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Coordination chemistry for antibacterial materials: a monolayer of a Cu^{2+} 2,2′-bipyridine complex grafted on a glass surface†

Piersandro Pallavicini,*[a] Giacomo Dacarro,[b] Pietro Grisoli,[c] Carlo Mangano,[a] Maddalena Patrini,[b] Federica Rigoni,[d] Luigi Sangaletti[d] and Angelo Taglietti[a]

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A propyltrimethoxysilane-modified 2,2′-bipyridine ligand is synthesized and its acetonitrile solutions are used to prepare monolayers of the molecule on glass surfaces. Absorption and X-ray photoelectron spectroscopy demonstrate that the modified glass surfaces bind Cu^{2+} with a 1 : 1 ratio with respect to the 2,2′-bipyridine moieties under the chosen preparative conditions, producing materials bearing 0.016 μg cm^{-2} of copper. Although in trace amounts, the bound Cu^{2+} cations exert a significant microbicidal effect against *Escherichia coli* and *Staphylococcus aureus*.

Introduction

Nosocomial infections are a serious and worldwide spread problem.[1] They are often due to bacteria adhering to and developing into colonies on the surface of medical devices, such as catheters, artificial prostheses or subcutaneous implants. An obvious solution is to coat the surfaces of such devices with antibacterial agents.[2] However, toxicity and local inflammatory events must be considered as possible drawbacks in using thick film coatings or polymeric coatings capable of sustained drug release.[3] Moreover, many different bacteria now exhibit multidrug resistance to antibiotics. Under these considerations, it appears particularly interesting to use coatings containing (and capable of releasing) only trace quantities of non-conventional antibacterial agents, thus exerting a local, surface-confined effect.

To this aim, silver nanoparticles have been widely studied, with many papers hypothesizing that it is the time-sustained release of Ag^+ cations from their surface that exerts an antimicrobial action.[4] We have recently demonstrated that monolayers of silver nanoparticles grafted on glass exert a highly efficient surface microbicidal effect against Gram− and Gram+ bacteria,[5] that is due both to the slow release of Ag^+ cations and to a membrane-breaking mechanical action, caused by the contact of bacteria with the silver nanoparticles surface.[5b,c] In the course of these studies, we have also prepared glass surfaces bearing covalently grafted monolayers of a Cu^{2+} tetraaza macrocyclic complex[6] and a layer of the polyamino polymer PEI, capable of binding Ag nanoparticles and also capable of complexing Cu^{2+}.[7] These are examples of traditional coordination complexes obtained from monolayers of ligands covalently bound to a surface. In both cases, Cu^{2+} was present in trace quantities on the surfaces ($<3 \times 10^{-10}$ mol cm^{-2}), from which it was released only in part if the material was exposed to aqueous solutions not containing competitive ligands. However, the surfaces exerted a significant microbicidal effect when in contact with floating cells of typical Gram+ and Gram− bacteria, namely *Staphylococcus aureus* and *Escherichia coli*. The effect is due to the release of Cu^{2+}, that is a well known antimicotic and antibacterial agent.[8]

It must be stressed that our approach required grafting on glass of a ligand and its complexes by means of a covalent Si–O–Si bond, obtained by reacting the –Si–OH surface groups with molecular fragments of the $-Si(OR)_3$ type (R = Me, Et). While the literature reports a large amount of self-assembled monolayers of coordination complexes physisorbed on bulk ordered surfaces,[9] the examples of monolayers of covalently-grafted complexes are scarce. Usually, the latter are kinetically inert metal complexes such as ferrocene[10] or 2,2′-bipyridine bound to inert metal centres, like Re(I),[11] Fe(II),[12] Ru(II) and

[a]*inLAB, Dipartimento di Chimica, Università di Pavia, viale Taramelli, 12, 27100 Pavia, Italy. E-mail: piersandro.pallavicini@unipv.it; Fax: +39 0382 568544; Tel: +39 0382 987336*
[b]*Dipartimento di Fisica, Università di Pavia, via Bassi, 6, 27100 Pavia, Italy*
[c]*Dipartimento di Scienze del Farmaco, Università di Pavia, viale Taramelli 12-14, 27100 Pavia, Italy*
[d]*I-Lamp and Dipartimento di Matematica e Fisica, Università Cattolica, via dei Musei 41, 25121 Brescia, Italy*

†Electronic supplementary information (ESI) available: Distribution diagrams of the bipy-PR complexes with Cu^{2+} and Cu^+ in acetonitrile solution; AFM images of surfaces; uncorrected absorption spectra of glass|bipy and glass|(bipy)Cu^{2+} on quartz slides; full range XPS spectra of glass|bipy and glass|(bipy)Cu^{2+}. See DOI: 10.1039/c2dt32607h

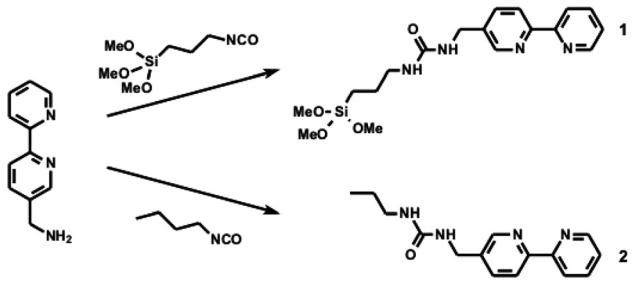

Fig. 1 Simplified synthetic scheme and formula of the molecules used in this paper: bipy-SIL (**1**) and bipy-PR (**2**).

