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Atomic force microscopy investigation of virus aggregation and assembly at chemical templates formed by scanned probe nanolithography

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Atomic force microscopy investigation of virus aggregation and assembly at chemical templates formed by scanned probe nanolithography

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Aggregation and assembly of macromolecules are important processes in a number of scientific fields including structural biology, medicine, and materials science. For example, growth of well-ordered two-dimensional (2-D) arrays and bulk crystals remains the rate-limiting step in macromolecular structure determination. Uncontrolled aggregation of proteins is the source of a number of devastating pathologies such as Creutzfeldt-Jakob syndrome. Moreover, the demonstrated ability of engineered viruses and proteins to act as templates for growth of inorganic nanostructures is driving a need for methods to deterministically pattern their assembly at surfaces in order to fabricate hierarchical materials and devices.

Because the nature of the interactions and rates of reaction in macromolecular systems are so different from those of atomic and small-molecule systems, one of the challenges associated with controlling macromolecular aggregation and assembly is to develop a fundamental understanding of the underlying physical principles. Here we use atomic force microscopy (AFM) as a tool both to create nanoscale chemical templates for organization of viruses and to investigate the dynamics of organization at these templates.

As a model system, we have chosen Cowpea Mosaic Virus (CPMV) genetically engineered to present either histidine (His) tags or cysteine (Cys) residues at specific sites on the capsid surface. Atomically-flat gold sub-

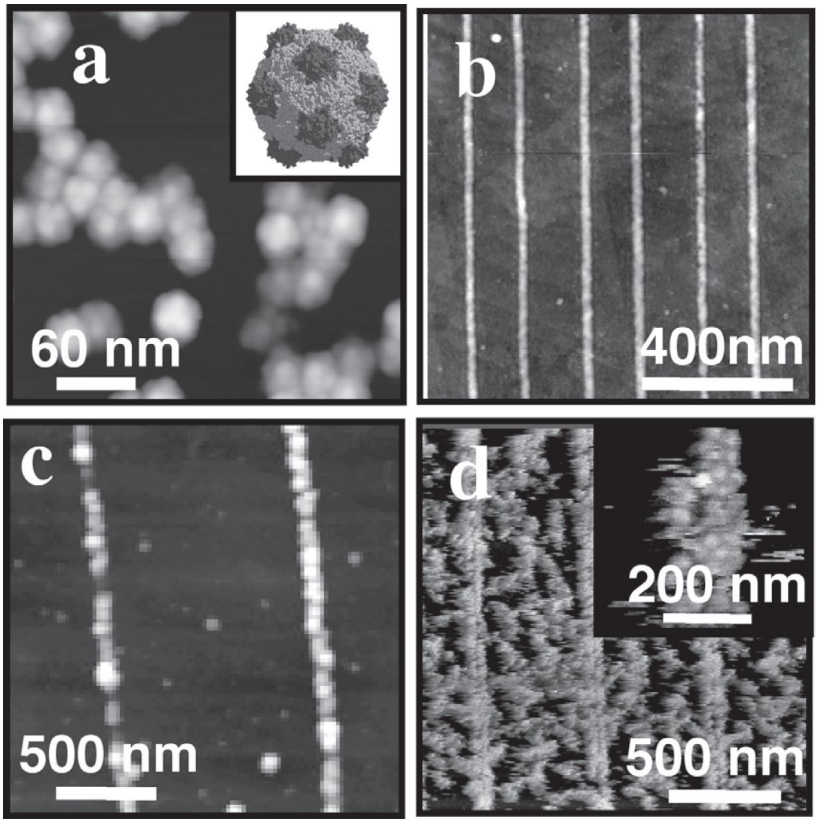

Figure 1. Atomic force microscopy images collected in height mode showing: (a) Cowpea Mosaic Virus viruses. Inset shows model of molecular structure. (b) Pattern of 30 nm wide lines formed by nanografting maleimide terminated alkane thiols into a polyethylene glycol (PEG) terminated self assembled monolayers (SAM). (c) Deposition of His-tag modified viruses onto Ni-NTA lines from solution with low virus concentration and no PEG in solution. Resulting arrays are one virus wide. (d) Same as (c) but with high concentration virus solution and moderate concentration of PEG. Inset is close-up of (d) showing rows are multiple viruses in width.

strates are prepared by first coating them with self assembled monolayers (SAMs) of polyethylene glycol (PEG) terminated alkane thiols. Nanometric patterns of alkane thiol-based chemical linkers are then made by scanned probe nanolithography, such that attachment to the Cys-residues and His-tags is through covalent bonding with maleimides groups or metal coordination complex linkage with nickel-chelating nitrilotriacetic acid (Ni-NTA) groups, respectively. In the latter case, to explore the effect of virus' mobility on assembly, we modulate the virus-surface binding strength by addition of competing metal coordinating ligands such as imidazoles. To investigate the role of virus flux and inter-viral interactions, we vary the virus and PEG concentration of the virus solution, respectively.

Force microscopy is then used to investigate the degree of ordering, packing geometry, assembly kinetics, and cluster-size distribution both on the regions patterned to bind chemoselectively with the virus, as well as the surrounding PEG-terminated region. Here we show the results for 2-D assembly on uniform surfaces and for one-dimensional (1-D) assembly at linear patterns of linkers for which the width of the lines is equal to the virus diameter.

We find that the degree of ordering depends on all parameters chosen: the surface chemistry, the virus concentration, the PEG concentration, and the feature size of the patterns. For example, as the PEG concentration is increased, 2-D arrays of viruses evolve from poorly-ordered to well-ordered rhombehedral to hexagonally close packed assemblies. For the 1-D patterns and the His-tag:Ni-NTA linkage, as virus flux is increased, the width of the virus arrays increases from one virus thick to multiple rows of viruses. With a moderate level of PEG and at moderate virus fluxes, the patterns tend towards double rows, each three viruses in width. Disordered clusters form on the PEG terminated regions, but do not grow appreciably in size with increasing time. As the virus concentration or PEG concentration is increased, the density and size of the clusters increases. The results are analyzed within the context of interaction potentials and within the framework of assembly principles borrowed from small molecule systems.

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