Electronic Supplementary information

Experimental

Chemicals and standards

LC-grade solvents (water, acetonitrile, methanol, ethanol) were purchased from Merck. Leucine enkephalin was purchased from Waters UK (Waters Corporation, Wilmslow, UK). Formic acid, bradykinin, angiotensin I, ubiquitin, beta-casein, and haemoglobin standards were purchased from Merck.

Radiofrequency-argon plasma

A custom handpiece (Ambimass, HU) with a corona pin was connected to a ForceTriad™ Energy Platform (Medtronic, Minneapolis, USA). The return electrode (Medtronic) was placed under a rubber return pad, with glass slides placed on top of the pad. The generator was run in 'coag spray' mode at power level 120 (representing approximately 5 kV peak to peak), and the distance between the corona pin tip and the glass slide was 12 mm. The activation of the device with 0.1 bar argon flowing generated a purple plasma, which covered a roughly 30 mm diameter area directly below the corona tip. Aqueous standards (1 µL at a concentration of 1 mg/mL) were applied to glass slides and allowed to dry. Leucine enkephalin was exposed to the plasma for 30 seconds continuously. Plasma was applied to ubiquitin, angiotensin I, beta-casein, and haemoglobin in pulses of around 1 Hz for 30 seconds. Standards were then dissolved in 3x20 µL 0.1% formic acid in water and stored in low volume insert vials before injection by LC-MS.

Mass spectrometry

LC-MS experiments were carried out using a Waters Acquity M-Class system (Waters Corporation, Wilmslow, UK) coupled to a Bruker 7T solariX FT-ICR (Bruker Daltonics, Bremen, DE; resolving power ≈ 80,000 FWHM at 662 m/z) in positive mode. 1 µL of sample was loaded onto a Kinetex 2.6 µm 100 Å XB-C18 150 x 0.3 mm column (Phenomenex, Torrance, US), with solvent A being 0.1% formic acid in water and solvent B being 0.1% formic acid in acetonitrile. The chromatography followed a 30 minute gradient at 10 µL/min, beginning at 3% solvent B and ramping to 60% over 20 minutes, followed by a 2 minute flush at 80% solvent B and a re-equilibration of 3% solvent B for 8 minutes.

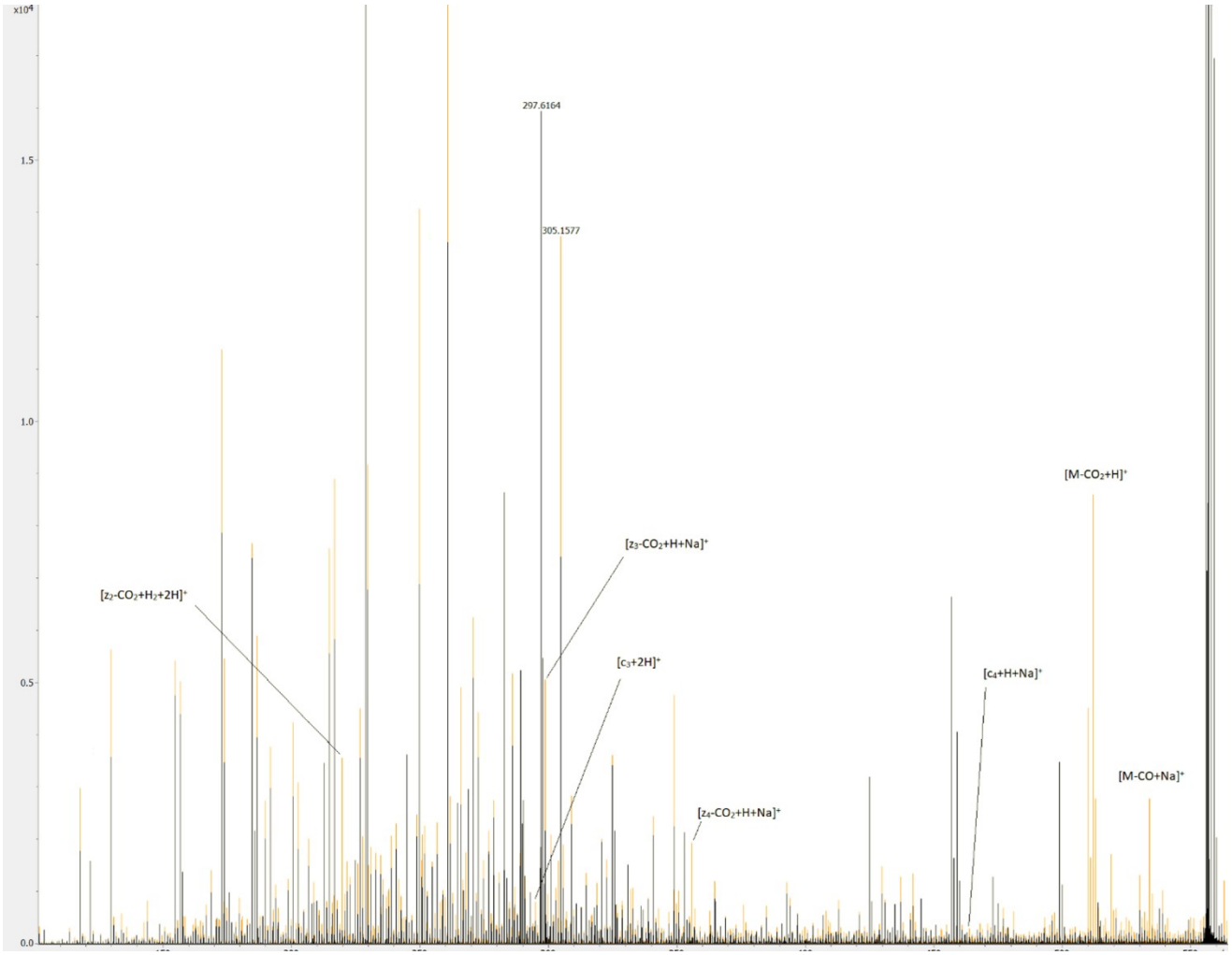
Time	*Solvent A (H_2O)*	*Solvent B (ACN)*
0.00	97	3
20.00	40	60
20.01	20	80
22.00	97	3
30	97	3

