Vitamin C prevents *S. aureus* infections of endothelial cells through its interaction with specific residues in the active site of gC1qR/p33 involved in direct entry, notably SER230, ASN345, ILE348, TYR224, ASN346, SER347, and GLU438, and its potential inhibitory effects on FnBPs, α5β1, and Protein A molecules

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Abstract

Staphylococcus aureus (*S. aureus*), often perceived as an extracellular pathogenic agent, exhibits a remarkable ability to penetrate host cells, including endothelial, epithelial, and osteoblastic cells, significantly contributing to the pathogenesis of infections. A significant pathway for this invasion appears to involve the bacterium's binding to the α5β1 integrin *via* a fibronectin bridge, followed by phagocytosis. Additionally, *S. aureus* presents staphylococcal protein A, a cell wall protein that binds to the Fc and Fab regions of immunoglobulins, playing a crucial role in virulence and immune evasion, and can also bind directly to the gC1qR receptor on endothelial cells. Furthermore, vitamin C is recognized for its antimicrobial and immunomodulatory properties, offering potential for reducing the risk of infection. Considering the aforementioned elements, our study focused on exploring the potential effects of vitamin C on the interactions between *S. aureus* and endothelial cells. Thus, we particularly examined two aspects: on the one hand, interactions involving fibronectin-binding proteins (FnBPs) proteins and human α5β1 integrin, and on the other hand, the interaction between vitamin C and the direct entry receptor (the globular heads of complement component 1q receptor [gC1qR/p33], also known as hyaluronic acid binding protein 1 [HABP1]). To achieve this, we utilized molecular modeling assays, primarily relying on molecular docking.

Keywords: antimicrobial properties, endothelial cells, fibronectin-binding proteins, gC1qR receptor, vitamin C, immunomodulatory effects, molecular docking, staphylococcal protein A, *Staphylococcus aureus*, α5β1 integrin

1. Introduction

S. aureus is an opportunistic pathogen capable of colonizing various cellular niches, endowing it with high adaptability and virulence, which can lead to various diseases in humans when it enters the bloodstream. Its ability to live inside host cells plays a crucial role in infection progression. Endovascular infections caused by *S. aureus*, such as infective endocarditis (IE), are associated with high morbidity and mortality rates. The molecular mechanisms underlying *S. aureus* colonization and tissue invasion are complex. Experimental data show that plasma proteins, endothelial cells, and components of the subendothelial matrix play an important role in the pathogenesis of endovascular infections caused by *S. aureus*. Bacterial adhesion to host cells and tissues is a crucial step in its pathogenesis, and it has become evident that many adhesins recognize more than one ligand. For example, it has been demonstrated that Staphylococcal protein A (SpA) binds to von Willebrand factor and another ubiquitously expressed cellular protein, designated gC1qR/p33 (gC1qR). gC1qR is highly expressed on activated platelets and endothelial cells [1]. Its virulence is indeed attributable to the presence of surface proteins, including protein A, which promotes immune system evasion by binding to the gC1qR receptor on endothelial cells.

Importantly, vitamin C (L-ascorbic acid), is one of the most common and essential vitamins. Additionally, beyond its protective role and ongoing exploration of novel functions, it stands as one of the most cost-effective treatment options capable of enhancing immune function, alleviating allergic reactions, fighting infections, and potentially inhibiting cancer development [2,3]. Although vitamin C itself is a potent antioxidant, its aerobic metabolism increases oxidative stress on bacterial cells [4]. Thus, vitamin C can be a safe and natural alternative to limit the growth of *S. aureus* when non-toxicity is required [5]. Moreover, in addition to its well-known immunomodulatory effects, it has recently been shown to possess immunomodulatory properties opposing interesting endothelial cell infection [6].

Molecular modeling has become a new technique for understanding chemical phenomena and a working tool in various fields other than structural chemistry, such as medicine, cell biology, biochemistry, immunology, etc., as well as in the calculation of potential energy surfaces of organic molecules [7,8]. Among the molecular modeling methods are docking and molecular dynamics (MD) [9,10]. These computational tools are very useful in biology, pharmacy, and medicine, as most active ingredients are small molecules (ligands) that interact with a biologically relevant target, usually a protein (receptor).

Considering aforementioned above, our objective was to employ molecular modeling methodologies to assess the potential impact of vitamin C on the interactions between *S. aureus* and human endothelial cells. This included examining the involvement of Staphylococcal protein A, FnBP proteins and human α5β1 integrin, as well as studying the interactions between vitamin C and the active site of the direct entry receptor gC1qR/p33.

2. Materials and Methods

This section aimed to predict the interactions between vitamin C and the target proteins FnBPs and α5β1 staphylococcal. Protein structures and ligands were obtained from the Protein Data Bank (PDB) [11] and PubChem databases [12]. Molecular docking was performed using ArgusLab software [13], with subsequent visualization of interactions carried out via Discovery Studio [14]. The steps of our virtual screening included protein and ligand selection and preparation, molecular docking, and post-docking analysis.

2.1 Microcomputers

We utilized a microcomputer with 16GB memory, an AMD Ryzen 5 3400g CPU 3.5GHZ processor, and Windows 11 operating system for studying the effects of vitamin C on interactions with FnBPs and α5β1. For exploring interactions between vitamin C and the direct entry receptor gc1qr/p33 of *S. aureus*, as well as studying the effect of vitamin C on staphylococcal protein A, we used a microcomputer equipped with 3GB RAM, an Intel(R) Core i5-480M processor clocked at 2.66 GHz with 3 MB cache, and Windows 7 version 2007 operating system.

2.2 Tools used for computational study

Several programs were employed for this work, including ArgusLab for molecular docking, which is based on quantum mechanics for electronic structure prediction, ChemSketch for designing 2D or 3D complex structures and modifying chemical structure images, Chem3D for visualizing and sharing three-dimensional (3D) structures of molecules, and Discovery Studio, a software suite used for simulating small and macromolecular systems, including simulation, ligand design, and pharmacophore modeling. It was used to visualize poses and interactions between vitamin C and protein active sites.

2.3 Databases

We utilized the PDB as the global archive for structural data of biological macromolecules, providing 3D structures of biological macromolecules, including proteins and nucleic acids obtained through various methods such as X-ray crystallography and NMR spectroscopy. Additionally, PubChem served as the American database of chemical molecules managed by NCBI, containing small molecules as well as larger molecules such as nucleotides, carbohydrates, and peptides.

