

## Electronic Supplementary Material

### **Self-assembled monolayers of a Cu<sup>2+</sup>-tetraazamacrocyclic complex on glass: an adhesive layer for silver nanoparticles grafting in the preparation of surface-active antibacterial new materials**

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## S1. Experimental

**S1a. Materials.** PBS was purchased from Sigma-Aldrich. 3-Isocyanatopropyltrimethoxysilane was purchased from Fluorochem. Silver nitrate (>99.8%), sodium borohydride (>99.0%), sodium citrate (>99.0%) and PBS were purchased from Sigma-Aldrich. Reagents were used as received. Solvents were purchased from Sigma-Aldrich and used as supplied. Microscopy cover glass slides (2.4×2.4cm) were purchased from Forlab (Carlo Erba). Quartz slides (Spectrosil 2000 fused silica, 1.4×1.4cm, 1mm thick) were purchased from UQG Optics Ltd. Glass cuvettes were standard optical glass cuvettes purchased from Hellma. Water was bidistilled, prepared from deionized samples. Silver NP ( $d = 7\text{ nm}$ ,  $\sigma = 4\text{ nm}$ ) were prepared as described in ref. 6 (main text).  $[\text{Cu}(\text{13aneN4})](\text{CF}_3\text{SO}_3)_2$  was prepared as described in reference 13

**S1b. Synthesis of  $\text{PTS}[\text{Cu}(\text{13aneN4})](\text{CF}_3\text{SO}_3)_2$ .**  $[\text{Cu}(\text{13aneN4})](\text{CF}_3\text{SO}_3)_2$  (55mg,  $9.5 \cdot 10^{-2}\text{ mmol}$ ) and (3-isocyanatopropyl)trimethoxysilane (19.6  $\mu\text{l}$ ,  $9.5 \cdot 10^{-2}\text{ mmol}$ ) were dissolved in 10ml refluxing acetone under nitrogen and kept under magnetic stirring. After 2 hours the mixture was cooled to room temperature and stirred overnight. After solvent removal under reduced pressure  $\text{PTS}[\text{Cu}(\text{13aneN4})](\text{CF}_3\text{SO}_3)_2$  was isolated in quantitative yield.

ESI-MS: 241.5 (2+)  $\text{PTS}[\text{Cu}(\text{13aneN4})]^{2+}$ , 632.3  $[\text{PTS}[\text{Cu}(\text{13aneN4})] + \text{CF}_3\text{SO}_3]^+$

FT-IR: see figure S2

**S1c. Functionalization of surfaces.** Glass and quartz slides were cleaned with piranha solution for 30', washed three times with ultrapure water under sonication and oven dried. Cover glass slides were deeped for 4 hours in a 0.1% solution of  $\text{PTS}[\text{Cu}(\text{13aneN4})]^{2+}$  in ethanol (prepared either from the solid product or diluting concentrated acetone solutions obtained by the synthesis described in paragraph S1b) and kept thermostatted at 40°C.

In a typical preparation, 8 glass slides were prepared at the same time, i.e. reacting in the same silane solution inside a 8-place holder (a microscope glass slides staining jar). After this, the slides were washed three times with ethanol in a sonic bath and blow-dried with nitrogen.

Quartz slides were prepared individually immersing the slide in 3 ml of silane solution in a vial. Glass cuvettes were prepared with the same preparation, filling the cuvettes with the described solution.

**S1d. Antibacterial activity tests.** The antibacterial activity of **SURF-PTS[Cu(13aneN4)]<sup>2+</sup>** and of **SURF-PTS[Cu(13aneN4)]<sup>2+</sup>-NP** monolayers was investigated against *Staphylococcus aureus* ATCC 6538 (Gram+) and *Escherichia coli* ATCC 10356 (Gram-). The microorganisms were grown overnight 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  $1 \times 10^8$  Colony Forming Units (CFU/ml) at 600 nm wavelength.

10  $\mu$ l of bacterial suspension was deposited on a standard glass slide (76x26 mm), then the microbial suspension was covered with a glass slide (24x24 mm) coated with the **SURF-PTS[Cu(13aneN4)]<sup>2+</sup>** or **SURF-PTS[Cu(13aneN4)]<sup>2+</sup>-NP** SAM, 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 reduction rate, Microbicidal effect (ME), was calculated using the formula:

**ME = log N<sub>C</sub> – log N<sub>E</sub>** (N<sub>C</sub> being the number of CFU/ml developed on the unmodified control glasses, and N<sub>E</sub> being the number of CFU/ml counted after exposure to modified glasses). The results expressed as ME represent the average of 3-5 equivalent determinations.

