

Mixed Mode Ligands for Isolation of Antibodies



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MIXED MODE LIGANDS



INDUSTRIAL

INDUSTRIAL THERAPEUTICS

BIOPHARMACEUTICALS

CONTRACT MANUFACTURERS



MIXED MODE LIGANDS



Metric Tonne Processes, Dairy, Nutraceuticals, Ind.
Enzymes

Plasma Fractionation & Recombinant Plasma Products

Recombinant Expression Systems

Disposable Capture Systems



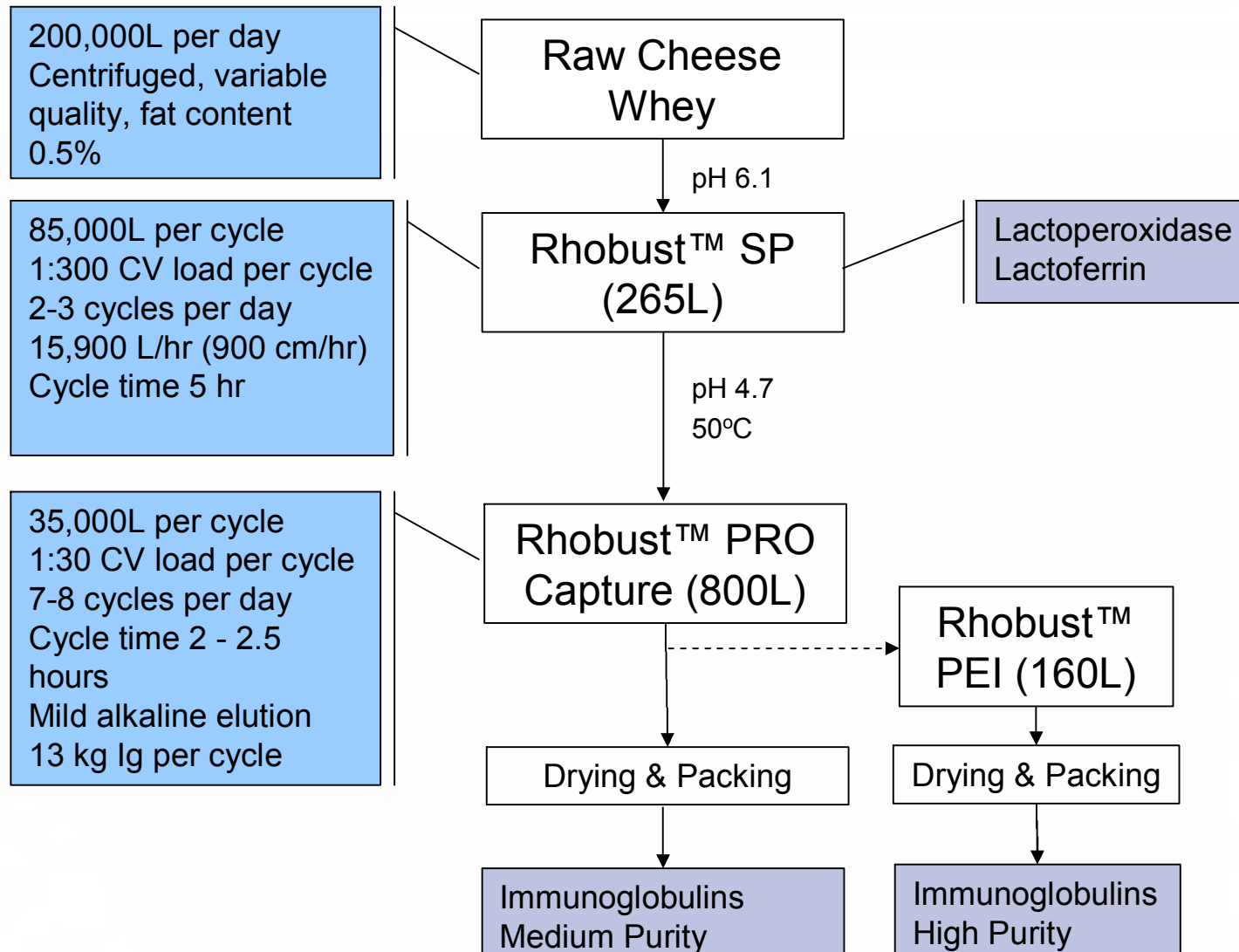
MIXED MODE LIGANDS



INDUSTRIAL



100 kg Immunoglobulins Per Day



Rhobust™ PRO (880L)



1.5 meter diameter

45 cm settled bed height

Operating 900 cm/hr

200,000L whey processed (7-8 cycle) per day

Clean 50°C, 0.5M NaOH + detergent

Working capacity 15-20 g Ig/L, >90% yield

12 kg Ig / cycle



Critical Operational Features



Robust Operation

No packing of adsorbent (self packing system)

