Supporting Information

Stabilization of a virus-like particle and its application as a nanoreactor at physiological conditions

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1 Experimental section

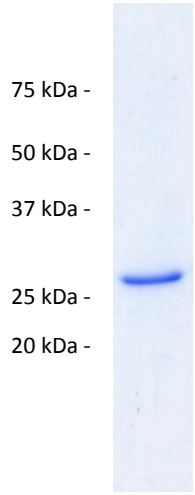
1.1 Buffers

Name	Composition
pH 7.5 dimer buffer	50 mM Tris·HCl, 500 mM NaCl, 10 mM $MgCl_2$, pH 7.5
pH 5.0 capsid buffer	50 mM NaOAc, 500 mM NaCl, 10 mM $MgCl_2$, pH 5.0
pH 7.5 capsid buffer	50 mM Tris·HCl, 2000 mM NaCl, 10 mM $MgCl_2$, pH 7.5
Sortase buffer	50 mM HEPES, 150 mM NaCl, 5 mM $CaCl_2$, pH 7.5
reaction buffer	10 mM Tris·HCl, 150 mM NaCl, 10 mM $MgCl_2$, pH 7.5

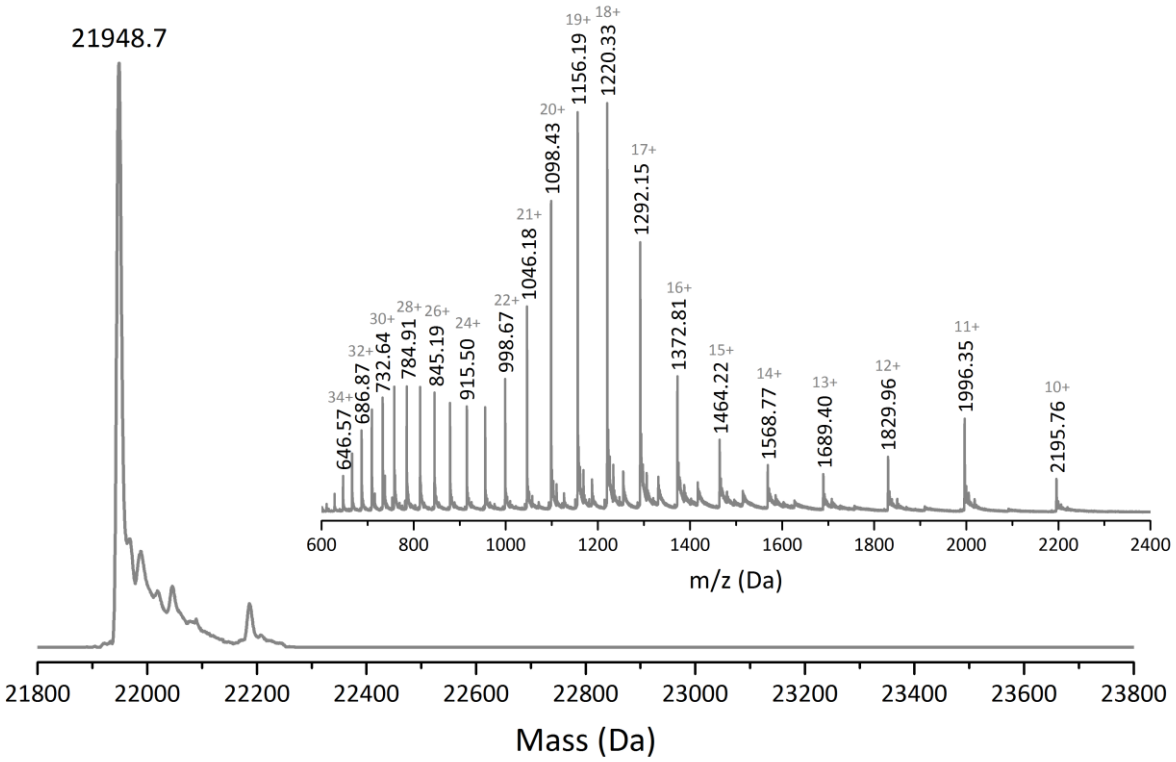
All buffers were filtered over a 0.2 micron filter prior to use.

1.2 Expression of Sortase A

The expression was performed according to a literature procedure.[1] The pQE30-H_6-SrtA vector encoding for the hexahistidine-tagged SrtA protein was previously constructed by the group of Geerten W. Vuister (Department of Biochemistry, University of Leicester) and kindly donated to our group. *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. 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 and 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 10-13 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.

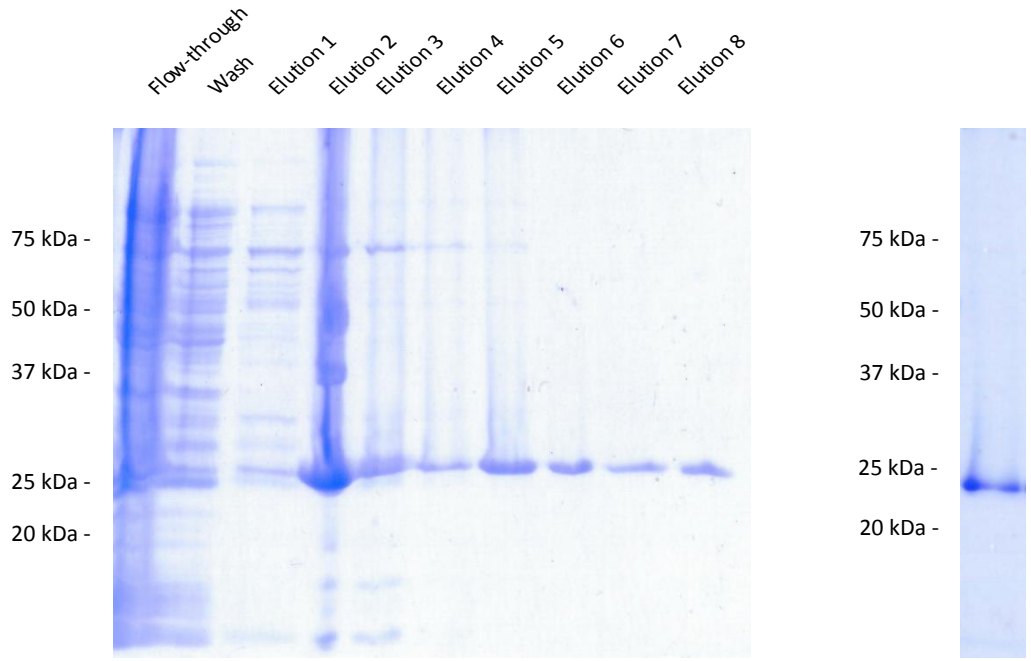


ESI-TOF mass spectrometry of purified Sortase A. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 21947.5 Da.

