Mixed Mode Separation of An Antibody Fragment from a Crude Lysate

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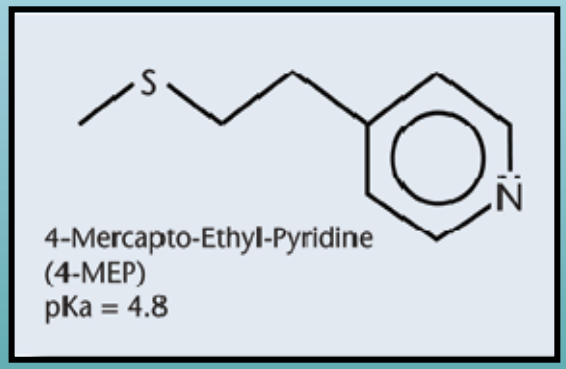
Feedstock

- Fab fragment containing lysate from *E. coli*
- Fab fragments are 50kDa fragments of IgG containing the antigen binding site
- Very stable and can be expressed to the periplasm
- Heat lysis of periplasm limits contaminants

MEP HyperCel

- 4-Mercaptoethylpyridine
- Mild hydrophobic adsorption between pH 4.8 and 9
- Porous cellulose bead between 80-100µm



MediaScout Columns

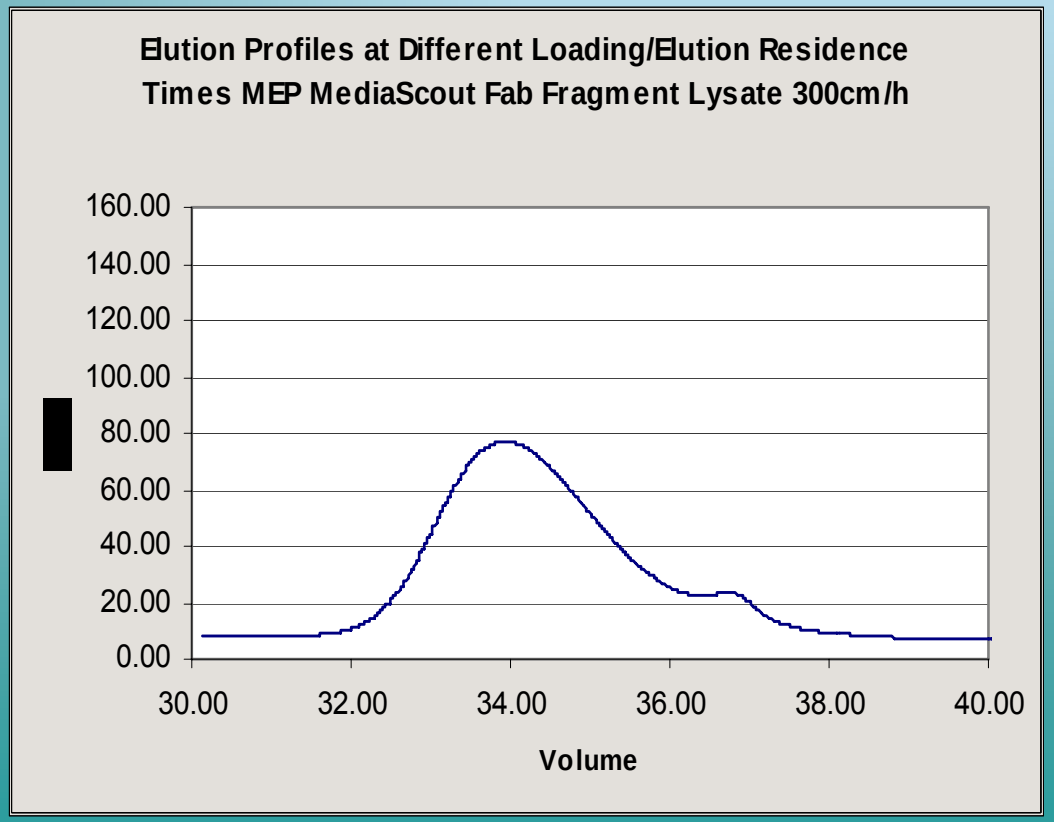
- 1ml internal volume
- Aspect ratio 1:10
- Well packed
- Fast to run
- Prelude to scale down work

