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In Silico Characterization of 14 – 3 – 3 Protein Identified In Peanut (Arachis Hypogaea L.) Under Drought Stress

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Abstract- ***Peanut, an important oil and food crop frequently encounter drought stress which limits its productivity. Of the many proteins synthesized in response to drought, 14-3-3 proteins are highly conserved regulatory proteins and involved in many biological processes. In the present investigation, peptides of 14-3-3 protein isolated and sequenced from ICGV 91114 peanut cultivar were employed. The physico-chemical and secondary structural properties indicated this protein as hydrophilic, soluble and stable. Since 3D structure of peanut 14-3-3 protein is not available in public domain to elucidate its regulatory role, the present investigation was initiated to build a homology model, using 2o98 protein of tobacco as a template and validated through Ramachandran plot. A hypothesis was built on the role of peanut 14-3-3 protein in regulating 3 other drought tolerant proteins in silico; Late Embryogenesis Abundant protein-1, Ascorbate peroxidase-1 and Calcium ion binding protein, by identifying protein binding sites, validating and molecular docking. The results indicated its maximum interaction with calcium binding protein indicating its probable role in signaling other proteins in silico during drought stress.***

Keywords ***Peanut, 14-3-3 Protein, Multiple Sequence Alignment, Homology Modeling, Ramachandran plot, Molecular Docking***

I. INTRODUCTION

Peanut plays an important role both as an oil and food crop, especially in the developing countries, due to high amounts of edible oil (36–55%) and protein (25–32%) content [1]. The productivity levels of this crop are lower due to a number of abiotic and biotic factors affecting almost all plant functions. Among various abiotic stresses, drought is the most important limiting factor at different stages of growth resulting in drastic reduction in its yield and quality [2]. Under drought stress, a large set of genes get activated transcriptionally leading to accumulation of new proteins conferring tolerance to drought stress [3, 4]. One of the major molecular responses to drought stress is the altered gene expression related to different pathways, up-regulating expression or by synthesizing novel proteins [5, 6]. Drought tolerance is a complex trait where several characteristics influence the plant growth and survival during the period of its life cycle [7].

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It is achieved by modulation of gene expression and accumulation of specific protective proteins and metabolites [8]. The regulatory and non-regulatory proteins play a significant role in coping with the oxidative stress and cellular abnormalities [9]. Among various proteins activated, 14-3-3 proteins are a group of highly conserved regulatory proteins found in eukaryotic cells, including several plant species [10]. They function as homo - / hetero - dimers and each monomer can bind to an interacting protein [11, 12]. More than one hundred proteins have been identified to be interacting partners with 14-3-3 protein and undergo changes in their activities or sub - cellular localization or mediate the formation of protein complexes [13, 14, 15]. In plants, 14-3-3 proteins were found to regulate enzymes involved in primary metabolism, ion transport, cellular and vesicle trafficking, signal transduction, chromatin function, gene expression and other 'housekeeping' functions [10, 16]. The 14-3-3 protein–protein interactions usually occur in response to signals transduced by protein kinases. These proteins themselves are not generally involved in signaling but their interactions with 14-3-3 proteins represent the ultimate step in signaling cascades. The 14-3-3 protein – protein interactions involve short amino acid motifs containing phosphor-serine or phosphor-threonine present in the conserved amphipathic grooves of the monomers of a dimeric 14-3-3 protein and the interactions are regulated by the phosphorylation status of one or two targets at the same time [17, 18]. Most of the 14-3-3 interactions possess two optimal binding motifs 'RSXpSXP' and 'RXY/FXpSXP' [18, 19]. The binding of 14 – 3 – 3 proteins with other proteins may directly alter protein activity or control nuclear-cytoplasmic shuttling or mediate protein import into mitochondria and chloroplasts, or form a scaffold to permit interactions between two different binding proteins [20]. In view of the above importance, the present study was undertaken to understand the regulatory role of peanut 14-3-3 protein during drought stress, using *in silico* approach. Hence, a 3-D homology model was constructed and docked with other drought tolerant proteins to study its interaction with other 3 proteins in conferring drought tolerance in peanut.

