

Supporting Information

Metabolomic profiling reveals developmentally regulated biosynthesis of polyprenols and dolichols in the malaria parasite

Flavia M. Zimbres^{1,2#}, Ana Lisa Valenciano^{1,2#}, Emilio F. Merino^{1,2}, Nicole R. Holderman¹, Anat Florentin^{3,2}, Guijuan He⁴, Katarzyna Gawarecka⁵, Karolina Skorupinska-Tudek⁵, Maria L. Fernández-Murga⁶, Ewa Swiezewska⁵, Xiaofeng Wang⁴, Vasant Muralidharan^{3,2}, Maria Belen Cassera^{1,2*}

From the ¹Department of Biochemistry & Molecular Biology, University of Georgia, Athens GA 30602; ²Center for Tropical and Emerging Global Diseases (CTEGD), University of Georgia, Athens GA 30602; ³Department of Cellular Biology, University of Georgia, Athens GA 30602; ⁴School of Plant and Environmental Sciences, Virginia Tech, Blacksburg VA 24061; ⁵Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland; ⁶Laboratory of Experimental Pathology, Health Research Institute Hospital La Fe, Valencia 46026, Spain

Running title: *Biosynthesis of cis-polyisoprenols in P. falciparum*

Contributed equally to this work

* To whom correspondence should be addressed: Maria Belen Cassera: Department of Biochemistry & Molecular Biology and Center for Tropical and Emerging Global Diseases (CTEGD), University of Georgia, Athens GA 30602; maria.cassera@uga.edu; Tel. (706) 542-5192.

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Table S1. Specific primers used for developing a PfPPRD conditional knockdown.

Primer label (localization)	Primer Sequence
P1 (3'-UTR -F)	5'-CTTTCGGGCGCGCCTTAAGATATATGATAACATTATTTTTATATATATATATAAATAC ATTGGTGTAACGTGTTTTTTAATAAATGCTG-3'
P2 (3'-UTR-R)	5'-AAATATATTAAGATATCCTTACATCATGCATTGTTGATTTAATAGGTGATAATTAC-3'
P3 (C-term-F)	5'-GATATCTTTAATATATTTTTCGTTCCTTATTAACAATAATATC-3'
P4 (C-term-R-HA)	5'-ACGTCATAAGGATAGACGTCTCAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAAT CTGGAACATCATATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAAT ATGGGAAGATTATTTTTCTGTTCTTATATTAAG-3'
P5 (C-term-R)	5'-ACGTCATAAGGATAGACGTCTCACAAAATATATGGGAAGATTATTTTTCTGTTCTTATA TTAAG-3'
P6 (guide RNA-F)	5'-AAGTATATAATATTTAACGTGTTTTTTAATAAAGTTTTAGAGCTAGAA-3'
P7 (guide RNA-R)	5'-TTCTAGCTCTAAAACCTTTATTAATAAACAACCGTTAAATATTATATACTTA-3'
P8 (5'-homology region-F)	5'-TTGACTCTCATCTTCGATTAGCTAGGCTACGCCCAAAAGG-3'
P9 (aptamer-R)	5'-GTAGACCCCATTTGTGAGTACATAAATATATTATATAAACTAGACTAGG-3'

Table S2. Time-course of growth of PfPPRD-TetR-DOZI knockdown parasites treated with BSD only or BSD+aTc.

Time (day)	Normalized parasitemia (%)	
	PfPPRD + aTc	PfPPRD - aTc
0	0.65 ± 0.01	0.66 ± 0.10
1	0.75 ± 0.09	0.71 ± 0.05
2	1.70 ± 0.06	1.71 ± 0.06
3	2.51 ± 0.09	2.32 ± 0.14
4	4.36 ± 0.10	4.19 ± 0.21
5	5.86 ± 0.66	4.97 ± 0.62
6	11.35 ± 0.42	8.76 ± 0.77
7	20.09 ± 0.48	15.25 ± 0.40
8	31.52 ± 0.34	23.57 ± 0.41

Figure S1. *De novo* biosynthesis of medium-long DOH in *P. falciparum*. Mass spectra of DOH detected in *P. falciparum* schizont stage. Natural isotopic distribution of DOH is indicated for the standard. ^{13}C -enrichment is detected as a bell-shaped envelope as indicated on each spectrum. A representative mass spectrum of DOH-15 ($[\text{M}+\text{NH}_4]=1058.9913$) and DOH-16 ($[\text{M}+\text{NH}_4]=1127.0604$) are shown.

