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

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Sartorius Stedim Biotech

# Agenda

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

# Introduction & Membrane Chromatography features

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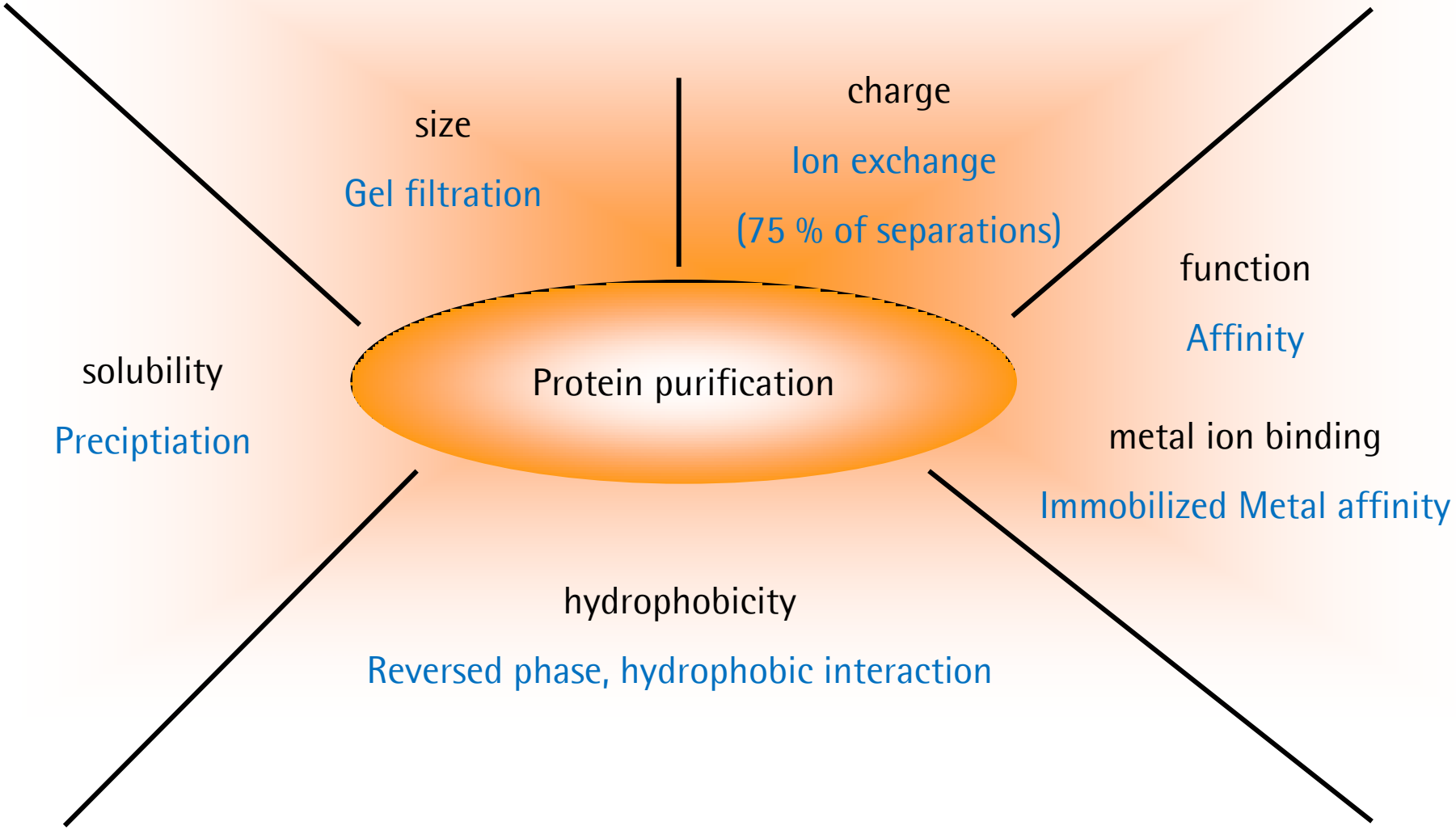
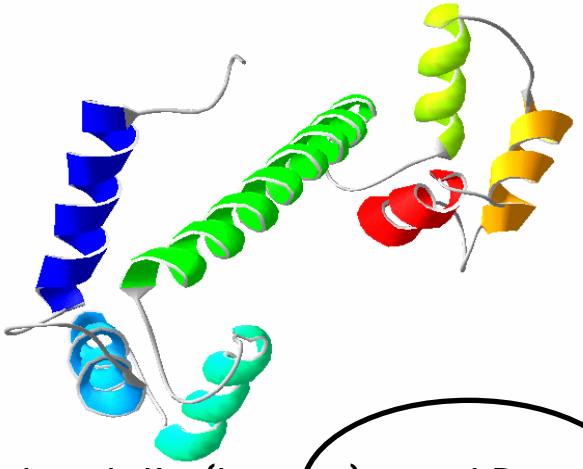
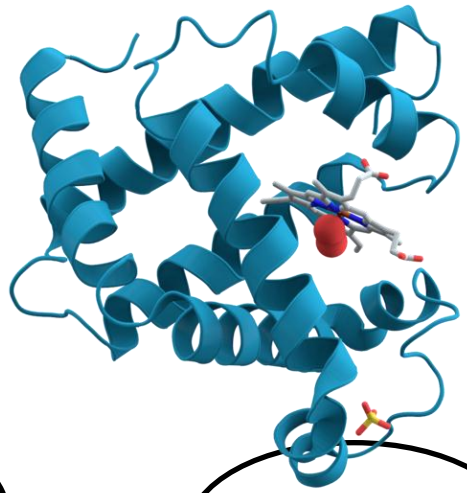
# Chromatography uses chemical and physical properties



