certain photonic band-gap materials, metal or semiconductor particles with potential catalytic or electronic properties, or even living biological cells and macrobiomolecules.

Experimental Section

Gold-coated substrates (60 nm Au, 10 nm Ti on Si) were prepared according to previously reported procedures.[8] For in situ imaging experiments requiring transparent substrates, coverslip glass (Corning No. 1 thickness, VWR, Chicago, IL) was cleaned with Ar/O_2 plasma for 1 min, then coated with 2 nm of Ti and 15 nm of Au.

All DPN patterning experiments and lateral force imaging experiments were carried out under ambient laboratory conditions (30% relative humidity, 23 °C) and as previously reported.[8]

Optical microscopy was performed using the optics on a Park Scientific CP AFM (Thermomicroscopes, Sunnyvale, CA) or, for in situ imaging, an inverted optical microscope (Axiovert 100A, Carl Zeiss, Jena, Germany) operated in differential interference contrast mode. Images were captured with a Penguin 600 CL digital camera (Pixera, Los Gatos, CA). Intermittant-contact imaging of particles was performed with a Thermomicroscopes M5 AFM using silicon ultralevers (Thermomicroscopes, spring constant = 3.2 $N m^{-1}$).

Suspensions of charged polystyrene latex particles in water were purchased from either Bangs Laboratories (0.93 μm, Fishers IN, USA) or IDC Latex (1.0 μm and 190 nm, Portland OR, USA). Particles were rinsed free of surfactant by centrifugation and redispersion (twice) in distilled deionized water (18.1 MΩ) purified with a Barnstead (Dubuque, IA) NANOpure water system.

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Orthogonal Assembly of Nanoparticle Building Blocks on Dip-Pen Nanolithographically Generated Templates of DNA**

Linette M. Demers, So-Jung Park, T. Andrew Taton, Zhi Li, and Chad A. Mirkin*

The development of methods for organizing nanostructures into functional materials with addressable nanoscopic components represents a significant challenge in nanoscience. A variety of methods have been employed to control the assembly of nanoparticles into ordered two- and three-dimensional (2D and 3D) architectures in solution and on surfaces. These involve three general approaches: 1) the use of organic linker molecules and covalent bonding to generate meso- and macroscopic architectures with control over particle placement within an assembled network of particles,[1] 2) the use of external physical forces (e.g. Langmuir – Blodgett techniques, electric fields) and weak interactions to form ordered 2D particle arrays,[1, 2] or 3) the use of biological molecules and their molecular-recognition properties to guide the assembly of polymeric-network structures either on a surface or in solution.[1, 3] However, at present there are no efficient methods for chemically directing the assembly of *multicomponent* nanostructures on surfaces with precise control over the placement of the nanoscale building blocks. An intriguing possibility for the biomolecule-based approach to particle assembly would be to learn how to pattern biological molecules on surfaces with nanoscale resolution, one could literally chemically program or encode such surfaces with information based upon the biorecognition elements used in the patterning process. For example, in the case of a synthetic sequence of DNA that is 20 bases long, there are 4^{20} possible recognition elements that could be used for guiding the assembly of nanoscale building blocks functionalized with the appropriate complementary sequences. As the length of the sequence increases, the number of recognition elements increases dramatically providing an almost limitless number of interaction pairs that can be designed to guide a given nanostructure-assembly process. In contrast, if ordinary organic molecules were used in either surface-modification chemistry or covalent organic methods for directing such processes, there are a limited and small number of interaction pairs that could be designed and employed. Indeed, Wrighton and co-workers, in studying the assembly of redox-active molecules on micron-scale electrode surfaces made of $In_2O_3 – SnO_2$ (ITO) and Au, respectively, showed that it is difficult to design even two interaction pairs by using coordination chemistry to guide such processes in a *perfectly orthogonal manner*.[4] Herein, we show how Dip-Pen

[*] Prof. C. A. Mirkin, L. M. Demers, S.-J. Park, T. A. Taton, Z. Li
Department of Chemistry and Institute for Nanotechnology
Northwestern University
2145 Sheridan Road, Evanston, Illinois 60208-3113 (USA)
Fax: (+1) 847-467-5123
E-mail: camirkin@chem.nwu.edu

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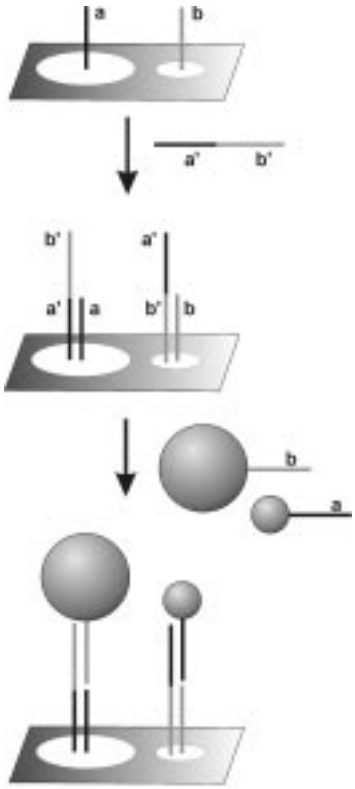
Nanolithography (DPN) can be used to create nanostructures of DNA on a surface, which can be used subsequently to guide the assembly of discrete nanoparticle building blocks with complementary DNA in an orthogonal manner. Significantly, this strategy could lead to a new and general way for preparing multicomponent nanostructures for a wide-range of applications ranging from biological diagnostics to nanoelectronics to the preparation of colloidal crystals for use in catalysis and photonics.[5]

