

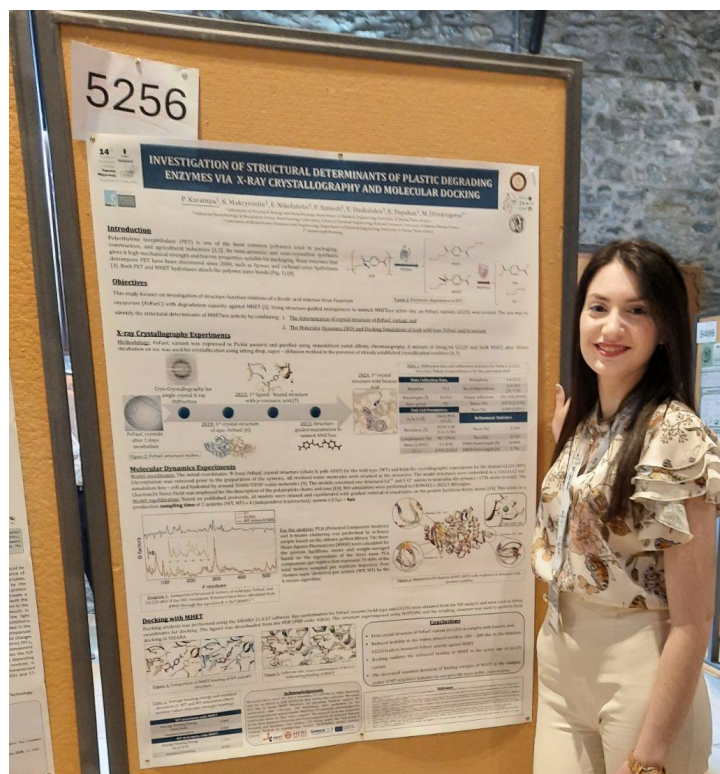
D4.3 Publication and/or presentation in International Conference of at least one determined structure

A series of dissemination activities were conducted to communicate the determined structures of enzymes targeting diverse scientific audiences through posters and publication. The results were also supported by structures' deposition in the PDBe.

Poster Presentations

- **14th Panhellenic Conference of Chemical Engineering**, Thessaloniki, Greece (29 – 31 May 2024)

A poster on *“Investigation of Structural Determinants of Plastics Degrading Enzymes via X-ray Crystallography and Molecular Docking”* was presented by Assistant Professor Maria Dimarogona and Ms. Panagiota Karampa (PhD candidate) to an audience of >250 researchers, related to biocatalysis and biotransformation.



- **7th LignoBiotech Symposium**, Toulouse, France (14–17 October 2024)

A poster on *“Harnessing the catalytic potential of ferulic acid esterase for MHET hydrolysis”* was presented by Assistant Professor Maria Dimarogona in a conference attended by over 100 researchers related to polymer degradation.

Harnessing the catalytic potential of a ferulic acid esterase for MHET hydrolysis

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Introduction
 Ferulic acid esterase (FAE) is one of the most common enzymes used in perfumery, cosmetics, and agricultural bioreactors [1]. Its wide substrate and substrate specificity gives a high molecular weight and hydrophobic nature suitable for producing biodegradable bioplastics. However, FAE have been found to be active on a broad range of substrates [2]. Both PET and MHET hydrolysis attack the polymer ester bonds [Fig. 1].

Objectives
 This study focuses on investigating the structure-function relation of a ferulic acid esterase from *Aspergillus fumigatus* with specific substrate specificity (FAE) for the hydrolysis of MHET. For this aim, a ferulic acid esterase, G1223, was isolated. The aim was to identify the structural determinants of FAE for its activity by carrying out: 1. **The determination of the crystal structure of FAE-Catalase** and 2. **Molecular dynamics (MD) and docking simulations** of both wild-type FAE and its mutant.

