



## Original Research Article

# Molecular docking approach towards targeting Leucine-rich alpha-2-glycoprotein (LRG1) through Phytochemicals to prevent bad angiogenesis of cancer

Rohit pritam Das<sup>1\*</sup> and Manaswini Jagadeb<sup>2</sup>

BIF Centre, Department of Bioinformatics, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India

\*Corresponding author

## ABSTRACT

### Keywords

Angiogenesis,  
Autodock,  
ERRATE2,  
LRG1,  
Phytochemicals,  
Interacting  
residues,  
H-bond

Among all deadliest disease cancer retain the most challenging factor for scientist and researchers. Out of all major causes one of the major causes of cancer is the formation and development of new bad blood vessels i.e. also known as bad angiogenesis. In this process or mechanism, some factors are responsible. Among many proteins, LRG1 (*Leucine-rich alpha-2-glycoprotein*) plays an important role to create bad blood vessels. So that targeting to this protein may be more effect towards treatment of cancer. The phytochemicals having anticancer property were docked with the protein to find out our result.

## Introduction

Angiogenesis is a physiological process which helps in new blood vessel devolvement and growth. It takes part in many major activity of human body like embryo formation, new blood vessel formation and wound healing etc. But in case of bad angiogenesis it may leads to pathogenic activity causes cancer or many inflammatory diseases.

The term bad angiogenesis describes the pathological behavior of blood vessels.

## Effect of pathological angiogenesis

Cancer is a deadliest disease spreading all over the world. The abnormal growth of certain cell leads to cancer. The tumor cells grow up to a certain stage approximately 1-2 mm<sup>3</sup>, then after a sufficient nutrition and blood supply required for the growth of those cells [1].

The tumor cells provide some angiogenesis signals to the endothelial cell passed near by

the tumor cell. Some receptor present in endothelial cell surface adopt the signal arrived from tumor cell. After receiving the signal a new blood supply is formed towards the tumor cells this phenomenon called as bad angiogenesis otherwise pathological angiogenesis.

### **Phytochemicals**

Phytochemicals are the chemical constituent of plant naturally present in plant. These chemical constituent also have some disease preventive capacity. Now a day's phytochemicals are playing an massive role towards disease prevention. There are many synthetic drugs are in use but these drugs have shown some side effects. So to avoid this, here in our study we used phytochemicals as our drug product. We have taken some phytochemicals which is having anticancer property.

In another study report it was clearly identify that genistein and curcumin has also some therapeutic target effect in diabetes [10]

### **Phytochemical properties**

Phytochemicals have some properties like mention bellow.

**Antioxidant properties:-** some phytochemicals have antioxidant properties which prevent us from oxidative risk , and also help to reduces the chances of growing cancer in our body.

**Anti bacterial and Anti cancer properties:-**phytochemical released from garlic shows the properties of anti bacterial activity. Also have some anti cancer property [2] phytochemicals like allicin (garlic) Showing the anticancer property also help full in anti angiogenesis process [3].

### **Related work**

As it is well known that angiogenesis is responsible for many important biological activity in our body like development, reproduction, wound healing, new blood vessel growth etc. however angiogenesis also helps in harm full angiogenesis where new blood formation may lead to tumor growth that leads to cancer. Tumor growth arrest after a certain stage then tumor cells are self capable of promote angiogenesis by producing several angiogenic factors, include vascular endothelial growth factor. The researcher discovered a protein named as LRG1 in mouse retinal model. Researcher found that LRG1 promote bad angiogenesis in abnormal retinal blood vessel disease. In their study report they believed that the inhibition of LRG1 is a therapeutic effect in future cancer treatment[4]. They also suggested that LRG1 play a vital role in bad angiogenesis rather than it has some less effective role in natural angiogenesis [4]. This information about LRG1 makes the protein an especially valid therapeutic target. On the other side Phytochemicals also play the same crucial role for disease target on the basis of their richness in diet [5]. Scientist now research about the effect of phytochemicals due to it's anticancer properties[6][7]. In the current study authors put their effort on targeting such dared full disease like Angiogenesis by taking Phytochemicals as a therapeutic target on LRG1.

