Supporting Information for "A Digital Microfluidic Platform for Human Plasma Protein Depletion"

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Supporting Information Contains

Additional Digital Microfluidic Chip Microfabrication Information

Device fabrication reagents included photoresist developer MF-321 from Rohm and Haas (Marlborough, MA), chromium etchant CR-4 from Cyantek (Fremont, CA), photoresist stripper AZ-300T from AZ Electronic Materials (Somerville, NJ), Teflon-AF from DuPont (Wilmington, DE) and Parylene C dimer from Specialty Coating Systems (Indianapolis, IN). Digital microfluidic devices were fabricated in the University of Toronto Nanofabrication Centre (TNFC) cleanroom facility as described previously[1,2] and summarized here. Bottom plates of DMF devices consisted of an array of 80 interdigitated working electrodes (2.2 mm x 2.2 mm) connected to 8 larger reservoir electrodes (16.4 mm x 6.7 mm) and 4 waste reservoirs (16.4 x 6.4 mm). Bottom plates were constructed with standard photolithography methods. Briefly, glass substrates (50.8 mm x 76.2 mm x 1.1 mm) coated with chromium (200 nm) and photoresist from Telic Co. (Santa Clarita, CA) were exposed to UV from a Suss MicroTec mask aligner (29.8 mW/cm^2, 10 seconds) under an acetate photomask printed at 20,000 dpi (Pacific Arts and Designs, Inc, Markham, ON). The exposed substrates were then developed in MF-321 (3-5 min.) and baked on a hot plate (125°C, 1 min.). Developed substrates were then etched in CR-4 chromium etchant for 3 minutes before being stripped of remaining photoresist in AZ-300T (5 min.). Substrates were rinsed in isopropanol, contact pads were covered in dicing tape and the substrates were coated with ~7 µm of Paralyene-C by vapor deposition. Subsequently, ~200 nm of Teflon-AF was applied to the devices by spin coating (1% w/w Teflon-AF in Fluorinert FC-40 at 2000 rpm for 45 seconds). Finally, the devices were baked on a hot plate (165°C, 10 min.) and the dicing tape removed from the contact pads. Top plates of DMF devices were fabricated by spin coating ~200 nm of Teflon-AF (as above) on unpatterned indium-tin oxide (ITO) coated glass substrate (Delta Technologies Ltd, Stillwater, MN) and then baking on a hot plate (225°C, 10 minutes).
Devices were assembled by attaching the ITO top plate to the electrode array bottom plate with a spacer formed from two layers of Scotch double-sided tape (3M, St. Paul MN). The total spacer thickness was ~180 µm."

(1) Choi, K.; Ng, A. H. C.; Fobel, R.; Chang-Yen, D. A.; Yarnell, L. E.; Pearson, E. L.; Oleksak, C. M.; Fischer, A. T.; Luoma, R. P.; Robinson, J. M.; Audet, J.; Wheeler, A. R. *Anal. Chem.* 2013, *85*, 9638-9646.
(2) Ng, A. H. C.; Choi, K.; Luoma, R. P.; Robinson, J. M.; Wheeler, A. R. *Anal. Chem.* 2012, *84*, 8805-8812.

Digital Microfluidic Chip Protein Depletion Followed by HPLC-nanoESI-MS/MS

To examine the compatibility of the DMF-based protein depletion protocol with nanoliquid chromatography mass spectrometry a small model protein, cytochrome C, was used in the protein mixture to replace hemopexin as it requires more complex denaturation steps prior to digestion. A sample containing 0.5 mg/mL cytochrome C was mixed with 2mg/mL IgG and 0.5 mg/mL HSA, and the double depletion process as described above was repeated on the mixture. Tryspin solution was then added to the depleted protein mixture (contains cytochrome C) after processing by DMF at an enzyme to substrate ratio of 1:5 (w/w). The digestion mixture was then incubated for 12 hours at 37 °C. The digested solution was then quenched by adding 5% formic acid. Three replicate samples were evaluated. Following in-solution enzymatic digestion of proteins, the tryptic peptides were analysed using an Orbitrap Velos Pro (Thermo Scientific, Bremen, Germany) mass spectrometer coupled to a nano-LC system, Easy LC, and nano-ESI source (Thermo Fischer Scientific, Bremen, Germany). Gradient Elution was employed for the LC separation where eluent A was aqueous formic acid (0.1%, v/v) and eluent B was formic acid (0.1%, v/v) in acetonitrile. The 10 µL samples were injected by the auto sampler onto the trap column (C18, internal diameter 100 µm, length 20 mm, particle diameter 5 µm). The peptides were then separated on an analysis column (C18, internal diameter 75 µm, length 100 mm, particle diameter 5 µm) with a flow rate

