Electronic Supplementary Information

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Grafted monolayers of the neutral Cu(II) complex of a dioxo-2,3,2 ligand: surfaces with decreased antibacterial action

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1.Materials and synthesis

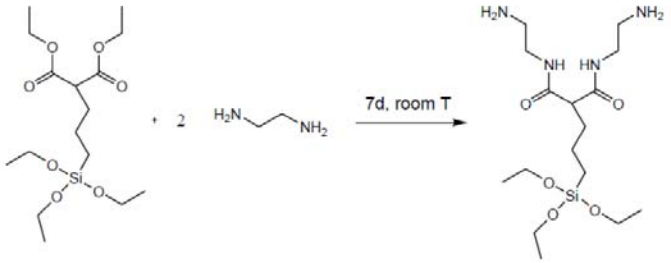
1.1 Materials.

Reagents. Diethyl-2-(3-triethoxysylilpropyl)malonate was purchased from Fluorochem and used without further purification. Ethylenediamine (>99.5%, Sigma-Aldrich) was distilled from CaH_2 prior to use. Diethyl butylmalonate, $Cu(CF_3SO_3)_2$, ethanol (>99.8%), CaH_2 (95%), Triethylamine (99%), FITC (Fluorescein 5-isothiocyanate, >97.5 %), hydrogen peroxide 30% v/v in water were purchased from Sigma-Aldrich and used without further purification.

Materials. Cover slides for microscopy ForLab 21x26 mm were purchased from Carlo Erba. Quartz slides (25x25x1mm) were purchased from UQG-Optics.

1.2 Syntheses

• Ligand **L1**H_2, N^1,N^3-bis(2-aminoethyl)-2-(3-triethoxysilylpropyl)malondiamide, was synthesized according to the following reaction scheme,



In a round-bottomed flask, 2.5 g diethyl-2-(3-triethoxysylilpropyl)malonate were dissolved in 50 ml of anhydrous ethylendiamine, freshly distilled from CaH_2. The obtained solution was kept under magnetic stirring for 7 days, under a N_2 atmosphere. Most of the excess ethylenediamine was removed by distillation under vacuum by heating at 50-60°C, yielding a dense yellow oil. This was further purified eliminanting residual ethylenediamine using a rotary oil pump (P = $5x10^{-2}$ torr) at 60°C. The final product, **L1**H_2, was a dense yellow oil (2.1 g, 79% yield).

Mass: m/z 393 [M + H^+]. ^{1}H NMR ($CDCl_3$): δ=0,6 (t, 2H, CH_2-Si); δ=1,2 (t, 9H, C**H**$_3$-CH_2-O-Si); δ=1,4 (m, 2H, C**H**$_2$-CH_2-Si); δ=1,9 (q, 2H, -C**H**$_2$-CH-(C=O)$_2$-); δ=2,77 (t, 4H, -C**H**$_2$-NH_2); δ=3,0 (t, 1H, -C**H**-(C=O)$_2$-); δ=3,3 (q, 4H, -(C=O)-NH-C**H**$_2$-CH_2); δ=3,85 (q, 6H, CH_3-C**H**$_2$-O-Si); δ=7,25 (t, large, 2H, -(C=O)-N**H**-CH_2-).

• Ligand **L2**H_2, N^1,N^3-bis(2-aminoethyl)-2-butyl malondiamide was prepared with an identical synthesis, starting from diethyl butylmalonate. Dense yellowish oil, 82% yield. Mass: m/z 245 [M + H^+]. ^{1}H NMR ($(CD_3)_2SO$): δ=0.84 (t, 3H, C**H**$_3$-CH_2-); δ=1,14-1,25 (m, 4H, CH_3-C**H**$_2$-C**H**$_2$-CH_2-); δ = 1.66 (q, 2H, -C**H**$_2$CH(C=O)$_2$); δ = 2.55 (t, 4H, NH_2); δ=2,57(t, 4H, -C**H**$_2$-NH_2); δ=3,0-3,1 (m, 5H, -C**H**-(C=O)$_2$- + -(C=O)-NH-C**H**$_2$); δ=7,93 (t, 2H, -(C=O)-N**H**-)

1.3 Glass and quartz slides functionalization

Glassware was filled with aqua regia for 30 minutes, then filled and washed with bidistilled water in a ultrasonic bath for 10 minutes. Water was discarded the washing cycle with bidistilled water/ultrasonic bath was repeated 2 more times. Finally, glasswate was dried in a oven at 140 °C for 1h.
Before reaction with silanes, glass and quartz slides were dipped for 30 min in a piranha solution (3:1 v/v 96% H_2SO_4 / 30% H_2O_2), then washed with bidistilled water in a ultrasonic bath for 10 minutes. Water was discarded and the washing cycle with bidistilled water/ultrasonic bath was repeated 2 more times. Finally, glasswate was dried in a oven at 140 °C for 1h.

