International Journal of Sciences

Research Article Volume 2 - October 2013 (10)

Interaction Model between HRSV G-Protein and Flavonoids

Fátima Pereira de Souza[1,2], Mariana Pella Sabbag[1], Gabriela Campos de Araujo[1,2], Haroldo Lima Pimentel Cravo[1], Thiago Pançonato Salen Teixeira[1,2], Deriane Elias Gomes[1,2], Valmir Fadel[1,2], Marcelo Andres Fossey[1,2]

[1]Departamento de Física
[2] Centro Multiusuário de Inovação Biomolecular (CMIB), Instituto de Biociências, Letras e Ciências Exatas, UNESP, Campus de São José do Rio Preto. Rua Cristóvão Colombo, 2265, São José do Rio Preto, 15054-000, São Paulo, Brazil.

Abstract

Background: Acute respiratory infections (ARI) are the leading cause of infant mortality in the world, and human respiratory syncytial virus (HRSV) is one of the main agents of ARI. One of the key targets of the adaptive host immune response is the RSV G-protein, which is responsible for attachment to the host cell. There is evidence that compounds such as flavonoids can inhibit viral infection *in vitro*. With this in mind, the main purpose of this study was to determine, using computational tools, the potential sites for interactions between G-protein and flavonoids.

Results: Our study allowed the recognition of an hRSV G-protein model, as well as a model of the interaction with flavonoids. These models were composed, mainly, of α-helix and random coil proteins. The docking process showed that molecular interactions are likely to occur. The flavonoid kaempferol-3-O-α-L-arabinopyranosil-(2 → 1)-α-L-apiofuranoside-7-O-α-L-rhamnopyranoside was selected as a candidate inhibitor. The main forces of the interaction were hydrophobic, hydrogen and electrostatic.

Conclusions: The model of G-protein is consistent with literature expectations, since it was mostly composed of random coils (highly glycosylated sites) and α-helices (lipid regions), which are common in transmembrane proteins. The docking analysis showed that flavonoids interact with G-protein in an important ectodomain region, addressing experimental studies to these sites. The determination of the G-protein structure is of great importance to elucidate the mechanism of viral infectivity, and the results obtained in this study will allow us to propose mechanisms of cellular recognition and to coordinate further experimental studies in order to discover effective inhibitors of attachment proteins.

Keywords: HRSV, G-protein, Molecular docking

Introduction

Human respiratory syncytial virus (hRSV) is the main causative agent of acute respiratory infections (ARI) in children [Nakamura et al., 2008; Morris et al., 2009], and is one of the major causes of respiratory disease in the adult population, especially in the elderly and immunocompromised people [Falsey et al., 200;Pavlova et al., 2009]. Following RSV infection, one of the key targets of the adaptive host immune response is the RSV attachment (G) protein which bears a genetically variable, extensively glycosylated ectodomain [Melero et al., 2004]. This protein is responsible for binding the virus to the host cell and also for mediating the consequent infection. It also plays an important role in antigen recognition as it is the target for the identification of RSV by antibodies.

There is evidence that flavonoids, substances which are present in the human diet, are potential inhibitors of viral infection [Wang et al., 1998]. Some of the viruses reported to be inhibited by flavonoids are herpes simplex virus, parainfluenza virus, adenovirus and RSV. The interactions of flavonoids at different stages in the virus replication cycle have been previously described [Kaul et al., 1985]. In these studies, it was shown that flavonoids in their glycone form seem to be more inhibitory regarding virus infectivity than flavonoids in their aglycone form [Bae et al., 2000].

The process of linking the virus to the host cell is accomplished through G-protein, an essential mechanism which allows the virus to enter into the cell. Knowing its structure will help to discover its functional characterization. On this basis, this study aimed to evaluate the interactions between G-protein

Fátima Pereira de Souza (Correspondence)
fatima@ibilce.unesp.br
+

and some candidate compounds which could work as potential inhibitors of G-protein activity. Here, we propose a computational study in order to find an effective anti-viral compound against hRSV G-protein. We also assessed the binding characteristics to understand the interaction mechanism.

