THE CRYSTAL STRUCTURE OF α-DIOXYGENASE PROVIDES INSIGHT INTO DIVERSITY IN THE CYCLOOXYGENASE-PEROXIDASE SUPERFAMILY

Christopher C. Goulah[1,a], Guangyu Zhu[1,a], Mary Koszelak-Rosenblum[b], and Michael G. Malkowski[a,b]

From [a]the Hauptman-Woodward Medical Research Institute and [b]Department of Structural Biology, The State University of New York at Buffalo, Buffalo, NY, 14203

SUPPLEMENTARY MATERIAL

Table S1. Primers utilized to generate mutant Ath αDOX constructs. Only forward primers are listed, with the mutation site highlighted in bold. All primers are listed from 5' to 3'.

Mutant	Forward primer
Q159N	GCAGCGAGCTGGATT**AAC**TTCATGATCCACGAC
Q159S	GCAGCGAGCTGGATT**TCT**TTCATGATCCACGAC
Q159V	GCAGCGAGCTGGATT**GTT**TTCATGATCCACGAC
H318A	GCGCGGTTGTGGCTAAAATC**GCG**ACTATCGACTGGACTGTCCAGC
H318Q	GCGCGGTTGTGGCTAAAATC**CAG**ACTATCGACTGGACTGTCCAGC
T323A	CCACACTATCGACTGG**GCT**GTCCAGCTGCTGAAAACC
T323L	CCACACTATCGACTGG**CTG**GTCCAGCTGCTGAAAACC
Y386F	CGGAGGATTTCACCAGCGTT**TTT**CGCATGCACTCTCTGCTGCCGG
R565A	CTTCCTGATCATGGCAACC**GCG**CGTCTGGAAGCTGATCGTTTC
R565K	CTTCCTGATCATGGCAACC**AAA**CGTCTGGAAGCTGATCGTTTC
R565L	CTTCCTGATCATGGCAACC**CTG**CGTCTGGAAGCTGATCGTTTC
R566A	CCTGATCATGGCAACCCGC**GCG**CTGGAAGCTGATCG
R566K	CCTGATCATGGCAACCCGC**AAA**CTGGAAGCTGATCG
R566L	CCTGATCATGGCAACCCGC**CTG**CTGGAAGCTGATCG

Figure S1. The Cyclooxygenase and α-Dioxygenase Reaction Mechanisms. Schematic representation of the reaction mechanisms carried out by COX and α-DOX enzymes. The 13pro-S hydrogen of arachidonic acid is abstracted by a radical formed on Tyr-385 in COX, while the 2pro-R hydrogen of linoleic acid is abstracted by a radical formed on Tyr-386 in α-DOX.

