Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

Ni(II) ions cleave and inactivate human alpha-1 antitrypsin hydrolytically, implicating nickel exposure as a contributing factor in pathologies related to antitrypsin deficiency

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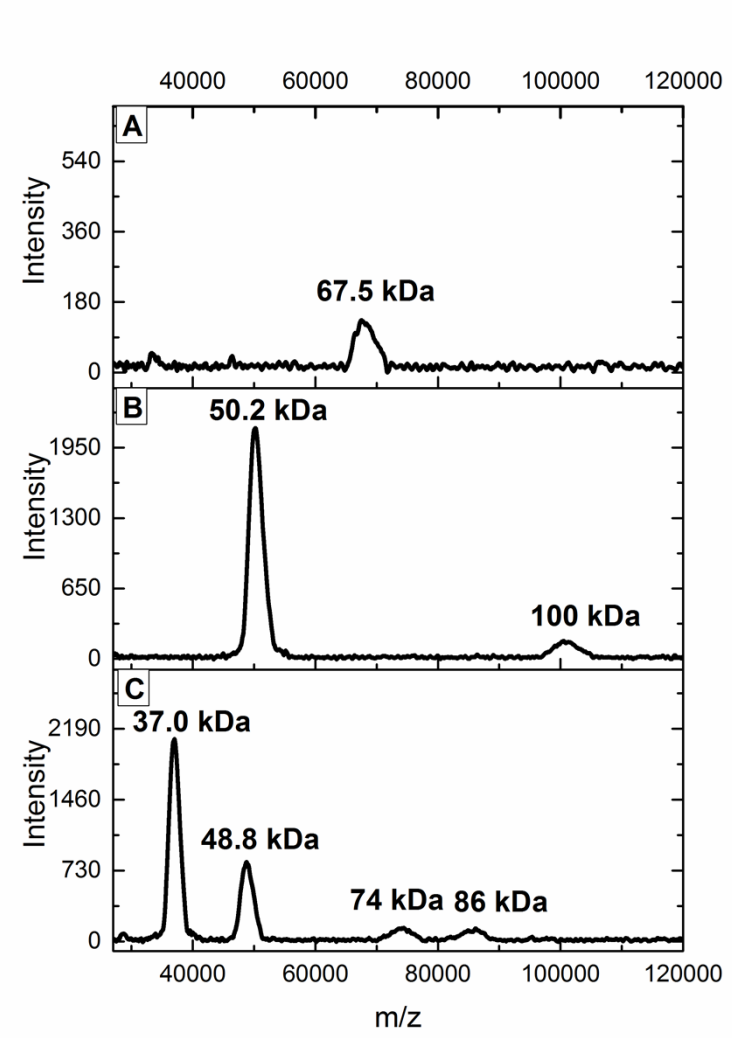


Fig. S1 MALDI-MS spectra of HPLC fractions of samples containing 50 µM AAT and 2.5 mM $Ni(NO_3)_2$ in 20 mM HEPES at pH 8.2. See Fig. 2 of the main text for HPLC details. (A) the peak at 57 min from the sample incubated for 30 days at 37 °C; (B) the peak at 67 min from the sample collected at the beginning of incubation; (C) the peak at 67 min from the sample incubated for 30 days.

