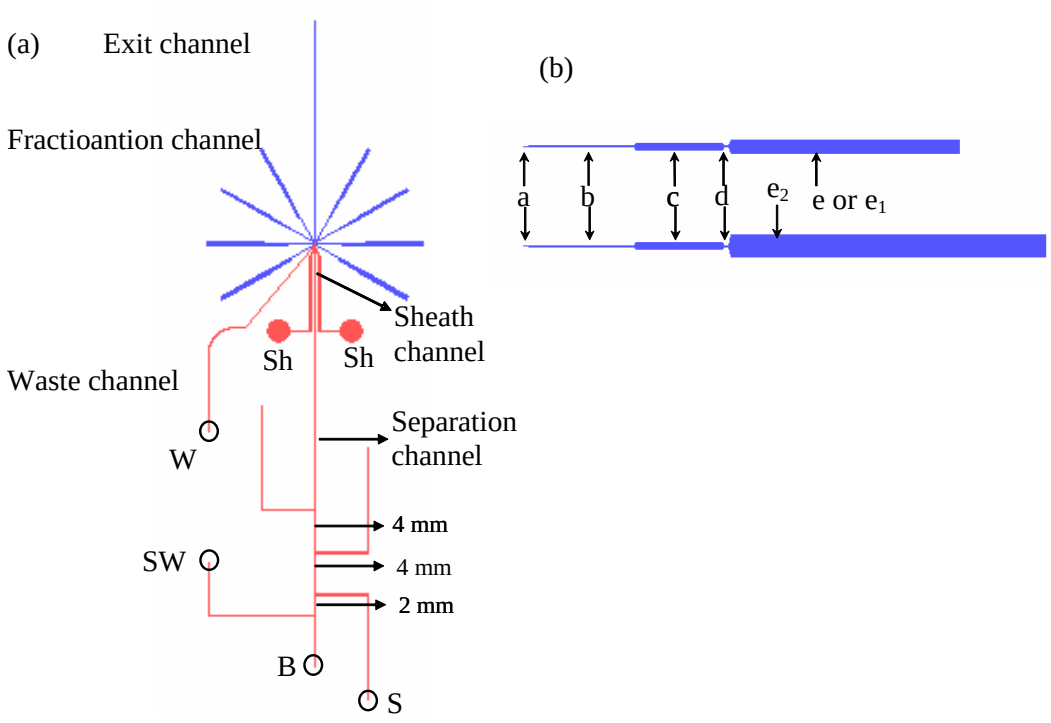
Supplementary Information

1. Detailed Dimension of the 6 and 8-channel devices

Detailed dimension of the devices used in the study are summarized in supplementary Table S1. The detailed structure of the 8-channel fractionation device is shown in Supplementary Figure S1, as an example. Each device comprises a separation channel with three or four sample injection arms, two sheath channels surrounding the separation channel as it enters the fractionation region, a waste channel connected to the bottom of the fractionation region to collect unwanted components during fractionation, six or eight collection channels evenly distributed on both sides of the fractionation zone for collecting the purified proteins, an exit channel at the end of the fractionation zone, through which analytes adsorbed on the SPE beds can be eluted and subjected to ESI-MS analysis. The injection element consists of three or four channels entering into the main separation channel to form different offsets, allowing geometric definition of various sample plug volumes. The waste channel is 10 μm wide and 25 mm long. Fraction collection channels are evenly distributed along the fractionation zone with a distance of 150 μm from center to center on one side. The five segements of the collection channels are marked a-e in Figure S1b. Segment “a” is a short and narrow channel used to connect the fractionation zone with the main part of the collection channel. Segment “b” is slightly wider than segment a, and is the main source of electrical impedance of the collection channel. Segment “c” is designed as an SPE bed structure with tapered geometries at both ends, where the hydrophobic monolith is prepared. Segment “d” is a small section of narrow channel used to define the bed structure. In Segment “e”, the channel is widened to 300 μm or 500 μm to lower the impedance of the collection channel. In the 6 channel device, two types of collection channels with different lengths (1.0 cm or 1.3 cm) are designed to provide enough space for drilling access holes and attaching reservoirs.

