Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

An improved capillary isoelectric focusing-mass spectrometry method for high-resolution characterization of monoclonal antibody charge variants

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Table S1. Comparison of theoretical and observed masses for the major glycoforms and PTMs in NISTmab, trastuzumab, cetuximab, and mAb1.

Antibody Name	Peak label	Glycoforms and PTMs	Theoretical Mass (Da)	Observed Mass (Da)	Mass Error (Da)	Mass Error (ppm)
NISTmab	M	G0F/G0F	148037.2	148038.0	0.8	5.4
	B1	G0F/G0F, +2K	148293.5	148294.4	0.9	5.8
	B2	G0F/G0F, +1K	148165.4	148165.8	0.4	2.9
	A	G0F/G0F, +Glycation	148199.3	148200.9	1.6	10.8
Trastuzumab	M	G0F/G0F	148056.6	148059.3	2.7	18.2
	B	G0F/G0F+PGK amidation	147998.6	147999.4	0.8	5.4
		G0F/G0F+PyroE	148038.6	148040.6	2.0	13.5
	A1	G0F/G0F, +1Deamidation	148057.6	148060.0	2.4	16.3
	A2	G0F/G0F, +2Deamidation	148058.6	148061.1	2.5	17.1
Cetuximab	M	Fab: G0F/G0F, Fc: G0F/G0F	151055.1	151055.7	0.6	4.0
	B	Fab: G0F/G0F, Fc: G0F/G0F, + 1K	151183.3	151182.8	-0.5	-3.1
	A1	Fab: G0F/G0F, Fc: G0F/G0F, + 1Deamidation	151056.1	151056.8	0.7	4.7
mAb1	M	G0F/G0F	147609.4	147610.8	1.4	9.8
	B1	G0F/G0F, +2K	147975.1	147974.6	-0.5	-3.2
	B2	+1K	147737.6	147738.1	0.5	3.6
		G0F/G0F, +PGK amidation	147551.4	147551.9	0.5	3.4
	A1	G0F/G0F, +2deamidation	147611.4	147612.5	1.1	7.7
	A5	G0F/G0F, truncation at C224/D225	100697.2	100697.3	0.1	0.1
		G0F/G0F, truncation at K226/T227	100453.9	100458.9	5.0	49.8
		G0F/G0F, truncation at H228/T229	100215.6	100214.6	-1.0	10.0

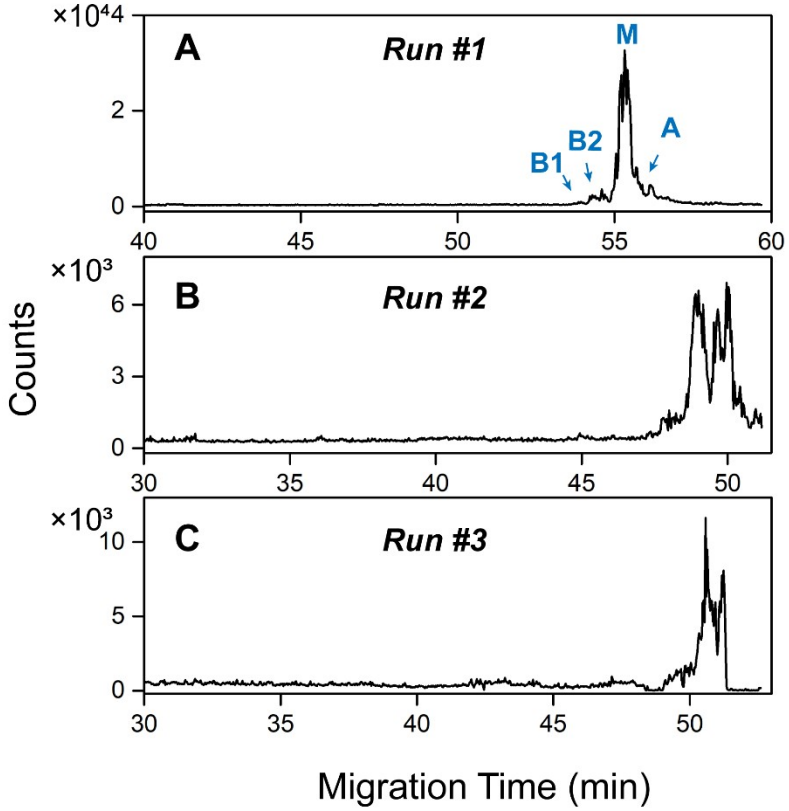

Figure S1. Triplicate cIEF-MS runs of NISTmAb with a high pH catholyte (pH 11.6) and an LPA-coated capillary (75 cm). Other parameters for cIEF separation include 12 cm catholyte plug, 63 cm sample plug, 0.2 mg/mL sample concentration, 1.5% (v/v) ampholyte mixture (pH range 3-10 and 8-10.5 with ratio of 1:4), 20 kV separation voltage, 10 mbar pressure at 20 min. The sample cannot focus well after the first cIEF-MS run because of deterioration of capillary coating by the high pH catholyte.

