

## CONTINUOUS COUNTER-CURRENT AFFINITY COLLOIDAL PURIFICATION

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Despite the significant efforts for development of continuous bioprocessing methods, the mainstay column-chromatography purification process has remained batch, with the exception of Simulated Moving-Bed chromatography (SMB) where we do not believe the savings is worth the complexity of the system. Affinity ultrafiltration with continuous modular counter-current tangential-flow filtration (CM-TFF) is a truly continuous process that uses commercial native soluble Protein A (sProA) and ultrafiltration TFF to affect the purification. A series of single-pass TFF operations are used, and the permeate from the cleaner downstream module is recycled as the diluent for a dirtier upstream feed. In this work, a Chinese Hamster Ovary (CHO) cell line expressing a monoclonal antibody IgG1 is used, where the cell culture is continuously harvested from days 0-14 in microporous TFF perfusion system. In order to purify the product through the CM-TFF process, first CM is concentrated up to 8 $\times$  using a 30-kDa filter. Second, the sProA is added continuously with the concentrated CM to antibody in a molar ratio of >0.3. This complex remains soluble, with a molecular weight of approximately 700 kDa. Third, the colloid is washed with relatively clean solutions recycled from downstream steps and retained by a 300 kDa filter to remove impurities and HCPs. And finally, the captured sProA-mAb complex is broken up with low pH in the presence a cation-exchange (CEX) resin slurry, which selectively binds the antibody. A SPTFF separates the sProA and antibody/CEX complex. The purified Protein A is recycled to the CM, while the antibody is eluted through a subsequent SPTFF.