Supporting Information

Copper binding and redox activity of α-Synuclein in membrane like environment

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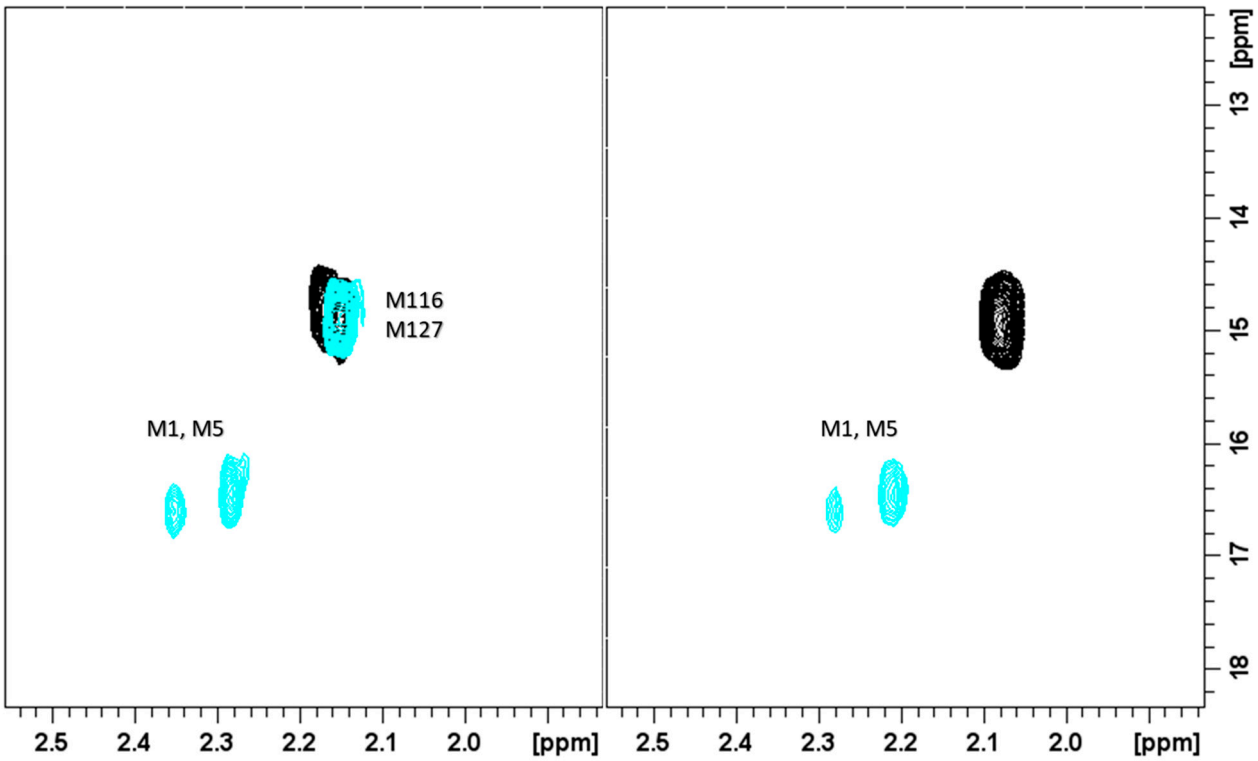


Figure S1. Overlaid ^{1}H-^{13}C HSQC spectra of micelles-bound $\alpha Syn_{1\text{-}140}$ 240 µM (left panel) and $\alpha Syn_{1\text{-}15}$ 450 µM (right panel) in absence and in presence of Ag^{+}: 0 eq (black), 0.8 eq (light blue). SDS-d_{25} 50mM, phosphate buffer 20 mM pH= 7.4, T =298 K.

