SUPPORTING INFORMATION

SUPPORTING METHODS

S1. Determination of cystathionine γ- and β-lyase activities of XometC

Cystathionine is degraded to cysteine and to homocysteine by γ- and β-elimination activities, respectively. Both cysteine and homocysteine were detected in the enzyme reaction with purified XometC through a method that measured DTNB-derivatized (*vide infra*) products (Bruinenberg *et al.*, 1997). The separation of the DTNB-derivatized products was conducted by reverse phase HPLC using an Agilent 1260 infinity liquid chromatography system with a TSK-GEL® ODS-80Ts 5-μm C_{18} Column (4.6 mm × 250 mm) (TOSOH). HPLC solvent A consisted of water and 0.2%(v/v) acetic acid, whereas solvent B consisted of water, 50%(v/v) acetonitrile, and 0.2%(v/v) acetic acid. The gradient program used 100% solvent A for 5 min after sample injection, a linear gradient of 0–100% solvent B over 35 min, and an isocratic step in 100% solvent B for 10 min and solvent A for 7 min. The flow rate was 0.8 $ml.min^{-1}$.

S2. Lyase activities of XometC for diverse substrates including L-serine dehydratase activity

The lyase activity including L-serine dehydratase activity was measured for diverse substrates. The lyase activity for L-cystathionine was determined by measuring the formation of free thiol groups in products (Uren, 1987). The lyase activity for L-o-succinyl homoserine, homoserine, and L-serine was measured by the production of keto acids (Esaki & Soda, 1987). The lyase activity for L-serine cleaves off the OH group from the Cβ carbon in serine, which is also called as L-serine dehydratase activity. The assay for the formation of thiol groups was performed in reaction buffer (100 μl) containing 0.1 *M* Tris pH 8.0, 0.2 m*M* 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), 0.01 m*M* PLP, various concentrations of substrates, and XometC (1 ug), and the reactions were conducted at 30°C

for 30 min. The quantity of thiol groups was measured by the absorbance at 412 nm. L-Cysteine was used as a standard. The assays for the production of keto acids were conducted in a solution (200 µl) containing 0.1 *M* Tris pH 8.0, 0.01 m*M* PLP, various concentrations of substrate, and XometC (1 ug). For L-serine substrate, 10 ug of XometC was used. The mixtures were incubated for 30 min at 30°C, and the reaction was stopped by adding 50% trichloroacetic acid (32.5 µl). The precipitated protein was removed by centrifugation. The supernatant (100 µl) was mixed with 0.5 *M* sodium acetate buffer (200 µl, pH 5.0) and 0.1% 3-methyl-2-benzothiazolinone hydrazone hydrochloride (80 µl) and incubated at 50°C for 30 min. The mixture was then cooled to room temperature, and the absorbance at A_{320} was measured. Pyruvic acid and 2-α-butyric acid were used as standards. Enzyme kinetic parameters were calculated using the assays indicated above and a Lineweaver-Burk plot. All measurements were performed in triplicate.

S3. UV-Vis spectroscopy study

UV-visible spectra were measured at room temperature with the XometC protein (6 $mg.ml^{-1}$) using a SpectraMax® 190 spectrophotometer (Molecular Devices, USA).

Supporting References

Bruinenberg, P. G., De Roo, G. & Limsowtin, G. (1997). *Appl Environ Microbiol* **63**, 561-566.

Esaki, N. & Soda, K. (1987). *Methods Enzymol* **143**, 459-465.

Uren, J. R. (1987). *Methods Enzymol* **143**, 483-486.

Supporting Table S1. Kinetic parameters of lyase activity of Xomet for substrates

Substrates	Kinetic parameters		
	*K*m (mM)	*kcat* (min^{-1})	*kcat*/*K*m (min^{-1}. mM^{-1})
L-cystathionine	0.27±0.08	73.0±0.7	270
L-o-succinyl homoserine	1.30±0.06	102.0±0.8	79
L-homoserine	6.80±0.28	84.3±1.0	12
L-serine	5.10±0.38	8.5±0.4	2

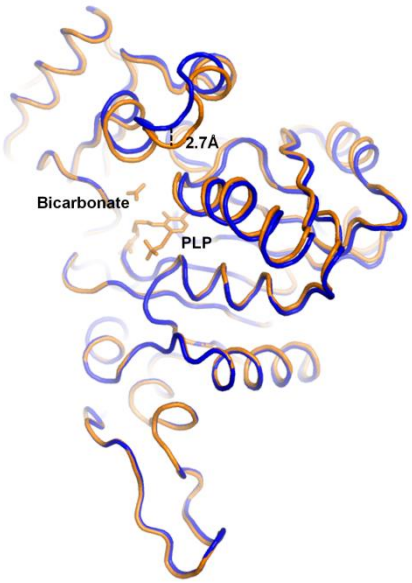
Supporting Table S2. Dihedral angles between pyridine ring and Schiff base linkage of PLP and of the terminal four atoms of Lys210 side-chain in different intermediates