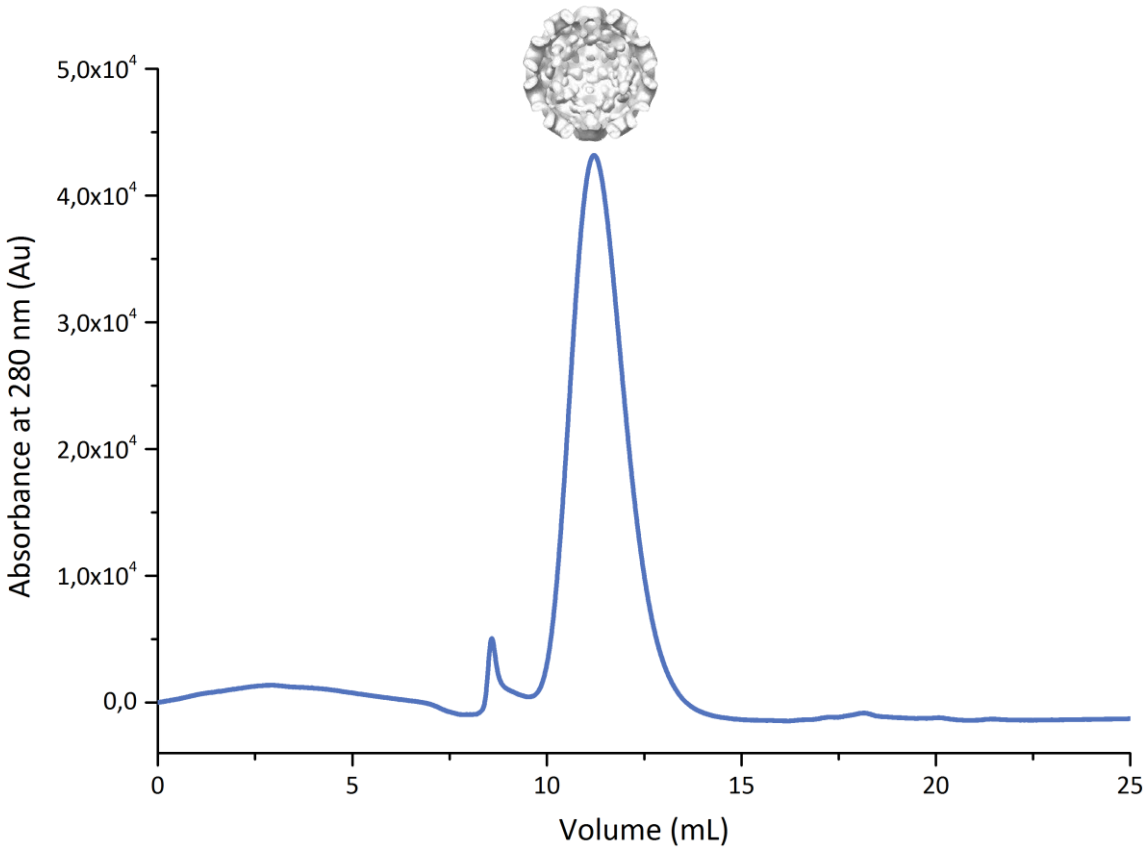
1.3 Expression of G-ELP-CCMV

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.*[2] The expression was performed according to a literature procedure.[1] 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 the pET-15b vector encoding for the desired CCMV capsid protein, and was incubated overnight at 37 °C. This overnight culture was used to inoculate 2x TY medium (1 L), supplemented with ampicillin (100 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.

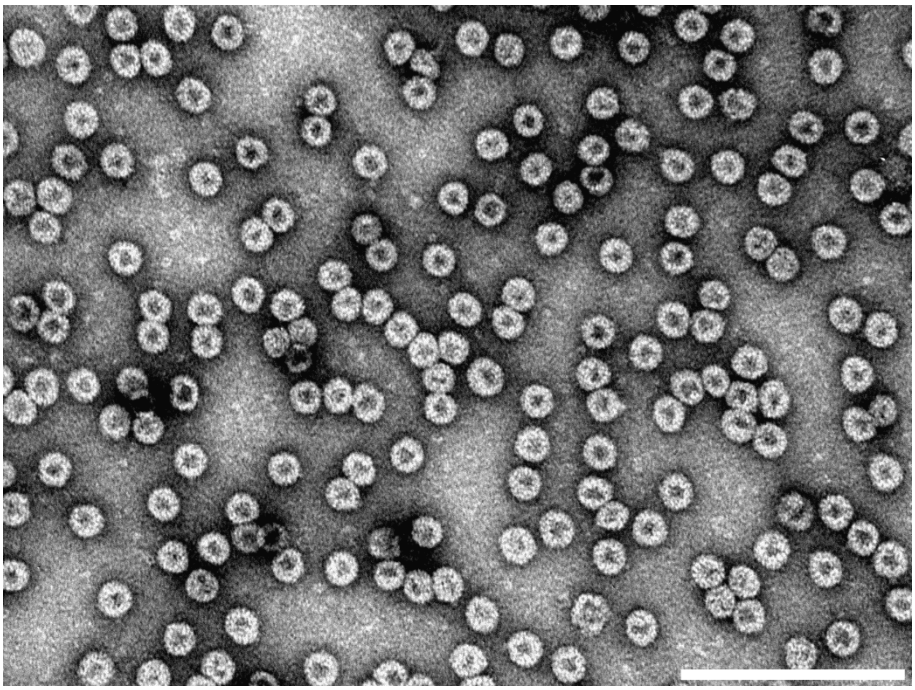
After thawing, the cell pellet was resuspended in lysis buffer (50 mM NaH_2PO_4, 1.3 M NaCl, 10 mM imidazole, pH 8.0; 25 mL). The cells were lysed by ultrasonic disruption (3 times 30 s, 100% duty cycle, output control 3, Branson Sonifier 250, Marius Instruments). Then the lysate was centrifuged (16.400 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, 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 the desired protein were combined and dialyzed against pH 7.5 dimer buffer to obtain the capsid protein dimers. For storage, the proteins were assembled by dialysis against pH 5.0 capsid buffer. The pure protein was obtained with a yield of 100 mg/L of culture. The purity of the proteins was verified by SDS-PAGE. The assembly properties of the capsid proteins and the geometry of the resulting capsids were analyzed by SEC using a Superose 6 GL 10/300 column with pH 5.0 capsid buffer as the eluent and TEM. ESI-TOF: calculated 22253.4 Da, found 22253.5 Da.