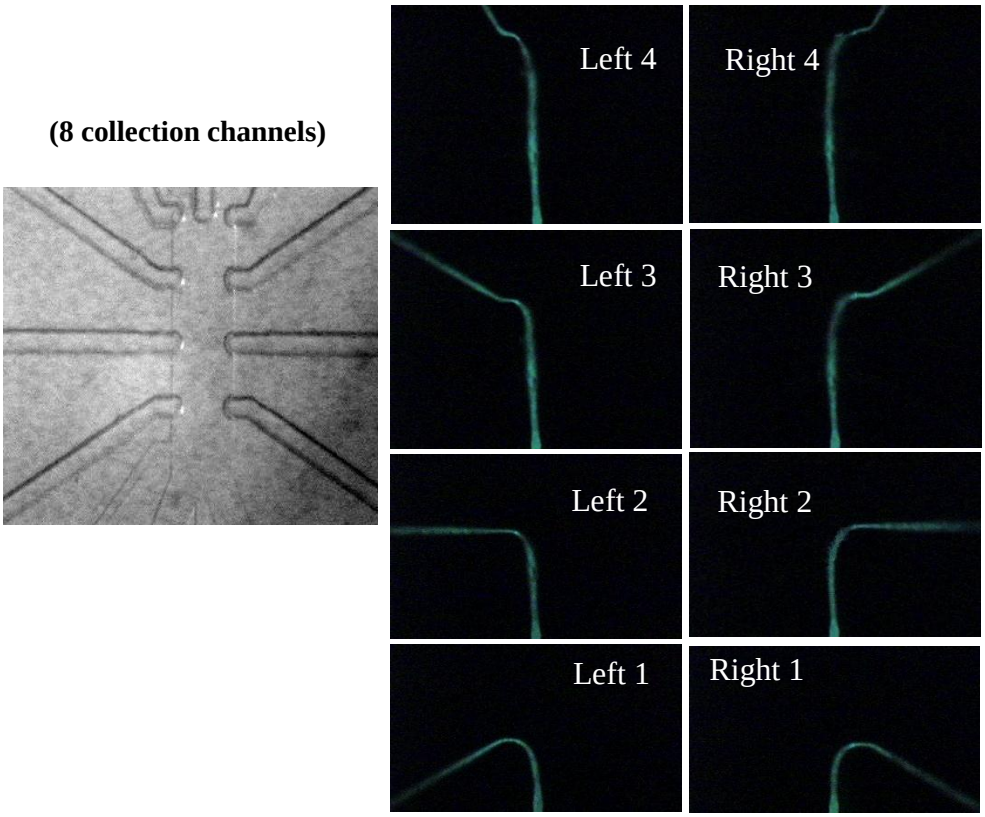
Table S1. Detailed mask dimensions of the 6 and 8 collection channel devices.

Device type	Fractionation Zone (μm)	Sheath channel	Collection channel		Sample channel	Exit channel	Injection arms
8 collection channel device	w =162 l =650	w =78 l =10 mm	Segment a w =10 μm l =100 μm	Segment b w =16 μm l =2.5 mm	W=20 μm l= 40 mm	w =16 μm l=17.5mm	w = 20 μm l =15 mm l_{offset} = 4 or 2 mm
			Segment c w =160 μm l =2 mm	Segment d w =16 μm l =100 μm			
			Segment e w =300 μm l =5.3 mm				
6 collection channel device	w =162 l = 500	w =78 μm l =11 mm	Segment a w =10 μm l =100 μm	Segment b w =16 μm l =2.5 mm	w=20 μm l =30 mm	w=16 μm l=12.5mm	w = 20 μm l=12.5 mm l_{offset} = 2 mm
			Segment c w =160 μm l =2 mm	Segment d w =16 μm l =100 μm			
			Segment e_1 w =300 μm l =5.3 mm	Segment e_2 w =500 μm l =8.5 mm			



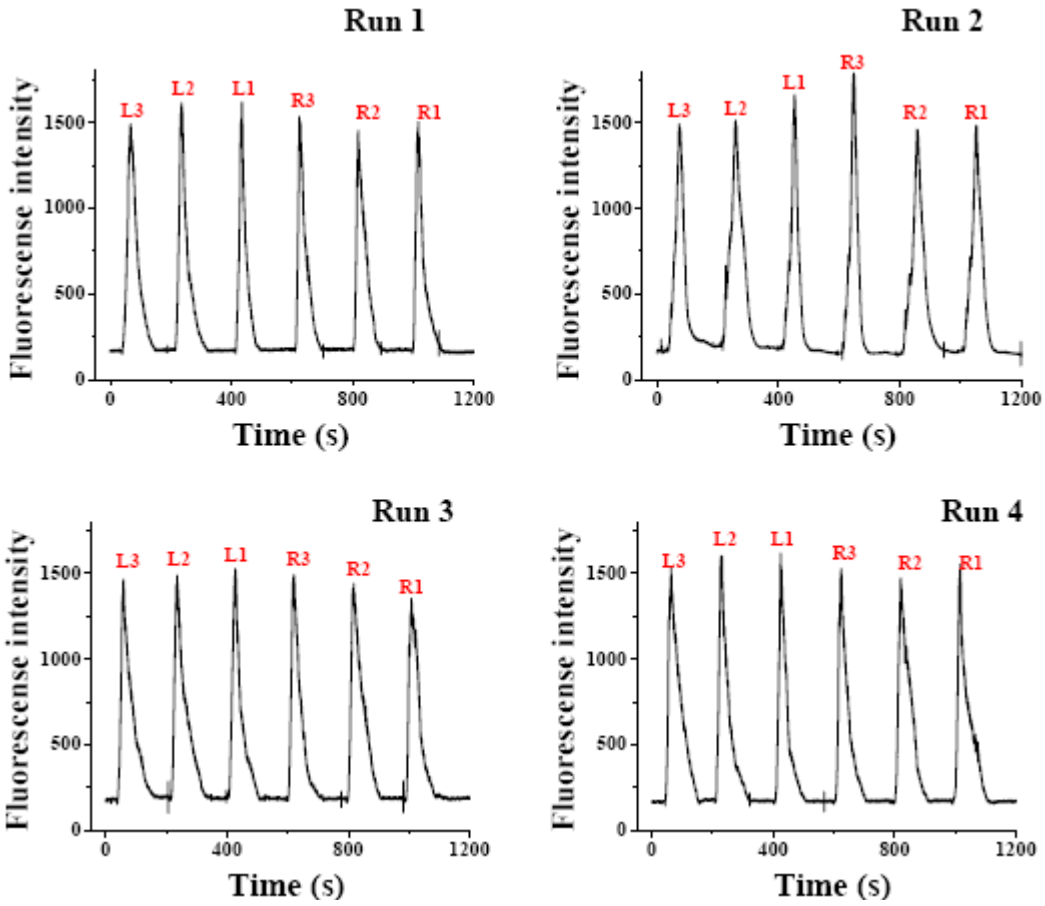
Supplementary Figure S1. (a) Detailed structure of the 8 channel device. Reservoirs S, Sh, SW, B and W represent reservoirs for sample, sheath buffer, sample waste, separation buffer and waste, respectively; (b) a close-up of the layout of the fraction collection channels; (c) layout of the collection channels in device A. The detailed dimensions of each portion of the collection channels are given in Table S1.

