Supporting information

Reaction mechanism of tetrathionate hydrolysis based on the crystal structure of tetrathionate hydrolase from *Acidithiobacillus ferrooxidans*

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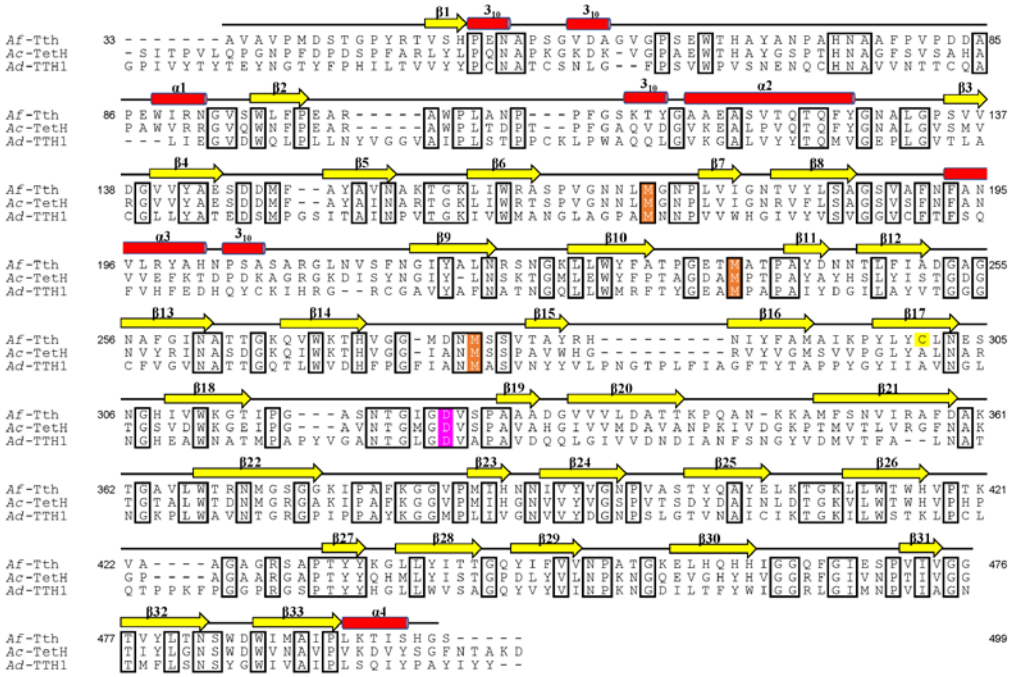
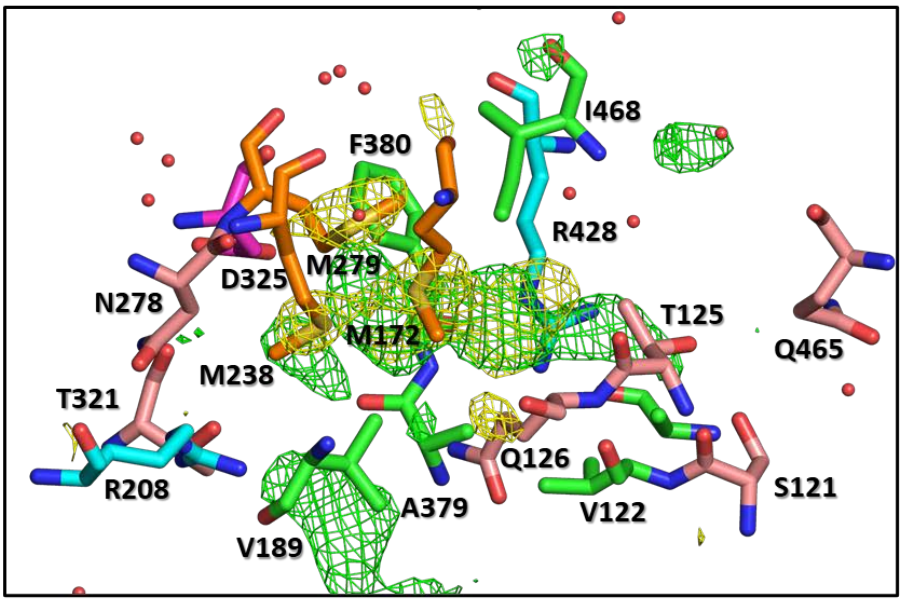


Figure S1. Sequence alignment among three tetrathionate hydrolases (4THases) from *Acidithiobacillus* species. *Af*-Tth, 4THase from *A. ferrooxidans*; *Ac*-TetH, 4THase from *A. caldus*; and *Ad*-TTH1: 4THase from *Ad. ambivalens*. Multiple alignment of protein sequences was done using ClustalX (Larkin *et al.*, 2007. *Bioinformatics*, 23, 2947-2948). Conserved amino acids are surrounded by rectangles. Three methionines (Met172, Met238, and Met279), Cys301 (only *Af*-Tth), and Asp325 are highlighted as orange, yellow, and magenta, respectively. The secondary structural elements of *Af*-Tth are drawn on the sequence alignment.

