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Electronic Supplementary Information for the Manuscript:

Dopamine Polymerization Promoted by a Catecholase Biomimetic $Cu^{II}(\mu$-OH)Cu^{II} Complex Containing a Triazine-based Ligand

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EXPERIMENTAL

All materials and solvents used in the syntheses were purchased from commercial sources. The reagents 2-piridinocarboxialdeído, ethylenediamine and pyridin-2-ylmethylamine were purified by distillation prior to use. The substrate 3,5- di-*terc*-butil catechol was purified in hot hexane, filtered off and washed with ethylether before use. Dopamine hydrochloride has used in the polymerization experiments.

Synthesis of $[LCu^{II}(\mu\text{-}OH)_2Cu^{II}].(ClO_4)_2$ (1)

The complex (**1**) was was synthesized by modifications to the procedure described previously by Massoud and co-workers.[1] 0.51 g (1.0 mmol, 509.99 g mol^{-1}) of the ligand L were dissolved in 20.0 ml of MeOH and 0.74 g of $Cu(ClO_4)_2 \cdot xH_2O$ (2.0 mmol, 370.54 g mol^{-1}) were added under magnetic stirring. To this solution was added 1.0 mL NaOH (1.0 mol L^{-1}).

The precipitate was filtered off and recrystallized in acetone/isopropanol (2:1, v/v). Yield: 0.45g (51.43 %). IR (KBr) in cm^{-1}: $\nu(O-H)_{bridge}$ 3575; ν(C-H_{ar} e C-H_{alif}) 3123-2809; ν(C=C and C=N) 1582-1484; ν(Cl-O) 1088; δ(C-H_{ar}) 766; δ(Cl-O) 622. ESI(+)-MS: ***m/z*** 335.50 $[(C_{27}H_{24}N_9Cl)Cu^{II}_2(OH)_2]^{2+}$.

Potentiometric Titration

The potentiometric studies were performed in solution using a titrator (Metrohm 848 Titrino Plus) using a combined glass electrode (reference Ag/AgCl) calibrated for direct reading of pH (pH = -log $[H^+]$. The system was calibrated using data obtained from a previously potentiometric titration of standard solutions of HCl/KOH in MeOH/H_2O (1:1 v/v) mixture (μ = 0.1 mol L^{-1} $NaNO_3$; pK_w = 14.30). The measurements were performed in a thermostatable cell at 25.00 ± 0.05 °C. The ligand L was titrated in duplicate at a concentration of 1.0 x 10^{-3} mol.L^{-1} in 50 mL MeOH/H_2O (1:1 v/v). The complex was titrated from [L] = 5.0 x 10^{-4} mol.L^{-1} and $[Cu^{2+}]$ = 1.0 x 10^{-3} mol.L^{-1} (previously standardized by FAAS). The final concentration of **1** in each experiment was [1] = 1.0 x 10^{-3} mol L^{-1} in MeOH/H_2O (1:1 v/v) mixture with $NaNO_3$ (μ = 0.1 mol.L^{-1}) as ionic strength (under argon atmosphere). The titration data were treated with the *BEST7*[2] program and species diagram obtained with *SPE* program. Species distribution of L (Figure S1) and complex (Figure S2) and logβ (Table 1) are shown below.

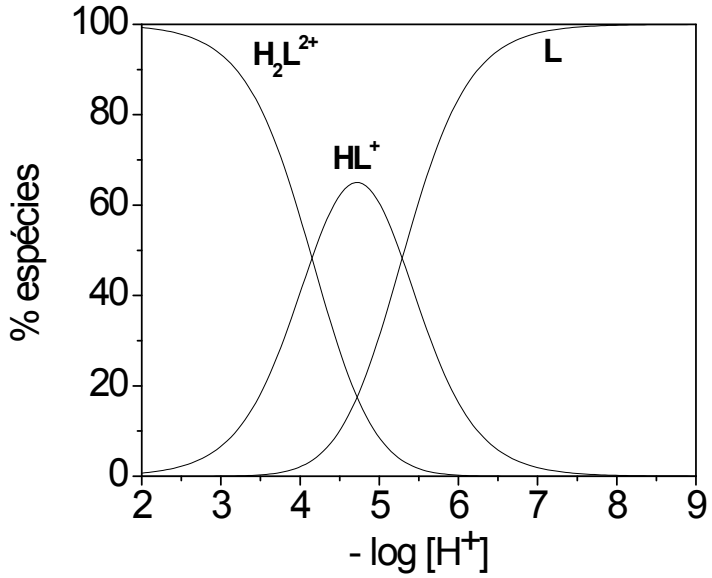

Figure S1. Species distribution curves of the L ligand. Conditions: [L] = 1.0 x 10^{-3} in an MeOH/H_2O solution, μ = 0.1 mol L^{-1} ($NaNO_3$) at 25°C

