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Templated Synthesis of DNA Nanotubes with Controlled, Predetermined Lengths

Pik Kwan Lo, Florian Altvater, and Hanadi F. Sleiman*

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Quebec H3A 2K6, Canada

Received March 2, 2010; E-mail: hanadi.sleiman@mcgill.ca

Abstract: We report a DNA-templated approach to construct nanotubes with controlled lengths and narrow molecular weight distribution, allowing the deliberate variation of this length. This approach relies on the facile and modular assembly of a DNA guide strand of precise length that contains single-stranded gaps repeating at every 50 nm. This is followed by positioning triangular DNA "rungs" on each of these single-stranded gaps and adding identical linking strands to the two other sides of the triangles to close the DNA nanotubes. The length of the guide strand can be deliberately changed. We show the use of this approach to produce nanotubes with lengths of 1 μm or 500 nm and narrow length distributions. This is in contrast to nontemplated approaches, which lead to long and polydisperse nanotubes. We also demonstrate the encapsulation of 20 nm gold nanoparticles within these well-defined nanotubes to form finite lines of gold nanoparticles with longitudinal plasmon coupling, with a number of potential nanophotonic applications. This guiding strand approach is a useful tool in the creation of DNA nanostructures, in this case allowing the use of a simple template generated by a minimal number of DNA strands to program the length and molecular weight distribution of assemblies, as well as to organize any number of DNA-labeled nano-objects into finite structures.

The controlled self-assembly of discrete nanostructures with well-defined shapes and narrow size distributions has attracted increased attention to meet the demand for advanced electronic and optical devices and tools for nanomedicine.[1] In this regard, considerable effort has been directed toward self-assembled nanotubes because of their high aspect ratio and encapsulation potential. Within the past decade, a number of noncovalent approaches have been used to obtain nanotubes composed of organic,[2] inorganic,[3] polymeric,[4] or biological molecules.[5] In particular, DNA has been used as a construction material for nanotubes,[6] enabling the precise control of their chirality,[7] geometry,[8] circumference,[9,14] persistence length,[10,11] and loading and positioning of materials.[11] A particularly attractive goal would be to access DNA nanotubes with a predetermined length and narrow molecular weight distribution. This would allow their use as templates for one-dimensional (1D) materials of preset lengths for nano-optics and nanoelectronics, discrete hosts for the encapsulation and release of guests, drug nanocarriers with controlled sizes, finite tracks for molecular motors, and liquid crystalline materials, among many other applications.

The 1D growth of rod-shaped viruses, such as the tobacco mosaic virus (TMV), is guided by a central nucleic acid template that controls and delimits the length of each virus.[12] We here report a DNA-templated approach to construct nanotubes with predetermined lengths and narrow molecular weight distribution, allowing the deliberate variation of this length. This approach relies on the facile assembly of a DNA guide strand of precise length that contains single-stranded gaps repeating at every 50 nm. This is followed by positioning triangular DNA "rungs" on each of these single-stranded gaps[8,11,13] and adding identical linking strands to the two other sides of the triangles to close the DNA nanotubes. The length of the guide strand can be deliberately changed. We show the use of this approach to produce nanotubes with lengths of 1 μm or 500 nm and narrow molecular weight distributions. We then demonstrate the encapsulation of 20 nm gold nanoparticles within these well-defined nanotubes to form finite lines of gold nanoparticles with longitudinal plasmon coupling.

For this approach to be successful, we needed to access linear DNA guide strands of arbitarily designed lengths, from the nanometer to the micrometer range. In principle, a DNA origami approach could be used to provide template strands.[14] However, in practice, this limits the generality of the approach to a handful of genomic templates (currently the M13 viral genome) and necessitates the use of a very large number (hundreds) of staple DNA strands, each with a different sequence. In examining the features of the guide strand, we noted that it could be composed of repeating DNA sequences **C** onto which the rungs are attached and that the only locations where unique sequences need to be included are in the strands that link these repeating DNA strands. Sequences **C** were constructed by assembling together DNA repeat strands **A** (*abc*) and **B** (*def*), using appropriate linker strands (Scheme 1; each letter in italics is a unique DNA sequence). Sequence *b* within the repeating unit **C** remained single-stranded and served as the attachment point for the triangular rungs along the length of the nanotube. To create a 1 μm template strand, 21 repeat units **C** are required: each unit **C** is equipped with linker strands that are complementary to its two sticky ends, *a* and *f*, and allow it to selectively hybridize to the next repeat unit (using 10-base sticky ends). These are the only sequences along the length of the nanotube that need to be varied (Scheme 1a). The two outer repeat units, **C1** and **C21**, are capped with blunt-ended DNA that terminates the assembly into a finite, 1 μm DNA template (Scheme 1b). This simple methodology allows deliberate control of the length of the DNA assembly, merely by changing the number of repeat units in the assembly, while using a minimal number of unique DNA sequences. This approach also allows the modification of each repeat unit down the length of the nanotube independently from the others and can result in longitudinally variable nanotubes.

