Electronic Supplementary Information

Synergistic Effect of Ag/MXene for Efficient Protein Ionization in Paper Spray Mass Spectrometry

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Experimental Details

Chemical and Materials. Deionized water was provided by Shanghai LiChen Instrument Technology Co. Methanol was obtained from Fisher Chemical. Formic acid, lysozyme and cytochrome c were bought from MACKLIN (Shanghai, China). Pyrogallol (PG), bicine and β-lactoglobulin (LGB) were purchased from Aladdin (Shanghai, China). $AgNO_3$ was purchased from CHRON CHEMICALS (Chengdu, China). $Ti_3C_2T_x$ (MXene) multilayer nanoflake (specification is shown in the Table 1 and Fig. S1) was bought from Jiangsu XFNANO Materials Tech. Co., Ltd. The Newstar quick qualitative filter paper with a diameter of 7 cm was purchased from Hangzhou Specialty Paper Co., Ltd.

Table 1 Detailed specification information for $Ti_3C_2T_x$ (MXene)

Material Name	$Ti_3C_2T_x$ (MXene) multilayer nanoflake
Thickness	100-200 nm
Diameter	2-10 μm
Purity	56-59 wt%
Ingredient	Ti_3C_2

Structure characterization. The scanning electron microscopy (SEM) images was carried out with TESCAN MIRA LMS scanning electron microscopy. X-ray photoelectron spectroscopy (XPS) was conducted by Thermo Scientific K-Alpha instrument. Hydrophobicity of nanomaterials was measured by JY-82C Contact Angle measuring instrument.

Conductivity test. Electrochemical workstation CHI760e was bought from Shanghai Chenhua Instrument Co. The qualitative filter paper was cut into circular paper base of 2 cm diameter and subsequently 200 μL of 2.5 mg/mL (based on MXene concentrations) AgMX, 2.5 mM (concentration of silver atom) Ag, 2.5 mg/mL MXene and 2.5 mg/mL Mix (based on MXene concentrations) were separately pipetted onto the paper base, which was dry before conductivity test.

Synthetics of Ag/MXene (AgMX) and AgNPs (Ag). AgMX was fabricated through a bio-inspired surface coating method[1]. Phillip B. Messersmith used a simple phenolic mimetic (pyrogallol,

PG) as the sole coating precursor to form colourless multifunctional coatings. This approach is a “green” surface modification strategy with simplicity and versatility. MXene was first suspended in ultrapure water with concentration of 10 mg/mL. Then 500 µL of MXene suspension is centrifuged to remove the supernatant. Next 500 µL 1 mg/mL PG, pH 7.8 (100 mM bicine) was added, and the suspension was sonicated for 40 min. The addition of 500 µL 10 mM $AgNO_3$ was then followed, and the suspension was sonicated for another 30 min. 10 µL of 12 mM acetic acid was added to stop the reaction. The final suspension was centrifugated and washed with 1 mL water several times, and finally suspended in 1 mL ultrapure water as a reserve solution. So the final concentration of AgMX was 5 mg/mL (based on MXene concentrations), and the concentration of silver (Ag) was 5 mM (concentration of silver atom). For comparison experiments, AgNPs were synthesized by the same procedure as preparing AgMX without adding MXene materials. The pure MXene was sonicated for the same time as described above and then used as a stock solution.

Preparation of AgMX, Ag, MXene and mixture of Ag and MXene coated paper. The qualitative filter paper was cut into isosceles triangles (base 9 mm, height 18 mm). 25 uL of 5 mg/mL AgMX (based on MXene concentrations) suspension, 5 mM Ag (concentration of silver atom), 5 mg/mL mixture of Ag and MXene (based on MXene concentrations) and 5 mg/mL MXene was pipetted onto a triangular paper to modify filter paper respectively, which was dry before use.

