Supplementary Figure S1

Alignments of the amino acid sequences of V2Hb and OliHb (Oli). Secondary-structure based numbering of important residues are indicated.

The figure was prepared with the program ESPript (Robert, X. and Gouet, P. (2014) "Deciphering key features in protein structures with the new ENDscript server". Nucl. Acids Res. 42(W1), W320-W324).
Supplementary Table S1

Refined B-factors and occupancies of the atoms of oxygen-surrounding residues.

| Data 1 (78% oxy) | Data 2 (69% oxy) |
|------------------|------------------|
| **Residue** | **atom** | **B-factor** | **Occ.** | **Residue** | **atom** | **B-factor** | **Occ.** |
| Heme | FE | 33.89 | 1 | Heme | FE | 29.13 | 1 |
| NA | 33.02 | | | NA | 30.66 | |
| NB | 35.18 | | | NB | 36.02 | |
| NC | 33.51 | | | NC | 30.65 | |
| ND | 34.15 | | | ND | 30.52 | |
| His94 (F8) | NE2 | 34.85 | | His94 (F8) | NE2 | 30.37 | |
| His62 (E7) | NE2 | 36.88 | | His62 (E7) | NE2 | 33.15 | |
| Phe46 (CE1) | CE1 | 37.07 | | Phe46 (CE1) | CE1 | 31.89 | |
| Val66 (E11) | CG2 | 35.50 | | Val66 (E11) | CG2 | 32.21 | |
| Trp32 (B10) | CH2 | 36.47 | | Trp32 (B10) | CH2 | 31.87 | |
| **Average** | | 35.05 | | **Average** | | 31.65 | |
| **S.D.** | 1.43 | | | **S.D.** | 1.92 | |
| **O2** | O1 | 35.20 | 0.7 | **O2** | O1 | 30.61 | 0.5 |
| | O2 | 35.68 | | | O2 | 31.61 | |
| **Data 1 (78% oxy)** | **Data 2 (69% oxy)** |
| **Residue** | **atom** | **B-factor** | **Occ.** | **Residue** | **atom** | **B-factor** | **Occ.** |
| Heme | FE | 53.41 | 1 | Heme | FE | 35.52 | 1 |
| NA | 51.07 | | | NA | 35.52 | |
| NB | 49.46 | | | NB | 31.05 | |
| NC | 52.03 | | | NC | 32.82 | |
| ND | 48.27 | | | ND | 35.28 | |
| His95 (F8) | NE2 | 57.02 | | His95 (F8) | NE2 | 35.11 | |
| His63 (E7) | NE2 | 50.19 | | His63 (E7) | NE2 | 32.04 | |
| Phe47 (CD1) | CE1 | 57.51 | | Phe47 (CD1) | CE1 | 39.18 | |
| Val67 (E11) | CG2 | 48.44 | | Val67 (E11) | CG2 | 28.03 | |
| Trp33 (B10) | CH2 | 62.57 | | Trp33 (B10) | CH2 | 39.20 | |
| **Average** | | 53.00 | | **Average** | | 34.38 | |
| **S.D.** | 4.67 | | | **S.D.** | 3.49 | |
| **O2** | O1 | 56.69 | 0.7 | **O2** | O1 | 36.17 | 0.7 |
| | O2 | 50.82 | | | O2 | 33.63 | |
| **Data 1 (78% oxy)** | **Data 2 (69% oxy)** |
| **Residue** | **atom** | **B-factor** | **Occ.** | **Residue** | **atom** | **B-factor** | **Occ.** |
| Heme | FE | 39.62 | 1 | Heme | FE | 36.00 | 1 |
| NA | 44.57 | | | NA | 36.93 | |
| NB | 40.41 | | | NB | 29.38 | |
| NC | 47.61 | | | NC | 36.19 | |
The figures were prepared using PYMOL software (http://www.pymol.org/).

