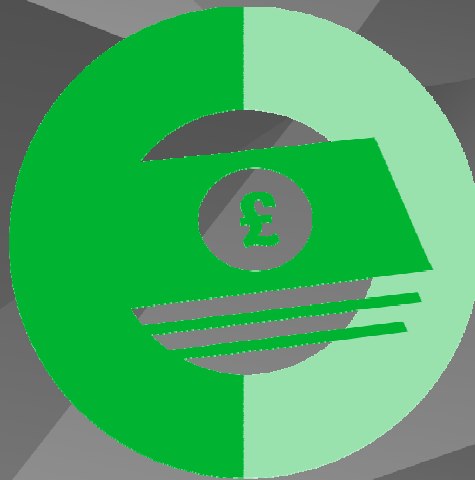
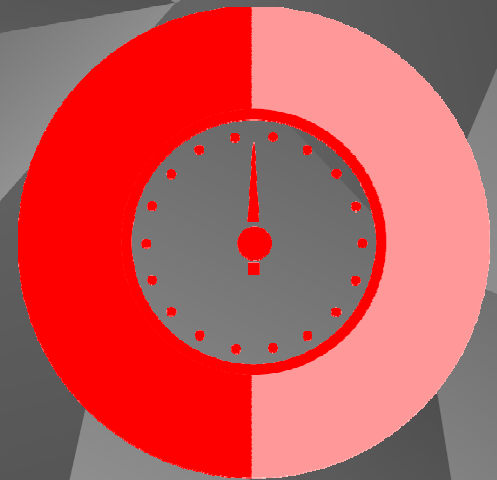


