Interaction of D-cycloserine with a D-amino acid transaminase from *Haliscomenobacter hydrossis*

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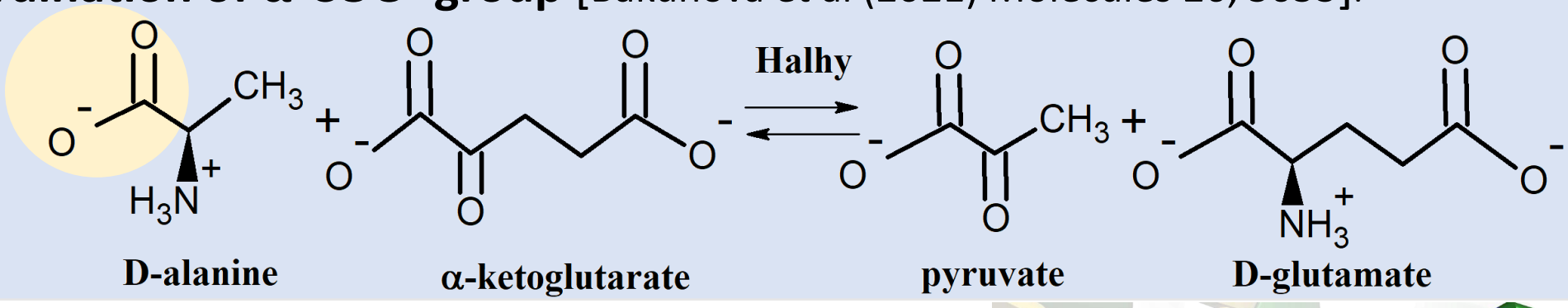
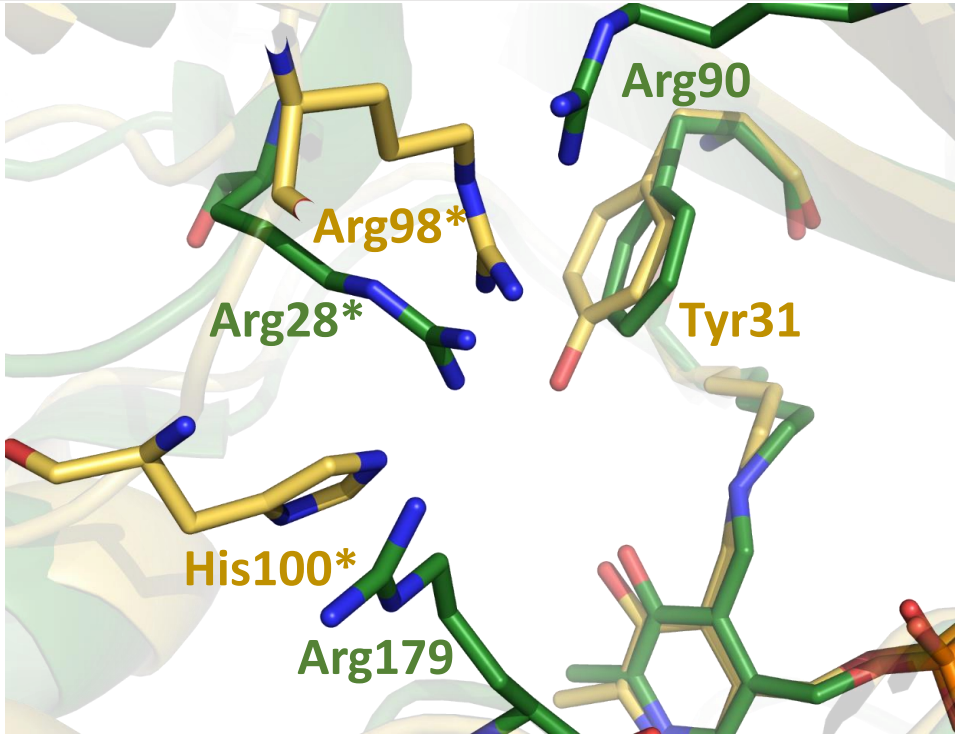
Pyridoxal-5'-phosphate (PLP)-dependent D-amino acid transaminase (DAAT) catalyses stereoselective reversible amination of α-ketoacids. We characterized DAAT from bacterium *Haliscomenobacter hydrossis* (Halhy) **with a new type of coordination of α-COO$^-$ group** [Bakunova et al (2021) Molecules 26, 5053].

