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

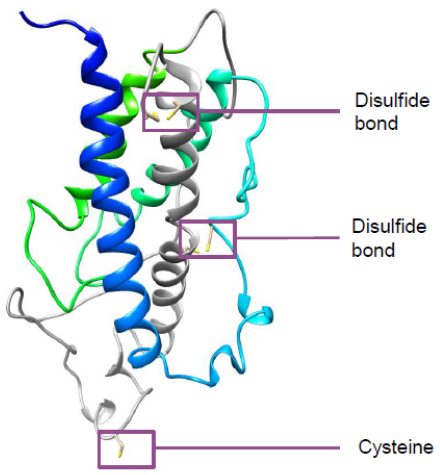
CHALLENGES

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-GSN℠ 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

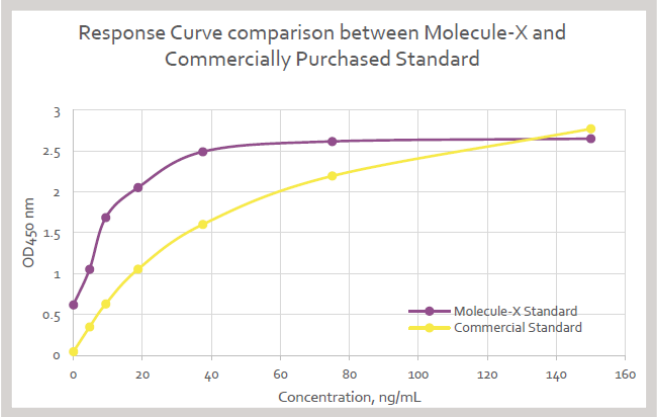
- Time savings for performing work in parallel with transient expression
- Developed a scalable purification process that did not rely on an affinity based resin.Developed a scalable purification process that could achieve target purity levels

DEVELOPMENT

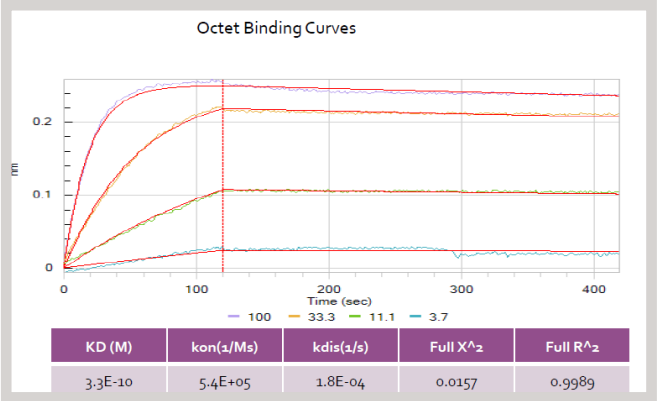
Cell Line Development:

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



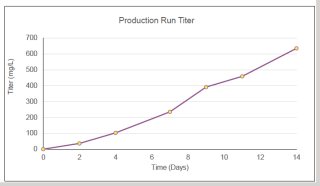
KD (M)	kon(1/Ms)	kdis(1/s)	Full X^2	Full R^2
3.3E-10	5.4E+05	1.8E-04	0.0157	0.9989

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

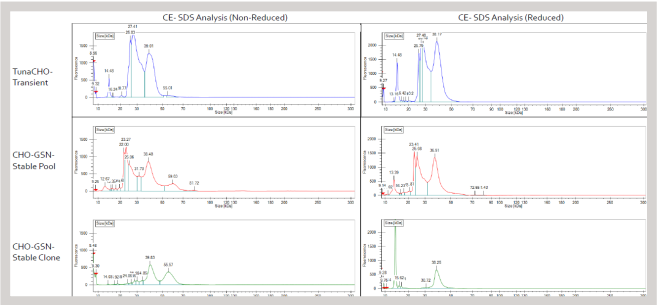
Expression Levels from various stages

Stage	Titer (mg/L)
Transient Expression	300 *
Stable Pool	330**
Stable Clone	660**

* Titer determined by IMAC Purification
** Titer analyzed by anti-Molecule-X ELISA



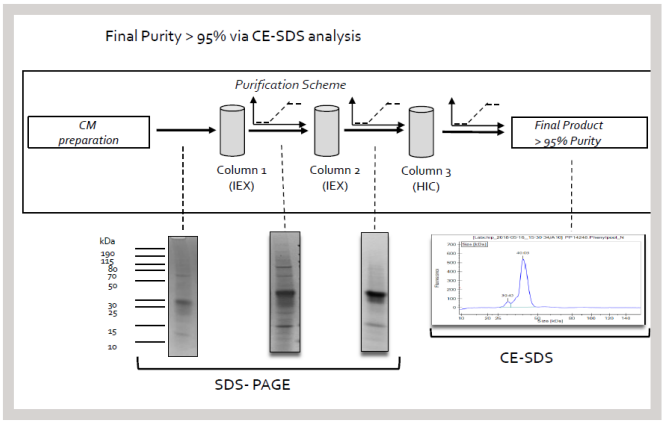
Target protein heterogeneity is minimized through clone selection and purification



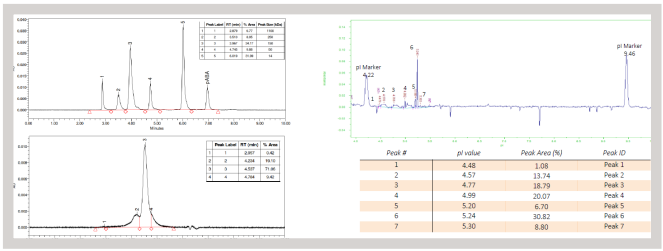
Downstream Process Development:

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



Peak #	pI value	Peak Area (%)	Peak ID
1	4.48	1.08	Peak 1
2	4.57	13.74	Peak 2
3	4.77	18.79	Peak 3
4	4.99	20.07	Peak 4
5	5.20	6.70	Peak 5
6	5.24	30.82	Peak 6
7	5.30	8.80	Peak 7

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-GSN^{SM} stable pool had slightly higher levels of expression material compared to the transient expression
- The CHO-GSN^{SM} 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