RESEARCH ARTICLE

Interaction of N-3-oxododecanoyl homoserine lactone with transcriptional regulator LasR of *Pseudomonas aeruginosa*: Insights from molecular docking and dynamics simulations [version 1; peer review: 1 approved, 1 approved with reservations, 1 not approved]

Hovakim Grabski¹, Lernik Hunanyan¹, Susanna Tiratsuyan^{1,2}, Hrachik Vardapetyan¹

¹Department of Medical Biochemistry and Biotechnology, Russian-Armenian University, Yerevan, 0051, Armenia

²Faculty of Biology, Yerevan State University, Yerevan, 0025, Armenia

V1 First published: 22 Mar 2019, 8:324
<https://doi.org/10.12688/f1000research.18435.1>

Latest published: 22 Mar 2019, 8:324
<https://doi.org/10.12688/f1000research.18435.1>

Abstract

Background: In 2017, the World Health Organization announced a list of the most dangerous superbugs. Among them is *Pseudomonas aeruginosa*, an opportunistic human pathogen with high levels of resistance to antibiotics that is listed as one of the 'ESKAPE' pathogens, which are the leading cause of nosocomial infections. A major issue is that it mostly affects vulnerable patients such as those suffering from AIDS, cystic fibrosis, cancer and severe burns. *P. aeruginosa* creates and inhabits surface-associated biofilms which increase resistance to antibiotics and host immune responses and contribute to the ineffectiveness of current antibacterial treatments. It is therefore imperative to find new antibacterial treatment strategies against *P. aeruginosa*. The LasR protein is a major transcriptional activator of *P. aeruginosa* and plays a pivotal role in biofilm formation and the activation of many virulence genes, although detailed characteristics of the LasR protein are not currently known. In the present study, we aimed to analyse the molecular properties of the LasR protein as well as its interactions with the signalling molecule N-3-oxododecanoyl homoserine lactone (3OC12-HSL).

Methods: We used a combination of molecular docking, molecular dynamics (MD) simulations and machine learning techniques to study the interaction of the LasR protein with the 3OC12-HSL ligand. We assessed conformational changes occurring upon their interaction and analysed the molecular details of their binding.

Results: A new possible interaction site for 3OC12-HSL and LasR was found, involving conserved residues from the ligand binding domain

Open Peer Review

Approval Status ✗ ✓ ?

	1	2	3
version 1	✗	✓	?
22 Mar 2019	view	view	view
1. Jon Paczkowski , Wadsworth Center, Albany, USA			
2. Faizul Azam , Unaizah College of Pharmacy, Qassim University, Unaizah, Saudi Arabia			
3. Kapil Dave , University of Illinois at Urbana-Champaign, Champaign, USA			

Any reports and responses or comments on the article can be found at the end of the article.

(LBD), beta turns in the short linker region (SLR) and the DNA-binding domain (DBD). This interaction is referred to as the LBD-SLR-DBD bridge or 'the bridge' interaction.

Conclusions: This study may enable future experimental studies to detect the interaction of signalling molecules with "the bridge" of the LasR protein and suggests a potential new interaction site to assist antibacterial drug design.

Keywords

Pseudomonas aeruginosa, transcriptional regulator, LasR, antibiotic resistant, ESKAPE, 3OC12-HSL, homology modelling, molecular docking, molecular dynamics, machine learning techniques

Corresponding author: Hovakim Grabski (hovakim.grabski@rau.am)

Author roles: **Grabski H:** Conceptualization, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Hunanyan L:** Supervision; **Tiratsuyan S:** Investigation, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Vardapetyan H:** Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The authors gratefully acknowledge financial support by the Russian-Armenian University [NIR 25/15]. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

Copyright: © 2019 Grabski H *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Grabski H, Hunanyan L, Tiratsuyan S and Vardapetyan H. **Interaction of N-3-oxododecanoyl homoserine lactone with transcriptional regulator LasR of *Pseudomonas aeruginosa*: Insights from molecular docking and dynamics simulations [version 1; peer review: 1 approved, 1 approved with reservations, 1 not approved]** F1000Research 2019, 8:324 <https://doi.org/10.12688/f1000research.18435.1>

First published: 22 Mar 2019, 8:324 <https://doi.org/10.12688/f1000research.18435.1>

Abbreviations

PDB, Protein Data Bank; MD, molecular dynamics; PCA, principal component analysis; 3OC12-HSL, N-3-oxododecanoyl homoserine lactone; AI, autoinducer; SLR, short linker region; BLAST, Basic local alignment search tool; DBI, David-Bouldin index; pSF, pseudo-F statistic; IQS, integrated quorum sensing; QS, quorum sensing; AHL, acyl homoserine lactone; HCN, hydrogen cyanide; HTH, helix-turn-helix; MSA, multiple sequence alignment; RMSD, root-mean-square-deviation; LBD, ligand binding domain; DBD, DNA binding domain

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative, monoflagellated, obligate aerobe^{1,2}. This species of bacteria is one of the 'ESKAPE' pathogens, which includes six bacterial pathogens which are commonly associated with antimicrobial resistance and are the leading cause of nosocomial infections throughout the world³. It can be found in many diverse environments such as in soil, plants and hospitals^{1,4}. *P. aeruginosa* is an opportunistic human pathogen because it rarely infects healthy people, mostly affecting patients suffering from AIDS, cystic fibrosis, cancer and burns^{2,5}. Most of the deaths caused by cystic fibrosis are due to this pathogen^{1,6}. The pathogenicity of *P. aeruginosa* is due to virulence factors such as: the synthesis of proteases, hemolysins, exotoxin A, pyocyanin, hydrogen cyanide (HCN) and rhamnolipids; the possession secretion systems types one (T1SS), two (T2SS), three (T3SS), four (T4SS)⁷, five (T5SS), and six (T6SS)⁸; the formation of biofilms^{7,9}.

Biofilm formation is a common characteristic of many bacteria as the bacterial population density increases in the body during pathology. These bacteria possess a system of regulating gene expression in response to population density, called quorum sensing (QS), that uses hormone-like molecules called autoinducers (AIs) which accumulate in the extracellular matrix. When a threshold is reached, the AIs bind to their cognate receptors and then a response regulator modulates the expression of QS virulence genes which regulate adaptation, colonization, antibiotic resistance, plasmid conjugation and other bacterial processes.

Pseudomonas aeruginosa has four QS systems¹⁰. The type one system is regulated by LuxI/LuxR-type proteins. AIs diffuse freely across the bacterial membrane and bind to the transcriptional activator LuxR. Type two and three systems are also LuxI/LuxR-type systems, the first LasI that produces 3OC12-HSL and the second, RhII that synthesizes C4-HSL; both acyl homoserine lactones (AHL) which regulate virulence and biofilm formation. A fourth integrated QS (IQS) system has been characterized very recently and the genes involved in IQS synthesis are non-ribosomal peptide synthase genes of the ambBCDE operon. The transcriptional regulator of IqsR is the IQS receptor¹¹.

QS regulates the expression of virulence factors such as HCN. Exposure to HCN can lead to neuronal necrosis through the inhibition of cytochrome c oxidase, the terminal component of the aerobic respiratory chain^{12,13}. In *P. aeruginosa*, the hcnABC operon is responsible for HCN biosynthesis by the enzyme HCN

synthase¹⁴. Three transcriptional regulators (LasR, ANR and RhIR¹⁵) control the transcription of the hcnABC gene cluster¹⁴ although it has been proposed that LasR is the crucial activator of hcnABC genes following mutagenesis experiments¹⁶.

The transcriptional activator protein LasR regulates target gene expression by recognizing a conserved DNA sequence termed a lux box^{16,17}. LasR has two domains: 1) a ligand binding domain at the N-terminus (LBD); 2) a DNA-binding domain at the C-terminus (DBD)¹⁸. LasR has a DNA-binding helix-turn-helix (HTH) motif¹⁹. Binding of the AI 3-O-C12HSL stabilizes LasR and promotes its dimerization. After that, the resulting LasR-AI homodimer complex binds to the promoter of the target DNA and induces gene transcription¹⁸.

During colonization and invasion, both the pathogen and the host are exposed to molecules released by each other, such as bacterial AIs or host stress hormones and cytokines. The mechanisms and receptors that might be involved in cross-talk between microbial pathogens and their hosts are not yet well described. LuxR homologue studies have demonstrated that LuxR-type activators are homodimers and consist of two domains. These two functional domains are joined by a short linker region^{20,21}.

There remains a need for a further understanding of the LasR monomer because to date there is no detailed information about LasR monomer interactions. Hence, we analysed the molecular details of the interactions of 3OC12-HSL with the LasR protein. So far, this is the first report that shows that 3OC12-HSL can interact with conserved amino acid residues from the ligand binding domain (LBD), beta turns in the short linker region (SLR) and the DNA-binding domain of LasR. For simplification, we refer to this region as the LBD-SLR-DBD bridge or "the bridge". This could mean that modulation of the transcriptional regulator LasR is more nuanced than previously thought. This study may be utilized for the development of new therapeutic agents against *P. aeruginosa* targeting both the LBD and the LBD-SLR-DBD bridge of LasR in order to inhibit the expression of virulence genes.

Methods

Figure 1. Flow chart providing an overview of the methodology used in this study.

Analysis of the LasR protein sequence

This part of the methodology was based on the work by Chowdhury *et al.* to reproduce the full structure of LasR protein¹. The amino acid sequence of the LasR protein of *P. aeruginosa* was obtained from UniprotKB²² (Uniprot ID: [P25084](#)). The crystal structure of the LasR protein LBD (amino acid residues 7 to 169) from *P. aeruginosa* was acquired from the Protein Data Bank²³ (PDB ID: [3IX3](#)). However, the entire structure of the LasR protein was required in order to have a better understanding of its molecular properties.

