Materials and Methods

Workflow

Proteins of interest were overexpressed in small bioreactors, harvested and the insoluble fraction (inclusion bodies) subsequently purified. A refolding screen (DoE matrix) provided optimal refolding parameters for the heterogeneous sample set tested. Samples were evaluated at 600 nm for turbidity of refolded protein solution; an indicator for precipitation and aggregation (Figure 1). Soluble proteins were captured using filter plate-based chromatography methods. Bound proteins were eluted based on DoE matrix results stated above. The eluted samples were then analyzed using microCE (Figure 2).

To confirm screening results, 3-4 refolding conditions were tested at bench-scale using two of the best solubilization conditions. The refolded proteins were then diluted and purified using suitable ion exchange chromatography. Small scale purification is performed using Atoll RoboColumns® on the JANUS® BioTx platform where all eight conditions can be purified in parallel. While microCE detects soluble protein, only through purification can one evaluate successful refolding conditions.

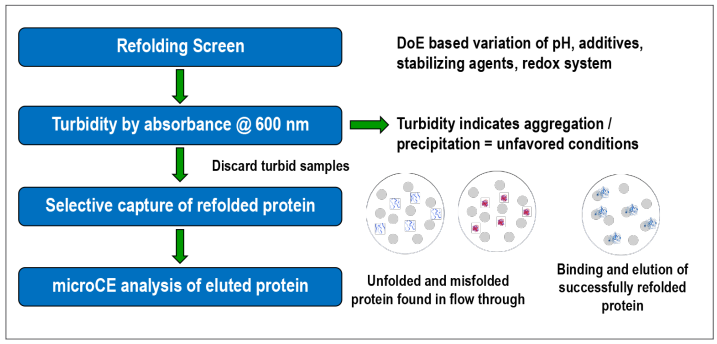

Figure 1. Refolding Screen Workflow.

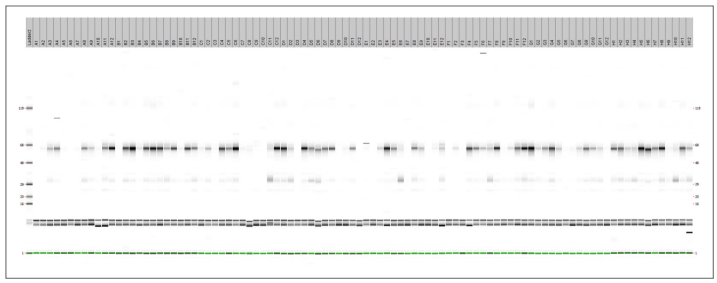

Figure 2. MicroCE results after capture of refolded protein. Successfully refolded samples are indicated by ~60kD protein bands. The visual absence of this band indicates unsuccessful or poor refolding under given condition.

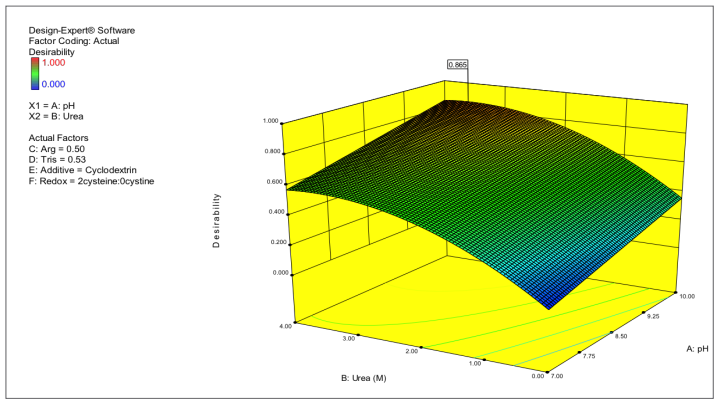

Figure 3. 3D Surface plot of analyzed DoE screen indicating optimal conditions at high urea concentration, high pH, 0.5 M Arg with 0.05 M Tris containing a beta-cyclodextrin additive.

Results and Discussion

Enabling High-throughput Protein Refolding Screens through MicroCE Characterization

Herein, we demonstrated that with LabChip microfluidics technology and the JANUS BioTx Workstation, researchers can purify and analyze in two days what typically takes weeks with alternative methods. This allows researchers to screen for optimal protein characteristics and integrate Quality by Design (QbD) or DoE initiatives into their biotherapeutics development workflows.

MicroCE results (Figure 2) were analyzed to determine optimal solubilization conditions and refolding yields. Determined yields were used for evaluation of the DoE (Figure 3) – high urea concentration and pH proved to be beneficial for refolding.

