Supplementary Information (SI) for Analyst.

Supporting Information for: Mass spectrometry reflects key aspects of copper-amyloid β chemistry

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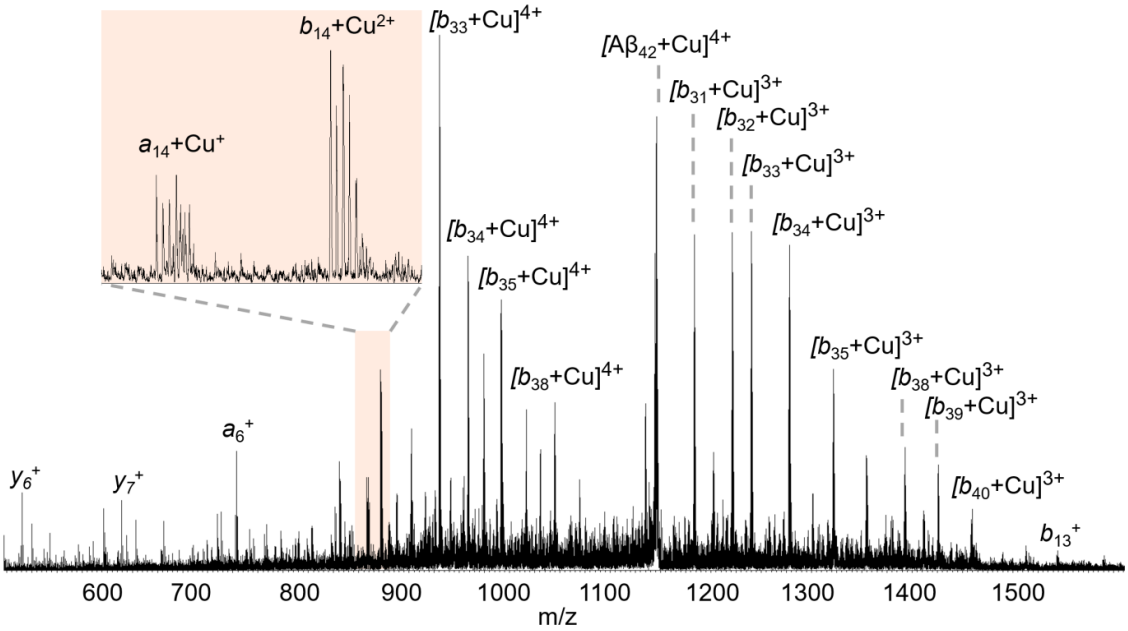

Figure S1. CID spectrum of [Aβ42+2H+Cu(II)]$^{4+}$ at a collision potential of 32 V (normalised collision energy *ca.* 0.03 eV/Da), showing similar results as in (Everett et al. 2021).