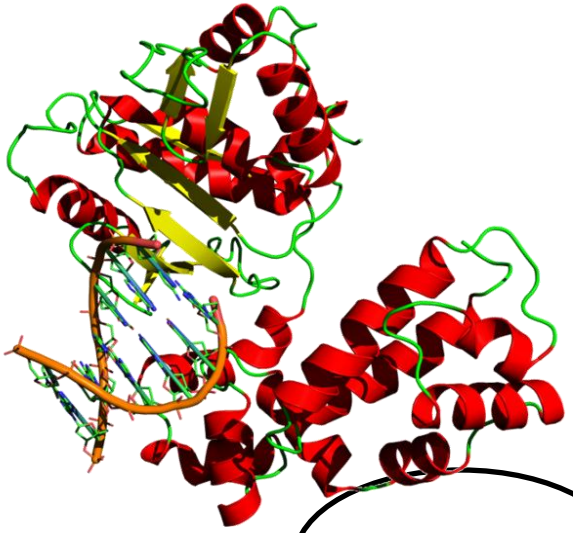
# Proteins vary in **size**



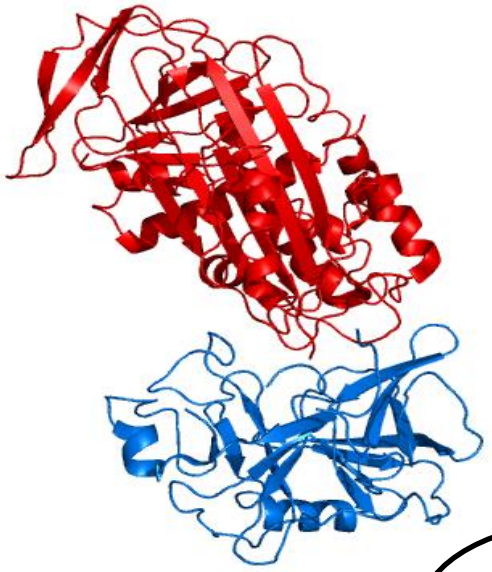
Calmodulin (human) 16.9 kDa



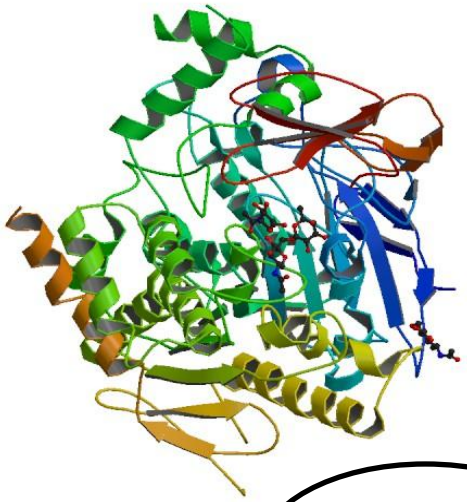
Myoglobin 17.7 kDa



DNA polymerase (human) 103 kDa



a-antitrypsin (human serum) 44.5 kDa



Acetylcholinesterase (64 kDa)

# Proteins vary in **shape and chemical properties**

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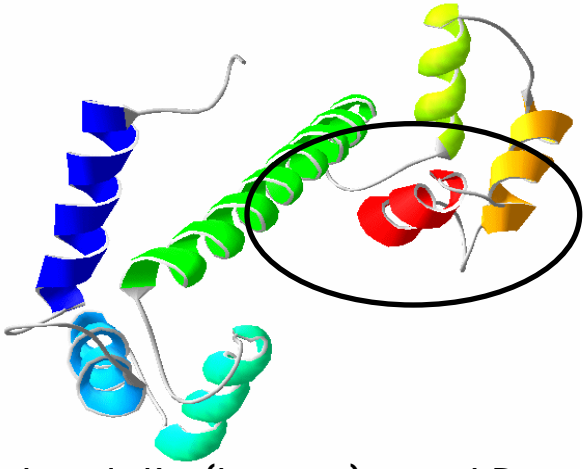

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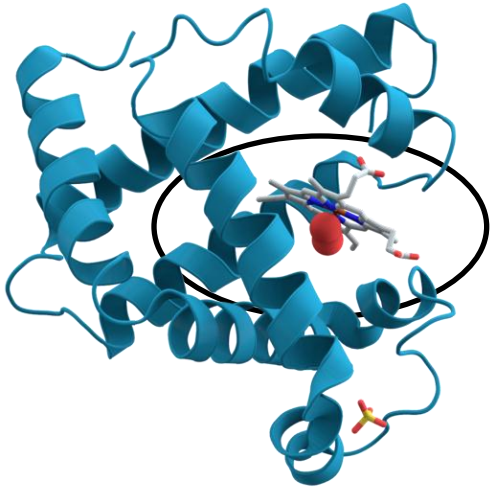

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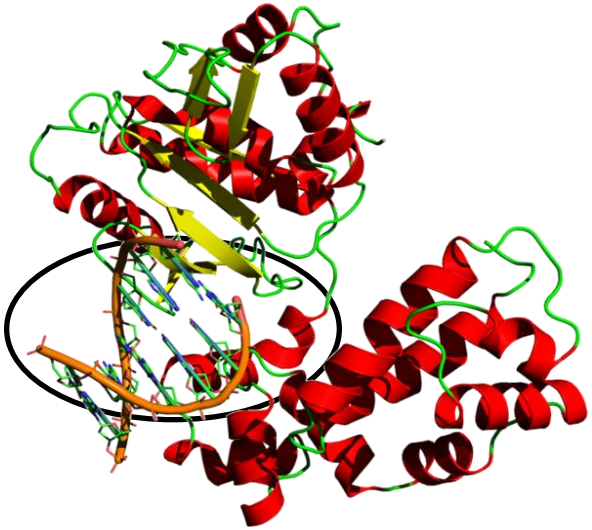

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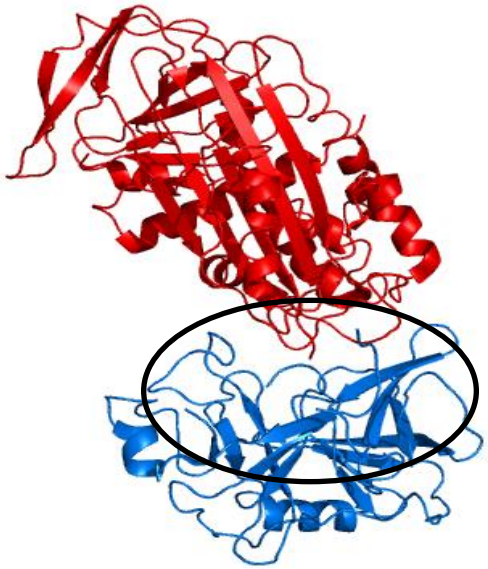