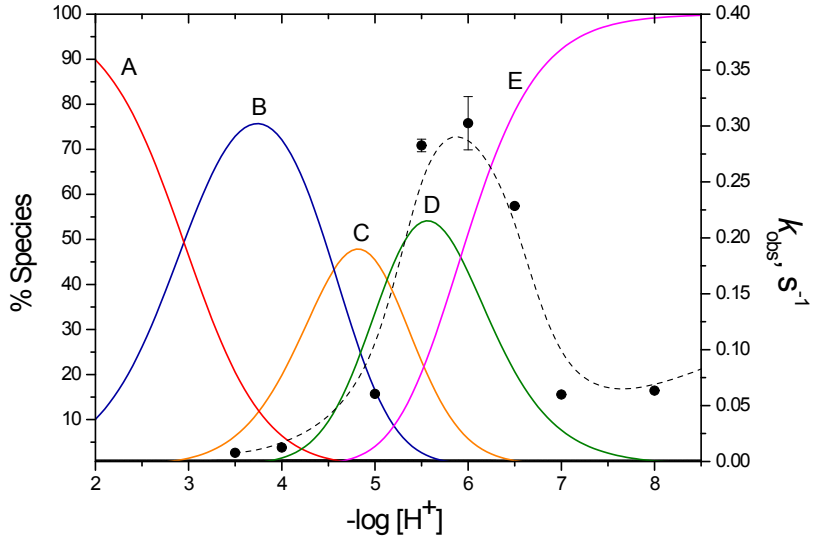

Figure S2. Solid lines representing the species distribution curves of the Cu^{II}-L system for dissolution of [Cu^{2+}] = 1.0 mmol and [L] = 0.5 mmol in a MeOH/H_2O solution (1:1 v/v), μ = 0.1 mol L^{-1} ($NaNO_3$) at 25°C. The dashed line corresponds to the observed rate constants for the oxidation of 3,5-dtbc as a function of the pH in a MeOH/H_2O solution (1:1 v/v). Conditions: [**1**] = 5.0 x 10^{-5} mol L^{-1}; [3,5-dtbc] = 6.0 x 10^{-3} mol L^{-1}; [B] = 0.1 mol.L^{-1}; at 25°C. A: $[Cu_2LH_2]^{6+}$; B: $[CuLH]^{3+}$; C: $[Cu_2L]^{4+}$; D: $[Cu_2H_{-1}L]^{3+}$; E: $[Cu_2H_{-2}L]^{2+}$.

Table 1. Logβ found for L and **1**. Conditions: [$NaNO_3$] = 0,1 mol.L^{-1} in MeOH/H_2O (1:1 v/v) mixture at 25 °C.

	Equilibrium	logβ
L	$L + H^+ \leftrightarrow HL^+$	5.29
	$HL^+ + H^+ \leftrightarrow H_2L^{2+}$	9.44
1	$L + Cu^{2+} \leftrightarrow CuL^{2+}$	15.25
	$CuL^{2+} + H^+ \leftrightarrow CuLH^{3+}$	18.12
	$2Cu^{2+} + L \leftrightarrow Cu_2L^{4+}$	26.02
	$L + 2Cu^{2+} + H^+ \leftrightarrow Cu_2LH^{5+}$	30.71
	$L + 2Cu^{2+} + 2H^+ \leftrightarrow Cu_2LH_2^{6+}$	33.64
	$2Cu^{2+} + L + H_2O \leftrightarrow Cu_2H_{-1}L^{3+} + H^+$	20.99
	$Cu_2H_{-1}L^{3+} + H_2O \leftrightarrow Cu_2H_{-2}L^{2+} + H^+$	14.96

Mass Spectrometry

The mass spectrum of **1** was registered using an amaZon Ion-Trap MS/MS spectrometer (Bruker Daltonics) in the positive mode ESI(+)-MS and coupled mode ESI(+)-MS/MS with following parameters: 298 K; capillary voltage between -400 and -500 V; 180 µL min^{-1} infusion flux and [**1**] = 0.5 ppm (MeOH/H_2O 1:1 v/v, pH 6.0). The scan range selected for the experiments was 200 to 900 m/z. pH of all samples was ajusted using diluted MeOH (Sigma-Aldrich Standard LC-MS) and H_2O (Millipore Milli-Q).