II. METHODOLOGY

A. Homology modeling and validation of 14-3-3 protein

The template structure of the 14-3-3 protein was generated using servers like 3D-PSSM (www.sbg.bio.ic.ac.uk/~3dpssm) and JPRED (www.compbio.dundbee.ac.uk).

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Of the many template structures generated by these servers, the best aligned structure showing 90 % sequence homology with PDB: 2o98 protein of tobacco plant (www. rcsb.org) was selected to construct a 3D model. 'Modeller9v10' software was used to design a homology model of 14-3-3 protein and the model with less geometric function (modeller objective function) was selected. The constructed 3D models were energy minimized in GROMACS force field using steepest descent minimization algorithms [21]. The overall stereo-chemical property of the 14-3-3 protein was assessed by Ramchandran plot [22]. The structural evaluation of the obtained model was performed by PROCHECK [23].

B. Molecular docking

The *Ah* 14-3-3 protein (modeled peanut 14-3-3 protein) was selected as a receptor protein and three other proteins with PDB IDs: (1xo8, 1APX and 1tiz) were considered as ligands. Protein binding/catalytic sites of *Ah* 14-3-3 protein and the three interacting proteins were identified using SYBYL-X (Tripos) software. The docking studies were performed using HEX software to find interactions between the *Ah* 14-3-3 protein with other three proteins. The interactions showing highest scores and docking energy were considered for protein-lignad complex structure. These results were analyzed and validated using PYMOL software.

C. Physico-chemical properties

The ProtParam server, in Expasy tools (http://us.expasy.org/tools/protparam.html) established by Swiss Institute of Bioinformatics (SIB) and European Bioinformatics Institute (EBI) was used to characterize the physico-chemical properties of 14-3-3 protein. Both Swiss-Prot and TrEMBL provide information related to the sequence, structure and function of a protein [24]. The physico-chemical properties include theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient [25], instability index [26], aliphatic index [27] and grand average hydropathy (GRAVY) [28]. CYS_REC was used to predict the disulphide- "SS" bonds in the protein sequence. SOPMA (Self Optimized Prediction Method with Alignment) is another programme used to predict the secondary structure of a protein which correctly predicts 69.5% of amino acids, whether a given amino acid lies in a helix, strand or coil [29].

III. RESULTS AND DISCUSSION

The 14-3-3 protein isolated from peanut cultivar, ICGV 91114 on MS/MS sequencing showed homology to the extent of 50% (155 amino acids of peanut matching to *Phaseolus angularis* (Q93XW1)) with a theoretical mass of 29.184 Da and pI value of 4.66, consisting of 259 amino acids. Protein identification was done based on matching peptide masses which require matching of at least 5 peptide masses for a confident identification (Fig. 1a & b).

A. Homology modeling

As there is no data available on three dimensional structure of peanut 14-3-3 protein, homology modeling was done to predict its 3-D structure based on the template structure deposited in PDB (http://www.rcsb.org). The query sequence from *Phaseolus* 14-3-3 protein was selected for homology based searching of the template structure against the structural database of PDB. The BLAST Sequences that displayed maximum identity with high score and low E value were considered for multiple sequence alignment. After performing the multiple sequence alignment, the sequence exhibiting high similarity and having the protein crystal structure were considered as the template structure for homology modeling of 14-3-3 protein. After performing the Jpred and 3D-PSSM, the protein sequences having the following values were obtained and these sequences were taken for the model construction. The protein having the PDB id 2o98a (a= A chain) showed 90% identity with sequence length of 231 with the query protein, the protein with PDB id 1qjba (a =A chain) showed 65% identity with sequence length of 228, the protein with PDB id 1s35a (A=chain) is showed 21% identity with sequence length of 211 and the protein having the PDB id 1eq1a (A chain) showed 21% identity with sequence length of 166. Out of these four template sequences, the protein showing more identity i.e., 90% with PDB id: 2o98 (14-3-3/H+ATPase plant complex protein of *Nicotianatabacum)* was selected as a template structure for comparative modeling as a high level of sequence identity promises more reliable alignment between the target sequence and the template structure. The initial model of 14-3-3 protein was built using Modeller 9v10 software which was based on an input sequence alignment between the target amino acid sequence to be modeled and a template protein with already known structure. The 'modeller9v10' software generated 40 models, among which the model with less geometric function (modeler objective function=1469.3707) was selected which is geometrically favorable (Fig. 2). Structural evaluation of the selected model was performed by PROCHECK. The model displayed 90% accuracy. The stereo chemical quality of the predicted models and accuracy of the protein model was evaluated after the refinement process using Ramachandran plot calculations computed with the PROCHECK program. The assessment of the predicted models generated by modeller was shown in Fig. 3. In the Ramachandran plot analysis, the residues were classified according to its regions in the quadrangle. The red regions in the graph indicate the most favored regions whereas the yellow regions represent additional allowed regions. Glycine is represented by triangles and other residues are represented by squares. The result revealed that the modeled structure has 90.0% residues in the most favored region (A, B, L), 9.6% in additional allowed regions (a, b, l, p), 0.5% in generously allowed region and 0.0% in disallowed region. The distribution of the main chain bond lengths and bond angles were found to be within the limits for these proteins. As most of the proteins were in the allowed region, the protein structure was accepted. The generated 3D structure of the 14-3-3 protein was optimized using SPDBV (Swiss-PdbViewer).

