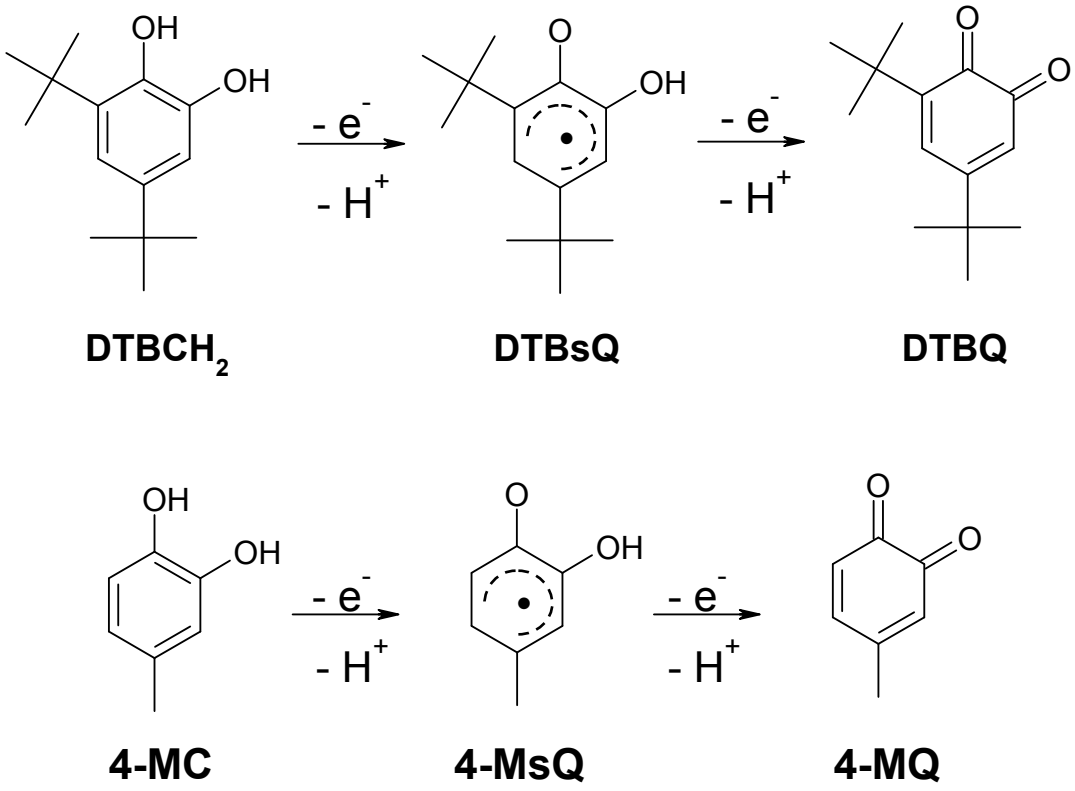
Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information

Reactivity of copper-α-synuclein peptide complexes relevant to Parkinson's disease

Simone Dell'Acqua,[a]* Valentina Pirota,[a] Cecilia Anzani,[a] Michela M. Rocco,[a] Stefania Nicolis,[a] Daniela Valensin,[b] Enrico Monzani,[a] and Luigi Casella[a]*

[a] Dipartimento di Chimica, Università di Pavia, Via Taramelli 12, 27100 Pavia, Italy
[b] Dipartimento di Biotecnologie, Chimica e Farmacia, Università di Siena, Via A. Moro 2, Siena, Italy



Scheme S1 – Oxidation of 3,5-di-*tert*-butylcatechol ($DTBCH_2$) (top) and 4-methylcatechol (4-MC) (bottom). The reaction proceeds through formation of 3,5-di-*tert*-butylsemiquinone (DTBsQ) and 4-methylsemiquinone (4-MsQ), and finally to 3,5-di-*tert*-butylquinone (DTBQ) and 4-methylquinone (4-MQ).