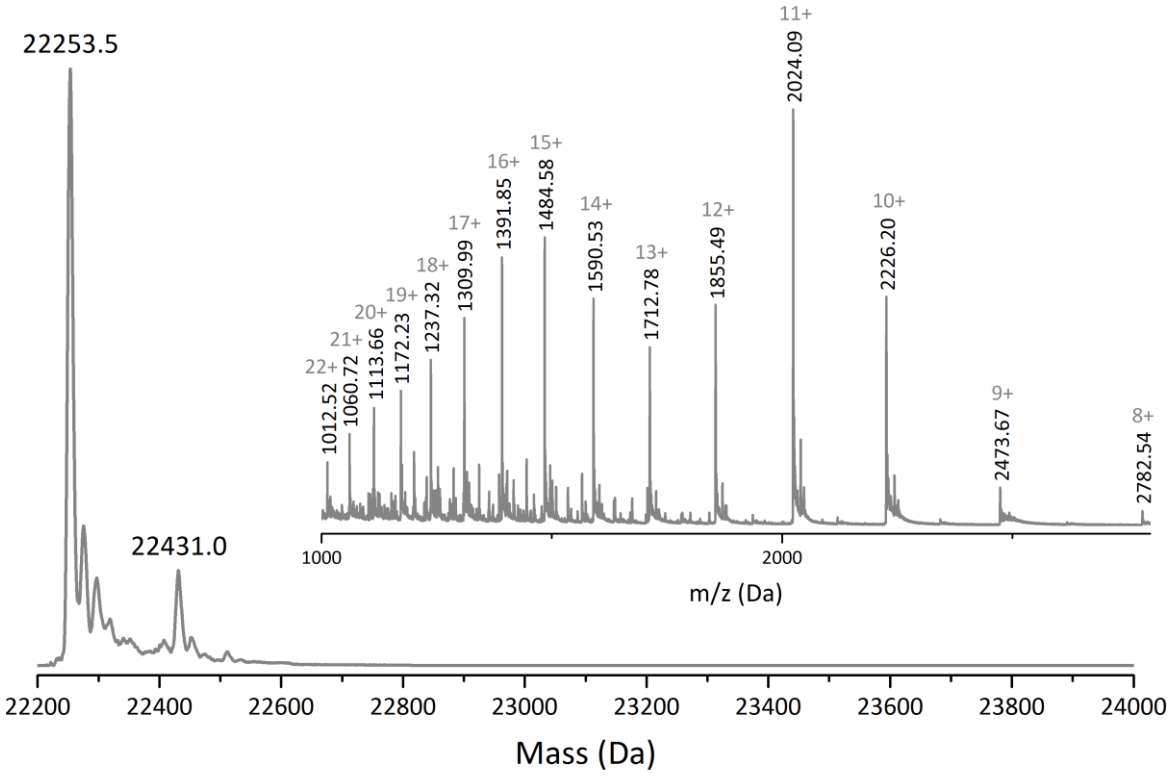
SDS-PAGE analysis of affinity purification of His_6-ELP-CCMV (left) and purified His_6-ELP-CCMV (right).



Size exclusion chromatogram of purified His_6-ELP-CCMV in pH 5.0 capsid buffer.



Uranyl acetate stained TEM micrograph of His_6-ELP-CCMV. Average particle size = 29.2 ± 1.5 nm. Scale bar corresponds to 200 nm.



ESI-TOF mass spectrometry of purified His_6-ELP-CCMV. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 22253.4 Da.

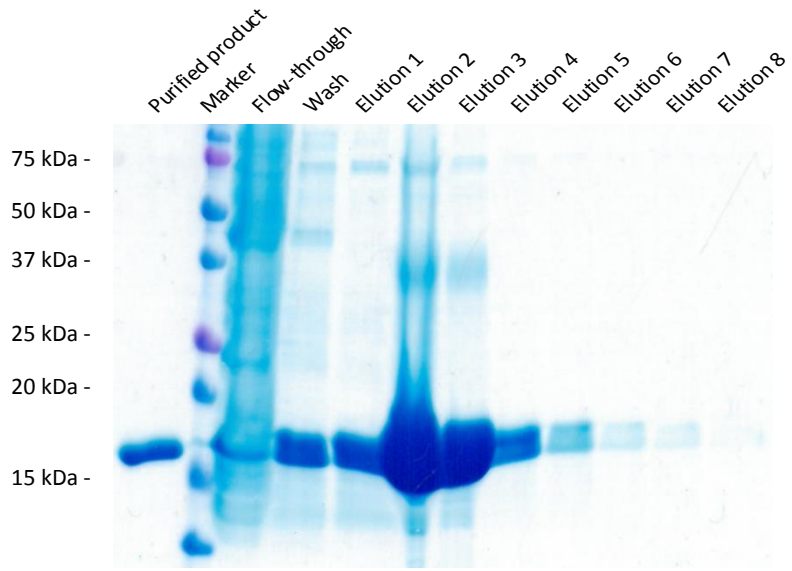
1.4 Cloning of T4L-H_6 and T4L-LPETG-H_6

T4 Lysozyme WT* was a gift from Brian Matthews (Addgene plasmid # 18111). For the introduction of a hexahistidine tag and an additional LPETG sequence, a set of DNA oligos was designed (Table S1). The oligos were annealed and the resulting insert encoded for the T4L protein, an LPETGG motif in case of T4L-LPETG and a hexahistidine tag with a 5' NcoI and a 3' BamHI restriction site. The products after PCR were purified by agarose gel electrophoresis. Both the purified inserts and a pET-15b vector were digested with NcoI-HF® and BamHI-HF® and the products were again purified by agarose gel electrophoresis. Subsequently, the inserts were ligated into the digested vector to yield pET-15b-T4L-H_6 and pET-15b-T4L-LPETG-H_6. The plasmids were transformed into *E. coli* XL1-BLUE cells, the DNA was extracted and the sequences were confirmed by DNA sequencing (Table S2). For expression, the plasmids were transformed into *E. coli* BLR(DE3)pLysS cells (Novagen, MERCK).

