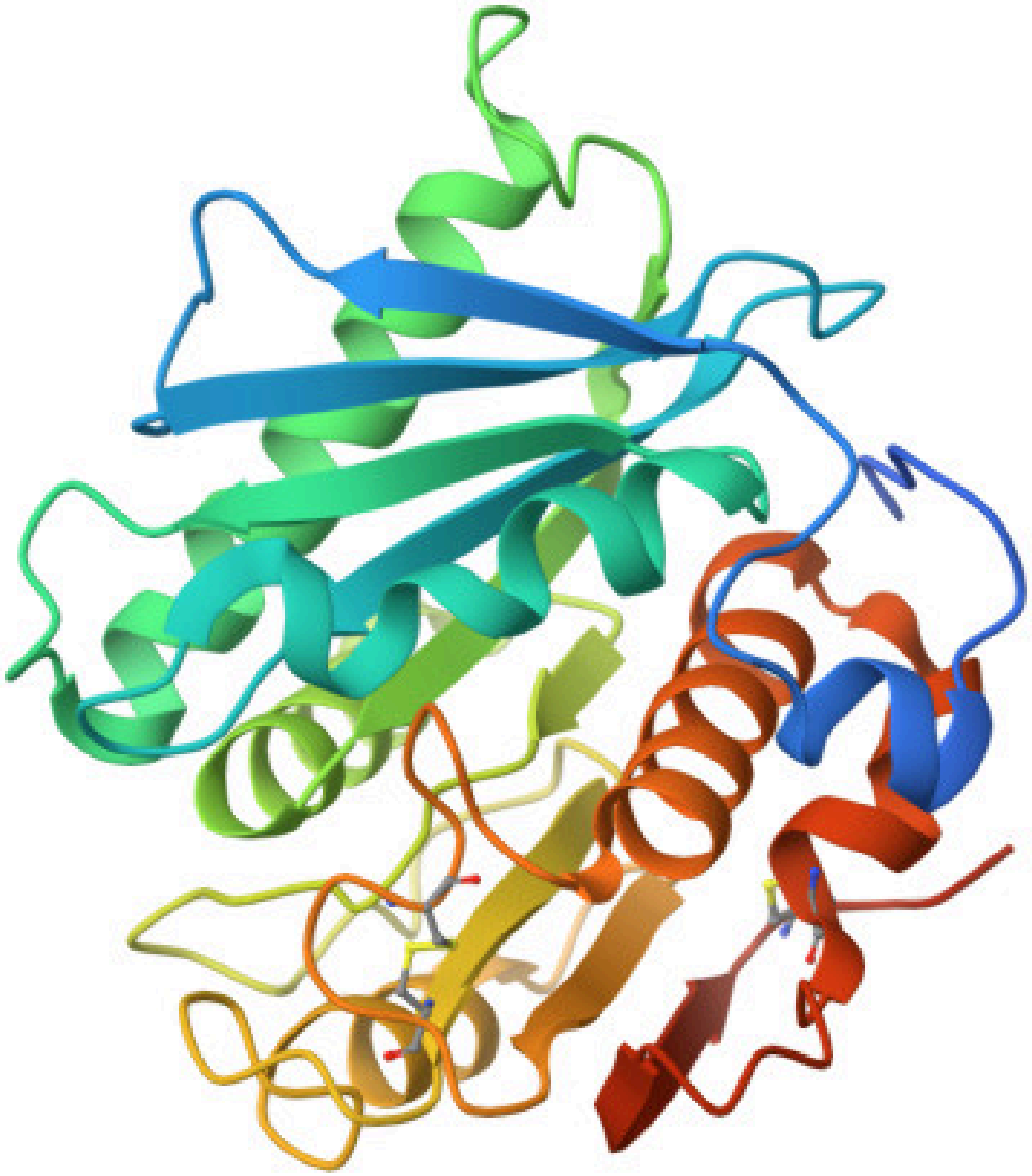
SOLVING THE PLASTIC PROBLEM

Improving PETase for Real World Environments

By Kian Khwaja



Background Research

What are Proteins and Amino Acids?

Proteins are large biomolecules made of chains of amino acid monomers. Different amino acids perform specific functions in enzymes like PETase.

Serine

Acts as reactive amino acid, breaks ester bonds to degrade PET plastic.

Aspartic Acid

Stabilizes histidine through hydrogen bonds and electrostatic interactions.

Histidine

Accepts proton from serine, activating it to break ester bonds.