Ir(III),[13] that have been grafted to the surfaces of silicon,[10] silver,[11a] inorganic oxides,[11b] gold[12] and carbon.[13]

In the present work, our aim is to form antibacterial surfaces bearing low quantities of the Cu^{2+} cation avoiding the use of polymeric ligands, and employing such a binding unit that allows prompt Cu^{2+} release. We thus stepped from PEI and macrocyclic ligands (forming kinetically inert complexes[14]) to a monolayer of 2,2′-bipyridine, a bidentate ligand that forms thermodynamically stable but kinetically labile complexes with Cu^{2+}. Here we present the modification of such a ligand with a terminal $-Si(OMe)_3$ group to form bipy-SIL (**1**, Fig. 1) and the grafting of the obtained molecules on conventional microscope cover-glass slides, forming glass|bipy surfaces (Fig. 2a). The modified surface is capable of complexing Cu^{2+} by simply dipping the slides in an acetonitrile solution of a Cu(II) salt, forming glass|(bipy)Cu^{2+} (Fig. 2b). The quantity of the complexed copper cation, the nature, and the stoichiometry of the complex, including copper release in water and acetonitrile, have been studied by means of UV-Vis spectrophotometry, XPS (X-ray photoelectron spectroscopy), and ICP-OES (inductively coupled plasma optical emission spectrometry). Comparison with the coordinative behaviour of the same molecules in solution has also been carried out, by means of spectrophotometric titrations, leading to the determination of the complexation constants with Cu^{2+} of the analogous, non-silylated ligand bipy-PR (**2**, Fig. 1). The microbicidal effect of the modified glasses has finally been studied, finding that these surfaces are efficient against *E. coli* and, although to a lesser extent, against *S. aureus*.

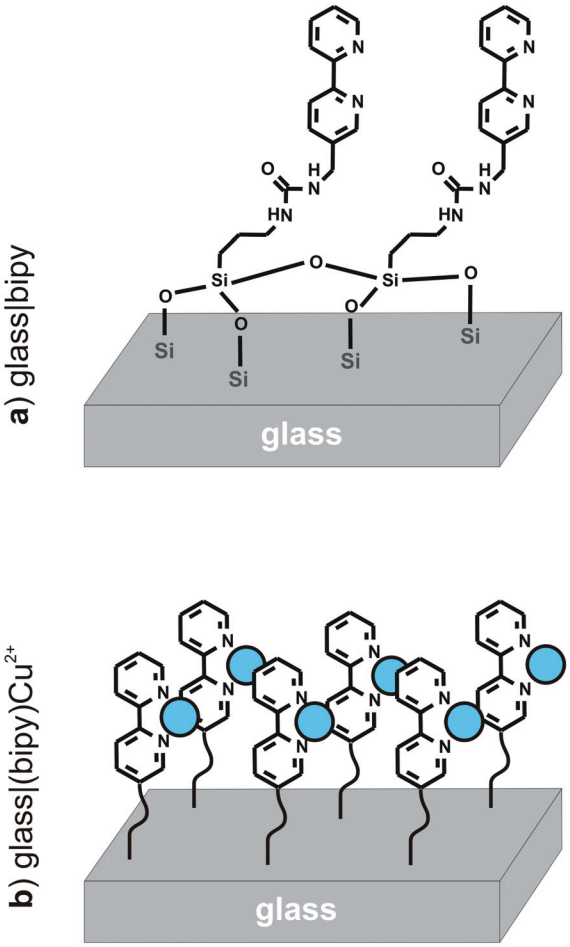

Fig. 2 Schematic representation of the glass|bipy (a) and glass|(bipy)Cu^{2+} (b) materials. In the latter case, the actual bonds and atoms connecting the bipy moiety to the glass surface have been represented with a simple curved line, for the sake of graphical clarity. The blue circles are Cu^{2+} cations.

Experimental section

Materials

Reagents and solvents were purchased from Sigma-Aldrich and used as supplied. Microscopy cover glass slides (2.1 × 2.6 cm) were purchased from Forlab (Carlo Erba). Quartz slides (Spectrosil 2000 fused silica, 2.5 × 2.5 cm, 1 mm thick) were purchased from UQGOptics Ltd. Water was deionized and then bidistilled. 5-Bromomethyl-2,2′-bipyridine was prepared according to the literature.[15]

Synthesis

5-(Aminomethyl)-2,2′-bipyridine. This molecule was prepared with a modification of a reported procedure.[16] 5-Bromomethyl-2,2′ bipyridine (1.21 g, 4.84 mmol) dissolved in $CHCl_3$ (3.5 mL) was added dropwise to a refluxing solution of hexamethylenetetramine (1.36 g, 9.7 mmol) in the same solvent (10 mL). The resulting mixture was heated to reflux (N_2 atmosphere) for an additional 4 hours. The white precipitate was filtered out, washed with two portions of cold $CHCl_3$, dried *in vacuo*, then treated with ethanol (20 mL) and 37% HCl (4 mL) and left to react at 80 °C (reflux) for 20 hours. After cooling to room temperature the solution was evaporated to dryness, the solid residue was dissolved in water (15 mL) and the resulting solution made basic (pH 12–13) by addition of concentrated aqueous NaOH and then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried on Na_2SO_4 overnight and finally evaporated to give 5-(aminomethyl)-2,2′-bipyridine (0.7 g, yield 77%) as a solid that was satisfactorily characterised by mass spectrometry (MS-ESI) and

NMR. The crude amine was used as such without further purification. ESI-MS: 186.1 $[M + H]^+$. ^{1}H-NMR (d-DMSO): δ 8.66 (s, 1H), δ 8.62 (d, 1H), δ 8.36 (d, 1H), δ 8.32 (d, 1H), δ 7.92 (m, 2H), δ 7.42 (t, 1H) bipyridine protons, 3.8 (s, 2H) methylene protons.

