cytiva

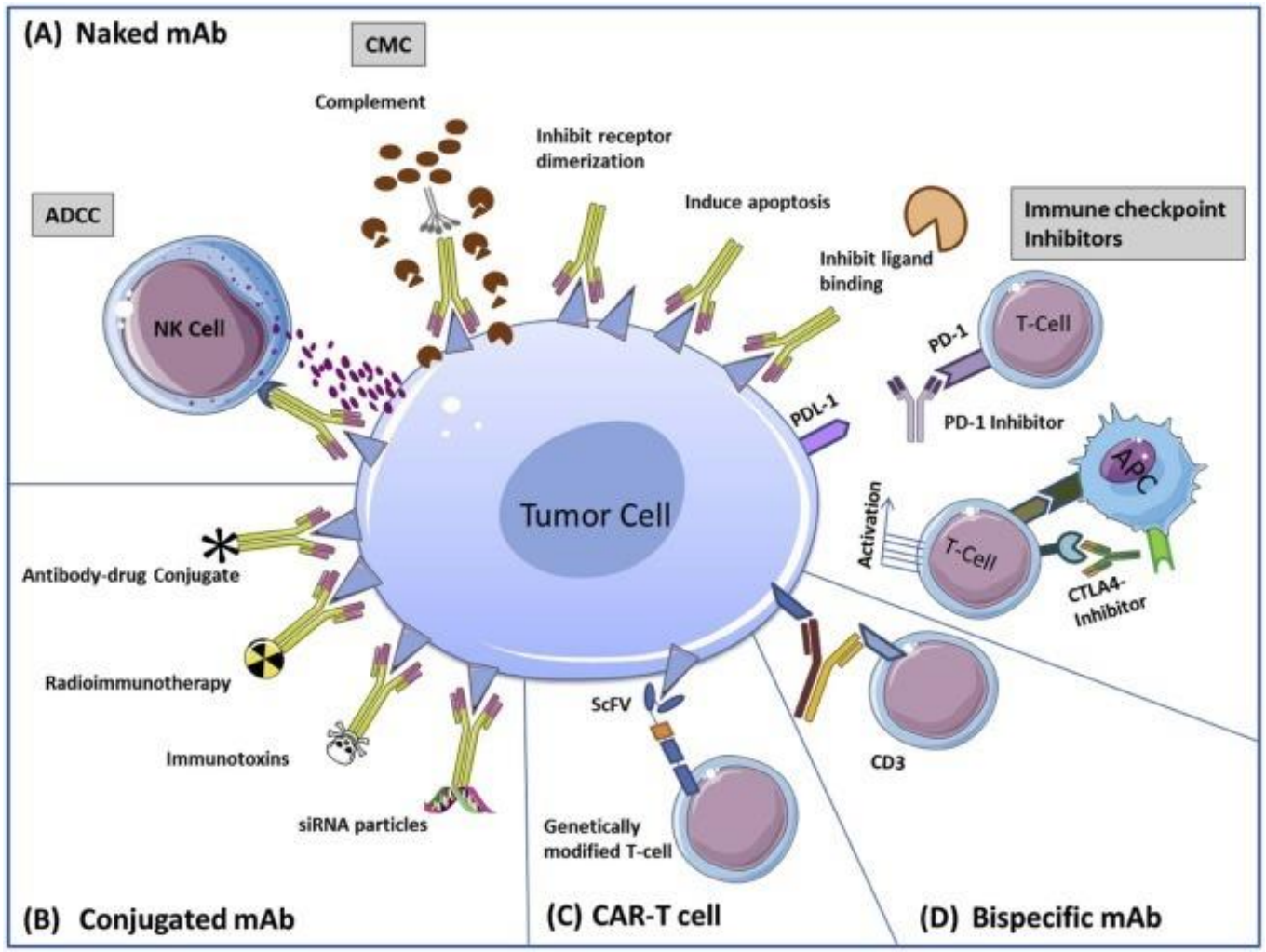
從純化的觀點到生產流程的策略與思維

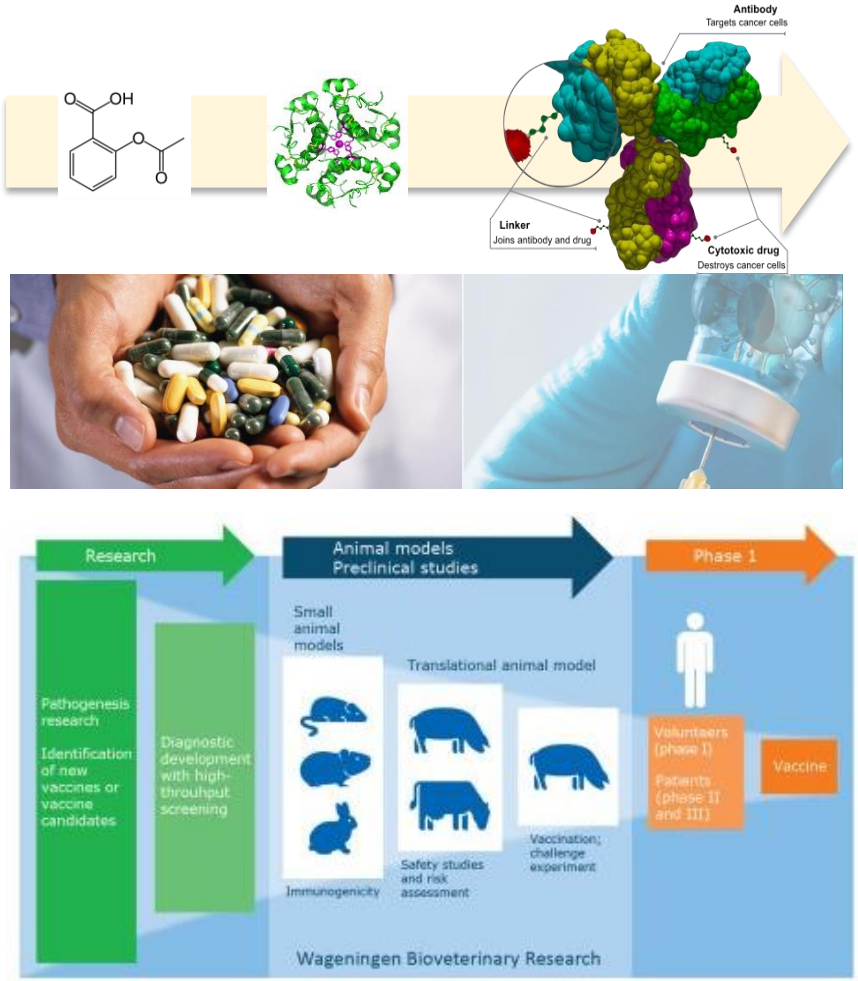
Introduction Biopharma development and Purification Strategy

Andy
9/19/2023

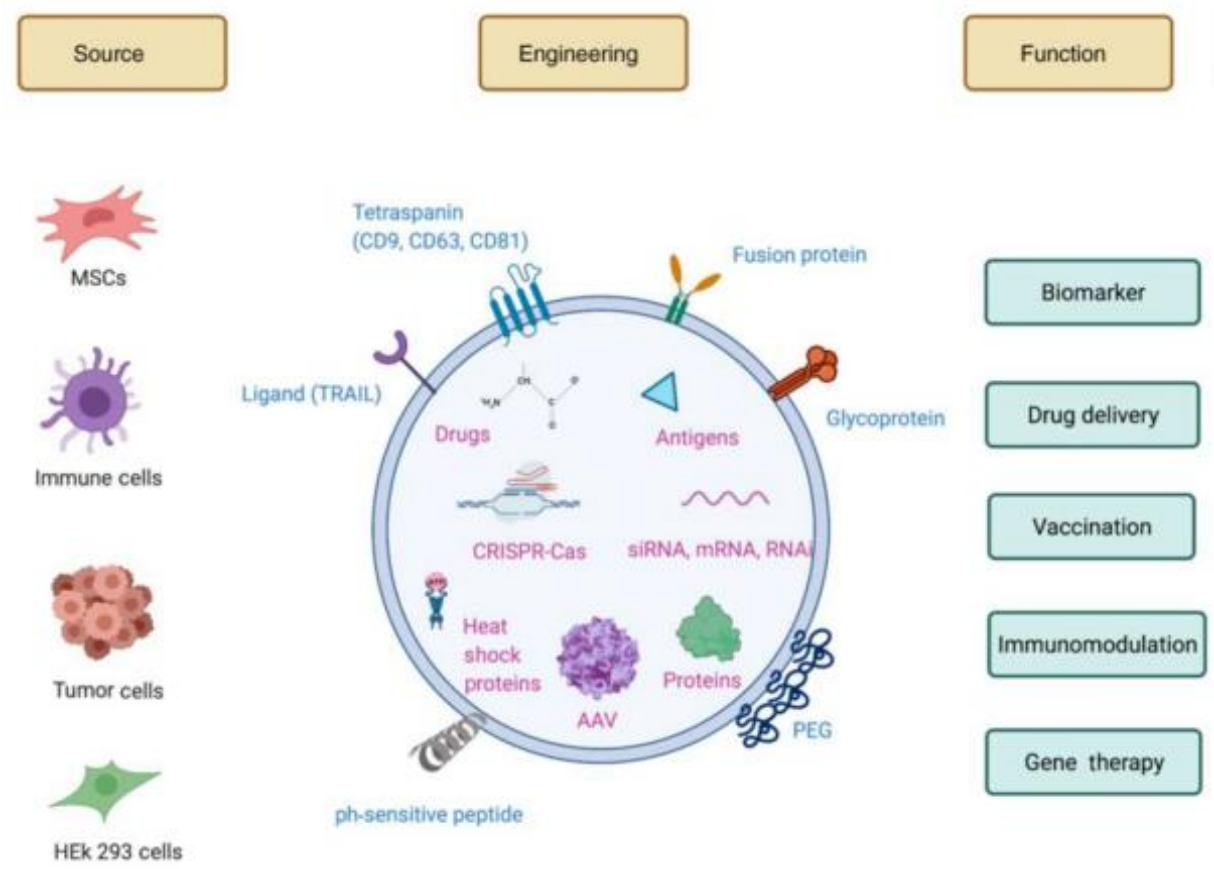
level
Discovering
new boundaries
進 階 生 物 科 技
Level Biotechnology Inc.

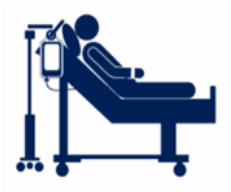
Precision medicine for cancer, Biologic Therapy





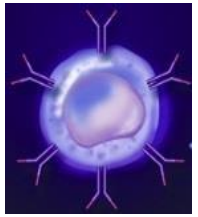
CAR T-cell Therapy, Exosome Therapy and Versus Stem Cell Therapy





Immune cells are collected by a procedure called apheresis

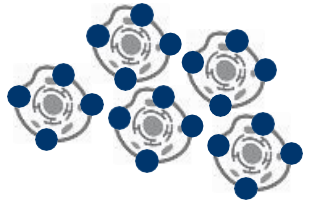
10^7 T cells
50-100mL



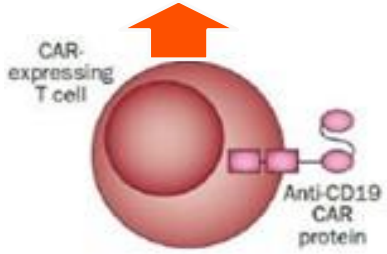
10^7 T cells
50-100mL

Cells are reinfused into the patient at clinical center

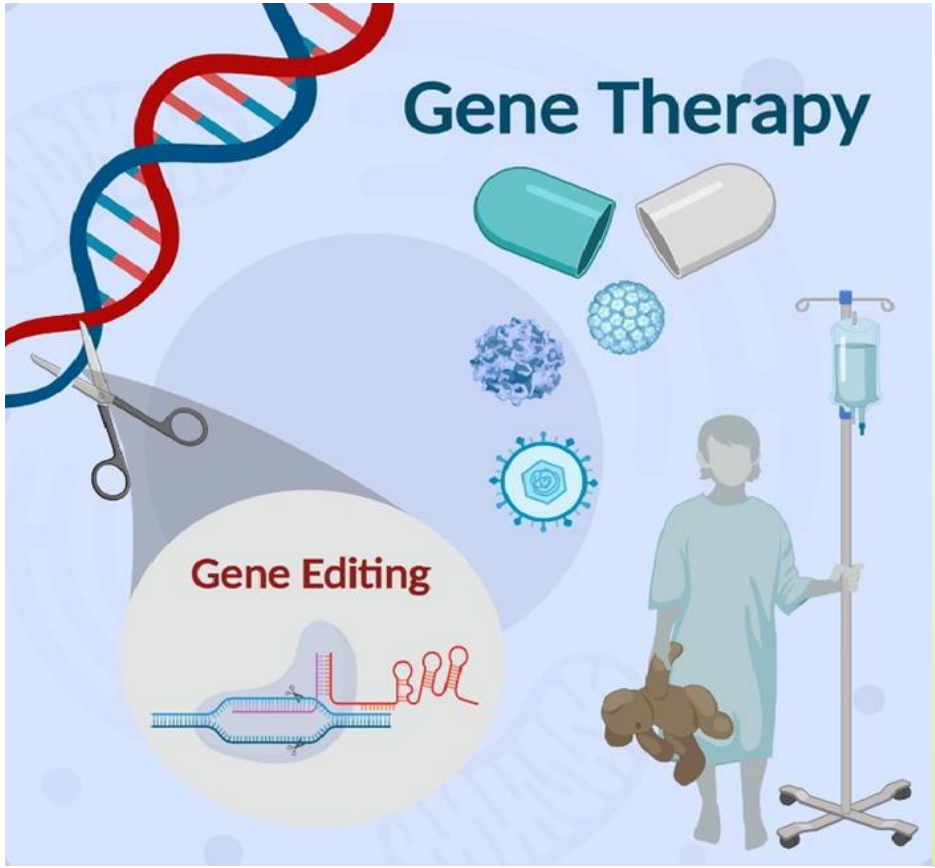
Cells are tested for safety and potency and packaged for injection back into the patient



10^8 T cells
50-100mL



Development of drug utilizing gene therapy technology



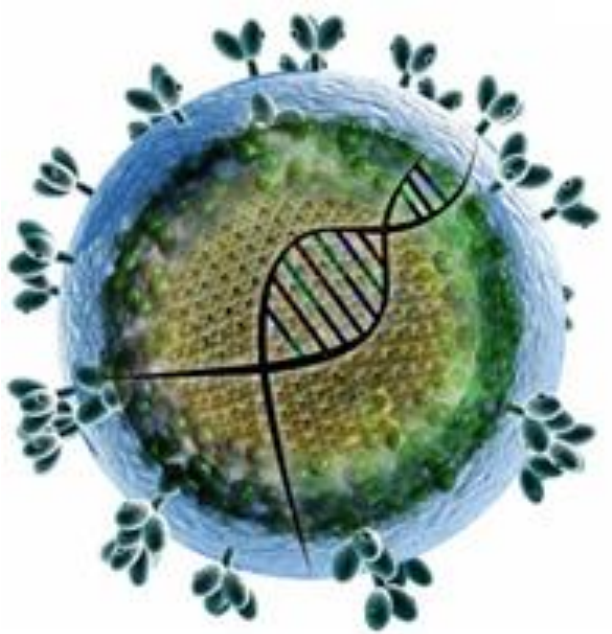
五種 COVID-19疫苗 的防護力及接種方式

HEHO

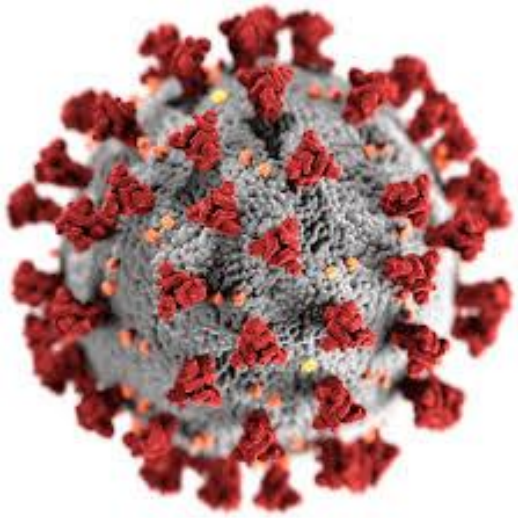
	牛津AZ疫苗	BNT輝瑞疫苗	莫德納疫苗	高端疫苗	聯亞疫苗
研發國家	英國/瑞典	美國/德國	美國	國產	國產
種類	腺病毒載體	mRNA	mRNA	重組蛋白	重組蛋白
儲存溫度	2-8度	零下70度	零下20度	2-8度	2-8度
防護力	70%	95%	94%	尚未公布	尚未公布
施打劑量	兩劑	兩劑	兩劑	兩劑	兩劑
目前狀況	已開打	未訂購	已開打	試驗階段	試驗階段

Ref.: https://m.match.net.tw/pc/life/health/20210612/5921991

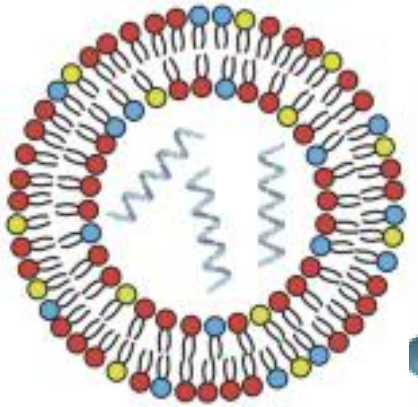
The sizes of viral vectors



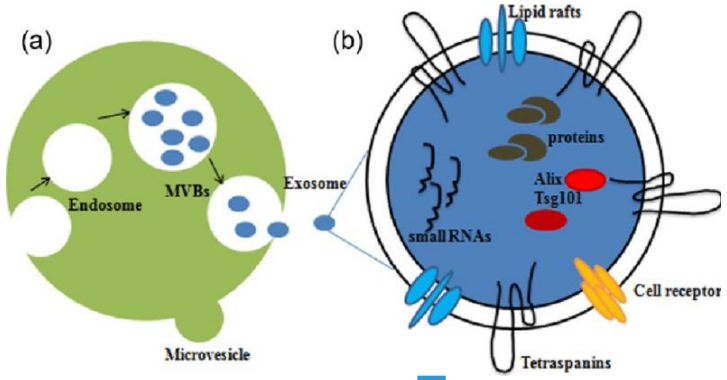
Lentivirus
~ 80–120 nm



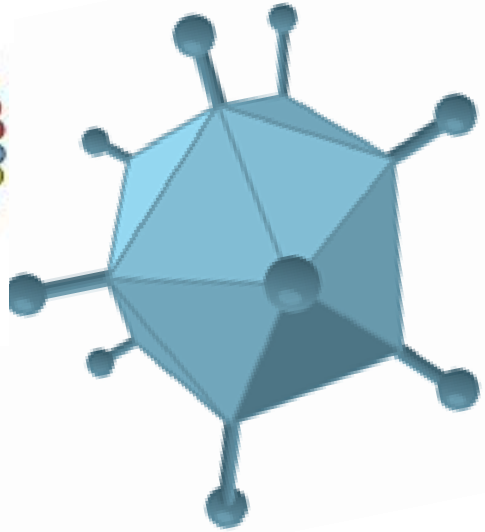
SARS-CoV-2 (COVID-19)
~ 60-140 nm



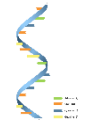
LNPs Vector for mRNA
~ 80-100 nm



Exosome
~ 60-120 nm



Adenovirus (AdV)
~ 90 nm



mRNA
~ 1-25 nm

AAV
~ 25 nm

Antibody Spike
~ 5 nm

Adenovirus vector platform

Upstream	Harvest	Clarification	Buffer exchange	Capture	Polishing	Formulation	Sterile filtration	Final product
HEK293 sus AdV5-GFP CDM4HEK293	Cell lysis, DNA degradation Tween™ 20 Benzonase	NFF ULTA™ 2 µm + 0.6 µm GF	TFF Hollow fiber 300 MWCO	AIEX Capto Q ImpRes	Capto Core 700	TFF Hollow fiber 300 MWCO	NFF ULTA 0.2 µm SG	AdV5 bulk

Scale-X™ carbo Bioreactor


ReadyCircuit™


ÄKTA™ flux 6


ÄKTA pure 150


ÄKTA flux s

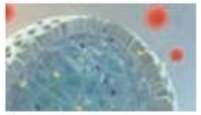

ULTA SG Filters


Biacore™ T200

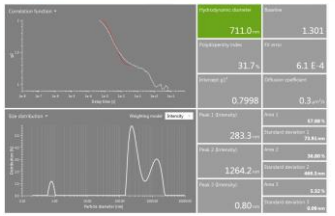

ReadyToProcess WAVE™ 25

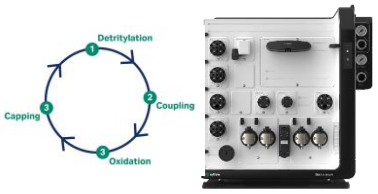

ReadyMate™
Aseptic Connector


ReadyToProcess™
hollow fibers


Capto™ Core 700


ReadyToProcess
hollow fibers





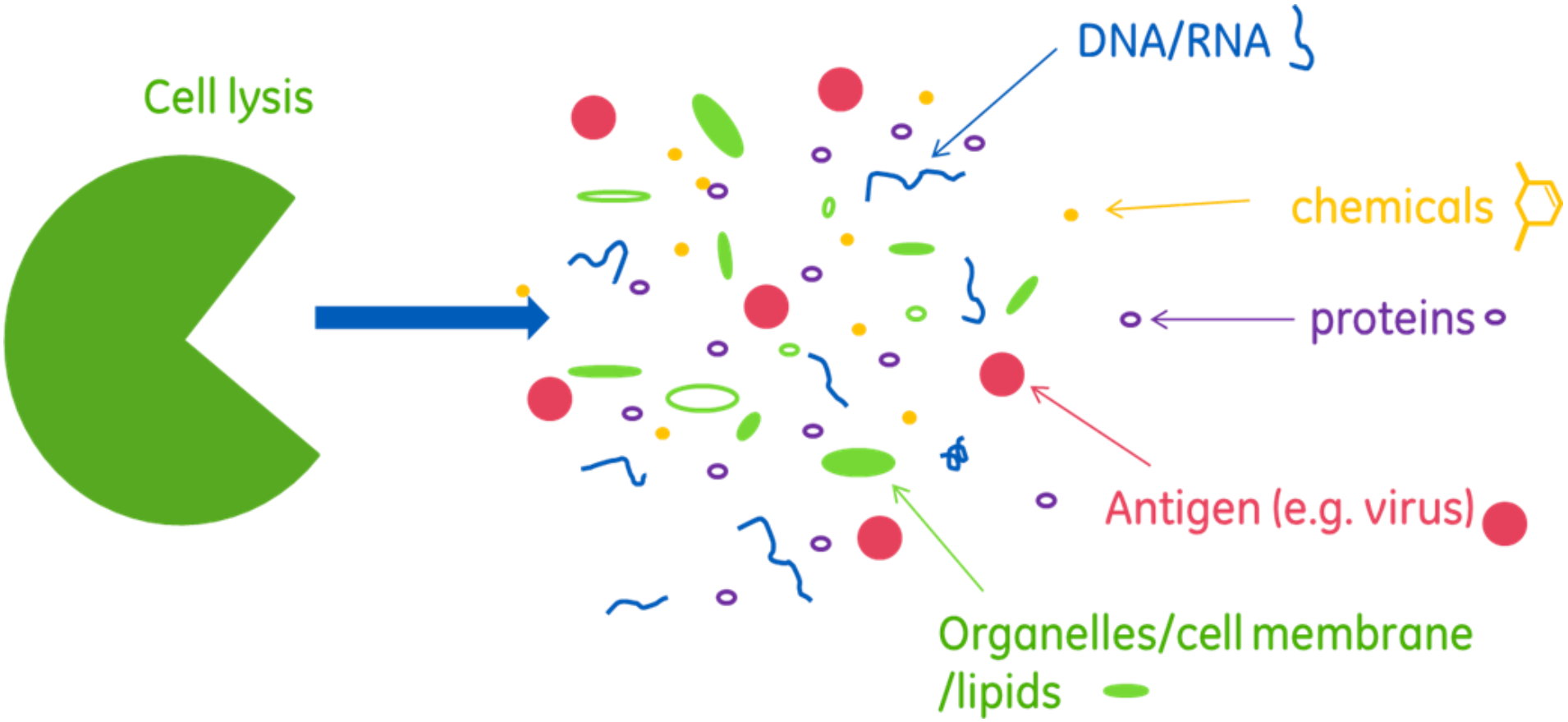
ÄKTA oligosynt™


ULTA NFF Filters

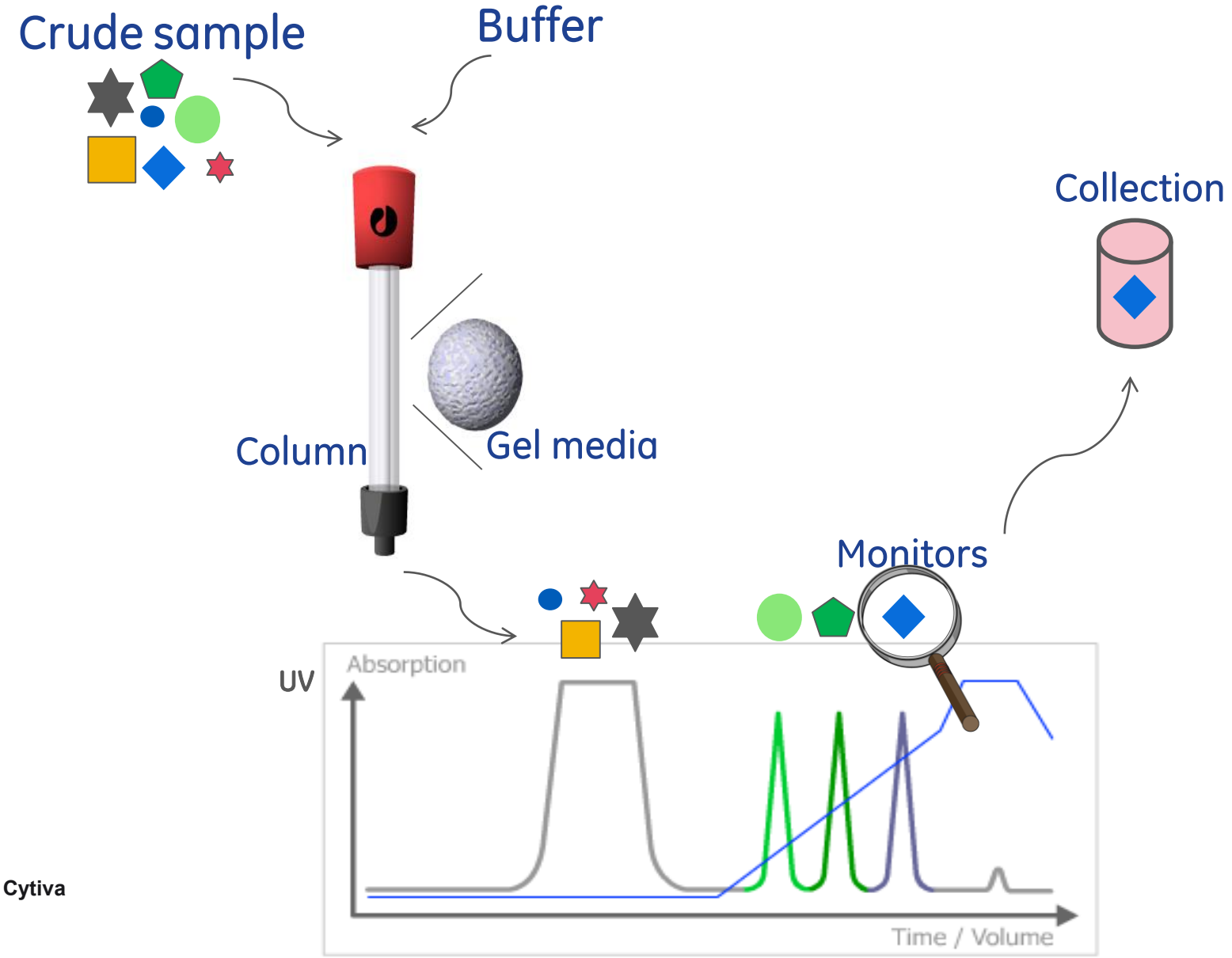

Pre-packed columns
and resins

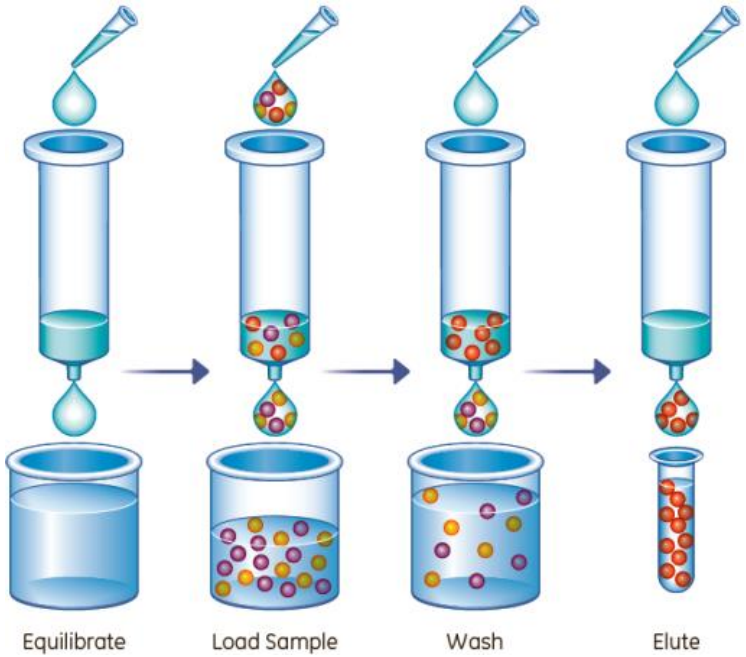
Introduction to protein purification

Sample purification and preparation

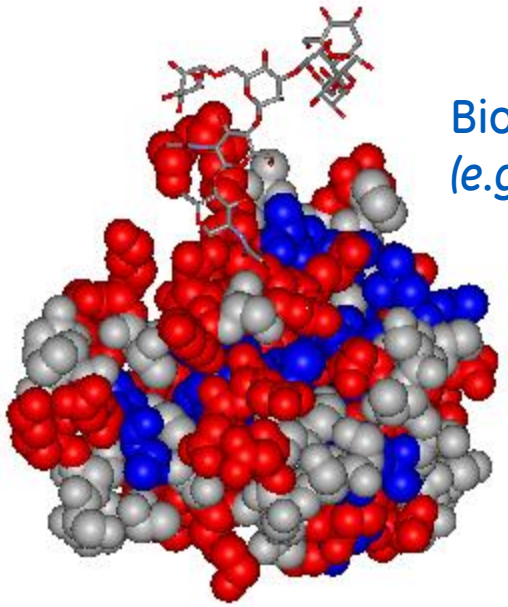


LC, Liquid Chromatography





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

The Principle of Affinity Chromatography

The Principle of Ion Exchange Chromatography

GE Healthcare
Life Sciences

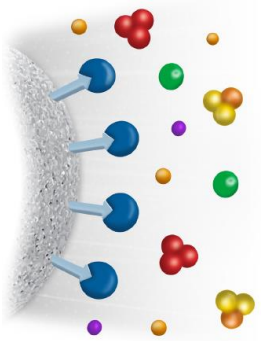
imagination at work

The Principle of Hydrophobic Interaction Chromatography

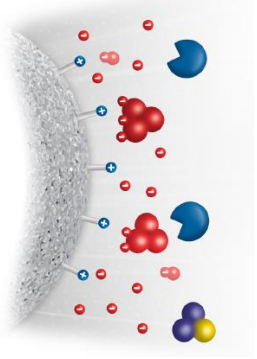
Principles of gel filtration chromatography- size exclusion chromatography

The principles of chromatography techniques

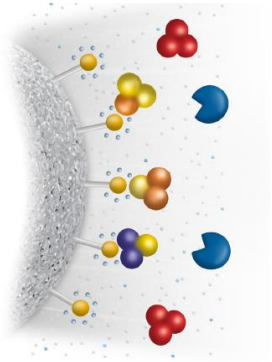
Affinity Chromatography (AC)



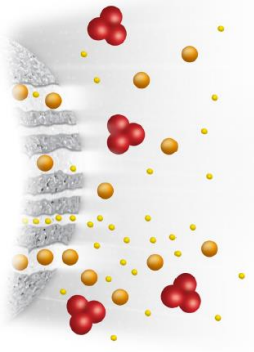
Ion exchange Chromatography (IEX)



Hydrophobic interaction Chromatography (HIC)



Size exclusion Chromatography (SEC)

