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Predicting Binding Modes for Carene Synthase - Using Alpha Fold and Rosetta Software to Probe Product Promiscuity

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Abstract: Terpenes are a class of molecules made by plants and some bacteria, they are very abundant and are responsible for many of the forest or soil smells we experience. Terpenes have played a crucial role in the flavor and fragrance industry, so manufacturing terpenes are economically relevant. Terpenes are made in plant and bacteria cells via terpene synthase enzymes, and many of these enzymes are currently being studied. Computational chemistry as an approach wields certain tools which could assist in understanding the mechanisms of these enzymes. "Molecular docking" is a technique used to predict interactions between the enzyme and the molecule. Evaluating these predictions can provide insights into lab experiments. For our study we focused on Carene synthase (CarS), an enzyme from Sitka spruce. This is a monoterpene synthase enzyme (a subtype of terpene synthase) that produces carene, as well as the side products sabinene and terpinolene. The aim of our study was to correlate binding orientations to products made by carene synthase. We were able to adapt the techniques from a 2019 study by Dr. Dean J. Tantillo and Dr. Justin B. Siegal, "Predicting Productive Binding Modes for Substrates and Carbocation Intermediates in Terpene Synthases - Bornyl Diphosphate Synthase as a Representative Case." We used Rosetta Commons software to dock two of the reaction intermediates and final products to find a location of convergence in the active site. We were able to find convergence for linalyl cation, terpinyl cation and carene which suggested tyrosine 544 likely plays an important role in the reaction. Our plans are to mutate this residue into a phenylalanine which would remove the hydroxyl group at this location while keeping the pi electron system. This is expected to alter the ability of CarS to produce carene and may lead to a preference for other monoterpene products. This will be tested with enzymatic assays of the CarS Y544F mutant. Future work is focused on finding convergence in our docking results on the other two main products (sabinene and terpinolene) made by carene synthase to gain additional insight into the active site and its impact on the product profile.