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Metal-catalyzed oxidation of Aβ and the resulting reorganization of the Cu binding sites promote ROS production

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Supporting Information

Experimental

Titration of $A\beta_{28}$, $AcA\beta_{28}$ and $A\beta_{40}$

All the synthetic peptides were bought from GeneCust (Dudelange, Luxembourg), with purity grade > 95%. Stock solutions of the $A\beta_{28}$ (sequence DAEFRHDSGYEVHHQKLVFFAEDVGSNK) and Ac-$A\beta_{28}$ (sequence AcDAEFRHDSGYEVHHQKLVFFAEDVGSNK), peptides were prepared by dissolving the powder in milliQ water (resulting pH ≈ 2). Peptide concentration was then determined by UV-visible absorption of Tyr10 considered as free tyrosine (at pH 2, $(\varepsilon_{276}-\varepsilon_{296})$ = 1410 M^{-1} cm^{-1}). Stock solution of $A\beta_{40}$ peptide (sequence DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV) was prepared by dissolving the powder in NaOH (50 mM) and purifying the solution in FPLC. The peptide concentration was then determined by UV-visible absorption of Tyr10, considered as free tyrosine ($(\varepsilon_{293}-\varepsilon_{360})$ = 2400 $M^{-1}cm^{-1}$) in NaOH (50 mM, resulting pH ≈ 13).

Proteolytic digestion

The solution of Aβ was filtered using Amicon 3 kDa centrifugal device (Millipore) by centrifugation for 15 min at 13500 rpm, then washed and centrifuged twice with 200 µL sodium hydrogenocarbonate (100 mM, pH 8). The concentrated sample (approx. 50 µL) was recovered and transferred to an Eppendorf ProteinLoBind 1.5 mL vial. Trypsin (0.05 ng/µL in formic acid 0.1%) was added to obtain a Aβ/trypsin ratio of 20/1 (w/w) and digestion was carried out at 37°C for 3h in a Thermomixer (Eppendorf), 10 s mixing at 750 rpm every min.

Mass spectrometry

High Performance Liquid Chromatography / Mass Spectrometry (HPLC/MS) analysis was performed on an ion-trap mass spectrometer (LCQ DECA XP Max, ThermoFisher), equipped with an electrospray ionization source, coupled to a SpectraSystem HPLC system. Sample (10 µL of Aβ tryptic digest) was injected onto the column (Phenomenex, Synergi Fusion RP-C18, 250 × 1 mm, 4 µm), at room temperature. Gradient elution was carried out with formic acid 0.1% (mobile phase A) and acetonitrile/water (80/20 v/v) formic acid 0.1% (mobile phase B) at a flow-rate of 50 $\mu L.min^{-1}$. The mobile phase gradient was programmed with the following time course: 12% mobile phase B at 0 min, held 3 minutes, linear increase to 100% B at 15 min, held 4 min, linear decrease to 12% B at 20 min and held 5 min. The mass spectrometer was used as a detector, working in the full scan positive mode between 50 and 2000 Da followed by data dependent scans of the two first most intense ions, with dynamic exclusion enabled. Isolation width was set at 1 Da and collision energy at 28% (units as given by the manufacturer), using wideband activation. The generated tandem MS data was searched using the SEQUEST algorithm against the human Aβ peptide sequence. Dynamic modifications were specified according to the expected mass shift due to the Aβ peptide oxidation (Supporting Information, Table s1). The same operating conditions (column and mobile phase gradient) were used to carry out high resolution mass spectrometry (HPLC/HRMS) experiments, by using a LTQ-Orbitrap XL mass spectrometer (ThermoFisher Scientific, Les Ulis, France) coupled to an Ultimate 3000 LC System (Dionex, Voisins-le-Bretonneux, France). The Orbitrap cell was operated in the full-scan mode at a resolution power of 60 000. HPLC/HRMS was also used to check for digestion efficiency, systematically found close to100 % (non-digested peptide not detected).

HPLC

High Performance Liquid Chromatography analysis was performed on an Agilent 1200 series device (Agilent technologies) equipped with a DAD detector. Sample (10 µL) was injected onto the column (Acclaim 120 C18, 50 × 3 mm, 3 µm, ThermoScientific), at room temperature. The gradient elution was carried out with formic acid 0.1% (mobile phase A) and acetonitrile/water (80/20 v/v) formic acid 0.1% (mobile phase B) at a flow-rate of 0.5 $mL.min^{-1}$. The mobile phase gradient was programmed with the following time course: 5% mobile phase

B at 0 min, held 3 minutes, linear increase to 55% B at 8 min, linear increase to 100% of B at 9 min, held 2 min, linear decrease to 5% B at 12 min and held 3 min. Aβ28 was detected with the absorption of Tyr10 at 276 nm.

