



Functional Characterization and Structure-Guided Mutational Analysis of The Transsulfuration Enzyme Cystathionine γ -Lyase from *Toxoplasma gondii*

Supplemental Figures

T. gondii	MASKQNDKDGAVRRDASFECGVKAGDWLPGFTPREETVYVHGGVEPDP-LTGAILPPIYQ	59
L. major	MSSQ-----QHLVSDFTAGSGSWLPQSQ-GFDTLQVHAGVRPDP-VTGAILTPIYQ	49
T. cruzi	MSSQ-----KHLVSDFTAGSGSWLDQTY-GFDTVLVHGGVVKPDP-VTGAVLTPVYQ	49
T. grayi	MSGA-----QHLFADFSEGGSGWQPQAQ-GFETLLVHGGVVKPDP-VTGAILTPVYQ	49
M. musculus	-----MQKDASLSGFLPSFQ-HFATQAIHVQDEPEQWNSRAVVLPISL	42
H. Sapiens	-----MQEKDASSQGFLPHFQ-HFATQAIHVQDEPEQWNSRAVVPPIISL	43
S. Cerevisiae	-----MTL-----QESD-KFATKAIHAGEHVD--VHGSVIEPISL	32
C. albicans	-----MTI-----ESSTNY-SFGTKAIHAGAPLDP-STGAVIEPISL	35
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T. gondii	NTTFVQESVENYLSKGFSSKSRTSNPTVLSLEKKIAEIEGGFGACCFATGMAATVTIFSAF	119
L. major	STTFVQESINSYQAKGYSKTRSANPTVAVLEQKLCALENGSYCTVYNTGMAATTTAISF	109
T. cruzi	STTFVQESIGIKYQSKGYSKTRCANPTVSLERKLCALENGDIATVYSTGMSATTTAISF	109
T. grayi	STTFVQESIERYQAKGYSKTRSANPTVSALEKLCALIEHGEYATVYSTGMSATTTAISF	109
M. musculus	ATTFKQDFPGQ-SSG-FEYSRSGNPTNRCLEKAVAAALDGAKHSLAFASGLAATITITH-L	99
H. Sapiens	STTFKQAGPQ-HSG-FEYSRSGNPTNRCLEKAVAAALDGAKYCLAFASGLAATVITH-L	100
S. Cerevisiae	STTFKQSSPAN-PIGTYEKSRSONPNRENLERAVAALENAQYGLAFSSGSAATTATILQ-S	90
C. albicans	STTFKQSEPSK-PLGIYEKSRSSNPNRDNFEITVAVALESAKYAIALLSSGSAATLVIQ-S	93
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T. gondii	LAPGDHCLVTNCSYGGTNRCARLHFSKYNIDFEFIDFRDPTNVEKAIRPQTKVVFSESPC	179
L. major	MNAGDHAILTNCYGGTNRACRVFFSRLGMEFTFVDMRDQPNVIDSIKPNTKLVISETPA	169
T. cruzi	MSAGDHAIITDCSYGGTNRACRVFFPRFGMEFTFVDMRDLKNVEAAIKPNTKLVSETPA	169
T. grayi	MSAGDHAIVTECSYGGTNRACRVFFTRLGMSFTFVDMRDVKNVEAAIKPNTKLVISEPA	169
M. musculus	LKAGDEIICMDEVYGGTNRVFRVASEFGLKISFVDCSKTKLLEAAITPQTKLVWIETPT	159
H. Sapiens	LKAGDQIICMDDVYGGTNRVFRVASEFGLKISFVDCSKIKLLEAAITPETKLVWIETPT	160
S. Cerevisiae	LPQGSHAVSIGDVGTHRYFTKVANAHGVETSFTNDLLN-DLPQLIKENTKLVWIETPT	149
C. albicans	LPINSHIVSSGDVYGGTHRYFTKVANHGVETAQVGNLVE-DLQALRENTRLVWLETPS	152
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T. gondii	NPTLYLADIEAISQICKEK-----KVLHVCDSTFATPYMMRPLDLGADIVVQSTRYVD	233
L. major	NPTLILIDVAAVSKICKER-----GIVHMCNTFATAYIMRPLDHGADVTLISTRYVD	223
T. cruzi	NPTLTLDTLDELKSLCKAK-----GLIHVCNTFATAFIMRPLDLGADVTLISTRYVD	223
T. grayi	NPTLTLTDIDALSILCKAK-----GIHMCNTFATAFIMRPLDHGADVTLISTRYVD	223
M. musculus	NPTLKLADIGACAQIVHKR-----GDIILVVDNTFMSAYFQRPLALGADICMSATRYMN	214
H. Sapiens	NPTQKVIDIEGCAHIVHKH-----GDIILVVDNTFMSPYFQRPLALGADISMSATRYMN	215
S. Cerevisiae	NPTLKVTDIQKVADLIKKAH--AGQDVILVVDNTFLSPYISNPLNFGADIVVHSATRYIN	207
C. albicans	NPTLQVTDIAKVKSIILVDHEAKTGNKVLAVDNTFLSPYLSNPLTHGADVHVHSVTRRYIN	212
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T. gondii	GHNCTLGGAVISSKEIHDKVFVFLRNVMGNIMSQAFTAFYTLTLKTLPIRVEKQSANAQK	293
