Expanding the scope of enzyme biocatalysis through chemical modification and ionic liquids

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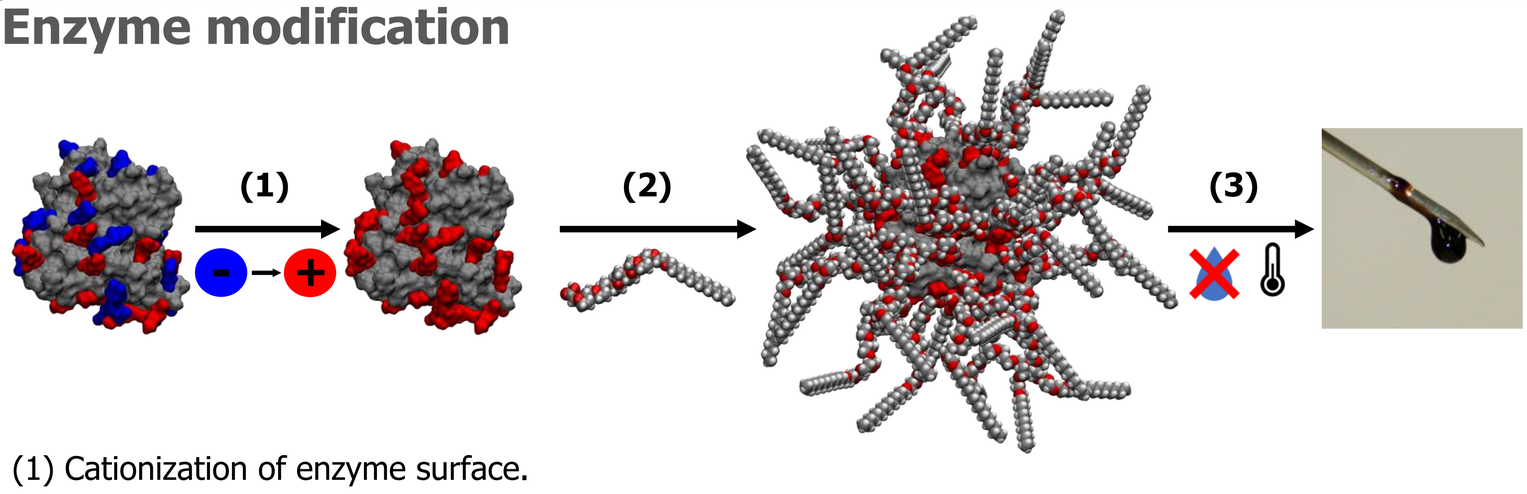
Introduction

Enzymes can perform many industrially relevant reactions with high specificity and efficiency. Recent successes in engineering have significantly broadened substrate scope. Despite this, effective enzyme-based biocatalysis largely remains limited by the aqueous solubility of substrates.

Ionic liquids are highly versatile solvents with tuneable and widely favourable properties. Particularly, ionic liquids can solvate a much larger range of substrates than conventional solvents, including otherwise recalcitrant polymers such as those involved in plastic production.

Here, we present a general chemical modification strategy for ionic liquid stability to **unlock new reactivities of enzymes towards otherwise recalcitrant molecules**. In doing so, we provide a blueprint for broad up-take of biocatalysis.

Enzyme modification

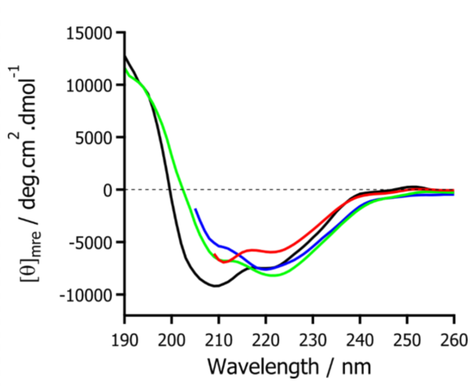


(1) Cationization of enzyme surface.

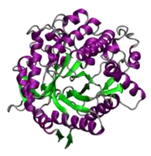
(2) Nanoconjugate formation *via* electrostatic complexation with anionic surfactants.

(3) Lyophilization and annealing to form solvent-free liquid enzyme, which is soluble in ionic liquids.

