Vaccine Downstream processing –an overview

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Imagination at work

Overview

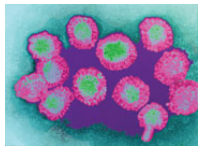
- Vaccines overview
- Demands on vaccine purification
- Common techniques for vaccine purification
- Example of a purification process
- Summary

Vaccine Overview

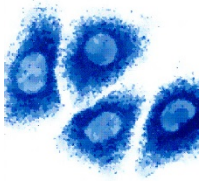
How Vaccines are manufactured

The Vaccines

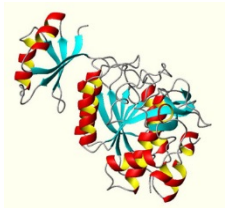
Bacteria based



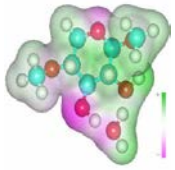
Virus based



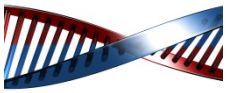
Protein based



Polysaccharide based



DNA based



The Manufacturing process

Cell culture / Fermentation

↓

Purification

↓

Fill and Finish

Analysis (QC/QA)

Number and order of the different steps depends on the specific vaccine production

Demands on vaccine purification

Safety and quality is priority

Regulatory requirements

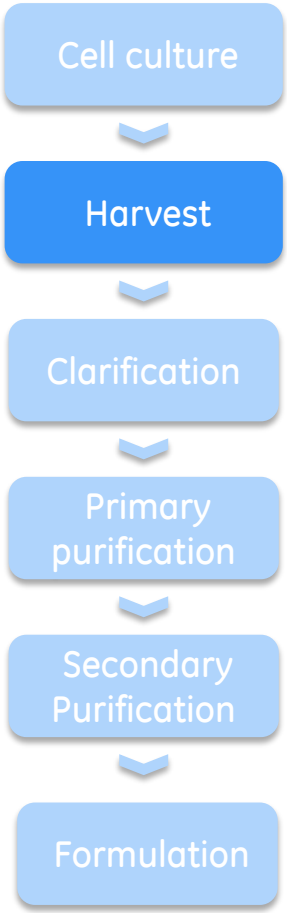
- Safe vaccine with no or minimal adverse effects
- Effective dose
- Stability
- Process control
- Reproducable process

Downstream purification of vaccines

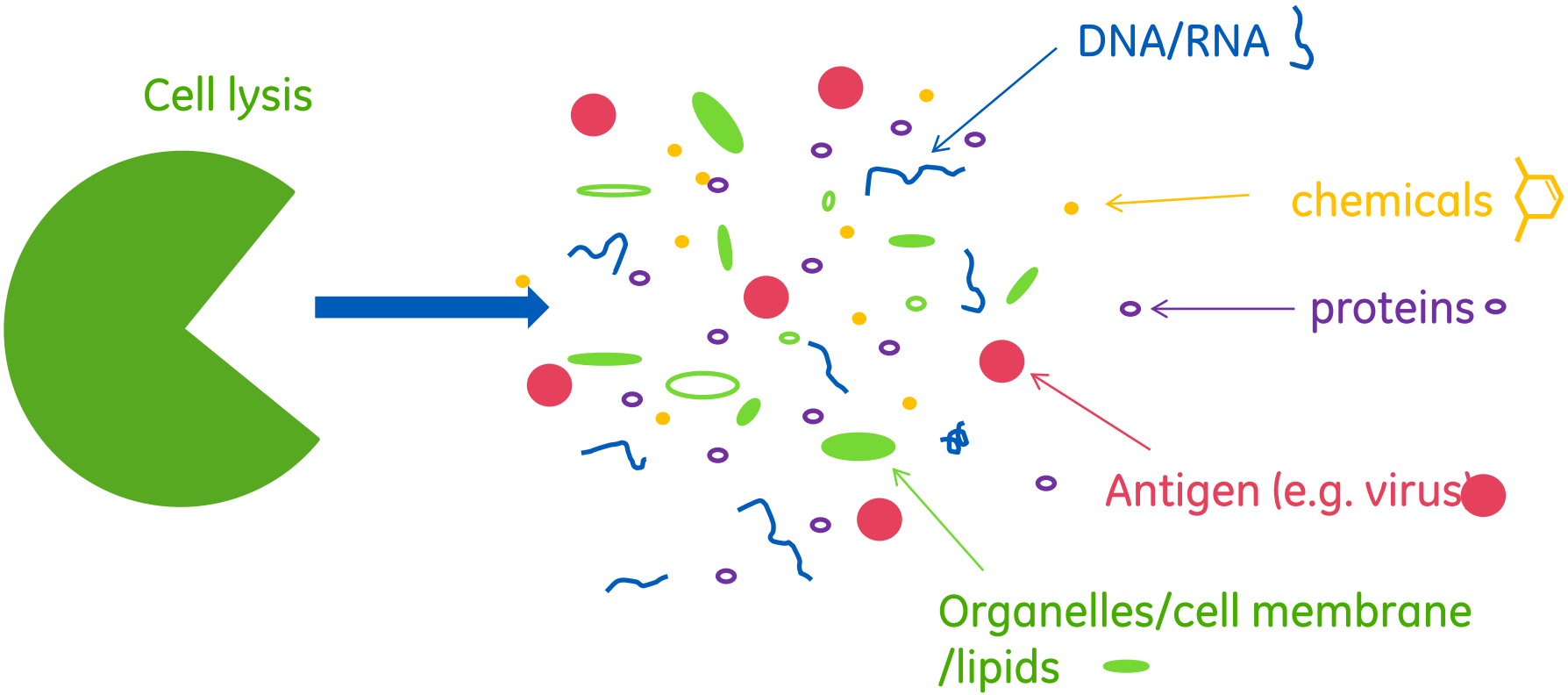
Downstream processing of viruses Available technologies

