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MODELING AND COMPUTATIONAL STRUCTURAL STUDIES OF PROTEIN 6-HYDROXYMETHYL-7,8-DIHYDROPTERIN PYROPHOSPHOKINASE FROM *Pseudomonas* aeruginosa

COMPUTATIONAL MODELING AND STRUCTURAL STUDIES OF THE PROTEIN 6-HYDROXYMETHYL-7,8-DIHYDROPTERIN PYROPHOSPHOKINASE FROM Pseudomonas aeruginosa

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SUMMARY

Due to the high mortality rate related to hospital infections caused by the bacterium *Pseudomonas aeruginosa,* it is important to conduct studies on proteins present in the bacteria that are therapeutic targets for the discovery of new antimicrobial drugs.
Enzymes that are not found in the human body, such as 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) play an important metabolic role in microorganisms and are therefore valuable potential targets (CHHABRA *et* al., 2012). The HPPK enzyme is part of the folate biosynthetic pathway, a very important compound for the proper functioning of *P. aeruginosa cells.* Therefore, this study performed computational homology modeling of *P. aeruginosa* HPPK (PaHPPK).
Specifically, models of PaHPPK were obtained in various conformational states, corresponding to each step of the pyrophosphokinase reaction, and the substrates and products were also modeled. The best modeled structures were selected based on the lowest DOPE score (SALI AND SHEN, 2006), and comparison of the models revealed a potential coupling mechanism between the adenine-binding region of ATP and the loop covering this substrate. It is hoped that this theoretical information can be added to biochemical knowledge to aid in the identification of ligands for HPPK as a therapeutic target.

Keywords: *Pseudomonas aeruginosa,* Homology Modeling, *Docking* Computational, 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, HPPK.

ABSTRACT

Due to the high mortality rate associated with hospital infections caused by the bacterium *Pseudomonas aeruginosa,* it is relevant to conduct studies on bacterial proteins that may serve as therapeutic targets for the discovery of new antimicrobial drugs. Enzymes not found in the human organism, such as 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK), play an important metabolic role in microorganisms and are therefore valuable potential targets (CHHABRA et al., 2012). HPPK is part of the folate biosynthetic pathway, a compound essential for the proper functioning of *P. aeruginosa* cells. Thus, in this work, homology-based computational modeling of *P. aeruginosa* HPPK (PaHPPK) was performed.
Specifically, models of PaHPPK were obtained in different conformational states corresponding to each step of the pyrophosphokinase reaction, in which substrates and products were also modeled. The best-constructed structures were selected based on the lowest DOPE score (SALI & SHEN, 2006), and comparison of the models suggested a potential coupling mechanism between the adenine-binding region of ATP and the loop that covers this substrate. It is expected that these theoretical insights may complement

biochemical knowledge and assistance in the identification of ligands targeting HPPK as a therapeutic candidate.

Keywords: *Pseudomonas aeruginosa,* Homology Modeling, Computational Docking, 6-Hydroxymethyl-7,8-Dihydropterin Pyrophosphokinase, HPPK.

1. INTRODUCTION

Hospital infection is such an old problem, dating back to the first places allocated to house sick people. Furthermore, both in ancient and modern society, modern, this type of infection has always caused impact and concern due to its high mortality rate (SANTOS, 2004). According to a WHO report carried out in 2014, the *Pseudomonas aeruginosa* is among the main species of bacteria resistant to antibiotics (BORDIGNON; LIMA, 2017). *P. aeruginosa* belongs to the family Pseudomonadaceae and is described as a straight or slightly rounded Gram-negative rod. curved, strictly aerobic, and can be observed as isolated cells, in pairs or in chains short, with the presence of flagella (FERREIRA, 2005). This species has clinical relevance due to its multiple resistance to antibacterial drugs and its difficult eradication; consequently, the bacteria is related to high rates of morbidity and mortality (NEVES *et* al., 2011). The difficulty in eradicating the bacteria is due to the fact that it survive for prolonged periods in humid environments, equipment and utensils hospitals – respirators and nebulizers -, as well as the fact that antiseptic solutions, disinfectants and therapeutic use serve as a reservoir (FERREIRA, 2005).