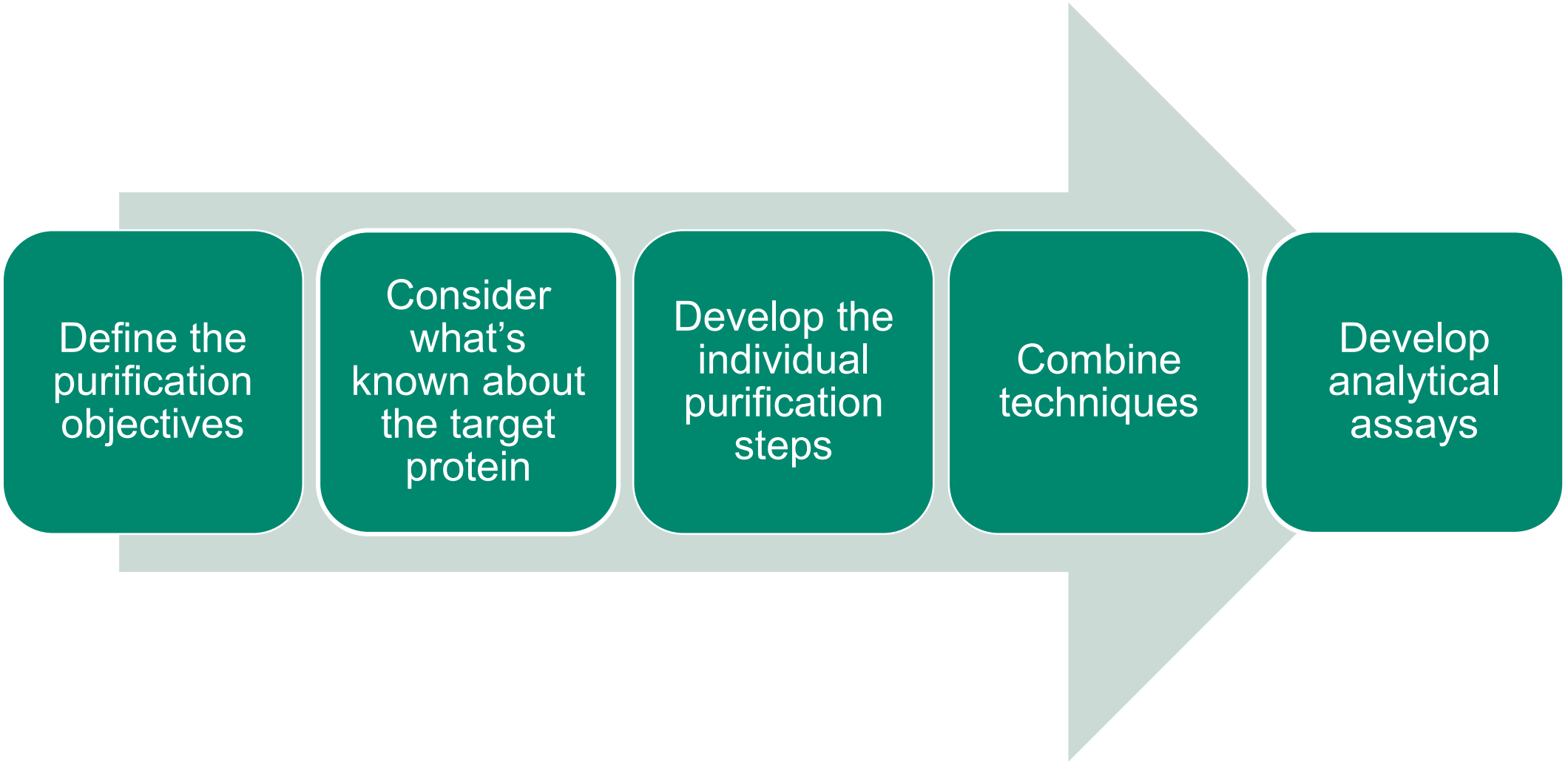


- Bind – elute principle
- Requires specific elution conditions
- Concentrating effect

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

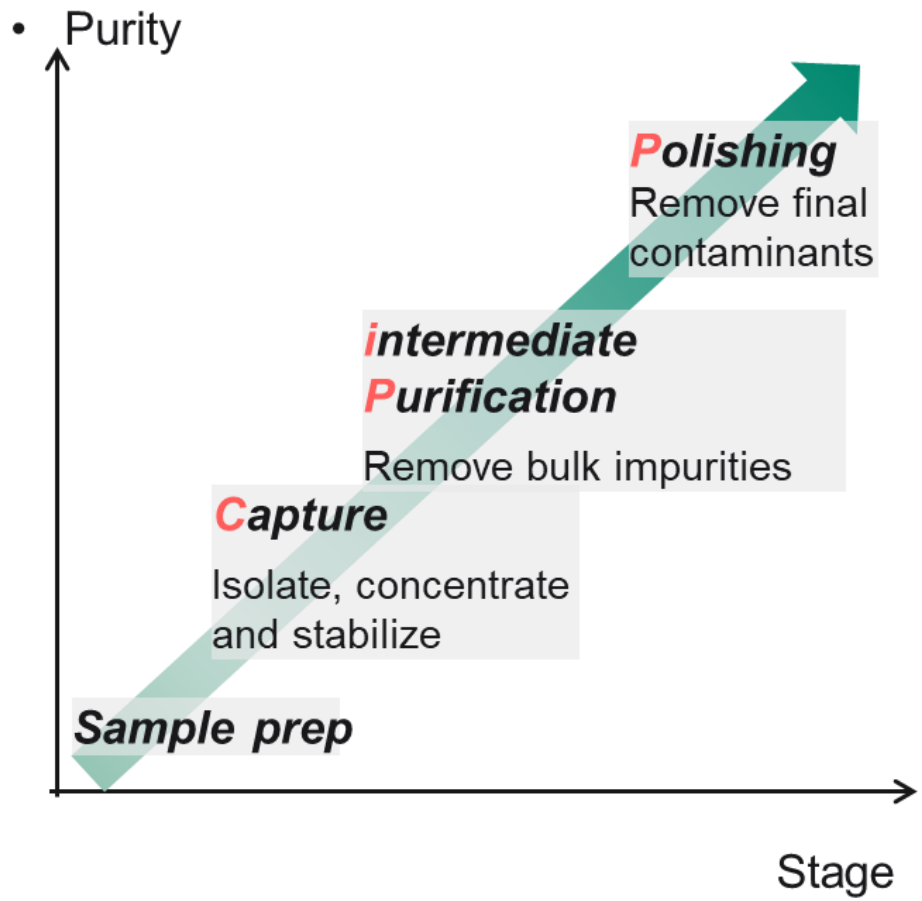
Purification strategy

Purification protocol development - step by step

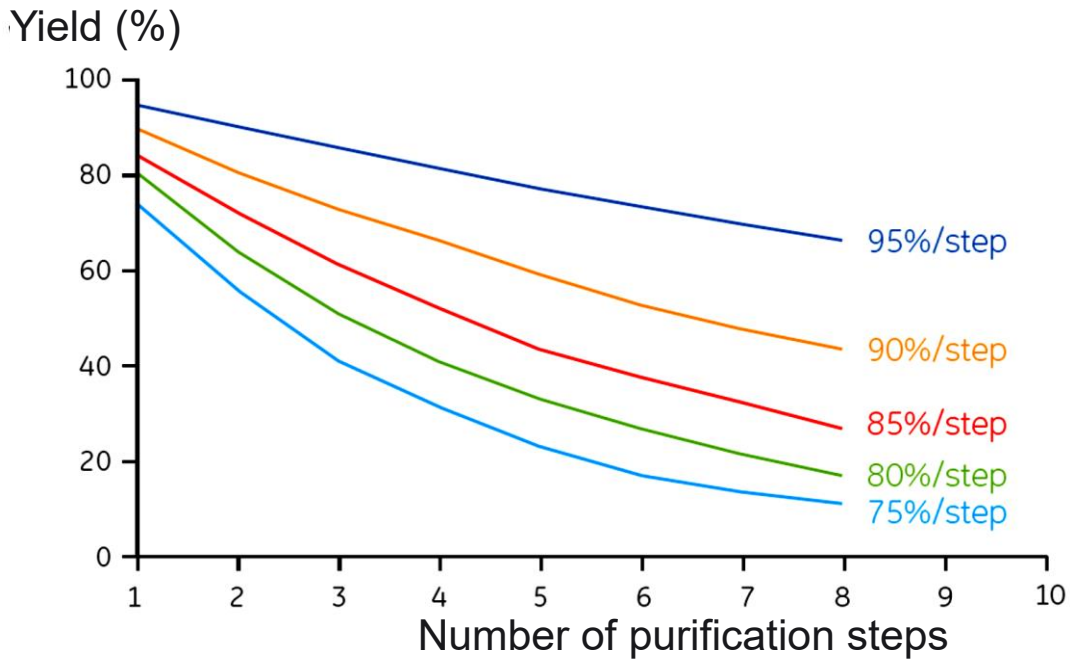


Introduction to CiPP purification strategy

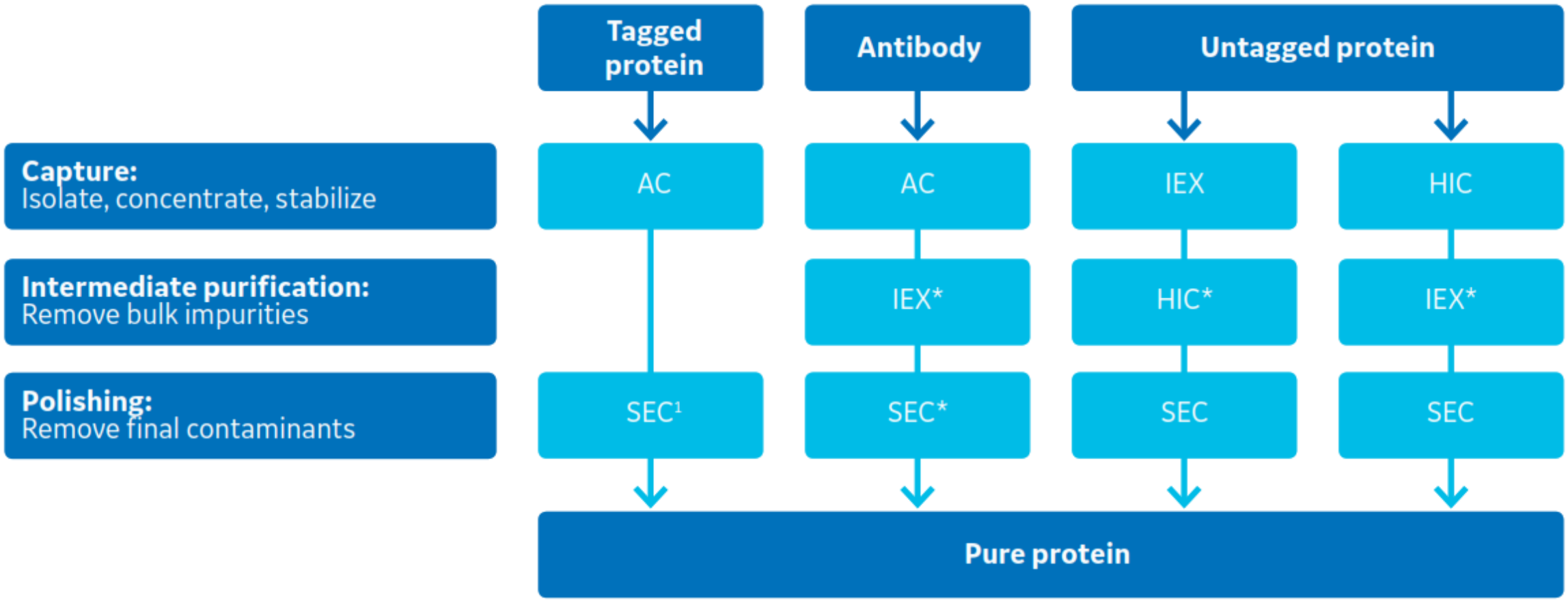
Purification strategy combining multiple steps



Protein recovery plotted against the number of purification steps

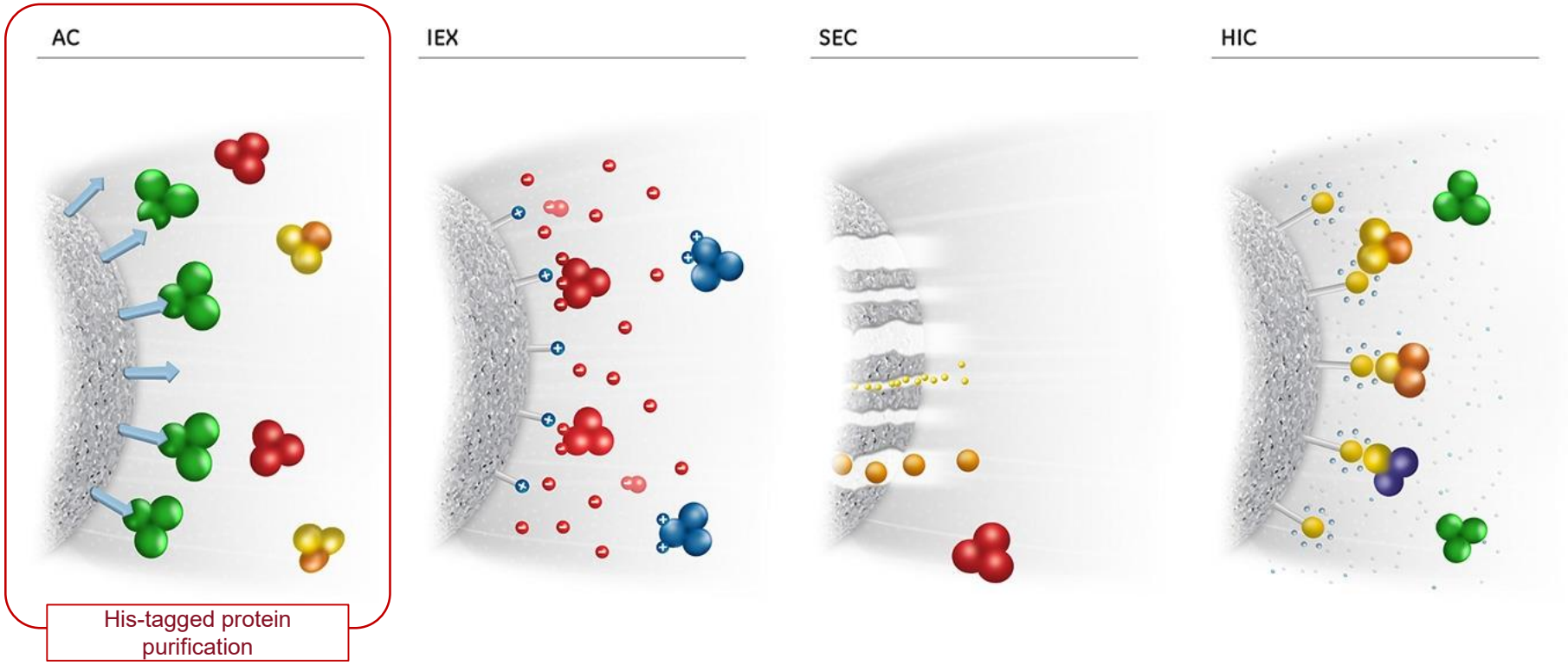


ÄKTA™ system provides efficient purification of your target protein



Application examples: Recombinant protein

How are affinity-tagged proteins purified?



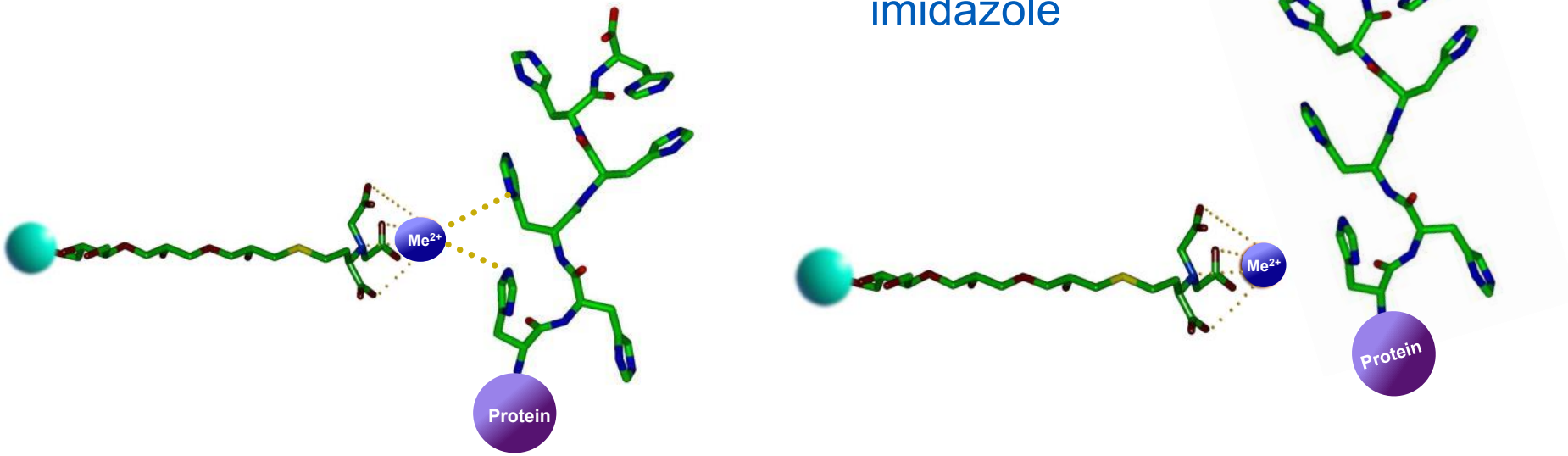
Chromatography techniques enable separation of proteins based on differences in specific properties.

Principle of histidine-tagged protein purification

IMAC resin

Binding of histidine-tagged protein

Elution of bound protein with imidazole



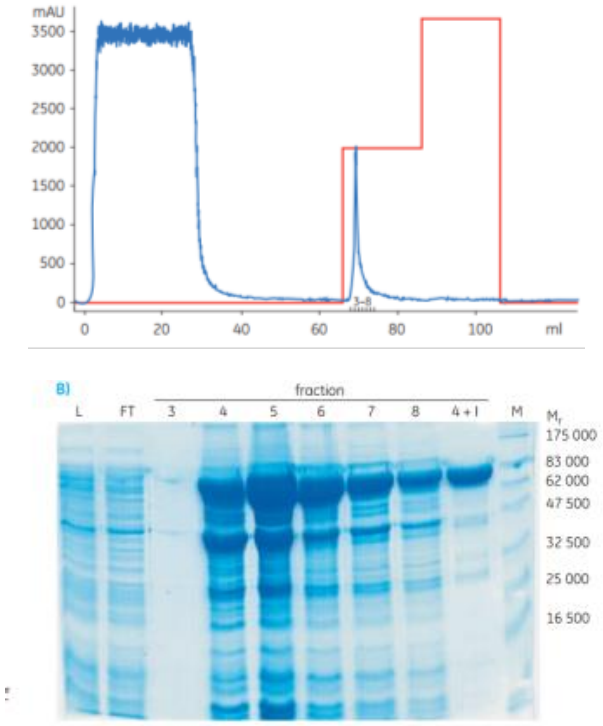
IMAC = Immobilized metal ion affinity chromatography

There are many ways to improve protein purity and yield

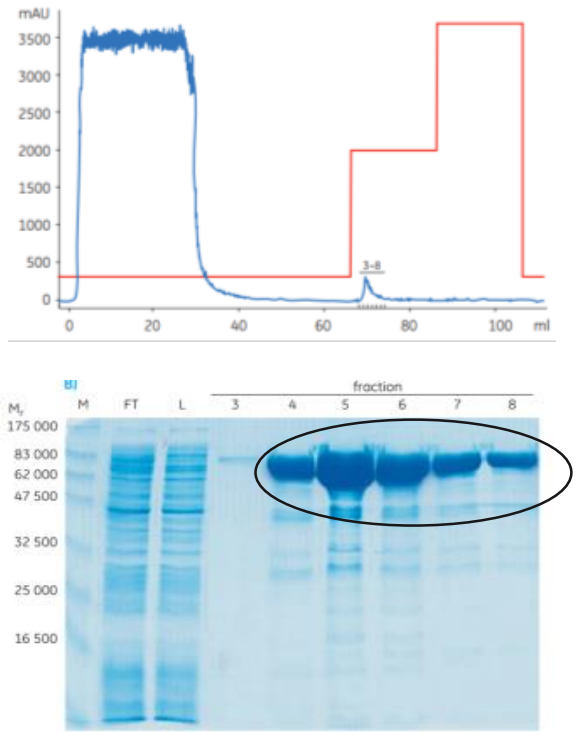
- Sample conditions
- Optimize binding conditions
- Optimize elution conditions
- Multi-step purification

Addition of imidazole to the binding buffer has a positive impact on protein purity*

No imidazole in the binding buffer



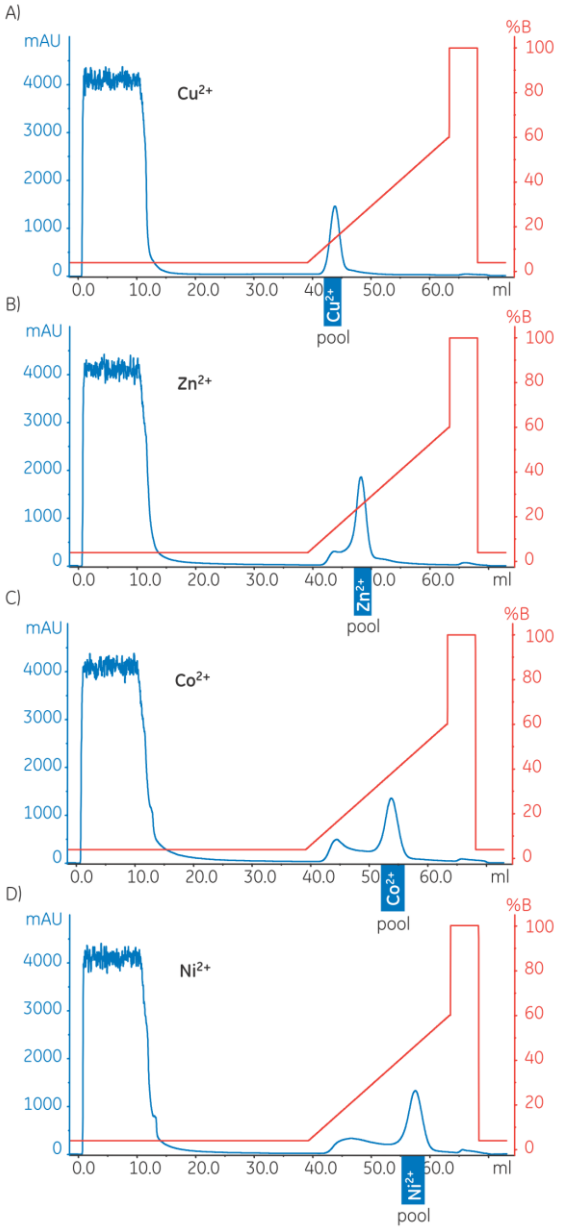
Imidazole in the binding buffer

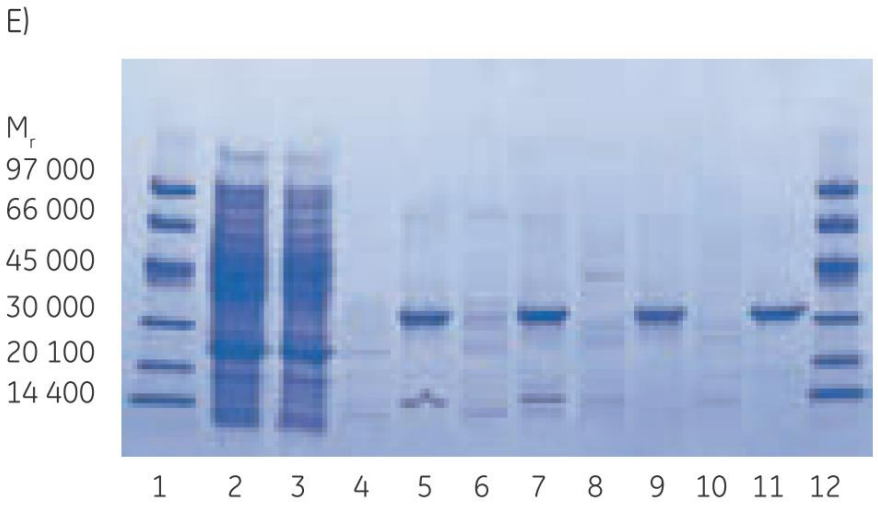


Adding imidazole to the binding buffer prevents nonspecific binding of other proteins that occur naturally in the host cell.

* In all application examples shared in this presentation, different ÄKTA™ chromatography systems from GE Healthcare's Life Sciences were used for purification.

Optimizing using different metal ions



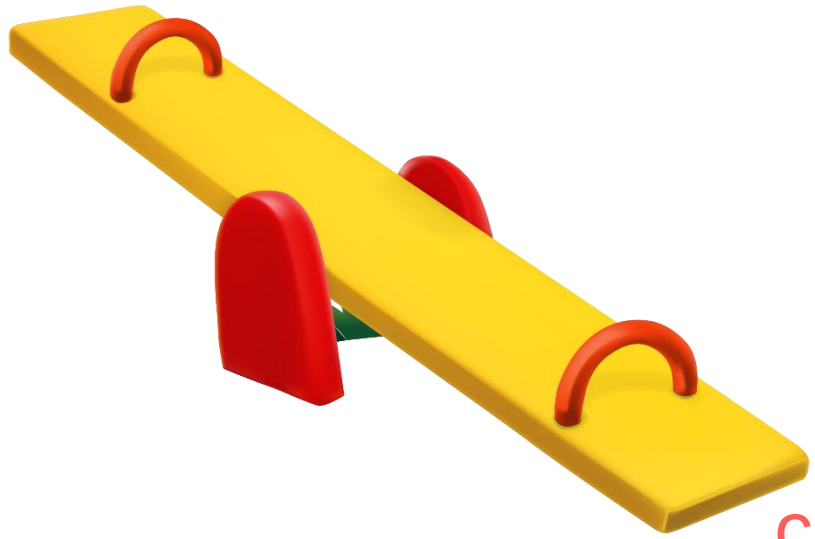


- Ni 2+ is generally used for histidine-tagged recombinant proteins.
- Co 2+ is also used for purification of histidine-tagged proteins, since it may allow weaker binding and reduce the amount of contaminants that may bind.

Method Optimization

- Increasing imidazole concentration
- Co ion

Selectivity



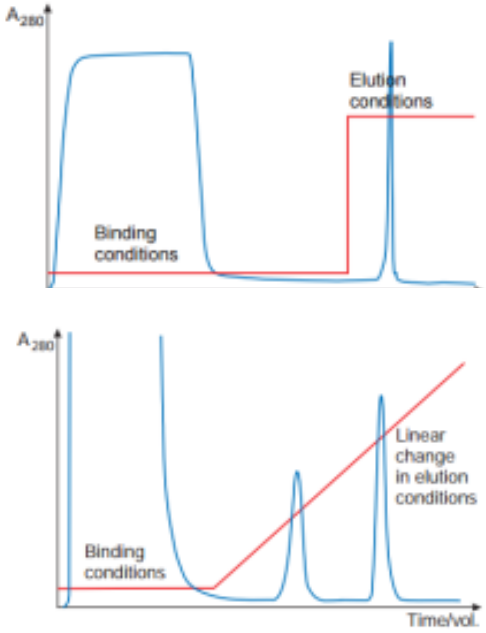
capacity

- Decreasing imidazole concentration
- Ni or Cu ion

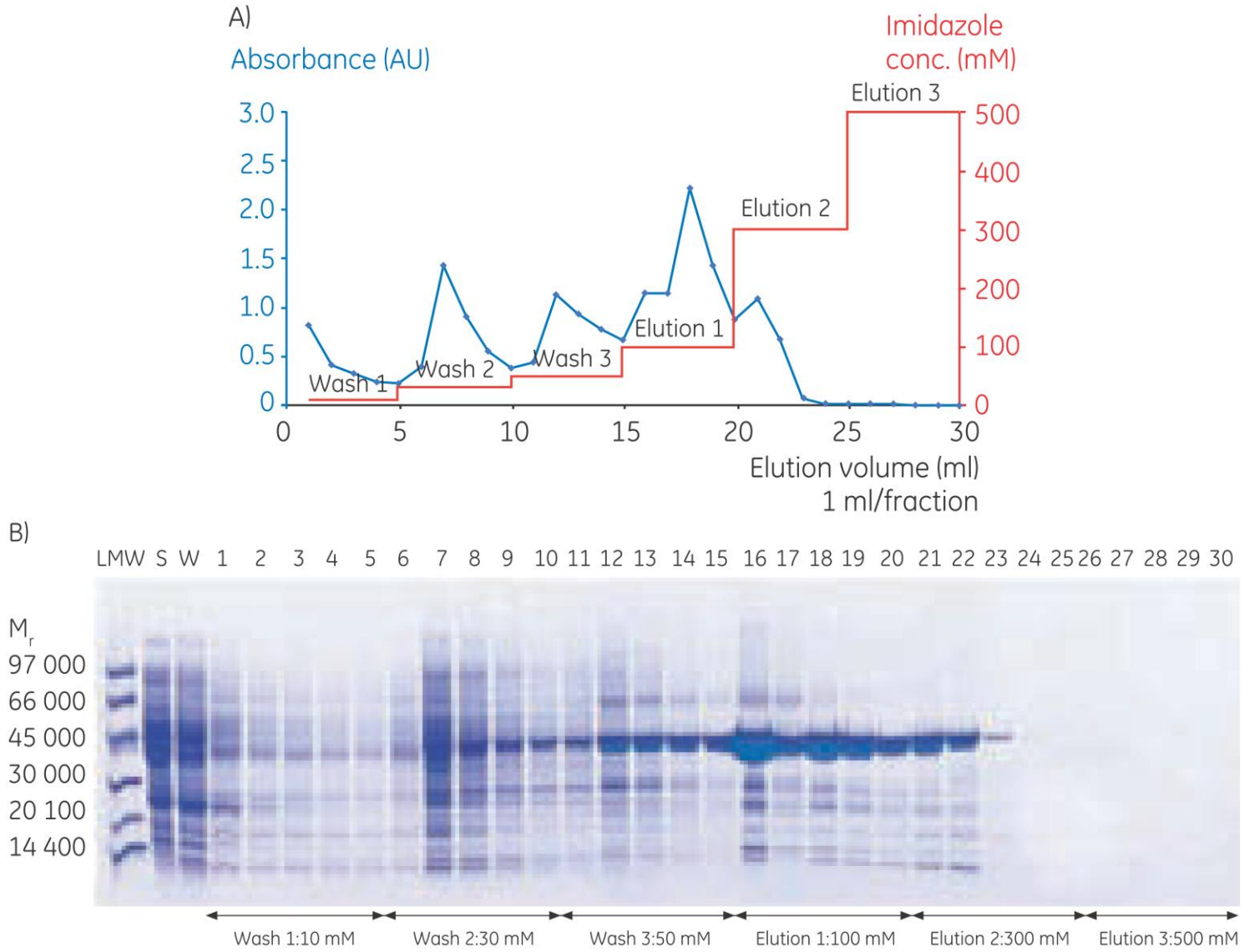
Different elution conditions can be used

- Bound histidine-tagged proteins are eluted by increasing the imidazole concentration
- This can be done by using linear gradient elution or step elution
- Linear gradients are conveniently created using a chromatography system
-

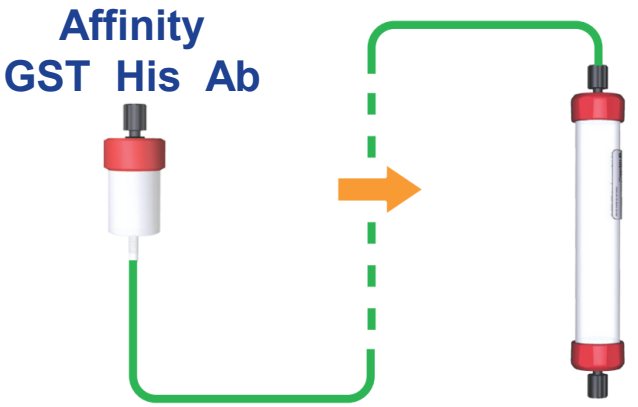
Method	Pros	Cons
Step elution	• Quick elution • Simple to perform with a single pump • Lower buffer volume needed • Concentrated eluate	• Eluted peak may contain several components
Linear gradient elution	• Better peak separation is achieved, high purity of target protein • Allows scanning for optimal elution conditions	• Eluted protein is more dilute

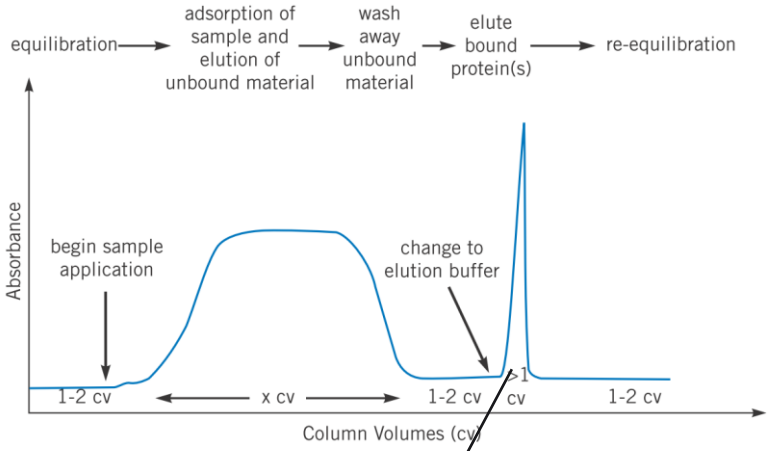


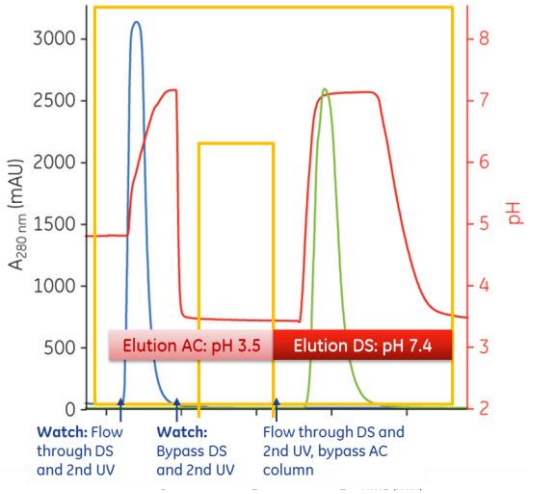
Different elution conditions can be used

