Engineering DXS for Improved Flux into the MEP Pathway

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Project Goals: Using machine learning trained on data from a high-throughput growth selection to design and characterize new DXS enzymes with enhanced activity for improving the overall flux into the MEP pathway.

The MEP pathway synthesizes isoprenoids, which are valuable next generation biofuels and bioproducts. The rate-limiting step of the pathway is the condensation of pyruvate and glyceraldehyde 3-phosphate by 1-deoxy-D-xylulose-5-phosphate synthase (DXS). In this work we engineered new DXS variants to increase flux into the MEP pathway. We designed, constructed, and screened a large library of chimeric DXS enzymes. We used nanopore sequencing to map how DXS sequence affects activity, and trained machine learning models to predict highly active DXS variants. We designed, synthesized, and tested four new DXS variants and confirmed their ability to complement Δ*dxs E. coli* strains. We also measured the designed enzymes activities in vitro and found the best DXS design was comparable to that of the best parental DXS. We will utilize insights from these experiments, combined with directed evolution, to further optimize the DXS variants. Future work will include biochemical and kinetic characterization of the designed enzymes, metabolomics to profile how they alter metabolite pools, and transferring the designed enzymes to other bioenergy organisms such as *Zymomonas mobilis*.

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