Supporting Information

S1. DNA Sequences

CelB-SP

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P22 Coat

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CelB

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S2. Protein Sequences

CelB-SP Amino Acid Sequence

MAKFPKNFMFGYSWSGFQFEMGLPGSEVESDWWVWVHDKENIASGLVSGDLPENGPAYWH
LYKQDHDIAEKLGMDCIRGGIEWARIFPKPTFDVKVDVEKDEEGNIISVDVPESTIKELE
KIANMEALEHYRKIYSDWKERGKTFILNLYHWPLPLWIHDPIAVRKLGPDRAPAGWLDEK
TVVEFVKFAAFVAYHLDDLVDMWSTMNEPNVVYNQGYINLRSGFPPGYLSFEAAEKAKFN
LIQAHIGAYDAIKEYSEKSVGVIYAFAWHDPLAEEYKDEVEEIRKKDYEFVTILHSKGKL
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Coat Protein Amino Acid Sequence

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CelB

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DWIGVNYYSRLVYGAKDGHLVPLPGYGFMSERGGFAKSGRPASDFGWEMYPEGLENLLKY
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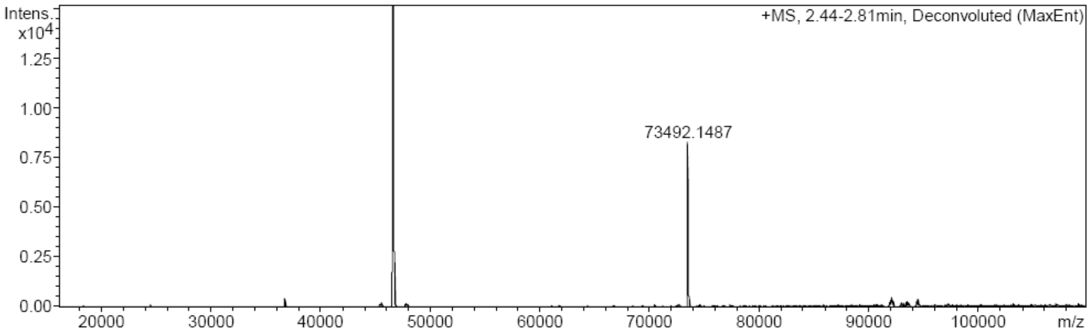
S3. Mass Spectrometry



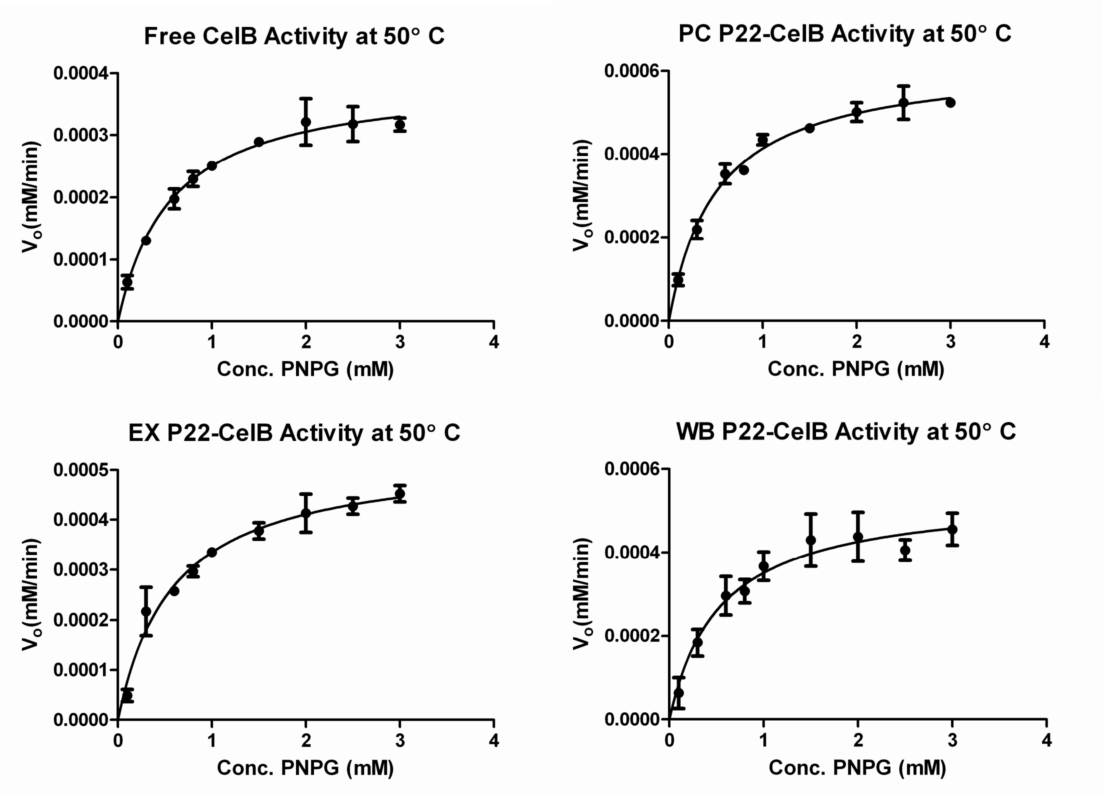
Deconvoluted mass spectra of CelB-SP P22 showing masses for CelB-SP fusion (lower intensity peak, mass 73492.15 Da; expected 73492.7 Da with loss of N-terminal methionine) and CP (major peak, mass of 46,594.62 Da; expected 46,592.62 with loss of N-terminal methionine). See materials and methods for experimental parameters.

