

## Supporting Information

### *Photoresponsive, reversible immobilization of virus particles on supramolecular platforms*

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## 1. Materials

All organic solvents were of analytical quality. Chemicals were used as received unless stated otherwise. 3-Azido-7-hydroxycoumarin (**2**) was obtained from Carbosynth (Berkshire, UK). SYBR Safe DNA Gel Stain 10,000X Concentrate in DMSO (Invitrogen™) was purchased from Thermo Fisher Scientific Inc. Deuterated solvents were obtained from Cambridge Isotope Laboratories Inc. All other chemicals were purchased from Sigma Aldrich.

Buffers were prepared with ultrapure water (Milli-Q® Advantage A10, Millipore, R = 18.2 MΩ·cm). For the EDC coupling, phosphate buffer (PB) containing 100 mM phosphate and 1 mM EDTA at pH 7.2 was used. For the click reaction and storage of native and modified CCMV, virus buffer (VB) containing 100 mM NaOAc, 1 mM EDTA and 1 mM NaN<sub>3</sub> at pH 5.0 was used. Buffers and virus samples were stored at 4 °C. Dialysis was performed using Spectra/Por® Dialysis Membranes with a 6-8 kDa MWCO.

Gold substrates for SPR and fluorescence microscopy were purchased from Ssens B.V. (Enschede, NL).

## 2. Instrumentation

Thin-layer chromatography (TLC) was carried out on Merck Silica Gel 60 F<sub>254</sub> aluminum sheets, with visualization by UV light. Column chromatography was carried out on silica gel (230–400 mesh). NMR spectra were recorded with a Bruker Ascend 400 at room temperature, using the specified solvent. Chemical shifts are given in ppm ( $\delta$ ) relative to tetramethylsilane. Mass analysis was performed using a Micromass LCT electrospray ionization time of flight (ESI-TOF) mass spectrometer equipped with a Harvard Apparatus syringe pump.

FPLC was 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 500  $\mu$ L aliquots of the samples were monitored by UV detection at  $\lambda$  = 260 and 280 nm for the virus and  $\lambda$  = 341 nm for the coumarin absorption. Buffers for FPLC were filtered over a Corning 0.2  $\mu$ m vacuum filter before use.

Samples for TEM analysis were obtained by deposition of 5  $\mu$ L aliquots onto 200-mesh carbon-coated copper grids. After 1 min 10 s, the excess liquid was blotted away with filter paper, followed by immediate staining for 16 seconds with 5  $\mu$ L of a 1% uranyl acetate solution. Images were obtained using a Philips CM300ST-FEG electron microscope operated at 300 kV.

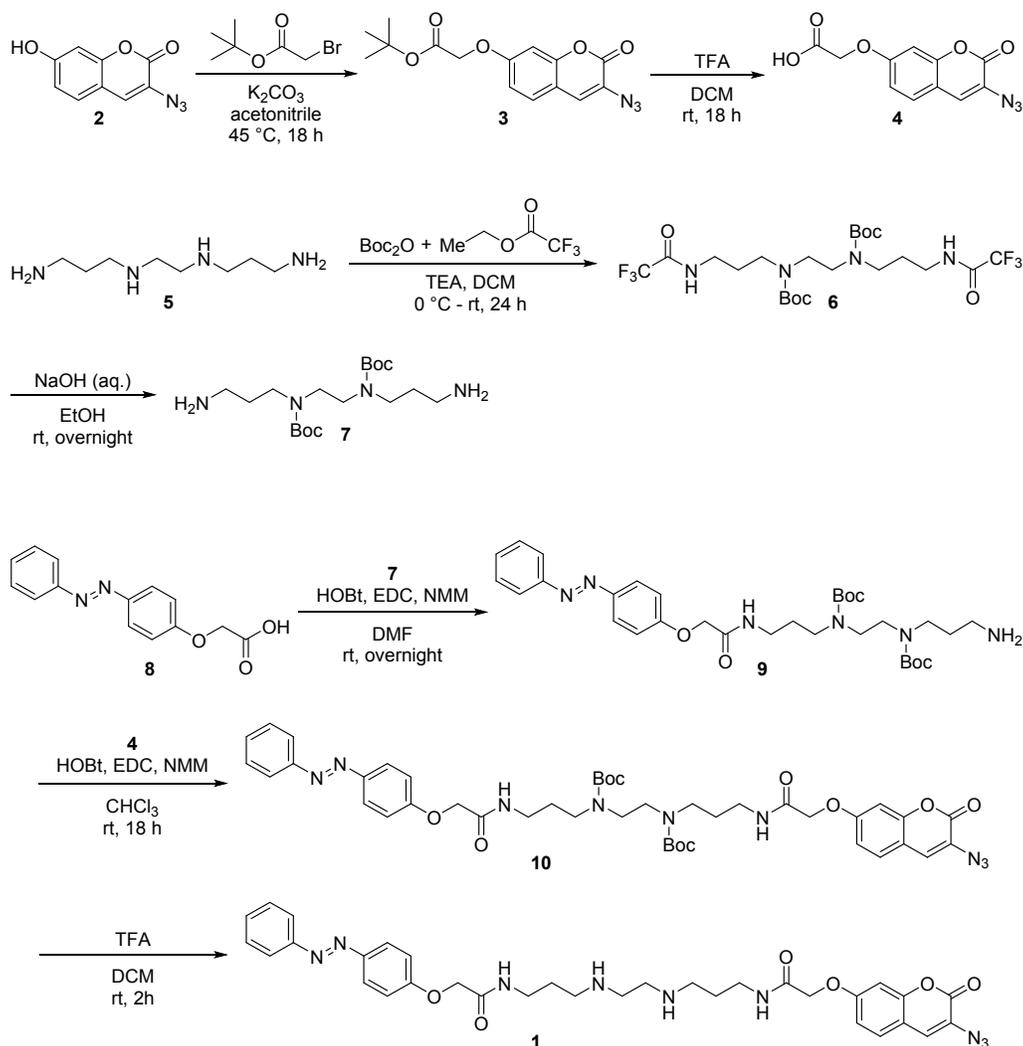
UV/Vis spectra were recorded using a Perkin Elmer Lambda 850 UV/Vis spectrophotometer at a slit width of 2 nm. Concentrations and RNA/protein ratios of native CCMV were determined using a Thermo Scientific NanoDrop 1000 Spectrophotometer. Fluorescence spectroscopy was performed on a Perkin Elmer LS 55 fluorescence spectrometer using an excitation slit width of 4.0 nm at a scan rate of 100 nm/min.