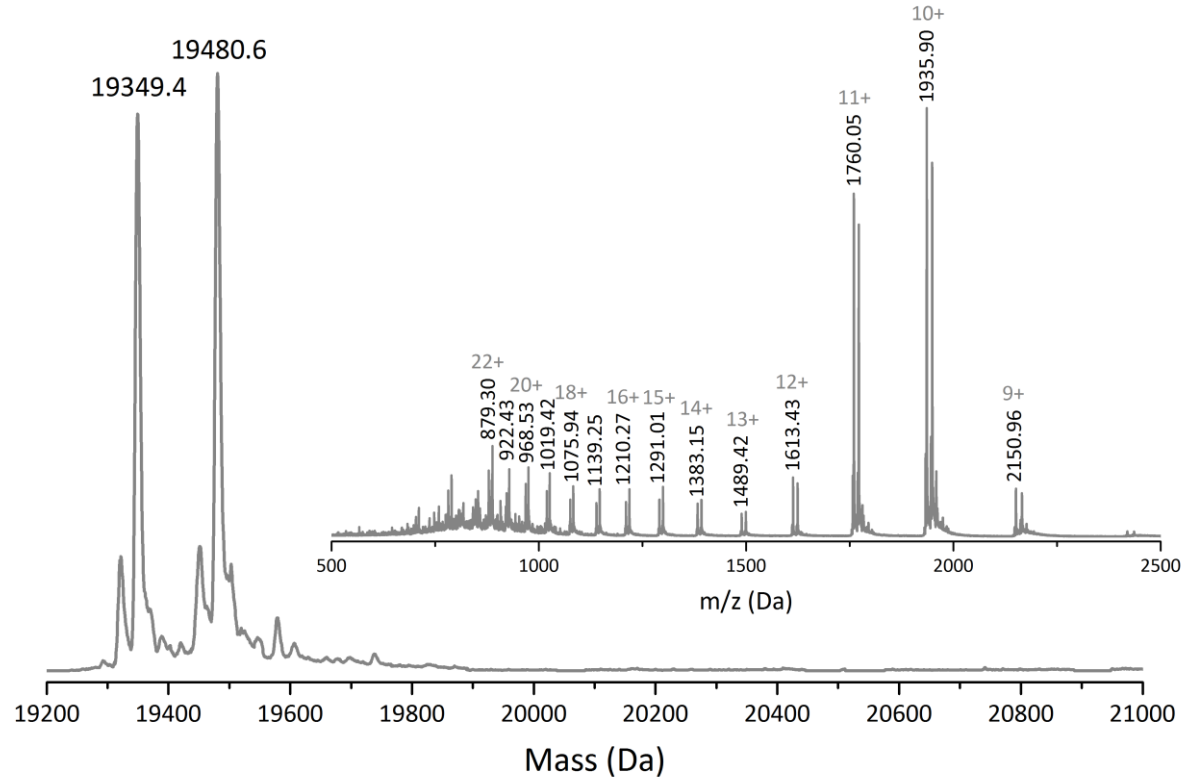
1.5 General protocol for the expression of T4 lysozyme

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 the pET-15b vector encoding for the desired T4 lysozyme protein, and was incubated overnight at 37 °C. This overnight culture was used to inoculate 2x TY 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.

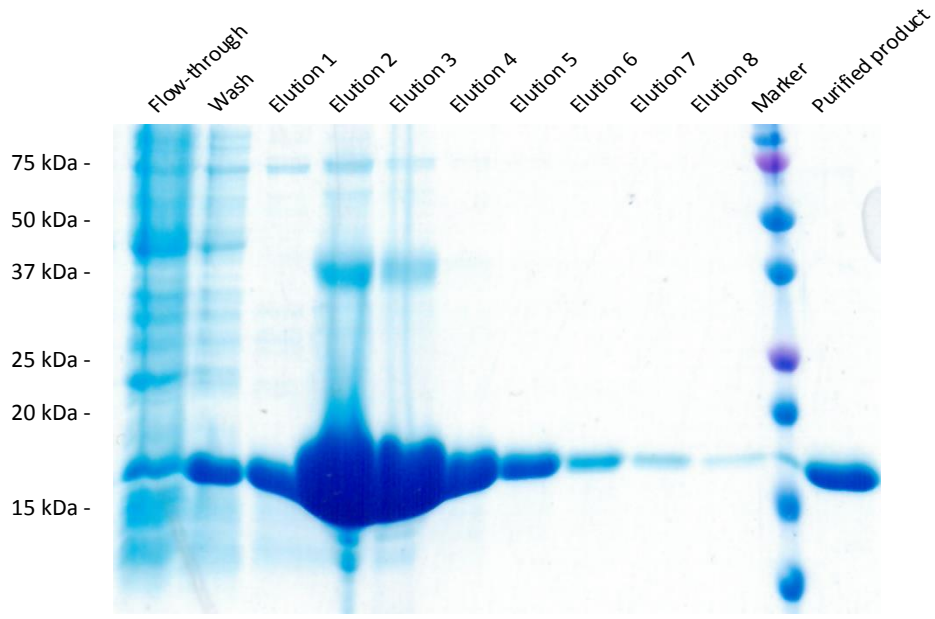
After thawing, the cell pellet was resuspended in lysis buffer (50 mM NaH_2PO_4, 1.5 M NaCl, 10 mM imidazole, pH 8.0; 25 mL). The cells were lysed by ultrasonic disruption (3 times 30 s, 100% duty cycle, output control 3, Branson Sonifier 250, Marius Instruments). Then the lysate was centrifuged (16.400 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, 1.5 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, 1.5 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. The fractions containing the desired protein were combined and dialyzed against PBS buffer to obtain the pure protein with a yield of 50 mg/L for T4L-H_6 and 60 mg/L for T4L-LPETG-H_6. The purity of the proteins was verified by SDS-PAGE. ESI-TOF T4L-H_6: calculated 19350.0 Da, found 19349.4 Da. ESI-TOF T4L-LPETG-H_6: calculated 19904.6 Da, found 19903.8 Da. For both proteins, also the mass of the desired protein with an additional methionine was found, indicating incomplete processing of the N-terminal methionine.