It is noteworthy that knowledge of the three-dimensional structure of proteins (mainly enzymes) is of great importance as it constitutes an auxiliary factor for biochemical information in the design of new compounds of pharmacological interest. Such structural information can be obtained experimentally through techniques X-ray diffraction and nuclear magnetic resonance, for example. However, when experimental failure, one can resort to computational methods in order to obtain a prediction of protein structure, for example, based on protein structures previously determined homologs (VERLI, 2014).

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2 THEORETICAL FRAMEWORK

Due to the relevance of these multidrug-resistant organisms in the hospital environment, studies of some of its enzymes, which are species-specific, may become valuable in pointing out potential therapeutic targets for drug development antimicrobials. One of these possible targets is the enzyme 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK), essential in the biosynthesis of folic acid and which is not produced in human organisms. These studies can stimulate research and development of specific inhibitors of HPPK (DERRICK, 2008) from *P. aeruginosa.* HPPK catalyzes the transfer of a pyrophosphate group from an adenosine triphosphate (ATP) molecule to 6-hydroxymethyl-7,8-dihydropterin (HP), resulting in adenosine monophosphate (AMP) and 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (HPPP) (BLASZCZYK *et* al., 2007).

2. MATERIAL AND METHOD

The amino acid sequence of the enzyme 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) from *Pseudomonas aeruginosa* was obtained from the gene portfolio of SSGCID *(Seattle Structural Genomic Center for Infectious Disease).* This sequence was used to build a sequence alignment, using the NCBI BLAST tool *(National Center for Biotechnology Information),* which assisted in the selection procedure of the structures of homologous template proteins (required in the model building step) based on degree of similarity, identity, function, and sequential completeness. Alignments structural elements produced with the SALIGN module of the MODELLER program (SALI E BLUNDELL, 1993) were used to improve the accuracy of the procedure modeling. 500 models were constructed using modeling by homology through satisfaction of spatial constraints as implemented in the program MODELLER. The choice of the best model was made based on the lowest value of DOPE score (SALI AND SHEN, 2006). All models were also superimposed theoretical data obtained, referring to the states of the HPPK enzyme, using as a basis for construction of the PDBs 1hka, 1rao, 1eq0, 1q0n and 1rb0. The models were evaluated for their stereochemical quality and folding (secondary, tertiary and quaternary structures). With

in order to highlight the differences and/or similarities between their structures, we used comparisons of the obtained model with the structures of homologous enzymes.

3. RESULTS AND DISCUSSION

The modeling was carried out in two stages and the sequence of complete amino acid sequence of PaHPPK (P. *aeruginosa HPPK protein).* In the first, with the In order to obtain a model of PaHPPK in apo form, a search was carried out for homologous structures with the BLAST tool, which provided 20 template proteins. The second stage consisted of using only one counterpart, representative of each of the states of the PaHPPK reaction (based on the following PDBs: 1HKA, 1RB0, 1EQ0, 1RAO and 1Q0N), to obtain a model of the PaHPPK complex with substrates and products in each case. The reason for this division is that these states exhibit quite significant conformational changes. large, characteristics of the conformation assumed by HPPK in each reaction step (YAN E JI, 2011), therefore, the objective was to avoid introducing spatial restrictions that were not specific to a particular state.

The stereochemical quality of the selected models was evaluated using the Ramachandran generated by the PROCHECK program (LASKOWSKI *et al.,* 1993). The figures 1-5 show the lowest energy model and the corresponding Ramachandran plot for the structural models of each of the reaction steps. The relative number of residues in each region of the Ramachandran chart is given in Table 1.

