Supplementary Material for

THE VIOLACEIN BIOSYNTHETIC ENZYME VIOE SHARES A FOLD WITH LIPOPROTEIN TRANSPORTER PROTEINS

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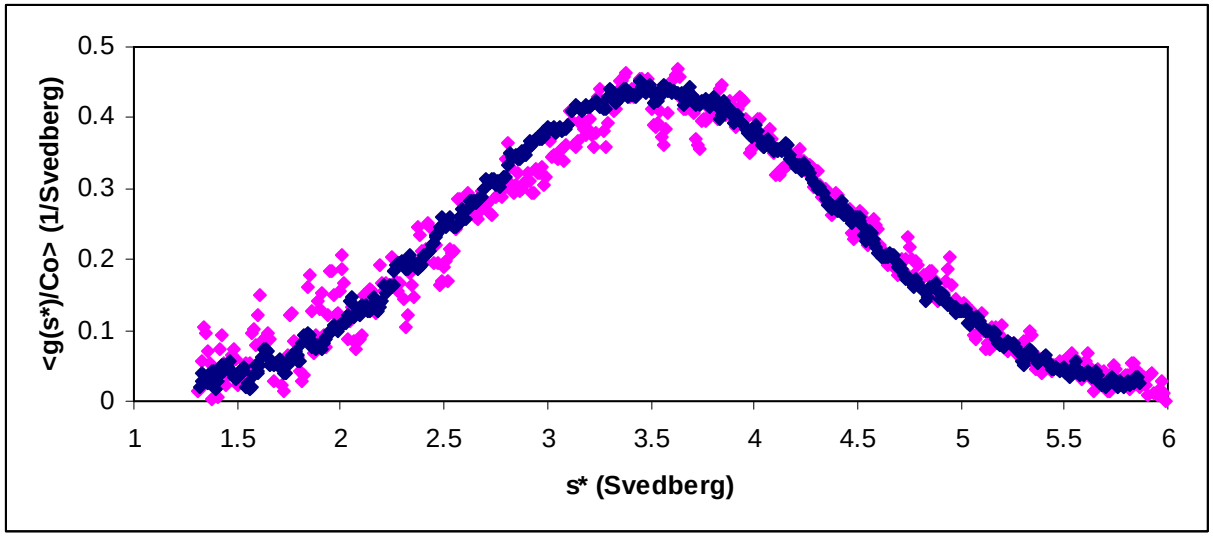
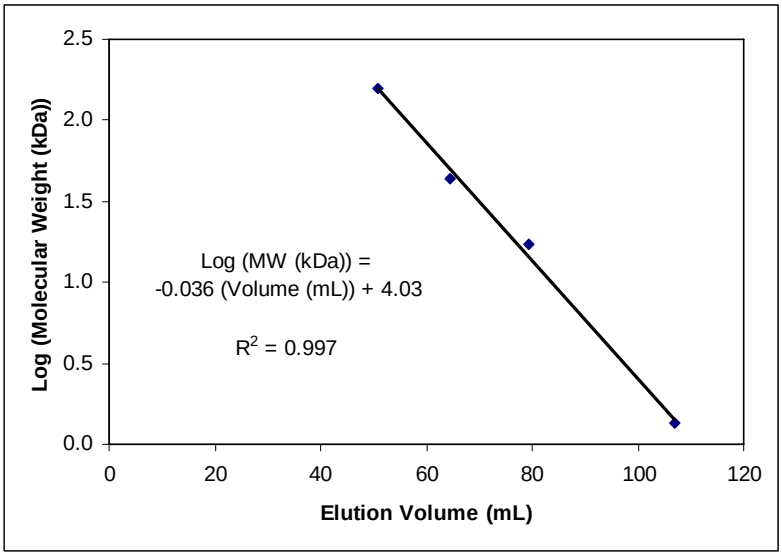


FIGURE S1. **Sedimentation velocity experiments.** Sedimentation velocity experiments were performed on VioE at observed loading concentrations of 8.6 μM and 2.8 μM. The single species model fit to this data using Sedanal gives a molecular mass of 45,879 g/mol (calculated molecular mass of C-terminally tagged VioE dimer is 45,685 g/mol). Determination of F-statistics gives a range of 41,200 to 51,000 g/mol for the molecular mass of the observed species, with a confidence level of 95%. These data are highly consistent with a VioE dimer in solution and are inconsistent with a VioE monomer or trimer in solution. Plots of the sedimentation distribution function normalized to loading concentrations are shown as a function of the sedimentation coefficient in Svedbergs, with the 8.6 μM sample shown in blue and the 2.8 μM sample shown in pink.

