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Predicting synergy of lysozyme with antibiotics – an *in silico* analysis of interaction of Amoxicillin and Cephalexin with hen-egg lysozyme

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Abstract

Lysozyme is the key enzyme of the innate immunity, more known because of the hydrolytic degradation of the peptoglycans. This is important for defense from bacteria and viruses, and also important for introducing this enzyme in drug discovery mechanisms. This *in silico* study aims to conduct deep research about the interaction of lysozyme with beta-lactams (amoxicillin and cephalexin) and to provide a basis for the hypothesis of synergistic antimicrobial effect. Molecular docking was done by a blind-docking protocol. Four complexes revealed significant results. Highest binding affinity was detected in the human lysozyme-cephalexin complex (-7.4 FEB). The binding of cephalexin was significantly stronger than that of amoxicillin. Comparative analysis of the sequences confirmed that subtle substitutions in contact spots LEU-PHE are the molecular basis for the higher affinity of human lysozyme binding. Strong binding of antibiotics to the catalytic gate of the enzyme reveals the possible transport complex formation, which can benefit the localization and better penetration of the antibiotics in bacterial cells.

Keywords: Lysozyme, amoxicillin, cephalexine, synergy

1. Introduction

1.1. Lysozyme – one of the most important enzymes for human health

Lysozyme was discovered in 1922 in Sir Alexander Fleming's laboratory at St. Mary's Hospital in London. Often in the shadow of penicillin, one of the greatest discoveries of modern medicine, lysozyme was only recognized at the end of the 20th century. I continued and increased my interest in medicinal properties and versatility with the development of medicine and microbiology. Alexander Fleming emphasized the antimicrobial potency of lysozyme in 1932 during the Royal Medical Meeting [1].

Lysozymes are a group of antimicrobial proteins whose group includes polysaccharide-degrading enzymes with similar structures. Lysozymes are divided into type C, type G, and type I according to differences in amino acid sequences and biological functions. Human lysozyme belongs to the group of type C lysozymes, as well as lysozyme isolated from chicken egg whites [1], [2].

Lysozyme is a hydrolytic enzyme from the group of glycoside hydrolases, and is described as a small and stable protein. As it is widely distributed in the human organism and in general in the environment, its availability makes it easier to collect and work in the laboratory. In humans, it can be found in tears, saliva, blood, urine, nasal secretions, stool, serum, and plasma, as well as other tissues [1], [3].

1.2. Structure of lysozyme

Lysozyme or muramidase, N-acetylmuramide glycohydrolase, is an enzyme from the group of glycoside hydrolases. At the molecular level, its structure is small, globular, and usually built from about 129 to 130 amino acid residues. For example, chicken egg white lysozyme has 129, and human lysozyme has 130. It is characterized as an enzyme of high stability with the presence of disulfide bonds [4].

1.3. Enzymatic activity

As one of the key enzymes in the human body, lysozyme is the body's first line of defense against bacterial infections. Its main function is to destroy gram-positive bacteria. Primarily, lysozyme targets peptoglycans, which are a vital component of the bacterial cell membrane, catalyzing the hydrolysis (cleavage with the help of water) of the chemical compound between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) molecules within peptoglycan. After the hydrolysis of these bonds, the cell membrane loses its integrity and strength, which sporadically leads to cell lysis and eventual destruction [5].

2. Research method

2.1.1. Retrieving the lysozyme structure

Crystallographic structure of the lysozyme, which was the receptor in this analysis, was retrieved from the RCSB Protein Data Bank (PDB). It was identified as 1HER on the PDB site, and it was gathered by X-ray crystallography. To avoid further steps of cleaning the lysozyme structure from water, this structure was uploaded directly to the online docking server that automatically cleans any heteroatoms from the receptor before docking [6].

2.1.2. Retrieving the ligand's structure

Two antibiotics from the penicillin origin – amoxicillin (PubChem CID: 33613) and cephalexin (PubChem: 27447) were downloaded in .sdf format from the PubChem database. The structures were then uploaded to the CB-Dock2 server [7].

