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Surface confined heteroleptic copper(II)–polypyridyl complexes for photonuclease activity†‡

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Heteroleptic copper(II)–polypyridyl complexes with extended π-conjugated, aromatic terminal units were immobilized on glass/Si substrates to intercalate DNA and cleave it upon photoexposure. Photonuclease activity is shown to be high, well reproducible and non-destructible towards the assembled complexes.

Cu(II)–polypyridyl complexes are frequently used for photoinduced DNA cleavage due to suitable redox, spectral, and structural properties.[1] The respective experiments are, however, usually performed in solution, whereas immobilization of these complexes on solid substrates for nuclease activity is largely unexplored.[2] Analogous studies have only been reported for fullerene[3] or lomefloxacin[4] based assemblies, whereas no supported systems containing metal–Cu(II) complexes were addressed in context of photonuclease activity. Moreover, the available DNA binding and photocleavage results are mostly based on optical (UV/Vis) experiments and lack most conclusive gel electrophoresis evidence for formation of DNA fragments–oligonucleotides.[3,4] In this context, we describe here two novel heteroleptic Cu(II)–polypyridyl complexes confined to glass/Si supports *via* robust siloxane based attachment chemistry.[5] These assemblies are utilized for DNA binding and subsequent photo-induced cleavage, with the process monitored optically *via* UV/Vis spectroscopy and gel electrophoresis.

The supported complexes **1** and **2** are shown in Fig. 1. They have a conduit imidazole spine and are terminated with 9-anthryl and 1-pyrenyl aromatic groups, respectively, which are basically

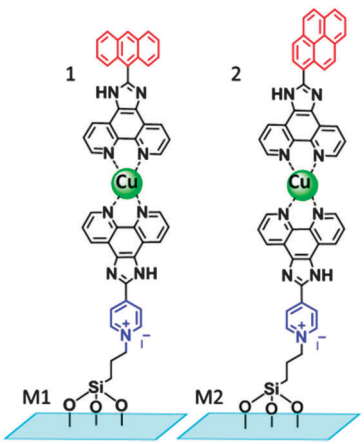

Fig. 1 Schematic of complexes **1** and **2** immobilized on Si/glass substrates *via* siloxane based attachment chemistry and forming monolayers **M1** and **M2**.

designed for efficient intercalation into a DNA strand. DNA binding studies in solution divulged that **1** and **2** are avid DNA binders as suggested by the high intrinsic binding constant, K_b = $\sim 10^5$ M^{-1} and the apparent binding constant, K_{app} = $\sim 10^6$ M^{-1} for both **1** and **2** (see Fig. S1–S6 in the ESI‡ for details). This binding affinity is comparable with those reported for other Cu(II)–polypyridyl complexes.[1,6] Gel electrophoresis studies implied formation of more than 90% nicked circles from supercoiled DNA for as little as 10 μM of the complexes upon UV-A light exposure for ~0.5 h (365 nm, 12 W). This indicates the involvement of metal-to-ligand charge transfer (MLCT) bands (350 nm to 400 nm for both **1** and **2**), associated with the Cu(II)–polypyridyl core, in the photoexcitation process. Photonuclease activity of **1** and **2** reduced drastically under an N_2 atmosphere, which suggested an important role of the reactive oxygen species (see Fig. S7 in the ESI‡ for details). Mechanistic analysis corroborated participation of singlet oxygen ($^1O_2$) in the cleavage reactions as NaN_3 was found to inhibit the DNA cleavage activity,[7] while the presence of D_2O increased the amount of cleaved DNA.[8] Note that involvement of hydroxyl radicals ($OH^\bullet$) or peroxide anion (O_2^{2-}) was neglected

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‡ Electronic supplementary information (ESI) available: Materials and methods, experimental details, UV-Vis data, DNA binding activity of **1** and **2**, the gel electrophoresis diagram for DNA cleavage in solution, XPS and NEXAFS data, and the FT-IR spectrum of ct-DNA in the buffer solution. See DOI: 10.1039/c4cc05063k

as DMSO–KI could not quench DNA cleavage (see Fig. S8 in the ESI‡ for the probable mechanism).[9]

Robust siloxane-based monolayers of **1** and **2** (abbreviated as **M1** and **M2**, respectively; see Fig. 1) were fabricated on glass/Si supports following the established procedures.[10] In brief, at first, a silane (3-iodo-*n*-propyl-trimethoxysilane) coupling layer (CL) was formed on the freshly cleaned substrate. The CL covered substrates were then immersed in a dry acetonitrile–toluene (3 : 7, v/v) solution of the complex **1** or **2** (0.5 mM) under an N_2 atmosphere and kept at 85 °C for 52 h. Subsequently, the functionalized substrates were rinsed thoroughly and sonicated gently in acetonitrile and isopropanol to remove any physisorbed material. UV/Vis measurements on **M1** and **M2** showed that the complexes adhered strongly to the substrate surface as they could be removed neither by the "scotch tape decohesion" test nor by a stream of critical carbon dioxide (snow-jet).[11] The assembly of **1** and **2** on the substrate was also evidenced by X-ray photoelectron spectroscopy (XPS) and near-edge X-ray absorption fine structure (NEXAFS) spectroscopy. The respective spectra exhibited emissions and absorption resonances characteristic of the building blocks and functional units of **1** and **2** (see Fig. S9 and S10 in the ESI‡ for details).