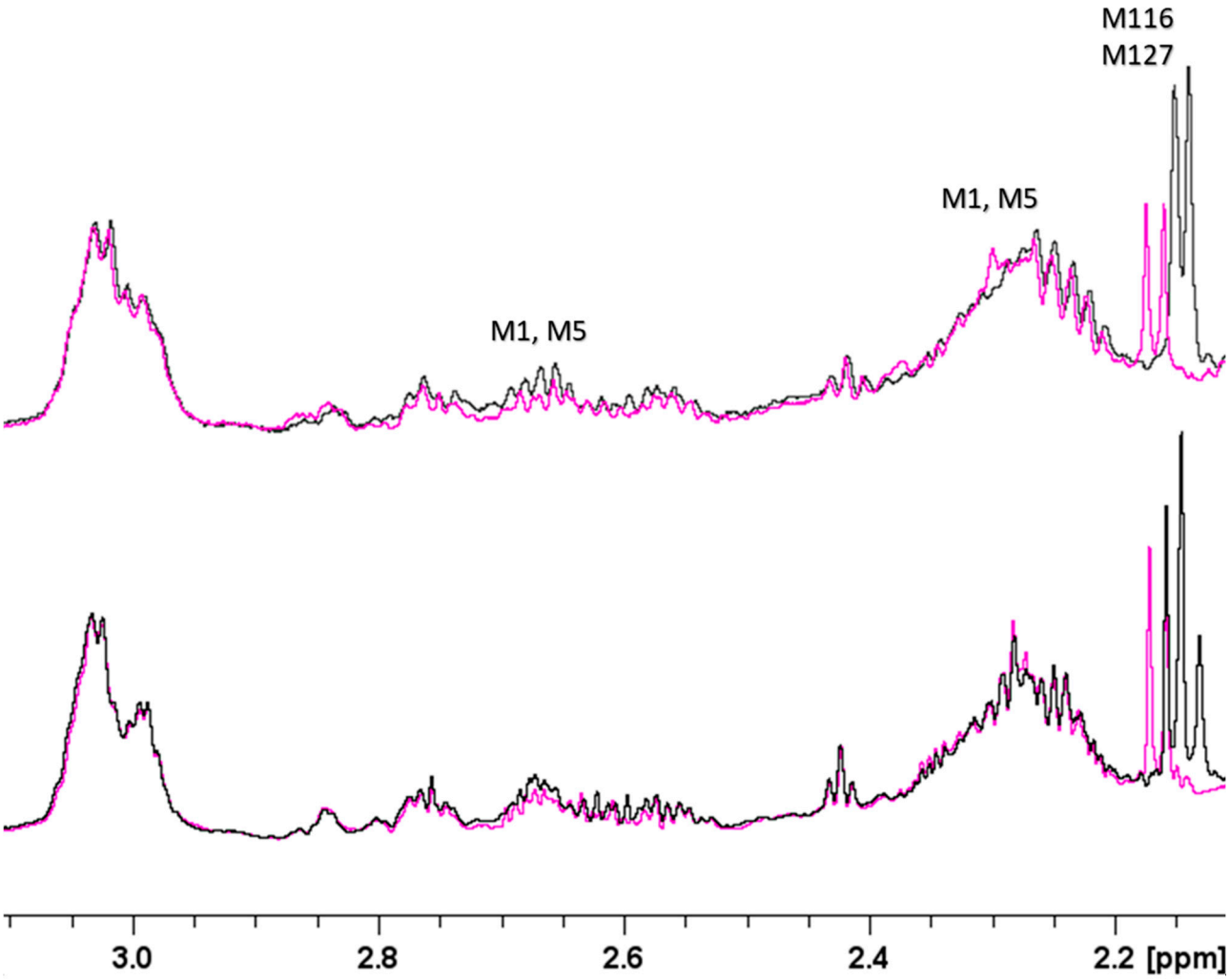


Figure S2. Overlaid ^{1}H 1D spectra of micelles-bound $\alpha Syn_{1\text{-}140}$ (lower trace) and acetylated $\alpha Syn_{1\text{-}140}$ in absence (black) and in presence of 0.8 Ag^{+} eq (magenta). Protein concentrations 240 µM, SDS-d_{25} 50mM, phosphate buffer 20 mM pH= 7.4, T =298 K.

Table S1. Fitting results of the EXAFS spectra including different models.

