Surface-modified hydrolytic enzymes for non-aqueous biocatalytic degradation of plastic polymers

Isabella Ewell, Jake H. Nicholson, Annika Samuel, Susana M. Meza Huaman and Alex P. S. Brogan.
Department of Chemistry, Faculty of Natural and Mathematical Sciences, King's College London, Britannia House, 7 Trinity Street, London, SE1 1DB, UK

Introduction and Project Aims

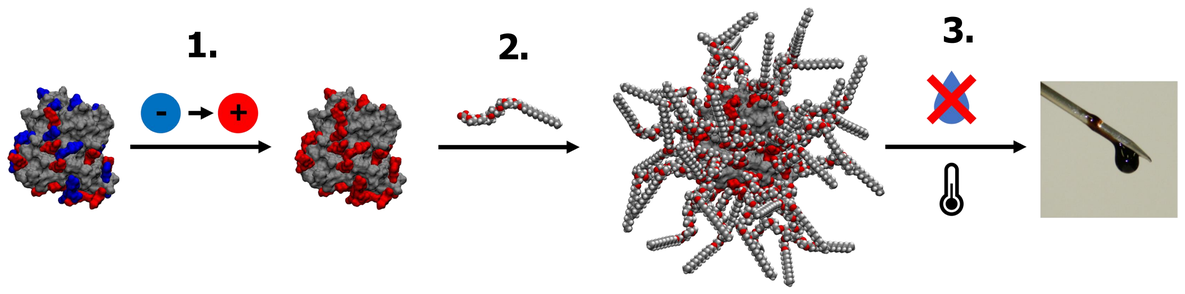
Enhancing enzymatic biocatalysis for plastic degradation has been explored through site-directed mutagenesis and enzyme immobilisation.[1,2] However, limited plastic substrate solubility and compromised enzyme activity hamper the scalability of these processes.

Previous work in the Brogan group has shown that enzyme-surfactant nanoconjugates, known as a 'solvent-free liquid proteins,' display catalytic activity at higher temperatures in non-aqueous solvents, such as ionic liquids (ILs).[3,4]

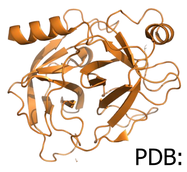
This project explores the modification of trypsin and pig liver esterase to investigate their plastic-degrading activity in ILs, for the breakdown the synthetic plastics polyurethane (PU) and polyethylene terephthalate (PET), respectively.

Enzyme Modification

1. Cationization of Glu and Asp residues on protein surface.
2. Electrostatic complexation with anionic surfactant to form protein-surfactant nanoconjugate.
3. Lyophilization and thermal annealing to form solvent-free liquid protein.

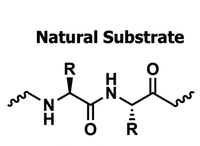


Trypsin from porcine pancreas



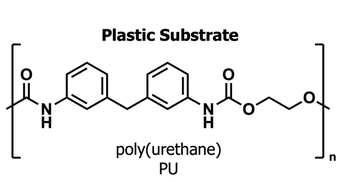
PDB: 1S81

Natural Substrate



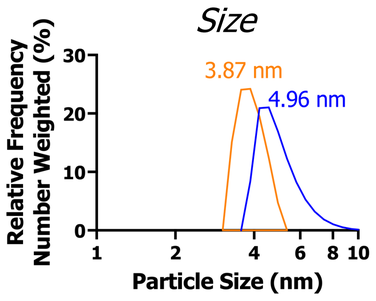
R = Arg, Lys

Plastic Substrate

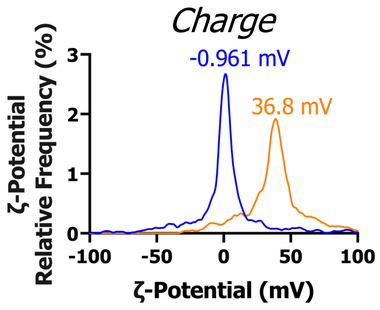


Effects of modification on enzyme **structure**:

