Separations in Pharmaceutical Biotechnology

BMGE KKFT. February 21, 2019

„BIOTECHNOLOGY” BY KÁROLY EREKY (1919)

Biotechnologie

der Fleisch-, Fett- und Milcherzeugung

im

landwirtschaftlichen Großbetriebe

für

naturwissenschaftlich gebildete Landwirte

verfaßt

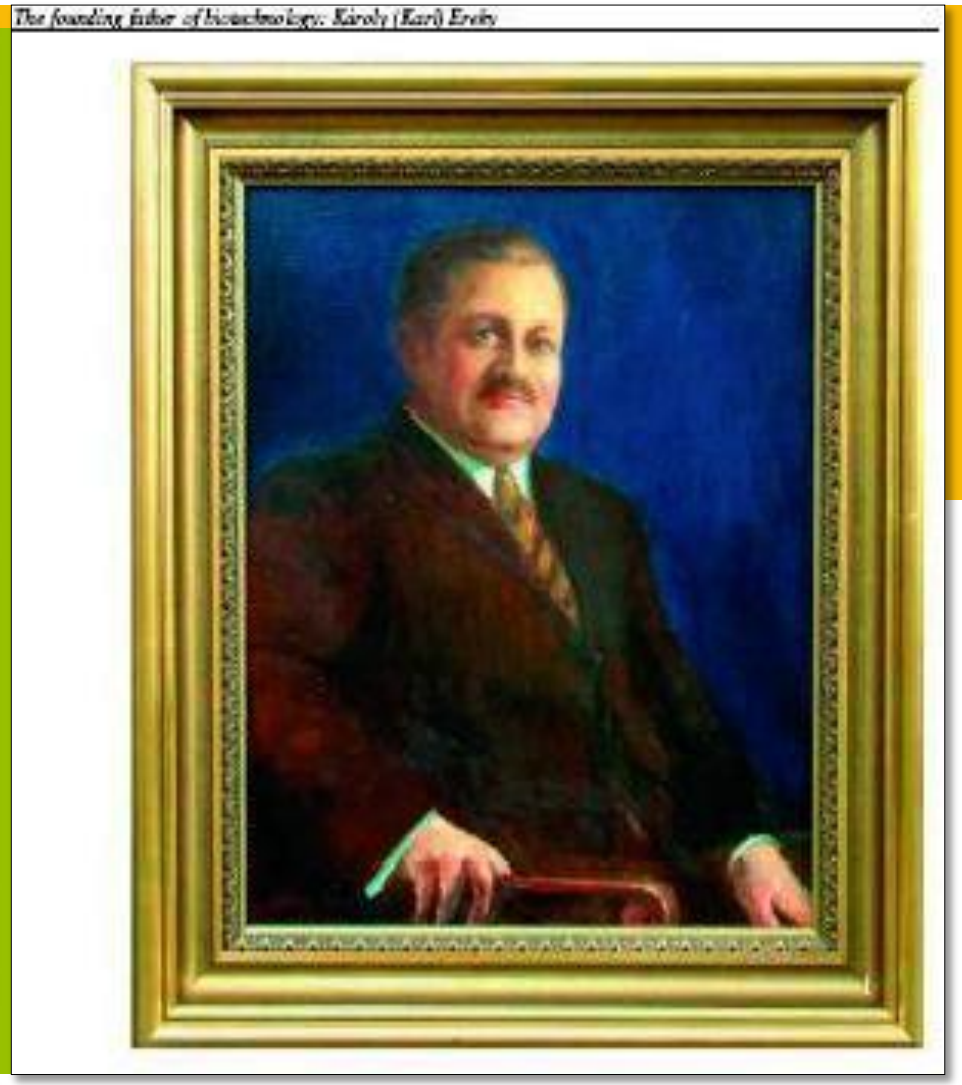
von

Dipl. Ing. Karl Ereky

Direktor der Viehverwertungsgenossenschaft ungarischer Großgrundbesitzer, Budapest.

VERLAGSBUCHHANDLUNG PAUL PAREY.

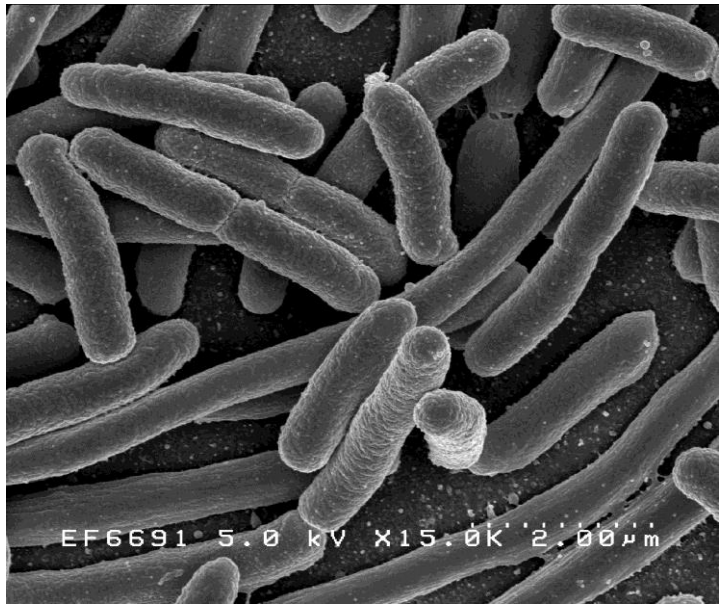
1919.



The founding father of biotechnology: Károly (Karl) Ereky

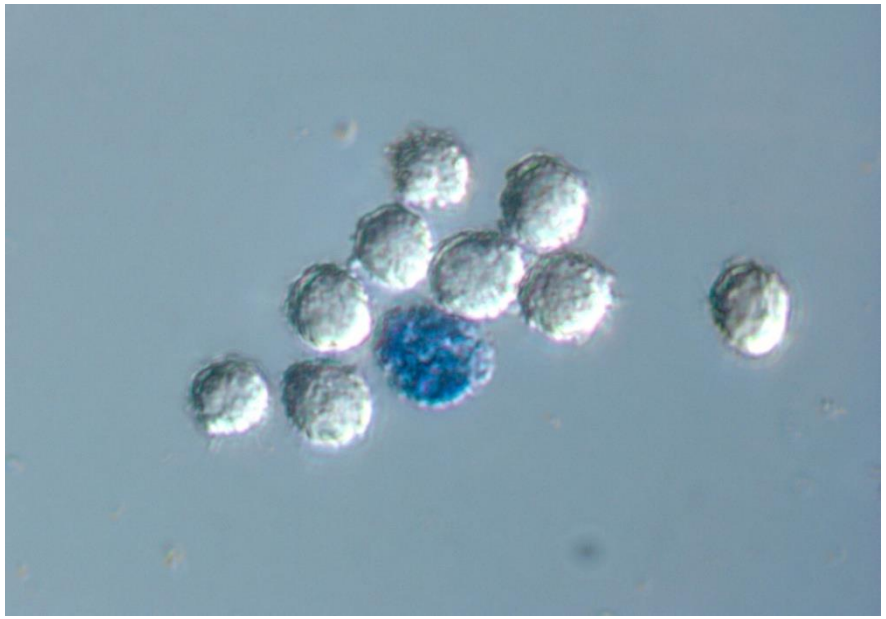
Generally applied host cells

E. coli



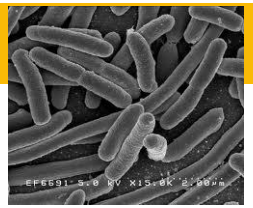
2 µm

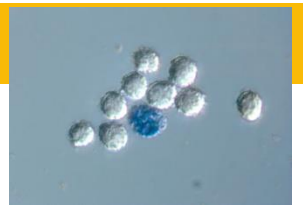
CHO

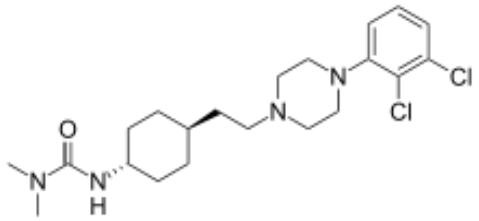


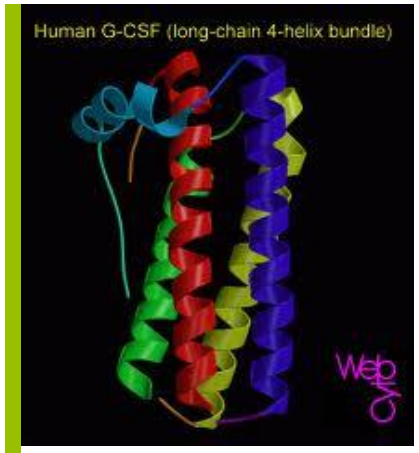
20 µm

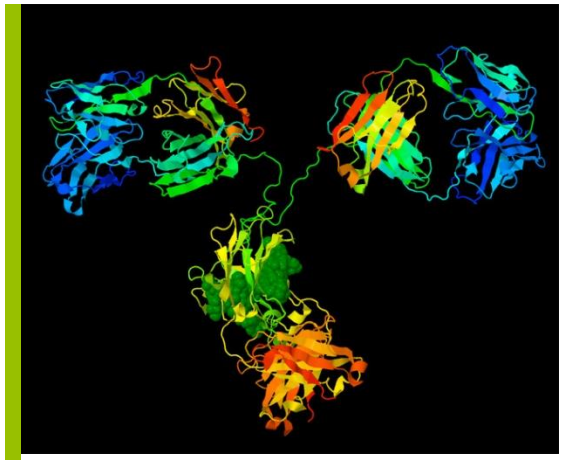
TYPICAL DRUG SUBSTANCE MOLECULES











Cariprazine
(RGH-188)
29 atoms

GCSF
20 kDa
~3000 atoms

Rituximab
150 kDa
~25000 atoms

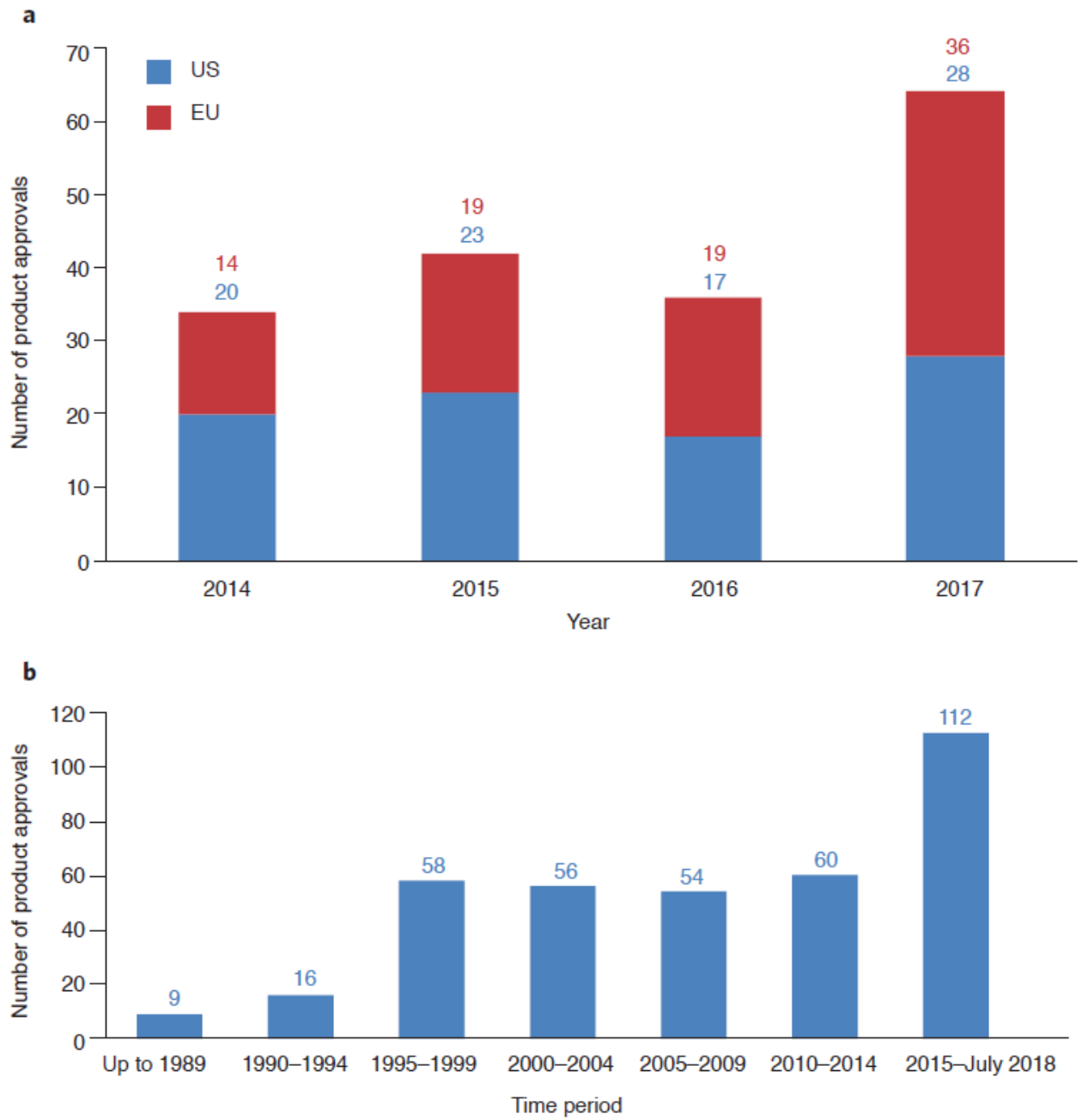


Figure 1 Product approvals profile. (**a**) Annual product approval numbers (by product trade name) by individual region. (**b**) Number of product approvals in one or both regions over the indicated periods.

