Process development and scale up of highly challenging biomolecules

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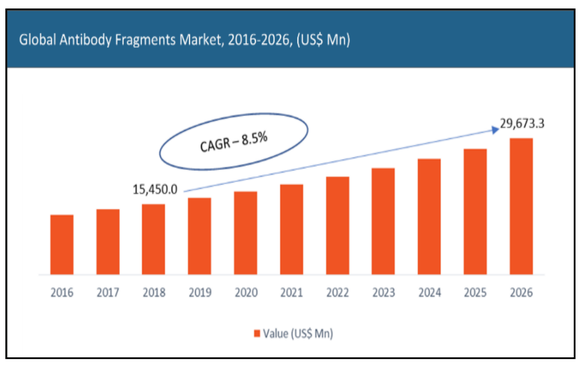
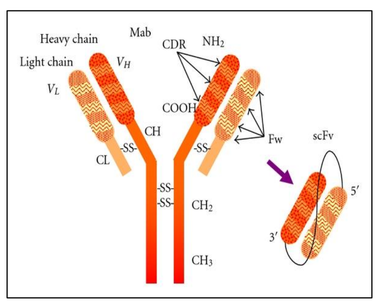
Syngene

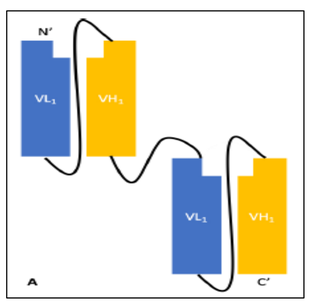
Case Study 1:

Modifying antibody properties such as molecular size, valency, binding affinity, and pharmacokinetics allows for the creation of antibody fragments with custom properties for a wide range of clinical applications. Several antibody fragments have already entered clinical trials, the majority of which are antigen-binding fragments (Fab, 50 kDa) and single-chain variable fragments (scFv, 28 kDa). Because of their high binding specificity, ease of production, low immunogenicity they are a very appealing option for targeting solid tumours. The global antibody fragments market was valued at **US$ 15,450.0 Mn 2018** and is expected to reach **US$ 29,673.3 Mn 2026**, growing at a CAGR of 8.5% during the forecast period.



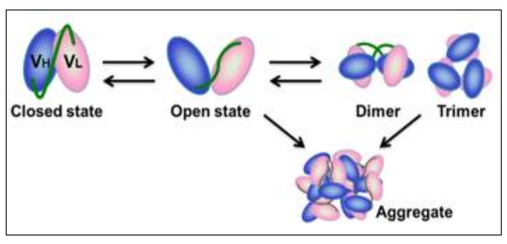
A single-chain variable fragment (scFv) is not actually a fragment of an antibody, but instead is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. Divalent (or bivalent) single-chain variable fragments (di-scFvs) are engineered by linking two scFvs. This is done by producing a single peptide chain with two VH and two VL regions.





Technical Challenge for the downstream process development of Antibody Fragments:

- Absence of the Fc region has been reported to render the antibody more aggregation-prone compared to the parental conventional immunoglobulins.
- Low recovery



Downstream platform process development for the antibody fragments at Synegene:

Because of the presence of bsAb-specific by-products, such as mispaired products, undesired fragments, and higher levels of aggregates, that are otherwise absent or present in lower levels in mAb cell culture supernatants, downstream processing of bsAbs often necessitates the development of additional purification strategies in order to obtain high purity products.

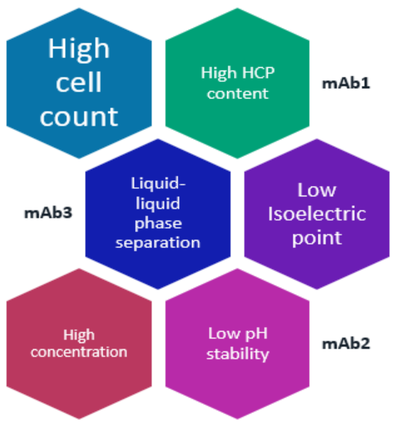
Conclusion: The developed process at 10L scale has been successfully scaled up to 500L scale. 200 gm of protein was generated from 500L batch with an overall recovery of around 60% with more than 97% of monomer purity.