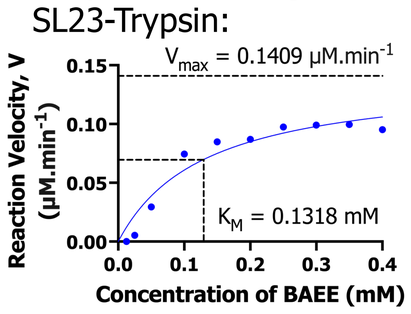
Size



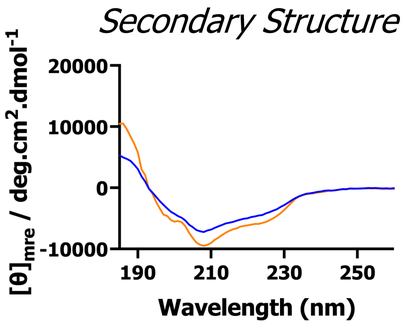
Charge



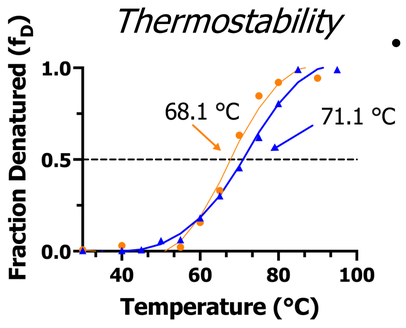
Enzyme **activity** of SL23-Trypsin:



Secondary Structure



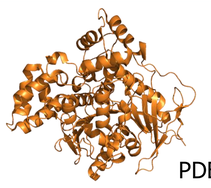
Thermostability



WT-Trypsin SL23-Trypsin

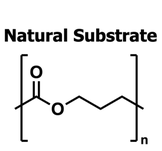
- Increase in particle size, retention of secondary structure features and slight increase in thermostability indicate successful modification of the enzyme.

Pig Liver Esterase (PLE)

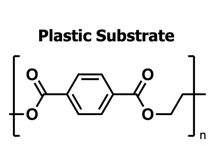


PDB: 5FV4

Natural Substrate

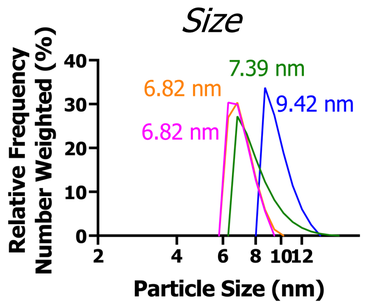


Plastic Substrate



Effects of modification on enzyme **structure**:

Size



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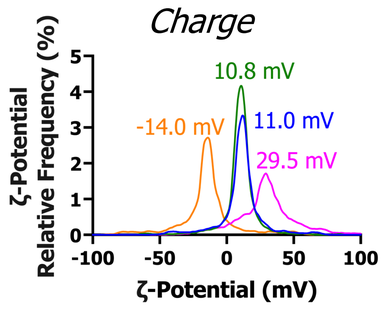
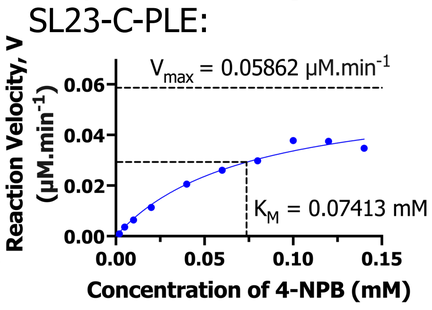
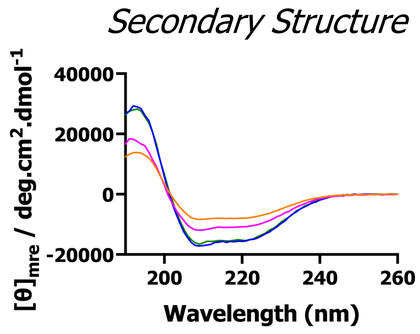


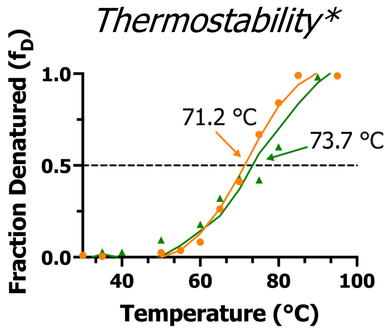
Enzyme **activity** of SL23-C-PLE:



