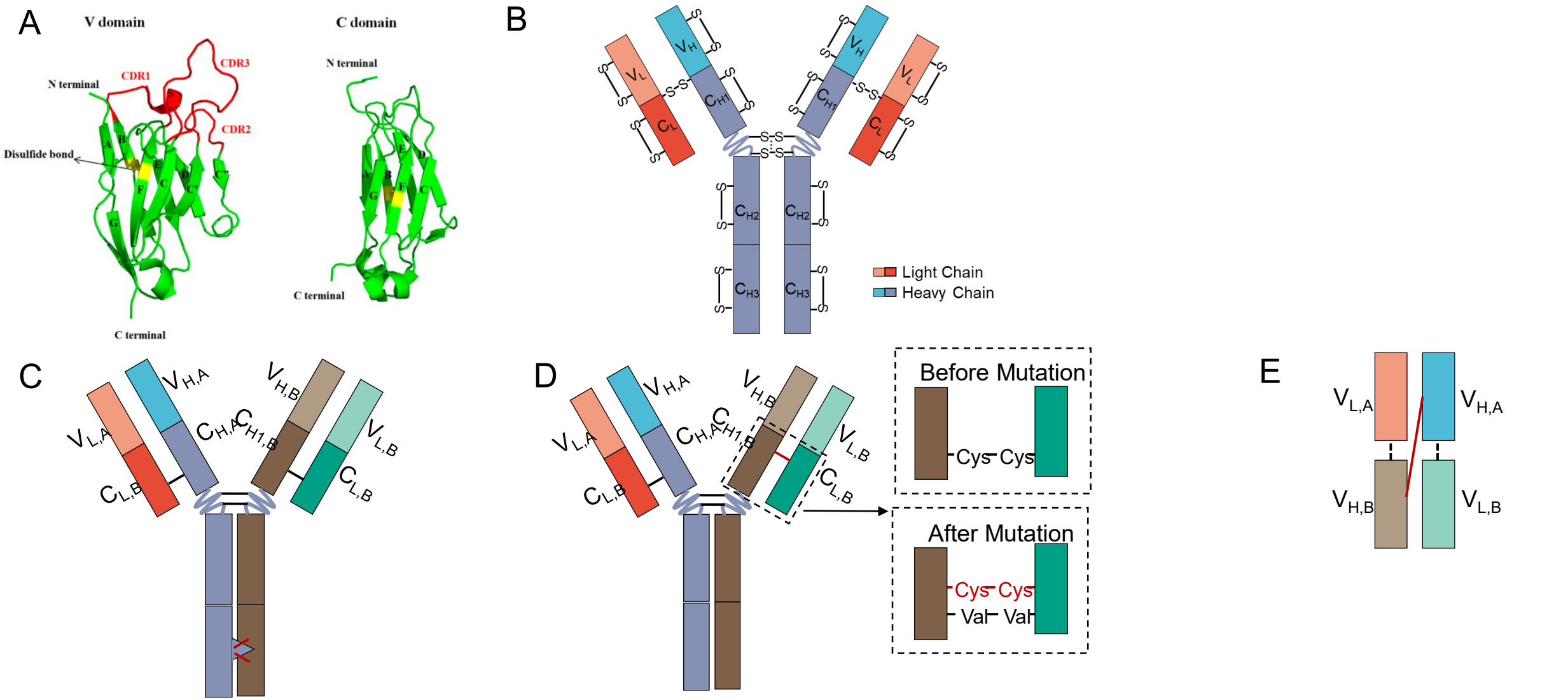

FIGURE 1 Illustrations of disulfide bond structure. (A) Intra-chain disulfide bond connects two beta-sheets in Ig domain(Li, Prabakaran et al. 2016). (B) Intra-chain and inter-chain disulfide bonds in a IgG monomer; (C) "knob-into-holes (Kih)" IgG-like bispecific antibody (BsAb) stability can be improved by having additional disulfide bonds (red line) formed in the Kih region; (D) Disulfide bond can improve correctly pairing IgG-like BsAb light chain and heavy chain: in one arm, the cysteine residues in C_H and C_L that originally formed disulfide were mutated into valine, and introduced an addition disulfide bond (marked as red) during the mutation; the other arm kept unmutated; (E) Dual Affinity Retargeting Molecules (DART), light chain variable region from one antibody is linked to the heavy chain variable region from the other antibody through small peptide (black dash line), and the two variable regions are linked through disulfide bond (red solid line)