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The superimposition of the modeled structure with the template showed homology between all the residues except for the amino acids THR208, LEU209, GLY210, GLU211, GLU212, SER213, THR214 and LYS215 (Fig. 4).

B. Molecular docking

Molecular docking was performed using Hex software which is based on algorithms like Fast Fourier Transform (FFT) and Critical Assessment of Prediction of Interactions (CAPRI). The modeled *Ah* 14-3-3 protein was energy minimized using Sybyl-X -Tripos force field.

The other three proteins (1xo8, 1APX and 1tiz) which are drought stress responsive, were selected as ligands for docking studies. Each ligand was docked inside the cavity of 14-3-3 protein and docking scores were obtained. Based on these docking scores, we opted for the interactions exhibiting highest score docking conformation with a docking energy of -5.313e+2 kcal/mol for protein ligand complex structure. The same procedure was followed for all the three ligand and protein interactions. The docking studies clearly indicated that the ligand and receptor were bound together closely to stabilize complex structure. To date many studies have demonstrated that the dimeric structure of 14-3-3 proteins contains many potential protein-binding sites and that these sites could be specific to its target's phosphorylation status. A high-resolution X-ray structure of 14-3-3 phosphoserine peptide complexes, identifying several binding sites for the 14-3-3-substrate interaction was proposed by [19].

The protein structure with PDB id: *1APX* represents the crystal structure of recombinant pea cytosolic ascorbate peroxidase. The closest interactions between *1APX* and *Ah* 14-3-3 protein were observed between the residues; glu29 (ligand) to ala55(receptor) with a distance of 7.34A^{o} (Fig. 5). Ascorbate peroxidase play an important role in protecting plants under oxidative stress and water deficit conditions by scavenging reactive oxygen species [30, 31]. It was proposed that *Arabidopsis* 14-3-3 protein (GF 14λ) interacts with APX under water stress conditions [32]. Hence, *Ah* 14-3-3 protein might also interact with *1APX* in peanut playing an important role in antioxidation metabolism under drought stress conditions.

Interaction of *Ah* 14-3-3 protein was also studied with *'1 tiz'* which represents the solution structure of a calmodulin-like binding domian of *Arabidopsis thaliana.* It is a polymer of 67 aminoacids with alpha helices and EF hand like foldings. The secondary structure is dominated by 56 % alpha-helices and 5 % beta sheets. On molecular docking of *Ah* 14-3-3 and calmodulin (1itz.pdb) proteins, the closest interactions were observed at residues lys14-glu23, phe10-phe27, arg5-met11, val4-leu111, glu28-ala33 and ile46-ala45 (Fig. 6). A large portion of interaction was observed between these two proteins with almost all the residues interacting with each other that clearly indicates the regulation of calmodulin by *Ah* 14-3-3 protein under drought stress. The CalM 42, a calcium ion binding protein of *Arabidopsis thaliana* is known to interact with calcium sensors. In the present investigation, this complete interaction might enable the peanut plant to rapidly sense and respond to environmental perturbations conferring better adaptation and survival under drought stress.