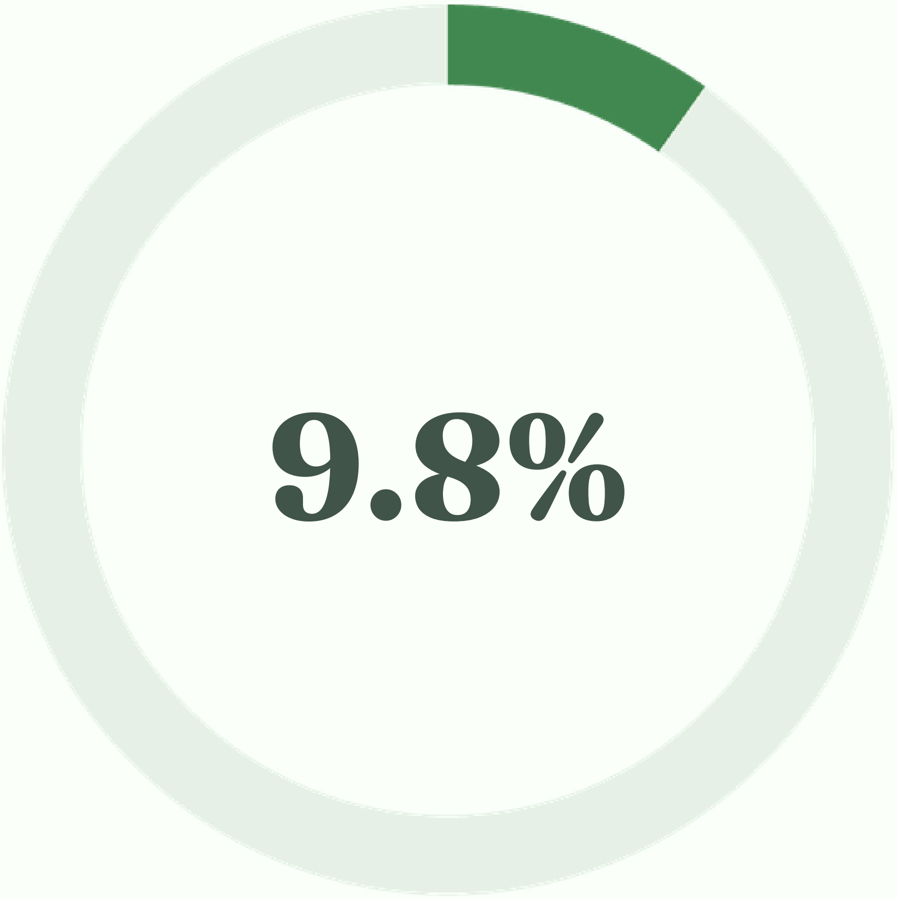
There are 20 different amino acids that combine to create protein sequences.

The Problem with Recycling

Current recycling methods fall short of true circular economy goals.

New Plastic

Plastic produced from newly made materials globally



Recycled Plastic

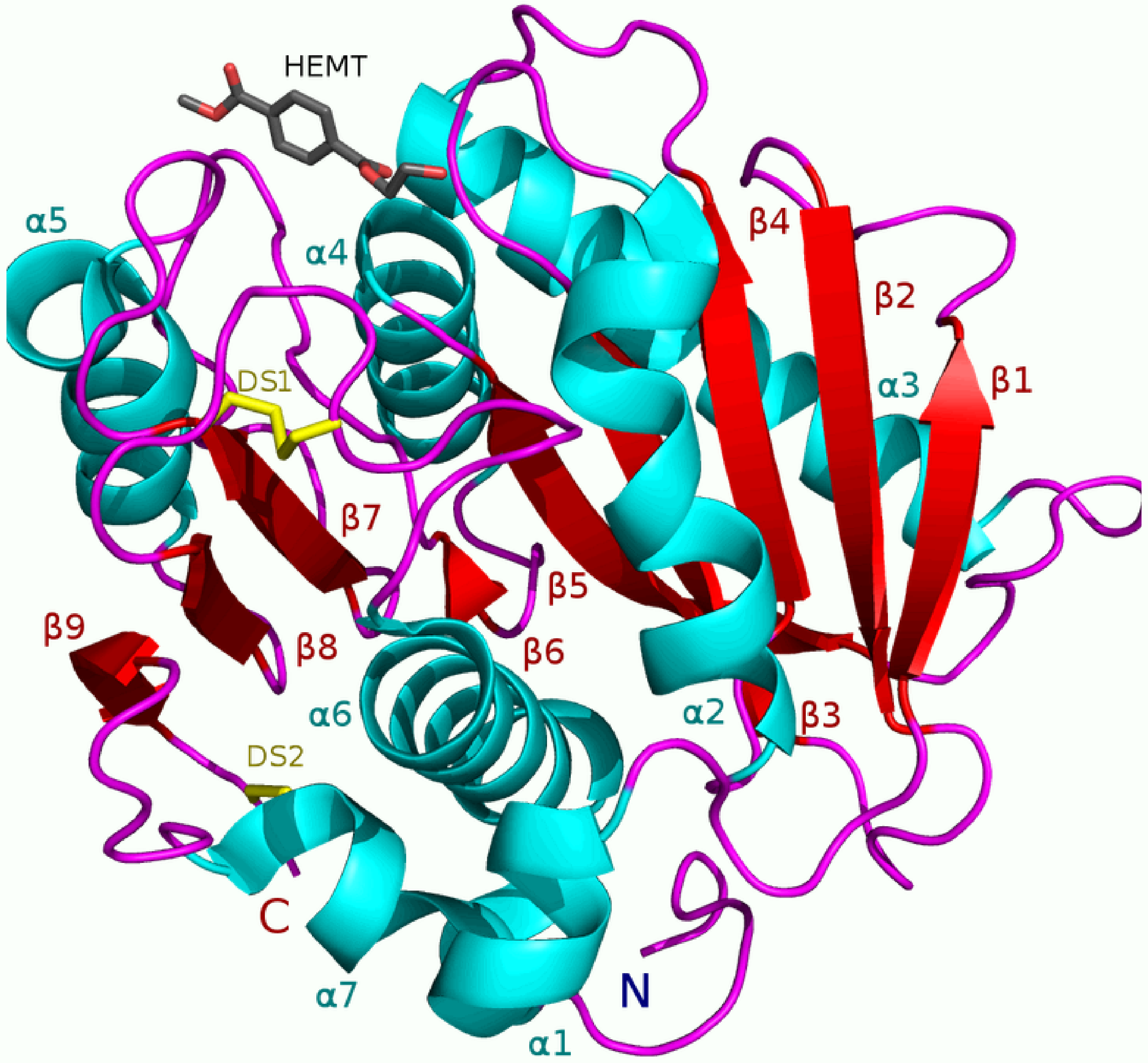
Actual recycled content in production

Quality and durability decrease with each recycling cycle, eventually making plastic unusable and destined for landfills.

