Supporting Information for

Photonic Crystal Enhanced Laser Desorption and Ionization Substrate for Stress Biomarkers Detection Under Atmospheric Pressure

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Contents

1. Results and discussion S2

1.1. The schematic diagram of preparation process S2

1.2. Characterization of PS photonic crystal template S2

1.3. The image of the SALDI-QTRAP400 MS system S4

1.4. Mass spectra of Arg at different laser intensity S4

1.5 Mass spectra of rats serum samoles S5

1.6 Mass spectra of rats serum samoles S6

1. Results and discussion

1.1 The schematic diagram of preparation process

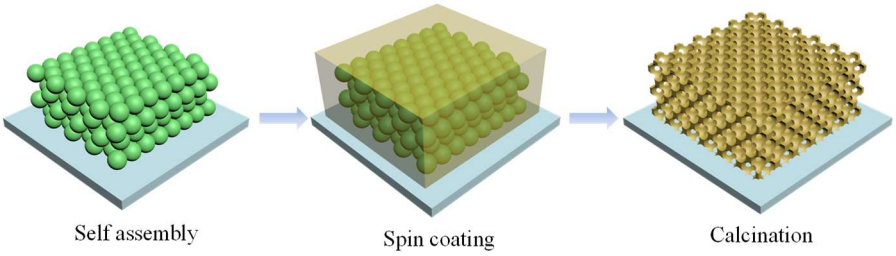


Fig.S1 The preparation of WO_3-TiO_2 inverse opal photonic crystals

1.2. Characterization of PS photonic crystal template

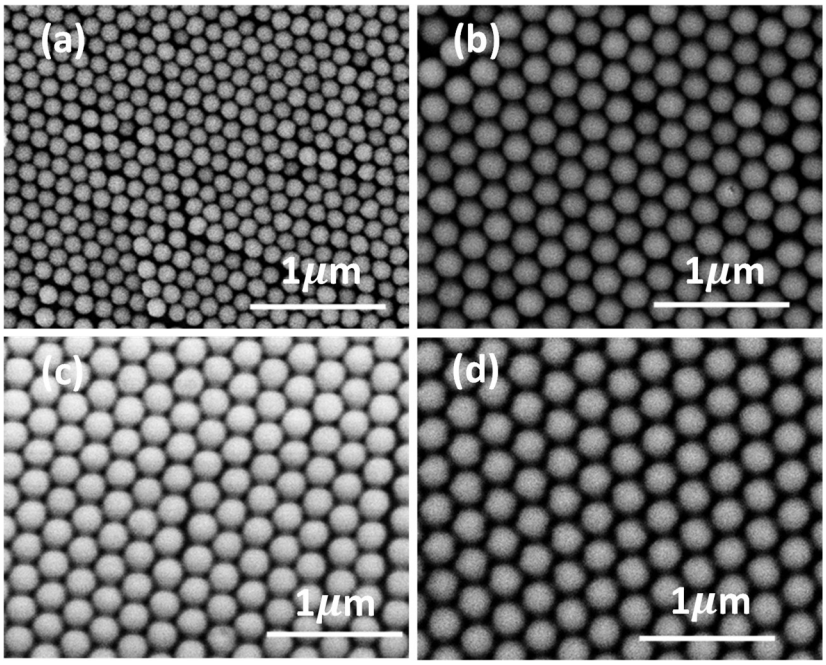


Fig.S2 SEM of PS photonic crystal templates. (a) Φ_{PS}=195nm; (b) Φ_{PS} =230nm; (c) Φ_{PS} =270nm; (d) Φ_{PS} =330nm.