- Large columns (fewer cycles) easy and safe to operate
- Minimal pre-treatment of feedstock; variations upstream are not fatal to downstream process
- Thorough cleaning-in-place



Critical Operational Features



No Backpressure Generated By Adsorbents

- Tall column
- High flow rates utilised

Columns Are Not Pressurised Systems

- High temperature cleaning
- Less expensive materials of construction



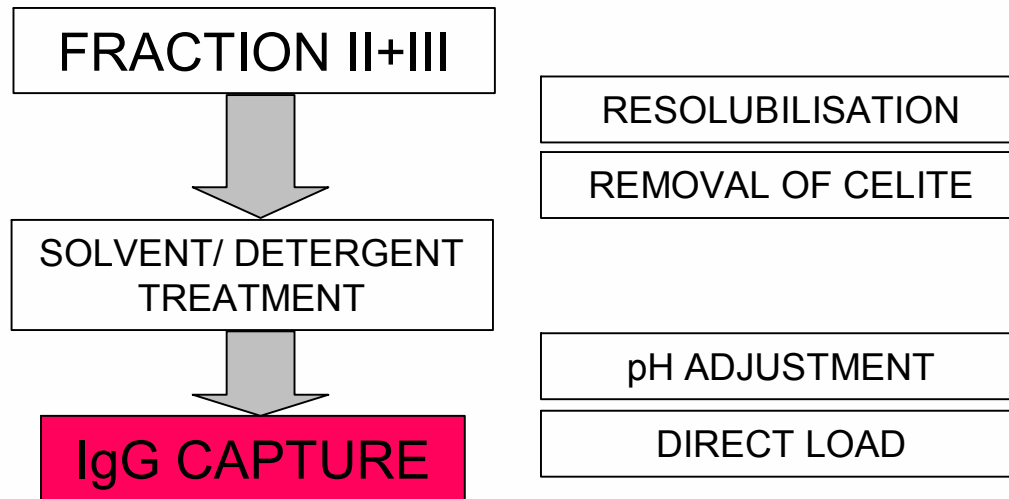
MIXED MODE LIGANDS



INDUSTRIAL THERAPEUTICS



FRACTION II+III PROCESSING

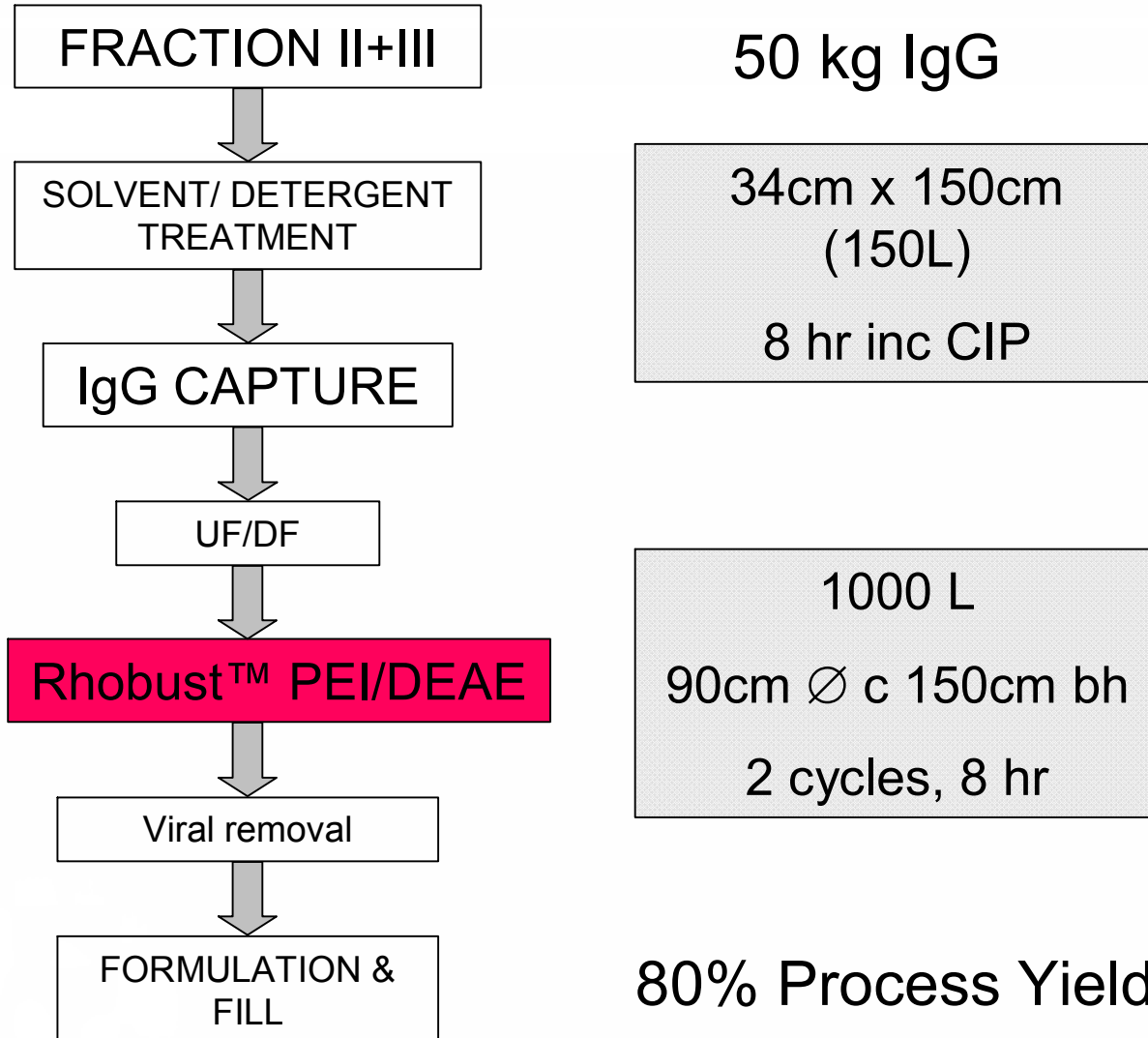


Binding capacity (IgG g/L)	IgG yield
28	86%
40	89%
100	72%
80	91%

Purity 94-100%
(IgG/Total Protein)



