Plasmodium IspD (2-C-methyl-D-erythritol 4-phosphate cytidyltransferase), an essential and druggable antimalarial target

Leah S. Imlay, Christopher M. Armstrong, Mary Clare Masters, Ting Li, Kathryn E. Price, Rachel L. Edwards, Katherine M. Mann, Lucy X. Li, Christina L. Stallings, Neil G. Berry, Paul M. O'Neill, and Audrey R. Odom

Supporting Information

10 pages

4 figures

1 table

Sequence information for codon-optimized genes

Codon-optimized PfIspD coding sequence:

ATGGCTCACCACCACCACCACCATATGATGCACATCTACGATAATAATAAAGAAAAC
GACATCTTCAACAAATACAACACGAAACAATACGAAAAAATCATCAAAAAAAAAAAC
ATTCATAGTATCCTGCTGTGCGGCGGTATTGGCAAACGTACCGAACTGATCGGCCC
GAAACAGTTTCTGAAACTGAACGATATTCCGCTGTTCATCTACAGTTTCAACCTGTT
TATCAAATGTAACCTGATCAAATCCCTGACCCTGGTTTGCGATAAAAAACATTTCAG
TTGTATCATCCACTCCATTAACGTCTATAATCAACTGCTGCTGAAACGCAAAATGAT
CAACTCTTTTCTGAAAAACGGCAACGATAAACTGAACATCAACCTGAAAGAATGCGA
TCAGGACGAAAAAGATAACATCAAAGACTTCAGCTCTCAAAAAATCAACCTGGGTGA
AAACCACTTTAACGATGACAGCAACATCATCGATTACATCCATAAAAACACCTACAT
CATCTACGATAACGAGAAAAACAAATGTATTCTGGACATGCAGGAACTGCTGAACG
ATCTGTCTAAAGACATTAAAATCCGTAAGAAAAAAGAAATCAACGAAATCAACGAAA
AACATGAAAAATACGAAATCCACGAGAAAAACGAAAAACATGAGAAAAACGAGAAAA
ACGAGAAAAACGAAAAAGATGAAAAAGACGAAAAAGACGAAAAAGATGAAAAACAC
ATCATCCAGATCAAACAAAAACATATCAACGTCAACCGTTATAAACTGCTGAAAATT
GTGGAATCAGGCAAAGAACGCCTGGATTCGTTCCTGAACGCAATGAAAAGCATTGA
TATTGAACTGGACTCTCAGATGTACATCTACGAACTGCTGAAAAAATACATCCAAGG
TAAAAACAACAAAAACGATAACATCAGCTATGAATTTGAAGACGTGAACATCAACAA
ATGCAACAAAAACTACAACAGCAACAACGATTCTATGCAGAACAACAACATCAAAGA
CAAAAAAGAAGAAAAGAAAAAGAAAATTCACATCACCAACATTCTGATCCATGATGG
CGCGCGTCCGTTCCTGTCAGAAATCGACTTTTTCAACCTGATCTACTACTCAACGCT
GGATAAAAACGTTATCCTGGGTTCGAAAGCCACCGACACGATTAAACTGATCCAGC
ACGAAGAAGAAAATAAAAAGAAAACCACGTCACCGTTTATTAAGAAAACCATCGATC
GCGACACGATTTTCCAGGCGCAAACCCCGCAGATTTTTGATTCGAAAACCCTGCAT
AATAACATTCTGACGTACATCCTGCCGATGAAAAACAACGAAAAGAAAATTAAACAA

ACGAACATCAATATTATCAATGATAATAACTATCCGAACAGTGAACAGCAACACAAT
AAACAGTTTACCGATACGAGTTCCCTGTATCAATACTTCAACAAAAGCAAGAAAAAG
AAAATTTTTGTCCTGCAATCTAACTTCCCGAACTTTAAAGTGACCACGCCGGAAGAT
GTGCTGCACTCCTTTTTCCTGATGAAATACATCTACAACTACAAATTCATCGATATTG
AAGAAAGTATCTTCAAAGACGAATACATCAACTCCCATTCATCGTACATCCTGAAAA
AACAGTTTAATAACTTTTTCTTTTATGACGCCCTGAACGAAAAACAAAAAATCCTGTA
TCACAAATTCTACTACTCCTCAAAATAA

PfIspD protein sequence:

MAHHHHHHMMHIYDNNKENDIFNKYNTKQYEKIIKKKNIHSILLCGGIGKRTELIGPKQFL
KLNDIPLFIYSFNLFIKCNLIKSLTLVCDKKHFSCIIHSINVYNQLLLKRKMINSFLKNGNDK
LNINLKECDQDEKDNIKDFSSQKINLGENHFNDDSNIIDYIHKNTYIIYDNEKNKCILDMQE
LLNDLSKDIKIRKKKEINEINEKHEKYEIHEKNEKHEKNEKNEKNEKDEKDEKDEKDEKHI
IQIKQKHINVNRYKLLKIVESGKERLDSFLNAMKSIDIELDSQMYIYELLKKYIQGKNNKND
NISYEFEDVNINKCNKNYNSNNDSMQNNNIKDKKEEKKKKIHITNILIHDGARPFLSEIDF
FNLIYYSTLDKNVILGSKATDTIKLIQHEEENKKKTTSPFIKKTIDRDTIFQAQTPQIFDSKT
LHNNILTYILPMKNNEKKIKQTNINIIINDNNYPNSEQQHNKQFTDTSSLYQYFNKSKKKKI
FVLQSNFPNFKVTTPEDVLHSFFLMKYIYNYKFIDIEESIFKDEYINSHSSYILKKQFNNFF
FYDALNEKQKILYHKFYYSSK

Codon-optimized PvIspD coding sequence:

ATGGCTCACCACCACCACCACCATCCGCGTCTGTTTGAAACCACCGCAAAAACCAA
TGATCATGACCGTGAAGAATGCAAAACCTATGAACAGAGCCTGCACAAAAAAAACA
TTCATGCAATTCTGCTGTGCGGTGGTATTGGTCAGCGTACCGAACTGGCAAGCCGT
AAACAGTTCCTGAAACTGAATGATGTTCCGCTGTTTGTGTATAGCTTCAACCTGTTT
GTTAAATGCAACCTGATTAAAGCCATTACCCTGGTTTGTGATCCGAACTATTTCCAG

CATGTGATTGAAAGCATTAATCGCCATAATGCAAGCCTGCTGCGTCGTAAACATCA

GCGTGGTTTTCTGCGTCGTGGTCATAGCAAAAATGTTCTGGCAAGCAGCGGTGAAC

AGAGTGAAGGTGATGCAAGCGGTGCACTGCATTTTCTGAAAAAAAACAAATATATCC

TGTACGACAACGAAAAAGGCAAATGCGTTACCAATCTGGATGAACTGCTGAGTGAT

GTTACCGCAACCAAAGGTCAGTATGTGAGCGAAGTTGATGAGGTTGATGAAGTTAA

ACCGGGTGATGTTAATAGCAACCGCTATAAACTGATTCGTCTGGTTGAAAGCGGTC

GTGAACGTGCAGATAGCCTGCTGAATGCACTGCGTGGTCTGGATCTGCGTGTTCA

GACCGGTGAATATATCAGCCAGCTGCTGCGTGGTGGTGGCACCGATGAAGCAGGT

AAAGGTGATGGTGCAGATAAAGGCGATAAATCAGATGGTGTTGATGGTGGTCATCC

GGCAGATTGTACCCCGATTAGCCATATTCTGGTTCATGATGGTGCCCGTCCGTTTC

TGAGCGAACTGGACCTGTTTAACCTGATTTATATGGCAACCATTGGTCGCAATGCC

ATTCTGGGTAGCCGTGCAACCGATACCATTAAACGTATTGGCACCGAACAGCAGGG

TGAAAGCTGTCCGCGTGTTAAAGCACATATGGATCGTCAGTTTATCTTTGCAGCACA

GACACCGCAGATTTTTAGCAGCCAGGCACTGCTGCAGGTTTGTGCAAAACTGCCGA

GCCGTCGCGGTGGTGAAACACCGGAAGGTAGTCGTGCATTTACCGATACCTCAAG

CCTGTTTCAGCATGTTACCAAAAAAAAAGTTTTTGCCCTGCAGGCGAAATTTCCGAA

CTTTAAAATCACCACCCCGACCGATGTTTTTCTGGCAATTTTTCTGATGGGCTGCAT

CTTTAAAACCAGCCATAGTGATGTTGATATGGGCATGTTTAAAGAAACCTTTGTGAA

TAGCCCGAGCAGCTGTGTTCCGGCAAATCAGCTGAACGATCATTTTTTCTATCATAG

CCTGGGTGGTAAACAGCGTGTTCTGTATCGTCATTTCTATTATGAATAA

PvIspD protein sequence:

MAHHHHHHPRLFETTAKTNDHDREECKTYEQSLHKKNIHAILLCGGIGQRTELASRKQF

LKLNDVPLFVYSFNLFVKCNLIKAITLVCDPNYFQHVIESINRHNASLLRRKHQRGFLRR

GHSKNVLASSGEQSEGDASGALHFLKKNKYILYDNEKGKCVTNLDELLSDVTATKGQY

VSEVDEVDEVKPGDVNSNRYKLIRLVESGRERADSLLNALRGLDLRVQTGEYISQLLRG

GGTDEAGKGDGADKGDKSDGVDGGHPADCTPISHILVHDGARPFLSELDLFNLIYMATI

GRNAILGSRATDTIKRIGTEQQGESCPRVKAHMDRQFIFAAQTPQIFSSQALLQVCAKLP

SRRGGETPEGSRAFTDTSSLFQHVTKKKVFALQAKFPNFKITTPTDVFLAIFLMGCIFKT

SHSDVDMGMFKETFVNSPSSCVPANQLNDHFFYHSLGGKQRVLYRHFYYE

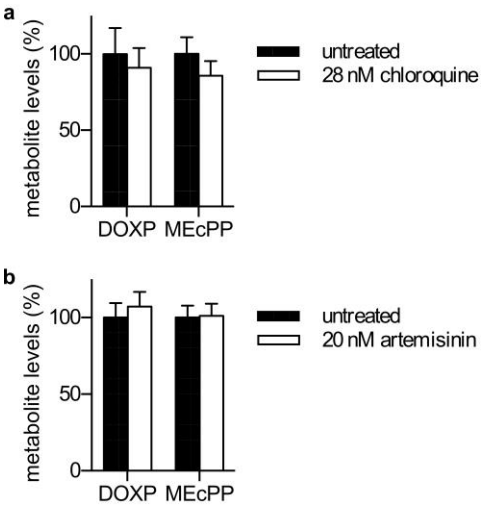

Figure S1. Artemisinin and chloroquine do not affect isoprenoid biosynthesis in malaria parasites.

MEP metabolites [deoxyxylulose phosphate (DOXP, upstream of IspD), and methylerythritol cyclic diphosphate (MEcPP, downstream of IspD)] were quantified by LC-MS/MS from *P. falciparum* parasites, following treatment with artemisinin (a) or chloroquine (b). Data are normalized to untreated controls and represent 3 independent biological replicates.

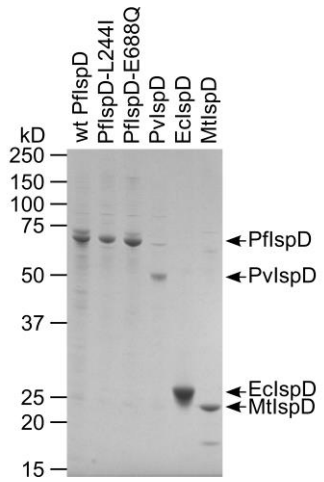

Figure S2. Purity of IspD enzymes used in enzyme assays.

Protein samples were separated by SDS-PAGE and stained with Coomassie Brilliant Blue R-250 dye. Anticipated molecular weights are as indicated. Estimated purity of protein preps is between 80-90% for each protein.

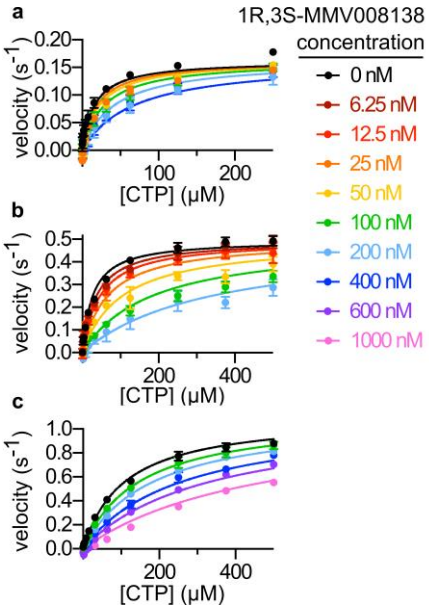

Figure S3. Competitive inhibition of additional IspD enzymes.

a) PfIspD-L244I kinetic parameters with respect to the CTP substrate.

b) Determination of PfIspD-E688Q kinetic parameters with respect to the CTP substrate.

c) Determination of PvIspD kinetic parameters with respect to the CTP substrate.

Each data point represents mean +/- S.E.M. from at least three independent experiments.

