Supporting Information

S1. DNA Sequences

AdhD-SP

ATGGCAAAAAGGGTAAATGCATTCAACGACCTTAAGCGTATAGGAGATGATAAGGTAACG
GCAATTGGAATGGGAACATGGGGAATAGGAGGGAGAGAGACCCCAGACTATTCTAGGGAT
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P22 Coat

ATGGCTTTGAACGAAGGTCAAATTGTTACACTGGCGGTAGATGAAATCATCGAAACCATC
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S2. Protein Sequences

AdhD-SP Amino Acid Sequence

MAKRVNAFNDLKRIGDDKVTAIGMGTWGIGGRETPDYSRDKESIEAIRYGLELGMNLIDT
AEFYGAGHAEEIVGEAIKEFEREDIFIVSKVWPTHFGYEEAKKAARASAKRLGTYIDLYL
LHWPVDDFKKIEETLHALEDLVDEGVIRYIGVSNFNLELLQRSQEVMRKYEIVANQVKYS
VKDRWPETTGLLDYMKREGIALMAYTPLEKGTLARNECLAKIGEKYGKTAAQVALNYLIW
EENVVAIPKASNKEHLKENFGAMGWRLSEEDREMARRCVPWLVPRGSCRSNAVAEQGRKT
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YHLGANPEKARQLLAMDGQSALIELTRLSERLTLKPRGKQISSAPHADQPITGDVSAANK
DAIRKQMDAAASKGDVETYRKLKAKLKGIR*

Coat Protein Amino Acid Sequence

MALNEGQIVTLAVDEIIETISAITPMAQKAKKYTPPAASMQRSSNTIWMPVEQESPTQEG
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NMAAEMGSLVITSPDAIGTNTADAWNFVADAEEIMFSRELNRDMGTSYFFNPQDYKKAGY
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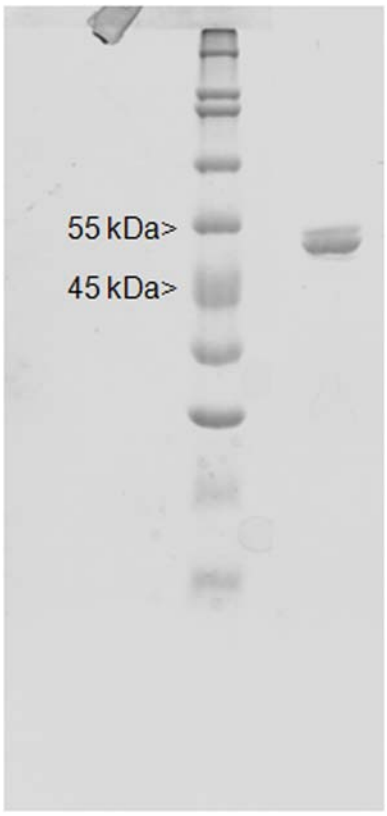
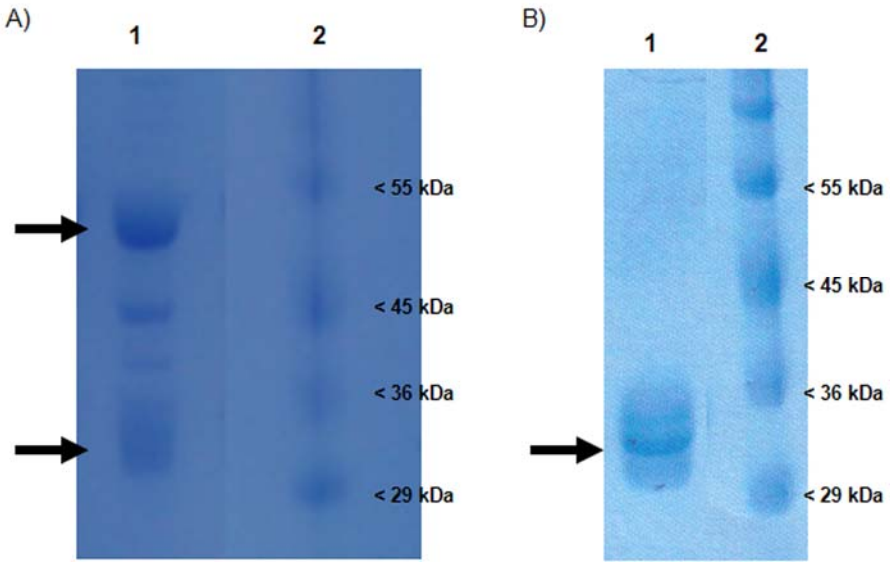
S3. SDS-PAGE and Densitometry



