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Hypothesis

# Molecular model of thylakoid membrane bound (*SlAPX6*) ascorbate peroxidase from *Solanum lycopersicum*

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**Abstract:**
Ascorbate peroxidase (E 1.11.1.11) acts as primary key component of plant defense against photo protection and photo-oxidative stress. Chloroplastic (APX) located in the thylakoid membrane (tAPX) and stroma (sAPX) have been thought to be key regulators of intracellular levels of $H_2O_2$. Therefore, it is of interest to study thylakoid membrane bound *SlAPX* from *Solanum lycopersicum* (tomato, a fleshy fruit). However, a structure model is not yet solved for tomato thylakoid membrane bound *SlAPX*. "Hence, a homology molecular model of *SlAPX6* from *S. lycopersicum* was constructed using a template structure (PDB ID: 1APX) from *Pisum sativum*. The model was further assessed using accessible surface area (ASA) calculations to identify surface residues for further characterization of active site regions. We further characterized the active site regions in the enzyme for functional inference. This information provides insights for the understanding of photo protection and photo-oxidative stress tolerant in *S. lycopersicum* during flower development and fruit ripening."

**Background:**
Tomato (*S. lycopersicum*) is fleshy fruit an important crop since of its great nutritive and commercial value of world, and also a good model plant for study fruit development with the release of the whole genome sequence of tomato. Now, it is very suitable to comprehensive structural and functional analysis of entire potential genes. Photooxidative stress is one of mainly important environmental factors that influence flower and fruit development stage (green, breaker and red). Photooxidative stress in the plant cell owing to higher reduced of electrons towards $O_2$ during photosynthesis and respiratory processes leading to enhancement to generation of reactive oxygen species. ROS can directly attack inactivates metabolic enzymes and DNA damage and membrane lipids leading to programmed cell death [**1**]. APX (EC, 1.11.1.11) is known play essential role in scavenging ROS and protecting cells against plant stress and it catalyze the conversion of $H_2O_2$ to $H_2O$ using ascorbate as the specific electron donor [**2**]. In tomato, complete genome information of *SlAPX* gene isoforms is comprised of genes encoding cytosolic (*SlAPX1*, and *SlAPX2*), peroxisomal (*SlAPX3*, and *SlAPX4*), and chloroplastic APX (*SlAPX5*, and *SlAPX6*). There is increasing interest in the chloroplastic thylakoid membrane bound *SlAPX6* genes in tomato for enhancing tolerance of photooxidative stress [**3**]; however, there is lacking information on the protein structure of the tomato *SlAPX6*.

In this paper reports structural modeling of thylakoid membrane bound (*SlAPX6*) from tomato was selected for which three dimensional structures were neither available at the protein data bank (PDB) and no sufficient information available in structural level. Hence, we studied the protein structure model of *SlAPX6* from tomato.

**Methodology:**
Template Selection and Protein sequence analysis:
The sequence of the tomato *SlAPX6* (*TbAPX*) was retrieved from Sol Genomics Network https://solgenomics.net/search/ database with accession number Solyc11g018550.2.1. Template selection was done using BLASTp [**4**] for the query sequence

