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Supporting information for article:

Structural and functional identification of the uncharacterized metallo-β-lactamase superfamily protein TW9814 as a phosphodiesterase with unique metal coordination

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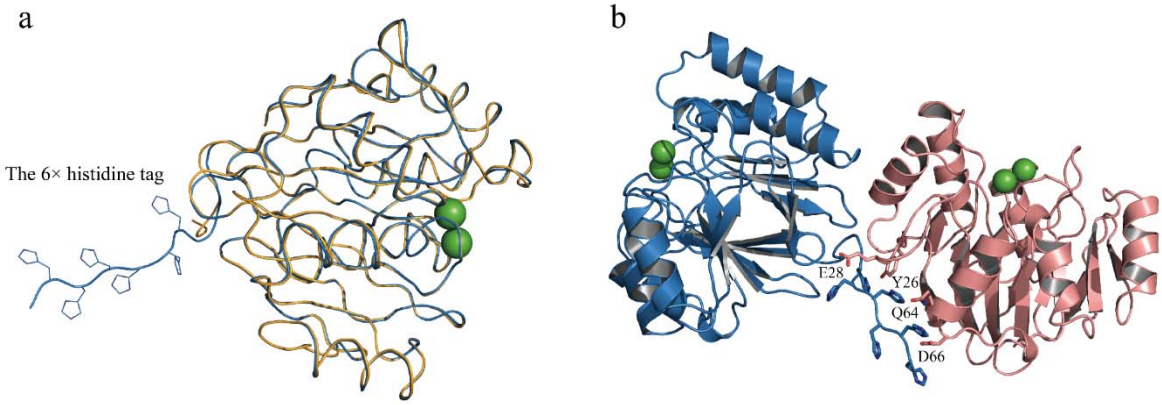

Figure S1 Ordered 6× His tag of TW9814. (a) TW9814 backbones and Mn(II) are shown as lines and spheres, respectively. TW9814 monomers are colored in blue (molecule A) and orange (molecule B), respectively. Two monomers are almost the same except for the N-terminal region. The 6× histidine residues at the N-terminal region of molecule B are disordered, while those of molecule A are well structured. (b) Interaction between the 6× His tag of molecule A and symmetry unit molecule B is shown as a stick model. The symmetry unit molecule B is colored in salmon. In this figure, CPK colors were used for nitrogen and oxygen atoms.

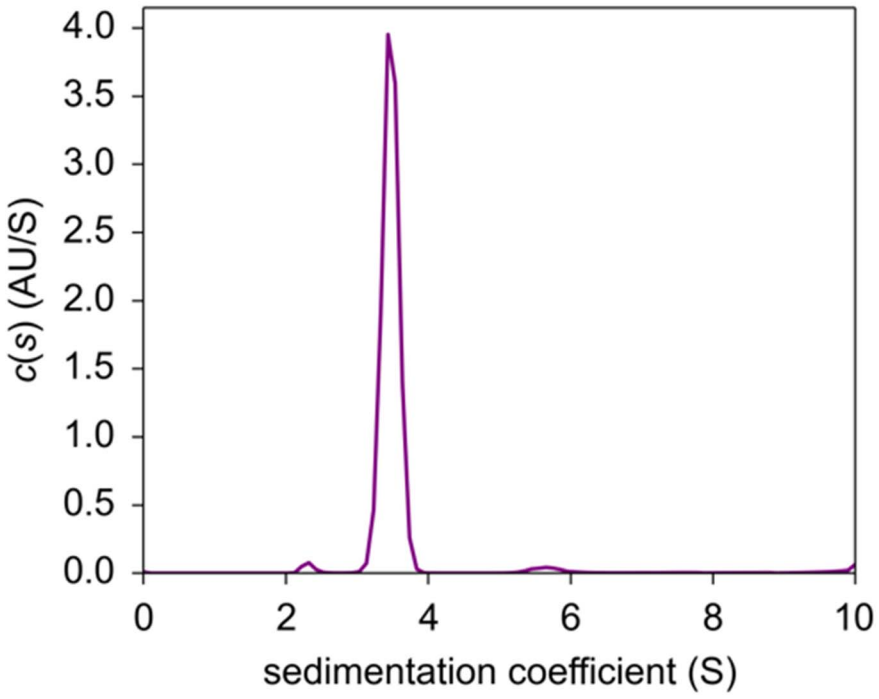

Figure S2 Analytical ultracentrifugation (AUC) result of TW9814. TW9814 peak from AUC is shown. The sedimentation coefficient of the peak is 3.47 S. The calculated molecular weight (MW) from the sedimentation coefficient is 50,931 Da. Considering that the MW of TW9814 is 27,467 Da, the AUC result suggests that TW9814 forms homodimers.

