Types of Ion Exchange Chromatography Media

Andy Masters
GE Healthcare, Life Sciences
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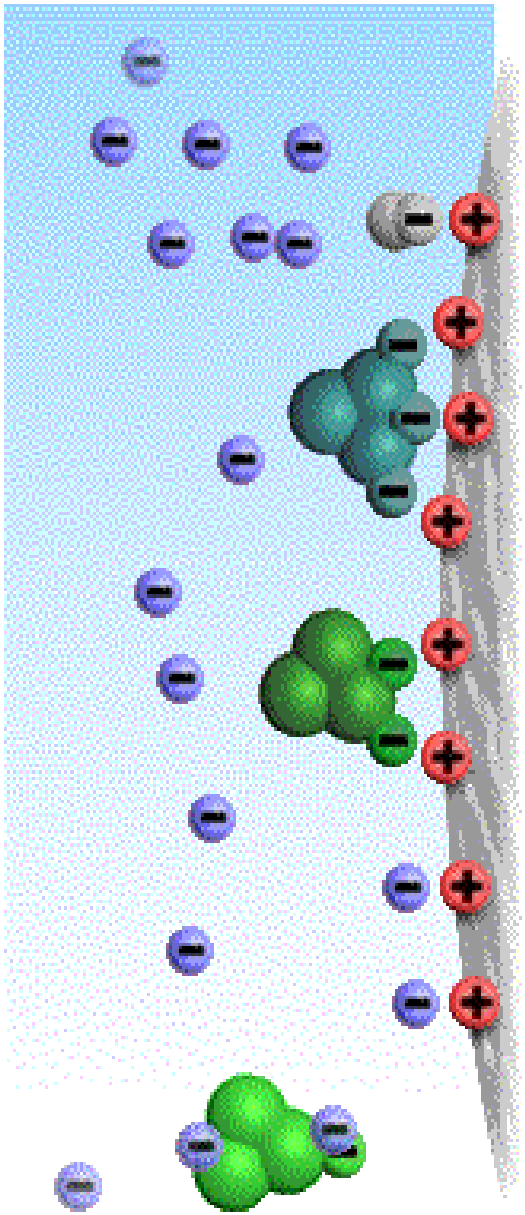
Chromatography Resins
www.gelifesciences.com/protein-purification

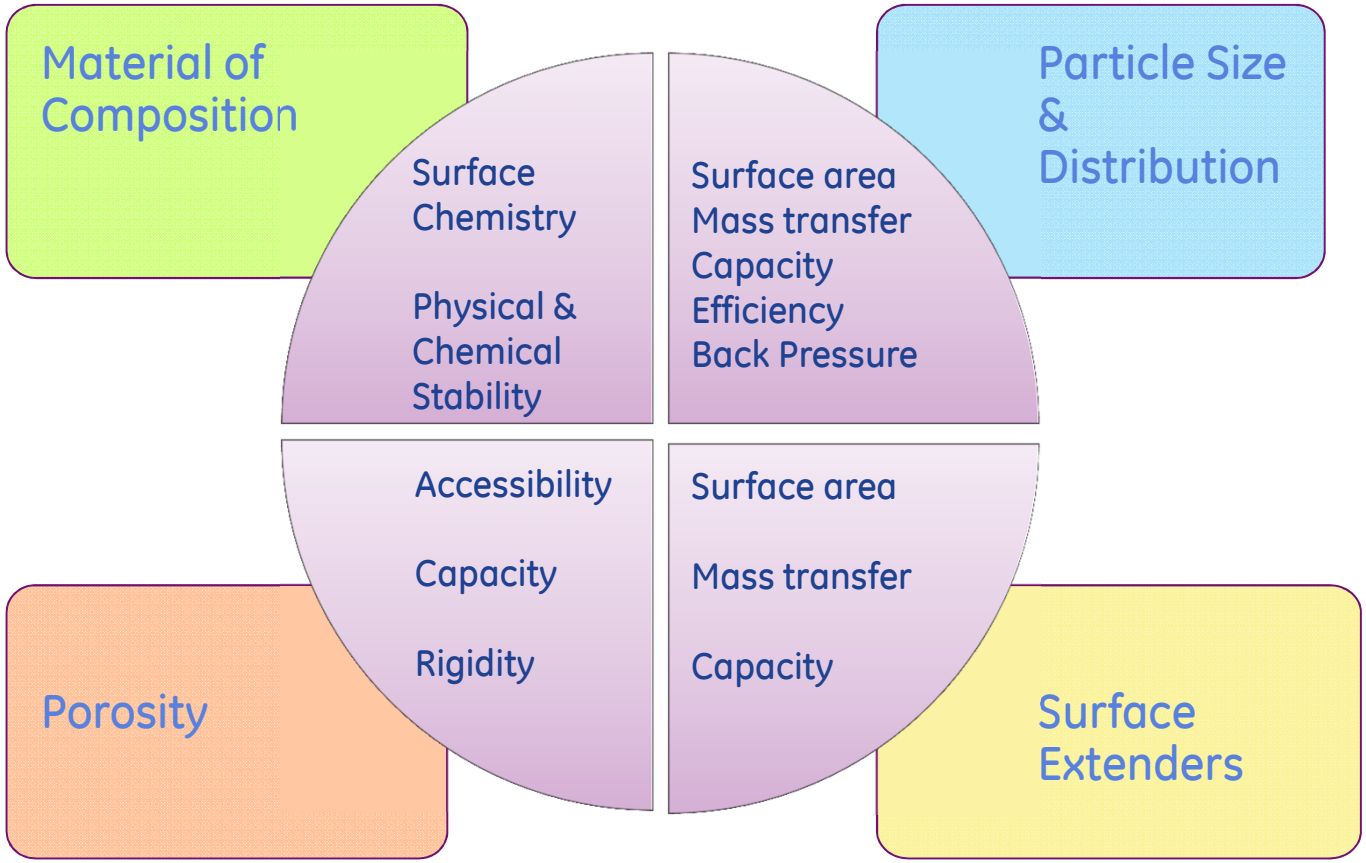


SCI IEX 2012: Technical Training
Ion Exchange Theory and Practice
for Bioprocessing
Tuesday 18 September 2012
Queens' College, University of Cambridge, UK

Content

- Properties of IEX chromatography media
- Classic ligands
- Strong and weak IEX and atypical behaviour
- Mixed mode ligands
- Future developments for IEX and MM
- Media selection

Base matrix key to properties of all chromatography media



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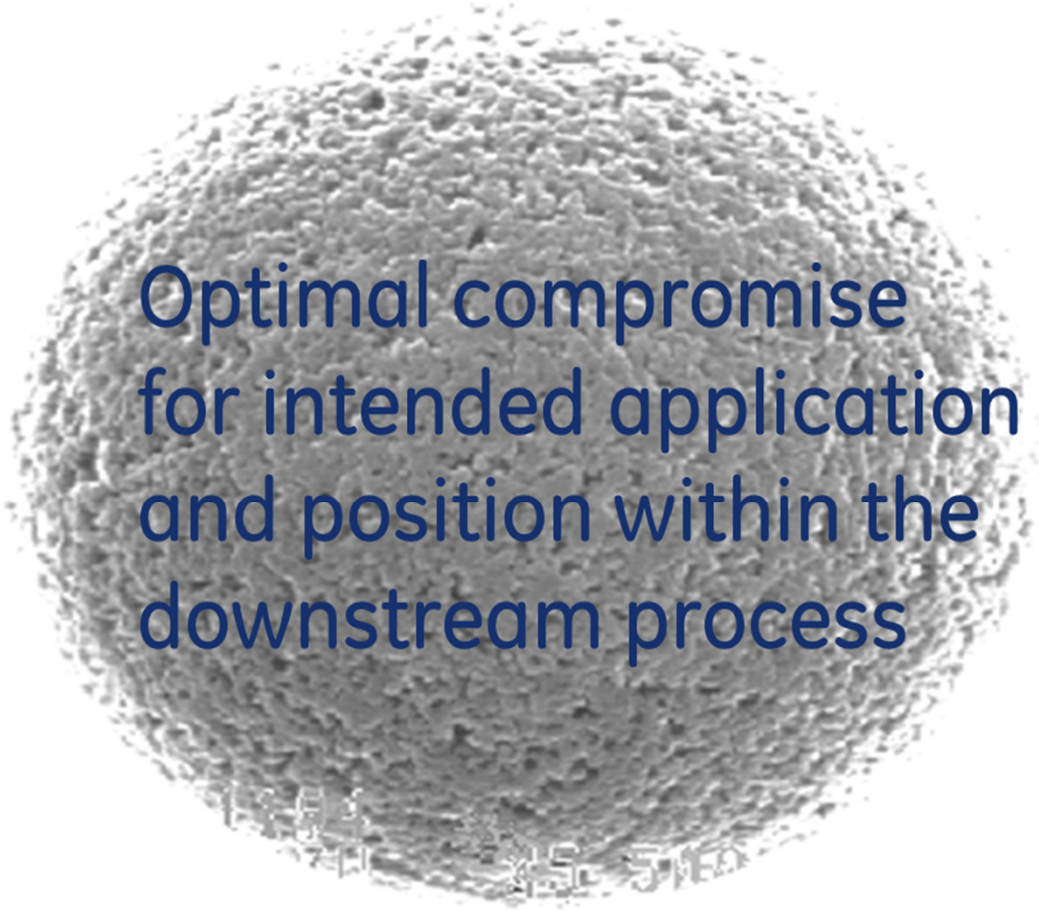
Base ... of all chron...



