## SUPPLEMENTARY INFORMATION

# Perfect Crystals: Microgravity capillary counterdiffusion crystallization of human manganese superoxide dismutase for neutron crystallography

William E. Lutz[1], Jahaun Azadmanesh[1], Jeffrey J. Lovelace[1], Carol Kolar[1], Leighton Coates[3], Kevin L. Weiss[4], and Gloria E. O. Borgstahl[1,2*]

[1] Eppley Institute for Cancer and Allied Diseases, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, USA
[2] Department of Biochemistry and Molecular Biology, 985870 Nebraska Medical Center, Omaha, NE 68198-5870, USA
[3] Second Target Station, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37831, USA
[4] Neutron Scattering Division, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37831, USA
*gborgstahl@unmc.edu

**Table S1: Duration of exposures to microgravity.**

| Box | Launch Date | Return Date | Time in Microgravity |
|---|---|---|---|
| SN001 | 2018-12-05 | 2019-01-14 | 40 days |
| SN002 | 2018-12-05 | 2019-08-27 | 263 days |
| SN003 | 2019-07-25 | 2019-08-27 | 33 days |
| SN004 | 2019-07-25 | 2020-01-07 | 164 days |

**Table S2. Box Contents**

| Box SN001 | | | | | | |
|---|---|---|---|---|---|---|
| **GCB #** | **Variant** | **[Protein]** | **[Reservoir]** | **Crystals** | **Gravity** | **Layout** |
| GCB 1 | WT | 25 mg/mL | 4M | Y | Earth | A |
| GCB 2 | WT | 25 mg/mL | 4M | Y | Earth | A |
| GCB 3 | WT | 25 mg/mL | 3M | N | Earth | A |
| GCB 4 | WT | 25 mg/mL | 4M | Y | Earth | C |
| GCB 5 | Y34F | 25 mg/mL | 4M | Y | Earth | A |
| GCB 6 | Y34F | 25 mg/mL | 4M | Y | Earth | A |
| GCB 7 | Y34F | 25 mg/mL | 3M | Y | Earth | D |
| GCB 8 | Y34F | 25 mg/mL | 3M | Y | Earth | D |
| GCB 9 | H30N | 25 mg/mL | 4M | Y | Earth | A |
| GCB 10 | H30Q | 25 mg/mL | 4M | Y | Earth | B |
| **Box SN002** | | | | | | |
| **GCB #** | **Variant** | **[Protein]** | **[Reservoir]** | **Crystals** | **Gravity** | **Layout** |
| GCB 1 | WT | 25 mg/mL | 4M | Y | Microgravity | A |
| GCB 2 | WT | 25 mg/mL | 4M | Y | Microgravity | A |
| GCB 3 | WT | 25 mg/mL | 3M | N | Microgravity | A |
| GCB 4 | WT | 25 mg/mL | 4M | Y | Microgravity | C |
| GCB 5 | Y34F | 25 mg/mL | 4M | Y | Microgravity | A |
| GCB 6 | Y34F | 25 mg/mL | 4M | Y | Microgravity | A |
| GCB 7 | Y34F | 25 mg/mL | 3M | N | Microgravity | A |
| GCB 8 | Y34F | 25 mg/mL | 3M | Y | Microgravity | D |
| GCB 9 | H30N | 25 mg/mL | 4M | Y | Microgravity | A |
| GCB 10 | H30Q | 25 mg/mL | 4M | Y | Microgravity | B |
| **Box SN003** | | | | | | |
| **GCB #** | **Variant** | **[Protein]** | **[Reservoir]** | **Crystals** | **Gravity** | **Layout** |
| GCB 1 | W161F | 25 mg/mL | 4M | Y | Earth | E |
| GCB 2 | W161F | 25 mg/mL | 3M | N | Earth | E |
| GCB 3 | W161F | 15 mg/mL | 4M | Y | Earth | E |
| GCB 4 | W161F | 15 mg/mL | 3M | N | Earth | E |
| GCB 5 | W161F | 15 mg/mL | 4M | N | Earth | A |
| GCB 6 | W161F | 15 mg/mL | 3M | N | Earth | A |
| GCB 7 | WT | 15 mg/mL | 4M | Y | Earth | E |
| GCB 8 | WT | 15 mg/mL | 3M | N | Earth | E |
| GCB 9 | Y34F | 15 mg/mL | 4M | Y | Earth | E |
| GCB 10 | Y34F | 15 mg/mL | 3M | N | Earth | E |
| **Box SN004** | | | | | | |
| **GCB #** | **Variant** | **[Protein]** | **[Reservoir]** | **Crystals** | **Gravity** | **Layout** |

| | | | | | | |
|---|---|---|---|---|---|---|
| GCB 1 | W161F | 25 mg/mL | 4M | Y | Microgravity | E |
| GCB 2 | W161F | 25 mg/mL | 3M | Y | Microgravity | E |
| GCB 3 | W161F | 15 mg/mL | 4M | Y | Microgravity | E |
| GCB 4 | W161F | 15 mg/mL | 3M | N | Microgravity | E |
| GCB 5 | W161F | 15 mg/mL | 4M | Y | Microgravity | A |
| GCB 6 | W161F | 15 mg/mL | 3M | N | Microgravity | A |
| GCB 7 | WT | 15 mg/mL | 4M | Y | Microgravity | E |
| GCB 8 | WT | 15 mg/mL | 3M | N | Microgravity | E |
| GCB 9 | Y34F | 15 mg/mL | 4M | Y | Microgravity | E |
| GCB 10 | Y34F | 15 mg/mL | 3M | N | Microgravity | E |