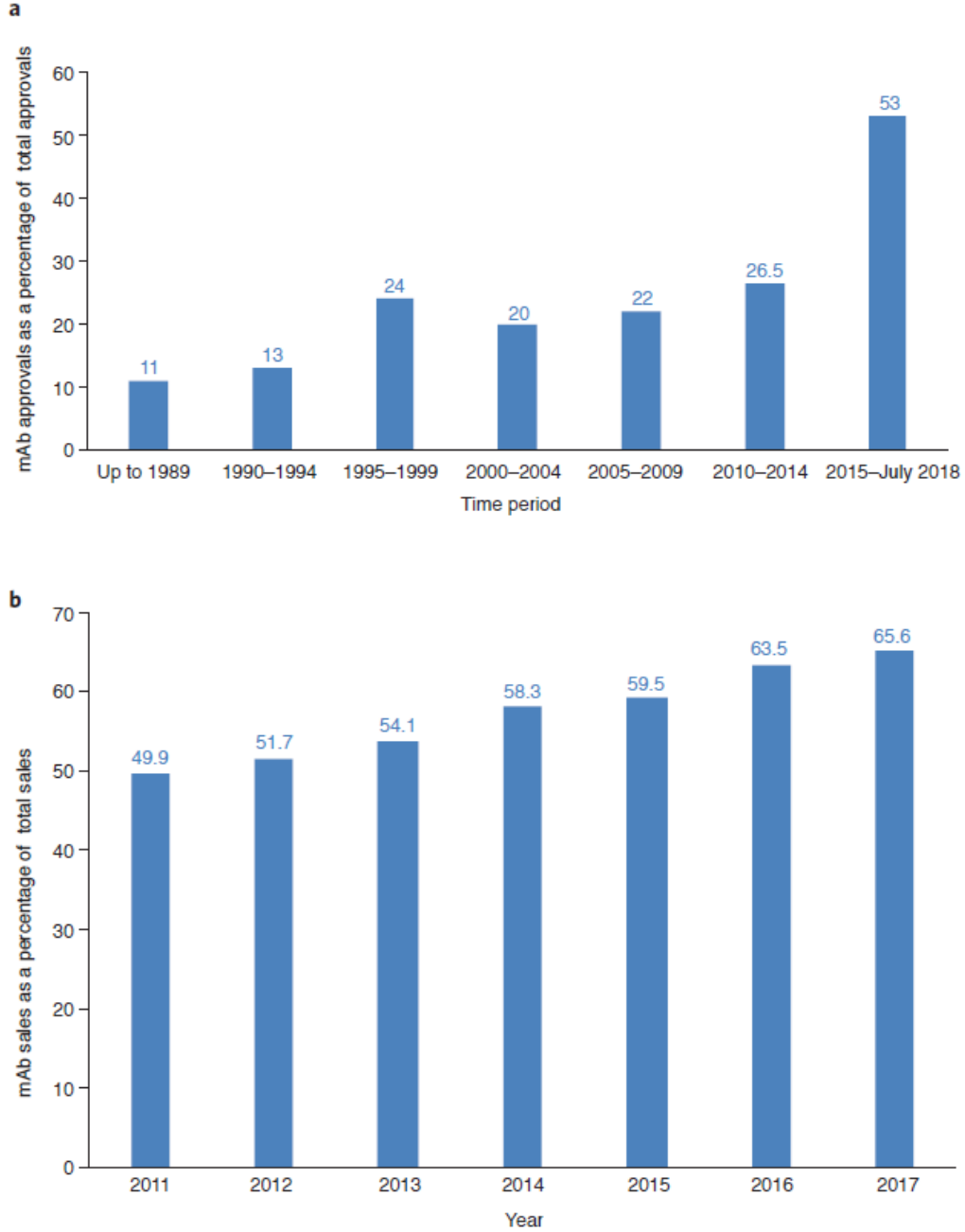


Figure 2 Overview of mAb approvals. (**a**) mAbs approved for the first time in the indicated periods, expressed as a percentage of total biopharmaceuticals approved for the first time in the same time period. (**b**) mAbs global annual sales value expressed as a percentage of total biopharmaceutical global sales for the indicated years. Financial data from La Merie Business Intelligence.

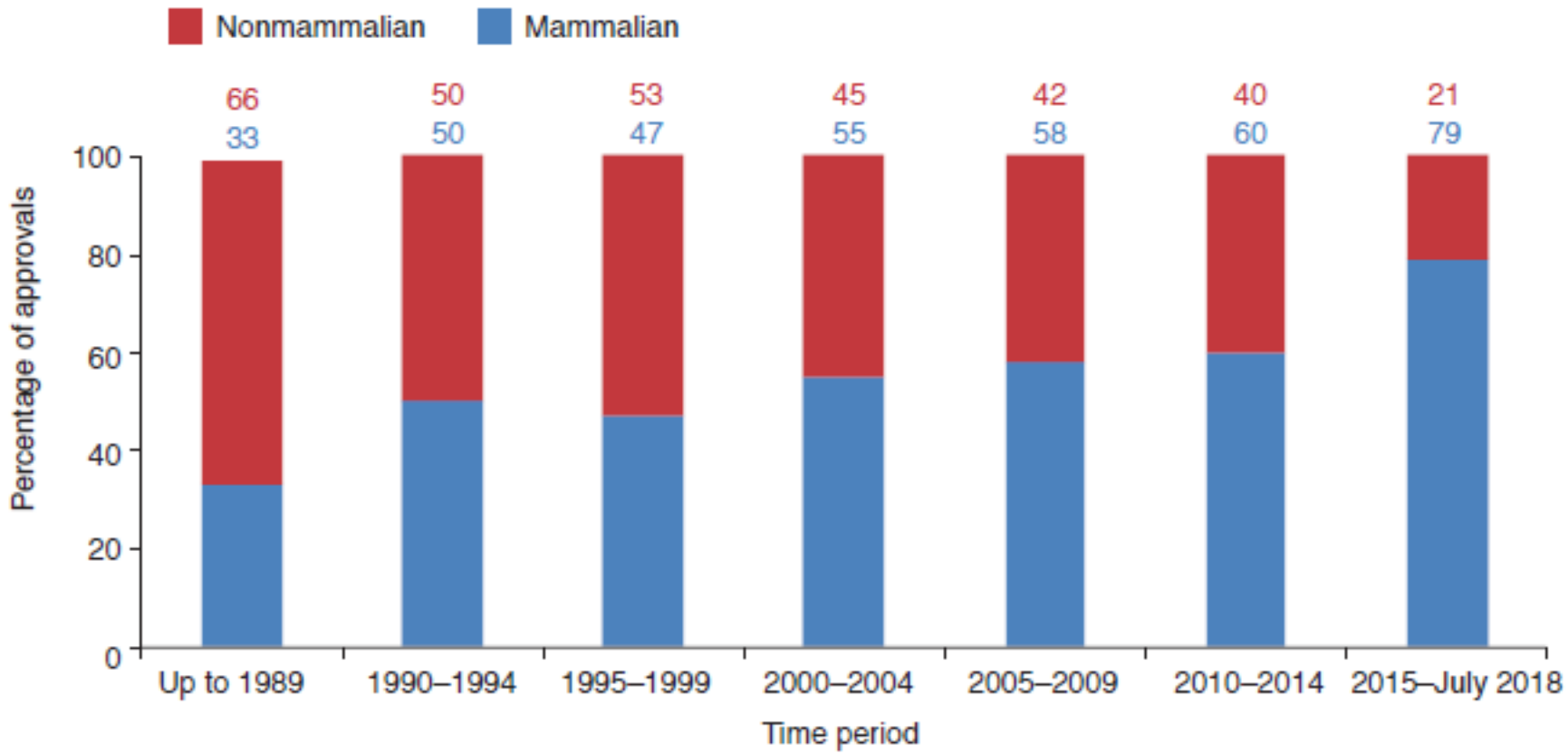
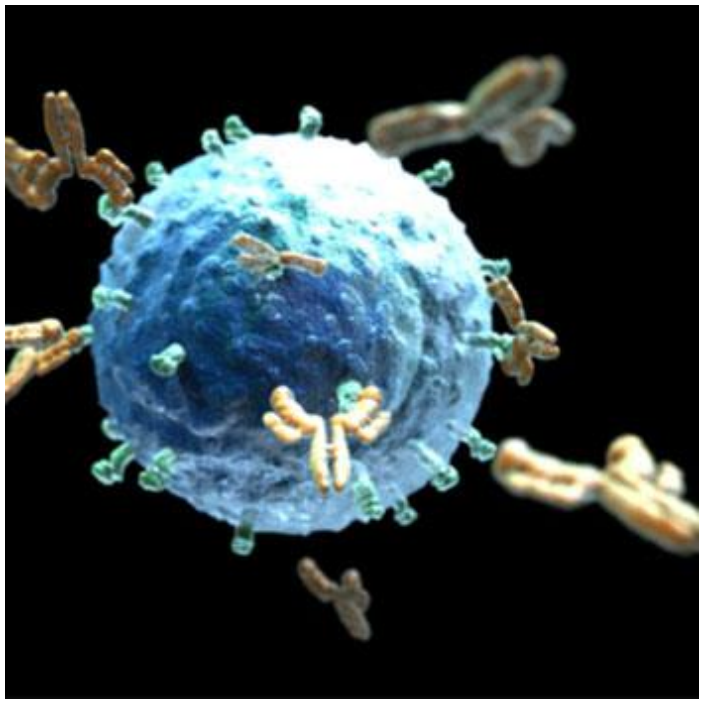


Figure 3 Relative use of mammalian- versus nonmammalian-based production cell lines in the manufacture of biopharmaceuticals approved over the indicated periods. Each dataset is expressed as a percentage of total biopharmaceutical product approvals for the period in question.

