Supplementary Information

Salt Segregation and Sample Cleanup on Perfluoro-coated Nanostructured Surfaces for Laser Desorption Ionization Mass Spectrometry of Biofluid Samples

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Experimental

NMR Spectroscopy

The human serum sample was deproteinated through ultrafiltration. Subsequently, 70 μl of D_2O and 30 μl of a standard buffer solution (11.7 mM sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), 730 mM imidazole, and 0.47% NaN_3 in H_2O) was added to 600 µl serum filtrate. The sample (700 μL) was then transferred to a standard NMR tube for spectral analysis. All ^{1}H-NMR spectra were collected on a 500 MHz Inova spectrometer (Varian Inc.) equipped with a 5 mm HCN Z-gradient pulsed-field gradient (PFG) room-temperature probe. ^{1}H-NMR spectra were acquired at 25°C using the first transient of the NOESY-pre-saturation pulse sequence, chosen for its high degree of quantitative accuracy. All FID's (free induction decays) were zero-filled to 64 K data points and subjected to line broadening of 0.5 Hz. The singlet produced by the DSS methyl groups was used as an internal standard for chemical shift referencing (set to 0 ppm) and for quantification. All ^{1}H-NMR spectra were processed and analyzed using the Chenomx NMR Suite Professional Software package version 7.1 (Chenomx Inc). The spectral fitting for metabolite was done using the standard Chenomx 500 MHz metabolite library. Sample spiking was used to confirm the identities of assigned compound, involving the addition of 20-200 μM of the suspected compound and examination of the resulting spectra to determine whether the relative NMR signal intensity changed as expected.

Taurine quantified by RPLC-MS/MS

The NMR peak of taurine overlaps with other components so it can not be reliably quantified by NMR. The NMR signal of malic acid is too weak to be detected. As a result, taurine in serum was quantified using a combination of the Absolute*IDQ*™ Kit (BIOCRATES Life Sciences AG) with RPLC-MS/MS, but the kit does not work for malic acid. The kit was used for derivatization and extraction of analytes. Isotope-labeled internal standards and other internal standards are integrated in the kit plate filter for metabolite quantification. The Absolute*IDQ* kit contains a 96 deep-well plate with a filter plate attached with sealing tape. The first 14 wells in the Kit were used for one blank, three zero samples, seven standards and three quality control samples provided with each Kit. All the serum samples were analyzed with the AbsoluteIDQ kit using the protocol described in the AbsoluteIDQ user manual. Briefly, serum samples were thawed on ice, vortexed and centrifuged at 13,000x g. 10 μL of each serum sample was loaded onto the center of the filter on the upper 96-well kit plate and dried in a stream of nitrogen. Subsequently, 20 μL of a 5% solution of phenyl-isothiocyanate was added for derivatization. After incubation, the filter spots were dried again using an evaporator. Extraction of the metabolites was then achieved by adding 300 μL methanol containing 5 mM ammonium acetate. The extracts were obtained by centrifugation into the lower 96-deep well plate, followed by a dilution step with MS running solvent. Mass spectrometric analysis was performed on an API4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with a solvent delivery system. The samples were delivered to the mass spectrometer by a LC method followed by a direct injection (DI) method. The Biocrates MetIQ software was used to control the entire assay workflow, from sample registration to automated calculation of metabolite concentrations to the export of data into other data analysis programs. A targeted profiling scheme was used to quantitatively screen for known small molecule metabolites using multiple reaction monitoring, neutral loss and precursor ion scans.

Quantitative Analysis

The error of estimating the concentration of an unknown, s_X, from the measured value y and an internal standard-based calibration curve were analyzed using the least-squares method:

$$s_X = \frac{s_y}{|m|}\sqrt{\frac{1}{k} + \frac{nx_u^2 + \sum(x_i^2) - 2x_u\sum x_i}{n\sum(x_i^2) - (\sum x_i)^2}} \quad \text{(Equation 1)}$$

where m is the slope, k is the number of measurements made of the unknown, n is the number of (x_i, y_i) data sets used in the calibration curve, x_u is the concentration of the unknown determined from the calibration curve and s_y is the standard deviation of the measurements, given by:

$$s_y = \sqrt{\frac{\Sigma(y-y_i)^2}{n-2}} \quad \text{(Equation 2)}$$

Table S-1: Artificial CSF (aCSF) composition and concentration (pH=7.3)