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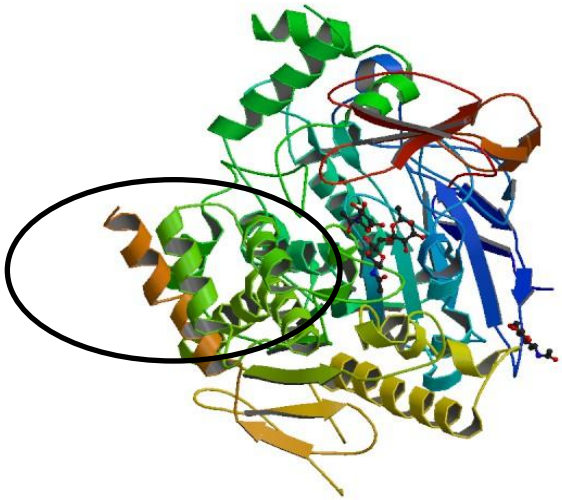
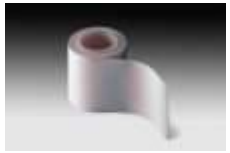
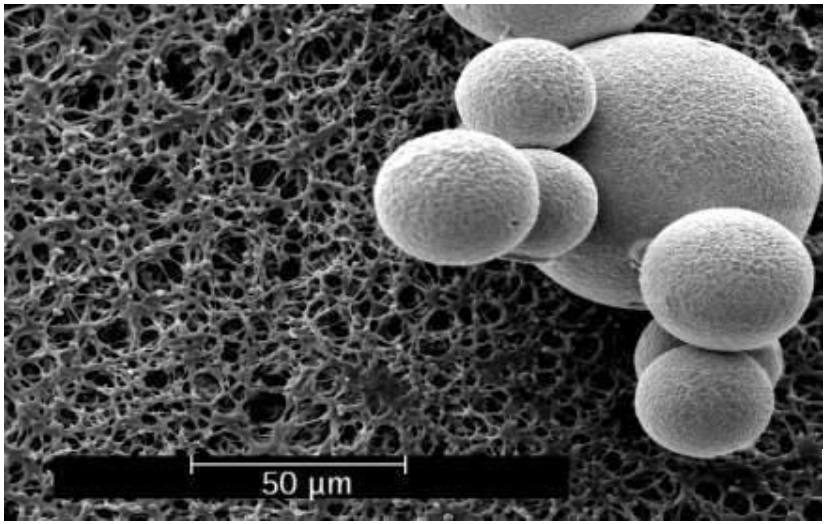


Acetylcholinesterase (64 kDa)

