Supporting Information

## Comparison of Inlet Geometry in Microfluidic Cell Affinity Chromatography

Peng Li, Yu Tian, Dimitri Pappas\*

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409

d.pappas@ttu.edu

\*Corresponding author



Fig. S1 Procedure of making parallel inlet device. 1) Three layers of Scotch tape are adhered on the inlet of separation channel. 2) Cast PDMS on the silicon mold, cure at 80°C for 1 h and peel off from mold. 3) Cut half of taller channel to expose inlet to the air. 4) Plasma seal on a clean glass slide for 60 seconds. 5) Insert a short capillary to the inlet end and sealed by adding a small portion of PDMS and curing agent mixture.



Fig. S2 Schematic of experiment setup. Syringe pump 1 is served as washing buffer loading pump. Syringe pump 2 is served as cell sample loading pump. Typical experiment procedure: 1) Turn on syringe pump 1 to fill system with separation buffer at flow rate 0.5 mL/h. 2) Turn off syringe pump 1 and turn on syringe pump 2 to load cell sample at flow rate 0.5 mL/h. 3) Change flow rate to 0.05 mL/h to perform continuous flow affinity cell separation. 4) Turn off syringe pump 2 and turn on syringe pump 1 to wash unbound cells away at flow rate 0.3-0.5 mL/h.



Fig. S3 Schematic of measured points using fluorescence correlation spectropy. a) Vertical inlet device; b) Parallel inlet device

	Diffusion time (ms)	Standard deviation
point 1	0.44803	0.04639
point 2	0.50814	0.04101
point 3	0.39343	0.06756
point 4	0.32789	0.02513
point 5	0.39715	0.03189
point 6	0.4694	0.03667
point 7	0.46625	0.03708
point 8	0.3948	0.03893
point 9	0.36187	0.03314
point 10	0.30397	0.02333
point 11	0.32092	0.02062
point 12	0.32402	0.02975
point 13	0.2983	0.02081

Table S1 Diffusion time and standard deviation of measured points in vertical inlet device

	Diffusion time (ms)	Standard deviation
point 1	0.28458	0.01987
point 2	0.25977	0.02314
point 3	0.32633	0.03465
point 4	0.49355	0.08311
point 5	0.35435	0.05448
point 6	0.34383	0.03761
point 7	0.39057	0.04938
point 8	0.33399	0.04031

Table S2 Diffusion time and standard deviation of measured points in parallel inlet device



b



Fig. S4 Images of actual devices used to measure diffusion time. a) Vertical inlet device (capillary is located on the left side of the channel); b) Parallel inlet device (capillary is located on the right side of the channel).



Fig. S5 Typical fitted correlation curve. a) Point 1 in vertical inlet device; b) Point 11 in vertical inlet device; c) Point 2 in parallel inlet device; d) Point 7 in parallel inlet device.

Video SI 1, 2. Inconsistent flow of cells in a vertical inlet device. The inconsistent flow arises from large numbers of captured cells at the inlet.

Video SI 3. Cell Capture at a vertical inlet, showing increased cell capture and cell-cell collisions.

Video SI 4. Cell capture downstream from the vertical inlet. 20 Minutes after separation, the majority of cells flowing in the channel are mouse endothelial cells (denoted by fluorescence), showing that enrichment is possible via negative selection.