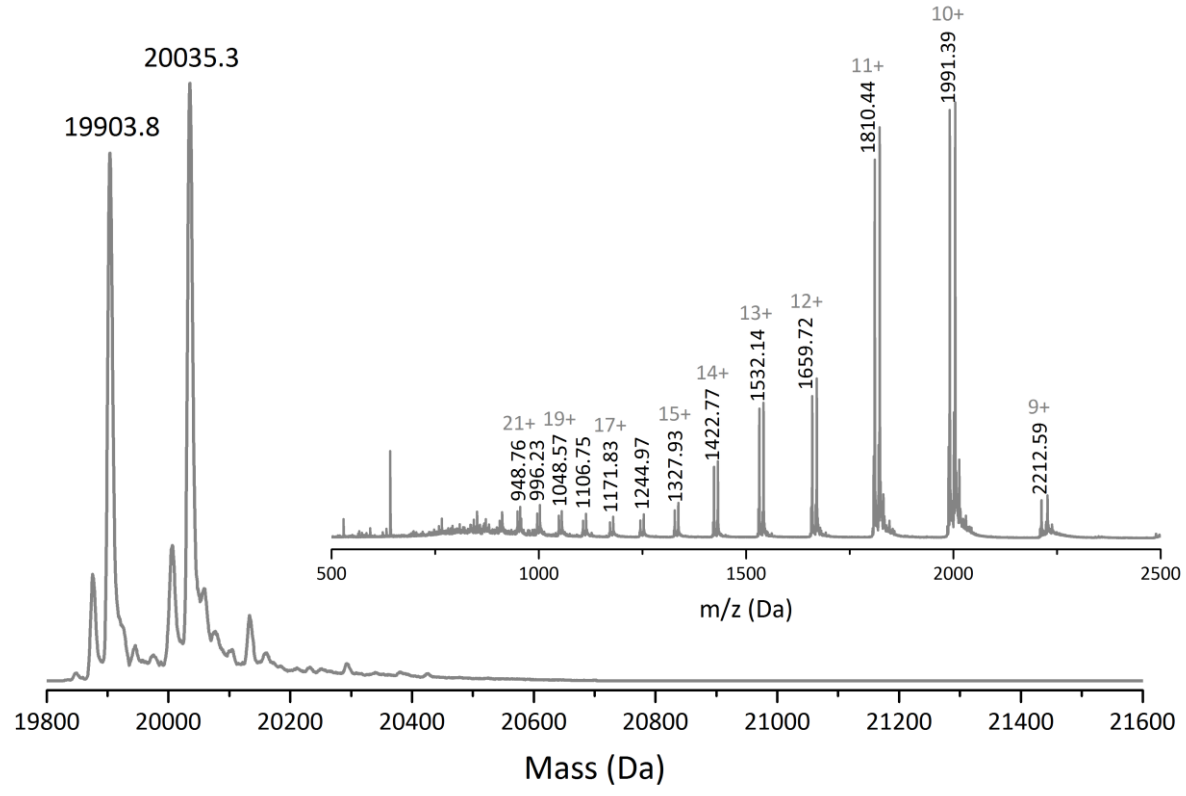
SDS-PAGE analysis of affinity purification of T4L-H_6 and purified T4L-H_6.



ESI-TOF mass spectrometry of purified T4L-H_6. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 19350.0 Da. The calculated molecular weight of M-T4L-H_6 is 19481.2 Da.



SDS-PAGE analysis of affinity purification of T4L-LPETG-H_6 and purified T4L-LPETG-H_6.



ESI-TOF mass spectrometry of purified T4L-LPETG-H_6. Deconvoluted total mass spectrum and multiply charged ion series (inset). The expected molecular weight is 19904.6 Da. The calculated molecular weight of M-T4L-LPETG-H_6 is 20035.8 Da.

1.6 Oligo and protein sequences

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

Name	Sequence
T4L-H_6 Forward	5′-ATATATCCATGGGCAATATATTTGAAATG-3′
T4L-H_6 Reverse	5′-ATATATGGATCCTTAGTGATGATGATGATGATGTAGATTTTTATACGC-3′
T4L-LPETG-H_6 Forward	5′-ATATATCCATGGGCAATATATTTGAAATG-3′
T4L-LPETG-H_6 Reverse	5′-ATATATGGATCCTTAGTGATGATGATGATGATGGCCGCCGGTTTCCGGCAGTAGATTTTTATACGC-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 T4L	MNIFEMLRIDERLRLKIYKDTEGYYTIGIGHLLTKSPSLNAAKSELDKAIGRNCNGVITKDEAEKLFNQDVDAAV RGILRNAKLKPVYDSLDAVRRCALINMVFQMGETGVAGFTNSLRMLQQKRWDEAAVNLAKSIWYNQTPNRAKRVI TTFRTGTWDAYKNL
T4L-H_6	GNIFEMLRIDEGLRLKIYKDTEGYYTIGIGHLLTKSPSLNAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAV RGILRNAKLKPVYDSLDAVRRAALINMVFQMGETGVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVI TTFRTGTWDAYKNLHHHHHH
T4L-LPETG-H_6	GNIFEMLRIDEGLRLKIYKDTEGYYTIGIGHLLTKSPSLNAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAV RGILRNAKLKPVYDSLDAVRRAALINMVFQMGETGVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVI TTFRTGTWDAYKNLLPETGGHHHHHH

