Electronic Supplementary Information

Peptide-based chemical models for lytic polysaccharide monooxigenases

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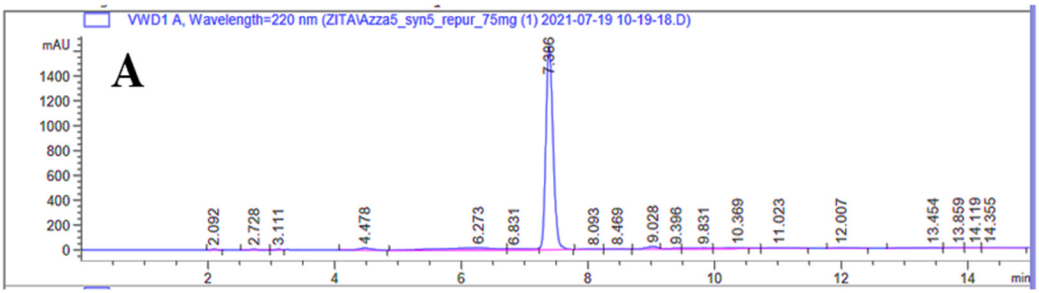

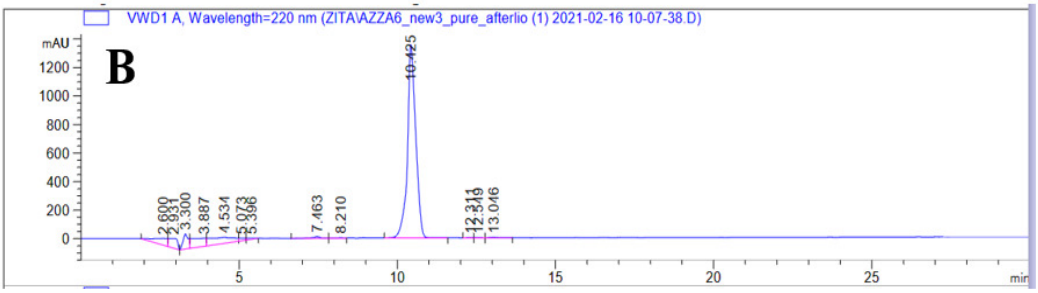

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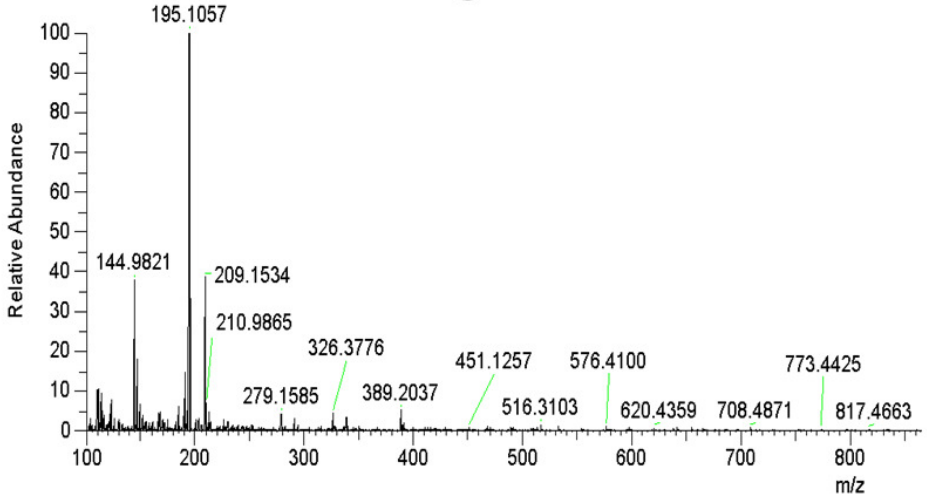
C



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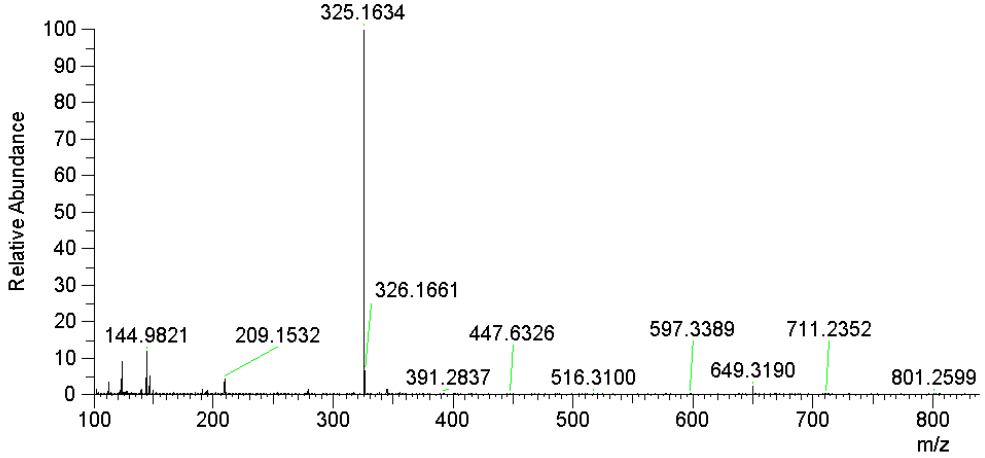