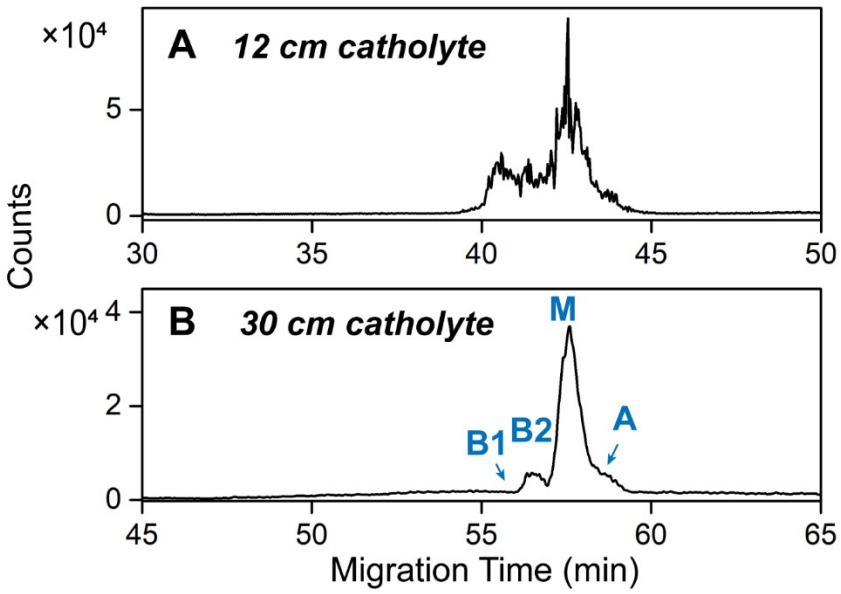

Figure S2. Base peak electropherograms of NISTmAb with 12 cm (A) and 30 cm catholyte (pH 10.0) (B). Other parameters for cIEF separation: 0.2 mg/mL sample concentration, 1.5% ampholyte mixture (pH range 3-10 and 8-10.5 with ratio of 1:4), 20 kV separation voltage, 10 mbar pressure applied at 20 min.

