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Developments in mixed mode chromatography in biopharmaceutical purification

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Outline

- Avecia background
- Review of mixed mode media chemistries
- Mixed mode behaviour
- Mixed mode application in biopharmaceutical purification processes
- Case studies
- Summary

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Businesses

Biologics

Vaccines

DNA Medicines

Biopharmaceutical API contract manufacturing

Development of Recombinant vaccines

Oligonucleotide API contract manufacturing

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Microbial and mammalian derived recombinant proteins

Customer Focus

US, Europe, Japan
Established Biotechs
Life-cycle involvement

Compliance

MHRA licensed
FDA inspection ready
Regular customer audits

Development

Process invention
Process development
Characterisation
Validation

Facilities

Activities aligned on one site
Pilot scale
Clinical manufacture (1000L)
Commercial manufacture (2x 5000L)
Net Assets > £60m

Dimensions

25 years experience; >35 biologics developed; >500 employees

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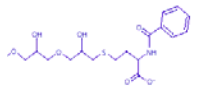
Mixed mode media

Media	Type	Ligand
MEP (Pall)	Hydrophobic binding near neutral pH with elution by pH reduction	4-Mercapto ethyl pyridine
HEA (Pall)	Hydrophobic binding near neutral pH with elution by pH reduction	Hexylamino
PPA (Pall)	Hydrophobic binding near neutral pH with elution by pH reduction	Phenylpropylamino
MBI (Pall)	Hydrophobic binding at slightly acid pH with elution by raising pH	2-Mercapto-5-benzamidazole sulfonic acid
Capto MMC (GEHC)	Cation exchanger with mixed mode functionality	
Capto adhere (GEHC)	Strong anion exchanger with mixed mode functionality	N-benzyl-N-methyl ethanolamine
CHT hydroxyapatite (BioRad)	Ion exchange with hydrophobic component	$(Ca_5(PO_4)_3OH)_2$
CHT fluoroapatite (Biorad)	Ion exchange with hydrophobic component	$(Ca_{10}(PO_4)_6F)_2$

Others

- Affinity media can display significant mixed mode character depending on spacer
- Thiophilic media

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Mixed mode media are new

But you may have been experiencing mixed mode media all the time……

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CIEX experimental studies

- Similar experimental configuration
- 80kD protein, pI 8.5
- Tested with four different cation exchange media
- All have same cation functionality
- Same experimental set up in all cases
- Loaded to 11mg/ml at 20mS/cm, pH 7.0
- 10CV linear elution gradient to 1M NaCl
- 14cm bed depth

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CIEX media characteristics

Media	Functionality	Matrix	Particle size (micron)
SP XL Sepharose	Sulphopropyl	Agarose (6% crosslink, dextran coating)	130
SP Sepharose	Sulphopropyl	Agarose (6% crosslink)	130
SP Sepharose HP	Sulphopropyl	Agarose (6% crosslink)	45
SOURCE 30S	Sulphopropyl	Polystyrene/ divinylbenzene	30

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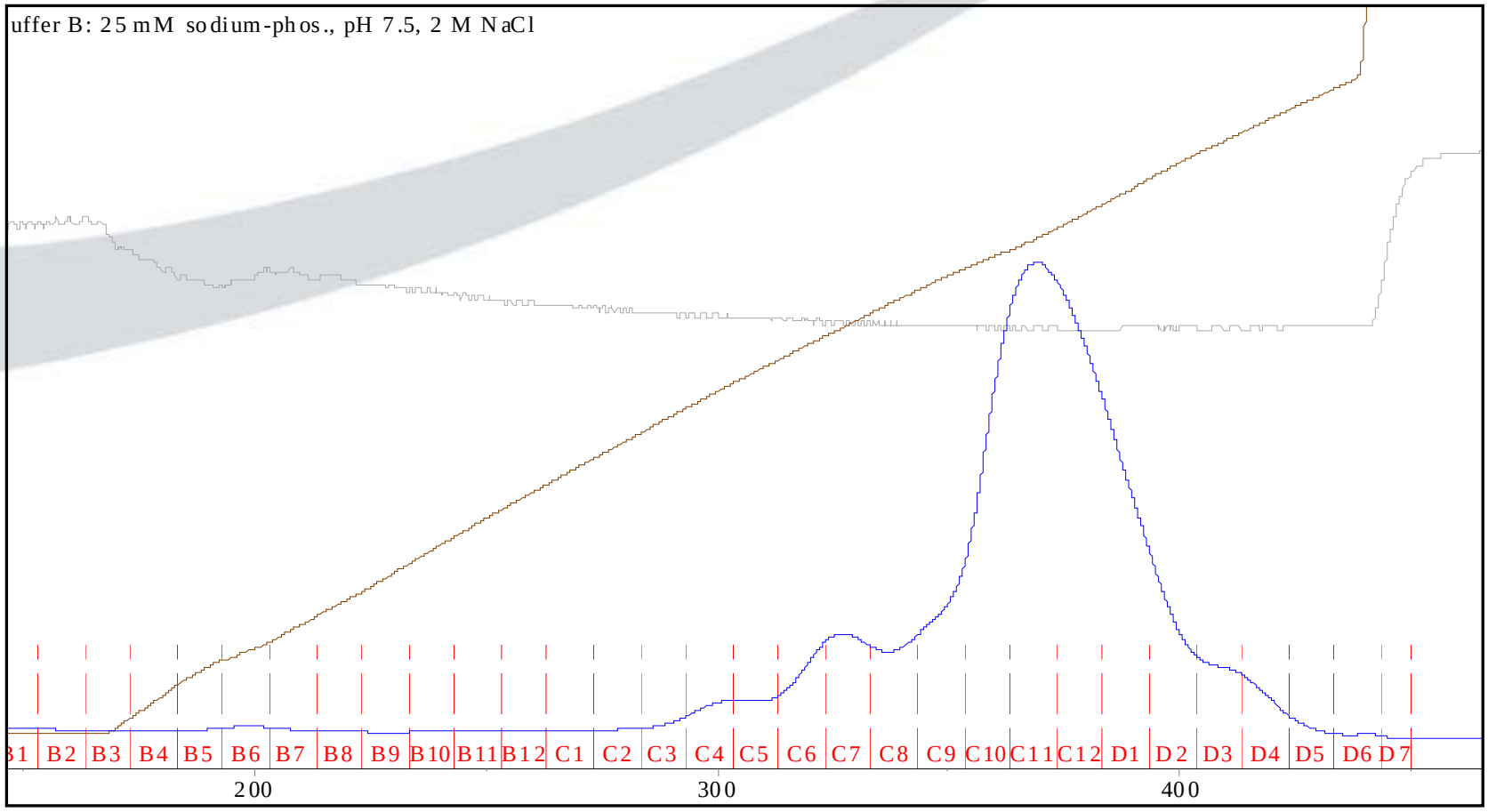
SP Sepharose FF



