Supporting Information

Tuning catalytic properties of P22 nanoreactors through compositional control

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Purification and characterization of AdhD-SP

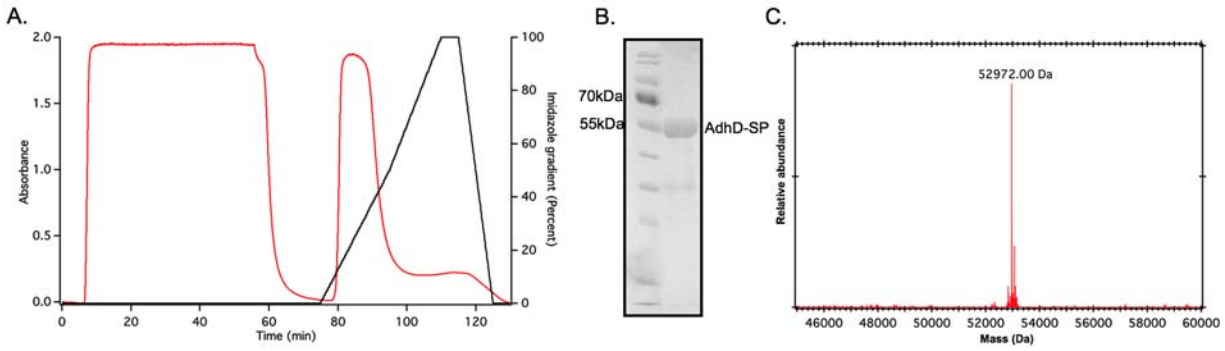

Figure S1. (A) Ni-NTA purification of AdhD-SP. The black line shows a gradient of imidazole (20 mM - 500 mM) used for the elution and the red line shows elution profile of the protein. (B) SDS-PAGE analysis of pooled fractions, eluted between 80 and 100 min, shows the band at expected molecular weight (~52kDa) and indicates the purity of AdhD-SP. (C) Mass-spectrometry reveals the MW of 52,972 Da, which is 741.76 Da higher than the expected molar mass (52230.24 Da). The higher mass is likely due to a covalent adduct formation of protein with $NADP^+$, as reported in the literature.[1-2]The adduct formed was found stable under our *in vitro* assembly conditions, as revealed by mass-spec analysis, and exhibited the expected kinetic behavior. The DNA sequence of this fusion protein matched with the expected sequence and is provided in the later sections.