In a typical experiment, a gold surface is patterned with 1,16-mercaptohexadecanoic acid (MHA) by DPN (contact force: 0.5 nN, contact time: 1 s per 100 nm dot radius, $r \sim t^{1/2}$, and 55% relative humidity).[6] The area surrounding the MHA pattern is modified with 1-octadecanethiol (ODT) by immersing the substrate in a 1 mM ethanolic solution of ODT for 5 min. This allows one to passivate the bare gold with minimal ODT exchange with the MHA patterns (not detectable by lateral force microscopy (LFM)). Alkylamine-modified DNA ("**a**" in Scheme 1) is then coupled to the MHA pattern by the formation of an amide bond. This linking is accomplished by immersing a patterned substrate in an aqueous buffer solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC; 10 mg mL^{-1} in 0.1M morpholine\ethanesulfonic acid (MES), pH 4.5) for 15 min to activate the surface carboxyl moieties, followed by rinsing with 0.1M sodium borate/boric acid buffer, pH 9.5. A 10 μL droplet of the alkylamine DNA (25 μM) in borate buffer is placed on the patterned substrate for 12 h. The substrate is rinsed with a phosphate buffered saline (PBS; 0.3M NaCl, 0.01M phosphate, pH 7), and then deionized water, dried in air and imaged by atomic force microscopy (AFM; Figure 1A).



Scheme 1. A schematic representation of orthogonal assembly with DNA nanostructures and nanoparticles functionalized with complementary DNA. From the 5′ end, sequences are **a**: TCTCAACTCGTAA$_{10}$, **b**: A$_{10}$CGCATTCAGGAT, **a′b′**: TACGAGTTGAGAATCCTGAATGCG; white elipsoid = MHA pattern.

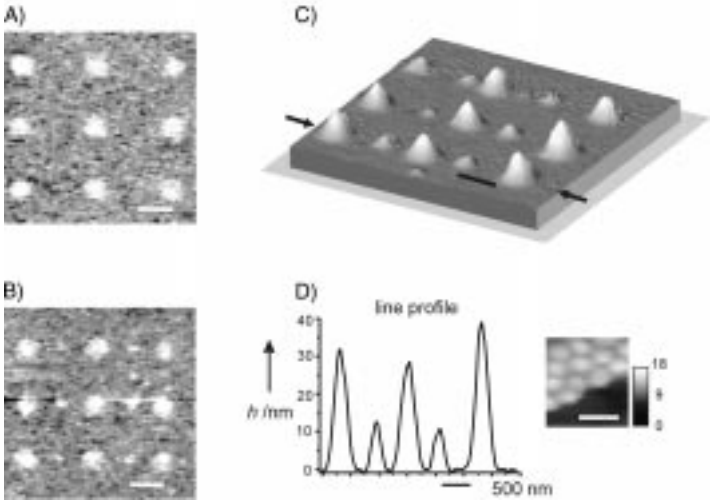


Figure 1. AFM images of patterned gold substrates. A) Topographical image (contact mode) of the substrate after DNA has been coupled to the DPN-generated MHA pattern. B) Topographical image of the two-component DNA pattern. C) Noncontact AFM topography image of particles after the orthogonal assembly process. Scale bars in A, B, and C are 1 μm. D) Line scan of the first row of particles in the image in Figure 1C. The inset shows a high resolution, tapping-mode image of one of the nanostructures comprising 13 nm particles (scale bar is 30 nm).

A second MHA pattern, offset from the first DNA pattern and with smaller features (200 nm), is generated by DPN. Significantly, in a DPN experiment MHA will replace the areas modified with ODT in this manner under normal writing conditions (0.5 nN, 55% humidity, 23°C), as evidenced by LFM. This allows a second nanopattern of MHA to be placed in near-perfect registration with the first pattern without chemically modifying the first pattern.[6b] A second alkylamine oligonucleotide ("**b**" in Scheme 1) can be immobilized on the second pattern of MHA by using the procedure described above. This creates two nanopatterns comprising two distinct oligonucleotide sequences that can guide the assembly of randomly dispersed nanoparticles, which are each functionalized with different oligonucleotides, Figure 1B and Scheme 1.

