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Free Energy Decomposition of CarO Outer Membrane Protein of *Acinetobacter baumannii*

Emrah Sariyer[1,a,*]

[1] *Medical Laboratory Techniques, Vocational School of Health Services, Artvin Çoruh University, Artvin, Turkey.*
**Corresponding author*

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ABSTRACT

The increase in the number of antibiotic-resistant microorganisms reported today has made this issue one of the main topics of all institutes. *Acineteobacter baumanni* is a species that is on the list of the WHO and plays an important role, especially in hospital-acquired infections. CarO outer membrane protein, which regulates the passage of small molecules and some antibiotics into the periplasmic space and is associated with carbapenem resistance, has been identified in *A. baumannii*. In this study, residues that contribute to the binding energy of imipenem to different types of CarO proteins were identified. In addition, energy decomposition was compared when Biapenem, Ertapenem, Imipenem, Faropenem, and Meropenem were docked to ATCC-17978 CarO protein separately. As a result of this study, it was determined that generally charged residues had a negative effect on binding affinity, but hydrophobic and uncharged residues had a positive effect. In addition, in ertapenem, faropenem, and meropenem-bound complexes, charged residues increased the affinity and caused the interaction between carbapenems and CarO to be continuous and tight. It was predicted that the residues determined in this study would be precursors to mutagenesis studies and could also be an example for similar studies.

Keywords: Acinetobacter baumannii, CarO, Imipenem, Binding affinity, Energy decomposition.

[a] *esaryer@yahoo.com* *https://orcid.org/0000-0003-1721-0314*

Introduction

Acineteobacter baumanni is a non-motile, aerobic, gram-negative bacterium and mostly causes infection in the liver, blood, urinary system, and wounds [1]. It is common in hospital-acquired infections in recent years and the World Health Organization has declared it among the ESKAPE organisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*) to be considered [2]. A carbapenem group of antibiotics is generally used against this bacterium, and multiple resistance mechanisms are developing rapidly against these antibiotics [3]. Carbapenem-Resistant *A. baumanni* was announced among the species that should be emphasized by the WHO in 2018 [4].

Since *A. baumanni* is a gram negative bacterium, it has a membrane consisting of an inner and an outer membrane, and the outer membrane has different structures and properties, including lipopolysaccharides (LPS) or lipooligosaccharides (LOS) [5, 6]. Thanks to the asymmetric outer membrane, it adheres to other cells or regulates the passage of small molecules, lipids or antibiotics from the outside into the periplasmic space [7]. The outer membrane contains the integral membrane proteins BamA, LptD, Omp33–36, OmpW, CarO and OprD, among which CarO has been related with the carbapenem resistance of the outer membrane protein [8]. CarO has a molecular weight of 29 kDa, consists of 8 β-barrel structures, and the 3-dimensional crystal structure of this protein has been defined for three different isoforms [9]. Decreased expression and structural changes of CarO have been reported to render *A. baumanni* resistant to Imipenem [10, 11].

There are clinical studies on the relationship of CarO-Imipenem resistance, but it was limited studies on the protein at the structural and molecular level [12]. Structural studies at the molecular level are both time-consuming and costly processes. For this reason, computational studies, which are powerful and useful methods of today, continue to dominate [13]. From these methods, protein ligand interactions can be analyzed quickly with molecular docking and molecular dynamics simulations [14]. Molecular dynamics simulations can simulate the movements of molecules at the atomic level according to classical mechanical rules. Generally, regional observations such as protein ligand interactions, loop mobility, secondary structure formation can be made in the ns time interval. However, computation times become excessively long in the case of large molecules. Today, this problem is overcome by using GPU processors instead of CPU processors. Simulations in the μs time interval have been reduced to 10-15 days with this technical infrastructure [15].

In our previous study, homology models of ATCC-17978, Type 1, Type 2, Type 3, and Type 4 CarO proteins were generated, carbapenems were docked to the binding site of CarO proteins, and complexes were simulated by molecular dynamics methods for the analysis of CarO-carbapenem interaction (unpublished data). In

this study, a free energy decomposition study, which is a post-process analysis, was performed following molecular dynamics simulations. In this context, the affinities of Imipenem for 5 different CarO isoforms ATCC-17978, Type 1, Type 2, Type 3, and Type 4, as well as the affinity of the different Carbapenems Biapenem, Ertapenem, Faropenem, and Meropenem to ATCC-17978 were analysed. This study aims to determine the important residues that will regulate the passage of carbapenems through the CarO channel by calculating the contribution of each residue to the binding energy. It is thought that the critical residues determined by this study will lead to mutation studies and also to lead to similar studies related to other CarO isoforms.