Densitometric analysis of standards for controlled loading experiments

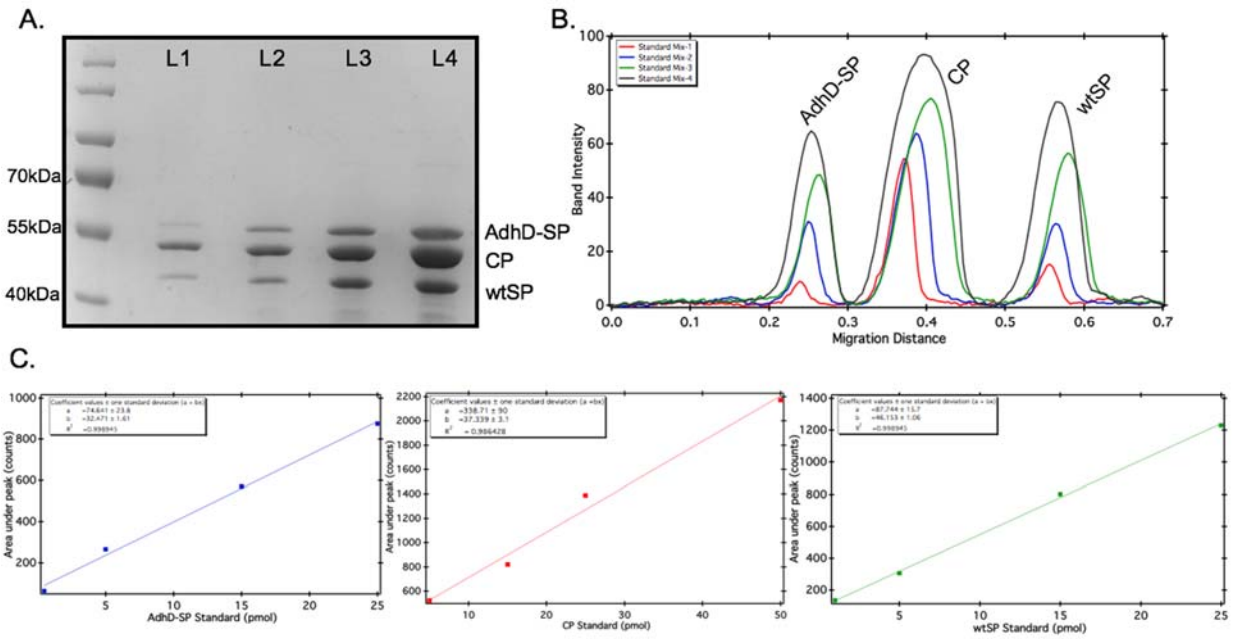

Figure S2. (A) SDS-PAGE analysis of standards (L1, L2, L3, and L4) containing mixture of AdhD-SP, CP and wtSP at different concentrations. (B) Line-scan densitometric profile of standards L1, L2, L3 and L4. (C) Calibration curve of each standard plotted between area under the peak (counts) and concentration (pmol).

SEC-MALS/QELS profiles of co-assembled capsids - P22-[AdhD-SP]$_x$[wtSP]$_y$

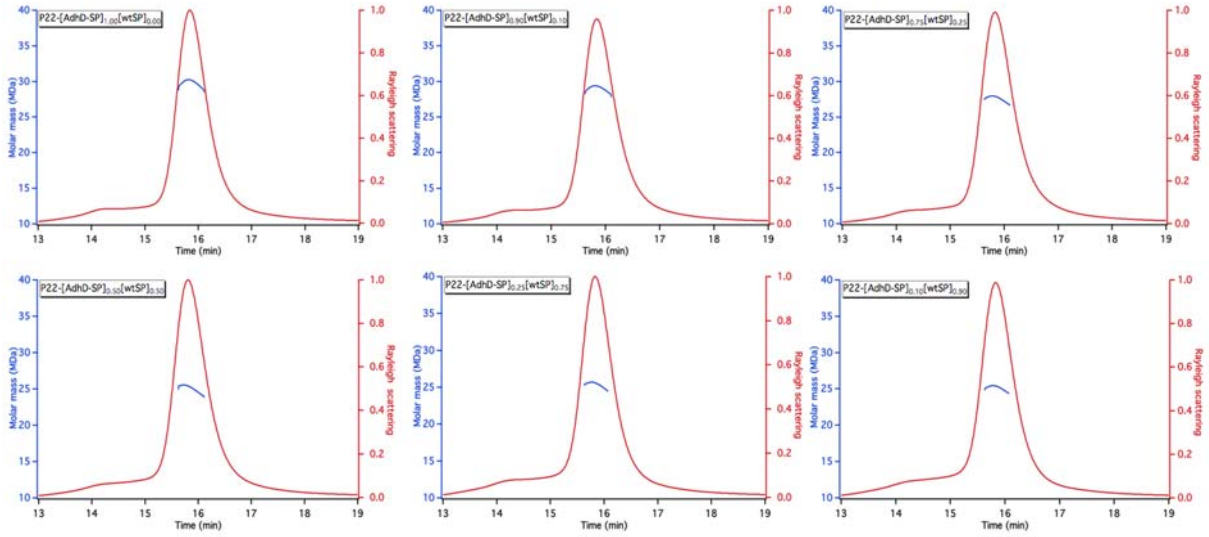

Figure S3. SEC-MALS/QELS analysis of co-assembled nanoreactors shows monodispersed population (red trace) and distribution of molar mass across the peak (blue trace). Subscripts in the legends show input stoichiometric ratio of AdhD-SP and wtSP used for co-assembly.