An IX71 Olympus microscope with filters was used for recording fluorescent images.

Surface plasmon resonance (SPR) experiments were conducted using 50 nm SPR gold substrates from Ssens B.V. on a Resonant-probes SPR. The reflectivity was measured at fixed angle at which point the linear region of the SPR curve stopped.

### 3. Experimental

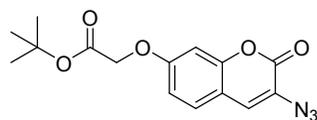
#### 3.1 Synthesis of compound 1



Scheme S1 Synthesis of 1

The syntheses of compounds 3 and 4 were carried out using a slightly modified literature procedure.<sup>S1</sup>

#### **tert-butyl 2-(3-azido-7-coumarinyl)oxyacetate (3)**



3-Azido-7-hydroxycoumarin (2) (300 mg, 1.48 mmol),  $K_2CO_3$  (815 mg, 5.92 mmol) and a minimum amount of KI were dissolved in 20 mL of acetonitrile. The mixture was stirred and heated to 45 °C and *tert*-butyl-2-bromoacetate (433 mg, 2.22 mmol) was added. The progress of the reaction was monitored by TLC in  $CHCl_3$ . After 18 h, the reaction was diluted with EtOAc (70 mL) and subsequently washed with water (2 × 60 mL). The organic phase was dried over anhydrous  $Na_2SO_4$ , filtered and the solvent was removed under vacuum. The residual brown oil

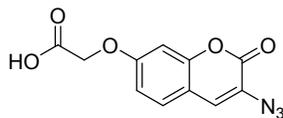
<sup>S1</sup> R. I. Jølick, H. Sun, R. H. Berg and T. L. Andresen, *Chem. Eur. J.*, 2011, 17, 3326.

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was dissolved in DCM (4 mL) and recrystallized from *n*-hexane (200 mL) to yield **3** as a brown solid (460 mg, 1.45 mmol, 98%).

<sup>1</sup>H-NMR (400 MHz, acetone-*d*<sub>6</sub>): δ 7.57 (d, <sup>3</sup>J = 8.7 Hz, 1H, CH<sub>arom</sub>), 7.47 (s, 1H, CH<sub>arom</sub>), 6.97 (dd, <sup>3</sup>J = 8.7 Hz, <sup>4</sup>J = 2.5 Hz, 1H, CH<sub>arom</sub>), 6.92 (d, <sup>4</sup>J = 2.4 Hz, 1H, CH<sub>arom</sub>), 4.76 (s, 2H, CH<sub>2</sub>), 1.47 (s, 9H, CH<sub>3</sub>).

**2-(3-azido-7-coumarinyl)oxyacetic acid (4)**

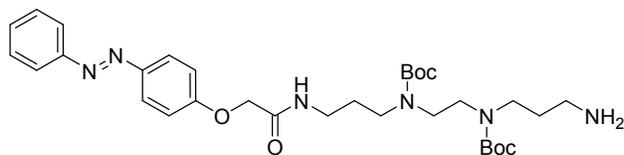


Trifluoroacetic acid (TFA) (1.17 mL, 15 mmol) was added to a stirring solution of **3** (460 mg, 1.45 mmol) in DCM (20 mL) and the mixture was left to react overnight at rt. Next, 100 mL of DCM were added and the mixture was washed with water (50 mL) and a 10 % aq. citric acid solution (50 mL). The organic phase was dried over anhydrous MgSO<sub>4</sub> and filtered. Removal of the solvent under vacuum yielded the pure product as an orange-brown solid (310 mg, 1.19 mmol, 82%).

<sup>1</sup>H NMR (400 MHz, acetone-*d*<sub>6</sub>): δ 7.58 (d, <sup>3</sup>J = 8.6 Hz, 1H, CH<sub>arom</sub>), 7.48 (s, 1H, CH<sub>arom</sub>), 7.00 (dd, <sup>3</sup>J = 8.6 Hz, <sup>4</sup>J = 2.5 Hz, 1H, CH<sub>arom</sub>), 6.96 (d, <sup>4</sup>J = 2.5 Hz, 1H, CH<sub>arom</sub>), 4.87 (s, 2H, CH<sub>2</sub>).

1,2-Bis(aminopropyl(tert-butoxycarbonyl)amino)ethane (**7**) and 2-(4-azobenzenoxy)acetic acid (**8**) were synthesized according to literature procedures.<sup>S2, S3</sup>

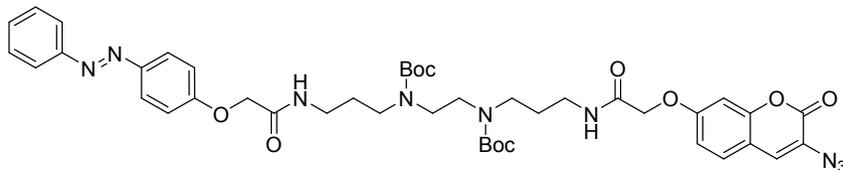
***N*-(3-aminopropyl)-*N'*-(3-(2-(4-azobenzenoxy)acetamido)propyl)-1,2-bis((tert-butoxycarbonyl)amino)ethane (9)**