2.4 Protein preparation

The three-dimensional structure of the FnBPs protein was downloaded from the Protein Data Bank (PDB), with the PDBID "1FNF" corresponding to the human fibronectin fragment including repetitions 7 to 10 of type III. The crystallographic structure of the gC1qR/P33 protein was obtained from the PDB under the code PDB: 6szw, and the 3D structure of the protein was examined and prepared using ArgusLab. The active site was identified, and water molecules and other ligands were removed. The three-dimensional structure of the SpA protein was downloaded from the PDB under the code PDBID "4WWI". This crystalline structure of the C domain of the staphylococcal protein A in complex with the FC fragment of human IGG was resolved by X-ray crystallography with a resolution of 2.3 Angstroms.

2.5 Ligand preparation

The ligand, ascorbic acid, was downloaded from the PubChem platform in 2D form, and its chemical structure was modeled using ChemSketch software. The ligand's availability was verified via the Molinspiration server.

114 **2.6 Molecular docking**

115 Molecular docking was performed to assess the effect of vitamin C on (i) FnBPs and α5β1
116 Staphylococcal molecules using a three-dimensional grid encompassing the active site, (ii)
117 intermolecular interactions between vitamin C and the active site of the direct entry receptor
118 gc1qr/p33 of *S. aureus*, and (iii) interactions between vitamin C and staphylococcal protein A,
119 using crystalline structures of the C domain of this protein. The protein A was embedded in a
120 three-dimensional grid largely encompassing the active site, with a center defined by
121 coordinates (X=20Å, Y=23Å, Z=21Å). Ligand-protein interactions were visualized using
122 Discovery Studio.

123 # 3. Results and Discussion

124 ## 3.1 Impact of vitamin C on *S. aureus*-FnBPs-endothelial cell interactions

125 ### 3.1.1 Insights from molecular docking analysis

126 The configuration of binding sites, spatial delineation of interaction zones, and dynamics of
127 molecular interactions between vitamin C and the FnBPs protein are respectively depicted in
128 Figures 1, 2, and 3.

129 The obtained results indicate that vitamin C meets Lipinski's criteria, suggesting adequate
130 bioavailability and potential favorable interaction with target proteins. Molecular docking
131 analysis reveals several plausible poses, with the most favorable showing significant
132 interactions with residues of the FnBPs protein's active site. Moreover, the interaction between
133 vitamin C and the FnBPs protein reveals the formation of hydrogen bonds, suggesting a possible
134 inhibitory effect on bacterial entry into endothelial cells. Complex 1FNF with the lowest Score
135 energy (-6.56802 kcal/mol) formed 4 interactions with our ligand. Hydrogen interactions are
136 formed with the following amino acids: SER1496:HN with a distance of 1.98Å, SER1496:O
137 with a distance of 2.19 Å, GLY1494:HA1 with a distance of 2.37 Å, as well as ARG1493:O
138 with a distance of 1.84 Å (Table 1), suggesting that vitamin C could have an effect on the
139 staphylococcal protein A. The top 10 results, detailing the ligand poses, are presented in Table
140 2. We determined the dimensions of the active site bounding box to be X = 40, Y = 40, Z = 60,
141 as shown in Table 3.

142 ### 3.1.2 Conclusions

143 This part of the study primarily focused on the use of various molecular modeling approaches
144 to predict interactions between vitamin C and staphylococcal FnBPs protein. Molecular
145 docking modeling was performed, followed by the revelation of interactions using Discovery

146 Studio software. The results showed four interactions, both classical and non-classical hydrogen
147 types. These results suggest that vitamin C blocked bacterial entry by binding to its receptor.

148 **3.2 Exploration of interactions between vitamin C and the active site of the direct entry**
149 **receptor gc1qr/p33 of *S. aureus***

150 Interactions between vitamin C and the amino acids of the active site were studied, revealing
151 both classical and non-classical hydrogen bonds as well as hydrophobic interactions. Vitamin
152 C exhibited good affinity with the gc1qR/p33 protein, in line with Lipinski's rule. The study of
153 the interaction between the amino acids of the gC1qR/p33 protein and vitamin C using Arguslab
154 software requires downloading the necessary structures from the databases mentioned earlier,
155 and the structures of the two molecules used in this study are depicted in Figure 4.

156 **3.2.1 Visualization of gC1qR/p33-ascorbic acid interaction**

157 The complex formation free energy between ascorbic acid and gC1qR/p33 is obtained using
158 the open-source software ArgusLab (Figure 5).

159 **3.2.2 Molecular docking protein-ligand circulation**

160 The characteristics of ligand-interaction circulation are listed in Table 4.

161 **3.2.3 Study of protein-ligand interactions**

162 Molecular docking calculation of interactions between the gC1qR/p33 protein sites and vitamin
163 C revealed an optimal interaction energy with gC1qR/p33 of -5.34639 kcal/mol (*pose 0
164 fitness), with a docking process runtime of 14 seconds (*Docking run: elapsed time) (Table 5).

165 **i. Interactions between ascorbic acid and the active site:**

166 Molecular docking allowed the study of interactions between ascorbic acid and the amino acids
167 of the active site of gC1qR/p33 during complex formation. These interactions were ensured

through various types of bonds, including classical hydrogen bonds, non-classical hydrogen bonds, and hydrophobic interactions. The list of amino acids involved in the active site and the bonds between ascorbic acid and the amino acids of the active site of gC1qR/p33 during complex formation are illustrated in Figure 6.

ii. Hydrogen and hydrophobic interactions

Hydrogen bonding occurs when a hydrogen atom, bound to an electronegative atom (the donor), is attracted to another electronegative atom (the acceptor), acting at very short distances (0.8 to 2.8 Å). These bonds, while few, accommodate well to molecular flexibility. Molecules devoid of charged groups or atoms capable of forming hydrogen bonds are termed hydrophobic substances, as they cannot hydrate and tend to aggregate through coalescence. During docking, ascorbic acid forms six classical hydrogen bonds with six amino acids of the active site of gC1qR/p33: SER230, ILE348, TYR224, ASN346, SER347, and GLU438. Additionally, three non-classical hydrogen bonds are established with two amino acids of the active site: SER230 and ASN345. Molecules lacking charged groups or atoms capable of forming hydrogen bonds are considered hydrophobic and tend to aggregate. A hydrophobic bond is also observed between ascorbic acid and the amino acid ILE348 of the active site, with a distance of 5.028796 A° (Table 6). Classical hydrogen bonds, non-classical hydrogen bonds, and hydrophobic interactions are depicted in Figure 7.

iii. Application of Lipinski's Rule

To assess ligand availability, Lipinski's rule of 5 was applied. The results of this evaluation are summarized in Table 7. It emerges that vitamin C fully meets Lipinski's rule criteria. Therefore, vitamin C could be considered a potential inhibitor of the direct entry receptor gc1qr/p33, without presenting absorption issues via oral administration.

