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Presented by: Atul Tyagi

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Docking and molecular dynamics simulation study of plant origin antifungal peptides with fungal protein of plant pathogen *Fusarium oxysporum*

- **Protein-peptide interactions play a key role in cell functions and their structural characterization is challenging for the discovery of new drugs**
- **CABS-dock introduces a successful docking for a potential therapeutic peptide to a target protein**
- **The CABS-dock web server provides an interface for modelling protein-peptide interactions using a highly efficient protocol for flexible docking of peptides to proteins**
- **The CABS-dock server peptide sequence takes only 4-30 amino acids in length as an input**
- **Our literature survey provided only 55 peptides out of 510 plant antifungal peptides based on above criteria**

Table: 1 Top fifty-five best models based on high cluster density and low RMSD using the CABS-dock server

Project Name	Cluster density	Average RMSD	Max RMSD	Number of elements
PHYTO1	43.0347	2.5096	18.6126	108
PHYTO2	34.744	3.10845	28.3374	108
PHYTO3	33.4868	4.68842	13.902	157
PHYTO4	41.221	3.85726	19.3698	159
PHYTO5	93.2059	1.20164	19.7809	112
PHYTO6	24.1826	5.37577	31.7782	130
PHYTO7	16.9603	7.07535	25.5927	120
PHYTO8	20.8742	5.98825	28.1455	125
PHYTO9	26.3897	4.39565	24.3733	116
PHYTO10	33.9131	3.56794	24.4612	121
PHYTO11	21.3125	7.78884	28.4952	166
PHYTO12	36.3688	3.79446	24.8309	138
PHYTO13	57.8178	1.43554	8.88006	83
PHYTO14	19.6346	4.78746	29.9645	94
PHYTO15	29.1095	6.01178	48.0312	175
PHYTO16	25.94	8.1727	32.9729	212
PHYTO17	55.6105	3.4346	24.3533	191
PHYTO18	26.2884	4.75494	24.9646	125
PHYTO19	26.6464	4.46589	32.0083	119
PHYTO20	23.0899	4.93723	19.6059	114
PHYTO21	37.8481	2.93277	20.2395	111
PHYTO22	39.6079	3.53465	32.153	140
PHYTO23	20.3506	7.46906	26.5846	152
PHYTO24	36.5879	3.52575	13.7793	129
PHYTO25	25.4886	4.78645	23.2311	122
PHYTO26	29.0369	4.99365	16.7709	145
PHYTO27	20.707	5.55368	25.6032	115
PHYTO28	30.3344	1.4505	11.5505	44
PHYTO29	31.871	3.7338	17.567	119
PHYTO30	20.4285	7.48954	27.1249	153
PHYTO31	25.1261	4.81571	27.5215	121
PHYTO32	38.7242	2.68566	21.5386	104
PHYTO33	31.8422	3.51735	35.6513	112
PHYTO34	28.7822	4.3777	23.3995	126
PHYTO35	44.7786	2.81384	15.6405	126
PHYTO36	22.3413	7.51969	27.9021	168
PHYTO37	28.2654	7.00504	26.7736	198
PHYTO38	24.1639	3.72457	13.3346	90
PHYTO39	20.8389	5.66248	31.6554	118
PHYTO40	20.8389	5.66248	31.6554	118
PHYTO41	22.252	5.48266	18.2149	122
PHYTO42	20.0459	7.83203	35.731	157
PHYTO43	16.6654	10.4408	44.9773	174
PHYTO44	20.8823	6.08171	36.7135	127
PHYTO45	20.305	10.1945	32.5832	207
PHYTO46	41.8317	2.10367	19.5646	88
PHYTO47	24.7336	4.16438	18.773	103
PHYTO48	23.2952	4.37858	17.0232	102
PHYTO49	35.1461	3.27206	20.1735	115
PHYTO50	35.6574	3.05687	9.72862	109
PHYTO51	37.6915	3.68784	19.4973	139
PHYTO52	38.6859	2.68832	21.2965	104
PHYTO53	27.9275	8.84433	27.7962	247
PHYTO54	55.8749	4.02685	28.1631	225
PHYTO55	34.2851	3.5584	29.3571	122

Table: 2 Top five best models based on high cluster density and low RMSD using the CABS-dock server

Project Name	Cluster density	Average RMSD	Max RMSD	Number of elements
PHYTO5 (A)	93.2059	1.20164	19.7809	112
PHYTO13 (B)	57.8178	1.43554	8.88006	83
PHYTO28 (C)	30.3344	1.4505	11.5505	44
PHYTO1 (D)	43.0347	2.5096	18.6126	108
PHYTO52 (E)	38.6859	2.68832	21.2965	104

