



Title Page

What specific amino acid mutations are needed to create PETase mutants with heat resistance, pH tolerance, and fast enough for large-scale plastic decomposition and how can I make it better?

By:	Kian Khwaja
School & Grade	Stem Innovation Academy Junior High Grade 7
CYSF Category	Life Sciences, and Biochemistry
Project Title	How can PETase be improved so that it can break down plastic in other environments other than labs?
Project Start Date	October, 29, 2025
Supervisor/Teacher	Janessa Bretner Email: j.bretner@stemia.ca

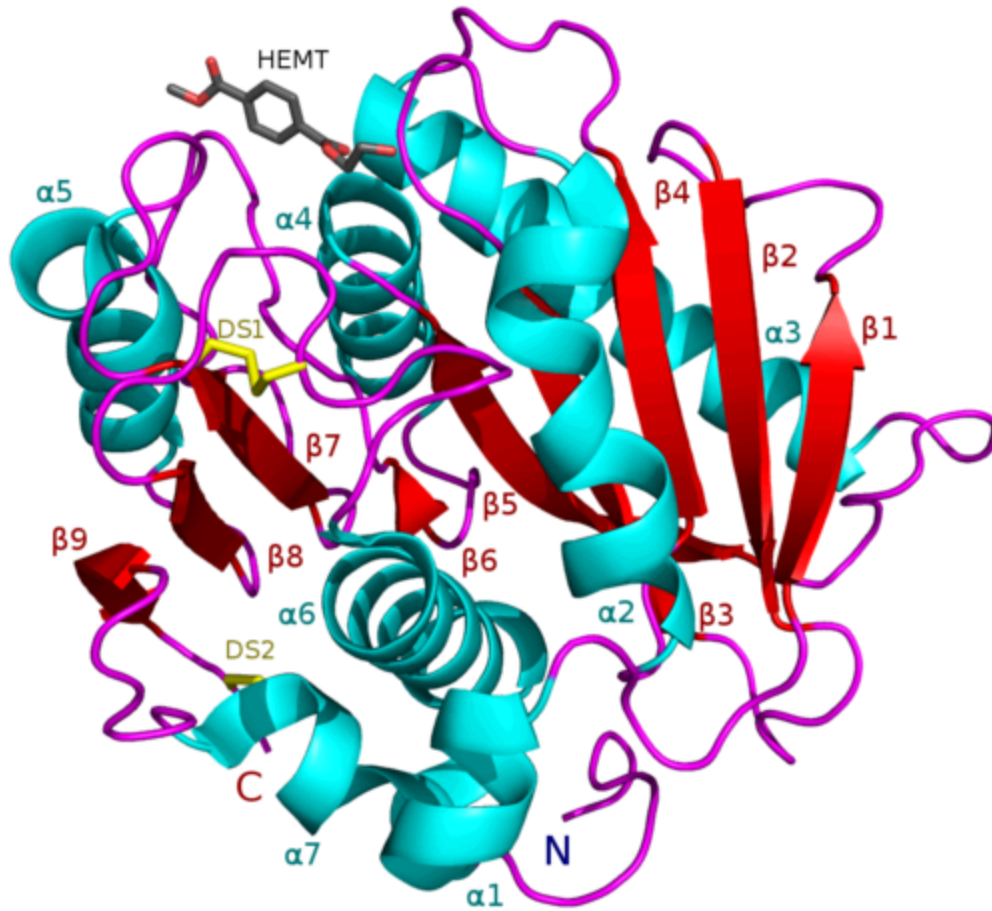


Image credit:
<https://en.wikipedia.org/wiki/PETase>

By: Kian Khwaja



Daily Notes

Oct 30th:

I researched some ideas and decided on an idea.

Nov 1th:

I got the idea approved

Nov 2th:

Filled basic information on CYSF

Nov 3th:

Filled ethics form on CYSF

Nov 10th:

Got ethics approved

Nov 11th:

Did some background research

Nov 12th:

Tweaked my question to be more specific.

Nov 13th:

Changed my basic information on CYSF

Nov 14th:

Changed my ethics on CYSF

Nov 22th:

Got ethics approved

Nov 24th:

I researched Proteins and what they are.

Nov 25th:

Researched amino acids and tweaked my question

Nov 26th:

Found out why plastic is problem

Nov 28th:

Added to my background research about Proteins and amino acids.

Nov 29th:

Read part of an article about a new PETase that is said to be the best one yet

Nov 30th:

Read some more of the article.

Dec 1th:

I read an article on LCC-ICCG-C09

Dec 2th:

Started to build up my hypothesis

Dec 3th:

Debated between thesis or hypothesis and which is best for my project

Dec 4th:

Found out that hypothesis is the best for my project

Dec 5th:

Finished my hypothesis

Dec 6th:

I found that my hypothesis should be more clear and specific, so I researched speeds of different PETases and found the speed of Fast-Petase which is 40% of 0.25mm thick plastic at 50 degrees celsius for 4 days or 0.025mm thick per day at 50 degrees celsius.

