UNIVERSITY OF PORTSMOUTH
CENTRE FOR ENZYME INNOVATION

Disposing of Plastic Nurdles - Are Enzymes the Answer?

Prof Andy Pickford, Director,
Centre for Enzyme Innovation, Portsmouth, UK

ITAC 2023

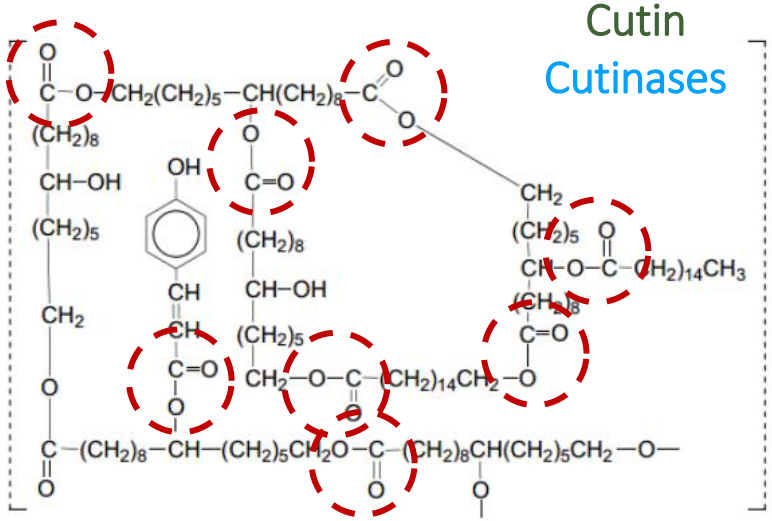
Nature Recycles Everything

Enzymes are Nature's Recyclers

- For every natural polymer, natural depolymerase enzymes exist to break them down.

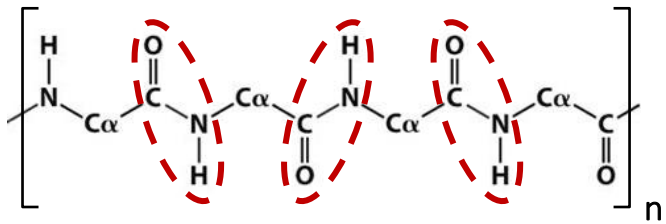
Cutin

Cutinases

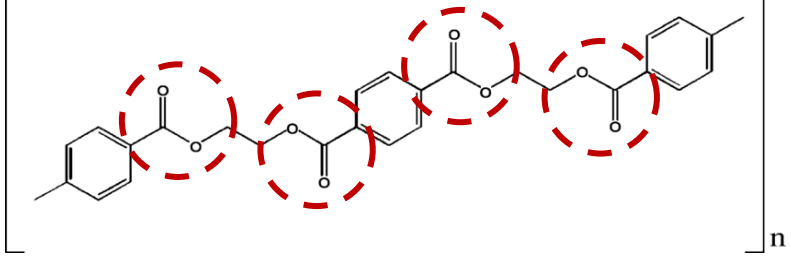


Protein

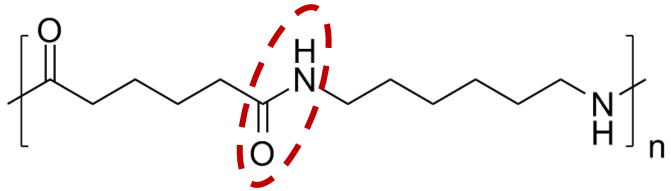
Proteases



polyethylene terephthalate (PET)

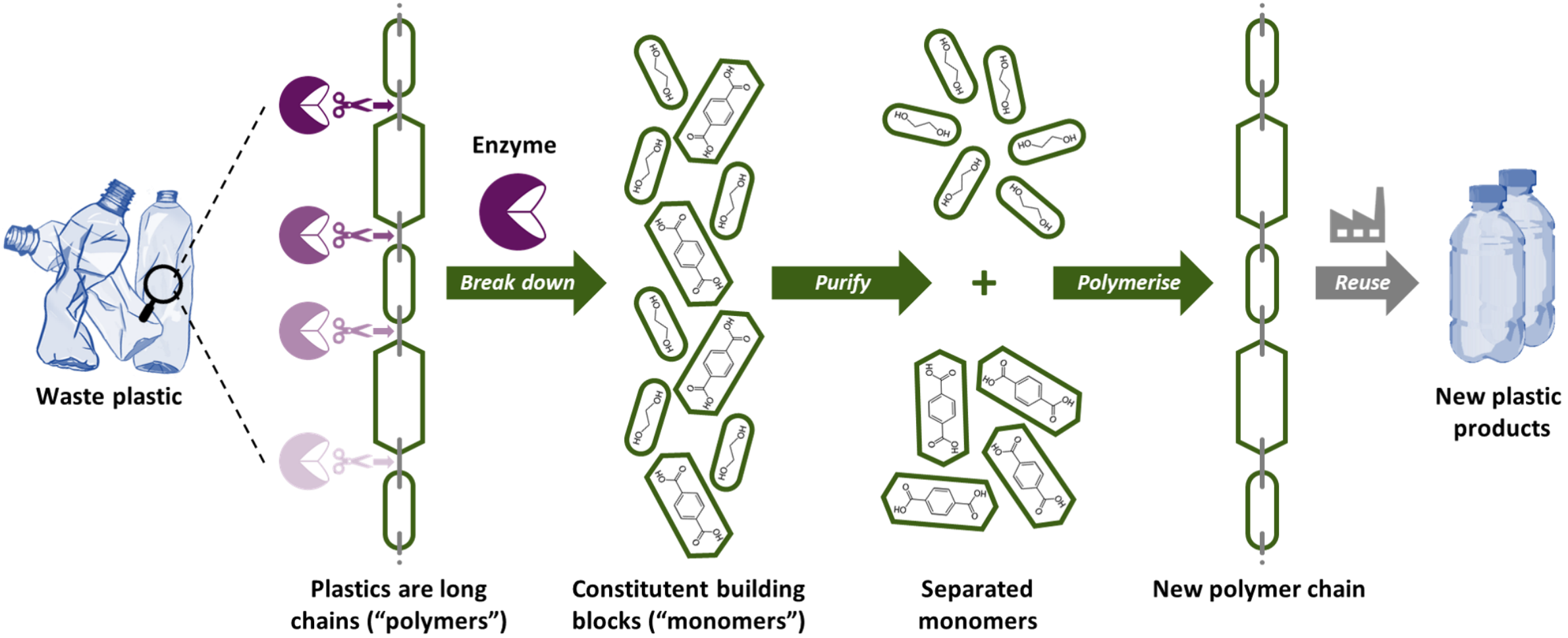


Nylon-6,6



- So, can we use enzymes to breakdown synthetic polymers with similar chemical bonds?

