Nanostructures

Control of Nanoparticle Assembly by Using DNA-Modified Diatom Templates**

*Nathaniel L. Rosi, C. Shad Thaxton, and Chad A. Mirkin**

Microorganisms have proven to be versatile templates for the organization of nanostructured materials into larger-scale functional architectures. In general, methods exist either to nonspecifically adsorb or nucleate the growth of nanoparticles onto template surfaces or to genetically manipulate the template organism to express desired functional groups that specifically interact with or nucleate nanoparticles.[1–5] An ideal biological template would be one that could be chemically modified in a versatile manner by using conventional bench-top methods so that the interaction between the template and the nanostructured materials could be understood and easily controlled. To this end, we have investigated the use of diatoms as templates for the assembly of prefabricated nanoparticles.[6–9] Diatoms are a diverse class of unicellular algae and are classified based upon the unique shapes and ornate structural features of their silica cell walls. There are literally thousands of readily available and taxonomically different diatoms.[10] Their cell walls have two halves (frustules), which fit together like a petri dish and generally have dimensions between 1 and 100 μm.

For classification purposes, the organic components of the diatoms are routinely digested in an acidic bath (Piranha) to allow more precise examination of their silica cell walls; the ability to use such corrosive conditions serves as a testament to their robust and stable nature. Because glass functionalization processes involve acidic activation followed by a reaction with silanes,[11] we reasoned that a similar protocol could be used to chemically program diatom surfaces to interact with specific nanoparticles of interest. Herein, we show that diatom cell walls can be covalently functionalized with DNA and then used as templates for the sequence-specific assembly of DNA-functionalized nanoparticles. We further demonstrate that DNA can program the assembly of multiple layers of nanoparticles onto the template.

In a typical experiment (Figure 1),[12] diatoms (either *Synedra* or *Navicula*; **1**) were cultured from a freshwater silicate-rich growth medium for two weeks under ambient cool-white fluorescent light (Carolina Biological Supply Company). A Piranha solution was used both to digest the organic component of the diatoms and to activate their cell walls (**2**) for amino-silane functionalization, which was in turn effected by using standard methods.[11] The amino-functionalized diatoms were then coupled to fluorophore-labeled thiolated DNA (3′-HS-C_3H_6-Cy3-AATATTGATAAGGAT-5′, **a**) by using succinimidyl 4-[*p*-maleimidophenyl]butyrate (SMPB, Pierce Biotechnology, Inc.), a hetero-bifunctional crosslinking agent. The DNA-functionalized diatoms (**3**) were stored as a suspension in phosphate-buffered saline (PBS; 1 mL, 0.3 M). Due to the presence of Cy3, they appeared pink and fluoresced at 563 nm upon excitation with a mercury lamp (Figure 2a).

