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Machine Learning Guided Directed Evolution of Unspecific Peroxygenases

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Epistasis, characterized by the interdependence of effects among various mutated positions, is frequently encountered in directed evolution (DE) campaigns, especially when employing multiple-site combinatorial mutagenesis libraries.[1] This phenomenon was notably observed in our prior work, which focused on engineering the unspecific peroxygenase (UPO) from *Myceliophthora thermophila* (MthUPO) for the enantiospecific hydroxylation of β-ionone.[2] To tackle the complexities introduced by epistasis and strive for a global optimum in DE campaigns, we have employed a data-driven approach, leveraging machine learning-guided directed evolution (MLDE).

A diverse library of mutants undergoes assay and sequencing to generate input data for training machine-learning models. These models are refined using various assessment metrics to accurately rank mutant activities. The most effective models guide the selection of additional mutants for assay, contributing to iterative model refinement. This process continues until predictions for untested mutants are consistently lower than those of the most active known mutants, indicating convergence. The final model's efficacy is confirmed through its accurate prediction of mutant activity levels. This approach showcases machine learning's capacity to enhance UPO engineering by effectively addressing epistasis challenges, leading to increased enzyme activity.