The Objective



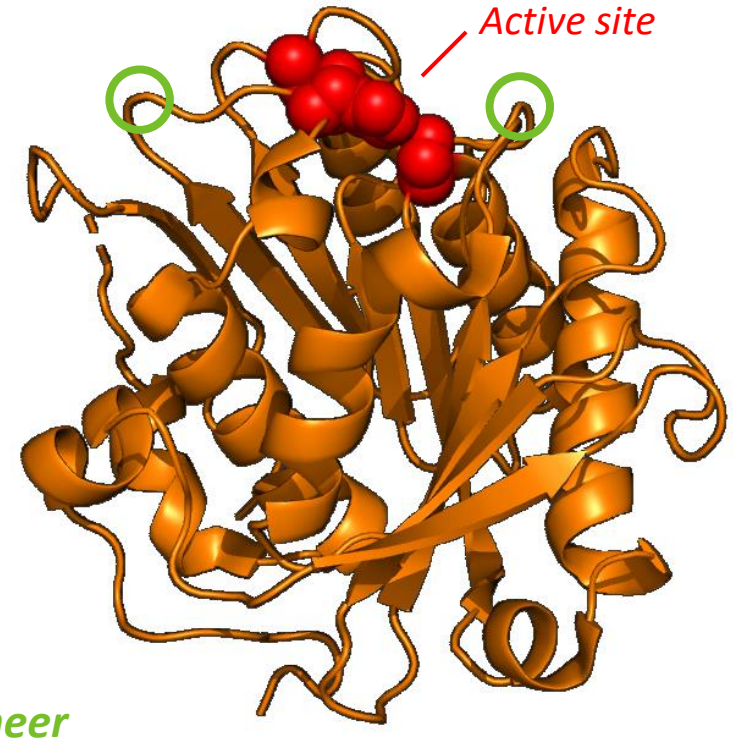
- For tackling plastic waste, we need to **engineer enzymes that are efficient and robust.**

Engineering Enzymes

- An enzyme's sequence determines its properties : structure, activity & stability.

```
atgcagaccaccgtgaccaccatgc
tcctcgcgtccgtagcattagcggc
ttgcgccggaggaggttccactcct
ctgcctctaccgcagcagcagccgc
ctcagcaggaaccgccacctcctcc
tgttccgctagccagtcgcgccgcg
tgtgaggcgctcaaagatggtaatg
gcgacatggtttggccgaatgccgc
cacggttgtagaggttgcagcctgg
cgtgatgcagcaccggccacggcat
cagccgcagccctgccggagcattg
cgaagtatcaggcgcgattgccaag
cgtactgggattgatgggtaccat…
```

```
ANPYERGPNPTDALLEARSGPFS
VSEERASRFGADGFGGGTIYYPR
ENNTYGAVAISGYTGTQASVAWL
GERIASHGFVVITIDTNTTLDQP
DSRARQLNAALDYMINDASSAVR
SRIDSSRLAVMGHSMGGGGTLRL
ASQRPDLKAAIPLTPWHLNKNWS
SVRVPTLIIGADLDTIAPVLTHA
RPFYNSLPTSISKAYLELDGATH
FAPNIPNKIIGKYSVAWLKRFVD
NDTRYTQFLCPGPRDGLFGEVEE
YRSTCPF
```



DNA sequence ← *Amino acid sequence* ← *Enzyme structure*

Engineer

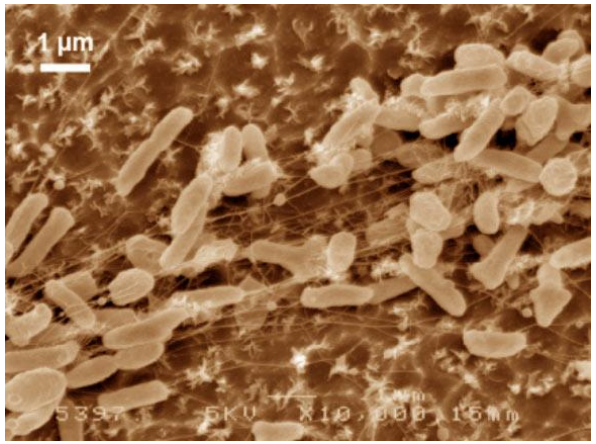
- Once we know the structure we can make informed changes to it via the sequence -> "structure-guided **enzyme engineering**".

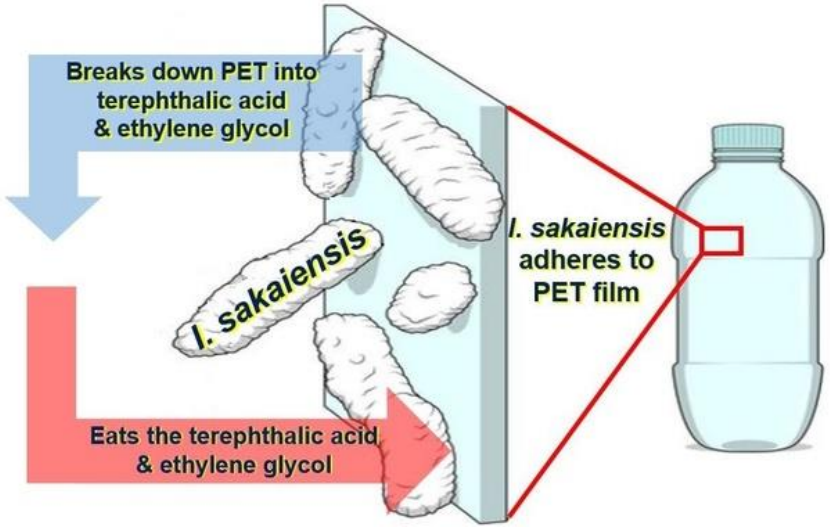
Natural Enzymes Can Break Down PET

- In 2016, a bacterium was discovered that had evolved the ability to “eat” PET.

