

Development of a Non-Standard Protein Therapeutic

BACKGROUND

Molecule X Technology:

This molecule was an engineered native protein containing a specific domain to improve its efficacy. Developed for an endocrine disease, Molecule X was administered through the intramuscular route.

Molecule X Characteristics:

- Theoretical Molecular Weight: 25 kDa
- Theoretical pI : 5.4
- (5) cysteins --(2) disulfide bonds
- Heavily O linked glycosylated

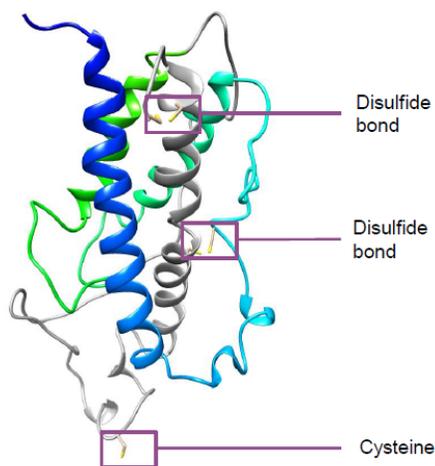
CHALLENGES

Client's Challenges:

- Client needed to generate material for early PK work quickly
- Client needed to find a solution around regulatory challenges with using a his tag
- Client needed to confirm proof of concept with an affinity tagless protein
- Client needed to generate CHO stable cell line and have a scalable purification process to proceed with clinical work

Molecule X Process Challenges:

This client asked Curia to address multiple challenges with this non-standard protein. First, they needed to generate material for early PK work quickly. That also required a solution around regulatory challenges with using a His-tag. A proof of concept with an affinity tagless protein would need to be confirmed. Finally, Curia needed to generate a CHO stable cell line and have a scalable purification process to proceed with clinical work.



Molecule-X Homology Model



APPROACH

Molecule X Project Scope:

TunaCHO™ transient expression with the his tagged construct of Molecule X with affinity tag removal

- Quickly generate representative material for pK studies to establish proof of concept
- Utilized the initial affinity tag to quickly enrich Molecule X
- Removal of affinity tag made the transiently produced product more representative of the desired final product
- Generate material to begin downstream PD work
- Confirm expression in CHO cells and CHO K1 cell line

Titer and functional assay development (proof of concept) using transiently produced material

- De-risk the Cell Time savings for performing work ahead of cell line development

Stable cell line generation in CHO-GSNSM stable cell line platform in parallel with transient work

- Time savings for performing work in parallel with transient expression
- Stable cell line generation resulted in higher levels of expression and more consistent product quality
- Utilized a stable cell line that had a track record for genetic stability

Downstream process development in parallel with transient work

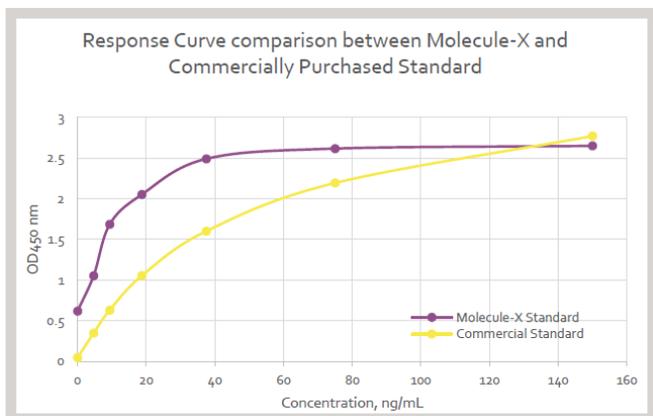
- Time savings for performing work in parallel with transient expression
- Developed a scalable purification process that did not rely on an affinity based resin. Developed a scalable purification process that could achieve target purity levels

DEVELOPMENT

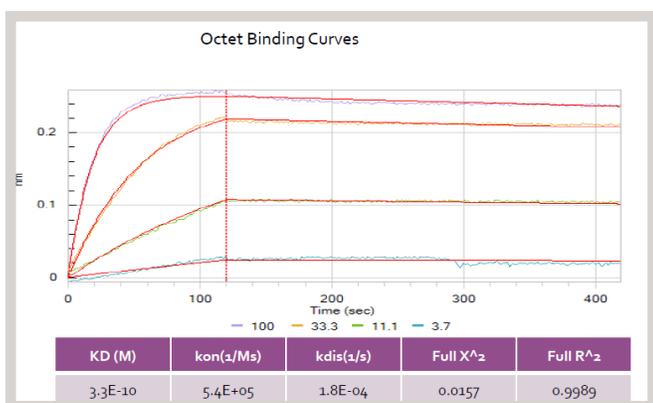
Cell Line Development:

