ELECTRONIC SUPPLEMENTARY INFORMATION

Chemically-coupled-peptide-promoted virus nanoparticle templated mineralization

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- **Quantification of Peptides Bound to Surface Lysines in Peptide$_{CoPt}$-CPMV**
- **Energy Dispersive X-ray Spectroscopy**
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- **Agarose Gel Electrophoresis**
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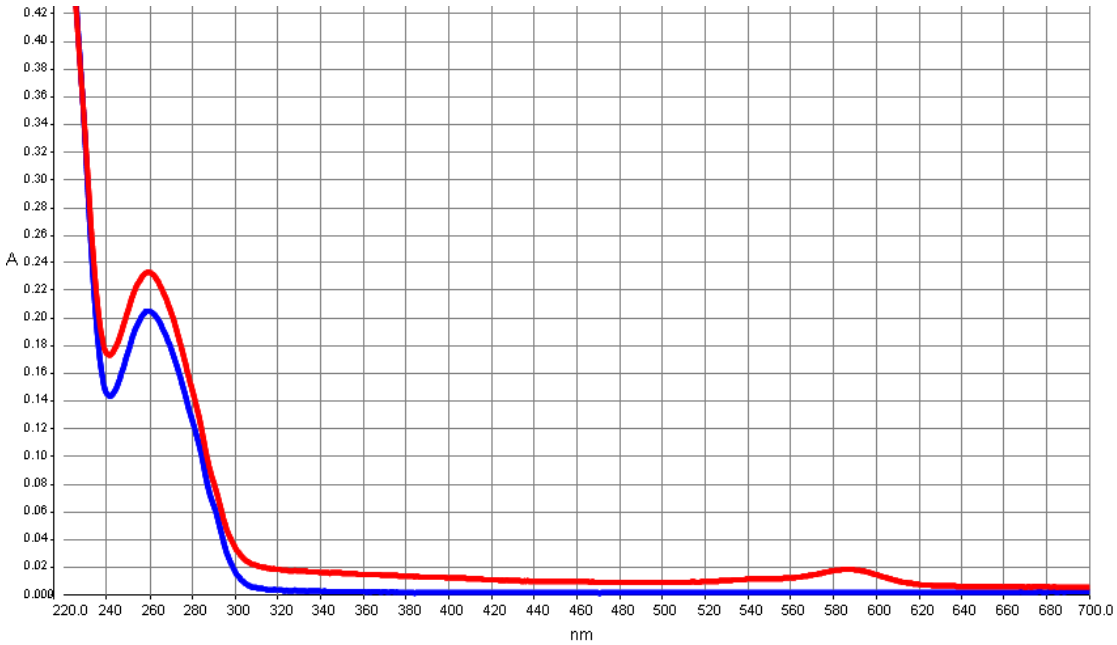
Quantification of Peptides Bound to Surface Lysines in Peptide$_{CoPt}$-CPMV

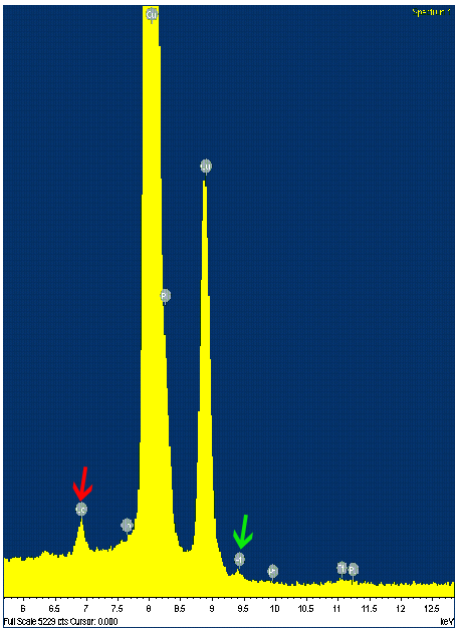
Peptide$_{CoPt}$-CPMV conjugate in sodium phosphate buffer pH7 was reacted with an excess of NHS ester-activated amine specific dye, DyLight594, and the number of free reactive lysines was calculated, from the absorbance after rigorous purification, to be ~66 per virion. This equates to approximately 180 peptides {maximum addressable amines (240) – free amines remaining in conjugate (66) = 174} being bound to each CPMV virus particle.

