

Supporting Information

Enhancing the Catalytic Activity of Glycolate Oxidase from *Chlamydomonas reinhardtii* through Semi-rational Design

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Table S1 The primers for the fusion expression of CreGO and GST

Primer	Sequence (5'-3')
pET22b-GST-F	AAGCTTGCGGCCGCACTC
pET22b-GST-GSG-R	TCCACTTCCACGCGGAACCAGATCGCTTT
GSG-CreGO-F	AAAAGCGATCTGGTTCCGCGTGGAAGTGGAATGGCAGACCTGAGCTTTCTGA
CreGO-R	CTCGAGTGCGGCCGCAAGCTTCAGTTTACACAGCTGTGCTGCC

Table S2 The primers for site-directed mutagenesis of CreGO in alanine scanning

Primer	Sequence (5'-3')
Y27A-F	TTTGATGCATATAGCACCGGTAGCGATACCTGT
Y27A-R	GCTATATGCATCAAATGCCATTTTCGGCATA
V111A-F	AGCACCGCAGCAACCAGCAGCCTGCAGG
V111A-R	GGTTGCTGCGGTGCTAAAGGTAAACGGAACACCT
L164A-F	CAGCGTGCAGGTAATCGTGAAGCAGATGCACG
L164A-R	ACGATTTGCACCACGCTGTGCATCAACGGTAACC
R167 A-F	GGTAATGCAGAAGCAGATGCACGTAATAAATTTACC
R167 A-R	TGCTTTGCGATTACCCAGACGCTGTGCATCAAC
F208A-F	AAACTGGCAACCAGCGAAGTTGATGATAGCCTG
F208A-R	GCTGGTTGCCAGTTTCATCAGACCGCTACCATCCTG
V212A-F	AGCGAAGCAGATGATAGCCTGACCTGGGAATTTATT
V212A-R	ATCATCTGCTTCGCTGGTAAACAGTTTCATCAGAC

Table S3 The primers for site-directed saturation mutagenesis of Y27 in CreGO

Primer	Sequence (5'-3')
Y27S-F	TTTGATAGCTATAGCACCGGTAGCGATACCTGT
Y27S-R	GCTATAGCTATCAAATGCCATTTTCGGCATA
Y27T-F	TTTGATACCTATAGCACCGGTAGCGATACCTGT
Y27T-R	GCTATAGGTATCAAATGCCATTTTCGGCATA
Y27C-F	TTTGATTGCTATAGCACCGGTAGCGATACCTGT
Y27C-R	GCTATAGCAATCAAATGCCATTTTCGGCATA
Y27N-F	TTTGATAATTATAGCACCGGTAGCGATACCTGT
Y27N-R	GCTATAATTATCAAATGCCATTTTCGGCATA
Y27Q-F	TTTGATCAGTATAGCACCGGTAGCGATACCTGT
Y27Q-R	GCTATA CTGATCAAATGCCATTTTCGGCATA
Y27H-F	TTTGATCATTATAGCACCGGTAGCGATACCTGT
Y27H-R	GCTATAATGATCAAATGCCATTTTCGGCATA
Y27K-F	TTTGATAAATATAGCACCGGTAGCGATACCTGT
Y27K-R	GCTATATTTATCAAATGCCATTTTCGGCATA
Y27R-F	TTTGATCGTTATAGCACCGGTAGCGATACCTGT
Y27R-R	GCTATAACGATCAAATGCCATTTTCGGCATA
Y27D-F	TTTGATGATTATAGCACCGGTAGCGATACCTGT
Y27D-R	GCTATAATCATCAAATGCCATTTTCGGCATA
Y27E-F	TTTGATGAATATAGCACCGGTAGCGATACCTGT
Y27E-R	GCTATATTCATCAAATGCCATTTTCGGCATA
Y27V-F	TTTGATGTTTATAGCACCGGTAGCGATACCTGT
Y27V-R	GCTATAAACATCAAATGCCATTTTCGGCATA
Y27L-F	TTTGATCTGTATAGCACCGGTAGCGATACCTGT
Y27L-R	GCTATACAGATCAAATGCCATTTTCGGCATA
Y27I-F	TTTGATATTTATAGCACCGGTAGCGATACCTGT
Y27I-R	GCTATAAATATCAAATGCCATTTTCGGCATA
Y27F-F	TTTGATTTTTATAGCACCGGTAGCGATACCTGT
Y27F-R	GCTATAAAAATCAAATGCCATTTTCGGCATA