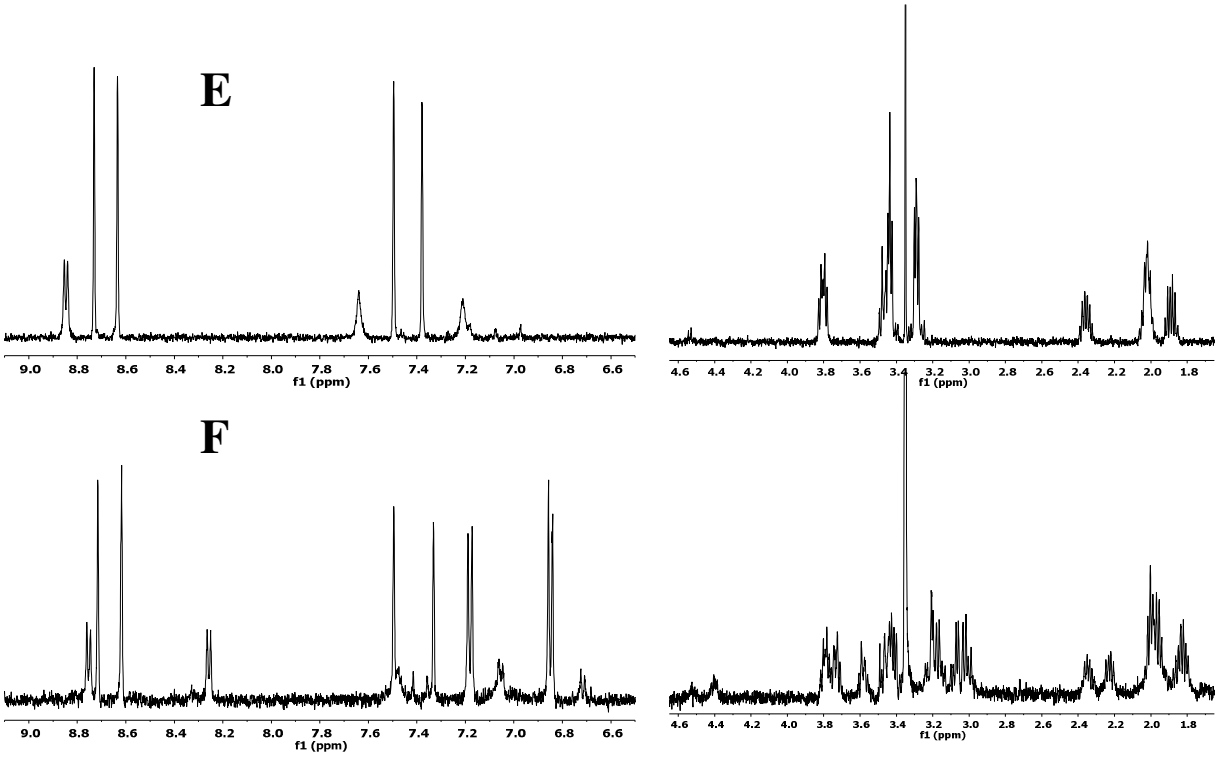


Figure S1 Analytical HPLC chromatograms (A,B), HR ESI-MS (C,D) and ^{1}H NMR (E,F) spectra of the purified HPH-NH_2 (**L^{1}**) (A,C,E) and HPHPY-NH_2 (**L^{2}**) (B,D,F) peptides.
Conditions of HPLC chromatograms: JUPITER C18 column (150 x 4.6 mm, 300 Å pore size, 5 μm particle size), mobile phase A: 0.1% TFA in water, mobile phase B: 0.1% TFA in acetonitrile/water, flow rate: 1.2 ml/min, detection:UV at 220 nm.
HR ESI-MS: (C) *m/z* calc for $C_{17}H_{24}N_8O_3$ $[M+H]^+$ 389.20, found $[M+1]^+$ 389.20 (z = 1) and $[M + 2H]^{2+}$ 195.11 (z = 2); (D) *m/z* calc for $C_{31}H_{40}N_{10}O_6$ $[M]^+$ 648.31, found $[M+1]^+$ 649.39 (z = 1) and $[M + 2H]^{2+}$ 325.16 (z = 2). For the assignment of the ^{1}H NMR peaks see the Experimental part.

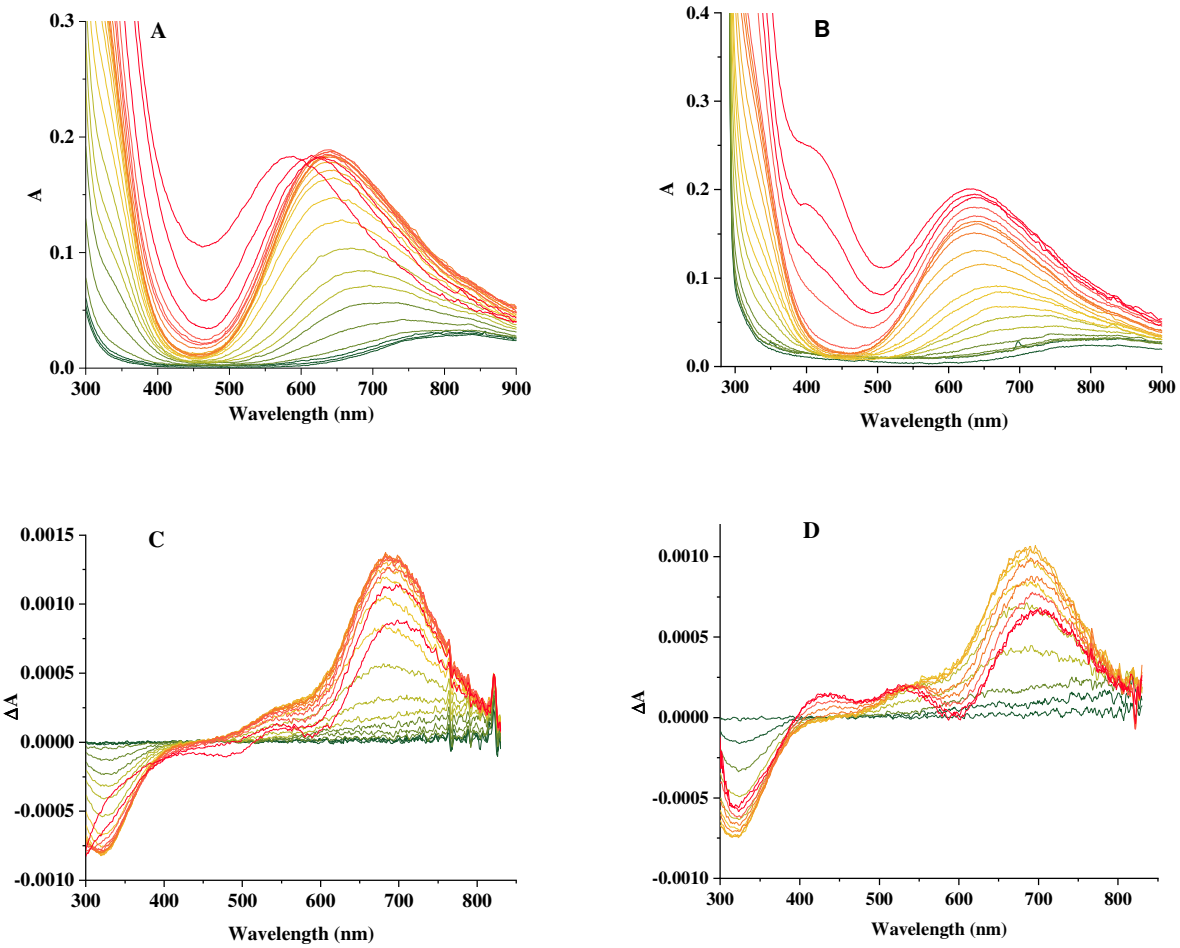


Figure S2 pH-dependent UV-Vis (A,B) and circular dichroism (C,D) spectra in the Cu(II)-$\mathbf{L^1}$ (A,C) and Cu(II)-$\mathbf{L^2}$ (B,D) systems at 1:2 metal-to-ligand ratios (the pH increases in the green-yellow-red direction, T = 298 K, I = 0.2 M KCl, $[L]_{tot}$ = 0.0014 M,).