Tables

Table S1: **Monoisotopic masses used for high resolution mass spectrometry**. Monoisotopic apparent masses (m/z in Th) of mono-, di- and triply-protonated ions of the tryptic peptides of oxidized $A\beta_{16}$; +16 accounts for the formal addition of one oxygen atom during oxidation (conversion of Histidine into oxohistidine).

Position	Peptide	$[M+H]^+$	$[M+2H]^{2+}$	$[M+3H]^{3+}$
1-5	$DAEFR_{dd}$*	592.2731	296.6405	198.0963
	$DAEFR_{ox}$**	548.2469	274.6274	183.4208
6-16	HDSGYEVHHQK+16	1352.5983	676.8031	451.5380

* $DAEFR_{dd}$: oxidative decarboxylation and deamination of Asp1

** $DAEFR_{ox}$: oxidative cleavage of Asp1

Table S2: **Identification of the oxidized residues of Aβ, when oxidized in the presence of H_2O_2**. Calculated (calc) and experimental (exp, obtained by HPLC/HRMS) monoisotopic masses of $DAEFR_{dd}$ and $DAEFR_{ox}$ and mass difference (in ppm) between experimental and calculated masses. Aβ peptide oxidized in the presence of H_2O_2 and digested by trypsin. Same experimental conditions as the ones of Figures S5 and S6.
Identification of Asp1 oxidation was performed by high-resolution mass spectrometry on monoisotopic masses of the tryptic peptides because MS/MS spectra were not enough informative: (1) too small peptides are usually fragmented as monoprotonated ions, leading to poor quality MS/MS spectra and (2) the terminal arginine residue of DAEFR blocks the proton mobility during MS/MS experiment, resulting in the absence of b ions (important for Asp1 mass shift determination). For the identification of His oxidation, see the MS/MS spectra in Figure S8 and S9.

Peptide	MH^+_{calc}	MH^+_{exp}	Δm (ppm)
$DAEFR_{dd}$	592.2731	592.2714	-2.9
$DAEFR_{ox}$	548.2469	548.2451	-3.3

Table S3: **Linear fitting of HO• production curves**. Gradient and determination coefficients of fits for each curve of Figures 7 and 8, for the two linear parts.

	First linear part			Second linear part		
	Gradient	Number of points	R^2	Gradient	Number of points	R^2
Free Cu(II)	7956	8	0.9998			
Cu-$A\beta_7$	6455	8	0.9997			
Cu-$A\beta_{16}$	1327	4	0.9972	3365	4	0.9991
Cu-$AcA\beta_{16}$	1259	4	0.9945	3602	4	0.9995
Cu-D7H- $A\beta_{16}$	1271	5	0.9954	2648	3	0.9996
Cu-$A\beta_{28}$	842	5	0.9926	2352	3	0.9984
Cu-$AcA\beta_{28}$	827	4	0.9938	3344	3	0.9992
Cu-$A\beta_{40}$	1275	5	0.9920	3103	4	0.9979
Cu-$A\beta_{16}$ 20/50	1153	8	0.9987			

Figures

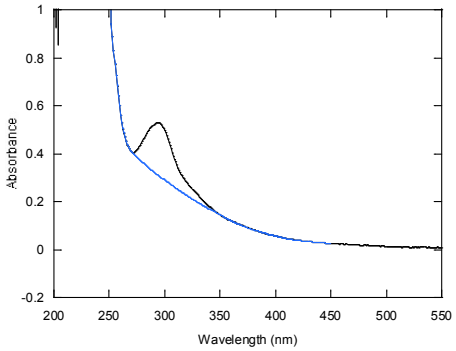


Figure S1: **Quantification of the oxidized Aβ peptide**. UV spectrum of oxidized $A\beta_{28}$ (black curve) and cubic spline interpolation (blue curve) to subtract the background absorbance of unknown origin from Tyr absorption.

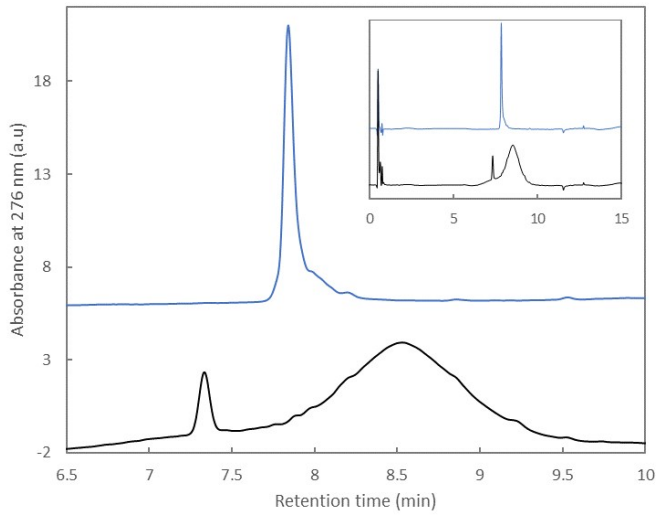


Figure S2: Trace chromatogram of Aβ28 (60 µM) after 30 min in the presence of Cu (50µM) (blue curve) or Cu (50 µM) and ascorbate (0.5 mM) (black curve) in phosphate buffer pH 7.4 (50 mM). $A\beta_{28}$ is detected by UV-Visible spectroscopy with the absorption of the Tyr10 at the wavelength of maximal absorption at acidic pH (276 nm).

