Intrinsic charge ladders of a monoclonal antibody in hydroxypropylcellulose-coated capillaries

Rohan D. Sanzgiri, Tracy A. McKinnon and Brian T. Cooper*

Electronic Supplementary Information: includes a scan of a pH 5–8 IEF gel showing mAb p*I* and charge heterogeneity, charge ladder electropherograms in pH 8.5 borate and Tris (as per Figs. 2–3), a tabular summary of our electrophoretic efficiency data, and chromatograms in pH 8.5 borate and pH 9.0 and 8.5 Tris (as per Fig. 4).

Antibody microheterogeneity

Fig. S1 shows the intrinsic charge heterogeneity of the mAb as revealed by isoelectric focusing, using the Bio-Rad apparatus, precast gels (pH 5–8), and reagents as described in the Experimental section.

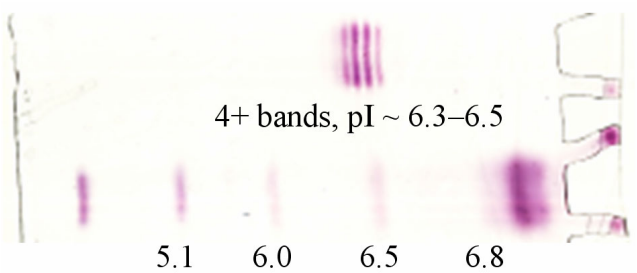

Fig. S1 IEF gel showing the p*I* and intrinsic charge heterogeneity of the model mAb. The peak distribution is essentially identical to that observed by CZE in HPC coated capillaries (Figs. 2–3 and S2–S3).

Figs. S2 and S3 are the pH 8.5 analogues of Fig. 2 (borate) and Fig. 3 (Tris). Despite the broader peaks, baseline artifacts, and significantly reduced sensitivity in the less-transparent Tris buffers, the electropherograms were reproducible enough to make us confident in our mAb peak assignments.

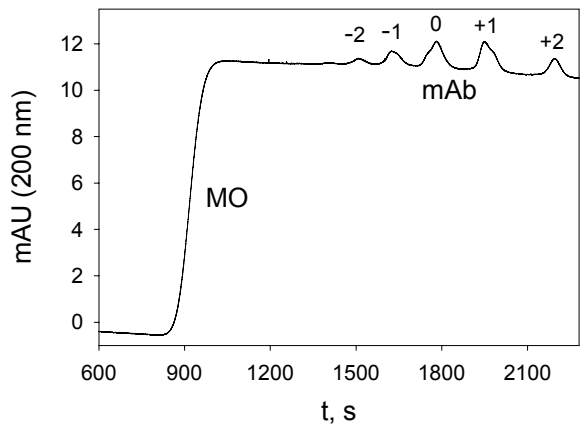

Fig. S2 Resolution of mAb charge variants in borate with simultaneous EOF measurement. Run buffer: 25 mM borate, pH 8.5; 25 °C. Sample: mAb stock diluted 10× into run buffer; electrokinetic injection for 60 s at the full −20 kV run voltage. EOF marker: ~0.02% MO in run buffer in destination vial. Run current: 5.0–4.6 μA from start to end. (Compare to pH 9.0 borate run in Fig. 2.)

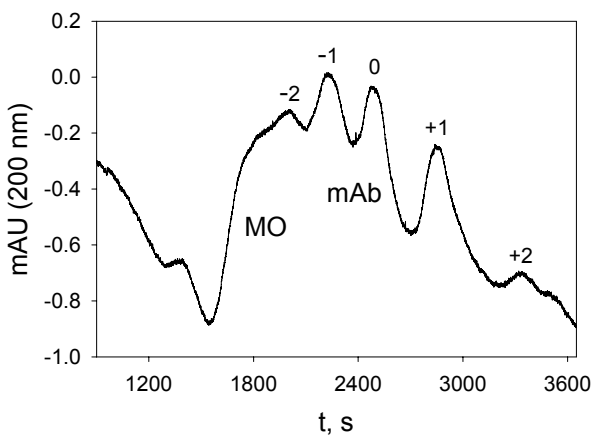

Fig. S3 Resolution of mAb charge variants in Tris with simultaneous EOF measurement. Run buffer: 25 mM Tris, pH 8.5; 25 °C. Sample: mAb stock diluted 10× into run buffer; electrokinetic injection for 80 s at the full −20 kV run voltage. EOF marker: ~0.01% MO in run buffer in destination vial. Run current: 7.1–6.4 μA from start to end. (Compare to pH 9.0 Tris run in Fig. 3.)

* *Department of Chemistry, University of North Carolina at Charlotte, 9201 University City Blvd., Charlotte, NC 28223-0001, USA. E-mail: btcooper@email.uncc.edu; Fax: +1-(704) 687-3151; Tel: +1-(704) 687-2526*

Electrophoretic efficiency and resolution

Table S1 summarizes our electrophoretic efficiency data for each buffer. These are the same numbers discussed in the paper, just presented here in tabular form for convenience.

Table S1 Electrophoretic efficiency[a] versus buffer conditions

Buffer (25 mM)	*N* (uncoated capillaries)	*N* (HPC-coated capillaries)
pH 9.0 Borate	8000 ± 1300	16 000 ± 1500
pH 8.5 Borate	3000 ± 600	10 000 ± 2200
pH 9.0 Tris	3000 ± 500	4 000 ± 400
pH 8.5 Tris	4000 ± 900	5 000 ± 500

[a] In uncoated capillaries, for the unresolved mixture of mAb charge variants. In coated capillaries, for the resolved "+2" variant.