Materials and Methods

Free Energy Decomposition

Molecular mechanics-generalized Born surface area (MMGBSA) method was used for free energy decomposition. This method is based on molecular dynamics simulation and is MD trajectory analysis. MD simulation was performed in the explicit water model, but in the MMGBSA method, implicit water was added by deleting the explicit water [16]. The binding energy is generally calculated by subtracting the apo-protein and ligand energies from the total bond energy in the complex (1). MMGBSA calculates the free energy of binding by combining gas phase energy (MM), electrostatic solvation energy (GB), and nonelectrostatic contribution to solvation energy (SA) (2). ΔE_{MM} shows gas-phase interaction energy between protein and ligand; ΔG_{GB} + $\Delta G_{nonpolar}$ shows the polar and nonpolar components of the desolvation free energy; $T\Delta S$ is the change of conformational entropy on ligand binding [17].

$$\Delta G_{bind} = G_{complex} - G_{protein} - G_{ligand} \quad (1)$$

$$\Delta E_{MM} + \Delta G_{GB} + \Delta G_{nonpolar} - T\Delta S \quad (2)$$

In this study, Imipenem docked to five different types of CarO and five different carbapenems docked to ATCC 17978 CarO were analyzed. Binding energy decomposition was performed on the trajectory files generated using 800 ns of simulation as a post processing MD. These binding energy contributions were calculated by taking a snapshot every 40 ns throughout the simulation using the MMPSA.py application [18]. The decomposition of the total account was calculated using the per-residue scheme. In the calculation, all residues that contributed or did not contribute to the binding energy were taken into account, and then those that did not contribute were eliminated and the results were given graphically.

Results and Discussion

The Energy Contribution per Residue in ATCC 17978-Carbapenem Complexes

In this part of the study, the trajectory of ATCC-17978 CarO-carbapenem complexes as a result of 800 ns simulation was analyzed. The residues that contributed to the binding energy were analyzed by making separately energy decomposition for the 5 types of carbapenems such as Biapenem, Ertapanem, Faropenem, Meropenem, and Imipenem. First of all, the contribution of all residues was carefully examined and only the residues that contributed to the binding energy were determined to simplify the graph. The energy contribution was given in kcal/mol in the drawn graph and residues were indicated on the x-axis (Figure 1).

According to these results, the residues that contributed most to the binding energy in the ATCC 17978-Biapenem complex were listed as Tyr54, Leu98, Lys100, Leu164, Glu185, and Ile189, respectively. Besides, Asp45, which is a negatively charged residue, stands out as the residue that reduces the binding affinity. In the ATCC 17978-Ertapenem complex, Leu47, Asp193, Lys194, Tyr195, Trp197, and Pro199 residues were the residues that contribute most to the binding energies. The most striking among these residues is the positively charged residue Lys194. The residues with the highest contribution to the binding energy in the ATCC 17978-Faropenem complex were listed as Tyr54, Leu98, Leu164, Glu185, Lys188, Ile189, and Lys194, respectively. The residue that contributed most to the binding of Faropenem to CarO was found to be positively charged Lys188 and Lys194, and also the residue that negatively affected the binding energy was expressed as negatively charged residues Lys100 and Asp192. In another complex, ATCC 17978-Meropenem, Arg44, Asp55, Asp59, Asp97, Thr99, Lys196, and Val200 were listed as residues that contributed to the binding energies. These residues usually stand out as positive and negative charged residues. In the last complex, ATCC 17978-Imipenem, it was noted that Tyr54, Val56, Tyr96, Leu98, Glu186, Arg190, Lys196, and Val200 residues contributed the most to the binding energy. These residues were generally hydrophobic and besides Lys100 residue reduced the binding affinity.

Although the residues that contribute to the binding energy vary according to the carbapenem type, the regions where the residues were located and the physical properties of the residues were similar. It was only seen as an Imipenem that differed from the others because the residues it interacted with were mostly hydrophobic. The residue that reduced the binding affinity was generally detected as the negatively charged residue Asp45-46.

