Native LESA-TWIMS-MSI: spatial, conformational and mass analysis of proteins and protein complexes.

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Supporting Information

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Table S1: Manual quadrupole profile. Times given are a percentage of each TOF scan (5 s)

m/z	Dwell Time (%)	Ramp (%)
1000	2	40
3000	3	55
4500	-	-

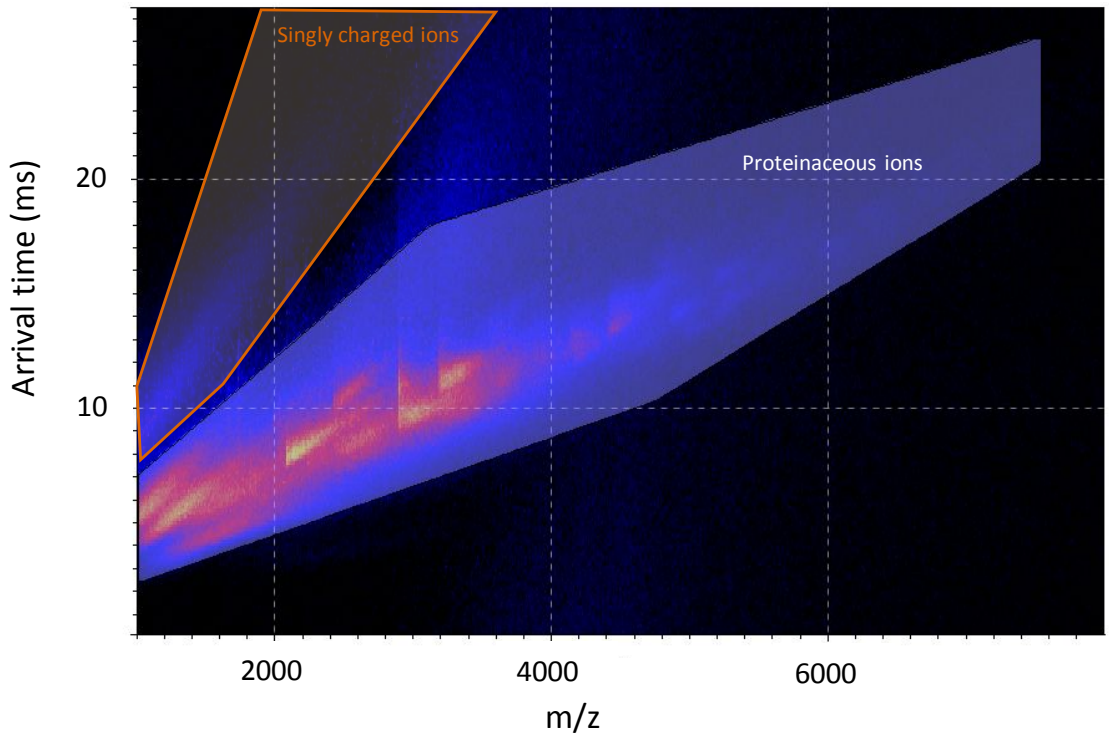

Figure S1: 2D heatmap plot of arrival time vs. m/z. Broad filtering arrival time selection rule for broad selection of proteinaceous ions is shown highlighted in blue. Singly-charged ion region is indicated in orange. The selection rule was exported as a text file and imported into in-house software and applied to each data file (pixel) in the imaging dataset.