The assembly of **A**−**C** by hybridization of their constituent strands was carried out at room temperature and was quantitative for all strands.[17] **C1**−**C21** were then sequentially combined at room temperature, to construct the 1 μm DNA guide strand **1**. The triangular DNA rungs **3**[8,11,13] were constructed by first synthesizing a single continuous DNA strand, embedded with the three rigid organic vertex molecules. Cyclization using a template DNA strand and chemical ligation using cyanogen bromide yielded single-stranded DNA triangle **2**. Rung **3** was then assembled from **2**, three complementary strands (**CS**) with sticky-end overhangs, and two rigidifying strands (**RS**). This assembly was quantitative at every step (Scheme 1c).[17]

With template **1** and unit **3** in hand, we proceeded to examine their potential to generate DNA nanotubes of controlled length. Every 50 nm along template **1** has sequence *b*, which can be hybridized to triangle **3**. Mixing **1** and **3** resulted in positioning these rungs on positions *b* to form partially open linear assemblies **4**. Two identical strands (**LS**) were then added to link together the two other vertical strands on rung **3**, thus closing the assemblies **4** to form nanotubes **5**

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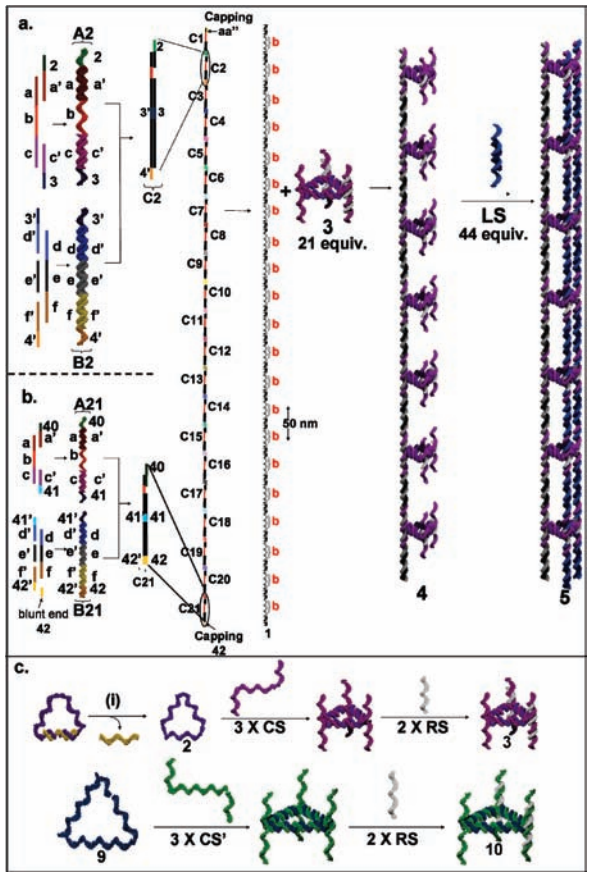
Scheme 1. Construction Approach[a]



[a] (a) Units **C** are constructed from **A** and **B**, each with a repeat strand (*abc* and *def*) and sticky ends of unique sequences; these allow **C1** to **C21** to selectively hybridize to each other into a 1 μm template sequence **1**. Triangular rungs **3** can hybridize to the repeat single-stranded units *b* of template **1**. Addition of linking strands **LS** give the closed nanotube **5**. (b) Capping units **C1** and **C21** have blunt ends that terminate the growth of **5**. Different colors represent different DNA sequences. Red lines (*b*) are single-stranded gaps along template **1**. (c) Construction of rungs **3** (7 nm sides) and **10** (14 nm).[17]