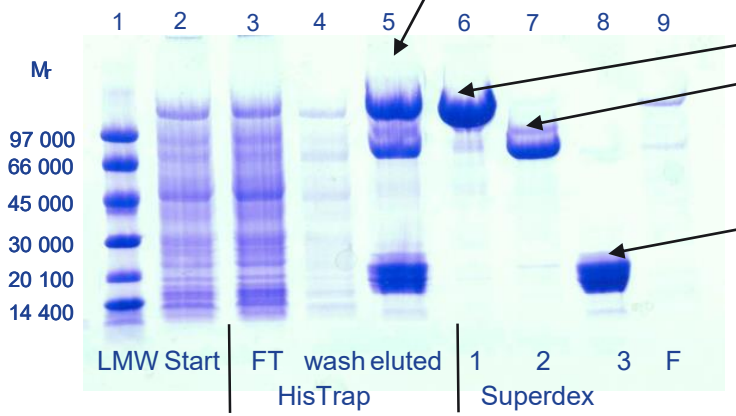


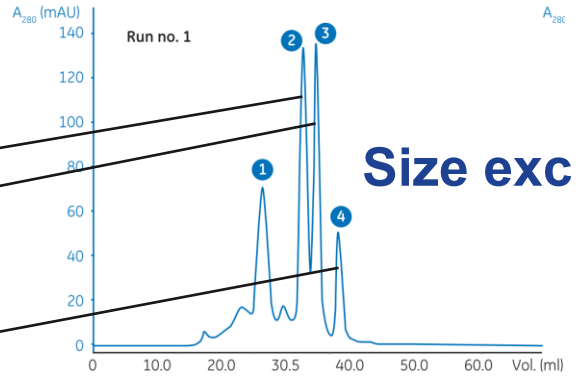
Impact of multi-step purification on protein purity







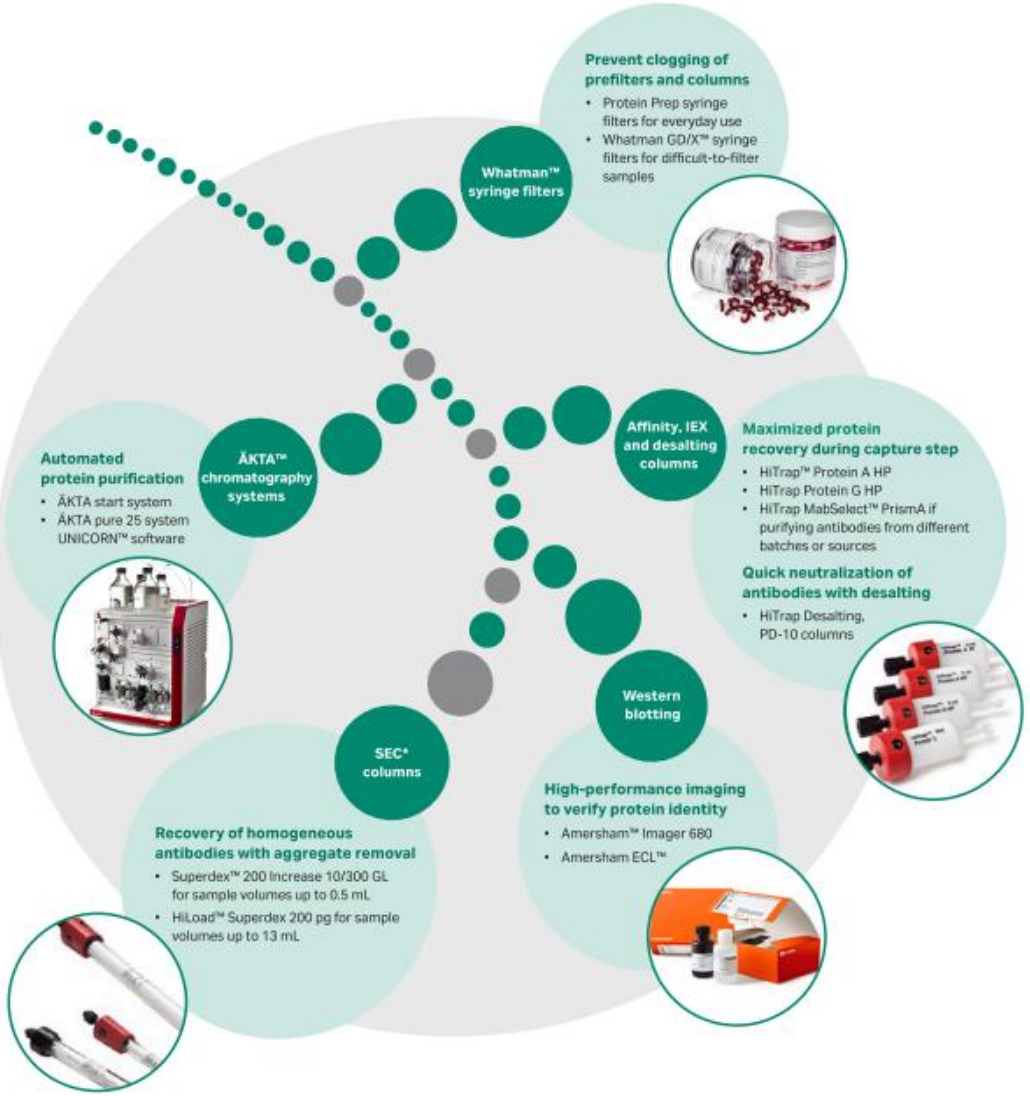




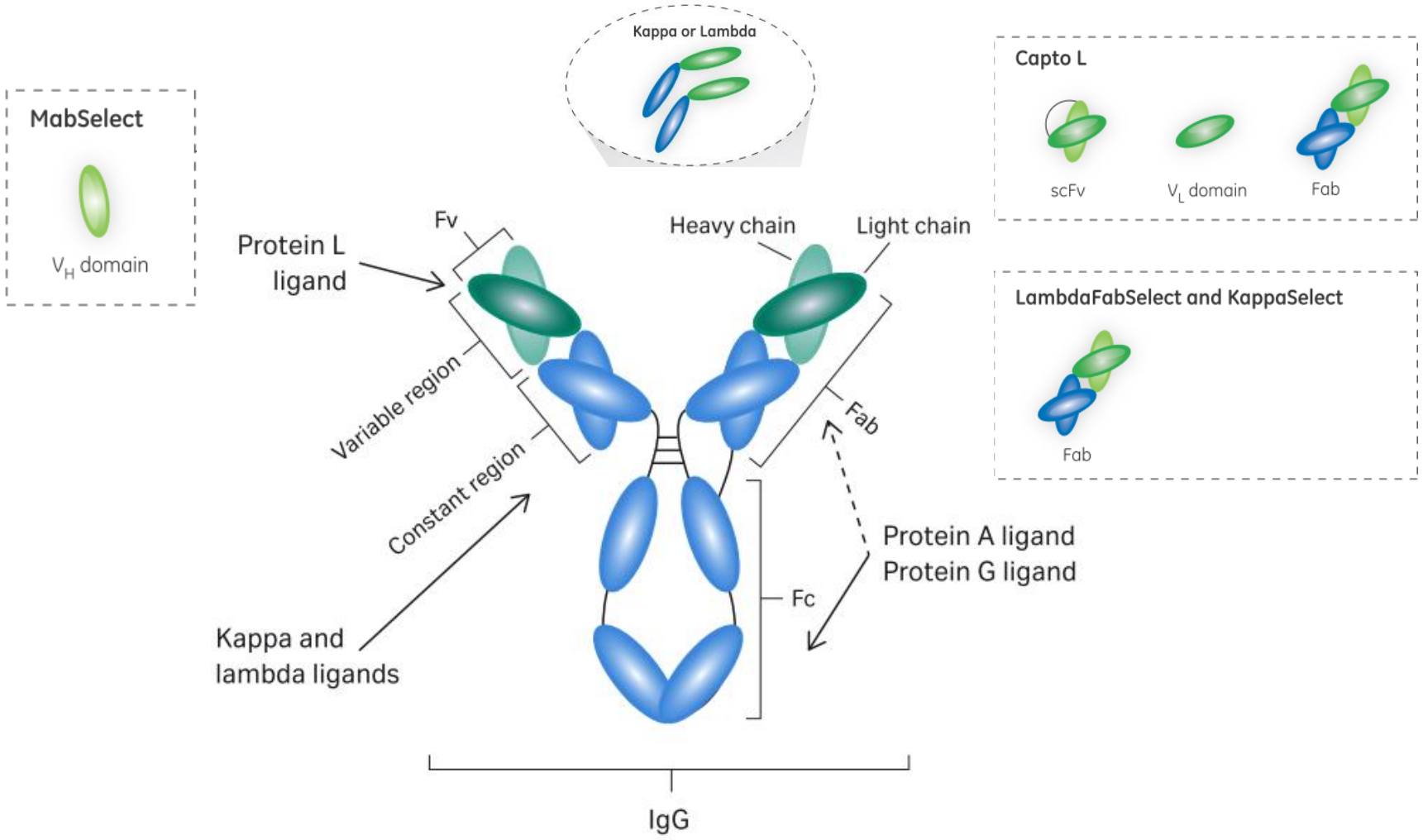
Size exclution

Application examples: Antibody purification

Introduction to antibody purification and analysis



Use of affinity chromatography for antibody purification

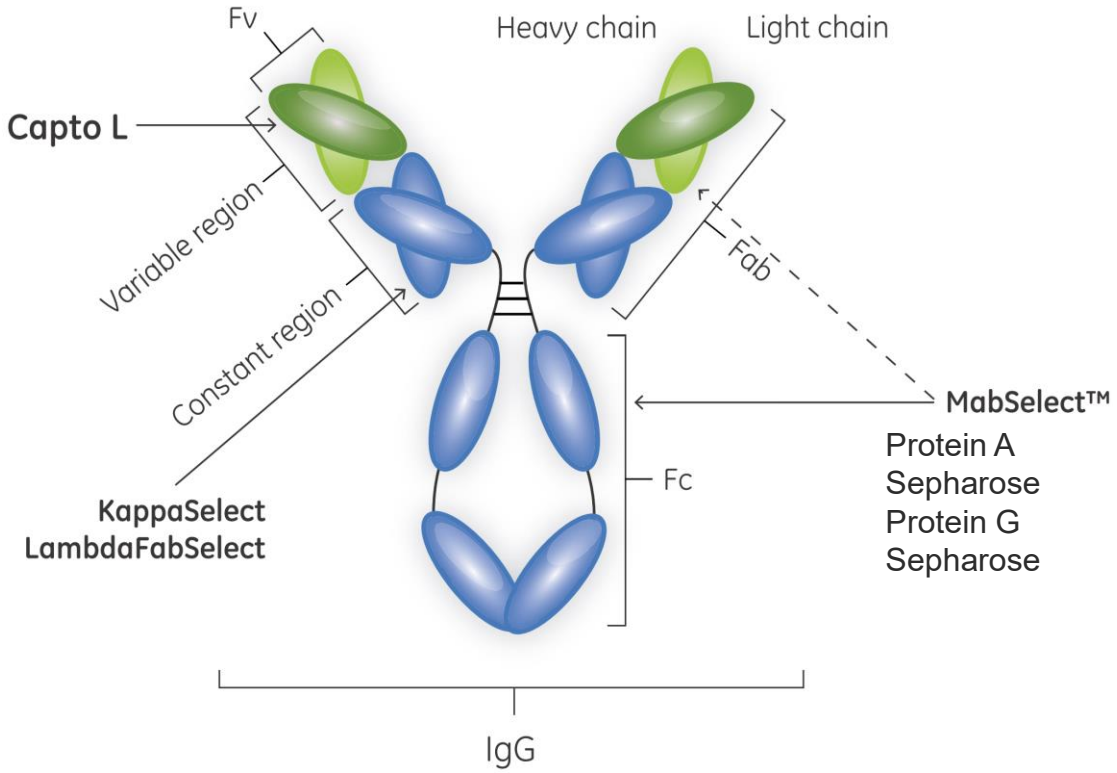


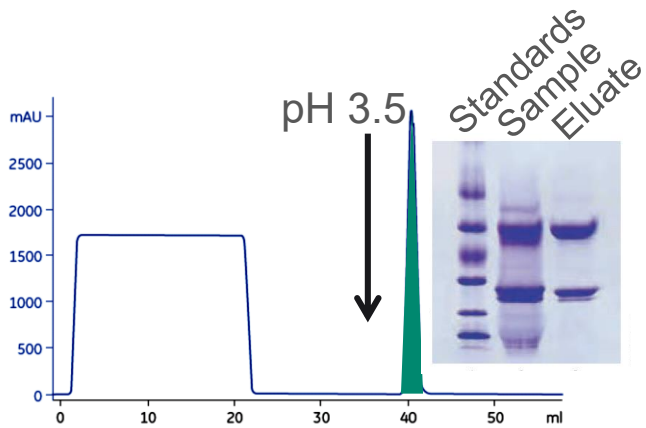
Use of affinity chromatography for antibody purification

Species	Antibody class	Affinity*		
		Protein A	Protein G	Protein L
Human	IgG_1	+++	+++	+++
	IgG_2	+++	+++	+++
	IgG_3	-	+++	+++
	IgG_4	+++	+++	+++
	IgA	Variable	-	+++
	IgD	-	-	+++
	IgE	-	-	+++
	IgM**	Variable	-	+++
Mouse	IgG_1	+	+++	+++
	IgG_{2a}	+++	+++	+++
	IgG_{2b}	+++	+++	+++
	IgG_3	+	+++	+++
	IgM**	Variable	-	+++
Rat	IgG_1	-	+	+++
	IgG_{2a}	-	+++	+++
	IgG_{2b}	-	+	+++
	IgG_{2c}	nd	nd	+++
	IgG_3	+	+	nd
Pig	Total IgG	+++	+++	+++
Dog	Total IgG	+	+	+
Cow	Total IgG	+	+++	-
Goat	Total IgG	-	+	-
Sheep	Total IgG	+/-	+	-
Chicken	Total IgG	nd	nd	-
Rabbit	Total IgG	+++	+++	nd
Avian egg yolk	IgY***	-	-	nd
Guinea pig	IgG_1	+++	+	nd
Hamster	Total IgG	+	+	nd
Horse	Total IgG	+	+++	nd
Koala	Total IgG	-	+	nd
Llama	Total IgG	-	+	nd
Monkey (rhesus)	Total IgG	+++	+++	nd
Other	Kappa light chain (subtypes 1,3,4)	nd	nd	+++
	Lambda light chain	nd	nd	-
	Heavy chain	nd	nd	-
	Fab	+/-	+/-	+++
	ScFv	nd	nd	+++
	Dab	nd	nd	+++

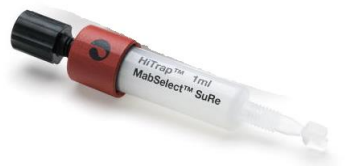
+++ = strong binding
+ = weak binding
- = no binding
+/- = weak binding in some cases
nd = no data available

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





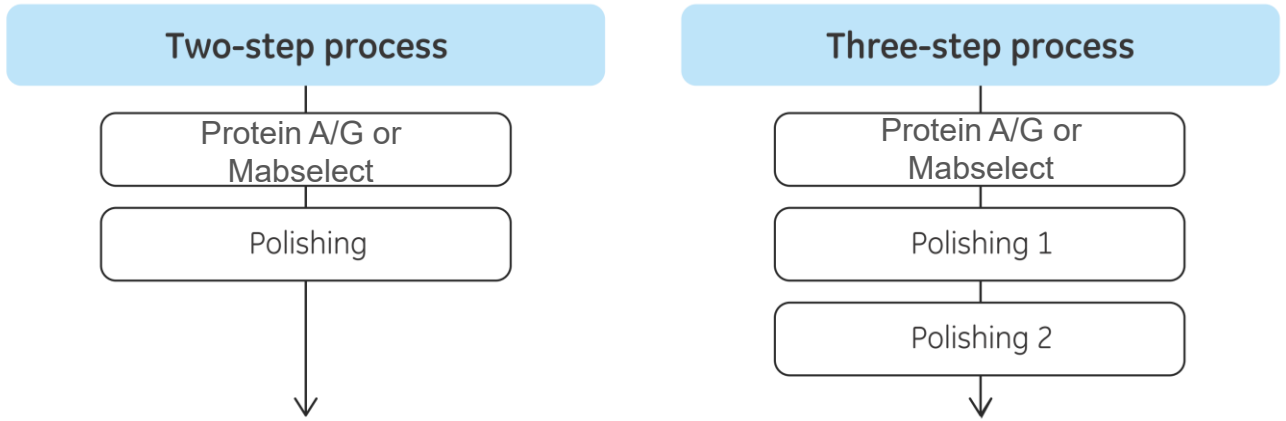
Column: HiTrap™ protein A 1 ml



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

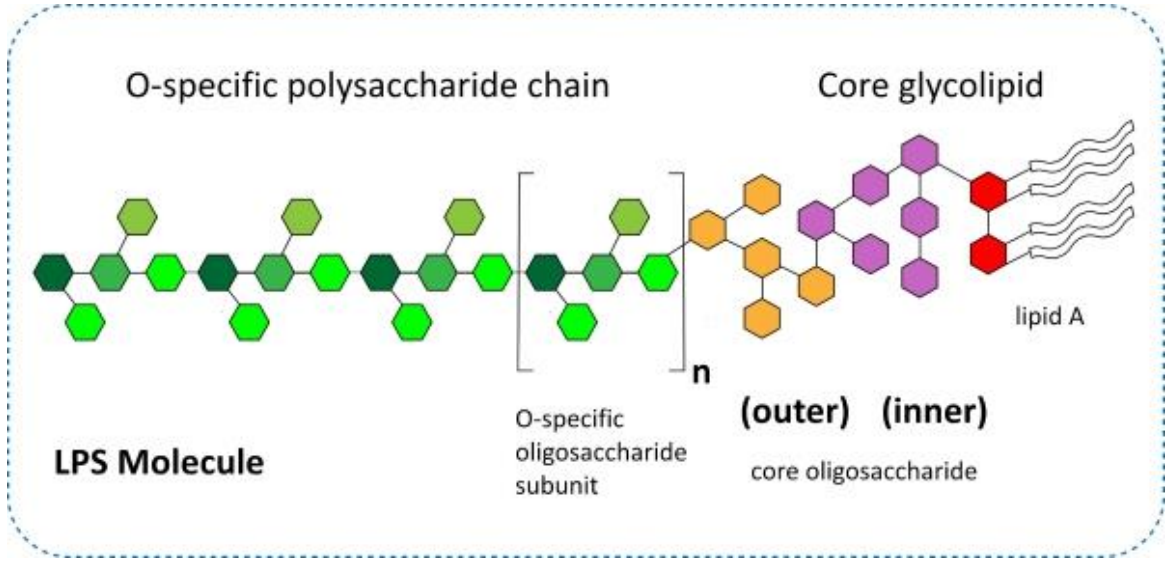
What is your associated contaminants?



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: Application: Endotoxins, DNA Cleaning



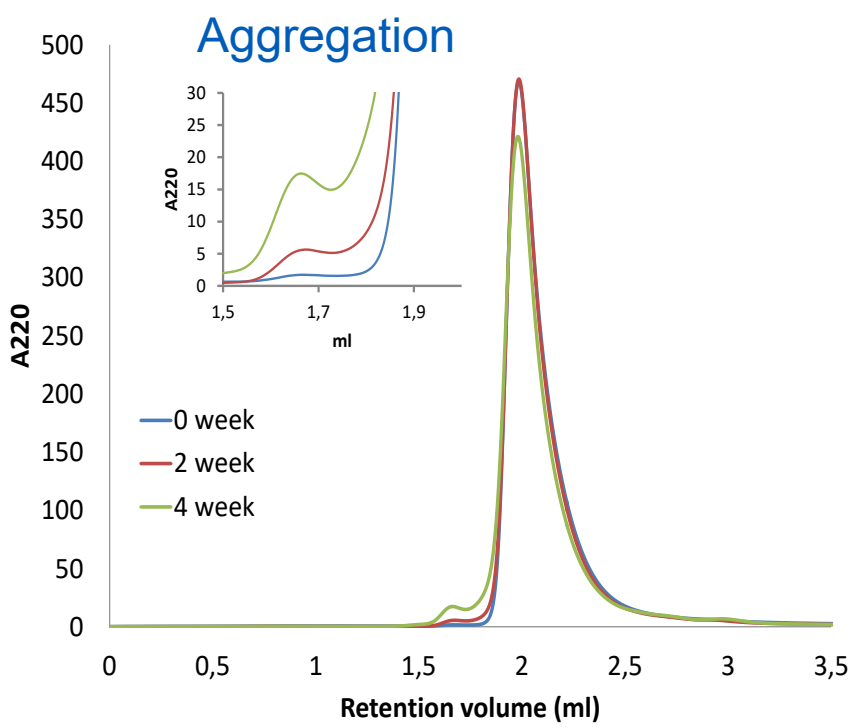
10~20 KD ~ 4 ×105 KD
< 0.25EU/ml

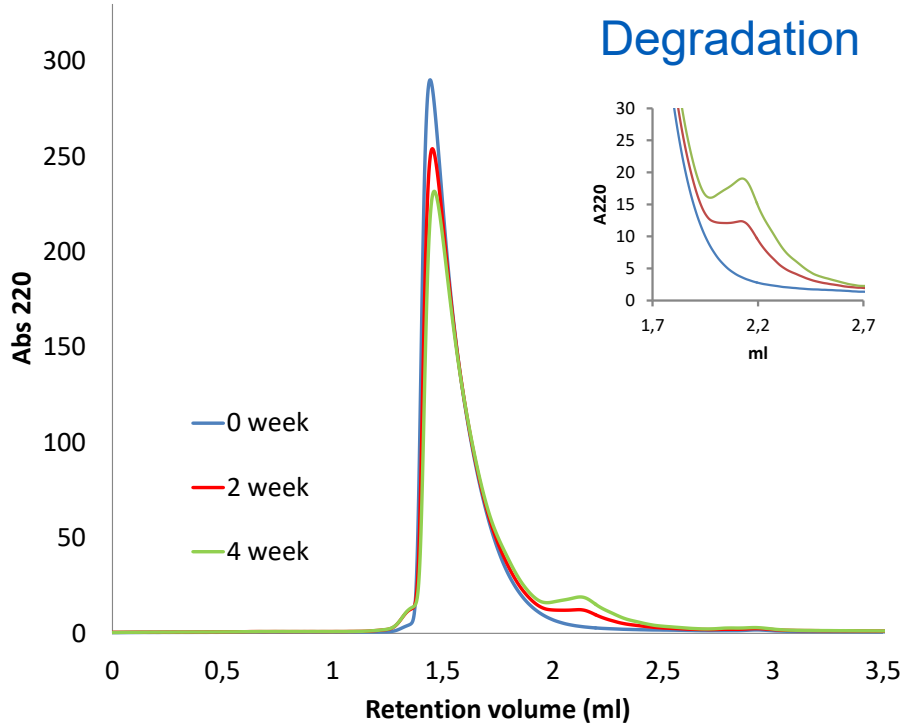
HiPrep DEAE FF 16/10

HiScreen™ Capto Q

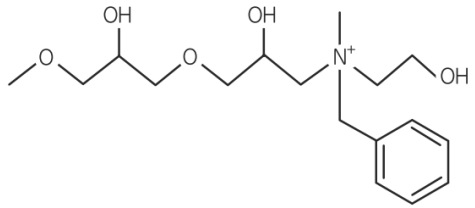
SEC: Product-related impurities

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



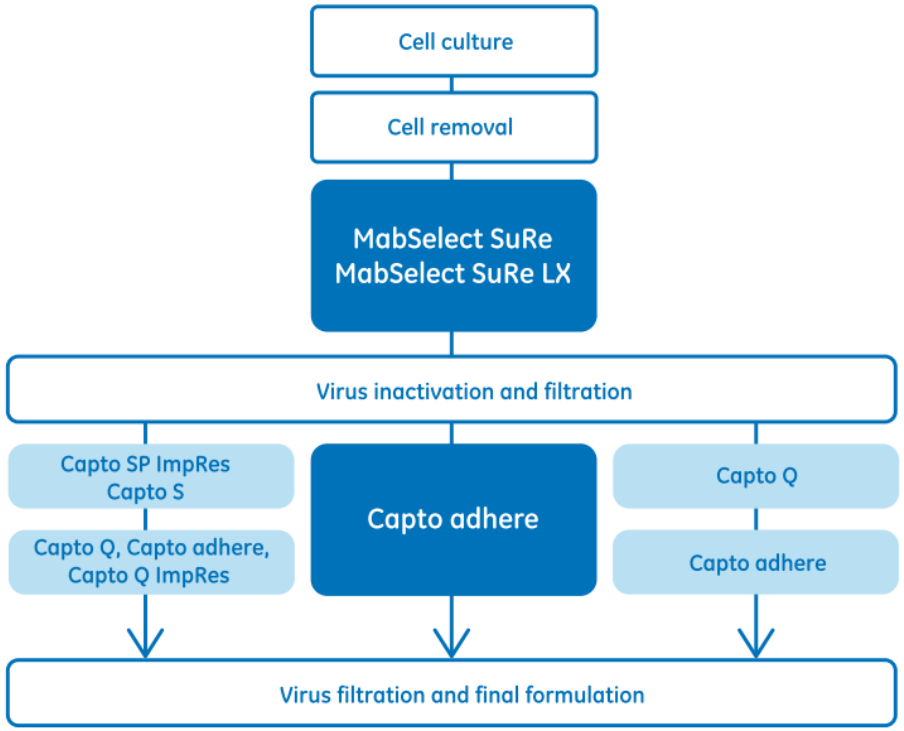


Example: Application: Multimodal functionality gel

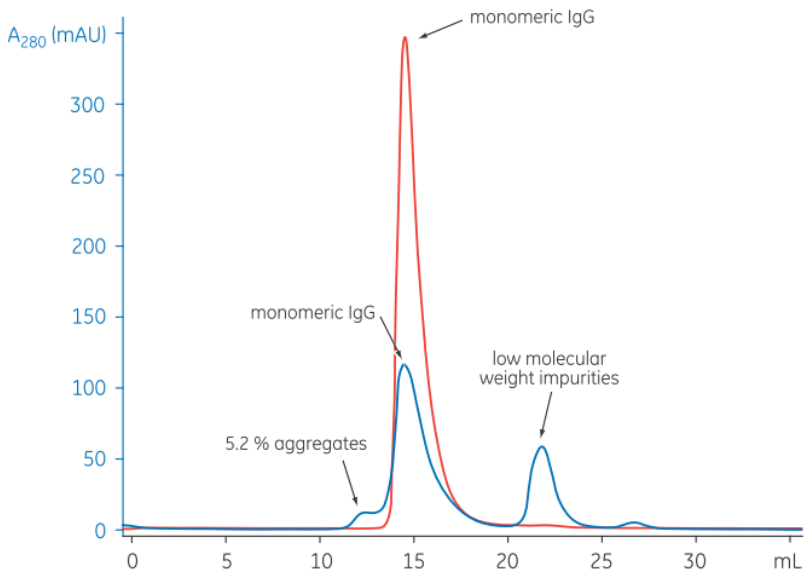


- aggregates
- DNA, viruses
- HCP
- endotoxin

Capto adhere

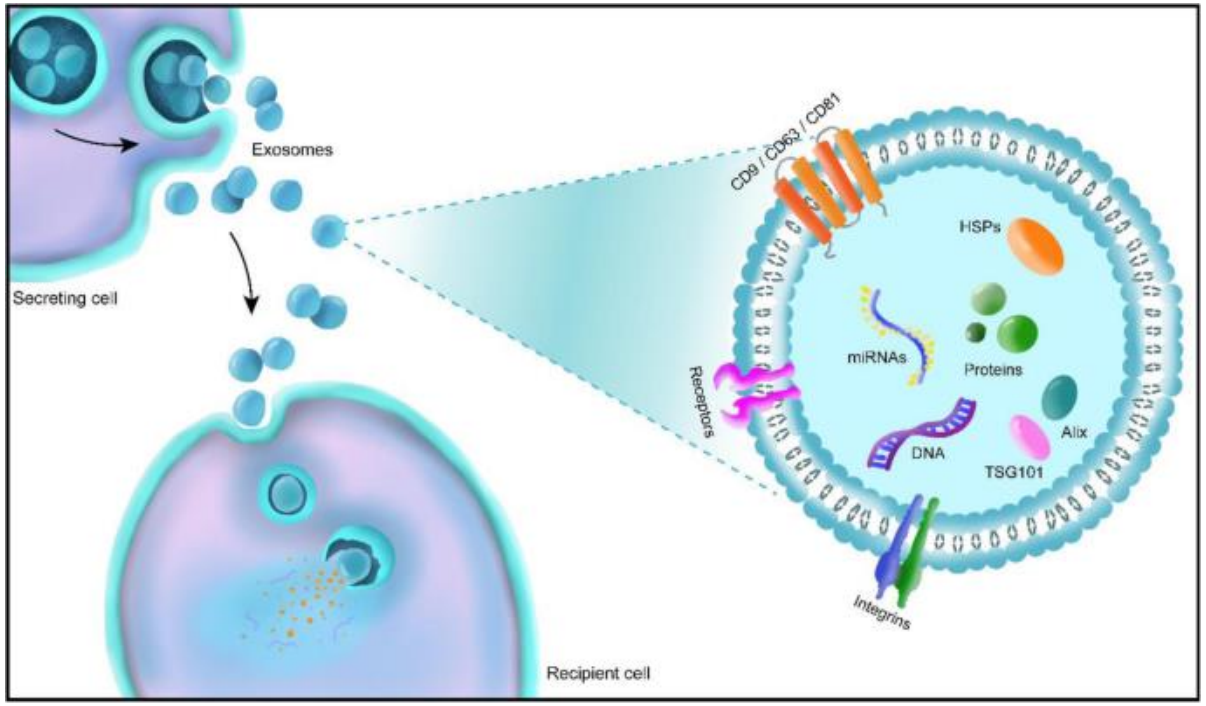


Column: Superdex 200 10/300
Sample: Flowthrough fraction (red) and eluate (blue) from the Capto adhere step
Sample load: 50 µL each
Loading buffer: 0.01 M sodium phosphate, 2.7 mM potassium phosphate, 137 mM sodium chloride, pH 7.4
Flow rate: 0.5 mL/min
System: ÄKTA chromatography system



Application examples: Exosome

Exosome
命名和歷史

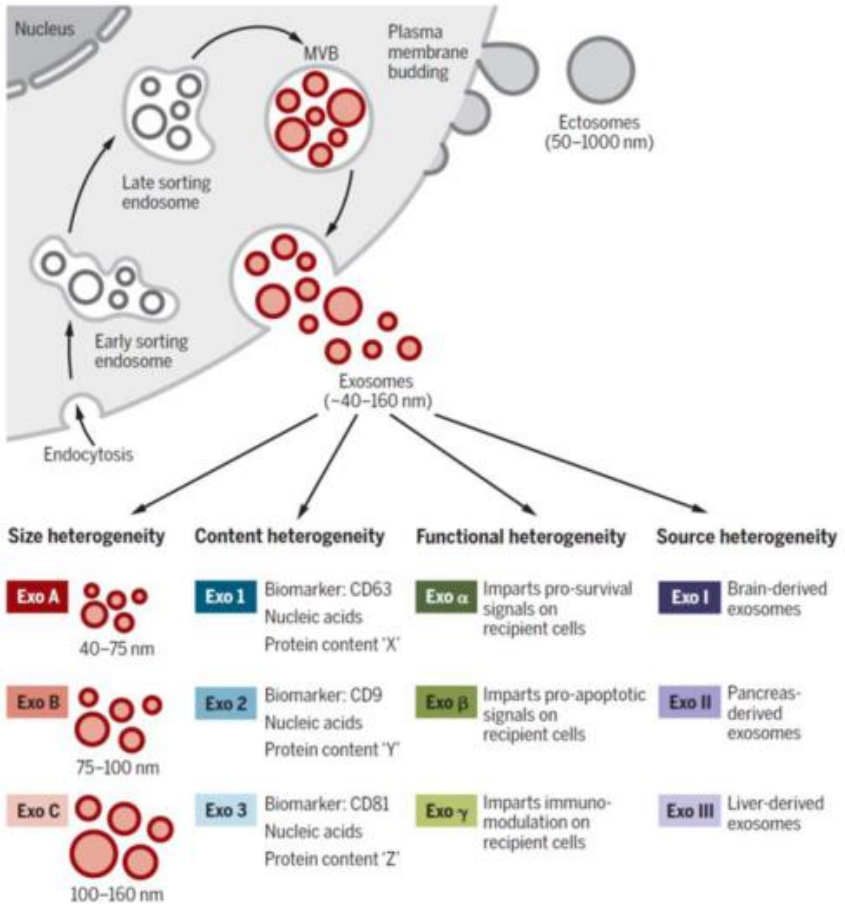


根據國際細胞外囊泡協會的說法，**細胞外囊泡（或 EV）是普遍存在的從細胞中自然釋放的納米顆粒，由脂質雙層分隔且無法複製**。

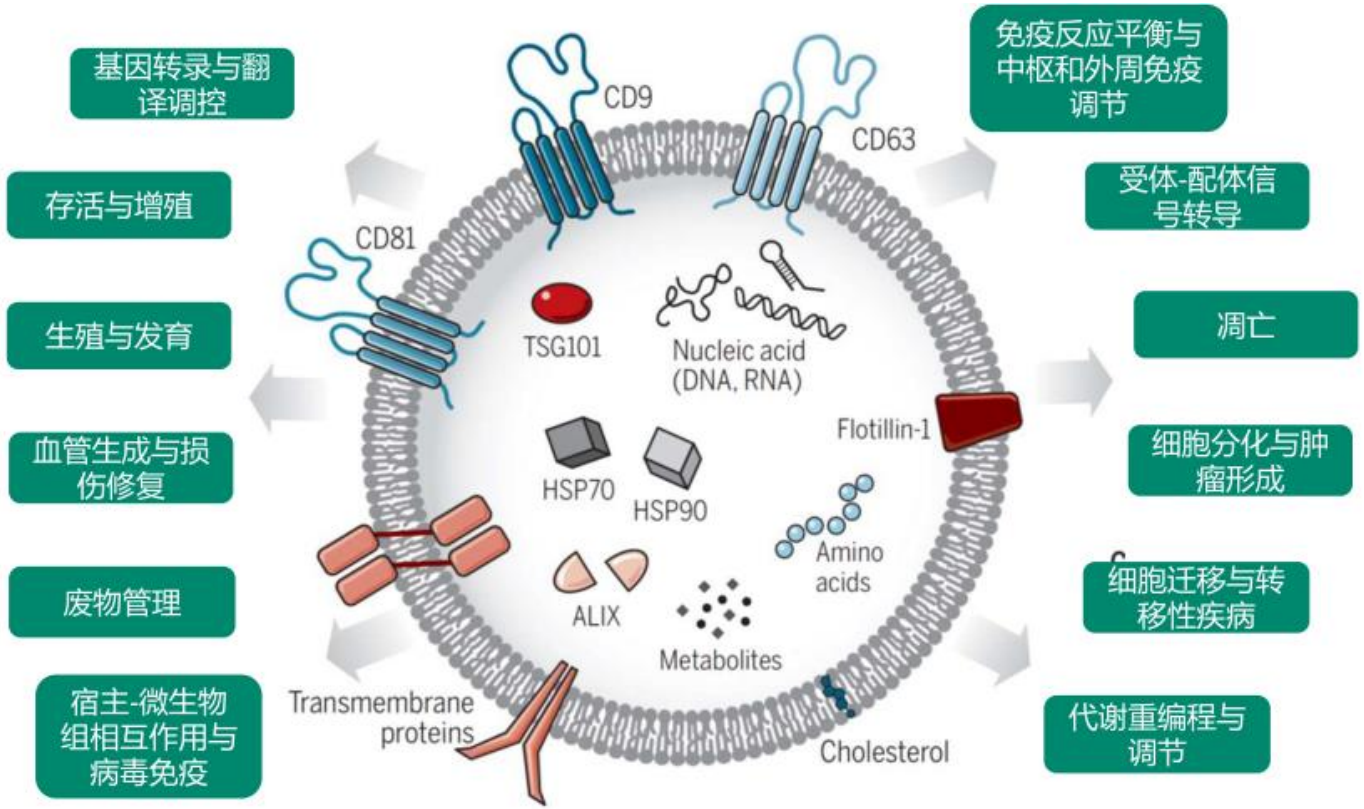
細胞外囊泡的不同亞型根據它們的大小、組成和生物發生來區分：**外泌體（小於 150 nm）**、微泡或膜外泌體（50 nm 和 500 nm 之間）和凋亡小體（直到 2,000 nm）。