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Base matrix composition

The ideal matrix for IEX

- Hydrophilic
- Large pore size/surface area
- Spherical (mono-sized) particle
- Rigid – optimal pressure/flow
- Easy to functionalize
- Chemically stable – SIP/CIP

Typical materials

- Agarose
- Cellulose
- Ceramics
- Dextran
- Polystyrene
- Polyacrylamide
- Silicas
- Synthetic/Organic Polymers

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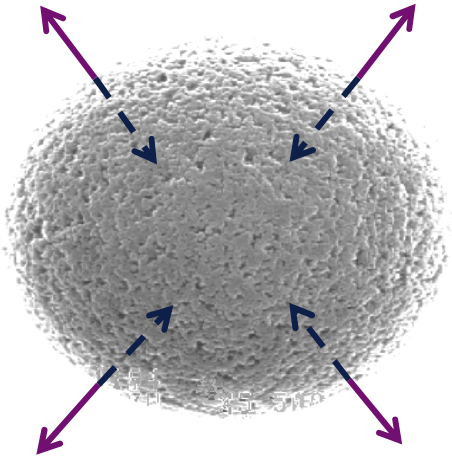
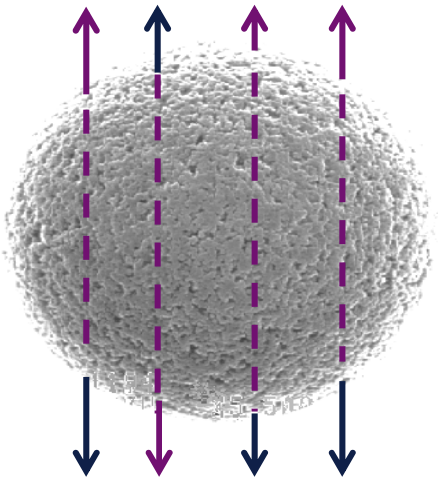
Porosity/surface area

Porous media



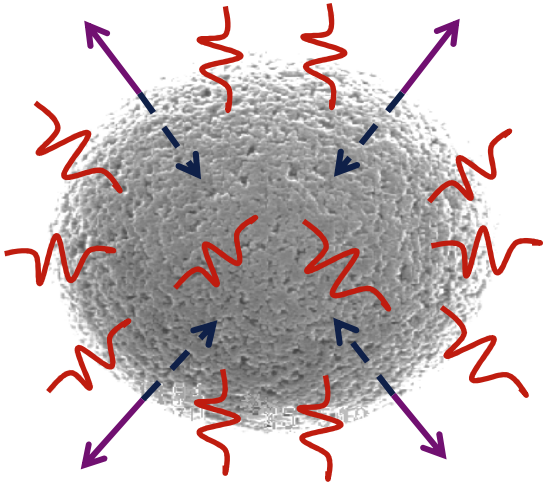
- Longer distances for mass transfer (diffusion)
- Good capacity require longer residence times
- Porosity impacts surface area and rigidity
- Pore size and porosity optimised for application

Perfusion/monoliths



- Shorter distances for mass transfer (convective flow)
- Good capacity shorter residence times
- Monoliths can be optimised for the binding of large molecules (e.g. viruses, plasmids)
- Structure can lead to limitations of scale

Surface Extenders



- Shorter distances for mass transfer
- Increased surface area without compromising rigidity
- High capacity even at short residence times
- Can demonstrate atypical IEX behaviour

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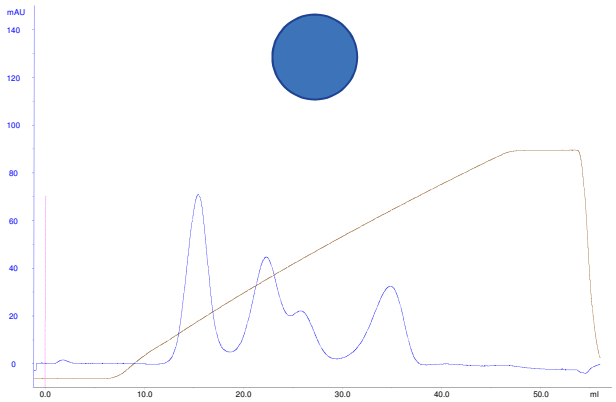
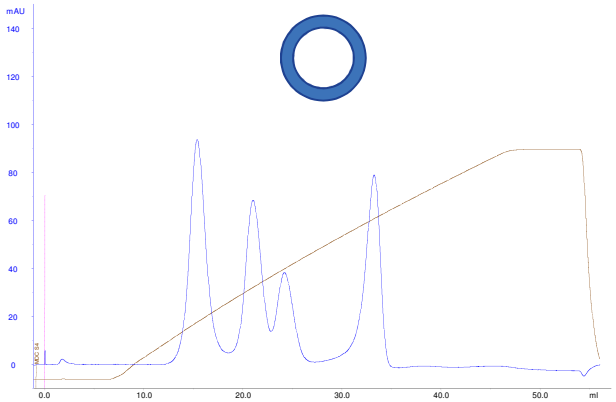
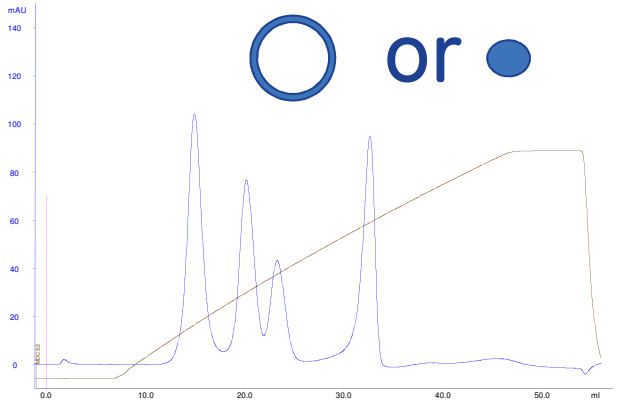
Speed of mass transfer and resolution

	Shell thickness (µm)	Fraction of bead volume functionalized (%)	BSA capacity (g/L)	BSA capacity (%)
	N/A	100	75	100
	11	65	61	81
	6	41	45	60







or

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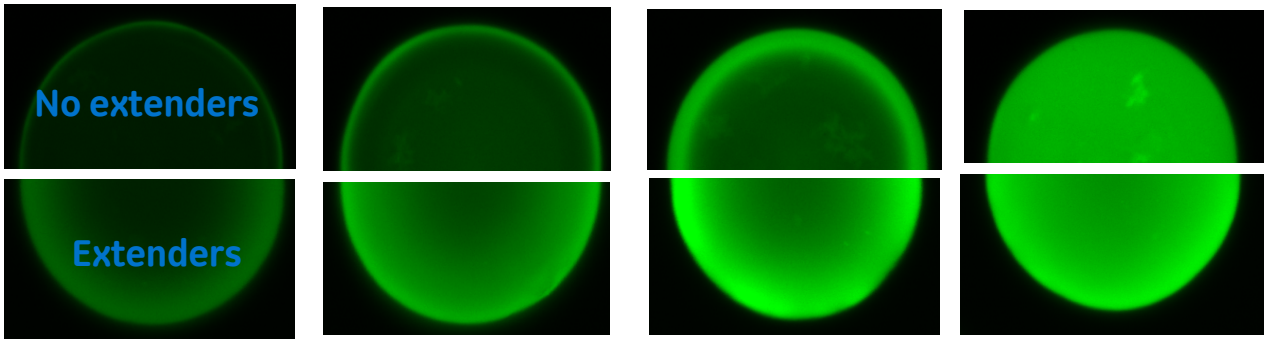
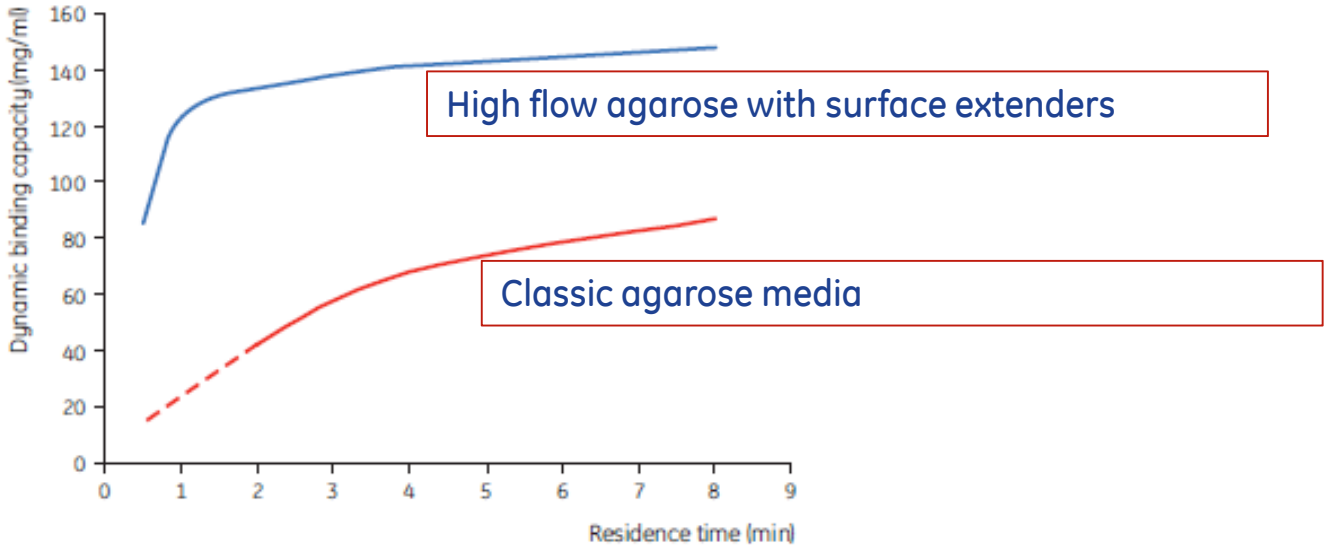
Effect of surface extenders on mass transfer and capacity



Confocal microscopy study showing enhanced protein uptake (increased mass transfer and equilibrium capacity) for IEX resin with ligand extenders

Time



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Porosity and surface extenders

