Virus-Templated Silica Nanoparticles**

Nicole F. Steinmetz, Sachin N. Shah, J. Elaine Barclay, Ghanasyam Rallapalli, George P. Lomonossoff, and David. J. Evans**

Silica nanoparticles are intriguing materials that have diverse applications including, for example, drug delivery, therapy and diagnosis, photonics, and bioanalysis.[1] Synthetic routes to such nanoparticles include the sol–gel Stöber process that involves the controlled hydrolysis of tetraethoxysilane (TEOS) using ammonia[2] and hydrolysis of TEOS in highly basic solutions in the presence of organic or inorganic cations.[3] More recently, TEOS hydrolysis in aqueous lysine solutions has been developed as a means to prepare suspensions of monodisperse silica nanoparticles within the 5–20-nm size range.[4] Here we describe, as proof-of-principle, the use of an engineered variant of the plant virus, *Cowpea mosaic virus* (CPMV), as a template for the controlled and designed fabrication of silica nanoparticles of ≈30-nm diameter at ambient temperature and in aqueous solvent. This is the first time that external mineralization of a viral cage-like structure has been reported.

Synthetic biology, that is, the exploitation of biomaterials for chemical design, has become an important area of materials design.[5] Organized biomolecular architectures offer a variety of morphologies, and many are robust enough to support reactions to produce novel organic/inorganic materials.[6–8] The filamentous bacteriophage M13 has been extensively studied as a template for mineralization and has been successfully utilized for the nucleation of different semiconductor and magnetic materials.[9,10] Belcher et al. have shown the potential of such an approach in the development of lithium ion battery electrodes using highly ordered M13-templated gold–cobalt oxide nanowires.[10] Besides utilizing the exterior of M13 as a template, the exterior and interior of the rod-shaped virus *Tobacco mosaic virus*,[7,11] as well as the interior cavity of the icosahedral virus *Cowpea chlorotic mottle virus*,[8,12] have also been used for the controlled entrapment and mineralization of materials.

Whereas the interior cavity of sphere-like viral particles has been exploited for mineralization,[8,12,13] to date there are no reports describing the controlled deposition and nucleation of inorganic materials on the exterior surface of such bionanoparticles. CPMV particles exhibit the characteristics of an ideal nanotemplate in terms of their size (≈28-nm diameter) and their regular symmetric structure. The genetic, biological, and physical properties are well-characterized and the structure of the virus particle is known.[14] Functional groups on the exterior surface of the virion makes CPMV a useful nanoscaffold, allowing attachment of different moieties.[15] Furthermore, infectious cDNA clones and chimaeric virus technology can be used to modify the surface and allow the presentation of foreign peptides on the particle surface.[16,17] To date, such genetically modified particles have been mainly used as immunogens with the principal aim of developing novel vaccines.[16–18] However, the recent observation that certain peptide sequences can stimulate the deposition of specific inorganic compounds[10,19,20] motivated us to explore whether CPMV-based chimaeric virus technology could be adapted for the production of silica nanoparticles.

It has been established that additional amino acids can be inserted into the highly surface exposed βB-βC loop of the S protein on the CPMV capsid.[21] The design of chimaeras that favored mineralization by silica involved the insertion of a specific peptide sequence (YSDQPTQSSQRP), previously selected for mineral nucleation by phage display,[19] between alanine-22 and proline-23 in the βB-βC loop. The peptide was engineered into the loop using established cloning procedures and the chimaeric $CPMV_{silica}$ construct was introduced into cowpea plants by agroinoculation (see Supporting Information).[16,22] Virus particles were produced after passaging the infection to further plants. After each passage and particle extraction from the infected plant tissue, the nucleotide sequence of the recombinant viral RNA was verified by reverse transcriptase polymerase chain reaction (RT-PCR) followed by sequencing through the region of the inserted peptide. Recombination or reversion to wild type was not observed and the chimaera was stable over several rounds of passaging. Yields were comparable to wild type CPMV, and typically 25 mg of $CPMV_{silica}$ particles were obtained from 100 g of infected leaf material. The integrity of the purified

[*] Dr. N. F. Steinmetz,[+] Dr. D. J. Evans, Dr. S. N. Shah, J. E. Barclay, G. Rallapalli, Prof. G. P. Lomonossoff
Department of Biological Chemistry, John Innes Centre
Norwich Research Park, Colney, Norwich, NR4 7UH (UK)
E-mail: nicoles@scripps.edu; dave.evans@bbsrc.ac.uk

[+] Present Address: Department of Cell Biology
Center of Integrative Molecular Biosciences
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)

[**] Dr. N. F. Steinmetz and Dr. S. Shah contributed equally to this research. The Biotechnology and Biological Sciences Research Council, UK (Core Strategic Grant to the John Innes Centre and Grant BB/E024939/1 {S.S}) and the EU Marie Curie Early Stage Training Scheme (MEST-CT-2004-504273 {N.F.S}) are thanked for funding. K. Findlay and S. Bunnewell, JIC, are thanked for assistance with TEM and R. Evans-Gowing, University of East Anglia, for EDX measurements, and A. Kirby, IFR, for NTA measurements.