(Scheme 1a).[17] Efficient generation of nanotubes **5** with controlled length was confirmed by atomic force microscopy (AFM).[17] Lateral cross-sectional analysis of the nanotubes showed them to be of nearly identical heights, ~1.2 nm.[17] In our initial studies, the DNA nanotubes were directly deposited on a mica surface from Mg^{2+}-containing solutions and the samples air-dried. These showed a large population of nanotubes of the expected 1 μm length (Figure 1a-*i*) for both open **4** and closed **5**.[17] However, some aggregation and some structures with reduced lengths were observed. Previous measurements of DNA contour lengths by AFM in air have been complicated by surface drying and conformational changes of DNA when in contact with the substrate surface.[15] As a result, we thought to introduce a streptavidin (STV) spacer between the nanotubes and the mica surface. A new template strand, **1-BL**, was created, in which four biotin moieties were precisely placed on its units **C2**, **C6**, **C12**, and **C18**, and it gave 1 μm biotinylated nanotubes **5-BL** (Scheme 2a). These were immobilized on a STV-functionalized mica surface. Almost 85% of the nanotubes were 0.8−1 μm long (Figure 1a-*ii*,*iii*)[17] using this approach. This method also illustrates the ability to precisely modify every unit of the nanotube independently, in this case by selective biotin labeling. In contrast, nontemplated synthesis of DNA nanotubes reported previously[8,11] yielded long nanotube architectures with polydisperse sizes (Figure 1a-*iv*). Thus, our DNA-templated approach generates uniform DNA nanotubes of controlled length, geometry, and diameter.

Importantly, this DNA-templated approach can be used to create nanotubes with any designed length by adding or subtracting repeat

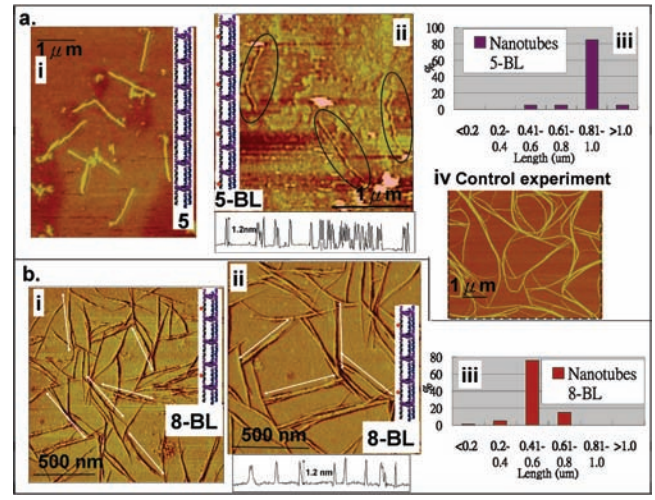
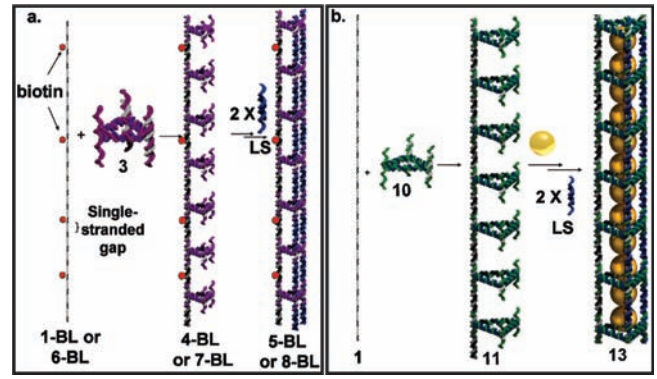


Figure 1. AFM characterization and length statistical analysis of DNA nanotubes. (a) AFM height images of (*i*) nanotubes **5** dried directly on mica and (*ii*) biotinylated nanotubes **5-BL** on streptavidin-coated mica (lower part, cross-section height analysis), (*iii*) length statistical analysis of **5-BL**, and (iv) AFM image of nontemplated synthesis of DNA nanotubes, which yields long nanotube architectures with polydisperse sizes. (b) AFM phase images of (*i*,*ii*) nanotubes **8-BL** on streptavidin-coated mica, including cross-section height analysis, and (*iii*) length statistical analysis of **8-BL**.

