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COMMUNICATION

Amphiphilic DNA-dendron hybrid: a new building block for functional assemblies†

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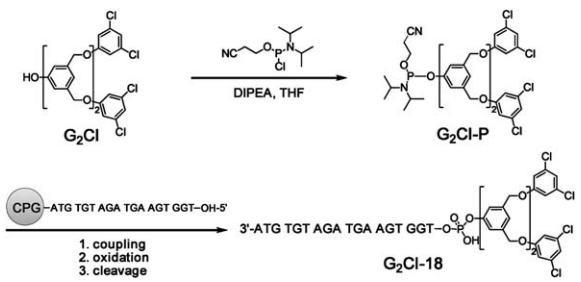
A new kind of amphiphilic DNA-dendron hybrid consisting of a highly hydrophobic dendron and a single stranded DNA is synthesized. The hybrid could assemble into long nanofibers in aqueous phase. The hybridization property of DNA at the shell of fibers associated with the encapsulation ability of dendron at the inner core enable further functionalization, offering a unique class of supramolecular building block.

Precise molecular design becomes more and more important to achieve well-defined supramolecular assemblies with functions. Besides designing completely new molecules, combining available components with different structures and functions, for example, biomacromolecules and organic polymers, to get new hybrid molecules is definitely efficient and consistently needed, and also challenging.[1] In the past decades, DNA with its inherent base-pairing fidelity, structure diversity, hydrophilicity and biocompatibility, has become an excellent supramolecular building block.[2] DNA based nanostructures,[3] nanomachines,[4] biosensors[5] and hydrogels[6] have been extensively studied. To achieve more versatile and multiple functions, DNA has also been combined with other materials,[7] in particular, DNA block copolymers composed of DNA and organic polymers, are examples of this exciting soft material.[8] Recently, some of them have been developed to assemble into tunable nanostructures with potential applications in nanoscience, diagnostics and biomedicine.[8a–c,f,g] However, owing to the wide molecular weight distribution of the polymeric segments, rational design of more controllable and precise DNA hybrids is highly desirable.

Dendrimers/dendrons are synthetic molecules with well-defined highly branched nanostructures and almost monodisperse molecular weights.[9] A variety of dendrimers with precisely designed cores and periphery groups have been constructed and widely applied in supramolecular chemistry and functional materials.[10] Covalently conjugation of both perfect architectures, DNA and dendrimer, certainly provides a new class of promising supramolecular building block with the potential to fabricate smart soft materials with controlled structures and functions.[11] Although a few kinds of DNA–dendrimer hybrids have been constructed,[12] these primary attempts mainly focused on fabrication methods,[12a–d] and mostly employed hydrophilic dendrimers. To the best of our knowledge, there is no report on the hierarchical assembly of such hybrids in aqueous solution. Herein, we report the synthesis of a new kind of amphiphilic DNA–dendron hybrid composed of a highly hydrophobic dendron and a hydrophilic single-stranded DNA (ssDNA). We studied their self- assembly behavior, a possible assembly mechanism and further investigated their functions.

First, we chose poly(benzyl ether) dendron peripherally functionalized with dichlorobenzene (G_2Cl, 2 represents the generation of the dendron) in this study. In addition to its hydrophobicity, this type of dendron may possess multiple interactions, such as π–π stacking,[13] van der Waals interactions[14] and halogen bonding interactions,[15] which are expected to play important roles in the self-assembly of such DNA–dendron hybrids. The synthesis route to our designed DNA–dendron hybrid is outlined in Scheme 1. The dendron was synthesized using the divergent method *via* repetitive ester reduction and Mitsunobu reaction (for detailed synthetic procedures, see ESI†). The resulting G_2Cl reacted with phosphoramidite chloride under room temperature for 30 min, after methanol precipitation, affording the key reagent, G_2Cl-P, for further coupling with DNA. In a typical solid phase synthesis experiment,[8b] a mixture containing G_2Cl–P, 5-ethylthiotetrazole (ETT) and the controlled pore glass (CPG) beads



Scheme 1 The solid phase synthesis of the G_2Cl-18 hybrid.

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loaded with DNA was transferred into a flask followed by addition of anhydrous tetrahydrofuran (THF). The reaction proceeded under nitrogen atmosphere overnight. Then the unreacted G_2Cl–P and ETT were removed from the system by rinsing the CPG beads with 15 mL anhydrous THF. The newly formed phosphite-triester group was oxidized with iodine solution for 30 s; the CPG beads were rinsed again with 15 mL THF and 15 mL acetonitrile. After the ammonium deprotection and cleavage from the CPG beads, the target product G_2Cl-18 (18 represents the DNA length) was purified by polyacrylamide gel electrophoresis (PAGE). This solid phase synthetic method can be used for preparation of DNA–dendron hybrids on a large scale. The molecular weight and structural assignment were confirmed by MALDI-TOF mass spectroscopy (Table S1†), and the obtained result was in good agreement with the calculated value. The purity of the product was confirmed by gel electrophoretic migration-shift assay (Fig. S1†), combined with the results above, indicating that the DNA–dendron hybrid was indeed formed with high purity.