The trace chromatogram of the oxidized $A\beta_{28}$ (Figure S2, black curve) shows the presence of several species unlike non-oxidized $A\beta_{28}$ (Figure S2, blue curve). Indeed, the single peak in $A\beta_{28}$ chromatogram indicates that only one species is present in the sample while several peaks including a broad one are observed in the oxidized $A\beta_{28}$ sample, indicating that several oxidized species are present in the mixture.

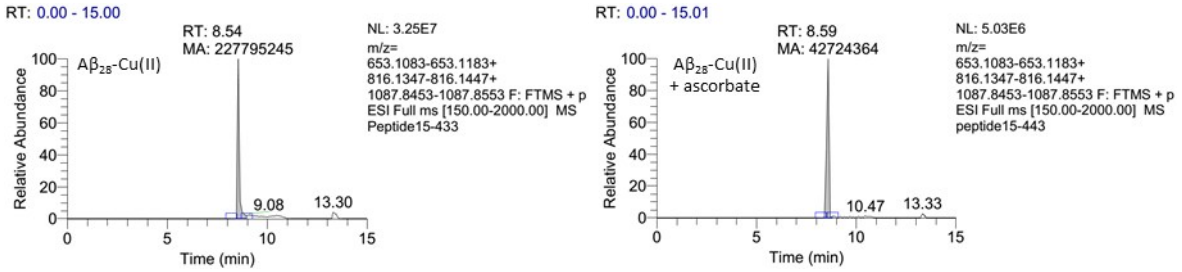
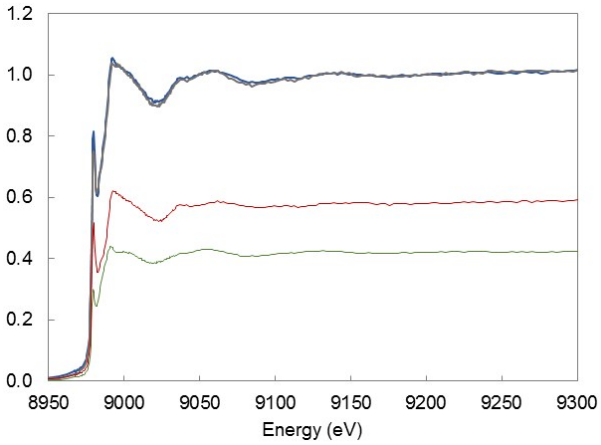


Figure S3: **Remaining $A\beta_{28}$ peptide.** Trace chromatograms of $A\beta_{28}$ (60 µM) after 30 min in the presence of Cu (50µM) (left panel) or Cu (50 µM) and ascorbate (0.5 mM) (right panel) in phosphate buffer pH 7.4 (50 mM). Aβ28 is detected with the $[M+3H]^{3+}$, $[M+4H]^{4+}$ and $[M+5H]^{5+}$ m/z ratio ions: 1087.8503, 816.1397 and 653.1133. Mass tolerance set at 5 ppm. Ratio

between the chromatogram area of $A\beta_{28}$-Cu(II) with ascorbate to chromatogram area of $A\beta_{28}$-Cu(II) without ascorbate is around 0.2, meaning that around 80% of $A\beta_{28}$ is oxidized (at least one amino acid residue is oxidized).



Quantitative results of linear combination fitting

Linear combination fitting carried out with the software Athena

R-factor = 0.0017131
Chi-square = 0.02825
Reduced chi-square = 0.0001733

standard	weight
HEPES-Cu(I)	0.000 (± 0.036)
$A\beta_{28}$-Cu(I)	**0.580** (± 0.028)
$A\beta_{7}$-Cu(I)	**0.420** (± 0.075)
sum	1.000

Figure S4: **Linear Combination Fitting of $A\beta_{28}$-Cu(I) and $A\beta_{7}$-Cu(I) to reproduce Aβox-Cu(I) spectrum**. The fitting allows deducing that around 40% of Cu(I) is bound the same way than $A\beta_{7}$ (bearing only one His), and that no Cu(I) is released in buffer (0% of Cu(I)-HEPES contribution). Quantitative results of linear combination fitting (Right panel) and the resulting XANES spectra with 42% Cu(I)-$A\beta_{7}$ and 58% Cu(I)-$A\beta_{28}$ XANES signatures (blue curve) compared to Cu(I)-Aβox XANES spectrum (grey curve). Cu(I)-$A\beta_{7}$ (green curve) and Cu(I)-$A\beta_{28}$ (red curve) XANES signatures, weighted according to the result found in the linear combination fitting, are also shown (Left panel).