# Chromatographic ligands are bound to a solid matrix

Membrane adsorber:
Sartobind Q



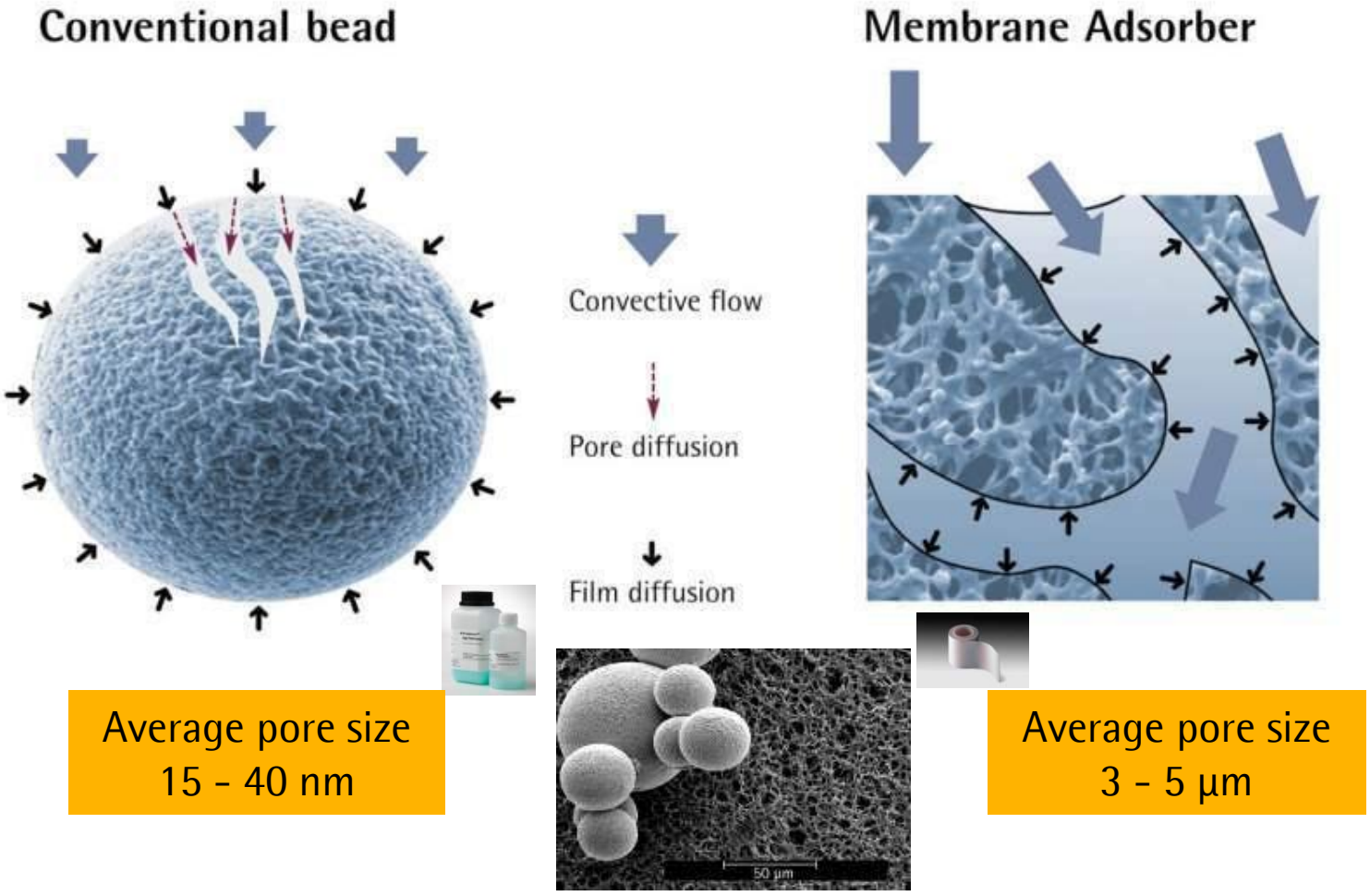



Gel particles:
Q Sepharose FF

Ready to use

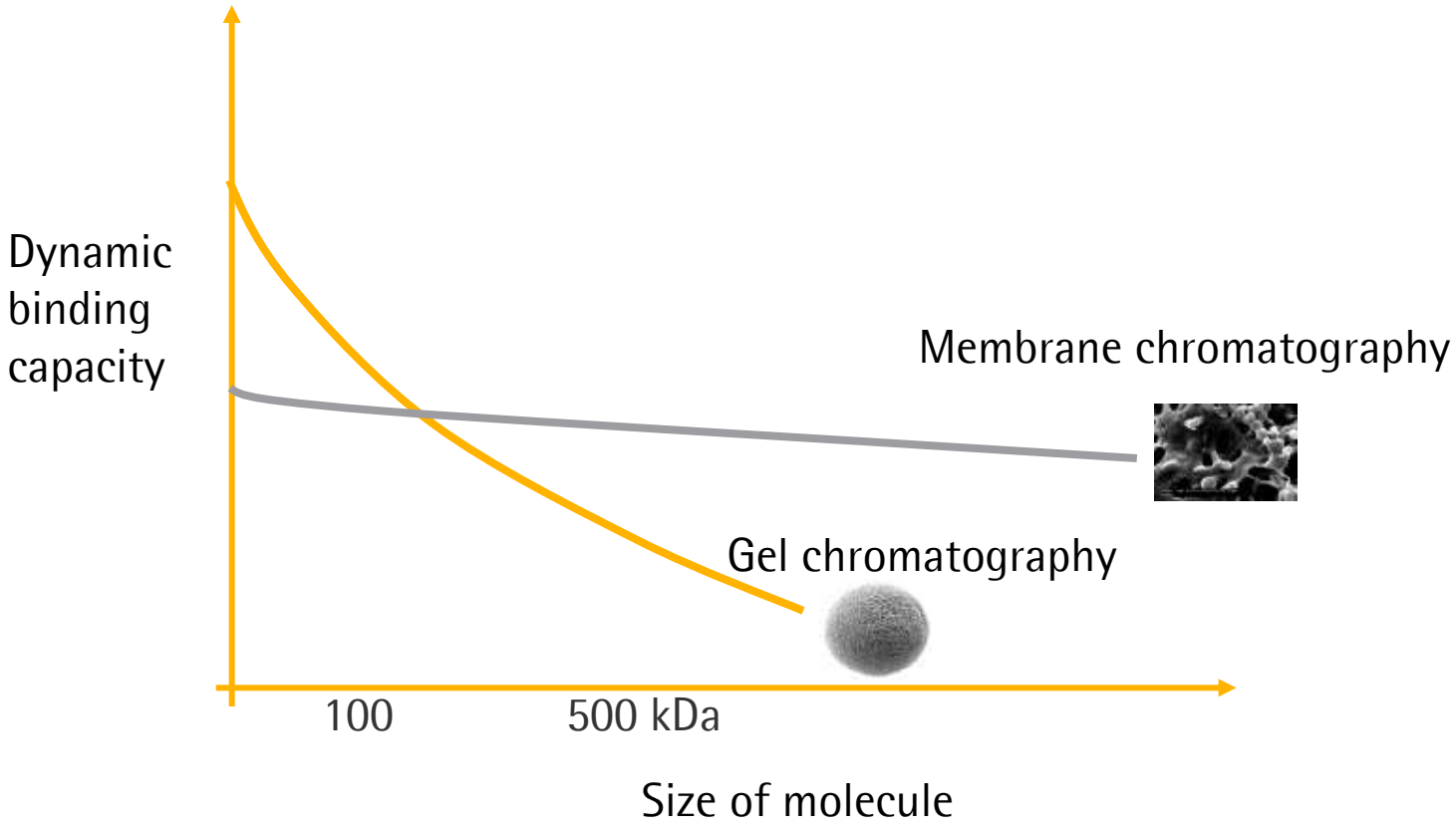
Filled into column by user

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



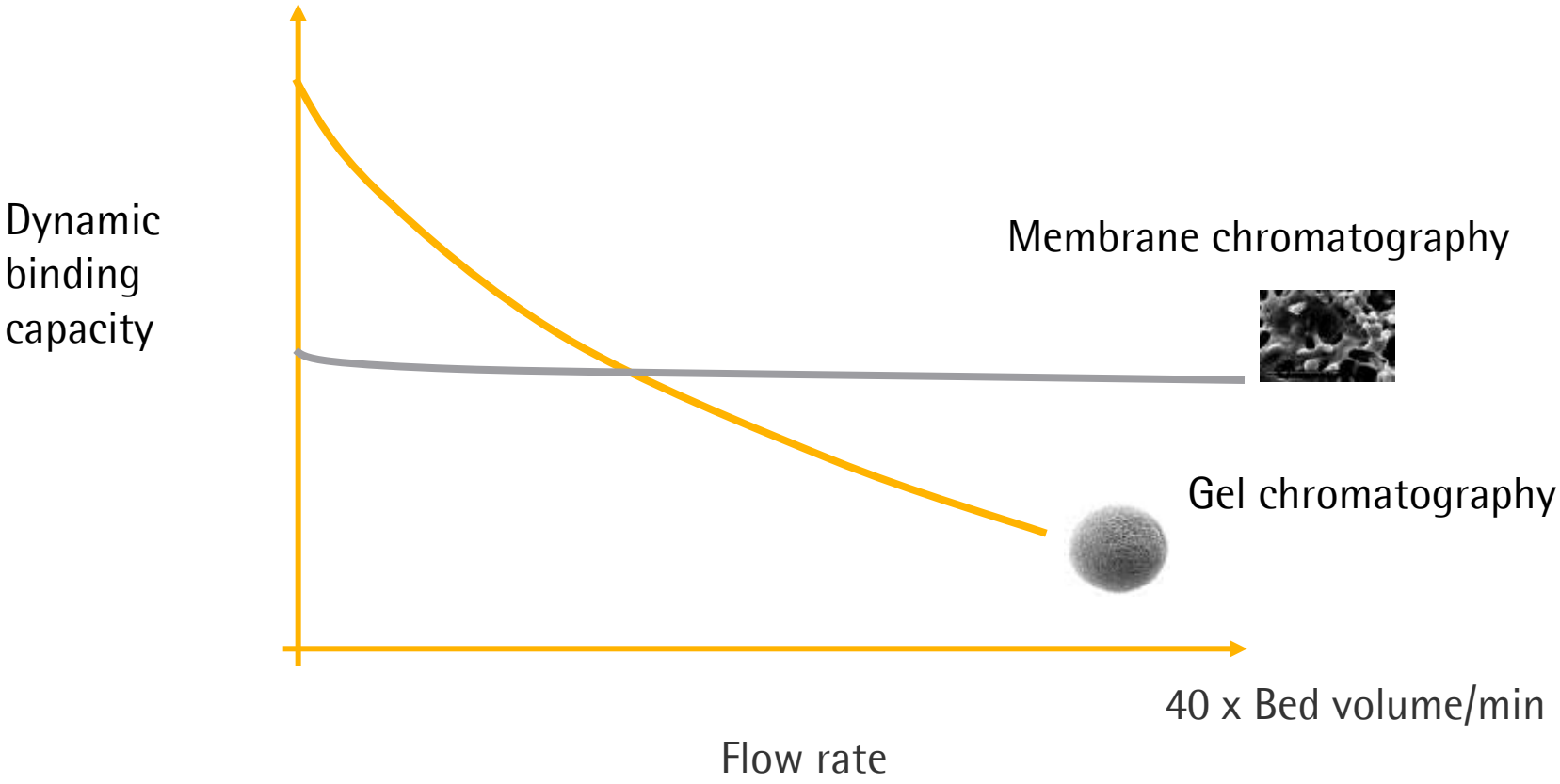
# Dynamic binding capacity ./. Size -> Capturing large molecules