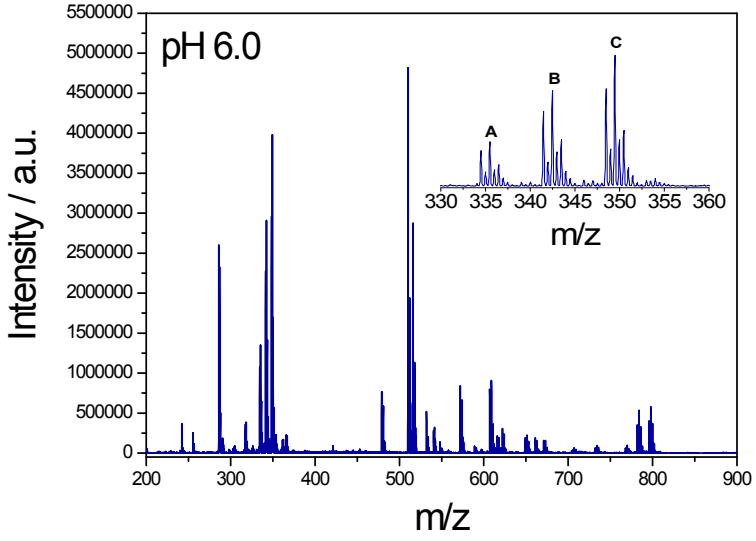

Figure S3. ESI(+)-MS of complex **1** in MeOH/H_2O (1:1 v/v) mixture at pH 6.0.