Importance of critical chromatography resin properties in the design of a high productivity immunoglobulin process

Joseph Bertolini, Chor Sing Tan, Karl McCann

CSL Biotherapies, 189 Camp Road, Broadmeadows VIC 3047, Victoria, Australia

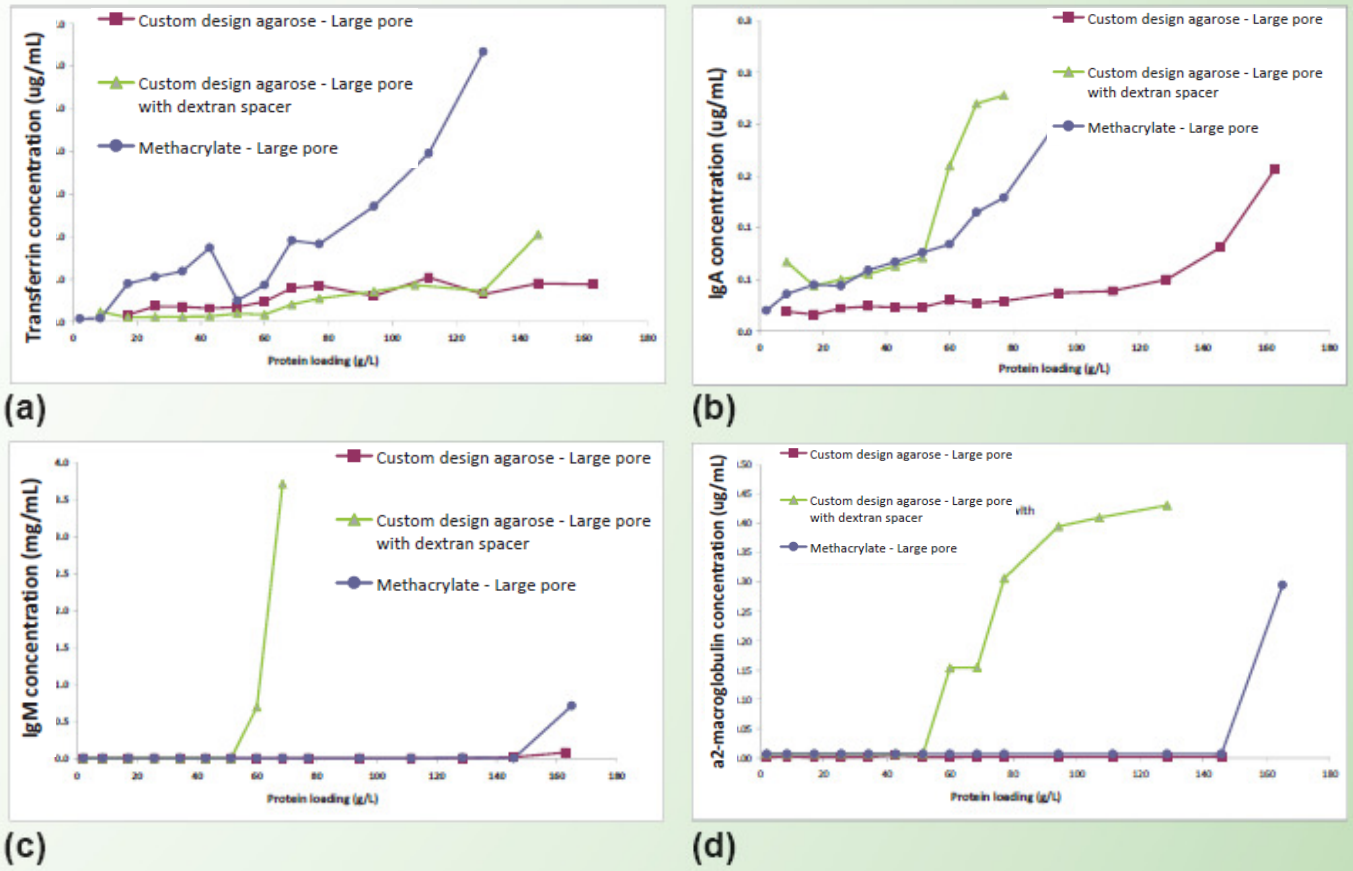

Figure 5 Impurity concentration of Pure IgG fractions derived from strong anion exchange resins loaded at 160 g/L of Crude IgG (a) transferrin (b) IgA (c) IgM (d) α_2-macroglobulin

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Presented at the Recovery of Biological Products conference 2010

Bead size/distribution and resolution

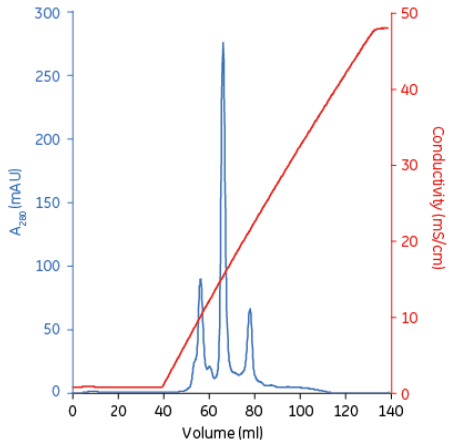
Small beads

- Fastest mass transfer
- Highest efficiency
- Best resolution
- Step and linear gradients used
- Higher back pressure
- Equipment and economy implications

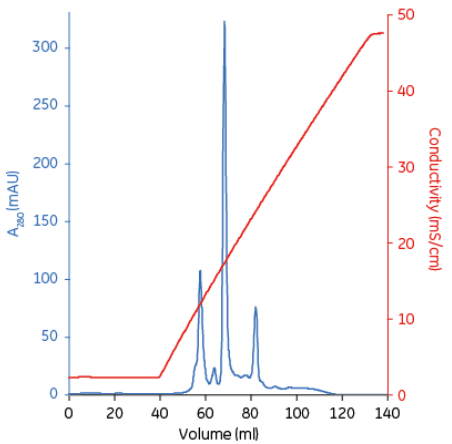
Large beads

- Good mass transfer
- Higher capacity
- Lower efficiency
- Less resolution
- High flow/capacity and step gradients for best productivity
- Low back pressure
- Most economical

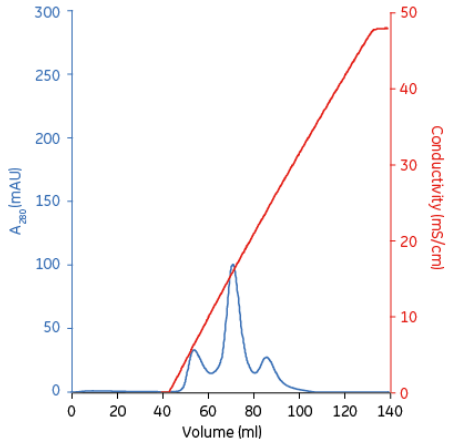
40um



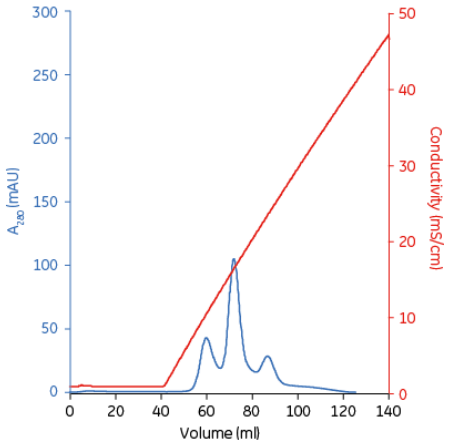
34um



90um surface extenders



90um



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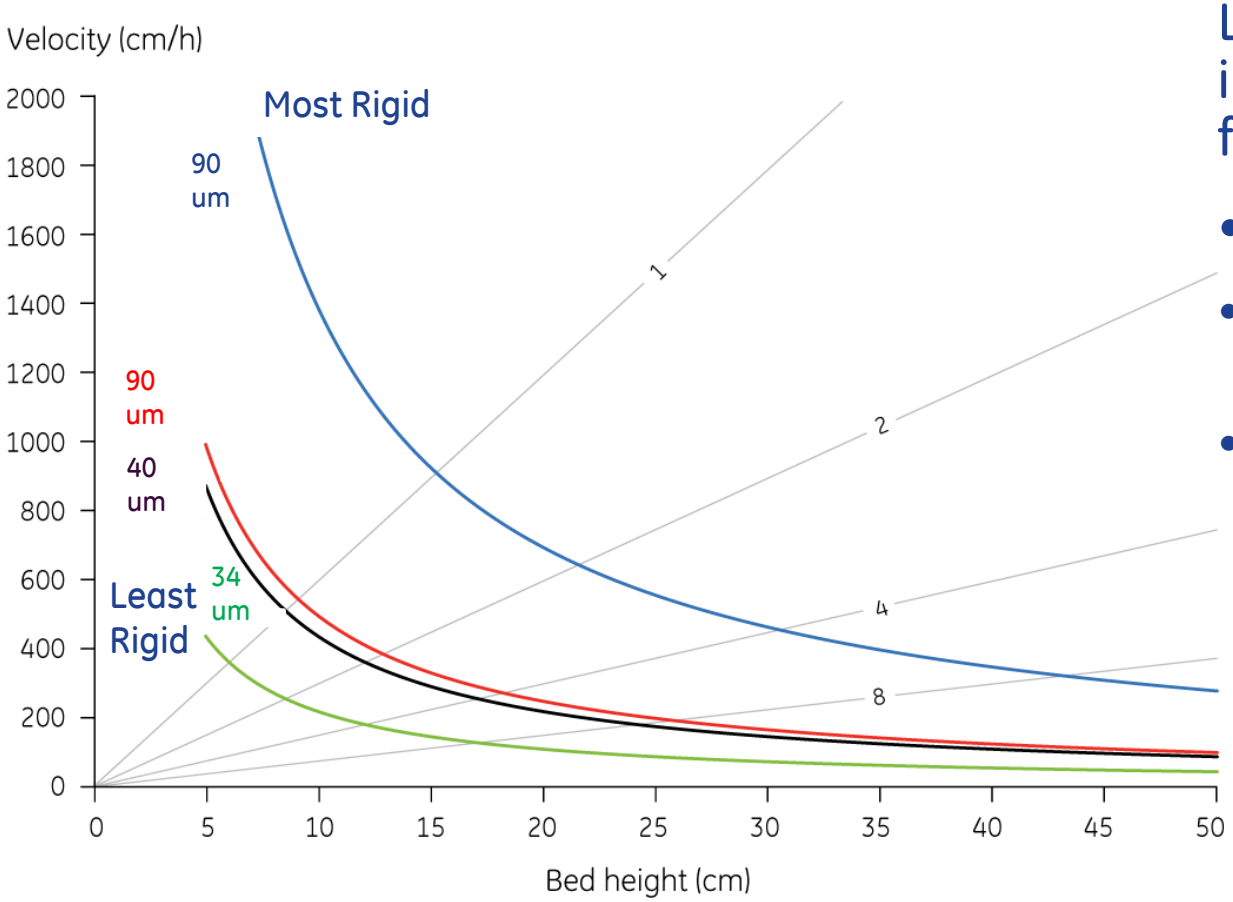
Rigidity

