

Structural and Functional Characterisation of the
Entry Point to Pyocyanin Biosynthesis in
Pseudomonas aeruginosa Defines a New 3-
Deoxy-D-*arabino*-heptulosonate 7-phosphate
Synthase Subclass

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SUPPORTING INFORMATION

MtuDAH7PS β_0 α_{0a} α_{0b}

1 10 20 30 40 50

MtuDAH7PS MNWTVVDIPIDQLPSLPLPTD L R L R L D A A L A K P A A O O P T W P A D Q A A M A R T V L E S V P P V T
PaeDAH7PS_PA2843 M S Q S W S P E S W R A K P I Q O O P E Y P D A A E H T A R V E Q T L A G Y P P L V
PaeDAH7PS_PA1901 M D D L L Q R V R R C E A I O O P E W G D P S R T R D V Q A Y T R G S P A L I
SacDAH7PS M S R Y P F P G A G H D P L I A S M V A A L P A R O O P Q W E P S R T A P V H E R L A R E S F L V
PchDAH7PS M E D I L K R V L S C E A F O O P Q W S E P S Q T H D A Q A Y L R D S A S L I
SfuDAH7PS_SFUL3326 M D N V V S E I R L R P A I O O P D W P D A E R T E E V R R T L S G R R A L V
SfuDAH7PS_SFUL5264 M D I D V R D I S S R V A L O O P D W A D D P R T E V R A Q T A R H D A L V

MtuDAH7PS α_{0c} β_1 α_1

60 70 80 90 100 110

MtuDAH7PS V P S E I V R L Q E Q L A Q V A K G E A F L L Q G G D C A E T F M D N T E P H I R G N V R A L L Q M A V V L T Y G A S M
PaeDAH7PS_PA2843 F A G E A R E L R R Q F A E V T A G R A F L L Q G G D C A E S F A E F S A A K I R D T F R V I L Q M A V V M T F A A G C
PaeDAH7PS_PA1901 R A C D I L A L R A T L A R V A R G E A L V V Q G G C A E D M D H H A E N V A R K A V I E L L A G A L R L A G R R
SacDAH7PS S Y T S V R E L R G L L A R A A E C E F C L I Q A G D C V E L T T E C E P C D V E R K V E M D D V L G D V M R T G S G L
PchDAH7PS R Y E D I L V L R A T L A R V A A C E A M V I Q S G D C A E D M D E S T P D H V V R K A V I L L A G T F R I V T Q Q
SfuDAH7PS_SFUL3326 R Y E D V R D L R Q I L A R V A R G E A Q V V Q G G C A E D P M E S S A G Y V A R K A A V I D L L A G A M K M A S H K
SfuDAH7PS_SFUL5264 R A E D V R R L R A V L A R V A A G E A H L V L A G D C A E D P A E C T S S Y V A R K A A I D V L A G T L K M V T R R

MtuDAH7PS β_2 TT

120 130 140 150 160 170

MtuDAH7PS P V V K V A R I A G O Y A K P R S A D I D A L G L R S Y R G D M I N G F A P D A A A R E H D P S R L V R A Y A N A
PaeDAH7PS_PA2843 P V V K V G R M A G O F A K P R S S G D E T Q N G V T L P A Y R G D I V N G I G F D E K S R V P D P E R L L Q A Y H Q S
PaeDAH7PS_PA1901 P V I R V G R I A G O F A K P R S K P H E Q V G E Q T L P V Y R G D M V N G R E A H A E Q R A D P P O R I L C Y A A A
SacDAH7PS P V V R V G R M A G O F A K P R S D D W E T V E G G R L P V F R G P V V N A P D A C E E A R T F P S R V I T G L E A A
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MtuDAH7PS α_2 α_{2a} α_{2b}

180 190 200 210 220 230

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PaeDAH7PS_PA2843 T A S L N L L R A F A Q G G F A D L H Q V H R W N L D F I A N S A L A E R Y Q O L A D R I D E T L A F M R A C G L D S A
PaeDAH7PS_PA1901 R N I M R H L G W D A A S G
SacDAH7PS R C A V T A L D R L G R G E
PchDAH7PS R D I M Q H L G W K A S A S Q
SfuDAH7PS_SFUL3326 G E M M G H L G W H S T A R E F
SfuDAH7PS_SFUL5264 A A V M A G L G R L P S G A