• Coating with [**L1**Cu].
$1x10^{-3}$ M, $5x10^{-4}$ M and $2.5x10^{-4}$ M solutions of **L1**H_2 were prepared by adding 31.2mg, 15.6 mg and 7.8 mg **L1**H_2, respectively, in 80 mL EtOH (> 99.8%). Then, 1:1 (mol/mol) $Cu(CF_3SO_3)_2$ was added to each solution as a solid and rapidly dissolved. After this, 2.2:1 (mol/mol with respect to **L1**H_2) Et_3N was added to each solution from a pre-prered 0.22 M Et_3N solution in EtOH. Finally, solutions were separated into two staining jars for microscopy, and 8 slides were made to react in vertical position in each jar, for 10 min at 40 °C on a reciprocating stirrer. After reaction, coated glasses were rapidly washed with 3 mL ethanol each and blow-dried with N_2.

2. Methods

• 2.1 Determination of total Cu^{2+} by ICP

Each slide was treated in a 50 mL beaker with 3.0 mL bidistilled water to which 0.180 mL 69 % HNO3 were added. The treated slides were left to react overnight and the solution was then analized by ICP, with a ICP-OES Optima 3300 DW Perkin Elmer instrument.

• 2.2 Determination of released Cu^{2+} at different pH values

An analogous procedure was used as for 2.2. Each slide was first dipped in a solution with pH regulated with microadditions of 0.1 M HNO_3 (acidic solutions) of 0.1 M NaOH (basic solution), then Cu^{2+} was analyzed by ICP.

• 2.3 UV-VIS-NIR absorption spectra

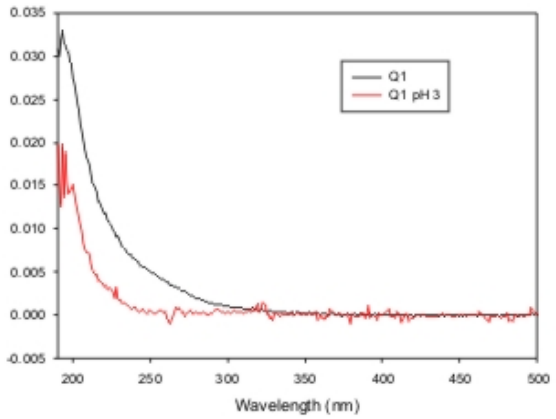
Absorption spectra in solution have been carried out in 1 cm glass cuvettes or in 1 mm quartz cuvettes using a Varian Cary instrument between 300nm and 900 nm (glass) or 190-900 nm (quartz).
Absorption spectra of functionalized slides were carried out using a Varian Cary 60 spectrophotometer equipped with a solid samples holder, in the 190- 900 nm range.

• 2.4 Potentiometric titrations

The protonation and Cu^{2+} complex formation equilibria for **L2**H_2 were studied in a water 0.1 M in $NaNO_3$ at 25 °C, by titrating with standard base a solution containing the chosen ligand and excess nitric acid (determination of protonation constants), or a solution containing **L2**H_2, one equivalent of $Cu(CF_3SO_3)_2$ plus excess nitric acid (determination of complexation constants). Potentiometric studies were carried out using an automated unit as previously described (V. Amendola, L. Fabbrizzi, P. Pallavicini, L. Parodi and A. Perotti, J. Chem. Soc., Dalton Trans., 1998, 2053). Solutions were typically of 40 mL volume, with 10^{-3} M ligand or ligand/Cu^{2+}.

• 2.5 Reloading of Cu^{2+} on demetallated surfaces.

Slides functionalized with [**L1**Cu] were first treated for 24 h in water at pH 3.0, then repeatedly washed with bidistilled water, finally blow-dried with N_2.
Quartz slides were also used to spectroscopically check the presence of ligand after the treatment. The following figure reports the absorption spectrum before treatment (black line, Q1) and after demetallation treatment (red line, Q1 pH3), showing in the latter case the expected residual ligand absorption.



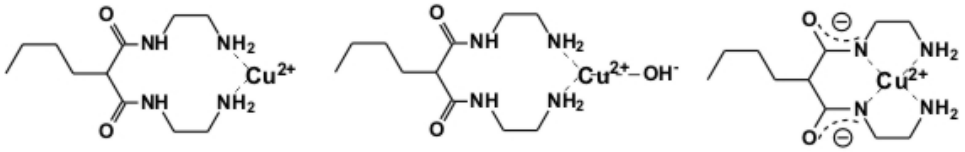
After this treatment reloading of Cu^{2+} was carried out by dipping the dried slides in 40 mL of 10^{-3} M $Cu(CF_3SO_3)_2$ in EtOH for 2 h, with the addition of 15μL of 0.22 M Et_3N, and workup as described in 1.3

