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Combinatorial Templates Generated by Dip-Pen Nanolithography for the Formation of Two-Dimensional Particle Arrays**

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A general method for organizing micro- and nanoparticles on a substrate could facilitate the formation and study of photonic band-gap arrays for analysis of the relationship between pattern structure and catalytic activity and enable formation of arrays of single protein molecules for proteomics research. While several methods have been reported for assembling collections of particles onto patterned surfaces,[1, 2] a major challenge lies in the selective immobilization of single particles into predetermined positions with respect to adjacent particles.

A strategy for chemically and physically immobilizing a wide variety of particle types and sizes with a high degree of control over particle placement calls for a soft lithographic technique capable of high-resolution patterning, as well as one with the ability to form patterns of one or more molecules with precise alignment. Dip-pen nanolithography (DPN) has emerged as one such tool.[3] DPN is a scanning probe nanopatterning technique in which an atomic-force microscopy (AFM) tip is used to deliver molecules to a surface through a water meniscus, which naturally forms in the ambient atmosphere. Significantly, DPN also can be used to generate many customized templates formed from the same or different chemical inks which can be screened under identical conditions for a particular application. Herein, we report an example of this new combinatorial approach, and focus on the problem of particle assembly in the context of colloidal crystallization.

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Recently, conventional sedimentation methods for preparing colloidal crystals consisting of close-packed layers of polymer or inorganic particles have been combined with polymer templates, fabricated by electron-beam lithography, to form high-quality single-component structures.[1] However, sedimentation or solvent evaporation routes do not offer the element of chemical control over particle placement.[4] Herein, we describe a DPN-based strategy for generating charged chemical templates to study the assembly of single particles into two-dimensional square lattices.

Our general method (Figure 1) is to form a pattern on gold composed of an array of dots of a "molecular ink" which will attract and bind a specific type of particle. For these studies we
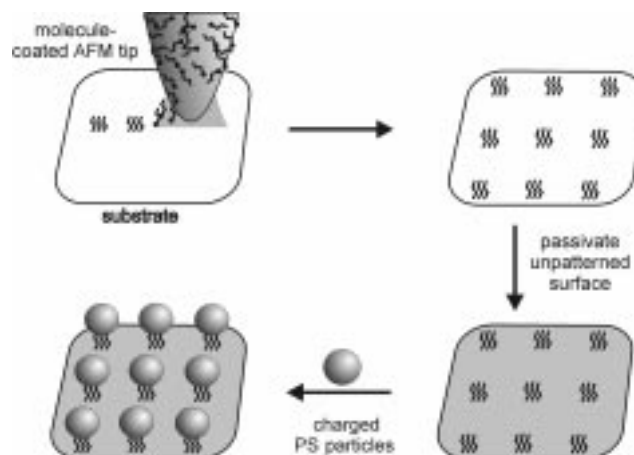

Figure 1. A schematic representation of the DPN-based particle organization strategy.

used 16-thiohexadecanoic acid (16-mercaptohexadecanoic acid, MHA) to make templates, and positively-charged protonated amine- or amidine-modified polystyrene (PS) spheres as particle building blocks. The unpatterned regions of the gold were passivated by immersing the substrate in a 1 mM ethanolic solution of another alkanethiol, such as 18-octadecanethiol (ODT), or cystamine for 9 min. Minimal, if any, exchange takes place between the MHA molecules and the ODT or cystamine in solution during this treatment, as evidenced by lateral force microscopy of the substrate before and after treatment with ODT (no change). Finally, particle assembly was accomplished by placing a 20-μL droplet of dispersed particles (10% wt/vol in deionized water) onto the horizontal substrate in a humidity chamber (100% relative humidity). Gentle rinsing with deionized water completes the process. In this particular study, it is important to note that the carboxylic acid groups in the MHA patterns are deprotonated, thus providing an electrostatic driving force for particle assembly.[5]

In a typical experiment involving 0.93 μm diameter particles, multiple templates are monitored simultaneously for particle assembly by optical microscopy. In these experiments, the diameter of the template dot is varied to search for optimal conditions for particle–template recognition (Figure 2). After 1 h of particle assembly, the substrates are rinsed with deionized water, dried under ambient laboratory conditions, and then imaged by optical microscopy (Figure 3).
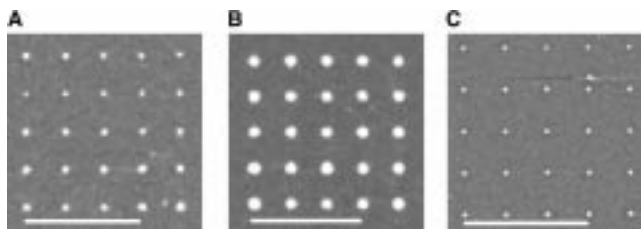
Figure 2. A–C) Patterns generated on gold thin films by DPN and imaged by lateral-force microscopy (MHA light areas, ODT dark areas). MHA dots (diameters: A: 540, B: 750, and C: 240 nm, center-to-center distance 2 µm) deposited by holding the AFM tip at a series of x,y coordinates (5, 10, and 15 s for A–C, respectively). Scale bars represent 6 µm.
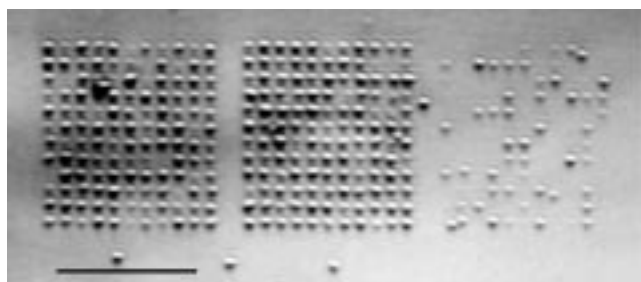