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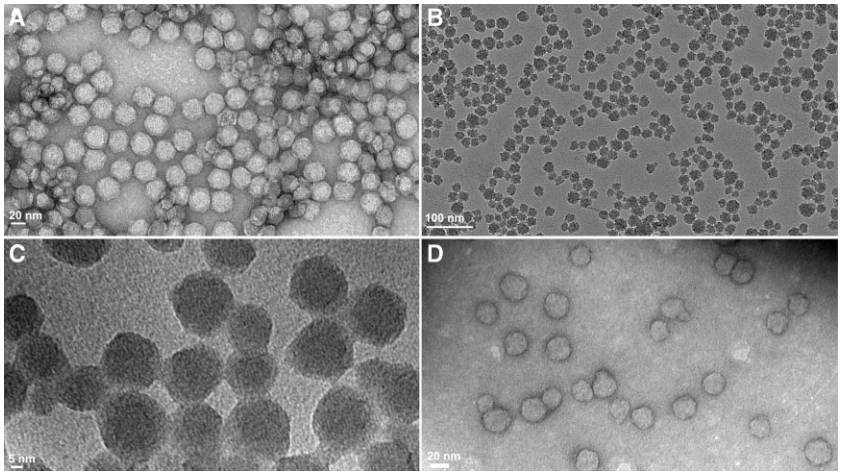

Figure 1. TEM images: A) $CPMV_{silica}$-chimaera particles before mineralization, stained with uranyl acetate. B, C) Unstained silicated-$CPMV_{silica}$ showing dense mineralized particles. D) Uranyl acetate-stained silicated-$CPMV_{silica}$.

$CPMV_{silica}$ virions was established by transmission electron microscopy (TEM) (Figure 1A) and native gel electrophoresis (not shown).

Having obtained the stable $CPMV_{silica}$-chimaera, silication was achieved by a sol–gel process. Chimaeric virus (15 mg mL^{-1}) in a 10 mM sodium phosphate buffer of pH 7 was treated with TEOS and aminopropyltriethoxysilane (90:10 mol%) and left to stand, without stirring, for two to four days at room temperature. The mixture was then heated for a further two days at 45 °C, a temperature at which CPMV is known to be stable. The silicated-$CPMV_{silica}$ nanoparticles were separated from nontemplated silica and purified by centrifugation and cut-off columns (see Supporting Information). The recovery of purified silicated-$CPMV_{silica}$ nanoparticles, for each method, was approximately 40% based on initial virus concentration.

TEM characterization indicated the presence of mineralized viral nanoparticles. While $CPMV_{silica}$ prior to mineralization is not visible in unstained TEM images (not shown), TEM images of unstained silicated-$CPMV_{silica}$ particles showed dense particles of about 30-nm diameter (Figure 1B and C), thus indicating that $CPMV_{silica}$ indeed served as a template for the mineralization process. Control reactions using wild type CPMV and buffer alone were also performed under identical conditions to those used to prepare silicated-$CPMV_{silica}$. In all cases, gel formation occurs but this is initially faster for $CPMV_{silica}$. After the purification process, the unstained TEM of the controls show no silica particles, consistent with the requirement for the presence of $CPMV_{silica}$ to achieve templated mineralization; negatively stained (uranyl acetate) TEM of wild type CPMV after being treated under the conditions for mineralization confirmed the presence of viral nanoparticles. TEM images (Figure 1D) of negatively stained silicated-$CPMV_{silica}$ revealed the presence of the silica coat and showed particles of a similar size to that seen with unstained material. However, uranyl acetate-stained silicated-$CPMV_{silica}$ particles have a different appearance in the TEM when compared to stained nonsilicated $CPMV_{silica}$-chimaera (Figure 1A) or wild type CPMV.

Energy dispersive X-ray (EDX) analysis unambiguously confirmed the presence of silicon and oxygen on the silicated-$CPMV_{silica}$ particles (Figure 2). Nanoparticle tracking analysis, based on laser light scattering microscopy, is also consistent with silication. The relative refractive index of silicated-$CPMV_{silica}$ is significantly increased compared to that of the nonsilicated $CPMV_{silica}$-chimaera (Figure 3), although by this method we were unable to estimate the size of the particles due to some nonspecific aggregation under the conditions employed. Aggregation may occur due to the propensity for silanol groups to form on the silica particle surface, with those on adjacent particles forming hydrogen bonds, leading to aggregate formation.[23]

Dynamic light scattering (DLS, see Supporting Information) was consistent with the findings from TEM studies and also showed successful mineralization. DLS of silicated-$CPMV_{silica}$ in buffer is consistent with

