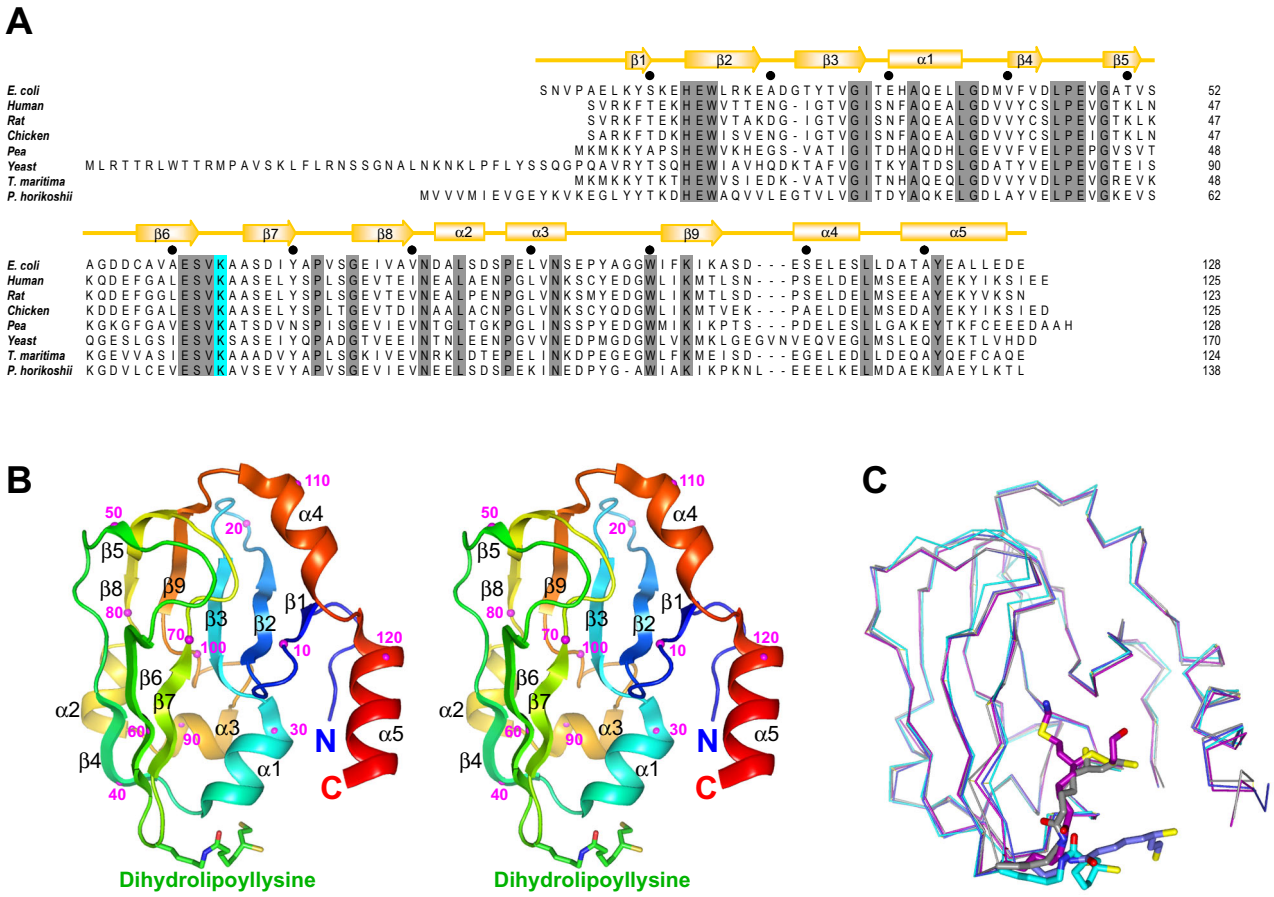
