Supplementary data for article:

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Electronic Supplementary Material (ESI) for Dalton Transactions.

Electronic Supplementary Information

Biliverdin-copper complex at physiological pH

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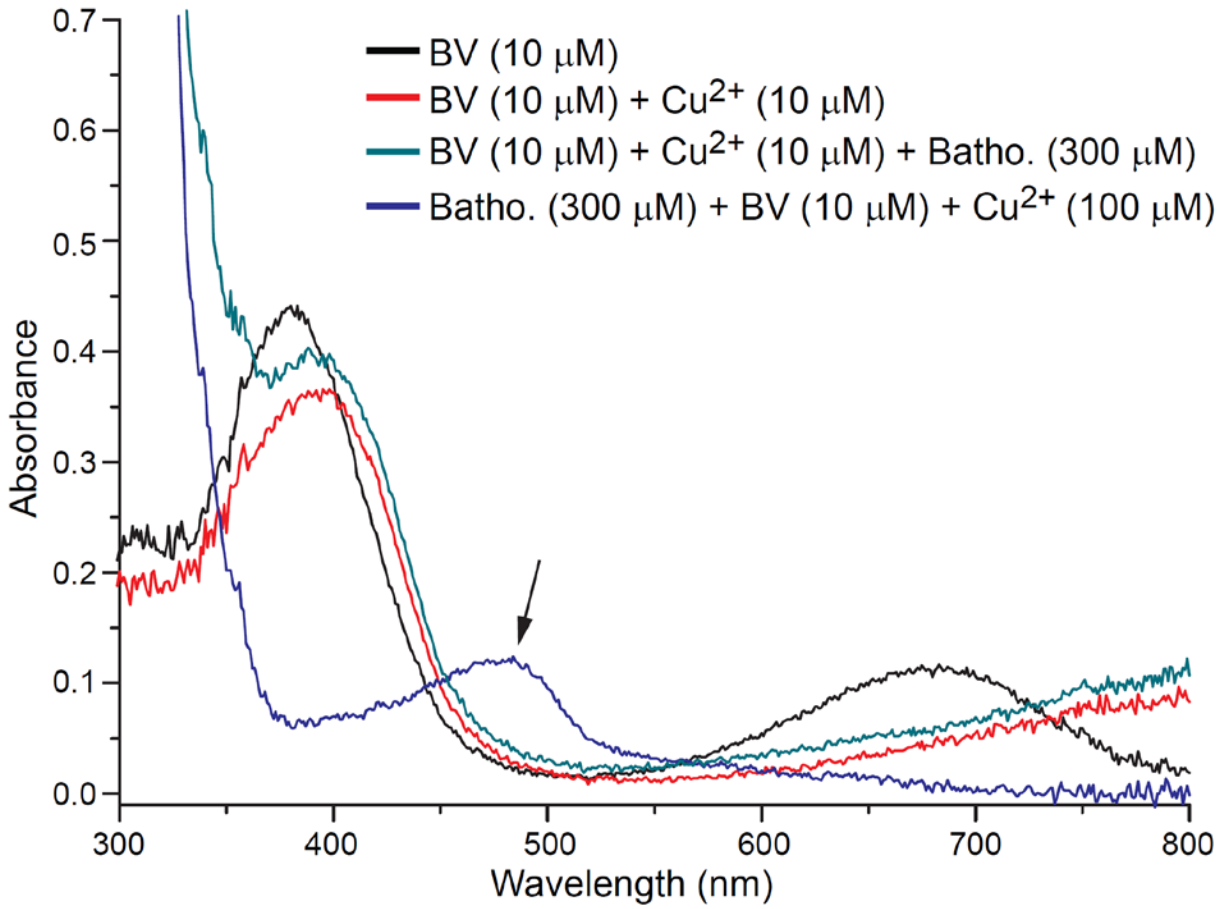

Figure S1. The stability of [BV]/[Cu^{2+}] = 1 system in the presence of copper chelating agent bathocuproine in phosphate buffer (50 mM; pH 7.4). Green line - BV and Cu^{2+} were incubated for 5 min before the addition of bathocuproine. Blue line - Cu^{2+} was added to the buffer after BV and bathocuproine (arrow – absorbance line of bathocuproine complex with copper). It is important to note that bathocuproine is a non-innocent copper chelator. In the presence of bathocuproine, the reduction potential for the Cu^{2+}/Cu^{1+} couple is raised by approximately 500 mV, making Cu^{2+} a powerful oxidant.[1] In the process, Cu^{2+} oxidizes BV-Cu complex, resulting in BV degradation (note that BV-related absorance is completely lost (blue line)) and Cu release.

