**Supplementary Materials**

**Supplementary Table S1** The relative activity of purified wild-type LATA and its mutants toward L-*allo*-threonine and L-threonine

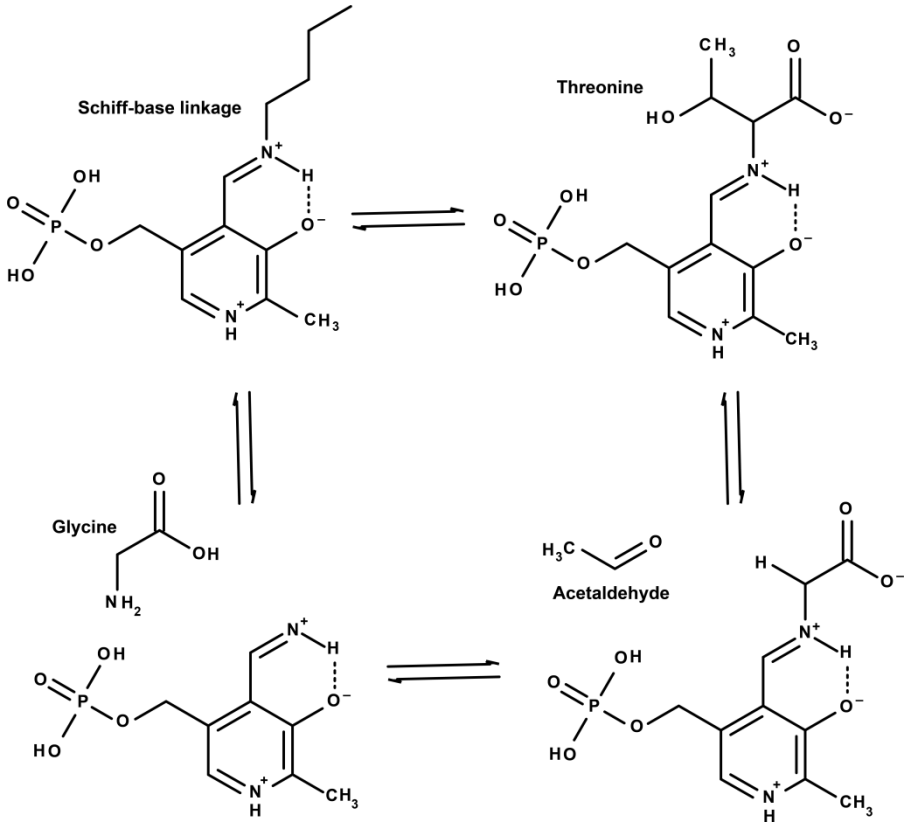
| Enzyme | Relative activity (%) | | Specificity ratio L-*allo*-Thr/L-Thr |
|---|---|---|---|
| | L-*allo*-Thr | L-Thr | |
| LATA_WT | 100 | 100 | 115 |
| LATA_E90A | 6.56 | 3.41 | 222 |
| LATA_D126A | 46.1 | 57.1 | 93.1 |

The activity of the wild-type LATA toward the each substrate is represented as 100, respectively.
The method for purification and activity assay of LATA and its mutants were described in the subsections 2.3. and 2.6., respectively. The purified LATA and its mutants were prepared and 8 m*M* substrates were added to the reaction mixture.

