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Supporting Information

Highly efficient enzyme encapsulation in a protein nanocage: towards enzyme catalysis in a cellular nanocompartment mimic

Lise Schoonen, Roeland J.M. Nolte, Jan C. M. van Hest

Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands.

List of contents

1 Materials and methods

1.1 Materials

Hot start II HF DNA polymerase, restriction enzymes, T4 DNA ligase and Antarctic phosphatase were obtained from New England Biolabs. The DNA oligos were synthesized by Biolegio. Ampicillin was purchased from MP Biomedicals. Chloramphenicol was obtained from Sigma-Aldrich. Isopropyl β-D-1-thiogalactopyranoside (IPTG) and DMSO were purchased from Acros. Ni-NTA agarose beads were obtained from Qiagen. CH_2Cl_2 was dried by purging it over an activated alumina column utilising an MBraun MB SPS800 system under nitrogen atmosphere. p-Nitrophenol and p-nitrophenyl acetate were purchased from Aldrich. EDC·HCl and MeO-PEG-NHCO-C_2H_4-COOH MW 750 and MW 2000 were obtained from Iris Biotech GmbH. DMSO-d_6 was purchased at Cambridge Isotope Laboratories. Endoproteinase Glu-C was obtained from BIOKÉ. Trypsin Gold was purchased from Promega.

1.2 Buffers

pH-induced assembly buffer: 50 mM NaOAc, 500 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 5.0.
Salt-induced assembly buffer: 50 mM Tris·HCl, 2 M NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.5.
pH 7.5 buffer: 50 mM Tris·HCl, 500 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.5.
CalB buffer: 50 mM NaH_2PO_4, 150 mM NaCl, pH 7.0.
Sortase buffer: 50 mM HEPES, 150 mM NaCl, 5 mM $CaCl_2$, pH 7.5.

All buffers were filtered over a 0.2 μM filter prior to use.

1.3 UV-vis absorbance measurements

Protein concentrations were measured on a Varian Cary 50 Conc UV-vis spectrometer using a quartz cuvette with a path length of 3 mm. Protein concentrations were calculated using the theoretical extinction coefficients.[1] Samples were centrifuged prior to the measurements.

1.4 Mass spectrometry

Protein mass characterization was performed by electrospray ionization time-of-flight (ESI-TOF) on a JEOL AccuTOF CS. Deconvoluted mass spectra were obtained using MagTran 1.03 b2. Isotopically averaged molecular weights were calculated using the 'Protein Calculator v3.4' at http://protcalc.sourceforge.net. Protein samples were desalted by spin filtration with MQ (final concentrations 10-150 μM).

1.5 Size exclusion chromatography (SEC)

SEC measurements were performed on a Superose 6 increase 10/300 column or a Superdex 75 PC 10/300 column (GE Healthcare). Analytical and preparative SEC measurements were executed on a Shimadzu LC-2010AHT HPLC and Agilent 1260 bio-inert HPLC, respectively. Samples (10-200 µg) were separated on the column with a flow rate of 0.5 mL/min.

1.6 Transmission electron microscopy (TEM)

TEM grids (FCF-200-Cu, EMS) were glow-discharged using a Cressington carbon coater and power unit. Protein samples (0.2-0.6 mg/mL, 5 µL) were applied on the glow-discharged grids and incubated for 1 min. The samples were carefully removed using a filter paper and the grid was allowed to dry for at least 15 minutes. Then the grid was negatively stained by applying 2% uranyl acetate in water (5 µL). The staining solution was removed after 15 seconds and the grid was allowed to dry for at least 15 minutes. The samples were analyzed on a JEOL JEM-1010 TEM.

1.7 Dynamic light scattering (DLS) measurements

DLS measurements were performed on a Zetasizer Nano S at 25 ^{o}C. Samples (1 mg/mL) were centrifuged prior to analysis. Organic solvents and buffers were filtered prior to use. All measurements were done in triplo.

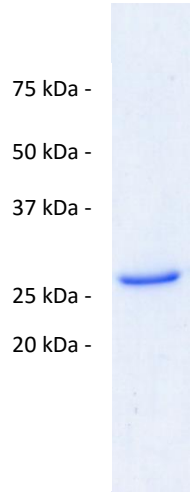
1.8 NMR measurements

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 (400 MHz) spectrometer in DMSO-d_6. The NMR solvent residual peak of DMSO-d_6 was used as the internal reference. Proton coupling constants (Hz) of the phenyl protons were determined by computer simulation using MestReNova.

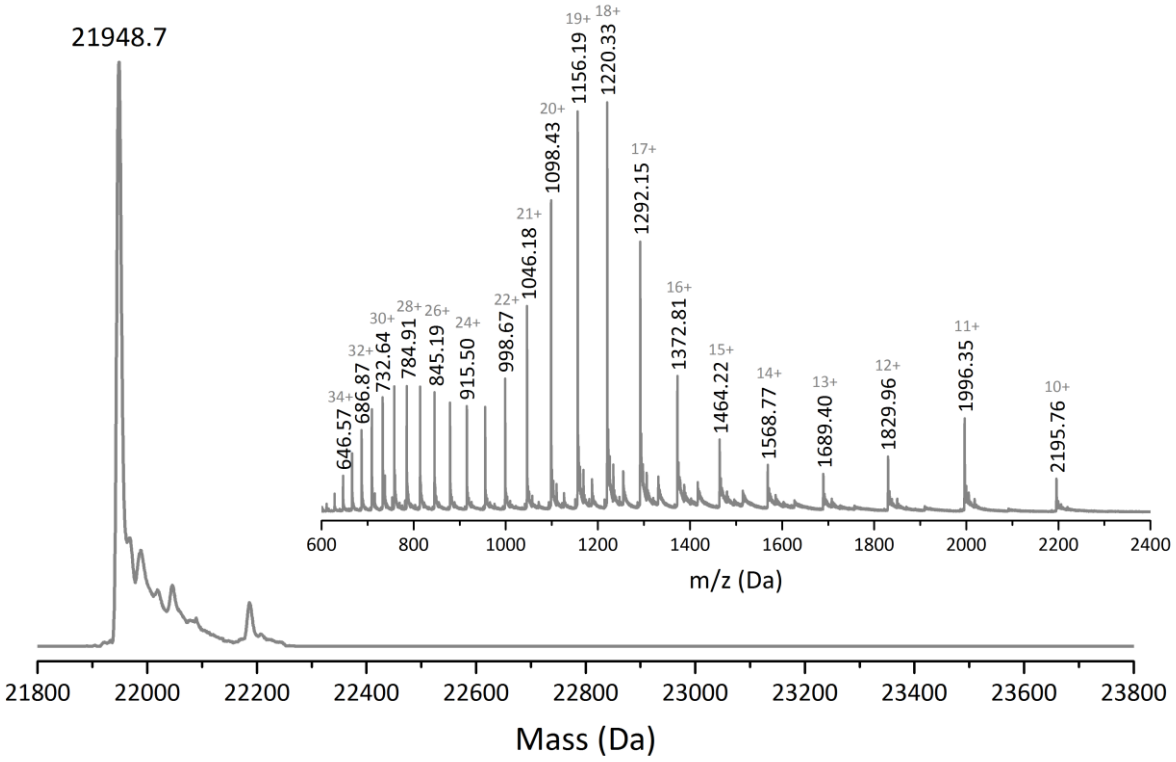
2 Experimental section

2.1 Expression of Sortase A

E.coli BL21 AI cells were transformed with a pQE30 plasmid carrying the Sortase gene, followed by incubation in LB medium (1 mL) for 1h at 37 °C.[2] After this short incubation phase, the cells were transferred into fresh LB medium (4 mL) with ampicillin (100 mg/L) and were incubated at 37 °C for 4h. This preculture was then transferred into TB medium (500 mL) with ampicillin (100 mg/L) and cells were incubated for 24h at 37 °C. Cells were pelleted and resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, supplemented with 1 mM phenylmethanesulfonyl fluoride, pH 8.0) and lysed by sonication. The lysate was centrifuged (14.000 g, 30 min, 4 °C) and the supernatant was incubated with Ni-NTA beads for 2 h at 4 °C. Ni-NTA beads were washed with wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) and the purified protein was eluted from the beads with elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 500 mM imidazole, pH 8.0). For storage the protein was dialyzed against Sortase buffer. The pure protein was obtained with a yield of 10-12.5 mg/L of culture. The purity was verified by SDS-PAGE. ESI-TOF: calculated 21947.5 Da, found 21948.7 Da.



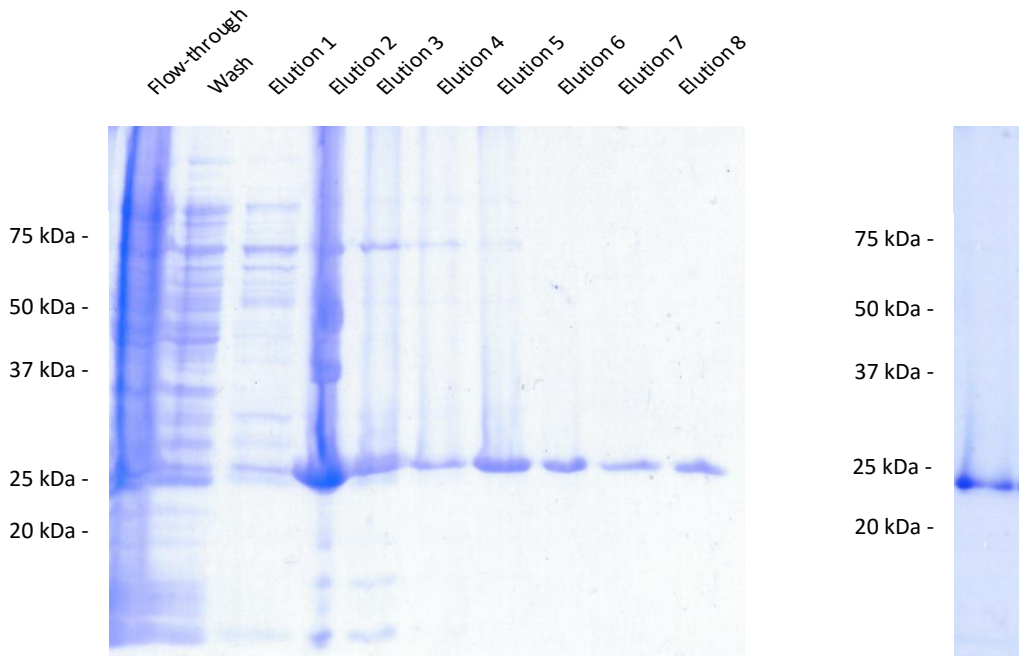
SDS-PAGE of purified Sortase A.