Table S1 – Standard reduction potential (E°) at pH = 7 of dopamine, 4-MC and $DTBCH_2$.

	E° ($HQ^{\bullet}/H_2Q$) (V)[a]	pK_{a1}	pK_{a2}	pK_s[b]
dopamine	0.53[c]	10.58[d]	12.07[d]	4.7[d]
4-MC	0.46[e]			
$DTBCH_2$	0.39[e]			

[a] E° ($HQ^{\bullet}/H_2Q$) represents the standard redox potential for the reaction: $HQ^{\bullet} + H^+ + e^- \rightleftarrows H_2Q$

[b] K_s represents the dissociation constant of the semiquinone: $HQ^{\bullet} \rightleftarrows H^+ + Q^{\bullet-}$

[c] Following the method used by Pham and Waite [S1], $E°_{pH=7}$ ($HQ^{\bullet}/H_2Q$) for dopamine was extrapolated from the equation: $E°_{pHi} = E°_{pH=0} + 0.059 \log (K_{a1} \times K_{a2} + K_{a1}[H^+] + [H^+]^2)/(K_s + [H^+])$, where $E°_{pH=0}$ = 1.076 V, obtained by the same equation giving $E°_{pH=13.5}$ = 0.018 V (Steenken and Neta [S2]).

[d] Sanchez-Rivera *et al*. [S3].

[e] Nemeikaite-Ceniene *et al*. [S4].

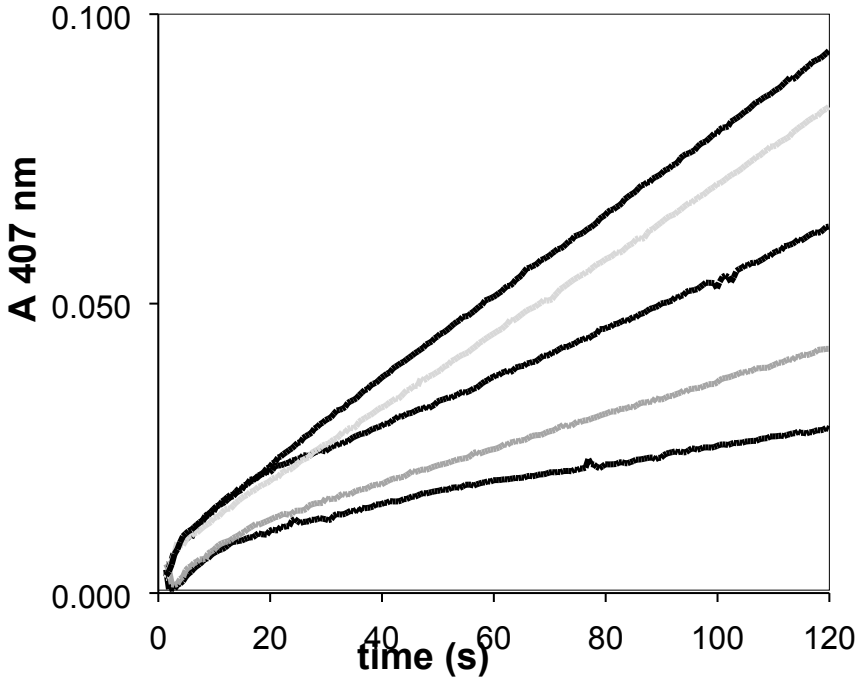


Figure S1 – Kinetic traces of absorbance at 407 nm *vs.* time for the oxidation of $DTBCH_2$ 4 mM (black continuous trace), 2 mM (light gray continuous trace), 1 mM (black dotted trace), 0.6 mM (dark gray continuous trace), 0.4 mM (black dashed trace), in a mixture of 80:20 = methanol:HEPES buffer (50 mM), pH 7.4, at 25 °C in the presence of free copper(II) (25 μM).

