* Abstract
  + Flow-through ion-exchange is widely used in mAb polishing
  + Impurity persistence is incompletely understood, partially due to the diversity of HCPs encountered
  + Transferrable insights into general behavior were sought
  + For impurities that persist via weak adsorption, the initial breakthrough volume was correlated with transport and thermodynamic properties
  + A correlation for thermodynamic data was also identified
  + These relationships provide novel insights into HCP chromatographic behavior, which has largely been experimentally inaccessible
* Introduction
  + Application of the flow-through operational mode in polishing
    - Ion-exchange, typically AEX
    - Impurities are intended to interact with the resin
  + Challenges with HCPs
    - Dilute
    - Large number of species
    - Biophysical diversity
  + Persistence mechanisms
    - Weak adsorption
    - Product association
  + Lack of transferrable insights/general behavioral understanding of impurities
    - Needed to
      * Expedite process development
      * Reduce costs
      * Increase consistency with quality by design principles
      * Improve column modeling efforts
  + Characteristics of flow-through IEX which make a general analysis possible
    - Isocratic
    - Expected fraction of the column saturated (back-of-the-envelope calculation)
      * Linear and independent adsorption equilibria
  + Value of this work
    - Resin retentivity characterization
    - Meta-correlation
    - Effects of transport and thermodynamic properties on the initial breakthrough volume
* Materials and methods
  + Chemicals and buffers
  + Protein solutions
  + Stationary phases
  + Chromatography instrumentation
  + Isocratic retention measurements
    - Parameter estimation (extra-column volume, non-adsorbing retention time, phase ratios)
    - Determination of IS/NaCl window (ref DePhillips)
  + Linear gradient elution measurements
  + Breakthrough simulations
  + Breakthrough measurements
    - Model parameter estimation
      * Bypass pulse, blue dextran pulse, lysozyme pulse at high ionic strength, breakthrough at high/low ionic strength
    - Lysozyme breakthrough
      * Identification of an ionic strength
      * Run order randomization
    - FITC-lysozyme breakthrough
      * Conjugation of FITC-lysozyme
      * Pre-purification (discussion of multi-component behavior)
      * pH set point and ionic strength decisions
      * Spiking in mAb
      * Breakthrough
* Results and discussion
  + Observations from the isocratic retention data
    - (Transition – looking for correlations for Keq)
    - Anion-exchange resin comparison
      * Retentivity: PXQ > CQ > PHQ
      * PXQ is ~4x more retentive than PHQ
      * Similar for each protein
    - Slope depends on the protein
      * Consistent with the idea of characteristic charge being protein-dependent
  + Characteristic charge correlation
    - From the isocratic data
    - Comparison to LGE data (both ours and from literature)
    - Implications – one measurement to get Keq, GH, and Ds as functions of IS (if the Ds Keq power law is known)
      * Possible utility with host cell proteins – required experiment
  + Comparison of characteristic charge correlation with literature data
    - Use of k’ data
    - Agreement with the electrostatics model
    - Noise/form problem
    - Difference between CEX and AEX
  + Resin retentivity ranking?