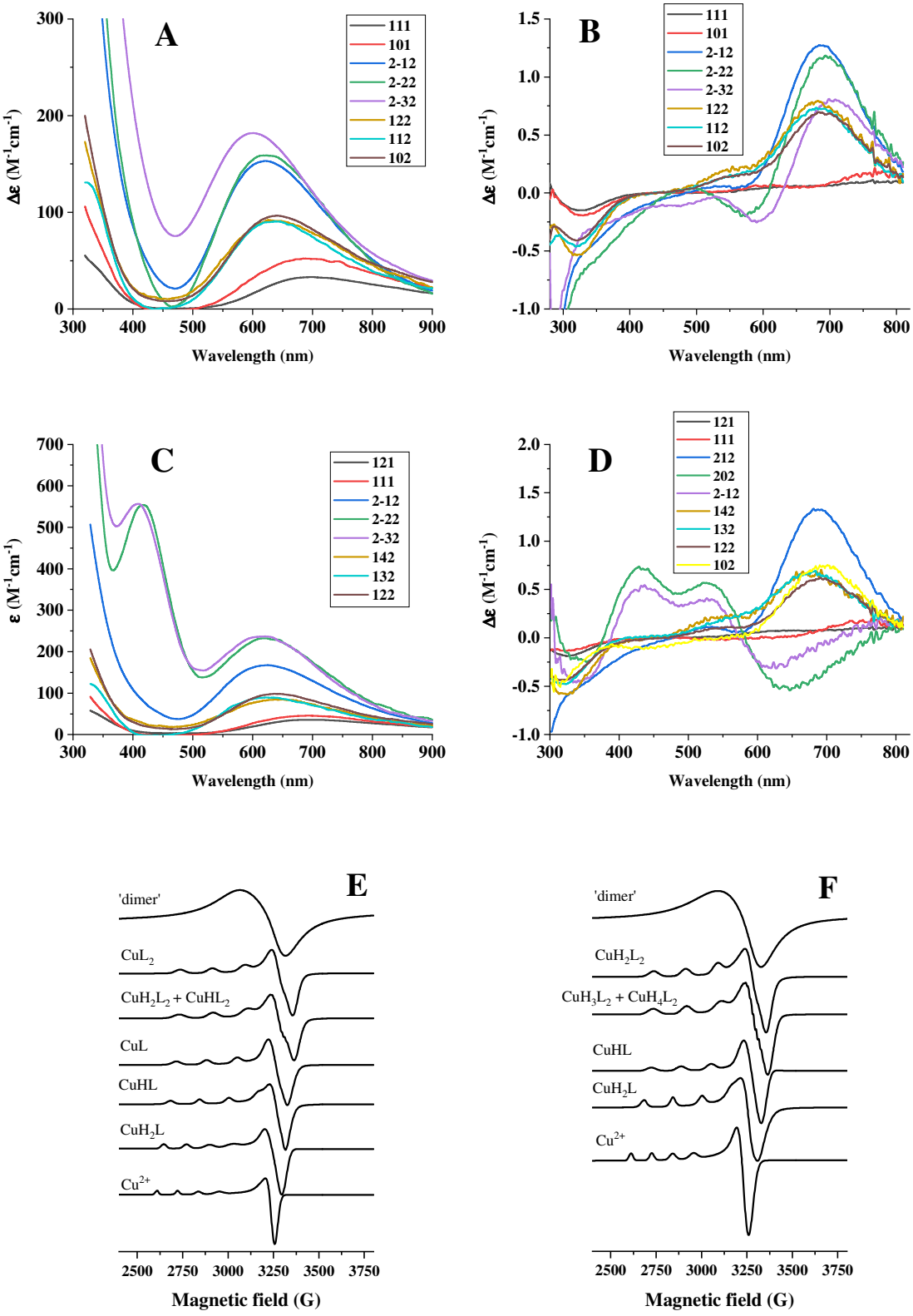


Figure S3 Individual UV-Vis (A,C), CD (B,D) and EPR (E,F) spectra of the complexes formed in the Cu(II)-**L**^{1} (A,B,E) and Cu(II)-**L**^{2} (C,D,F) systems. UV-Vis and CD spectra were calculated by the computer program PSEQUAD, while the component EPR spectra were obtained using the 'epr' software.

