

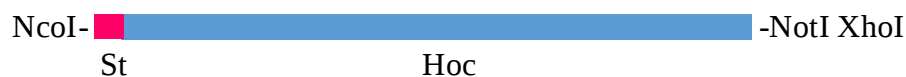
Supplementary information

A. Primer sequences

Table S1. Primer sequences for cloning enzymes into pET28b and Hoc into pACYCduet.		
Name	Sequence (5'-3')	Cloning vector
*Amylase F	CCTTTT ACCATGGGCGCGGTGAATGG	pET28b
Amylase R	CCTTTTGGATCCAGAACCGCCACCGCCGCTGCCACCCCC TCCCCATGAAGAAGTATGG	pET28b
Maltase F	CCTTTTCCATGGGCATGACCATCTCTGACCAC	pET28b
Maltase R	CCTTTTGGATCCAGAACCGCCACCGCCGCTGCCACCCCC TCCTTTAACCAGGTAGATACG	pET28b
Glucokinase F	GGAATTCCATGGCCGAATTCGCCAGATCTATGACAAAG TATGCATTAGTCG	pET28b
Glucokinase R	AGCGGTCTCGAGGCCGGATCCAGAACCGCCACCGCCGC TGCCACCCCCCTCCCAGAATGTGACCTAAGGTCTGG	pET28b
#St-Hoc F1	AAATATCCATGGGCGCGCATATTGTGATGGTGGATGCG TATAAACCGACCAAAATGACTTTTACAGTTGATATAACT CC	pACYC duet
#Hoc Not Xho R1	TGTGTCTCGAGAGTGC GGCCGCCTTATGGATAG1. GTATAGATGATAC	pACYC duet
*The amplified fragments, Aml, Mal and GK using three primer sets, were digested with NcoI and BamHI and then inserted into the compatible sites into our existing pET28-sdAb in BglII format (Goldman, Broussard 2017). # The amplified Hoc gene fragment using these two primers were digested with NcoI and NotI and inserted into pACYCduet with the compatible ends.		

B. Gene maps

1. Spyttag(St)-Hoc



2. Enzymes (Amylase, Maltase, and Glucokinase)



3. Spycatcher



4. Enzyme-SpyCatcher (SC)



C. Gene sequences

>St-Hoc in pACYCduet

ATGGGCGCGCATATTGTGATGGTGGATGCGTATAAACCGACCAAAATGACTTTTAC
AGTTGATATAAACTCCTAAAACACCTACAGGGGTTATTGATGAACTAAGCAGTTTA
CTGCTACACCCAGTGGTCAAACCTGGAGGCGGAACCTATTACATATGCTTGGAGCGTAG
ATAATGTTCCACAAGATGGAGCTGAAGCAACTTTTAGTTATGTACTAAAAGGACCTG
CCGGTCAAAAGACTATTAAAGTAGTTGCAACAAATACACTTTCTGAAGGAGGCCCG
GAAACGGCTGAAGCGACAACAACCTATCACAGTTAAAAATAAGACACAGACGACTAC
CTTAGCCGTAACTCCTGCTAGTCCCGCGGCTGGAGTGATTGGAACCCAGTTCAATT
TACTGCTGCCTTAGCTTCTCAACCTGATGGAGCATCTGCTACGTATCAGTGGTATGTA
GATGATTACAAGTTGGTGGAGAACTAACTCTACATTTAGCTATACTCCAACCTACA
AGTGGAGTAAAAAGAATTAAATGCGTAGCCCAAGTAACCGCGACAGATTATGATGC
ACTAAGCGTTACTTCTAATGAAGTATCATTAACGGTTAATAAGAAGACAATGAATCC
ACAGGTTACATTGACTCCTCCTTCTATTAATGTTTACGCAAGATGCTTCGGCTACATTT
ACGGCTAATGTTACGGGTGCTCCAGAAGAAGCACAAATTACTTACTCATGGAAGAA
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GGAAGTCAAACCTATTGAAGTTACTGCAACTGTTACTGCTGCAGATTATAACCTGTAA
ACCGTTACCAAAACTGGTAATGTAACAGTCACGGCTAAAGTTGCTCCAGAACCAGA
AGGTGAATTACCTTATGTTTCATCCTCTTCCACACCGTAGCTCAGCTTACATCTGGTGC
GGTTGGTGGGTTATGGATGAAATCCAAAAAATGACCGAAGAAGGTAAAGATTGGAA
AACTGACGACCCAGATAGTAAATATTACCTGCATCGTTACACTCTCCAGAAGATGAT
GAAAGACTATCCAGAAGTTGATGTCCAAGAATCGCGTAATGGATACATCATTATA
AAACTGCTTTAGAACTGGTATCATCTATACCTATCCATAA