191 ### 3.2.4 Conclusions

192 This section focused on the molecular interactions between vitamin C and the gc1qr/p33
193 direct entry receptor of *S. aureus* in endothelial cells. Analysis of these interactions revealed a
194 specific binding between vitamin C and the active site of gc1qr/p33, involving residues
195 SER230, ASN345, ILE348, TYR224, ASN346, SER347, and GLU438. Additionally,
196 examination of ΔG bind values and application of Lipinski's rule of 5 confirmed the favorable
197 affinity of vitamin C for the gc1qr/p33 protein. Based on these in silico results, this strong
198 affinity suggests a potential role in preventing endothelial cell infection by *S. aureus*.
199 However, further validation through in vitro and/or in vivo tests may be necessary to confirm
200 these findings.

201 ## 3.3 Study of the effect of vitamin C on staphylococcal protein A

202 ### 3.3.1 Molecular docking

203 The active site of the C domain of staphylococcal Protein A and the binding site bounding box
204 are presented in Figure 8. We filled in the dimensions of the binding site bounding box (X = 20,
205 Y = 23, Z = 21) (Table 8). Molecular docking modeling with "ArgusLab" allowed for the
206 reconstruction of the vitamin C ligand, revealing 48 poses with the best score, presenting a ΔG
207 equal to -5.17065 kcal/mol (Table 9). This score results from the formation of hydrogen bonds
208 in the protein's catalytic cavity (Figure 9). These hydrogen bonds are established with amino
209 acids at distances ranging from 1.661660 to 3.398596 Å. Additionally, numerous hydrophobic
210 interactions were observed, including hydrophobic Pi and Pi/alkyl mixed interactions with
211 distances of 3.557828 Å and 5.322754 Å (Table 10 and Figure 9). These interactions contribute
212 to the stability of the vitamin C - Protein A complex, involving amino acid residues constituting
213 the active site of the protein's C domain, present on helices 1 (PHE5, GLN9, GLN10, ASN11,
214 PHE13, TYR14, LEU17) and 2 (ASN28, ILE31, GLN32, LYS35).

3.3.2 Interaction between vitamin C and Protein A

Complex 4WWI, displaying the lowest energy score (-5.17065 kcal/mol) (Table 8), established 6 interactions with our ligand. Hydrogen bonds are formed with the following amino acids: PHE13 at a distance of 3.4 Å, GLN9: HE22 at a distance of 2.2 Å, GLN9: HE21 at a distance of 2.8 Å, as well as with ASP2 at a distance of 1.6 Å. It is also noteworthy to mention the presence of hydrophobic interactions with amino acid LYS35 at a distance of 3.5 Å, as well as a second interaction with PHE13 at a distance of 5.3 Å (Table 9). These interactions suggest that vitamin C could have an impact on staphylococcal Protein A.

3.3.3 Lipinski's rule of five

Each ligand must meet several fundamental criteria, including affordable production cost, adequate solubility and stability, as well as absorption, distribution, metabolism, excretion, and toxicity characteristics. In this regard, we evaluated the bioavailability of our ligand using the Molinspiration Checker. This evaluation was based on the criteria established by Lipinski's rules. The evaluated parameters include a molecular weight below 500 daltons, a partition coefficient (logP) or lipophilicity between -2 and 5, a maximum of five hydrogen bond donors, a maximum of ten hydrogen bond acceptors, and a number of rotation angles less than or equal to five. Our results show that our ligand fully satisfies Lipinski's criteria, which bodes well for good interaction even without ligand optimization. The results of applying these rules to our ligand are presented in Table 11.

3.3.4 Conclusions

This study primarily focused on the use of several theoretical molecular modeling approaches to predict interactions between vitamin C and staphylococcal Protein A. Molecular docking modeling was performed, followed by the revelation of interactions using Discovery Studio software. The six identified interactions included both classical and non-classical hydrogen

239 bonds, as well as Pi and mixed Pi/alkyl hydrophobic interactions. These results suggest that
240 vitamin C could have an inhibitory effect on Protein A, a virulence factor of the bacterium
241 Staphylococcus aureus.

4. Conclusions and Future Prospects

It is evident to conclude that vitamin C has strong potential to interfere with interactions between *S. aureus* and endothelial cells. This conclusion arises from the results obtained from various molecular modeling approaches, including molecular docking, which revealed specific molecular interactions between vitamin C and *S. aureus* cell receptors. Thus, vitamin C specifically interacts with staphylococcal FnBPs and α5β1 proteins, as well as with the active sites of target proteins, including the gC1qR/p33 direct entry receptor and staphylococcal Protein A, thereby inhibiting *S. aureus* adhesion and entry into endothelial cells (Table 12). These interactions involve different residues, confirming a favorable affinity of vitamin C for these proteins. Indeed, in the study on gC1qR/p33, classical and non-classical hydrogen bonds were observed with key residues such as SER230, ASN345, ILE348, TYR224, ASN346, SER347, and GLU438, as well as hydrophobic interactions, demonstrating diversified interaction with the active site. Similarly, in the study on staphylococcal Protein A, hydrophobic interactions of Pi and mixed Pi/alkyl types were identified, highlighting the complexity of the interaction between vitamin C and this protein. This affinity could play a crucial role in preventing endothelial infections by *S. aureus*. This affinity was corroborated by the analysis of ΔG bind values and the conformity of vitamin C to Lipinski's rule of 5 (Table 13), which evaluates the bioavailability of pharmaceutical compounds. Molecular modeling studies have identified active sites and key residues involved in these interactions, thus highlighting the potential mechanism by which vitamin C exerts its preventive effect against bacterial infections.