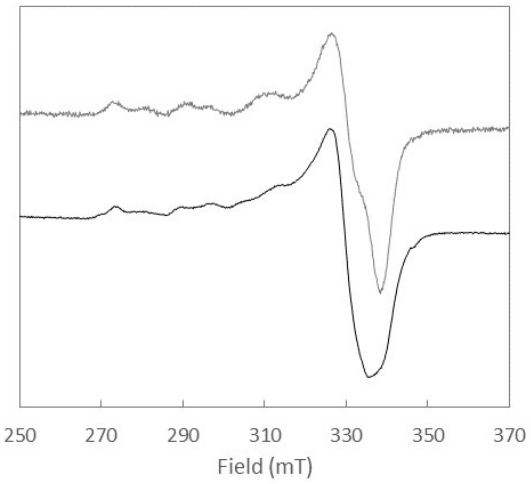


Figure S5: EPR spectra of Cu(II)-Aβox (black line) and Cu(II)-$A\beta_{28}$ (grey line) at pH 7.4. Aqueous solution with 10% of glycerol containing ^{65}Cu (450 µM) and peptide (500 µM). T = 120 K, ν = 9.5 GHz, microwave power = 20.5 mW, Amplitude modulation = 10.0 G, Modulation frequency = 100 kHz.

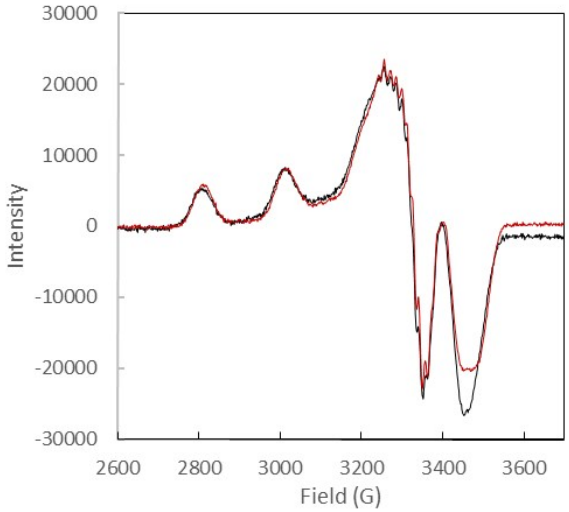


Figure S6: Linear combination fitting of 60% Cu(II)-Aβ_{28} and 40% Cu(II)-AcAβ_{28} (red line) compared to Cu(II)-Aβox signature (black line).