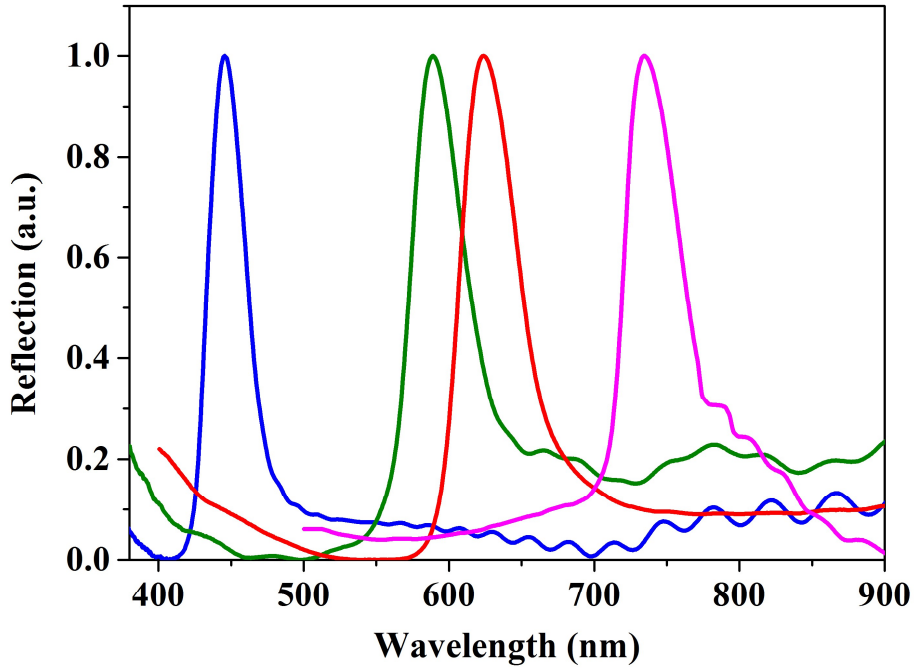


Fig.S3 Reflectance spectra of PS photonic crystal templates with PS particle diameters of 195, 230, 270, 330 nm.

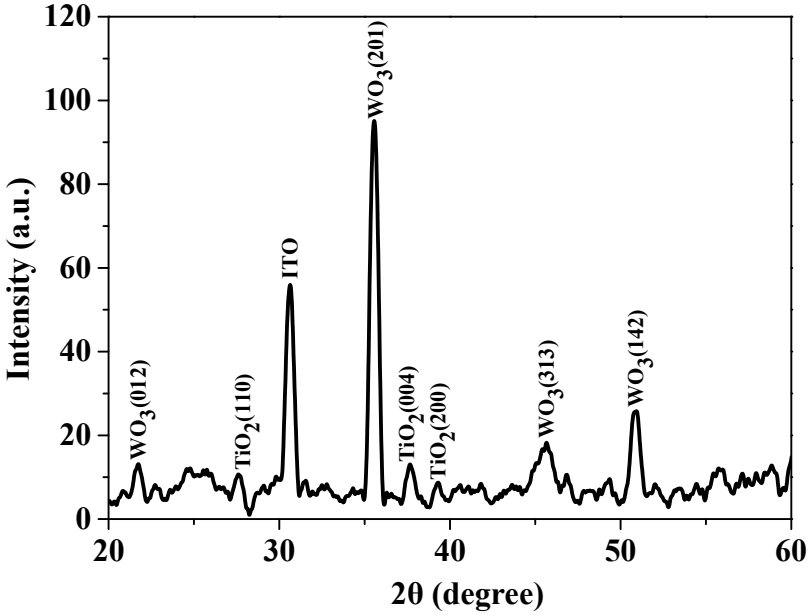


Fig.S4 XRD pattern of the WO_3-TiO_2 inverse opal photonic crystal films.

1.3. The image of the SALDI-QTRAP400 MS system

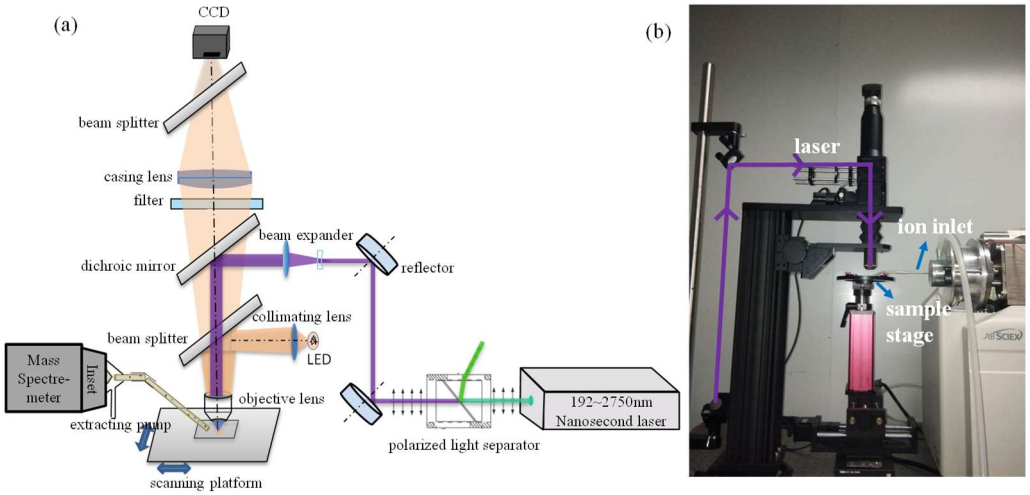


Fig.S5 (a) The schematic diagram of the SALDI-QTRAP400 MS system; (b) The actual image of the SALDI-QTRAP400 MS system.