Paper Spray Ionization Mass Spectrometry (PSI-MS) analysis. Lysozyme, β-lactoglobulin and Cytochrome C were dissolved in methanol/water (1:1) with 0.1% (v/v) formic acid respectively. Paper loaded with AgMX, Ag, MXene and mixture of Ag and MXene are clamped onto conductive copper clips respectively, and then 25 µL of the protein solution was directly pipetted onto the paper to complete the sample addition. All experiments were performed by the Quattro Premier XE triple quadrupole mass spectrometry (Waters Corp). The distance between paper spray tip and MS inlet was about 8 mm and the cone voltages was 100 V. A positive voltage of 4 kV was applied. The data sampling time was set to 3.5 minutes for all proteins. Mass spectra were generated by combining 180 scans over a scanning mass range of 800-2000 for lysozyme and β-lactoglobulin. Mass spectra were generated by combining 180

scans over a scanning mass range of 700-2000 for Cytochrome C. The width of Extracted Ion Chronograms (EIC) was set to 1.0000 Da.

Detection of LGB in urine. The urine sample was taken from a healthy male volunteer and informed consent was obtained from the volunteer. The experiment was performed in compliance with relevant laws and guidelines. The experiment was approved by the Medical Ethics Committee of Guangxi University. LGB of different amounts were added to urine for AgMX PSI. Methanol/water (1:1) with 0.1% (v/v) formic acid was mixed with urine containing different concentrations of LGB at a volume ratio of 960:40. The absolute intensity of $[M + 13H]^{13+}$ (m/z 1416.4) of LGB was selected to make correlation curve.

Optimisation of experimental conditions. As shown in Fig. S3, we mainly optimized the spray voltage, spray solvent and loading concentration of AgMX. For solvent optimization, 0.1% formic acid was added to the four solvents, and the solvents were used to prepare a 0.5 mg/mL lysozyme solution. The protein was then detected using AgMX PSI-MS. For optimizing the voltage, 0.5 mg/mL of lysozyme using methanol/water (1:1) with 0.1% (v/v) formic acid as solvent was detected by varying the voltage (3 kV, 3.5 kV, 4 kV, 4.5 kV). For AgMX concentration optimization, the 25 μL different concentrations (5 mg/mL, 2.5 mg/mL, 1 mg/mL) of AgMX (based on MXene) were dripped uniformly onto paper, which was subsequently dried and used for lysozyme analysis under 4 kV. The intensity of $[M + 10H]^{10+}$ (m/z 1431.6) of lysozyme was calculated for choosing optimal experimental conditions.

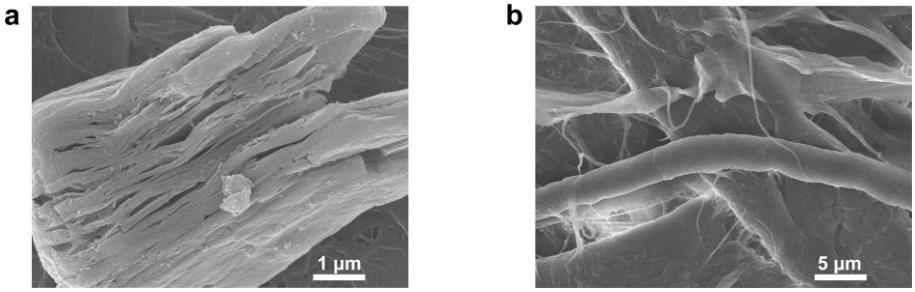

Figure S1. SEM image of (a) $Ti_3C_2T_x$ (MXene) multilayer nanoflake and (b) filter paper before modification.