For this reason, the amino acid sequence (Uniprot ID: [P25084](#)) was used to search the PDB databank using BLASTp²⁴ in order to identify suitable templates for homology modelling. The

Figure 1. Flowchart of the research.

crystal structure of QscR bound to 3OC12-HSL was found to be the best fit based on sequence identity, query coverage, and E-value from the BLAST results (PDB ID: 3SZT)²⁰. The full list of similar structures obtained from the BLAST search are shown in Table 1.

Reconstruction of the LasR monomer

As the current available crystal structures of LasR only contain the ligand binding domain¹⁸, we needed to reconstruct the full structure of LasR with the missing DNA-binding domain (DBD). The HHPred web server²⁵ was used for homology modelling of the missing LasR DBD as was performed by Chowdhury *et al.*¹. The four templates used for homology modelling of the LasR DBD have the PDB identifiers 3SZT, 3IX3, 1FSE and 3ULQ. The experimental structure of LasR¹⁸ and these templates were used to reconstruct the final model of LasR that contains both the LBD and the DBD. The final reconstructed model of the LasR protein was verified using PROCHECK²⁵ (Figures S1 and S2, Extended data²⁶), Verify3D²⁶ and Ramachandran plots. More than 96.65% of the amino acid residues in the final reconstructed model had a 3D–2D score > 0.2 as indicated by Verify3D (Figure S3, see Extended data²⁶). This score indicates a suitable computational model²⁷. The Ramachandran plot (Figure S1, see Extended data²⁶) revealed that no amino acid residues were located in the disallowed regions. The ProSA²⁸ value of this model was -6.69 which suggests that the quality of the homology model is high²⁸. Although the obtained model is slightly different from that obtained by Chowdhury *et al.*¹ it is still viable.

Analysis of docking conformations and trajectories

The analysis of docking conformations and trajectories was performed using custom Python scripts (see Extended data²⁶). These scripts use pandas v0.8.1²⁹, scikit-learn v0.18.2³⁰ and MDTraj v1.9³¹ libraries. pandas²⁹ facilitates data manipulation and analysis.

In particular, it offers data structures and operations for manipulating numerical tables and time series. scikit-learn³⁰ is a machine-learning library, which provides a set of common algorithms to Python users through a consistent interface. The MDTraj Python library³¹ facilitates the combination of machine learning libraries such as scikit-learn for trajectory analysis. MDTraj serves as a bridge, connecting molecular dynamics (MD) data with statistics and graphics libraries developed for general data science users.

Diagrams of the interaction of 3OC12-HSL with LasR were obtained using LigPlot+ v.1.4³² using default parameters, which automatically generates 2D diagrams of ligand–protein interactions from 3D coordinates by loading the coordinate files in pdb format. Plot visualization was carried out using matplotlib v2.0.1^{32,33} with seaborn v0.8.1³⁴. The seaborn library aims to make visualization a central part of exploring and understanding data. It also provides a concise, high-level interface for drawing statistical graphics. Figures and videos were prepared with PyMOL v1.9.0.0³⁵, VMD 1.9.3³⁶ and UCSF Chimera v1.11.2³⁷. VMD³⁶ is often used for this purpose as it has the ability to read trajectory files created during simulations in formats produced by many different software packages. PyMOL³⁵ and UCSF Chimera³⁷ are also commonly used by researchers and produce high quality images but are typically less straightforward to use for viewing and making videos of the trajectories.

The analysis protocol was similar to the work of Wolf *et al.*³⁸ and Zamora *et al.*³⁹, using PCA and cluster analysis modules within scikit-learn v0.18.2. Principal component analysis (PCA) is an unsupervised statistical technique that is often used to make data easy to explore and visualize. PCA tries to explain the maximum amount of variance with the fewest number of principal components⁴⁰. The process of applying PCA to a protein trajectory is called essential dynamics (ED)^{41,42}. PCA

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

PDB ID	Sequence identity (%)	Query coverage (%)	E-value	Structure
3SZT	30	97	2×10^{-26}	Quorum Sensing Control Repressor, QscR, Bound to N-3-oxo-dodecanoyl-L-Homoserine Lactone
1FSE	37	28	2×10^{-4}	Crystal structure of Bascillus subtilis regulatory protein gene
4Y15	30	76	3×10^{-16}	Crystal structure of Sdia in complex with 3-oxo-c6-homoserine Lactone
3ULQ	32	25	0.09	Crystal structure of the Anti Activator Rapf complexed with the response regulator coma DNA binding domain

analysis, performed using Cartesian coordinates, has become an important tool for the examination of conformational changes. Default parameters within the scikit-learn library were used. Cluster analysis is another unsupervised technique that tries to identify structures within data. It is a data exploration tool for dividing a multivariate dataset into groups. Clustering algorithms can be grouped into partitional and hierarchical clustering approaches⁴³. kmeans algorithm^{44,45} was used for molecular docking analysis. Agglomerative clustering using Ward's linkage⁴⁶ was used for molecule dynamics trajectory analysis with the connectivity matrix built on the basis of the k-Neighbors graph³⁰. Default parameters within the scikit-learn library were used.

Molecular dynamics simulations of LasR protein systems

Molecular dynamics (MD) simulations of all systems were conducted with the GROMACS suite, version 5.1.2⁴⁷, utilizing the Amber ff99SB-ILDN force field⁴⁸, which exhibits considerably better agreement with NMR data than other forcefields. In all cases, short-range non-bonded interactions were cut off at 1.4 nm. The Particle mesh Ewald (PME) method^{49,50} was used for the calculation of long-range electrostatics.

LasR monomer simulation. In order to generate the starting structure of the LasR monomer before docking, the Las R molecule was placed in a dodecahedron box of TIP3P water⁵¹. After that, 100 mM NaCl was added, including neutralizing counterions. Following two minimizations by the method of steepest descent algorithm, the LasR monomer was equilibrated in two stages. The first stage involved simulating for 200 picoseconds under a constant volume (NVT) ensemble. The second stage involved simulating for 200 picoseconds under a constant pressure (NpT), maintaining pressure isotropically at 1.0 bar. Production simulation was conducted for 100 nanoseconds in the absence of any restraints. The temperature was sustained at 300 K using the v-rescale⁵² algorithm. For isotropic regulation of the pressure, the Parrinello-Rahman barostat⁵³ was used. This combination of thermostat and barostat use ensures that an adequate NpT ensemble is sampled. Finally, the trajectory of the LasR monomer simulation was used for the calculation of chemical shifts. SPARTA+ v2.90⁵⁴ and SHIFTX2 v1.10⁵⁵ were used to predict the chemical shifts of the protein backbone atoms with the help of the MDTraj wrapper functions³¹. Both programs have been proven to be remarkably accurate, especially for predicting

¹³C shifts⁵⁴. This was used to validate whether the force field is appropriate for further investigation. The final extracted structure that was used for docking and MD simulations was also verified with Gaia⁵⁶ from the Chiron webserver⁵⁷, which compares the intrinsic structural properties of *in silico* protein models to high resolution crystal structures.

Molecular dynamics simulations using docking poses. The 3D model of 3OC12-HSL was obtained from PubChem (CID: 127864) (Figure 2)⁵⁸. It has been shown that docking has its limitations⁵⁹. For this reason, after finishing the molecular docking simulations of 3OC12-HSL with LasR, the docking poses were extracted to perform molecular dynamics (MD) simulations. The ligand parameters for the molecular dynamics simulations were calculated for the General Amber Force Field (GAFF)⁶⁰ using the ACPYPE tool⁶¹ with AM1-BCC partial charges⁶² and generation of a GAFF topology. A time step of 2 femtoseconds was used during heating and the production run and coordinates were recorded every 1 picosecond. Three simulations of 300 nanoseconds were performed. Further details about the simulation protocol can be found in 'LasR monomer simulation' section.

LasR-3OC12-HSL ligand blind docking experiments

To build the LasR-ligand complex, the ligand 3OC12-HSL was docked with the LasR monomer using AutoDock Vina v1.1.2⁶³. However, AutoDock Vina currently uses several simplifications that affect the obtained results. The most notable simplification, as the creators note⁶³, is the use of a rigid receptor. The amount of computation used during a docking experiment is determined by a parameter called 'exhaustiveness' in AutoDock Vina^{63,64}. However, the default exhaustiveness value is 8 and the creators claim that increasing this value leads to an exponential increase in the probability of finding the energy minima^{63,64}. The conformational space of the whole protein was searched using grid box dimensions 60×62×48Å. The following exhaustiveness values were tested in this study: 8, 16, 32, 64, 128, 256, 512, 1024, 2048 and 4096. The ligand and target structures containing hydrogen atoms were prepared using the AutoDockTools⁶⁵ from MGLTools v1.5.6.

Principal component analysis (PCA)⁴⁰ and cluster analysis using k-means clustering^{44,45} was performed (Figures S4 and S5, Extended data²⁶). The results demonstrated that the number of

Figure 2. The stick representation of the native ligand of LasR (3O-C12-HSL).

interaction sites does not change for exhaustiveness values from 1024 to 4096. An exhaustiveness value of 1024 was chosen as it provides good results, good speed and thorough sampling of the docked configurations. The exhaustiveness value was set to 1024 and the maximum number of binding modes to generate was set to 20. After that, 100 independent docking calculations were carried out with random initial seeds. The target was taken from the molecular dynamics simulation of the LasR monomer. Water molecules were removed from the structure.