Figure 3. Optical micrograph of particle arrays in a combinatorial experiment on a MHA-patterned substrate (as shown in Figure 2). Scale bar represents 20 µm.

The combinatorial experiment reveals that the optimum size of the template pad with which to immobilize a single particle of this type in high registry with the pattern is approximately 500–750 nm. It is important to note that drying the substrate tends to displace the particles from their preferred positions on the template, an effect that has been noted by others with larger scale experiments.[6] Indeed, evidence for better, in fact near-perfect, particle organization (using 700-nm template dots) is obtained by in situ imaging of the surface after the template has been treated with 1-µm amine-modified particles for 1 hour (Figure 4).

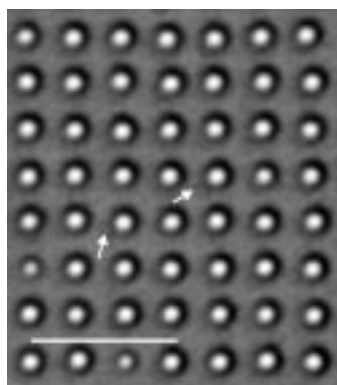
Figure 4. In situ optical micrograph of 1.0 µm diameter amine-modified polystyrene particles organized into a square array with a lattice constant of 2 µm. The dark fuzzy dots are particles in solution that have not reacted with the template (white arrows). Scale bar represents 6 µm.

Spatial organization of single particles on the micron length-scale has been achieved by physical means, for example, by using optical tweezers,[7] or by sedimentation

onto electron-beam lithographically patterned polymer films.[1] However, the DPN-based method described here offers an advantage over previous methods because it provides flexibility of length scale and pattern type as well as a means to achieve more robust particle array structures. For example, we have used DPN to construct chemical templates which can be utilized to prepare square arrays of 190 nm diameter amidine-modified polystyrene particles. Screening of the dried particle arrays by noncontact AFM or scanning electron microscopy (SEM) imaging after 3 hours of particle drying reveals that 300-nm template dots of MHA, spaced 570 nm apart, with a surrounding repulsive monolayer of cystamine are suitable for immobilizing single particles at each site in the array (Figure 5A). However, MHA dots of 700 nm diameter and 850-nm spacing result in immobilization of multiple particles at some sites (Figure 5B).
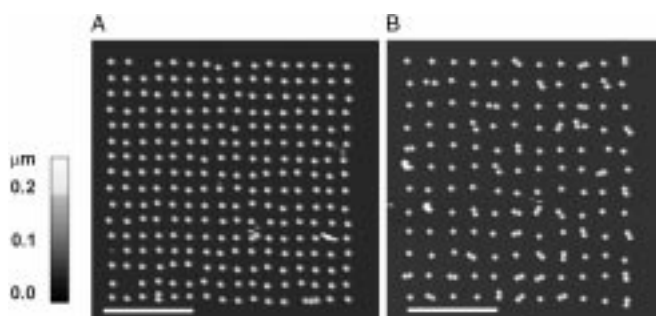

Figure 5. Two regions of a gold substrate with 190 nm diameter amidine-modified polystyrene particles selectively organized on MHA regions of the patterned surface, imaged by intermittent-contact AFM. Particle arrays formed on: A) 300 nm diameter MHA dots and B) 700 nm diameter dots. Also, note that the AFM tip in some case drags the particles from their preferred locations. Scale bars represent 3 µm (A) and 4 µm (B), respectively.

Similar particle assembly experiments conducted at pH < 5 or > 9 result in random, nonselective particle adsorption, presumably as a result of protonation of the surface acid groups, or deprotonation of the amine or amidine groups on the particles. These experiments suggest that the particle assembly process is induced by electrostatic interactions between charged particles and patterned regions of the substrate.

We have demonstrated that DPN can be used as a tool for generating combinatorial chemical templates with which to position single particles in two-dimensional arrays. It is important to note that the DPN approach will allow one to systematically vary the chemical composition of the array, spot size, spot shape, and interfeature distances. The specific example of charged alkanethiols and latex particles described here will provide a general approach for creating two-dimensional templates for positioning subsequent particle layers in predefined crystalline structures that may be composed of single or multiple particle sizes and compositions. In a more general sense, the combinatorial DPN method will allow researchers to efficiently form patterned substrates with which to study particle–particle and particle–substrate interactions of dielectric spheres which comprise

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**

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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

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