The laser path was comprised of the Nd:YAD nanosecond laser, a polarizing beam splitter, reflectors, a dichroic mirror and an objective lens, which focused the laser on the sample. Thus, the analytes were desorbed and ionized. And the produced ions were introduced to the mass spectrometer for mass analysis.

1.4. Mass spectrometry of Arg at different laser intensity

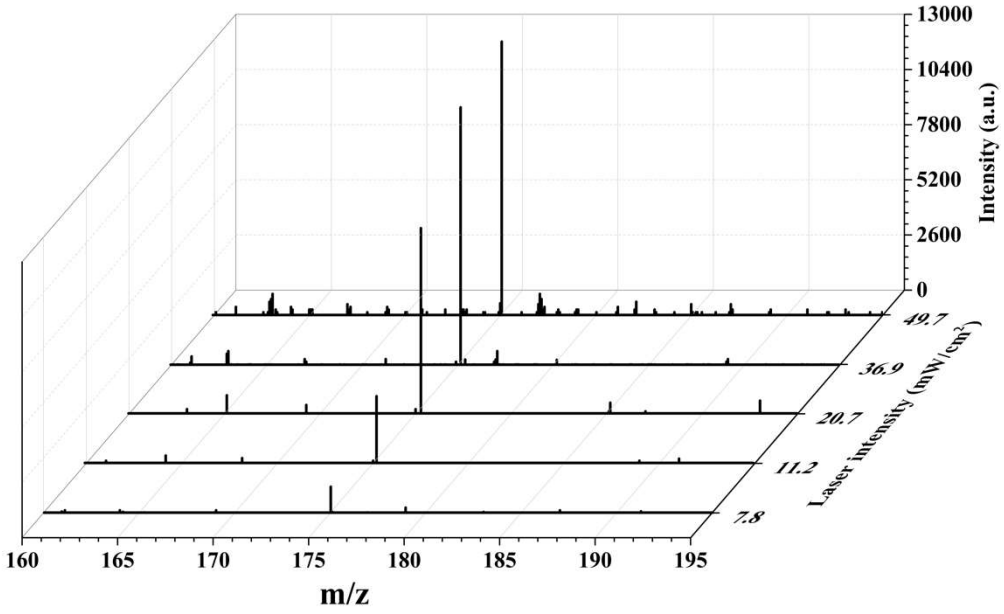
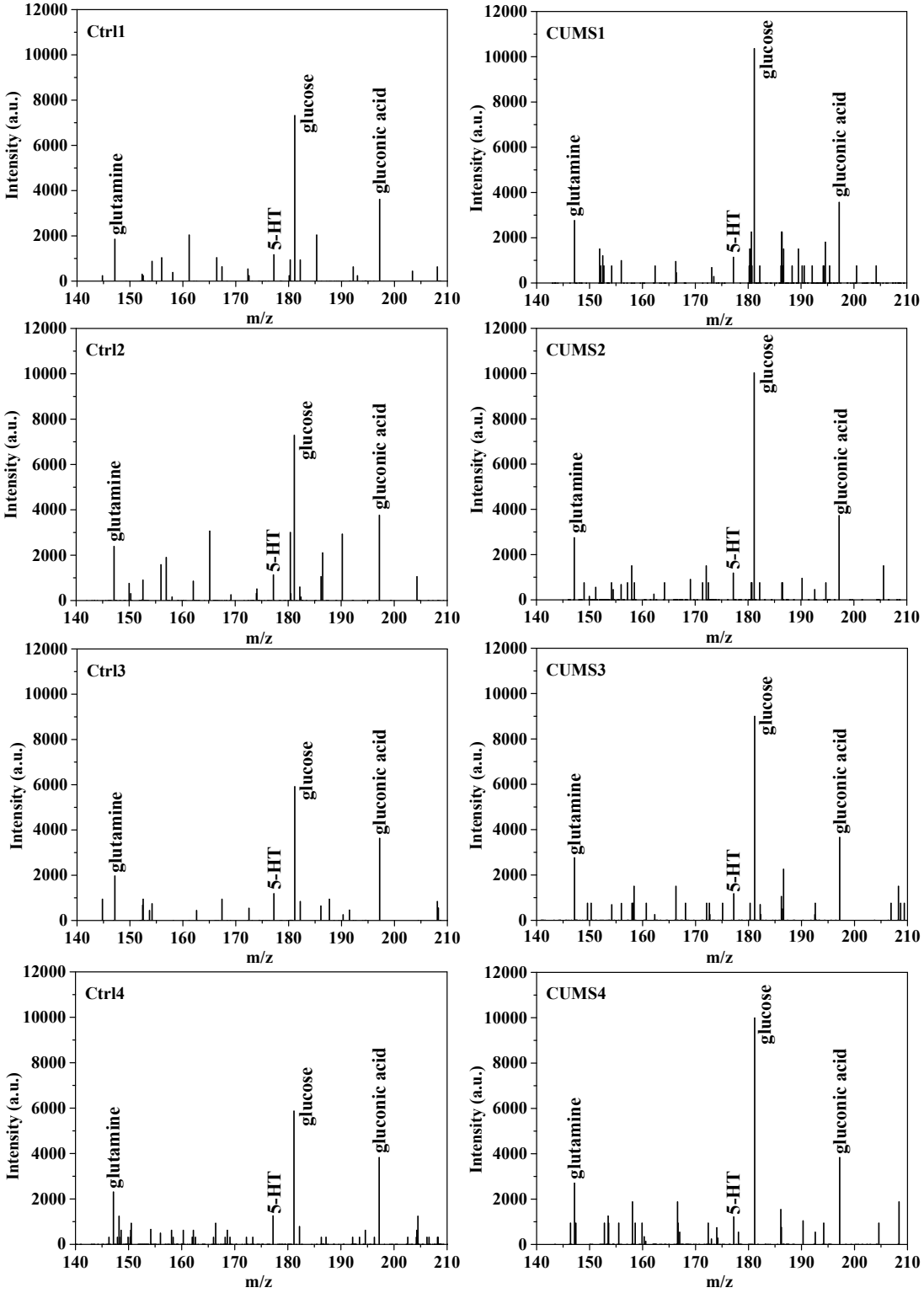