	Nº Fit	Atom type	N*	Distance (Å)	$2\sigma^2$ (Å^2)	R - factor	χ_v^2	ΔE_0 (eV)
$\alpha S_{1\text{-}15}$ SDS-Cu^+	1	S	2	2.16 (1)	0.002 (1)	0.023	12	-5 (3)
		S	2	2.68 (1)	0.005 (1)			
	2	S	4	2.22 (1)	0.008 (1)	0.066	54	3 (4)
	3	N/O	1	2.05 (1)	0.003 (1)	0.039	32	8 (4)
		S	3	2.24 (1)	0.006 (1)			
	4	S	3	2.23 (1)	0.004 (1)	0.033	27	5 (3)
	5	N/O	1	2.05 (1)	0.001 (1)	0.034	28	10 (4)
		S	2	2.25 (1)	0.002 (1)			
αSyn SDS-Cu^+	1	S	2	2.11 (1)	0.007 (1)	0.041	11	-3(3)
		S	1	2.99 (1)	0.013 (1)			
	2	N/O	1	1.94 (1)	0.002 (1)	0.045	12	5 (3)
		S	1	2.15 (1)	0.005 (1)			
		S	1	3.04 (1)	0.007 (1)			
	3	S	3	2.10 (1)	0.012 (2)	0.078	20	-6 (4)
	4	S	1	2.12 (2)	0.001 (2)	0.107	27.5	0 (5)
		S	1	2.99 (2)	0.002 (2)			
	5	S	2	2.11 (1)	0.007 (1)	0.063	16	-5 (4)
	6	S	4	2.09 (2)	0.017 (2)	0.125	32	-9 (5)
αSyn UV-Cu^+	1	S	3	2.27 (1)	0.008 (1)	0.026	36	6 (3)
	2	N/O	1	2.06 (1)	0.005 (1)	0.060	62	5
		S	3	2.25 (1)	0.009 (1)			
	3	N/O	1	2.06 (1)	0.003 (1)	0.097	100	5
		S	2	2.25 (1)	0.005 (1)			
	4	S	2	2.21 (2)	0.005 (2)	0.066	89	-2 (6)
		S	2	2.74 (2)	0.010 (2)			

N – coordination number.*

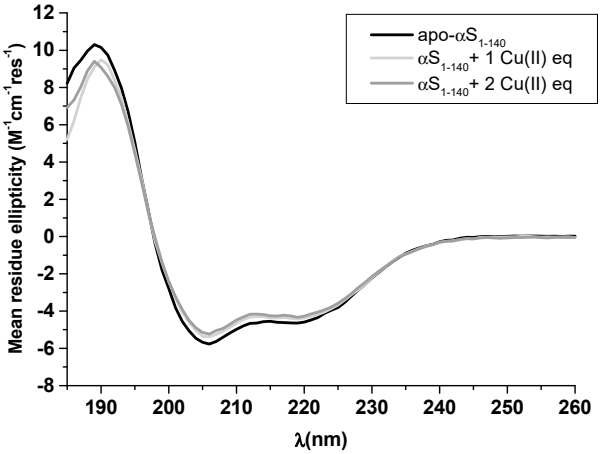


Figure S3. Far UV CD spectra at 298K of 10 μM full length αSyn at pH 7.4, in the presence of 50 mM SDS. Spectra were recorded at different Cu^{2+} concentration: 0 eq (black), 1 eq (light gray), 2 eq (dark grey).

Table S2. Anisotropic magnetic parameters of micelle bound αSyn_{1-140}, αSyn_{1-15} and αSyn_{45-15} copper(II) complexes.

	$g_{\parallel}$[a]	$g_{\perp}$[a]	$A_{\parallel}$(mT)[b]	$A_{\perp}$(mT)[b]	n°N[c]	A_N(mT)[b]
αSyn_{1-140}	2.236	2.056	17.8	0.5	2	1.4
αSyn_{1-15}	2.247	2.058	18.0	0.65	2	8
αSyn_{45-55}	2.2	2.076	122	7.5	-	-

[a] *Estimated error ±0.001.* [b] *Coupling constant are given in mT. Estimated error for A_{Cu} = ±0.1mT and for A_N = ±0.05mT.* [c] *Number of equatorial nitrogen donor atoms.*

