Supporting Information for

Interface of physics and biology: engineering virus-based nanoparticles for biophotonics

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Supplementary Figures

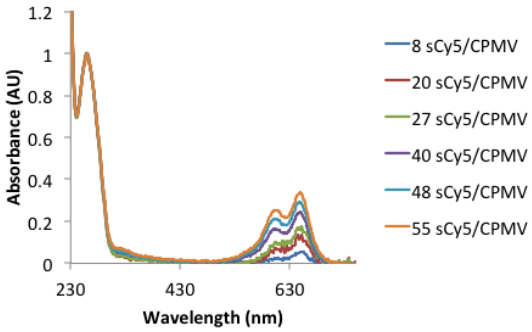

Figure S1. UV/visible spectra of CPMV-sCy5 used for quantification of dye labels, with absorbance at 260 nm normalized to 1.

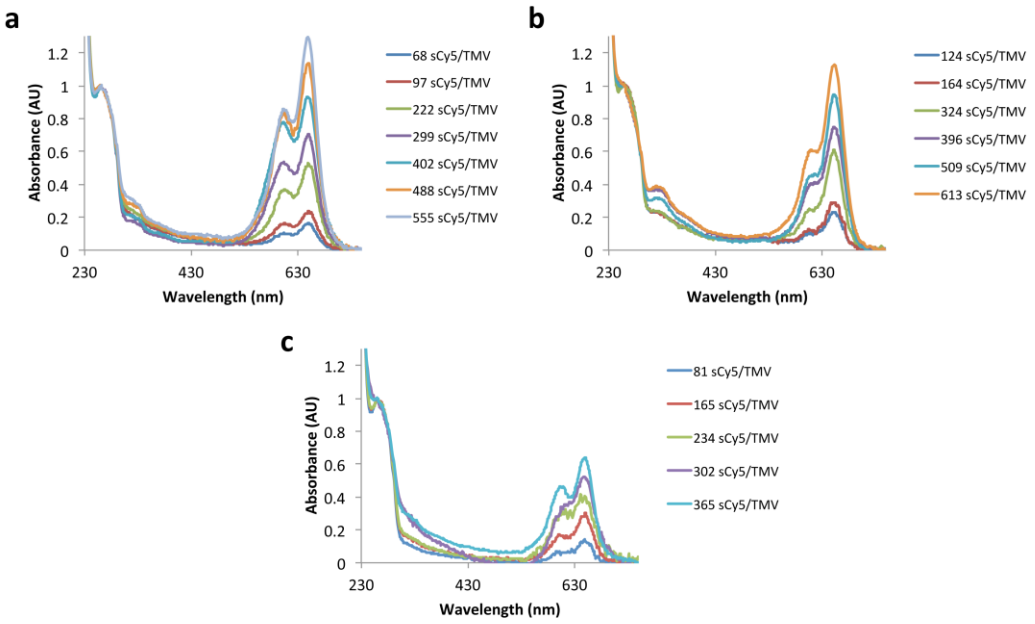

Figure S2. UV/visible spectra of TMV-sCy5 formulations normalized at 260 nm. a) Interiorly labeled TMV. b) Exteriorly labeled TMV. c) TMV-lys mutant.