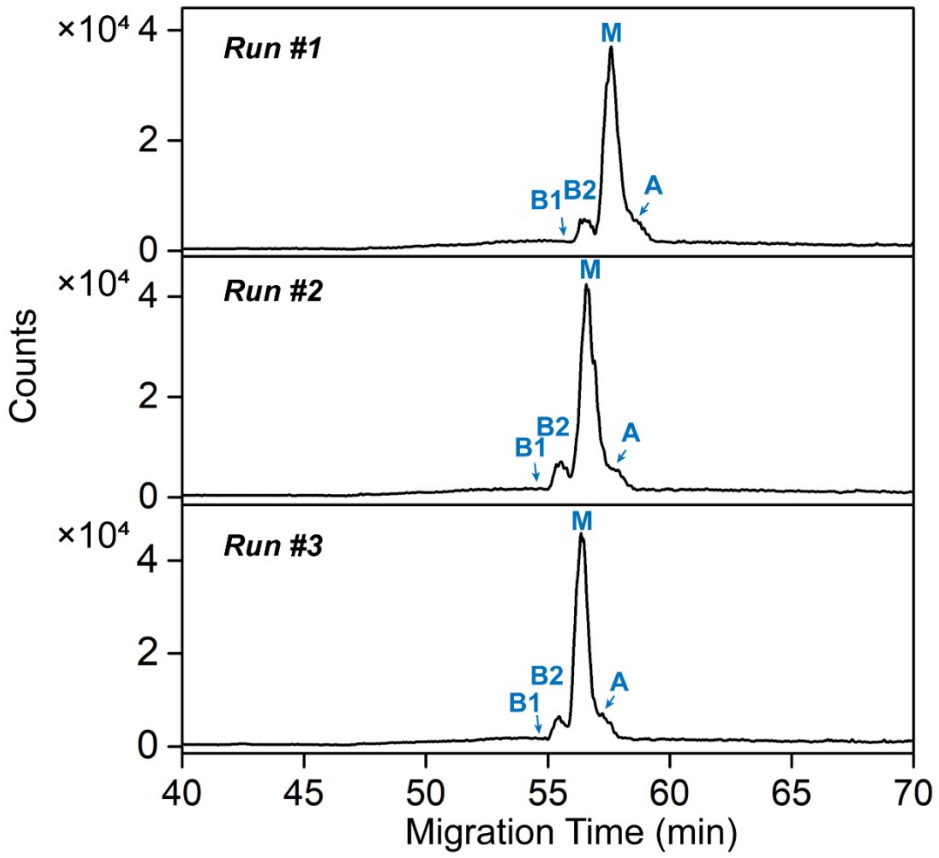

Figure S3. Triplicate cIEF-MS runs of NISTmAb with a pH 10.0 catholyte and an LPA-coated capillary (75 cm). Other parameters for cIEF separation were 30 cm catholyte plug, 45 cm sample plug, 0.2 mg/mL sample concentration, 1.5% ampholyte mixture (pH range 3-10 and 8-10.5 with ratio of 1:4), 20 kV separation voltage, 10 mbar pressure applied at 20 min.

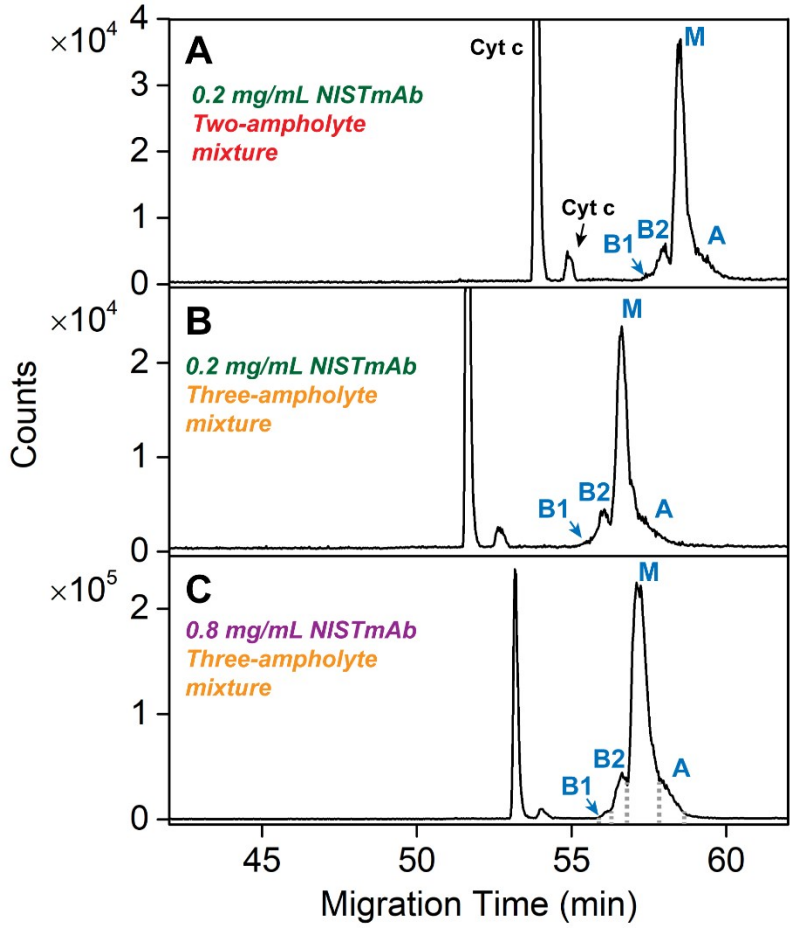

Figure S4. (A, B) Comparison of cIEF separation of NISTmAb between using a two-ampholyte mixture and a three-ampholyte mixture. The two-ampholyte mixture contains 1.5% ampholytes with pH range of 3-10 and 8-10.5 (ratio 1:4). The three-ampholyte mixture comprises 2% ampholytes with pH range of 3-10, 5-8 and 8-10.5 (ratio: 1:2:4). (B, C) Comparison of cIEF separation and MS signal of NISTmAb between using 0.2 mg/mL and 0.8 mg/mL sample concentration. Other parameters for separation: 75 cm LPA-coated capillary, 30 cm catholyte plug, 45 cm sample plug, 0.05 mg/mL cytochrome c, 20 kV separation voltage, 10 mbar pressure applied at 20 min.