Bipy-SIL (1). 20.2 mg (0.11 mmol) of 5-(aminomethyl)-2,2′-bipyridine were dissolved in 10 mL anhydrous acetonitrile under N_2. 22.4 μL (0.11 mmol) of 3-(trimethoxysilyl)propyl isocyanate were added and the reaction mixture was heated to reflux. After 2 hours, the mixture was cooled to room temperature and stirred overnight. In a typical preparation the mixture was diluted with acetonitrile to a volume of 40 mL, obtaining a 0.1%w solution suitable for glass functionalization. The mass spectrum on this solution revealed a significant peak only for bipy-SIL (ESI-MS: 391.2 $[M + H]^+$), supporting the expected ~100% yield of the amine/isocyanate coupling reaction. ^{1}H-NMR (CD_3CN): δ 8.65 (d, 1H), δ 8.56 (s, 1H), δ 8.39 (d, 1H), δ 8.35 (d, 1H), δ 7.87, (t, 1H), δ 7.77 (d, 1H), δ 7.36 (t, 1H) bipyridine protons; δ 4.3 (d, 2H) bpy-CH_2 protons; δ 5.5 (s, 1H), δ 5.1 (s, 1H) urea protons; δ 4.3 (d, 2H) bpy-CH_2 protons; δ 3.5 (s, 9H) $SiOCH_3$; δ 3.1 (dt, 2H), δ 1.5 (m, 2H), δ 0.6 (t, 3H) propyl protons.

Bipy-PR (2). 51.7 mg (0.28 mmol) of 5-(aminomethyl)-2,2′-bipyridine were dissolved in 2 mL ethanol in a vial. 23.8 μL (0.28 mmol) of propyl isocyanate were added and the mixture was kept under stirring at room temperature overnight. The solvent was concentrated and a pale yellow precipitate formed. The solid was filtered and dried under vacuum. Yield: 73.0 mg (96%).

ESI-MS: 271.1 $[M + H]^+$. ^{1}H-NMR (d-CH_3CN): δ 8.65 (d, 1H), δ 8.56 (s, 1H), δ 8.40 (d, 1H), δ 8.36 (d, 1H), δ 7.87, (t, 1H), δ 7.78 (d, 1H), δ 7.37 (t, 1H) bipyridine protons; δ 4.3 (d, 2H) bpy-CH_2 protons; δ 5.5 (s, 1H), δ 5.1 (s, 1H) urea protons; δ 3.1 (dt, 2H), δ 1.4 (m, 2H), δ 0.9 (t, 3H) propyl protons.

ESI-MS: 271.1 $[M + H]^+$.

[Cu(bipy-PR)](CF$_3$SO$_3$)$_2$. 19.1 mg (0.071 mmol) of bipy-PR were dissolved in 3 mL dichloromethane. A solution of 25.7 mg (0.071 mmol) copper trifluoromethanesulfonate in a 10 mL dichloromethane–ethanol mixture 9 : 1 was slowly added under stirring. The mixture was kept under stirring overnight at room temperature. The solvent was evaporated under reduced pressure to a volume of 2 mL. A blue precipitate formed and it was isolated by filtration, washed with diethyl ether and dried under vacuum.

Glass|bipy surfaces. Glass and quartz substrates were cleaned for 30 min in freshly prepared piranha solution (3 : 1 v/v H_2SO_4 : H_2O_2 (30%). *Caution! Piranha solution is a strong oxidizing agent and should be handled with care*), washed three times with ultrapure water while being sonicated, and oven-dried. The slides were then fully immersed for 4 hours in a 0.1% (w/w) solution of bipy-SIL in acetonitrile at 40 °C, while being kept in a vertical position (surface functionalization takes place on both sides of the slides). In a typical preparation, 8 glass slides were prepared at the same time, *i.e.* reacting in the same silane solution inside an 8-place holder (a microscope glass slides staining jar). After this, the slides were washed three times with acetonitrile in a sonic bath and blow-dried with nitrogen.

Glass|(bipy)Cu^{n+} surfaces (n = 1, 2). Eight glass|bipy slides were fully immersed in 30 mL of a 10^{-3} M acetonitrile solution of the chosen copper salt, *i.e.* copper(II) trifluoromethanesulfonate or copper(I) tetra acetonitrile perchlorate, for 4 hours, while being kept in a vertical position inside a microscope glass slides staining jar. Glass slides were then washed three times with a small volume of acetonitrile (2 mL) and blow dried with nitrogen.

Spectrophotometric titrations

Solutions of bipy-PR in acetonitrile (concentrations in the 2×10^{-5}–5×10^{-5} M range) were titrated by adding small volumes of Cu^{2+} (as $Cu(CF_3SO_3)_2$) or Cu^+ (as $[Cu(CH_3CN)_4]ClO_4$) solutions (concentrations in the 2×10^{-3} M–10^{-2} M range). After each addition, a portion of the solution was transferred into a quartz cuvette and its absorption spectrum recorded. After the spectrum was recorded, the solution was transferred back to the bulk solution. Formation constants were calculated with the Hyperquad® software package.[17]

Antibacterial activity tests

The antibacterial activity of functionalized cover glasses 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 re-suspended in Dulbecco's PBS (phosphate buffer saline) and optical density (OD) was adjusted to 0.2, corresponding approximately to 1×10^8 Colony Forming Units (CFU) ml^{-1} at 650 nm wavelength. 10 μL of bacterial suspension was deposited on a standard glass slide (76 × 26 mm), then the microbial suspension was covered with a functionalized cover glass slide (21 × 26 mm), 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 the control sample. After the times of contact, 9 mL of PBS were introduced in each Falcon test-tube under 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

$$ME = \log NC - \log NE$$

(NC being the number of CFU mL^{-1} developed on the unmodified control glasses, and NE being the number of CFU mL^{-1} counted after exposure to modified glasses). The results

expressed as ME represent the average of 3 equivalent determinations.