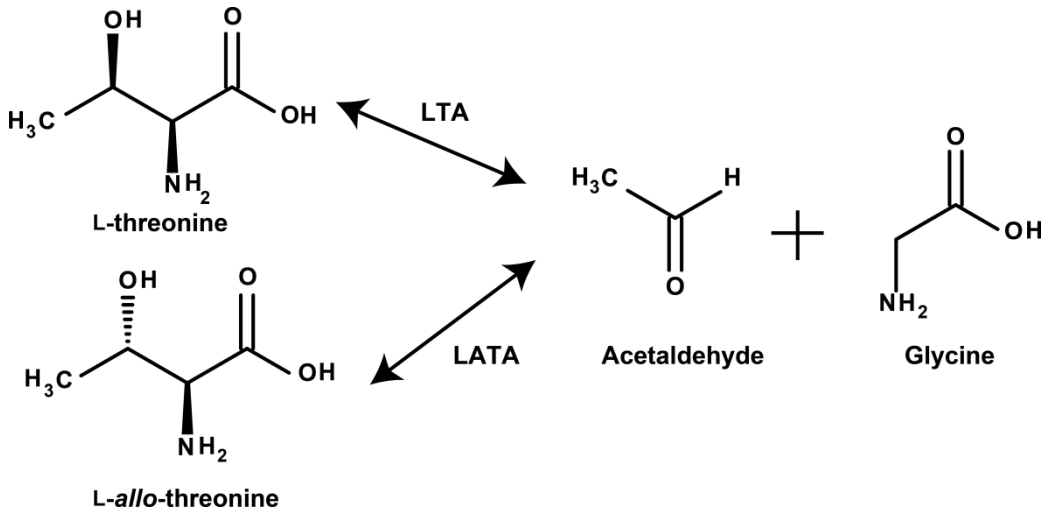
**Supplementary Table S2** The relative activity of His128 saturation mutagenesis toward L-*allo*-threonine and L-threonine

| Enzyme | Relative activity (%) | | Specificity ratio L-*allo*-Thr/L-Thr |
|---|---|---|---|
| | L-*allo*-Thr | L-Thr | |
| WT | 100 | 100 | 74.6 |
| H128A | 48.5 | 83.6 | 43.3 |
| H128C | 31.3 | 79.3 | 29.4 |
| H128D | 9.01 | 4.90 | 136 |
| H128E | 9.24 | 13.6 | 50.8 |
| H128F | 140 | 833 | 12.5 |
| H128G | 27.1 | 46.6 | 43.4 |
| H128I | 48.3 | 199 | 22.7 |
| H128K | 57.6 | 165 | 32.7 |
| H128L | 95.5 | 476 | 18.7 |
| H128M | 115 | 297 | 36.1 |
| H128N | 23.7 | 55.1 | 40.2 |
| H128P | 35.4 | 64.1 | 51.8 |
| H128Q | 23.5 | 53.5 | 42.8 |
| H128R | 15.1 | 37.7 | 39.2 |
| H128S | 19.7 | 453 | 4.24 |
| H128T | 25.4 | 68.1 | 36.3 |
| H128V | 33.8 | 111 | 29.6 |
| H128W | 61.7 | 208 | 28.9 |
| H128Y | 201 | 844 | 17.8 |

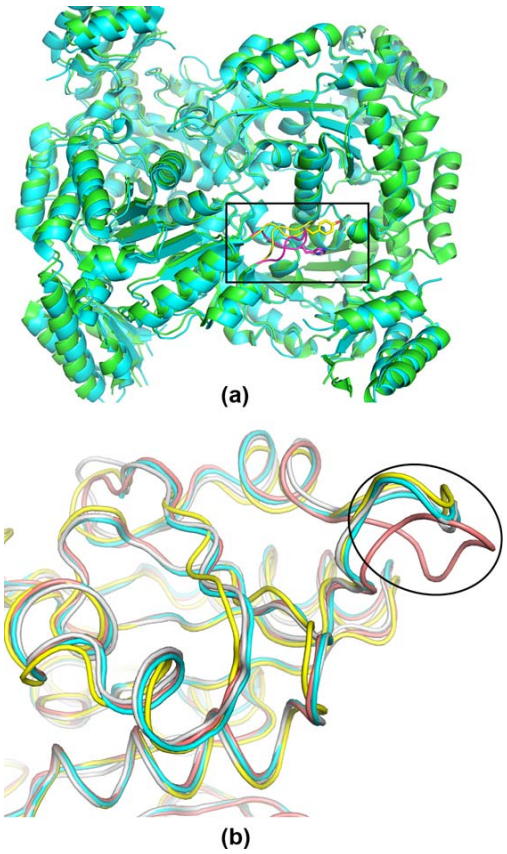
The activity of the wild-type LATA toward each substrate is taken as 100%. The method for measuring threonine aldolase activity of random mutagenesis of LATA was described in the subsection 2.1. The cell-free extracted LATA and its mutants were prepared and 4 m*M* substrates were added to the reaction mixture.