In terms of prospects, it is important to note that these results require further validation through in vitro and/or in vivo experiments to fully confirm their clinical relevance. Further studies could also explore the underlying molecular mechanisms of vitamin C action and its potential efficacy as a therapeutic agent against bacterial infections. These researches could include studies on bacterial resistance mechanisms to vitamin C and the identification of synergies with

267 other antimicrobial agents. These results could pave the way for new strategies for the
268 prevention and treatment of infectious diseases, thus offering new perspectives in the fields of
269 public health and medicine.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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327 ## Figure legends

328 **Figure 1. Binding site on ArgusLab.** The program provided us with 150 poses, and we selected
329 pose 1, which was the most significant with an energy of -6.56802 kcal/mol.

330 **Figure 2. Binding sites.**

331 **Figure 3. Interaction between vitamin C and the FnBPs protein.**

332 **Figure 4. Structure of the gC1qR/p33 protein and the ascorbic acid (AA) ligand.**

333 **Figure 5. Visualization of the ascorbic acid molecule and gC1qR/p33.**

334 **Figure 6. Active site amino acids of gC1qR/p33 and their interactions with ascorbic acid.**

335 **Figure 7. Classic, non-classic hydrogen bonds, and hydrophobic interactions between**
336 **ascorbic acid and the active site amino acids of gC1qR/p33 during molecular complex**
337 **formation.**

338 **Figure 8. Active site of the C domain of the staphylococcal Protein A and the site bounding**
339 **box.** We specified the dimensions of the site bounding box (X = 20, Y = 23, Z = 21).

340 **Figure 9. Interactions of the ligand with the protein, including hydrogen bonds, non-**
341 **classic hydrogen bonds, hydrophobic Pi interactions, and mixed Pi/alkyl hydrophobic**
342 **interactions.**

343

344 **Table 1. Types of bonds formed between the protein and the ligand.**

Molecule	**Interaction hydrogéne**	
	Involved residu	**Distance (Å)**
Vitamine C	SER1496:HN	1.98112
	SER1496:O	2.19359
	GLY1494:HA1	2.37902
	ARG1493:O	1.84572

345

346

347 **Table 2. Ligand poses.**

Pose	Ligand
Pose 1	-6.56802
Pose 2	-6.50183
Pose 3	-6.37257
Pose 4	-6.30471
Pose 5	-6.28464
Pose 6	-6.28156
Pose 7	-6.28156
Pose 8	-6.24117
Pose 9	-6.21076
Pose 10	-6.20253

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349

350 **Table 3. Protein-ligand docking calculation.**

Box	**Docking energy**	**Ligand type**
X: 40 **Y: 40** **Z: 60**	ArgusDock	Semi-flexible

351

352

353 **Table 4. Circulation Characteristics (Ligand-Interaction).**

Box	**Docking ernergy**	**Ligand type**
X: 34.000000 **Y: 30.000000** **Z: 25.000000**	ArgusDock	Semi-flexible

354

355

356 **Table 5. Top 10 molecular docking poses, relative to interactions between the binding sites**

357 **of the gC1qR/p33 protein and vitamin C.**

Pose	Pose 1	Pose 2	Pose 3	Pose 4	Pose 5	Pose 6	Pose 7	Pose 8	Pose 9	Pose 10
Score (Kcal/mol)	-5.34639	-5.34637	-5.34621	-5.19833	-5.18274	-5.13107	-5.12681	-5.12669	-5.11563	-5.11511

358

359

360 **Table 6. Hydrogen and Hydrophobic interactions in the protein-ligand complex along with**

361 **their distances.**

Ascorbic acid	**Hydrophobic interactions**	
	Involved residus	**Distance (A°)**
	ILE348	5.028796
Ascorbic acid	**H-bonding**	
	Involved residus	**Distance (A°)**
Classic	SER230	3.048348
	GLU438	2.848861
	TYR224	1.546703
	ASN346	1.981419
	SER347	2.081011
	ILE348	1.718322
Non-classic	ASN345	2.438632
	SER230	2.325086
	SER230	3.052368

362

363

364 **Table 7. Lipinski rule results on ascorbic acid.**

Compound	**MM**	**logP**	**HB**	**HA**	**FB**
Ascorbic acid	176.12	-1.40	4	6	2

365 MM = Molecular Weight (≤ 500 g/mol), LogP = Water/Octanol Partition Coefficient (-2 ≤ logP

366 ≤ 5), HA = Hydrogen Acceptor (nO, N) (≤ 10), HB = Hydrogen Bond Donor (nOH, NH) (≤ 5).

367 LF = Flexible Bond (nrotb) (≤ 15).

368

369

370 **Table 8. Protein-Ligand Docking Calculation.**

Box	**Docking energy**	**Ligand type**
X : 20.000000 **Y : 23.000000** **Z : 21.*000000***	ArgusDock	Semi-flexible

371

372

373 **Table 9. Poses de ligand poses.**

Pose	Score (kcal/mol)
Pose 1	-5.17
Pose 2	-4.92
Pose 3	-4.91
Pose 4	-4.81
Pose 5	-4.78
Pose 6	-4.76
Pose 7	-4.76
Pose 8	-4.72
Pose 9	-4.71
Pose 10	-4.65
Pose 11	-4.64
Pose 12	-4.62
Pose 13	-4.60
Pose 14	-4.60
Pose 15	-4.57
Pose 16	-4.57
Pose 17	-4.56
Pose 18	-4.55

Pose 19	-4.54
Pose 20	-4.52
Pose 21	-4.50
Pose 22	-4.49
Pose 23	-4.48
Pose 24	-4.48
Pose 25	-4.47
Pose 26	-4.47
Pose 27	-4.46
Pose 28	-4.43
Pose 29	-4.43
Pose 30	-4.41
Pose 31	-4.41
Pose 32	-4.39
Pose 33	-4.39
Pose 34	-4.39
Pose 35	-4.38
Pose 36	-4.38
Pose 37	-4.35
Pose 38	-4.34

Pose 39	-4.19
Pose 40	-4.07
Pose 41	-4.05
Pose 42	-3.95
Pose 43	-3.92
Pose 44	-3.70
Pose 45	-3.01
Pose 46	-2.97
Pose 47	-2.85
Pose 48	-1.99

376 **Table 10. Types of Bonds Established between the Protein and the Ligand.**

Molecule	Hydrogen interaction		hydrophobic interaction	
	Involved residus	Distance (Å)	Involved residus	Distance (Å)
Vitamine C	PHE13	3.398596	PHE13	5.322754
	GLN9: HE22	2.208768	LYS35	3.557828
	GLN9: HE21	2.897263		
	ASP2	1.661660		

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379 **Table 11. Lipinski's Rules Application on Vitamin C.**