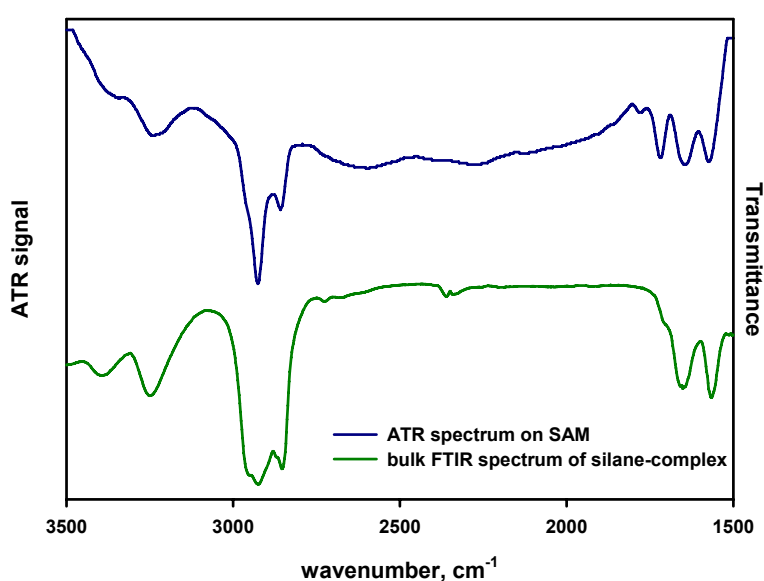
**S1e. Instrumentation and methods.** Absorbance spectra of colloidal suspensions were taken with a Varian Cary 100 spectrophotometer in the 200-1000 nm range. Spectra of functionalized glasses or quartzes were obtained placing the slides on the same apparatus equipped with a dedicated Varian solid sample holder, or directly using modified cuvettes in their standard holder. Measurements of Absorbance vs time on modified cuvettes were carried out by filling the cuvette with the chosen solution and keeping it stoppered and in the dark between successive spectra.

Atomic force microscope images were obtained with a Thermo Microscopes CPII AFM, operated in tapping mode with NT-MDT silicon tips NSC05\_10° and NSG01. Data

analysis (manual width and height calculation on line profiles) was carried out with Image Processing and Data Analysis software - version 2.1.15 by TM Microscopes. Inductively coupled plasma atomic emission spectroscopy data were collected with an ICP-OES OPTIMA 3000 Perkin Elmer instrument. The total Ag or Cu content on glasses with NP monolayers was determined by quantitatively oxidizing / demetallating the monolayers on a single slide (24×24 mm or 12×24 mm) by deeping it in 5ml ultrapure concentrated HNO<sub>3</sub> diluted 1:5 with water (13%w as final concentration) in a vial, and keeping it at RT on a Heidolph Promax 1020 reciprocating platform shaker overnight. The Ag and Cu content in solution was then determined by ICP.

## S2. IR characterization of the copper(II) complex and surface

FTIR-ATR (Fourier transform infrared – attenuated total reflectance) spectra were measured on **SURF-PTS[Cu(13aneN4)]<sup>2+</sup>** SAM by a Bruker IFS113v spectrometer, equipped with an home-made setup for multiple reflection ATR. Samples were prepared directly on double-polished Silicon ATR crystals (Korth Kristalle GmbH, 10x50x3 mm<sup>3</sup>, trapezoidal shape with 45° bevels). Each spectrum is collected with 256 scans at a spectral resolution of 4 cm<sup>-1</sup>. The sample compartment is kept under vacuum (10<sup>-3</sup> torr).

