Selection-Based Discovery and Mechanistic Analysis of Self-Cleaving Peptides

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Amide bonds are remarkably stable, which presents a major challenge for their cleavage despite its critical role in protein degradation and numerous biotechnological processes. In nature, proteases overcome this challenge by adopting highly intricate three-dimensional structures that enable them to catalyze amide bond cleavage. However, short peptides generally lack the structural complexity required to form such catalytic active sites. While significant progress has been made in developing peptide-based catalysts for a variety of chemical reactions, no peptide catalysts have yet been demonstrated to cleave amide bonds effectively.

In this study, we present a novel approach to identify peptides with amide bond cleaving activity using an mRNA display-based selection strategy. We synthesized a molecular library composed of biotin, a Cys-Pro-amide bond as the substrate domain, a random peptide as the catalytic domain, and mRNA/cDNA encoding the peptide sequence. Following the cleavage reaction, active species that lost biotin were separated from inactive ones and selectively amplified to reconstruct the library. Remarkably, this selection campaign serendipitously led to peptides with self-cleaving activity. We will discuss the characterization and catalytic properties of these active peptides, which provide new insights into peptide-based catalysis and suggest promising directions for future research.