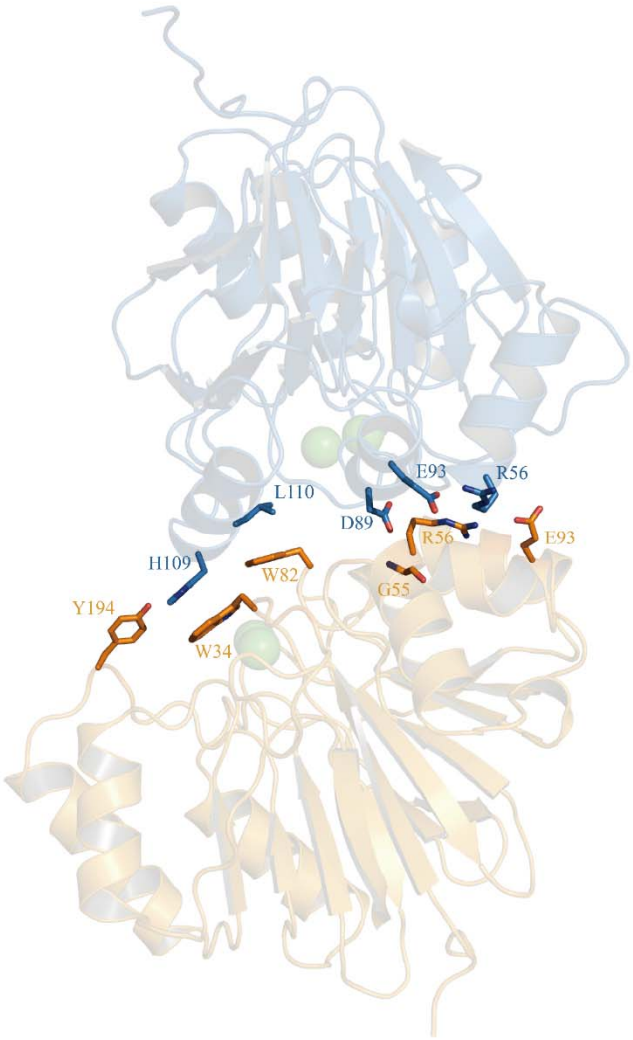

Figure S3 Interaction between monomers in dimeric TW9814. The residues involved in the interaction between monomers are shown as sticks. They contain five residues of molecule A (colored in blue) and six residues of molecule B (colored in orange). In this figure, CPK colors were used for nitrogen and oxygen atoms.