PURITY



ECONOMY



EFFICIENCY

Options for Clarification

Centrifuge



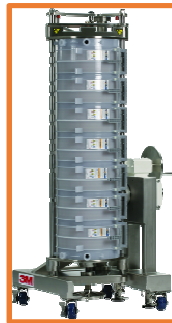
Clarification based on density

ZetaPlus™



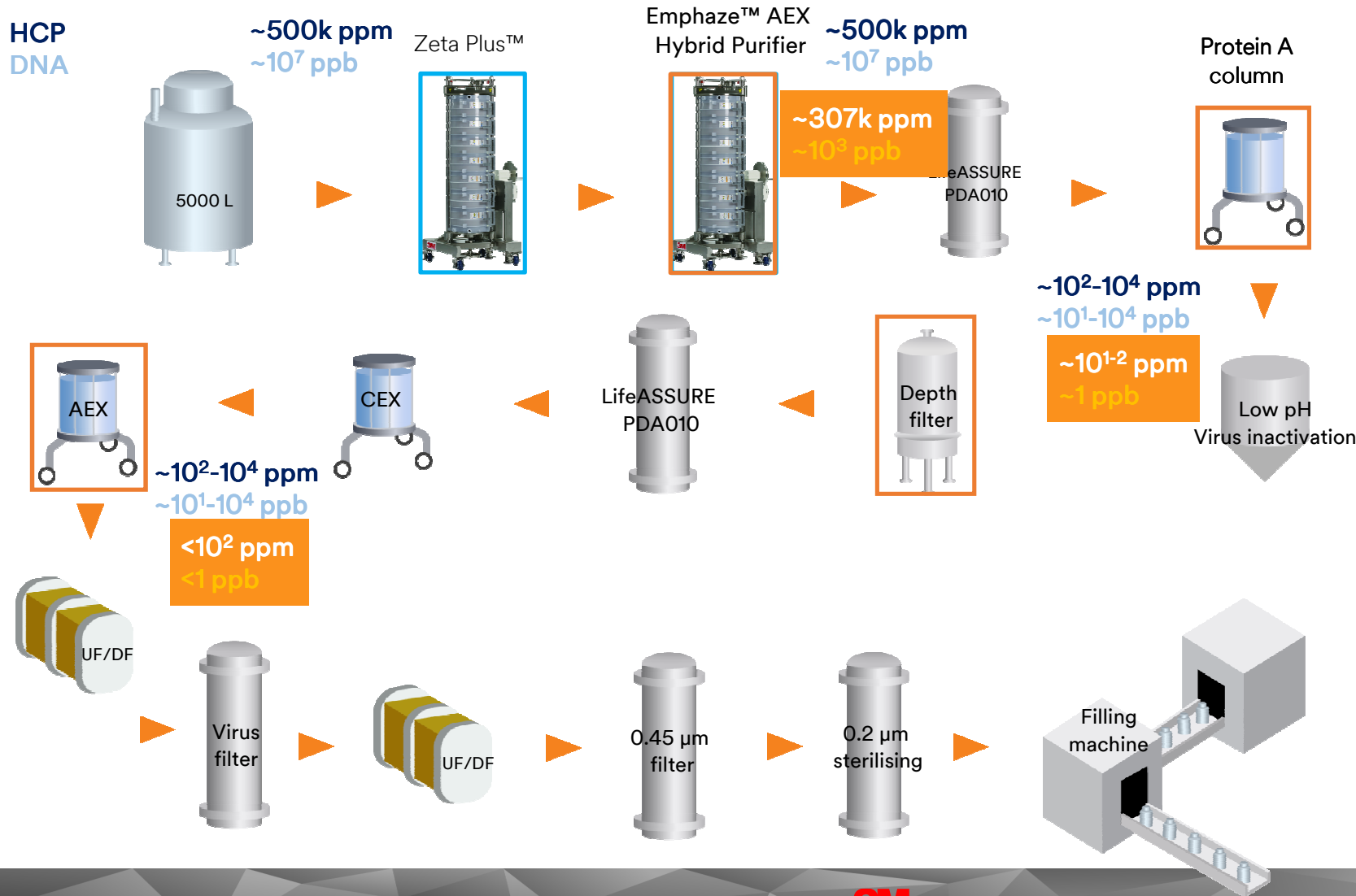
Clarification based on tortuous path

Emphaze™ AEX
Hybrid Purifier



Clarification based on charge

The Impact of the Emphaze™ AEX Hybrid Purifier on a Process

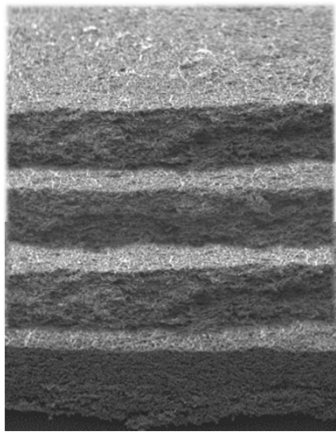


mAb process with chromatographic clarification

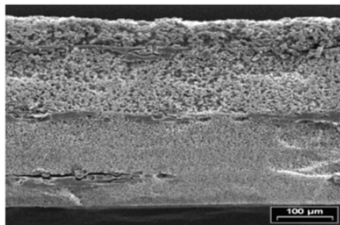
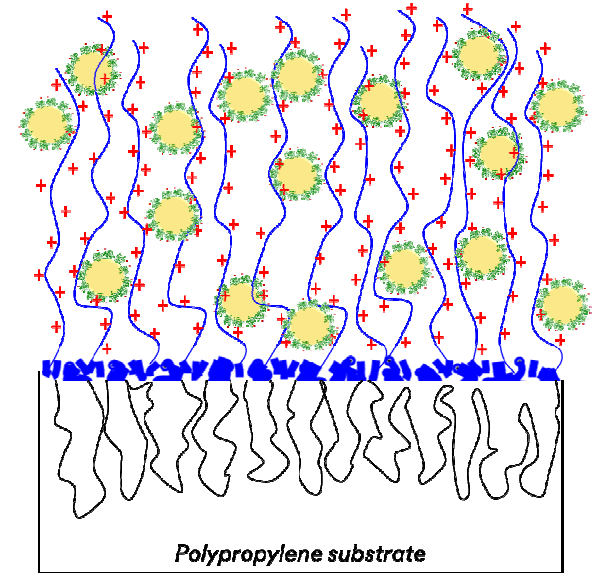
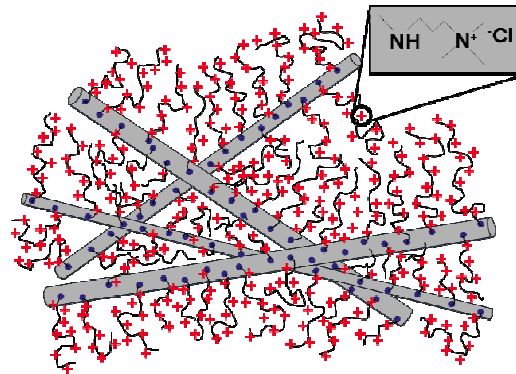
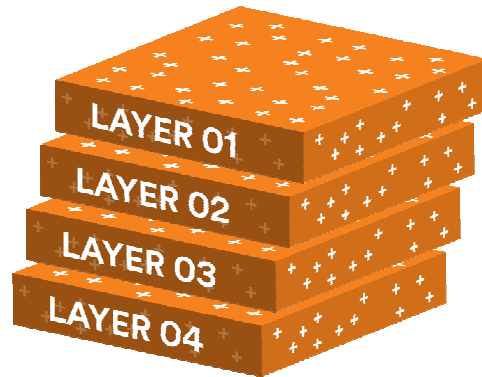
- ▶ Highly controlled output turbidity and particle sizing
- ▶ Significant reduction of soluble and insoluble contaminants
- High purity early in the process:
 - ▶ Increases performance of Protein A
 - ▶ Increases robustness of Pro A step
 - ▶ Reduces aggregation propensity
 - ▶ Allows downsizing of the AEX column

Structure of the Emphaze™ AEX Hybrid Purifier

Direction of flow



Tilted cross-section of 4 layers of Q-functional nonwoven



**0.2 micron 9-zone
polyamide size exclusion
membrane**

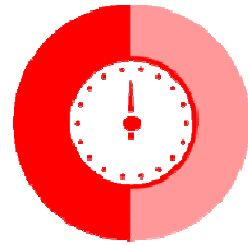
- ▶ Turbidity & bioburden control

All-synthetic construction

- ▶ Well defined organic and ultralow elemental extractables

4 layers of Q-functional nonwoven

- ▶ Soluble and insoluble contaminant reduction



Efficiency

Stabilisation of the cell culture fluid can
address turbidity problems at various points in
the downstream processing