Analysis of the gel pictured above gave an intensity ratio of 43:77 for AdhD-SP to CP. The capsid is composed of 420 subunits of CP and based of the previously stated ratio of AdhD-SP to CP, there are approximately 235 enzymes per capsid (see calculations below). The absence of a band for scaffold protein (expected molecular weight of is ~18 kDa; lowest molecular weight marker band is 20 kDa) suggest that expression yields AdhD-SP fusion that is encapsulated before any cleavage occurs between the AdhD and SP fusion, which is observed during the purification process of "free" AdhD-SP.



SDS-PAGE of Ion Exchange Chromatography Purification of AdhD-SP. A) First pass purification of AdhD-SP over Mono Q IEC column. Lane 1: Molecular weight marker; Lane 2: AdhD-SP. B) Second pass purification of AdhD-SP over Mono Q IEC column. Lane 1: AdhD (SP lost). The AdhD-SP fusion has a calculated molecular weight of 50 kDa and AdhD alone has a calculated molecular weight of 32 kDa. The upper arrow in A indicates the intact fusion protein, AdhD-SP, and the lower arrows in A and B indicate the degradation product that has had the SP cleaved away. After a second purification by IEC all of the SP is cleaved from AdhD. Cleavage of the SP from non-encapsulated cargo proteins is commonly observed.

S4. MALS Data and Radius Discussion

Sample	MW1	MW2	MW3	Average	Std. Dev.	R1	R2	R3	Average	Std. Dev.
PC	3.23E+07	3.29E+07	3.16E+07	3.23E+07	650025.6	21.8	21.7	21.8	2.18E+01	0.057735
EX	3.13E+07	3.39E+07	3.24E+07	3.25E+07	1288966	22.6	22.5	22.4	2.25E+01	0.1
WB	2.92E+07	2.94E+07	2.98E+07	2.94E+07	293995.5	22.6	23	23	2.29E+01	0.23094

Table providing the molecular weights (MW1-3, in units of Daltons) and Radii (R1-3, in units of nm) and the averages and errors for three separate runs.

Radius of Gyration and Hydrodynamic Radius

Although the radius of a protein structure may be known by crystal structures and cryo-electron microscopy reconstruction studies, the radius determined in solution studies can vary based on the technique used. Two main techniques are dynamic light scattering and static light scattering (utilized in multiangle laser light scattering, MALS) which provide the hydrodynamic radius (R_H) and radius of gyration (R_g) respectively. The R_H is a dependant on shape of the particle and solvent effects and is given by the Stokes-Einstein equation:

$$D = \frac{KT}{6\pi\eta R_H}$$

where D is the diffusion coefficient, K is Boltzmann's constant, T is the temperature, η is the solution viscosity, and R_H is the hydrodynamic radius.

The R_g on the other hand is not shape and solvent dependent, but is depend on the center of mass according to the equation:

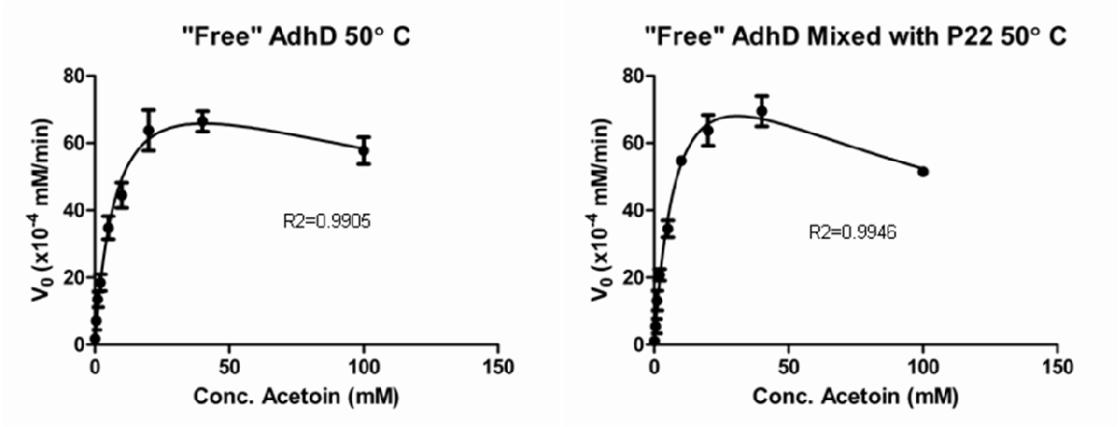
$$R_g^2 = \frac{\Sigma m_i r_i^2}{\Sigma m_i}$$

