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Supporting information for article:

Dose-resolved serial synchrotron and XFEL structures of radiation-sensitive metalloproteins

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**Table S1**  Data collection and processing statistics for the SFX structure and MSS series 1.

All structures are in space group P2₁.

| Dataset       | SACLA SFX | MSS1-ds1 | MSS1-ds2 | MSS1-ds3 | MSS1-ds4 | MSS1-ds5 |
|---------------|-----------|----------|----------|----------|----------|----------|
| **Data Collection** |           |          |          |          |          |          |
| Cumulative dose / kGy | N/A | 32.8 | 65.6 | 98.4 | 131.2 | 164.0 |
| Number of integrated frames | 73281 | 9751 | 9833 | 10002 | 9801 | 9243 |
| Number of images used* | 72615 | 8596 | 8608 | 8700 | 8342 | 7787 |
| **Data processing** |           |          |          |          |          |          |
| Space Group | P2₁ | | | | | |
| Cell dimensions / Å/deg. | a=72.72, b=68.18, c=74.62, beta=105.58 | a = 72.95 (0.33) | a = 72.96 (0.35) | a = 72.97 (0.36) | a = 72.99 (0.39) | a = 73.01 (0.42) |
| Resolution / Å | 41.46-1.88 | 44.64 - 1.78 | 44.63 - 1.78 | 44.62 - 1.83 | 44.63 - 1.90 | 44.64 - 1.98 |
|---------------|------------|--------------|--------------|--------------|--------------|--------------|
| (1.81 - 1.78) | 44.64 - 1.78 | 44.63 - 1.78 | 44.62 - 1.83 | 44.63 - 1.90 | 44.64 - 1.98 | |
| Number of reflections | 57351 | 67891 | 67896 | 62552 | 55954 | 49513 |
| R_{split} | 7.22 (58.29) | 18.92 (47.72) | 18.03 (51.62) | 16.97 (56.25) | 16.40 (61.71) | 16.73 (62.72) |
| CC_{1/2} | 99.27 (0.722) | 94.43 (67.71) | 95.34 (67.20) | 95.99 (66.77) | 96.63 (59.07) | 96.94 (60.18) |
| Multiplicity | 380.6 (212.6) | 27.12 (11.36) | 28.36 (10.48) | 32.21 (10.79) | 34.48 (10.19) | 35.48 (9.39) |
| Completeness (%) | 100 (100) | 100 (99.9) | 100 (99.9) | 100 (99.9) | 100 (99.8) | 100 (99.8) |

**Refinement**

| Number of reflections | 57312 | 67891 | 67896 | 62551 | 55953 | 49512 |
| R_{work}/R_{free} | 0.132/0.167 | 0.1647 / 0.2032 | 0.1662 / 0.2114 | 0.1668 / 0.2142 | 0.1670 / 0.2120 | 0.1720 / 0.2322 |
|                      | 0.009 | 0.006 | 0.006 | 0.006 | 0.007 | 0.007 |
|----------------------|-------|-------|-------|-------|-------|-------|
| RMSD bond lengths    |       |       |       |       |       |       |
| (Å)                  |       |       |       |       |       |       |
| RMSD bond angles     | 0.92  | 0.81  | 0.82  | 0.84  | 0.87  | 0.89  |
| (deg)                |       |       |       |       |       |       |
| Ramachandran plot    |       |       |       |       |       |       |
| Most favoured (%)    | 98.5  | 98.3  | 98.2  | 98.1  | 97.5  | 96.9  |
| Allowed (%)          | 1.50  | 1.67  | 1.81  | 1.81  | 2.50  | 3.06  |
| PDB accession code   | 6I43  | 6I7Z  | 6I8E  | 6I8I  | 6I8J  | 6I8K  |

* Number of merged patterns in CrystFEL.
**Table S2**  Data collection and processing statistics for MSS series 2.

All structures are in space group P2₁.