To a stirring solution of 2-(4-azobenzenoxy)acetic acid (**8**) (200 mg, 0.78 mmol) in dry DMF (10 mL) at rt, 1-hydroxybenzotriazole hydrate (HOBt) (158.1 mg, 1.17 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbo-diimide (EDC) (224.3 mg, 1.17 mmol) were added. The solution was subsequently stirred for 45 min at rt before adding **7** (1.17 g, 3.12 mmol) in dry DMF (5 mL). Next, *N*-methylmorpholine (NMM) (130 μL, 1.17 mmol) was added and the reaction mixture was stirred overnight. Most of the solvent was then removed under vacuum. Water was added and the product was extracted with DCM. The organic phase was washed with brine four times and then the crude product was purified by silica gel column chromatography (CHCl<sub>3</sub>:MeOH:NH<sub>4</sub>OH, 90:9:1) to afford pure **9** as an orange oil (280 mg, 0.46 mmol, 59%).

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 7.94-7.87 (m, 4H), 7.53-7.43 (m, 3H), 7.12-7.06 (m, 2H), 4.58 (s, 2H), 3.34-3.20 (m, 10H), 2.72-2.66 (m, 2H), 1.72-1.61 (m, 4H), 1.46-1.45 (m, 18H). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ 167.66, 159.79, 156.23, 155.42, 152.61, 147.60, 130.57, 129.04, 124.77, 122.61, 115.07, 79.78, 67.44, 45.11, 43.79, 43.62, 39.39, 39.06, 34.95, 29.26, 28.43, 27.75, 27.61. ESI-MS (m/z): calculated for [C<sub>32</sub>H<sub>48</sub>N<sub>6</sub>O<sub>16</sub>H]<sup>+</sup>: 613.3722, found: 613.3708.

***N*-(3-((2-(3-azido-7-coumarinyl)oxy)acetoamido)propyl)-*N'*-(3-(2-(4-azobenzenoxy)acetamido)propyl)-1,2-bis-((tert-butoxycarbonyl)amino)ethane (10)**



2-(3-azido-7-coumarinyl)oxyacetic acid (**4**) (11.2 mg, 0.043 mmol) was dissolved in 1.5 mL CHCl<sub>3</sub> and 5 drops of THF. The solution was stirred and

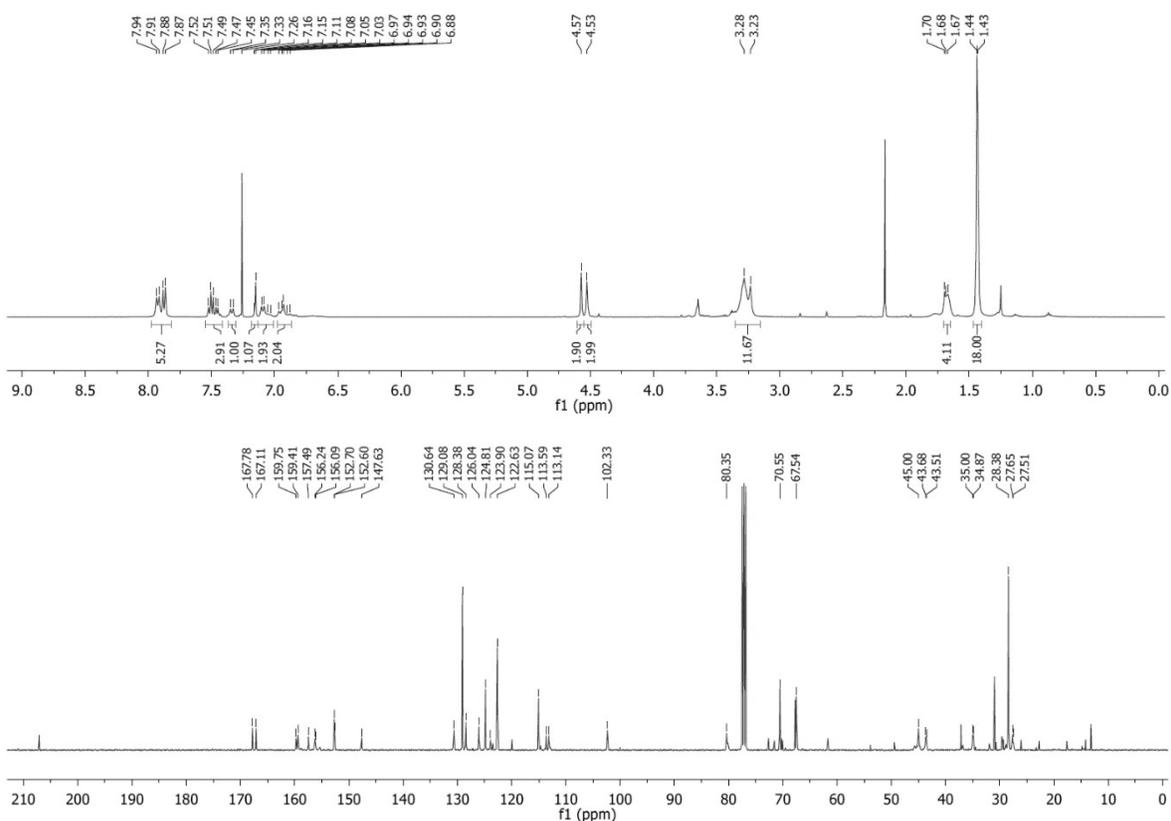
<sup>S2</sup> A. Samanta and B. J Ravoo, *Chem. Eur J.*, 2014, **20**, 4966.

<sup>S3</sup> B. Zhang, L. Yue, Y. Wang, Y. Yang, L. Wu, *Chem. Commun.*, 2014, **50**, 10823.