Improving pressure/flow properties for modern media



Large operational window in terms of bed heights and flow rates

- *Facility fit*
- *Higher bed heights – reduced footprint*
- *Higher flow rates – increased throughput*

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Functional groups used on ion exchangers

Cation exchangers	Functional group
Carboxymethyl (CM)	$-OCH_2COO^-$
Sulphopropyl (SP)	$-CH_2CH_2CH_2SO_3^-$
Methyl sulphonate (S)	$-CH_2SO_3^-$

Anion exchangers	Functional group
Diethylaminopropyl (ANX)	$-CH_2CHOHCHH_2N^+H(CH_2CH_3)_2$
Diethylaminoethyl (DEAE)	$-OCH_2CH_2N^+H(CH_2CH_3)_2$
Quaternary aminoethyl (QAE)	$-OCH_2CH_2N^+(C_2H_5)_2CH_2CH(OH)CH_3$
Quaternary ammonium (Q)	$-CH_2N^+(CH_3)_3$

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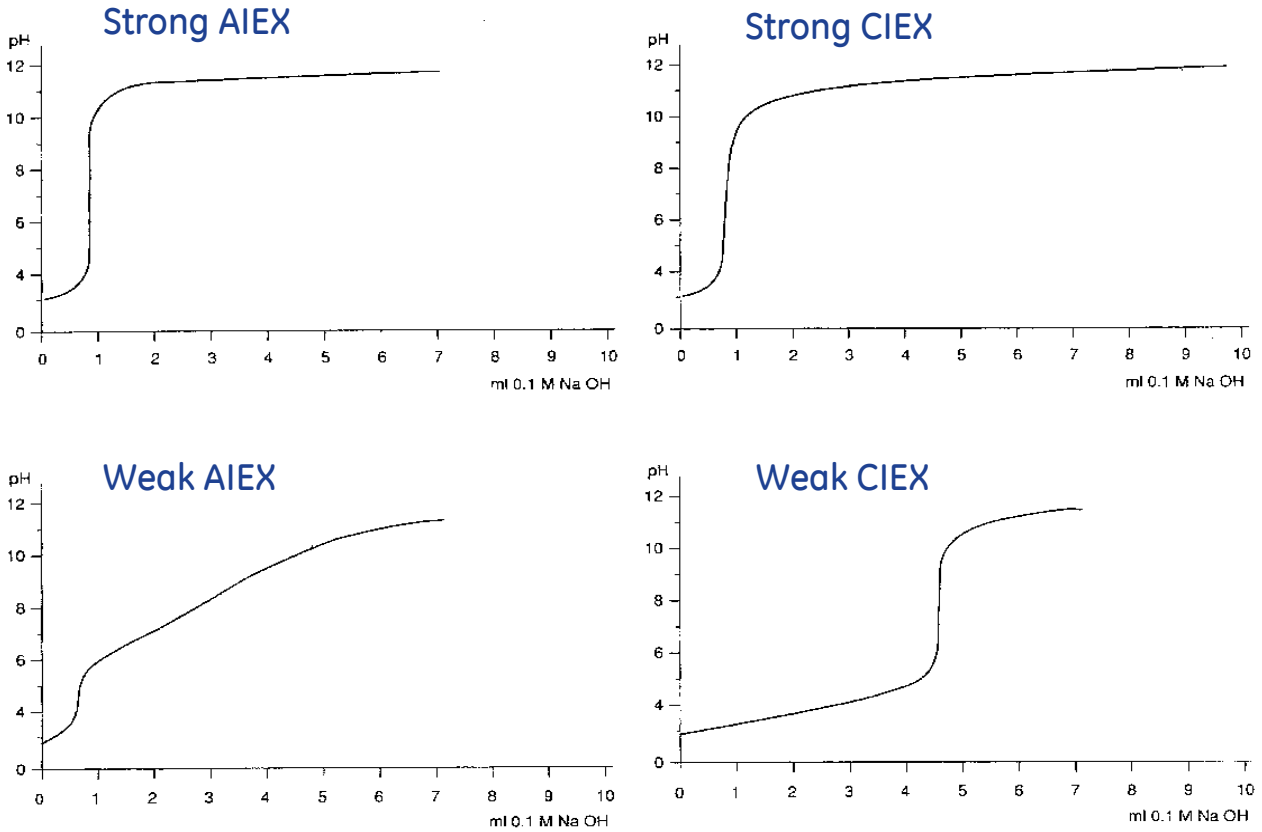
Strong and weak ion exchangers

strong ion exchangers: capacity is constant over a wide range of pH



weak ion exchangers: capacity varies with pH

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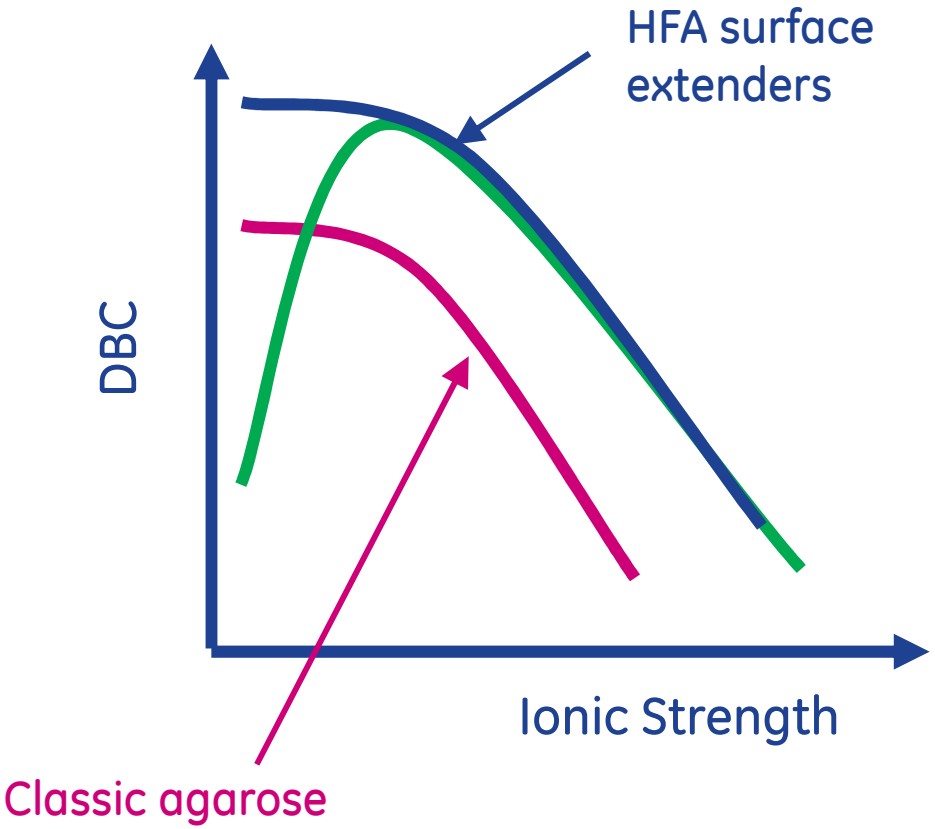
Surface extenders and non-traditional AIEX behavior!

Effect of conductivity on DBC



- Normally the highest DBC is obtained at low ionic strength (conductivity)
- Sometimes there is an optimal ionic strength for absorption of a certain sample under certain loading conditions (pH) so called non-traditional behavior
- Non-traditional behavior seems to occur more often on surface enhanced ion exchangers.