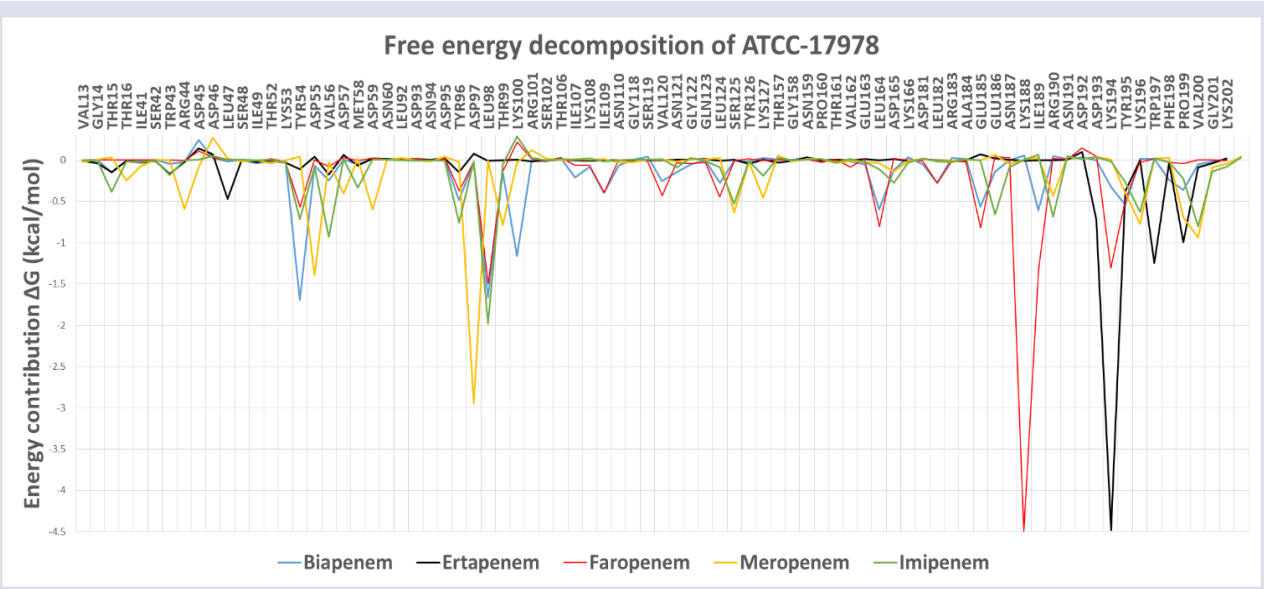

Figure 1. Per-residue free energy decomposition of ATCC 17978-Carbapenem complexes

The Energy Contribution Per Residue in Imipenem-CarO's Complexes

The common residues contributing to the binding energy in ATCC-17978 CarO-carbapenem complexes were determined, then the same properties were analysed in different CarO isoforms. Only Imipenem docked CarO types were used in these analyses because clinically an Imipenem-CarO resistance relationship has been reported [12]. For this reason, residues affecting the binding affinity of Imipenem were determined separately in Type 1, Type 2, type 3, and Type 4 CarO isoforms.

First of all, the energy decomposition in the Type 1-Imipenem complex was listed as Leu98, Lys100, Ser109, Val126, Met130, Leu170, Glu192 and Glu196 respectively (Figure 2). Among these residues, hydrophobic residues Leu98 and Ile196, positively charged Lys100, and negatively charged Glu192 were found to contribute the most. Charged residues Lys191 and Arg197 were residues that reduced the binding affinity of Imipenem to Type 1 CarO.