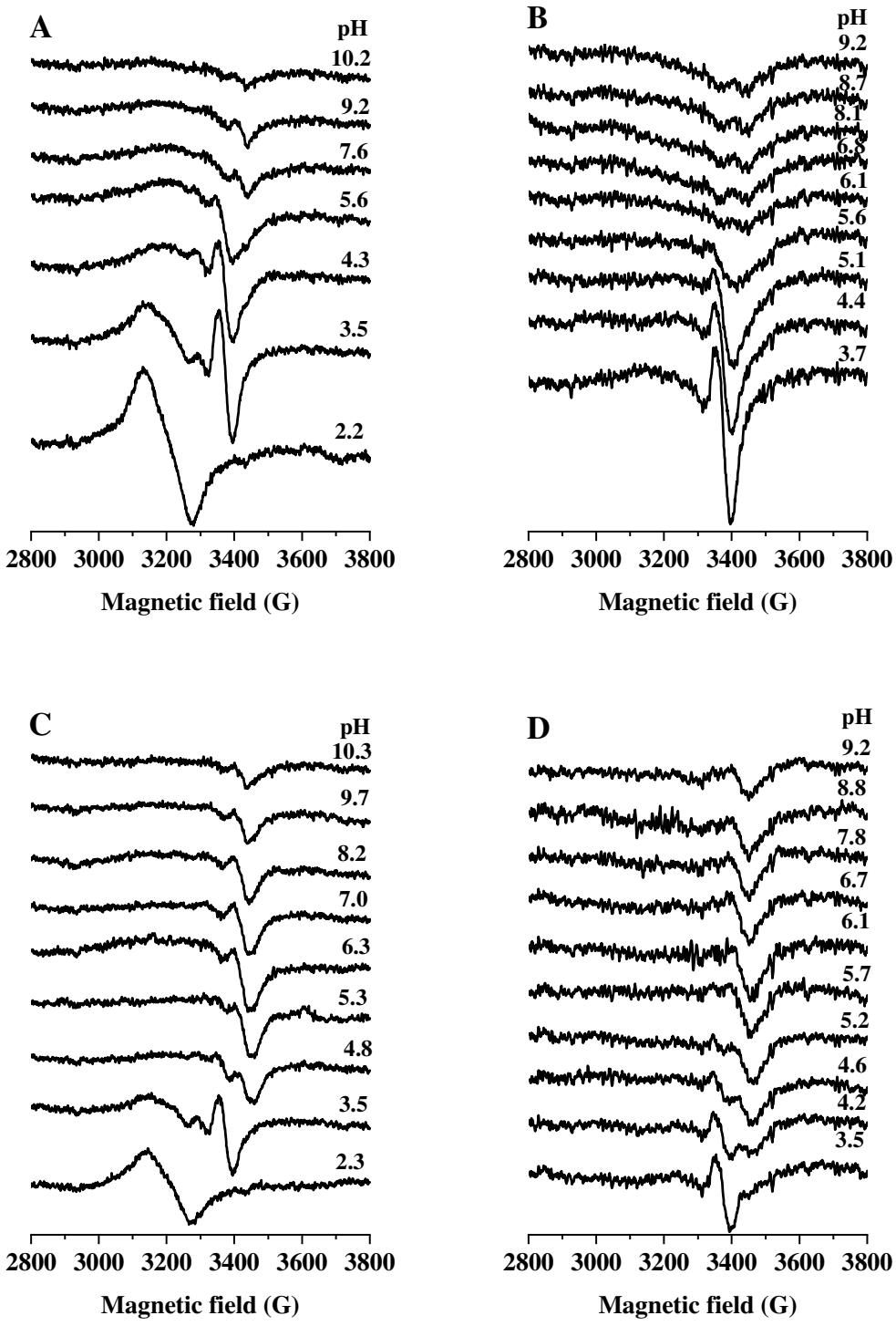


Figure S4 Experimental X-band CW-EPR spectra recorded at room temperature in the Cu(II)-$\mathbf{L^1}$ (A,C) and Cu(II)-$\mathbf{L^2}$ (B,D) systems at 1:1 (A,B) and 1:2 (C,D) metal-to-ligand ratios (A: $[Cu(II)]_{tot}$ = 1.24 mM, $[\mathbf{L^1}]_{tot}$ = 1.30 mM, B: $[Cu(II)]_{tot}$ = 0.65 mM, $[\mathbf{L^2}]_{tot}$ = 1.30 mM, C: $[Cu(II)]_{tot}$ = 1.80 mM, $[\mathbf{L^1}]_{tot}$ = 1.90 mM, D: $[Cu(II)]_{tot}$ = 0.95 mM, $[\mathbf{L^2}]_{tot}$ = 1.90 mM)

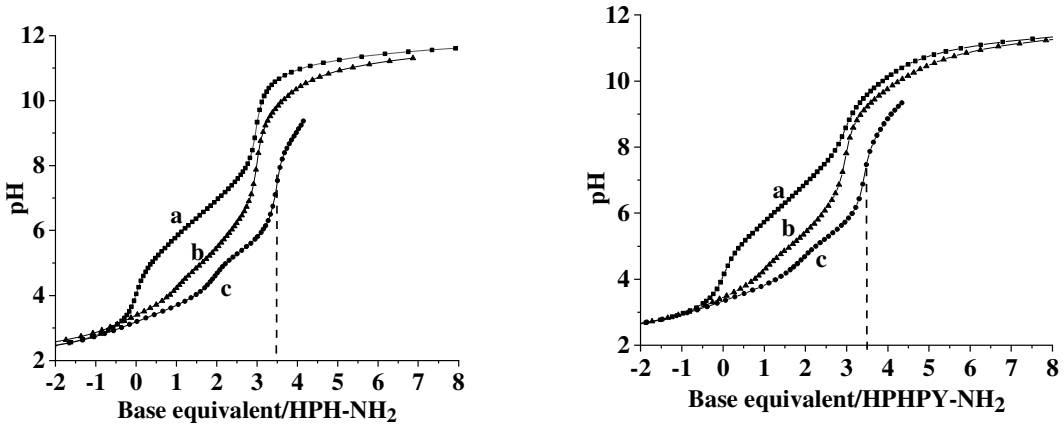


Figure S5.Titration curves of the free ligand (a), copper - ligand (II) 1:2 (b) and 1:1 (c) systems as a function of added base equivalent relative to the ligand $\mathbf{L^1}$ (left) and $\mathbf{L^2}$ (right).