Objectives

- Compare and contrast data from separation of Fab fragment lysate by chromatographic means
- Compare an ion exchanger and a mixed mode sorbent on a small column

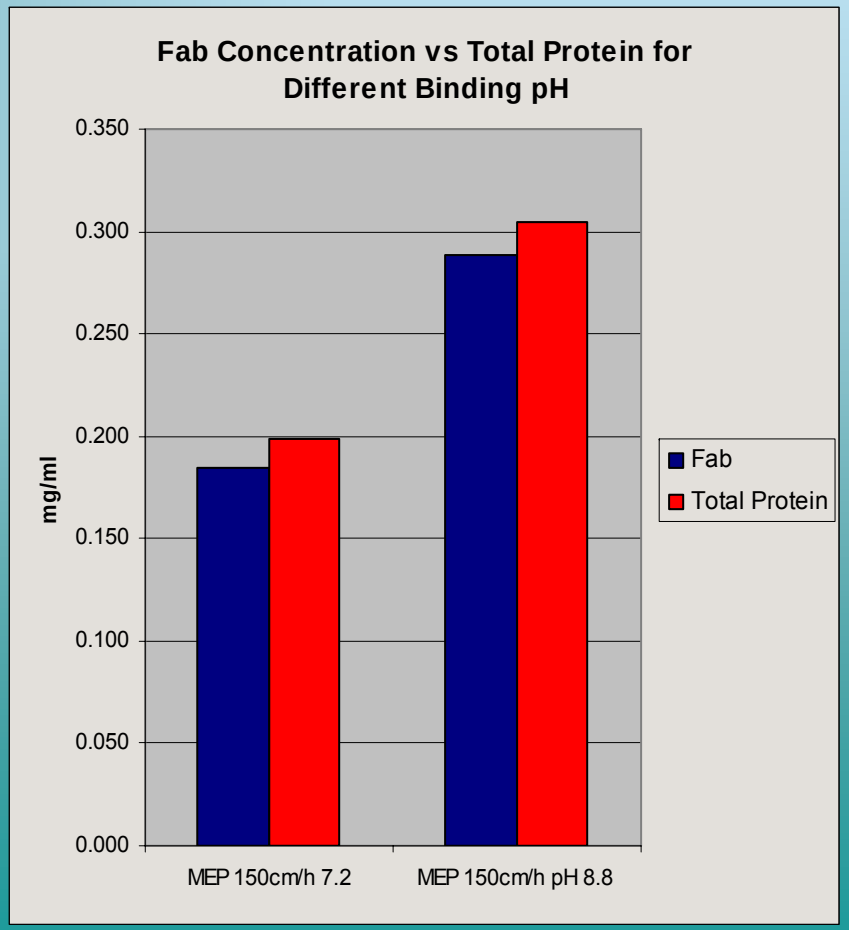
MEP HyperCel Separation

- 30 CV pH gradient using pH 2.2 sodium citrate
