# Supplementary Information

**Table S1.** Data Collection and Refinement Statistics for select pH=6.2 CDO structures [a]

| CDO form | **C93A** | **Y157F** | **Y157F** | **Wild-type** |
|---|---|---|---|---|
| soak[b] | **none** | **none** | **dt[b]** | **thiosulfate** |
| pH | **6.2** | **6.2** | **6.2** | **6.2** |
| *Data collection* | | | | |
| Resolution (Å) | 39-1.40[f] | 39-1.35 | 29-1.25 | 34-1.30 |
| | (1.48-1.40) | (1.37-1.35) | (1.27-1.25) | (1.32-1.30) |
| Unique Obs. | 37237 (4823) | 40658 (995) | 49712 (1202) | 51666 (2596) |
| Multiplicity | 24.6(13.4) | 27.5 (22.5) | 24.0 (7.6) | 25.7 (13.6) |
| Completeness | 88.3(79.9) | 88.6 (45.1) | 85.4 (41.9) | 99.9 (97.5) |
| $\langle I/\sigma \rangle$ | 13.9 (1.0) | 30.1 (2.8) | 29.3 (1.4) | 16.7 (0.7) |
| $R_{meas}$[c] (%) | 16.2 (417) | 8.9(129) | 7.7 (155) | 16.2 (459) |
| $R_{pim}$ (%) | 3.2 (110) | 1.7 (26.4) | 1.5 (55.1) | 3.1 (118) |
| $CC_{1/2}$[d] (%) | 1.0 (0.25) | 1.0 (0.77) | 1.0 (0.47) | 1.0 (0.22) |
| *Refinement* | | | | |
| $R_{cryst}$ / $R_{free}$ (%) | 16.6/20.3 | 14.9/17.8 | 12.1/16.4 | 17.4/20.1 |
| No. Obs | 36553 | 40635 | 49645 | 51392 |
| No. residues | 186 | 186 | 186 | 186 |
| No. waters | 143 | 311 | 344 | 329 |
| No. atoms | 1717 | 1873 | 1938 | 1955 |
| rmsd angles ($^o$) | 1.27 | 1.26 | 1.18 | 1.00 |
| rmsd lengths (Å) | 0.011 | 0.013 | 0.011 | 0.008 |
| φ,ψ-favored (%)[e] | 99.0 | 99.0 | 99.0 | 100 |
| φ,ψ-outliers (%)[e] | 0 | 0 | 0 | 0 |
| $\langle B \rangle$ protein (Å$^2$) | 23 | 17 | 16 | 23 |
| $\langle B \rangle$ ligand (Å$^2$) | 24 | 11 | 16 | 18 |
| PDB code | 4PIX | 4XFC | 4XFF | 5I0S |

[a] All refinements used space group $P4_32_12$ with a=b=57.60 Å and c= 122.40 Å. Numbers in parentheses refer to the highest resolution bin.
[b] dt-dithionite, Hcy-homocysteine
[c] $R_{meas}$ is the multiplicity-weighted merging R-factor [1]
[d] $CC_{1/2}$ is the correlation between two datasets each based on half of the data as defined in Karplus & Diederichs [2]
[e] Ramachandran statistics as defined by Molprobity [3]
[f] Refinements carried out at 1.35 Å resolution (CC1/2=0.11), but per referee request we truncated the data set to 1.4 Å resolution for final maps and statistics.