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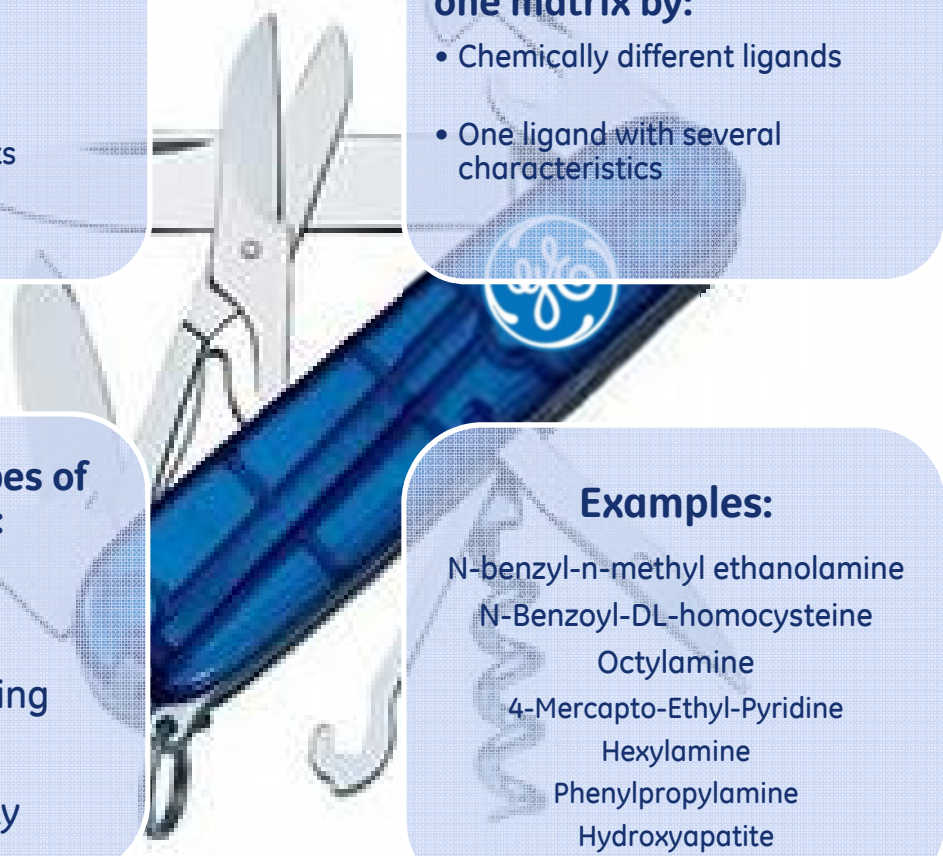
Multimodal chromatography

Multimodal benefits:

- Unique selectivity
- Use with complex feeds
- Purify challenging targets
- De-colourise feed stocks

Different techniques on one matrix by:

- Chemically different ligands
- One ligand with several characteristics



Utilizes many types of interactions:

Ionic
Hydrophobic
Hydrogen bonding
Thiophilic
Pseudo-affinity

Examples:

N-benzyl-n-methyl ethanolamine
N-Benzoyl-DL-homocysteine
Octylamine
4-Mercapto-Ethyl-Pyridine
Hexylamine
Phenylpropylamine
Hydroxyapatite

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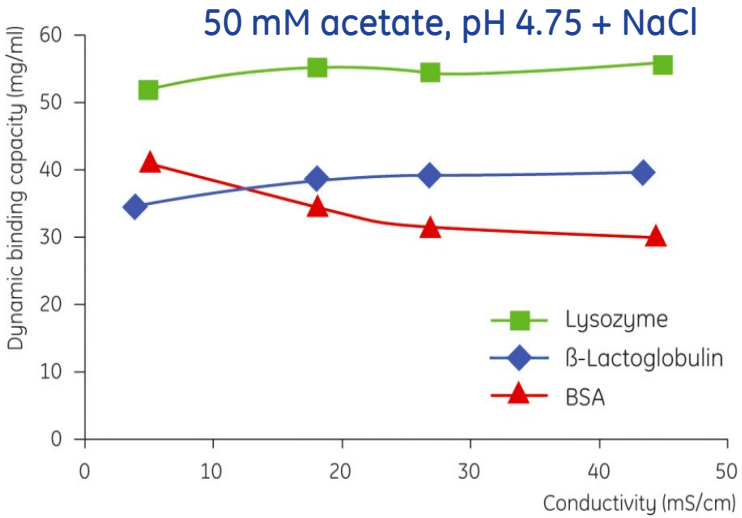
MM designed for specific applications

Capture in high salt

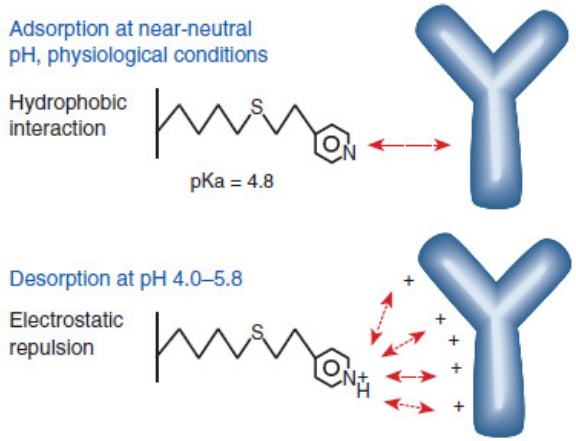
(N-Benzoyl-DL-homocysteine)



- Capture from undiluted feedstocks.
- Remove requirement for dilution or diafiltration
- Increase productivity and process economy

Purification of antibodies

(4-Mercapto-Ethyl-Pyridine)*



- A no-salt/low-salt alternative to Hydrophobic Interaction Chromatography (HIC)
- Monoclonal and polyclonal IgG capture and intermediate purification (aggregate, DNA and HCP removal)
- Enhanced process economics

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*Pall Life Sciences product note USD 2629

But gaining widespread applicability and used as a development platform

Table 1. The use of mixed-mode materials in the downstream processes of various therapeutic proteins

Protein	Source	Resin	Process step	Binding	Elution	Load (mg/mL)	Purity after process step (%)
Cytokine mutein	*E. coli*	CHT type I	Intermediate	5 mM K-phosphate, pH 6.5	5–500 mM K-phosphate gradient, pH 6.5	5–18	70–80
Recombinant protease inhibitor	Yeast supernatant	Lewatit CNP105	Capture	Supernatant adjusted to pH 6	0.1 N HCl	7	35–40
Recombinant protease inhibitor mutein I	Yeast supernatant	Capto MMC	Capture	20 mM Na acetate, pH 5	20 mM Tris, 2 M NaCl, pH 8.5	9–17	90
Recombinant protease inhibitor mutein I	Yeast supernatant	HEA Hypercel	Intermediate	20 mM Tris, 2 M NaCl, pH 8.5	40 mM Na citrate, pH 2.7	15–27	>90
Recombinant protease inhibitor mutein II	Yeast supernatant	Capto MMC	Capture	20 mM Na citrate, pH 3	20 mM Tris, pH 8.5	10	85–90
Human protease inhibitor	*E. coli* lysate	Capto MMC	Capture	50 mM Na citrate, pH 6.5	50 mM Tris, 0.5 M NaCl, pH 7.5	5	85
Antibody fragment	Yeast supernatant	MEP Hypercel	Capture	25 mM Tris, pH 7.5	25 mM Na citrate, 150 mM NaCl, pH 3.7	15–26	72
Antibody fragment	Mammalian cell line	CHT type I	Polishing	15 mM K-phosphate, pH 7	15 mM K-phosphate, pH 7, NaCl gradient	10	>98

Mixed-Mode Chromatography in Downstream Process Development
Salt-tolerant adsorption and unique selectivity are the major advantages of mixed-mode materials over single-mode resins. Mar 2, 2010
By: Felix Oehme, PhD, Joerg Peters, PhD data from Bayer Pharma, Biotech Development
BioPharm International Supplements

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Complete process using only MM media for challenging target proteins

Figure 2. Flow scheme of the purification process for the recombinant protease inhibitor mutein I

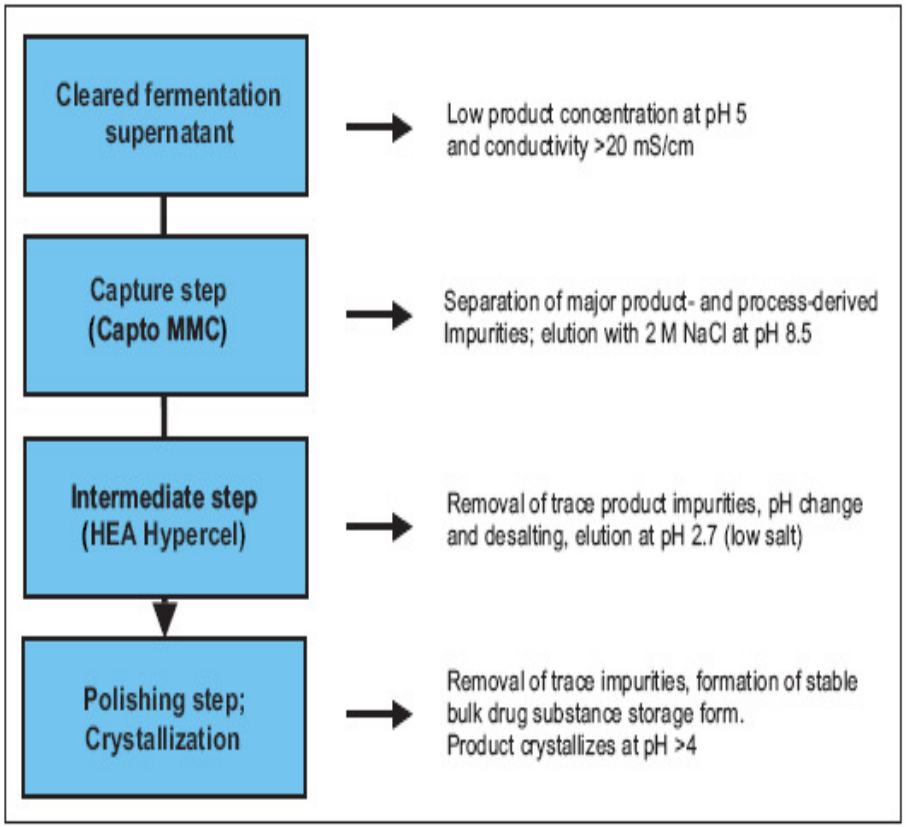

Table 3. Purification data from a typical Capto MMC capture run of the recombinant protease inhibitor mutein I

Step	Volume (L)	Mutein concentration (mg/L)	Total amount mutein (g)	Yield (%)	Purity (%)
Starting material	30	267	8.0	-	<10
Flow through	30	0	0	0	–
Wash 1 + 2	10	0	0	0	–
Wash 3	7.5	933	0.7	10	36
Elution	2.8	2,600	7.3	90	90