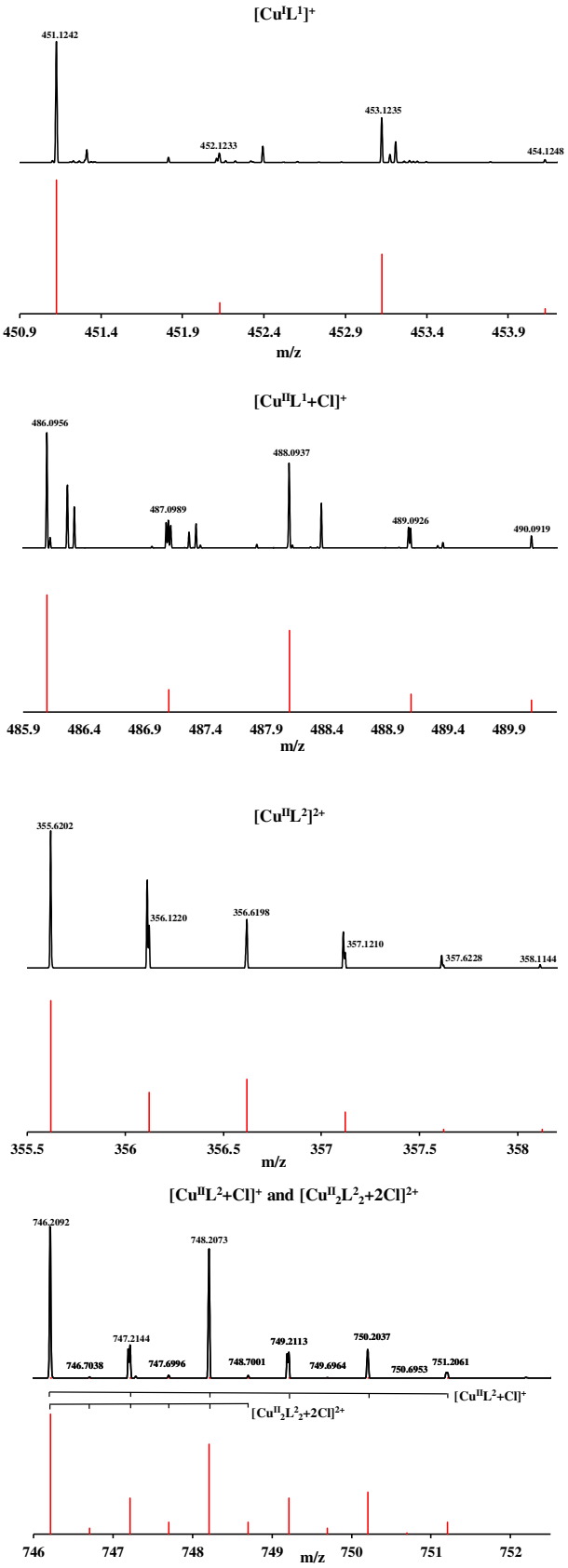


Figure S6 Monomer and dimer complexes identified on the ESI-MS spectra. The peaks related to the dimer $[Cu^{II}_2\mathbf{L^2}_2+2Cl]^{2+}$ complex (bottom) have notable lower intensity than those of the monomer species.

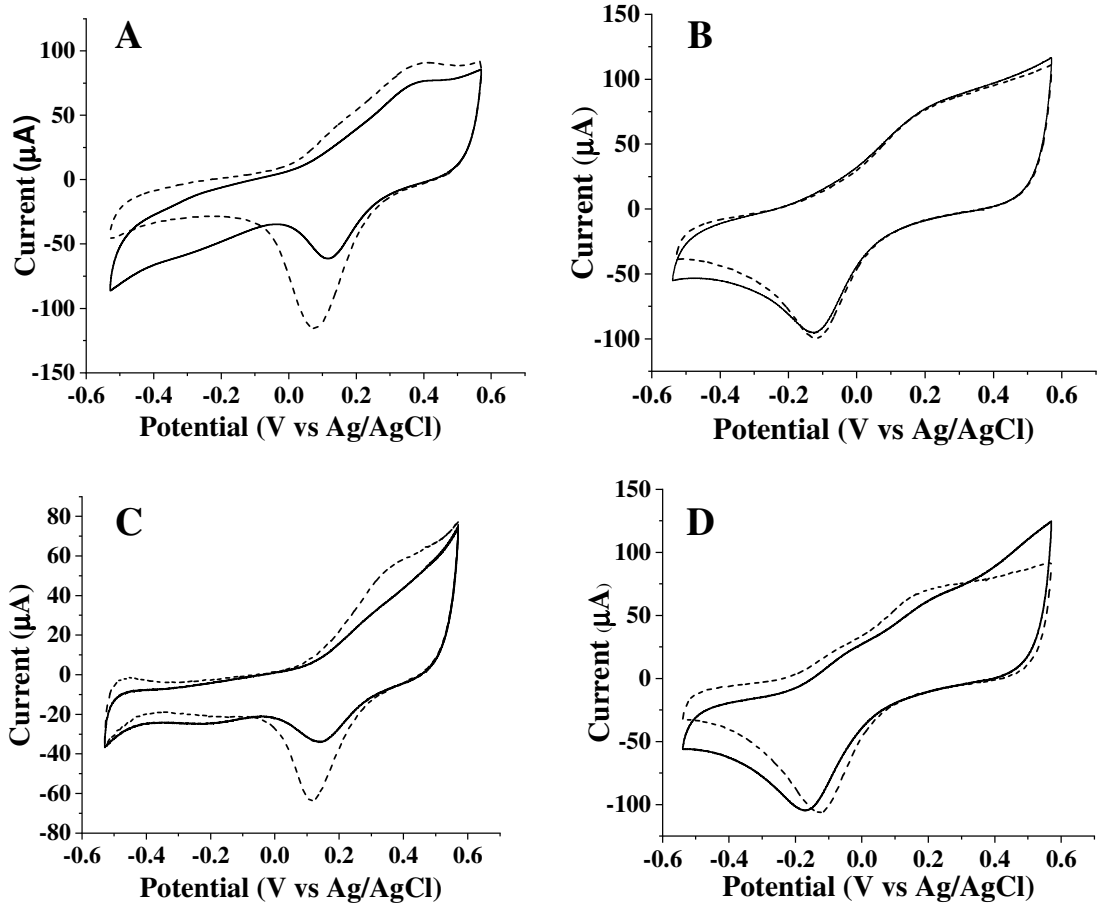


Figure S7. Cyclic voltammograms of the Cu(II)-**L**^{1} (A,B) and Cu(II)-**L**^{2} (C,D) 1:1 systems at pH= 7.4 (A,C) and pH = 10.5 (B,D) in absence (continuous lines) and in presence (dashed lines) of 1.1 equivalent H_2O_2 (T = 298 K, I = 0.2 M KCl, $[L]_{tot}$ = 0.6 mM).

