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# Copper(II)-Thymine Coordination Polymer Nanoribbons as Potential Oligonucleotide Nanocarriers

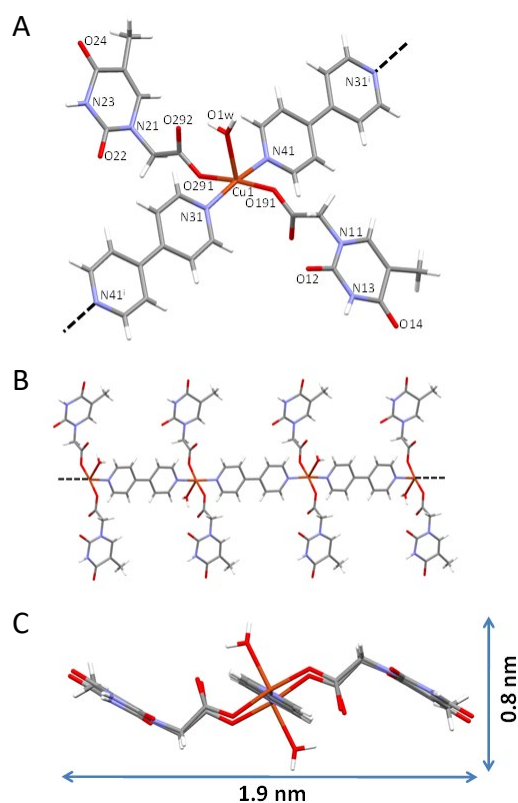
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Dedicated to Prof. Bernhard Lippert for his developments and teaching in the field of metal-nucleobase compounds

**Abstract:** The direct reaction between copper nitrate, thymine-1-acetic acid and 4,4'-bipyridine in water leads to the formation of a blue colloid comprising homogeneous over micron length crystalline nanoribbons (width ~ 150-185 nm and diameter ~ 15-60 nm) of a coordination polymer that displays a thymine-based structure freely available for supramolecular interactions. These nanostructures show significant selective interaction with single-stranded oligonucleotides based on adenine. Remarkably, they present low cell toxicity in three cell lines, despite the Cu(II) content, and can be used as nanocarriers of oligonucleotides. These results suggest the potential of this type of nanostructures towards several biological applications.

Coordination polymers (CPs) comprise well-ordered structures in which organic ligands and metal ions are connected by coordination bonds.<sup>[1]</sup> The architectures and properties of CPs are mainly determined by the features of these building blocks.<sup>[2]</sup> Indeed physico-chemical properties of these modular materials can be pre-designed by the suitable selection of the metal entities and the organic connectors. Eventually, CPs give rise to multifunctional materials combining several physical properties, such as optical with magnetic or optical with electrical properties.<sup>[1]</sup> On the other hand, non-toxic porous CPs have shown potential applications in medicine for drug delivery or as contrast agents.<sup>[3]</sup> The use of biological ligands, such as peptides and nucleobases for CPs preparation have gained increasing attention,<sup>[4]</sup> but the number of examples is still very limited. Recently, some examples have been reported in a new class of

highly tailorable nanomaterials from CPs, named nanoscale coordination polymers (NCPs).<sup>[5]</sup> However, it is somehow surprisingly that despite the high scientific interest<sup>[4a]</sup> and potential applications that the scaling-down of these CP materials can bring, they are still little developed.<sup>[5d, 6]</sup>



**Figure 1.** Lateral (a) and frontal (b) views of a fragment of  $[\text{Cu}(\text{TAcO})_2(4,4'\text{-bipy})(\text{H}_2\text{O})]_n \cdot 2\text{H}_2\text{O}$  (**1**). (c) Fragment of the crystal structure showing lateral dimensions for a single chain.

Therefore our aim was the preparation of CPs based on nucleobases as a new source of biocompatible nanomaterials able to incorporate some of the DNA abilities, such as molecular recognition.<sup>[7]</sup> Thus, we have focused our efforts on the synthesis of one-dimensional coordination polymer (1D-CP) nanostructures based on copper(II) and thymine 1-acetic acid (TAcOH). The selected building blocks have allowed us to prepare crystals, of formula  $[\text{Cu}(\text{TAcO})_2(4,4'\text{-bipy})(\text{H}_2\text{O})]_n \cdot 2\text{H}_2\text{O}$  (**1**) (4,4'-bipy = 4,4'-bipyridine), showing electric and magnetic properties. Interestingly, the fine tuning of the synthetic