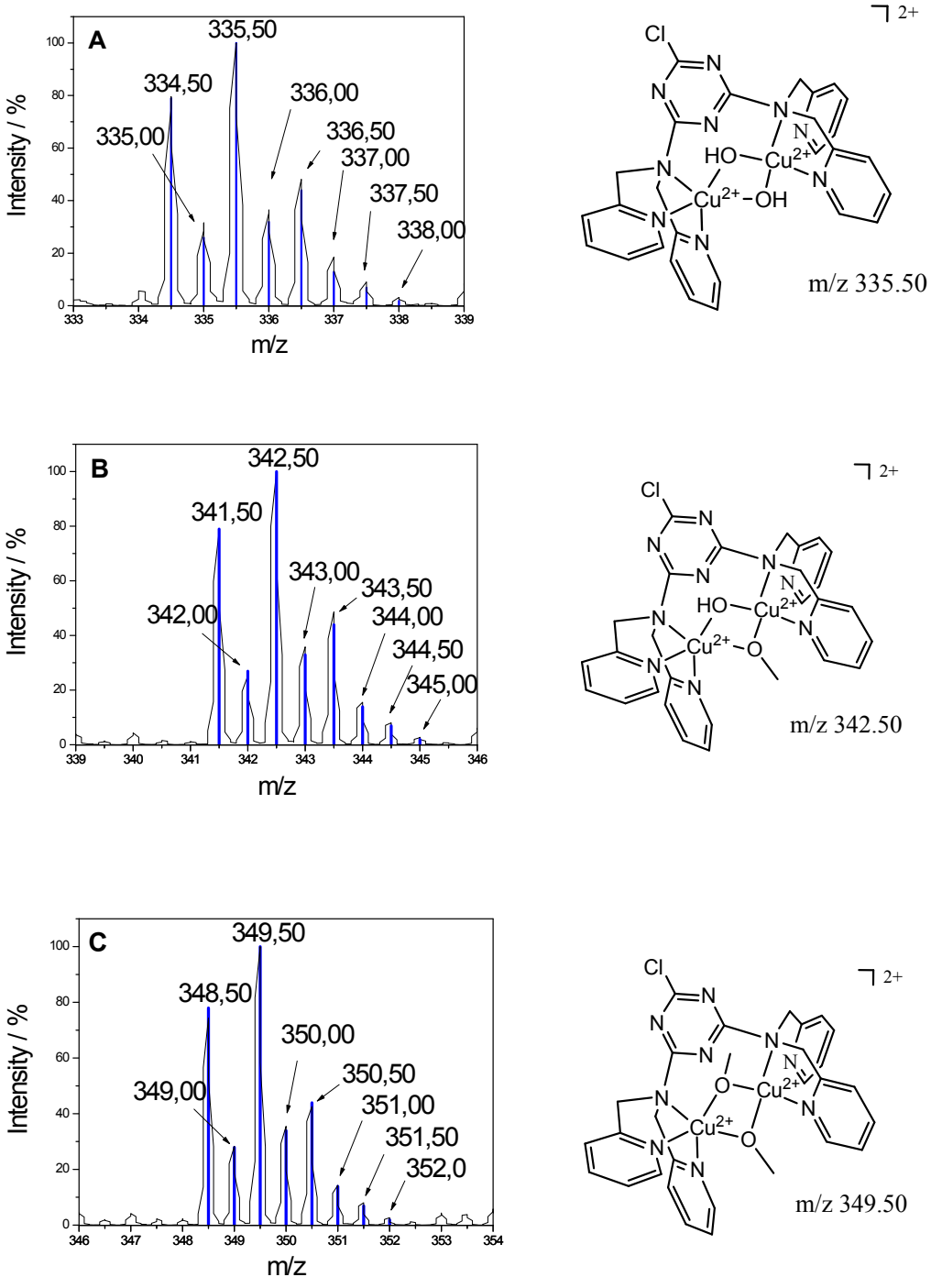

Figure S4. Simulation of the obtained species: **A**, *m/z* 335.50 $[(C_{27}H_{24}N_9Cl)Cu^{II}_2(OH)_2]^{2+}$, **B**, *m/z* 342.50 $[(C_{27}H_{24}N_9Cl)Cu^{II}_2(OH)(MeO)]^{2+}$, and **C** *m/z* 349.50 $[(C_{27}H_{24}N_9Cl)Cu^{II}_2(MeO)_2]^{2+}$. Black lines are experimental and blue lines are simulated data.

Knectic Assays

Kinetic experiments for the oxidation of the model substrate 3,5-di-*terc*-butilcatecol (3,5-dtbc) were followed spectrophotometrically for the absorbance increase at 400 nm (ε = 1570 L mol^{-1} cm^{-1} in MeOH/H_2O 50:50 v/v) because of the formation of 3,5-dtbq (3,5-di-*tert*-buthylquinone) over time, under conditions of excess substrate at 25.0 °C. In these experiments, all of the solution mixtures were in an MeOH/H_2O (1:1 v/v) medium. The systems showed high catalytic activity, and their study was only possible through the stopped-flow technique. The knectic assays were performed with KinetAsyst Transtech, Model SF- 61DX2 equipment, the optical path of 1.0 cm and using the diode array (Hi-Tech KnetaScan Diode Array) as the main detector coupled to a thermostated bath (25.0 °C). A total of 50.0 μL was used in each reaction solution (complex/buffer and substrate/buffer, both in MeOH/H_2O 1:1 v/v) at each shooting. The pressure of the shots was set at 0.5 MPa. For each kinetic run have been processed 200 and 300 readings until the maximum time of 6.0 seconds for the complex reaction. Studies on the effects of pH on the oxidation reaction were performed in the pH range of 3.0-8.0: (MES pH 3,5 at 6,5), and HEPES (pH 7,0 at 8.0) under a 120-fold excess of substrate, at 25 °C (Figure 2). The concentration of complex, substrate and buffer were: $[\mathbf{1}]_{final}$ = 5,0 x 10^{-5} mol.L^{-1}, $[S]_{final}$ = 6,0 x 10^{-3} mol.L^{-1} e $[T]_{final}$ = 0,1 mol L^{-1} in MeOH/H_2O 50:50 v/v. Experiments to determine the dependence of the reaction rate on the substrate concentration (1.5 x 10^{-4} to 5.0 x 10^{-3} mol.L^{-1}) were carried out at 25 °C, pH 6.0, with $[\mathbf{1}]_{final}$ = 5.0 x 10^{-5} mol.L^{-1} (Figure S4)

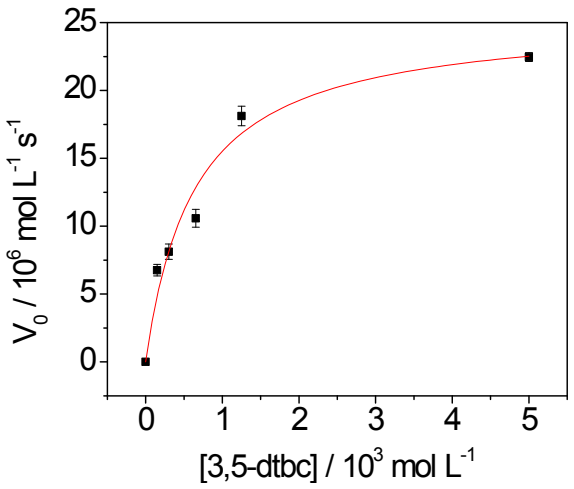

Figure S5. Dependence of the reaction oxidation rate according to the concentration of the substrate 3,5-dtbc by complex 1 under following experiental conditions: H_2O/MeOH solution 1:1 v/v [**1**] = 5.0 x 10^{-5} mol.L^{-1} [3,5-dtbc] = (0,15-5.0) x 10^{-3} mmol.L^{-1} [B] = 0.05 mmol L^{-1} (buffer MES pH 6.0) at 25 °C.