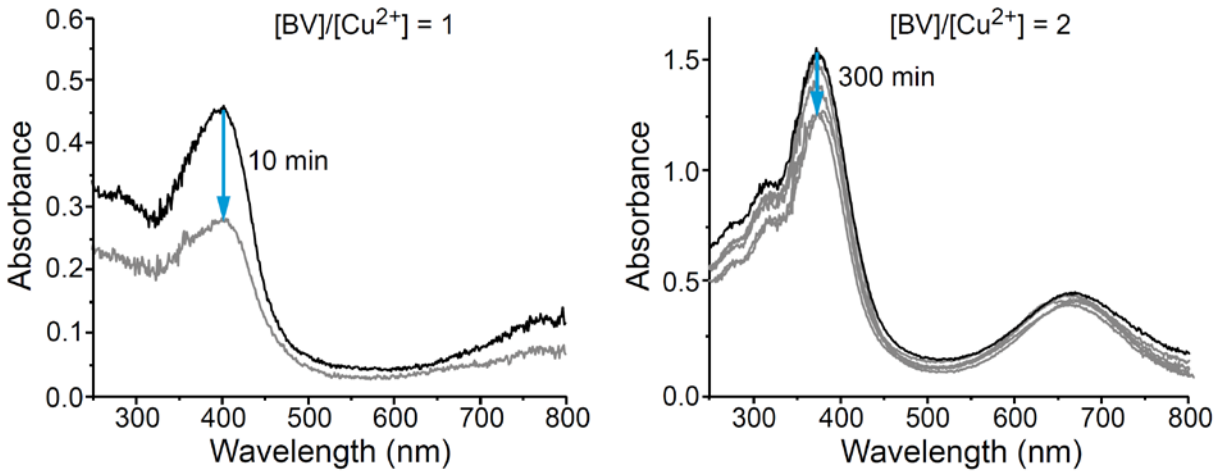

Figure S2. Changes in UV-Vis spectra of BV-Cu^{2+} systems prepared at high concentrations in 50 mM phosphate buffer, pH 7.4. Left: $[BV]/[Cu^{2+}] = 1$; $[BV] = [Cu^{2+}] = 0.3$ mM. Right: $[BV]/[Cu^{2+}] = 2$; [BV] = 2mM; $[Cu^{2+}] = 1$ mM. Aliquots were taken from each system and diluted to lower final concentrations (10 or 40 μM, respectively), to allow spectra acquisition. It can be observed that the $[BV]/[Cu^{2+}] = 1$ system underwent degradation within 10 min, whereas the $[BV]/[Cu^{2+}] = 2$ system was relatively stable for 5 h.

