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Biofunctionalized nanoarrays of inorganic structures prepared by dip-pen nanolithography

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Abstract
A dip-pen nanolithography based strategy for fabricating and functionalizing Au nanostructures on a semiconductor substrate is reported. The generation of arrays of nanoscale features functionalized with inorganic nanoparticles and proteins (rabbit IgG) is reported. In the case of rabbit IgG, the bioactivity of the array was demonstrated by monitoring its reaction with fluorophore-labelled anti-rabbit IgG. The methodology reported herein points towards ways of making raised optically active and bioactive nanostructures that could prove useful in stamping methodologies or biosensing applications.

(Some figures in this article are in colour only in the electronic version)

Methods for fabricating nanostructures are essential for developing and capitalizing upon the emerging field of nanoscience. In particular, methods are needed that offer the capability of working with both hard and soft matter on a scale of sub-100 nm to many micrometres. Several methods, such as e-beam lithography [1], ion beam lithography [2a] and nanoimprint lithography [3a] offer one the ability to build hard structures on this scale of length, but they do not allow one to deposit molecule-based nanostructures directly on a surface. Microcontact printing (μCP) [4a] allows one to directly deposit molecules onto a surface in a massively parallel fashion, but it does not allow one to generate structures made of different inks with nanoscale registration capabilities. Dip-pen nanolithography (DPN) [5a] has emerged as a tool that allows one to make multicomponent nanostructures on a surface with near-perfect registration capabilities. Although it does not offer the massively parallel capabilities of photolithography [1] and some of the stamping methods [4a], it has been transformed into a parallel writing tool through the use of cantilever arrays consisting of multiple pens [5c, 6]. This makes DPN a unique tool that can be used to fabricate both hard and soft structures with nanoscale precision.

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Inorganic nanostructures functionalized with biomolecules are having a major impact in the field of biodiagnostics [7a] and beginning to find use in other areas, including materials synthesis [8a], optics [9a], and electronics [10]. Nanostructures functionalized with oligonucleotides [7a, 11] and proteins [12] have been shown to be particularly useful in the development of high sensitivity and selectivity detection systems for DNA, small molecules, and protein structures.

In our previous manuscripts [13], we developed a method for fabricating arrays of Au nanostructures on a SiO_x/Si surface based on DPN and wet chemical etching [14a]. Herein, we show how the etched Au nanopatterns, created via this methodology, can be used as templates to adsorb and/or assemble Au nanoparticles and proteins from solution to form functionalized inorganic/biological nanostructures. These structures are potentially interesting for their optical and biological sensing capabilities.

In a typical experiment, a substrate suitable for patterning experiments was prepared by thermally evaporating 8–10 nm of Au on a Ti-coated (1 nm) oxidized silicon (∼500 nm of oxide) substrate (pressure $< 1 \times 10^{-7}$ Mbar). These fresh Au substrates were then patterned with 16-mercaptohexadecanoic acid (MHA) by DPN according to procedures given in the literature [5a, 13]. A MHA-coated tip was prepared by immersing a commercial Si_3N_4 tip (spring constant =

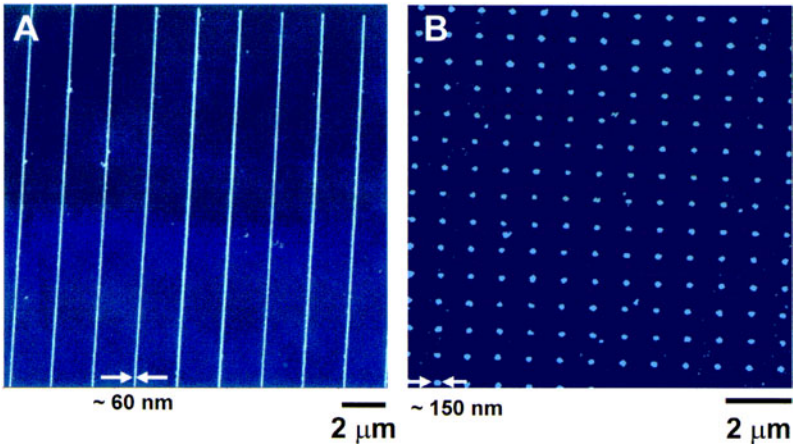


Figure 1. TMAFM topographic images of the etched MHA/Au/Ti/SiO_x/Si nanostructures, lines (A) and dots (B), based on the DPN of MHA on an Au surface.

