

Supporting Information to manuscript:

Improving Mass Spectral Quality of Monoclonal Antibody Middle-Up LC-MS Analysis by Shifting the Protein Charge State Distribution

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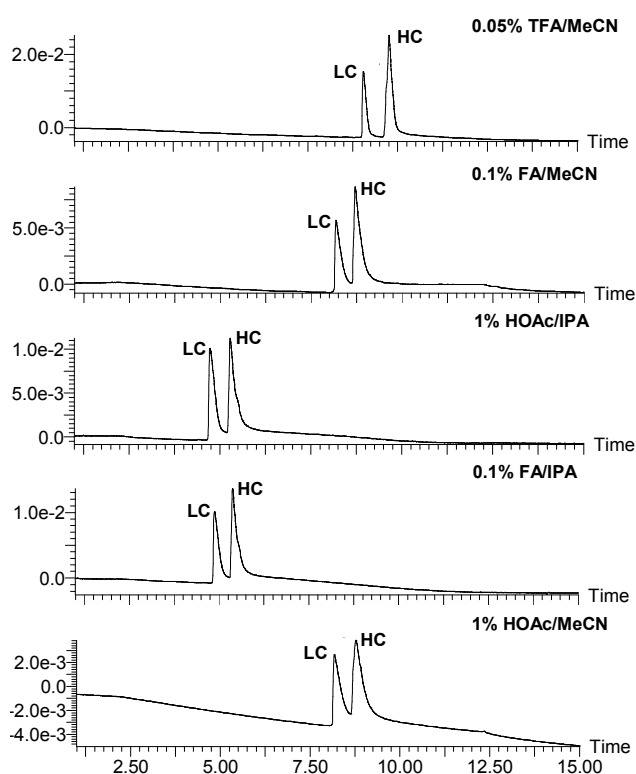


Figure S-1. Chromatograms of denatured, reduced and alkylated IgG1 heavy and light chains (by Reduction Procedure III) at 280 nm eluted with five different mobile phase (MP) systems.

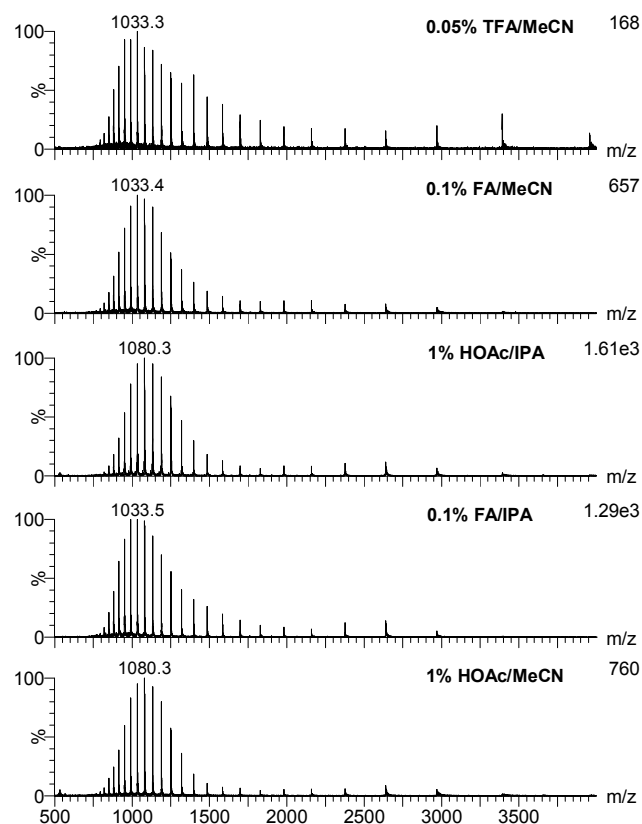


Figure S-2. Mass spectra of denatured, reduced and alkylated IgG1 light chain (by Reduction Procedure III) eluted with five different MP systems. Labeled at the upper right corner are intensities of the most abundant ion in each spectrum.

