

Supporting Information

“Turn on” electron-transfer-based selective detection of ascorbic acid via copper complexes immobilized on glass

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Materials and methods: 2-Acetyl pyridine, 4-pyridine carboxaldehyde, 1-10, phenanthroline, 3-iodo-n-propyltrimethoxysilane and sodium perchlorate were purchased from Sigma-Aldrich and were used as received. Copper acetate monohydrate was purchased from s. d. fine chemicals (India). 1,10-Phenanthroline was purchased from Spectrochem (India). Methanol and ethanol were purchased from Merck and was distilled over activated magnesium turnings and iodine before use. 30% aq. Ammonia, H₂O₂, dry n-pentane, toluene, dichloromethane and acetonitrile (All AR grade) were purchased from s. d. fine chemicals (India). DL-dopamine, D-glucose, L-cysteine, succinimide, uric acid, urea and ascorbic acid was procured from SRL (India). Glass slides having thickness ~1.0-1.2 mm were purchased from Pearl India and Single-crystal silicon [100] substrates were purchased from Georg Albert PVD- Beschichtungen (Silz, Germany). Hydrothermal bombs (25mL & 50mL) were purchased from Prakash Scientific Works (India). Water used for the experiment was double distilled.

Glass slides were cleaned by immersing them in *piranha* solution (7:3 (v/v)), Conc.H₂SO₄: 30% H₂O₂) for 1h and subsequent washing with ample amount of de-ionized (DI) water. (*Caution: Piranha is an extremely strong and dangerous oxidising agent and all protective measures must be adopted to prevent any harm.*) Then the slides were kept in RCA solution (1:5:1 (v/v), aq. NH₃:H₂O:H₂O₂) for 1h followed by rinsing with ample amount of de-ionized water, dried with N₂ stream and kept in oven for 2h at 110 °C. Silicon wafers were cleaned by sonication for 15 minutes each in n-hexane, acetone and isopropanol followed by drying under N₂ stream before placing in oven at 110 °C for 2h. The monolayer formation was carried out under an inert atmosphere using standard Schlenk/cannula techniques.

Physical measurements: All ¹H NMR spectra were recorded on Jeol JNMECX 400P spectrometer. Single crystal data were collected using CCD diffractometer (Oxford) at 195K. The unit cell parameters and the intensity data of a crystal mounted on a glass fiber using epoxy cement were obtained using a Kappa CCD diffractometer, equipped with sealed tube Mo-K α ($\lambda = 0.71073$) graphite monochromator, with increasing ω (width 0.5 deg frame⁻¹) at a scan speed of 1.0 deg per 125 sec. The data were processed with

Denzo-scalepack and corrected for absorption. Structure solution and refinement were done using SHELXS system of programs using direct methods. Hydrogen atoms were placed at their calculated positions and refined using a riding model. The non-hydrogen atoms were refined anisotropically. Crystal data and structure refinement parameters are given in table S1. Detailed crystallographic data in CIF format have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 810672. Infrared spectra were recorded on Perkin-Elmer FT-IR spectrometer. Elemental analyses were performed on a GMBH VarioEL elemental analyzer. All the above data collection was performed at Central Instrumentation Facility (CIF), University of Delhi. Electronic absorption spectra were recorded using JASCO UV-Vis-NIR spectrophotometer (660D). Mass spectra were recorded on THERMO Finnigan LCQ Advantage max ion trap mass spectrometer (MSAIF, CDRI, Lucknow, India).

Preparation of 4'-pyridyl-2,2':6',2''-terpyridine(Pytpy): The 4'-pyridyl-2,2':6',2''-terpyridine was synthesized following reported procedure^{s1} and purity was checked by ¹HNMR and Mass spectrometry. ¹HNMR (400 MHz, CDCl₃); δ/ppm: 8.76 (s, 2H), 8.78 (d, 2H), 8.68 (d, 2H), 7.8(d, 2H), 7.89 (t, 2H), 8.75 (d, 2H), 7.39 (t, 2H). ESI-MS: *m/z*. 310 (M⁺).

Preparation of [Cu(OOCH₃)₂(Pytpy)] (1)^{s2}: Copper acetate monohydrate (0.193g, 0.96 mmol) was dissolved in 10 mL methanol at room temperature. A methanolic solution of 4'-pyridyl-2,2':6',2''-terpyridine (0.30g, 0.96 mmol) was added dropwise in 30 minutes span time and the solution was kept for stirring at 60° C for 2h. The solution was filtered when hot. The filtrate was cooled to room temperature and slow diffusion of diethyl ether to this solution yield needle shaped green colour single crystals suitable for X-ray diffraction. Yield: 0.28g (60%). UV-Vis: λ_{max}/nm (ε/M⁻¹ cm⁻¹): 220 (12139), 270 (12917), 336 (4676), 347 (5891), 626 (63). FT-IR (KBr disk) (cm⁻¹): 421, 1090, 1553, 1585, 1609, 1618. (ESI-MS): *m/z* = 246 (M²⁺).