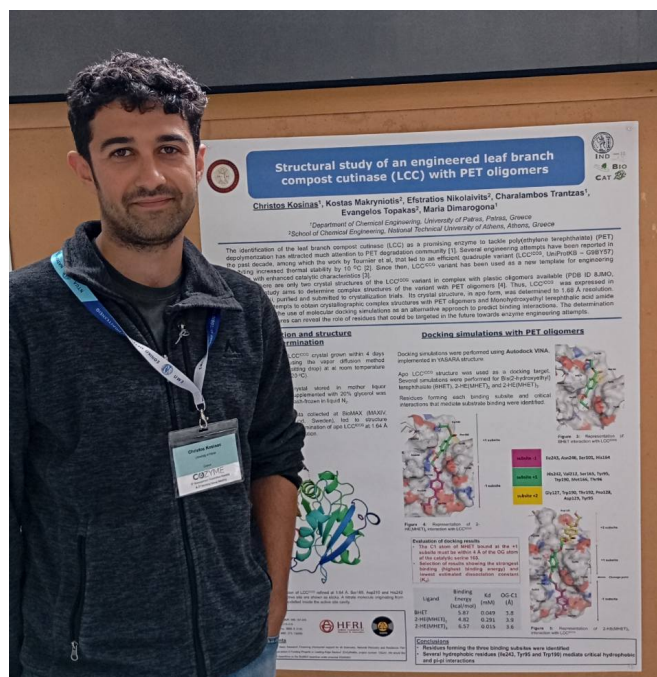
X-ray Crystallography
 Mutations of FAE were expressed in *Escherichia coli* and purified using ion-exchange and size exclusion chromatography. The protein structure was determined using X-ray diffraction. The structure was refined to an R-factor of 19.8% and an R-free of 24.8%. The structure was deposited in the Protein Data Bank (PDB) under the accession number 7J11.

Molecular Dynamics Simulations
 Molecular dynamics (MD) simulations were performed using GROMACS 5.1.4. The protein structure was prepared for the simulation using the CHARMM36 force field. The simulation was performed for 100 ns. The results of the simulation are shown in Fig. 2. The RMSD of the protein backbone atoms is shown in Fig. 3. The RMSF of the protein backbone atoms is shown in Fig. 4. The interaction energy between the protein and the substrate is shown in Fig. 5. The interaction energy between the protein and the water molecules is shown in Fig. 6. The interaction energy between the protein and the ions is shown in Fig. 7. The interaction energy between the protein and the lipid molecules is shown in Fig. 8. The interaction energy between the protein and the other molecules is shown in Fig. 9. The interaction energy between the protein and the other molecules is shown in Fig. 10.

Docking with MHET
 Docking studies were performed using the AutoDock Vina software. The protein structure was prepared for docking using the CHARMM36 force field. The substrate structure was prepared for docking using the CHARMM36 force field. The docking studies were performed for 100 ns. The results of the docking studies are shown in Fig. 11. The interaction energy between the protein and the substrate is shown in Fig. 12. The interaction energy between the protein and the water molecules is shown in Fig. 13. The interaction energy between the protein and the ions is shown in Fig. 14. The interaction energy between the protein and the lipid molecules is shown in Fig. 15. The interaction energy between the protein and the other molecules is shown in Fig. 16.

