Butelase 1 for C-terninal-specific Ligation and Macrocyclization of Peptides and Proteins

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Proteases are ubiquitous whiereas ligaes, peptide-forming enzymes that catalyze the revere reaction are rare. Here we present the discovery and application of butelase 1, a C-terminal Asn/Asp (Asx)-specific ligase for ligation and macrocyclization of peptides and proteins. Butelase 1, isolated from *Clitoria ternatea* of the legume family, requires an Asx as the recognition residue in a sorting signal such as a tripeptide motif Asx-HisVal at the C-terminus of a peptide or protein substrate with HisVal dipeptide as a leaving group. Butelase 1 accepts most amino acids as a nucleophile to form an Asx-Xaa bond. Among the known ligases including Sortase A, TraF, PATG and PCY1, butelase 1 is the fastest ligase with *Kcat* values as high as $17s^{-1}$ and catalytic efficiencies 542,000 $M^{-1}s^{-1}$. These favorable properties: broad specificity, fast kinetics and traceless ligation product, bode well for butelase 1 in ligation, macrocyclization and labeling of petides, proteins and live cells.

Macrocyclization often enhances metabolic stability and has been used as a strategy to stabilize peptides and proteins. In addition, the covalent closure of the amide backbone induces a constrained structure that may improve biological activity. In this presentation, we show butelase 1 efficiently cyclizes various peptides and proteins ranging in size from 10 to 300 residues, including non-cysteine-containing peptides

and a green fluorescent protein (GFP). In addition, we will present examples in designing and engineering macrocyclic peptides in the under-appreciated druggable natural product space of the "bigger, better and orally-active, small molecule-like peptides".