ESI Figure 1. UV-visible spectrum of wild-type CPMV (blue line) and peptide$_{CoPt}$-CPMV conjugate labelled with amine-specific DyLigh594 dye (red line).

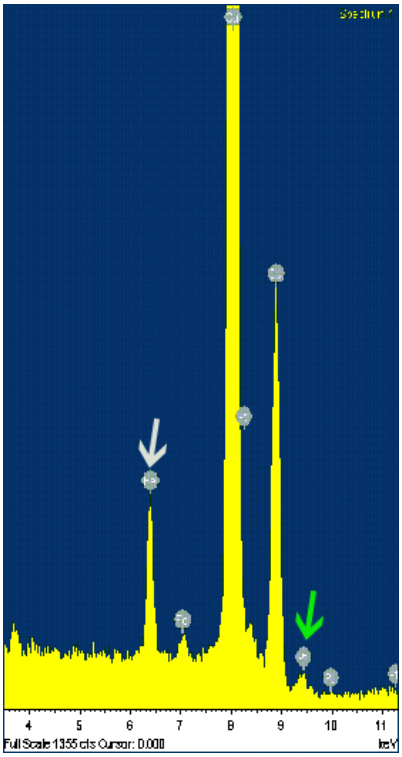


Energy Dispersive X-ray Spectroscopy (EDXS)

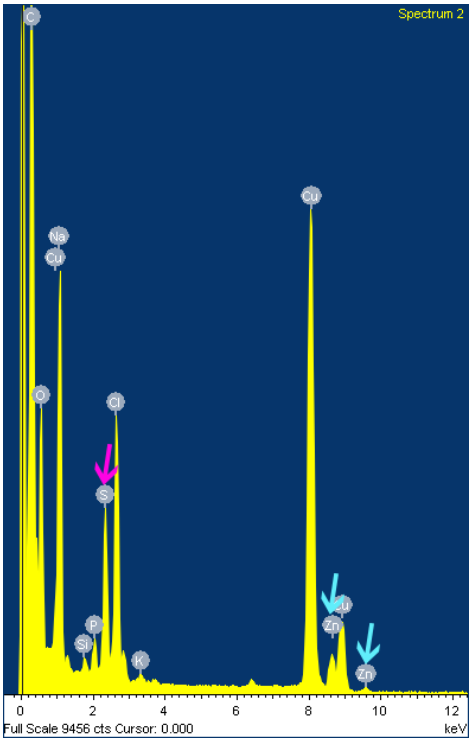
ESI Figure 2. EDX spectrum of CoPt-CPMV mineralized particles. Major peaks for each metal indicated by arrows.



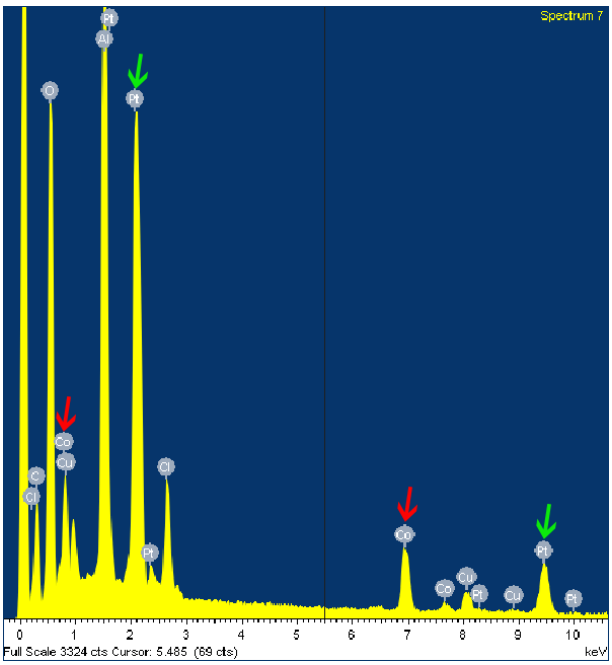
ESI Figure 3. EDX spectrum of FePt-CPMV mineralized particles. Major peaks for each metal indicated by arrows.