細胞外囊泡**富含蛋白質、核酸和脂質，通常在表面發現並用作細胞外囊泡標記的蛋白質是四跨膜蛋白tetraspanins（CD9、CD63、CD81、CD82）和主要組織相容性複合物。**

Exosome



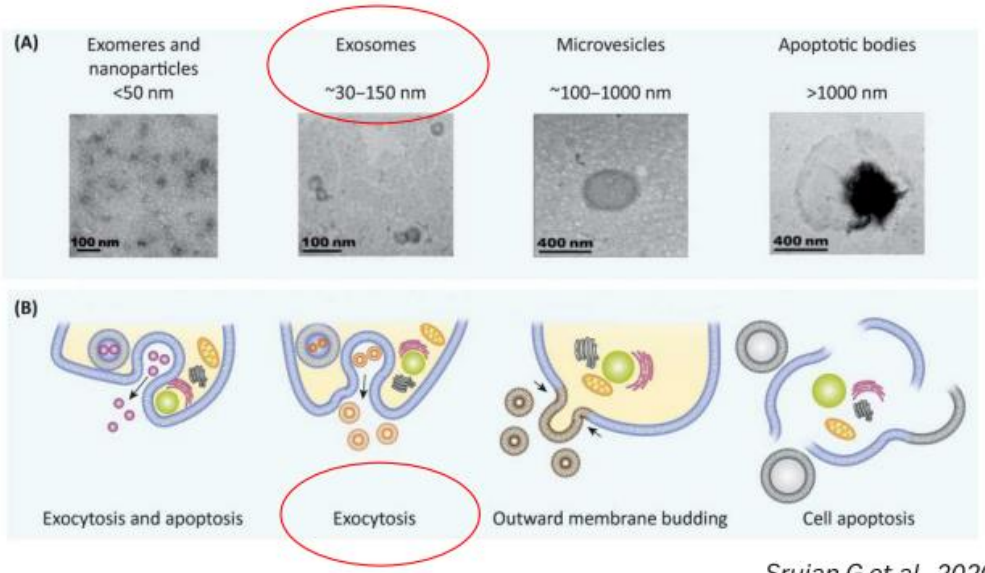
Kalluri et al., Science 367, (2020)



Cytiva

Kalluri et al., Science 367, (2020)

Exosome

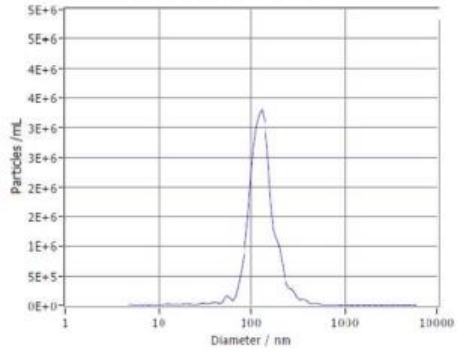


Srujan.G et al., 2020

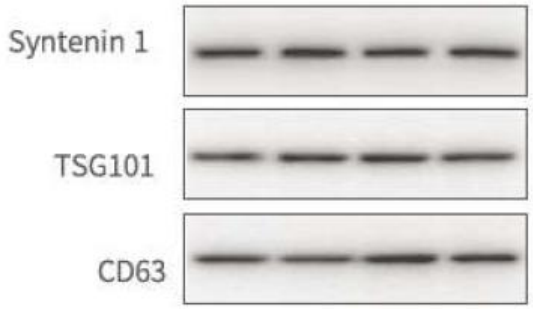
Table 1 Comparison between extracellular vesicles

Types	Exosomes	Microvesicles	Apoptotic bodies
Origin	Endocytic pathway	Plasma membrane	Plasma membrane
Size	30–150 nm	50–1000 nm	500–2000 nm
Function	Intercellular communication	Intercellular communication	Facilitate phagocytosis
Markers	Alix, Tsg101, tetraspanins (CD81, CD63, CD9), flotillin	Integrins, selectins, CD40	Annexin V, phosphatidylserine
Contents	Proteins and nucleic acids (mRNA, miRNA, and other non-coding RNAs)	Proteins and nucleic acids (mRNA, miRNA, and other non-coding RNAs)	Nuclear fractions, cell organelles

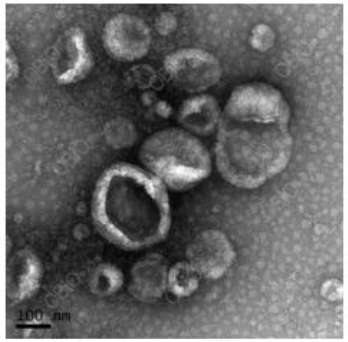
粒徑分析 NTA/DLS



西方點墨 WB



電顯 TEM



Integrated Bioprocess

EFFECTIVE BIO-PROCESSING EXOSOME PLATFORM

CORNING cytiva PEPROTECH. OUR SUPPORT, YOUR DISCOVERY Part of Thermo Fisher Scientific MERCK STILLA

level Discovering new boundaries 進階生物科技 Level Biotechnology Inc.

Upstream

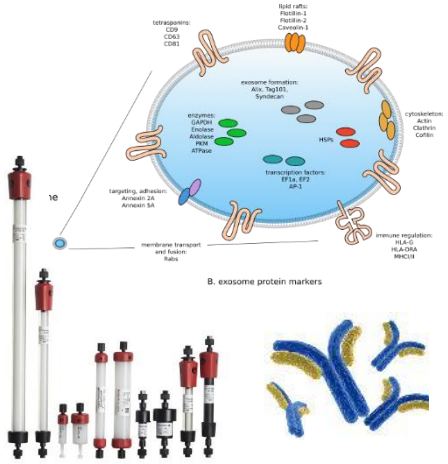
Scale-X™ carbo Bioreactor

Cytiva ReadyToProcess WAVE™ 25

Harvest / Clarification

ÄKTA flux tangential flow filtration

Downstream



ÄKTA Pure FPLC Purification

Analyze & Quality

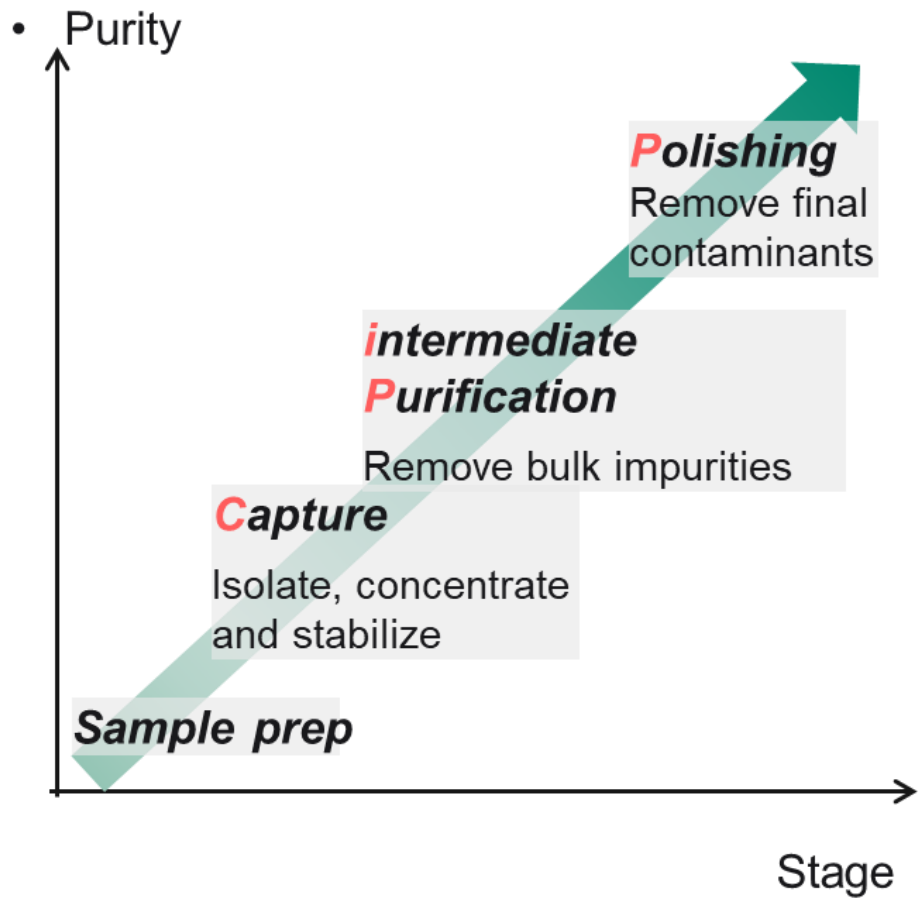
Naica® 6-color Digital PCR-Gene absolute quantitation

Cytiva Biacore™ SPR systems for titer analysis

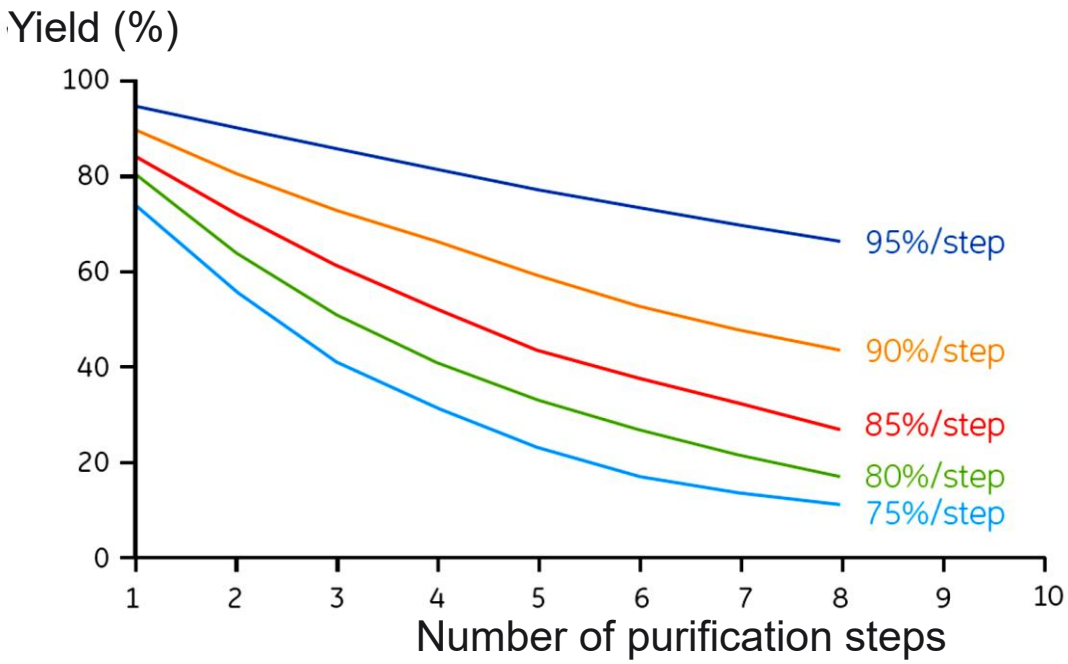
Bioprocess Downstream

Introduction to CiPP purification strategy

Purification strategy combining multiple steps



Protein recovery plotted against the number of purification steps

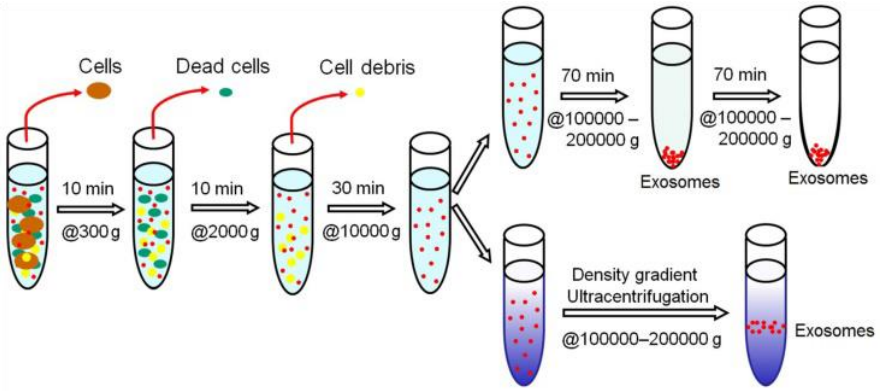


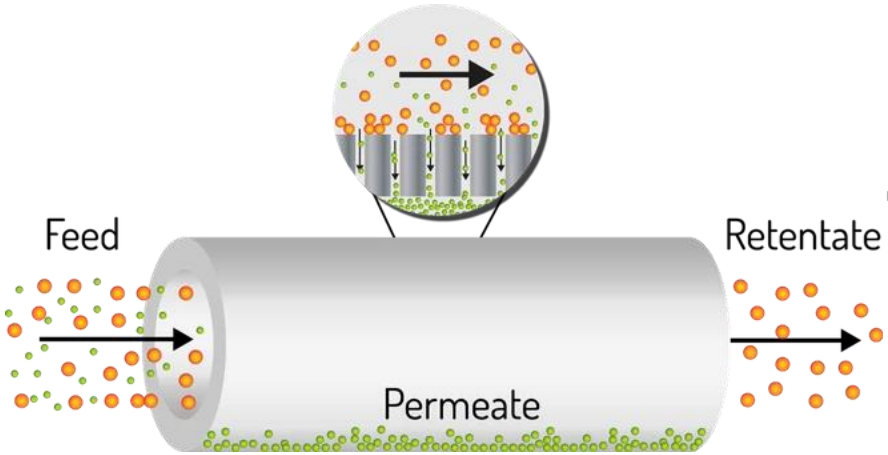
Exosome

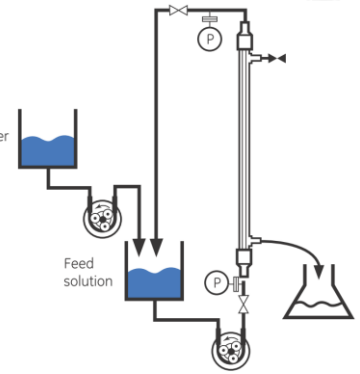
Isolation method	Tangential Flow Filtration	Differential Ultracentrifugation	Size exclusion chromatography	Density gradient ultracentrifugation	Ultrafiltration	Anion exchange chromatography	Immunoaffinity capture
Concentration of the EVs	++	++	-	±	++	+	+
Host-cell proteins purification	-	+	++	++	+	++	+
Recovery	++	±	+	-	±	+	+
Scalable	++	-	+	-	-	++	?

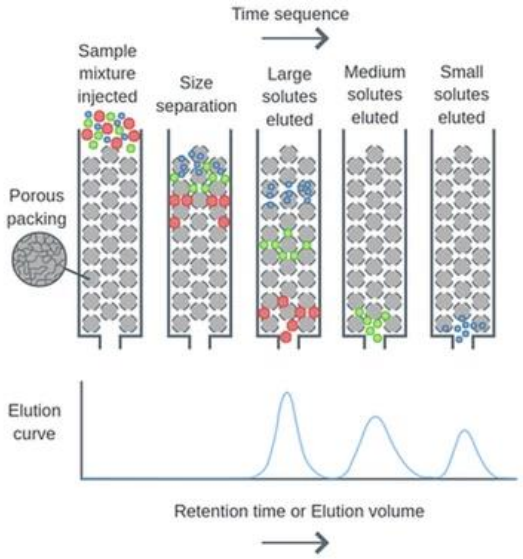
++: Best method; +: correct method; ±: variable; -: insufficient

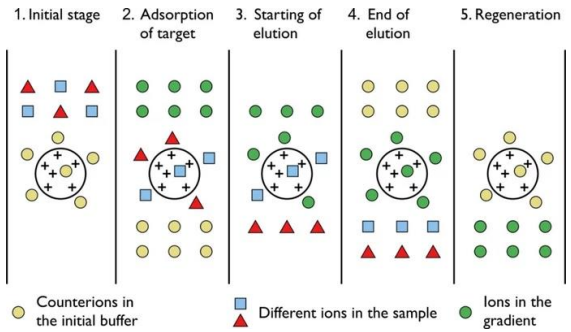
Exosome

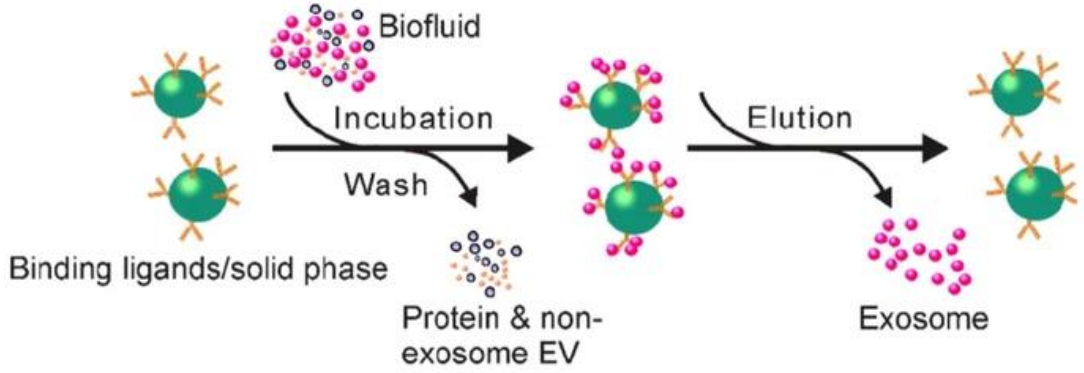

Cells
Dead cells
Cell debris
10 min
@300 g
10 min
@2000 g
30 min
@10000 g
70 min
@100000 – 200000 g
Exosomes
70 min
@100000 – 200000 g
Exosomes
Density gradient
Ultracentrifugation
@100000–200000 g
Exosomes


Feed
Retentate
Permeate

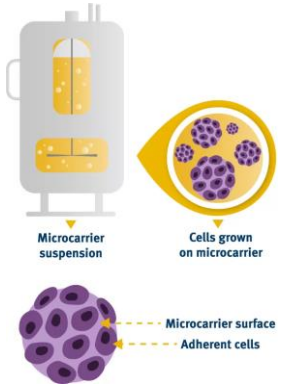

Buffer
Feed solution
P
P

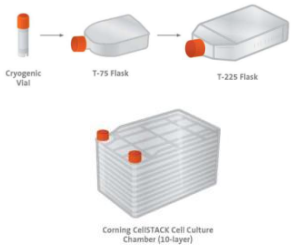

Time sequence
Sample mixture injected
Size separation
Large solutes eluted
Medium solutes eluted
Small solutes eluted
Porous packing
Elution curve
Retention time or Elution volume


1. Initial stage
2. Adsorption of target
3. Starting of elution
4. End of elution
5. Regeneration
Counterions in the initial buffer
Different ions in the sample
Ions in the gradient


Biofluid
Incubation
Wash
Elution
Binding ligands/solid phase
Protein & non-exosome EV
Exosome

Exosome Purification





MSC	樣品前處理	Exosome Isolation	Protein removal	Exosome Purification
懸浮培養微載體	離心/去除細胞	高超速離心	SEC	Streptavidin sepharose + Biotin Ab
	0.22 filter	PEG 沉澱	IEX	Mag Separose + Ab
3D/2.5培養	RNAseA 去除 non-EV	TFF 100/300KDa	Capto Core	IEX

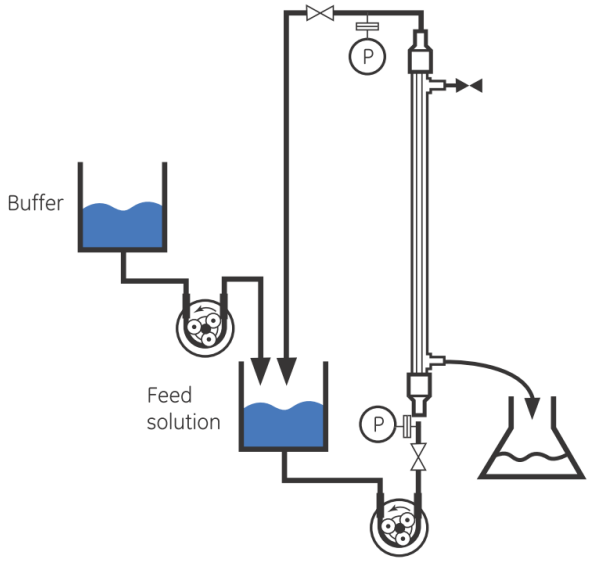
- 3000rpm 30min
- 2000rpm 2min

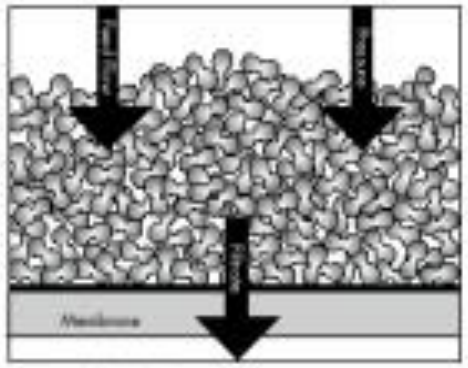
膜過濾系統 AKTAflux

NFF vs. TFF

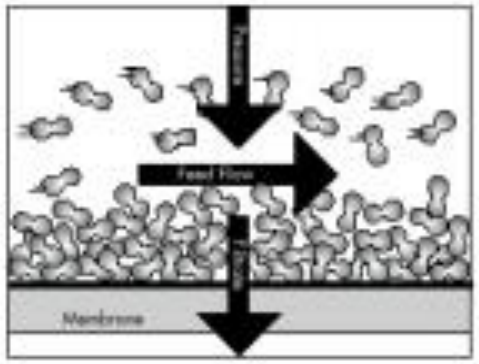
- **Normal Flow Filtration (NFF)**
  Fluid is pumped directly toward the membrane under a applied pressure.

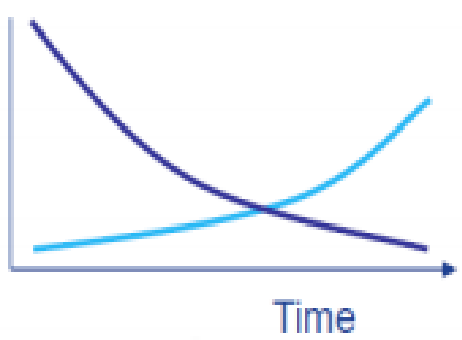
- **Tangential Flow Filtration (TFF)**
  The fluid is pumped tangentially along the surface of membrane.

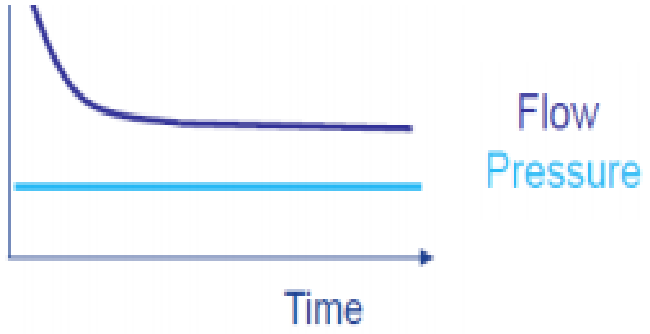




Normal Flow Filtration

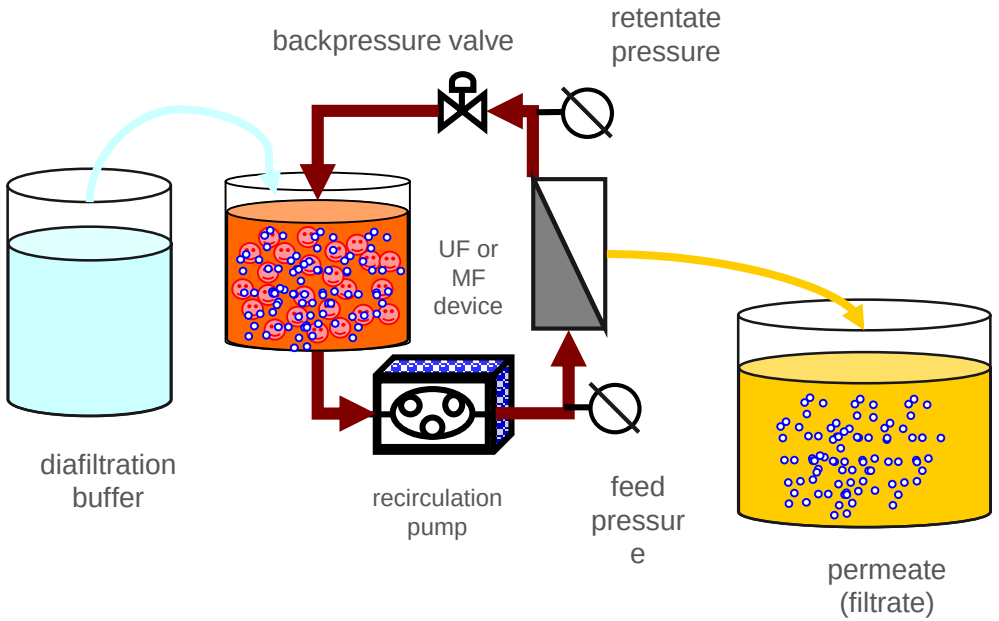




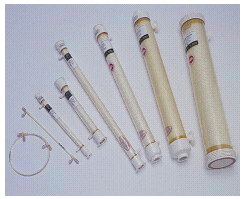
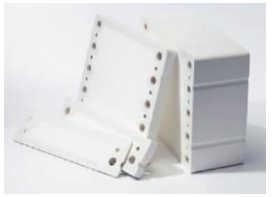


ÄKTA™ flux: a new cross flow filtration system

- Concentration
- Diafiltration (buffer exchange)
- Cell harvest
- Clarification



MF 0.1, 0.2, 0.45, 0.65 µm Depth filter
UF 1, 3, 5, 10, 30, 50, 100, 300, 500, 750 kDa


ÄKTA™ flux: a new cross flow filtration system

- Concentration
- Diafiltration (buffer exchange)
- Cell harvest
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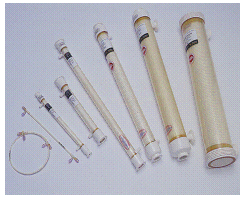
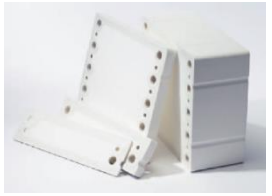
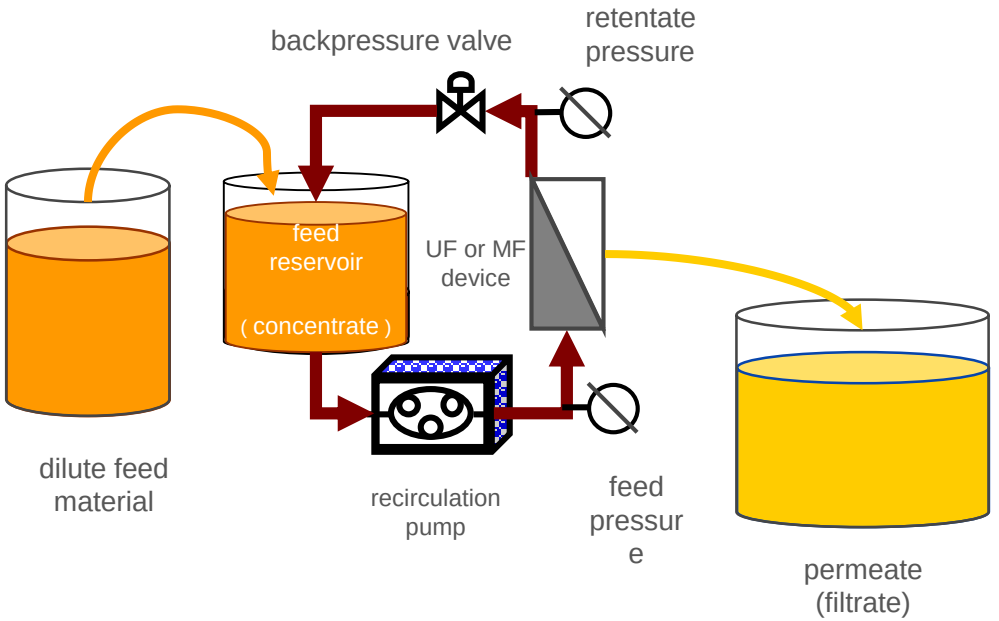
MF 0.1, 0.2, 0.45, 0.65 µm Depth filter
UF 1, 3, 5, 10, 30, 50, 100, 300, 500, 750 kDa







ÄKTA™ flux: Filter selection

For target passage:

Pore size should be 5-10X larger than the molecule.

For target retention:

Pore size should be 3-5X smaller than the molecule.

For species separation:

A 10X size difference is usually required.