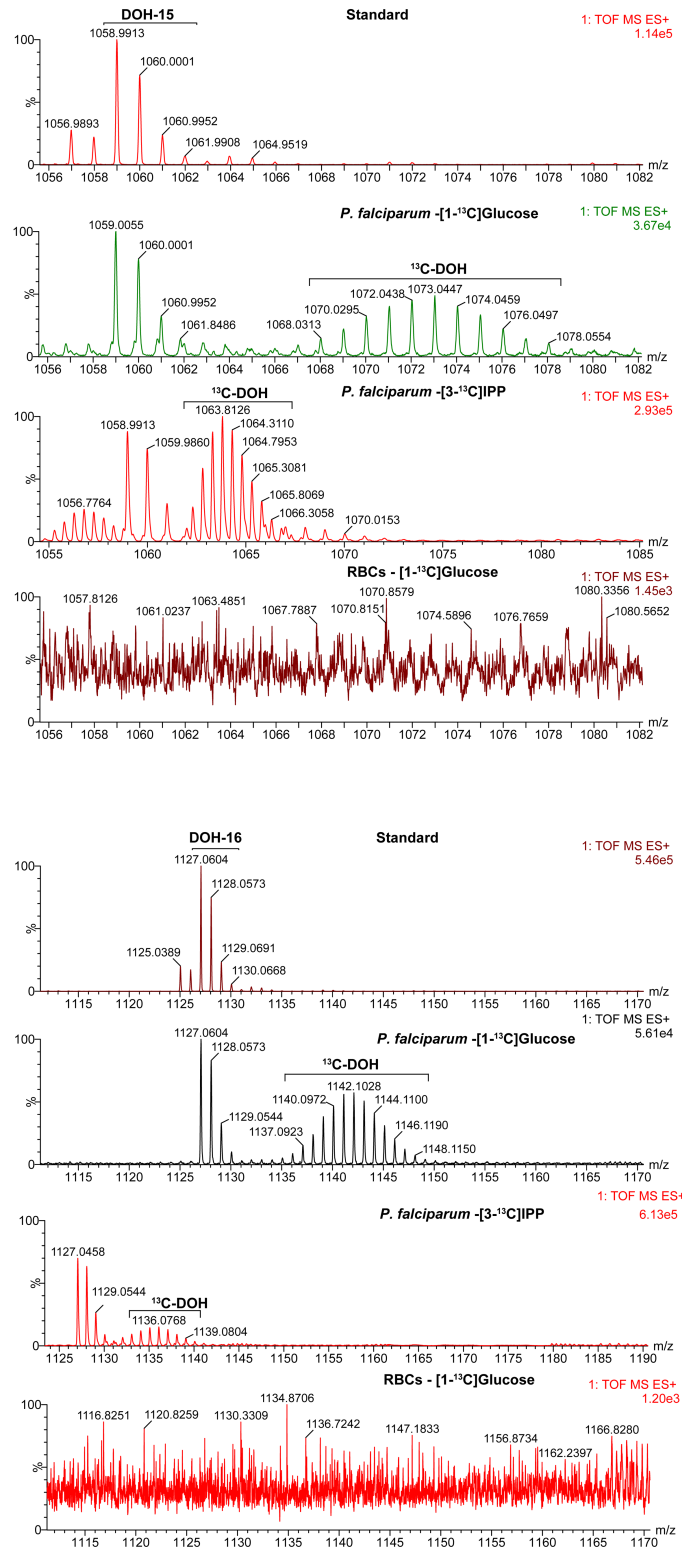


Figure S1 (continuation). De novo biosynthesis of medium-long DOH in *P. falciparum*. Mass spectra of DOH detected in *P. falciparum* schizont stage. Natural isotopic distribution of DOH is indicated for the standard. ^{13}C -enrichment is detected as a bell-shaped envelope as indicated on each spectrum. A representative mass spectrum of DOH-18 ($[\text{M}+\text{NH}_4]=1263.1897$) and DOH-19 ($[\text{M}+\text{NH}_4]=1331.2476$) are shown.

