An Unprecedented Function for A Tungsten-Containing Oxidoreductase

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Supporting Information

Table S1
Figures S1 – S6

Table S1, Matthew et al. Purification of WOR5

Step	Specific Activity (U/mg)	Total Activity (U)	Total Protein (mg)	Yield (%)	Fold Purification
Cytoplasmic Extract	0.79[a]	682	857	100	1
Ni-NTA	34.8[a]	312	9.0	45	43
Q-HP	26.2[a]	126	4.8	18	33

Step	Specific Activity (U/mg)	Total Activity (U)	Total Protein (mg)	Yield (%)	Fold Purification
Cytoplasmic Extract	0.42[b]	360	857	100	1
Ni-NTA	9.8[b]	88	9.0	25	23
Q-HP	13.4[b]	65	4.8	18	32

[a] BV to Hexanal (500 uM)
[b] BV to Taurine (500 uM)

Figure S1, Mathew et al.

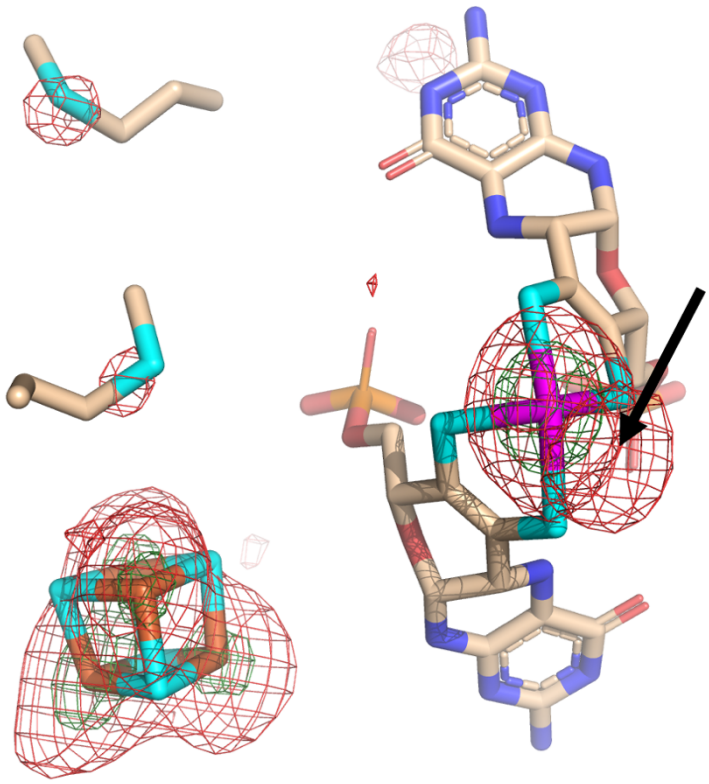

Figure S1. F_o-F_c anomalous difference map generated from data collected at the iron edge (7,112 eV), contoured at 5 σ (red cage) and 20 σ (green cage), as well as a stick representation of the tungsto-bispyranopterin cofactor (PTE), the [4Fe-4S] cofactor (SF4) as well as the side chains of amino acids M174 and M194. As can be seen, a significant anomalous peak (black arrow) was observed in front of (coming out of the page) the tungsten ion within the active. Carbon, oxygen, nitrogen, sulfur, phosphorus, iron, and tungsten ions are colored tan, red, blue, cyan, light orange, dark orange, and magenta respectively.

Figure S2, Mathew et al.