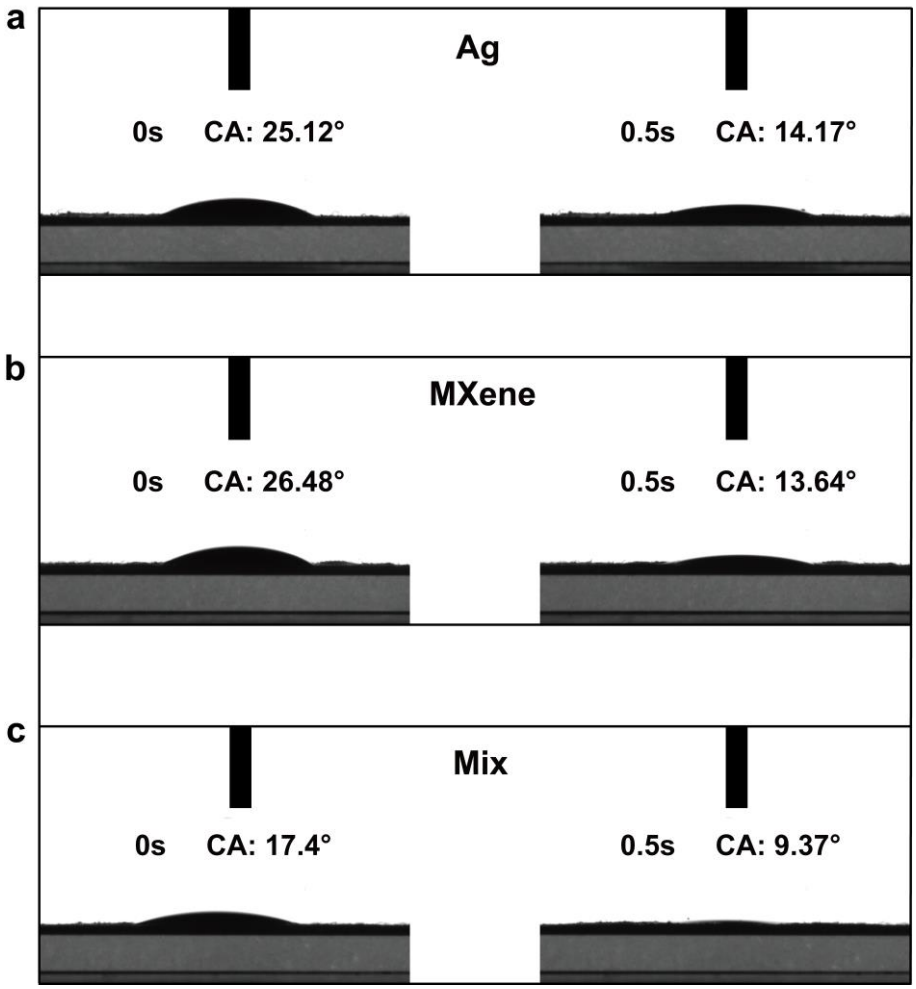

Figure S2. Contact angles of filter paper modified by (a) Ag, (b) MXene and (c) Mix.

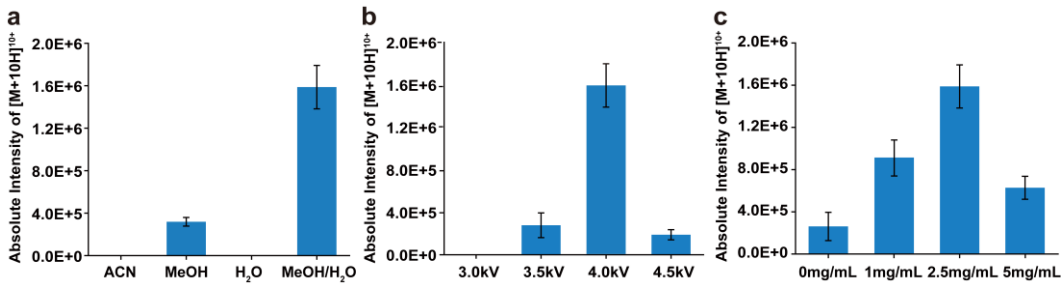

Figure S3. Optimization of (a) spray solvent (No signal using ACN and H_2O), (b) spray voltage (No signal at 3 kV) and (c) AgMX concentration.