Ligand	Log P	PSA	Natcoms	MW	nON	Nohnh	nviolation	nrot
Vitamine C	-1.40	107.22	12	176.12	6	4	0	2

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382 **Table 12. Molecular docking score. interactions and bond length of GC1qR/p33. SpA.**
383 **FnBPs/α5β1 with vitamin C.**

Proteins	Binding Affinity (kcal/mol)	Hydrophobic bonds		Hydrogen bonds	
		Involved residu	Distance (A°)	Involved residu	Distance (A°)
GC1qR/p33 (PDB: 6szw)	-5.34639	ILE348	5.028796	SER230	3.048348
				GLU438	2.848861
				TYR224	1.546703
				ASN346	1.981419
				SER347	2.081011
				ILE348	1.718322
				ASN345	2.438632
				SER230	2.325086. 3.052368
SpA (PDB: 4WWI)	-5.17	PHE13	5.322754	PHE13	3.398596
		LYS35	3.557828	GLN9	2.208768. 2.897263
				ASP2	1.661660
FnBPs/α5β1 (PDB: 1FNF)	-6.56802			SER1496	2.19359. 1.98112
				GLY1494	2.37902
				ARG1493	1.84572

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385 **Table 13. Physicochemical properties and Lipinski's 'rule of 5'.**

	Log *P*	**H-bond donors**	**H-bond acceptors**	**Molecular weight**	**Rule of 5' criteria met**
Rule	< 5	≤ 5	≤ 10	< 500	At least 3
Vitamin C	-1.40	4	6	176.12	2

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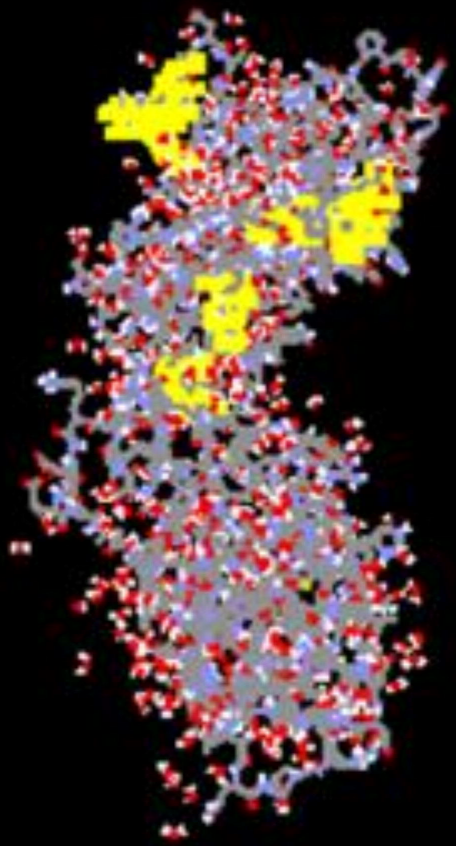
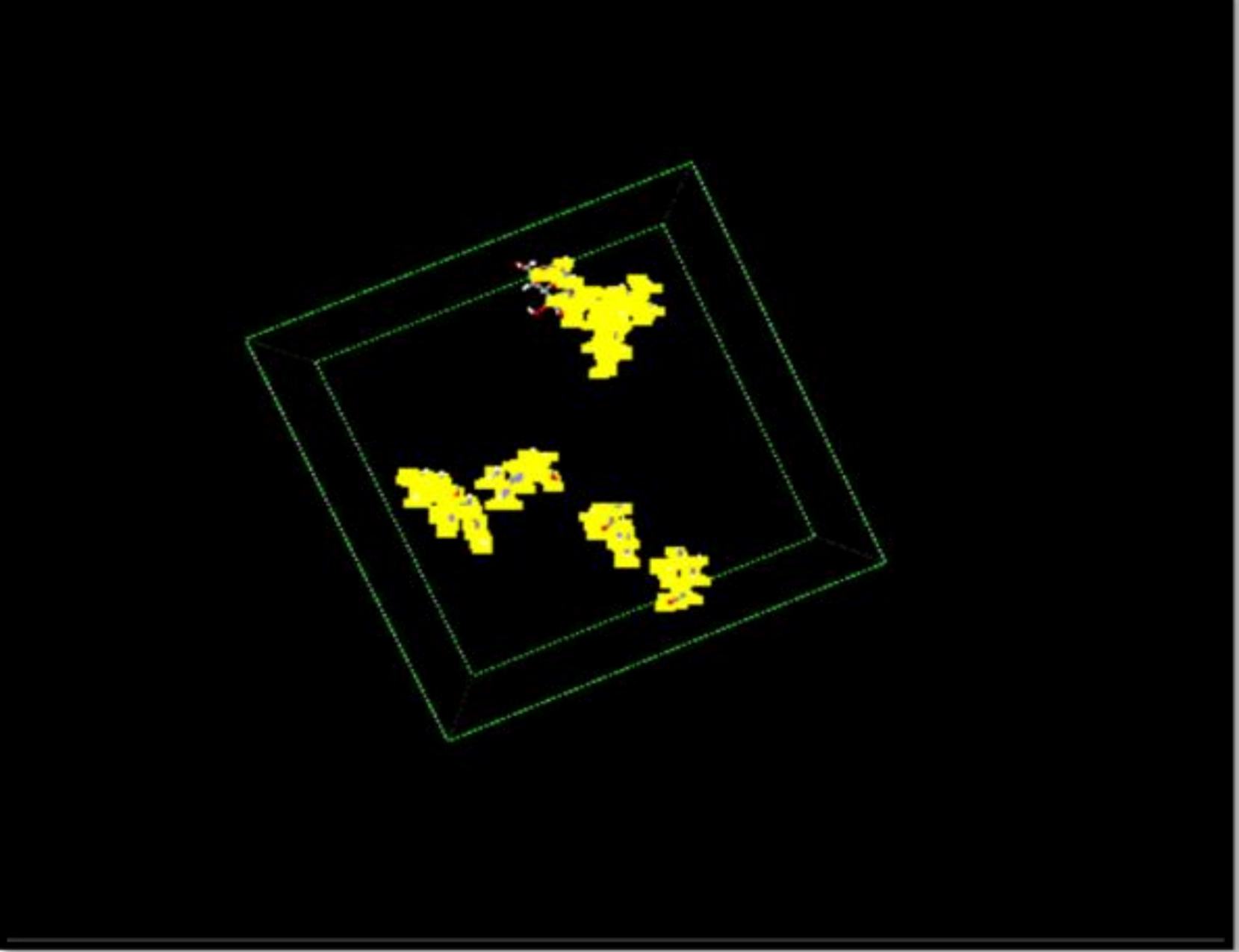
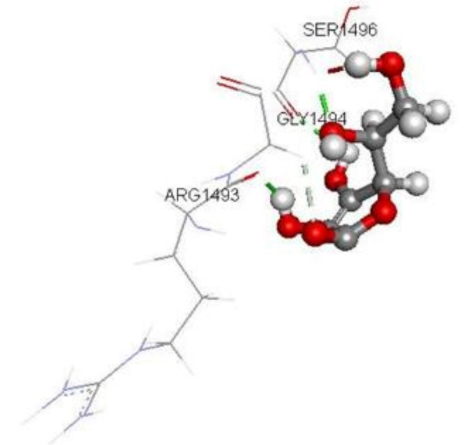
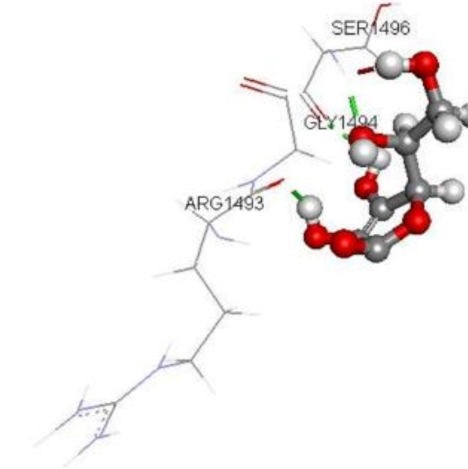