Before docking, CB-Dock prepared ligands by adding hydrogen atoms, defining partial atomic charges, and rotating bonds.

2.1.3. Hen-egg and human lysozyme structure prediction

In order to have a complete analysis, it was necessary to predict 3D structures of human and hen-egg lysozyme using the SWISSMODEL online tool. Models with the highest sequence identity and coverage were selected. Three-dimensional structures of predicted models are presented in the results section [8].

2.1.4. Molecular docking

Molecular docking for this *in silico* analysis was performed using the CB-Dock2 online tool. The CB-Dock2 tool is an online platform that detects cavities automatically following the AutoDock Vina algorithm. CB-Dock2 uses an algorithm for the detection of cavities (Curvature-based Cavity Detection) in order to automatically identify potential docking sites or gaps [9]. For every ligand, this tool automatically finds the optimal center and dimensions of the grid box, which is adapted to the size of the ligand. Docking was performed on the 5 largest, the most promising cavities, to have complete blind docking in various sites.

2.1.5 Pairwise sequence alignment

For this step, the EMBOSS-needle online software tool was used to perform pairwise sequence alignment. FASTA sequences of human and hen-egg lysozyme were retrieved from the Protein Data Bank (PDB) and introduced into the software for sequence alignment to confirm the similarity of the sequences and to explain the slightly higher affinity of cephalexin to bind to both lysozymes [10].