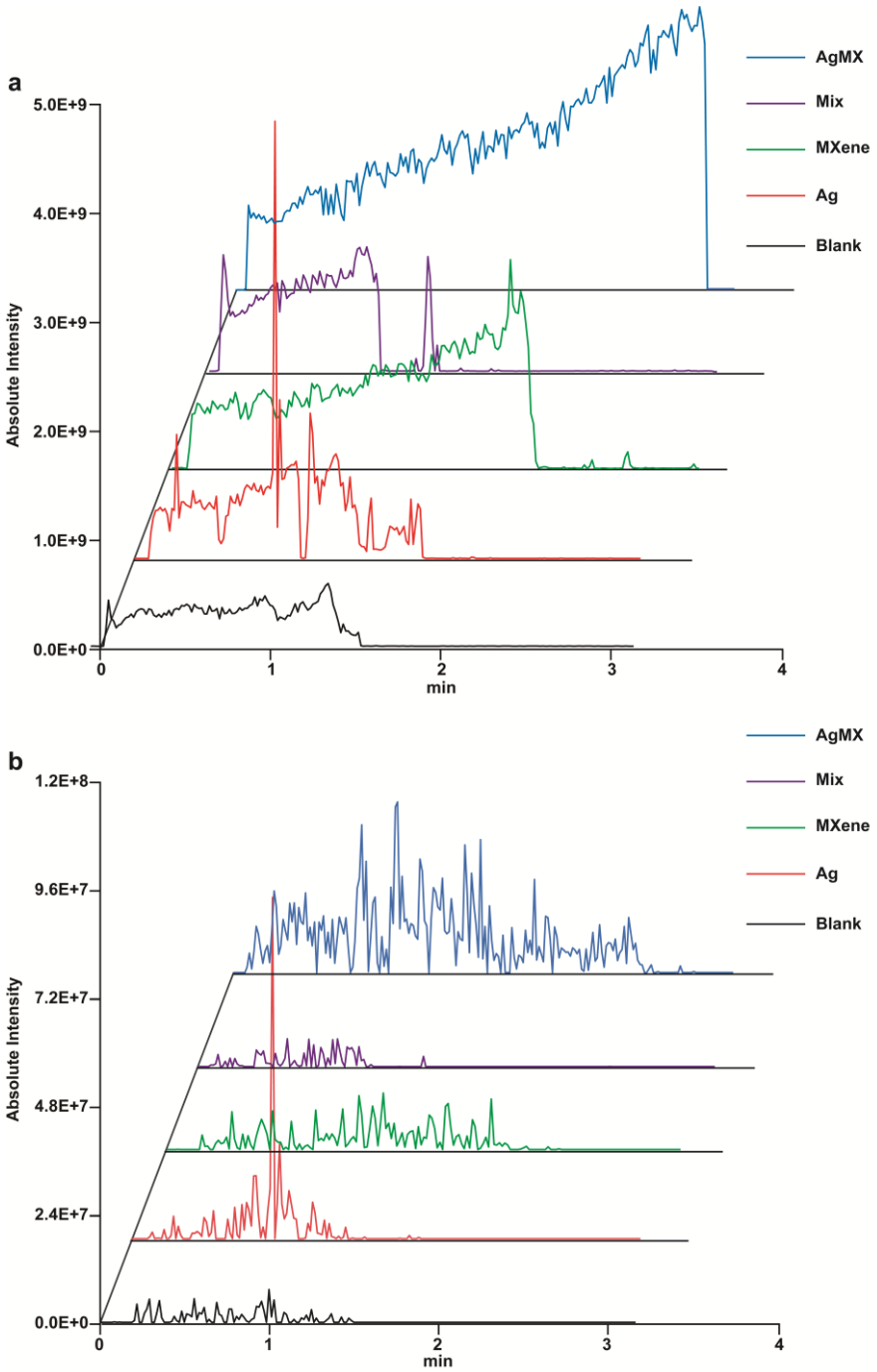

Figure S4. (a) Total Ion Chromatograms (TIC) and (b) Extracted Ion Chronograms (EIC) of lysozyme ($[M + 10H]^{10+}$, m /z 1431.6) detected by conventional filter paper, Ag-loaded paper, MXene-loaded paper, Mix-loaded paper and AgMX-loaded paper.