Briefly, refinements were performed for the oxygen-omitted model; then, oxygen molecules were added to the model, and the XYZ coordinates, atomic displacement parameters (B-factors), and group occupancies for the oxygen molecule were manually adjusted, and the individual

### Table S1

| Residue (A1') | atom | B-factor | Occ. | Residue (A1') | atom | B-factor | Occ. |
|--------------|------|----------|------|--------------|------|----------|------|
| Heme         | FE   | 40.14    |      | Heme         | FE   | 30.97    |      |
|              | NA   | 36.29    |      |              | NA   | 32.23    |      |
|              | NB   | 42.82    |      |              | NB   | 28.74    |      |
|              | NC   | 39.70    |      |              | NC   | 33.55    |      |
|              | ND   | 44.73    |      |              | ND   | 35.37    |      |
| His94 (F8)   | NE2  | 39.65    |      | His94 (F8)   | NE2  | 27.91    |      |
| His62 (E7)   | NE2  | 41.64    |      | His62 (E7)   | NE2  | 33.04    |      |
| Phe46 (CE1)  | CE1  | 40.55    |      | Phe46 (CE1)  | CE1  | 34.23    |      |
| Val66 (E11)  | CG2  | 38.33    |      | Val66 (E11)  | CG2  | 29.23    |      |
| Trp32 (B10)  | CH2  | 41.55    |      | Trp32 (B10)  | CH2  | 39.06    |      |
|              |      | 40.54    |      |              |      | 32.43    |      |
|              | S.D. | 2.35     |      |              | S.D. | 3.40     |      |
| O₂           | O1   | 40.40    | 0.8  | O₂           | O1   | 32.51    | 0.8  |
|              | O2   | 41.40    |      |              | O2   | 35.12    |      |

### Table S1 continued

The final models were validated by MOLPROBITY [19]. The statistics for data collection and refinement are

| Residue (A2') | atom | B-factor | Occ. | Residue (A2') | atom | B-factor | Occ. |
|--------------|------|----------|------|--------------|------|----------|------|
| Heme         | FE   | 40.14    |      | Heme         | FE   | 30.97    |      |
|              | NA   | 36.29    |      |              | NA   | 32.23    |      |
|              | NB   | 42.82    |      |              | NB   | 28.74    |      |
|              | NC   | 39.70    |      |              | NC   | 33.55    |      |
|              | ND   | 44.73    |      |              | ND   | 35.37    |      |
| His94 (F8)   | NE2  | 39.65    |      | His94 (F8)   | NE2  | 27.91    |      |
| His62 (E7)   | NE2  | 41.64    |      | His62 (E7)   | NE2  | 33.04    |      |
| Phe46 (CE1)  | CE1  | 40.55    |      | Phe46 (CE1)  | CE1  | 34.23    |      |
| Val66 (E11)  | CG2  | 38.33    |      | Val66 (E11)  | CG2  | 29.23    |      |
| Trp32 (B10)  | CH2  | 41.55    |      | Trp32 (B10)  | CH2  | 39.06    |      |
|              |      | 40.54    |      |              |      | 32.43    |      |
|              | S.D. | 2.35     |      |              | S.D. | 3.40     |      |
| O₂           | O1   | 40.40    | 0.8  | O₂           | O1   | 32.51    | 0.8  |
|              | O2   | 41.40    |      |              | O2   | 35.12    |      |
Incubated for 3-180s. The crystals were immediately flash-frozen under a nitrogen gas stream at -183°C.

HEPES-NaOH pH 7.5. The dissociation of the bound oxygen is not solely dependent on the concentration of sodium then soaked in the final buffer containing 50 mM sodium hydrosulfite, 20% PEG-400, 18-20% PEG-3350, and 100 mM and 10 mM CaCl2 or MgCl2. Bright red crystals were obtained by the sitting-drop vapor diffusion method at 20°C using Biophysics and Physicobiology Vol. 19. Subsequent milestones of the theoretical Monod−Wyman−Ch has been available to date. One of the major reasons for this lack of information is that it remains challenging to prepare however, only a small amount of intact structural information about intermediates between oxy and deoxy forms [7−9].

This nature impacts the behavior of protein molecules broadly, affecting metabolism and signaling pathways, and providing clues to drug development. Arguably the most exhaustively studied allosteric protein is hemoglobin (Hb), beginning with this nature impacts the behavior of protein molecules broadly, affecting metabolism and signaling pathways, and providing clues to drug development. Arguably the most exhaustively studied allosteric protein is hemoglobin (Hb), beginning with.

Materials and Methods

Oxygen Equilibrium Measurements

The absorption spectra of the soaked crystals were measured and corrected for the air blank baseline under a nitrogen-helium and 0.95% butane gas was injected into the cell and 3 ml of a 50 µM of V2Hb solution (pH 6.6) was added and absorbance of around 2.8 at 416 nm to 280 nm were collected.