UV/Vis spectra of the monolayers showed deviations from the respective spectra of **1** and **2** in CH_3CN. For **M1** and **M2**, MLCT bands appeared at λ_{max} = 372 and 379 nm, respectively, exhibiting a red shift of ~5 and ~12 nm with respect to the spectra of **1** and **2** in CH_3CN (see Fig. 3 and ESI‡). This could be a consequence of quaternization of pendent pyridyl N-atoms[10c] and/or related to cofacial orientation of the molecules within the purview of exciton theory.[12a] Other factors can be involved as well.[12b,c] For both **M1** and **M2**, the average surface density was estimated at ~2 molecules per 100 Å^2 of the substrate area, which is reasonable for such species.[13] Fourier-transform infrared (FT-IR) spectra of **M1** and **M2** (Fig. 2) revealed the characteristic N–H out-of-plane stretch at ~820 cm^{-1}, the out-of-plane aromatic C–H stretch at ~900 cm^{-1}, the $Si_{surface}$–O–Si_{CL} stretch at ~1110 cm^{-1}, aromatic C–H wagging at ~1450 cm^{-1}, as well as Si_{CL}–CH_2-stretches at ~1300 and 970 cm^{-1}.[14] Along with the UV/Vis, XPS, and NEXAFS data, characteristic stretches in the FT-IR spectra are indicative of the covalent attachment and efficient assembly of **1** and **2** on the substrates.

Unlike DNA binding in solution, where different possibilities of aggregation of the complexes with DNA exist, only the terminal aromatic groups of **1** and **2** are exposed to DNA for the supported complexes. To prove the activity of these functional groups towards DNA, **M1** and **M2** were immersed in 50 mM tris-HCl–NaCl buffer solution of ct-DNA (pH = 7.2) for 4 h at room temperature inside an N_2 filled glovebox (O_2 < 0.5 ppm). The monolayers were then washed several times with fresh buffer solution to remove free/physisorbed ct-DNA and subsequently dried at room temperature under an N_2 stream. UV-Vis spectra of **M1** and **M2** showed a bathochromic shift of ~6 and 12 nm, respectively, upon DNA binding, along with a hypochromism of ~13 and ~28% with respect to their MLCT bands (see Fig. 3). This suggests the intercalation as the preferred binding mode of DNA to supported **1** and **2**, similar to the analogous interaction in solution (see Fig. S4, ESI‡). As a negative control, **M1** and **M2** were kept in DNA-free tris-HCl–NaCl buffer solution. After prolonged storage, no changes in the UV-Vis spectra were observed.

The intercalation process was additionally monitored by FT-IR spectroscopy which has already been successfully used for detection of DNA immobilized on surfaces like diamond.[15] At first, the FT-IR spectrum of ct-DNA in tris-HCl–NaCl buffer solution was recorded after preliminary baseline adjustments (see Fig. S11, ESI‡).[16] The characteristic bands of the DNA moieties were observed at ~1100 cm^{-1}, assigned to the symmetric PO_2^- stretching mode and C–O backbone stretching vibration of the ribose–phosphate bond, and at ~1210 cm^{-1}, assigned to asymmetric PO_2^- stretching. Bands in the range of 1300–1800 cm^{-1} are ascribed to the ring stretching modes in the base residues, *viz.* guanine (G), cytosine (C), adenine (A) and thymine (T); they are comprised of C=O, C=C, and C=N stretching vibrations in the base planes (1746 cm^{-1} for G, 1691 cm^{-1} for T, 1655 cm^{-1} for A).[13]

In the second step, FT-IR spectra of the monolayer–DNA assemblies were measured and compared to the spectra of **M1** and **M2**. The latter spectra served as a baseline, so that only the stretches corresponding to DNA could be expected in the spectra

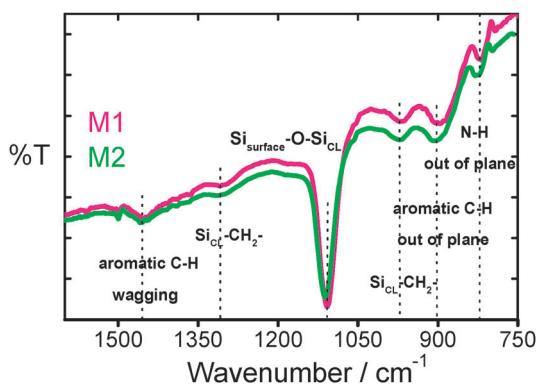

Fig. 2 FT-IR spectra of **M1** (red curve) and **M2** (green curve) assembled on silicon supports. A suitable baseline was recorded using a bare, freshly cleaned silicon substrate. Vertical dotted lines indicate the positions of the characteristic IR stretches. The respective assignments are given.

