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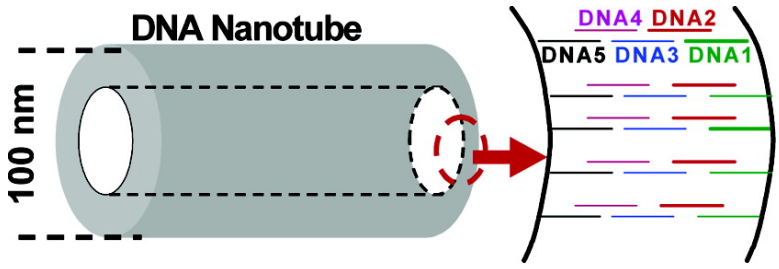

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Template-Synthesized DNA Nanotubes

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There is considerable interest in DNA-functionalized nanotubes with proposed applications that include use as gene delivery vehicles,[1] in DNA-assisted separation and assembly of carbon nanotubes,[2] and in nanotube-based DNA sensing[3a] and separations.[3b] In all of these previous cases, the DNA was attached to a nanotube composed of a second material; it might also be advantageous to have nanotubes composed entirely, or predominately, of DNA itself. We describe here a template synthesis[4a] method for preparing such DNA nanotubes.

The synthetic strategy builds on prior work,[4b] where we used Mallouk's alternating α,ω-diorganophosphonate (α,ω-DOP) Zr(IV) chemistry[5] to deposit layered nanotubes along the pore walls of an alumina template membrane. The layers are held together by bonds between the phosphonate groups and the Zr(IV) ions, and the polyvalency of this interaction provides cross-linking that imparts structural integrity to these layered nanotubes.[6] The DNA nanotubes described here have an outer skin of one or more of these α,ω-DOP/Zr(IV) layers, again to provide structural integrity, surrounding an inner core of multiple double-stranded DNA layers held together by hybridization between the layers (Figure 1).

The template membrane was a nanopore alumina, 36 μm thick, 1 cm^2 area, with ~100 nm diameter pores.[4b] As per our prior work,[4b] both faces of the membrane were first sputtered with a ~5 nm film of Au.[7] The outer α,ω-DOP/Zr(IV) nanotube skin was prepared by immersing the membrane into a 1.25 mM solution of 1,10-decanediylbis(phosphonic acid) (the α,ω-DOP) and then into a 5.0 mM solution of $ZrOCl_2$.[4b,7] While DNA nanotubes with an outer skin consisting of only one such α,ω-DOP/Zr(IV) layer were used for most of the studies described here, analogous data were obtained for DNA tubes with three-layer α,ω-DOP/Zr(IV) outer skins.[7]

The DNA core that comprises the majority of the nanotube was then synthesized within this α,ω-DOP/Zr(IV) skin. A 1 M NaCl solution that was 50 μM in both DNA1 and DNA2 (Figure 1) was prepared, and 24 h were allowed for the two 15-base segments (shown in black font) to hybridize. The membrane containing the α,ω-DOP/Zr(IV) skin was then immersed into this solution for 30 h to allow the phosphonate end of DNA1 to bind to the Zr(IV) on the inner surface of the skin.[4b,8] This results in a nanotube composed of (from outer to inner layer) α,ω-DOP/Zr(IV)/DNA1/DNA2 — a two-layer DNA nanotube. To make a three-layer DNA nanotube, this membrane was subsequently immersed into an analogous solution of DNA3. This results in hybridization of the 15-base segments of DNA2 and DNA3 (green font in Figure 1) to yield a nanotube composed of α,ω-DOP/Zr(IV)/DNA1/DNA2/DNA3. DNA4 and DNA5 were subsequently added to make the four- and five-layer nanotubes. The DNA layers in these tubes are held together by hybridization between DNA segments that are 15 bases long. We have also made nanotubes held together by hybridization between DNA segments 12 and 8 bases long (vide infra).

The nanotubes were liberated by dissolution of the template — in a solution that was 1.5% in H_3PO_4 and 2 M in NaCl at 0 °C — and collected by centrifugation.[7] The high salt content and low temperature ensure that dehybridization does not occur during membrane dissolution.[9] Figure 2A shows a transmission electron micrograph (TEM) of liberated five-layer DNA nanotubes that had an outer skin of only one α,ω-DOP/Zr(IV) layer. The nanotube length is much shorter than the thickness of the template. This indicates that the tubes were broken during membrane dissolution and centrifugation. This problem can be mitigated by preparing nanotubes with outer skins that are three α,ω-DOP/Zr(IV) layers thick. Because this outer skin provides structural integrity, these thicker-skinned nanotubes are less susceptible to breakage (Figure 2B).