Ion in artificial CSF	**Concentration (mM)**
Na^+	150
K^+	3.0
Ca^{2+}	1.4
Mg^{2+}	0.8
PO_4^{3-}	1.0
Cl^-	155

Source: http://www.alzet.com/products/guide_to_use/cfs_preparation.html

Table S-2: Instrument Settings of MALDI-TOF-MS

	Acceleration voltage (kV)	**Grid Voltage (%)**	**Guide Wire Voltage (%)**	**Delay Time (ns)**	**Laser Intensity(a.u.)**
Negative Ion Mode	18	73	0.05	150	2100
Positive Ion Mode	20	69	0.07	350	2000

Results and Discussion

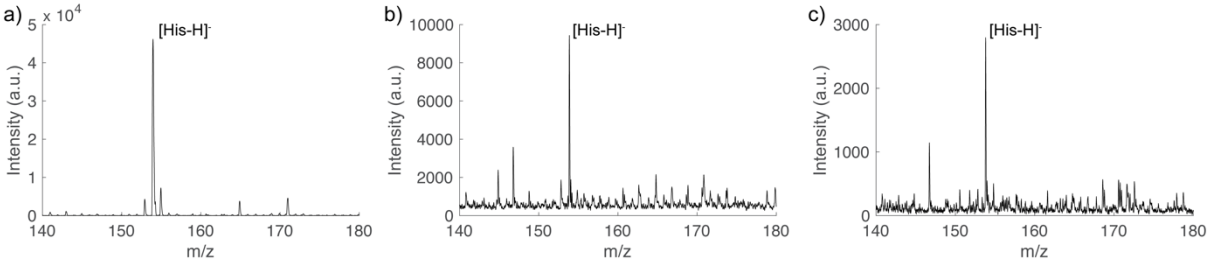

Figure S-1: Mass spectra of a) 10 μM (8 pmol) b) 2 μM (1.6 pmol) and c) 1 μM (800 fmol) pure histidine solution obtained on a $pFSiCl_3$ coated (1% coating concentration, ~140° contact angle)) SMALDI chip.

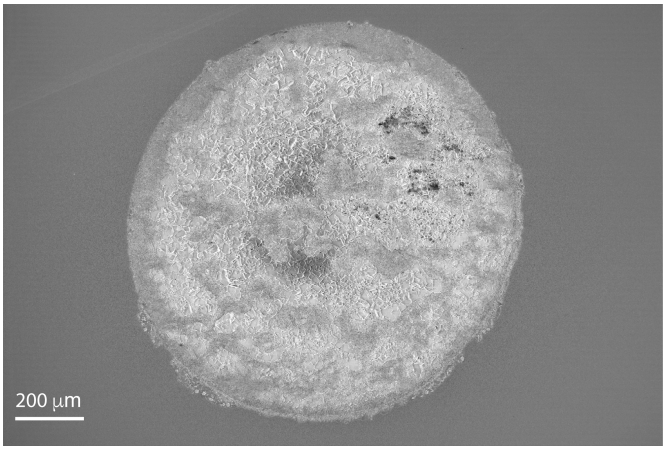

Figure S-2: SEM image of the entire sample spot shown in Figure 2b, showing artificial cerebrospinal fluid salt deposits over the entire spot surface, when working with a 1% $pFSiCl_3$ coated GLAD surface with a contact angle of 150°.

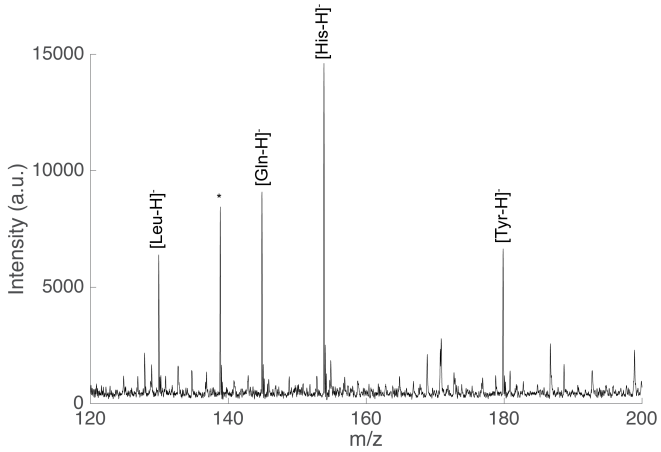

Figure S-3: A mass spectrum of artificial cerebrospinal fluid (aCSF) sample containing leucine, glutamine, histidine, and tyrosine, 20 μM for each, obtained on $pFSiCl_3$ coated (0.3% coating concentration, 120 ° contact angle) SMALDI chip with slow air drying of the sample spot.

