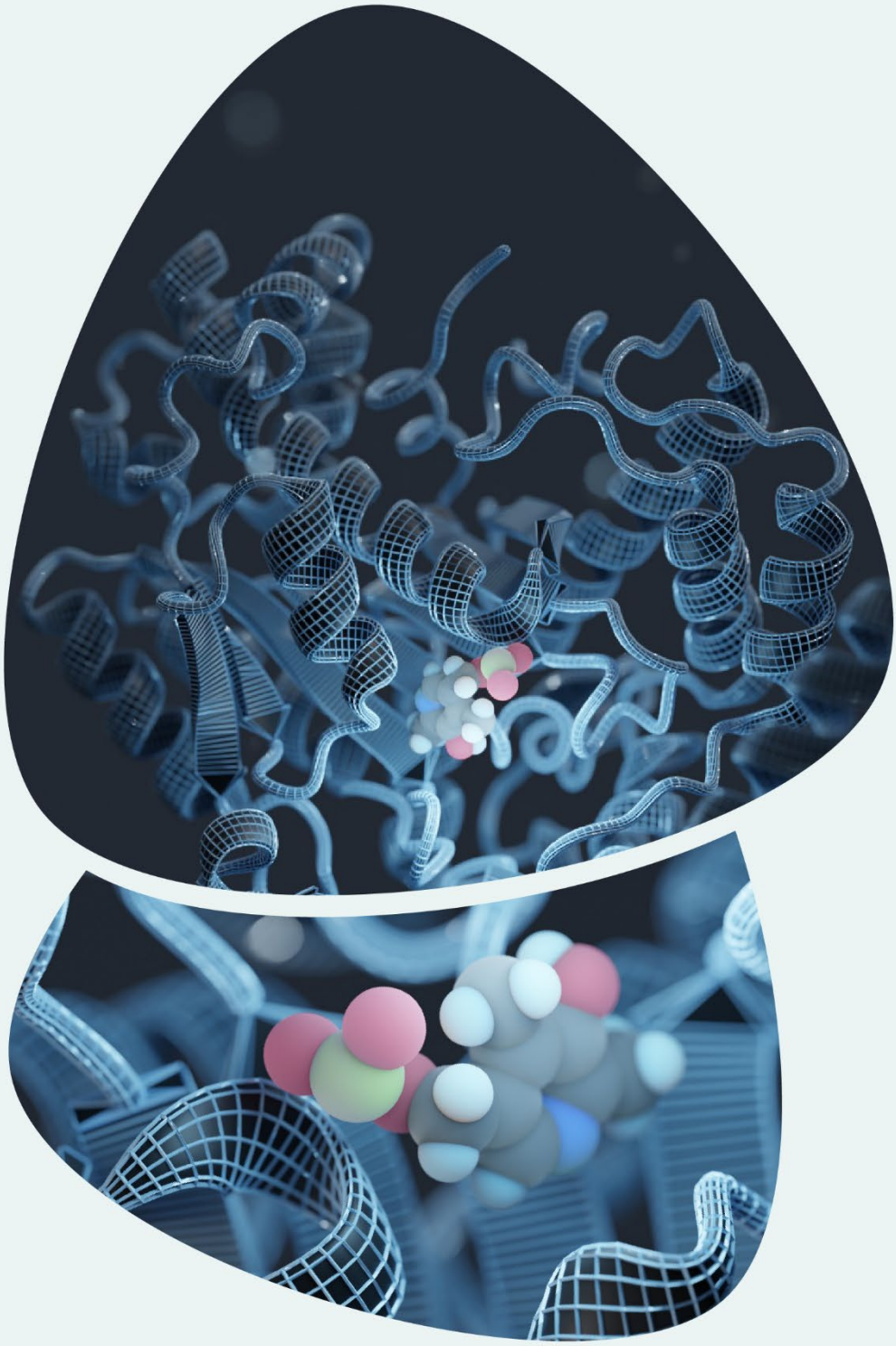

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BOOK OF ABSTRACTS

Working group meeting | April 24th–25th, 2025 | Athens, Greece

Poster presentation (26): WG3

Structural studies and molecular dynamics simulations of a ferulic acid esterase variant active on PET oligomers

