Electronic Supplementary Information

Mixed-mode electrokinetic and chromatographic peptide separations in a microvalve-integrated polymer chip

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S1. Chip fabrication

The retention of particle-based stationary phases in microfluidic devices can be achieved with physical constrictions (weirs) fabricated into the channels.[1-3] To confine the 5 μm diameter silica beads used as a stationary phase in the current 2-D chips, it is desirable to fix the dimensions of the constriction below 15 μm to exploit the so-called "keystone effect" for particle trapping.[4] Unfortunately, reliably fabricating channel dimensions at this scale by micromilling is highly challenging. To avoid this constraint, a 1 mm long RP BMA-EDMA monolith plug was photopolymerized near the column exits to act as porous frits for column packing, with a weir structure (Fig. S1) fabricated within the channels to help physically retain the monolith plug without relying on chemical bonding of the monolith to the thermoplastic channel sidewalls. The porous monolith plug was found to have relatively high permeability and low flow resistance, allowing 5 μm silica particles to be readily trapped by slurry packing using a syringe under manually-applied pressure. No deformation of the plugs are observed after packing the 42 mm long RPLC columns.

Fluidic connection between the IEF-*m*RPLC chip and the off-chip LC pump and capillary flow splitter were established using a high pressure and low dead volume needle interface shown to withstand high pressures (40 MPa/5800 psi), well above the delamination limit (24 MPa/3480 psi) of solvent-bonded COP substrates,[5-6] and exceeding the typical working pressures required for efficient on-chip HPLC using the packed microcolumns (4~6 MPa/580~870 psi). Overall the needle ports were found to provide a highly convenient, robust, and low cost interfacing method.

Another essential component of the IEF-*m*RPLC chip is a novel on-chip microvalve,[7] which allows hydrodynamic isolation between the separation dimensions and thus uniform sample transfer and gradient elution from each RPLC column. The reported microvalve has a small footprint and introduces low dead volume, essential to the fabrication of compact and high performance microfluidic analysis systems. Instead of pneumatic valving control often used in traditional PDMS valves,[8] the high-pressure microvalve consisted of a small plug of PDMS elastomer integrated into the microfluidic chip, with a

threaded needle used to deform the PDMS into the underlying COP microchannel to close the flow path. The high mechanical strength of the metal needle and the high bonding strength of the COP substrates enabled the compressed PDMS elastomer to withstand high pressures above 12 MPa/1740 psi over 100 open/close cycles without leakage, with a maximum operational pressure of 24 MPa/3480 psi.[7]

With regard to device disposability, the current IEF-mRPLC chips were fabricated using inexpensive thermoplastics (COP), standard stainless steel hyperdermic needles and the common silicone elastomer PDMS. Therefore, the material cost of a virgin device is low.. In contrast, the RPLC stationary phase is relatively expensive. However, since only very small amount (< 5 mg) is required to pack one chip, the average additional cost for each device is small. Thus chip costs are likely to be driven primarily by manufacturing costs, involving chip patterning, bonding, photolithographic formation of the packing frit, and bead packing. Each of these steps is amenable to batch fabrication, potentially reducing costs such that devices can be disposed after one run if sample cross-contamination is a concern. However, it must be noted that the chips are reusable. The IEF channel is regenerated by coating the channel with 0.4% HPMC solution prior to experiments, while RPLC columns can be revived through 1-hour acetonitrile rinsing followed by 1-hour equilibration with water or aqueous buffers. Unless clogged by PDMS material from damaged on-chip PDMS valves or severely contaminated by species with strong adsorption toward channel surface and RP beads, the IEF-mRPLC chips can be used at least 50 times.

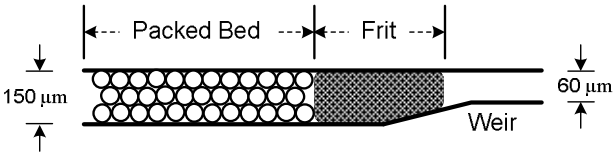

Fig. S1 Silica beads packed against monolith frit.

S2. Reproducibility of IEF-mRPLC chips

Reproducibility of the chips using FITC-protein digests was evaluated using relative standard deviation (RSD) of major IEF band location and retention time of main peaks in gradient RPLC chromatograms, which were measured from 3 different IEF-mRPLC runs of a single chip (run-to-run RSD) and 3 individual chips (chip-to-chip RSD). The resulting reproducibility data are shown in Table S1. Relatively low reproducibility was found in IEF dimension of the prototype chip in comparison to the mRPLC dimension. We believe that the low IEF reproducibility originated from the formation of irreproducible pH gradients during IEF. In the IEF-mRPLC chips channels in the upper channel network are interconnected. To prevent current leakage and thus electromigration of sample ions through the upper channel network, low-conductivity DI water was filled in the network prior to IEF. The approach

effectively contained most migrating sample ions in IEF channel. Unfortunately, hydrogen ions, hydroxyl ions and small ampholyte ions can still diffuse from the IEF channel to the upper channel network overtime, resulting in the disturbance of local pH gradients and formation of an irreproducible pH gradient. Approaches toward segregating the upper channel network from the IEF channel are currently under development.

Table S1 Reproducibility of chip IEF-mRPLC separations

	Run-to-Run (RSD)	Chip-to-Chip (RSD)
IEF band location	< 30%	< 40%
Gradient LC retention time	< 3%	< 7%

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