Understanding Clarification at Molecular Level and Stability of CCCF

Primary Clarification Pool

~ 41 NTU
~ 10^5 ppm HCP
~ 10^8 ppb DNA
~ 1.5 g/ L mAb

ZetaPlus™



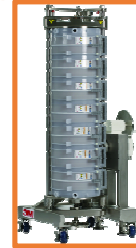
Conventional
approach

ZetaPlus™

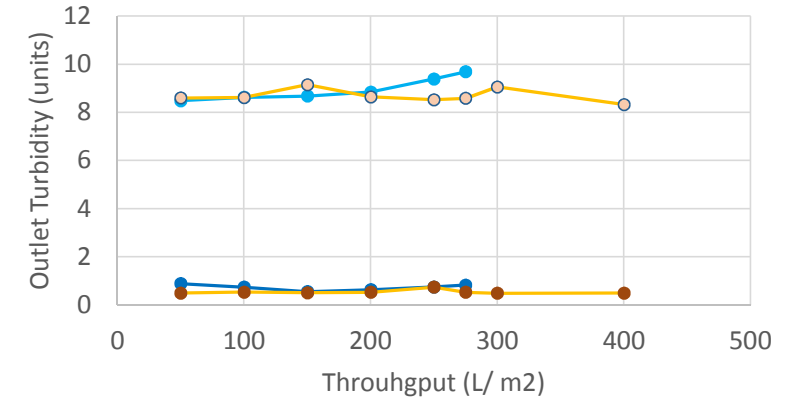


Chromatographic
clarification

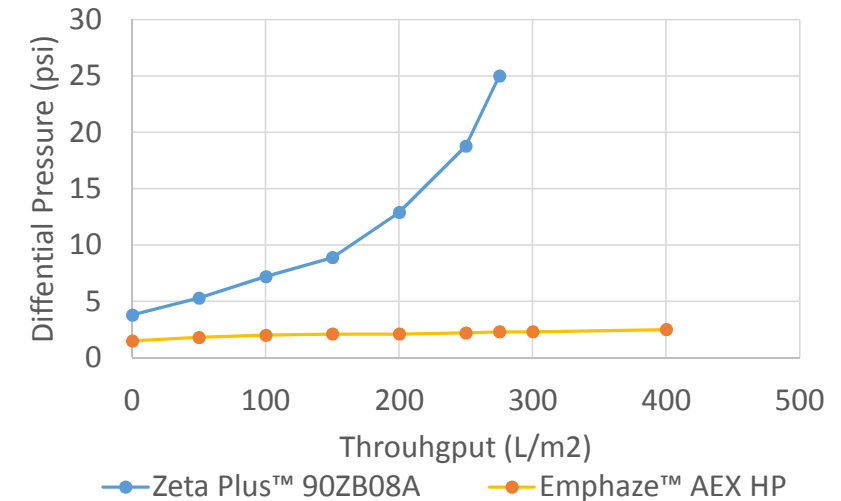
Emphaze™ AEX
Hybrid Purifier



NTU: 400-680 nm – affected by visible color of solution
FNU: 700-900 nm – not affected by visible color

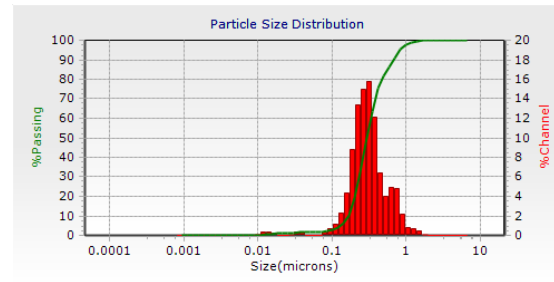


—●— Zeta Plus™ 90ZB08A (NTU) —●— Zeta Plus™ 90ZB08A (FNU)
—○— Emphaze™ AEX HP (NTU) —●— Emphaze™ AEX HP (FNU)

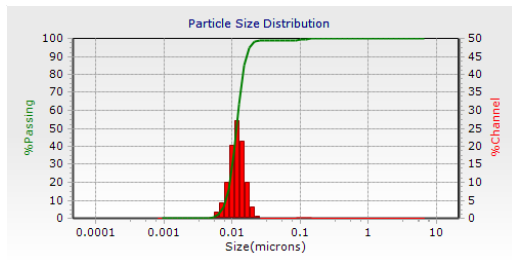


Different Particle Size Distributions Result in Similar Turbidity

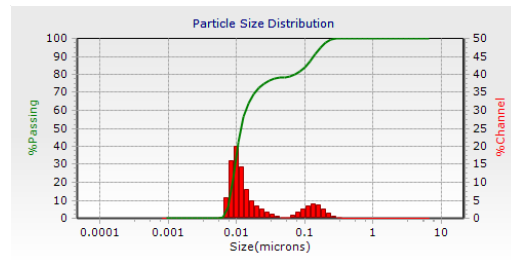
Feed material
~41NTU



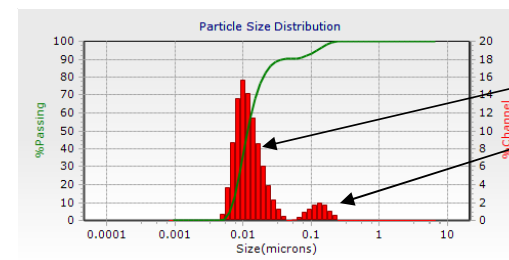
3M Zeta Plus™ 90ZB08A + 0.2 µm PES ~ 8 NTU



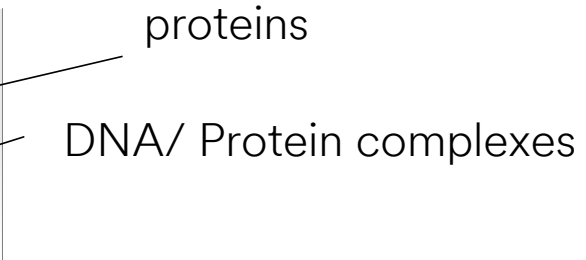
100 L/ m²



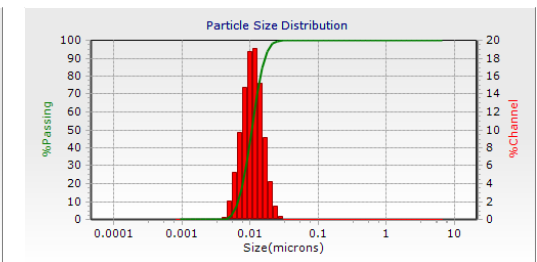
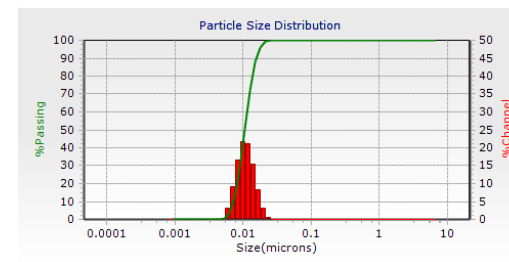
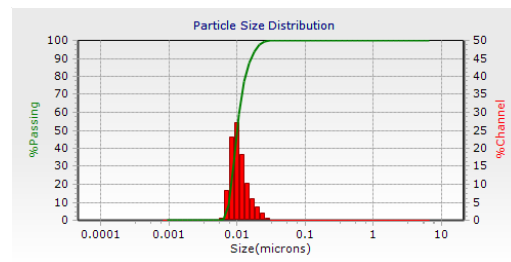
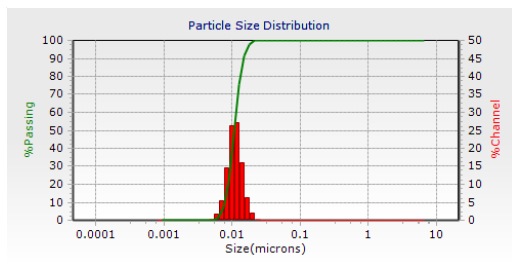
200 L/ m²