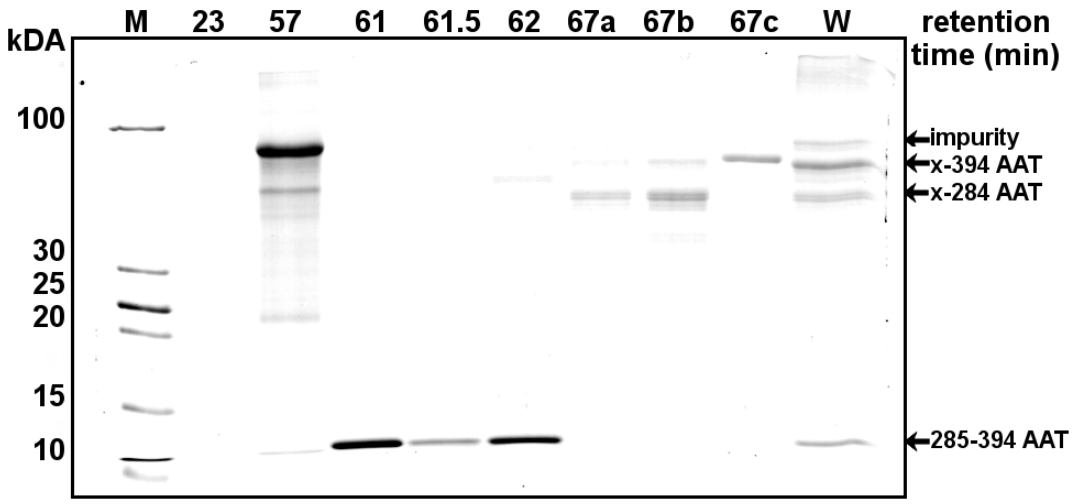


Fig. S2 SDS-PAGE gels of HPLC fractions of samples containing 50 μM AAT and 2.5 mM $Ni(NO_3)_2$ in 20 mM HEPES at pH 8.2 and 37 °C after 30 days of incubation, unless stated otherwise. The HPLC retention time of each fraction is presented above the gel. The fraction at 67 min was diluted ten times (67a), and five times (67b). The ninth lane (67c) contains the fraction at 67 min collected at the beginning of incubation. The last lane (W) contains the sample incubated for 11 days which was not separated by HPLC. The first lane contains molecular weight markers with weights indicated on the left side of the gels. Labels on the right side indicate protein fragments yielded by Ni(II)-dependent hydrolysis of AAT: x-394 AAT – native 1-394 AAT and 13/14-394 AAT; x-294 AAT – 1-284 and 13/14-394 AAT; and 285-394 AAT. Proteins were visualized by SYPRO Ruby Protein Gel Stain.

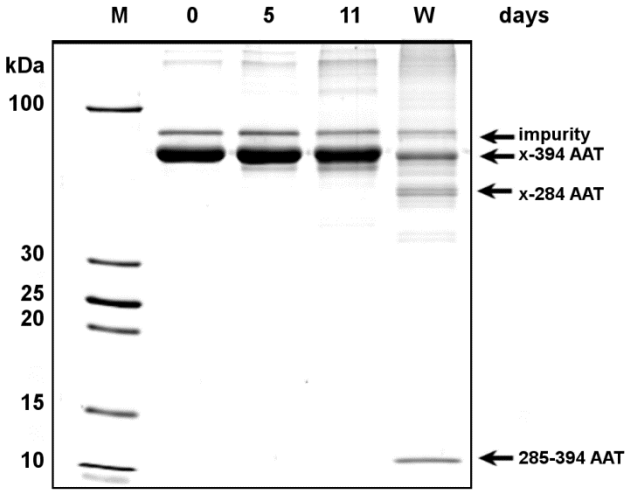


Fig. S3 SDS-PAGE gels of AAT incubated in the absence of Ni(II) ions, in 20 mM HEPES at 37 °C, and pH 8.2. Labels above the lanes indicate incubation time in days (0, 5, and 11, respectively). For a comparison, lane W presents the sample incubated in the presence of 0.5 mM Ni(II) ions for 11. The first lane (M) contains molecular weight markers with weights indicated on the left side of the gels. Labels on the right side indicate protein fragments yielded by Ni(II)-dependent hydrolysis of AAT: x-394 AAT – native 1-394 AAT and 13/14-394 AAT; x-284 AAT – 1-284 and 13/14-394 AAT; and 285-394 AAT. Proteins were visualized by SYPRO Ruby Protein Gel Stain.

