# SUPPORTING INFORMATION

# Development of viral nanoparticles for efficient intracellular delivery

**Zhuojun Wu,[d,e ‡] Kevin Chen,[a] Ibrahim Yildiz,[a] Anouk Dirksen,[d] Rainer Fischer,[e] Philip E. Dawson,[d] Nicole F. Steinmetz[a,b,c]***

5 ***Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX***
**DOI: 10.1039/b000000x**

**Conjugation of CPMV with FLAG-tag peptide:**

In order to investigate the reactivity of 4FB groups and the optimal hydrazone reaction conditions applicable to CPMV particles, a 10 negatively-charged FLAG-tag peptide containing a *C*-terminal hydrazinopyridine group was conjugated to the VNP surface in the presence of different concentrations of the nucleophilic catalyst aniline. At neutral pH, hydrazinopyridines react readily with benzaldehyde groups. With no aniline present, up to 80 hydrazone bonds were formed after 2 h and up to 240 bonds were formed after 24 h (Figure S1). The number of hydrazone bonds per particle was determined *in situ* and in real time by UV/vis spectrometry using the absorption coefficient of the hydrazone bond (ε 354 nm = 29,000 $M^{-1}$ $cm^{-1}$) and the molar extinction coefficient of CPMV (ε 260 nm = 15 8.1 $g^{-1}$ L $cm^{-1}$) (Figure S1). When aniline was added to a final concentration of either 10 mM or 100 mM, the maximum coverage of all 300 addressable benzaldehyde groups was achieved after just 1 h (Figure S1). Maximum labeling efficiency, i.e. 300 peptide per particle, was achieved using 10 mM aniline. with a 2:1 molar excess of hydrazinopyridine groups over 4FB. Increased aniline concentrations resulted in comparable labeling efficiency, however the data were less reproducible.

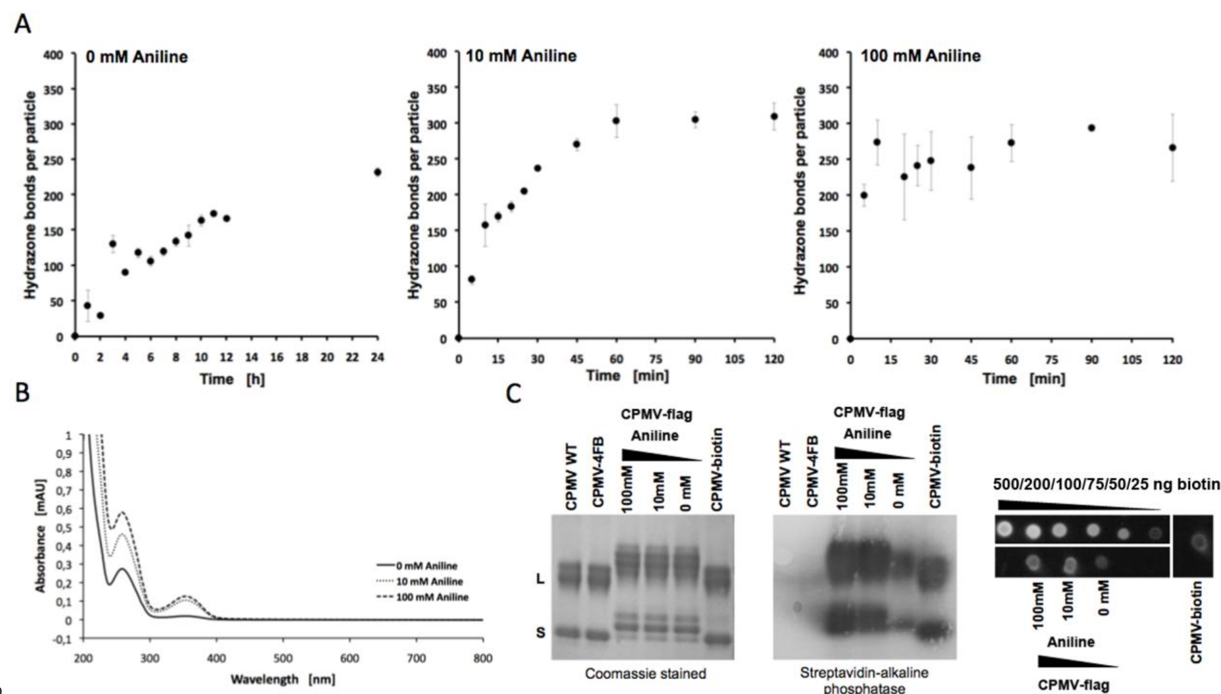


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**Figure S1.** Characterization of CPMV labeling with the biotinylated FLAG-peptide. (A) Real-time reaction kinetics of hydrazone bond formation with no aniline, 10 mM aniline and 100 mM aniline. Error bars represent the average standard deviations. (B) UV/vis spectrometry data of purified CPMV-4FB-FLAG particles after 2 h incubation with 0, 10 and 100 mM aniline as the catalyst. (C) 25 Denaturing gel electrophoresis of intact CPMV particles (10 µg) using a 4–12% Bis-Tris gel. Western blotting using streptavidin-alkaline phosphatase was used to detect the *N*-terminal biotin tag. (D) ECL dot blot of purified FLAG-tagged CPMV particles. The number of biotin labels (FLAG peptide displays biotin) per particle was determined using standardized biotin concentrations and Chemidoc XRS software.