>Amylase-SC in pET28b

ACCATGGGCGCGGTGAATGGGAAAGGGATGAATCCAGATTATAAAGCGTATTTAAT
GGCACCGCTCAAAAAAATCCCGGAAGTAACCAACTGGGAGACCTTCGAAAACGACC
TGCGCTGGGCGAAACAGAATGTTTTTATGCCATTACTGTAGATTTTTGGTGGGGGG
ACATGGAAAAAACGGGGACCAACAATTCGATTTCTCTTACGCTCAACGCTTTGCTC
AGAGCGTTAAGAACGCAGGTATGAAAATGATCCCGATCATTAGCACCCACCAGTGC
GGTGGTAACGTAGGTGACGATTGCAATGTGCCAATTCGAGCTGGGTATGGAATCA
GAAATCTGATGATAGCTTATACTTCAAATCGGAGACAGGTACCGTCAATAAAGAAA
CCCTGAATCCGCTGGCGAGTGATGTAATTCGTAAAGAGTATGGCGAATTGTATACCG
CTTTCGCAGCCGCAATGAAACCGTATAAAGATGTTATTGCAAAGATTTATCTCTCCG
GCGGACCAGCTGGTGAGCTGCGTTACCCAAGCTACACGACCAGTGACGGGACGGGT
TATCCAAGCCGCGGCAAGTTTCAGGCGTACACAGAATTTGCAAAATCAAATTCGT
TTATGGGTTCTGAACAAATACGGAAGCCTGAACGAAGTCAATAAGGCGTGGGGAAC
CAAACCTGATTTTCAAGAACTGGCTATCTTACCGCCGAGCGATGGCGAACAGTTCCCTTAT
GAATGGTTATCTGAGCATGTATGGCAAAGATTATTTGGAATGGTACCAAGGCATCCT

GGAGAATCACACCAAGCTCATCGGGGAACTGGCTCATAATGCTTTTGACACTACGTT
CCAAGTACCGATCGGTGCGAAGATTGCGGGCGTCCATTGGCAATACAACAATCCAA
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GACGCATTCAAATCTGCTAAATTGGACGTCACGTTACCTGTCTGGAAATGACCGAT
AAAGGTAGCTATCCAGAGTACTCCATGCCGAAAACCTTGGTGCAGAACATCGCCAC
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ATGAGGAGGAATACAAGCGTGTGGCGGAGATGGCATTAACTATAACTTTGCAGGC
TTCACCCTGCTGCGTTATCAGGACGTTATGTACAATAACTCTCTCATGGGAAAAATTT
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ACTACCATCGGGGACACAGTTTATATCACAGGGAATCGTGCGGAGCTCGGGTCCTG
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CGGTAATGTGGTTCTGCCGGCAGAACGCAATATTGAGTTTAAGGCCTTTATTAAATC
AAAAGACGGTACGGTTAAGAGCTGGCAGACTATCCAACAATCCTGGAACCCGGTTC
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CGGTTCTGGATCTGTTGATACCTTATCAGGTTTATCAAGTGAGCAAGGTCAGTCCG
GTGATATGACAATTGAAGAAGATAGTGCTACCCATATTAAATTCTCAAACCGTGATG
AGGACGGCAAAGAGTTAGCTGGTGCAACTATGGAGTTGCGTGATTTCATCTGGTAAA
ACTATTAGTACATGGATTTTCAGATGGACAAGTGAAAGATTTCTACCTGTATCCAGGA
AAATATACATTTGTGCAAACCGCAGCACCAGACGGTTATGAGGTAGCAACTGCTATT
ACCTTTACAGTTAATGAGCAAGGTCAGGTTACTGTAAATGGCAAAGCAACTAAAGG
TGACGCTCATATTGGATCCGGCCTCGAGCACCACCACCACCACCACTGA