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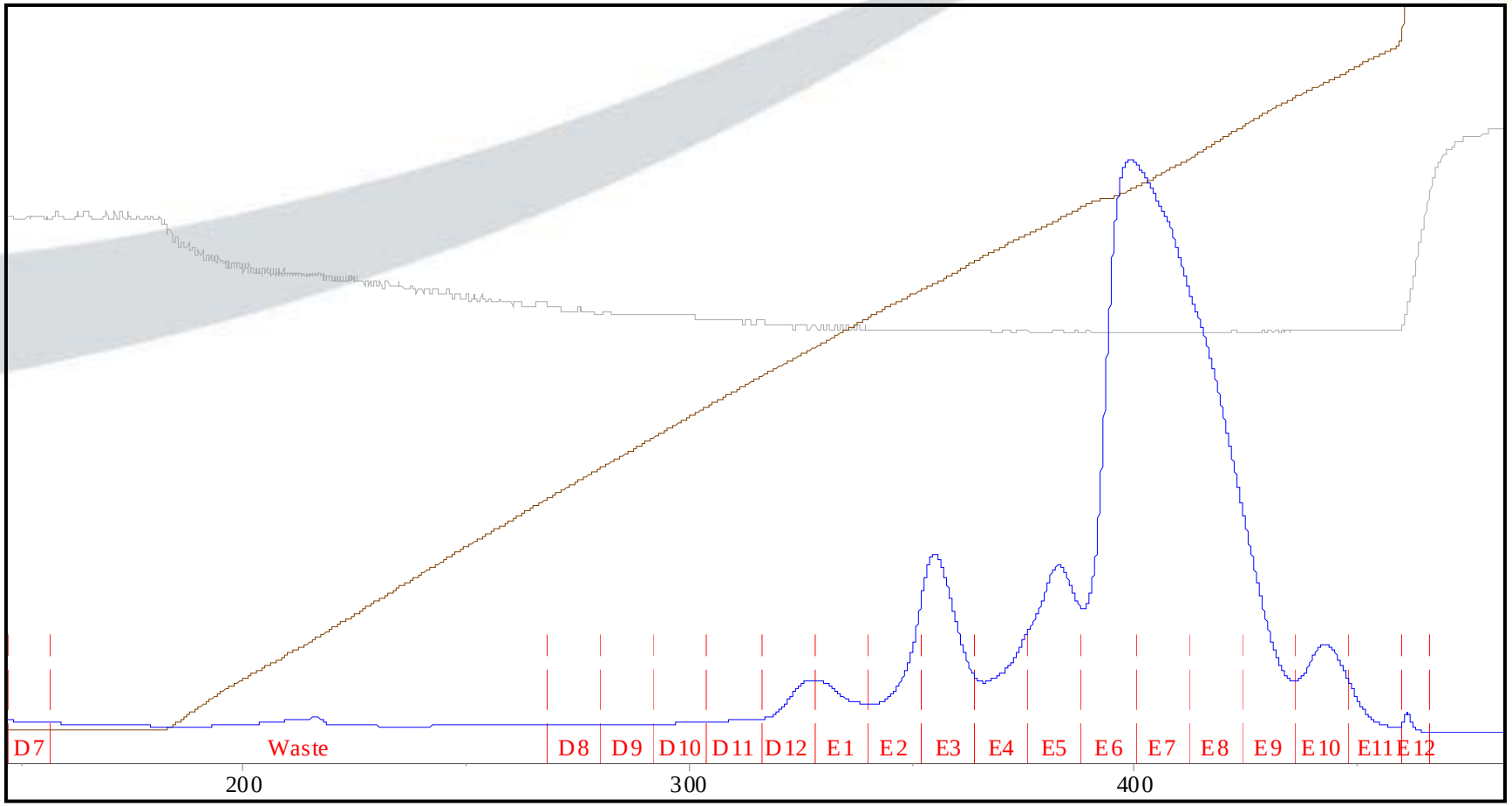
SP Sepharose HP