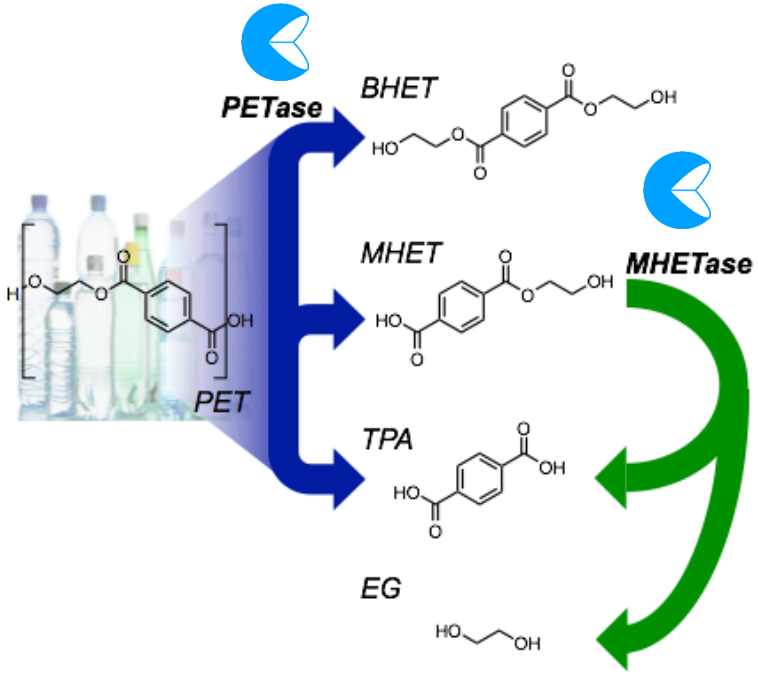
A bacterium that degrades and assimilates poly(ethylene terephthalate)

Shosuke Yoshida,[1,2]* Kazumi Hiraga,[1] Toshihiko Takehana,[3] Ikuo Taniguchi,[4] Hironao Yamaji,[1] Yasuhito Maeda,[5] Kiyotsuna Toyohara,[5] Kenji Miyamoto,[2]† Yoshiharu Kimura,[4] Kohei Oda[1]†



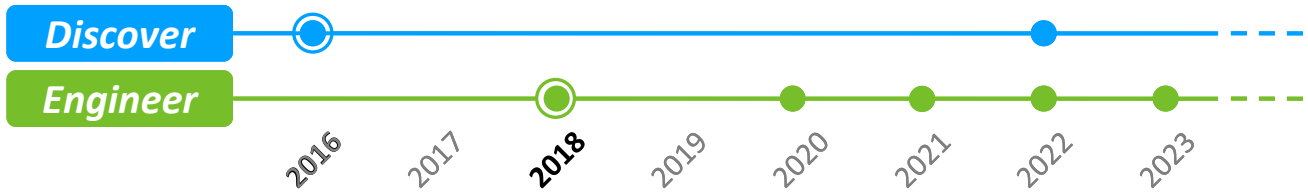


Yoshida *et al.* (2016) *Science*

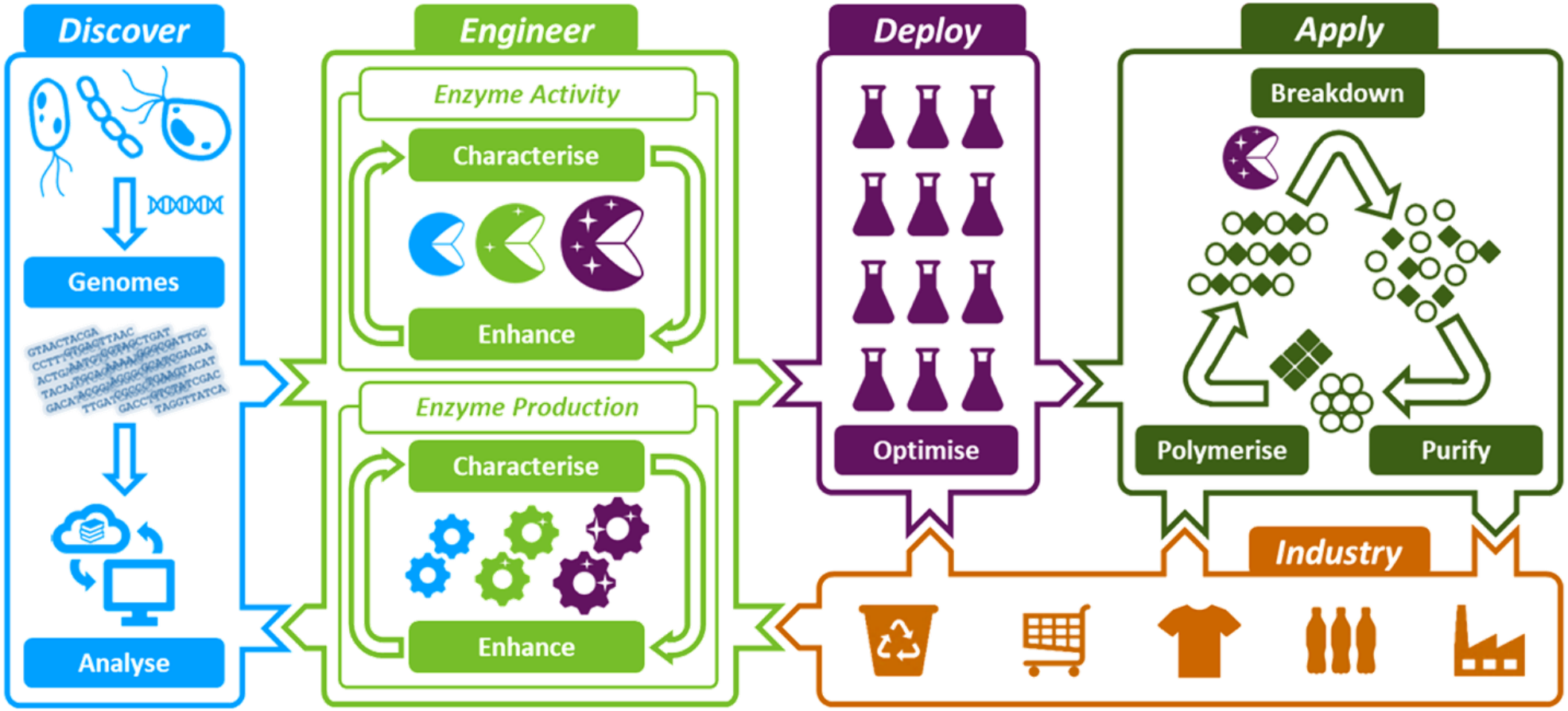


Austin *et al.* (2018) *PNAS*

- In 2018, we engineered its PETase enzyme to make it 20% faster (by accident).



