# Nanofiber formation from sequence-selective DNA-templated self-assembly of a thymidylic acid-appended bolaamphiphile†

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**Quaternary self-assembly of a thymidylic acid-appended bolaamphiphile, with heteropolymeric DNA as a template, produced supramolecular helical nanofibers in the presence of specific target DNA.**

The complementarity of nucleobases enables DNA to act as a powerful and skillful biomolecular building block for the fabrication of well-defined nanostructures and nanomaterials.[1] For example, programmed self-assembly of DNA molecules can fabricate nano-tiles,[2] -cubes,[3] or -lattices.[4] DNA can also arrange organic compounds including metallosalens,[5] crown ethers,[6] and conjugated molecules.[7–9] DNA-hybridized nanoparticles are useful as tools for the detection of target DNA, since the aggregation of these particles upon hybridization can cause colorimetric changes in sample solutions.[10–12] These DNA-derived nanoarchitectures are comprised of DNA or utilize organic compounds connected covalently to single-stranded DNA (ssDNA) and nanoparticles connected *via* thiol–gold bonds.

The combination of DNA science and supramolecular chemistry contributes to the construction of well-defined nanostructures.[13,14] DNA functions as a template to form lipid–oligonucleotide-complexed bilayers,[15] one-dimensional fibrous structures of low-molecular-weight cholesterol gelators,[16] and chromophores.[17] We have also reported the self-assembly of helical nanofibers from a thymidylic acid-appended bolaamphiphile **1** (Fig. 1(a)),[18] whereby a single-stranded DNA functions as a template. However, all reports on DNA supramolecular assemblies stabilized by complementary base-pairing are based on the use of homopolymeric DNA. In the present study we provide the first description of heteropolymeric DNA-directed and DNA sequence-selective supramolecular self-assembly of bolaamphiphile **1**. In this system, bolaamphiphile **1** and template oligonucleotides **2** and **3** self-assemble to form helical nanofibers only in the presence of a target oligonucleotide **4** of a specific sequence (Fig. 1(b)). The resultant helical nanofiber is clearly detected by atomic force microscopy (AFM).

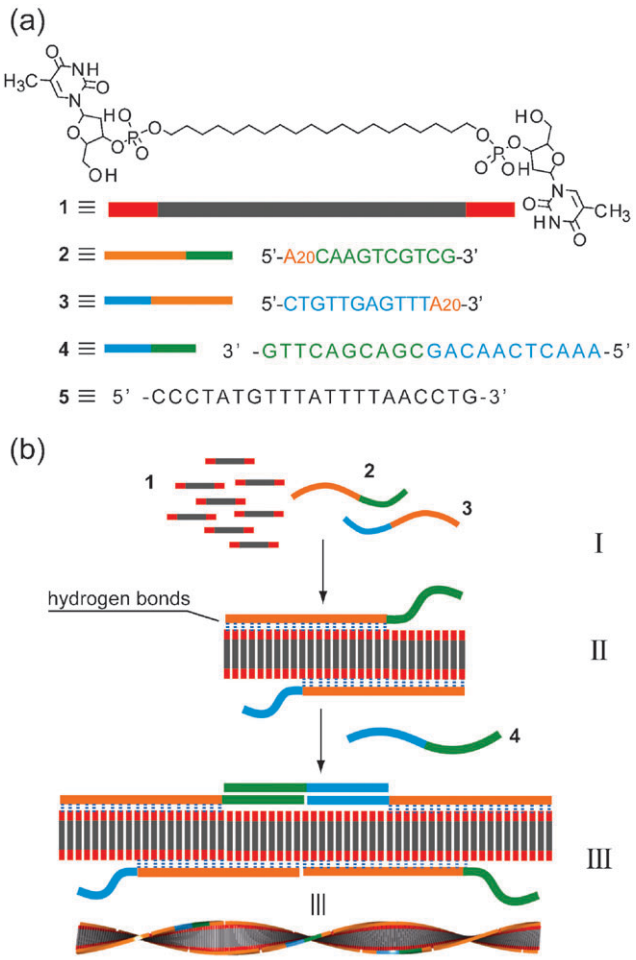


**Fig. 1** (a) Molecular formula of compound **1**, the nucleobase sequences used as templates (**2** and **3**), and the target **4** and non-target **5** oligonucleotides. The green and the blue sequences in **2** and **3**, respectively, can hybridize with **4**. (b) Triggering of helical nanofiber formation in the presence of the target oligonucleotide. (I) Compounds **1**, **2** and **3** were mixed in a buffered solution. (II) Precursors form by complementary base pairing between the thymine moieties in **1** and the adenine moieties in probe oligonucleotides **2** and **3**. Note that many types of precursors could form in addition to that in the illustration. (III) Hybridization of **4** with the set of **2** and **3** templates elongated the nanofiber structure, resulting in helical nanofiber formation. The stoichiometry of the **1**, **2**, **3** and **4** in the illustration does not reflect the experimental conditions used.