Y27W-F	TTTGATTGGTATAGCACCGGTAGCGATACCTGT
Y27W-R	GCTATACCAATCAAATGCCATTTTCGGCATA
Y27M-F	TTTGATATGTATAGCACCGGTAGCGATACCTGT
Y27M-R	GCTATACATATCAAATGCCATTTTCGGCATA
Y27P-F	TTTGATCCGTATAGCACCGGTAGCGATACCTGT
Y27P-R	GCTATACGGATCAAATGCCATTTTCGGCATA

Table S4 The primers for iterative saturation mutagenesis of V212 in CreGO

Primer	Sequence (5'-3')
V212S-F	AGCGAAAGCGATGATAGCCTGACCTGGGAATTTATT
V212S-R	ATCATCGCTTTCGCTGGTAAACAGTTTCATCAGAC
V212T-F	AGCGAAACCGATGATAGCCTGACCTGGGAATTTATT
V212T-R	ATCATCGGTTTCGCTGGTAAACAGTTTCATCAGAC
V212C-F	AGCGAATGCGATGATAGCCTGACCTGGGAATTTATT
V212C-R	ATCATCGCATTTCGCTGGTAAACAGTTTCATCAGAC
V212N-F	AGCGAAAATGATGATAGCCTGACCTGGGAATTTATT
V212N-R	ATCATCATTTTCGCTGGTAAACAGTTTCATCAGAC
V212Q-F	AGCGAACAGGATGATAGCCTGACCTGGGAATTTATT
V212Q-R	ATCATCCTGTTTCGCTGGTAAACAGTTTCATCAGAC
V212H-F	AGCGAACATGATGATAGCCTGACCTGGGAATTTATT
V212H-R	ATCATCATGTTTCGCTGGTAAACAGTTTCATCAGAC
V212K-F	AGCGAAAAAGATGATAGCCTGACCTGGGAATTTATT
V212K-R	ATCATCTTTTTTCGCTGGTAAACAGTTTCATCAGAC
V212R-F	AGCGAACGTGATGATAGCCTGACCTGGGAATTTATT
V212R-R	ATCATCACGTTTCGCTGGTAAACAGTTTCATCAGAC
V212D-F	AGCGAAGATGATGATAGCCTGACCTGGGAATTTATT
V212D-R	ATCATCATCTTCGCTGGTAAACAGTTTCATCAGAC
V212E-F	AGCGAAGAAGATGATAGCCTGACCTGGGAATTTATT
V212E-R	ATCATCTTCTTCGCTGGTAAACAGTTTCATCAGAC
V212L-F	AGCGAACTGGATGATAGCCTGACCTGGGAATTTATT
V212L-R	ATCATCCAGTTCGCTGGTAAACAGTTTCATCAGAC
V212I-F	AGCGAAATTGATGATAGCCTGACCTGGGAATTTATT
V212I-R	ATCATCAATTTTCGCTGGTAAACAGTTTCATCAGAC
V212F-F	AGCGAATTTGATGATAGCCTGACCTGGGAATTTATT
V212F-R	ATCATCAAATTCGCTGGTAAACAGTTTCATCAGAC
V212W-F	AGCGAATGGGATGATAGCCTGACCTGGGAATTTATT
V212W-R	ATCATCCCATTTCGCTGGTAAACAGTTTCATCAGAC