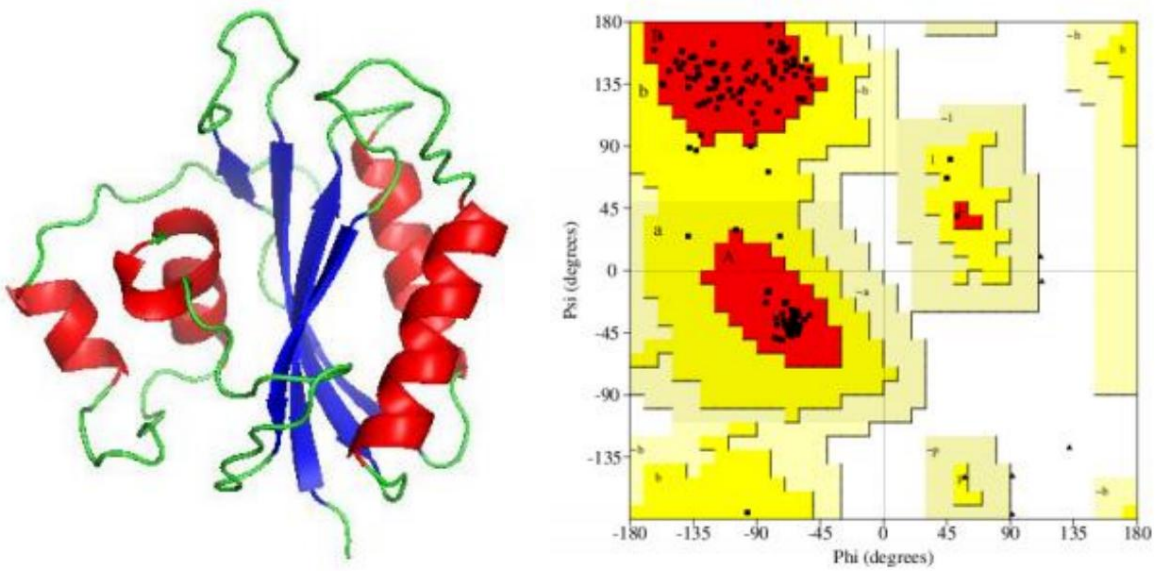

Figure 1. Apo-PaHPPK structure and Ramachandran plot. The types of secondary structures were colored as follows: alpha-helices in red, beta-strands in blue and loops in green.

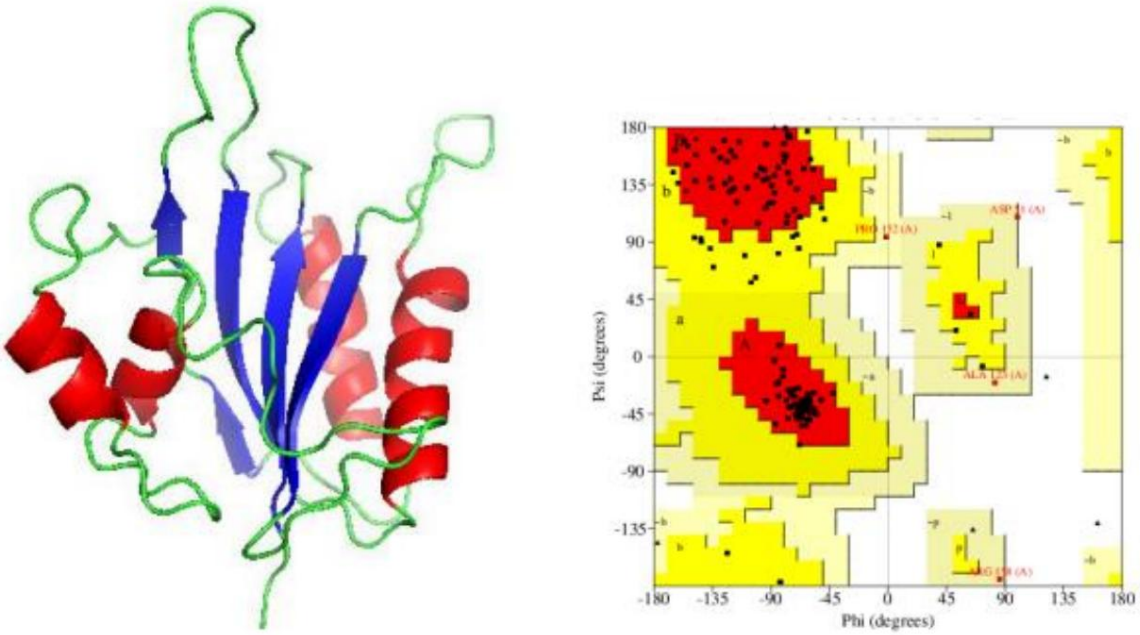

Figure 2. HPPK-MgAMPPCP structure and Ramachandran plot. Secondary structure types are colored as follows: alpha-helices in red, beta-strands in blue, and loops in green.