Case study 2:

Syngene carried process development of three broadly neutralizing antibodies, promising agents to prevent HIV infection and achieve HIV remission without antiretroviral therapy (ART). bNAbs are unique in that they target conserved epitopes of the virus, meaning the virus may mutate, but the targeted epitopes will still exist. bnAbs have a window of opportunity to prevent infection in the absence of an established cellular HIV reservoir and potentially in concert with effector functions of the immune system. Beyond neutralization, bNAbs exert Fc-dependent functions including antibody-dependent cellular cytotoxicity and activation of the complement.

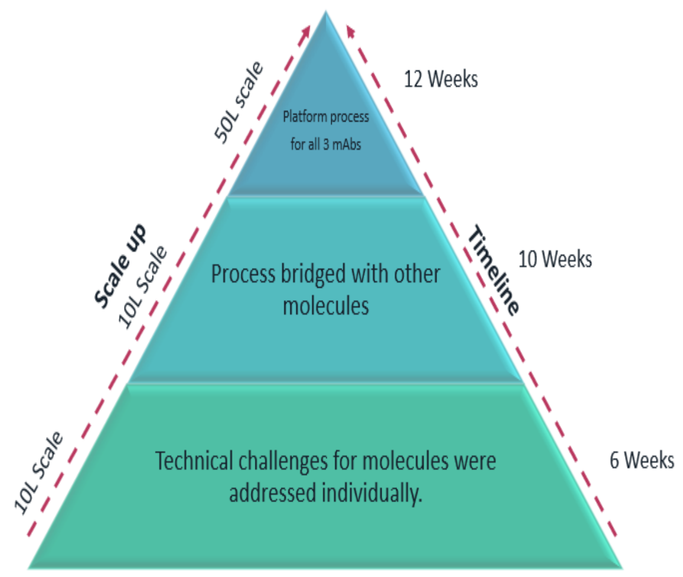
The major focus for HIV vaccine design is the elicitation of broadly neutralizing antibodies (bNAbs), capable of preventing entry by diverse viruses by binding to conserved regions on the HIV envelope glycoprotein trimer, which is the sole entry complex for HIV.

Technical Challenge for the downstream process development

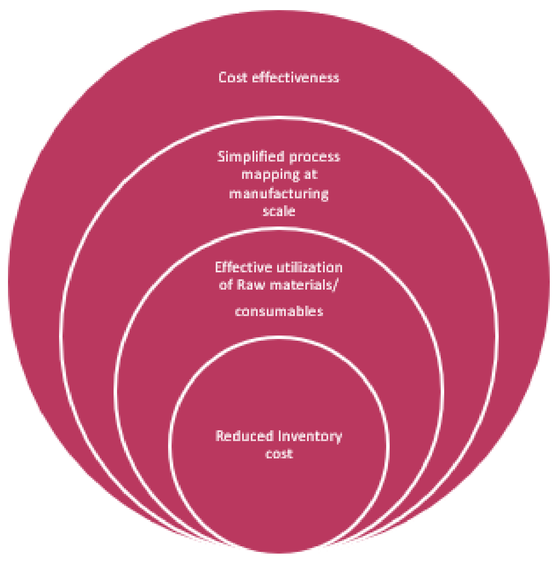


Syngene downstream team worked in parallel to address the technical challenges for all 3 molecules and developed a platform process for all three broadly neutralizing antibodies with defined timeline.

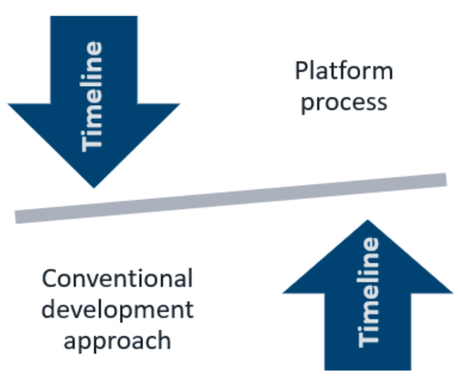
Process development Approach



Potential outcome of platform process



Impact



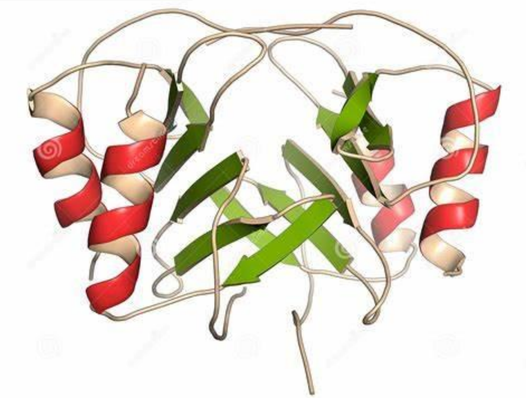
Conclusion: Platform downstream process has benefitted the client in achieving cost effectiveness and process was developed in short time frame and successfully scaled up to 2KL. Syngene team has shown the capability to adopt new molecules to platform process, address the technical challenge and successfully scale up the process for multiple clients.