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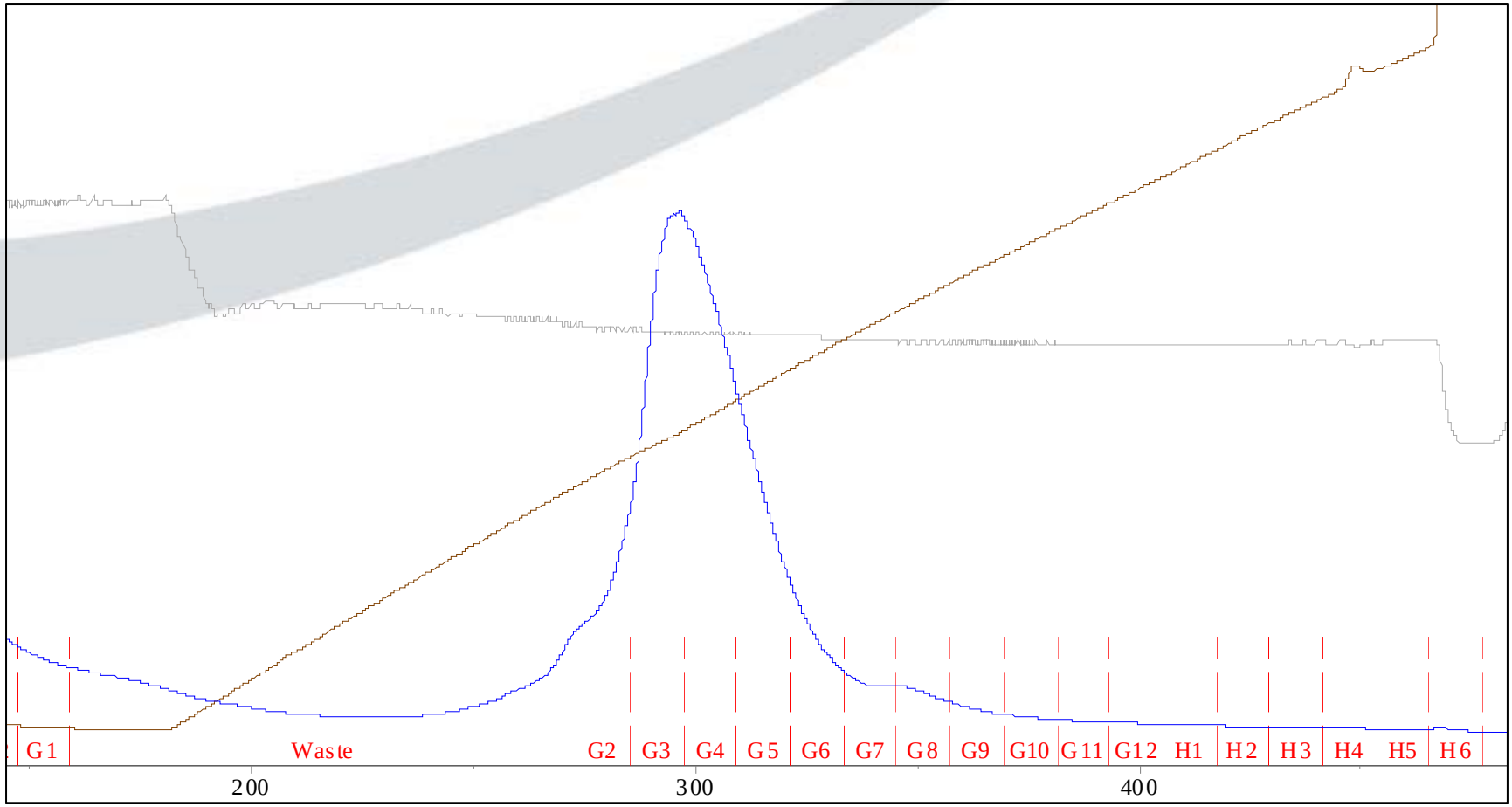
SOURCE S