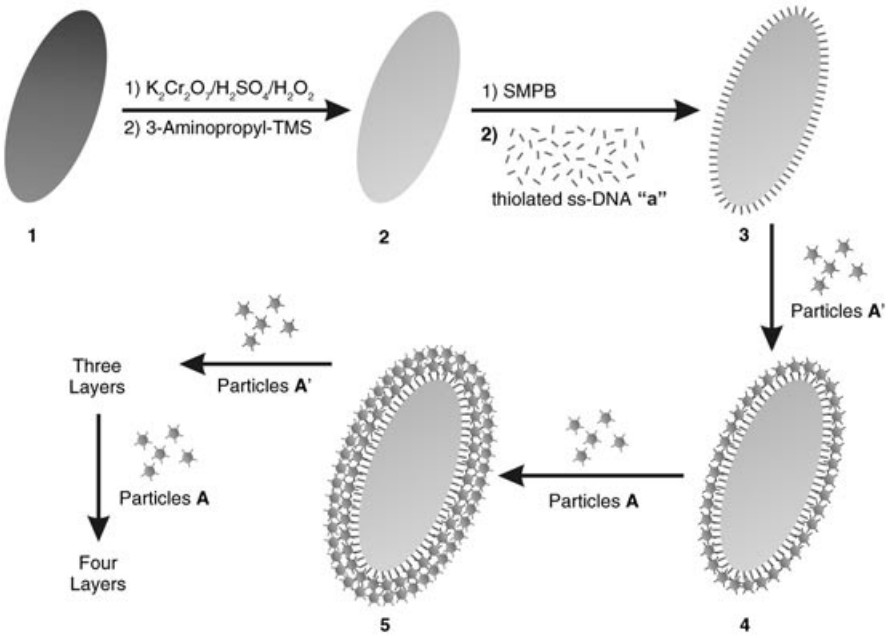

Figure 1. Scheme depicting the functionalization of diatom templates with DNA and DNA-functionalized nanoparticles. A Piranha solution was used to digest the organic components of the diatoms (**1**) and activate their cell walls for reaction with 3-aminopropyltrimethoxysilane (amino-propyl-TMS). The amino-functionalized diatoms (**2**) were coupled to thiolated DNA by using succinimidyl 4-[*p*-maleimidophenyl]butyrate (SMPB) to generate a DNA-functionalized template (**3**). **3** was coated with 13 nm gold particles functionalized with complementary DNA (Particles A′) to form a three-dimensional material (**4**) that adopts the size and shape of the diatom template. A bilayer material (**5**) was generated from the reaction with particles **A**; up to seven nanoparticle layers were added by iterative reactions with particles **A**′ followed by particles **A**.

[*] N. L. Rosi, C. S. Thaxton, Prof. C. A. Mirkin
Department of Chemistry and
Institute for Nanotechnology
Northwestern University
2145 Sheridan Road, Evanston, IL 60208-3113 (USA)
Fax: (+1) 847-467-5123
E-mail: chadnano@northwestern.edu

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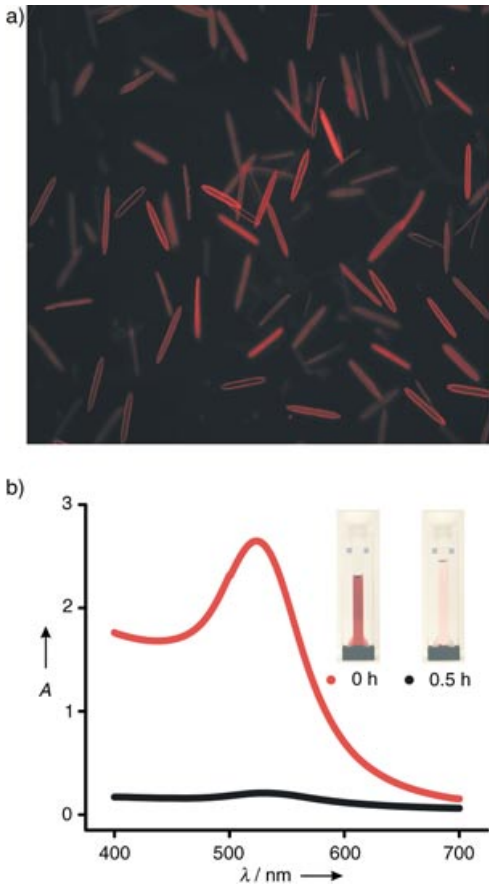

Figure 2. Upon covalent immobilization of fluorophore-labeled thiolated DNA onto their surfaces, the diatoms can be visualized by using fluorescence microscopy (a). The reaction of DNA-functionalized diatoms with complementary DNA-functionalized nanoparticles can be monitored unaided with the naked-eye or by UV/Vis spectroscopy (b). At 0 h, the DNA-functionalized 13 nm gold colloid appears dark red (left, inset) and exhibits a strong extinction band at 520 nm. After 0.5 h of reaction with the DNA-functionalized diatom templates, the solution appears light-pink (right, inset), and there is a dramatic decrease in the extinction peak at 520 nm. This is due to the complementary DNA hybridization that directs the assembly of gold nanoparticles onto the diatom templates.