### **Materials and Method**

#### **Structure prediction and structure validation**

The primary sequence of LRG1 (entry name, A2GL\_HUMAN) was taken from SWISS-PROT/UniProt KB (ID, P02750). The secondary structure of LRG1 was

predicted by using SOPMA server ([https://npsa-prabi.ibcp.fr/cgi-bin/secpred\\_sopma.pl](https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl)). The structure has shown that 42.94% is occupied by alpha helix. Tertiary structure prediction was formed but Phyre2 server (<http://www.sbg.bio.ic.ac.uk/phyre2/>) with 100% confidence. After the tertiary structure was performed, the predicted model was gone through energy minimizing process by ModRefiner (<http://zhanglab.ccmb.umich.edu/ModRefiner/>). Then it was validated by Errat2 of the Saves web server and Rampage (<http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>). 3D structure quality was verified by ProSA (<http://prosa.services.came.sbg.ac.at/prosa.php>)

### **Molecular docking of LRG1 model with phytochemicals**

After validation process molecular docking between energy minimized predicted structure of LRG1 and phytochemicals selected on the basis of their involvement effect upon various cancer [8][9] was accomplished using the AutoDock-4.2 algorithm (<http://autodock.scripps.edu/>). For doing AutoDock, we were gone through different protocols like Kollman charges, docking parameters, and hydrogens atom were added to polar region into the LRG1

PDB files. The structures of phytochemicals were taken from Pubchem (<http://ncbi.nlm.nih.gov/pccompound>) database of the National Center of Biotechnology information (NCBI). The phytochemicals structures were downloaded in SDF format, converted into PDB format by using PyMol software. A grid was set by covering the whole protein as it allows the ligands to docked with each amino acid present in protein. For each ligand, one hundred positions run were performed. The ligands/phytochemicals selected for the current study are:-  $\beta$ -carotene, anthocyanins, genistein, ellagic acid, limonene, allicin, curcumin.

The primary sequence of LRG1 was collected from UniProt (PO2750). It has observed that the protein is 347 amino acid in length and extracellular in location. Secondary structure were predicted by SOPMA SERVER [Fig 2]The tertiary structure was predicted by using Phyre2 with 100% confidence[Fig 3]. The energy minimization was done by using ModRefiner. The RMSD was found as 0.291 [Fig 4]which gives an impression of a better result. Then the predicted structure was validated with the help of Ramachandran plot [Fig 5].

**Table.1** Molecular Docking analysis of phytochemicals with LRG1 protein

Sl no.	Drugs name	Docked energy	KI value	Inter molecular energy
1	Beta-carotene	-8.28	854.01uM	-11.26
2	Anthocyanins	-7.92	1.57uM	-8.22
3	Genistein	-8.06	1.23uM	-9.25
4	Elargic acid	-7.52	3.08uM	-8.71
5	Limonene	-6.27	25.31uM	-6.57
6	Allicin	-5.11	179.15uM	-6.6
7	Curcumin	-10.0	46.6nM	-12.99

**Table.2** Interacting residues with H-bond with bond length and bond energy

Interacting residues	H-bond residue	Bond length	Bond Energy
TYR65, GLY64, LEU66, PRO67, ALA68, PRO63, ILE62, LEU87, PRO84, GLU61, HIS82, THR81, LEU80	-	-	-
LEU131, PRO133, PRO132, LUE131, PHE136, GLN137, TRP159, HIS161, GLY162	LEU131(O..H)	2.885	-
PRO133, GLY134, PRO132, LEU131, PHE136, GLU156, VAL154, TRP159, HIS169	GLU156(O..H)	2.161	-3.689
PRO133, LUE131, PHE136, TRP159, HIS161, SER158, VAL157, GLU156	GLU156(H..O)	1.853	-3.36
PRO133, GLY134, PRO132, LEU131, PHE136, TRP159, GLY162, HIS161	-	-	-
PHE136, PRO133, LEU131, GLY134, LEU135, PHE136, GLY162, TRP159, HIS161, LEU160	PHE136(O..H)	2.076	-5.874
PRO133, GLY130, THR129, LEU131, PRO132, GLY134, LEU135, PHE136, TRP159, HIS161, VAL157, GLU156, VAL154, GLU153	LEU131(H..O) GLY134(O..H) PHE136(O..H) GLU153(O..H)	2.184 2.09 1.847 1.911	-1.575 -5.036 -2.652 -6.904