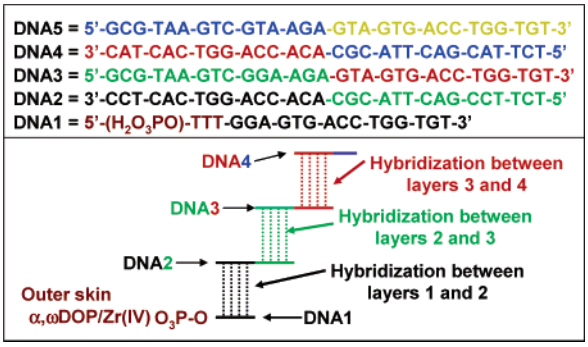

Figure 1. DNAs used to prepare nanotubes where hybridization between segments 15 bases long hold the layers together, and schematic of the hybridization-based layer-by-layer DNA nanotube concept.

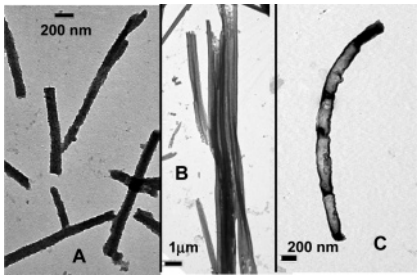

Figure 2. TEM images of five-layer DNA tubes with (A) one-layer and (B) three-layer α,ω-DOP/Zr(IV) skins. (C) Same as that for A, but after heating to 85 °C. The nanotube diameter appears larger than the pore diameter of the template because the tubes collapse on the TEM grid surface.

Because these nanotubes are held together by hybridized (double-stranded) DNA chains (Figure 1), heating the tubes above the melting point of the dsDNA should release the dehybridized ssDNA chains from the tubes; that is, thermal decomposition should provide a route for releasing the "DNA payload" from the these nanotubes. TEM provides the first evidence that this is, indeed, possible. Figure 2C shows an image of a DNA nanotube identical to that in Figure 2A, but after heating in a buffer solution[7] at 85 °C. While the nanotubes that had not been heated are electron opaque (Figure 2A), the heated nanotube is nearly electron transparent (Figure 2C).

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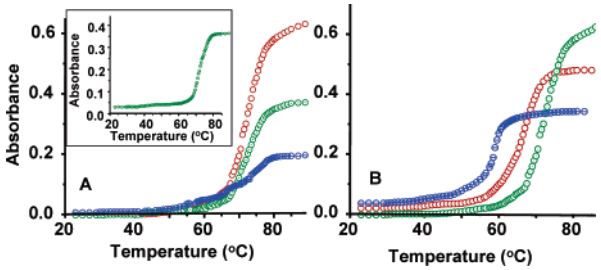

Figure 3. Thermal decomposition curves for (A) nanotubes composed of the DNAs in Figure 1. Number of DNA layers: blue = 2, green = 3, red = 5. The nanotubes were embedded within the pores of the template. Inset shows five-layer tubes that had been liberated from the template. (B) Five-layer nanotubes composed of hybridized 8 base (blue), 12 base (red), and 15 base (green) duplexes.

This is because the DNA payload has been released, and this "ghost" tube consists only of the outer α,ω-DOP/Zr(IV) skin.

To explore this issue quantitatively, samples of DNA-nanotube-containing alumina membranes were immersed into buffer solution and heated from 23 to 85 °C.[7] Because the nanotubes were sequestered within the pores of the template, there was initially no DNA present in the solution. However, melting of the dsDNA duplexes released the component ssDNA chains into the solution, where they were detected by UV absorbance (260 nm). Analogous data were obtained for nanotubes that had been liberated from the template membrane. In this case, the nanotubes were present as a compacted film on the bottom of the centrifuge tube. Buffer was added, and the UV absorbance of the solution above this compacted film was measured as a function of temperature.[7]

Figure 3A shows thermal decomposition curves for two-, three-, and five-layer DNA nanotubes (outer skin = 1 α,ω-DOP/Zr(IV) layer) that were sequestered within the pores of the template membrane. At lower temperatures, no DNA is present in the solution above the membrane, and at higher temperatures, DNA is released with a temperature profile similar to[7] that of a DNA melting curve.[10] Indeed, the duplexes obtained by hybridizing the various 15-base segments in Figure 1 have calculated melting temperatures (T_m) between 70 and 74 °C.[7,10] Correspondingly, the thermal decomposition curves in Figure 3A have temperatures at half-maxima (apparent T_m, $T_{m,app}$)[7] of ~73 °C. $T_{m,app}$ values for nanotubes that were liberated from the template were identical (inset Figure 3A).