Preparation of [Cu(ph)(pytpy)].(ClO₄)₂ (2): A solution of 1, 10-phenanthroline (0.192g, 0.96mmol) was added to a copper acetate monohydrate (0.193g, 0.96mmol) (dissolved in 10 mL methanol) dropwise in 30 min span time. Subsequently, a solution of

4'-pyridyl-2,2':6',2''-terpyridine (0.30g, 0.96mmol, in methanol) was added dropwise again in 30 min span time. The solution was kept at stirring for 6h at room temperature. Addition of saturated solution of sodium perchlorate (in 5 mL methanol) to the reaction mixture yields blue colour precipitate which was filtered and washed with diethylether. Yield: 0.48 g (66.67%). In an alternative method, a solution of 1,10-phenanthroline (0.2 g, 1.0 mM) in methanol was added dropwise to the solution of complex **1** (0.49 g, 1.0 mM) at room temperature with stirring for 6h. Addition of saturated solution of sodium perchlorate (in 5 mL methanol) followed by washing the precipitate with diethyl ether yield complex **2**. Yield: 54.12 %. (ESI-MS): $m/z = 277$ ($M^{2+}-2ClO_4$). FT-IR (KBr disk)(cm^{-1}): 416, 1090, 1415, 1476, 1550, 1572, 3049. UV-Vis: λ_{max}/nm ($\epsilon/M^{-1} cm^{-1}$): 229 (9648), 269 (10632), 333 (4503), 349 (4398), 642 (113). Anal. Found (%): C, 51.08; H, 3.06; N, 11.21 Calc. (%) for $CuC_{32}H_{22}Cl_2N_6O_8$: C, 51.04; H, 2.94; N, 11.16.

Preparation of complex 3: An excess of 3-iodo-n-propyl-1-trimethoxysilane (45 μ L, 0.23 mmol) was added to acetonitrile/toluene (3:7 (v/v)) solution of (**1**) (24.5 mg, 0.050 mmol) under N_2 in a 50mL pressure vessel (hydrothermal bomb). The reaction mixture was kept in a programmed oven for 72h at 85 °C. Afterwards, solvent was evaporated under high vacuum and residue was washed with ample amount of dry n-pentane (5 \times) and dried under vacuum to yield pale green colour precipitate. Yield: 28 mg (71.5 %). Anal. Found (%): C, 47.00; H, 4.43; N, 7.01 Calc. (%) for $CuC_{30}H_{35}IN_4O_7Si$: C, 46.70; H, 4.51; N, 7.16. (ESI-MS): $m/z = 655$ (M^+-I). UV-Vis: λ_{max}/nm ($\epsilon/M^{-1} cm^{-1}$): 218 (10234), 260 (11737), 337 (4786), 348 (7734). FT-IR (Nujol)(cm^{-1}): 1021, 1115, 1208, 1293, 1617, 2854, 2923.

Preparation of complex 4: Complex **4** was prepared following same procedure as for complex **3**. 3-iodo-n-propyl-1-trimethoxysilane (45 μ L, 0.23 mmol) was added to acetonitrile/toluene (3:7 (v/v)) solution of **2** (37.6 mg, 0.050 mmol) under N_2 in a 50mL pressure vessel (hydrothermal bomb). The reaction mixture was kept in a programmed oven for 72h at 85 °C. Afterwards, solvent was evaporated under high vacuum and residue was washed with ample amount of dry n-pentane (5 \times) and dried under vacuum to yield pale green colour precipitate. Yield: 24 mg (46 %). Anal. Found (%): C, 41.26; H, 3.39;

N, 7.72 Calc. (%) for $\text{CuC}_{38}\text{H}_{37}\text{I}_3\text{N}_6\text{O}_3\text{Si}$: C, 41.56; H, 3.40; N, 7.65. (ESI-MS): $m/z = 422$ ($\text{M}^{2+}-2\text{I}$), 238 ($\text{M}^{3+}-3\text{I}$). FT-IR (Nujol)(cm^{-1}): 1029, 1483, 1476, 1572, 3049. UV-Vis: $\lambda_{\text{max}}/\text{nm}$ ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$): 217 (11342), 260 (9876), 334 (4356), 349 (5876).

Formation of monolayers^{S3}: The freshly cleaned glass slides ($2.5 \times 0.9 \times 0.1 \text{ cm}^3$) and silicon wafers ($1.0 \times 1.2 \text{ cm}^2$) were immersed in the solution of **3** or **4** (0.5 mM, acetonitrile/toluene (v/v), 3:7) in a Teflon pressure vessel under N_2 atmosphere using Schlenk/cannula techniques. The vessel was kept in a programmed oven for 52h at 85 °C. The reaction vessels were then cooled to room temperature and the functionalised substrates were rinsed with acetonitrile and dichloromethane. Further, these substrates were sonicated for 3 minutes each in acetonitrile and isopropanol. The substrates were then dried and stored in desiccator with exclusion of light and under inert atmosphere.

Increasing the reaction time from 52 to 64h does not affect the intensity and peak position of the bands at $\lambda_{\text{max}} = 356 \text{ nm}$ and 354 nm for **3**- and **4**- based monolayer respectively, indicating the formation of a fully formed monolayer. Shortening the monolayer deposition time to 20 h decreases the intensity ($\sim 20\text{-}25\%$) of the absorption band in both cases.

Characterization of the monolayers: The monolayers were characterized by a combination of aqueous contact angle measurements, semicontact atomic force microscopy (AFM), optical (UV/vis) transmission spectroscopy, spectroscopic ellipsometry, and X-ray photoelectron spectroscopy.

The XPS measurements were performed using a Mg $\text{K}\alpha$ X-ray source and a hemispherical analyzer. The spectra acquisition was carried out in normal emission geometry with an energy resolution of $\sim 0.9 \text{ eV}$. The X-ray source was operated at a power of 260 W and positioned $\sim 1.5 \text{ cm}$ away from the samples. The binding energy (BE) scale of the XPS spectra was referenced to the Si 2p doublet (appearing as a single peak) at 99.15 eV.^{S4} The spectra were fitted by symmetric Voigt functions and Shirley-type background. The peak fits were carried out self-consistently, i.e., the similar peak parameters were used for identical spectral regions. The intensity ratios of the Cu 2p and N 1s signals, corrected for the atomic sensitivity factors, are close to the values expected from the molecular

composition, which underlines the intact adsorption of the molecules. In contrast, the C 1s/Cu 2p and C 1s/N 1s ratios are little higher than expected suggesting that there is a residual contamination on the surface, parallel to the molecular adsorption.

