

New binding site of the quorum sensing molecule N-3-oxododecanoyl homoserine lactone with the transcriptional regulator LasR of *Pseudomonas aeruginosa*

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Introduction

In 2017 World Health Organization announced the list of the most dangerous superbugs and among them is *Pseudomonas aeruginosa*, which is an antibiotic-resistant opportunistic human pathogen. This organism attacks human patients suffering from such diseases as AIDS, cancer, cystic fibrosis, etc. Current therapies lack efficacy because *P. aeruginosa* creates and inhabits surface-associated biofilms conferring increased resistance to antibiotics and host immune responses. Biofilm formation is controlled through cell-cell communication system called quorum sensing. The main quorum sensing system of *P. aeruginosa* includes receptor protein LasR. The transcriptional regulator LasR acts as a dimer with the help of its native autoinducer ligand and binds to the promoter DNA. However, there remains a need for the understanding of the LasR protein, because till date there is no molecular detail information.

Objective

In the present study, we tried to analyze the molecular properties of the receptor protein LasR monomer as well as the mode of its interactions with signal molecule N-3-oxododecanoyl homoserine lactone (3-O-C12-HSL).

Methods

The whole methodology is presented as a flowchart for a better comprehension:

- LasR protein sequence taken from UniprotKB (sequence ID P25084) and performed blastp of LasR to identify suitable templates for reconstruction.
- HH-pred web server was used for the reconstruction of LasR monomer structure and validated using Verify 3D, Procheck, Prosa.
- 100ns MD simulation of the reconstructed LasR monomer using AMBER99SB-ILDN forcefield.
- The 3D model of 3-O-C12-HSL acquired from PubChem web server.
- Docking of 3-O-C12-HSL with the LasR monomer performed using Autodock Vina and 2000 docked poses were generated. PCA and cluster analysis performed on docking conformations.
- Extraction of centroid conformations from cluster analysis.
- Ligand parameters generated using Acypype interface in the framework of the AMBER force field.
- Using centroid conformations as starting points for molecular dynamics simulations using Gromacs.
- 900ns of MD data used for the analysis of the interaction.

Results

Part 1: Reconstruction of LasR Structure

LasR is a 239 residue long protein. The crystal structure of the N-terminal ligand binding domain (LBD) of LasR contains residues 1 to 169 (see Fig.1a). However, in order to find the mode of interactions between the LasR and 3-O-C12-HSL, the structure of the entire LasR protein was needed. The homology modeling of LasR, including DNA binding domain (DBD), was done with HHpred web server using the following templates (see Table 1 and Fig. 1b). We performed MD simulation run of 100 ns for the assessment of conformational changes of LasR (see Fig. 1c).

Table 1 List of similar structures to LasR obtained from BLAST search.

PDB ID	Identity (%)	Query(%)	E-value	Structure
3SZT	30	97	2×10^{-26}	Quorum Sensing Control Repressor, QscR
1FSE	37	28	2×10^{-4}	Bacillus subtilis regulatory protein gene
4Y15	30	76	3×10^{-16}	Sdia in complex with 3-oxo-c6-homoserine
3ULQ	32	25	0.09	Anti Activator Rapf complexed with DNA DBD

