Report on Ph.D. thesis
of
MSc. Zeenat Zara

"Computational modeling of biomolecular interactions in proteins using MD simulations"

The dissertation of MSc. Zeenat Zara is a valuable contribution to studies of protein-ligand and protein-protein interactions. The thesis document consists of ~80 pages organized in four main chapters. The first chapter - Introduction - familiarizes reader with studied enzymes that are involved in degradation of plastics and with medicinally important G-protein coupled receptors (GPCRs). The second chapter describes used methods - homology modeling and molecular dynamics (MD) simulations. Next chapter presents main results, which are as follows:

a) MD simulations were used to study interactions between the aqueous solution of ionic liquids and PETase - the most efficient PET degrading enzyme. The stability of its complex with bis-(hydroxyethyl)-terephthalate (BHET) ligand in the presence of either water or different ionic liquids was analyzed in detail. It was found that only cholinium phosphate has a minimal impact on the structure of this enzyme-ligand complex.

b) Further, computational modeling was employed to explore the protein-protein interactions between serotonin GPCR that is involved in sleep, mood, anxiety, learning etc. and Gs protein in the state prior to activation. The interface of GPCR and Gs protein in the preassembly and active complexes was compared using long MD simulations. It was found that most of interacting residues are different.

c) Finally, the role of ionic liquid-based nanomaterials for drug delivery was reviewed.

The dissertation itself, as far as I can judge, is written in good and understandable English. A significantly larger amount of images would be beneficial. The reader often has to open the protein structure using a software package and find the mentioned amino acids, otherwise it is difficult to follow and understand the text.

The whole work represents very suitable combination of various computational methods used to shed light on studied subjects. It is evident that MSc. Zeenat Zara masters various modeling techniques using several different software packages (GROMACS, Modeller etc.). Apparently, she knows to suitably apply them to get valuable data.

There are enclosed two papers and one manuscript that was submitted. In addition, one more paper and one other manuscript were not included in the text of this dissertation. Moreover, MSc. Zeenat Zara has several other publications from earlier years, which are based on her diploma thesis. In total, she is now the co-author of ten papers.

In my opinion, her work fulfils the requirements for Ph.D. thesis, and I recommend it for the defense.

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Questions:

Q1 - Regarding MD simulations of PETase. I'm not sure what ligand was studied because in paper 1 (Zara et al. Molecules 2022, 27, 119) this ligand is not shown. The text states:

p. 6 "BHET molecule starts bending from the central carbonyl carbon due to non-bonding interaction between two sites of BHET rings".

p. 12 "The ligand presented in our study has two TPA rings, which are called BHET."

But this does not correspond to Figure 6 in the thesis, where the BHET structure has just one ring, or to Supplementary Figure 3 in the paper: Joo et al. **Structural insight into molecular mechanism of poly(ethylene terephthalate) degradation** Nature Communications 9 (2018) 382.

Show Supplementary Figure 3 from this paper and make clear which ligand was used.

Q2 - How was the initial structure of the PETase-ligand complex obtained? In the dissertation and paper 1 (Zara et al. Molecules 2022, 27, 119) this point is not described or I skimmed it. The initial 5xjh crystal structure does not contain any ligand.

Q3 - Which of the binding sites presented in Joo et al. Nature Communications 9 (2018) 382, where they used flexible and covalent docking for the larger substrate 2-HE(MHET)$_4$, was occupied by the ligand in the complex investigated in the dissertation? Show the complex from the dissertation and Figure 2 from Joo et al. side by side and compare them.

Q4 - Show the conformation of the ligand at the beginning and end of the MD simulation. How was the ligand bending stimulated by ionic liquids? Was it a direct interaction of ionic liquids with the ligand? Or an indirect interaction where ionic liquids influenced PETase, which subsequently caused ligand bending?

Q5 - Alphafold structure AF-Q91559-F1 (https://alphafold.ebi.ac.uk/entry/Q91559) contains a very different model for ICL3 of 5-HT7R - well-structured alpha-helices. Could this be an artifact due to the x-ray structures like 7WC4 and 4IAQ actually being fusions of a GPCR and an unrelated protein in the ICL3 region to facilitate crystallization that confused AI?

Q6 - Show the most important amino acids from the heat maps in Figure 6 and 7 in the context of the complex consisting of GPCR and Gs structures, so that the distinction between active and inactive complex is clear.

Q7 - What are the main results of the paper: Borthakur et al. Journal of Environmental Management 1(4) article 111989 that was not included in your thesis?