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Fabrication of Assembled Virus Nanostructures on Templates of Chemoselective Linkers Formed by Scanning Probe Nanolithography

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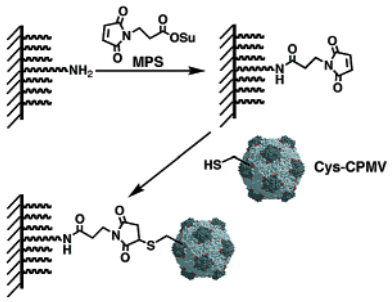
In recent years, methods for fabricating nanometric biomolecular arrays have attracted intense interest due to their great potential in numerous applications including protein chips for proteomic analysis, DNA chips for genomic analysis, and 2D or 3D crystalline arrays for determination of protein structure.[1] Because supramolecular assemblies of macromolecules, such as viruses, are often on the order of tens of nanometers in size and can be produced with atomic precision, they are ideal monodisperse, nanometric building blocks for the study of molecularly directed assembly of biomolecular arrays.

Here we present a multistep approach to biomolecular assembly, which combines scanning probe nanolithography (SPN) with chemoselective protein-to-surface linkers to create nanometric chemical templates for fabricating virus arrays. The strategy for the assembly of these arrays is to introduce unique target chemical groups on the virus surface and to design a chemoselective linker and reaction scheme to covalently bind the virus onto a patterned chemical template created by SPN.

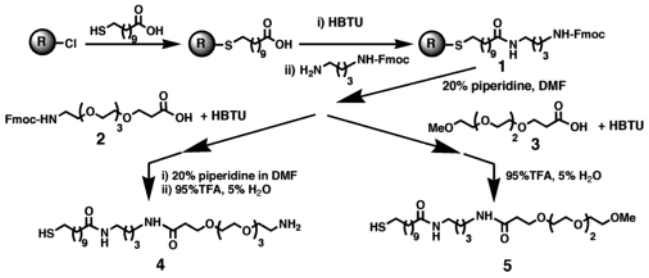
Our model system consists of gold substrates, functionalized alkanethiols as the linkers, and genetically modified cow pea mosaic virus (Cys-CPMV)[2] as the adsorbate. The virus was genetically engineered to present Cys residues at geometrically equivalent positions on the viral capsomer as shown in the inset to Figure 2a.[2] Long aliphatic thiols are well-known to form high-density self-assembled monolayers[3] and are well suited to fabrication of nanometric patterns on gold surfaces by SPN. Moreover, the reaction between sulfhydryl groups on the virus and maleimides is highly chemoselective.[4] Thus, we have devised a sulfhydryl-maleimide chemoselective reaction scheme with functional thiols for linking the mutated virus onto chemical templates where triethylene glycol-terminated thiols are applied as "protein resist" to the surrounding regions for prevention of nonspecific adsorption[5] (Scheme 1).

The synthesis of chemically modified long alkanethiol linkers was accomplished by a new and efficient solid-phase approach developed by our group[4] (Scheme 2). Briefly, 11-mercaptoundecanoic acid was first immobilized on a trityl chloride resin through the selective formation of thioether bond to the solid-support. The free carboxylic function was acylated with mono-Fmoc-1,4-diaminobutane and converted into a more versatile amino group. Afterward, this group was acylated with various activated acids to yield the two different thiols used in this work. In both cases, the final products were fully deprotected and cleaved from the solid support by acidolytic cleavage. The whole synthetic process was extremely efficient in both cases with overall yields of ca. 90% and thus required minimal purification.

Scheme 1



Scheme 2



Nanometric chemical templates of the amine terminated alkanethiol **4**[6] with dimensions close to the diameter of the mutated virus (ca. 30 nm) were created by two SPN techniques: dip-pen nanolithography (DPN)[7] and nanografting.[8] To generate chemical templates by DPN an atomic force microscope (AFM) probe coated with thiol **4** was used to deposit the "ink" onto the gold "paper" by operating an AFM in contact mode. The suitability of this ink for DPN at the 10−100-nm-length scale is demonstrated by Figure 1a. A variation of conventional nanografting[8] was also used to create large area line patterns. An AFM probe was used to displace the "protein resist" thiol **5** precoated on flat gold substrates under high load and high scan speed to create linear "trenches". The samples were subsequently functionalized with thiol linker **4**, thus yielding a functionalized pattern of parallel lines. Figure 1b shows that the resulting sample has patterns of ca. 30-nm-width lines filled with an organic film layer of thickness of 0.5−2.0 nm above the background.