- Peak eluted at pH 5.5

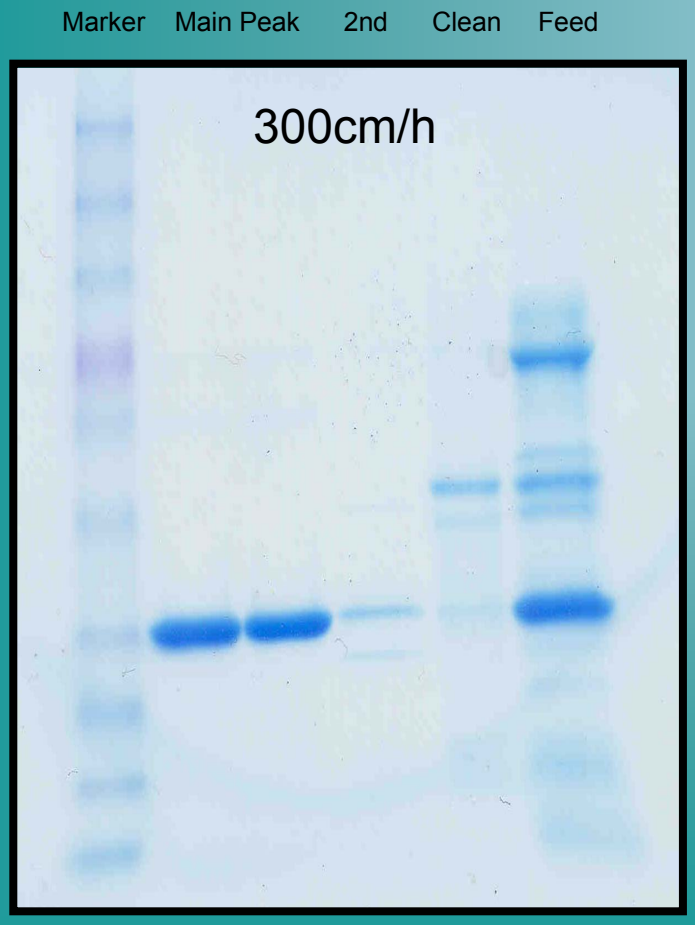


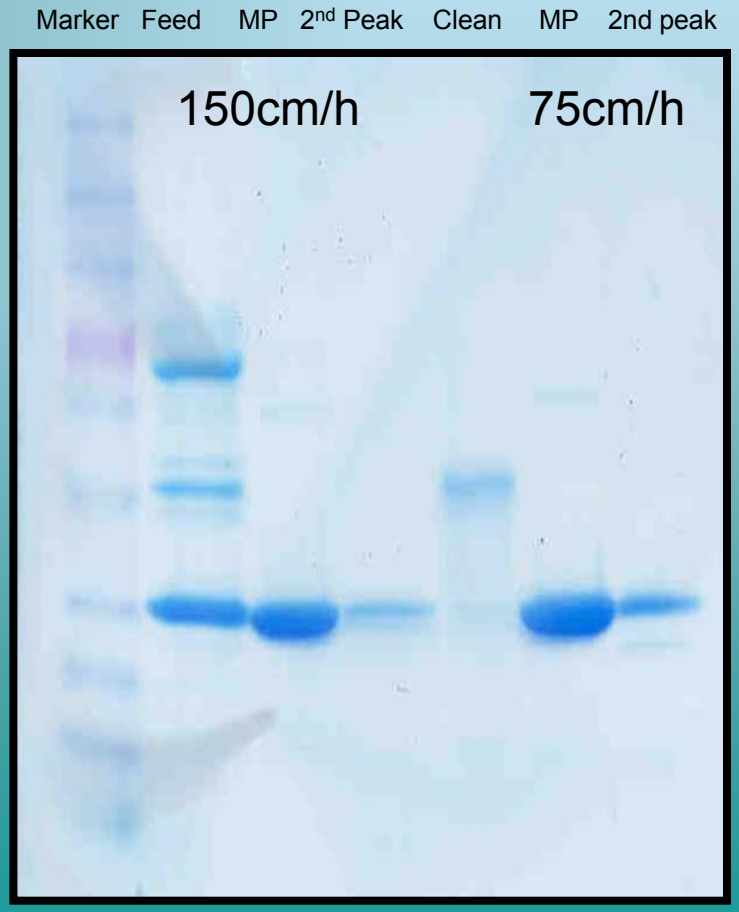
Binding Conditions - MEP

- Fab has a pI of approximately 9
- Previous work on HEA and PPA sorbents higher pH buffer leads to increased yield
- 1ml fraction taken from peak