Table 5. Purification data from a typical HEA Hypercel intermediate step of the recombinant protease inhibitor mutein I

Step	Volume (L)	Mutein concentration (mg/L)	Total amount mutein (g)	Yield (%)	Purity (%)
Starting material	1.15	2,600	3.0	–	90
Flow through	1.15	0	0	0	–
Wash	0.8	0	0	0	–
Elution	0.35	7,372	2.6	87	91

Mixed-Mode Chromatography in Downstream Process Development
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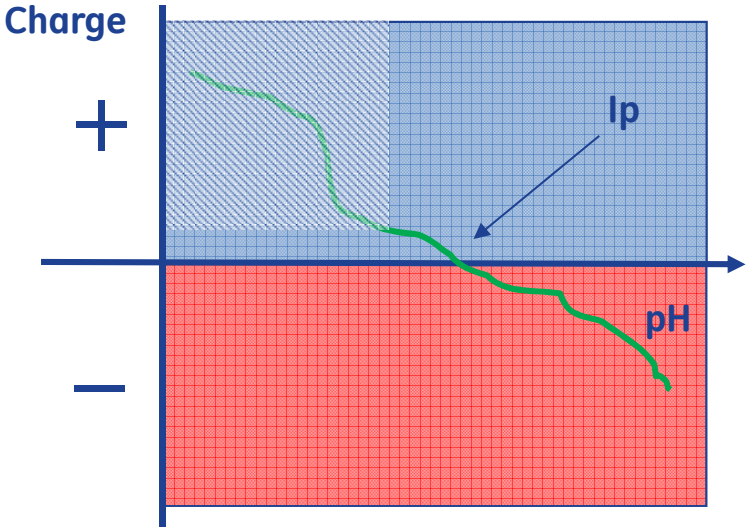
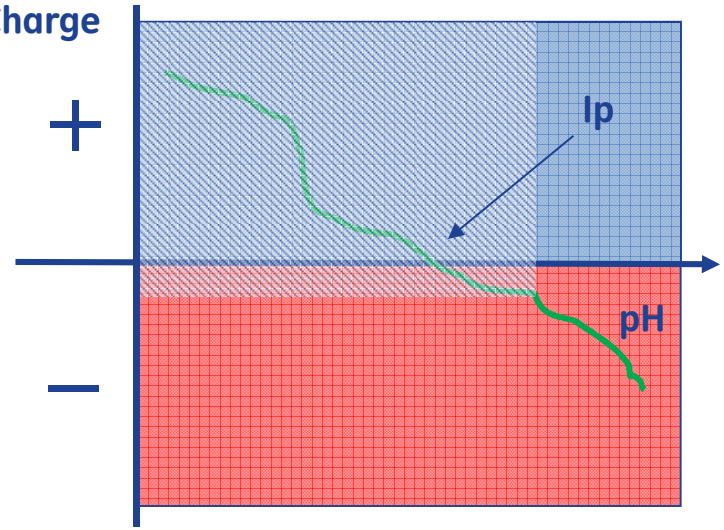
Cation exchangers vs. Multimodal

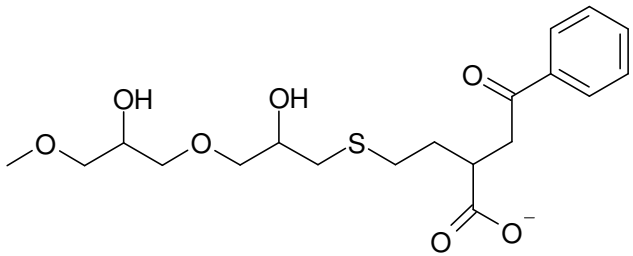
Isoelectric point vs. loading pH

Tradional cation exchange



N-Benzoyl-DL-homocysteine





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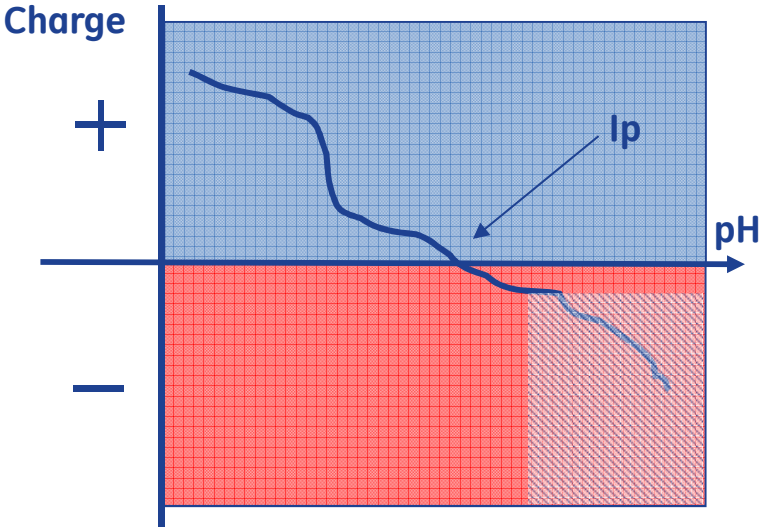
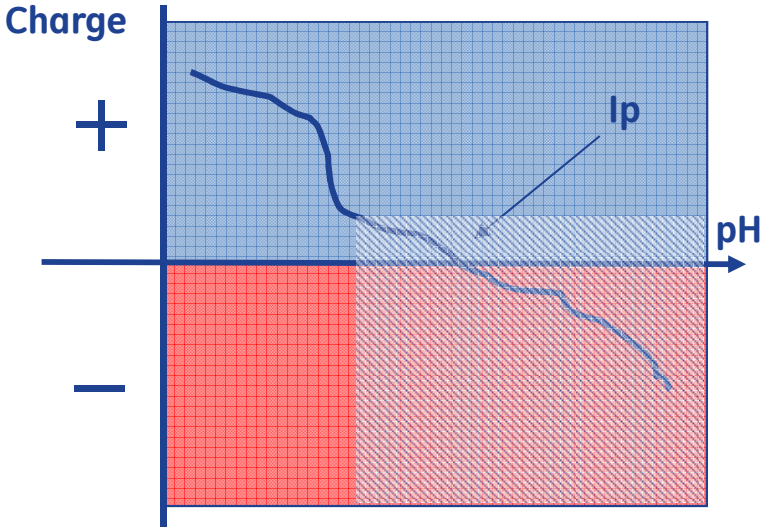
Anion exchangers vs. Multimodal

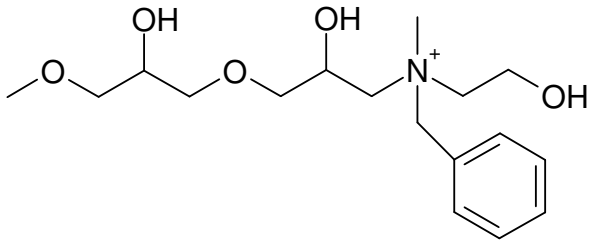
Isoelectric point vs. loading pH

Tradional anion exchange



N-benzyl-n-methyl ethanolamine





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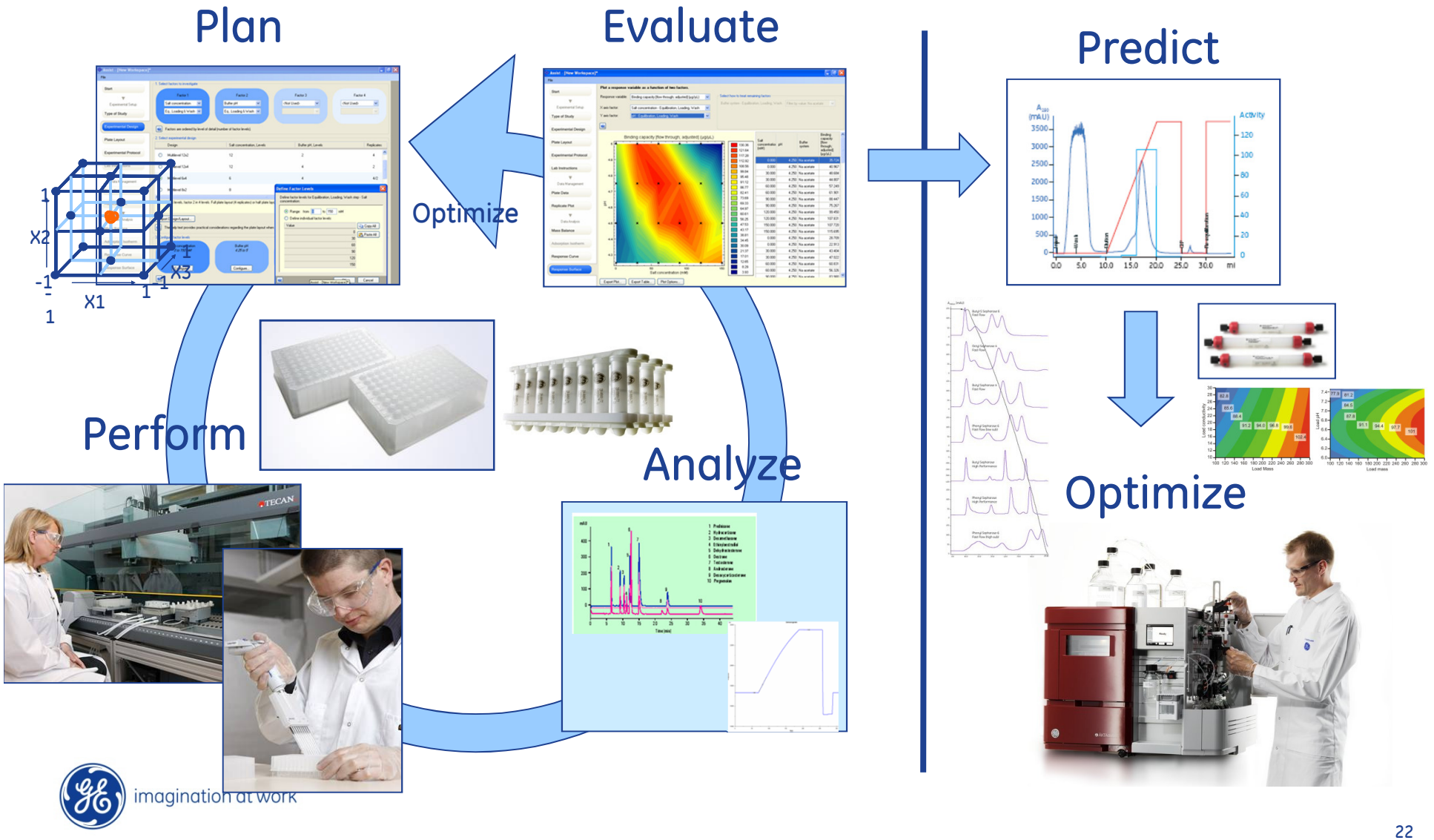
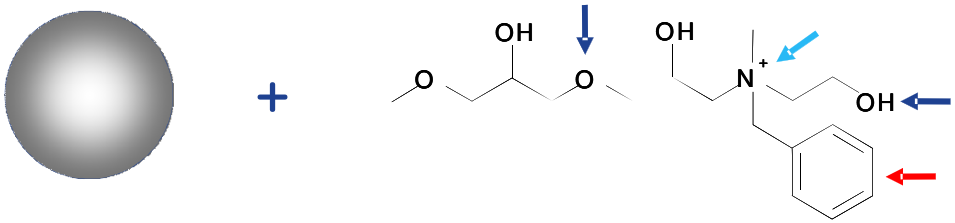
HTPD & DoE ideal for optimisation