Task of antibodies in the nature (polyclonals):

Recognition and neutralization of foreign objects such as

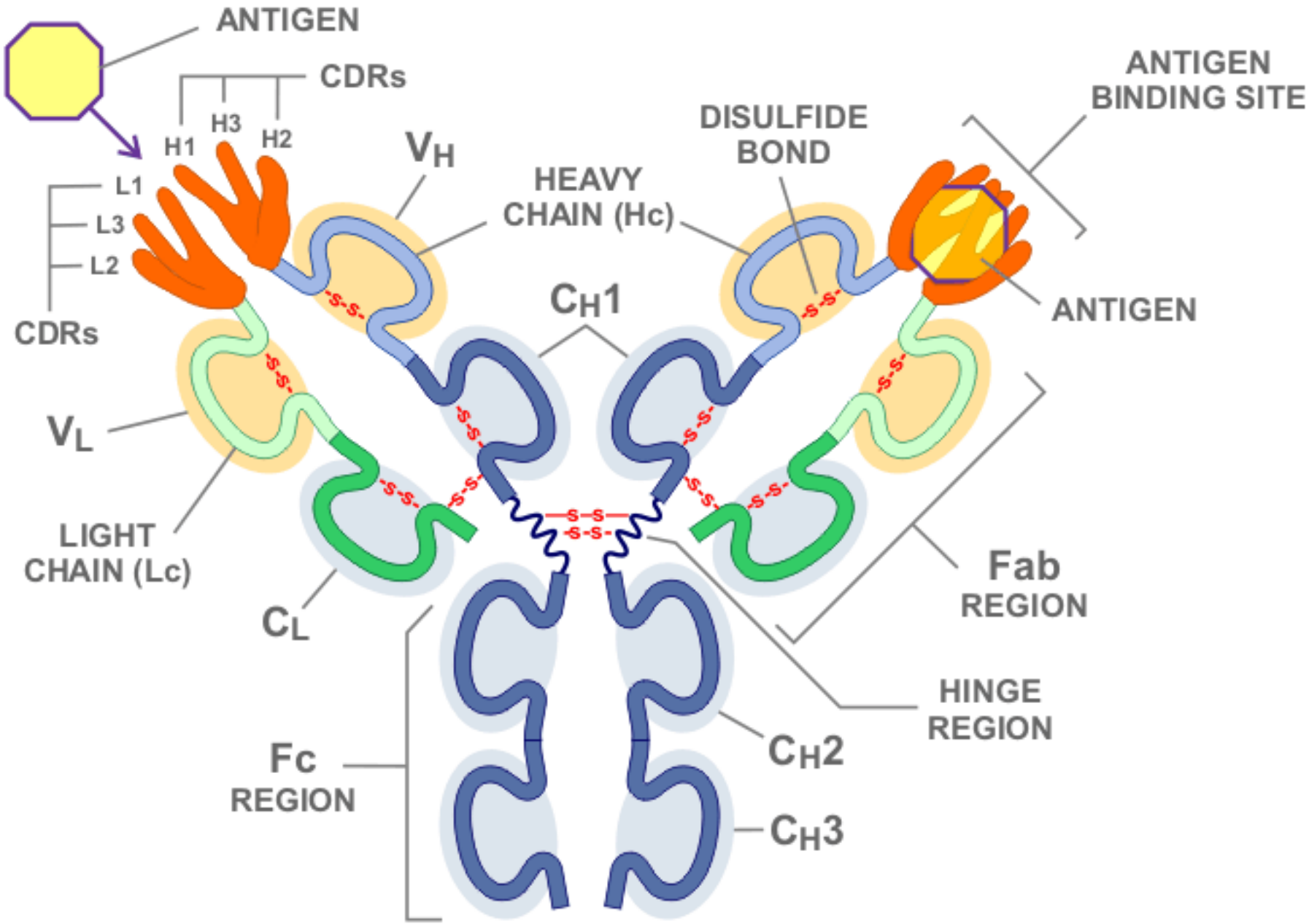
- Bacteria
- Viruses
- Parasites
- Toxins



How they are prepared?

B-cells of the immune system

10 billion different antibodies can be produced


ANTIGEN
CDRs
H1
H3
H2
L1
L3
L2
CDRs
V_H
HEAVY CHAIN (Hc)
DISULFIDE BOND
ANTIGEN BINDING SITE
ANTIGEN
C_H1
V_L
LIGHT CHAIN (Lc)
C_L
Fab REGION
HINGE REGION
Fc REGION
C_H2
C_H3

Monoclonal antibodies – therateutic applications

Immunology
- Overactive immune system → Antibody binds and blocks specific components
- Autoimmun deseases
- Organ rejection

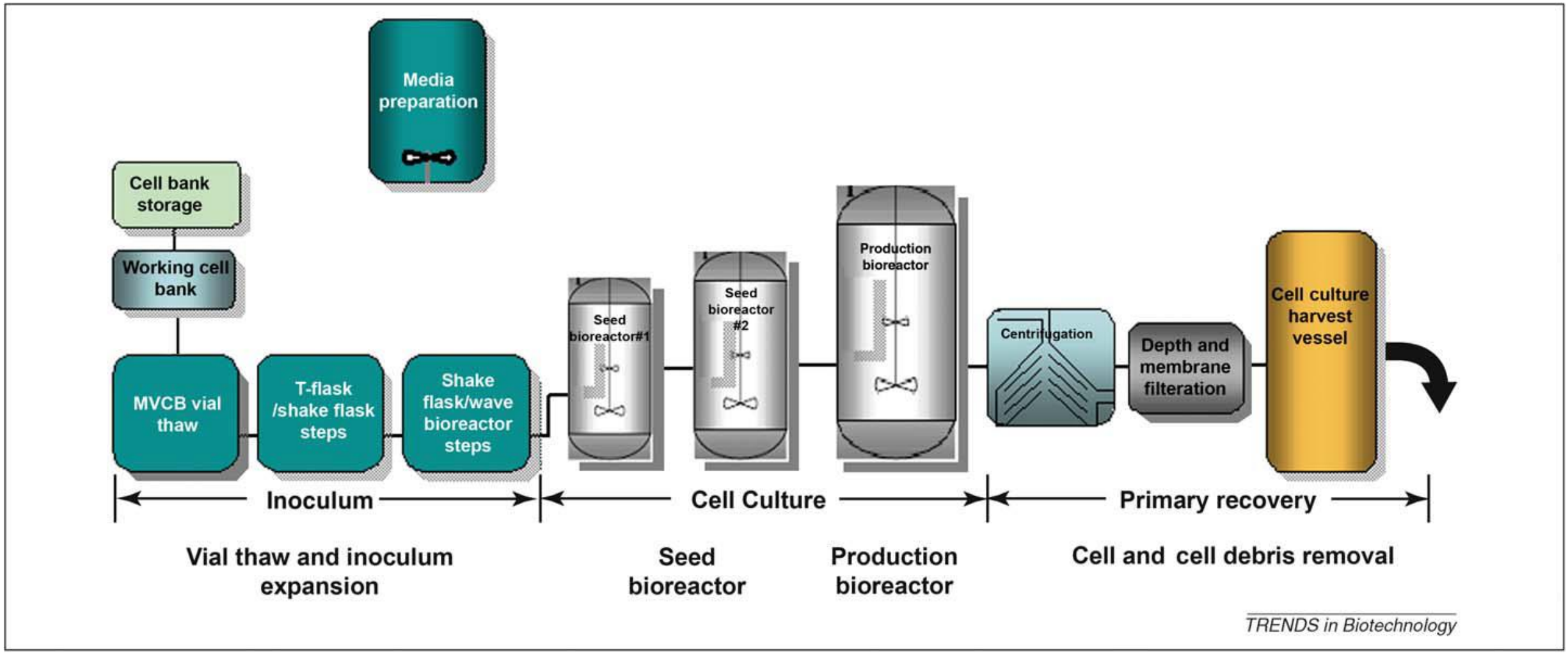
Oncology
- Cancer cells have high O2 demand → antibodies affect on blood vessel growth and formation factors
- Cancer develops resistance over treatment → antibodies can block cancer's resistance factors
- Her-2 binding

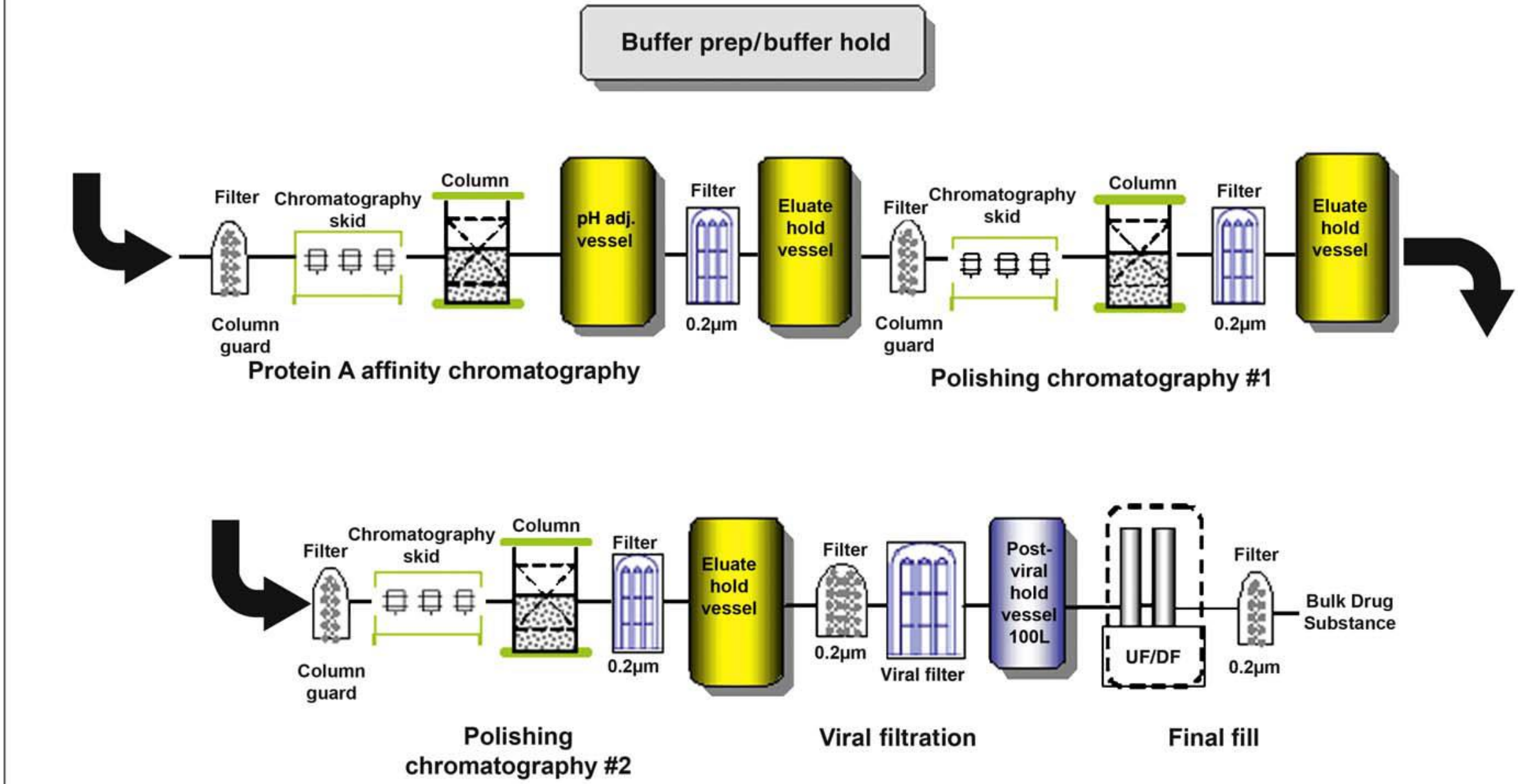
Elderly blindness
- Failed vision by damaged vessel system → antibodies affect angiogenic factors
- Wet age related macular degeneration

Miscellaneous
- Hypercholesterolaemia
- Migrain
- Haemophilia
- Asthma

BASIC STEPS OF MANUFACTURING

- **Upstream**
  - Inoculum train
  - Cultivation
  - Separation, clarification filtration
- **Downstream**
  - Chromatography steps (Affinity, IEX)
  - Membrane filtration
  - Virus inactivation, filtration
  - Bulk filtration and filling


Media preparation
Cell bank storage
Working cell bank
MVCB vial thaw
T-flask /shake flask steps
Shake flask/wave bioreactor steps
Seed bioreactor#1
Seed bioreactor #2
Production bioreactor
Centrifugation
Depth and membrane filteration
Cell culture harvest vessel
Inoculum
Cell Culture
Primary recovery
Vial thaw and inoculum expansion
Seed bioreactor
Production bioreactor
Cell and cell debris removal
TRENDS in Biotechnology