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



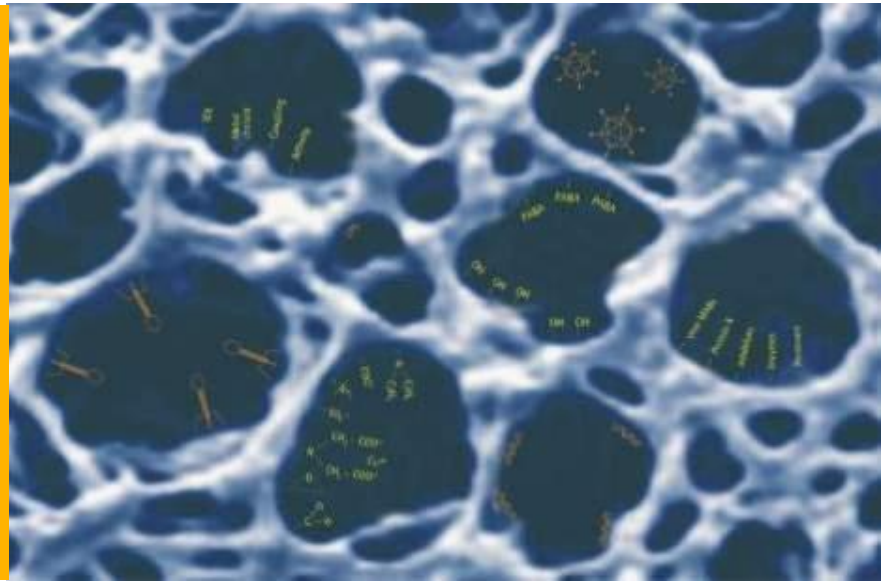

*E. Karlsson, L. Rydén and John Brewer, Protein Purification, Principles, Jan-Christer Janson, Lars Rydén Eds., VCH Weinheim, pp 123, 1989

# Dynamic binding capacity ./. Flow rate -> Contaminant removal



# Formats of Membrane Adsorbers

# Membranes



| | |
|---|---|
| Ion exchange | Strong: Q, S |
| Anion exchange | Weak: D |
| Anion exchange | Sartobind STIC® PA (Primary Amine)<br>Salt Tolerant Interaction Chromatography |
| HIC | Phenyl |
| Metal chelate | Iminodiacetic acid (IDA) |

| | |
|---|---|
| Coupling | Aldehyde |
| Affinity | Protein A |

# Typical binding capacities

| Membrane | Description | Dynamic binding capacity 10 %* |
|---|---|---|
| Quaternary ammonium (Q) | Strong basic anion exchanger | 29 mg/ml (0.8 mg/cm²) |
| Sartobind STIC primary amine (PA) | Weak basic anion exchanger | 50 mg/ml (1.4 mg/cm²) in TRIS buffer+150 mM NaCl |
| Sulfonic Acid (S) | Strong acidic cation exchanger | 26 mg/ml (0.7 mg/cm²) |
| Phenyl | Hydrophobic Interaction Chromatographic membrane | 14.6 mg/ml (0.4 mg/cm²) |

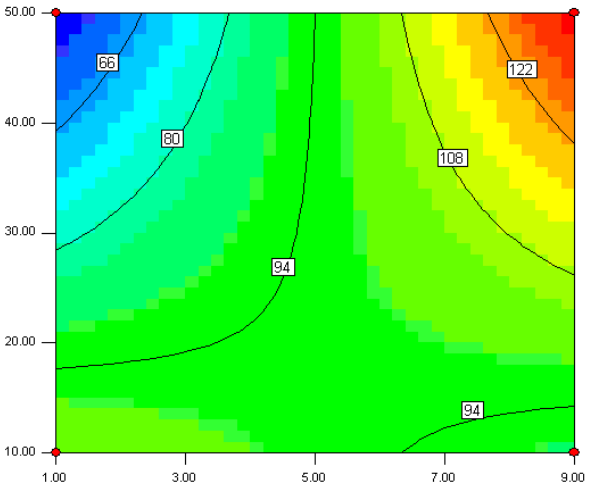
*Standard proteins: BSA (Sartobind Q and STIC) 20 mM TRIS/HCl pH 7.5
lysozyme (S) 10 mM KPi pH 7,
HIC: globulin, 0.9 M $(NH_4)_2SO_4$

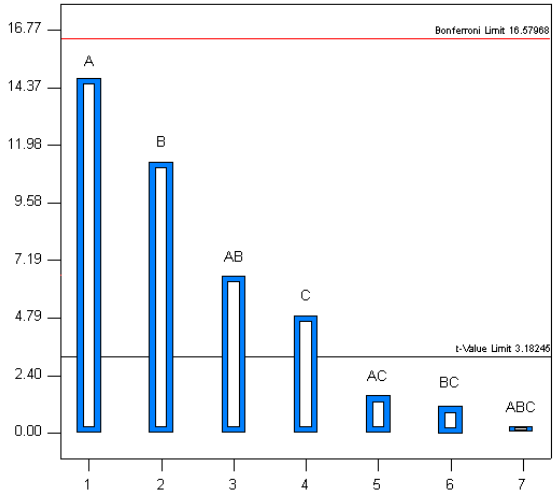
Membrane area: 36.4 cm² = 1 ml volume

# 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 |

- Sceening 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

Q

S

STIC

| Size | pico | nano | mini | 5" | 10" | 20" | 30" | mega |
|---|---|---|---|---|---|---|---|---|
| ml | 0.08 | 1 | 7 | 70 | 180 | 360 | 540 | 1620 |