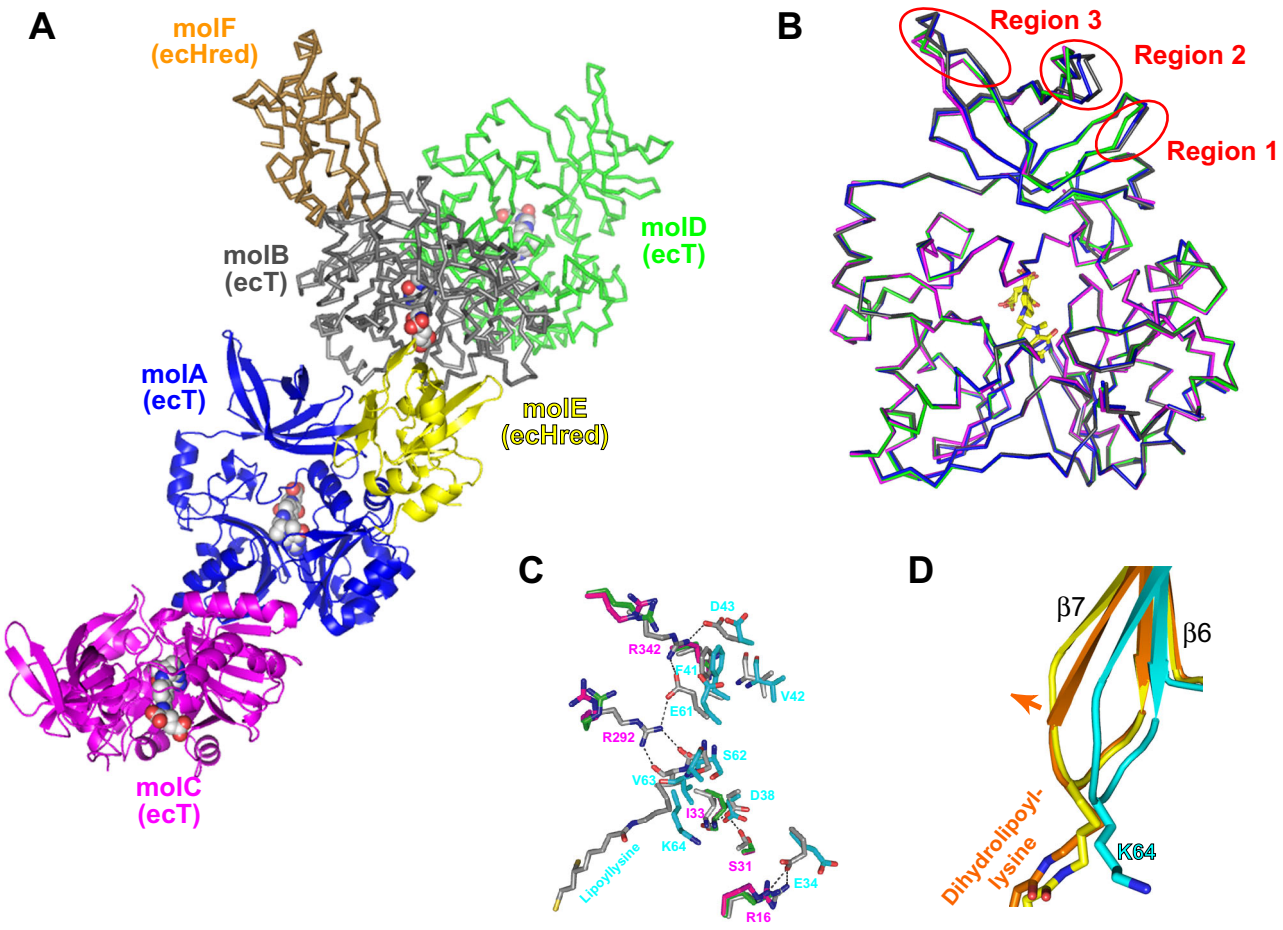