Examples

MAb concentration: 30-50 kD

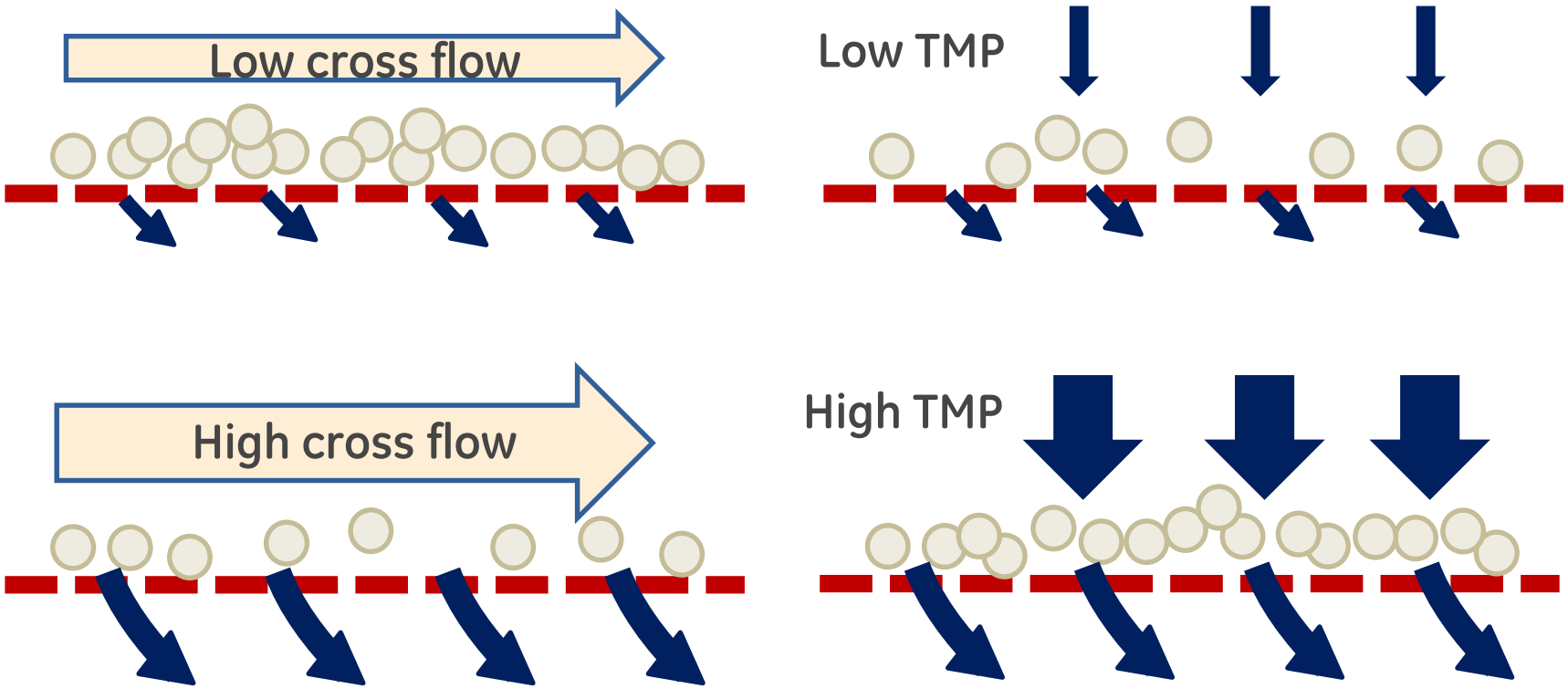
Virus concentration: 300-700 kD

Exosome concentration: 100-500 kD

Plasmid concentration: 300 kD

LNPs and Liposome concentration: 100-300 kD

ÄKTA™ flux: Effect of cross flow and TMP



ÄKTA™ flux: CFF process parameters

pressures

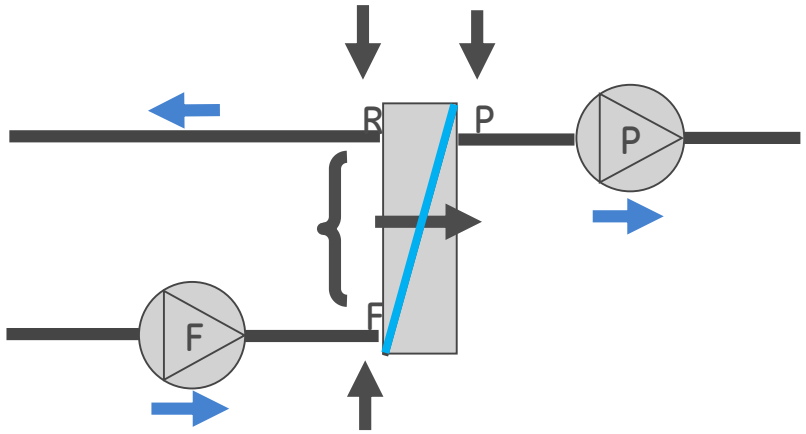
feed

retentate

permeate

deltaP

TMP



flow rates

feed

retentate

permeate

flux

pressure drop (ΔP)

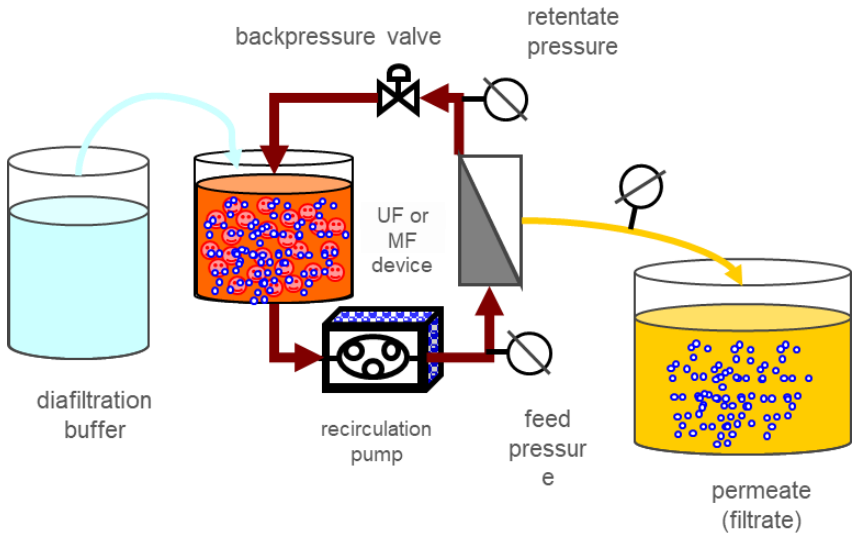
$\Delta P = p_f - p_r$

transmembrane pressure (TMP)

$TMP = [(p_f + p_r)/2] - p_p$

permeate flux

permeate flow divided by membrane area [LMH] *(L/m²h)*



ÄKTA™ flux: Effect of cross flow

Shear rate $r = (4*q)/\pi*R^3$

r = shear rate, sec^{-1}

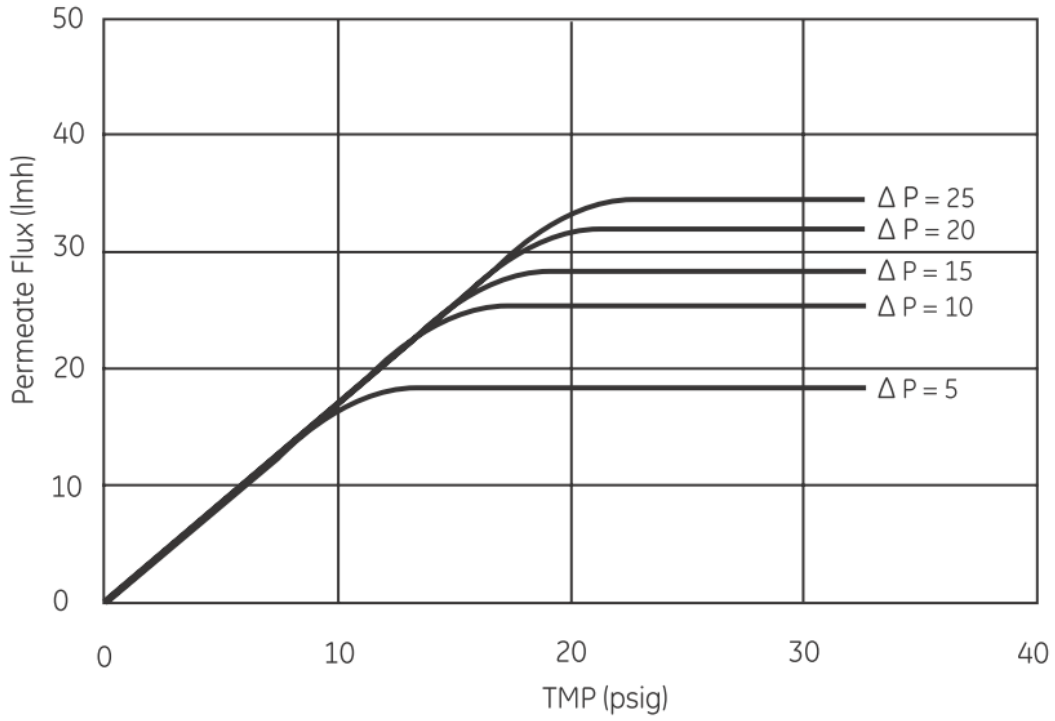
q = flow rate through the fiber lumen, cm^3/sec

R = fiber radius, cm

Nominal lumen ID (mm)	Shear rate ~2,000 sec^{-1}	Shear rate ~4,000 sec^{-1}	Shear rate ~8,000 sec^{-1}	Shear rate ~16,000 sec^{-1}
0.5	0.06	0.12	0.25	0.5
0.75	0.1	0.2	0.4	0.8
1	0.15	0.3	0.6	1.2

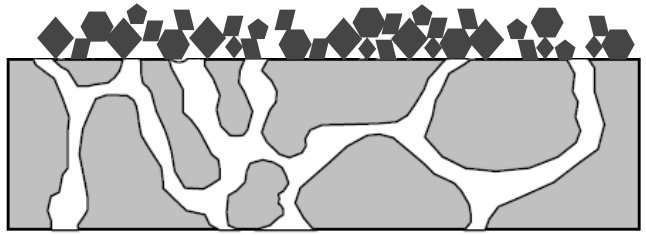
剪切力（迴圈速率）	樣品類型
2000-4000sec^{-1}	適用于易破碎的哺乳動物細胞和病毒
6000-8000 sec^{-1}	適用于高粘度的酵母
8000-16000 sec^{-1}	適用於細菌細胞，細胞裂解液，和絕大多數蛋白質

ÄKTA™ flux: Effect of cross flow and TMP



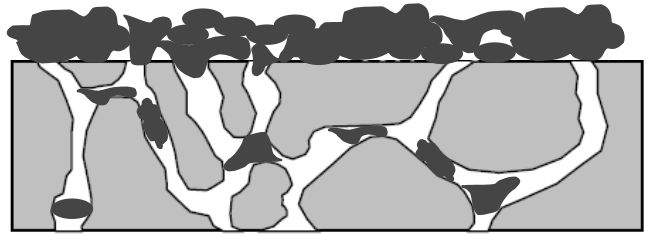
Hard (non-deformable)

- easy to filter



Soft (deformable)

- difficult to filter



ÄKTA™ flux: a new cross flow filtration system

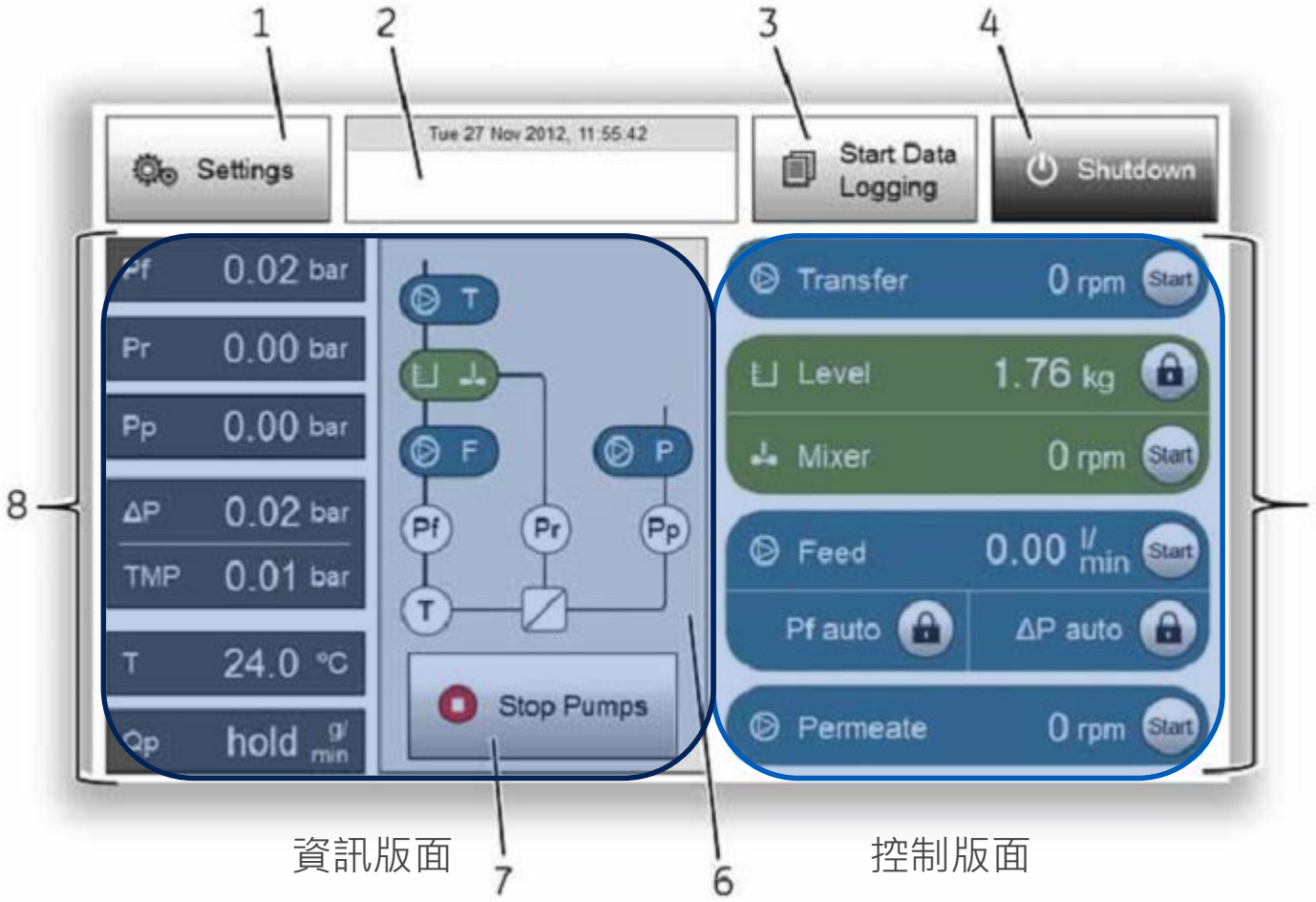
ÄKTA flux s

20-350 ml/min

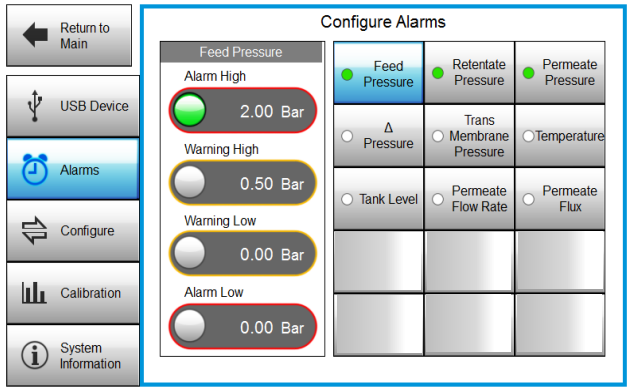
ÄKTA flux 6

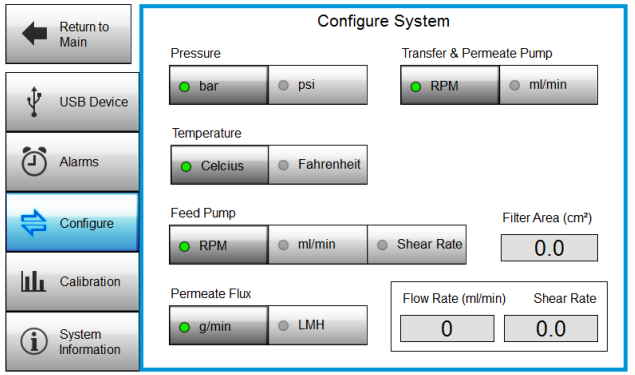
0.1-6 l/min

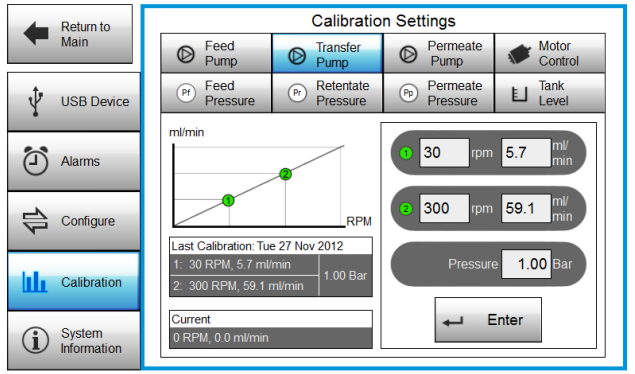
ÄKTA™ flux: a new cross flow filtration sys



1. 設置按鈕
2. 信息欄
3. 資料記錄
4. 關機鍵
5. 控制版面
6. 流路圖
7. 停泵按鈕
8. 資訊版面







膠體純化系統 AKTApure

New Generation of ÄKTA System

New System

Transition from manual to automated purification
Education in protein purification

ÄKTA™ start

Achieve desired purity with ease in routine purifications
Make the most of valuable bench/cold room space

ÄKTA™ go

Flexibility in research
Match most current and future purification challenges

ÄKTA™ pure

Productivity in process development
Fast and secure development of purification processes

ÄKTA™ avant

Functions & Price

Classic System

ÄKTAprime™

ÄKTApurifier™ UPC

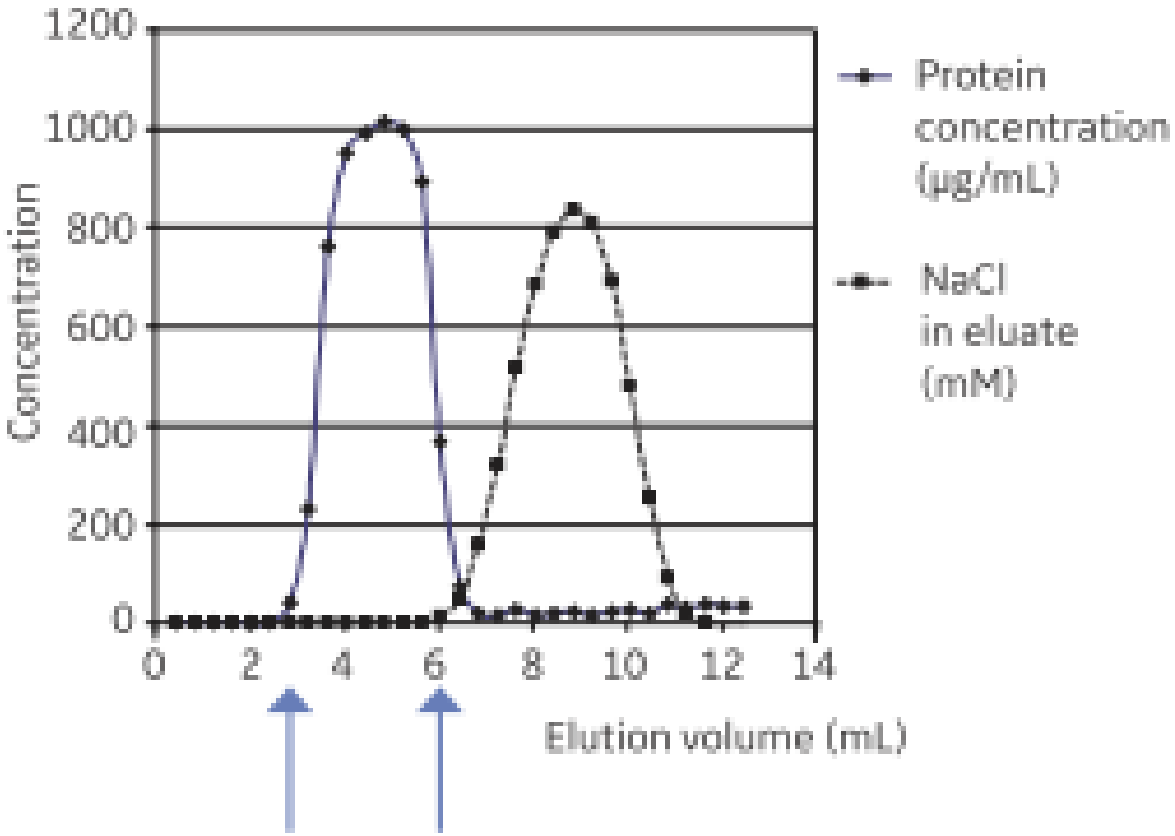
ÄKTAFPLC™

ÄKTApurifier™

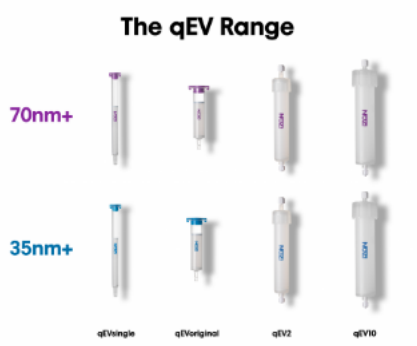
ÄKTAexplorer™

Desalting/Buffer exchange & Size Exclusion Chromatography

凝膠過濾填料

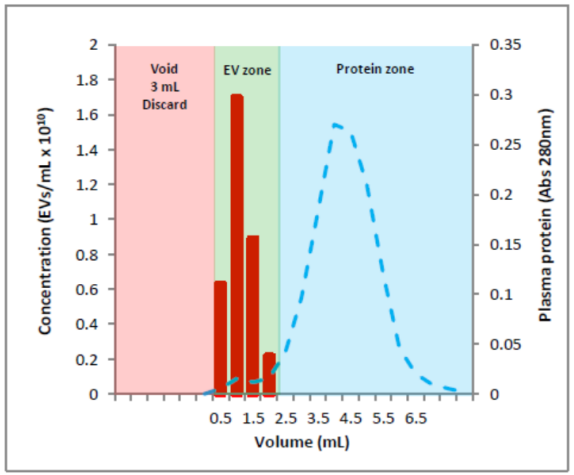


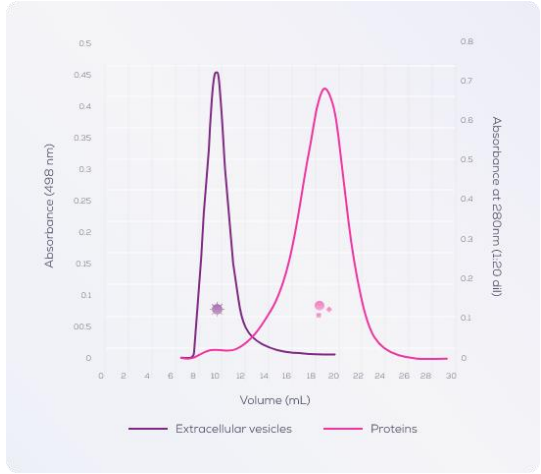
qEV Columns Gen 2 規格



1. 樣品注入體積 : 150 µL- 100 mL
2. 操作溫度 : 15-25 °C (室溫)
3. 適定流速 : 一般為 0.8-1.2 mL/min (18 °C)
4. 通過的最小顆粒 : 35 nm / 70 nm (2種尺寸)
5. 通過的最大顆粒 : 1 µm
6. 頂端和底部的過濾器尺寸 : 20 µm
7. 可穩定運作的 pH 值範圍 : 3-13
8. 可穩定再生的 pH 值範圍 : 2-14
9. 初次使用前的保值期 : 1 year
10. 初次使用後的保值期 : 依使用和保存狀態而定, 建議3個月內

※ 全新改良型的 Agarose resin 填充的 "Gen 2" 型號的columns 已開始發售, 可大幅提升回收率與純度





SEC on Sepharose 4 Fast Flow

(A)

V_{sample}/V_{column}	0.05
Virus recovery (%)	69
DNA removal (%)	96
Total protein removal (%)	67
Ratio areas (virus peak/total area)	37

(B)

V_{sample}/V_{column}	0.1
Virus recovery (%)	65
DNA removal (%)	94
Total protein removal (%)	80
Ratio areas (virus peak/total area)	34

(C)

V_{sample}/V_{column}	0.2
Virus recovery (%)	70
DNA removal (%)	97
Total protein removal (%)	72
Ratio areas (virus peak/total area)	42

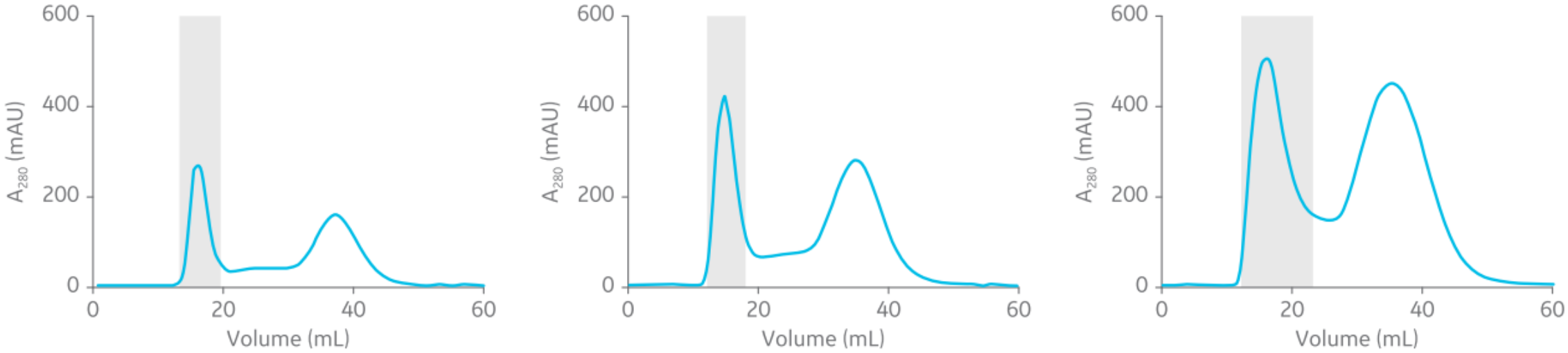


Fig 6. Summary of virus recovery and impurity removal by SEC on Sepharose 4 Fast Flow using SV-to-CV ratios of (A) 0.05, (B) 0.1, and (C) 0.2. The shadowed area represents the collected fractions. The recovery and impurity removal results are presented in the table on top.

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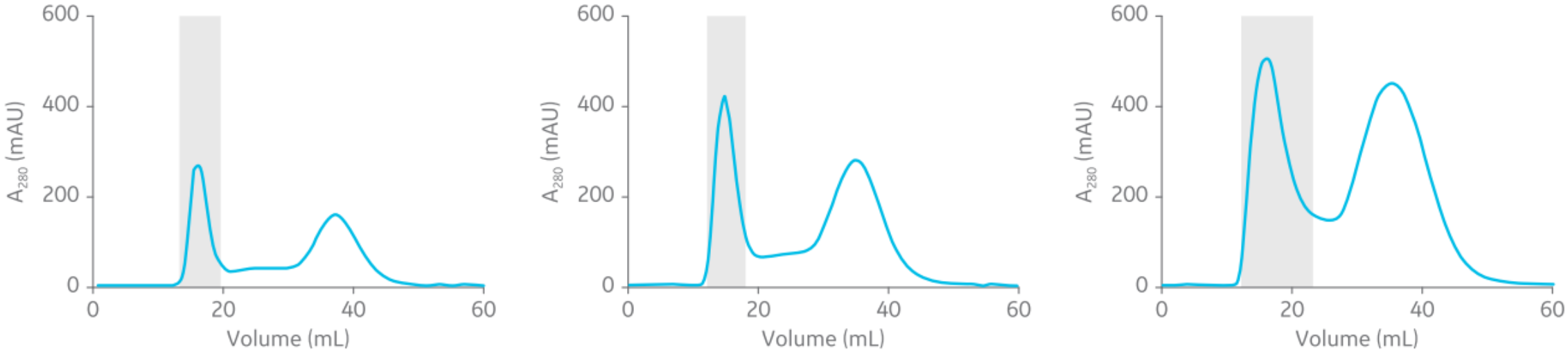

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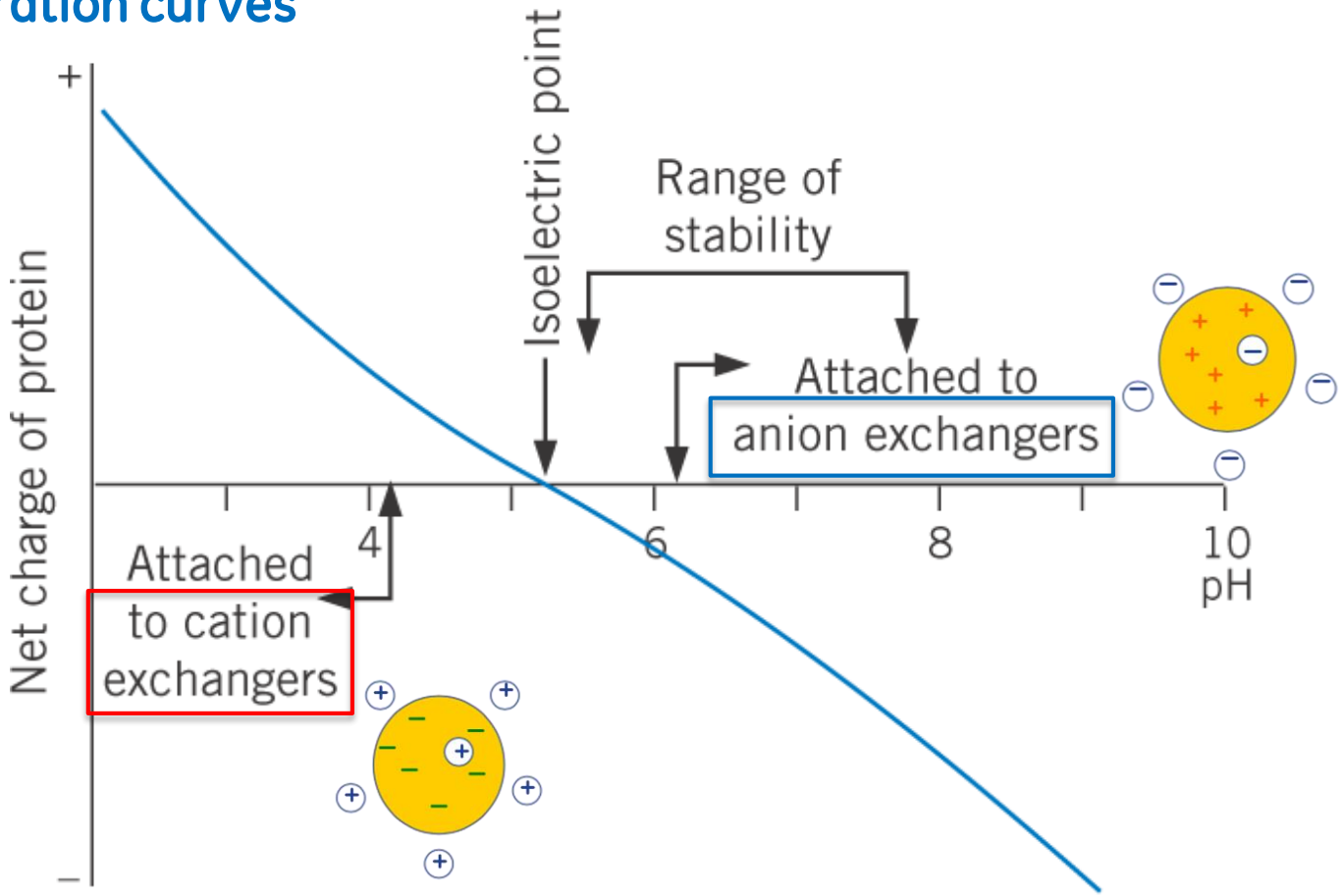
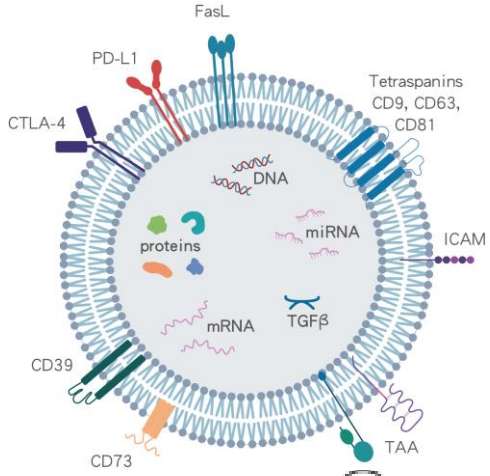
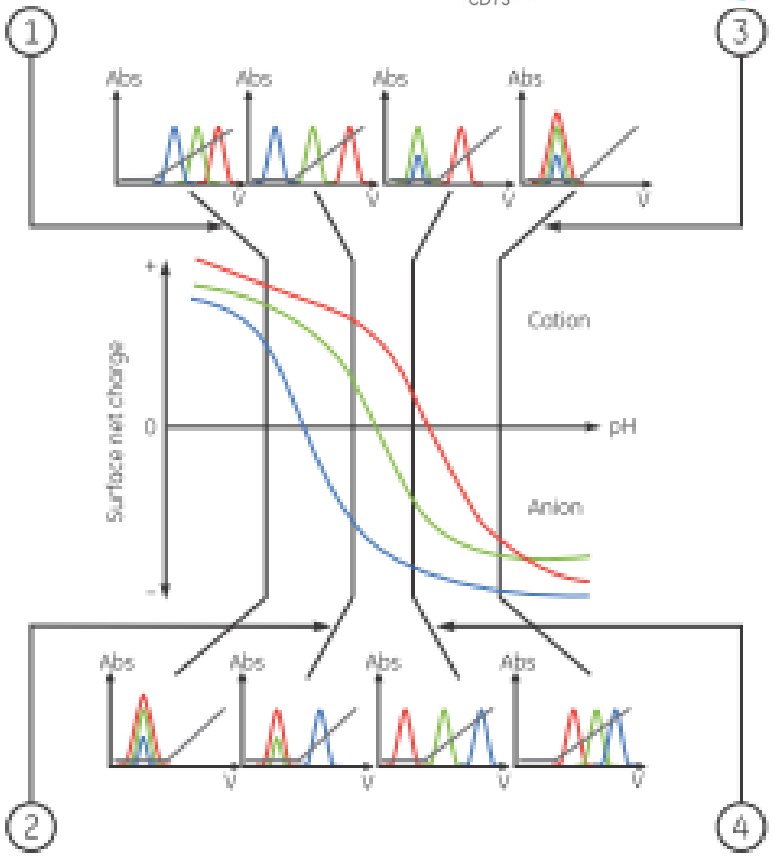
Ion exchange chromatography