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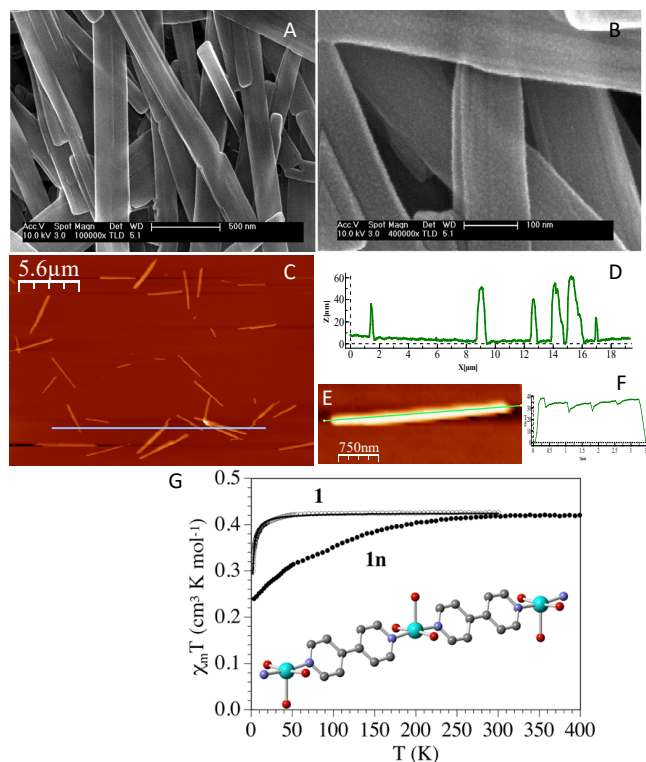
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conditions has enabled the preparation of crystalline ultra-large nanoribbons of  $[\text{Cu}(\text{TAcO})_2(4,4'\text{-bipy})(\text{H}_2\text{O})]_n \cdot 2\text{H}_2\text{O}$  (**1n**), as a stable colloid in water, with the same structure than that found in the bulk material. Moreover, **1n** has shown molecular recognition capability towards oligonucleotides based on adenine, low cytotoxicity, and cell translocation.

The preparation of blue crystals of **1** suitable for X-ray diffraction requires the hydrothermal reaction between Cu(II) nitrate, thymine-1-acetic acid and 4,4'-bipyridine in water (pH 4.8) at 140 °C. Similar results are obtained when the reaction is carried out at 25 °C and pH 6.1, but the formation of a microcrystalline material of **1** is isolated instead of relatively large single crystals. The crystal structure of **1** consists of linear chains in which the metal centers are bridged by 4,4'-bipyridine ligands that impose a Cu...Cu distance of 11.124 Å (Figure 1 and Figure S1). The coordination sphere of the copper(II) metal center adopts an axially elongated square pyramid geometry (Tables S1 and S2). The basal plane being occupied by the two carboxylate oxygen atoms from two thymine-1-acetate ligands in the *trans* arrangement. The apical position is occupied by a water molecule with a significantly longer coordination bond distance than those placed in the basal plane (2.31 Å vs 1.97-2.01 Å). The metal center is displaced 0.140 Å from the basal plane towards the apical position. The 4,4'-bipyridine bridging ligand is twisted 61.20 ° with respect to the mean basal plane. The shape of the coordination polymer in the solid state is best described as a linear ribbon of dimensions  $1.9 \times 0.8$  nm (Figure 1).

Interestingly, a fast precipitation preparation carried out under ambient conditions and at a higher concentration of reactants to that used for the bulk synthesis (Supporting Information), produces a blue water stable colloid **1n** in high yield. The isolation and morphological characterization of **1n** shows a nanoribbon-like structure highly homogeneous in dimensions as confirmed by the scanning electron microscopy (FESEM) (Figure S2). Thus, Figures 2A and 2B clearly show that **1n** consists of nanoribbons with *ca.* 150-185 nm width with over tens of microns length. The thickness of the nanoribbons corresponding to the polymer **1n** has been studied by AFM (Figure 2C-F). The AFM images obtained upon deposition by drop casting of a diluted suspension of the initial colloid on  $\text{SiO}_2$  (Supporting Information) confirmed the width of the nanoribbons observed by FESEM, and a minimum height of *ca.* 15 nm (Figure 2D). Interestingly a very smooth roughness is measured on the top of these nanoribbons (Figures 2E and 2F). The observed typical height suggests a range from *ca.* 15 to 60 nm. Therefore this is consistent with the isolation of ribbons comprise of *ca.* 12-50 single chains as deduced from the X-ray diffraction data (Figure 1C).