Static contact angles of water were measured on a Rame-Hart goniometer. Atomic force microscopy (AFM) images were recorded using a Solver P47 (NTMDT) operated in the semicontact mode. OTESP-type Si SPM probes were used with a resonance frequency of tip ~ 250 kHz and a tip radius of ~ 10 nm. Roughness data were extracted from $500 \text{ nm} \times 500 \text{ nm}$ images.

Spectroscopic ellipsometry measurements were carried out using an M 2000V (J. A. Wollam Co. Inc.) spectroscopic ellipsometer over a range of 370 to 1000 nm. Measurements were done at incident angles of 60° , 70° and 80° . The data were simulated using Wvase32 software over the entire range to fit the experimental parameters. A Cauchy model was used to fit the data for the organic layer, assuming model parameters of $A=1.45$, $B=0.01 \text{ nm}^2$, $C=0$ and $\exp=0$. The native oxide layer was estimated to be 20 \AA , for the purpose of the calculation, based on ellipsometry measurements of freshly cleaned Si(100) pieces.

Preparation of ppm solutions of ascorbic acid (AA) and other analytes: A stock solution of 1.0 mM (176 ppm) of Ascorbic Acid (AA) was prepared by dissolving 17.6 mg of AA in 100 mL water and degassed with N_2 . This solution was further diluted to generate different solutions containing 0.5, 1.0, 2.0, 3.5, 5.5, 6.5 and 10 ppm of AA. Stock solutions 1.0 mM of other analytes *viz* DL-Dopamine, L-Cysteine, Glucose, Sucrose, Uric acid, Succinimide and Urea were also prepared by dissolving their calculated amounts in water.

Optical sensing of ppm-levels of AA in aqueous medium: The Cu(II)-based monolayer on glass was treated with a series of N_2 -saturated aqueous solutions (N_2 gas was purged for 10 min in each solution) containing 0.5 – 10 ppm of AA. The reaction of AA with **3**-based and **4**-based monolayer on glass was monitored both *in-situ* and *ex-situ* by UV/vis spectrophotometry in the transmission mode. In a particular set of experiments, the monolayers were placed in the cuvette containing 5.5 ppm AA (in N_2 -saturated water) for

5 min. The substrates were washed with absolute ethanol and dried under N₂ stream before recording UV-Vis spectra. Experiments with other solutions were carried out in similar way. The Cu(II)-based monolayer was reset to its original state by keeping it in air for five minutes or to the O₂ saturated aqueous solution containing 0.5 mM sodium acetate for 2 min. Full recovery of the formal metal oxidation state was confirmed by UV/vis measurements. In another experiment **3**-based monolayer was placed in a cuvette containing 6.5 ppm AA in 3 mL N₂-saturated water and the reaction were monitored by *in-situ* UV/vis measurements as a function of time. An identical glass substrate without monolayer was used to compensate for the background absorption in all cases. A similar experiments were repeated without purging N₂ in aqueous solution (Figure S7). All measurements were performed at room temperature (~25 °C) unless stated otherwise.

Reversibility test: Alternate treatment of the monolayer-based sensor with AA

and air/O₂: Alternate sensing/resetting cycles were obtained by immersion of glass substrates functionalized on both sides with a Cu (II)-based monolayer for 5 min in aqueous solution of AA (10 ppm) and flow of air (~5 min), respectively. Next, the substrate was rinsed with absolute ethanol, gently cleaned with task paper, and then dried at room temperature under a gentle stream of N₂ before the absorption spectrum was recorded. The experiment was repeated for four alternating cycles of AA exposure, and recovery with air. In another set of experiment the recovery experiment was performed in O₂-saturated aqueous solution (O₂ gas was purged for 10 min in solution) containing 0.5 mM of NaOOCCH₃ for ~2 min. It was observed that the presence of ions accelerate the recovery process. Notably the redox process in monolayer requires uptake/release of anion from surrounding to balance the charge.

Selectivity test: The selectivity of the sensor was demonstrated *ex-situ* using each of the following common interfering chemicals *viz.* D-glucose, DL-dopamine, L-cysteine, succinimide, uric acid and urea. A solution of each of these chemicals was prepared in water (0.5 mM) and **3**- and **4**-based monolayers were treated separately in each solution of these analytes for 30 min. The monolayers were washed with water and ethanol

followed by drying with N₂ stream before recording UV/vis spectra. Similar experiments were performed with equimolar mixture of all the above analytes (see Figure 3). In all cases UV/vis spectra measurements did not show any optical responses. Under similar condition presence of AA show a large optical change in UV and visible region. In another experiment, equal amount of AA were added to the mixture of all the above analytes and monolayer were treated for 30 min. Only **3**-based monolayer show optical response in presence of AA. The monolayers were reset using O₂ purged water solution containing 0.5 mM of sodium acetate.

Thermal stability of the monolayers: **3**-based monolayer on glass substrates were subjected to thermal stress. The samples were placed inside a sealed glass pressure tube under air and heated for 48h at 120 °C in temperature controlled oven (accuracy ± 2 °C). The samples were allowed to attain room temperature, rinsed with ethanol, gently wiped with task paper before *ex-situ* UV/Vis measurements.

DFT Calculation: DFT calculation for complex **1** and its reduced form have been performed using density functional theory DFT calculated with the Orca package. For the structure optimization, the PBE0 exchange-correlation functional has been used together with the Basis set 6-311G** (Adds polarization functions to hydrogen's of the 6-311G* basis set). See Figure S5. There is significant changes in bond length and bond distances once Cu(II) reduces to Cu(I).

