



## The use of Chromatography Membranes for the development and production of biopharmaceuticals.

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# Agenda

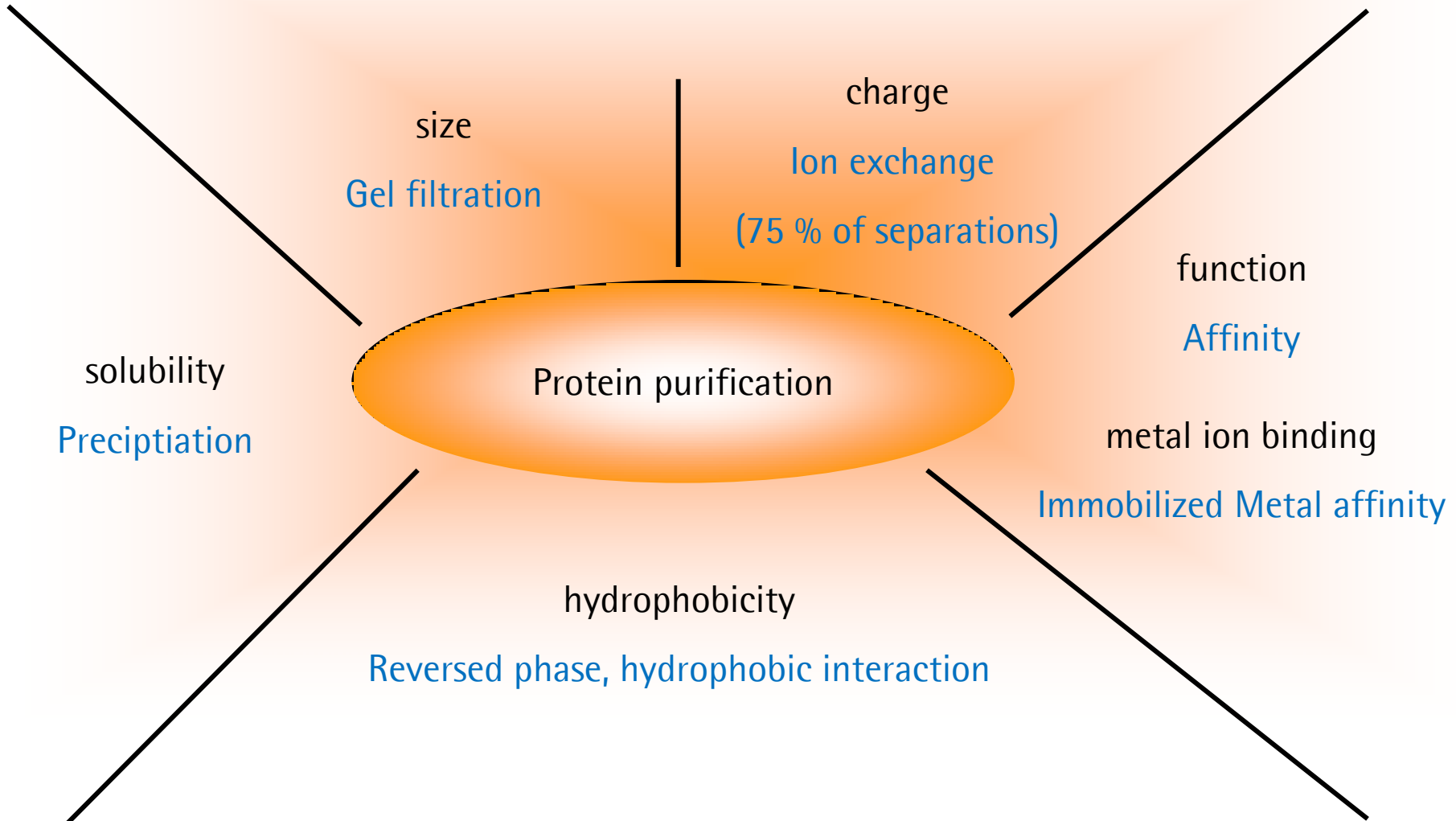


1. Introduction & Membrane Chromatography features
2. Formats of Membrane Adsorbers
3. Applications



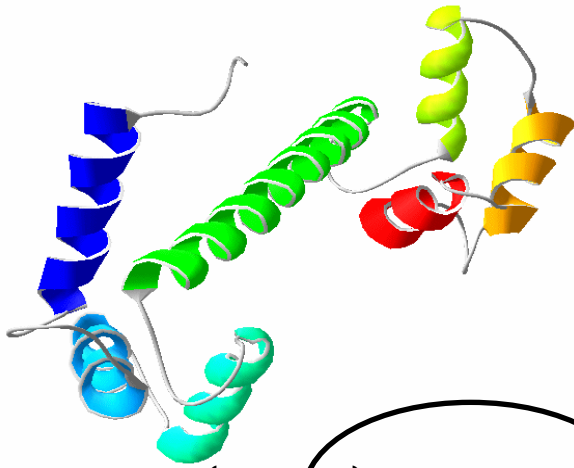
## Introduction & Membrane Chromatography features

