# DIRECTED EVOLUTION AND PREDICTIVE MODELLING OF GALACTOSE OXIDASE TOWARDS BULKY BENZYLIC AND UNACTIVATED SECONDARY ALCOHOLS

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In the field of alcohol oxidation, galactose oxidase (GOase) is one of the most established enzymes capable of this important chemical transformation under benign conditions.[1] Yet, the applicability of GOase towards more complex molecules such as those frequently found in the pharmaceutical, or agrochemical industries remains restricted. By employing a combined approach of directed evolution and computational modelling, we have identified new GOases with significantly expanded substrate specificity toward both bulky benzylic and unactivated secondary alcohols, showing activity enhancements of up to 2,400-fold compared to the reported benchmark M3-5 mutant.[2] Beneficial mutations conveying relaxed substrate enantioselectivity biases (R/S ratios down to 1.05) and higher thermostabilities (up to 20-fold versus benchmark) have also been identified. Our improved mutants have been applied in the synthesis of functional molecules such as UV-curing photo initiators (100% yield) and carbonyl reductase inhibitors (99% yield).[3] We have also developed models based on computational tools that are well correlated with features related to enzyme structure, selectivity, protein stability, enzyme kinetics, and catalytic activity. These predictive models yield valuable insights that can aid exploration of enzyme diversity in silico, providing in-roads to accelerate enzyme engineering workflows.

## References

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