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**Running head:** Cowpea mosaic virus gold nanoparticles

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## **“Polyelectrolyte-modified Cowpea mosaic virus for the synthesis of gold nanoparticles”**

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### **Summary**

Polyelectrolyte surface–modified Cowpea mosaic virus (CPMV) can be used for the templated synthesis of narrowly dispersed gold nanoparticles. Cationic polyelectrolyte, poly(allylamine) hydrochloride, is electrostatically bound to the external surface of the virus capsid. The polyelectrolyte–coated CPMV promotes adsorption of aqueous gold hydroxide anionic species, prepared from gold(III) chloride and potassium carbonate, that are easily reduced to form CPMV–templated gold nanoparticles. The process is simple and environmentally benign using only water as solvent at ambient temperature.

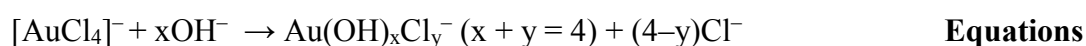
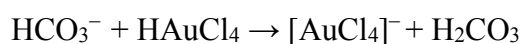
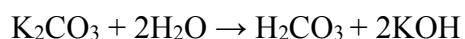
**Key words:** Nanoparticles, Gold, Cowpea mosaic virus, Polyelectrolyte, Templated mineralization

## 1. Introduction

Gold nanoparticles have recently generated considerable interest because of their potential for applications in the optical sensing of biological and chemical analytes (1), diagnostics, drug delivery and photothermal therapy (2). Particles of the 30 nm diameter icosahedral plant virus, Cowpea mosaic virus (CPMV), provide an ideal nanotemplate for mineralization. The genetic, biological and physical properties of the virus are well characterised and its structure is known to near atomic resolution (3). Groups on the exterior surface of the virus capsid enable the attachment of many different chemicals and make CPMV a useful nanoscaffold (4–7). Over the past few years we have investigated methods for the synthesis of CPMV–templated, inorganic, monodisperse, nanoparticles. The approaches include: (i) using genetically modified CPMV chimaeras that display peptides on their outer surface which promote specific mineralization processes, to create silica (8) and iron–platinum (9) nanoparticles; (ii) chemically–coupled–peptide–promoted mineralization of wild–type CPMV, for iron–platinum, cobalt–platinum and zinc sulfide (10); (iii) an electroless deposition technique, for iron, cobalt, nickel, platinum, cobalt–platinum and nickel–iron (11); (iv) templated mineralization by charge–modified CPMV, for cobalt and iron oxide (12), see Chapter X.

Here, we describe the use of polyelectrolyte–modified CPMV for the synthesis of gold nanoparticles (Fig. 1) (13). CPMV at neutral pH can be considered as an anionic macromolecule with an isoelectric point ( $pI$ ) of  $4.3 \pm 0.1$  (14). At physiological pH (pH 7.4), aspartic and glutamic acid residues on the external surface of the virus capsid are deprotonated so the surface presents an overall negative charge. Incubation

of CPMV with cationic polyelectrolyte, poly(allylamine) hydrochloride (PAH), led to electrostatic adsorption of a thin layer of polyelectrolyte onto the capsid surface, <sup>PA</sup>CPMV. The <sup>PA</sup>CPMV was characterised by a range of techniques (13) including uranyl acetate stained transmission electron microscopy (TEM) (Fig. 2). An aqueous gold hydroxide solution was prepared from gold(III) chloride and potassium carbonate (Equations) by a published procedure (15, 16). Treatment of <sup>PA</sup>CPMV with this solution initially electrostatically adsorbs anionic gold(III) hydroxyl species onto the cationic surface of the <sup>PA</sup>CPMV particles. Mild reduction gave metallic gold at the surface; the reaction was accompanied by a colour change from colourless to light purple. After purification, the gold coated CPMV nanoparticles (Au–CPMV) were characterised by a range of techniques (13) including TEM (Fig. 2), dynamic light scattering (DLS), energy dispersive X–ray spectroscopy and zeta potential measurements.



“[Fig 1 near here]”

The Au–CPMV particle size, as measured by DLS, showed an average diameter of *ca.* 67 nm; the gold coating has a thickness of approximately 20 nm. The particles were narrowly dispersed, in agreement with the observed TEM images, and the CPMV particles were mineralized on their outer surfaces. The thickness of the gold coating can be controlled. For example, by reducing the volume of the gold solution aliquot

by half gave particles of *ca.* 40 nm diameter, whereas doubling the gold solution aliquot gave larger particles of *ca.* 300 nm diameter, when all other conditions were kept constant. The method is simple and environmentally benign, as only aqueous solvent and ambient temperature and pressure are required.