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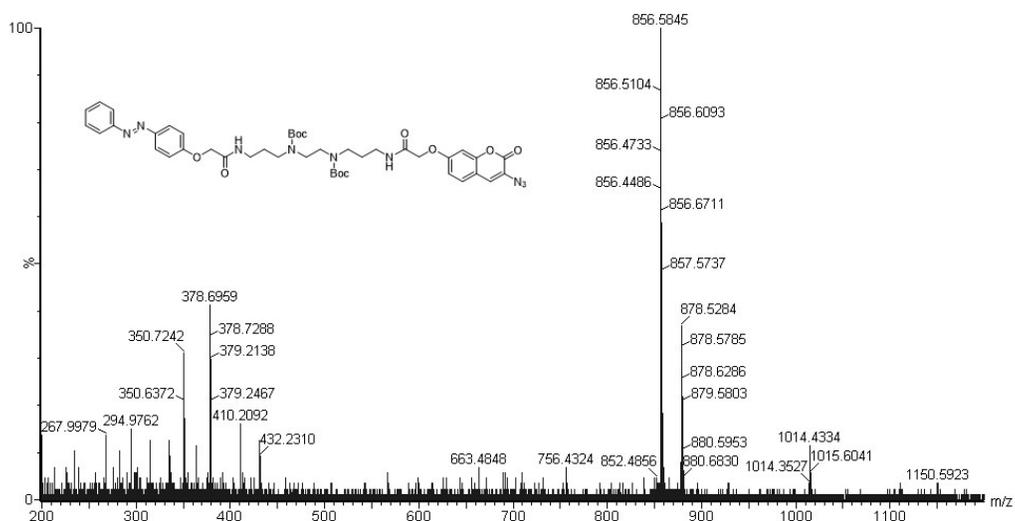
HOBt (6.6 mg, 0.049 mmol) and EDC (9.4 mg, 0.049 mmol) were added. After 30 min, **9** (30 mg, 0.049 mmol) dissolved in 0.5 mL CHCl<sub>3</sub> was added dropwise, followed by NMM (5 mg, 0.049 mmol), and the reaction mixture was stirred overnight. Subsequently, the solvent was removed under vacuum and the crude product was purified by silica gel column chromatography (CHCl<sub>3</sub>:MeOH:NH<sub>4</sub>OH, 90:9:1) to obtain pure **10** (33.7 mg, 0.039 mmol, 92%).

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 7.94-7.87 (m, 4H, CH<sub>arom</sub>), 7.52-7.47 (m, 3H, CH<sub>arom</sub>), 7.34 (d, *J* = 8.5 Hz, 1H, CH<sub>arom</sub>), 7.16-7.15 (m, 1H, CH<sub>arom</sub>), 7.11-7.03 (m, 2H, CH<sub>arom</sub>), 6.97-6.87 (m, 2H, CH<sub>arom</sub>), 4.57 (s, 2H, CH<sub>2</sub>), 4.53 (s, 2H, CH<sub>2</sub>), 3.28-3.23 (m, 12H, CH<sub>2</sub>), 1.70-1.67 (m, 4H, CH<sub>2</sub>), 1.44-1.43 (m, 18H, CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ 167.78, 167.11, 159.75, 159.41, 157.49, 156.24, 156.09, 152.70, 152.60, 147.63, 130.64, 129.08, 128.38, 126.04, 124.81, 123.90, 122.63, 115.07, 113.59, 113.14, 102.33, 80.35, 70.55, 67.54, 45.00, 43.68, 43.51, 35.00, 34.87, 28.38, 27.65, 27.51. ESI-MS (*m/z*) C<sub>43</sub>H<sub>53</sub>N<sub>9</sub>O<sub>10</sub>: calcd. 855.3915; found 856.5845 [M + H]<sup>+</sup>, 878.5284 [M + Na]<sup>+</sup>, 756.4324 [M – Boc + H]<sup>+</sup>, 378.6959 [M – Boc + 2H]<sup>2+</sup>, 350.7242 [M – 2Boc + 2Na]<sup>2+</sup>.



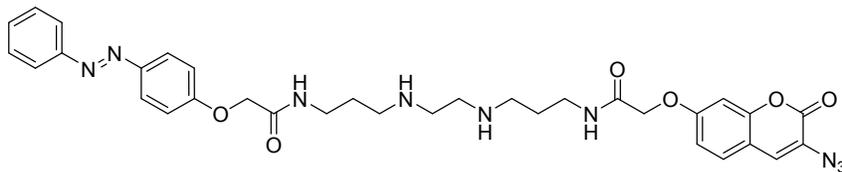
**Fig. S1** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of **10**

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**Fig. S2 ESI-MS of 10**

***N*-(3-((2-(3-azido-7-coumarinyl)oxy)acetoamido)propyl)-*N'*-(3-(2-(4-azobenzenoxy)acetamido)propyl)-1,2-bis-aminoethane (1)**



A solution of **10** (25 mg, 0.029 mmol) in DCM (1 mL) was cooled to 0 °C and 1 mL of TFA was added. The mixture was stirred at rt and the progress of the reaction