We also performed another set of docking simulations using AutoDock Vina v1.1.2⁶³, rDock v2013.1⁶⁶ and FlexAID v2.48⁶⁷. We performed 10 docking simulations using each program. Each individual run was set to generate 20 docked poses. For AutoDock Vina, we performed one docking run for each exhaustiveness value: 8, 16, 32, 64, 128, 256, 512, 1024, 2048 and 4096.

Docking was also performed using the rDock program⁶⁶ which was previously found to be successful in predicting the binding mode for the CCDC-Astex set of 85 diverse protein-ligand complexes in approximately 80% of cases⁶⁶. A cavity with a radius of 36 Å was set and centred on the LasR monomer to cover the whole protein surface and define the docking volume. AutoDock Vina and rDock were chosen based on the performance of these academic programs⁶⁸.

FlexAID (parameters are available in Extended data²⁶) was also used as, unlike other programs, it is robust against increasing structural variability⁶⁷. FlexAID performs better than AutoDock Vina in all scenarios against non-native complex structures⁶³. FlexAID uses a soft and smooth scoring function based on contact surfaces which is different from Autodock Vina and rDock. FlexAID was run using default parameters.

Binding energy calculation

g_mmpbsa v1.6⁶⁹ was used for the evaluation of binding energies. It was also used for the estimation of the energy contribution of each residue to the binding energy. g_mmpbsa calculates the relative binding energy rather than the absolute binding energy, so the entropy contribution (TAS) is not evaluated⁶⁹. From the two simulations with length of 300 nanoseconds, we cut out 10 nanoseconds with an interval of every 120 picoseconds for the calculation of binding energies. g_mmpbsa uses the MM-PBSA approach, which has grown to be one of the most widely used methods to compute interaction energies and is often employed to study biomolecular complexes⁶⁵.

Sequence conservation analysis

To find out whether 3OC12-HSLs interact with conserved amino acid residues, we performed multiple sequence alignment (MSA) using the R msa package v1.4.5⁷⁰. ClustalW⁷¹, Clustal Omega⁷² and MUSCLE⁷³ within the msa package were used to carry out multiple sequence alignments. ClustalW and MUSCLE are commonly and widely used, while Clustal Omega is a more recent and powerful method that is used when aligning a large number of sequences⁷². We performed multiple sequence alignment of the LasR protein and closest homologue sequences, based on the work by Chowdhury *et al.*¹. The consensus threshold parameter in msa was set to 75, the rest was set to default. Table 2 shows the sequences used for sequence alignment.

Protein-protein docking

When docking homology models, it is preferable to have experimental evidence suggesting the general interaction site (within ~10 Å). Representative structures from molecular dynamics simulations were used for protein-protein docking using ClusPro v2.0⁷⁴. ClusPro was chosen because it gave equivalent results to the best human predictor group according to the latest CAPRI experiments carried out in 2013⁶⁸.

From the experimental X-ray data, it was found that 'Trp152', 'Lys155' and 'Asp156' play an important role during dimerization. The distance between 'Trp152' from chain A and 'Asp156' from chain B of the crystallographic structure was 0.276 nm and the distance between 'Asp156' from chain A and 'Lys155' from chain B was 0.279 nm. These residues were used as attraction constraints.

Results

Conformational changes of LasR without 3OC12-HSL

We performed a simulation run of 100 nanoseconds using a standard MD protocol for the assessment of conformational changes of the LasR monomer. The overall stability of the molecule was assessed using the root-mean-square-deviation (RMSD) of the protein atoms. RMSD was calculated with reference to the initial frame through the time of the MD run (Figure S6, Extended data²⁶). Another suitable measurement for the stability of the LasR monomer structure is the radius of gyration, which also shows the structure is stable (Figure S7, Extended data²⁶).

Table 2. LasR and LasR homologue sequences from closely related species used for multiple sequence alignment.

Nº Reference Sequence	Nº Accession	Name	Species
1. WP_054058449.1	WP_054058449	LuxR family transcriptional regulator	<i>Pseudomonas fuscovaginae</i>
2. NP_250121.1	NP_250121	Transcriptional regulator LasR	<i>Pseudomonas aeruginosa</i> PAO1
3. KFC75736.1	KFC75736	LuxR-type transcriptional regulator	<i>Massilia</i> sp. LC238
4. WP_018433960.1	WP_018433960	LuxR family transcriptional regulator	<i>Burkholderia</i> sp. JPY251
5. WP_042326260.1	WP_042326260	LuxR family transcriptional regulator	<i>Paraburkholderia ginsengisoli</i>
6. WP_012426170.1	WP_012426170	LuxR family transcriptional regulator	<i>Paraburkholderia phytofirmans</i>
7. WP_027776298.1	WP_027776298	LuxR family transcriptional regulator	<i>Paraburkholderia caledonica</i>
8. WP_027214716.1	WP_027214716	LuxR family transcriptional regulator	<i>Burkholderia</i> sp. WSM2232
9. WP_041729325.1	WP_041729325	LuxR family transcriptional regulator	<i>Burkholderia</i> sp. CCGE1003
10. CBI71275.1	CBI71275	UnaR protein	<i>Paraburkholderia unamae</i>
11. CAP91064.1	CAP91064	BraR protein	<i>Paraburkholderia kururiensis</i>
12. WP_003082999.1	WP_003082999	Transcriptional activator protein LasR	<i>Pseudomonas</i>
13. WP_012076422.1	WP_012076422	LuxR family transcriptional regulator	MULTISPECIES: <i>Pseudomonas</i>
14. WP_050376898.1	WP_050376898	LuxR family transcriptional regulator	MULTISPECIES: <i>Pseudomonas</i>
15. WP_050395760.1	WP_050395760	LuxR family transcriptional regulator	<i>Pseudomonas aeruginosa</i>

During the examination of MD trajectories, principal component analysis (PCA)⁴⁰ is usually used for the visualization of the motion of the system (Figure 3a). Generally, in order to capture more than 70% of the variance in the trajectory data, the first handful of components are sufficient³⁹. PCA can uncover the fundamental movements contained in an MD trajectory, however, it does not group the snapshots into different clusters⁴³. This can be accomplished by clusterization of the PCA data.

Cluster analysis was performed on the LasR monomer simulation for the selection of an initial starting structure to study docking. Identifying a distinct, representative structure of the cluster allows blind docking to be performed on the whole structure. It should be also noted that cluster analysis allows the evaluation of the frequent conformations of LasR. For the clustering analysis, we chose agglomerative clustering using Ward's linkage⁴⁶. This algorithm appears to be the most appropriate as it is deterministic, allowing for reproducibility of the resulting clusters, thus minimizing the amount of bias.

We performed several rounds of agglomerative clustering using Ward's linkage. The accuracy of the clustering was assessed with the help of the Davies–Bouldin index (DBI)⁷⁵, Dunn index⁷⁶, silhouette score⁷⁷ and Calinski-Harabasz pseudo-F statistic (pSF)⁷⁸ metrics. An optimal number of clusters were chosen, simultaneously accounting for low DBI, high silhouette, high Dunn index and high pSF values.

The distribution of clusters over the simulation is visualized in Figure 3b and the four clusters are: cluster 4 (black) at the beginning of the simulation (after equilibration), cluster 2 (green) in the middle, cluster 1 (light green) and cluster 3 (dark blue)

at the end. The clusterization defined by the first two principal components (Figure 3b) provides a coherent picture and it is also supported by good DBI, Dunn index, silhouette score and pSF values (Figure S8, Extended data²⁶). Figure 3c shows clearly that the simulation has converged.

For validation of the molecular dynamics simulation quality, theoretical NMR shifts were calculated using Sparta⁵¹ and ShiftX2⁵². For the NMR shifts calculation, snapshots from cluster 3 (Figure 3b) were used because of their stability. We compared NMR values of C-alpha atoms from the experimental NMR of the LBD with theoretical NMR values of the LBD from our molecular dynamics simulations (BMRB: 6271)¹⁸. Unfortunately, the current available NMR information only includes the 173 amino acid residues of the LBD. In Figure 4, one can see that simulated NMR shift values from the two programs are close to the experimental data, thus demonstrating the quality of the MD simulation of the LasR monomer.

It should be noted this protein becomes partially soluble when the ligand interacts with it¹⁸. The differences between the theoretical chemical shifts and experimental values could be attributed to: 1) the theoretical model does not involve the interaction with the ligand 2) the experimental NMR only includes information about the LBD and not the DBD, which can affect the LBD.

After that, the representative structure (Figure 5c) was extracted from cluster 3 (Figure 3c) to study docking. The representative structure of the LasR system and its differences from the homology modelled structure (Figure 5b) are visible in Figure 5c. Secondary structure analysis of the representative structure

Figure 3. LasR monomer simulation analysis **a)** Simulation trajectory projected onto its first two principal components. The black scale indicates the time progression from 0 ns (white) to 100 ns (black) **b)** Clustering results of ward-linkage algorithm formed by first two PCs **c)** Colour-coded RMSD of the simulation obtained from Ward-linkage cluster analysis.

Figure 4. Plot of chemical shifts of C α -atom vs. residue number.