Table S1: DLS analysis of nanoreactors (co-assembled and SP depleted) at 50°C

Nanoreactor	Co-assembled, Diameter (nm)	SP depleted, Diameter (nm)
P22-[AdhD-SP]$_{0.10}$ [wtSP]$_{0.90}$	59.9 ± 0.51; PDI 0.13	59.4 ± 0.78; PDI 0.13
P22-[AdhD-SP]$_{0.25}$ [wtSP]$_{0.75}$	60.0 ± 0.99; PDI 0.13	59.4 ± 0.53; PDI 0.15
P22-[AdhD-SP]$_{0.50}$ [wtSP]$_{0.50}$	59.7 ± 0.53; PDI 0.14	59.1 ± 1.01; PDI 0.15
P22-[AdhD-SP]$_{0.75}$ [wtSP]$_{0.25}$	58.1 ± 2.23; PDI 0.14	56.0 ± 0.06; PDI 0.07
P22-[AdhD-SP]$_{0.90}$ [wtSP]$_{0.10}$	57.6 ± 0.99; PDI 0.12	55.7 ± 0.04; PDI 0.04
P22-[AdhD-SP]$_{1.00}$ [wtSP]$_{0.00}$	57.5 ± 0.27; PDI 0.09	57.6 ± 0.34; PDI 0.08

Concentration dependent activities of AdhD-SP and AdhD

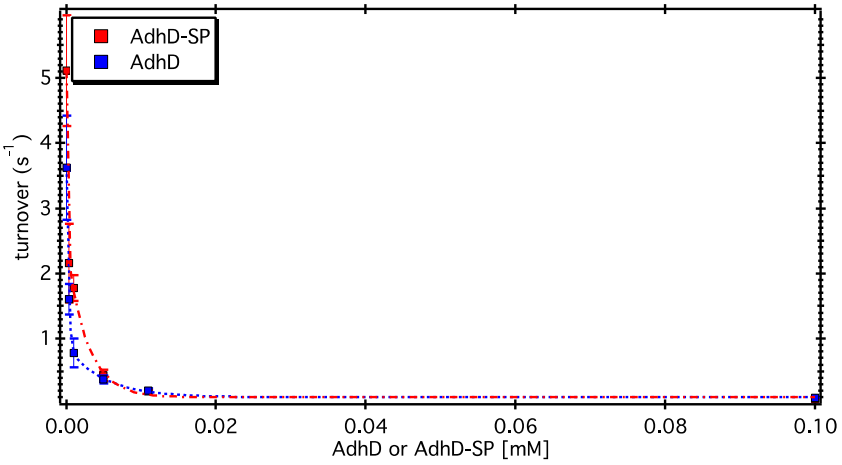

Figure S4. Plot showing the concentration dependent activity of enzyme with and without SP fusion.

Activity of free enzyme in the presence of polyethylene glycol (PEG 8k) crowding agent

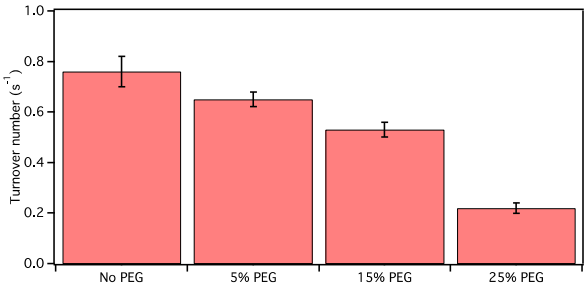

Figure S5. Dependence of enzyme activity on the concentration of PEG 8k. The activity was monitored at different concentrations (% w/v) of PEG 8k.