Chromatography uses chemical and physical properties

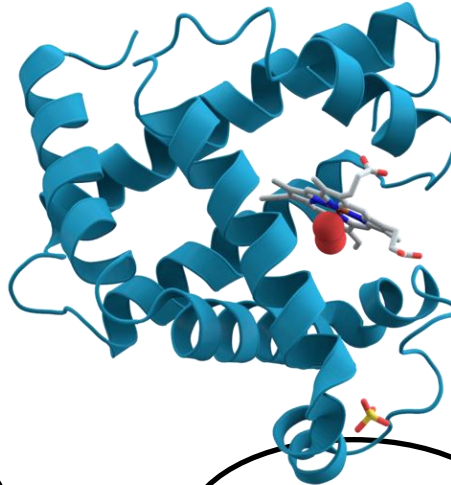




## Proteins vary in size



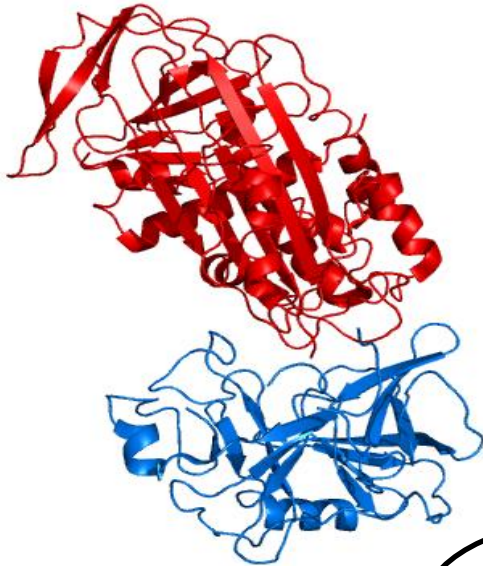
Calmodulin (human) 16.9 kDa



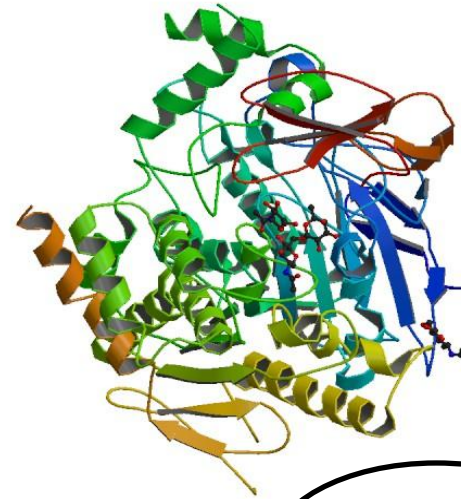
Myoglobin 17.7 kDa



DNA polymerase (human) 103 kDa

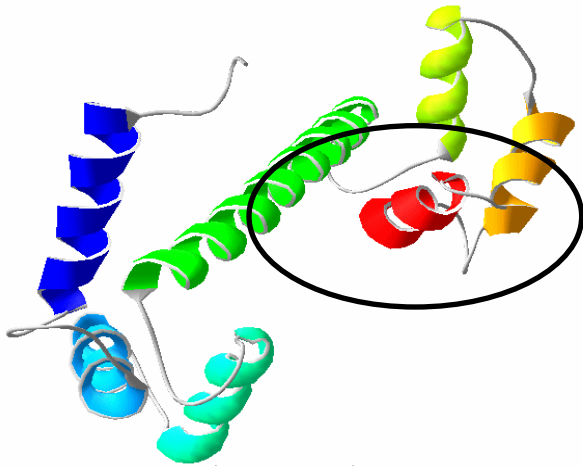


$\alpha$ -antitrypsin (human serum) 44.5 kDa

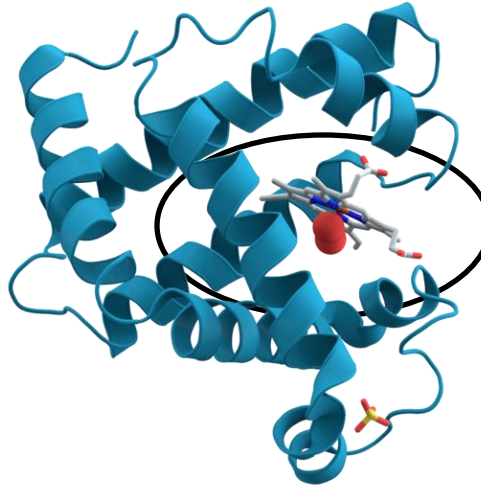


Acetylcholinesterase (64 kDa)

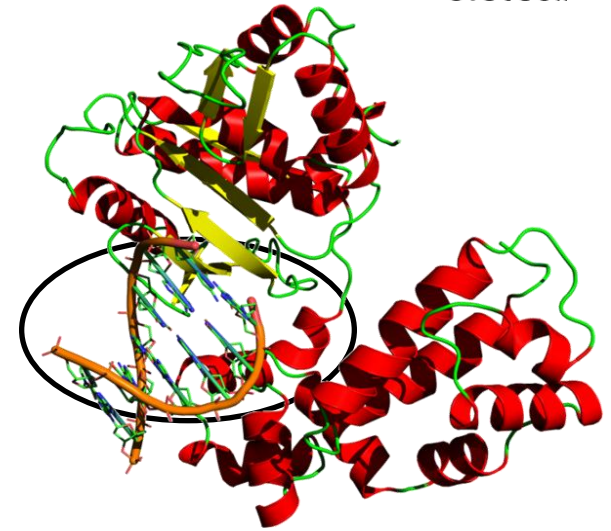
# Proteins vary in shape and chemical properties



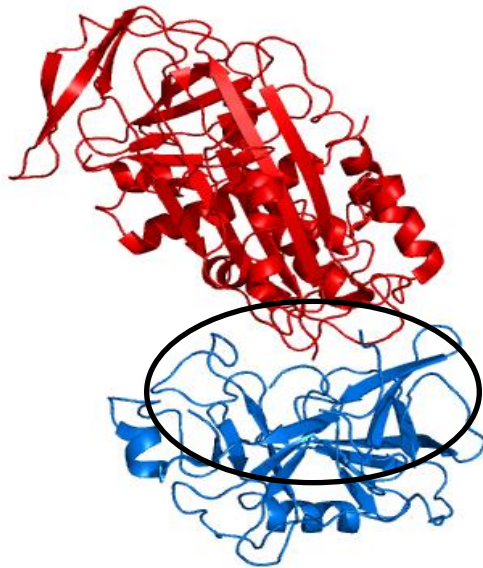
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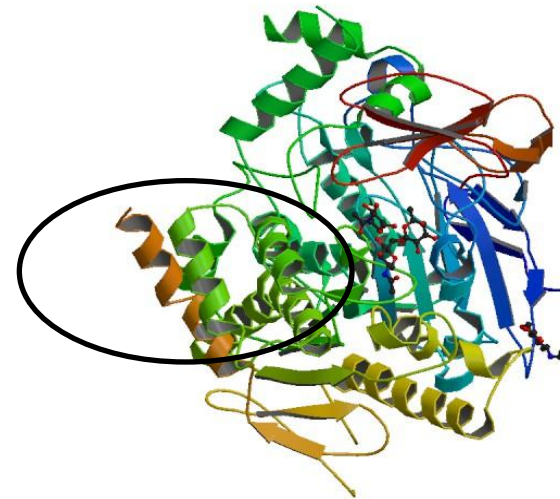
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Acetylcholinesterase (64 kDa)