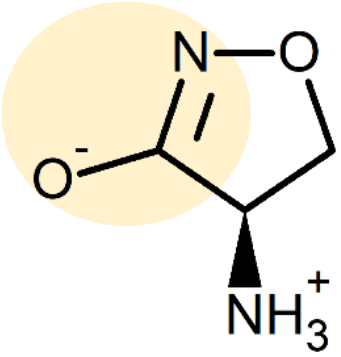


The superposition of the crystal structures of Halhy (green) and canonical DAAT from *Bacillus* sp. YM1 (yellow)



Halhy (PDB ID: 7P7X):
a new type of coordination of α-COO$^-$ group

Canonical DAAT from *Bacillus* sp. YM1 (PDB ID: 5DAA):
"carboxylate trap" Tyr … Arg x His for coordination of α-COO$^-$ group

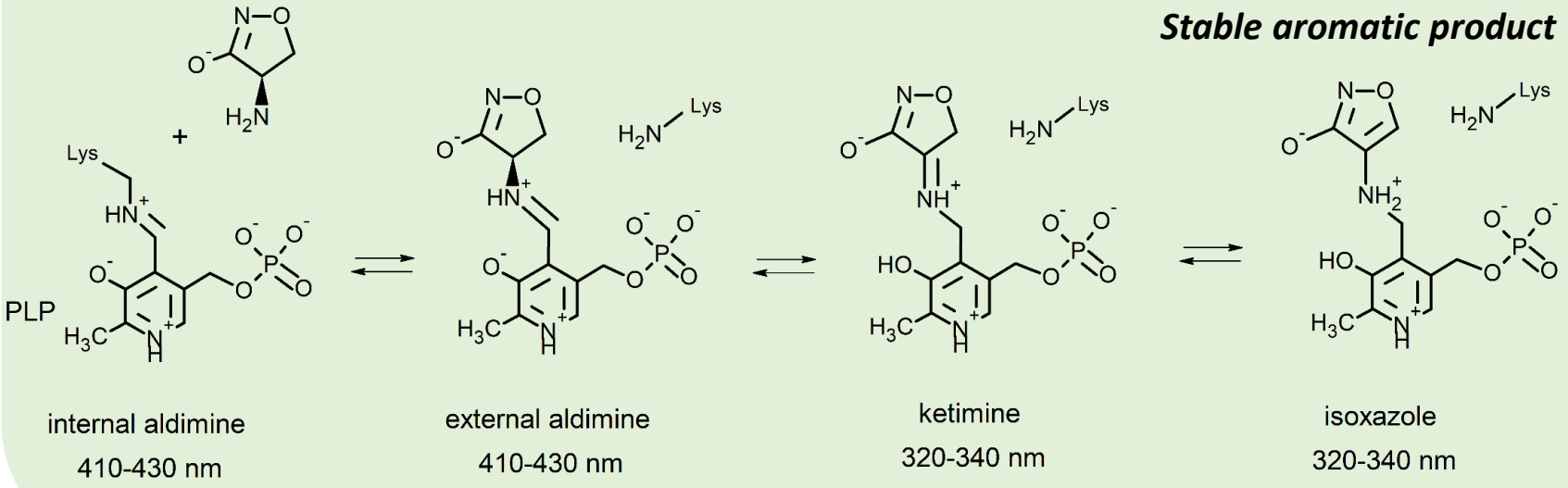


D-cycloserine (D-CS) has an analog of α-COO^- group

- D-CS is a strong inhibitor of Halhy with IC_{50} value of 3 µM

General mechanism of D-cycloserine inhibition of transaminase:

Canonical sequence of DAAT reaction steps

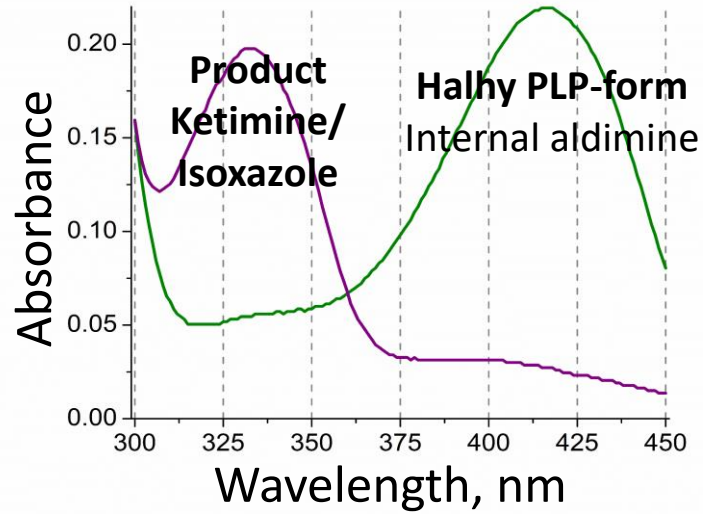


Stable aromatic product

The spectral changes after the half reaction Halhy PLP-form (green) with D-cycloserine