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

Singe-use

## 8 mm bed height

Q

S

HIC

| | nano | 150 ml | 1200 ml | Jumbo |
|---|---|---|---|---|
| | 3 | 150 | | 5000 ml |

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

Single-use / intra-batch use

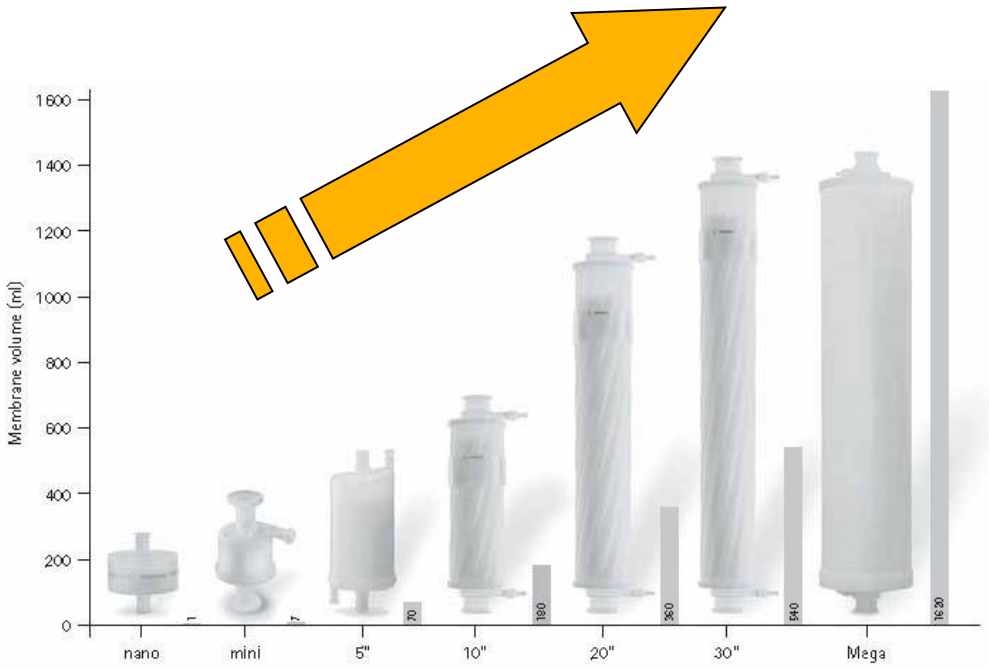
# General scale-up rules remain unchanged