Fig.S6 Mass spectra of the protonated molecule generated at different laser intensity by using Iopal-195 as substrate.

1.5 Mass spectra of rats serum samples



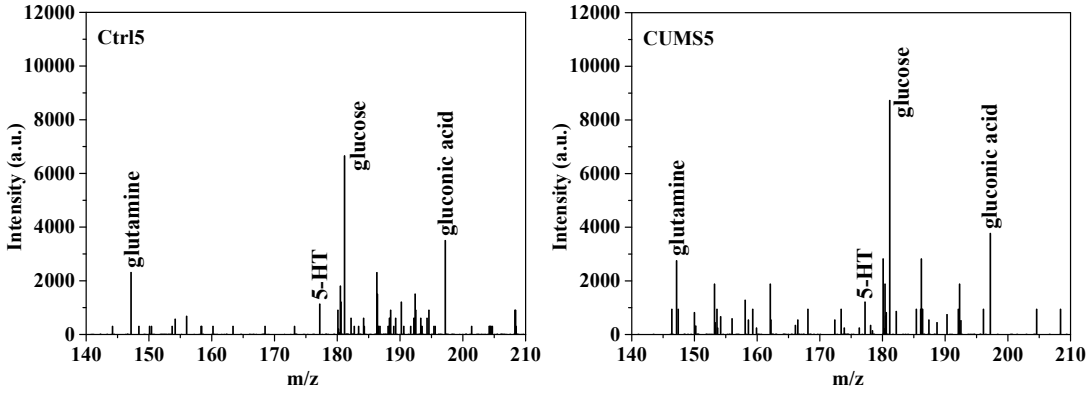


Fig.S7 Mass spectra of the serum samples containing internal standard (Ctrl: control group, CUMS: CUMS model group)