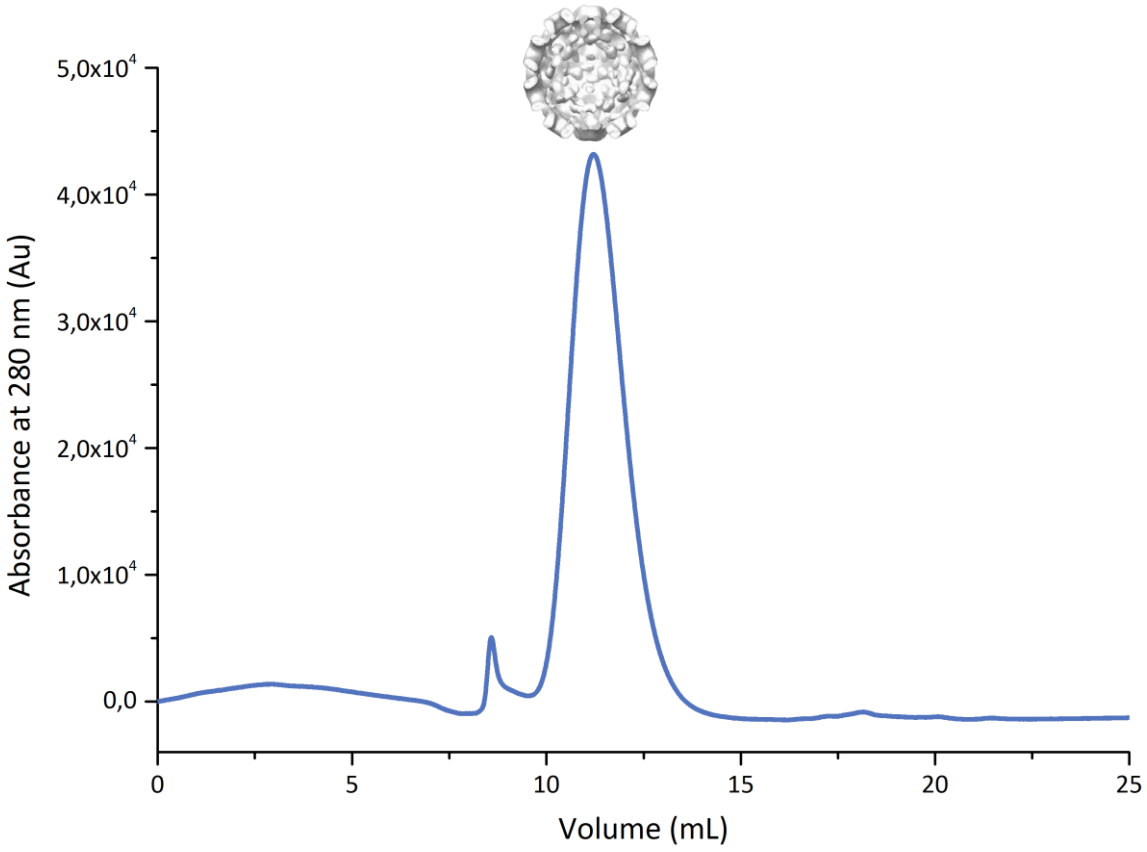
ESI-TOF mass spectrometry of purified Sortase A. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 21947.5 Da.

2.2 Expression of G-ELP-CCMV

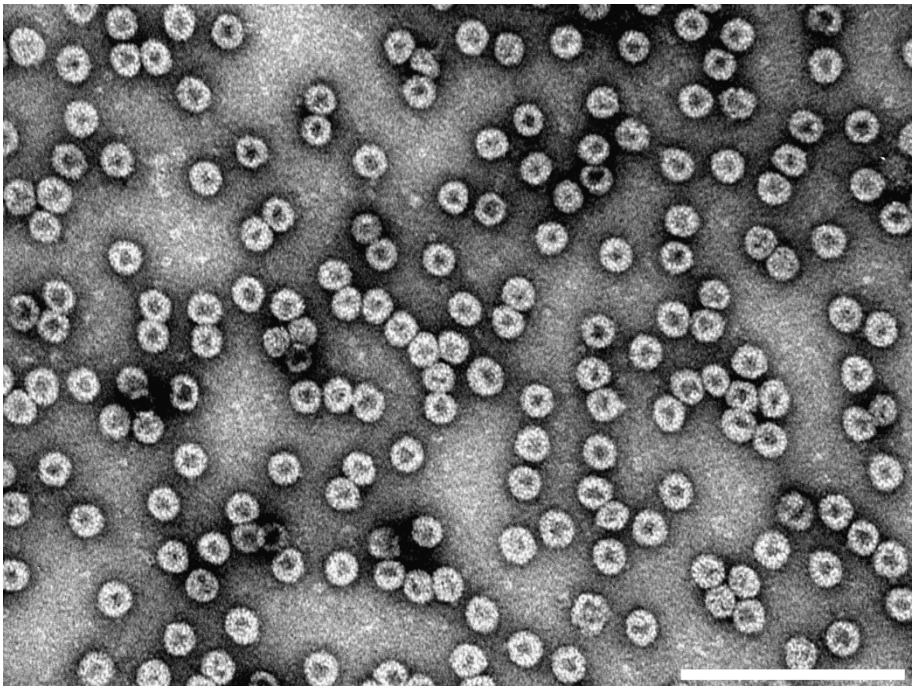
The pET-15b-G-H_6-[$V_4L_4G_1$-9]-CCMV(ΔN26) vector encoding for the hexahistidine-tagged ELP-CCMV protein was previously constructed as described by van Eldijk *et al.*[3] For a typical expression, LB medium (50 mL), containing ampicillin (100 mg/L) and chloramphenicol (50 mg/L), was inoculated with a single colony of *E. coli* BLR(DE3)pLysS containing pET-15b-G-H_6-[$V_4L_4G_1$-9]-CCMV(ΔN26), and was incubated overnight at 37 °C. This overnight culture was used to inoculate 2xTY medium (1 L), supplemented with ampicillin (100 mg/L) and chloramphenicol (50 mg/L). The culture was grown at 37 °C and protein expression was induced during logarithmic growth (OD_{600} = 0.4-0.6) by addition of IPTG (1 mM). After 6 h of expression at 30 °C, the cells were harvested by centrifugation (2700 g, 15 min, 4 °C) and the pellets were stored overnight at -20 °C.
After thawing, the cell pellet was resuspended in lysis buffer (50 mM NaH_2PO_4, 1.3 M NaCl, 10 mM imidazole, pH 8.0; 25 mL). The cells were lysed by ultrasonic disruption (5 times 30 s, 100% duty cycle, output control 3, Branson Sonifier 250, Marius Instruments). The lysate was incubated with DNase (10 mg/L) and RNase A (5 mg/L) for 10 min at 4 °C. Then the lysate was centrifuged (16.400 g, 15 min, 4 °C) to remove the cellular debris. The supernatant was incubated with Ni-NTA agarose beads (3 mL) for 1 h at 4 °C. The suspension was loaded onto a column, the flow-through was collected and the beads were washed twice with wash buffer (50 mM NaH_2PO_4, 1.3 M NaCl, 20 mM imidazole, pH 8.0; 20 mL). Then, the protein of interest was eluted from the column with elution buffer (50 mM NaH_2PO_4, 1.3 M NaCl, 250 mM imidazole, pH 8.0; 1 time 0.5 mL, 7 times 1.5 mL). The purification was analyzed by SDS-PAGE. The fractions containing G-ELP-CCMV were combined and dialyzed against pH 7.5 buffer to obtain the protein dimers. For storage the proteins were assembled by dialysis against pH-induced assembly buffer. The pure protein was obtained with a yield of 100 mg/L of culture. The purity was verified by SDS-PAGE. The geometry and assembly properties were analyzed by SEC using a Superose 6 increase 10/300 column with pH-induced assembly buffer as the eluent and TEM. ESI-TOF: calculated 22253.4 Da, found 22253.5 Da.



