Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Material

The Unique Functional Role of the C-H···S Hydrogen Bond in the Substrate Specificity and Enzyme Catalysis of Type 1 Methionine Aminopeptidase

Ravikumar Reddi[1], Kiran Kumar Singarapu[2], Debnath Pal[3]* and Anthony Addlagatta[1]*

[1]*Centre for Chemical Biology*
[2]*Centre for NMR and Structural Chemistry*
CSIR-Indian Institute of Chemical Technology
Hyderabad 500 007, Telangana
INDIA
[3]*Department of Computational and Data Sciences*
Indian Institute of Science
Bangalore 560 012, Karnataka
INDIA
**Contact: dpal@cds.iisc.ac.in, anthony@iict.res.in*

Table S1: Crystallographic Information Table

Cell parameters	C105S
Space group	$P2_1$
a (Å)	49.22
b (Å)	48.66
c (Å)	55.72
β (deg)	95.16
Data collection	
Resolution range (Å)	28.52 - 2.00 (2.11-
(Highest res. shell)	2.00)
Collected reflections	
Total	85,375
Unique (High. Res.)	14,906 (798)
Completeness (%)	87.8 (90.8)
$I/\sigma(I)$	5.6 (2.1)
*R*sym (%)	12.7 (39.9)
Refinement statistics	
R (*R*-free) (%)	21.42 (27.37)
Δbonds (Å)	0.021
Δangles (deg)	1.924
PDB code	3ROR

Table S2: **Mean electrostatic potential values (kT/e) obtained at various residue locations**

	***Ec*MetAP1a**				***Mt*MetAP1c**	
PDB	**1C23**	**1C23**	**1C27**	**PDB**	**1YJ3**	**1YJ3**
Residue	**Ligand Present?**			**Residue**	**Ligand Present?**	
	No	Yes	Yes		No	Yes
CYS59	-17.29	-22.62	-30.74	Thr94	-31.48	-54.49
TYR62	-2.78	-7.66	-2.14	TYR97	-28	-33.97
TYR65	-14.38	-8.23	-17.5	Phe100	-23.93	-26.69
CYS70	-38.15	-57.81	-53.1	CYS105	-49.92	-81.65
HIS79	2.2	-3.55	-4.07	HIS114	-15.29	-25.92
F177	-4.02	-15.41	-17.88	F211	-35.39	-68.83
W221	-14.5	-15.71	-14.17	W255	-23.41	-23.59
Co401	-176.79	-262.51	-210.39	Co401	-213.9	-283.66
Co402	-204.3	-286.6	-270.96	Co402	-229.35	-328.91
MPH300	-	-91.99	-59.35*	Met500	-	-148.07

*NLP300 (noleucine phosphonate) is the ligand in PDB:1C27
MPH = Methionine aminophosphonate

Table S3: List of primers used for site-directed mutagenesis

Mutant	Primers
C105S fwd	5’AAG TCG TGC TCC ACG TCC CTC AAC GAG GTC ATC TGC3’
C105S rev	5’GAG GGA CGT GGA GCA CGA CTT CGG GAA TCC CTT GTA3’
C105T fwd	5’AAG TCG TGC ACC ACG TCC CTC AAC GAG GTC ATC TGC 3’
C105T rev	5’GAG GGA CGT GGT GCA CGA CTT CGG GAA TCC CTT GTA3’
C105V fwd	5’AAG TCG TGC GTC ACG TCC CTC AAC GAG GTC ATC TGC 3’
C105V rev	5’GAG GGA CGT GAC GCA CGA CTT CGG GAA TCC CTT GTA 3’
C105L fwd	5’AAG TCG TGC CTC ACG TCC CTC AAC GAG GTC ATC TGC 3’
C105L rev	5’GAG GGA CGT GAG GCA CGA CTT CGG GAA TCC CTT GTA 3’