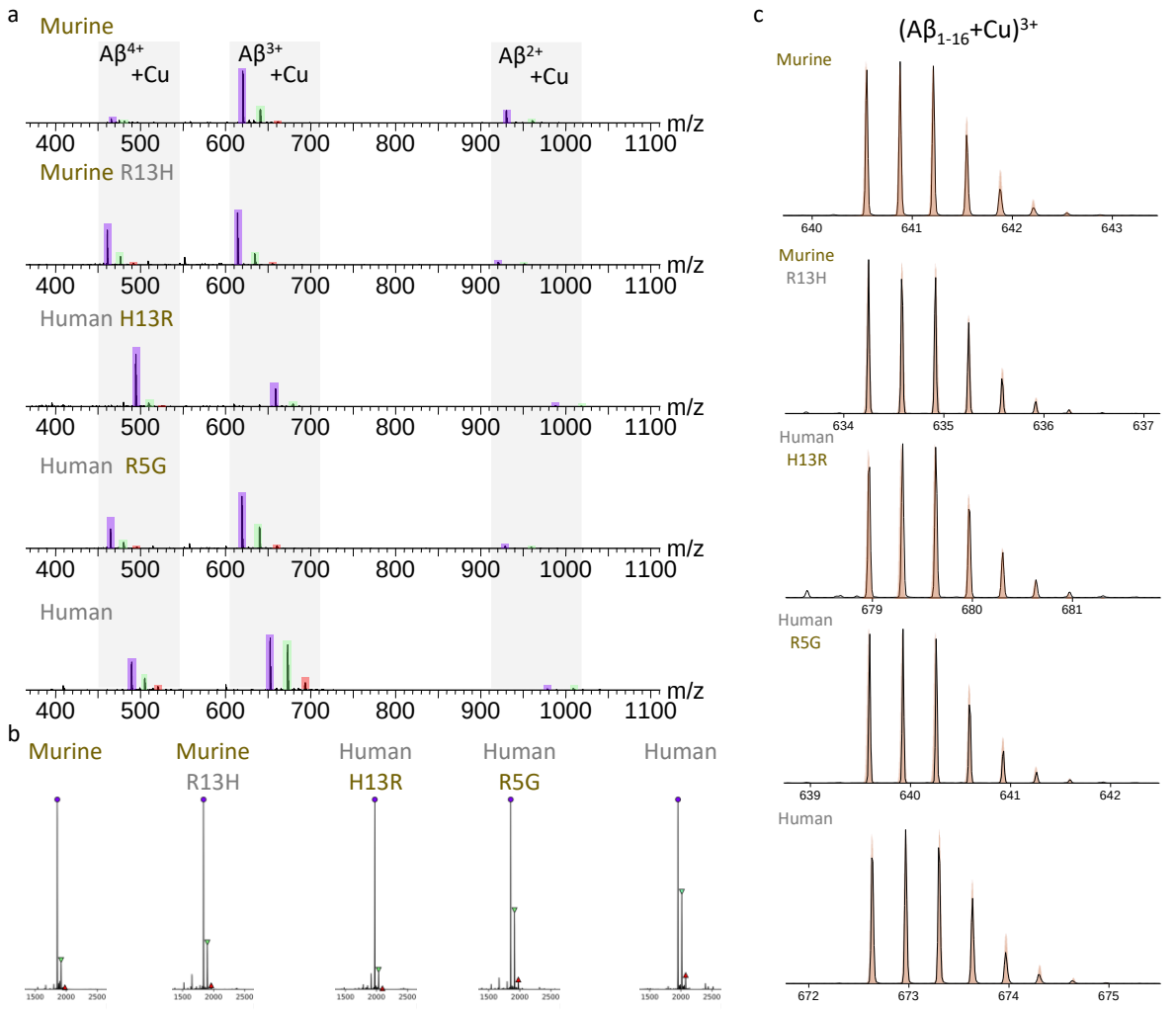

Figure S2. (a) Full MS spectra of 10 µM $A\beta_{1\text{-}16}$ construct and 5 µM $CuSO_4$. The metal-free (violet), Cu-bound (green), and double Cu-bound (red) $A\beta_{1\text{-}16}$ are present as doubly, triply and quadruply charged ions. **(b)** Charge-deconvolution of the spectra in Panel (a). Deconvolution was carried out with UniDec (Marty et al. 2015; Reid et al. 2019), and peptides with zero, one, and two bound copper ions are indicated with a blue circle, green triangle, and red triangle, respectively. **(c)** Isotopic distribution of Cu-bound $A\beta_{1\text{-}16}$ at charge state 3+. Simulated distributions assuming 100% Cu(II) are overlaid in orange.

