Supporting Information

Site-specific copper catalyzed oxidation of alpha-synuclein: Tightening the link between metal binding and protein oxidative damage in Parkinson´s disease

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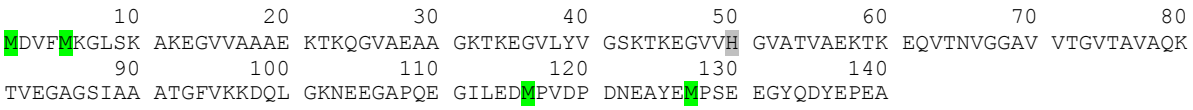


Figure S1. Primary sequence of AS. Letters shaded in green indicate methionine residues while histidine residue is shaded in grey.

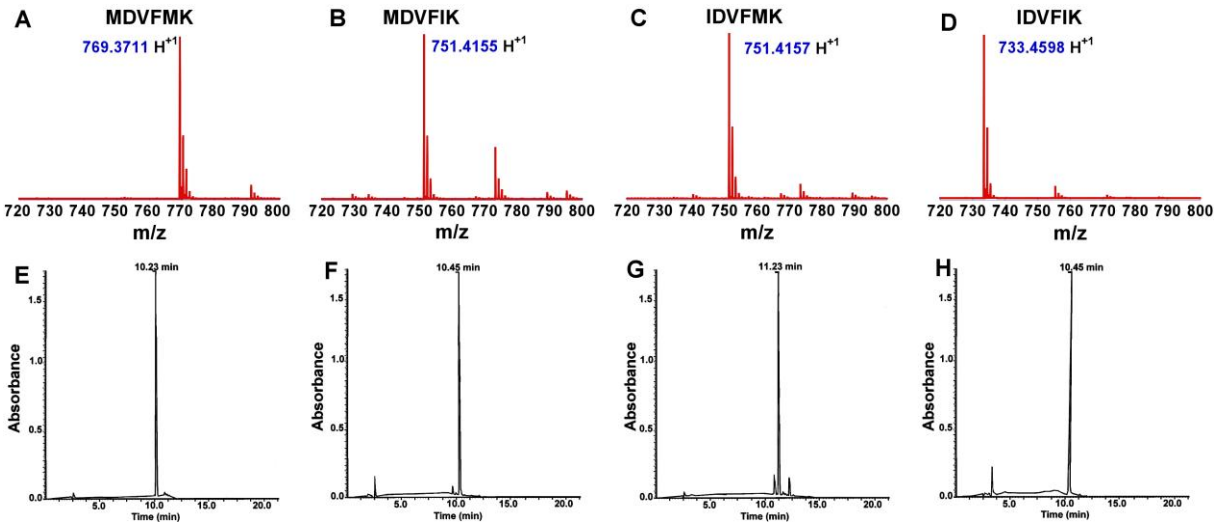
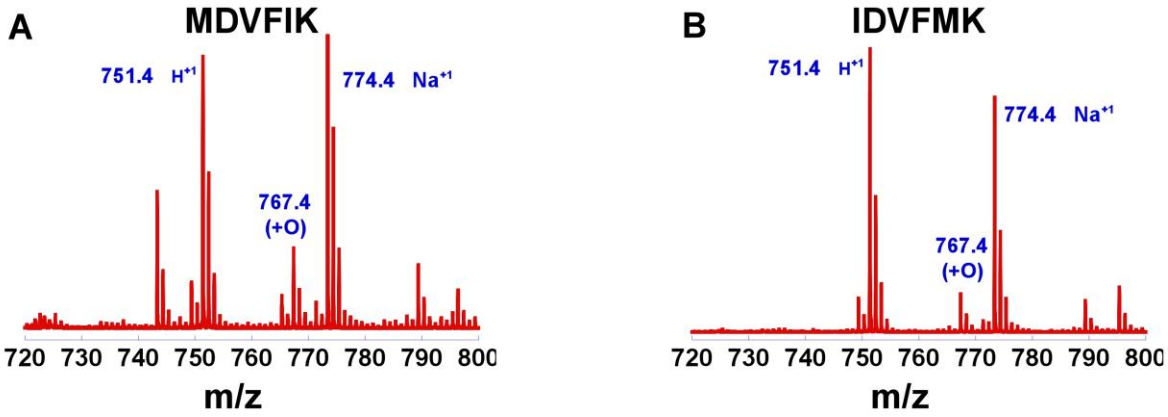