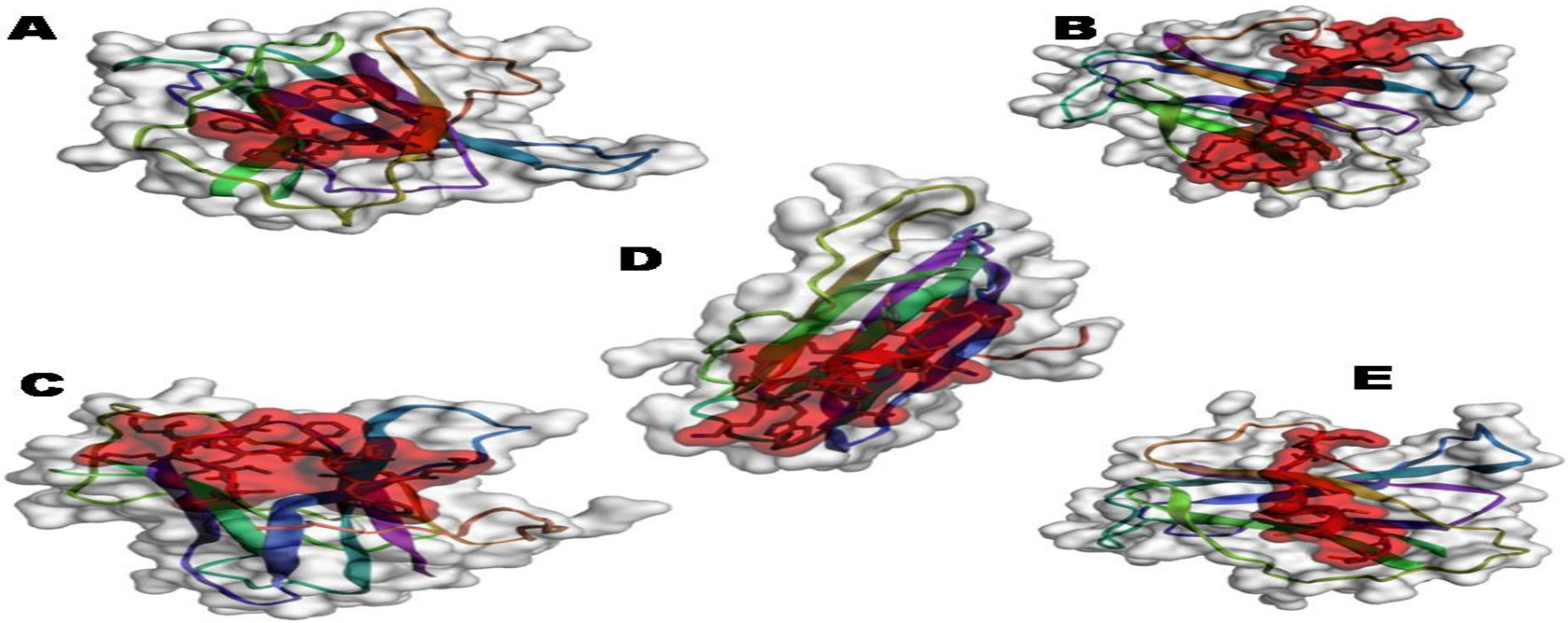
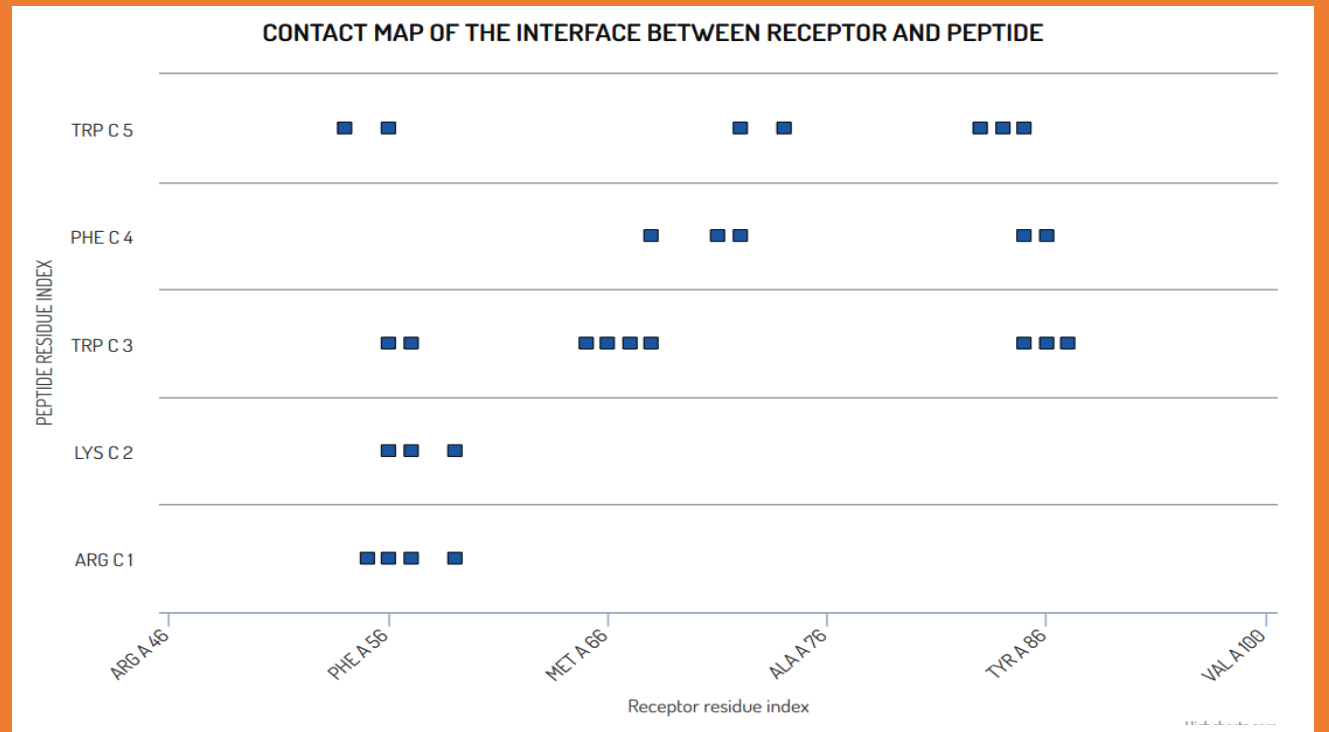


Figure:1 CABS-dock based top-5 models have been described in A (PHYTO5) B (PHYTO13) C (PHYTO28) D (PHYTO1) and E (PHYTO52) in this figure.