### Structure prediction and validation of LRG1 protein model

SOPMA result for : P02750

10            20            30            40            50            60            70

|            |            |            |            |            |

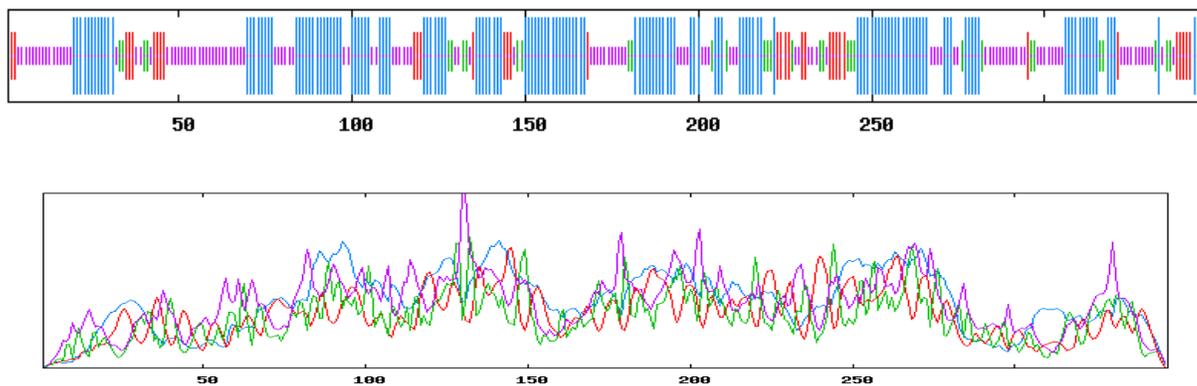
MSSWSRQRPKSPGGI QPHVSR TLFLL LLLAASAWGV T LSPKDCQVFRSDHGSS I SCQPPAE I PGYLPADT  
hceccccccccccccccccchhhhhhhhhhhhhctteeccttceeecccccccccccccccccccccccccccc  
VHLAVEFFNLTHLPANLLQGASKLQELHLS SNGLESLSPEFLRPVPQLRVLDLTRNALTGLPPGLFQASA  
hhhhhhhhccccchhhhhhhhhhhhhhhcchhhhhhhcchhhhccccceehhhhhhhhhhhhhhhhhhhhhhh  
TLDTLV LKENQLEVLEVSWLHGLKALGHL DLSGNRLRKLPPGLLANFTLLR TLDLGENQLETLPD L LRG  
hhhhheectthhh  
PLQLERLHLEGNKLQVLGKDLLLPQPDRLYLFLNGNKLARVAAGAFQGLRQLDMLDLSNNSLASVPEGLW  
cchhh  
ASLGQPNWDMRDGF D I SGNPWI CDQNLS DLYRWLQAQKDKMFSQNDTRCAGPEAVKGQTLLAVAKSQ  
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hhtccccccccccccccettccccccccchhh

Sequence length : 347

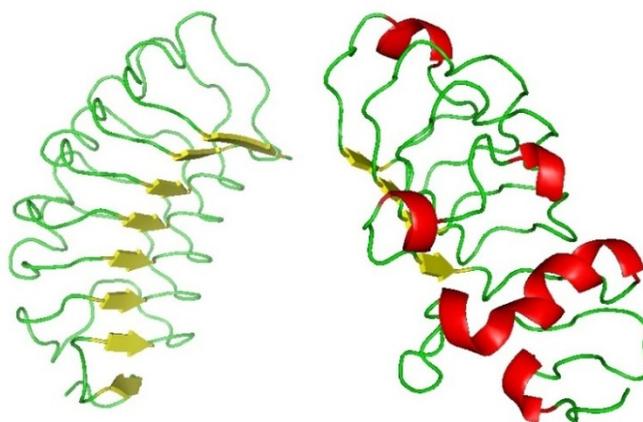
SOPMA :