Small-scale purification provided an efficient and material minimal approach for initial purification and evaluation of the DoE screen. Bench-scale verification confirmed small-scale results, demonstrating the capability of generating predictive data for high- throughput acceleration of biotherapeutic protein development.

Bench-Scale Confirmation

Fundamental to its utility, is that the platform provides predictive data of larger-scale workflows. To verify scale-up, 3-4 conditions were evaluated by bench-scale capture; confirming small-scale capture results. Based on the DoE results, four refolding buffers were selected to test at bench-scale. The inclusion bodies were solubilized using two different solubilization conditions, and evaluated for refolded soluble protein yields. The obtained chromatograms (Figure 4) combined with SDS-PAGE analysis showed that different refolding conditions generated various refolded and capture yields, as well as different chromatography peak shapes. Refolding condition 4 showed the lowest refold and capture yields and an additional shoulder in front of the main peak. No significant differences were found between the two different solubilization conditions.

Benefits of High-throughput (HT) Approaches

High-throughput small-scale purification methods were preferred because, at bench-scale, screening 96 refolding conditions would require ~100 mg of solubilized protein necessary for 1 mL column capture and elution. The experimental execution would take 60 days with one bench-scale instrument.

The presented HT-approach using robotic systems alleviated the throughput and time obstacles stated above. The entire DoE experiment was carried out in four days using less than 4 mg of solubilized protein. The reduced material and time savings, enabled the comprehensive screening of all 96 refolding conditions, which in turn, yielded a broader knowledge of the proteins studied.

Furthermore, the refolded proteins can be processed at bench-scale in parallel (eight at a time) under optimized conditions using the JANUS BioTx Workstation. Confirmation of the DoE screen was performed in two days instead of at least five days with conventional bench-scale instrument for capture.

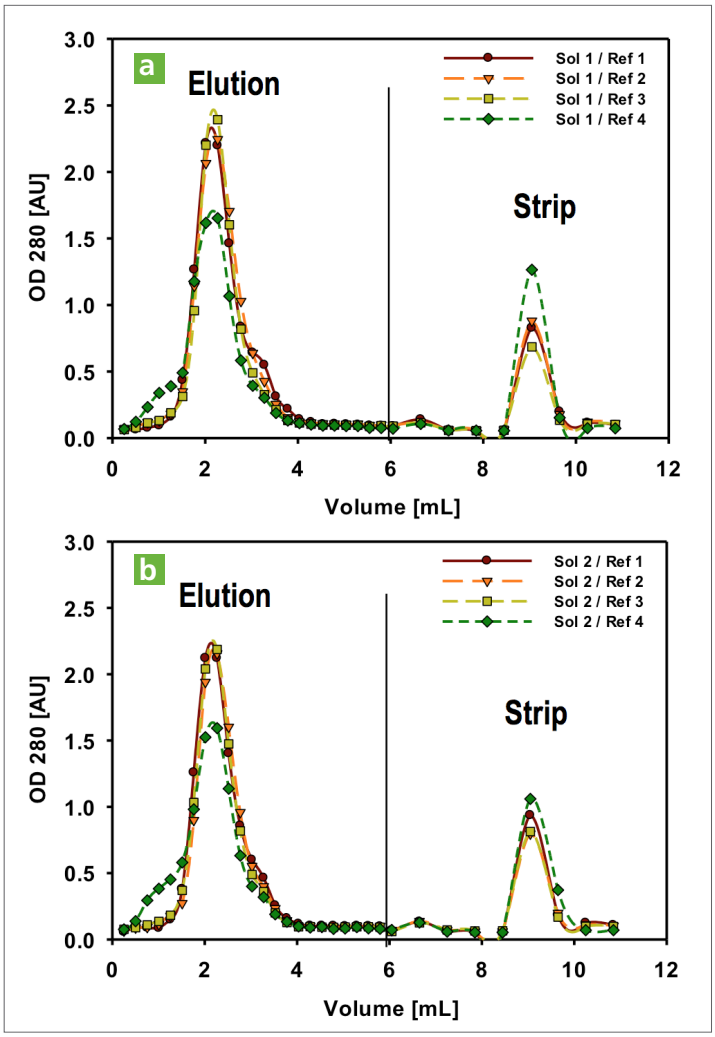

Figure 4. Capture chromatograms for various refolding and solubilization conditions obtained using Atoll RoboColumns® on JANUS BioTx Workstation. Figure 4A and 4B represent solubilization buffer 1 and 2, respectively.

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