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Original Research Article

Homology Modeling and Molecular Docking Studies of Selected Substituted Tetradecane on VlsE *Borrelia spielmanii*

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Abstract

VlsE is the key enzyme in antibacterial and suicide antigenic variation. While the VlsE of *Borrelia burgdorferi* sensu lato complex causes Lyme disease. Therefore, VlsE is considered a significant drug target for Lyme disease. In this paper, we report the model of the three-dimensional structure of VlsE resulting from a homology modeling study. Homology modeling was developed using three different software and evaluating the best model. Subsequent docking studies of the natural substrate tetradecane and known antibacterial drugs were performed with SwissDock and shed new light on the binding characteristics of the enzyme. Binding energies ranged from -2024.12 to -2032.17 kcal/mol. As a result, they might be synthesized further and developed into active commercial antibacterial drugs.

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INTRODUCTION

A recent Emerging Infectious Disease issue published an assessment of data on this Borrelia species in patients with early Lyme borreliosis. This elaborated that Lyme disease is the most common tick-borne human infection in the northern hemisphere and central Europe[1]. The first instances of Lyme borreliosis were documented in Sweden in 1910 by Afzelius, then in Austria in 1914 by Lipschutz. These researchers recorded the first examples of individuals with single or numerous erythematous plaques of centrifugal expansion, which they called Erythema Chronicum Migrans (ECM)[2]. Following the first findings, more instances of ECM were discovered in various European nations, primarily in Central Europe. Hollström[3] used procaine penicillin to treat patients with ECM in 1958, leading him to propose a bacterial origin for the condition. At least five species of the *Borrelia burgdorferi* sensu lato complex such as *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. bissettii*, and *B. lusitaniae*, have a pathogenic role in human Lyme disease[4,5]. In 1977, many occurrences of arthritis in young individuals with skin lesions comparable to ECM were recorded in the city of Lyme, Connecticut, US[6]. Lyme illness, Lyme arthritis, or Lyme borreliosis, was named from the relationship between ECM and arthritis (LB)[7].

In vitro, several Lyme disease genospecies are resistant to complement-mediated death[8]. The majority of *B. afzelii* isolates are serum resistant, while *B. burgdorferi* isolates are moderately serum-resistant, and *B. garinii* isolates are susceptible to complement-mediated death[9-11]. The medications for Lyme disease are now amoxicillin, doxycycline, cefuroxime axetil, and ceftriaxone[12]. Although the medicines employed in the current therapy are clinically successful in the vast majority of instances, treatment failures for most of these compounds have been consistently recorded. Although various therapeutic approaches have been employed to treat Lyme disease, the recurrence of clinical symptoms even after the active infection has ended the need for research to develop new, powerful alternate therapeutic techniques[13]. Some experts say *B. burgdorferi* cannot be entirely eradicated *in vitro* conditions. These antibiotic-tolerant Borrelia cells are not resistant mutants but rather persisters[14,15].

A14S, a sixth pathogenic strain, was identified in two Dutch and two German patients with erythema migrans (a circular rash often appearing in the early stages of Lyme disease)[16]. A14S was also found in four questing *Ixodes ricinus* ticks in Germany and one in the Czech Republic[17]. It was recently reported as a novel species, *B. spielmanii*, and its major reservoir host is most likely the garden dormouse (*Eliomys quercus*)[18]. While *B. spielmanii* was not found in mice or voles, Richter *et al*.[19] found no ticks carrying *B. spielmanii* in three of the five regions studied in Germany. They were nearly all found in a single location, where the incidence of infection with this genotype was 6%[20]. *Borrelia spielmanii* sp. nov. has recently been identified as a new human pathogenic genospecies responsible for Lyme disease in Europe. Borreliae's capacity to persist in their natural cycle and overcome the innate and adaptive immune responses in distinct reservoir hosts necessitates various mechanisms for survival in diverse habitats[21].

Recombination events within VlsE have been found in mice as early as four days after infection and continue to occur during the disease[22]. Furthermore, antibodies specific to the variable regions of VlsE are generated during experimental mouse infection[23]. Interestingly, VlsE antigenic flipping in *B. spielmanii* and *B. burgdorferi* is only visible during mammalian infections, indicating that host factors may be necessary to promote antigenic variation[24,25]. There is no known job for the VlsE protein in addition to antigenic variation. However, it has been postulated that the protein may act in other kinds of immune evasion[26,27].