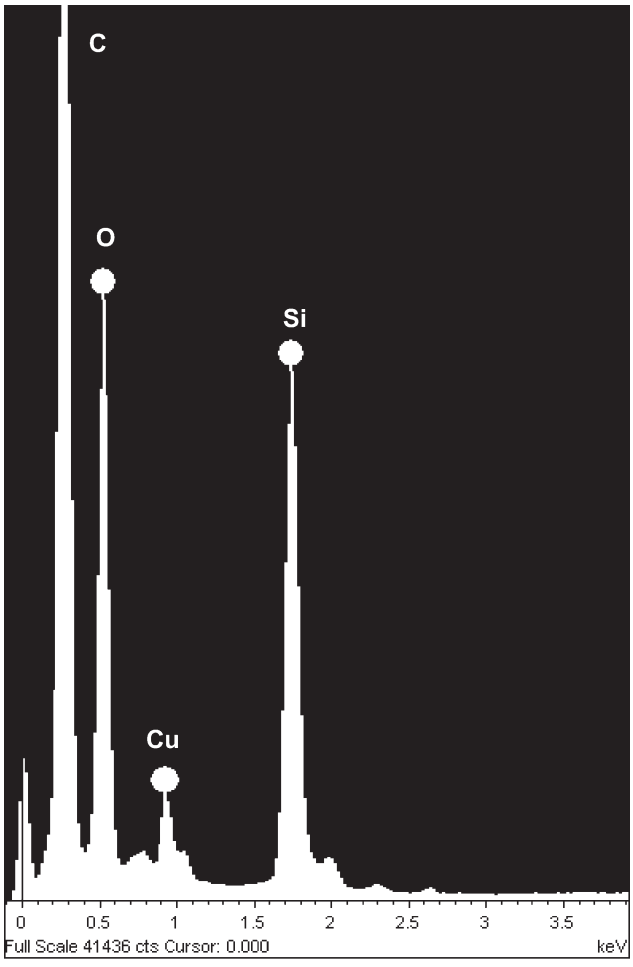

Figure 2. EDX spectrum of silicated-$CPMV_{silica}$ mounted on a TEM copper grid showing peaks characteristic of silicon and oxygen and confirming that silicated-$CPMV_{silica}$ particles are mineralized with silica.

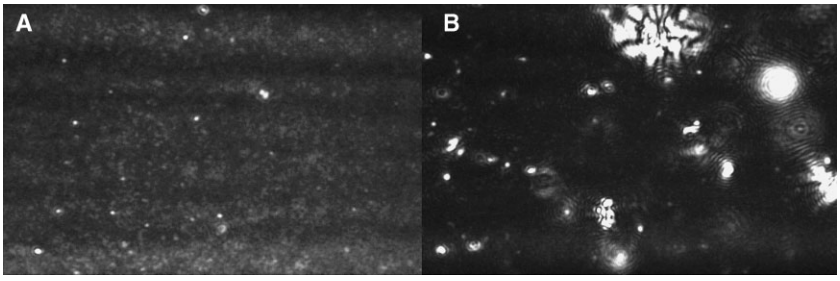

Figure 3. Nanoparticle tracking analysis showing an increase in the refractive index of $CPMV_{silica}$-chimaera particles after mineralization with silica. A) $CPMV_{silica}$-chimaera before mineralization has low intensity; the particles appear individually as point scatterers under Brownian motion. B) Silicated-$CPMV_{silica}$ particles show much higher intensity.

monodisperse nanoparticles, with an average radius of ≈16 nm. Prior to mineralization, $CPMV_{silica}$ has a radius of ≈14 nm, implying that the average coating of silica on each particle is approximately 2 nm. The DLS polydispersity of $CPMV_{silica}$ and silicated-$CPMV_{silica}$ are 7% and 14%, respectively, indicating that, by this criterion, the particles are monodisperse. Further support for the conclusion that a robust silica shell has been deposited on the surface of the $CPMV_{silica}$ was obtained by comparison of the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel of silicated and nonsilicated CPMV particles (see Supporting Information). Even under harsh conditions (denaturation with lithium dodecyl sulfate at 100 °C for 30 min), there is no denaturation of silicated-$CPMV_{silica}$, as shown by the absence of coat proteins on the SDS-PAGE gel. This is the result that would be expected if the denaturing reagent is either unable to penetrate the silica coat and/or the coat proteins cannot be released from the silica shell.

In conclusion, through the use of a CPMV-chimaera that contains a short peptide that favors mineralization with silica, we have produced templated silicated nanoparticles. Since this method facilitates the preparation of the silica nanoparticles at ambient temperature and in aqueous media, no organic solvents are required and the method produces little waste. This is the first time that mineralization of the external surface of a cage-like virus has been reported. We are now exploring how these and other mineralized-CPMV-chimaeras may be exploited in nanotechnological applications such as biomedicine and catalysis.

Keywords:
bionanotechnology · mineralization · nanoparticles · silica · viruses

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