Quantitative determination of copper on glass and Cu release

The total Cu content on glasses loaded with metal complex monolayers (glass|(bipy)Cu^{n+} surfaces) was determined by quantitatively dissolving the copper grafted on a single slide (21 × 26 mm) by dipping it in 3 mL ultrapure 4% HNO_3 in a beaker, and keeping it overnight at RT on a Heidolph Promax 1020 reciprocating platform shaker. In Cu release experiments in water the functionalized glasses (glass|(bipy)Cu^{n+}) were dipped in 3.0 mL of bidistilled water and shaken. After the proper release time (5 h or 24 h) the glass was removed, 0.18 mL of concentrated HNO_3 were added (final concentration 4%v) and the Cu content in solution was in all cases determined by inductively coupled plasma (ICP) atomic emission spectroscopy (ICP-OES).

In acetonitrile release experiments glasses were dipped in 3 mL of solvent and shaken. After the proper release time (5 h or 24 h) the glass was removed and acetonitrile was evaporated with a nitrogen stream and replaced with 3 mL ultrapure water 4%v in HNO_3.

Instrumentation and instrumental methods

Absorbance spectra of solutions were recorded with a Varian Cary 50 spectrophotometer in the 200–1100 nm range. Spectra of functionalized glasses or quartzes were obtained by placing the slides on the Varian Cary 100 spectrophotometer equipped with a dedicated Varian solid sample holder.

NMR spectra (400 MHz) were taken on a Bruker AMX400 instrument.

Static contact angle determinations were made with a KSV CAM200 instrument, with the water sessile drop method.

AFM images were carried out with an Auto Probe CP Research Thermomicroscopes scanning system in tapping mode with a Au coated Si probe with a theoretical spring constant k = 2.5–10 N m^{-1} (NSG01 probes from NT-MDT). Images were analyzed using Image Processing 2.1 provided by Thermomicroscopes.

Inductively coupled plasma optical emission spectroscopy (ICP-OES) was carried out with an ICPOES OPTIMA 3000 Perkin Elmer instrument.

The XPS data have been collected with the Al Kα line ($h\nu$ = 1486.6 eV) of a non-monochromatized dual-anode PsP X-ray source. The analyzer for XPS was a SCIENTA R3000, operating in the transmission mode, which maximizes the transmittance and works with a 30 degrees acceptance angle. The system was operated with a base pressure of 2×10^{-10} mbar. For the stoichiometry analysis, we referred to the survey spectra of each sample, collected with a pass energy of 100 eV. For each element the integrated peak intensity was normalized with respect to the specific cross section and analyzer transmission. While the monolayers on glass were examined by directly fixing the glass slide on the sample holder in the XPS chamber, for the powder samples a hollow cavity on the sample holder was prepared, to host the powders, gently pressed to fill the cavity itself.

Results and discussion

Coordination in solution

The coordination properties of the 2,2′-bipyridine ligand, bearing an –H_2C–NH–(CO)–NH–R moiety in the 5-position, have been studied on ligand bipy-PR (**2**, Fig. 1). Bipy-PR was prepared with the aim of obtaining the same binding unit as bipy-SIL (**1**), but avoiding the –$Si(OMe)_3$ group, as it does not participate in coordination and may hydrolyze and polymerize in solution *e.g.* due to adventitious water absorbed from air. Complexation studies were carried out with Cu^{2+} and Cu^{+} cations in acetonitrile by means of spectrophotometric titrations, from which the formation constants were calculated with the Hyperquad package.[17] $Cu(CF_3SO_3)_2$ and $[Cu(CH_3CN)_4]ClO_4$ were used as the Cu^{2+} and Cu^{+} salts, respectively, due both to the negligible coordination ability of the anions and to their anhydrous nature. Coordination of Cu^{+} was studied for the sake of comparison and as a support to the hypotheses on the surface-confined coordination complexes made in the section dedicated to X-ray photoelectron spectroscopy (XPS, *vide infra*).

In the UV region ligand **2** has a strong band due to the bipyridine moiety, with λ_{max} = 286 nm (ε 18 600 M^{-1} cm^{-1}). Cu^{2+} addition makes this band decrease, with the increase of a new band at 318 nm (ε 18 580 M^{-1} cm^{-1}) and two isosbestic points at 267 and 296 nm, Fig. 3a. The expected weak d–d absorption typical of Cu(II) complexes with the 2,2′-bipyridine ligand[18] is also found at ~690 nm, ε < 200 M^{-1} cm^{-1} (at the low concentrations used for the spectrophotometric titrations it is strongly affected by background noise, and the extinction coefficient cannot be exactly calculated). In the case of Cu^{+} addition, Fig. 3b, the band at 286 nm decreases and shifts to 290 nm (ε 14 490 M^{-1} cm^{-1}), with an isosbestic point at 267 nm and with the rise of a large charge transfer band at ~360 nm (ε 1660 M^{-1} cm^{-1}, metal to ligand), typical of Cu(I) interacting with the 2,2′-bipyridine framework. In both cases, by fitting the data of absorbance *vs.* mol of added metal, two complex species were found, *i.e.* one ligand/one metal and two ligand/one metal complexes. The relative formation equilibria and constants follow.

$$\text{bipy-PR} + Cu^{2+} = [(\text{bipy-PR})Cu]^{2+} \quad \log K_{11,Cu(II)} = 7.9\ (0.1)$$

$$2\text{bipy-PR} + Cu^{2+} = [(\text{bipy-PR})_2Cu]^{2+} \quad \log K_{12,Cu(II)} = 14.7\ (0.1)$$

and

$$\text{bipy-PR} + Cu^{+} = [(\text{bipy-PR})Cu]^{+} \quad \log K_{11,Cu(I)} = 3.28\ (0.02)$$

$$2\text{bipy-PR} + Cu^{+} = [(\text{bipy-PR})_2Cu]^{+} \quad \log K_{12,Cu(I)} = 7.33\ (0.02)$$

