Supplementary Material (ESI) for Chemical Communications

Separation of denatured proteins in free solution based on differential binding of alkyl sulfates with different carbon chain lengths

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Microchip Electrophoresis:

The microchips were fabricated on Pyrex 7740 wafers using photolithography, plasma etching, and thermal bonding techniques. The design of the microchip is shown in Figure S1. The microchannels had a depth of 3.8 μm and a half-depth width of 26 μm. The serpentine separation channel had a total length of 12 cm. The channel width at the turns was one half of the straight channel. The structure was previously reported for the minimization of geometric dispersion of analyte bands [1,2]. The microfluidic chip was used with uncoated channel walls and conditioned by flowing water and then 0.1 M NaOH for 20 minutes each before the first use. The running buffer was flowed for 20 minutes each time before the labeled protein solution was loaded. After the experiment, the microchip was cleaned by running water, 0.1 M NaOH and then water again, each for 20 minutes. The microchip was stored with water in the channel and the reservoirs sealed with parafilm. A plug of labeled protein mixture was injected at the cross of the microfluidic channels using a gated injection scheme previously described [3,4]. The protein bands were detected with laser-induced fluorescence. An inverted microscope (Olympus, IX 70) was used in all the experiments. An air-cooled argon ion laser provided the excitation at 488 nm. Fluorescence was collected by a 60x, 0.70 NA microscope objective, filtered with a 615DF45 bandpass filter (Omega Optical, Brattleboro, VT) to remove scattered light. The light was imaged onto a photomultiplier tube (R1477, Hamamatsu, Bridgewater, NJ) biased at 1.2 kV. The resulting signal was amplified by a low-noise preamplifier (SR 560, Stanford Research Systems, Sunnyvale, CA) and digitized by an I/O board (PCI-MIO-16E-4, National Instruments). Software written in LabVIEW controlled the injection time and plotted the data. The microchip generated highly reproducible data. Figure S2 shows that the migration times of the peaks had a reproducibility with a smaller than 0.5% relative standard deviation during consecutive 11 runs. There was no sign of serious adsorption of the proteins to the microchannel walls.

References

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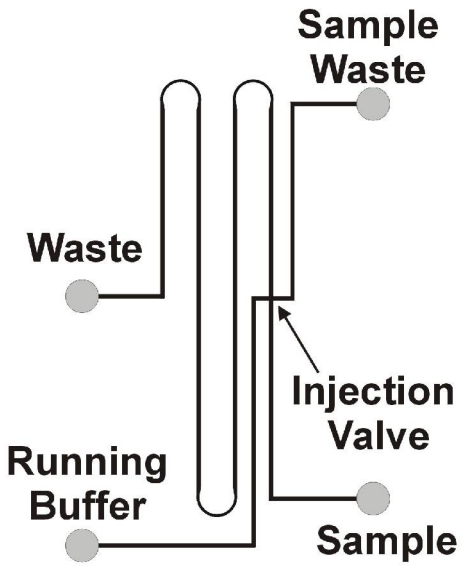

Figure S1. The layout of the microchip

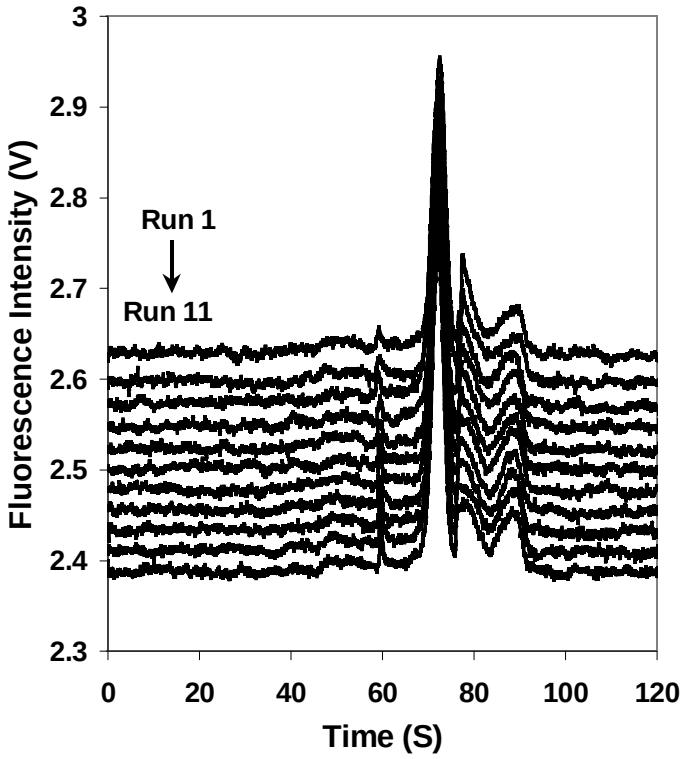

Figure S2. Free solution electrophoresis of denatured proteins on a microchip. The electrophoresis was done with 500V/cm and 3s injection time. The proteins were denatured and labeled according to the protocol in the main text. The peaks were assigned as follows: β-Galactosidase (60s); Conalbumin (73s); α-Lactalbumin (78s); Ovalbumin (90s). The relative standard deviations of the migration times from 11 runs were within 0.5% (0.37% (60s), 0.28%(73s), 0.27%(78s), 0.43%(90s)).