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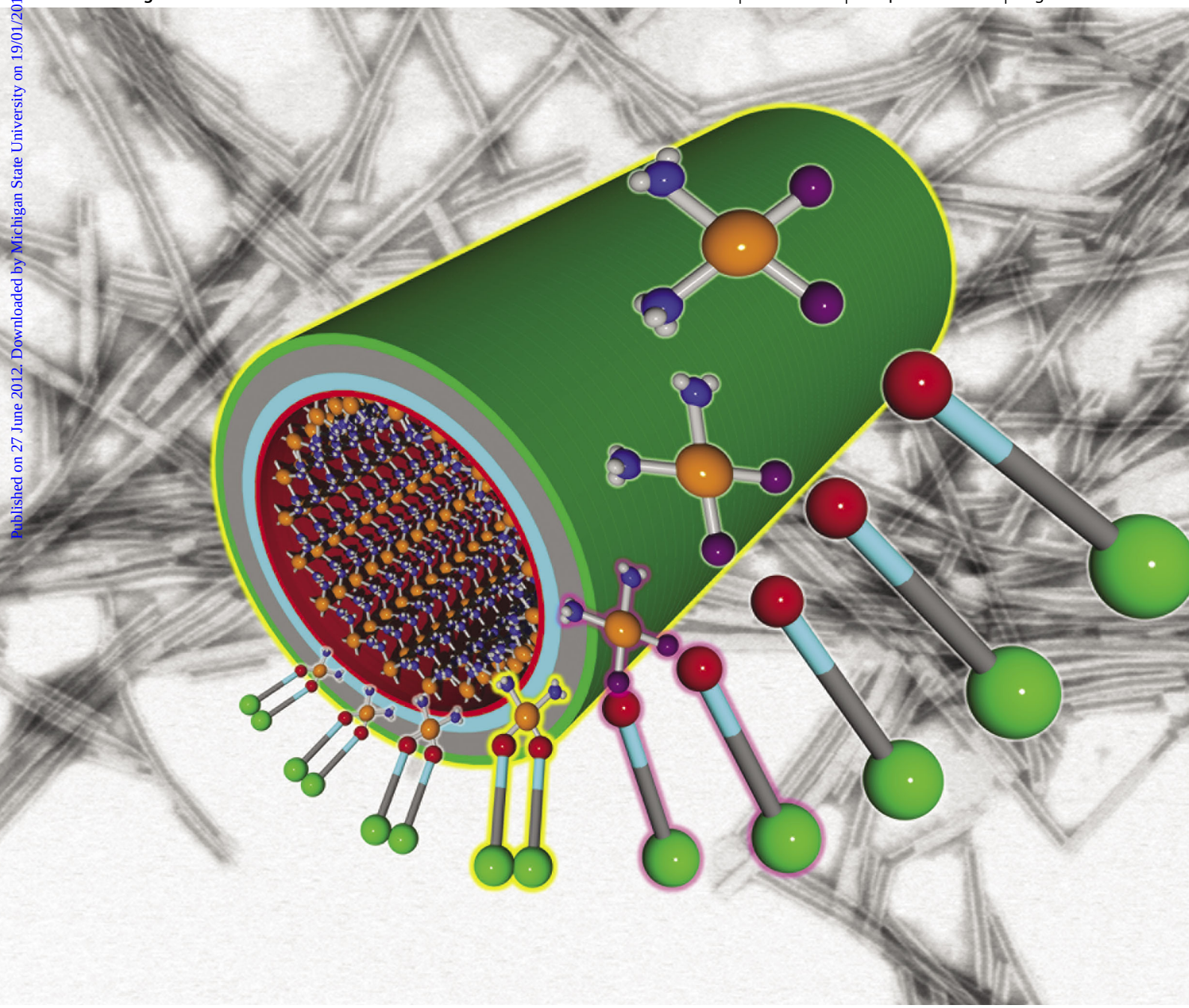

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COMMUNICATION

Cisplatin-encapsulated organic nanotubes by *endo*-complexation in the hollow cylinder†

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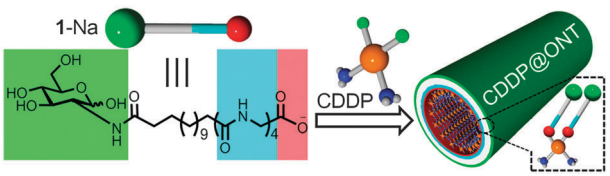
A bipolar glycolipid self-assembles into organic nanotubes upon its chelation with an anticancer drug *cis*-dichlorodiamineplatinum(II) (CDDP). The facile synthesis of glycolipid, chelation-assisted formation of the nanotubes, and efficient loading and prolonged release of CDDP demonstrate a new approach to high-axial supramolecular drug nanocarriers.