    - As observed from the consolidated data
    - POROS through-pores increase intra-particle diffusivity, thereby increasing retention
  + Correlation of simulated breakthrough volumes
    - CADET simulations to elucidate the behavior of dilute species
    - Effects of thermodynamic and transport parameters
      * Yamamoto’s dimensionless variable
      * Combined intraparticle diffusivity
    - Problematic Keq/k’
      * Load CVs that are typical
  + Comparison with experiment
    - Agreement (qualitative and quantitative) with the lysozyme data
    - Agreement (qualitative) with the FITC-lysozyme data
* Conclusions
* Competing interests, author contributions, acknowledgements, supplementary materials, references, corresponding author, and contact information
* Random thoughts
  + Column modeling
    - Consistent parameters were fit to the data (i.e. parameters that should be the same across measurement conditions were fit globally)
      * Need to give details for global estimation procedure
    - FITC-lysozyme not modeled due to its multi-component behavior
  + 10 mm flow cell on the Akta
  + Common point implied by the meta-correlation
  + May be interesting to include the comparison of axial dispersions fit for lysozyme with Carbonell correlation
* To-do
  + Direct comparison of lysozyme results to the transport correlation with point overlay
  + Rigorously test the effect of polymer derivatization in the meta-correlation
* Notable presentations
  + 2021-01-11: charge depth in protein structures – motivation for MD to account for hydration effects
  + 2021-02-08 BMS: post-hoc grouping of resin retentivity
  + 2021-04-05 BMS: Pe effect for lysozyme breakthrough
  + 2021-04-19 and 2021-04-26 and 2021-05-03: fitting lysozyme dilute breakthrough data
    - 2021-05-03: comparison of fit axial dispersions for lysozyme data with Carbonell correlation
  + 2021-07-19: FITC-lysozyme re-fractionation and breakthrough data
  + 2021-07-26 BMS: column saturation fraction back-of-the-envelope
  + 2021-08-02: column modeling for the FITC-lysozyme conjugate/estimation of F/P
  + 2021-08-09: recent developments with the Pe correlation/discovery of problems
  + 2021-08-16: current Pe correlation, demonstration of multicomponent FITC-lysozyme behavior
  + 2021-08-23: estimation of problematic Keq/k’ for flow-through with the Pe correlation
* Novelty of this work
  + Transferrable insights into flow-through behavior – transport correlation
  + Novel characteristic charge correlation
  + Note: show the novelty in the intro, don’t explicitly state it
  + I think the description of a lack of general insights, followed by “we correlated the initial breakthrough volume of weakly adsorbing impurities with transport and thermodynamic properties” would suffice
* Back-of-the-envelope calculations
  + Expected fraction of the column saturated by HCPs (2021-07-26)
  + Column loading (~50 CV) (2021-08-16)
* Should the following be included?
  + Why have Poros resins been anecdotally observed to be more selective in flow-through?
    - Large through-pores
      * Should increase pore diffusivity, thus decreasing the dimensionless group and increasing the slope term
    - Consistent with the post-hoc grouping of resin retentivity in Staby’s data
      * Response is ln k’, factors are resin, protein, and pH, covariate is ln IS (see 2021-02-08 BMS, slide 4)
      * POROS 50 HQ resin retentivity ranked 5, 6, and 9 for myoglobin, lipolase, and mAb, respectively
      * Need to check the same pH was used within a given protein’s measurements
      * Also need to note significance/lack thereof (and how the analysis of covariance was performed/the general linear model was fit)
  + Should our electrostatics model be described, and should predictions be shown?
  + Should we explicitly state the model fits can be improved when not constraining ourselves to a global set of parameters?
* J Chrom A notes
  + Intro, principles, materials and methods, results and discussion, conclusions
    - Principles may be optional – there is some flexibility in terms of how the intro and materials and methods are structured
  + “**Particular scrutiny will be placed on the degree of novelty and significance of the research and the extent to which it adds to existing knowledge in separation science**”
  + “As part of the Introduction section to each manuscript, authors must address the question of how their proposed methodology compares with previously reported methods and this comparison must show that significant advances are proposed.”
  + <https://www.elsevier.com/journals/journal-of-chromatography-a/0021-9673/guide-for-authors>
    - <https://www.journals.elsevier.com/journal-of-chromatography-a>
  + Intro, theory/calculation, materials and methods, results and discussion, conclusions, appendices
* Problems with mean-field models
* Anisotropic model with discrete charges
* Approximate accounting for nonlinearity
  + Numerical Derjaguin approximation
  + Solution of the nonlinear Poisson-Boltzmann equation in 1D with orthogonal collocation
  + Energy interpolation surface to expedite calculations