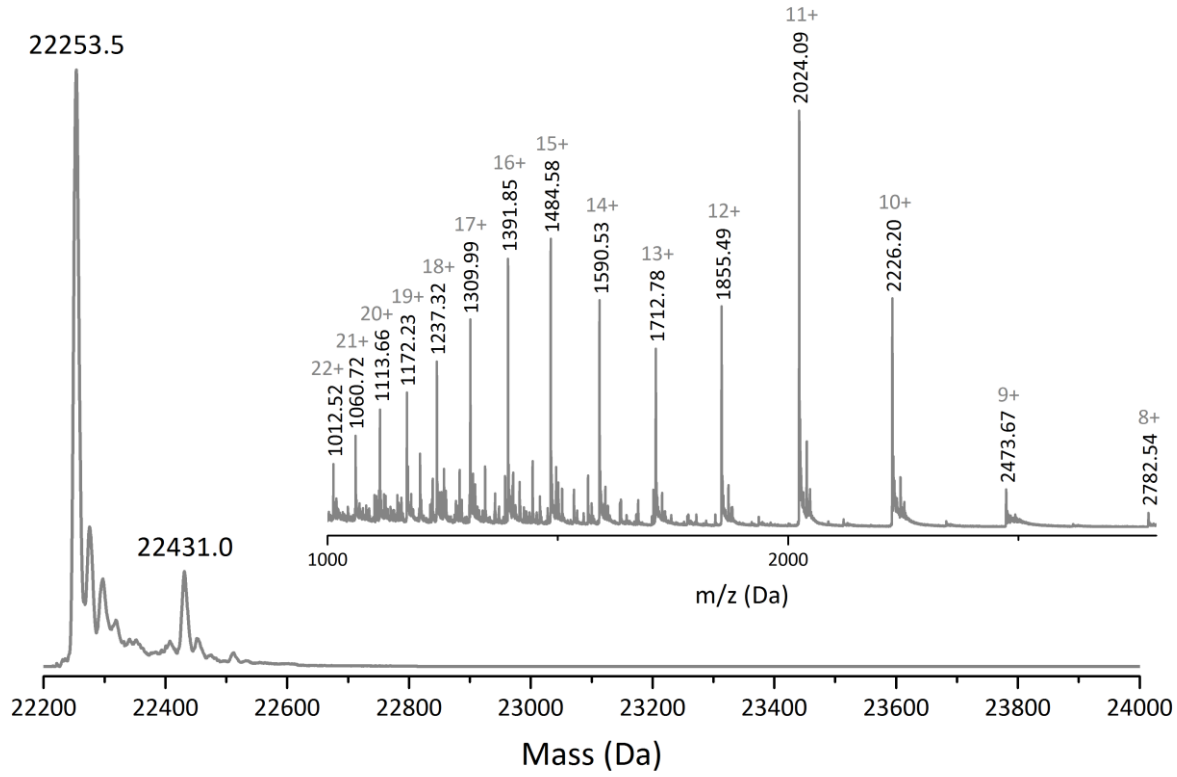
SDS-PAGE of affinity purification of G-ELP-CCMV (left) and purified G-ELP-CCMV (right).



Size exclusion chromatogram of purified G-ELP-CCMV in pH-induced assembly buffer.



Uranyl acetate stained TEM micrograph of G-ELP-CCMV. Average particle size = 29.2 ± 1.5 nm. Scale bar corresponds to 200 nm.

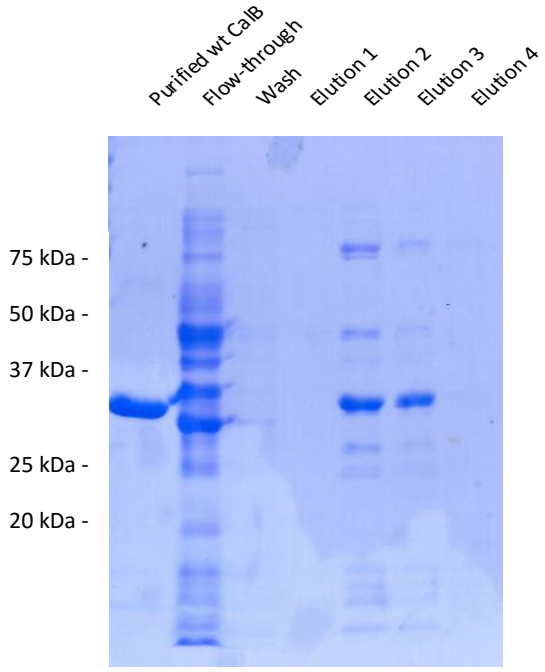


ESI-TOF mass spectrometry of purified G-ELP-CCMV. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 22253.4 Da.

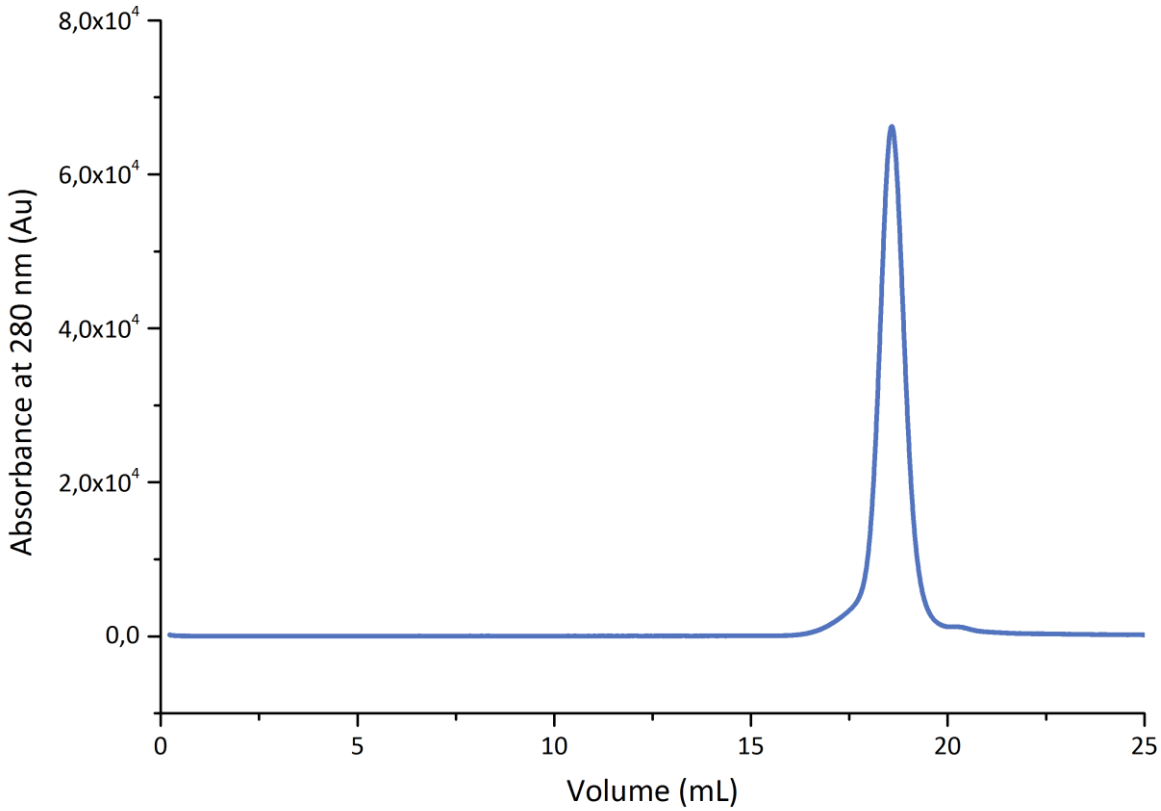
2.3 Expression of wild type CalB

The pET-22b-wtCalB vector encoding for bacterial expression of the histidine-tagged wild type CalB protein was previously constructed as described by Schoffelen *et al.*[4] For a typical expression, LB medium (50 mL), containing ampicillin (100 mg/L) and chloramphenicol (50 mg/L), was inoculated with a single colony of *E. coli* B834(DE3)pLysS containing pET-22b-wtCalB and was incubated overnight at 37 °C. This overnight culture was used to inoculate 2xTY medium (1 L), supplemented with ampicillin (100 mg/L) and chloramphenicol (50 mg/L). The culture was grown at 37 °C and protein expression was induced during logarithmic growth (OD_{600} = 0.4-0.6) by addition of IPTG (1 mM). After 25 h of expression at 25 °C, the cells were harvested by centrifugation (2700 g, 15 min, 4 °C) and the pellets were stored overnight at -20 °C.

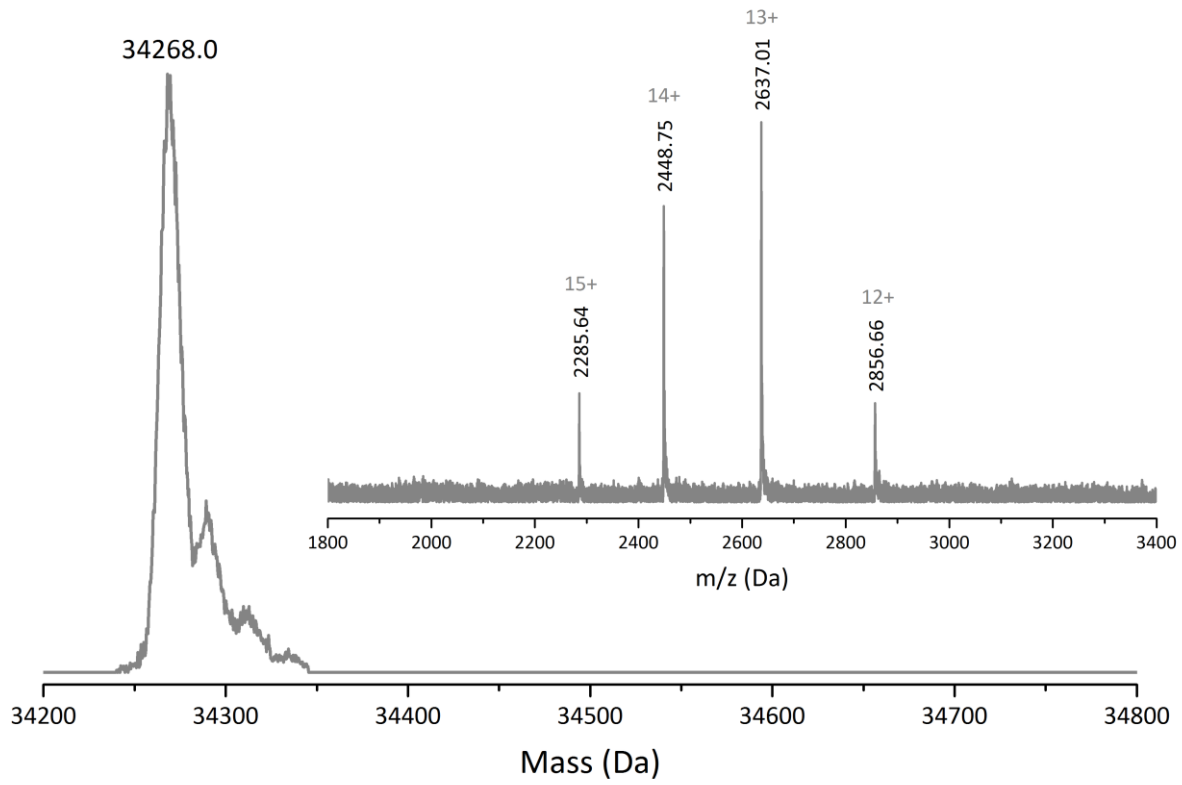
After thawing, the cell pellet was resuspended in lysis buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 10 mM imidazole, pH 8.0; 25 mL). The cells were lysed by ultrasonic disruption (5 times 30 s, 100% duty cycle, output control 3, Branson Sonifier 250, Marius Instruments). Then the lysate was centrifuged (16.400 g, 15 min, 4 °C) to remove the cellular debris. The supernatant was incubated with Ni-NTA agarose beads (3 mL) for 1 h at 4 °C. The suspension was loaded onto a column, the flow-through was collected and the beads were washed twice with wash buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 20 mM imidazole, pH 8.0; 20 mL). Then, the protein of interest was eluted from the column with elution buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 250 mM imidazole, pH 8.0; 1 time 0.5 mL, 7 times 1.5 mL). The purification was analyzed by SDS-PAGE. Elution fractions containing CalB were combined and concentrated (Amicon® Ultra-4 Centrifugal Filter Device 10.000 NMWL). Further purification was performed by preparative SEC using a Superdex 75 PC 10/300 column and CalB buffer as the eluent. The pure protein was obtained with a yield of 1.2-1.9 mg/L of culture. The purity was verified by SDS-PAGE and SEC using a Superose 6 10/300 GL column and CalB buffer as the eluent. ESI-TOF: calculated 34269.7 Da, found 34268.0 Da.



