# Poster Presentations

**[MS5-P28] A Novel Cellulase for Biofuels Production: Structure of a Marine GH7 Cellobiohydrolase** John E. McGeehan[1], Simon D. Streeter[1], Richard N.A. Martin[1], Amaia Etxabe[1], Graham P. Malyon[1], Simon M. Cragg[1], Marcelo Kern[2], Katrin Besser[2], Luisa Elias[2], Will Eborall[2], Neil C. Bruce[2], Simon J. McQueen-Mason[2], Christina M. Payne3, Gregg T. Beckham[4], Michael E. Himmel[4], Kirk Schnorr[5]

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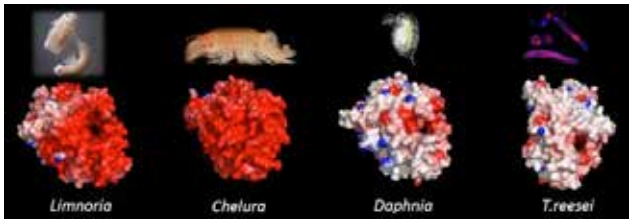
There is strong pressure to diversify feedstocks used for biofuel generation, to avoid competition with food crops. In particular, there is increasing emphasis on the utilisation of woody (lignocellulosic) materials that are recalcitrant to degradation. Prospecting for enzymes capable of overcoming this recalcitrance has focused on the relatively few types of microorganisms and animals that have wood-degrading capability. Of particular interest are the GH7-family enzymes that convert cellulose polymers to cellobiose units, the central step in the production of glucose for downstream fermentation to bioethanol. Currently, fungal GH7 enzymes represent the main hydrolytic component in the majority of industrial cocktails, however, their high cost represents a significant barrier to the commercial viability of large-scale biofuel production. We are therefore exploring the rich resource of endogenous lignocellulose-degrading enzymes in wood boring crustaceans that achieve breakdown without the help of microbial mutualists. Unlike animals such as termites that employ a complex community of microbial flora to produce digestive enzymes, the marine crustacean *Limnoria quadripunctata* has a sterile gut and produces all the necessary enzymatic machinery to efficiently digest these difficult substrates. A successful collaboration between Portsmouth and York Universities, NREL in Golden, USA and Novozymes in Denmark has resulted in the detailed characterisation of the first animal cellobiohydrolase, LqCel7B. Following a transcriptomic analysis [1], we selected a GH7 family enzyme that was highly expressed in the hepatopancreas digestive gland. Biophysical analysis revealed a stable monomeric protein and extensive crystallisation trials produced well diffracting crystals. Four structures have been solved to date, one apo form and three with various bound ligands occupying the active site tunnel. A complex with the inhibitor thiocellobiose diffracted to 1.1 Å resolution. These structures provided the basis for structural comparisons and molecular dynamic simulations and have revealed a host of novel features with industrial potential [2].




The figure shows the surface charge comparisons of LqCel7B, homology models of *Chelura terebrans* and *Daphnia magna* GH7s (based on the Limnoria structure) and the well characterised structure of the industrially significant fungal *Trichoderma reesei* Cel7A. The electrostatic potential between -7kT/e and 7kT/e is shown as a colored gradient from red (acidic) to blue (basic). The marine enzymes from Limnoria and Chelura reveal a highly acidic surface, a characteristic often observed in halophilic enzymes. The Daphnia example, although closely related phylogenetically, displays a more common neutral surface with basic and acidic patches, possibly a consequence

of its fresh water origin. We have shown that the Limnoria enzyme has extreme salt tolerance and stability, remaining active at 4M NaCl concentrations, a likely consequence of evolution in the marine environment. From an industrial standpoint, robust marine enzymes that degrade recalcitrant lignocellulosic substrates represent a new space for prospecting enhanced properties. This and the other novel properties of this enzyme will be discussed.

[1] King, A.J. et al (2010) *PNAS* **107**(12):5345-5350
[2] Kern, M, McGeehan, J. et al (2013) *PNAS* (in press)

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