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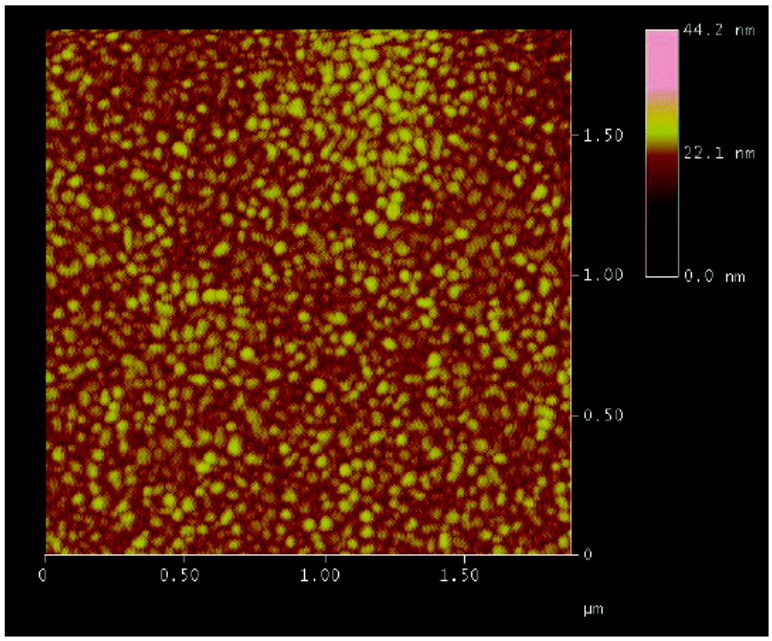

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2-D Array Formation of Genetically Engineered Viral Cages on Au Surfaces and Imaging by Atomic Force Microscopy

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Biomimetic approaches to materials chemistry have provided a new avenue for the synthesis and assembly of nanomaterials.[1−5] There is growing interest in materials chemistry to take advantage of the physical and chemical properties of biomolecules for development of the next generation of nanoscale materials.[6] Viral protein cages (virions) exist in a variety of sizes and shapes, and the protein surfaces can be used as synthetic platforms for chemical modification.[7,8] The ability of some protein cages to form self-assembled arrays on a variety of substrates is of significant interest as possible precursors to interesting nanomaterials such as magnetic semiconductors.[9,10]

Previous work has relied on forming arrays at the liquid−air interface,[11] functionalized Au surfaces,[12] modified glass surfaces,[13] particle lithography,[14] two-photon holography,[14b] and dip-pen nanolithography.[16] Electron microscopy has been a major tool in imaging protein arrays, but tapping mode atomic force microscopy (AFM) is emerging as a useful tool in imaging biomolecules.[17] The present work employs the use of an Au surface to bind genetically and chemically altered viral capsids that behave as self-assembled monolayers.

We have genetically modified the cowpea chlorotic mottle virus (CCMV) protein cage for directing array formation. The CCMV protein cage is composed of 180 identical 20 kDa coat protein subunits (190 residues) that assemble into a virion having icosahedral $T = 3$ symmetry whose outer diameter is 28 nm.[18] The protein cage is 2−4 nm thick, which defines a central cavity in which the viral RNA is packaged. In this work, an alanine was replaced by a cysteine residue at position 163 of the coat protein (Figure 1, A163C) using site directed mutagenesis (Supporting Information). The mutated protein cage was expressed in a yeast-based heterologous expression system and purified to homogeneity. This mutant provides 180 exposed sulfhydryls on the exterior surface surrounding the pseudo 6-fold and 5-fold axes of the cage. The exposed nature of the sulfhydryls demanded the presence of a reducing agent such as 2-mercaptoethanol to prevent interparticle cross-linking via disulfide bond formation.

The engineered cysteines are symmetrically presented over the entire surface of the virion due to the very high symmetry (Figure 1). Because of this, it is difficult to control directionality in the orientation due to the multivalent presentation, which can lead to uncontrolled aggregation. We have used a solid-phase synthetic approach to break the symmetry of the engineered cysteine-rich CCMV particle (Figure 2). Briefly outlined, the A163C mutant was bound to a Sepharose resin containing an activated thiol. All unbound virus was removed by extensive washing, and all remaining free thiols on the virus were chemically passivated by treatment with iodoacetic acid (IAA). The bound CCMV A163C was subsequently eluted by reduction of the disulfide bond holding it to the resin, through addition of 2-mercaptoethanol. After elution, only those thiols previously bound to the resin were available for subsequent derivatization. As a control, unbound A163C was treated with IAA to passivate all 180 engineered cysteines.

