Supporting Information

Structure of a Novel Phosphotriesterase from *Sphingobium* sp. TCM1; a Familiar Binuclear Metal Center Embedded in a 7-Bladed β-Propeller Protein Fold

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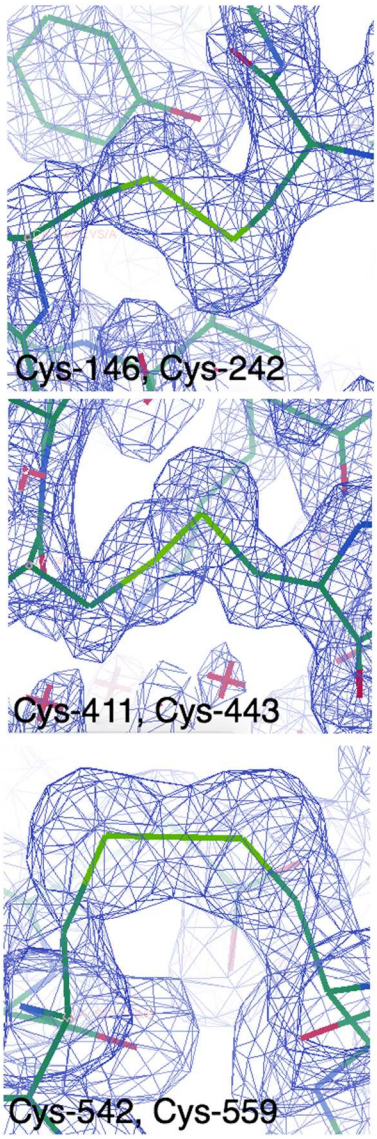

Figure S1. $2F_o$-F_c maps of *Sb*-PTE (PDB id: 5IOJ) contoured at 1.0σ showing intact disulfide bridges involving Cys-146/Cys-242, Cys-411/Cys-443, Cys-542/Cys-559.

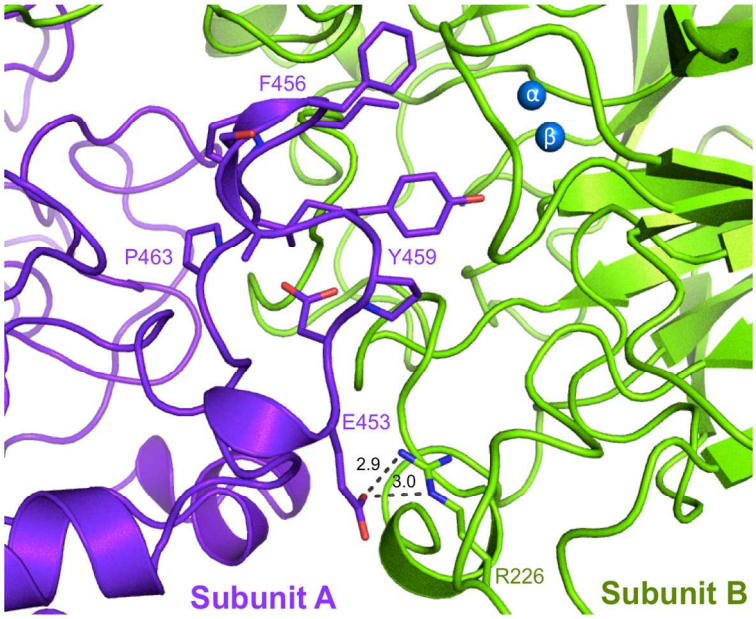

Figure S2. A close look at the *Sb*-PTE dimer interface formed between the hydrophobic active site of one subunit and the hydrophobic coil of the other. The hydrophobic coil encompasses residues 447-463 and consists of three prolines, two phenylalanines, two isoleucines, and one each of tyrosine and alanine. The side chain of Phe-456 extends into the leaving group pocket, while that of Tyr-459 extends into the small group pocket. The interaction is strengthened by a salt bridge between Glu-453 and Arg-226 located in separate subunits. The active site is shown by the location of the two Mn centers, represented by blue spheres.