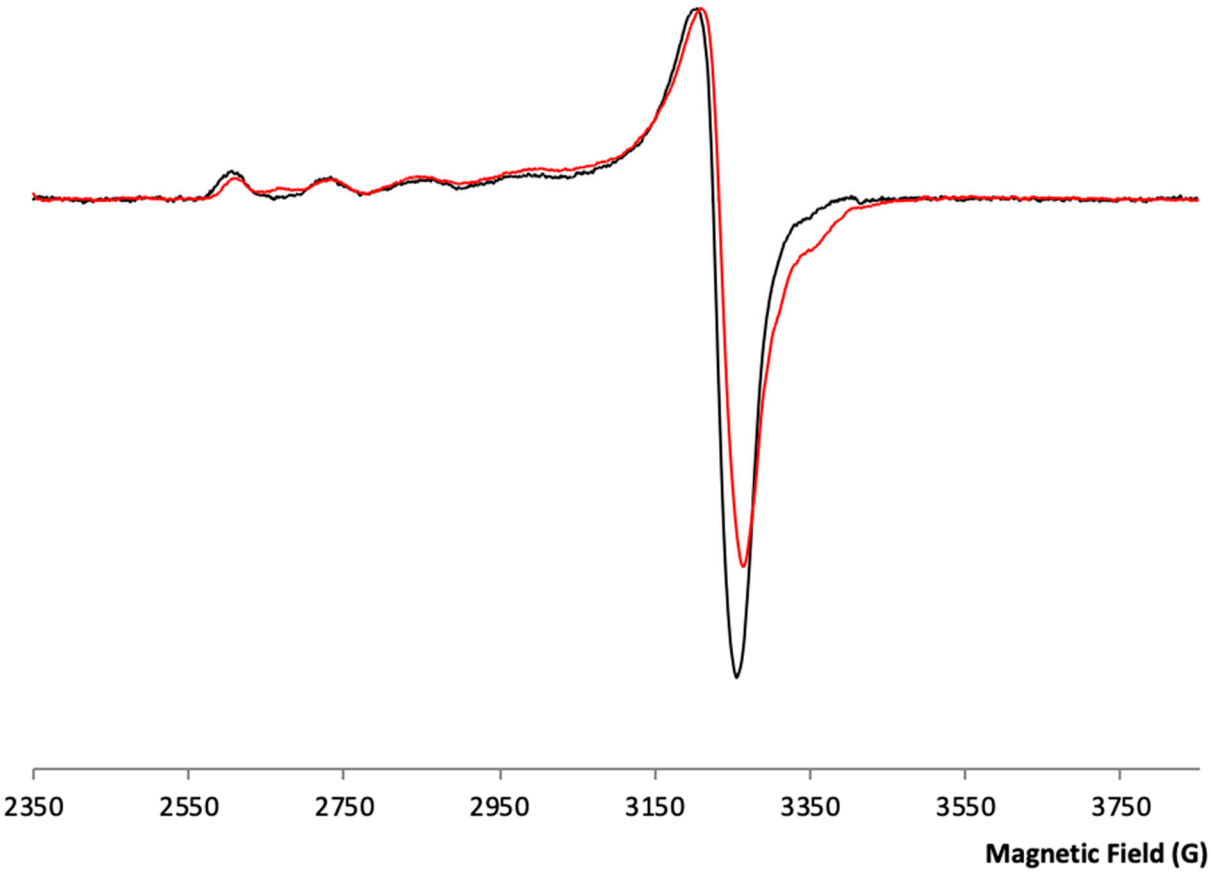


Figure S4. Comparison of EPR spectra of micelle bound $\alpha Syn_{45\text{-}55}$ Cu^{2+} complex (red line) and hexaaquacopper(II) ion (black line). $\alpha Syn_{45\text{-}55}$ 500 µM, Cu^{2+} µM, SDS 50mM, phosphate buffer 20 mM pH= 7.4.