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**Table S1:** Optimization of the CPP attachment reaction by hydrazone chemistry

| Buffer | pH | mM NaCl | Excess R5:Lys | CPMV conc. (mg/ml) | CPMV conc (mol/L) | Peptide (mol/L) | Aniline (mM) | Linker | Aggregation Precipitate observed by eye | Aggregation Elevated baseline in UV | Yield after purification [%] | FPLC Result | Quantification of labels attached |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.1M Phophate Buffer | 7.4 | - | 600 | 1 | 1.786E-11 | 1.07E-08 | 10 | 4FB | yes | yes | <10 | No Peak at 354, FPLC no elution fraction | UV (elevated base level) |
| 0.1M Phophate Buffer | 7.4 | - | 300 | 1 | 1.786E-11 | 5.35E-09 | 10 | 4FB | yes | yes | NA* | No Peak at 354, FPLC no elution fraction | UV (elevated base level) |
| 0.1M Phophate Buffer | 7.4 | - | 300 | 0.5 | 8.929E-12 | 5.35E-09 | 10 | 4FB | yes | yes | NA* | No Peak at 354, FPLC no elution fraction | UV (elevated base level) |
| 0.1M Phophate Buffer | 7.4 | - | 120 | 0.5 | 8.929E-12 | 2.568E-09 | 10 | 4FB | no | yes | NA* | NA | UV (elevated base level) |
| 0.1M Phophate Buffer | 7.4 | - | 120 | 0.5 | 8.929E-12 | 2.568E-09 | 5 | 4FB | no | yes | NA* | NA | UV (elevated base level) |
| 0.1M Phophate Buffer | 7.4 | - | 120 | 0.5 | 8.929E-12 | 2.568E-09 | 0 | 4FB | no | no | NA* | NA | No detecable peak via UV |
| 0.1M Phophate Buffer | 7.4 | - | 60 | 0.5 | 8.929E-12 | 1.28E-09 | 10 | 4FB | no | no | NA* | NA | No detecable peak via UV |
| 0.1M Phophate Buffer | 7.4 | 1000 | 120 | 0.5 | 8.929E-12 | 2.568E-09 | 10 | 4FB | no | NA | NA* | No Peak at 354, FPLC no elution fraction | NA |
| 0.1M Phophate Buffer | 7.4 | 100 | 120 | 0.5 | 8.929E-12 | 2.568E-09 | 10 | 4FB | no | NA | NA* | No Peak at 354, FPLC no elution fraction | NA |
| 0.1M Phophate Buffer | 7.4 | 1000 | 120 | 0.5 | 8.929E-12 | 2.568E-09 | 10 | PFB | no | NA | NA* | No Peak at 354, FPLC no elution fraction | NA |
| 0.1M Phophate Buffer | 7.4 | 100 | 120 | 0.5 | 8.929E-12 | 2.568E-09 | 10 | PFB | no | NA | NA* | No Peak at 354, FPLC no elution fraction | NA |
| 0.1M Tris-HCl Buffer | 8 | - | 300 | 0.5 | 8.929E-12 | 5.35E-09 | 10 | 4FB | yes | yes | NA* | NA | UV (elevated base level) |
| 0.1M Tris-HCl Buffer | 8 | - | 300 | 0.5 | 8.929E-12 | 5.35E-09 | 10 | PFB | yes | yes | NA* | NA | UV (elevated base level) |
| 0.1M Tris-HCl Buffer | 9 | - | 300 | 0.5 | 8.929E-12 | 5.35E-09 | 10 | 4FB | yes | yes | NA* | NA | UV (elevated base level) |
| 0.1M Tris-HCL Buffer | 9 | - | 300 | 0.5 | 8.929E-12 | 5.35E-09 | 10 | PFB | no | no | >70% | Peak at 354, 260:280 = 1,9 | No detecable peak via UV |
| **0.1M Tris-HCl Buffer** | **9** | **10** | **360** | **0.5** | **8.929E-12** | **6.419E-09** | **10** | **4FB** | **no** | **no** | **>70%** | **NA** | **ECL: 40** |
| **0.1M Tris-HCl Buffer** | **9** | **10** | **360** | **0.5** | **8.929E-12** | **6.419E-09** | **10** | **PFB** | **no** | **no** | **>70%** | **NA** | **ECL: 10** |
| 0.1M Tris-HCL Buffer | 9 | - | 450 | 0.5 | 8.929E-12 | 8.024E-09 | 10 | 4FB | yes | yes | NA* | NA | UV (elevated base level) |