| Dataset | MSS2-1 | MSS2-2 | MSS2-3 | MSS2-4 | MSS2-5 | MSS2-6 | MSS2-7 | MSS2-8 |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|
| **Data Collection** | | | | | | | | |
| Cumulative dose / kGy | 39.2 | 78.4 | 117.6 | 156.8 | 196 | 235.2 | 274.4 | 313.6 |
| Number of integrated frames | 15294 | 14719 | 14032 | 13307 | 12464 | 11524 | 10058 | 8492 |
| Number of images used | 13024 | 12513 | 11889 | 11121 | 10210 | 9070 | 7678 | 6221 |
| **Data processing** | | | | | | | | |
| Cell dimensions / Å | a = 72.99 (0.54) | a = 73.01 (0.55) | a = 73.03 (0.55) | a = 73.04 (0.56) | a = 73.06 (0.59) | a = 73.07 (0.63) | a = 73.07 (0.67) | a = 73.07 (0.73) |
|                        | b = 68.36 (0.31) | b = 68.36 (0.31) | b = 68.36 (0.32) | b = 68.37 (0.33) | b = 68.37 (0.35) | b = 68.38 (0.37) | b = 68.38 (0.40) | b = 68.39 (0.45) |
|                        | c = 74.95 (0.61) | c = 74.94 (0.61) | c = 74.94 (0.62) | c = 74.97 (0.63) | c = 74.97 (0.63) | c = 75.03 (0.66) | c = 75.08 (0.70) | c = 75.06 (0.80) |
| Resolution / Å  | β = 105.63 (0.43) | β = 105.67 (0.44) | β = 105.72 (0.44) | β = 105.76 (0.47) | β = 105.81 (0.52) | β = 105.84 (0.54) | β = 105.85 (0.60) | β = 105.82 (0.65) |
|----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 44.71 - 1.70 (1.73 - 1.70) | 44.69 - 1.73 (1.76 - 1.73) | 44.67 - 1.74 (1.77 - 1.74) | 44.68 - 1.78 (1.81 - 1.78) | 44.70 - 1.82 (1.85 - 1.82) | 44.71 - 1.93 (1.96 - 1.93) | 44.72 - 2.03 (2.07 - 2.03) | 44.71 - 2.18 (2.22 - 2.18) |
| Number of reflections | 78148 | 74214 | 72957 | 68910 | 64459 | 54263 | 46140 | 37330 |
| Rsplit | 18.24 (73.81) | 18.31 (71.41) | 18.15 (73.88) | 18.05 (71.35) | 17.38 (74.98) | 17.17 (79.11) | 17.90 (78.30) | 18.79 (74.51) |
| CC1/2 | 95.78 (49.99) | 95.87 (49.15) | 95.09 (51.04) | 95.59 (52.06) | 96.56 (50.64) | 96.63 (54.40) | 96.92 (50.62) | 96.87 (53.87) |
| Multiplicity | 52.99 (12.44) | 51.36 (13.38) | 48.90 (11.86) | 47.42 (11.65) | 45.97 (10.87) | 46.51 (12.73) | 44.54 (11.42) | 41.36 (11.78) |
| Completeness (%) | 100 (99.9) | 100 (100) | 100 (99.8) | 100 (99.8) | 100 (99.5) | 100 (99.8) | 100 (99.6) | 100 (99.5) |
| Refinement | | | | | | | | |
| Number of reflections | 78148 | 74203 | 72955 | 68181 | 63833 | 53667 | 46136 | 37329 |
| Rwork/Rfree | 0.1805 / 0.2346 | 0.1774 / 0.2337 | 0.1780 / 0.2304 | 0.1784 / 0.2321 | 0.1798 / 0.2359 | 0.1803 / 0.2355 | 0.1816 / 0.2492 | 0.1778 / 0.2555 |
| RMSD bond lengths (Å) | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.008 | 0.008 |
|----------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| RMSD bond angles (deg) | 0.85  | 0.84  | 0.85  | 0.85  | 0.86  | 0.91  | 0.94  | 0.98  |
| Ramachandran plot | Most favoured (%) | 97.91 | 98.05 | 98.05 | 97.08 | 97.22 | 96.66 | 96.52 | 94.44 |
| | Allowed (%) | 2.09  | 1.95  | 1.95  | 2.78  | 2.78  | 3.34  | 3.34  | 5.15  |
| PDB accession code | 6I8O  | 6I8P  | 6I8Q  | 6Q31  | 6Q34  | 6Q3D  | 6Q3E  | 6IBN  |
### Table S3

(a) Heme site parameters for SFX and MSS-1 datasets.

| Structure | SFX  | MSS1-1 | MSS2-2 | MSS2-3 | MSS2-4 | MSS2-5 |
|-----------|------|--------|--------|--------|--------|--------|
| Resolution (Å) | 1.88 | 1.78   | 1.78   | 1.83   | 1.90   | 1.98   |
| Fe-His (Å)    | 2.19 | 2.24   | 2.25   | 2.29   | 2.25   | 2.23   |
| Fe-W1 (Å)     | 2.40 | 2.48   | 2.70   | 2.77   | 2.71   | 2.97   |
| PDB accession code | 6I43 | 6I7Z   | 6I8E   | 6I8I   | 6I8J   | 6I8K   |

(b) Heme site parameters for MSS-2 datasets.

| Structure | SFX  | MSS2-1 | MSS2-2 | MSS2-3 | MSS2-4 | MSS2-5 | MSS2-6 | MSS2-7 | MSS2-8 |
|-----------|------|--------|--------|--------|--------|--------|--------|--------|--------|
| Resolution (Å) | 1.88 | 1.70   | 1.73   | 1.74   | 1.78   | 1.82   | 1.93   | 2.03   | 2.18   |
| Fe-His (Å)   | 2.19 | 2.27   | 2.27   | 2.31   | 2.30   | 2.28   | 2.22   | 2.13   | 2.23   |
| Fe-W1 (Å)    | 2.40 | 2.50   | 2.67   | 2.64   | 2.93   | 2.91   | 3.16   | 3.37   | 3.76   |
| PDB accession code | 6I43 | 6I8O   | 6I8P   | 6I8Q   | 6Q31   | 6Q34   | 6Q3D   | 6Q3E   | 6IBN   |
**Figure S1** DtpAa amplification primers.

- **2602-F**
  5’-CTAACATATGGACCCGCTGCCTGGCCGACGCG-3’

- **2602-R**
  5’-CCGAAGCTTTTACGCCTTGGCCGAAACAGCG-3’

**Figure S2** Unit cell volume vs. absorbed dose for MSS data collected at Diamond beamline I24.
Figure S3 2Fo-Fc electron density maps contoured at 1σ for the haem environment of DtpAa in (A) the SFX dataset from SACLA, (B-F) selected structures from the low dose MSS-1 series; (G) Superposition of selected MSS-1 structures revealing the dose-dependent migration of the water molecule W1 away from the haem Fe.
Figure S4  2Fo-Fc electron density maps contoured at 1σ for the haem environment of DtpAa in (A) the SFX dataset from SACLA, (B-F) selected structures from the higher dose MSS-2 series; (G) Superposition of selected MSS-2 structures revealing the dose-dependent migration of the water molecule W1 away from the haem Fe.
Figure S5  $2Fo-Fc$ electron density maps contoured at 1σ for the haem propionate environment of DtpAa in the SFX dataset from SACLA (blue) and from the highest dose (313.6 kGy) structure from the MSS-2 series (red), showing corresponding waters between the ‘damage free’ and highest dose structure shift, a potential cause or effect of the flip in haem propionate(s).