Electronic Supplementary Material (ESI) for Analyst.
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Supporting Information

Narrow open tubular column for high efficiency liquid chromatographic separation

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Assembling of apparatus. Briefly, the 488-nm laser beam was produced by an argon ion laser (LaserPhysics, Salt Lake City, UT), directed by a dichroic mirror (Q505LP, Chroma Technology, Rockingham VT) and focused onto the detection window of the narrow capillary via an objective lens (20×and 0.5 NA, Rolyn Optics, Covina, CA). The emitted fluorescence was collimated by the same lens, went through the same dichroic mirror, an interference band-pass filter (532 nm, Carlsbad, CA) and a 1-mm pinhole, and finally collected by a photosensor module (H5784-04, Hamamatsu). A measurement computing USB-1208FS (Measurement Computing, Norton, MA) was used to measure the output of the photosensor module. The data were acquired and analyzed using a program written in-laboratory with labview (National Instruments, Austin, TX). The alignment of the detection window with the LIF detector was achieved via an x-y-z translation stage.

Flow Rate Measurement. We filled a short plug of mineral oil in a 60-cm-long and 20-μm-i.d. capillary and attached this capillary to the end of the OTLC column via a union. An air bubble was allowed to be present between the solution out of the OTLC column and the mineral oil in the 20-μm-i.d. capillary. The same apparatus as shown in Figure 1 was used for this experiment and a stereo microscope was used to monitor the bubble movement. Figure S7 presents the measurement results.

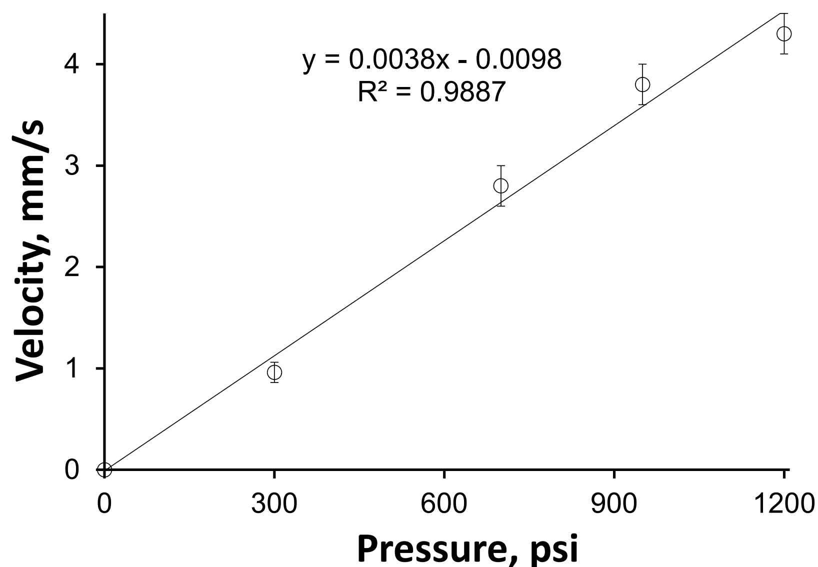

Figure S1. Flow rate measurement results.