Figure 5. 3D LasR protein structures: **a)** Crystal structure of the N-terminal LBD of LasR protein **b)** Full structure of the protein with the missing DBD, which was modelled using homology modelling **c)** Representative structure after 100ns MD run. Images generated with UCSF Chimera³⁶.

was performed using PDBSum⁷⁹ (Figure S9, Extended data²⁶). Finally, the extracted centroid structure was validated using Gaia⁵⁶ (Figure S10, Extended data²⁶), which also shows the model is of a high quality. Therefore, in this study, a model has been developed to include the dynamics of the full-length LasR molecule (residues 1 to 239) and it has been shown that the dynamics of the complete C-terminal region of LasR modulate the N-terminal region, as will be discussed later.

Docking analysis of 3OC12-HSL with LasR monomer

In this study, a molecular docking approach was used to inspect the possible binding modes of 3OC12-HSL with the LasR monomer. PCA and cluster analysis were performed on docking data (Figure 6a, b) and the results show three binding sites. Cluster 2 corresponds to experimental data, while cluster 1 and cluster 3 do not (Figure 6c). These results clearly support the findings of Bottomley *et al.*¹⁸, who also demonstrated that 3OC12-HSL binds to the LBD.

We performed several rounds of k-means clustering. The accuracy of the cluster analysis was evaluated using the DBI⁷⁵, Dunn index⁷⁶, silhouette score⁷⁷ and pSF⁷⁸ metrics (Figure S11, see Extended data²⁶). An optimal number of clusters were chosen for docking analysis, simultaneously accounting for low DBI, high Dunn, high silhouette and high pSF values. We generated 2000 docked poses and performed representative structure extraction for use in MD simulations of the LasR 3OC12-HSL binding sites. The representative structures were produced by identifying the centroid conformations of the clusters using the following algorithm:

1. Compute all pairwise root mean square deviations (RMSDs) between the conformations.
2. Transform the distances into similarity scores and they are calculated as

$$S_{ij} = e^{-\beta d_{ij}/d_{scale}} \quad (\text{eq. 1})$$

Where s_{ij} is the pairwise similarity, d_{ij} is the pairwise distance, and d_{scale} is the standard deviation of the values of d to make the computation scale invariant.

3. Then the centroid is defined with $\beta=1$ as

$$\operatorname{argmax}_i \sum_j S_{ij} \quad (\text{eq. 2})$$

Representative structures from each cluster were extracted for further use in MD simulations. The binding energy of the representative structure of cluster 1 is -5.4 kcal/mol and the mean binding affinity for the whole cluster is -5.257 ± 0.233 kcal/mol. Cluster 1 contains 839 docked poses from a total 2000 (41.95%). For cluster 2, the binding affinity of the representative structure is -5.1 kcal/mol and the mean for the whole cluster is -5.593 ± 0.386 kcal/mol. These results correspond to the experimental binding site data¹⁸. Cluster 2 contains 864 docked poses from a total of 2000 (43.2%). For cluster 3, the representative structure features the highest binding affinity of -5.7 kcal/mol and the mean binding affinity for the whole cluster is -5.264 ± 0.27 kcal/mol. Cluster 3 contains 297 docked poses from 2000 (14.85%) which does not correspond to the experimental binding site.

It has been suggested that molecular docking is not appropriate for the prediction of binding affinity or binding poses of protein-ligand complexes. However, docking analysis can still provide important information regarding potential binding sites and the resulting poses can be used for MD simulations^{59,80}.

We also ran another set of simulations to corroborate the blind docking with other molecular docking software, including AutoDock Vina⁶³, rDock⁶⁶ and FlexAID⁶⁷ (Figure 7). All three programs, which each use different algorithms, suggest that there are two potential binding sites. One is within the LBD, which corresponds to experimental data¹⁸, and the other is within the 'bridge'. PCA of the results from these docking programs was performed for easier comprehension (Figure S12, Extended data²⁶).

Binding modes of 3OC12-HSL by MD simulations

In total, 900 nanoseconds of MD simulations have been performed and used for the analysis of 3OC12-HSL interaction with the LasR monomer. The representative structures were taken from docking results (Figure 6c) and used as starting points for MD simulations with LasR. Simulations were conducted for a sufficient time to allow the positions of 3OC12-HSL to reach equilibrium in the LasR molecule.

The stability of the molecule was evaluated by calculating the RMSD of the backbone atoms. RMSD was calculated with reference to the initial snapshot. Figure 8a shows that Simulation 2 and 3 experience a substantial RMSD deviation from the initial starting point. Simulation 2 corresponds to cluster 2 in the docking simulations, while Simulation 3 corresponds to cluster 3 (Figure 8a). For Simulation 1, which corresponds to cluster 1, the 3OC12-HSL molecule did not fixate and reach equilibrium and therefore no further analysis was performed (Figure 8a) for this simulation. Simulation 2 shows that after 230 nanoseconds, the structure becomes stable. For Simulation 3, the structure changes its conformation at 100 nanoseconds and becomes stable between 260 nanoseconds and 300 nanoseconds.

Figure 6. Analysis of Autodock Vina docking results **a)** Silhouette plot analysis of clusterization quality **b)** Clustering results using k-means algorithm formed by first two PCs **c)** 3D visualization of the analysed docking data with their representative structures and clusters.

Figure 7. Blind docking with various molecular docking programs. Red circles - binding interactions that are characteristic to all programs **a)** Autodock Vina **b)** rDock **c)** FlexAid.

Simulation 2 and 3 represent the binding sites predicted by all docking programs from Figure 7. Calculation of the root-mean-square fluctuation (RMSF) was used to evaluate the flexibility of the LasR monomer. The average per-residue RMSF was calculated for each simulation run (Figure 8b).

It is visible in Figure 8b that the residues from 165 to 176, which correspond to beta turns in the SLR of LasR, are highly mobile. Simulations 2 and 3 show that 3OC12-HSL has two binding modes, one with the LBD (Figure 9a), which corresponds to experimental data and one with the LBD-SLR-DBD bridge of LasR (Figure 9d).

PCA and cluster analysis were performed on simulation 2. Hydrogen bond analysis was also performed, based on cut-offs for distance and angles according to the Wernet-Nilson criteria⁸¹ using MDTraj³¹. During the simulation, 3OC12-HSL forms hydrogen bonds with Tyr56, Ser129 and Trp60, which is in agreement with experimental data¹⁸ (Figure S14, Extended data²⁶). Over the course of simulation 2, 3OC12-HSL also establishes a large number of hydrophobic contacts with the amino acid side chains of the LasR LBD (Figure 9b). This phenomenon is not unexpected, given the large hydrophobic surface area of the LasR LBD and the low solubility of 3OC12-HSL in water. 3OC12-HSL has hydrophobic interactions mainly with amino acids from helix 3, helix 5 and sheet 1 side chains. RMSD analysis of the conformations of LasR with and without 3OC12-HSL bound to the LBD gives a value of 7.027 Å between the conformations (Figure S16, Extended data²⁶). RMSD of the modelled full structure of LasR with ligand superimposed onto the experimentally obtained tertiary structure of LBD of LasR with ligand gives a value of 1.692 Å, where RMSD < 2 Å in relation to the experimentally studied structure (Figure 9c). Residues that participate in hydrophobic interactions and hydrogen bonding are shown in Figure 9e and Figure S18 and 19 (Extended data²⁶).

The second binding mode involves the interaction of 3OC12-HSL with the LBD-SLR-DBD bridge. PCA, cluster and hydrogen bond analysis were also performed on simulation 3. Over the course of simulation 3, 3OC12-HSL establishes hydrogen bonds and a large number of hydrophobic contacts with amino acid side chains in the beta turns of the LasR SLR. In simulation

Figure 8. Analysis for 3 independent runs of 300 ns using representative structures from 3OC12-HSL docking poses as starting points **a)** RMSD evolution **b)** RMSF of $C\alpha$ -atoms of LasR for each individual run.

Figure 9. Visualizations of the interactions of 3OC12-HSL with LasR. Conformations taken from centroids from cluster analysis **a)** 3OC12-HSL with LasR LBD **b)** Insertion of 3OC12-HSL in LBD of LasR **c)** Superimposition of the modelled interaction (red) of 3OC12-HSL with LasR LBD and crystallographic structure (green) **d)** 3OC12-HSL with the “bridge” of LasR **e)** Putative hydrogen bonds of 3OC12-HSL with Lys182 and Leu177.

3, 3OC12-HSL forms hydrogen bonds mainly with the ‘Lys182’ and ‘Leu177’ beta turn residues. RMSD analysis of the two conformations of the LasR monomer and when LasR is bound to 3OC12-HSL via the DBD gives a value of 1.677 Å (Figure S20, see Extended data²⁶). Residues that participate in hydrogen bonding and hydrophobic interactions are shown in Figure S16. Complete data sets for MD simulations are available as extended data (Figures S13-S20²⁶).

Binding energy of 3OC12-HSL to LasR and sequence conservation

In order to analyse the binding sites in detail, MM-PBSA⁶⁹ binding energy calculations were performed for each binding site based on the MD trajectories. The binding energy calculations demonstrated that the interaction of the ligand with the LBD-SLR-DBD bridge is not competitive with its interaction with the LBD (Table 3). Analysis of the energy terms demonstrated that only polar solvation energy contributes positively. However, for the interaction with the “the bridge”, the electrostatic interaction energy is 3.6 times higher than the energy for the LBD interaction.

This suggests that a combination of Van der Waals, electrostatic interaction and non-polar solvation energy contribute to the stability of the LasR-3OC12-HSL binding complex. To assess which amino acids are involved, we performed analysis of the energy contribution of residues to binding for both simulations obtained from MM-PBSA calculations using *g_mmpbsa*. Sequence alignment was performed using the R *msa* package⁷⁰. ClustalW⁷¹, Clustal Omega⁷² and Muscle⁷³ algorithms were used for sequence alignment. Full alignments using these algorithms are available as extended data²⁶. Analysis suggested that LasR residues Tyr64, Tyr56, Trp88, Leu36 and Trp60 contribute the most to binding with 3OC12-HSL (Figure 10a), with a binding energy of approximately -128.578 kJ/mol (Table 3).