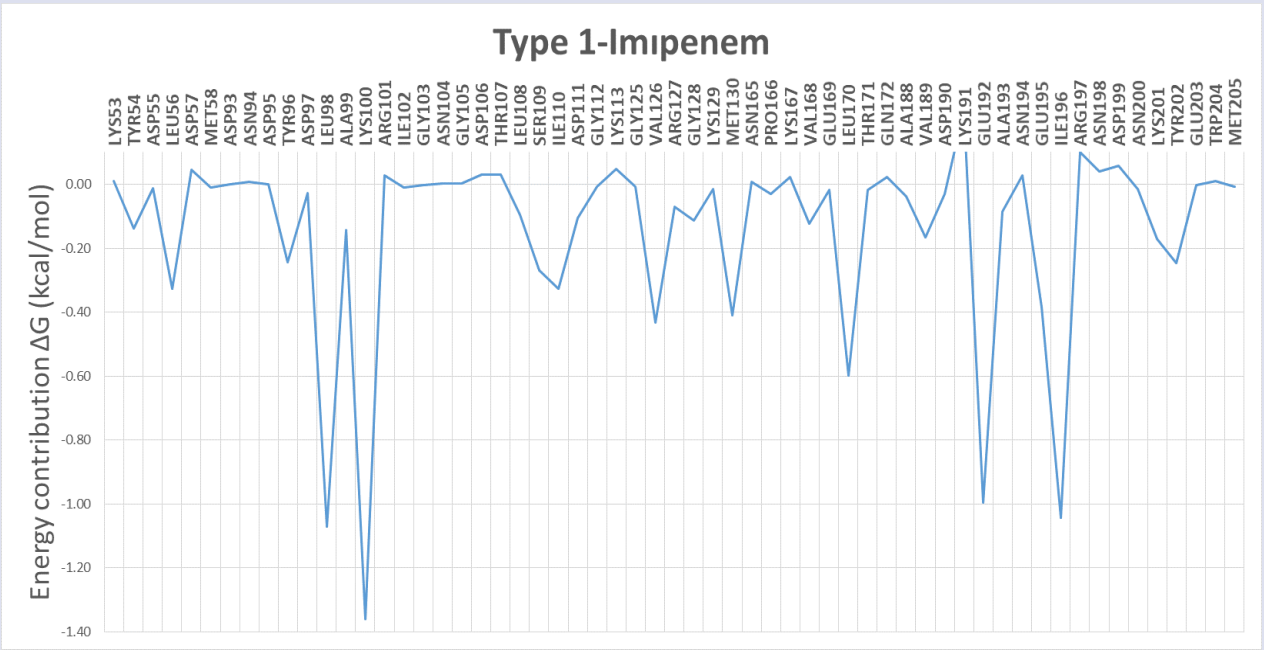

Figure 2. Per-residue free energy decomposition of Type 1-Imipenem

The residues contributing to the binding energy in the Type 2-Imipenem complex were Trp44, Ser45, Tyr55, Met57, Pro205, and Val206, respectively (Figure 3). All of these residues were uncharged and on the other hand, the charged residues Glu110, and Lys208 negatively affected the binding affinity of Imipenem to Type 2 CarO.