Alpha helix	(Hh)	:	149	is	42.94%
3 <sub>10</sub> helix	(Gg)	:	0	is	0.00%
Pi helix	(Ii)	:	0	is	0.00%
Beta bridge	(Bb)	:	0	is	0.00%
Extended strand	(Ee)	:	35	is	10.09%
Beta turn	(Tt)	:	32	is	9.22%
Bend region	(Ss)	:	0	is	0.00%
Random coil	(Cc)	:	131	is	37.75%
Ambiguous states (?)		:	0	is	0.00%
Other states		:	0	is	0.00%

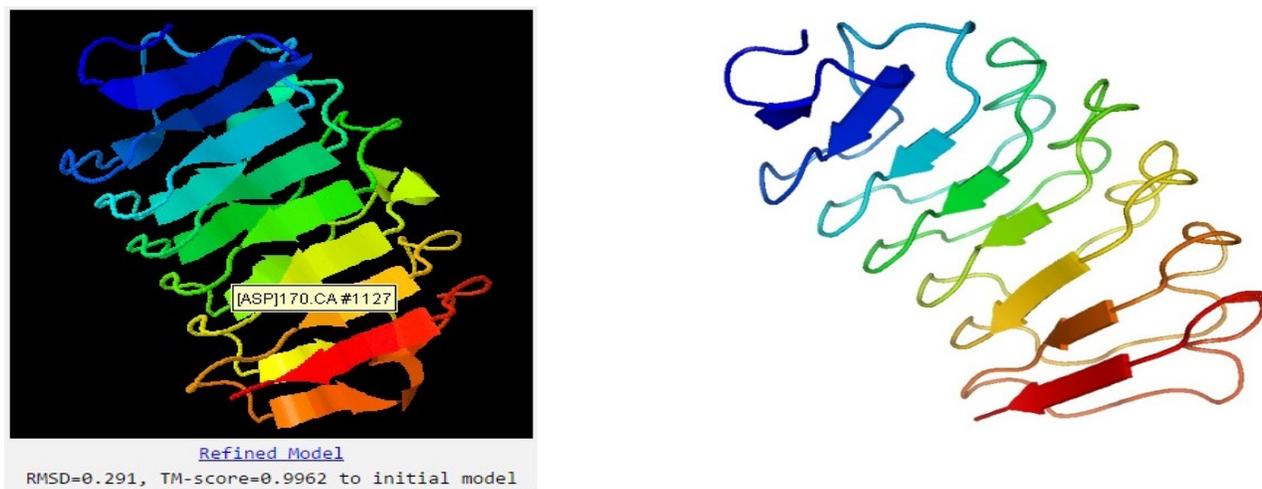
**Fig.2** Secondary structure prediction by SOPMA