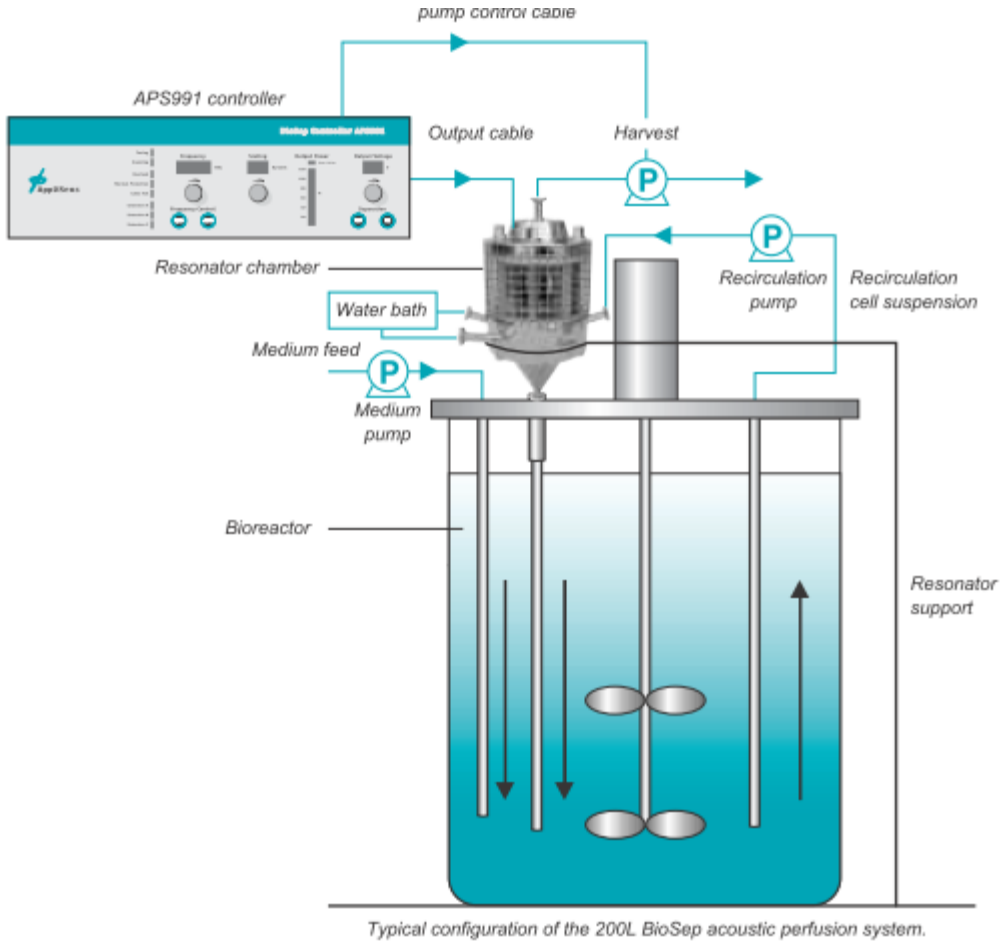

Buffer prep/buffer hold
Filter
Chromatography skid
Column
pH adj. vessel
Filter
Eluate hold vessel
Column guard
0.2µm
Protein A affinity chromatography
Filter
Chromatography skid
Column
Filter
Eluate hold vessel
Column guard
0.2µm
Polishing chromatography #1
Filter
Chromatography skid
Column
Filter
Eluate hold vessel
Column guard
0.2µm
Polishing chromatography #2
Filter
Post-viral hold vessel 100L
Viral filter
0.2µm
Viral filtration
UF/DF
Filter
Bulk Drug Substance
0.2µm
Final fill
TRENDS in Biotechnology

Main goals of a typical mAb production

- Upstream – Biosynthesis of the product by cultivation of CHO cells
- Midstream – Removal of cells and cell debris
- Downstream – Recovery and purification of the product by removal of
  - Process related impurities
  - Product related impurities or variants
  - Contaminants or adventitious agents

Techniques used in the upstream process

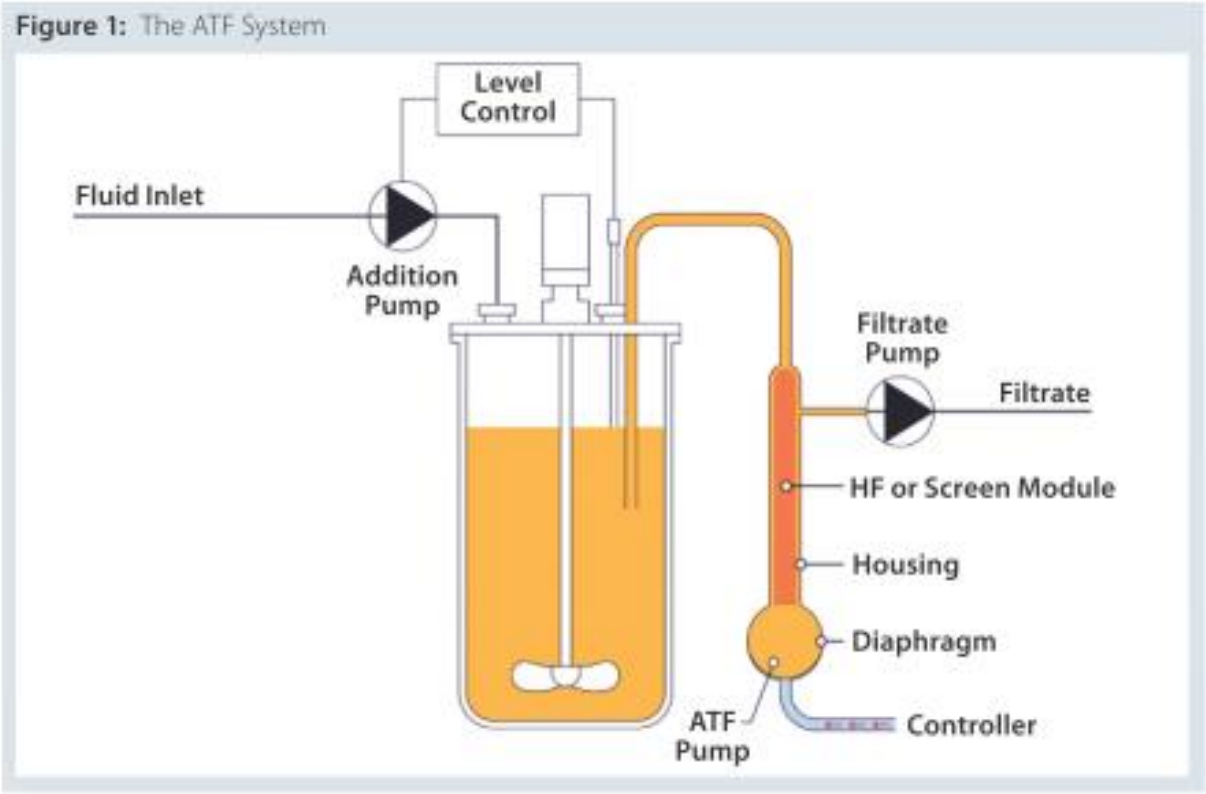
- Batch cultivation
- Fed-batch cultivation
- Continuous cultivation
- Cell retention methods
  - Perfusion cultivation
  - Concentrated fed-batch cultivation



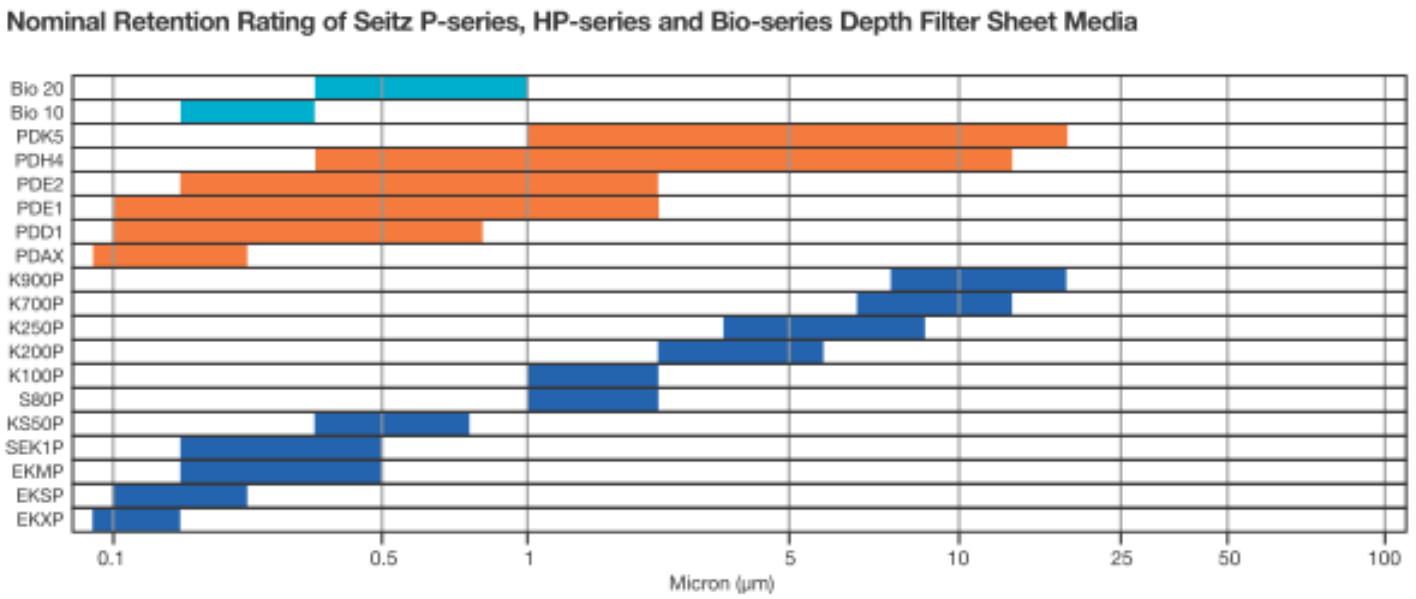
Typical configuration of the 200L BioSep acoustic perfusion system.

Alternating Tangential Flow

An Xcellerex bioreactor used in an intensified perfusion process with ATF System equipment (WWW.REFINETECH.COM)



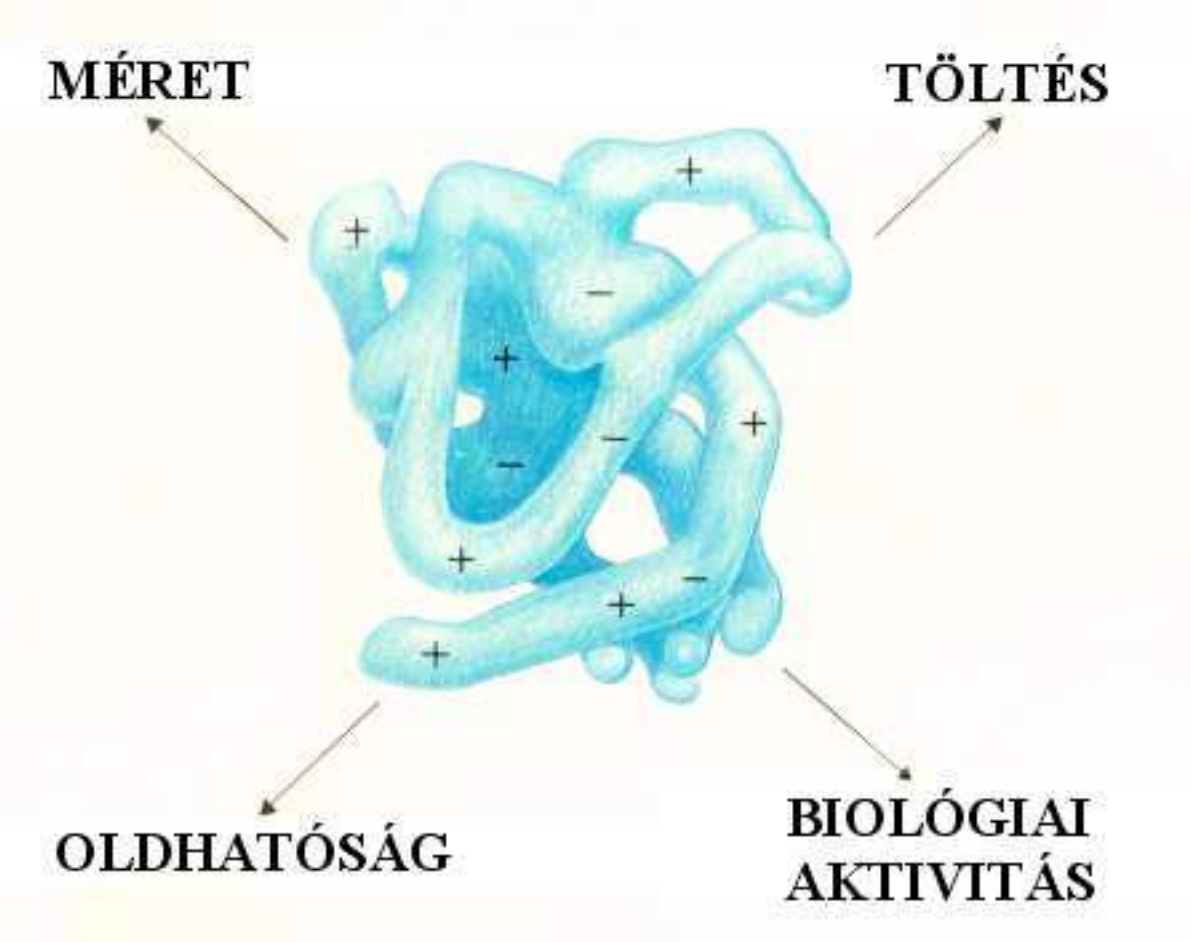
Midstream: depth filtration



Typical unit operations of the downstream process

- Capture chromatography
- Virus inactivation
- Intermediate purification chromatography
- Polishing chromatography
- Viral filtration
- Ultrafiltration and diafiltration (concentration and buffer exchange)
- Sterile filtration