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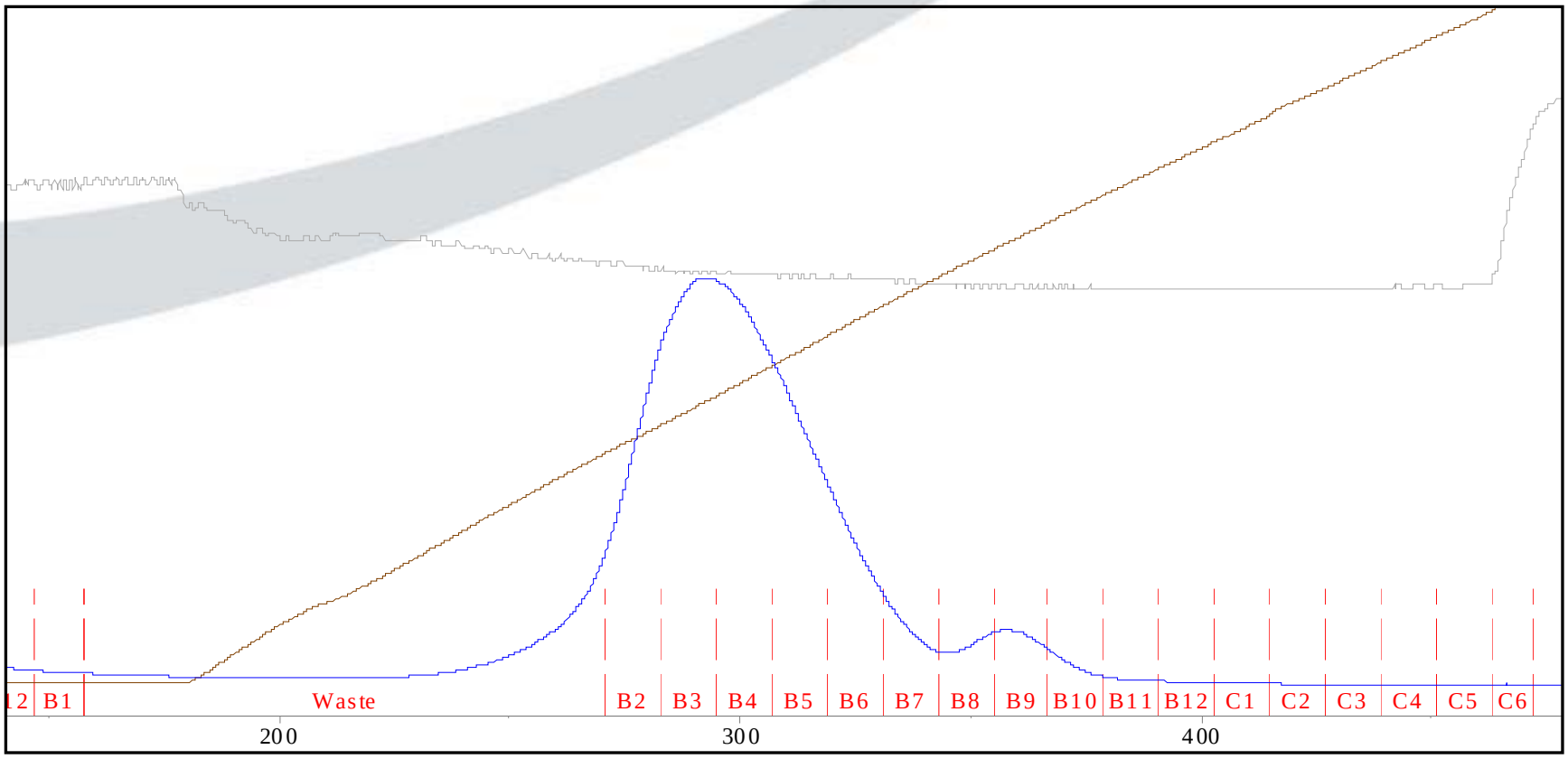
SP XL Sepharose



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Some observations….

- Capacity: SP FF/SP HP > SOURCE 30S > SP XL
- Resolution: SP HP > SP FF > SOURCE ≅ SP XL
- Variable position of elution in gradient
- Resolution not always related to particle size
- Mixed mode behaviour – matrix and ligand interaction