Dec 7th:

Searched more for speed of Wild-PETase and LCC-ICCG-C09.

Dec 8th:

Emailed Dr. Bhattacharya, Emilio Parisini, and Alfonso Gautieri to ask them about the LCC-ICCG-C09 and maybe even Wild-PETase.

Dec 9th:

Added things about PETase to the protein section in background research.

Dec 10th:

Added to background research PETase part about why we need it.

Dec 11th:

Started my main research

Dec 15th: I started looking deeper into the specific amino acid swaps for the 15 mutations I want to use . I need to make sure they all fit into the wild-type PETase without breaking the protein structure.

Dec 20th: Spent time researching how pH levels affect enzymes in landfills. I found that adding stability points from all three parent enzymes could widen the working window from pH 6.0 to 10.5.

Jan 5th: I used AlphaFold visualizations to see how different PETase enzymes look. It's cool to see the 3D shape and how the molecules are arranged .

Jan 12th: I researched more about the 15 mutations and checked for conflicts. I had to make sure two mutations weren't trying to change the same spot on the enzyme's blueprint.

Jan 20th: I realized that my original heat math was a bit low . I went back to my research on LCC-ICCG-C09 and saw that its actual melting limit is 97.1°C.

Jan 28th: I decided to push my heat math further. I realized that I did that math wrong since the LCC-ICCG-C09 is 97.1°C .So I edited that.

Jan 30th: I sat down and calculated the new heat total: $97.1 + 21 + 31 = 149.1^{\circ}\text{C}$ This is way past the wild-type's weak limit of 46°C and ensures the enzyme stays active in industrial recycling centers.

Feb 3rd: I updated my Applications section to focus on Calgary's landfills. I realized that having an enzyme that survives at 149.1°C is perfect because it hits a good spot for softening plastic (80°C to 90°C) without the enzyme melting.

Feb 5th: I finished my Conclusion. My research and math prove that Trident-PETase is 600 times faster and can handle extreme heat and messy pH levels, so it can actually work in the real world .

Feb 8th: Finalized the logbook and checked all my references . I made sure they were all about PETase research instead of the old template links.

 Choosing an idea

Choosing an Idea

- **Date:** 28, October, 2025
- **Initial Ideas Explored:**
 - What specific amino acid mutations are needed to create PETase mutants with heat resistance, pH tolerance, and fast enough for large-scale plastic decomposition?

Why are there so many types of different PETases, and no large-scale degrading yet?
- **Final Selection:** What specific amino acid mutations are necessary to engineer PETase variants with enhanced stability and catalytic activity suitable for large-scale plastic decomposition in industrial and landfill settings?
 - This problem is important because it can help us degrade plastic easily in just under a month, and so we wouldn't have to wait 500 years for it to degrade. Also because, then we can use plastic all we want without feeling guilty of harming mother earth.
 - I am focusing on this PETase field because I have done projects on it in the past, so I have knowledge in this field. I am also choosing this because I love to learn about how we can save the earth.
 - I have chosen this project over the other one, because the other project is just a general question with some research needed, and I wanted to do a project that could actually challenge me this year, and actually make a difference.
 - What will I be covering in this project?:
This year I will be researching different kinds of PETases and what mutations they have. Then I will use the program STELLA to modify PETase using what I learned from my research and try to change it so that it can be used in large-scale degradation and real life applications.



Background Research

Background Research

History of plastic

Plastic was invented in 1869 as a cheaper option besides ivory, which was made from cellulose of plants. Then fully synthetic plastic came out 1907 which was named as Bakelite. Bakelite was liked and praised for its price in the early 20th century. Then when people started to throw it away, when they were done using it, they saw that it took years and years to degrade. Not long after the people started to realise how harmful it is to the environment by making plastic and throwing it away they are causing more pollution and global warming in the world.

What is a Protein and an Amino Acid?

Proteins are large biomolecules and macromolecules that have chains of monomers called amino acids in them. Different types of amino acids can help with different things such as Serine, Aspartic acid, and Histidine, which are the most important amino acids in PETase. In PETase serine acts as a reactive amino acid and breaks ester bonds to help break down the PET. Aspartic acid stabilizes histidine by forming a hydrogen bond and electrostatic interaction with it to keep histidine in the correct position and helps it accept a proton from serine. Histidine accepts a proton from serine, activating serine so it can break the ester bonds in PET plastic. There are 20 different amino acids like these that repeat to create a sequence. These amino acids are Alanine, Valine, Leucine, Isoleucine, Methionine, Phenylalanine, Tryptophan, Proline, Serine, Threonine, Asparagine, Glutamine, Tyrosine, Cysteine, Aspartic acid, Glutamic acid, Lysine, Arginine, Histidine, and Glycine. All of these together make PETase degrade PET.