>Maltase-SC in pET28b

ACCATGGGCATGACCATCTCTGACCACCCGGAAACGGAGCCAAAATGGTGGAAGA
AGCCACCATCTACCAGATTTACCCGGCGAGCTTCAAGGATTCTAATAACGATGGTTG
GGGTGATCTGAAAGGCATTACCTCCAAACTGCAGTACATCAAAGATCTGGGTGTTGA
TGCTATTTGGGTATGCCCGTTCTACGACAGCCCGCAGCAGGACATGGGCTACGATAT
TTCTAACTATGAAAAAGTATGGCCGACCTATGGTACCAACGAGGACTGCTTCGAGCT
GATCGATAAAACGCACAAACTGGGCATGAAATTTATCACGGATCTGGTTATTAACCA
CTGCTCTACTGAACACGAGTGGTTCAAAGAATCTCGCTCTAGCAAAACGAACCCGA
AACGTGACTGGTTCTTTTGGCGCCCGCCTAAAGGTTACGACGCGGAAGGTAAACCG
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ACGACGAACGAATTCTACCTGCGTCTGTTTCGCTCTCGTCAGGTTGACCTGAACCTGG
GAGAACGAAGACTGTCGTCGTGCGATCTTTGAATCCGCGGTTGGCTTCTGGCTGGAT
CACGGCGTAGATGGCTTCGCGATTGATACGGCAGGTCTGTATTCCAAACGCCCGGGC
CTGCCGGACTCTCCGATCTTCGACAAAACCTCCAAACTGCAGCACCCAAACTGGGGT
TCCCACAACGGCCCGCGTATCCACGAGTATCATCAGGAACTGCACCGCTTCATGAAA
AACCGCGTTAAAGACGGCCGTGAAATCATGACTGTCGGTGAAGTAGCTCACGGTTCT
GACAACGCTCTGTACACTTCTGCGGCTCGTTATGAGGTTAGCGAGGTATTTTCCTTCA
CCCACGTTGAACTGGGCACCTCTCCGTTTTTCCGTTATAACATCGTACCGTTTACGCT
GAAGCAGTGGAAGAGGCAATCGCATCTAACTTCCTGTTTCATCAACGGTACTGACTC
CTGGGCAACGACCTATATCGAGAACCATGATCAGGCTCGCTCTATCACTCGTTTCGC
AGACGATTCCCCGAAGTATCGTAAAATTTCTGGTAAACTGCTGACTCTGCTGGAATG
CTCCCTGACCGGCACCCTGTATGTGTATCAGGGTCAAGAGATCGGTCAAATTAACCT
TAAAGAATGGCCGATCGAAAAATACGAAGACGTTGACGTTAAAAACAACCTACGAGA
TCATCAAAAAGTCCTTCGGTAAAAACTCCAAAGAAATGAAAGACTTTTTCAAAGGT

ATCGCCCTGCTGAGCCGCGATCACTCTCGCACGCCGATGCCGTGGACCAAGGATAA
ACCTAACGCGGGTTTTACCGGCCCGGACGTAAAGCCGTGGTTTTTCTGAATGAATC
TTTTGAACAGGGCATCAATGTAGAGCAGGAGAGCCGTGATGATGACAGCGTGCTGA
ACTTCTGGAAACGTGCACTGCAGGCCCGCAAAAAGTACAAAGAGCTGATGATTTAC
GGCTATGATTTCCAGTTCATTGACCTGGACTCCGACCAGATCTTCTCTTTCACCAAAG
AATACGAAGATAAACTCTGTTTGGCGCTCTGAACTTCTCTGGCGAAGAAATTGAGT
TCTCCCTGCCGCGTGAAGGCGCCTCCCTGTCTTTCATCCTGGGTAACTATGATGACA
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GAGGGGGTGGCAGCGGCGGTGGCGGTTCTGGATCTGTTGATACCTTATCAGGTT
TATCAAGTGAGCAAGGTCAGTCCGGTGATATGACAATTGAAGAAGATAGTGCTACC
CATATTAATTTCTCAAAACGTGATGAGGACGGCAAAGAGTTAGCTGGTGCAACTAT
GGAGTTGCGTGATTCATCTGGTAAAACTATTAGTACATGGATTTTCAGATGGACAAGT
GAAAGATTTCTACCTGTATCCAGGAAAATATACATTTGTGCGAAACCGCAGCACCAGA
CGGTTATGAGGTAGCAACTGCTATTACCTTTACAGTTAATGAGCAAGGTCAGGTTAC
TGTAATGGCAAAGCAACTAAAGGTGACGCTCATATTGGATCCGGCCTCGAGCACC
ACCACCACCACCACTGA