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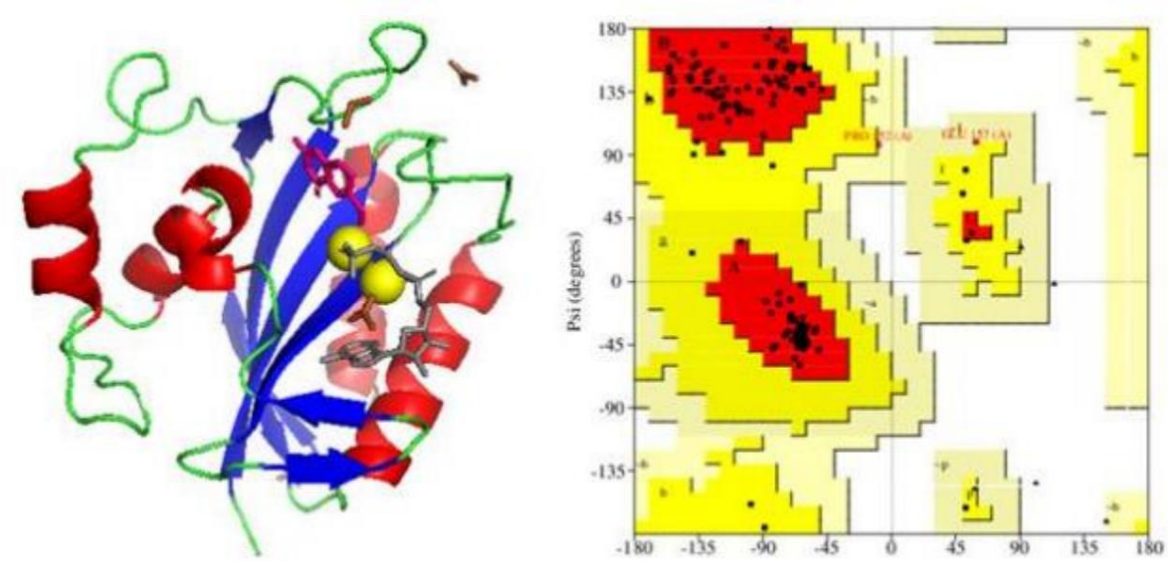

Figure 3. HPPK-MgAMPPCP-HP structure and Ramachandran plot. Secondary structure types were colored as follows: alpha-helices in red, beta-strands in blue, loops in green, magnesium ions in yellow, ACT in brown, APC in gray, and PH2 in pink.

