Protein modelling for enzyme engineering

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***Keywords*— laccase, aniline, protein engineering, arylamines, esterases, promiscuity, accessible volume**

EXTENDED ABSTRACT

ACTIVITY PREDICTION

Laccases (EC 1.10.3.2) are a polifacetic enzyme type with multiple applications in various fields: food industry, manufacture of anticancer drugs or ingredients in cosmetics, bioremediation, etc.. Their ability to oxidize a great variety of molecules and the versatility of laccase-mediator systems open interesting perspectives to this biocatalyst. Moreover, laccases require only oxygen to function and produce water as the reaction byproduct, which makes them a perfect green catalyst for sustainable industrial applications. Furthermore, soft buffers, like phosphate, can be used which are less aggressive for the industrial material and safer for the personnel. Arylamines, such as aniline, are a group of compounds with a large industrial applications, for example aniline polymers (PANI) have a broad range of uses. Actually PANI production requires hard chemicals as ammonium peroxydisulfate and strongly acid conditions. Some laccases are able to perform aniline oxidation for PANI production but with a low efficiency and requires protein engineering for improve activity above aniline.

By experimental directed evolution it was possible to produce an improved laccase variant for aniline oxidation and PANI production. Using computational modeling, we aim at further improve this initial design for a higher PANI production.. In particular, two positions were selected, 207 and 263, and mutated to serine and aspartic acid respectively. The aim of both mutations is to modify the binding cavity environment, improving the substrate oxidation potential. Computational predictions indicated an activity increase in the double mutant in comparison with the improved laccase variant from directed evolution. Experimental validation of double mutation confirmed a 2-fold Kcat increase for aniline oxidation and a maintenance of protein stability parameters [1].

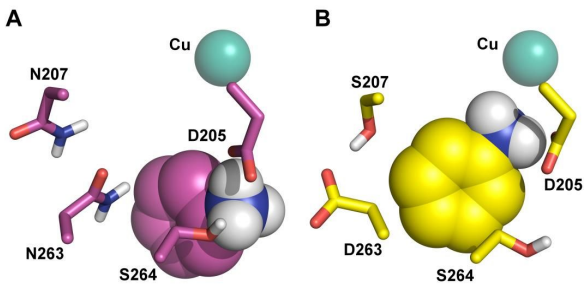
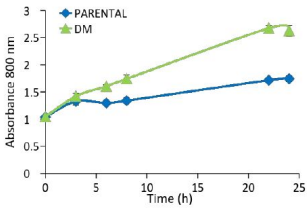


Fig. 1 Representative binding mode for aniline interaction with parent laccase



(A) and double mutant (B).

Fig. 2 On the left the enzymatic polymerization of 15 mM aniline along time (with 5 mM SDBS as template) as shown by the increase of absorbance at 800 nm, and on the right green PANI produced in (C) by parental and DM laccases.

PROMISCUITY PREDICTION

Enzyme promiscuity characterizes the range of substrates that react with a biocatalyst. The growing industrial needs in sustainable chemistry drives the search for new biocatalysts capable to perform a large variety of reactions. Deciphering an enzyme promiscuity, however, requires a huge amount of experimental tests and it's really difficult to obtain in an accurately manner. In this part of the work, by combining structural parameters, including the active site volume and solvent accessible surface area (SASA), we show that it is possible to predict substrate promiscuity. For the analysis we used esterases (EC 3), a well-known and widely used (including industry) hydrolase enzyme that splits esters into an acid and an alcohol, by means of a chemical reaction with water called hydrolysis.

Our previous studies revealed that the SASA and the cavity volume had key roles in substrate diffusion and enzyme-substrate interaction in different esterases with opposite promiscuity profiles. Based on this knowledge the analysis was expanded to a list of 98 esterases tested with 96 substrates each. Figure 3 presents the Accessible (or Effective) Volume, the active site volume divided by the catalytic triad SASA, for all 98 esterases versus the promiscuity, the total number of compounds hydrolyzed. The data reveals that for an Accessible Volume higher than 62,5 A^3 the esterase will be capable to hydrolyze more than 20 substrates. The base idea behind is the cavity volume as a major component of enzyme promiscuity, the bigger is the cavity the more substrate can accommodate. At some some point, however, the cavity is too large, becoming too solvent exposed and unable to accommodate (retain) substrates. SASA (adimensional value) corrects the volume and allow us to obtain the Accessible Volume. That test has a precision of 95% becoming a useful tool to predict esterases promiscuity

and opening the possibility to extent the analysis to other enzymatic types.

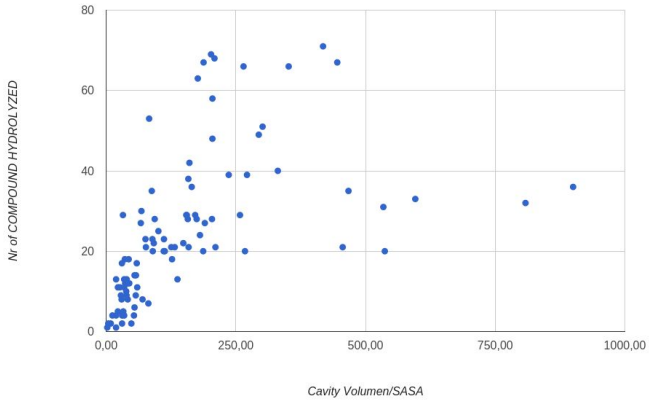


Fig. 3 The figure shows the relationship between the reaction cavity volume and catalytic triad SASA (accessible volume) with enzyme promiscuity (number of substrates hydrolyzed). When the esterase is capable to hydrolyze more than 20 substrates has an accessible volume (Catalytic Volume/Triad SASA) higher than 62.5.

ACKNOWLEDGEMENT

This study was supported by the INDOX (KBBE-2013-7-613549) EU-project and the OxiDesign (CTQ2013−48287-R) Spanish project. G.S. acknowledges an FPI grant from the Spanish Ministry of Competitiveness.

References

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Author biography

Gerard Santiago obtained his Biochemistry degree by University of Barcelona (UB) in 2013, and a Bioengineering Master by Institut Químic de Sarrià (IQS) in 2015. He is currently a PhD candidate in Barcelona Supercomputing Center (BSC-CNS). His scientific career revolves around different levels of Green Biotechnology, such as: new enzyme discovery for industrial applications in extreme environments, production of high-value compounds on eukaryotic strains grow it in waste materials and enzyme design (focused on oxidoreductases) for eco-friendly industry.