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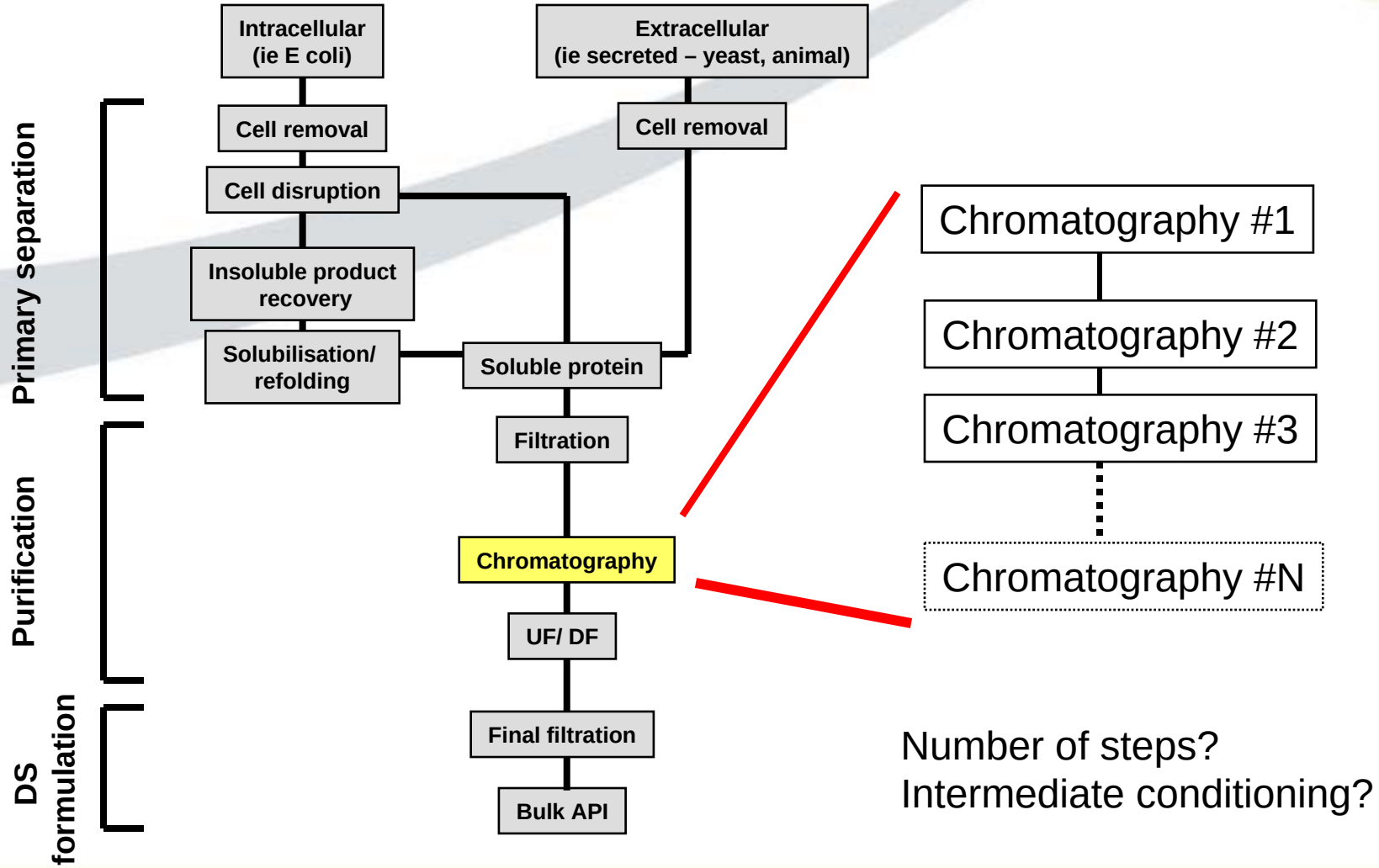
General biological separation scheme



Number of steps?
Intermediate conditioning?

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Analysis of chromatography step order

Separation basis	Chromatography #1	Chromatography #2
Ion exchange	78	52
Hydrophobic	7	48
Affinity	11	0
Metal chelation	4	0

- Based on biopharmaceutical processes at early 2006 (in house data)
- Ion exchange as step #1, HIC as step #2 a frequent pattern
- Good practical reasons
  - Loading capacity
  - IEX→HIC rather than HIC→IEX

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Mixed mode media and purification processes

- Depends on the mix of the modes
- Provides new process options
- Hydrophobic media which can produce a low ionic strength eluant
- Highly hydrophobic media from which protein can be recovered quantitatively
- Selectivity control through control of binding or elution pH, ionic strength, salt type
- Potentially any chromatography step – advantage of matching to subsequent chromatography step
- Capture steps may have most potential
- Highlighted in some examples

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Mixed mode and process development

- More complex development
- Different from IEX and HIC
- More adjustable parameters
  - Elution profile - pH step, pH gradient
  - Load - ionic strength, pH, salt type
  - Elution - ionic strength, pH, salt type
- Experimental design approaches and beyond