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**Colocalization with transferrin and EEA1:**

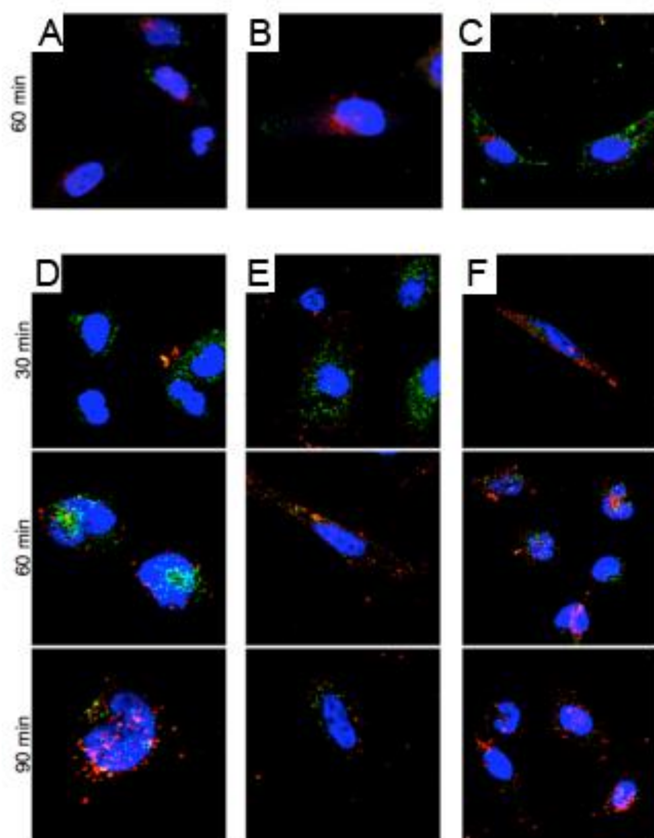


**Figure S2:** (A-C) Evaluating the co-internalization of transferrin-Texas Red and immunofluorescence-labeled CPMV/CPMV-R5 using HeLa cells and confocal microscopy. Transferrin-Texas Red (Invitrogen, in red) and CPMV particles were incubated with HeLa cells for 1 h and detected using a primary rabbit anti-CPMV-antiserum and a secondary goat anti-rabbit-AF488 antibody (in green). The nucleus was stained with DAPI (in blue). (A) Wild type CPMV; (B) CPMV-R5L; (C) CPMV-R5H. Imaging was performed using a Biorad 2100 confocal microscope with a 60x oil objective. (D-F) Evaluating the co-localization of immunofluorescence-labeled CPMV/CPMV-R5 with early endosome antigen 1 (EEA1) in HeLa cells by confocal microscopy. Time course of CPMV uptake. CPMV particles detected using a primary rabbit anti-CPMV-antiserum and a secondary goat anti-rabbit-AF555 antibody (red). EEA1 detected using a primary mouse anti-EEA1 antibody and a secondary goat anti-mouse-AF488 antibody (green). Cell nuclei are stained with DAPI (blue). (D) Wild type CPMV; (E) CPMV-R5L; (F) CPMV-R5H. Imaging was performed using a Biorad 2100 confocal microscope with a 60x oil objective.

**Acknowledgements:** This work was supported by NIH/NIBIB grants R00 EB009105 (to Steinmetz) and P30 EB011317 (to Steinmetz), a student fellowship from the Undergraduate Research Opportunities Program (UROP, to Wu) and the Adam-Schall-Gesellschaft (to Wu). We would like to thank Prof. Manchester (UCSD) for helpful discussions.

[a] Department of Biomedical Engineering, [b] Radiology, [c] Materials Science and Engineering, Case Western Reserve University School of Medicine, 10900 Euclid Ave., Cleveland, OH 44106-5056, USA, Tel: +1 216 368 5590, E-mail: nicole.steinmetz@case.edu, * corresponding author
[d] Department of Cell Biology and Chemistry, Center for Integrative Molecular Biosciences, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
[e] Institute of Biology VII, Molecular Biotechnology, RWTH Aachen University, Worringer Weg 1, 52074 Aachen, Germany
‡ *Present address: Institute for Molecular Cardiovascular Research, RWTH Aachen University, Pauwelstrasse 30, 52074 Aachen, Germany*