ESI Figure 4. EDX spectrum of ZnS-CPMV mineralized particles. Major peaks for each element indicated by arrows.



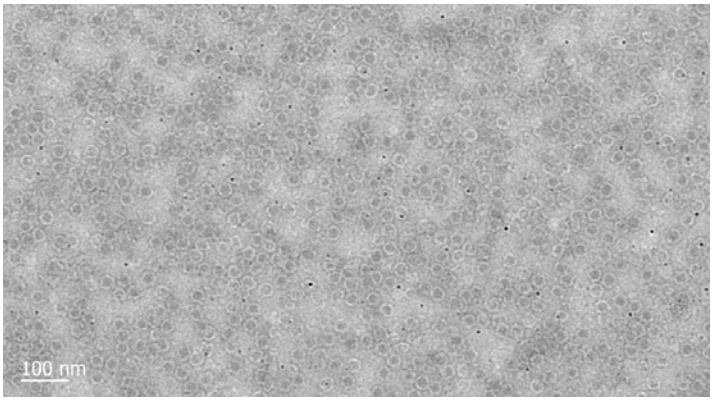
ESI Figure 5. EDX spectrum of CoPt mineralized MWCNT. Major peaks for each metal indicated by arrows.



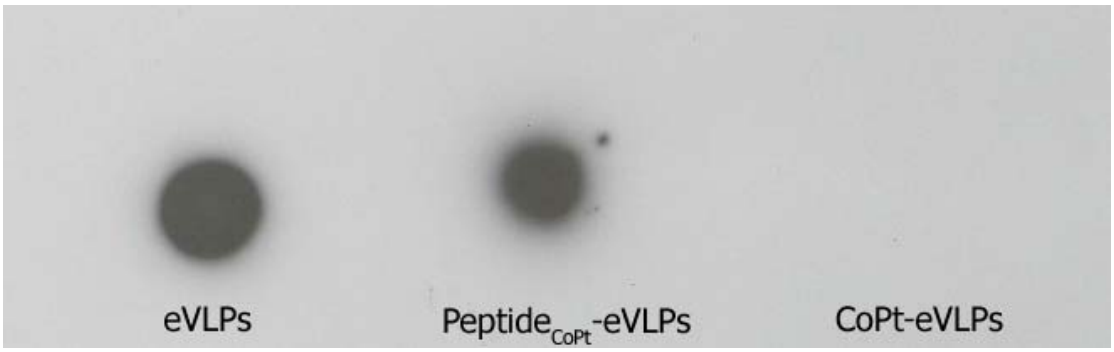
Mineralization of Peptide$_{CoPt}$-Modified Empty CPMV Particles

Peptide$_{CoPt}$ was incubated with a 2000 molar excess of 1:4 EDC/sulfo-NHS in water and left to react at room temperature while gently stirring for 2 hours. The esterified peptide was next incubated with (0.7 mg/mL, 1mL) of natural infection empty (RNA free) CPMV particles (eVLPs, top component, see N. F. Steinmetz, D. J. Evans and G. P. Lomonossoff, *ChemBioChem*, 2007, **8**, 1131-1136); the reaction was left to proceed overnight at 4 °C while gently stirring. Peptide$_{CoPt}$-eVLPs were purified on PD-10 columns; the eluted fractions were collected, concentrated on 100 kDa cut-off columns. The particles were incubated with a freshly prepared 1:1:1 mixture of $CoCl_2$ (0.05 mol dm^{-3}), H_2PtCl_6 (0.05 mol dm^{-3}) and $NaBH_4$ (0.1 mol dm^{-3}) each in Milli-Q water (600 µL). The reaction was allowed to proceed at ambient temperature with gentle stirring. After 24 hours the reaction mixture was centrifuged at 14000 rpm (bench top) for 20 minutes, the supernatant was purified further on a 100 kDa cut-off column and washed thoroughly with Milli-Q water. Mineralized particles were then examined by TEM and antibody detection. The unstained TEM image is consistent with both internal and external mineralization and shows only one morphological form. Immunological detection shows the presence of accessible coat protein for eVLPs and peptide$_{CoPt}$-eVLPs but not for mineralized particles.