Supplemental Figure S1. Structure of ecHred.

A, Sequence alignment of H-proteins from various species. The sequence of ecH (*E. coli)* was aligned with H-proteins from *Homo sapiens* (human), *Rattus norvegicus* (rat), *Gallus gallus* (chicken), *Pisum sativum* (pea), *Saccharomyces cerevisiae* (yeast), *Thermotoga maritima* MSB8 (*T. maritima*), and *Pyrococcus horikoshii* OT3 (*P. horikoshii)* using ClustalW2. The yeast sequence includes the mitochondrial targeting presequence. Identical residues and lysine residues to which lypoic acid is covarently attached are shaded in gray and cyan, respectively. Secondary structural elements of ecH are indicated above the alignment and every ten residues are marked with filled circles.

B, Stereoview of a cartoon representation of the structure of ecHred. The a-helices, b-strands, and every ten residues are numberd and colored from blue to red from the N- to C-termini. The dihydrolipoyllysine is shown in stick.

C, Comparison of the structure of ecHred and peaH. Backbone structures of ecHred (cyan), peaH bearing aminomethyllypoillysine (purple) (PDB code 1HTP), and two molecules of peaH bearing dihydrolipoyllysine in an asymmetric unit (gray and light blue) (PDB code 1DXM) are superimposed.



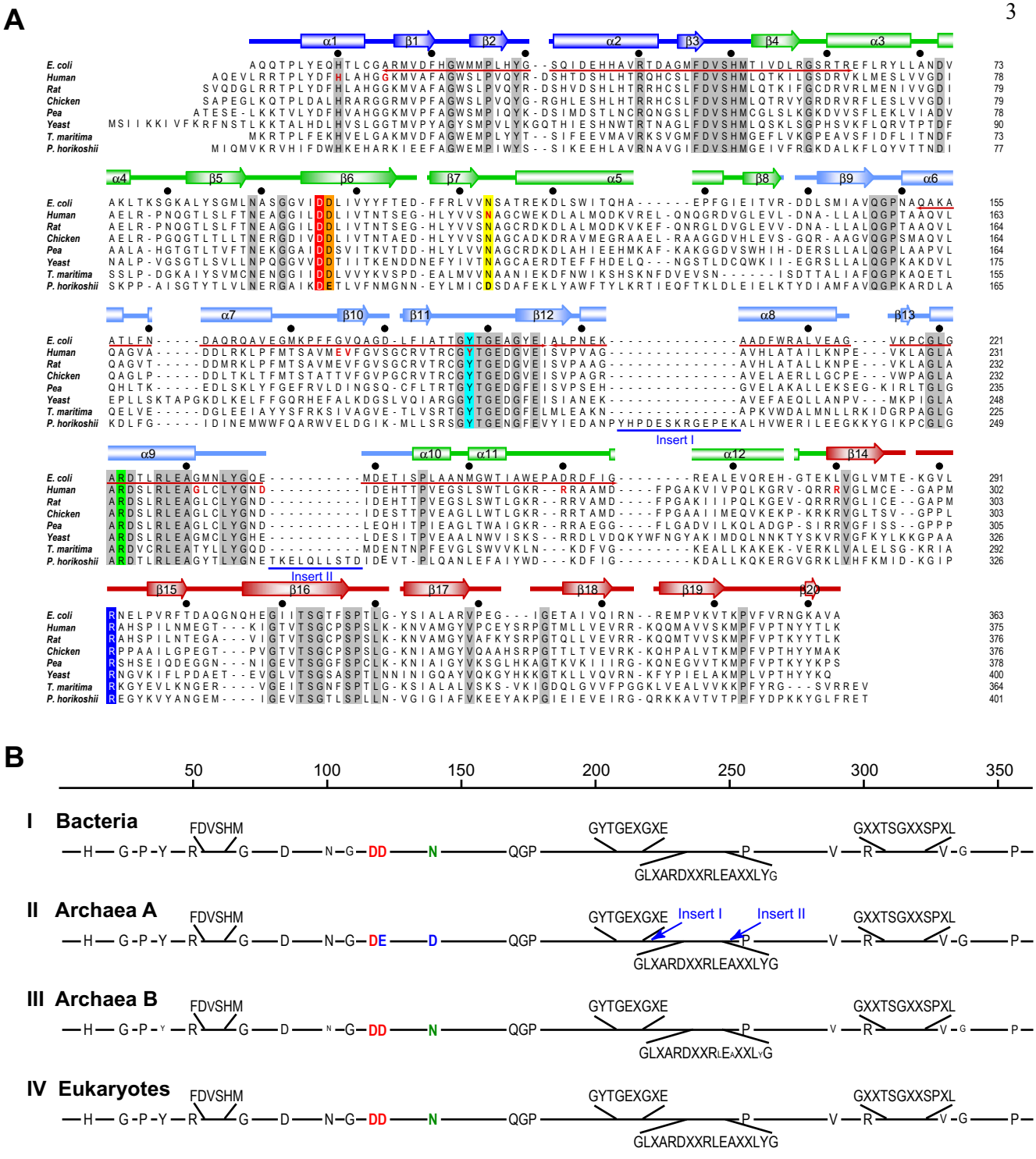
Supplemental Figure S2. Structure of the ecT-ecHred complex.