LC-MS gradient for all experiments.

For ECD of haemoglobin, a 5 mg/mL solution of haemoglobin in 50% acetonitrile with 0.1% formic acid was injected by direct infusion at a rate of 2 µL/min. ECD pulses were applied for 0.006 seconds. For ECD of ubiquitin, a 1 mg/mL solution of ubiquitin in 50% acetonitrile with 0.1% formic acid was injected by direct infusion at a rate of 2 µL/min. ECD pulses were applied for 0.03 seconds.

Direct infusion and CID MS/MS experiments were carried out using a Bruker TIMS-ToF fleX (Bruker Daltonics, Bremen, DE; resolving power ≈ 45,000 FWHM at 556 m/z) in positive mode. For CID, the isolation window was 0.5 m/z and the collision energy was 25 eV.

Data was analysed in Compass DataAnalysis (Bruker). Chromatograms were integrated and mass lists generated using the SNAP algorithm. Identified peaks were then annotated using Protein Prospector (UCSF, http://prospector.ucsf.edu) for terminal ions, and with an in-house script for internal ions.



Supplementary 1.1: Overlaid direct infusion mass spectra of leucine enkephalin after exposure to the radiofrequency cold argon plasma. The control spectrum is black and the 30 second exposure spectrum is orange.

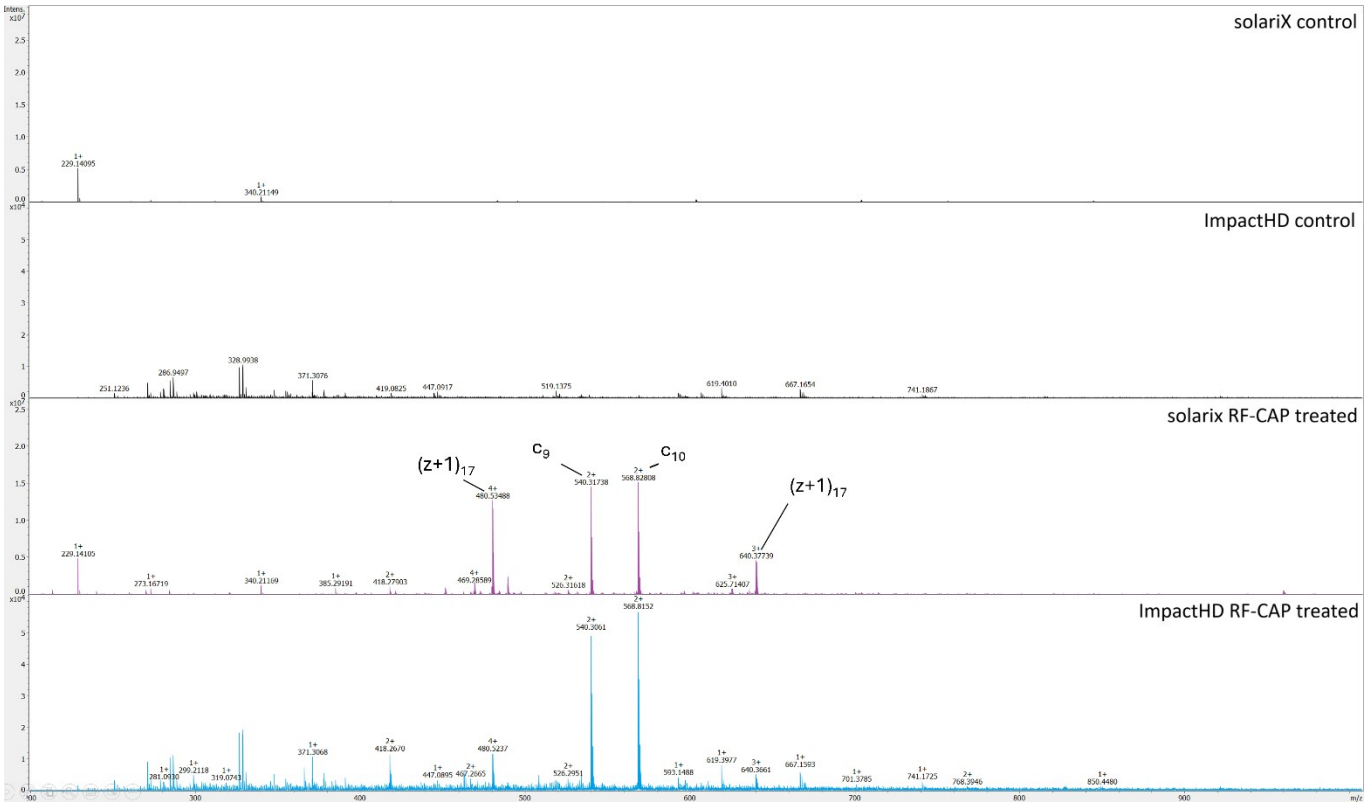
Data

Peak	Error (ppm)	Annotation	0 s	5 s	30 s	60 s
556.28	-0.50	$[M+H]^+$	2.9e5	5.3e5	2.7e5	5.4e4
550.26	-3.63	$[M-CO+Na]^+$	-	5.3e2	5.6e2	-
534.27	-0.19	$[M-CO_2+Na]^+$	-	6.7e2	1.2e3	1.4e2
532.25	1.88	$[M-CO_2-H_2+Na]^+$	-	1.9e2	2.7e2	-
528.28	1.89	$[M-CO+H]^+$	-	1.8e2	1.5e2	3.7e1
512.29	0.59	$[M-CO_2+H]^+$	-	3.0e3	3.0e3	9.1e2
510.27	0.20	$[M-CO_2-H_2+H]^+$	-	2.2e3	1.7e3	4.8e2
464.19	0.00	$[c_4+H+Na]^+$	-	2.7e2	3.8e2	2.4e1
442.21	0.00	$[c_4+2H]^+$	1.9e1	1.5e3	1.3e3	1.6e2
400.18	2.25	$[z_4+Na]^+$	6.0e0	4.8e2	6.1e2	2.1e1
356.19	0.28	$[z_4-CO_2+H+Na]^+$	-	1.9e3	4.3e3	-
332.20	-3.91	$[z_4-CO_2+2H]^+$	-	2.0e2	2.3e2	-
299.17	1.00	$[z_3-CO_2+H+Na]^+$	-	3.2e3	8.0e3	-
295.14	1.69	$[c_3+2H]^+$	-	1.3e3	1.2e3	8.4e1
277.19	-1.44	$[z_3-CO_2+2H]^+$	-	9.2e2	1.2e3	3.1e1
264.16	-1.89	$[z_2+2H]^+$	-	1.4e2	1.0e2	-
242.15	-0.83	$[z_2-CO_2+H+Na]^+$	-	6.2e2	1.9e3	1.1e2
238.12	2.52	$[c_2+2H]^+$	-	9.4e2	1.0e3	-
235.18	2.55	$[y_2-CO_2+2H]^+$	-	3.8e2	3.5e2	3.3e1
220.17	-1.36	$[z_2-CO_2+2H]^+$	1.5e1	8.5e2	1.2e3	1.8e2
218.15	4.13	$[z_2-CO_2-H_2+2H]^+$	-	1.9e2	2.0e2	7.0e1
193.10	4.14	$[a_2]^+$	-	1.4e2	1.5e2	3.0e1
122.10	-4.10	$[Phe-CO_2+H]^+$	9.0e0	4.3e1	3.0e1	1.8e1

