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Homology modeling and docking studies of a plasmid partition protein, ParF: Flavonoids as anti-plasmid agents

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ABSTRACT

ParF is a plasmid partition protein of 206 amino acids, responsible for the active segregation of plasmid pOLA52 in Escherichia coli. In this in silico study the physiochemical properties and secondary structure were determined. The tertiary structure of the protein was predicted and refined using PHYRE2 and by GalaxyRefine servers respectively. 120 compounds were collected from Drug bank and ZINC data bases and were docked with the best model using Hex 8.0.0. The best ten compounds were docked again by Autodock 4.2.6. Five models were generated by GalaxyRefine software and the best model, Model 5, was evaluated by RAMPAGE, ERRAT, QMEAN6, and ProSA validation tools. Quality assessment indicated that Model 5 was the best reliable model having an overall quality of 99.49% in ERRAT and its QMEAN6 score was 0.729. 99% of its residues were in the favored region, therefore, Model 5 was submitted into Protein Model Data Base. Docking with Hex 8.0.0 and Autodock 4.2.6 showed that six flavonoids; rutin, amentoflavone, hinokiflavone, vicenin, silybin and scutellarin were better in docking than the previously used anti-plasmids drugs; phenoxybenzamine, verapamil, chloropromazine and octoclothepin. These flavonoids could be used to eliminate the antimicrobial resistance plasmids in pathogens to improve the antibiotic action.

Keywords: Anti-plasmids, ATPase, Homology modeling, Rutin

INTRODUCTION

Accurate distribution of the genetic material to daughter cells in cell division is crucial for organisms. Therefore, plasmids contain systems to ensure faithful DNA segregation during mitosis [1]. Low copy number plasmids have partition systems. These systems consist of three components: a nucleotide triphosphate-dependent filament forming protein (ParA), a DNA binding adaptor (ParB) and a centromere-like DNA region [2]. ParB binds the centromere-like region then ParA is brought via interactions with ParB to create a segregation complex. This complex directs the newly synthesized plasmids to their specific locations in the daughter cells [3].

Homology modeling basically consists of four steps a) the identification of templates of known structure; b) the alignment of the target (the unknown) with the template; c) building of the models; and d) the quality estimation of the model [4]. Several methods have been developed to analyze the correctness of the protein models proposed. These methods use stereochemical checks and molecular mechanics energy approaches to identify problems in the structure of these models. *In silico* models were used to predict protein function, to locate binding sites and to design of enzymes, antibodies and various drugs [5].

The spread of resistance among bacterial pathogens to almost all antibiotics is one of the most serious public health issues. Recent advances in molecular biology have significantly increased the ability to discover new antibacterial targets. Such possible targets are quorum sensing systems, the shikimate pathway, isoprenoid biosynthesis and plasmid maintenance systems [6].

The observation that patients receiving the phenothiazine chlorpromazine as antipsychotic had lower infection rate motivate researchers to screen antihistamines, anti-inflammatory agents, antipsychotics and cardiovascular drugs for possible antimicrobial properties [7, 8]. Flavonoids are secondary metabolites found in medicinal plants. They act as antioxidant, anti-inflammatory, anticancer agents and can eliminate free radicals [9]. In this study, the three dimensional structure of a plasmid partition protein, ParF is proposed, evaluated by various methods and virtually docked with flavonoids since eliminating antibiotic-resistance plasmids could resolve the increasing antimicrobial resistance.

MATERIALS AND METHODS

Sequence retrieval

ParF is a partitioning protein in plasmid pOLA52 isolated from of *Escherichia coli* [10]. NCBI Reference sequence is YP_001693223.1 (http://www.ncbi.nlm.nih.gov/).

Physiochemical properties

These include the molecular weight, amino acid composition, theoretical isoelectric point (pI), extinction coefficient [11], instability index [12], aliphatic index [13], and grand average hydropathy (GRAVY) [14]. All were computed using the ProtParam tool of ExPAsy server (http://web.expasy.org/protparam/) [15].

Secondary structure determination

The secondary structure of the protein was predicted by PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/) [16].

Homology modeling and refinement

Protein tertiary structure was determined by PHYRE2 (Protein Homology/anoloY Recognition Engine version 2) (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) [17]. The generated structure was refined by GalaxyRefine server (http://galaxy.seoklab.org/) [18].

