Supporting Information for:

Viral nanoparticles can elude protein barriers: exploiting rather than imitating nature

Alberto Berardi\textsuperscript{a,b,\*}, Francesca Baldelli Bombelli\textsuperscript{c}, Eva C. Thuenemann\textsuperscript{b} and George P. Lomonossoff\textsuperscript{b}.

a Department of Pharmaceutical Sciences and Pharmaceutics, Faculty of Pharmacy, Applied Science Private University, Amman 11931, Jordan.

b Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK.

c Laboratory of Supramolecular and BioNano Materials (SupraBioNanoLab), Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Milano, Italy

* Corresponding author: Department of Pharmaceutical Sciences and Pharmaceutics, Faculty of Pharmacy, Applied Science Private University, Amman 11931, Jordan Mail: a_berardi@asu.edu.jo, Tel. +962 6 5609999; Fax: (+962) 65515017.
Supplementary Figures

Figure S1. Characterisation of purified CPMV. (a) SDS-PAGE. L and S protein are visible as 39 kDa and 22 kDa bands. (b) Coomassie-stained (left – for protein) and ethidium bromide stained (right – for nucleic acid) native agarose gel. The red arrow indicates the main CPMV band. Stained protein capsid and its encapsidated nucleic acid have the same electrophoretic mobility, consistent with previous reports 1–3. (c) DLS showing the size distribution and the diameter of the particles (n = 3; mean, ±S.D.). (d) TEM.
Figure S2. Characterisation of purified BTV CLPs. (a) SDS-PAGE. GFP-VP3 and VP7 proteins are visible as 131 kDa and 39 kDa bands, respectively. (b) Coomassie-stained (left – for protein) and agarose gel and fluorescence detection following UV excitation (right). Stained protein capsid and the encapsidated GFP have the same electrophoretic mobility. (c) DLS showing the size distribution and the diameter of the particles (n = 3; mean, ±S.D.). (d) TEM.
Figure S3. Particle size distribution of CPMV (a) and PS-COOH NP (b) after 1 hour exposure to different concentrations of BSA in PBS. The negative control represents the size distribution of 20 mg/ml BSA (without NPs).
Figure S4. Density gradient ultracentrifugation for the separation on NPs from the excess serum protein. CPMV was pre-incubated in FBS (or PBS control), separated by density gradient ultracentrifugation and then fractions corresponding to the 30-60% sucrose were collected and analysed by Coomassie-stained SDS-PAGE. (a) positive control - pristine CPMV in PBS banded mainly in the 40 and 50% fractions. (b) negative control - FBS (without NPs) remained mainly confined in the 30% fraction of the gradient. (c) CPMV banded mainly in the 40 and 50% fractions (as the positive control) and FBS remained in the 30% fraction (as the negative control). (d) Synthetic NPs control - PS-COOH NP in FBS banded mainly in the 40% fraction (this could be easily detected by shining UV light on the test tube to visualise the fluorescent NPs). The 40% fractions here contains more protein than the corresponding fraction in the negative control [in (b)], suggesting PC formation on PS-COOH NP. To better highlight these findings all samples from the 40 and 50% fraction from (a), (b), (c) and (d) were dialysed and concentrated and analysed again by SDS-PAGE (see Figure 4a).
Figure S5. Pepsin binding to PS-COOH NPs and CPMV. NPs are exposed to increasing concentrations of pepsin at pH 3 to investigate the extent of protein adsorption to the NP surface. 1x indicates the standard pepsin dilution of simulated gastric fluids from Pharmacopeia⁴. In (a) PS-COOH NP shows reduced electrophoretic mobility only at the highest pepsin concentration. In (b) CPMV is free for pepsin binding at all pepsin concentrations. N = negative control.
Figure S6. Nucleic acid binding to PS-COOH NPs and CPMV. NPs are exposed to increasing concentrations of DNA to investigate the extent of adsorption to the NP surface. In (a) PS-COOH NPs shows reduced electrophoretic mobility at all DNA concentration and even aggregation at concentration of DNA $\geq 5$ mg/ml. In (b) CPMV is stable and free from PC in DNA at concentrations up to 5 mg/ml. N = negative control.
Figure S7. Mucin binding to NPs at pH 4.0. NPs are exposed to increasing concentrations of mucin to investigate the extent of protein adsorption to the NP surface. (a): agarose gel of PS-COOH NPs. PS-COOH NPs shows reduced electrophoretic migration as a function of mucin concentration. (b): agarose gel of CPMV. The binding of mucin to CPMV is minimal.
Figure S8. Particle size distribution of CPMV (a) and PS-COOH NP (b) after 1 hour exposure to different concentrations of mucin in 0.2 M sodium phosphate buffer (PB) at pH 7.4. The negative control represents the size distribution of 20 mg/ml mucin (without NPs).
Figure S9. One of three Coomassie-stained SDS-PAGE gel relative to the CPMV transport through mucin gels. Starting from the left: the first four lanes (“Transport through mucus”) are the samples collected from the acceptor compartment of the Transwell at different time points, after overlaying CPMV on the mucin layer in the donor compartment. The next four lanes (“Positive”) are the samples collected from the acceptor compartment of Transwell at different time points, after overlaying CPMV on plain phosphate buffer solution in the donor compartment and corresponding to unhindered transport. The last four lanes contain a serial dilution of known concentrations of CPMV. The amount of CPMV in all lanes was determined from the gel by measuring the intensity of the S protein.
Supplementary references