Uncertainties affecting the log K values are reported in parentheses. The log K values are in agreement with what was reported in the literature for the interaction of Cu^{2+} and Cu^{+} with 2,2′-bipyridine: *e.g.* for plain 2,2′-bipyridine in water log K_{11} = 8.70 and log K_{12} = 14.4 for Cu(II)[19] and for a 2,2′-bipyridine ligand bearing substituents in the 6,6′ and 4,4′

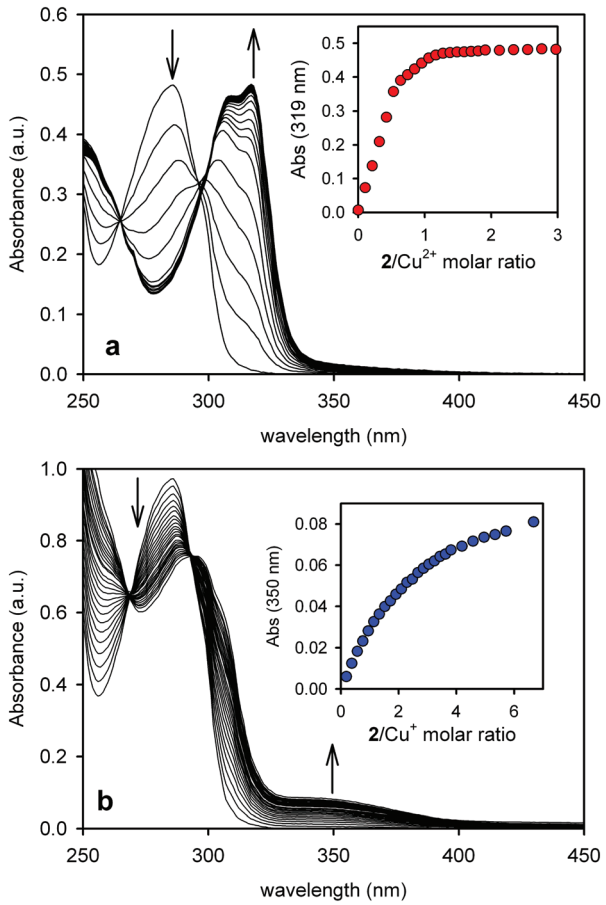

Fig. 3 Spectra obtained in the spectrophotometric titration of ligand **2** with addition of Cu^{2+} (a) and Cu^{+} (b). Ligand **2** concentration was 2.5×10^{-5} M and 5.1×10^{-5} M, respectively (solvent = acetonitrile). Insets display the variation of absorbance on the maximum of increasing bands, reported as a function of the ligand/metal molar ratios.

positions, in acetonitrile, log K_{11} = 4.6 and log K_{12} = 8.2 for Cu (I).[20] Distribution diagrams can be drawn from the log K values, expressing the % of each complex species as a function of the concentration of the metal cation at a chosen ligand concentration (see ESI Fig. S1†).

Surface functionalization: glass|bipy

To prepare glass|bipy slides, bipy-SIL has been grafted on glass or quartz surfaces with a well-developed synthetic approach, *i.e.* the reaction of the Si–OH surface groups with $(RO)_3$-Si-spacer-X molecules, forming Si–O–Si linking moieties, such as those sketched in Fig. 2a. This approach is commonly used to obtain monolayers on glass or other SiO_2 surfaces of molecules bearing a remote X function such as –SH[21] or $–NH_2$,[22] or bulkier groups, *e.g.*, PEI[7] or metal complexes of macrocyclic ligands.[6,23] These cannot be considered proper "self-assembled monolayers", as they are obtained by the formation of covalent bonds and not of softer, reversible bonds (*e.g.* $–S^-\cdots Au$[24]) that allow the maximization of intermolecular interactions and the attainment of ordered, quasi-crystalline

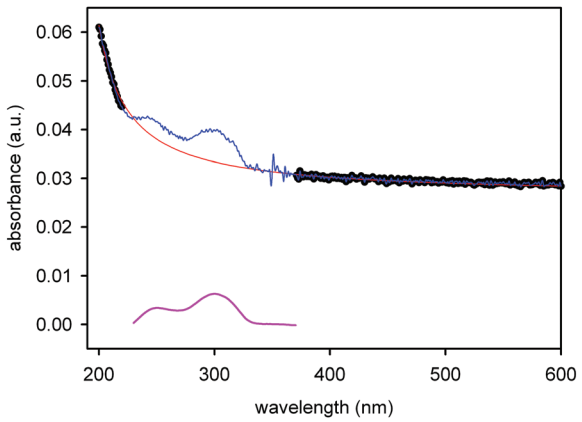

Fig. 4 Blue spectrum: original spectrum of a glass|bipy surface (on a quartz slide). Black circles are the experimental points (range $200 < \lambda < 220$ and $370 < \lambda < 900$) fitted to calculate the background (red solid line). In the lowest part of the figure, the violet solid line is the spectrum of the glass|bipy surface after background subtraction.