ESI Figure 6. Unstained TEM image of peptide$_{CoPt}$-eVLPs that have been mineralized both internally and externally (the black spots are non-templated CoPt nanoparticles).



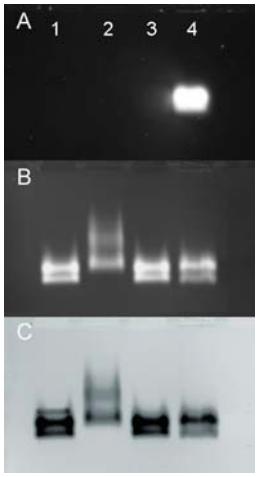
ESI Figure 7. Immunological detection of coat protein for eVLPs, peptide$_{CoPt}$-eVLPs and mineralized CoPt-eVLP.



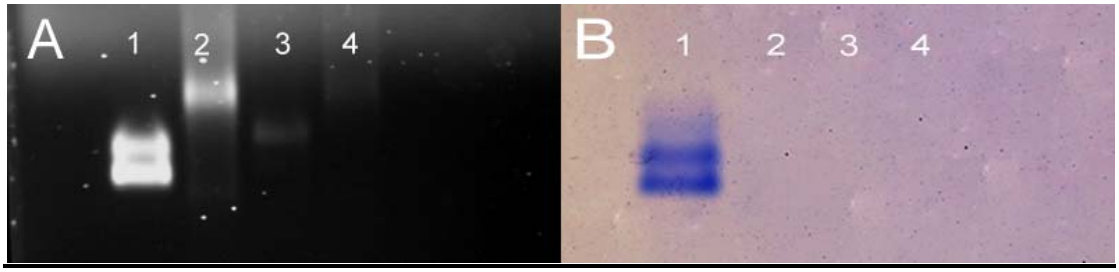
Agarose Gel Electrophoresis

5-10 µg of CPMV particles suspended in 10 mM sodium phosphate buffer pH 7.0 with 2 µL of loading dye (Coomassie staining solution or MBI Fermentas dye) were analysed on 1.2 % (w/v) agarose gel in an electric field of 60 V for 1-2 hours. For ethidium bromide staining (of nucleic acid), 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 (50 % (v/v) methanol; 20 % (v/v) acetic acid in Milli-Q water) overnight. Gel images were recorded using camera or scanner.

ESI Figure 8. Native 1.2% agaraose gel (A) unstained; (B) ethidium bromide stained; (C) Coomassie Blue stained. Lane 1, wild-type CPMV; 2, NHS esterified-CPMV; 3, peptide$_{CoPt}$-CPMV conjugate; 4, peptide$_{CoPt}$-CPMV conjugate dyed with DyLight594 dye.



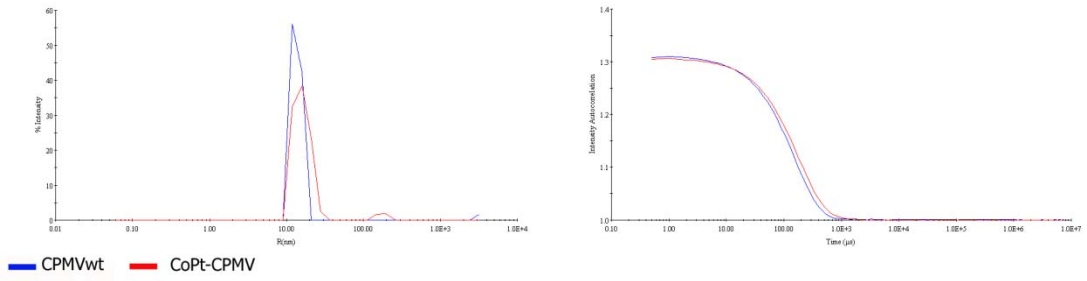
ESI Figure 9. Native 1.2% agaraose gel stained with (A) ethidium bromide and (B) Coomassie Blue. Lane 1, wild-type CPMV; 2, CoPt-CPMV; 3, FePt-CPMV; 4, ZnS-CPMV.