against Protein Data Bank) database [**5**]. The target subsequently selected was the x ray crystal structure with PDB code 1APX. Sequence alignments were completed employing ClustalW [**6**]. ScanProsite [**7**] was used to identify motif.

```
SlAPX6          1 MTSLTGATSHLLPSATIAAISASTTARLAISFSSSSSSSLKCIRSSPLLP    50

PsAPX           1 ------------------------------------------------      0

SlAPX6         51 HIFRYQKRSLIGTTSSGRFSTFASPKCAASDPDQLKSAREDIKELLKTTF   100
                                |:.....||....:    ::.|:..::.:....
PsAPX           1 ---------------GKSYPTVSPDYQKA----IEKAKRKLRGFIAEKK    30

SlAPX6        101 CHPILVRLGWHDAGTYNKNIEDWPQRGGANGSLRFEVELKHGANAGLVNA   150
                  |.|:::||.||.|||::..    .:.||..|:::.:.||.||||.||..|
PsAPX          31 CAPLILRLAWHSAGTFDSK----TKTGGPFGTIKHQAELAHGANNGLDIA    76

SlAPX6        151 LKLLQPIKDKYAGVTYADLVQLASATAIEEARGPKIPMKYGRIDVSGPDE   200
                  ::||:|||:::..|:|||..|||...|:|...||::|...||.|...|
PsAPX          77 VRLLEPIKEQFPIVSYADFYQLAGVVAVEITGGPEVPFHPGREDKPEP--   124

SlAPX6        201 CPEEGRLPDAGPPNPSSHLRDVFYR-MGLNDKEIVALSGAHTLGRSRPER   249
                  |.|||||||  ...|.||||||.: |||:|::||||||.||:|.:..||
PsAPX         125 -PPEGRLPDA--TKGSDHLRDVFGKAMGLSDQDIVALSGGHTIGAAHKER   171

SlAPX6        250 SGWGKPETRYTKDGPGSPGGQSWTVQWLKFDNSYFKDIKEQRDEDLLVLP   299
                  ||:         :||       ||...|.||||||.::.....:.||.||
PsAPX         172 SGF---------EGP-------WTSNPLIFDNSYFTELLTGEKDGLLQLP   205

SlAPX6        300 TDAVLFEDSSFKEYAEKYAVNQDVFFKDYAEAHAKLSNLG---AKFDPPE   346
                  :|..|..||.|:...||||.::||||.||||||.|||.||   |
PsAPX         206 SDKALLTDSVFRPLVEKYAADEDVFFADYAEAHLKLSELGFAEA------   249

SlAPX6        347 GFSIDNNPTQVQPEKFVAAKYSTGKRELSDAMKQKIRAEYEGLGGTPDKP   396

PsAPX         250 ------------------------------------------------   249

SlAPX6        397 LPTNYFLNIIIVIGVLAILTYLLGN    421

PsAPX         250 -------------------------   249
```

**Figure 1**: Sequences alignment SlAPX6 (target) and PsAPX (template) showing conserved patterns of protein sequences

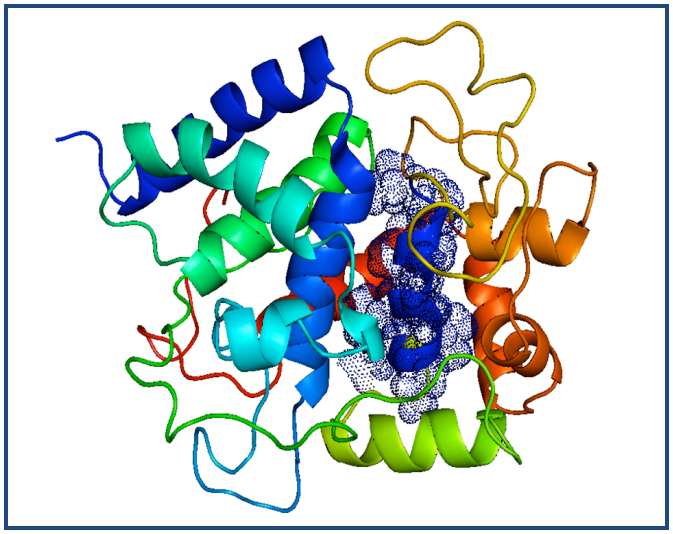

**Figure 2:** The final 3D model structure of SlAPX6 protein. This was obtained by Modeller 9v5 and verified employing PROCHECK, Verify3D and ProSA servers

*Homology modeling*

The 3D structure model of tomato *SlAPX6* was generated by Modeller 9.16 [**8**]. Five models were generated with the lowest DOPE (Discrete Optimized Protein Energy) score was considered for further refinement and validation. Simultaneously another model was also generated by online web server employing Swiss model using same template [**9**]. The energy minimization was carried out employing GROMOS96 implemented incorporated in Swiss PDB Viewer [**10**] Protein models and active site outputs were viewed by PyMOL molecular visualization tool.

