

Supplementary Information

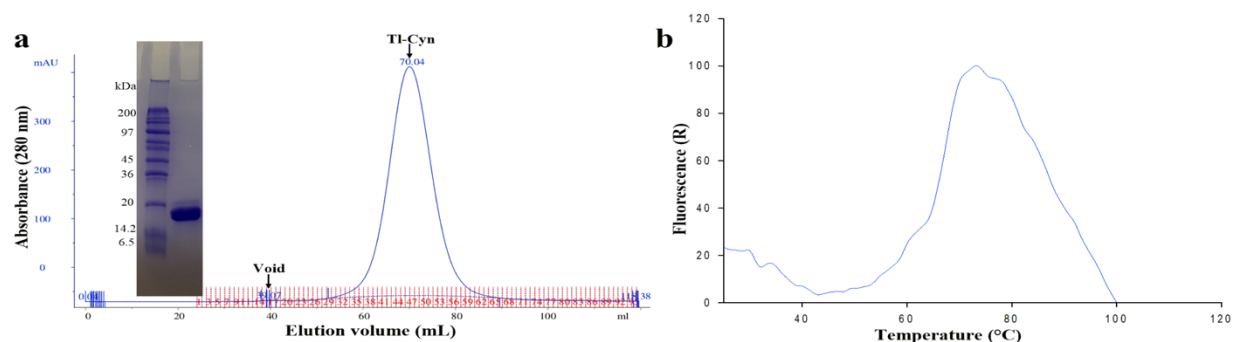
Crystal structure of a thermophilic fungal cyanase and its implications on the catalytic mechanism for bioremediation

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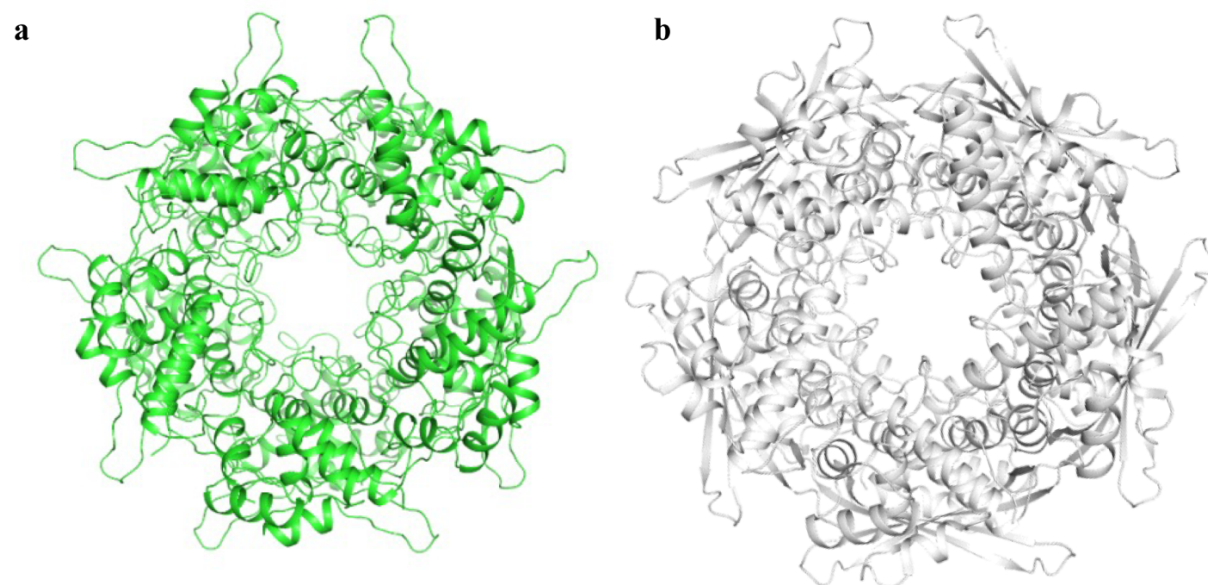
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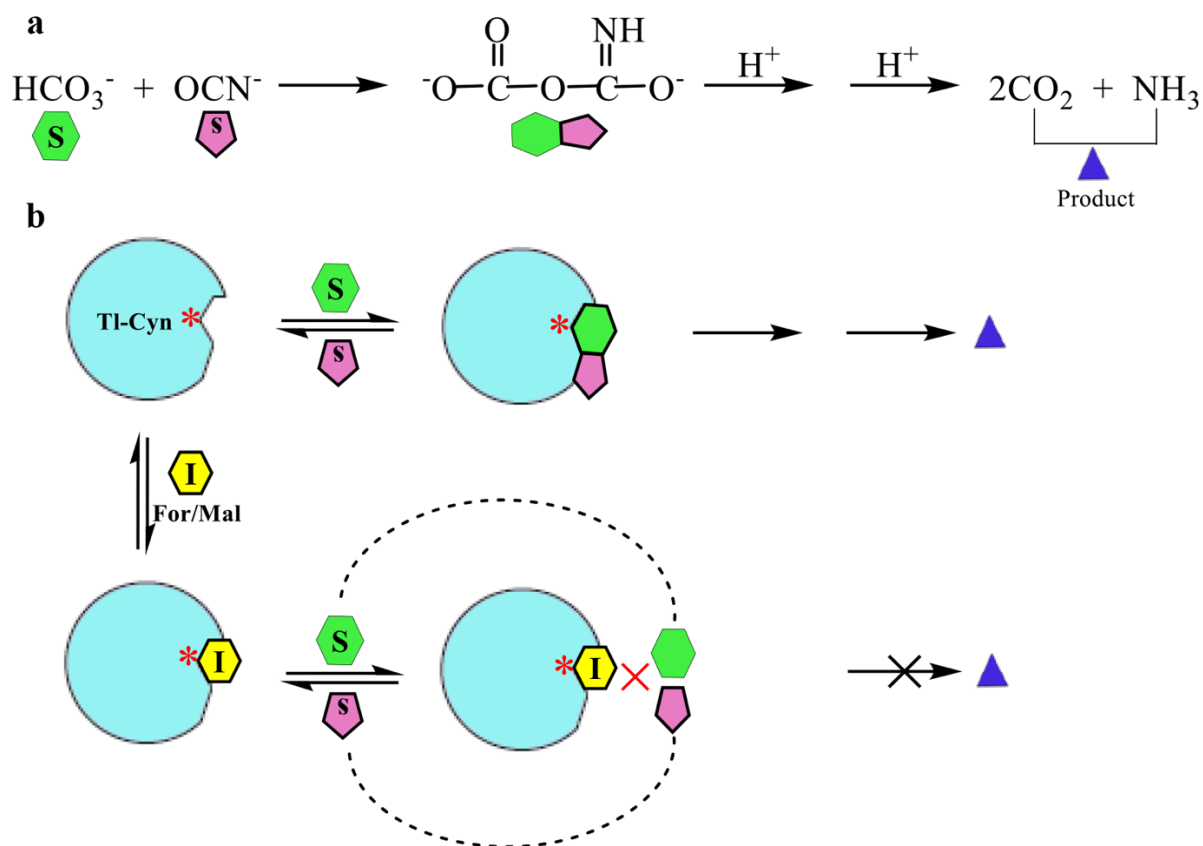
Supplementary Figures