Secondary Structure



*Thermostability**



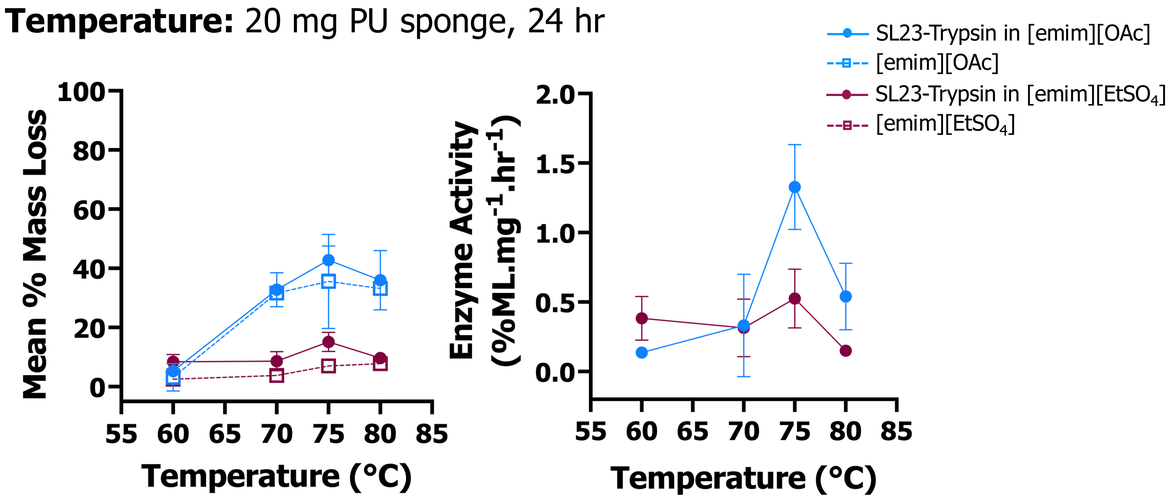
WT-PLE C-PLE SL23-C-PLE GAELE-C-PLE

- PLE modifications were more successful for SL23 than GAELE.
- SL23 surfactant selected for plastic degradation studies.

*C-PLE and SL23-C-PLE did not denature in temperature range.

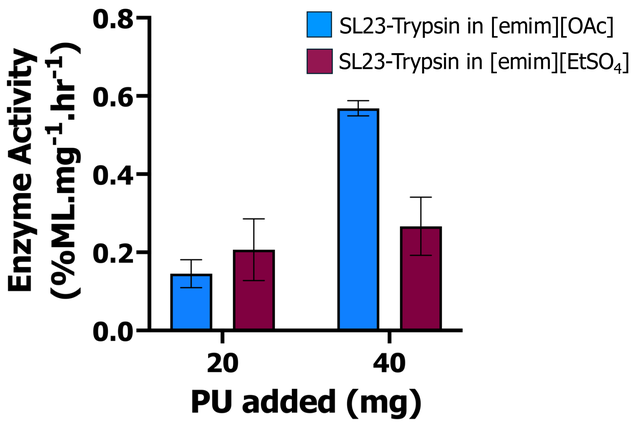
PU degradation by SL23-Trypsin

Temperature: 20 mg PU sponge, 24 hr



- Enzyme displays greater polymer degradation and enzyme activity in the more hydrophilic IL, [emim][OAc], in line with previous studies, particularly at 75 °C.[3]