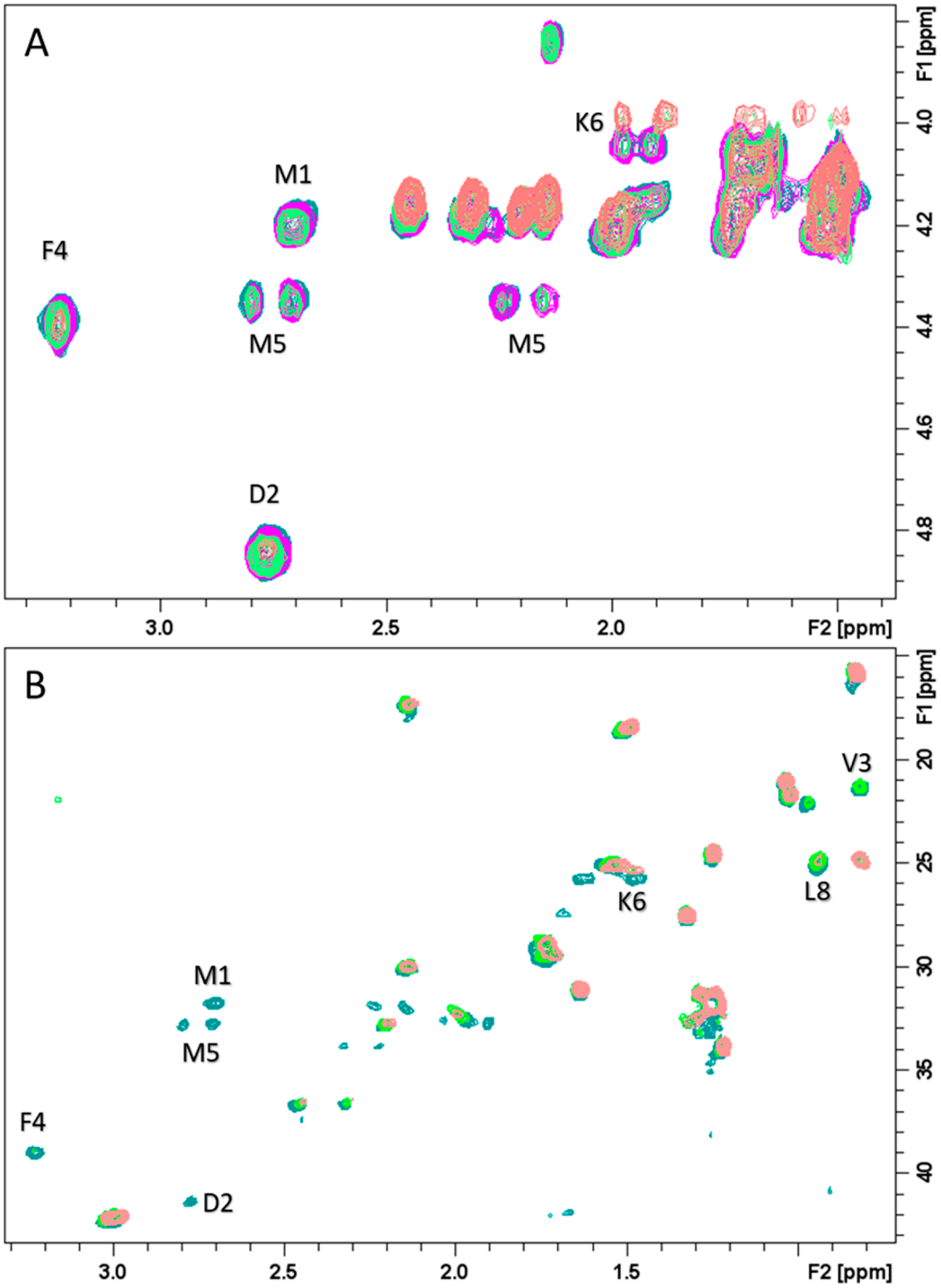


Figure S5. Overlaid ^{1}H-^{1}H TOCSY (A) and ^{1}H-^{13}C HSQC (B) spectra of αSyn_{1-15} before (sea-green) and after the addition of increasing Cu^{2+} equivalent: 0.2 Cu^{2+} equiv. (magenta), 0.4 Cu^{2+} equiv. (green), 0.6 Cu^{2+} equiv. (pink). αSyn_{1-15} 450 µM, SDS-d_{25} 50mM, phosphate buffer 20 mM pH= 7.4, T =298 K.

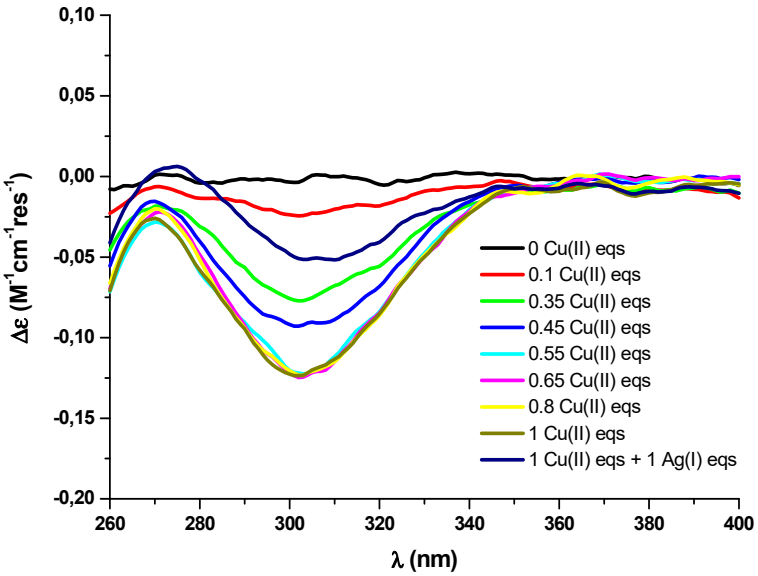


Figure S6. CD spectra of Cu^{2+}/Ag^{+} αSyn_{1-15} micelle bound system. αSyn_{1-15} 450 µM, SDS 50mM, phosphate buffer 20 mM pH= 7.4, T =298 K.