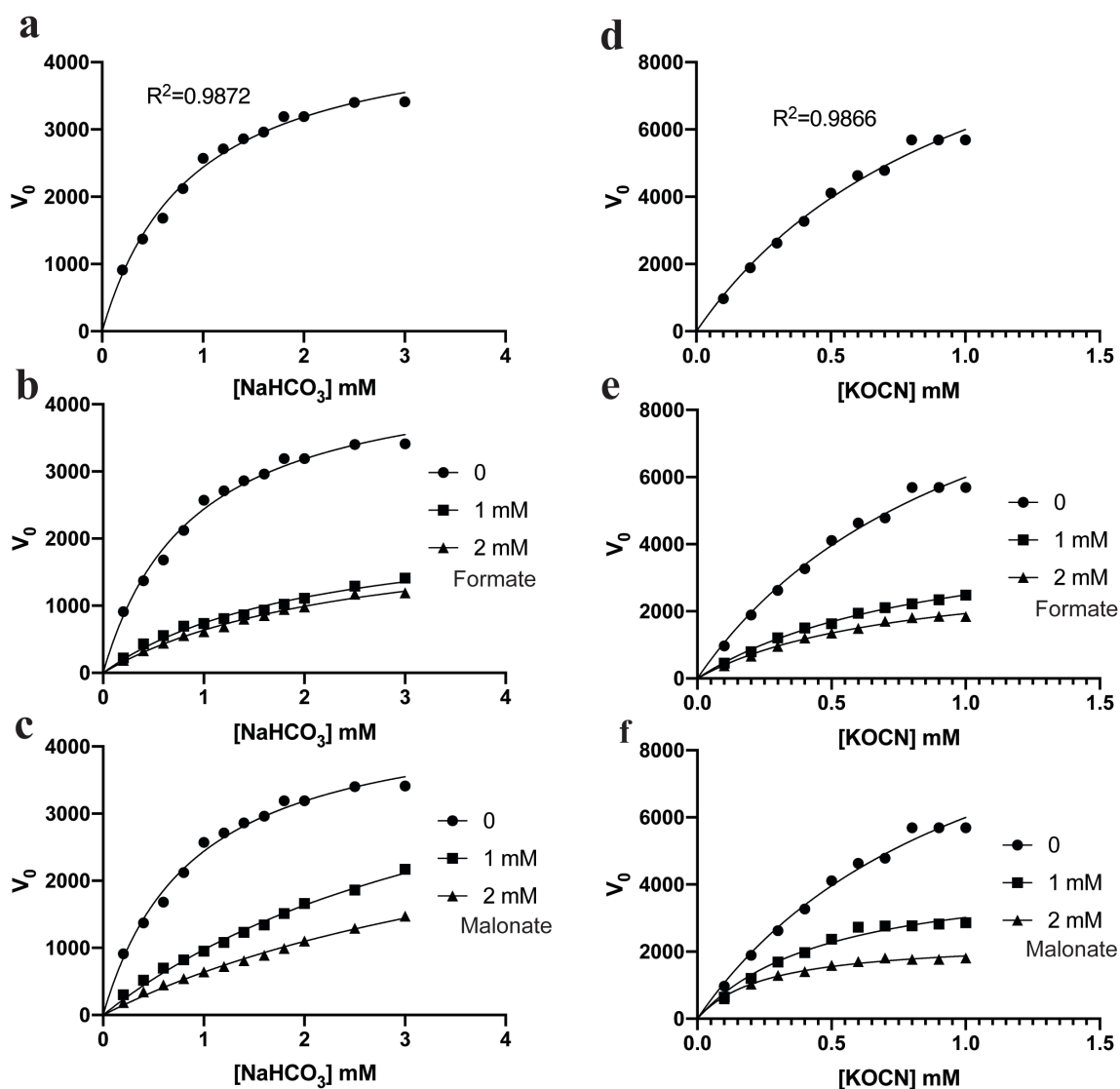
Supplementary Figure 1. Purification and thermal unfolding analysis of TI-Cyn. a, Gel filtration chromatogram of the purified TI-Cyn. Inset depicts SDS-PAGE image of a purified TI-Cyn. **b,** Thermal shift assay to examine the thermostability of TI-Cyn.