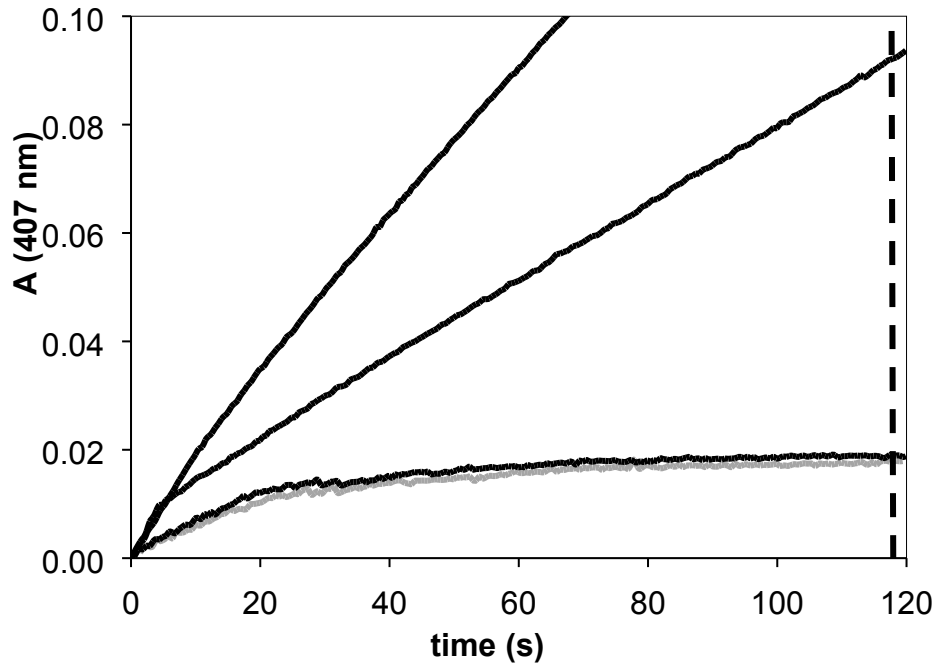


Figure S2 – Kinetic traces of absorbance at 407 nm *vs.* time for the oxidation of $DTBCH_2$ (4 mM) in a mixture of 80:20 = methanol:HEPES buffer (50 mM), pH 7.4, at 25 °C in the presence of free copper(II) (25 μM) (black continuous trace), and copper(II) (25 μM) and αSyn6 (50 μM) (gray continuous trace). The same experiment has been carried out in the same conditions with the solvent mixture saturated with pure oxygen with both free copper(II) (black dashed trace) and copper-αSyn6 1:2 complex (black dotted trace).

