

SUPPLEMENTAL INFORMATION

Secreted Heme Peroxidase from *Dictyostelium discoideum*: Insights into Catalysis, Structure and Biological Role

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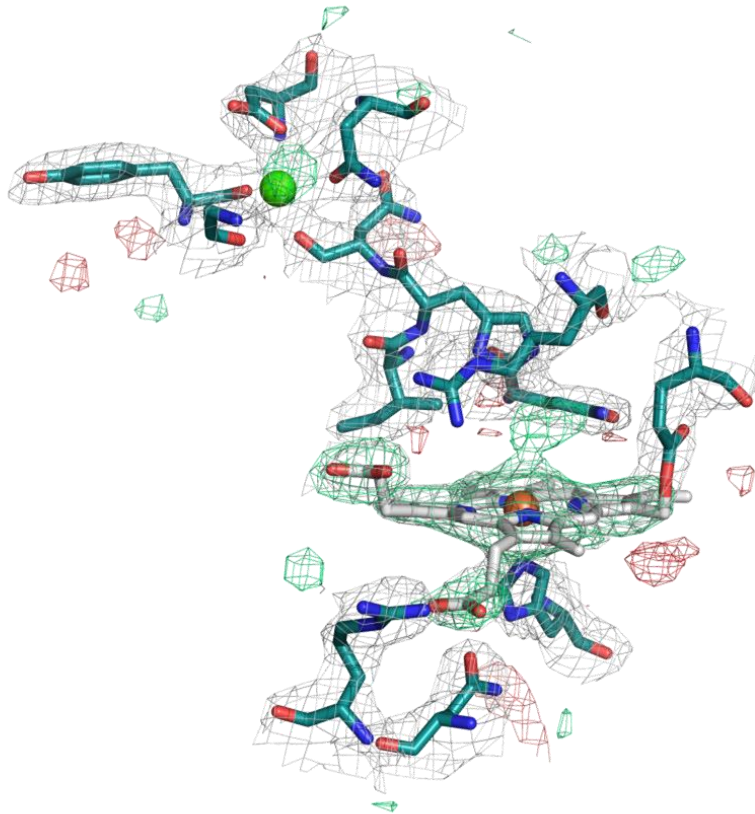
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Running title: Secreted heme peroxidase from *Dictyostelium discoideum*