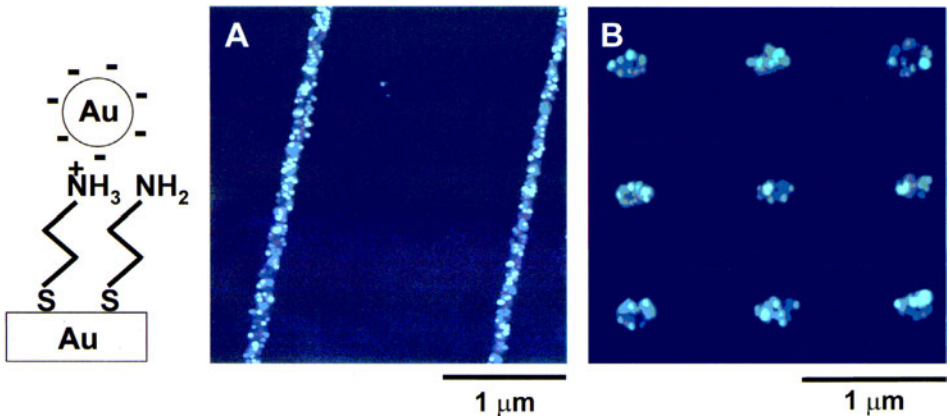


Figure 2. TMAFM topographic images of individual Au nanoparticles adsorbed on NH_2-SAM-modified nanopatterns of lines (A) and dots (B).

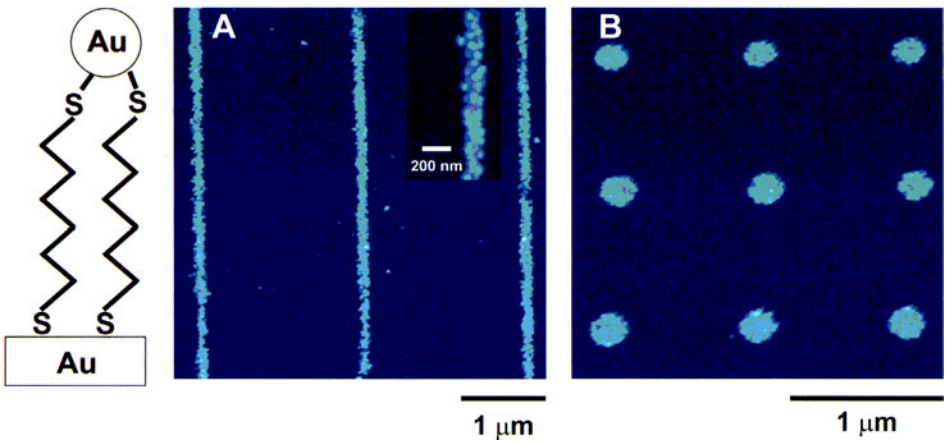


Figure 3. TMAFM topographic images of individual Au nanoparticles adsorbed on HS-SAM-modified nanopatterns of lines (A, high-resolution image inserted) and dots (B).

0.05 N m^{-1}, TM Microscopes, Sunnyvale, CA) into a 5 mM MHA acetonitrile solution for ∼15 s and then drying it with compressed difluoroethane (Dust-off, Ted Pella, Inc., Redding, CA). The DPN experiments were carried out under ambient conditions (set point = 0.5 nN, 22–24 °C, 30–36% relative humidity) by using an AutoProbe CP AFM (TM Microscopes, Sunnyvale, CA) and commercial lithography software (DPNWrite^{TM}, DPN System-1, NanoInk Inc., Chicago, IL) with a MHA-coated tip. Each DPN dot feature was generated by holding a MHA-coated tip in contact with an Au surface for 0.25 s (figures 1(B), 2(B) and 3(B)) and 3.5 s (figure 4), and each line was generated by moving a MHA-coated tip on an Au surface at a rate of 1 μm s^{-1} (figures 1(A), 2(A) and 3(A)).