was monitored by TLC. After 3 h, the reaction was concentrated under vacuum and alkalinized with aq. NaOH (3% w/w) to pH  $\geq$  11. The solution was extracted with DCM (2  $\times$  10 mL) and the combined organic phases were subsequently washed with aq. sat. NaHCO<sub>3</sub> (2  $\times$  10 mL) and brine (2  $\times$  10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent was removed under reduced pressure. The crude product was then dissolved in a minimum amount of MeOH (~2 mL) and one drop of acetyl chloride was added. Precipitation occurred and pure **1** was isolated by filtration (7.9 mg, 0.012 mmol, 42%). <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O):  $\delta$  7.67-7.65 (m, 4H, CH<sub>arom</sub>), 7.54 (m, 3H, CH<sub>arom</sub>), 7.33 (d, *J* = 8.7 Hz, 1H, CH<sub>arom</sub>), 7.27 (s, 1H, CH<sub>arom</sub>), 7.02-7.00 (m, 2H, CH<sub>arom</sub>), 6.86-6.84 (m, 1H, CH<sub>arom</sub>), 6.69 (br s, 1H, CH<sub>arom</sub>), 4.62 (s, 2H, CH<sub>2</sub>), 4.43 (s, 2H, CH<sub>2</sub>), 3.45-3.36 (m, 8H, CH<sub>2</sub>), 3.13-3.08 (m, 4H, CH<sub>2</sub>), 1.98-1.91 (m, 4H, CH<sub>2</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  167.55 (C<sub>q</sub>), 167.31 (C<sub>q</sub>), 160.38 (C<sub>q</sub>), 159.70 (C<sub>q</sub>), 157.19 (C<sub>q</sub>), 152.36 (C<sub>q</sub>), 151.98 (C<sub>q</sub>), 146.55 (C<sub>q</sub>), 130.98 (CH<sub>arom</sub>), 129.44 (2CH<sub>arom</sub>), 128.89 (CH<sub>arom</sub>), 127.18 (CH<sub>arom</sub>), 124.51 (2CH<sub>arom</sub>), 122.58 (C<sub>q</sub>), 122.31 (2CH<sub>arom</sub>), 115.45 (2CH<sub>arom</sub>), 113.42 (CH<sub>arom</sub>), 113.10 (C<sub>q</sub>), 101.64 (CH<sub>arom</sub>), 69.81 (CH<sub>2</sub>), 67.10 (CH<sub>2</sub>), 44.85 (2CH<sub>2</sub>), 43.39 (2CH<sub>2</sub>), 35.59 (2CH<sub>2</sub>), 26.17 (2CH<sub>2</sub>). ESI-MS (*m/z*) C<sub>33</sub>H<sub>37</sub>N<sub>9</sub>O<sub>6</sub>: calcd. 655.2867; found 656.4758 [M + H]<sup>+</sup>, 328.7383 [M + 2H]<sup>2+</sup>, 314.7337 [M - CO]<sup>2+</sup> or [M - N<sub>2</sub>]<sup>2+</sup>.