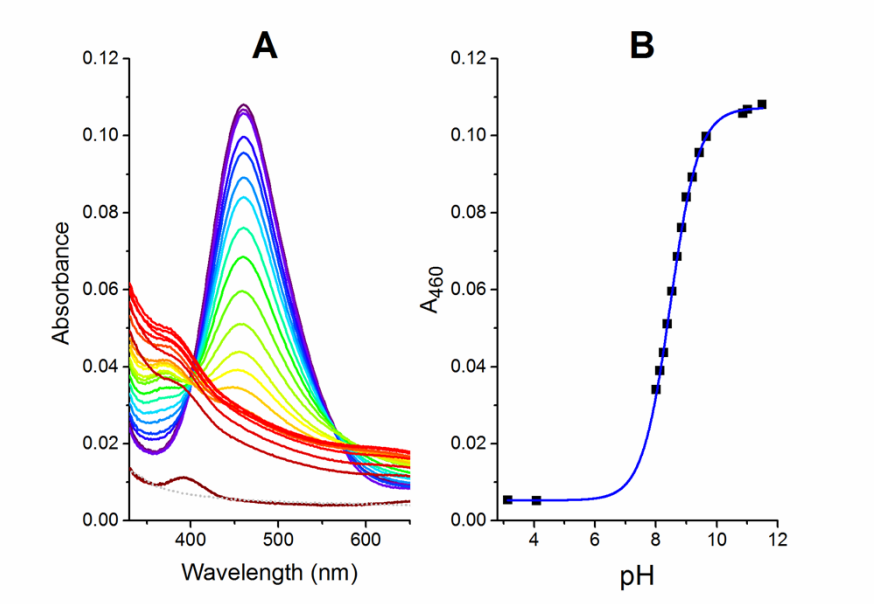


Fig. S4 (A) UV-vis spectra of Ni(II) complexes of the Ac-KTDTSHHDQ-am peptide, at different pH values, coded with rainbow colors from red (the lowest pH 3.5) to dark blue (the highest pH 11.5); (B) pH-dependence of the absorbance at 460 nm, derived from the spectra shown in panel A. The spectrum of the peptide in the absence of Ni(II) ions is shown as dotted grey line. The formation of poorly soluble high-spin Ni(II) complexes (red lines) in the pH range 5.7-7.9 resulted in the baseline elevation. These spectra were not included in the pH-dependence analysis. The spectra were recorded at 25 °C, for samples containing 0.95 mM peptide and 0.9 mM $Ni(NO_3)_2$.