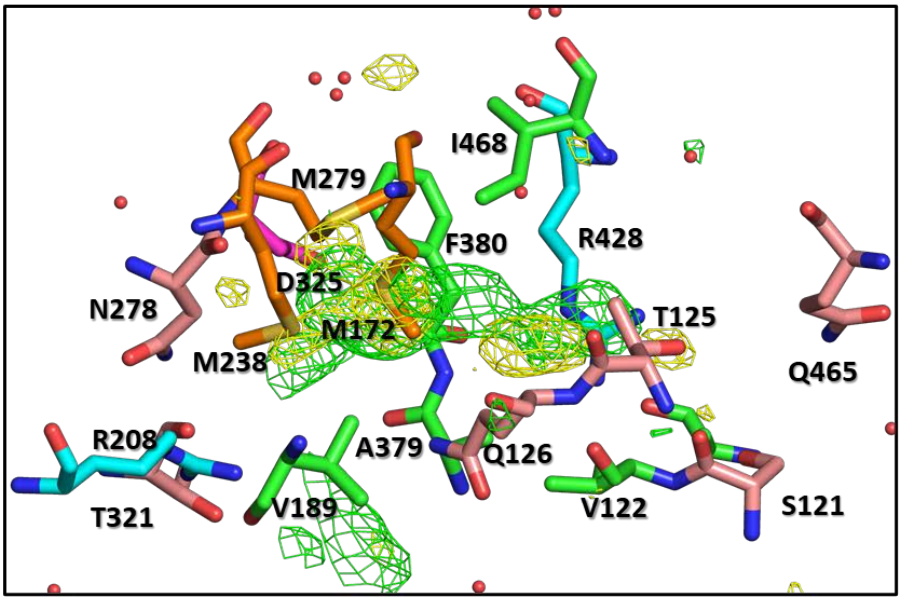
Blade I, β1 and β3–β6; blade II, β7–β10; blade III, β11–β14; blade IV: β15–β18; blade V, β19–β22; blade VI, β23–β26; blade VII, β27–β30; blade VIII, β2 and β31–β33.

Disordered residues in the wild-type crystal: Leu197–Pro203, Ala345–Lys348, and Gly498–Ser499 (in chain A); Ala33, Lys342–Ala349, and Gly498–Ser499 (in chain B); Ala33, Phe193–Ala205, Ser319–Gly322, Lys342–Phe351, Gly374–Ile377, Val422–Ala424, and His497–Ser499 (in chain C); Ala33 and Gln344–Met350 (in chain D); Ala33–Val34, Val57–Ala59, Pro343–Ala349, and His497–Ser499 (in chain E); and Ala33, Ser319–Gly324, Lys347–Lys348, and Ser499 (in chain F).

(a)