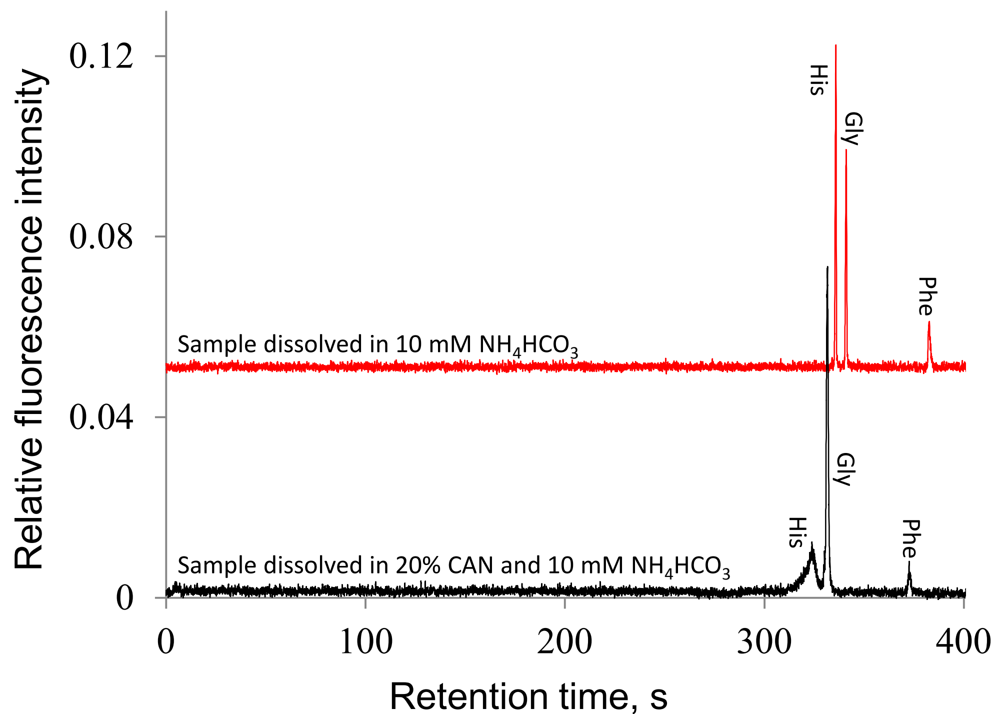

Figure S2. Comparison between isocratic runs. A separation column of 2-µm-i.d had an effective length of 40 cm. Mobile phase was 20% CAN and 10 mM NH_4HCO_3 in DDI water. Injection volume was ca. 7.1 pL. Sample contained histidine (His), glycine (Gly), and phenylalanine (Phe); each at 10 µM. An elution pressure of 600 psi was applied for the 2-µm-i.d.-capillary experiment and 100 psi for the 5-µm-i.d.-capillary experiment.

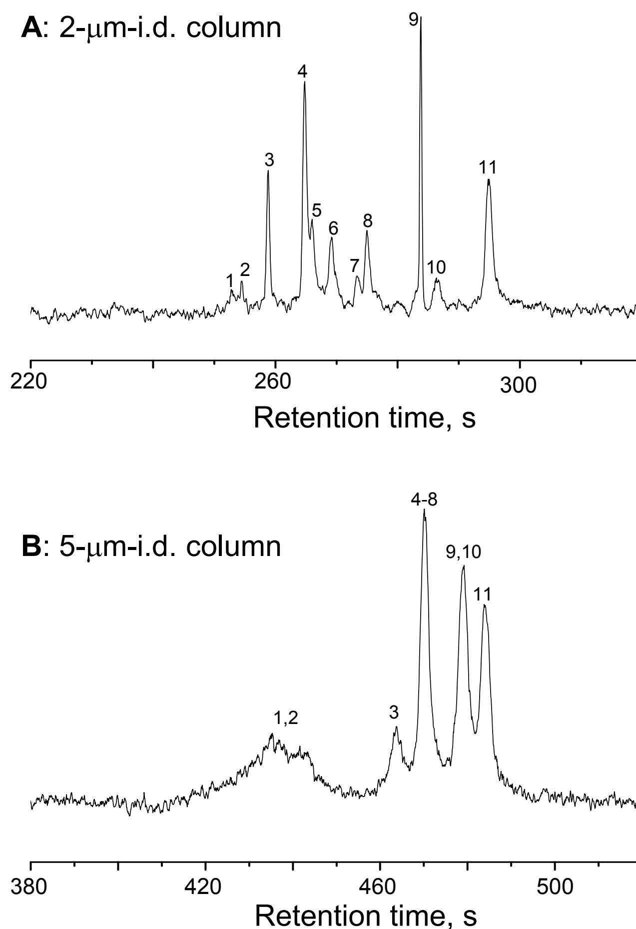

Figure S3. Effect of inner diameter on resolution. A separation column of 5-µm-i.d. or 2-µm-i.d had a total length of 48 cm (44 cm effective), and an o.d. of 150-µm. Mobile phase A was 10 mM NH_4HCO_3in DDI water and mobile phase B was acetonitrile. Isocratic elution was carried out using 20% mobile phase B and 80% mobile phase A. Injection volume was ca. 7.1 pL. Sample contained histidine (1), asparagine (2), glycine (3), tyrosine (4), arginine (5), alanine (6), tryptophan (7), valine (8), isoleucine (9), phenylalanine(10), and leucine(11); each at 6.5 µM for the 2-µm-i.d.-capillary experiment and 0.3 µM for the 5-µm-i.d.-capillary experiment. An elution pressure of 600 psi was applied for the 2-µm-i.d.-capillary experiment and 100 psi for the 5-µm-i.d.-capillary experiment.