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† Electronic supplementary information (ESI) available: Details of sample preparation and experimental methods. Fig. S1: First-derivative curves of UV melting measurements for the **1**/**2**/**3**/**4**, **1**/**2**/**3** and **2**/**3**/**4** systems. Fig. S2: UV melting and first-derivative curves of the **1**/**2**/**3**/**5** system. See DOI: 10.1039/b813592d

 

We utilized bolaamphiphile **1**[19] and oligonucleotide templates **2** and **3** (Fig. 1(a)) to form helical nanofibers. The sequences of oligonucleotide templates **2** and **3** were designed to act as templates for the formation of helical nanofibers[18] and as hybridization probes for the target oligonucleotide **4**. For hybridization, 20-mer adenine regions were connected to the 5′- and 3′-ends of **2** and **3**, respectively. The set of probe regions of **2** and **3** (green and blue sequences in Fig. 1(a)) can also hybridize with the target oligonucleotide **4**. Compound **1** was dissolved in a 0.1× TE buffer solution (1.0 mM Tris-HCl and 0.1 mM EDTA, pH = 8.0) by sonication for 30 min. The templates (**2** and **3**) were added to the solution, and the resulting solution was cooled to room temperature to form a whitish viscous solution (abbreviated **1**/**2**/**3** hereafter). To the aqueous solution of **1**/**2**/**3** we added a solution of the target oligonucleotide **4** dissolved in 0.1× TE buffer; the mixture was then kept at 20 °C for one day. The final concentrations of **1**, **2**, **3** and **4** were $1.8 \times 10^{-2}$, $9 \times 10^{-4}$, $9 \times 10^{-4}$ and $1 \times 10^{-4}$ M, respectively. This solution (abbreviated **1**/**2**/**3**/**4**) was subjected to AFM analysis. For comparison, we prepared a solution containing the non-target oligonucleotide **5** ([**5**] = $1 \times 10^{-4}$ M, abbreviated **1**/**2**/**3**/**5**) by using the same procedure described for the preparation of **1**/**2**/**3**/**4**.

Helical nanofiber structures with lengths ranging from 300 nm to 3 μm and heights ranging from 7 to 8 nm, which had self-assembled from the **1**/**2**/**3**/**4** system, were observed in AFM images. The helical morphology was clearly visualized with a pitch of *ca.* 52 nm (Fig. 2(a) and (b)). Interestingly, the 52-nm pitch of the helical nanofiber formed from the 61-mer template oligonucleotides (30-mer + 31-mer = 61-mer, after hybridization) is consistent with the pitch observed in our previous report.[20] In these investigations the helical pitch of the nanofiber proved to be strongly dependent on the template length. The observed pitches were 11 and 40 nm for 10- and 40-meric template oligoadenylic acids, respectively. In contrast, we were unable to observe any nanofiber structures in the **1**/**2**/**3** and **1**/**2**/**3**/**5** systems (Fig. 2(c) and (d)). Instead, spherical objects with heights of *ca.* 15 nm were observed. These results strongly suggest that target oligonucleotide **4** was crucial to the formation of helical nanofibers, whereas the non-target oligonucleotide **5** had no effect on the morphology of the structures formed in the **1**/**2**/**3** assembly.

To gain insight into the hybridization behavior of **4**, we performed melting experiments with ultraviolet (UV) spectroscopy of the **1**/**2**/**3**/**4**, **1**/**2**/**3** and **2**/**3**/**4** systems in 0.1× TE buffer (Fig. 3). The melting temperatures for the **2**/**3**/**4**[21] and **1**/**2**/**3** systems were 35 and 60 °C, respectively. The melting curve of the **1**/**2**/**3**/**4** system was biphasic, with melting temperatures at 37 and 57 °C, close to those observed individually for the **2**/**3**/**4** and **1**/**2**/**3** systems (see ESI†). However, the UV absorbance for **1** alone in 0.1× TE buffer barely changed upon heating or cooling at the concentration used in these experiments (data not shown). These results strongly support the hybridization of the target oligonucleotide **4** with **2** and **3** (Fig. 3, (b) → (a)), in which a complementary base pair formed between thymine in **1** and adenine in **2** and **3** (Fig. 3, (c) → (b)) in the **1**/**2**/**3**/**4** system.