V212M-F	AGCGAAATGGATGATAGCCTGACCTGGGAATTTATT
V212M-R	ATCATCCATTTGCTGGTAAACAGTTTCATCAGAC
V212P-F	AGCGAACCGGATGATAGCCTGACCTGGGAATTTATT
V212P-R	ATCATCCGGTTGCTGGTAAACAGTTTCATCAGAC
V212Y-F	AGCGAATATGATGATAGCCTGACCTGGGAATTTATT
V212Y-R	ATCATCATATTCGCTGGTAAACAGTTTCATCAGAC
V212A-F	AGCGAAGCAGATGATAGCCTGACCTGGGAATTTATT
V212A-R	ATCATCTGCTTCGCTGGTAAACAGTTTCATCAGAC

Table S5 The primers for iterative site-saturation mutagenesis of V111 in CreGO

Primer	Sequence (5'-3')
V111S-F	AGCACCAGCGCAACCAGCAGCCTGCAGG
V111S-R	GGTTGCGCTGGTGCTAAAGGTAAACGGAACACCT
V111T-F	AGCACCACCGCAACCAGCAGCCTGCAGG
V111T-R	GGTTGCGGTGGTGCTAAAGGTAAACGGAACACCT
V111C-F	AGCACCTGCGCAACCAGCAGCCTGCAGG
V111C-R	GGTTGCGCAGGTGCTAAAGGTAAACGGAACACCT
V111N-F	TTTGATAATTATAGCACCGGTAGCGATACCTGT
V111N-R	GGTTGCATTGGTGCTAAAGGTAAACGGAACACCT
V111Q-F	AGCACCCAGGCAACCAGCAGCCTGCAGG
V111Q-R	GGTTGCCTGGGTGCTAAAGGTAAACGGAACACCT
V111H-F	AGCACCCATGCAACCAGCAGCCTGCAGG
V111H-R	GGTTGCATGGGTGCTAAAGGTAAACGGAACACCT
V111K-F	AGCACCAAAGCAACCAGCAGCCTGCAGG
V111K-R	GGTTGCTTTGGTGCTAAAGGTAAACGGAACACCT
V111R-F	TTTGATCGTTATAGCACCGGTAGCGATACCTGT
V111R-R	GGTTGCACGGGTGCTAAAGGTAAACGGAACACCT
V111D-F	TTTGATGATTATAGCACCGGTAGCGATACCTGT
V111D-R	GGTTGCATCGGTGCTAAAGGTAAACGGAACACCT
V111E-F	AGCACCGAAGCAACCAGCAGCCTGCAGG
V111E-R	GGTTGCTTCGGTGCTAAAGGTAAACGGAACACCT
V111V-F	TTTGATGTTTATAGCACCGGTAGCGATACCTGT
V111V-R	AGCACCTGGCAACCAGCAGCCTGCAGG
V111L-F	TTTGATCTGTATAGCACCGGTAGCGATACCTGT
V111L-R	GGTTGCCAGGGTGCTAAAGGTAAACGGAACACCT
V111I-F	AGCACCATTGCAACCAGCAGCCTGCAGG
V111I-R	GGTTGCAATGGTGCTAAAGGTAAACGGAACACCT
V111F-F	AGCACCTTTGCAACCAGCAGCCTGCAGG
V111F-R	GGTTGCAAAGGTGCTAAAGGTAAACGGAACACCT

V111W-F	AGCACCTGGGCAACCAGCAGCCTGCAGG
V111W-R	GGTTGCCCAGGTGCTAAAGGTAAACGGAACACCT
V111M-F	AGCACCATGGCAACCAGCAGCCTGCAGG
V111M-R	GGTTGCCATGGTGCTAAAGGTAAACGGAACACCT
V111P-F	AGCACCCCGGCAACCAGCAGCCTGCAGG
V111P-R	GGTTGCCGGGGTGCTAAAGGTAAACGGAACACCT
V111Y-F	AGCACCTATGCAACCAGCAGCCTGCAGG
V111Y-R	GGTTGCATAGGTGCTAAAGGTAAACGGAACACCT
V111A-F	AGCACCGCAGCAACCAGCAGCCTGCAGG
V111A-R	GGTTGCTGCGGTGCTAAAGGTAAACGGAACACCT

