Creating the next generation LPMOs for biofuel production – to start October 2019

Christine Desty Scholarship, fully-funded (Home/EU fees £4630 plus stipend of £15,009) for an MSc by Dissertation (MSD) in the School of Biological Sciences, University of Essex

Background

Deconstructing plant and non-plant (e.g. fungal) biomass to value added products will become a major route to obtain sustainable materials in many industrial processes like food, feed manufacturing, non-food and renewable bioenergy. A significant proportion of the polysaccharides in biomass resists deconstruction with the currently available enzyme cocktails for cellulose, chitin and starch. New enzymes and/or improved versions of existing enzymes will be needed to include this fraction of the biomass to the product stream. This project is concerned with the latter and is focused on a family of copper (Cu) containing enzymes called lytic polysaccharide monooxygenases (LPMOs).[1,2] These enzymes are now known to perform the oxidative pre-treatment of the biomass to make it readily available for further breakdown by hydrolases such as cellulases and amylases.[3]

Starch liquefaction is the starting process of the starch, sweetener, syrup, and alcohol industries, thus being of great relevance for the food and bioethanol sector. Starch is a mixture of amylose and amylopectin, with the ratio variable depending on source (e.g. corn, wheat, rice etc). The AA13 family of LPMOs have been shown to have an enhancing effect on α-amylase activity with retrograde starch.[4] In contrast to α-amylases, AA13 LPMOs can bind directly to the substrate, oxidatively disrupt the crystallinity and in theory facilitate the action of amylases through making more sites available for hydrolysis. Currently, there is no commercial application of AA13 LPMOs in starch liquefaction processes.

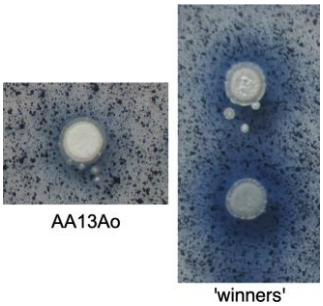

Fig. 1: Left, a *S. lividans* colony over-expressing wild-type AA13 LPMO and indicating minimal activity on AZCL-amylose (absence of blue halo). Right, two *S. lividans* colonies expressing 'winners' from the loop-1 library screen (note the intensity and size of the blue halo indicating cleavage and release of AZCL-dye).

In collaboration with a German company, WeissBioTech, Leiden University (Netherlands) and funded through a BBSRC Business Interaction Voucher we have developed an *in vivo* library screen for a AA13 starch LPMO with the goal to identify better performing LPMOs and test whether these can be used in industrial starch liquefaction. Libraries are based on a series of loops that create an active surface for substrate interaction surrounding the active site Cu. By re-engineering these loops through creation of synthetic loop-saturation-libraries and screening in a *Streptomyces* host, 'winners' i.e. a better performing AA13 LPMO variants with enhanced substrate activity, can be identified.

In the first screening round using wheat amylose, 'winners' have been identified (**Fig. 1**). Testing these and 'winners' from subsequent screening in small–scale industrial pilot liquefaction processes is now required and is the aim of this MSD project.

Work plan
The MSD student will work towards meeting the following objectives:

- Create expression constructs for heterologous production of AA13 LPMO 'winners' identified from the *in vivo* screen using established methods. **(months 1-3)**
- Purify and characterise the 'winners' using structural (X-ray crystallography) and biophysical techniques (differential scanning fluorimetry). **(months 3-12)**
- Test the purified 'winners' during a secondment at WeissBioTech, Zwingenberg, using their Brabender Viscograph apparatus for assaying liquefaction. **(month 8)**

References
1. G. Vaaje-Kolstad, *et al. Science*, 2010, 330
2. R. J. Quinlan *et al. Proc Natl Acad Sci U S A*, 2011, 108, 15079
3. S. J. Horn, *et al. Biotech Biofuels*, 2012, 5, 45
4. L. Lo Leggio, *et al. Nat Commun*, 2015, 6, 5961

Entry requirements and application procedures
Highly motivated applicants with, or expecting, a good degree in the broad area of Life Sciences are encouraged to apply.
Applications should be submitted electronically by **24th April 2019** see here for details https://www.essex.ac.uk/pgapply/enter.aspx

You are encouraged to contact the supervisor before application: jworrall@essex.ac.uk and mahough@essex.ac.ukIf you have any queries with the online application process, please contact ecrix@essex.ac.uk

For general information about the School of Biological Sciences at the University please visit our webpages http://www.essex.ac.uk/bs/.

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