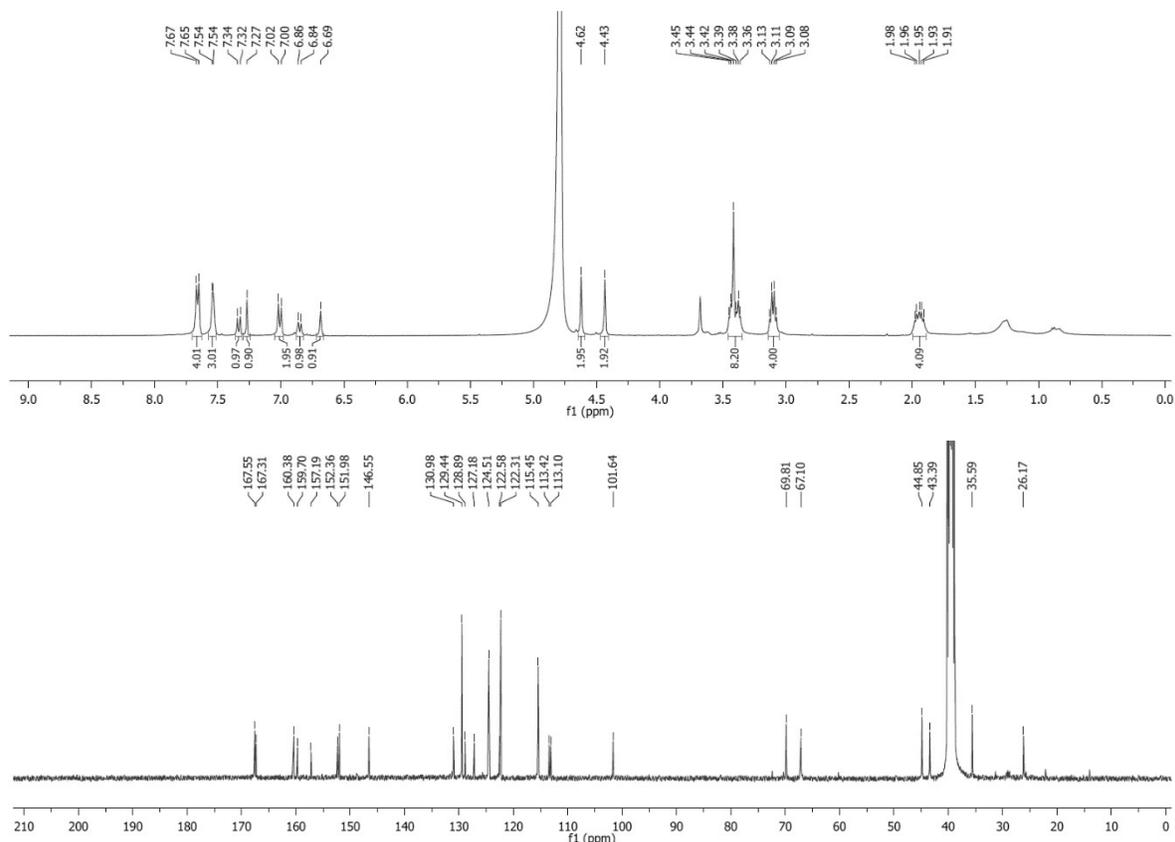


Fig. S3  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra of **1**

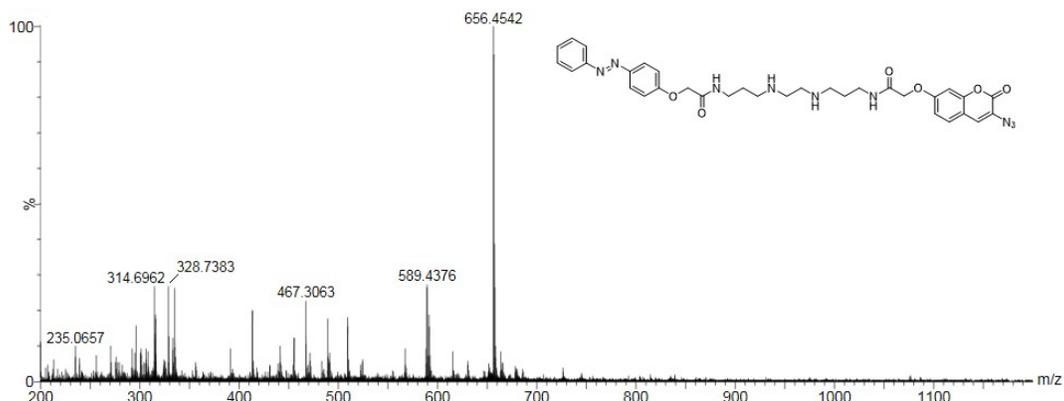


Fig. S4 ESI-MS of **1**

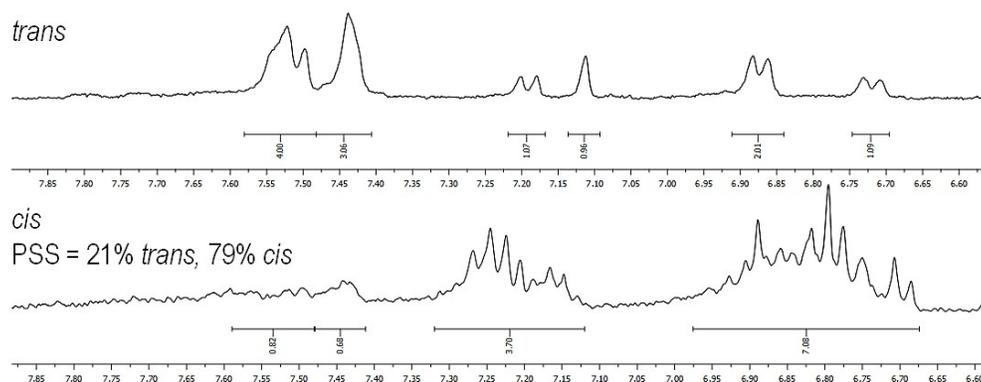


Fig. S5 Initial state (top) and photostationary state (bottom) of compound **1** in  $\text{D}_2\text{O}$  (10 min irradiation,  $\lambda = 365 \text{ nm}$ )

### 3.2 CCMV isolation and purification

CCMV was isolated and purified from cowpea plants with a 10-day old infection, as described previously.<sup>S4</sup> The obtained virus was analyzed by UV/vis spectroscopy, SDS-PAGE and FPLC.

### 3.3 Functionalization of CCMV

The functionalization of CCMV with linear alkyne groups was performed with eight equiv. of both EDC and NHS, and seven equiv. of propargylamine per solvent accessible carboxyl group.<sup>S5</sup> The reaction was incubated in PB for 18 h at 4 °C and subsequently dialyzed to VB and purified by preparative FPLC to obtain alkyne functionalized CCMV (CCMV-A).

<sup>S4</sup> 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.

<sup>S5</sup> E. Gillitzer, D. Willits, M. Young and T. Douglas, *Chem. Commun.*, 2002, 2390.

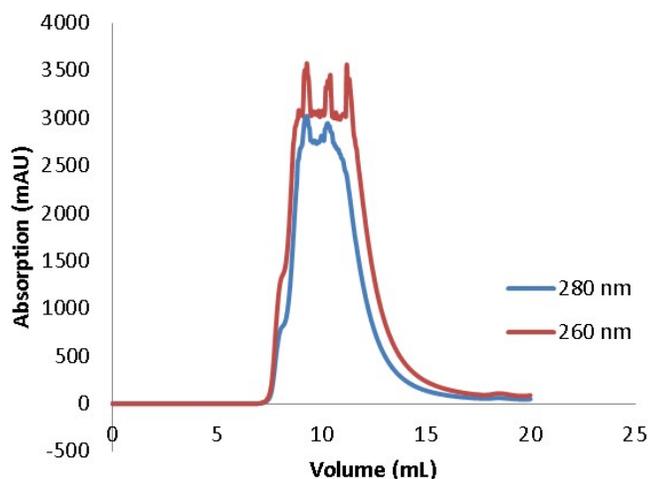


Fig. S6 FPLC chromatogram of alkyne-modified CCMV (CCMV-A)

### 3.4 Post-functionalization of CCMV

The post-functionalization of CCMV-A (3.04 mg, 0.7 nmol) was performed with 0.42 mg of copper sulfate (22 equiv. per CP), 1.35 mg of L-ascorbic acid (63 equiv. per CP) and 3.84 mg of **1** (48 equiv. per CP). The reaction was incubated in VB for 18 h at rt. The modified virus was purified by several centrifugation and dialysis steps, followed by preparative FPLC.