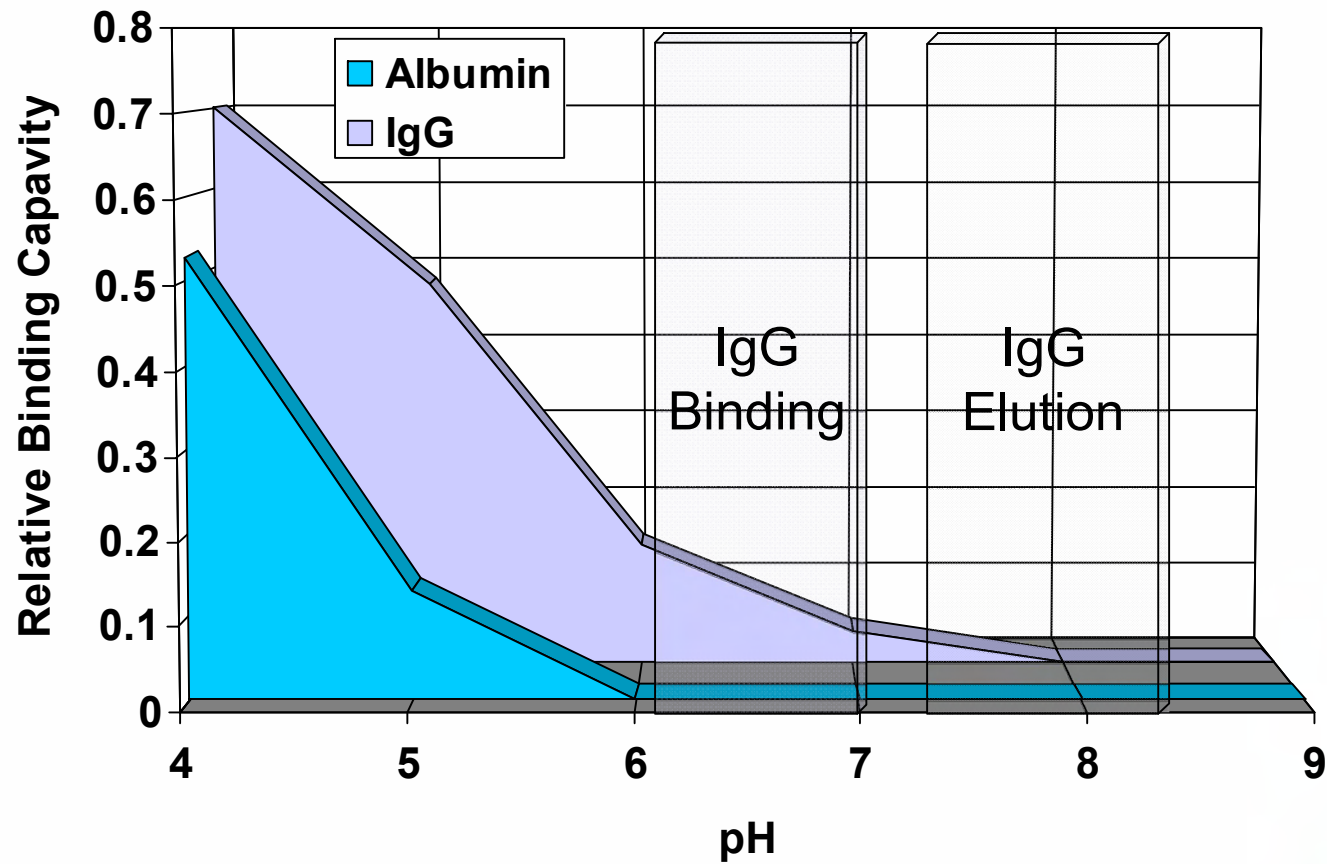
FRACTION II+III PROCESSING



Heteroaromatic Ligands With Acidic Substituents



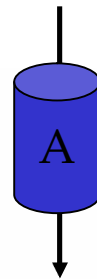
Selectivity Profile For IgG & Albumin



Mixed Mode Ligands – Multiple Elution Steps

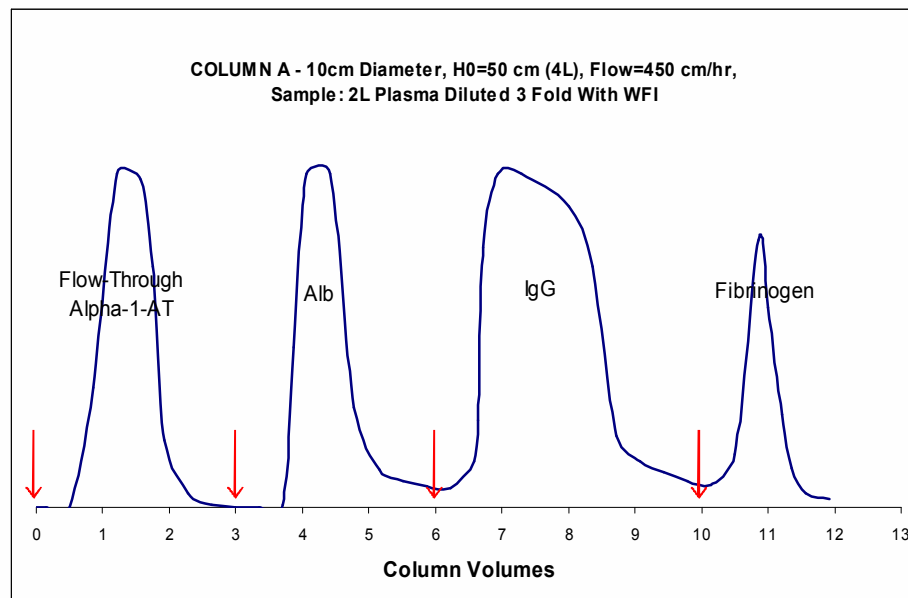


Crude plasma (diluted 1+2, pH 5.1)



- EBA fraction I (Albumin)
- EBA fraction II (Immunoglobulin G, Transferrin)
- EBA fraction III (Fibrinogen)

Alpha-1-proteinase inhibitor
Alpha-1-acid glycoprotein



MIXED MODE LIGANDS



BIOPHARMACEUTICALS



Rhobust™ Pilot Scale Set Up – Crude Feedstock Processing



LOAD

— <0.1 bar, >500 cm/hr



WASHDOWN



ELUTION



GMP Pilot Scale Column & Operating Systems For High Throughput Adsorption



Rhobust™ Platform – Mixed Mode + 2nd GEN EBA



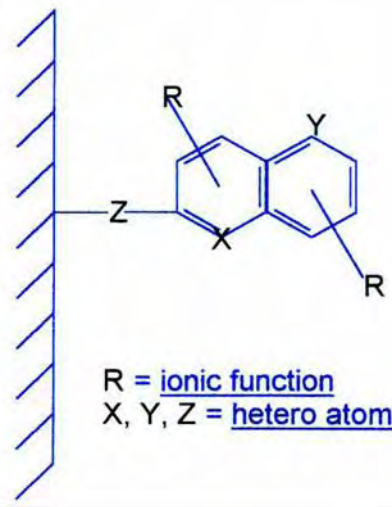
- 1. Robust processing of crude feedstocks**
- 2. Thorough Cleaning-in-place**
- 3. Simple operation of large columns**
- 4. Increased flow rates (500-2000 cm/hr)**



MIXED MODE LIGAND INTERACTIONS



2-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 3-amino-4,5-dimethoxybenzoic acid, 5-sulfosalicylic acid, nitrobenzoic acid, 2-aminobenzoic acid, 3-aminobenzoic acid, 2-mercaptobenzoic acid, 2-mercaptopyridylhydroxymethanesulfonic acid, 4-acetamidophenol, 2-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, 2,6-dicarboxylic acid, 4-pyridylthioacetic acid, 2-pyrimidinylmercaptimidazole, 2-mercaptoimidazole, 2-mercapto-1-methylimidazole, 2-mercapto-1-methyltetrazole, 2-mercaptothiazoline, 2-mercapto-1,3,4-thiadiazole, benzimidazole, 2-hydroxybenzimidazole, 2-mercapto-5-nitrobenzimidazole, 2-amino-6-nitro-benzothiazole, 2-amino-6-ethoxybenzothiazole, 2-mercaptobenzothiazole, 6-amino-2,5-dihydroxybenzoxazole, 2-(2-hydroxyphenyl)benzoxazole