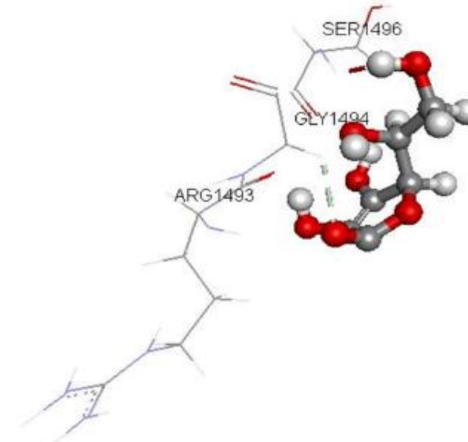
Vitamin C interaction with FnBPs protein

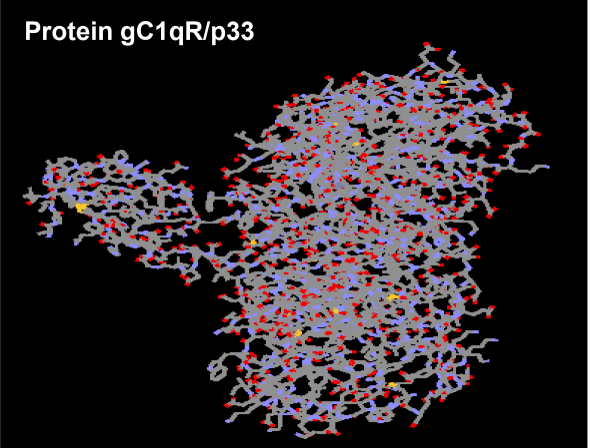


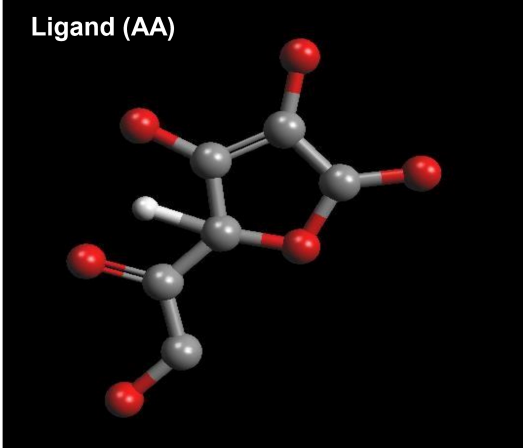
Classical hydrogen interactions

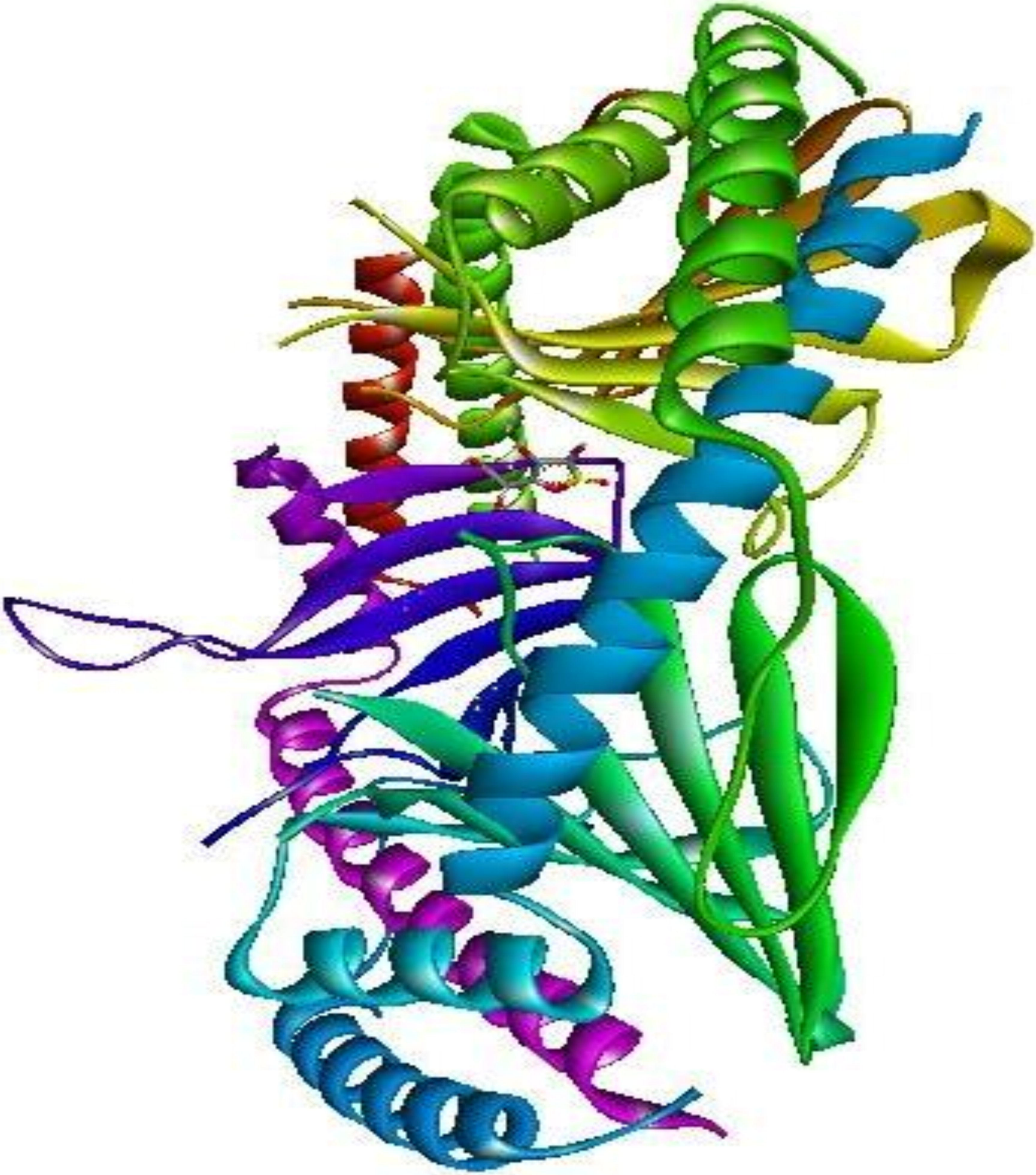


Non-classical hydrogen interactions

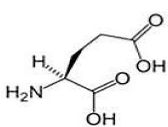



Protein gC1qR/p33

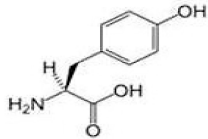

Ligand (AA)



