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The kinetic characterization of the small laccase from Streptomyces coelicolor using EnzymeML: a technical lesson in reproducibility

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PURPOSE OF THE ABSTRACT

One of the main challenges in enzyme characterisation is the availability of reliable and reproducible results [1]. Studies have shown that there is a large discrepancy in the ability of researchers to reproduce their own results [2], as well as reproduce the findings of others. The STRENDA commission [3] has outlined a set of guidelines useful for the thorough reporting of enzymology data, including but not limited to protein information, assay conditions, detailed experimental methods as well as processing of results.

The small laccase (SLAC) from Streptomyces coelicolor is a two-domain laccase that has been extensively described in both biochemical and structural nature [4, 5, 6], and was selected for use in this study. Using a ThermoScientific Multiskan 1500 microtiter plate reader, the enzymatic oxidation of 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was monitored at 340nm (substrate utilisation) between pH values of 3.0 – 5.5, across a substrate concentration range of 0-200µM, and over a temperature gradient of 25-45˚C. Oxidation was monitored for 15 minutes. In total, 30 different assay conditions were performed which yielded more than 100 000 individual reads.

In order to process such a large dataset accurately, and subsequently, circumvent the likelihood of erroneous data curation through human error, an EnzymeML [7] workflow was implemented contained in a single Jupyter Notebook. Experimental metadata was captured in an EnzymeML Excel spreadsheet. Photometric measurements for standard curves and oxidation measurements were parsed into individual EnzymeML documents, and the calibration data was used to convert the oxidation measurement into concentration.

Michaelis-Menten rate equations, along with different inhibition models were tested to determine which best fits the experimental data, scored based on the lowest Akaike's Information Criterion (AIC). The kinetic parameters, along with a detailed statistical report are produced.

The best catalytic efficiency for SLAC was observed at 45˚C and pH 3.0, at the edge of the parameter space which indicates these are not necessarily the optimal conditions and could therefore be expanded upon. A correlation between the kcat¬ and Km was observed, suggesting too low of an initial substrate concentration was utilised in relation to the true Km of the enzyme. An enzyme half-life of only 10 minutes at the highest catalytic efficiency was observed and further experimentation is required to determine the cause of this inactivation, likely by expanding enzyme concentrations and the use of different substrates.

Currently, the SLAC experiment is being expanded to address the problems in the experimental procedure to determine the biochemical characteristics of the enzyme more accurately – namely by increasing enzyme concentration 5- and 10-fold, as well as testing mutants to better understand the nature of the catalytic activity. The robustness of the workflow will also be tested on an expanded set of multicopper oxidases (MCOs), including three-domain MCOs of fungal and bacterial origin, as well as a multicopper polyphenol oxidoreductase laccase (MPOL).

This pipeline demonstrated the use of computational biology to describe the kinetics of SLAC, rapidly and accurately, within the limitations of the experimental setup. It is useful for not only describing the biochemical metrics of the enzyme, but also to indicate flaws in the experimental design and allows the end-user to make discriminating choices regarding the technical procedures involved in wet-lab preparation.

FIGURES

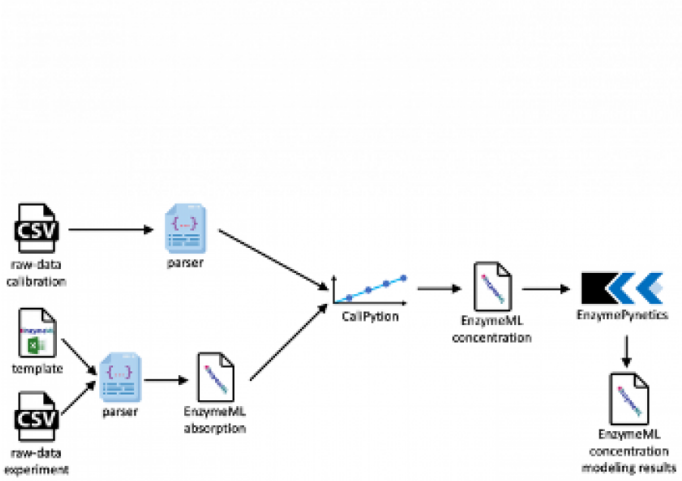

FIGURE 1

FIGURE:

Schematic of the EnzymeML workflow

FIGURE 2

KEYWORDS

MULTICOPPER OXIDASE | COMPUTATIONAL BIOLOGY | ENZYME CHARACTERISATION | BIOCATALYSIS

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