3. Results

3.1. Three-dimensional predicted models with SWISSMODEL online software

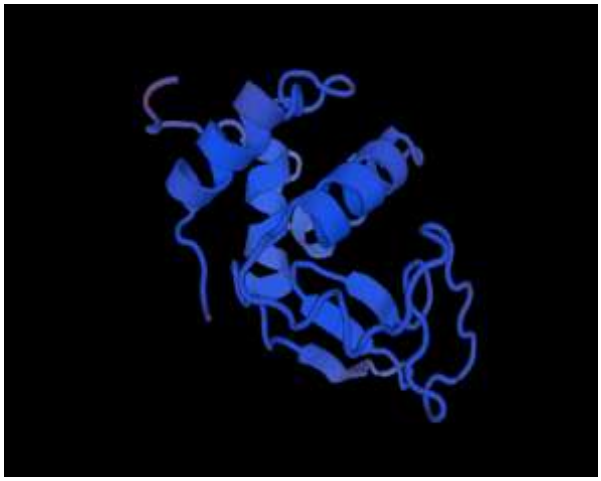

Figure 1. Predicted 3D model of hen-egg lysozyme by SWISSMODEL

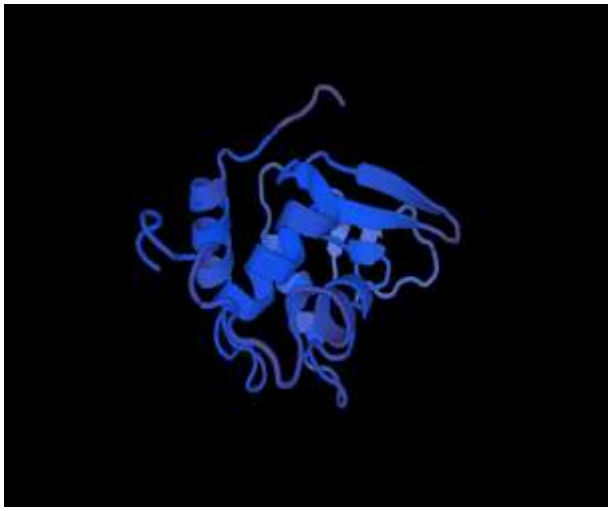

Figure 2. Predicted 3D model of human lysozyme by SWISSMODEL

3.2. Three-dimensional models of the ligands retrieved from PubChem

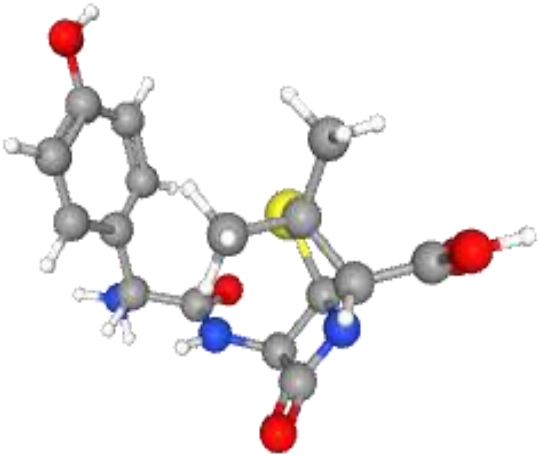

Figure 3. Three-dimensional model of Amoxicillin from PubChem

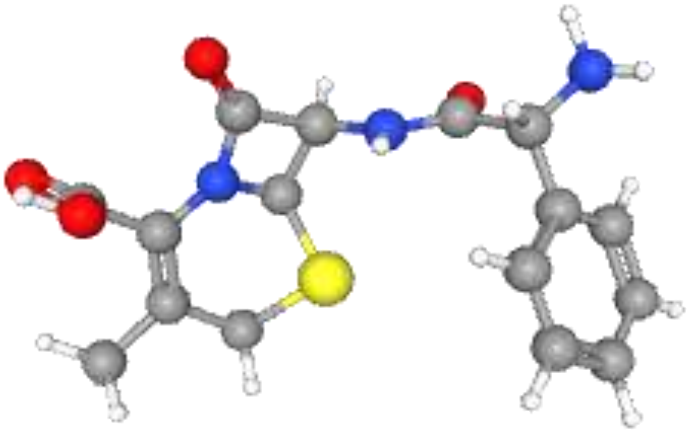

Figure 4. Three-dimensional model of the cephalexin retrieved from PubChem

3.2. Molecular docking of the human lysozyme with cephalexin and amoxicillin

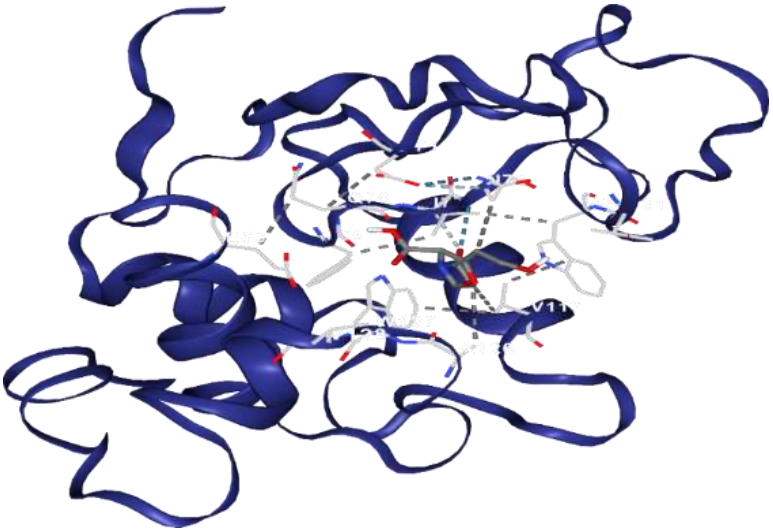

Figure 5. Human lysozyme and amoxicillin molecular docking by the CB-Docking online tool

