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: (3S)-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** |                                 |                                        |                                      |                           |                           |
| 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_1,2,2_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 | Resolution (Å) | R_split | <I> | <I>/σ(I)> | CC-1/2 | Completeness (%) | Multiplicity | Wilson B value (Å²) | Refinement | PHENIX |
|--------------|----------------|----------------|---------|-----|------------|--------|------------------|-------------|---------------------|------------|--------|
|              | 27077 (2700)   | 59.69-1.86 (1.93-1.86) | 0.232 (0.820) | 74992 (6733) | 28.8 (2.3) | 0.94 (0.28) | 100 (100) | 86.89 (10.86) | 22.73 | 0.1886/0.2283 |
|              | 36065 (3574)   | 50.69-1.70 (1.76-1.70) | 0.215 (0.841) | 59601 (4048) | 34.2 (2.3) | 0.95 (0.26) | 99.9 (99.4) | 62.41(10.54) | 19.93 | 0.1819/0.2103 |
|              | 20346 (1028)   | 35.67-1.75 (1.81-1.75) | 0.178 (0.789) | 6663 (660)  | 37.2 (2.8) | 0.98 (0.26) | 100 (100) | 57.57 (10.60) | 18.39 | 0.1622/0.1914 |
|              | 11994 (593)    | 39.35-2.10 (2.13-2.10) | 0.333 (0.617) | 11298 (1965) | 48.4 (8.795) | 0.87 (0.41) | 100 (99.9) | 25.8 (12.92) | 23.04 | 0.2195/0.2506 |
|              | 16880 (600)    | 60.85-2.20 (2.24-2.20) | 0.235 (1.004) | 65916 (16988) | 15.0 (1.4) | 0.96 (0.18) | 97.7 (99.8) | 130.22 (7.96) | 24.74 | 0.2088/0.2474 |
|              | 27077 (2700)   | 59.69-1.86 (1.93-1.86) | 0.232 (0.820) | 74992 (6733) | 28.8 (2.3) | 0.94 (0.28) | 100 (100) | 86.89 (10.86) | 22.73 | 0.1886/0.2283 |
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|              | 16880 (600)    | 60.85-2.20 (2.24-2.20) | 0.235 (1.004) | 65916 (16988) | 15.0 (1.4) | 0.96 (0.18) | 97.7 (99.8) | 130.22 (7.96) | 24.74 | 0.2088/0.2474 |

- **Enzyme**
- **Ligand**
- **Water**

**Average B-factors**
- **Enzyme**: 32.91
- **Ligand**: 53.12
- **Water**: 37.73

**R.m.s deviations**
- **Bond lengths (Å)**
- **Bond angles (°)**
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 \( \text{O}_2 \) 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 \( \sigma \) 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:(3S)-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 \( \sigma \) contour level, carved around the (3S)-hydroxy-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 \( \alpha \)-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:(3S)-hydroxy-l-Arg complex (PDB: 6Y12, this study) structures reveals small apparent differences in the conformation of (3S)-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).