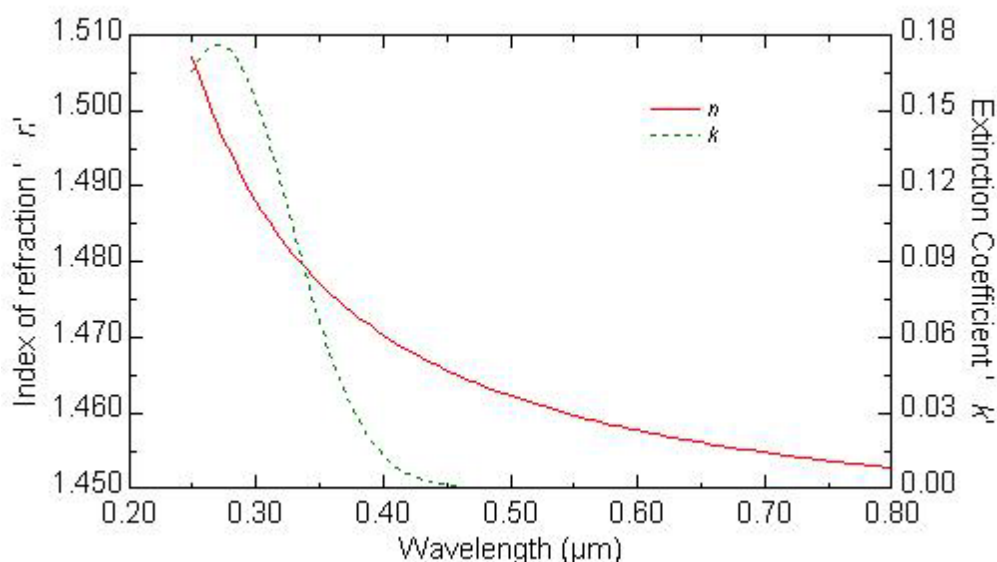


**Figure S2.** Green profile: FT-IR of **PTS[Cu(13aneN4)](CF<sub>3</sub>SO<sub>3</sub>)<sub>2</sub>**, obtained as a film on NaCl cells by evaporation of a concentrated solution of the complex in ethanol. Blue profile: ATR-FTIR spectrum of **SURF-PTS[Cu(13aneN4)]<sup>2+</sup>**, obtained by coating the SiO<sub>2</sub> native layer of a Si prism with **PTS[Cu(13aneN4)]<sup>2+</sup>** (silicon prisms were plasma-cleaned with argon and oxygen and silanized in the same conditions described in S1c). The additional peak at 1720cm<sup>-1</sup> is due to SiO<sub>2</sub> (J. Electrochem. Soc., 1985, Vol. 132, No. 12, 3034)

### S3. Ellipsometry

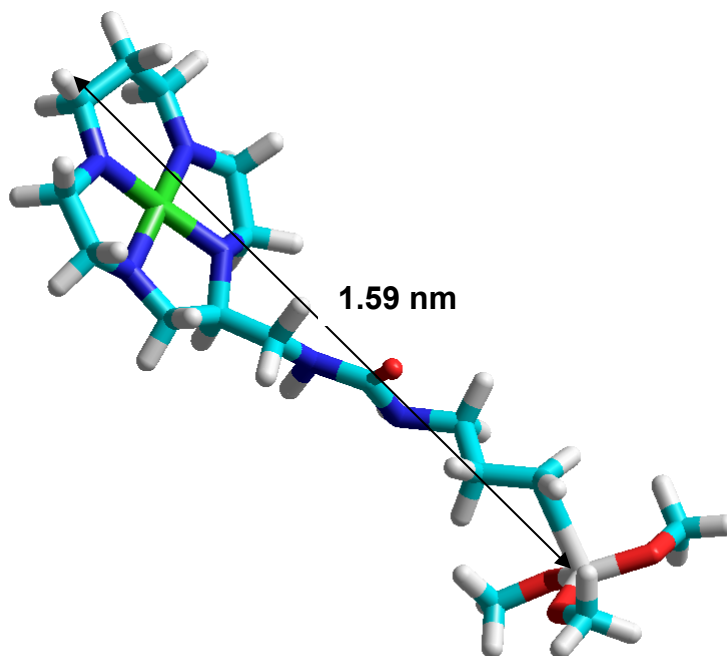
Ellipsometric spectra on **SURF-PTS[Cu(13aneN4)]<sup>2+</sup>** were measured from 250 to 900 nm with an SOPRA ES4G rotating polarizer ellipsometer, equipped with a single-photon-counting photomultiplier detector, at angles of incidence of 70 and 75 degrees. Experimental results were analyzed with the WVASE32 software from J.A. Woollam Inc. Samples were prepared on silicon crystals, with a procedure identical to that applied to the glass slides. Spectroscopic ellipsometry (SE) measurements in the 250 to 900 nm range have been performed on the same Silicon crystals used for ATR measurements. Reference measurements on the different crystals before functionalization allow to determine the native SiO<sub>2</sub> oxide thickness of 2.7 nm.