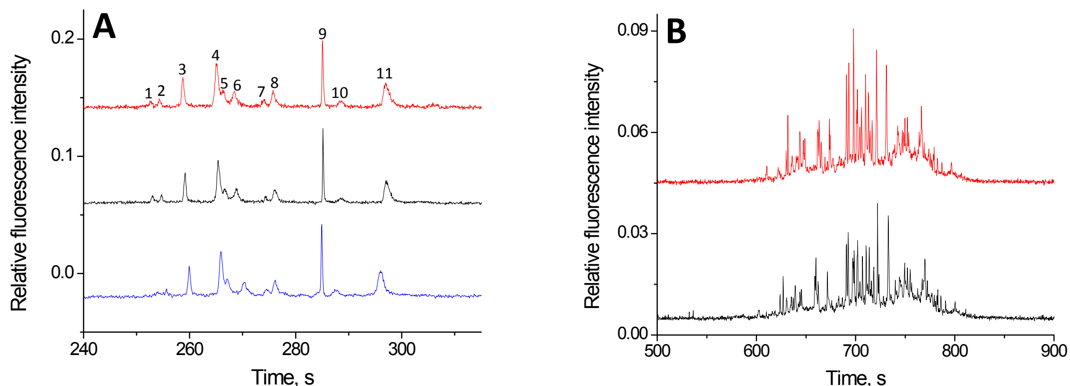

Figure S4. Repetitive test. (A) Chromatograms of repetitive isocratic separations of 11 amino acids. The separation column had a total length of 48 cm (44 cm effective), an o.d. of 150 µm and an i.d. of 2 µm. 10 mM NH_4HCO_3 in DDI water was used as mobile phase A, and acetonitrile was used as mobile phase B. Isocratic elution was performed using 20% mobile phase B and 80% mobile phase A. The external loop of the injection valve had a volume of 2 µL. The sample was a mixture of histidine (1), asparagine (2), glycine (3), tyrosine (4), arginine (5), alanine (6), tryptophan (7), valine (8), isoleucine (9), phenylalanine (10) and leucine (11). (B) Chromatograms of repetitive gradient separations of BSA digest. Gradient profile was mobile phase B increasing from 0 to 82.5% from 0 to 10 min. Sample was trypsin-digested BSA, each at 0.62 mg/mL. Elution pressure was 675 psi. All other conditions were the same as in (A).