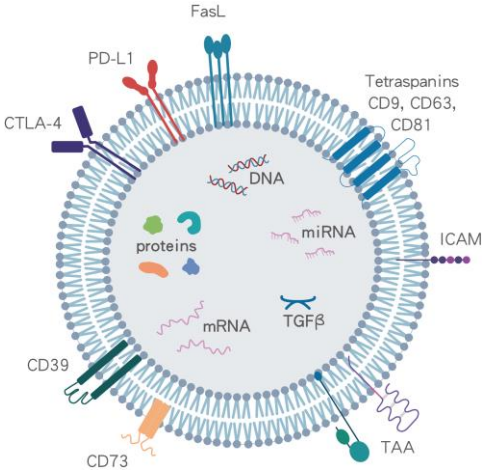
Titration curves

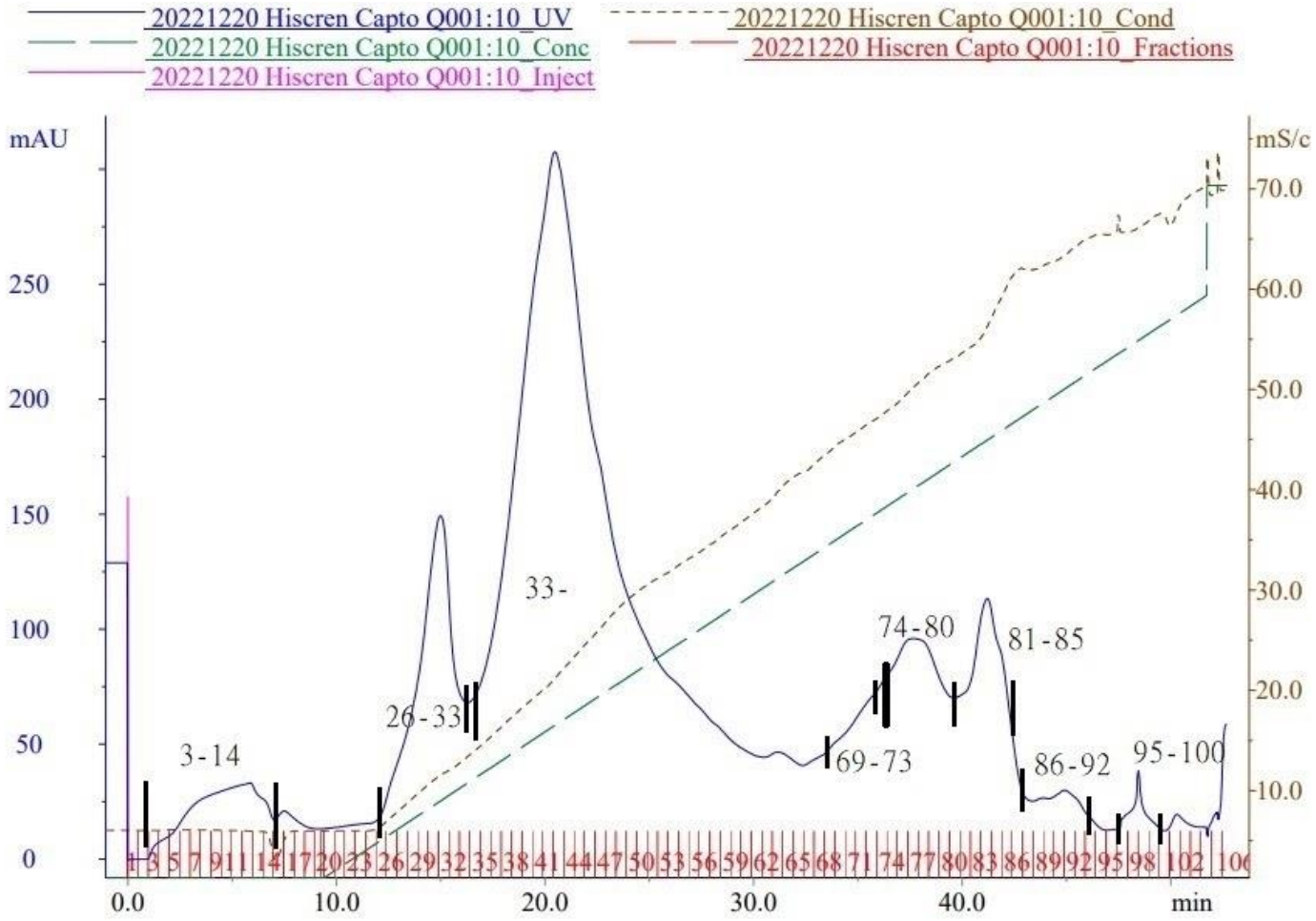




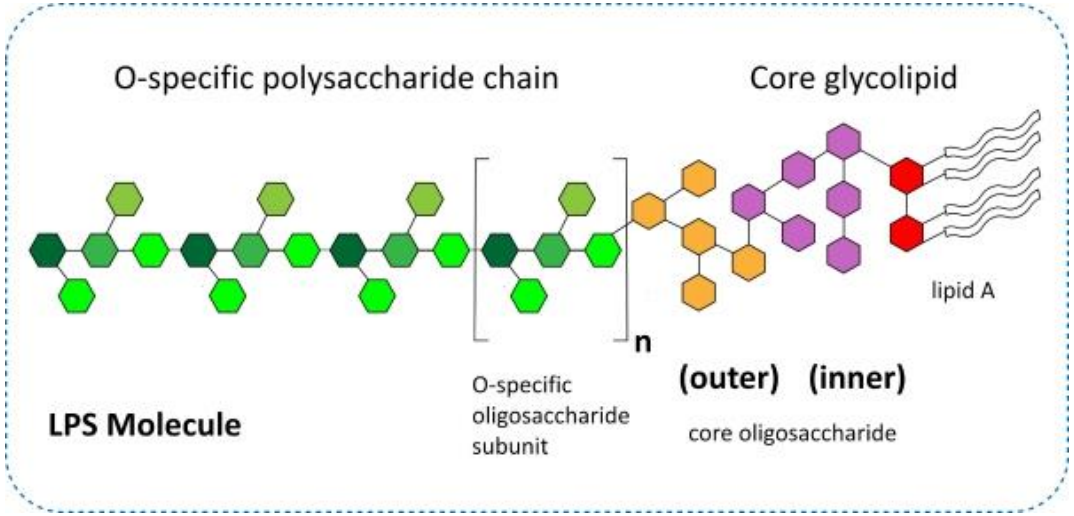


Ion exchange chromatography/Exosome

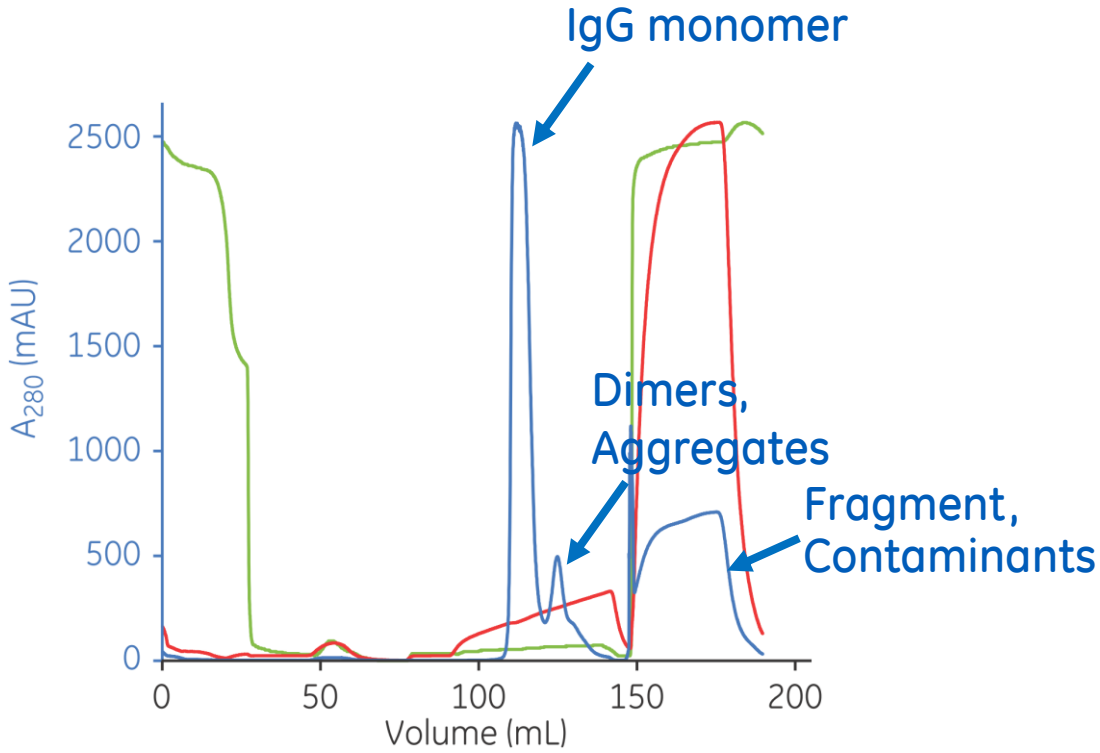




Ion exchange chromatography

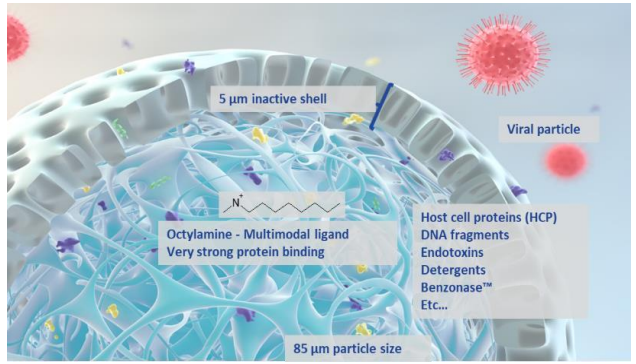


10~20 KD ~ 4 ×105 KD
< 0.25EU/ml



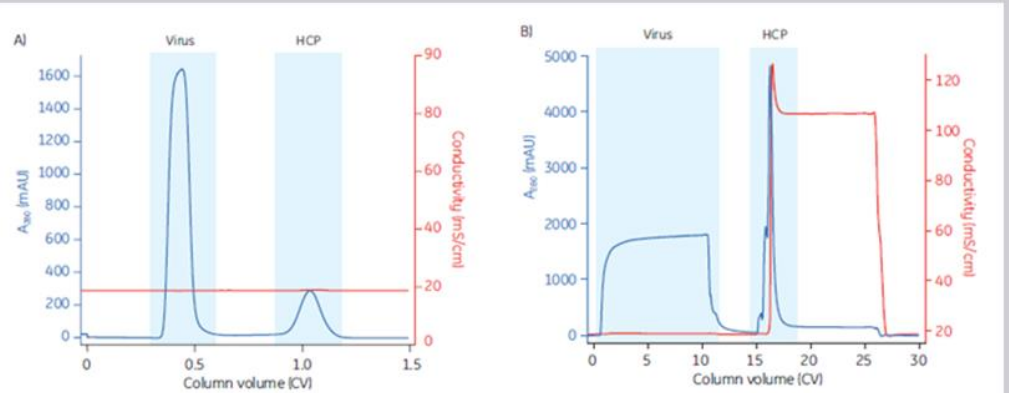
Column: Capto™ SP ImpRes

LNPs, Virus, VLPs, Exosome Multimodal chromatography

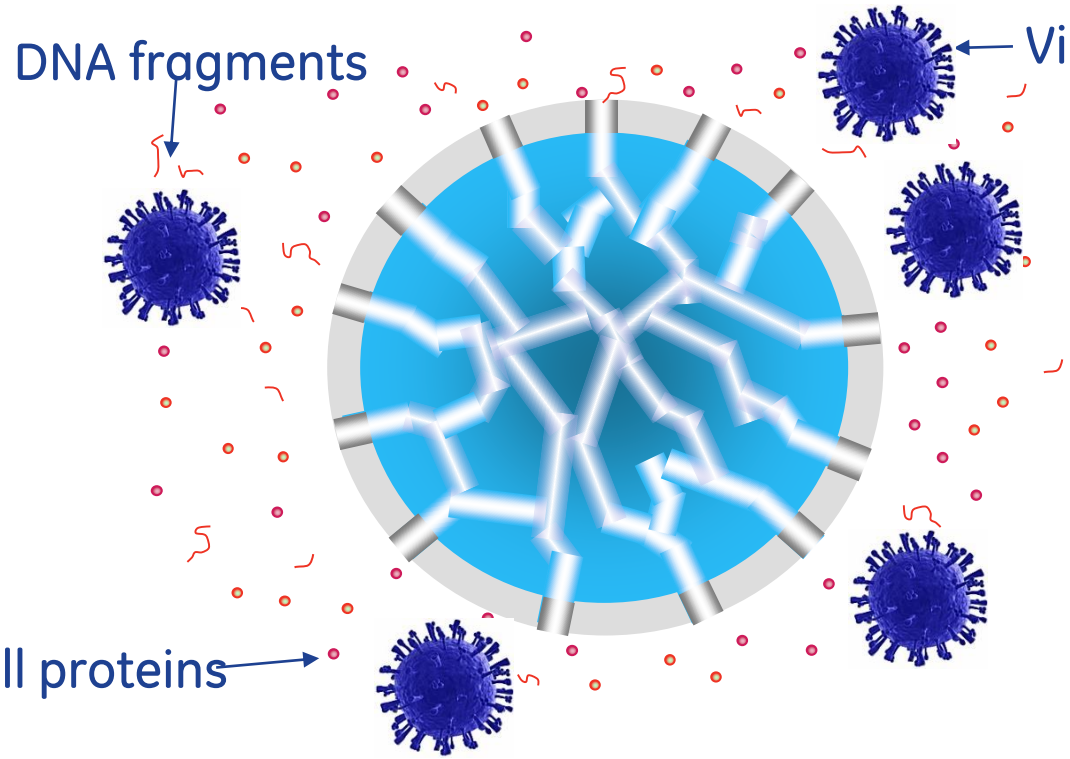


- aggregates
- DNA, RNA
- HCP
- endotoxin

Gel Filtration vs. Capto Core 700 performance

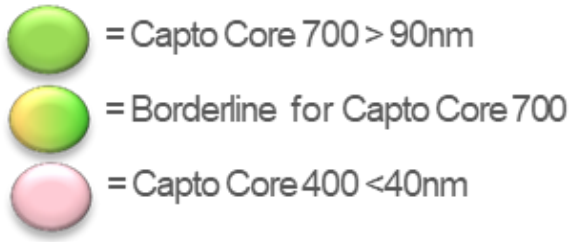


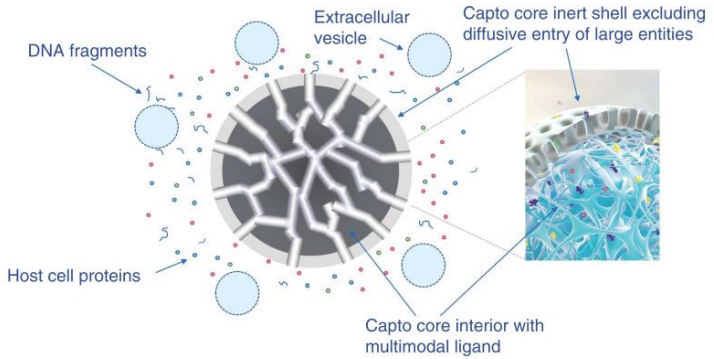
Media	Virus Recovery [%]	HCP Removal [%]	Column load [CV]	Dilution factor
Sepharose™ 4 FF	100	95	0.1	~2
Capto™ Core 700	97	95	10	1



Core 700 or Core 400?

	Retroviridae	Flaviviridae	Picornaviridae
Size	100 nm	50 nm	30 nm
Type	Enveloped, RNA	Enveloped, RNA	Naked, RNA
Sample tested	Clarified harvest	Clarified harvest	Purified virus suspension
Columns	HiScreen 4.7 mL (0.77 cm i.d, 10 cm bed height)		
Sample and load	Clarified harvest, 10 CV	Clarified harvest, 20 CV	Purified virus, 6 CV
Equilibration/wash	50 mM Tris, pH 7.3 + 50 mM NaCl	50 mM Tris, pH 7.5 + 150 mM NaCl	35 mM phosphate buffer, pH 7
Flow velocity during loading	150 cm/h (Capto Core 400) 300 cm/h (Capto Core 700)	150 cm/h (Capto Core 400) 400 cm/h (Capto Core 700)	150, 325, and 500 cm/h (Capto Core 400 and Capto Core 700)
Elution	50 mM Tris, pH 7.3 + 1.2 M NaCl	50 mM Tris, pH 7.5 + 1 M NaCl	35 mM Phosphate buffer, pH 7
CIP (cleaning in place)	1 N NaOH, 30% isopropanol		
System	ÄKTA™ pure		





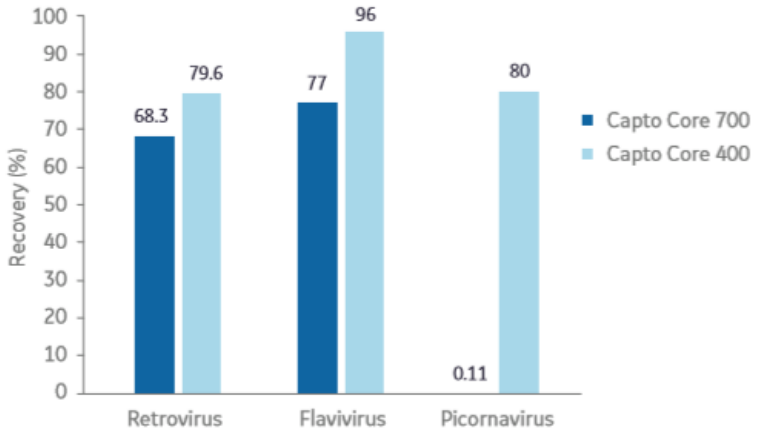


Fig 6. ELISA results from study with Capto Core 400 and Capto Core 700 on flavivirus, retrovirus, and picornavirus.

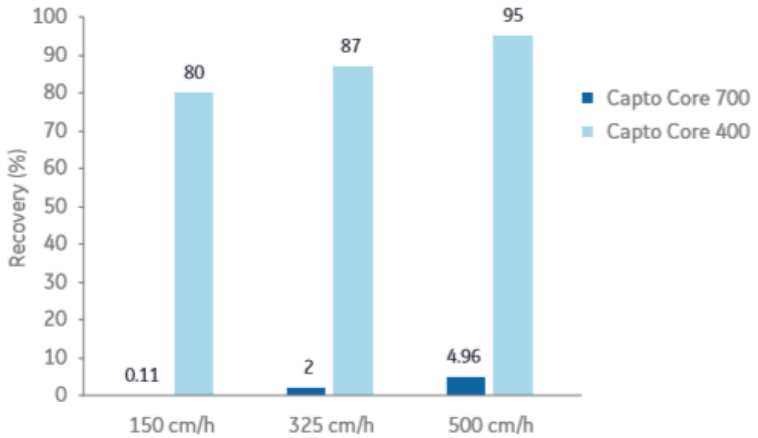
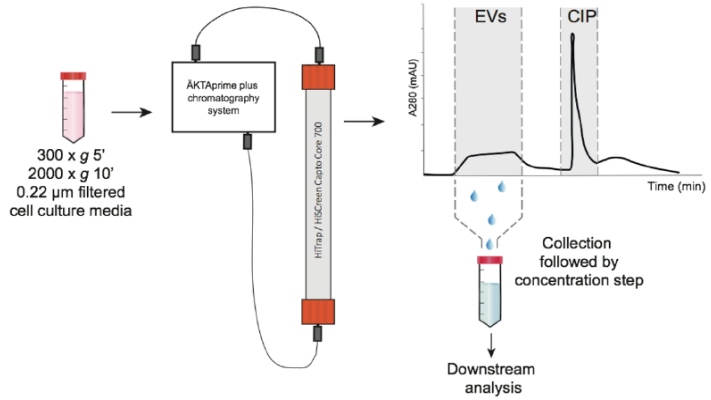


Fig 7. ELISA results from study with Capto Core 400 and Capto Core 700 on picornavirus at three different flow velocities: 150, 325, and 500 cm/h.

Core 700 or Core 400?



SCIENTIFIC REPORTS

OPEN

Reproducible and scalable purification of extracellular vesicl using combined bind-elute and si exclusion chromatography

Received: 9 May 2017
Accepted: 11 August 2017
Published online: 14 September 2017

Giulia Corso[1], Imre Mäger[2,5], Yi Lee[1], André Görgens[1,3], Jarred Bultema[4], Bernd Gie
Matthew J. A. Wood[2,6], Joel Z. Nordin[1,6] & Samir EL Andaloussi[2,6]

Extracellular vesicles (EVs) play a pivotal role in cell-to-cell communication and have been shown take part in several physiological and pathological processes. EVs have traditionally been purifie by ultracentrifugation (UC), however UC has limitations, including resulting in, operator-depend yields, EV aggregation and altered EV morphology, and moreover is time consuming. Here we s that commercially available bind-elute size exclusion chromatography (BE-SEC) columns purify with high yield (recovery ~ 80%) in a time-efficient manner compared to current methodologies. technique is reproducible and scalable, and surface marker analysis by bead-based flow cytomet revealed highly similar expression signatures compared with UC-purified samples. Furthermore, of eGFP labelled EVs in recipient cells was comparable between BE-SEC and UC samples. Hence, SEC based EV purification method represents an important methodological advance likely to fa robust and reproducible studies of EV biology and therapeutic application.

Extracellular vesicles (EVs) are nanosized cell-derived vesicles[1–3] delimited by a lipid bilayer and typicall into three subgroups, according to their biogenesis pathways; exosomes, microvesicles (MVs) and apopt ies[4]. In this article, the term EVs will refer to exosomes and MVs only. Exosomes are 70–150 nm in size: inate from the endocytic pathway[5] whereas MVs are generally larger, 100–1000 nm in diameter and bu from the plasma membrane[6,7]. They carry proteins and RNAs, both miRNAs and mRNAs, and have be to transfer their cargo to recipient cells[3,8,9]. EVs are of fundamental importance in conveying critical int messages[8,10] both in physiological and pathological processes, such as taking part in the coagulation c immune response[11–14] as well as aiding the spread of malignancies[8,15] and viral infections[16,17].

Because of their small size, physicochemical properties and the complexity of the surrounding fl fication of EVs is a great challenge. The gold standard in the field is to purify EVs by sequential centr followed by an ultracentrifuge (UC) step to pellet the EVs at 110,000 × g[18]. We and others have previou that the UC step damages the vesicles and leads to aggregation[18–20], which can ultimately affect dow analysis[21] or application of EV[18,22]. Furthermore, this technique is time consuming and prone to variat due to the diverse protocols and equipment used in different laboratories[23]. To overcome these issue other promising purification techniques have been proposed, such as precipitation kits[24,25] and size chromatography (SEC)[18,26].

In this article, we have evaluated a novel liquid chromatography technique for EV purification: u bead chromatography. The technology combines both size separation with bind-elute chromatography (where large molecules bypass these beads while molecules smaller than 700 kDa penetrate the inert o and bind to hydrophobic and positively charged octylamine ligands within the core. We hypothesise

[1]Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden. [2]Department of Ph Anatomy and Genetics, University of Oxford, Oxford, United Kingdom. [3]Institute for Transfusion Medicine, U Hospital Essen, University of Duisburg-Essen, Essen, Germany. [4]Department of Chemistry and Bioc University of Colorado, Colorado Springs, USA. [5]Institute of Technology, University of Tartu, Tartu, [6]Evox Therapeutics, King Charles House, Park End Street, Oxford, United Kingdom. Giulia Corso and Im contributed equally to this work. Correspondence and requests for materials should be addressed to J.Z.N joel.nordin@ki.se) or S.E.A. (email: samir.el-andaloussi@ki.se)

Journal of Extracellular Vesicles

ISSN: (Print) 2001-3078 (Online) Journal homepage: http://www.tandfonline.com/loi/zjev20

Large-scale, cross-flow based isolation of highly pure and endocytosis-competent extracellular vesicles

Ryan P. McNamara, Carolina P. Caro-Vegas, Lindsey M. Costantini, Justin T. Landis, Jack D. Griffith, Blossom A. Damania & Dirk P. Dittmer

To cite this article: Ryan P. McNamara, Carolina P. Caro-Vegas, Lindsey M. Costantini, Justin T. Landis, Jack D. Griffith, Blossom A. Damania & Dirk P. Dittmer (2018) Large-scale, cross-flow based isolation of highly pure and endocytosis-competent extracellular vesicles, Journal of Extracellular Vesicles, 7:1, 1541396, DOI: 10.1080/20013078.2018.1541396

To link to this article: https://doi.org/10.1080/20013078.2018.1541396

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Separation of CHO-derived vesicles through multimodal and heparin affinity chromatography

TÉCNICO LISBOA

Ana Francisca Dias Gomes C
Instituto Superior Técnico, Lisbon, Octob

Extracellular vesicles comprise a heterogeneous mixture including exosomes and microvesicles and they are m intercellular communication. Their role in a cell culture process for production of a monoclonal antibody is not clear, but a major process-related impurity. In a CHO-S strain producing trastuzumab, 2.2x10[11] particles/mL with size of 30-300 r detected after centrifugation of the broth. A downstream process was then applied to separate different types of extr vesicles from this supernatant using a 2-step chromatography. The first multimodal purification ensured 49.3% particle r in the flow through and capture of smaller impurities. Discrimination between populations was achieved using heparir chromatography with overall recovery of 45.8%. Two different populations of extracellular vesicles were separated (74.1 flow through and 25.9% in the elution). Typical exosome markers did not allow classification according to esti denominations. This chromatographic method is a step towards a better understanding of extracellular vesicles, being an alternative to common separation techniques. Identification of diverse protein signatures in the different populations conf complexity of these vesicles and it is shown that their heterogeneity and amount depend on cell type, time of harvest stress factors.

Keywords: extracellular vesicles, exosomes, microvesicles, multimodal chromatography, heparin affinity chromatc purification, separation, exosome markers, Capto™ Core 700, Capto™ Heparin

1. Introduction

Previously, extracellular vesicles (EVs) were thought to serve only as means for carrying cellular waste. [1] However, their therapeutic potential is now being studied, since they represent a novel class of drug delivery systems [2] and also biomarkers for various diseases. [3] These and other applications are now recognized due to the biological information transported by these vesicles. EVs are membrane-enclosed vesicles released by both normal and pathologically altered cells to the extracellular space and their cargoes comprise proteins, RNA, DNA and lipids. [2, 4] By exchanging these cargoes between cells, EVs have a role in intercellular communication and are thought to mirror information of their secreting cells. [3] Their signals are transmitted either by direct interaction between the vesicle membrane protein and the recipient membrane protein or by internalization of their content by the recipient cell, leading to functional changes in the latter. [2]

Besides coming from different cell sources, EVs heterogeneity is noticeable on size, structure and intracellular origin, which are the differences that separate them in types. **Exosomes** are known as the smallest, most characterized, homogeneous and of endosomal origin, having sizes between 30 and 120 nm. **Microvesicles** are a more heterogeneous group shedding directly from the membrane, that can go from 100 nm to 1000 nm. Other type of vesicles comprehend the ones produced from cells undergoing apoptosis, called **apoptotic bodies**, bigger than 1 µm. [2, 4, 5] Their heterogeneity and amount encountered are said to be dependent on pathological state and enviromental conditions. [6]

Search for specific protein markers is ongoing to distinguish one EV type from another and understand which vesicles are valuable. Exocarta is a compendium created to gather the molecular data (lipid, RNA and proteins) identified in exosomes. [7] A broader database is Vesiclepedia that gathers these data for different classes of EVs. [8] For exosomes, common markers are tetraspanins (e.g. CD63 and CD9), Alix and TSG101, while integrins, matrix metalloproteinases and tissue factor are markers for microvesicles. DNA content, histones and annexin V are often associated with apoptotic bodies. [2, 4, 5] However, it is unlikely that any surface marker will alone define exosomes or other EVs. Also, the markers are often expressed in other processes and not specific which is a concern when their source is a mixture of b material. Therefore, the displayed markers will depen EVs' source and the discovery of more selective ma needed for a better characterization. [9]

Concerning EVs' isolation, there are no stanc techniques proven to work across all applications, but t commonly used protocol for their purification is dif ultracentrifugation (DUC). [10] Exosomes have been the EVs research and, consequently, the isolation proto frequently directed towards enrichment of these vesicl

Most of the common isolation methods were pe successfully in different settings but do not provide a platform for efficient and scalable EV purification. In D overlap between exosomes (30-120 nm) and micro size (100-1000 nm), does not fully allow separation final pellet contains a mixed population. [11] Moreove reports have previously shown that high-speed steps damage EVs and lead to aggregation, which can aff downstream analysis or application. [12] This technique time-consuming and prone to variable results due to settings in different laboratories. Density gradients an techniques allow separation of different EV populati have pure yields, but are low throughput, time-consun provide widely variable results depending on the type studied. In ultrafiltration and precipitation-based i purity of the material is generally low and many impurities are co-isolated. It was also shown that preci based isolation has an effect on structure and compo the EVs. On the contrary, size-exclusion chromatogr not seem to alter the size and vesicular characteristics suggests that chromatographic approaches sho considered, especially if the forth coming applicatio purified EVs requires their full integrity.

Chromatography is the most employed and essential in downstream processing of biopharmaceuticals. purification of EVs, Corso *et al.* claimed a reproduci scalable process from mouse neublastoma and myob culture using Capto™ Core 700 with consistent re *circa* 80%. [12] Capto™ Core 700 is a multimodal resin in a negative mode, i.e. the product is collected in

JOURNAL OF EXTRACELLULAR VESICLES
2019, VOL. 7, 1541396
https://doi.org/10.1080/20013078.2018.1541396

Taylor & Francis

RESEARCH ARTICLE

OPEN ACCESS

Large-scale, cross-flow based isolation of highly pure and endocytosis-competent extracellular vesicles

Ryan P. McNamara, Carolina P. Caro-Vegas, Lindsey M. Costantini, Justin T. Landis, Jack D. Griffith, Blossom A. Damania and Dirk P. Dittmer

Lineberger Comprehensive Cancer Center and Department of Microbiology and Immunology, The University of North Carolina, Chapel Hill, NC, USA

ABSTRACT

Isolation of extracellular vesicles (EVs) from cell culture supernatant or plasma can be accomplished in a variety of ways. Common measures to quantify relative success are: concentration of the EVs, purity from non-EVs associated protein, size homogeneity and functionality of the final product. Here, we present an industrial-scale workflow for isolating highly pure and functional EVs using cross-flow based filtration coupled with high-molecular weight Capto Core size exclusion. Through this combination, EVs loss is kept to a minimum. It outperforms other isolation procedures based on a number of biochemical and biophysical assays. Moreover, EVs isolated through this method can be further concentrated down or directly immunopurified to obtain discreet populations of EVs. From our results, we propose that cross-flow/Capto Core isolation is a robust method of purifying highly concentrated, homogenous, and functionally active EVs from industrial-scale input volumes with few contaminants relative to other methods.

ARTICLE HISTORY
Received 18 June 2018
Revised 2 October 2018
Accepted 12 October 2018

KEYWORDS
Extracellular vesicles; industrial scale; tangential flow; cross-flow; capto core

Introduction

The role of extracellular vesicles (EVs) has received considerable attention in recent years, in particular the class of EVs with a diameter < 120 nm. MicroRNA (miRNA), messenger RNA (mRNA), long non-coding RNA (lncRNA) molecules, as well as proteins (both host and virus-encoded), lipids, and small metabolites have all been found in EVs. EVs have demonstrated roles in cancer progression, metastasis, the response to invading pathogens, and normal development and physiology, such as synaptic transmission and immune responses (reviewed in [1]). Modified EVs are under consideration as vaccine and drug delivery modalities (reviewed in [2]). This principle borrows from the successful encapsulation of the anticancer drugs doxorubicin and paclitaxel into liposomal vehicles and albumin nanoparticles, to yield the clinically approved formulations Doxil and Abraxane [3–5]. Lastly, EVs have gained popularity as disease biomarkers, and substantial efforts are underway to translate EVs profiling into a clinical modality, termed "liquid biopsy" [1,6–10].

Exosomes are distinguished from other EV because of their intracellular origin. Exosomes arise from the inward budding of endosomes into multivesicular bodies (MVB). The exosome-loaded MVB then traffic to the plasma membrane and releases the exosomes into the cell surroundings. Exosomes and other classes of EVs are eventually taken up by recipient cells and the packaged contents are unloaded. Whereas the majority of EVs are likely involved in modulating the proximal microenvironment, akin to synaptic vesicles, a significant fraction circulates systemically and can affect distant organs/tissues throughout the body [7,11–13]. EVs are present in bodily secretions such as milk, urine, semen or saliva. Despite significant progress, many of the fundamental molecular details of EVs biogenesis, uptake and trafficking remain unknown [14,15]. This represents a gap in our understanding that prevents successful applications in medicine.

Central to the study of EVs function is the ability to isolate robust quantities of EVs, reproducibly and from a variety of sources and volumes. This includes isolation under enhanced biosafety measures, e.g. in order to study the role of EVs in human immunodeficiency virus (HIV) infection [15–21]. Here, we present a workflow that improves upon this essential first step in EVs research. Cross-flow filtration (CFF)-based EVs enrichment offers several benefits compared to prior methods, and as we describe here, can be used to rapidly, continuously and automatically isolate pure populations of EVs from large volumes of fluids

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Supplemental data for this article can be accessed here.

- Exosomes

SCIENTIFIC REPORTS

OPEN

Reproducible and scalable purification of extracellular vesicles using combined bind-elute and size exclusion chromatography

Received: 9 May 2017
Accepted: 11 August 2017
Published online: 14 September 2017

Giulia Corso[1], Imre Mäger[2,5], Yi Lee[1], André Görgens[1,3], Jarred Bultema[4], Bernd Giebel[3], Matthew J. A. Wood[2,6], Joel Z. Nordin[1,6] & Samir EL Andaloussi[1,2,6]

Extracellular vesicles (EVs) play a pivotal role in cell-to-cell communication and have been shown to take part in several physiological and pathological processes. EVs have traditionally been purified by ultracentrifugation (UC), however UC has limitations, including resulting in, operator-dependant yields, EV aggregation and altered EV morphology, and moreover is time consuming. Here we show that commercially available bind-elute size exclusion chromatography (BE-SEC) columns purify EVs with high yield (recovery – 80%) in a time-efficient manner compared to current methodologies. This technique is reproducible and scalable, and surface marker analysis by bead-based flow cytometry revealed highly similar expression signatures compared with UC-purified samples. Furthermore, uptake of eGFP labelled EVs in recipient cells was comparable between BE-SEC and UC samples. Hence, the BE-SEC based EV purification method represents an important methodological advance likely to facilitate robust and reproducible studies of EV biology and therapeutic application.

Extracellular vesicles (EVs) are nanosized cell-derived vesicles[1–3] delimited by a lipid bilayer and typically divided into three subgroups, according to their biogenesis pathways; exosomes, microvesicles (MVs) and apoptotic bodies[4]. In this article, the term EVs will refer to exosomes and MVs only. Exosomes are 70–150 nm in size and originate from the endocytic pathway[5] whereas MVs are generally larger, 100–1000 nm in diameter and bud directly from the plasma membrane[6,7]. They carry proteins and RNAs, both miRNAs and mRNAs, and have been shown to transfer their cargo to recipient cells[3,8,9]. EVs are of fundamental importance in conveying critical intercellular messages[8,10] both in physiological and pathological processes, such as taking part in the coagulation cascade[11], immune response[12–14] as well as aiding the spread of malignancies[9,15] and viral infections[16,17].