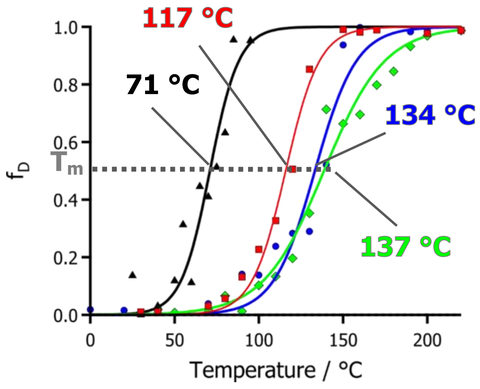
Biomass processing



Key: **Aqueous**
[bmpyrr][OTf]
[bmpyrr][NTf$_2$]
[bmpyrr][OAc]

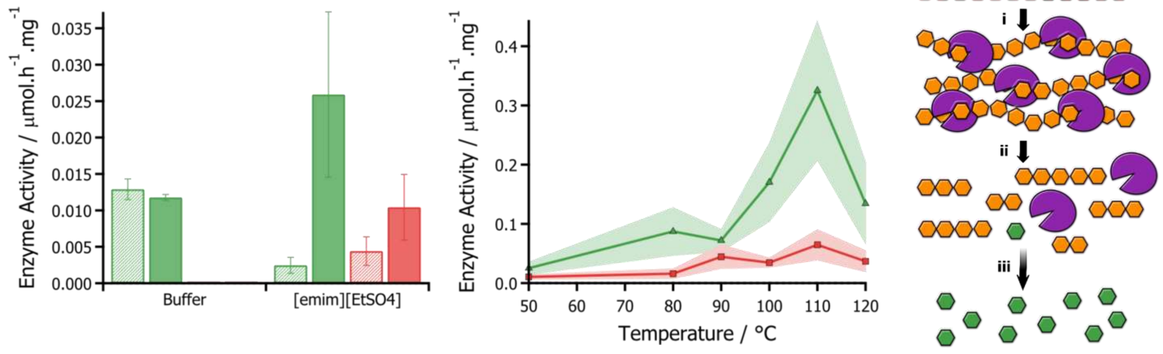


Glucosidase



- Chemical modification maintains enzyme structure in ionic liquids.
- Thermal stability drastically improved after modification and in ionic liquids.

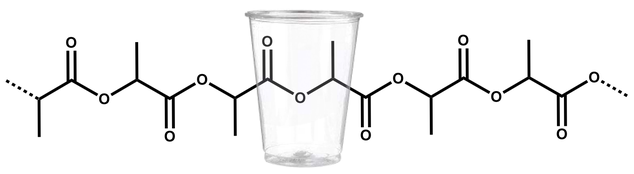
Substrates: **Cellobiose** **Cellulose**



- Ionic liquid unlocks unseen reactivity of modified glucosidase towards cellulose.
- Optimum operating temperature shifts to 110 °C with 30x increase in activity.

Plastic depolymerisation

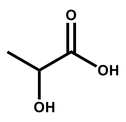
Lipase (CaLB)



Poly(lactic acid)