A custom assays were developed to measure the titer and in vitro binding activity to guide the selection of the stable cell line

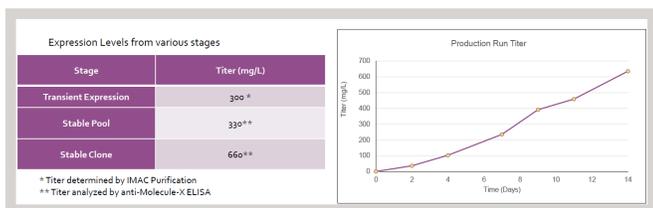
Titer Assay Development



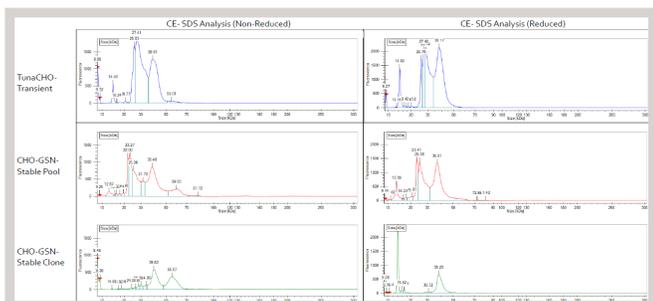
InVitro Binding Assay Development



Molecule X expressing CHO GSN stable cell line achieved titers > 600 mg/mL



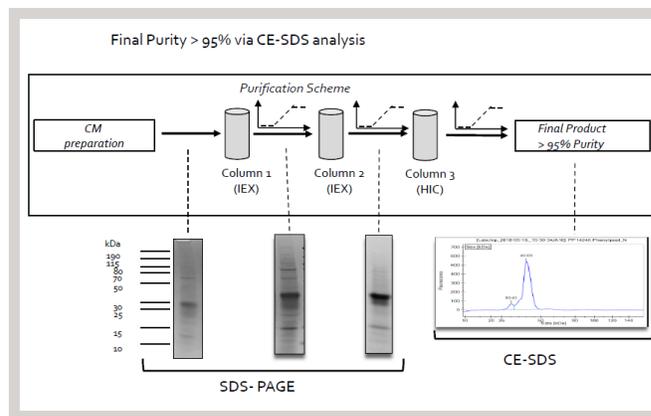
Target protein heterogeneity is minimized through clone selection and purification



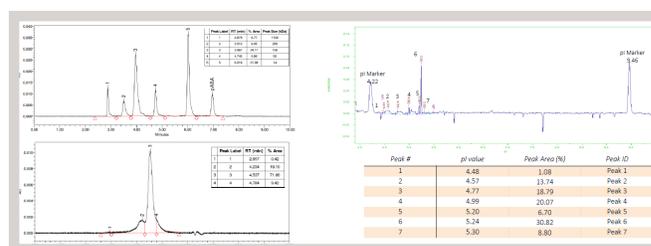
Downstream Process Development:

The charge profile of molecule was exploited to selectively enrich glycosylated species from impurities and process related impurities

A (3) step chromatography process achieved > 95% purity



Analytics reveal a highly uniform population that was selectively purified from the batch



SOLUTIONS

Recap:

- Transiently produced Molecule X was sufficiently consistent with the stable clone generated material to begin pK and downstream process development work
- Titer and binding assays were critical in supporting the cell line development program
- The CHO-GSNSM stable pool had slightly higher levels of expression material compared to the transient expression
- The CHO-GSNSM stable clone had 2x the expression of the stable clone
- Critical Product Quality profiles were selected at the clone selection stage
- Purification profiles were designed to leverage the charge properties of the glycosylated protein

Lessons Learned:

- Transient productions performed in parallel are a critical tool in reducing the development timeline
- Titer assay development should initiate before stable cell line production programs initiate
- For non standard proteins, construct liability and developability assessments are critical in streamlining development
- Clone selection plays a critical role in simplifying the downstream development process