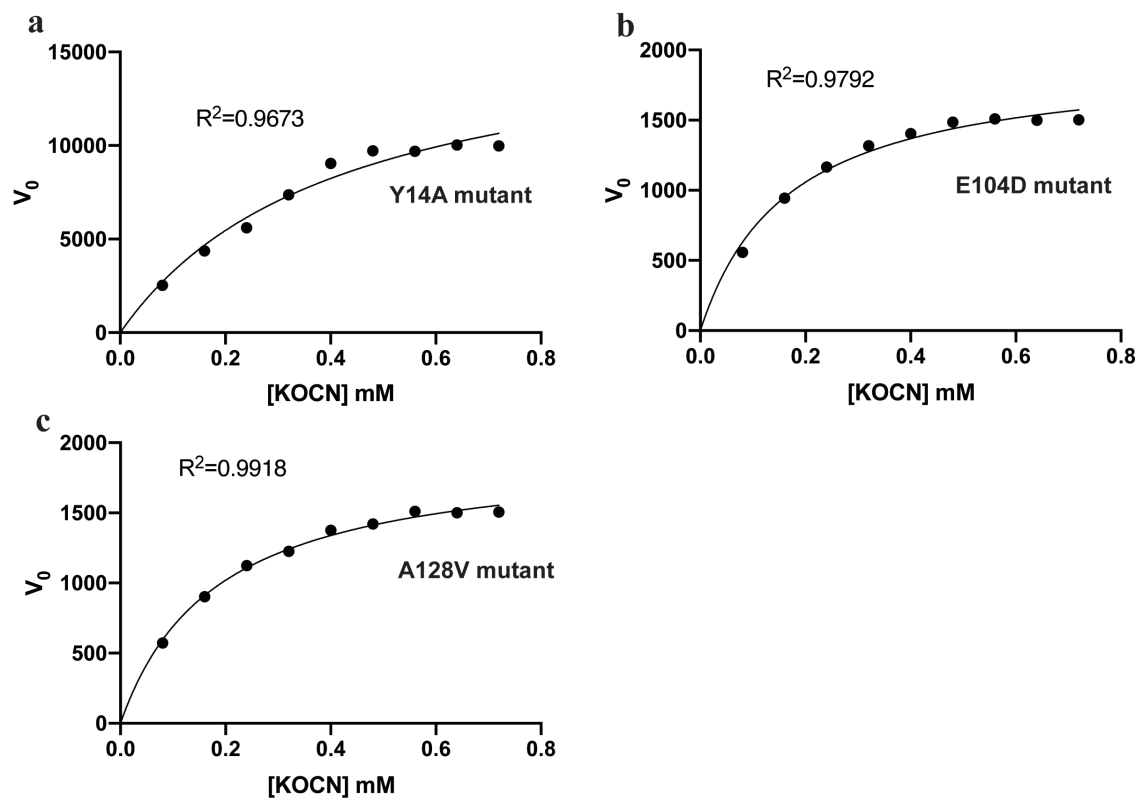
Supplementary Figure 2. Overall appearance of the two cyanase decamers. a TI-Cyn. **b,** *E. coli*.