What is PETase?

An enzyme discovered in 2016 near a Japanese PET bottle recycling site. PETase can degrade plastic and convert it into its basic form.

Timeframe: 24 hours to 4 weeks depending on plastic density, thickness, quantity, and molecular structure.



How PETase Works

PET Plastic

Starting material

Degration

PETase breaks the ester bonds at molecular level

MHET

After the bonds are broken it turns into MHET

Original Materials

MHETase degrades MHET terephthalic acid and ethylene glycol

The Problem with PETase

pH Sensitivity

Alkaline water levels cause enzyme malfunction and death.

Production Cost

Difficult and expensive to manufacture at scale.

Slow Processing

Fast-PETase (5× faster) degrades only 40% of 0.25mm PET in 4 days at 50°C.

Heat Limitation

Wild PETase max: 40°C. Plastic requires 70-80°C to soften for degradation.

New Versions of PETase

LCC-ICCG-C09

- 600× faster than wild PETase
- Resists up to 97.1°C
- Optimal pH: 7.5-9.5

FAST-PETase

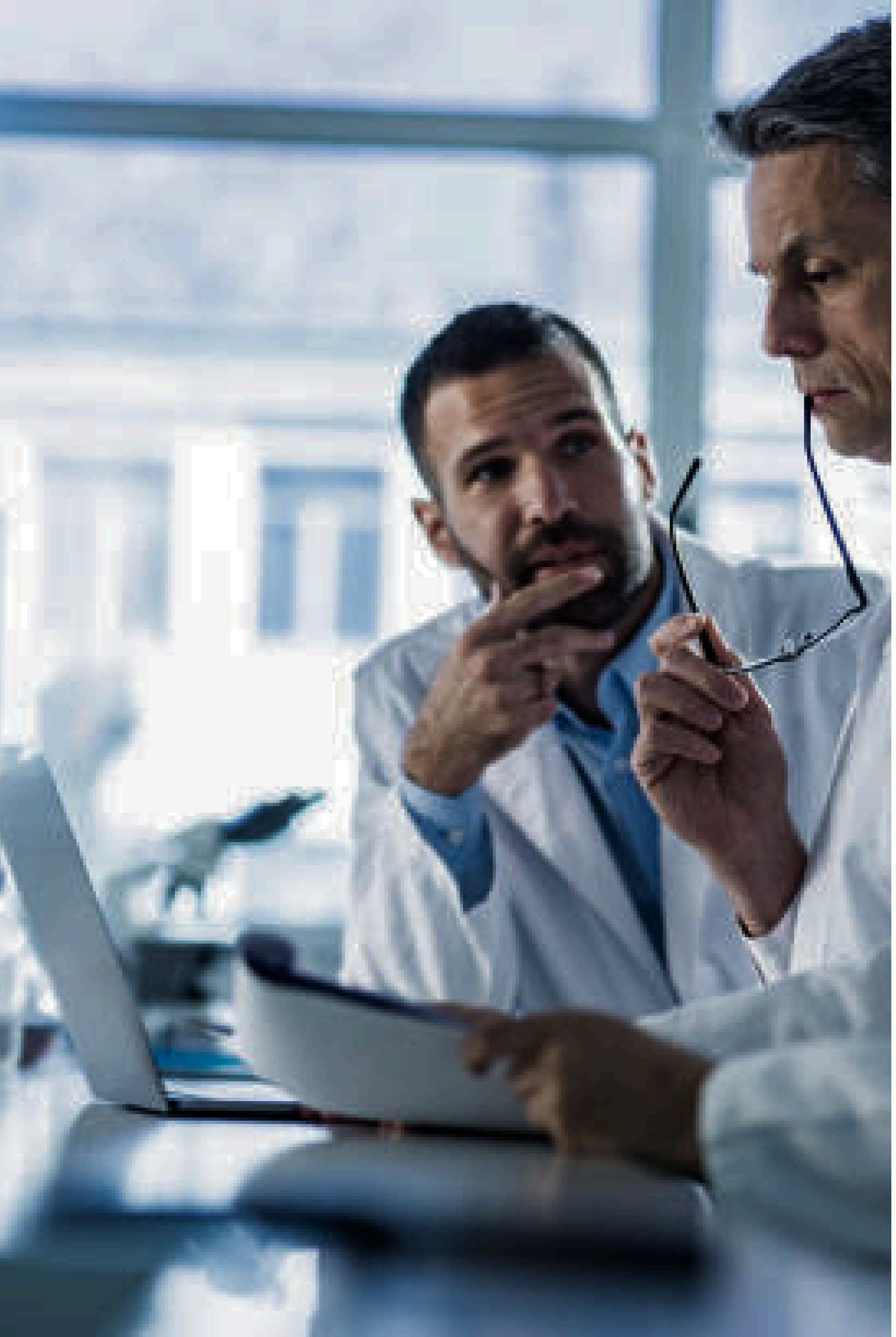
- 5× faster than wild type
- Survives up to 67°C
- Resists pH 6.5-9.5

Dura-PETase

- 300× faster than wild type
- Resists up to 77°C
- Resists pH 8.0-9.0

Research Question

What specific amino acid mutations are needed to create a PETase mutant with heat resistance, pH tolerance, and fast enough for large-scale plastic decomposition and how can it be improved?



