Supporting Information

An unusual route for *p*-aminobenzoate biosynthesis in *Chlamydia trachomatis* involves a probable self-sacrificing diiron oxygenase

Yamilet Macias-Orihuela[a], Thomas Cast[b†], Ian Crawford[b], Kevin J. Brandecker,[b‡] Jennifer J. Thiaville[c§], Alexey G. Murzin[d], Valérie de Crécy-Lagard[c], Robert H. White[a], Kylie D. Allen[a,#]

[a]Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

[b]Department of Chemistry and Biochemistry, Gonzaga University, Spokane, WA, USA

[c]Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences and Genetics Institute, University of Florida, Gainesville, FL, USA

[d]MRC Laboratory of Molecular Biology, Cambridge, UK

Running Head: An unusual route for pABA biosynthesis in *Chlamydia*

[#]Address correspondence to Kylie Allen, kdallen@vt.edu

[†]Current address: Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO, USA

[‡]Current address: Hackensack Meridian School of Medicine at Seton Hall University, Nutley, NJ, USA

[§]Current address: Viral Vector Services, Thermo Fisher Scientific, Alachua, FL, USA

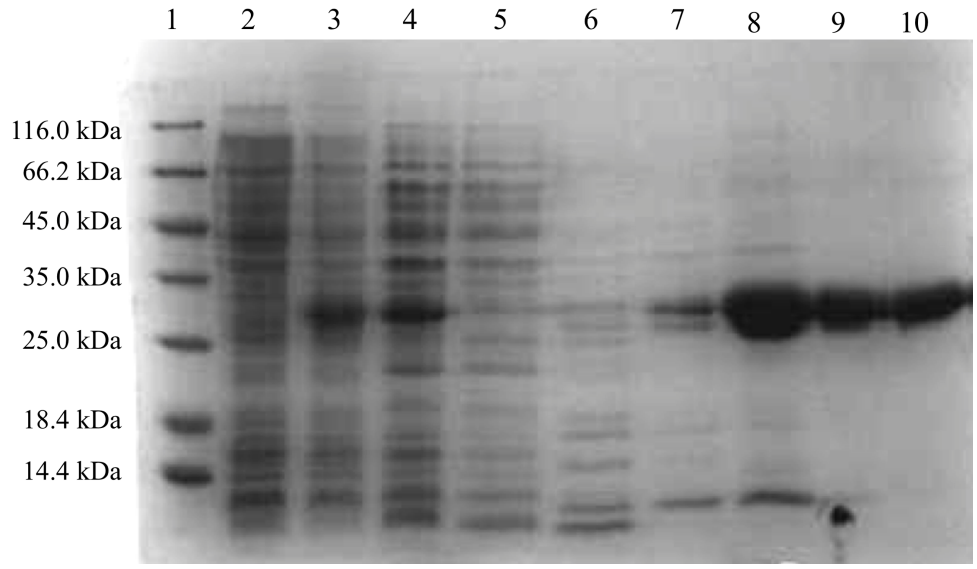

Figure S1: SDS-PAGE gel stained with Coomassie showing purified his-tagged CT610 expressed from pET19b. Lane 1- protein ladder, Lane 2- *E. coli* total proteins before induction, Lane 3- *E. coli* total proteins 4 hours after induction with IPTG, Lane 4- soluble supernatant after sonication, Lane 5- 25 mM imidazole wash from IMAC purification, Lane 6- 100 mM imidazole wash from IMAC purification, Lane 7- Fraction 1 from 250 mM imidazole elution, Lane 8- Fraction 2 from 250 mM imidazole elution, Lane 9- Fraction 3 from 250 mM imidazole elution, Lane 10- final purified CT610 after exchange into 100 mM Tris, 8 mM $MgCl_2$, 10% glycerol, pH 8.8.

