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 available (PDB ID 8JMO, 8JMP). 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 1.68 Å resolution. Unsuccessful attempts to obtain crystallographic complex structures with PET oligomers and Monohydroxyethyl terephthalic acid amide (MHETA), led to the use of molecular docking simulations as an alternative approach to predict binding interactions. The determination of complex structures can reveal the role of residues that could be targeted in the future towards enzyme engineering attempts.

Crystallization and structure determination

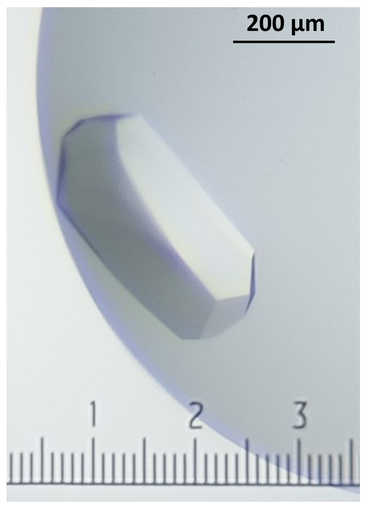

Figure 1: *Crystal of* LCC^{ICCG} developed by sitting drop method.

LCC^{ICCG} crystal grown within 4 days using the vapor diffusion method (sitting drop) at at room temperature (20 ^{o}C).

Crystal stored in mother liquor supplemented with 20% glycerol was flash-frozen in liquid N_2.

Data collected at BioMAX (MAXIV, Lund, Sweden), led to successful structure determination of apo LCC^{ICCG} at 1.64 Å resolution.

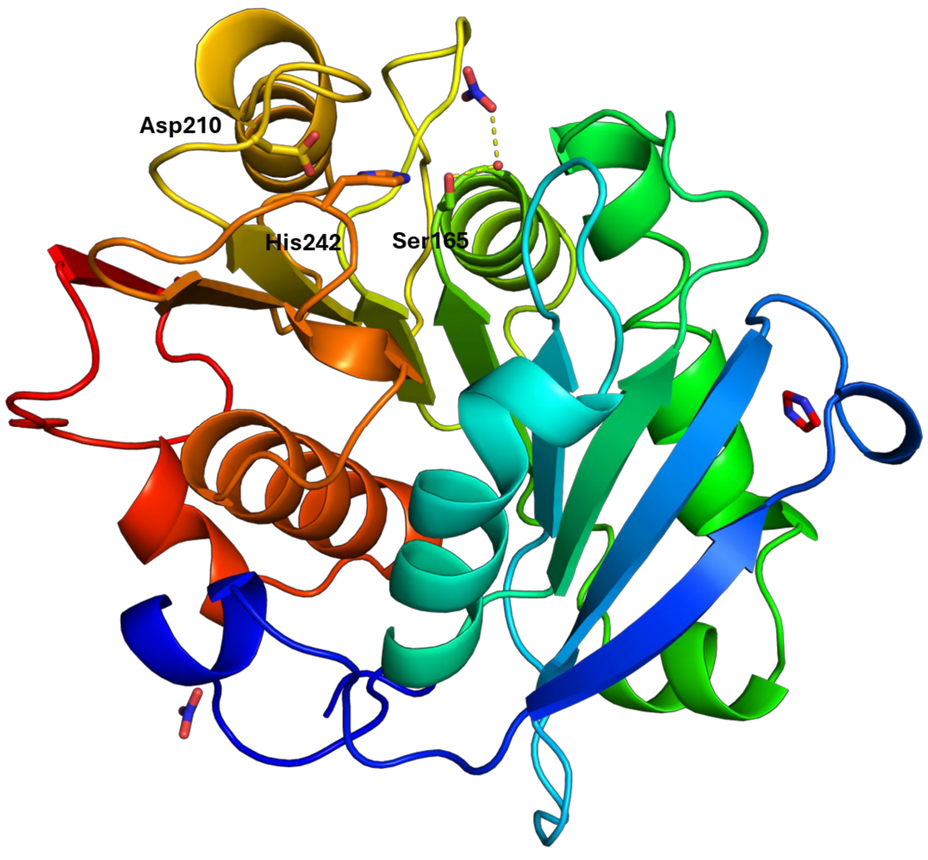

Figure 2: Cartoon representation of LCC^{ICCG} refined at 1.64 Å. Ser165, Asp210 and His242 that constitute the enzyme's active site are shown as sticks. A nitrate molecule originating from the crystallization buffer was modelled inside the active site cavity.

Docking simulations with PET oligomers

Docking simulations were performed using **Autodock VINA**, implemented in YASARA structure.

Apo LCC^{ICCG} structure was used as a docking target. Several simulations were performed for Bis(2-hydroxyethyl) terephthalate (BHET), 2-HE$(MHET)_2$ and 2-HE$(MHET)_3$.

Residues forming each binding subsite and critical interactions that mediate substrate binding were identified.