Methods

Analysis by bioinformatics programs

In this study, 62 sequences of the hRSV G-protein were selected, taken from the International Database GenBank [Wheeler et al., 2007]. Through the program BioEdit [Hall, 1999], multiple alignments were performed in order to verify possible mutations between the sequences and also to choose one mutation which could represent the whole set of sequences. Tests of the secondary structure prediction were done, using the program PREDICTPROTEIN [Rost et al., 2004]. This program was also used to determine possible post-translational modifications.

Modeling the three-dimensional structure of G-protein and flavonoids

Since a G-protein model has not yet been proposed, protein modeling techniques were used to obtain a protein tertiary structure model. Since G-protein has low homology with other proteins, the modeling technique employed was *ab initio*, because it was the method that provided the best results for a complete protein structure. We used the program Rosetta [Chivian et al., 2003] to perform protein modeling. The quality of the model was evaluated by conventional validation methods, and it was analyzed by SAVES (Structural Analysis and Verification Server), contained in a single analysis tool, PROCHECK [1Laskowski et al., 1996].

Most of the flavonoids studied in this work have no three-dimensional structures solved by experimental techniques. For this reason, *in silico* modeling of these structures was required. FROG2 [Miteva et al., 2010] was used to calculate the three-dimensional structure of molecules through the Monte Carlo method and also through energy minimization via the AMMOS force field [Pencheva et al., 2008].

Molecular docking of G-protein with flavonoids

Determining the possible interactions between G-protein and flavonoids was the main objective of this study. After identifying sites that could probably interact with the compound, through the program Q-SiteFinder [Laurie et al., 2005], the structures of the complexes were prepared, using the Protein Preparation Wizard [Schrödinger, 2005]. Explicit hydrogens were added to the protein, followed by a careful investigation of the protonation state of ionizable residues. Restrained minimization, using the OPLS-AA 2005 force field, was performed for the refinement of each complex structure, until the average RMS deviation of the non-hydrogen atoms reached the specified default of 0.30 Å. Finally, all heteroatoms were removed.

It is necessary to mention that the binding site, which was used to generate the energy grids, was defined by an outer cubic box centered at residues 180-200, with an edge length of 25 Å, and by an inner box with an edge length of 10 Å.

All docking calculations were performed using Glide XP [Schrödinger, 2005; Friesner et al., 2004]. The calculations were set to dock flexibly, to sample ring conformations and to penalize non-planar amide bonds. For the Glide screens, 5,000 poses per ligand were specified to be kept for the initial phase of the docking calculation. The scoring window for keeping the initial poses was set at 100 kcal/mol. The 800 best poses per ligand were kept for energy minimization, using expanded sampling. The maximum number of minimization steps was set to 1000. The default settings of Glide were used for the remaining parameters.

Results And Discussion

Prediction and modeling of the G-protein secondary structure

From the selected sites, we could predict the particular characteristics of the protein through the program PREDICTPROTEIN. Some information about the composition of secondary structures is important to comprehend the function and the type of interactions in which this specific protein performs. Predicted protein analysis showed that the G-protein secondary structure is mostly composed of random coils (85%) and some α-helical regions.

Generally, hydrophobic regions that traverse the membrane have an α-helix conformation, as the side chains of these amino acid residues protrude from the outer surface of the helix and interact with the lipid membrane, forming a very stable structure. As G-protein is a transmembrane protein, the region between amino acid 38 and 66 [Sullender, 2000] probably assumes this type of conformation. The major conformation assumed by G-protein was irregular coils without a defined secondary structure; therefore, it is more difficult to describe it. These regions are associated with protein sequences that undergo post-translational modifications and have multiple cellular functions.