L. major	GHDMTVGGALVTNSKELDAKVRILTQNILGNVMSQVAFVLQQLTVKTMRLVTKQSHNAQK	283
T. cruzi	GHNMTVGGALVTKRKLDEKVRILTQNILGNMSPFVAYLQQLTVKTMRLVTKQSHNAQK	283
T. grayi	GHNMTVGGALVTKSKELDKVRLTQNILGNMSPFVAYLQQLTVKTMRLVTKQSHNAQK	283
M. musculus	GHSDDVMGLVSVNSDDLNSRLRFLQNSLGAVSPFDCYLCRGLKTLQVRMEKHFKNGMA	274
H. Sapiens	GHSDDVMGLVSVNCSLHNRLRFLQNSLGAVSPIDCYLNRGLKTLHVRMEKHFKNGMA	275
S. Cerevisiae	GHSDDVVLGVLATNNKPLYERLQFLQNAIGAIPSPFDLWLRGLKTLHLRVRQAALSANK	267
C. albicans	GHSDDVMGLVATNDSQLHERFRFLQNAIGSIPSPFDLWLAHRGLKTLHLRVRQAASNAQR	272
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T. gondii	IAEFLSKH-HKVEHVIYPGIPSPQKELALKQHK-NVHGGMLAFEVKGTEAGIRMMNHV	351
L. major	IAEFLETH-RAVDRVYVYPLASHPQKELADRQHRNHLHGGMLWFEVKGTEAGRRMMDTV	342
T. cruzi	VAEFLETH-PAVEKVMYPGLKSPQKALADRQHLNHLHGGMLWFEVKGTEAGRRMMDTV	342
T. grayi	VAEFLETH-PAVERVMYPGLKSPQKALADRQHANHLHGGMLWFEVKGTEAGRRMMDTV	342
M. musculus	VARFLETH-PRVEKVMYPGLSPHQLAKRQCSG--CPGMVSYIKGALQHAQKAFI-KN	330
H. Sapiens	VAQFLESN-PWVEKVIYPGLSPHQLAKRQCTG--CTGMVTFYIKGTLQHAQKAFI-KN	331
S. Cerevisiae	IAEFLAADKENVAVNYPGLKTHPNYDVLKQHRDALGGGMSIFRIKGGAEASKFA-SS	326
C. albicans	IAEYLSQH-SAVLKVNYPGLKSHRNHDVVLQRQDGLGGGMSIFRIAGGAKGAAVFT-SS	330
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T. gondii	PRPWSLCENLGACESIITCPAVFTHANMLREDRLKVGITDGFIRVSVGIEDVNDLIDGLD	411
L. major	PRPWSLCENLGASESIITCPSVMTHANMTSEDRMKVGITDGFVRSVCGIEDVDDLIAALK	402
T. cruzi	QRPWSLCENLGAEESIITCPSVMTHANMTKEDRLKVGITDGFVRSVCGIEEAKDLITALK	402
T. grayi	QRPWSLCENLGATESIITCPSVMTHANMTEDRMKVGITDGFVRSVCGIEDAADLISALK	402
M. musculus	LKLFTLAESLGGYESLAEIPAIMTHASVPEKDRATLGINDTLIRLSVGLDEQDLEDLD	390
H. Sapiens	LKLFTLAESLGGFESLAEIPAIMTHASVPEKDRATLGINDTLIRLSVGLDEQDLEDLD	391
S. Cerevisiae	TRLFTLAESLGGIESLLEVPAMTHGGIPKEAREASGVFDDLVRSVGIETDLEDLKE	386
C. albicans	TCLFTLAESLGGIESLIEVPAIMTHGGIPKEEREANGVDDLVRSVGIETDLEDLKE	390
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T. gondii	YALSKA----- 417	
L. major	VAMDALV----- 409	
T. cruzi	TALDAL----- 408	
T. grayi	AALDALGK----- 410	
M. musculus	RALKAHP----- 398	
H. Sapiens	QALKAHPSPGSHS 405	
S. Cerevisiae	QALKQATN----- 394	
C. albicans	QALQKAASV----- 399	
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Figure S1. Sequence alignment of CGL from different organisms. Black shading indicates the PLP-binding lysine in the active site. The target residues for mutational analysis are indicated by arrows. The CGLs used in this alignment (NCBI accession number) are XP_002364505.1, *Toxoplasma gondii* ME49; XP_003722717.1, *Leishmania major*; EKG03141.1, *Trypanosoma cruzi*; XP_009313447.1, *Trypanosoma grayi*; NP_666065.1, *Mus musculus*; NP_001893.2, *Homo sapiens*; NP_009390.1, *Saccharomyces cerevisiae*; XP_716241.1, *Candida albicans*. All sequence alignments were carried out using the Clustal OMEGA program.