**Table S2.** Data Collection and Refinement Statistics for select Cys- and Hcy-bound CDO forms at pH 6.2[a]

| CDO form | **C93A** | **Y157F** | **Wild-type** | **C93A** | **Y157F** |
|---|---|---|---|---|---|
| Soak | **Cys** | **Cys** | **Hcy[b]** | **Hcy[b]** | **Hcy[b]** |
| pH | **6.2** | **6.2** | **6.2** | **6.2** | **6.2** |
| *Data collection* | | | | | |
| Resolution (Å) | 34-1.40 (1.42-1.40) | 28-1.35 (1.37-1.35) | 34-1.40 (1.48-1.40) | 42-1.60 (1.69-1.60) | 33-1.40 (1.42-1.40) |
| Unique Obs. | 41405 (2011) | 45246 (1785) | 42028 (6012) | 28457 (4040) | 39877 (1434) |
| Multiplicity | 23.7 (13.4) | 23.0 (8.6) | 7.6 (5.6) | 13.8 (13.7) | 23.3 (9.0) |
| Completeness | 100 (100) | 97.8 (80.7) | 100 (100) | 99.6 (99.0) | 94.7 (67.8) |
| $\langle I/\sigma \rangle$ | 11.3 (0.8) | 17.8 (0.7) | 12.5 (0.6) | 12.1 (1.1) | 24.7 (1.1) |
| $R_{meas}$[b] (%) | 18.2 (275) | 11.7 (276) | 7.6 (287) | 27.9 (495) | 8.3 (253) |
| $R_{pim}$ (%) | 3.7 (74) | 2.4 (92) | 2.7 (119) | 7.4 (132) | 1.7 (82) |
| $CC_{1/2}$[c] (%) | 1.0 (0.19) | 1.0 (0.20) | 1.0 (0.18) | 1.0 (0.26) | 1.0 (0.36) |
| *Refinement* | | | | | |
| $R_{cryst}$ / $R_{free}$ (%) | 16.3/19.7 | 16.2/19.3 | 17.1/19.8 | 16.4/20.9 | 16.2/19.2 |
| No. Obs | 41323 | 45159 | 41879 | 27819 | 39392 |
| No. residues | 186 | 186 | 186 | 186 | 186 |
| No. waters | 299 | 319 | 247 | 279 | 281 |
| No. atoms | 1870 | 1907 | 1823 | 1856 | 1884 |
| rmsd angles ($^o$) | 1.28 | 1.26 | 1.30 | 1.28 | 1.32 |
| rmsd lengths (Å) | 0.015 | 0.011 | 0.011 | 0.012 | 0.014 |
| φ,ψ-favored (%)[d] | 99.0 | 99.0 | 99.0 | 98.5 | 99.0 |
| φ,ψ-outliers (%)[d] | 0 | 0 | 0 | 0 | 0 |
| $\langle B \rangle$ protein (Å$^2$) | 20 | 20 | 27 | 24 | 21 |
| $\langle B \rangle$ ligand (Å$^2$) | 15.2 | 22 | 42 | 38 | 22 |
| PDB code | 4XFG | 4XFH | 4PIZ | 4PIY | 4XFI |