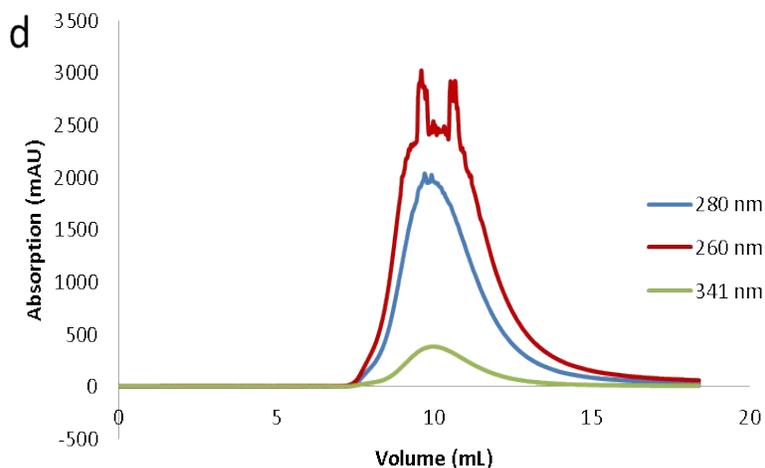


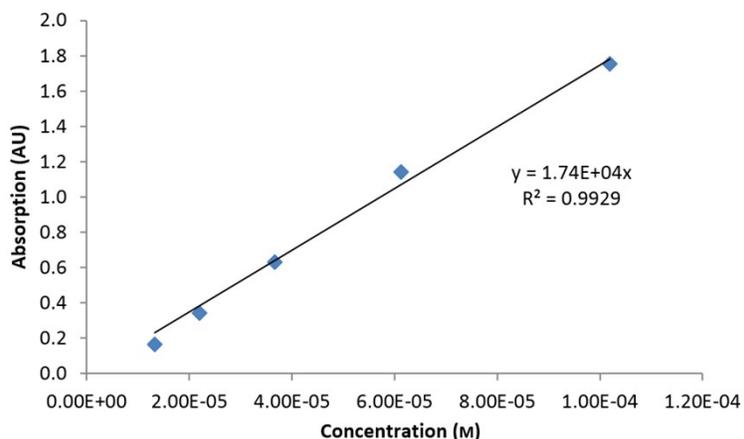
Fig. S7 FPLC chromatogram of CCMV-CoumAzo

### 3.5 UV/Vis spectroscopy

#### Degree of functionalization

To quantify the amount of azobenzene switches on the outer surface of CCMV-CoumAzo, a calibration curve was fitted for compound **1**. Different concentrations of **1** are plotted against the absorption at  $\lambda = 337$  nm. The determined extinction coefficient is  $17.4 \times 10^3 \text{ M}^{-1}\cdot\text{cm}^{-1}$ , which gives a degree of functionalization of approximately 125 switches per capsid.

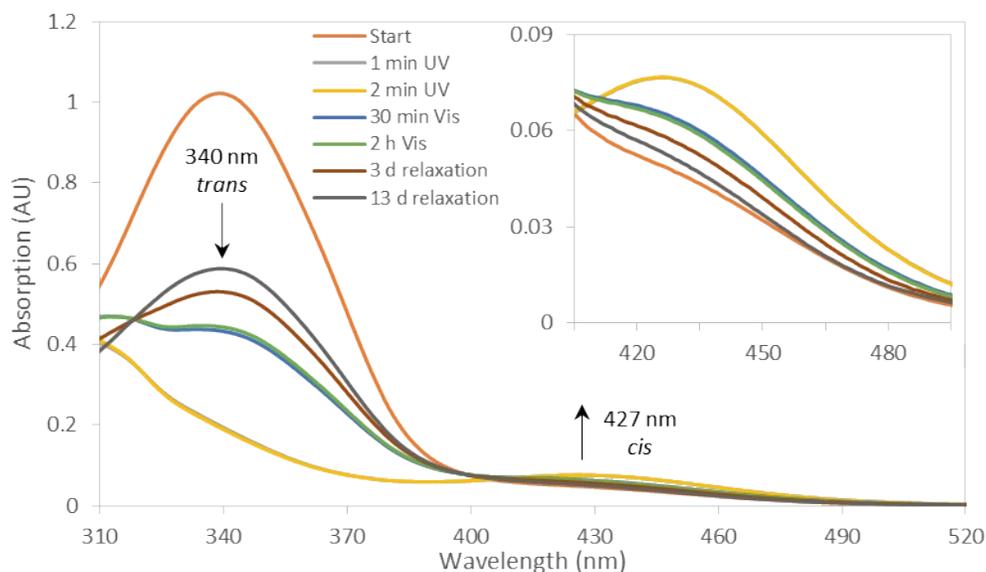
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**Fig. S8** Calibration curve of **1** at  $\lambda = 337$  nm, extinction coefficient  $\epsilon = 17.4 \times 10^3 \text{ M}^{-1}\cdot\text{cm}^{-1}$ .

### **Cis-trans** switching of **1**

In order to study the photoisomerization of **1**, UV/Vis spectra were measured at the initial state, after 1 min irradiation, after 2 min irradiation and after relaxation for 30 min. The light source used was a 30 mW lamp at  $\lambda = 365$  nm and the samples were irradiated at a distance of 10 cm.



**Fig. S9** Photoisomerization of compound **1** by UV/Vis spectroscopy. Orange: initial absorption; grey and yellow: after irradiation for 1 and 2 min resp.; blue: after 30 min relaxation under visible light; green: after relaxation for two hours under visible light; brown: after relaxation for three days; anthracite: after relaxation for 13 days. Inset shows enlarged region between 405 and 495 nm.

## **3.6 Reversible immobilization of CCMV-CoumAzo with cucurbit[8]uril**

### **Preparation of self-assembled monolayers on gold substrates**

Gold substrates were first washed with piranha solution ( $\text{H}_2\text{SO}_4 + 30\% \text{H}_2\text{O}_2$ , 70:30 v/v), and copious amount of Milli-Q water afterwards. Substrates were then immersed overnight in a 1 mM ethanolic solution of 11-(1,4,7,10,13-pentaoxatridecyl)-undecyl disulfide and (18-(4-maleimido-butaneamido)-*N*-

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1,4,7,10,13,16-hexaoxaoctadecyl)-undecyl 11-(1,4,7,10,13-pentaoxatridecyl)-undecyl disulfide at a molar ratio of 99:1 at room temperature in the dark. The substrates were then cleaned thoroughly with ethanol, Milli-Q water and dried with a stream of nitrogen gas. They were then immediately incubated with 1 mM MV-SH in 50 mM phosphate buffer at pH 6.4 for 1 h. The substrates were then washed thoroughly with Milli-Q water, dried with nitrogen gas and used for further supramolecular assembly experiments.