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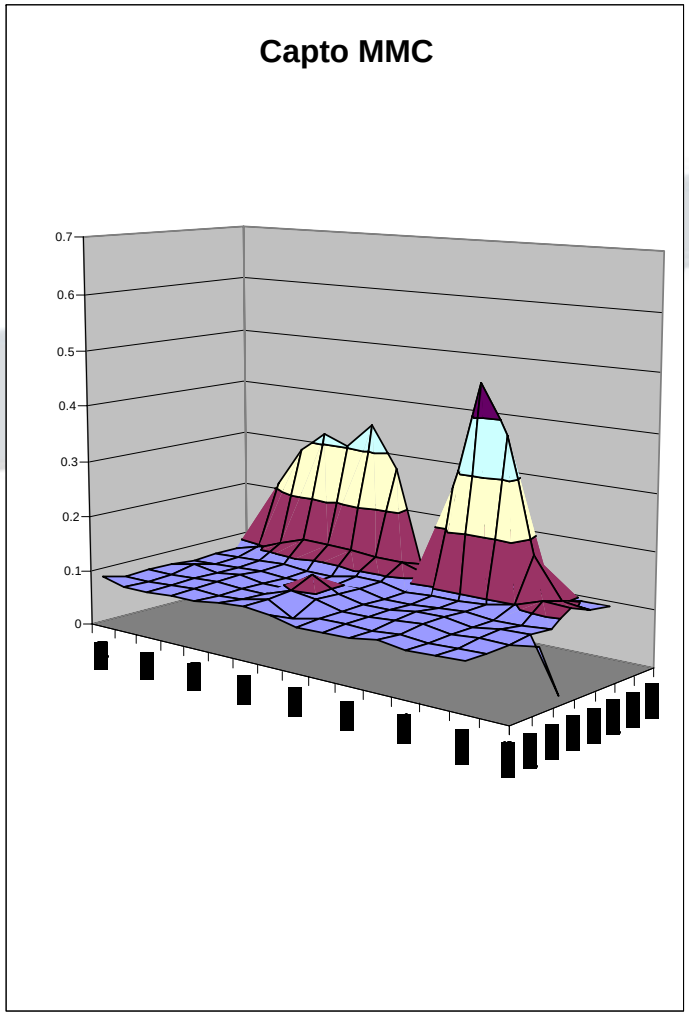
Potential for high throughput techniques



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Example 1 - background

- Evaluation of Pall mixed mode chromatography sorbents in initial capture step for recombinant proteins from *E. coli* homogenate
- Capture the target without conditioning the homogenate
- Elute via a simple pH gradient at low ionic strength
- Provide an eluate suitable for simply pH titration and load onto an IEX column without intermediate UF/DF

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Test system

- Recombinant protein
- Intracellular expression in E coli
- Expressed as fusion protein
- Initial studies with post cleavage, partially purified protein
- 37.7kDa
- pI 5.57
- Aliphatic index 90.75
- GRAVY –0.549

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Strategy

1. Screening:
   - Establish loading, washing and elution conditions
   - Sample loaded without feed conditioning, washed with the equilibration buffer
   - Eluted via a 20 CV pH gradient from 7 to 3 into a low conductivity buffer.
2. Binding capacity:
   - As screening, but loading protein until breakthrough is seen.
3. Optimisation:
   - Depending on previous data and observations,
   - Parameters such as load rate, load condition, wash buffer, elution strategy varied systematically
   - Maximise data but minimise experimentation.

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Experimental protocol

Step	Buffer	Volume
Equilibration	20mM PB pH 7.4	5 CV
Load	7.0 $mg.ml^{-1}$ product in PBS pH 7.4	1 CV
Wash 1	20mM PB pH 7.4	4 CV
Wash 2	Buffer A: 100mM Phosphate-50mMCitrate Buffer pH 7	4 CV
Linear gradient elution	Buffer A: 100mM Phosphate-50mMCitrate Buffer pH 7 Buffer B: 100mM Phosphate-50mMCitrate Buffer pH 2.6	20 CV
Wash 3	Buffer B: 100mM Phosphate-50mMCitrate Buffer pH 2.6	2 CV

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Preliminary results

Ligand	pKa	Recovery (%)	Capacity at 10% breakthrough ($mg.ml^{-1}$ resin)	Elution pH
MEP HyperCel	4.8	58	Not tested	< 5.5
HEA HyperCel	6 and 9	88	>>7	< 4.5
PPA HyperCel	6 and 9	64	66.5	< 3.5