A



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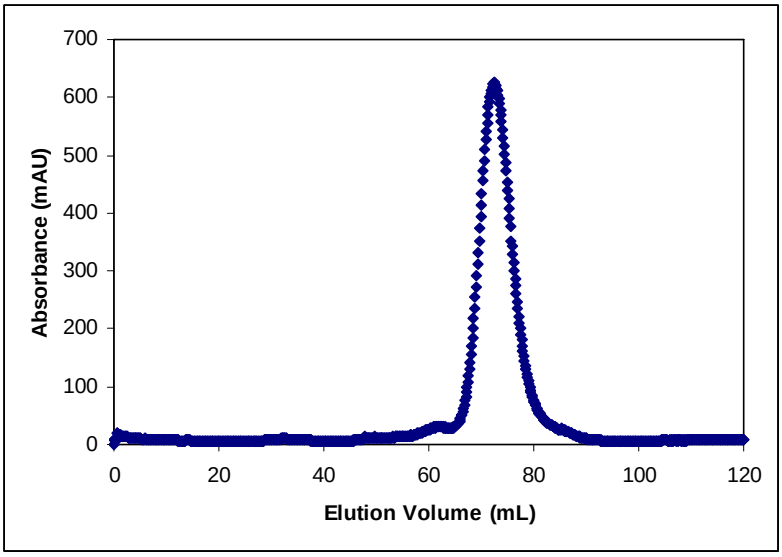

FIGURE S2. **Gel filtration of VioE**. Protein purified from a nickel (II) loaded Chelating Sepharose™ Fast Flow column was loaded onto a HiLoad Superdex™ 75 prep grade column. This column was previously calibrated using gel filtration standards, with bovine thyroglobulin (670 kDa) eluting at 46.8 mL, bovine γ-globulin (158 kDa) eluting at 51.0 mL, chicken ovalbumin (44 kDa) eluting at 64.6 mL, horse myoglobin (17 kDa) eluting at 79.4 mL, and Vitamin B_{12} (1.4 kDa) eluting at 107.0 mL. *A.* A standard curve was generated for the column using these standards, using the logarithm (base-10) of the molecular weight (in kDa) as a function of elution volume (in mL), excluding the value for bovine thyroglobulin, which is not on the linear portion of the curve. The linear regression equation and R^2 value are shown. *B.* VioE elutes as one major peak at 72.5 mL. This corresponds, based on the standard curve, to a calculated molecular weight of 26.3 kDa. The molecular weight of VioE (including the C-terminal tag) is 22.8 kDa, and the calculated dimeric molecular weight is 45.7 kDa. The peak corresponding to the calculated molecular weight of 26.3 kDa is more likely to represent monomeric VioE.

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JLD             ---MPTHVSPPLLPMQWSSAYVSYWTPMQADDQVTSGYCWFDYARNICRIDGLFNPWSEK 57
JLB             ---MSIQVAPPLLPMKWSSAYISYWTPMQEDDQVTSGYCWFDYARNICRIDGLFNPWPEK 57
CV              ---MENRE-PPLLPARWSSAYVSYWSPMLPDDQLTSGYCWFDYERDICRIDGLFNPWSER 56
PT              MQLSKITKKPPLLPAQWSSSYISYWMPMQPDDDITSGYCWFDYTKNVCRIDGIFNPWPEI 60
                         ***** :***:*:*** **  **::********* :::*****:****.*