2 Supplemental figures

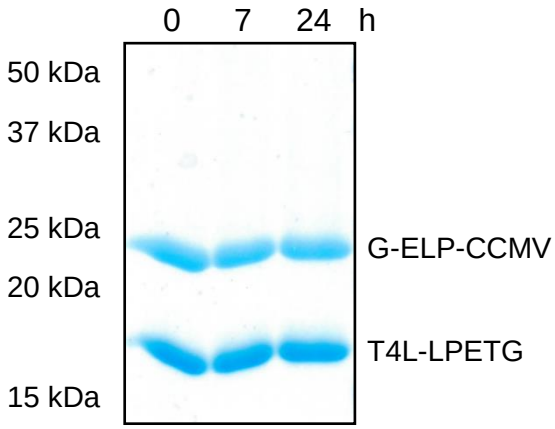
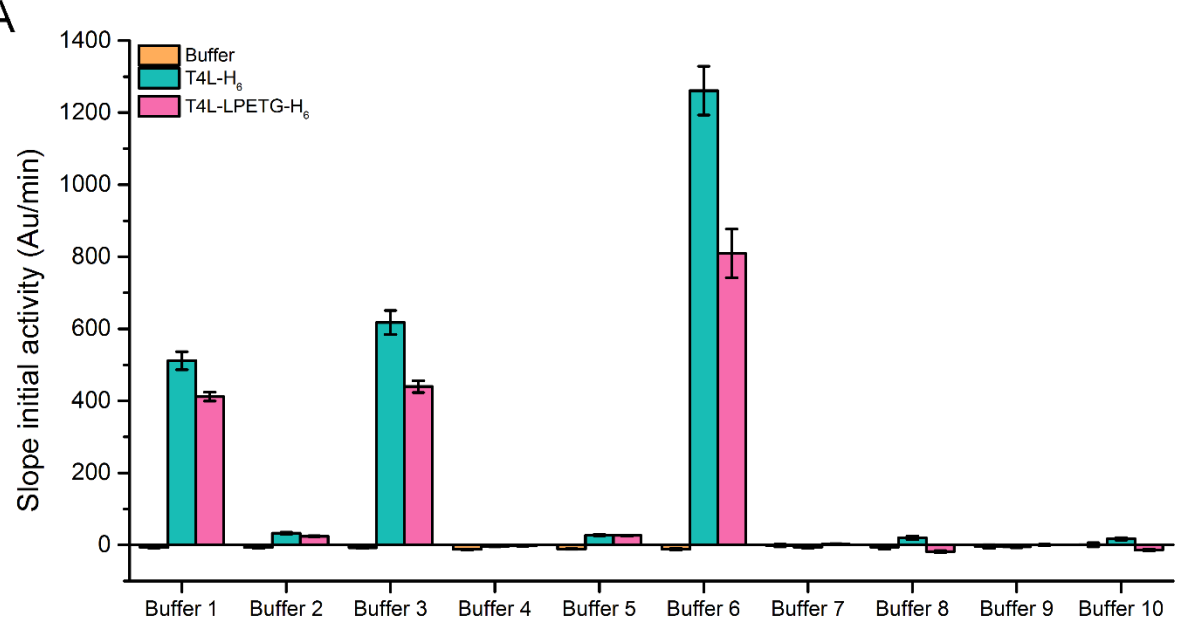


Figure S1. SDS-PAGE analysis of the coupling of T4L-LPETG to G-ELP-CCMV without the presence of Sortase. Protein bands were visualized by Coomassie blue staining.

A



B

	Buffer	Additives		pH
Buffer 1	100 mM Na_3PO_4	100 mM NaCl	2 mM NaN_3	7.5
Buffer 2	50 mM Tris·HCl	500 mM NaCl	10 mM $MgCl_2$	7.5
Buffer 3	50 mM Tris·HCl	150 mM NaCl	10 mM $MgCl_2$	7.5
Buffer 4	50 mM Tris·HCl	2000 mM NaCl	10 mM $MgCl_2$	7.5
Buffer 5	10 mM Tris·HCl	500 mM NaCl	10 mM $MgCl_2$	7.5
Buffer 6	10 mM Tris·HCl	150 mM NaCl	10 mM $MgCl_2$	7.5
Buffer 7	50 mM NaOAc	500 mM NaCl	10 mM $MgCl_2$	5.0
Buffer 8	50 mM NaOAc	150 mM NaCl	10 mM $MgCl_2$	5.0
Buffer 9	10 mM NaOAc	500 mM NaCl	10 mM $MgCl_2$	5.0
Buffer 10	10 mM NaOAc	150 mM NaCl	10 mM $MgCl_2$	5.0

Figure S2. (A) Catalytic activity of T4L-H_6 and T4L-LPETG-H_6 in different buffers. The activity was measured using the EnzChek® lysozyme assay. The initial slopes of the fluorescence intensity curves were taken as a measure of the enzymatic activity. (B) List of the components of each buffer used in the buffer screening shown in A. Buffer 2: pH 7.5 dimer buffer, buffer 4: pH 7.5 capsid buffer, buffer 6: reaction buffer, buffer 7: pH 5.0 capsid buffer.

