

HAMA: A multiplexed LC-MS/MS assay for specificity profiling of adenylate-forming enzymes.

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Abstract

Adenylation enzymes selecting substrates for ribosomal and nonribosomal protein and peptide biosynthesis have been popular targets of enzyme engineering. Previous standard assays for adenylation specificity have been cumbersome and failed to reflect the competition conditions inside a cell because they measure substrates one at a time. We have developed an adenylation assay based on hydroxamate quenching and LC-MS/MS detection of hydroxamate products testing dozens of competing amino acid substrates in parallel. Streamlined specificity profiling of adenylation enzymes will

facilitate engineering and directed evolution of ribosomal and nonribosomal peptide synthesis.

Beteiligte Forschungseinheiten

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