Hypothesis

> If I genetically edit PETase by combining most of the mutations from FAST-PETase, DuraPETase, as well as LCC-ICCG-C09 into one PETase, then it should theoretically be able to decompose PET 600 times faster, survive temperatures of 149.1°C, and handle a pH level of 6.0 to 10.5.

This is because FAST-PETase, DuraPETase, and LCC-ICCG-C09 each provide their own mutations to help with heat resistance, pH stability, and speed boosts. Putting them all together can fix the main weaknesses of PETase.

Research Overview

LCC-ICCG

The current gold standard for PETases

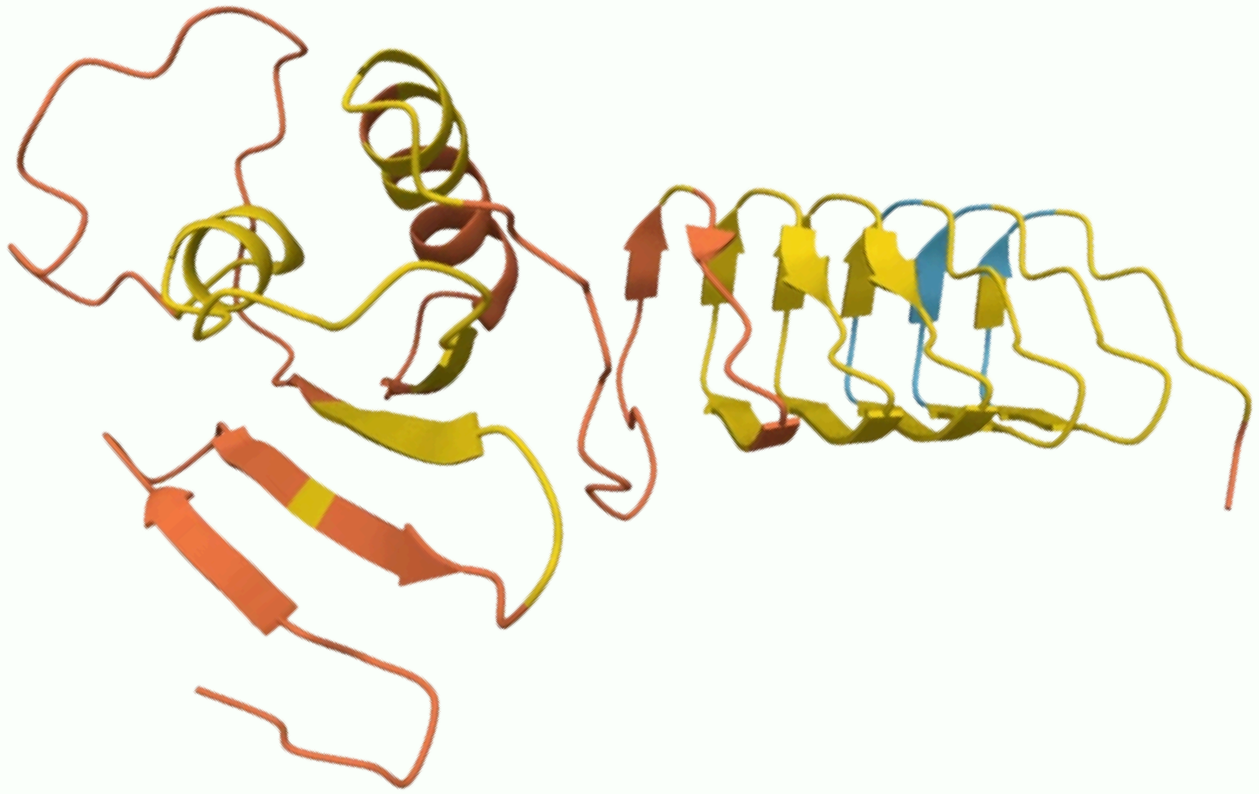
Fast-PETase

An AI made enzyme developed in the University of Texas

Dura-PETase

A PETase Primarly made to be further Heat-resistant

LCC-ICCG: Breakthrough PETase Architecture



Thermal Resilience

LCC-ICCG represents a new generation of PET-hydrolyzing enzymes engineered using in silico protein design methods. This mutant withstands temperatures up to 68°C, significantly exceeding wild-type PETase limits of 46°C.

C09 Enhancement

The LCC-ICCG-C09 variant achieves 2× greater efficiency through three additional strategic mutations: V212H, A131G, and Q189R.

Y127G

Large bulky Tyrosine replaced with small Glycine at position 127. Removes steric hindrance blocking PET entry into degradation site.

D238C

Aspartic acid → Cysteine creates disulfide bonds, enhancing structural stability and heat resistance.

F243I

Phenylalanine → Isoleucine optimizes active site geometry for faster catalysis.

S283C

Serine → Cysteine pairs with D238C to form second disulfide bond, maximizing thermal stability.

LCC-ICCG-C09: Three Additional Mutations

The C09 variant introduces three mutations that dramatically enhance plastic binding and processivity:

1

V212H

Valine → Histidine creates sticky surface that doubles enzyme residence time on PET plastic. Histidine acts as molecular adhesive, preventing enzyme detachment and enabling continuous degradation cycles.

