DIVISION OF BIOCHEMICAL TECHNOLOGY OF THE AMERICAN CHEMICAL SOCIETY

BIOT

The Best of BIOT Awards: September 27, 2017

Date	Area	Time	Presenter	Institution
Wednesday, September 27th	Downstream Process Development	12:00-12:30 PM	**Julie Robinson**	Rensselaer Polytechnic Institute/Merck & Co.
		Investigating domain contributions to antibody retention in chromatography systems		
		12:30-1:00 PM	**Chloe Andersen**	Amgen
		Overcoming limitations of high throughput scale chromatography columns to facilitate the evaluation of extended resin lifetime		

Webinar registration can be made at ACS BIOT WebEx Webinars

"Investigating domain contributions to antibody retention in chromatography systems"

Julie Robinson, Rensselaer Polytechnic Institute, Troy, NY/Merck & Co., Kenilworth, NJ

Although a platform process has been established for purification of antibodies, a deep, fundamental understanding of how these molecules interact with chromatography resins has yet to be developed. The increasing prevalence of antibody-related therapeutics and associated purification challenges further motivate research into these molecular level interactions. The objective of this work is to understand the nature (i.e. size and properties) of preferred protein-ligand binding regions for large, multi-domain molecules such as antibodies. In this work, three antibodies with different charge and hydrophobicity were enzymatically digested to create $(Fab)_2$, Fab, and F_C domains. The retention of the constituent domains was compared to that of the full mAbs in linear gradient chromatography experiments to identify how each domain contributed to binding of the full mAb in the different resin systems. Different selectivity trends were observed for three intact antibodies in the various multimodal systems. Unique domain contributions were also observed for each mAb in the multimodal systems. While some mAbs were dominated by contribution from the F_C constant region, other mAbs were primarily driven by $(Fab)_2$ interactions. Comparison with domain contribution results for single mode HIC and IEX systems demonstrated that merely summing the IEX and HIC interactions is not indicative of the behavior in multimodal systems. The unique behavior observed for each mAb was connected to the protein surface properties using novel surface property clusters recently developed by our group along with electrostatic potential (EP) and spatial aggregation propensity (SAP) maps. This work lays the foundation for identifying the key surface patches on antibodies and other protein therapeutics that are important interaction sites in multimodal systems. This work also has important implications for the separation of product related variants as well as the design of complex therapeutics for biomanufacturability.

“Overcoming limitations of high throughput scale chromatography columns to facilitate the evaluation of extended resin lifetime”

Chloe Andersen, Amgen, Cambridge, MA

High throughput screening (HTS) is a proven way to quickly evaluate multiple operating conditions for early development work, and is now increasingly being applied to late-stage process characterization activities. Resin lifetime studies require intensive material and labor resources at bench scale and there has not yet been a robust alternative at the high throughput scale due to limitations in the column hardware. By nature HTS chromatography columns are only intended for a minimal number of reuses. The columns typically fail due to leakage near the inlet adapter after about 10 reuses. In this study, a method of removing and replacing the inlet adapter of HTS columns without altering column integrity was developed. Column integrity was evaluated by connecting the HTS column to an AKTA system and measuring HETP and asymmetry by injecting a salt spike and measuring conductivity profiles. This method has allowed for lifetime studies to be performed at the high throughput scale. Using a TECAN robotic liquid handler, evaluation of up to 8 cleaning regimens can be evaluated in parallel to the end of resin lifetime. This allows determination of resin lifetime under optimal regeneration conditions before moving to bench and manufacturing scale, saving an extensive amount of operator time and materials, as well as building a robust design space. Additionally, by qualifying high throughput columns as small scale models, it may be possible to eliminate bench scale lifetime studies altogether.