20 mkM Halhy PLP-form + 20 mkM D-cycloserine

In 50 mM K-phosphate buffer, pH 8.0, 40 °C

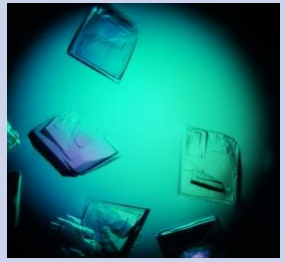


The complex with D-cycloserine was obtained by soaking crystals of Halhy PLP-form in inhibitor solution during 10 s.

Crystal growth conditions:
0.1 M sodium acetate, pH 4.8
+ 18% PEG 3350, at 4 °C

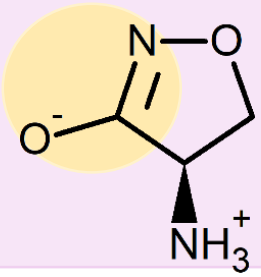
The X-ray diffraction data were collected at the BL41XU beamline of the SPring-8 synchrotron (Hyogo Prefecture, Japan)

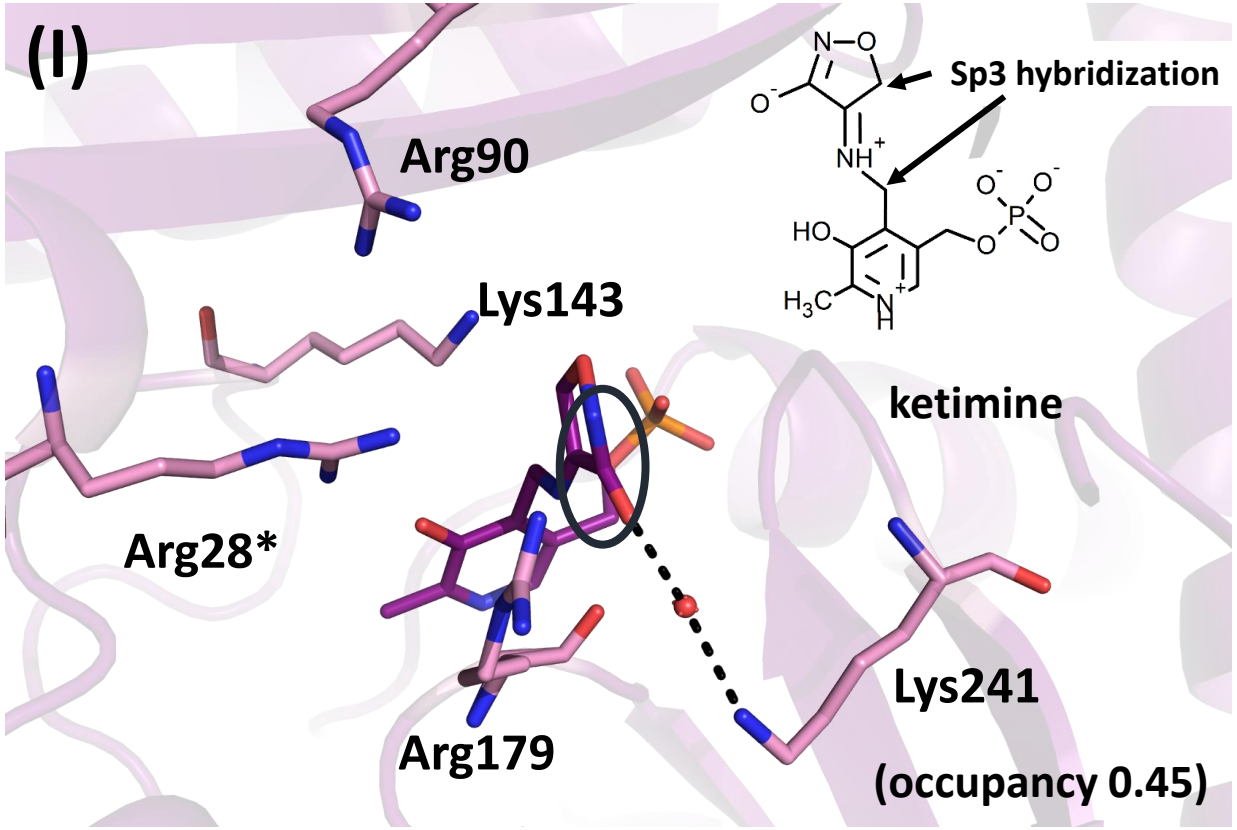
Resolution: 1.4 Å

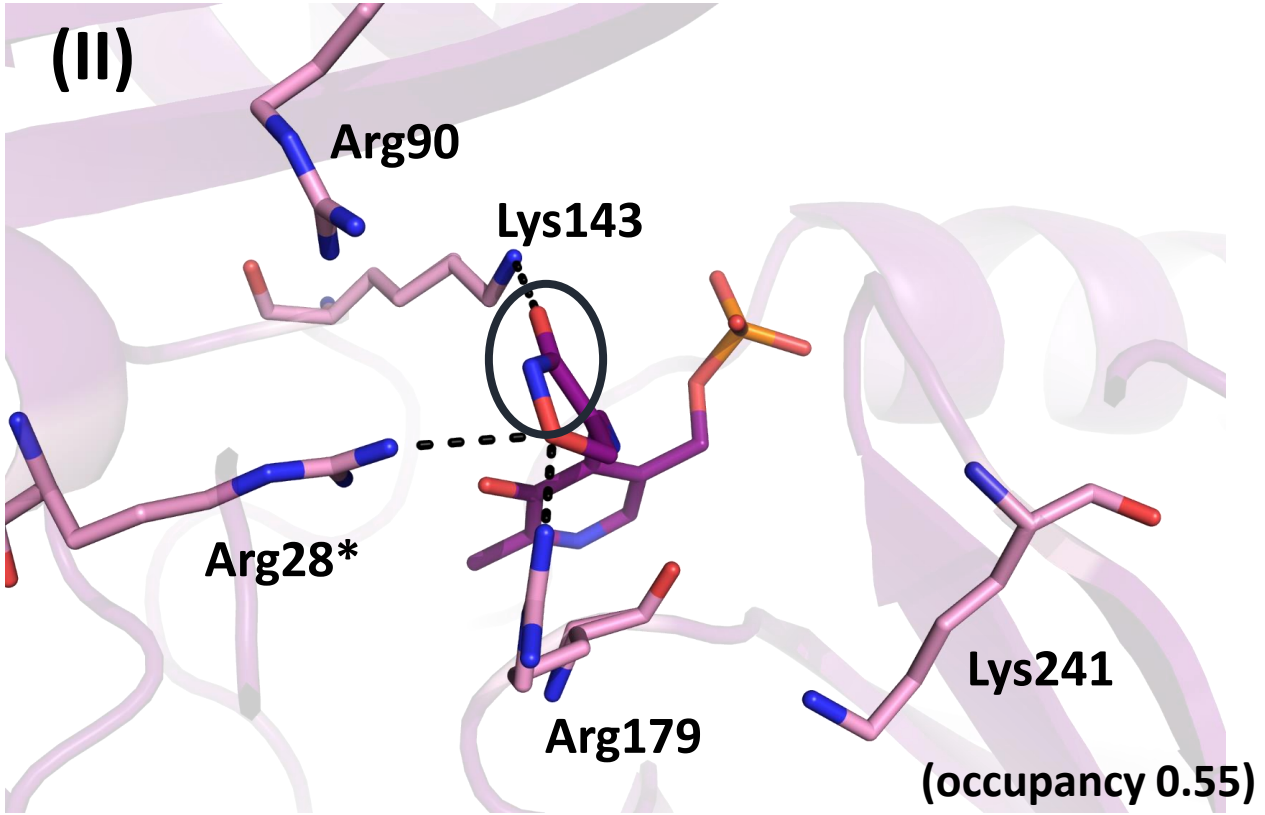