Proposed mechanism for electron-transfer-based AA detection^{S5,S6}:

Sensing: (i) Ascorbic acid exist as ascorbate anion in aqueous solution. (ii) Ascorbate anion substitute acetate ion of **3**-based monolayers (iii) Ascorbate moiety transfers an electron to the metal (Cu²⁺) resulting in formation of radical and reduction of Cu²⁺ to Cu⁺ in penta-coordination (iv) The second O-H bond of ascarbate radical dissociates leading to the formation of dehydroascorbic acid and resulting in more stable tetrahedral conformation around Cu⁺.

Recovery: (i) Dioxygen binds to the metal (Cu^+) centre (ii) Cu^+ transfer an electron to O_2 which in the presence of H^+ forms H_2O_2 and Cu^+ oxidizes to Cu^{2+} (iii) Presence of acetate ions accelerate the reset the monolayer into its original state, thus making it reusable.

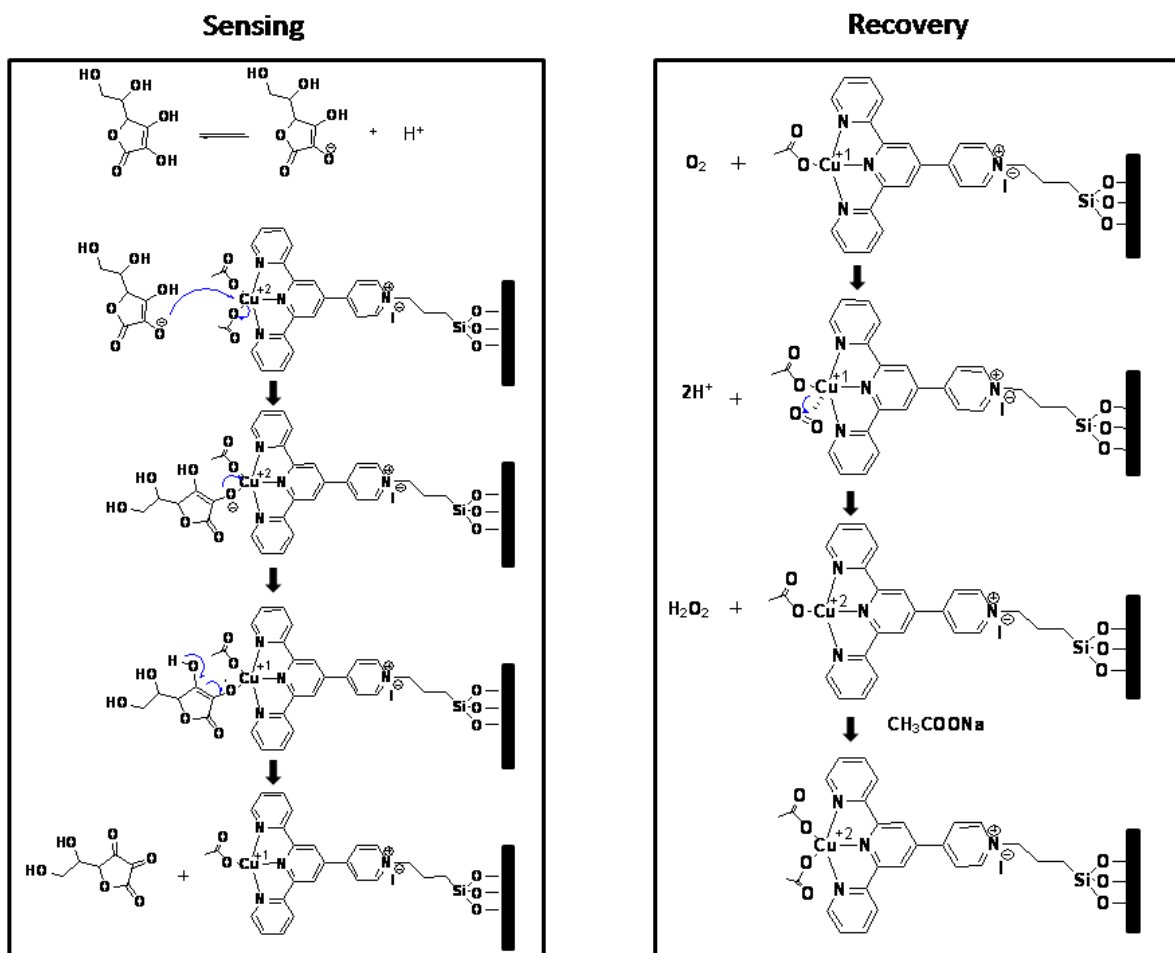


Figure S1. Proposed mechanism for electron-transfer-based AA detection (at the left) and subsequent recovery (at the right) for the **3**-based monolayer sensor.^{S5,S6}