Chromatography techniques



Separation by SIZE:
Gel permeation
Chromatography, SEC

Separation by CHARGE:
Ion exchange
chromatography

Separation by SOLUBILITY:
partitioning,
adsorption, HIC

Separation by BIOLOGICAL
ACTIVITY:
Affinity chromatography

Capture step

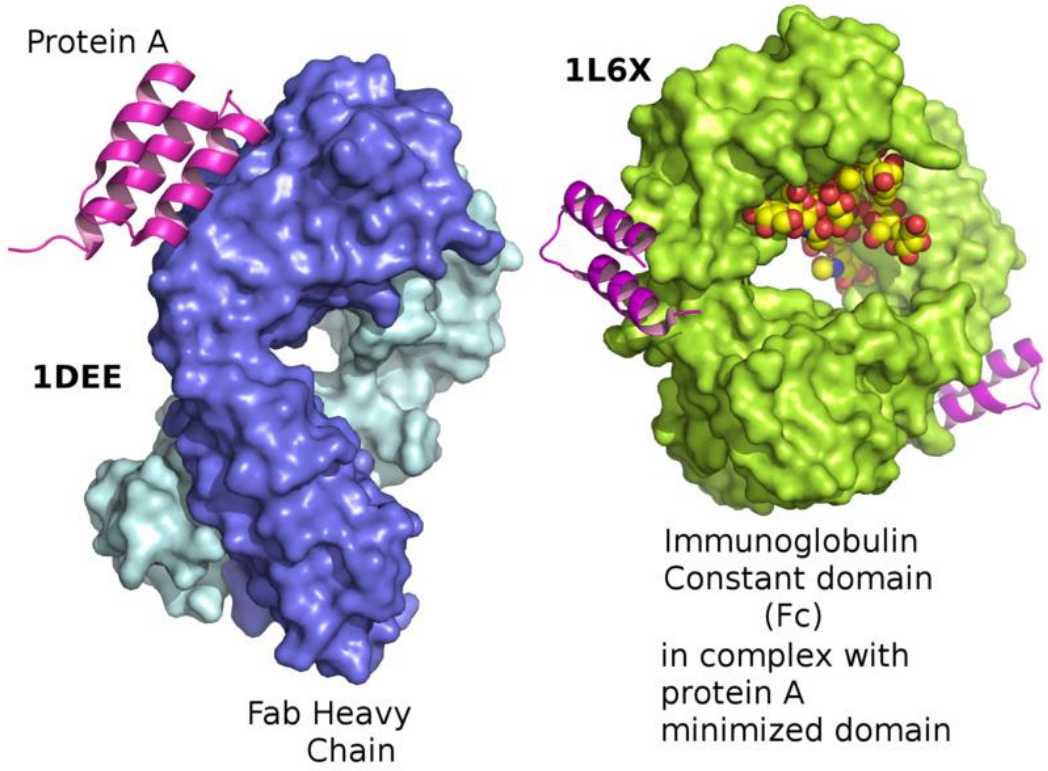
- Selective binding of antibodies to a chromatography medium
- Main goals
  - Concentrate the target antibody
  - Reduce sample volume
  - Removal of medium components
  - Removal of host cell proteins and DNA
- Suitable chromatographic methods:
  - Affinity Chromatography (AC)
  - Hydrophobic Interaction Chromatography (HIC)
  - Hydrophobic Charge-induction Chromatograhy (HCIC)
  - Cation Exchange Chromatography (CEX)

Affinity chromatography

- Proteins are separated and purified using techniques that exploits the unique biological property of the proteins to bind to ligands specifically and reversibly.
- The technique offers high selectivity , hence high resolution and usually high capacity for protein of interest.
- The first step involves the determination of the availability of a suitable ligand.
- The ligand is applied to the matrix of chromatographic resin creating the affinity medium.
- Sample is applied under conditions that favour specific binding of the target protein or protein of interest to the ligand. The protein of interest binds specifically but reversibly to the ligand.
- The protein of interest is removed by a process of elution using buffers. The elution process involves the breaking of the ligand-protein bond or substitution

Protein A

Protein A **is a 42-kDa protein anchored in the cell wall of Staphylococcus aureus …**

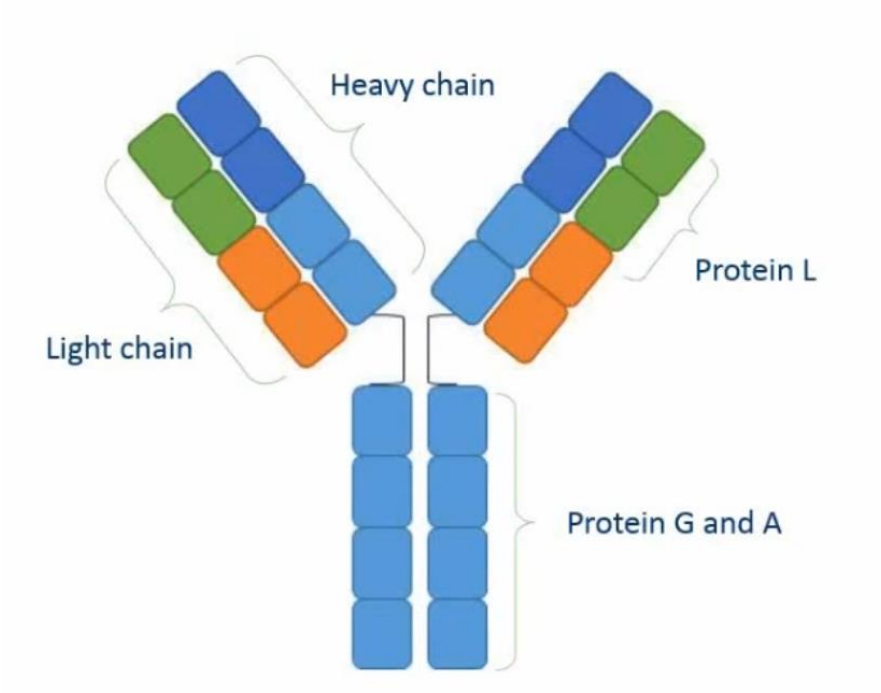


… with the ability to selectively interact with immunoglobulins (IgGs)

Protein A and G

Another affinity ligand, also used for IgG-purification and originating from a bacterial species, is the streptococcal protein G.

Despite the widespread use of protein A and protein G in antibody purification, the **ligand stability** is an issue that has limited their use.



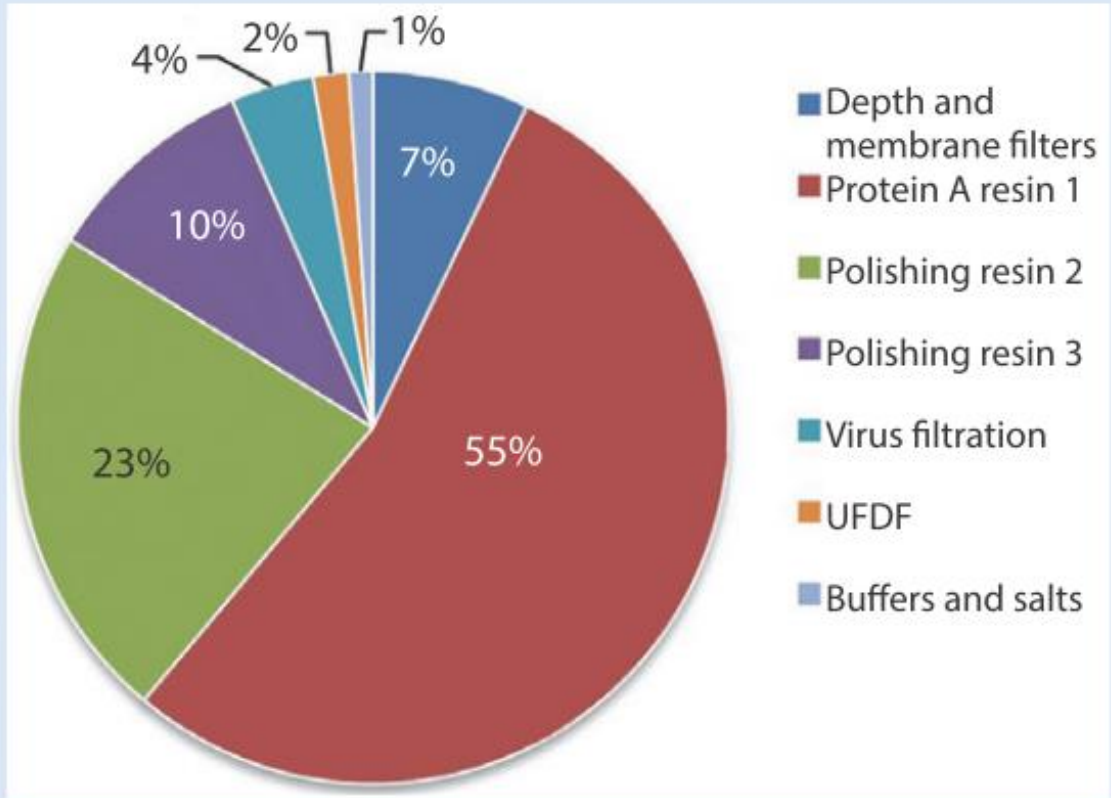
Protein A and G

Due to the **superior stability of protein A** compared to protein G, the use of protein G in industrial purifications is very restricted.

However, affinity purification using protein G is normally the first choice for the purification of serum and human IgG subclass 3, due to **protein A's low affinity** towards that subclass.