Average

| Residue     | atom | B-factor | Occ. | Residue     | atom | B-factor | Occ. |
|-------------|------|----------|------|-------------|------|----------|------|
| Heme        | FE   | 49.57    | 1    | Heme        | FE   | 36.03    | 1    |
|             | NA   | 43.19    |      |             | NA   | 31.11    |      |
|             | NB   | 42.87    |      |             | NB   | 30.96    |      |
|             | NC   | 43.95    |      |             | NC   | 32.83    |      |
|             | ND   | 53.69    |      |             | ND   | 30.12    |      |
| His95 (F8)  | NE2  | 53.12    |      | His95 (F8)  | NE2  | 33.67    |      |
|            |       |          |      | His63 (E7)  | NE2  | 30.87    |      |
|            |       |          |      | His63 (E7)  | NE2  | 30.87    |      |
| Phe47 (CD1)| CE1  | 47.64    |      | Phe47 (CD1)| CE1  | 35.47    |      |
| Val67 (E11)| CG2  | 46.34    |      | Val67 (E11)| CG2  | 27.00    |      |
| Trp33 (B10)| CH2  | 53.17    |      | Trp33 (B10)| CH2  | 39.81    |      |
| Average    |      | 47.51    |      | Average    |      | 32.79    |      |
|            | S.D. | 4.66     | 0.8  |            | S.D. | 3.62     | 0.8  |
| O2         | O1   | 48.32    | 0.8  | O2         | O1   | 33.63    | 0.8  |
|            | O2   | 46.36    |      |            | O2   | 33.19    |      |

B1’

| Residue  | atom | B-factor | Occ. | Residue  | atom | B-factor | Occ. |
|----------|------|----------|------|----------|------|----------|------|
| Heme     | FE   | 35.84    | 1    | Heme     | FE   | 29.20    | 1    |
|          | NA   | 40.01    |      |          | NA   | 30.61    |      |
|          | NB   | 39.60    |      |          | NB   | 27.05    |      |
|          | NC   | 47.86    |      |          | NC   | 31.76    |      |
|          | ND   | 43.23    |      |          | ND   | 35.66    |      |
| His99 (F8)| NE2 | 41.09    |      | His99 (F8)| NE2 | 31.48    |      |
| Phe47 (CD1)| CE1| 42.00    |      | Phe47 (CD1)| CE1 | 26.59    |      |
| Val71 (E11)| CG2| 35.20    |      | Val71 (E11)| CG2 | 24.84    |      |
| Phe37 (B10) | CZ | 33.99    |      | Phe37 (B10)| CZ | 26.87    |      |
| Average  |      | 39.89    |      | Average  |      | 29.47    |      |
|          | S.D. | 4.14     | 0.4  |          | S.D. | 3.21     | 0.4  |
| O2       | O1   | 39.10    | 0.4  | O2       | O1   | 31.07    | 0.4  |
|          | O2   | 37.78    |      |          | O2   | 31.47    |      |

B2’

| Residue  | atom | B-factor | Occ. | Residue  | atom | B-factor | Occ. |
|----------|------|----------|------|----------|------|----------|------|
| Heme     | FE   | 53.41    | 1    | Heme     | FE   | 38.82    | 1    |
|          | NA   | 52.76    |      |          | NA   | 39.56    |      |
|          | NB   | 53.78    |      |          | NB   | 38.71    |      |
|          | NC   | 56.98    |      |          | NC   | 42.98    |      |
|          | ND   | 58.76    |      |          | ND   | 38.44    |      |
| His99 (F8)| NE2 | 59.25    |      | His99 (F8)| NE2 | 36.96    |      |
| Phe51 (CD1)| CE1| 46.17    |      | Phe51 (CD1)| CE1 | 37.62    |      |
| Val71 (E11)| CG2| 46.80    |      | Val71 (E11)| CG2 | 30.98    |      |
| Phe37 (B10) | CZ | 64.19    |      | Phe37 (B10)| CZ | 36.86    |      |
| Average  |      | 53.76    |      | Average  |      | 37.97    |      |
Oxygenation Properties of V2Hb

Results

The Hill plot of the oxygen equilibrium (Figure 1B) clarified that the oxygen affinity (slope of the black line) of V2Hb is remarkably higher than that of Hb (maximum 0.19 mmHg), though the cooperativity (n, max value of 1.4) of V2Hb is lower than that of Hb (maximum 0.19 mmHg), though the cooperativity (n, max value of 1.4).