To confirm the results obtained by PREDICTPROTEIN analysis, computational modeling was performed to predict the G-protein model through the *ab initio* method. Homology modeling was discarded in this work since the RSV G-protein does not share reasonable homology with other proteins. The structure obtained through our modeling studies had similar characteristics related to

the secondary structure, which increases the reliability of the applied methods and reinforces the idea that this novel protein sequence is one of this type.

With the structure in PDB format (Figure 1), a comparative analysis was conducted with the previously obtained predicted results, only with the primary sequence being observed. We noted that, in the model predicted by Rosetta, G-protein showed a large percent of random coils, followed by α-helix structures.

The occurrence of disulfide bonds between conserved cysteine residues (173, 176, 182, 186) was also predicted; however, the distances between the atoms were greater than 3 Å(in both models), showing a low probability of binding. According to some studies, the conserved cysteine residues present in the G-protein ectodomain should lead to disulfide bonds in a loop structure [Gorman et al., 1997,Johnson et al., 1987,Langedijk et al., 1998]. This would involve residues at positions 173-186 and 176-182; this region can be interpreted as the recognition sites for cellular interactions. However, some studies have shown that this region is not necessary for *in vitro* infection, which leads us to believe that the role of this conserved region of 13 amino acids is still unclear [Teng et al., 2001,Teng et al., 2002]. The absence of disulfide bonds in our theoretical study may be explained by considering only the protein sequence and not its insertion in an *in vitro* environment. Therefore, the results do not consider all the possible conformations that can exist in a real environment.

The Ramachandran plot for the model (Figure 2A), which was calculated by the PROCHECK program [Laskowski et al., 1996], returned 97% of amino acids in the allowed regions of the target proteins, 1.9% of amino acids in generously allowed regions and 1.1% of amino acids in disallowed regions. Some of the residues which were found in disallowed regions are glycines, and, sometimes, they were found in different G-protein sequence endings. Therefore, the plot demonstrates the quality of the main chain torsion angles and also reveals good validation of the model.

Prediction of post-translational modifications

Possible sites of post-translational modifications were analyzed by PREDICTPROTEIN, in order to better understand the type of changes G-protein may undergo, and how the secondary structure may be affected by these changes. The G-glycoprotein ectodomain has a high content of serine and threonine, and possible carbohydrate binding sites for O-linked or N-linked glycosylation [Wertz et al., 1985]. It has been shown that hRSV infectivity is sensitive to the removal of glycosylation sites by endoglycosidases, indicating the need for carbohydrates which may help G-glycoprotein play its role. The highly glycosylated sites found for G-protein may explain the large percentage of random coils predicted by PREDICTPROTEIN and by other molecular docking programs, since carbohydrate groups added to the protein sequence make modeling more difficult by generating irregular structures without defined forms (coils).

For many other viral glycoproteins, studies have shown that N-glycans are important structural components which influence the processes of folding and transporting, as well as the activity, stability and immunological properties of proteins [Zimmer et al., 2001]. Scientists believe that selective pressures act to maintain a minimum number of N-glycosylation sites [Esteves et al., 2002], and, since the pattern of glycosylation may be important in viral replication and transmissibility, the recognition of highly glycosylated regions may also be important in order to elucidate the strategies by which the virus escapes the host immune system [Lole et al., 1999].

The results show that glycosylation sites are conserved in the ectodomain, suggesting a significant role in G-protein structure and function. However, few studies have reported the true role of N-glycosylation for hRSV G-protein. It is thought that these modifications are necessary to protect the virus against antibody recognition, may help in maintaining its correct conformation or even allow conformational changes after proteolytic processing. Similar functions have been proposed for conserved glycosylation sites in the influenza virus [Ohuchi et al., 1997].

Molecular docking between G-protein and flavonoids

The models created for hRSV G-protein were subjected to Q-Site Finder, which tried to identify interaction sites. Figure 3 shows the protein models, allowing the identification of the main binding sites.