Supplementary figures

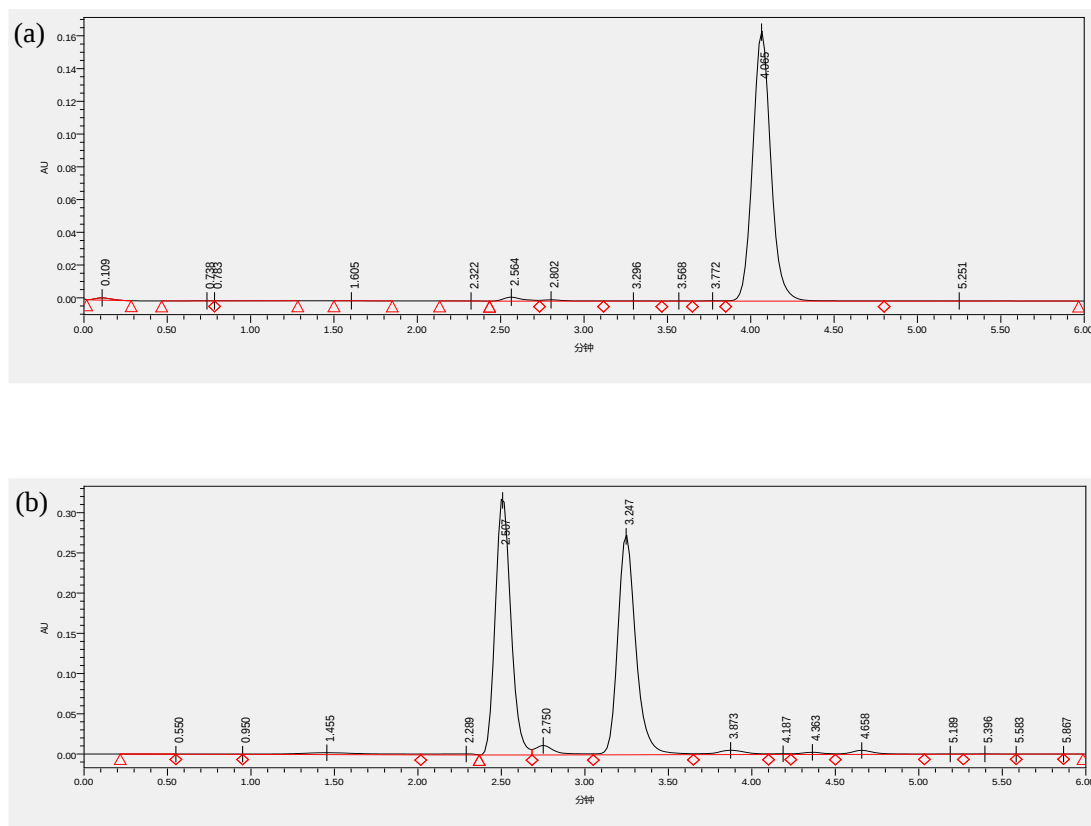


Figure S1 HPLC analyses of methyl glycolate (a) and methyl glyoxylate (b). Methyl glycolate, 4.07 min; methyl glyoxylate, 2.51 min; toluene as solvent, 3.25 min.

