Supporting information

Versatile post-functionalization of the external shell of Cowpea Chlorotic Mottle Virus by using click chemistry

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Instrumentation

FPLC analyses were performed on a GE Healthcare ÄKTApurifier™ system equipped with a Superose 6 10/300 GL column from GE Healthcare (flow rate 0.5 mL/min) and a fractionating device. Injections of 100-500 µL aliquots of the samples were monitored using UV detection at λ = 260 and 280 nm for the virus and λ = 340 or 404 nm for the coumarin absorption. Buffers for FPLC were filtered over a Corning 0.2 µm vacuum filter before use.

Samples for TEM analysis were obtained by deposition of 5 µL aliquots onto 100-mesh carbon-coated copper grids. After 1 min, the excess liquid was blotted away with filter paper, followed by immediate staining for 15 seconds with 5 µL of a 1% uranyl acetate solution. Images were obtained using a Philips CM300ST-FEG electron microscope operated at 300 kV.

UV-Vis spectra were recorded using a Perkin Elmer Lambda 850 UV spectrophotometer. Concentrations and RNA/protein ratios of native CCMV were determined using a Thermo Scientific NanoDrop 1000 Spectrophotometer.

NMR spectra were recorded with a Bruker Ascend 400 at room temperature, using the specified solvent. Chemical shifts are given in ppm (δ) relative to tetramethylsilane.

SDS-PAGE was carried out according to standard procedure using a 12% acrylamide gel. Protein bands were visualized by staining with Bio-Safe Coomassie brilliant blue G 250.

CCMV isolation and purification

CCMV was isolated and purified from cowpea plants with a 10-day old infection, as described previously.[1] The obtained virus was analyzed by UV/Vis spectroscopy, SDS-PAGE and FPLC.

General procedure for CCMV VLP formation

The removal of the viral RNA from the modified CCMV capsids was performed by dialyzing a virus sample in VB overnight against protein isolation buffer (PIB; 500 mM $CaCl_2$, 50 mM Trizma Base, 1 mM DTT, pH 7.5; 1000 times excess in volume). The precipitated RNA was pelleted by ultra-centrifugation (2 h, 47000 rpm, 10 °C) using a Thermo Scientific Sorvall WX80 Ultra Centrifuge. The top three-quarters of the supernatant were dialyzed against three changes of clean buffer (CB; 500 mM NaCl, 50 mM Trizma Base, 1 mM DTT, pH 7.5; 100-1000 times excess in volume, 3 h per change). The obtained CP, free of RNA, was associated into empty virus-like-particles by dialysis to capsid storage buffer (CSB; 1.0 M NaCl, 50 mM NaOAc, 1 mM NaN_3, pH 5.0; 100–1000 times excess in volume, 3 h per change), and the VLP was stored at 4°C.

Experimental

Synthesis of 4-azido-4'-cyanobiphenyl (**4**) (two steps)

This compound was synthesized according to literature.[2] Potassium 4-iodophenyltrifluoroborate (930 mg, 2.9 mmol), NaN_3 (195 mg, 3.0 mmol), CuBr (10 mol%), Cs_2CO_3 (954 mg, 2.9 mmol) and *N,N'*-dimethylethylenediamine (20 mol%) were dissolved in DMF (12 mL) under atmospheric conditions.

[1] M. Comellas-Aragonès, H. Engelkamp, V. I. Claessen, N. A. J. M. Sommerdijk, A. E. Rowan, P. C. M. Christianen, J. C. Maan, B. J. M. Verduin, J. J. L. M. Cornelissen and R. J. M. Nolte, *Nat. Nanotechnol.*, 2007, **2**, 635

[2] Y. A. Cho, D.-S. Kim, H. R. Ahn, B. Canturk, G. A. Molander and J. Ham, *Org. Lett.* **2009**, *11*, 4330-4333

The reaction mixture was heated in an oil bath at 90 °C for 16 h (^{1}H NMR analysis indicated 90% conversion). The reaction was precipitated in 250 mL of stirred Et_2O. The residual product was filtered, concentrated and redissolved in acetone (18 mL), and the insoluble salts were removed by filtration over Hyflo®. The filtrate was concentrated to 20 mL on a rotary evaporator and the product was precipitated in 300 mL of cold, stirred Et_2O. The product was filtered and dried *in vacuo* to afford potassium 4-azidophenyltrifluoroborate in 32% yield (210 mg, a yellow solid). ^{1}H NMR (400 MHz, CD_3CN) δ 7.44 (d, 2H, *J* = 7.90 Hz), 6.88 (d, 2H, *J* = 7.78 Hz); ^{13}C NMR (100 MHz, CD_3CN) δ 137.6, 133.9.

