



Chemistry and Biochemistry

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Mutagenesis Study of Limonene Synthase to Alter Cyclization Products

Speaker:

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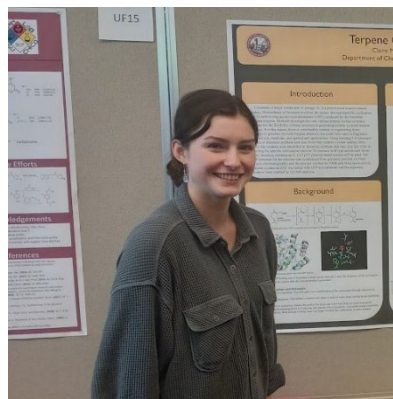
Honors Seminar

When:

Friday, 03/29/24

1pm in Glenn Hall

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Limonene, a major component of orange oil, is a prototypical terpene natural product. Biosynthesis of limonene involves the stereo- and regiospecific cyclization of a 10-carbon long geranyl-pyrophosphate (GPP), catalyzed by the limonene synthase enzyme. Multiple investigations into various terpene cyclase enzymes have shown the flexibility of these enzymes in generating diverse cyclized terpene products. For this reason, there is considerable interest in engineering these enzymes to generate cyclized terpene products that could have uses in fragrance, essential oils, medicine, and agricultural applications. Using existing 3-D structural models of limonene synthase and experimental data from other terpene cyclase studies, three active site residues were identified in limonene synthase that may play key roles in directing the specific cyclization reaction. To produce wild type and mutant forms of the limonene synthase an *E. Coli* pET plasmid-based system was used. The GPP substrate for the enzyme was chemically synthesized from geraniol and purified via flash column chromatography. Terpene cyclase activity was tested with GPP as a substrate and the products were identified with GC-MS analysis. GC-MS analysis showed expected production of limonene with purified wild type enzyme. The purification and the study of terpene production with mutated limonene synthases will be presented.

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