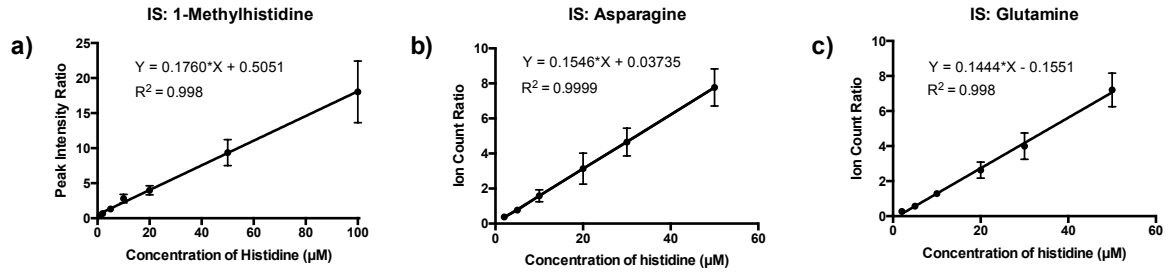

Figure S-4: The internal standard calibration curves of histidine in aCSF using 20 μM a) 1-methylhistidine, b) glutamine and c) asparagine as internal standards. SMALDI chips were coated with a 1.2% concentration of $pFMe_2SiCl$, 120 ° contact angle. The linearity ranges are 1-100 μM in a) and 2-50 μM in b) and c). The error bar represents SD of 10 individual measurements.

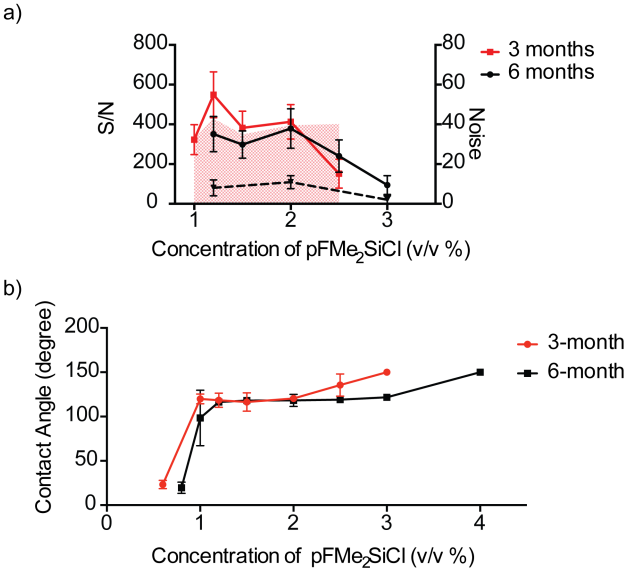

Figure S-5:

A) The relationship between S/N (lines), noise (shadow) and coating concentration is shown. The S/N of 10 μM histidine spiked in aCSF were tested on $pFMe_2SiCl$ coatings. Red represents films 3-month old and black represents films 6-months old before perfluorosilane coating. The samples were dried in a humid chamber at 4°C (solid lines) or room temperature (dashed line), and the duration of drying was ~5 h and ~1 h, respectively. Slow air drying amplifies the desalting performance, resulting in much higher signal. Referring to Figure 6b, the S/N significantly decreases on superhydrophobic surfaces, independent of film age and dying time. Comparing S/N and the noise range shown in pink in Figure S-5A, the decrease of S/N at high coating concentration arises from loss of signal. Error bars show the standard deviation of 9 measurements.

B) A plot of contact angle versus coating concentration for a subset of data in Figure 6, extending to lower $pFMe_2SiCl$ concentrations and lower contact angles. Contact angles below 50 degrees are indicative of very low quality coatings.

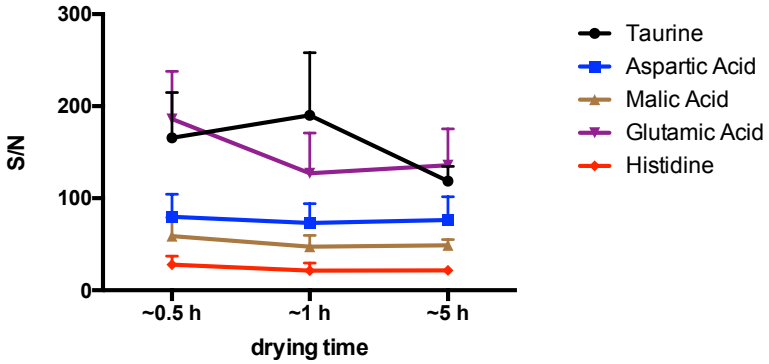

Figure S-6: S/N of serum metabolites under different drying conditions. The serum samples spotted on 1.2% $pFMe_2SiCl$ coated SALDI surface were air dried either rapidly (~0.5 h) at ambient conditions, ~1 h in a humidity chamber at ambient temperature, or ~5 h in a humidity chamber at 4°.

