Supporting Information

Femtomolar Concentration Detection Limit and Zeptomole Mass Detection Limit for Protein Separation by Capillary Isoelectric Focusing and Laser-induced Fluorescence Detection

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Laser power optimization. The photostability of Chromeo P540 has not been reported previously. We continuously infused a solution of 1.3-nM labeled β-lactoglobulin in a CHES-Tris SDS buffer (100-mM, 100 mM, 3 mM) and measured the fluorescence signal as a function of laser power, which was adjusted by means of a polarizing filter. We fit the photobleaching signal to a simple three-level saturation model.

$$I(P) = \frac{I_{\max} P}{P_{PhotoBleach} + P} \qquad \text{Eq. S1}$$

where *I(P)* is the observed fluorescence intensity, P is the laser power, I_{max} is the asymptotic signal at high laser power, and $P_{photobleach}$ is the power necessary to generate a signal that is one-half the maximum value. The results of the study are shown in figure S1. The saturation behavior

was similar to Chromeo P503 labeled proteins;[s1] 3-mW of laser power generated a signal one-half of the limiting signal.

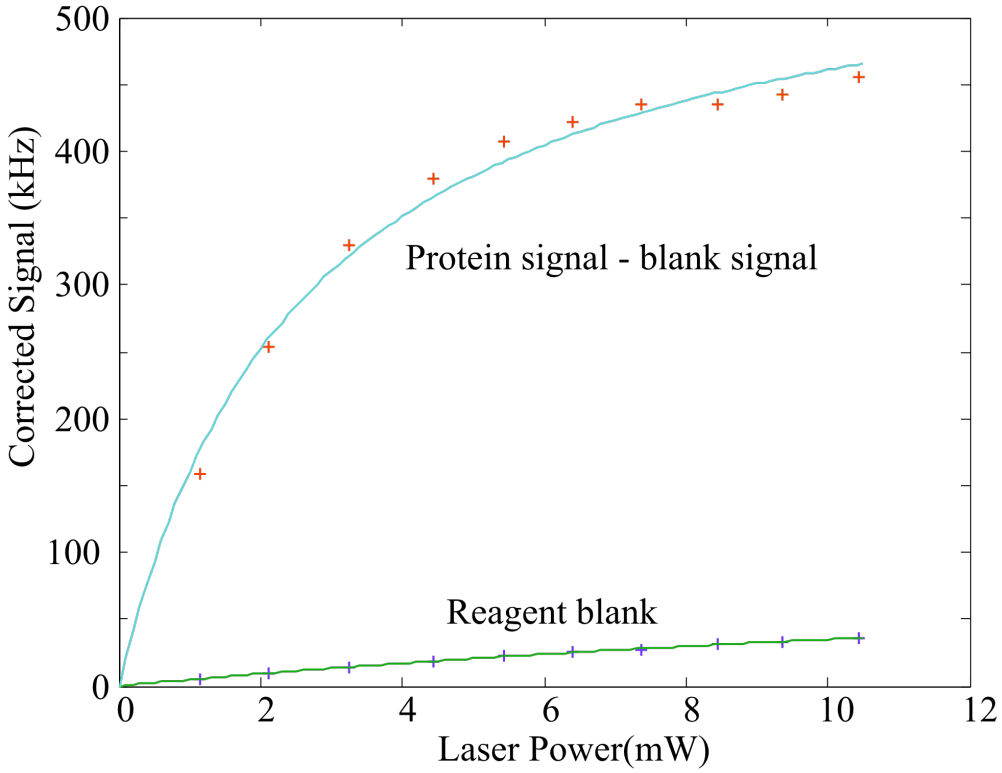

Figure S1. Photobleaching curve for Chromeo P540-labeled β-lactoglobulin. A 7 nM solution of labeled β-lactoglobulin was prepared in a CHES:Tris:SDS (100 mM:100mM:3.5mM) separation buffer. Solutions were continuously infused at a voltage of 15 kV into a 30-µm ID, 150 µm OD, 23 cm long capillary. The bottom curve was generated from a reagent blank while the top curve was generated by subtracting the blank signal from the labeled protein signal. Laser power was adjusted by means of a polarizing filter. Crosses are the data and the smooth curves are the result of regression analysis using equation S1.

Performance of Chromeo P540-labeled β-lactoglobulin. To validate the sensitivity of the Py 540 dye and insure the detection limits were similar to the previously reported Py503 dye, capillary zone electrophoresis experiments were performed. β-lactoglobulin was labeled with Py-540 and diluted in a CHES:Tris:SDS (100 mM:100mM:3.5mM) separation buffer. A solution was injected at 1 kV for 1 s and separated at 20 kV in a 30-µm ID, 150 µm OD, 25 cm long capillary, figure S2. The data were passed through a 3 point median filter and convoluted with a Gaussian function with 0.25 s standard deviation. Noise was estimated as the standard deviation of the signal between 165 and 185 s. Detection limits (3 s) were 2.6 ± 0.3 (n = 2) zmole. The separation efficiency was 200,000 theoretical plates.