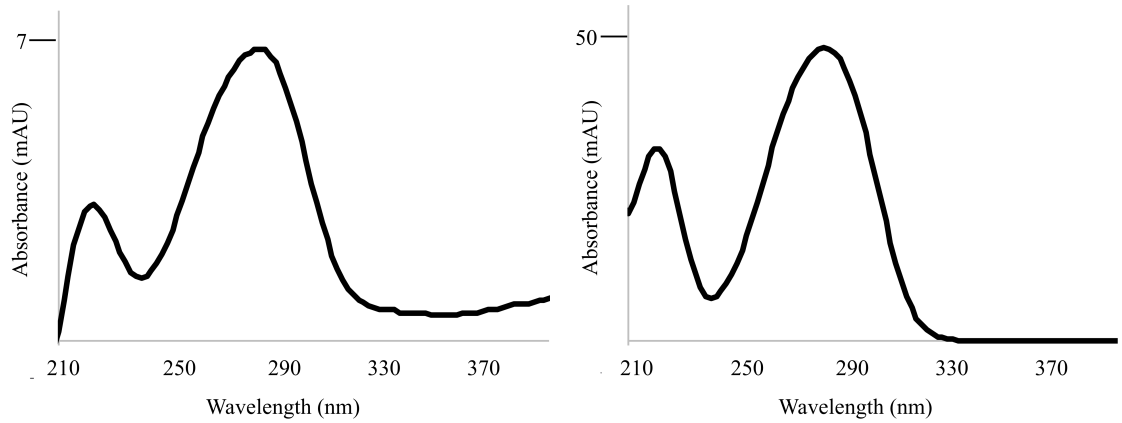

Figure S2. UV-Vis spectrum of the pABA peak from HPLC analysis of a CT610 reaction (left) compared to the spectrum of a 5 μM pAB standard (right).

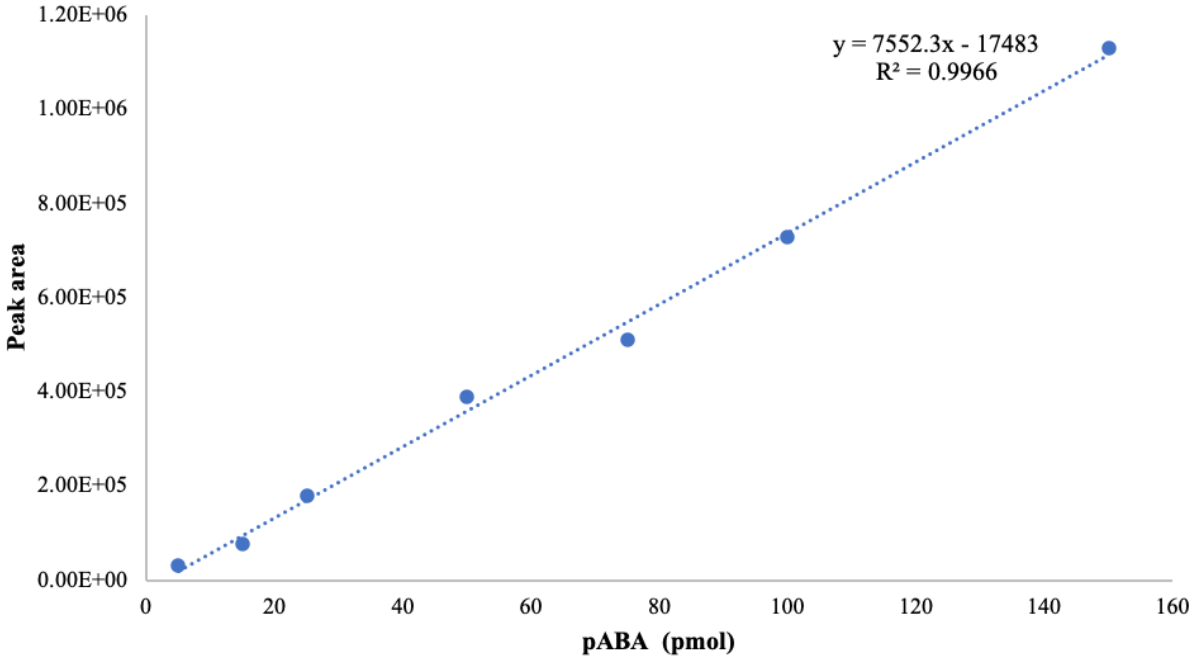

Figure S3. Standard curve for pABA quantitation. The peak area refers to the pABA peak area from the LC-MS extracted ion current chromatogram. See manuscript for a complete description of the LC-MS methods.