A, Cartoon and ribbon representations of the four ecT molecules (molA, molB, molC, and molD) and two ecHred molecules (molE and molF) in an asymmetric uint. A heterotrimer comprising molA (blue), molC (magenta), and molE (yellow) is in cartoon representation, and the other one comprising molB (gray), molD (green), and molF(orange) is in ribbon form. Atoms of the bound 5-CH_3-THF represented in CPK are colored in gray (carbon), blue (nitrogen), and red (oxygen).

B, Ribbon representation of the backbone superimposition of four ecT molecules in an asymmetric unit. Backbone colors are as in (A) and 5-CH_3-THF is shown in stick colored in yellow. Pairwise structural comparisons gave r.m.s.d. ranging from 0.11 Å to 0.32 Å, with an average value of 0.23 Å. Three regions showing conformational changes upon ecHred binding are circled.

C, Superimposition of MolBF, MolC, MolD, and ecHred monomer. Residues from ecHred (labeled in cyan) and ecT (labeled in magenta) contributing to the heterodimer interface of molBF are compared with those in molC, molD and ecHred monomer, representing in stick with carbon atoms colored in gray, magenta, green, and cyan, respectively. Hydrogen bonds are depicted as broken lines.

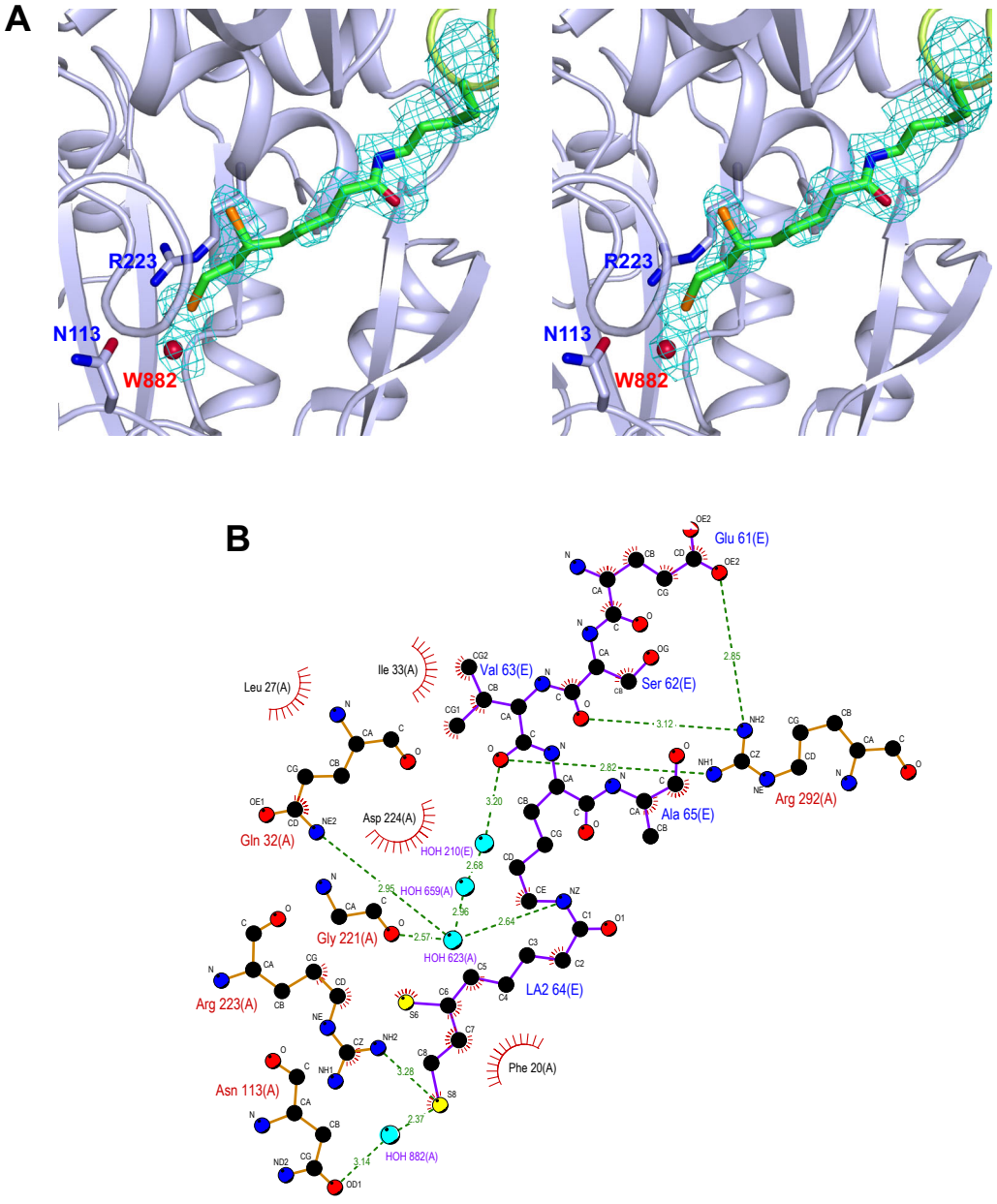
D, Close-up view of the hairpin b-motif region of superimposed molE, molF, and ecHred monomer. The orange arrow indicates the conformational rearrengement of β7 upon binding to ecT.



