GPCR Affecting Fatty Acid Amide produced by Non-Ribosomal Peptide Synthetase Cluster

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Non-ribosomal peptide synthetases (NRPS) are multi-domain modular enzymes that catalyze the synthesis of small molecules using amino acids or non-proteinogenic amino acid substrates. The 3 core domains that all NRPS contain include condensation (C), adenylation (A), and thiolation (T). The A domain serves to activate the loaded substrate which is then transported by the T to either the C or other catalytic domains which modify the substrate. This occurs in an assembly line process until a functional molecule is formed and completely constructed. We are examining an NRPS pathway from the bacterium Corprococcus eutactus which is found in the human gut and produces the fatty acid amide: oleoyl aminovaleric acid. Unlike canonical NRPS which have multidomain architectures, each domain from C. eucactus exists as a free-standing protein (OaaA, OaaC, OaaT). To explore the structure and function of this biosynthetic pathway, we have successfully expressed and purified OaaA and OaaC. We have performed solution scattering (SS) experiments with the OaaA and OaaC in liganded and unliganded states and are currently being processed using singular value decomposition in order to extract the pure protein signal. We will also utilize the processed SS difference profiles and apply an algorithm named density from solution scattering (DENSS). This will allow us to validate the ligand pose obtained through x-ray crystallography and probe the protein in a more physiologically relevant state.