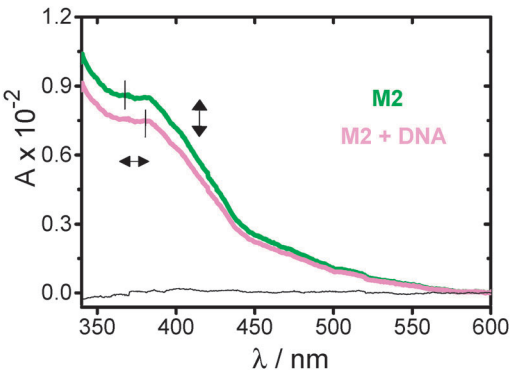

Fig. 3 Representative UV/Vis spectra of **M2** (green curve) and **M2** + DNA (magenta curve) on glass supports. Suitable baselines were recorded using freshly cleaned bare glass substrates (thin black curves). Black vertical lines indicate absorption maxima while double headed arrows emphasize the extent of bathochromic/hypochromic shifts upon DNA binding.

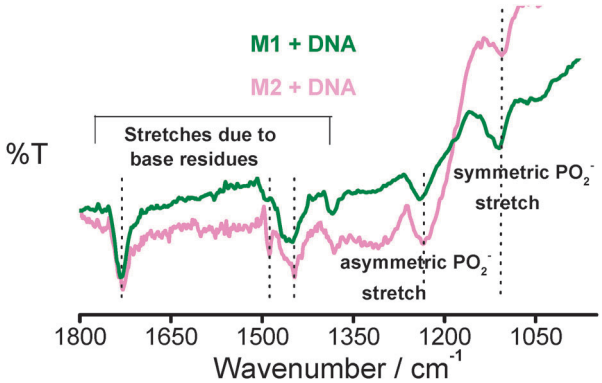

Fig. 4 FT-IR spectra of **M1** + DNA (olive curve) and **M2** + DNA (magenta curve) assemblies on the silicon substrate recorded after preliminary baseline adjustments. The spectra exhibit IR stretches associated with the immobilized DNA only. Significant variations are observed as compared to the spectra of ct-DNA in solution. Vertical dotted lines indicate the positions of characteristic IR stretches. The respective assignments are given.

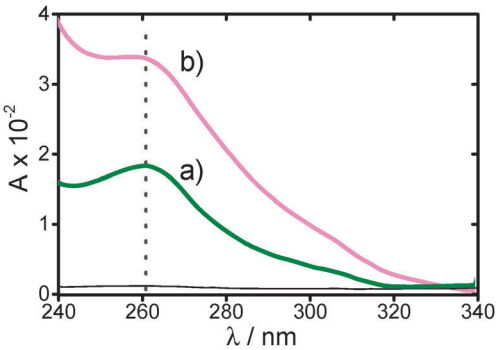

Fig. 5 UV/Vis spectra of tris-HCl–NaCl buffer solution recorded after irradiating either the **M1** + DNA (a; olive curve) or the **M2** + DNA (b; magenta curve) assembly immersed in buffer for 0.5 h. The horizontal black line represents UV/Vis spectra of the buffer solution when the samples were kept in the dark. The vertical dotted line indicates the peak position of the spectra.

of the monolayer–DNA assemblies (Fig. 4). In these spectra, shifts of ~10–12 cm^{-1} for the 1746 cm^{-1} peak, ~4–10 cm^{-1} for the 1691 cm^{-1} peak, and ~0–2 cm^{-1} for the 1602 cm^{-1} peak were observed in the ring stretching modes of the base residues as compared to the spectrum of ct-DNA in solution, which is indicative of monolayer intercalation mainly into the G–C base pairs. In addition, the shift of the asymmetric as well as symmetric PO_2^- modes (~1222 and ~1064 cm^{-1}, respectively) by ~2–4 cm^{-1} indicates electrostatic interactions between the complexes on the Si substrate and the phosphate backbone of the ct-DNA. Thus, FT-IR spectroscopy data provide clear and plausible evidence for the DNA attachment to the monolayers, predominantly *via* the intercalation binding mode.