where m_i is the mass of the i^{th} atom, r_i is the distance from the center of mass of the i^{th} atom, and R_g is the hydrodynamic radius.

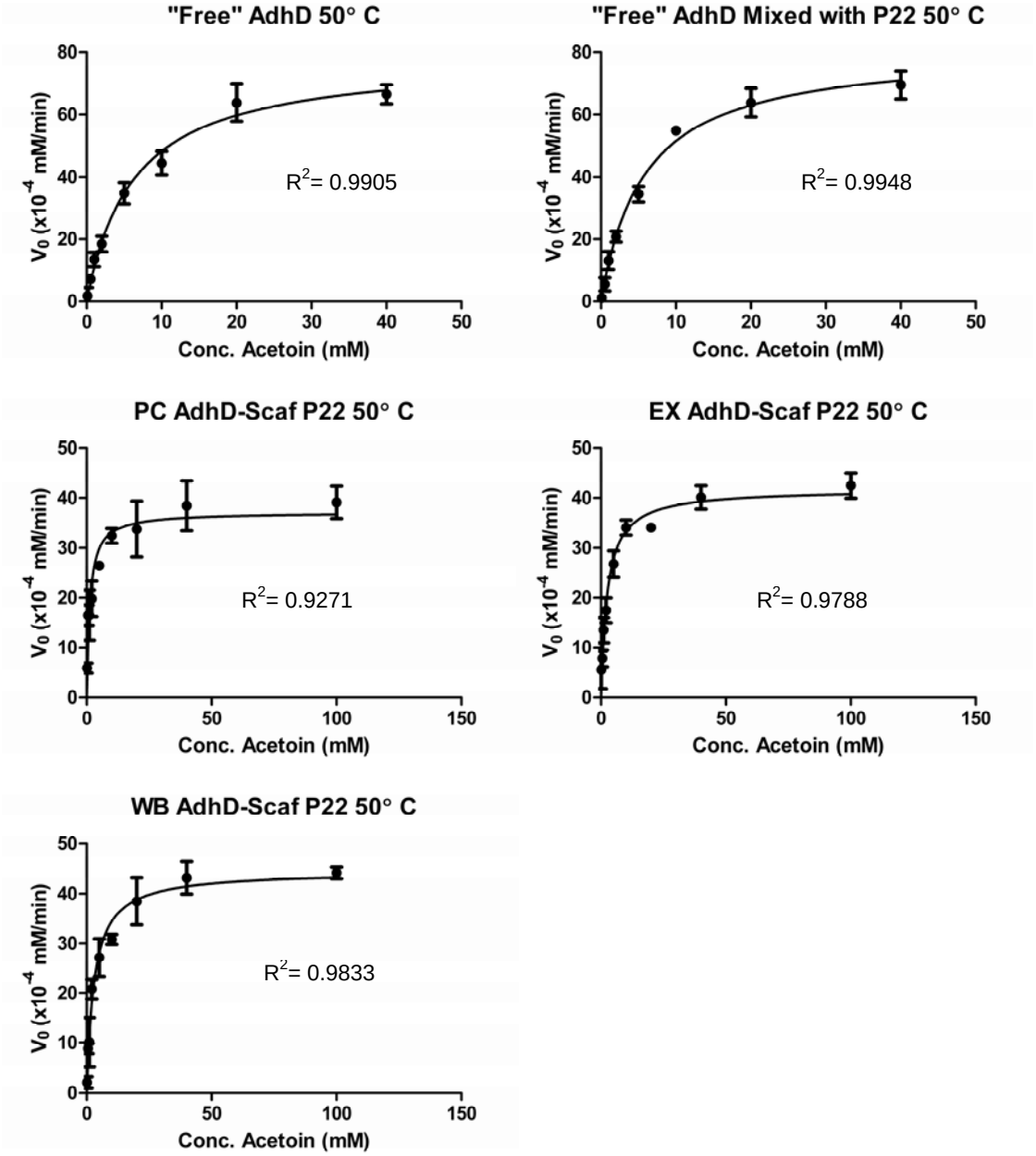
Due to the mass dependency, R_g can therefore be smaller than one would expect, as we observe for MALS. In addition, R_H can yield larger values than expected from a molecular structure due to, for example, ordered solvent shells that form around the protein structure.