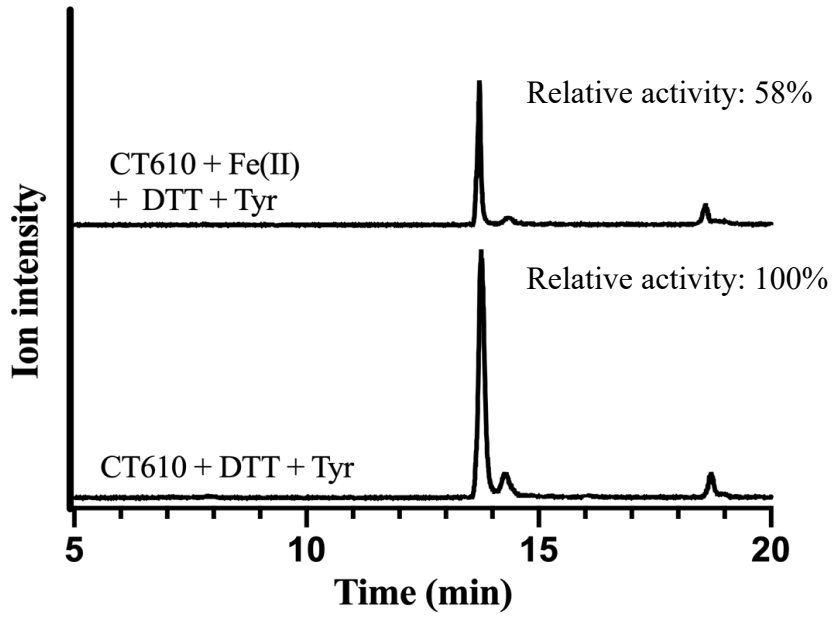

Figure S4. LC-MS analysis of CT610 enzyme reactions in the presence (top) or absence (bottom) of added Fe(II), which was added as ferrous ammonium sulfate (100 μM). The relative activity reported is based on the pABA peak area.

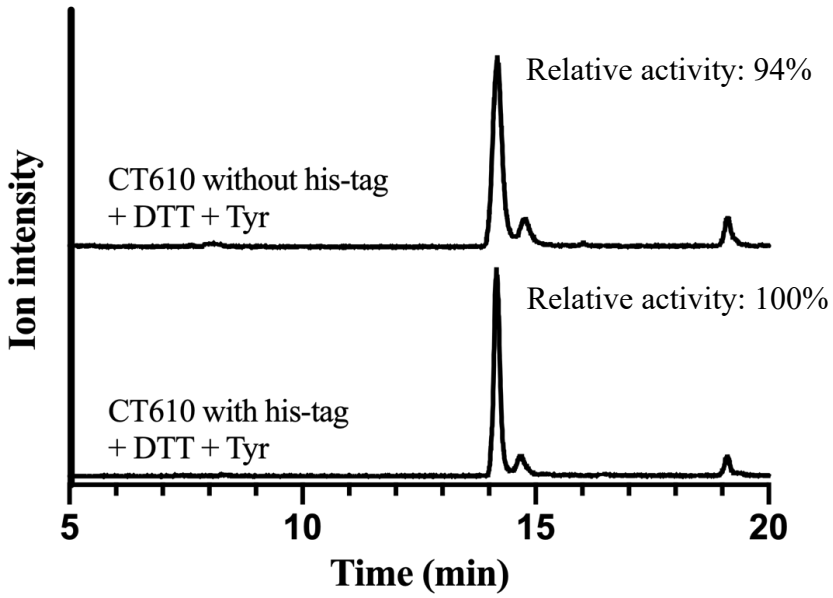

Figure S5. LC-MS analysis of CT610 enzyme reactions with or without the his-tag. The N-terminal His_{10} tag was encoded by pET19b and was removed by protease cleavage with EKMax™ Enterokinase (Thermo Fisher Scientific) according to the manufacturer's instructions. The relative activity reported is based on the pABA peak area.