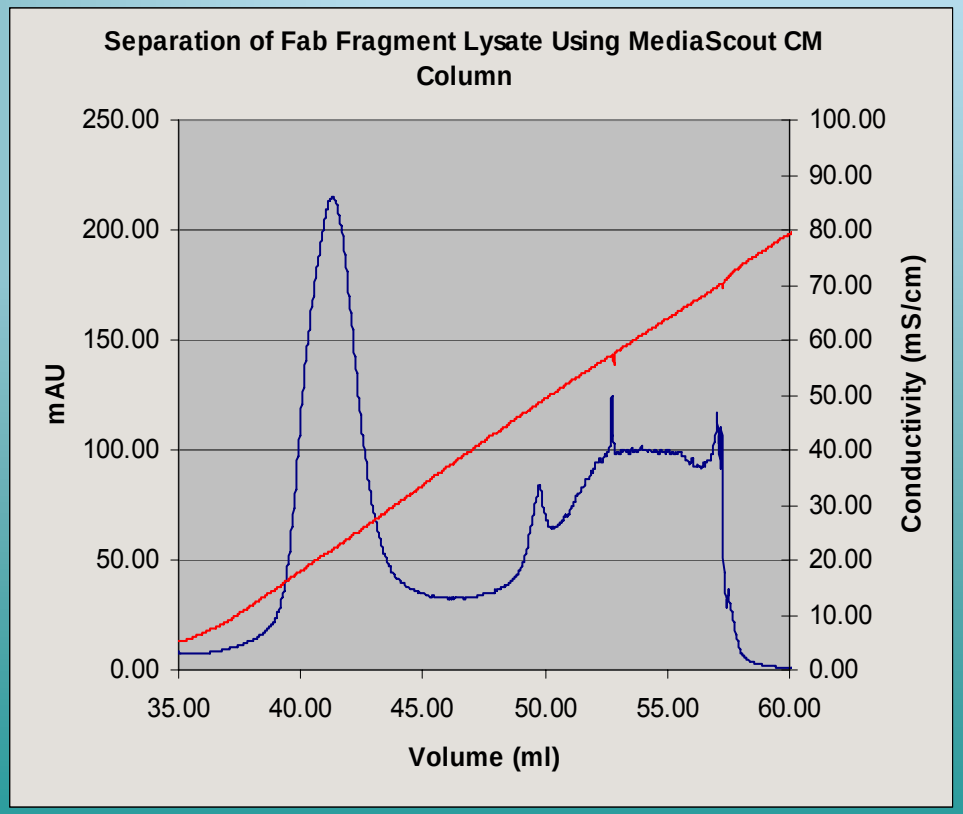
Peak Analysis MEP – SDS-PAGE



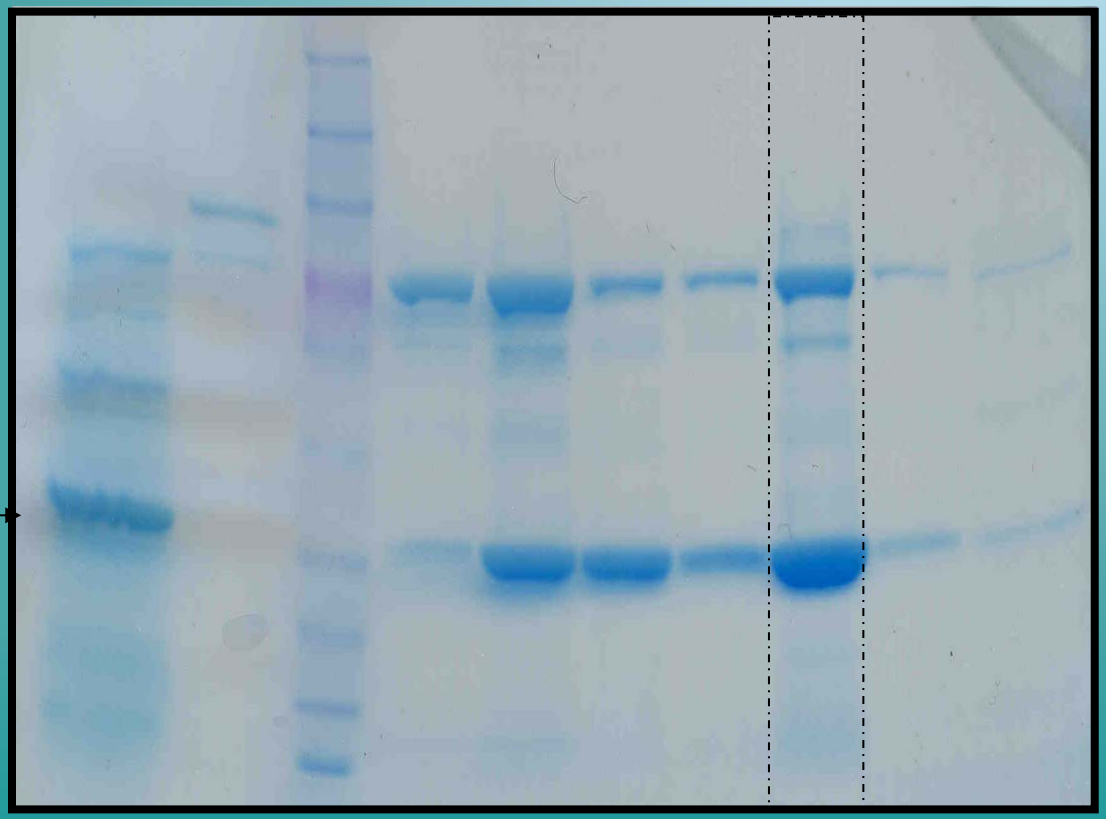


CM Separation of Lysate