Intermediate structure	Average dihedral angle (°)		Dihedral angle (°) of each monomer			
	PLP (pyridine ring and Schiff base linkage)	Lys210 sidechain (terminal four atoms)	Crystal name	Monomer ID	PLP (pyridine ring and Schiff base linkage)	Lys210 sidechain (terminal four atoms)
Native	34	44	Native	A	40	10
				B	24	77
				C	38	63
				D	33	25
PGD	52	5	PGD_EAA_IAI	D	52	5
EAS	25	n/a	EAS_EAA	B	34	n/a
				C	18	n/a
				D	24	n/a
EAA	22	n/a	EAS_EAA	A	22	n/a
			PGD_EAA_IAI	A	21	n/a
IAI	46	18	PGD_EAA_IAI	B	43	16
			PGD_EAA_IAI	C	48	20

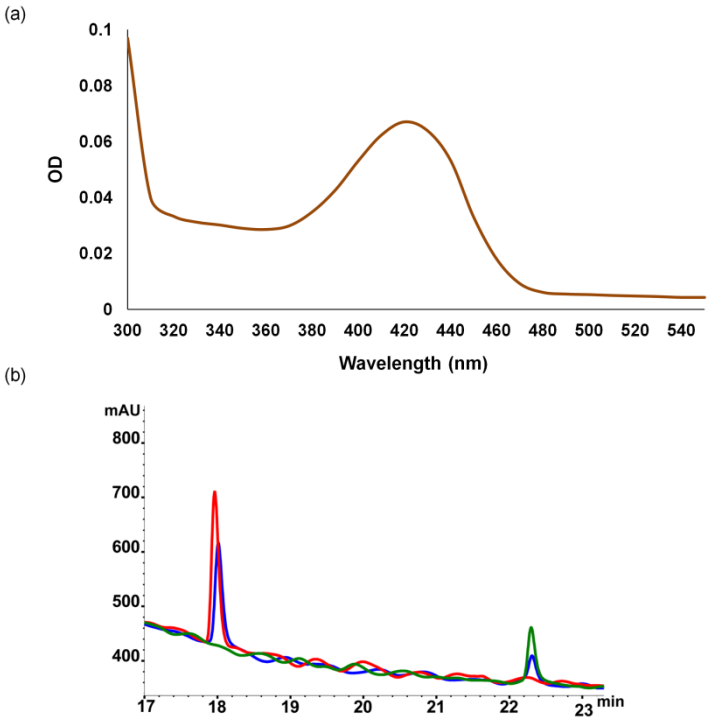
Supporting Table S3. RMSD between 12 protomers in XometC crystals

Crystal	Moving molecule A	Fixed molecule B	Aligned length (a.a)	RMSD
Native (AIXZ)	A (381)	Native (monomer B, 381)	381	0.601
	B (381)		381	0.000
	C (378)		378	0.312
	D (378)		378	0.320
EAS_EAA (4IY7)	A (EAA, 383)		381	0.611
	B (EAS, 383)		381	0.612
	C (EAS, 383)		381	0.574
	D (EAS, 385)		381	0.606
PGD_EAA_IAI (4IYO)	A (EAA, 383)		381	0.602
	B (IAI, 382)		381	0.588
	C (IAI, 382)		381	0.513
	D (PGD, 386)		381	0.591
Crystal	Moving molecule A	Fixed molecule B	Aligned length (a.a)	RMSD
Native (AIXZ)	A (381)	EAS_EAA (monomer A, EAA)	381	0.219
	B (381)		381	0.611
	C (378)		378	0.528
	D (378)		378	0.541
EAS_EAA (4IY7)	A (EAA, 383)		381	0.000
	B (EAS, 383)		381	0.168
	C (EAS, 383)		381	0.163
	D (EAS, 385)		381	0.138
PGD_EAA_IAI (4IYO)	A (EAA, 383)		381	0.066
	B (IAI, 382)		381	0.180
	C (IAI, 382)		381	0.212
	D (PGD, 386)		381	0.157

Numbers in parentheses show the number of amino acids.