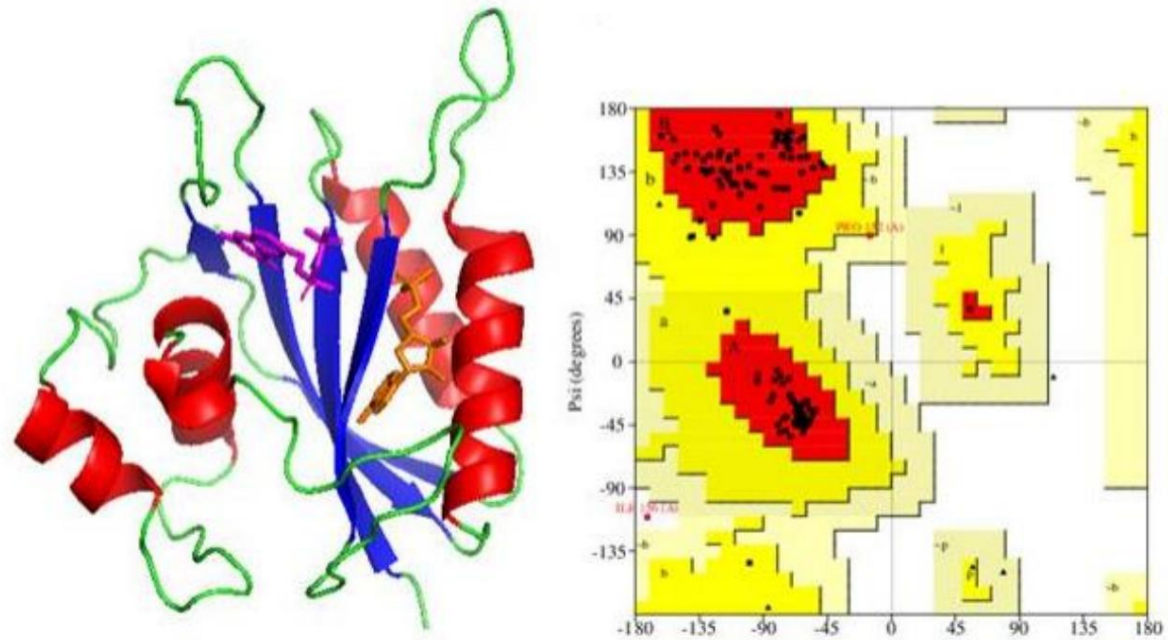

Figure 4. HPPK-AMP-HPPP structure and Ramachandran plot. Secondary structure types are colored as follows: alpha-helices in red, beta-strands in blue, loops in green, AMP in orange, and HH2 in pink.

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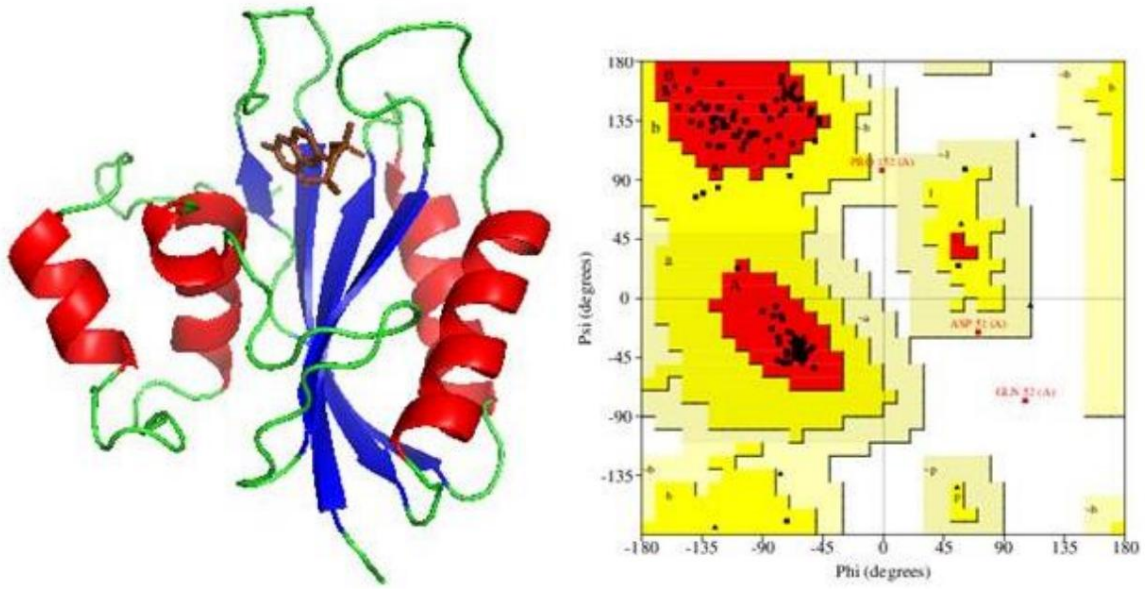

Figure 5. HPPK-HPPP structure and Ramachandran plot. Secondary structure types are colored as follows: alpha-helices in red, beta-strands in blue, loops in green, and HH2 in brown.

Table 1. Ramachandran plot. The percentage values refer to each of the conformational states of the PaHPPK model.

