Virus templated metallic nanoparticles.

Alaa A. A. Aljabali, J. Elaine Barclay, George P. Lomonossoff and David J. Evans*

Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Colney,
Norwich, NR4 7UH (United Kingdom)

Fax: +44-(0)1603-450018
dave.evans@bbsrc.ac.uk

Supplementary Information

Instrumentation

- Dynamic Light Scattering (DLS) – DynaPro Titan, Wyatt Technology Corporation.
- Zeta Potential (ZP) – Malvern Instruments Zetasizer-Nano ZS.
- AFM Asylum Research MFP 3D mounted on Olympus IX71 inverted optical microscope
- UV-vis Spectrometer – Perkin Elmer Lambda 25

General Methods

Cowpea mosaic virus (CPMV) purification

CPMV was purified from infected Vigna unguiculata (black-eyed pea) leaves using a modified protocol (http://www.dpvweb.net/dpv/showdpv.php?dpvno=197). Particle integrity was investigated by TEM and agarose gel electrophoresis and the concentration was determined photometrically; the molar extinction coefficient of CPMV at a wavelength of $\lambda = 260$ nm is $\varepsilon = 8.1 \, \text{ml mg}^{-1} \, \text{cm}^{-1}$. 
Transmission Electron Microscopy (TEM)
Purified samples were transferred into Milli-Q water using 100 kDa cut-off columns (Microcon), allowed to settle briefly onto pyroxylin and carbon-coated copper grids and then blotted dry. For negative staining, 2% uranyl acetate solution was used. Grids were viewed at 200 kV in an FEI Tecnai20 TEM (FEI UK Ltd, Cambridge) and images were obtained using a bottom-mounted AMT XR60 CCD camera (Deben UK Ltd, Bury St. Edmunds).

Dynamic Light Scattering (DLS)
Diluted samples (13 µl), at approximately 0.5 mg/ml in sodium phosphate buffer (10 mM) pH 7.0 were filtered through 0.1 µm filters (Millipore) prior to analysis. Measurements were taken every 10 seconds, and 10 measurements were averaged from 3 runs at 21 °C.

Zeta potential
1 ml of 0.5 mg/ml metallized-CPMV particles dispersed in sodium phosphate buffer (10 mM) pH 7.2 was prepared. Zeta cells were equilibrated at 21 °C for two minutes before recording three measurements each of ten runs. Data was collected with automatic attenuation selected and analysed using the Smoluchowski module.

Agarose Gel Electrophoresis
5-10 µg of CPMV particles suspended in sodium phosphate buffer (10 mM) pH 7.0 with 2 µl of loading dye (Coomassie staining solution) were analysed on 1.2 % (w/v) agarose gel in an electric field of 60 V for 1-2 hours. For ethidium bromide staining, 0.1 µg/ ml (4-5 µl) in 1x TBE buffer was added to the gel. Particles were visualised on a UV transilluminator at 302 nm using Gene Genius Bio Imaging System with software Gene Snap (Syngene). For coat protein staining, gels were treated with Coomassie staining solution (50 % (v/v) methanol; 10 % (v/v) acetic acid; 0.25 % (w/v) Coomassie Brilliant Blue G-250) for 1 hour followed by destaining solution (20 % (v/v) methanol; 7.5 % (v/v) acetic acid in MilliQ water) overnight. Gel images were recorded using camera or scanner.
Supplementary Information Figures

**Figure S-1.** DLS, hydrodynamic radius (left) and correlation plots (right) comparing wild-type CPMV (blue line) and Pd⁰-CPMV particles (red line).

**Figure S-2.** EDX spectrum of Pd⁰-CPMV particles. Main palladium peak indicated by arrow.
Figure S-3. Zeta potential measurement in buffer at pH 7.0 of (A) Pd$^{2+}$-CPMV particles, (B) Pd$^{0}$-CPMV particles.

Figure S-4. Agarose gel (1.2%) of CPMV particles visualised by (A) ethidium bromide staining, (B) Coomassie staining. Lane 1, wild-type CPMV; 2, Pd$^{0}$-CPMV.
Figure S-5. EDX spectrum of Co-CPMV, Fe-CPMV, Ni-CPMV, Pt-CPMV and NiFe-CPMV metallized particles. Major metal peaks indicated by arrows.
Figure S-6. EDX spectrum of CoPt-CPMV metallized particles. Major metal peaks indicated by arrow.

Figure S-7. DLS comparing wild-type CPMV to Ni-CPMV, Co-CPMV, Pt-CPMV, and Fe-CPMV particles. Hydrodynamic radius plot (left) and correlation graph (right).
Figure S-8. DLS for wild-type CPMV, NiFe-CPMV and CoPt-CPMV particles. Hydrodynamic radius plot (left) and correlation graph (right).
Figure S-9. Zeta potential measurement of (A) Co-CPMV, (B) Fe-CPMV, (C) Ni-CPMV, (D) Pt-CPMV, (E) CoPt-CPMV and (F) NiFe-CPMV particles suspended in buffer at pH 7.0.
Figure S-10. Negative image of a freeze-frame from nanoparticle tracking analysis for metallized-CPMV nanoparticles showing that the particles appear individually as point scatterers under Brownian motion with a high refractive index. (A) Co-CPMV; (B) Ni-CPMV; (C) CoPt-CPMV.

Figure S-11. AFM image for Ni-CPMV dried on glass slide.