MtuDAH7PS β_3 α_3 β_4 α_4

240 250 260 270 280 290

MtuDAH7PS . N L Q T A E I Y A S H E A L V L D Y E R A M L S L S D G D D G E P Q L F D L S A H T V W I G E R T R O I D C A H T A F
PaeDAH7PS_PA2843 P Q L R E T S F F T A H E A L L N Y E A L T R R D S I T C E W Y D C S A H M L W I G D R T R O I D C A H V E M
PaeDAH7PS_PA1901 E . A N A S P V W T S H E M L L D Y E L S M R E D E Q R R V Y L G S T H W P W I G E R T R O V D C A H V A L
SacDAH7PS G A A P E E R V W T S H E A L L D Y E L P Q V R R H T D C G S Y I A S T H W P W I G E R T R O A D A H V R L
PchDAH7PS E Q L S G S P A W T S H E M L V L D Y E L P Q R R D E Q C R V F L G S T H W P W I G E R T R O L T C A H V T L
SfuDAH7PS_SFUL3326 G R A I D P P V W T S H E A L L D Y E L P M L R R D E S G A R W L A S T H L P W I G E R T R A L D C A H V E L
SfuDAH7PS_SFUL5264 P R Q I D P D V W T S H E A L L D Y E V P M L R R E A G C Q L L I G S T H W P W I G E R T R O V E C A H V A L

MtuDAH7PS β_5 α_5 β_6 α_6

300 310 320 330 340 350

MtuDAH7PS A Q V I A N P V G V K L G P N M T P E L A V E Y V E R L D P H N K P G R I T L V S R M G N H K V R D L L P P I V E K V Q
PaeDAH7PS_PA2843 I R G V G N P I G V K V G S M D S E E L I R L I D I L N P D N D P G R I N L I V R M G A D K V G D H I P R L I Q A I Q
PaeDAH7PS_PA1901 L A E V L N P V A C K V G P E I G R D Q L L A L C E R L D P R R E P G R I T L I A R M G A Q V G E R L P P I V E A V R
SacDAH7PS M T E L D N P V A C K V G P E A T V D E V L D L C A V L D P H R T P G R I T L I A R F G A D R V A . A L A P L V R A V R
PchDAH7PS I S E V I N P V A C K V G P D I T R D Q L L S L C E R L D S K R E P G R I T L I A R M G A Q V A D R L P P I V E A V R
SfuDAH7PS_SFUL3326 F A S I A N P V A C K V G P S M T V A D L L G L C E R L D P D R E P G R I T L I S R M G A G A V G E R L P A L V A A V R
SfuDAH7PS_SFUL5264 L A A V V N P V A C K V G P R M A P D E L V A L C A R L D P G R E P G R I T L I A R M G A G A V A G A L P P I V T A V R

MtuDAH7PS β_7 α_7 β_8

360 370 380 390 400 410

MtuDAH7PS A T C H Q V I W Q C D P M H G N T H E S S T G K T R H F D R I V D E V Q G F F E V H R A L G T H P G G I H V E I T G E
PaeDAH7PS_PA2843 R E C R Q V I W S S D P M H G N T I K A S S G Y K T R D F A R V L A E V R Q F F E V H Q A E G S Y A G G I H F M T G Q
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SacDAH7PS Q A C H P V L W M C D P M H G N T V K T P D G L K T R R L D T I M S E I R Q C V D V L A E N G E R C A G L H L E A S P D
PchDAH7PS Q A C H K I I W L S D P M H G N T I V A P C G N K T R M V Q A I T E I T A F K H A V T S A G G V A A G L H L E T T P D
SfuDAH7PS_SFUL3326 A A C H P V S W L C D P M H G N T V T T G E G L K T R Y L E H V E R E V R G F L T A V R S A D G T A G G I H L E T T P E
SfuDAH7PS_SFUL5264 E A C H P V I W L T D P M H G N T V T T A T G V K T R F V E T V V E E V R Q F Q L A V R Q A G G V A G L H L E A T P D