Supplemental Figure S3. Sequence alignment of T-proteins.

A, Sequence alignment using ClustalW2 of ecT (*E. coli*) with T-proteins from *Homo sapiens* (human), *Rattus norvegicus* (rat), *Gallus gallus* (chicken), *Pisum sativum* (pea), *Saccharomyces cerevisiae* (yeast), *Thermotoga maritima* MSB8 (*T. maritima*), and *Pyrococcus horikoshii* OT3 (*P. horikoshii*). The yeast sequence includes the mitochondrial targeting presequence. Secondary structural elements of ecT are indicated above the alignment, and domain 1, 2, and 3 are colored in blue/lightblue, green, and red, respectively. Identical residues are shaded and NKH-related residues in human T-protein are in red. Residues subjected to mutation analysis are shaded with colors. The regions used as the training datasets for the HMM profiles are underlined in red.

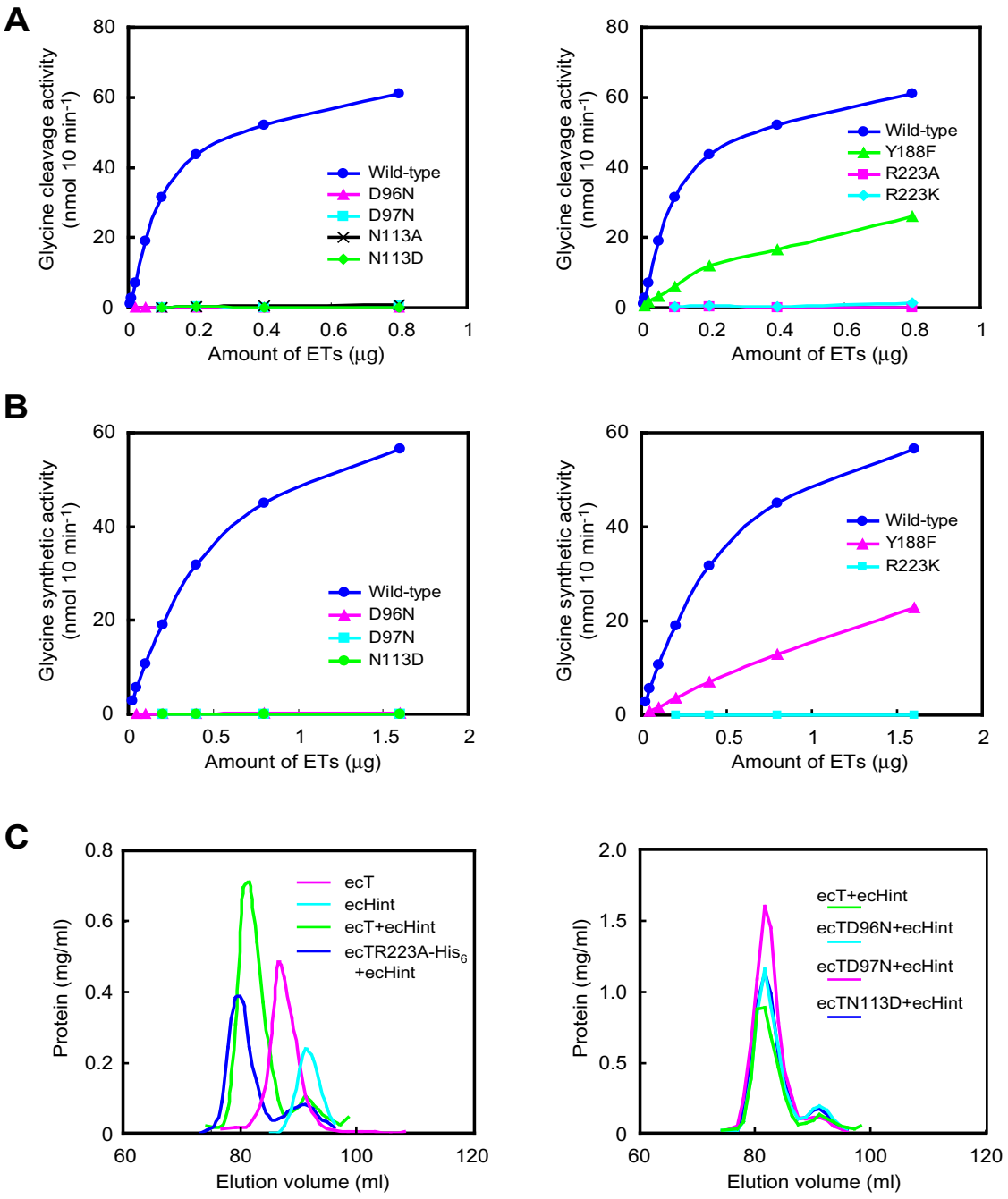
B, Sequence comparison of T-proteins from 136 organisms. T-protein sequences are classified into four groups: I, bacteria (97 sequences); II, archaea A (6); III, archaea B (6), and IV, eukaryotes (27). T-proteins belonging to Archaea A have extra sequences as shown in *P. horikoshii* T-protein (insert I and II). *T. maritima* T-protein belongs to archaea B. The top line shows the residue number of ecT. Identical or highly conserved residues are shown in different size caracters depending on diversity. The notable residues are shown in red, blue, and green.



Supplemental Figure S4. Structure of the dihydrolipoyllysine binding site in the molAE heterodimer.

A, Stereoview of omit F_o-F_c electron density (in cyan) for dihydrolipoyllysine contoured at 1.6 σ.

B, Schematic drawing of the interactions between dihydrolipoyllysine from ecH and surrounding residues in the active site. Atoms in dihydrolipoyllysine with short red lines are involved in hydrophobic interactions with the respective facing ecT residues also outlined with short red lines. Hydrogen bonds are represented by green broked lines with distances in Å. (A) and (E) show molA and molE, respectively