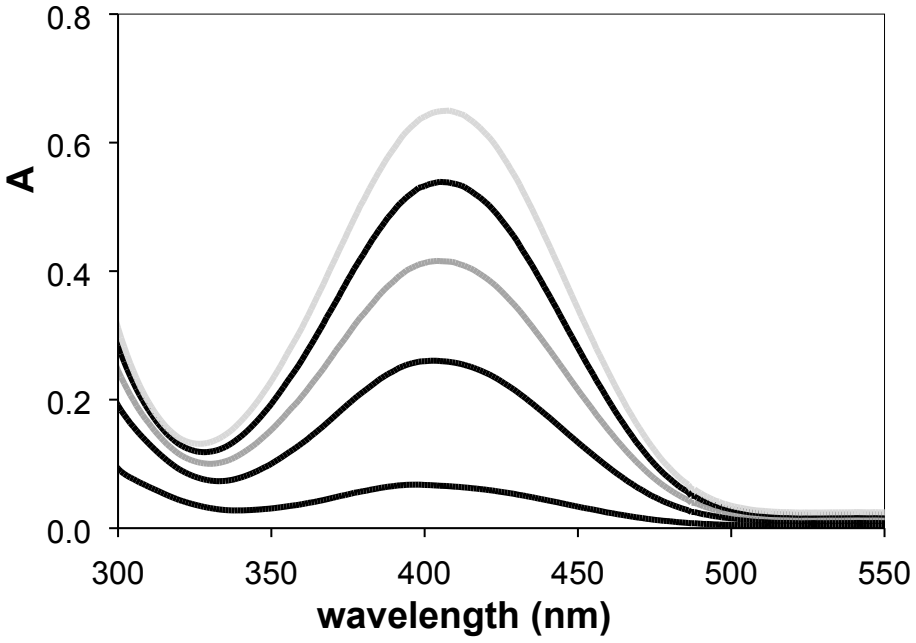


Figure S3 – Selected absorption spectra of the oxidation reaction of $DTBCH_2$ (4 mM) in a mixture of 80:20 = methanol:HEPES buffer (50 mM), pH 7.4, at 25 °C in the presence of free copper(II) (25 μM) at the following reaction times: 5 min (black dotted line), 20 min (black continuous line), 40 min (dark gray continuous line), 60 min (black dashed line), 100 min (light gray continuous line).

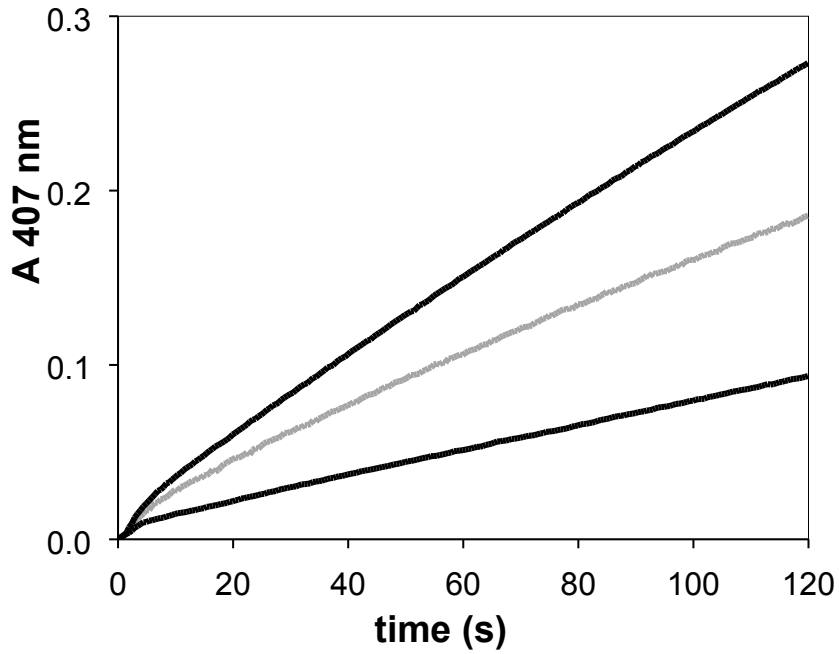


Figure S4 – Kinetic traces of absorbance at 407 nm *vs.* time for the oxidation of $DTBCH_2$ (4 mM) in a mixture of 80:20 = methanol:HEPES buffer (50 mM), pH 7.4, at 25 °C in the presence of free copper(II) 25 μM (black dashed trace), 50 μM (gray continuous trace) and 75 μM (black continuous trace).