Energy calculations to assess residue interactions and sequence alignment show that 3OC12-HSL interacts with highly conserved amino acid Trp60, which agrees with experimental data⁸². The interaction also involves nine other conserved amino acids; Tyr64, Asp73, Pro74, Val76, Phe102, Phe103, Ala105 and Gly113 (Figure 10b). In total, 16 amino acids that contribute to the interaction have over 75% conservation.

Analysis suggested that ligand residues Leu236, Leu177, Val176, Phe219, Lys182 and Trp19 contribute the most (Figure 10c) to the newly described binding state involving ‘the bridge’ of LasR, with a binding energy of approximately -166.456 kJ/mol (Table 3). The analysis of residue sequence alignment shows that 3OC12-HSL interacts with highly conserved amino acids such as Leu236, Leu177, Phe219 and Trp19 (Figure 10d). The 3OC12-HSL interacts with 12 fully conserved amino acids in this case. In total, 16 amino acids participate in the interaction, where amino acid conservation is more than 75%. In this new binding site, Trp19 and Asp156 of the LBD, Leu177 of the SLR and Arg180, Glu181, Glu196, Lys218, Arg224 and Lys236 of the DBD of LasR participate in the interactions with 3OC12-HSL. A lactone head group interacts with the conserved Trp19 of the LBD.

Protein docking of MD models

It is known that LasR binds to the corresponding promoter as a dimer¹ after interacting with the autoinducer. Thus, monomeric LasR-3OC12-HSL complexes were subjected to dimerization as an additional experiment. The protein docking experiments using structures from MD runs were performed using ClusPro v2.0^{74,83–85} because of its success in the CAPRI (Critical Assessment of Predicted Interactions) scoring experiment. We used the centroid conformations of the LasR bridge-3OC12-HSL and LasR LBD-3OC12-HSL complexes from MD

Table 3. Relative binding energies calculated from simulation data using g_mmpbsa.

Sim №	Binding sites	Van der waals (kJ/mol)	Electrostatic interactions (kJ/mol)	Polar salvation (kJ/mol)	Non-polar salvation (kJ/mol)	Binding energy (kJ/mol)
2	LBD	-215.673 ±8.007	-39.586 ±18.817	147.522 ±13.978	-20.840 ±0.794	-128.578 ±18.757
3	LBD-SLR- DBD bridge	-205.141 ±11.803	-142.974 ±29.032	201.889 ±20.117	-20.230 ±0.842	-166.456 ±20.492

Figure 10. Energy contributions of residues and multiple sequence alignments demonstrating the conserved amino acid residues of LasR that interact with 3OC12-HSL: **a)** All amino acids that interact with LBD **b)** Conserved amino acids that interact with LBD. Blue boxes and arrows point the amino acid residues that interact with 3OC12-HSL **c)** All amino acids that interact with the "bridge" **d)** Conserved amino acids that interact with "bridge". Black boxes and arrows point the residues that interact with 3OC12-HSL.

simulations. Then we performed protein docking using protein conformations without the ligands and used constraints based on the residue distances between monomers from the experimental data¹⁸. For the selection of the model, we used an approach recommended by the authors of ClusPro⁷⁴, which suggests finding the most populated clusters. For LasR bridge-3OC12-HSL complex docking, the top model contained 80 members and

the scores by ClusPro for the docking model were -951.4 for the centre and -1332.0 and for the lowest energy region, suggesting a favourable binding mode (Figure 11a). For the LBD LasR-3OC12-HSL complex, the top model contained 122 members and the scores for the docking model were -1440.7 for the centre and -1517.9 for the lowest energy region (Figure 11b). Both docking structures feature negative energy and this could

Figure 11. LasR monomer protein docking results and crystallographic dimer structure of quorum sensing control repressor QscR bound to 3OC12-HSL **a)** using centroid conformation from the LBD LasR-3OC12-HSL complex **b)** using centroid conformation from the “bridge”-3OC12-HSL complex **c)** crystallographic dimer structure of QscR, which is a homologue of LasR.

suggest the possibility of multiple docking conformations. It is possible that the multiple binding modes of 3OC12-HSL imply that LasR has multiple dimerization interfaces like QscR (Figure 11c)²⁰, which is a post-translational repressor of LasR. In the absence of 3OC12-HSL, it forms a heterodimer with LasR. It appears this protein may have a much more delicate regulation than was initially thought.

Discussion

Pseudomonosa aeruginosa is one of the ESKAPE pathogens³, which are the leading cause of nosocomial infections throughout the world. However, the molecular mechanisms of the LasR protein in regulating quorum sensing machinery have not been fully elucidated. LasR has been problematic to structurally analyse for the last decade because of the insolubility of the full protein¹⁸. All of the available crystallographic structures contain only the LBD and lack the DBD. The lack of a full crystal structure has prevented us from furthering our understanding of LasR as knowledge of its full 3D structure is vital. It is therefore very important to study the interaction of the autoinducer with the full structure of the LasR protein. Adequate knowledge of this interaction would provide the opportunity to conduct improved structure-based drug discovery studies.

Results from our analysis show that the interaction of 3OC12-HSL with LasR has two binding modes. The results were cross-checked using multiple docking programs, molecular dynamics simulations and machine learning techniques. The 3OC12-HSL has a long hydrophobic tail and is therefore capable of binding to both the LBD and the “bridge” of LasR. These results suggest that both the C-terminal and the N-terminal regions of LasR interact with 3OC12-HSL. The binding of 3OC12-HSL provokes conformational transitions in the structure of LasR. Binding energy analysis showed that the “bridge” does not compete with LBD. An MM-PBSA approach was used for the calculation of relative binding energy⁶⁹, although this finding needs confirmation through further research. The calculation of absolute binding energy could give more reliable results, though this type of calculation is more time consuming⁶². The results of our *in silico* experiments for the interaction of 3OC12-HSL with the LasR LBD is comparable with the results of other

authors^{18,82}, although currently there are no studies investigating the DBD.

This work is connected to recent experimental studies concerning LasR regulation. It should be noted that the LasR quorum-sensing system of *P. aeruginosa* is regulated by pre-quorum and post-quorum regulating systems⁸⁶. The first system is controlled by post-translational repressors QscR, QslA and QteE^{20,86–89}. In the absence of 3OC12-HSL, they can each form a heterodimer with LasR. Structural and functional studies of bacterial QS anti-activators revealed three different modes of action for LuxR-type transcriptional regulation: the destruction of the LBD dimerization interface in *P. aeruginosa*, the occupation of the AHL binding pocket and binding to the DBD¹¹. All these interactions indirectly change the conformation of the DBD, thus allosterically preventing DNA binding. It was previously shown that QslA can bind to LasR and thereby inhibit its DNA-binding capability⁸⁶. Moreover, it has been shown that QslA suppresses formation of the LasR homodimer complex by occupying the same surface area that is used for dimerization^{88,89}.

It has also been shown that QscR interacts with 3OC12-HSL²⁰. QscR has multiple dimerization interfaces and possesses both an LBD and DBD²⁰. The heterodimer QslA-LasR affects the dimerization interface and prevents receptor activation and DNA promoter binding⁸⁸. According to one of the mechanisms, the anti-activator QslA blocks the regions that are necessary for transcription factors multimerization¹⁵. Moreover, 3OC12-HSL does not overcome the repression of LasR exerted by QslA. This indicates that 3OC12-HSL interacts with helices that form part of these dimerization interfaces. It may be that the multiple binding modes of 3OC12-HSL imply that LasR has multiple dimerization interfaces like QslA and QscR.

There is also another aspect to be discussed. One major strategy for LasR inhibition is the development of small-molecule antagonists that mimic the native autoinducer and inactivate LasR. However, the mechanism by which they inactivate LasR is unknown⁹⁰. It has been suggested that LasR interactions with these antagonists promote a robust, unnatural fold of LasR that

does not permit DNA binding⁹⁰. Further research studying flavonoids as potential inhibitors shows that they do not bind in the ligand-binding pocket of LasR⁹¹. It has also been shown that they prevent LasR from binding to promoter DNA, but do not stop dimerization⁹¹. Unfortunately, in this study it was not possible to assay the LasR DBD due to insufficient protein yields⁹¹. The author suggest that the AI and flavonoid inhibitors can simultaneously bind to LasR and therefore postulate that flavonoids do not use the canonical AI binding site. We suggest that flavonoids and other inhibitors may interact with the bridge, thereby preventing LasR from binding to promoter DNA.

Our model might explain the molecular mechanisms proposed by recent experimental studies involving the study of flavonoids and other inhibitors which suggest the possibility of another binding site^{90,91}. It may also explain why computational drug design has not been successful in targeting this protein. However, the lack of a full, experimentally-derived structure for LasR is problematic and requires further experimental and computational research. We expect that our molecular insights on LasR can shed more light on this protein and assist in the development of new treatments against *P. aeruginosa*.