The Contact maps allows investigating the interaction between the receptor and peptide

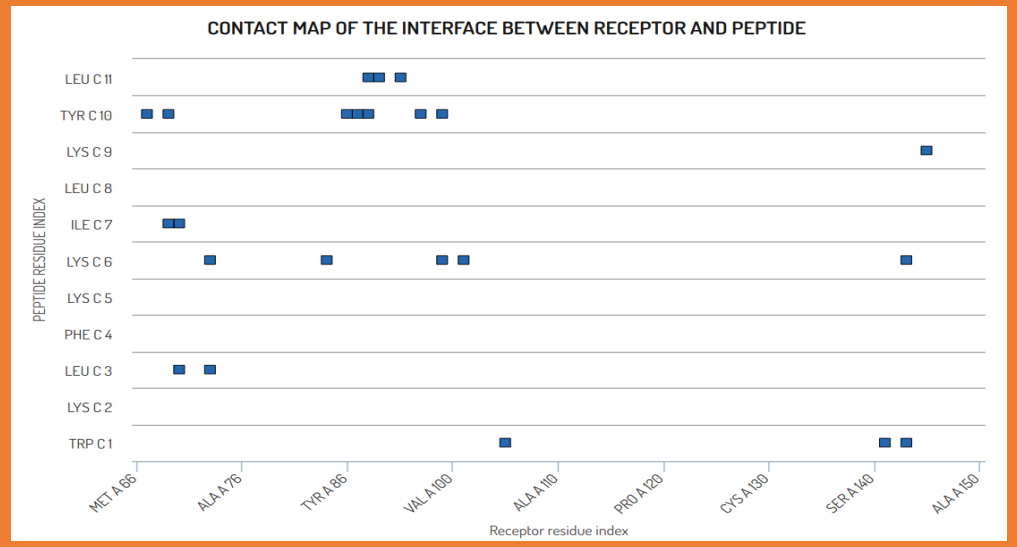
In the **PHYTO5** dataset model contact map shows that peptide made of 5 amino acid chains in which **ARG C1** have interacting residue is **SER A 55**, **PHE A 56, SER A 57** and **GLU A 59** of the receptor protein residue

Same in the last **TRP C5** have interacting residue is **THR A 54, PHE A 56, PRO A 72, GLU A 74, HIS A 83, ARG A 84** and **ILE A 85** amino acid residues

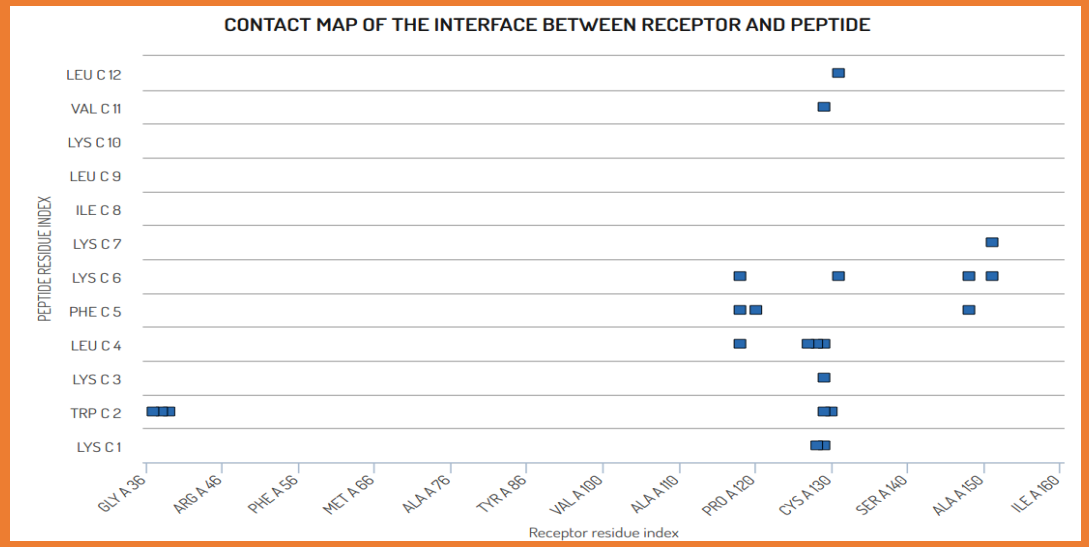


Peptide residue	Receptor residue	Peptide residue	Receptor residue
ARG C 1	SER A 55	TRP C 3	TYR A 86
ARG C 1	PHE A 56	TRP C 3	GLU A 87
ARG C 1	SER A 57	PHE C 4	HIS A 68
ARG C 1	GLU A 59	PHE C 4	PRO A 71
LYS C 2	PHE A 56	PHE C 4	PRO A 72
LYS C 2	SER A 57	PHE C 4	ILE A 85
LYS C 2	GLU A 59	PHE C 4	TYR A 86
TRP C 3	PHE A 56	TRP C 5	THR A 54
TRP C 3	SER A 57	TRP C 5	PHE A 56
TRP C 3	ARG A 65	TRP C 5	PRO A 72
TRP C 3	MET A 66	TRP C 5	GLU A 74
TRP C 3	LEU A 67	TRP C 5	HIS A 83
TRP C 3	HIS A 68	TRP C 5	ARG A 84
TRP C 3	ILE A 85	TRP C 5	ILE A 85