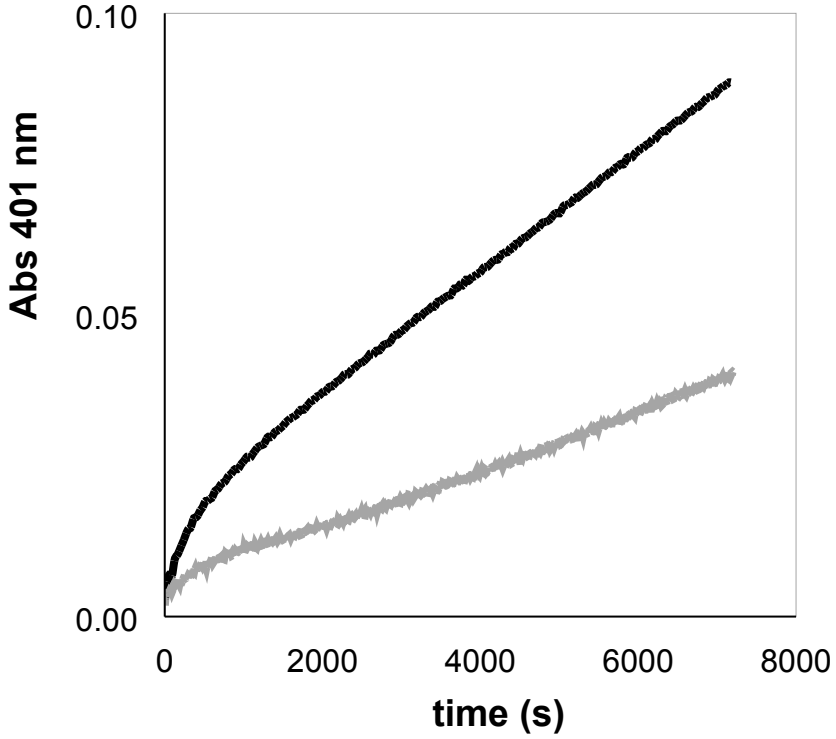


Figure S5 – Kinetic traces of absorbance at 401 nm vs. time for the oxidation of 4-MC (3 mM) in a mixture of 80:20 = methanol:HEPES buffer (50 mM) at pH 7.4 and 25 °C in the presence of free Cu^{2+} (25 μM) (black trace), and Cu^{2+} (25 μM) and αSyn15 (50 μM) (gray trace).

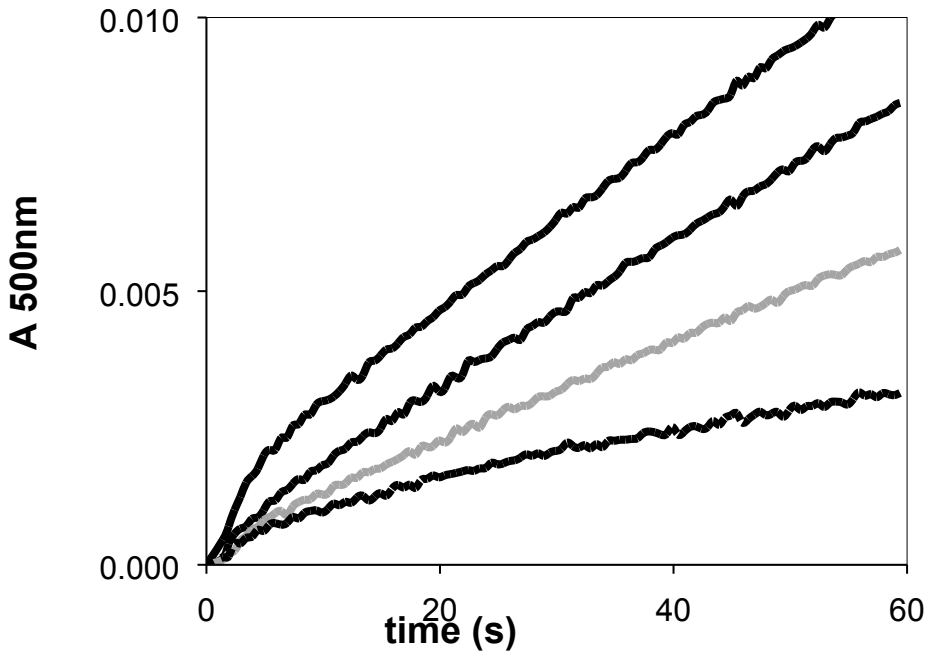


Figure S6 – Kinetic traces at 500 nm in the initial phase of reaction for the formation of the MBTH-quinone adduct by oxidation of phenol (2 mM) in the presence of Cu^{2+} (5 μM) and MBTH (2 mM) (black continuous trace) and variable amounts of αSyn6 (0.2 equiv. - black dashed trace; 0.5 equiv. – gray continuous trace; 1.5 equiv. - black dotted trace), in HEPES buffer (50 mM) at pH 7.0.