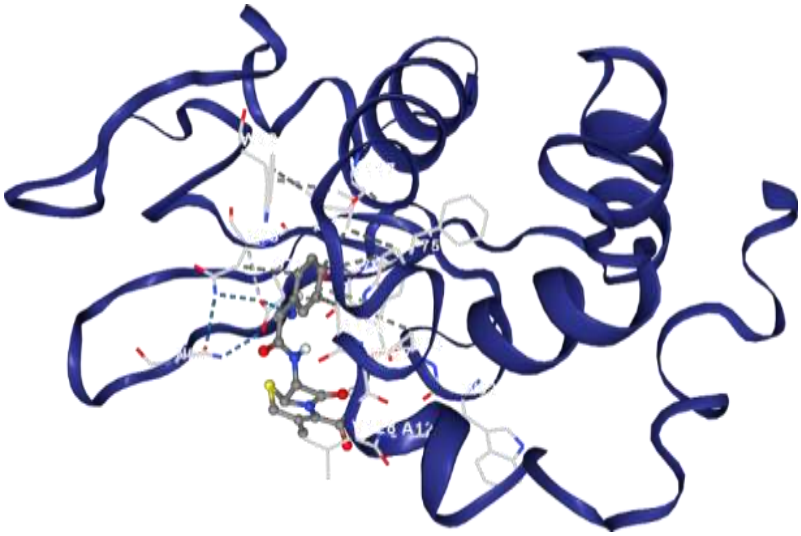

Figure 6. Human lysozyme and cephalexin molecular docking by the CB-Docking online tool

3.3. Molecular docking of the hen-egg lysozyme with cephalexin and amoxicillin

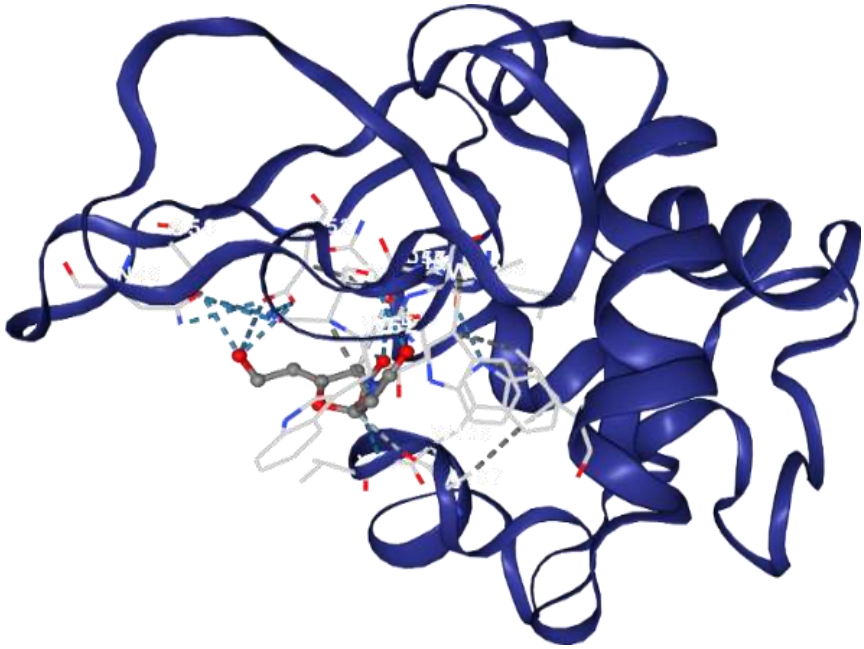

Figure 7.. Hen-egg lysozyme and amoxicillin molecular docking by the CB-Docking online tool

