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Supporting information for article:

Anaerobic fixed-target serial crystallography

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Table S1 LIC primers used in the cloning of the codon-optimised VioC into pNIC28-Bsa4 vector.

Oligo name	Sequence (5´-3´)	Tm (°C)
LIC_VioC fw	TACTTCCAATCCATGATGACCGAAAGT CCGACCACACATCATGG	85.5
LIC_VioC rev	TATCCACCTTTACTGGCGCTGACCCAG AACACGGC	83.3

Table S2 Data collection and refinement statistics. Absorbed doses were calculated using RADDOSE-3D (Bury *et al.*, 2018).

Datasets	**VioC:Fe:2OG:Arg** (PDB ID: 6Y0N)	**VioC:Fe:SIN:(3*S*)-OH-Arg** (PDB ID: 6Y12)	**AlkB:Fe:2OG:T-1meA-T**(PDB ID: 6Y0Q)	**AlkB:Fe:2OG** (PDB ID: 6YPV)	**IPNS:Fe:ACV** (PDB ID: 6Y0O)
Data Collection					
Beamline (Wavelength, Å) and detector	DLS I24 (0.9686), Pilatus3 6M				
Data Processing	DIALS (integration), cctbx.xfel (merging)				
Diffraction weighted dose / kGy	36	36	68	68	38
Number of images used	12318	16594	11778	4640	9449
Space group	C 2	C 2	P 1	P1	$P2_12_12_1$
Cell dimensions					
a, *b*, *c* (Å)	82.0, 66.6, 63.6	82.4, 67.1, 64.0	36.4, 39.1, 40.8	37.0, 39.1, 40.9	41.9, 75.8, 102.0
α, β, γ (°)	90, 110.2, 90	90, 110.2, 90	79.0, 78.0, 66.9	78.1, 76.0, 66.5	90, 90, 90
No. of	1	1	1	1	1

molecules/ASU					
No. reflections	27077 (2700)	36065 (3574)	20346 (1028)	11994 (593)	16880 (600)
Resolution (Å)	59.69-1.86 (1.93-1.86)	50.69-1.70(1.76-1.70)	35.67-1.75 (1.81-1.75)	39.35-2.10 (2.13-2.10)	60.85-2.20 (2.24-2.20)
R_{split}	0.232 (0.820)	0.215 (0.841)	0.178 (0.789)	0.333 (0.617)	0.235 (1.004)
<I>	74992 (6733)	59601 (4048)	6663 (660)	11298 (1965)	65916 (16988)
<I/σ(I)>	28.8 (2.3)	34.2 (2.3)	37.2 (2.8)	48.4 (8.795)	15.0 (1.4)
CC-1/2	0.94 (0.28)	0.95 (0.26)	0.98 (0.26)	0.87 (0.41)	0.96 (0.18)
Completeness (%)	100 (100)	99.9 (99.4)	100 (100)	100 (99.9)	97.7 (99.8)
Multiplicity	86.89 (10.86)	62.41(10.54)	57.57 (10.60)	25.8 (12.92)	130.22 (7.96)
Wilson B value ($Å^2$)	22.73	19.93	18.39	23.04	24.74
Refinement	PHENIX				
R_{work}/R_{free}	0.1886/0.2283	0.1819 /0.2103	0.1622/0.1914	0.2195/0.2506	0.2088/0.2474
No. atoms	2677	2787	1738	1593	2761
- Enzyme	2515	2574	1548	1510	2614
- Ligand	11	9	54	11	36
- Water	151	204	136	72	112
Average B-factors	33.26	26.87	24.24	26.83	30.09
- Enzyme	32.91	26.08	22.75	26.44	29.94
- Ligand	53.12	23.44	38.27	33.70	40.68
- Water	37.73	36.93	35.65	33.98	30.21
R.m.s deviations					
- Bond lengths (Å)	0.003	0.004	0.004	0.004	0.002
- Bond angles (°)	0.62	0.71	0.68	0.54	0.49