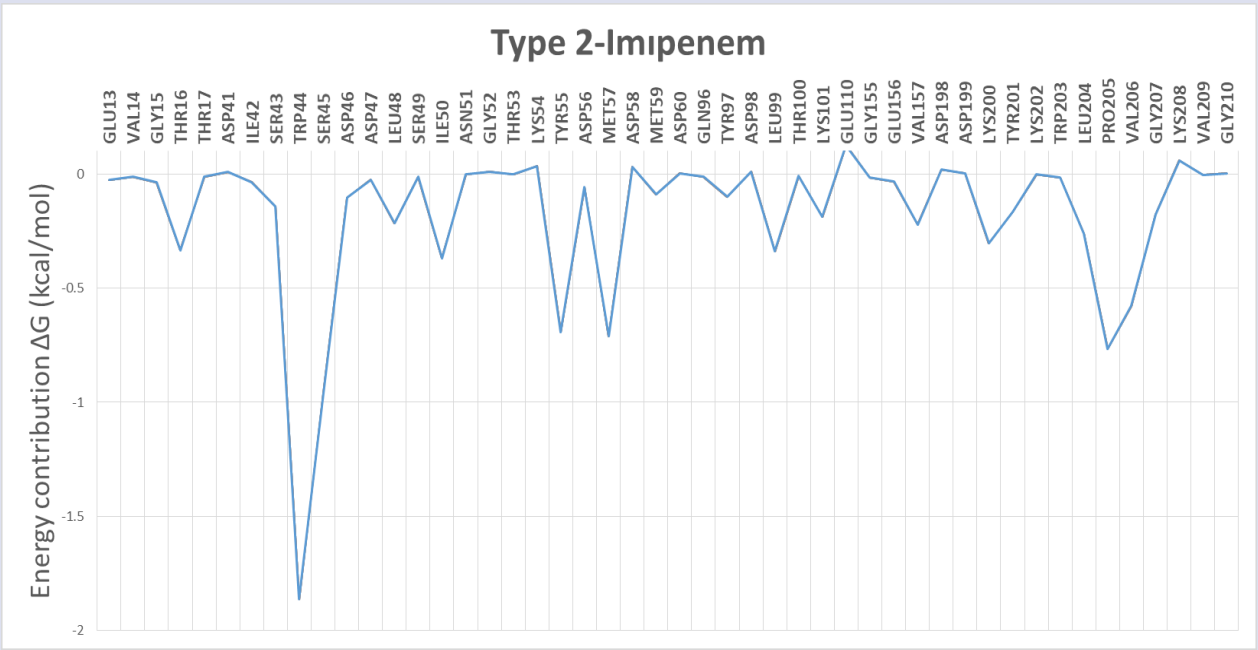

Figure 3. Per-residue free energy decomposition of Type 2-Imipenem

In the Type 3-Imipenem complex, the residues contributing to the binding energy of Imipenem were listed as Val50, Asn51, Ser53, Arg101, Phe109, and Arg110 (Figure 4). Similar to Type 1 and Type 2, the charged residues that reduce the binding affinity of Imipenem were Asp46, Glu190, and Lys193.

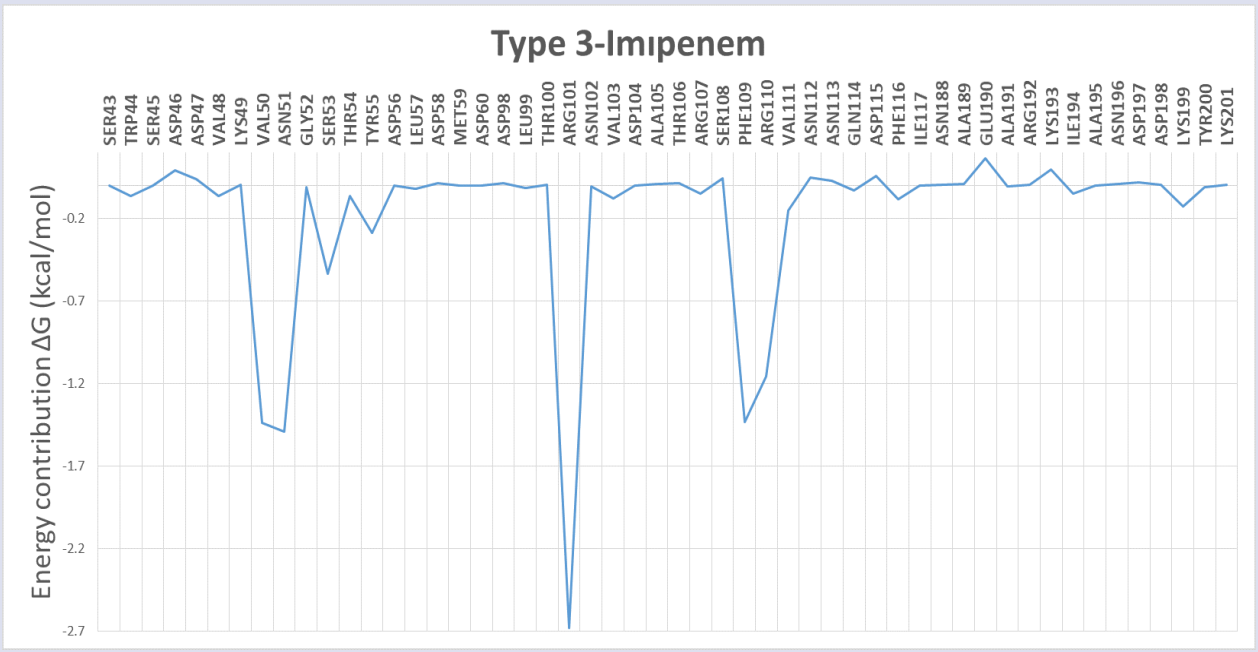

Figure 4. Per-residue free energy decomposition of Type 3-Imipenemenem

The residues contributing to the binding energy of Imipenem in the Type 4-Imipenem complex were listed as Trp44, Ile50, Tyr55, Lys101, Ile190, Lys195, and Tyr196, respectively (Figure 5). Except for the Lysine residue, the residues were hydrophobic and the charged residues Lys54, Glu186, and Asp193 were residues that weakened the binding affinity of Imipenem to Type 4 CarO

