A novel class of metal-directed supramolecular DNA-delivery systems†

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The bis-complexes $[Cu(L_{dt})_2](OTf)_2$ (1) and $[Cu(L_{ot})_2](OTf)_2$ (2), where L_{dt} = 1-dodecyl-1,4,7-triazacyclononane, L_{ot} = 1-octadecyl-1,4,7-triazacyclononane and OTf = trifluoromethanesulfonate, formed a novel class of metallo-liposomes in water that transfect pEGFP-N1 plasmids into HEK 293-T cells at 38% and 4% efficiency, respectively.

Synthetic materials that function as DNA-delivery systems for mammalian cells are expected to play critical roles in gene therapy.[1] They can potentially overcome the fundamental problems that viral vectors present with respect to costs and health risks, as well as avoiding the immune response that the viral vectors elicit, which diminish their effectiveness.[2] On the other hand, there needs to be improvements over synthetic delivery agents that are based on quaternary ammonium surfactants because of problems with toxicity and instability of their DNA-condensates.[1,3] This has stimulated research in the development of innovated transfection systems that provide better control over the stability and programmability of the DNA lipo-complexes, which may lead to better gene delivery systems for *in vivo* applications. Recently, several new materials have been developed with these goals in mind such as dendrimeric polyamido-amines,[4] porous silica nanoparticles,[5] and gold nanoparticles.[6] Herein we report the first examples of a new class of metal-mediated supramolecular materials that can deliver large fragments of DNA into eukaryotic cells. The molecular design consists of the formation of amphiphilic Cu(II) complexes that self-assemble into metallo-liposomes in water and condense DNA plasmids into deliverable structures programmed to react with intracellular components *via* redox- and ligand-exchanged reactions. Molecular and supramolecular characterization of the materials was carried out with X-ray crystallography, extended X-ray absorbance fine structure (EXAFS) spectroscopy, transmission electron microscopy (TEM), dynamic light scattering (DLS), and fluorescent optical microscopy. Transfection studies were carried out with pEGFP-N1 DNA (4.7 kb) plasmid, which encodes for the enhanced green fluorescent protein (EGFP), and human embryonic kidney (HEK) 293-T cells.

Natural coordination-capable lipids are present in marine bacteria[7] and other organisms,[8] where they are known to function as siderophores. These molecules have the ability to self-assemble into micelles in water and upon binding to transition metal ions, they undergo phase transitions and form metallo-liposomes.[7,9] Early uses of synthetic metallo-lipids were applied to the study of electron-transfer reactions across membranes.[10] To our knowledge, the role of metallo-liposomes as gene carrying agents is unprecedented.

In this study, we synthesized two coordination-capable lipids that form bis-complexes with Cu(II) ions, namely $[Cu(II)(L_{dt})_2](OTf)_2$ (**1**) and $[Cu(II)(L_{ot})_2](OTf)_2$ (**2**), where L_{dt} = 1-dodecyl-1,4,7-triazacyclononane, L_{ot} = 1-octadecyl-1,4,7-triazacyclononane, and OTf = trifluoromethanesulfonate, Fig. 1. The non-amphiphilic model system $[Cu(II)(L_{tacn})_2](OTf)_2$ (**3**), where L_{tacn} = 1,4,7-triazacyclononane, was prepared in order to support the characterization analysis of **1** and **2**.

The ligands were prepared from the corresponding nucleophilic addition of one equivalent of 1-bromoalkane to 1,4,7-triazacyclononane (L_{tacn}) in dry THF over sodium hydride at 60 °C for 12 h. After flash column purification, they were reacted with half equivalents of $Cu(OTf)_2$ in acetonitrile at room temperature to form the corresponding amphiphilic Cu-complexes **1** and **2**, which were isolated by fractional crystallization with diethyl ether at −40 °C (~75% yield).

The corresponding non-amphiphilic Cu-complex **3** was obtained under the same reactions conditions, except by using the unmodified tacn ligand. Single-crystals suitable for X-ray diffraction analysis were obtained from diethyl ether diffusion into a concentrated CH_3CN solution of the complex at room temperature. The molecular structure of **3** is shown in Fig. 1. Cu-complex **3** crystallized in the trigonal crystal system with the $P3_121$ space group.‡ The structure reveals that the Cu atom lies on a twofold axis and is coordinated to two tacn units in an octahedral configuration with equatorial Cu–N distances of 2.053(2) and