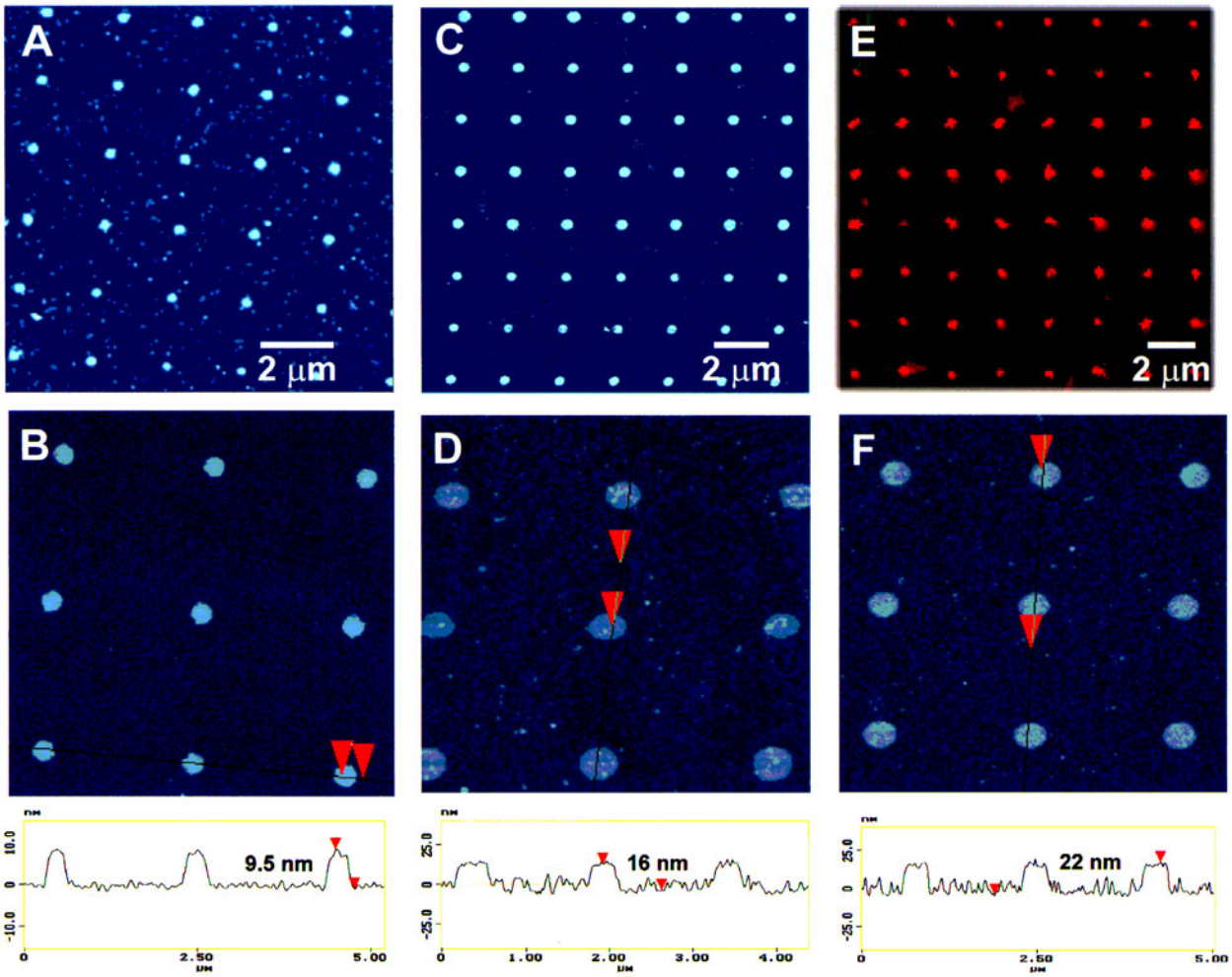
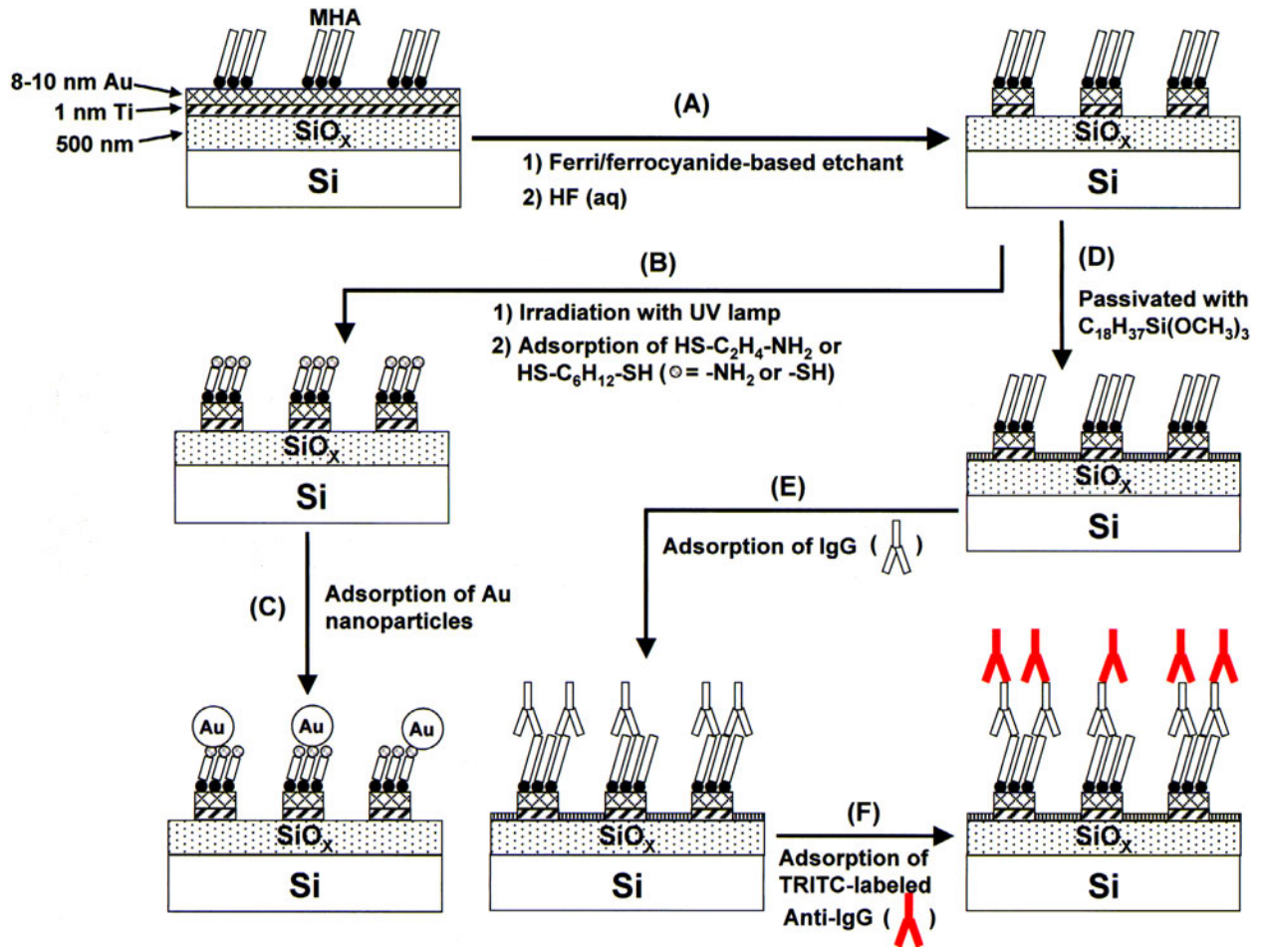