Using the highly selective thiol-maleimide reaction, Cys-CPMV virus was chemoselectively attached on chemical templates containing the maleimido functionality according to Scheme 1. First, the Cys-CPMV sample deposited on poly-Lys-coated mica surface was characterized by AFM. The positions of the virions as well as their relative orientation to the surface can be readily resolved by AFM (Figure 2a). To demonstrate a proof of principle for the virus assembly scheme, we fabricated a micrometer-

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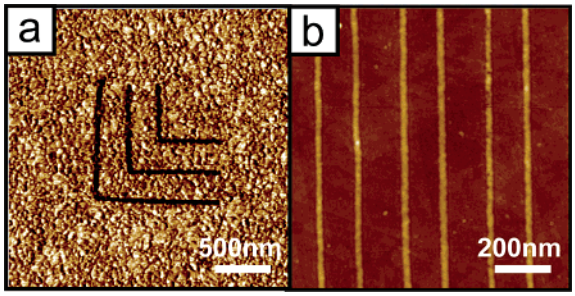

Figure 1. (a) Lateral friction image of an "LLL" symbol of 50-nm-line-width patterned with amino thiol linker **4** by DPN on an e-beam evaporated gold surface. (b) AFM height image[9] of parallel lines of amino thiol **4** with background coated by poly(ethylene glycol) terminated thiol **5** on a mica-peeled flat gold surface patterned by nanografting.

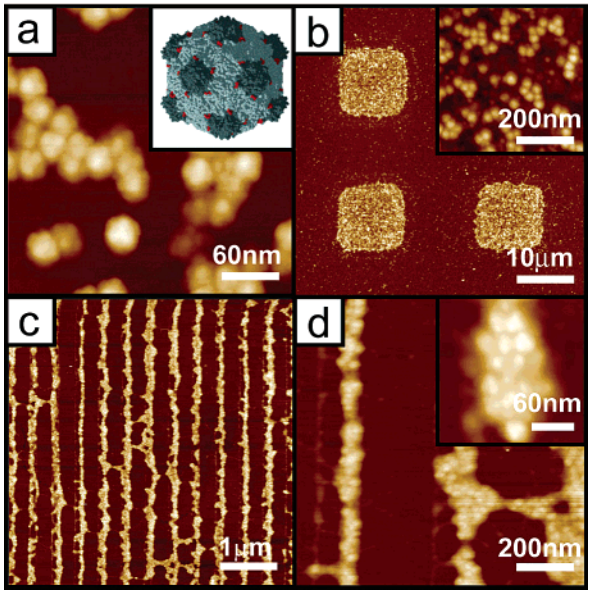

Figure 2. (a) AFM height image of cow pea mosaic virus. (Inset) Model of genetically modified CPMV virus with unique cysteine residues (Cys-CPMV). Red dots indicate the locations of mutated cysteine residues. (b) AFM height image of Cys-CPMV virus assembled on micrometer-sized template. (inset) Zoom-in section of the functionalized square shown in Figure 2b. (c) AFM height image of a monolayer-thick virions assembled on a parallel line pattern created by nanografting with the chemoselective linkers. (d) Zoom-in section of Figure 2c. (inset) Zoom-in image of another section of the same sample for Figure 2c.

scale chemical template by microcontact printing[10] squares of the amino linker thiol **4** on gold substrates and subsequent filling of the background with "protein resist" thiol **5**. The maleimido function was then introduced by acylating the patterned amino groups with 3-maleimidopropionate *N*-hydroxysuccinimide ester (MPS) (Scheme 1). The chemical template was then treated with freshly reduced Cys-mutated CPMV in phosphate buffer at pH 7.0 for 2 h. After washing the unbound virus, the surface was imaged by AFM in tapping mode. As shown in Figure 2b, the Cys-CPMV preferentially attached to the squares containing the maleimido function. Few virions were found in the background area. However, neither spatial correlation, short-range order, nor long-range order between the viruses was observed within the squares.

When the dimension of the chemical template was reduced from the micrometer-scale to a size comparable to that of a CPMV, the morphology of virus assembly changed dramatically. Figure 2c shows an assembly of a dense population of the Cys-CPMV virions on most of the 30-nm-wide patterned lines made by nanografting and processed as the micrometer-sized templates. Probably due to size exclusion and a significant intervirion attractive interaction, the virions started to pack together into a close-packed morphology. Not only did the lines themselves act as templates for attachment of the virions, but the lines of assembled virions then also acted as templates for lateral growth of the viral assembly. As other virions "stuck" to these lines, the lines "grew" laterally at the resulting step-edge and, in some cases, contacted adjacent lines of virions to form a pseudo-2D assembly (Figure 2d). Nevertheless, presumably because the attractive intervirion interaction was too large, the lateral expansion did not proceed in a smooth and well-ordered manner. By taking a lesson from the science of bulk crystallization of macromolecules, modulation of this strong intervirion interaction by altering solution conditions such as pH and ionic strength could assist the creation of 1D nucleation templates for growth of ordered 2D crystalline films.

In summary, we have developed a general approach which combines nanolithography techniques with chemoselective linkers to fabricate virus assemblies. These nanometric templates are now being used to investigate the role of intervirion interactions on assembly morphology and kinetics.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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