JLD             EHGHLLWMSEIGDARREQSRKQKVAYARQAEAAGEQLQGTALADEVTPFHELFLPQAVLL 117
JLB             EHGHLLWMSEIGDARREQSRKQKVAYARQAGATGEQLQGTALADEVTPFHELFLPQAVLL 117
CV              DTGYRLWMSEVGNAASGRTWKQKVAYGRERTALGEQLCERPLDDETGPFAELFLPRDVLR 116
PT              KMGNRLWMSEIMYPNTDESFKSKVAYAREDMKSISEFSAQVLDDEIDPCHELILTQKVLI 120
                . *  *****:  .   .: *.****.*:     .::    * **  *  **:*.: **

JLD             DGGARHDGRHTVLGREADAWVVERA-GKPPSVFYLEAGGNRLLRMVTGNDPQHLSVRDFP 176
JLB             DGSARHDGRHTVLGQEADAWVMERA-GKPPSVFYLEAGGNRLLRMVTGNDPQHLSVRDFP 176
CV              RLGARHIGRRVVLGREADGWRYQRP-GKGPSTLYLDAASGTPLRMVTGDEASRASLRDFP 175
PT              ECNAQYMGIETVLGHQAEKWLFQRPDNKGPATYYFINGTNHLVRMITGDPKICASVRDFP 180
                  .*:: * ..***::*: *  :*. .* *:. *:  . .  :**:**:     *:****

JLD             NLFVSDIPDSVFTSCNT-- 193
JLB             NLFVGDIPDSVFTSCNT-- 193
CV              NVSEAEIPDAVFAAKR--- 191
PT              NFNTYKIDNEIFKPEPLKK 199
                *.   .* : :* .
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FIGURE S3. **Homologues of VioE.** A ClustalW alignment of the closest homologues of VioE from *Chromobacterium violaceum* ATCC 12472 (indicated as 'CV') is shown. The homologue from *Janthinobacterium lividum* DSM 1522 is indicated as 'JLD,' the homologue from *Janothinobacterium lividum* BP is indicated as 'JLB,' and the homologue from *Pseudoalteromonas tunicata* D2 is indicated as 'PT.' Identical residues are indicated with *****, and similar residues are indicated with one or two dots, according to the conventions of ClustalW.

```
VioE  86  RTALGEQLCERPLDDETGPFAELFLPRDVLRRLGARHIGRRV 127
RifG   2  RTTIPVRLAERSYDVLVGPGVRAALP-EVVRRLGAR---RAV  39

VioE 128 VLGREADGWRYQRPGKGPSTLYLDAASGTPLRMVTGDE     165
RifG  40 VVSARPADWV---PGTGVETLLLQARDGEPTKRLSTVE      74
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FIGURE S4. **Comparison of VioE to RifG.** RifG, a 351 amino acid protein from *Amycolatopsis mediterranei*, shows similarity to VioE, a 191 amino acid protein. RifG aligns at its N-terminus to the central region of the VioE sequence. Shown are only the residues aligned from a BLAST search. Based on the VioE structure, the highest identity is in the loop that connects β6 to β7, bridging one side of the protein to the other. RifG is a putative 5-amino 3-dehydroquinate synthase (1), thought to carry out a cyclization of 3,4-dideoxy-4-amino-D-*arabino*-heptulosonic acid 7-phophate (2). Heterologous expression studies have shown that RifG is involved, together with RifJ and RifH, in the synthesis of 3-amino-5-hydroxybenzoic acid (1). It is unclear what RifG's similarity to VioE reveals about either protein. Yellow highlighting indicates identical residues, whereas gray highlighting indicates similar residues.