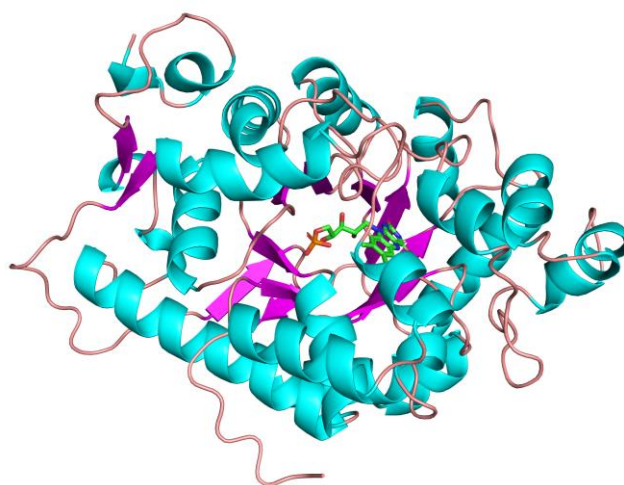


Figure S2 Modeling of glycolate oxidase CreGO

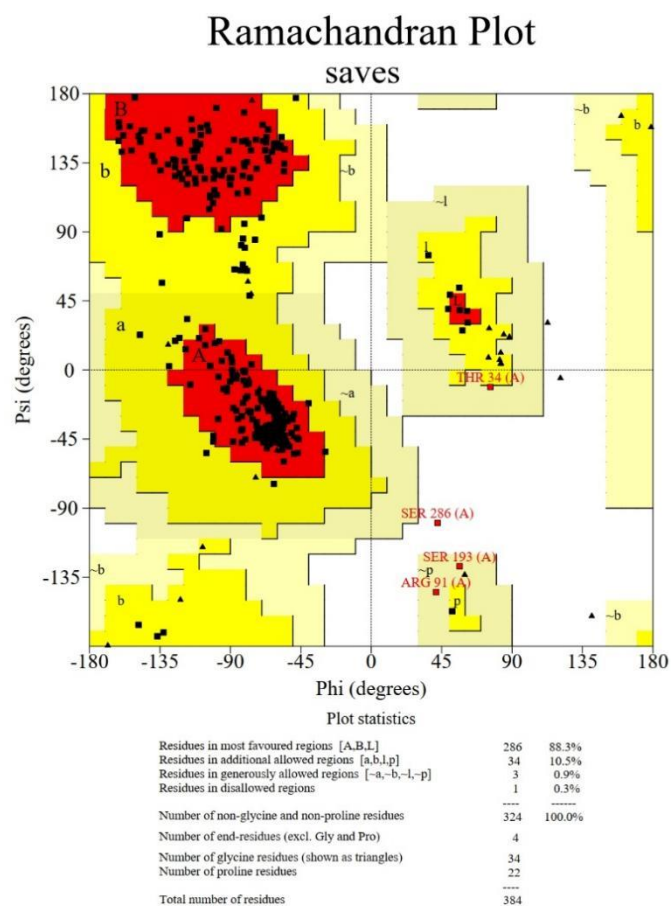


Figure S3 Ramachandran plot analysis of CreGO modeling

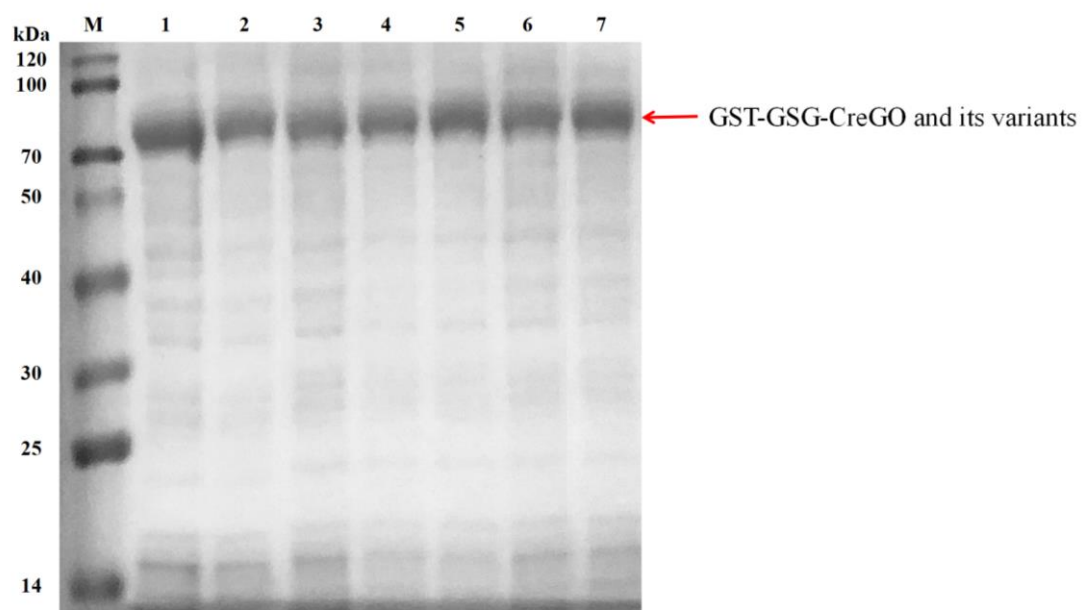


Figure S4 SDS-PAGE (12%) analysis of the fusion enzyme GST-GSG-CreGO and its variants in alanine scanning. Lane M, standard molecular mass proteins; lanes from 1 to 7 represent the variants (from left to right): GST-GSG-CreGO, Y27A, V111A, L164A, R167A, F208A and V212A. The proteins were visualized by staining with Coomassie brilliant blue R-250.