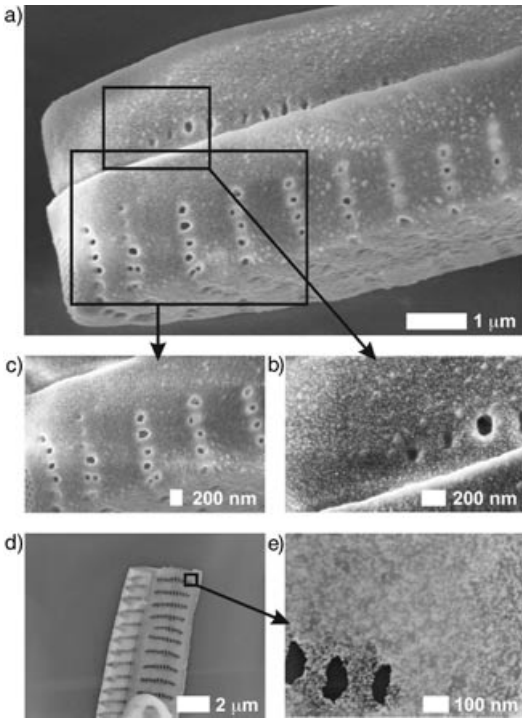

Figure 3. Electron microscope images of DNA-functionalized *Synedra* diatoms densely coated with one layer of DNA-modified 13 nm gold particles. SEM images (a–c), at different magnifications, reveal a portion of a diatom where the two frustules remain attached. Note the regularly patterned nanoscale pores on the *Synedra* surface. In some cases, the diatom frustules are detached from one another. This is exemplified in the TEM images (d,e), which show the inside wall of a detached frustule. The definition of the nanoparticle coverage is best illustrated in b), exterior wall coverage, and e) interior wall coverage.

To demonstrate their utility as templates for the assembly of nanoparticles, a sample of DNA-functionalized diatoms (typically ≈250 μL) was exposed to 13 nm Au particles (particles **A′**) functionalized with complementary 3′-propylthiol-capped oligonucleotide strands (3′-HS-C_3H_6-ATCCTTATCAATATT-5′; **a′**; Figure 1).[13] After about 30 min at room temperature followed by brief centrifugation (3000 rpm), most of the nanoparticles assembled onto the diatom surfaces (**4**) as evidenced by both the naked-eye and UV/Vis spectroscopy (Figure 2b). Fresh colloid was added iteratively until the solution remained red for at least 24 h to fully saturate available surface binding sites. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of the coated diatoms revealed nearly saturated monolayer coverage for both *Synedra* (Figure 3) and *Navicula* (Figure 4), with the nanoparticle coating adopting the surface morphology and shape of the diatom templates. In some cases, the diatom frustules remained attached and together (Figure 3a–c and Figure 4a–c) while in other cases they were either separated (Figure 3d,e) or displaced from one another (Figure 4d,e). From these examples, we can conclude that both the interior and exterior of the cell walls are coated with nanoparticles. It is important to note that the assembly process is directed by the sequence specific interactions between the DNA-functionalized diatoms and the DNA-functionalized nanoparticles-no assembly was observed when nanoparticles modified with noncomplementary DNA were used.

Because DNA is used to direct the template–nanoparticle assembly process, raising the temperature beyond the melting point of the duplex DNA results in release of the nanoparticles from the template. This process can be monitored using UV/Vis spectroscopy by measuring the increase of the characteristic nanoparticle surface plasmon band at 520 nm as a function of increasing temperature. The resulting "melting" curves (Figure 5) provide further evidence of the hybridization-directed assembly. Moreover, the sharp melting transitions, which result from a cooperative melting effect routinely observed for aggregates of DNA-functionalized nanoparticles,[14] corroborate the dense surface coverage observed by electron microscopy. The observed melting transitions are slightly broader (≈8–10 °C) than those reported for monolayers of 13 nm gold particles on glass