In the past decade, the potential utility of supramolecular nanostructures as drug nanocarriers has been intensively studied.[1] Recently, high-axial-ratio nanostructures received special attention due to their shape effect, such as long blood circulation and high tumoral accumulation capability.[2] A number of high-axial-ratio nanostructures have been reported as novel drug nanocarriers,[2a,3] although post loading of drugs into the preformed nanocarriers often suffered from multiple processes or limited encapsulation efficiency. These issues can be resolved by a drug-assisted self-assembly process,[4] which could effectively encapsulate the drugs during the formation of nanostructures.

Cisplatin (*cis*-dichlorodiamineplatinum(II), CDDP) is a well-known metal complex exhibiting high antitumor activity.[5] Several studies have attempted, so far, to form a CDDP–nanostructure complex by substituting CDDP chloride ligands with carboxylate groups.[6] Most of these CDDP–nanostructures, however, have been limited to spherical shape except the CDDP–peptide nanofibers.[7]

Organic nanotubes (ONTs) self-assembled from amphiphiles are one of the promising novel nanocarriers.[8] Their high-axial-ratio nanostructures with hollow cylinders can efficiently encapsulate various guests, release from their open ends, and may exhibit prolonged blood circulation due to the shape effect.[2] The construction of ONTs *via* metal-complex formation has been reported by Kogiso *et al.* and Zhang *et al.*[9] Both of these ONTs, however, associated with the metal ions on both the inner and outer surfaces. We report herein the first example of high-axial-ratio CDDP-encapsulated ONT (denoted CDDP@ONT) in which a platinum(II) complex selectively localized in the hollow cylinder and a hydrophilic glucose covers the outer surface of ONT. The formation of CDDP@ONT was assisted by the *endo*-complexation of CDDP with the $-CO_2^-$ group in a bipolar glycolipid at a high CDDP encapsulation efficiency (Scheme 1). The CDDP@ONT possesses uniform morphology, and shows a sustained release of CDDP in biological medium.



Scheme 1 CDDP-assisted assembly of organic nanotubes.

The glycolipid (**1**) consists of three segments, a large headgroup of 2-glucosamine, a small headgroup of tetraglycine with carboxylic acid terminal, and a hydrophobic spacer of the dodecamethylene chain. In contrast to our previously reported glycolipids,[10] **1** has the advantage of easy synthesis in three steps with a high yield (see ESI†), and it is readily soluble in water as sodium salt (**1**-Na) at room temperature. Dynamic light scattering (DLS) measurements indicated that the solution of **1**-Na consisted of self-assemblies with a hydrodynamic size of around 60 nm (Fig. S3 in ESI†), which is much larger than spherical micelles (generally around 10 nm). The scanning transmission electron microscopic (STEM) observation revealed that rapidly evaporated solution of **1**-Na gave fibrous assembly morphology with several nanometers in width (Fig. S4 in ESI†). Therefore, we tentatively conclude that **1**-Na self-assembled into nanofibers in water, although the details, such as molecular arrangement and their phase diagram, were unclear.

Surprisingly, upon the addition of CDDP solution, the nanofibers of **1**-Na gradually transformed into tubular nanostructure, so called organic nanotubes (ONTs). Based on the STEM images of different incubation time (Fig. S5 in ESI†), the transformation into ONTs gradually started after 8 h, and completed after 48 h. Comparison of stained and unstained images of ONTs (Fig. 1) revealed that CDDP was encapsulated into the hollow cylinder of ONTs, named "CDDP@ONT". The negatively stained image of CDDP@ONT with phosphotungstate visualized a uniform tubular nanostructure with outer diameter 14 nm, inner diameter 6.7 nm, and membrane thickness

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† Electronic supplementary information (ESI) available: Synthesis, experimental methods, DLS measurement, time-dependent STEM images, gel formation, STEM images of nanofiber and bare ONT, IR of **1** as acidic form, pH change, XRD patterns, STEM images after release. See DOI: 10.1039/c2cc33970f

