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Polysaccharide–porphyrin–fullerene supramolecular conjugates as photo-driven DNA cleavage reagents†

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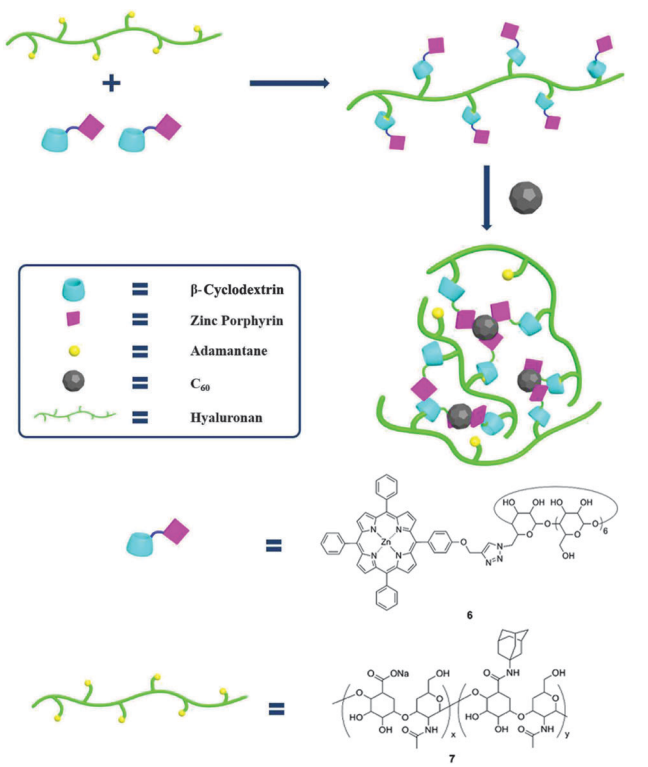
Two water-soluble polysaccharide–porphyrin–fullerene supramolecular conjugates were constructed from the non-covalent incorporation of triphenyl Zn-porphyrin-modified β-cyclodextrins, adamantyl-modified hyaluronate and C_{60}. Significantly, these supramolecular conjugates, which exist as cross-linked or discrete nanoparticles with a diameter of 50–200 nm, can completely cleave closed supercoiled DNA to nicked DNA under light irradiation.

Construction of nanometer-scaled functional materials based on porphyrins and their derivatives is being intensely investigated due to their attractive electronic, photochemical, and photophysical properties.[1–3] In the past two decades, porphyrin-based nanomaterials with various topologies, such as spheres,[4] wires,[1,5,6] rings,[7] have been widely reported. Among them, the porphyrin–fullerene conjugate has attracted more attention owing to its good performance in photo-induced electron transfer and photodynamic therapy.[8–13] On the other hand, cyclodextrins (CDs) and hyaluronic acid (HA) are widely regarded as two important polysaccharide building blocks in the construction of bioactive supramolecular assemblies. Cyclodextrins, a class of cyclic oligosaccharides with six to eight D-glucose units linked by 1,4-glucose bonds, are water-soluble, non-toxic, and commercially available at a low price, and their hydrophobic cavities can bind various inorganic/organic/biological molecules and ions both in aqueous solution and in the solid state to form functional supramolecular systems.[14] Whereas, hyaluronic acid, an anionic glycosaminoglycan, has been widely used for various medical applications such as drug delivery,[15] tissue engineering,[16] and bioimaging diagnosis[17] due to its biocompatibility, biodegradability, non-toxicity, non-immunogenicity and non-inflammatory properties. Interestingly, HA can also be used as a targeting agent for cancer therapy because various tumor cells are known to over-express HA receptors such as cluster determinant 44 (CD44)[18] and receptor for hyaluronate-mediated motility (RHAMM).[19] Therefore, one can hypothesize that the combination of polysaccharides with porphyrin–fullerene conjugates may bring a breakthrough in many fields of chemistry and biomaterials science. Herein, we wish to report a facile method to construct water-soluble nanometer-scaled supramolecular conjugates composed of polysaccharides (β-CDs and adamantyl-modified HA), triphenyl Zn-porphyrins and C_{60} (Scheme 1). Compared with the reported



Scheme 1 Construction of polysaccharide–porphyrin–fullerene supramolecular conjugates.

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porphyrin–fullerene conjugates, the inherent advantages of these supramolecular conjugates are as follows: (1) the presence of numerous CD units can greatly improve the water solubility and the biocompatibility of porphyrin–fullerene conjugates. (2) Introduction of hyaluronate will endow the porphyrin–fullerene conjugates with targeting properties. (3) The supramolecular non-covalent conjugation among polysaccharides, porphyrins and fullerenes can provide a convenient and versatile approach to overcome the disadvantages of direct covalent conjugation which are as follows: it usually requires a large amount of organic solvents, a long time course, repeated separation and purification, and does harm to the environment. Interestingly, these polysaccharide–porphyrin–fullerene supramolecular conjugates showed good DNA cleavage abilities under light irradiation.