CEI Research & Innovation Pipeline



Research England

Biotechnology and Biological Sciences Research Council

Natural Environment Research Council

SOLENT LOCAL ENTERPRISE PARTNERSHIP

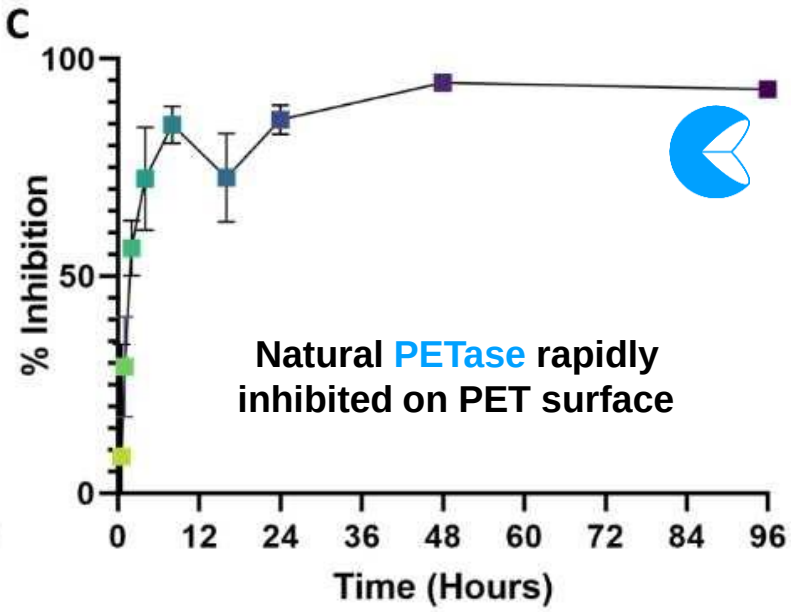
HM Government

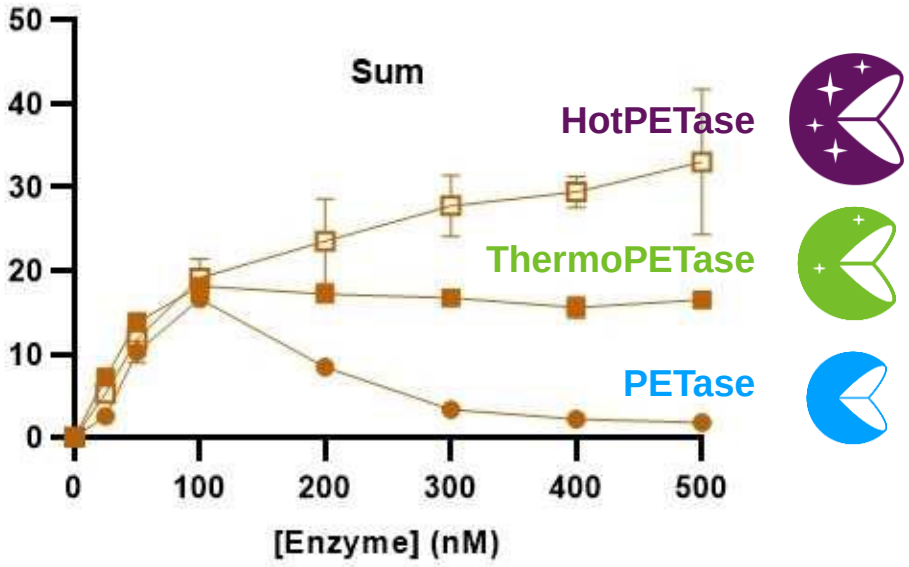
bottle
changing the way we recycle

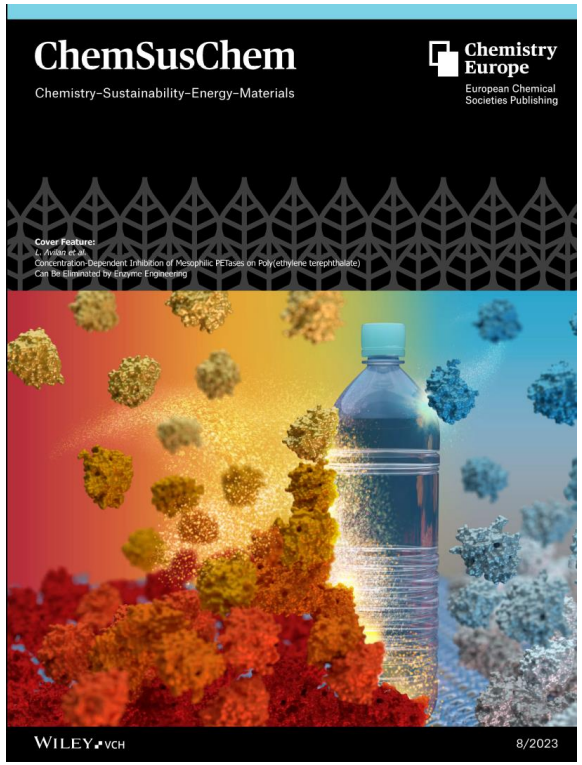
- Awarded £5.8m from Research England (2019) to establish a centre focussed on developing enzymes for plastics recycling, and £1m from Solent LEP (2021) to expand.

Engineered Enzymes Perform Better

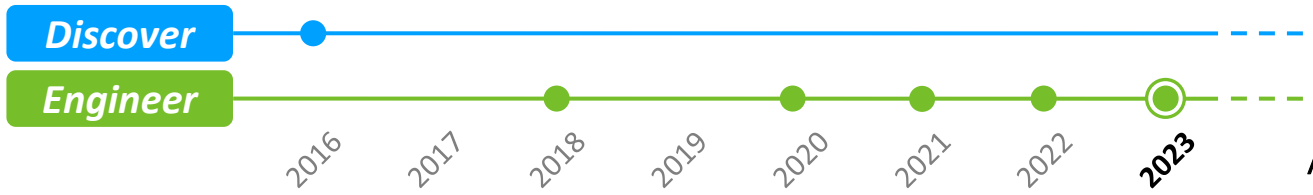
- Natural enzymes, like PETase, often exhibit poor performance on plastic surfaces…







- …but enzymes engineered for thermostability are better at plastic breakdown.