Evaluation of the 3D structure

The refined models were evaluated by several tools to select the best model and to assess the quality of that model. Ramachandran plot obtained from RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) [19]. ERRAT is a protein's structure algorithm for evaluating the model building (http://services.mbi.ucla.edu/ERRAT/). ERRAT detects incorrect regions in the 3D structure on the basis of heavy atomic-pair distributions (CC, CN, CO, NN, NO, OO) in the amino acid residues [20]. The Z-score measures the deviation of the model in respect to an energy distribution derived from random experimental structures. The Z-score was determined by PROSA web tool (https://prosa.services.came.sbg.ac.at/prosa.php) [21].QMEAN6 in SWISS-MODEL workspace server (http://swissmodel.expasy.org/workspace/) [4]. The QMEAN6 server (Qualitative Model Energy Analysis) estimates the quality of the models by six descriptors. The raw score of QMEAN6 should lie between 0-1 [22]. These six descriptors are a) solvation potential which estimates the residue burial; b) torsion angle potential measures the local geometry of the protein; c) two distance-dependent potentials based on β-atoms and all atoms to evaluate atomic interactions; and d) two terms describing the agreement between the predicted and calculated secondary structure and solvent accessibility [22].

Submission of the model

The best model was submitted into the protein model database (PMDB) (http://bioinformatics.cineca.it/PMDB) [23].

Active site Determination

Binding site was predicted using Computed Atlas of Surface Topography of protein server (http://sts.bioe.uic.edu/castp) [24] and Active Site Predictor (http://www.scfbio-iitd.res.in/) [25].

Molecular Docking

The compounds used in screening for ParF inhibition were obtained either from Drug bank (http://www.drugbank.ca) [28] or ZINC databases (http://zinc.docking.org/) [27]. Rigid protein-ligand docking was carried out using Hex 8.0.0. Hex 8.0.0 uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations [28, 29]. The settings were: Grid dimension = 0.6, docking solutions = 500, an initial Steric Scan at N = 18, followed by a Final Search at N = 25, receptor and ligand range 180 degrees. AutoDock 4.2.6 [30] was also used to dock ligands using a Grid of 60×60×60 A° and box center -4.0×30.0×54.0 for x, y, z respectively.

RESULTS AND DISCUSSION

Primary and Secondary properties

ParF belongs to the ParA family of Walker-type ATPases which is related to the hydrolases of SIMIBI superfamily of ATPases and GTPases [31]. This molecule consists of 206 amino acids with a molecular weight of 220563 Daltons and pI of 5.61 hence this protein is acidic. The instability index of the protein is computed to be 27.27. This classifies the protein as stable [12]. The aliphatic index is 99.42 and the grand average of hydropathicity (GRAVY) is 0.199. It contains 24 (11.65%) negatively charged amino acids and 23 (11.16%) positive amino acids. Alanine is the most abundant amino acid 24 (11.65%). There is no cysteine in the protein since intracellular proteins have lower number of cysteine residues but higher number of aliphatic and charged amino acids [32]. The intracellular proteins have higher content of the negative charged amino acids than extracellular proteins [33]. These extracellular proteins contain more disulphide bridges and cysteine residues [34].

PSIPRED secondary prediction server predicted that ParF has eight α-helices and six β-strands (Fig. 1). The α-helices are α1 (15-28), α2 (43-50), α3 (65-72), α4 (89-97), α5 (109-126), α6 (144-156), α7 (169-177) and α8 (189-205). The β-strands are β1 (2-7), β2 (34-38), β3 (57-59), β4 (78-81), β5 (100-103) and β6 (133-137).