CARACTERIZATION OF POLY(DOPAMINE)

UV-Vis spectrophotometry

The spectral monitoring of dopamine oxidation activity of **1** was evaluated spectrophotometrically on a Varian Cary50 Bio spectrophotometer through the appearance of the large semiquinone band (λ_{max} = 609 nm) at 25 °C. The experiments was monitored using MeOH/H_2O (1:1, v/v) solution, at the following concentrations: [B] = 0,1 mol L^{-1} (MES pH 6,0), [**1**] = 1,0 x 10^{-4} mol L^{-1}, [dopamine] = 1,0 x 10^{-2} mol L^{-1} at 25 °C for 20 minutes.

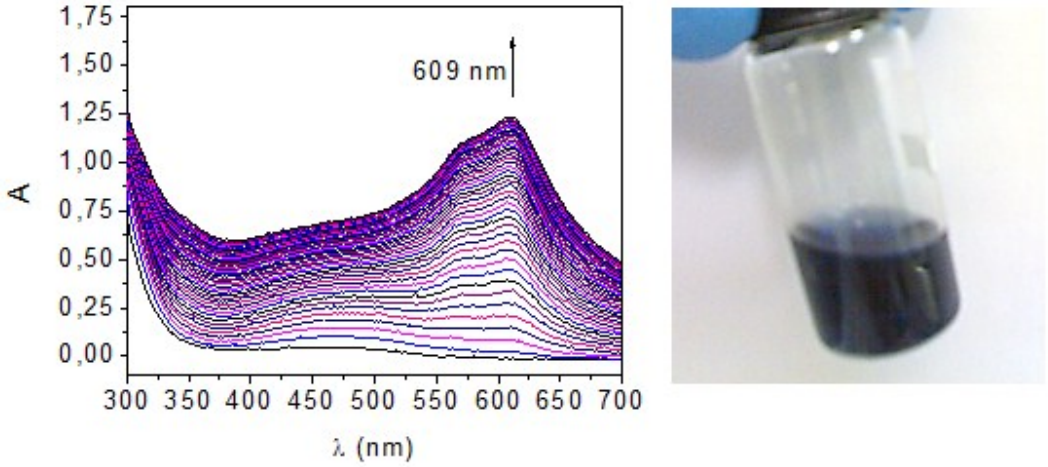

Figure S6. Spectral monitoring of dopamine oxidation reaction (left) and picture showing deep blue solution took after 20 minutes from the start of reaction. Conditions: MeOH/H_2O (50:50, v/v), [B] = 0,1 mol L^{-1} (MES pH 6,0), [**1**] = 1,0 x 10^{-4} mol L^{-1}, [dopamine] = 1,0 x 10^{-2} mol L^{-1} at 25 °C for 20 minutes.

FT-IR analysis

After 20 minutes of reaction ([B] = 0,1 mol L^{-1} (MES pH 6,0), [**1**] = 1,0 x 10^{-4} mol L^{-1}, [dopamine] = 1,0 x 10^{-2} mol L^{-1} at 25 °C) the black powder was centrifuged and washed several times with deionized water to remove residual buffer and complex. The same spectrum was obtained for the reaction under similar conditions (pH 6.0), but without addition of buffer.