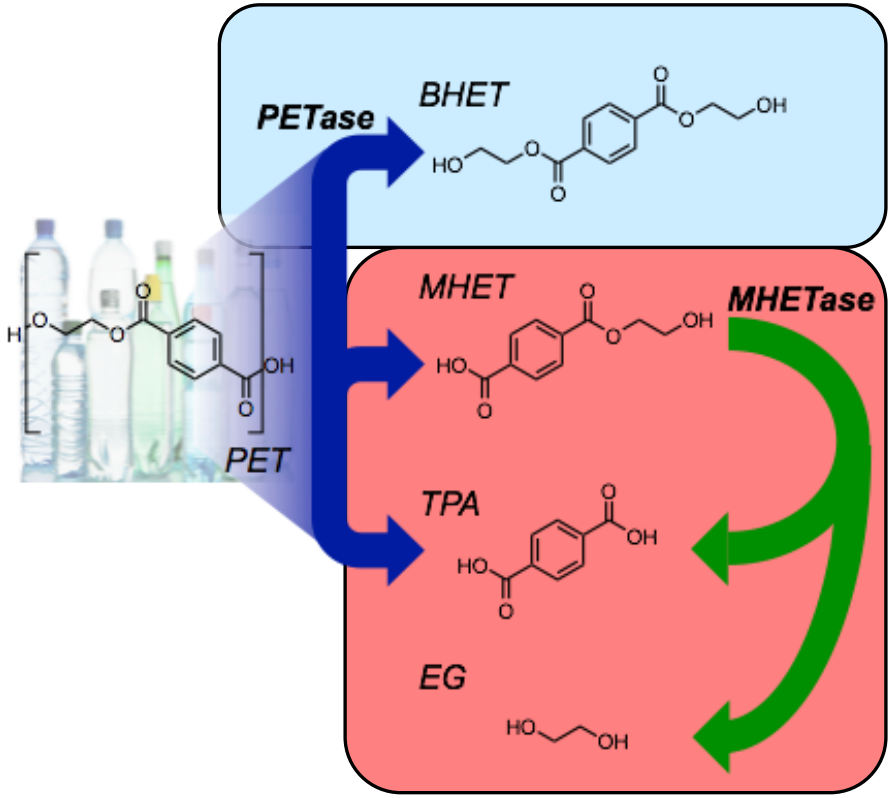


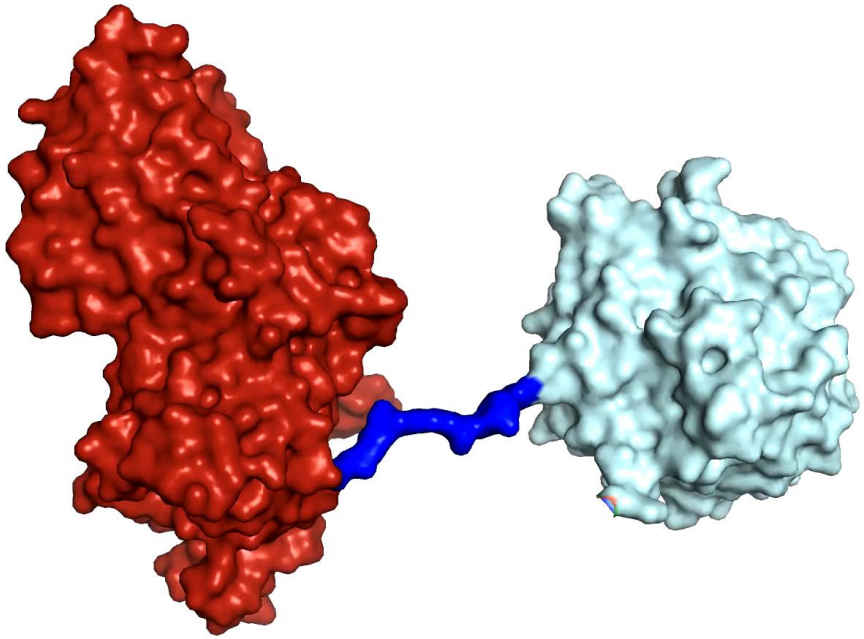
Avilan *et al.* (2023) *ChemSusChem*

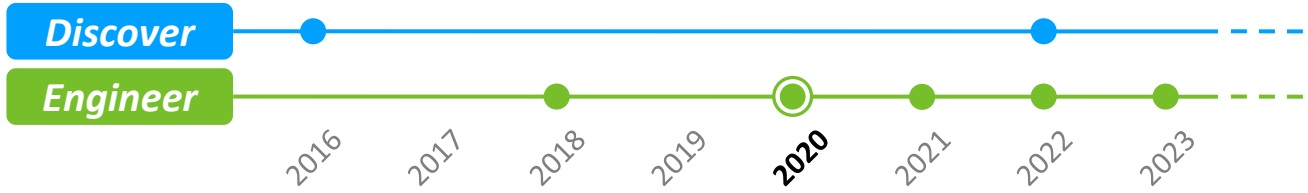
bottle
changing the way we recycle

Development Timeline - PETase

- By linking PETase to MHETase, we increased the rate of PET breakdown 6-fold.







