Supporting information

Fabrication of monolithic frits and columns for microfluidic chip-based multidimensional separation devices.

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S-1 Scheme of a glass chip design

The glass chips (as shown in Figure 1A) are featured with a flow distributor that branched into channels with progressively decreasing internal diameters (from 1000 to 300 µm), a first-dimension separation channel (300 µm × 70 mm long), followed by 17 parallel second-dimension channels (300 µm × 31.2 mm long). Figure 1B shows an enlarged section of the glass chip. The channels of the flow distributors that are close to the first-dimension column end in a tapered connection with a width of 20 µm also for the second-dimension channels

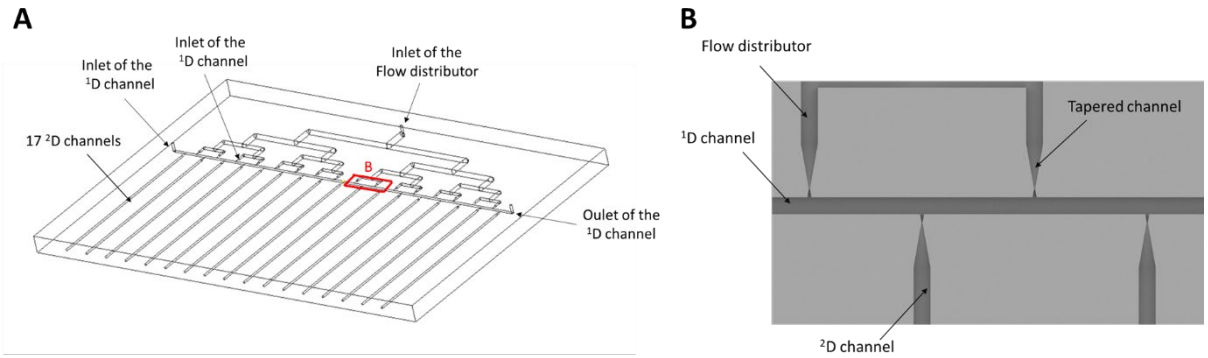

Figure S-1: A) glass chip design, B) enlarged section of the glass chip

S-2 Fabrication of monoliths with the use of glycerol

Monoliths in the glass chip were first fabricated by filling the chip with the monolith solution. The ^{1}D channel was flushed with one column volume of glycerol to wash out the monolith solution. Figure S1-A shows that the monolith solution and the glycerol are immiscible. Both the monolith solution and glycerol are the transparent solution. Therefore, for visualization, the monolith solution contains a blue dye and glycerol contains red dye. Also during polymerization, the glycerol was colored with a red dye, see Figure S1-B. After the polymerization, it can be observed that the ^{1}D channel contains small globules. It suggests that flushing with one column volume is not sufficient to keep the channel empty, see Figure S1-C. However, flushing the channel with more than column volume the glycerol will flow to the ^{2}D channel and interrupt the polymerization as can be seen in Figure S1-D