Data 3 (55% oxy)

| Residue | atom | B-factor | Occ. | S.D. |
|---------|------|----------|------|------|
| Heme    | FE   | 32.91    | 1    | 6.23 |
|         | NA   | 31.22    |      |      |
|         | NB   | 32.30    |      |      |
|         | NC   | 32.35    |      |      |
|         | ND   | 31.60    |      |      |
| His94 (F8) | NE2 | 35.08    |      |      |
| His62 (E7) | NE2 | 35.58    |      |      |
| Phe46 (CE1) | CE1 | 31.89    |      |      |
| Val66 (E11) | CG2 | 31.95    |      |      |
| Trp32 (B10) | CH2 | 30.60    |      |      |
| Average |      | 32.55    |      | 1.60 |

| Residue | atom | B-factor | Occ. | S.D. |
|---------|------|----------|------|------|
| O2      | O1   | 54.00    |      | 55.83 |
|         | O2   | 53.19    |      | 56.95 |

Data 4 (26% oxy)

| Residue | atom | B-factor | Occ. | S.D. |
|---------|------|----------|------|------|
| Heme    | FE   | 34.63    | 1    | 3.00 |
|         | NA   | 30.96    |      |      |
|         | NB   | 38.94    |      |      |
|         | NC   | 38.06    |      |      |
|         | ND   | 34.25    |      |      |
| His94 (F8) | NE2 | 33.15    |      |      |
| His62 (E7) | NE2 | 40.05    |      |      |
| Phe46 (CE1) | CE1 | 42.48    |      |      |
| Val66 (E11) | CG2 | 37.30    |      |      |
| Trp32 (B10) | CH2 | 40.42    |      |      |
| Average |      | 37.02    |      | 3.66 |

| Residue | atom | B-factor | Occ. | S.D. |
|---------|------|----------|------|------|
| O2      | O1   | 32.54    | 0.3  | 51.17 |
|         | O2   | 33.06    |      | 54.83 |

Data 5 (55% oxy)

| Residue | atom | B-factor | Occ. | S.D. |
|---------|------|----------|------|------|
| Heme    | FE   | 44.28    | 1    | 4.32 |
|         | NA   | 43.76    |      |      |
|         | NB   | 40.36    |      |      |
|         | NC   | 42.84    |      |      |
|         | ND   | 42.81    |      |      |
| His95 (F8) | NE2 | 47.20    |      |      |
| His63 (E7) | NE2 | 42.90    |      |      |
| Phe47 (CD1) | CE1 | 43.64    |      |      |
| Val67 (E11) | CG2 | 37.81    |      |      |
| Trp33 (B10) | CH2 | 51.23    |      |      |
| Average |      | 43.68    |      | 3.61 |

| Residue | atom | B-factor | Occ. | S.D. |
|---------|------|----------|------|------|
| O2      | O1   | 46.41    | 0.6  | 52.60 |
|         | O2   | 41.56    |      | 55.83 |

Data 6 (26% oxy)

| Residue | atom | B-factor | Occ. | S.D. |
|---------|------|----------|------|------|
| Heme    | FE   | 51.51    | 1    | 51.51 |
|         | NA   | 52.60    |      |      |
|         | NB   | 56.50    |      |      |
|         | NC   | 50.14    |      |      |
|         | ND   | 55.83    |      |      |
**Microspectrometry**

Deoxygenation of the Oxy Crystals

The crystals were immediately flash-frozen under a nitrogen gas stream at -183°C.

Recently we reported that bound oxygen of the crystals can gradually dissociate thorough the soaking method [11,12], and that the oxygen saturation of each molecule converged within the standard deviation of the refined coordinates and experimental data. Multiple molecules at this resolution converged within the standard deviation of the refined coordinates and experimental data. No symmetry restraint was applied in the structure.

The oxygen molecules were manually adjusted, and the individual adjustments and changes are smaller with local perturbations than those observed in Oli Hb. Our intermediate structures of V2Hb together with six copies of each of the four subunits (Figure 1A). Recently we reported that bound oxygen of the crystals has been available to date. One of the major reasons for this lack of information is that it remains challenging to prepare sheep blood of the worm. The allosteric nature of proteins has been widely studied for over half a century [1,2] as the "second secret of life" [3]; and that the oxygen saturation of each molecule converged within the standard deviation of the refined coordinates and experimental data. No symmetry restraint was applied in the structure.

The atomic coordinates and experimental data (PDB ID: 3wct or 3wcu) were used as the search models to determine the initial phases by performing using COOT [17] and PHENIX [18], respectively. No symmetry restraint was applied in the structure.