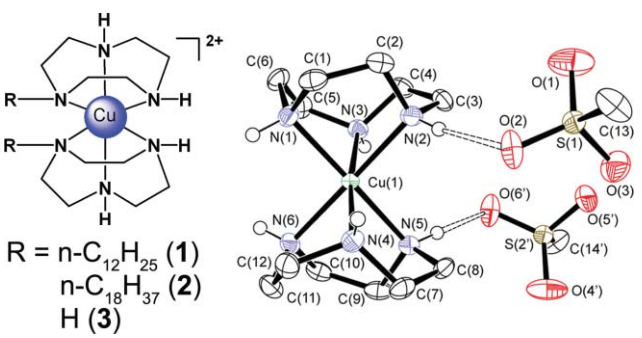

Fig. 1 ORTEP drawing for $[Cu(L_{tacn})_2](OTf)_2$ (**3**) with ellipsoids drawn at 50% probability levels. F atoms omitted for clarity.

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† Electronic supplementary information (ESI) available: Detailed synthesis and characterization of complexes **1**, **2** and **3**, and crystallographic files for **3**. See DOI: 10.1039/b703201c

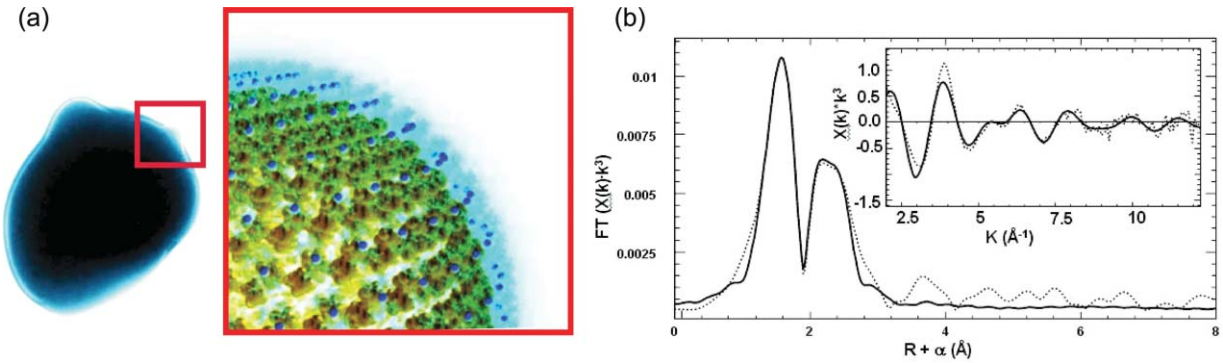

Fig. 2 (a) TEM image (color enhanced for clarity) of unilamellar metallo-liposomes of **2** and a computer model of their surface structure. (b) Fourier transform of experimental EXAFS oscillations (black line) and simulation (dashed line) of **2**.

2.075(2) Å and an axial Cu–N distance of 2.338(2) Å. The Cu-complex **3** exhibits hydrogen-bonding interactions (~2.1 Å) with the $^{-}$OTf anions with bond angles of approximately 160°.

The amphiphilic complexes **1** and **2** self-assemble into metallo-liposomes in water and exhibit an intrinsic affinity to bind the PO_4 groups of DNA *via* electrostatic and hydrogen-bonding interactions, possibly *via* similar interactions to those observed between complex **3** and $^{-}$OTf anions.

The metallo-liposomes were produced by the reverse phase methodology previously established.[11] Water was added to chloroform solutions of the complexes ($CHCl_3$: H_2O 1 : 100 v/v) and the dispersion was sonicated for 15 minutes while the organic solvent was removed in vacuum. The final volume was adjusted with water to produce 1 mM solutions. The solutions were then forced through a 0.4 μm pore-size polycarbonate filter using an extruder apparatus (Avanti Polar Lipids). The size of the metallo-liposomes was verified from DLS, which showed a narrow distribution near the pore size of the filter (400 nm). TEM analysis corroborated the size of the metallo-liposomes and revealed that their membrane had a lipid bilayer composition. EXAFS spectroscopy of the vesicles was most consistent with six N atoms as the coordination sphere of the Cu(II) center, of which two exhibit Jahn–Teller distortions, Fig. 2. Similar EXAFS data from complex **3** further confirmed this coordination structure on the surface of the metallo-liposomes.

The critical micelle concentration (cmc) values for **1** and **2** were determined using fluorescent spectroscopy,[12] and the obtained results were 50 μM and 800 μM, respectively (supporting information†). This difference may be attributed to the presence of more London dispersion forces in **2**, which may stabilize the lamellar phase that precedes vesicle formation.