2

A131G

Alanine → Glycine at position 131 widens enzyme entrance channel. Replaces larger amino acid with smallest possible residue, allowing PET chains easier access to catalytic core.

3

Q189R

Glutamine → Arginine introduces positive charge that attracts negatively charged PET chains. Functions as molecular magnet, drawing plastic toward degradation zone.

Critical Mutation Constraints

Position-Specific Limitations

Key mutations from LCC-ICCG cannot be directly transferred to wild-type PETase due to structural differences:

- D238C + S283C disulfide pair is specific to LCC-ICCG scaffold
- Must substitute N233C for equivalent stabilization
- F243I impossible—no phenylalanine at position 243
- Use W159H instead to accelerate catalysis

These constraints highlight the importance of scaffold compatibility when combining mutations from different PETase variants.

FAST-PETase: Speed Optimization

500

Years

Natural PET degradation in environment

20

Days

Wild-type PETase degrades 0.25mm plastic

4

Days

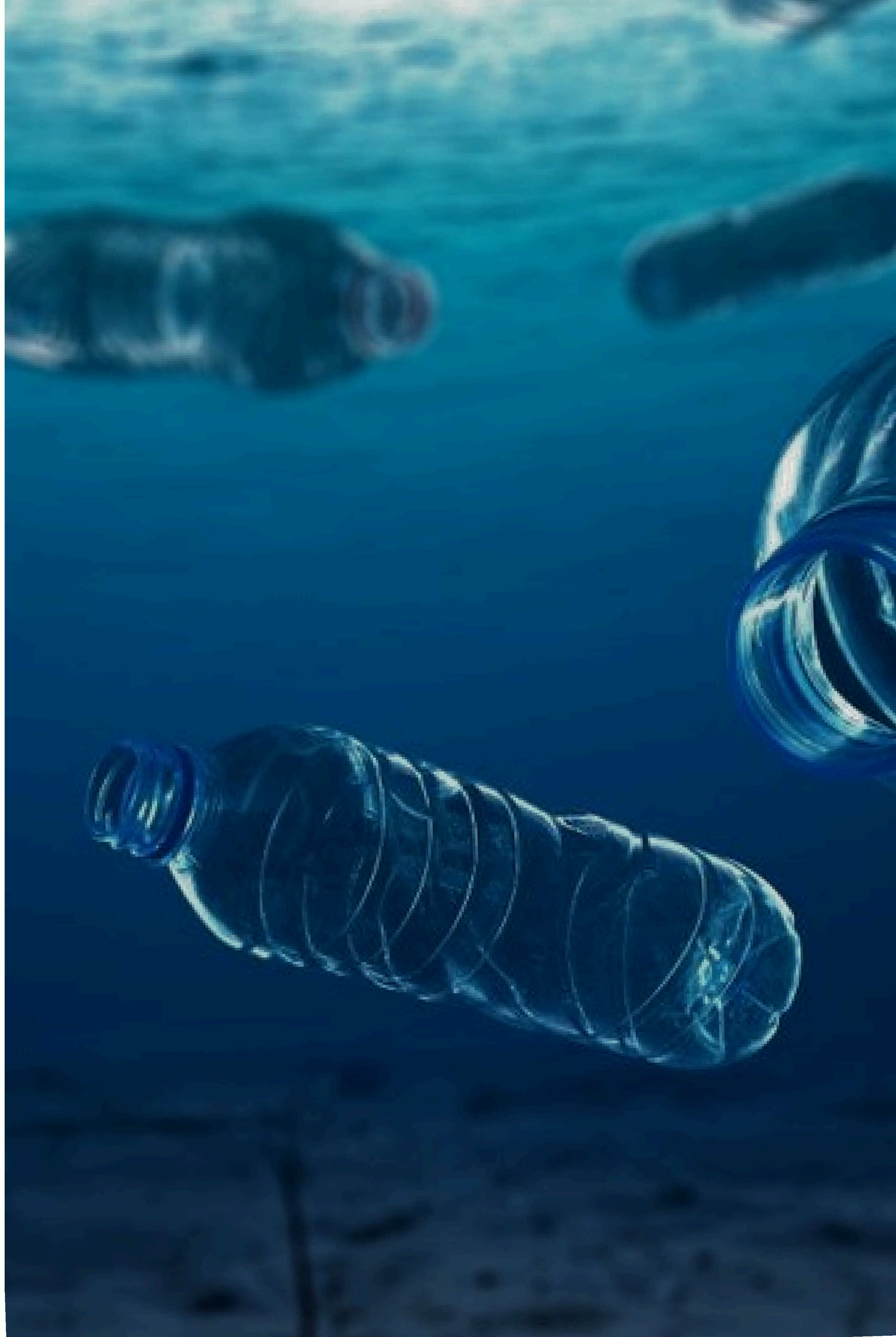
FAST-PETase achieves same degradation

5×

Speedup

Improvement over wild-type enzyme

Developed at University of Texas, FAST-PETase employs genetic engineering to create thermally stable enzyme that maintains function across variable temperatures. Five mutations collectively enable 5× acceleration by optimizing substrate binding, catalytic efficiency, and structural rigidity.



FAST-PETase Mutation Mechanisms

01

N233K: Electrostatic Attraction

Asparagine → Lysine adds positive charge. PET's negative charge attracts to degradation zone, accelerating substrate capture.

02

R224Q: Active Site Streamlining

Arginine → Glutamine removes bulky amino acid obstructing catalytic site. Smoother pathway enables faster polymer chain processing.