With this hybrid in hand, we then investigated its self-assembly behavior. G_2Cl-18 was dissolved into 100 μL water to get a concentration of 20 μM. In the presence of 5 μL dichloromethane, the solution was heated to 90 °C for 30 min and subsequently cooled to room temperature in 2 h. The TEM results clearly showed that nanofibers existed almost exclusively and extended tens of microns in length (Fig. 1A). An amorphous network would be formed without annealing (Fig. S2†), indicating the heating process can destroy these undesired aggregates, and the hybrids could reassemble during cooling. Therefore, the annealing process was necessary for the fiber formation. In order to make sure that the nanofibers were not formed during the drying or the negative staining process of the TEM sample preparation, cryo-TEM (Fig. 1B) and tapping mode AFM (Fig. 1C) were also performed. The morphologies observed are in good agreement with the TEM results. We also measured the critical micelle concentration (cmc) of G_2Cl-18 by using Nile Red as a fluorescence probe (Fig. S3†), and it is shown that the cmc value is 0.7 μM, similar to other DNA block copolymer systems.[8a,c] Interestingly, G_2Cl-18 nanofibers were all observed at the concentration range of 1 to 50 μM (Fig. S4†), and the fibers exhibited high stability, retaining the same morphology even after six months under 4 °C.

The average diameter of the G_2Cl-18 fibers was 16.5 ± 1.3 nm in cryo-TEM, about twice of the molecule length, 9.2 nm (the contour length of the 18-mer ssDNA is approximately 7.7 nm at a 0.43 nm rise per base,[16] and the G_2Cl was 1.5 nm in the CPK model). Due to the electrostatic repulsion, the DNA strands are almost completely stretched.[8c] We propose that in the nanofiber the dendrons aggregate to form a hydrophobic core while the hydrophilic DNA strands assemble at the corona. The average diameter of the fibers in TEM image was similar to that in the cryo-TEM images. We note that the height of the fibers in AFM images was only 9.4 ± 1.7 nm (Fig. 1C), about 7 nm smaller than the diameters observed in TEM and cryo-TEM, possibly due to sample compression by the AFM tip.

In order to explore the universality of the designed DNA–dendron system, other hybrids with different dendron generations and DNA lengths were also synthesized and assembled in aqueous solution (Chart S2†). Under the same assembly conditions as G_2Cl-18, the more sterically demanding G_3Cl-18 could also assemble into nanofibers with a diameter of 17.3 ± 0.9 nm (Fig. S5†). Notably, G_1Cl-18, with such a small hydrophobic part (hydrophobic/hydrophilic weight ratio equals to 0.079), could also form long nanofibers (Fig. S5†), suggesting that other interactions besides hydrophobic aggregation existed in the dendrons as mentioned previously. On the other hand, as the DNA length became shorter, from 18 to 9 bases, nanofibers still predominantly existed in the aqueous solution of G_2Cl-9 (Fig. S5†). Certainly, the diameter was smaller than that of the G_2Cl-18 fibers. These results demonstrate the aggregation behavior of such designed amphiphilic DNA–dendron hybrids is general due to structure similarity.

To verify the proposed assembly mechanism of DNA–dendron hybrids, we employed DNA modified gold nanoparticles (AuNPs) containing complementary strands to hybridize with G_2Cl-18 (Fig. 2A). The ssDNA was first modified with thioctic acid and then conjugated with 5 nm AuNPs adopting the published method and characterized by agarose gel electrophoresis.[17] AuNPs modified with multiple complementary ssDNA (5′-TACACATCTACTTCA-3′) were mixed with the pre-formed G_2Cl-18 nanofibers in 50 mM Tris-HCl buffer (pH 8.0) and 50 mM NaCl at room temperature overnight to ensure the hybridization. As shown in Fig. 2B, AuNPs

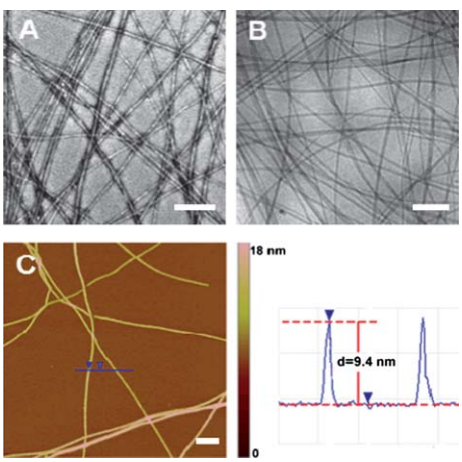

Fig. 1 Images of nanofibers formed by self-assembly of G_2Cl-18 in aqueous solution. (A) Negatively stained TEM image, (B) cryo-TEM image and (C) AFM image, including cross-section height analysis. The scale bar is 200 nm.