Potassium 4-azidophenyltrifluoroborate (100 mg, 0.43 mmol), 4-bromobenzonitrile (78 mg, 0.43 mmol), Cs_2CO_3 (3 equiv.), and $PdCl_2(dppf)\cdot CH_2Cl_2$ (10 mol%) were added to a dry two-neck flask equipped with a stirring bar and a condenser. The vessel was sealed with a septum, and methanol (7 mL) was added *via* syringe. The reaction was heated in an oil bath at 80 °C. After bromobenzonitrile was completely consumed (the reaction was monitored by TLC), the reaction mixture was cooled to rt. The solvent was removed *in vacuo*. The residual compound was dissolved in ethyl acetate (<9.0 mL), and the insoluble salts were filtered through a thin pad of silica gel. The solution was concentrated on a rotary evaporator, and the crude product was purified by preparative TLC, using Merck 2 mm coated silica gel Kieselgel 60 F_{254} plates (33% ethyl acetate in heptane), to give the desired product in 39% yield (37 mg, an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, 2H, *J* = 8.55 Hz), 7.65 (d, 2H, *J* = 8.55 Hz), 7.58 (d, 2H, *J* = 8.64 Hz), 7.14 (d, 2H, *J* = 8.64 Hz). ^{13}C NMR (100 MHz, CDCl3) δ 144.6, 140.8, 135.9, 132.8, 128.7, 127.5, 119.8, 119.0, 111.1.

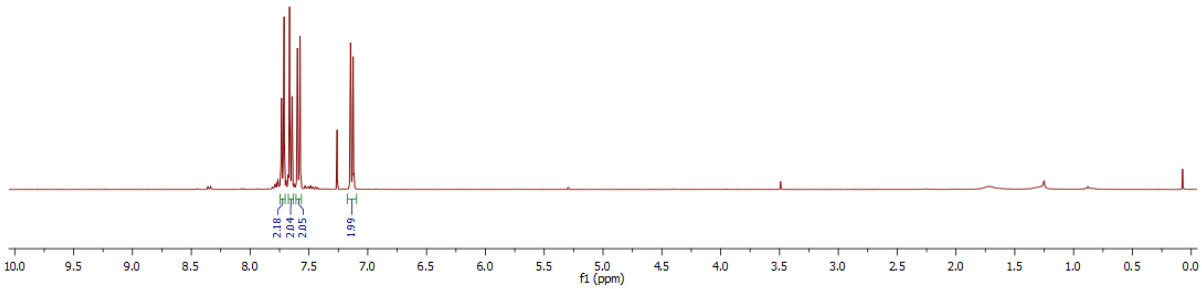

Figure S1. 400 MHz ^{1}H NMR spectrum of **4** in $CDCl_3$.

Synthesis of 3-(4-(aminomethyl)-1*H*-1,2,3-triazol-1-yl)-7-hydroxycoumarin (**5**)

This compound was synthesized according to literature.[3] 3-azido-7-hydroxycoumarin (**3**) (17 mg, 0.16 mmol) and propargylamine (**1**) (18 µL, 0.48 mmol) were dissolved in a 2:1 mixture (v/v) of THF and MeOH (3 mL in total). A solution of tris[(*N*-benzyltriazolyl)methyl]amine (TBTA) (8 mg, 16 µmol) and $Cu(CH_3CN)_4PF_6$ (6 mg, 16 µmol) in MeOH (1 mL) was added and the reaction was allowed to stir overnight at rt, while shielded from light. The filtrate was then removed and the remaining solid was dissolved in a minimum amount of MeOH. Addition of EtOH led to further precipitation of undesired side product, which were filtered off. After evaporation of the solvent and drying under vacuum, compound **5** could finally be obtained pure as a pale yellow solid. ^{1}H NMR (400 MHz, D_2O): δ 8.44 (s, 1H), 8.31 (s, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 6.71 (d, *J* = 8.1 Hz, 1H), 6.58 (s, 1H), 4.34 (s, 2H), 1.30 (m, 2H); ESI-TOF: $[M + H + (H_2O)_2]^+$ calcd 295.10. Found 294.84. UV λ_{max}(VB)/nm 342 (ε = 4600 $M^{-1}\cdot cm^{-1}$).