## Maintain

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

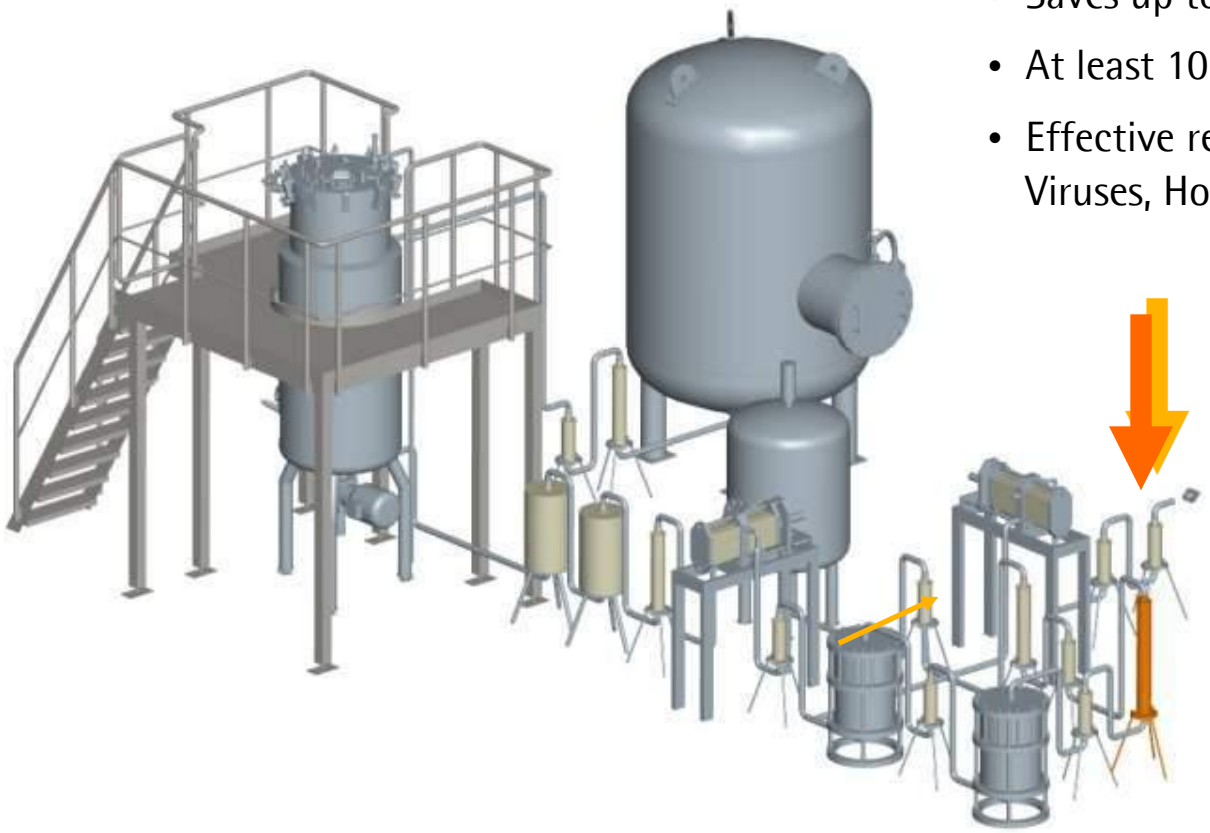
## Increase

- Membrane volume
- Volumetric flow rate
- Sample loading



# Applications: Polishing (Flowthrough)

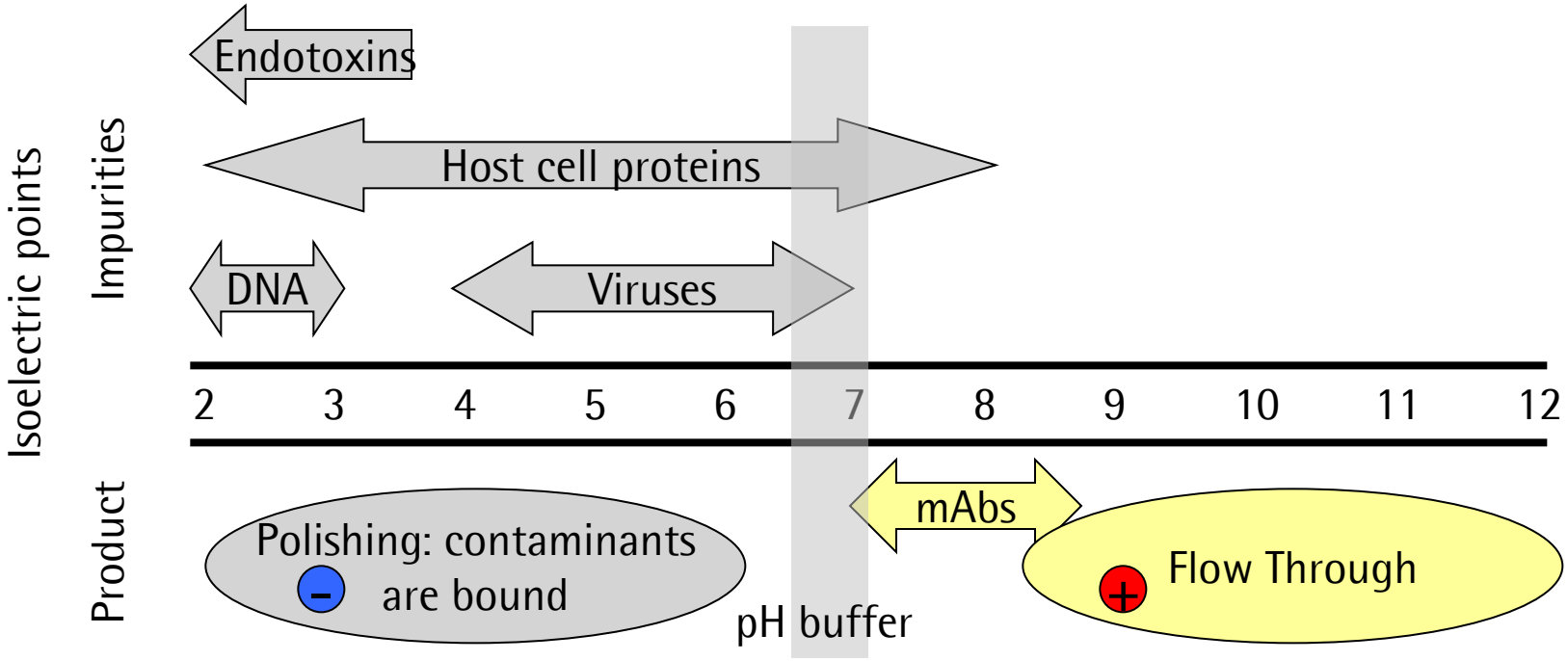
# Why use Membrane Adsorber downstream in mAb production – major drivers



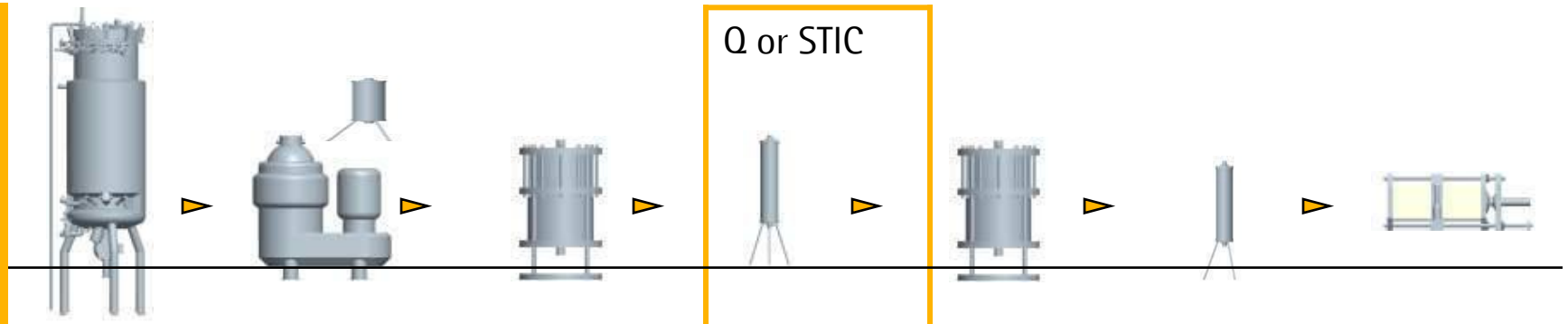
- No validation
- Saves up to 95 % buffer
- At least 10 times faster
- Effective removal of DNA, Endotoxins, Viruses, Host Cell Proteins