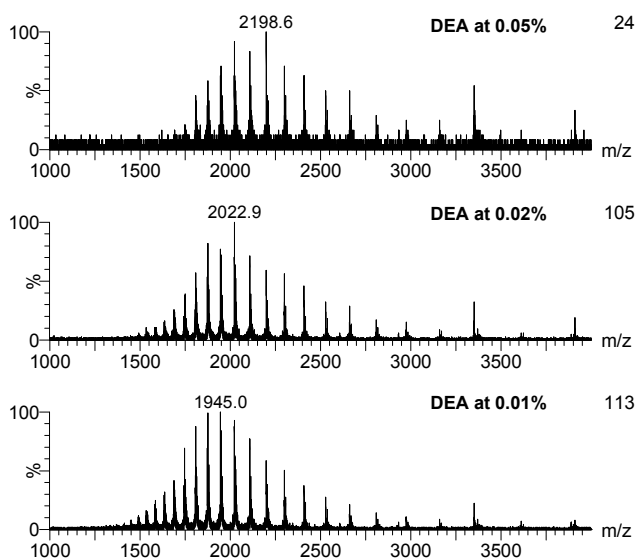


Figure S-3. Mass spectra of partially reduced IgG1 heavy chain eluted with MPs of 1% HOAc in H₂O and IPA with base additive DEA at three levels. Labeled at the upper right corner are intensities of the most abundant ion in each spectrum.

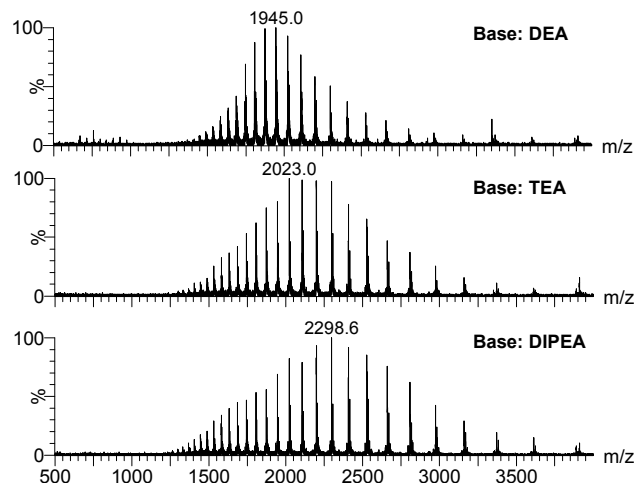


Figure S-4. Mass spectra of partially reduced IgG1 heavy chain eluted with three MP systems containing 1% HOAc in H₂O and IPA with three base additives at 0.01%.