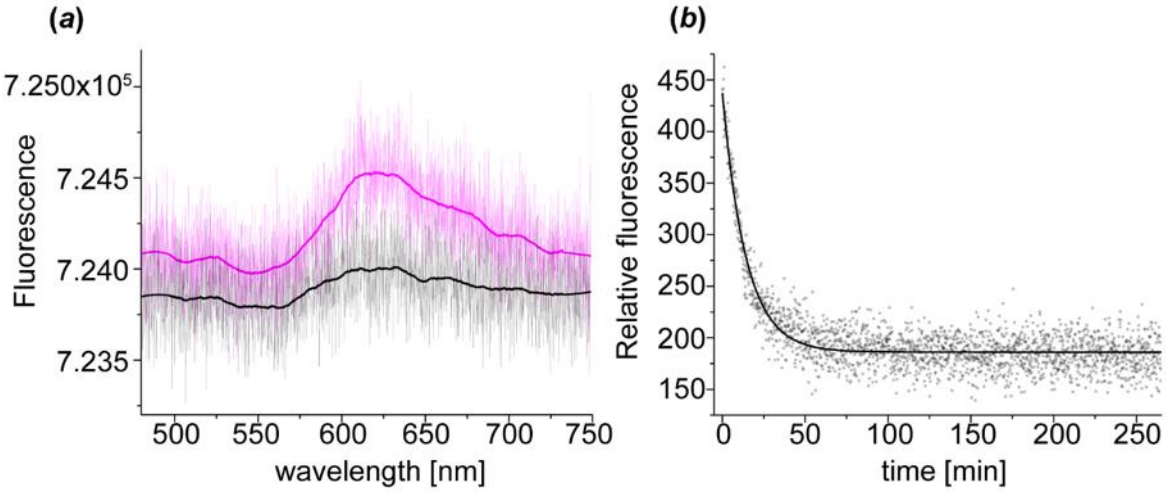

Figure S1 Fluorescence measurements. (*a*) Exemplary raw and smoothened accumulated optical spectra taken at the beginning (t_0, magenta) and the end of the kinetic series (t = 265 min, black) for the "5" type of setup indicated in Fig. 2(*d*). (*b*) Exemplary plot showing decay of the fluorescence signal for the "3" type of setup indicated in Fig. 2(*d*) plotted as the absolute difference between the number of counts at 615 nm with 550 nm (dots) fitted to a single exponential decay model (solid line), from which a $t_{0.5}$ = 13.7 minutes was obtained.