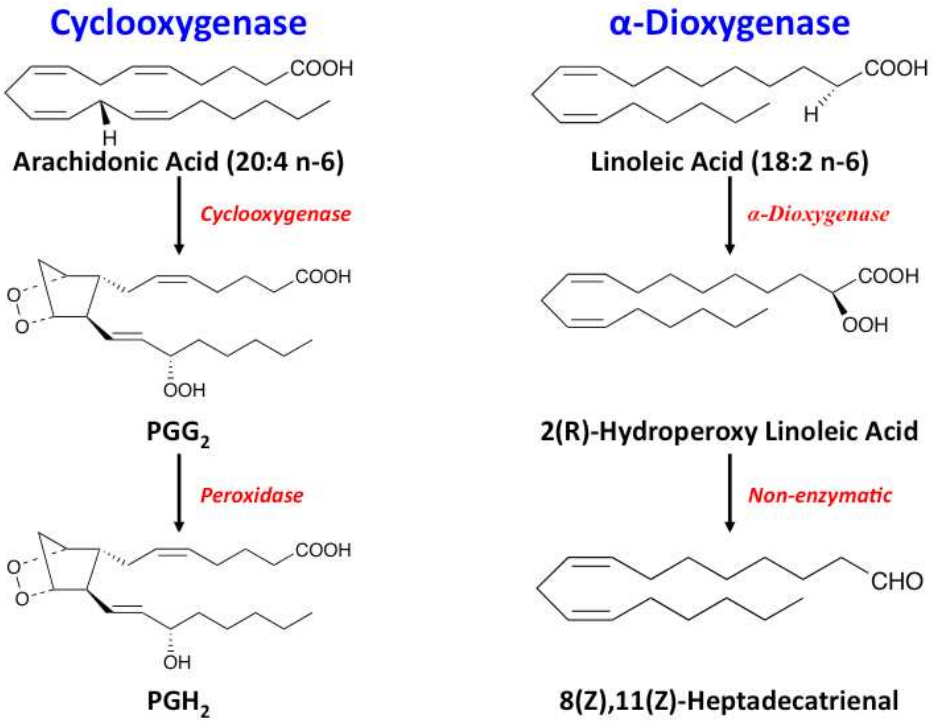


Figure S2. Secondary Structure Alignment of Ath α-DOX with murine COX-2. The amino acid sequence of Ath αDOX along with the corresponding secondary structure derived from the crystal structure of α-DOX is shown aligned with the sequence of murine COX-2 and the corresponding secondary structure derived from monomer B of the crystal structure of PDB 3HS5 (1). Boxes depict secondary structural elements in each structure, while dots (.) represent regions with no secondary structure and dashes (-) represent gaps in the alignment. The secondary structural elements that are equivalent between α-DOX and COX-2 are colored in red and labeled as defined in Ref. (2). Secondary structural elements that are unique to α-DOX or COX-2 are colored light blue and green, respectively. The numbers above the sequence of COX-2 correspond to residue numbering for ovine COX-1, which is standard in the cyclooxygenase field, whereas the numbers below the sequence of α-DOX correspond to residue numbering from the *A. thaliana* protein sequence. The proximal and distal histidine residues, the catalytic tyrosine, and residues mutated within this study are shown in bold. h, helix; s, strand.