VlsE Protein complex of *B. spielmanii* causes Lyme disease. This disease often begins with symptoms such as a rash, fever, headache, and exhaustion. However, if the infection is not treated correctly, it can spread to your joints, heart, and nervous system[28]. This disease needs extensive investigation, especially when no experimental 3D structure prediction or docking investigations of VlsE are available. Therefore, VlsE is regarded as an important therapeutic target for various disorders. The present study intends to construct a homology model and docking analysis of the VlsE protein and evaluate the best model. Each subunit has a large N-terminal extracellular domain, a transmembrane domain, and a small C-terminal cytoplasmic portion. Some integrin subclasses share a common beta chain but have distinct alpha chains. The experimental 3D structure of VlsE[22] in *B. spielmanii* is not accessible, and a homology model must be created. The 3D structure of the integrin VlsE protein from *B. spielmanii* was created using three independent software: trRosetta[29], SWISS-MODEL[30], and Modeller[31]. PROCHECK, ProSA-web, ERRAT, Verify3D, and WHAT IF were used to ensure the highest quality model. Among all the models, the best model will be selected based on the overall scores[32]. Protein-ligand docking was performed between the disease-stimulating protein and the drugs used to inhibit the production of that stimulating protein. Ten different drugs were selected from the drug bank for analysis. These computational methods can give homology modeling and docking, which can then be employed in molecular dynamics simulations. We describe the homology and docking studies of this novel Lyme disease spirochete, *B. spielmanii*.

The major drawback of this research was investigating the mechanisms associated with the generation of VlsE variation, single-nucleotide polymorphism, and subsequent DNA sequence analyses on the VlsE gene and its paralog, BBJ51, a related gene with a frameshift mutation. Then molecular docking is due to the lack of confidence in the ability of scoring functions to give accurate binding energies. This stems from the fact that some intermolecular interaction terms are hardly predicted accurately, such as solvation effect and entropy change. The fundamental biological concept of "Sequence implies structure and structure implies function" deciphers that an increase in sequence knowledge has no biological significance until the protein structure is identified[33]. A protein's biological function is entirely dependent on its native 3D structure. Although commonly used protein structure determination techniques such as XRD or NMR are accurate, they are also costly and time-consuming. However, this rate is unmatched by the way the structures of proteins have been deciphered experimentally during the same tenure.

MATERIALS AND METHODS

Sequence Collection and Alignment

The VlsE stimulating protein sequence was obtained from NCBI (https://www.ncbi.nlm.nih.gov/protein/?term=vlse).The protein is 322aa, a *B. spielmanii* VlsE protein found on locus CAQ52815. For further investigation, the sequences were downloaded in FASTA format. The basic local alignment

similarity search is commonly used to locate matching amino acid and nucleotide sequences (**Figure 1**). There were five types of BLAST[34]. The sequence was then run via BLAST-P (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) to determine the percentage of identity with other species. The best five hits recovered by the BLAST-P program are appeared in **Table I**.