# Removal of contaminants during monoclonal antibody production

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



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

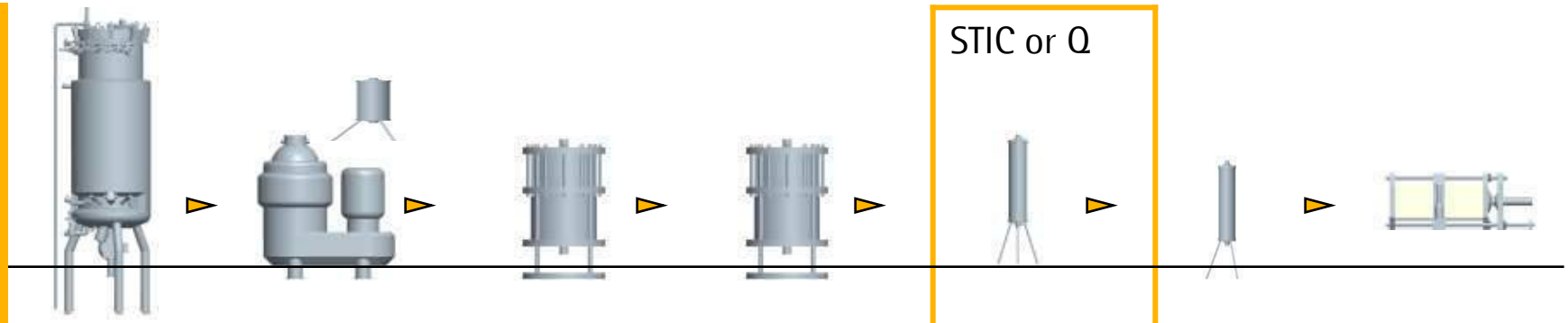



| Bioreactor | Centrifuge /Dept filter | Protein A | AEX step Membrane | CEX | Virus filter | Cross Flow |
|---|---|---|---|---|---|---|
| Production | Clarification | Capturing | FT Polishing | Capturing | Virus removal | UF / DF |

Reduces:

- Viruses
- DNA
- HCP
- Leached ligand
- Endotoxins

# Anion exchange step in flowthrough polishing 3rd step: Position for Sartobind STIC




| Bioreactor | Centrifuge /Dept filter | Protein A | CEX | AEX step Membrane | Virus filter | Cross Flow |
|---|---|---|---|---|---|---|
| Production | Clarification | Capturing | Capturing | FT Polishing | Virus removal | UF / DF |

Reduces:

Viruses

DNA

HCP

Leached ligand

Endotoxins

# 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

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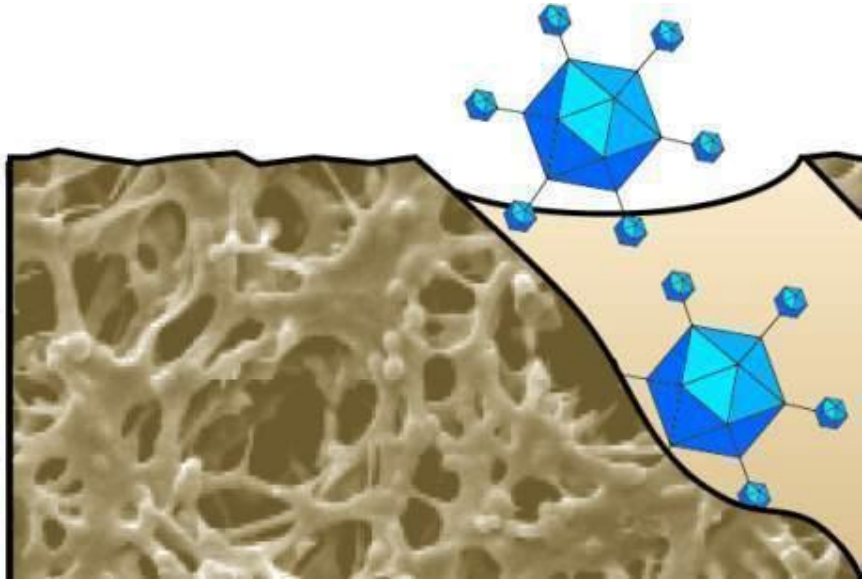
# Application: Capture

# Applications Capture

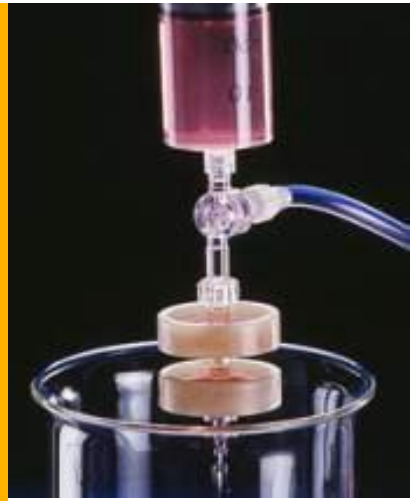
- Large molecules such as Factor VIII
- Vaccines speeds up in importance of sales

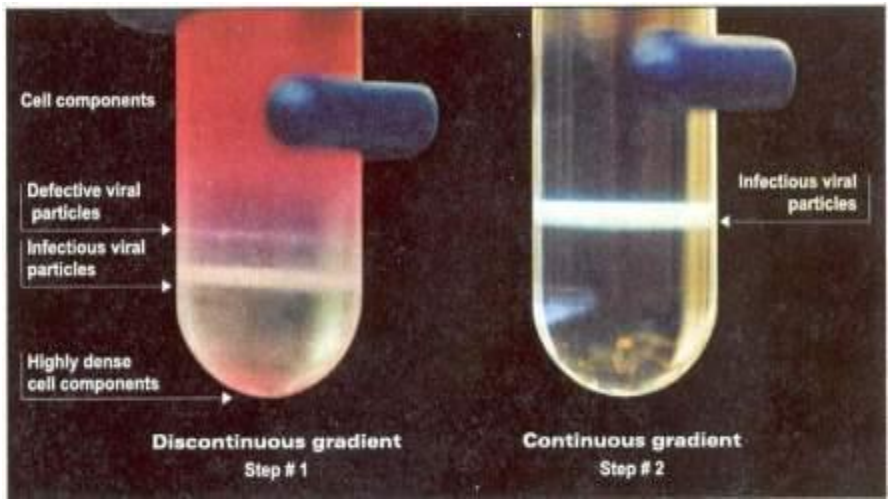
# Sartobind: successful in virus/vaccine purification



- Adenoviruses
- Lentiviruses
- Adenoassociated viruses
- Baculoviruses
- Densonucleosis viruses
- Foot and mouth desease v.
- Influenza viruses
- Alphaherpes viruses
- Rabies viruses
- Conjugated vaccines
- Phages

# Membrane Adsorbers vs. Density Gradient – Adenovirus purification






| Membrane Adsorbers | Density gradient ultra centrifugation |
|---|---|
| 2 hours | 36 hours |
| Up to $10^{13}$ VP/ml | $10^{6}$ VP/ml |
| No carryover, disposable, no validation, simple | Carryover validation |
| No contaminants | Toxic $CsCl_2$, sucrose removal from finished product |

# Membrane Adsorbers vs. Columns – Adenovirus purification


| Membrane Adsorbers | Gel columns |
|---|---|
| Time for processing 1 h | 25 h |
| Size used 5 liter | 50 liter gel needed |
| No dilution by void volume optimization | 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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