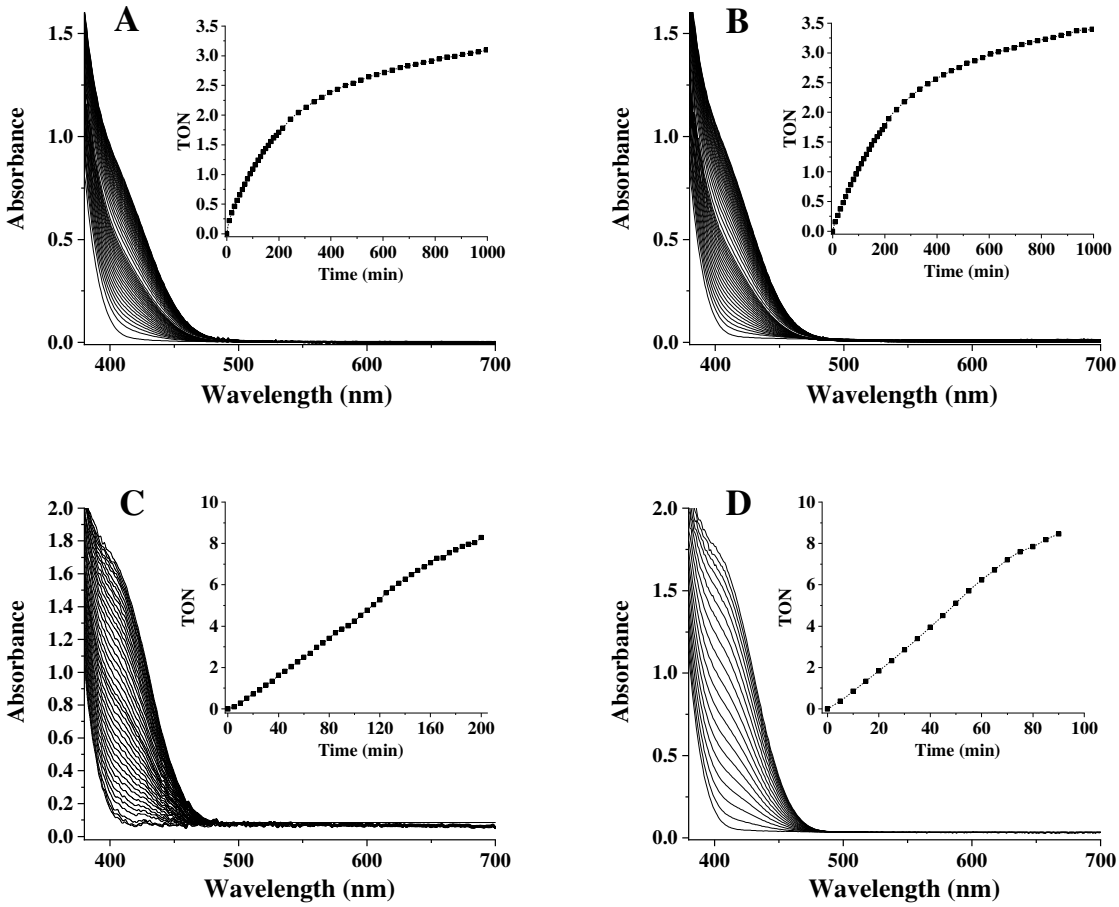


Figure S8 Time-dependent formation of *p*-nitrophenolate during the oxidation of PNPG catalysed by the Cu(II)-$\mathbf{L^1}$ (A,C) and Cu(II)-$\mathbf{L^2}$ (B,D) 1:1 systems at pH 7.4 (A,B) and pH 10.5 (C,D); the inserts show the plot of turnover number (TON) as a function of time. (At pH = 7.4: $[H_2O_2]$ = [PNPG] = 20 mM, $[Cu(II)]_{tot}$ = 0.1 mM, 5 mm cuvette; at pH = 10.5 $[H_2O_2]$ = 10 mM, [PNPG] = 5 mM, $[Cu(II)]_{tot}$ = 0.01 mM, 10 mm cuvette).

The reason of saturation behaviour at pH 7.4 is unknown, but maybe related to the oxidation of the peptides during such long-term kinetic studies.

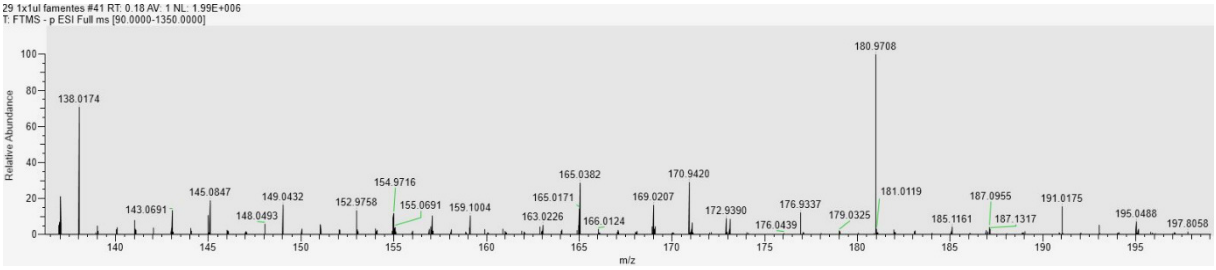


Figure S9 ESI-MS spectrum in negative mode of the reaction mixture of PNPG oxidation promoted by the Cu(II)-**L**^{1} system after 4h reaction time (pH = 10.5 [H_2O_2] = 10 mM, [PNPG] = 1 mM, $[Cu(II)]_{tot}$ = 0.05 mM). The peaks at m/z = 138.0174 and 195.0488 corresponds to *p*-nitrophenolate (calc. m/z = 138.0191) and gluconate (calc. m/z = 195.0505) anions, respectively.

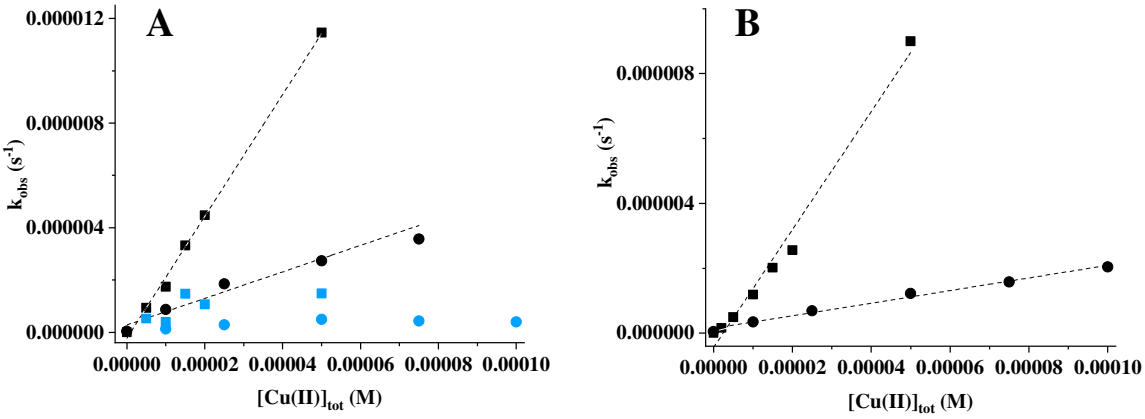


Figure S10 Dependence of k_{obs} of PNPG oxidation on the copper(II) concentration in the Cu(II)-**L**^{1} (A) and Cu(II)-**L**^{2} (B) 1:1 systems at pH = 7.4 (●) and 10.5 (■); [PNPG] = [H_2O_2] = 10 mM. Blue signs in figure A show the effect of $CuCl_2$ concentration, in the absence of peptides.