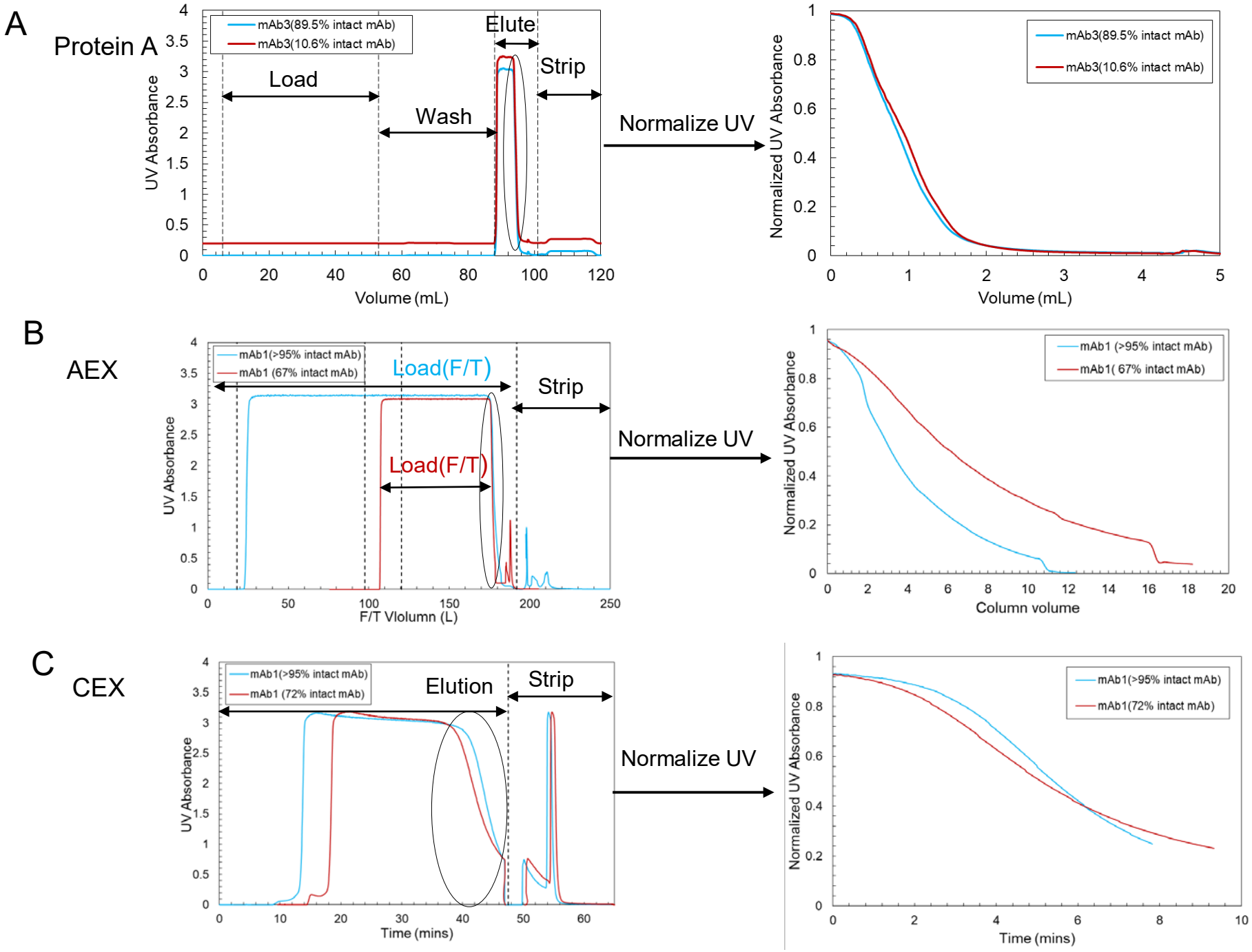

FIGURE 2 (A) Protein A chromatography profile from mAb3 purification process (B) AEX (Flow through mode) from mAb1 purification process; (C) CEX (bind and elute mode) from mAb1 purification process. Blue line represented the load sample that had no significant level disulfide bond reduction, red line represented the load sample that had disulfide bond reduction. The tail part for each step were normalized and enlarged for comparison purpose.