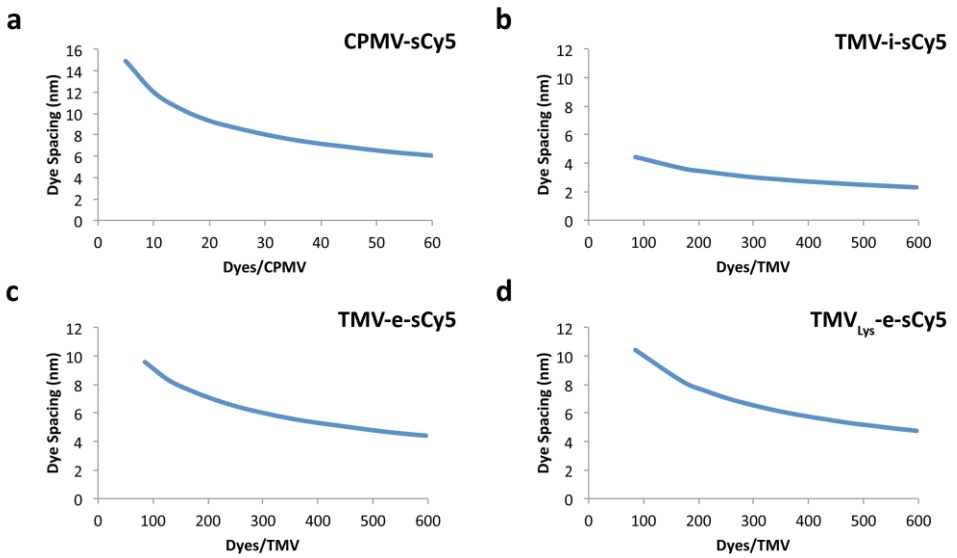

Figure S3. Dye interdistances for CPMV-sCy5 (a), TMV-i-sCy5 (b), TMV-e-sCy5 (c), and TMV$_{Lys}$-e-sCy5 (d). The distances were calculated from simulations where the dyes were randomly positioned on the particles at their reactive groups. The coordinates of the side chains were determined from the crystal structures of viruses (viperdb.scripps.edu and www.rcsb.org, files 1NY7 and 3J06 – a cryo EM reconstruction).