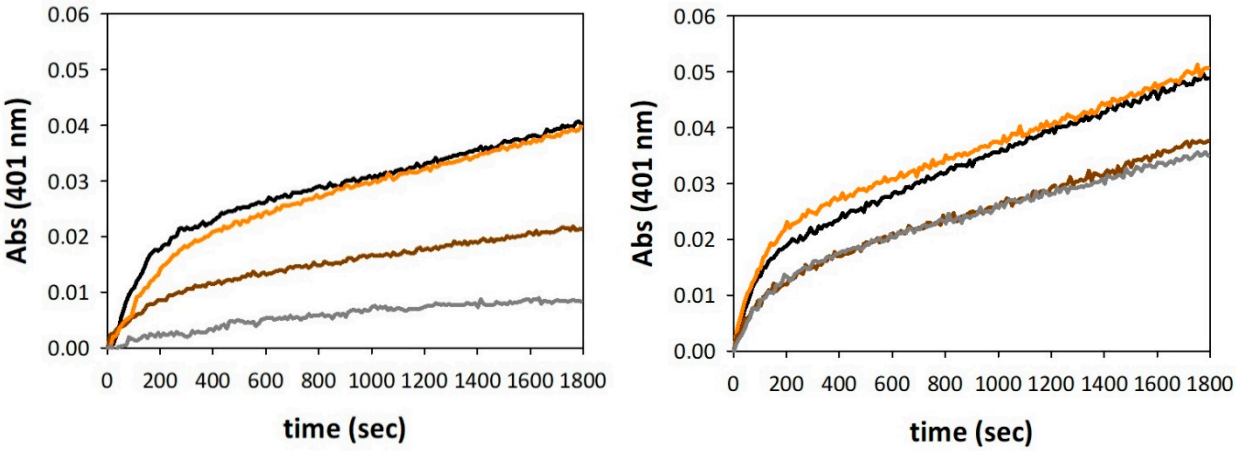


Figure S7. Kinetic profiles of 4-MC (3 mM) oxidation with time in 50 mM HEPES buffer at pH 7.4 and 20 °C in the presence of Cu^{2+} (25 μM) without SDS (black trace) and with SDS (20 mM) (orange), and with the addition of αSyn (50 μM, left panel and 25 μM, right panel) without SDS (brown) and with SDS (20 mM) (grey).