2. Fractionation of fluorescein in the 8-channel device coated with PolyE-323 but with no monolithic bed in the collection channels.



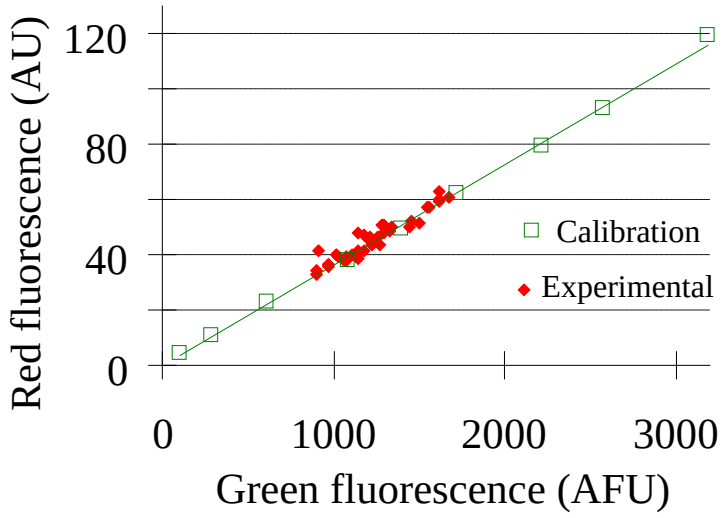
Supplementary Figure S2. Sheath images illustrating that the focused fluorescein stream was delivered into individual collection channels under the protection of sheath buffers. On the left side of the sheath images is a picture of the fractionation zone of the device. Sample voltage: -1.0 kV, sheath voltage: -1.5 kV. Collection channel was grounded individually.

3. Reproducibility of the sequential elution of FITC-BSA adsorbed on the 6-collection device



Supplementary Figure S3. Elution traces of four different runs illustrating the sequential elution of FITC-BSA off six monolithic SPE beds in a 6-channel device. Elution channel: 0.5 kV; collection channel for elution and focusing: ground, sheath reservoirs: ground. The corresponding channel is labeled above each peak.

4. Determination of sample carryover effect in multichannel SPE device



Supplementary Figure S4. Graphical interpretation of possible presence of sample carryover of carboxy SNARF for elution of Fluorescein from the SPE beds. The green line with open square symbols represents the amount of optical and electrical leakage; the red solid diamonds represent 40 experimental data points from 10 separate runs.

The amount of residual adsorbed material is estimated from the following equation. For example, the amount of residual carboxy SNARF remained in the SPE bed left after elution can be estimated from

$$SNARF_{residual}\% = \frac{(\frac{I_{R,leakage}}{I_{G,Fluor\_elution}} - 0.0364) \times I_{G,Fluor\_elution}}{I_{R,SNARF\_elution}} \times 100\%$$

where $I_{R,leakage}$ and $I_{G,Fluor\_elution}$ are the intensity of red fluorescence (the total leakage including electrical, optical and chemical leakage) and green fluorescence recorded during the elution of fluorescein, respectively. 0.0364 represents the ratio of optical and electrical leakage to signal. $I_{R,SNARF\_elution}$ is intensity of the SNARF elution peak.