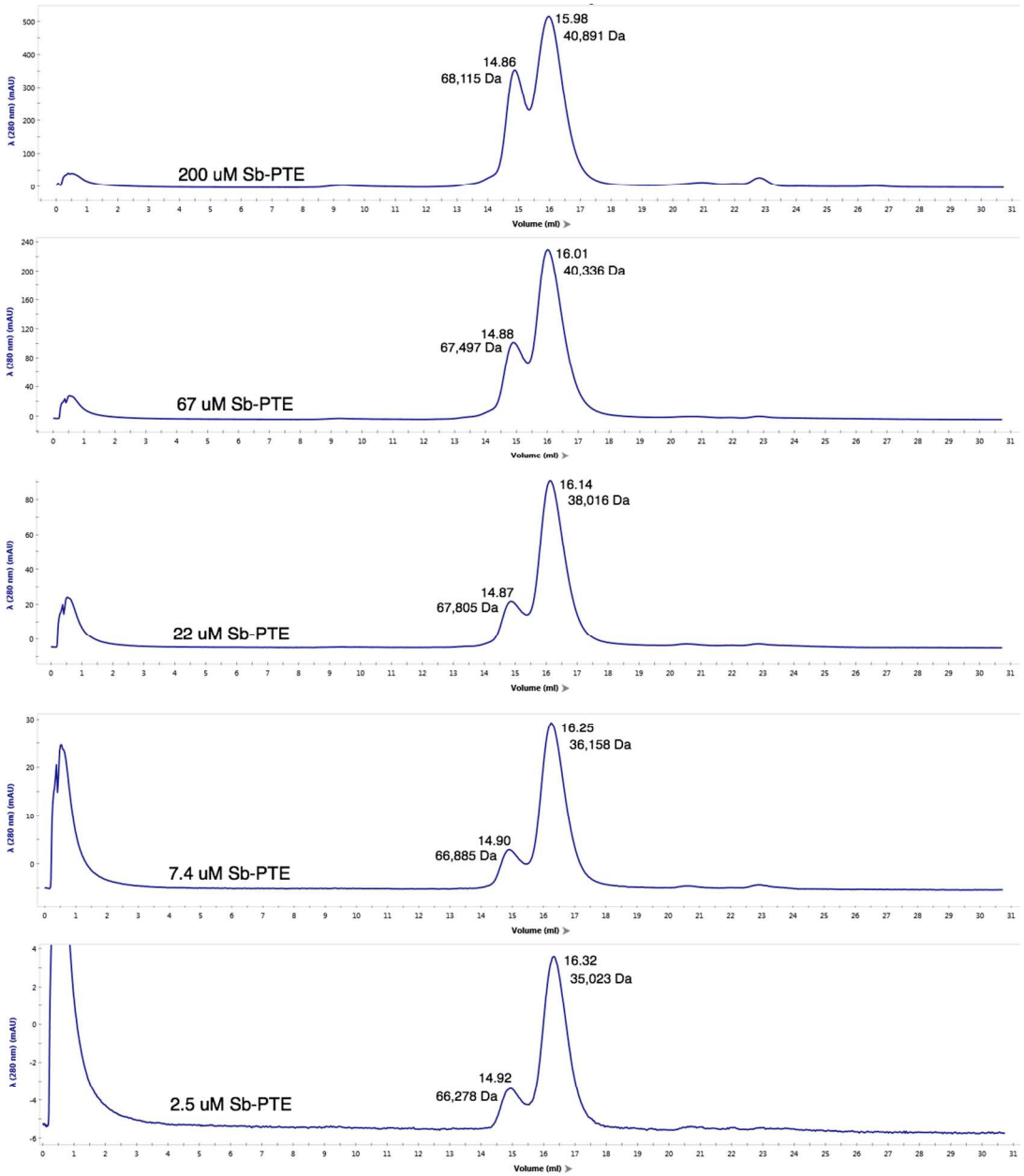

Figure S3. Determination of the *Sb*-PTE oligomerization state by size exclusion chromatography. At the range of concentrations used in this experiment, two peaks were consistently observed. The first peak corresponding to the larger species, presumed to be the homodimer, has an apparent molecular mass range of 66-68 kDa. The smaller peak presumed to correspond to the monomer has an apparent molecular mass range of 35-41 kDa.

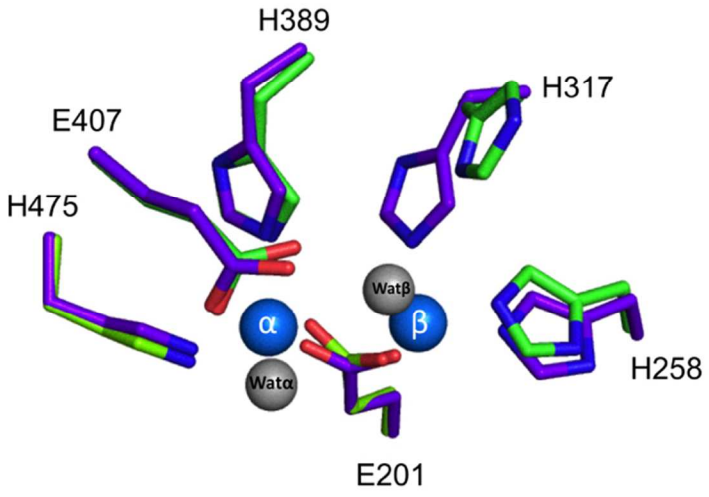

Figure S4. Superposition of the coordination spheres of the binuclear Mn *Sb*-PTE and