Although fibrous morphology was not observed in the AFM images for the **1**/**2**/**3** system, the results of the UV melting experiments suggested that complementary base pairing occurred between bolaamphiphiles **1** and **2**, as well as between and **1** and **3** (Fig. 3, (b)). Therefore, we propose that the assembly of **1**, **2** and **3** represents the formation of precursors (Fig. 1(b), parts I and II). In addition, the melting behavior of the **1**/**2**/**3**/**4** system was characterized by a steeper curve and larger changes in absorbance at temperatures ranging from 30 to 40 °C (Fig. 3) than those observed for the **2**/**3**/**4** system. This finding suggests that stacking occurs between the nucleobase moieties in **2**, **3** and **4** and the thymines of bolaamphiphile **1**. Therefore, the hybridization of **4** with **2** and **3** should accompany the columnar arrangement of bolaamphiphile **1**.[18,19] Moreover, such hybridization is likely to support the assembly of the **1**/**2**/**3** precursor structure, which

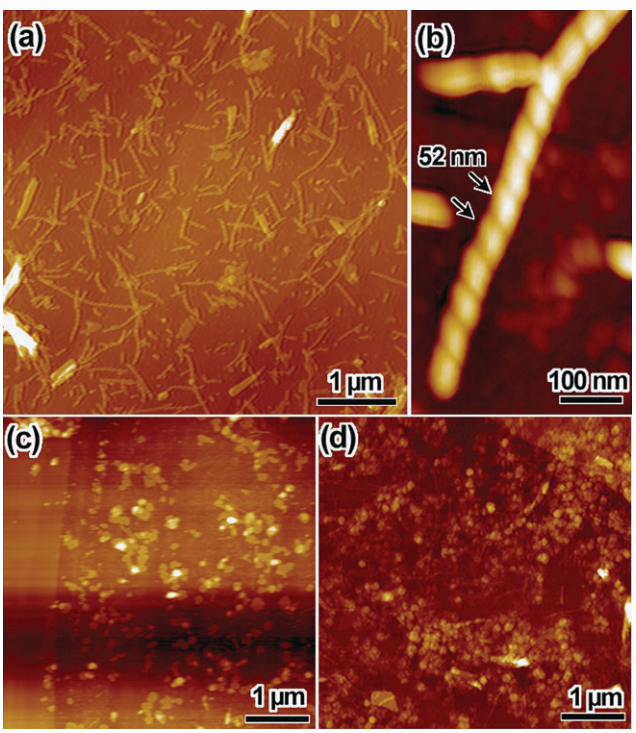


**Fig. 2** AFM images of (a, b) helical nanofibers formed in the **1**/**2**/**3**/**4** system, and spherical objects formed in the (c) **1**/**2**/**3** and (d) **1**/**2**/**3**/**5** systems.

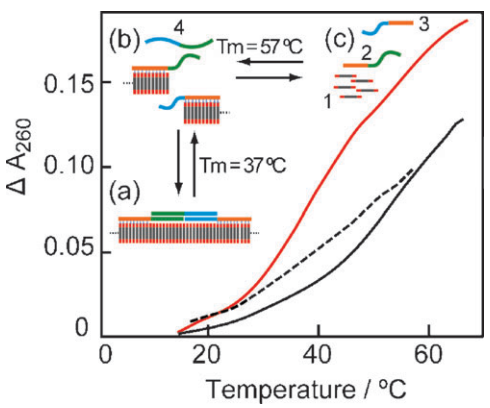


**Fig. 3** UV melting curves recorded by changes in UV absorption spectroscopy at a wavelength of 260 nm ($\Delta A_{260}$) for the **1**/**2**/**3**/**4** (red), **1**/**2**/**3** (black) and **2**/**3**/**4** (dotted) systems in 0.1× TE buffer (pH = 8.0).

propagates nanofiber growth in the length dimension (Fig. 1(b), part III), because the rigid double-helix DNA, which extends over ~150 base pairs,[22,23] probably serves as a template for controlled nanofiber growth.

In conclusion, DNA sequence-selective supramolecular assembly was achieved by the self-assembly of thymidylic acid-appended bolaamphiphile **1**, with heteropolymeric single-strand DNA as a template. In this system, hybridization of the target DNA with the template DNA induces helical nanofiber formation. We believe that our results will contribute greatly to the development of nanotechnology through the application of supramolecular chemistry.

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