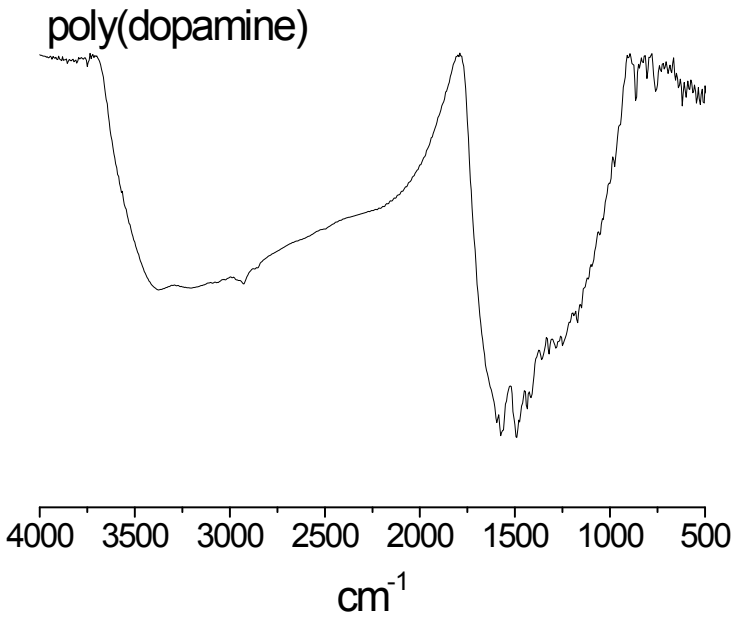

Figure S7. FT-IR spectra (KBr, cm^{-1}) of poly(dopamine).

Mass spectrometry

Conditions: the initial concentration of [**1**] = 5,0 x 10^{-5} mol.L^{-1} and [dopamine] = 6,0 x 10^{-3} mol.L^{-1} were diluted with MeOH/H_2O solution (1:1, v/v). The pH of a stock metanol/H_2O solution was previously adjusted with LiOH and $HClO_4$ and then diluting the reaction mixture stock solution (Figures S7, S8 and S9).

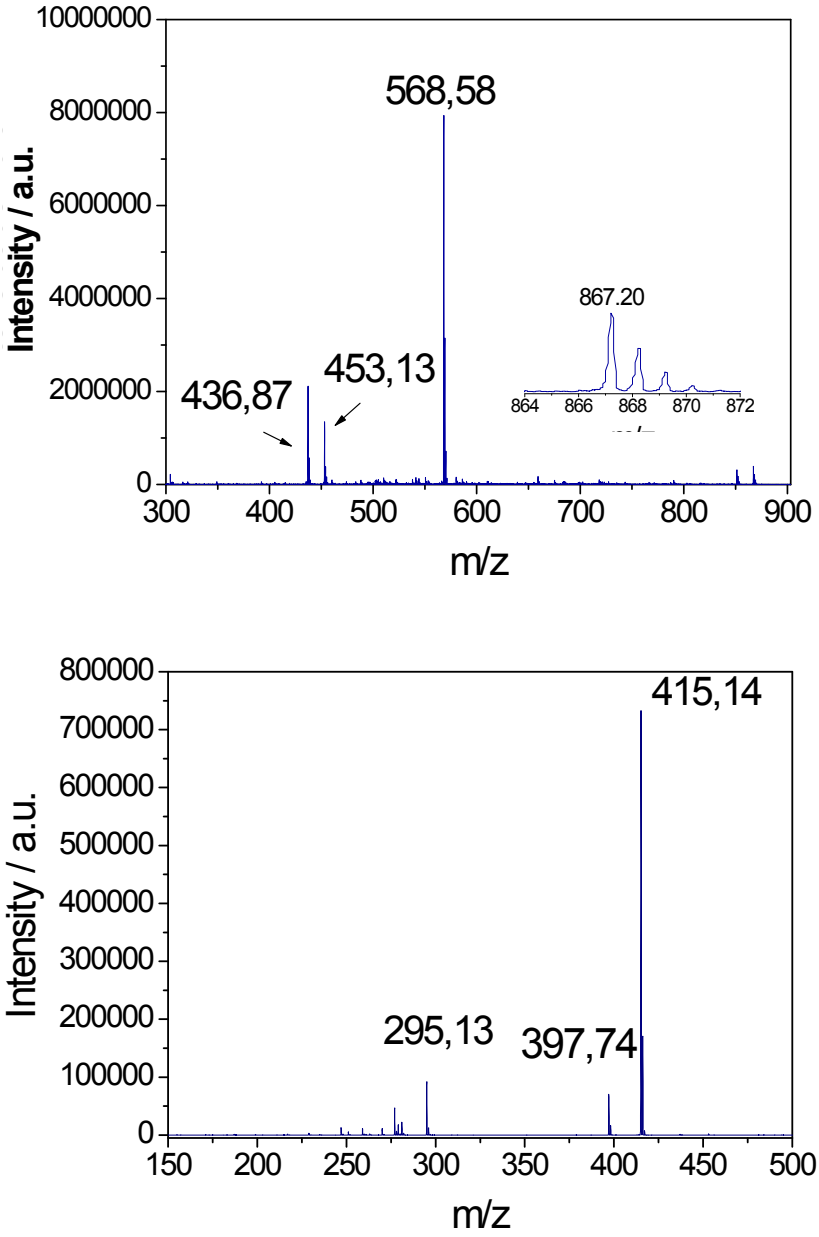

Figure S8. ESI(+)-MS spectra of dopamine polymerization promoted by **1** with O_2 (in MeOH/H_2O 1:1 v/v; pH 6.0) after 15 minutes of reaction (above) and ESI(+)-MS/MS of m/z 568.58 main peak (below).