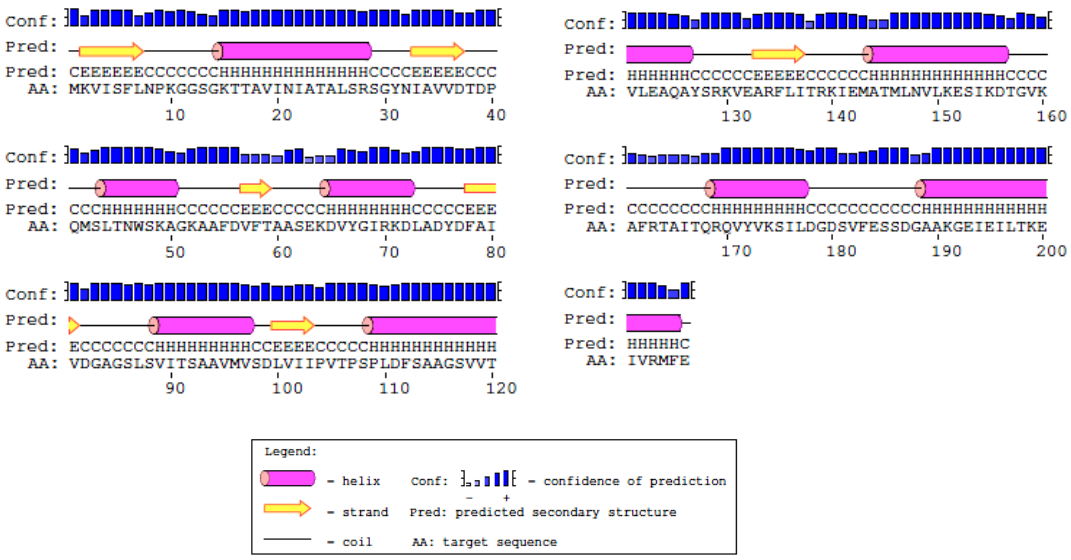

Figure1: Secondary structure of ParF predicted by PSIPRED, eight α-helices and six β-stands

Homology modeling, models evaluation, active site prediction

The 3D model of ParF was built by PHYRE2 server. This server uses powerful loop modeling techniques to model insertions and deletions. An *ab initio* folding process is integrated to model regions that do not have a homology with known protein structures [17]. In addition GalaxyRefine server can also detect unreliable regions and perform *ab initio* modeling process to improve the quality of the model as demonstrated by CASP9 (9th critical assessment of techniques for protein structure prediction) [18]. Five refined models were generated (Table 1).

Table 1: The refined models produced by GalaxyRefine with their scores

Model	Quality by ERRAT (%)	QMEAN6 score	Ramachandran Plot by RAMPAGE		
			FA[a]	AR[b]	DR[c]
Model 1	95.67	0.725	201(98.5%)	3(1.5%)	0(0%)
Model 2	98.98	0.720	201(98.5%)	3(1.5%)	0(0%)
Model 3	98.98	0.705	201(98.5%)	3(1.5%)	0(0%)
Model 4	95.43	0.698	202(99%)	2(1%)	0(0%)
Model 5	99.49	0.729	202(99%)	2(1%)	0(0%)

[a]Number of residues in favored region (%), [b]Number of residues in allowed region (%), [c]Number of residues in disallowed region (%).

Model 5 (Fig. 2) had the best quality as indicated by ERRAT, QMEAN6 and RAMPAGE validation tools. ERRAT analysis (Fig. 3) shows that Model 5 has the best overall quality (99.49%). The generated Ramachandran plot [35] by RAMPAGE indicates that the models are of the best stereochemistry where no residues lie in the outlier region. The Models 4 and 5 have 99% of the residues in the favored regions and only 1% in the allowed region where 98% and 2% expected in good models respectively in standard configurations (Fig. 4). ProSA web tool is used to calculate Z-score. The Z-score of Model 5 is (-6.43), lies within the range characteristic of native proteins (Fig. 5). This model was submitted successfully into the Protein Model Database with PMDB ID: PM0079891.

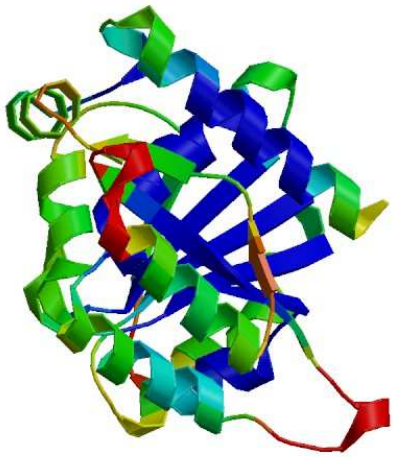

Figure 2: Three dimensional structure of ParF Model 5 produced by PHYRE2 and refined by GalaxyRefine server