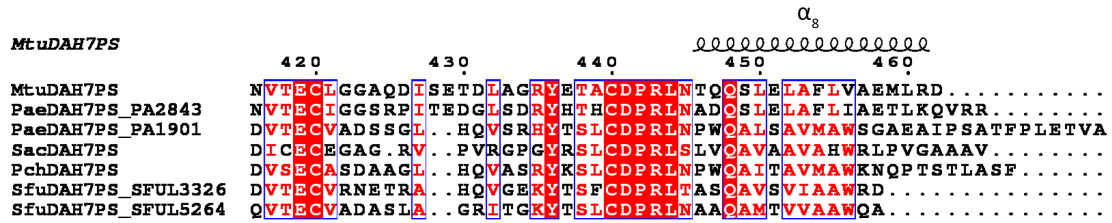


Figure S1: Sequence Alignment among the type II DAH7PSs, indicating the relative positions of β strands and α helices based on MtuDAH7PS (PDB 2B7O). Mtu, *Mycobacterium tuberculosis*; Pae_PA2843, *Pseudomonas aeruginosa*; Pae_PA1901, *Pseudomonas aeruginosa*; Sac, *Streptomyces achromogenes*; Pch, *Pseudomonas chlororaphis*; Sfu_SFUL3326, *Streptomyces fulvissimus*; Sfu_SFUL5264, *Streptomyces fulvissimus*.

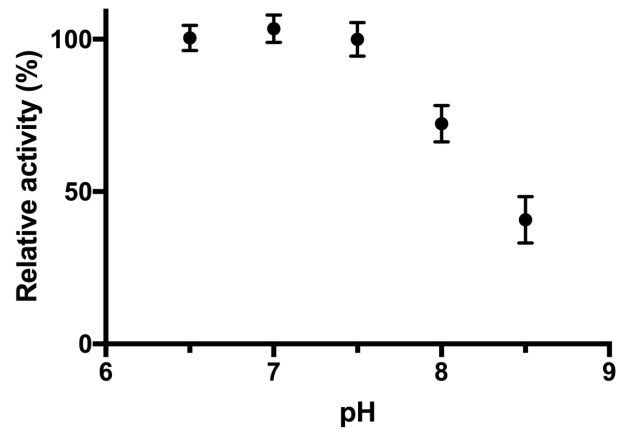


Figure S2: Activity of *PaeDAH7PS*^{PA1901} measured at a range of pH, relative to the activity observed at pH 7.5, indicates that this enzyme is active over a range of pH between pH 6.5 and 7.5. Substrate concentrations were held at 150 μ M each for PEP and E4P.

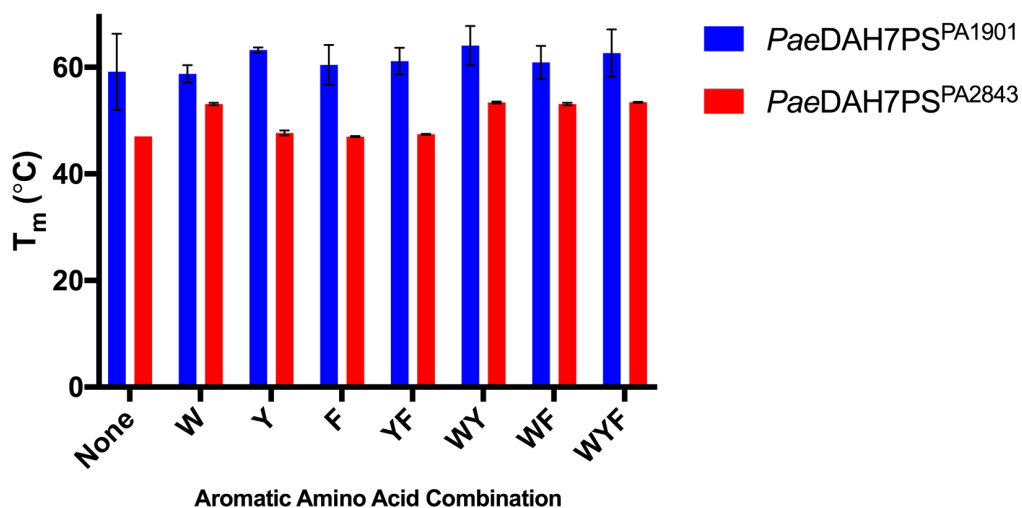


Figure S3: The melting temperature of *PaeDAH7PS*^{PA1901} and comparison to that previously reported for *PaeDAH7PS*^{PA2843} [1] in the presence of various combinations of aromatic amino acids. Each single letter code corresponds to 200 μ M of the appropriate amino acid.