Figure S2. ESI-TOF-MS data (top row) and analytical HPLC Chromatograms (bottom row) for AS peptides: MDVFMK(**A** and **E**), MDVFIK (**B** and **F**), IDVFMK (**C** and **G**), and IDVFIK (**D** and **H**). Peptides were purified using a reverse phase chromatography (Waters HPLC Photodiode Array) with column C18 and a gradient 0 to 30 % ACN/H_2O in 20 minutes. As shown in the Figure, retention times were 10.23 min for MDVFMK, 10.45 min for MDVFIK, 11.23 min for IDVFMK and 10.45 min for IDVFIK at wavelength 214 nm.



Sequence	**Mass (H^+)**	**1 Met-Ox (H^+)**
MDVFMK	769.35	785.33[a]
IDVFMK	751.41	**767.41**
MDVFIK	751.41	**767.41**

Figure S3. MALDI-TOF data of AS peptides after exposure of Cu(I)-AS complexes to oxygen. **A** corresponds to MDVFIK, and **B** corresponds to IDVMFK. In both cases, a peak is observed at the mass that corresponds to the peptide with one oxidized Met residue, according to the table. Oxidation of methonine residues to sulphones (m/z 783.4) was not observed under the experimental conditions of our studies.

[a] As reported in Ref. 44 in the manuscript.

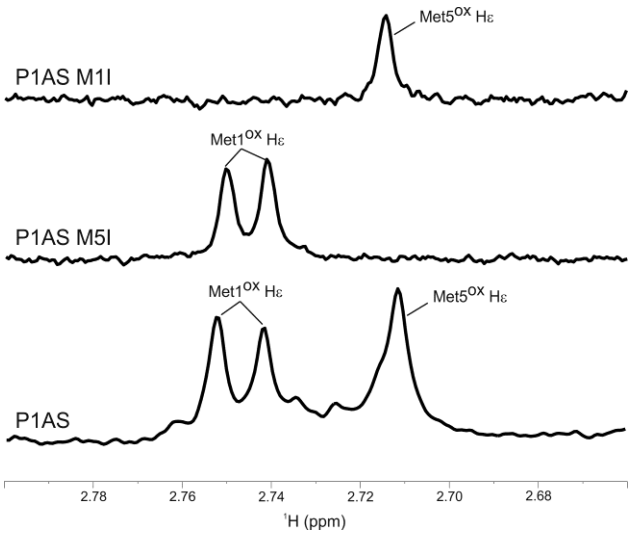


Figure S4. 1D ^{1}H NMR spectra of oxidized P1AS, M5I P1AS and M1I P1AS variants. The S-CH_3 resonances of methionine sulfoxides fall in a particularly well-resolved region of the ^{1}H NMR spectrum of the peptide variants studied and can be assigned unambiguously. NMR spectra were recorded after 24h of air exposure of samples containing peptide-Cu(I) complexes (100 μM), followed by addition of 5mM EDTA.