**Table S3. Neutron data collection statistics.**

| | Y34F $Mn^{3+}$SOD |
|---|---|
| Diffraction Source | MaNDi |
| Wavelengths (Å) | 2-4 |
| Temperature (K) | 296 |
| Detectors | 40 SNS Anger Cameras |
| Crystal-to-detector distance (mm) | 450 |
| Rotation along Φ between images (°) | 20 |
| Total rotation range (°) | 220 |
| No. of images collected | 11 |
| Exposure time per image (hrs) | 20 |
| Space group | $P6_122$ |
| *a*, *b*, *c* (Å) | 79.3, 79.3, 240.6 |
| *α*, *β*, *γ* (°) | 90, 90, 120 |
| Resolution range (Å) | 14.39-2.28 (2.36-2.28) |
| Total No. of reflections | 161187 |
| No. of unique reflections | 20703 |
| Completeness (%) | 97.78 (88.40) |
| Multiplicity | 7.79 (6.15) |
| I/σ(I) | 7.4 (3.7) |
| $R_{merge}$ | 0.246 (0.323) |
| $R_{meas}$ | 0.263 (0.349) |
| $R_{p.i.m.}$ | 0.090 (0.127) |

**Table S4. Purification yields for deuterated wild-type MnSOD and its variants.**

| MnSOD type | Pellet weight (g) | Purified Protein (mg) | Protein/Pellet (mg/g) |
|---|---|---|---|
| Wild-type | 120 | 1530 | 12.8 |
| Tyr34Phe | 62 | 276 | 4.5 |
| His30Gln | 35 | 31 | 0.9 |
| His30Asn | 38 | 94 | 2.5 |
| Trp161Phe | 41 | 887 | 21.6 |

**Table S5. GCB Layouts**

| GCB Layout A (1 mL agarose slug) | | |
|---|---|---|
| **Capillary #** | **Content** | **Agarose Plug Length (cm)** |
| 1 | Protein | 2 |
| 2 | Reservoir | 3 |
| 3 | Protein | 2.5 |
| 4 | Reservoir | 3 |
| 5 | Protein | 3 |
| 6 | Reservoir | 3 |
| 7 | Protein | 3.5 |
| 8 | Reservoir | 3 |
| 9 | Protein | 4 |
| 10 | Reservoir | 3 |

| GCB Layout D (1 mL agarose slug) | | |
|---|---|---|
| **Capillary #** | **Content** | **Agarose Plug Length (cm)** |
| 1 | Reservoir | 2 |
| 2 | Reservoir | 3 |
| 3 | Reservoir | 2.5 |
| 4 | Reservoir | 3 |
| 5 | Protein | 3 |
| 6 | Reservoir | 3 |
| 7 | Protein | 3.5 |
| 8 | Reservoir | 3 |
| 9 | Protein | 4 |
| 10 | Reservoir | 3 |

| GCB Layout C (1 mL agarose slug) | | |
|---|---|---|
| **Capillary #** | **Content** | **Agarose Plug Length (cm)** |
| 1 | Reservoir | 3 |
| 2 | Protein | 2.5 |
| 3 | Reservoir | 3 |
| 4 | Protein | 3 |
| 5 | Reservoir | 3 |
| 6 | Protein | 3.5 |
| 7 | Reservoir | 3 |
| 8 | Protein | 4 |
| 9 | Reservoir | 3 |
| 10 | Protein | 3 |

| GCB Layout E (0.4 mL agarose slug) | | |
|---|---|---|
| **Capillary #** | **Content** | **Agarose Plug Length (cm)** |
| 1 | Protein | 2 |
| 2 | Reservoir | 3 |
| 3 | Protein | 2.5 |
| 4 | Reservoir | 3 |
| 5 | Protein | 3 |
| 6 | Reservoir | 3 |
| 7 | Protein | 3.5 |
| 8 | Reservoir | 3 |
| 9 | Protein | 4 |
| 10 | Reservoir | 3 |

| GCB Layout C (1 mL agarose slug) | | |
|---|---|---|
| **Capillary #** | **Content** | **Agarose Plug Length (cm)** |
| 1 | Protein | 2 |
| 2 | Protein | 2 |
| 3 | Protein | 2.5 |
| 4 | Protein | 2.5 |
| 5 | Protein | 3 |
| 6 | Protein | 3 |
| 7 | Protein | 3.5 |
| 8 | Protein | 3.5 |
| 9 | Protein | 4 |
| 10 | Protein | 4 |

**Table S6. Precipitant Dilution Factors for GCB Layouts**

| | Volumes (mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| **GCB Layout** | **Quartz** | **Agarose** | **Protein** | **Reservoir** | **GCB (sans lid)** | **GCB-Quartz** | **Precipitant Dilution Factor*** |
| Layout A | 0.5 | 1.94 | 0.94 | 6.62 | 10 | 9.5 | **1.44** |
| Layout B | 0.5 | 1.97 | 0.91 | 6.62 | 10 | 9.5 | **1.44** |
| Layout C | 0.5 | 1.94 | 1.88 | 5.67 | 10 | 9.5 | **1.67** |
| Layout D | 0.5 | 1.94 | 0.52 | 7.04 | 10 | 9.5 | **1.35** |
| Layout E | 0.5 | 1.34 | 0.94 | 7.22 | 10 | 9.5 | **1.32** |

*dilution factor= (GCB-Quartz)/(Reservoir)