monolayers. However, it has been thoroughly demonstrated that $(RO)_3$-Si-[spacer]-X molecules form authentic *mono*layers on glass or glass-like surfaces (quartz, native SiO_2 on Si, ITO), with a density of molecules cm^{-2} typically in the 10^{13}–10^{14} range.[21d,22d,25] In this work, the number of bipy units per cm^2 (n_s) in glass|bipy has been indagated by absorption spectroscopy on quartz slides, measuring the absorbance on the intense UV band of the 2,2′-bipyridine moiety, falling at 286 nm in solution (ε = 18 600 M^{-1} cm^{-1}). This band can be seen in the absorption spectra of coated quartz glasses directly measured on a standard UV-Vis spectrophotometer, see Fig. 4, blue line. As has already been noted by us[21d] and other authors,[22d] untreated slides or quartz slides with transparent molecular monolayers display background absorptions varying from sample to sample, so it is problematic to define a unique sample for background subtraction. According to an established procedure,[21d,22d] here we simulated the background for each spectrum as a smooth function of the wavelength, $f(\lambda) = [c1/(c2 + \lambda)] + c3$, by fitting experimental points in the range $200 < \lambda < 220$ and $370 < \lambda < 900$, *i.e.* out of the absorption peak of interest (black circles in Fig. 4). The simulated background (red solid line, Fig. 4) was subtracted from each spectrum, obtaining corrected spectra as is illustrated in Fig. 4 for a representative case (violet solid line). The average λ_{max} is 295(±4) nm, obtained on 6 experiments.

From the background-subtracted spectra, the surface concentration of bipy units (n_s) is calculated using relation (1):

$$n_s\ (cm^{-2}) = 6 \times 10^{20}\ A/2\varepsilon \qquad (1)$$

where A is the absorbance at the wavelength of the maximum of absorption on glass and ε is the molar extinction coefficient of the same molecule at its maximum absorption in solution[21d,26] (the factor 2 is used as our samples are covered on both sides; ε = 18 600 M^{-1} cm^{-1}). An average value of n_s = 1.04 (±0.04) × 10^{14} cm^{-2} has been found on 5 samples. It must be stressed that after background subtraction, the spectra of

glass|bipy display the absorption maximum of the bipy moiety shifted with respect to solution (298 instead of 286 nm). Moreover, in eqn (1) we use the extinction coefficient of the maximum of absorption of bipy-PR in solution and this surely introduces an error. Accordingly, n_s calculation with eqn (1) should be considered just the best possible optical evaluation of the real value. However, calculation of complexed Cu^{2+} (*vide infra*) with a reliable and independent method confirms that 1.04×10^{14} molecules cm^{-2} is a good approximation of the real value.

AFM images (ESI, Fig. S2†) show that the obtained glass|bipy surfaces are flat, with a 0.2535 nm roughness well compatible with a monolayer of bipy-SIL molecules lying disorderly on the surface.

X-ray photoelectron spectroscopy (XPS, see ESI Fig. S4†) was used to further ascertain the presence of the bipy molecule. An N1s peak is found at binding energy 399.8 eV, which can be confidently assigned to the N atom functions in the neutral 2,2′-bipyridine moiety, on the basis of closely comparable literature BE values for differently substituted pyridines.[27] The C : N atom ratio was investigated by comparing the areas under C1s and N1s peaks, finding a C/N ratio value of 4.31, slightly higher than the 3.75 (15 : 4) value expected from the structure of the molecular monolayer sketched in Fig. 2A. This is most probably due to carbon contamination during the preparation of the samples, which were manipulated (although as carefully as possible) with lattice gloves.

Coordination on surface: glass|(bipy)Cu^{2+}

Eight glass|bipy slides (2.1 × 2.6 cm) were exposed to 30 mL of a 10^{-3} M $Cu(CF_3SO_3)_2$ solution in acetonitrile for 4 hours. Approximating the number per cm^2 of 2,2′-bipyridine units to 1×10^{14}, the total number of bipy units reacting with Cu^{2+} is 8.7×10^{15} (1.4×10^{-8} mol). Being in contact with a 30 mL solution, a formal bipy concentration of 4.8×10^{-7} M is calculated. Using the distribution diagrams obtained for bipy-PR with Cu^{2+} in solution (ESI Fig. S1†), at [bipy-PR] = 4.8×10^{-7} M the 1 : 1 complex is >99.98% when $[Cu^{2+}] = 10^{-3}$ M. Although in this calculation we are using the formation constants obtained for a free ligand in solution, on its basis it can be reasonably argued that by exposing the surface-grafted ligands to such a large excess of Cu^{2+} the 1 : 1 complex is formed, as sketched in Fig. 2b. Preparation of glass|(bipy)Cu^{2+} on quartz slides allows us to indagate the obtained surface-grafted complex by absorption spectrophotometry. After background subtraction, the spectra display the expected red-shift of the LMCT band, as is found for bipy-PR in solution, see Fig. 6, green line (see also ESI, Fig. S3† for uncorrected spectra). We obtained an averaged λ_{max} = 312(±3) nm on 6 experiments. From relation (1), using the ε value at the maximum of absorbance of the $[Cu(bipy\text{-}PR)]^{2+}$ complex in acetonitrile solution, a surface density $n_s = 1.08(\pm0.07) \times 10^{14}$ cm^{-2} is calculated. The value is reassuringly very similar to what was found for the uncomplexed ligand in glass|bipy slides. However, a more affordable value of the Cu^{2+} surface density has to be calculated with an independent experiment, *i.e.* by ligand protonation and cation release in a 4% HNO_3 solution, followed by Cu^{2+} determination by ICP-OES analysis. From 8 samples we calculated $n_s = 1.5(\pm0.1) \times 10^{14}$ cm^{-2} (corresponding to 0.016 μg cm^{-2} of copper). The value is slightly higher than what was determined with the spectrophotometric method. This has to be attributed to an underestimation error of the latter, as errors of this type and magnitude are common in the determination of surface density by absorption spectra, as we have already pointed out.[26]

To emphasize the correspondence between the coordination chemistry of the chosen ligand in solution and on the surface, we also prepared glass|(bipy)Cu^{2+} slides using more concentrated and more diluted Cu^{2+} acetonitrile solutions (5×10^{-3} M and 5×10^{-4} M $Cu(CF_3SO_3)_2$, respectively). On the basis of the distribution diagrams drawn from formation constants determined in solution (ESI, Fig. S1c†) the 1 : 1 complex is formed in all cases. ICP-OES analysis confirmed this, showing no difference in the copper surface concentration with respect to the standard preparation. Finally, we checked the release of the Cu^{2+} cation in water. A glass|(bipy)Cu^{2+} slide was dipped in 3.0 mL water for 24 hours. We found that ~80% Cu^{2+} is released. On the other hand, a release experiment in 3.0 mL acetonitrile disclosed <20% Cu^{2+} release. This low value is compatible with the lower affinity of Cu^{2+} for CH_3CN with respect to H_2O.