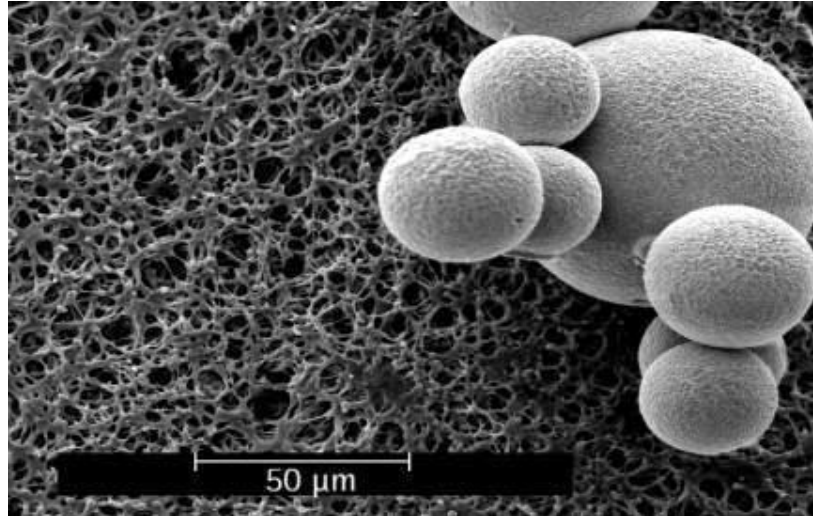


Chromatographic ligands are bound to a solid matrix

Membrane adsorber:  
Sartobind Q



Ready to use



Gel particles:  
Q Sepharose FF

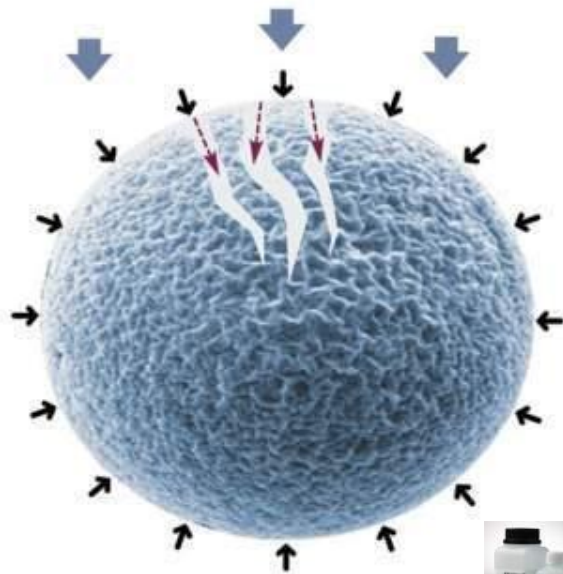


Filled into column by user



## Diffusion limited gels (time) versus convection limited (flow rate)

**Conventional bead**

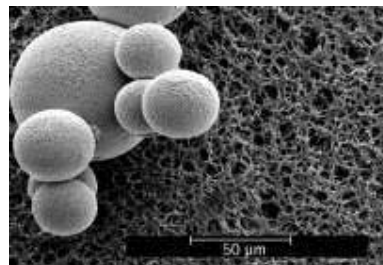


Average pore size  
15 - 40 nm

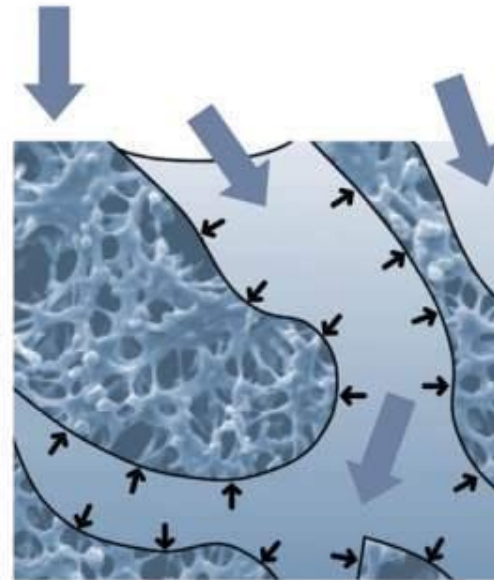
Convective flow

Pore diffusion

Film diffusion