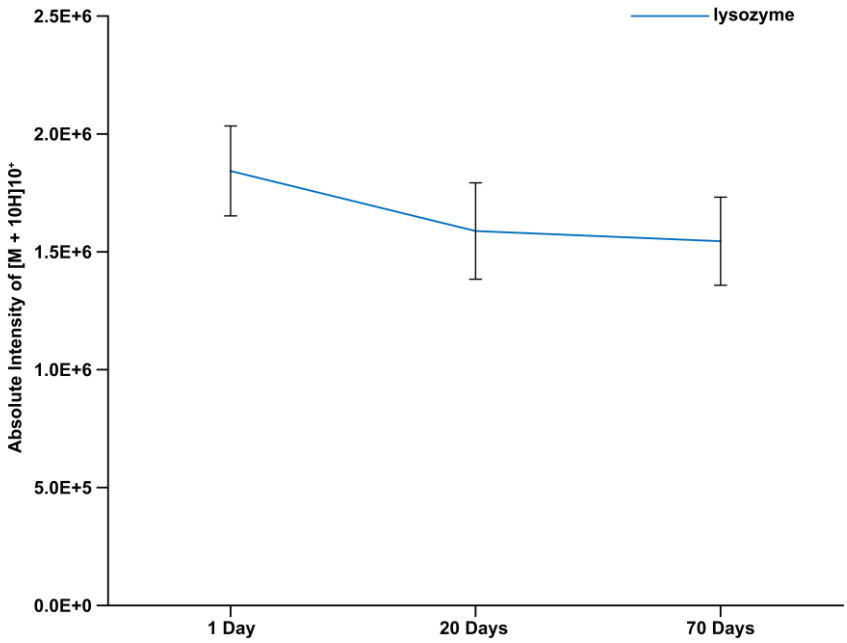

Figure S5. Stability of AgMX for protein measurements by PSI-MS over 70 days. (The error bars indicate the standard error of five replicates.)

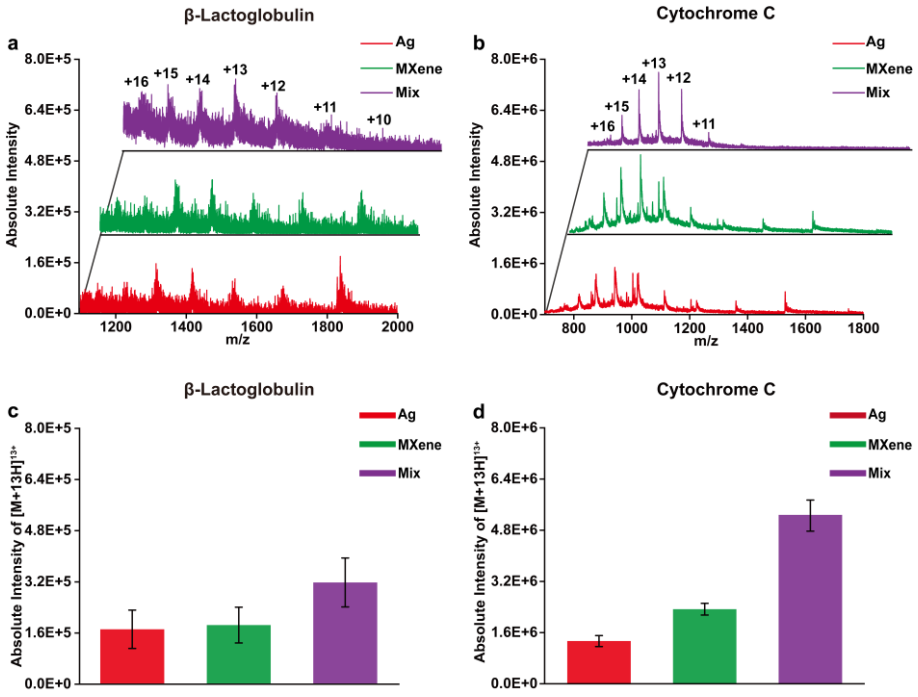

Figure S6. Mass spectra of (a) LGB and (b) Cytochrome C detected by Ag-loaded paper, MXene-loaded paper and Mix-loaded paper. Absolute intensity of $[M + 13H]^{13+}$ (m/z 1416.4) of (c) LGB and $[M + 13H]^{13+}$ (m/z 941.4) of (d) Cytochrome C measured by Ag-loaded paper, MXene-loaded paper and Mix-loaded paper. (The error bars indicate the standard error of five replicates.)