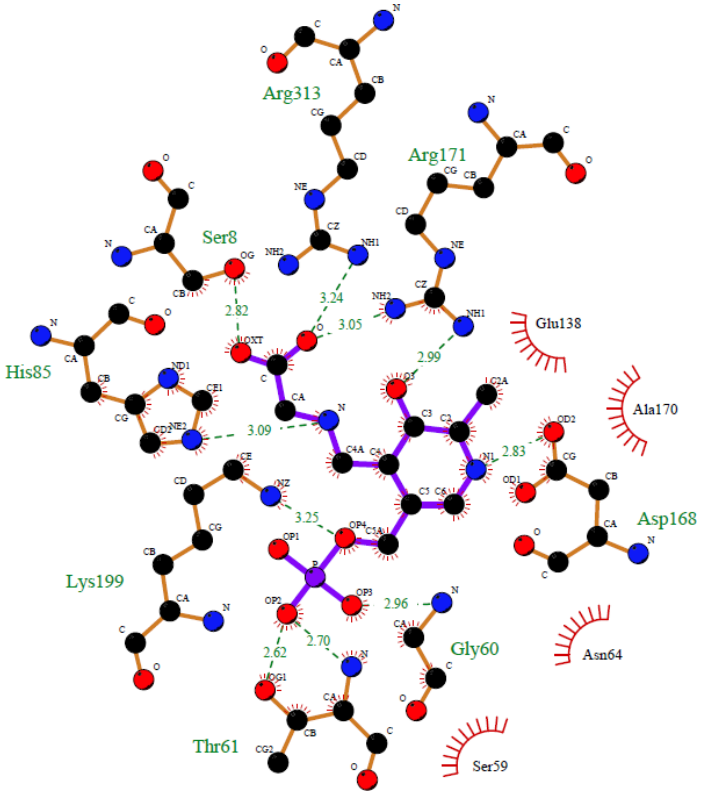

**Supplementary Fig. S1** Proposed mechanism of the reaction catalyzed by threonine aldolases.



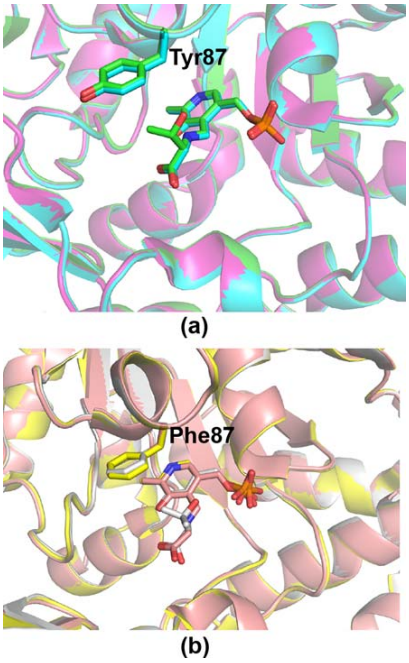

**Supplementary Fig. S2** Enzymatic reaction scheme of threonine aldolases for the reversible interconversion of L-*allo*-threonine/L-threonine to glycine and acetaldehyde.




**Supplementary Fig. S3** (a) The movable loop of Ala123-Pro131 in the tetrameric structures of LATA (green) and LATA_H128Y/S291R (cyan). (b) Superposition of the structures of LATA (cyan), LATA_H128Y/S291R (salmon), TMTA (yellow) and eTA (white). The movable loop is denoted in black cycle.




**Supplementary Fig. S4** Schematic diagram of protein-ligand interactions generated by LIGPLOT. The hydrogen bonds are shown as green dashed lines. Hydrophobic contacts to the ligand are represented by red semi-circles with radiating spokes.




**Supplementary Fig. S5** (a) The superposition of Tyr87 in TMTA structures (TMTA-PLP, magenta; TMTA-PLP-Gly, cyan; TMTA-PLP-Thr, green). (b) The superposition of Phe87 in eTA structures (eTA-PLP, yellow; eTA-PLP-Ser, salmon; eTA-PLP-Thr, white).