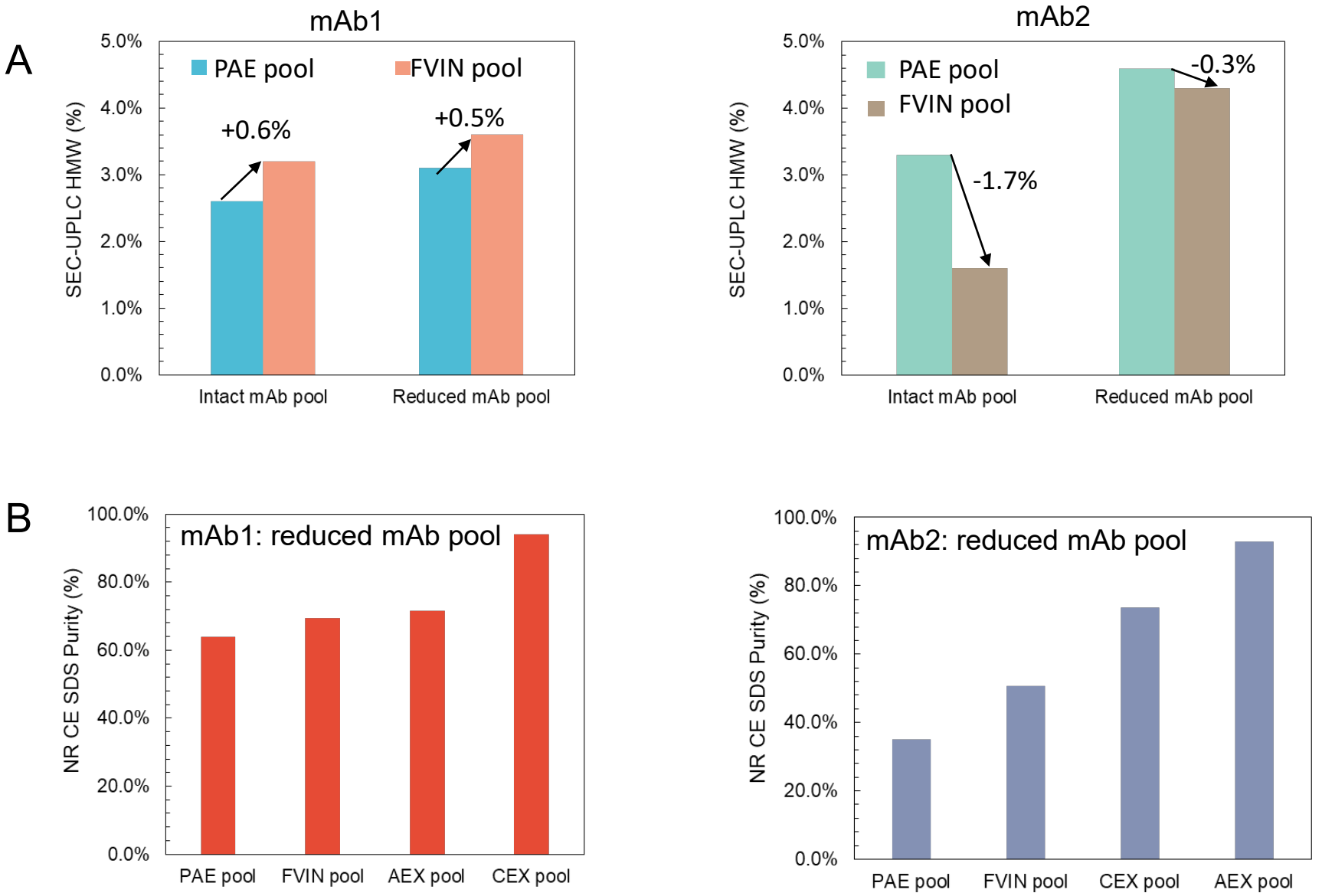

FIGURE 3 Qualifications of product quality attributes for mAb1 and mAb2 during downstream processing. (A) During the low pH viral inactivation step, for mAb1, the HMW% for intact mAb pool and reduced mAb pool increased at similar level (0.5~0.6%); for mAb2, the HMW% decreased more for intact mAb pool (-1.7%) than reduced mAb pool (-0.3%); (B) For both mAb1 and mAb2, reduced mAb pool purity increased during downstream process, showed the possibilities that reduced mAb can re-oxidize.