of 30 nL/min using a two-step gradient, 5 to 50% eluent B over 70 min, followed by increasing eluent B to 100% over 45 min where it is maintained at 100% for an additional 28 min. The transfer capillary temperature was set to 270 °C. An ion spray voltage of 2.0 kV was applied to a PicoTip™ on-line nano-ESI emitter (New Objective, Berlin, Germany). Precursor ion survey scans were acquired at an Orbitrap resolution of 60,000 for m/z range 200 to 2,000. Data were acquired using Xcalibur™ software, and processed by Sequest search engine (Proteome Discover 1.4, Thermo Fischer) against the SwissProt database, allowing up to two missed cleavage sites and a mass tolerance of 10 ppm for precursor ion scans and 0.8 u for product ion scans.

After repeating the analysis three times, sequence coverage was found to be 89% for cytochrome C, which suggests the entire depletion and digestion process using the magnetic bead depletion and DMF was very efficient. Table S1 summarizes peptides found and other related parameters.

Table S1. Sequence Peptides of Trypsin Digested Cytochrome C using LC-MS/MS

A3	Sequence	q-Value	Charge	MH+ [Da]	ΔM [ppm]
High	KTGQAPGFSYTDANKNK	0	3	1826.91726	7.69
High	KTGQAPGFSYTDANK	0	2	1584.77823	8.15
High	HKTGPNLHGLFGRK	0	2	1561.88294	7.60
High	EETLmEYLENPKK	0	2	1639.80069	7.47
High	GITWKEETLmEYLENPK	0	2	2097.04155	9.81
High	TGPNLHGLFGR	0	2	1168.62871	5.56
High	KTEREDLIAYLK	0	2	1478.82998	5.81
High	TGQAPGFSYTDANK	0	2	1456.68292	8.63
High	TGQAPGFSYTDANKNK	0	2	1698.82109	7.56
High	EETLmEYLENPK	0	2	1511.70673	8.77
High	KYIPGTKmIFAGIK	0	3	1582.91380	7.06
High	EETLMEYLENPK	0	2	1495.71135	8.56
High	HKTGPNLHGLFGR	0	3	1433.78747	7.93
High	TEREDLIAYLK	0	2	1350.73733	8.08
High	MIFAGIKK	0	2	907.55052	7.81
High	mIFAGIKK	0	2	923.54544	7.68
High	GGKHKTGPNLHGLFGR	0	4	1675.92532	6.75
High	mIFAGIK	0	2	795.45102	9.61
High	YIPGTKmIFAGIK	0	2	1454.81778	6.96
High	KYIPGTKMIFAGIK	0.001	3	1566.91741	6.19
High	TGPNLHGLFGRK	0.006	2	1296.72638	7.09
Medium	TEREDLIAYLKK	0.036	3	1478.83082	6.39
Low	EDLIAYLKK	0.1	2	1092.63980	8.99
Low	CAQCHTVEK	0.103	2	1018.45439	9.71
Low	KYIPGTK	0.214	2	806.48480	9.56

Low	EETLmEYLENPKKYIPGTK	0.268	3	2299.17240	8.56
Low	IFVQK	0.269	2	634.39791	8.84
Low	KIFVQK	0.335	2	762.49372	8.45
Low	MIFAGIKKK	0.349	3	1035.64629	7.62
Low	MIFAGIK	0.414	2	779.45461	7.89
Low	EDLIAYLK	0.426	2	964.54332	8.62
Low	YIPGTK	0.449	2	678.38850	9.40
Low	YIPGTKMIFAGIKK		3	1566.91741	6.19
Low	mIFAGIKKK		3	1051.64138	7.66
Low	KKTER		2	661.40571	9.87