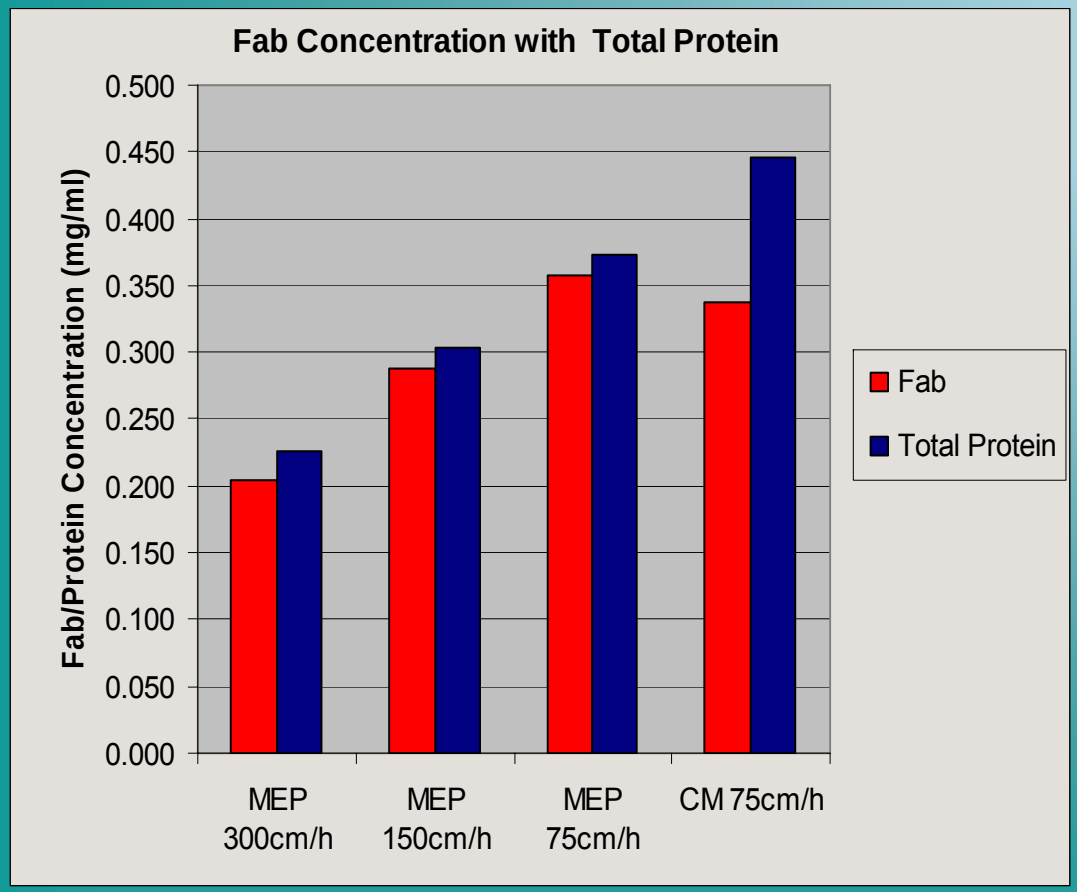
- Bound pH 7.2 50mM Tris-HCl
- Elution difficult
- Eluted with pH 12 50mM diethylamine/1M NaCl with gradient