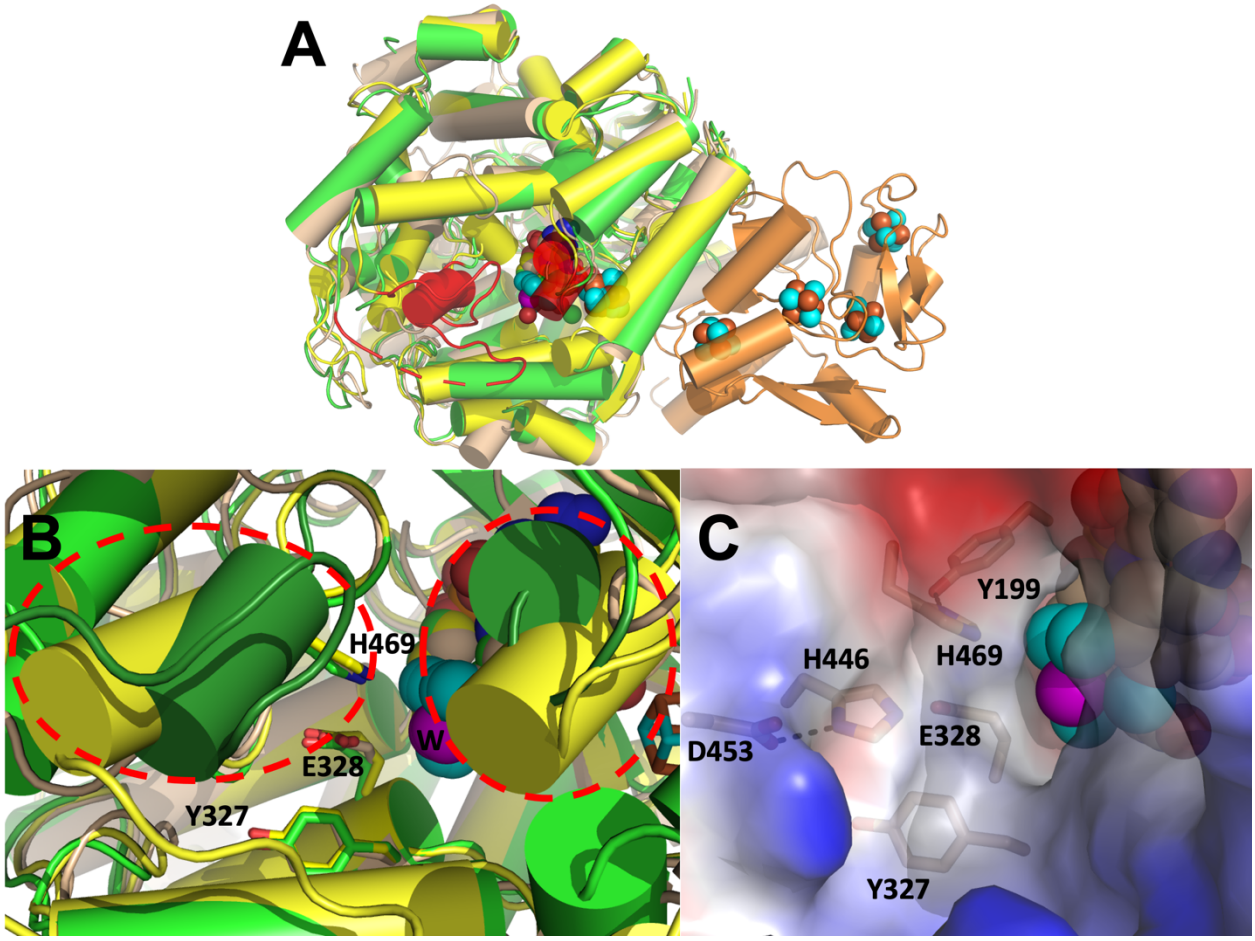

Figure S2. Structural comparison of WOR5, AOR (PDB ID – 1AOR), and FOR (PDB ID – 1B4N). *Panel A;* Cartoon representation showing the large (tan) and small subunit (brown) of the WOR5 model aligned with the models of AOR (green) and FOR (yellow). Two helices in AOR and FOR occlude the active when compared to WOR5 (highlighted with red helices). *Panel B;* Blowup of the active site highlighting the conserved active site residues in all three enzymes (WOR5 numbering) relative to the tungsten atom (W). *Panel C;* Surface potential map around the open active site of WOR5. Y199, H446, and D453 are unique to the WOR5 active site. Y199 and D453 are within hydrogen bonding distance of H469 (2.8 Å) and H446 (2.7 Å), respectively.

Figure S3, Matthew et al.

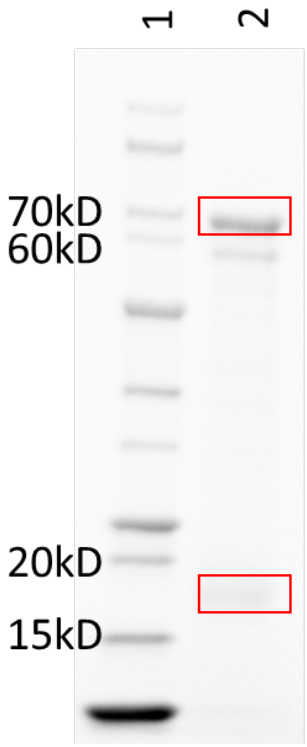

Figure S3. SDS-PAGE showing molecular weight standards (*Lane 1*) and purified WOR5 (*Lane 2*). The WOR5 large subunit (WOR5-L) has a calculated mass of 68 kDa while the WOR5 small subunit has a calculated mass of 19 kDa. The two WOR5 subunits were confirmed by MS analysis. A third minor band near 60 kDa was identified as a TCP-1/cpn60 chaperonin family protein and is not likely to contribute to the aldehyde oxidation activity of the WOR5 samples.

Figure S4, Mathew et al.