250 L/ m²



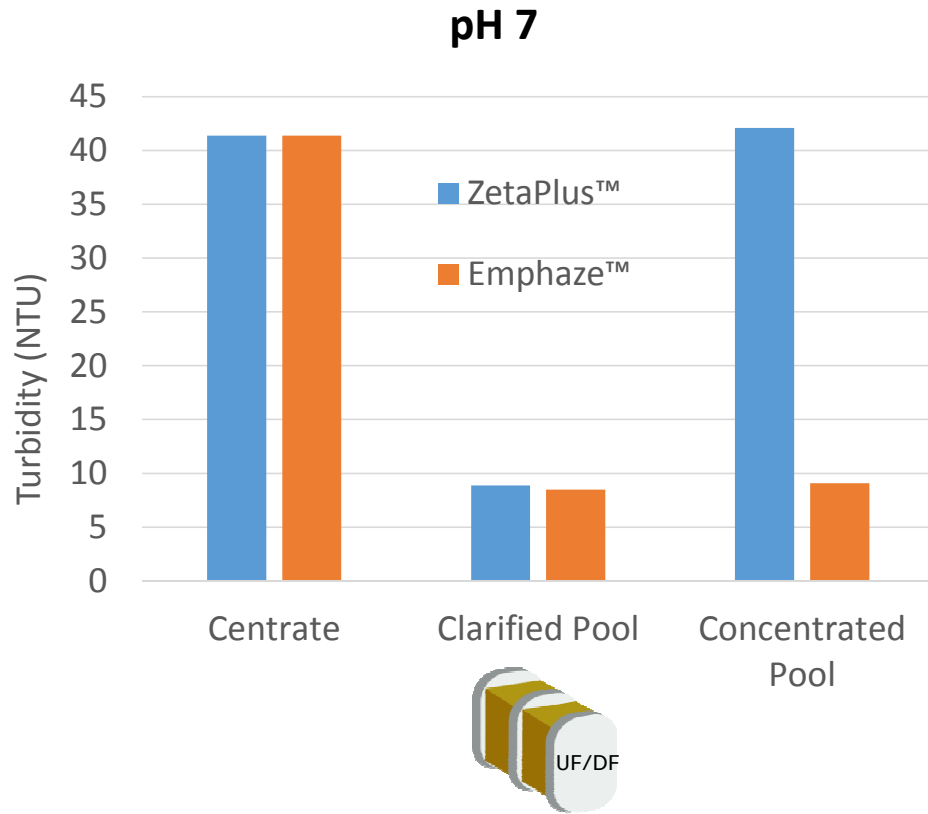
400 L/ m²



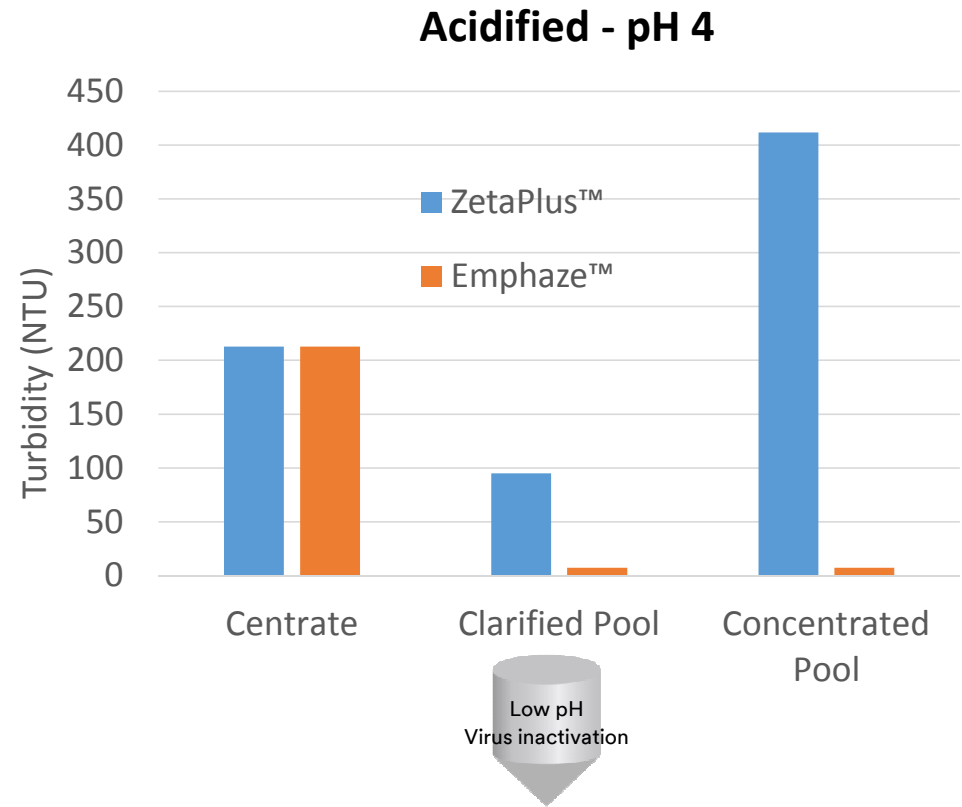
3M Emphaze™ AEX Hybrid Purifier + 0.2 µm PES ~ 8 NTU

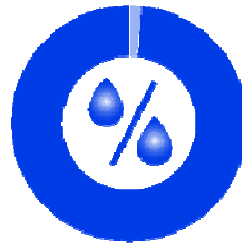
3M

Emphaze™ Stabilisation of CCF Improves Substantially Reduces Turbidity on Concentration and Acidification