Harvest

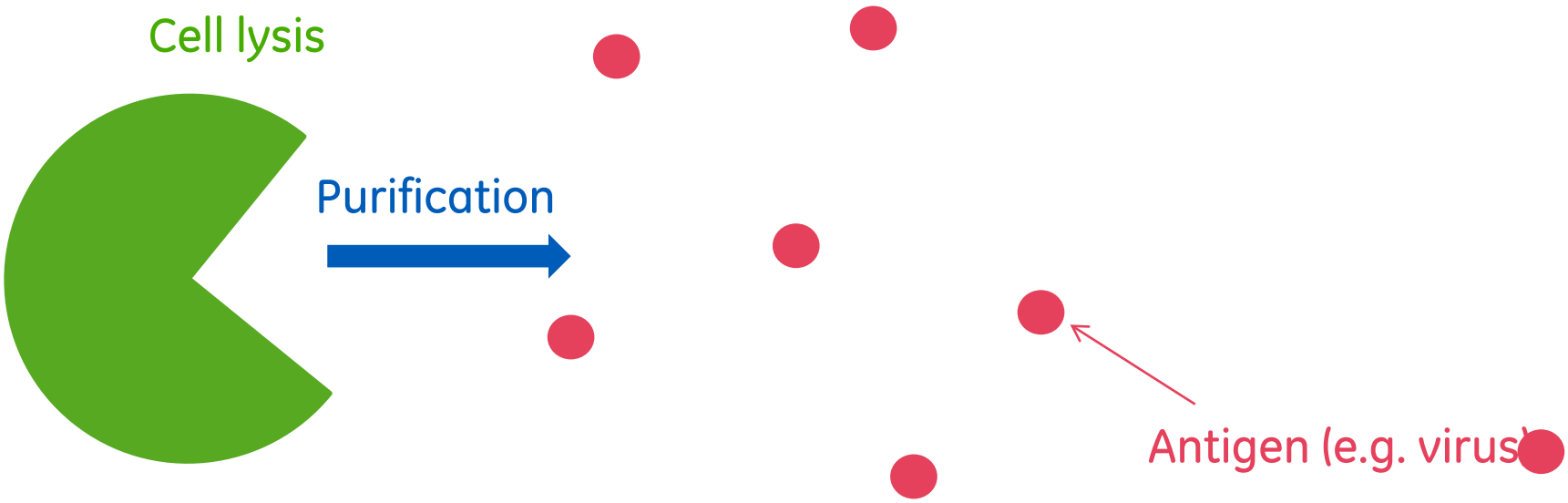
- Lytic virus
- Non-lytic virus
  - Detergent
  - Mechanical disruption / Homogenization
  - Osmotic shock
  - Freeze-thaw