In further experiments, a set of 8 identical glass slides (3.0 cm × 1.0 cm × 1.25 mm), grafted with either **M1** or **M2**, was dipped in ct-DNA solution in 50 mM tris-HCl–NaCl buffer (pH = 7.2) for ~4 h using a Teflon slide holder. Afterwards, the slides were thoroughly washed with buffer solution to remove free/physisorbed ct-DNA and subsequently dried under an N_2 stream. The slides were then kept in fresh buffer solution and irradiated with UV-A light (365 nm, 12 W) for either 0.5 or 1 h. Finally, the container was shaken thoroughly and 2 mL of the solution were extracted and tested by UV-Vis spectroscopy (200–400 nm range) in transmission mode. As a negative control, similar experiments were performed under dark conditions and the UV/Vis spectra of the extracted solution were recorded thereafter. In the case of exposure of the monolayer–DNA assembly to UV-A light, UV-Vis spectra of the solution showed a strong signal with a maximum at ~260 nm indicating the presence of DNA fragments (see Fig. 5). In contrast, analogous UV-Vis spectra of the samples kept in the dark did not show any band characteristic of DNA (see Fig. 5). Additional control experiments, in which **M1** and **M2** immersed into DNA-free buffer solution were subjected to UV-A light for 0.5 h, showed no effect of the irradiation on the monolayers themselves, with no evidence for their damage or fragmentation. The above results suggest that the DNA chains bound to the monolayers were cleaved into shorter fragments such as oligonucleotides under UV-A light irradiation, with subsequent diffusion into the bulk phase. Note that the amount of DNA fragmented is higher for **M2** as compared to **M1**, because the absorbance at λ_{max} = ~260 nm in the **M2** case is almost twice as large as that for **M1** (see Fig. 5). This can be tentatively explained by the superior π-conjugation, aromaticity, and planarity of the pyrenyl moiety of **M2**, which eventually makes this system a better monolayer-based photonuclease. In this context, the most plausible role of the assembled **1** and **2** appears to be that of a photosensitizer, where the photosensitized Cu(II)–polypyridyl cores of **1** and **2** transfer energy to activate oxygen available in the buffer solution from its stable triplet state ($^3O_2$, $^3\Sigma_g^-$) to a highly reactive singlet state ($^1O_2$, $^1\Delta_g$) in the near vicinity of DNA leading to its rupture.[1a,17]

In addition to the UV/Vis studies, agarose gel electrophoresis experiments were conducted using the same buffer extraction procedure, following the exposure of the monolayer–DNA assemblies to UV-A light (see Fig. 6). 20 μL solution of the buffer containing DNA fragments and 2 μL of the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol, were used for running the 0.8% agarose gel in 1× tris-acetate–EDTA buffer at a potential of 20 V for 15 minutes. As seen in the gel pictures, DNA fragmentation has indeed occurred as supercoiled DNA (SC) has moved only slightly while the fragmented DNA has moved much farther. It is also evident that **M2** cleaves DNA with higher efficiency as the intensity of DNA fragments for **M2** is higher than that for **M1**. These findings agree with the results of the UV/Vis measurements (*vide supra*). Moreover, as can be expected, an increase of the irradiation time to 1 h resulted in an increase of the intensity of the bands associated with the DNA fragments, as shown in Fig. 6.

We demonstrated that the Cu(II)–polypyridyl complexes **1** and **2** immobilized on silicon/glass supports in a monolayer fashion (**M1** and **M2**) are able to intercalate into DNA and cleave it efficiently upon exposure to UV-A light without the need of any additional reducing or oxidizing chemicals. No photo-degradation of the monolayer assemblies was observed. The DNA cleavage activity was monitored by UV/Vis spectroscopy and gel-electrophoresis experiments. The terminal aromatic moieties in **M1** and **M2**

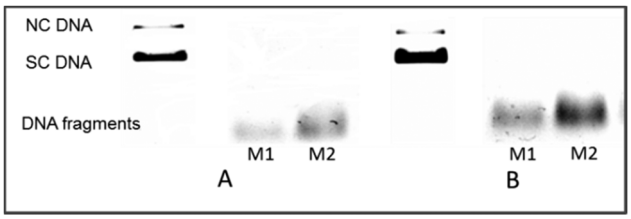

Fig. 6 Gel electrophoresis diagram of photonuclease activity of **M1** and **M2**. NC and SC refer to nicked circular (form II) and supercoiled (form I) forms of ct-DNA, respectively. 'A' and 'B' correspond to a UV-A irradiation time of 0.5 and 1 h, respectively.

are assumed to play a key role in DNA binding and cleavage activities, with the pyrenyl group (**M2**) performing better than 9-anthryl (**M1**). The fabricated monolayers as well as other analogous systems represent efficient model systems for controlled DNA cleavage. In addition, they can be potentially utilized for fibre-optics based DNA cleavage *in vivo*.

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