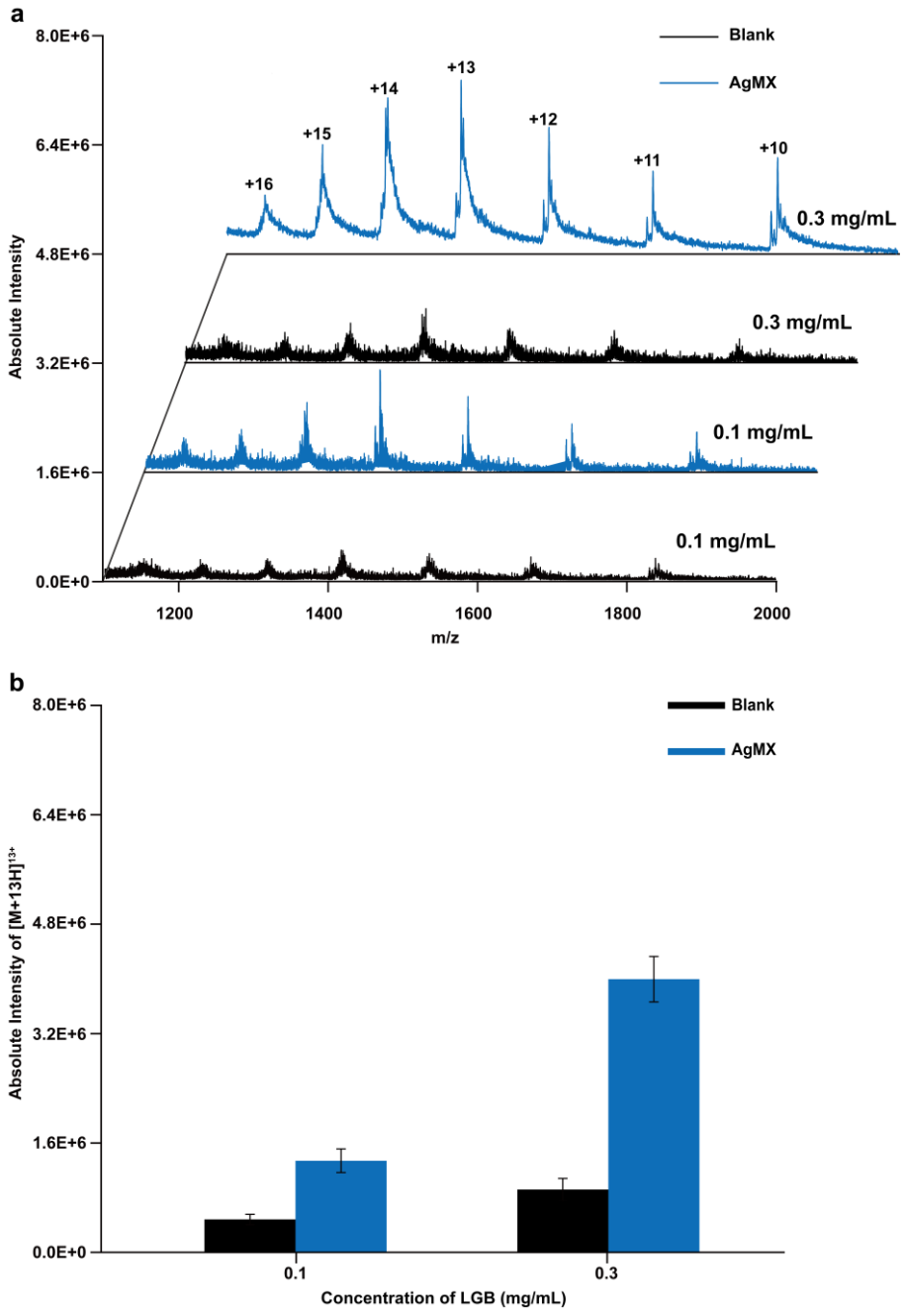

Figure S7. (a) Mass spectra and (b) absolute intensity of $[M + 13H]^{13+}$ (m/z 1416.4) of LGB at 0.1 mg/mL and 0.3 mg/mL detected by conventional filter paper and AgMX-loaded paper.