SDS-PAGE of affinity purification of wild type CalB and purified wild type CalB.



Size exclusion chromatogram of purified wild type CalB.



ESI-TOF mass spectrometry of purified wild type CalB. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 34269.7 Da.

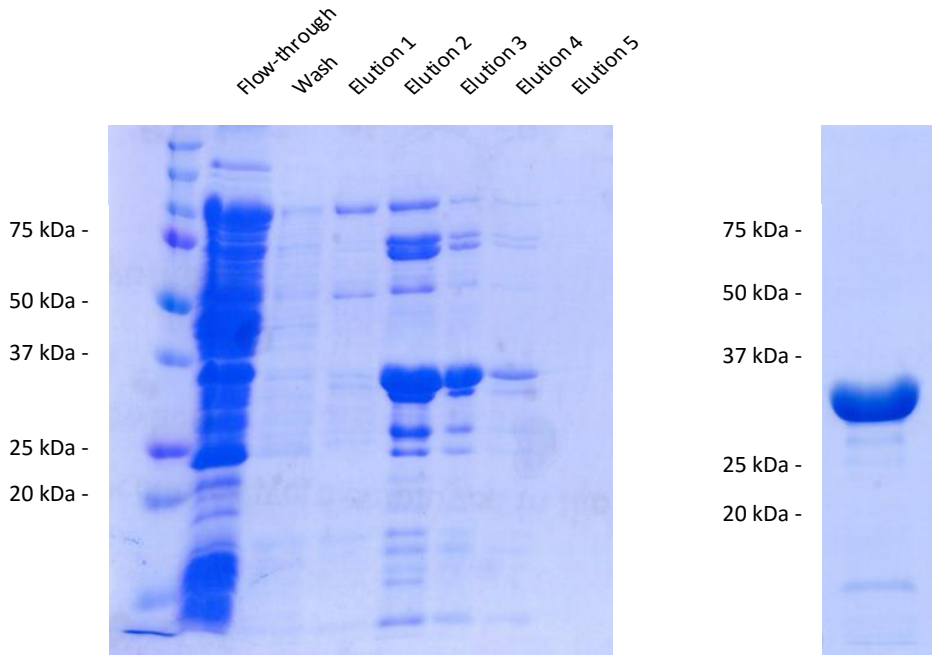
2.4 Cloning of CalB-LPETG-H_6

The pET-22b-wtCalB vector encoding for bacterial expression of the histidine-tagged wild type CalB protein was previously constructed as described by Schoffelen *et al.*[4] For the introduction of the LPETG-tag, a set of DNA oligos was designed (Table 1). The oligos were annealed and the resulting insert encoded for the CalB protein, an LPETGG tag and a histidine tag with a 5' NcoI and a 3' XhoI restriction site. The product after PCR was purified by agarose gel electrophoresis. Both the purified insert and the pET-22b-wtCalB vector were digested with NcoI-HF® and XhoI and the products were again purified by agarose gel electrophoresis. Subsequently, the inserts were ligated into the digested vector to yield pET-22b-CalB-LPETG-H_6. This plasmid was transformed into *E. coli* XL1-BLUE cells, the DNA was extracted and the sequence was confirmed by DNA sequencing (Table 2). For expression of CalB-LPETG-H_6, the plasmid was transformed into *E. coli* BLR(DE3)pLysS cells (Novagen, MERCK).

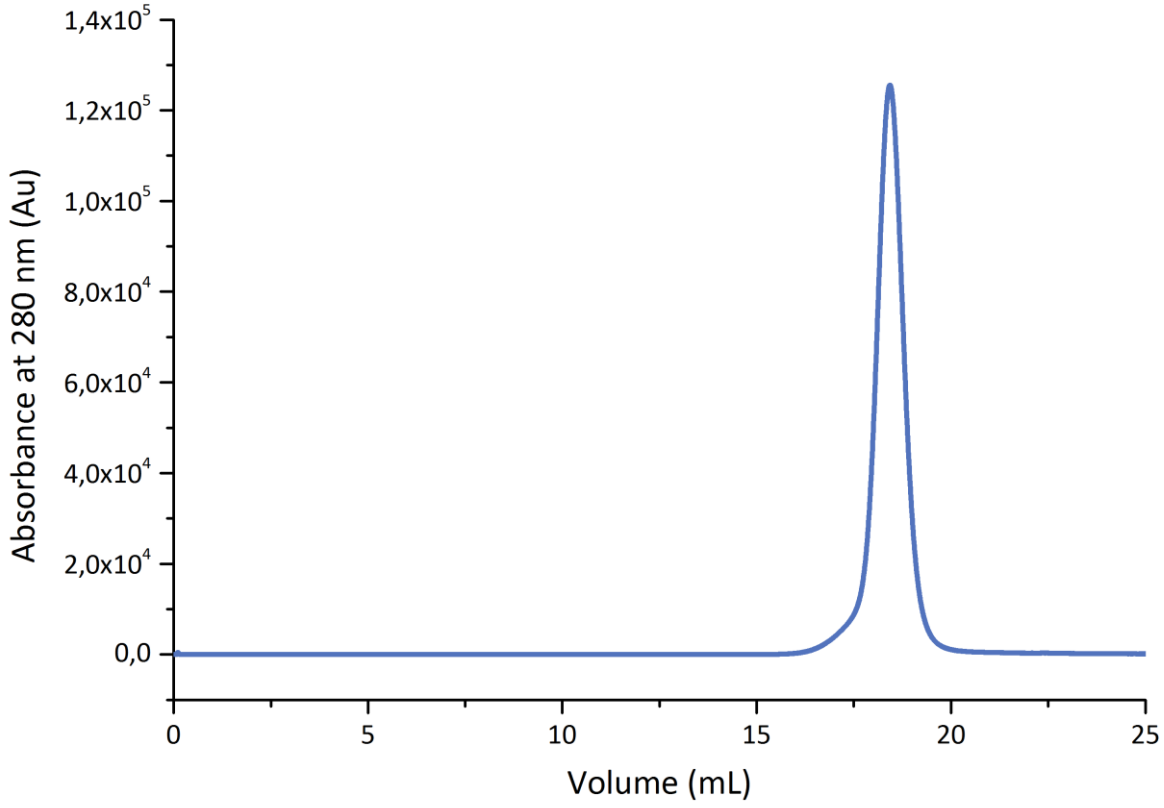
2.5 Expression of CalB-LPETG-H_6

For a typical expression, LB medium (100 mL), containing ampicillin (100 mg/L) and chloramphenicol (50 mg/L), was inoculated with a single colony of *E. coli* BLR(DE3)pLysS containing pET-22b-CalB-LPETG-H_6 and was incubated overnight at 30 °C. This overnight culture was used to inoculate 2xTY medium (1 L), supplemented with ampicillin (100 mg/L) and chloramphenicol (50 mg/L). The culture was grown at 37 °C and protein expression was induced during logarithmic growth (OD_{600} = 0.4-0.6) by addition of IPTG (1 mM). After 6 h of expression at 30 °C, the cells were harvested by centrifugation (2700 g, 15 min, 4 °C) and the pellets were stored overnight at -20 °C.