Because of their small size, physicochemical properties and the complexity of the surrounding fluid, purification of EVs is a great challenge. The gold standard in the field is to purify EVs by sequential centrifugation followed by an ultracentrifuge (UC) step to pellet the EVs at 110,000 × g[18]. We and others have previously shown that the UC step damages the vesicles and leads to aggregation[18–20], which can ultimately affect downstream analysis[21] or application of EVs[19,22]. Furthermore, this technique is time consuming and prone to variable results due to the diverse protocols and equipment used in different laboratories[23]. To overcome these issues, several other promising purification techniques have been proposed, such as precipitation kits[24,25] and size exclusion chromatography (SEC)[19,26].

In this article, we have evaluated a novel liquid chromatography technique for EV purification: using core bead chromatography. The technology combines both size separation with bind-elute chromatography (BE-SEC) where large molecules bypass these beads while molecules smaller than 700 kDa penetrate the inert outer shell and bind to hydrophobic and positively charged octylamine ligands within the core. We hypothesised that the

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Harvest CM(貼壁細胞)

↓

離心去除細胞碎片

↓

0.22 μm 過濾

↓

大體積50~200 mL樣品，UF/DF(濃縮透析)，進一步去除雜質。

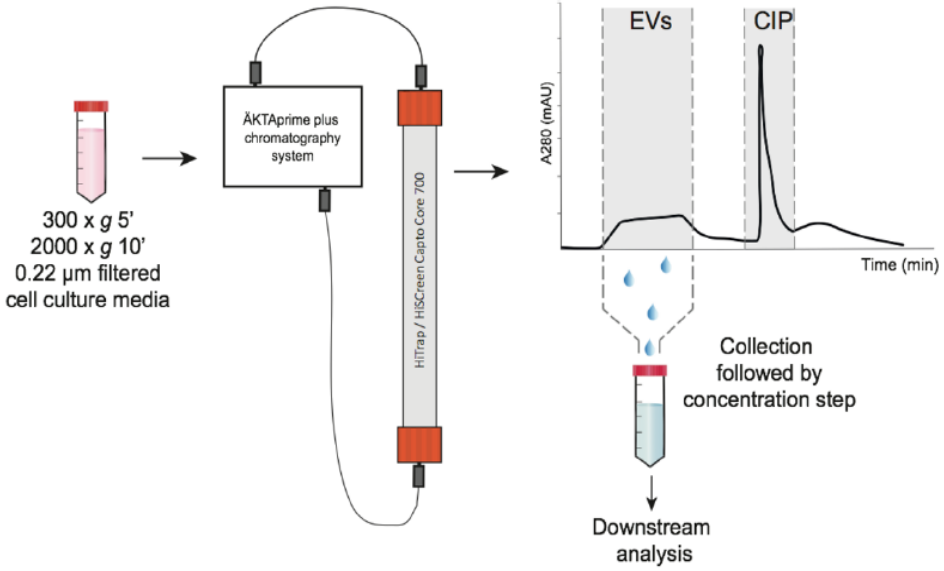
↓

管柱純化

HiScreen Capto Core 700

↓

檢測顆粒大小、完整性、濃度、纯度；蛋白Markers；標靶確認。



- Exosomes

Journal of Extracellular Vesicles

ISSN: (Print) 2001-3078 (Online) Journal homepage: http://www.tandfonline.com/loi/zjev20

Large-scale, cross-flow based isolation of highly pure and endocytosis-competent extracellular vesicles

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To cite this article: Ryan P. McNamara, Carolina P. Caro-Vegas, Lindsey M. Costantini, Justin T. Landis, Jack D. Griffith, Blossom A. Damania & Dirk P. Dittmer (2018) Large-scale, cross-flow based isolation of highly pure and endocytosis-competent extracellular vesicles, Journal of Extracellular Vesicles, 7:1, 1541396, DOI: 10.1080/20013078.2018.1541396

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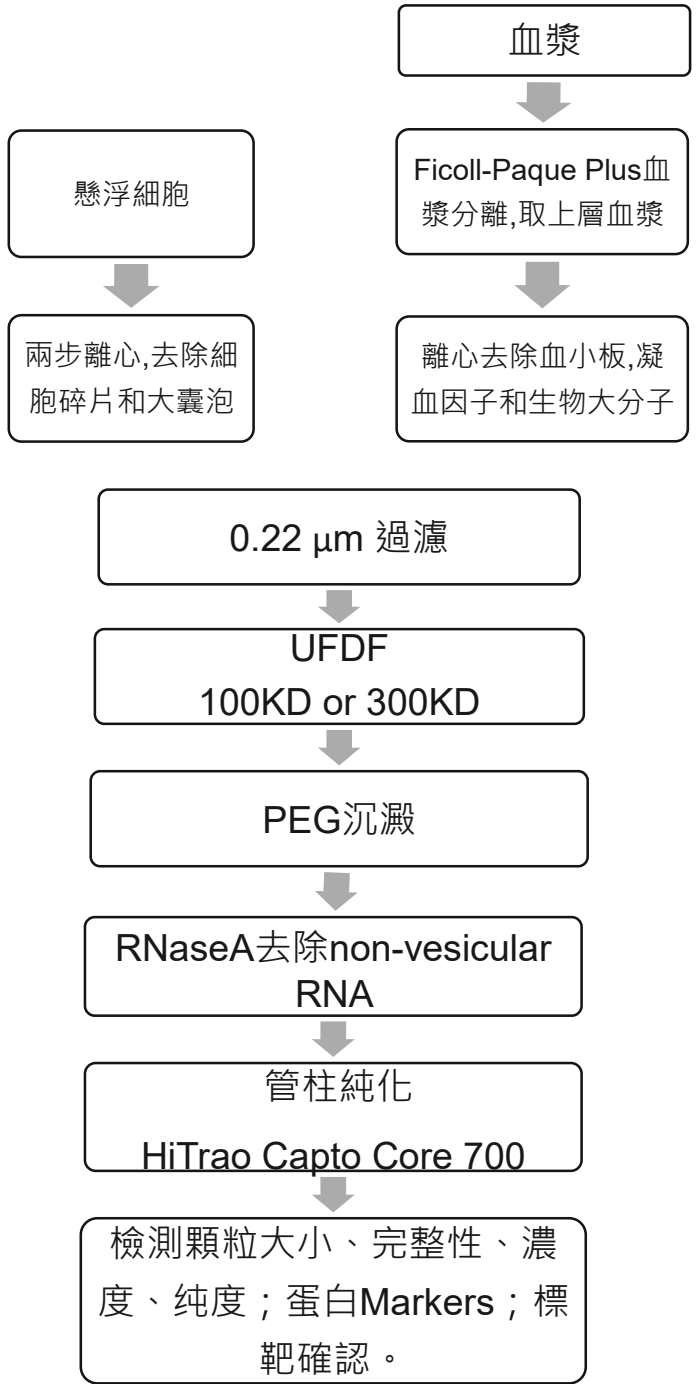
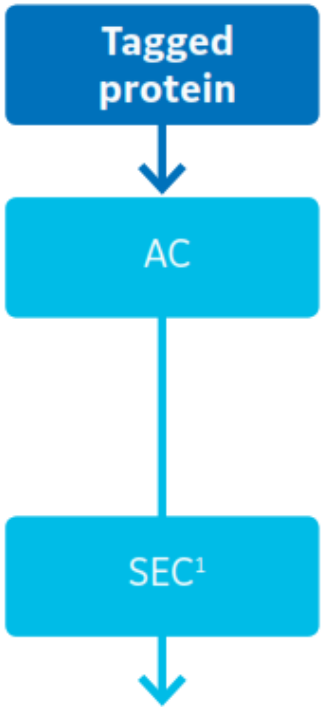
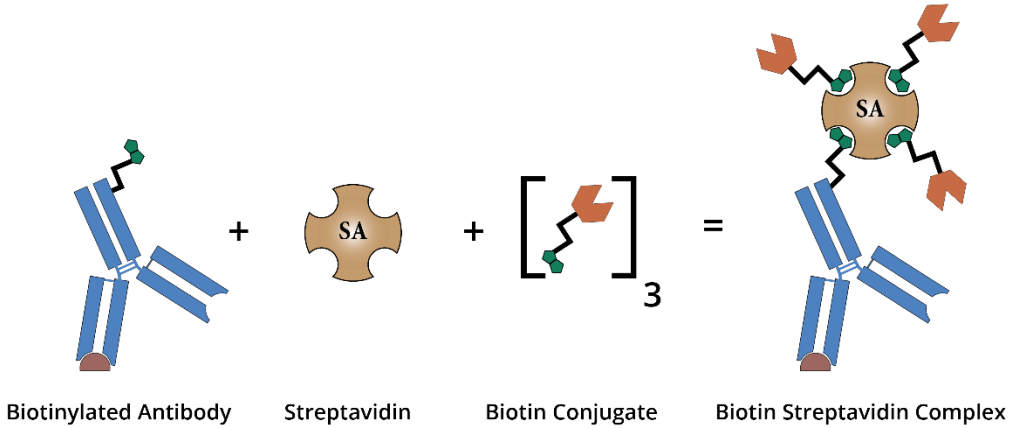
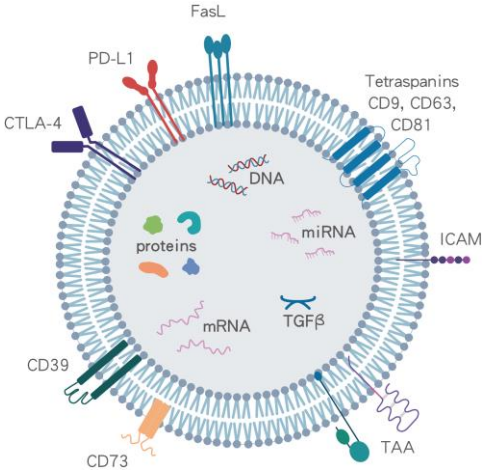
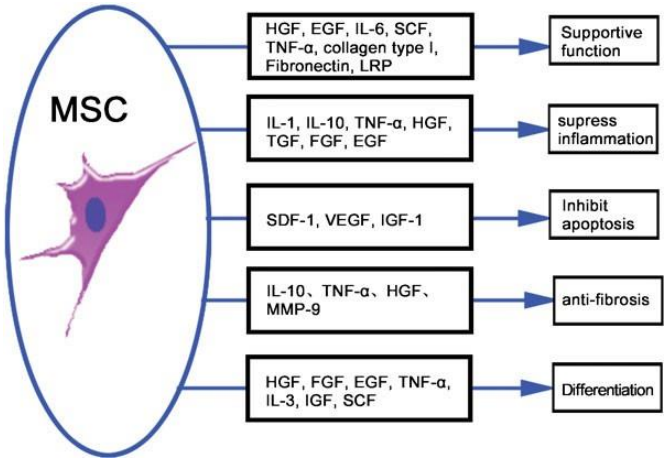


ÄKTA™ system provides efficient purification of your protein

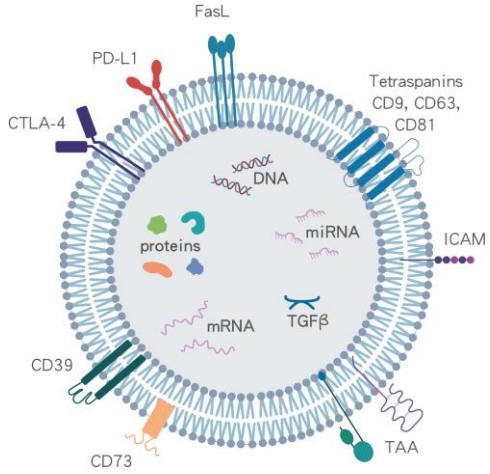


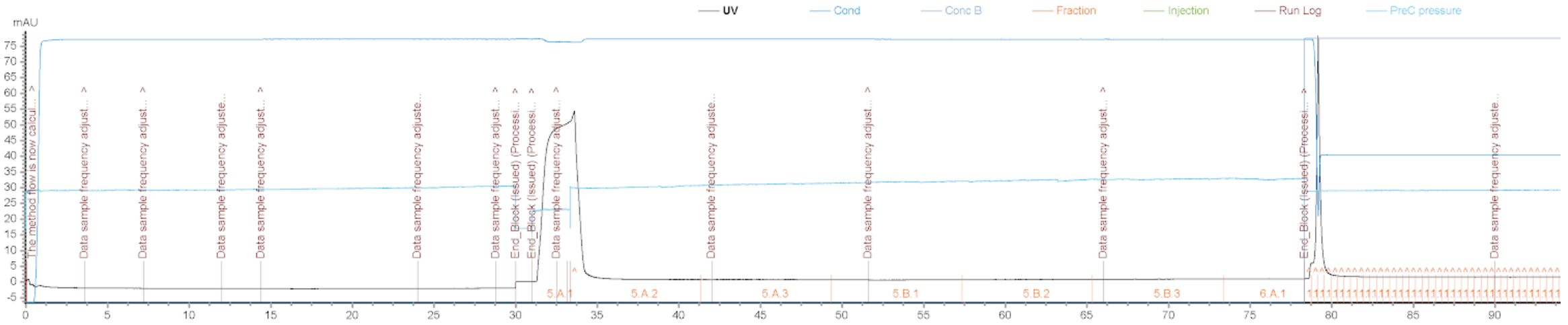






ÄKTA™ system provides efficient purification of your protein





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

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

1982—FPLC™ system released, predecessor to ÄKTA systems

2019—ÄKTA™ go : provides efficient purification of your target protein

- **2012** - ÄKTA pure: protein purification your way
- **2015 upgrade**

1996—ÄKTAexplorer™, single platform for all chromatography techniques

New Generation of ÄKTA System

New System

Transition from manual to automated purification
Education in protein purification

ÄKTA™ start

Achieve desired purity with ease in routine purifications
Make the most of valuable bench/cold room space

ÄKTA™ go

Flexibility in research
Match most current and future purification challenges

ÄKTA™ pure

Productivity in process development
Fast and secure development of purification processes

ÄKTA™ avant

Functions & Price

Classic System

ÄKTAprime™

ÄKTApurifier™ UPC

ÄKTAFPLC™

ÄKTApurifier™

ÄKTAexplorer™

ÄKTA™
Productivity in process development

Flexibility in research
Match most current and future purification challenges

ÄKTA™ pure

Productivity in process development
Fast and secure development of purification processes

ÄKTA™ avant

Prepare for GMP environment.
Smaller GMP batches for early clinical studies Scale-up and robustness testing

ÄKTA pilot 600

ÄKTAprocess is a versatile platform providing thousands of configuration possibilities. The system is available in three flow rate ranges that extend up to 2000 L/h.

ÄKTA process

Functions & Price & Scale-up

Hitrap

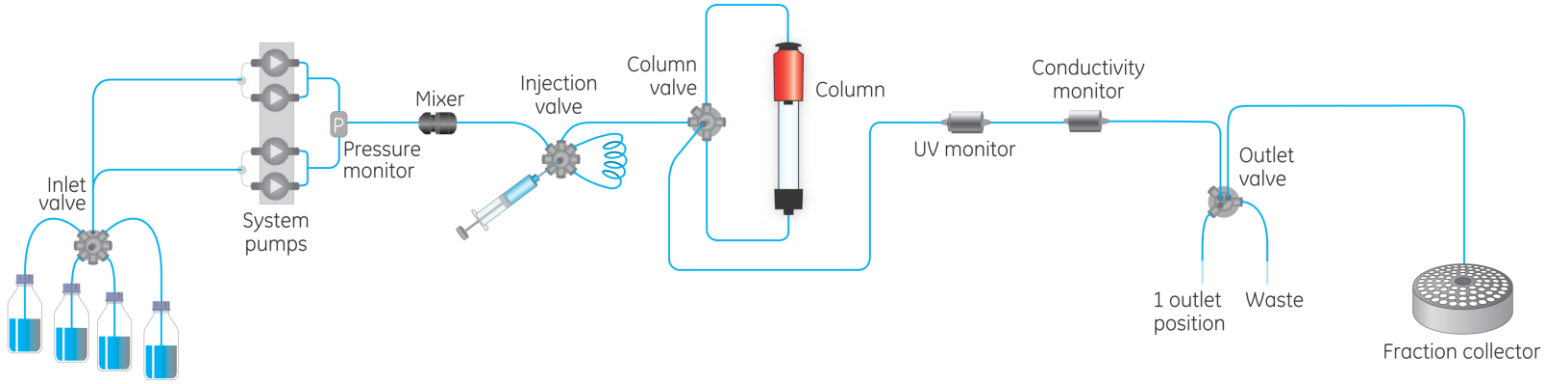
HiScreen

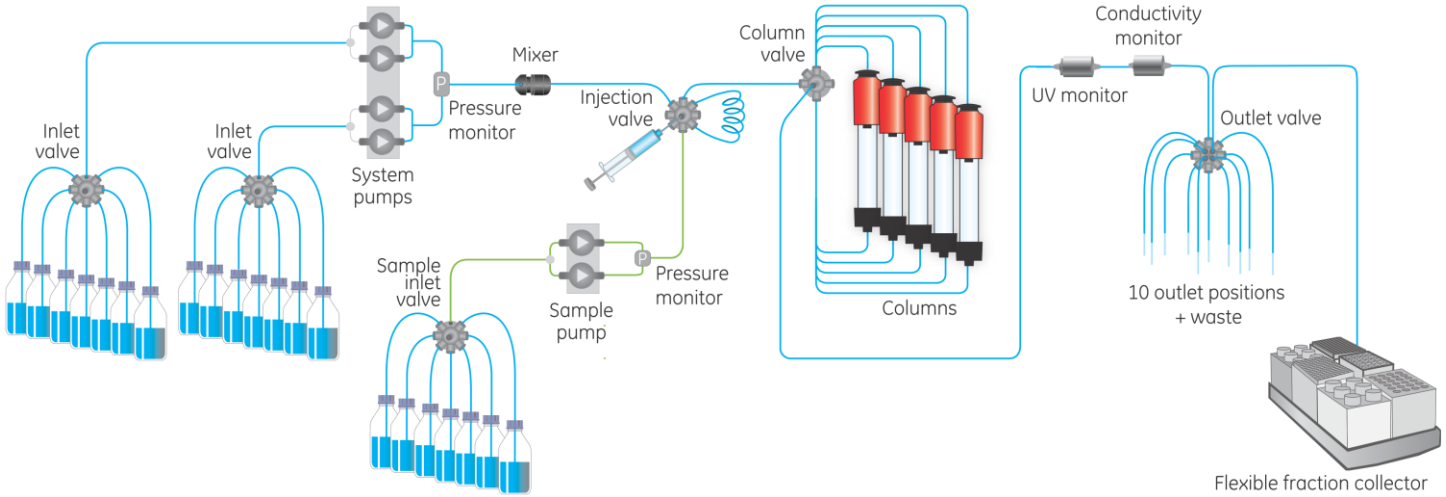
XK/Hiscal e

BPG

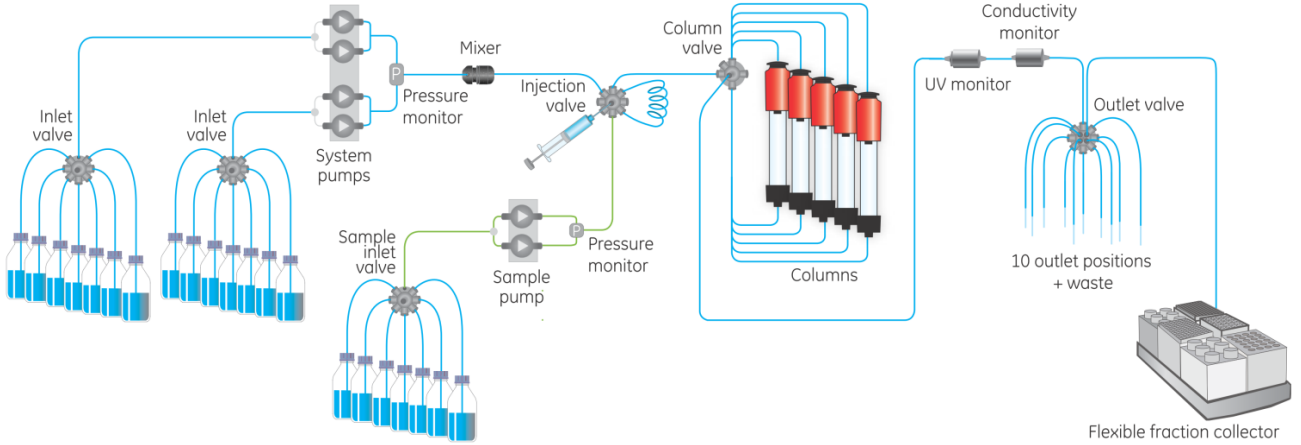
AxiChrom

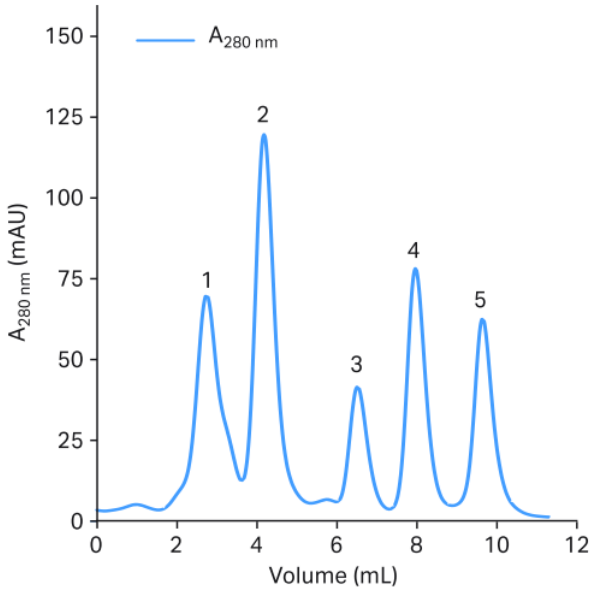
ÄKTA Pure

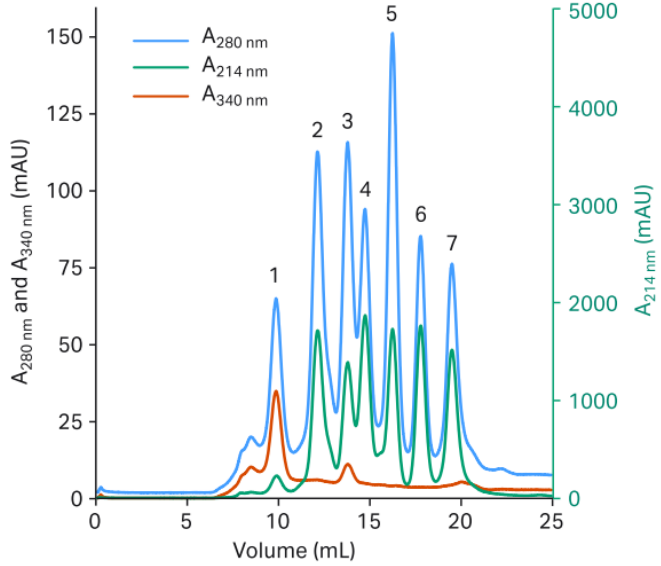

Inlet valve
System pumps
Pressure monitor
Mixer
Injection valve
Column valve
Column
UV monitor
Conductivity monitor
Outlet valve
1 outlet position
Waste
Fraction collector

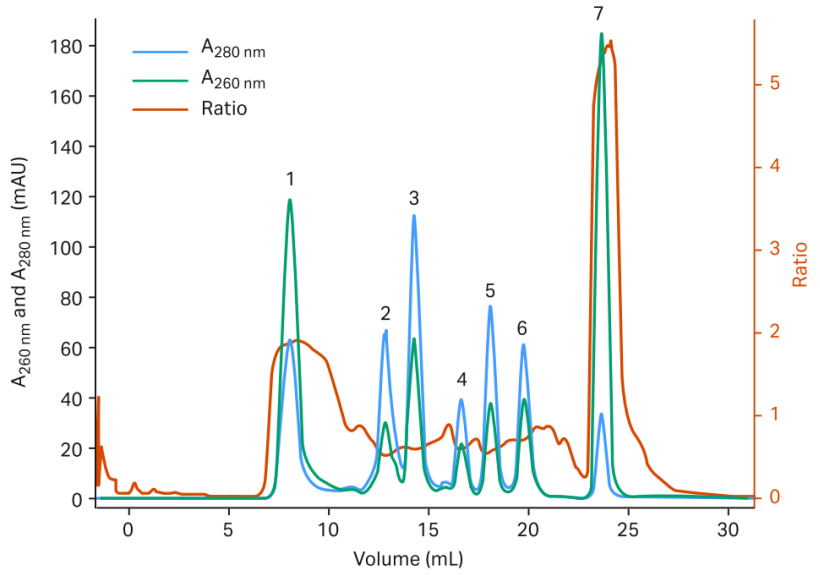

Inlet valve
Inlet valve
System pumps
Pressure monitor
Mixer
Injection valve
Column valve
Columns
Conductivity monitor
UV monitor
Outlet valve
10 outlet positions + waste
Sample inlet valve
Sample pump
Pressure monitor
Flexible fraction collector

ÄKTA Pure


Inlet valve
Inlet valve
System pumps
Mixer
Pressure monitor
Injection valve
Column valve
Columns
Conductivity monitor
UV monitor
Outlet valve
10 outlet positions + waste
Flexible fraction collector
Sample inlet valve
Sample pump
Pressure monitor

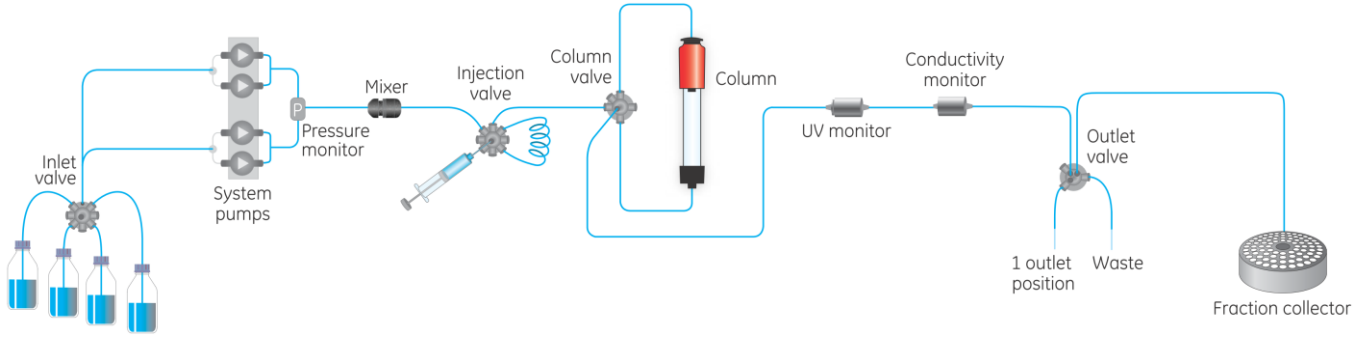

A280 nm
A280 nm (mAU)
Volume (mL)

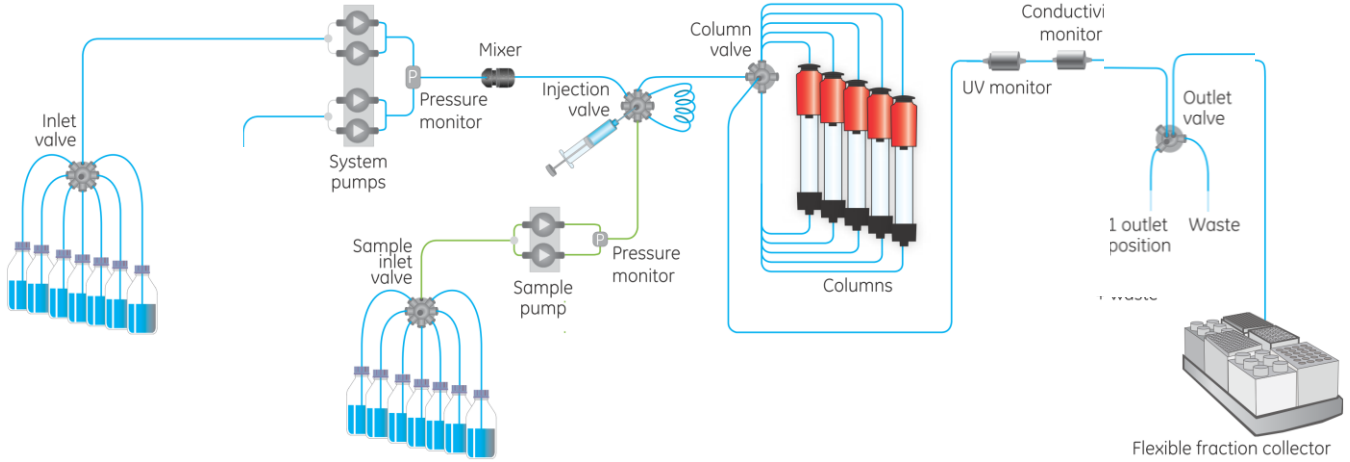

A280 nm
A214 nm
A340 nm
A280 nm and A340 nm (mAU)
A214 nm (mAU)
Volume (mL)


A280 nm
A260 nm
Ratio
A260 nm and A280 nm (mAU)
Ratio
Volume (mL)

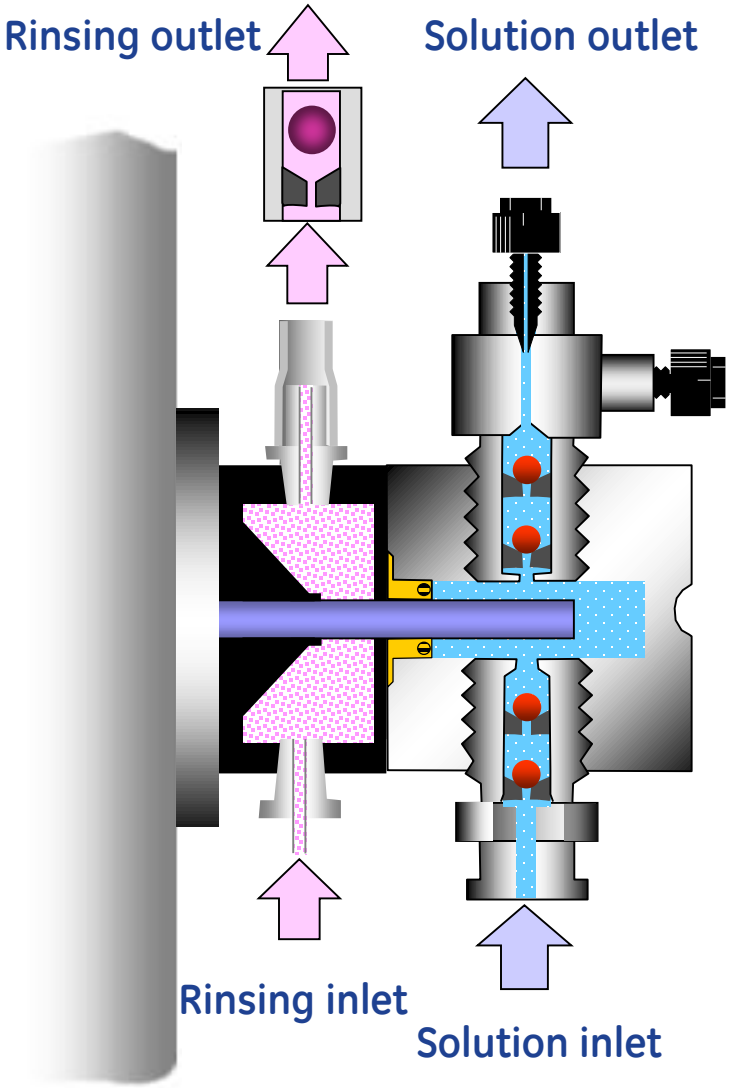
ÄKTA™

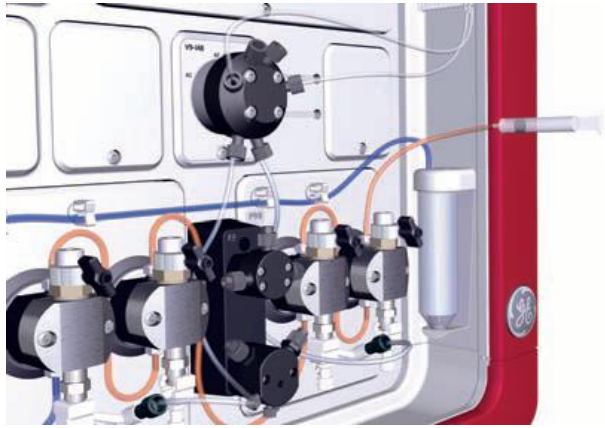
ÄKTA 機型	流速範圍	耐壓	應用	軟體
Avant & Pure 25	0.001-25 ml/min	20 MPa	分析到製備級	UNICORN 7.3