3. Cu^{2+} complex formation in solution

Table 3.1 - Protonation and complexation constants for **L2**H_2 and **L2**H_2/Cu^{2+}, determined by potentiometric titrations

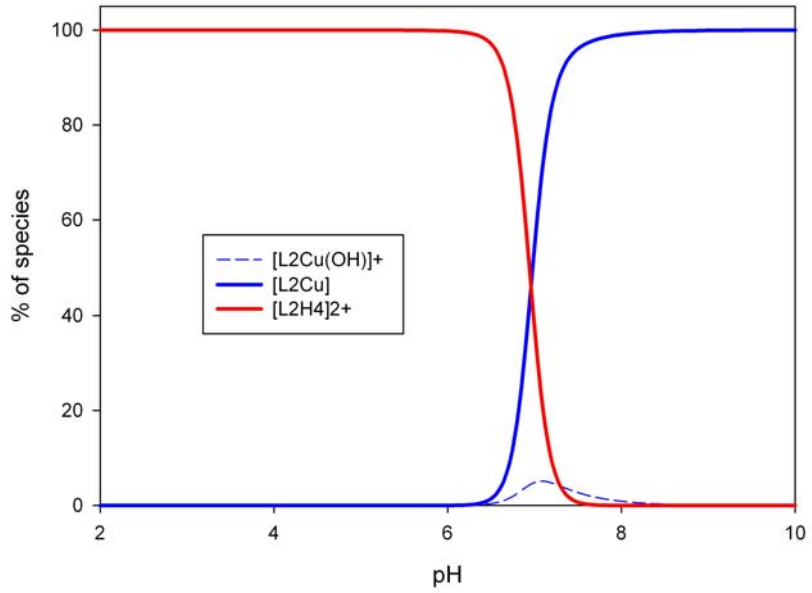
equilibrium	formed species	log K
L2H_2 + H^+ = **L2**H_3^+	101	8.74
L2H_2 + $2H^+$ = **L2**H_4^{2+}	102	17.73
L2H_2 + Cu^{2+} = [Cu(**L2**H_2)]$^{2+}$	110	8.03
L2H_2 + Cu^{2+} + H_2O = [Cu(**L2**H_2)OH]$^+$ + H^+	11-1	1.24
L2H_2 + Cu^{2+} = [Cu**L2**] + $2H^+$	11-2	-4.71

Sketches of the formula of the 3 copper complexes



[**L2**H_2Cu]$^{2+}$ = 110 [**L2**H_2Cu(OH)]$^+$ = 11-1 [**L2**Cu] = 11-2

Distribution diagram for 1:1 **L2**H_2:Cu^{2+}, recalculated for $1.5x10^{-6}$ M concentration



The [Cu(**L2**H_2)]$^{2+}$ species forms only in negligible quantities at this concentration and it is not reported in the diagram.

4. Antibacterial activity tests

The antibacterial activity of functionalized surfaces was investigated against *Staphylococcus aureus* ATCC 6538 (Gram+) and *Escherichia coli* ATCC 10356 (Gram-). The microorganisms were grown overnigth in Tryptone Soya Broth (Oxoid; Basingstoke, Hampshire, England) at 37°C. Washed cells were resuspended in *Dulbecco's PBS* and optical density (OD) was adjusted to 0.1, corresponding approximately to $1x10^8$ Colony Forming Units (CFU/ml) at 600 nm wavelength.

10 µl of bacterial suspension was deposited on a standard glass slide (76x26 mm), then the microbial suspension was covered with a glass slide (21x26 mm) coated with [**L1**Cu] (or **L1**H_2, after release of Cu^{2+} at acidic pH), forming a thin film between the slides that facilitates direct contact of the microorganisms with the active NP surface. The two assembled glasses were introduced in a Falcon test-tube (50 ml) containing 1 ml of PBS to maintain a damp environment. For each bacterial strain two equivalent modified glasses were prepared; the slides were maintained in contact with the liquid films containing bacteria at room temperature for 5 and 24 hours, respectively; for each time of contact an unmodified glass slide was treated in the same way as control sample. After the times of contact, 9 ml of PBS were introduced in each Falcon test-tube under a gentle shaking to detach the assembled glass slides. Bacterial suspensions were then grown in Tryptone Soya Agar (Oxoid; Basingstoke, Hampshire, England) to count viable cells.

The decimal-log redution rate, Microbicidal effect (ME), was calculated using the formula

$$\mathbf{ME = \log N_C - \log N_E}$$

($\mathbf{N_C}$ being the number of CFU/ml developed on the unmodified control glasses, and $\mathbf{N_E}$ being the number of CFU/ml counted after exposure to modified glasses). The results expressed as ME (Table 1, main text) represent the average of 3-5 equivalent determinations.