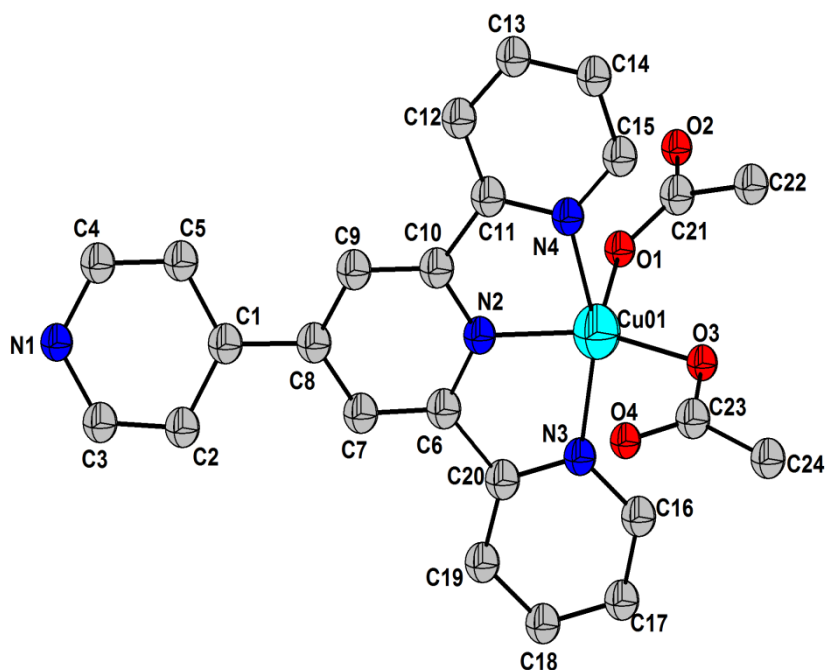


Figure S2. ORTEP diagram of the complex **1** drawn in 50% thermal probability ellipsoids showing atomic numbering. H atoms are omitted for simplicity. Selected bond lengths [\AA] and angles [deg]: N(2)-Cu(01), 1.9508(16); N(3)-Cu(01), 2.0355(17); N(4)-Cu(01), 2.0381(16); O(1)-Cu(01), 2.1446(15); O(3)-Cu(01), 1.9511(14); C(23)-O(3), 1.278(3); C(23)-O(4), 1.246(2); C(21)-O(1), 1.269(3); C(21)-O(2), 1.240(3); C(10)-N(2)-Cu(01), 119.12(13); C(6)-N(2)-Cu(01), 119.47(13); C(16)-N(3)-Cu(01), 125.92(15); C(20)-N(3)-Cu(01), 115.30(13); O(3)-Cu(01)-N(2), 155.34(7); O(3)-Cu(01)-N(3), 96.25(6); N(2)-Cu(01)-N(3), 79.29(7); O(3)-Cu(01)-N(4), 100.25(6); N(2)-Cu(01)-N(4), 79.62(6); N(3)-Cu(01)-N(4), 157.97(7); O(3)-Cu(01)-O(1), 105.70(6); N(2)-Cu(01)-O(1), 98.87(7); N(3)-Cu(01)-O(1); 95.13(6); N(4)-Cu(01)-O(1), 94.36(6).

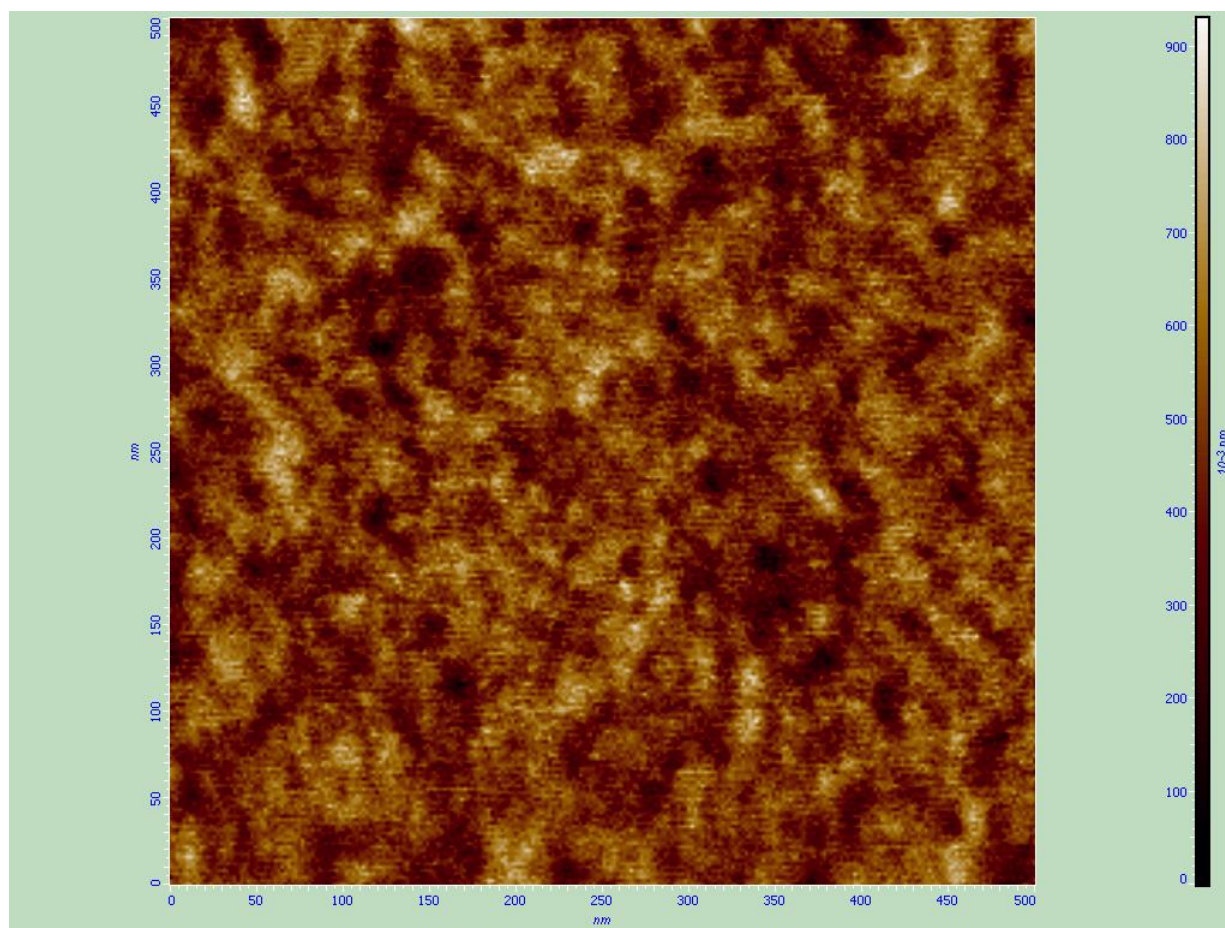


Figure S3: Representative AFM image of the monolayer of complex **3** on Si(100) recorded on a Solver P47 (NT-MDT) operated in semi-contact mode using a Si OTESP-type SPM probe with a resonance frequency of tip ~ 250 kHz. Scan area 500×500 nm, resolution 300×300 pts. Scan speed ~ 0.5 Hz.

