NC STATE UNIVERSITY

LigaTrap®

# Clearance of host cell proteins from CHO cell culture harvests via "Flow-through affinity chromatography" using LigaGuard™ resins

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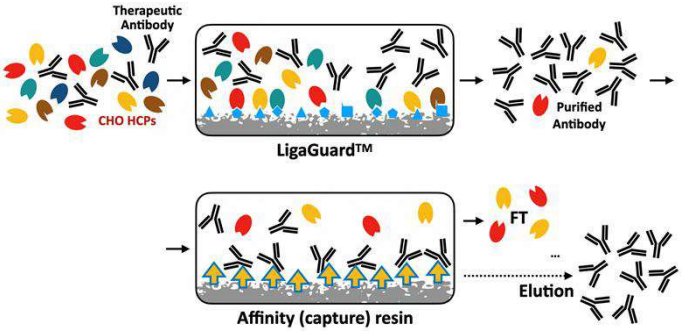
## - Introduction -

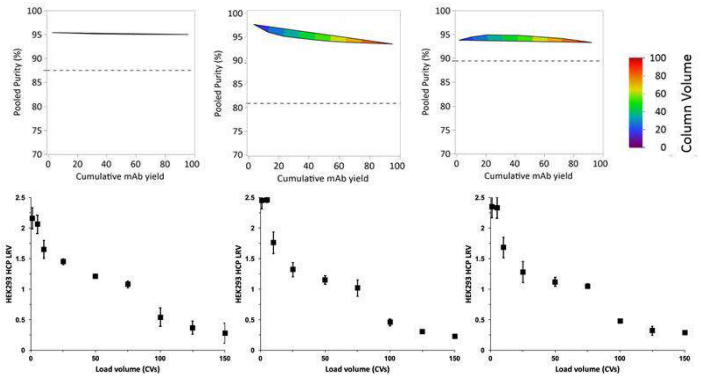
- Purification of therapeutic monoclonal antibodies (mAbs) consists of the removal of product-related (aggregates, fragments, charged variants, etc.) and process-related (host cell proteins (HCPs) and DNA etc.) impurities;
- Chinese Hamster Ovary harvested cell culture fluids (CHO HCCFs) contain, together with the mAb product, a substantial amount of HCPs (up to 0.5 mg/mL), which are diverse in titer, biophysical properties, and safety profile;
- HCP clearance poses major challenges due to the ability of highly immunogenic HCPs to associate with and/or degrade the mAb product;
- These "high-risk HCPs" impose taxing optimization of both Protein A purification and polishing;

## - LigaGuard™ -

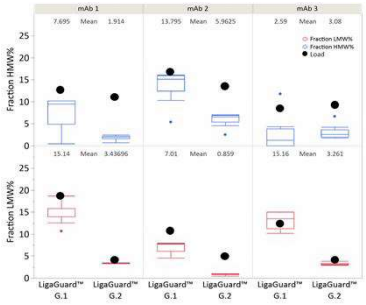



- Our work demonstrates that LigaGuard resin has the potential to:
  - De-risk biomanufacturing: by effectively clearing all HCPs, including the HR-HCPs that commercial resins may struggle to remove, LigaGuard resin provides a significant contribution towards safeguarding the stability and safety of special mAb products;
  - Increase process flexibility and robustness by widening the "space of design" where high mAb yield and purity are achieved and minimizing the impact of variability of HCP titer and composition in the harvest;
  - Reduce CAPEX and OPEX by enabling an agile purification sequence fully operated in flow-through mode, which are prized for their compact footprint and reduced volume of aqueous service streams;
  - Enable purification processes of emerging therapeutic modalities, such as gene therapy and cell therapies.