SEC-MALS/QELS profiles of capsids depleted of wtSP

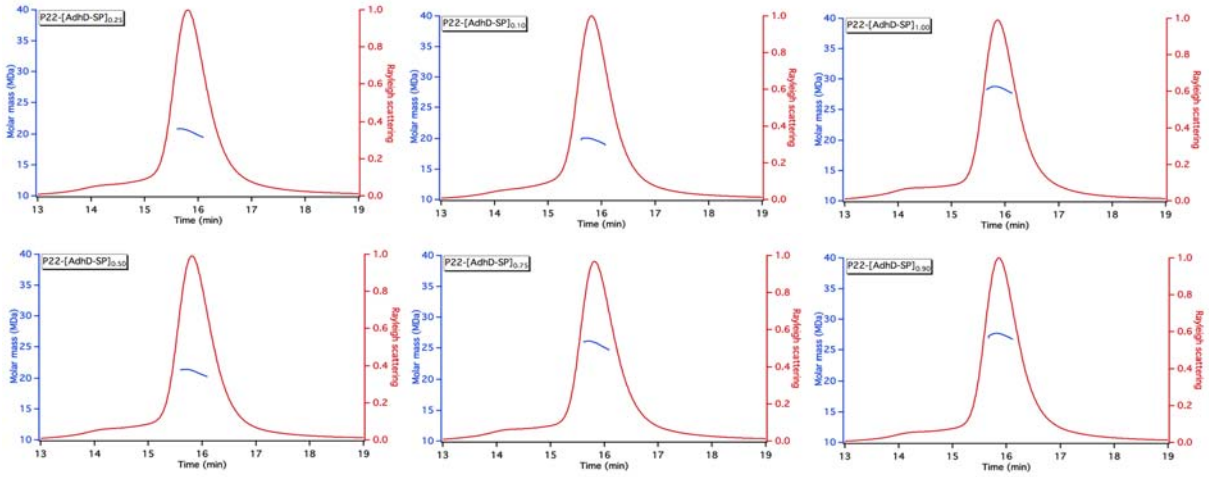

Figure S6. SEC-MALS analysis of capsids depleted of wtSP shows distribution of the population (red trace) and molar mass across the peak (blue trace).

Michaelis-Menten constant K_M and catalytic efficiency (k_{cat}/K_M) of AdhD encapsulated in co-assembled capsids P22-[AdhD-SP]$_x$[wtSP]$_y$ and in capsids depleted of wtSP

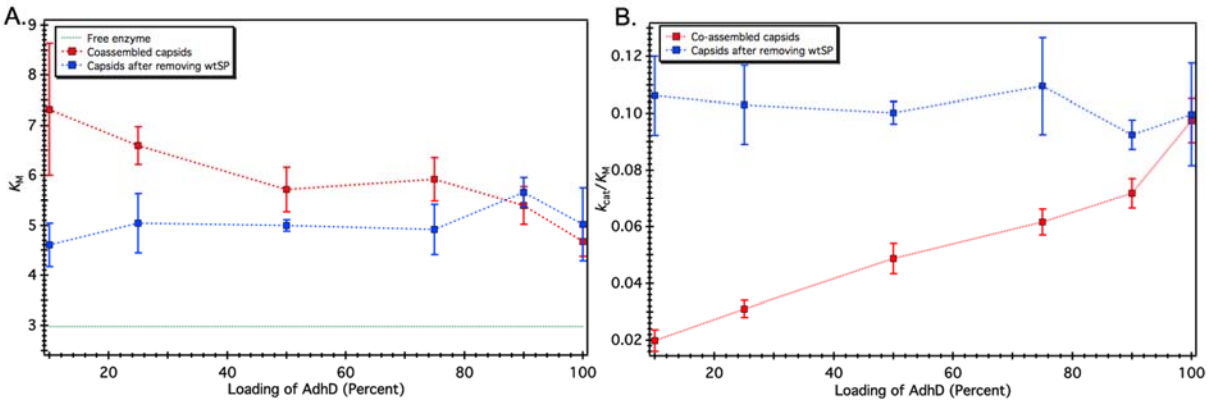

Figure S7. (A) Michaelis-Menten constant K_M and (B) catalytic efficiency of AdhD encapsulated in the co-assembled capsids (red trace) and in the capsids depleted of wtSP (blue trace) were plotted as a function of capsid composition. The K_M of free enzyme (green trace) was also plotted for comparison.

