Chemical Engineering Doctoral Defense

Structural and Functional Studies of Nonribosomal Peptide Synthases

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Abstract

One element at the heart of environmental health debates today is plastics. Finding biodegradable alternatives for synthetic petroleum derivatives is a necessity when planning for planetary longevity. One NRPS, Cyanophycin synthetase (CphA), can produce cyanophycin grana protein (CGP), a polymer composted of a poly-aspartic acid backbone decorated with arginine side chains. The aspartic acid backbone has the potential to replace synthetic polyacrylate, although the current production costs are prohibitive. In Chapter 2 of these works, we characterized a CphA variant from Tatumella morbirosei that produces up to 3x more CGP than other known variants, and shows significant potential for scale-up into a bioreactor-type production setting. Future work on this topic will include analyzing the CGP amino acid content and standardizing the enzyme coefficients and characteristics. We also examine another CphA variant, this one from Acinetobacter baylyi, where we used rational protein design to create two novel mutants. One, G217K, is 34% more productive than the wild type, while G163K produces a CGP with shorter individual chain lengths. We also refined the current structure from 4.4Å to 3.5Å. Future work will involve characterizing the mutant CGP amino acid content and evaluating the potential of this mutation across other CphA variants.Another exciting application of NRPSs is in healthcare. They can be used to generate novel peptides such as complex antibiotics without the necessity of manual chemical synthesis for each step of a production process. A recently discovered iterative polyketide synthase (IPTK), dubbed AlnB, produces an antibiotic called allenomycin. One of the modular subunits, a dehydratase named AlnB_DH, was the focus of a highly collaborative study. As part of this study, discussed in Chapter 4, we were able to crystallize AlnB_DH and solve the structure to 2.45Å. We designed, created, and tested several mutations in multiple active site residues to help understand the functional mechanism of AlnB_DH. We also used cryo-EM to generate a preliminary holoenzyme AlnB structure at 3.8Å although the large disorganized regions demonstrated an incomplete structure.

October 27, 2022; 12 PM; BDC 335;
Zoom Link: https://asu.zoom.us/j/85361824408