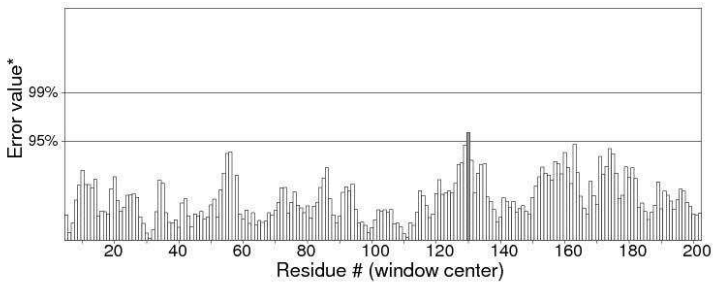

Figure 3: ERRAT result of ParF, Model 5 On the error axis two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value

Computed Atlas of Surface Topography of proteins (CASTp) provides an online resource for locating and measuring concave surface regions of proteins [24]. The result indicated 30 pockets of which the largest pocket had an area of 195 $A^{\circ 2}$ and a volume of 186 $A^{\circ 3}$ and formed by the residues: G^{12}, S^{13}, G^{14}, T^{17}, A^{18}, N^{21}, P^{104}, T^{106}, L^{136}, T^{138}, I^{166}, T^{167}, Q^{168} and Y^{172} while the Site Prediction Sever predicted 14 cavities of which the largest had a volume of 455 $A^{\circ 3}$ and consists of the amino acid residues: P^{9}, K^{10}, G^{11}, G^{12}, S^{13}, G^{14}, K^{15}, T^{16},T^{17}, A^{18}, Q^{41}, S^{43}, G^{85}, P^{104}, V^{105}, T^{106}, P^{107}, S^{108}, P^{109}, L^{110}, D^{111}, F^{112}, A^{114}, F^{135}, L^{136}, T^{138}, R^{139}, K^{140}, I^{141}, M^{146}, L^{147}, I^{166},T^{167}, Q^{168}, R^{169}, Q^{170}, Y^{172}, Q^{173} and I^{176}. Schumacher *et al*. [36] proposed a binding pocket for ADP formed by the residues 9-16, 37-49

and 166-177. The active sites predicted share common residues; G^{12}, S^{13}, G^{14}, T^{17}, A^{18}, I^{166}, T^{167}, Q^{168} and Y^{172} suggesting that these amino acid residues are essential **constituents of the active site.**

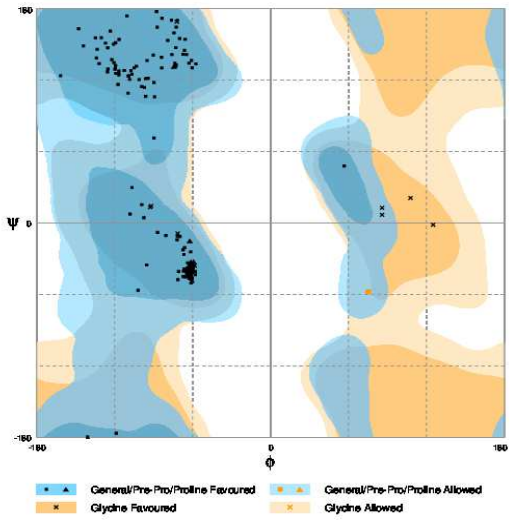

Figure 4: Ramachandran plot of the predicted ParF, Model 5 using RAMPAGE

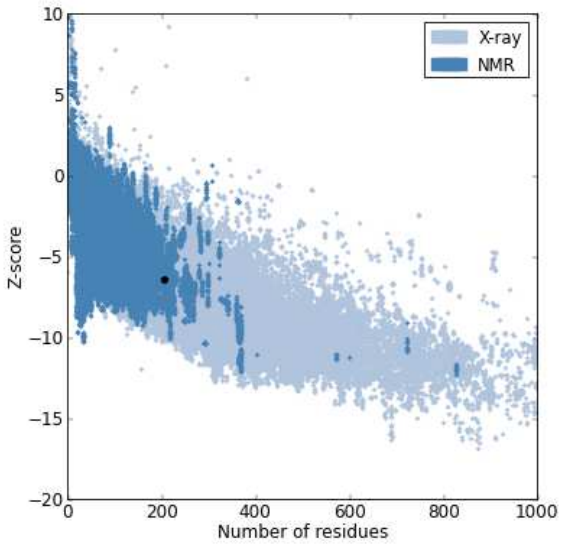

Figure 5: Z-score of ParF, Model 5 (black dot) computed by ProSA web tool compared with Z-scores of the experimentally determined proteins by NMR spectroscopy and X-ray crystallography