>Glucokinase-SC in pET28b

ATGGCCGAATTCGCCAGATCTATGACAAAGTATGCATTAGTCGGTGATGTGGGCGGC
ACCAACGCACGTCTTGCTCTGTGTGATATTGCCAGTGGTGAAATCTCGCAGGCTAAG
ACCTATTCAGGGCTTGATTACCCAGCCTCGAAGCGGTCATTTCGCGTTTATCTTGAA
GAACATAAGGTCGAGGTGAAAGACGGCTGTATTGCCATCGCTTGCCCAATTACCGGT
GACTGGGTGGCGATGACCAACCATACTGGGCGTTCTCAATTGCCGAAATGAAAAA
GAATCTCGGTTTTAGCCATCTGGAAATTATTAACGATTTTACCGCTGTATCGATGGC
GATCCCGATGCTGAAAAAAGAGCATCTGATTCAGTTTGGTGGCGCAGAACCGGTCG
AAGGTAAGCCTATTGCGGTTTACGGTGCCGGAACGGGGCTTGGGGTTGCGCATCTGG
TCCATGTGATAAGCGTTGGGTAAGCTTGCCAGGCGAAGGCGGTCACGTTGATTTTG
CGCCGAATAGTGAAGAAGAGGCCATTATCCTCGAAATATTGCGTGCGGAAATTGGT
CATGTTTCGGCGGAGCGCGTGCTTTCTGGCCCTGGGCTGGTGAAATTTGTATCGCGCA
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CGTGCCGCATTTGAAGATAAAGGGCGCTTTAAAGAATATGTCCATGATATTCGGGTG
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ACCTTAGGTCACATTCTGGAGGGGGTGGCAGCGGCGGTGGCGGTTCTGGATCT
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GAAGAAGATAGTGCTACCCATATTAATTTCTCAAAACGTGATGAGGACGGCAAAGA
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GATTTTCAGATGGACAAGTGAAAGATTTCTACCTGTATCCAGGAAAATATACATTTGT
CGAAACCGCAGCACCAGACGGTTATGAGGTAGCAACTGCTATTACCTTTACAGTTAA
TGAGCAAGGTCAGGTTACTGTAAATGGCAAAGCAACTAAAGGTGACGCTCATATTG
GATCCGGCCTCGAGCACCACCACCACCACCACTGA

>Synthesized SC fragments (NcoI-EcoRI-BglI at 5' and BamHI-XhoI)

CCATGGGCGAATTCAGATCTGTTGATACCTTATCAGGTTTATCAAGTGAGCAAGGTC
 AGTCCGGTGATATGACAATTGAAGAAGATAGTGCTACCCATATTAAATTCTCAAAAC
 GTGATGAGGACGGCAAAGAGTTAGCTGGTGCAACTATGGAGTTGCGTGATTCATCT
 GGTA AAACTATTAGTACATGGATTTTCAGATGGACAAGTGAAAGATTCTACCTGTAT
 CCAGGAAAATATACATTTGTGCGAAACCGCAGCACCAGACGGTTATGAGGTAGCAAC
 TGCTATTACCTTTACAGTTAATGAGCAAGGTCAGGTTACTGTAAATGGCAAAGCAAC
 TAAAGGTGACGCTCATATTGGATCCGGCCTCGAGCACCA

D. Construction of enzyme-SC fusions and St-Hoc plasmids

We used the BglBrick cloning strategy (Anderson, Dueber et al. 2010) to produce the enzyme-SC fusions. First a BglBrick compatible version of the pET28b expression vector was constructed by using the quick change mutagenesis kit (Agilent) to remove the unique BglII site. Each enzyme (Aml, Mal, GK) was amplified by polymerase chain reaction (PCR) to include a 10-amino acid glycine-serine linker on their 3' ends. Resulting enzyme gene fragments were subsequently digested with NcoI and BamHI and inserted into similarly digested pET28b. Next, the resulting enzyme BglBricks pET28b DNA was digested with BamHI and XhoI and ligated with SC fragments flanked with BglII and XhoI to produce the enzyme-SC pET28b fusions (Goldman, Broussard et al. 2017). St-Hoc fragments were amplified using PCR with primers containing sequences encoding St, A H I V M V D A Y K P T K, and NcoI site at 5' end and NotI site at 3' end. The resulting St-Hoc fragments containing NcoI and NotI overhang sequences were then inserted into pACYCduet via the compatible sites. The resulting St-Hoc pACYCduet did not have 6xHis. All clones were verified by sequencing. All of the enzymes for cloning were purchased from New England Biolabs (Ipswich, MA)

