Molecular Dynamics Investigation into the binding mechanisms of PET polymer - enzyme PETase Complex

Athina Karaoli,[1] Co-Authors: Dimitris G. Mintis,[1,2] Haralampos Tzoupis,[1] Iseult Lynch,[2,4] Georgia Melagraki,[5] Antreas Afantitis[1,2,3]

1. Introduction

Plastic pollution has reached a point where traditional waste management can no longer mitigate the plastic waste problem effectively. The UN estimates that the annual global plastic production exceeds 400 million tonnes and is expected to triple by 2060. A significant portion of plastics (~85 %) is being disposed of in unregulated landfills or left unmanaged, and only 9 % is currently recycled [1].

Enzyme-based methodologies have emerged as an alternative for addressing plastic waste [2]. Advances in enzyme-based approaches hold great promise, as their integration into plastic degradation processes offers a sustainable and energy-efficient alternative to traditional mechanical recycling [3].

A breakthrough achieved in 2016 [4], showed that polyethylene terephthalate (PET), can be hydrolysed. During this process, two enzymes PETase and MHETase catalyse the degradation of PET to mono(2- hydroxyethyl) terephthalate (MHET) and then into terephthalic acid (TPA) and ethylene glycol (EG) (Figure 1) [5]. Building on these findings, the PhD project presented here aims to investigate the chemical processes involved in the degradation of plastic polymers such as PET using molecular simulation techniques.

[1] Department of ChemoInformatics, NovaMechanics Ltd., Nicosia CY-1070, Cyprus: info@novamechanics.com
[2] Entelos Institute Ltd, Larnaca 6059, Cyprus
[3] NovaMechanics MIKE, Piraeus 18545, Greece
[4] School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom
[5]Division of Physical Sciences and Applications, Hellenic Military Academy, Vari 16672, Greece

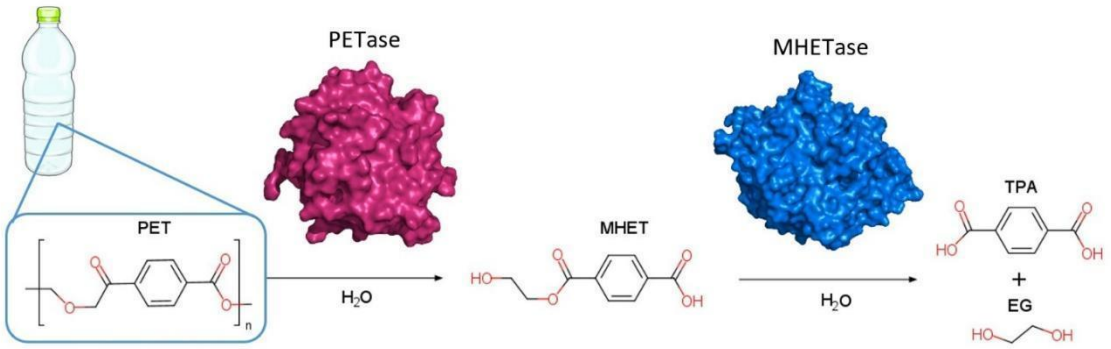

Figure 1. Representation of the degradation of PET into TPA and EG.

2. Methodology

Methodological Enhancement through Automation: The integration of Enalos Asclepios KNIME nodes (https://novamechanics.com/products/asclepios-knime-nodes/) with molecular dynamics (MD) studies represents a significant advance, offering a robust automation tool for complex computational chemistry tasks. The Asclepios KNIME nodes facilitate the streamlining and high-throughput processing of MD, which has been particularly instrumental in the field of small molecule drug design research.

A PET dimer was docked in PETase (PDB ID: 6EQE) using AutoDock Vina [6]. The Amber ff14sb and the GAFF2 force fields were employed to build the parameters for the protein and ligand, respectively, for the MD simulations. All MD simulations were performed using GROMACS [7]. Two production MD simulations were performed under constant temperature/pressure ensemble (T=300 K and P=1 atm) for: (i) the unrestrained complex and (ii) the complex with positional restraints on the ligand.