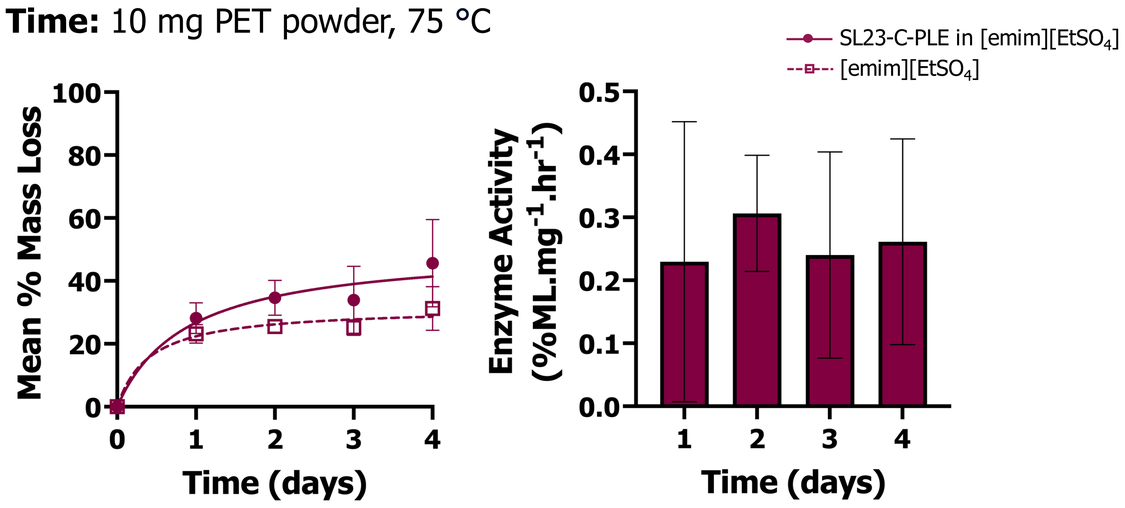
Substrate Loading: PU sponge, 75 °C, 48 hr



- Increasing substrate loading more than doubles enzyme activity in [emim][OAc] after 2 days.
- This suggests a lag phase occurs during the first day of the degradation where the IL is dissolving polymer.

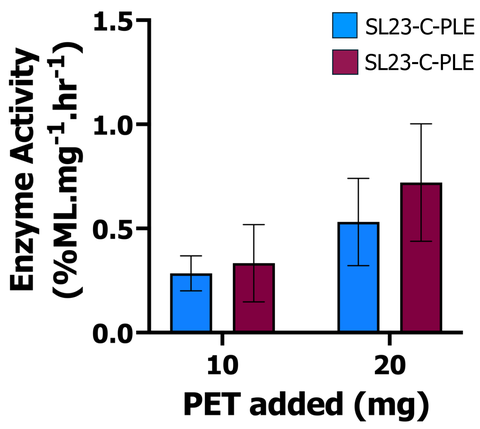
PET degradation by SL23-C-PLE

Time: 10 mg PET powder, 75 °C



- Enzyme activity generally increases with longer time points, perhaps due to enzyme acting on oligomers, which will be investigated by HPLC.

Substrate Loading: PET powder, 75 °C, 24 hr



- Increasing substrate loading doubles enzyme activity in [emim][$EtSO_4$] after only 1 day.
- Future work will investigate whether enzyme activity continues increasing with increased substrate loading at longer time points.

Conclusions and Future Work

- Two hydrolytic enzymes, trypsin and PLE, have been successfully modified and exhibit some ability to degrade PU and PET, respectively.
- While the more hydrophilic IL, [emim][OAc], is more effective at solubilising plastic substrates, it may disrupt enzyme activity, particularly of SL23-C-PLE.
- Future work will focus on characterising degradation products by HPLC and MALDI-TOF to determine a biocatalytic mechanism of plastic degradation.

References

1. Y. H. Chai *et al.* Immobilized Enzyme/Microorganism Complexes for Degradation of Microplastics: A Review of Recent Advances, Feasibility and Future Prospects. *Sci. Tot. Environ.* **2022**, 832, 154868.
2. H. Zhao *et al.* Directed Evolution: Past, Present, and Future. *AIChE Journal.* **2013**, 59(5), 1432-1440.
3. S. M. Meza Huaman *et al.* A General Route to Retooling Hydrolytic Enzymes toward Plastic Degradation. *Cell Rep. Phys. Sci.* **2024**, 5(2), 101783.
4. J. H. Nicholson *et al.* Enhancing the reactivity of a P450 decarboxylase with ionic liquids. *Green Chem.* **2025**, 27, 517-526.