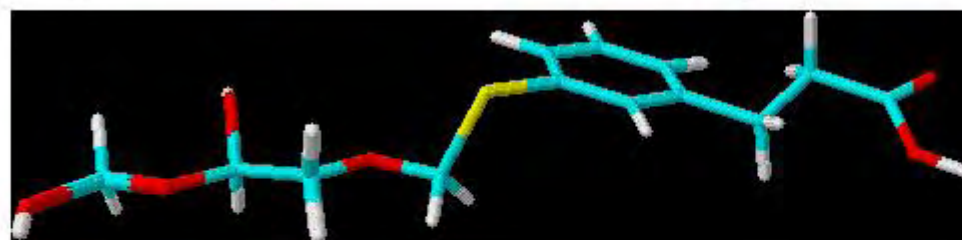
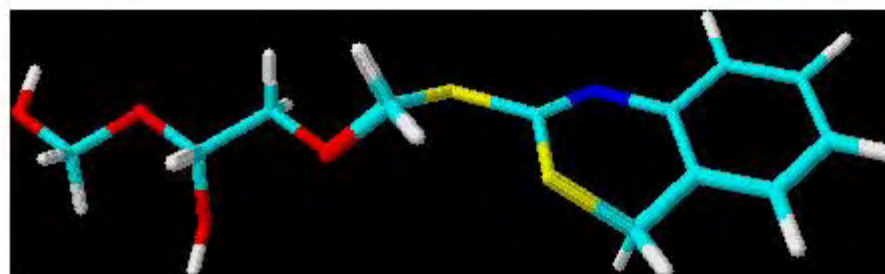
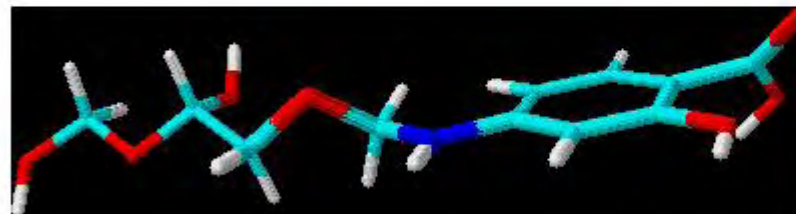
- Stable to high [NaOH] at high temperatures
- Low leakage – ether linkage
- Low toxicity
- Off-the-shelf chemistry; defined chemical syntheses



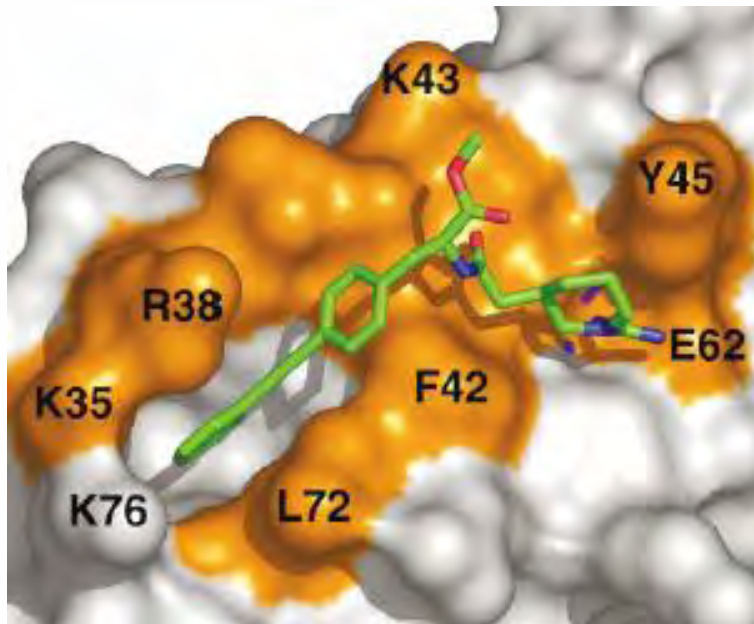
MIXED MODE LIGAND INTERACTIONS



- ❖ SALT BRIDGE
- ❖ HYDROGEN BONDING
- ❖ VAN DER WAALS
- ❖ $\pi - \pi$ STACKING
- ❖ $\pi - \text{CATION}$
- ❖ D orbital – aromatic ring ('thiophilic')
- ❖ HYDROPHOBIC (WATER SHIELDING)



