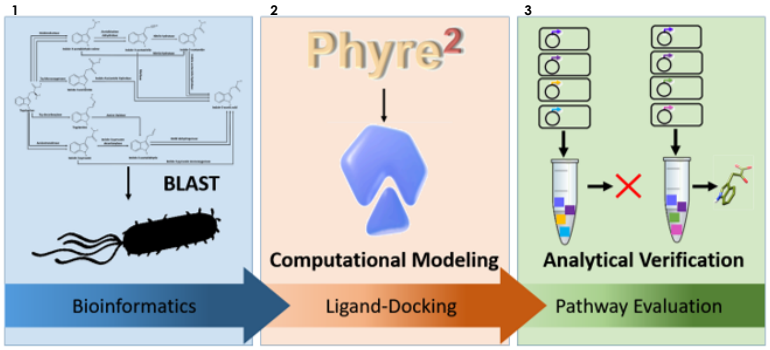
PMI SFA Publication Highlight

U.S. DEPARTMENT OF ENERGY | Office of Science

Novel Results: Rapid and scalable approach allows for rapid elucidation of secondary metabolite pathways

Objective	• To develop an integrative computational and experimental approach to elucidating the production of natural products in microorganisms.
New science	• *Pantoea* sp. YR343 produces the phytohormone indole-3-acetic acid (IAA) from tryptophan through an uncharacterized metabolic pathway. • To elucidate this pathway and test the effectiveness of using computationally guided discovery, a scalable, multi-step process was developed (figure at right). • Using BLAST analyses on the sequenced genome of *Pantoea* sp. YR343, relevant enzymes and potential IAA metabolic pathways were defined. The only complete pathway was through the production of indole-3-pyruvate. • Phyre 2, a protein structure prediction tool, was used to generate a computational homology model and predict ligand binding sites for selected enzymes. Proteins capable of binding the ligand were then tested experimentally. • Small-scale, heterologous expression and *in vitro* reactions in a crude extract were performed to verify the contribution of individual enzymes to the predicted pathways. Redundant enzymatic capabilities were observed which provided insights into IAA production and "underground" metabolic capabilities.
Impact	• These results illustrate the scalable integration of computational tools and cell-free enzymatic reactions to identify and validate metabolic pathways without the need for time-consuming and expensive genetic systems or even culturable organisms.



A scalable method for predicting and verifying metabolic pathways has been developed. The primary steps are shown in the schematic. **1.** Literature-derived sequences are used to create a query pool of enzymes and generate putative metabolic pathways. **2.** Computational docking of ligands to protein models is used to screen for those enzymes most likely to be connected to the pathway of interest. **3.** Proteins proposed to be capable of docking the ligand are expressed *in vitro,* individually and in combination, to generate potential pathways. These enzymes and pathways are then tested for their ability to produce the metabolite(s) of interest.

Computationally-guided discovery and experimental validation of indole-3-acetic acid synthesis pathways Garcia, D. C. et al. (2019). *ACS Chemical Biology,* doi: 10.1002/ajb2.1373

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