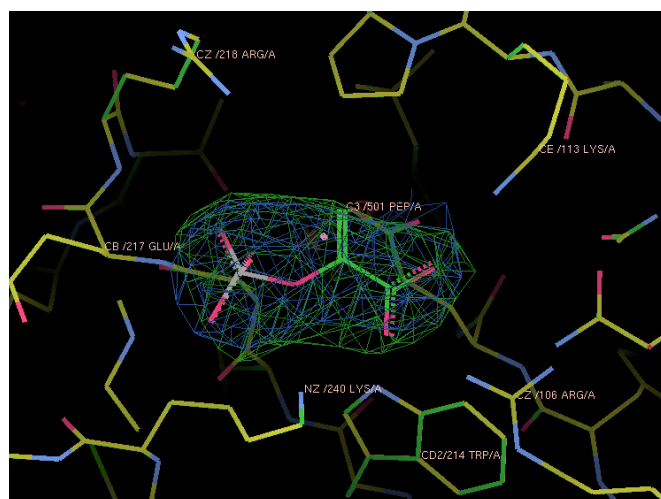


Figure S4: Electron density omit maps for PEP at the active site of *PaeDAH7PS*^{PA1901} (PDB **6BMC**). 2FoI-FcI is represented by the blue mesh contoured at 1.5 σ and FoI-FcI is represented by the green mesh contoured at 3 σ .

Table S1: SEC-SAXS sample and data collection parameters.

Sample Details	<i>PaeDAH7PS</i>^{PA1901}
Organism	<i>Pseudomonas aeruginosa</i>
UniProt sequence ID	G3XCJ9
Extinction coefficient [A_{280} , 0.1 %]	1.22
M from chemical composition (Da)	44, 468
SEC-SAXS column	5 x 150 mm, Superdex S200
Loading concentration (mg mL ⁻¹)	1.0, 5.0 or 8.0
Injection volume (μ L)	20
Flow rate (mL min ⁻¹)	0.2
Solvent	50 mM bis-Tris propane pH 7.5, 100 μ M cobalt chloride, 200 μ M PEP, 5 % glycerol
SAXS data collection parameters	
Instrument	Australian synchrotron SAXS/WAXS beamline
Detector	Dectris PILATUS 1M
Sample environment	SEC-SAXS with sheath-flow cell
Wavelength (\AA)	1.0332
Camera length (mm)	1600
Set temperature ($^{\circ}$ C)	20