S4. Tabulation of SEC Characterization

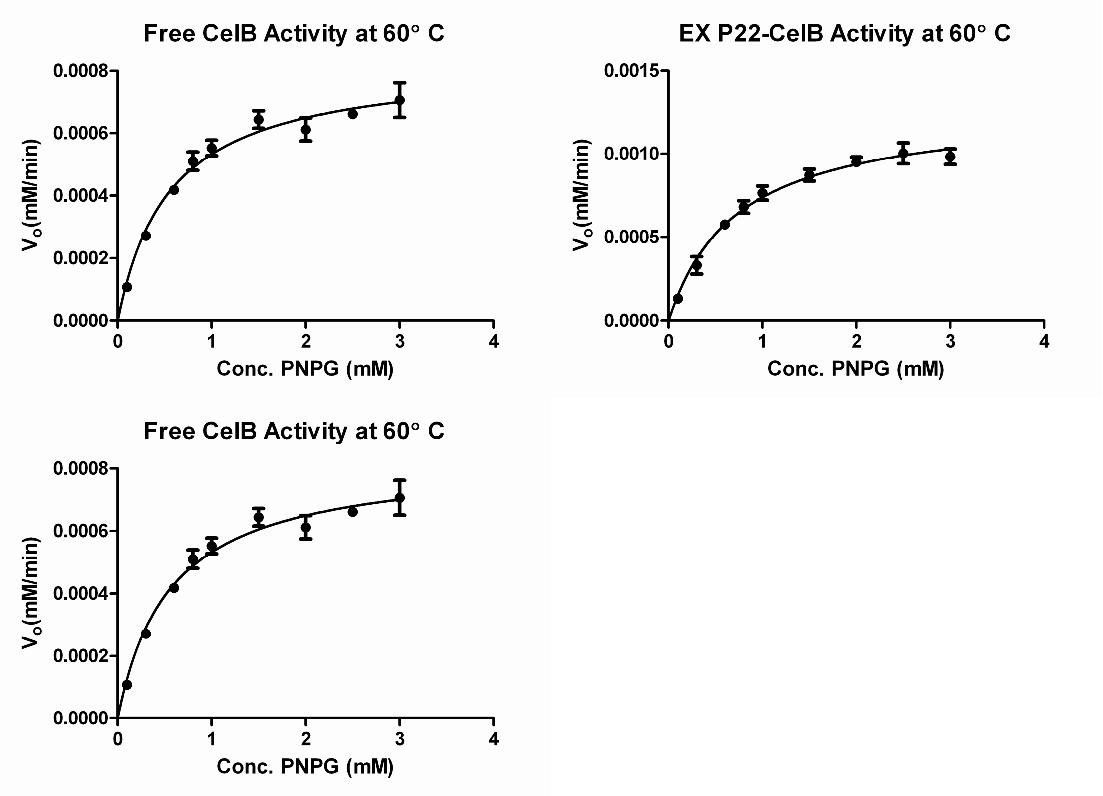
Construct	M_N (MDa)	Mass Cargo (MDa)	R_g (nm)	R_h (nm)	R_g/R_h
PC CelB-SP P22	26.0 ± 0.26	6.4	23.5	27.5	0.86
EX CelB-SP P22	24.9 ± 0.30	5.3	26.3	30.4	0.87
WB CelB-SP P22	23.0 ± 0.18	6.2	26	30.1	0.86

Values given are for the averages of three measurements.