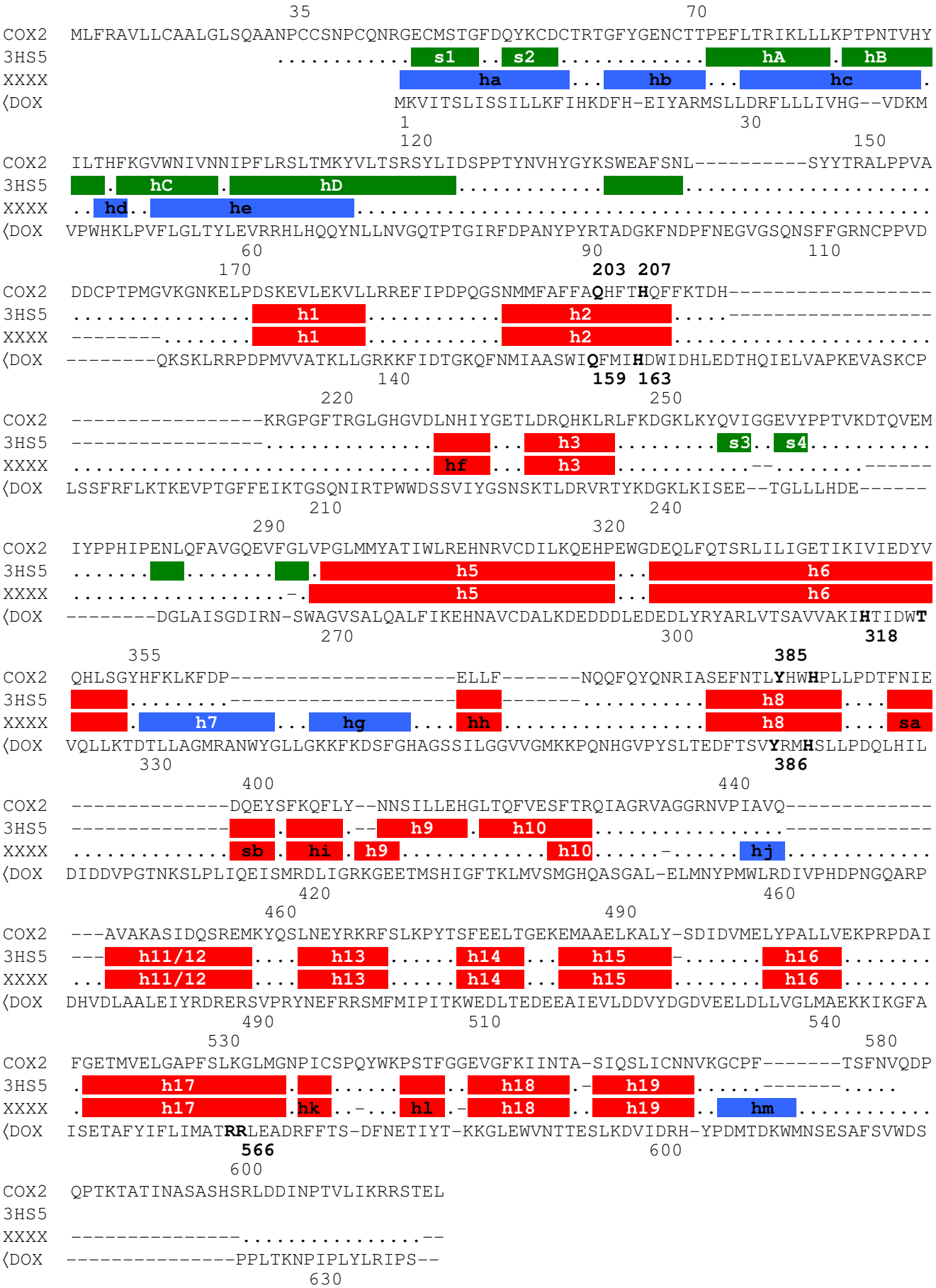

COX2 MLFRAVLLCAALGLSQAANPCCSNPCQNRGECMSTGFDQYKCDCTRTGFYGENCTTPEFLTRIKLLLKPTPNTVHY
3HS5 s1 s2 hA hB
XXXX ha hb hc
⟨DOX MKVITSLISSILLKFIHKDFH-EIYARMSLLDRFLLLIVHG--VDKM
COX2 ILTHFKGVWNIVNNIPFLRSLTMKYVLTSRSYLIDSPPTYNVHYGYKSWEAFSNL----------SYYTRALPPVA
3HS5 hC hD
XXXX hd he
⟨DOX VPWHKLPVFLGLTYLEVRRHLHQQYNLLNVGQTPTGIRFDPANYPYRTADGKFNDPFNEGVGSQNSFFGRNCPPVD
COX2 DDCPTPMGVKGNKELPDSKEVLEKVLLRREFIPDPQGSNMMFAFFAQHFTHQFFKTDH----------
3HS5 h1 h2
XXXX h1 h2
⟨DOX --------QKSKLRRPDPMVVATKLLGRKKFIDTGKQFNMIAASWIQFMIHDWIDHLEDTHQIELVAPKEVASKCP
COX2 ----------KRGPGFTRGLGHGVDLNHIYGETLDRQHKLRLFKDGKLKYQVIGGEVYPPTVKDTQVEM
3HS5 h3 s3 s4
XXXX hf h3
⟨DOX LSSFRFLKTKEVPTGFFEIKTGSQNIRTPWWDSSVIYGSNSKTLDRVRTYKDGKLKISEE--TGLLLHDE------
COX2 IYPPHIPENLQFAVGQEVFGLVPGLMMYATIWLREHNRVCDILKQEHPEWGDEQLFQTSRLILIGETIKIVIEDYV
3HS5 h5 h6
XXXX h5 h6
⟨DOX --------DGLAISGDIRN-SWAGVSALQALFIKEHNAVCDALKDEDDDLEDEDLYRYARLVTSAVVAKIHTIDWT
COX2 QHLSGYHFKLKFDP----------ELLF-------NQQFQYQNRIASEFNTLYHWHPLLPDTFNIE
3HS5 h8
XXXX h7 hg hh h8 sa
⟨DOX VQLLKTDTLLAGMRANWYGLLGKKFKDSFGHAGSSILGGVVGMKKPQNHGVPYSLTEDFTSVYRMHSLLPDQLHIL
COX2 ----------DQEYSFKQFLY--NNSILLEHGLTQFVESFTRQIAGRVAGGRNVPIAVQ----------
3HS5 h9 h10
XXXX sb hi h9 h10 hj
⟨DOX DIDDVPGTNKSLPLIQEISMRDLIGRKGEETMSHIGFTKLMVSMGHQASGAL-ELMNYPMWLRDIVPHDPNGQARP
COX2 ---AVAKASIDQSREMKYQSLNEYRKRFSLKPYTSFEELTGEKEMAAELKALY-SDIDVMELYPALLVEKPRPDAI
3HS5 h11/12 h13 h14 h15 h16
XXXX h11/12 h13 h14 h15 h16
⟨DOX DHVDLAALEIYRDRERSVPRYNEFRRSMFMIPITKWEDLTEDEEAIEVLDDVYDGDVEELDLLVGLMAEKKIKGFA
COX2 FGETMVELGAPFSLKGLMGNPICSPQYWKPSTFGGEVGFKIINTA-SIQSLICNNVKGCPF-------TSFNVQDP
3HS5 h17 h18 h19
XXXX h17 hk h1 h18 h19 hm
⟨DOX ISETAFYIFLIMATRRLEADRFFTS-DFNETIYT-KKGLEWVNTTESLKDVIDRH-YPDMTDKWMNSESAFSVWDS
COX2 QPTKTATINASASHSRLDDINPTVLIKRRSTEL
XXXX
⟨DOX ---------------PPLTKNPIPLYLRIPS--