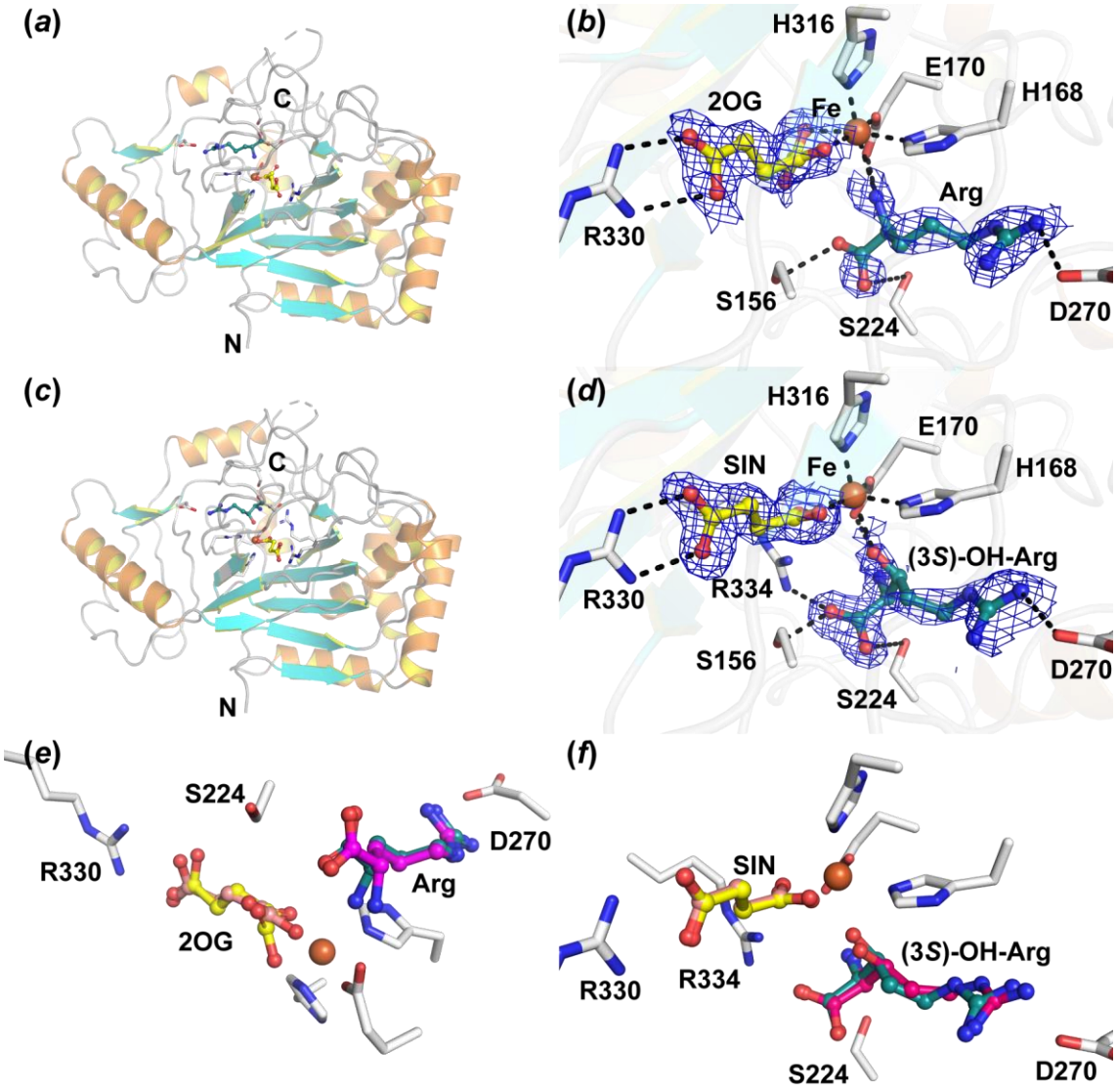

Figure S2 Structural comparison of the anaerobic and O_2 exposed VioC structures. (*a*) Ribbons representation and (*b*) close-up view of the active site from the VioC:Fe:2OG:Arg complex structure reported in this study (PDB: 6Y0N). The N- and C-termini are labelled as N and C, respectively. Alpha-helices and beta-strands are shown in gold and cyan, respectively. The arginine substrate, 2OG cosubstrate and coordinating residues are shown in sticks. Panel (*b*) shows the composite omit map to 1.86 Å resolution displayed at 0.7 σ contour level, carved around the arginine substrate and 2OG. (*c*) Ribbons representation and (*d*) close-up view of the active site of VioC:Fe:succinate:(3*S*)-hydroxy-l-Arg complex after dioxygen exposure (PDB: 6Y12). Panel (*d*) shows the composite omit map to 1.70 Å resolution displayed at 0.7 σ contour level, carved around the (3*S*)-hydoxy-l-Arg and succinate. (*e*) Superimposition of the cryogenic (magenta, and salmon, PDB: 6ALM) and serial room temperature (cyan, and yellow, PDB: 6Y0N, this study) VioC:Fe:2OG:Arg complex structures reveals small apparent differences in the bidentate coordination of the metal by 2OG, as well as a change in the conformation of the arginine substrate (including the α-amino group), possibly reflecting the flexible nature of the VioC active site (Mitchell *et al*., 2017). (*f*) Superimposition of the cryogenic (magenta

and salmon, PDB: 2WBP) and serial room temperature (cyan and yellow) VioC:Fe:succinate:(3*S*)-hydroxy-l-Arg complex (PDB: 6Y12, this study) structures reveals small apparent differences in the conformation of (3*S*)-hydroxy-l-Arg, whereas the binding of succinate is consistent with that in the reported cryogenic structure (Helmetag *et al*., 2009; Mitchell *et al*., 2017).