# The Downstream Data Browser – Enhancing Data-Driven Decisions via Customized Data Handling and Visualization

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## Introduction

- The Just-Evotec Biologics platform-in-plate (PiP) concept is an automated high throughput batch-binding workflow which evaluates several chromatography resins in a single 96-well plate (Gillespie, 2017)[1].
- High-throughput screening methodologies have accelerated downstream development for monoclonal antibodies (mAbs) by enabling parallelized evaluation of chromatographic resins across a range of conditions. However, scientists must now interpret results in a meaningful and consistent way.

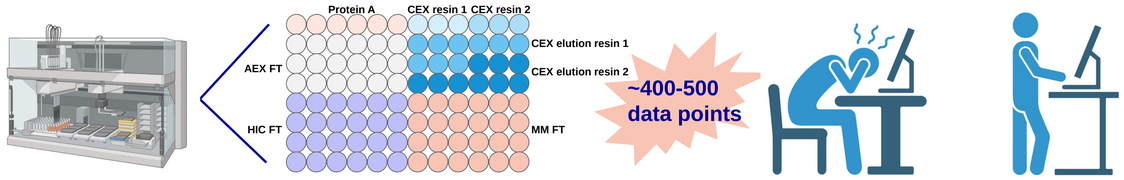
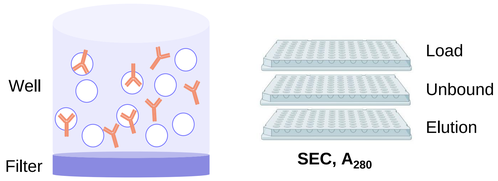



**Figure 1: Workflow to automate visualization of high throughput datasets to standardize results reporting**

- The Downstream Data Browser offers a flexible web-based visualization and analysis tool. This tool automates visualization of high-throughput datasets, fits response surface statistical models, standardizes report results from a high-throughput screening method and facilitates comparison across molecules.

## Methods







$$Kp = \frac{Q}{C} = \frac{[Bound]}{[Free/Unbound]}$$

- $[Free/Unbound]$ measured directly in PiP
- $[Bound] = [Load] - [Unbound]$

$$S = \log_{10} \frac{Total\ HMW\ Kp}{Total\ Monomer\ Kp}$$

| Mode | Kp | Log Kp |
|---|---|---|
| True flow through | < 0.1 | -1.0 |
| Flow through limit | 3 | 0.5 |
| Effective weak partitioning limit | 10 | 1.0 |
| Bind and elute | 20 | 1.3 |

- For **flow-through polishing resins**, 24 buffer conditions to evaluate protein-resin interactions and compatibility with flow through, weak-partition, or bind-elute chromatography operations.
- Log Total Kp is calculated based on UV Absorbance at 280 nm to capture resin interaction with all product species. Separation factors (S) provide selectivity between monomer and total HMW and are calculated based on monomer and high molecular weight (HMW) Kp based on size exclusion (SEC) signals.
- The downstream data browser automatically fits pH and NaCl factors to a response surface model and reports outcome measures including partition coefficients, separation factor of contaminant species, and solution stability, which are visualized by a pre-defined set of contour plots.

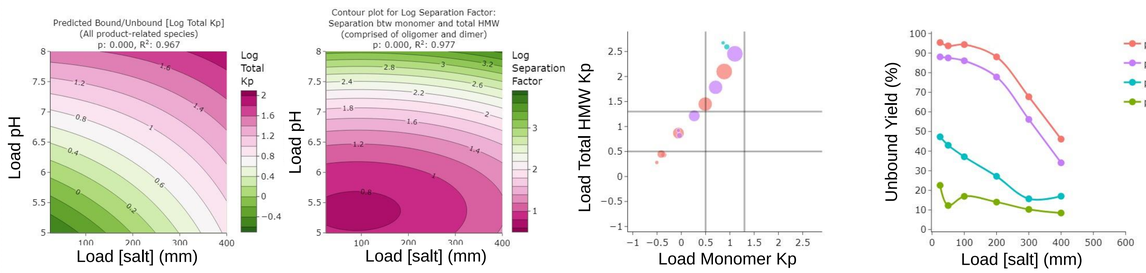



**Figure 3: Pre-defined set of plots from mixed mode polishing resin showing Log Kp, Separation factor, Log Total HMW Kp vs. Log Monomer Kp, and unbound yield (%)**