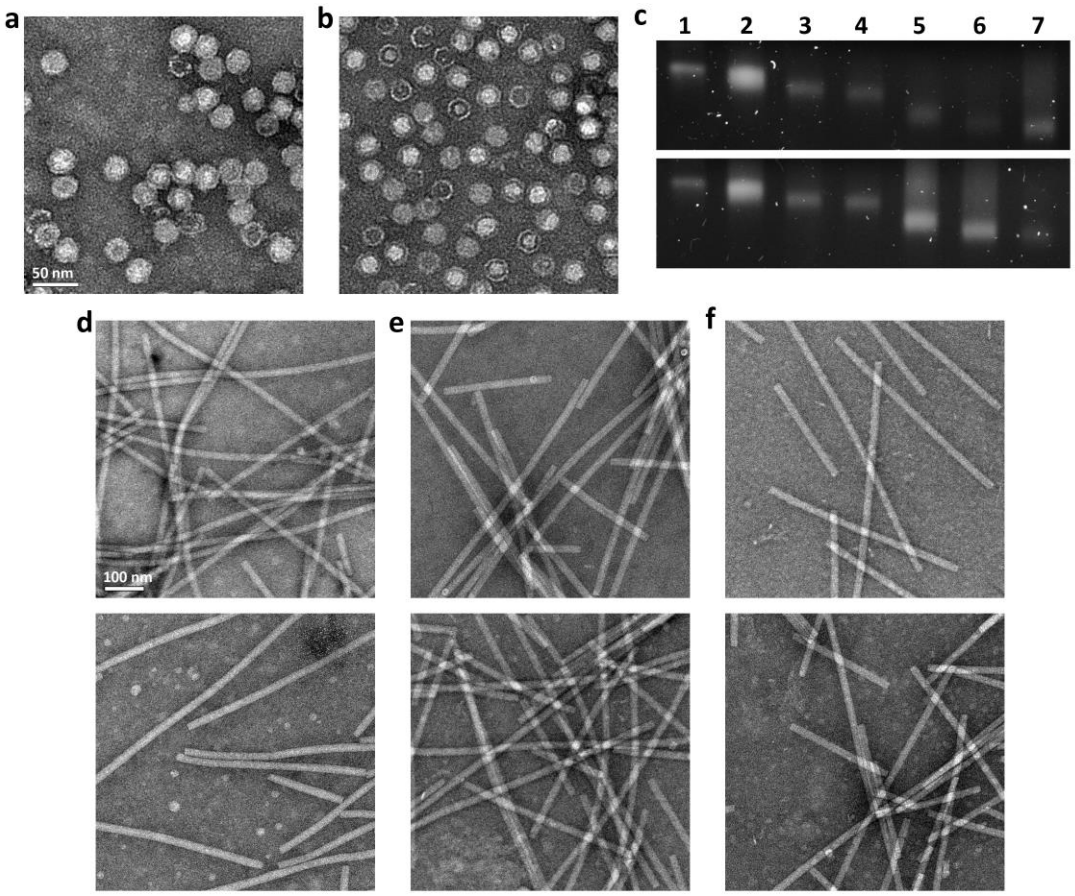

Figure S4. Particle integrity after fluorescence and lifetime measurements. a,b) TEM images of CPMV-sCy5 with 27 and 55 dyes, respectively. c) CPMV particles run on a 1.2% agarose gel visualized with UV light before (top) and after (bottom) measurements. 1 = CPMV, 2 = CPMV-sCy5 with 8 dyes, 3 = CPMV-sCy5 with 20 dyes, 4 = CPMV-sCy5 with 27 dyes, 5 = CPMV-sCy5 with 40 dyes, 6 = CPMV-sCy5 with 48 dyes, and 7 = CPMV-sCy5 with 55 dyes. d) TMV-i-sCy5 with 222 dyes per particle (top) and 488 dyes per particle (bottom). e) TMV-e-sCy5 with 164 dyes per particle (top) and 613 dyes per particle (bottom). f) TMV_{Lys}-e-sCy5 with 165 dyes per particle (top) and 365 dyes per particle (bottom).

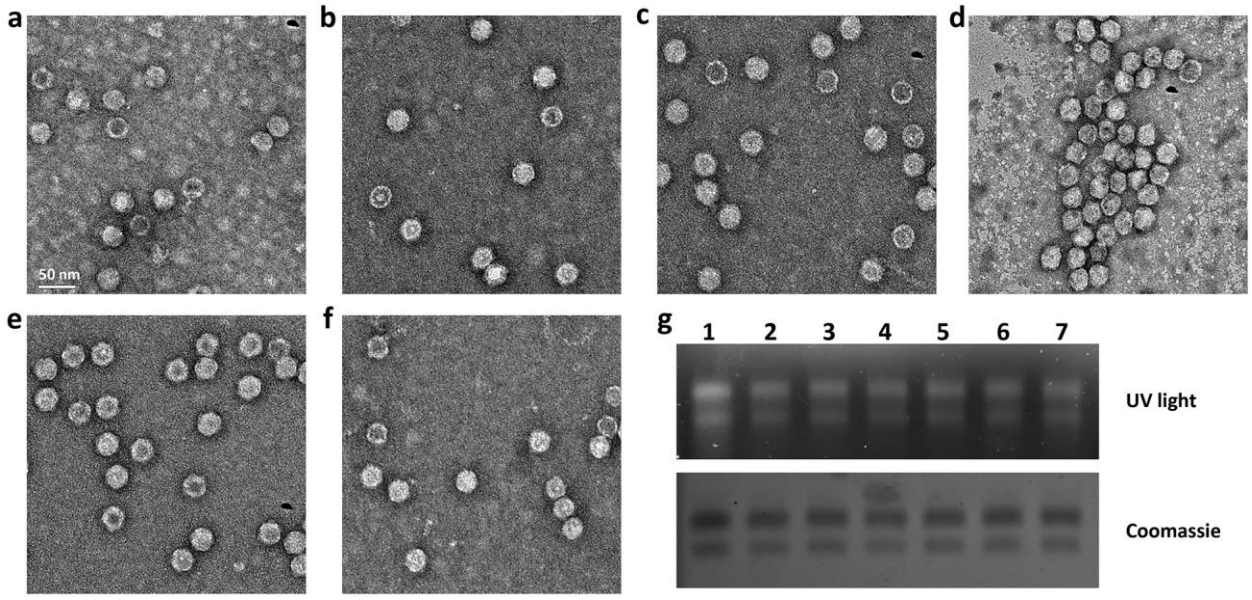

Figure S5. Stability of CPMV tested for various pulse and power settings. a-f) TEM images of CPMV after optical excitation with laser pulses show they remain stable. The particles were treated with a Nd:YAG pulsed laser (pulse duration 5 ns, rep. rate 20 Hz, 10 s of exposure at 532 nm) at 320 μJ/pulse (a) as well as 450 μJ/pulse (b). They were also exposed to a Ti:Sa pulsed laser (pulse duration 120 fs, rep. rate 80 MHz, 30 s of exposure) at powers of 15 mW (c) and 50 mW (d) at 400 nm and at powers of 50 mW (e) and 100 mW (f) at 750 nm. g) Particles run on a 1.2% agarose gel visualized under both UV light (top) and after Coomassie staining (bottom) also demonstrate intactness. 1 = CPMV; 2-7 = CPMV particles from (a)-(f).