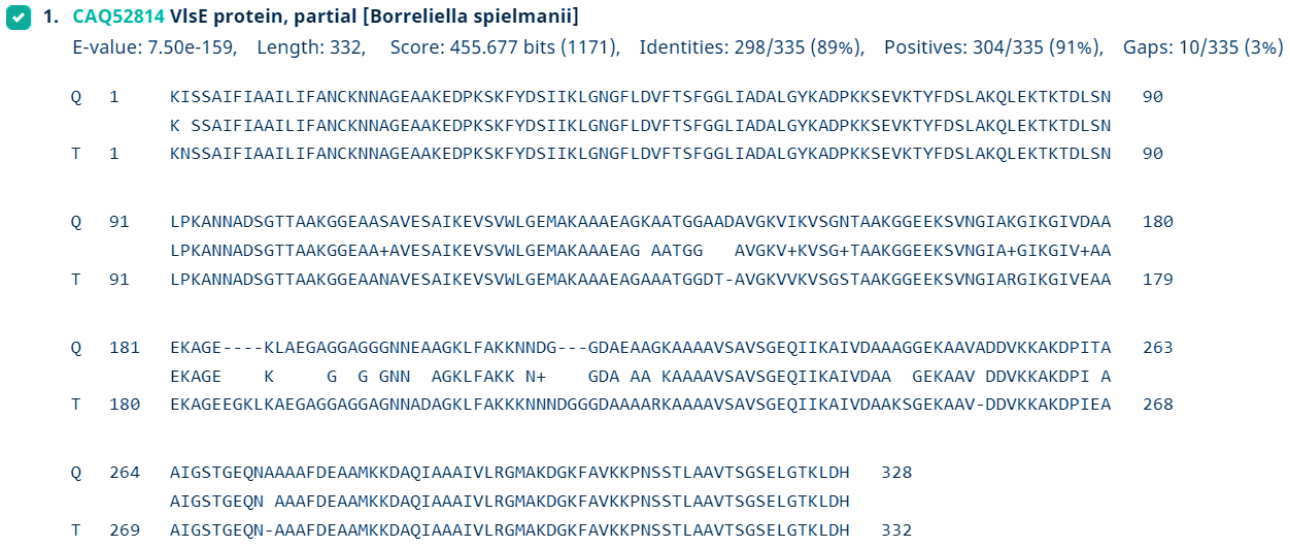


Figure 1. Multiple sequence alignments of the VlsE target sequence with the template sequence using BLAST.

Table I. Best hit obtained by PSI-BLAST with the VlsE Sequence

Description	Scientific name	Common name	Taxid	Max score	Total score	Query cover	E value	Per. ident	Acc. Len	Accession
VlsE protein [*Borreliella spielmanii*]	*Borreliella spielmanii*	NA	88916	338	338	95%	7.00E-112	100	328	CAQ52815.1
VlsE protein [*Borreliella spielmanii*]	*Borreliella spielmanii*	NA	88916	294	294	95%	1.00E-94	82.19	326	CAQ52813.1
VlsE protein [*Borreliella spielmanii*]	*Borreliella spielmanii*	NA	88916	256	344	70%	2.00E-79	87.73	332	CAQ52814.1
VlsE protein [*Borreliella spielmanii*]	*Borreliella spielmanii*	NA	29519	185	185	49%	1.00E-53	68.86	202	WP_215540979.1
VlsE protein [*Borreliella spielmanii*]	*Borreliella spielmanii*	NA	29519	182	182	49%	6.00E-52	69.46	264	WP_215540978.1

Prediction of 3D Structure

The protein's tertiary structures were produced using various bioinformatics tools, including trRosetta[29], SWISS-MODEL[30], and Modeller[31]. These three alternative models were created to identify the best model based on the stereo-chemical evaluation[35].

Generating Model using trRosetta

The trRosetta is one of the most popular web-based platforms for rapid and accurate protein structure prediction. Rosetta design can identify sequences compatible with a given protein backbone and include the design of a novel protein fold, redesigning an existing protein for more excellent stability and increased binding affinity between proteins. It attempts approximately 30000 nine-residue fragment insertions, followed by another 10000 three-residue fragment insertions to generate a protein model. Usually, 20000-50000 models are folded for each protein[36].

Generating Model using SWISS-MODEL

SWISS-MODEL represents the homology modeling tool for the protein. The Expasy webserver (https://swissmodel.expasy.org/) can generate the model for a particular protein. It provides the distinct tertiary structure model of protein appearing in the regions of α-helix, β-sheet, β-turn, coils, and helix. The generation of the models took place by submitting the FASTA format sequence in the workspace[37].

Generating Model using Modeller 9.12

The FASTA format sequence was performed by the BLAST—the template and target sequence lined up with the Modeller. Then the 3D model of the protein was built based on the template and target. Modifying the files should take place on the priority of the internal scoring function and the DOPE score of the Modeller. We can select the best model for the target by using various validation software; we can validate the quality of the final model[38].

Docking Protein-Ligands using SwissDock

The molecular docking was performed by the SwissDock (http://www.swissdock.ch/) after proper preparation of the protein-ligand structure, which was the organization with the compliant ligand and rigid protein. The ligand and protein PDB file were uploaded by adjusting the specific criteria. The best protein-ligand binding model was obtained based on energy and utilizing a particular scoring function. The particular binding position of the protein was identified by the P server. The energy that binds to the substrate in a specific position was represented as a binding (or) active site[39].