Impurity challenges after lysis



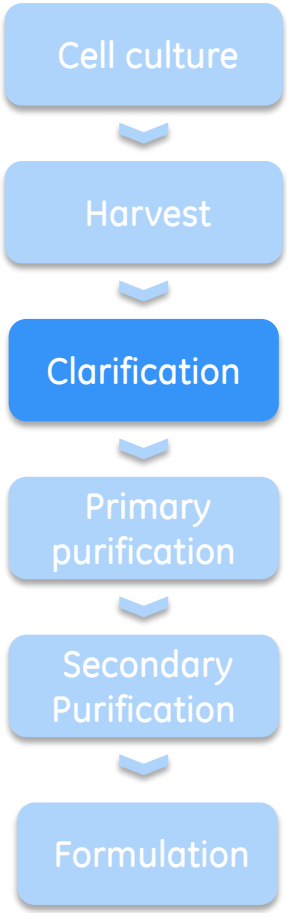
Goal with purification



Downstream processing of viruses
Available technologies

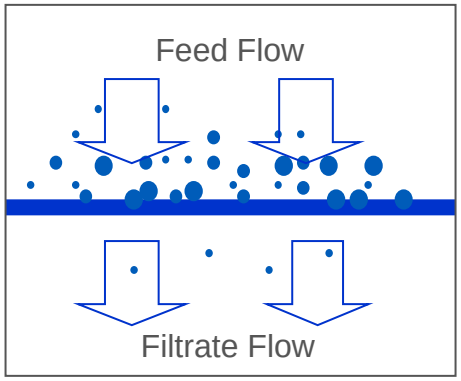
Clarification

- Filtration
  - Normal flow
  - Tangential flow
- Centrifugation



Normal flow filtration

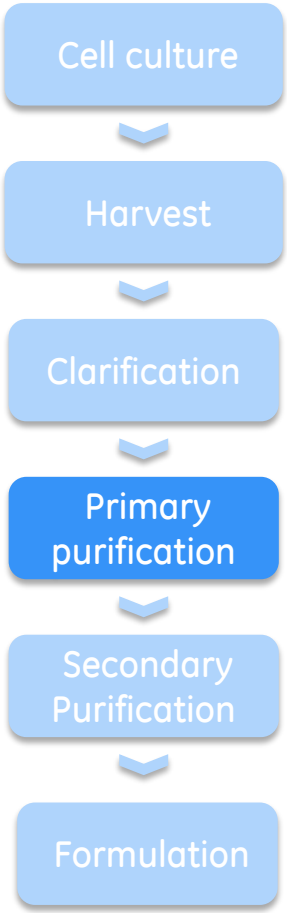
- Removal of cell debris and larger particulates
- Porosities from 0.2 - 20 µm
- Scalable
- Single-use
- Straight forward process set up
- Not recommended for harvest with high particulate content



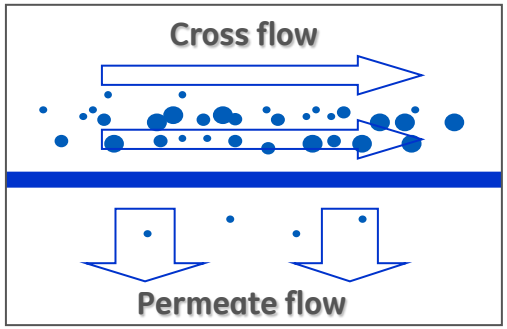
Downstream processing of viruses
Available technologies