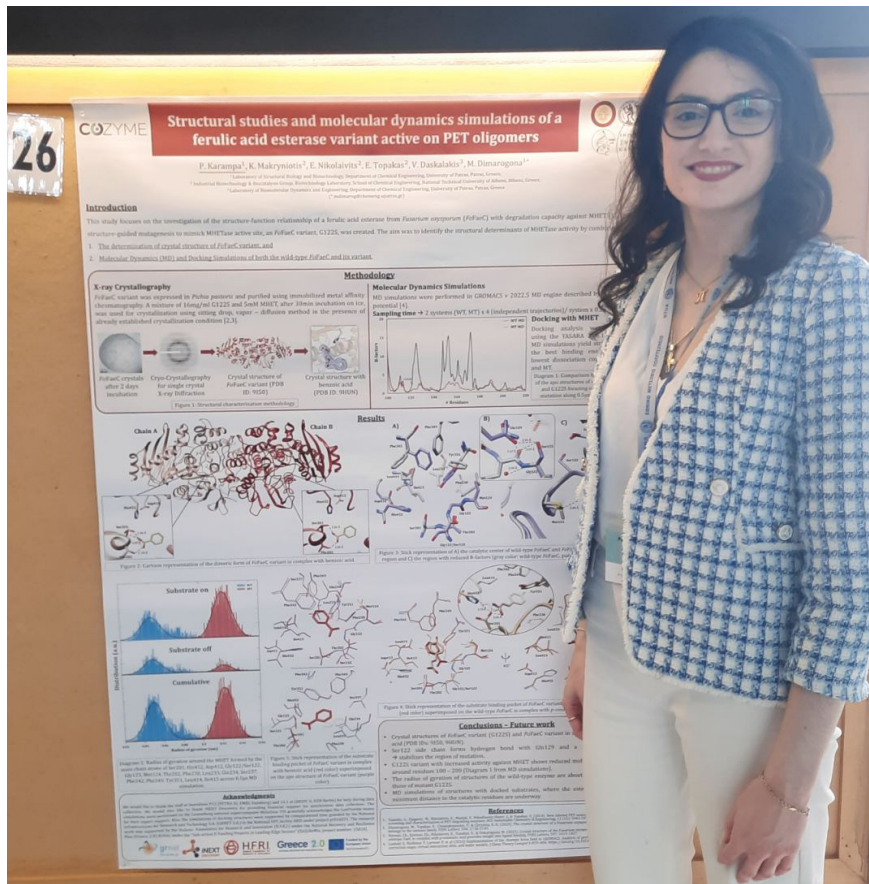
Conclusions - Future work
 • The crystal structure of FAE-Catalase (G1223) is complex and will be used to identify the structural determinants of FAE for its activity.
 • Docking studies for the substrate MHET and its mutant G1223 were performed.
 • The docking studies of FAE with MHET and its mutant G1223 are ongoing.

- **5th Management Committee Meeting and 3rd Working Group Meeting of the COZYME, Athens, Greece (24 – 25 April 2025)**
 A poster on “*Structural study of an engineered leaf branch compost cutinase (LCC) with PET oligomers*” was presented by Dr. Christos Kosinas in a conference attended by over 200 researchers related to biocatalysis and biotransformation.



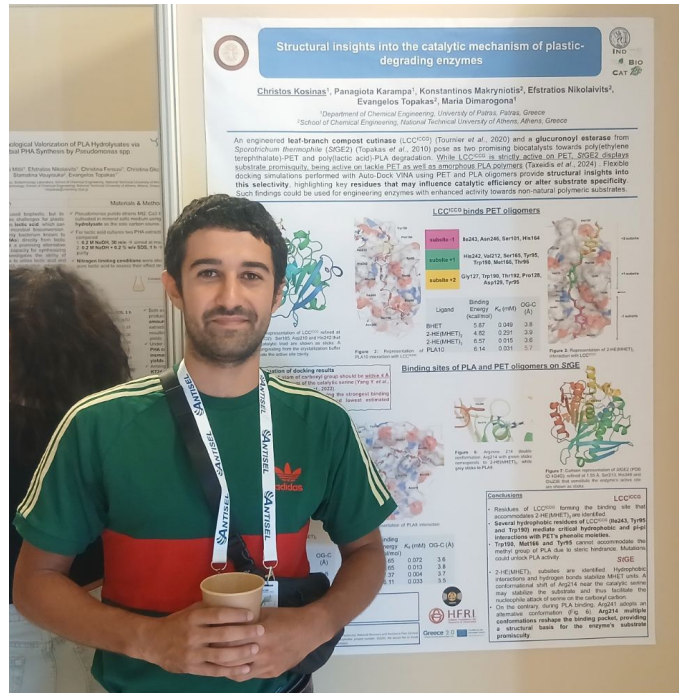
- **5th Management Committee Meeting and 3rd Working Group Meeting of the COZYME, Athens, Greece (24 – 25 April 2025)**

A poster on “*Structural studies and molecular dynamics simulations of a ferulic acid esterase variant active on PET oligomers*” was presented by Ms. Panagiota Karampa (PhD candidate) in a conference attended by over 200 researchers related to biocatalysis and biotransformation.