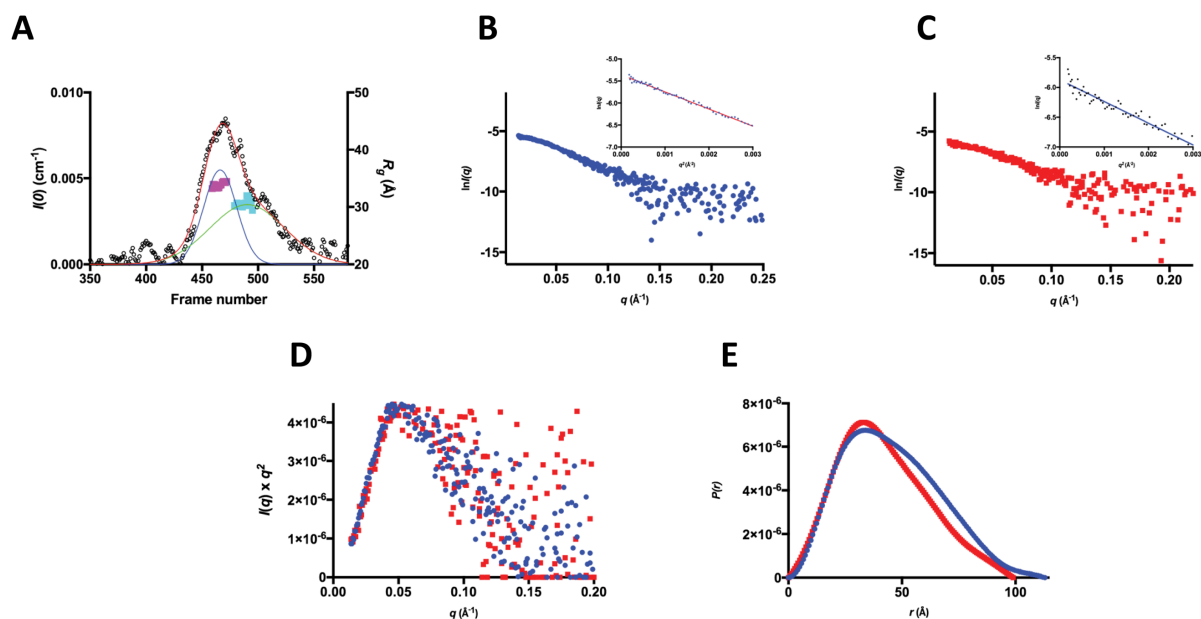


Figure S5: SEC-SAXS analysis for *PaeDAH7PS*^{PA1901} from the 5.0 mg/mL SEC-input concentration. (A) Deconvolution of the SEC-SAXS data indicates two Gaussian components (peak A, blue line and peak B, green line. Sum, red line). The R_g values across each peak are indicated as magenta or cyan squares respectively. (B) – (C) the SAXS profiles for the deconvoluted data peak A and peak B respectively. Guinier plots are inset. (D) Kratky plots of the deconvoluted data in (B) and (C) (peak A, blue circles and peak B, red squares). (E) $P(r)$ plots for the deconvoluted data in (B) and (C) (peak A, blue circles and peak B, red squares).

Table S2: SEC-SAXS parameters determined for *PaeDAH7PS*^{PA1901} from the 5.0 mg/mL SEC-input concentration.

	5.0 mg/mL Peak A	5.0 mg/mL Peak B
<i>Guinier Analysis</i>		
R_g (Å)	34.0 ± 1.6	32.0 ± 3.8
$I(0)$ (cm ⁻¹)	$0.004 \pm 5e^{-5}$	$0.004 \pm 1e^{-4}$
q_{\min}	0.013	0.013
Correlation coefficient, r^2	0.992	0.928
<i>Pair Wise Distribution Analysis</i>		
R_g (Å)	35.25	32.5
$I(0)$ (cm ⁻¹)	0.00	0.00
d_{\max} (Å)	113.1	99.0
V_p (Å ³)	167,000	126,000
q range (Å ⁻¹)	0.013 – 0.25	0.013 – 0.25