When *Ah* 14-3-3 protein was docked with 1xo8.pdb, that represents the solution structure of Late Embryogenesis Abundant protein of *Arabidopsis thaliana*, the closest interactions were obseved at residues; ala512 (ligand) to ala58 (receptor) with a distance of 4.31A^{o} (Fig. 7). Among a diversity of responses, plants adapt to water deficit by the induction of specific genes such as the gene family encoding for a group of proteins called late embryogenesis abundant (LEA) proteins [33]. In the present investigation, the interaction of *Ah* 14-3-3 protein with LEA proteins implicate its role in triggering LEA that play an important role in the maintenance of structure of other proteins, endo-membrane structures, in the sequestration of ions such as calcium, in binding or replacement of water and functioning as molecular chaperones under water stress [34]. Our docking studies clearly indicated that this modeled *Ah* 14-3-3 protein which interacts with other proteins closely, may regulate other functional proteins in conferring tolerance to the drought conditions.

C. Physico-chemical properties

The *Ah* 14-3-3 protein is an acidic protein which tends to be negatively charged at the physiological pH, with an isoelectric point (pI) of 4.66. These proteins are unstable in nature with an instability index of 49.92. A negative GRAVY value (-0.545) showed that 14-3-3 protein is hydrophilic in nature indicating the possibility of better interaction with water. The molar extinction co-effiecient of 27390 $M^{-1}cm^{-1}$ at 280 nm revealed the existence of more no. of cystine, tyrosine and tryptophan residues. The computed extinction coefficients help in the quantitative study of protein–protein and protein–ligand interactions in solution. The aliphatic index which is defined as the relative volume of a protein occupied by aliphatic side chains (alanine, valine, isoleucine and leucine) is regarded as a positive factor for the increase of thermal stability of globular proteins. This protein can be stable for a wide range of temperature as indicated by high aliphatic index value (83.40). This protein has cysteine at 2 positions (103 and 198), indicating the formation of functional linkages. The secondary structure analysis indicated that 14-3-3 protein is dominated by alpha helices followed by random coils and extended strands while beta turns are highly negligible.

IV. CONCLUSION

The 14-3-3 protein which is a highly conserved regulatory protein is known to interact with a number of proteins expressed under various metabolic pathways. In our study, we have shown the interaction of this protein with three other most common drought tolerant proteins. This protein displayed maximum interaction with calcium ion binding protein which is an important second messenger under drought stress. This study would be useful for predicting interactions with other drought tolerant proteins *in vivo.*

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FIGURES

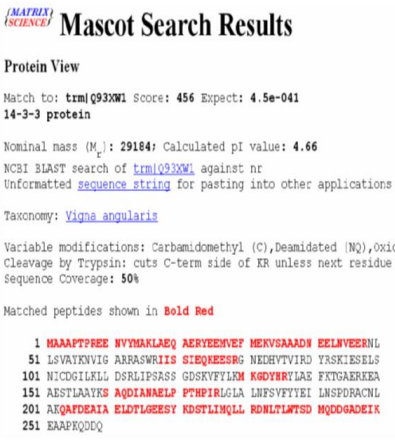

Fig 1a. Sequence coverage map of identified 14-3-3 like protein in peanut. Red amino acids correspond to those that were matched to experimental data on MALDI-TOF/TOF sequencing, here in this case, indicating the matching peptides of peanut 14-3-3 like protein with 14-3-3 like protein of *Phaseolus angularis*