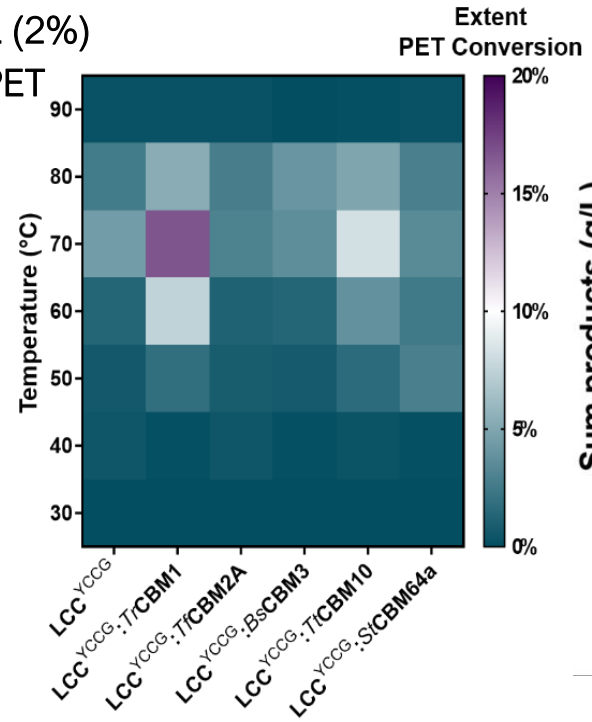
Knott *et al.* (2020) *PNAS*

bottle
changing the way we recycle

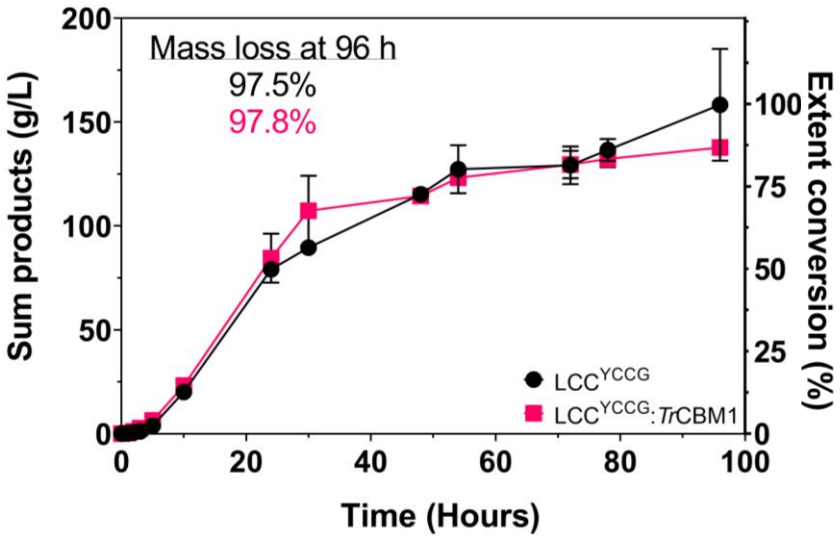
Enzyme under Industrial-like Conditions

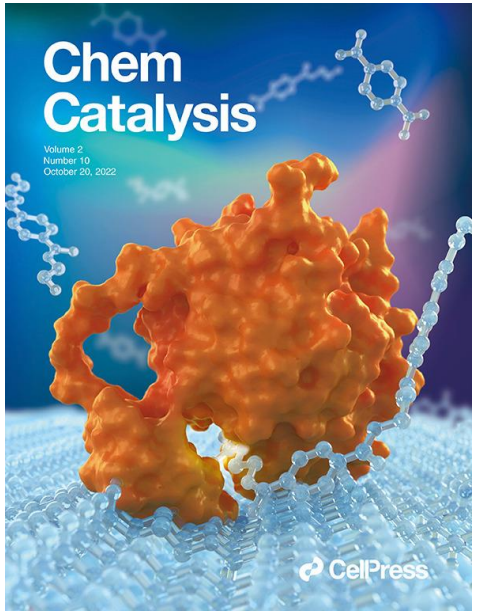
- We assess the PET breakdown at high solids loading (SL) in lab-scale bioreactors.

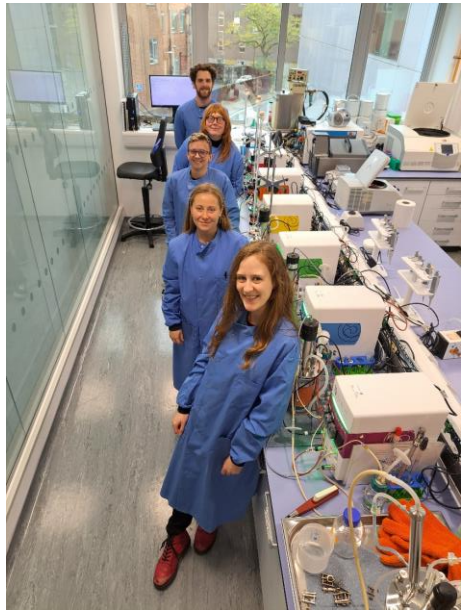
Low SL (2%)
amPET



High SL (20%)
amPET

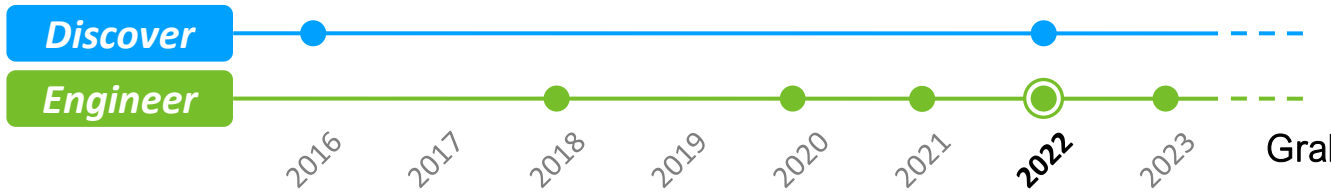






CEI Biorecycling Team

- Sometimes, promising enzymes don't work well under industrial conditions.