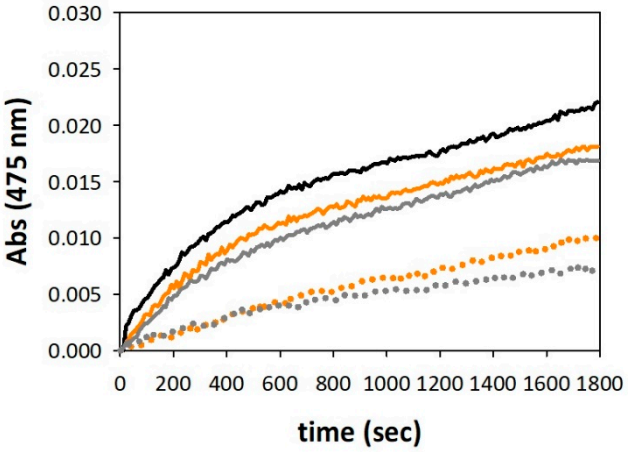
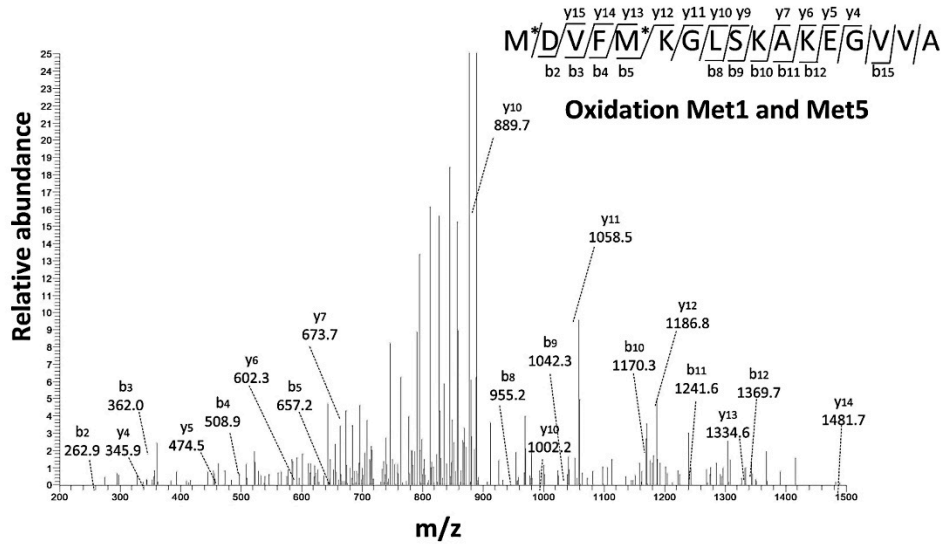
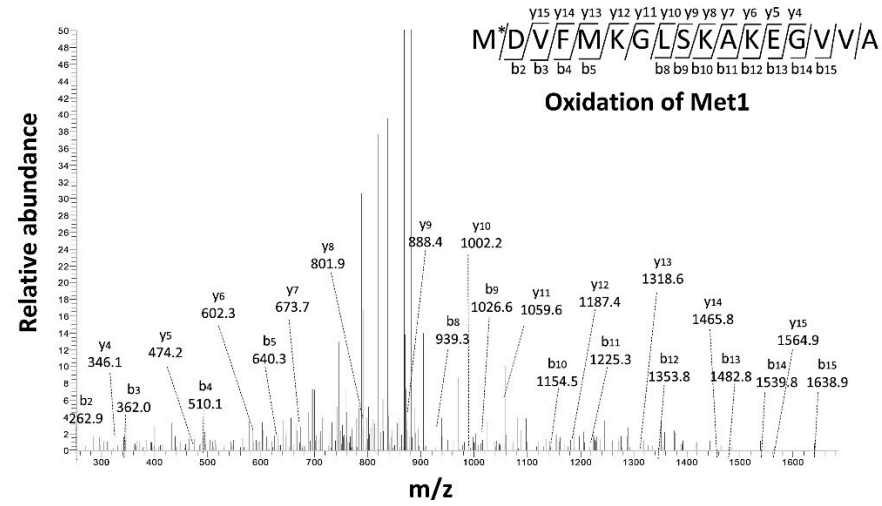
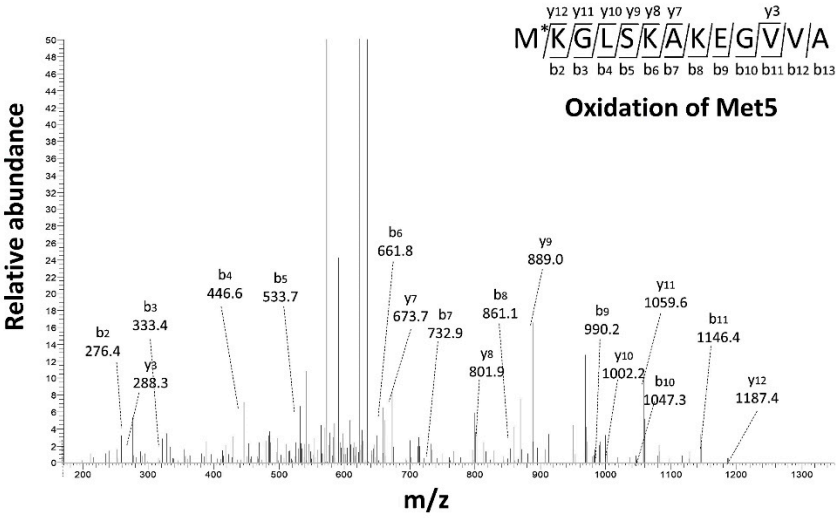


Figure S8. Kinetic profiles of dopamine (3 mM) oxidation with time in 50 mM HEPES buffer at pH 7.4 and 20 °C in the presence of Cu^{2+} (25 μM) (black trace) and upon the addition of 25 (orange) and 50 μM (grey) αSyn in buffered solution alone (solid traces) or in SDS micelles (dotted traces).