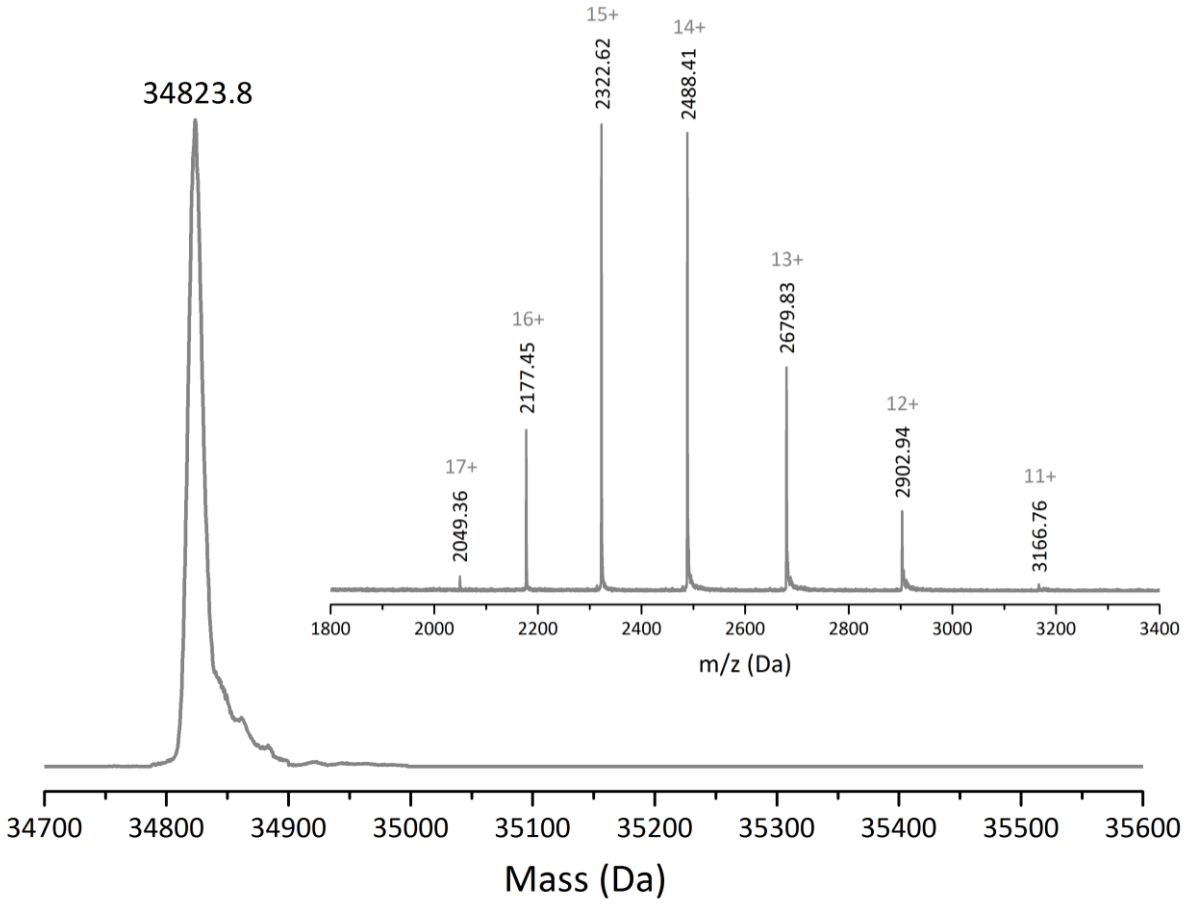
After thawing, the cell pellet was resuspended in lysis buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 10 mM imidazole, pH 8.0; 25 mL). The cells were lysed by ultrasonic disruption (5 times 30 s, 100% duty cycle, output control 3, Branson Sonifier 250, Marius Instruments). Then the lysate was centrifuged (16.400 g, 15 min, 4 °C) to remove the cellular debris. The supernatant was incubated with Ni-NTA agarose beads (3 mL) for 1 h at 4 °C. The suspension was loaded onto a column, the flow-through was collected and the beads were washed twice with wash buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 20 mM imidazole, pH 8.0; 20 mL). Then, the protein of interest was eluted from the column with elution buffer (50 mM NaH_2PO_4, 0.5 M NaCl, 250 mM imidazole, pH 8.0; 1 time 0.5 mL, 8 times 1.5 mL). The purification was analyzed by SDS-PAGE. Elution fractions containing CalB were combined and concentrated (Amicon® Ultra-4 Centrifugal Filter Device 10.000 NMWL). Further purification was performed by preparative SEC using a Superdex 75 PC 10/300 column and CalB buffer as the eluent. The pure protein was obtained with a yield of 2.0-3.3 mg/L of culture. The purity was verified by SDS-PAGE and SEC using a Superose 6 10/300 GL column and CalB buffer as the eluent. ESI-TOF: calculated 34824.3 Da, found 34823.8 Da.



SDS-PAGE of affinity purification of CalB-LPETG-H_6 (left) and purified CalB-LPETG-H_6 (right).



Size exclusion chromatogram of purified CalB-LPETG-H_6.



ESI-TOF mass spectrometry of purified CalB-LPETG-H_6. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 34824.3 Da.

2.6 Oligo and protein sequences

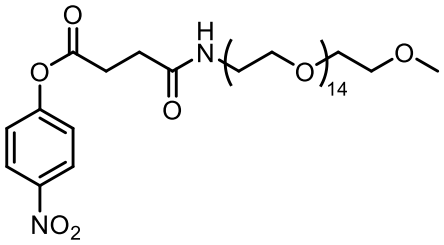
Table S1 - DNA sequence of the oligos used for the construction of expression vectors in this study.

Name	Sequence
CalB-LPETG Forward	5′-ATATATCCATGGGACTACCTTCCG-3′
CalB-LPETG Reverse	5′-ATATATCTCGAGGCCGCCGGTTTCCGGCAGGGGGGTGACGATGCC-3′

Table S2 - Amino acid sequences of the proteins used in this study.

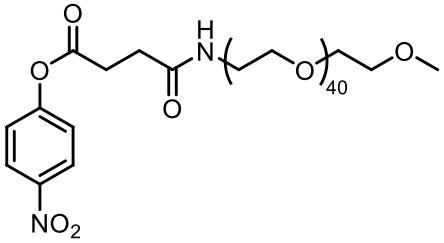
Name	Sequence
Sortase A	TGSHHHHHHGSKPHIDNYLHDKDKDEKIEQYDKNVKEQASKDKKQQAKPQIPKDKSKVAGYIEIPDADIKEPVYP GPATPEQLNRGVSFAEENESLDDQNISIAGHTFIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRDVKPT DVGVLDEQKGKDKQLTLITCDDYNEKTGVWEKRKIFVATEVK
wild type CCMV	MSTVGTGKLTRAQRRAAARKNKRNTRVVQPVIVEPIASGQGKAIKAWTGYSVSKWTASCAAAEAKVTSAITISLP NELSSERNKQLKVGRVLLWLGLLPSVSGTVKSCVTETQTTAAASFQVALAVADNSKDVVAAMYPEAFKGITLEQL TADLTIYLYSSAALTEGDVIVHLEVEHVRPTFDDSFTPVY
G-ELP-CCMV	GHHHHHHVPGVGVPGLGVPGVGVPGLGVPGVGVPGLGVPGGGVPGVGVPGLGLEVVQPVIVEPIASGQGKAIKAW TGYSVSKWTASCAAAEAKVTSAITISLPNELSSERNKQLKVGRVLLWLGLLPSVSGTVKSCVTETQTTAAASFQV ALAVADNSKDVVAAMYPEAFKGITLEQLTADLTIYLYSSAALTEGDVIVHLEVEHVRPTFDDSFTPVY
wild type CalB	MGLPSGSDPAFSQPKSVLDAGLTCQGASPSSVSKPILLVPGTGTTGPQSFDSNWIPLSAQLGYTPCWISPPPFML NDTQVNTEYMVNAITTLYAGSGNNKLPVLTWSQGGLVAQWGLTFFPSIRSKVDRLMAFAPDYKGTVLAGPLDALA VSAPSVWQQTTGSALTTALRNAGGLTQIVPTTNLYSATDEIVQPQVSNSPLDSSYLFNGKNVQAQAVCGPLFVID HAGSLTSQFSYVVGRSALRSTTGQARSADYGITDCNPLPANDLTPEQKVAAAALLAPAAAAIVAGPKQNCEPDLM PYARPFAVGKRTCSGIVTPLEHHHHHH
CalB-LPETG	MGLPSGSDPAFSQPKSVLDAGLTCQGASPSSVSKPILLVPGTGTTGPQSFDSNWIPLSAQLGYTPCWISPPPFML NDTQVNTEYMVNAITTLYAGSGNNKLPVLTWSQGGLVAQWGLTFFPSIRSKVDRLMAFAPDYKGTVLAGPLDALA VSAPSVWQQTTGSALTTALRNAGGLTQIVPTTNLYSATDEIVQPQVSNSPLDSSYLFNGKNVQAQAVCGPLFVID HAGSLTSQFSYVVGRSALRSTTGQARSADYGITDCNPLPANDLTPEQKVAAAALLAPAAAAIVAGPKQNCEPDLM PYARPFAVGKRTCSGIVTPLPETGGLEHHHHHH

2.7 Synthesis of substrate 2



p-Nitrophenol (54 mg, 0.39 mmol, 1.5 eq.), MeO-PEG-NHCO-C_2H_4-COOH MW 802 (203 mg, 0.25 mmol, 1.0 eq.) and EDC·HCl (77 mg, 0.40 mmol, 1.6 eq.) were dissolved in anhydrous CH_2Cl_2 (20 mL) under Ar and stirred overnight at room temperature. An aqueous 2M HCl solution (50 mL) and CH_2Cl_2 (30 mL) were added, the layers were separated, and the aqueous phase was extracted again with CH_2Cl_2 (2x 50 mL). The combined organic layers were washed with aqueous 0.5 M K_2CO_3 (3 x 50 mL) and brine (1 x 50 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* yielding ester **2** as a yellow solid (229 mg, 0.25 mmol, quant.). R_f = 0.49 (MeOH/CH_2Cl_2 1:9, *v/v*). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.33 – 8.25 (m, 2H), 8.01 (t, *J* = 5.5 Hz, 1H), 7.42 – 7.36 (m, 2H), 3.49 (m, 66H)*, 3.43 – 3.36 (m, 4H), 3.22 (s, 3H), 3.21 – 3.16 (m, 2H), 2.78 (t, *J* = 6.7 Hz, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.8, 170.6, 155.5, 144.9, 137.4, 136.4, 125.3, 123.1, 71.3, 69.8 (b) (multiple PEG carbons), 69.7, 69.6, 69.1, 58.0, 54.9, 29.7, 29.3 ppm.