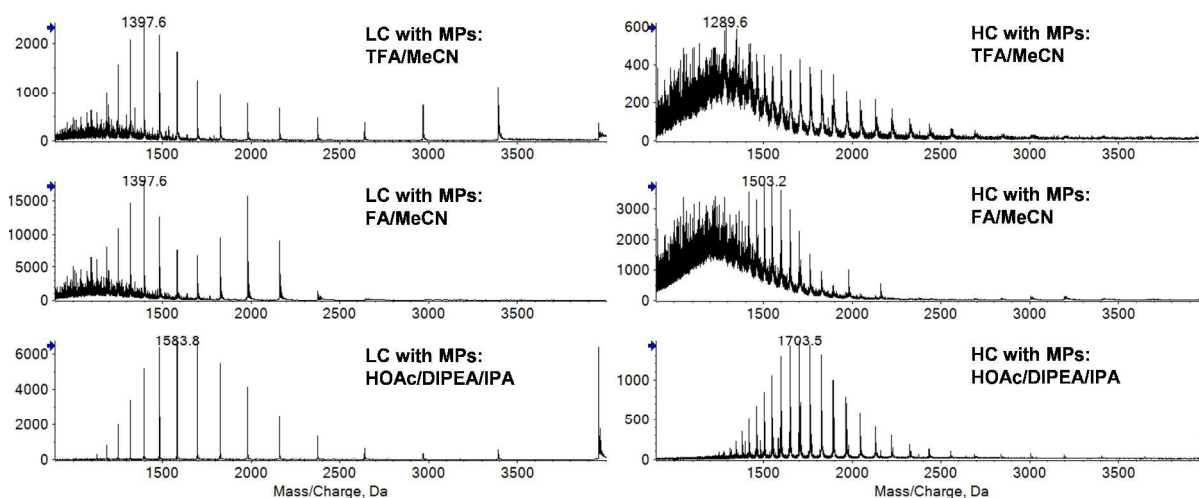


Figure S-5. Mass spectra of denatured, reduced and alkylated IgG1 light and heavy chains (by Reduction Procedure III) eluted with three different MP systems and acquired with a Sciex triple TOF 6600.

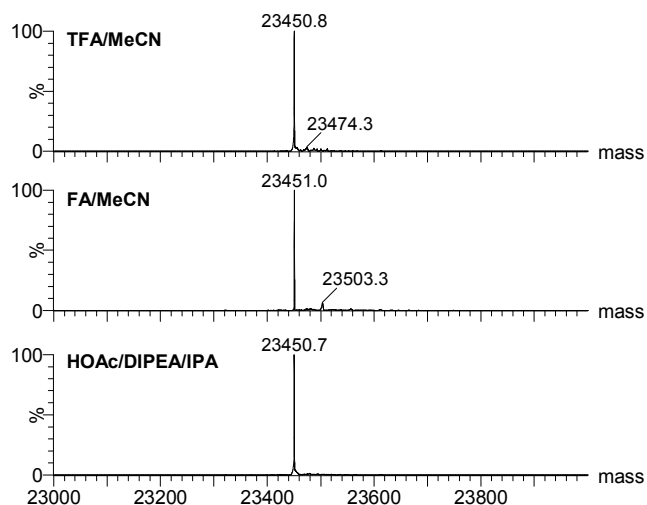


Figure S-6. Deconvoluted spectra of partially reduced IgG1 light chain (by Reduction Procedure II) with three different MP systems. Scans across the peak base were summed for data processing and a 0.1 Da resolution was used in deconvolution. The theoretical mass of partially reduced IgG1 light chain is 23451.1 Da.

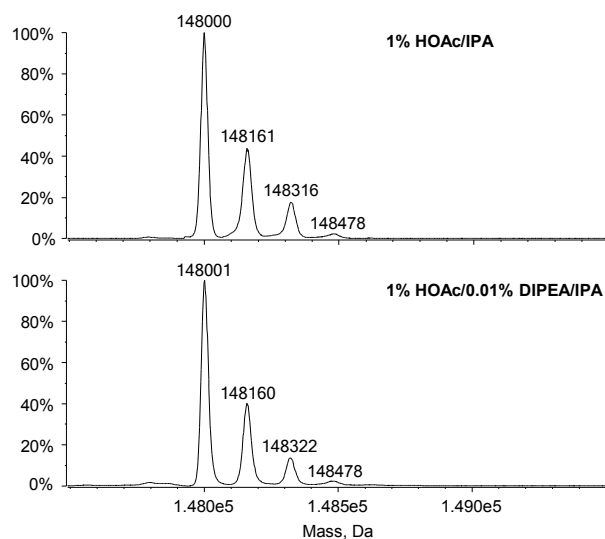


Figure S-7. Deconvoluted spectra of IgG1 intact protein eluted using two different MP systems. Data were acquired with a Sciex Triple TOF 6600. Deconvolution was performed by Sciex BioPharmaView.

