Supporting Information for:

**Electrospun Nanofibers as Substrates for Surface-Assisted Laser
Desorption/Ionization and Matrix-Enhanced Surface-Assisted Laser
Desorption/Ionization Mass Spectrometry**

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Experiment

Materials.

SU-8 2100, an epoxide-based negative photoresist, was purchased from MicroChem Corporation (Newton, MA). PVA (99%+ hydrolyzed, M_w 89 000-98 000), PAN (M_w 150 000) and NaHCO_3 were purchased from Sigma-Aldrich (St. Louis, MO). SU-8 was diluted to 75 (v/v) % with cyclopentanone (Sigma Aldrich, St. Louis, MO). PVA was dissolved in deionized water (8%, w/w). Glutaraldehyde (GA, 25% in water, Baker) and HCl (0.1 M) were added to the aqueous PVA solution prior to electrospinning as a crosslinker and catalyst, respectively.¹ PAN was dissolved in N, N-dimethylformamide (DMF, Sigma Aldrich, St. Louis, MO) with a concentration of 10% (wt%). Angiotensin I, D-(+)-glucose, L-arginine monohydrochloride, crystal violet, polystyrene 4000 (PS 4000, standard, M_w 4130), polystyrene 2000 (PS 2000, standard, M_w 2360), poly(ethylene glycol) (PEG 3400, M_n 3400), poly(ethylene glycol) (PEG 900 000, M_w 900 000),

CHCA, 2,5-dihydroxybenzoic acid (DHB), trifluoroacetic acid (TFA), silver trifluoroacetate, and sodium trifluoroacetate were purchased from Sigma-Aldrich (St. Louis, MO). Dithranol (DR) was purchased from Fisher Scientific (Hanover Park, IL). Methanol and tetrahydrofuran (THF) were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ).

Electrospinning.

PAN (10% and 12.5%, wt%) was electrospun using previously optimized conditions of 20 kV, a distance between the tip and the collector of 20 cm, and a flow rate of 1.5 mL/h with relative humidity less than 50%.² Porous PAN was prepared following a reported procedure.³ Briefly, 5% or 10% of NaHCO₃ (wt%) was mixed and homogeneously dispersed in 10% PAN solution. The electrospinning condition was the same as the one used for pure PAN. After electrospinning the fiber mat was peeled off from the collector and immersed into 10% HCl solution to remove NaHCO₃ and create pores. Then the mat was washed with nano pure water thoroughly to remove the salt and HCl. When the mat was still wet it was spread onto the target plate. The fiber mat adhered to the plate when it was dry. The *in-situ* crosslinked PVA was electrospun after adding glutaraldehyde (GA:PVA, 90:1, mol/mol) and HCl (HCl:GA, 1:5, mol/mol). PVA was also electrospun using previously optimized conditions of 20 kV, a distance between the tip and the collector of 20 cm, a flow rate of 0.5 mL/h, and relative humidity of less than 30%.¹ The SU-8 was electrospun in a dark room due to its UV sensitivity. The previously optimized conditions consisted of a voltage of 9 kV, a distance between the tip and the collector of 10 cm, and a flow rate of 1.2 mL/h.⁴ The obtained SU-8 mat was crosslinked under UV light (Sunray 400SM UV flood light, Uvitron International West Springfield, MA) for

about 5 min.⁵ For all polymers, the electrospinning time varied from 5 min to 25 min to obtain a homogeneous coverage of the target plate (dimension app. 53×41 mm) and fibrous mats of $2 \text{ mg} \pm 0.5 \text{ mg}$. For PAN and SU-8, electrospinning about 5 min yielded 2 mg mats, while for crosslinked PVA about 20 min electrospinning time was needed. Because of the mass loss during the pyrolysis, electrospinning times of 15, 25 and 35 min were used to produce 2 mg nanofibrous mats for the carbon nanofibers processed to 600, 800 and 900 °C, respectively.

Fabrication of Carbon Substrates.

The carbon nanofibrous substrates were made by pyrolysis of the crosslinked SU-8 precursor. The target plate with electrospun and crosslinked SU-8 was placed in a tube furnace (Lindberg/Blue M Asheville NC, Model: STF55346C-1) and heated under forming gas (5% H₂ in N₂) at a ramp rate of 1 °C/min for final temperatures of 800 °C and 900 °C and 2 °C/min for 600 °C. The final pyrolysis temperature was held for 6 h and cooled to room temperature.⁵

Sample Preparation.