Activity of free (AdhD-SP) and encapsulated (P22-[AdhD-SP]$_{1.00}$[wtSP]$_{0.00}$) enzyme

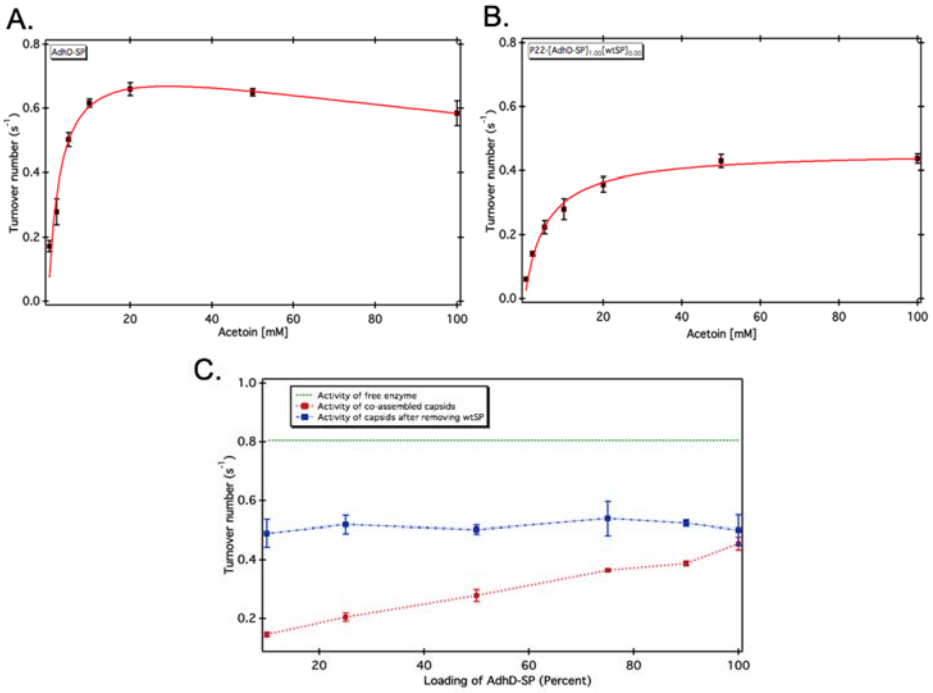

Figure S8. (A) Activity of free enzyme, AdhD-SP. Data was fitted with a model for substrate inhibition (red line). (B) Activity of encapsulated enzyme. Data was fitted with standard Michaelis-Menten model. The kinetic parameters obtained from fit are as follows: a) free enzyme, k_{cat} = 0.80 ± 0.01 s^{-1} and K_M =2.98 ± 0.53 mM, k_{cat}/K_M =0.27 ± 0.05 s^{-1} mM^{-1}; Encapsulated enzyme k_{cat} = 0.46 ± 0.02 s^{-1} and K_M =4.67 ± 0.30 mM, k_{cat}/K_M =0.1 ± 0.01 s^{-1} mM^{-1}. (C) Plot comparing the activity of free enzyme with enzymes encapsulated in co-assembled capsids and in capsids depleted of wtSP. The nanoreactors containing AdhD only (100 percent loading level) showed similar activities before and after GuHCl treatment, indicating that GuHCl does not affect the enzymatic activity and the capsid porosity.