Zeta Plus™ pool concentrated ~ 4 X
Emphaze™ pool concentrated ~ 16 X

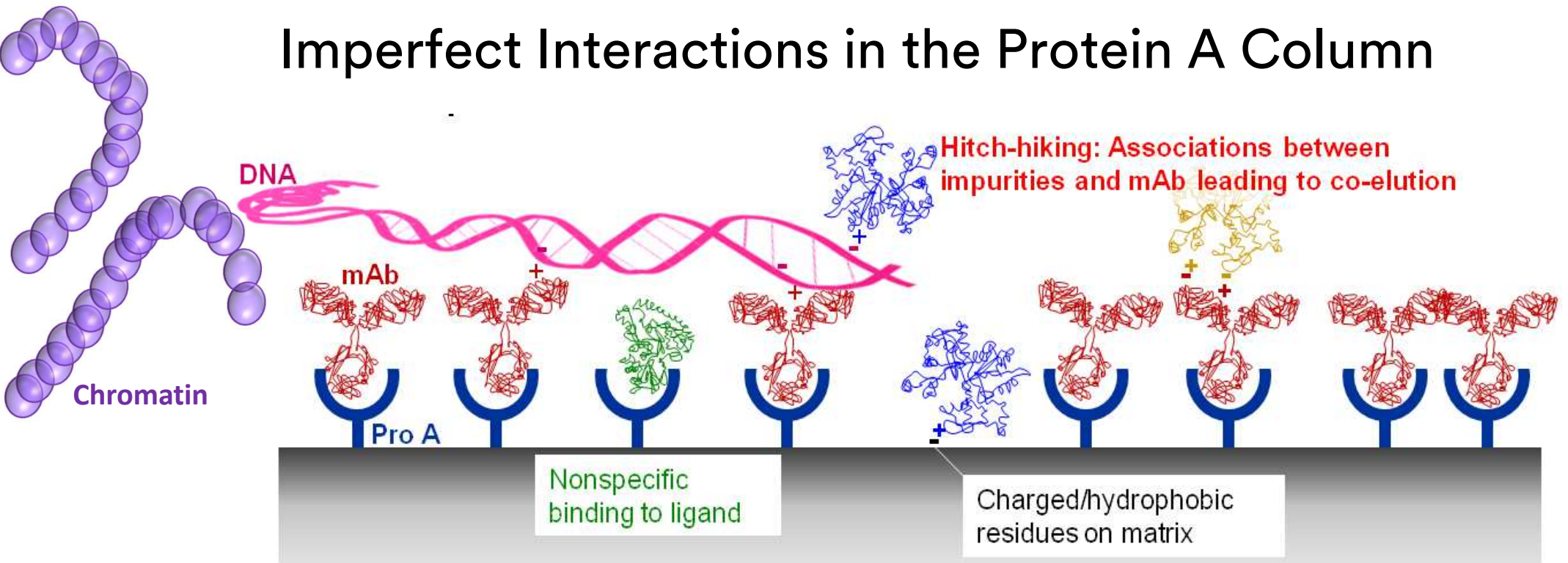




Purity

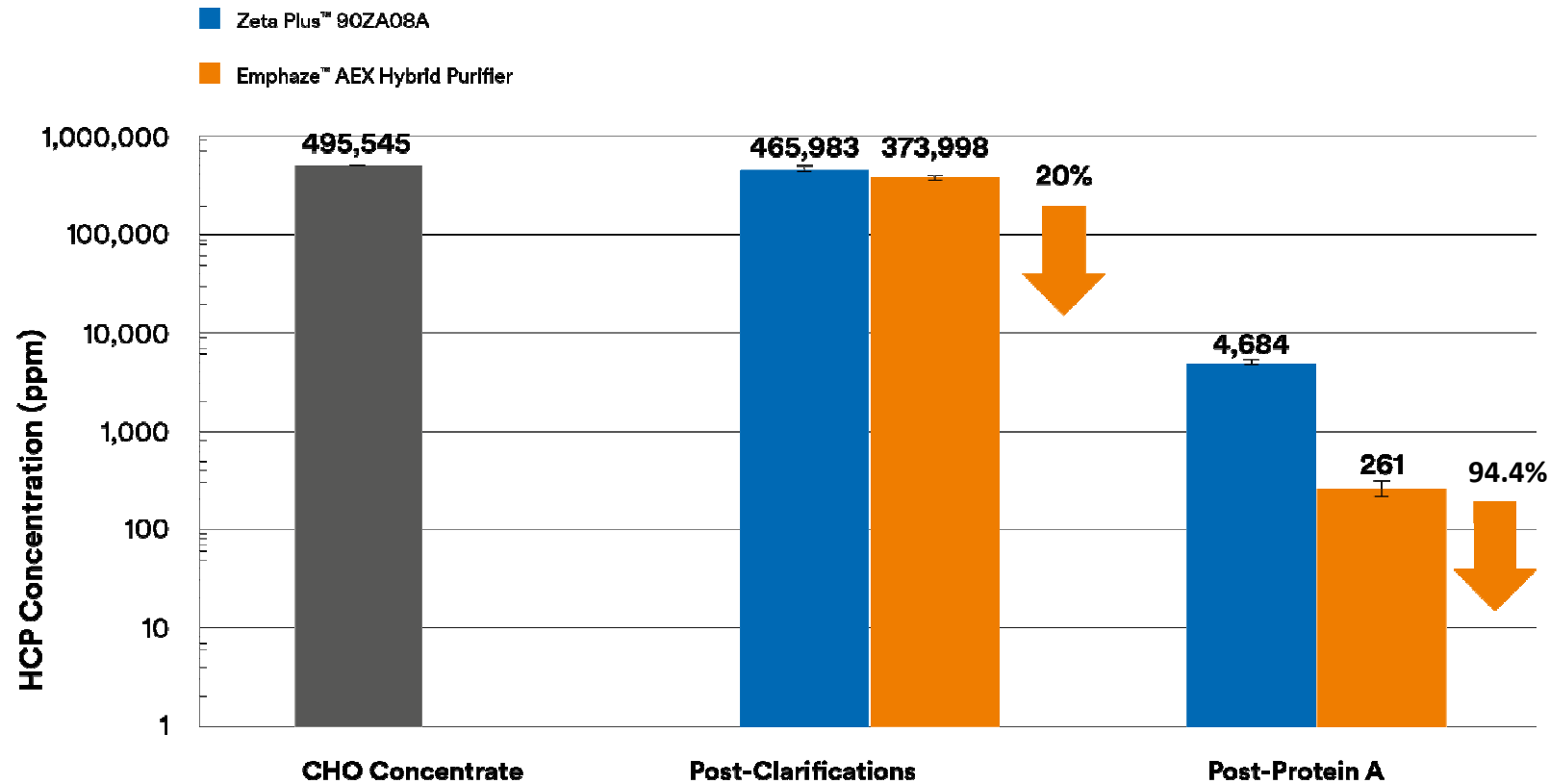
Reduction in soluble impurity levels during clarification can **enhance Protein A column performance**