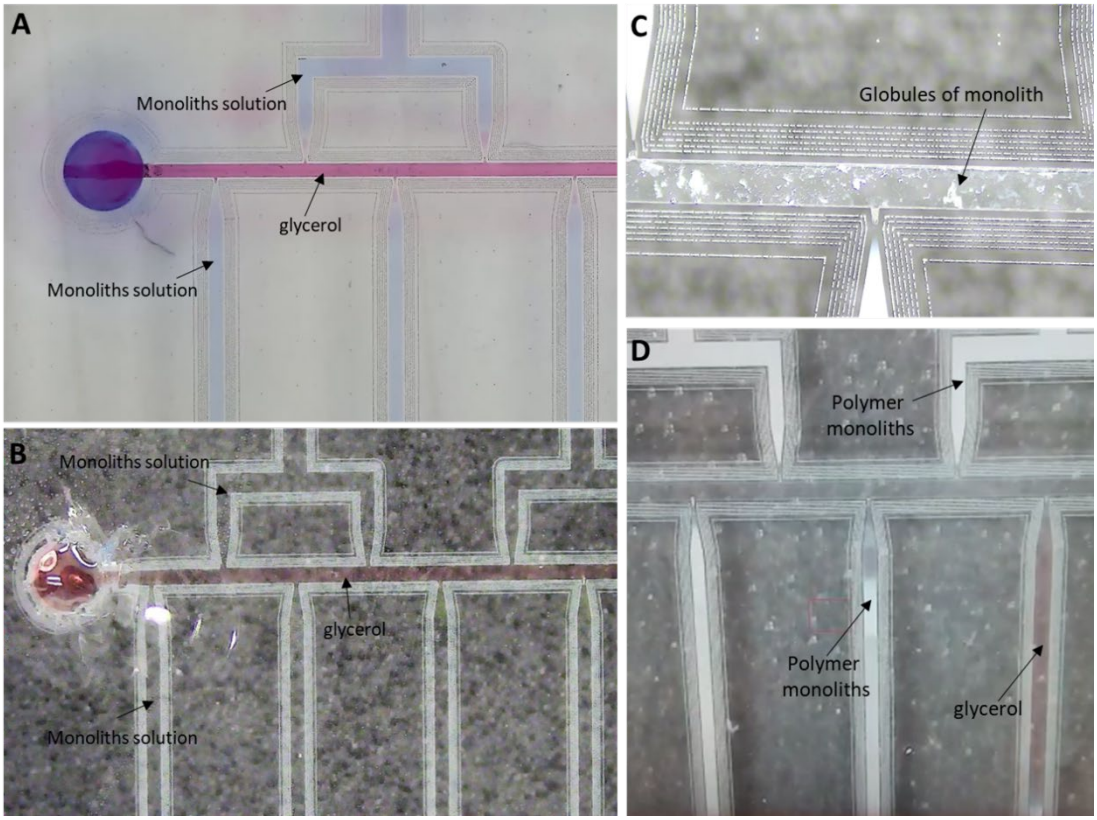

Figure S-1: Microscope images of the fabrication of monoliths with the use of glycerol.

S-3 Thermal polymerization

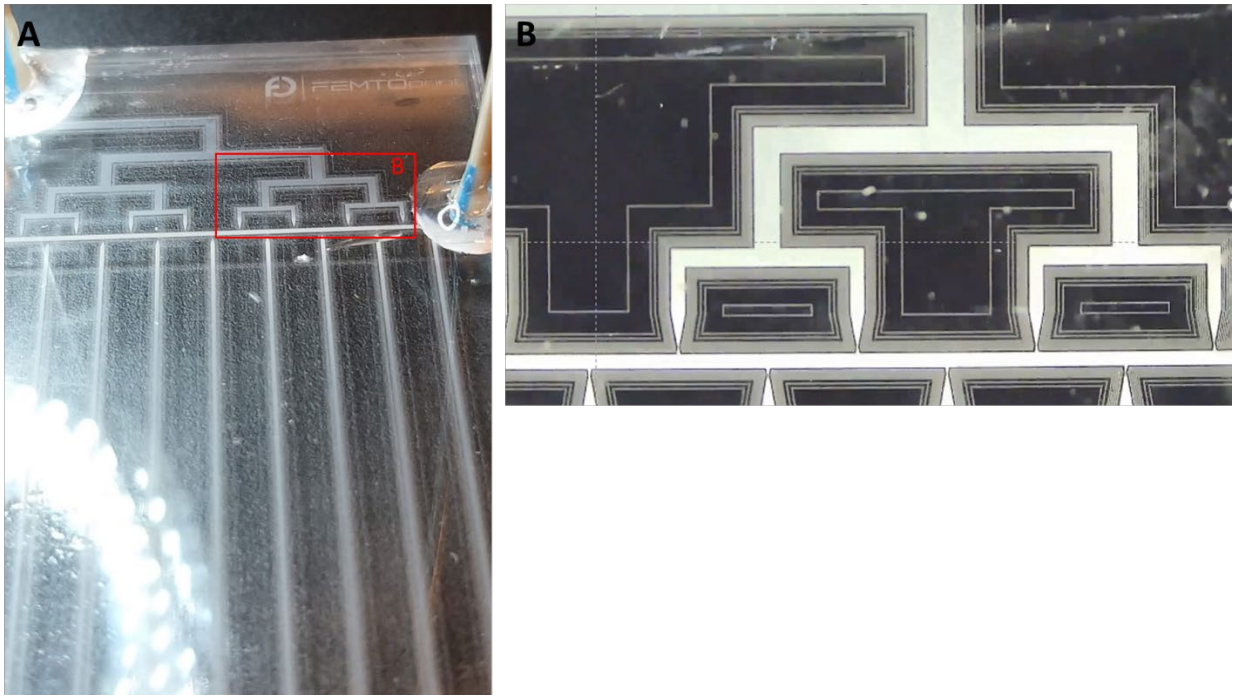

Figure S2, A) Image of a fully polymerized glass chip B) Microscope image of an enlarged section (red square) showing the monolith that is formed in all channels.

S-4 Repeatability measurements

Table S1 RSD values of the retention times of the repeated measurements for all peptides

Number of runs	Retention times (min)				
	Gly-Tyr	**Val-Tyr-Val**	**Met-Enkephalin**	**Leu-Enkephalin**	**Angiotensin II**
1	4.55	10.48	21.85	29.02	37.54
2	4.58	10.55	21.90	29.06	37.59
3	4.77	10.73	22.18	29.32	37.85
Average	4.63	10.59	22.98	29.13	37.66
RSD%	2.1	1.0	0.7	0.5	0.4