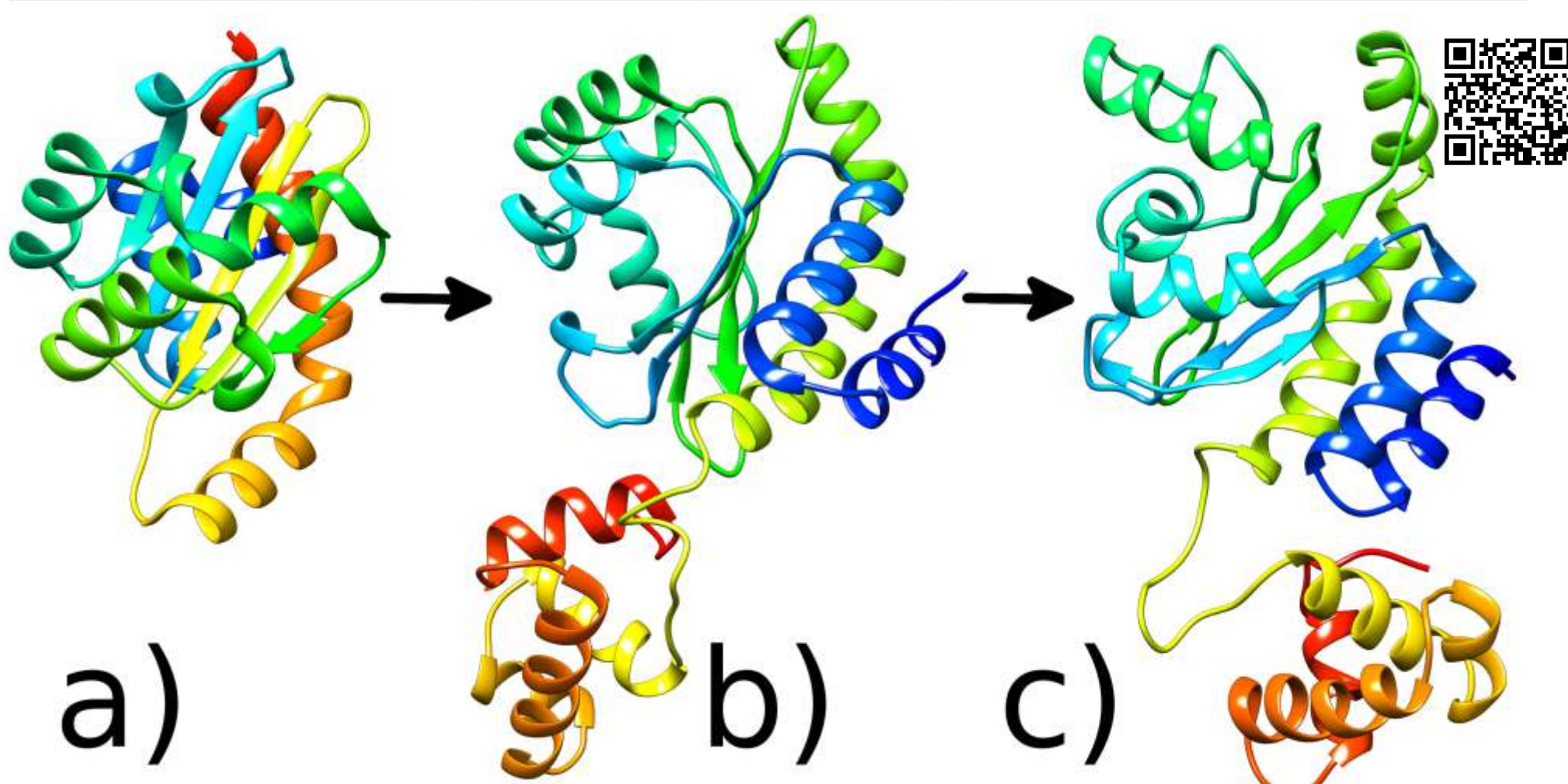


Fig. 1 3D LasR structures: a) Crystal structure of the LBD of LasR (PDB id: 3IX3) b) Reconstructed structure with DBD c) Representative structure after 100ns MD run.

Part 2: Docking analysis of 3-O-C12-HSL with LasR monomer

Molecular docking approach was used to inspect the possible binding modes of 3-O-C12-HSL in LasR. PCA and cluster analysis were performed on docking data. The results show three binding sites (see Fig. 2). An optimal number of clusters were chosen for docking results, simultaneously accounting for a low Davies-Bouldin Index, high Dunn, high Silhouette and high pSF values (see Fig. 2).