Imperfect Interactions in the Protein A Column



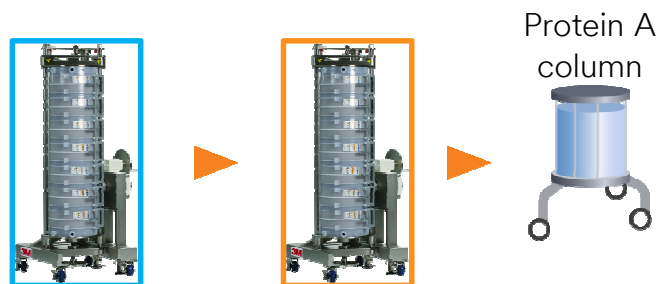
- “Hitch-hiking” interactions are the dominant cause of HCP co-elution with product
A. A. Shukla & P. Hinckley; *Biotechnology Progress* **2008**, 24, 1115-1121.
- Select sub-populations of HCP may be particularly strong “hitch-hikers”
B. Nogal, K. Chhiba & J. C. Emery; *Biotechnology Progress* **2012**, 28, 454-458.
- DNA-protein complexes may strongly facilitate “hitch-hiking” interactions
P. Gagnon, et al.; *Journal of Chromatography A* **2013**, 1291, 33-40.

Emphaze™ AEX Hybrid Purifier: effect on Protein-A pool HCP reduction



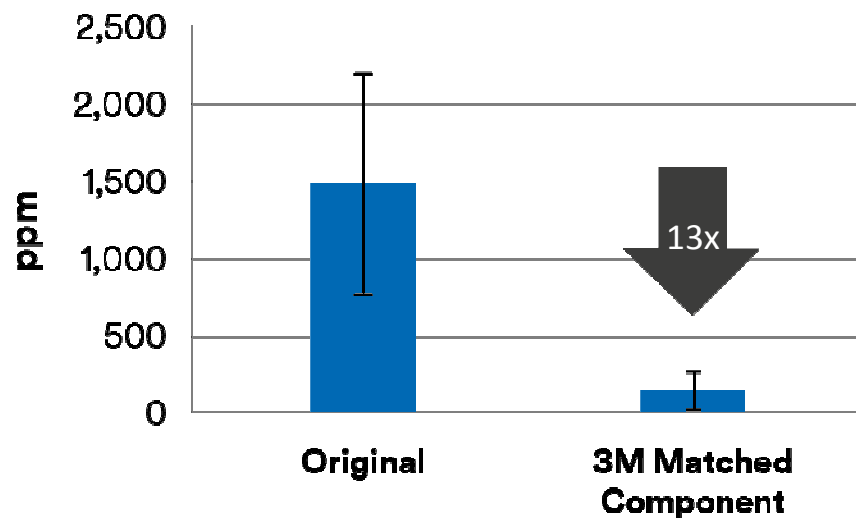
Compilation of published collaboration data