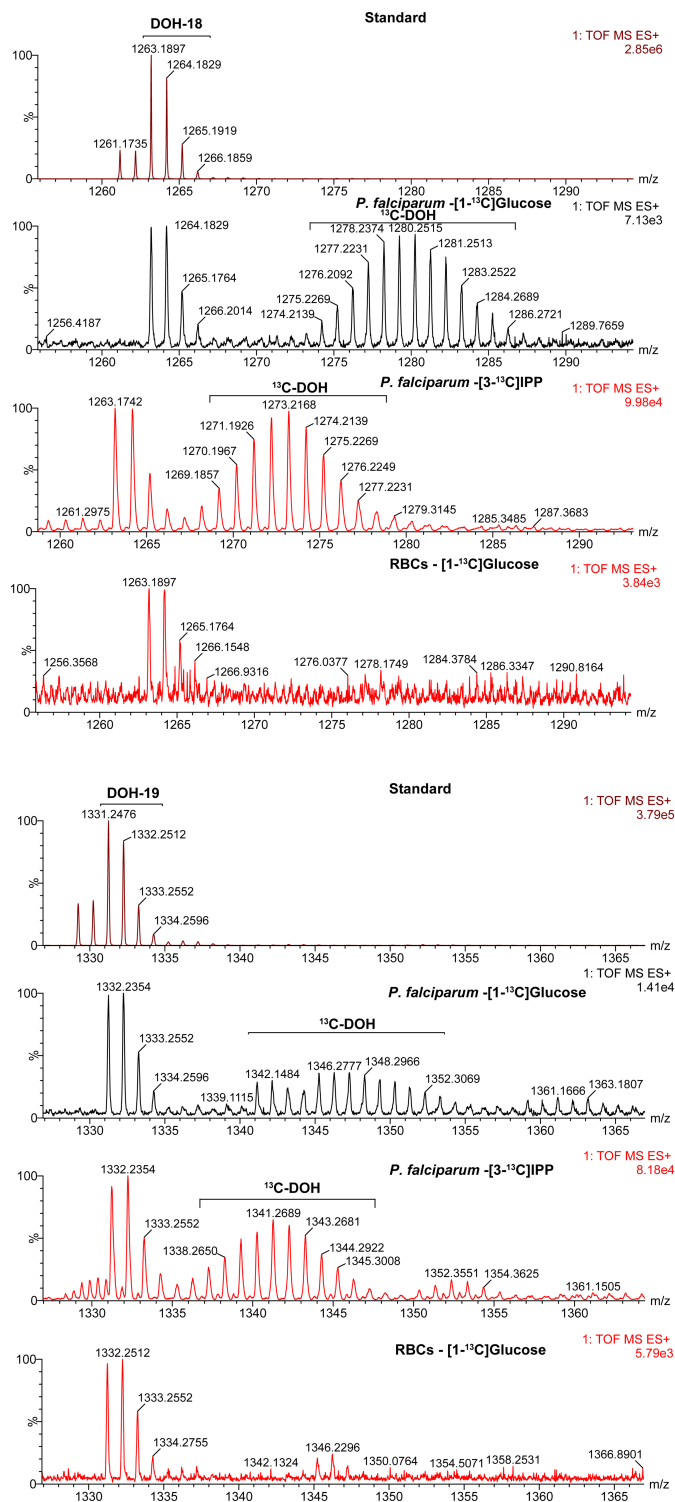


Figure S2. PCR analysis confirming lack of integration at the recombinant locus after multiple attempts to tag *PfPPRD* at the C-terminus with the TetR-DOZI-HA. Initial integration of the linearized TetR-DOZI-HA plasmid was observed in the first week of transfection using primers P8 and P9 (see Fig. 6 and Table S1), but then it was lost suggesting a fitness cost. Wild type parasites were detected using primers P8 and P2 (Table S1).

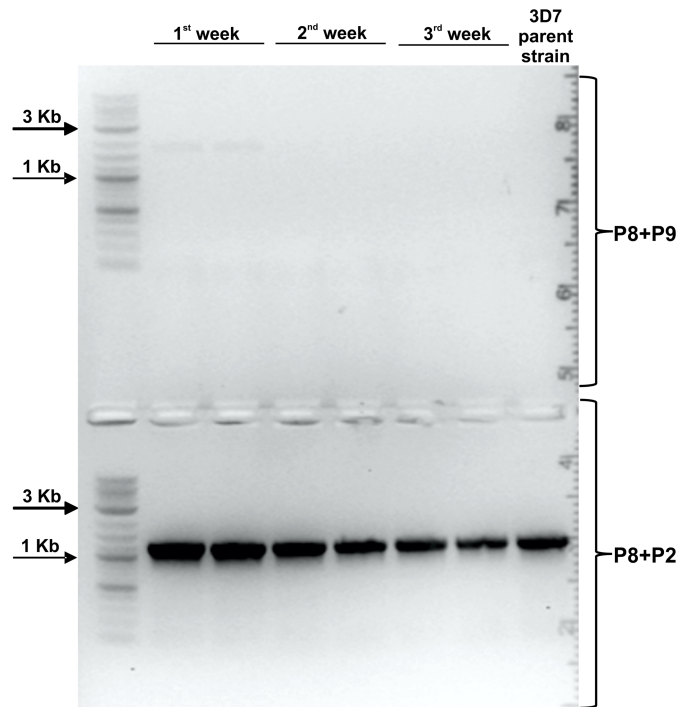


Figure S3. ^{13}C -DOH was not detected in parasites metabolically labeled with $[1-^{13}\text{C}]$ glucose after 10 days of aTc removal. ^{13}C -enrichment was observed in POH 18 supporting that PfCPT is active. ^{13}C -enrichment in DOH 18 was reduced in the absence of aTc, thus, the detected DOH 18 was not biosynthesized *de novo* by parasites under our experimental conditions. Metabolic labeling was performed as indicated in figure 3A (scheme) and as described in the methodology section. Natural isotopic distribution for POH and DOH is indicated for the standard. ^{13}C -enrichment is detected as a bell-shaped envelope as indicated in the PfPPRD+aTc spectrum. A representative mass spectrum of POH-18 ($[M+\text{NH}_4]=1261.1712$) and DOH-18 ($[M+\text{NH}_4]=1263.1742$) is shown. A representative Giemsa-stained smear is shown for each condition at the time that parasites were recovered for LC-HRMS analysis.

