**Materials and methods**

**Buffers**

Sodium hydroxide, hydrochloric acid, sodium chloride, monobasic sodium phosphate, dibasic sodium phosphate, acetic acid, sodium acetate, tris, and sodium carbonate were purchased from Fisher (Fair Lawn, NJ). Ethanolamine was purchased from Sigma (St. Louis, MO). All buffer constituents were used without any purification beyond their supplied states. Buffer solutions were prepared at room temperature with deionized water from an EMD Millipore Milli-Q system (>18.2 MXXX cm). Equilibration and elution buffers were prepared with 0 and 1 M NaCl, respectively. Buffer pH and conductivity were measured with a Cole-Parmer PC200 meter, and pH adjustments were made with concentrated sodium hydroxide and hydrochloric acid solutions. Buffer solutions were filtered with Fisher 0.2 µm aPES membranes (Pittsburgh, PA).

**Proteins**

Lyophilized bovine liver catalase, bovine milk β-lactoglobulin, hen egg white lysozyme, hen egg white ovalbumin, and *S. cerevisiae* alcohol dehydrogenase were purchased from Sigma (St. Louis, MO). Proteins were dissolved in the desired equilibration buffers at 10 mg/ml, and lyophilized excipients were removed with three buffer exchange cycles using EMD Millipore Amicon centrifugal filter units (Cork, Ireland) and a Cole-Parmer MP centrifuge. Protein solutions were filtered using Thermo Scientific 0.2 µm PVDF membranes (Rockwood, TN), and concentrations were measured with a Thermo Scientific NanoDrop Lite spectrophotometer.

A mAb sample was also obtained from the viral inactivated and neutralized protein A eluate of a manufacturing process at Bristol-Myers Squibb (Devens, MA). The mAb solution was supplied at 18 mg/ml and stored at -80 C. It was thawed immediately prior to use and buffer-exchanged into the desired equilibration buffer using a GE Sephadex G25 desalting column.

**Chromatography equipment**

All chromatographic measurements were performed with an Amersham Biosciences Äkta Explorer equipped with a P-960 sample pump, fraction collector, 10 mm UV flow cell, and in-line pH and conductivity meters. Capto Q and SP Sepharose FF resins were purchased from GE Healthcare (Upsala, Sweden). Poros 50 HQ and Poros XQ resins were purchased from Applied Biosystems (Foster City, CA). Prior to column packing, resin storage solutions were exchanged three times into a pH 7, 20 mM sodium phosphate equilibration buffer by centrifugation and decantation. SP Sepharose FF was slurry packed into a 0.5 cm ID Waters AP chromatography column to a final bed volume of 2.0 ml. All other resins were flow packed at 600 cm/h in the pH 7 equilibration buffer. POROS XQ and POROS 50 HQ were packed into 0.5 cm ID Waters AP columns to final bed volumes of 0.8 ml. Capto Q was packed into a 1 cm ID GE C column to a final bed volume of 2.9 ml. Column packing was validated at pH 7 by equilibrating with 5 column volumes (CVs) at 0.3 M NaCl, injecting a 100 µl pulse of 1 M NaCl, and washing the column with 0.3 M NaCl. Columns with conductivity trace asymmetries between 0.8 and 1.6 were accepted.

**Isocratic retention measurements**

Lysozyme retention measurements were performed at pH 5, 7, and 9 in 75 mM sodium acetate, 25 mM sodium phosphate, and 25 mM ethanolamine buffers, respectively. Retention measurements with all other proteins were performed at pH 7 in 20 mM sodium phosphate.

**Linear gradient elution measurements**

**Breakthrough simulations**

**Breakthrough measurements**

Lysozyme breakthrough measurements were performed at pH 7 in 20 mM sodium phosphate, and FITC-lysozyme breakthrough measurements were performed at pH 9.5 in 25 mM sodium carbonate.

Note

* 2000 kDa blue dextran from Sigma (St. Louis, MO)

**Need**

* tris(?), sodium carbonate, FITC, and FITC solution suppliers
* AEX resin suppliers
* mAb D information
* GE Sephadex G25 desalting column
* Detailed discussion of resin morphologies/chemistries

**Abbreviations**

* CV
* FITC
* mAb

Chemicals and buffers

Sodium hydroxide, hydrochloric acid, sodium chloride, monobasic sodium phosphate, dibasic sodium phosphate, acetic acid, and sodium acetate were purchased from Fisher (Fair Lawn, NJ). Ethanolamine and 2000 kDa blue dextran were purchased from Sigma (St. Louis, MO). Bovine liver catalase, bovine milk β-lactoglobulin, hen egg white lysozyme, hen egg white ovalbumin, and *S. cerevisiae* alcohol dehydrogenase were also purchased from Sigma (St. Louis, MO). mAb D was kindly provided by Bristol-Myers Squibb (Devens, MA) as viral inactivated and neutralized protein A eluate.