Polystyrene with silver trifluoroacetate (1:1, w/w) and dithranol were dissolved in THF. Poly(ethylene glycol) with sodium trifluoroacetate (1:1, w/w) and DHB were dissolved in methanol. For dynamic range study PS 4000 solution (250 mg/mL in THF with 250 mg/mL silver trifluoroacetate) and PS 4000/PS 2000 blend (250 mg/mL in THF with 250 mg/mL silver trifluoroacetate and 5% PS 2000, mol%) were prepared. Glucose and arginine were dissolved in water (1 mg/mL). Crystal violet was dissolved in methanol (5 mg/mL). Angiotensin I and matrix CHCA were dissolved in acetonitrile/water (1:1, v/v) with 0.1% trifluoroacetic acid. Dried droplet by the multiple-layer spotting method was

used for sample preparation.⁶ 0.1 μ L of the analyte solution was applied on the target plate for SALDI analysis. Matrix solution was applied over the dried analyte. Due to the limit of the solubility of CHCA and dithranol, multiple layers of the solution were applied to reach different matrix/analyte ratios (Table S-1). The electrospun substrates were removed from the target plate after use, and the target plate was reused after thoroughly cleaning the surface with THF, acetone, methanol, and water followed by sonication in methanol for 15 min.

Instrumentation.

The mass spectra were collected using a Microflex MALDI-TOF Bruker mass spectrometer in positive ion mode. A nitrogen laser at 337 nm with a pulse width of 3 ns was used. The ion acceleration potential was 20 kV. The laser power used to obtain the spectra and to optimize the S/N ratios was slightly above the ionization threshold. For PEG 900 000 linear mode was used. For all the other samples reflector mode was used. Each spectrum was the summation of 120 shots, unless otherwise noted. All of the results were repeated at least three times. FlexAnalysisTM software was used for data analysis. For angiotensin I, SNAP was used for the peak pick and S/N determination due to its isotopic pattern, and for all the other analytes CENTROID was used.⁷

A Hitachi S-300 (Hitachi High Technologies, America, Inc., Pleasanton, CA) scanning electron microscope (SEM) was used to obtain images of the nanofibrous substrates. A stainless steel plate of the same dimension as the target plate was used as the collector during electrospinning for the samples used in SEM imaging.

Table S-1. Average diameters of the electrospun nanofibers.

	Diameter (nm)
Carbon (600 °C)	195 ± 80
Carbon (800 °C)	150 ± 55
Carbon (900 °C)	140 ± 50
PAN (10%)	460 ± 90
PAN (12.5%)	880 ± 90
PAN (10% with 5% NaHCO ₃)	470 ± 100
PAN (10% with 10% NaHCO ₃)	430 ± 80
PVA	190 ± 50
SU-8	420 ± 90

Table S-2. S/N of ME-SALDI-TOF spectra with different substrates.

Analyte (mg/mL)	Matrix (mg/mL)	Carbon (600 °C)	Carbon (800 °C)	Stainless steel
PEG 3400 (5)	DHB (40)	48 ± 7	15 ± 10	80 ± 10
PEG 3400 (5)	DHB (80)	52 ± 5	55 ± 8	78 ± 8
PEG 3400 (5)	DHB (160)	50 ± 3	110 ± 20	130 ± 10
PEG 3400 (10)	DHB (40)	36 ± 6	50 ± 9	70 ± 15
PEG 3400 (10)	DHB (80)	61 ± 7	80 ± 20	100 ± 20
PEG 3400 (10)	DHB (160)	77 ± 8	90 ± 20	130 ± 10
PEG 3400 (20)	DHB (40)	40 ± 9	60 ± 9	70 ± 20
PEG 3400 (20)	DHB (80)	39 ± 4	100 ± 12	100 ± 10
PEG 3400 (20)	DHB (160)	57 ± 6	100 ± 20	100 ± 9
PS 4000 (25)	DR (40×1)	90 ± 20	13 ± 6	67 ± 9
PS 4000 (25)	DR (40×3)	80 ± 20	18 ± 4	90 ± 10
PS 4000 (25)	DR (40×5)	95 ± 15	40 ± 10	95 ± 10
PS 4000 (50)	DR (40×1)	90 ± 10	20 ± 7	62 ± 9
PS 4000 (50)	DR (40×3)	130 ± 20	150 ± 20	120 ± 20
PS 4000 (50)	DR (40×5)	105 ± 20	50 ± 10	90 ± 20
PS 4000 (100)	DR (40×1)	70 ± 15	70 ± 20	45 ± 9
PS 4000 (100)	DR (40×3)	80 ± 20	80 ± 7	60 ± 15
PS 4000 (100)	DR (40×5)	190 ± 20	160 ± 17	125 ± 7
Angiotensin I (0.5)	CHCA (10)	1500 ± 300	4300 ± 400	1500 ± 100
Angiotensin I (1)	CHCA (5)	1200 ± 200	4000 ± 1000	1800 ± 300
Angiotensin I (1)	CHCA (10)	3400 ± 200	4700 ± 800	2000 ± 150
Angiotensin I (1)	CHCA (10×2)	3300 ± 400	2100 ± 200	2400 ± 300
Angiotensin I (2)	CHCA (5)	1600 ± 500	1200 ± 500	1700 ± 300
Angiotensin I (2)	CHCA (10)	4400 ± 200	2400 ± 300	2000 ± 200
Angiotensin I (2)	CHCA (10×2)	3400 ± 350	5400 ± 650	1300 ± 300