Scheme 2. Construction of Biotinylated DNA Nanotubes **5-BL** and **8-BL** and Gold Nanoparticle-Encapsulated **13**[a]



[a] (a) Formation of **5-BL** and **8-BL** using 1 or 0.55 μm guide strands **1-BL** and **6-BL**, each site-specifically labeled with biotin (red circles). (b) Rungs **10** (14 nm sides) were added to template **1** to form the open assemblies **11**, and then 20 nm AuNPs and linking strands **LS** were added to generate nanotubes **13**.

units **C** to or from the template strand. We generated a shorter biotinylated template, **6-BL** (~0.55 μm), by adding only the first 11 pieces of the segments **C1**−**C11** in order to direct the assembly of shorter triangular DNA nanotubes **8-BL** (with two biotin units at **C2** and **C8**; Scheme 2a).[17] Statistical length analysis on individual tube **8-BL** shows a narrow length distribution, with a yield of ~78% nanotubes between 0.4 and 0.6 μm (Figure 1b-*i*−*iii*). The phase images clearly show association of two or more nanotubes by "coiling together". Interestingly, this forms a network with relatively angular features that are spaced by ~500 nm, i.e., the expected length of a nanotube. Thus, the population of nanotubes that are longer than the template is the result of this nanotube association.

The ability to encapsulate and position cargo within these nanotubes is a prerequisite for integrating them into functional devices and drug delivery tools.[11] For these studies, we constructed a triangular nanotube, **12**, with a larger diameter (length of triangle arm, ~14 nm) by hybridizing the larger triangular rung **10** to the 1 μm template strand **1** and adding the **LS**.[17] To test the encapsulation potential of these structures, 20 nm gold nanoparticles (AuNPs) coated with a citrate

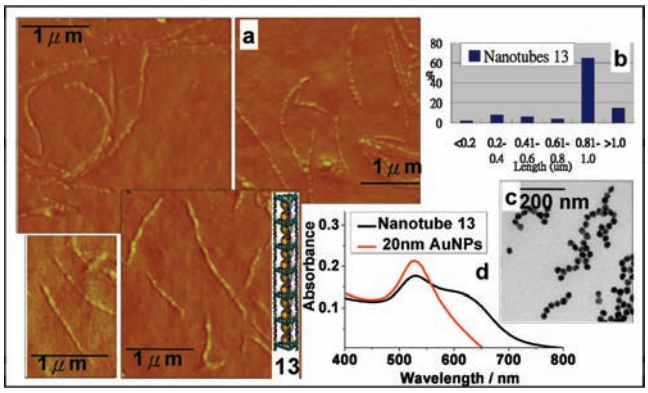


Figure 2. (a) AFM phase images, (b) length statistical analysis, (c) TEM images, and (d) UV−vis measurement of gold nanoparticle encapsulated nanotubes **13**.

shell were added to the open form **11** (Scheme 2b, in Mg^{2+}-containing solutions). This was followed by addition of the linking strands **LS**, which would close the nanotube and trap these particles (Scheme 2b).[11a] AFM and transmission electron microscopy (TEM) studies confirmed the encapsulation of AuNPs in these nanotubes **13**, showing many finite lines of particles in close proximity (Figure 2a,c). Nanotubes **13** were significantly greater in height (~6 nm) than unfilled nanotubes **12**.[17] While the finite lines of AuNPs appeared to be rigid on the hydrophilic mica surface in the AFM experiment, distortions were noted by TEM, using hydrophobic carbon-coated grids. Statistical length analysis was conducted on individual gold-encapsulated nanotubes **13**, showing a yield of ~65% nanotubes of length 0.8−1 μm (Figure 2b). Encapsulated nanotubes **13** had a more rigid architecture toward imaging. A total of 15% of the encapsulated nanotubes were longer than 1 μm, likely due to end-to-end association upon drying. UV−vis spectra of **13** showed a decrease in the 525 nm plasmon band, along with a red-shifted and low-energy shoulder peak at 630 nm, which was ascribed to a longitudinal plasmon band (Figure 2d).