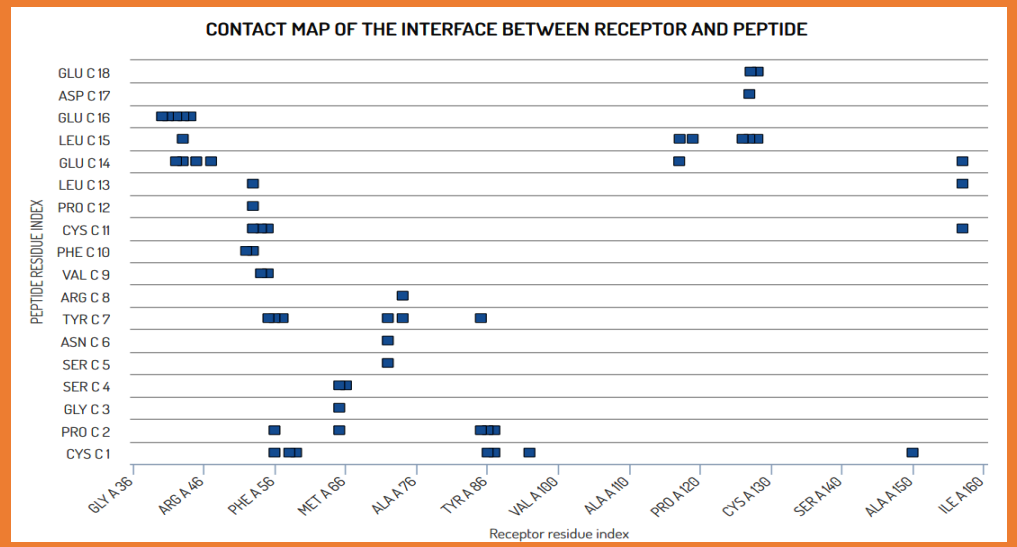
Figure: 2 PHYTO5 dataset model in Contact maps



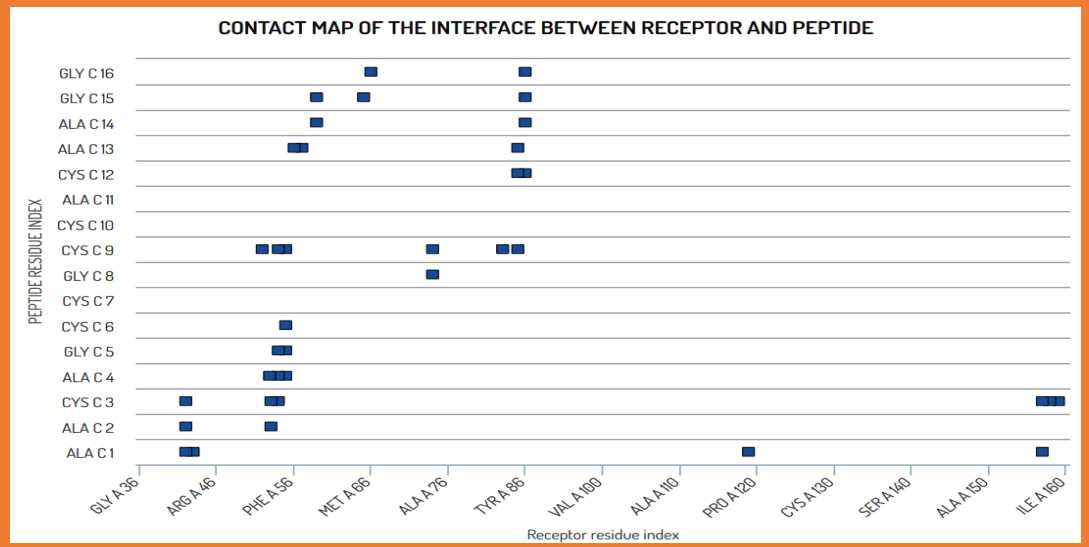
PHYTO13(B)



PHYTO28(C)



PHYTO1(D)



PHYTO52(E)

Figure: 3 Dataset models B (PHYTO13), C (PHYTO28), D (PHYTO1) and E (PHYTO52) in Contact maps

Molecular dynamics has become an important research method, covering millions of atomic-level systems.

It's most important to consider hydrogen bonds' properties in drug design because they are essential for drug specificity, metabolism, and adsorption.

We have performed molecular dynamics studies on the top 5 models, but we have only described our most important PHYTO5 model.

Our study with contact maps supported by simulations for the top model PHYTO5 suggests that **SER57, GLU59, ARG65, HIS68, GLU74,** and **GLU 87** are significant hydrogen bonds of the peptides.

Our best model project PHYTO5 has an RMSD value of **1.20164**, a good starting point for more precise modeling.

