# Supplemental Data

# Separations in poly(dimethylsiloxane) microchips coated with supported bilayer membranes

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EXPERIMENTAL

**Methods**

*Chip fabrication*. PDMS chips were formed on silicon molds.[27] All channels were produced in silicon wafers by reactive ion etching and were measured to be 30 μm deep. For cell adhesion and $\mu_{eo}$ measurement, the mold possessed a 30 μm wide channel that was 3 cm long. For separations, a cross design with 30 μm wide channels was used. The separation channel was 3.5 cm long and intersected at 1.5 cm from one end by a bisected 2 cm injection channel. To make PDMS, a 10:1 ratio of Dow Corning Sylgard 184 PDMS elastomer and curing agent were mixed, degassed, and cured at 70° C for at least one hour. A Harrick (Ithaca,NY) plasma cleaner was used for PDMS oxidation. The PDMS piece with imprinted channels and a glass slide were plasma oxidized on high for 45 s and pressed together. Wells made from silicon tubing were attached at the channel inlets and outlets. Solutions containing lipid vesicles were placed in the wells immediately after device assembly while the PDMS was still hydrophilic from oxidation. This enabled to lipid solutions to spontaneously fill the channels by capillary action. The vesicles were incubated in the channels for 15 min, the channels were rinsed with Tris-sep for 10 min and fresh Tris-sep was placed in the four wells.

*$\mu_{eo}$ measurements*. The current monitoring method[28] was used to measure the electroosmotic mobility ($\mu_{eo}$). A 30 μm volume of 10 mM PB was placed in both channels and 400 V potential applied. Then the 10 mM PBS in one channel was replaced with 9 mM PB. A difference of ~10% with a neutral marker method has been observed in the measurement of electroosmotic flow by the current monitoring method using similar concentrations.[29] To perform stability measurements, 133 V/cm was continuously applied and the current monitoring method was used at regular time points to measure the $\mu_{eo}$.

*Separations*. The chip was placed on an inverted Nikon (Melville,NY) Eclipse TE300 microscope in a custom-made teflon holder that secured the four electrodes while allowing observation of the channels through the bottom slide with an objective. Separation potentials were generated by a LabSmith (Livermore,CA) HVS448 power supply connected to a laptop computer. Four SHV connectors were terminated with

platinum electrodes for insertion into microchip wells. Current was monitored with an Agilent (Santa Clara, CA) 34401A multimeter. Unless specified otherwise, Tris-sep buffer was used in all wells and the analytes were prepared and diluted in Tris-sep. A simple pinched-injection routine was used.[30] For PC and ePC50–coated channels, 500 V was applied at the analyte waste and 0 V on other channels. With PCPG, 500 V was applied to the sample, 0 V to the sample waste, 700 V to the buffer waste, and 475 V to the buffer reservoir. Depending on the analyte, a positive or negative potential was applied across the injection channel to load sample for 30 s to 1 min. Next, the fields were switched and the analyte plug was driven into the separation channel. Either 1000 V was applied to the buffer reservoir with 0 V on the other reservoirs, or 1000 V was applied to the buffer waste reservoir while 500 V was applied to the analyte and analyte waste reservoirs and 0 V to the buffer reservoir. Alternatively where noted, 700 V was applied to the buffer waste reservoir and 400 V to each of the analyte reservoirs. A typical electrokinetic loading volume was 26 pL. Gravity driven flow was also used to load dye through the cross arms by removing buffer from the analyte waste reservoir. This procedure, henceforth referred to as gravity loading, generated a 40 pL plug at the cross intersection. A microscope objective was positioned over the separation channel 1 mm to 1 cm from the channel intersection to collect fluorescent light. For OG and FL,separations shown in Figure 1, the separation distance (d) =5 mm and separation voltage (V)=-235 V/cm. For the OG and FL separations shown in Table 1, d=2 mm and V=-235 V/cm. For FL/OG separations on PCPG-coated microchannels, d=5 mm and V=118 V/cm. For OG/FL separations on ePC50-coated channels, d=2 mm and V=-235 V/cm. For F-PKB/pF-PKB separations on PC-coated microchannels, d=2 mm and V=118 V/cm, while on PCPG-coated channels d=3 mm and V=118 V/cm. For F-ABL/pF-ABL separations on PC coatings, d=2 mm and V=-129 V/cm, while on PCPG coatings d=5 mm and V=99 V/cm. eGFP/GFP-CRAKL were separated on PC coatings with d=2 and -235 V/cm. For gravity loading the analyte waste buffer volume was reduced to 10 μL while the other buffer wells were kept at 30 μL. The resulting injection plug had a volume of 40 pL, slightly larger than the 25 pL electrokinetically pinched plug. F-ABL and pF-ABL were loaded by gravity and separated on PC-coated microchips in ECB/glucose, with d=5mm and V=-235 V/cm. For streptavidin and avidin separations on

PC, d=5 mm, V=-235 V/cm. Peak efficiencies were calculated using the method of full width at half of the maximum signal height.

| | PC (+-) | PCPG (-) | EPC (+) |
|---|---|---|---|
| sep. eff. **OG** | 611,000 ± 39,000 | 312,200 ± 54,000 | 88,000 ± 5,000 |
| sep. eff. **FL** | 499,000 ± 46,000 | 182,800 ± 37,000 | 111,000 ± 16,000 |
| resolution (R) | 2.4 ± 0.1 | 1.7 ± 0.1 | 2.7 ± 0.1 |
| migration time (OG) | 1.26 ± 0.06 | 7.78 ± 0.12 | 2.05 ± 0.02 |
| migration time (FL) | 1.68 ± 0.09 | 6.36 ± 0.07 | 4.48 ± 0.09 |
| sep. eff. F-**ABL** | 183,000 ± 34,000 | 166,000, ± 20,000 | |
| sep. eff. **pF-ABL** | 365,000 ± 55,000 | 413,000, ± 40,000 | |
| resolution (R) | 1.7 ± 0.1 | 1.6 ± 0.1 | |
| migration time (F-ABL) | 5.84 ± 0.26 | 5.86 ± 0.01 | |
| migration time (pF-ABL) | 4.24 ± 0.16 | 7.18 ± 0.01 | |
| sep. eff. **PKB** | 239,000 ± 83,000 | 219,000 ± 56,000 | |
| sep. eff. **pPKB** | 341,000 ± 28,000 | 328,000 ± 31,000 | |
| resolution (R) | 1.7 ± 0.1 | 1.9 ± 0.1 | |
| migration time (F-PKB) | 6.24 ± 0.33 | 4.9 ± 0.06 | |
| migration time (pF-PKB) | 8.24 ± 0.52 | 6.42 ± 0.11 | |

**Table S1**. Average separation efficiency (theoretical plates per meter) obtained for separated dye and peptide analytes, average resolution for each analyte pair, and average migration times (s) (n=3).

| | **[conc]** | **migration time** (s) | **efficiency** (plates/m) |
|---|---|---|---|
| avidin-FITC | 588 pM | 11.24 ± 0.32 | 155,000 ± 41,000 |
| streptavidin-AF | 340 pM | 7.44 ± 0.26 | 272,000 ± 39,000 |
| eGFP | 115 pM | 4.87 ± 0.32 | 358,000 ± 6,000 |
| GFP-CrakI | N.A. | 3.49 ± 0.31 | 278,000 ± 64,000 |

**Table S2**. Separation results for four proteins separated on PC membrane-coated microchips (n=3).