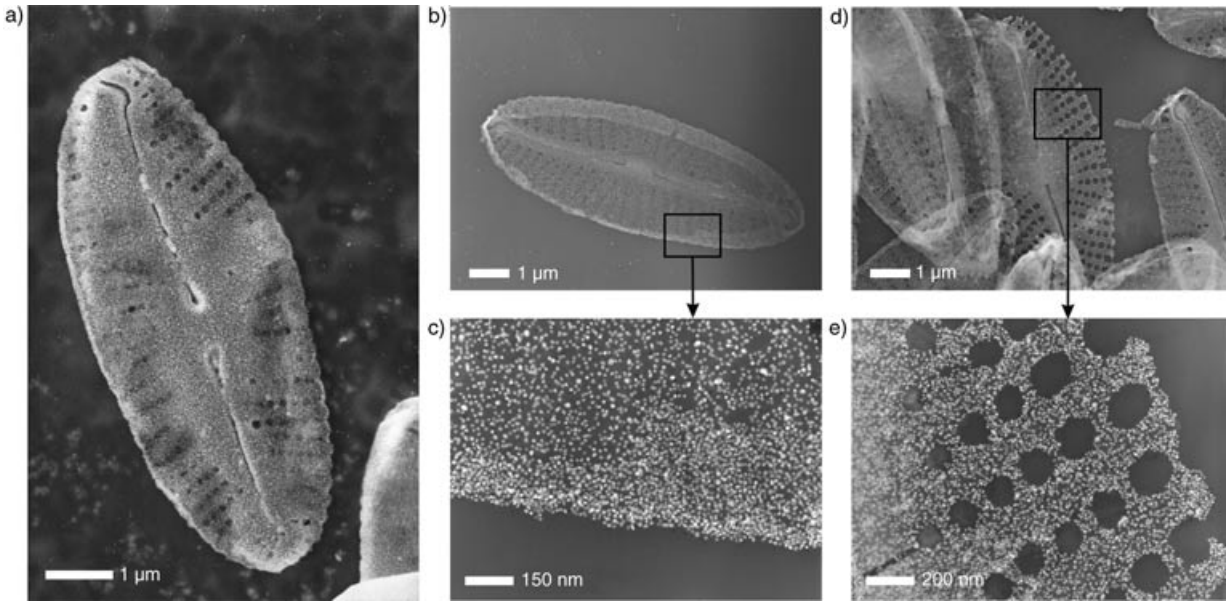

Figure 4. Electron microscope images of DNA-functionalized *Navicula* diatoms densely coated with one layer of DNA-modified 13 nm gold particles. SEM (a) and TEM images (b,c) both reveal a dense outer coating of nanoparticles on the *Navicula* surface. The homogeneous nanoparticle coating of the highly symmetric and nanoscopically detailed *Navicula* template is further illustrated in d) and e), which are different magnifications of a single frustule.

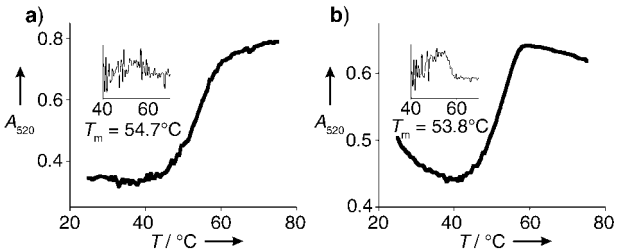

Figure 5. The release of the gold nanoparticles from the *Synedra* (a) and *Navicula* (b) surfaces is monitored using UV/Vis spectroscopy by measuring the increase in absorbance at 520 nm as a function of temperature. The melting temperatures (inset) of the DNA-duplex structures on *Synedra* and *Navicula* were determined to be 54.7 °C and 53.8 °C, respectively.

microscope slides (5 °C).[15] We attribute these differences to the irregular, undulating diatom surfaces that may play a role in decreasing the net cooperative melting effect.