S5. Activity Assay Plots

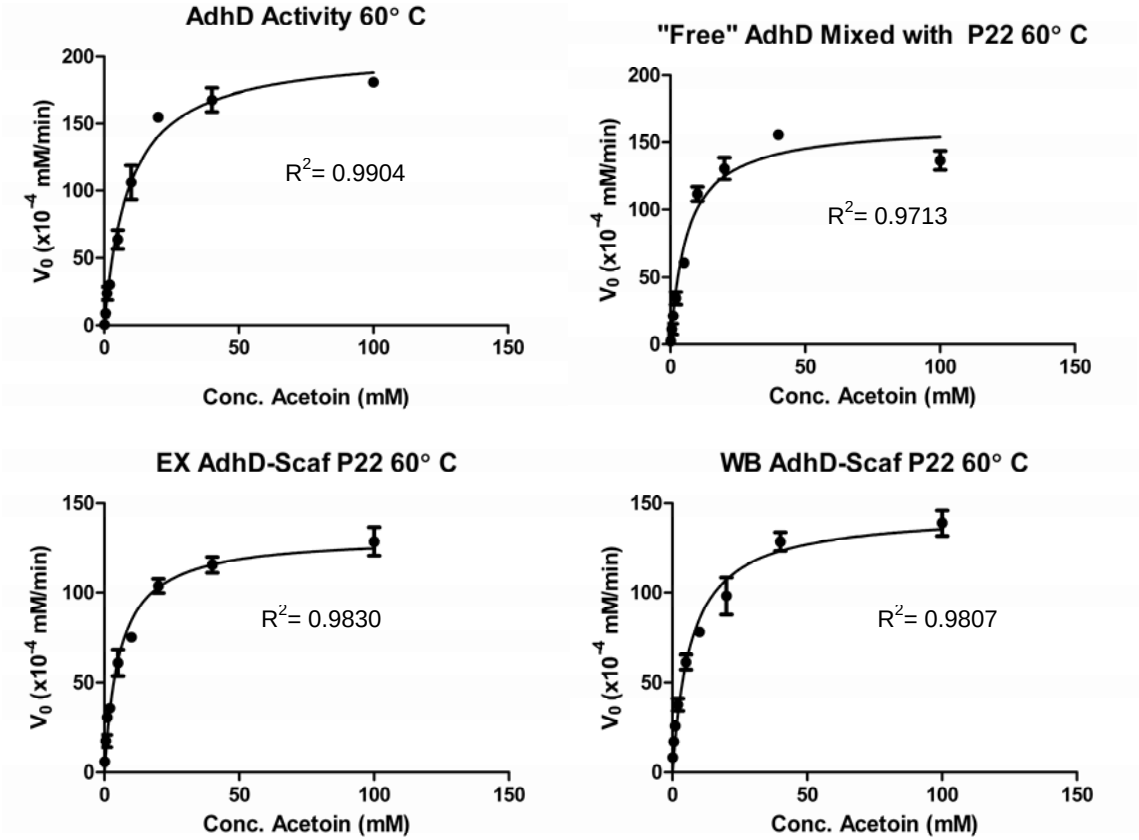
Substrate Assays



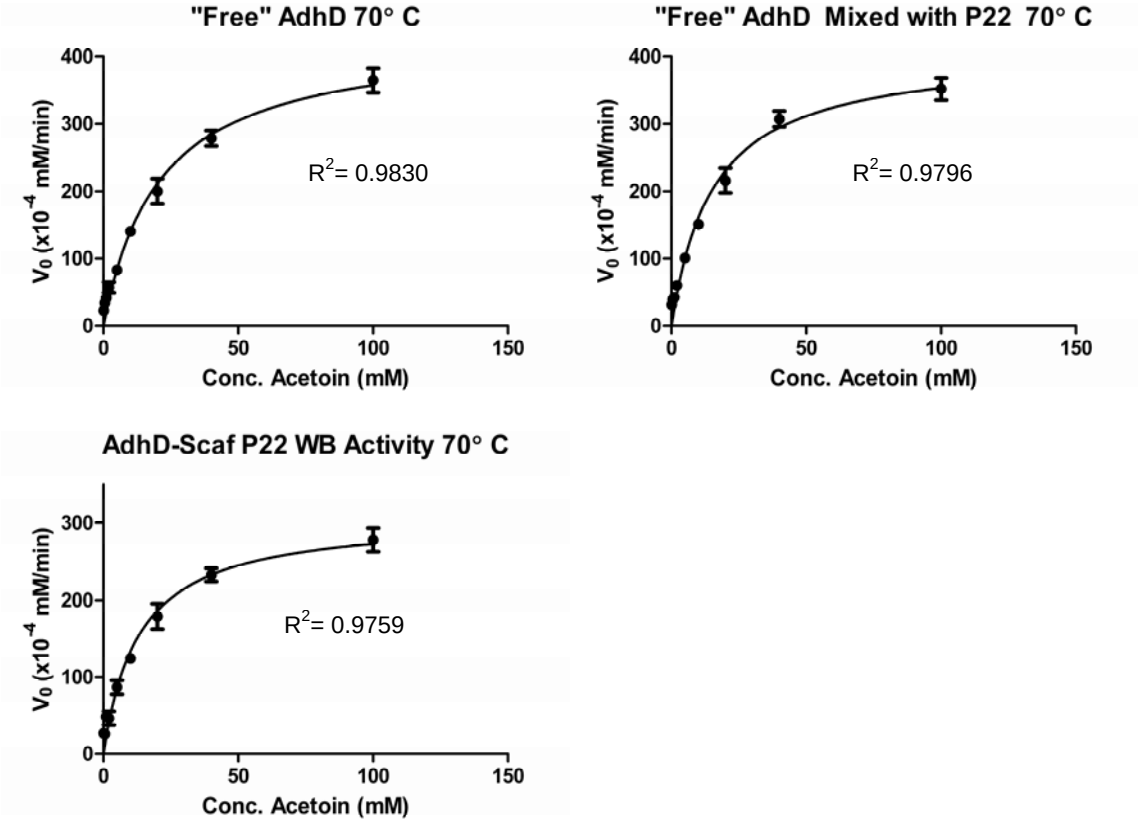
S5-A. Plots of data and substrate inhibition fits for substrate (acetoin) dependence activity assays performed with temperature controls set to 50º C. The fitting results gave $K_{M,Ace,App}$ of 8.35 mM and 9.54 mM for AdhD and AdhD mixed with P22 respectively. The k_{cat} values for AdhD and AdhD mixed with P22 were 0.911 sec^{-1} and 1.08 sec^{-1}, respectively.