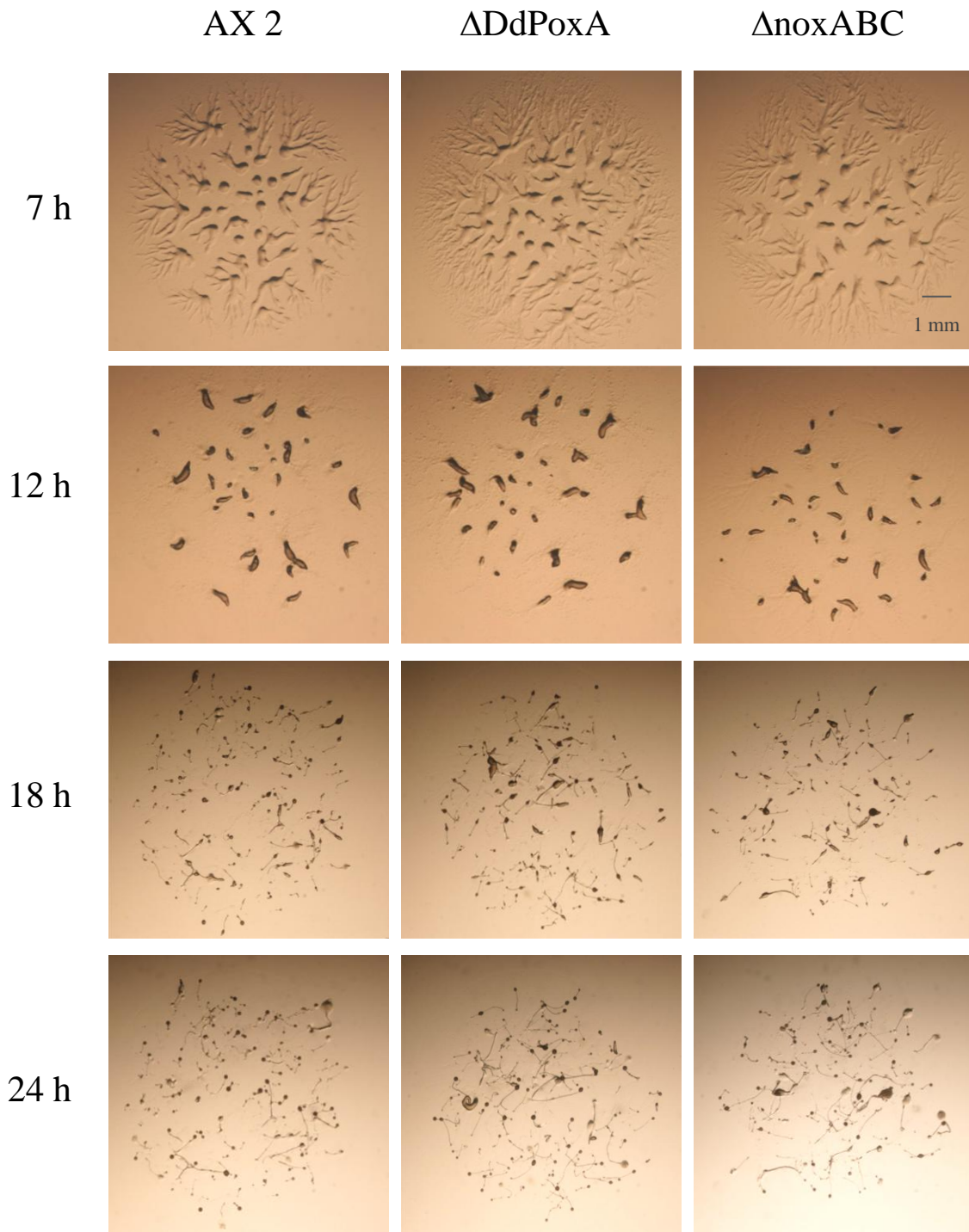
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DdPoxA	-----		0			
goat LPO	-----MLVCLHLQVFLASVALFEV--AASDTIAQAASTTISDAVSKVKTQVNKAFLDSRRLKALSS		62			
human EPO	-----MHLLPALAGVLATLVLAQPCGTDPA SPGAVETSVLRDCIAEAKLLVDAAYNWTQKSIKQRLRS		64			
human MPO	MGVPPFFSRLRCMVDLGPWCAGGLTAEMLKLLALAGLAILATPQPSEGAAPVLGEVDTSLVLSMEEAKQLVDKAYKERRRESIKQRLRS		90			
DdPoxA	-----MRLNLLISFF--IIFITLVISINSQEFRSYITGE		30			
goat LPO	EAPTTRQLSEYFKHAKGRTRTAIRNGQVWEESLKRLLRD-----T-TLTNVTDPSELELTALSWEVGGGAPVPLVTCDEQSPYRTITGD		144			
human EPO	GSASPMDLLSYFKQVAAATRTVVRAADYMHVALGLLEEKLPQRSRPFNVTDVL-TEPQLRLLSQASGCALRDAQERCS--DKYRTITGR		151			
human MPO	GSASPMELLSYFKQVAAATRTAVRAADYLHVALDLLERKLSLWRRPFNVTDVL-TPAQLNVLSKSSGCAYQDVGVTCPEQDKYRTITGM		179			
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	S1	H1	H2			
DdPoxA	GNKKQNPKQGSITPFIRLAN-----PI----KFNKNGFPNITNQPSRAISNIIFDQQT-HIGSKEHLTDMFNMMWQFLIHNMAALS		106			
goat LPO	CNNRRSPALGAANRALARWLPAEYEDGLAVPFGWTQKTRNGFRVLP---LAREVSNKIVGYLDEEGVLQDNRSLLFMQWQIIVHDLDFD		231			
human EPO	CNNKRRPLLGASNQALARWLPAEYEDGLSFPFGWTPSRRRNGFLLP---LVRAVSNQIVRFPPNERLTSDRGRALMFMQWQFIDHDLDFS		238			
human MPO	CNNRRSPPLGASNRAFRWLPAEYEDGFSLPYGTWPGVKNRPFVFA---LARAVSNEIVRFPTDQLTPDQERSLMMFMQWQLLDHDLDFD		266			
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DdPoxA	KPE-----PNSWPIKVPKCDQYFDPACIGNKTMNYFRTRADEVPCDVGKTVDVEDGKCYEQINSLGSYIDGNVLYG		177			
goat LPO	PETELGSSEHSKQVCEEYCVQDECFPIPFKNDPKLKT---QGKMPFFRAGFV---CPTP---PYQSLARDQINAVTSLFDASLVYG		311			
human EPO	PESPARVAFTAGVDCERTCAQLPPCFPIKIPNDPRIKN---QRDCIPFFRSAPS---CPQN---K---NRVRNQINALTSEVFDASMYG		316			
human MPO	PEPAARASFVTVGNVCEVQVPPCFPLKIPNDPRIKN---QADCIFFRSAPCA---CPGS---N---ITIRNQINALTSEVFDASMYG		344			
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	H4	H5	H6			
DdPoxA	NSEETCKNLRSLSGGEMKMTVDV---G--DLPPKNVPGVPMNDANLFPIDQLYSVGERRGNENPGLLSIHTLLLRDHNRLARKFARL		261			
goat LPO	SEPSLASRLRNLSPLGLMAVNGEAWDHGLAYPPFNKVPSPCEF---INTTAHVPCFQAGDSRASQIILLATVHTLLREHNRLARELKR		400			
human EPO	SEVLSLRLLRNRTNYLGLLAINQRFDQNGRALLPFDNLHDDPCLL---TNRSARIPCFLAGDTRSTTPKLAAMHTLFMRHNRLATELRRL		405			
human MPO	SEEPRLARNLRNMSNLGLLAVNQRFQDNGRALLPFDNLHDDPCLL---TNRSARIPCFLAGDTRSSMPELTSMHTLLREHNRLATELKS		433			
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DdPoxA	HPWEDDERVFQQRSCIIEQIQKITIDEYLPTTLGS-----FPSYTGVDANVNAQVSNNEFTTAFRFGSEVGPFMEEYSENGTRLQP-		344			
goat LPO	NPHWDGEMLYQEARKILGAFIQIITFRDYLPVLGS-EMQKWIPPYQGYNNSVDPRIANVFT-FAFRFGMEVPSTVSRLDENYQPWGPE		488			
human EPO	NPRWNGDKLYNEARKIMGAMVQIITYRDYLPVLGKARARTLGHYRGYCSNVDPRIANVFT-LAFRFGTMLQPFMFRDLSQYRASAPN		494			
human MPO	NPRWDGERLYQEARKIVGAMVQIITYRDYLPVLGPTAMRKYLPYRSYNSVDPRIANVFT-NAFRYGTLLIQPFMFRDNRYPQMEPN		522			
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	H9	H10	H11	H12	H13	H14