Figure 4. A TMAFM topographic image of etched MHA/Au/Ti/SiO_x/Si (A, SiO_x passivated by OTS; B, high-resolution image and section analysis), and rabbit IgG adsorbed on MHA SAM on the etched dot nanoarrays (C, D: high-resolution image and section analysis). Fluorescence image (E) and high-resolution TMAFM image and section analysis (F) of fluorophore (TRITC)-labelled anti-rabbit IgG adsorbed on IgG nanoarrays.

The ferri/ferrocyanide etchant used in this experiment was prepared by mixing 0.1 M $Na_2S_2O_3$, 1.0 M KOH, 0.01 M $K_3Fe(CN)_6$ and 0.001 M $K_4Fe(CN)_6$ in a 1:1:1:1 (v:v:v:v) ratio. After being patterned with MHA, the Au substrates were immersed in the ferri/ferrocyanide etching solution for ~20 min under constant stirring to remove the exposed Au areas. Then they were treated with 0.5% aqueous HF (obtained by diluting 48% HF (Aldrich)) for 15–30 s to remove the Ti layers [13] (step A in scheme 1). After rinsing with Milli-*Q* H_2O and drying with N_2, the MHA-modified Au nanopatterns of lines and dots were characterized by tapping mode AFM (TMAFM) (Nanoscope IIIa, Digital Instruments, Santa Barbara, CA) (figure 1). The distances between the lines (figure 1(A)) and dots (figure 1(B)) are 2 and 1 μm, respectively. The width of the Au lines (figure 1(A)) and the diameter of the Au dots (figure 1(B)) are ~60 and 150 nm, consistent with the values given in the literature for similar experiments [13a].