Table S-3: The putative metabolites for peaks detected in human serum in negative ion mode by SMALDI-MS

Observed m/z	Assigned metabolite*	Adduct MW (Da)	Δ m/z	CV (%)
108.8923	N/A			53.40
110.8816	Thiosulfate	110.9216	0.0400	32.73
119.9362	Cysteine	120.0125	0.0763	40.64
121.9334	Isonicotinic acid/ Nicotinic acid/ Picolinic acid	122.0248	0.0914	53.96
124.0001	Taurine	124.0074	0.0073	18.06
128.0315	Pyroglutamic acid	128.0353	0.0038	10.58
132.0302	Aspartic acid	132.0302	0	20.9
133.0057	Malic acid	133.0142	0.0085	24.99
135.0202	Erythronic acid/ Threonic acid	135.0299	0.0097	24.54
135.9220	N/A			48.50
137.9042	N/A			32.11
145.0376	Adipic acid/ Methylglutaric acid/ 2-Methylglutaric acid	145.0506	0.0160	15.07
146.0439	Glutamic acid	146.0459	0.0020	20.74
146.9736	2-Hydroxyglutaric acid/ 3-Hydroxyglutaric acid/ Citramalic acid	147.0299	0.0563	32.11
148.9814	4-Ethylbenzoic acid/ Hydrocinnamic acid	149.0608	0.0794	28.78
154.0698	Histidine	154.0622	0.0076	29.10
154.9306	2,5-Furandicarboxylic acid	154.9986	0.0680	21.91
162.9517	Coumaric acid/ 4-Hydroxycinnamic acid/ Phenylpyruvic aicd/ 2-Oxo-3-phenylpropanoic aicd	163.0401	0.0884	35.44
167.0301	Homogentisic acid/ Vanillic acid/ 3-Hydroxymandelic acid/ p-Hydroxymandelic acid/ 5-Methoxysalicylic acid/ 3,4-Dihydroxybenzeneacetic acid	167.0350	0.0050	26.28
174.0042	N-Acetyl-L-aspartic acid	174.0408	0.0366	36.65
181.9689	4-Pyridoxic acid	182.0459	0.0770	23.91
182.9579	4-O-Methylgallic acid/ 3,4-Dihydroxymandelic acid	183.0299	0.0720	18.93
183.9653	Phosphoserine	184.0016	0.0363	18.58
185.9969	N/A			15.48
187.0194	Azelaic acid	187.0976	0.0782	24.50
189.9928	5-Hydroxyindoleacetic acid	190.0510	0.0582	49.34
191.0197	Citric acid/ Isocitric acid	191.0197	0	18.94
192.0240	4-Anilino-4-oxobutanoic acid/ 2-Methylhippuric acid	192.0666	0.0426	40.88
197.9291	O-Phosphothreonine	198.0173	0.0882	28.18
201.0420	Sebacic acid	201.1132	0.0712	28.57
208.9871	N/A			29.37
215.0313	Bisnorbiotin/ 6-Aminopenicillanic acid	215.0496	0.0183	25.27
217.0264	3-Hydroxysebacic acid/ 2-Hydroxydecanedioic acid	217.1081	0.0817	11.80
224.9676	3-Nitrotyrosine	225.0517	0.0841	30.15
249.0001	Gamma-Glutamylcysteine	249.0551	0.0550	25.49

* Peaks were assigned to metabolites using Human Metabolome Database (HMDB, www.hmdb.ca). The adduct type is limited to $[M-H]^-$ which is the most likely ionization form in negative ion mode using pSi-LDI chips. The molecular weight tolerance is ±0.1 Da. Aspartic acid and citric acid were used as references for mass calibration, so Δm/z at 132.0 and 191.0 are zero. When assigning possible metabolites to peaks detected, we only considered the metabolites that have been detected in blood. 5 of 37 peaks were not assigned, among which the peak at m/z 186.00 is a known background peak.