Optical functions for the **PTS[Cu(13aneN4)]<sup>2+</sup>** monolayer have been modelled with a Sellmeier-type behaviour for the refractive index  $n$  joined to gaussian-like extinction coefficient  $k$  associated to the UV absorption edge. From the best-fit to the SE spectra, three important features must be stressed. First, the refractive index of **PTS[Cu(13aneN4)]<sup>2+</sup>** (determined in the transparent part of the spectrum) is similar to the silicon dioxide one. Second, the absorption fingerprint of **PTS[Cu(13aneN4)]<sup>2+</sup>** clearly appears, centered at 270 nm.. Finally, the functionalization process produces an effective overlayer thickness ranging from 1.26-1.32 nm.

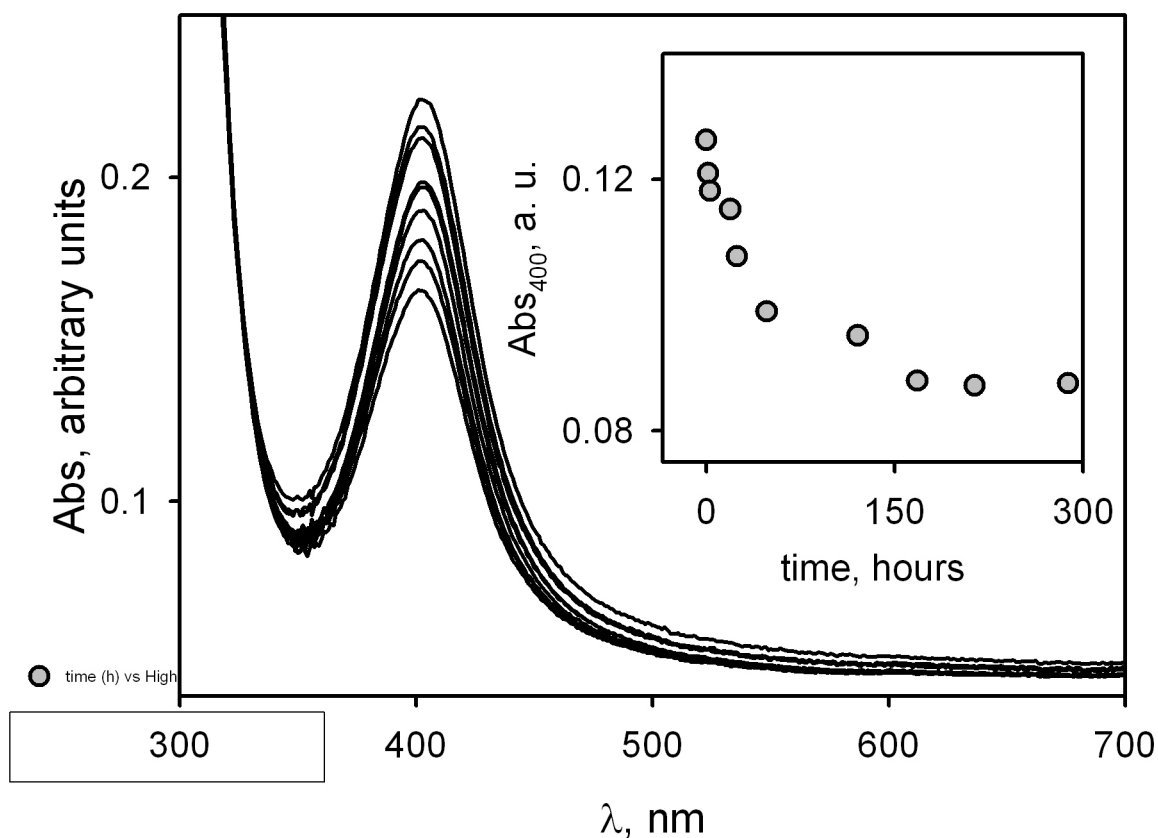


#### S4. Molecular modelling

PTS[Cu(13aneN4)]<sup>2+</sup> modelling (semi-empirical PM3), obtained with Hyperchem 6.03 software.



**S5. UV-Vis spectra on a cuvette with internal walls modified as SURF-PTS[Cu(13aneN4)]<sup>2+</sup>-NP, filled with PBS.**