**Membrane Adsorber**

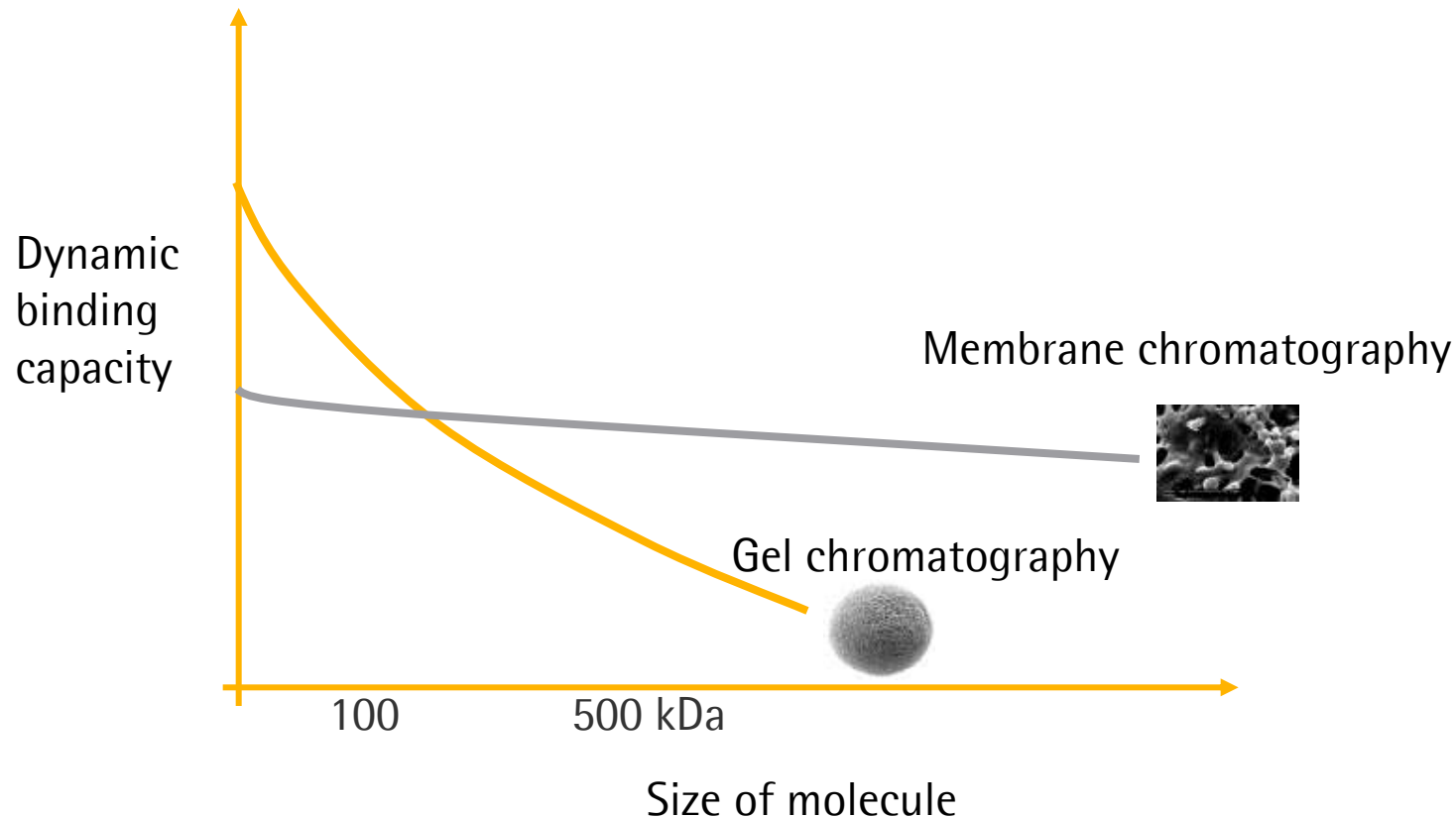


Average pore size  
3 - 5 µm



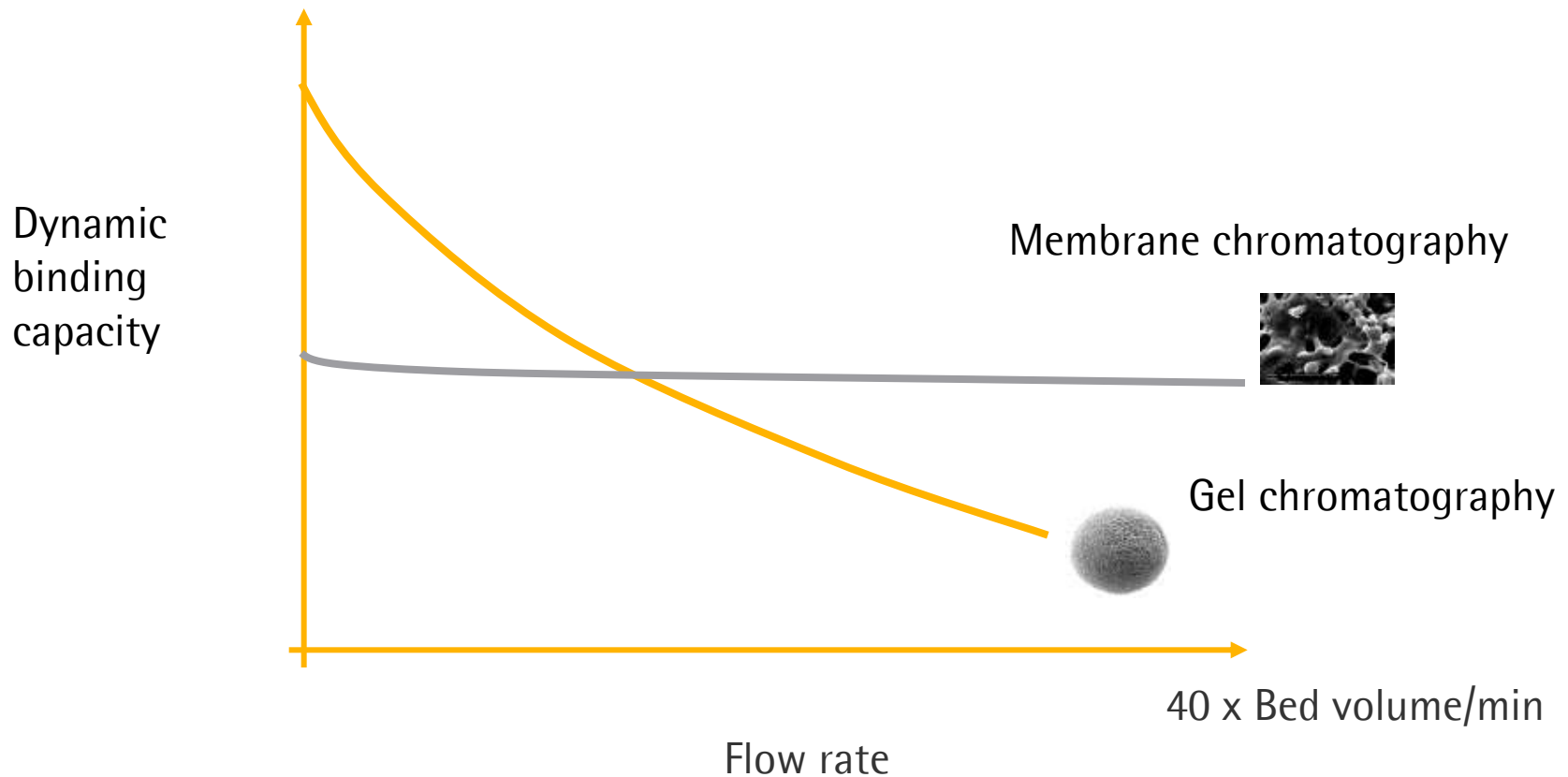
Dynamic binding capacity ./ Size -> Capturing large molecules

Size exclusion for proteins >100 kDa when using gel matrix of microporous 30-50 nm\*



\*E. Karlsson, L. Rydén and John Brewer, Protein Purification, Principles, Jan-Christer Janson, Lars Rydén Eds., VCH Weinheim, pp 123, 1989

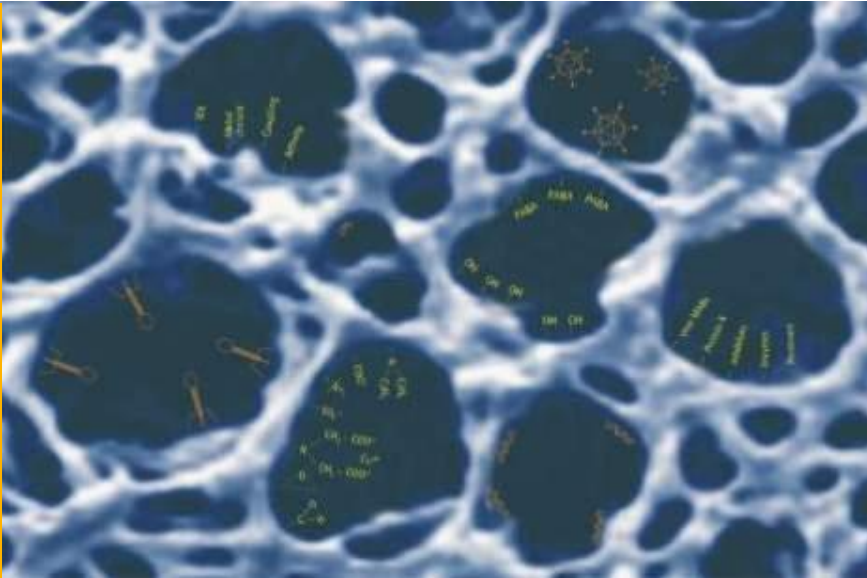
Dynamic binding capacity ./ Flow rate  $\rightarrow$  Contaminant removal