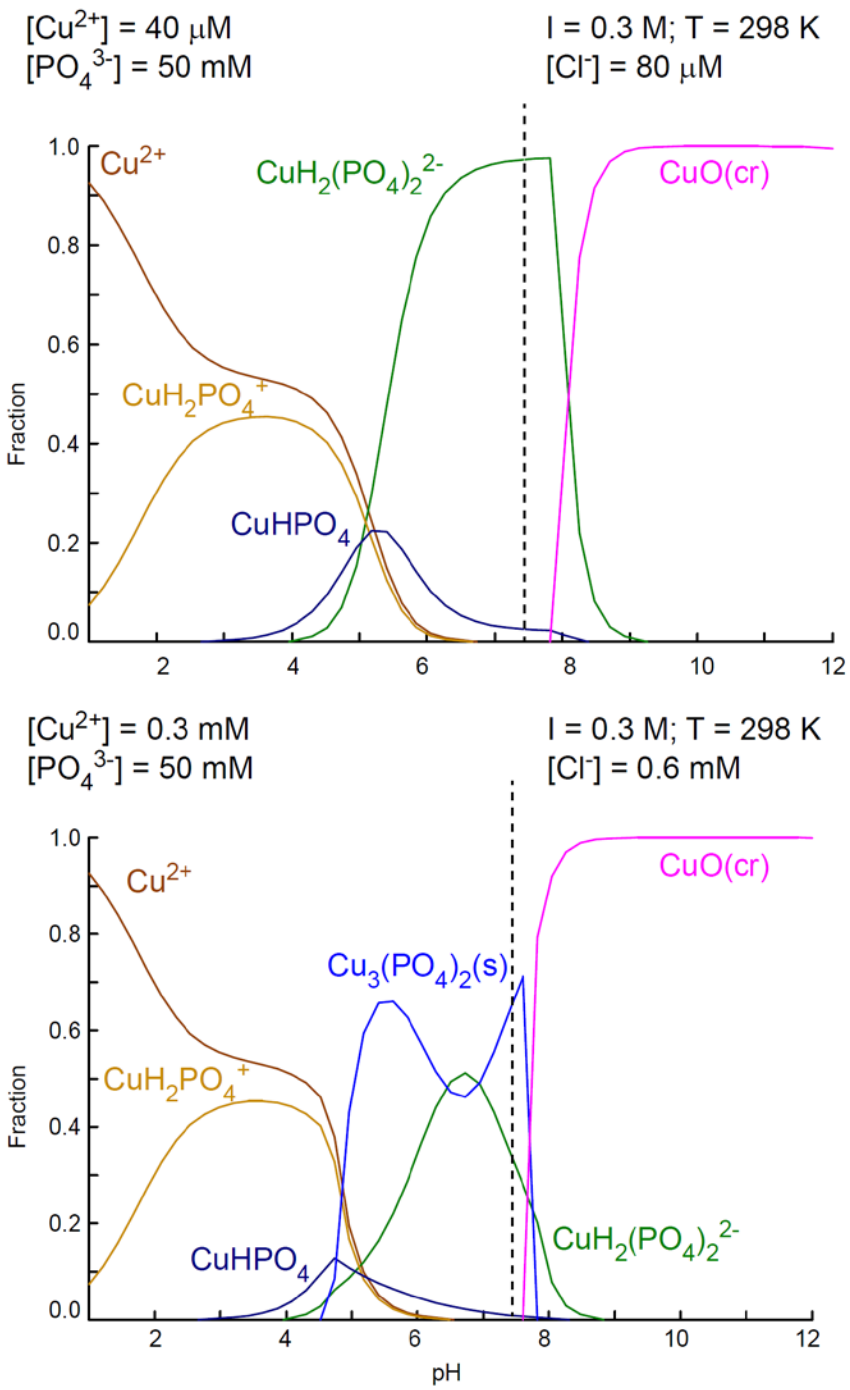

Figure S3. Speciation diagrams of Cu^{2+} in phosphate buffer (50 mM) at two concentrations – 40 μM (top) and 300 μM (bottom). Diagrams were prepared in Hydra-Medusa Software, using the presented parameters.