Peak Analysis CM – SDS-PAGE



Fab Concentration and Purity



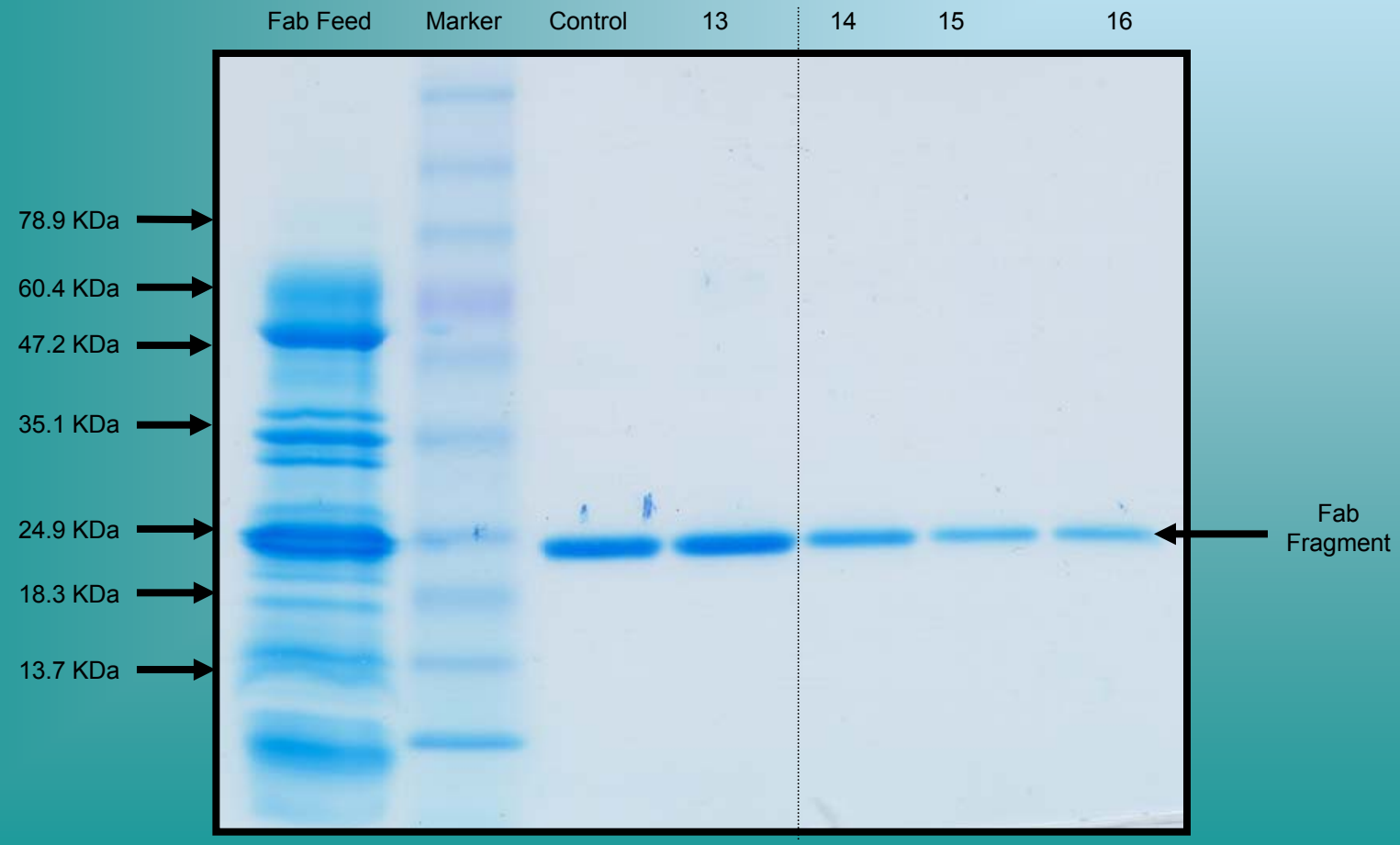
- By Protein G HPLC
- Purity range between 90-95% for MEP
- CM purity 76%
- Feedstock 18% Fab fragment

Mixed Mode Separation of Lysate Containing Fab Fragment

UCL Biochemical Engineering

Pall Inc

PPA Separation



Conclusions

- Mixed mode separation of Fab fragment crude lysate is very effective at lower linear velocities
- Potential to resolve closely related contaminants
- Binding is most effective for Fab fragment at high pH
- CM ion exchange separation is less effective both in yield and purity

Acknowledgements

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Thank you
Any questions?