Electronic supplementary information

Development of the C12Im-Cl -assisted method for rapid sample preparation in proteomic application

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Extraction of FFPE tissue proteins

A FFPE cancer tissue section was put into in a polypropylene Eppendorf tube. Then incubated with xylene twice for 15 min each time for deparaffinization. Then the following series of ethanol bath was performed for 15 min respectively: 100% (v/v), 95% (v/v), 70% (v/v), and 50% (v/v) ethanol. Lysis buffer (4% (w/v) C12Im-Cl, 100 mM dithiothreitol (DTT), 1% (v/v) protease inhibitor cocktail, 100 mM Tris-HCl, pH 8.0) was added to the deparaffinized FFPE tissues, and the sample was placed in constant temperature shaking on the device (600 rpm). It was heated at 99°C to de-crosslink for 60 min. The sample was sonicated on ice at 200 W for 2 s with a 1-s

break for a total of approximately 5 min, and then centrifuged at 4°C for 15 min at 14,000g. The supernatant was transferred to the tube and the protein concentration was measured with the BCA kit (Thermo Fisher Scientific, Waltham, MA, USA).

The traditional filter-aided sample preparation (FASP) method

20 μg proteins in a 4% (w/v) C12Im-Cl lysis buffer from liver cancer FFPE tissue were added to a 30 KD ultrafiltration tube. After centrifugation at 14,000g for 15 min, 200 μL urea was added, and the buffer was separated by centrifugation at 14,000g for 15 min. Another 200 μL aliquot of 8 M urea was added to the ultrafiltration tube. Subsequent to the reduction reaction, 100 μL of 20 mM DTT dissolved in 8 M urea was added and incubated at 37 °C for 4 h. After completion of the reduction reaction, the sample was centrifuged at 14,000g for 15 min to remove the waste liquid. 100 uL of 50 mM IAA dissolved in 8 M urea was added to the ultrafiltration tube at room temperature for 30 min in the dark for alkylation reaction. After the alkylation reaction was completed, the sample was centrifuged at 14,000g for 15 min to remove the waste liquid. After 15 min of reaction in the dark, 100 μL of 20 mM DTT was added to the ultrafiltration tube, which was then centrifuged at 14,000g for 15 min, and the waste liquid removed. A further 200 μL of 8 M urea was added to the ultrafiltration tube, which was centrifuged at 14,000g for 15 min, and the process was repeated once to remove excess reagents. 200 μL of 50 mM NH_4HCO_3 was added to the ultrafiltration tube, and after centrifugation at 14,000g for 15 min, this step was repeated, to replace the urea. After the new cannula was placed, 150 uL digestion solution (0.4 μg trypsin and 1 mM calcium chloride in 50 mM NH_4HCO_3 buffer) was added to the ultrafiltration tube and reacted overnight (16 h) in a 37°C incubator. After the overnight reaction was complete, the sample was centrifuged at 14,000g for 15 min to collect the centrifugal fluid. The peptide mixtures were lyophilized and re-dissolved in 0.1% (v/v) formic acid before MS analysis.

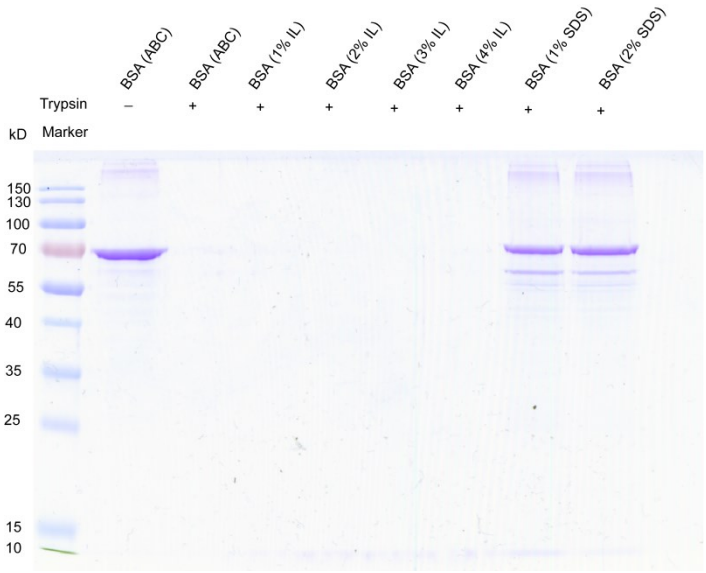

Fig. S1 Digestion of Bovine albumin in different concentrations of ionic liquids and SDS, with 50 mM ammonium bicarbonate buffer as the control experiment.