Attempts to prepare glass slides bearing the Cu^{+} complex were also carried out, under the same experimental conditions as for Cu^{2+}. However, in agreement with the much lower formation constants found in solution with bipy-PR, the quantity of Cu^{+} coordinated on glass was very low, *i.e.* <0.005 μg cm^{-2} (with the procedure used to determine copper by its release in HNO_3, the actual amount falls under the limit of quantification of ICP-OES, that is 20 μg L^{-1} for this element).

XPS spectra on glass|(bipy)Cu^{2+}

XPS spectra were carried out also on glass|(bipy)Cu^{2+} surfaces. For each element the integrated peak intensity was normalized with respect to the specific cross section and analyzer transmission. The Cu : N atom ratio was indagated by comparing the areas under the Cu **2p** and N 1s peaks, finding an N/Cu value of 4.1 in agreement with the formation of 1 : 1 copper–bipy complexes. It must be stressed that the analysis of the peak shape and of the BE in the 920–980 eV range shows that Cu is in oxidation state (I),[28] even if samples are prepared with Cu^{2+}. We believe that reduction of Cu(II) to Cu(I) is a consequence of the conditions of the XPS experiments, namely X-ray irradiation during data collection and high vacuum exposure, which may lead to the removal of adsorbed and coordinated solvent, leaving Cu^{2+} with an incomplete coordination sphere. Moreover, the preparation of glass|(bipy)Cu^{2+} samples by complexation with $Cu(CF_3SO_3)_2$ implies the presence of triflate as the counter anion. The absence of the F and S peaks in the XPS spectrum indicates that decomposition of $CF_3SO_3^-$ has taken place, reasonably with electron release to Cu^{2+}. To further prove this, we analyzed bulk powder samples of

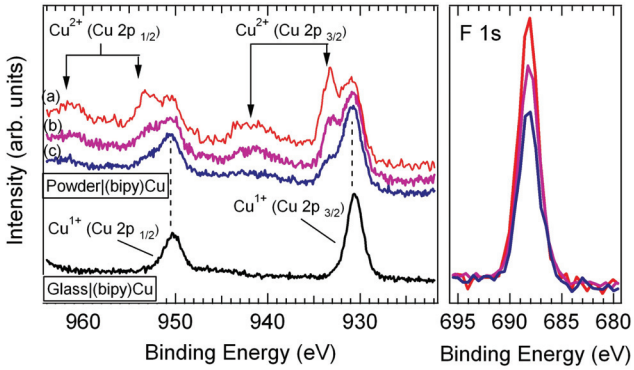

Fig. 5 Left panel: Cu 2p XPS spectra of the $[Cu(bipy)](CF_3SO_3)_2$ powders (coloured spectra a, b, c) and of glass|(bipy)Cu^{2+} (black spectrum). The vertical arrows identify the Cu^{2+} spectral weight. Right panel: F 1s XPS spectra of the $[Cu(bipy)](CF_3SO_3)_2$ powder measured after 2 (top), 4 (middle), and 6 (bottom) hours irradiation.

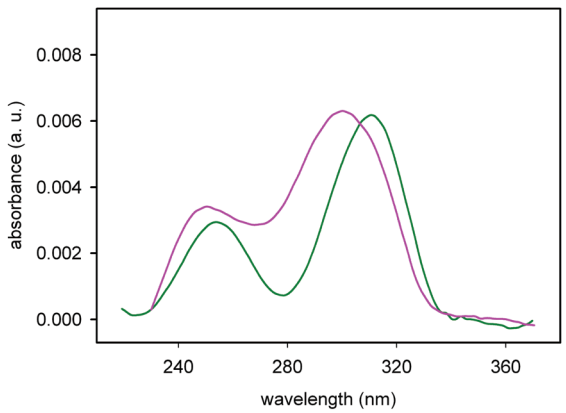

Fig. 6 The green line is the background-subtracted spectrum of the copper complex, *i.e.* of glass|(bipy)Cu^{2+}. The violet line is the profile of the parent glass|bipy surface (same as in Fig. 4, bottom). Both have been obtained on functionalized quartz slides.

$[Cu(bipy)](CF_3SO_3)_2$ and the copper(II) reduction was clearly detected also in this case, as shown in Fig. 5.

In the figure, the Cu 2p XPS spectra collected from the powder samples of the Cu(II) complex are compared with the spectrum collected from the glass|(bipy)Cu^{2+} monolayer. For the powder sample, three spectra (labelled as a, b, and c) are shown, corresponding to 2, 4, and 6 hours X-ray irradiation, respectively. The spectra have been normalized to the intensity of the Cu $2p_{3/2}$ peak of Cu^+ at 931 eV. As the X-ray exposure time increases, the spectral features assigned to Cu^{2+} (vertical arrows) decrease. Likewise, the F 1s intensity decreases (right panel), confirming the hypothesis of the $CF_3SO_3^-$ decomposition. In the case of glass|(bipy)Cu^{2+}, as just a monolayer of bipy–Cu^{2+} complex is involved, copper reduction reasonably occurs well before the completion of the XPS scan, and no signal from Cu^{2+} was ever detected in any of the measured glass|(bipy)Cu^{2+} samples. On the other hand, for the powder samples, the much larger thickness allowed us to probe both the topmost layers, where Cu(II) reduction mainly occurred, and the underlying layers where the reduction process was hindered. Moreover, it has to be remembered that in the complexation of bipy-PR with Cu^+ in acetonitrile solution (Fig. 3b) the ligand band at 286 nm decreases in intensity and shifts to 290 nm, with a large LMCT band arising in the 330–400 nm range. In contrast, spectra recorded on glass|(bipy)Cu^{2+} samples (Fig. 6 green line) show a band at 312 nm of the same intensity as the band of the uncomplexed ligand and no absorption over 330 nm, as is found in solution for complexation of bipy-PR with Cu^{2+} (Fig. 3a).