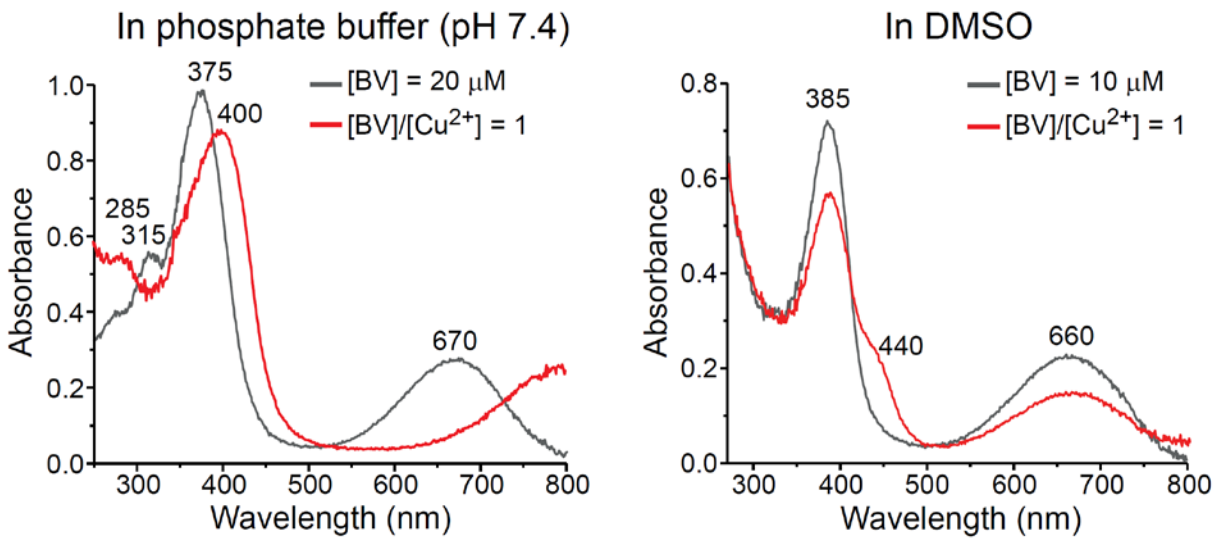

Figure S4. Comparison of UV-Vis spectra of biliverdin (BV) in the absence and the presence of Cu^{2+} in phosphate buffer (50 mM; pH 7.4) and in DMSO. Spectra were recorded after 5 min incubation period.

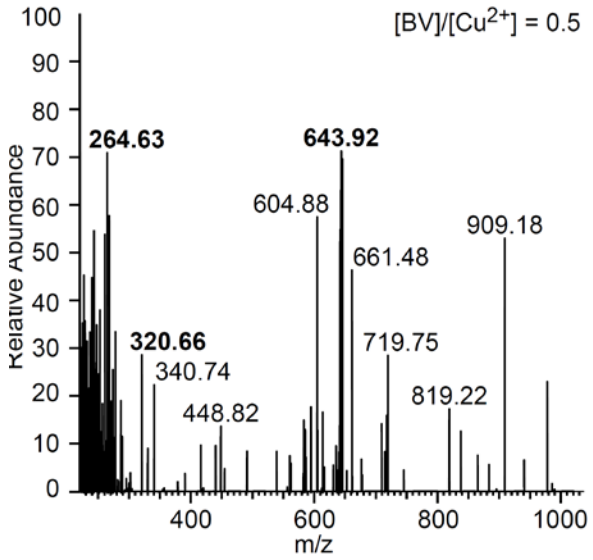

Figure S5. HESI-MS spectrum (full scan mode) of the system with [BV] = 20 μM and [Cu] = 40 μM ([BV]/[Cu] = 0.5). Assignation: *m/z* 643, BV-Cu complex; *m/z* 264, propentdyopent; *m/z* 320, propentdyopent complex with copper.[2]