Docking studies

Mainly flavonoids, 120 compounds were screened via docking against ParF, Model 5 in Hex 8.0.0 using ATP as control. Table 2 shows the compounds having total energy of binding lower than ATP. These compounds were further docked by AutoDock 4.2.6 (Table 3). Their physiological characteristics are presented in Table 4 where Lipinski rule of five states that a candidate drug to be absorbed efficiently should have a molecular weight less than 500 Daltons, less than 5 hydrogen bonds as donors and 10 hydrogen bond acceptors and log P less than 5 [37].

Table 2: Results of docking (in total energy of binding) by Hex 8.0.0

Compound	Data base ID	Total energy (Kcal/mol)
ATP	DB00171	-297.36
Phenoxybenzamine	DB00925	-326.80
Verapamil	Z03871832	-319.32
Chlorpromazine	DB00477	-301.42
Octoclothepin	Z19362651	-300.48
Rutin	Z59764511	-378.46
Hinokiflavone	Z04098521	-337.55
Amentoflavone	Z03984030	-337.13
Silybin	Z02033589	-326.90
Scutellarin	Z21992916	-298.71
Vicenin	Z98369451	-298.61

Phenoxybenzamine, verapamil, chloropromazine and octoclothepin docking results were higher than ATP. Nisa *et al*. [38] targeted ParA, a chromosome partition protein to identify drugs acting against tuberculosis. Phenoxybenzamine inhibited 50% of the ATPase activity while the antipsychotic octoclothepin inhibited 20% of ATPase activity of ParA.

Being a proton pump inhibitor, Spenlger [39] used a combination of verapamil (5µg/mL) and the antiplasmid compound, trifluoperazine (concentration 50µg/mL) which had a marked increase in plasmid curing ratio from 6.76% to 25.6% of tetracycline resistance of *E. coli* K12 LE 140.The subinhibitory concentrations of chlorpromazine, thioridazine, promethiazine, trimeprazine and acridine orange eliminated plasmids from *E. coli* K12 LE140 strain in the percentage of 29%, 34%, 25%, 22% and 20% respectively [40]. Phenothiazines may inhibit the generation of hydronium ions from ATP hydrolysis by ATP synthase activity. This will affect the efflux pump mediated resistance to antibiotic [41].

In Hex docking the flavonoids; rutin, amentoflavone, hinokiflavone and silybin were superior to phenoxybenzamine, verapamil and the antipsychotics chloropromazine and octoclothepin. Fig. 6 shows the ligand rutin in the binding site. Despite the violations in Lipinski rule of five, rutin is the highest to all other compounds tested by Hex 8.0.0 and AutoDock 4.2.6. Rutin is a glycoside of quercetin found in tea, onions, fruits and berries [42]. Rutin possess antioxidant activity and potentiates glutathione peroxidase and reductase enzymes [43].

Table 3: Results of docking by AutoDock4

Compound	Binding energy (Kcal/mol)	Intermolec-ular energy (Kcal/mol)	Internal energy (Kcal/mol)	Docking energy (Kcal/mol)	Inhibition constant (µm)
Phenoxybenzamine	-3.93	-6.31	-1.67	-7.98	1.02
Verapamil	-2.08	-6.26	-1.26	-7.52	29.81
Octoclothepin	-6.58	-6.88	-0.43	-7.31	15.02
Chlorpromazine	-5.46	-6.65	-0.45	-7.10	99.51
Rutin	-2.81	-7.58	-11.24	-18.82	8.71
Vicenin	-3.58	-8.35	-6.10	-14.45	2.38
Amentoflavone	-3.80	-6.49	-5.12	-11.61	1.63
Scutellarin	-3.23	-6.22	-4.46	-10.68	4.26
Silybin	-4.08	-6.76	-3.43	-10.19	1.03
Hinokiflavone	-3.70	-6.39	-3.75	-10.14	1.93