The model analyzed by Q-SiteFinder showed 10 interaction sites, and the region between amino acids 180-200 was chosen to perform docking assays, since this site is located in the ectodomain and has a random coil structure, favoring interactions with the protein (Figure 4). According to some studies, this region is the binding domain for host cells [Feldman et al., 2000].

The fact that glycosylated flavonoids have a higher interaction energy than non-glycosylated flavonoids was verified in our work (Table 1). Analyzing the hydrophobic, electrostatic and hydrogen interaction contributions, we observed that for non-glycosylated flavonoids, the hydrophobic contribution is more significant than for the others, except for quercetin

and myricetin. On the other hand, for glycosylated flavonoids, the hydrogen interaction contribution prevails over the others, due to the OH groups derived from glycosylation.

The analysis of the electrostatic contributions showed that non-glycosylated flavonoids produced a little less energy than glycosylated flavonoids. The reason for this behavior is that the electrons of the heteroatoms in the flavonoids core structure delocalize over the electron cloud of the aromatic rings, giving the structure a hydrophobic core.

The structures docked into G-protein had energy interactions ranging from -9.74 to -2.10 kcal/mol (Figure 5). Most of the compounds had interaction energies between -7 and -6 kcal/mol; kaempferol-3-O-α-L-arabinopyranosil-(2 → 1)-α-L-apiofuranoside-7-O-α-L-rhamnopyranoside had the lowest interaction energy, -9.74 kcal/mol. This compound showed hydrogen interactions with the amino acid residues Thr16, Asn160, Asp162, Asn169, Arg188, Asp214, Thr259, Ser260 and Gln261 (Table 2).

The non-glycosylated flavonoids were located in a region with more polar residues (Thr198, Thr200, Thr203, Thr211, Lys213, Asp214, Thr259 and Gln261) than non-polar ones (Pro202 and Pro206). The same was observed for glycosylated flavonoids, with more polar (Thr16, Asn160, Asp162, Asn169, Arg188, Asn191, Lys192, Thr203, Lys204, Lys205, Lys213, Asp214, Gln218, Thr259, Ser260 and Gln261) than non-polar residues (Leu20, Ile185, Pro202 and Pro206).

The interaction between non-glycosylated flavonoids with G-protein was weaker than for glycosylated flavonoids, because non-glycosylated molecules are more hydrophobic than glycosylated flavonoids, and the protein surface is more hydrophilic. The protein pockets are shallow and those which are more hydrophobic are also small, thereby accommodating the flavonoid. Moreover, glycosylated flavonoids interact more through hydrogen bonds, mainly via their sugars. These results indicate that the interaction with flavonoids occurs in an important G-protein region, regardless of the program used to generate the protein model, or the program used for molecular docking. Therefore, some scientists suggest that flavonoids, when interacting with G-protein, are suitable inhibitors of interactions with other types of proteins.

Conclusion

The results obtained in this work contribute to the possible development of a viral infection inhibitor based on the protein interaction process. We have brought together key information about the hRSV G-protein, demonstrating that it has many features yet to be discovered, especially through experimental studies. We also noted that the results can be used in future studies on respiratory syncytial virus neutralization. We demonstrated that G-protein is highly glycosylated and has many functions. We discovered that flavonoids such as kaempferol have great potential for interaction with G-protein, and thus these compounds may be promising as viral inhibitors. This finding encourages future experimental studies which will provide new information and lead to more effective hRSV infection inhibition.

Competing Interests

The authors declare they have no competing interests.

Author Contributions

All authors have read and approved the final manuscript.

Acknowledgments

This work was supported by FAPESP grants (Grant # 2010/50444-4 to FPS) and by a CNPq grant (Grant # 306342/2010-3 to FPS). FPS is supported by a FAPESP and CNPq fellowship.