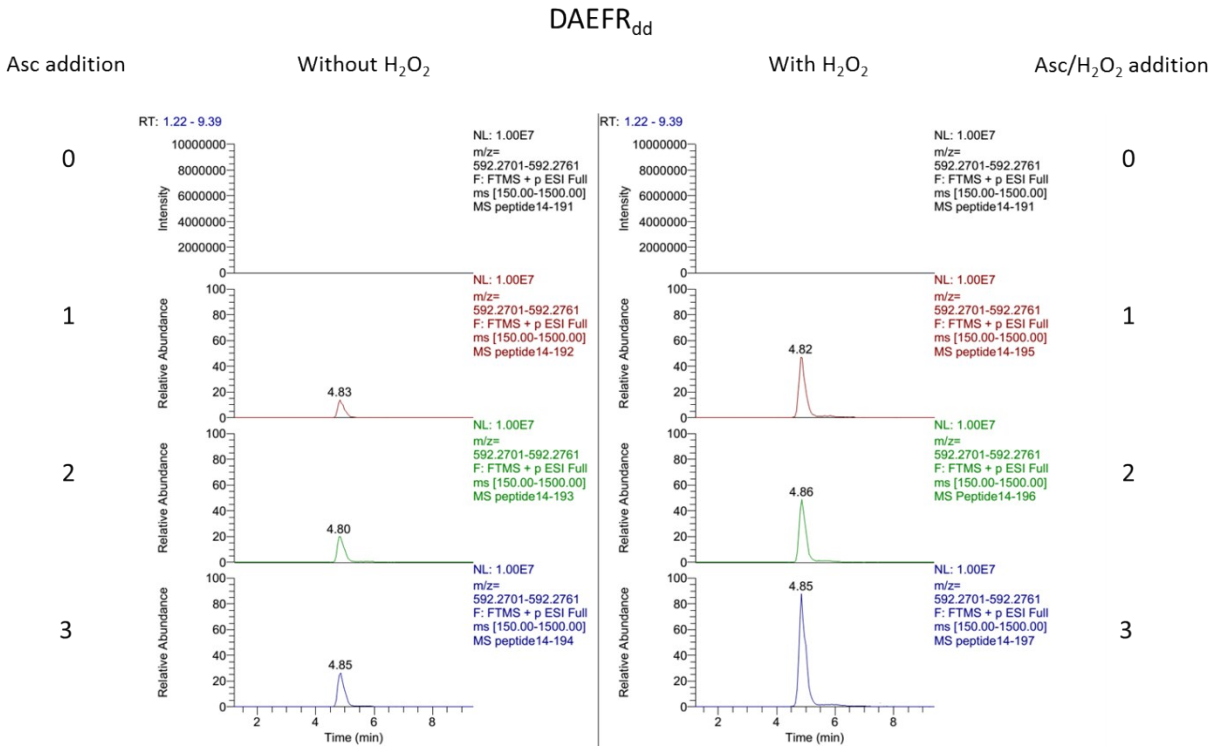


Figure S7: **Oxidation of Asp1 (DAEFR$_{dd}$) in the presence or absence of H_2O_2.** Trace chromatograms of the Aβ oxidized tryptic peptide DAEFR$_{dd}$ (decarboxylation and deamination of Asp1), after successive additions of ascorbate (4 nmol, final conc. 20 µM; left panel) or ascorbate/H_2O_2 (4/10 nmol, final conc. 20/50 µM; right panel). Aβ_{28} 25 µM and Cu(II) 20 µM, phosphate buffered pH 7.4 (50 mM). Mass tolerance set at 5 ppm; m/z ratios used for detection are specified in Table S1.

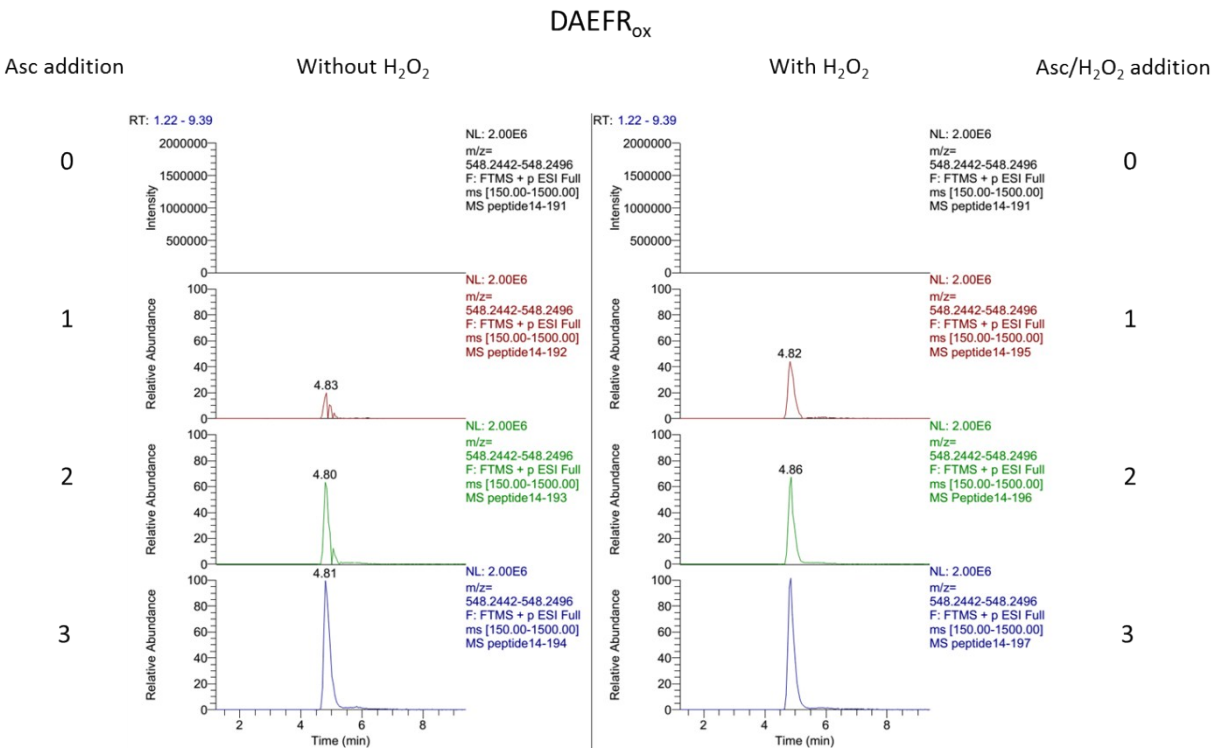


Figure S8: **Oxidation of Asp1 (DAEFR$_{ox}$) in the presence or absence of H_2O_2**Trace chromatograms of the oxidized Aβ tryptic peptide DAEFR$_{ox}$ (oxidative cleavage of Asp1), after successive additions of ascorbate (4 nmol, final conc. 20 µM; left panel) or ascorbate/H_2O_2 (4/10 nmol, final conc. 20/50 µM; right panel). Aβ_{28} 25 µM and Cu(II) 20 µM, phosphate buffered pH 7.4 (50 mM). Mass tolerance set at 5 ppm; m/z ratios used for detection are specified in Table S1.