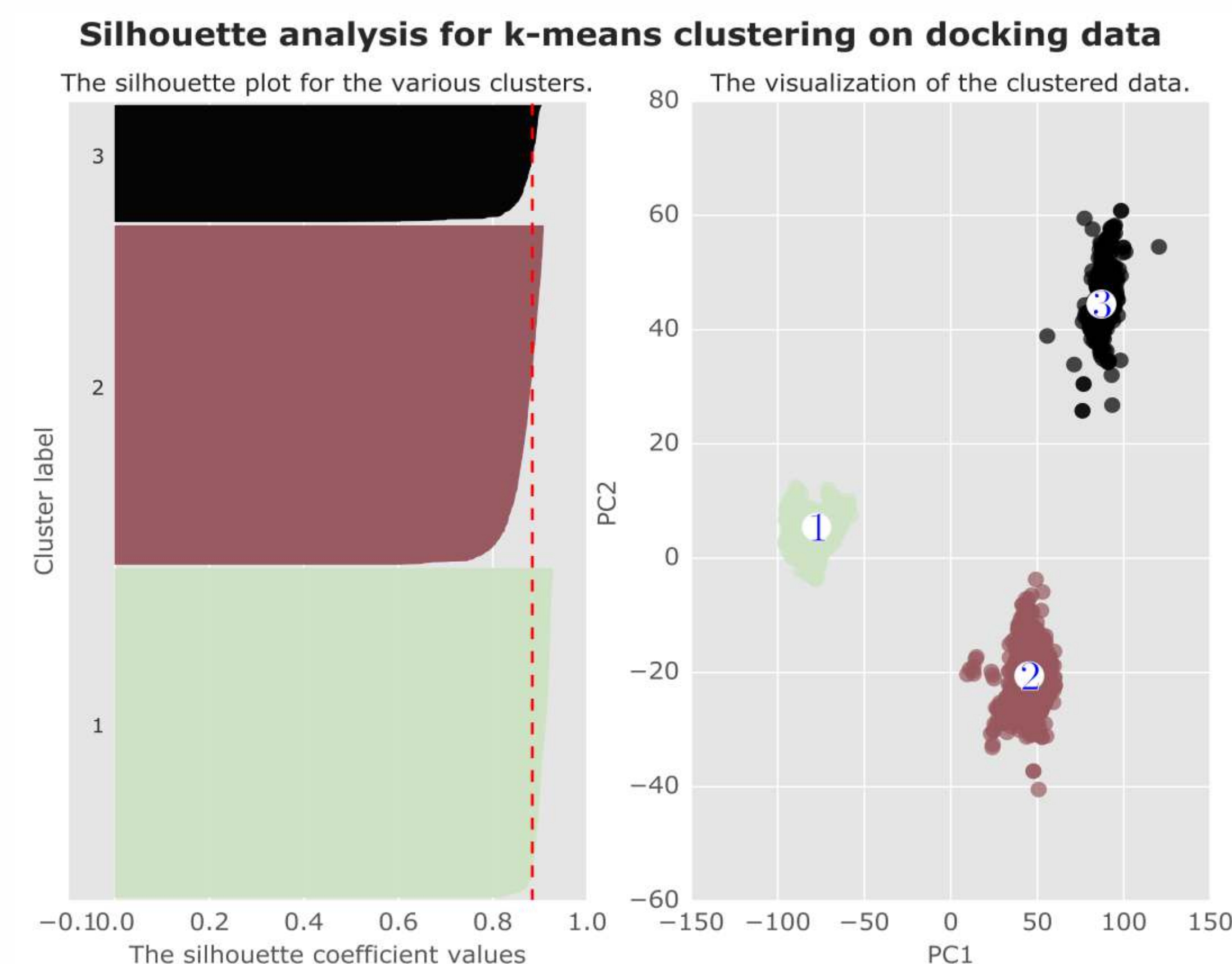


Fig. 2 Silhouette plot and clustering results on the docking data using k-means algorithm formed by first two PCs.