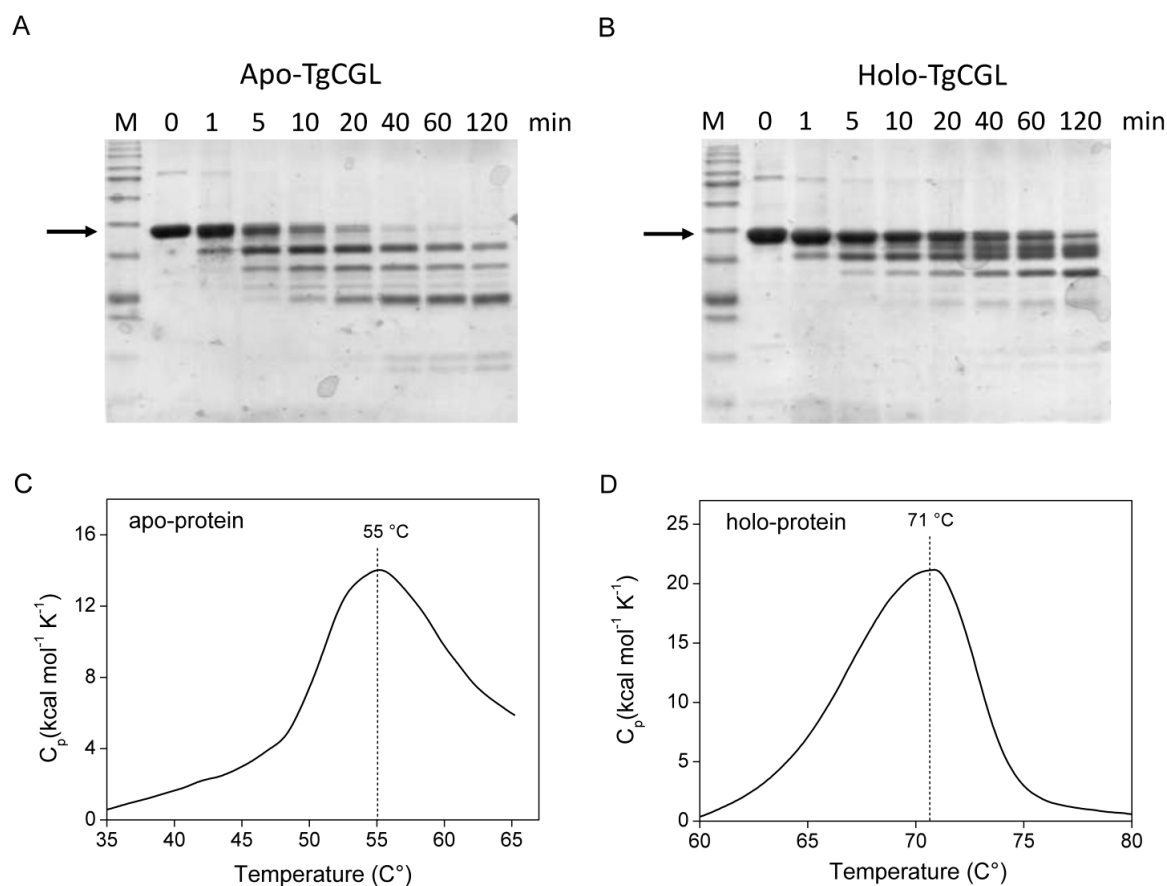


Figure S2. Properties of apo-TgCGL. (A) and (B). Trypsin digestion profile of apo- (A) and holo-TgCGL (B) after incubation of TgCGL with trypsin 1:200 (*w/w*) for 0, 1, 5, 10, 20, 40, 60 and 120 min, respectively. The intensity of the untreated with trypsin TgCGL band (lane 0 min) was assumed as 100%. The arrow indicates the untreated 46 kDa band. Lane M represents a molecular mass marker. (C) and (D). Representative DSC thermograms of apo- (C) and holo-TgCGL (D), respectively, after baseline-correction.