## Formats of Membrane Adsorbers

## Membranes



Ion exchange	Strong: Q, S
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Anion exchange	Weak: D
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Anion exchange	Sartobind STIC® PA (Primary Amine) <u>S</u> alt <u>T</u> olerant <u>I</u> nteraction <u>C</u> hromatography
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HIC	Phenyl
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Metal chelate	Iminodiacetic acid (IDA)
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Coupling	Aldehyde
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Affinity	Protein A
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## Typical binding capacities

Membrane	Description	Dynamic binding capacity 10 %*
Quaternary ammonium (Q)	Strong basic anion exchanger	29 mg/ml (0.8 mg/cm <sup>2</sup> )
Sartobind STIC primary amine (PA)	Weak basic anion exchanger	50 mg/ml (1.4 mg/cm <sup>2</sup> ) in TRIS buffer+150 mM NaCl
Sulfonic Acid (S)	Strong acidic cation exchanger	26 mg/ml (0.7 mg/cm <sup>2</sup> )
Phenyl	Hydrophobic Interaction Chromatographic membrane	14.6 mg/ml (0.4 mg/cm <sup>2</sup> )

\*Standard proteins: BSA (Sartobind Q and STIC) 20 mM TRIS/HCl pH 7.5

lysozyme (S) 10 mM KPi pH 7,

HIC: globulin, 0.9 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

Membrane area: 36.4 cm<sup>2</sup> = 1 ml volume

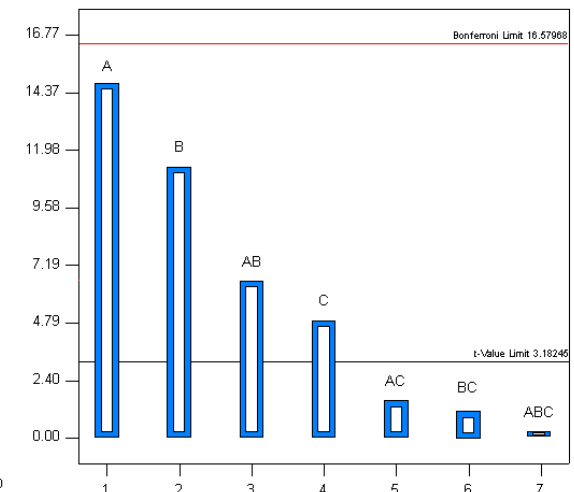
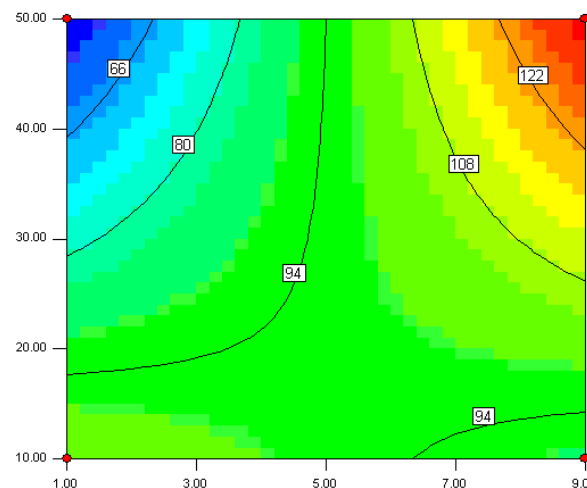
# 96 well-plates for screening



## 96 well plates equipped with 8 strips

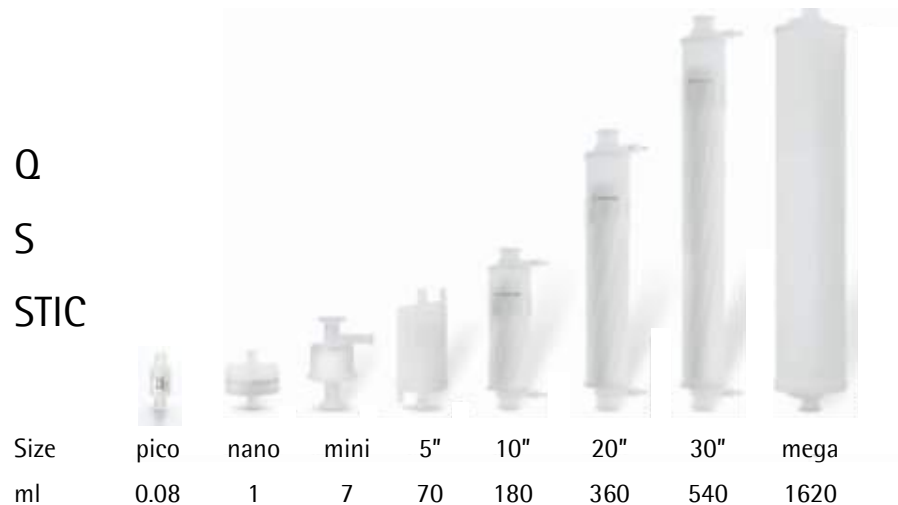
Application	Screening for process development work
Membrane ligands	S, Q, STIC PA and HIC Phenyl
Housing material	Polypropylen
Number of layers	3
Bed height	0.8 mm
Membrane volume	0.019 ml
Membrane diameter	5.5 mm
Maximum well volume [ml]	0.5 ml
Collection plate volume [ml]	2 ml

- Screening of conditions (e.g. pH, conductivity, load)
- DoE
- Avoid to use too much product and too much Sartobind
- Available with Q, S, Phenyl and STIC



