# Development of a calcium -responsive ZZ-CSQ-based affinity precipitation process with Tangential flow filtration (TFF) for enhanced monoclonal antibody purification

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Protein A-based affinity chromatography is a conventional method for antibody purification; however, its scalability is constrained by extended purification times, decreased activity of both protein A and antibody, and high costs. Affinity precipitation offers an alternative solution that overcomes these challenges by delivering high selectivity, straightforward scalability, and a simplified process. In previous study, we developed an antibody purification method using calcium-responsive precipitation with ZZ-CSQ, a fusion protein of human calsequestrin 1 and the antibody-binding protein ZZ. However, it was unsuitable for industrial applications due to its reliance on centrifugation, which is impractical for scaling up to large volumes. Here, we report a ZZ-CSQ-based antibody purification system using a Tangential Flow Filtration (TFF) system. In this method, purification time was reduced by over 4 -fold compared to standard protein A chromatography, while significantly decreasing impurities such as host cell protein (HCP) and DNA (HCD) by 2.8 -fold and 12.4 -fold, respectively. Consequently, a high purity monoclonal antibody (> 97%) was achieved with a 90 % recovery yield, and the entire purification process was finished within 80 minutes. These results suggest that the ZZ-CSQ-based mAb purification method using the TFF system offers greater efficiency, reduced time, and simplified approach.