“[Fig 2 near here]”

## 2. Materials

Poly(allylamine hydrochloride) (PAH, MW ~15000), poly(fluorescein isothiocyanate allylamine hydrochloride) (FITC-PAH, MW ~9291) were purchased from Sigma-Aldrich; potassium carbonate from BDH; hydroxylamine hydrochloride, 99 %, from Lancaster Synthesis; Sephadex G-25 PD-10 columns from GE Healthcare; 100 and 300 kDa molecular weight cut-off membranes from Spectrum Labs. All reagents were used without further purification and all experiments were performed using Milli-Q water with resistivity of 18.2 MΩ cm (Millipore). The propagation and purification of wild-type CPMV particles were performed by standard procedures (17). Purified virions were stored at 4 °C in 10 mM sodium phosphate buffer pH 7.0. (1 litre of 0.2 M Na<sub>2</sub>HPO<sub>4</sub> (27.0 g) and 1 litre of 0.2 M NaH<sub>2</sub>PO<sub>4</sub> (28.39 g) were prepared in Milli-Q water and autoclaved before mixing. To prepare 0.1 M buffer solution; 305 mL of Na<sub>2</sub>HPO<sub>4</sub> solution and 195 mL of NaH<sub>2</sub>PO<sub>4</sub> solution was added to 500 mL Milli-Q water that gave a pH of 7.0).

## 3. Methods

### Poly(allylamine)hydrochloride-CPMV (<sup>PA</sup>CPMV)

1. Before and after each experimental step UV-visible absorbance was recorded at 260 nm (*see Note 1*).

2. Just prior to use, CPMV particles were suspended in Milli-Q water by either buffer exchange on disposable gel filtration columns (PD-10) pre-equilibrated with Milli-Q water or by dialysis using 100 kDa cut-off membranes for 4–6 hours, changing Milli-Q water at 2 hourly intervals. Typically 10 mg mL<sup>-1</sup> CPMV particles were suspended in 2.5 mL; the void volume of the gel filtration column.
3. CPMV particles suspended in Milli-Q water (1 mg mL<sup>-1</sup>, 1 mL) were added dropwise over 5–10 minutes, with continuous and vigorous stirring on a vortex mixer at 2000 rpm, to a freshly prepared solution of polyallylamine hydrochloride (PAH) (1 mg mL<sup>-1</sup>) dissolved in aqueous 0.25 M NaCl (1 mL).
4. The cationic polyelectrolyte was allowed to adsorb onto the virus capsid for 30 minutes while gently shaking (800 rpm) at 4 °C (cold room).
5. The <sup>PA</sup>CPMV particles were washed 3–4 times with Milli-Q water (15 mL) on 100 or 300 kDa cut-off membranes.
6. Quality control: to ensure modified-virus particle integrity before proceeding to the next step, <sup>PA</sup>CPMV particles were run on an agarose electrophoresis gel and stained with ethidium bromide or Coomassie blue (*see Note 2*).

**“[Fig 3 near here]”**

7. The washing was followed by dialysis against 10 mM sodium phosphate buffer pH 7.0 for 3 hours at ambient temperature or in the cold room (4 °C).
8. <sup>PA</sup>CPMV was obtained in approximately 50% yield, based on initial virus concentration, as calculated by measuring the UV–visible absorbance at 260 nm.

### **Gold hydroxide solution**

1. Gold(III) chloride trihydrate (17.4 mL of 25 mM solution) was diluted with Milli–Q water (982.6 mL) in a 1 litre brown Duran bottle.
2. Potassium carbonate (249 mg, 1.8 mM) was added to the solution.
3. The solution was aged for 1–2 days in the dark (in brown foil wrapped vial) at 4 °C, during which it changed colour from yellow to colourless due to the formation of gold hydroxide solution (*15, 16*).
4. Gold hydroxide solution was used within 2 weeks

### **Gold coated CPMV nanoparticles (Au–CPMV)**

1. Freshly prepared <sup>PA</sup>CPMV particles (1–1.5 mg mL<sup>-1</sup>, 1mL) suspended in Milli–Q water were incubated with gold hydroxide solution (1 mL) as prepared above. The thickness of the gold coating was controlled by varying the incubation time with the gold hydroxide solution (*see Note 3*).
2. The reaction solution, in a vial wrapped in foil, was left stirring at 600 rpm at ambient temperature for 2 hours.

3. A freshly prepared aqueous solution of hydroxylamine hydrochloride was added to a final concentration of 20 mM.
4. The reduction reaction was left to proceed for a further 20 minutes at ambient temperature.
5. Au-CPMV particles were spun at 14000 rpm (bench top centrifuge) for 20 minutes to remove any large aggregates.
6. The supernatant was collected and layered onto 5 mL 10%–50% sucrose gradients (*see Note 4*). Sucrose fractions containing Au-CPMV particles (bluish colour) were collected and dialysed against 10 mM sodium phosphate buffer pH 7.0 for 15 hours using 100 kDa molecular weight cut-off membranes.