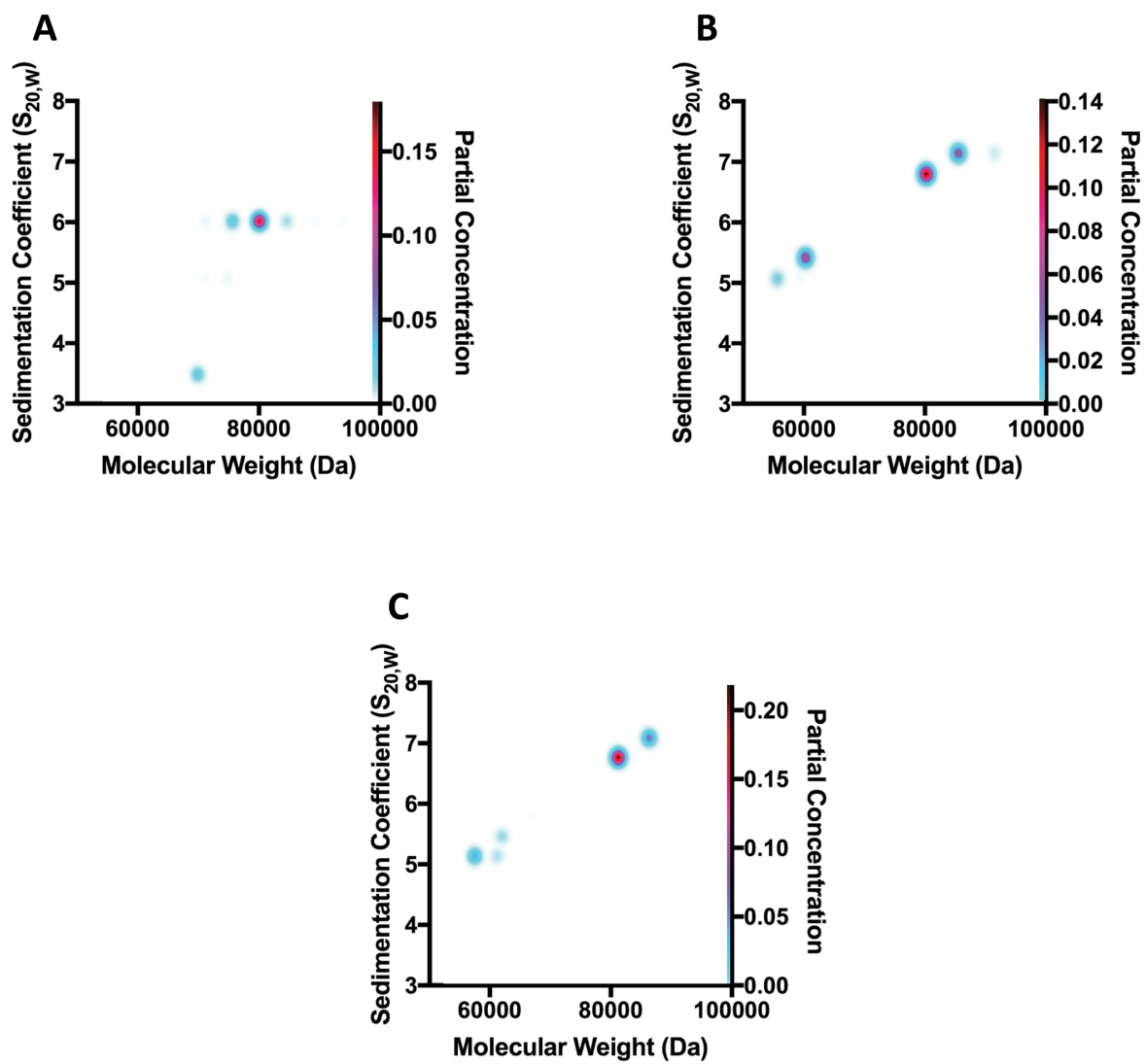


Figure S6: Pseudo-3D plots for distributions of the 2DSA Monte Carlo results shown in Figure 10 for (A) 8 μ M, (B) 23 μ M, and (C) 30 μ M concentrations of *PaeDAH7PS*_{PA1901}.

Table S3: RMSD values obtained for the alignment between the structures of *PaeDAH7PS*^{PA1901} and either *PaeDAH7PS*^{PA2843} or *MtuDAH7PS*.

Alignment between		RMSD (Å)
Single chain		
6BMC_chain A	5UXO	0.816
6BMC_chain A	5UXN	0.830
6BMC_chain A	5UXM	0.822,
6BMC_chain A	2B7O_Chain A	0.775
6BMC_chain A	2B7O_Chain B	0.821
6BMC_chain B	5UXO	0.816
6BMC_Chain B	5UXN	0.817
6BMC_chain B	5UXM	0.840
6BMC_chain B	2B7O_Chain A	0.754
6BMC_chain B	2B7O_Chain B	0.776
Crystallographic Dimer		
6BMC	5UXO	25.740
6BMC	5UXN	26.020
6BMC	5UXM	26.100
6BMC	2B7O	25.100
Crystallographic Tetramer		
6BMC	5UXO	27.850
6BMC	5UXN	28.610
6BMC	5UXM	28.800
6BMC	2B7O	34.460

References

1. Sterritt OW, Kessans, S. A., Jameson, G. B., Parker, E. J. A Pseudoisostructural Type II DAH7PS Enzymes from *Pseudomonas aeruginosa*: alternative evolutionary strategies to control shikimate pathway flux. *Biochemistry*. 2018;57(18):2667 - 78.