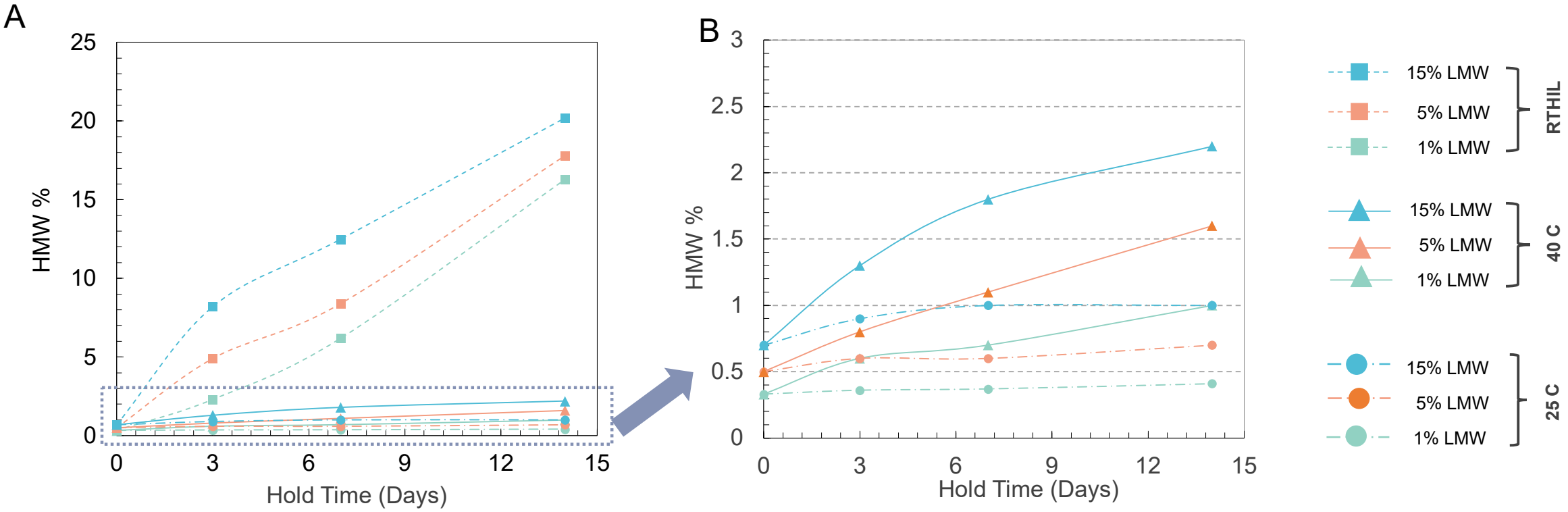

FIGURE 4 Stability of antibody samples with varied initial disulfide reduction levels under the conditions of room temperature (25 ºC), high temperature (40 ºC), and light exposure at 25 ºC for total 14 days. A, Aggregation level as a function of time for all studied conditions; B, Zoom-in profile of aggregation level as a function of time for 25 C and 40 C.