NW12A at KEK PF-AR. All data were processed and scaled using XDS [14] and truncated by the CCP4 software suite.

### Table 1

| Residue          | atom | B-factor | Occ. | Residue          | atom | B-factor | Occ. |
|------------------|------|----------|------|------------------|------|----------|------|
| Heme             | FE   | 47.55    | 1    | Heme             | FE   | 47.14    | 1    |
|                  | NA   | 46.30    |      |                  | NA   | 49.01    |      |
|                  | NB   | 46.58    |      |                  | NB   | 56.24    |      |
|                  | NC   | 47.80    |      |                  | NC   | 45.74    |      |
|                  | ND   | 49.44    |      |                  | ND   | 50.99    |      |
| His99 (F8)       | NE2  | 46.03    |      | His99 (F8)       | NE2  | 52.70    |      |
| His67 (E7)       | NE2  | 45.03    |      | His67 (E7)       | NE2  | 51.71    |      |
| Phe51 (CD1)      | CE1  | 47.54    |      | Phe51 (CD1)      | CE1  | 56.57    |      |
| Val71 (E11)      | CG2  | 43.06    |      | Val71 (E11)      | CG2  | 49.82    |      |
| Phe37 (B10)      | CZ   | 40.61    |      | Phe37 (B10)      | CZ   | 61.70    |      |

**Average**

|          | S.D. |          |          |
|----------|------|----------|----------|
| O2       | 0.9  | O2       | 0.3      |
| O2       | 0.7  | O2       | 0.3      |

**A1’**

| Residue          | atom | B-factor | Occ. | Residue          | atom | B-factor | Occ. |
|------------------|------|----------|------|------------------|------|----------|------|
| Heme             | FE   | 37.09    | 1    | Heme             | FE   | 53.13    | 1    |
|                  | NA   | 33.86    |      |                  | NA   | 42.48    |      |
|                  | NB   | 35.38    |      |                  | NB   | 42.83    |      |
|                  | NC   | 36.61    |      |                  | NC   | 48.07    |      |
|                  | ND   | 34.06    |      |                  | ND   | 46.04    |      |
| His94 (F8)       | NE2  | 34.68    |      | His94 (F8)       | NE2  | 41.58    |      |
| His62 (E7)       | NE2  | 39.93    |      | His62 (E7)       | NE2  | 46.06    |      |
| Phe46 (CE1)      | CE1  | 37.48    |      | Phe46 (CE1)      | CE1  | 45.96    |      |
| Val66 (E11)      | CG2  | 35.26    |      | Val66 (E11)      | CG2  | 43.97    |      |
| Trp32 (B10)      | CH2  | 43.68    |      | Trp32 (B10)      | CH2  | 42.04    |      |

**Average**

|          | S.D. |          |          |
|----------|------|----------|----------|
| O2       | 0.6  | O2       | 0        |
| O2       | 0.6  | O2       | 0        |

**A2’**

| Residue          | atom | B-factor | Occ. | Residue          | atom | B-factor | Occ. |
|------------------|------|----------|------|------------------|------|----------|------|
|                  |      |          |      |                  |      |          |      |

|          | S.D. |          |          |
|----------|------|----------|----------|
| O2       | 0    | O2       | 0        |
| O2       | 0    | O2       | 0        |
### Table S1: Oxygenation Properties of V2Hb

The oxygen affinity of V2Hb is remarkably higher than that of Hb. Briefly, refinements were performed for the oxygen-omitted model; then, oxygen molecules were added to the structure. The occupancies of the bound oxygen molecules at each subunit were estimated as described in our previous study of Hb [12].

#### Data Collection, Model Building, and Refinement

The atomic coordinates and experimental data (PDB ID: 7vlc, 7vld, 7vle, and 7vlf for the structures of 

#### Gas Cryostream

Gas cryostream using an online microspectrophotometer at beamline BL38B1 [13]. All data were processed and scaled using XDS [14] and truncated by the CCP4 software suite NW12A at KEK PF-AR.

#### Refinement

The refinements were performed using COOT [17] and PHENIX [18], respectively. No symmetry restraint was applied in the structure refinement though the asymmetric unit contains two copies of each of the four subunits. Average R-factors were refined. Iterative occupancy of the oxygen molecules were manually adjusted, and the individual molecules converged within the standard deviation of the refined refinement.

#### Figures

The figures were prepared using PYMOL software (http://www.pymol.org/).