Amentoflavone and hinokiflavone initially extracted from *Selaginella* spp., but amentoflavone is found in variety of plants e.g. *Ginko biloba* and *Hypericum perforatum* [44, 45]. Amentoflavone has been shown to possess antimicrobial effect against *Staphylococcus aureus*, *E. coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa.* Their MICs ranged between 8-100 microg/mL except for *P. aeuroginsa* whom MIC was higher than100 microg/mL [46]. Carbonezi *et al*. [47] found that four biflavonoids that were isolated from *Ouratea multiflora*; heveaflavone, amentoflavone-7″,4‴-dimethyl ether, podocarpusflavone-A and amentoflavone had antimicrobial activity against *S. aureus* and *Bacillus subtilis.*

All the flavonoids tested by Autodock 4.2.6 showed docking energy lower than the previously used drugs in this study. Vicenin followed rutin in this respect. Vicenin has a high hydroxyl radical elimination activity *in vitro* [48].Vicenin is extracted from *Ocimum sacntum* (the Indian Holy Basil) is used as antidiabetic, antibacterial and

analgesic [49]. Silybin extracted from the seeds of *Silybum marianum* interacts with multidrug resistance-associated protein 1 (MRP1). Silybin derivatives were found to be potent inhibitors of the NorA MDR efflux pump in *S. aureus* [50, 51].Scutellarin (4,5,6-trihydroxyflavone-7-glucuronide) extracted from *Erigeron breviscapus* and is used in China for treatment of cerebrovascular diseases due to antioxidant activity [52, 53].

CONCLUSION

The results suggest that the ATPase activity of plasmid partition proteins may be one of the targets for anti-plasmids. ATPase activity is essential for the function of partition proteins to direct the segregation of plasmids and interruption of such activity may cause plasmid loss. These flavonoids could be used as anti-plasmid agents since they have higher docking scores.

Table 4: Physiological Properties of the compounds

Compounds	Weight (g/mol)	Log P	H-bond donors	H-bond acceptors	Rotatable bonds
Phenoxybenzamine	303.63	4.26	0	2	8
Verapamil	455.62	4.55	1	6	13
Chlorpromazine	318.86	5.18	0	2	4
Octoclothepin	345.92	4.38	1	2	1
Rutin	610.52	-1.06	10	16	6
Hinokiflavone	538.46	5.18	5	10	4
Amentoflavone	538.46	5.16	6	10	3
Silybin	482.44	1.47	5	10	4
Scutellarin	461.36	0.07	6	12	4
Vicenin	594.52	-2.10	11	15	5

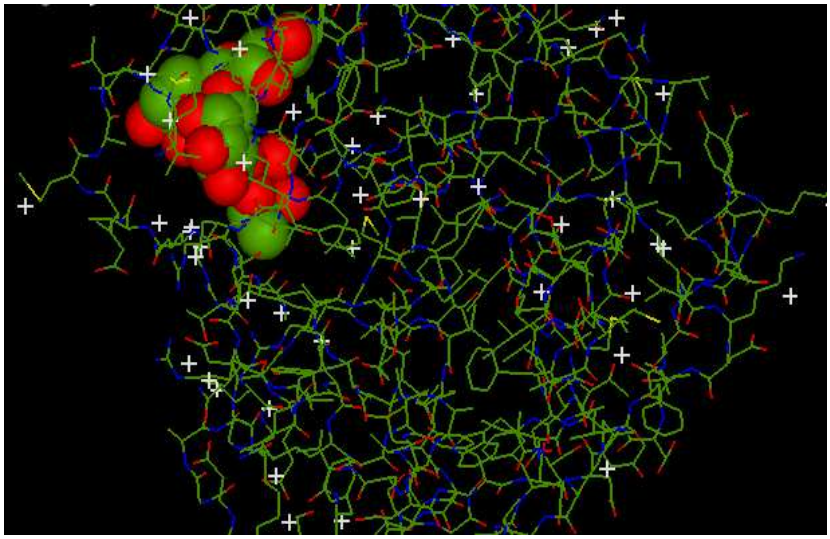

Figure 6: Docking of ParF, Model 5 with Rutin which appear colored spheres at the binding side of the wired-configuration molecule of ParF

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