Species	Immunoglobulin	Binding to Protein A	Binding to Protein G	Binding to Protein L
Human	IgG1	Strong	Strong	Strong
	IgG2	Strong	Strong	Strong
	IgG3	Negligible	Strong	Strong
	IgG4	Strong	Strong	Strong
Mouse	IgG1	Weak	Strong	Strong
	IgG2a	Strong	Strong	Strong
	IgG2b	Medium	Medium	Strong
	IgG3	Weak/medium	Medium	Strong
Rat	IgG1	Negligible	Weak	Strong
	IgG2a	Negligible	Strong	Strong
	IgG2b	Negligible	Medium	Strong
	IgG2c	Negligible	Medium	Strong
Goat	IgG	Weak	Medium	Negligible
Rabbit	IgG	Strong	Medium	Weak
Sheep	IgG	Weak	Medium	Negligible

Figure 1: Estimated percentage of raw material costs for monoclonal antibody downstream process



Pros and cons

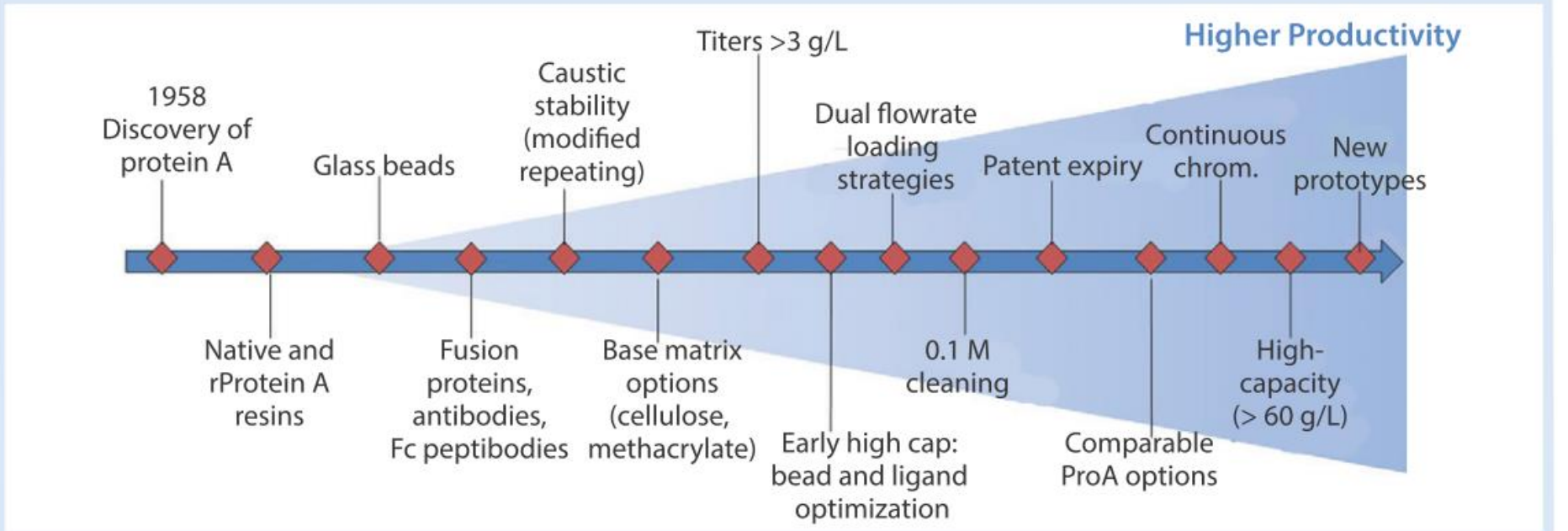
Very expensive processes

- one way to save money is to use better matrix

Sorbent	Ligand	Manufacturer	Bead Matrix	Mean Particle Diameter	DBC at 3 min Residence	Working Flow Rate	Caustic Tolerance	Estimated Price*
CaptivA	Recombinant native protein A	Repligen	4% agarose 4FF	90 µm	~38 mg/mL	300 cm/h	0.1 N NaOH	$5,800/L
MabSelect Xtra	Recombinant protein A	GE Healthcare	Highly cross-linked agarose	75 µm	35 mg/mL	100–300 cm/h	50 mM	$12,803/L
MabSelect SuRe	Tetramer alkali-stabilized Z-domain	GE Healthcare	Highly cross-linked agarose	85 µm	≥30 mg/mL	100–500 cm/h	0.1–0.5N NaOH	$15,850/L
MabSelect SuRe LX	Tetramer alkali-stabilized Z-domain	GE Healthcare	Highly cross-linked agarose	85 µm	45 mg/mL	100–500 cm/h	0.1–0.5N NaOH	$17,157/L
Prosep Ultra Plus	Recombinant native protein A	EMD Millipore	Controlled pore glass	60 µm	~48 mg/mL	800 cm/h	None	$14,440/L
Poros Mab-Capture A	Recombint native protein A	Life Technologies	Polystyrene divenyl-benzene	45 µm	>45 mg/mL	700 cm/h	0.1 N NaOH	$13,750/L
TOYOPEARL AF-rProtein A-650F	Tetramer alkali-stabilized C domain	Tosoh Bioscience	Polymeth-acrylate	45 µm	>30 mg/mL	≤1,000 cm/h	0.1–0.5N NaOH	$12,240/L

* 2013 list prices in US dollars (from websites or direct sales inquiries) listed as fair comparison without discounts (e.g., for large-volume orders)

Figure 2: Protein A step schematic of improvements to process, equipment, and materials to meet demands of higher productivity

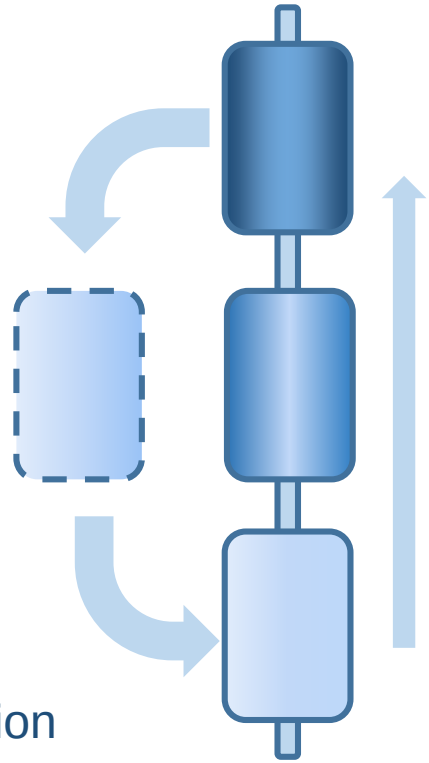


Pros and cons

Pre-packed single use columns
- Ready to use from vendors
- Disposable after use (campaign)
- Maybe expensive, but easy to use and needs no preparations

Multiple cycles
- Smaller volume
- Increased process time

Multiple column methods for semi-continuous application



Periodic counter-current chromatography (PCC)

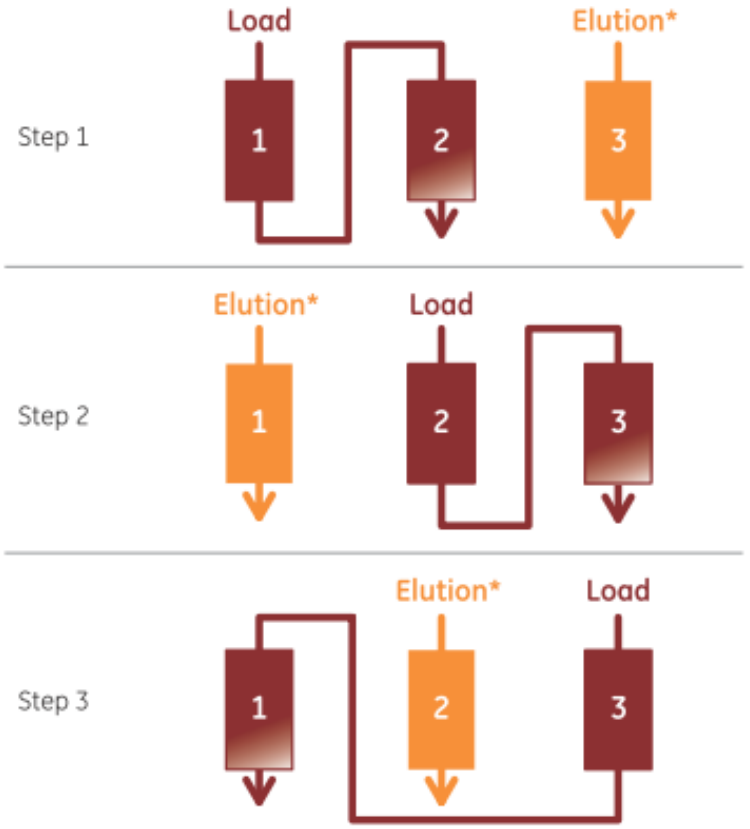


Fig 1. The principle of 3C PCC. Step 1: column 1 and 2 are loaded with sample (red). Step 2: column 1 has reached the defined level of breakthrough (BT) and is disconnected from the loading zone to be eluted, while column 2 becomes the first column in the loading zone. Step 3: column 2 has reached the defined BT level and column 3 becomes the first column in the loading zone. This procedure is repeated in a cyclic manner to achieve a continuous operation. *Elution phase in this figure includes wash, elution, strip, CIP, and re-equilibration.

Hydrophobic Interaction Chromatography

- Different hydrophobicity of proteins
- Interaction can be enhanced by ionic strength
- Proteins bind to the media
- Elution: decreasing ionic strength (salt concentration)
  - Different proteins will elute at different ionic strength
- Advantage
  - It can remove protein aggregates
- Disadvantage
  - The hydrophobicity of a protein is difficult to predict

Additional chromatography steps

- Intermediate purification
- Final polishing
- Suitable chromatographic methods:
  - Ion Exchange Chromatograpy (IEX)
  - Size Exclusion Chromatography (SEC)
  - Hydrophobic Interaction Chromatography (HIC)
  - Mixed-mode Ion Exchange Chromatography (MM-IEX)