```
VioE   1  --------------------------------MENREP  6
LolA   1  ------------------------------DAAS-----D  5
LolB   1  ASVTTPKGPGKSPDSPQWRQHQQDVRNLNQ----------  30

VioE 7    PL--L--PARWSSAYVS-Y-WSPMLPDD----------Q-  29
LolA 6    LKSRLDKVSSFHASFTQ-KVT--------DG--SGAAVQ-  33
LolB 31   ---------YQTRGAFAY--I-------SD-QQ-----KV  46

VioE 30   LTSGYCWFDY-E-R-DICRIDG----LFNPWSERDTGYRL  62
LolA 34   EGQGDLWVKR-P---NLFNWHMTQPD--------------  55
LolB 47   -YARFFWQQ-TGQ-DRYRLLLTN-----------------  66

VioE 63   -----WMSEVGNAAS--GRTWKQ--KVAYGRERTALGEQL  93
LolA 56   -----ESILVSD-GK---TLWFY--NPF------------  72
LolB 67   PLGST-ELELNAQ--PGNVQLV-DNK--------------  88

VioE 94   CERP-L--DDETGP-FA--EL-------------------  108
LolA 73   ----VEQ---ATATWLKDAT-GNTPF--------------   90
LolB 89   -------G-QRYTAD-D---------AEEMIGKLTGMPIP  110

VioE 109  F--LP-RD------VLR---R-----------LGARHI-G  124
LolA 91   M-LIARNQ---------SSDWQQYN---------IKQ--N  109
LolB 111  LNSLR-Q-WILGLPG-----D-----------ATDYK-L-  130

VioE 125  RRVVLGREA-------DGWRYQRPGK-------GPS-TL-  148
LolA 110  ---------G------DDFVLTP----KSNGNLKQF-TI-  129
LolB 131  ----------DDQYRLSEITYS-QN-G------KNWKV-V  151

VioE 149  YLDAA----------SG-T-PLRMVTGDEASR----ASLR  172
LolA 130  NVG--R---------DG-T-IHQFSAV-----EQDQRSSY  152
LolB 152  Y-----GGYDTKTQPA-MPA-NMELTDG---G----QRIK  177