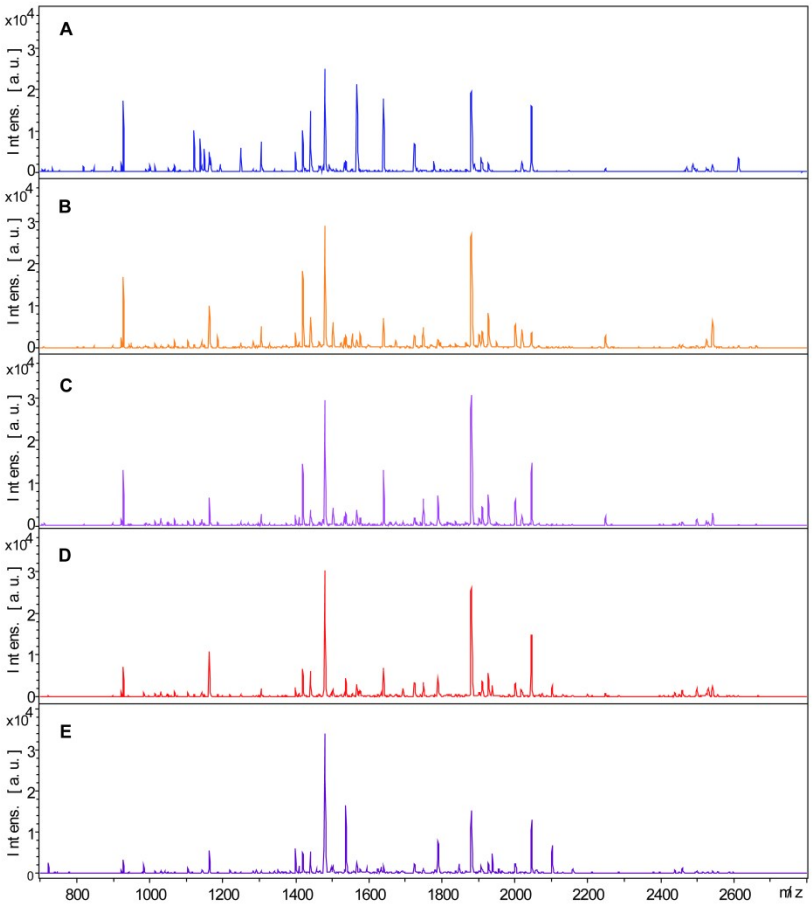

Fig. S2 MALDI-TOF MS spectra of BSA product digested with trypsin (A) in 50 mM NH_4HCO_3, (B) with addition of 1% (w/v) C12Im-Cl, (C) 2% (w/v) C12Im-Cl, (D) 3% (w/v) C12Im-Cl, and (E) 4% (w/v) C12Im-Cl.

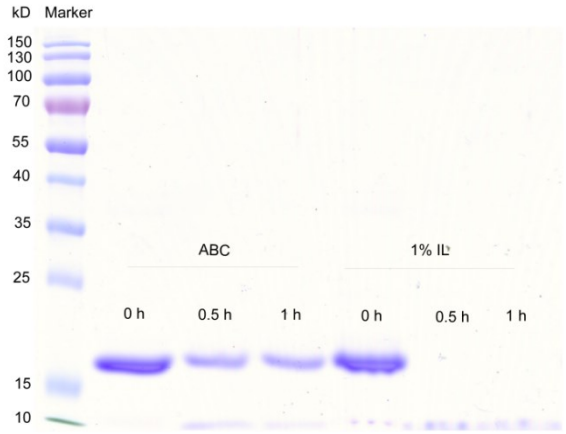

Fig. S3 Digestion of myoglobin monitored by SDS-PAGE and Coomassie blue staining.

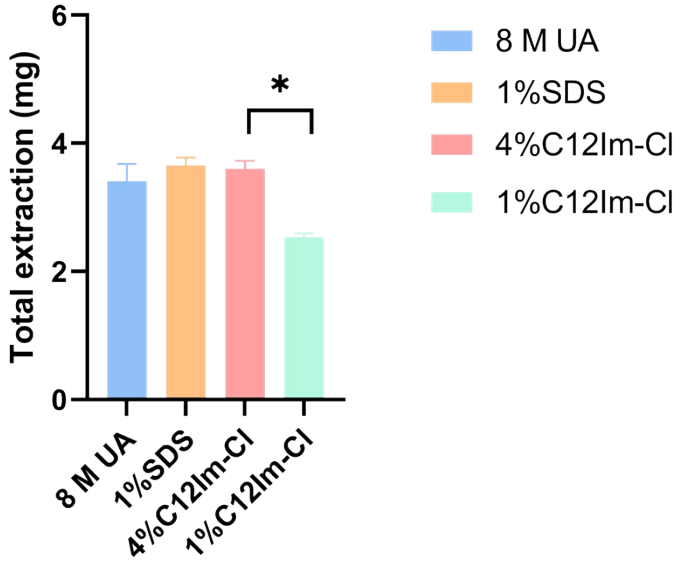

Fig. S4 Total proteins extraction from HeLa cells in different solubilizer systems, ∗, $p<0.05$, two-tailed, unpaired T-test.