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## Structure of a novel S8 keratinase

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### 1. Introduction

Keratin is a hardy fibrous protein found in all vertebrates with many varying types, and  $\alpha$  and  $\beta$  keratin are the most abundant.  $\alpha$ -Keratin is found in all vertebrates and consists of  $\alpha$ -helix secondary structural elements. In contrast,  $\beta$ -keratin is found only in sauropsids such as reptiles and birds, and is formed out of  $\beta$ -sheet secondary structural elements [1]. Because of their resilient nature, many industries (such as the poultry industry) produce keratin waste, with very few methods to be upcycled.

Thermal degradation methods utilize heat and pressure to break down keratin into nutrient-rich products. These methods are expensive, destroy valued amino acids, and produce toxic gases [2, 3]. Chemical methods are very effective and utilize strong acids and bases to degrade keratin. These methods, however, have a high risk factor for the environment, while only producing low-nutrient products. The last method is enzymatic degradation, in which proteases with the capability to target peptide bonds in keratin are used to degrade keratin into smaller peptides and single amino acids. This method produces nutrient-rich products, including essential amino acids, with minimal risk to the environment. The largest drawback of this method is that very few keratinolytic organisms are known and even fewer stable keratinolytic enzymes identified, with only five known keratinase structures having been solved [1, 2].

### 1. Results

The current project is part of the ThermoK consortium and focuses on identifying novel keratinases from the keratinolytic thermophilic organism *Fervidobacterium pennivorans*, as well as solving their tertiary structures by X-ray crystallography [4]. A novel protease from the S8 family has been isolated and heterologously expressed in *E. coli*. This protease has shown high levels of keratinolytic activity using both milled feather and keratin azure assays. The tertiary structure of this S8 protease has been solved at a resolution of 1.63 Å and possibly matured in the crystallization droplet, as the structure contained the cleaved pro-domain still attached to the catalytic domain. This S8 structure can be used to further elucidate the substrate specificity of keratinases. Understanding the structure-function relationship of this enzyme could aid in the identification of additional keratinases for the upcycling of keratin-laden waste.

### 1. References

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