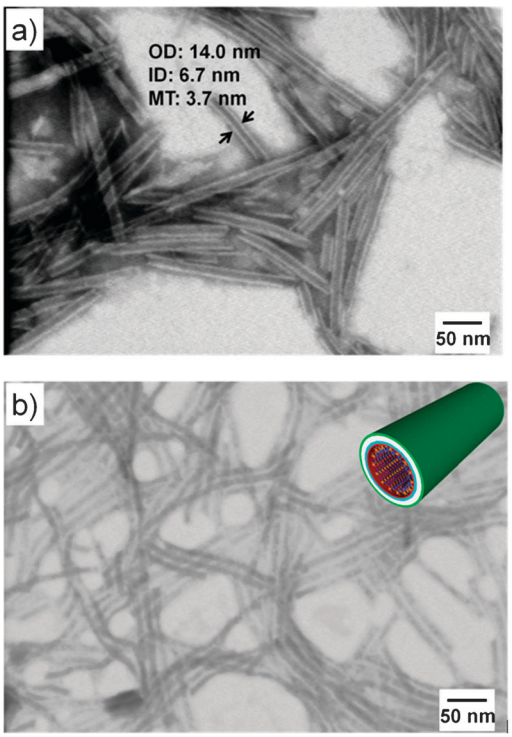

Fig. 1 STEM images of CDDP@ONT (a) with and (b) without staining. Sample in (a) was negatively stained with 2%-phosphotungstate. OD, ID, and MT: outer diameter, inner diameter, and membrane thickness of ONT, respectively. The inset showed the schematic model of CDDP@ONT.

3.7 nm (Fig. 1a). Without the staining treatment, the hollow cylinder was still visible as a darker image, indicating the platinum localized in the nanospace (Fig. 1b). However, the membrane of unstained CDDP@ONT was unidentifiable. The CDDP@ONT was considered to be over several micrometers long since it formed a brittle gel after 24 h incubation (Fig. S6 in ESI†).

We supposed that the formation of CDDP@ONT was induced by metal-complex formation of platinum in CDDP with the $-CO_2^-$ group of **1**-Na, as indicated by infrared (IR) spectra (Fig. 2) and time-dependent association (Fig. 3). In the IR spectra, the shoulder peak assigned to the $-CO_2^-$ stretching vibration[9a] (about 1610 cm^{-1}, shoulder peak B in Fig. 2) of **1**-Na disappeared after the formation of CDDP@ONT at 48 h. Further, the corresponding protonated form ($-CO_2H$), which is observed for

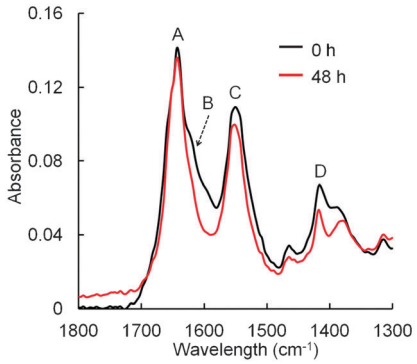

Fig. 2 IR spectra of mixture solution containing **1**-Na and CDDP (molar ratio of 1/1) after 0 and 48 h incubation. (A) Amide I; (B) C=O stretch of $-CO_2^-$; (C) amide II; (D) C–H deformation of polyglycine.

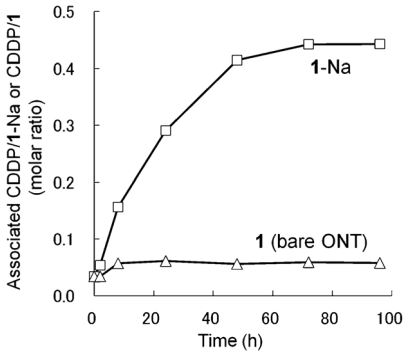

Fig. 3 Time-dependent association of CDDP with **1**-Na solution or **1** (dispersion of bare ONT) at initial molar ratios of CDDP/**1**-Na = or CDDP/**1** = 1/1.

the acidic form of **1** (1706 cm^{-1}, Fig. S7 in ESI†), was absent. Thus, the formation of a carboxylate–platinum(II) complex in **1**–CDDP might have shifted the carboxylate peak and made the peak overlap with the amide I band of amide groups (peak A).

The time-dependent association of CDDP with **1**-Na was investigated over a period of 96 h (Fig. 3, open square) with a colorimetric method to determine unreacted CDDP (see ESI†). The values of associated CDDP–**1**-Na steadily increased with the incubation time, and reached a constant value of approximately 0.44 after 48 h. The value of 0.44 and its slow dynamic behaviour implied that one CDDP molecule shall associate with approximately two $-CO_2^-$ groups *via* the metal-complex formation, rather than the physical association. Namely, 1 g of ONT can retain 220 mg of CDDP in their hollow cylinders in the form of highly ordered carboxylate–platinum(II) complex. The slightly smaller value of the CDDP–**1**-Na (0.44) than the ideal value (0.5 in 1 : 2 complexation) was ascribable to the transformation of **1**-Na into its acidic form. Indeed, the mixture solution of **1**-Na and CDDP showed a slight decrease in pH from 7.7 to 6.0 (Fig. S8 in ESI†) along the incubation period (up to 96 h) owing to the spontaneous hydrolysis of excess CDDP.[11]

Note that **1** alone, the acidic form of **1**-Na, formed bare ONT with a similar morphology to the aforementioned CDDP@ONT upon neutralization of **1**-Na to acidic conditions (Fig. S9 in ESI†). Loading of CDDP into the hollow cylinder of the preformed bare ONT, however, resulted in very low efficiency (CDDP/**1** value of about 0.05) as shown in Fig. 3 (open triangle). These highly ordered $-CO_2H$ groups on the inner surface of ONTs seemed to be weak or slow in the formation of a metal-complex with CDDP. All these results indicated that an effective loading of CDDP into the ONT is attainable only in the nanotube formation assisted by the coordination of CDDP with the $-CO_2^-$ group of **1**-Na during the nanotube formation.