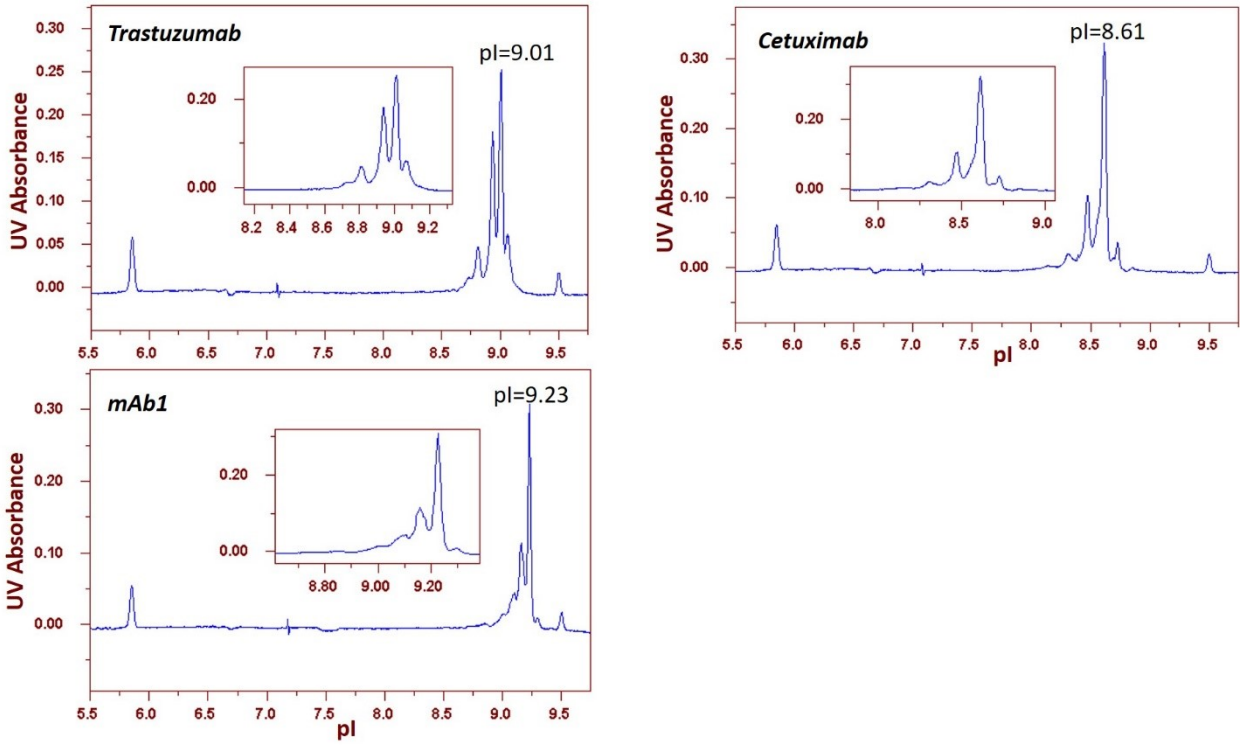

Figure S5. icIEF-UV data of three mAbs (trastuzumab, cetuximab, and mAb1). Method conditions are as follows. Cartridge: FC-Coated cIEF Capillary Cartridge, ProteinSimple Catalog No. 101701. Pharmalyte: Pharmalyte 3-10. Final prepared sample: 0.8 M Urea, 0.28% methyl cellulose, 4% carrier ampholytes, 0.2% pI 5.85 marker, 0.2% pI 9.50 marker, ~1.0 mg/mL protein. Focusing period 1: 1500V for 1 min; Focusing period 2: 3000V for 8 min. UV detection wavelength: 280 nm. System: iCE3 (ProteinSimple). All the reagent except Urea were purchased from ProteinSimple. Urea was bought from Sigma.