## Documentation and Standardization

**Inputs**
- Automated robotics for experiment execution
- Custom HT tools for sample management and assay submission

**Processing**
- Assay results, data transformation, models built in real-time without user intervention
- Detailed documentation and guidelines for interpretation

**Outputs**
- Standardized results and reporting
- Historical datasets for comparison
- Data quality metrics to confirm experimental integrity

## Results

- **Affinity capture** step is evaluated to select Protein A elution pH for any molecular format (IgG1, IgG4, Fc-fusion, bi-specifics) to maximize yield and minimize HMW. Simultaneously, sensitivity to low pH for viral inactivation is evaluated with eluate from each condition.
- Opportunities for platform improvement are readily detected, optimal elution pH.
- Across five molecules with unique molecular formats, only one required modification from platform elution pH.

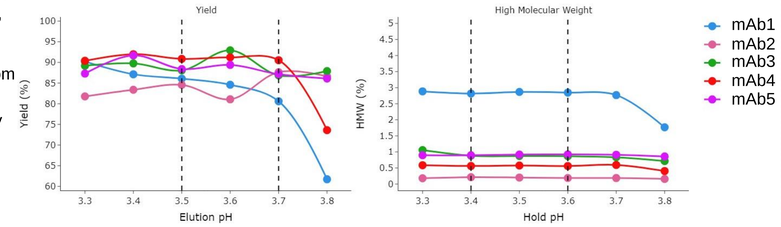
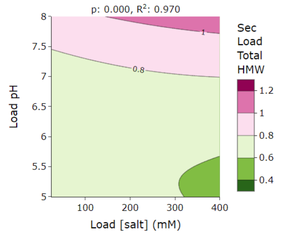



**Figure 4: Protein A yield and HMW plots, manufacturing elution pH and VI range in dashed lines**

| Attributes Measured | Method |
|---|---|
| Colloidal Stability | Absorbance at 405 nm |
| Aggregation Propensity | SEC |
| Thermal Stability | Differential Scanning Fluorimetry |




- **Solution behavior** of product provides insight into optimal solution conditions for downstream processing.
- Objective: detect potential process liabilities in terms of pH and salt during HT screening.
- Commonly observe HMW generation at pH > 7 and high salt.

**Figure 5: Predicted total HMW by SEC across solutions**

## Model Quality

- The tool also provides fit parameters to assess model quality, and a residual plot to interrogate the model – these features have helped identify cases where additional modeling with more terms has been necessary. The downstream data browser allows users to download all data in tidy format[3].

**Figure 6: low impurity feed, model sufficient**

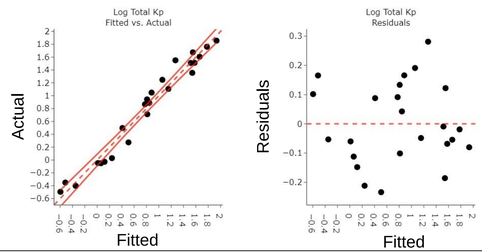
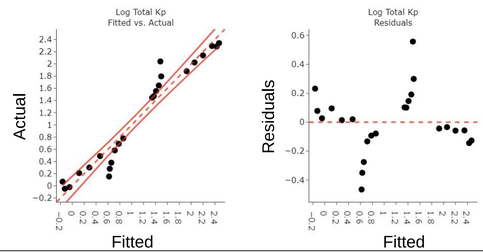



**Figure 7: high impurity feed, additional model terms required**




- For **bind and elute CEX chromatography**, static binding capacity and elution moment are screened
- Rapid detection of on-column aggregation as well as prediction of cases in which the product likely will co-elute with XMuLV (predicted virus elution denoted by dashed lines).

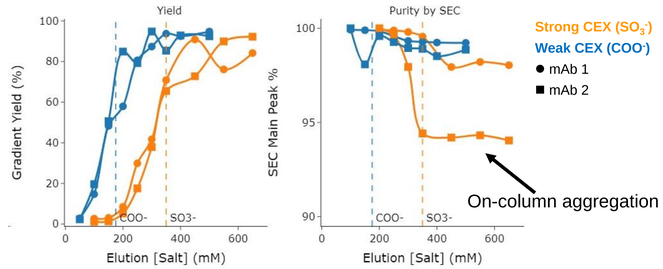



**Figure 8: Yield and purity profiles for CEX Chromatography. Dashed lines denote predicted elution of xMulV**

## References

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