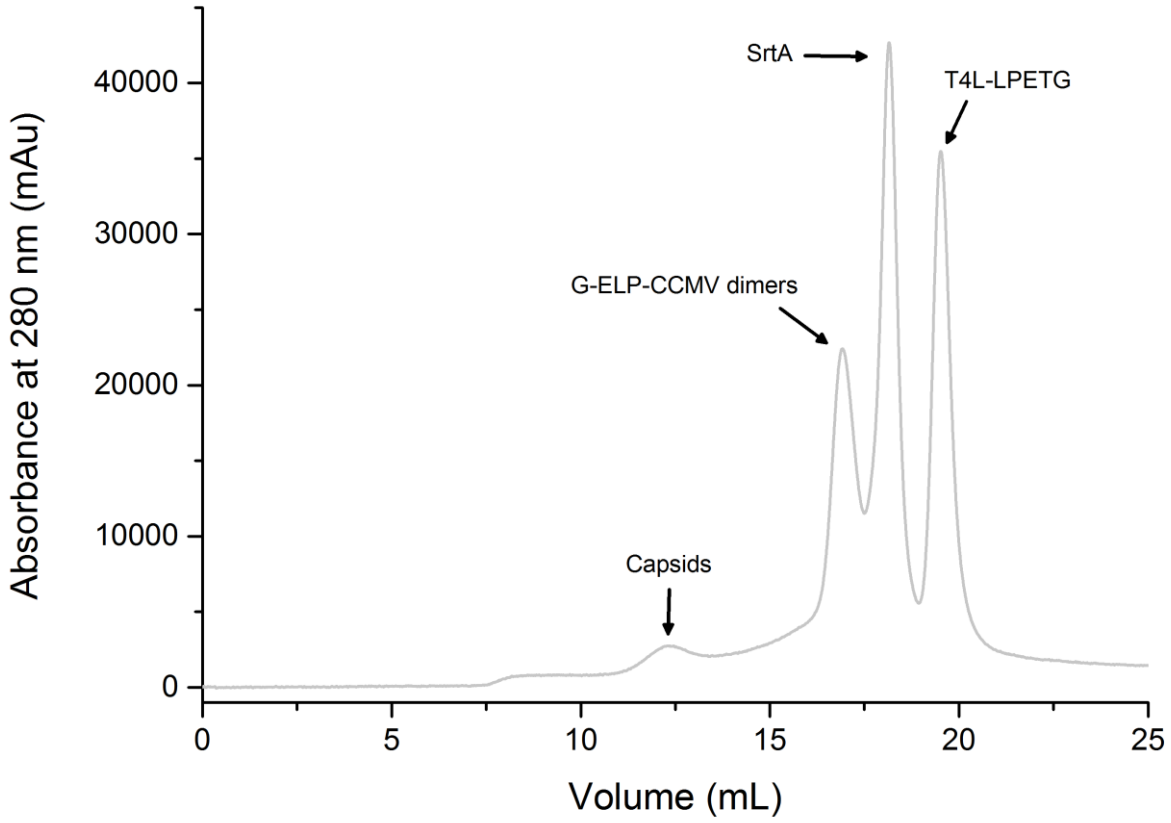


Figure S3. SEC chromatogram of a SrtA reaction mixture containing T4L-LPETG (50 μM), G-ELP-CCMV (50 μM) and SrtA (50 μM), after the addition of Ni^{2+} (450 μM, equal volume as the reaction mixture), meant to induce capsid assembly. Very little capsids were observed and tailing of the capsid peak was observed.

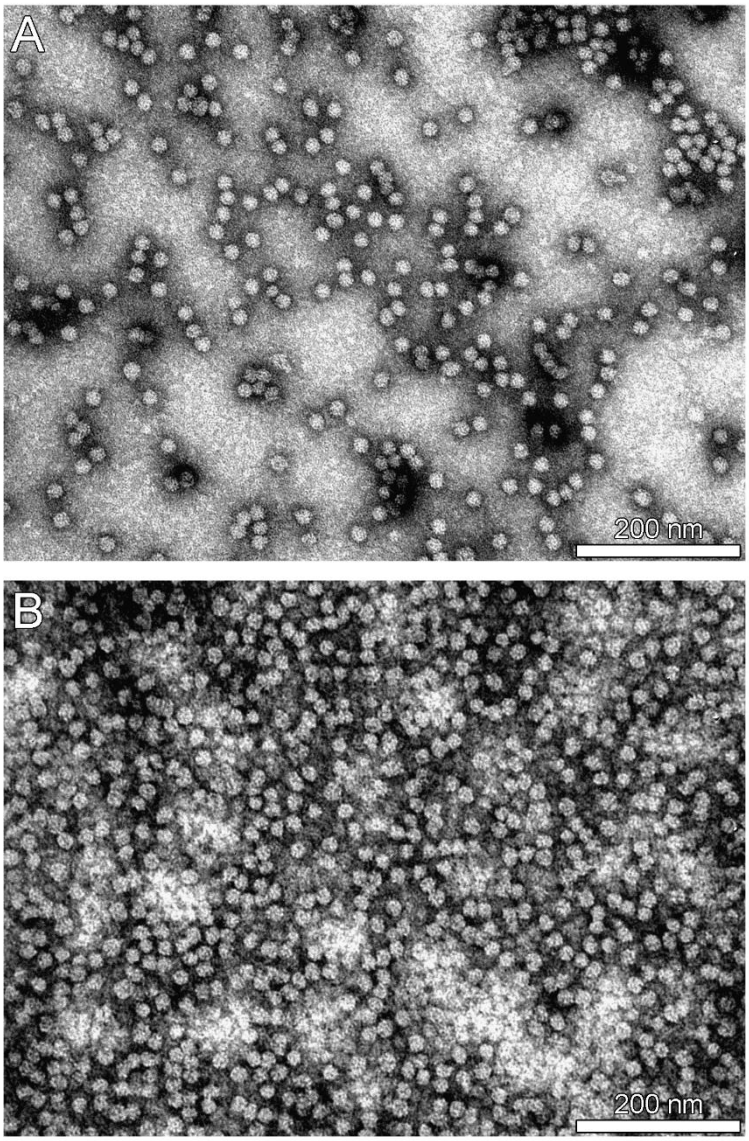


Figure S4. Uranyl acetate-stained TEM micrographs of G-ELP-CCMV after reaction with T4L-LPETG. Scale bars correspond to 200 nm. A) Particles after dialysis to pH 7.5 capsid buffer and subsequent purification using preparative SEC. Size = 19.6 nm ± 1.3 nm. B) Particles shown in A, after stabilization with Ni^{2+} ions and subsequent dialysis to reaction buffer. Size = 19.6 nm ± 1.4 nm.

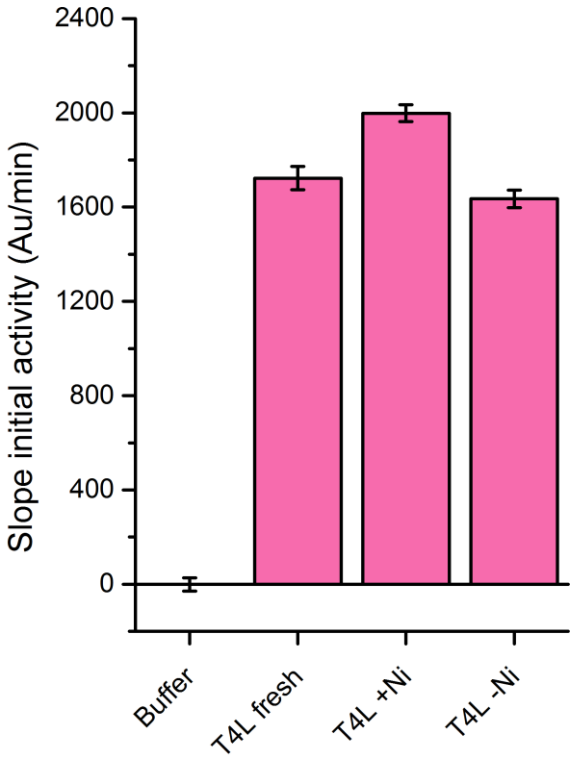


Figure S5. Catalytic activity of free T4L-LPETG (T4L fresh) and free T4L-LPETG after being subjected to the protocol used to form T4L-modified ELP-CCMV capsid in reaction buffer, compared to the background reaction in reaction buffer. See experimental section for the protocol used to prepare the capsids. In short, T4L-LPETG was dialyzed to Sortase buffer and incubated in this buffer for three hours at 21 °C. Subsequently, the protein was dialyzed to pH 7.5 capsid buffer and stored in this buffer overnight at 4 °C. The next day, the protein solution was split into two equal parts and either $NiCl_2$ (+Ni) or buffer (-Ni) was added and incubated, followed by dialysis to reaction buffer and dilution to 2 µM to ensure equal lysozyme concentration in all samples. The activity was measured using the EnzChek® lysozyme assay.