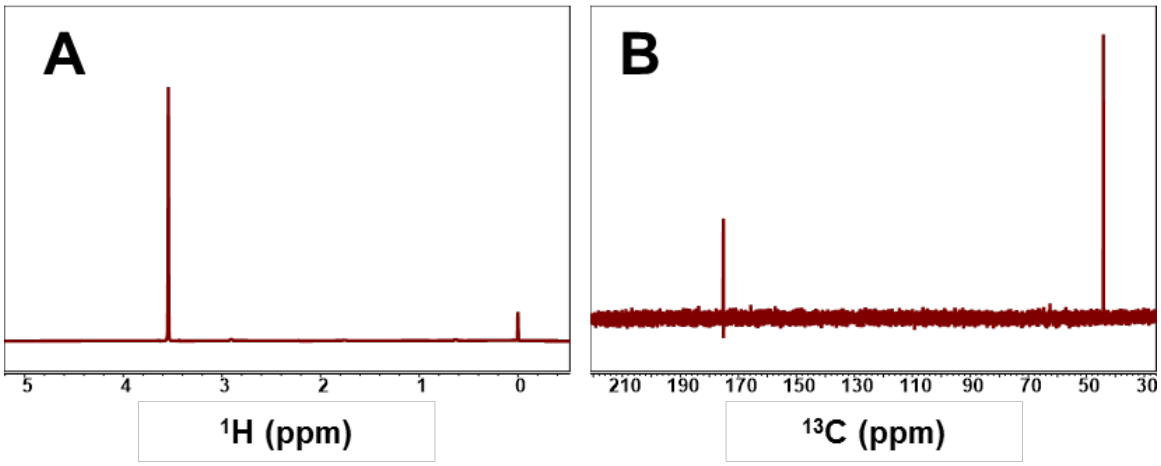

Figure S4. (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of a 10 mM glycine standard in PBS buffer (pH 7.4) prepared in D_2O. Chemical shift values for glycine: δ_H=3.54 ppm *singlet*, δ_C=44.13 and 175.22 ppm.

Figure S5, Mathew et al.

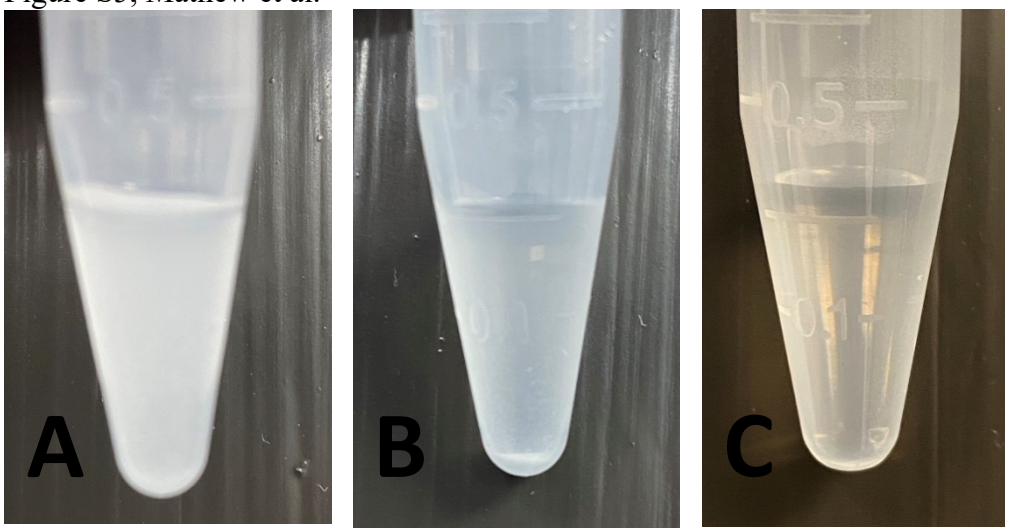

Figure S5. Images captured of T_f sample after precipitation of sulfate: (A) precipitate suspended in solution (B) precipitate pelleted at bottom of micro-centrifuge tube, and (C) the control sample T_0

Figure S6, Mathew et al.

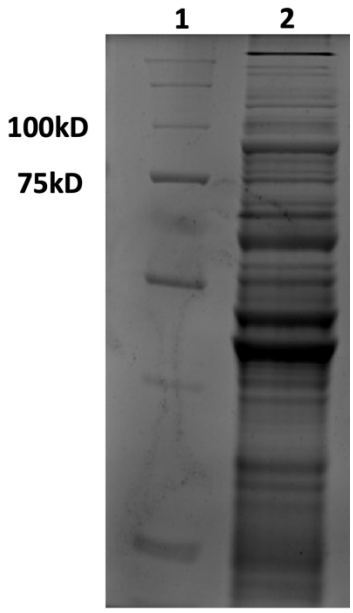

Figure S6. SDS-PAGE showing molecular weight standards (*Lane 1*) and WOR5 from the over expression strain (*Lane 2*). The WOR5 large subunit (WOR5-L) has a calculated mass of 68 kDa while the WOR5 small subunit has a calculated mass of 19 kDa. The relative location of the 100 kDa and 75 kDa standards are labeled.