[a]See Table S1 footnotes for definitions related to Table entries

## Supplementary Figure S1

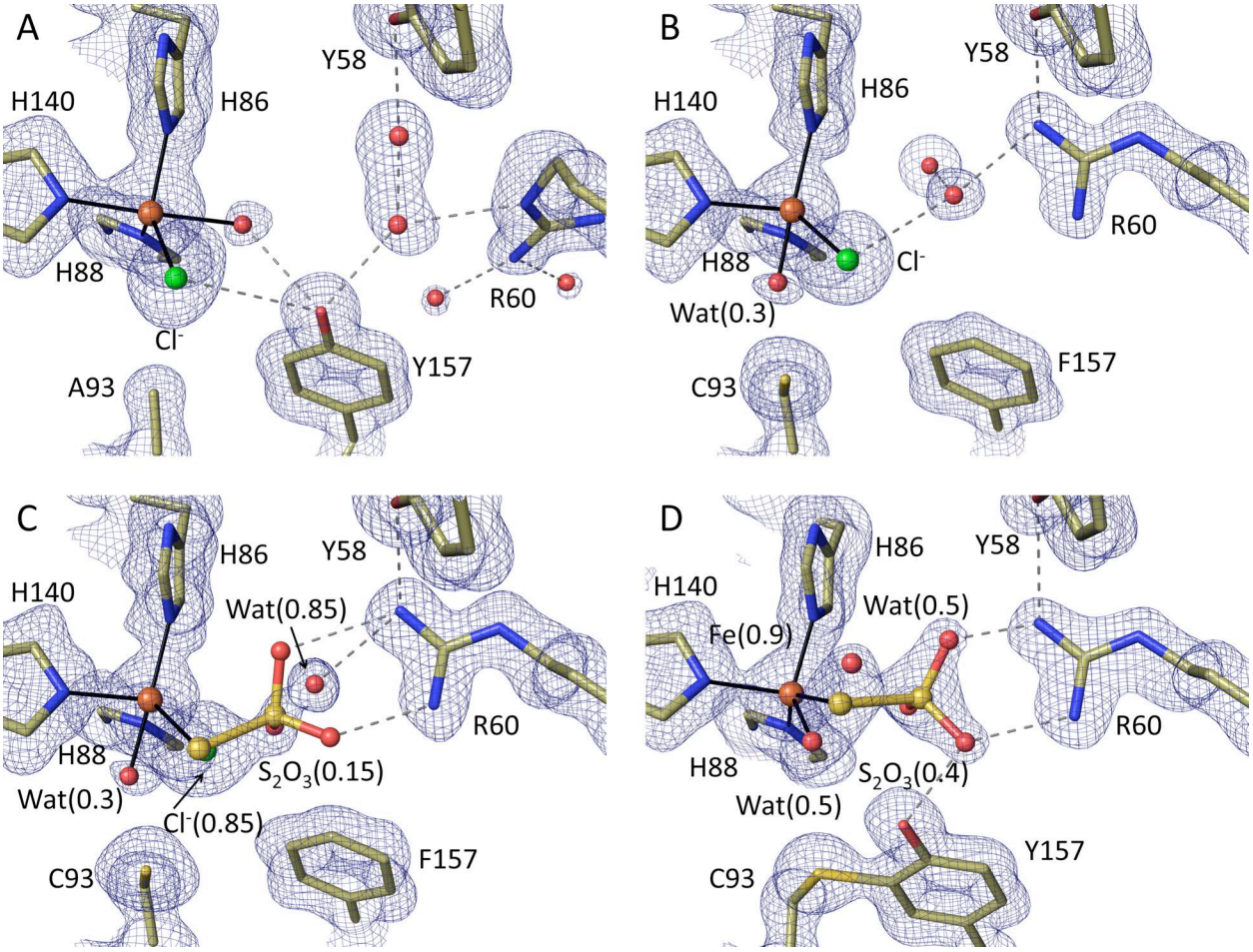


**Figure S1.** High resolution views of the Y157F and C93A active sites and of thiosulfate binding at pH 6.2. All $2F_o$-$F_c$ density is contoured at 1.4 $\rho_{rms}$ and stick models and interactions are shown as in Figure 1 and with chloride atoms green. (A) unliganded C93A at pH 6.2 (PDB code 4PIX), (B) unliganded Y157F at pH 6.2 (PDB code 4XFC), with an iron bound water at 30% occupancy (C) Y157F soaked in dithionite at pH 6.2 (PDB code 4XFF), showing thiosulfate bound at 15% occupancy. (D) wild-type CDO soaked with thiosulfate at pH 6.2 (PDB code 5I0S), with thiosulfate bound at 40% occupancy.