Lamellar single crystals, size ~ 100x100x10 µm

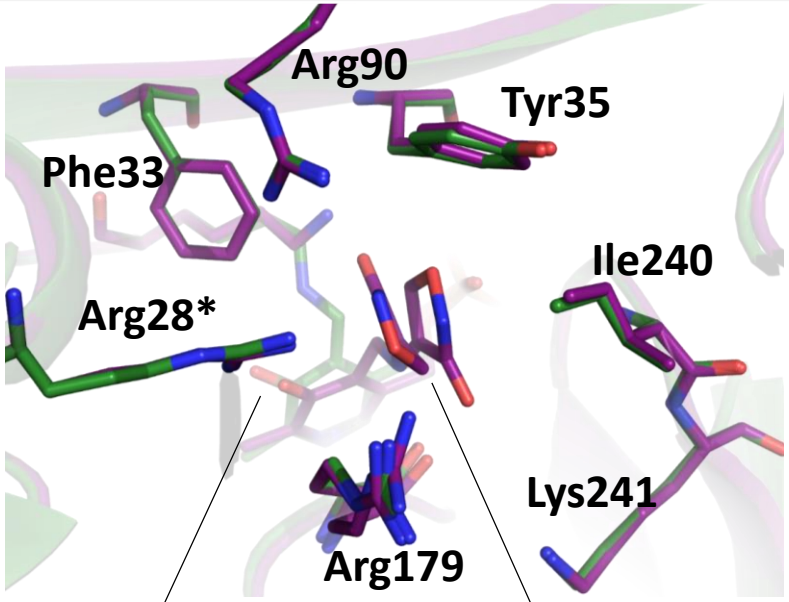
In the crystal structure D-CS is covalently attached to the cofactor forming a ketimine intermediate.
The ketimine occupies two position: **(I)** and **(II)**.
No interaction between the analog of α-COO⁻ group and the residues of the recognition site



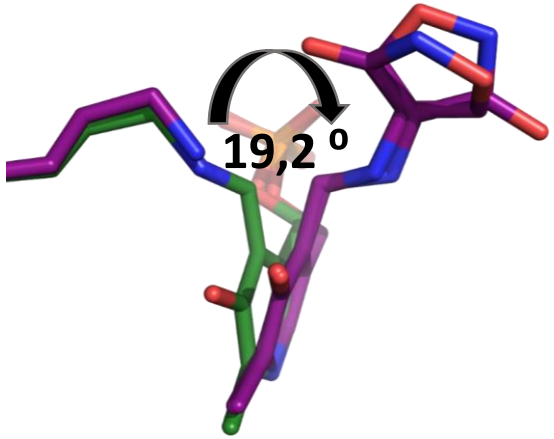




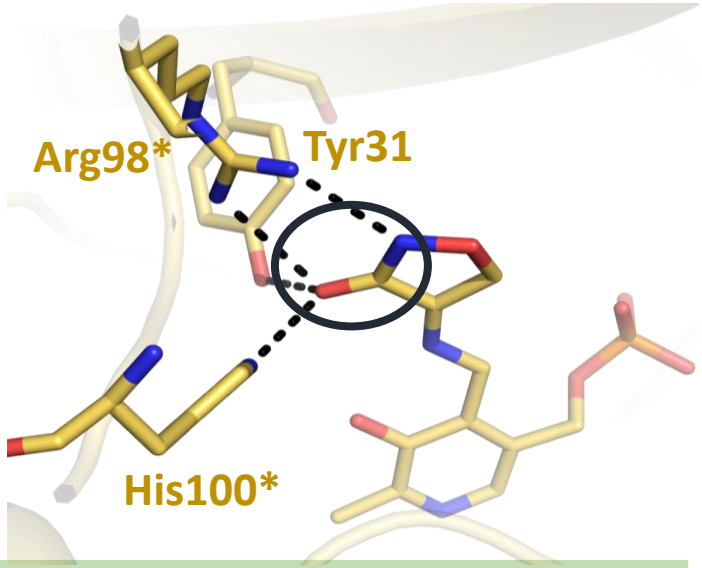
The superposition of the crystal structures of Halhy PLP-form (green) and the complex with D-cycloserine (purple)



Cofactor rotation after reaction:



In the crystal structure of canonical DAAT from *Bacillus* sp. YM1 (PDB ID: 2DAA) "carboxylate trap" (Tyr … Arg x His) coordinates the analog of α-COO$^-$ group of D-cycloserine



Conclusions:

- D-CS binds PLP irreversibly; however, the excess of PLP restores the activity of Halhy via substitution of PLP-D-CS adduct in the active site.
- The adduct of D-CS and PLP is observed in the active site of Halhy, the analog of a-COOH group is coordinated via Lys143 and Lys241.
- Pyridine moiety of PLP is shifted on 19.2 ° in D-CS-PLP adduct comparing to PLP orientation in the holo form of Halhy.

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