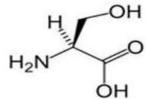
Active site amino acids



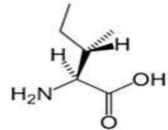
Glutamic acid



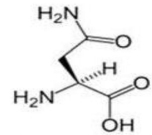
Tyrosine



Serine

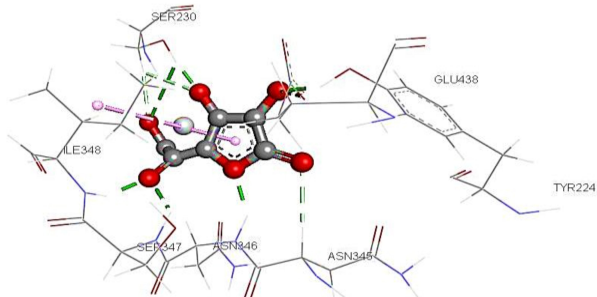


Isoleucine

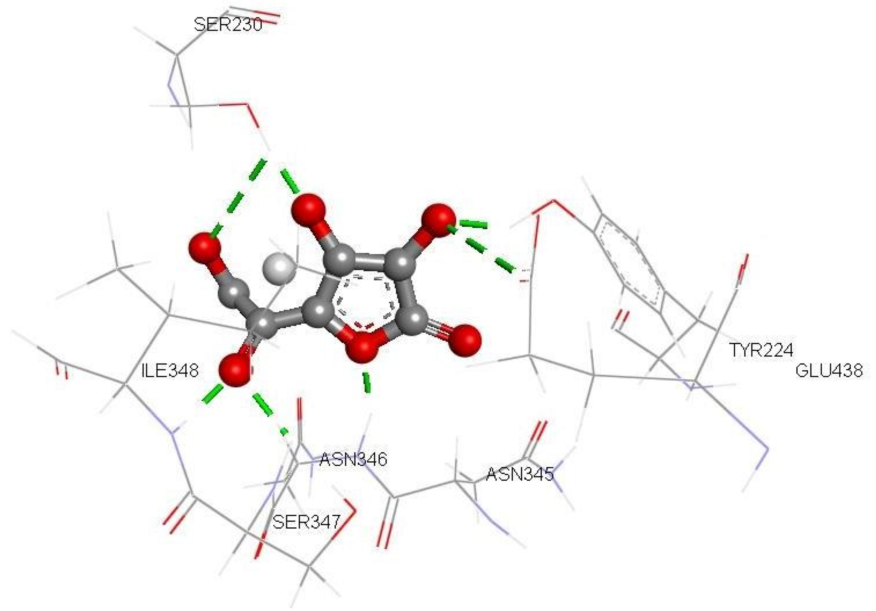


Asparagine

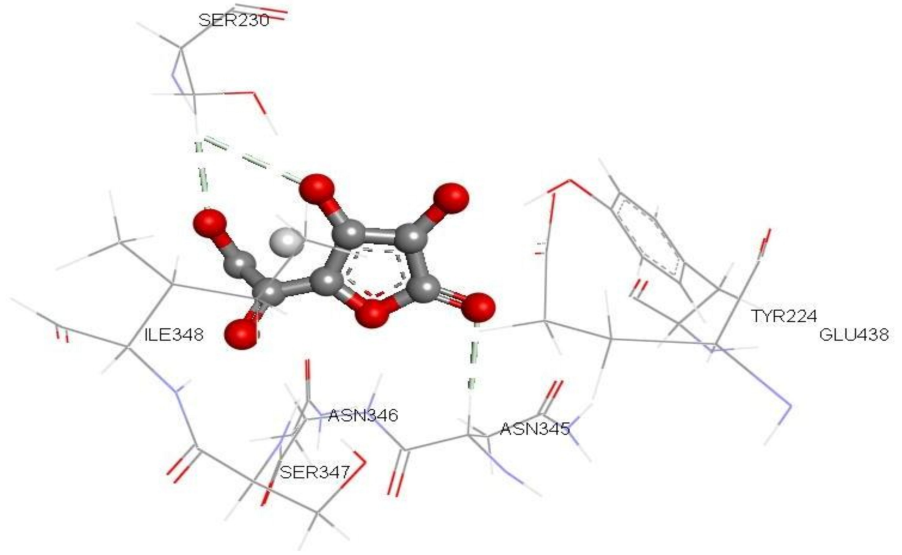
Ascorbic acid binding to gC1qR/p33 active site