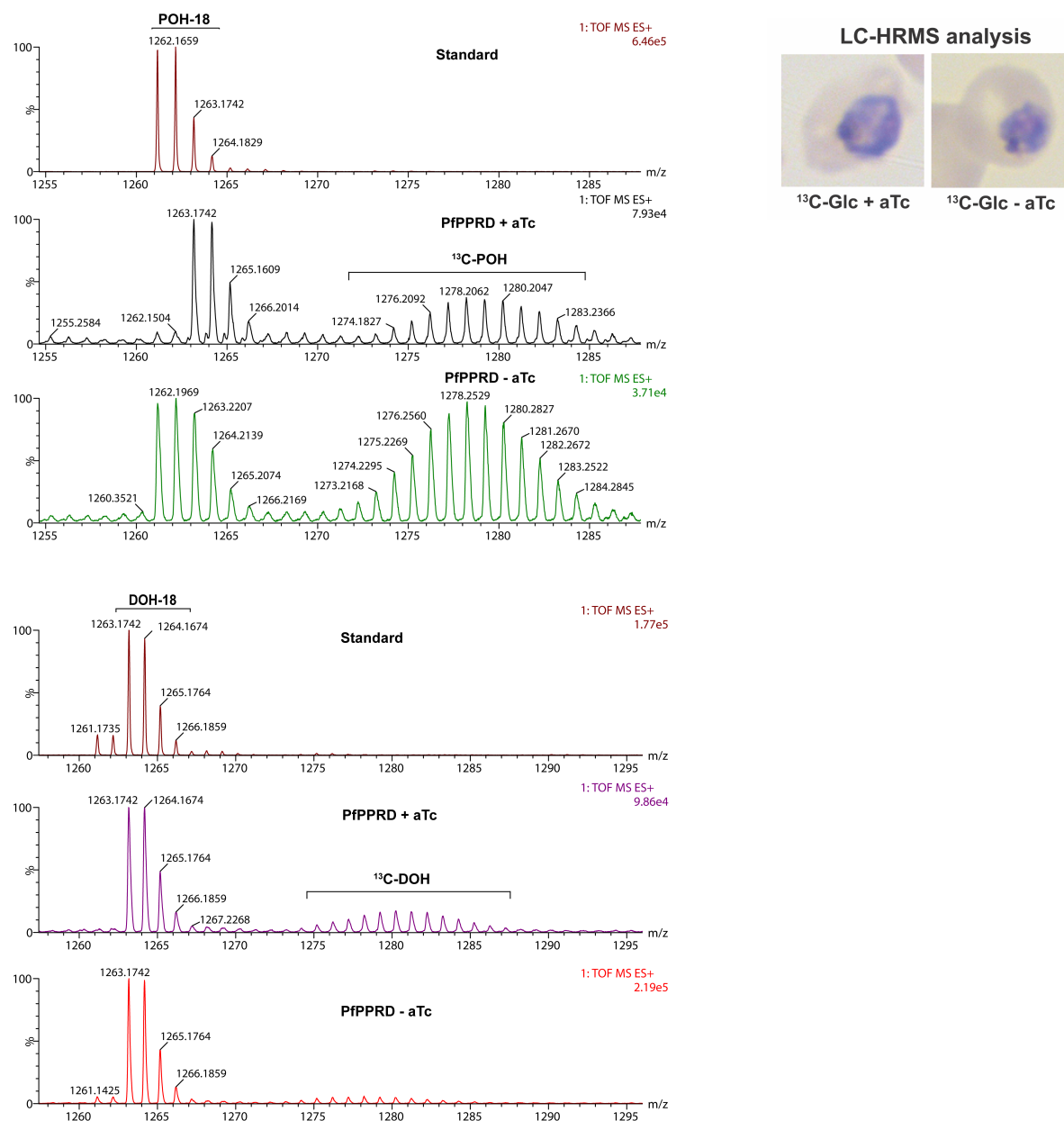


Figure S3 (continuation). A representative mass spectrum of DOH-19 ($[M+NH_4]^+$ = 1331.2476) and DOH-20 ($[M+NH_4]^+$ = 1399.2943) is shown. Natural isotopic distribution for POH and DOH is indicated for the standard. The area in the spectrum where ^{13}C -enrichment is expected to appear as a bell-shaped envelope is indicated as ^{13}C -DOH.