apo-*Sb*-PTE. Significant distortion of the coordination sphere occurs in the absence of the divalent metals (blue spheres), replaced by two water molecules (gray spheres) whose positions are shifted relative to the metal ions.

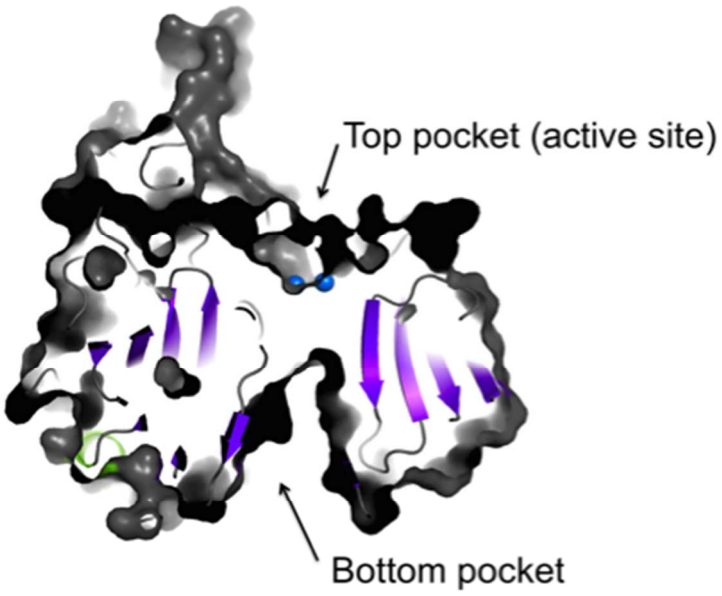

Figure S5. Sliced surface representation of *Sb*-PTE showing the two major pockets. The top pocket corresponds to the active site bearing the binuclear Mn^{2+} center (blue spheres). The bottom pocket has an inverted funnel shape and is filled with solvent molecules. The core β-sheet scaffolding of the 7-bladed β-propeller is shown in purple.

