In the manufacture of therapeutic proteins such as monoclonal antibodies (mAbs), host cells produce not only the product of interest but also many other host-cell proteins (HCPs) that are required for the biological functions of the cell. To remove such process-related impurities, a typical mAb process employs multiple sequential purification steps to enable an acceptable level of product purity in terms of total HCP concentration. However, HCP control is still of particular interest because certain individual HCPs may persist during the purification, contributing to specific challenges for patient safety or drug quality. Three main mechanisms have been proposed to explain persistence of HCPs: 1) HCP-mAb binding; 2) HCP-resin interactions; 3) non-specific interactions between chromatin-derived complexes and resin. While the first two mechanisms focus on biophysical properties of HCPs that determine the interactions of individual HCPs with mAb or resin, the third considers the charge properties of chromatin, histones and DNA to be the major factor causing the formation of heterogeneous DNA-HCP-mAb aggregates and the persistence of such complexes.

This study aims to deepen the current understanding and knowledge of HCP persistence in mAb processes. Four key process streams, i.e., harvested cell culture fluid (HCCF), protein A eluate, and two polishing pools, of seven different mAbs were used as the base materials for the analyses in this study. The results obtained from LC-MS/MS-based characterization of HCPs in the processing steps show a great degree of variability in HCP identities and quantities. However, for the most abundant HCPs in HCCF, extensive commonality is observed in both. Analysis of HCP abundance in HCCF shows a likely connection between abundance and the reproducibility of quantitation measurements and suggests that some groups of HCPs may reduce the accuracy of the characterization. Measurements of HCPs persisting through purification steps coupled with the findings from the HCCF analysis indicate HCP abundance as a major factor contributing to the persistence of individual HCPs.

Another mechanism for HCP persistence was proposed and investigated, namely the role of HCP-rich aggregates, the formation of which has been attributed previously to the presence of chromatin. Analyses performed using fractionation by size-exclusion chromatography (SEC) in
tandem with protein detection and quantitation by LC-MS/MS show HCPs involved in protein synthesis and the unfolded protein response (UPR) to be core-conserved groups of proteins among the HCPs found in HCP-mAb aggregates and those co-eluting with the product during SEC. Such HCPs are likely associated with the product and vulnerable to protein aggregate formation due to functional complementarity with the product. In addition, the aggregates are identified as a major source of these process-related impurities, i.e., HCPs in the samples, not only with many HCPs detected in the aggregates but also with significant amounts of HCPs appearing in the aggregates. The incorporation of the proteomics data from different processing steps enables a more comprehensive tracking of the origins and persistence of HCPs through successive processing operations.

The three main mechanisms previously associated with HCP persistence are revisited with an emphasis on HCP-mAb binding, which has received most attention in the literature as a likely cause of HCP persistence. The implementation of LC-MS/MS-based quantitation of HCPs in binding affinity assays using immobilized mAb resin and a null HCCF enables the estimation of binding strengths between the mAbs and all HCPs identified in the HCCF. Interestingly, strong binding itself is not identified as a primary driver for an HCP to be considered difficult to remove but joint consideration of the binding strength and the HCP abundance is more likely to aid the assessment of the likely extent of clearance. Further characterization of the null HCCF by SEC in tandem with the quantitative proteomics reveals that many HCPs are present in aggregated form, which may impair their binding to mAbs. The results from the affinity assays are further supported by protein A chromatography experiments with different SEC fractions of a mAb-containing HCCF. HCP-resin interactions are also examined using a commercial protein A resin and recombinant CHO HCPs prepared in this work. All these factors are considered in seeking to understand how they contribute to HCP persistence in mAb processing.

The results of this work lay out a basis to revisit and recalibrate current understanding and knowledge of mechanisms of HCP persistence in biopharmaceutical development and manufacturing. Multiple analyses suggest that abundance of HCPs should receive more attention as it plays a key role in driving HCP persistence, albeit with other factors (e.g., HCP-mAb binding, HCP-resin interaction) playing a role. The work can also help guide the adoption of improved strategies and techniques to characterize HCPs in different systems, which are significant in extending understanding of mechanisms of HCP persistence and thus developing more robust control strategies.