E. Protein production and purification:

Both enzyme-SC fusion and St-Hoc plasmids were transfected into *E.coli* cells, Tuner (DE3) (*F⁻ompT hsdS_B (r_B⁻ m_B⁻) gal dcm lacY1*(DE3), and plated onto LB supplemented with kanamycin and chloramphenicol. Next day, one colony from the plate was inoculated into 3 ml of LB containing both kanamycin and chloramphenicol and grown overnight in a 37 °C shaker. One mL of the overnight culture was then inoculated into 500 mL TB supplemented with both antibiotics and shook for 7 to 8 hrs at 37 °C before switching to 25 °C and adding 0.25 mM IPTG. The induced culture was then shook overnight. Cell pellets were obtained the next day by centrifugation at 10,000 x g for 10 min and resuspended in approximately 30 mL phosphate buffer saline (PBS). One mg of Lysozyme was then added to the cell suspension and shook for 5 min at room temperature (RT). Cells were then subjected to sonicating on ice for 2 min with the output cycle 50% (ThermoFisher Scientific, Waltham, MA). The cell suspension were then spun at 20,000 x g for 15 min and the supernatant was transferred to a new 50 mL conical tube. Subsequently, PBS equilibrated Nickel resin (GE Healthcare) was added and the mixture was rotated in a cold room for 1-2 hrs before pelleting at 1200 x g for 5 min. The resin was washed once with 1x IMAC buffer (20 mM phosphate buffer [pH 7.4], 0.4 M NaCl, and 0.02 M imidazole) and the protein was then eluted with 0.25 M of Imidazole (except for Malt-SC which was eluted with 0.1M Imidazole) and subsequently purified by fast protein liquid chromatography (FPLC) using Enrich SEC 650/70 column with the flow rate 0.5 mL/min and

0.75 mL/fraction. All the eluted fractions were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (ThermoFisher Scientific, Waltham, MA) to confirm the sizes of enzyme-Hoc fusions. The confirmed enzyme-Hoc fractions were then aliquoted into 3 tubes and stored at -80 °C.

The protein concentration for each fraction was determined by abs.280 nm/molar extinction coefficient (ϵ) based on Beer's law ($A_\lambda = \epsilon cL$, where c =[protein]; L =width of light path). The value of ϵ for each fusion is determined by the composition of Tryptophan, Tyrosine, and Cysteine amino acid and was calculated via free web tool (<https://www.novoprolabs.com/tools/protein-extinction-coefficient-calculation>).

F. Preparation of tailless Δ Hoc T4 phage

Fifteen mL overnight culture grown from a single colony was inoculated into 300 mL of M9 medium supplemented with 0.5% of glucose, 0.1% Casamino acid 2 mM $MgSO_4$, 0.1 mM $CaCl_2$ and 50 μ g/mL Tryptophan and shook for 4 hrs at 37 °C until reaching OD 0.45 at 600 nm. *E.coli* cells were then infected with Δ HocT4 phage at Moi (multiple of infection) 2-5 and superinfect again in 7 min followed by the addition of 9-aminoacridine (9-AA) in 30 min. The cells continued shaking for 0.5 hr and the cell pellet was obtained by centrifuging at 10,000 xg for 10 min and resuspended in SM buffer (10 mM, Tris-HCl, NaCl 100 mM, $MgSO_4$ 50 mM, Gelatin 0.01%) pH 7.5 supplemented with 1% of $CHCl_3$, 20 μ g/mL DNase I, 20 μ g/mL RNase A and 10 μ g/mL Lysozyme. The suspension was shaken at 37 °C for 1 hr before subjecting to centrifugation at 20000 xg for 30 min. The supernatant was then filtered through Amicon ultra centrifuge filter with cutoff 100 kDa (MilliporeSigma, Burlington, MA) and washed with PBS 6 times to get rid of small proteins, DNA and RNA fragments and concentrate the supernatant. The resulting supernatant was then purified using Superose 6, 10/300 GL (GE Healthcare Life Sciences, Pittsburgh, PA) running on BioRad NGC medium pressure chromatography system equipped with ChromLab 6.0 software (Bio-Rad, Hercules, CA) with the flow rate 0.5 mL /min and 0.75 ml/fraction. The fraction right after void volume was collected.