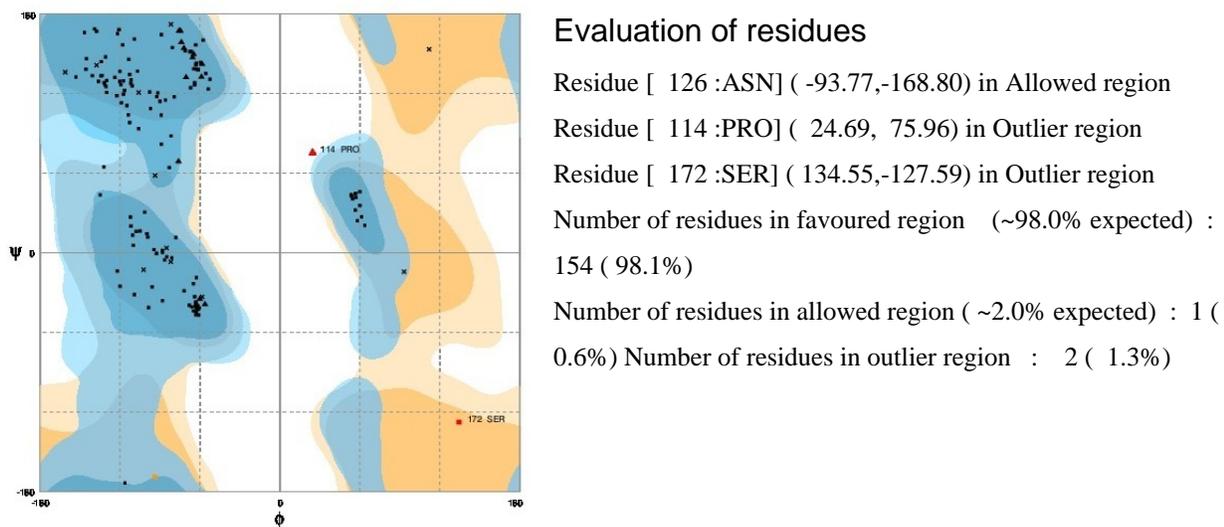
**Fig.3** Predict tertiary structure of LRG1 protein



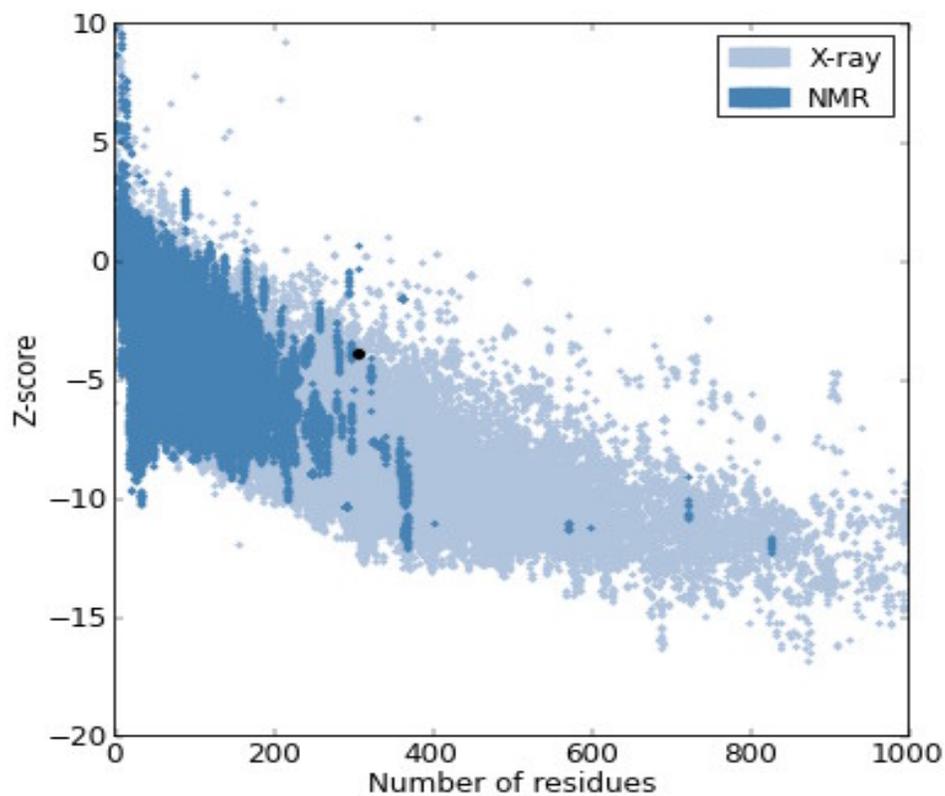
**Fig.4** Refined(energy minimized) structure of LRG1 produced by ModRefiner