The X-ray powder diffraction, spectroscopic and analytical data confirms the composition and structure of **1n** which is in agreement with those observed for **1** in bulk (Figure S3). We assessed the stability of the nanoribbons **1n**, by monitoring morphological changes under different concentrations and pH over time (Figures S4-S7). Remarkably, the nanoribbons were very stable, and no significant variations in neither shape nor spectroscopic features were detected.



**Figure 2.** (A,B) FESEM images of **1n** with different magnifications. (C) Large AFM topographic image of nanoribbons from **1n** on  $\text{SiO}_2$  obtained upon deposition by drop casting of a diluted suspension of the initial colloid. (D) Height profile along the blue line in (C). (E) A zoom of an isolated nanoribbon of (C) with its height profile along the green line (F). (G) Thermal variation of  $\chi_m T$  per Cu(II) ion for a crystalline sample of **1** and of the same compound prepared as nano-ribbons (**1n**). The solid line is the best fit to the model (see text). The inset shows the different orientation of the apical Cu-O bonds of neighboring Cu(II) ions along the chain.

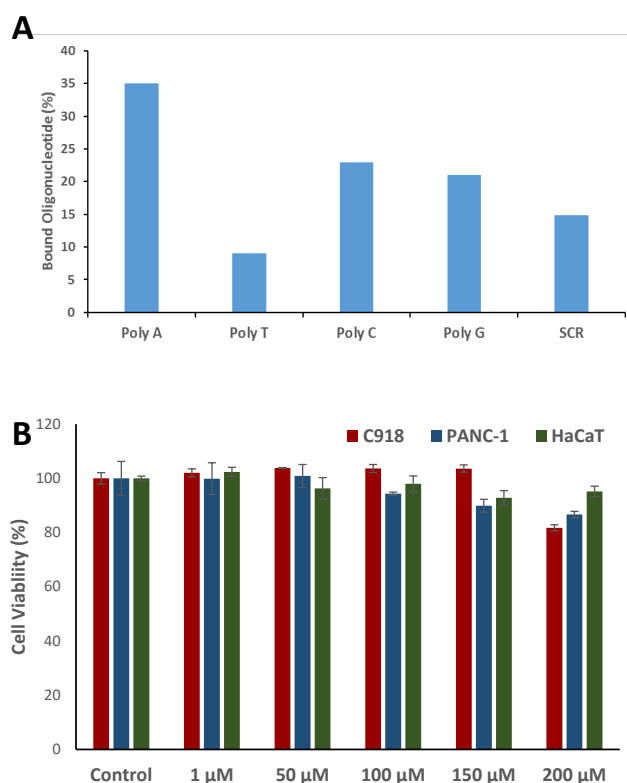
Thus, the length of the nanoribbons became shorter when the concentration of the reactants increased going from  $3 \mu\text{m}$  at 0.05 M to  $1 \mu\text{m}$  at 0.2 M (Figure S4). This can be rationalized assuming that an increase in the concentration produces a larger number of nucleation centers.<sup>[6a]</sup> Additionally, variations in the pH of the synthesis from 6.1 to 7.4 (values within the intracellular pH range) produce only a slight increase in the width going from 185 to 235 nm (Figure S5). Finally, we have observed that upon standing the colloid in contact with solution, at 25 °C, from freshly prepared colloid (time 0) to an aged colloid of 1 month, the width only increases from 185 to 290 nm (Figure S6) indicating a slow aggregation of the nanoribbons with long periods of time.

CPs based on the combination of copper with aromatic N donor ligands have shown interesting electronic properties.<sup>[8]</sup> The electrical characterization of single crystals of **1** shows an electrical conductivity value of  $10^{-6} \text{ Scm}^{-1}$  at 300 K, similar to that found in analogous 1D-CPs with bridging ligands based on N-containing aromatic molecules.<sup>[8b]</sup> To evaluate the size effect in the magnetic properties, we have characterized **1** as both crystalline bulk material and as microcrystalline nano-ribbons (**1n**). The  $\chi_m T$  product for a crystalline sample of **1** shows at 300 K a value of *ca.*  $0.40 \text{ cm}^3 \text{K mol}^{-1}$ , the expected one for a  $S = \frac{1}{2} \text{ Cu(II)}$  ion with  $g \approx 2$ . When the temperature is decreased,  $\chi_m T$  remains