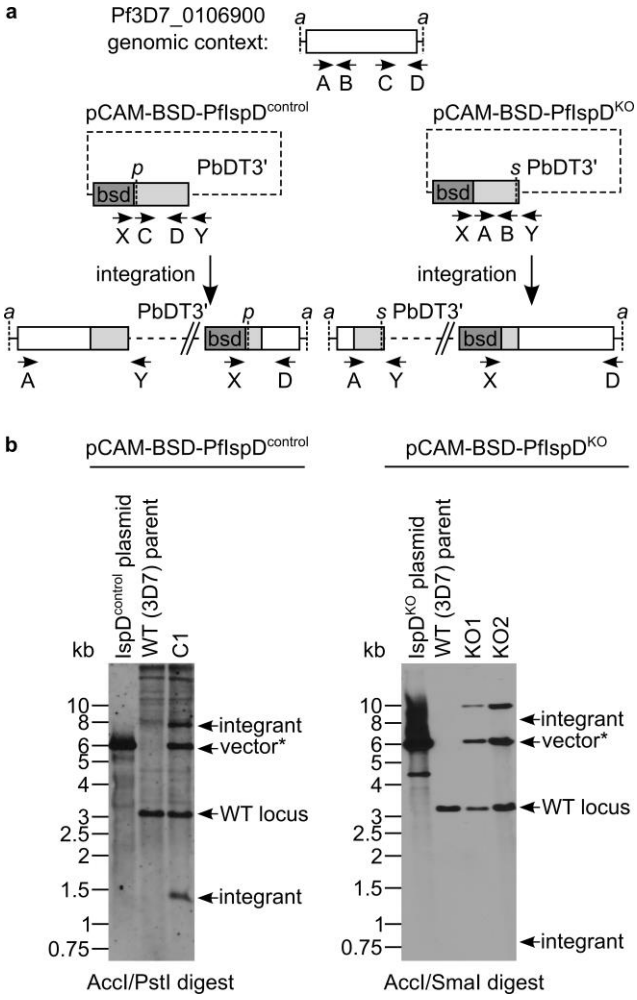

Figure S4. PfIspD locus is resistant to genetic disruption.

a) Strategy for single-crossover disruption of the *ISPD* locus, PF3D7_0106900. Transfection plasmids contain inserts homologous to the coding sequences for either the C-terminus (positions 1543-2205; pCAM-BSD-PfIspD^{control}; left) or an N-terminal section (positions 17-645; pCAM-BSD-PfIspD^{KO}; right) of PfIspD. Integration of the control vector is expected to recapitulate normal gene function, while integration of the knockout vector would disrupt functional PfIspD expression. Primers used in diagnostic PCRs are shown as arrows. AccI restriction sites are depicted with the letter *a*, PstI restriction sites are depicted with the letter *p*, and SmaI restriction sites are depicted with the letter *s*.

b) Southern blots to assess integration at the genomic *P. falciparum ISPD* locus, PF3D7_0106900. For transfection with pCAM-BSD-IspD^{control} (left), genomic and plasmid DNA was digested with AccI and PstI, transferred to membrane, and probed with the IspD fragment used as insert in the pCAM-BSD-IspD^{control} vector. If vector is present, a band is expected at 6000 bp; if the genomic locus is intact, a band is expected at 3100 bp; if integration has occurred, bands are expected at 7700 and 1400 bp. For transfection with pCAM-BSD-IspD^{KO} (right), genomic and plasmid DNA was digested with AccI and SmaI, transferred to membrane, and probed with the IspD fragment used as insert in the pCAM-BSD-IspD^{KO} vector. If vector is present, a band is expected at 6000 bp; if the genomic locus is intact, a band is expected at 3100 bp; if integration has occurred, bands are expected at 8300 and 800 bp (arrows). *, vector bands may indicate either episomal plasmids or integrated concatamers.

Table S1. Kinetic parameters of *Plasmodium* IspD enzymes with respect to the CTP substrate.

	K_m[CTP] (µM)	k_{cat}[CTP] (s^{-1})	K_i[CTP] (nM)
wt PfIspD	59 +/- 4.3	0.43 +/- 0.0084	14 +/- 1.3
PfIspD-L244I	16 +/- 1.9	0.16 +/- 0.0045	130 +/- 26
PfIspD-E688Q	27 +/- 2.1	0.50 +/- 0.0078	17 +/- 1.7
PvIspD	110 +/- 5.6	1.1 +/- 0.019	230 +/- 17