References

1. Bae EA, Han MJ, Lee M, Kim DH. **In vitro inhibitory effect of some flavonoids on rotavirus infectivity.** *Biol Pharm Bull* 2000, **23**:1122–4.
2. Chivian D, Kim DE, Malmstrom L, Bradley P, Robertson T, Murphy P, Strauss C. E, Bonneau R, Rohl CA, Baker D. **Automated prediction of CASP5 structures using the Robetta server**. *Proteins* 2003, **53**(Suppl 6):524-533.
3. Esteves A, Parreira R, Venenno T, *et al*. **Molecular epidemiology of HIV type 1 infection in Portugal: high prevalence of non-B subtypes**, *AIDS Res Hum Retroviruses* 2002, **18**(5):313-325.
4. Falsey AR, Wash EE. **Respiratory syncytial virus infections in adults.** *Clin Microbiol Rev* 2000. **13**(3):371-84.
5. Feldman SA, Audet S, Beeler JA. **The fusion glycoprotein of human respiratory syncytial virus facilitates virus attachment and infectivity via an interaction with cellular heparan sulfate**. *J Virol* 2000, **74**:6442–6447.
6. Friesner RA, Banks JL, Murphy RB, *et al*. **S: Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy.** *J Med Chem* 2004, **47**:1739–1749.
7. Gorman JJ, Ferguson BL, Speelman D, *et al*. **Determination of the disulfide bond arrangement of human respiratory syncytial virus attachment (G) protein by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.** *Protein Sci* 1997, **6**:1308-1315.
8. Hall TA. **BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.** *Nucl Acids Symp Ser* 1999, **41**:95-98.
9. Johnson PR, Spriggs MK, Olmsted RA. *et al.* **The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins.** *Proc Natl Acad Sci USA* 1987, **84**:5625–5629.
10. Kaul TN, Middleton E Jr, Ogra PL**. Antiviral effect of flavonoids on human viruses.** *J Med Virol* 1985, **15**:71–9.
11. Langedijk JP, De Groot BL, Berendsen HJ, *et al.* **Structural homology of the central conserved region of the attachment protein G of respiratory syncytial virus with the fourth subdomain of 55-kDa tumor necrosis factor receptor.** *Virology* 1998, **243**:293-302.
12. Laskowski RA, Rullmannn JA, Macarthur MW, *et al.* **AQUA and PROCHECK-NMR: programs for checking**