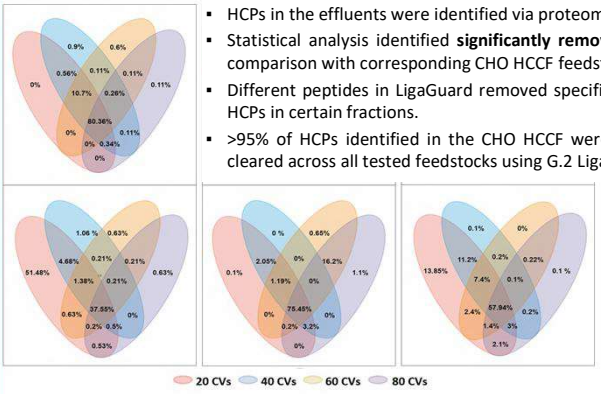
## - Results -




- Static CHP HCP binding capacity of LigaGuard resin > 20 g HCP per L resin;
- LigaGuard resin affords mAb yield of 93 - 96% irrespective of HCCF properties;
- HCP clearance (logarithmic removal value, LRV):
  - Average LRV > 1.5 for loading up to 30 CVs;
  - Average LRV ~ 1.3 – 1.4 for loading up to 75 CVs;
  - Average LRV ~ 1.1 – 1.2 for loading up to 100 CVs;



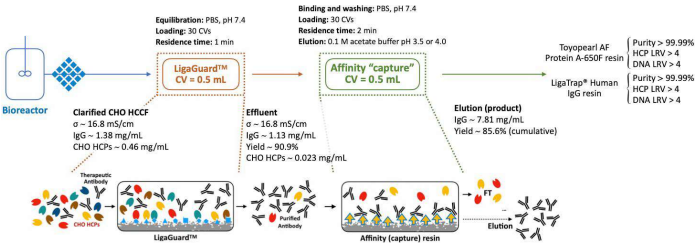

- Analysis of LigaGuard effluents via SEC HPLC demonstrated the selective recovery of mAb monomers, while mAb aggregates are cleared; we hypothesize that the capture of HCPs favors the removal of HCP-associated mAb aggregates.




- HCPs in the effluents were identified via proteomics analysis.
- Statistical analysis identified **significantly removed HCPs** in comparison with corresponding CHO HCCF feedstocks.
- Different peptides in LigaGuard removed specific subsets of HCPs in certain fractions.
- >95% of HCPs identified in the CHO HCCF were effectively cleared across all tested feedstocks using G.2 LigaGuard

- **Effective clearance of high-risk HCPs** was documented, including phospholipase B-like 2 protein (PLBL-2), Heat shock protein 70 (HSP70), Cathepsins A, B, D, Z, serine proteases, carboxypeptidases, glutathione transferases, metalloproteases, etc.
- **LigaGuard resin can be regenerated**, making it amenable to both single-use or multiple use formats.

## - LigaGuard™-based Process -




- **Documented removal of Chromatin-based and HCP-laden aggregates** prior to the affinity capture column, thus reducing HCP persistence;
- Two-Step **HCP LRV > 4 and DNA LRV > 4**;
- Improved Protein A performance (elution yield and lifetime);
- Improved stability of the mAb product (long-term colloidal stability of the mAb under cold storage).

## - Publications -


- S. Sripada, […], R.G. Carbonell, A.M. Lenhoff, S.M. Cramer, J. Bill, D. Roush, and S. Menegatti. "Towards continuous mAb purification: clearance of host cell proteins from CHO cell culture harvests via "flow-through affinity chromatography" using peptide-based adsorbents." *Biotechnology and Bioengineering* (2022).
- A. R. Lavoie, […], R.G. Carbonell, and S. Menegatti. "Removal of host cell proteins from cell culture fluids by weak partitioning chromatography using peptide-based adsorbents." *Separation and Purification Technology*257 (2021): 117890.
- A. R. Lavoie, […], R.G. Carbonell, and S. Menegatti. "Targeted capture of Chinese hamster ovary host cell proteins: Peptide ligand binding by proteomic analysis." *Biotechnology and Bioengineering* 117, no. 2 (2020): 438-452.
- A. R. Lavoie, […], R.G. Carbonell, and S. Menegatti. "Targeted capture of Chinese hamster ovary host cell proteins: peptide ligand discovery." *International journal of molecular sciences* 20, no. 7 (2019): 1729.
- Peptide ligands for capture of host cell proteins (US20220009959A1).