I468
F380
R428
M279
D325
N278
T125
M172
Q465
M238
T321
Q126
R208
A379
S121
V122
V189

(b)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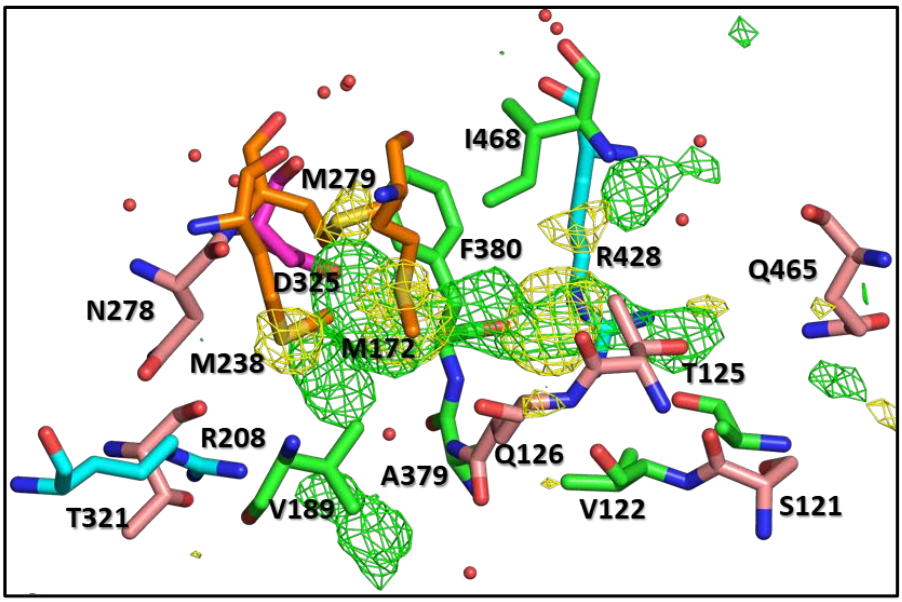
T321
V189
V122
S121

(c)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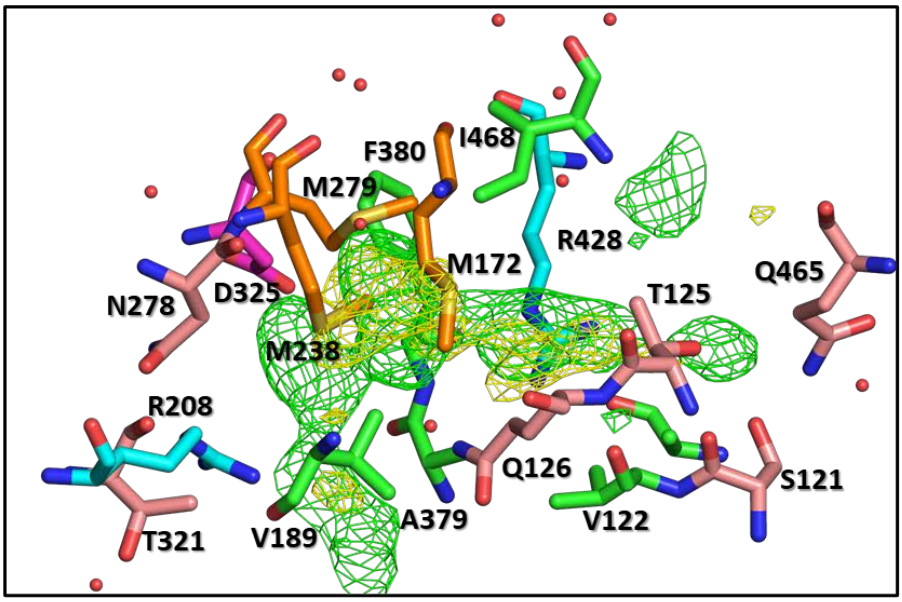
V189
V122
S121

(d)


F380
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A379
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V189
V122

(e)

