LYTAG-DRIVEN PURIFICATION STRATEGIES AS A KEY TO INTEGRATE AND INTENSIFY THE DOWNSTREAM PROCESSING OF MONOCLONAL ANTIBODIES

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Monoclonal antibodies (mAbs) are currently the most important class of recombinant protein therapeutics in the biotechnological and biopharmaceutical industry with more than 250 mAbs currently undergoing clinical trials. High titer producing cultures and complex mixtures containing high cell densities, together with an increasing growing demand for highly pure mAbs is making recovery and purification processes hot targets for improvement and opens important technological challenges in mAbs manufacturing platforms.

This work explores the use of an affinity dual ligand based on a choline binding polypeptide tag (LYTAG) fused with the synthetic antibody Z domain (LYTAG-Z) as a tool to integrate and optimized the downstream processing of mAbs. Upon addition of this ligand to an animal cell culture broth, antibody-LYTAG-Z complexes are formed which can be easily captured and separated from host cell impurities by affinity partitioning in aqueous two-phase systems (ATPS) composed of polyethylene glycol –PEG, as PEG molecules have the ability to binding to the choline binding sites of LYTAG. Integration of clarification and primary mAbs recovery was successfully accomplished using a system composed of 6% PEG 3350 Da and 7% dextran 500,000 Da in which an extraction yield of 89% and a clarification higher than 95% were achieved. IgG-rich phases were further processed by chromatography, using three different strong anion exchange matrices charged with quaternary methyl amines (a choline analogue) – CIMmultus QA, HiTrap Q FF and gPore NW Q. A two-elution method was developed for the separation of the antibody-LYTAG-Z complexe, allowing simultaneous purification of the antibody and recovery of the ligand.

The process was successfully scale-up 10000 times allowing a global antibody recovery of 70% with a purity of 89% and enabling 100% cell removal.