# Sartobind membrane adsorber portfolio process scale

4 mm bed height



**Contaminant removal:** flowthrough mode to remove DNA, Host cell proteins, endotoxins, viruses

Singe-use

8 mm bed height



**Purification:** bind & elute of viruses and virus like particles, large proteins

Single-use / intra-batch use

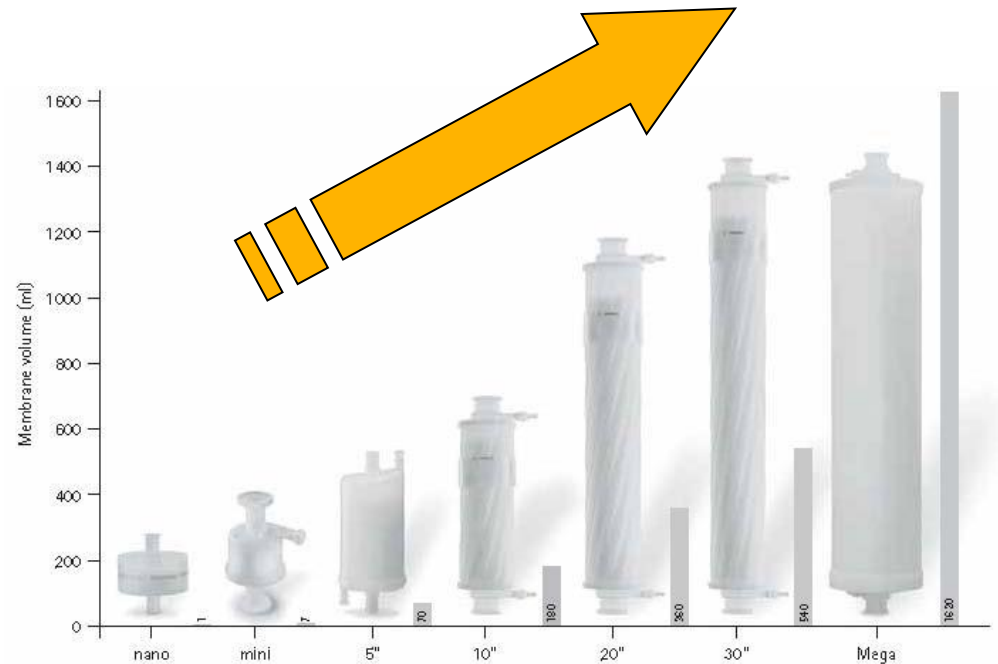
## General scale-up rules remain unchanged

### Maintain

- Bed height (4mm or 8mm)
- Linear flow
- Sample concentration
- Gradient volume: media volume

### Increase

- Membrane volume
- Volumetric flow rate
- Sample loading







Applications: Polishing (Flowthrough)

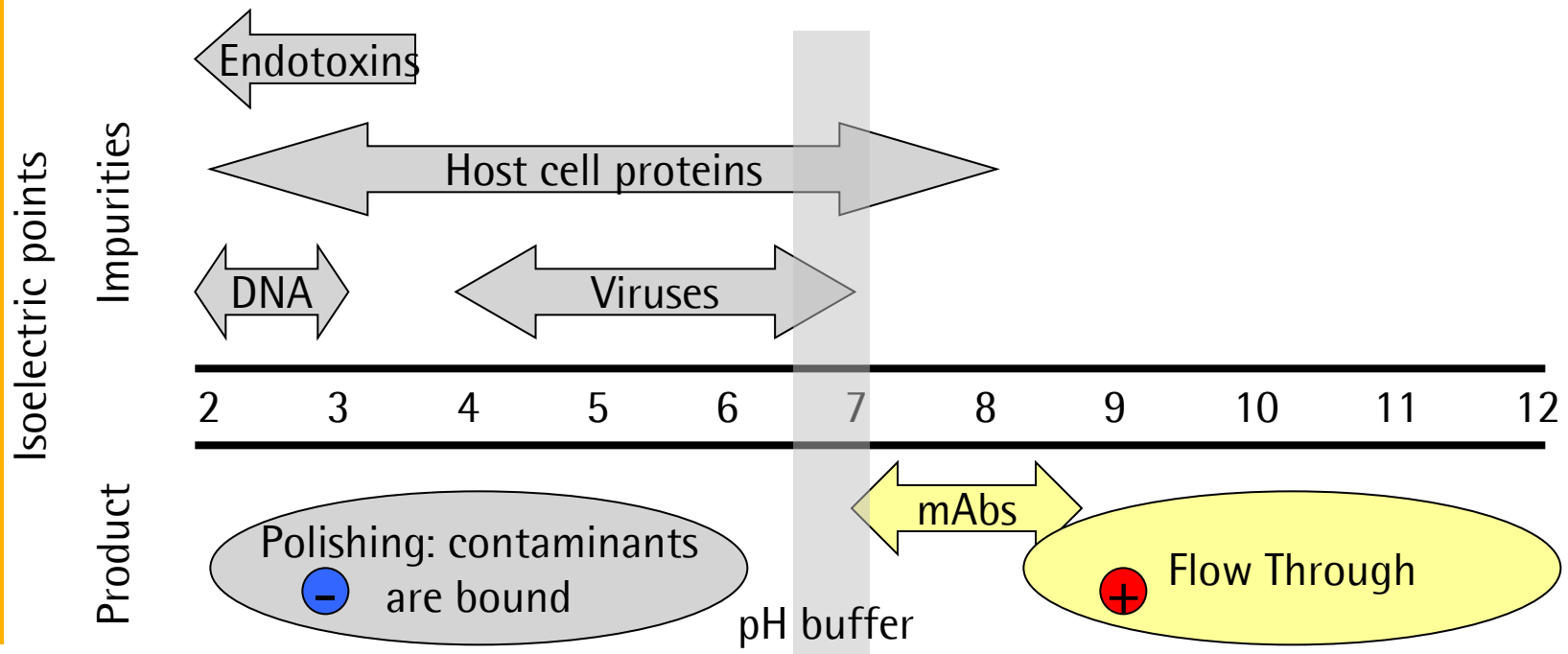
## Why use Membrane Adsorber downstream in mAb production – major drivers

- No validation
- Saves up to 95 % buffer
- At least 10 times faster
- Effective removal of DNA, Endotoxins, Viruses, Host Cell Proteins