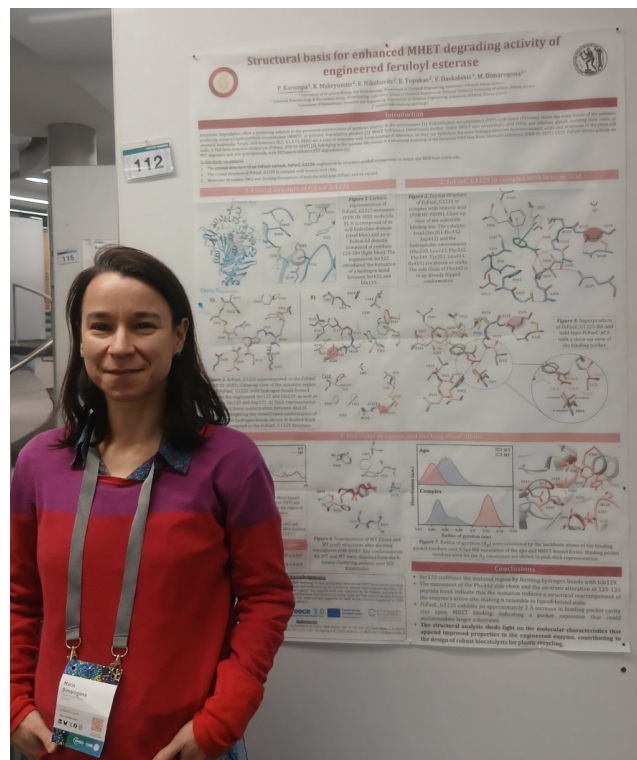
- **Mikrobiokosmos & CEESME Central and Eastern Europe Symposium on Microbial Ecology, Thessaloniki, Greece (22 – 24 September 2025)**

A poster on “*Structural studies into the catalytic mechanism of plastic-degrading enzymes*” was presented by Assistant Professor Maria Dimarogona and Dr. Christos Kosinias in a conference attended by over 200 researchers in the field of microbiology.



- **EMBO Workshop: Computational Structural Biology**, Heidelberg, Germany (2 – 5 December 2025)

A poster on “*Structural basis for enhanced MHET degrading activity of engineered feruloyl esterase*” was presented by Assistant Professor Maria Dimarogona in a conference attended by over 200 researchers in the field of bioinformatics.



Manuscript Publication

A research article titled “*Structural investigation of an engineered feruloyl esterase with improved MHET degrading properties*” was posted as a preprint on bioRxiv (doi: <https://doi.org/10.64898/2026.01.05.697840>) and is currently accepted for publication at FEBS Letters journal (*in Press*).

PDBe depositions

Two crystal structures have been deposited in the PDBe. Both are currently in “hold for publication” status.

- **PDB ID: 9RD2**, Online (30 May 2025)

A crystal structure was deposited titled “*Crystal structure of a leaf-branch compost cutinase variant (LCC_ICCG)*” in the PDBe (PDB link: <https://www.rcsb.org/structure/unreleased/9RD2>)

- **PDB ID: 9TTZ**, Online (8 January 2026)

A crystal structure was deposited titled “*Crystal structure of an esterase from Aspergillus parasiticus MM36 (AparEst)*” in the PDBe (PDB link: <https://www.rcsb.org/structure/unreleased/9TTZ>)