the quality of protein structures solved by NMR. *J Biomol NMR* 1996, **8:**477-486.
13. Laurie AT, Jackson RM. **Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites.** *Bioinformatics* 2005, **21:**1908-1916.
14. Lole KS, Bollinger RC, Paranjape RS, *et. al*. **Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination.** *J Virol* 1999, **73**(1):152-160.
15. Melero JA, *et. al.* **The soluble form of human respiratory syncytial virus attachment protein differs from the membrane-bound form in its oligomeric state but is still capable of binding to cell surface proteoglycans.** *J Virol* 2004, **78**(Suppl l):3524-3532.
16. Miteva MA, Guyon F, Tufféry P. **Frog2: Efficient 3D conformation ensemble generator for small compounds.** *Nucleic Acids Res* 2010, **38**:622-627.
17. Morris S K, Dzolganovski B, Beyene J, *et. al.* **A meta-analysis of the effect of antibody therapy for the prevention of severe respiratory syncytial virus infection.** *BMC Infect Dis* 2009, **9,** n 106.
18. Nakamura M, Itokazu K, Taira K, et al. **Genotypic and phylogenetic of the G gene of respiratory syncytial virus isolates in Okinawa, Japan, 2008.** *Japan J Infect Dis* 2009, **62**:326-327.
19. Ohuchi M, Ohuchi R, Feldmann A, Klenk HD. **Regulation of receptor binding affinity of influenza virus hemagglutinin by its carbohydrate moiety.** *J Virol* 1997, **71**:8377–8384.
20. Pavlova S, Hadzhiolova T, Abadjieva P, *et. al.* **Aplication of RT-PCR for diagnosis of respiratory syncytial virus and human metapneumovirus infection in Bulgaria, 2006-7 and 2007-8**. *Eurosurveillance* 2009,14, n. 23.
21. Pencheva T, Lagorce D, Pajeva I, *et al.* **AMMOS: Automated Molecular Mechanics Optimization tool for in silico Screening.** *BMC Bioinformatics* 2008**, 9**:438.
22. Rost B, Yachdav G, Liu J. **The PredictProtein server.** *Nucleic Acids Res* 2004, **32**(Web Server issue):W321-6.
23. Schrödinger; LLC. New York, 2005; http://www.schrodinger.com.
24. Sullender WM. **Respiratory syncytial virus genetic and antigenic diversity.** *Clin Microbiol Rev* 2000. **13**(1):1-15.
25. Teng MN, Collins PL. **The central conserved cysteine noose of the attachment G protein of human respiratory syncytial virus is not required for efficient viral infection in vitro or in vivo.** *J Virol* 2002**, 76**:6164-6171.
26. Teng MN, Whitehead SS, Collins PL. **Contribution of the respiratory syncytial virus G glycoprotein and its secreted and membrane-bound forms to virus replication in vitro and in vivo.** *Virology* 2001, **289**:283–296.
27. Wang HK, Xia Y, Yang ZY, Natschke SL, Lee KH. **Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents.** *Adv Exp Med Biol* 1998, **439**:191–225.
28. Wertz GW, Collins PL, Huang Y, *et al.* **Nucleotide sequence of the G protein gene of human respiratory syncytial virus reveals an unusual type of viral membrane protein**. *Proc Natl Acad Sci U S A* 1985, 82(12):4075-4079.
29. Wheeler DL, Barrett T, Benson DA, *et. al.* **Database resources of the National Center for Biotechnology Information.** *Nucleic Acids Res* 2007, **35**:5-12.
30. Zimmer M, Danko JP, Pennings SC, *et al.* **Hepatopancreatic endosymbionts in intertidal isopods (Crustacea: Isopoda), and their contribution to digestion.** *Mar Biol* 2001, **138**:955–963.

Figure Legends

Figure 1: *RSV G-protein model obtained by Rosetta. Regions composed of random coils are highlighted in green; regions composed of α-helix are highlighted in red.*

Figure 2: *Ramachandran plot of the RSV G-protein model predicted by Rosetta. Red: residues in favorable regions; dark yellow: residues in allowed regions; light yellow: residues in generously allowed regions; white: residues in disallowed regions.*

Figure 3: *Results obtained with the Q-Sitefinder method applied to the hRSV G-protein model generated by Rosetta. Interaction sites obtained by the program are colored and sites described in the literature as flavonoid binding sites are highlighted.*

Figure 4: *Molecular docking of flavonoids in the G-protein. A) G-protein region indicating the docking site; B) Magnified view of non-glycosylated docked flavonoids; C)Magnified view of glycosylated docked flavonoids.*

Table 1: *Docking energy of flavonoids. The Gscore is the sum of all the forces involved in the interaction.*

Table 2: *Hydrogen interaction between the G-protein and the flavonoid kaempferol-3-O-α-L-arabinopyranosil-(2 → 1)-α-L-apiofuranoside-7-O-α-L-rhamnopyranoside.*