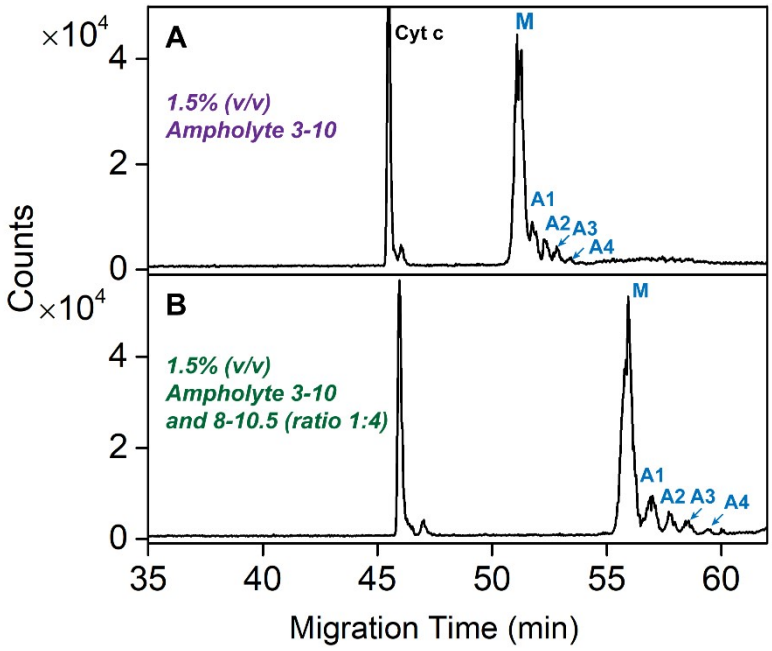

Figure S6. Comparison of cIEF separation of cetuximab and cytochrome c between using a single ampholyte (1.5%, pH range of 3-10) (A) and a two-ampholyte mixture (1.5%, pH range of 3-10 and 8-10.5, ratio: 1: 4) (B). Other parameters for separation: 75 cm LPA-coated capillary, 30 cm catholyte plug, 45 cm sample plug, 0.2 mg/mL cetuximab, 20 kV separation voltage, 10 mbar pressure applied at 20 min.

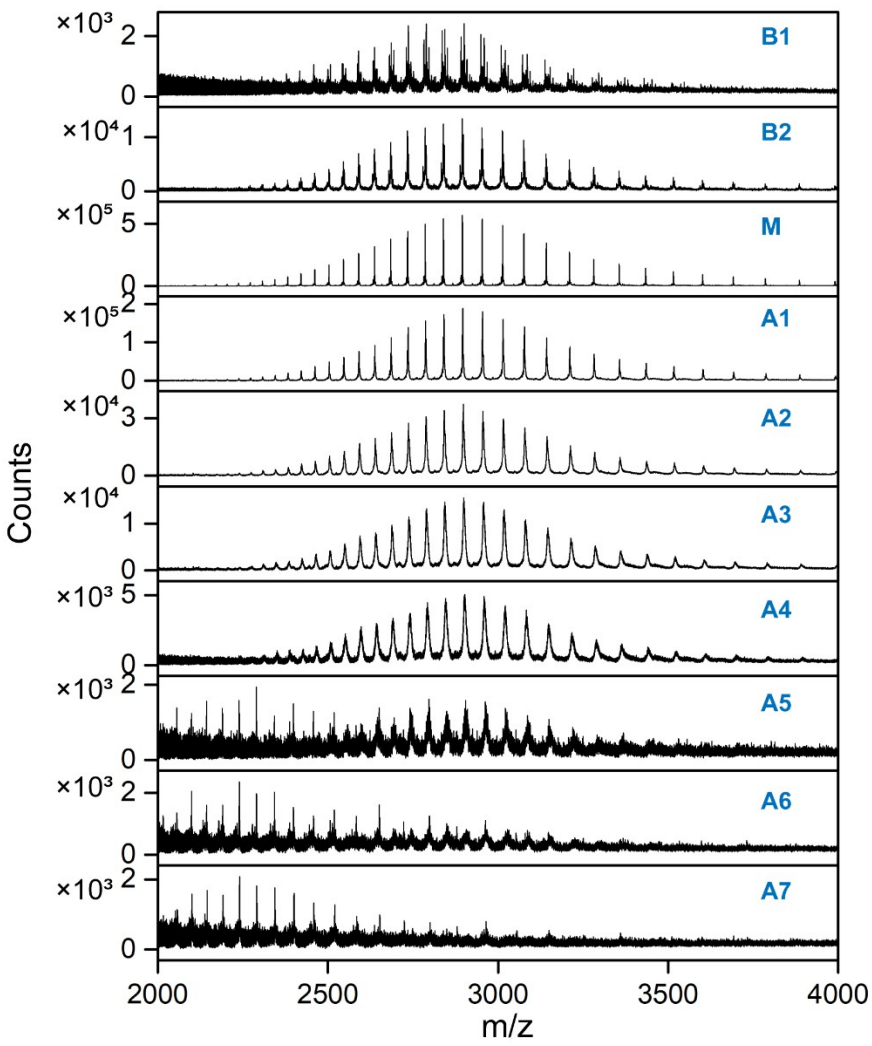

Figure S7. Mass spectra of ten charge variants of mAb1.