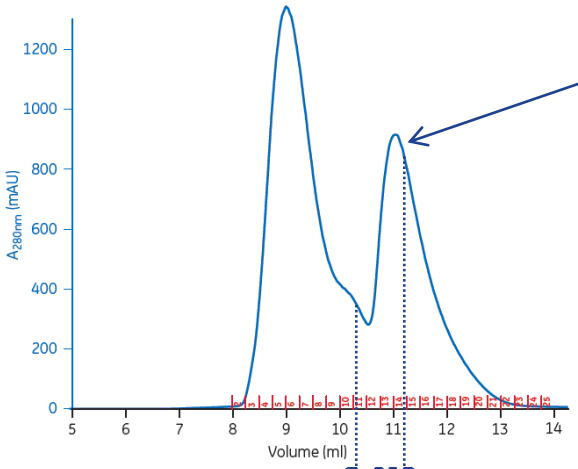


Column: Superdex™ 200 5/150 GL
System: ÄKTA pure 25

imagination at work

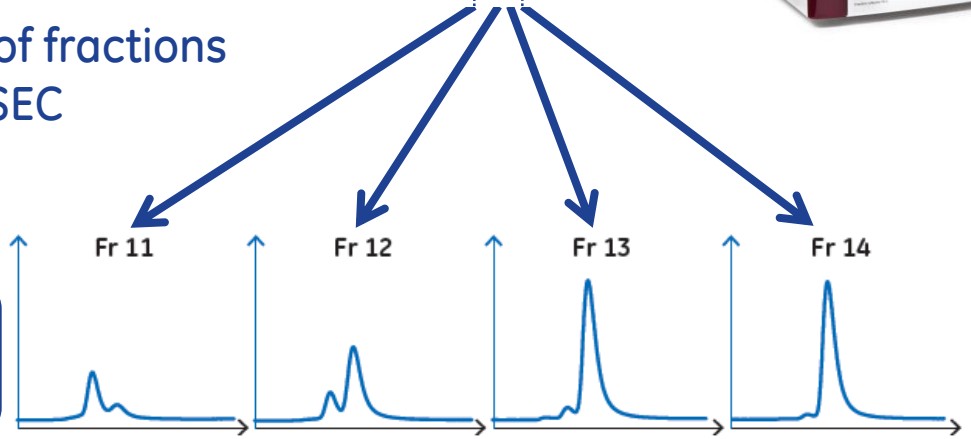
SEC: Analysis for pooling decisions

Step 1: Purification step (preparative SEC)



Protein of interest

Step 2: Identification of fractions to pool using SEC



Column:
Superdex™ 75 Increase 5/150 GL
System: ÄKTA™ pure

imagination at work

Summary

Summary

- The purification objectives & the target protein properties
  define the purity requirements and the selection of tools and methods
- The strategy Capture, intermediate Purification and Polishing (CiPP)
  provides a proven framework for developing the purification scheme
- Tools
  Manual purification, LC system purification, analytical
- Keep it simple!

Do you want to know more?
Handbooks from GE Healthcare

For guidance on choosing the right chromatography column, download the Purify App – www.gelifesciences.com/Purify

What is ÄKTA™ club?

ÄKTA club is an online platform for ÄKTA system users

It delivers:

Easy and quick access to information about ÄKTA systems and protein purification

Exclusive forum for ÄKTA system users

Customized My accounts page

https://proteins.gelifesciences.com/discussion-forum/akta-club/

ÄKTA accessories

GE Purify

Thank you