Figure S3. Imidazole Bound to the Heme. Stereo view of the distal face of the heme with imidazole bound to the iron atom. $2F_O$-F_C electron density covering imidazole and the heme, contoured at 1σ, is shown in blue. The proximal (His-389) and distal (His-163) histidines are labeled accordingly.

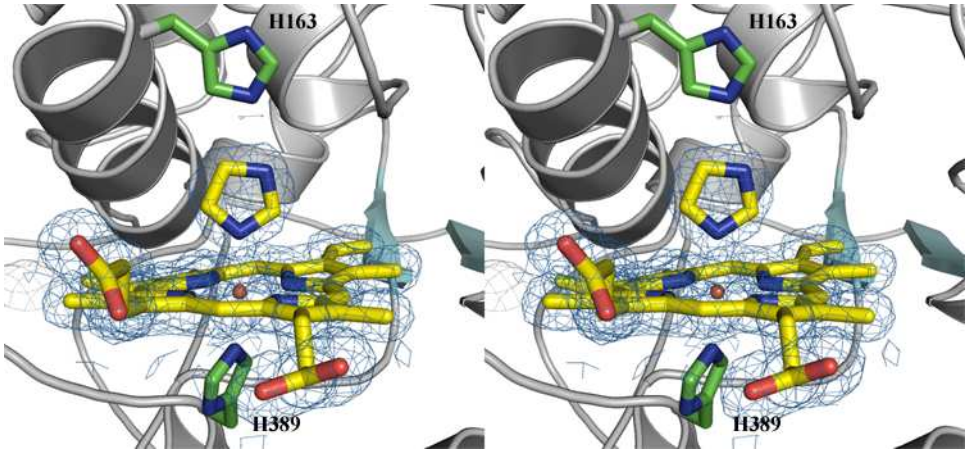


Figure S4. Purity of Wild Type and Mutant Constructs of Ath α-DOX. SDS-PAGE analysis of wild type and mutant constructs of **(A)** Gln-159; **(B)** His-318 and Thr-323; and **(C)** Arg-565 and Arg-566.