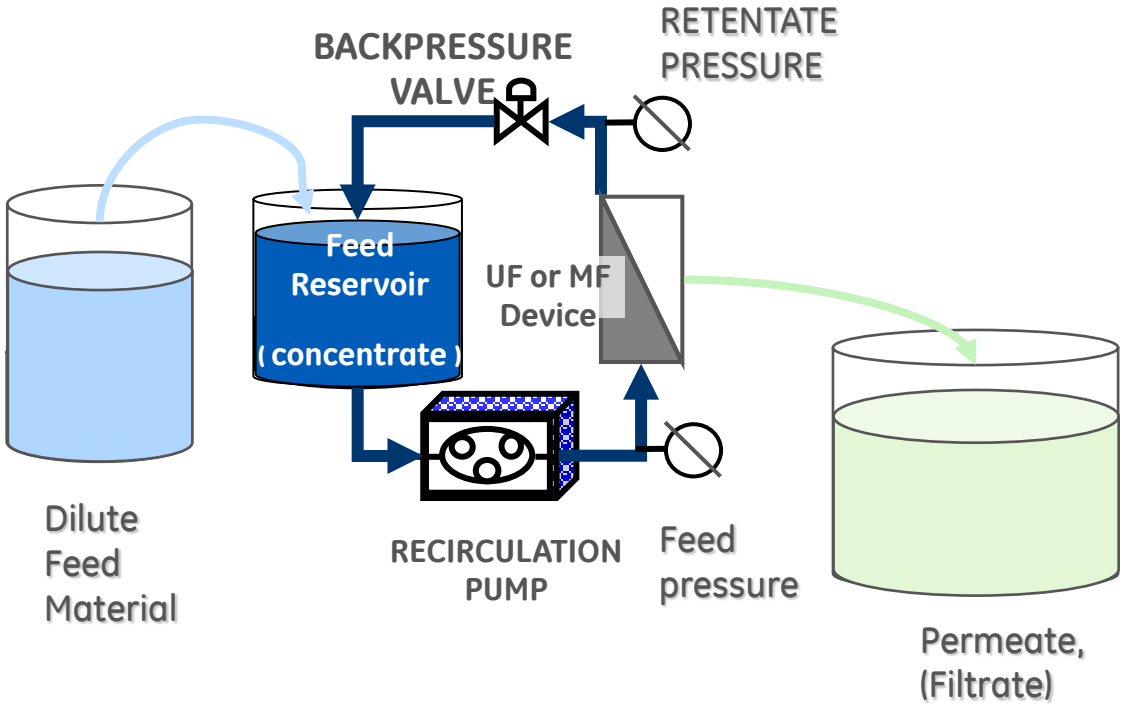
Primary purification

- Tangential flow filtration (TFF)
- Density gradient centrifugation
- Precipitation
- Chromatography



Tangential flow filtration





- Sweeping effect clean filter surface
- Allow greater throughput on smaller surface area

Tangential flow filtration

Hollow fiber filters

- Hollow fiber cartridge consists of tubular fibers
- Concentration/ diafiltration
- Microfiltration
- Suitable for shear sensitive material
- Possible handle high particle loads (ex cell harvest)
- Defined pore sizes
- Re-usable
- Scalable

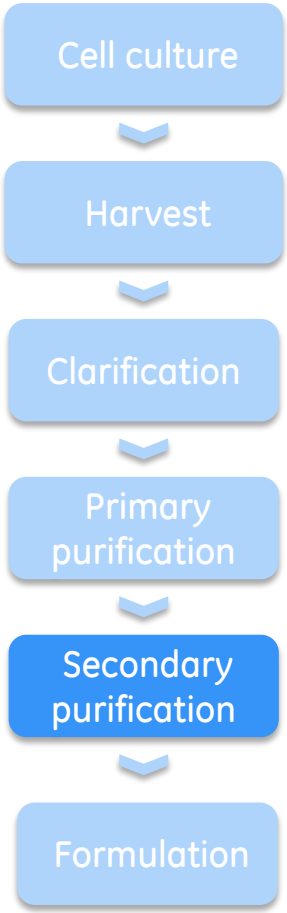
Flat sheet cassettes

- Cassettes consists of sheet membranes
- Concentration/ diafiltration
- Defined pore sizes
- Re-usable
- Scalable

Downstream processing of viruses Available technologies

Secondary purification

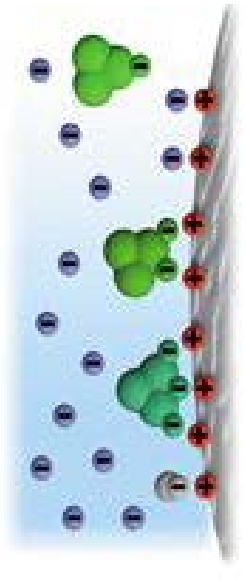
- Density gradient centrifugation
- Selective precipitation
- Chromatography
  - IEX, MM, AC, HIC, SEC
  - Bead format (Packed bed)
  - Membrane format (Capsule)