The applied volume of sample and matrix solution was 0.1 µL. 20 shots were used to acquire each spectrum. “Sweet spot” were searched all over the sample spot to obtain the optimum signal S/N ratios. Five replicates were performed for each sample. Since the standard deviation listed in Table S-1 was calculated from five spectra obtained only from “sweet spots” by searching all over the sample, the standard deviation cannot be used to represent the shot-to-shot reproducibility like that discussed in the SALDI section.

Figure S-1. SEM image of the electrospun (a) PAN (10%) substrate, and digital photographs of target plates with (b) PAN and (c) carbon nanofibers processed to 600 °C as substrates,

All the other carbon and polymeric substrates have similar nanofibrous structures. PVA and SU-8 also showed the circles on the stainless steel target plate after electrospinning. Due to the high temperature treatment those circles disappeared after pyrolysis of the carbon substrates.

Figure S-2. SALDI mass spectrum of PEG 3400 (20 mg/mL) using carbon processed to 800 °C substrate. Laser intensity 30 μ j.

Figure S-3. Optimization of laser energy of PEG 3400 (20 mg/mL) with carbon substrate processed to 800 °C.

Figure S-4. SALDI-TOF mass spectra of (a) glucose (1 mg/mL), (b) arginine (1 mg/mL) and (c) crystal violet (5 mg/mL) using the carbon substrate processed to 800 °C. The peak at m/z of 114 in (b) is from impurity.

Figure S-5. Optimization of laser energy of PS 4000 (50 mg/mL) with PAN (10%) substrate.

Figure S-6. S/N dependence with number of laser radiations on the same spot of the PS 4000 using PVA substrate. Each laser radiation contains 20 shots. (●) 100 mg/mL, (■) 50 mg/mL, (◆) 25 mg/mL.

Figure S-7. SALDI-TOF mass spectra of (a) 5% PS 2000 mixed with PS 4000 (mol %) and the expanded low mass region of (b) PS 4000 and (c) 5% PS 2000 mixed with PS 4000 (mol %) using the PAN substrate.

Figure S-8. S/N of PS 4000 (50 mg/mL) on PAN substrates with different diameters, pore sizes and pore density.

Figure S-9. SEM image of porous PAN prepared by adding 10% NaHCO_3 .

Figure S-10. Optimization of S/N of angiotensin I (2 mg/mL) using CHCA (2×10 mg/mL) as matrix with (a) carbon substrate processed to 800 °C and (b) PVA substrate.

Figure S-11. S/N ratios of (a) PEG 3400, (b) PS 4000, and (c) angiotensin I on different substrates: (■) stainless steel, (■) carbon processed to 800 °C, (■) carbon processed to 600 °C, (■) PAN, (■) PVA, (■) SU-8. S/N ratios calculated from five spectra with 20 shots each. The error bars show the standard deviations.

Figure S-12. ME-SALDI spectra of angiotensin I using carbon substrate processed to 800 °C with concentrations of 2 mg/mL (a), 1 mg/mL (b), 0.5 mg/mL (c), stainless steel substrate with concentration of 0.5 mg/mL (d), and PVA substrate with concentration of 1 mg/mL (e). The matrix/analyte ratios were kept the same for all samples (1:10, w/w).

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