constant down to *ca.* 10 K and below this temperature it shows a sharp decrease to reach a value of *ca.* 0.30 cm<sup>3</sup>Kmol<sup>-1</sup> at 2 K (Figure 2G). This behavior indicates that **1** is essentially paramagnetic with a weak antiferromagnetic Cu-Cu coupling. Given the chain structure of **1**, we have used the regular  $S = \frac{1}{2}$  antiferromagnetic chain model of Hatfield *et al.*<sup>[9]</sup> to fit the magnetic properties of **1**. This model reproduces very satisfactorily the magnetic properties of **1** in the whole temperature range with  $g = 2.130$  and  $J = -0.87$  cm<sup>-1</sup>. **1n** also shows a  $\chi_m T$  value close to 0.40 cm<sup>3</sup> K mol<sup>-1</sup>, but on cooling  $\chi_m T$  decreases from *ca.* 250 K and reaches a value of *ca.* 0.24 cm<sup>3</sup> K mol<sup>-1</sup> at 2 K (Figure 2G). This behavior could not be fit to a chain model even with inter-chain interactions, suggesting that the magnetic structures of **1** and **1n** are different. We attribute this different behavior to the large shape-anisotropy of the nano-ribbons that gives rise to a preferential orientation of these nano-ribbons (with the ribbon or chain axis always located perpendicular to the magnetic field). A closer look at the structure of **1** shows that the Cu(II) ions are not located exactly on the chain axis, giving rise to *zig-zag* magnetic chains. Furthermore, the apical oxygen atoms of the square pyramids of neighboring Cu(II) centers present a different orientation along the chain (inset in Figure 2G) that results in a different orientation of the  $g$  tensors of neighboring Cu(II) ions, giving rise to an anisotropic magnetic chain, already observed in other Cu(II) regular chains.<sup>[10]</sup>

**Table 1.** Oligonucleotides containing different base sequences used in this work.

Name	Sequence
PolyA	5'-dAdAdAdAdAdAdAdAdAdA-3'
PolyC	5'-dCdCdCdCdCdCdCdCdCdC-3'
PolyG	5'-dGdGdGdGdGdGdGdGdG-3'
PolyT	5'-dTdTdTdTdTdTdTdTdTdT-3'
SCR	5'-dTdCdGdTdAdAdGdCdAdT-3'



**Figure 3.** (A) The affinity of the different sequences represented as % of the bound material. The PolyA sequence has the highest affinity for the polymer due to the efficient interaction between thymines and adenines. (B) Cell viability in C918, PANC-1 and HaCaT cell lines at different concentrations of **1n**.

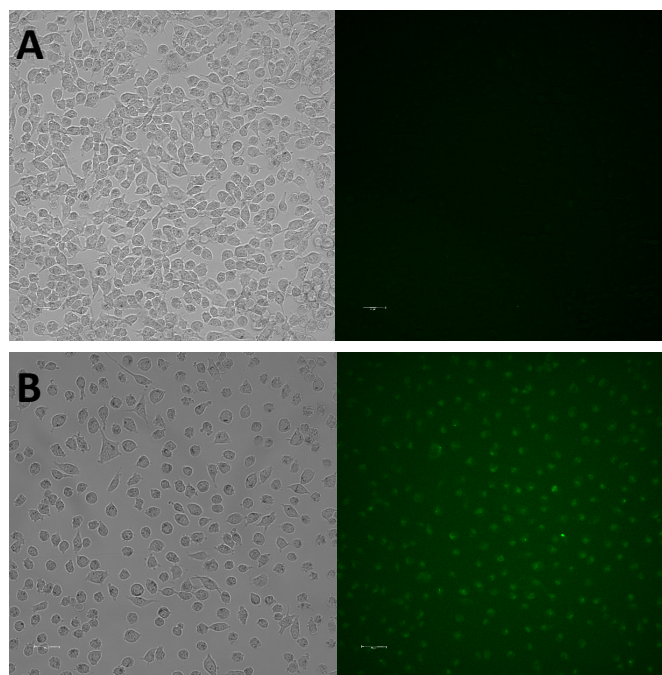
The presence of a free thymine residue decorating the [Cu(4,4'-bipy)-]<sub>n</sub> chain (Scheme S2) prompted us to evaluate the interaction of the nanoribbons **1n** with oligonucleotides. Due to the selective interaction between complementary sequences we expected some selectivity towards adenine-rich sequences. In this sense, and as a proof-of-concept, we have studied the interaction of **1n** with oligonucleotides containing different base sequences (Table 1).