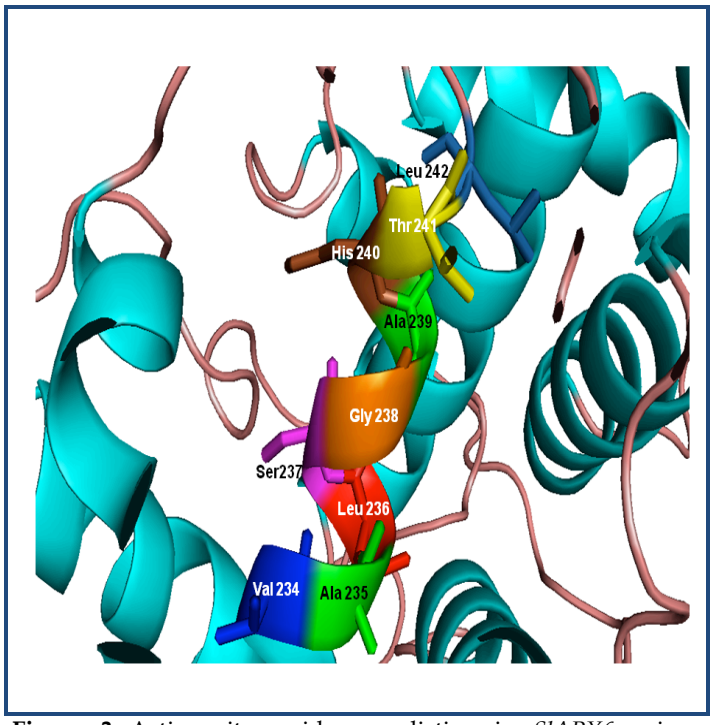


**Figure 3** Active site residue prediction in *SlAPX6* using discovery studio, active site mark with different colors val234 blue, ala235 green, leu236 red, Ser237 magenta, gly238 orange, ala239 green, his240 brown, thr241 yellow and leu242 skyblue.

*Validation of model*

The model was validated on the basis of stereochemically and geometrically constraints employing PROCHECK [**11**], ProSA-Web [**12**] and Verify 3d [**13**]. Root Mean Square Deviation (RMSD) investigation of the predicted structural model from its template was calculated by SUPERPOSE [**14**].

*Structural sequences alignment*

Structural sequences alignment of tomato *SlAPX6* (Solyc11g018550.2.1) proteins and along with other PDB such as 1IYN, 2VCF, 2XIF, 2Y6B and template (*PsAPX*) was done using structure prediction of tomato *SlAPX6* using PROMALS3D (http://prodata.swmed.edu/promals3d/promals3d.php) was performed [**15**].

**Result & Discussion:**

The length of *SlAPX6* protein sequences is 421 amino acid residues long. Sequence search using BLAST identified the crystal structure with PDB ID: 1APX. The *Pisum sativum SlAPX6* (PDB ID: 1APX) showed 45% sequence identity to the query sequence with an E-value of 1e-59. ScanProsite server predicted the fragment VALSGAHT from 234- 242 residues as a motif (**Figure 1**). The sequence alignment revealed that the substrate binding interacting residues valine, alanine, leucine, serine, glycine alanine histidine and threonine were conserved both in *SlAPX6* as target and *PsAPX* as template (**Figure 2**). These highly conserved residues might be functional in plant stress defense during fruit ripening and development in tomato (**Figure 3**).

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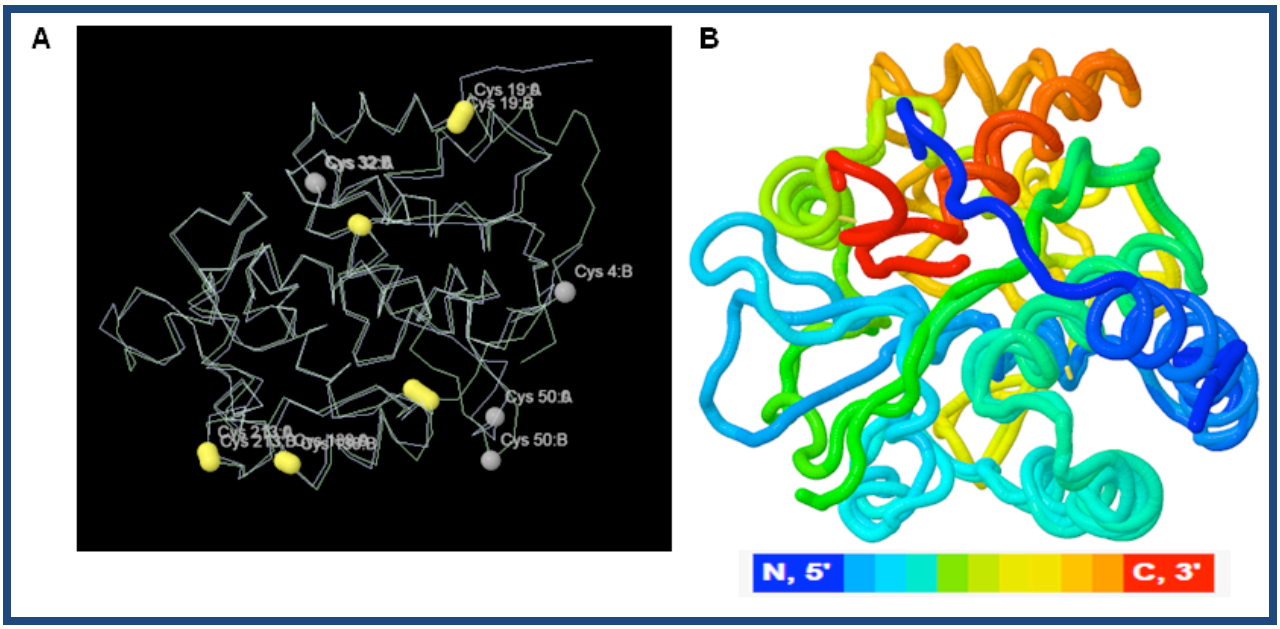