To demonstrate the concept of orthogonal assembly of nanostructures by using DPN-generated oligonucleotide templates, a droplet of an oligonucleotide linker strand (0.3 μM in PBS buffer) was placed on top of the patterned substrate for 2.5 h. This linker strand was designed to bind in an orthogonal manner to the two different oligonucleotide patterns (3′–5′ and 5′–3′, respectively from the surface) generating floppy ends that are complementary to the predesigned oligonucleotide-modified particles (Scheme 1). The substrates are rinsed with PBS buffer prior to particle-assembly experiments. Then, a droplet of solution containing DNA-derivatized 13 and 30 nm particles (particle concentrations ~1 nM) was placed on the substrate for 4 h then rinsed

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with PBS buffer and 0.3 M aqueous ammonium acetate, pH 7. The latter, which is a volatile salt, is used to prepare the substrates for imaging by reducing imaging complications that result from salt crystallization on the substrate. AFM images of the resulting structures show that the 13 and 30 nm particles are only immobilized on the complementary oligonucleotide patterns (Figure 1C). Note the line scan shows the expected change in height as the tip traverses the nanoparticle-modified areas (Figure 1D). Significantly, under the conditions used, there is no evidence of cross-reactivity between the nanoparticles and the DNA nanopatterns (see inset, Figure 1, no 30 nm particles are evident.) Indeed, when the patterning is performed in the reverse order, that is, the DNA patterns that bind the 13 nm particles are formed first, followed by patterns that bind the 30 nm particles, AFM images after particle assembly show no evidence of the larger particles bound to the first DNA pattern. In addition, control experiments without the linker show no particle assembly takes place, and that with the linker, each particle can be introduced separately to analogous substrates to generate one component nanostructures (either 13 or 30 nm particles) on the complementary regions of the substrate (Figure 2A and B).

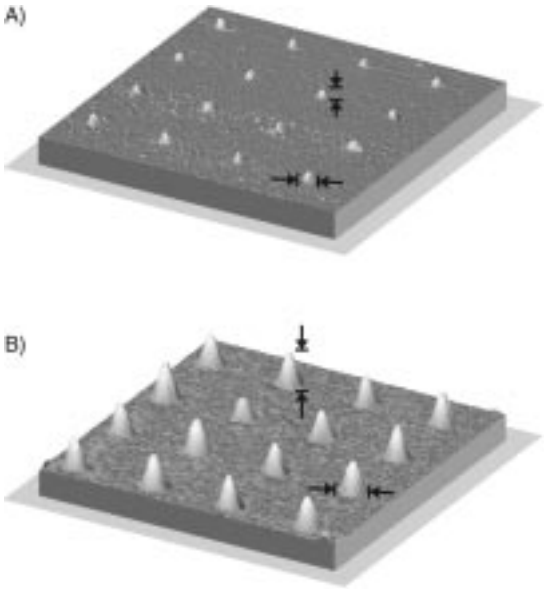

Figure 2. Noncontact AFM topography images of A) 13 nm and B) 30 nm diameter gold particles hybridized to complementary DNA patterns in the presence of linker DNA. Horizontal arrows indicate widths of 200 and 400 nm, and vertical arrows indicate heights of approximately 13 and 31 nm, for A and B, respectively.

Noteworthy is that the choice of conditions and modification procedures used in these experiments are important for reducing nonspecific particle adsorption. Indeed, although this process is clearly dominated by the specific interactions between the DNA patterns and complementary nanoparticles, it is not perfect. High resolution AFM images show that a few stray particles nonspecifically bind to the areas surrounding the oligonucleotide patterns. Indeed, in any sort of experiment geared towards controlling nanostructure placement on a surface, non-specific binding of the nanoscale building blocks to the patterned surface will dictate the quality of the structures that can be generated. This is why it is essential to use direct-write methods such as DPN to immobilize the strong-binding agents on the substrate to be patterned. Indirect methods that rely on resists or exchange chemistry will ultimately lead to surface contamination and could increase particle nonspecific binding. This work points toward the need to develop ways of using oligonucleotides as inks that are directly patterned by the DPN experiment. Efforts in this direction are underway.[5]

Experimental Section

Gold coated substrates were prepared according to reported procedures.[7] All DNA was synthesized and purified by literature methods.[8] ODT, MHA, and EDAC were purchased from Aldrich (Milwaukee, WI). MES was purchased from Sigma (St. Louis, MO). Gold nanoparticles were purchased from Ted Pella (Redding, CA; 30 nm diameter) or prepared by reduction of $HAuCl_4$ as previously described (13 nm diameter).[9] Nanoparticles were modified with thiol-functionalized DNA as previously described.[8]

Noncontact and lateral-force imaging experiments were performed with a Park Scientific Instruments CP (Thermomicroscopes, Sunnyvale, CA) with silicon ultralevers (Thermomicroscopes, spring constant = 3.2 $N m^{-1}$) or silicon nitride microlevers (Thermomicroscopes, spring constant = 0.3 $N m^{-1}$). Tapping-mode images of nanoparticles were obtained using a Digital Instruments (Santa Barbara, CA) Nanoscope IIIa with silicon cantilevers from Digital Instruments, spring constant = ~ 40 $N m^{-1}$. All AFM images were processed by applying first-order flattening and a low-pass filter when needed.

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