We generated 2000 docked poses and performed representative structure extraction for use in MD simulations of the LasR 3-O-C12-HSL binding sites. The results show three binding sites, cluster 2 corresponds to experimental data, while cluster 1 and cluster 3 do not (see Fig 3).

Representative structures from each cluster were extracted. These cluster representative structures were produced by finding the centroid conformations. The binding energy for the centroid conformation of cluster 1 is -5.4 kcal/mol. Cluster 1 contains 839 docked poses from 2000 (41.95%). For cluster 2 the binding affinity is -5.1 kcal/mol and this one corresponds to the experimental binding site data. Cluster 2 contains 864 docked poses from 2000 (43.2%). For cluster 3, the representative structure features the highest binding affinity -5.7 kcal/mol. Cluster 3 contains 297 docked poses from 2000 (14.85%), which is a rather unstudied area.

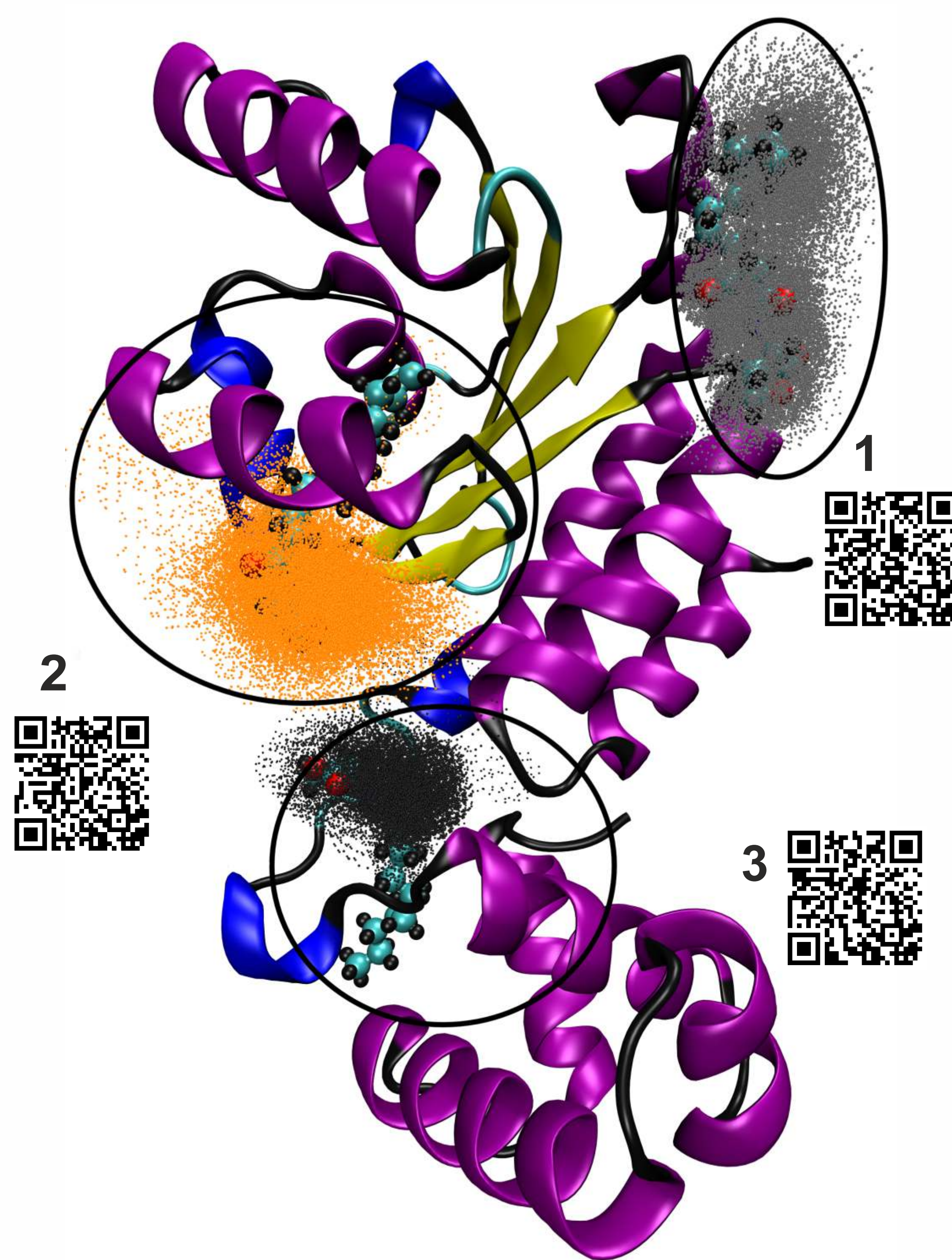


Fig. 3 3D visualization of the analyzed docking data with their representative structures and clusters. Mean binding affinities: cluster 1 - $\Delta G = -5.257 \pm 0.233$ kcal/mol, cluster 2 - $\Delta G = -5.593 \pm 0.386$ kcal/mol, cluster 3 - $\Delta G = -5.267 \pm 0.233$ kcal/mol.

Part 3: Binding Modes of 3-O-C12-HSL

We performed three 300 ns simulations using standard MD protocol. The representative structures were taken from docking results (see Fig. 3) and used as starting points for MD simulation with LasR. The overall stability was assessed using the RMSD of the backbone atoms (see Fig. 4). In simulation 1, which corresponds to cluster 1, the molecule of 3-O-C12-HSL did not fixate and reach equilibrium, so further research was not performed. Simulation 2 and 3 correspond to clusters 2 and 3 of docking data.