Supplementary Figures

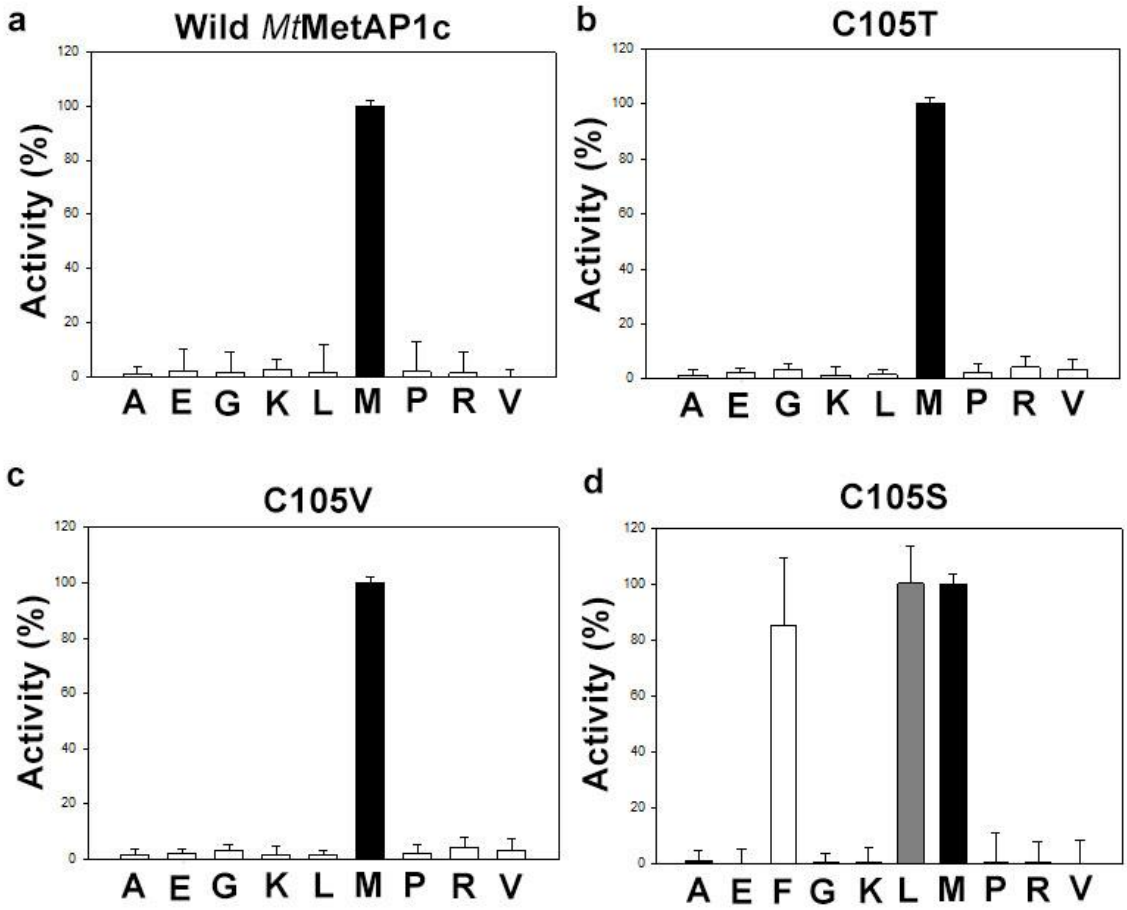

Figure S1. Substrate specificity of *Mt*MetAP1c and its C105 mutants expressed relative to N-terminal Methionine cleavage activity. **(a).** Wild *Mt*MetAp1c is showing strict MetAP activity. **(b).** C105T. **(c).** C105V also showing strict MetAP activity. (**d).** C105S is showing relaxed substrate specificity, cleaving methionine (M), in addition to leucine (L) and phenylalanine (F). Sharp reduction in catalytic efficiency of the mutants can be seen from Table 1 in the main text.