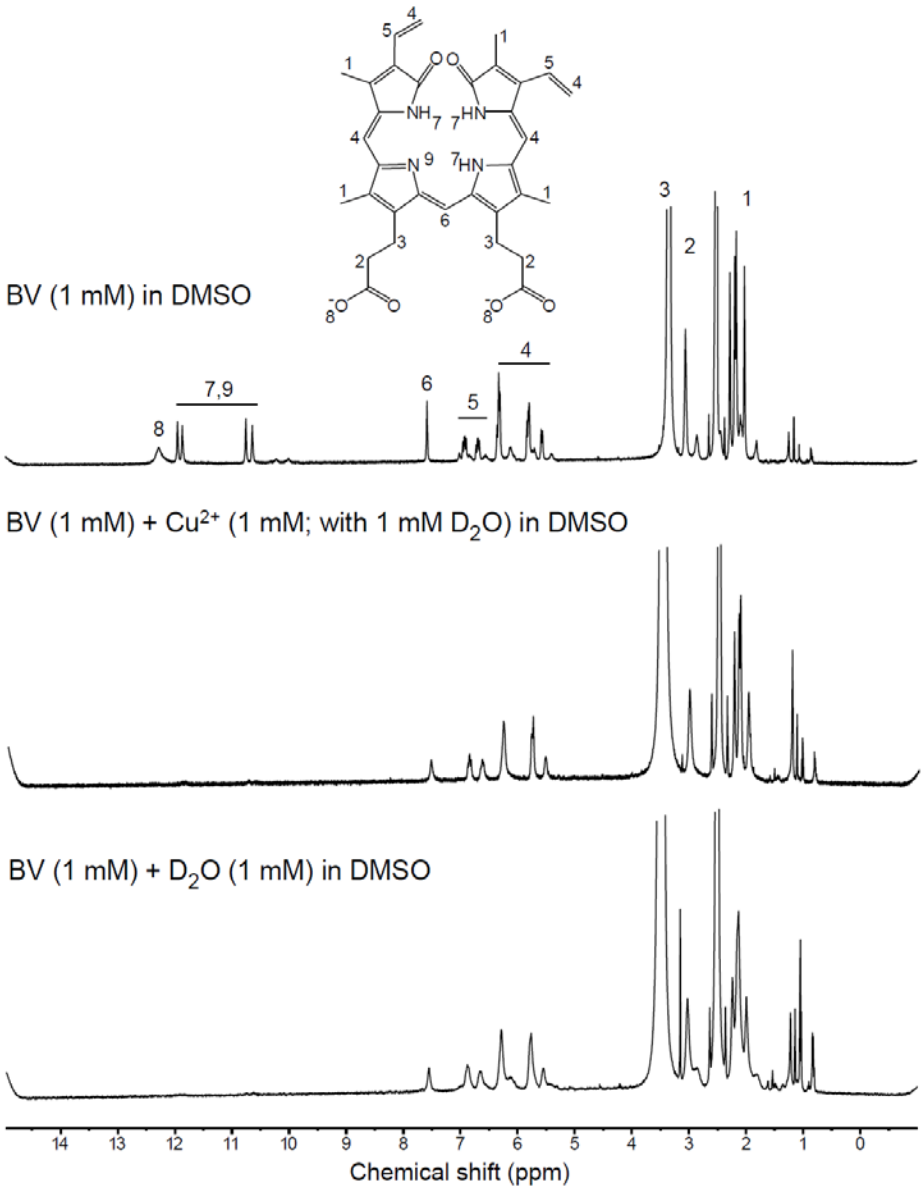

Figure S6. ^{1}H NMR spectra of biliverdin (0.3 mM) in DMSO-d6 in the absence or the presence of Cu^{2+} at equimolar concentration. The bottom spectrum was recorded in a copper-free system, with the equimolar amount of D_2O as in experiments with copper. It can be observed that Cu^{2+} and D_2O induced similar (but not identical) changes. Changes in NH signals could not be observed in the presence of D_2O because of the chemical exchange. The peaks were assigned in accordance to previous reports.[3] The signals labeled with 4 come from two types of protons (-CH= and $=CH_2$). The spectra were collected within 20 min after sample preparation.