## Supplementary Figure S2

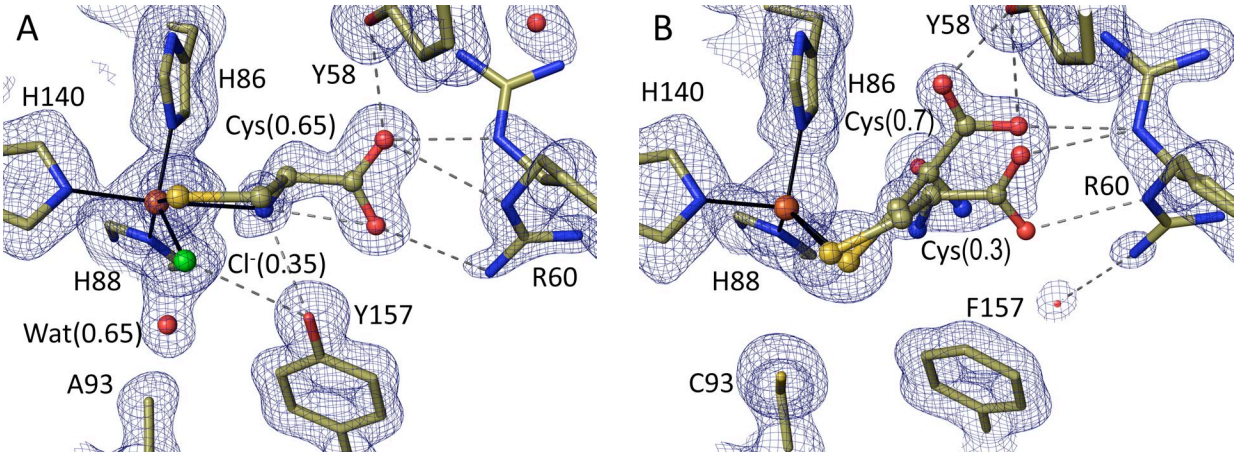


**Figure S2.** High resolution views of the cysteine-complexes of Y157F and C93A CDO at pH 6.2. $2F_o$-$F_c$ density and models and interactions are shown as in Figure S1 with occupancies of the bound chloride and Cys provided in parentheses. (A) Cys soak of C93A at pH 6.2 (PDB code 4XFG). Cys binds at ~65% occupancy, with chloride remaining at ~35% occupancy. Cys coordinates the iron via its thiol group (~2.4 Å) but the α-amino group is further from the iron (~2.6 Å) than was seen the pH 8.0 or wild-type structures. This shift in Cys is correlated with a slight shift in Tyr58 as well as Arg60 in its standard position. There is also evidence for a minor conformation (~35%) of the Arg60 side chain adopts a position not seen before this work. This new Arg60 position allows its NE atom to hydrogen bond to the Cys carboxylate as it is bound in this structure. (B) Cys soak of Y157F at pH 6.2 (PDB code 4XFH). Cys binds in two conformations with the minor conformation (30%) being similar to the minor conformation for the Y157F mutant, and the major conformation (70%) having the carboxylate moved more toward Tyr58 and differing from any mode seen previously. Both the minor and major conformations bind to the iron via only the thiol, located roughly where the displaced chloride was, and have their α-amino atom 3.3 Å and 4.3 Å away from the iron, respectively. Associated with two positions of Cys, Arg60 adopts a mix of a new position (70%) and the standard position (30%). Tyr58 adopts a single conformation that is shifted ~0.7 Å relative to wild-type, and forms a short ~2.4 Å hydrogen bond to the Cys in the major conformation and a ~3.0 Å hydrogen bond with Cys in the minor conformation.

## Supplementary Figure S3

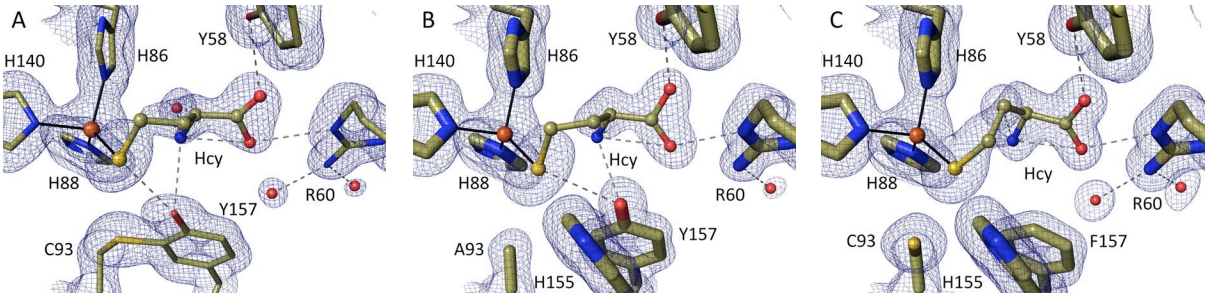


**Figure S3.** High resolution views of the homocysteine-complexes of wild-type, C93A and Y157F CDO. $2F_o$-$F_c$ density and models and interactions are shown as in Figure S1. (A) Homocysteine soak of wild-type CDO at pH 6.2 (PDB code 4PIZ). (B) Homocysteine soak of C93A CDO at pH 6.2 (PDB code 4PIY), and (C) Homocysteine soak of Y157F CDO at pH 6.2 (PDB code 4XFI).