Dynamic light scattering (DLS) of free enzyme monitored at different concentrations

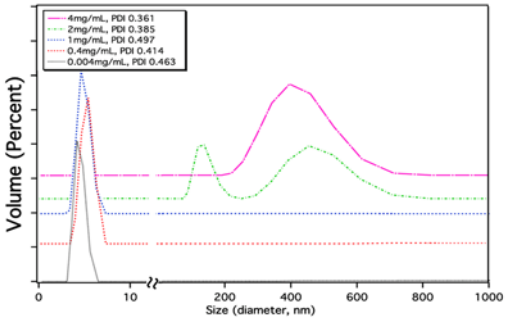

Figure S9. DLS measurement of free enzyme at different concentrations. The measurement was performed under the buffer and temperature conditions that were used for kinetics.

Molar confinement calculation[3]

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

M_{conf} of AdhD in P22-$[AdhD\text{-}SP]_{1.00}$ $[wtSP]_{0.00}$ is as follows:
M_{conf} = 219 ÷ 6.022 x 10^{23} x 1000 mmol/mol ÷ 5.8 x 10^{-17} cm^3 = 0.00627 mmol/cm^3 = 6.3 mM

Table S2: Units conversion

Loading	#AdhD-SP	AdhD-SP (mM)	AdhD-SP (mg/ml)	#wtSP	wtSP (mM)	wtSP (mg/ml)
P22-$[AdhD\text{-}SP]_{1.00}$ $[wtSP]_{0.00}$	219	6.3	334	—	—	—
P22-$[AdhD\text{-}SP]_{0.90}$ $[wtSP]_{0.10}$	200	5.7	302	3	0.1	4
P22-$[AdhD\text{-}SP]_{0.75}$ $[wtSP]_{0.25}$	154	4.4	233	22	0.6	21
P22-$[AdhD\text{-}SP]_{0.50}$ $[wtSP]_{0.50}$	98	2.8	148	74	2.1	74
P22-$[AdhD\text{-}SP]_{0.25}$ $[wtSP]_{0.75}$	43	1.2	64	143	4.1	144
P22-$[AdhD\text{-}SP]_{0.10}$ $[wtSP]_{0.90}$	14	0.4	21	183	5.4	190
P22-$[AdhD\text{-}SP]_{0.00}$ $[wtSP]_{1.00}$	0	—	—	194	5.5	193

Concentration of AdhD-SP in assembled capsids[3]

To determine the concentration of AdhD-SP in capsids, the number of copies of AdhD-SP encapsulated in P22 VLPs was determined from SEC-MALS. To get the number of copies, the molar mass of empty coat protein shell (19.7 MDa) was subtracted from the molar mass of P22-AdhD (31.1 MDa). The mass obtained was then divided by the molecular weight of AdhD-SP, to give 319 copies of AdhD per capsid. Using the number of copies and absorbance at 280 nm, the concentration of AdhD-SP was calculated as follows:

The total absorbance from P22-AdhD can be written as follows $A_T = A_{CP} + A_{AdhD\text{-}SP}$...……...(1)

where A_T is the total absorbance, A_{CP} is the absorbance contribution from CP, and $A_{AdhD\text{-}SP}$ is the absorbance from AdhD-SP. According to the Lambert-beer's law

$A_T = C_{CP}\_{CP}\, l + C_{AdhD\text{-}SP}\_{AdhD\text{-}SP}\, l$……..……...……....(2)

Where C_{CP} and $C_{AdhD\text{-}SP}$ are the concentrations of CP and AdhD-SP, respectively, $\_{CP}$ and $\_{AdhD\text{-}SP}$ are the extinction coefficients of CP and AdhD-SP, respectively, and l is the path length of the cuvette. The concentration of CP in relation to AdhD-SP, as determined by SEC-MALS, can be described by the equation:

$C_{CP} = 1.32 * C_{AdhD\text{-}SP}$……...……...…...(3)

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

$A_T = 1.32 * C_{AdhD\text{-}SP} \_ _{CP} + C_{AdhD\text{-}SP} \_ _{AdhD\text{-}SP}$……..…….....…..(4)

In this equation, both extinction coefficients are known (calculated from ExPASy ProtParam tool), the path length is known, and A_T is measured, leaving only $C_{AdhD\text{-}SP}$.

