Towards PET degradation engineering

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I. Extended Abstract

Plastics and their massive production are a world threat that needs to be dealt as soon as possible. Polyethylene terephthalate (PET) is one of the most widely used plastic polymers in the globe and its stability leads to a long lifetime in nature. Thus, its biodegradation was not considered until recently when a PET hydrolyzing bacteria was found in a PET bottle recycling site on Japan [1]. From this bacteria, two novel enzymes were found called PETase and MHETase. PETase degraded the polymer into subpolymers, while the second enzyme fully cleaved the monomer into subunits that the bacteria used as carbon source.

The following years consisted in elucidating the structure of the enzyme to decipher the exact catalytic mechanism of action against the PET polymer. Several research groups obtained X-ray data to generate different models of the WT enzyme and even mutant engineered versions of it [2], [3], [4], [5].

Our research group has previous experience on enzyme engineering and had a recent paper regarding the addition of an extra active site to a functional enzyme, what we call plurizymes [6].

Therefore, the main goal of this research is to rationally design the current PETase enzyme to contain an extra active site for the polymer increasing its activity.

A. PELE simulations

To design a new active site in the enzyme, we have to explore the surface of the protein for unknown binding sites first. To accomplish this, we use PELE software, which is a Monte Carlo technique combined with side chain prediction [7].

The PDB codes for the used crystal structures in the simulations are 6EQE and 5YNS for the WT and the R280A mutant enzymes, respectively [2], [5]. The R280A mutant version is also studied, since it is more efficient than the WT protein.

Regarding the ligands, we have used the monomer (MHET), the dimer ($(MHET)_2$), the dimer with an extra tail of ethylene glycol ($2\text{-HE}(MHET)_2$) and the tetramer with an extra tail of ethylene glycol ($2\text{-HE}(MHET)_4$) due to their common use in the structural studies of the PETase (see Figure 1 to visualize both the enzyme and the ligands).

The analysis of the results consists in mainly plotting the different parameters/metrics stored during the simulation to find the the local minima, which are the structures of the protein with the ligand bound to it.

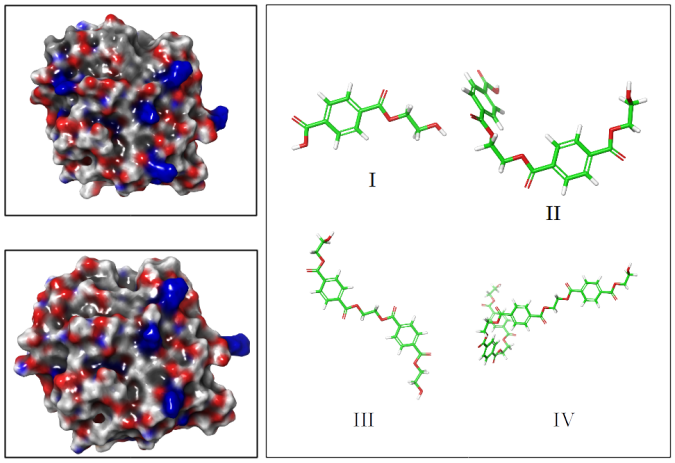

Fig. 1. Left: Surfaces of 6EQE (top) and 5YNS (bottom) crystal structures. Right: I; MHET, II; $(MHET)_2$, III; $2\text{-HE}(MHET)_2$, IV; $2\text{-HE}(MHET)_4$.

Once new binding sites are found, they are functionalized according to different concepts related with chemistry and statistical mechanics.

B. Results

After running PELE simulations for all the mentioned ligands with specific parameters, we see different unknown binding sites that correspond to local minima or are metastable as it can be seen in Figure 2.

For instance, the local minima found 20 Å away from the serine residue (S) in the main active site shows nice properties that enable its functionalization. It shows a S near the binding of the ligand and two near residues that can be easily changed by histidine and an acidic residue. Besides, the binding site resembles a cavity and it presents a tryptophan residue that interacts with the benzene ring in the substrate.

Hence, we rationally mutated some of the near residues to create the catalytic triad and to still accommodate the substrate as well.

Once we have the selected mutations, we rerun PELE on those mutations to see if the binding site is explored with the inserted mutations. Once we have done this and we see that the ligand still explores the new potential active site, we run a MD simulation of some ns to see the stability of the complex with the mutations.

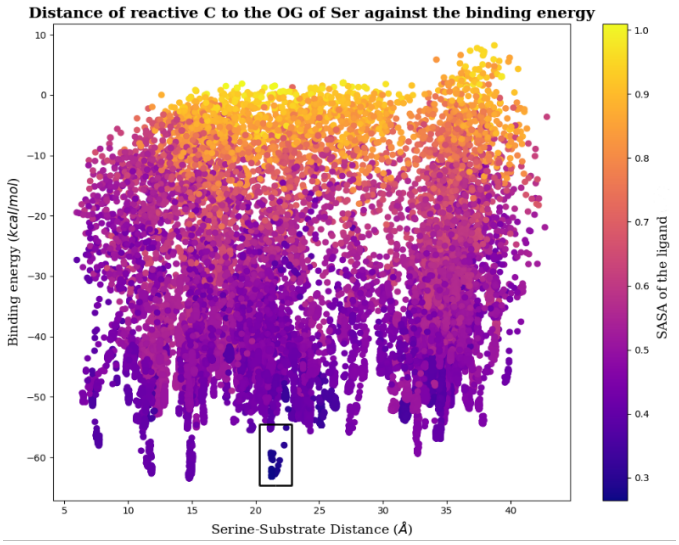

Fig. 2. Plot that represents the distance of the reactive C in the substrate to the nucleophile O_γ in the serine residue in the main active site against the binding energy of the substrate with the enzyme. The colorbar refers to the normalized solvent accessible surface area (SASA) of the ligand.

We have created 3 potential active sites but we are still currently working on the MD simulations and exploring all the PELE results.

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Sergi Rodà is a native of Terrassa and is currently a MSc research student at the BSC in the EAPM group led by Víctor Guallar. He got his BSc degree in Biochemistry at the "Universitat Autònoma de Barcelona". He has worked in the group of molecular bases of disease at the "Institut Investigació Biomèdica - Sant Pau" as a researcher. He started his MSc studies in Bioinformatics on October of 2018 with the "Becas Másteres de Excelencia - Fundació Catalunya-La Pedrera" grant.