Info:

I got the information for all the amino acids in PETase from:

<https://www.uniprot.org/uniprotkb/A0A0K8P6T7/entry>. I got the three important amino

acids and their functions, serine, histidine, and aspartic acid, from scientific research on how PETase works form: <https://pubmed.ncbi.nlm.nih.gov/38538850/>

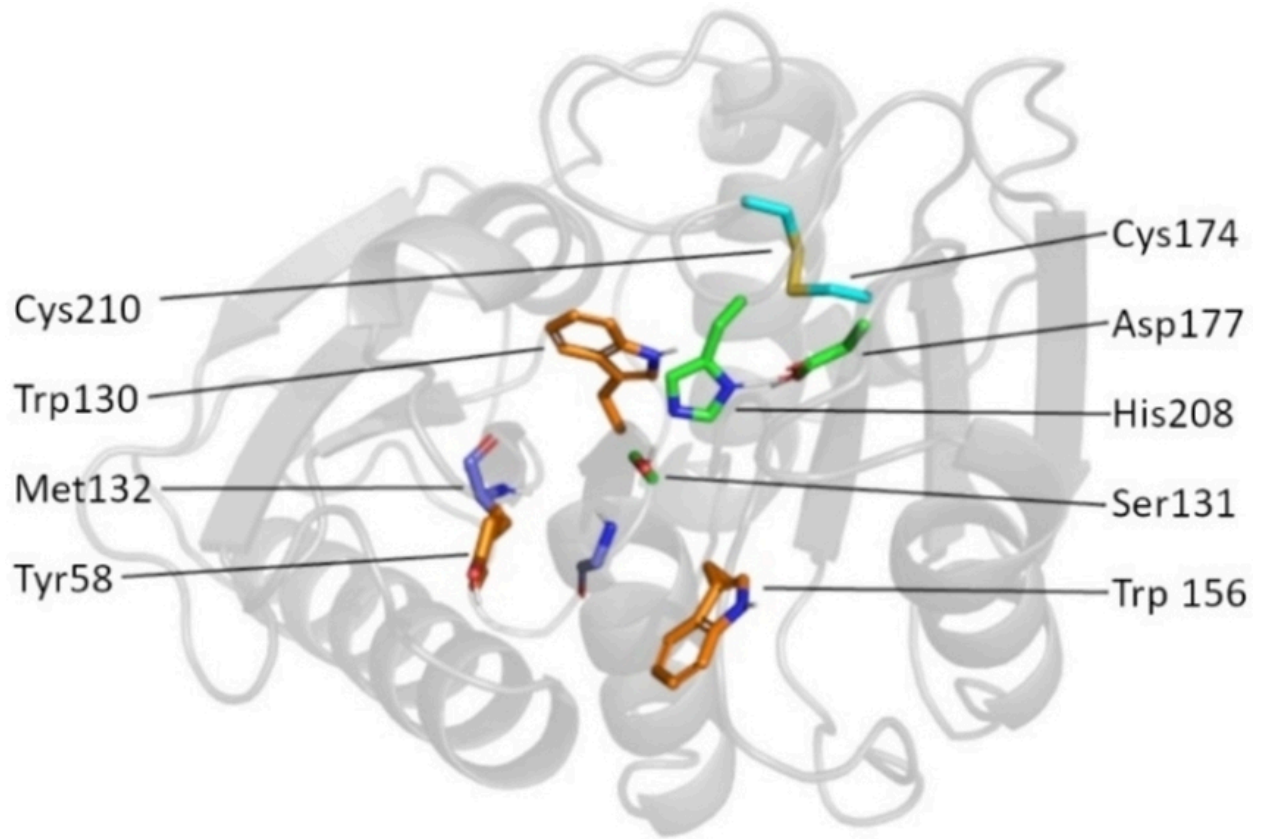


Image Credit:
<https://en.wikipedia.org/wiki/PETase>

PETase

What is PETase?

What is PETase? Petase is an enzyme that was found in 2016 near a Japanese PET bottle recycling site. It is an enzyme that can degrade plastic and convert it into its basic form.

When scientists found it, they tried to make it better, and their task was accomplished because now there is an even faster and more efficient enzyme called Fast-petase.

It takes 24 hours to 4 weeks to degrade plastic. It depends on how close together the molecules of the plastic are, how thick the plastic is, how much plastic there is, and the molecular structure of the PET (Plastic).

Why do we need to use PETase to degrade PET, and not just recycle all the PET?

Well first of all we are not fully re-cycling all plastics and we are creating lots of new plastic which is shown because 90.2% of the plastic used around the world comes from newly made plastics and only 9.8% is actually coming from recycled plastics. Second of all the quality and durability of the plastic decreases when recycled and so eventually that plastic will go through multiple recycles and become not usable, which will cause it to be through in the land fill.

How does PETase work?