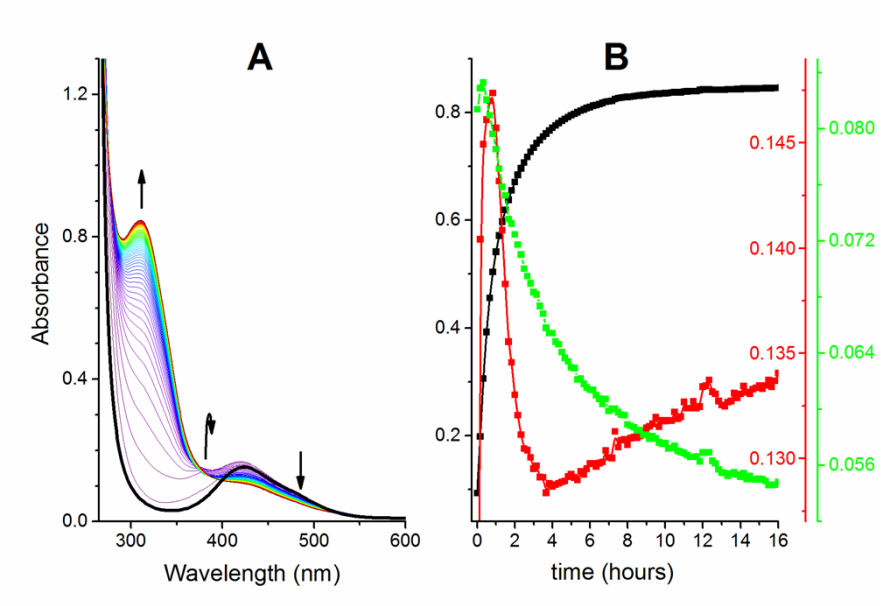
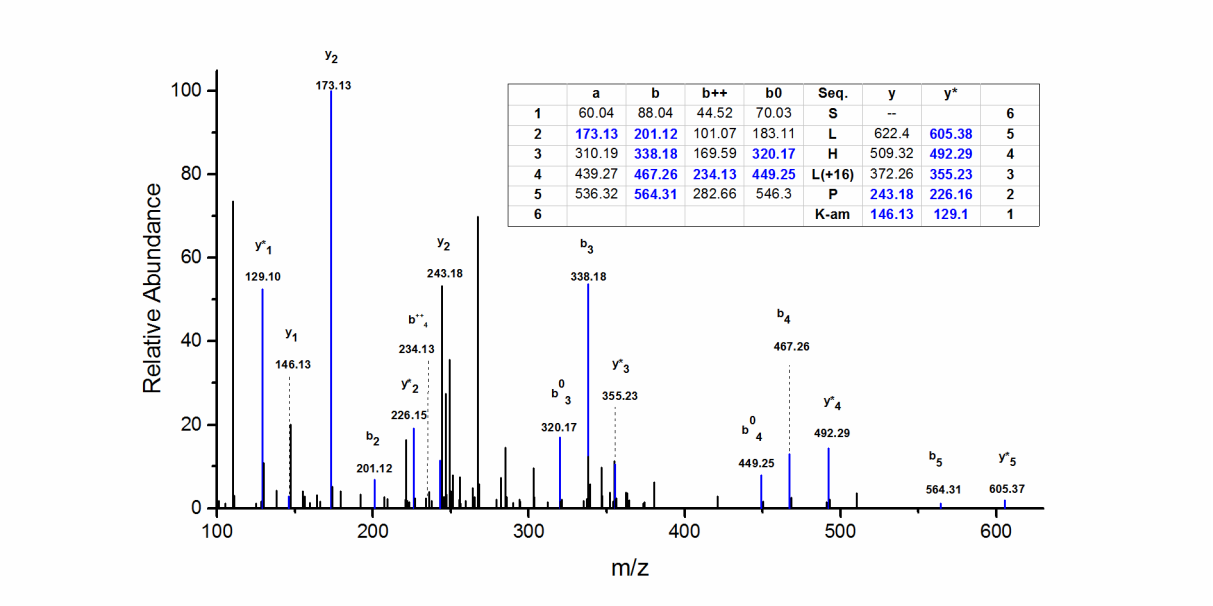


Fig. S5 (A) UV-vis spectra of nickel complexes of the C-terminal product of hydrolysis of Ac-KTDTSHHDQ-am (1.0 mM), incubated with 10 mM mM H_2O_2, for 16 hours at 37 °C. The spectra were recorded every 10 min. The spectrum recorded prior to H_2O_2 addition is marked with a thick black line. Arrows indicate the direction of changes at 310 nm, 383 nm, and 480 nm; (B) Time course of spectral changes at 310 nm (black), 383 (red), and 480 nm (green).



	a	b	b++	b0	Seq.	y	y*	
1	60.04	88.04	44.52	70.03	S	--		6
2	173.13	201.12	101.07	183.11	L	622.4	605.38	5
3	310.19	338.18	169.59	320.17	H	509.32	492.29	4
4	439.27	467.26	234.13	449.25	L(+16)	372.26	355.23	3
5	536.32	564.31	282.66	546.3	P	243.18	226.16	2
6					K-am	146.13	129.1	1

Fig. S6 ESI-MS/MS spectrum of SLHLPK-am (the C-terminal product of Ni(II)-dependent hydrolysis of Ac-RSASLHLPK-am) after a 30 min incubation of the hydrolytic reaction mixture (initially containing 1.2 mM SLHLPK-am, 1.2 mM Ac-RSA and 1 mM Ni(II)) with 10 mM H_2O_2. The oxidation of leucine at position 4 (L(+16)) was observed. The theoretical m/z values matched with peaks present in the spectrum are highlighted in blue.

Table S1 The list of PDB codes for structures of non-mutated human alpha-1 antitrypsin

PDB codes	Fragments of AAT with the structure resolved
1QLP	23-394
1EZX	24-358
2D26	24-358
2QUG	24-392
3CWL	23-394
3CWM	24-393
3NE4	24-393