Ion exchange chromatography

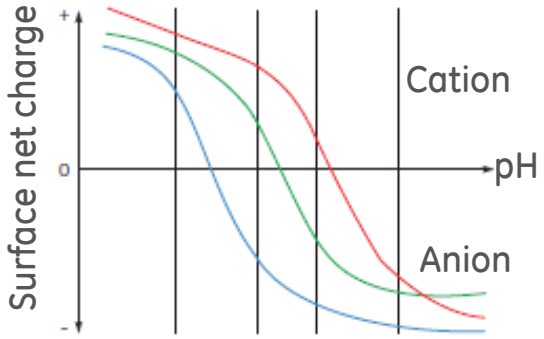
Anion exchange chromatography

- (-) Negatively charged molecules binds to (+) positively charged ligands



Cation exchange chromatography

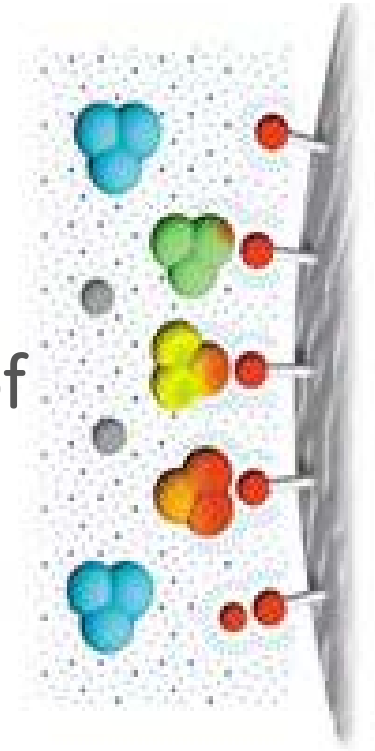
- (+) Positively charged molecules binds to (-) negatively charged ligands



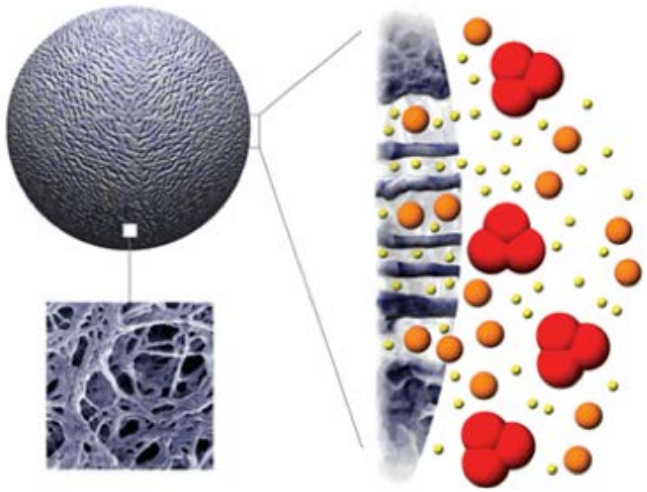
Hydrophobic interaction chromatography

Separation by hydrophobicity

- Hydrophobic surfaces of proteins interact with the ligand in precence of salts
- High salt content enhance and low salt weakens the interaction