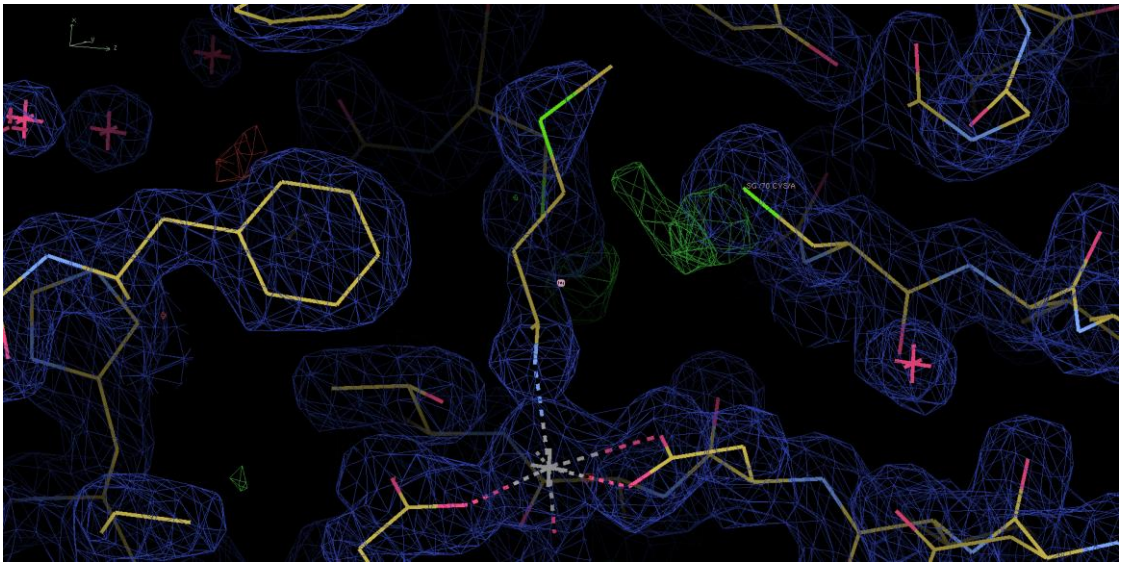

Figure S2. Figure showing the electron density map around the Cys70 residue and the Met ligand in *Ec*MetAP1c. The carbon atoms are shown in yellow, nitrogen in blue, oxygen in pink and sulfur in green. Metal ion coordinate bonds are shown in ash blue dashed lines. The additional density at 3σ level is shown in green and is in close proximity to the Cys thiol group.

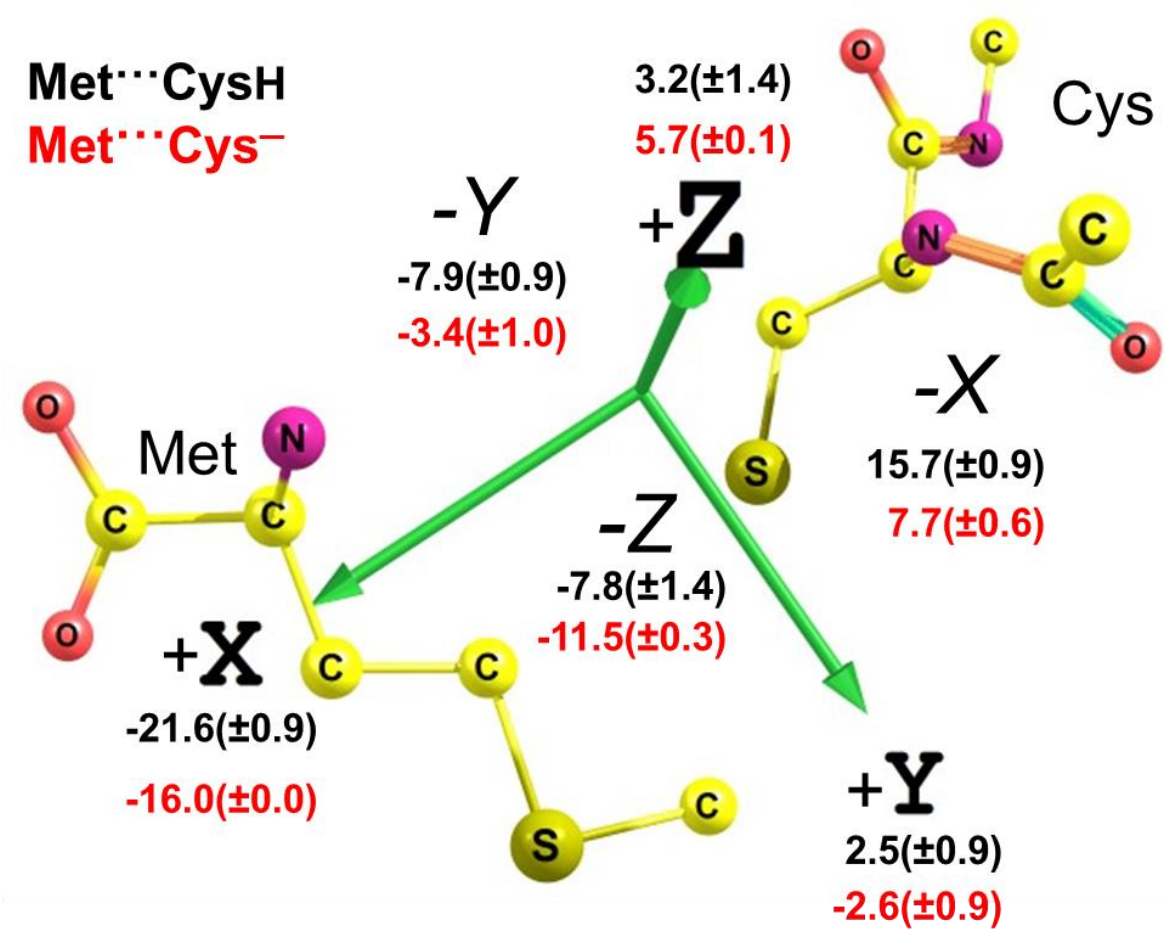

Figure S3: Figure showing the change of the self consistent Field (SCF) energy (kcal/mol) of the model Met-CysH and Met-Cys$^-$ system when an electric field of 0.006 AU is introduced in the six ±X, ±Y, ±Z directions of the coordinate axes against no electric field. The base coordinates are taken from the crystal structure of *Mt*MetAP1c (PDB: 1YJ3) and suitably end capped before calculations.