

Acidic substrate tunnel redesign by loop transplant to enhance SES7 selectivity towards base amino acids

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The X-ray crystallographic structure of the mature form of wild-type subtilisin E-S7 (SES7) at 1.90 Å resolution and its mutants M5 at 1.96 Å resolution are reported here. Using normalized B-factor evaluation and a novel bioinformatics analysis strategy, we identified the key area related to substrate selectivity and screened the suitable acidic motifs sequence to transplant loops in this area. Totally 11 amino acids in two loops are replaced to reconstruct new SES7 substrate tunnel. To further investigate the protease substrate selectivity mechanism, we used SES7 to hydrolyze skim milk and analyzed the hydrolysates by LC-MS for peptide identification. The results based on the peptide analysis are consistent with our design and simulation, which is instrumental in future protein engineering. Furthermore, the selectivity was determined to assess the utility of SES7 for further industrial applications; The SES7 selectivity towards base amino acids are raised from 11% to 50%.

Keywords: Subtilisin E-S7, crystal structure, normalized B-factor, motifs, loop transplant, LC-MS, selectivity