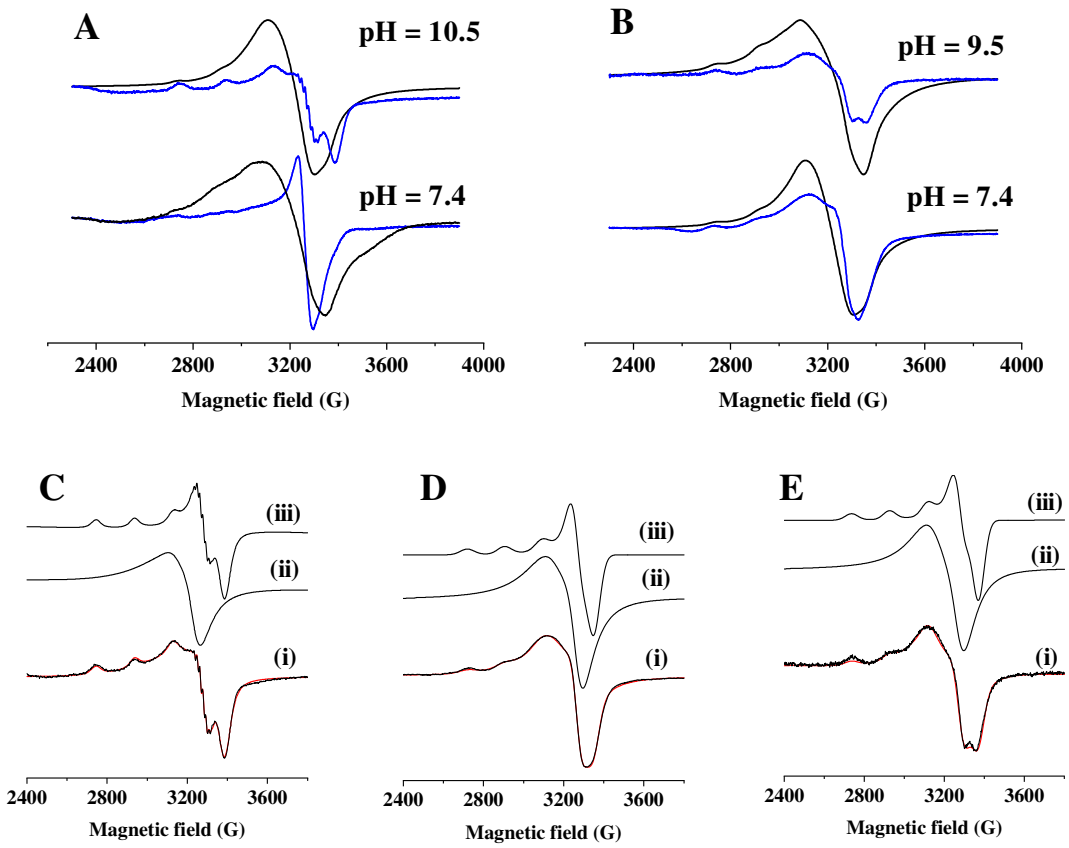


Figure S11. A,B: The change of 77K EPR spectra upon addition of 25 eq. H_2O_2 in the Cu(II)-**L**^{1} (A) and Cu(II)-**L**^{2} (B) 1:1 systems. C,D,E: Deconvolution of the EPR spectra obtained after addition of H_2O_2 to the (C) Cu(II)-**L**^{1} at pH 10.5, (D) Cu(II)-**L**^{2} at pH 7.4, (E) Cu(II)-**L**^{2} at pH 9.5; (i) experimental (black) and simulated (red) spectra, (ii) and (iii) are the component spectra of the dimer and monomer complexes, respectively.

The EPR parameters of the mononuclear (hydro)peroxo complexes: (C) $g_{\perp}$ = 2.045(2), $g_{\parallel}$ = 2.222(2), $A_{\perp}$ =14(2) G, $A_{\parallel z}$=189(2) G, $A_{N,\perp}$ = 15(2) G; (D) $g_{\perp}$ = 2.058(2), $g_{\parallel}$=2.248(2), $A_{\perp}$=12(5)G, $A_{\parallel}$=182(2)G; (E) $g_{\perp}$ = 2.050(2), $g_{\parallel}$=2.231(2), $A_{\perp}$ = 10(5) G, $A_{\parallel}$ =186(2) G. Isotropic components were described with singlet lines.