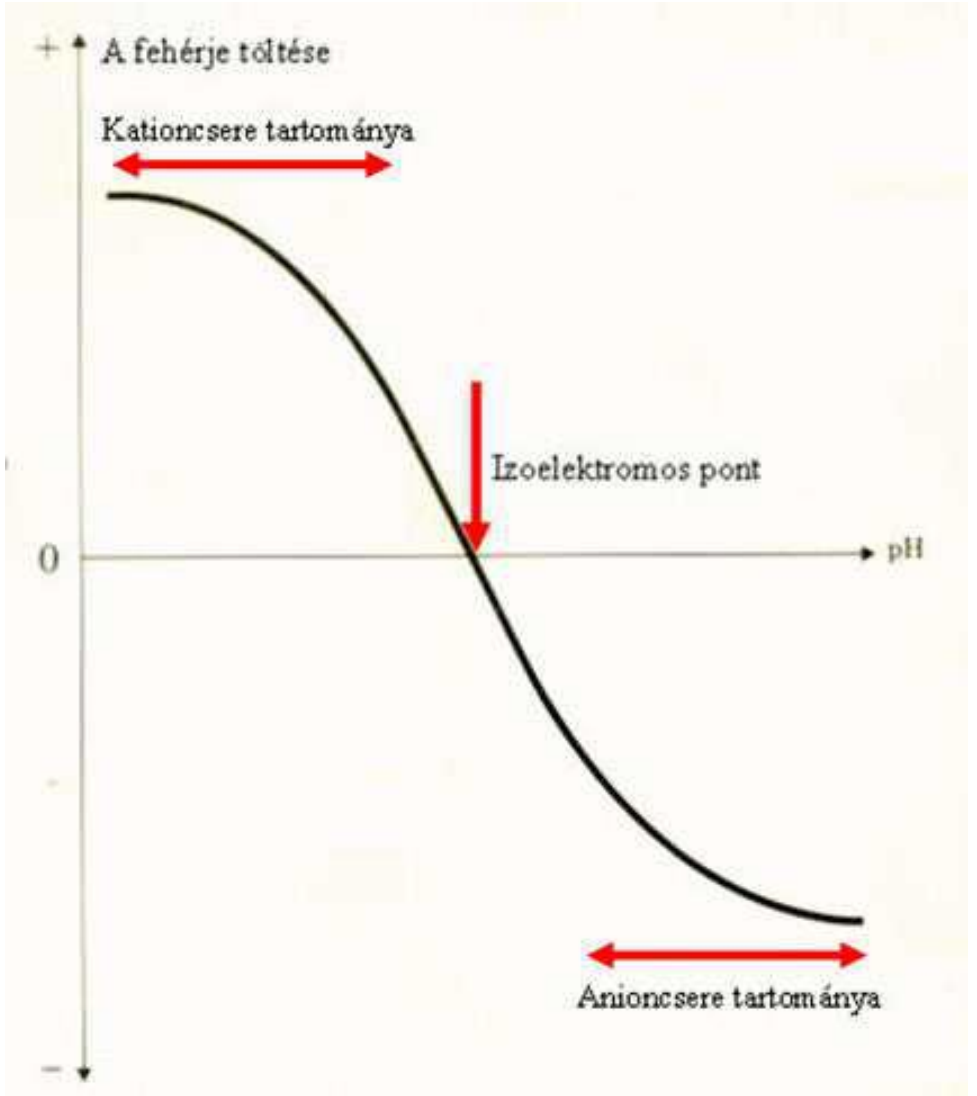
Ion-Exchange Chromatograpy

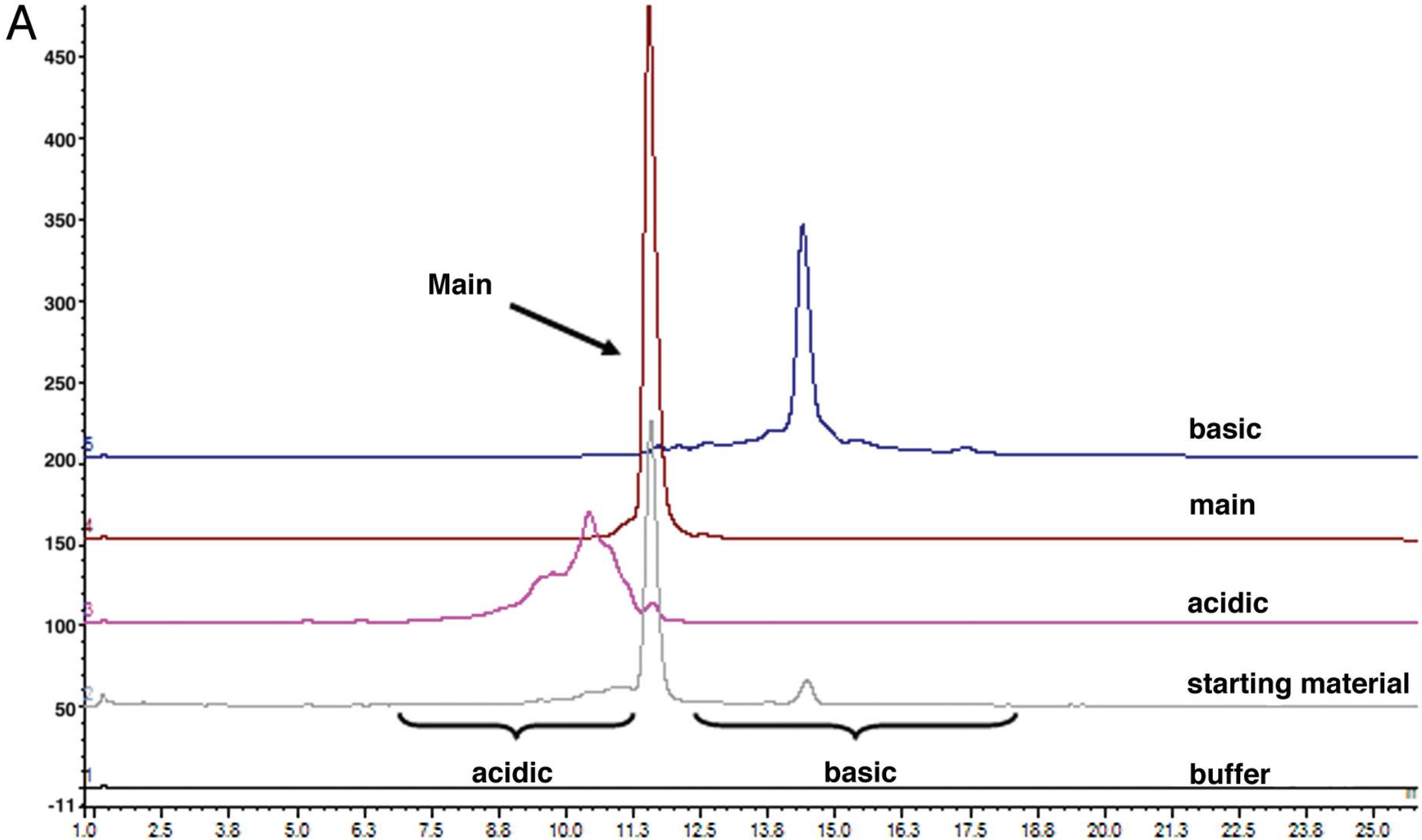
- Difference between pI of proteins (antibodies have higher pI)
- Separation of charge variants
- Different modes:
  - Bind-elute
  - Flowthrough
  - Membrane adsorbers
- It can remove DNA, leached protein A, host cell proteins, endotoxins

Charge of proteins

The charge of a protein depends on the pH of the medium:
Any protein can bind in either cation or anion exchange mode at proper pH value.

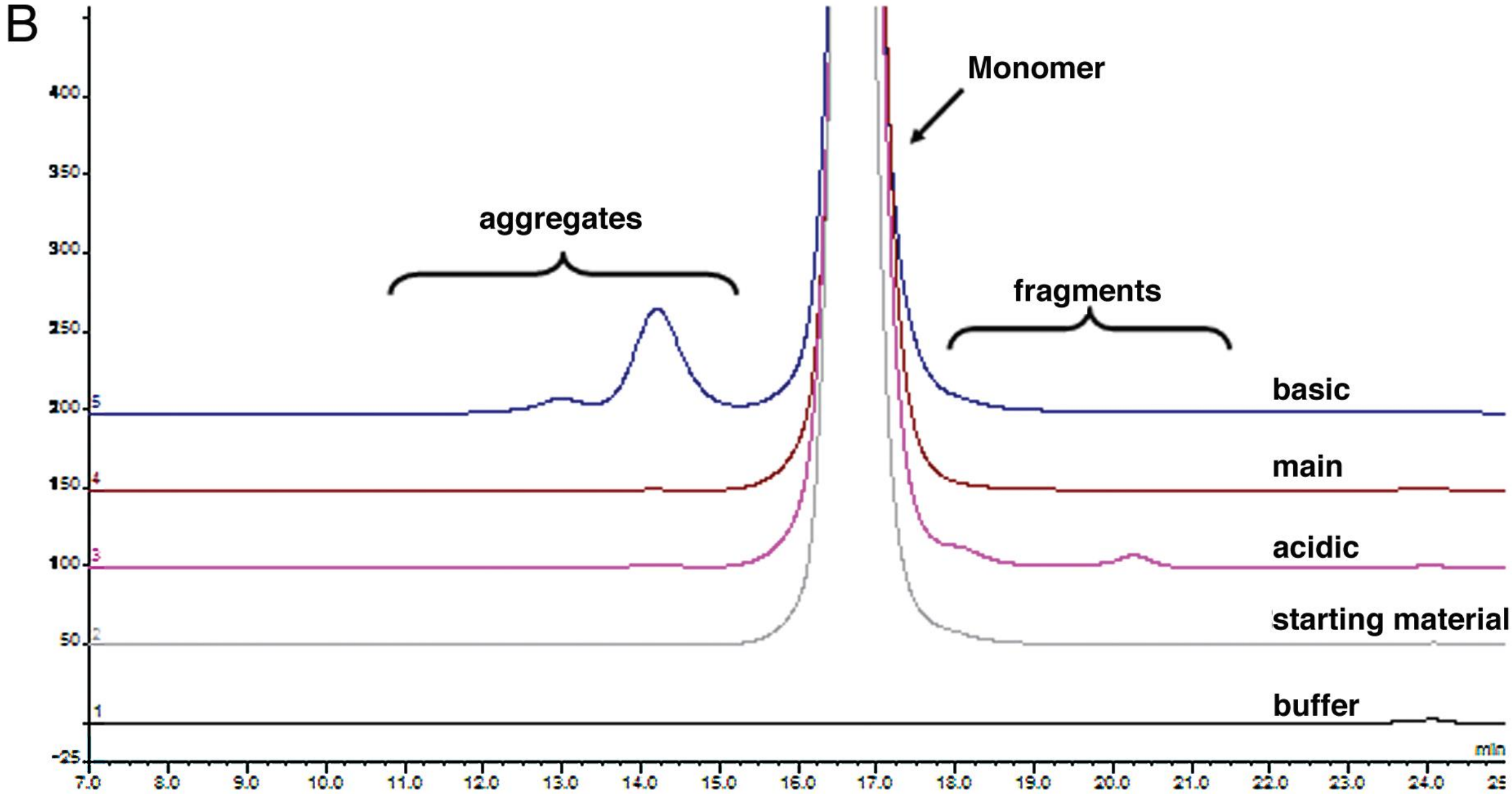
In the close vicinity of the isoelectric point (pI) no binding occur → the protein can be removed from the IEX medium by adjusting the pH towards the pI value




A
Main
basic
main
acidic
starting material
buffer
acidic
basic
450
400
350
300
250
200
150
100
50
-11
1.0
2.5
3.8
5.0
6.3
7.5
8.8
10.0
11.3
12.5
13.8
15.0
16.3
17.5
18.8
20.0
21.3
22.5
23.8
25.0

Size exclusion chromatography

- Difference between the size of molecules
- Removal of aggregates and fragments
- Reduced sample volume is applicable
  - Extremely slow technique
- Advantage
  - The buffer usually does not have effects on separation


B
Monomer
aggregates
fragments
basic
main
acidic
starting material
buffer
5
4
3
2
1
400
350
300
250
200
150
100
50
-25
min
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
24.0

Cadence™ BioSMB Process System

Continuous Single-Use, Multi-Column Chromatography

Cadence BioSMB

ChromaCon®
A new dimension in purification

The MCSGP process principle and Contichrom® equipment

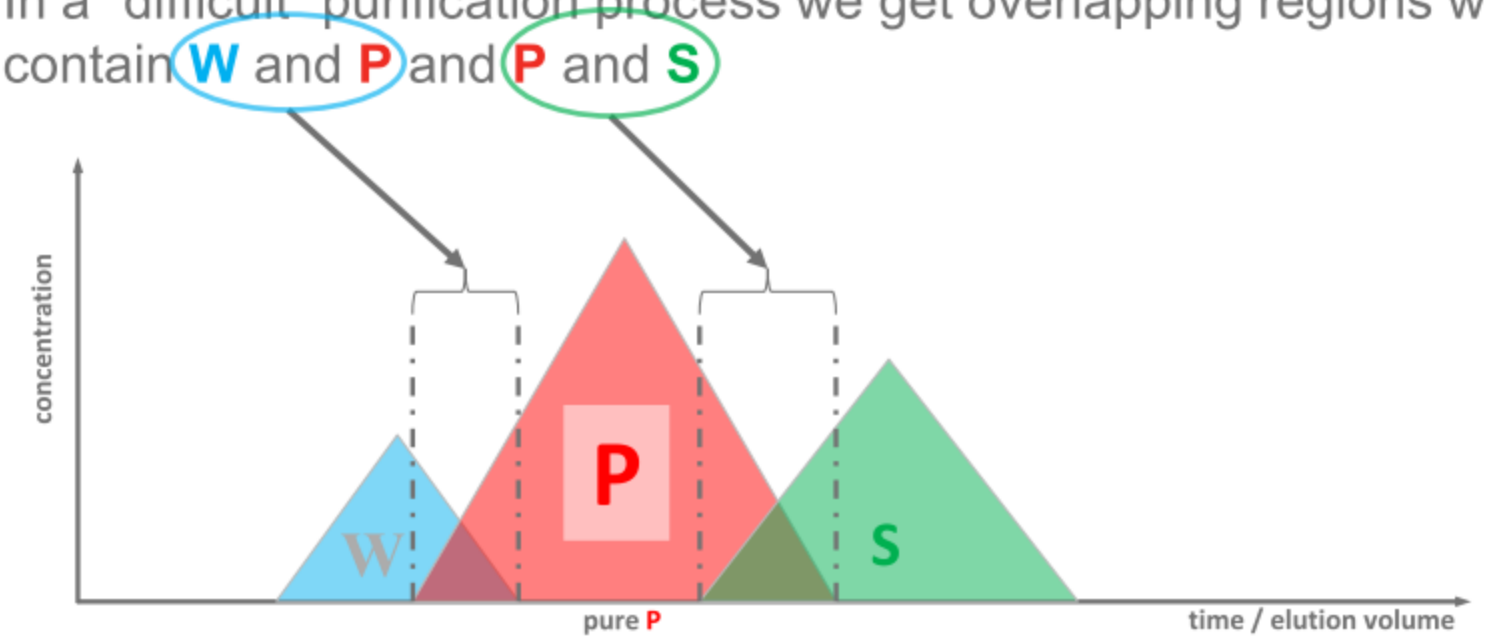
MCSGP

- MCSGP (multi-column solvent gradient purification) is a high resolution chromatographic process, employing two columns

- It is the optimal process solution for any complex sepration, where early and late eluting impurities overlap with the target product