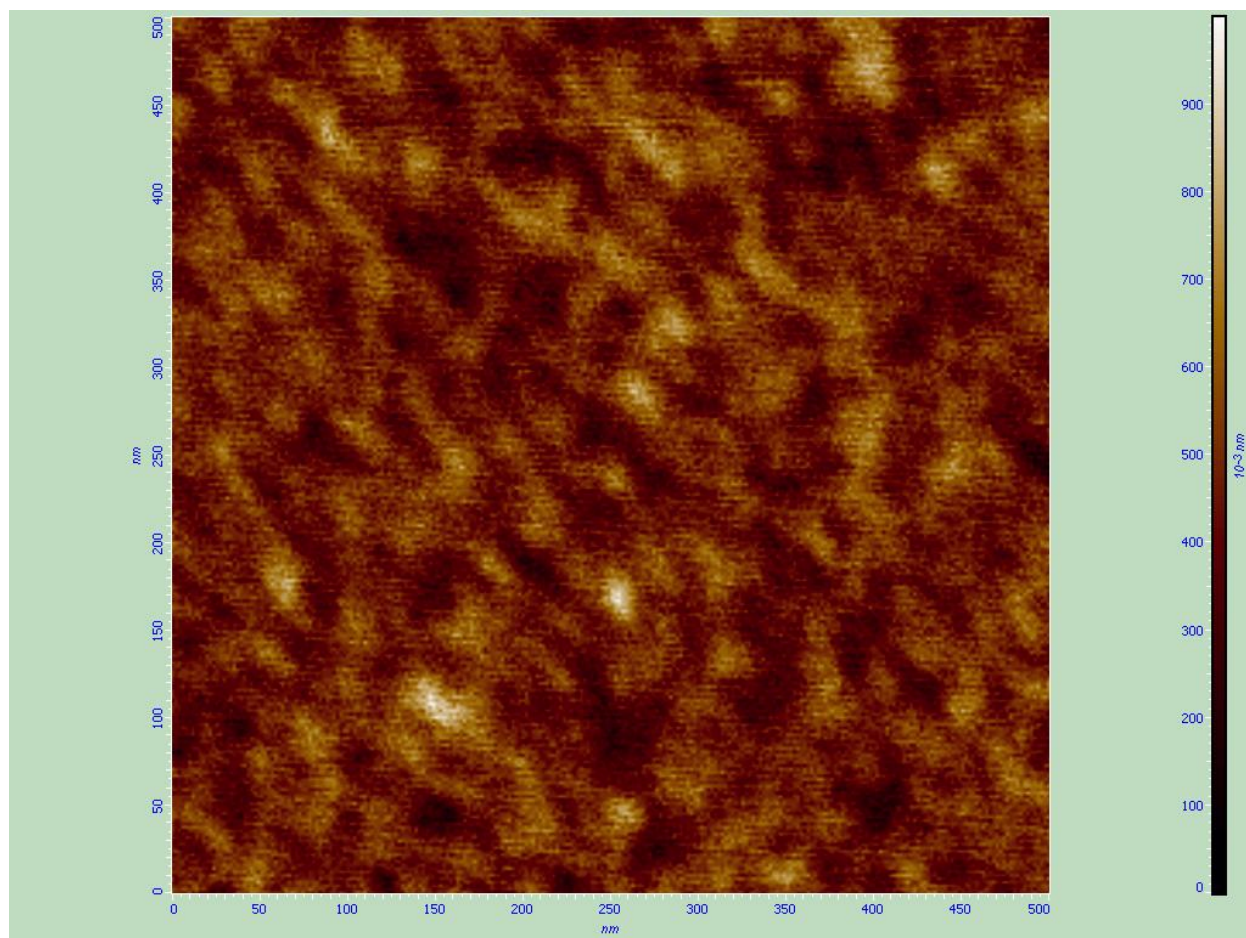


Figure S4: Representative AFM image of the monolayer of complex **4** on Si(100) recorded on a Solver P47 (NT-MDT) operated in semi-contact mode using a Si OTESP-type SPM probe with a resonance frequency of tip ~ 250 kHz. Scan area 500×500 nm, resolution 300×300 pts. Scan speed ~ 0.5 Hz.