The sequence-specific assembly properties conferred by DNA also can be used to build hierarchical structure and complexity into the templated materials. As a first step in this direction, we assembled multiple alternating layers of 13 nm gold particles onto the diatom surfaces (**5**, Figure 1) by using particles **A′** and complementary particles **A** (13 nm Au functionalized with 3′-HS-C_3H_6-AATATTGATAAGGAT-5′; **a**). A total of seven layers were added to both *Synedra* and *Navicula* diatoms; no interdiatom aggregation was observed during the assembly processes. TEM images of two nanoparticle layers on both *Navicula* and *Synedra* are shown in Figure 6 (a and b, respectively).[16] A comparison of Figure 6 with Figure 3 and 4 reveals denser nanoparticle coverage, and the melting curves for four and seven layers (Figure 6 c,d and

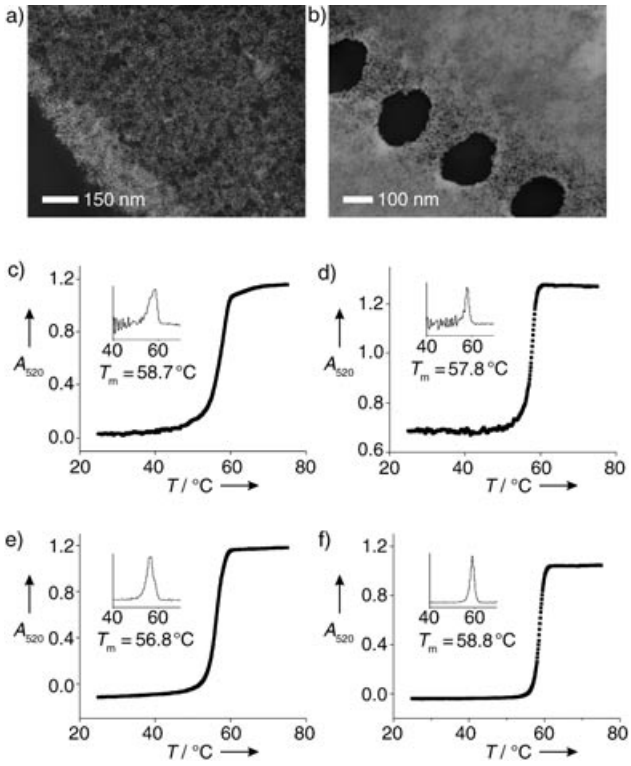

Figure 6. Multiple layers of nanoparticles can be assembled onto the diatom templates. Here, TEM images of two layers of nanoparticles on both *Navicula* (a) and *Synedra* (b) are illustrated. The melting curves for both four and seven layers of nanoparticles along with the corresponding melting temperatures are shown for *Navicula* (c, four layers; e, seven layers) and *Synedra* (d, four layers; f, seven layers).

e,f, respectively) are much sharper and shifted to higher temperatures in comparison to the melting curve for only one layer (Figure 5). A similar phenomenon is observed on glass slides and is due to increased cooperativity as the aggregate size and number of DNA linkages is increased.[15]

Throughout this report, we have understated the unique structural and surface aspects of the diatom templates. Unlike the surfaces of some biological templates, the surface of the diatoms exhibit remarkable species-specific nanoscopic details that include pores, grooves, and ridges that cannot be rendered or synthesized by using conventional material fabrication techniques. We expect that these aspects, in combination with the unique properties of nanomaterials, might prove useful for various applications in catalysis and optics. Additionally, we anticipate that the nanoparticle-coated diatoms may be effective as substrates for surface enhanced Raman scattering (SERS)—preliminary studies are currently underway in our lab.

In conclusion, we emphasize that this novel approach is extraordinarily basic and general. Using straightforward reactions, we can easily modify the diatom surfaces with many different functional groups designed specifically to interact with nanostructures of interest or to perform desired chemistry. Similar strategies will be adopted for other microorganism templates that have surfaces amenable to facile chemical functionalization.

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