M*DVFM*KGLSKAKEGVVA
Oxidation Met1 and Met5
Relative abundance
m/z


M*DVFMKGLSKAKEGVVA
Oxidation of Met1
Relative abundance
m/z


M*KGLSKAKEGVVA
Oxidation of Met5
Relative abundance
m/z

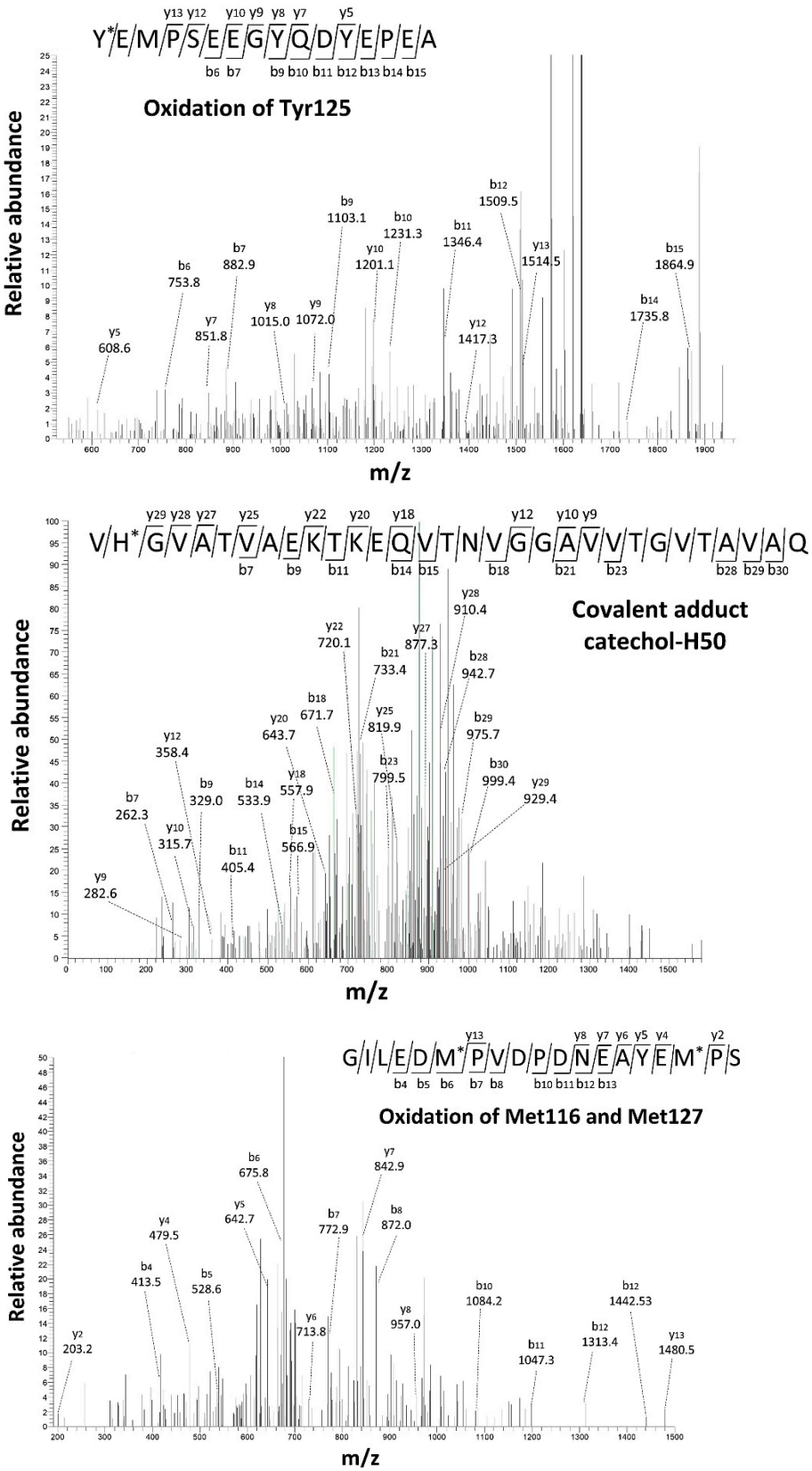


Figure S9. MS/MS spectra of more relevant fragments containing an insertion of one O-atom or of a catechol molecule obtained from HPLC-MS analysis of pepsin-digested αSyn in the reaction mixture. The assignments of the y and b ion series is shown. At the top of each spectrum, the residues which have undergone the modification are shown as star-marked and the summary of the y and b ions found in the spectrum for each fragment is also presented.