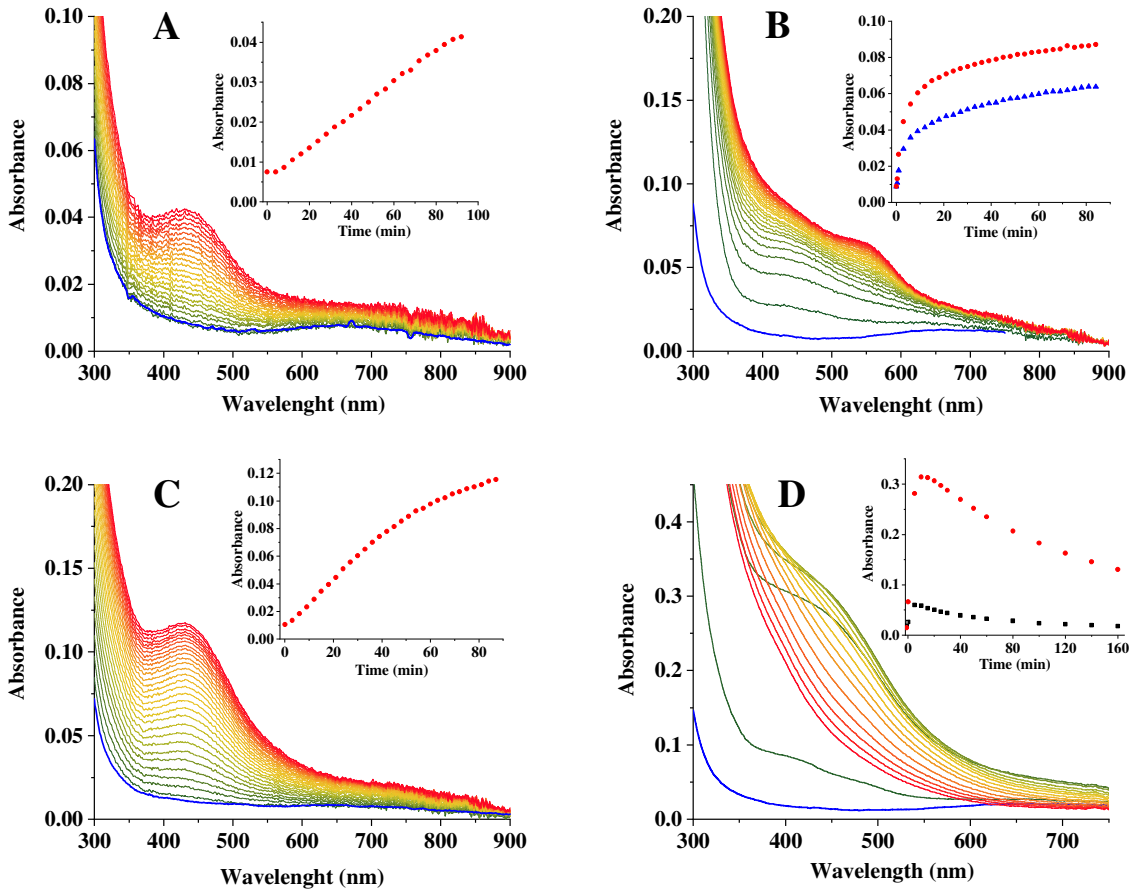


Figure S12 Time dependence of UV-Vis spectra of the Cu(II)-$\mathbf{L^2}$ 1:1 systems upon addition of 1.1 eq. (A,B) 10 eq. (C) and 200 eq. H_2O_2 (D) at pH 7.4 (A,C,D) and at 10.5 (B). Blue lines: the initial spectra, green-to-yellow-to-red lines changes upon addition of H_2O_2. Inserts show the time dependence of spectrum intensity at 440 nm (●), 550 nm (▲) and 650 nm (■). ([Cu(II)] = 0.05 mM, 20 mm cuvette (A,B,C) and 50 mm cuvette (D));

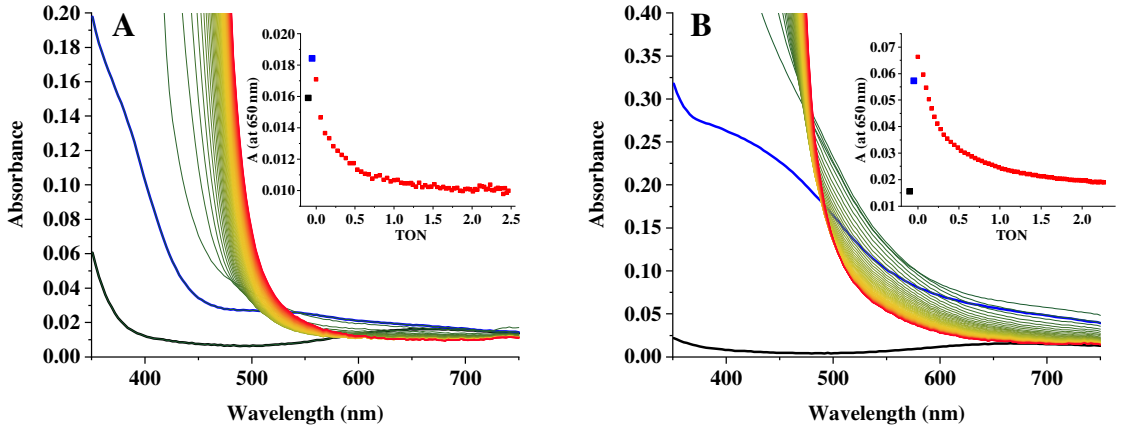
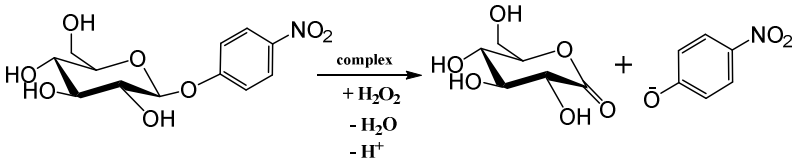


Figure S13 UV-Vis spectra of the Cu(II)-$\mathbf{L^1}$ (A) and Cu(II)-$\mathbf{L^2}$ (B) 1:1 systems at pH 7.4 and at [Cu(II)] = [HPH] = 0.05 mM in 50 mm cuvette (black line), + 10 mM H_2O_2 (blue line), + 10 mM PNPG time dependence up to 650 minutes (green-to-red lines); insert show the change of spectrum intensity at 650 nm as a function of turn-over number (TON); initial spectrum (■), + 10 mM H_2O_2 (■), time (TON) dependent changes after addition of 10 mM PNPG (■). TONs were calculated based on the absorbances at 450 nm where $\varepsilon_{\text{p-nitrophenolate}}$ (450nm) ~ 0.2*$\varepsilon_{\text{p-nitrophenolate}}$ (400nm).



Scheme S1: Scheme for the oxidative conversion of p-nitrophenyl-β-D-glucopyranoside

Table S1 Cathodic and anodic peaks observed by cyclic voltammetry in the Cu(II)-**L^1** and Cu(II)-**L^2** 1:1 systems ([Cu(II)]$_{tot}$ = 0.6 mM, [H_2O_2] = 0.66 mM, [buffer] = 0.1 M, I = 0.2 M KCl). Potentials are given in V vs. Ag/AgCl electrode.

peptide	pH	1.1 eq. H_2O_2	Ep^c (V)	Ep^{mi*} (V)	Ep^{ma**} (V)
L^1	7.4	-	0.11	~ 0.16	0.39
		+	0.07	~ 0.16	0.40
	10.5	-	-0.13	-	0.21
		+	-0.12	-	0.21
L^2	7.4	-	0.14	-	0.31
		+	0.11	-	0.35
	10.5	-	-0.18	~ -0.03	0.19
		+	-0.13	~ -0.07	0.17

*minor anodic peak
** major anodic peak