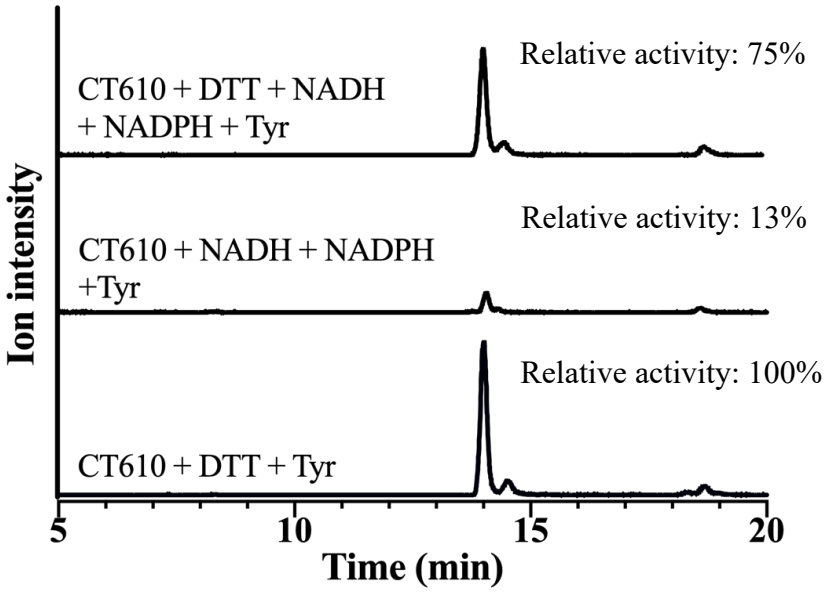

Figure S6. LC-MS analysis of CT610 enzyme reactions in the presence (top and middle) vs. absence (bottom) of NADH and NADPH. The relative activities reported are based on the pABA peak areas.

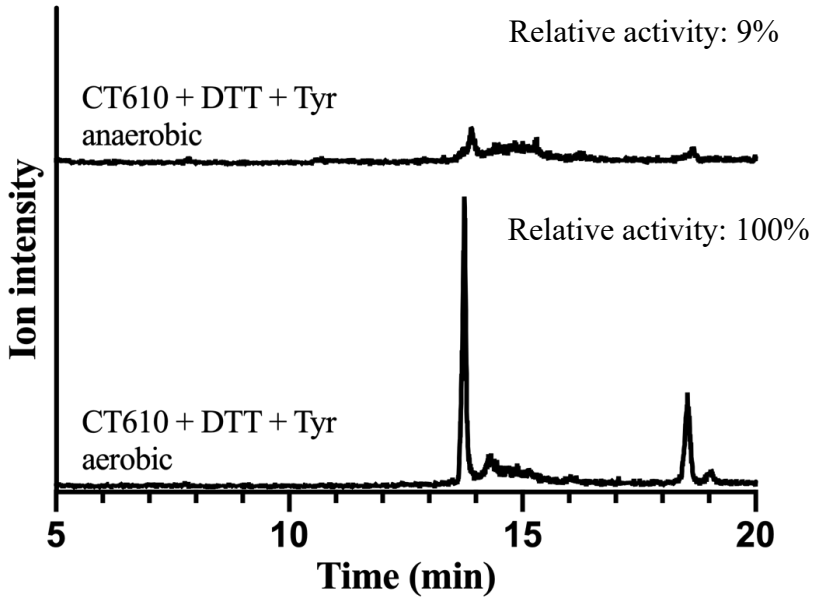

Figure S7. LC-MS analysis of CT610 enzyme reactions in the presence (bottom) vs. absence (top) of O_2. The enzyme solution was made anaerobic by gentle stirring in an anaerobic chamber for ~4 hours followed by addition of DTT and tyrosine. The aerobic sample was from the same protein purification batch and was treated in the same manner except under normal atmospheric oxygen conditions. The relative activity reported is based on the pABA peak area.