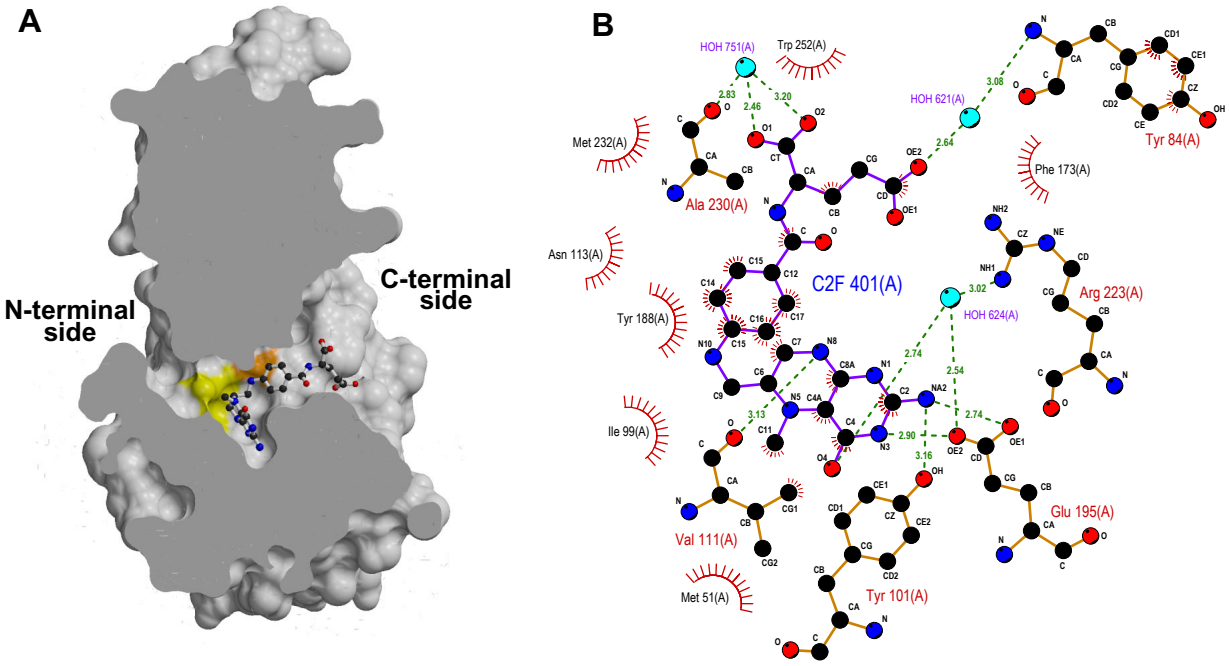


Supplemental Figure S5. Mutational analysis of ecT residues in the active site.

A, Glycine cleavage activity in the overall reaction in the presence of THF with saturating amounts of ecH, ecP, and L-protein. Limited amount of ecTs with C-terminal His_6-tag (0-0.8 μg) were employed.

B, Glycine synthesis activity in overall reaction in the presence of 5,10-CH_2-THF with saturated amounts of auxiliaries. ecTs with C-terminal His_6-tag (0-1.6 μg) are employed.

C, Heterodimer forming ability of ecTs with ecHint. ecTs with or without C-terminal His_6-tag were incubated with 1.2-1.5-fold molar excess of ecHint at 25 ℃ for 30 min, and subjected to gel-filtration column chromatography. All mutants formed heterodimers. Wild-type ecT with a C-terminal His_6-tag was eluted a slight earlier than that without a His_6-tag. This was also the case for the heterodimer comprising ecTR223A-His_6 and ecHint.



Supplemental Figure S6. Structure of the 5-CH_3-THF binding site in the molAE heterodimer structure.

A, Cross-section of the molecular surface of ecT with bound 5-CH_3-THF in stick form. The positions of Asp97 (orange) and Asn113 (yellow) of ecT are colored. Atom colors of the ligand are as in supplemental Fig. S2*A*.

B, Schematic drawing of the interactions between 5-CH_3-THF and residues in ecT active site. Atoms in the ligand with short red lines are involved in hydrophobic interactions with the respective facing ecT residues also outlined with short red lines. Hydrogen bonds are represented by green broked lines with distances in Å. (A) shows molA.