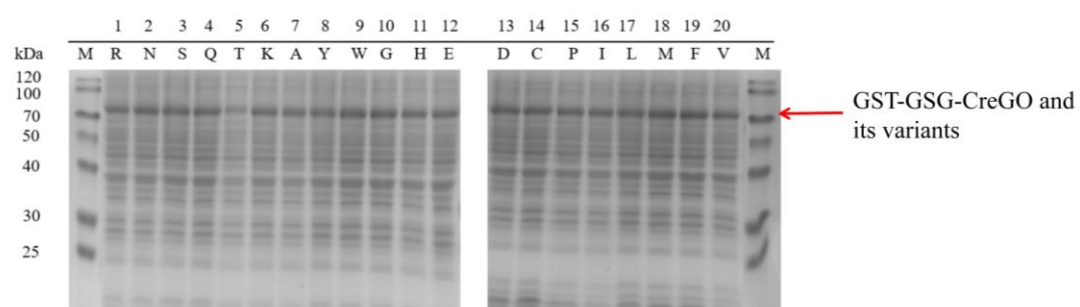


Figure S5 SDS-PAGE (12%) analysis of the fusion enzyme GST-GSG-CreGO and its variants in the saturation mutagenesis of the residue Y27. Lane M, standard molecular mass proteins; lanes from 1 to 20 represent the variants (from left to right): Y27R, Y27N, Y27S, Y27Q, Y27T, Y27K, Y27A, wild type, Y27W, Y27G, Y27H, Y27E, Y27D, Y27C, Y27P, Y27I, Y27L, Y27M, Y27F and Y27V. The proteins were visualized by staining with Coomassie brilliant blue R-250.