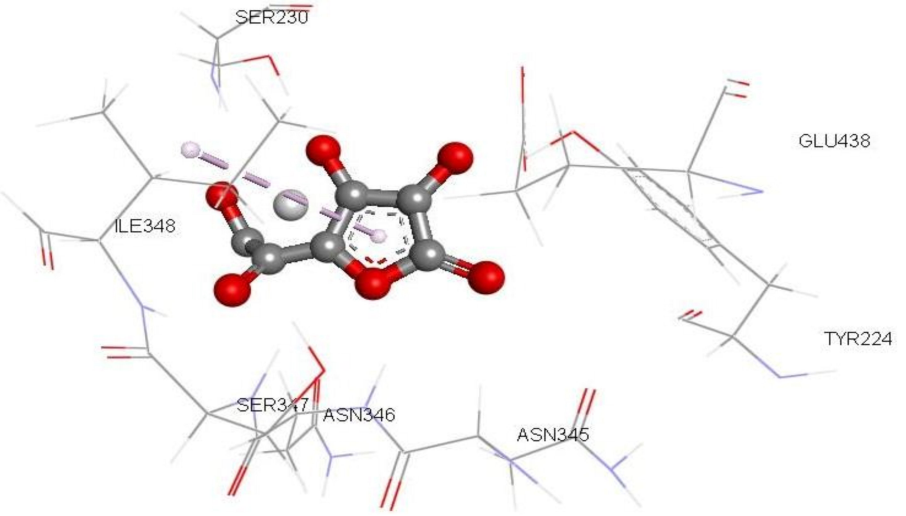
Classical hydrogen interactions

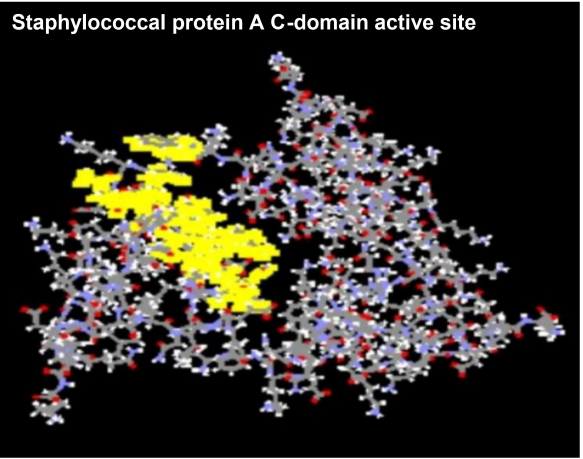


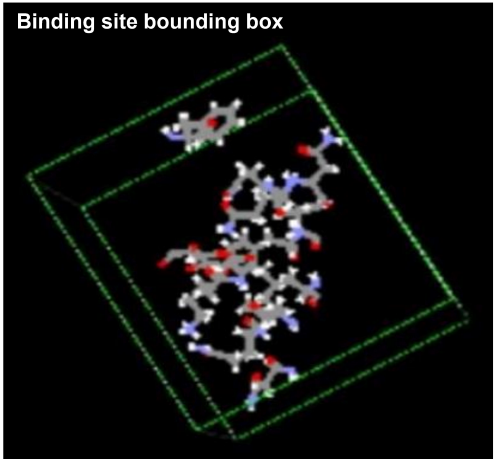
Non-classical hydrogen interactions



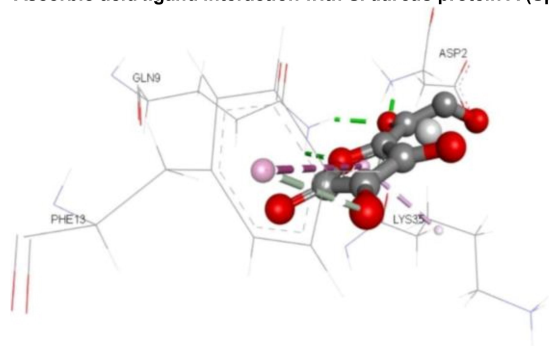
Hydrophobic interactions



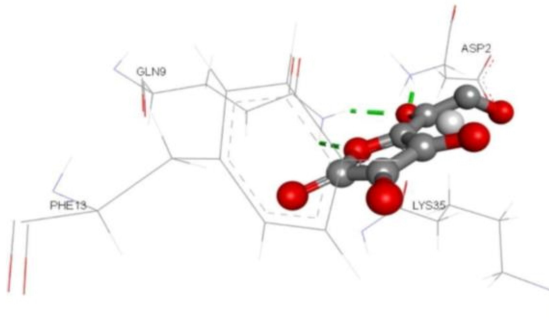

Staphylococcal protein A C-domain active site


Binding site bounding box

Ascorbic acid ligand interaction with *S. aureus* protein A (SpA)



Classical hydrogen interactions



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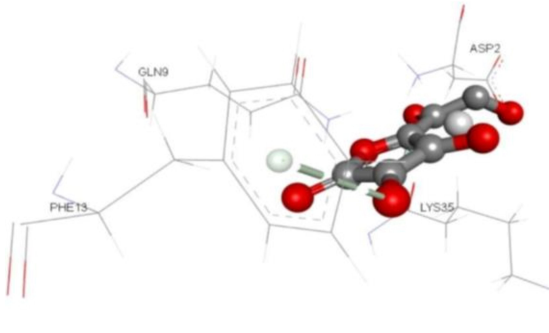
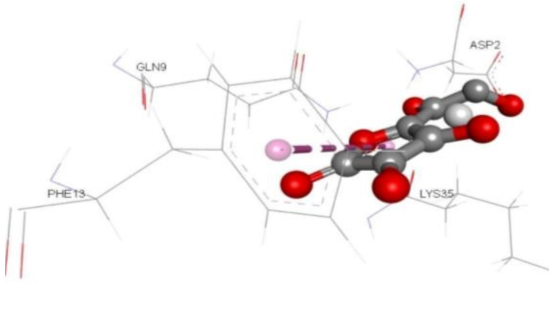
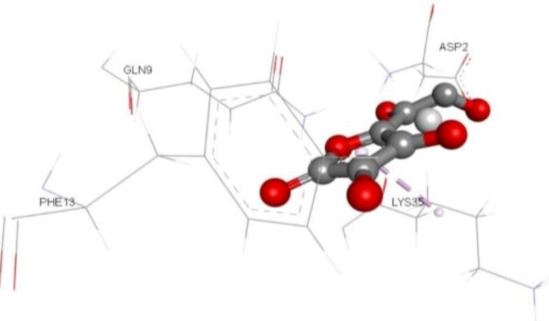


Hydrophobic Pi interaction



Mixed Pi/Alkyl hydrophobic interaction