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Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
1 - 8	830.4257	829.4184	829.4116	8	0	-.MAAPTPR.E Oxidation (M) (No match)
1 - 8	830.4257	829.4184	829.4116	8	0	-.MAAPTPR.E Oxidation (M) (No match)
1 - 16	1794.7830	1793.7757	1793.8440	-38	1	-.MAAPTPREEAVDNAK.L Oxidation (M) (No match)
1 - 16	1794.7830	1793.7757	1793.8440	-38	1	-.MAAPTPREEAVDNAK.L Oxidation (M) (No match)
17 - 23	816.4107	815.4034	815.4137	-13	0	K.LAEQAER.Y (No match)
17 - 23	816.4107	815.4034	815.4137	-13	0	K.LAEQAER.Y (Ions score 35)
17 - 33	2164.9070	2163.8997	2163.9340	-16	1	K.LAEQAERYEEMVEFMEK.V Deamidated (NQ); 2 Oxidat
24 - 33	1366.5187	1365.5114	1365.5468	-26	0	R.YEEMVEFMEK.V 2 Oxidation (M) (Ions score 14)
24 - 33	1366.5187	1365.5114	1365.5468	-26	0	R.YEEMVEFMEK.V 2 Oxidation (M) (No match)
24 - 48	2995.2881	2994.2808	2994.2634	6	1	R.YEEMVEFMEKVSAADKEELVKEER.N 2 Deamidated (NQ)
34 - 48	1646.7592	1645.7519	1645.7431	5	0	K.VSAADKEELVKEER.N Deamidated (NQ) (Ions score
34 - 48	1646.7592	1645.7519	1645.7431	5	0	K.VSAADKEELVKEER.N Deamidated (NQ) (No match)
68 - 75	917.5062	916.4989	916.5229	-26	0	R.IISSIEQK.E (Ions score 17)
68 - 75	917.5062	916.4989	916.5229	-26	0	R.IISSIEQK.E (No match)
68 - 79	1419.6970	1418.6897	1418.7252	-25	1	R.IISSIEQKEESR.G Deamidated (NQ) (No match)
68 - 79	1419.6970	1418.6897	1418.7252	-25	1	R.IISSIEQKEESR.G Deamidated (NQ) (No match)
130 - 136	906.4154	905.4081	905.4178	-11	1	K.MKGDYHR.Y (No match)
130 - 136	922.4051	921.3978	921.4127	-16	1	K.MKGDYHR.Y Oxidation (M) (Ions score 25)
130 - 136	922.4051	921.3978	921.4127	-16	1	K.MKGDYHR.Y Oxidation (M) (No match)
160 - 176	1829.9210	1828.9137	1828.9431	-16	0	K.SAQDIANAELSPTHPIR.L (No match)
160 - 176	1829.9210	1828.9137	1828.9431	-16	0	K.SAQDIANAELSPTHPIR.L (Ions score 55)
203 - 221	2128.9473	2127.9400	2127.9848	-21	0	K.QAFDEAIAELDTLGEESYK.D (Ions score 100)
203 - 221	2128.9473	2127.9400	2127.9848	-21	0	K.QAFDEAIAELDTLGEESYK.D (No match)
222 - 231	1189.6433	1188.6360	1188.6536	-15	0	K.DSTLIMQLLR.D (No match)
222 - 231	1189.6433	1188.6360	1188.6536	-15	0	K.DSTLIMQLLR.D (Ions score 50)
222 - 231	1205.6300	1204.6227	1204.6485	-21	0	K.DSTLIMQLLR.D Oxidation (M) (Ions score 29)
222 - 231	1205.6300	1204.6227	1204.6485	-21	0	K.DSTLIMQLLR.D Oxidation (M) (No match)
222 - 250	3338.7136	3337.7063	3337.5694	41	1	K.DSTLIMQLLRDNLTLWTSDMQDDGADEIK.E Deamidated (N

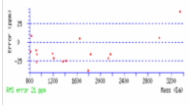

Fig 1b. List of peptides identified by MALDI TOF-TOF (those with matching MS/MS data have an "ion score" while those that are identified only by mass have "no match").