Compare with

Hydrophobic interaction media (phenyl) capacity of 10 mg/ml

AIEX capacity ~20 $mg.ml^{-1}$

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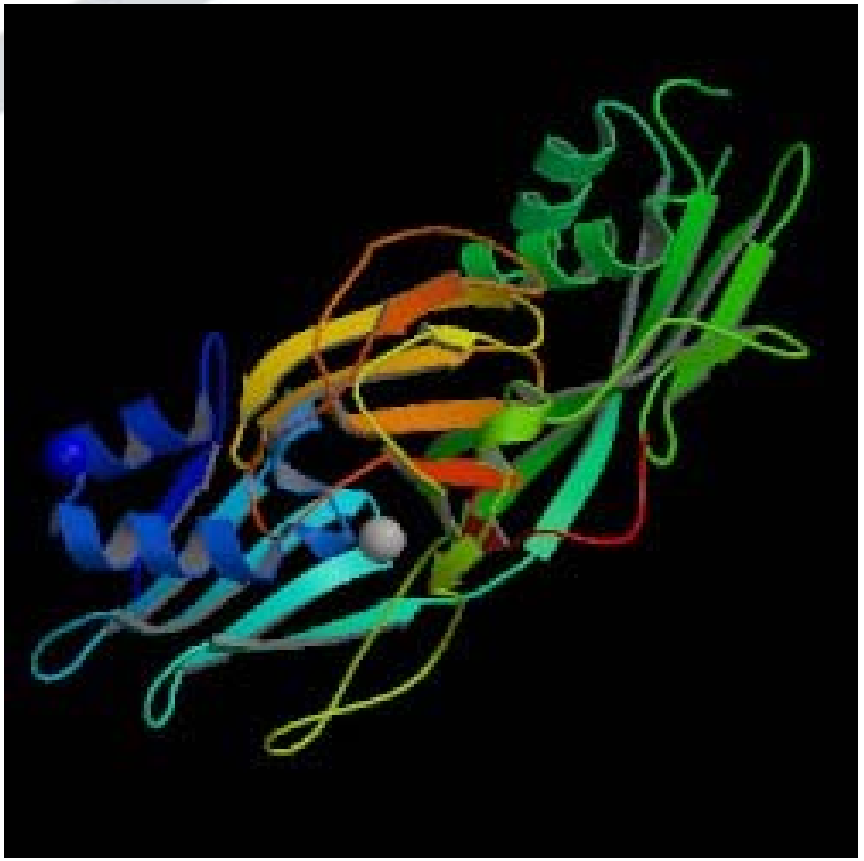
Example 2 - background

- Monomeric
- 26kDa
- pI 10.5
- No cysteines
- Aliphatic index 60.61
- GRAVY –0.509



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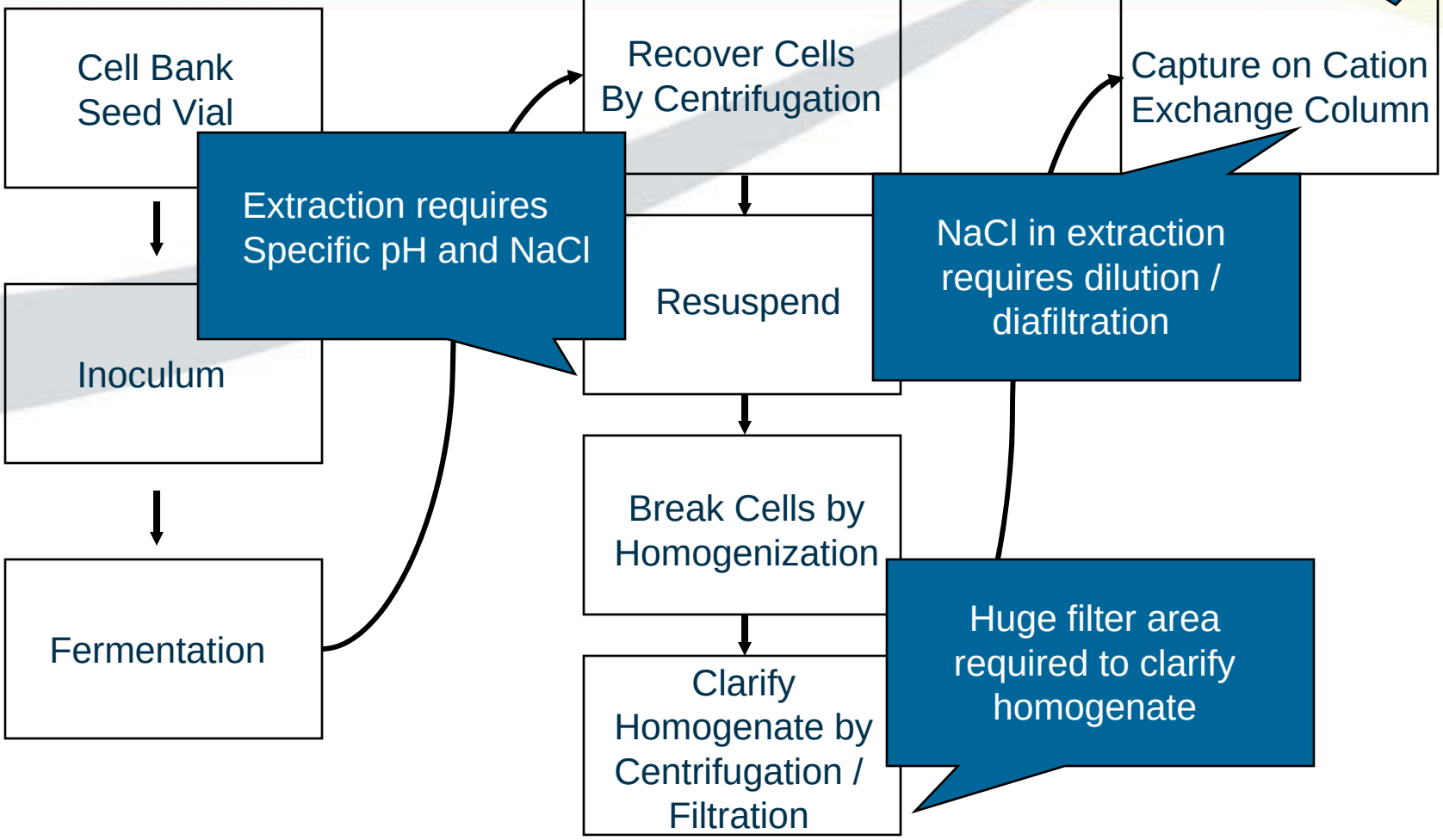
Original Capture Step arrangement