Figs. S4–S6 (analogous to Fig. 4) demonstrate that chromatographic retention of the mAb by the HPC coating could *not* be detected in any of the four buffers tested. In all cases, a wider protein peak overlapped a narrower, slightly retarded azide peak. The relative size of the azide component in Fig. S6 is smaller than in the other runs because of the way the sample was prepared in that case.

As shown by the overlap of the red and heavy black lines, fits of the composite peak profile to a sum of two Gaussians were excellent. The fit in Fig. S5 was less good than the others because the injection time was too long relative to the observed band dispersion, thus flattening the Gaussian peaks.

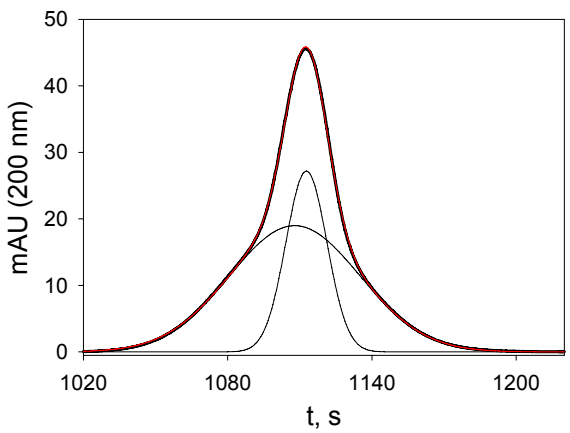

Fig. S4 Testing for chromatographic retention in HPC-coated capillaries. Run buffer: 25 mM borate, pH 8.5; 25 °C. Sample: mAb stock (includes 0.1% sodium azide) diluted 10× into run buffer; pressure injection for 2 s at ~4 psig. Gravity pumping by raising inlet reservoir level ~20 cm above outlet. Heavy black line: baseline-subtracted absorbance signal. Thin black lines: best-fit Gaussians for mAb (wide curve: t_R = 1107.9 s, σ = 27.0 s, N = 1700) and azide (narrow curve: t_R = 1112.8 s, σ = 8.53 s, N = 17 000). Red line: sum of the best-fit curves, which overlays the data almost perfectly. (Compare to pH 9.0 borate run in Fig. 4.)

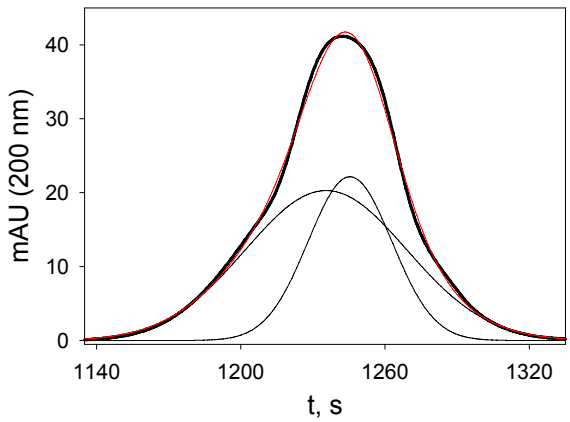

Fig. S5 Testing for chromatographic retention in HPC-coated capillaries. Run buffer: 25 mM Tris, pH 9.0; 25 °C. Sample: mAb stock (includes 0.1% sodium azide) diluted 10× into run buffer; pressure injection for 2.5 s at ~4 psig. Gravity pumping by raising inlet reservoir level ~20 cm above outlet. Heavy black line: baseline-subtracted absorbance signal. Thin black lines: Gaussian fits for mAb (wide curve: t_R = 1235.5 s, σ = 33.5 s, N = 1400) and azide (narrow curve: t_R = 1245.4 s, σ = 17.4 s, N = 5100). Red line: sum of the best-fit curves. The fit is not as good as the other runs because the injection was a little too long, making the actual peaks flatter than pure Gaussians. (Compare to pH 9.0 borate run in Fig. 4.)

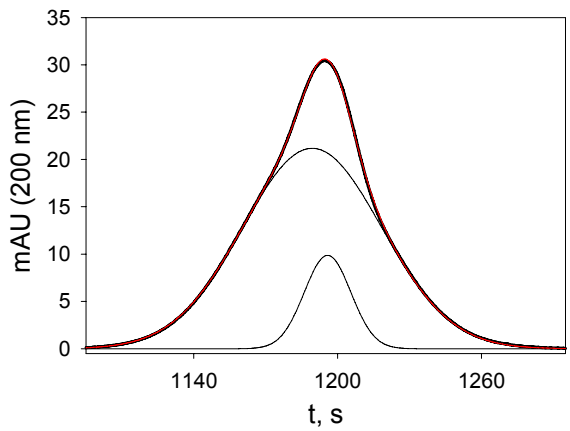

Fig. S6 Testing for chromatographic retention in HPC-coated capillaries. Run buffer: 25 mM Tris, pH 8.5; 25 °C. Sample: mAb stock (includes 0.1% sodium azide) diluted 10× into run buffer then concentrated to ~5× (relative to the original stock) using a spin filter and redilution; pressure injection for 1.5 s at ~4 psig. Gravity pumping by raising inlet reservoir level ~20 cm above outlet. Heavy black line: baseline-subtracted absorbance signal. Thin black lines: best-fit Gaussians for mAb (wide curve: t_R = 1189.4 s, σ = 29.0 s, N = 1700) and azide (narrow curve: t_R = 1195.8 s, σ = 9.70 s, N = 15 000). Red line: sum of the best-fit curves, which overlays the data almost perfectly. (Compare to pH 9.0 borate run in Fig. 4.)