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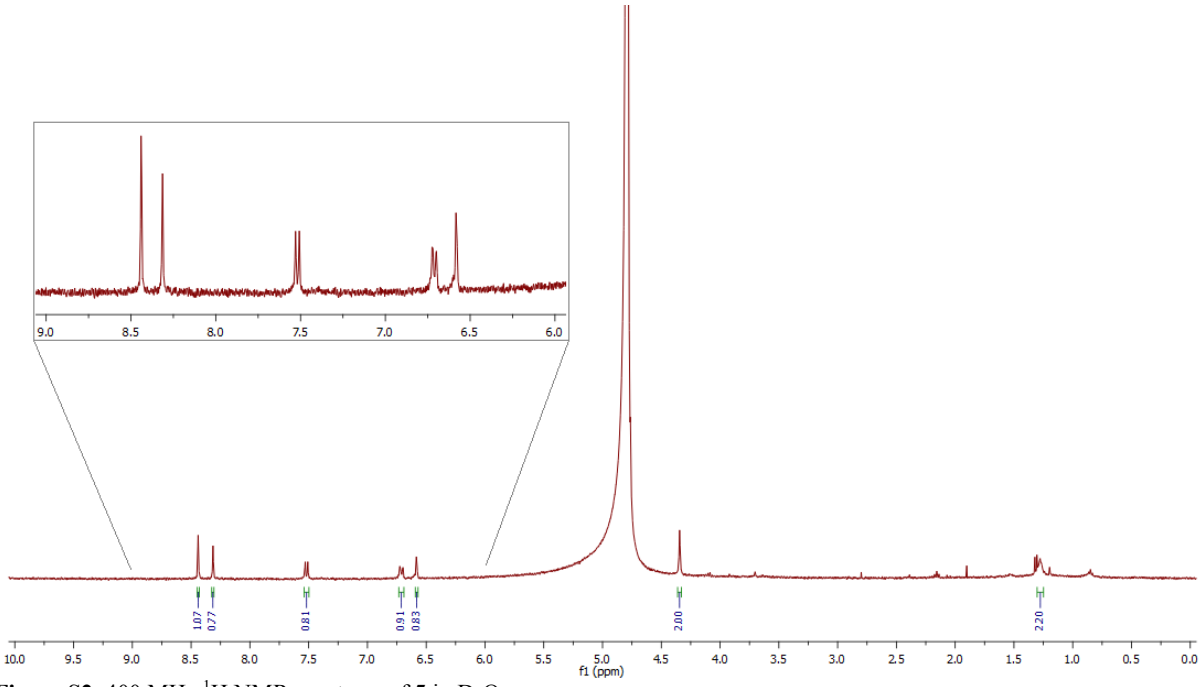

Figure S2. 400 MHz ^{1}H NMR spectrum of **5** in D_2O.

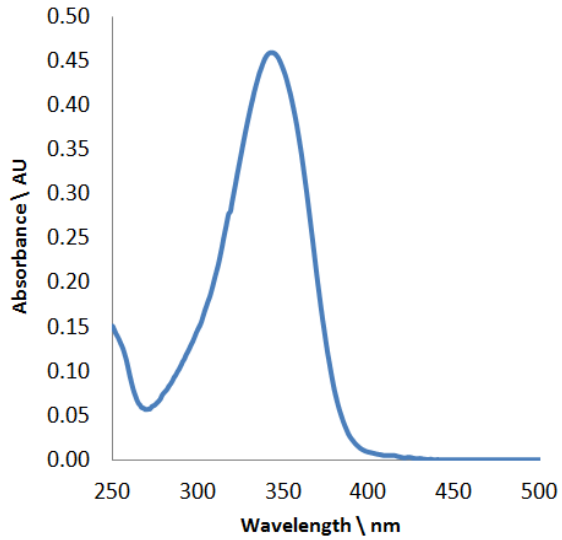

Figure S3. UV/Vis absorbance spectrum of **5**.