It works by breaking down the PET into MHET at the molecular level, this is done by breaking the ester bonds between the PET. Then the MHETase breaks down the MHET into the original materials that PET was actually made of or monomers, terephthalic acid, and ethylene glycol by cleaving all of the MHET. Which sounds really simple but is really hard to use in large scale usage, because sometimes the PH of the plastic changes to abnormally acidic levels, which causes PETase to malfunction and die. Second of all PETase is really hard to make and costs a lot of money. Another reason we do not use PETase is that it is too slow. Results have shown that the fast version of PETase, Fast-PETase, which is 3-4 times faster can only break down 40% of 0.25mm thick PET in 4 days at 50 degrees celsius heat. Lastly, it cannot withstand enough heat to be used outside or in decomposing centers, because the maximum limit of wild PETase is only 40°C.

Why don't we use PETase in colder countries such as Canada?

It cannot function in the cold properly either because its optimal temperature is 30°C to 40°C. In the process of degradation it gets really hot which can cause it to not degrade the PET. To create a PETase that can withstand the heat, can withstand the pH level and is fast enough we will need to genetically edit this PETase protein and add different amino acids to it. An amino acid is a monomer of a protein and it basically makes up a protein. So we would need to add different kinds of monomers or amino acids into PETase to change it and make it how we want it to be. This is what is done in examples such as Fast- PETase, Turbo- PETase and many more.

Speed, Heat resistance, and pH resistance of Dura-PETase, LCC-ICCG-C09, Fast-PETase

- LCC-ICCG-C09's speed is 600 times faster than the wild type of PETase because it is twice as efficient as the previous best version, LCC-ICCG, which was 300 times faster.
- LCC-ICCG-C09's heat resistance lets it work in temperatures up to 97.1 degrees celsius because it uses extra chemical bridges to lock the protein together.
- LCC-ICCG-C09's pH resistance makes it work best at pH 8.0 because of a special mutation that changes the charge of the enzyme's surface.
- FAST-PETase's speed is 5 times faster than the wild type because it was designed by AI to fix the slow parts of the enzyme.
- FAST-PETase's heat resistance lets it survive up to 67 degrees because it uses hydrogen bonds to stay strong instead of melting.
- FAST-PETase pH resistance keeps the enzyme working in a window from pH 6.5 to 9.5 so it can handle different types of water.
- Dura-PETase's speed is 300 times faster than The wild type of PETase because it clears out the bulky amino acids that were blocking the entrance.
- Dura-PETase's heat resistance lets it survive up to 77 degrees celsius because it strengthens the core so the enzyme stays active for many days.
- Dura-PETase's pH resistance keeps the enzyme from malfunctioning in a range of pH 7.0 to 8.0.

Polymer and Ester bonds

Polymer is a large molecule that contains monomers, terephthalic acid, and ethylene glycol. Ester bonds is the bonds between the molecules and it connects all the molecules that create PET plastic.

? Research Question

Problem

PETase is a solution that we have found to care for PET, but we have not used it for degradation anywhere except for the lab which is infuriating because the PET is increasing and is finding new ways to harm, which can be really dangerous to mankind. In this project we will explore why we are not using PETase, and What things we should add to PETase to make it be able to degrade plastic outside of the lab.

Research Question

What specific amino acid mutations are needed to create PETase mutants with heat resistance, pH tolerance, and are fast enough for large-scale plastic decomposition?

Hypothesis

Hypothesis

If I genetically edit PETase by combining most of the mutations from FAST-PETase, DuraPETase, and LCC-ICCG-C09 into one PETase, then it will theoretically be able to decompose PET 600 times faster, survive temperatures of 149.1°C degrees celsius, and handle a pH level of 6.0 to 10.5. Because right now PETases are way too slow and stop working when it gets too hot or the pH changes, and since 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 fixes major weakness of PETase at once so the enzyme can work in the real world instead of just in a lab.



Materials + Methods

Materials

List of all softwares used:

- **Protein data bank:** I used this to find the sequence for different PETase enzymes.
- **Alphafold:** I used this to visualizations of different PETase enzymes using their sequences. Finally, I utilized my knowledge to incorporate different mutations into a single enzyme and create protein structure for Trident PETase.

Procedure

1. I researched different kinds PETases and focused on Fast-PETase, Dura-PETase, LCC-ICCG, and LCC-ICCG-C09
2. Found Mutations for different kinds of PETases
3. Calculated speed of Trident PETase by
4. I checked the wild-type PETase model to make sure the 15 mutations didn't try to change the same spot , and I swapped F243I for W159H so the plastic can go deeper inside to attack the bonds.
5. I used math to calculate the final results by multiplying the speed boosts and adding the heat and pH points from the three PETases to the wild-type model .
6. I used the Alphafold model to see the final 3D shape and make sure all 15 mutations worked together to fix the heat, pH, and speed without the enzyme unfolding.
7. I compared the math results of my Trident-PETase model against the wild-type to prove it can theoretically solve the plastic problem much faster in the real world .