MIXED MODE LIGAND INTERACTIONS

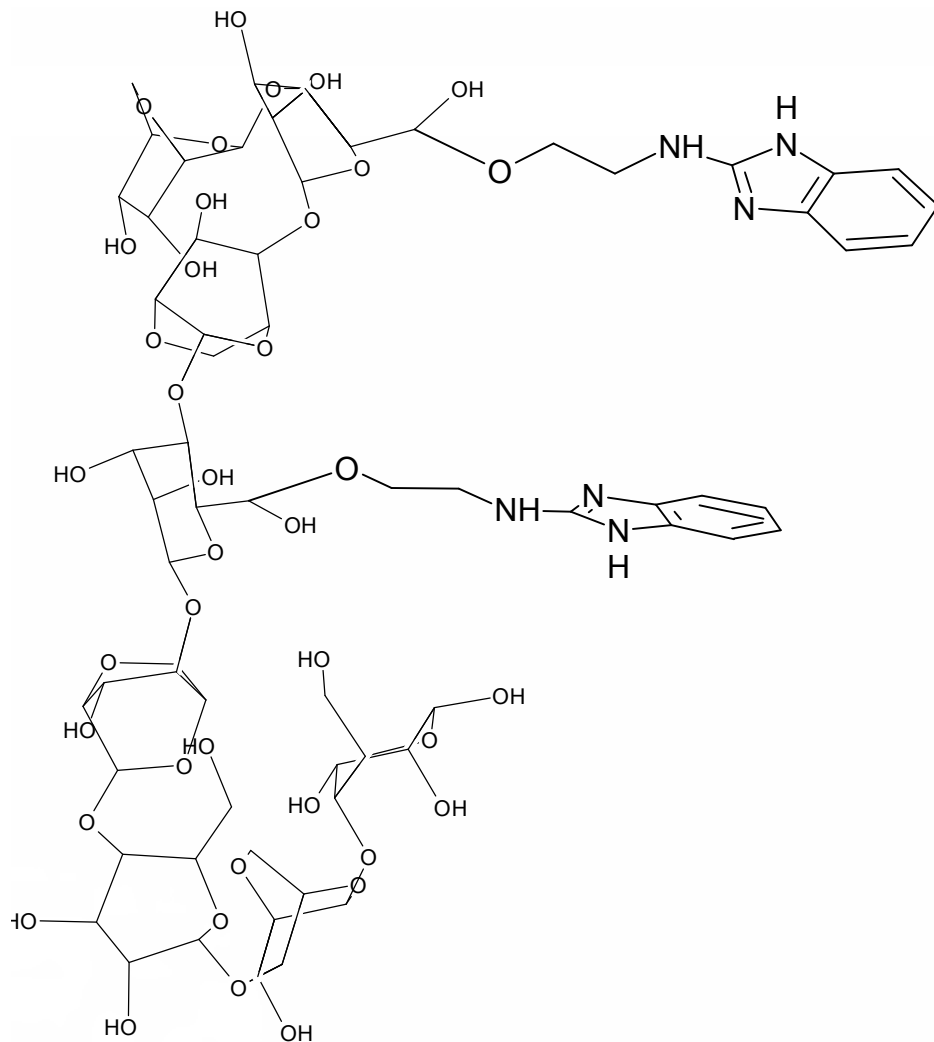


X-ray structure of the complex between IL-2 and 1. Residues established as important for IL-2Ra binding are labeled and colored orange. E62 forms a critical bidentate salt bridge and the adaptive hydrophobic pocket is created around residues F42, L72, K35, R38, and K76. Taken from Erlanson, Wells and Braisted, *Annu. Rev. Biophys. Biomol. Struct.* 2004, 33, 199)

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MIXED MODE LIGAND INTERACTIONS



- Enhanced differentiation of protein binding (compared to IEX). Greater variation of displacing molecules.
- Isoelectric point of ligand in operating region (pH elution)
- Salt tolerant
- $K_d < 1\mu\text{M}$ ($< 100\mu\text{g/ml}$)
- Low ligand density