Protein RMSD Findings:

The plot shows the RMSD evolution of a protein (left Y-axis). All protein frames are first aligned on the reference frame backbone, and then the RMSD is calculated based on the atom selection

- RMSD analysis can indicate if the simulation has equilibrated — its fluctuations towards the end of the simulation are around some thermal average structure
- Our Simulation shows the RMSD value is large, but its acceptable because the protein is big, and the system has started to equilibrate at the end
- Most importantly during the entire run, the amino acid of the proteins binding site has overlapped with the peptide for bond formation

Ligand RMSD Findings:

- Ligand RMSD (right Y-axis) indicates how stable the ligand is with respect to the protein and its binding pocket.
- The ligand values observed are significantly lower than the RMSD of the protein, then it is likely that the ligand has not diffused away from its initial binding site

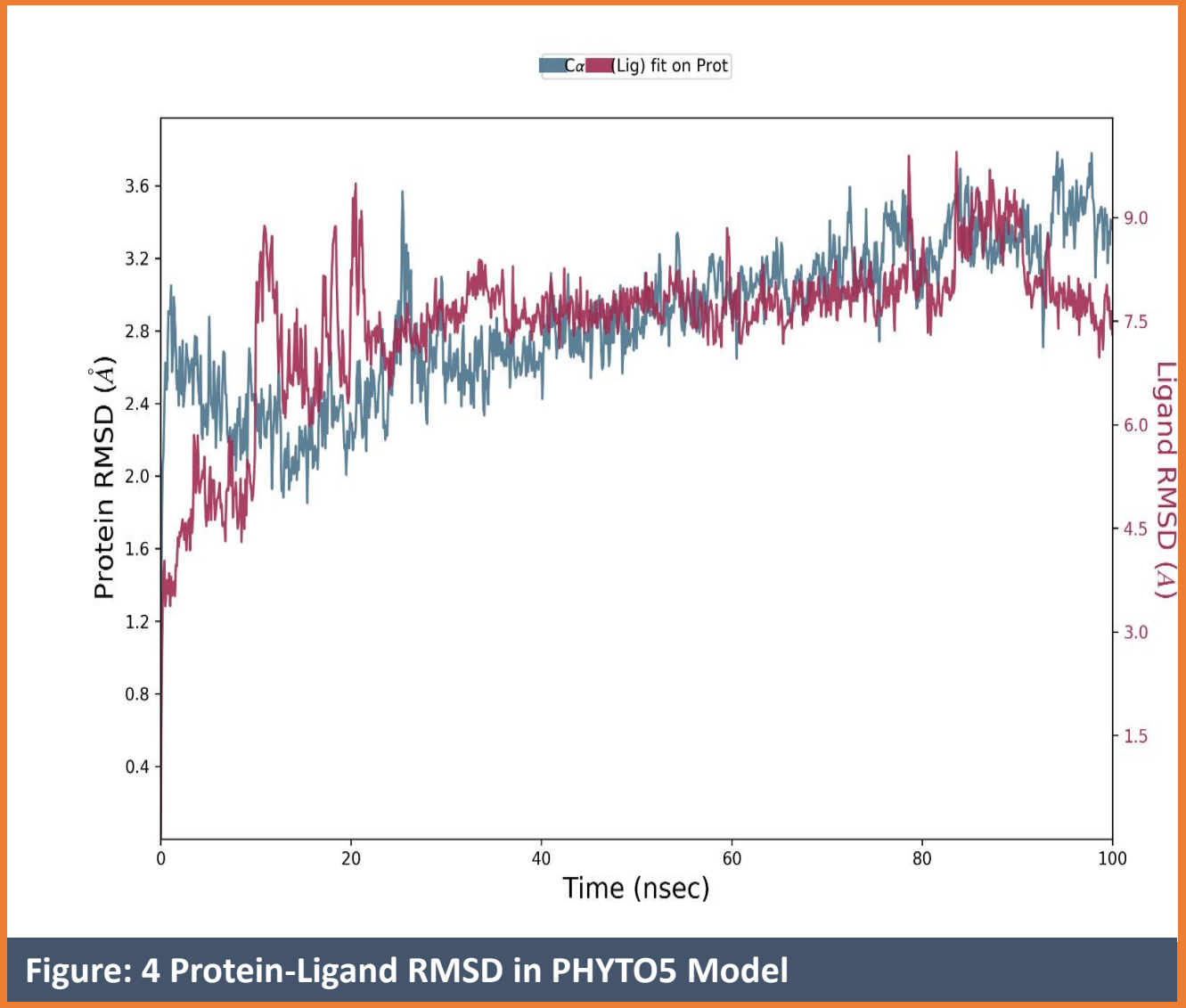


Figure: 4 Protein-Ligand RMSD in PHYTO5 Model

Protein RMSF:

- The Root Mean Square Fluctuation (RMSF) is useful for characterizing local changes along the protein chain
- On this plot, peaks indicate areas of the protein that fluctuate the most during the simulation
- The observation shows that the tails (N- and C-terminal) fluctuate more than any other part of the protein.
- Secondary structure elements like alpha helices and beta strands are usually more rigid than the unstructured part of the protein, and thus fluctuate less than the loop regions