Explanation of results:

TEM imaging (**Figure S5A-F**) indicated that particles remained intact after each exposure condition tested. Native gel electrophoresis further confirmed stability of the particles (**Figure 5G).** Intact CPMV particles exist in two electrophoretic forms, slow and fast, both of which are detectable on the native gels. The change in the electrophoretic mobility of intact CPMV particles from the slow to the fast form is a result of loss of the C-terminal 24 amino acids of the small coat protein by proteolysis in plants.[1] The slow form has the full-length small coat protein and the fast form has the truncated version.[2] Whether the slow and fast form are detected and resolved on the gels depends on the time of harvest and purification; the cleavage occurs in the plant, and we found that whether the fast form is present varies from batch to batch. This batch showed the slow and fast form at a 1:1 ratio; while the CPMV batch analyzed in **Figures 4 and S4** showed mostly the cleaved, fast electrophoretic form. It should be noted that on a macroscopic scale, the fast and slow electrophoretic forms exhibit similar material's properties; their diameters and number of reactive surface lysines remains consistent.

The stability results are very interesting since this study clearly proves that viral nanoparticles can be exposed to pulsed and CW optical excitation in a wide range of energy and average powers, allowing the study of their optical properties before and after labeling processes.

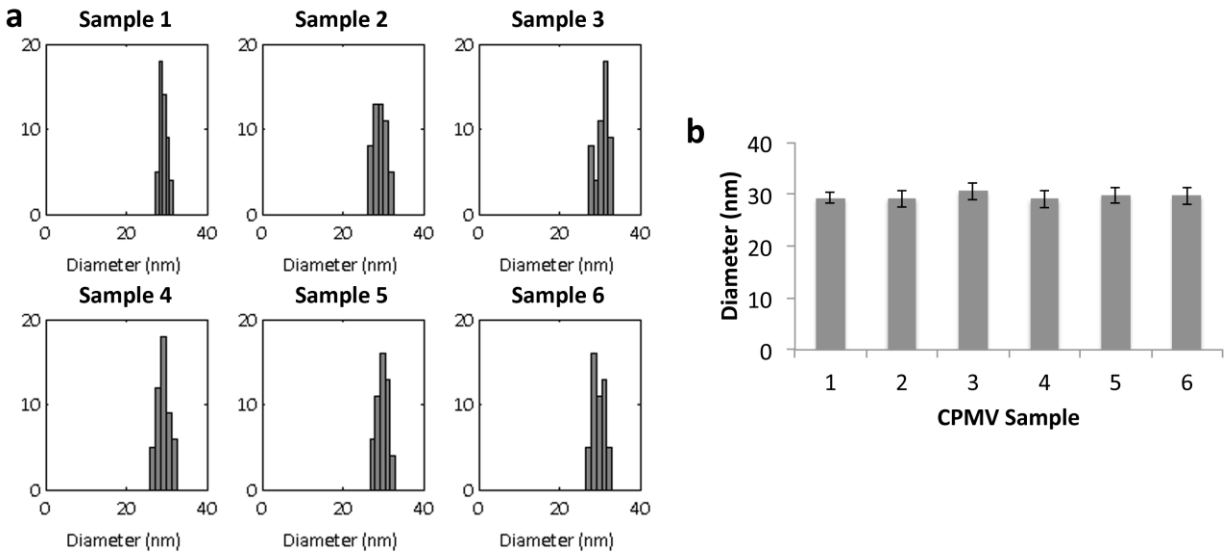

Figure S6. Size distribution of CPMV after treatment with laser (*n*=50) as measured from TEM images (see **Figure 5**). Samples 1-6 correspond to the particles from **Figure 5a-f**, respectively. a) Histograms of particle diameters measured for each sample. b) Average and standard deviation of particle diameters.

References

(1) Lomonossoff, G. P.; Johnson, J. E. *Prog. Biophys. Mol. Biol.* **1991,** *55*, 107-137.
(2) Steinmetz, N. F.; Evans, D. J.; Lomonossoff, G. P. *Chembiochem* **2007,** *8*, 1131-1136.