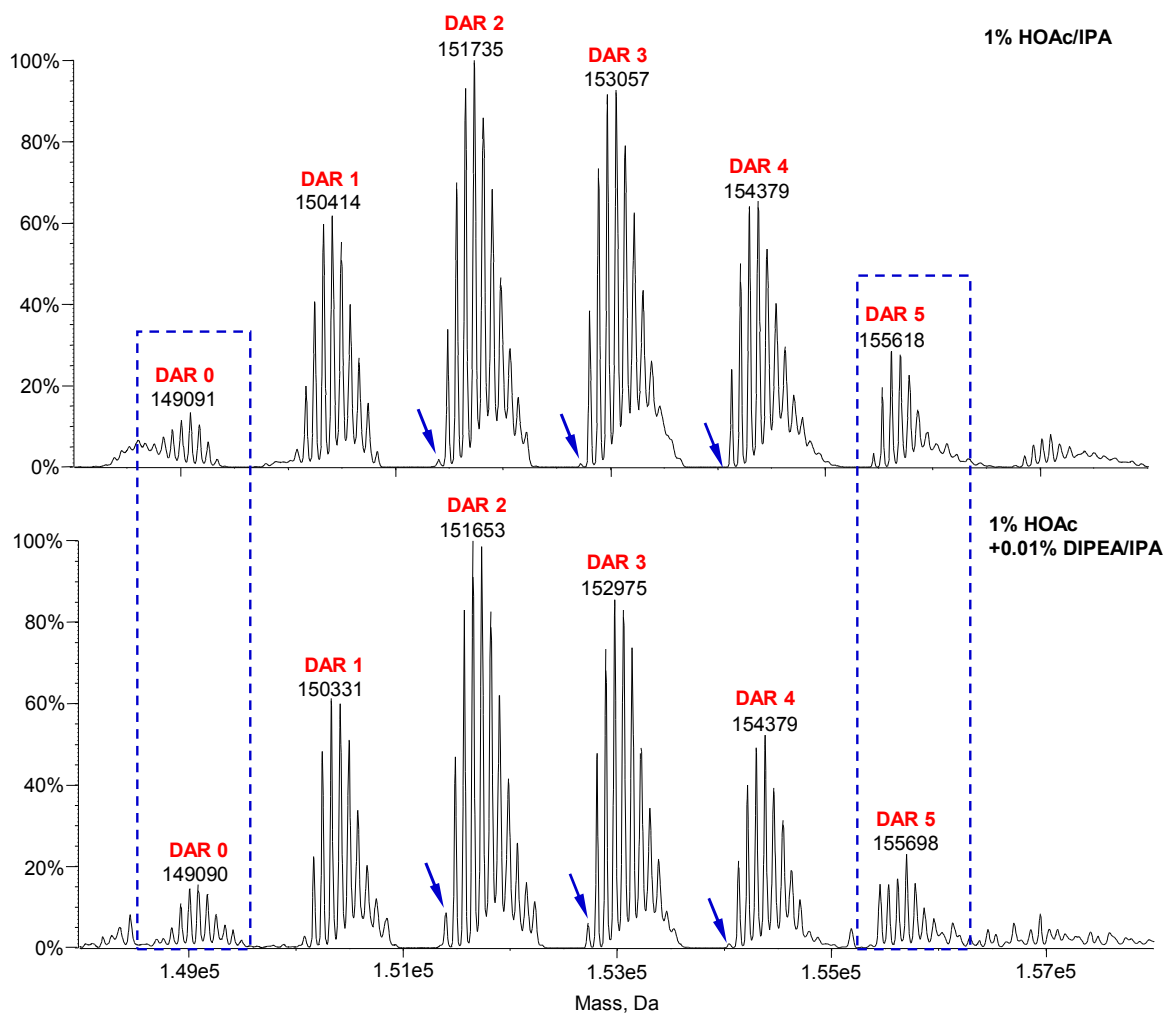


Figure S-8. Deconvoluted spectra of intact ADC (random lysine conjugated) using two different MP systems without and with DIPEA. Data were acquired with a Sciex Triple TOF 6600. Deconvolution was performed by Sciex BioPharmaView. The dotted boxes illustrate resolution improvement that gas-phase charge stripping brought to minor ADC glycan/linker by-product species in the minor DAR 0 and DAR 5 groups. The arrows pointing to the minor species in DAR 2, DAR 3 and DAR 4 groups demonstrated further resolution and/or detectability using this methodology.