Structural studies of the bacterial cytochrome P450 DitU using serial crystallography towards time-resolved studies

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Cytochrome P450s (P450s) are heme monooxygenase enzymes found amongst all branches of life, which catalyze C-H bond hydroxylations as well as a wide variety of other oxidations.[1] Bacteria found in tree bark are able to degrade toxic compounds in the bark, especially terpenoids such as resin acids. However, there is relatively little understood regarding the structure and function of the P450s involved. Elucidation of these enzymes is of interest in biotechnology and may in the future increase efficiency for forestry and paper industries.[2]

Previous research shows that bacteria expressing these proteins can grow on media containing the toxic extractives.[3] The recent discovery and genome assembly of the resin acid-degrading bacterium, *Pseudomonas abieticivorans*, provides an opportunity to study this system in greater biochemical detail. *P. abieticivorans* produces several P450s in its *dit* cluster, including *Pa*DitU, from the understudied family CYP226. To obtain structure-function information into this understudied P450 system, we are using X-ray crystallography, both traditional at cryogenic temperatures and room-temperature serial crystallography, aiming at time-resolved serial crystallography.[4]

Within our collaboration, the first structure of *Pa*DitU has been solved, and we are currently optimizing multiple crystallization conditions to obtain microcrystals suitable for ligand binding studies using room temperature serial crystallography.

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