Supplemental Table S1. Data collection and refinement statistics

	ecHred	ecT–ecHred	ecT–ecHred–5-CH_3-THF	ecTD97N–ecHred
Data collection				
Space group	$P4_32_12$	$P1$	$P1$	$P1$
Cell dimensions				
a, *b*, *c* (Å)	60.3, 60.3, 68.6	54.0, 88.5, 97.2	54.0, 88.9, 98.0	54.1, 88.4, 97.9
α, β, γ (°)	90, 90, 90	91.5, 102.2, 89.7	91.5, 102.5, 89.6	91.6, 102.4, 89.6
Resolution (Å)	50.0-1.65 (1.67-1.65) [a]	50.0-1.98 (2.07-2.00)	30.0-1.99 (2.07-1.99)	50.0-1.95 (1.98-1.95)
R_{merge} [b]	5.5 (36.1)	5.9 (34.6)	9.3 (37.9)	7.1 (44.2)
$I / \sigma I$	79.8 (8.6)	15.3 (2.4)	9.78 (2.86)	20.9 (3.2)
Completeness (%)	100.0 (100.0)	98.0 (97.1)	85.1 (88.0)	98.3 (97.4)
Redundancy	14.1 (14.2)	2.1 (2.1)	3.1 (2.9)	4.0 (3.9)
Refinement				
Resolution (Å)	20-1.65	44.4-1.98	29.8-1.99	44.0-1.95
No. reflections	14,972	112,820	97,181	119,927
R_{work} [c] / R_{free} [d](%)	20.4 / 21.6	17.2 / 23.8	18.2 / 24.4	17.1 / 22.9
No. atoms				
Protein	968	13,251	13,258	119,927
Ligand/ion	0/2	0/0	137/5	0/0
Water	46	1362	1124	1495
B-factors (Å^2)				
Protein	24.6	27.1	23.9	24.6
Ligand/ion	/26.5		27.8/39.4	
Water	32.5	35.4	31.3	33.9
r.m.s. deviations				
Bond lengths (Å)	0.022	0.022	0.024	0.023
Bond angles (°)	1.991	1.799	1.971	1.867
Ramachandran plot (%)				
Favored	87.5	90.0	88.6	90.2
Additional allowed	11.6	9.2	10.4	9.1
Generously allowed	0.9	0.6	0.5	0.4
Disallowed	0	0.1	0.5	0.3

[a]Values in parentheses are for the highest-resolution shell.

[b]$R_{merge} = \Sigma\Sigma_j|\langle I(h)\rangle - I(h)_j| / \Sigma\Sigma_j|\langle I(h)\rangle$, where $\langle I(h)\rangle$ is the mean intensity of symmetry-equivalent reflections.

[c]$R_{work} = \Sigma |F_o - F_c| / \Sigma F_o$ for reflections of working set.

[d]$R_{free} = \Sigma |F_o - F_c| / \Sigma F_o$ for reflections of test set (5% of total unique reflections).

Supplemental Table S2. The list of gcvT sequences engaged as the training datasets for the HMM profile construction

KEGG GENES entry name	organism	NCBI Gene ID
ath:AT1G11860	*Arabidopsis thaliana*	837733
cko:CKO_04269	*Citrobacter koseri* ATCC BAA-895	5584765
cre:CHLREDRAFT_196242	*Chlamydomonas reinhardtii*	5718707
dda:Dd703_3311	*Dickeya dadantii*	8089258
ddi:DDB_0231218	*Dictyostelium discoideum*,	3386367
eco:b2905	*Escherichia coli* K-12 MG1655	947390
ent:Ent638_3324	*Enterobacter* sp. 638	5111838
hal:VNG1606G	*Halobacterium* sp. NRC-1	1448168
hma:rrnAC1500	*Haloarcula marismortui*	3127617
hsa:275	*Homo sapiens*	275
kpe:KPK_0759	*Klebsiella pneumoniae* 342	6939543
mmu:434437	*Mus musculus*	434437
nph:NP4774A	*Natronomonas pharaonis*	3702746
pho:PH1146	*Pyrococcus horikoshii*	1443465
plu:plu3598	*Photorhabdus luminescens*	2803610
rno:306586	*Rattus norvegicus*	306586
sbc:SbBS512_E3326	*Shigella boydii* CDC 3083-94	6272620
sce:YDR019C	*Saccharomyces cerevisiae*	851582)
see:SNSL254_A3290	*Salmonella enterica* subsp. enterica serovar Newport	6483955
sgl:SG2002	*Sodalis glossinidius*	3867083
tac:Ta0010m	*Thermoplasma acidophilum*	1456956
yen:YE3393	*Yersinia enterocolitica*	4713786