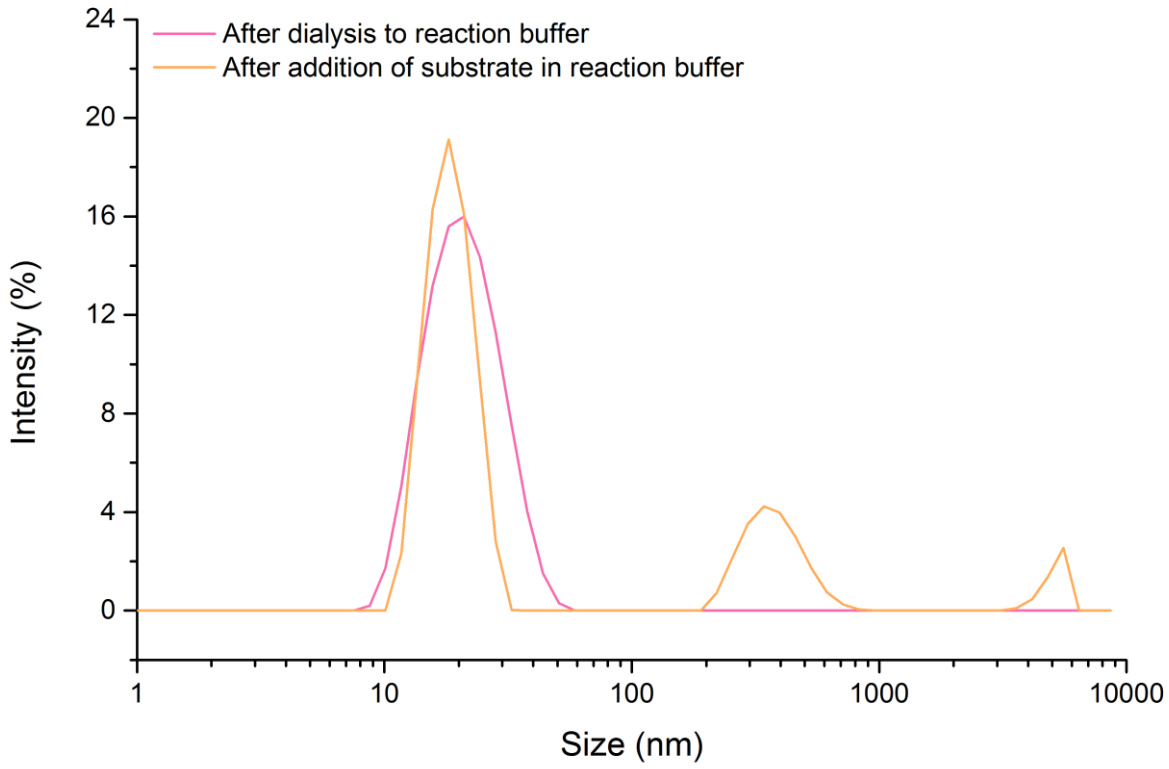


Figure S6. DLS measurements of T4L-modified ELP-CCMV *T = 1* capsids after stabilization with Ni^{2+} ions and dialysis to reaction buffer (pink line) and subsequent addition of fluorescently labeled *M. luteus* cell walls (orange line).

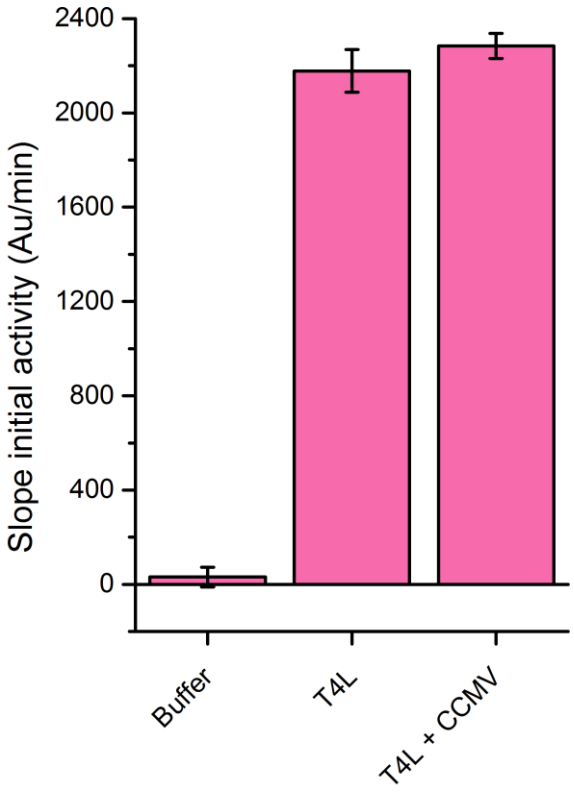


Figure S7. Catalytic activity of free T4L-LPETG and free T4L-LPETG in the presence of free G-ELP-CCMV, compared to the background reaction in reaction buffer. The activity was measured using the EnzChek® lysozyme assay.

3 References

1. Schoonen, L.; Pille, J.; Borrmann, A.; Nolte, R. J. M.; van Hest, J. C. M. *Bioconjug. Chem.* **2015**, *26*, 2429–2434.

2. van Eldijk, M. B.; Wang, J. C.-Y.; Minten, I. J.; Li, C.; Zlotnick, A.; Nolte, R. J. M.; Cornelissen, J. J. L. M.; van Hest, J. C. M. *J. Am. Chem. Soc.* **2012**, *134*, 18506–18509.