Conclusion

From the simulations, it appears that the AI ligand 3OC12-HSL can bind to both the LBD and the “bridge” of the transcriptional regulator LasR. The interaction with the “bridge” is a novel site. Binding energy analysis shows that the interaction of 3OC12-HSL with the “bridge” and its interaction with the LBD is not competitive. Conserved amino acids such as Leu236, Phe219, Leu177, Lys182 and Trp19 contribute the most during the interaction with “bridge”. This could suggest that the interaction of 3OC12-HSL with the “bridge” is necessary for the DNA binding capability of LasR. One possible explanation for these multiple binding sites is that LasR may have multiple dimerization interfaces. This study may reveal new insights into the interactions of the native 3OC12-HSL ligand with transcriptional regulator LasR in *P. aeruginosa*. Results from this study may aid future drug development endeavours.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Zenodo: Interaction of N-3-oxododecanoyl homoserine lactone with transcriptional regulator LasR of *Pseudomonas aeruginosa*:

Insights from molecular docking and dynamics simulations.
<https://doi.org/10.5281/zenodo.2586559>²⁶

This project contains the following extended data:

- AdditionalFile1_SupportingInformation.pdf (Figures S1-20)
- AdditionalFile2_ClustalW.pdf (Full sequence alignment of LasR using ClustalW algorithm)
- AdditionalFile3_ClustalOmega.pdf (Full sequence alignment of LasR using Clustal Omega algorithm)
- AdditionalFile4_Muscle.pdf (Full sequence alignment of LasR using Muscle algorithm)
- HSL_dock.zip (Molecular docking simulations input and output for AutoDock Vina)
- HSL_exhaustiveness.zip (Molecular docking simulations input and output for the determination of exhaustiveness (AutoDock Vina))
- LasR_HSL_multidock.zip (Molecular docking simulations input and output for AutoDock Vina, rDock and FlexAID)
- MD100ns.tar.gz (Molecular dynamics simulation data of LasR protein for 100 nanoseconds)
- HSL_LasR_simulation_1.zip (1st MD simulations data for 3OC12-HSL with LasR) HSL_LasR_simulation_2.zip (2nd MD simulation data for 3OC12-HSL with LasR)
- HSL_LasR_simulation_3.zip (3rd MD simulation data for 3OC12-HSL with LasR)
- scripts.zip (Scripts that were used for the analysis of the data based on the molmolpy set of scripts⁹²)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Grant information

The authors gratefully acknowledge financial support by the Russian-Armenian University [NIR 25/15].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We thank Yerevan Physics Institute for providing time on the cluster for the molecular dynamics simulations.

References

1. Chowdhury N, Bagchi A: **Molecular insight into the activity of LasR protein from *Pseudomonas aeruginosa* in the regulation of virulence gene expression by this organism.** *Gene*. 2016; **580**(1): 80–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. Botzenhart K, Döring G: **Ecology and Epidemiology of *Pseudomonas aeruginosa*.** In: Campa M, Bendinelli M, Friedman H, editors. *Pseudomonas Aeruginosa Opportunistic Pathog*, Boston, MA: Springer US; 1993: 1–18.
[Publisher Full Text](#)
3. Pendleton JN, Gorman SP, Gilmore BF: **Clinical relevance of the ESKAPE pathogens.** *Expert Rev Anti Infect Ther*. 2013; **11**(3): 297–308.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Lederberg J: **Infectious history.** *Science*. 2000; **288**(5464): 287–93.
[PubMed Abstract](#) | [Publisher Full Text](#)
5. Irvin RT: **Attachment and Colonization of *Pseudomonas aeruginosa*: Role of the Surface Structures.** In: Campa M, Bendinelli M, Friedman H, editors. *Pseudomonas Aeruginosa Opportunistic Pathog*, Boston, MA: Springer US;

- 1993; 19–42.
[Publisher Full Text](#)
6. Govan JR, Deretic V: **Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and Burkholderia cepacia.** *Microbiol Rev.* 1996; **60**(3): 539–74.
[PubMed Abstract](#) | [Free Full Text](#)
 7. Coggan KA, Wolfgang MC: **Global regulatory pathways and cross-talk control *Pseudomonas aeruginosa* environmental lifestyle and virulence phenotype.** *Curr Issues Mol Biol.* 2012; **14**(2): 47–70.
[PubMed Abstract](#) | [Publisher Full Text](#)
 8. Filloux A: **Protein Secretion Systems in *Pseudomonas aeruginosa*: An Essay on Diversity, Evolution, and Function.** *Front Microbiol.* 2011; **2**: 155.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 9. Van Delden C, Iglewski BH: **Cell-to-cell signaling and *Pseudomonas aeruginosa* infections.** *Emerg Infect Dis.* 1998; **4**(4): 551–60.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 10. Lee J, Wu J, Deng Y, *et al.*: **A cell-cell communication signal integrates quorum sensing and stress response.** *Nat Chem Biol.* 2013; **9**(5): 339–43.
[PubMed Abstract](#) | [Publisher Full Text](#)
 11. Lee J, Zhang L: **The hierarchy quorum sensing network in *Pseudomonas aeruginosa*.** *Protein Cell.* 2015; **6**(1): 26–41.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 12. Solomonson LP: **Cyanide as a metabolic inhibitor.** *Cyanide Biol B Vennesland Al.* 1981.
[Reference Source](#)
 13. Niquet J, Baldwin RA, Allen SG, *et al.*: **Hypoxic neuronal necrosis: protein synthesis-independent activation of a cell death program.** *Proc Natl Acad Sci U S A.* 2003; **100**(5): 2825–30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 14. Laville J, Blumer C, Von Schroetter C, *et al.*: **Characterization of the *hcnABC* gene cluster encoding hydrogen cyanide synthase and anaerobic regulation by ANR in the strictly aerobic biocontrol agent *Pseudomonas fluorescens* CHA0.** *J Bacteriol.* 1998; **180**(12): 3187–96.
[PubMed Abstract](#) | [Free Full Text](#)
 15. Pessi G, Haas D: **Transcriptional control of the hydrogen cyanide biosynthetic genes *hcnABC* by the anaerobic regulator ANR and the quorum-sensing regulators LasR and RhIR in *Pseudomonas aeruginosa*.** *J Bacteriol.* 2000; **182**(24): 6940–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 16. Fuqua C, Greenberg EP: **Self perception in bacteria: quorum sensing with acylated homoserine lactones.** *Curr Opin Microbiol.* 1998; **1**(2): 183–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 17. Eglund KA, Greenberg EP: **Quorum sensing in *Vibrio fischeri*: elements of the *luxI* promoter.** *Mol Microbiol.* 1999; **31**(4): 1197–204.
[PubMed Abstract](#) | [Publisher Full Text](#)
 18. Bottomley MJ, Muraglia E, Bazzo R, *et al.*: **Molecular Insights into Quorum Sensing in the Human Pathogen *Pseudomonas aeruginosa* from the Structure of the Virulence Regulator LasR Bound to Its Autoinducer.** *J Biol Chem.* 2007; **282**(18): 13592–600.
[PubMed Abstract](#) | [Publisher Full Text](#)
 19. Fukushima J, Ishiwata T, Kurata M, *et al.*: **Intracellular receptor-type transcription factor, LasR, contains a highly conserved amphipathic region which precedes the putative helix-turn-helix DNA binding motif.** *Nucleic Acids Res.* 1994; **22**(18): 3706–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 20. Lintz MJ, Oinuma K, Wysoczynski CL, *et al.*: **Crystal structure of QscR, a *Pseudomonas aeruginosa* quorum sensing signal receptor.** *Proc Natl Acad Sci U S A.* 2011; **108**(38): 15763–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 21. Costas C, López-Puente V, Bodelón G, *et al.*: **Using surface enhanced Raman scattering to analyze the interactions of protein receptors with bacterial quorum sensing modulators.** *ACS Nano.* 2015; **9**(5): 5567–76.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. UniProt Consortium: **UniProt: a worldwide hub of protein knowledge.** *Nucleic Acids Res.* 2019; **47**(D1): D506–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 23. Berman HM, Westbrook J, Feng Z, *et al.*: **The Protein Data Bank.** *Nucleic Acids Res.* 2000; **28**(1): 235–42.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 24. Altschul SF, Gish W, Miller W, *et al.*: **Basic local alignment search tool.** *J Mol Biol.* 1990; **215**(3): 403–10.
[PubMed Abstract](#) | [Publisher Full Text](#)
 25. Söding J, Biegert A, Lupas AN: **The HHpred interactive server for protein homology detection and structure prediction.** *Nucleic Acids Res.* 2005; **33**(Web Server issue): W244–248.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 26. Grabski H, Hunanyan L, Tiratsuyan S, *et al.*: **Interaction of N-3-oxododecanoyl homoserine lactone with transcriptional regulator LasR of *Pseudomonas aeruginosa*: Insights from molecular docking and dynamics simulations.** 2019. <http://www.doi.org/10.5281/zenodo.2586559>
 27. Eisenberg D, Lüthy R, Bowie JU: **VERIFY3D: assessment of protein models with three-dimensional profiles.** *Methods Enzymol.* 1997; **277**: 396–404.
[PubMed Abstract](#) | [Publisher Full Text](#)
 28. Wiederstein M, Sippl MJ: **ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins.** *Nucleic Acids Res.* 2007; **35**(Web Server issue): W407–10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 29. McKinney W: **pandas: a foundational Python library for data analysis and statistics.** *Python High Perform Sci Comput.* 2011; **14**.
[Reference Source](#)
 30. Pedregosa F, Varoquaux G, Gramfort A, *et al.*: **Scikit-learn: Machine Learning in Python.** *J Mach Learn Res.* 2011; **12**: 2825–30.
[Reference Source](#)
 31. McGibbon RT, Beauchamp KA, Harrigan MP, *et al.*: **MDTraj: A Modern Open Library for the Analysis of Molecular Dynamics Trajectories.** *Biophys J.* 2015; **109**(8): 1528–32.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 32. Laskowski RA, Swindells MB: **LigPlot+: multiple ligand-protein interaction diagrams for drug discovery.** *J Chem Inf Model.* 2011; **51**(10): 2778–86.
[PubMed Abstract](#) | [Publisher Full Text](#)
 33. Droettboom M, Caswell TA, Hunter J, *et al.*: **Matplotlib/Matplotlib V2.0.1.** 2017.
[Publisher Full Text](#)
 34. Waskom M, Botvinnik O, O’Kane D, *et al.*: **Mwaskom/Seaborn: V0.8.1 (September 2017).** 2017.
[Publisher Full Text](#)
 35. DeLano WL, others: **Pymol: An open-source molecular graphics tool.** *CCP4 Newsl Protein Crystallogr.* 2002; **40**: 82–92.
[Reference Source](#)
 36. Humphrey W, Dalke A, Schulten K: **VMD: visual molecular dynamics.** *J Mol Graph.* 1996; **14**(1): 33–8, 27–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 37. Pettersen EF, Goddard TD, Huang CC, *et al.*: **UCSF Chimera—a visualization system for exploratory research and analysis.** *J Comput Chem.* 2004; **25**(13): 1605–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
 38. Wolf A, Kirschner KN: **Principal component and clustering analysis on molecular dynamics data of the ribosomal L11-23S subdomain.** *J Mol Model.* 2013; **19**(2): 539–49.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 39. Zamora JE, Papadaki M, Messer AE, *et al.*: **Troponin structure: its modulation by Ca²⁺ and phosphorylation studied by molecular dynamics simulations.** *Phys Chem Chem Phys.* 2016; **18**(30): 20691–707.
[PubMed Abstract](#) | [Publisher Full Text](#)
 40. Hotelling H: **Analysis of a complex of statistical variables into principal components.** *J Educ Psychol.* 1933; **24**(6): 417–41.
[Publisher Full Text](#)
 41. Hayward S, de Groot BL: **Normal modes and essential dynamics.** *Methods Mol Biol.* In: Kukol A, editor. *Mol Model Proteins.* Totowa, NJ: Humana Press; 2008; **443**: 89–106.
[PubMed Abstract](#) | [Publisher Full Text](#)
 42. Amadei A, Linssen AB, Berendsen HJ: **Essential dynamics of proteins.** *Proteins.* 1993; **17**(4): 412–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
 43. Jain AK, Murty MN, Flynn PJ: **Data Clustering: A Review.** *ACM Comput Surv.* 1999; **31**(3): 264–323.
[Publisher Full Text](#)
 44. Arthur D, Vassilvitskii S: **K-means++: The Advantages of Careful Seeding.** *Proc Eighteenth Annu ACM-SIAM Symp. Discrete Algorithms*, Philadelphia, PA USA: Society for Industrial and Applied Mathematics; 2007; 1027–1035.
[Reference Source](#)
 45. Hartigan JA, Wong MA: **Algorithm AS: 136: A K-Means Clustering Algorithm.** *Appl Stat.* 1979; **28**(1): 100–108.
[Publisher Full Text](#)
 46. Johnson SC: **Hierarchical clustering schemes.** *Psychometrika.* 1967; **32**(2): 241–54.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. Abraham MJ, Murtola T, Schulz R, *et al.*: **GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers.** *SoftwareX.* 2015; **1–2**: 19–25.
[Publisher Full Text](#)
 48. Lindorff-Larsen K, Piana S, Palmo K, *et al.*: **Improved side-chain torsion potentials for the Amber f99SB protein force field.** *Proteins.* 2010; **78**(8): 1950–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 49. Darden T, York D, Pedersen L: **Particle mesh Ewald: An N -log(N) method for Ewald sums in large systems.** *J Chem Phys.* 1993; **98**: 10089–92.
[Publisher Full Text](#)
 50. Essmann U, Perera L, Berkowitz ML, *et al.*: **A smooth particle mesh Ewald method.** *J Chem Phys.* 1995; **103**: 8577–93.
[Publisher Full Text](#)
 51. Jorgensen WL, Chandrasekhar J, Madura JD, *et al.*: **Comparison of simple potential functions for simulating liquid water.** *J Chem Phys.* 1983; **79**: 926–35.
[Publisher Full Text](#)
 52. Bussi G, Donadio D, Parrinello M: **Canonical sampling through velocity rescaling.** *J Chem Phys.* 2007; **126**(1): 014101.
[PubMed Abstract](#) | [Publisher Full Text](#)