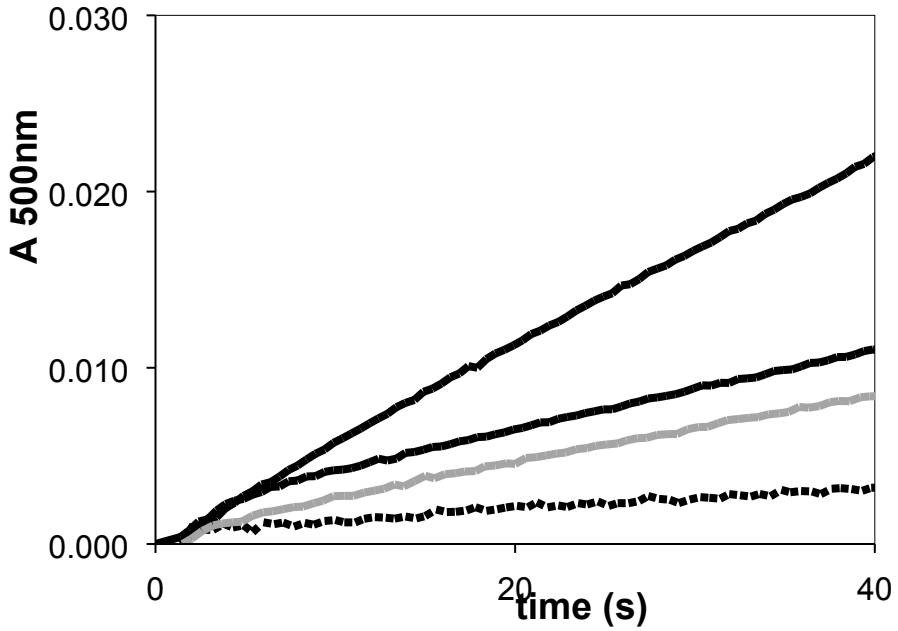


Figure S7 – Kinetic traces of absorbance at 500 nm *vs.* time for the formation of the MBTH-quinone adduct by oxidation of phenol (2 mM) in HEPES buffer (50 mM) at pH 7.0 in the presence of copper(II) (5 μM) and MBTH (2 mM) (black continuous trace) and copper(II) (5 μM) and αSyn15 (7.5 μM) (black dotted trace). The same experiment has been carried out in the same conditions with the solvent mixture saturated with pure oxygen with both free copper(II) (black dashed trace) and copper-αSyn6 1:1.5 complex (gray continuous trace).