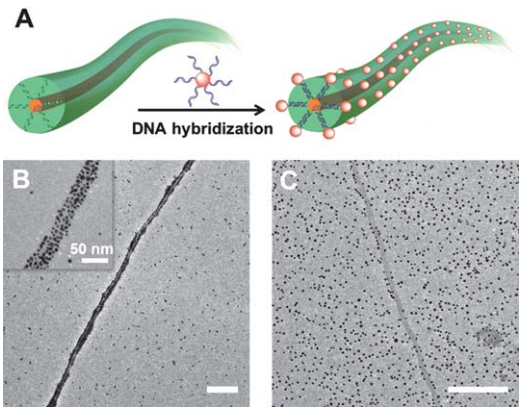

Fig. 2 (A) Schematic illustration of the hybridization of DNA modified AuNPs with G_2Cl-18 nanofibers. TEM images of the hybridization of G_2Cl-18 nanofibers with 5 nm AuNPs containing (B) complementary ssDNA and (C) non-complementary ssDNA. Inset is the enlarged image. The scale bar is 200 nm.

particularly aggregated around G_2Cl-18 nanofibers and AuNP chains as long as several microns were observed; in contrast, the control experiment used non-complementary ssDNA (5′-TTTCGCAATGACTGTACT-3′) modified AuNPs in Fig. 2C, resulting in a random dispersion of AuNPs. Both observations suggest that the accumulation of AuNPs along G_2Cl-18 nanofibers was induced by DNA hybridization instead of non-specific interactions. The diameter of the chains was about 25 nm, approximately 10 nm wider than that of the G_2Cl-18 nanofiber, this is reasonable considering the 5 nm diameter of AuNPs. These results support the assembly mechanism that the hydrophilic ssDNA forms a shell surrounding the hydrophobic core. In addition, it provides a new platform for functionalization and fabrication of DNA–dendron nanofibers through DNA hybridization. For example, we employed a carboxyfluorescein modified complementary strand FAM-15 to hybridize with G_2Cl-18, and a large amount of green nanofibers were observed under fluorescence microscopy, indicating the fluorescent FAM group was successfully loaded onto the fiber through DNA hybridization (Fig. S6†). Due to the peculiar sequence programmable and modifiable feature of DNA, we believe other functional groups/species could also be incorporated into the fiber with the same strategy, realizing a smart multifunctional system.

We make one final investigation regarding the assembly mechanism and functionality of DNA–dendron. We employed G_2Cl-18 nanofibers as carriers for hydrophobic species, *e.g.*, Nile Red.[18] As shown in Fig. 3A, the remarkable fluorescence emission at 621 nm indicated that Nile Red indeed entered into the G_2Cl-18 nanofibers. However, in the control experiment, Nile Red in the solution of 18-mer DNA with the same sequence as that in G_2Cl-18 showed no observable emission. The fluorescence microscopy measurement under green light excitation provided direct evidence for the encapsulation of Nile Red into the hydrophobic core of nanofibers (Fig. 3B). The size and the shape of the red fibers were in accordance with the TEM characterization (Fig. S7†). This experiment additionally verifies the existence of the hydrophobic dendron core in G_2Cl-18 nanofibers. We note that it has been reported that nanofibers might be more effective in drug delivery than spherical micelles,[19] moreover, DNA hybridization has advantages to integrate targeting molecules, *e.g.*, folic acid,[8f] onto the corona, therefore, this new class of materials would have great potential in drug delivery.

In conclusion, we have successfully developed a new kind of amphiphilic DNA–dendron hybrid composed of a highly hydrophobic dendron and a hydrophilic ssDNA. These hybrids with different dendron generations and DNA lengths all self-assembled into uniform long nanofibers in aqueous solution. We proposed and verified that the nanofiber contained a hydrophobic dendron core and a hydrophilic DNA shell. By taking advantage of unique recognition and modification properties of DNA, gold nanoparticles and fluorescent molecules were particularly decorated onto fibers; in addition, the hydrophobic core was capable of encapsulating hydrophobic species as a reservoir; taken together, we believe the DNA–dendron hybrids could be used as promising building blocks for constructing functional materials and open up a wide range of applications in drug delivery, supramolecular templates, biosensors and hydrogels.

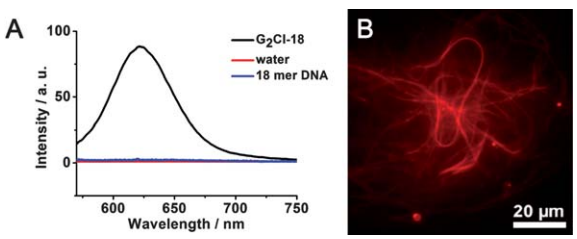

Fig. 3 (A) The fluorescent emission spectra of Nile Red in aqueous solution in the presence or absence of G_2Cl-18 hybrid and (B) the fluorescent image of the nanofibers after Nile Red encapsulation.

Acknowledgements

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