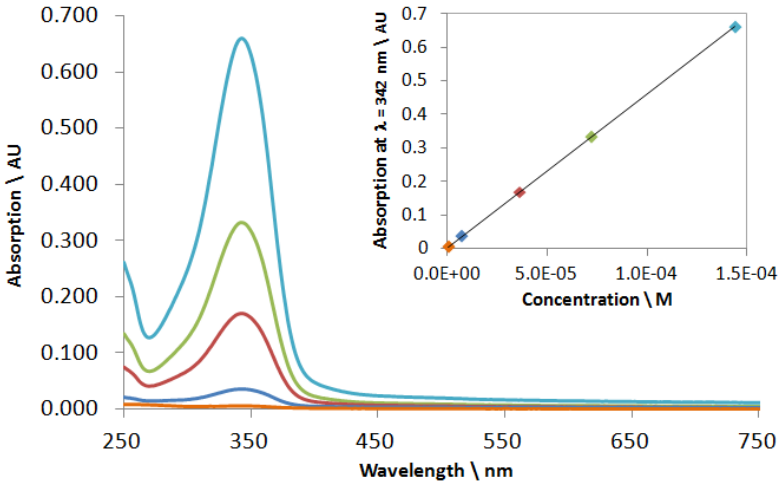

Figure S4. The UV/Vis absorbance shows a linear dependence to the concentration of **5**.