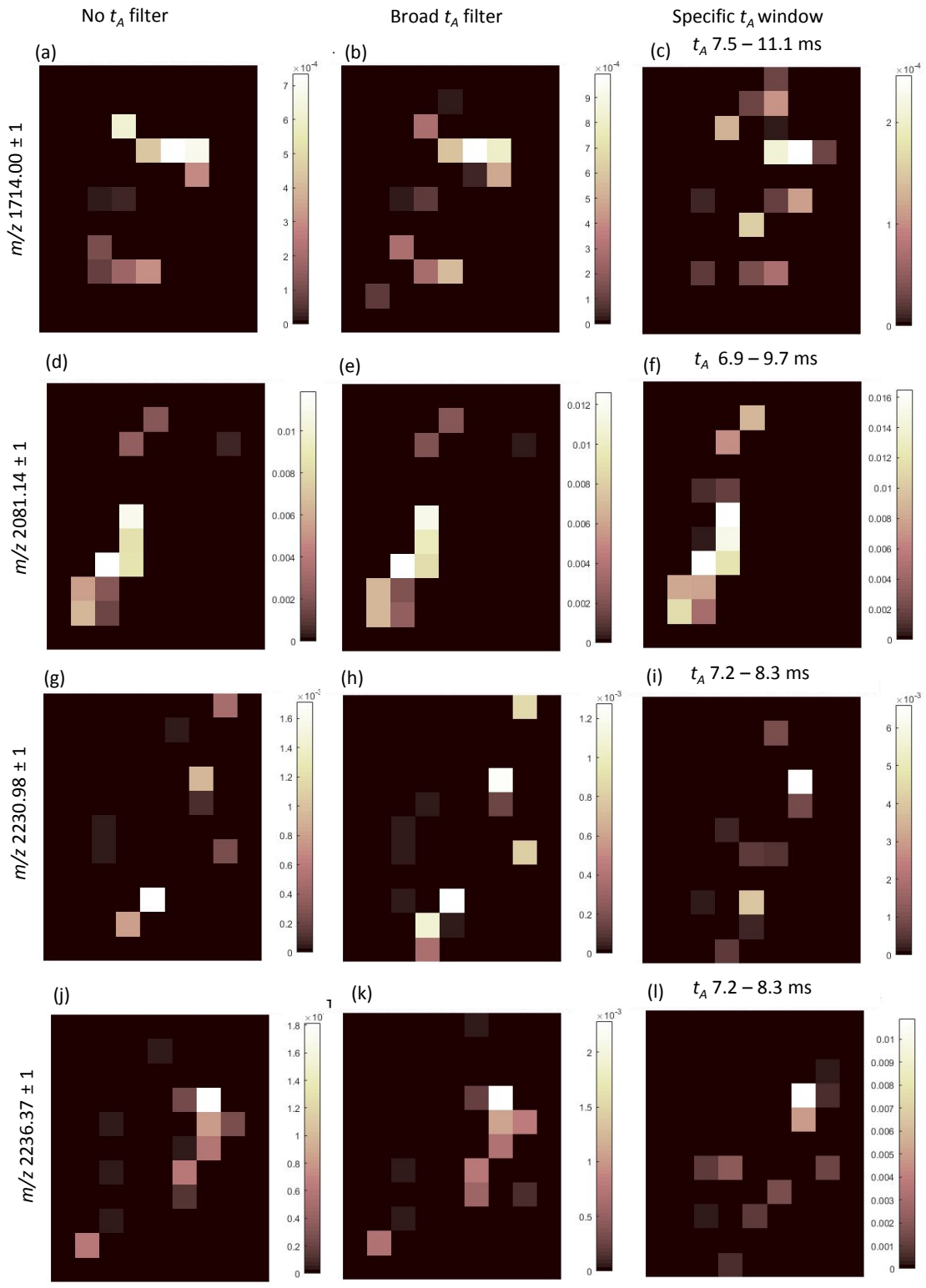

Figure S2: Ion images for small proteins detected by LESA-TWIMS-MSI. Ubiquitin (8560 Da, m/z 1714, 5^+ charge state) was found to be generally homogenously distributed, particularly when specific t_A filtering was performed. The unknown protein with m/z 2081 (~ 14568 Da, 7^+ charge state) was distributed towards the left side of the image, coinciding with location of the large blood vessels. CID MS/MS (not shown) of the protein suggests the presence of heme, i.e., that it is a haemoglobin-related species. The ions with m/z 2230.98 and m/z 2236.37 (unknown mass; charge state unresolvable) featured more intense signals in the renal cortex.

