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## **Rational design of a thermostable FAOX enzyme**

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Fructosyl Amino Acid Oxidases (FAOX) are enzymes that can de-glycate amino acids, cleaving the sugar unit from the amino acidic moiety. These enzymes have been used as biosensors for diabetes monitoring, by measuring the concentration of glycated haemoglobin in blood samples. However, the used FAOX enzymes show relatively low stability levels, which affect their conditions of use. In this work, we show a rational design method based on molecular dynamics simulations through which we screen a library of potentially stabilizing mutants. The most promising mutants are then experimentally produced and tested. Using this approach we obtained two thermostable FAOX I mutants showing a significantly higher  $T_{50}$  (55.3°C and 60.6°C, respectively) compared to the wild-type enzyme (52.4°C). In addition, one mutant exhibit hyperstabilization, showing residual activity up to 95°C, while the wild-type enzyme becomes inactive at  $T > 55^\circ\text{C}$ . In conclusion, the computational method presented here represents a useful approach to expedite the design of thermostable enzymes.