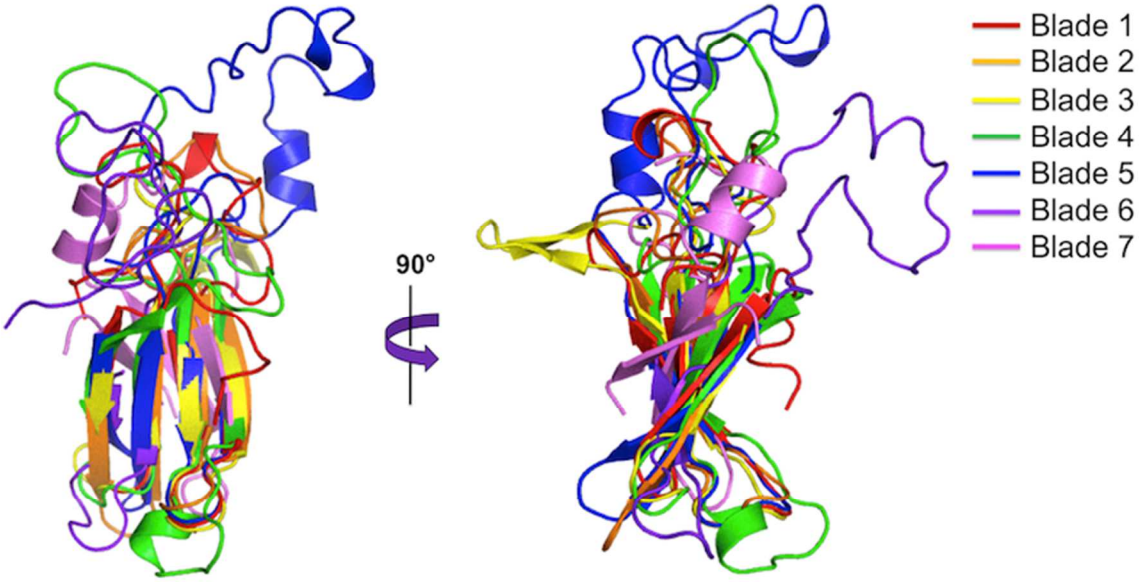

Figure S6. Superposition of the seven individual blades making up the *Sb*-PTE β-propeller. The first blade (Blade 1, red) was designated as the reference structure for superposition of the six remaining blades. Each blade is chosen to start at the first residue of the D-A coil preceding its β-strand A and terminates at the last residue of its β-strand D. The illustration shows structural conservation of the core β-sheet structures, while divergence in length and presence of pendant secondary structures occur among the coils. The B-C and D-A coils located on the top of the β-propeller are longer than the A-B and C-D coils located at the bottom.