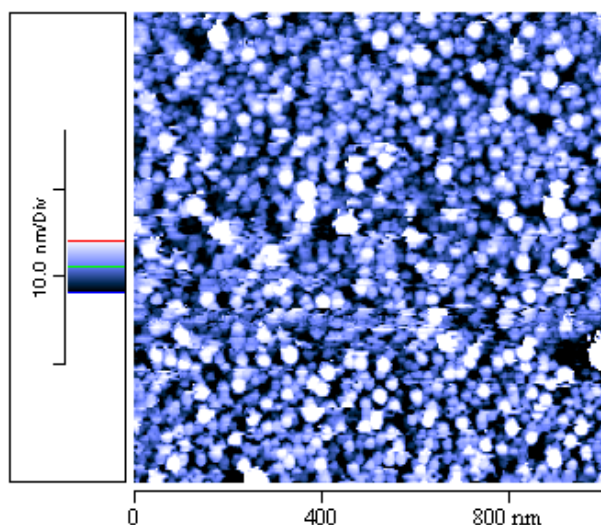


**Figure S5** - UV-Vis absorbance spectra as a function of time on a cuvette modified on the internal walls as SURF-PTS[Cu(13aneN4)]<sup>2+</sup>-NP, filled with Phosphate Buffered Saline. Inset displays the variation of absorbance at  $\lambda_{\text{max}}$  vs time (Abs values are from background-subtracted spectra)

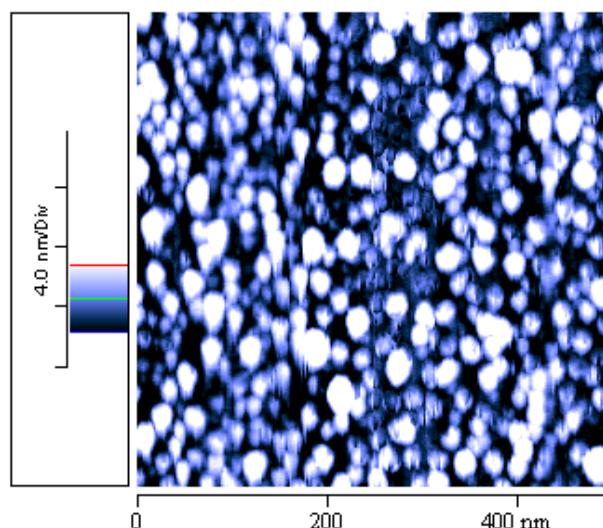


## S6. Apparent and real dimensions of Ag NP in AFM imaging.

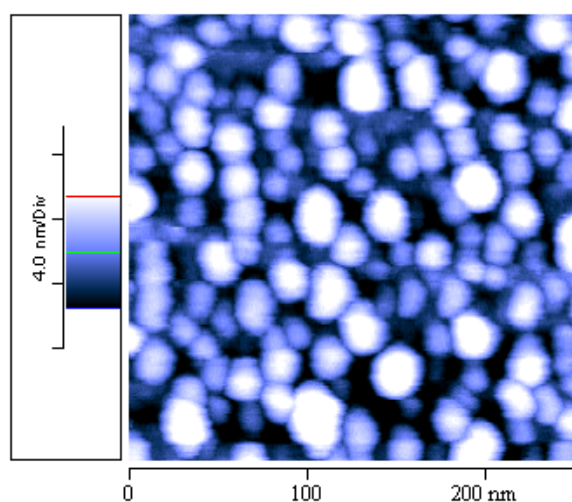
The following figures are AFM images recorded at different magnification



**Figure S7 a:** dimensions = 1  $\mu$ m  $\times$  1  $\mu$ m



**Figure S7b:** dimensions = 500 nm  $\times$  500 nm



**Figure S7c:** dimensions = 250 nm  $\times$  250 nm

It should be pointed out that the apparent dimensional increase of NP (average  $d = 7$  nm in solution,  $\sim 22$  nm in Figure 2B) is an AFM artifact. In tapping-mode, convolution of the finite tip size (10 nm curvature radius in our case) with true surface morphology results in overestimated in-plane dimensions of an object, if its width is comparable with the tip curvature (apparent  $> 3 \times$  real dimensions) [K.C. Grabar, K.R. Brown, C.D. Keating, S.J. Stranick, S.L. Tang and M.J. Natan, *Anal.Chem.*, 1997, **69**, 471]

Empirical calculations relating the tip curvature radius, the object radius and the visualized width of the imaged object [C. Bustamante, J. Vesenka, C.L. Tang, W. Rees, M. Guthold

and R. Keller, *Biochemistry*, 1992, **31**, 22] when applied to our case, would result in an observed width of  $20 \pm 4$  nm, that is compatible with what displayed in Figure 2B. It should be noticed that the measured height must instead correspond to the real one [C. Bustamante, J. Vesenska, C.L. Tang, W. Rees, M. Guthold and R. Keller, *Biochemistry*, 1992, **31**, 22]. We measured a  $6 \pm 2$  nm height, that is perfectly comparable with the real NP dimensions.