Research

Different types of PETase

LCC-ICCG

finished an article and found a new type of PETase called LCC-ICCG and LCC-ICCG-C09. In that article it states that LCC-ICCG and LCC-ICCG-C09 are the best PETases yet to be developed. It also says that it can stand up to 68 °C because they have added silico protein design methods to make a PET-hydrolyzing enzyme. Their mutant, the LCC-ICCG-C09 is 2 times more efficient than the LCC-ICCG. The LCC-ICCG used Y127G, D238C, F243I, S283C to make it better. Y127G is when they change Tyrosine to Glycine at position 127. This helps increase the speed of LCC-ICCG because it helps make the PET enter the enzyme into the degrading area because Tyrosine is a large bulky amino acid that can block the way so it takes time for the PET to get inside and Glycine is a small amino acid making it easy for the PET enter into the degradation area. D238C is when aspartic acid residue at position 238 is changed to cysteine. This helps create disulfide bonds which stabilizes PETase and makes it stronger giving it further heat resistance. F243I is when phenylalanine at position 243 is changed to isoleucine. This helps with making it faster and making it stronger. S283C is when Serine is changed into Cysteine at position 283. This together with D238C creates a disulfide bonds also helping with stabilization, and heat resistance.

Compared to LCC-ICCG, the LCC-ICCG-C09 has three more amino acid changes and they are V212H, A131G, and Q189R.

- At position 212, they convert valine to histidine as the change V212H. This is very good for the enzyme because histidine acts as a hand or tiny hook that makes it much easier for the enzyme to pick up and stick to the PET plastic surface than valine can. It is twice as good because the enzyme can continue to degrade and consume the bottle for a much longer time without stopping because it allows the enzyme to stick to the plastic rather than simply floating in the water.

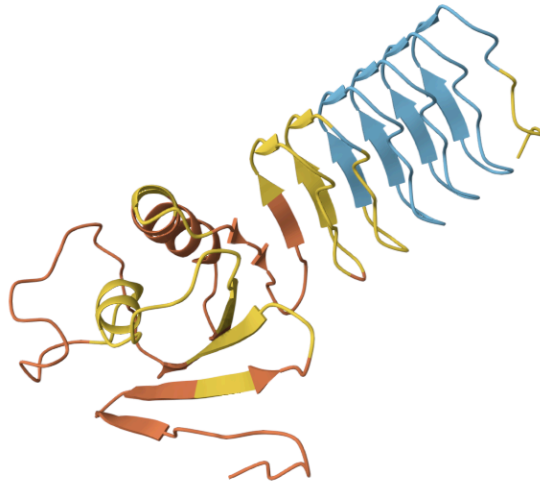
- A131G is when we change the amino acids alanine to glycine at position 131. This is similar to the Y127G mutation we showed above, as it replaces a larger amino acid with the smallest amino acid possible. By using the amino acid glycine, it allows the entrance more room so that the enzyme can let come inside the enzyme and the plastic chains can break more easily and also faster.

- Q189R is when we change the amino acid glutamine to arginine at position 189. This is a very cool mutation because the amino acid arginine has a positive charge, so it acts like a magnet to the PET, drawing the plastic chains towards the area to be degraded more effectively, so the enzyme can be degraded.

LCC-ICCG



LCC-ICCG-C09



Extra information for LCC-ICCG and LCC-ICCG-C09 and what mutations can be used.

I was researching more on LCC-ICCG and LCC-ICCG-C09 and found that the mutation combination D238C, and S283C works only for that specific PETase type and should not be used for any other PETase, because I am focusing on improving the wild type PET and you cannot do D238C. Instead I found that you have to use N233C.

I also found that the change F243I cannot be done either because there is no Phenylalanine in position 243 to change. I have to use W159H instead to increase the speed.

Fast-PETase

Fast-PETase is a special fast version of PETase that scientists made to help get rid of plastic faster than the wild PETase. Plastic usually takes a long time to degrade such as 500 years but with PETase it takes 20 days to degrade a 0.25mm thick plastic piece. To make this process faster scientists genetically edited the wild to make it faster at the University of Texas. The main change scientists made was making the enzyme much tougher. The wild type PETase would not work if the water got too hot or if the conditions weren't exactly in the optimal range. The Fast-Petase is very stable and can keep working even when temperatures slightly change, and in the process they made it faster. This makes the Fast-Petase able to degrade plastic way faster like a 0.25mm thick plastic in just 4 days. That's 5 times faster than Wild type PETase.



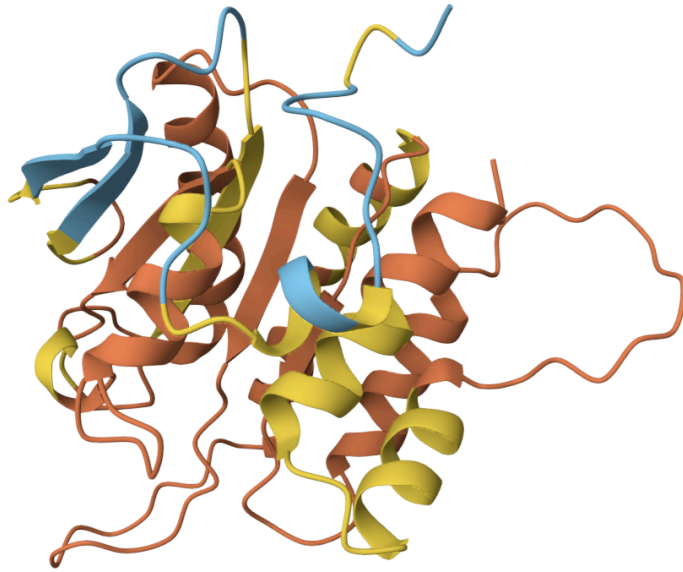
Genetic Mutation of Fast-PETase

- N233K is a swap from Asparagine to Lysine. N233K helps in Speed by adding positive electrical charge to the enzyme. The PET has a slightly negative charger