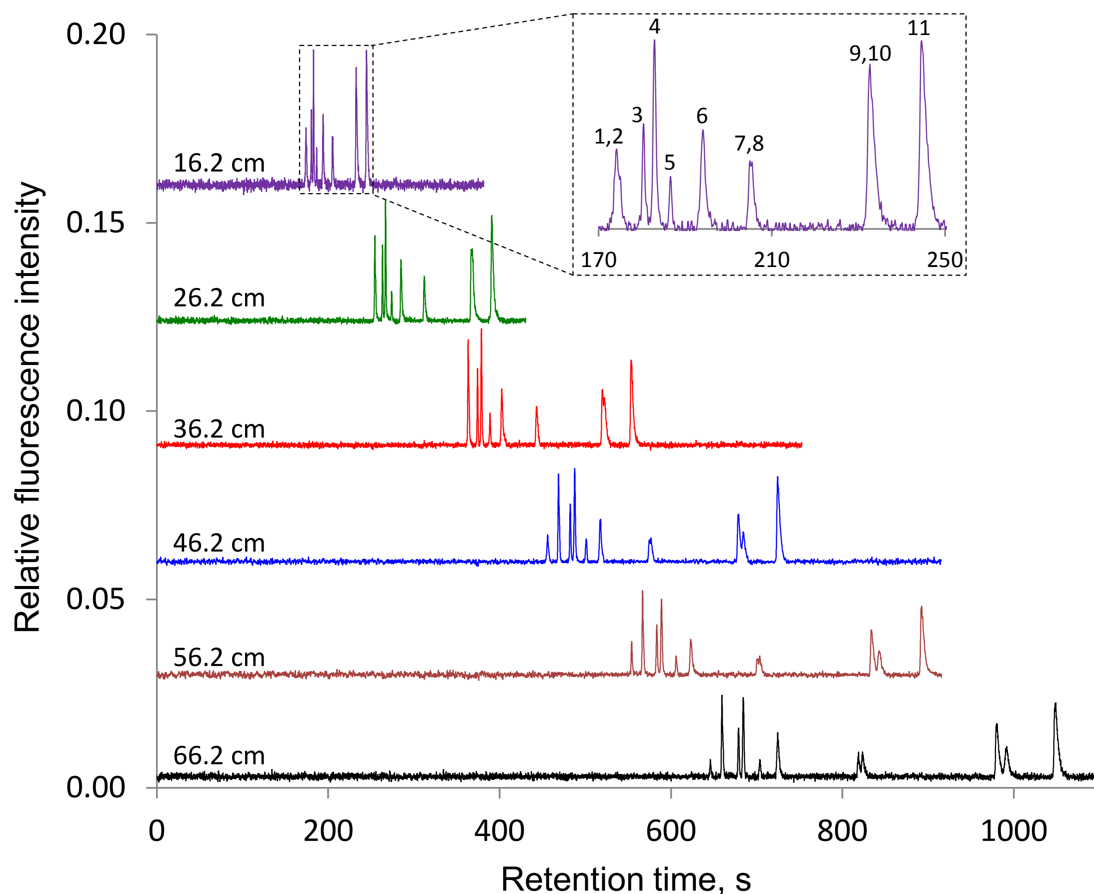

Figure S5. Effect of column length on resolution. Separation column had a total length of 75 cm and an i.d. of 2 µm. The effective lengths were marked on the corresponding chromatograms. Mobile phase A was 10 mM NH_4HCO_3 in DDI water and mobile phase B was acetonitrile. Isocratic elution was carried out using 20% mobile phase B and 80% mobile phase A under an elution pressure of ca. 600 psi. Injection volume was ca. 7.1 pL. Sample contained histidine (1), asparagine (2), glycine (3), tyrosine (4), arginine (5), alanine (6), tryptophan (7), valine (8), isoleucine (9), phenylalanine(10), and leucine(11); each at 6.5 µM.

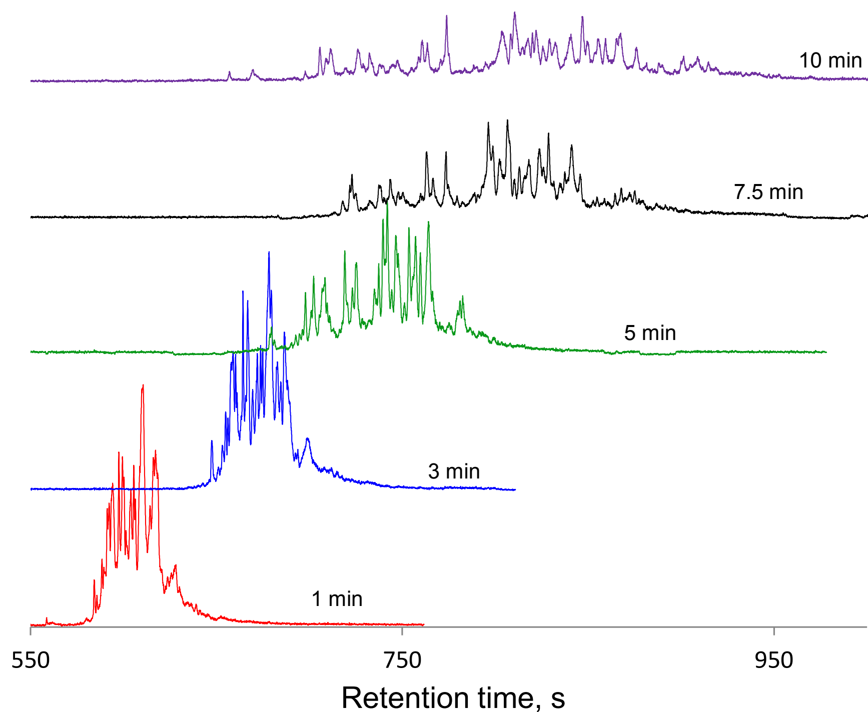

Figure S6. Effect of gradient profile on resolution. Separation column had a total length of 48 cm (44 cm effective), 150-µm o.d. and 2-µm i.d.. Mobile phase A was 10 mM NH_4HCO_3 in DDI water and mobile phase B was acetonitrile. All elutions started with 0% and ended with 82.5% mobile phase B in a time as indicated for each chromatogram under an elution pressure of ca. 600 psi. Injection volume was ca. 7.1 pL. Sample was trypsin-digested BSA at a concentration of 0.62 mg/mL.

Table S1. Typical plate numbers

Peak No.[a]	Efficiency-measured[b]
1	3.3×10^6
2	2.3×10^6
3	3.9×10^6
9	1.1×10^7
10	1.6×10^6
11	8.5×10^5

[a]See Figure 2 for peak identification. [b]The bottom chromatogram in Figure 2A was for these efficiency measurements.