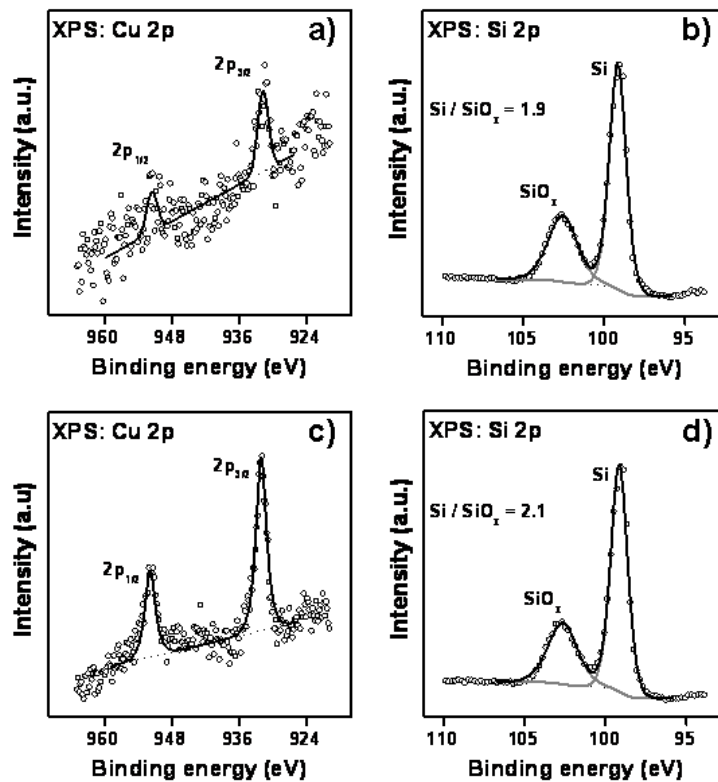


Figure S5: Cu 2p (a,c) and Si 2p (b,d) XPS spectra of the **3**- (a,b) and **4**- (c,d) based monolayers. The relative intensity of the SiO_x emission in (b) and (d) increases with decreasing sampling depth of XPS suggesting that the oxidation involves only the topmost part of the Si substrate (as expected).

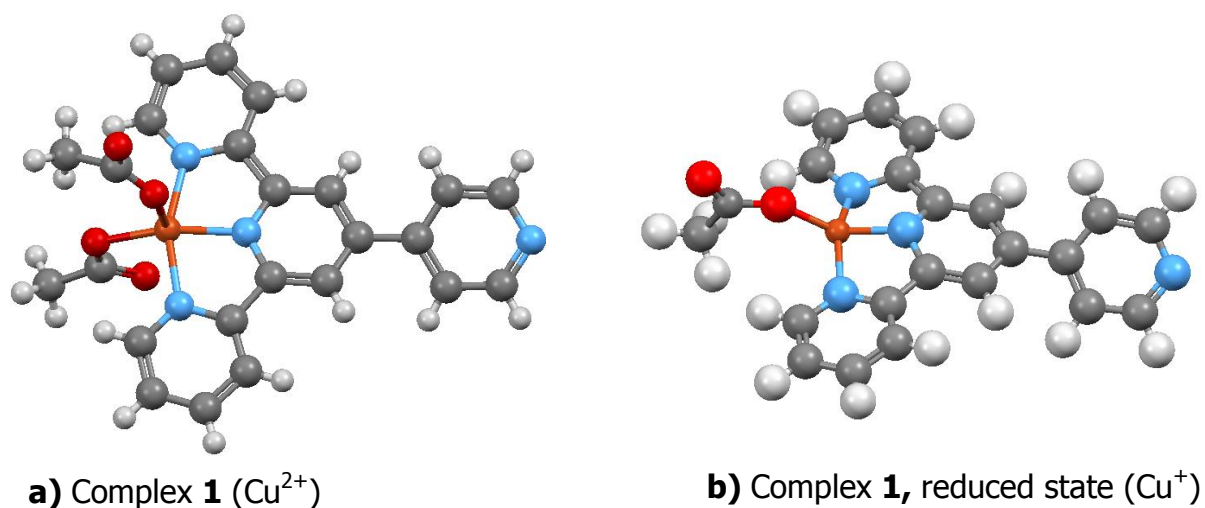


Figure S6: a) Optimized molecular structure of complex **1** as calculated at the PBE0/6-311G** level of theory. DFT geometry optimization suggest distorted square pyramidal geometry for complex **1**. N-Cu-N bond angles are 79.29, 79.63, 157.97 degree. Cu atom is in the plane of the molecule. b) The optimized structure suggests distorted tetrahedral geometry for the reduced form. N-Cu-N bond angles are 84.43, 84.52 and 150.47 degree while the Cu atom is out of the plane.

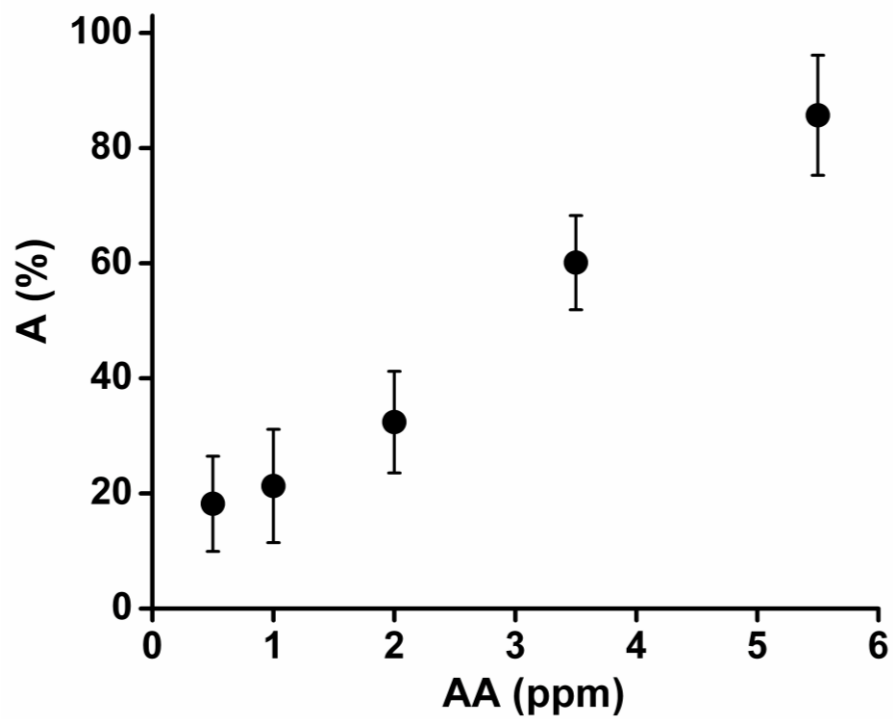


Figure S7: Absorption intensity changes of the sensor at $\lambda = 423$ nm after 5 min exposure as a function of AA concentration in aqueous solution ($R^2 = 0.98$ for linear fit) in absence of N_2 .