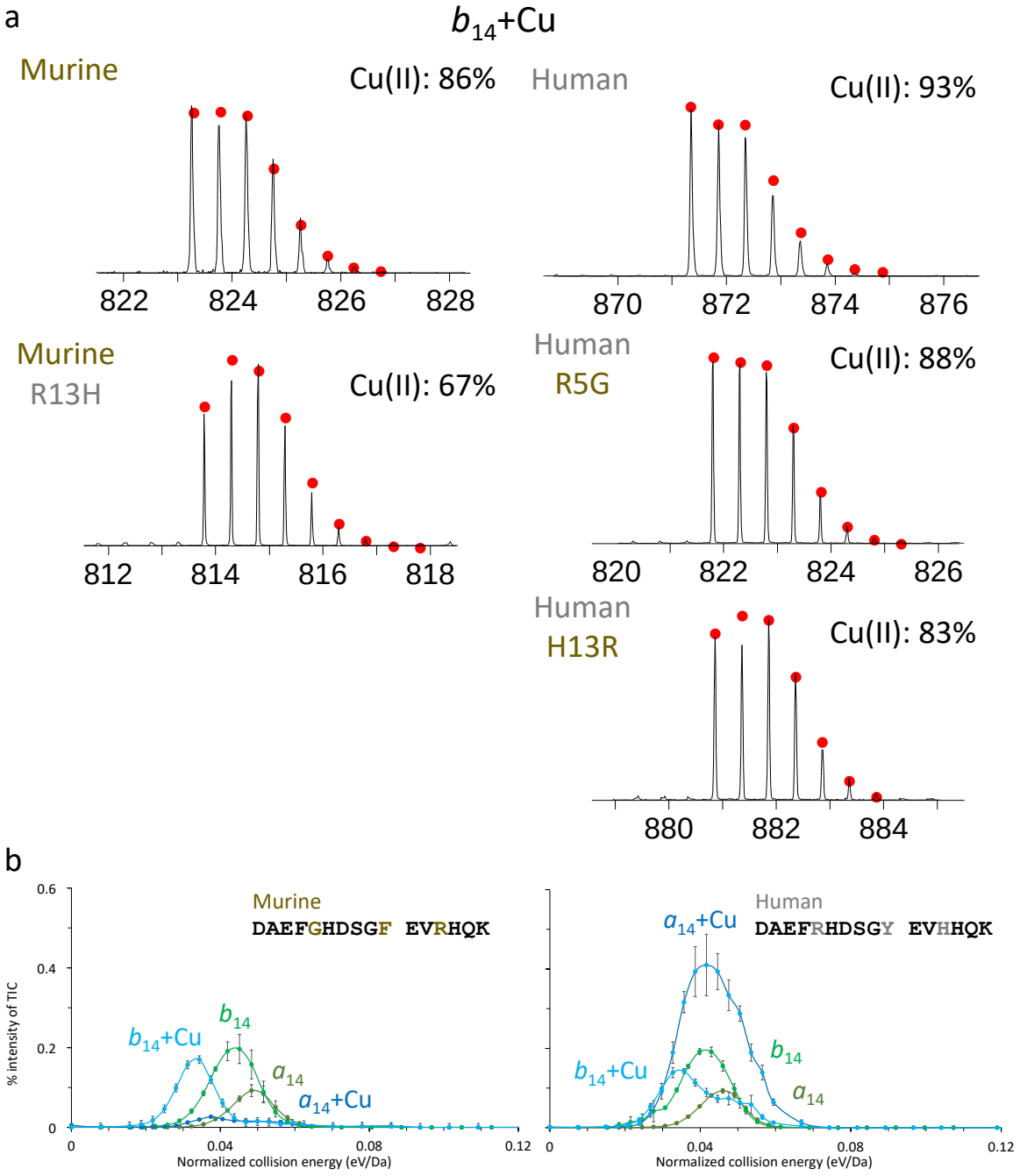

Figure S3. (a) Isotope distribution of the b_{14} fragment at a collision voltage of 22 V (equivalent to a normalised collision energy of ca. 0.035 eV/Da) and the calculated percentage of Cu(II) (red dots show the best-fit simulation of the Cu(I)/Cu(II) ratio. **(b)** Energy-dependence of the relative intensity of a_{14} and b_{14} fragments after collisional activation of metal-free murine and human $A\beta_{1\text{-}16}$ (dark and light green curves for a_{14} and b_{14}, respectively) and of Cu(II)-complexes of murine and human $A\beta_{1\text{-}16}$ (dark and light blue curves for a_{14} and b_{14}, respectively). Note that no copper-free a_{14} or b_{14} fragments were detected in the spectra of the metal-free precursor. Error bars show the standard deviation of triplicate experiments, illustrating excellent reproducibility and consistency.

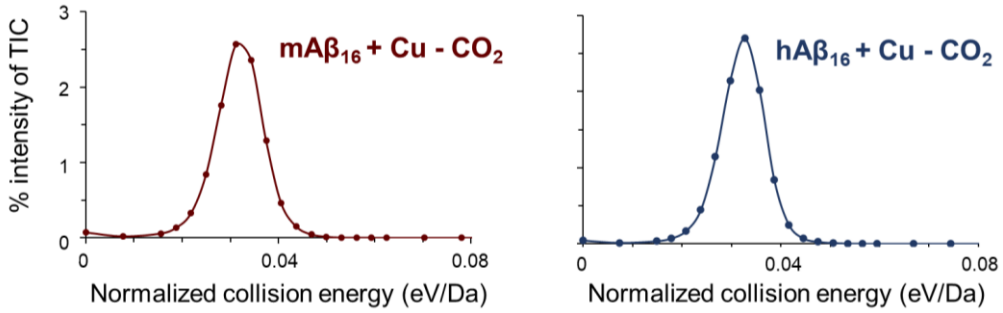

Figure S4. Energy-dependence of the relative intensity of the decarboxylated precursor after collisional activation of Cu(II)-bound murine and human $A\beta_{1-16}$.

Experimental Section

Full-length $A\beta_{1-42}$ was purchased from Bachem AG (Bubendorf, Switzerland). LC-MS grade water was obtained from Fisher Scientific (Schwerte, Germany), and other reagents, including custom peptides, from Merck (Darmstadt, Germany). MS measurements were performed with a Synapt XS instrument. Samples were loaded into glass emitters produced in-house with a Sutter P-97 Flaming/Brown micropipette puller and transferred into the gas phase with nano-electrospray ionisation in direct-infusion positive-ion mode. For full-length amyloid β and its copper complex, the 4+ charge state was isolated in the quadrupole of the instrument; for the smaller custom peptides, we selected the 3+ precursor. Collisional activation was performed in the Trap cell. In fragmentation experiments, we acquired data for 3 minutes per spectrum and averaged the fragment intensities over this time. We subsequently normalised this average intensity to the total ion current to allow comparison between experiments.

References

Everett, James; Lermyte, Frederik; Brooks, Jake; Tjendana-Tjhin, Vindy; Plascencia-Villa, Germán; Hands-Portman, Ian et al., Biogenic metallic elements in the human brain?, *Science advances*, 2021, **7**, eabf6707.

Marty, Michael T.; Baldwin, Andrew J.; Marklund, Erik G.; Hochberg, Georg K. A.; Benesch, Justin L. P.; Robinson, Carol V., Bayesian deconvolution of mass and ion mobility spectra: from binary interactions to polydisperse ensembles, *Analytical chemistry*, 2015, **87**, 4370–4376.

Reid, Deseree J.; Diesing, Jessica M.; Miller, Matthew A.; Perry, Scott M.; Wales, Jessica A.; Montfort, William R.; Marty, Michael T., MetaUniDec: High-Throughput Deconvolution of Native Mass Spectra, *Journal of the American Society for Mass Spectrometry*, 2019, **30**, 118–127.