Droplet-Based Compartmentalization of Chemically Separated Components for Two-Dimensional Separations

Supplementary Material

Apparatus

A Waters Quanta 4000E Capillary Electrophoresis system (Waters, Milford, MA, USA) was used as a high voltage supply for the 2nd dimension CE separation. Harvard PHD 2000 syringe pumps were utilised for delivery of flows. An Alliance Model 500 UV/VIS Detector (GenTech, Arcade, NY, USA) was used to perform UV absorbance detection (at 214 nm) for both the first and second dimensions.

Chemicals & Materials

Phosphate buffer, 100 mM sodium phosphate, pH 3.0; Standard peptide mixture, containing Gly-Tyr, Val-Tr-Val, Leu enkephalin, Met enkephalin and angiotensin II, were obtained from Sigma-Aldrich (Poole, UK). Acetonitrile was purchased from Thermo Fisher Scientific (Loughborough, UK). Fused silica capillary, 100 μm i.d., 365 μm o.d. was obtained from Composite Metal Services (Ilkley, UK). PS-DVB particulate packing material (PLRP 10 μm, 1000 A) was obtained from Varian (Church Stretton, UK). PTFE tubing, 300 μm i.d. was purchased from Sigma-Aldrich. FC 40 oil obtained from 3MTM was used as the continuous phase in all experiments. Droplet storage tubing was obtained from Harvard Apparatus (Item No. 598324).

Capillary column

Due to the heat-sensitive nature of the PS-DVB polymer particles used for the chromatographic separation, a single particle fritting method was used to fabricate the end frits in a sinter-free way [1], and a transparent coated fused silica capillary, 100 μm id, 365 μm od, was slurry packed with PLRP particles (10 μm in diameter). The capillary has a packed length of 10 cm and a total length of 15 cm.

Capillary liquid chromatography separation, droplet fractionation and collection

A 0.5 ml plastic syringe was connected to the inlet end of the capillary column via a 1 cm long PTFE sleeve, id 300 μm. During operation, the syringe was used as the mobile phase reservoir. Once a sample was loaded on to the head of the column, the syringe was mounted to a Harvard PhD 2000 syringe pump, which was used as the high pressure supply for capillary LC separation. The outlet end of the capillary column was inserted into the T junction chip, as shown in Figure 1a, for droplet fractionation of the capillary LC effluent. Tubing filled with FC40 was connected to the outlet end of the T junction chip, and a syringe pump was operated in refill mode for aspirating the droplets into the tubing. Figure S1 shows a photographic image of the tubing with the collected droplets from the first device. Coloured dye was added to the effluent from the additive channel as indicated in Figure 1a to increase the contrast of the droplets to the background of the tubing. However, other denaturing reagents such as SDS TTB, affinity ligands or derivatives such as fluorescent dyes could also be added depending on the specific application or separation mechanism.

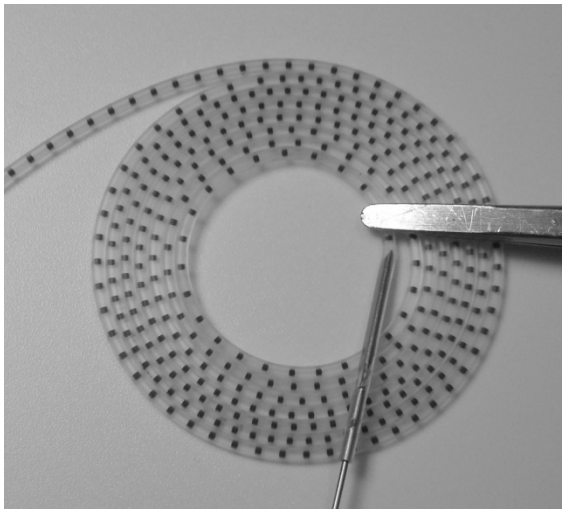

Figure S1: Tubing containing segmented droplets

CE separation of the droplets

Figure S2 shows the UV absorbance electropherogram of a representative droplet from the second dimension separation described in Figure 3.

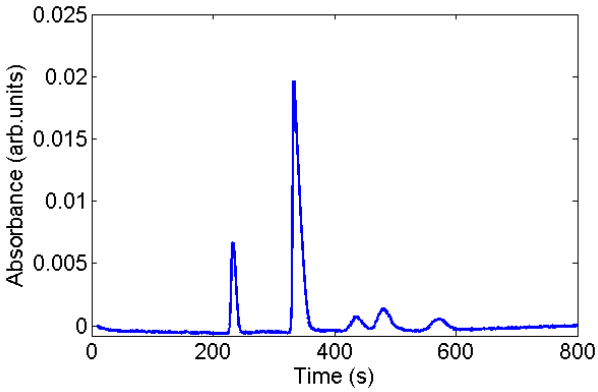

Figure S2: 11th droplet in Figure 3

"Heart-cutting" 2D separation of proteins from yeast cells

Figure 4 shows a heart-cutting 2D separation of crude lysate of yeast cells[2]. A stepwise gradient separation was conducted in the first dimension LC separation. Four plugs of mobile phase with increasing AcN concentrations, 20, 40, 60 and 80% were utilized sequentially. The effluent was segmented within 40 minutes into 260 droplets, containing proteins that ranged from highly hydrophilic to highly hydrophobic. We selectively chose droplets in elution plugs of 20% and 40% AcN, (containing hydrophilic proteins) to perform the second dimension CE separation.

References

1 B. Zhang, E. T. Bergstrom, D. M. Goodall and P. Myers, *Anal. Chem.*, 2007, **79**, 9229-9233.

2 G. Pappenberger, E. A. McCormack and K. R. Willison, J. Mol. Biol. 2006, **360**, 484-496