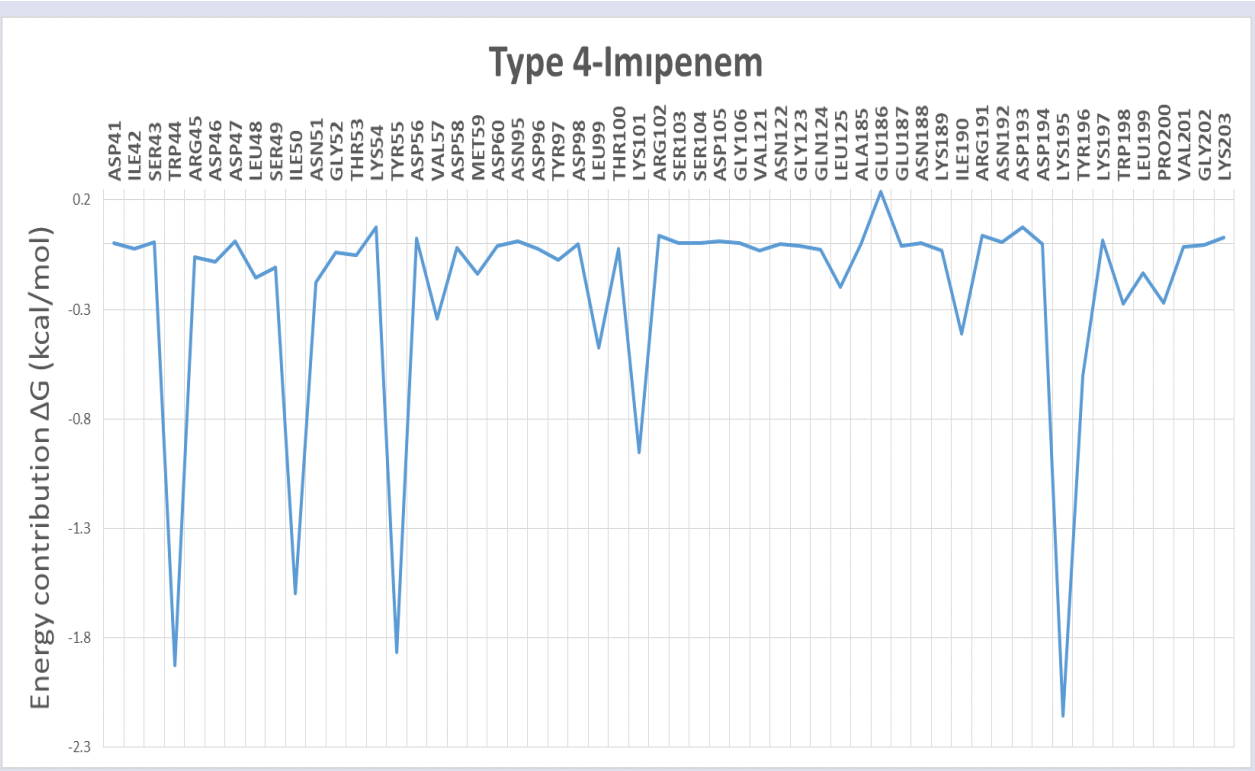

Figure 5. Per-residue free energy decomposition of Type 4-Imipenem

Conclusion

In this study, 9 different energy decompositions were performed and these were given comparatively for themselves. The residues contributing to the binding energy of carbapenems in the ATCC-17978 CarO outer membrane protein were evaluated in general. Theoretically, in order for a compound to be taken into the periplasmic space after binding to the outer membrane protein, it should not contain very tight and continuous bonds [10]. For this reason, the physical properties of the residues expressed in this study and their contribution to the binding energies were critical. To simplify the comparison, the residues that contribute to the binding energy in each complex and their physical properties are given in Table 1. As can be seen from this table, almost all of the residues were negatively or positively charged and hydrophobic residues. On the other hand, all of the residues that reduced the binding affinity were found to be negatively or positively charged residues.