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Enzymatic degradation represents a promising approach to address the continuous accumulation of synthetic plastics in the environment.[1] Since 2000, numerous polyethylene terephthalate (PET) hydrolases, known as PETases, have been discovered. These enzymes target the polymer's ester bond, producing mono-(2-hydroxyethyl) terephthalate (MHET), as the primary degradation product.[2] MHET esterases (MHETases) then cleave the ester bonds of MHET, yielding terephthalic acid (TPA) and ethylene glycol as end products, enabling their utilization as feedstocks. On the other hand, ferulic acid esterases (FAEs) are enzymes of biotechnological interest, that cleave the ester bonds between hydroxycinnamic acids and arabinose in the plant cell wall. A FAE from *Fusarium oxysporum* (*Fo*FaeC; PDB ID: 6FAT),[3] belonging to tannase-like family, is a structural homolog of a well-studied bacterial MHETase from *Ideonella sakaiensis* (PDB ID: 6JTT).[4,5] This enzyme shows activity on PET oligomers and demonstrates synergistic effect for PET degradation when combined with PETases.[6] The present work focuses on an *Fo*FaeC variant, G122S, that was created by structure-guided mutagenesis, in an effort to mimic the MHETase active site. G122S exhibits increased catalytic activity against MHET compared to wild-type *Fo*FaeC. The crystal structure of G122S, in apo and benzoic acid bound form, were determined (PDB codes 9I50 and 9HUN, respectively), to 1.90 and 1.71Å resolution, respectively. These were subsequently used for molecular dynamics and docking simulations, in order to gain a deeper understanding of the biochemical findings. Analysis of the experimental and computational results sheds light on the structural determinants of PET active enzymes.

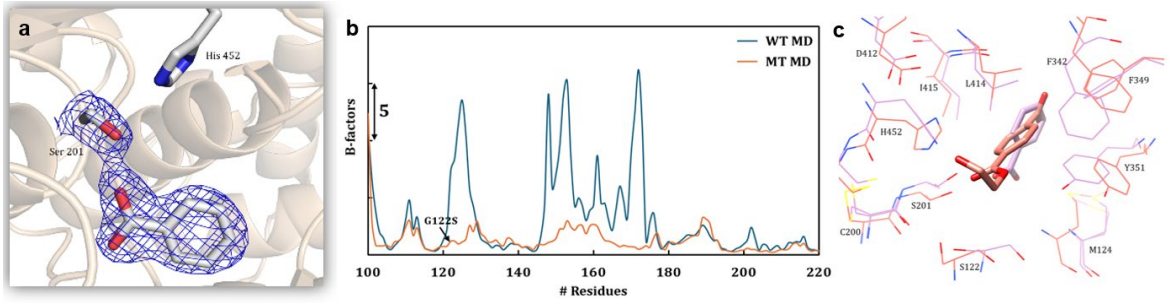

Figure 1. a) Catalytic center of G122S in complex with benzoic acid, b) Comparison between B-factors of wild-type FoFaeC and G122S focusing on the region carrying the mutated residue after 0.5 μs MD simulation and c) Superposition between crystal structure of G122S in

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Funded by the European Union

complex with benzoic acid and G122S MD structure in complex with MHET after docking simulations.

Acknowledgements:

We would like to thank the staff at beamlines P13 (PETRA III, EMBL Hamburg) and 14.1 at (BESSY II, HZB Berlin) for help during data collection. We would also like to thank iNEXT Discovery for providing financial support for synchrotron data collection. The research work was supported by the Basic Research Financing (Horizontal support for all Sciences), National Recovery and Resilience Plan (Greece 2.0), Greece 2.0, Action, under the “Sub-action II Funding Projects in Leading-Edge Sectors” (EnZyReMix, project number: 15024).

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