MIXED MODE LIGANDS



CONTRACT MANUFACTURERS



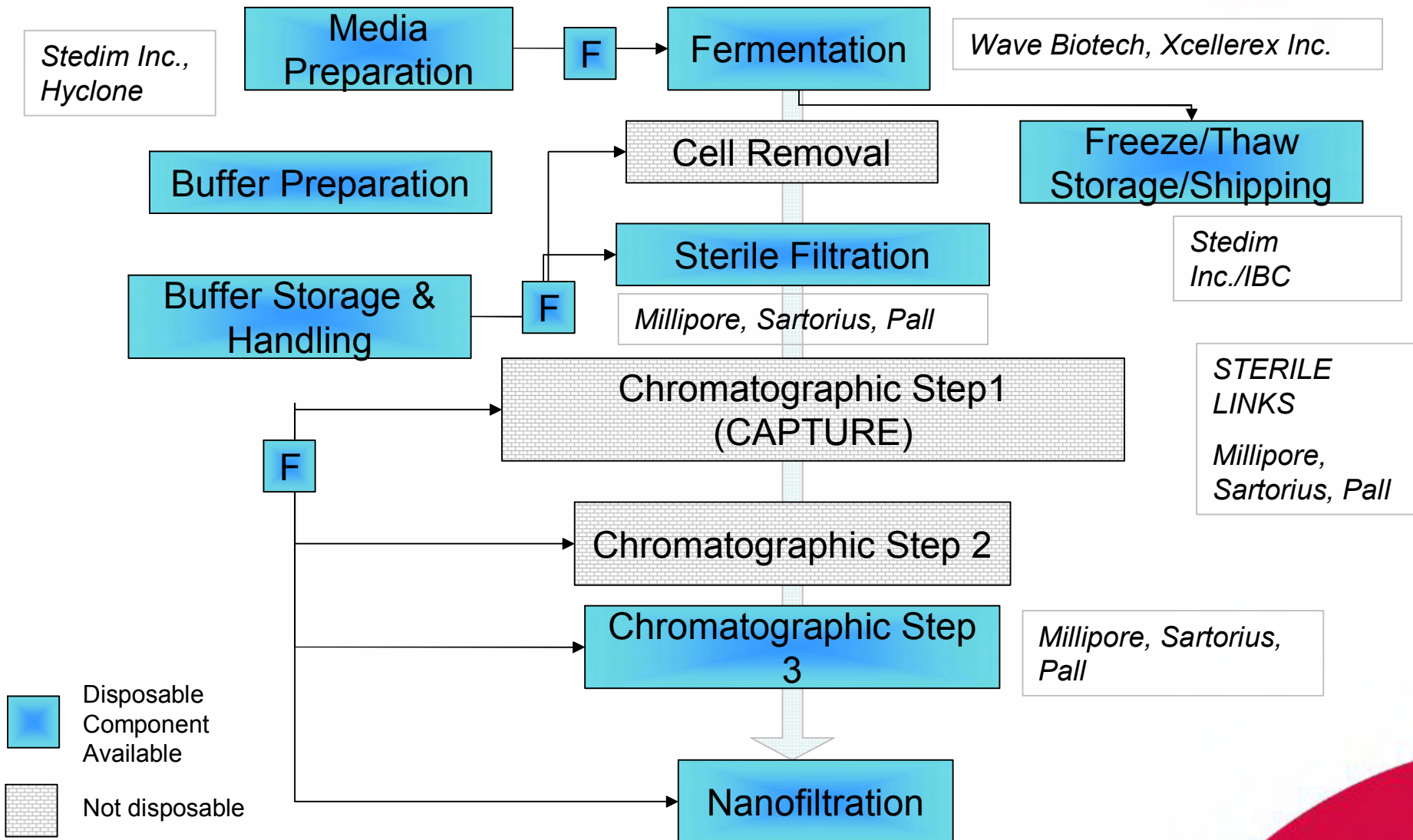
Mixed Mode Adsorbents for Antibody Therapeutics DSP



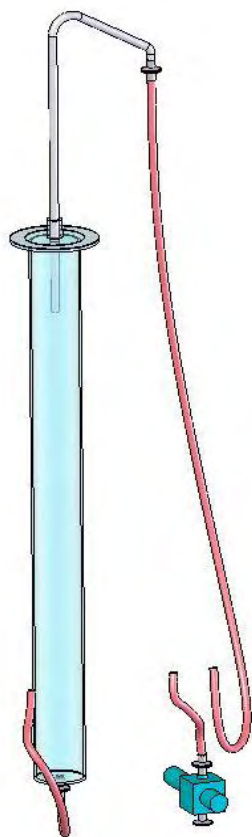
- No acidic elution step → processing of pH sensitive MAB's
- Universal process step
 - All sub-classes bind and antibody fragments
 - Short process development time (standardized conditions)
- Cleaning with 1 M NaOH, 60°C
- No Protein A leakage