03

S121E: Surface Adhesion

Serine → Glutamic acid creates chemical bond with plastic surface. Prevents enzyme detachment, maintaining catalytic engagement.

04

D186H: Thermal Lock

Aspartic acid → Histidine forms hydrogen bond inside enzyme. Prevents unfolding at elevated temperatures.

05

R280A: Structural Relaxation

Arginine → Alanine removes internal tension point. Enzyme maintains functional conformation under thermal stress.

DuraPETase: Heat Resistance Architecture

W159H

Tryptophan → Histidine at position 159. Replaces a large aromatic side chain that blocked PET entry with a smaller residue, widening the access channel and accelerating substrate binding and degradation.

I168R

Isoleucine → Arginine introduces a positive charge that forms stabilizing salt bridges with nearby negative residues, reinforcing structural weak points that are vulnerable at ~60°C.

S188Q

Serine → Glutamine creates additional hydrogen bonds complementary to I168R, providing a dual-reinforcement network that helps maintain enzyme conformation under heat stress.

DuraPETase: Surface Engineering Mutations

S214H: Aromatic Stacking

Serine → Histidine introduces large aromatic ring at position 214. Rings connect through π-stacking, strengthening enzyme core. Prevents vibrational breakdown at high temperatures.

L117F: Pi Stacking

Leucine → Phenylalanine adds aromatic ring complementary to PET's carbon rings. Creates "molecular magnet" effect through π-stacking interactions, enhancing surface adhesion.

Q119Y: Hydrophobic Matching

Glutamine → Tyrosine replaces water-loving amino acid with hydrophobic ring structure. Creates favorable environment for PET binding, preventing premature substrate release.

A121E: Charge Engineering

Alanine → Glutamic acid adds negative surface charge. Optimizes enzyme orientation at water-plastic interface, maintaining catalytic domain positioning.

Trident-PETase: Unified Mutation Strategy

Combining compatible mutations from FAST-PETase, DuraPETase, and LCC-ICCG-C09 creates synergistic enzyme architecture:

Speed Mutations

Y127G, A131G, W159H, R224Q

Heat Resistance

N233C, D186H, R280A, I168R, S188Q, S214H

Surface Binding

V212H, L117F, Q119Y

Charge Optimization

S121E, Q189R

Critical Decision: Retained N233C over N233K for scaffold compatibility, S121E over A121E for superior binding. Total of 15 non-overlapping mutations.

Research Methodology: The Power of Synergy

After analyzing FAST-PETase, DuraPETase, and LCC-ICCG-C09, I discovered that combining their best mutations creates exponential improvements rather than simple additions. Most scientists focus on single improvements, but real-world PETase must address multiple limitations simultaneously.

Speed Multiplication

Rather than adding speed boosts, the mutations multiply their effects as each enhancement enables better performance from others.

Heat Resistance

Combining thermal stability from all three variants creates an enzyme that withstands extreme temperatures without melting or deactivating.

pH Flexibility

Expanding the working pH range allows operation in diverse environments from acidic landfills to alkaline industrial settings.

Speed Calculation: 600× Faster Than Nature

01

FAST-PETase Foundation

Provides 5× speed boost, reducing degradation time from 20 days to 4 days compared to wild-type PETase.

02

DuraPETase Enhancement

Adds 60× improvement (300× better than wild-type divided by FAST-PETase's 5× boost), creating robust baseline performance.

03

LCC-ICCG-C09 Optimization

Contributes 2× faster rate by widening enzyme entrance for easier plastic access, building on existing 300× LCC foundation.

04

Trident-PETase Total

5 × 60 × 2 = 600× speed multiplier, theoretically degrading plastic in days instead of centuries.

Thermal Stability: 149.1°C Heat Resistance

Wild-PETase melts at only 46°C, but combining mutations from all three variants creates extraordinary thermal tolerance. The enzyme can easily handle the 80–90°C sweet spot where PET plastic becomes softest and most vulnerable to enzymatic breakdown.

46°C

Wild-Type Baseline

Natural PETase starts melting at this temperature

+51.1°C

LCC-ICCG-C09 Contribution

97.1°C limit minus wild-type baseline (97.1 - 46)

+21°C

FAST-PETase Boost

Additional thermal stability from FAST variant

+31°C

DuraPETase Addition

Enhanced heat resistance from Dura mutations

149.1°C

Trident-PETase Total

46 + 51.1 + 21 + 31 = Final heat resistance

Extended pH Range: 6.0–10.5 Operating Window

Wild-PETase operates only within narrow pH 7.0–8.0 range, but combining stability points from all three PETases expands functionality by 3.5 points total. This creates working window of pH 6.0–10.5, enabling survival in variable chemistry environments like landfills where pH constantly shifts.

Baseline: pH 7.0–8.0

Wild-PETase operates in narrow, pH-neutral range only

Expanded: +3.5 Points

FAST-PETase adds 2.0, LCC-ICCG-C09 adds 1.5, DuraPETase adds 0

Trident: pH 6.0–10.5

Works in acidic landfills and alkaline industrial conditions

Compatibility Analysis: 15 Strategic Mutations

Ensuring mutations work together without conflicts required careful analysis of protein structure and function. Key insights prevented incompatible changes while optimizing enzyme performance.

Problem: D238C/S283C Limitation

These mutations only work with LCC-type structure, not wild-type PETase blueprint.

Solution: N233C Selection

Chose N233C mutation to maintain enzyme stability while remaining compatible with wild-type foundation structure.