Solid masses were measured with Ohaus Scout Pro 4000 g and Denver Instrument 110 g balances. Solutions were prepared using deionized water further purified with an EMD Millipore Synergy UV system. Lysozyme retention measurements were performed at pH 5, 7, and 9 in 75 mM sodium acetate, 25 mM sodium phosphate, and 25 mM ethanolamine buffers, respectively. Retention measurements with all other proteins were performed at pH 7 in 20 mM sodium phosphate. Lysozyme breakthrough measurements were performed at pH 7 in 20 mM sodium phosphate, and FITC-lysozyme breakthrough measurements were performed at pH 9.5 in 25 mM sodium carbonate. All buffer solutions were prepared with 0 and 1 M NaCl.

Solution pH and conductivity were measured with a Cole-Parmer PC200 pH/conductivity meter, and pH adjustments were made with the addition of concentrated sodium hydroxide or hydrochloric acid. Buffer solutions were filtered using Fisher 0.2 µm aPES membranes (Pittsburgh, PA), and protein solutions were filtered using Thermo Scientific 0.2 µm PVDF membranes (Rockwood, TN). Prior to retention measurements, lyophilized excipients were removed from protein solutions with three buffer exchange cycles using EMD Millipore Amicon centrifugal filter units (Cork, Ireland) and a Cole-Parmer MP centrifuge. Protein concentrations were measured with a Thermo Scientific NanoDrop Lite spectrophotometer.

Resins and columns

GE Healthcare Capto Q, Applied Biosystems Poros 50 HQ, and Applied Biosystems Poros XQ were kindly provided by Bristol-Myers Squibb (Devens, MA). SP Sepharose FF was purchased from GE Healthcare (Upsala, Sweden). Resin storage solutions were exchanged three times with the appropriate packing buffer using an Eppendorf MiniSpin Plus centrifuge. SP Sepharose FF was gravity packed into a 0.5 x 10 cm Waters AP chromatography column to a final bed volume of 2.0 ml. All other resins were flow packed at 600 cm/h. POROS XQ and POROS 50 HQ were packed into 0.5 x 5 cm Waters AP columns to final bed volumes of 0.8 ml. Capto Q was packed into a 1 x 20 cm GE C column to a final bed volume of 2.9 ml. Column packing was validated at pH 7 by equilibrating with 5 column volumes (CVs) at 0.3 M NaCl, injecting a 100 µl pulse of 1 M NaCl, and washing the column with 0.3 M NaCl. Columns with conductivity trace asymmetries between 0.8 and 1.6 were accepted.

Retention measurements

All chromatographic measurements were made with an Amersham Biosciences Äkta Explorer equipped with a 10 mm UV flow cell.

The isocratic retention of lysozyme on SP Sepharose FF was measured at pH 5, 7, and 9 over a range of ionic strengths. Isocratic retention measurements of mAb D on SP Sepharose FF were performed at pH 7. Likewise, isocratic retention measurements of alcohol dehydrogenase, β-lactoglobulin, catalase, and ovalbumin were performed at pH 7 on Capto Q, Poros 50 HQ, and Poros XQ. For all retention measurements, at least a 5 CV equilibration period in the appropriate buffer preceded a 100 µl sample injection. To approximate linear chromatographic conditions, sample concentrations of at most 10 mg/ml were used. Absorbance was measured at 280 and 215 nm, and each measurement was followed by a 5 CV wash at 1 M NaCl. Columns were regenerated at least once every ten measurements with 0.5 M NaOH. Requisite NaCl concentrations were achieved for isocratic measurements through inline mixing of 0 and 1 M NaCl buffers. Lysozyme retention on SP Sepharose FF was also measured with linear gradient elutions at pH 5, 7, and 9, where the linear gradient lengths ranged from 10 to 50 CVs. Gradient elution began after sample injection and proceeded from 0 to 1 M NaCl. All retention measurements were performed at a superficial velocity of 300 cm/h.

Breakthrough measurements

An Äkta P-960 sample pump was used to load samples during breakthrough profile measurements.

To do:

* need tris(?), sodium carbonate, FITC (and FITC acronym), and FITC solution suppliers
* Need AEX resin suppliers
* include lithium nitrate? (for ionic capacity determination)
* change how I reference BMS