INTRODUCTION
To meet purification challenges of more diversified classes of biomolecules, a series of Hydrophobic Interaction Chromatography (HIC) resins were developed with extensive user input. Resin design goals focused on addressing typical pain points for current HIC separations such as resolution, capacity and product recovery. In monoclonal antibody (mAb) purification processes, HIC offers orthogonal selectivity to ion exchange chromatography for clearance of aggregate, host cell proteins (HCP) and other process and product-related impurities. Here we present application data for a process development study using POROS HIC in Bind-Elute mode with a model high-aggregate containing (>10%) mAb molecule. The general approach presented herein can be used to solve similarly complex downstream challenges in other therapeutic modalities.

RESIN DESIGN
A new 55µm POROS™poly(styrene-divinylbenzene) base bead was created to meet design goals. Novel coating and functionalization procedures were used to graft unique alkyl or alicyclic ligands onto the bead structure to provide a differentiated range of hydrophobicity compared to current market offerings. Design features include:

- Differentiated selectivity & improved resolution
- High performance with lower salt concentrations
- Improved recovery, higher capacity, and superior pressure-flow characteristics
- Consistent lot-to-lot performance
- Robust chemical stability

Figure 1. Hydrophobicity of POROS-HC Resins. POROS Ethyl and POROS Benzyl bracket the commercially available range of HIC resins, while POROS Benzyl Ultra is considerably more hydrophobic than HIC resins currently on the market. A deep understanding of POROS resin chemistry enables build-to-suit custom hydrophobicity resins to meet unique purification challenges.

AGGREGATE CONTENT IS A CRITICAL QUALITY ATTRIBUTE FOR BIOTHERAPEUTICS
A critical quality attribute in the final formulation of biotherapeutics is the lowest possible concentration of soluble product aggregates. HIC is a uniquely positioned tool for aggregate removal; especially for challenging molecules such as Fc fusion proteins, bispecific antibodies, antibody-drug conjugates, and select monoclonal antibody processes. In the following case study, a highly efficient HIC bind-elute process was developed for a model mAb A containing >10% aggregate. High-Throughput Screening (HTS) was performed to characterize the effect different salts on binding capacity and aggregate partitioning. Retention and selectivity curves were constructed using small prepacked columns, followed by process development. Robust aggregate removal and process improvements were achieved compared to an established clinical process for mAb A.

Figure 2. Maximal Static Capacity of mAb A on POROS HIC resins. Static capacity on both resin hydrophobicity and salt type. Consistent with the Hofmeister series, ammonium sulfate and sodium citrate were strongest promoters of hydrophobic interaction. Interestingly, up to 55g/L resin was achieved on POROS Ethyl resin using ammonium sulfate.

Table 1. High-Throughput Screen of mAb A on POROS HIC resins. HTS was performed in 96-well filter plate format, by varying resin type, salt type, and salt concentration, while keeping pH constant at 7.0. (66mg/mL, resin, 10L total resin, 1hr binding). Protein concentration was determined on the Varioskan™ LUX microplate reader at A300 and aggregate analysis was determined using a MAOpac™ SEC-1 analytical column on the Ultimate 3000 HPLC system. Maximum Salt concentrations used were determined using the Salt Tolerability Window

Table 2. Partition Selectivity Ratio by HTS to approximate Optimal Elution Partition Selectivity Ratio (PSR) = (logKpAggregate / logKpMonomer), where Kp is the Partition Coefficient: Kp = qc / ([Resin-bound] / [Flow-through]) The higher PSR conditions (blue) is considered to be optimal elution conditions (green encoding). Conditions for which both monomer/aggregate bound strongly (logKp>1.0, white), or both monomer/aggregate eluted strongly (logKp>1.0, gray) were not included in the analysis. Sodium Citrate was chosen for column scale-up.

Figure 4. Selectivity Curves for mAb A on POROS HIC resins. Cumulative Monomer Purity was plotted against Cumulative Yield across fractions collected across the gradient elution peak. POROS Benzyl resin displayed highest selectivity and chosen for SDM process optimization.

Figure 5. HIC Step Elution Optimization for mAb A on POROS Benzyl Resin. Pre-packed Column: 0.5cm x 5cm, CV=1mL Loading Density: 33mL/mg resin. Elution Sodium Citrate: 180-280mM Load Monomer Purity: 30%

Figure 6. Step Elution Optimization for mAb A on POROS Benzyl Resin. Pre-packed Column format: 0.5cm x 5cm CV=1mL Loading Density: 33mL/mg resin; 2min Residence Time; Load Purity: 90%; Purity decreased and recovery increased with decreasing sodium citrate concentration used for elution. CV conductivity transition was achieved by FPLC pumps A/B pre-mixing and washing.

Figure 7. Retention Analysis of mAb A with Sodium Citrate Gradient Elution. 3CV Fractions were collected across each peak to generate selectivity curves in Figure 5. Column: 0.5 cm x 5 cm, CV=1mL Residence Time: 2min; Buffer A: 600 mM Sodium Citrate, Tri-Acetate pH 7.0, Buffer B: Tri-Acetate pH 6.5 Linear Gradient: 15CV. Retention of mAb A on POROS HIC resins in pre-packed column format closely paralleled the elution conditions predicted by HTS Partition Selectivity Ratio in Table 2

Figure 8. Monomer Purity Eluate Pool (%). Diagram shows the effect of resin type, washing and elution conditions on mAb A purity and recovery. Monomer Purity Eluate Pool (%) was measured using ProteinSEQ Immunoassay.

Table 3. Dynamic Binding Capacity of mAb A Monomer on POROS Benzyl Resin. CVv1mL Column: 0.5cm x 5cm Salt: 575mM Sodium Citrate. Maximal Static Capacity: 446mL/mg resin

Table 4. Process Improvement for mAb A. Pre-packed 0.8x10cmL pre-packed column scale-up confirmation run: CV=5mL, Elution: 265mM Citrate, pH7.0, 50mAu/50mAu pool. Mass Balance: 100%. Load Density set ~90% of %breakthrough at 2min residence time. HCP reduction: 96% to 12ppm. IX reduction (ProteinSEQ Immunoassay).

CONCLUSIONS
- Significant reduction of mAb aggregates on POROS Benzyl Ultra was achieved in a highly robust bind-elute polypeptide process (10% Aggregation Reduction). Robust and optimal elution conditions were predicted by HTS.
- POROS convective flow allows fast mass transfer and high performance at high flow rates (High resin loading density at sub 2min residence time in bind-elute mode possible).
- High-throughput screening strategy accurately predicts optimal binding and elution conditions for column scale-up confirmation

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Monoclonal Antibody Aggregate Removal using POROS Hydrophobic Interaction Chromatography (HIC) resins in Bind-Elute Mode

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