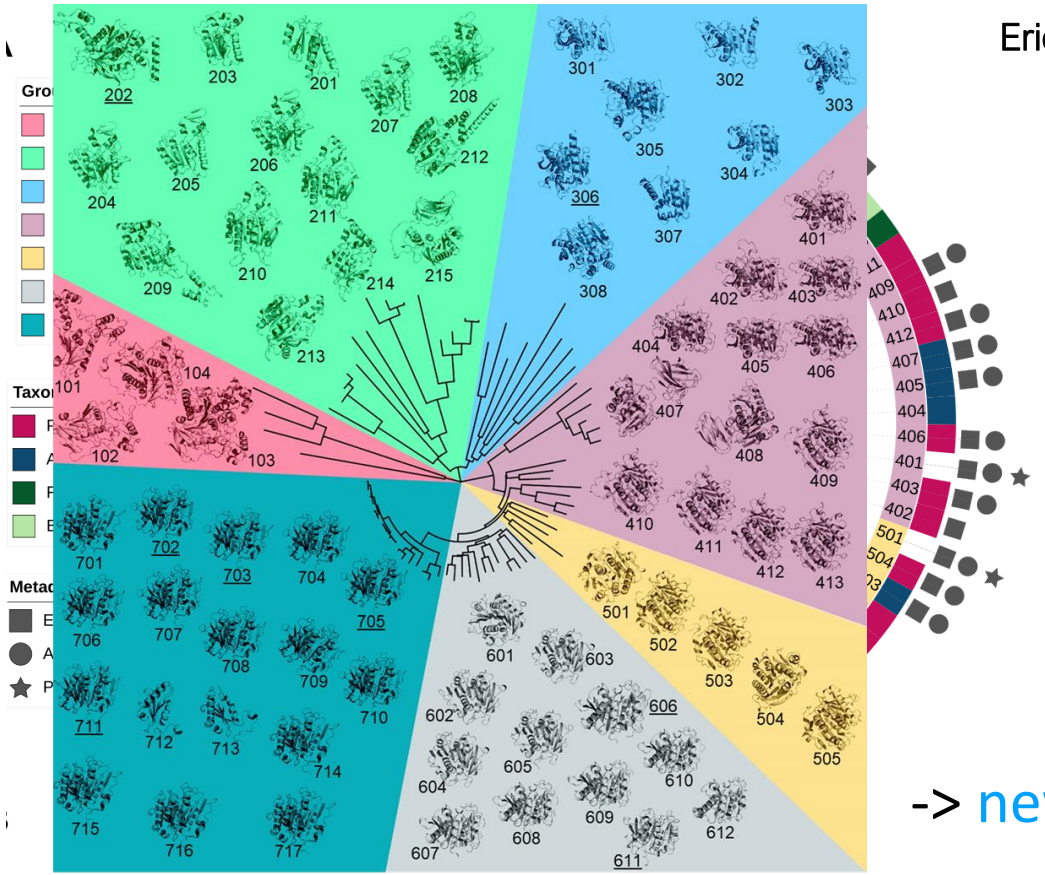


Graham *et al.* (2022) *Chem Catalysis*

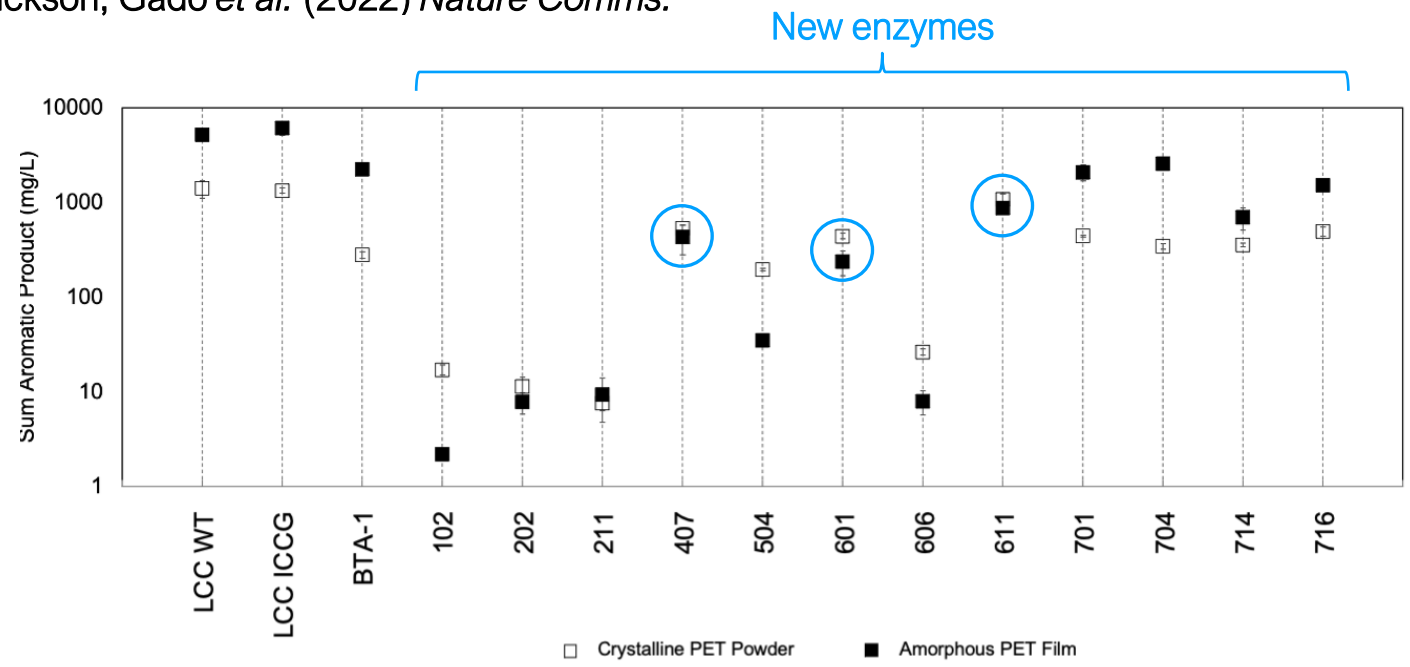
bottle
changing the way we recycle

Are there Crystalline-Tolerant PETases?

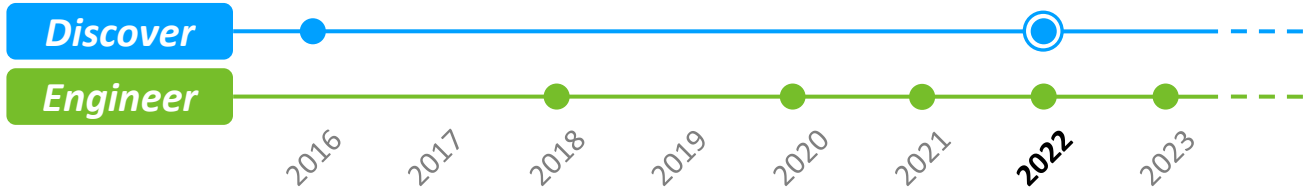
- We used machine learning to find PET depolymerases from their amino acid sequence.



Erickson, Gado *et al.* (2022) *Nature Comms.*



-> new enzymes, some with good activity on crystalline PET.



DeepMind

bottle
changing the way we recycle

Commercialised Enzymatic Recycling

- Pilot plant open. Industrial plant to recycle 50,000 tonnes p.a. PET to open in 2025.

The X-Press Pearl Nurdle Spill

- In 2021, X-Press Pearl sank off Sri Lanka, creating the world's worst nurdle spill.

Source: Sri Lanka Ports Authority

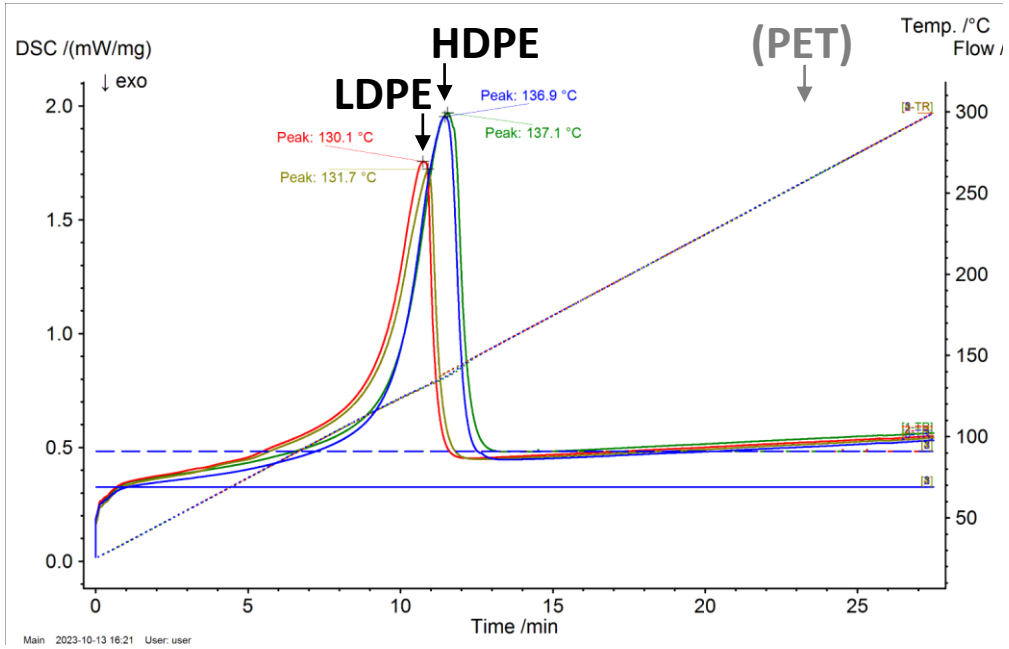
Source: EIA

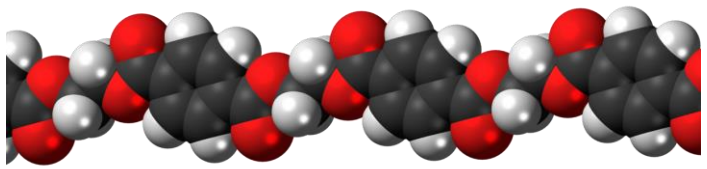
- The ship was carrying 87 containers of plastic nurdles, estimated at ~1,700 tons.
- **Question: Can our PETase enzymes break down the collected nurdles into monomers?**

Enzymes are Polymer-Specific

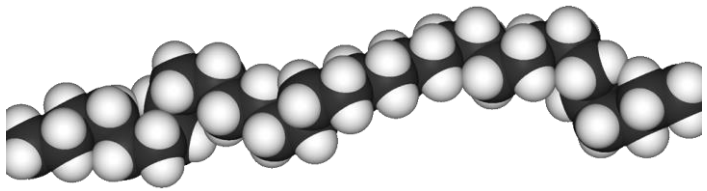
- Our tests revealed the beach-collected nurdles to be polyethylene (PE).