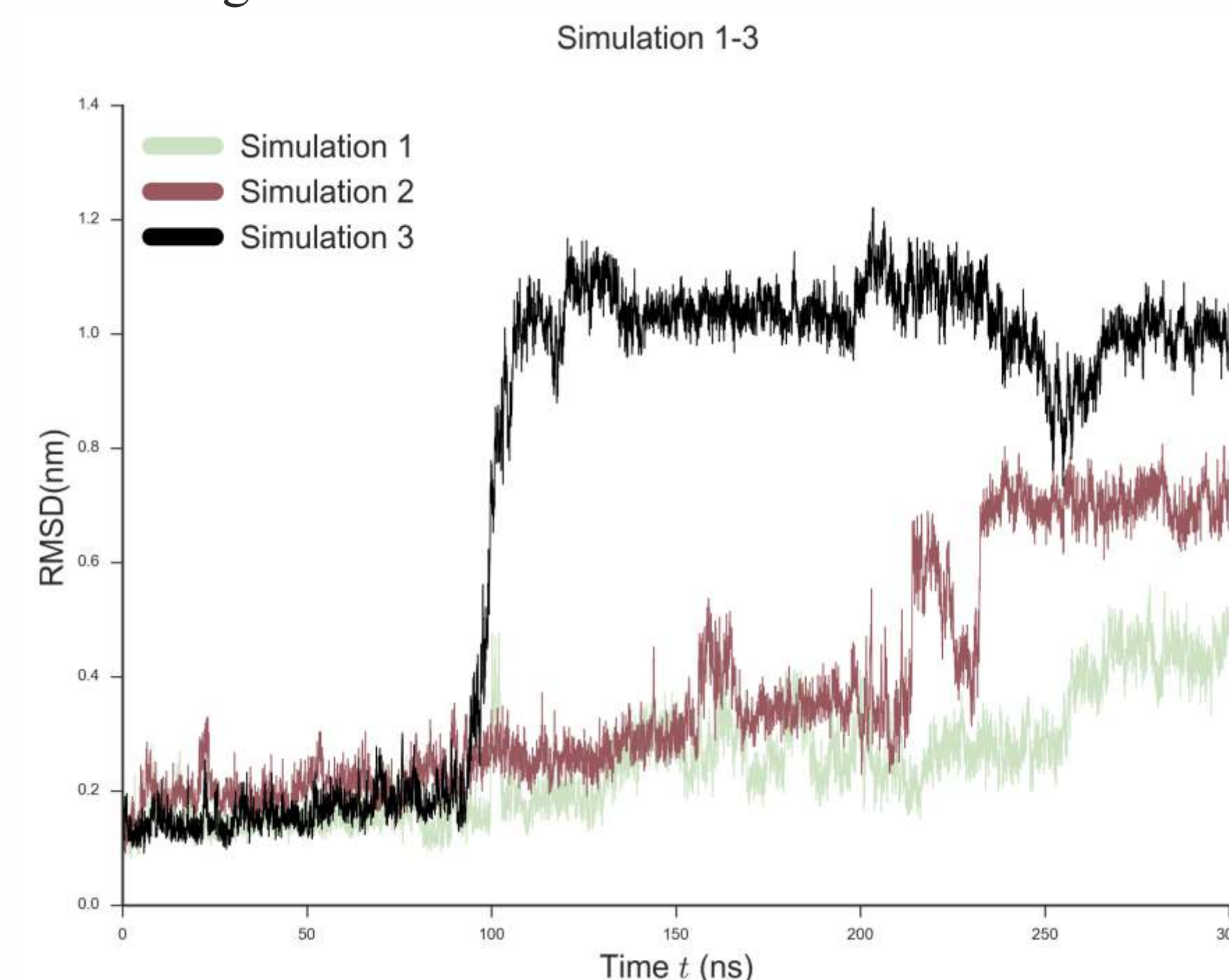


Fig. 4 RMSD evolution during 300 ns of simulation for 3 independent runs using representative structures of 3-O-C12-HSL docking poses as starting points.

Over the course of simulation 3, 3-O-C12-HSL establishes hydrogen bonds and a large number of hydrophobic contacts with amino acid side chains in the beta turns of the short linker region (SLR) of LasR. SLR connects LBD and DBD together. 3-O-C12-HSL forms 2 hydrogen bonds: O21 of C20 with Lys182 and O11 of C10 with Leu177 of the beta turns in the SLR of LasR. During the 300 ns trajectory, it was observed that the interaction between 3-O-C12-HSL and the Leu177 residue in LasR persisted for more than 93.64% of this simulation (from the period of 0-300ns).