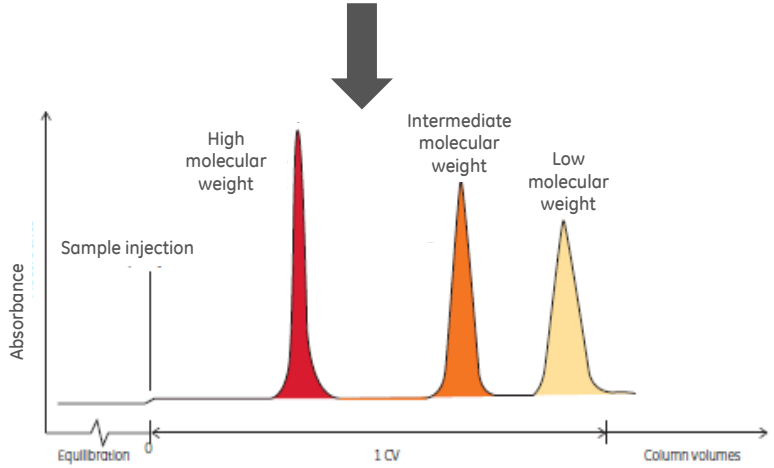
Size exclusion chromatography



Exluded from pores

Enter a fraction of the pores

Enter all pores



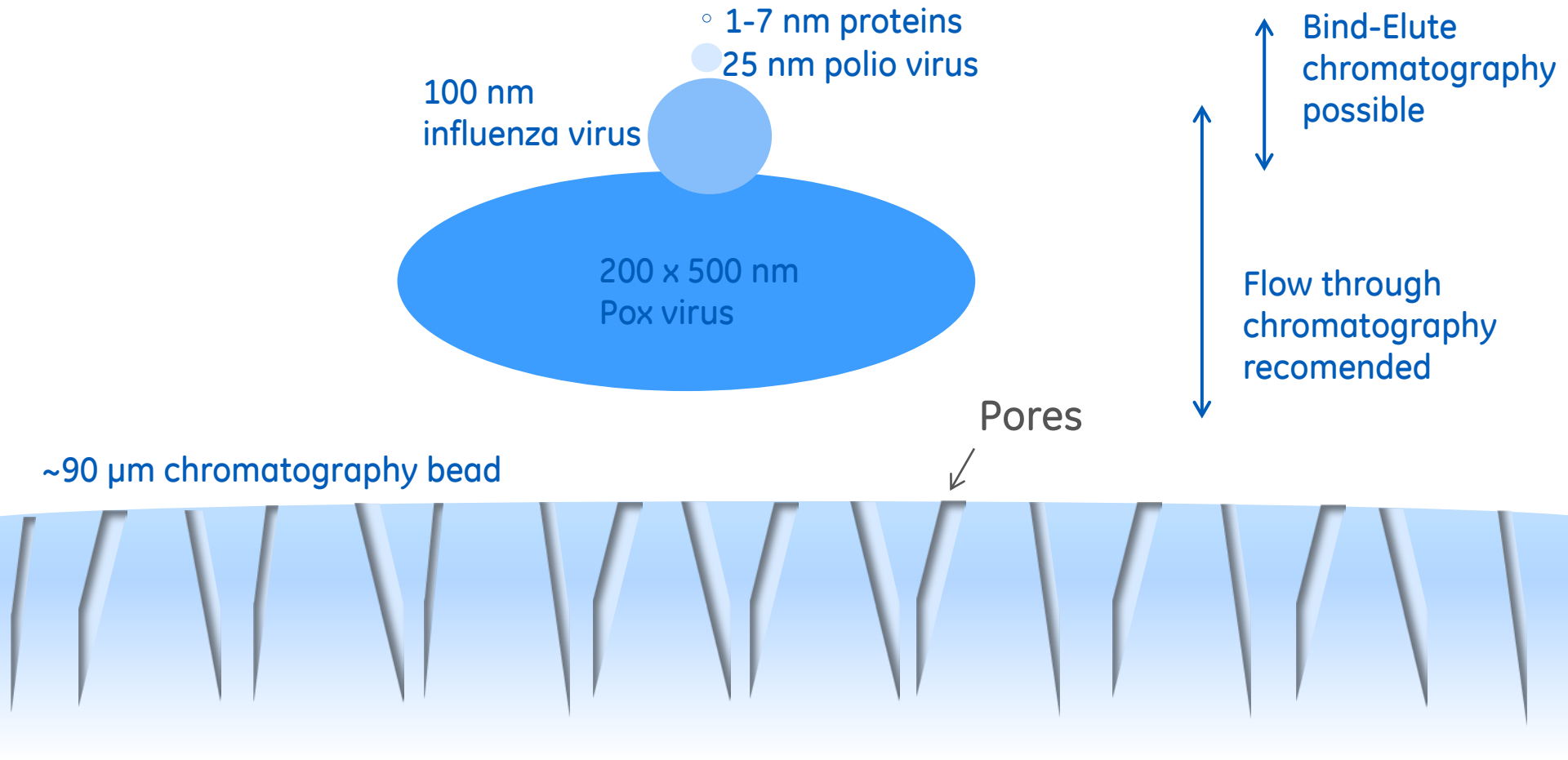
Affinity chromatography

Specific binding

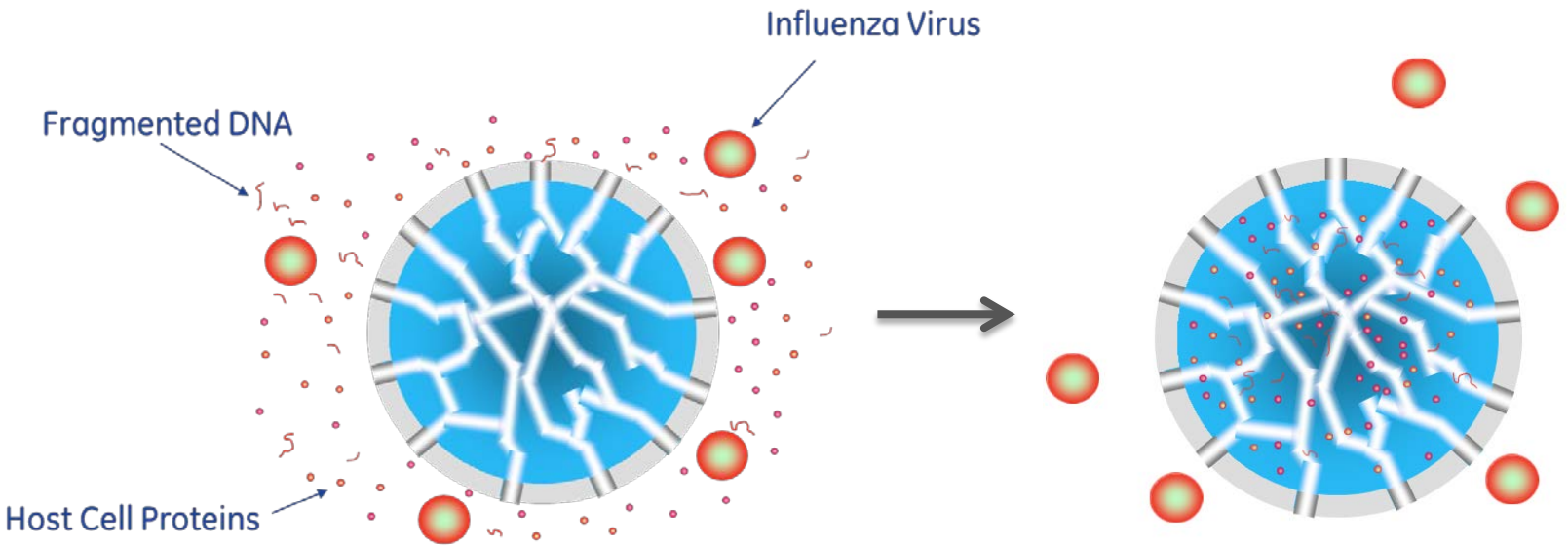
Few affinity resins available for vaccines

- Agarose based affinity resin for adeno associated virus
- Pseudo affinity resins for influenza
  - sulphated cellulose
  - sulphated dextrane

Chromatograpic purification of large molecules can be challenging



Core bead chromatography



- Host cell proteins and DNA fragments bind to the core and viruses stay in the void.

Process example

Polio IPV

Seed N-2 Cell expansion

Seed N-1 Cell expansion

Production bioreactor Virus propagation

Clarification NFF Removal of cell debris and large particles

TFF Conc of polio virus

SEC Separation of polio virus from small molecular compounds

AIEX (FT) DNA removal. Polio virus in flow through

Virus inactivation formaldehyde

Formulation Sterile filtration, mixing with other strains

Summary

- Robust downstream process can ensure consistent high quality
- Most vaccines have unique purification processes
- Preferably use scalable techniques when developing new processes
- Purification of particles in binding mode can be difficult with classic chromatography
- Core bead chromatography suitable for purification of particles of sufficient size

Thank you

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