3. Results

The PET degradation process initiates through interactions between residues Ser160-His237-Asp206 [5, 8-10]. Table 1 shows a comparative analysis of the observed distances with those described in the literature. Figure 2 shows the conformation of PET with respect to the active site residues. Our results agree with those reported by Han et al (2017). PET forms hydrogen bonds with Tyr87 and Met161 residues that stabilise it in the active site.

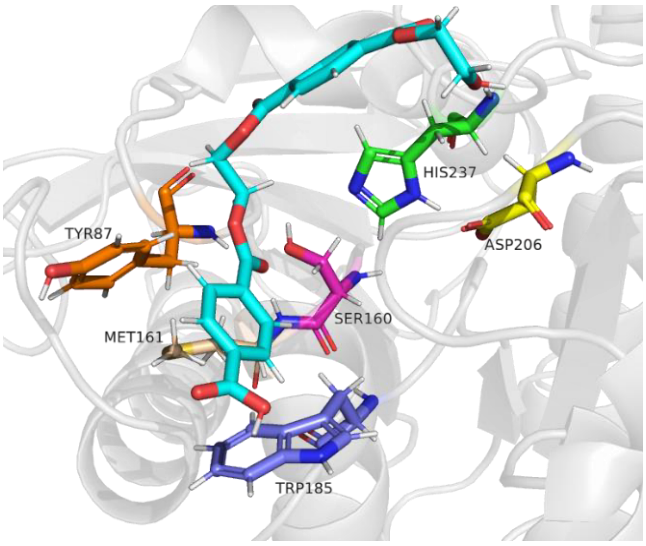

Figure 2. Close-up view of the PETase active site with the PET substrate.

Our MD simulations highlight the proximity between Ser160 and His237 that facilitates deprotonation of Ser160 by His237. Furthermore, our analysis showed the formation of an oxyanion hole involving the amine groups of Met161 and Tyr87 along with the O_3 of the PET in the restrained system, characterised by short H−O_3 contacts. Conversely, the unrestrained system displayed a disruption in this oxyanion hole.

Table 1. Average Distances as calculated by molecular docking and MD simulations.

		Distances (Å)	SER160 HG - HIS237 NE2	HIS237 HD1 - ASP206 OD2	TYR87 NH - PET O3	MET161 NH - PET O3
Molecular docking		present study	3.70	1.70	2.30	3.00
		Han et al., (2017) [5]	3.70	1.70	1.90	2.30
		da Costa et al., (2021) [8]	2.64	1.79		
Molecular Dynamics	Unrestraint system	present study	2.24 ± 0.49	1.81 ± 0.10	11.53 ±0.66	10.92 ± 0.82
		da Costa et al., (2021) [8]	4.18 ± 0.78	7.97 ± 0.37		
	Restraint system	present study	3.56 ± 0.24	1.82 ± 0.10	3.40 ± 0.42	3.58 -± 0.26
		Jerves et al., (2021) [9]	1.76 ± 0.15	1.62 ± 0.15	2.68 ± 0.57	3.07 ± 0.44
		Garcia et al., (2023) [10]	1.92 ± 0.14	3.10 ± 0.30	1.94 ± 0.14	2.20 ± 0.20

4. Future work

The initial results highlight the importance of PETase in PET degradation and lead us to further analyse the system through Density Functional Theory (DFT) calculations. This approach allows us to model the reaction mechanisms, providing insights into the catalytic processes.

Future plans include investigating ThermoPETase [11] and FAST-PETase [12]. ThermoPETase has improved thermostability and longer activity at high temperatures, while FAST-PETase exhibits a more efficient degradation process at 40 °C. The limited data available regarding FAST-PETase and ThermoPETase highlight the importance of employing quantum mechanics/molecular mechanics (QM/MM) models to investigate the application of these enzymes for PET degradation in an industrial setting.

5. Conclusions

The findings show the presence of an oxyanion hole and the dynamic facilitation of proton transfer between the SER and HIS residues in the active site. The understanding of the principles in enzymatic degradation at the molecular level serves as a crucial step towards our aim, which entails the development of a structure-based machine learning algorithm for engineering resilient and highly efficient enzymes capable of rapidly decomposing environmentally persistent plastics.

The use of Enalos Asclepios KNIME nodes in this study underscores a commitment to enhancing computational efficiency and accuracy in MD investigations, especially in contexts requiring high-throughput data processing.

6. Acknowledgments

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7. References

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