Analyses of functionalized capsids

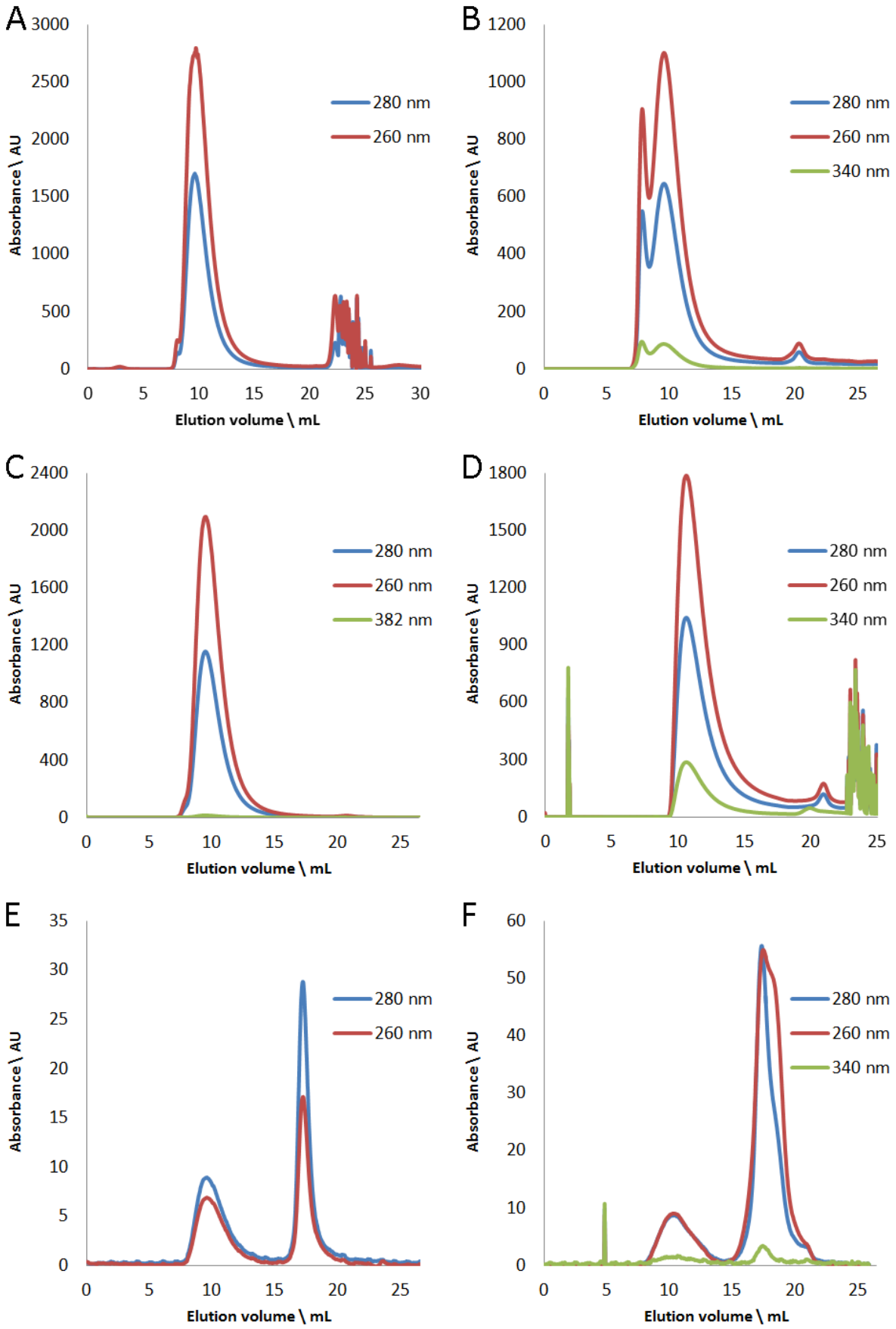

Figure S5. FPLC chromatograms of modified CCMV capsids. A) CCMV-A; B) CCMV-A-Coum; C) CCMV-A-CNBP; D) CCMV-BCN-Coum; E) VLP-A; F) VLP-Coum

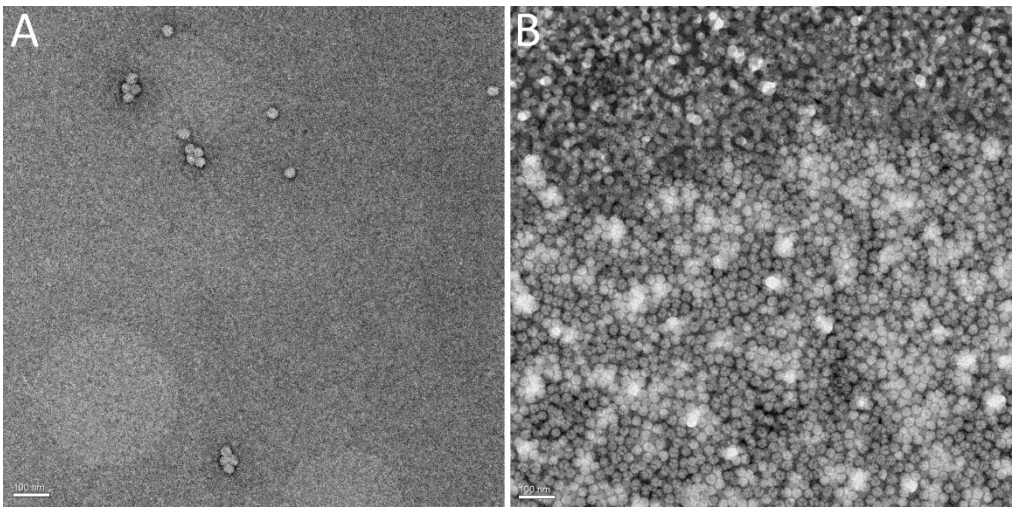

Figure S6. TEM micrographs of uranyl acetate stained capsids (scale bar = 100 nm).
A) CCMV-A; B) CCMV-BCN-Coum.

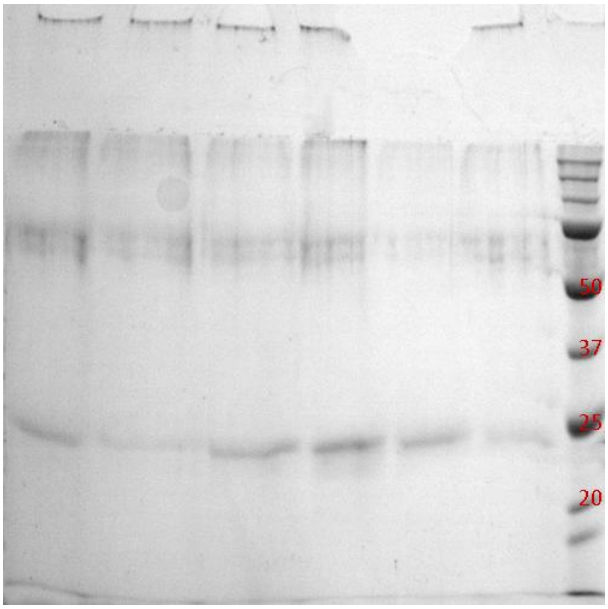

Figure S7. SDS-PAGE analysis. Lane 1, CCMV-A; lane 2 + 3, VLP-A; lane 4, CCMV-A-Coum; lane 5, CCMV-A-CNBP; lane 6, CCMV-BCN-Coum, lane 7, Bio-Rad Precision Plus Protein™ marker.