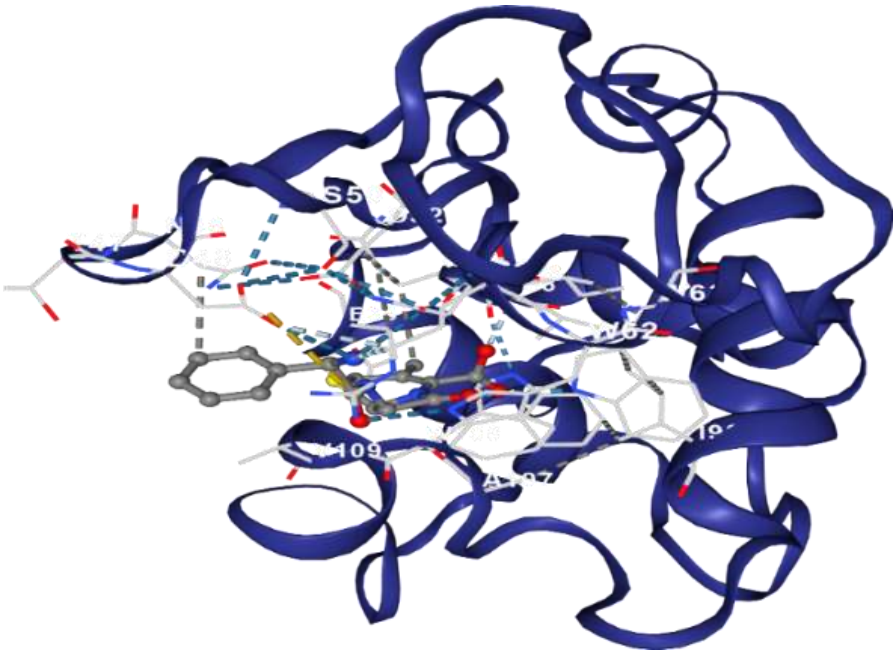

Figure 8. Hen-egg lysozyme and cephalexin molecular docking by the CB-Docking online tool

3.4. Pairwise sequence alignment results

Results of the pairwise sequence alignment are presented in Table 1.

Table 1. Results of pairwise sequence alignment performed with EMBOSS-needle

Length	Identity	Similarity	Gaps
130	77/130 (59.2%)	100/130 (76.9 %)	1/130 (0.8%)

4. Discussion

4.1. Molecular docking of amoxicillin to hen-egg lysozyme

Molecular docking of amoxicillin to hen-egg lysozyme (PDB ID: 1HER) was done by the CB-Dock2 web docking tool. This software detected and identified 5 the five most promising cavities for amoxicillin docking. Ranking of the results was presented in Table 2.

Table 2. Five of the most promising cavities for amoxicillin docking

CurPocket ID	Cavity volume (A˚3)	Center (XYZ)	Vina score (kcal/mol)	Contact residues
1	159	8.18, 28.40, -1.71	**-5.6**	GLU:35, ASN:44, ASP:52, TRP:62, TRP:63, TRP:108}
5	25	7.13, 16.14, -10.35	-4.8	ASN:19, TYR:23, ASN:27, TRP:111
2	66	-10.76, 21.09, 3.66	-4.4	PHE:3, GLU:7, HIS:15, ILE:88
4	30	7.47, 10.17, -3.24	-4.3	CYS:30, PHE:34, ARG:114, TRP:123
3	38	-1.62, 13.24, -10.40	-4.1	ASP:18, TYR:23, CYS:115

The best result of the docking for amoxicillin was detected in pocket 1 with the lowest Vina score or free-binding energy with 5.6. kcal/mol. Since this result is significantly negative, the lysozyme-amoxicillin complex can be declared as conformationally stable. That implies that there is a high thermodynamic affinity of amoxicillin towards the lysozyme *in silico.*Cavity 1, which showed the highest docking affinity and highest volume of the pocket, is in high correlation with the known active site of hydrolysis of lysozyme according to the position of the docking pocket (Center: X=8.18, Y=28.40, Z=-1.71)

Contact residues in pocket 1 include the key catalytic amino acids of lysozyme. GLU:35 and ASP:52 are critical amino acids for the catalytic mechanism of the lysozyme, which function as an acid and nucleophile alongside the stabilization of the intermediate. TRP:62, TRP:63, and TRP:108 as a triptophane residue are typical for active sites of lysozyme, which form the hydrophobic surface and play a key role in the substrate binding.

4.2. Molecular docking of cephalexin to hen-egg lysozyme

Molecular docking of cephalexin to hen-egg lysozyme (PDB ID: 1HER) was done by the CB-Dock2 web docking tool. This software detected and identified 5 the five most promising cavities for amoxicillin docking. Ranking of the results was presented in Table 3.