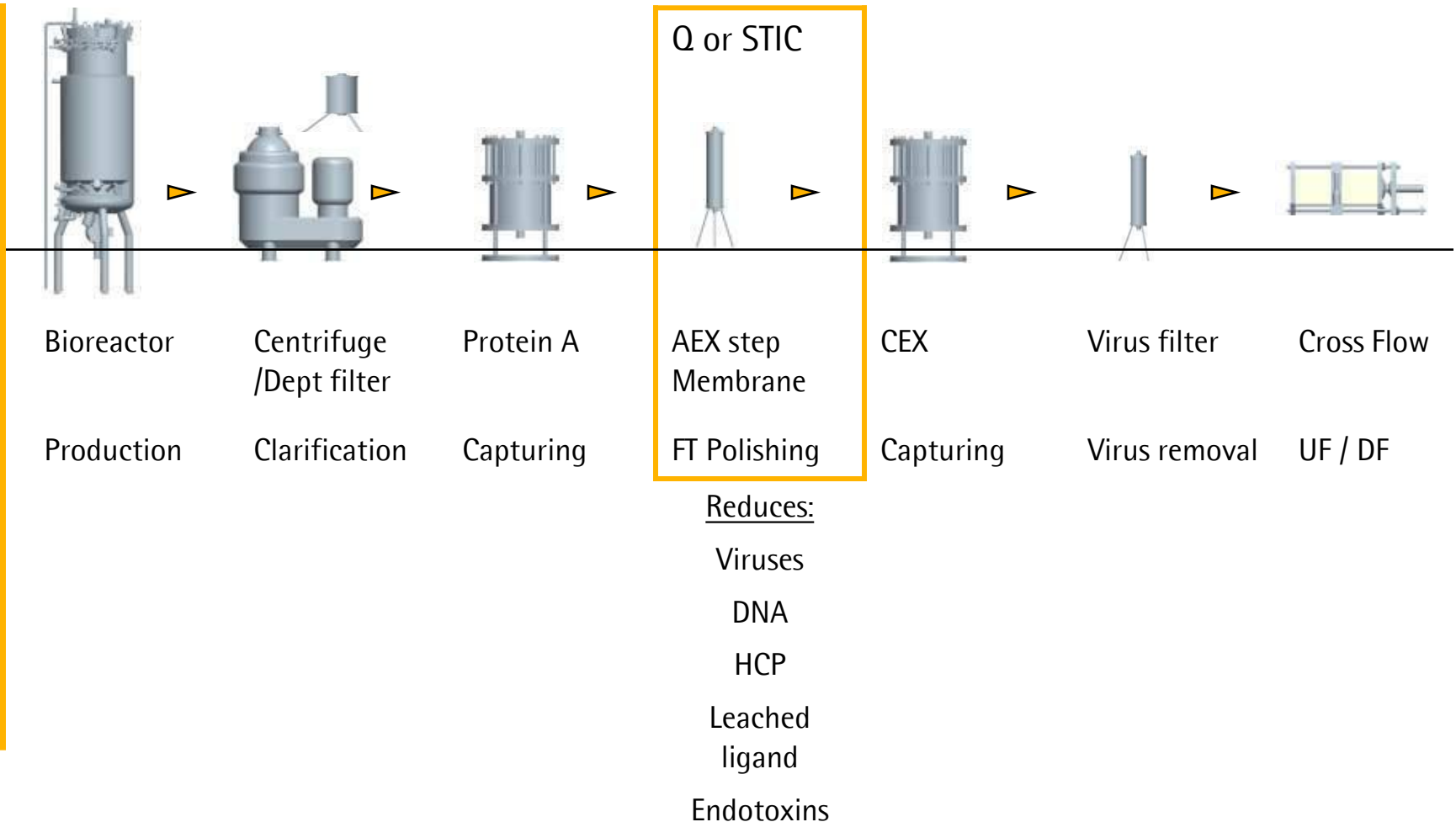


# Removal of contaminants during monoclonal antibody production

Positively (+) charged adsorber binds negatively (-) charged contaminants

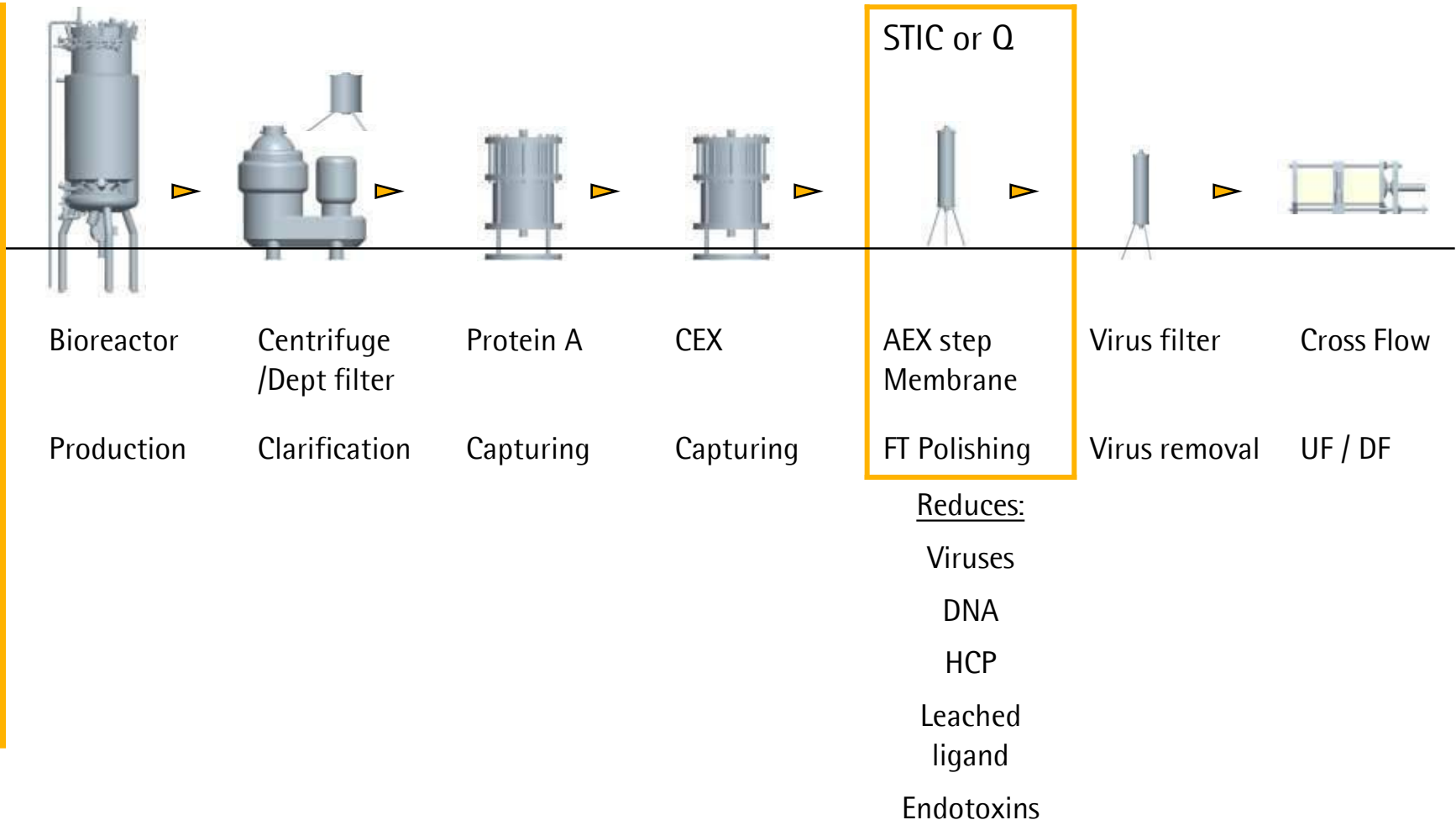


Anion exchange step in flowthrough polishing 2<sup>nd</sup> step:  
Sartobind Q may be sufficient, mAb dependent – both are options





# Anion exchange step in flowthrough polishing 3<sup>rd</sup> step: Position for Sartobind STIC



## Purification bottleneck – Facility / Tank size limitation at high mAb titers:



**5 g/L mAb**

**10000 Liters**



**200 L Protein A 30 g/L, 8 cycles, 2.5 CV Pool**

**4000 Liters**



**500 L CEX 100 g/L, 6 CV Pool, 12-15 mS/cm**

**3000 Liters**



**Q needs 4-7 mS/cm, dilution 1:1**

**6000 Liters**

**AEX 10 kg/L FT**



Purification bottleneck – Facility / Tank size limitation at high titers, e.g.:



**5 g/L mAb**

**10000 Liters**



**200 L Protein A 30 g/L, 8 cycles, 2.5 CV Pool**

**4000 Liters**



**500 L CEX 100 g/L, 6 CV Pool, 12-15 mS/cm**

**3000 Liters**



**Sartobind STIC AEX 10 kg/L FT**

**no need for 6000 Liter tank**





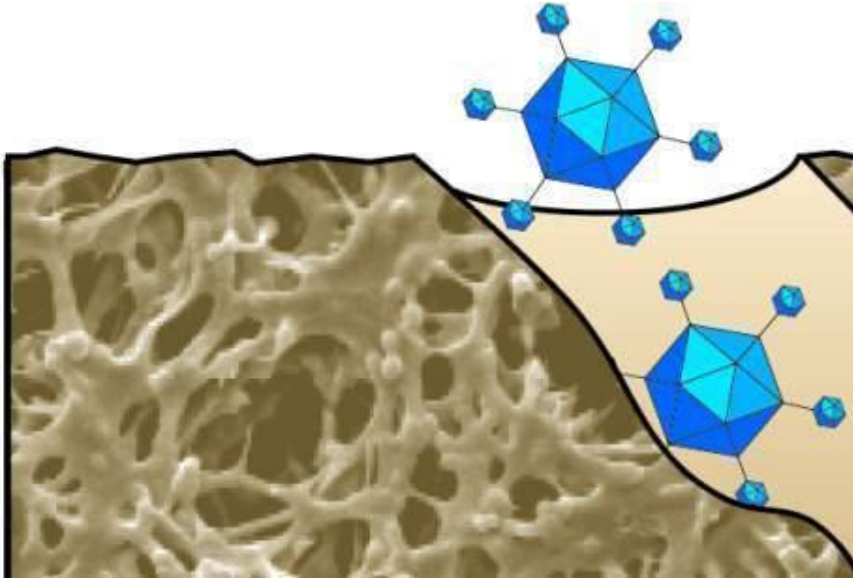
Application: Capture



## Applications Capture

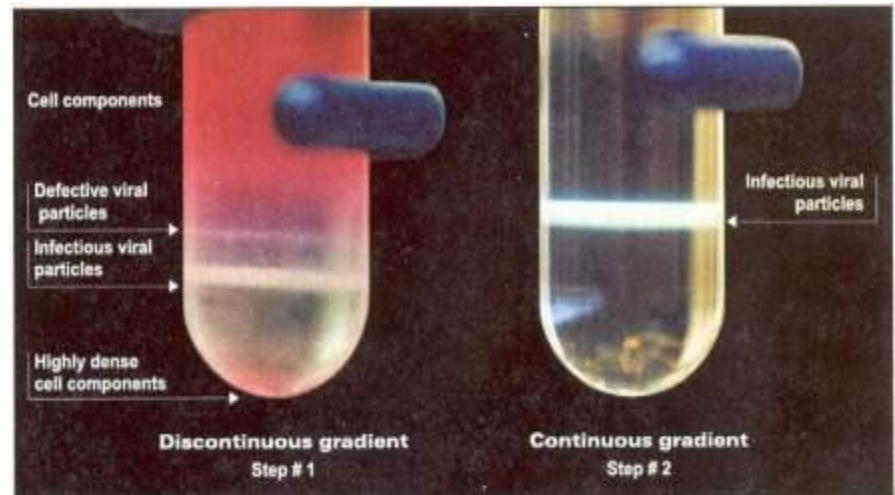
- Large molecules such as Factor VIII