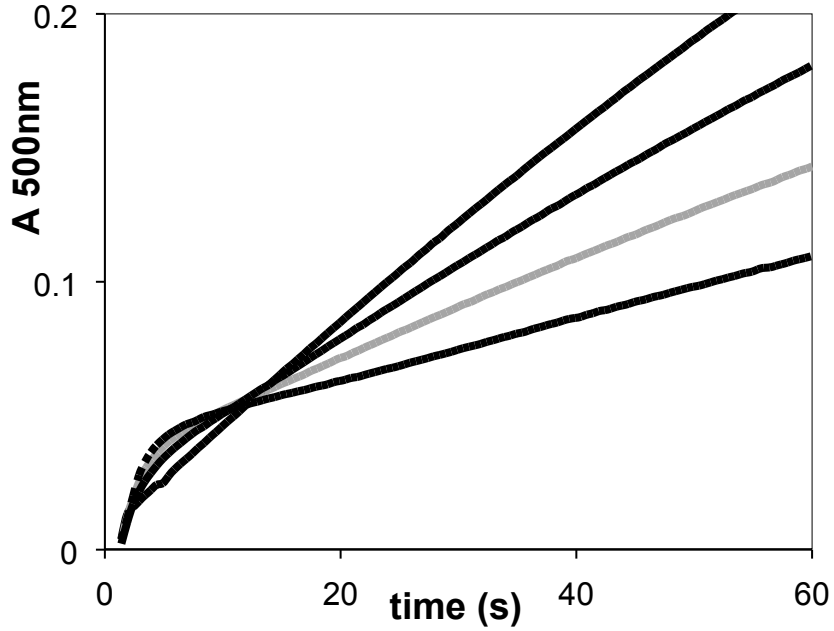


Figure S8 – Kinetic traces at 500 nm in the initial phase of oxidation of 4-MC (2 mM), with formation of MBTH-quinone adduct, in the presence of Cu^{2+} (25 μM) (black continuous trace), variable amounts of αSyn6 (0.8 equiv. - black dashed trace; 1.5 equiv. – gray continuous trace; 3.0 equiv. – black dotted trace), and MBTH (2 mM), in HEPES buffer (50 mM) at pH 7.0.

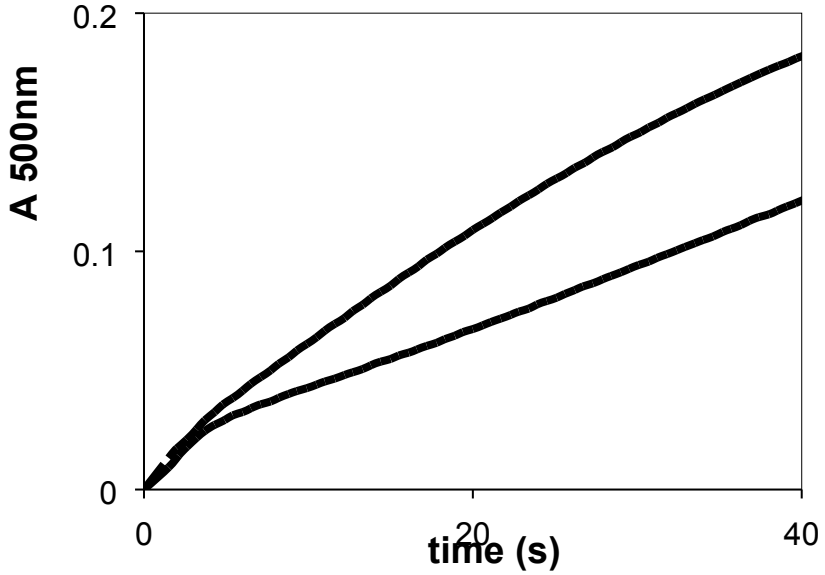


Figure S9 – Kinetic traces of absorbance at 500 nm in the initial phase of oxidation of 4-MC (2 mM), with formation of MBTH-quinone adduct, in the presence of Cu^{2+} (25 μM) and αSyn15 (25 μM) (black continuous trace) in HEPES buffer (50 mM) at pH 7.0. The same experiment has been carried out in the same conditions with buffer saturated with pure oxygen (black dashed trace).

References:

[S1]. A. N. Pham, T. D. Waite, *J Inorg Biochem*, **2014**, 137, 74-84.
[S2]. S. Steenken, P. Neta, *J. Phys. Chem.*, **1982**, 86, 3661–3667.

[S3]. A.E. Sanchez-Rivera, S. Corona-Avendano, G. Alarcon-Angeles, A. Rojas-Hernandez, M.T. Ramirez-Silva, M.A. Romero-Romo, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **2003**, 59, 3193–3203.
[S4]. A. Nemeikaite-Ceniene, A. Imbrasaite, E. Sergediene and N. Cenas, *Arch. Biochem. Biophys.*, **2005**, 441, 182-190.