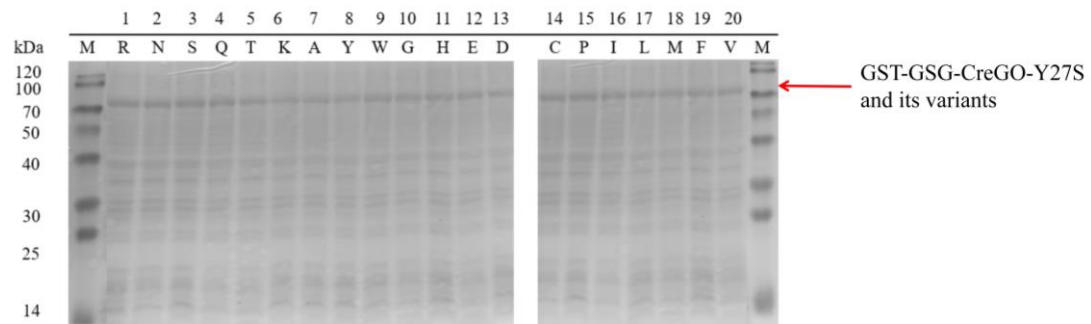


Figure S6 SDS-PAGE (12%) analysis of the glycolate oxidase GST-GSG-CreGO-Y27S and its variants in the iterative saturation mutagenesis of the residue V212. Lane M, standard molecular mass proteins; lanes from 1 to 20 represent the variants (from left to right): V212R, V212N, V212S, V212Q, V212T, V212K, V212A, V212Y, V212W, V212G, V212H, V212E, V212D, V212C, V212P, V212I, V212L, V212M, V212F and M₁. The proteins were visualized by staining with Coomassie brilliant blue R-250.

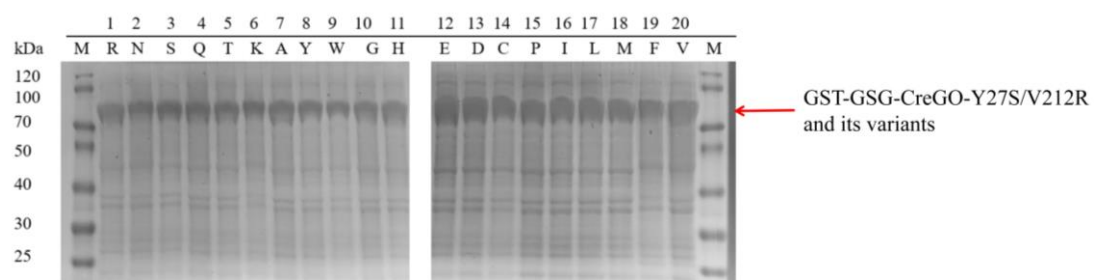


Figure S7 SDS-PAGE (12%) analysis of the glycolate oxidase GST-GSG-CreGO-Y27S/V212R and its variants in the iterative saturation mutagenesis of the residue V111. Lane M, standard molecular mass proteins; lanes from 1 to 20 represent the variants (from left to right): V111R, V111N, V111S, V111Q, V111T, V111K, V111A, V111Y, V111W, V111G, V111H, V111E, V111D, V111C, V111P, V111I, V111L, V111M, V111F and M₂. The proteins were visualized by staining with Coomassie brilliant blue R-250.