Ionic liquid

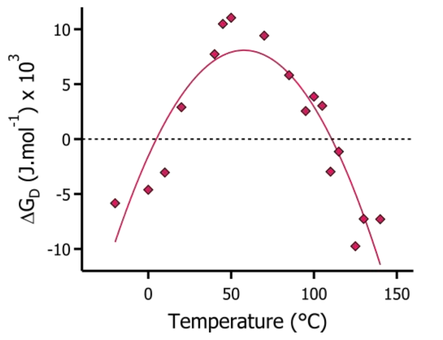
T > T_g

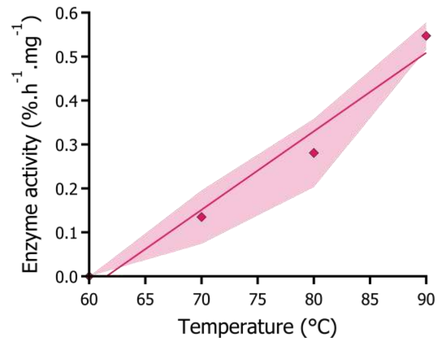


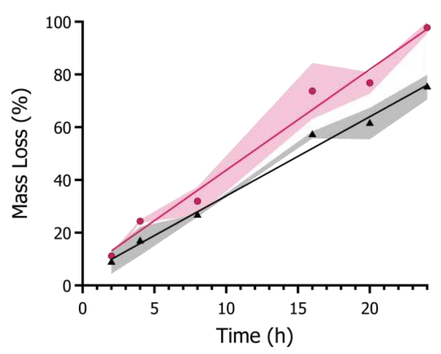
Lactic acid

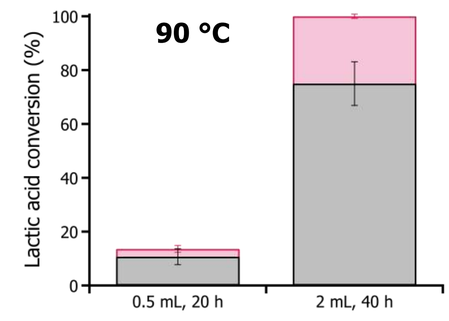
- Enzyme stabilization strategy allows for high activity in ionic liquids at high temperatures.
- Modified lipase in [emim][OAc] can degrade PLA within 24 h with full depolymerisation within 40 h.
- Synergistic effect of enzyme and ionic liquid for plastic recycling.

Key: **[emim][OAc]**
Enzyme + IL



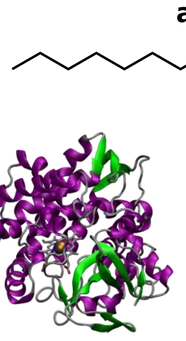






Biofuel production

Long chain fatty acid (>C16)



Decarboxylase ($OleT_{RN}$)

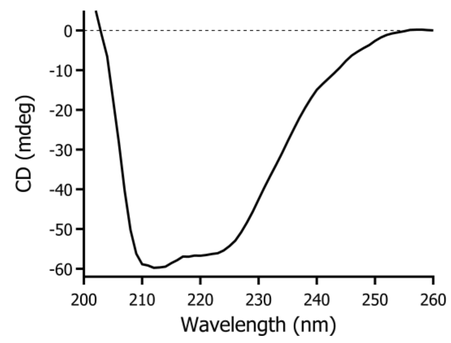
[emim][OAc]
365 nm
>60 °C

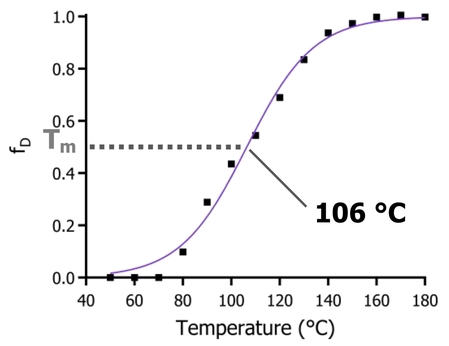
+ CO_2

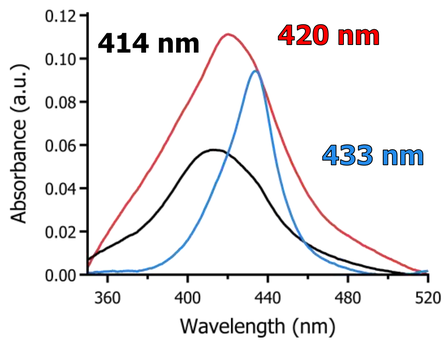
Drop-in biofuel

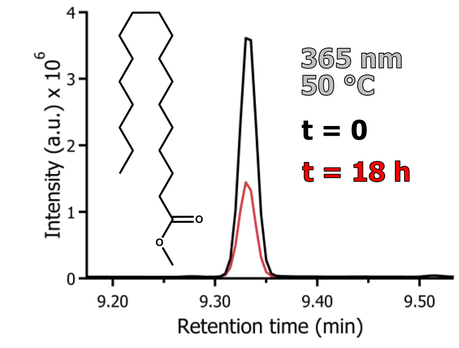
- [C-OleT_{RN}][S] soluble with retained secondary structure in ionic liquids.
- High thermal stability in [emim][OAc]

- UV reduction of heme in [emim][OAc].
- Active enzyme in presence of O_2
- Decarboxylation of fatty acid in ionic liquid yields alkene.
- Ionic liquid allows for substrate concentrations far higher than possible in water—process intensification.









Conclusions

Chemical modification of enzymes to yield solvent-free liquids have shown to be a robust methodology for stabilizing enzymes against temperature and non-aqueous environments. Here, solubilizing stabilized enzymes in ionic liquids has been shown as a blueprint for significantly enhancing biocatalysis.

In particular, we have shown that chemical modification of the ubiquitous enzyme lipase allows for highly efficient depolymerisation of post-consumer PLA. Furthermore, ionic liquids allow for the P450 decarboxylase $OleT_{RN}$ to operate at significantly higher substrates, and remarkably bypassing the requirement for H_2O_2 through UV reduction of heme group.

References

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