Região do gráfico de Ramachandran / %	Porcentagem de resíduos				
	apo-HPPK	HPPK-MgAMPPCP	HPPK-MgAMPPCP-HP	HPPK-AMP-HPPP	HPPK-HPPP
Mais favoráveis	93,3	81,5	89,6	94,1	94,1
Adicionalmente permitidas	6,7	16,3	9,6	5,2	4,4
Generosamente permitidas	0,0	2,2	0,7	0,0	0,7
Não permitidas	0,0	0,0	0,0	0,7	0,7

Regarding the interaction of residues with the substrate itself, it is observed that 6-hydroxymethyl-7,8-dihydropterin (HP) interacts with nine residues of the HPPK enzyme *Escherichia coli:* G8, T42, P43, L45, Y53, N55, W89, D95 and F123. There are interactions hydrophobic due to residues G8, L45, Y53, W89 and F123. On the other hand, twelve residues

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are involved in the binding of AMPCPP (an ATP analogue): Q74, E77, R84, R88, W89, R92, I98, R110, T112, H115 and Y116 (BLASZCZYK *et al.,* 2000).

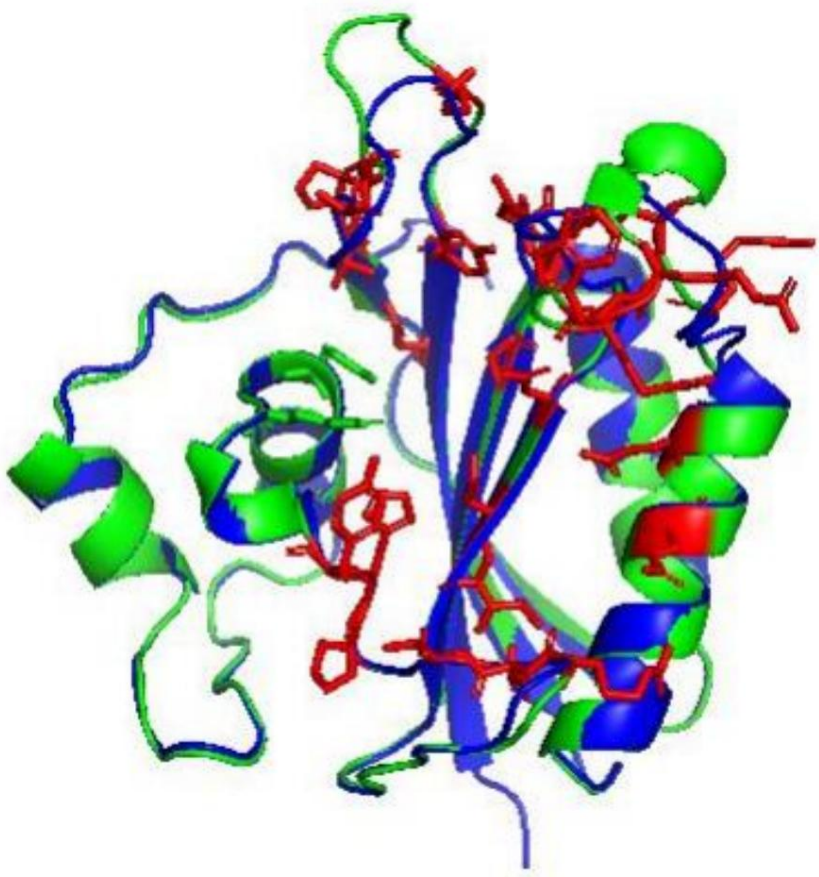

Figure 6. Overlay performed by the PyMOL program of apo-HPPK from *Escherichia coli* (PDB: 1hka) to the best theoretical model of PaHPPK obtained by the Modeller program. In red, the residues that make up the active site of this conformation.

FINAL CONSIDERATIONS

From the superposition of the models in the different states (Figure 7), it is observed that ATP binding induces conformational changes in a *hairpin-like region,* which is responsible for polar contacts with adenine (in addition to *loop* 3). According to (BLASZCZYK *et al.,* 2000), when ATP is bound, the plane containing the two positioned ÿ-strands approximately in the horizontal direction in the image. However, in the states corresponding to the apo form and in the two subsequent to the occurrence of the reaction, the plane undergoes a

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tilt. This tilt increases the distance between adenine and the residues that hold it in the its site and consequently reduces the affinity of AMP allowing it to be released into the solution as *loop* 3 assumes the open conformation. Furthermore, the movement of the *hairpin* affects the positioning of the R111 side chain (located in *hairpin)* that makes ionic and polar interactions with the side chains of D73 and Q76 (both in ÿ-helix immediately below), respectively. The interactions between these three residues could represent a way in which *loop* 3 and the *hairpin,* mediated by the ÿ-helix, couple during the ATP recognition event (BLASZCZYK *et al.,* 2000).