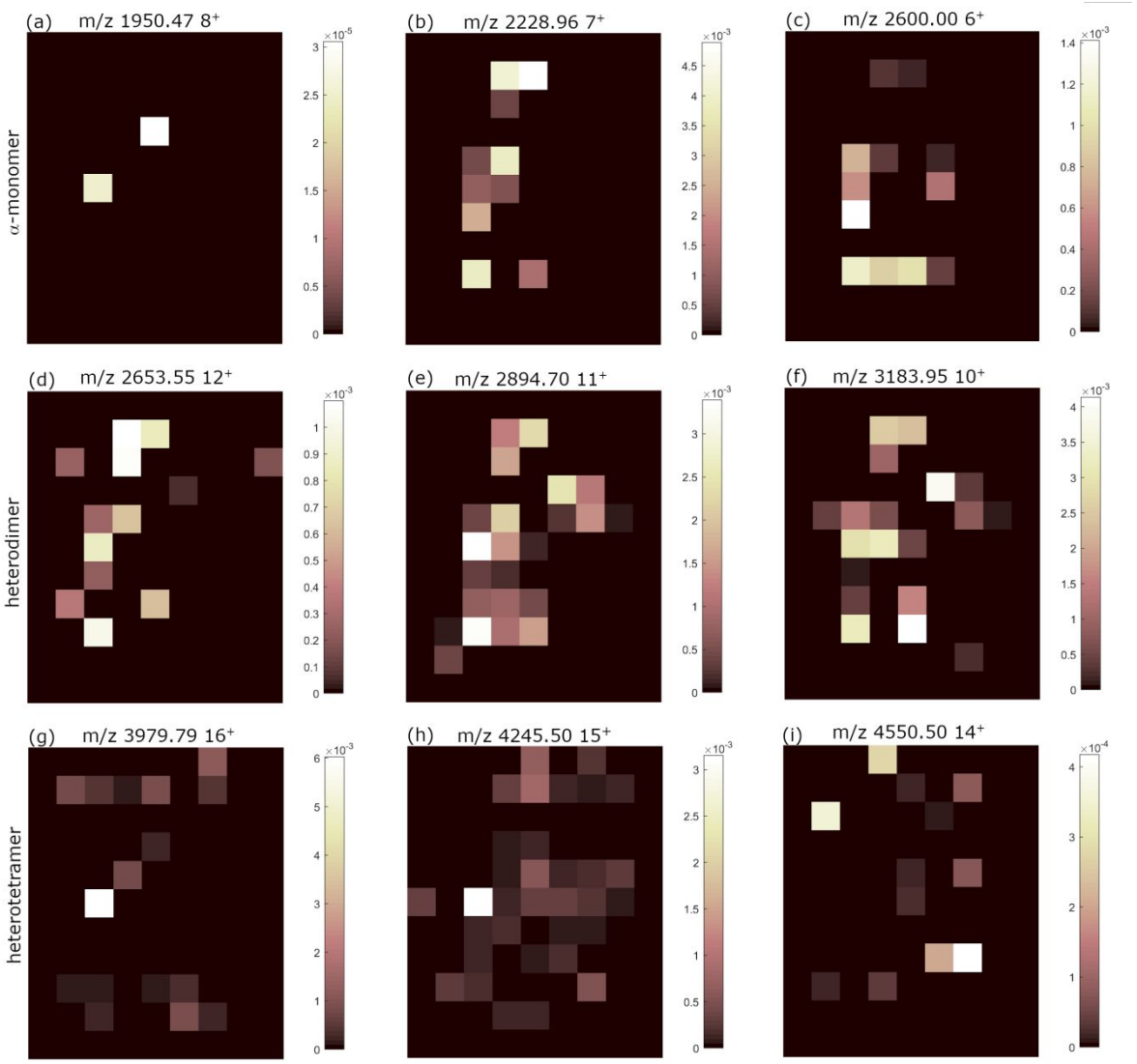

Figure S3: Arrival time filtered images for hemoglobin ions. (a-c) heme-bound α-monomer 8^+–6^+, (d-f) heterodimer 12^+–10^+, (g-i) heterotetramer 16^+–14^+. The middle column represents the image for the most intense charge state.