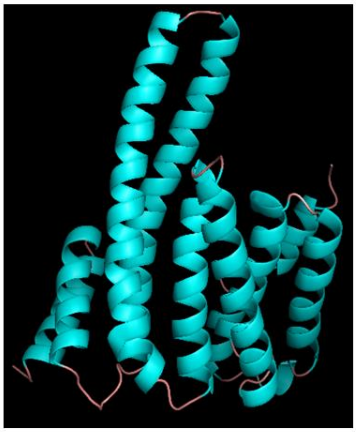

Fig 2. A three dimensional (3D) homology model of 14-3-3 protein

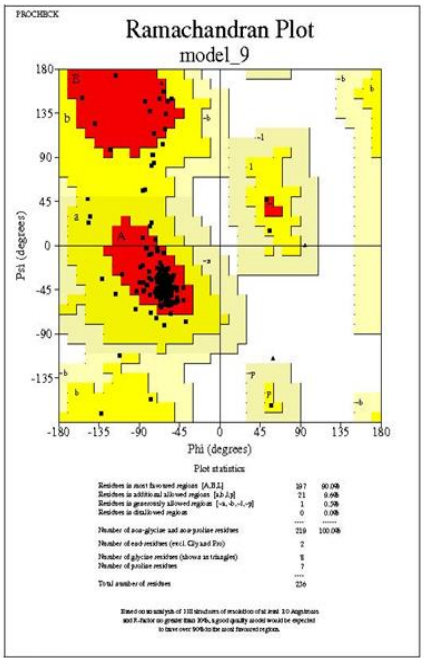

Fig 3. Ramachandran plot of 14-3-3 protein

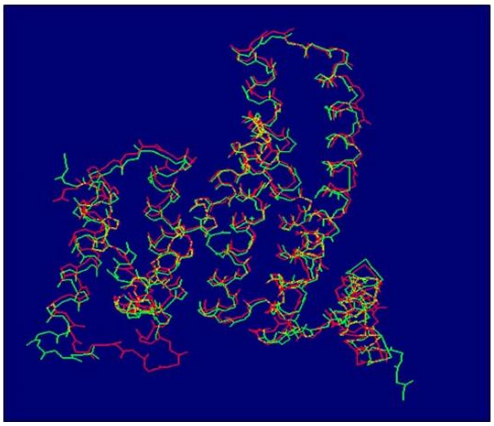

Fig 4. Superimposition of 3D structure of *Ah* 14-3-3 protein of peanut (green) with 14-3-3 protein (2o98) of *Nicotiana tabacum* (red).

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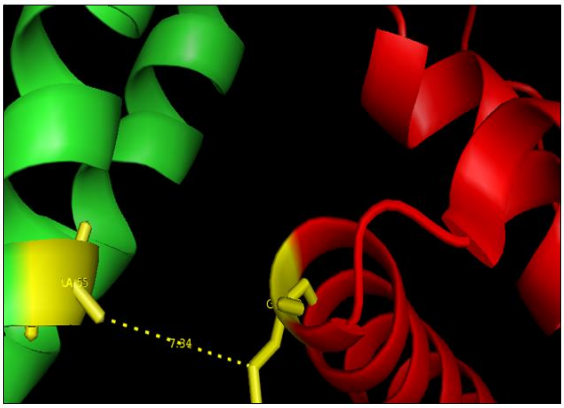

Fig 5. Docking of *Ah* 14-3-3 protein of peanut (green) with 1APX (Ascorbate peroxidase)

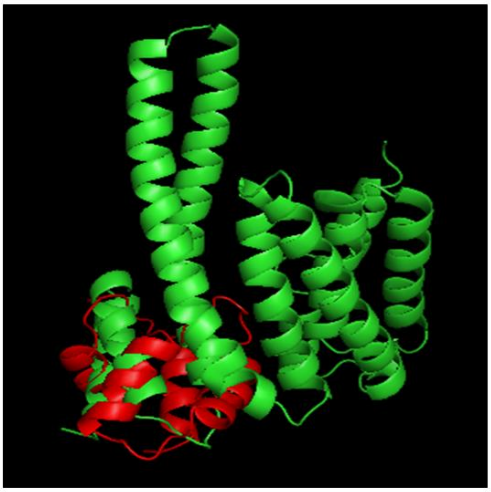

Fig 6. Docking of *Ah* 14-3-3 protein of peanut (green) with 1tiz (Calcium ion binding protein)

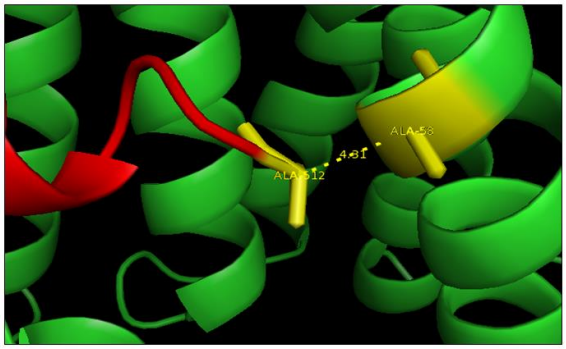

Fig 7. Docking of *Ah* 14-3-3 protein of peanut (green) with 1xo8 (Late embryogenesis abundant protein)

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