1.6 Reliability verification of the SALDI analysis

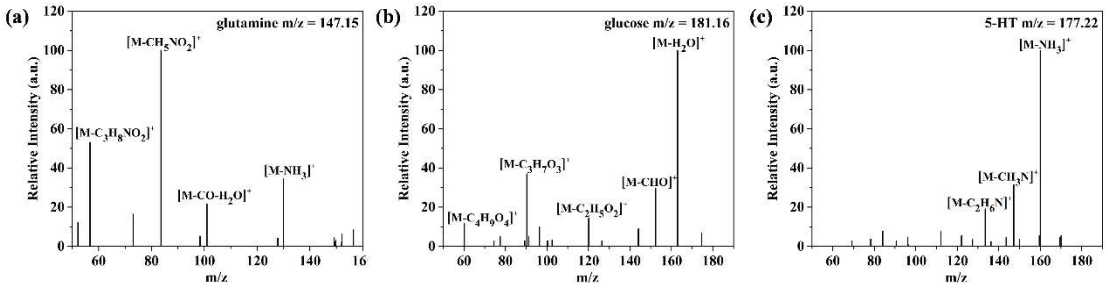


Fig.S8 The MS/MS spectra of (a) glutamine; (b) glucose; (c) 5-HT.

As shown in Figure S8, the MS/MS spectra of three ionized molecules were obtained. And, the scanning results confirmed the reliability of three ionized molecules.

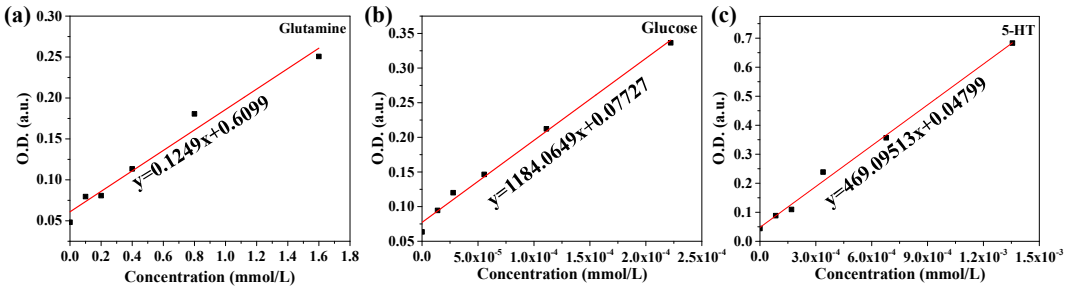


Fig.S9 The standard cure of each CUMS markers.

Table S1 The comparison of quantitative results between SALDI analysis and ELISA kit

	glutamine	glucose	5-HT
SALDI analysis	0.681±0.052 mmol/L	7.214±0. 621 mmol/L	0.029±0. 003 mmol/L
ELISA kit	0.732±0. 064 mmol/L	6.285±0. 593 mmol/L	0.025±0. 004 mmol/L
quantitation error	7.49%	12.88%	13.79%

In order to assess the performance of the proposed method, the biomarkers levels of the control group-1 serum sample was detected using the traditional method, which is enzyme-linked immunosorbent assay (ELISA) kit detection method. The glutamine ELISA kit (YX-071214M), glucose ELISA kit (YX-071221M) and 5-hydroxytryptamine ELISA kit (YX-050820M) were purchased from HePeng Biotech (Shanghai, China). The standard cure of each CUMS markers was generated by plotting the average O.D. (450nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (Figure S9). Then, the concentration of each markers in diluted serum sample was calculated from the O.D. value according to the curve. Finally, the concentration of each markers in original serum sample was calculated by multiplying the concentration by corresponding dilution factor. The calculation result showed that the concentrations of glutamine, glucose and 5-hydroxytryptamine were 0.732mmol/L, 6.285mmol/L and 0.025mmol/L, respectively. And the results indicated that the quantitation error of the SALDI analysis was within 15%. The quantitation error was calculated by $\frac{|C_{SALDI}-C_{ELISA}|}{C_{SALDI}}$, where C_{SALDI} and C_{ELISA} were the biomarker concentrations detected by SALDI analysis and ELISA kit, respectively.

Table S2 The comparison between current method and MALDI & SELDI

	sample preparation	salt tolerance	low-mass region interference	mechanism
CHCA/DHB[1]	co-crystallization	bad	strong	light absorption
metallic nanoparticles[2, 3]	matrix was deposited with analytes solution	good	very little	localized surface plasmon
WO_3-TiO_2 IOPCs	analytes solution was dripped onto substrate	good	very little	slow photon effect

Reference

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3 Y. Gholipour, S. L. Giudicessi, H. Nonami and R. Errabalsells, Anal. Chem., 2010, 82, 5518-5526.