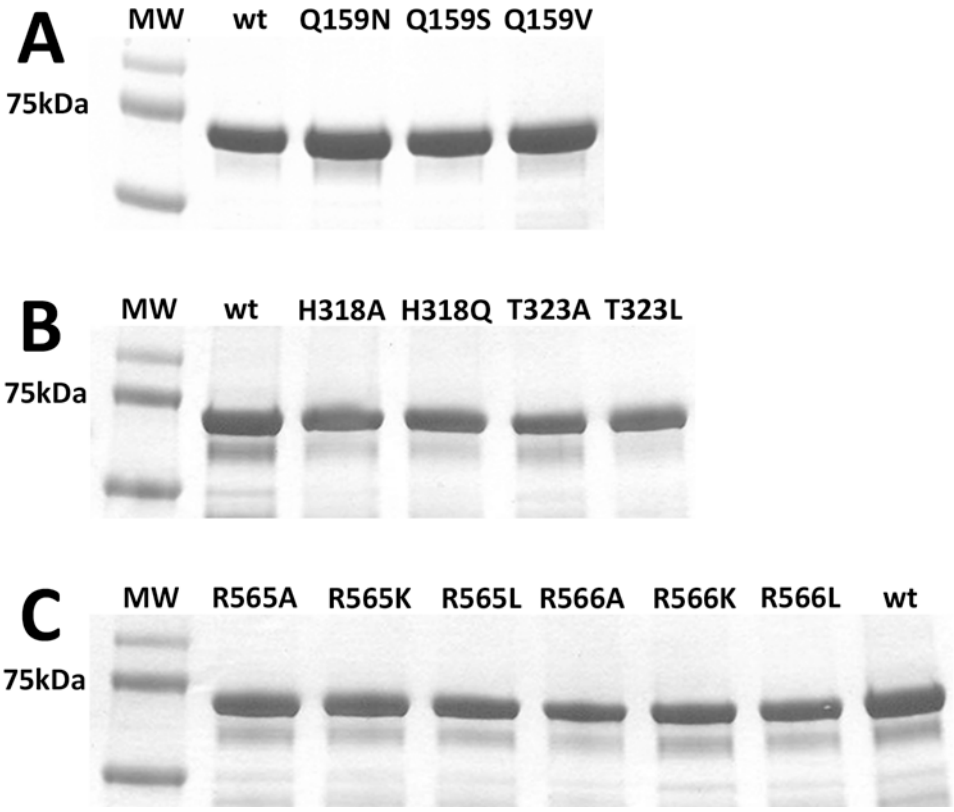
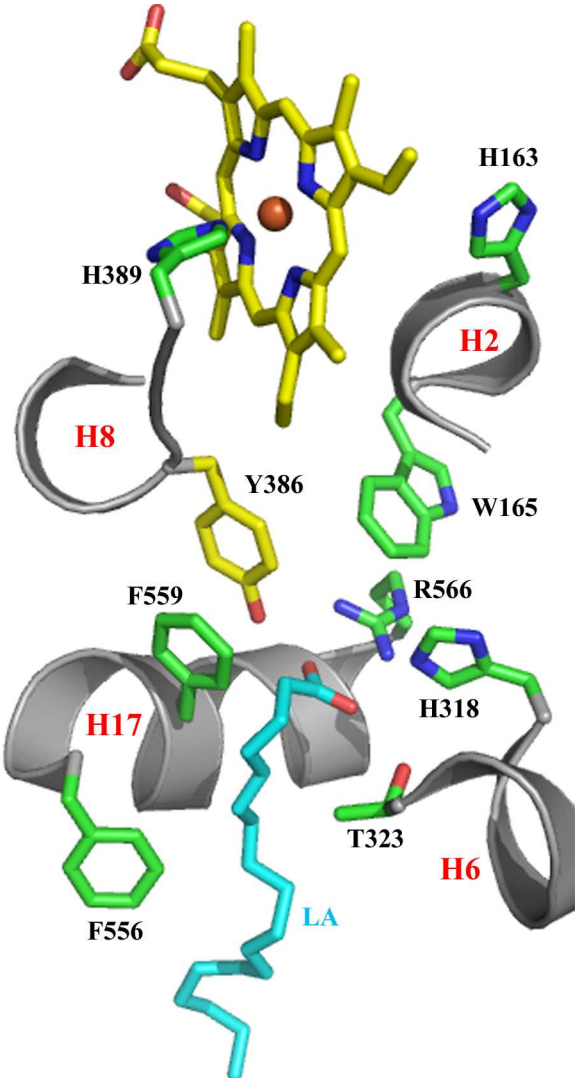


Figure S5. Model of LA Bound Within the Active Site Channel of α-DOX. Linoleic acid (LA; 18:2 ω-6) is depicted in stick representation (light blue) bound between helices H6 and H17 in the active site channel of α-DOX. Carbon-2 of LA lies ~2.8Å below the catalytic tyrosine, Tyr-386, which is poised for abstraction of the 2*proR* hydrogen. The carboxylate of LA is positioned to interact with the side chains of His-318, Thr-323, and Arg-566. Portions of helices H2, H6, H8, and H17 were removed for clarity.



REFERENCES

1. Vecchio, A. J., Simmons, D. M., and Malkowski, M. G. (2010) Structural basis of fatty acid substrate binding to cyclooxygenase-2, *J Biol Chem 285*, 22152-22163.
2. Picot, D., Loll, P. J., and Garavito, R. M. (1994) The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1, *Nature 367*, 243-249.