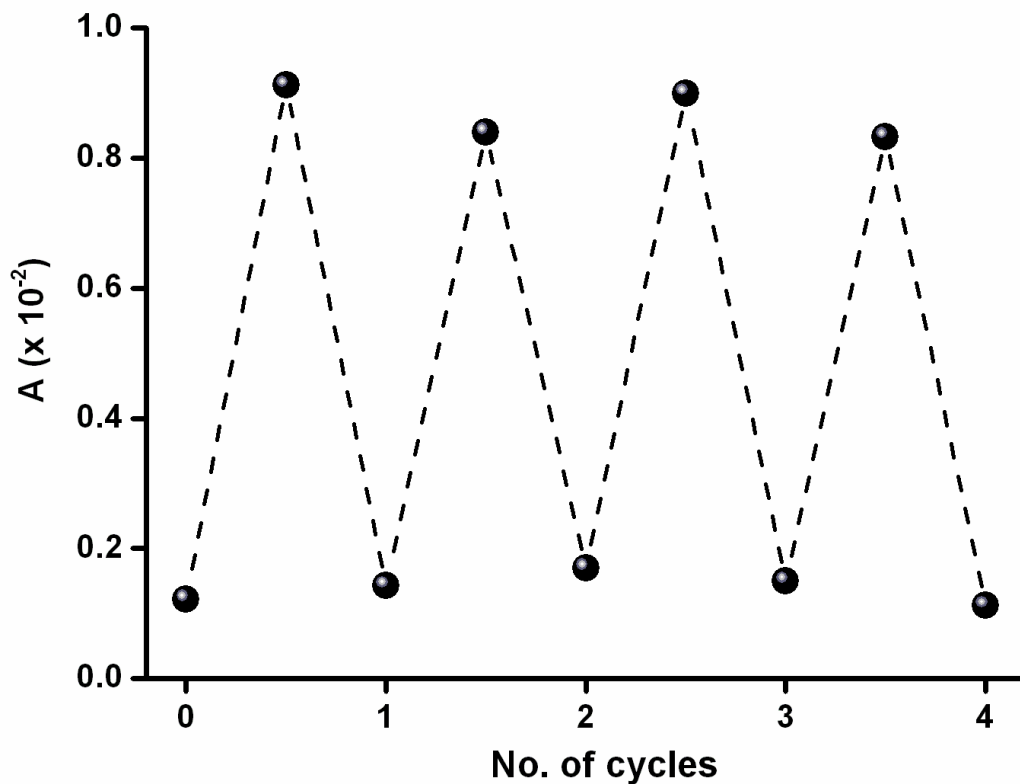


Figure S8: Absorption intensity changes of MLCT band at $\lambda = 423$ nm after thermal treatment for 48h at 120 °C for **3**-based monolayer on glass. The MLCT band was observed after exposing monolayer to 10 ppm AA in H₂O for ~5 min, followed by regeneration in air saturated aqueous solution containing 0.5 mM sodium acetate for ~2 min. Dotted lines are guide to the eyes.

Table 1. Selected crystallographic data and structure refinement for complex **1**

Empirical formula	C ₂₄ H ₂₂ CuN ₄ O ₅
Formula weight/g mol ⁻¹	510.00
Temperature/K	120(2)
Wavelength (λ)	0.71073 Å
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 8.7402(4) Å α = 73.598(4)°. b = 10.6570(6) Å β = 81.236(4)°. c = 13.3251(5) Å γ = 66.007(5)°.
Volume (Å ³)	1086.77(9)
Z	2
Density (calculated)	1.559 Mg/m ³
Absorption coefficient	1.051 mm ⁻¹
F(000)	526
Crystal size	0.24 x 0.18 x 0.12 mm ³
Theta range for data collection	3.19 to 26.00°.
Index ranges	-10 ≤ h ≤ 10, -13 ≤ k ≤ 13, -16 ≤ l ≤ 16
Reflections collected	9453
Independent reflections	4254 [R(int) = 0.0210]
Completeness to theta	26.00° 99.8 %
Absorption correction	Semi-empirical from equivalent
Max. and min. transmission	1.0000 and 0.8444
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	4254 / 0 / 317
Goodness-of-fit on F ²	1.141

Final R indices [$I > 2\sigma(I)$]	R1 = 0.0303, wR2 = 0.0804
R indices (all data)	R1 = 0.0355, wR2 = 0.0815
Largest diff. peak and hole	0.367 and -0.380 e.Å ⁻³

References:

- S1 A. Winter, A. M. J. van den Berg, R. Hoogenboom, G. Kickelbick, U. S. Schubert, *Synthesis*, 2006, **17**, 2873.
- S2 W. –J. Shi, L. Hou, D. Li, Y. –G. Yin, *Inorgan. Chim. Acta*, 2007, **360**, 588–598.
- S3 T. Gupta, M. Altman, A. D. Shukla, D. Freeman, G. Leitus, M. E. Van der Boom, *Chem. Mater.*, 2006, **18**, 1379.
- S4 J. F. Moulder, W. E. Stickle, P. E. Sobol, K. D. Bomben, *Handbook of X-ray Photoelectron Spectroscopy*; J. Chastian, Ed.; Perkin-Elmer Corp.: Eden Prairie, MN, **1992**.
- S5 (a) N. H. Williams, J. K. Yandell, *Aust. J. Chem.*, 1982, **35**, 1133; (b) M. M. Khan, A. E. Martell, *J. Am. Chem. Soc.*, 1967, **89**, 4176.
- S6 (a) S. A. Mills, J. P. Klinman, *J. Am. Chem. Soc.*, 2000, **122**, 9897; (b) M. E. G. Lyons, W. Breen, J. Cassidy, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 115.