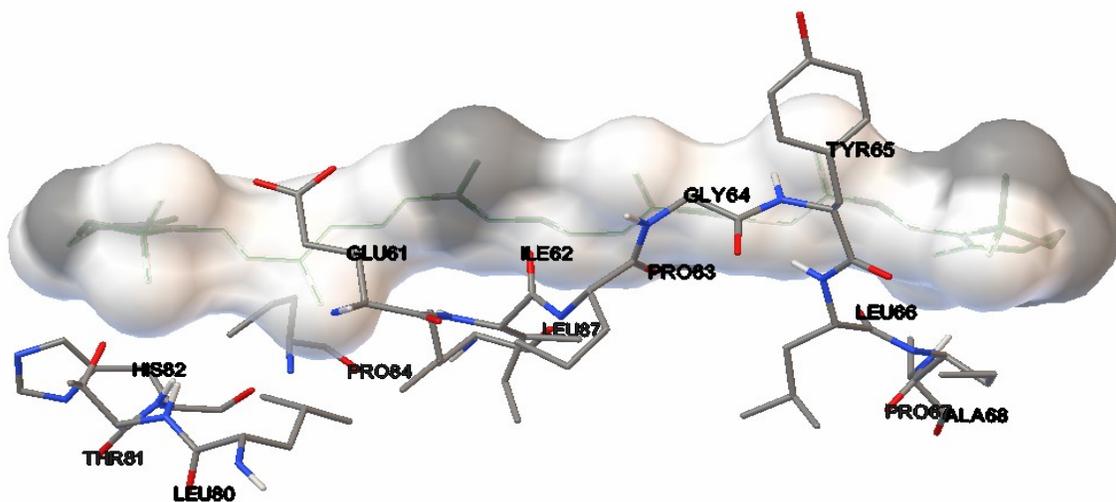
**Fig.5** Tertiary structure validation by Ram page



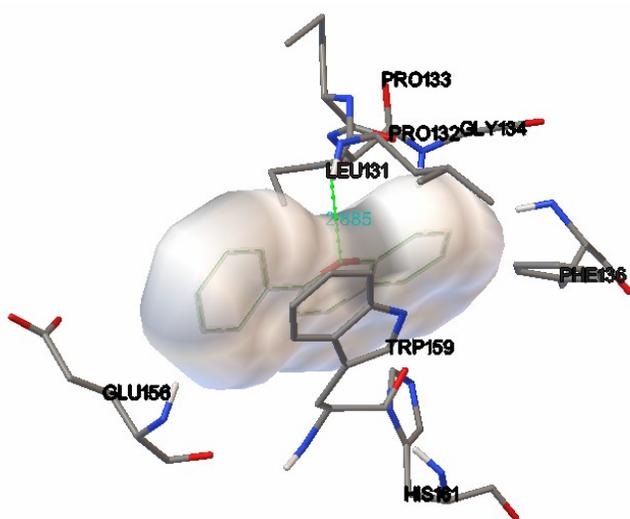
**Fig.6** Validation of predicted structure by ERRAT2



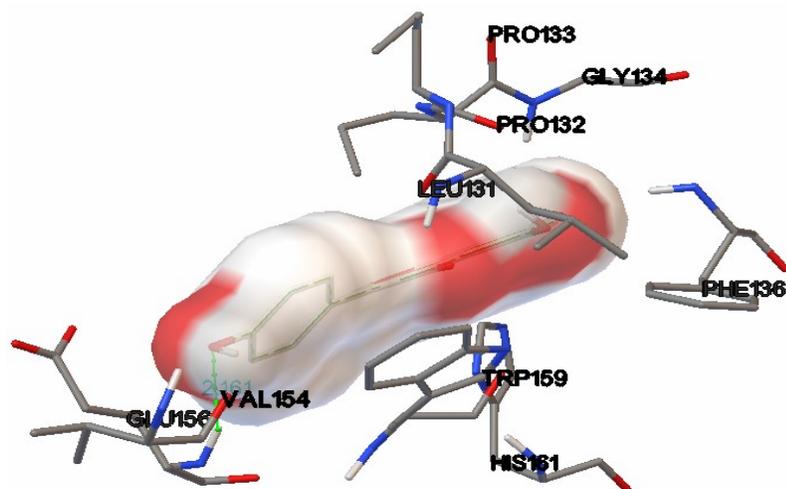
**Fig.8** Showing the close interacting residues and H-Bond of phytochemicals (solid surface) with LRG1 (a) beta-carotene (b) anthocyanins (c) genistein (d) ellagic acid (e) limonene (f) allicin (g) curcumin