To ascertain encapsulation, control experiments were performed by (i) assembling nanotube **12** in the presence of 10 nm AuNPs that would be too small to stay trapped on its inside, (ii) assembling smaller nanotube **5** (Scheme 1) in the presence of 20 nm AuNPs that would be too large to be encapsulated, or (iii) adding 20 nm AuNPs to preformed nanotubes **12**. All these showed no particle lines and absence of the band at 630 nm.[17] This confirms that the observed particle lines arise from encapsulated particles, rather than particles bound to the nanotubes' exterior. It also suggests a "sieving" capacity of these nanotubes, allowing them to encapsulate nanoparticles of one unique size that matches their cavity dimensions.[11a] The creation of finite gold nanoparticle lines within these nanotubes can result in a number of applications, including for direct optical modulation of conductivity, for guiding light on the nanometer scale below its diffraction limit, and as templates for finite metallic nanowires of deliberately variable dimensions.[16]

In summary, we have presented a facile and general method for the formation of DNA nanotubes with controlled, predetermined lengths. This was achieved using a finite linear DNA template that limits the 1D growth of DNA nanotubes. The template is assembled in a modular manner that allows deliberate variation of its length using a minimal number of DNA sequences. Using this approach, nanotubes with well-defined lengths of 0.55 and 1 μm and two different inner diameters were readily prepared. The two-step process for the preparation of these nanotubes, which involves positioning of the rungs on the DNA guide strand followed by closure of the nanotubes, allows for the encapsulation of gold nanoparticle guests within these structures. This gives rise to gold "nano-peapod" lines with controlled length, an associated longitudinal plasmon band, and a number of potential nanophotonics applications. This guiding strand approach is thus a useful tool to program the length and molecular weight distribution of assemblies, as well as to organize any number of DNA-labeled nano-objects into finite structures. Ongoing efforts in our laboratory focus on positioning rungs of different shapes on these guiding strands to create longitudinally variable nanotubes for the directional transport of materials.