Protein-Ligand Interactions

In the Discovery Studio Visualizer software, we could study the interaction of the protein-ligand. The ligands were dispatched in **Table II**. The evaluation of the interaction and distance between protein and ligand was done by the Discovery Studio, and protein-ligand interaction was typically done by executing a highly generated model[40].

Table II. Ligands considered for docking

No	Ligands	Name	Formula
1	C14	Tetradecane	$C_{14}H_{30}$
2	C15	N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	$C_{17}H_{38}NO_3S$
3	C16	4-(4-methyl-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)benzonitrile	$C_{16}H_{10}N_2O_2$
4	C19	3-(2-aminoquinazolin-6-yl)-1-(3,3-dimethylindolin-6-yl)-4-methylpyridin-2(1H)-one	$C_{24}H_{23}N_5O$
5	C13	1-{3-[(3,5-dichlorobenzyl)amino]propyl}-3-phenylurea	$C_{17}H_{19}Cl_2N_3O$
6	C12	2-(1-amino-2-hydroxypropyl)-4-(4-hydroxybenzyl)-1-(2-oxoethyl)-1H-imidazol-5-olate	$C_{15}H_{18}N_3O_4$
7	N11	6-[(7-nitro-2,1,3-benzoxadiazol-4-yl)sulfanyl]hexan-1-ol	$C_{12}H_{15}N_3O_4S$

RESULTS AND DISCUSSION

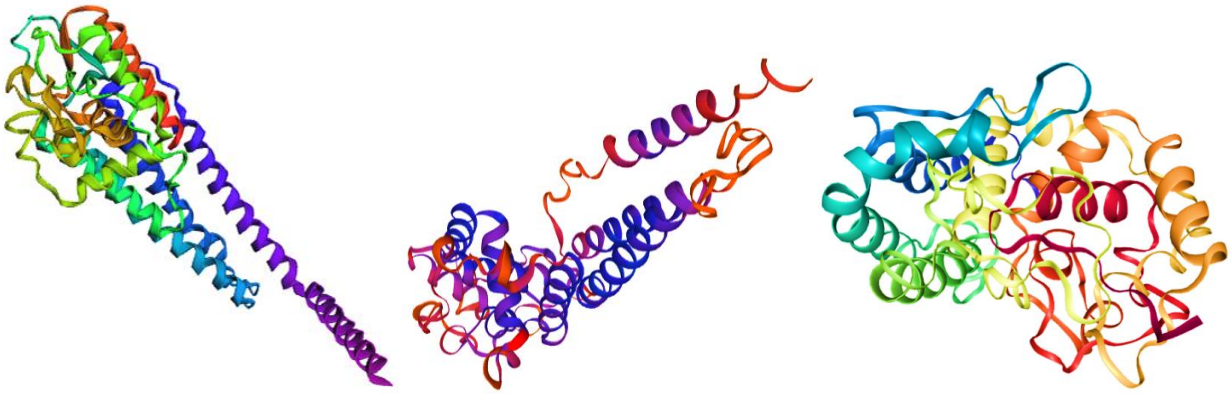
Tertiary Structure

SWISS-MODEL, trRosetta, and Modeller generate three models. Compared to other software models, trRosetta created the finest model selected based on the lowest DOPE score by refinement of the loops, ultimately determining the protein's unique three-dimensional shape. Structure amino acid sequence that drives the folding and intra-molecular bonding of the linear amino acid chain[41]. The structures were then visualized using PyMOL, Discovery Studio Visualizer, and Swiss PDB Viewer to analyze the models more clearly (**Figure 2**). It also represented the JS Models for every software model PDB file (**Figure 3**).