2.8 Synthesis of substrate 3



p-Nitrophenol (24 mg, 0.17 mmol, 1.6 eq.), MeO-PEG-NHCO-C_2H_4-COOH MW 1931 (212 mg, 0.11 mmol, 1.0 eq.) and EDC·HCl (30 mg, 0.16 mmol, 1.4 eq.) were dissolved in anhydrous CH_2Cl_2 (20 mL) under Ar and stirred overnight at room temperature. An aqueous 2M HCl solution (50 mL) and CH_2Cl_2 (30 mL) were added, the layers were separated, and the aqueous phase was extracted again with CH_2Cl_2 (2x 50 mL). The combined organic layers were washed with aqueous 0.5 M K_2CO_3 (3 x 50 mL) and brine (1 x 50 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* yielding ester **3** as a yellow solid (221 mg, 0.11 mmol, quant.). R_f = 0.47 (MeOH/CH_2Cl_2 1:9, *v/v*). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.35 – 8.28 (m, 2H), 8.03 (t, *J* = 5.5 Hz, 1H), 7.45 – 7.37 (m, 2H), 3.51 (m, 200H)*, 3.44 – 3.38 (m, 5H), 3.24 (s, 3H), 3.23 – 3.18 (m, 2H), 2.80 (t, *J* = 6.7 Hz, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.8, 170.6, 155.5, 144.9, 125.3, 123.1, 71.3, 69.8 (b) (multiple PEG carbons), 69.7, 69.6, 69.1, 58.1, 54.9, 29.7, 29.3 ppm.

* The peak belonging to the PEG protons did not match with the mass that was given by the supplier. During further experiments, the mass given by the supplier was used in the calculations.

2.9 Analysis of capsid stability in the presence of organic solvents

DLS analysis: Two solutions of G-ELP-CCMV in buffer C (1 mg/mL, 100 µL) were prepared. To one solution, DMSO (1 µL) was added, to the other solution, buffer (1 µL) was added. The solutions were stored at room temperature in a cuvette. After 0, 1, 2, 3, and 4 hours, the solutions were analyzed by DLS.

UV-vis analysis: Two solutions of G-ELP-CCMV in buffer C (1 mg/mL, 300 µL) were prepared. To one solution, DMSO (3 µL) was added, to the other solution buffer (3 µL) was added. The solutions were shaken at 21 ^{o}C. After 0, 1, 2, 3, and 4 hours, a 50 µL aliquot of each solution was removed, centrifuged to precipitate any aggregated material (13.000 rpm, 1 min, 4 ^{o}C) and analyzed by UV-vis spectroscopy.

2.10 SrtA-mediated coupling experiments

For a typical SrtA-mediated coupling experiment, stock solutions of SrtA, G-ELP-CCMV and CalB-LPETG were prepared in Sortase buffer. If a component had been dissolved in another buffer, it was spin filtrated to Sortase buffer (10 kDa MWCO, 3 x 10 min). The components were added together to final concentrations of 0-100 µM SrtA, 50 µM G-ELP-CCMV and 50 µM CalB-LPETG. The solutions were shaken at 21 ^{o}C for 24 hours. The reaction progress was followed by SDS-PAGE analysis.

2.11 SrtA-mediated coupling experiments, followed by capsid purification

For a typical SrtA-mediated coupling experiment, stock solutions of SrtA, G-ELP-CCMV and CalB-LPETG were prepared in Sortase buffer. If a component had been dissolved in another buffer, it was spin filtrated to Sortase buffer (10 kDa MWCO, 3 x 10 min). The components were added together to final concentrations of 0/100 µM SrtA, 50 µM G-ELP-CCMV and 0/50 µM CalB-LPETG. The solutions were shaken at 21 ^{o}C for 3 hours. The capsids were assembled by spin filtration to either pH-induced assembly buffer (buffer B) or salt-induced assembly buffer (buffer C) (10 kDa MWCO, 3 x 10 min). Then the capsids were isolated using preparative SEC. The capsid fractions were analyzed and used for further experiments.

2.12 Activity assay of non-encapsulated CalB

The lipase activity of wild type CalB and CalB-LPETG was analyzed by the hydrolysis of p-nitrophenol acetate (p-NPA). CalB (111.0 nM, 45.0 µL) in the desired buffer was added to p-NPA (10 mM in DMSO, 5.0 µL) in triplo. The background hydrolysis was measured by the addition of the desired buffer without CalB to the substrate. The production of p-nitrophenol was monitored for 5 minutes at 25 °C by measuring the absorbance at 405 nm on a Tecan infinite M200 Pro microplate reader. The slopes of the curves were taken as a measure of the hydrolytic activity. Calibration curves of the absorbance of p-nitrophenol in the different buffers were measured, in order to convert the measured relative activity to U/mg.

Buffers

Buffer A	50 mM NaH_2PO_4, 150 mM NaCl, pH 7.0
Buffer A + 350 mM NaCl	50 mM NaH_2PO_4, 500 mM NaCl, pH 7.0
Buffer A + 1.85 M NaCl	50 mM NaH_2PO_4, 2000 mM NaCl, pH 7.0
Buffer A + 10 mM $MgCl_2$	50 mM NaH_2PO_4, 150 mM NaCl, 10 mM $MgCl_2$, pH 7.0
Buffer A + 1 mM EDTA	50 mM NaH_2PO_4, 150 mM NaCl, 1 mM EDTA, pH 7.0
Buffer B	50 mM NaOAc, 500 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 5.0
Buffer C	50 mM Tris.HCl, 2000 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.5

Calculation enzyme activity in U/mg

The enzyme activity was calculated and expressed in U/mg. The definition of 1U is:

$$1U = \text{conversion of 1 µmol substrate per minute}$$

The measured slope of background hydrolysis of p-NPA to p-nitrophenol was subtracted from the CalB-catalyzed conversion and multiplied by a factor of 60, to convert the units into minutes instead of seconds:

$$\Delta Abs = (\Delta Abs_{CalB} - \Delta Abs_{BG}) * 60$$

Next, the calibration curves were used to convert this absorbance difference into an amount of converted p-nitrophenol per minute, which is equal to U:

$$\Delta substrate\ (\mu M) = \frac{\Delta Abs}{\text{slope calibration curve}} = U_{total}$$

Finally, U_{total} was divided by the amount of CalB present in the reaction mixture to obtain the enzyme activity in U/mg:

$$Actvity\ (U/mg) = \frac{U_{total}}{\text{amount of CalB (mg)}}$$

2.13 Activity assay of encapsulated CalB

A capsid fraction of CalB-ELP-CCMV capsids (49.5 µL) was added to p-NPA, substrate **2** or substrate **3** (100 mM in DMSO, 0.5 µL) in duplo. The background hydrolysis was measured by the addition of salt-induced assembly buffer (buffer C) to the substrate. The production of p-nitrophenol was monitored for 5 minutes at 25 ^{o}C by measuring the absorbance at 405 nm on a Tecan infinite M200 Pro microplate reader. The slopes of the curves were taken as a measure of the hydrolytic activity.

2.14 Protease degradation assay

Protease Glu-C (1 µg/µL in MQ, 1.0 µL) and trypsin (1 µg/µL in 50 mM acetic acid, 1.0 µL) were added to a capsid fraction (2 x 120 µL). As a control, the proteases were added to salt-induced assembly buffer (buffer C) (2 x 120 µL) or non-encapsulated CalB (same concentration as the encapsulated CalB, 2 x 120 µL). The solutions were shaken at 21 ^{o}C for 5 hours. After 0 and 5 hours, samples (49.5 µL) were removed and added to p-NPA (100 mM in DMSO, 0.5 µL). The production of p-nitrophenol was monitored for 5 minutes at 25 ^{o}C by measuring the absorbance at 405 nm on a Tecan infinite M200 Pro microplate reader. The slopes of the curves were taken as a measure of the hydrolytic activity.

3 Supplemental figures

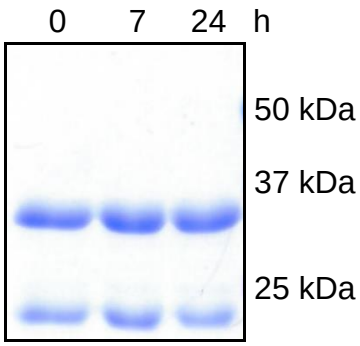
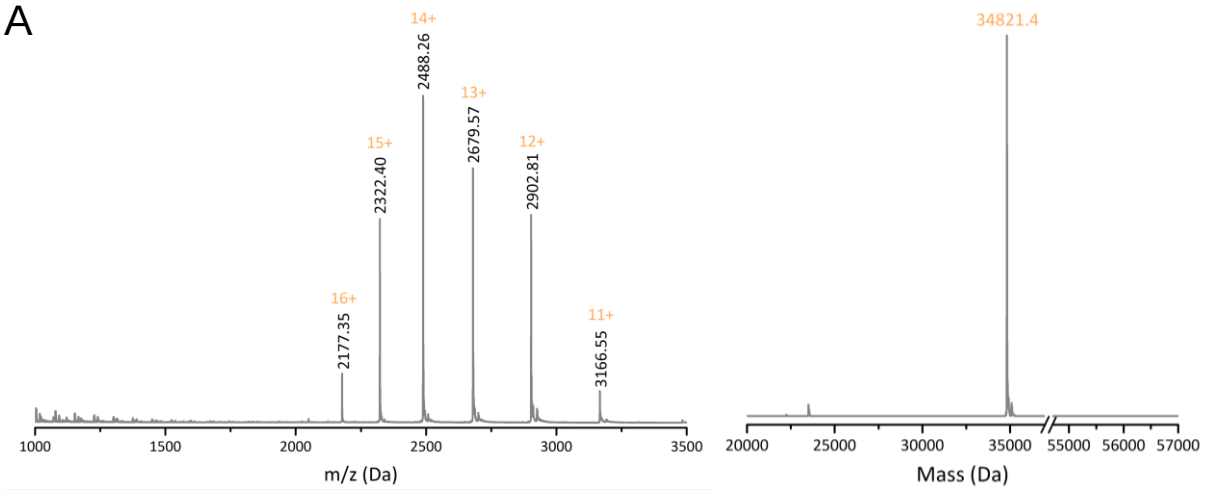