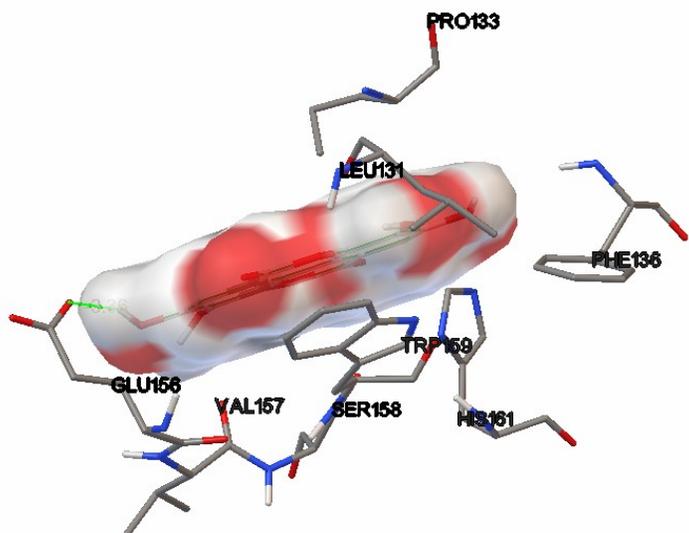
(a)



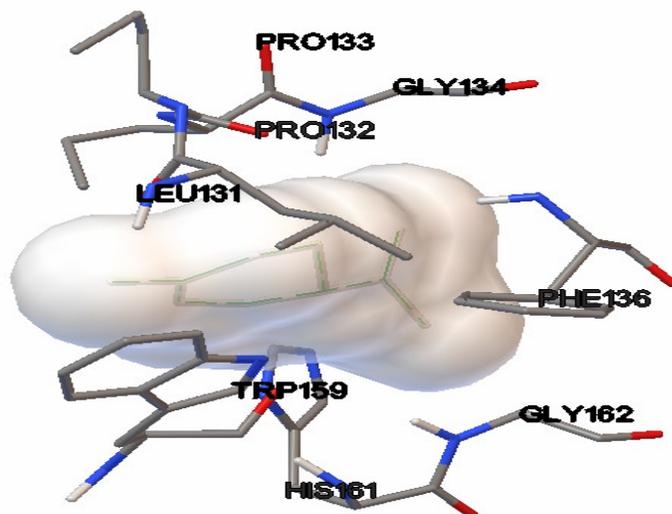
(b)



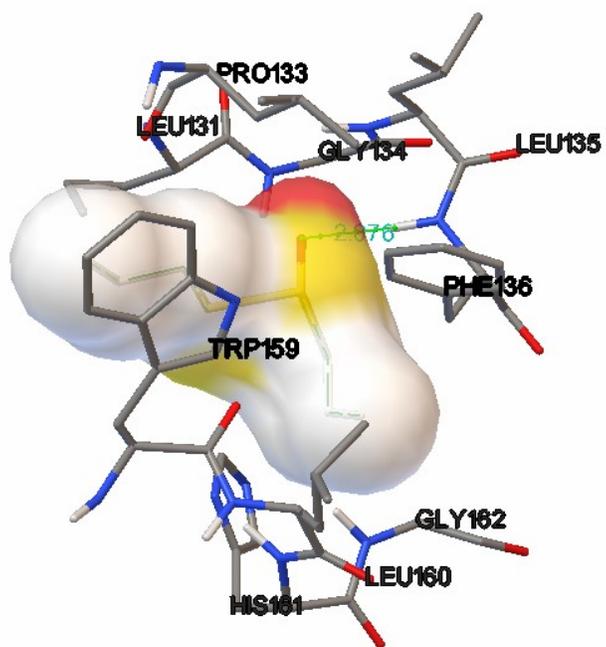
(c)