- Vaccines speeds up in importance of sales

## Sartobind: successful in virus/vaccine purification



- Adenoviruses
- Lentiviruses
- Adenoassociated viruses
- Baculoviruses
- Densonucleosis viruses
- Foot and mouth disease v.
- Influenza viruses
- Alpha herpes viruses
- Rabies viruses
- Conjugated vaccines
- Phages

## Membrane Adsorbers vs. Density Gradient – Adenovirus purification



### Membrane Adsorbers

2 hours

Up to  $10^{13}$  VP/ml

No carryover, disposable, no validation, simple

No contaminants

### Density gradient ultra centrifugation

36 hours

$10^6$  VP/ml

Carryover validation

Toxic  $\text{CsCl}_2$ , sucrose removal from finished product

## Membrane Adsorbers vs. Columns – Adenovirus purification



Jumbo 5 l



### Membrane Adsorbers

Time for processing 1 h

Size used 5 liter

No dilution by void volume  
optimization

### Gel columns

25 h

50 liter gel needed

Loss by long process times

## Summary

- Membranes Adsorbers are powerful tools in the biopharmaceutical industry to
  - remove contaminants from antibodies
  - capture large molecules, vaccines
  - simplify processes, and reduce cost.
- Sartorius has great experience in the market
  - most sizes and largest customer base

Thank you!



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