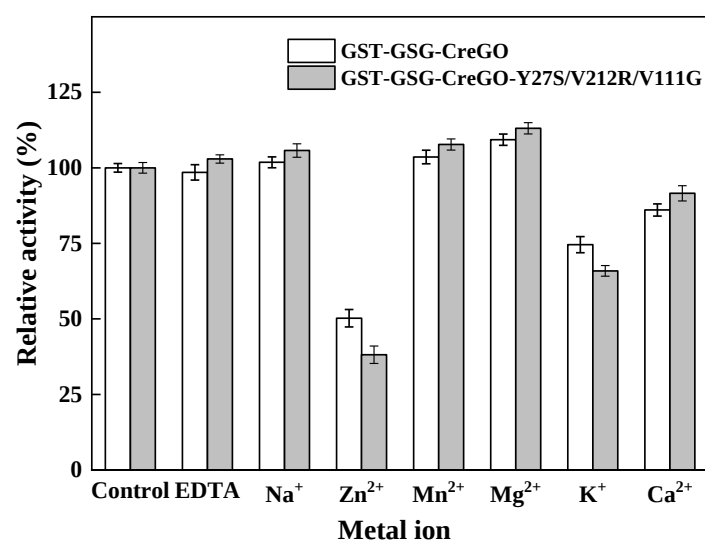


Figure S8 The effects of metal ions and EDTA on activity of GST-GSG-CreGO and its variant GST-GSG-CreGO-Y27S/V212R/V111G. The assay mixture (1 mL) consisted of 1 μ g purified enzyme, 10 mM phenylhydrazine hydrochloride, 1 mM metal ion or EDTA, 10 mM sodium glycolate and 100 mM PBS buffer (pH 6.5). The activity was measured in triplicate at 40 $^{\circ}$ C by monitoring changes in the absorbance at 324 nm. The relative activity of 100% for GST-GSG-CreGO and GST-GSG-CreGO-Y27S/V212R/V111G represents 6.9 U/mg and 24.9 U/mg, respectively. Standard deviations are indicated in the diagram ($n = 3$).

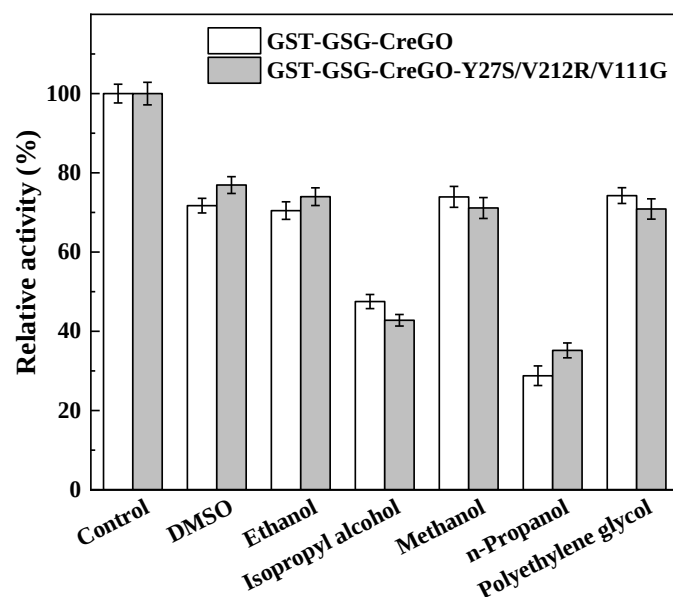


Figure S9 The effects of organic solvents on activity of GST-GSG-CreGO and its variant GST-GSG-CreGO-Y27S/V212R/V111G. The assay mixture (1 mL) consisted of 1 μ g purified enzyme, 10 mM phenylhydrazine hydrochloride, 10% (v/v) organic solvents, 10 mM sodium glycolate and 100 mM PBS buffer (pH 6.5). The activity was measured in triplicate at 40 °C by monitoring changes in the absorbance at 324 nm. The relative activity of 100% for GST-GSG-CreGO and GST-GSG-CreGO-Y27S/V212R/V111G represents 6.9 U/mg and 24.9 U/mg. Standard deviations are indicated in the diagram ($n = 3$).

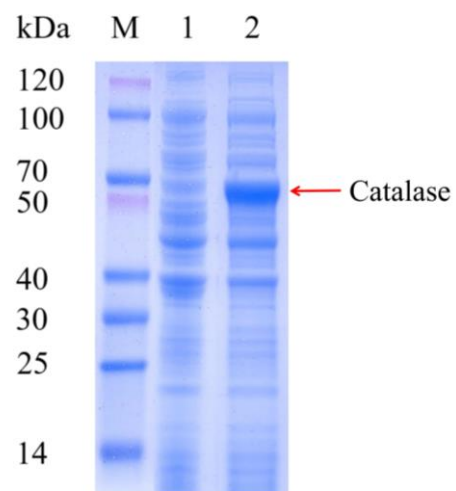


Figure S10 SDS-PAGE (12%) analysis of recombinant catalase from *Acinetobacter* sp. YS0810 expressed in *E. coli*. The proteins were visualized by staining with Coomassie brilliant blue R-250.