The surface coverage of 21-mer DNA duplexes on a nanopore alumina like that used here was found to be $\sim 3\times10^{12}$ per cm^2.[11] Densities on other surfaces are nearly identical.[7] From this number, and the total surface area of our template, we calculate that ~2.7 nmol of DNA2 could be present in 1 cm^2 of our two-layer DNA nanotube membrane;[7] 1.9 ± 0.2 nmol was obtained experimentally. Also, the quantity released from the three-layer nanotubes should be twice that from the two-layer tubes, and an experimental ratio of 1.9 is obtained (Figure 3A).

Thermal decomposition data were also obtained for nanotubes where the DNA layers were held together by hybridized chain segments 12 and 8 bases long (Figure 3B). In both cases, the nanotubes were five DNA layers thick, and the first DNA layer was DNA1 (Figure 1). To make nanotubes held together by hybridized 12-base segments, the second layer was the sequence 3′CAC-TGG-ACC-ACA-ATT-CAG-CCT-TCT5′, termed DNA6. Because the first 12 bases at the 3′ end of DNA6 are complementary to the last 12 bases at the 3′ of DNA1, DNA1 and DNA6 form a 12-base duplex.[7] The third layer was 5′TAA-GTC-GGA-AGA-GTG-ACC-TGG-TGT3′, termed DNA7. The fourth layer was DNA6, and the fifth layer was DNA7. A similar route was used to prepare the nanotubes held together by hybridization between segments 8 bases long.[7]

Figure 3B compares thermal decomposition curves for five-layer DNA nanotubes where the layers were held together by duplexes 8, 12, and 15 bases long. The $T_{m,app}$ values obtained from these curves were 8 base = 55 °C, 12 base = 62 °C, and 15 base = 73 °C. These $T_{m,app}$ values are in good agreement with calculated T_m values for the various duplexes comprising these DNA nanotubes.[7,10] Finally, Figure 3B also shows, as expected, that the total quantity of oligonucleotide released increases with the length of the DNA chains comprising the nanotubes.

We have described a new family of layer-by-layer template-synthesized nanotubes where the bulk of the tube is composed of DNA, and the layers are held together by hybridization of complementary DNA strands. The DNA molecules comprising these tubes can be varied at will, and the DNA can be released from the tube by melting of the duplexes.

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Supporting Information Available: Details of experimental procedures (PDF). Complete author list for ref 2a is also there. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Pantarotto, D.; Singh, R.; McCarthy, D.; Erhardt, M.; Briand, J.-P.; Prato, M.; Kostarelos, K.; Bianco, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5242−5246. (b) Kam, N. W. S.; Jessop, T. C.; Wender, P. A.; Dai, H. *J. Am. Chem. Soc.* **2004**, *126*, 6850−6851.
(2) (a) Zheng et al. *Science* **2003**, *302*, 1545−1548. (b) Xin, H.; Woolley, A. T. *J. Am. Chem. Soc.* **2003**, *125*, 8710−8711.
(3) (a) Wang, J.; Liu, G.; Jan, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 3010−3011. (b) Kohli, P.; Harrell, C. C.; Cao, Z.; Gasparac, R.; Tan, W.; Martin, C. R. *Science* **2004**, *305*, 984−986.
(4) (a) Martin, C. R.; Kohli, P. *Nat. Rev. Drug Discovery* **2003**, *2*, 29−37. (b) Hou, S.; Harrell, C. C.; Trofin, L.; Martin, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 5674−5675.
(5) Lee, H.; Kepley, L. J.; Hong, H. G.; Akhter, S.; Mallouk, T. E. *J. Phys. Chem.* **1988**, *92*, 2597−2601.
(6) Katz, H. E.; Wilsin, W. L.; Scheller, G. *J. Am. Chem. Soc.* **1994**, *116*, 6636−6640.
(7) See Supporting Information for details.
(8) Nonglaton, G.; Benitez, I. O.; Guisle, I.; Pipelier, M.; Léger, J.; Dubreuil, D.; Tellier, C.; Talham, D. R.; Bujoli, B. *J. Am. Chem. Soc.* **2004**, *126*, 1497−1502.
(9) The low temperature also minimizes hydrolytic depurination known to occur at low pH. (See, for example: Lehninger, A. L *Biochemistry*; Worth Publishers: New York, 1970; p 256.) That we minimized such damage is proven by the fact that the nanotubes that were left embedded in the template (no exposure to acid) have the same melting points as the nanotubes liberated from the template.
(10) Zuker, M. *Nucleic Acids Res.* **2003**, *31*, 3406−3415.
(11) Vlassiouk, I.; Krasnoslobodtsev, A.; Smirnov, S.; Germann, M. *Langmuir* **2004**, *20*, 9913−9915.

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