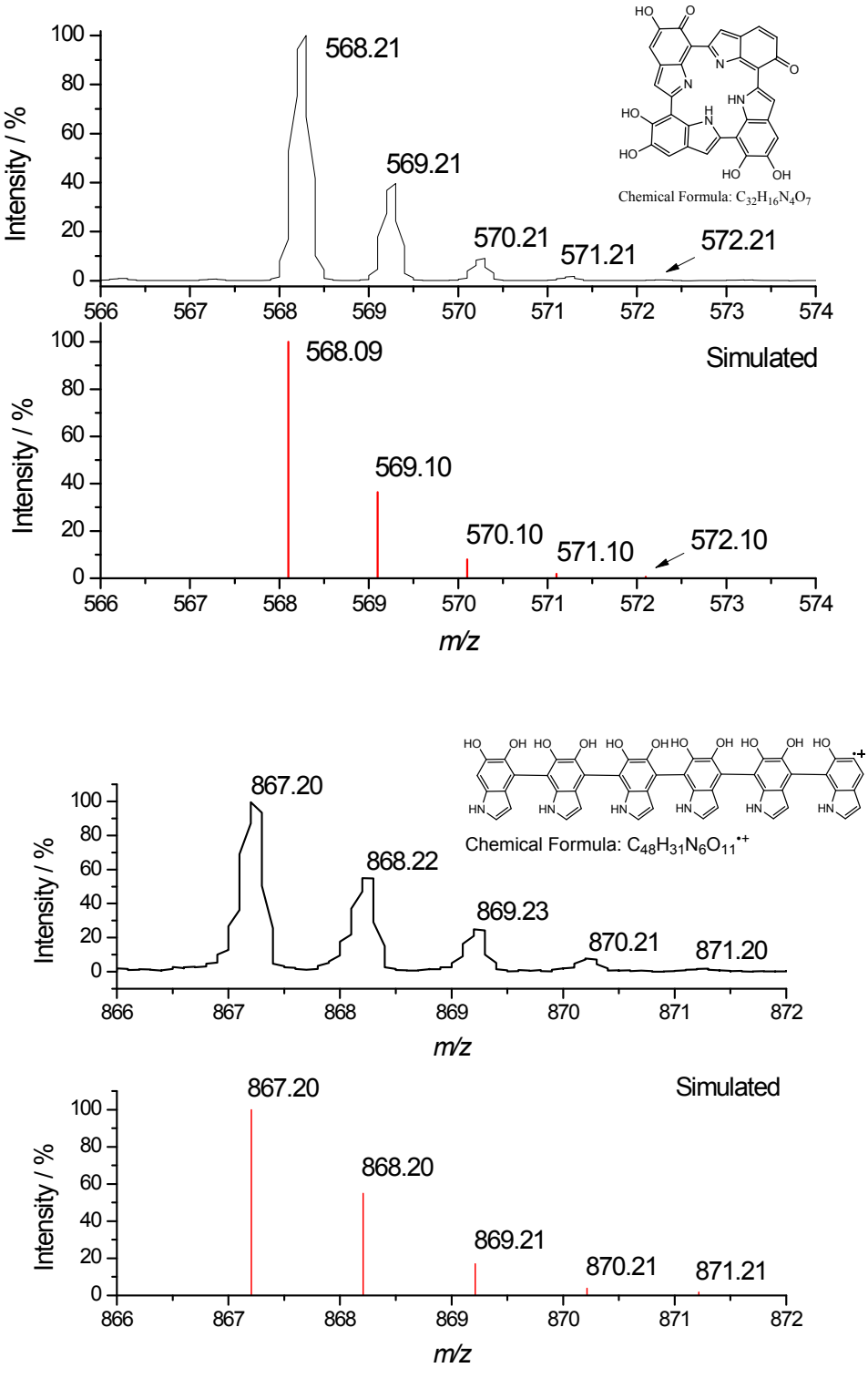

Figure S9. Experimental and simulated (red lines) ESI(+)-MS spectra of $(DHI)_2$/quinone-methide/indolone structure (*m/z* 568.21) and the oligomer $[(DHI)_6 - OH]^+$ (*m/z* 867.20).