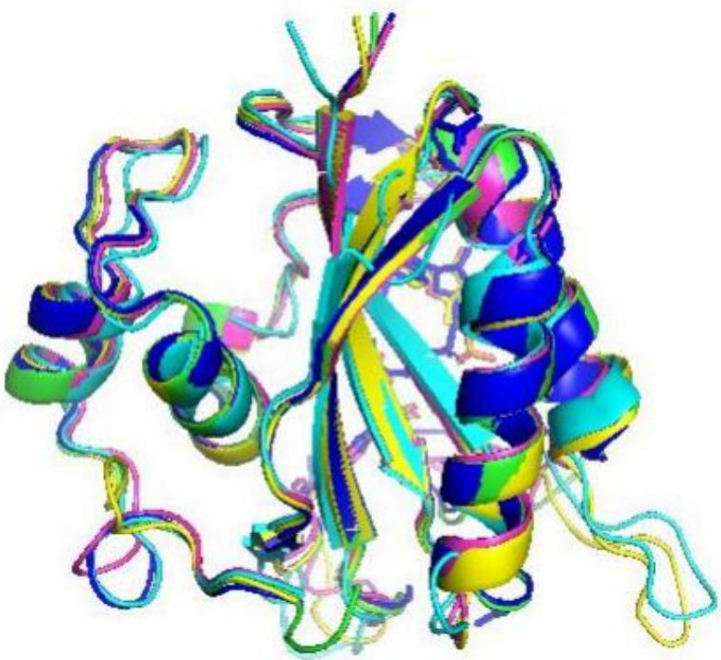

Figure 7. Superposition of theoretical models of all states of the PaHPPK enzyme.

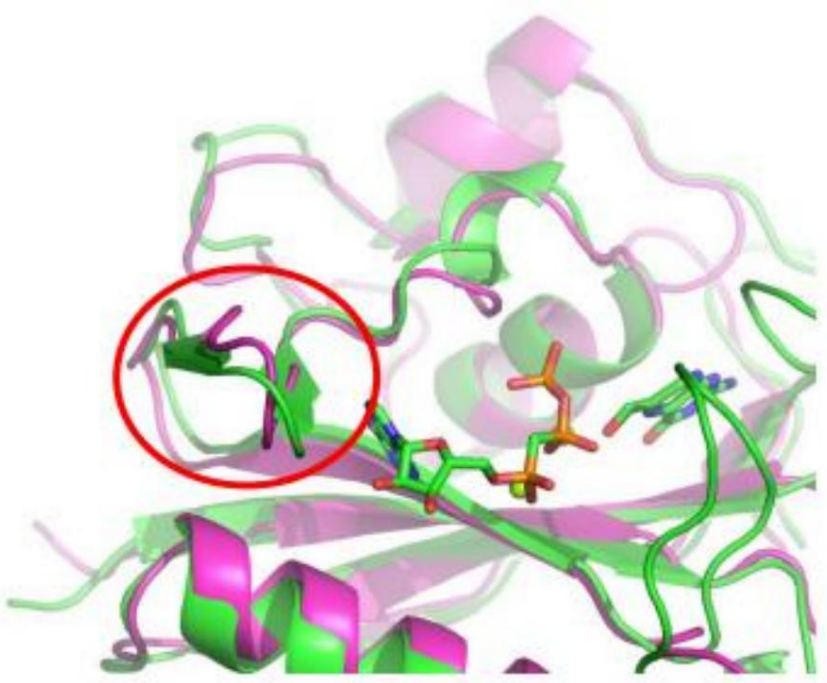

Figure 8. Overlay of the *hairpin* region (marked in red). In green, the PaHPPK-MG-AMPCPP-HP model (the cofactor is represented in green spheres (Mg2+) and AMPCPP and HP are represented in sticks); in magenta, the apo-PaHPPK model.

The structural information acquired from the models will be useful, especially those referring to the amino acids that make contact with the substrates, during the steps subsequent related to the choice of ligands for *docking*.

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