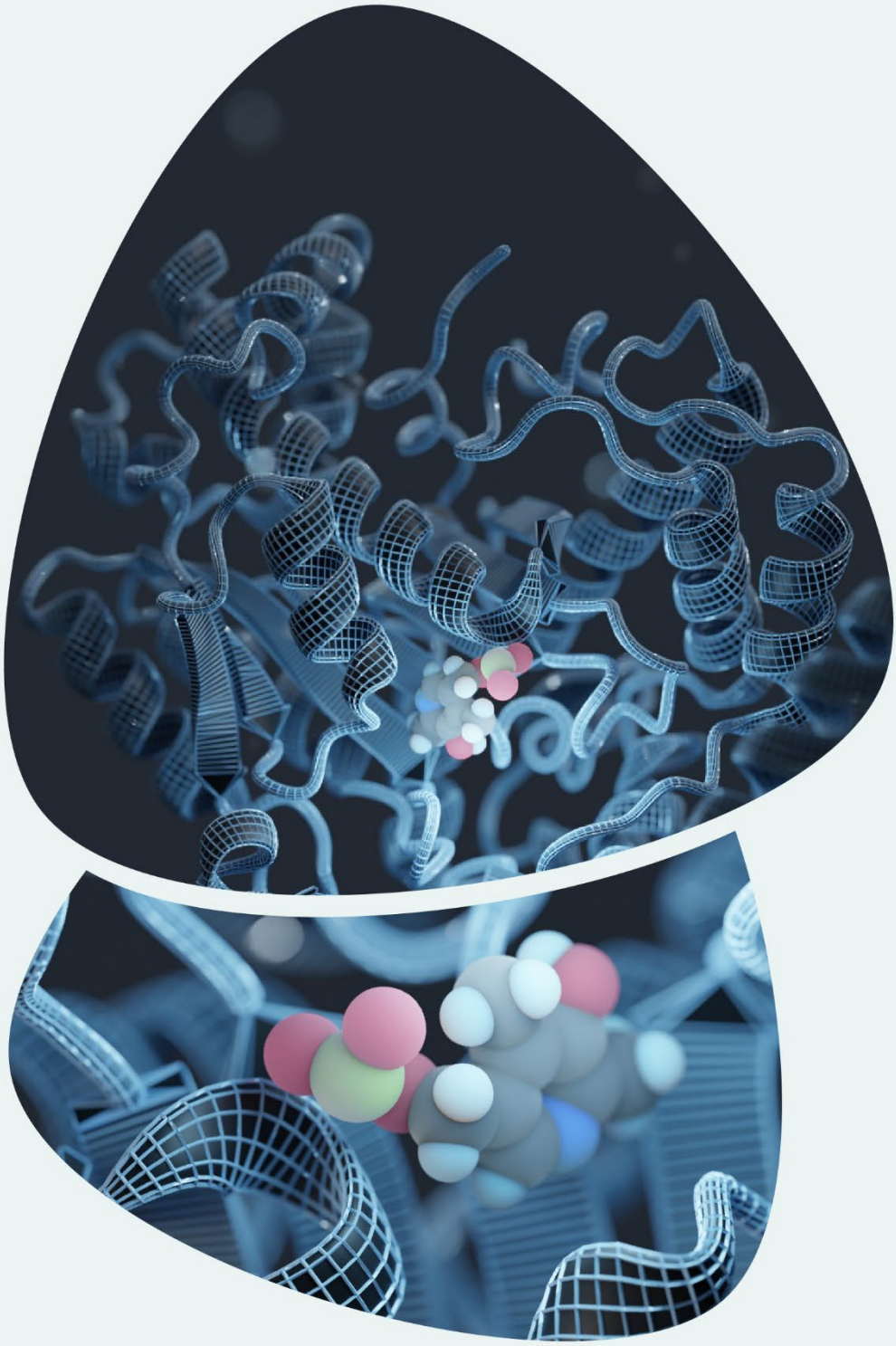

COZYME

BOOK OF ABSTRACTS

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

Poster presentation (16): WG3

Structural study of an engineered leaf branch compost cutinase (LCC) with PET oligomers

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The identification of the leaf branch compost cutinase (LCC) as a promising enzyme to tackle poly(ethylene terephthalate) (PET) depolymerization has attracted much attention to PET degradation community.[1] Several engineering attempts have been reported in the past decade, among which the work by Tournier *et al,* that led to an efficient quadruple variant ($LCC^{ICCG,}$ UniProtKB – G9BY57) exhibiting increased thermal stability by 10 ^{O}C.[2] Since then, LCC^{ICCG} variant has been used as a new template for engineering attempts with enhanced catalytic characteristics.[3]

Currently, there are only two crystal structures of the LCC^{ICCG} variant in complex with plastic oligomers (PDB ID 8JMO, 8JMP), available in the PDB. Our study aims to determine complex structures of the variant with PET oligomers.[4] Thus, LCC^{ICCG} was expressed in *Escherichia coli*, purified and submitted to crystallization trials. Its crystal structure, in apo form, was determined to 1.83 Å resolution. Attempts to obtain complex structures with PET oligomers and Monohydroxyethyl terephthalic acid amide (MHETA) are currently underway. As an alternative to protein crystallography, docking simulations of the aforementioned substrates with the enzyme will be also presented. The determination of complex structures can reveal the role of residues that could be targeted in the future towards enzyme engineering attempts.

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References:

1. S. Yoshida, K. Hiraga, I. Taniguchi and K. Oda, *Methods Enzymol.*, **2021**, 648, 187205.
2. V. Tournier, C. M. Topham, A. Gilles, B. David, C. Folgoas, E. Moya-Leclair, E. Kamionka, M.-L. Desrousseaux, H. Texier, S. Gavalda, M. Cot, E. Guémard, M. Dalibey, J. Nomme, G. Cioci, S. Barbe, M. Chateau, I. André, S. Duquesne and A. Marty, *Nature*, **2020**, 580, 216-219.
3. S. Fritzsche, F. Tischer, W. Peukert, and K. Castiglione, *React. Chem. Eng.*, **2023**, 8, 2156.
4. M. Djapovic, D. Milivojevic, T. Ilic-Tomic, M. Lješević, E. Nikolaivits, E. Topakas, V. Maslak, and J. Nikodinovic-Runic, *Chemosphere*, **2021**, 275, 130005