

## A FULLY CONTINUOUS DOWNSTREAM PROCESS CONCEPT WITHOUT COLUMN CHROMATOGRAPHY

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We propose a continuous coupled precipitation-filtration process concept as the centerpiece of a fully continuous, column chromatography-free downstream process for recombinant protein manufacture. We expect the process to be generalizable to a wide range of proteins and to offer performance, raw material usage, simplicity and cost advantages relative to packed bed chromatography-based processes, thereby forming the basis for a new, integrated continuous downstream manufacturing paradigm for the production of recombinant proteins.

The process is aimed at high-titer products and is based on target pre-concentration and precipitation in the capture step followed by precipitate washing and re-dissolution. A novel format consisting of tubular contactor - hollow fiber filter pairs is used, with controlled precipitation in the contactor and removal of soluble components in the hollow fiber membrane. Subsequent orthogonal, flow-through polishing operations are conducted with either membrane chromatography units or with chromatographic media slurries in contactor-filter pairs. This format allows for fully continuous and counter-current operation of several process steps, increasing efficiency and productivity.

We have demonstrated the central precipitation and precipitate filtration capture operation with several proteins and are developing the thermodynamic, kinetic and operational models necessary for de novo process design and selection of optimal operating conditions. We have demonstrated: the effectiveness of synergistic pairings of volume excluding and cross-linking precipitants with mAbs and other globular proteins, the existence of yield-purity synergies rather than trade-offs, the ability to produce different precipitate particle morphologies (size distributions and fractal dimensions), the ability to scale precipitations up by x20 and down by x50 while maintaining particle morphology, the ability to dewater precipitates in hollow fiber membrane modules and the dependence of filtration critical flux performance in hollow fiber membrane modules on precipitate morphology.