Table 3. Five of the most promising cavities for cephalexin docking

CurPocket ID	Cavity volume (A˚3)	Center (XYZ)	Vina score (kcal/mol)	Contact residues
1	159	8.18, 28.40, -1.71	**-7.2**	GLU:35, ASP:52, TRP:62, TRP:63, TRP:108, ASN:113
3	38	-1.62, 13.24, -10.40	-5.8	ASP:18, TYR:23, TRP:111, ASP:119
5	25	7.13, 16.14, -10.35	-5.5	ASN:19, TYR:23, TRP:111, ARG:114

4	30	7.47, 10.17, -3.24	-5.0	PHE:34, TRP:123, ARG:125
2	66	-10.76, 21.09, 3.66	-4.7	PHE:3, GLU:7, HIS:15

The best result of the docking for amoxicillin was detected in pocket 1 with the lowest Vina score or free-binding energy with – 7.2 kcal/mol. Since this result is significantly negative, the lysozyme-amoxicillin complex can be declared as conformationally stable. That implies that there is a high thermodynamic affinity of cephalexin towards the lysozyme *in silico.* Pocket 1 showed a promising field for the docking of cephalexin, and it was characterized by the highest volume of the cavity with 159 Å^3. Coordinates of this pocket (Center: X=8.18, Y=28.40, Z=-1.71) showed high affinity towards the main catalytic site of the lysozyme. The best conformational pose of the cephalexin is positioned deep inside the active site of the lysozyme while making contacts with key residues GLU:35 and ASP:52. Also, dominant contact with hydrophobic residues implies tryptophan rings TRP:62, TRP:63, and TRP:108. These residues form a hydrophobic pocket, which is the key to the stabilization of the nonpolar parts of the ligand.

4.3. Molecular docking of the human lysozyme and cephalexin

Molecular docking of cephalexin to human lysozyme (PDB ID: 1SF2) was done by the CB-Dock2 web docking tool. This software detected and identified 5 the five most promising cavities for amoxicillin docking. Ranking of the results was presented in Table 4.

Table 4. Five of the most promising cavities for cephalexin docking with human lysozyme

CurPocket ID	**Cavity volume (A˚3)**	**Center (XYZ)**	**Vina score (kcal/mol)**	**Contact residues**
1	238	13.35, 71.35, 25.25	**-7.4**	TRP:52, GLU:53, ASP:67, ASP:71, TRP:82, TRP:127
2	56	13.24, 72.82, 4.10	-6.0	PHE:21, GLU:22, ARG:32, THR:58, ARG:59
5	30	11.65, 61.77, 14.99	-5.4	TYR:38, ARG:39, CYS:95, ARG:116
4	35	7.84, 88.43, 22.85	-5.2	CYS:48, TRP:52, ARG:133, TYR:142
3	50	24.18, 61.10, 22.91	-5.0	TYR:72, ASP:85, CYS:99

Cephalexin revealed the highest docking affinity in pocket 1 with the highest free docking energy of -7.4 kcal/mol. This result depicts a great enhancement of docking when compared to the hen-egg lysozyme, which suggests better geometric and chemical complementarity with human lysozyme. All docking results are negative, which reveals that complex human lysozyme-cephalexin can be classified as conformationally stable.

Pocket 1 once again revealed as a primary position of docking with the highest cavity volume of 238 Å^3. Docking occurs in the region of catalytic activity of human lysozyme. The best docking pose of the cephalexin reveals the key contact with the most important catalytic amino acids of human lysozyme GLU:53 and ASP:7. Docking is furthermore stabilized by strong hydrophobic interactions with TRP:52, TRP:82, and TRP:127. The presence of these tryptophan residues makes an ideal hydrophobic microsystem for cephalexin binding.

4.4. Molecular docking of amoxicillin with human lysozyme

Molecular docking of amoxicillin to human lysozyme (PDB ID: 1SF2) was done by the CB-Dock2 web docking tool. This software detected and identified 5 the five most promising cavities for amoxicillin docking. Ranking of the results was presented in Table 5.