MCSGP process principle

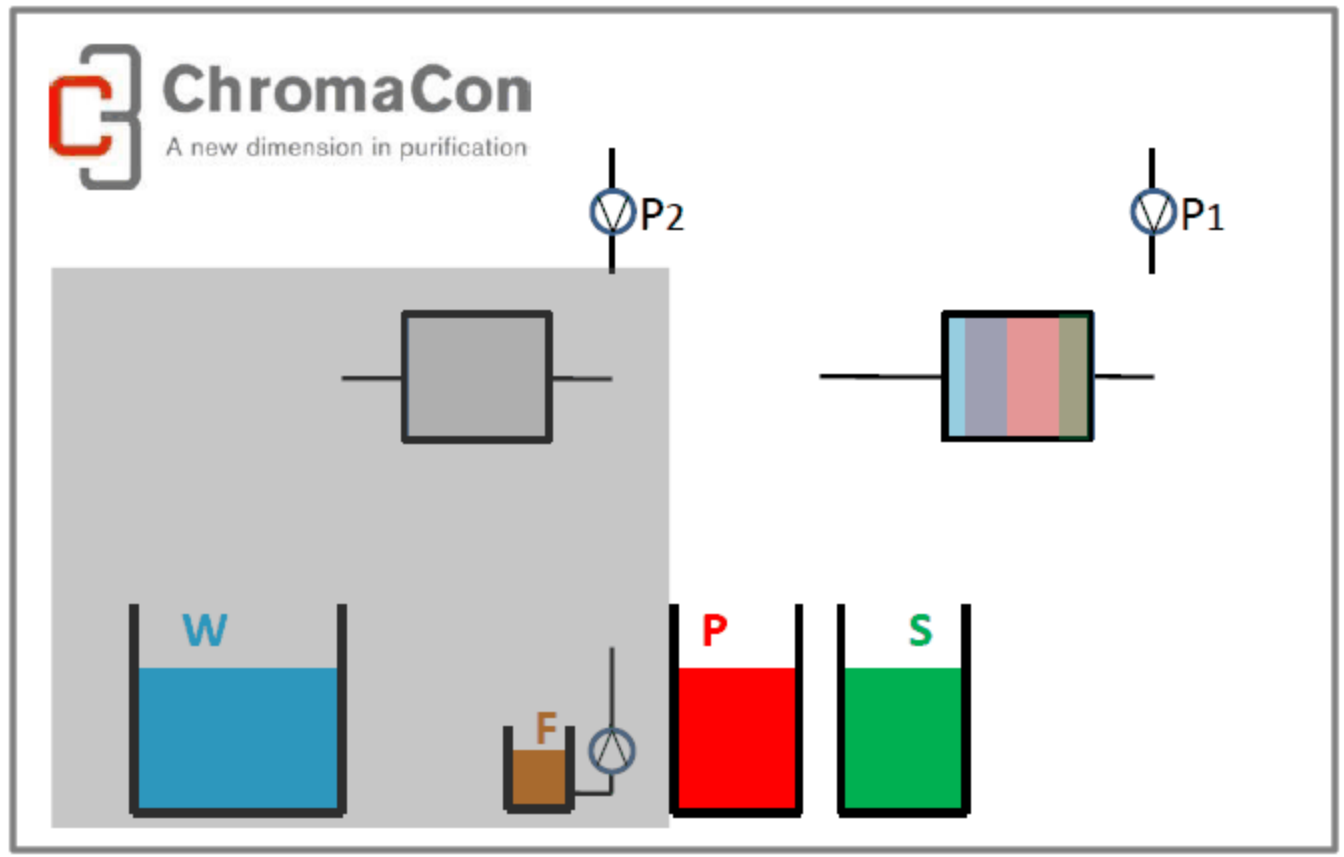
- “Purification“ means, that one target component (**P**) is isolated from weakly adsorbing impurities (**W**) and strongly adsorbing impurities (**S**)
- In a “difficult“ purification process we get overlapping regions which contain **W** and **P** and **P** and **S**



⇨in batch chromatography, the overlap is lost

2-MCSGP Animation (Contichrom®)

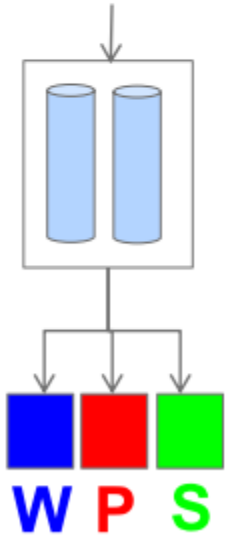
In 2-column MCSGP the overlapping fraction (W+P & P+S) are recycled **sequentially**.



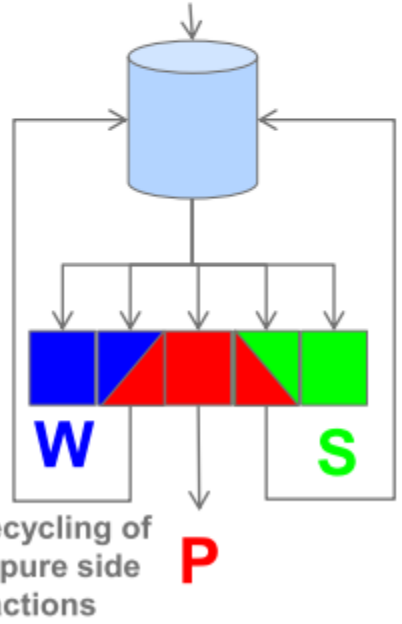
Animations can be downloaded at *www.chromacon.ch*

Comparison of different systems

MCSGP:
- Productivity 10 g/L/h
Yield 95%

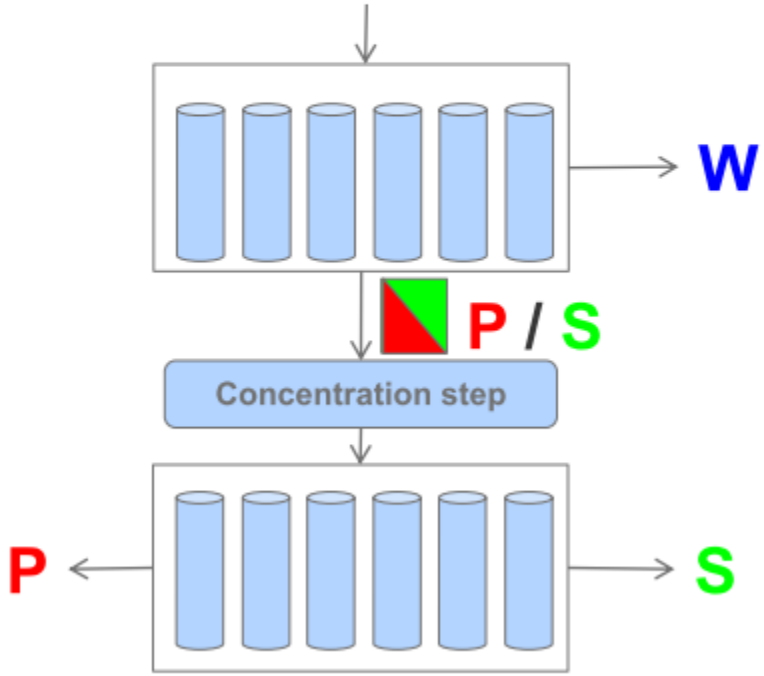


Batch with recycling:
- Productivity 2 g/L/h for 80% yield
- Productivity 1 g/L/h for 90% yield



Assumption: batch productivity = 20% of MCSGP productivity

SMB cascade:
- Productivity 1 g/L/h for 90-95% yield (without conc. step)



Assumption: SMB productivity = 1/3 MCSGP prod. due to flow rate limitations (6 columns instead of 2)

Virus retentive filtration

- Nanofiltration (20 nm pore size)
- Flat membrane casettes
- Hollow fiber cartridges
- Prefilters

Ultrafiltration and diafiltration

- Tangential flow filtration
- Flat membrane casettes
- Hollow fiber cartridges
- 30 kDa MW cut-off value
- Concentration
- Buffer exchange
  (continuous mode, 8 – 10 diavolumes)

DEBRECEN - BIOTECHNOLOGY FACILITY

Opened in 2012

Greenfield investment

Expansion is underway

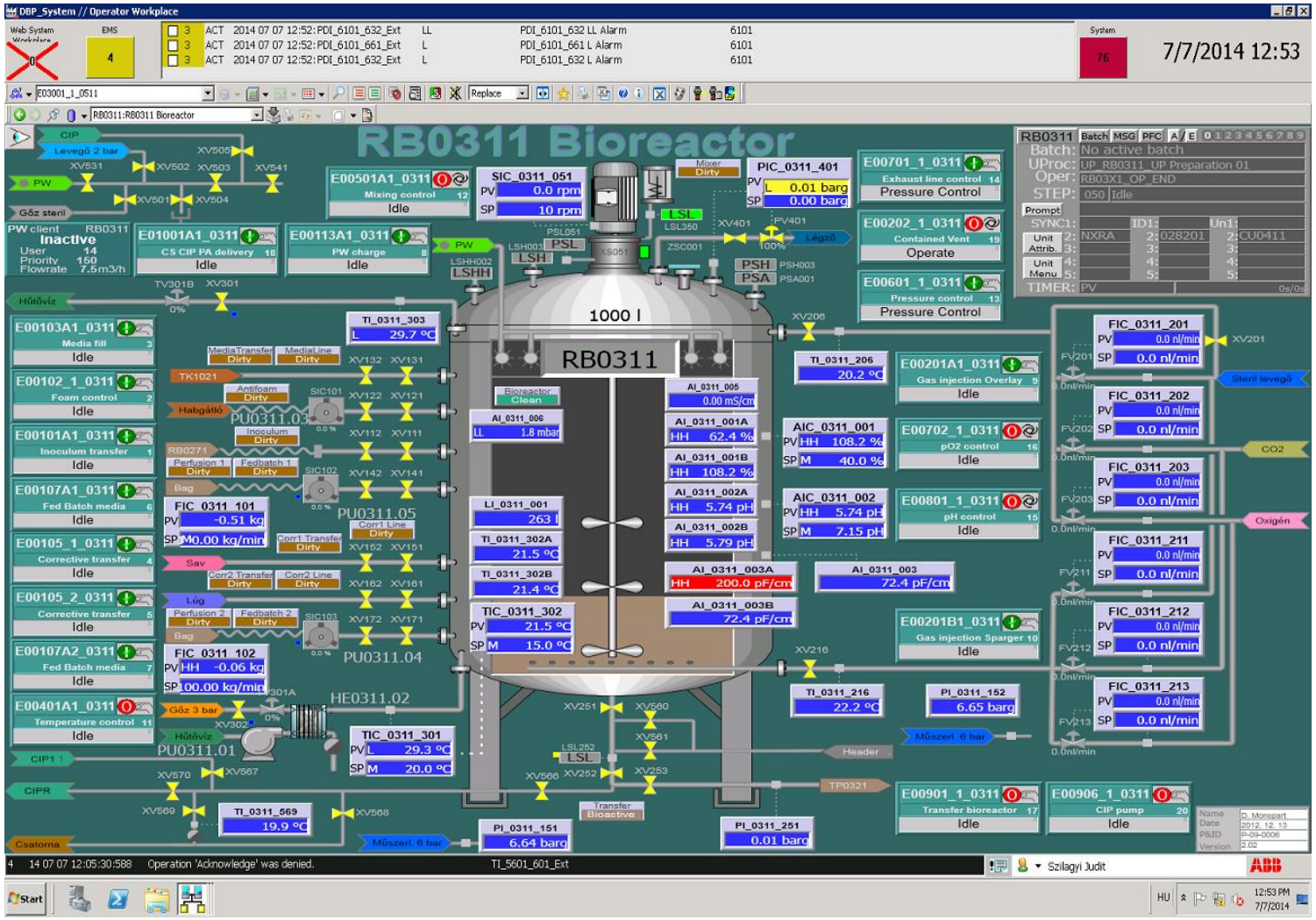
- **Mammalian cell fermentation & downstream processing**
- **Analytical services support**
- **2* 5000 l fermentor capacity, ongoing capacity increase**
- **Hybrid production lines (stainless steel & single-use)**
- **Currently manufacturing monoclonal antibodies for clinical trials**
- **Multipurpose facility**

OPENING CEREMONY – 2012.04.19.


B.OENGINEERING

BIOTECH PLANT

- Deeply integrated process automation system (PAS)




PALL

Thank you for your attention!

GEDEON RICHTER

Delivering quality therapy since 1901