so the PET is attracted to the degrading zone inside of PETase. This makes the process faster because PETase doesn't have to get the PETase inside of itself in another way that takes more time.

- R224Q is a swap from Arginine to Glutamine. R224Q helps in speed by changing the shape of the degradation zone. It removes a bulky part or amino acid that was in the way and replaces it with a smaller, smoother and less bulky one, this makes the enzyme able to move more freely, letting it move through plastic chains without getting stuck. This allows it to reduce the time because it removes an obstacle that takes significant time for the enzyme to navigate by.
- S121E is a swap from Serine to Glutamic Acid. S121E helps in binding by creating a chemical bond with the surface of the plastic. PET is very slippery but this change creates a sticky surface. This makes it so that the PET stays in place and makes it for the enzyme to be able to degrade it and not have to keep looking for it because it doesn't stay in place.
- D186H is a swap from Aspartic Acid to Histidine. D186H helps in Heat endurance by creating a hydrogen bond inside the enzyme. This extra connection prevents the enzyme from unfolding or breaking when the water gets too hot, keeping it stable and strong.
- R280A is a swap from Arginine to Alanine. R280A helps in Heat endurance by removing a part that was causing internal tension in the enzyme's structure. By swapping it for a better suited piece like Alanine the enzyme becomes stronger without so much tension. This allows it to keep its shape and still work in hot temperatures.

Dura-PETase



Dura-PETase's Genetic Mutations

- W159H is a mutation in which Tryptophan is changed to Histidine and it helps with making the degradation faster. In the wild type PETase, Tryptophan acts like a big amino acid that blocks the entrance to get inside PETase. Tryptophan has a very big side chain so it blocks the PET from entering the degradation site making the process much slower. By changing this to Histidine, which is a smaller amino acid this helps the PET get inside without facing the obstacle. This makes it so that the plastic can go inside the enzyme faster making the process faster and can go deeper inside which makes the degradation much faster because it can attack the bonds better.
- I168R is a mutation in which Isoleucine is changed to Arginine and it helps with Heat Resistance. Isoleucine is a neutral amino acid that doesn't make many bonds with the amino acids near it. By changing it to Arginine which has a positive charge, so the amino acid is able to form a bond with nearby negatively charged amino acids. This makes it so that the weak spot becomes stronger, and makes it so that the PETase melts when it is put into the hot water which is 60°C and it is required to soften plastic for degradation.

- S188Q is a mutation in which Serine is changed to Glutamine and it helps make the PETase stronger giving it heat resistance and a bit of pH resistance too. This mutation is a similar change to I168R change. While Arginine provides the positive charge for a new bond in I168R, Glutamine makes a hydrogen bond. These two changes create a good combined support. These bonds are important because if the enzyme's shape changes even a little bit because of heat, the enzyme will lose its ability to take the plastic. S188Q's bond makes it so that the functions of the PETase don't malfunction due to the heat and make it so that they all work properly making the enzyme able to work and degrade the plastic.

- S214H is a mutation in which Serine is changed to Histidine and it helps with thermal stability or heat resistance. This change replaces a simple and small amino acid with one that has a large aromatic ring. These rings are excellent at connecting against other rings to create a solid foundation. By placing a Histidine at position 214, the core of the enzyme is strengthened. This prevents the core of the protein from vibrating too much at high temperatures, so that the enzyme can stay active for days in an industrial use rather than the enzyme getting destroyed in a matter of minutes.
- L117F is a mutation in which Leucine is changed to Phenylalanine and it helps with Plastic Binding. This mutation is made to make the enzyme stickier toward plastic. PET plastic is made of aromatic carbon rings, and Phenylalanine also contains a large aromatic ring. By changing this the enzyme develops a close liking for the plastic surface through a process called pi stacking where the rings of the enzyme and the rings of the plastic create a bond like magnets. This helps the enzyme stay attached to the plastic while the degradation is happening
- Q119Y is a mutation in which Glutamine is changed to Tyrosine and helps with Hydrophobic Interaction. Similar to the L117F mutation, this change replaces a water amino acid with Tyrosine, which is stickier and contains a ring structure. Since plastic hates water based amino acids, and like to interact with hydrophobic molecules. By placing Tyrosine on the surface of the enzyme's binding pocket which is a place where the PET attaches, making an environment that is more

inviting, so that the PET can stay locked in place. This prevents the PET from sliding out of the PETase before the ester bonds can be fully broken.