**Figure 4**: **A**) Superpose of model with disulphide linkage in protein chain; **B**) N- C Rainbow represent the protein chain with amino N terminal of protein are blue and carboxy C terminal are red.

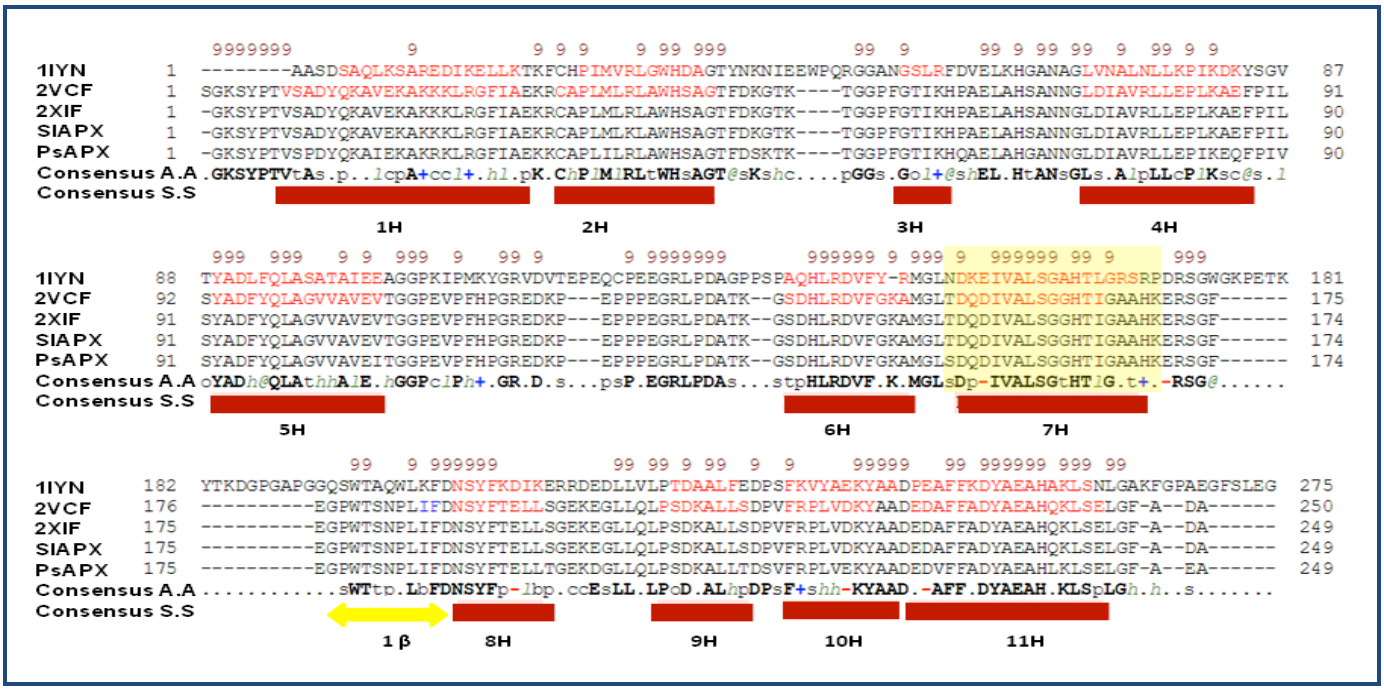


**Figure 5** Structural sequences alignment of chloroplastic tomato *SlAPX6* with its various homologous PDB such as 1IYN, 2VCF, 2XIF, 2Y6B and template (*PsAPX*) revealed that 11 alpha helices and 1 beeta strands were conserved in all the selected PDB id such as 1IYN, 2VCF, 2XIF, 2Y6B and template (*PsAPX*). This was generated using the **PROMALS3D** online web tools. The secondary structure elements were marked in colors red (helices) and yellow (beta sheet).

After that we have buildup structure for tomato *SlAPX6* employing MODELLERTM. Five models were generated with the lowest Discrete Optimized Protein Energy, a statistical potential used to determine homology *SlAPX6* models score of -24028.06 was considered to be thermodynamically stable and chosen for further refinement and validation. The stereochemically quality and accuracy of the predicted *SlAPX6* model was evaluated by Procheck tool through Ramachandran plot. The MODELLERTM created model produced 94.6% residue found in most favored region, 4.9% residues in additionally allowed region, 0.5% residues in generously allowed and with no residues found in the disallowed region

following the Ramachandran plot. This model is also compared with the Swiss Model with 95.0% residues in most favored region, 4.3% residues in additionally allowed region and 1.7% residues in generously allowed region. Consequently the model structure produced employing MODELLERTM was used for further analysis.

To further analyze the overall model quality target (*SlAPX6*) and template (*PsAPX*) for compare check by ProSA web analysis server revealed a Z score value measure of model quality total energy of the both structures such as template and target respectively of -8.78 of *PsAPX* and -8.32 of *SlAPX6*, Z-score analysis revealed a very good accuracy of our *SlAPX6* model. Verify3D use for the determines the compatibility of an atomic model (3D) with its own residues by assigning a structural class based on its alpha, beta, loop, polar, non-polar. Therefore its comparing the results to good structures revealed 98.91% of the