Case study 3:

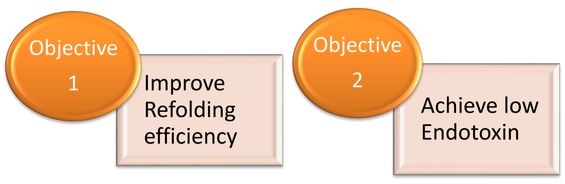
Syngene carried process development of new class of regenerative implant used for treatment of degenerative disease. The molecule belongs to TGF-β super family of protein, that plays an important role in the development of bone, and cartilage.

Molecule is expressed in *E.coli* as inclusion bodies (IB). Protein (monomer) contains 7 cysteine residues, refolding (dimerization) to the active form, happens by 3 three intramolecular and one intermolecular disulfide bond formation.

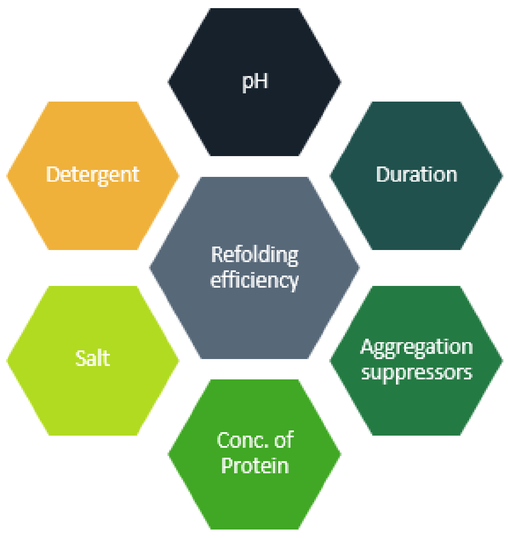
Molecular structure



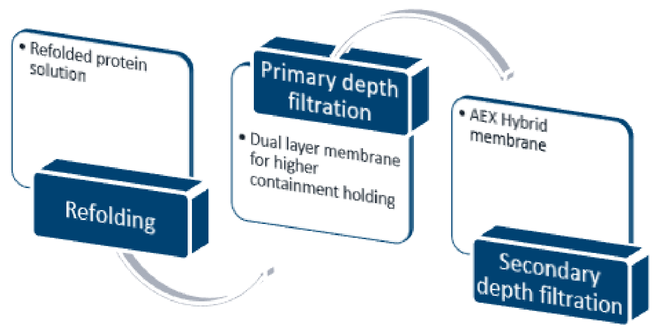
Objectives of downstream process development



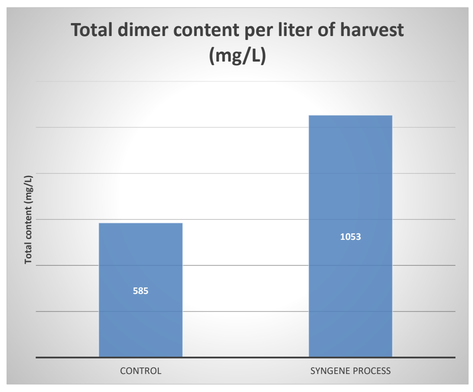
Syngene downstream team addressed the technical challenge by optimizing refolding process where in multiple parameters were screened in parallel and optimal solubilization and refolding condition were identified.



Refolded protein solution was subjected to primary clarification using dual layer cellulose depth filter for clearance of larger particles in top layer followed by trapping of smaller particles in bottom layer, the filtrate then passed through AEX hybrid membrane having quaternary amine for clearance of Endotoxin.



Outcome



Conclusion: Optimized downstream process resulted in 2x fold increase in productivity and endotoxin content of <3EU/mg. The process was successfully assessed at 10L scale followed by scale up to 200L fermentation. Product quality attribute of dimer purity of >95% was achieved.

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