The oligonucleotides were incubated with **1n** and the unbound oligonucleotides removed by dialysis. We found that **1n** binds better PolyA (35 %) in comparison to PolyT (9 %), PolyC (23 %), PolyG (21 %) and a scramble oligonucleotide sequence SCR (15 %) (Figure 3A). We have also observed that the interaction between **1n** and PolyC or PolyG is higher than that with PolyT. This might be due to the high affinity of cytosine residues for Cu(II)<sup>[11]</sup> and the formation of stable wobble base pairs between guanines and thymines.<sup>[12]</sup>

These results support the potential use of CPs bearing modified nucleobases as platforms for the interaction with oligonucleotides *via* conventional Watson-Crick base pairs and/or more complex interactions such as non-canonical base pairs and metal-nucleobase interactions. To test the biocompatibility of **1n** we have evaluated its toxicity in different cell lines using the resazurin assay.<sup>[13]</sup> We have studied the effect of **1n** in uveal melanoma (C918), pancreatic cancer (Panc-1) and non-tumoral



(HaCaT) cell lines. We have observed some toxicity when the concentration of **1n** was as high as 200  $\mu$ M. Concentrations below this threshold did not affect significantly the cells (Figure 3B).



**Figure 4.** (A) C918 cells incubated with PolyA labeled with fluorescein. (B) C918 cells incubated with **1n** treated with PolyA labeled with fluorescein.

Additionally, we have evaluated the capability of **1n** as a cell vehicle of nucleic acids using as model a derivative of PolyA labeled with fluorescein. The cells were incubated with the fluorescent oligonucleotide with and without **1n** for 3 hours and then washed with phosphate buffered saline. The fluorescent images reveal that the naked oligonucleotide is unable to interact with the cells (Figure 4A). However, the cells treated with **1n** incubated with the same fluorescent oligonucleotide showed a clear fluorescent signal in all the cells (Figure 4B). When the experiment was done using a PolyT derivative we did not observe any fluorescence (Figure S8). Motivated by these results we decided to evaluate the use of a PolyA sequence as an anchoring motif to ease the translocation of different sequences using our system. We used DNA duplexes with and without a PolyA tail and we observed a significant increase of fluorescence when the PolyA was present (Figures S9 and S10). This result suggests that other DNA structures can be delivered using a PolyA as an anchoring motif to promote the interaction between the DNA structure and **1n**.

In summary, the facile preparation of the nanoribbons **1n** by a one-pot reaction in water under ambient conditions and the control of the shape and dimensions of the nanostructures renders this CP as an interesting nanomaterial. Moreover, the presence of nucleobases in its structure has proven useful for the selective recognition of oligonucleotides bearing multiple adenine residues, which can be exploited in the preparation of more complex nanostructures or biological applications. Furthermore,

its low cytotoxicity and the capability to transport oligonucleotides to cells represent and added value. Despite these results represent just a proof-of-concept, they suggest a high potential for CP nanostructures bearing nucleobases as ligands for biological uses.

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**Keywords:** Coordination Polymers • Nanoscale Coordination Polymers • Nanoribbon • Oligonucleotides • Nanocarriers

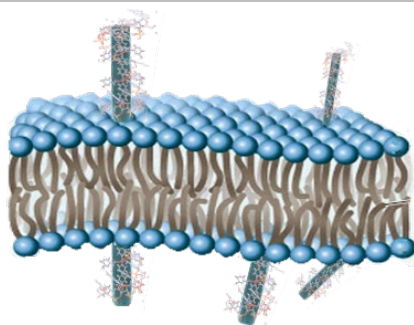
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## Entry for the Table of Contents

### COMMUNICATION

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We report on the one-pot preparation of crystalline copper-thymine coordination polymer nanoribbons. These nanostructures show preferential binding to oligonucleotides containing adenine, low cytotoxicity and can be used as carriers of oligonucleotides in cells.



Verónica García, Romina Lorca, Ana Latorre, Khaled Hassanein, Carlos J. Gómez-García, Oscar Castillo, Álvaro Somoza,\* Félix Zamora\* and Pilar Amo-Ochoa\*

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**Copper(II)-Thymine Coordination Polymer Nanoribbons as Potential Oligonucleotide Nanocarriers**