7 mAb /
adjacencies
products

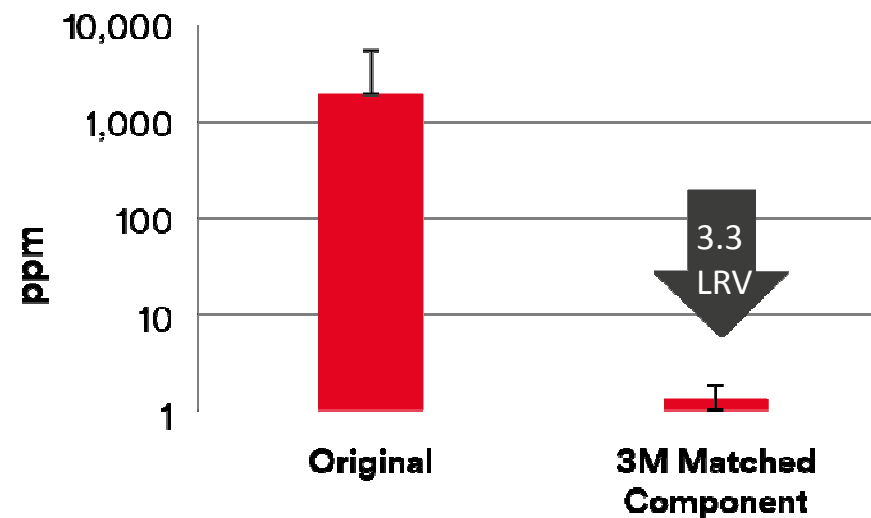


Impurity Content of Protein A Pool

Average Post-Protein A HCP

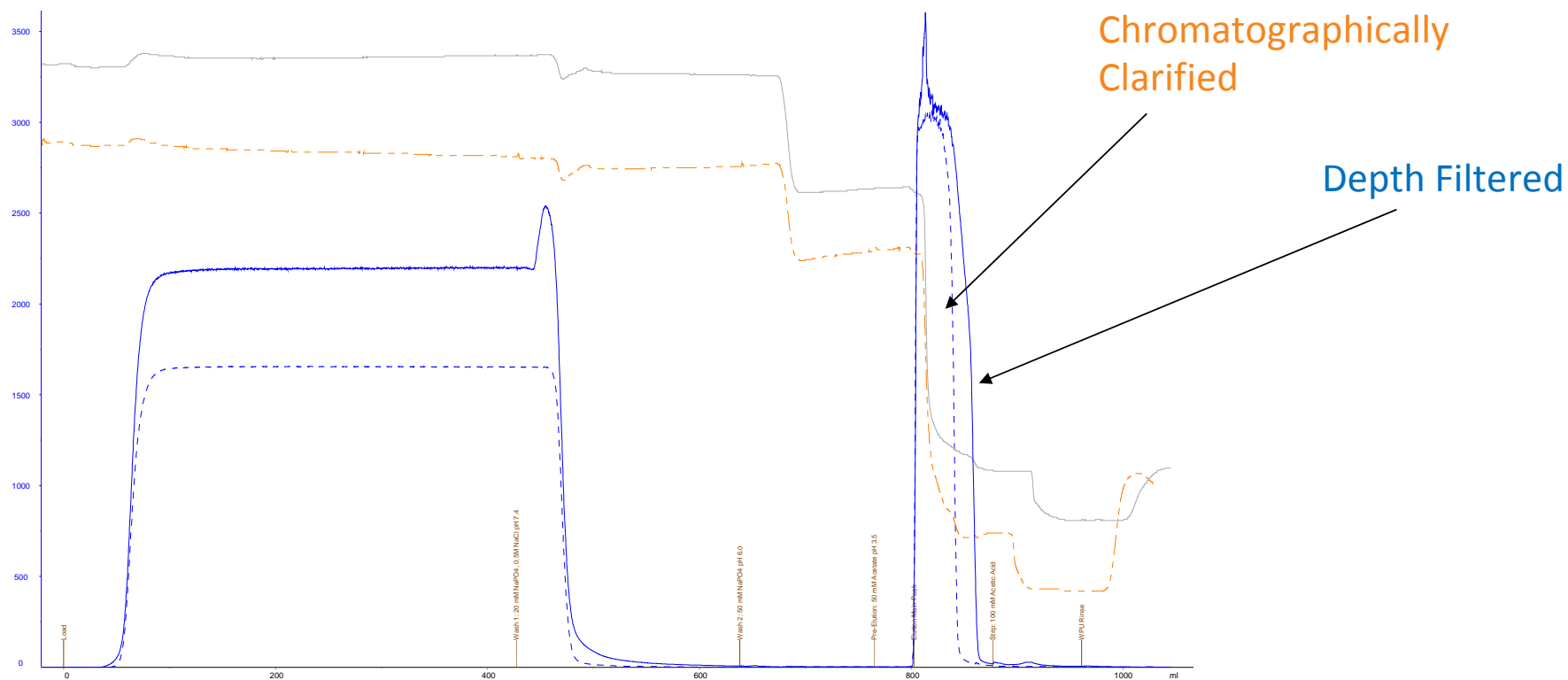


Average Post-Protein A DNA



Chromatographic Clarification Approach Enables Narrower Elution Peak with ~ 30% More Concentrated Product