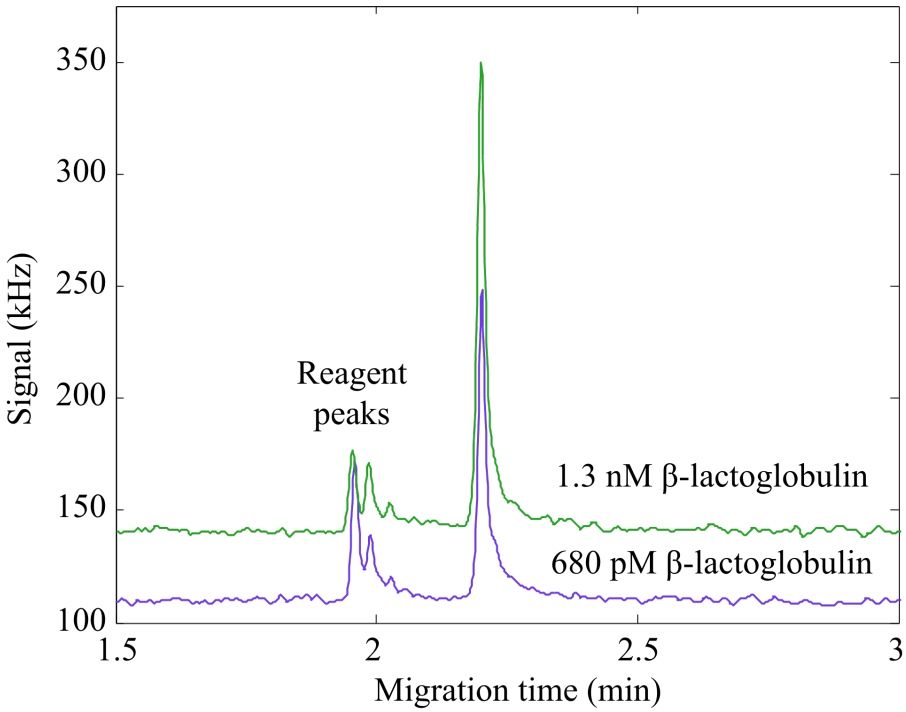

Figure S2. Capillary zone electrophoresis of 1.3 nM (top) and 680 pM (bottom) β-lactoglobulin.

Separation of standard proteins. Two standard proteins were separated using capillary isoelectric focusing with both untreated and photobleached ampholytes, figure S3. β-lactoglobulin generated sharp peaks, whereas ovalbumin generated a much broader set of peak.