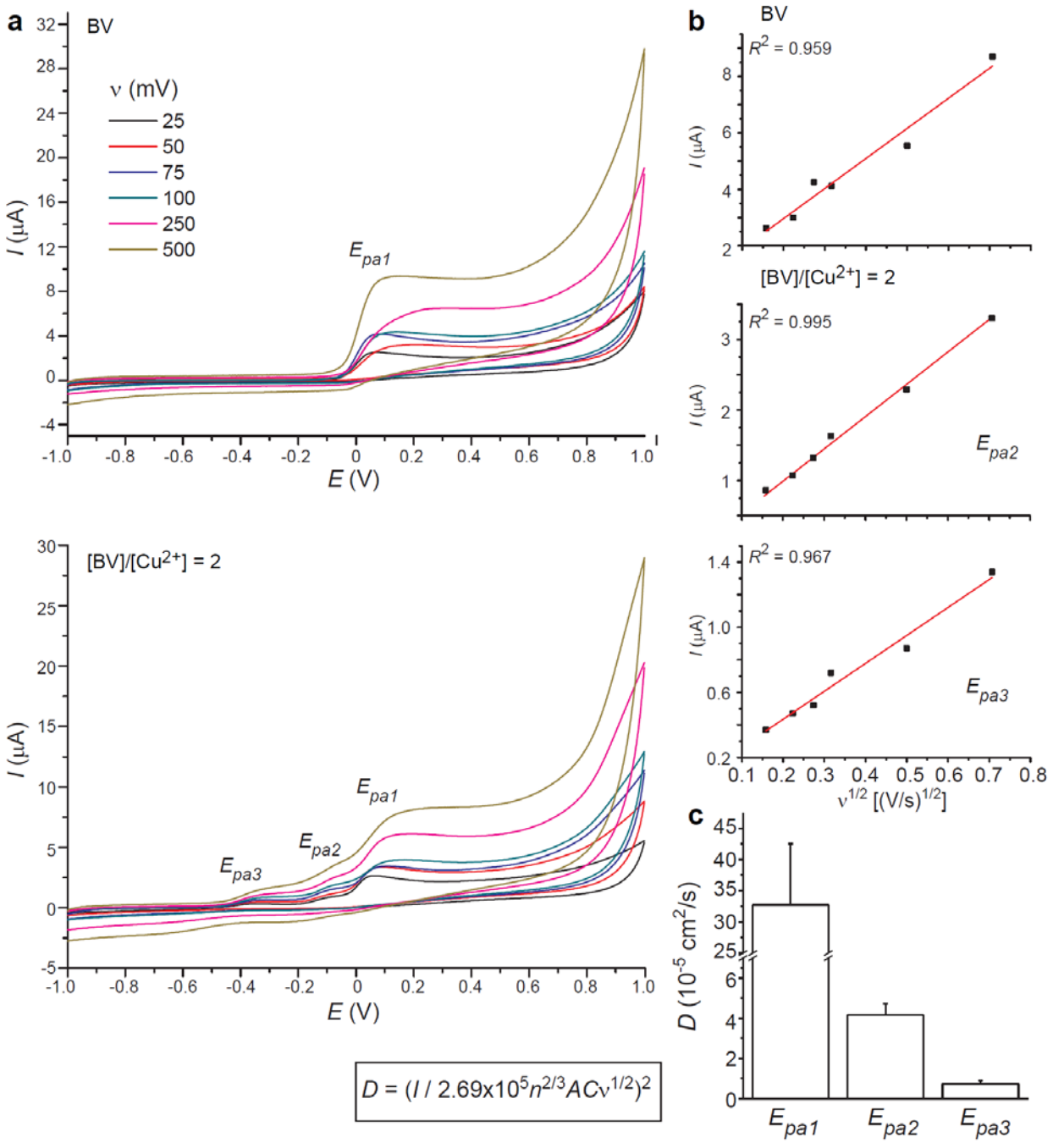

Figure S7. Scan rate analysis of BV and BV-Cu complex in phosphate buffer (50 mM; pH 7.4). (a) Cyclic voltammograms of BV (0.4 mM) in absence or presence of Cu^{2+} (0.2 mM) at the boron doped diamond electrode obtained at different scan rates (ν = 0.025–0.5 V/s). (**b**) The dependence between anodic peak currents *I* at potentials E_{pa1} (oxidation of BV), and E_{pa2} and E_{pa3} (oxidation of BV-Cu complex(es)) and $\nu^{1/2}$. Linear fit and R^2 values are presented. (**c**) *D* for BV and BV-Cu complex(es). Randles–Sevick equation (in the box): *n*, number of transferred e^-

(1e^- for all peak currents), *A*, area of the working electrode (0.0707 cm^2); *C*, concentration of redox species in solution ([BV] = 0.4 mM; [BV-Cu] = 0.2 mM). Results are presented as means (± standard deviation) of measurements made at various ν. All three *D* values were statistically different ($p < 0.001$; ANOVA with *post hoc* Duncan's test).

Table S1. Raman spectral lines that were observed for BV (1 mM), using the λ = 532 nm laser excitation line.

Line [cm^{-1}]	**Assignment**	**References**
1619	Lactam stretching	4
1470	C–C deformation, likely between rings	4,5
1443	Stretching CC, stretching CN	6
1393	CH3 asymmetric deformation	6
1362	CH3 deformation	6
1331	In plane bending CH(CH3)	6
1303	CH wagging	6
1254	Lactam ring	6
1179	C–H twisting	4
1101	Stretching C–C, stretching C–N	6
1003	Asymmetric CH_3 deformation	6
971	C–C stretching mixed with C–H rocking	4
954	Stretching C–C–O	7
844	Stretching ring	6
767	In plane ring deformation	6
717	Out of plane ring deformation	6
684	Out of plane bending C=O	6

References

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