The grafting of adamantyl groups on the backbone of HA was confirmed by FT-IR spectra (Fig. S1, ESI†), where the stretching vibration band of the carbonyl group (amido bond) at 1557 cm^{-1} in **7** was stronger than that in the unmodified HA, attributing to the partial amidation of carboxylic acid groups in HA. The degree of substitution (D_S) of adamantyl groups in **7**, defined as the ratio of adamantyl groups per 100 carboxyl units of HA, was quantitatively calculated as 25.0% from the integration ratio of the characteristic peaks of adamantane in the range of 1.4–1.8 ppm and HA at 1.9 ppm ($-CH_3$) in the ^{1}H NMR spectra (Fig. S2, ESI†). In addition, the UV-Vis spectra of supramolecular conjugates **6**/C_{60} and **6**/**7**/C_{60} exhibited broad bands at 265 and 339 nm ascribed to C_{60} (Fig. 1a).[20] In aqueous solution, the UV-Vis spectrum of **6** showed a maximum at 430 nm assigned to the Soret band of the porphyrin unit, and this maximum shifted to a longer wavelength (439 nm for **6**/C_{60} and 441 nm for **6**/**7**/C_{60}) in the UV-Vis spectrum of **6**/C_{60} or **6**/**7**/C_{60}, accompanied by a slight bathochromic shift of the Q-band, indicating the electronic interaction between **6** and C_{60}.[21] Moreover, the broad absorption in the range of 650–800 nm in the UV-Vis spectrum of **6**/C_{60} or **6**/**7**/C_{60} also indicated the charge transfer (CT) interactions between fullerenes and porphyrins.[22] In addition, the fluorescence emission of **6**/C_{60} and **6**/**7**/C_{60} also revealed significant quenching (Fig. 1b) as compared with that of free **6**, indicating an efficient electron transfer between porphyrins and C_{60}.[6] These phenomena jointly demonstrated the fullerene–porphyrin interactions.

Considering the difference in solubility of C_{60} between DMSO and toluene, the content of C_{60} in the supramolecular conjugates was determined by UV-Vis spectroscopy. A certain amount of the supramolecular conjugate was dissolved in DMSO; the insoluble substance was separated by centrifugation and dissolved in toluene. The UV-Vis spectra of DMSO and toluene solution were recorded (Fig. S3, ESI†), and the content of C_{60} in **6**/**7**/C_{60} was calculated to be 5.98×10^{-5} mol g^{-1}. In addition, the molar ratio between **6** and **7** in **6**/**7**/C_{60} was determined by ^{1}H NMR spectra (Fig. S4, ESI†). Since the ^{1}H NMR signals in the range of 3.0 to 6.0 ppm, which were assigned to the glucose groups in both **6** and **7**, were complex and overlapped with each other, we only analyzed the signals in the low field and the high field, which were assigned to the porphyrin group in **6** and the adamantyl group in **7**, respectively, to calculate the molar ratio between **6** and each adamantyl unit of **7** in **6**/**7**/C_{60}, which is given as 1 : 1.

The morphological information of the supramolecular conjugates was obtained from the electron microscopy measurements. Fig. 2 shows the TEM images of **6**/C_{60} and **6**/**7**/C_{60}. **6**/C_{60} existed as irregular nanoparticles with a diameter of 50 to 130 nm, and these nanoparticles tended to crosslink with the neighboring ones (Fig. 2a). In contrast, **6**/**7**/C_{60} existed as spherical nanoparticles with a diameter of 50 to 200 nm and did not show the obvious crosslinking and transmogrification (Fig. 2b). A similar phenomenon was also observed in the AFM images. As shown in Fig. 3a, **6**/C_{60} mainly existed as crosslinked aggregates, while **6**/**7**/C_{60} mainly existed as discrete nanoparticles. These phenomena demonstrated that the introduction of HA prevented the crosslinking of neighboring **6**/C_{60} aggregates to some extent.