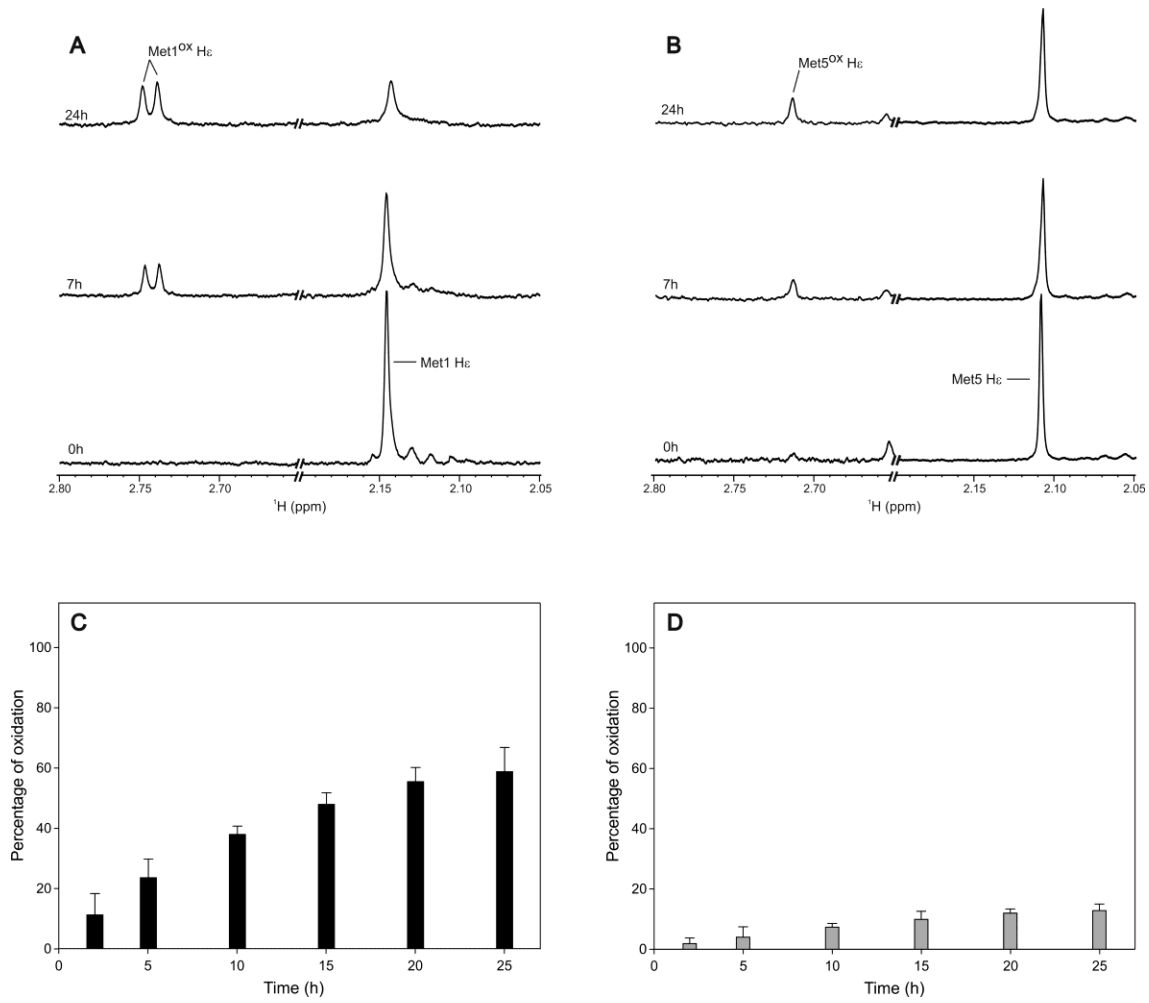


Figure S5. Metal catalyzed oxidation of P1AS peptide mutants monitored by NMR. Time evolution of 1D ^{1}H NMR spectra of (A) M5I P1AS (100 μM) and (B) M1I P1AS (100 μM) in the presence of 1 equivalent of Cu(I) under aerobic conditions. Oxidation of Met residues as a function of time was monitored by integrating the signals corresponding to the Hε protons of (C) Met-1 of M5I P1AS and (D) Met-5 of M1I P1AS in their sulfoxide states.

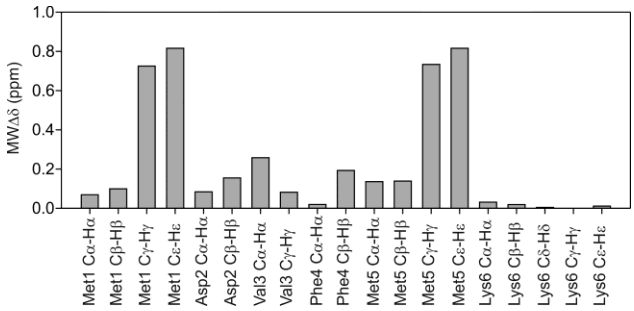


Figure S6. Mean weighted chemical shift displacements (MW ^{1}H-^{13}C Δδ) of 1mM ^{1}MDVFMK^{6} signals induced by the presence of 0.8 equivalents of Cu(I). NMR experiments were recorded in buffer A at 15ºC.