### **Surface Plasmon Resonance**

After obtaining a stable baseline, the prepared MV substrates were first saturated with 50  $\mu\text{M}$  CB[8] in VB. After equilibrium was reached, the solution was changed to CCMV-CoumAzo in the presence of 50  $\mu\text{M}$  CB[8] in VB at five different concentrations, starting from 0.26  $\mu\text{M}$  of azobenzene switch up to 2.64  $\mu\text{M}$ . After saturation with the highest CCMV-CoumAzo concentration, the solution was changed back to 50  $\mu\text{M}$  CB[8] in VB and back to VB. To fully dissociate the CB[8] from the MV surface, (ferrocenylmethyl)trimethyl-ammonium ( $\text{FcTMA}^+$ ) was used, which shows a higher binding affinity to CB[8] than MV. Finally, the sample was flushed with VB for several minutes and the measurement was stopped. The flow rate was set to 0.1 mL/min. As a negative control, native CCMV with CB[8] and CCMV-CoumAzo with CB[7] were measured, all at the same conditions.

### **Fluorescence microscopy**

To visualize optical control over the complex formation of CB[8] with post-functionalized CCMV, microcontact printing was performed onto 20 nm thick gold substrates which were functionalized with 1% MV as described above. PDMS stamps were prepared with dot-patterns of 100  $\mu\text{m}$  diameter and 100  $\mu\text{m}$  spacings. Before using, PDMS patterns were plasma treated for 30 s in order to get a hydrophilic surface. PDMS stamps were briefly washed with water and dried before use. A 50  $\mu\text{M}$  CB[8] in VB solution was printed onto the MV surfaces for 20 min with approximately 15 g of weights. Next, 100  $\mu\text{L}$  CCMV-CoumAzo (2.6  $\mu\text{M}$  of CoumAzo on CCMV) in VB was incubated on the CB[8] surface for 20 min. The patterned surface was then incubated for 20 min with a solution of 1 mM SYBR Safe in VB (100  $\mu\text{L}$ ) to stain the viral RNA. After incubation, the samples were washed with Milli-Q water, dried and imaged.

To manifest the *trans-cis* switching of the azobenzene on CCMV-CoumAzo and therefore also its presence on the virus capsids, MV-gold substrates were imprinted with CB[8] and incubated with CCMV-CoumAzo, and stained with SYBR Safe as explained. For photoisomerization, a filter cube with excitation of 350-360 nm was used with maximum illumination intensity from an X-Cite<sup>®</sup> 120 light source. Localized release experiments were performed by closing the field diaphragm in the UV excitation path (epi). Irradiation was always performed for 5 min.

## 4. Additional experimental data

### 4.1 UV/Vis spectra of native CCMV and CCMV-CoumAzo

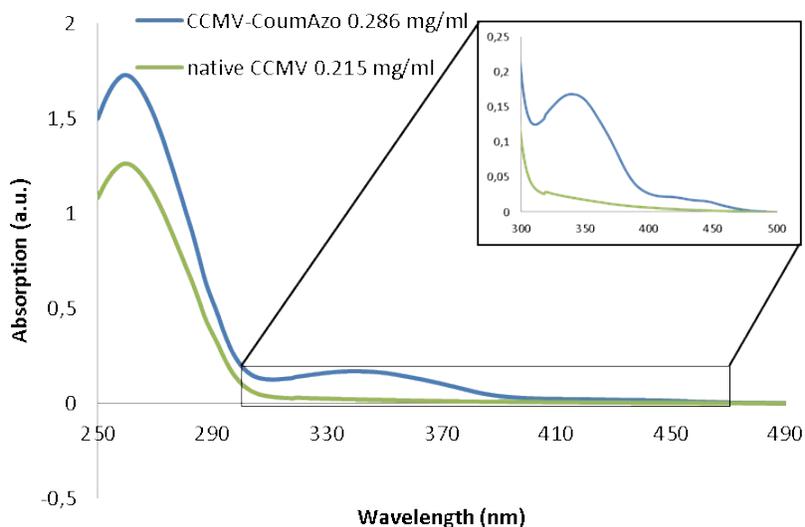


Fig. S10 Comparison of native CCMV and CCMV-CoumAzo by UV/Vis

### 4.2 Fluorescence spectrum of CCMV-CoumAzo

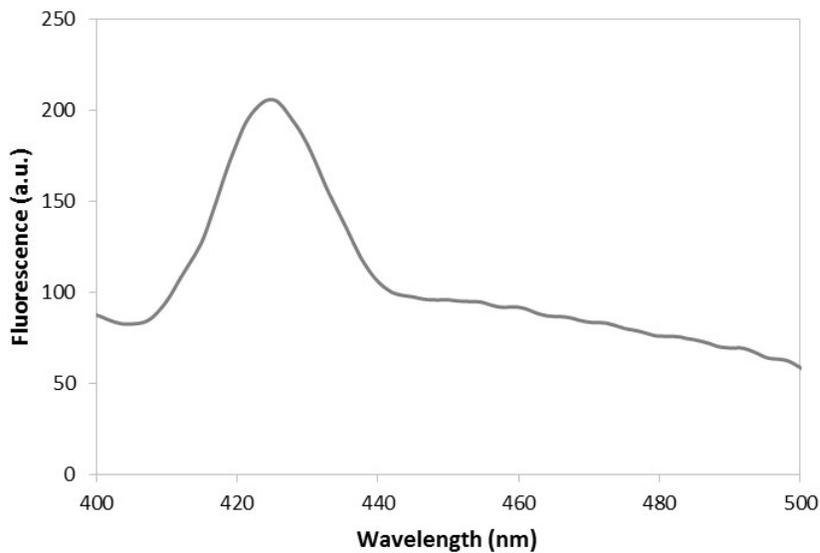


Fig. S11 Fluorescence spectrum of CCMV-CoumAzo upon excitation at  $\lambda = 370$  nm;  $\lambda_{em,max} = 425$  nm