

Poster Presentation

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NRPS condensation domain—substrate studies using X-ray crystallography

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Nonribosomal peptide synthetases (NRPSs) are a family of large multimodular enzymes that synthesize structurally and functionally diverse peptides including siderophores, toxins, agriculturally-important compounds and pharmaceutically-important compounds. The condensation (C) domain is responsible for peptide bond formation, the central chemical step in nonribosomal peptide synthesis. Here we present the crystal structure of the first condensation domain of the calcium-dependent antibiotic (CDA) synthetase (CDA-C1) from *Streptomyces coelicolor* soaked with a small molecule compound representing the acceptor substrate. To increase the likelihood of complex formation, we designed the compound to contain a free thiol group in order to form a covalent bond between the substrate and a cysteine residue of the CDA-C1. The tethering of the substrate to the active site mimics delivery of substrate to the active site by the NRPS PCP domain, and the disulfide bond it forms with the protein will ensure a high local concentration of substrate. Initial maps, calculated from diffraction datasets collected at the home source, indicated density corresponding to the presence of substrate at the active site. This result, along with activity assay data, will help implicate residues important to enzyme catalysis and substrate specificity. In all, these studies will help characterize C domain function in NRPSs and potentiate the use of NRPSs in bioengineering experiments to produce novel or improved therapeutics.

Keywords: Non-ribosomal peptide synthetase, Condensation domain, Enzyme