

## Engineering substrate specificity in galactose oxidase

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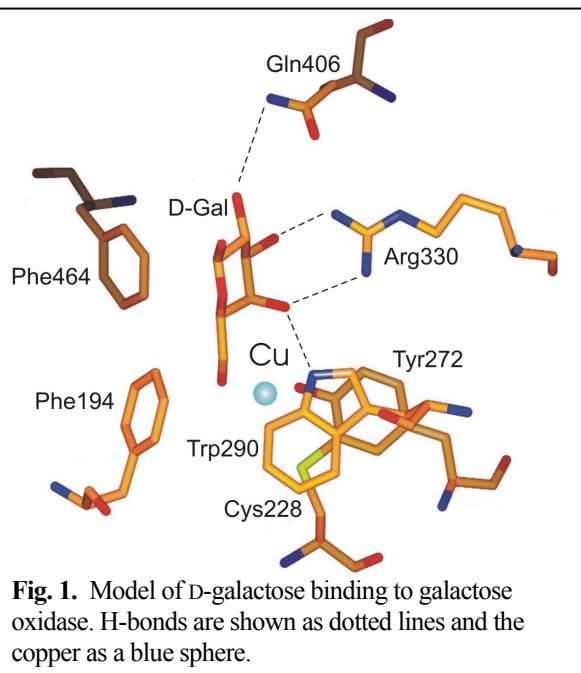
Galactose oxidase (GO; E.C. 1.1.3.9), is a 68kDa mononuclear copper-containing enzyme that oxidises primary alcohols to the corresponding aldehyde with coupled reduction of molecular oxygen to hydrogen peroxide according to the reaction scheme:



A copper ligand, Tyr 272, is covalently bonded through C<sub>ε</sub> to the sulphur of Cys 228 and is the site of the catalytic free radical. The enzyme shows broad substrate specificity and displays a high  $K_M$  for substrates, including D-galactose (~70 mM). There is no crystallographic structure of substrate bound enzyme, but molecular modeling suggests a binding site consistent with known substrate specificity. For example, the enzyme is essentially inactive towards D-glucose, and the model indicates this would be due to a steric clash between the axial ligand Tyr495 and the O4 of glucose. We are interested in testing the substrate binding model and in altering substrate specificity of this enzyme, and one potential target substrate is fructose, against which the enzyme displays a very low activity.

The proposed site comprises Arg330, Gln406, Trp290, Phe 194 and Phe 464 (Fig. 1). We have introduced mutations at some of these residues to investigate the properties of the resulting proteins. To facilitate these studies we also developed an expression system based on the methylotrophic yeast, *Pichia pastoris*, to overcome some of the issues with the existing system based on the filamentous fungus *Aspergillus nidulans*. The crystal structure, spectroscopic properties and kinetic measurements show essentially no difference between the enzymes isolated from different expression hosts.

Analysis of mutational variants revealed effects on kinetic parameters with substantial increases in  $K_M$ , indicating the importance in substrate binding of the residues tested (R330A and K, F464A).



The kinetic parameters for fructose oxidation by wild-type GO indicate a very high  $K_M$  ~2.5M and low  $k_{cat}$  (~9 M<sup>-1</sup>s<sup>-1</sup>). All the variants exhibited lower  $K_M$  values for fructose with the lowest (R330K) at ~1M. R330K was also the most interesting variant displaying an 8.2-fold increase in  $k_{cat}/K_M$  for fructose compared with wild-type GO, and a reduced level of discrimination between the substrates. Further studies are required to improve the kinetic parameters exhibited by this variant, but even at this stage the enzyme represents a better fructose oxidase than wild-type GO.

### Publication

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