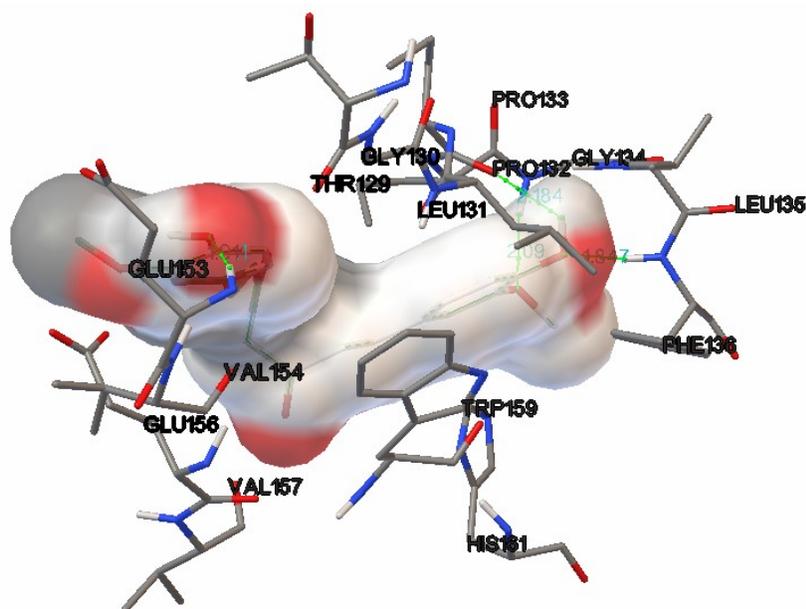
(d)



(e)



(f)



(g)

This statistics show 98.0% residues in allowed region which proves it as better quality. The non bonded interaction between different atoms were plotted in ERRAT [Fig 6] to calculate the overall quality factor of LRG1. The quality factor of our given model id 51.839. It was justified the improvement in quality of LRG1 protein. The structural model of LRG1 is publically available in the Protein Model DataBase (PMID ID, PM0079937). For statically score i.e Z-score for LRG1 predicted by ProSA is -3.97[Fig 7][11].

### **Autodock analysis of LRG1 with Phytochemicals**

Here for the study of molecular docking several phytochemicals were taken on the priority to inhibit the disease protein.

The modeled tertiary structure, after being energy minimized was taken for Autodock analysis. It was done with all 7 phytochemicals.[fig 8] The protein was prepared by adding hydrogen and kollman charges and with a parameter of 100 runs. It was identified that all the phytochemicals have better binding affinity and KI value with a high H-bond energy. But usually curcumin, Genestein and  $\beta$ -carotinoid were shown a high docked energy and intermolecular energy [table1]. The H-bond shows a strong interaction with phytochemicals as all were found within 3  $\text{A}^0$ . There are so many amino acids were found near phytochemicals interaction.[table 2]

LRG1 is identified as a better target to cure cancer as it helps in production of bad blood vessels. The 100% confidence of predicted structure and its validation may helpful to predict new drugs against it. The energy minimization which provides stability helping to perform better docking score with

phytochemicals. Overall, all phytochemicals are showing a very good docked energy but among them mostly Curcumin(-12.99), Beta-carotene(-11.26), Genistein (-9.25) have proved them as better. All the H-bond length and energy shows a close and strong interaction with the ligands/ phytochemicals. So the predicted, refined and validated structure of LRG1 along with these information about docked energy, KI value, internal energy, H-bond, interacting residues, H-bond length, H-bond energy etc with these phytochemicals can give a better knowledge to create a novel drug to win over cancer.

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