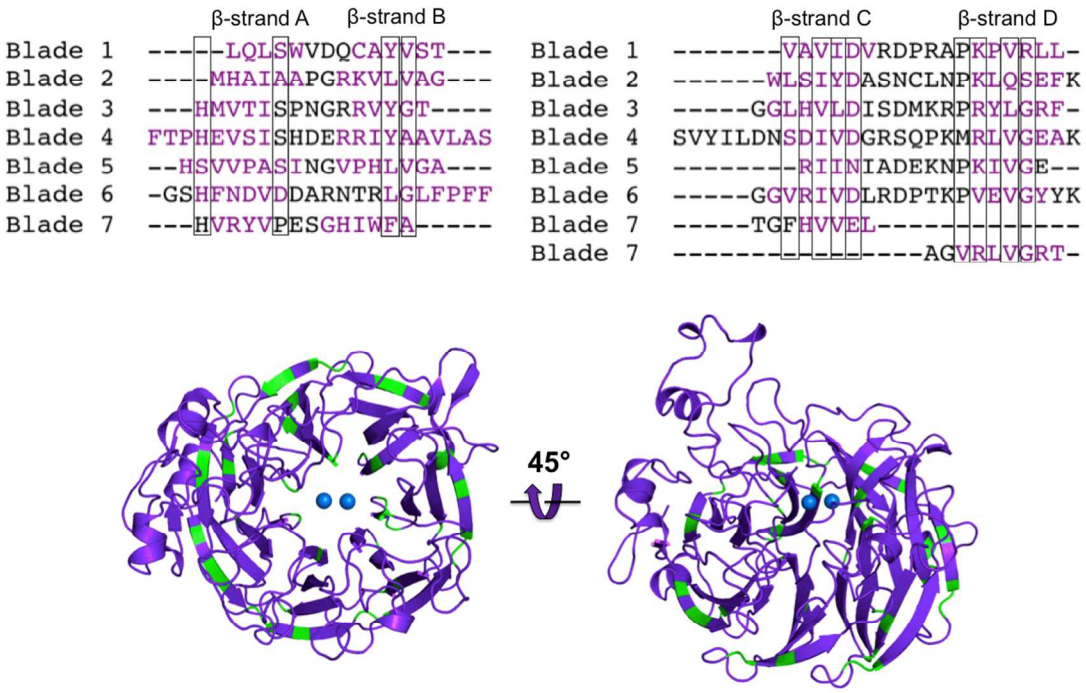

Figure S7. Sequence alignment of β-strands A, B, C, and D (purple letters), and the intra-blade A-B and C-D coils of *Sb*-PTE. Due the varied lengths of the intra-blade B-C and inter-blade D-A coils, they were not included in the alignment. Otherwise, no meaningful alignments could be achieved among the sequences. Greater conservation is apparent in β-strands C and D compared with β-strands A and B. Each amino acid found in four out of seven β-strands in the alignment are mapped in the model of *Sb*-PTE and are found to concentrate at the periphery of the β-propeller rather than the center of the torus.

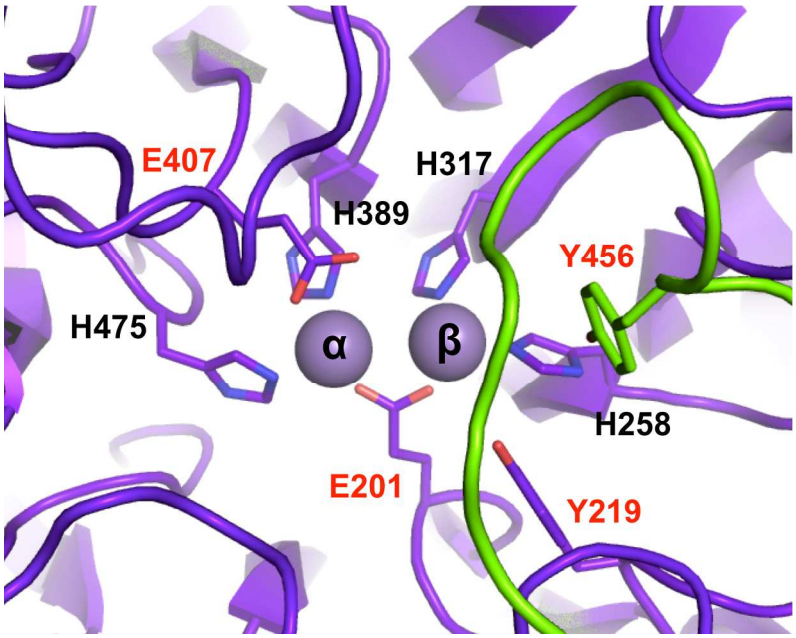

Figure S8. The active site of *Sb*-PTE showing the locations of amino acid residues mutated in this study (red labels). Residues Glu-201 and Glu-407 ligate the Mn^{2+} metals. The residues Tyr-219 of one subunit and Tyr-456 (green sticks) of the other subunit were mutated to determine the source of protonation of the unactivated leaving group of the substrate.