#### 4. Notes

1. UV-visible spectroscopy of CPMV particles: Virus concentration was determined by photometrical measurement using either a Perkin Elmer Lambda 25 UV-VIS spectrophotometer with UVWINLab Software or a NanoDrop® ND-1000 spectrophotometer and ND-1000 software, at room temperature using a quartz cuvette (light path 1 cm). CPMV particles have an absorption maximum at a wavelength of  $\lambda = 260$  nm (derived from the encapsidated RNA molecules) with molar extinction coefficient of  $\epsilon = 8.1 \text{ mL mg}^{-1} \text{ cm}^{-1}$ . The law of Beer-Lambert can be used to calculate the concentration:

$$A = c d \epsilon$$



Where  $A$  is the absorbance,  $c$  is the concentration of the particles in  $\text{mg mL}^{-1}$ ,  $d$  is the length of the light path in  $\text{cm}$  and  $\epsilon$  the molar extinction coefficient in  $\text{mL mg}^{-1} \text{cm}^{-1}$ .

2. Confirmation of adsorption of polyelectrolyte to the surface was by the use of fluorescently labelled polyelectrolyte, poly(fluorescein isothiocyanate allylamine hydrochloride (FITC-PA). FITC-PA CPMV particles were prepared following the same method as described for PA CPMV except that the incubation was for 15 minutes.

3. Treatment of PA CPMV under our standard conditions, as described, gave a particle size of *ca.* 67 nm. Keeping all other conditions the same but reducing the volume of the gold solution aliquot by half gave particles of *ca.* 40 nm diameter, whereas doubling the gold solution aliquot gave much larger particles of *ca.* 300 nm diameter.

4. Sucrose Gradients: Sucrose solutions of 50%, 40%, 30%, 20%, and 10% (w/v) in 10 mM sodium phosphate buffer pH 7.4 were prepared and used within 2 weeks. Gradients were prepared by underlying sucrose solutions of decreasing density (175 or 500  $\mu\text{L}$ ) in a centrifuge tube of (2.1 mL or 5 mL, respectively) and carefully overlaying the sample to fill the tube. Gradients were ultracentrifuged in a swing-out rotor (AH-650) and centrifuged at 137000  $g$  for 1.5–2.5 hours at 4 °C; 175–300  $\mu\text{L}$  fractions containing modified particles were collected and buffer exchanged for 10 mM sodium phosphate buffer pH 7.4.

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## Figure Legends:

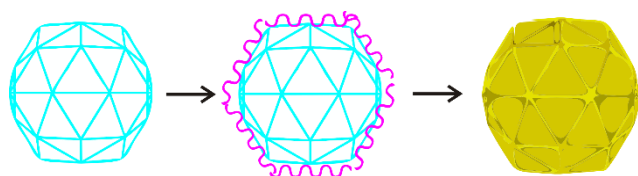
**Figure 1.** Schematic representation of polyelectrolyte surface–modified CPMV templated synthesis of gold nanoparticles (Reprinted with permission from *Biomacromolecules*, 2011, **12**, 2723-2728. Copyright 2011 American Chemical Society).

**Figure 2.** TEM images of (A) uranyl acetate stained <sup>PA</sup>CPMV and (B) unstained Au–CPMV with high magnification image shown in inset (Reprinted with permission from *Biomacromolecules*, 2011, **12**, 2723-2728. Copyright 2011 American Chemical Society).

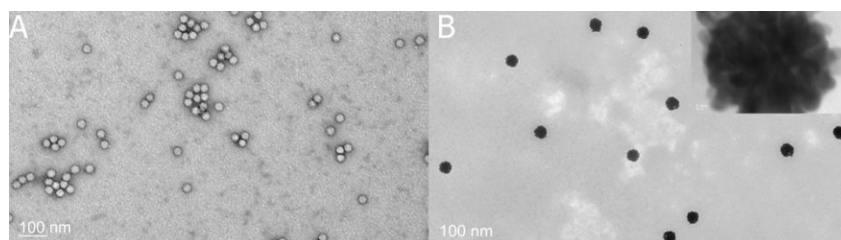
**Figure 3.** Agarose gel electrophoresis (1.2%). (A) unstained, (B) ethidium bromide stained and (C) Coomassie blue stained. Lane 1, CPMVwt, Lane 2, <sup>PA</sup>CPMV and Lane 3, <sup>FITC-PA</sup>CPMV (*see Note 2*). Gel C is a photographic image at different scale to A and B (Reprinted with permission from *Biomacromolecules*, 2011, **12**, 2723-2728. Copyright 2011 American Chemical Society).

## Figures.

**Figure 1.**



**Figure 2.**



**Figure 3.**