53. Parrinello M, Rahman A: **Polymorphic transitions in single crystals: A new molecular dynamics method.** *J Appl Phys.* 1981; **52**: 7182–90.
[Publisher Full Text](#)
54. Shen Y, Bax A: **SPARTA+: a modest improvement in empirical NMR chemical shift prediction by means of an artificial neural network.** *J Biomol NMR.* 2010; **48**(1): 13–22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
55. Han B, Liu Y, Ginzinger SW, *et al.*: **SHIFTX2: significantly improved protein chemical shift prediction.** *J Biomol NMR.* 2011; **50**(1): 43–57.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
56. Kota P, Ding F, Ramachandran S, *et al.*: **Gaia: automated quality assessment of protein structure models.** *Bioinformatics.* 2011; **27**(16): 2209–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
57. Ramachandran S, Kota P, Ding F, *et al.*: **Automated minimization of steric clashes in protein structures.** *Proteins.* 2011; **79**(1): 261–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
58. Bolton EE, Wang Y, Thiessen PA, *et al.*: **PubChem: Integrated Platform of Small Molecules and Biological Activities.** *Annu Rep Comput Chem.* Elsevier; 2008; **4**: 217–41.
[Publisher Full Text](#)
59. Chen YC: **Beware of docking!** *Trends Pharmacol Sci.* 2015; **36**(2): 78–95.
[PubMed Abstract](#) | [Publisher Full Text](#)
60. Wang J, Wolf RM, Caldwell JW, *et al.*: **Development and testing of a general amber force field.** *J Comput Chem.* 2004; **25**(9): 1157–74.
[PubMed Abstract](#) | [Publisher Full Text](#)
61. Sousa da Silva AW, Vranken WF: **ACPYPE - AnteChamber PYthon Parser interfacE.** *BMC Res Notes.* 2012; **5**: 367.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
62. Jakalian A, Jack DB, Bayly CI: **Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation.** *J Comput Chem.* 2002; **23**(16): 1623–41.
[PubMed Abstract](#) | [Publisher Full Text](#)
63. Trott O, Olson AJ: **AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading.** *J Comput Chem.* 2009; **31**(2): 455–61.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
64. Forli S, Huey R, Pique ME, *et al.*: **Computational protein-ligand docking and virtual drug screening with the AutoDock suite.** *Nat Protoc.* 2016; **11**(5): 905–19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
65. Morris GM, Huey R, Lindstrom W, *et al.*: **AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility.** *J Comput Chem.* 2009; **30**(16): 2785–91.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
66. Ruiz-Carmona S, Alvarez-Garcia D, Foloppe N, *et al.*: **rDock: a fast, versatile and open source program for docking ligands to proteins and nucleic acids.** *PLoS Comput Biol.* 2014; **10**(4): e1003571.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
67. Gaudreault F, Najmanovich RJ: **FlexAID: Revisiting Docking on Non-Native-Complex Structures.** *J Chem Inf Model.* 2015; **55**(7): 1323–36.
[PubMed Abstract](#) | [Publisher Full Text](#)
68. Pagadala NS, Syed K, Tuszynski J: **Software for molecular docking: a review.** *Biophys Rev.* 2017; **9**(2): 91–102.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
69. Kumari R, Kumar R, Open Source Drug Discovery Consortium, *et al.*: **g_mmpbsa—a GROMACS tool for high-throughput MM-PBSA calculations.** *J Chem Inf Model.* 2014; **54**(7): 1951–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
70. Bodenhofer U, Bonatesta E, Horejš-Kainrath C, *et al.*: **msa: an R package for multiple sequence alignment.** *Bioinformatics.* 2015; **31**(24): 3997–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
71. Thompson JD, Gibson TJ, Higgins DG: **Multiple sequence alignment using ClustalW and ClustalX.** *Curr Protoc Bioinformatics.* 2002; Chapter 2: Unit 2.3.
[PubMed Abstract](#) | [Publisher Full Text](#)
72. Sievers F, Wilm A, Dineen D, *et al.*: **Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega.** *Mol Syst Biol.* 2011; **7**(1): 539.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
73. Edgar RC: **MUSCLE: multiple sequence alignment with high accuracy and high throughput.** *Nucleic Acids Res.* 2004; **32**(5): 1792–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
74. Kozakov D, Hall DR, Xia B, *et al.*: **The ClusPro web server for protein-protein docking.** *Nat Protoc.* 2017; **12**(2): 255–78.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
75. Davies DL, Bouldin DW: **A cluster separation measure.** *IEEE Trans Pattern Anal Mach Intell.* 1979; **1**(2): 224–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
76. Dunn JC: **A Fuzzy Relative of the ISODATA Process and Its Use in Detecting Compact Well-Separated Clusters.** *J Cybern.* 1973; **3**(3): 32–57.
[Publisher Full Text](#)
77. Rousseeuw PJ: **Silhouettes: A graphical aid to the interpretation and validation of cluster analysis.** *J Comput Appl Math.* 1987; **20**: 53–65.
[Publisher Full Text](#)
78. Calinski T, Harabasz J: **A Dendrite Method for Cluster Analysis.** *Commun Stat.* 1974; **3**(1): 1–27.
[Publisher Full Text](#)
79. Laskowski RA, Hutchinson EG, Michie AD, *et al.*: **PDBsum: a Web-based database of summaries and analyses of all PDB structures.** *Trends Biochem Sci.* 1997; **22**(12): 488–90.
[PubMed Abstract](#) | [Publisher Full Text](#)
80. Warren GL, Andrews CW, Capelli AM, *et al.*: **A critical assessment of docking programs and scoring functions.** *J Med Chem.* 2006; **49**(20): 5912–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
81. Wernet P, Nordlund D, Bergmann U, *et al.*: **The structure of the first coordination shell in liquid water.** *Science.* 2004; **304**(5673): 995–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
82. Zheng Y, Sintim HO: **Molecular insights into how ligands activate or inactivate LasR.** *Chem Biol.* 2014; **21**(10): 1261–3.
[PubMed Abstract](#) | [Publisher Full Text](#)
83. Comeau SR, Gatchell DW, Vajda S, *et al.*: **ClusPro: a fully automated algorithm for protein-protein docking.** *Nucleic Acids Res.* 2004; **32**(Web Server issue): W96–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
84. Kozakov D, Brenke R, Comeau SR, *et al.*: **PIPER: an FFT-based protein docking program with pairwise potentials.** *Proteins.* 2006; **65**(2): 392–406.
[PubMed Abstract](#) | [Publisher Full Text](#)
85. Comeau SR, Gatchell DW, Vajda S, *et al.*: **ClusPro: an automated docking and discrimination method for the prediction of protein complexes.** *Bioinformatics.* 2004; **20**(1): 45–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
86. Bondí R, Messina M, De Fino I, *et al.*: **Affecting *Pseudomonas aeruginosa* phenotypic plasticity by quorum sensing dysregulation hampers pathogenicity in murine chronic lung infection.** *PLoS One.* 2014; **9**(11): e112105.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
87. Ledgerham F, Ventre I, Soscia C, *et al.*: **Interactions of the quorum sensing regulator QscR: interaction with itself and the other regulators of *Pseudomonas aeruginosa* LasR and RhlR.** *Mol Microbiol.* 2003; **48**(1): 199–210.
[PubMed Abstract](#) | [Publisher Full Text](#)
88. Fan H, Dong Y, Wu D, *et al.*: **QsIA disrupts LasR dimerization in antiactivation of bacterial quorum sensing.** *Proc Natl Acad Sci U S A.* 2013; **110**(51): 20765–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
89. Seet Q, Zhang LH: **Anti-activator QsIA defines the quorum sensing threshold and response in *Pseudomonas aeruginosa*.** *Mol Microbiol.* 2011; **80**(4): 951–65.
[PubMed Abstract](#) | [Publisher Full Text](#)
90. Suneby EG, Herndon LR, Schneider TL: ***Pseudomonas aeruginosa* LasR-DNA Binding Is Directly Inhibited by Quorum Sensing Antagonists.** *ACS Infect Dis.* 2017; **3**(3): 183–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
91. Paczkowski JE, Mukherjee S, McCreedy AR, *et al.*: **Flavonoids Suppress *Pseudomonas aeruginosa* Virulence through Allosteric Inhibition of Quorum-sensing Receptors.** *J Biol Chem.* 2017; **292**(10): 4064–76.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
92. Grabski H: **molmolpy.** 2019.
<http://www.doi.org/10.5281/zenodo.2585392>