Problem: F243I Incompatibility

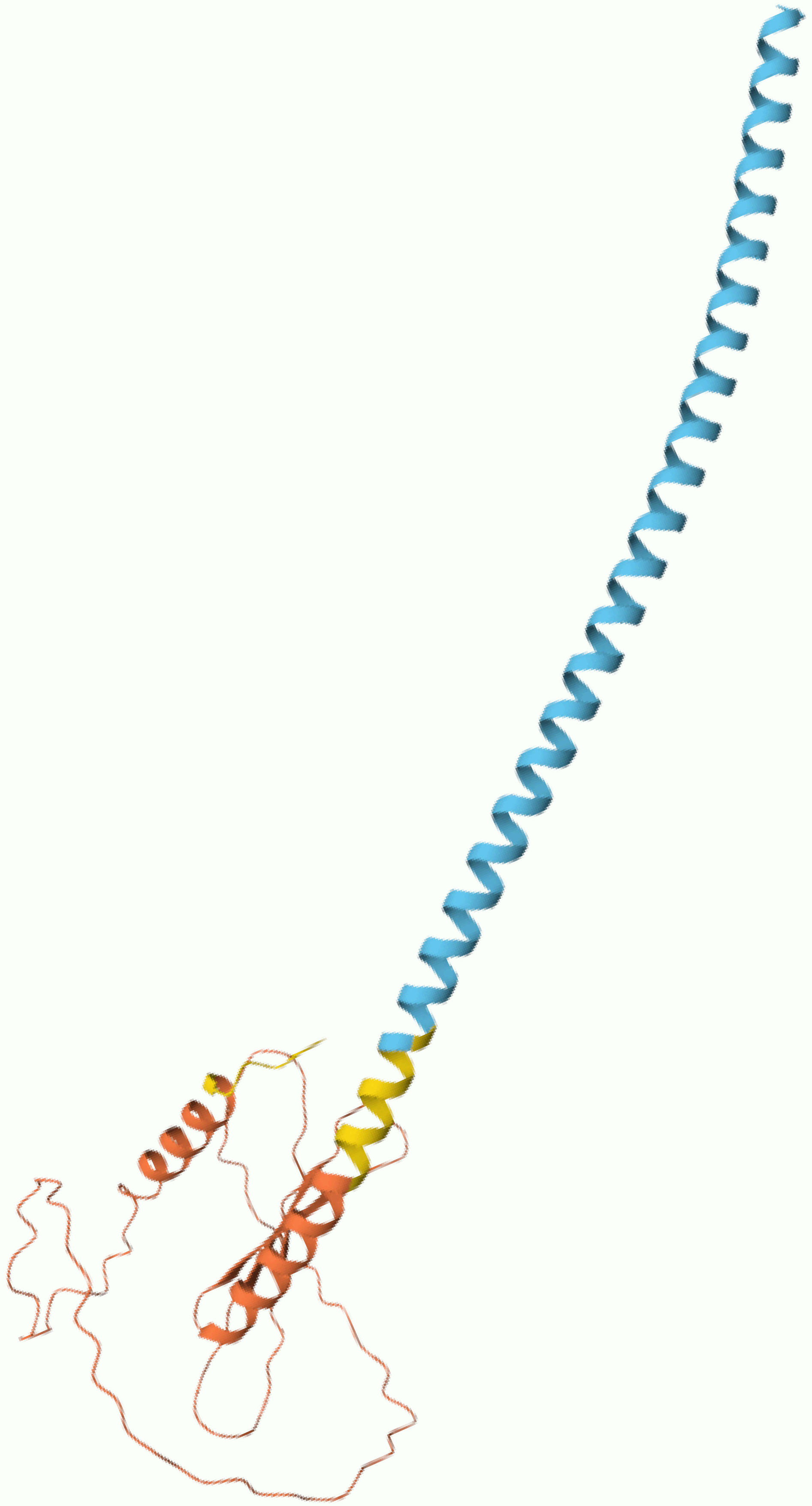
No phenylalanine residue exists at that position in wild-PETase, making this mutation impossible to implement.

Solution: W159H Substitution

Selected W159H instead to clear bulky entrance, allowing plastic deeper access to attack chemical bonds more effectively.