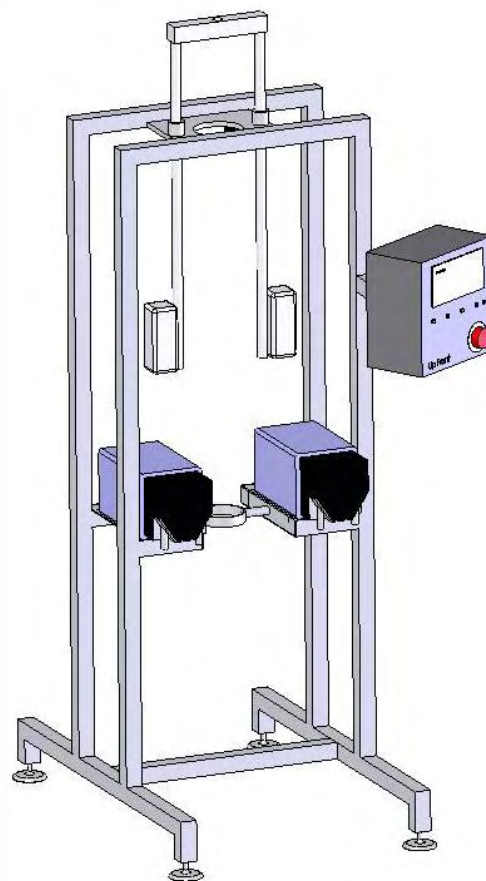
CURRENT DISPOSABLE PRODUCTION UNITS



Concept for Disposable Chromatography



Disposable column unit

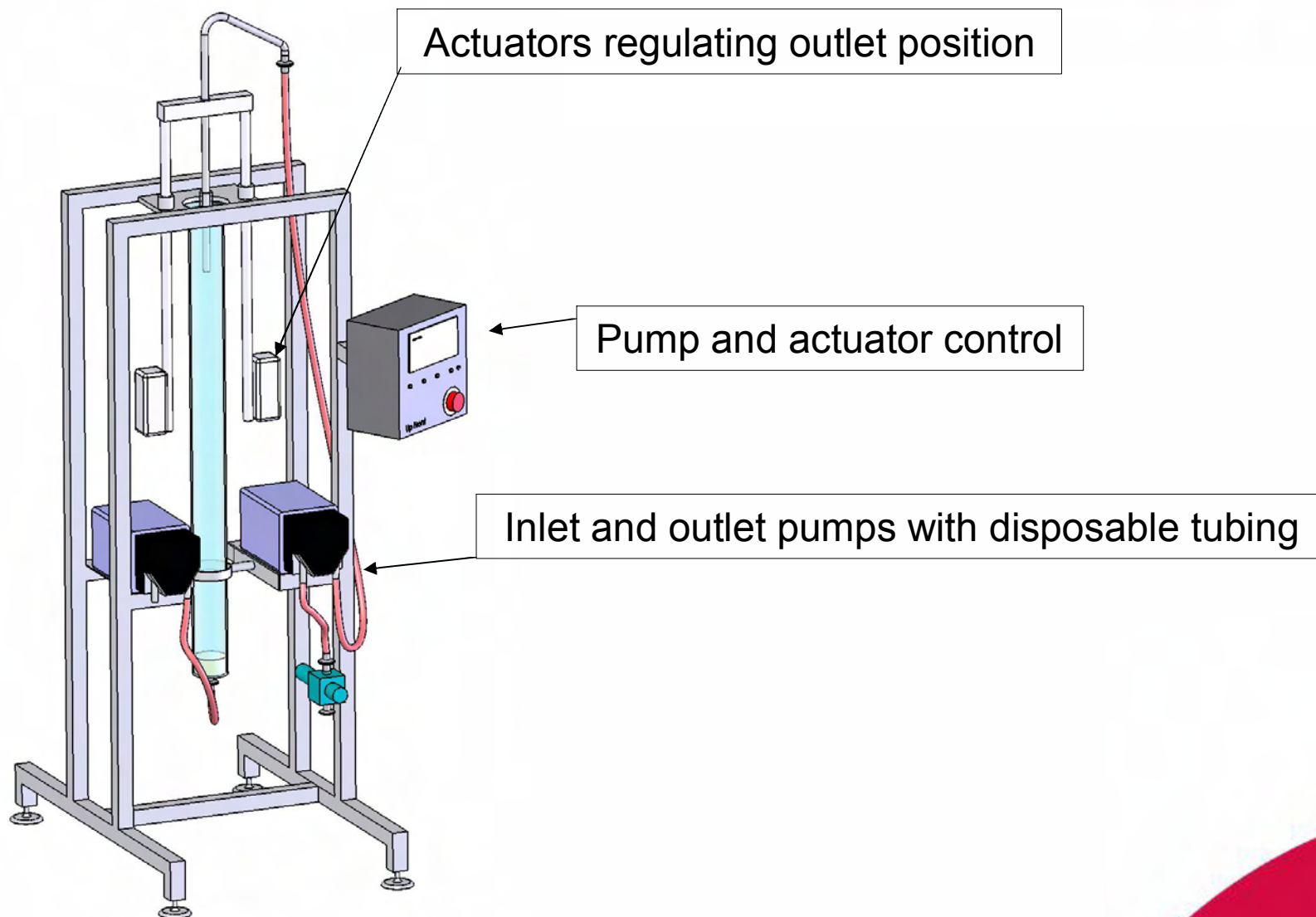


Column stand with
pumps and outlet
regulator

(no product
contact)



Concept for Disposable Chromatography



- Selective, Stable, Versatile
- Platform Important For Application
(Rhobust 2nd Generation Expanded
Bed, Disposable, Packed Bed)
- Polishing (Flow-through)
Capability



Acknowledgements



-
- UpFront R&D – Allan Lihme, Marie Bendix-Hansen, Inga Vaast and staff
 - Photo's of cGMP equipment – Morton Orlander
 - Our clients



Rhobust Bioprocess Technology