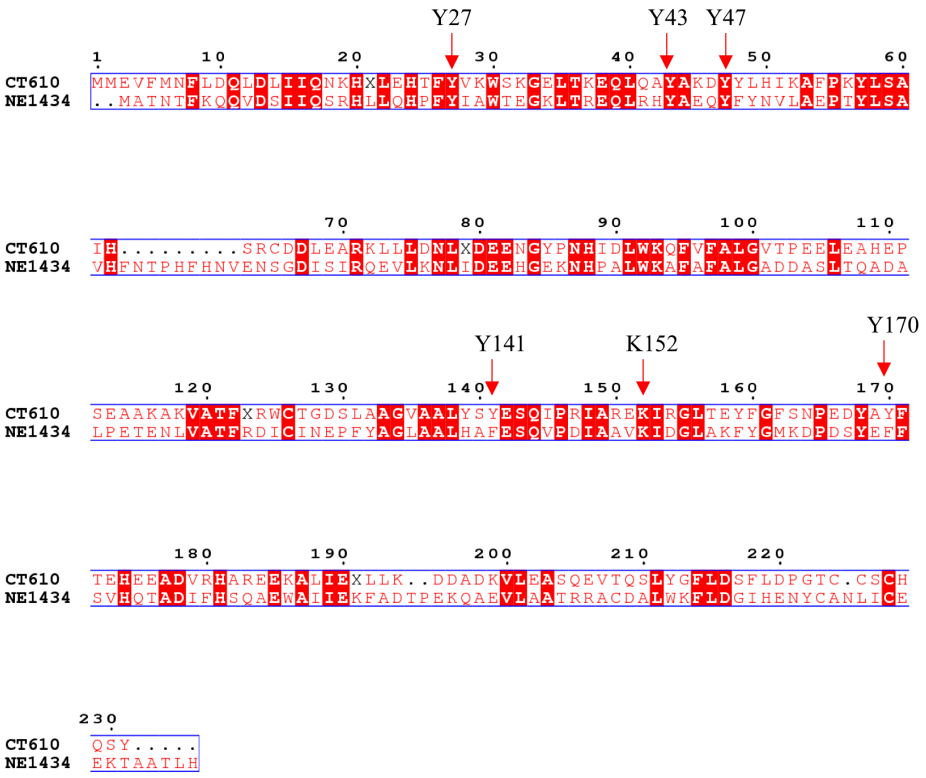

Figure S8. Sequence alignment of CT610 from *Chlamydia trachomatis* (UniProt ID O84616) and NE1434 from *Nitrosomonas europaea* (UniProt ID Q82UP8). Numbering corresponds to CT610. Alignment was performed with MultAlin (1) and visualized with ESPript (2).

Table S1. Primers used to generate the CT610 site-directed mutants. The mutation is highlighted in bold and underlined. All primers were 5'phosphorylated.

Mutation	Primer Sequence
Y27F	Forward- gaacacacgttt**ttt**gtgaaatggtcg
	Reverse- tagcatatgcttattttgaataattaaatc
Y43F	Forward- gcaattacaggcg**ttt**gccaaagactatt
	Reverse- tctttagtaagctccccttcgaccatttc
Y47F	Forward- tatgccaaagac**ttt**tatttacatatc
	Reverse- cgcctgtaattgctctttagtaagctcccc
K152R	Forward- atcgctagagag**aga**attcgtggattg
	Reverse- acgtggaatttgactctcataagaatac
Y141F	Forward- tgctttgtattct**ttt**gagagtcaaattc
	Reverse- gccactcctgcagctaaagaatctcctg
Y170F	Forward- tgaagactatgca**ttt**ttcacagaacatg
	Reverse- ggattggaaaatccaaagtactcagtc

References:

1. Corpet F. 1988. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 16:10881-90.
2. Robert X, Gouet P. 2014. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res 42:W320-4.