Trident-PETase: The Name Explained

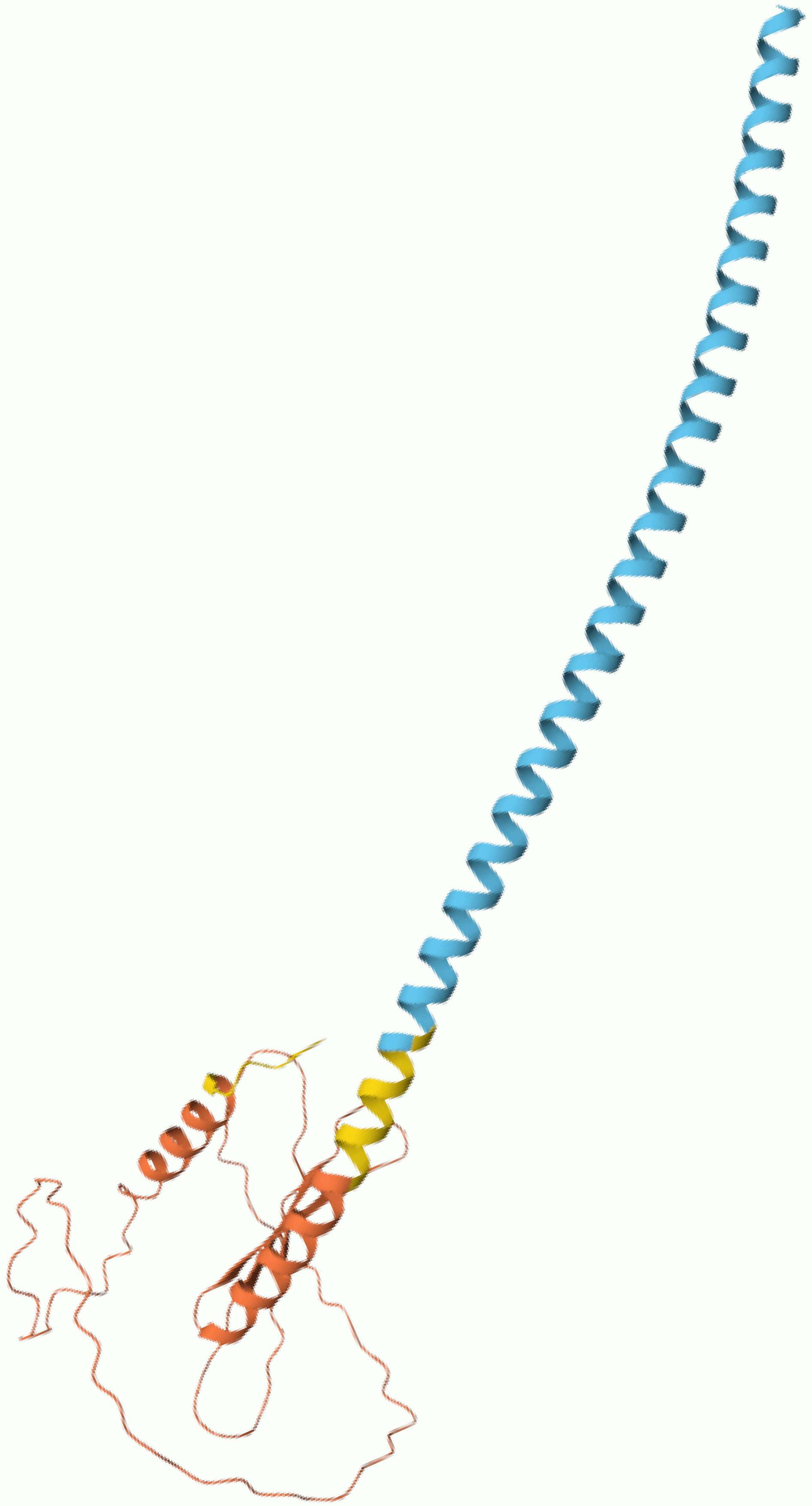
The name **Trident-PETase** reflects the three-pronged approach targeting the enzyme's critical weaknesses. Like a trident's three prongs working in unison, this engineered enzyme simultaneously addresses heat sensitivity, pH limitations, and slow degradation speed—the three barriers preventing real-world PETase deployment.



Conclusion

My research and math show that my hypothesis was right because my version of PETase, the Trident-PETase, fixes the three main things that stop us from using enzymes outside of a lab. By putting together the best mutations from FAST-PETase, DuraPETase, and LCC-ICCG-C09, my model can theoretically survive heat up to 149.1°C degrees Celsius and a pH range from 6.0 to 10.5.

Because these mutations help each other, the speed multiplies and becomes 600 times faster than the wild type. This means we could break down plastic in just a few days instead of waiting hundreds of years. My math and analysis in my research section proves that this new enzyme is strong enough and fast enough to work in hard places like landfills or recycling centers without melting or stopping. This makes it so that we can solve the plastic problem by making one powerful enzyme that works in the real world.



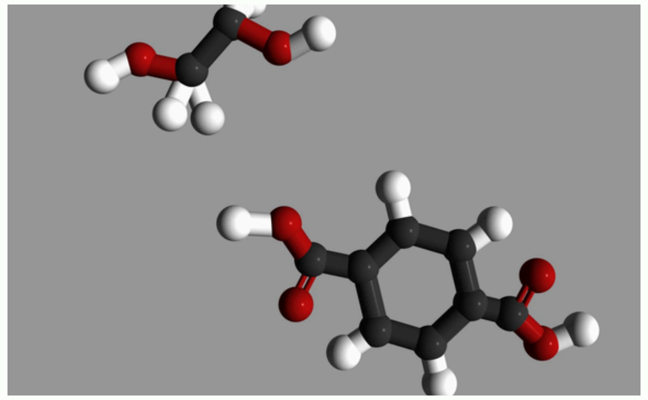
Real-World Applications

Landfill Remediation

Operates in Calgary's landfills and similar sites where messy, acidic environments kill conventional enzymes. pH 6.0–10.5 range survives variable chemistry.

Large-Scale Recycling

Functions at high temperatures (80–90°C) where PET softens most easily. Processes material continuously without quality loss.



Circular Economy

Recycles PET indefinitely in days instead of 500 years. Reduces reliance on new plastic production.

Future Directions & Next Steps

Machine Learning Analysis

Model how 15 mutations interact to break down PET under varying weather and environmental conditions

Laboratory Validation

Test Trident-PETase model in real lab settings to verify 1,000g PET per hour degradation rate calculations

Scientific Collaboration

Partner with researchers to synthesize enzyme variant and validate mathematical predictions experimentally

Global Deployment

Scale proven technology for worldwide implementation across recycling centers and landfill operations