Supplementary information

"A novel approach for the determination of exchangeable copper in serum using protein precipitation"

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Table S 1: Instrumental setup and analytical parameters for size-exclusion chromatography employed in this study

size exclusion chromatography	
instrument	Agilent 1260 Infinity HPLC System
column	YMC-Pack Diol 200, 250 x 4.6 mm, 20 nm, S-5µm
flow rate	0.1 mL/min
run-time/sample	40 min
injection volume	10 µl
column temperature	38°C
eluent	100 mM NH_4NO_3 50 mM KCl pH 6.8
ICP-MS/MS parameter	
instrument	Agilent 8800 ICP-QQQ-MS
plasma RF power	1550 W
nebulizer	MicroMist (Glass Expansion, Melbourne Australia)
spray chamber	Scott-type
plasma gas flow	15 L/min
auxiliary gas flow	0.9 L/min
nebulizer gas flow	1 L/min
spray chamber temperature	2°C
cones	Ni
sample depth	8.0 mm
gas mode	O_2
cell gas flow	0.4 mL/min
mass transitions	32→48 ($S→SO^+$) & 34→50 ($S→SO^+$); 63→63 ($^{63}Cu^+$) & 65→65 ($^{65}Cu^+$)
integration time	0.1 s/point

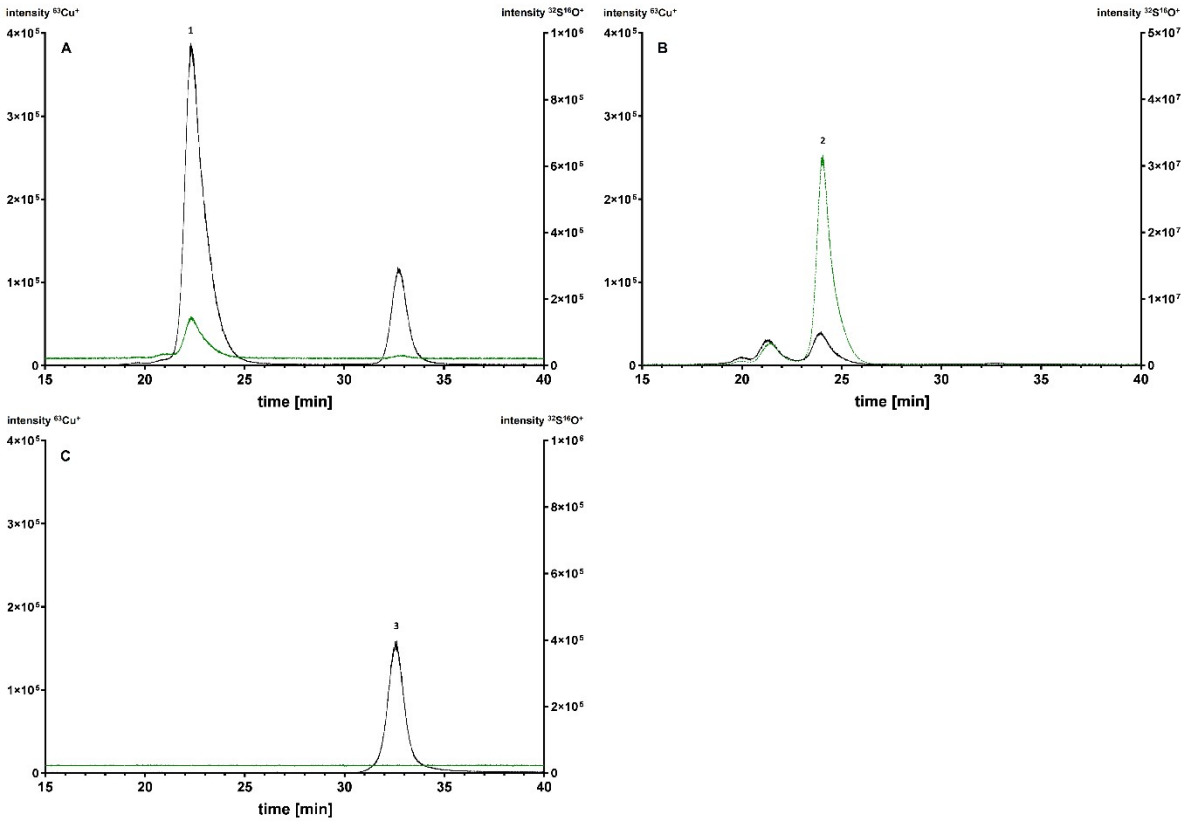

Figure S 1: Size exclusion chromatograms of commercially available protein standards (Sigma-Aldrich, Saint-Louis, MO, USA). The black solid line shows the $^{63}Cu^{+}$ signal and the green dotted line shows the $^{32}S^{16}O^{+}$ signal. Solutions of 50 g/L human serum albumin and 0.5 g/L ceruloplasmin were prepared in PBS buffer at pH 7.4 and diluted further 1:3 for SEC/ICP-MS/MS measurements. The chromatograms show (A) ceruloplasmin (B) human serum albumin (HSA) and (C) a 100 µg Cu/L $CuCl_2$ standard solution.

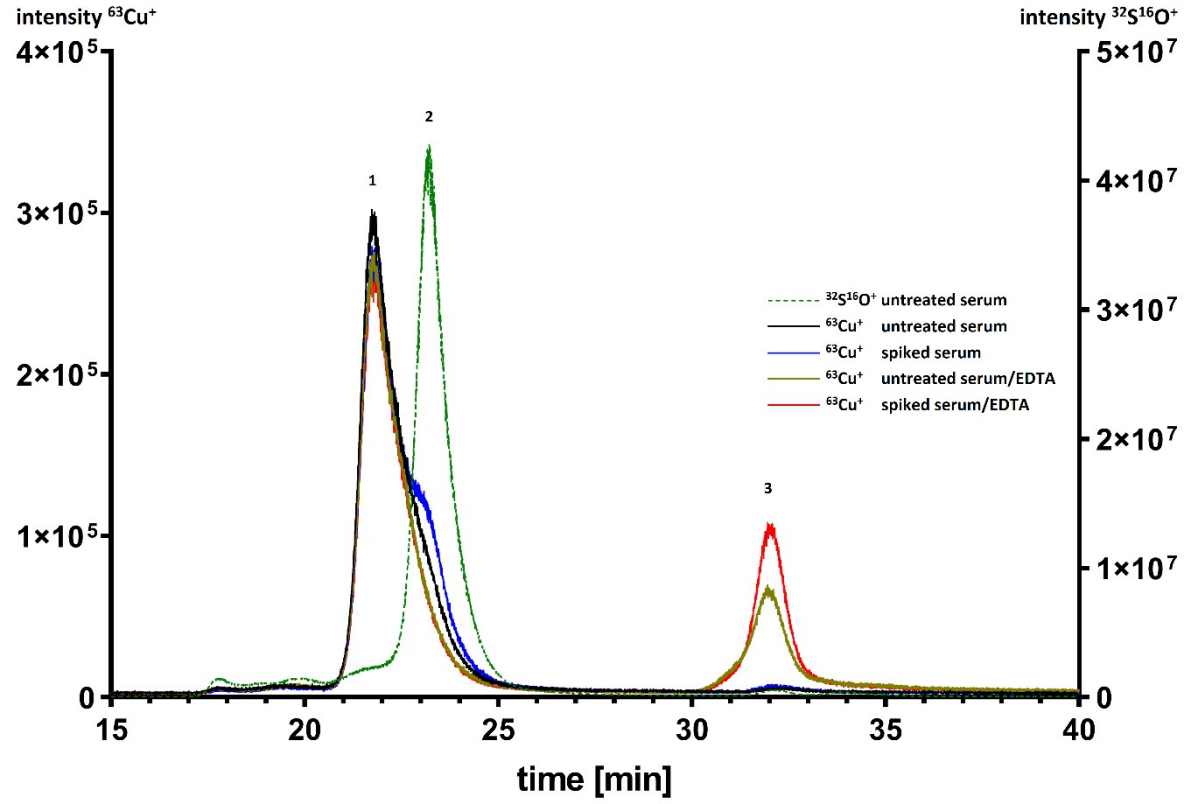

Fig. S 2: Comparison of size exclusion chromatograms of independent injections of a human serum sample.