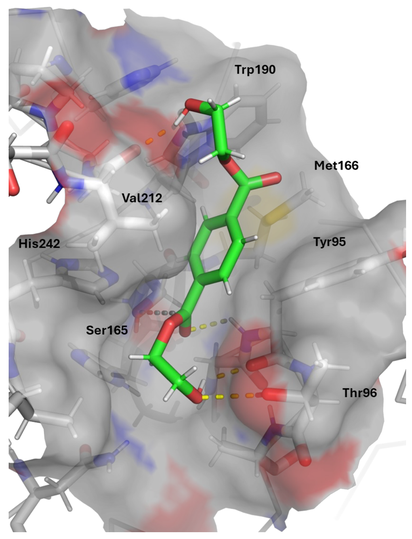

Figure 3*:* Representation of BHET interaction with LCC^{ICCG}

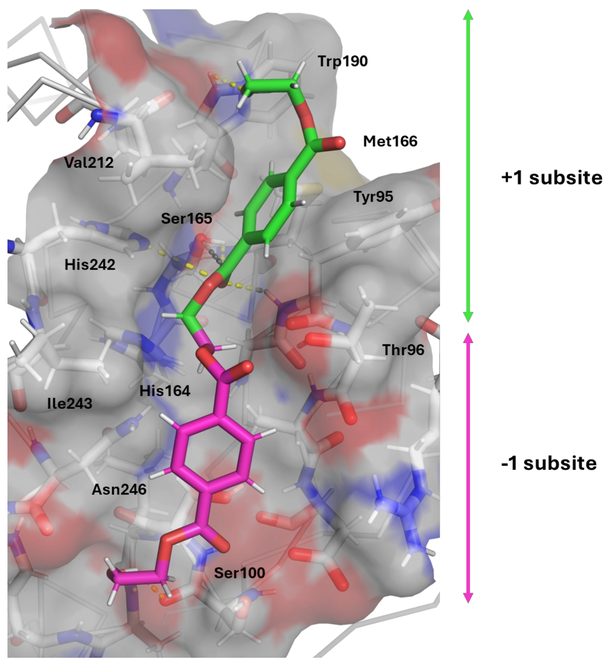

Figure 4*:* Representation of 2-HE$(MHET)_2$ interaction with LCC^{ICCG}

subsite -1	Ile243, Asn246, Ser101, His164
subsite +1	His242, Val212, Ser165, Tyr95, Trp190, Met166, Thr96
subsite +2	Gly127, Trp190, Thr192, Pro128, Asp129, Tyr95

Evaluation of docking results

- **The C1 atom of MHET bound at the +1 subsite must be within 4 Å of the OG atom of the catalytic serine 165.**
- **Selection of results showing the strongest binding (highest binding energy) and lowest estimated dissociation constant (K_d).**

Ligand	Binding Energy (kcal/mol)	Kd (mM)	OG-C1 (Å)
BHET	5.87	0.049	3.8
2-HE$(MHET)_2$	4.82	0.291	3.9
2-HE$(MHET)_3$	6.57	0.015	3.6

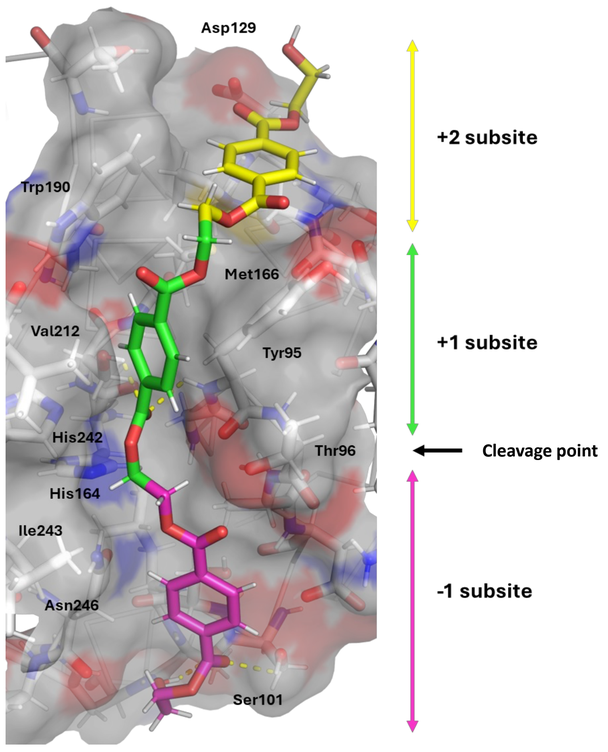

Figure 5*:* Representation of 2-HE$(MHET)_3$ interaction with LCC^{ICCG}

Conclusions

- **Residues forming the three binding subsites were identified**
- **Several hydrophobic residues (Ile243, Tyr95 and Trp190) mediate critical hydrophobic and pi-pi interactions**

References

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