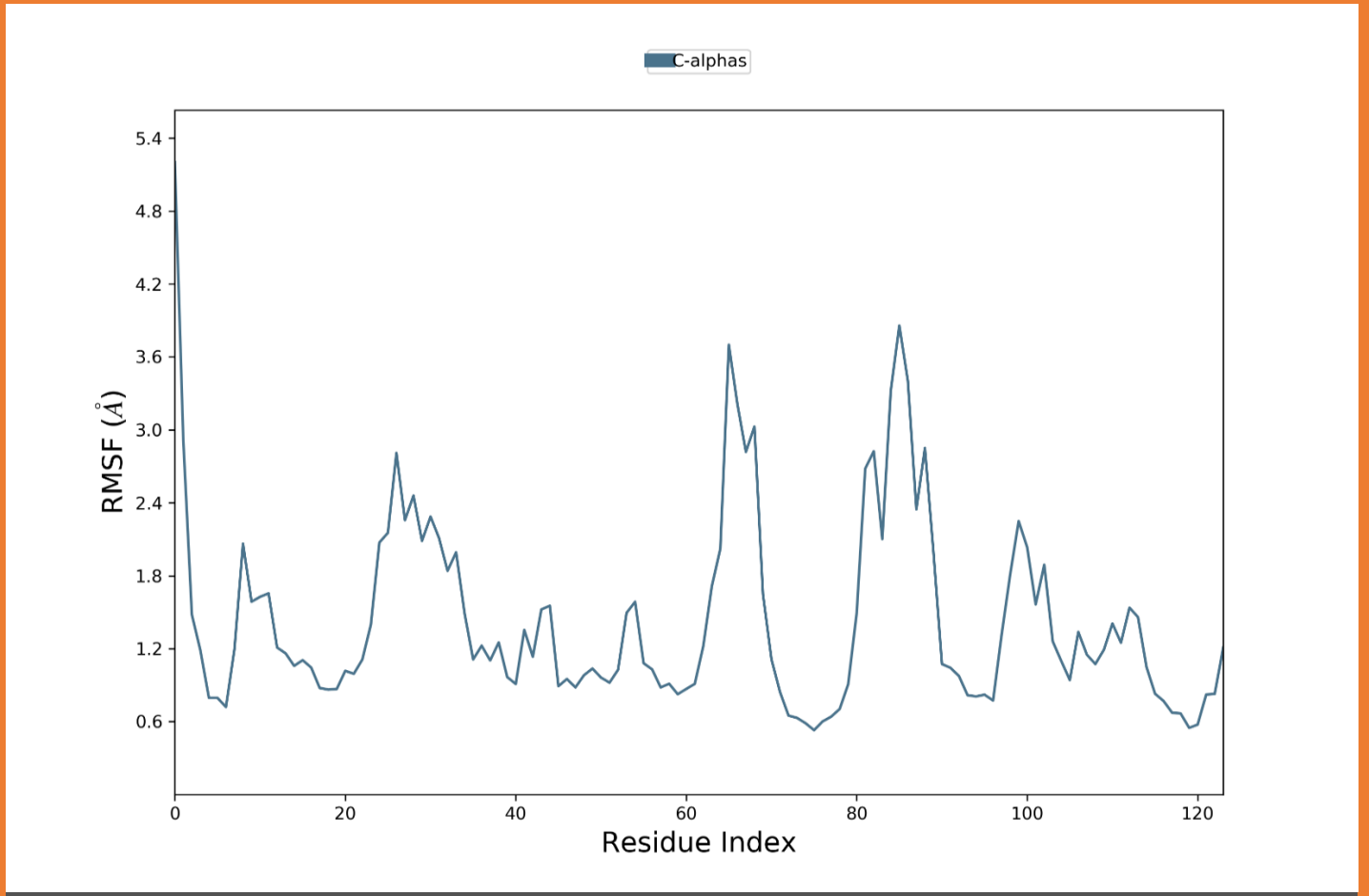


Figure: 5 Protein-RMSF in PHYTO5 Model

Protein Interaction:

- Protein interactions with the ligand can be monitored throughout the simulation
- Protein-ligand interactions (or 'contacts') are categorized into four types: Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges
- The stacked bar charts are normalized over the course of the trajectory: for example, a value of 0.7 suggests that 70% of the simulation time the specific interaction is maintained
- Values over 1.0 are possible as some protein residue may make multiple contacts of same subtype with the ligand
- **In our case GLU59 shows multiple interactions exceeding 100%.**