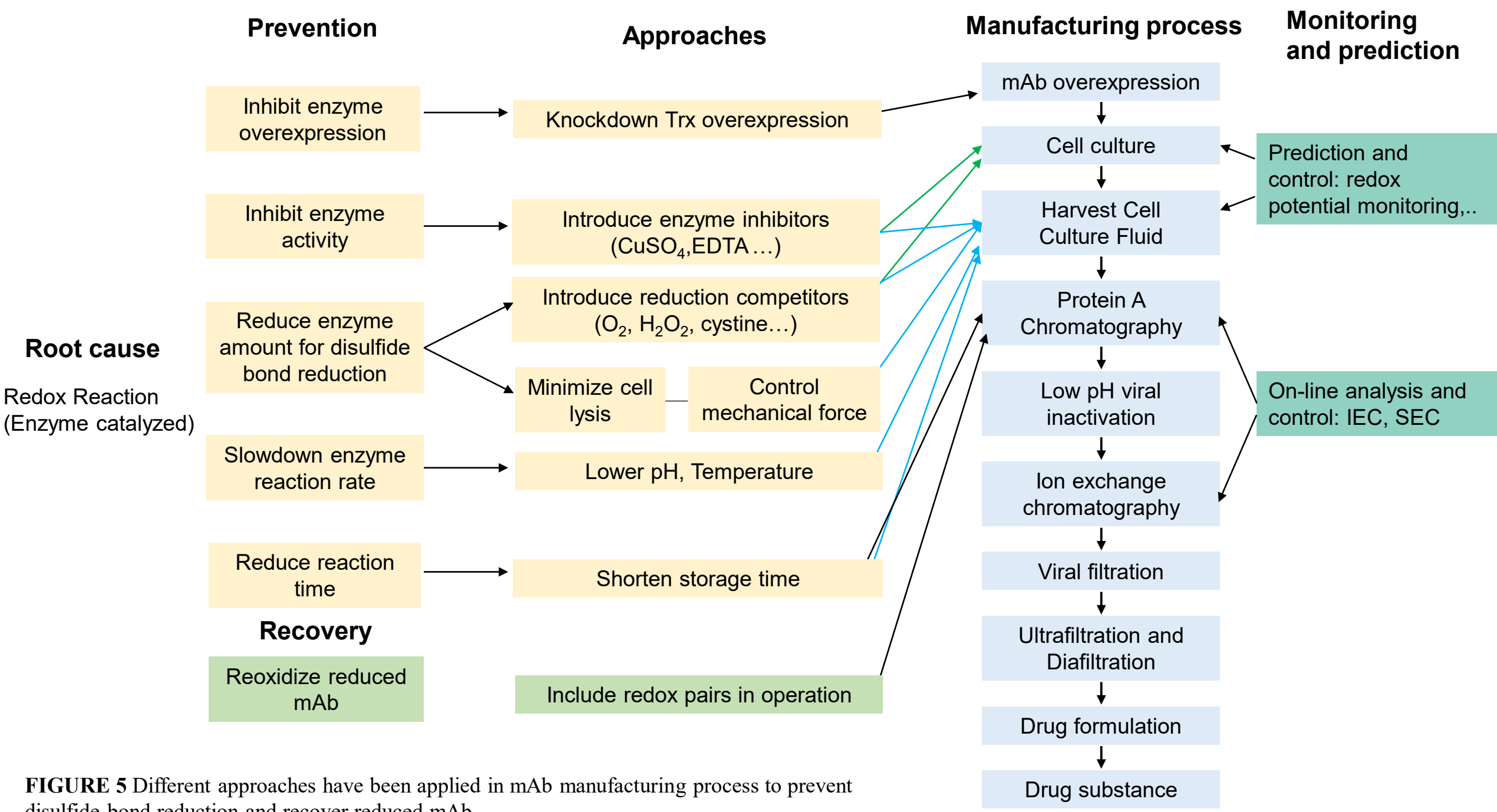

FIGURE 5 Different approaches have been applied in mAb manufacturing process to prevent disulfide bond reduction and recover reduced mAb.