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 25 October 2021

<https://doi.org/10.5256/f1000research.20169.r97391>

© 2021 Dave K. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Kapil Dave

Center for Biophysics and Quantitative Biology, University of Illinois at Urbana-Champaign, Champaign, IL, USA

In this manuscript, authors have employed computational methods (Docking and MD simulations) to study the interaction of autoinducer molecule, 3OC12-HSL, and LasR protein (*Pseudomonas aeruginosa*). The study revealed a second interaction site "bridge" for 3OC12-HSL and LasR protein. This bridge interaction involves the ligand-binding domain (LBD), beta turns in the short linker region (SLR), and the DNA-binding domain (DBD). The in-silico experiments are well defined with sufficient details provided for the computational methods. Overall, the data support the conclusion made in this manuscript. However, the authors should address the comments and concerns below.

- The authors need to expand the introduction to include the PQS system. Authors should also include recent references from work conducted by Bagchi *et al.*
- Authors mentioned their LasR protein model (with LBD and DBD domains) was different from that previously reported model by Chowdhury *et al.* Authors should briefly discuss the prime reasons that resulted in differences between their and previously reported LasR model.
- The manuscript demonstrated Cluster 2 and 3 (15% of total structures) as the binding interactions from various molecular docking programs in Figure 7. However, Cluster 1, which constituted 41.95% of the total structure as reported in the docking analysis, was not observed in any other molecular docking software results. Figure 7a (Autodock Vina) does show cluster 1 interactions. The authors should provide clarification regarding these findings.
- In Table 3 binding energies numbers are reported past 3 decimal places, is that accurate? If not authors should report numbers with significant decimal places.
- Page 13 the reference to Figure 11a and Figure 11b are not consistent with the figure's caption. Please correct the manuscript text or figure caption.

- The authors should discuss the tendency of LasR to form heterodimer when 3OC12-HSL is interacting at LBD vs the bridge site. What implications an additional interacting site can have on the binding of the LasR complex to the target DNA?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Protein misfolding and aggregation, Molecular dynamics simulations, Protein-protein interactions, ultrafast-spectroscopy, Molecular Biophysics, Cellular Biophysics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 20 October 2021

<https://doi.org/10.5256/f1000research.20169.r92461>

© 2021 Azam F. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Faizul Azam

Department of Pharmaceutical Chemistry & Pharmacognosy, Unaizah College of Pharmacy, Qassim University, Unaizah, Saudi Arabia

The manuscript entitled "Interaction of N-3-oxododecanoyl homoserine lactone with transcriptional regulator LasR of *Pseudomonas aeruginosa*: Insights from molecular docking and

dynamics simulations [version 1]" employs several computational techniques to study the interactions of N-3-oxododecanoyl homoserine lactone with transcriptional regulator LasR protein.

The manuscript is well designed and robust experimental protocols were implemented. However, following issues must be rectified:

1. Recent citations from 2021, 2020 and recent years should be included in the introduction/discussion sections.
2. In the methods section, Analysis of docking conformations and trajectories should be separated. So, the docking and MD simulation should not be mixed.
3. PCA analysis of docking poses? Please provide more details.
4. In Figures, please remove the background color.
5. In total, how many snapshots were included in mm/pbsa binding energy calculations?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Drug design and discovery, medicinal chemistry, molecular docking, molecular dynamics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 22 May 2020

<https://doi.org/10.5256/f1000research.20169.r63083>

© 2020 Paczkowski J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Jon Paczkowski

New York State Department of Health, Division of Genetics, Wadsworth Center, Albany, NY, USA

In this study, Grabski et al. use molecular docking techniques to define a second binding site for the LasR autoinducer molecule, 3OC12-HSL. The researchers use several computational platforms to confirm that 3OC12-HSL binds to a "bridge" domain that links the ligand binding domain and the DNA binding domain in LasR.

I am no expert in computational modeling or docking. I have some familiarity with the tools used in this study and I do not find them particularly compelling in "this new finding", especially calling what they do "machine learning." Otherwise, the methods were quite detailed and well-written.

The authors have some factual errors in their introduction. They seem to imply that there are three LuxI/R QS systems in *Pseudomonas aeruginosa*. That is not the case. They entirely omit the PQS system in their introduction. Furthermore, it is not entirely clear why they focus on HCN in the introduction. They never come back to it and it is not the focus of the study. It seems irrelevant.

During the introduction they mention that LasR requires its AI to fold, function, and dimerize. Next, they state they're interested in studying LasR in the monomeric state. There is no evidence that LasR exists as a stable monomer on its own, let alone plays any functional role.

Inexperience with docking simulations aside, it's particularly intriguing to discover that there might be a second AI binding site. The statistics seem fine. Docking a hydrophobic molecule with a protein that contains a hydrophobic patch will result in a positive result. That doesn't necessarily mean that the interaction occurs in nature. Furthermore, the primary binding site for 3OC12-HSL is a mixture of hydrophilic interactions with the lactone head group and hydrophobic interactions with the acyl tail. The residues defined as the second binding site do not appear particularly hydrophilic to account for stabilizing the lactone head group. How do the authors account for this discrepancy? Additionally, there is no hydrophobic "core" that stabilizes the tail in its entirety. Simply put, I think there is too much solvent exposed surface on 3OC12-HSL for this binding to occur. Can the authors compare the canonical binding site to the secondary binding site in this regard?

Furthermore, what role does this second binding site even play? LasR has been purified in the presence of excess 3OC12-HSL and the ratio is 1 LasR:1 AI. How do the authors reconcile this? There needs to be additional biochemical or cell-based assays performed to a) show that this second binding site actually exists and is not an artifact of the limitations of molecular docking and b) determine what its potential role is.

It would be an interesting exercise if the authors performed similar docking experiments with other LuxR-type proteins with their partner AI. Would they get the same results?

While I am sure the data are sound, I am not convinced of its relevance or importance in the

context of LasR signaling. There needs to be some other data to prove that the second binding site is biologically relevant.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: X-ray crystallography, biochemistry, signal transduction, and bacterial cell-cell communication.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research