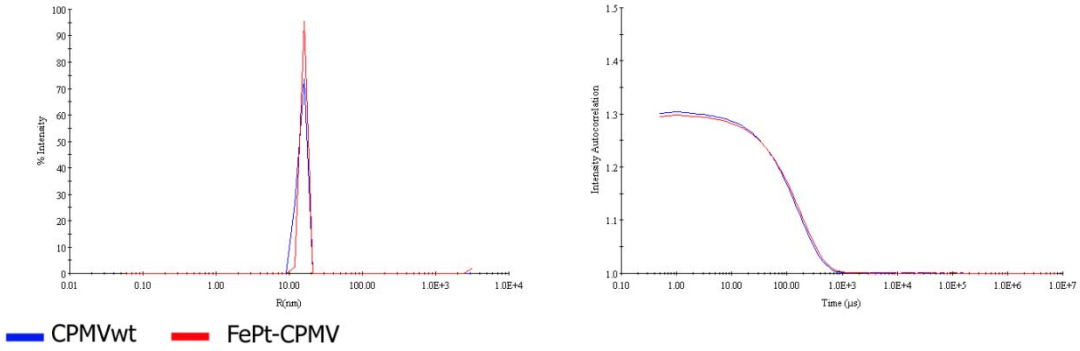
Dynamic Light Scattering (DLS)

Hydrodynamic radius (left) and raw correlation plots (right) are shown comparing wild-type CPMV (blue line) with mineralized-CPMV nanoparticles (red line). Particles at approximately 0.5 mg/mL in 10 mM sodium phosphate buffer pH 7.0 were filtered through 0.1 μm filters (Millipore) immediately prior to analysis. Measurements were taken every 10 seconds and 10 measurements were averaged from 3 runs at 21 °C.

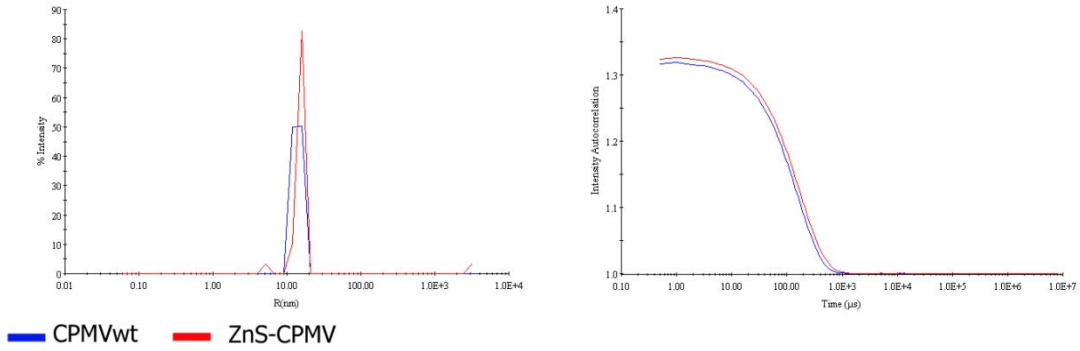
ESI Figure 10. DLS comparing wild-type CPMV (blue line) and CoPt-CPMV mineralized particles (red line).



ESI Figure 11. DLS comparing wild-type CPMV (blue line) and FePt-CPMV mineralized particles (red line).