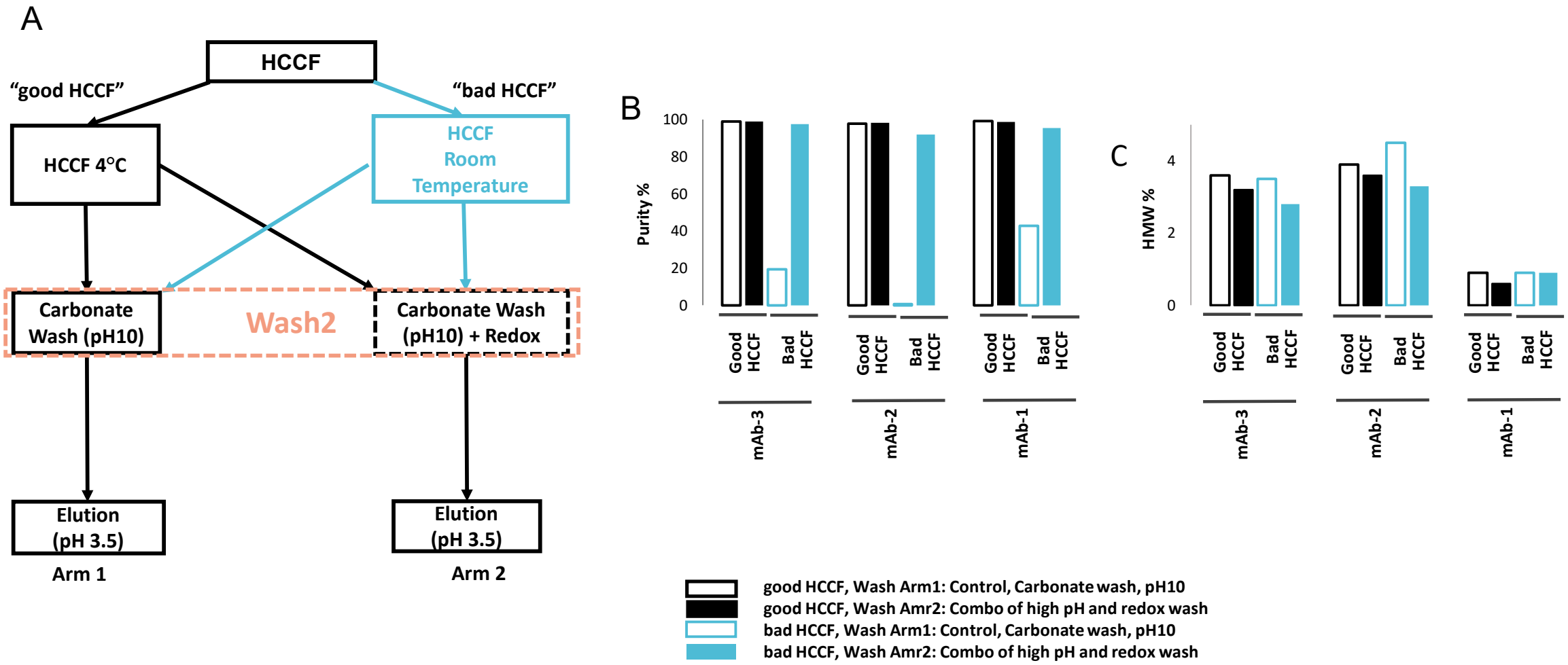

FIGURE 6 Comprehensive evaluation of using redox wash system in the platform Protein A chromatography(Tan, Ehamparanathan et al. 2020). The study was performed using three mAb harvest cell cultures according to the design including three arms. The protein A pools from each run was tested for product quality attributes. (A) Comprehensive study: Arm 1, control; Arm 2, combined wash step; (B) Intact mAb impurity; (C) Aggregates (HMW%).