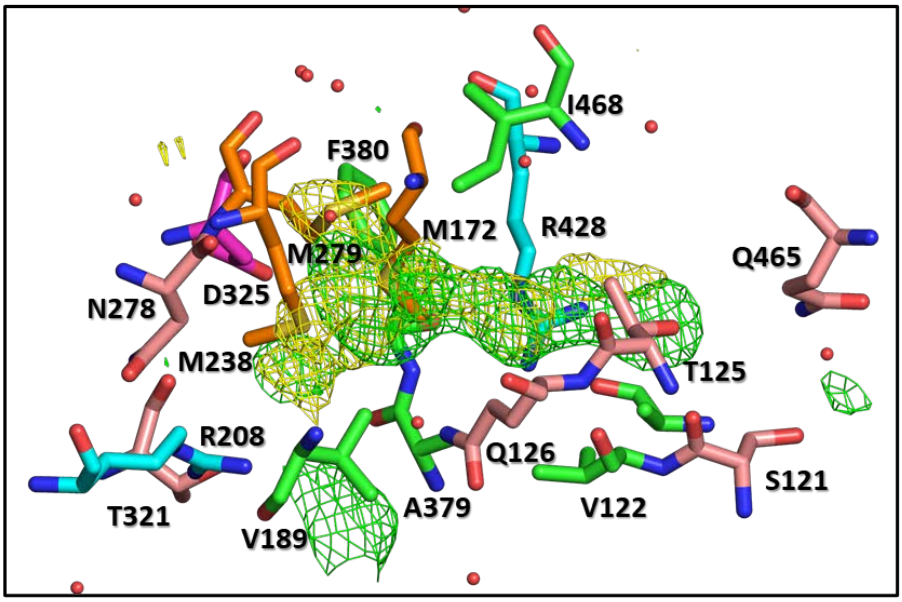

Figure S2. Electron densities in the cavity of the substrate-soaked structure. (a) Chain A, (b) chain B, (c) chain D, (d) chain E, and (e) chain F. Side chains of Val122, Thr125, Gln126, Met172, Val189, Arg208, Met238, Asn278, Met279, Thr321, Asp325, Ala379, Phe380, Arg428, Gln465, and Ile468 are indicated as a stick model. Water molecules are indicated as a sphere and ball model. Methionines, arginines, aspartic acid, hydrophobic residues (Val, Ala, Phe, Ile), and other residues (Ser, Thr, Asn, Gln) are colored orange, cyan, magenta, green, and wheat, respectively. The green and yellow contour shows the $F_o - F_c$ omit map (3.0σ, λ = 1.0 Å) and anomalous difference map (3.0σ, λ = 1.9 Å), respectively.

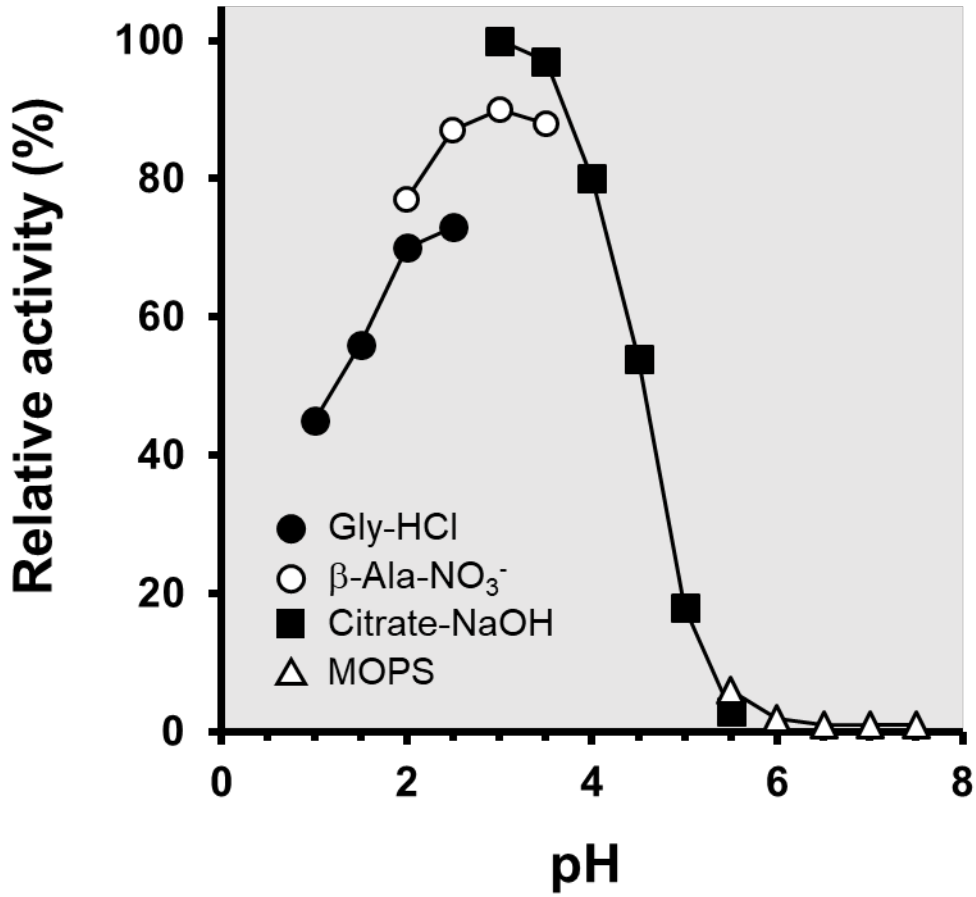

Figure S3. pH profile of tetrathionate hydrolase from *A. ferrooxidans* (*Af*-Tth) activity. *Af*-Tth activity was measured under various pH values. Symbols: closed circle, glycine-HCl buffer; open circle, β-alanine NO_3^- buffer; closed square, citrate-NaOH buffer; and open triangle, MOPS.