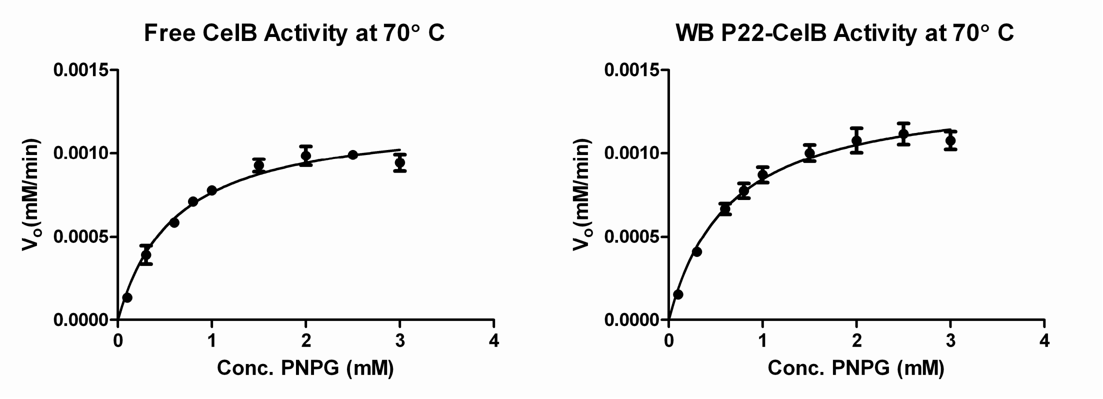
S5. Activity Assay Plots



S5-A. Plots of data and Michaelis-Menten fits for substrate (PNPG) dependence activity assays performed with temperature controls set to 50º C.



S5-B. Plots of data and Michaelis-Menten fits for substrate (PNPG) dependence activity assays performed with temperature controls set to 60º C.



S5-C. Plots of data and Michaelis-Menten fits for substrate (PNPG) dependence activity assays performed with temperature controls set to 70º C.

S6. Calculations

Number and Concentration of CelB-SP

Total concentration of CelB-SP in encapsulated samples was determined using size exclusion chromatography (coupled with multiangle light scattering). From size exclusion chromatography, molar mass data determined from MALS and RI detectors showed that P22 samples were homogenous, as indicated by the polydispersity index values (all near 1.00) and the molar mass distributions across the elution peak (Figure 2.D). The total mass of CelB-SP per P22 VLP was calculated from the number average molar mass, M_n, by subtracting the calculated molecular weight of the P22 VLP coat (420 CP/capsid * kDa/CP), which was in agreement with the experimental value obtained by SEC for the empty shell (no scaffold or cargo) P22 VLP, from the Mn average of three runs. The total mass of CelB-SP encapsulated was then divided by the theoretical molecular weight of CelB-SP (calculated using Protein Calculator v3.3, Chris Putnam, Scripps) to give the total number of CelB-SP per capsid, ~85 enzymes per capsid and the ratio of coat monomers to CelB-SP monomers is 4.95.

Using the total number of CelB-SP per capsid one can calculate the concentration of enzymes inside the capsid (see below) or it can be used in conjunction with absorption data to calculate the total concentration as follows:

The total absorbance of the CelB-SP P22 VLP is described by the equation:

$$A_T = A_{CP} + A_{CelB\text{-}SP} \qquad (1)$$

Where A_T is the total sample absorbance, A_{CP} is the absorbance contribution from CP, and $A_{CelB\text{-}SP}$ is the absorbance contribution from CelB-SP. Which can be rewritten according to the Beer-Lambert Law as:

$$A_T = C_{CP}\, \varepsilon_{CP}\, l + C_{CelB\text{-}SP}\, \varepsilon_{CelB\text{-}SP}\, l \qquad (2)$$

Where C_{CP} and $C_{CelB\text{-}SP}$ are the concentrations of CP and CelB-SP, respectively, ε_{CP} and $\varepsilon_{CelB\text{-}SP}$ are the extinction coefficients of CP and CelB-SP, respectively, and l is the pathlength of the cuvette.

The concentration of CP in relation to CelB-SP, as determined by MALS, can be described by the equation:

$$C_{CP} = 4.95 * C_{CelB\text{-}SP} \qquad (3)$$

Therefore, equation 3 allows replacement of C_{CP} in equation 2 to give:

$$A_T = 4.95 * C_{CelB\text{-}SP}\, \varepsilon_{CP}\, l + C_{CelB\text{-}SP}\, \varepsilon_{CelB\text{-}SP}\, l \qquad (4)$$

In this equation both extinction coefficients are known (calculated), the pathlenth is known, and A_T is measured leaving only $C_{CelB\text{-}SP}$. From equation 4 the concentration of CelB-SP used in the experiments was calculated.

Calculation of VLP volume

Using the equation for the volume of a sphere, $v=(4/3)\pi r^3$, and the internal radius determined from the cryo-EM reconstruction image of the different morphologies of P22, the volume of P22 in PC and EX (also corresponding to WB) were calculated as follows:

For PC r =24 nm; $(4/3)\pi(24)^3 = 58{,}000\ \text{nm}^3$

For EX and WB r =30; $(4/3)\pi(30)^3 = 113{,}000\ \text{nm}^3$

Molar confinement (M_{conf}) calculations.

Enzymes in cage $\div$ 6.022 x 10^{23} enzymes/mole enzyme $\div$ Internal Volume of Capsid = M_{conf}

Example PC calculation:

85 CelB $\div$ 6.022 x 10^{23} x 1000 mmol/mol $\div$ 5.8 x 10^{-17} cm^3 = 0.002434mmol/cm^3 = 2.4 mM CelB