residues had a score > = 0.2 for a best quality of our *SlAPX6* model. The degree of structure similarity is measured using root-mean-square distance (RMSD) between corresponding atom pairs. RMSD examination of the tomato *SlAPX6* model was measured from its template employing tools web server SuperPose (**Figure 4**). The RMSD between alpha carbon (Cα) and backbone deviation of Target (*SlAPX6*) and the template (*PsAPX*) crystal structure were 1.06Å, and 1.08Å respectively. Thus, the MODELLERTM developed model was evaluated using several methods for reliability and precision.

Structural sequences alignment of chloroplastic tomato *SlAPX6* with its various homologous PDB such as 1YN, 2VCF, 2XIF, Y6B and template (*PsAPX*) revealed that 11 alpha helices and 1 beeta strands were conserved in all the selected PDB id such as 1YN, 2VCF, 2XIF, Y6B and template (*PsAPX*) (**Figure 5**). It illustrated high sequence likeness at the N terminal to C terminal with high helical region in whole protein sequences 11 helix and only one β sheets. Thus, the model provides molecular insight to metal binding molecular function towards the understanding of photooxidative stress during flower to fruit development and ripping stage in tomato. The predicted model could be further explored for identification of substrate ligand binding sites, site directed mutagenesis and alter for better protein engineering which may be useful to understand specific role of functional site residues during catalysis.

**Conclusion:**

The thylakoid membrane bound tomato ascorbate peroxidase (*SlAPX6*) plays a major role in flower and fruit development in fleshy fruit during tomato ripening stage green, breaker and red against photo-oxidative stress. Therefore, it is of interest to deduce its molecular function and propose mechanism of action. Thus, a homology structural model of the protein was constructed and analyzed further to infer molecular function. The model data in addition to other relevant post model analysis data proposes to provide molecular insight to heme binding molecular function towards the understanding of photo-oxidative stress tolerant in fleshy fruit tomato. Data highlights the importance of the heme-binding site in scavenging ROS from flesh fruit tomato during ripening.

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