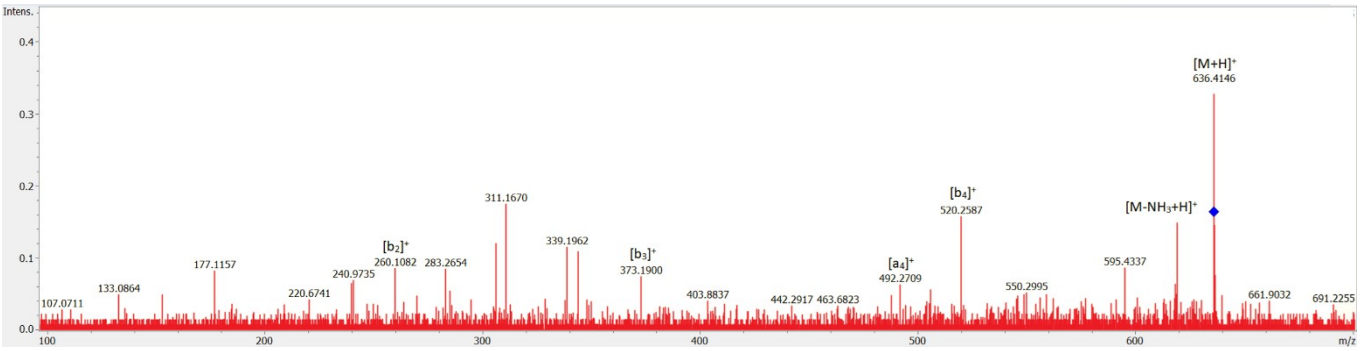
Supplementary 1.2: Notable fragments, their annotations, and their absolute intensities from direct infusion analysis of leucine-enkephalin exposed to the RF-AP source. Mass error is calculated from the 30s peak.

Peak	Error (ppm)	Annotation	Control	5 secs	30 secs	1 min
1060.57	-1.23	$[M+H]^+$	5.1e5	4.3e3	9.0e2	5.1e2
530.79	-1.88	$[M+2H]^{2+}$	1.3e6	3.1e5	1.0e5	5.4e5
572.33	-3.49	$[c_5+2H]^+$	2.0e2	6.8e2	4.1e2	1.2e3
491.26	-2.85	$[z_4+2H]^+$	1.6e2	5.0e2	2.9e2	7.1e2
452.25	-0.44	$[c_8+3H\ ]^{2+}$	-	2.9e2	1.2e2	3.8e2
447.27	-3.35	$[z_4\text{-}COOH+3H]^+$	-	1.9e3	9.9e2	4.2e3
425.26	-4.23	$[c_4+2H]^+$	2.0e2	3.5e2	3.3e2	7.2e2
378.71	-2.11	$[c_7+3H]^{2+}$	5.2e1	1.6e2	1.3e2	4.3e2
307.18	-3.58	$[z_2+2H]^+$	-	1.2e2	2.7e1	1.9e2
297.66	-3.36	$[c_5+2H+Na]^{2+}$	1.4e1	2.6e1	1.9e1	4.6e1
286.67	-2.09	$[c_5+3H]^{2+}$	2.3e1	1.7e2	1.4e2	4.5e2

Supplementary 1.3: Notable fragments, their annotations, and their absolute intensities from direct infusion analysis of bradykinin exposed to the RF-AP source. Mass error is calculated from the 30s peak.