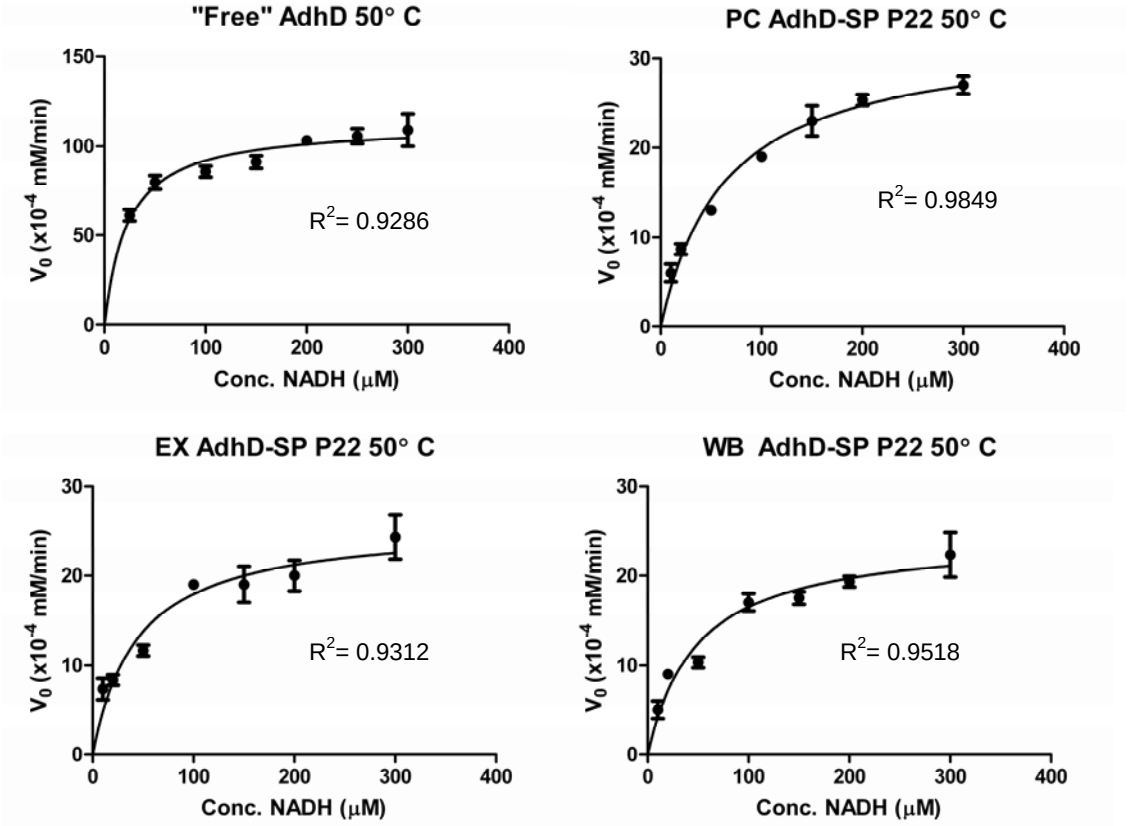
S5-B. Plots of data and Michaelis-Menten fits for substrate (acetoin) dependence activity assays performed with temperature controls set to 50º C.



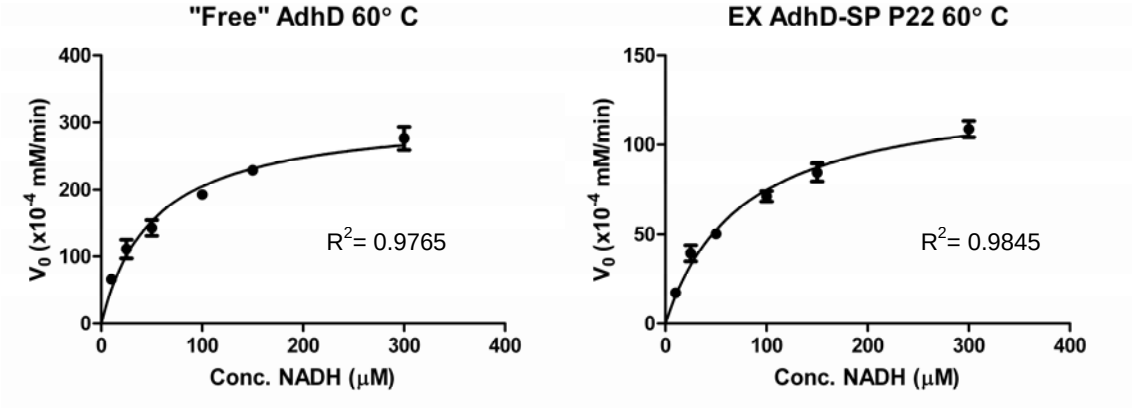
S5-C. Plots of data and Michaelis-Menten fits for substrate (acetoin) dependence activity assays performed with temperature controls set to 60º C.