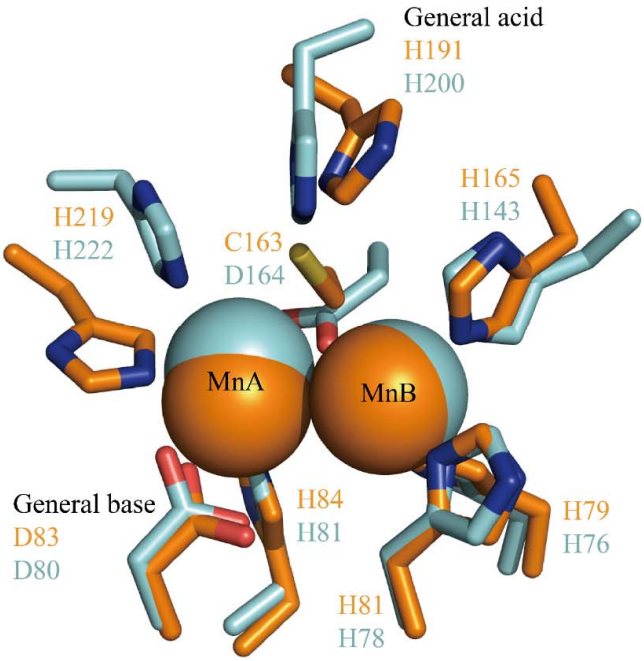

Figure S4 Active sites comparison between TW9814 and PhnP. Active sites of TW9814 and PhnP (PDB code: 3P2U). TW9814 and PhnP are colored in orange and cyan, respectively. Mn(II) and residues are shown as spheres and sticks, respectively. His191/His200 and Asp83/Asp80 serve as general acid and base, respectively. In this figure, CPK colors were used for sulfur, nitrogen, and oxygen atoms.

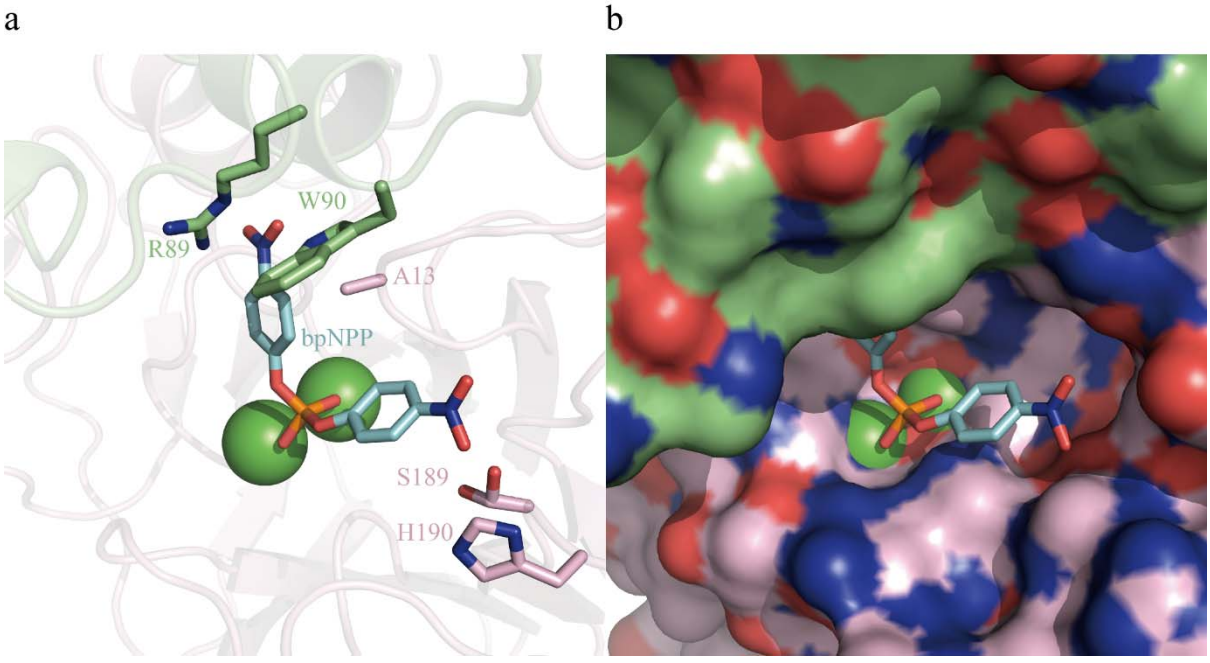

Figure S5 A docking model of PhnP and bpNPP. (a) Interactions between PhnP (PDB code: 3P2U) and bpNPP. Each monomer of PhnP was colored in pale green and light pink, respectively. Mn(II) and bpNPP are shown as spheres and sticks, respectively. (b) Surface model of PhnP and bpNPP. In this figure, CPK colors were used for phosphorus, sulfur, nitrogen, and oxygen atoms.

TW9814+bpNPP

TW9814+bpNPP+Zn(II)

TW9814+bpNPP+Mn(II)

TW9814+bpNPP+Ni(II)

Figure S6 Metal dependence of TW9814 activity. The color change was detected in the presence of Mn(II) or Ni(II), but not in the presence of Zn(II).