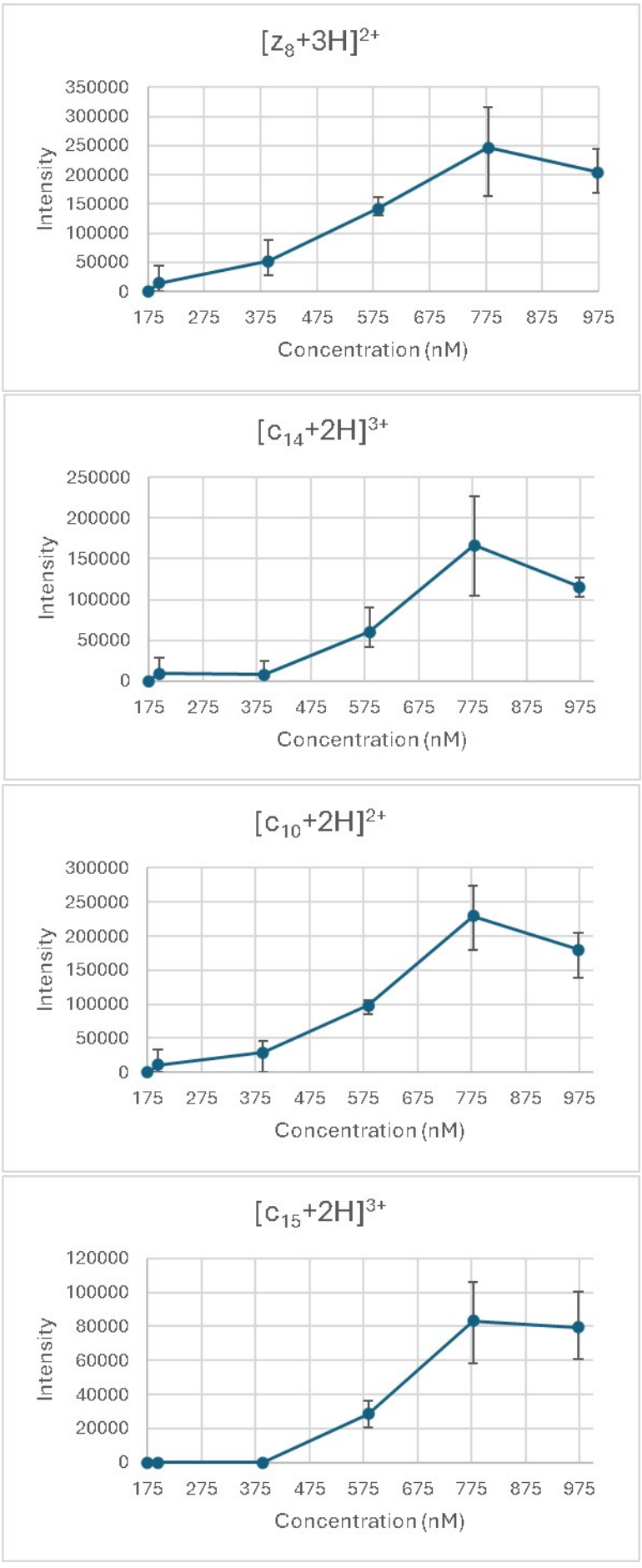
Supplementary 1.4: Mass spectra showing analytes eluting at ~8.0-8.5 minute retention time for ubiquitin control (top) and after exposure to plasma (bottom). The purple spectrum was recorded on a Bruker 7T solariX FT-ICR, and the blue spectrum was recorded on a Bruker Impact HD (Bruker Daltonics, Bremen, DE; resolving power ≈ 8500 FWHM at 540 m/z) approximately four months later. Peaks at 480.5, 540.3, 568.8, and 640.4 are annotated as $(z+1)_{17}$, c_9, c_{10}, and $(z+1)_{17}$ respectively.