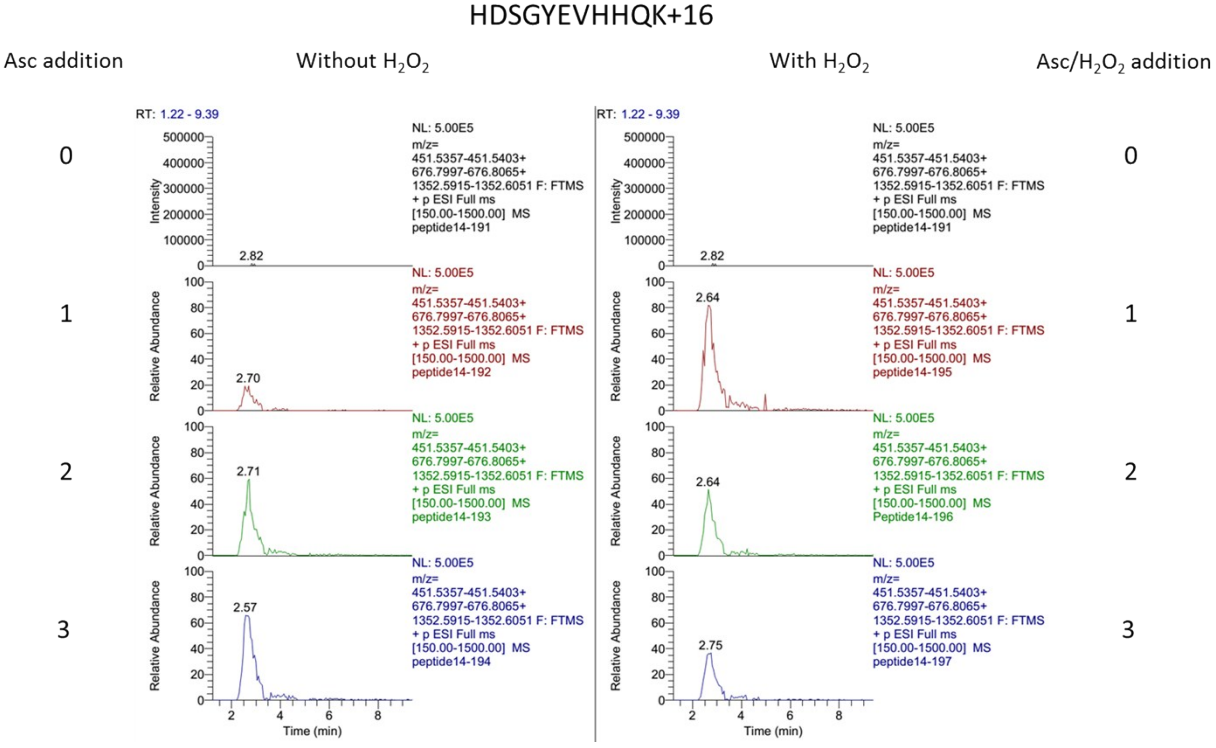


Figure S9: **Oxidation of His (HDSGYEVHHQK+16) in the presence or absence of H_2O_2.**Trace chromatograms of the oxidized Aβ tryptic peptide HDSGYEVHHQK+16 (formal addition of an oxygen atom), after successive additions of ascorbate (4 nmol, final conc. 20 µM; left panel) or ascorbate/H_2O_2 (4/10 nmol, final conc. 20/50 µM; right panel). Aβ_{28} 25 µM and Cu(II) 20 µM, phosphate buffered pH 7.4 (50 mM). Mass tolerance set at 5 ppm; m/z ratios used for detection are specified in Table S1.

DTA for scans: 105-108
Precursor ion: 677.43
Mass type: mono
Mod's: (DAEFRHSGYVQKLFNM* +16.00000) (DAEFRHSGYVQKLFNM# +32.00000) (DAEFRHSGYVQKLFNM@ -45.00000) (DAEFRHSGYVQKLFNM^

Ion series for charge: +1

AA	A ions	B ions	B* ions	Bo ions	C ions	Y ions	Y* ions	Yo ions	Z ions
H		138.07							
D		253.09				1215.54			
S		340.13				1100.52			
G		397.15				1013.49			
Y		560.21				956.46			
E		689.25				793.40			
V		788.32				664.36			
H*		941.38				565.29			
H		1078.44				412.23			
Q		1206.50				275.17			
K						147.11			