Table 1 Microbicidal effect (ME)

	ME			
	S. aureus		*E. coli*	
	5 h	24 h	5 h	24 h
Glass|bipy[a]	0.12	0.15	0.00	0.07
Glass|(bipy)Cu^{2+} [a]	0.68	1.73	1.71	2.77
Glass|(13aneN4)Cu^{2+} [b]	0.98	1.65	1.02	2.46
Glass|(PEI)Cu^{2+} [c]	1.34	1.60	0.65	2.24

[a] This paper. [b] Ref. 6. [c] Ref. 7.

Antibacterial activity

Antibacterial activity tests were run on glass|bipy and on glass|(bipy)Cu^{2+} slides. We have used a test developed in our laboratories[5a] that measures the ME (microbicidal effect)[29] by simulating real-life conditions of use, *i.e.* it allows the evaluation of the bactericidal effect in a thin liquid film in contact with the surface (see the Experimental section). Gram-positive *Staphylococcus aureus* and Gram negative *Escherichia coli* were used as the bacterial strains. They have been chosen because they are commonly considered representative for the evaluation of antibacterial activity of drugs.[30] Moreover, these are the same strains that we used in previous papers to check the antibacterial activity of a macrocyclic Cu^{2+} complex and of Cu^{2+} complexed by PEI on a glass surface, so that a direct comparison is possible.[6,7] ME effects are summarized in Table 1.

While the glass|bipy slides have no significant ME, excluding a microbicidal action played by the molecular monolayer, the slides bearing the Cu^{2+} complex are remarkably bactericidal. The effect is higher on Gram– *E. coli* coherently with a recurring difference between the two strains, that has been attributed to the fact that Gram+ bacteria (like *S. aureus*) have a thicker and more rigid peptidoglycane membrane, disfavouring the internalization of bactericidal cations.[5b] Table 1 also reports the ME values found for the other ligand/Cu^{2+} monolayers on glass that we have previously studied. Glass slides coated with the Cu^{2+} complex of the tetraaza macrocycle 13aneN4[6] and glass slides coated with the Cu^{2+} complex of a PEI layer[7] display a slightly lower ME effect. The copper content is ~16 ng cm^{-2} for glass|(bipy)Cu^{2+}, ~14 ng cm^{-2} for glass|(13aneN4)Cu^{2+} and ~19 ng cm^{-2} for glass|(PEI)Cu^{2+}. The surface concentration is slightly different although in the same range for the three cases, but there is no correlation

between the total quantity of copper and ME. However, if we consider the release of Cu^{2+} when the slides are dipped in water, we observe that only ~8% and ~5% Cu^{2+} is released in 24 h from glass|(13aneN4)Cu^{2+} and glass|(PEI)Cu^{2+}, respectively. In the same time glass|(bipy)Cu^{2+} releases 80% of its load, and this corresponds to a higher observed ME. A further comparison can be made with ME values determined with the same test on glass slides bearing monolayers of citrate-stabilized spherical silver nanoparticles, grafted on MPTS monolayers. We have found ME values of 5.54 and 5.90 on *S. aureus* and *E. coli*, respectively (same strains used in the present paper).[5a] The ME of Ag NP is thus ~3 orders of magnitude higher with respect to what was found with our Cu^{2+} glasses. However, much higher quantities of noble metal are grafted on the surfaces, Ag being 35.7 μg cm^{-2} with respect to the 0.016 μg cm^{-2} of Cu in the present paper. Moreover, the risk of nanoparticles detachment and dispersion in the organism must be carefully taken into account, while it is obviously avoided in the present case.

Finally, also glass|bipy slides treated with Cu^{+} were examined with the antibacterial activity test. The quantity of loaded Cu(I) is negligible, as described in the "Coordination on surface" section. Accordingly, null activity (ME < 0.1) was found against *E. coli* and *S. aureus*.

Conclusions

In this paper we have prepared SiO_2 bulk surfaces (glass, quartz) coated with a covalently grafted monolayer of a modified 2,2′-bipyridine ligand. The same ligand, bearing no grafting function, has also been indagated in solution for its coordinative interactions with Cu^{2+} and Cu^{+}. The obtained formation constants and the observed changes in the absorption spectrum have been successfully used to predict and interpret the coordinative behaviour of the ligand when grafted on glass. We were thus able to use the ligand-coated SiO_2 materials to bind Cu^{2+} from acetonitrile solutions, that in a large range of concentrations yield surfaces bearing monolayers with the predicted 1 : 1 Cu^{2+}–bipy stoichiometry. The kinetically labile complexes release Cu^{2+} and exert a significant microbicidal effect *vs*. strains representative of Gram+ and Gram− bacteria. Significantly, this is obtained with trace amounts of copper, as its surface concentration is <20 ng cm^{-2}. We believe that such a small quantity rules out any toxicity risk for patients and also avoids the possibility of local inflammatory reactions. This candidates our Cu^{2+}-modified surfaces as coatings for internalized medical devices, capable of fast local Cu^{2+} release, imparting an antibacterial protection to the device during the implantation process.

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