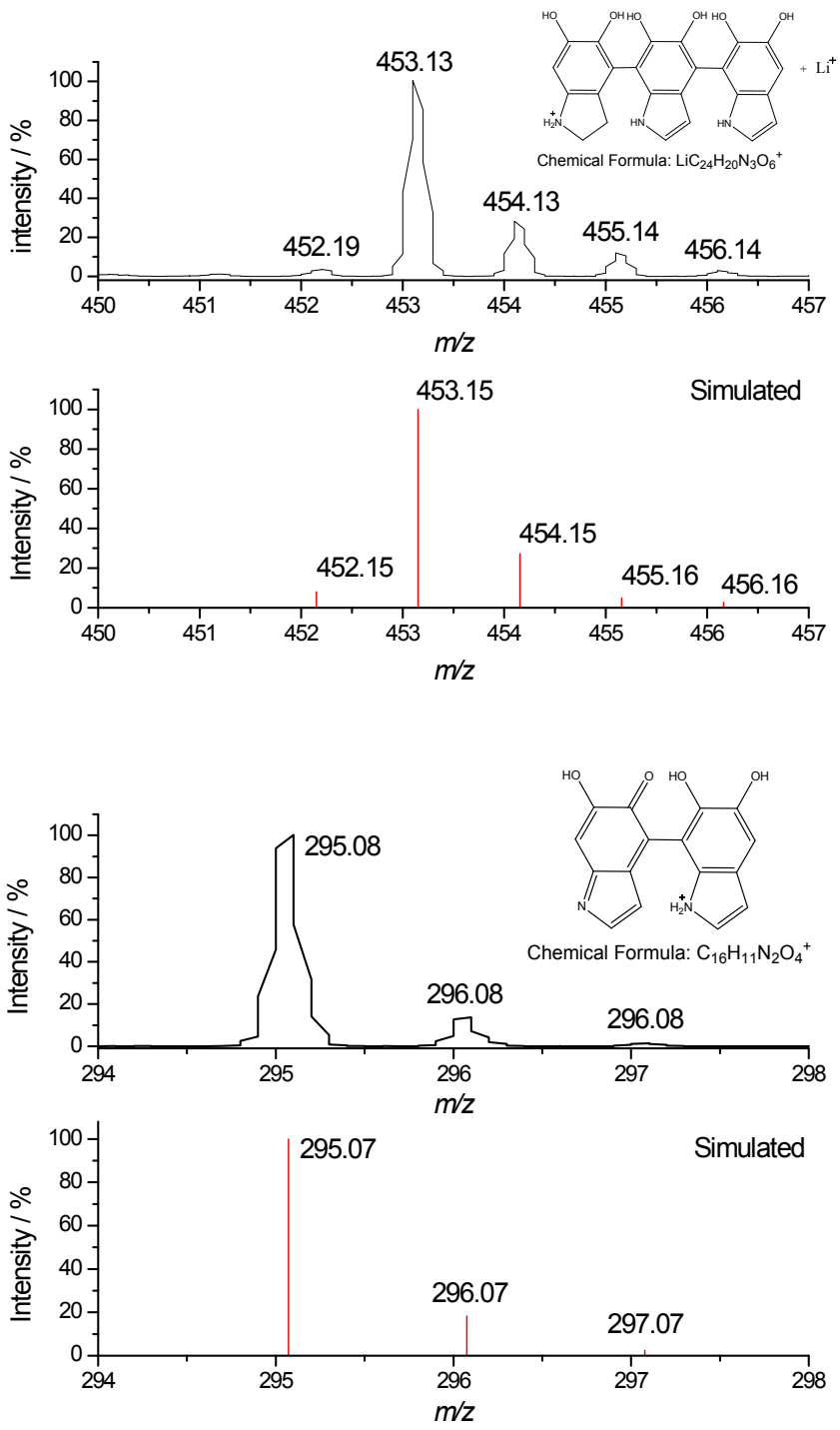

Figure S10. Experimental and simulated (red lines) ESI(+)-MS spectra of dihydroxyindoline/$(DHI)_2$ trimer (*m/z* 453.13) and DHI/quinone-methide dimer (*m/z* 295.08) obtained from couple mode ESI(+)-MS/MS of *m/z* 568.68 father main peak.

References

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(2) A. Martell and R. J. Motekaitis, *Determination and Use of Stability Constants*, 2nd ed.; VCH: New York, **1992**.