Table 5. Five of the most promising cavities for amoxicillin docking with human lysozyme**.**

CurPocket ID	Cavity volume (A˚3)	Center (XYZ)	Vina score (kcal/mol)	Contact residues
1	238	13.35, 71.35, 25.25	**-5.8**	GLU:53, ASP:67, ASP:71, TRP:82, TRP:127
2	56	13.24, 72.82, 4.10	-4.6	PHE:21, GLU:22, THR:29, ARG:59
3	50	24.18, 61.10, 22.91	-4.6	ASP:85, LYS:87
4	35	7.84, 88.43, 22.85	-4.6	TRP:52, ARG:133, TYR:142
5	30	11.65, 61.77, 14.99	-4.3	TYR:38, CYS:95, ARG:116

According to the Vina score of -5.8 and other negative results, complex human lysozyme and amoxicillin can be classified as conformationally stable. Pocket ID1 is the biggest cavity and reflects the main catalytic surface of the human lysozyme. Key interactions involve the contact with catalytic residues GLU:35 and ASP:71, which strongly suggests that amoxicillin can interfere with the hydrolytic function of the enzyme, but not in every case, since their synergetic manner should be confirmed in the laboratory.

4.5. Comparison of the highest docking affinity for amoxicillin and cephalexin to the lysozyme of hen-egg and human

In Table 6. results of the lowest Vina scores or docking affinity scores are presented.

Table 6. The lowest Vina scores of every docking performed

Complex	Lysozyme	Ligand	Vina score (kcal/mol)
I	Hen-egg lysozyme (HEWL)	Amoxicillin	-5.6
II	Human Lysozyme (h-Lyz)	Amoxicillin	**-5.8**
III	Hen-egg lysozyme (HEWL)	Cephalexin	-7.2
IV	Human Lysozyme (h-Lyz)	Cephalexin	**-7.4**

First of all, in order to have a complete comparative analysis, it is necessary to first compare ligands and types of enzymes and then ensure that their results are covered. Analysis revealed that cephalexin has a higher affinity for binding to both types of lysozymes when compared to amoxicillin.

4.6. Comparison of the ligands and receptors

This *in silico* analysis revealed that cephalexin showed a higher affinity for docking to both types of lysozymes when compared to amoxicillin. This difference suggests that the presence of various substituents on the cephalosporine ring in cephalexin is making a better stabilizing interaction possible. When it comes to the

receptors, this analysis revealed that both antibiotics have higher docking affinity to human lysozyme than hen-egg lysozyme.

This consistency reveals that smaller structural differences in the catalytic pocket of human lysozyme (possibly in the distribution of anions, cations, and side-chains) have a positive impact on binding both beta-lactam structures.

4.7. Pairwise sequence alignment – confirming the affinity of antibiotic docking to lysozyme

Results of molecular docking revealed stable complex formation in all 4 tested systems, since all VinaScores are below zero. Cephalexin constantly showed higher affinity to dock to both lysozymes, when compared to Amoxicillin. The lowest VinaScore (highest affinity) was detected for complex human-lysozyme-cephalexin (-7.4 kcal/mol), but the lowest affinity was detected in hen-egg lysozyme-amoxicillin (-5.6 kcal/mol). Sequence alignment revealed high homology between human and hen-egg lysozyme, with approximately 77% of similarity. Also, it revealed high amino acid substitutions in the peripheral region of the active site. Substitutions in contact points of human lysozyme, like Leucine-Fenylalanine, explained a slightly higher affinity to dock to human lysozyme. These substitutions are making a slightly more hydrophobic surrounding for packing the beta-lactane ring of cephalexine.

5.Conclusion

Results of molecular docking revealed that amoxicillin and cephalexin successfully dock to the main catalytic point of human and hen-egg lysozyme with conformationally stable affinity. Cephalexine revealed a slightly higher affinity for docking, which makes it a potent candidate for synergetic activity or potential inhibition of lysozyme. This docking confirms that strong complexes are formed when lysozyme interacts with antibiotics and also that there can be slight modulation in the enzymatic activity of lysozyme. This *in silico* research opens the field for *in vitro* and *in vivo* analysis to confirm synergetic antimicrobial effect of lysozyme and antibiotics, enabling the formulation of new medications that can minimize antibiotic resistance.

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