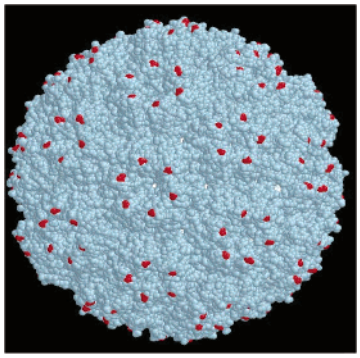

Figure 1. Space-filling representation of CCMV A163C with the engineered cysteines shown in red based on the PDB 1CWP.[18]

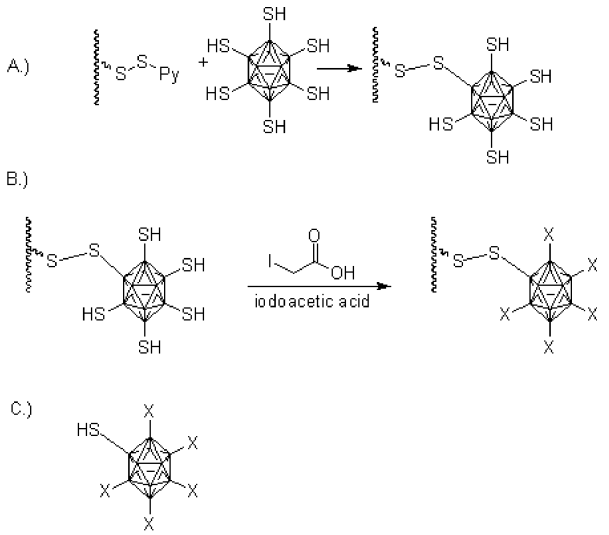

Figure 2. Symmetry breaking of the A163C viral cage: (A) reaction scheme for the thiol binding of the viral cage to an activated resin; (B) passivation of unbound cysteine residues with IAA; (C) removal of the symmetry-broken A163C by reduction.

The surface-exposed cysteines provide a mechanism to attach the viral cage to a gold substrate. An atomically flat Au surface was prepared by a controlled vacuum deposition of Au onto an oriented Si wafer (111). A solution of virus was placed on top of the gold surface and allowed to incubate for 20 min. Excess virus was removed by centrifugation, and the substrate was washed with water and allowed to air dry before imaging with AFM. All AFM imaging was performed in tapping mode on a Nanoscope III

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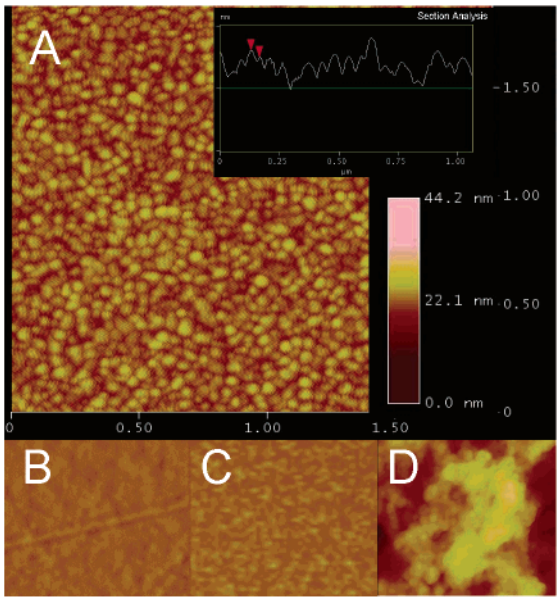

Figure 3. Tapping mode AFM imaging on CCMV Au substrate: (A) symmetry-broken A163C with distance and height profile (inset); (B) wild-type CCMV with no exposed thiols; (C) A163C treated with IAA to passivate all exposed thiols; (D) untreated A163C with surface-exposed thiols. All scans shown are 2 μm in length.

Multimode SPM (Digital Instruments, Inc., Santa Barbara, CA) using Tap300 cantilevers (NanoDevices, Santa Barbara, CA).

Four different viral samples were investigated. These were wild-type virus with no surface-exposed thiols, A163C with all 180 exposed thiols, A163C in which all exposed thiols have been passivated with IAA, and symmetry-broken A163C with a limited number of exposed thiols per virion.

The symmetry-broken A163C viral capsids, unlike the other three samples, form the beginnings of a monolayer array on the Au surface (Figure 3A). Some ordering appears to occur over 2−3 μm. An examination of the height and spacing profiles gives a height of approximately 33 nm above the Au substrate and a center-to-center spacing of 33 nm, which is in agreement with the expected 28 nm diameter of the viral cage (Figure 3A, inset). A Fourier transform of the viral array yields an average spacing (1/*k*) of 32 nm. In the controls, with particles having no introduced cysteines or the completely chemically passivated A163C, no virus was detected to be bound to the Au substrate (Figure 3B,C). In contrast, A163C (with up to 180 thiols exposed) formed large irregular aggregates bound to the surface of the Au (Figure 3D). These results clearly indicate that a limited number of reactive sites per cage is required for controlled monolayer formation at the Au surface.