DdPoxA	--LPIKFSYFNP--HALNRGVEPLIRGLIINEEE--NIDIYMI\$DLRNFLFGKPGQ-GGLDLASRLQCRDRHGIPPYNSLRRQLGLRPV					427
goat LPO	AELPLHTLFFNTWRIKIDGGIDPLVRGLLAKNSKLMNQNMVTELRNKLFPQTHKVVHGFDLAAIQLQCRDHGMPGYNSWRGFCGLSQP					578
human EPO	SHVPLSSAFFASWRIVYEGGIDPILRGLMATPAKLNQDAMLVDELDRDLFRQVRR-IGLDLAALMQSRDHGLPGYNAWRRFCGLSQP					583
human MPO	PRVPLSRVFFASWRVVLGEGIDPILRGLMATPAKLNQNIQIIVAEIRERLFEQVMR-IGLDLPAALMQSRDHGLPGYNAWRRFCGLSQP					611
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	H15	H16	H17	H18	H19	H20
DdPoxA	QTWS--DITSDPQIQNRLKNAYKSVDDIDSYVGGLEAEDHMEGSCVQTFYLIIEYQFFRTRAGDRFWYETPEMRMNVNREC--ETTTFAE					512
goat LPO	KTLKGLQAVLNKVKLAKLLDLKTPDNIDIWIGGNAEPMVERGVGPELLACLGRQFQQIRDGDRFVWENPGVTEKQRDSLQKVFSFR					668
human EPO	RNLQLSRVLKNQDLARKFLNLYGTPDNIIDIWIGAIAPLLPGARVGPGLLACLFENQFRARDDGDRFVWQKRGVFTKRQRKALSRI SLSR					673
human MPO	ETVQGLTVLRNKLARKLMEQYGTPNNDIWMGGVSEPLKRKRGVGPELLACIIGTQFRKLRDGDGRFVWENEGVFSMQQRQALAI SLPR					701
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	H21					
DdPoxA	VIKRTSNIIGYVQPNVFRK*-----					531
goat LPO	LICDNTHITKVP-L-HAFQANNYPHDFVDCSAVDKLDLSPWASREN*					712
human EPO	IICDNTGITTVSR-DIFRANIYPRGFVNC\$RI PRLNLSAWRGT*--					715
human MPO	IICDNTGITTVSKNNIFMSNSYPRDFVNC\$TLPALNLSAWREAS*--					745
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Supplemental Figure S1. Structural alignment of DdPoxA in comparison to mammalian peroxidases. Total sequence alignment of DdPoxA with goat LPO, human EPO and human MPO. Secondary structure elements of DdPoxA are highlighted in grey (α -helices) and orange (β -strands). The native signal sequence of DdPoxA is depicted in blue. Important distal residues are shown in yellow, the conserved glutamate forming the heme to protein bond is highlighted in green. The distal isoleucine of DdPoxA is depicted in pink. The conserved proximal triad is shown in red. Fully conserved residues are marked with an asterisk, a colon indicates residues with strongly similar properties, and a period shows residues with weakly similar properties.



Supplemental Figure S2. OMIT map of DdPoxA active site structure. OMIT map (A) of the active site structure of DdPoxA. The 2.5 Å resolution mF_o-DF_c maps contoured at $\pm 3 \sigma$ positive (green) and negative (red) and $2mF_o-DF_c$ maximum-likelihood omit map contoured at 1σ (grey) are shown. Maps were calculated for a refined model with the heme atoms omitted.



Supplemental Figure S3. Comparative cell development. Representative images of *D. discoideum* wild-type (AX2), Δ DdPoxA and Δ noxABC mutants throughout the development cycle (7, 12, 18 and 24 h after start of starvation) recorded using a stereomicroscope. Cell density in all images is 1×10^6 cells per drop.