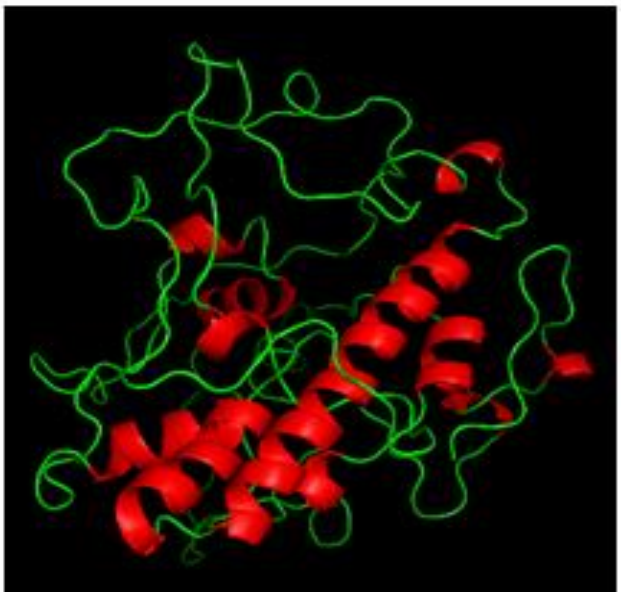

Figure 1

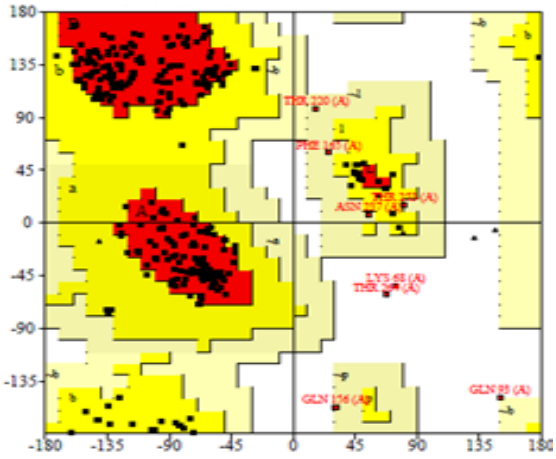

Figure 2

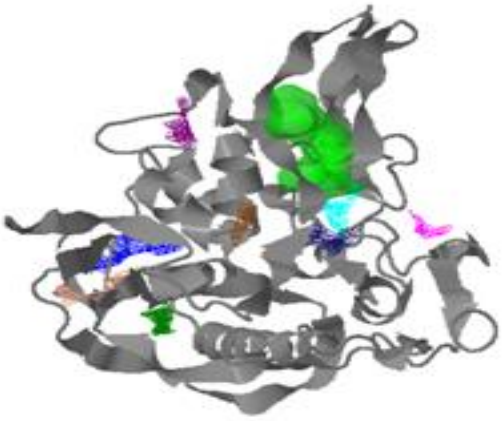

Figure 3

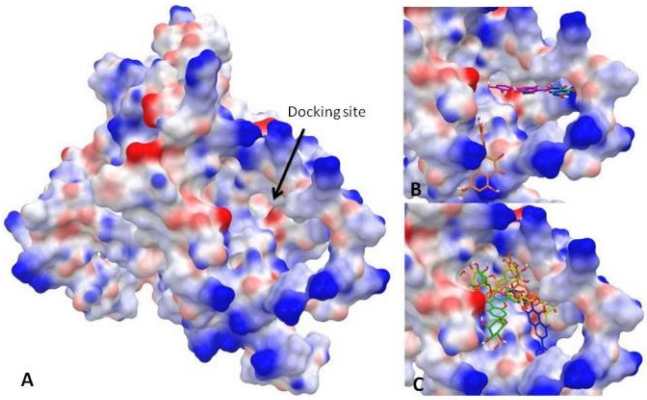

Figure 4

Table 1

	Gscore	Hydrophobic interaction	Hydrogen interaction	Electrostatic interaction
Kaempferol	-2.10	-1.54	-1.15	-0.57
Kaempferol 54	-9.74	-2.10	-6.37	-2.00
Quercetin	-4.11	-1.73	-2.17	-0.80
Rutin	-8.78	-2.64	-4.67	-1.70
Apigenin	-2.48	-1.57	-0.59	-0.60
Apigenin 59	-6.28	-1.03	-5.03	-1.67
Myricetin	-3.36	-1.18	-1.48	-0.63
Myricetin 17	-7.29	-1.69	-5.34	-1.79