DTA for scans: 105-108
Precursor ion: 677.43
Mass type: mono
Mod's: (DAEFRHSGYVQKLFNM* +16.00000) (DAEFRHSGYVQKLFNM# +32.00000) (DAEFRHSGYVQKLFNM@ -45.00000) (DAEFRHSGYVQKLFNM^

Ion series for charge: +2

AA	A ions	B ions	B* ions	Bo ions	C ions	Y ions	Y* ions	Yo ions	Z ions
H		69.54							
D		127.05				608.28			
S		170.57				550.76			
G		199.08				507.25			
Y		280.61				478.74			
E		345.13				397.20			
V		394.66				332.68			
H*		471.19				283.15			
H		539.72				206.62			
Q		603.75				138.09			
K						74.06			

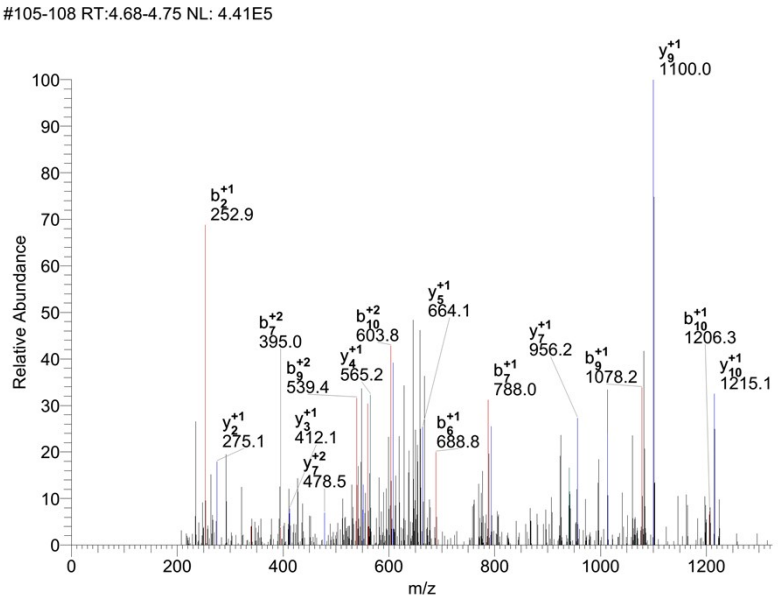


Figure S10: **MCO of Aβ in the presence of H_2O_2, identification of oxidized His13**. Series of b and y ions (charge 1+ and 2+) used for the identification of the oxidation of His13, and corresponding MS/MS spectrum of the doubly protonated ion of HDSGYEVHHQK+16. Same experimental conditions as the ones of Figure S8.

DTA for scan: 131
Precursor ion: 677.06
Mass type: mono
Mod's: (DAEFRHSGYVQKLFNM* +16.00000) (DAEFRHSGYVQKLFNM# +32.00000) (DAEFRHSGYVQKLFNM@ -45.00000) (DAEFRHSGYVQKLFNM^

Ion series for charge: +1

AA	A ions	B ions	B* ions	Bo ions	C ions	Y ions	Y* ions	Yo ions	Z ions
H		138.07							
D		**253.09**				**1215.54**			
S		340.13				**1100.52**			
G		**397.15**				**1013.49**			
Y		**560.21**				**956.46**			
E		**689.25**				**793.40**			
V		**788.32**				**664.36**			
H		**925.38**				**565.29**			
H*		**1078.44**				**428.23**			
Q		**1206.50**				275.17			
K						147.11			

DTA for scan: 131
Precursor ion: 677.06
Mass type: mono
Mod's: (DAEFRHSGYVQKLFNM* +16.00000) (DAEFRHSGYVQKLFNM# +32.00000) (DAEFRHSGYVQKLFNM@ -45.00000) (DAEFRHSGYVQKLFNM^

Ion series for charge: +2

AA	A ions	B ions	B* ions	Bo ions	C ions	Y ions	Y* ions	Yo ions	Z ions
H		69.54							
D		127.05				**608.28**			
S		170.57				**550.76**			
G		199.08				507.25			
Y		**280.61**				**478.74**			
E		345.13				**397.20**			
V		**394.66**				332.68			
H		**463.19**				283.15			
H*		**539.72**				214.62			
Q		**603.75**				138.09			
K						74.06			