Figure S1. SDS-PAGE analysis of the coupling of CalB-LPETG to G-ELP-CCMV without the presence of Sortase. The gel was stained by Coomassie blue staining.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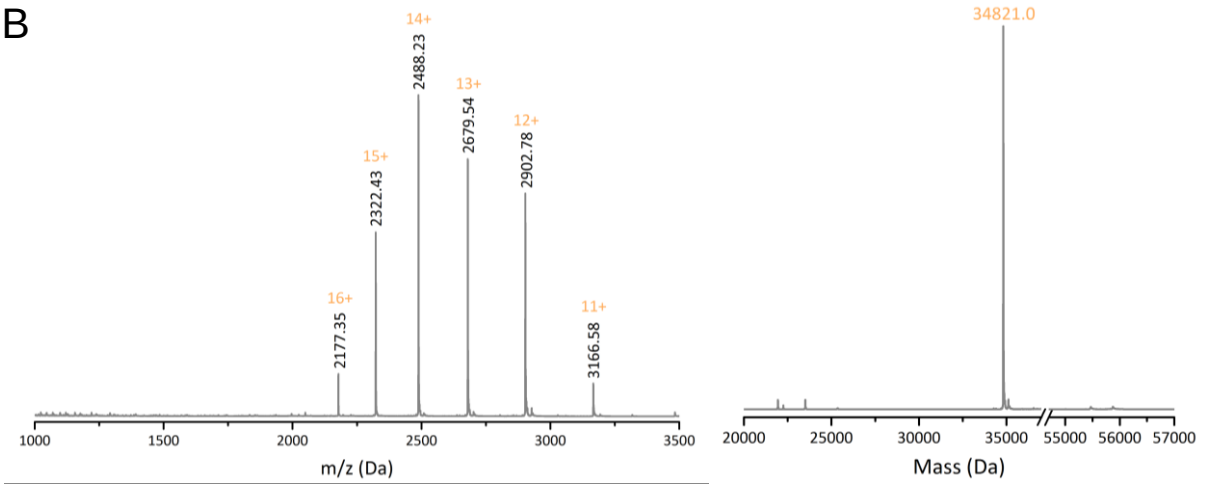
Mass (Da)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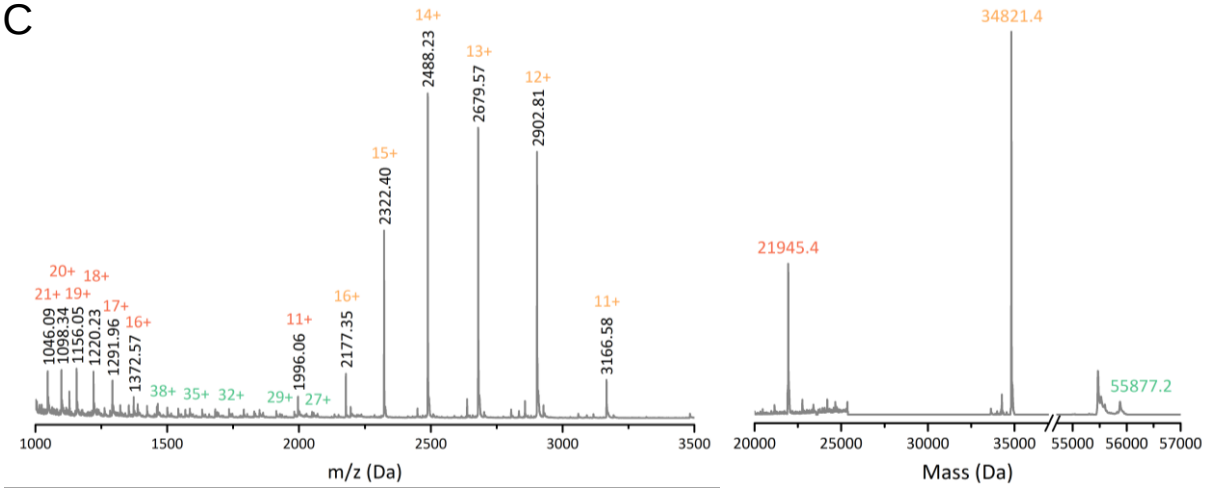
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Mass (Da)

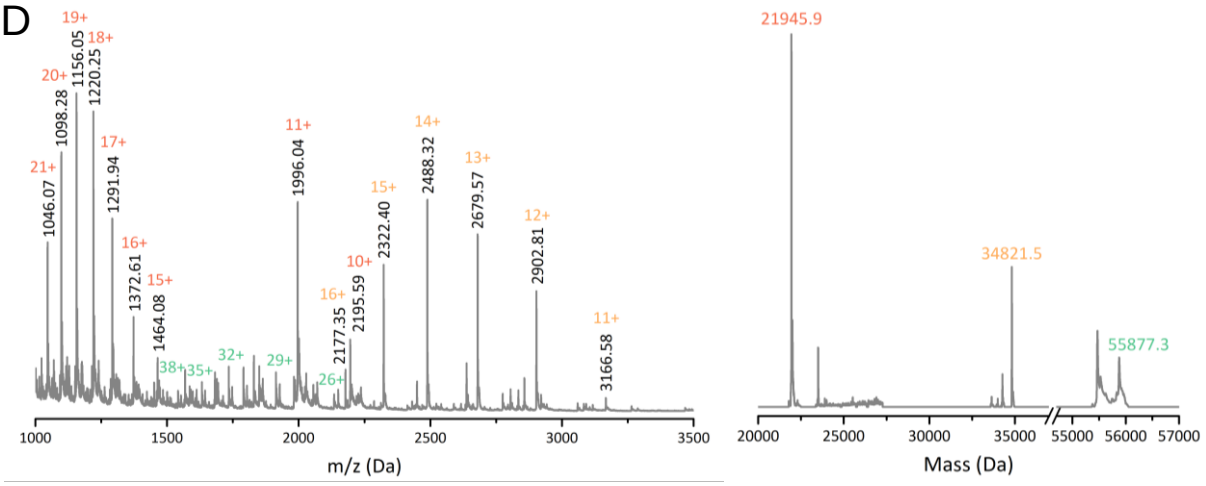

Figure S2. ESI-TOF mass spectrometry of SrtA-catalyzed modifications of G-ELP-CCMV with CalB-LPETG. Multiply charged ion series (left) and deconvoluted total mass spectra (right). The expected molecular weights are 21947.5 Da (SrtA, red), 22253.4 Da (G-ELP-CCMV, blue), 34824.3 (CalB-LPETG, orange) and 55880.5 (CalB-LPETG-ELP-CCMV, green). A: 0 eq. SrtA. B: 0.2 eq. SrtA. C: 1.0 eq. SrtA. D: 2.0 eq. SrtA.

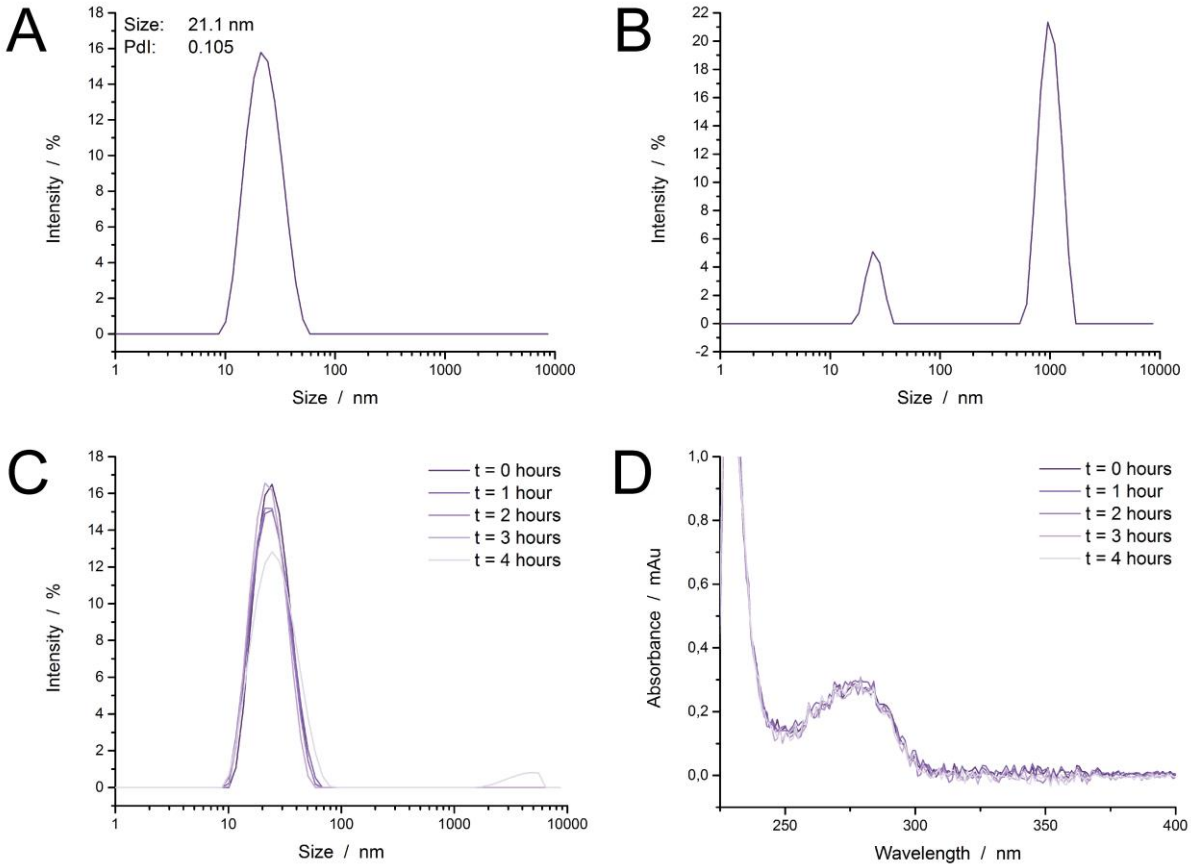

Figure S3. Analysis of ELP-CCMV T=1 capsid stability in salt-induced assembly buffer (buffer C) in the presence of DMSO. (A) DLS measurement of ELP-CCMV T=1 capsids in buffer C. (B) DLS measurement of ELP-CCMV T=1 capsids in buffer C with 10% DMSO after 15 minutes. (C) DLS measurement of ELP-CCMV T=1 capsids in buffer C with 1% DMSO. The capsid stability was followed for a period of 4 hours. (D) UV-vis absorbance of ELP-CCMV T=1 capsids in salt-induced assembly buffer with 1% DMSO.