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Supporting Information Available: Sequences, self-assembly, and AFM analysis of DNA nanotubes. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Wang, Z. M., Ed. *One-Dimensional Nanostructures: Lecture Notes in Nanoscale Science and Technology*; Vol. 3; Springer: New York, 2008; p 19. (b) Cao, G. *Nanostructures and Nanomaterials—Synthesis, Properties and Applications*; Imperial College: London, 2004; p 110.
(2) (a) Zhang, L.; Rakotondradany, F.; Myles, A. J.; Fenniri, H.; Webster, T. J. *Biomaterials* **2009**, *7*, 1309–1320. (b) Yamamoto, T.; Fukushima, T.; Aida, T. *Adv. Polym. Sci.* **2008**, *220*, 1–27. (c) Shimizu, T. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 2601–2611.
(3) Tominaga, M.; Fujita, M. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 1473–1482.
(4) (a) Yoog, H.; Yoon, H.; Lee, S. H.; Kwon, O. S.; Song, H. S.; Oh, E. H.; Park, T. H.; Jang, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 2755–2758. (b) Liu, G. *Adv. Polym. Sci.* **2008**, *220*, 29–64.
(5) (a) Zhou, Y. *Crit. Rev. Solid State Mater. Sci.* **2008**, *33*, 183–196. (b) Zhou, Y.; Shimizu, T. *Chem. Mater.* **2008**, *20*, 625–633. (c) Shao, H.; Parquette, J. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 2525–2528. (d) Gazit, E. *Chem. Soc. Rev.* **2007**, *36*, 1263–1269. (e) Ngweniform, P.; Li, D.; Mao, C. *Soft Matter* **2009**, *5*, 954–956.
(6) (a) Ko, S. H.; Liu, H.; Chen, Y.; Mao, C. *Biomacromolecules* **2008**, *9*, 3039–3043. (b) Mathieu, F.; Liao, S.; Mao, C.; Kopatsch, J.; Wang, T.; Seeman, N. C. *Nano Lett.* **2005**, *5*, 661–665. (c) Kuzuya, A.; Wang, R.; Sha, R.; Seeman, N. C. *Nano Lett.* **2007**, *7*, 1757–1763. (d) Wang, R.; Liu, W.; Seeman, N. C. *Chem. Biol.* **2009**, *16*, 862–867.
(7) Mitchell, J. C.; Harris, J. R.; Malo, J.; Bath, J.; Turberfield, A. J. *J. Am. Chem. Soc.* **2004**, *126*, 16342–16343.
(8) Aldaye, F. A.; Lo, P. K.; Karam, P.; McLaughlin, C. K.; Cosa, G.; Sleiman, H. F. *Nature Nanotechnol.* **2009**, *4*, 349–352.
(9) (a) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882–1884. (b) Yin, P.; Hariadi, R. F.; Sahu, S.; Choi, H. M. T.; Park, S. H.; LaBean, T. H.; Reif, J. H. *Science* **2008**, *321*, 824–826.
(10) Rothemund, P. W. K.; Ekani-Nkodo, A.; Papadakis, N.; Kumar, A.; Fygenson, D. K.; Winfree, E. *J. Am. Chem. Soc.* **2004**, *126*, 16344–16352.
(11) (a) Lo, P. K.; Karam, P.; Aldaye, F. A.; McLaughlin, C.; Hamblin, G.; Cosa, G.; Sleiman, H. F. *Nature Chem.* **2010**, *2*, 319–328. (b) Sharma, J.; Chhabra, R.; Cheng, A.; Brownell, J.; Liu, Y.; Yan, H *Science* **2009**, *323*, 112–114.
(12) (a) Klug, A. *Philos. Trans. R. Soc. London, Ser. B* **1999**, *354*, 531–535. (b) Bull, S. R.; Palmer, L. C.; Fry, N. J.; Greenfield, M. A.; Messmore, B. W.; Meade, T. J.; Stupp, S. I. *J. Am. Chem. Soc.* **2008**, *128*, 8975–8979.
(13) (a) Aldaye, F. A.; Palmer, A. L.; Sleiman, H. F. *Science* **2008**, *321*, 1795–1799. (b) Aldaye, F. A.; Sleiman, H. F. *Angew. Chem., Int. Ed.* **2006**, *118*, 2204–2209. (c) Aldaye, F. A.; Sleiman, H. F. *J. Am. Chem. Soc.* **2007**, *129*, 4130–4131. (d) Aldaye, F. A.; Sleiman, H. F. *J. Am. Chem. Soc.* **2007**, *129*, 13376–13377.
(14) (a) Rothemund, P. W. K. *Nature* **2006**, *440*, 297–302. (b) Douglas, S. M.; Chou, J. J.; Shih, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 6644–6648. (c) Stearns, L. A.; Chhabra, R.; Sharma, J.; Liu, Y.; Petuskey, W. T.; Yan, H.; Chaput, J. C. *Angew. Chem.* **2009**, *48*, 8494–8496.
(15) Sanchez-Sevilla, A.; Thimonier, J.; Marilley, M.; Rocca-Serra, J.; Barbet, J. *Ultramicroscopy* **2002**, *92*, 151–158.
(16) (a) Meital, R.; Ehud, G. *Science* **2003**, *300*, 625–627. (b) Hu, M. S.; Chen, H. L.; Shen, C. H.; Hong, L. S.; Huang, B. R.; Chen, K. H.; Chen, L. C. *Nat. Mater.* **2006**, *5*, 102–106. (c) Wang, C. J.; Lin, L. Y. *Nanoscale Res. Lett.* **2007**, *2*, 219–229. (d) Liu, D.; Park, S. H.; Reif, J. H.; LaBean, T. H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 717–722. (e) Lin, H. Y.; Tsai, L. C.; Chen, C. D. *Adv. Funct. Mater.* **2007**, *17*, 3182–3186. (f) Warner, M. G.; Hutchison, J. E. *Nat. Mater.* **2003**, *2*, 272–277. (g) Luo, J.; Huang, Z.; Zhao, Y.; Zhang, L.; Zhu, J. *Adv. Mater.* **2004**, *16*, 1512–1515. (h) Nakao, H.; Shiigi, H.; Yamamoto, Y.; Tokonami, S.; Nagaoka, T.; Sugiyama, S.; Ohtani, T. *Nano Lett.* **2003**, *3*, 1391–1394.
(17) See Supporting Information.

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