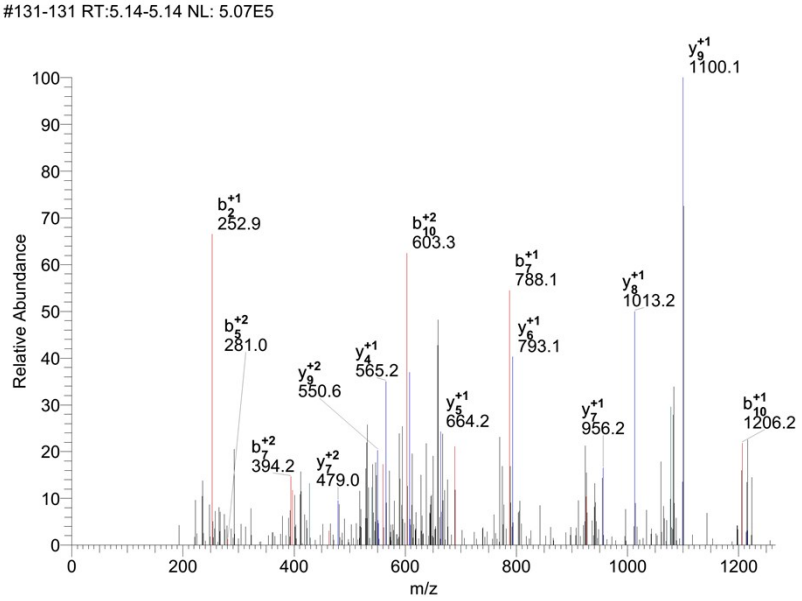


Figure S11: **MCO of Aβ in the presence of H_2O_2, identification of oxidized His14**. Series of b and y ions (charge 1+ and 2+) used for the identification of the oxidation of His14, and corresponding MS/MS spectrum of the doubly protonated ion of HDSGYEVHHQK+16. Same experimental conditions as the ones of Figure S8.

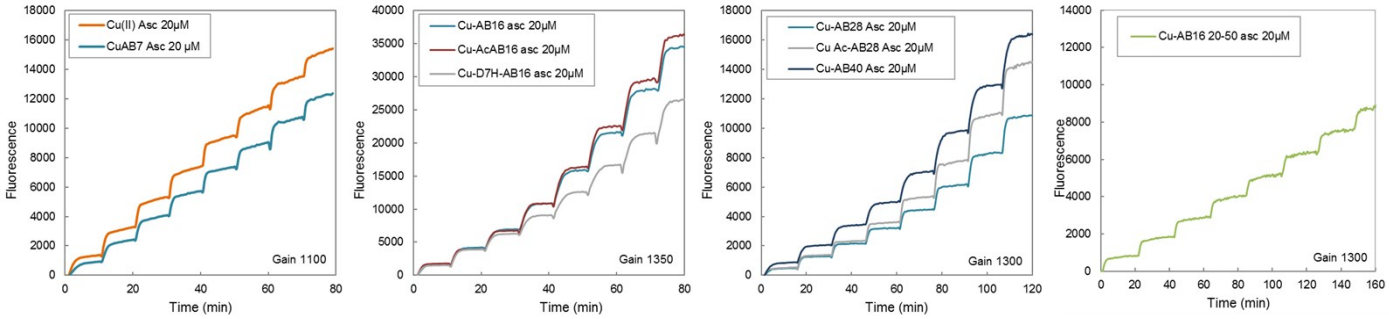


Figure S12: Fluorescence curves of phosphate buffered solutions (50 mM) containing Cu (20 µM), Aβ peptide (25 µM, except the green curve: 50 µM), CCA (0,5 mM) with additions of ascorbate (20 µM) and hydrogen peroxide (50 µM) realized every 15 min during 2 hours.

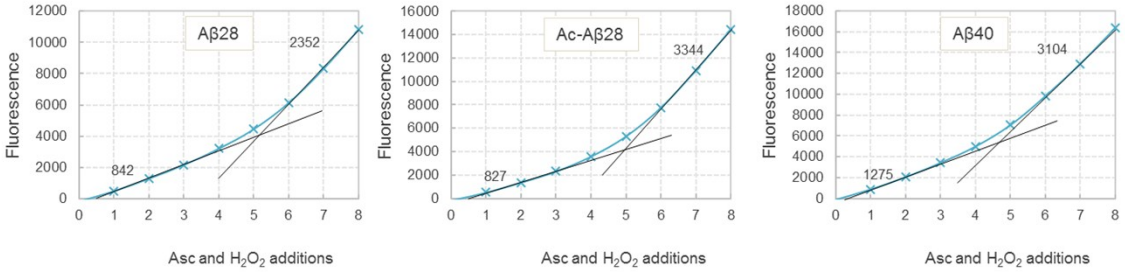


Figure S13: Fluorescence at the plateau of phosphate buffered solution (50 mM) containing Cu (20 µM), Aβ peptide (25 µM), CCA (0,5 mM) as a function of the number of ascorbate and H_2O_2 additions. A total of 8 additions of 2 µL ascorbate (2 mM) and 2 µL hydrogen peroxide (5 mM) are realized, i.e. respectively 4 and 10 nmol for each addition (initial concentrations reaching 20 and 50 µM for the first addition, respectively).