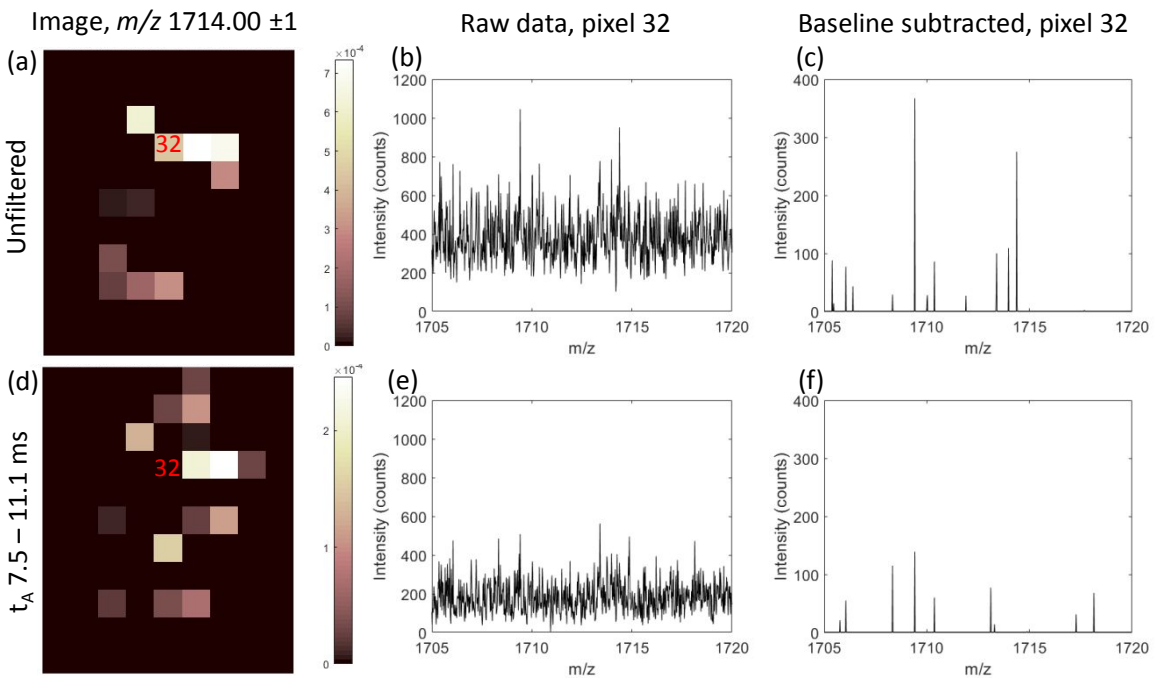

Figure S4: Unfiltered and t_A filtered ion images for ubiquitin $[M+5H]^{5+}$. The unfiltered image (a) features an intense signal for the selected m/z in pixel 32, whereas the arrival time filtered image does not (d). In the raw mass spectra (b, e), peaks for ubiquitin $[M+5H]^{5+}$ were not present, yet the noise in the unfiltered mass spectrum results in peaks in the baseline-subtracted mass spectrum (c). That was not the case for the t_A filtered mass spectrum (f).

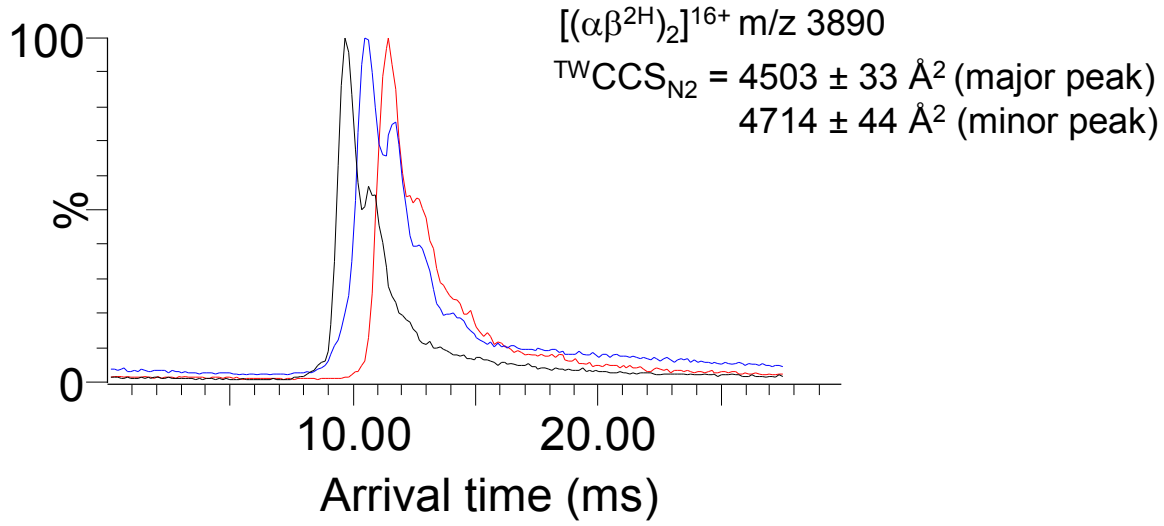

Figure S5: Arrival time distribution for 16^+ Hb tetramer ions measured at three wave heights, 24 V (red), 25 V (blue) and 26 V (black). A minor peak with a later arrival time was detected, corresponding to a 200 Å^2 larger $^{TW}CCS_{N2\rightarrow N2}$ than for the major peak.