DoE ensures a more robust process

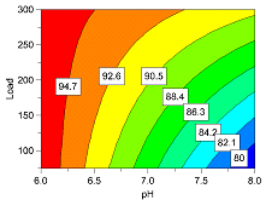
Multimodal media



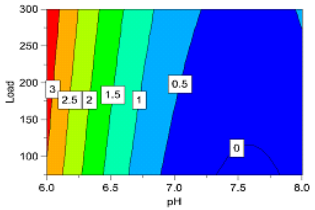
Base matrix
High flow agarose
75 µm (average)

N-benzyl-n-methyl ethanolamine
Multimodal strong anion ligand

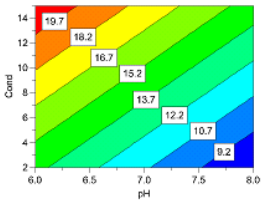
Yield



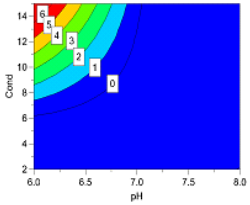
D/A



Protein A



HCP



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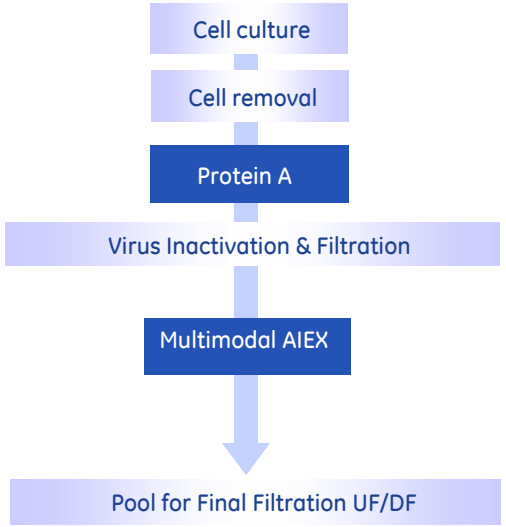
Two-chromatographic step process

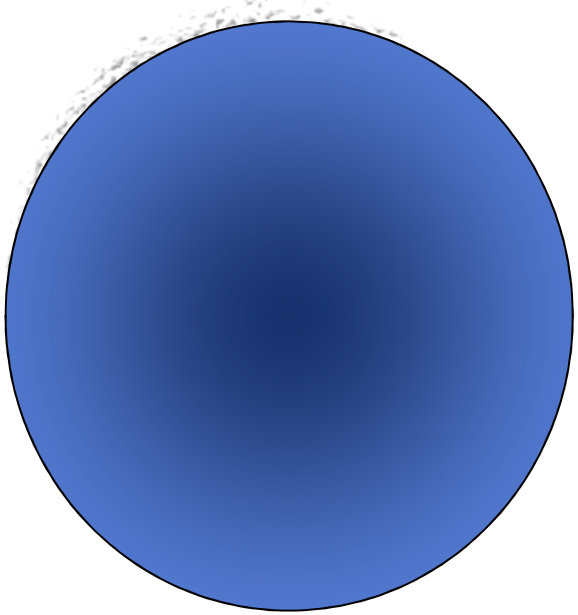


Viral clearance

Virus	Conductivity	Log_{10} red. factor
MVM	10	5.8
MVM	30	5.9
MuLV	10	4.5
MuLV	30	3.6

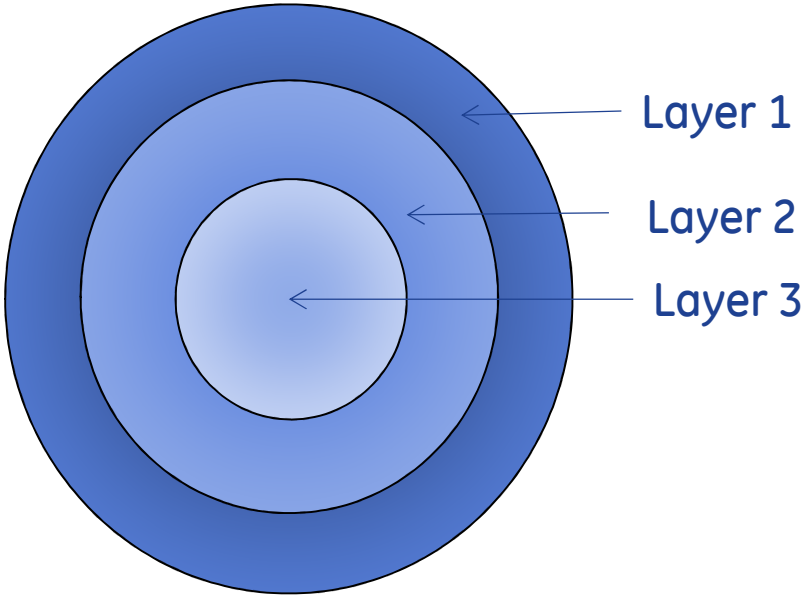
What's next for AIEX?

Concept
Layered beads
More dimensions to each bead



Design variables

-Particle size
-Pore size
-Type of ligand
-Ligand density



Additional design variables

-Number of layers
-Layer thickness
-Porosity modification
-Type of ligand

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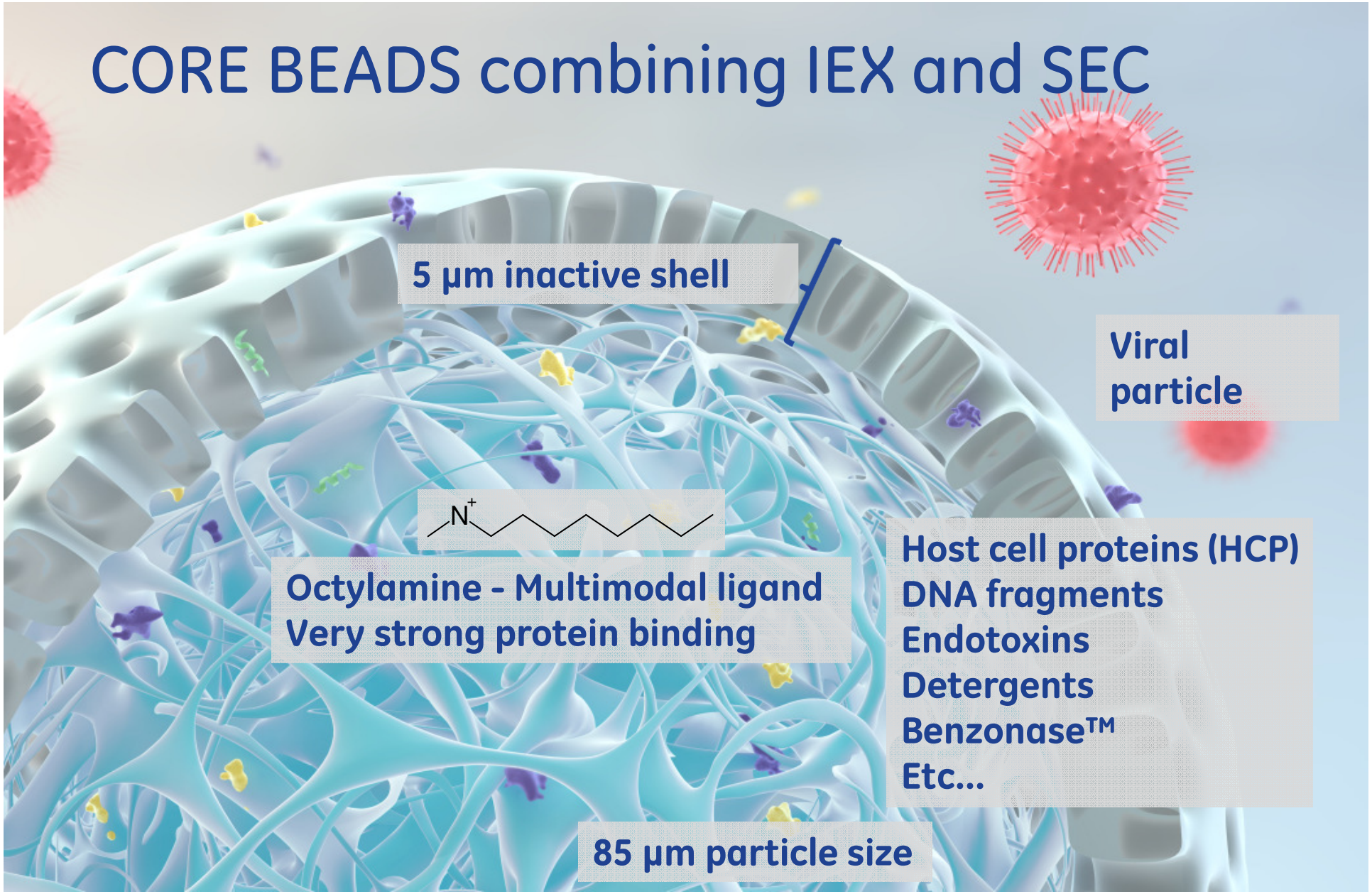
CORE BEADS combining IEX and SEC