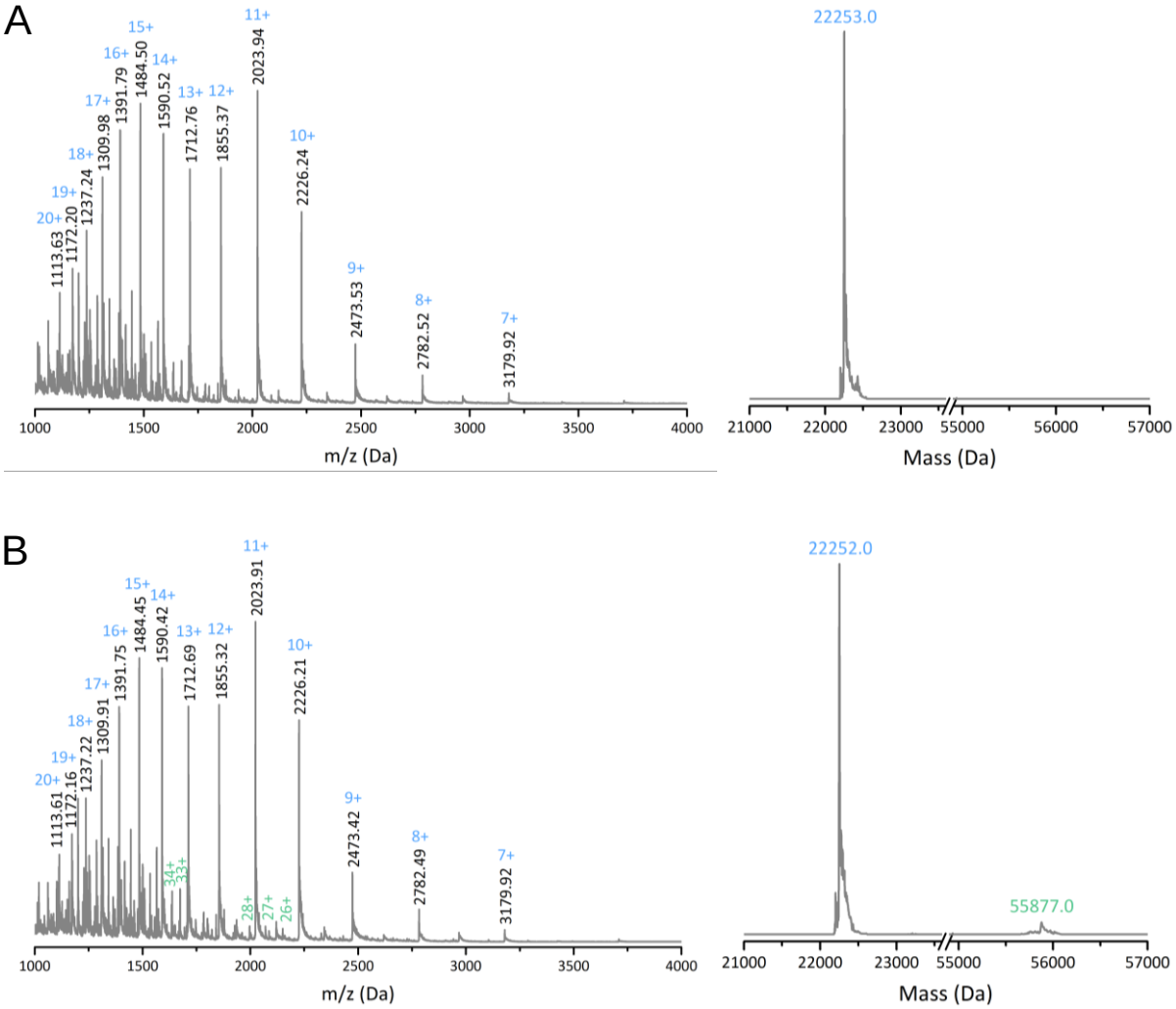

Figure S4. ESI-TOF mass spectrometry of SrtA-catalyzed modifications of G-ELP-CCMV with CalB-LPETG after capsid purification. Multiply charged ion series (left) and deconvoluted total mass spectra (right). The expected molecular weights are 22253.4 Da (G-ELP-CCMV, blue) and 55880.5 (CalB-LPETG-ELP-CCMV, green). A: 0 eq. SrtA. B: 2.0 eq. SrtA.

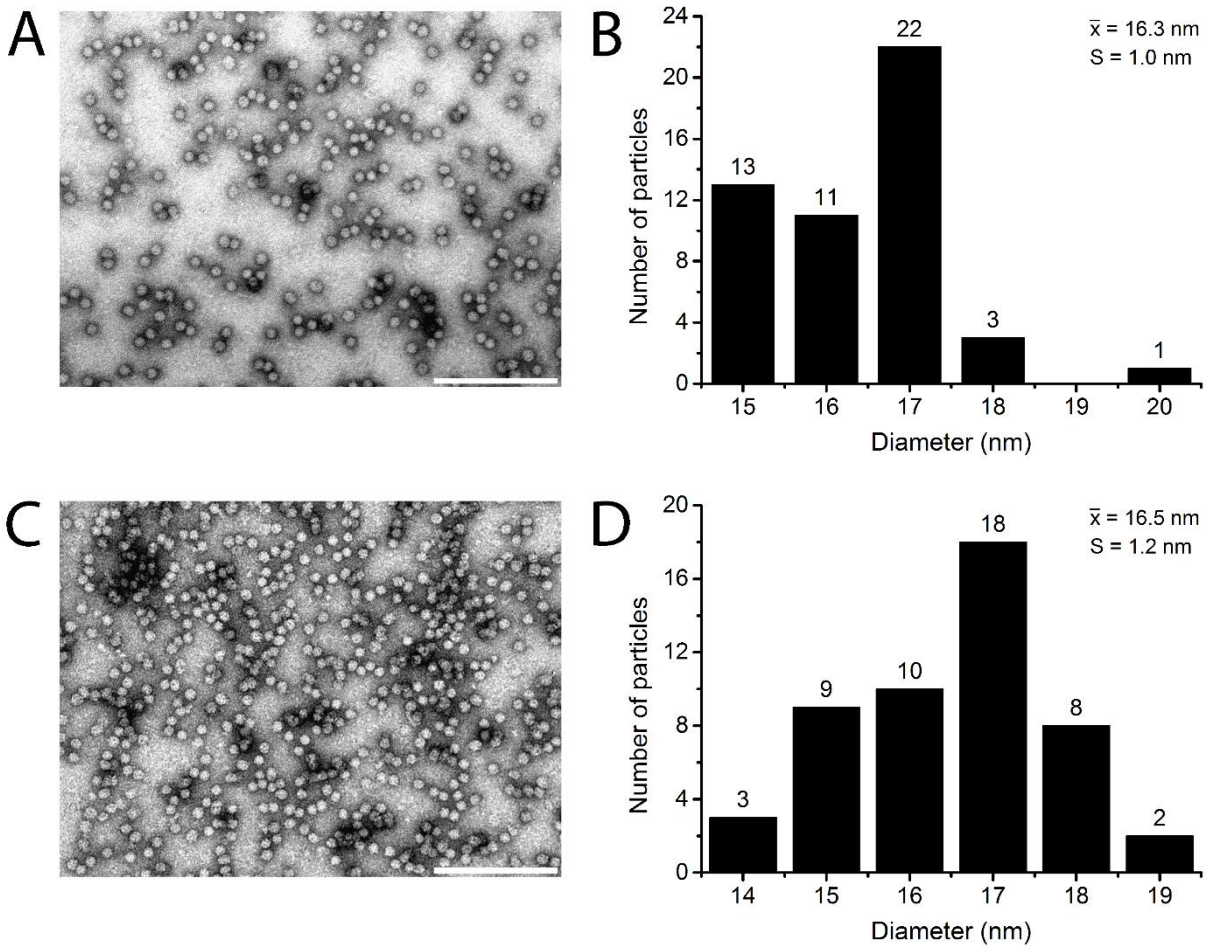

Figure S5. A) Uranyl acetate-stained TEM micrograph of G-ELP-CCMV after reaction with CalB-LPETG (0 eq. SrtA). Scale bar corresponds to 200 nm. B) Size distribution of CCMV particles shown in A. C) Uranyl acetate-stained TEM micrograph of G-ELP-CCMV after reaction with CalB-LPETG (2.0 eq. SrtA). Scale bar corresponds to 200 nm. D) Size distribution of the CalB-modified ELP-CCMV particles shown in C.

4 References

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