System Pump Design



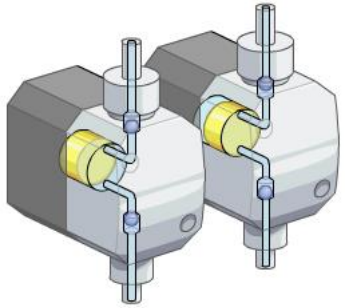


Pump head rinsing system

Function：

--Rinse piston；

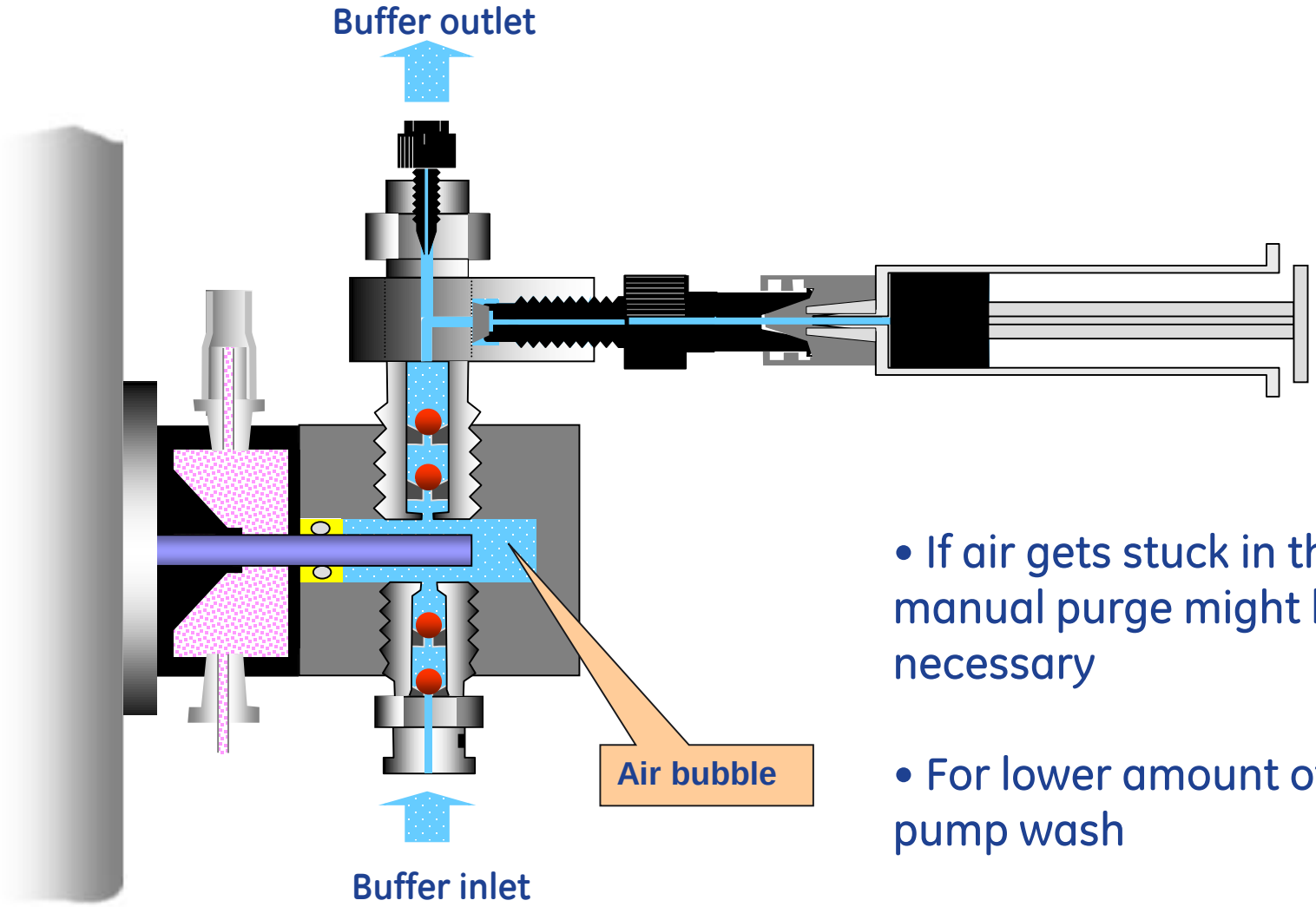
--Rinse out buffer salt；

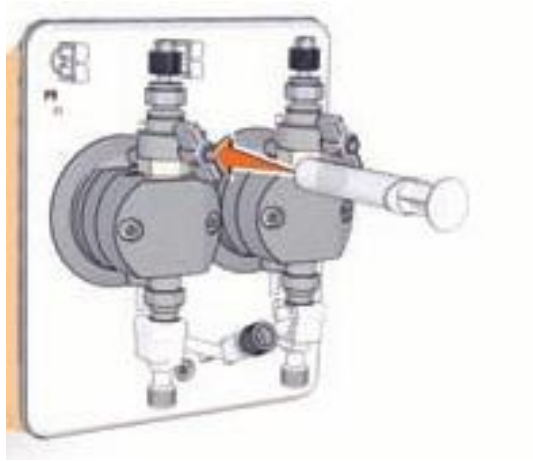


Maintenance： 20% EtOH

（change rinsing solution every week）

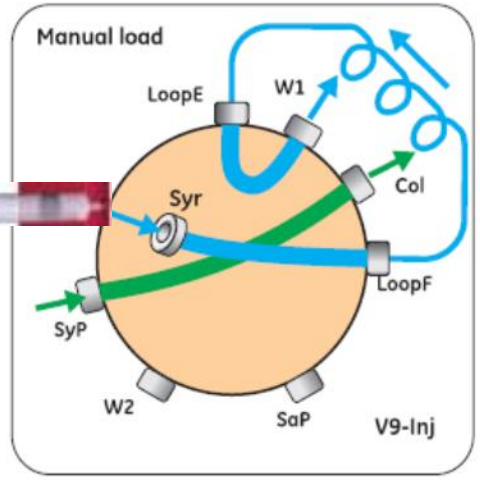
System Pump Design

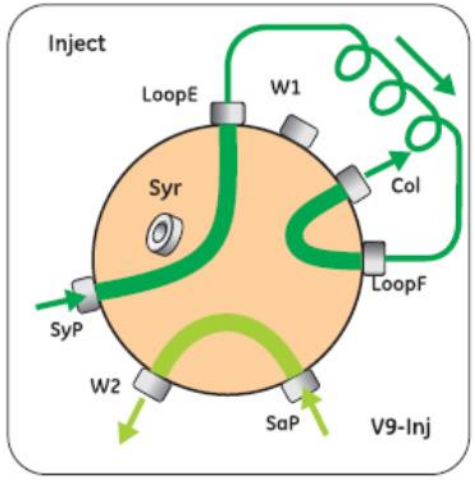


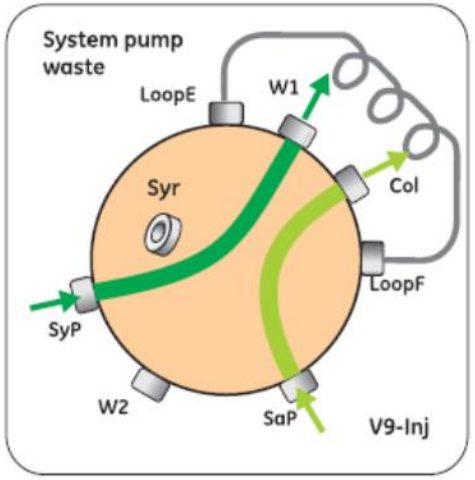


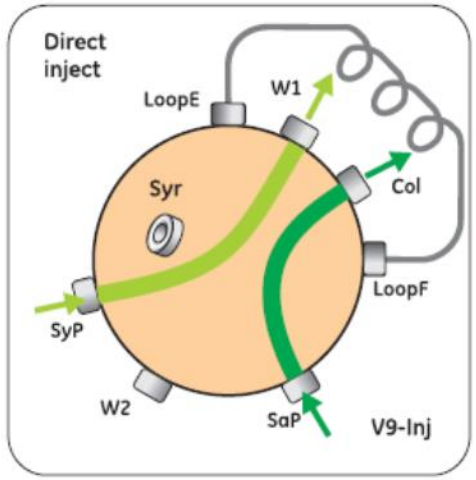
- If air gets stuck in the pump, manual purge might be necessary
- For lower amount of air, use pump wash

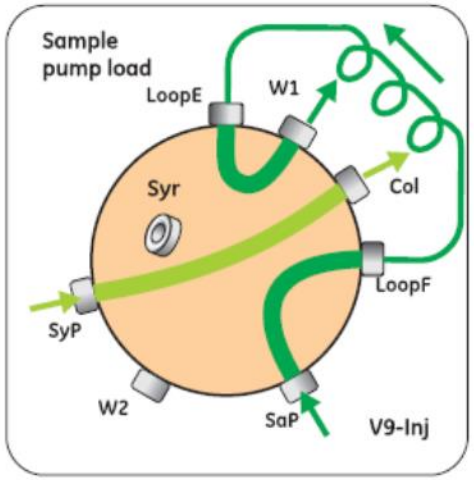
Injection Valve

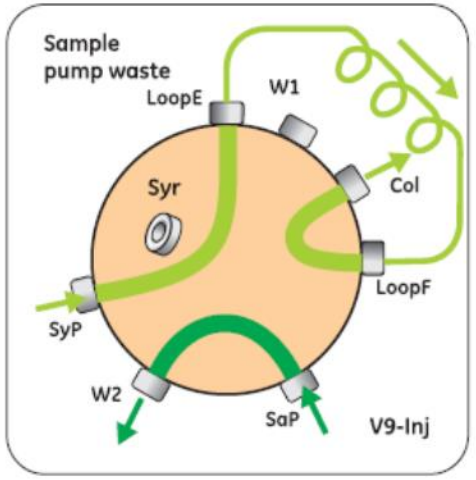






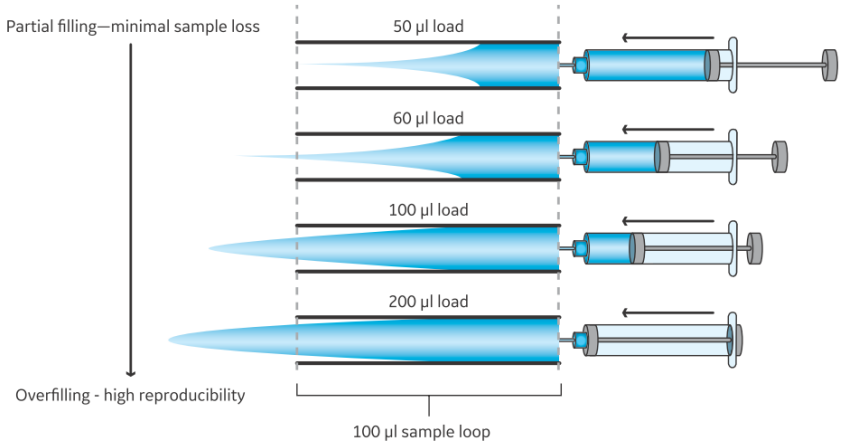




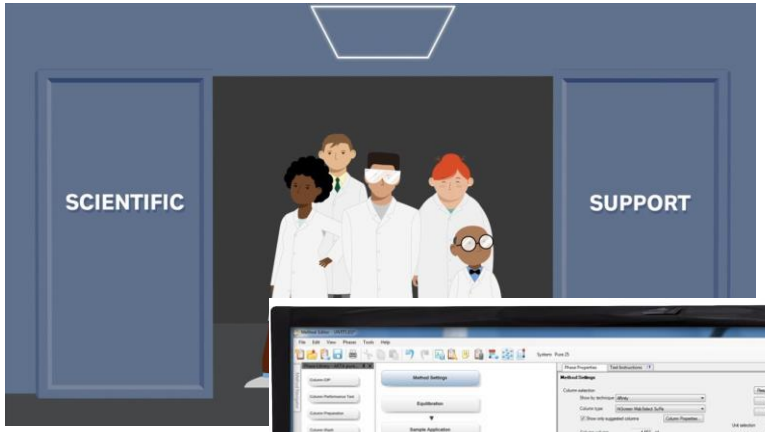


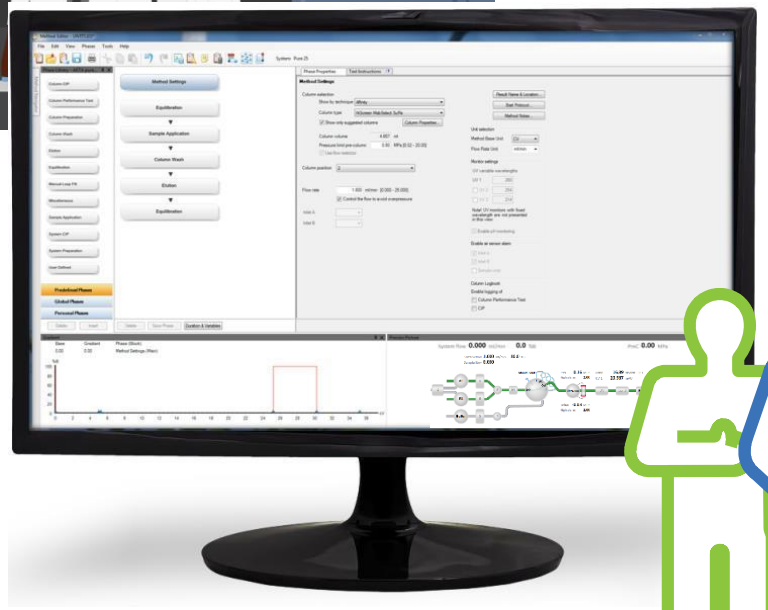
Injection Valve-Loop

- Sample loop- 10µL, 100µL, 500µL, 1mL, 2mL,5mL
- Superloop- 10mL, 50mL, 150mL



ÄKTA™
Productivity in process developmer

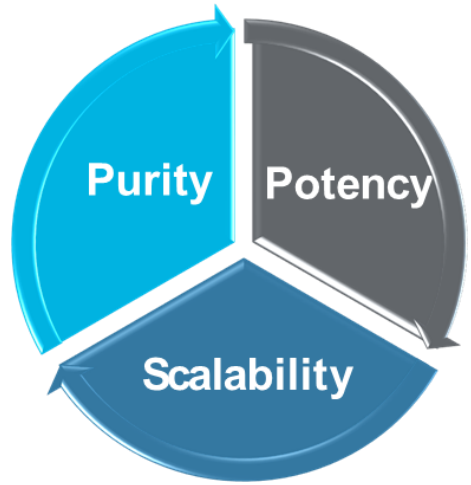




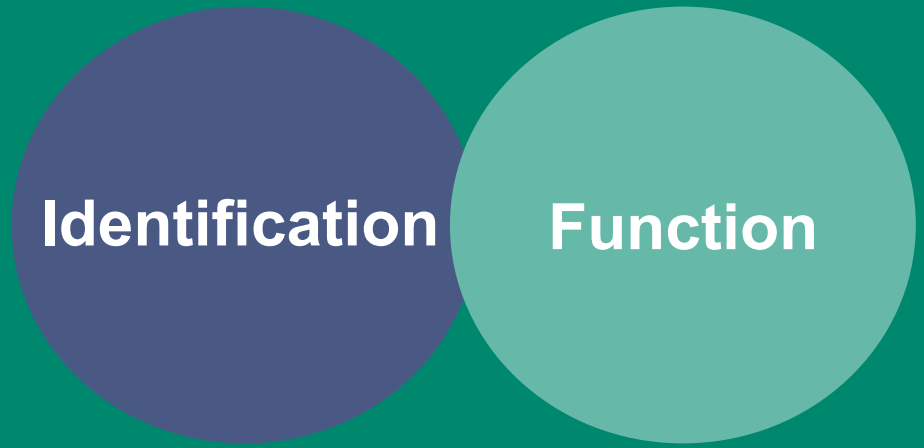
Standardization

Automation

Documentation



Analyze, Qualitative, Quantitative



Litesizer 500

Dimensions (mm):
D460 x W485 x H135

Weight (kg): 18 kg

Anton Paar

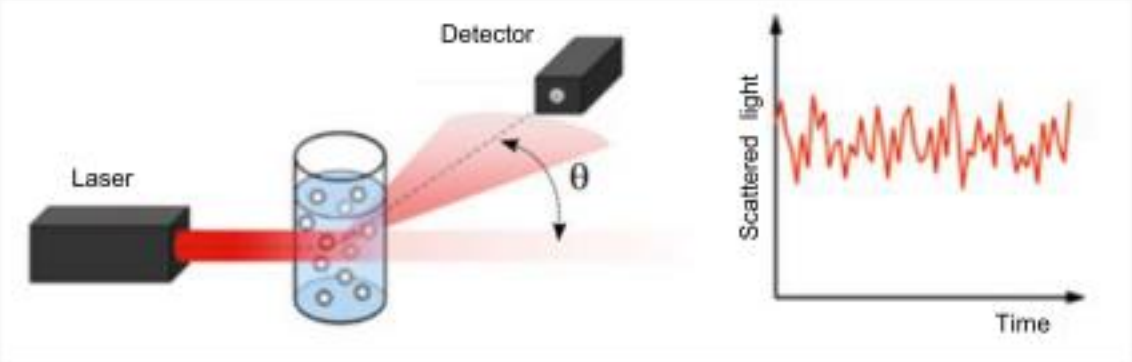
Measurement Mode	Method	Highlights & Specification
Particle Size & Particle Size Distribution **粒徑分佈量測**	Dynamic Light Scattering (DLS) **動態光散射射分析**	• 3 detection angles with automatic angle selection • Measurement range: 0.3 nm – 10 µm • Min. sample volume: 12 µL
Zeta Potential **界達電位量測**	Electrophoretic Light Scattering (ELS) **電泳光散射射分析**	• signal processing via patented cmPALS (patented) • Anton Paar Omega cuvettes (patented) • Measurement range: ± 1000 mV
Molecular Mass **分子量量測**	Static Light Scattering (SLS) **靜態光散射射分析**	• Measurement range: 980 Da – 20 MDa • Measuring range (particle size): up to 40 nm
Transmittance **穿透率量測**	Light Permeability **透光率分析**	• continuous measurement for monitoring of sedimentation & aggregation • Measurement range: 1.28 - 1.50
Refractive Index **折射率量測**	Focus-dependent Scattering Intensity **Focus-dependent散射強度分析**	• required input parameter for DLS and ELS • only DLS based instrument on the market (patented)

21 CFR part 11 software: Audit trail, customizable user management, electronic or hard copy signing

Dynamic Light Scattering – DLS

Basic Setup

- Sample is contained in a cuvette
- If particles are present an incident laser light gets scattered in all directions
- **Time dependent changes of the intensity of the scattered light** are detected



DLS is also named: Photon Correlation Spectroscopy (PCS), Quasi-Elastic Light Scattering (QELS);

Litesizer 500

Dimensions (mm):
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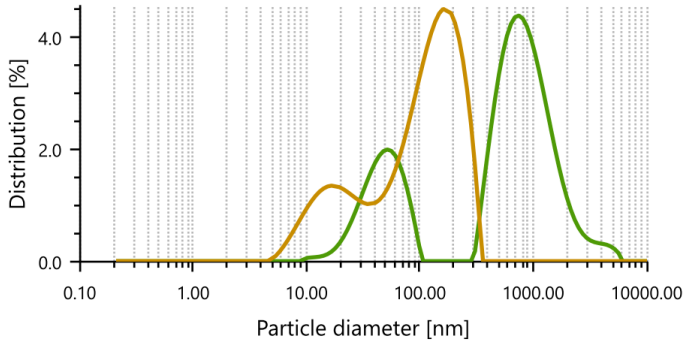
Anton Paar

Particle size distribution (intensity)



Statistics table

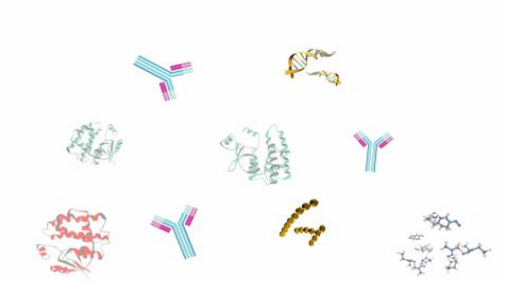
Name	Hydrodynamic diameter [nm]	Polydispersity index [%]	Peak 1 [nm]	Peak 2 [nm]	Peak 3 [nm]	Transmittance [%]	Diff. coeff. [µm²/s]
Mean value	253.8	33.8	597.2 (Intensity)	33.56 (Intensity)	- (Intensity)	0.5	3.4
Standard deviation	239.1	6.5	633.8 (Intensity)	22.01 (Intensity)	- (Intensity)	0.3	3.2
Rel. standard deviation	94.21	19.15	106.13 (Intensity)	65.60 (Intensity)	- (Intensity)	61.2	94.2

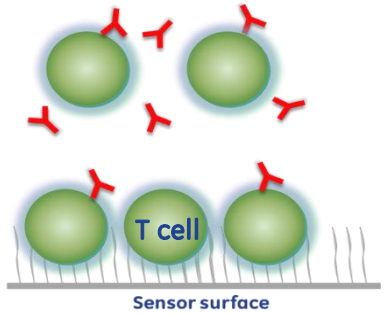
Measurements (intensity)

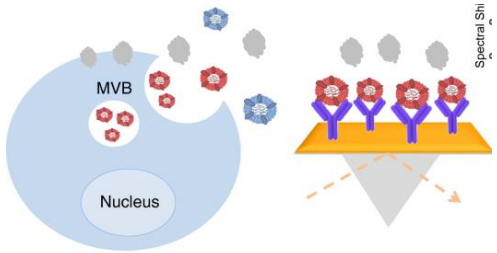
Name	Color	Hydrodyn. diam. [nm]	Polydispersity index [%]	Peak 1 [nm]	Peak 2 [nm]	Peak 3 [nm]	Transmittance [%]	Diff. coeff. [µm²/s]
PEG		422.8	38.4	1045.4 (Intensity)	49.12 (Intensity)	- (Intensity)	31.04	1.1
TFF		84.72	29.2	149.02 (Intensity)	17.99 (Intensity)	- (Intensity)	78.38	5.7

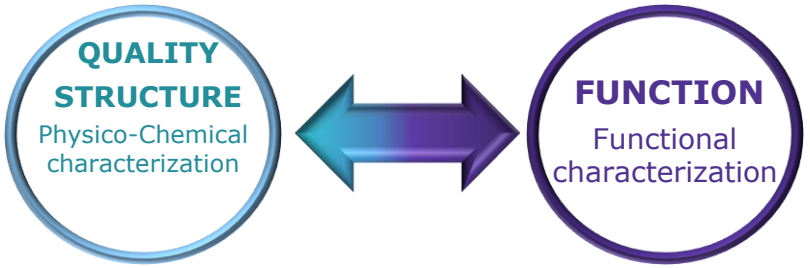
21 CFR part 11 software: Audit trail, customizable user management, electronic or hard copy signing

Function assay, Quality control



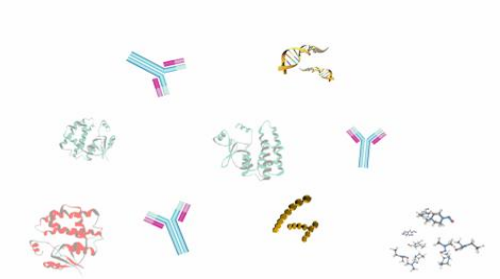


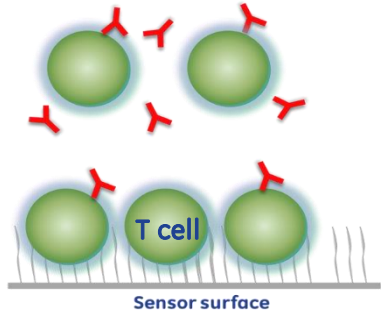


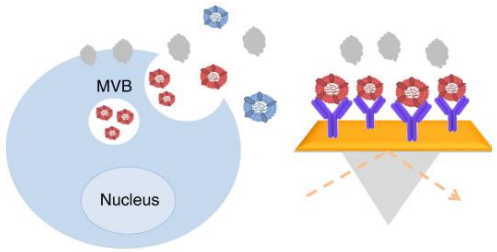


Dedicated functional analytical panels for each drug

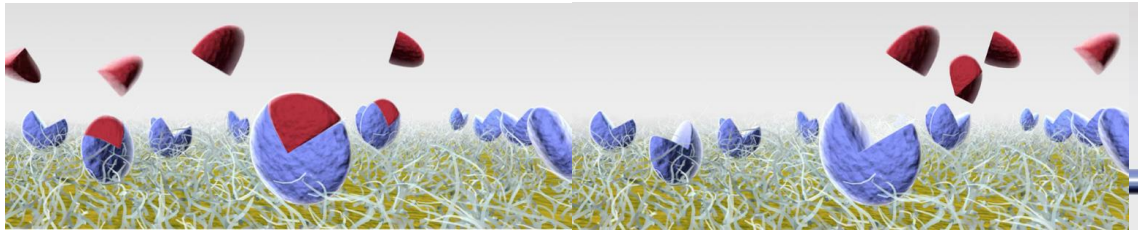
Real-time monitoring of binding events

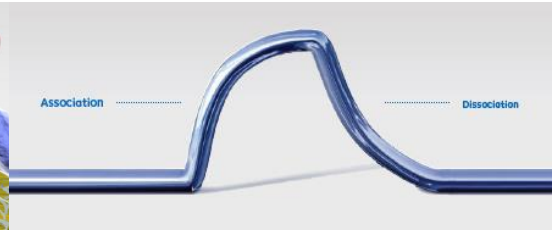






Association **Dissociation** **Sensorgram**





Antibodies
Proteins
Peptides
Compounds and fragments
Nucleic acids
Ions
Virus
Exome, Whole Cell, T Cell

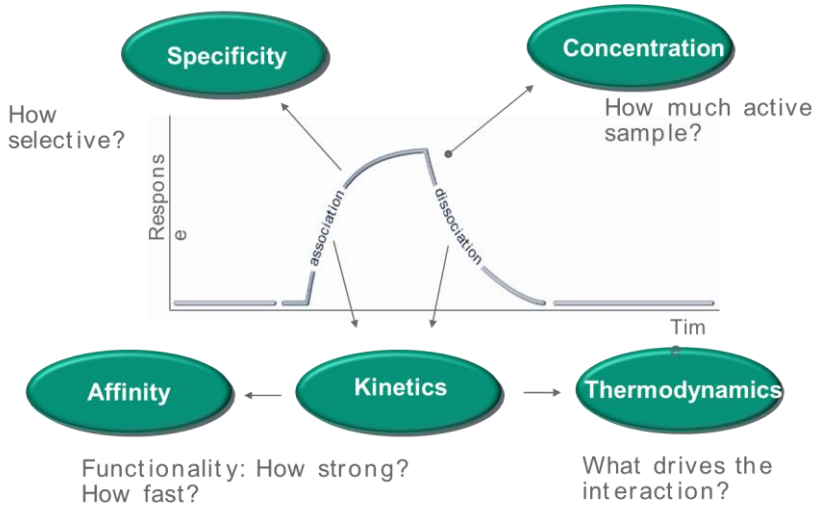
Affinity
Kinetics
Screening
Specificity
Concentration
Epitope binning
Immunogenicity
Thermodynamics

Biacore

Screening

Characterization

Yes/No Binding → **Kinetic Screen** → Epitope binning → Kinetic analysis → Active conc.



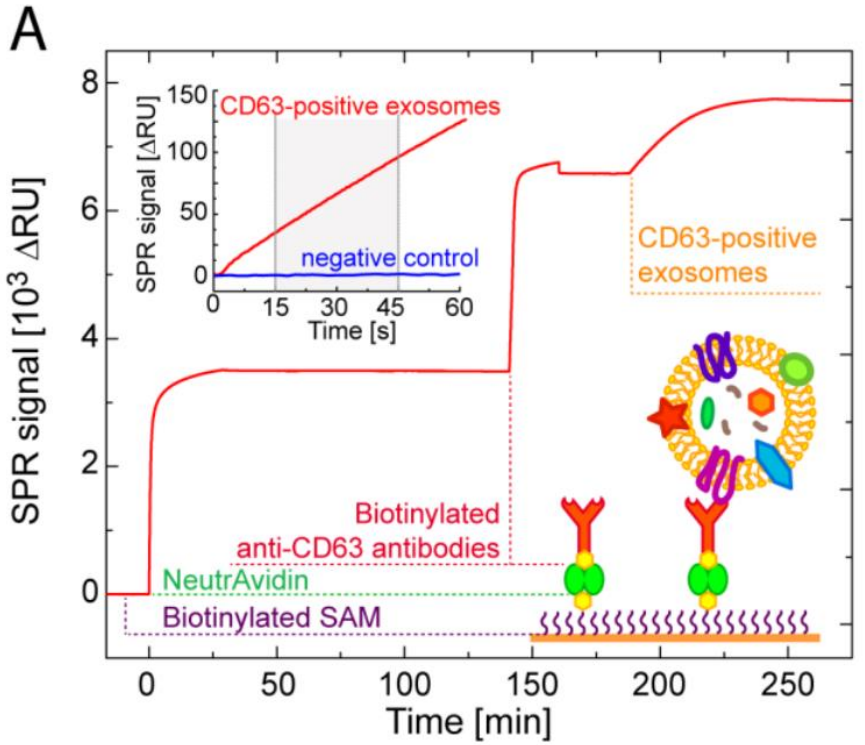
analytical chemistry

Article

Determination of exosome concentration in solution using surface plasmon resonance spectroscopy

Deborah L. M. Rupert, Cecilia Lässer, Maria Eldh, Stephan Block, Vladimir P. Zhdanov, Jan Lotvall, Marta Bally, and Fredrik Höök

Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/ac500931f • Publication Date (Web): 22 May 2014



https://pubs.acs.org/doi/10.1021/ac500931f

Biacore™ 1 series
Our next generation one needle SPR systems

Biacore™ 1K

Your research — streamlined

Upgradeable

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Your development — advanced

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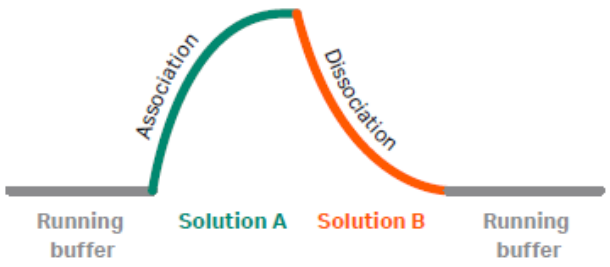
Your discovery — elevated

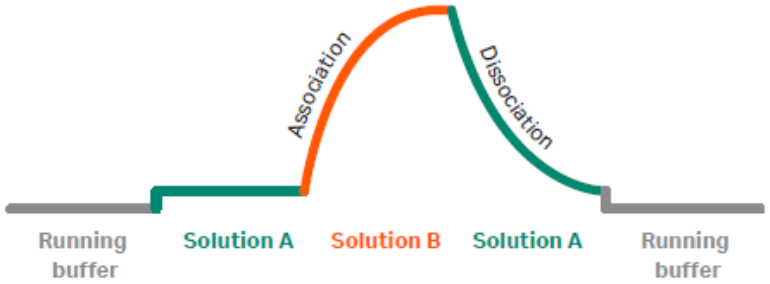
Biacore™ 1 series — three system configurations

Expand application versatility — more injection tools developed

Dual injection



ABA injection



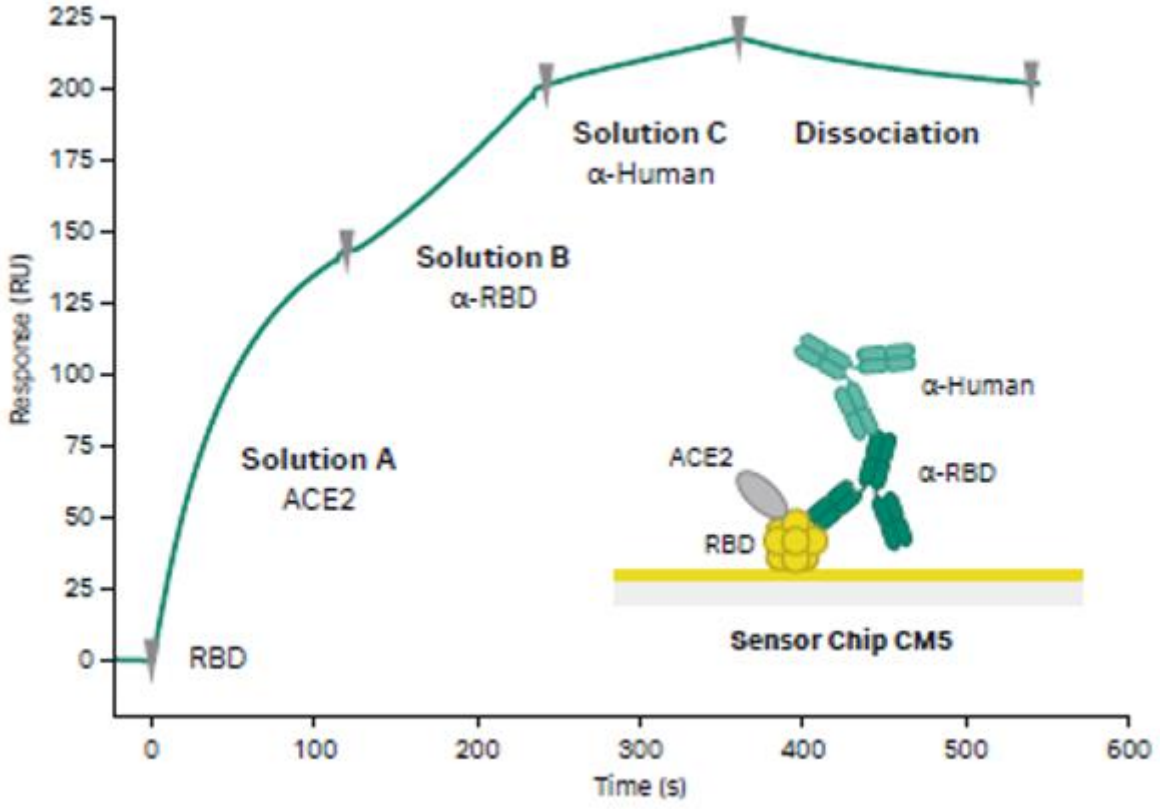
Poly injection (3- 5 injections)

Faster buffer screening, and enables competition assays and multi-complex analysis in a novel way

Build Covid protein complex using ***Poly*** injections

Example:

Three ***Poly*** injections to study Covid proteins complex formation




cytiva