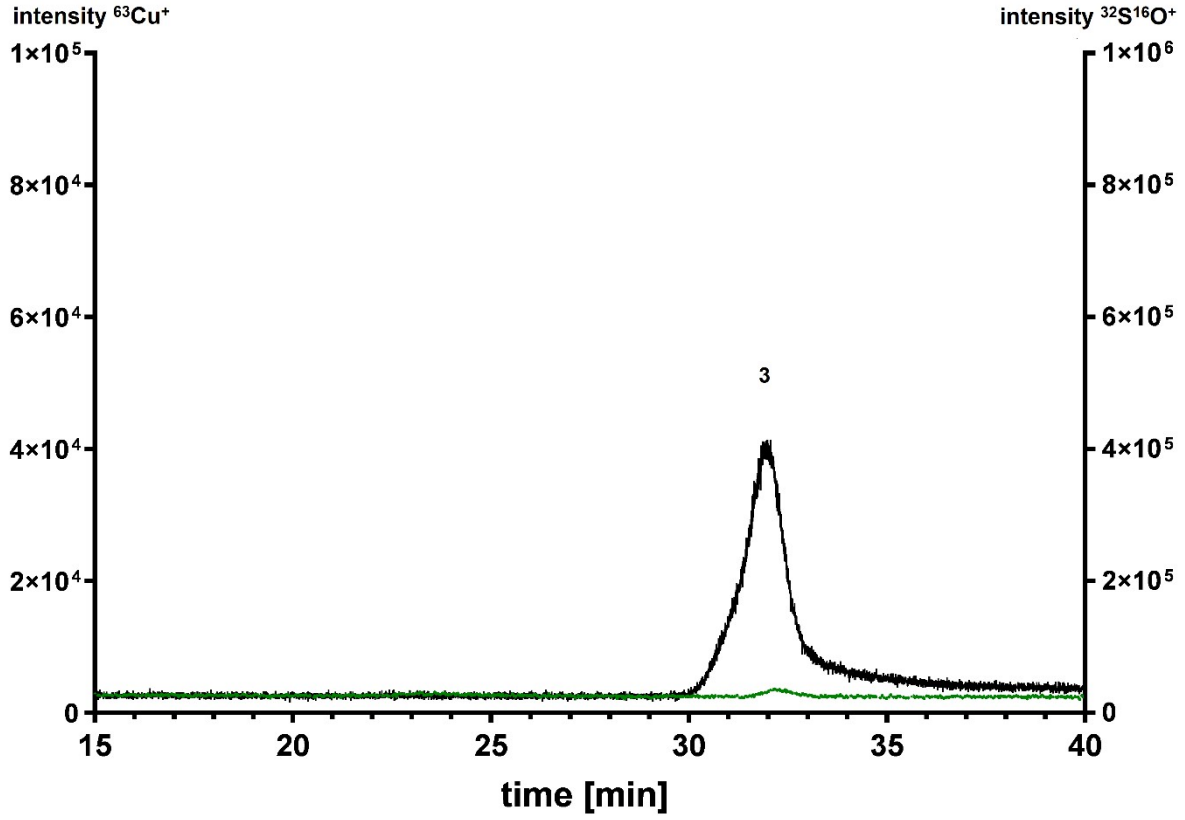

Fig. S 3: Size exclusion chromatogram of a human serum sample (V1) after application of the final method for the determination of CuEXC. black line: $^{63}Cu^{+}$ signal, green dotted line: $^{32}S^{16}O^{+}$ signal