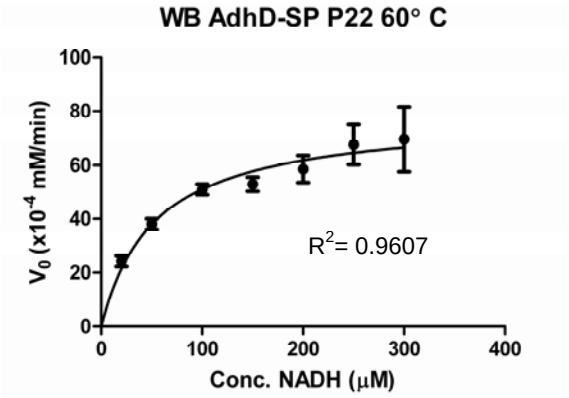


S5-D. Plots of data and Michaelis-Menten fits for substrate (acetoin) dependence activity assays performed with temperature controls set to 70º C.

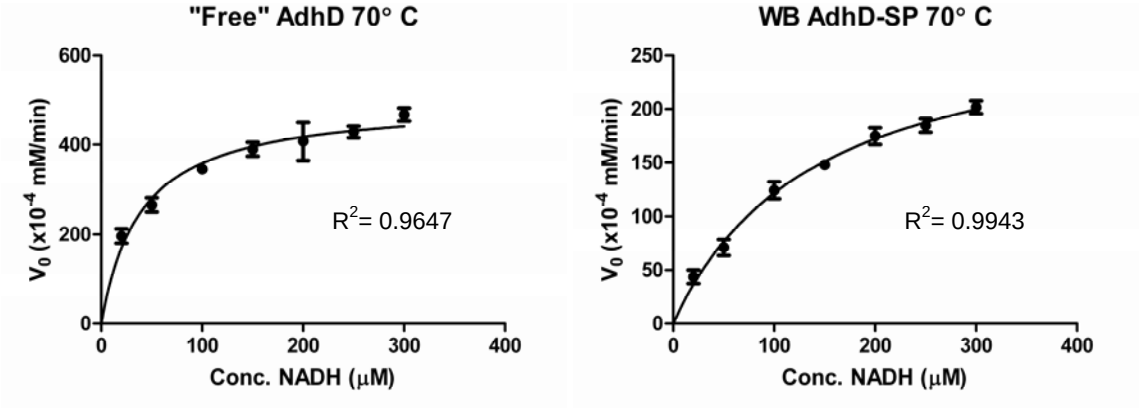


S5-E. Plots of data and Michaelis-Menten fits for cofactor (NADH) dependence activity assays performed with temperature controls set to 50º C.





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

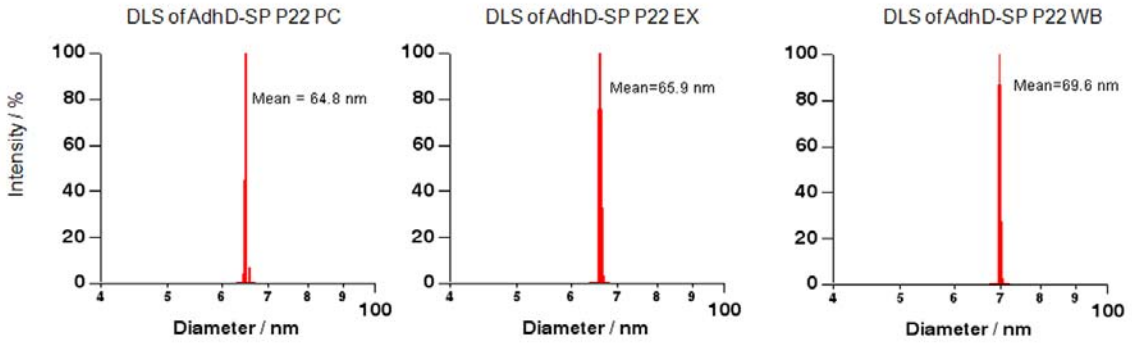


S5-G. Plots of data and Michaelis-Menten fits for cofactor (NADH) dependence activity assays performed with temperature controls set to 70º C.