An Ellmann's assay was performed on the viral films to estimate the number of free exposed thiols.[19] Free thiols were detected only in the aggregated A163C sample, consistent with the observed three-dimensional cross-linking. In the symmetry-broken A163C monolayers, no free thiols were detected, consistent with complete attachment to the Au from a single face of the virus.

To further illustrate the ability to pattern the genetically and chemically modified viral cage onto a substrate, we created a patterned Au surface using a 300 mesh grid as a mask (Figure 4). After removal of the mask, the symmetry-broken A163C binds selectively to the Au substrate (Figure 4A) but not to the underlying Si (Figure 4B).

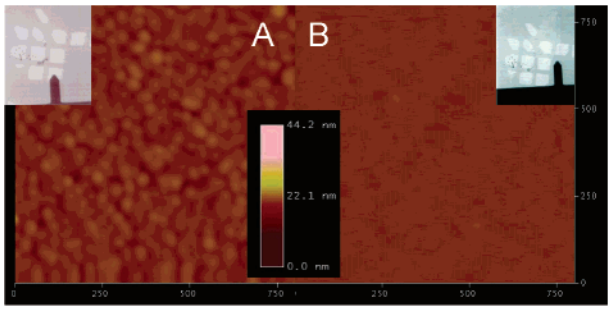

Figure 4. Selective binding of a modified viral protein cage to a patterned Au substrate: (A) symmetry-broken A163C binding selectively to patterned Au; (B) underlying Si with no detectable virus. Insets indicate the position of the cantilever relative to the patterned surface.

In conclusion, this work highlights three important concepts. First, through a combination of genetic and chemical modifications, we can engineer chemical functionality to symmetry-related sites on the viral capsid. Second, through a solid-phase synthetic approach, we can selectively block functional groups in a spatially controlled manner, thereby breaking the symmetry of the particle. Finally, viral protein cages with asymmetric functional groups can impart the ability to direct patterned monolayer formation of the viral capsids on a Au substrate.

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Supporting Information Available: Details on the site-specific mutagenesis, resin preparation, and symmetry-breaking protocols (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665−668.
(2) Lee, S.-W.; Mao, C.; Flynn, C. E.; Belcher, A. M. *Science* **2002**, *296*, 892−895.
(3) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nature Mater.* **2002**, *1*, 169−172.
(4) *Biomimetic Materials Chemistry*; Mann, S., Ed.; VCH: New York, 1996.
(5) Mann, S.; Archibald, D. D.; Didymus, J. M.; Douglas, T.; Heywood, B. R.; Meldrum, F. C.; Reeves, N. J. *Science* **1993**, *261*, 1286.
(6) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128.
(7) Douglas, T.; Strable, E.; Willits, D.; Aitouchen, A.; Libera, M.; Young, M. *Adv. Mater.* **2002**, *14*, 415.
(8) Wang, Q.; Lin, T. W.; Tang, L.; Johnson, J. E.; Finn, M. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 459−462.
(9) Stucky, G. D.; Dougal, J. E. M. *Science* **1990**, *247*, 669.
(10) Murray, C. B.; Norris, D. J.; Bawendi, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 8706.
(11) Nagayama, K. *Adv. Biophys.* **1997**, *34*, 3.
(12) Wagner, P.; Zaugg, F.; Kernen, P.; Hegner, M.; Semenz, G. *J. Vac. Sci. Technol. B* **1996**, *14*, 1466.
(13) Johnson, C. A.; Yaun, Y.; Lenhoff, A. M. *J. Colloid Interface Sci.* **2000**, *223*, 261.
(14) Garno, J. C.; Nabil, A. A.; Kapila, W.-M.; Gang-Yu, L. *Langmuir* **2002**, *18*, 8186.
(15) Brott, L. L.; PPikas, D. J.; Naik, R. R.; Kirkpatrick, S. M.; Tomline, D. W.; Whitlock, P. W.; Clarson, S. J.; Stone, M. O. *Nature* **2001**, *413*, 291.
(16) Lee, K.-B.; Park, S.-J.; Mirkin, C. A.; Smith, J. C.; Mrksich, M. *Science* **2002**, *295*, 1702.
(17) Kuznetsov, Y. G.; Malkin, A. J.; Lucas, R. W.; Plomp, M.; McPherson, A. *J. Gen. Virol.* **2001**, *82*, 2025.
(18) Speir, J. A.; Munshi, S.; Wang, G.; Baker, T. S.; Johnson, J. E. *Structure* **1995**, *3*, 63.
(19) Ellman, G. L.; Courtney, K. D.; Merrified, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 3102.

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