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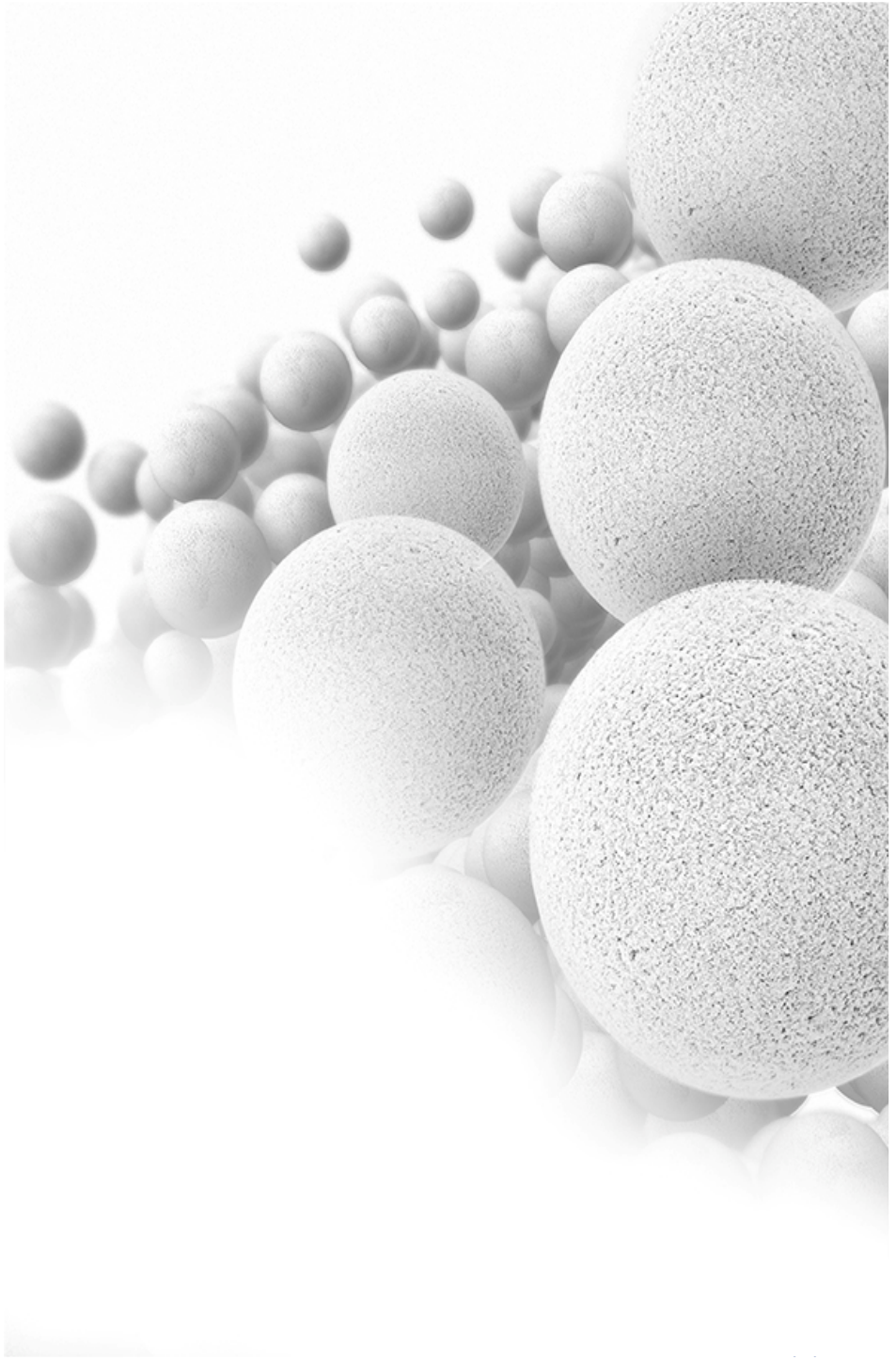
Selecting an appropriate IEX resin

- Where is the step in the downstream process?
  - Capture, Intermediate purification, polishing
  - Influence bead size, flow rates, capacity, salt tolerance
- What's the goal of the step?
  - Bulk separation, concentration of target, removal of contaminants (binding or flow through mode), resolution.
  - Influence bead size, flow rates, capacity (target or contaminants) ligand selectivity
- What are the characteristics of the target molecule vs. contaminants?
  - pI and charge, stability, size
  - Influence choice of ligand, porosity, capacity, bead size
- What are the characteristics of the feed?
  - Clarity, viscosity, composition
  - Influence choice of ligand, porosity, capacity, bead size and distribution, rigidity, flow rates
- Compatibility with other techniques in the process (AF, HIC, SEC etc.)?
  - Minimise sample handling, diafiltration, and or dilution,
  - Influence number of U/O, process economy, robustness
- Scalability of process conditions within facility?
  - Flow rates, capacity, pressure, packing, buffers
  - Influence column dimensions, system and column requirements, process tanks

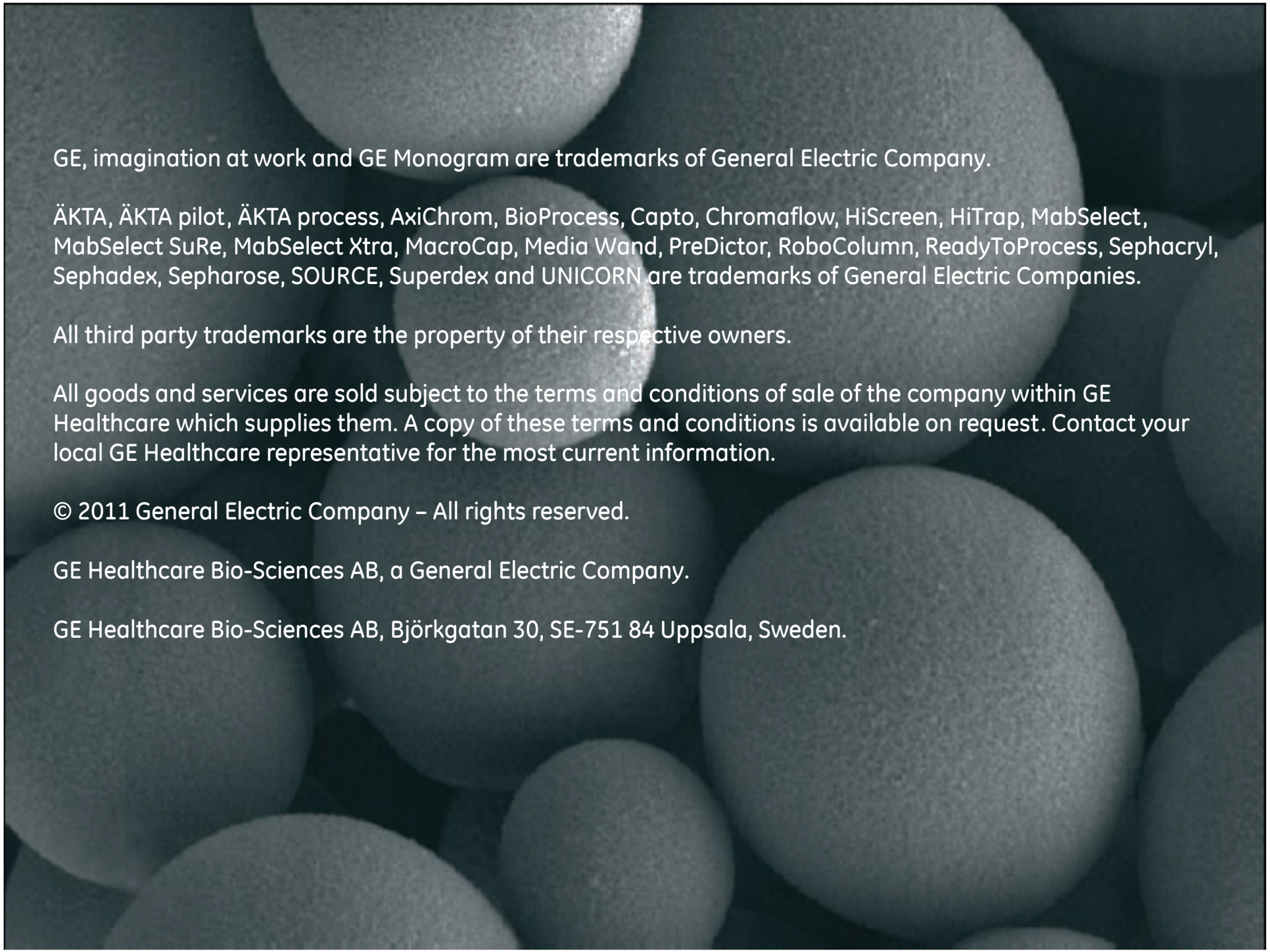
Handbooks from GE Life Sciences

imagination at work

Thank you!



imagination at work



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