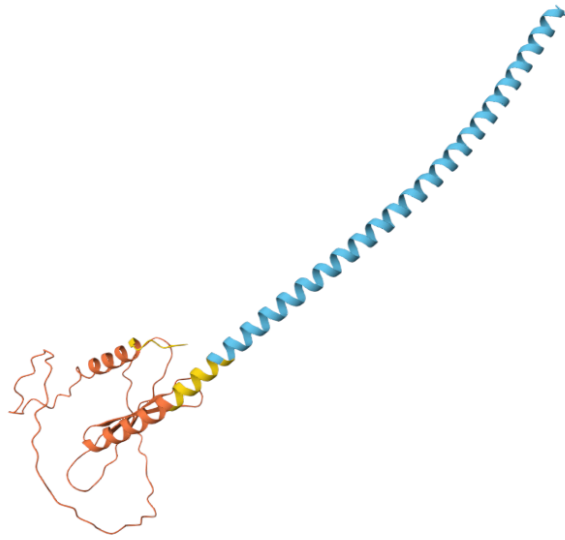
- A121E is a mutation in which Alanine is changed to Glutamic Acid and helps with Surface Charge. Alanine is a very small, neutral amino acid that doesn't do much on the surface of the protein. By changing it to Glutamic Acid, which is negatively charged, we have altered the surface of the enzyme. This change helps the enzyme manage the tricky boundary between the water it lives in and the plastic it is trying to degrade. It stabilizes the enzyme's orientation, ensuring that the cutting part of the enzyme stays pointed toward the plastic rather than flipping away into the water.


All mutations research that will work and do not occupy
same spots

- Y127G
- V212H
- A131G
- Q189R
- N233C (Kept this for stability over N233K)
- W159H
- R224Q
- S121E (Kept this over A121E)
- D186H
- R280A
- I168R
- S188Q
- S214H
- L117F
- Q119Y

Why I chose the name Trident-PETase

I have named Trident-PETase like that because I have focused on 3 points of weakness: heat, ph levels, and speed to focus on for this PETase and a trident has prongs suggesting this.



 Analysis

My Research Analysis

After I looked at all the research on FAST-PETase, DuraPETase, and LCC-ICCG-C09, I had to figure out how they would work if I put them all together. Most of the time, scientists only fix one thing like how hot it can get or just the speed but for PETase to actually work in the real world it has to fix everything at the same time.

The way the math works for finding out the 600x speed is that the speeds don't just add up, they actually multiply because each part helps the other parts do a better job. FAST-PETase gives the first 5x speed boost so it can degrade plastic in 4 days instead of 20 from the wild type. Then DuraPETase gives a 60x boost because it is 300x better than wild type and the FAST-PETase is 5x better so if you divide 300 by 5 it gives you 60. Finally, LCC-ICCG-C09 is the newest one I found that is 2 times faster and better than the LCC-ICCG because it makes the entrance wider for the plastic to get in. So LCC-ICCG is 300x the wild type and 2x that is LCC-ICCG-C09 and Dura-PETase is also 300x so 600x divided by 300x is 2. So the total calculation comes out to, 5 times 60 times 2 equals 600, which means my Trident-PETase can theoretically degrade plastic 600 times faster than nature.

The way the math works for finding out the 149.1°C limit is that Wild-PETase is way too weak and starts to melt at only 46°C, but I found that combining the heat resistance from all three PETases makes it way tougher. I calculated that LCC-ICCG-C09 adds 51.1°C of extra strength because its limit is 97.1°C and the wild type is only 46°C ($97.1^{\circ}\text{C} - 46^{\circ}\text{C} = 51.1^{\circ}\text{C}$). When I add the other boosts, where FAST-PETase adds 21°C and Dura-PETase adds 31°C, the final math becomes $46^{\circ}\text{C} + 51.1^{\circ}\text{C} + 21^{\circ}\text{C} + 31^{\circ}\text{C} = 149.1^{\circ}\text{C}$ This proves the enzyme can easily handle the 80°C to 90°C range without melting, which is the perfect spot where the plastic is softest and easiest to degrade.

The way the math works for finding out the pH resistance of 6.0 to 10.5 is that Wild-PETase is very pH picky and only works between pH 7.0 and 8.0, but I added up the stability points of the 3 PETases to widen that pH level. FAST-PETase adds 2.0, DuraPETase adds 0, and LCC-ICCG-C09 adds 1.5, which expands the original range by 3.5 points total. This creates a new working window of pH 6.0 to 10.5, which is the math that lets the enzyme survive in messy places like landfills where the chemistry is always changing.