To remove the monolayer 'etch resist', the etched substrate was irradiated with a UV lamp (365 nm, 21.7 mW cm^{-2}, B-100AP, Fisher Scientific, Pittsburgh, PA) for ~10 h. It is known that UV-irradiation of an alkylthiol-coated Au substrate under ambient conditions results in photooxidation of the surface-bound monolayer and its desorption from the surface, if immersed in the appropriate solvent [15a]. After removing the oxidized MHA SAM by rinsing with water, the fresh Au nanostructures can be functionalized with other thiol-containing molecules (step B in scheme 1). Two kinds of thiol functionalized molecules, 2-aminoethanethiol ($HS–C_2H_4–NH_2$) (AET) and 1,6-hexanedithol ($HS–C_6H_{12}–SH$) (HDT) (Aldrich), were used to modify these fresh Au nanopatterns. After immersing the Au nanopatterns in a 1.0 mM ethanolic solution of AET or HDT for 24 h, amino (NH_2) or thiol (HS)-terminated SAMs formed on the Au nanofeatures. These structures can be used to assemble additional nanostructures by electrostatic or covalent interactions to form Au-nanoparticle arrays (step C in scheme 1). For example, after immersing the array of AET-modified nanostructures into a solution of citrate-stabilized gold nanoparticles (~15 nm, ~15 nM) [8b, 16] for ~10 h, with subsequent rinsing with Milli-*Q* water, a monolayer of Au particles localized on each of the



Scheme 1. The procedure for preparing biofunctionalized DPN-generated nanostructures.

nanofeatures within the array (figure 2). The TMAFM measured height of the individual Au nanoparticles was ∼15 nm and in excellent agreement with the TEM-measured diameter of Au nanoparticles that comprise the colloid. This observation is also consistent with the conclusion that the MHA monolayers on each of the nanofeatures has been removed and replaced with AET. Magnesium oleate capped Au particles (∼15 nm, ∼10 nM) [17a], were used to modify the SH-rich surface of HDT–SAM modified nanofeatures (figure 3). In both cases, collections of individual Au nanoparticles on the Au nanofeatures are observed with very little nonspecific binding to the underlying silicon oxide substrate.

Protein arrays of elevated Au nanofeatures can also be fabricated via modification of the aforementioned approach. To inhibit nonspecific adsorption of the protein on the exposed SiO_x layer of the etched substrate, an array of MHA-coated nanofeatures was initially immersed into a 0.5–2% (w/w) n-octadecyltrimethoxysilane (OTS)/toluene solution for 1.5 h [18] (step D in scheme 1). OTS is known to chemisorb to silicon oxide [18]. After rinsing with toluene and drying with N_2, the MHA-modified Au patterns were characterized by TMAFM (figures 4(A) and (B)). The height and diameter of each dot within the array are ∼9.5 and 340 nm, respectively. The resulting set of nanostructures then can be used as a template to adsorb proteins to form a protein nanoarray. As proof-of-concept, an etched array of dot features was immersed into a 10 mM PBS buffer solution containing 10 μg ml^{-1} of rabbit IgG (pH ∼ 7) for 45 min [19] (step E in scheme 1). The substrate was then rinsed with 10 mM PBS buffer, 0.05% Tween-20 solution and Milli-Q water, and immediately characterized by TMAFM. Rabbit IgG and the broad class of IgG proteins in general are known to have a high affinity for COOH-terminated SAMs at pH 7 and a relatively weak affinity for hydrophobic surfaces coated with alkanesilane as compared to the carboxylic acid-terminated surfaces [20]. TMAFM images (figures 4(C) and (D)), show an increase in height of 6.5 nm, which is consistent with the formation of an IgG monolayer on the dot arrays [19]. In order to test the bioactivity of the IgG array, the sample was immediately immersed into a 10 mM PBS buffer solution of fluorophore (TRITC)-labelled anti-rabbit IgG (10 μg ml^{-1}; pH ∼ 7) for 1 h (step F in scheme 1). After rinsing the substrate with 10 mM PBS buffer, 0.05% Tween-20 solution and Milli-Q water, confocal fluorescence microscopic image shows that the TRITC-labelled anti-rabbit IgG specifically binds to the IgG-immobilized nanofeatures (figure 4(E)). The TMAFM image shows that the height of this nanopattern increases by 6 nm, consistent with the adsorption of a monolayer of the anti-rabbit IgG [19] (figure 4(F)).

In summary, we have provided a straightforward method for creating arrays of inorganic nanostructures that can be

selectively modified with adsorbates that provide additional and desirable functionality. Importantly, using this strategy we can use adsorbates like HDT and cysteamine, which are not easily transported via DPN, as modification agents for the structures fabricated in the DPN process.

Acknowledgments

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