

SUPPLEMENTARY INFORMATION

Cystathionine β -synthase is involved in cysteine biosynthesis and H₂S generation in *Toxoplasma gondii*

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Table S1. Steady-state kinetic parameters of TgCBS for canonical reactions in the presence of SAM^a.

	- SAM	+ SAM 0.5 mM
L-Ser + L-Hcys → L-Cth + H₂O		
k_{cat} (s ⁻¹)	6.3 ± 0.4	5.9 ± 0.2
$K_{\text{m}}^{\text{L-Ser}}$ (mM)	0.42 ± 0.04	0.47 ± 0.03
$K_{\text{m}}^{\text{L-Hcys}}$ (mM)	0.23 ± 0.03	0.28 ± 0.04
$K_{\text{i}}^{\text{L-Hcys}}$ (mM)	1.0 ± 0.1	1.1 ± 0.2
L-OAS + L-Hcys → L-Cth + H₂O		
k_{cat} (s ⁻¹)	5.5 ± 0.1	5.7 ± 0.3
$K_{\text{m}}^{\text{L-OAS}}$ (mM)	1.3 ± 0.2	1.6 ± 0.2
$K_{\text{m}}^{\text{L-Hcys}}$ (mM)	0.20 ± 0.05	0.24 ± 0.04
$K_{\text{i}}^{\text{L-Hcys}}$ (mM)	1.4 ± 0.2	1.7 ± 0.3

^a Reactions were carried out in MBP buffer pH 9 containing 0.2 mM NADH, 2 μ M LDH, 1.5 μ M CBL, and 0.1-10 mM L-Ser (or 1-100 mM L-OAS), 0.1-10 mM L-Hcys and 0.2-2 μ M TgCBS at 37°C. Data were fit to Eq. (1). Data are mean ± s.e.m.

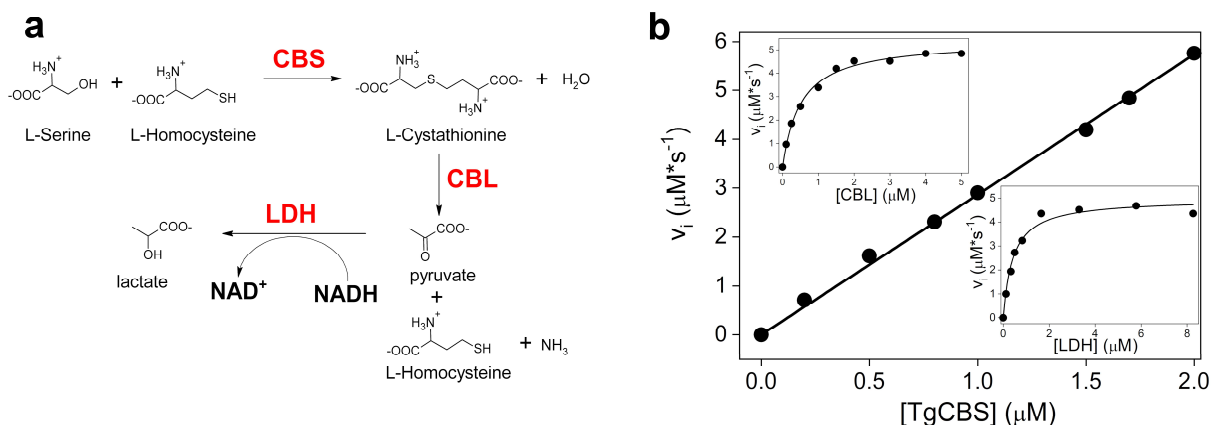


Figure S1. Coupled-coupled CBS assay. (a) Scheme of CBL/LDH coupled-coupled assay for CBS. CBS catalyzes condensation of L-Ser with L-Hcys to form L-Cth which is then converted to pyruvate, L-Hcys, and ammonia by CBL. Next, the conversion of pyruvate to lactate by LDH is accompanied by NADH oxidation, which is followed spectrophotometrically at 340 nm. (b) Dependence of the rate of NADH oxidation in the CBL/LDH assay as a function of TgCBS, CBL, and LDH concentration. Reactions were carried out in MBP pH 9 containing 0.2 mM NADH, 20 μ M PLP, 1 mM L-Ser, 0.8 mM L-Hcys, and: *main panel*, 0.2-2 μ M TgCBS, 1.5 μ M CBL and 2 μ M LDH; *upper inset*, 1.5 μ M TgCBS, 0.1-5 μ M CBL and 2 μ M LDH; *lower inset*, 1.5 μ M TgCBS, 1.5 μ M CBL and 0.1-8.2 μ M LDH.

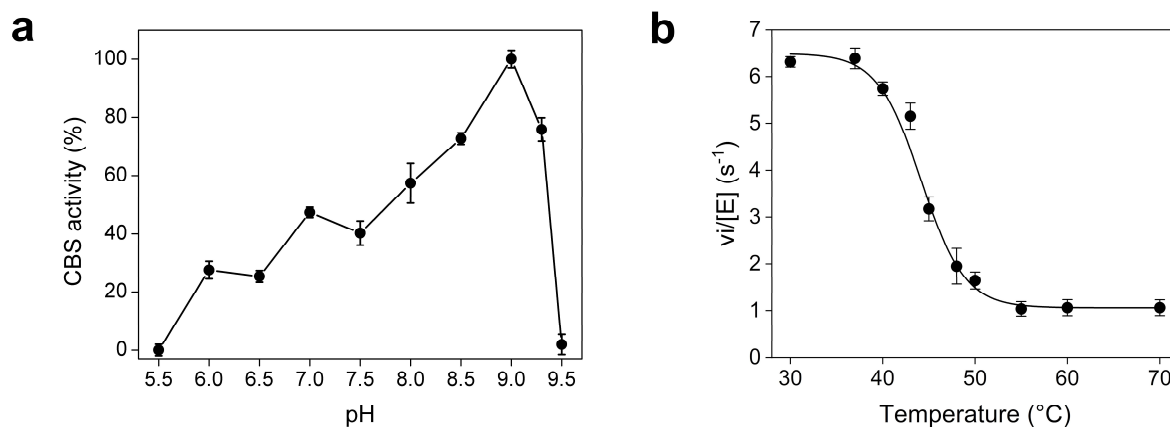


Figure S2. Effect of pH and temperature on CBS canonical activity of TgCBS. (a) pH-dependent activity profile for TgCBS performed at constant substrate concentrations (10 mM L-Ser and 0.8 mM L-Hcys) in the pH range of 5.5-9.5 at 37 $^{\circ}$ C. (b) Effect of thermal pre-treatment of TgCBS (10 min at temperatures between 30 $^{\circ}$ C and 70 $^{\circ}$ C) on its steady-state initial velocity at saturating concentration of L-Ser and L-Hcys in the canonical reaction. Each data set is relative to four replicas and error bars represent s.e.m.