For the compatibility benchmark, I also had to make sure the 15 mutations I picked wouldn't fight each other or try to change the same spot . I found that D238C and S283C only work for the LCC type and you can't use them for the wild-type PETase's blueprint, so I analyzed this and found that N233C is a better choice to keep the enzyme stable . I also realized F243I wouldn't work because there is no Phenylalanine in that spot for wild-PETase, so I used W159H instead to clear the bulky entrance so the plastic can go deeper inside and attack the bonds better .

By fixing the three big weaknesses, Heat, pH, and Speed all at once and in the same PETase. This Trident-PETase can finally work where the wild type PETase would just malfunction and die. My analysis proves that we can theoretically take the plastic degradation problem and solve it in a few weeks or days instead.



Results + Conclusion

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.

Applications + Extensions

Applications

My math and analysis proves that Trident-PETase could be used in the real world to fix the PET problem in places like Calgary's landfills. Since the enzyme can handle a pH of 6.0 to 10.5, it won't happen to die in the messy and acidic environment of the recycling plants where there is a lot of PET.

We could also use it in large scale recycling centers because it works at high temperatures up to 149.1°C where the PET is soft and easiest to degrade. Instead of waiting 500 years for PET plastics to degrade, this enzyme could finish the job in just a few days because it is 600 times faster than the wild type PETase. This would let us recycle PET over and over again without losing quality or harming our great mother earth.

Extensions

To make my project even better, I want to use more machine learning to see exactly how the 15 mutations work together to break down PET in different weather. I also want to test my Trident-PETase model in a real lab one day to see if it actually eats 1000g of PET per hour like I calculated.

Another thing I could do is try to get help from scientists and actually make this PETase outside on the internet. I could also test it to see if my math is true and then it could be duplicated and be used in large scale uses around the world.



References

Acknowledgements

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References

- Bhattacharya, S., Castagna, R., Estiri, H., Upmanis, T., Ricci, A., Gautieri, A., & Parisini, E. (2025). Development of a highly active engineered PETase enzyme for polyester degradation. *The FEBS Journal*. <https://doi.org/10.1111/febs.70228>
- Burgin, T., Pollard, B. C., Knott, B. C., Mayes, H. B., Crowley, M. F., McGeehan, J. E., Beckham, G. T., & Woodcock, H. L. (2024). The reaction mechanism of the *Ideonella sakaiensis* PETase enzyme. *Communications Chemistry*, 7(1), 1–14. <https://doi.org/10.1038/s42004-024-01154-x>
- Cui, Y., Chen, Y., Liu, X., Dong, S., Tian, Y., Qiao, Y., Mitra, R., Han, J., Li, C., Han, X., Liu, W., Chen, Q., Wei, W., Wang, X., Du, W., Tang, S., Xiang, H., Liu, H., Liang, Y., & Houk, K. N. (2021). Computational Redesign of a PETase for Plastic Biodegradation under Ambient Condition by the GRAPE Strategy. *ACS Catalysis*, 11(3), 1340–1350. <https://doi.org/10.1021/acscatal.0c05126>
- Diao, H., Chen, N., Wang, K., Zhang, F., Wang, Y.-H., & Wu, R. (2020). Biosynthetic Mechanism of Lanosterol: A Completed Story. *ACS Catalysis*, 10(3), 2157–2168. <https://doi.org/10.1021/acscatal.9b05221>
- Lee, S. H., Seo, H., Hong, H., Park, J., Ki, D., Kim, M., Kim, H.-J., & Kim, K.-J. (2023). Three-directional engineering of IsPETase with enhanced protein yield, activity,

and durability. *Journal of Hazardous Materials*, 459, 132297.

<https://doi.org/10.1016/j.jhazmat.2023.132297>

Lu, H., Diaz, D. J., Czarnecki, N. J., Zhu, C., Kim, W., Shroff, R., Acosta, D. J., Alexander, B. R., Cole, H. O., Zhang, Y., Lynd, N. A., Ellington, A. D., & Alper, H. S. (2022). Machine learning-aided Engineering of Hydrolases for PET Depolymerization. *Nature*, 604(7907), 662–667.

<https://doi.org/10.1038/s41586-022-04599-z>

Tournier, V., Topham, C. M., Gilles, A., David, B., Folgoas, C., Moya-Leclair, E., Kamionka, E., Desrousseaux, M.-L. ., Texier, H., Gavalda, S., Cot, M., Guémard, E., Dalibey, M., Nomme, J., Cioci, G., Barbe, S., Chateau, M., André, I., Duquesne, S., & Marty, A. (2020). An engineered PET depolymerase to break down and recycle plastic bottles. *Nature*, 580(7802), 216–219.

<https://doi.org/10.1038/s41586-020-2149-4>

UniProt. (2026). UniProt. <https://www.uniprot.org/uniprotkb/A0A0K8P6T7/entry>.

Yip, A., McArthur, O. D., Ho, K. C., Aucoin, M. G., & Ingalls, B. P. (2024). Degradation of polyethylene terephthalate (PET) plastics by wastewater bacteria engineered via conjugation. *Microbial Biotechnology*, 17(9).

<https://doi.org/10.1111/1751-7915.70015>