To determine the concentration of AdhD-SP in co-assembled capsids, the total amount of cargo (AdhD-SP and wtSP) was obtained by subtracting the molecular weight of capsid alone from the observed molecular weight of co-assembled capsids. The number of copies of each component was determined by the ratio of the two SP components, as determined by the densitometric analysis of the SDS-PAGE. The concentrations of CP and wtSP in relation to AdhD-SP, as determined by SEC-MALS and densitometry, were plugged into equation (6) to determine the concentration of AdhD-SP in co-assembled capsids.

$A_T = C_{CP} \_ _{CP} l + C_{AdhD\text{-}SP} \_ _{AdhD\text{-}SP} l + C_{wtSP} \_ _{wtSP} l$……...(5)

Where $\_ _{wtSP}$ is the extinction coefficient of wtSP, obtained from ExPASy ProtParam.

From equation (4) and (5) the concentration of AdhD-SP was calculated under denatured conditions. Using this concentration, the extinction coefficient was obtained for the capsid under non-denaturing conditions, which was then used to calculate the concentration of AdhD-SP in P22 VLPs.

Model used to fit substrate inhibition data

$f(S) = k_{cat} * S/(Km + S (1+S/Ki))$

where,
k_{cat} is the turnover number
S is the substrate concentration
Km is the Michaelis-Menten constant
Ki is the enzyme-inhibitor dissociation constant

The data were fit in Igor pro 6.37.

Sequence information of AdhD-SP construct

DNA sequence

ATGGGCAGCTCGCACCATCATCACCATCACAGCGGCGCAAAACGTGTCAATGCTT
TTAACGACCTGAAGCGCATCGGCGACGACAAGGTTACCGCTATCGGGATGGGAA
CCTGGGGAATCGGCGGACGTGAGACGCCAGATTATAGTCGTGACAAGGAAAGTA
TCGAGGCCATCCGCTATGGTCTGGAGCTGGGCATGAATTTGATTGACACGGCTGA
ATTTTACGGGGCAGGTCATGCCGAAGAAATTGTTGGAGAGGCAATTAAAGAGTTT
GAGCGTGAAGATATTTTCATCGTTAGTAAGGTTTGGCCAACCCACTTTGGCTACGA
GGAAGCGAAGAAGGCCGCGCGCGCGTCGGCAAAGCGTTTGGGAACATACATTGA

CTTGTACCTTCTTCACTGGCCTGTCGATGATTTTAAGAAAATCGAAGAAACTCTTC
ATGCTCTTGAAGATTTGGTTGACGAGGGTGTCATTCGTTACATCGGAGTCAGCAAT
TTTAACCTGGAACTTCTTCAACGTTCACAGGAAGTTATGCGTAAGTACGAAATCGT
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TTAGATTACATGAAGCGTGAGGGCATTGCCTTGATGGCTTATACTCCTTTAGAGAA
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ACCGCAGCTCAGGTCGCTTTGAACTACCTTATCTGGGAGGAGAATGTCGTGGCAA
TCCCTAAGGCATCAAATAAGGAGCACTTAAAAGAGAACTTTGGGGCCATGGGGTG
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TGCCCGCAAACACTATGACGCGGCGGAAAAGCTCAACATCCCTGACTATCAGGAG
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CTCGACTATCCGAACGCTTAACTCTCAAGCCTCGCGGTAAACAAATCTCTTCCGCT
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Amino acid sequence

MGSSHHHHHHSGAKRVNAFNDLKRIGDDKVTAIGMGTWGIGGRETPDYSRDKESIEA
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MRKYEIVANQVKYSVKDRWPETTGLLDYMKREGIALMAYTPLEKGTLARNECLAKIGE
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EKEDAFMQLVPPAVGADIMRLFPEKSAALMYHLGANPEKARQLLAMDGQSALIELTRL
SERLTLKPRGKQISSAPHADQPITGDVSAANKDAIRKQMDAAASKGDVETYRKLKAKL
KGIR

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