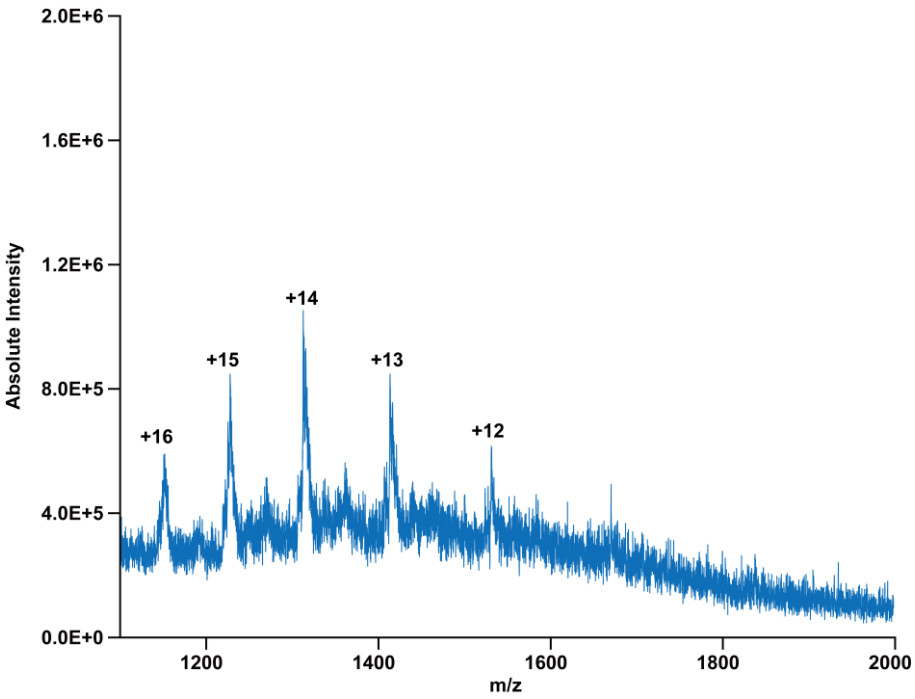

Figure S8. AgMX PSI-MS of 0.03 mg/mL LGB.

Table 2 Coefficient of variation (CV) for the signal replicates for the same sample.

		Blank	AgMX	Ag	MXene	Mix
CV (%)	Lysozyme	51.30	12.88	25.96	11.39	N
	β-lactoglobulin	11.15	16.64	35.50	30.64	24.23
	Cytochrome C	9.14	4.83	12.95	7.91	9.15

(N means no protein signal.)