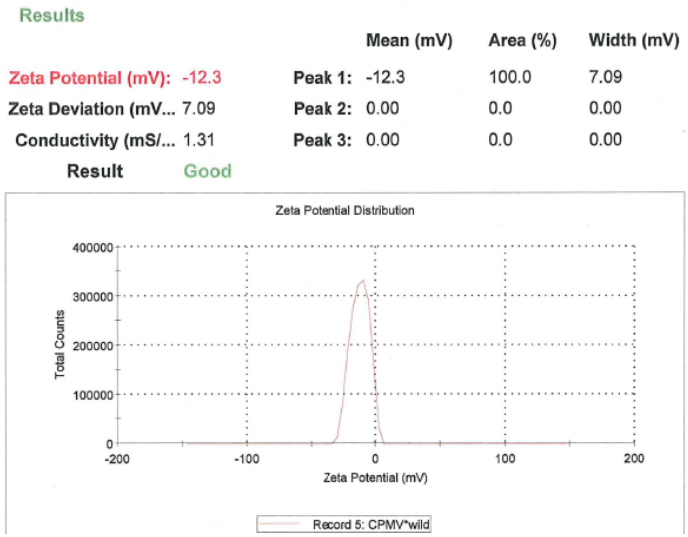
ESI Figure 12. DLS comparing wild-type CPMV (blue line) and ZnS-CPMV mineralized particles (red line).



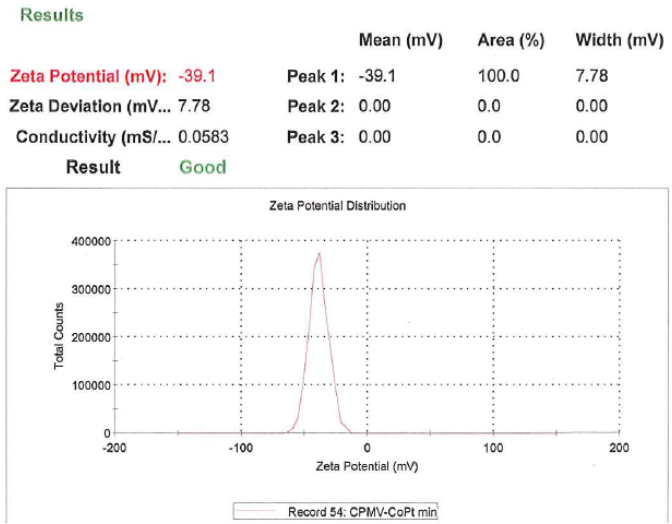
Zeta Potential Measurement

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

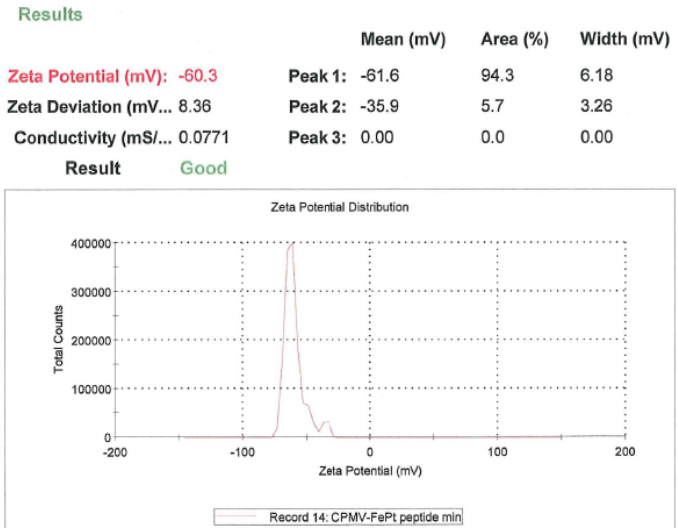
ESI Figure 13. Zeta potential measurement of wild-type CPMV suspended in buffer at pH 7.2.



ESI Figure 14. Zeta potential measurement of CoPt-CPMV mineralized particles suspended in buffer at pH 7.2.



ESI Figure 15. Zeta potential measurement of FePt-CPMV mineralized particles suspended in buffer at pH 7.2.



ESI Figure 16. Zeta potential measurement of ZnS-CPMV mineralized particles suspended in buffer at pH 7.2.