VioE 173  DFPNVSEA--EIPDAVFAAKR----------           191
LolA 153  QLKSQQNG--AVDAAKFTFTPQGVTVDDQRK          182
LolB 178  LKMDNW-IVK--------------------           186
```

FIGURE S5. **Structure-based sequence alignment of VioE, LolA, and LolB.** The structure of VioE was individually aligned using LSQMAN (3) to the structures of LolA and LolB (4), which, based on analysis using DALI (http://www.ebi.ac.uk/dali/), had significantly higher structural homology to VioE than did any other published protein structures. A final structural alignment was generated where distances between Cα carbons were within 3.5 Å. Structurally aligned sequences of VioE, LolA (PDB code 1UA8), and LolB (PDB code 1IWM) are shown with secondary structure assignments (blue text indicates β-sheets and red text indicates α-helices and 3_{10} helical turns). Gray highlighting indicates residues disordered in the crystal structures.

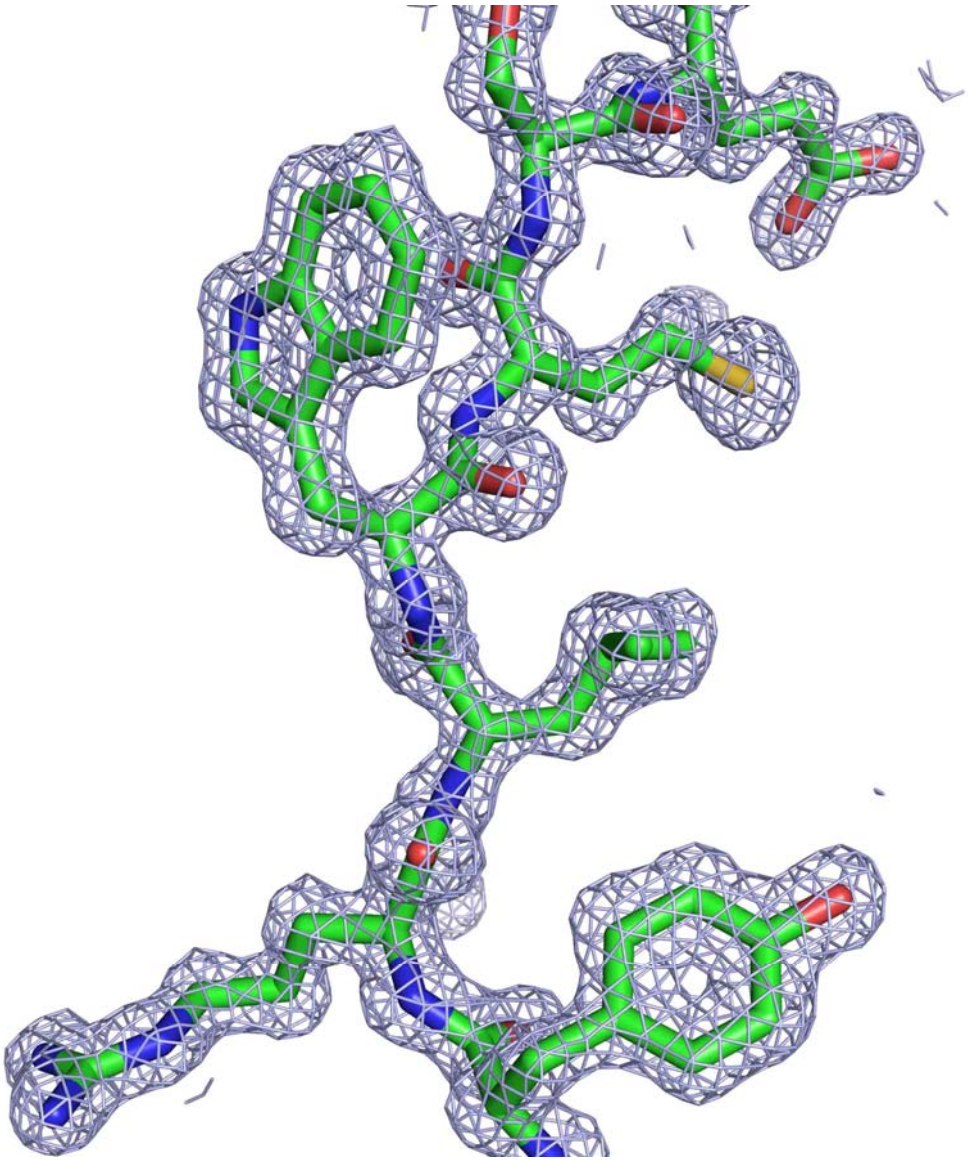
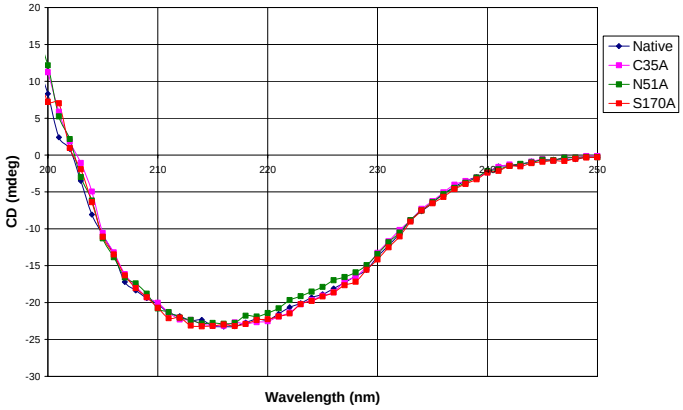


FIGURE S6. **Electron density for VioE**. Seven residues (Tyr^{60}, bottom, to Glu^{66}, top) are shown in a 1.5 σ sigmaA-weighted $2F_o$-F_c map. The high quality of the electron density over most of the structure, including this segment, is in contrast to the lower quality of density surrounding the PEG molecules.

A



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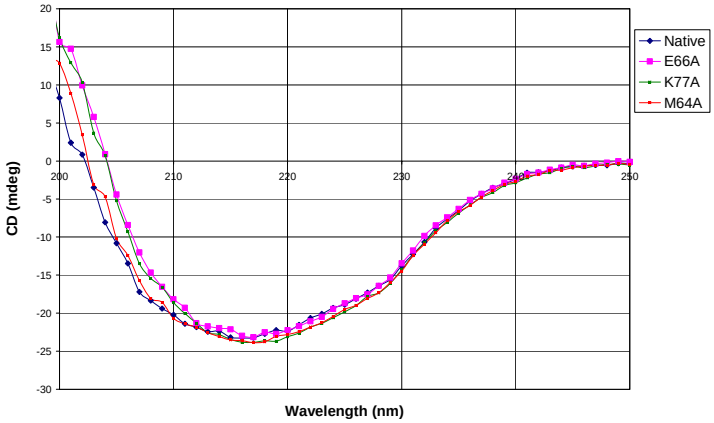

C

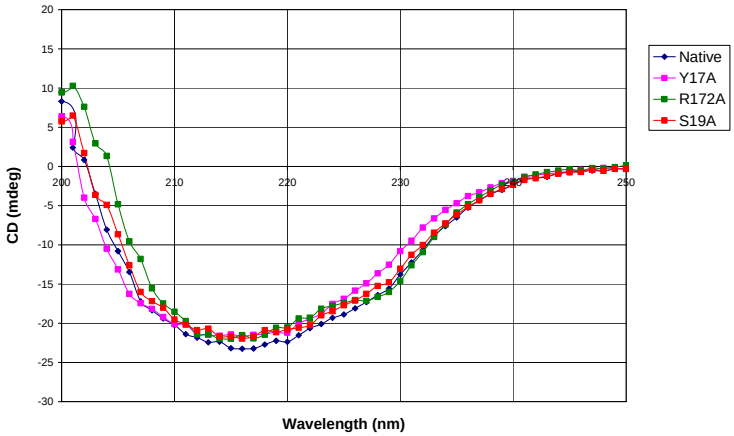

FIGURE S7. **CD Spectra of mutant proteins.** Circular dichroism spectra of all mutants appear similar to that of wildtype VioE, suggesting that all mutants are properly folded. a) Native versus Cys35Ala, Asn51Ala, and Ser170Ala. b) Native versus Glu66Ala, Lys77Ala, and Met64Ala. c) Native versus Tyr17Ala, Arg172Ala, and Ser19Ala.