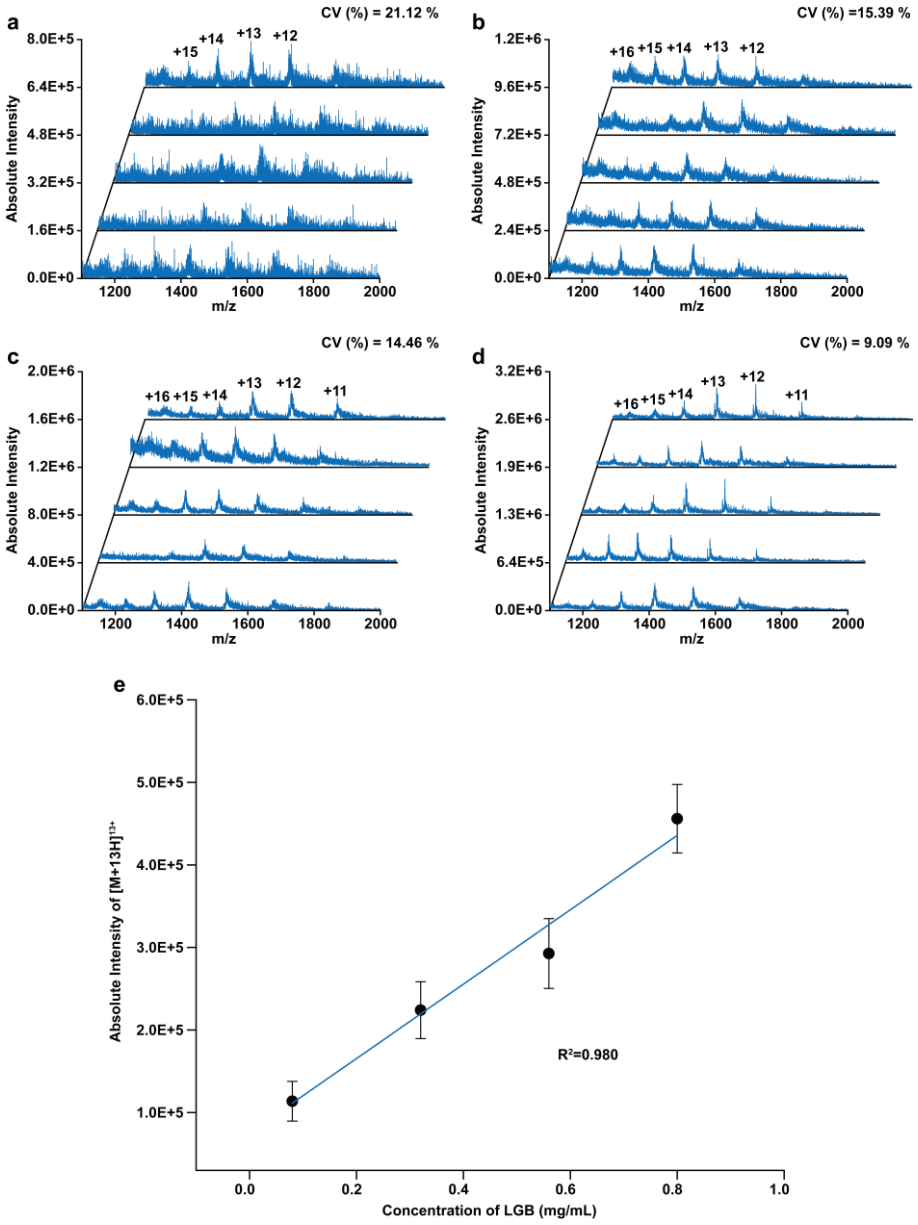

Figure S9. Mass spectra of repeated tests of (a) 0.08 mg/mL, (b) 0.32 mg/mL, (c) 0.56 mg/mL and (d) 0.8 mg/mL LGB in urine. (e) Semi-quantitative curve of LGB in urine. (The error bars indicate the standard error of five replicates.)

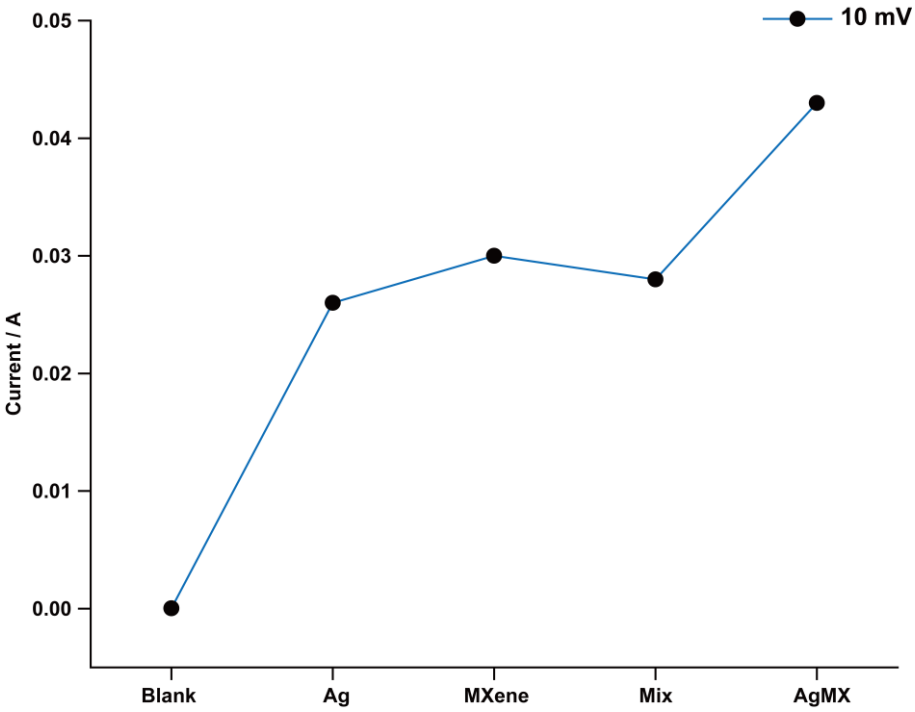

Figure S10. Conductivity test on filter paper modified by different materials.

References

1 T. S. Sileika, D. G. Barrett, R. Zhang, K. H. A. Lau and P. B. Messersmith, *Angewandte Chemie International Edition*, 2013, 52, 10766-10770.