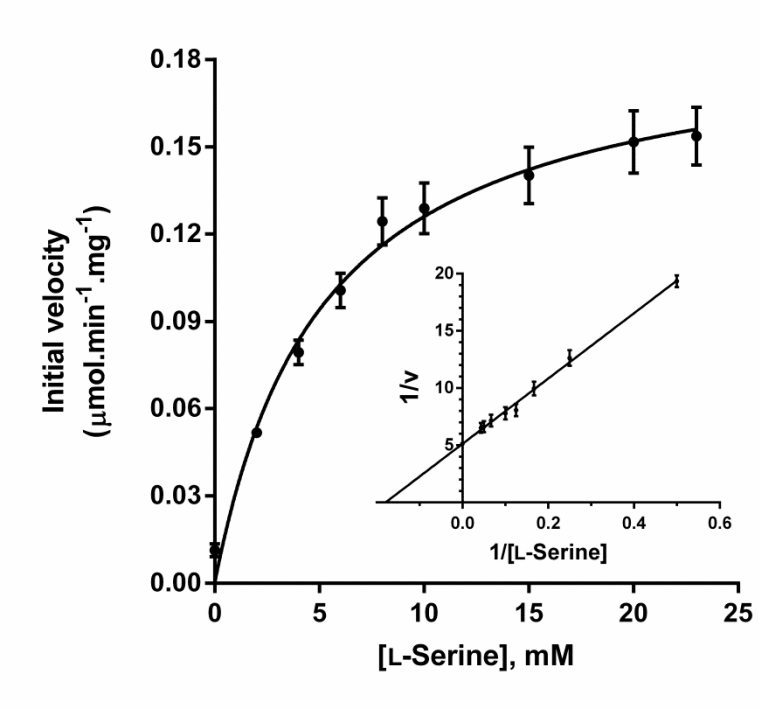


Supporting Figure S1. Superimposed structures of bicarbonate-bound (orange) and unliganded (blue) protomers in native XometC crystals. Bound bicarbonate and PLP-linked lysine residue are shown as stick presentation.

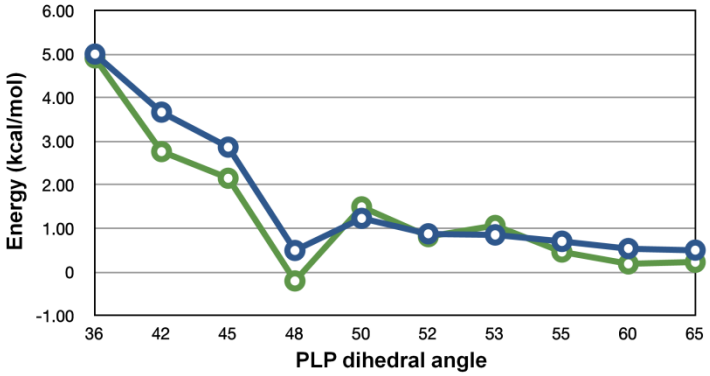


Supporting Figure S2. UV-Vis absorbance spectrum of native XometC and the measurement of CGL and CBL activity for CTT. (**a**) The absorption spectrum of native XometC showed a major peak centered at 423 nm of the internal aldimine structure. Absorption spectra were measured in the solution containing 6 mg.ml^{-1} of XometC, 25 m*M* Tris pH 7.5, and 3 m*M* β-mercaptoethanol. (**b**)

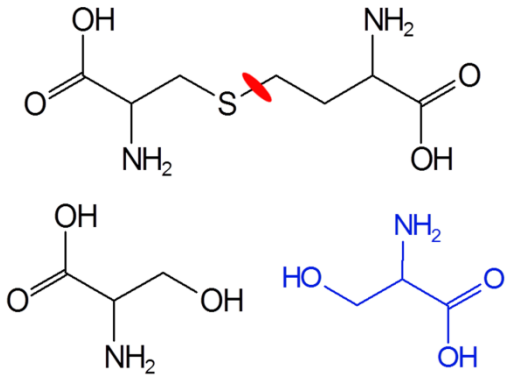
Separation of DTNB-derivatives of reaction products of CTT by XometC in reverse-phase HPLC. Red and green lines show the standard equal molar amounts of Cys-TNB and HomoCys-TNB, respectively. Blue line shows DTNB-derivatives of reaction products of CTT. The molar ratio of Cys-TNB (γ-elimination reaction product) to HomoCys-TNB (β-elimination reaction product) is 1.4.



Supporting Figure S3. The initial velocity of L-serine dehydratase activity of XometC for L-serine substrate.



Supporting Figure S4. The calculated activation energy (Ea) and reaction energy (Er) from IA to GD at various fixed dihedral angles. Ea is shown as blue line and Er is shown as green line.



Supporting Figure S5. The chemical structure of cystathionine which is a pseudo-symmetric substrate is shown with a red oval at its pseudo-symmetric center. Two serine molecules are also shown that mimic the half molecule of the pseudo-symmetric cystathionine.