When we look at Table 1 in conclusion, it appears to be different residues as the protein and carbapenem types change. If the imipenem-bound complexes were evaluated within themselves, it was observed that the meropenem-bound complex had a different interaction motif. Mostly, these residues were different than the others with charged residues. Biapenem and faropenem showed nearly identical interactions, with the most similar partially imipenem-bound complex.It was also observed that Faropenem, Ertapenem, and Meropenem made strong bonds with ATCC17978 CarO residues. However, Imipenem generally travels in a hydrophobic pocket and, unlike the others, charged residues reduce the binding energy of Imipenem. Faropenem and Ertapenem. Charged residues such as Lys188 in the faropenem complex, Lys194 in the ertapenem complex, and Asp97 in the meropenem complex contributed highly to the affinity of these carbapenems for CarO. However, hydrophobic residues such as Tyr54 in the biapenem complex and Leu98 in the imipenem complex contributed relatively more to their affinity for CarO. The most outstanding residue was the result that Leu98 contributed significantly to the binding energy in all complexes except the Meropenem complex.

As a further comparison, different types of CarO outer membrane protein were compared with each other. In this comparison, Imipenem, which is the Carbapenem-CarO resistance relationship was reported [19], was analysed.

When the residues that contributed to each complex were compared, it was noticed that the Type2 CarO complex was differentiated from the others. The residues that increased the binding affinity were hydrophobic and polar, while those that decreased it were charged residues. In this sense, when all the complexes were examined, it was observed that the residues that increased the binding energy were generally Lysine and Arginine residues. The result in the graphics is residues Lys100, which contributes highly to the binding affinity in Type1 CarO. While Trp44 and Ser45 were in Type 2, Arg101 in Type 3 stood out, and finally, it was expressed as Lys195 in Type 4.

Table 1 The energy contribution of residues in the complex. (Common residues were shown as an underlined and R indicates "Reducing affinity")

Complexes	Hydrophobic	Charged	Polar and uncharged
ATCC17978-Biapenem	Tyr54, Leu98, Leu164, and Ile189	Lys100, Glu185 and Asp45(R)	
ATCC17978-Ertapenem	Leu47, Tyr195 and Trp197	Asp193, Lys194, Asp45(R)	Pro199
ATCC17978-Faropenem	Tyr54, Leu98, Leu164 and Ile189	Glu185, Lys188, Lys194, Lys100(R) and Asp192(R)	
ATCC17978-Meropenem	Val200	Arg44, Asp46(R) Asp55, Asp59, Asp97, Arg101(R) and Lys196	Thr99
ATCC17978-Imipenem	Tyr54, Val56, Tyr96, Leu98, and Val200	Lys100(R), Glu186, Arg190 and Lys196	
Type 1-Imipenem	Leu98, Val126, Met130 and Leu170	Glu192, Glu196, and Arg197, Lys100, Lys191(R) and Arg197(R)	Ser109
Type 2-Imipenem	Trp44,Tyr55, Met57 and Val206	Glu110(R), and Lys208(R)	Ser45 and Pro205
Type 3-Imipenem	Val50 and Phe109	Asn51, Arg101, Arg110 Asp46(R), Glu190(R) and Lys193(R)	Ser53
Type 4-Imipenem	Trp44, Ile50, Tyr55,Ile190 and Tyr196	Lys101, Lys195, Lys54(R), Glu186(R), and Asp193(R)	

The literature says the extracellular glove-shaped extensions do not have a specific binding motif and it consist of cationic channel [10]. When all the results are evaluated together, in order for antibiotics or other molecules to be taken up by the outer membrane proteins, they must first bind to this channel and then move towards the channel and be taken into the periplasmic space. For this reason, strong and fixed bonds either completely stop this progress or slow it down. When the samples in this study were compared, it was concluded that Imipenem was most likely to pass through the channel and that other carbapenem may or may not pass more difficult. In the comparison between CarO isoforms, it was observed that the more charged residues in the protein, the tighter and more stable bonds were formed. Among these types, it was concluded that the most likely ATCC-17978 CarO outer membrane protein Imipenem would get into the periplasmic space. In addition, it was predicted that the residues expressed in this study would be a source for mutagenesis studies. Moreover, it was thought that it would lead to studies related to different carbapenems or different types of CarO proteins.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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