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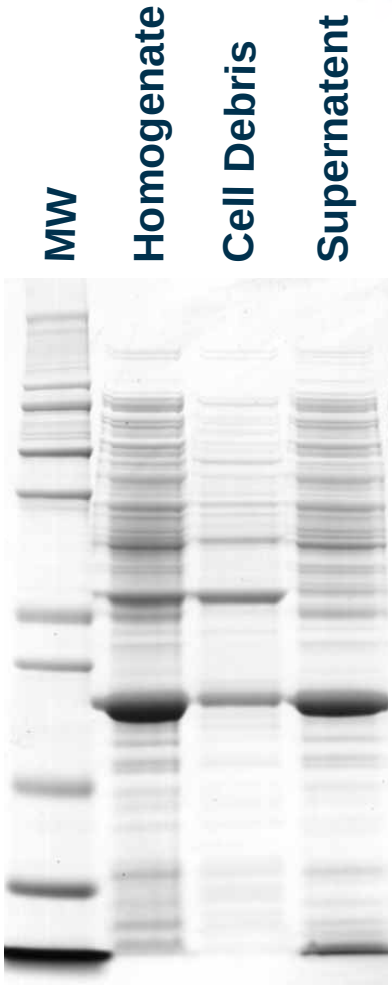
Homogenate Clarification

- Polyethylenimine (PEI) used to improve clarification
- Cationic polymer
- Highly effective flocculating agent
- Filter requirements substantially reduced and filter use consistent
- Little loss of product to debris phase



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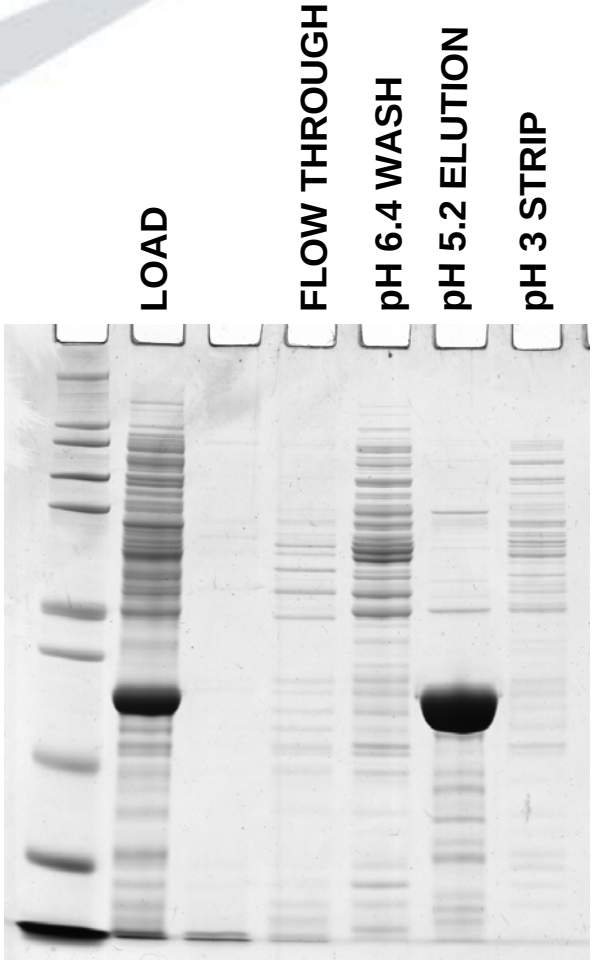
MEP Hypercel Capture

- Effective binding directly from extraction conditions
- High yield ~80%
- Substantial purification
- Low conductivity elution
- High capacity >50g/L



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Added Benefits

SAMPLE	ENDOTOXIN CONTENT
Fermenter Culture	>>10 000 EU/ml
Homogenate	>>10 000 EU/ml
PEI Conditioned Supernatant	1190 EU/ml
MEP Eluate	**1.8 EU/ml**

- Effective endotoxin elimination

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Added Benefits

- Excess PEI binds tightly to cation exchange media
- Limits re-use
- Excess PEI cleared from process
- Majority cleared in load flowthrough

SAMPLE	PEI MASS BALANCE
Load	100% (0.1% v/v)
Flow Through	81%
Wash	5%
pH 6.4 Wash	2%
pH 5.2 Elution	0.75%
Strip	5%

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Conclusions

- Mixed mode media covers a range of effects
- Significant scope for application in biopharmaceutical processes
- Highlighted by the examples and increasing seen applied in processes
- Additional parameters compared to single mode media to be characterised in development
- Development likely to be more complex

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Acknowledgements

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