TABLE S1: **Primers used for generation of site-directed mutants.** Bold text within oligonucleotide sequences indicates restriction digest sites, italicized text indicates modified overhangs, and underlined text indicates the modified codon.

Protein	Fragment	Sequence
All mutants	a – 5' forward	*caagtta***catatg**gttaaccgcccgtacgc
	b – 3' reverse	*caagtta**ctcgag***ctcgctatctgtaatcacgtc
VioE Y17A	a – 3' reverse	catcggactccagtacgacacgcggcgctgctcca
	b – 5' forward	ccggcgcgctggagcagcgccgcggtgtcgtactgg
VioE S19A	a – 3' reverse	cggcagcatcggactccagtacgccacataggcgct
	b – 5' forward	cgctggagcagcgcctatgtggcgtactggagtccg
VioE C35A	a – 3' reverse	gtcgcgctcgtagtcgaaccacgcgtagccggacgt
	b – 5' forward	gaccagctgacgtccggctacgcgtggttcgactac
VioE N51A	a – 3' reverse	ggtgtcgcgctccgaccagggcgcgaacaggccgtc
	b – 5' forward	tgtcggatagacggcctgttcgcgccctggtcggag
VioE M64A	a – 3' reverse	ggcggcgttgccgacctcggacgcccacagccggta
	b – 5' forward	gacaccggctaccggctgtgggcgtccgaggtcggc
VioE E66A	a – 3' reverse	gccgctggcggcgttgccgaccgcggacatccacag
	b – 5' forward	ggctaccggctgtggatgtccgcggtcggcaacgcc
VioE K77A	a – 3' reverse	gcggccataggccaccttctgcgcccaggtgcggcc
	b – 5' forward	gccgccagcggccgcacctgggcgcagaaggtggcc
VioE S170A	a – 3' reverse	gacgttggggaaatcgcgcagcgccgcgcgcgacgc
	b – 5' forward	ggggacgaggcgtcgcgcgcggcgctgcgcgatttc
VioE R172A	a – 3' reverse	ctcgctgacgttggggaaatccgccagcgacgcgcg
	b – 5' forward	gaggcgtcgcgcgcgtcgctggcggatttccccaac

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