#### Table

| Residue | atom | B-factor | Occ. | Residue | atom | B-factor | Occ. |
|---------|------|----------|------|---------|------|----------|------|
| Heme    | FE   | 42.12    | 1    | Heme    | FE   | 51.13    | 1    |
|         | NA   | 34.72    |      | NA      | 49.21 |
|         | NB   | 37.25    |      | NB      | 48.42 |
|         | NC   | 36.11    |      | NC      | 48.79 |
|         | ND   | 40.47    |      | ND      | 53.87 |
| His95 (F8) | NE2 | 41.22    |      | His95 (F8) | NE2 | 53.2 |
| His63 (E7) | NE2 | 38.99    |      | His63 (E7) | NE2 | 58.79 |
| Phe47 (CD1) | CE1 | 44.82    |      | Phe47 (CD1) | CE1 | 60.43 |
| Val67 (E11) | CG2 | 38.08    |      | Val67 (E11) | CG2 | 51.03 |
| Trp33 (B10) | CH2 | 44.34    |      | Trp33 (B10) | CH2 | 65.2 |
| **Average** |     | 39.81    |      | **Average** |     | 54.01    |
|          | S.D. | 3.39     |      | S.D.    | 5.66 |

| Residue | atom | B-factor | Occ. | Residue | atom | B-factor | Occ. |
|---------|------|----------|------|---------|------|----------|------|
| O₂      | O1   | 38.82    | 0.7  | O₂      | O1   | 51.02    | 0.4  |
|         | O2   | 39.83    |      |         | O2   | 51.63    |      |

#### B1' Residue

| Residue | atom | B-factor | Occ. | Residue | atom | B-factor | Occ. |
|---------|------|----------|------|---------|------|----------|------|
| Heme    | FE   | 32.32    | 1    | Heme    | FE   | 49.40    | 1    |
|         | NA   | 33.74    |      | NA      | 50.51 |
|         | NB   | 32.41    |      | NB      | 45.79 |
|         | NC   | 37.26    |      | NC      | 51.01 |
|         | ND   | 32.40    |      | ND      | 51.33 |
| His99 (F8) | NE2 | 35.91    |      | His99 (F8) | NE2 | 48.21 |
| His67 (E7) | NE2 | 33.10    |      | His67 (E7) | NE2 | 47.98 |
| Phe51 (CD1) | CE1 | 34.59    |      | Phe51 (CD1) | CE1 | 51.59 |
| Val71 (E11) | CG2 | 27.78    |      | Val71 (E11) | CG2 | 49.30 |
| Phe37 (B10) | CZ  | 28.77    |      | Phe37 (B10) | CZ  | 54.24 |
| **Average** |     | 32.83    |      | **Average** |     | 49.94    |
|          | S.D. | 2.90     |      | S.D.    | 2.34 |

| Residue | atom | B-factor | Occ. | Residue | atom | B-factor | Occ. |
|---------|------|----------|------|---------|------|----------|------|
| O₂      | O1   | 33.01    | 0.2  | O₂      | O1   | 50.02    | 0    |
|         | O2   | 33.43    |      |         | O2   | 51.63    |      |

#### B2' Residue

| Residue | atom | B-factor | Occ. | Residue | atom | B-factor | Occ. |
|---------|------|----------|------|---------|------|----------|------|
| Heme    | FE   | 48.07    | 1    | Heme    | FE   | 57.67    | 1    |
|         | NA   | 47.12    |      | NA      | 59.07 |
|         | NB   | 46.86    |      | NB      | 55.81 |
|         | NC   | 48.26    |      | NC      | 55.38 |
|         | ND   | 48.89    |      | ND      | 57.76 |
| His99 (F8) | NE2 | 50.03    |      | His99 (F8) | NE2 | 59.78 |
| His67 (E7) | NE2 | 43.79    |      | His67 (E7) | NE2 | 51.61 |
| Phe51 (CD1) | CE1 | 43.78    |      | Phe51 (CD1) | CE1 | 59.02 |
| Val71 (E11) | CG2 | 43.19    |      | Val71 (E11) | CG2 | 53.80 |
| Phe37 (B10) | CZ  | 51.13    |      | Phe37 (B10) | CZ  | 56.80 |
| **Average** |     | 47.11    |      | **Average** |     | 56.67    |
|          | S.D. | 2.74     |      | S.D.    | 2.57 |
**Deoxygenation of the Oxy Crystals**

Crystallization

Microspectrometry

Oxygen Equilibrium Measurements

Results