Polymer DSC Analysis





poly(ethylene terephthalate) (PET)



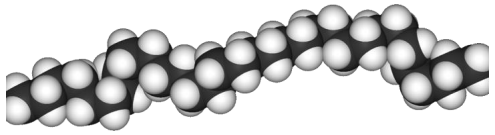
polyethylene (PE)

PETase?

- Unlike PET, PE doesn't have any ester bonds, so PETase-like enzymes won't hydrolyse it.
- **Question: So is there an enzyme that will break down PE into its monomers?**

Does Nature Have a Solution?

- Salivary enzymes from wax worms were reported to degrade PE.



✓?

Article

https://doi.org/10.1038/s41467-022-33127-w

Wax worm saliva and the enzymes therein are the key to polyethylene degradation by *Galleria mellonella*

Received: 11 May 2022

Accepted: 2 September 2022

Published online: 04 October 2022

A. Sanluis-Verdes[1,9], P. Colomer-Vidal[1,9], F. Rodriguez-Ventura[1], M. Bello-Villarino[1], M. Spinola-Amilibia[2], E. Ruiz-Lopez[3], R. Illanes-Vicioso[3], P. Castroviejo[4], R. Aiese Cigliano[5], M. Montoya[6], P. Falabella[7], C. Pesquera[8], L. Gonzalez-Legarreta[8], E. Arias-Palomo[2], M. Solà[3], T. Torroba[4], C. F. Arias[1] & F. Bertocchini[1]

- The enzymes fragment the PE polymer into a soup of chemicals (rather than breaking it into ethylene monomers), so its a less attractive solution from an industrial perspective.
- The natural enzymes are very inefficient but they might be improved by engineering.

Our OSRL-Funded Research Project

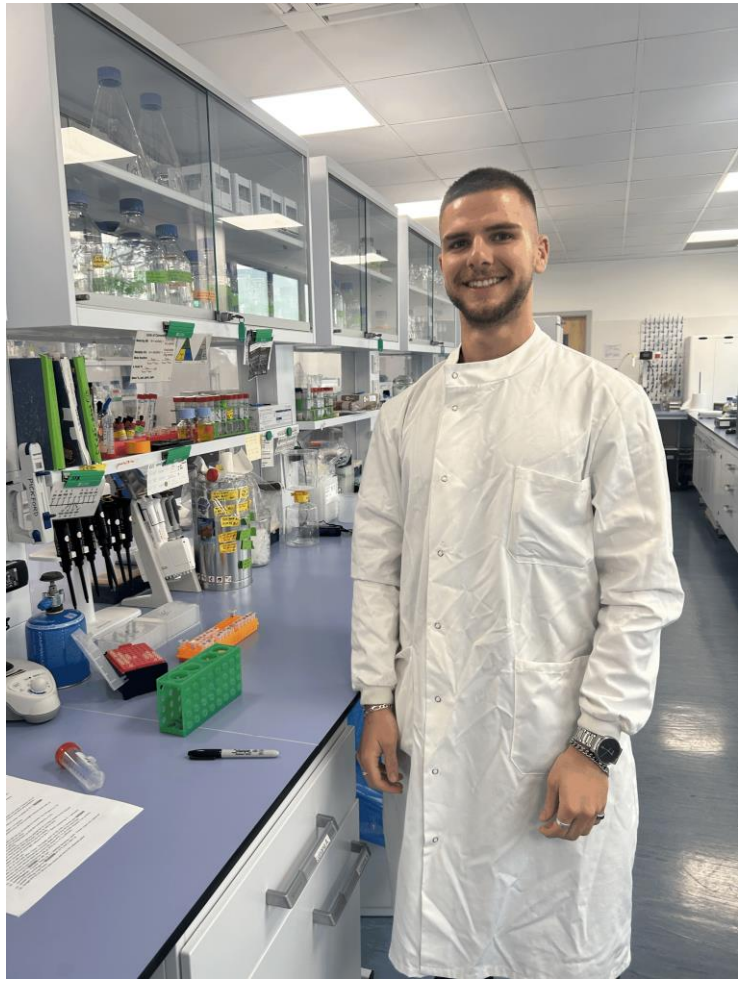
- We've started a 12-month project, funded by OSRL, to explore the potential of wax worm enzymes to breakdown polyethylene (PE).

- The project aims to:-

1. Genetically engineer yeast so that they make the wax worm saliva enzymes.

2. Explore the enzymes' ability to breakdown PE (both LDPE and HDPE).

3. Start engineering the enzymes to improve their ability to breakdown PE nurdles.



Haydon Ross, MRes student

Oil Spill Response

UNIVERSITY OF PORTSMOUTH
CENTRE FOR ENZYME INNOVATION

Thank you for Listening!

- **CEI, Nurdle Project**
  Louise Farrand
  Haydon Ross
  Victoria Bemmer
  Bruce Lichtenstein
  Elaine Rudge, Kerry Green
- **OSRL**
  Rhea Shears, Rob Holland
- **CEI, other projects**
  Luisana Avilan, Tilly Clark, Paul Cox
  Raj Gill, Rosie Graham, Gerhard Koenig,
  John McGeehan, Liliana Oliveira Pessoa,
  Tom Shakespeare, Brooke Wain, Michael Zahn
- **BOTTLE / NREL**
  Greg Beckham, Erika Erickson, Japheth Gado
- **DeepMind**
  Kathryn Tunyasuvunakool, John Jumper

UKRI Research England
UKRI Biotechnology and Biological Sciences Research Council
UKRI Natural Environment Research Council
Oil Spill Response
bottle changing the way we recycle
NREL NATIONAL RENEWABLE ENERGY LABORATORY
diamond
SOLENT LOCAL ENTERPRISE PARTNERSHIP