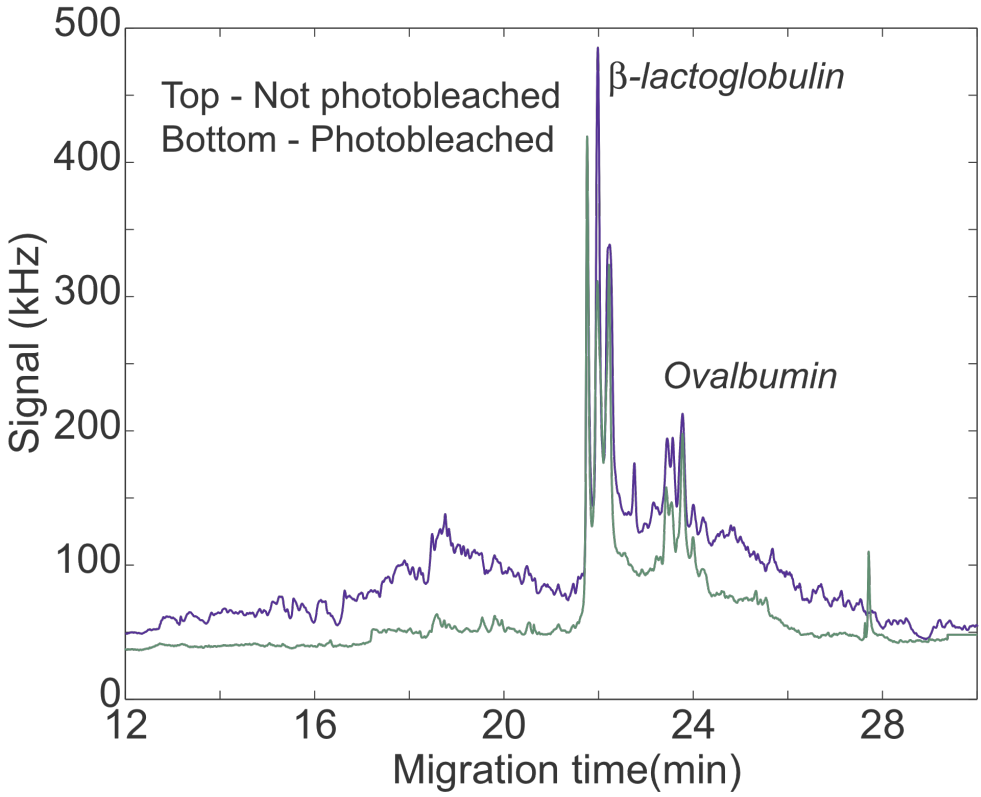

Figure S3. Separation of Chromeo P540-labeled β-lactoglobulin (400 pM) and ovalbumin (3 nM) by capillary isoelectric focusing in an acrylamide coated capillary with pH 3-10 ampholytes. The top curve was generated with untreated ampholytes and the bottom curve was generated with photobleached ampholytes. The data are not offset.

Ampholyte photobleaching. Figure S4 presents the results of photobleaching the pH 3-10 ampholytes. The capillary length was 28 cm coated with Guarant (id 50 micrometers, od 150

micrometers). Five hours illumination was required to reduce the background to negligible levels.

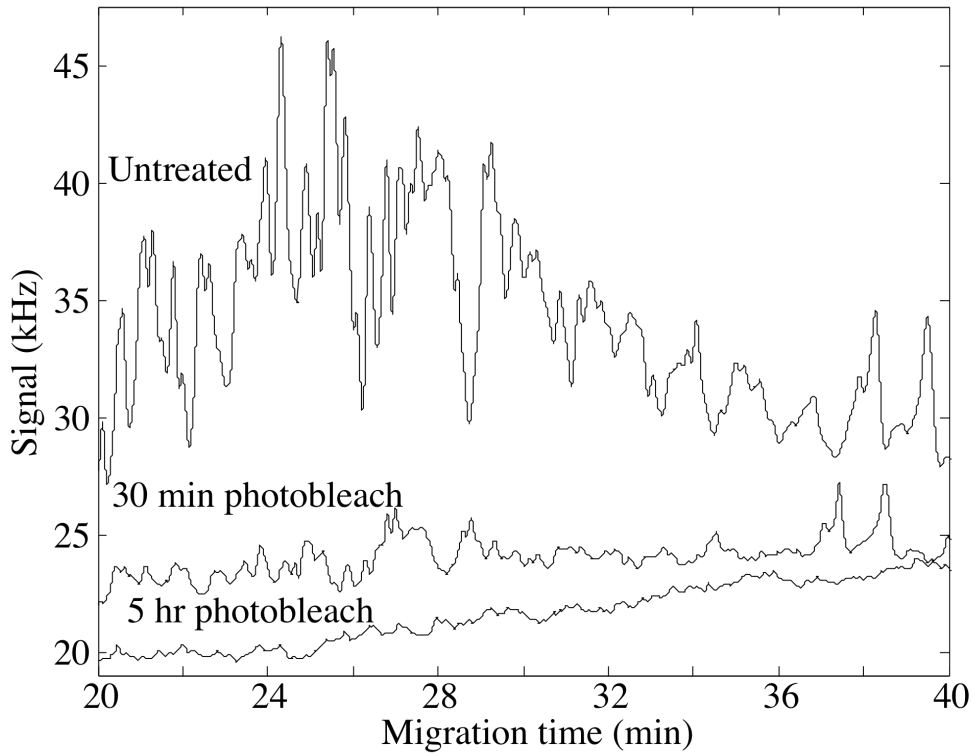

Figure S4. Electropherograms of pH 3-10 ampholyte blanks. The top curve is the untreated blank, the middle curve is after 30 minutes of photobleaching, and the bottom trace is after 5 hours of photobleaching. Electrophoresis was performed in a Gaurant-coated capillary. Data not offset.

Ampholyte Tanning Booth. A 9 by 9 by 13 cm box with 1 by 1 by 4 cm slot for a cuvette holder was made at the electronic shop in the University of Washington Chemistry Department.

The box consisted of two Atlas Series high intensity LED lights (Lamina, Westampton, NJ, USA) with a wavelength min/max of 515/535 and typical power output of 4.8 W. Each LED light was connected to 1" diameter heatsink (Lamina) and AFB brushless fan to control the temperature (Delta). Finally all features were connected to a 700 mA BuckPuck LED power supply (LED Supply).

References

S1 - Ramsay, L.; Dickerson. J.; Dovichi N. J. *Electrophoresis* **2008**, *in press*.