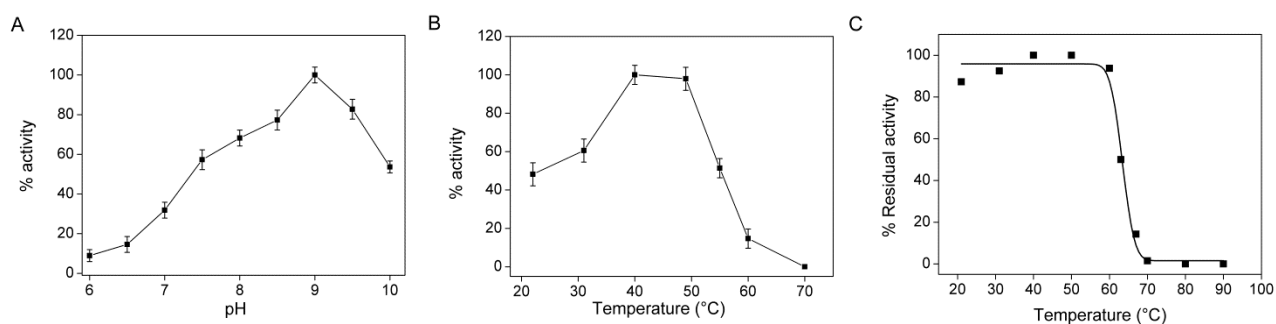


Figure S3. pH and temperature optima for L-cystathionine hydrolysis of TgCGL. (A) Effect of pH on purified TgCGL in MBP buffer in the pH range of 6–10 at 37 °C. (B) Effect of temperature on the activity of purified TgCGL at pH 9. (C) Representative thermal stability curve for purified TgCGL,

held at temperature range from 20 to 90 °C for 15 min. L-cystathionine hydrolysis was estimated using the DTNB assay.

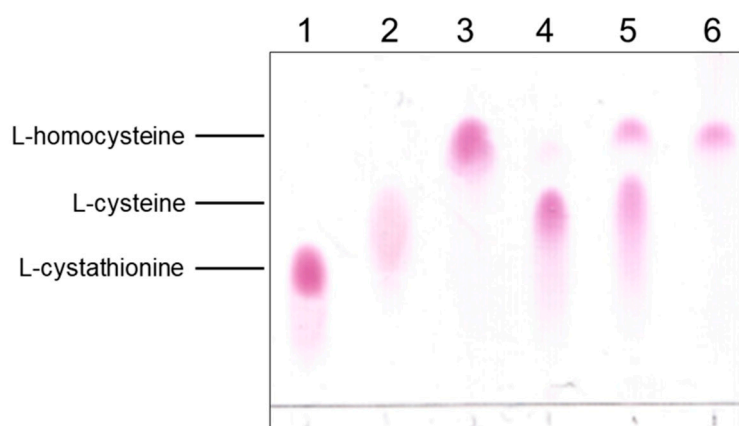


Figure S4. TLC analysis of the amino acid products of L-cystathionine hydrolysis by wt and N360S TgCGL variants. Reaction products and amino acid standards were separated by TLC and derivatized with ninhydrin. Spotting volume is 1 μ L. Lane 1–3, amino acids standards. Lane 1: 1 mg/mL L-cystathionine; lane 2: 1 mg/mL L-cysteine; lane 3: 1 mg/mL L-homocysteine; lane 4: 5 mM L-cystathionine + 100 μ M wt TgCGL; lane 5: 5 mM L-cystathionine + 100 μ M N360S TgCGL; lane 6: 5 mM L-cystathionine + 10 μ M cystathionine β -lyase from *Corynebacterium diphtheriae* which catalyzes the β -elimination of L-cystathionine to generate ammonia, pyruvate, and homocysteine [34].