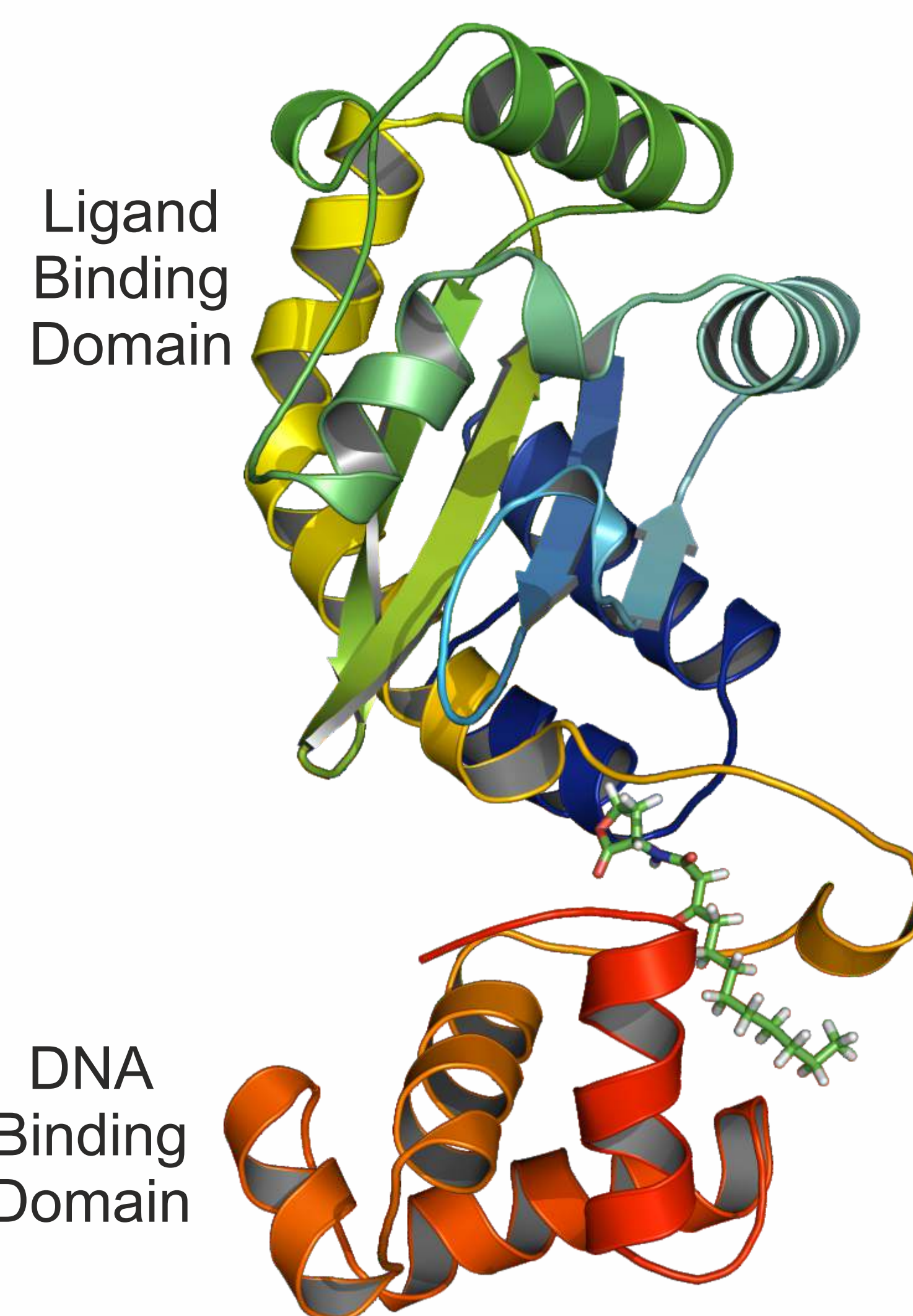


Fig. 5 Interaction of 3-O-C12-HSL with the SLR of LasR.

Summary

- It can be safely concluded that 3-O-C12-HSL can bind both to ligand binding domain and the short linker region of the transcriptional regulator LasR of human opportunistic pathogen *P. Aeruginosa*.
- So far this is the first report that shows that the native ligand 3-O-C12-HSL can interact with the beta turns in the short linker region (SLR) of receptor protein LasR, which is a new binding site.
- Results from this study may be used for future drug development endeavors, which are based on a new anti-infective strategy against quorum sensing.

Acknowledgements

- This work was supported by grant NIR 25/15 of the Russian-Armenian University.
- We thank Yerevan Physics Institute for providing time on the cluster for the molecular dynamics simulations.
- We also thank for the 13th Student Council Symposium (SCS) Travel Fellowship Award and Calouste Gulbenkian Foundation.