Powder X-ray diffraction (XRD, Table 1, and Fig. S10 in ESI†) measurement revealed the molecular packing in CDDP@ONT, the nanofibers of **1**-Na and bare ONT. The measured membrane thickness, *d*, of CDDP@ONT was well compatible with that estimated by STEM observation (3.7 nm shown in Fig. 1). The slightly smaller *d* values than the calculated *L* values suggested that the nanotubes consisted of a single monolayer membrane (MLM) in which the molecules tilted by 32.8°.[10a] In the IR spectrum (Fig. 2), the C–H deformation band of tetraglycine (peak D) suggested the formation of polyglycine-II-type hydrogen-bonding networks which

Table 1 The molecular length of lipid (L), the membrane thickness (d) in the assemblies, tilt (θ), and plausible molecular packing

	Molecular length L (nm)[a]	Membrane thickness (nm)[b]	Molecular packing and tilt θ[c]
CDDP@ONT	4.58	3.85	
Nanofibers (**1**-Na)	4.17	3.56	
Bare ONT	4.18	3.27	

[a] Values were calculated from molecular modeling. [b] Thickness was measured by XRD. [c] Tilt degree was calculated on the basis of L and d values.

ensures parallel molecular packing within the MLM (Table 1).[10b] The parallel molecular packing and large glucose headgroup of **1** should bend the resultant MLM to form tubular structures for CDDP@ONT. Consequently, the carboxylate–platinum(II) complex should be localized on the inner surface in CDDP@ONT. All these data agreed well with the relatively darker image of cylindrical nanospaces in Fig. 1b. The nanofibers from **1**-Na were also estimated to consist of the MLM; however, the exact molecular packing was still unclear. Bare ONT from **1** displayed a similar molecular packing to that of CDDP@ONT with glucose headgroups on the outer surface and the $-CO_2H$ groups on the inner surface, except the molecules tilted in a larger degree.

The release of CDDP from CDDP@ONT was studied in HEPES and HBS (HEPES-buffered saline) buffers to see the effect of chloride ions. The CDDP@ONT was purified by a membrane filtration (100 nm pore size) to remove residual CDDP. The isolated CDDP@ONT was redispersed in Milli-Q as a fine dispersion and used for the release experiment. The open square marks in Fig. 4 show the sustained release of CDDP in HBS buffer, with a release amount of 37% at 96 h. Similar to other CDDP–nanostructure complexes,[6] the release of CDDP from CDDP@ONT was essentially triggered by the chloride ions since the release in HEPES buffer was negligible ($<5\%$). In line with the release behaviour described above, the CDDP@ONT remained the tubular nanostructures in HEPES buffer, whereas resultant ONT partially transformed into nanofibers after 96 h release in HBS buffer (Fig. S11 in ESI†). This

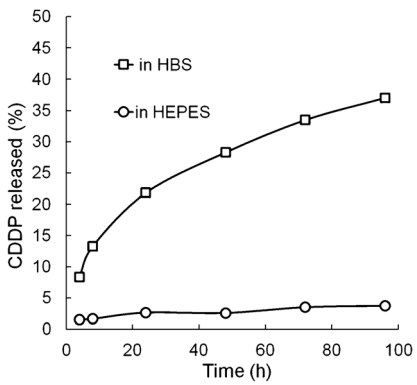

Fig. 4 Release of CDDP from CDDP@ONT in HBS (150 mM of sodium chloride) and HEPES buffer at 37 °C.

prolonged release of CDDP under physiological conditions can reduce its toxic side effect *in vivo*. Details of its pharmacokinetics and anticancer effects are now under investigation.

In conclusion, we demonstrated the formation of organic nanotubes assisted by chelation of one CDDP molecule with two glycolipids *via* carboxylate–platinum(II) complexation. CDDP molecules were selectively encapsulated into the cylindrical nanospaces during the formation of ONT, whereas the preformed bare ONT was ineffective in the CDDP encapsulation. The CDDP@ONT system demonstrated a remarkable slow release of CDDP under physiological conditions. This novel high-axial-ratio CDDP@ONT may provide a new approach in cancer therapy.

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