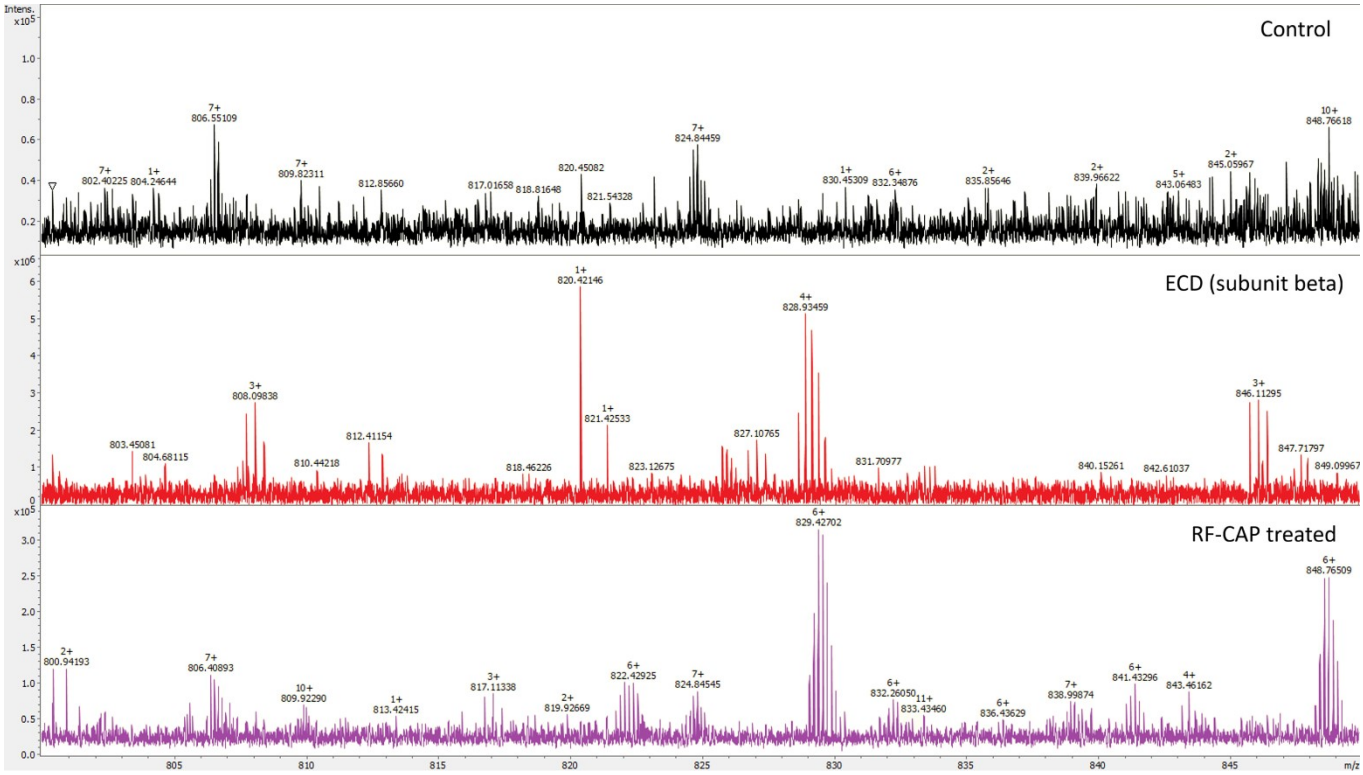
Supplementary 1.5: Annotated CID spectrum of plasma-exposed ubiquitin product peak at 636.35 m/z (annotated as $[c_5+2H]^+$). A series of b-ions is identifiable.

Concentration (nM)	$[z_8+3H]^{2+}$	$[c_{10}+2H]^{+}$	$[c_{14}+2H]^{+}$	$[c_{15}+2H]^{+}$
973	243405	194389	126723	100475
973	168084	205313	103583	77128
973	201300	139396	115893	60751
778	163944	179527	104009	58567
778	316045	273297	226781	106044
778	259876	235129	169428	85119
584	162294	103563	41859	20576
584	133998	105004	90514	36045
584	130032	85741	48307	29108
389	39971	40882	0	0
389	89225	45814	24688	0
389	28180	0	0	0
195	0	0	0	0
195	0	0	0	0
195	44819	33179	28645	0
175	0	0	0	0
175	0	0	0	0
175	0	0	0	0

Supplementary 1.6a: Concentration curve demonstrating intensities of selected fragments. Fragments were not detected below 195 nM (195 fmol injection on column).



Supplementary 1.6b: Average intensity graphs of selected fragments in concentration curve.



Supplementary 1.7: Comparison of 800-850 m/z range of untreated haemoglobin (top, LC-MS, 11.71-11.95 minutes), ECD spectrum of haemoglobin (middle), and haemoglobin post-plasma (bottom, LC-MS, 11.71-11.95 minutes). Several new peaks are present in the post-plasma spectrum, of which the peaks at 809, 822, 829, 838, 841, and 843 were not annotatable as abc-xyz fragments. There is a great wealth of new fragments unique to haemoglobin throughout the chromatogram.

Supplementary 1.8: Comparison of 875-925 m/z range of untreated beta-casein (top) and RF-CAP-treated beta-casein (bottom) at ~8.35-8.50 min retention time. The phosphorylated fragment is highlighted in red. The neutral -HPO_3 loss at 856.45 is absent in the spectra and across the entire chromatogram.
Insert: Comparison of untreated beta-casein (top), RF-CAP-treated beta-casein (middle), and simulation of $[c_{15}+2H]^{2+}$, incorporating phosphorylated Ser_{15}; the isotope distribution for this product shows good agreement with predictions (-0.56 ppm error).
Diagram: Proposed structure for $[c_{15}+2H]^{2+}$.