The metallo-liposomes derived from **1** and **2** exhibited the ability to bind and condense the pEGFP-N1 DNA plasmid. Two DNA-condensates were observed in solution. TEM imaging revealed the formation of spherical multi-lamellar metallo-liposomes of 500 nm in diameter. Fluorescent optical microscopy on SYBR-Green stained samples revealed that double-stranded DNA is encapsulated within the spherical and multi-liposomal ensembles, Fig. 3.

The transfection ability of these DNA-condensates was tested using HEK 293-T cells and the transfection efficiency was determined to be 37% ± 4 for **1** and 4% ± 3 for **2**, Fig. 4. Solutions of complex **3** or metal-precluded solutions of **1** or **2** in EDTA-containing media showed no observable transfection.

Interestingly, the transfection efficiency of **1** is comparable with the transfection ability of some commercial liposomal transfection agents such as transfectin (Bio-Rad Laboratories), which suggests

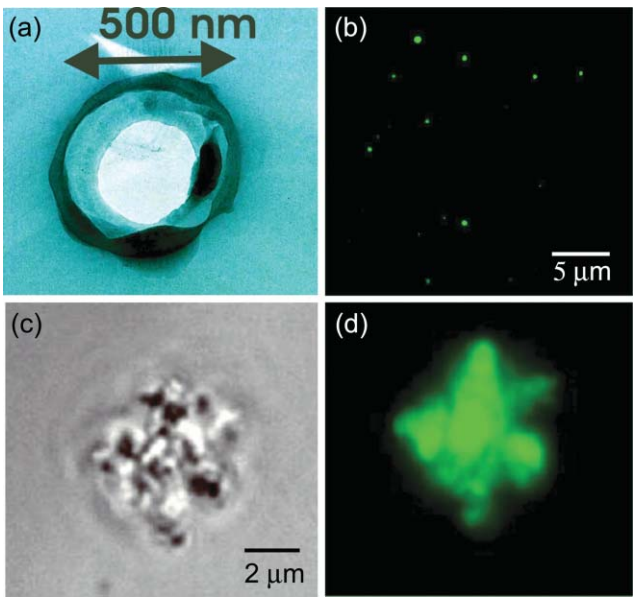

Fig. 3 (a) TEM image of spherical multi-lamellar phase condensates of pEGFP-N1 and metallo-vesicles of **2**. (b) Fluorescent light micrograph of (a) stained with SYBR-Green. (c) Bright field light micrograph of largest observable DNA condensate with metallo-liposomes of **1**. (d) Fluorescent micrograph of (c) stained with SYBR-Green.

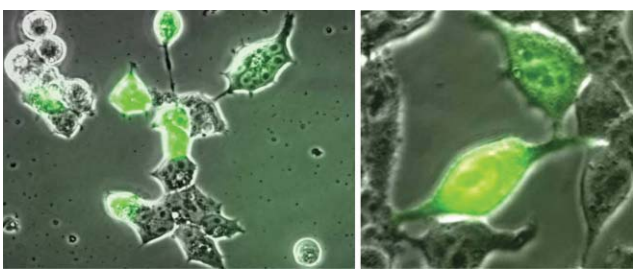

Fig. 4 Overlays of bright field light and fluorescent light micrographs of HEK 293-T cells transfected with pEGFP-N1 DNA plasmid *via* the Cu-complex **1** (left) and Cu-complex **2** (right).

that under optimal conditions related to cell- and plasmid-types the transfection ability of **1** and **2** may be higher.

The intracellular mechanism of DNA release by the Cu-complexes is not yet known. However, given the fact that Cu-complex **3** exhibits an irreversible reduction potential of −940 mV in acetonitrile,[13] redox and ligand-exchange reactions are likely to occur during transfection, particularly in intracellular reducing environments that exist along ubiquitous mechanisms for metal regulation. The released metal ions may also play a role during transfection, as evident from recent reports of DNase II deactivation in the presence of transition metals.[14] Future designs that fine-tune the redox potential and ligand exchange kinetics of metallo-liposomes could exploit these new avenues for gene delivery.

In conclusion, we report the first example of a metal-mediated gene transfection system. The transfection ability of the Cu-complexes was associated to their cmc values, with the compound having the lowest cmc exhibiting the greatest transfection efficiency. Current studies to understand the role of the metals and to investigate structure–function relationships related to transfection ability are ongoing.

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‡ CCDC 638805. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b703201c

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