Sample	k_{cat} (sec^{-1})	$K_{M,Ace}$ (mM)	$K_{M,NADH}$ (μM)	$k_{cat}/K_{M,Ace}$ (sec^{-1} mM^{-1})
50°C				
AdhD	0.77 ± 0.031	6.23 ± 0.87	22.6 ± 4.31	0.124
AdhD + P22	0.80 ± 0.027	6.20 ± 1.39	NA	0.129
60° C				
AdhD	2.01 ± 0.081	9.26 ± 1.22	52.1 ± 9.82	0.217
AdhD + P22	1.60 ± 0.081	8.82 ± 1.18	NA	0.181
70° C				
AdhD	4.23 ± 0.280	20.8 ± 3.65	39.2± 6.62	0.204
AdhD + P22	4.06 ± 0.54	15.9 ± 4.77	NA	0.255

S5-H. Table comparing the kinetic parameters for free AdhD with and without empty P22 added to the same concentration as found in encapsidated samples. NADH data was not obtained since we did not see any statistical difference for the substrate assays.

S6. Dynamic Light Scattering



Dynamic light scattering (DLS) plots for PC, EX, and WB forms of P22 samples encapsulating AdhD-SP.

S7. Calculations

Number and Concentration of AdhD-SP

Total concentration of AdhD-SP in encapsidated samples was determined using two independent methods, size exclusion chromatography (coupled with multiangle light scattering) and densitometry from gel electrophoresis. For SEC based calculations, 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 3.C). The total mass of AdhD-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 AdhD-SP encapsulated was then divided by the theoretical molecular weight of AdhD-SP

(calculated using Protein Calculator v3.3, Chris Putnam, Scripps) to give the total number of AdhD-SP per capsid, ~250 enzymes per capsid. Alternatively, densitometry gave a intensity ratio of 43 to 77 for AdhD-SP and CP respectively. Dividing the total number of coat proteins per capsid (420) by the ratio of CP to AdhD-SP (1.79) gives the reported 235 enzymes per capsid.

Using the total number of AdhD-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 AdhD-SP P22 VLP is described by the equation:

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

Where AT is the total sample absorbance, ACP is the absorbance contribution from CP, and AAdhD-SP is the absorbance contribution from AdhD-SP. Which can be rewritten according to the Beer-Lambert Law as:

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

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

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

$$C_{CP} = 1.68 * C_{AdhD\text{-}SP} \qquad (3)$$

Therefore, equation 3 allows replacement of CCP in equation 2 to give:

$$A_T = 1.68 * C_{AdhD\text{-}SP}\,\varepsilon_{CP}\,l + C_{AdhD\text{-}SP}\,\varepsilon_{AdhD\text{-}SP}\,l \qquad (4)$$

In this equation both extinction coefficients are known (calculated), the pathlenth is known, and AT is measured leaving only $C_{AdhD\text{-}SP}$. From equation 4 the concentration of AdhD-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; $(4/3)\pi(24)^3 = 58{,}000\ nm^3$

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

Molar confinement (M_{conf}) calculations.

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

Example PC calculation:
250 AdhD ÷ 6.022 x 10^{23} x 1000 mmol/mol ÷ 5.8 x 10^{-17} cm^3 = 0.007158 mmol/cm^3 = 7.16 mM AdhD

Percent Occupancy Calculations

The percent occupancy (P.O.) is calculated according to the following equation:

(# of Enzymes encapsulated) x V_{enzyme} ÷ V_{capsid} x 100% = P.O.

where V_{capsid} is the volume of the capsid (calculations shown above) and V_{enzyme} is the volume of the enzyme. The volume of an enzyme/protein can be approximated using the partial specific volume (v_{bar}) calculated for the protein from the sequence using the program Sednterp (Hayes, Laue, and Philo; 1995-2012) or other programs with this built in function, using the equation:

$$\frac{v_{bar} \times (10^{20} nm^3 / cm^3) \times MW}{6.033 \times 10^{23} molecules / mol} = V_{enzyme}$$

where v_{bar} is the partial specific volume in mL per gram (cubic centimeters), MW is the molecular weight of the enzyme/protein in grams per mol, and $V_{enzyme\ is}$ the volume of the enzyme/protein.

The partial specific volume (v_{bar}) of the AdhD-SP fusion was calculated to be 0.7387 mL/g and the molecular weight 50,722 g/mol. Using these values in the equation above yields a V_{enzyme} of 62.219 nm^3 for AdhD-SP. Placing the enzyme volume, internal volume of the capsid and number of enzymes encapsulated calculated above into the percent occupancy equation gives the following calculation:

(250 Enzymes encapsulated) x 62.219 nm^3 ÷ 58,000 nm^3 x 100% = 26.8%