Protein A Chromatogram Comparison

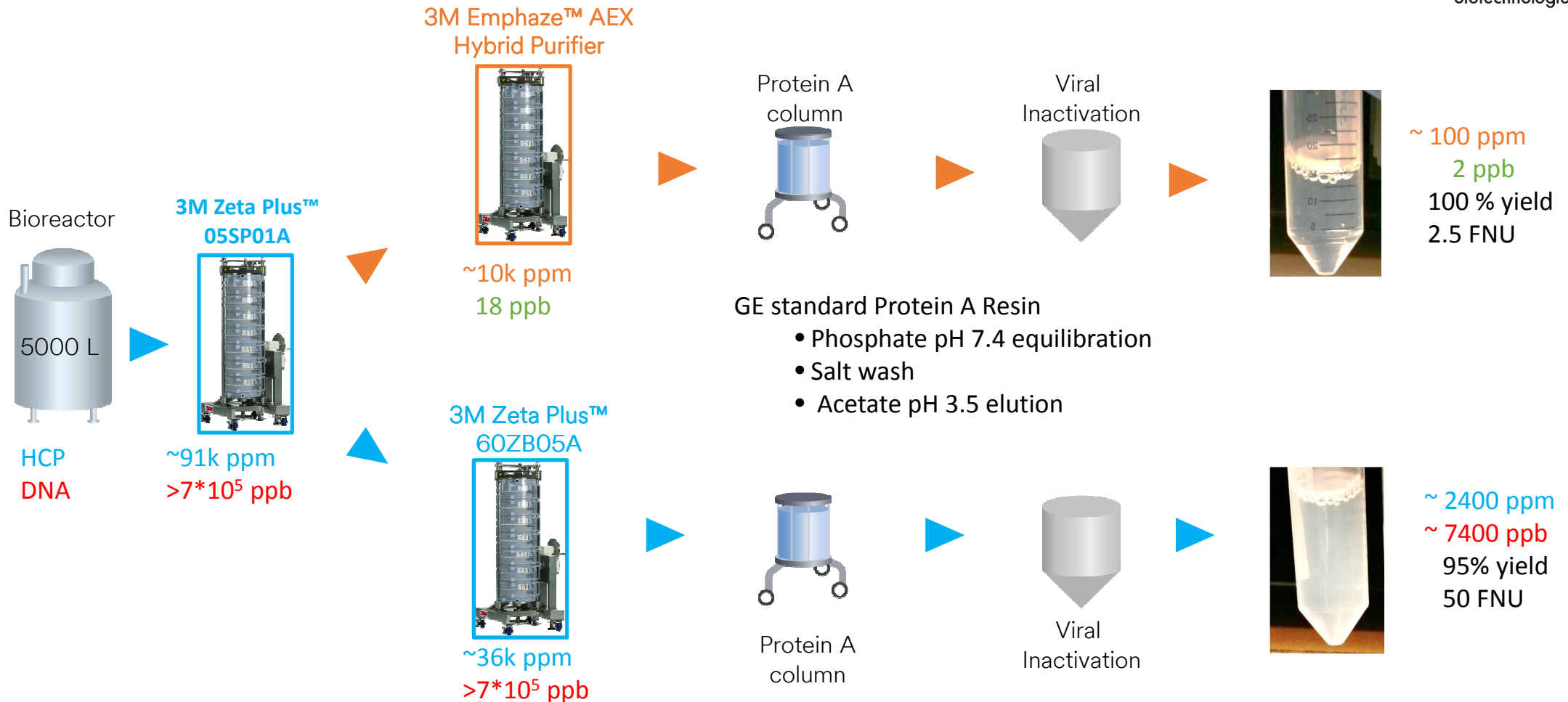




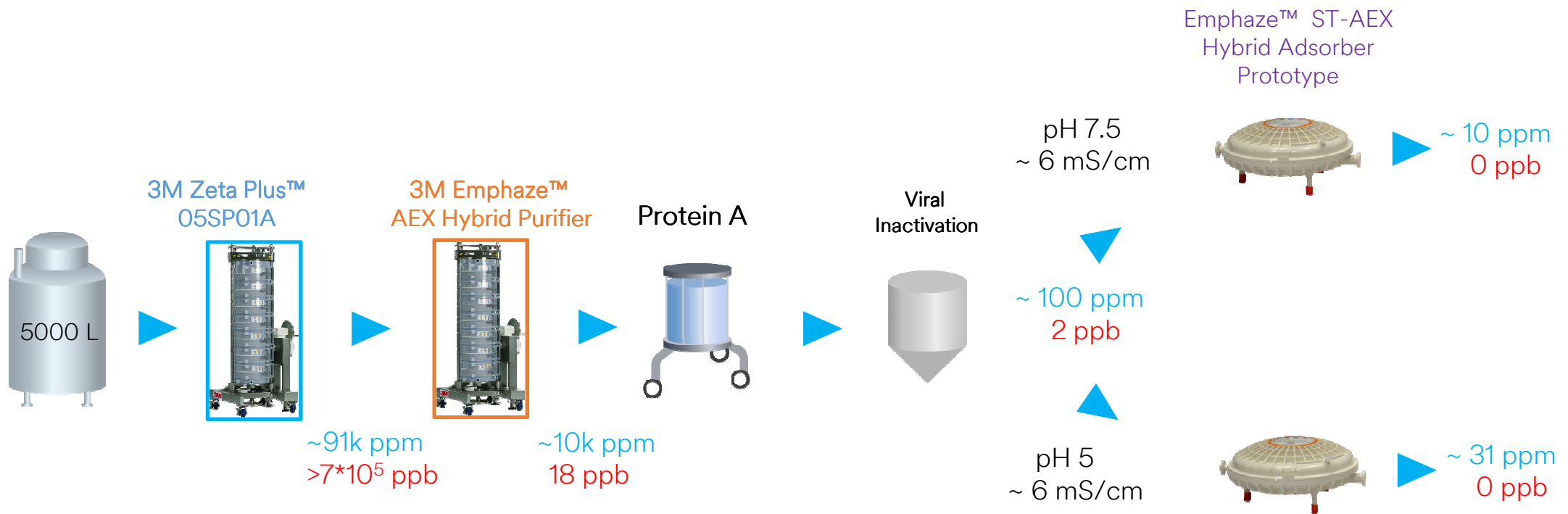
Economics

Reduction in soluble impurity levels during clarification can enable **downsizing of polishing steps**

Significantly Higher Protein A Column Performance and Post Capture Process Stream Stability



Can Emphaze™ Enable Downstream Process Compression?



High purity/ performance of the capture step enables:

- Highly compact polishing trains
- High degree of fault-tolerance
- Focus on key polishing attributes (viral clearance, HMWAs)



Economics

All of this can significantly **reduce
manufacturing costs**

Impact of the EMPHAZE AEX HP on manufacturing costs

mAb production
cost per gram

Assumptions

1

\$115

Standard process

2

\$108

Throughput — $>400\text{L/m}^2$ &
Recovery — 90 to 97%

3

\$105

Increase in Protein A dynamic
binding capacity by 15%

4

\$93

Removed Depth Filter post VI

5

\$87

AEX step reduced by 4X

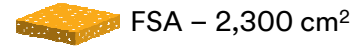
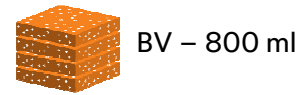
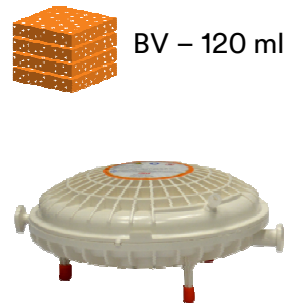
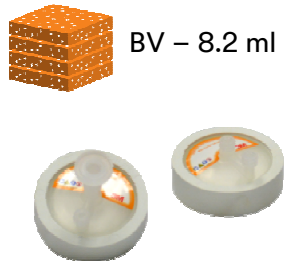
6

\$86

Replace centrifuge recovery to 90%

Product range

Lab scale			Pilot Scale	Production Scale
0.5 - 2L	5 - 25L	20 - 70L	> 50L	300 - 25000L



BV – bed volume | FSA – frontal surface area |

3M

Summary

- Stabilisation of the cell culture fluid can eliminate turbidity problems at various points in the DSP
- Reduction in soluble impurity levels during clarification can enhance the protein a column performance
- Reduction in soluble impurity levels during clarification can enable downsizing of polishing steps
- All of this can significantly reduce manufacturing costs



Questions?

3M



Thank you

3M