The photo-induced DNA cleavage ability of the supramolecular conjugates was detected by the agarose gel electrophoresis assay. As shown in Fig. 4, both **6**/C_{60} and **6**/**7**/C_{60} showed significant DNA cleavage abilities, and **6**/**7**/C_{60} showed higher DNA cleavage ability than **6**/C_{60}. For example, closed supercoiled DNA (form I) was completely cleaved to nicked DNA

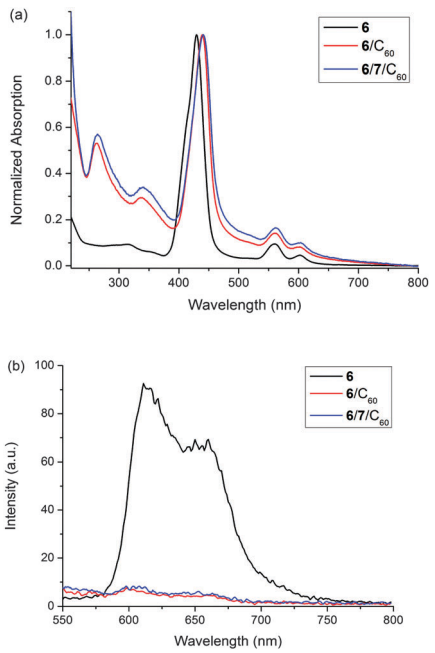

Fig. 1 (a) Normalized UV-Vis spectra of **6**, **6**/C_{60} and **6**/**7**/C_{60} in water at 25 °C; (b) fluorescence spectra of **6**, **6**/C_{60} and **6**/**7**/C_{60} in water at 25 °C.

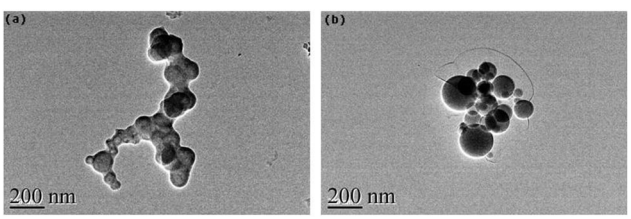

Fig. 2 TEM images of (a) **6**/C_{60} and (b) **6**/**7**/C_{60}.

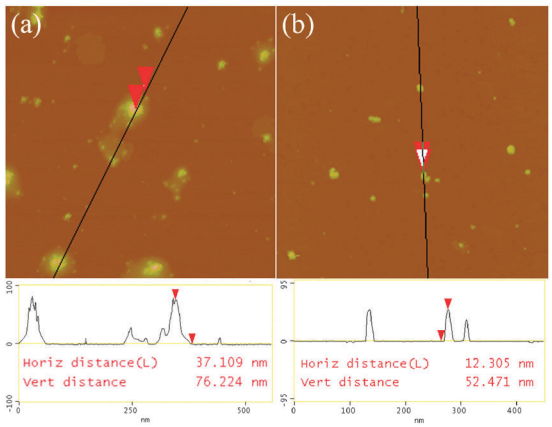

Fig. 3 AFM images of (a) **6**/C_{60} and (b) **6**/**7**/C_{60}.

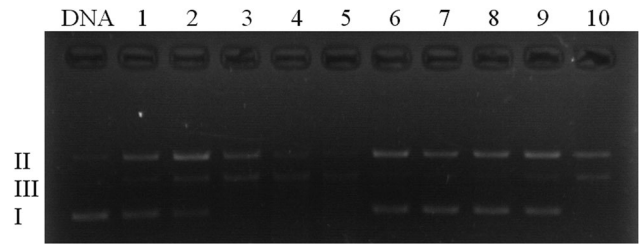

Fig. 4 Agarose gel electrophoresis assay of pBR 322 DNA (5 ng μL^{-1}). [**6**/**7**/C_{60}] = 50, 100, 300, 500, 700 ng μL^{-1} for lanes 1 to 5, respectively; [**6**/C_{60}] = 50, 100, 300, 500, 700 ng μL^{-1} for lanes 6 to 10, respectively.

(form II) by **6**/**7**/C_{60} at a mass concentration of 300 ng μL^{-1} (Fig. 4, lane 3); the mass concentration of **6**/C_{60} should be 700 ng μL^{-1} to achieve the same result (Fig. 4, lane 10). The control experiments showed that the content of C_{60} in **6**/**7**/C_{60} was 5.6 times lower than the corresponding value in **6**/C_{60}; we deduced that the DNA cleavage ability of **6**/**7**/C_{60} should be fairly higher than that of **6**/C_{60}.

In summary, several water-soluble polysaccharide–porphyrin–fullerene supramolecular conjugates, which have application potential as DNA reagents, have been constructed from β-CDs, triphenylporphyrins and adamantyl-modified hyaluronate. As an example of the possible applications, these supramolecular conjugates displayed a good ability of cleaving DNA under visible light irradiation. Considering the good photophysical and photochemical properties of porphyrins and fullerenes as well as the targeting capability of hyaluronate, this achievement would not only provide a new access to modifying polysaccharides with functional groups but also extend the possible applications of polysaccharide-based supramolecular species in many fields of pharmaceutical chemistry and biological technology.

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