## Supplementary Figure S4

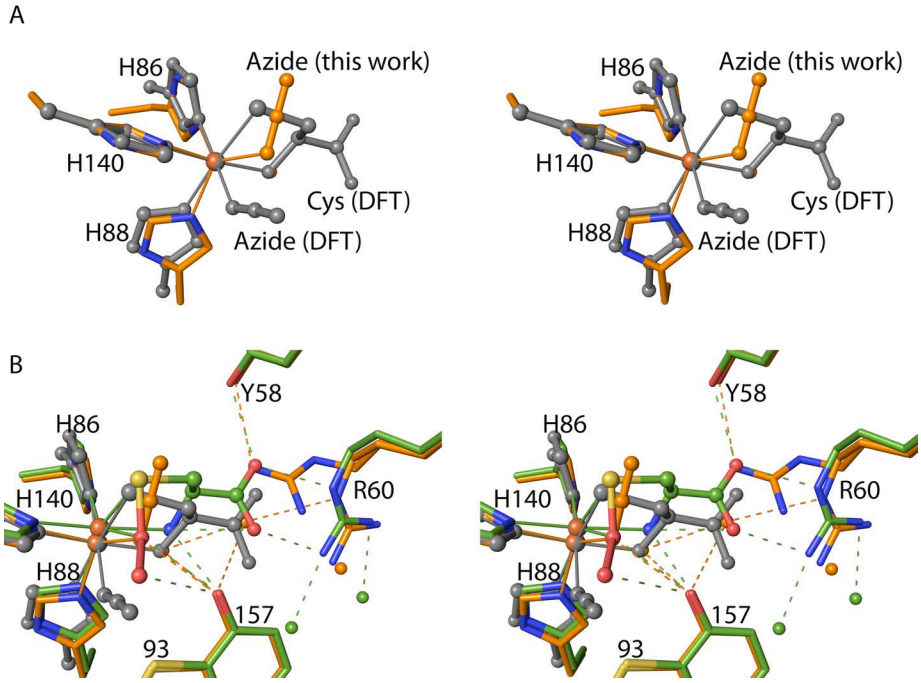


**Figure S4.** Comparisons of DFT optimized Cys/azide to azide and Cys-persulfenate bound CDO. (A) Stereo view overlay of the azide complex reported in this study (PDB code 4PJY; orange carbons/waters/azide) with the DFT model of the low-spin azide/Cys-Fe(III)CDO complex[4] (grey atoms). (B) Stereo view overlay of Cys-persulfenate bound CDO (green carbons/waters, PDB code 3ELN [5]), the azide complex reported in this study (orange carbons/waters/azide), PDB code 4PJY), and the DFT model of the low-spin azide/Cys-Fe(III) complex[4] (grey atoms). The DFT model overlay with our structures puts the azide close to Cys93, with the iron-proximal to iron-distal atoms of the azide being 2.6 Å, 2.5 Å, and 2.9 Å from Cys93-SG, respectively. The overlay also puts the DFT azide within hydrogen bonding distance of Tyr157, with the iron-proximal to iron-distal atoms of the azide being 3.4 Å, 3.2 Å, and 3.5 Å from Tyr157-OH, respectively.

## References


1. Diederichs, K. & Karplus, P. A. (1997). Improved R-factors for diffraction data analysis in macromolecular crystallography. *Nat Struct Biol* **4**, 269-75.
2. Karplus, P. A. & Diederichs, K. (2012). Linking crystallographic model and data quality. *Science* **336**, 1030-3.
3. Word, J. M., Lovell, S. C., LaBean, T. H., Taylor, H. C., Zalis, M. E., Presley, B. K., Richardson, J. S. & Richardson, D. C. (1999). Visualizing and quantifying molecular goodness-of-fit: small-probe contact dots with explicit hydrogen atoms. *J Mol Biol* **285**, 1711-33.
4. Blaesi, E. J., Fox, B. G. & Brunold, T. C. (2015). Spectroscopic and Computational Investigation of the H155A Variant of Cysteine Dioxygenase: Geometric and Electronic Consequences of a Third-Sphere Amino Acid Substitution. *Biochemistry* **54**, 2874-84.
5. Driggers, C. M., Cooley, R. B., Sankaran, B., Hirschberger, L. L., Stipanuk, M. H. & Karplus, P. A. (2013). Cysteine dioxygenase structures from pH4 to 9: consistent cys-persulfenate formation at intermediate pH and a Cys-bound enzyme at higher pH. *J Mol Biol* **425**, 3121-36.