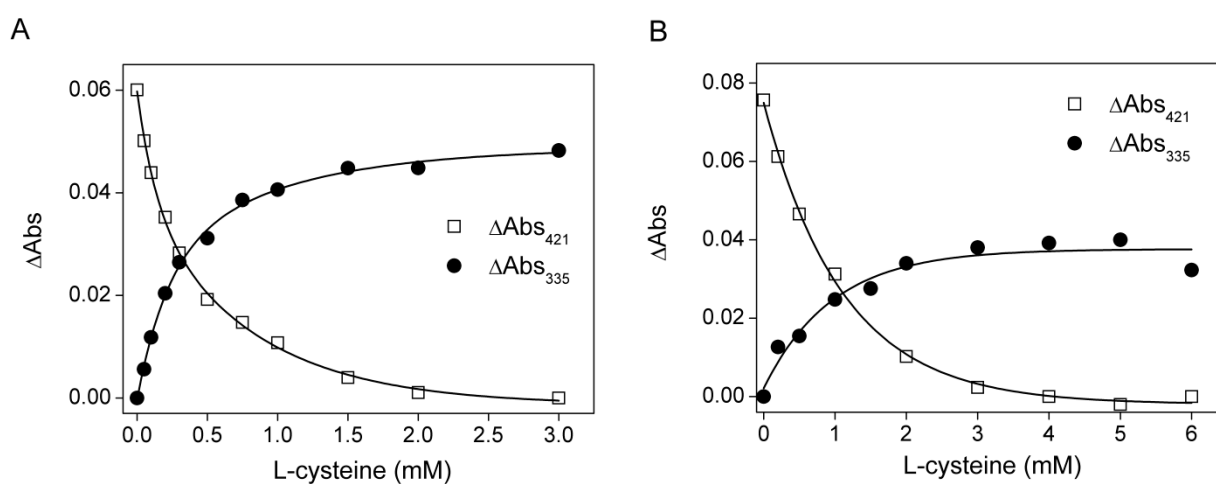


Figure S5. Spectral characterization of the interaction between TgCGL N360S and S77E variants and L-cysteine. Absorbance changes of N360S (A) and S77E (B) variants at 335 (solid circle) and 421 nm (open square) plotted against L-cysteine concentration. The continuous lines represent the theoretical curves according to Eqn. (3): 335 nm (K_{app} N360S = 335 ± 15 μ M; K_{app} S77E = 677 ± 180 μ M), and 421 nm (K_{app} N360S = 331 ± 20 μ M; K_{app} S77E = 874 ± 87 μ M).

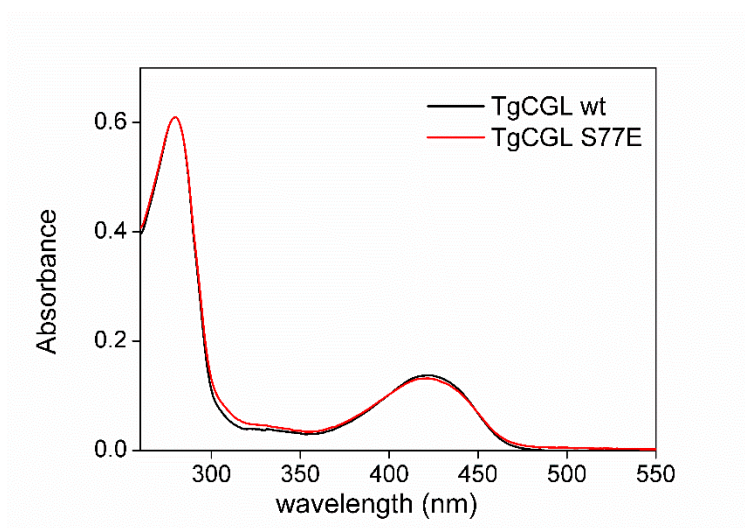


Figure S6. UV–visible absorption spectra of TgCGL wt (black line) and S77E variant (red line).

Table S1. List of mutagenic primers used to generate TgCGL active site variants. PF = Primer Forward, PR = Primer Reverse.

Mutation	Type of Primer	Primer Sequence
S77E	PF	GAGCAAAGGGTTCGAATATTCGCGAACTAGTAACCCG
	PR	CGGGTTACTAGTTCGCGAATATTCGAACCCTTGCTC
S77A	PF	CTATTTGAGCAAAGGGTTCGCATATTCGCGAACTAGTAAC
	PR	GTTACTAGTTCGCGAATATGCGAACCCTTGCTCAAATAG
N360S	PF	GGTCTTTGTGTGAAAGCCTGGGAGCGTGCG
	PR	CGCACGCTCCCAGGCTTTCACACAAAGACC