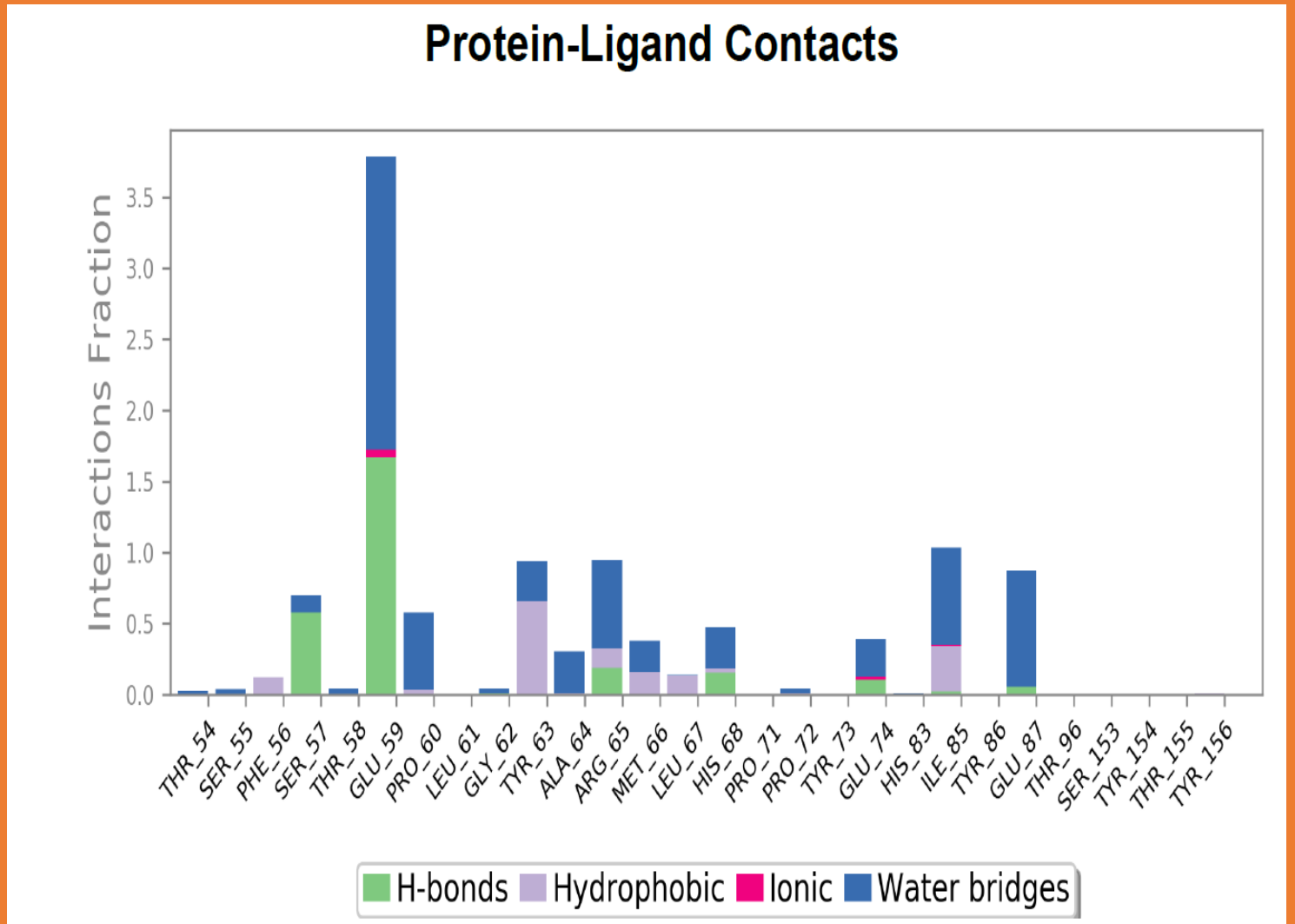


Figure: 6 Protein-Ligand contacts in PHYTO5 Model

Ligand RMSF:

- The Ligand Root Mean Square Fluctuation (L-RMSF) is useful for characterizing changes in the ligand atom positions

- The ligand RMSF may give you insights on how ligand fragments interact with the protein and their entropic role in the binding event

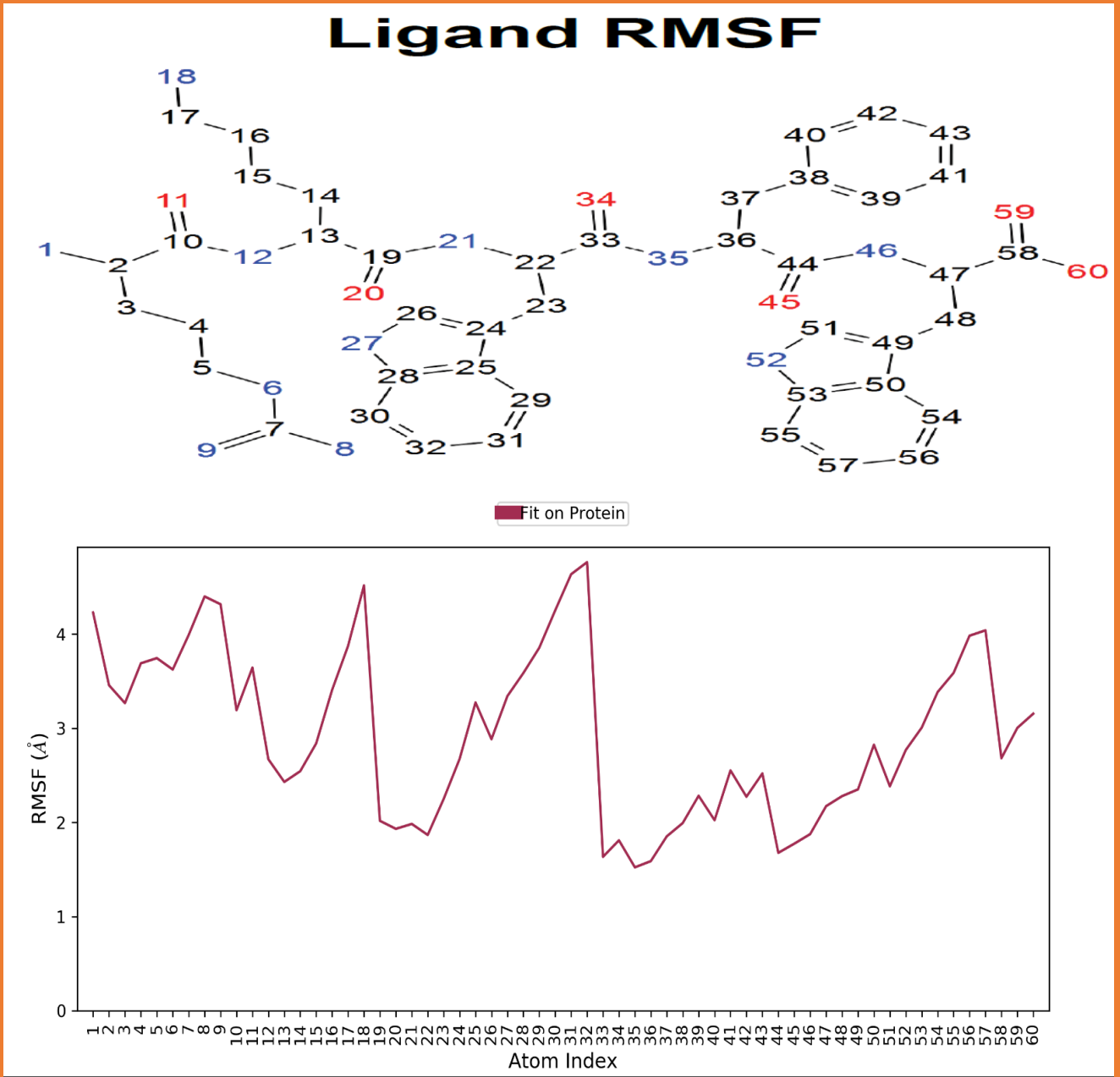


Figure: 7 Ligand-RMSF in PHYTO5 Model

Ligand Protein Contacts:

- A schematic of detailed ligand atom interactions with the protein residues.
- Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown

- Note: it is possible to have interactions with >100% as some residues may have multiple interactions of a single type with the same ligand atom

- In our current findings, **SER57, GLU59, PRO60, ARG65 and ILE85** of the receptor fungal protein are responsible in forming multiple interactions with the peptide ligand exceeding 30% contacts
- **Consideration of hydrogen-bonding properties in drug design is most important because of their strong influence on drug specificity, metabolization and adsorption**

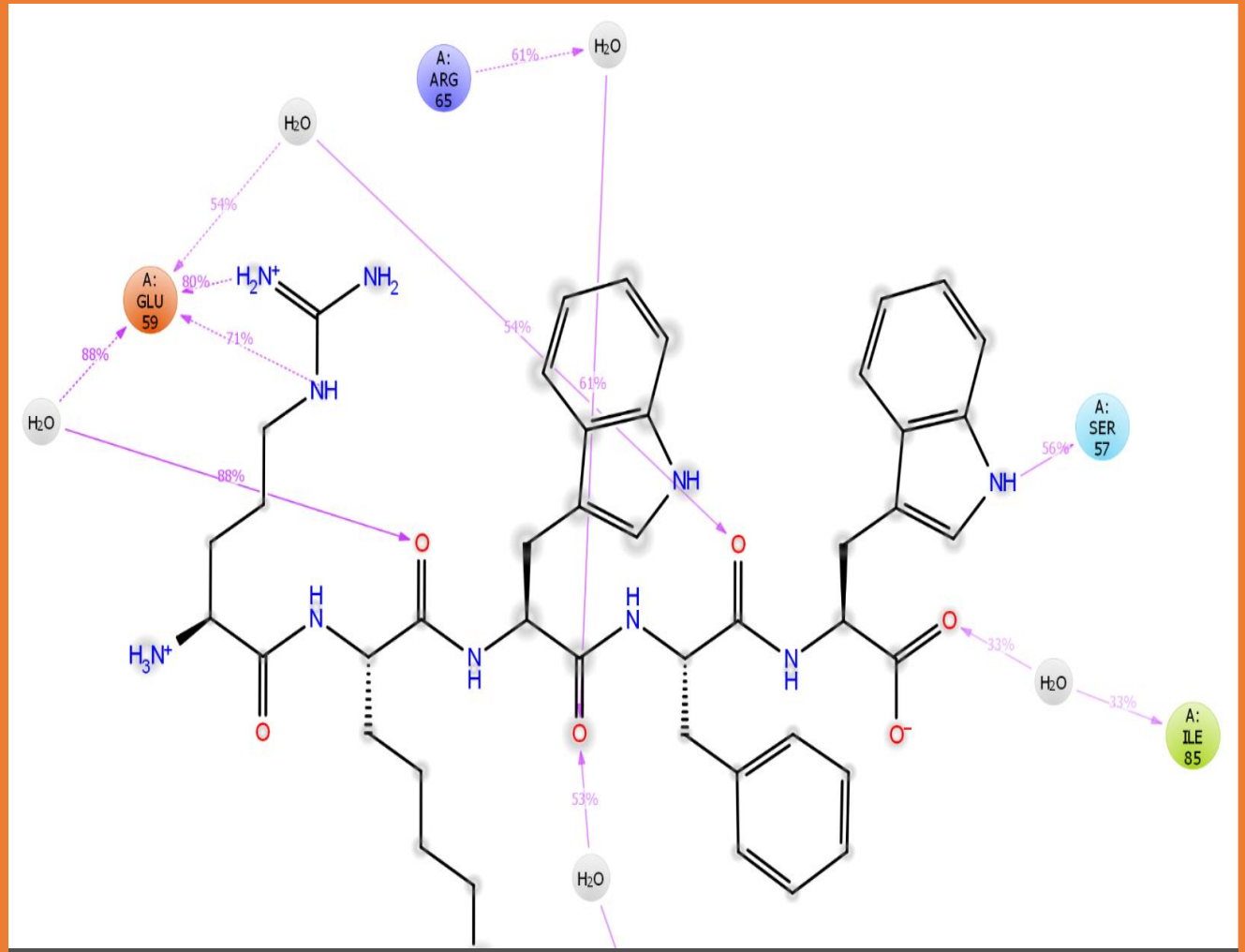


Figure: 8 Protein-Ligand contacts in PHYTO5 Model

Conclusions

- **Our study suggests, that out of 55 project dataset models, top 5 project dataset models such as PHYTO5, PHYTO13, PHYTO28, PHYTO1 and PHYTO52 might be used as antifungal inhibitor**

- **Our study with contact maps supported by simulations for the top model PHYTO5 suggests that SER57, GLU59, ARG65, HIS68, GLU74 and GLU 87 are involve in major hydrogen bond with the peptides**

- **Consideration of hydrogen-bonding properties in drug design is most important because of their strong influence on drug specificity, metabolization and adsorption**

- **To the best of our knowledge, there is no investigation of molecular interaction study of plant origin antifungal peptides with target fungal proteins of microbes infecting plants and animals**

Thank you