	Quercetin		Rutin		Miricetin		Miricetin 70		Kaempferol		Kaempferol 54		Apigenin		Apigenin 59	
Residues	Atom	Distance	Atom	Distance	Atom	Distance	Atom	Distance	Atom	Distance	Atom	Distance	Atom	Distance	Atom	Distance
$OH_{(sc)}$ Thr16	-	-	OH_5	2.64	-	-	-	-	-	-	$OH_{arabino}$	2.61	-	-	-	-
$NH_{1(SC)}$ Asn160	-	-	-	-	OH_8	2.36	-	-	-	-	$O_{rhamino}$	2.08	-	-	-	-
$NH_{2(sc)}$ Asn160	-	-	-	-	OH_8	2.57	-	-	-	-	-	-	-	-	-	-
$O_{\delta2(sc)}$ Asn160	-	-	-	-	OH_9	2.18	-	-	-	-	-	-	-	-	-	-
$O_{\delta2(sc)}$ ASP162	-	-	-	-	-	-	OH_{gluco}	1.70	-	-	OH_9 $OH_{rhamino}$	1.71 2.46	-	-	-	-
$O_{\delta1(sc)}$ Asn169	-	-	-	-	-	-	OH_{gluco}	2.19	-	-	-	-	-	-	-	-
$NH_{(sc)}$ Asn169	-	-	-	-	-	-	OH_{gluco} OH_{gluco}	2.64 2.33	-	-	O_1 OH_7	2.57 2.41	-	-	-	-
$NH_{(sc)}$Arg188	-	-	O_1	2.06	-	-	OH_{gluco}	2.18	-	-	O_1	2.08 2.47	-	-	-	-
$O_{(bb)}$Asn191	-	-	-	-	OH_2	2.09	-	-	-	-	-	-	-	-	-	-
$O_{(bb)}$ LYS192	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OH_9	2.23
$OH_{(sc)}$ Thr200	-	-	-	-	-	-	-	-	OH_7	2.21	-	-	OH_7	2.24	-	-
$OH_{(sc)}$ Thr203	OH_5	2.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$NH_{(bb)}$ Thr203	-	-	$O_{rhaminose}$	2.37	-	-	-	-	-	-	-	-	-	-	-	-
$O_{(bb)}$ Thr203	-	-	OH_{10}	2.15	-	-	-	-	-	-	-	-	-	-	-	-
$O_{(bb)}$ Lys204	-	-	OH_9	1.82	-	-	-	-	-	-	-	-	-	-	-	-
$NH_{(sc)}$ Lys205	-	-	-	-	-	-	OH_9	1.86	-	-	-	-	-	-	-	-
$NH_{(sc)}$ Lys213	-	-	-	-	-	-	-	-	O_1	2.09	-	-	OH_1	2.14	-	-
$O_{\delta2(sc)}$ Asp214	OH_8	1.74	OH_{gluco} OH_{gluco}	1.88 1.93	-	-	OH_7 OH_7	2.05 2.62	OH_9	1.97	OH_9	1.52	OH9	2.02	OH_{gluco}	1.65 2.11 2.18
$NH_{(sc)}$ Gln218	-	-	-	-	-	-	OH_7	2.18	-	-	-	-	-	-	O_{gluco}	1.92
$OH_{(sc)}$ Thr259	-	-	-	-	-	-	OH_9	1.91	-	-	$O_{arabino}$	2.14	-	-	-	-
$O_{(bb)}$Thr259	OH_9	2.18	OH_{gluco}	2.50	-	-	OH_{gluco}	1.93	-	-	-	-	-	-	OH_{gluco}	1.66
$HO_{(sc)}$ SER260	-	-	-	-	-	-	-	-	-	-	OH_{apio} OH_{apio}	2.23 1.87	-	-	-	-
$NH_{(sc)}$ Gln261	OH9	1.96	OH_{gluco} OH_{gluco}	1.85 2.48	-	-	-	-	-	-	-	-	-	-	O_{gluco} O_{gluco}	1.99 2.57
$NH_{(bb)}$ Gln261	-	-	O7	2.61	-	-	-	-	-	-	O_{apio}	1.71	-	-	-	-