Further reading:

Grabski, H., Hunanyan, L., Tiratsuyan, S., Vardapetyan, H. 2017. Interaction of N-3-oxododecanoyl homoserine lactone with transcriptional regulator LasR of *Pseudomonas aeruginosa*: Insights from molecular docking and dynamics simulations. bioRxiv doi:10.1101/121681

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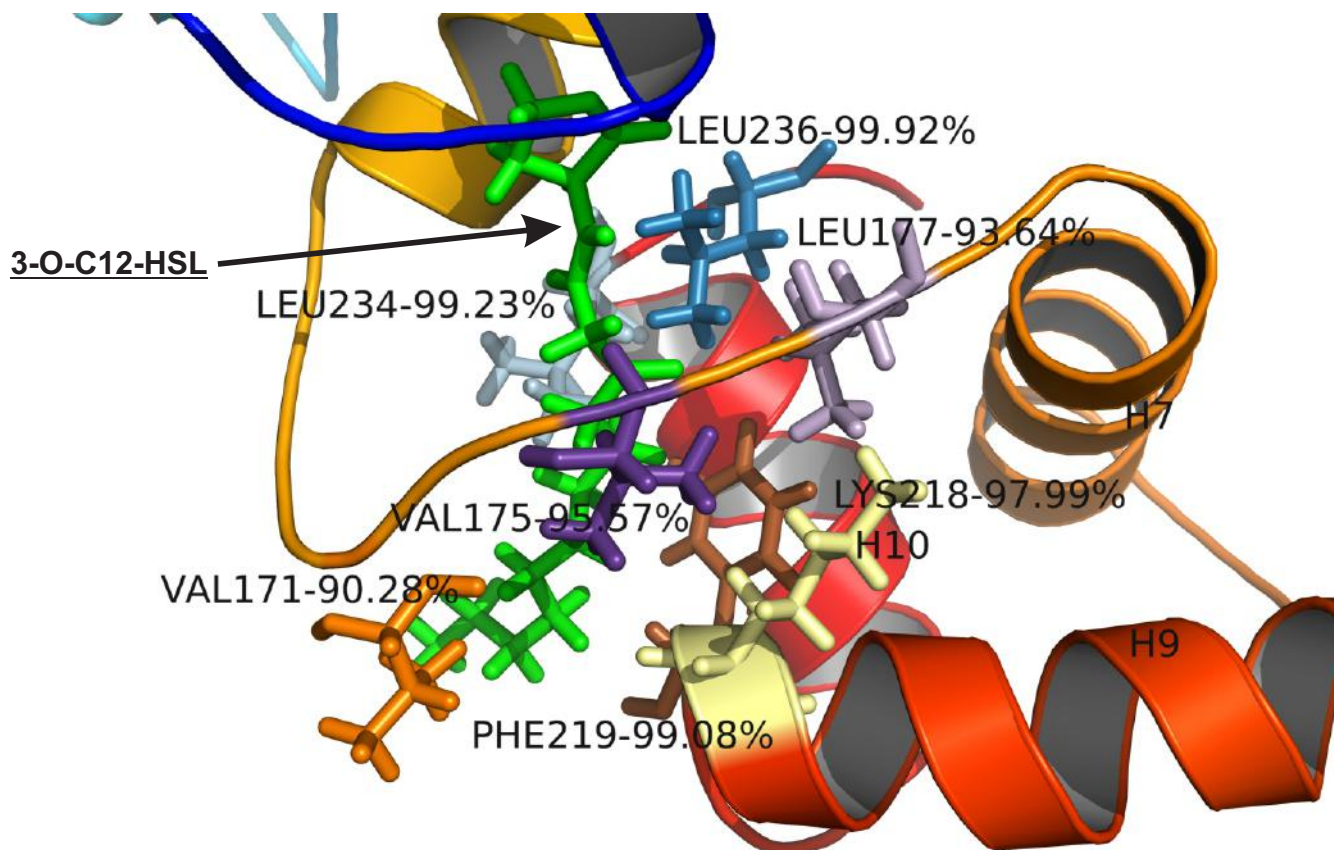


Fig. Interaction of 3-O-C12-HSL with the SLR of LasR.



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Further reading:

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