Supplementary Figure 3. Possible mechanism of the TI-Cyn catalysis and inhibition. a, Schematic drawing for the mechanism of cyanate decomposition catalyzed by cyanase. The substrates are shown with different colors and shapes, bicarbonate green hexagon, and cyanate purple pentagon. **b,** A cartoon representation for the catalysis and kinetic inhibition of the TI-Cyn. The active site is indicated with the red *asterisk*. The presence of the inhibitor at the active site of the TI-Cyn has hindered substrate binding. The substrates are given separate colors as in **a**. The inhibitor molecule is shown in yellow hexagon.



Supplementary Figure 4. Kinetic studies on the inhibition of TI-Cyn by Non-linear curve-fitting using Michaelis-Menten plots. **a**, Titration of NaHCO_3 , while keeping KOCN constant in the absence of inhibitors. **b**, Titration of NaHCO_3 , while keeping KOCN constant in the presence of inhibitor (Formate). **c**, Titration of NaHCO_3 , while keeping KOCN constant in the presence of inhibitor (Malonate). **d**, Titration of KOCN , while keeping NaHCO_3 constant in the absence of inhibitors. **e**, Titration of KOCN , while keeping NaHCO_3 constant in the presence of inhibitor (Formate). **f**, Titration of KOCN , while keeping NaHCO_3 constant in the presence of inhibitor (Malonate). The Non-linear curve-fittings and data prediction were performed using Prism 8 software.



Supplementary Figure 5. Kinetic studies of Tl-Cyn mutants by Non-linear curve-fitting using Michaelis-Menten plots. a, Titration of KOCN, while keeping NaHCO_3 constant for Y14A mutant. **b,** Titration of KOCN, while keeping NaHCO_3 constant for E104D mutant. **c,** Titration of KOCN, while keeping NaHCO_3 constant for A128V mutant.

Supplementary Tables

Supplementary Table 1. Primer sequences for site directed mutagenesis

Primer	Sequence (5'→3')*
BRF_Y14	GACGTC ACT CAGCATCCAG GC TCTACCCGCCTACTCCAA
BRR_Y14	TTGGAGTAGGCGGGTAGAG GC TGGATGCTGAGTGACGTC
BRF_R101	AAGGAACCCTTGATTTAT AA ATTGTATGAGATTGTGCA
BRR_R101	TGCACAATCTCATAACAAT TT TATAAATCAAGGGTTCCTT
BRF_E104	GATTTATCGATTGTATGAC CA TTGTGCAGAATTATGGA
BRR_E104	TCCATAATTCTGCACAAT GT CATAACAATCGATAAATC
BRF_S127	TCGGGGACGGTATCATGAC CT GCGATCAGCTTTTCAAC
BRR_S127	GTTGAAAAGCTGATCGCA GT CATGATACCGTCCCCGA
BRF_A128	GGGACGGTATCATGAGTGT GT GATCAGCTTTTCAACGTC
BRR_A128	GACGTTGAAAAGCTGATC AC ACTCATGATACCGTCCC

*Substituted nucleotides are indicated in bold.

Supplementary Table 2. Summary of kinetic data for wild-type and mutant cyanases from Non-linear curve-fitting using Michaelis-Menten plots

Protein	k_{cat} (s ⁻¹)	K_m (mM) (for cyanate)	k_{cat}/K_m (s ⁻¹ M ⁻¹)
Wild-type	3.52×10^4	0.72 (from 95% CI)	4.88×10^7
Y14A	4.78×10^4	0.41 (from best-fit)	11.66×10^7
R101K	NA ^a	NA ^a	NA ^a
E104D	5.51×10^3	0.17 (from best-fit)	3.24×10^7
S127T	NA ^a	NA ^a	NA ^a
A128V	5.54×10^3	0.18 (from best-fit)	3.08×10^7
A128V/ R101K	NA ^a	NA ^a	NA ^a

^aNo activity was observed under the same conditions.