Model Validation

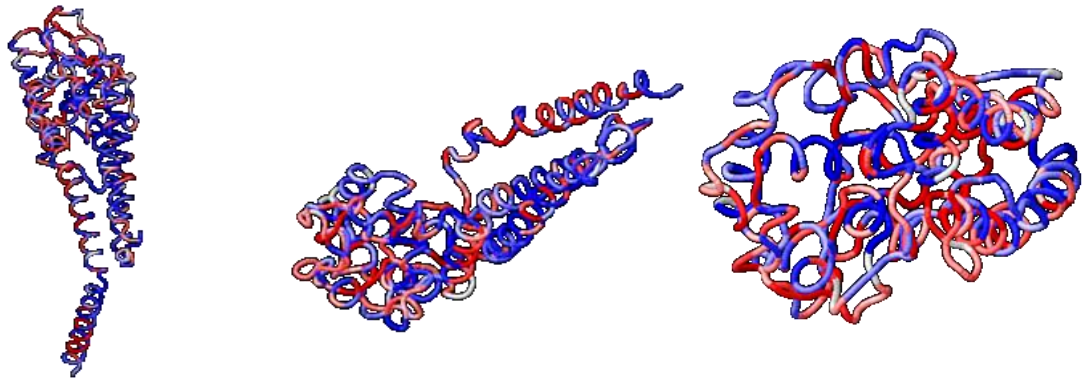
The number of amino acids that are determined showed in the phi-psi dissemination which is analyzed by the program. The PROCHECK program was calculated by the Ramachandran plot (**Figure 4**). The trRosetta generate 94.9%. The most allowed region is 7.4%; disallowed and generously allowed is 0.4% (**Table III**). Verify3D and ERRAT program, trRosetta generated 95.9% and 98.7% scores (**Figures 5** and **6**). By comparing the SWISS-MODEL and Modeller, it came to know that the two models' output didn't perform as well as trRosetta. The trRosetta creates a good Z score of -6.1 (**Figure 7**) which is archived and demonstrated by the was excellent. The trRosetta generates

compared to the other two models that come with standard and good with LG score of 2 and ERRAT Score 97.5 (**Figure 8**). SWISS-MODEL also generated 85.9% of the favored region, and Modeller 9.12 generated 65.7% of the favored region (**Table IV**). The trRosetta generates models with the highest percentage of the favored region. Hence, trRosetta generates the finest model. The binding areas of the protein were derived from the active site prediction (**Figure 9**). The largest volume was used to get the best binding sites[42].



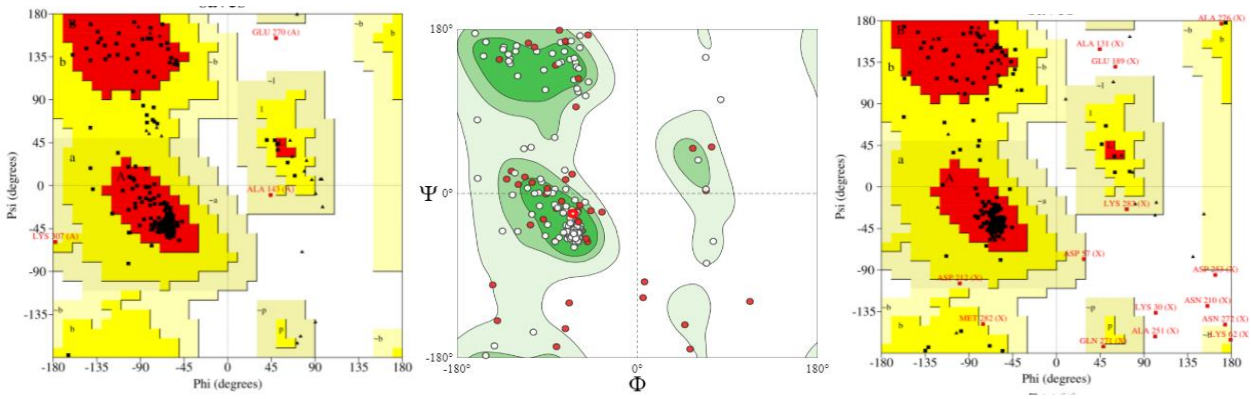
a **b** **c**

Figure 2. Homology modeling on VlsE protein of *B. spielmanii* using (**a**) trRosetta, (**b**) SWISS-MODEL, and (**c**) Modeller.



a **b** **c**

Figure 3. JS Model of VlsE from *B. spielmanii* using (**a**) trRosetta, (**b**) SWISS-MODEL, and (**c**) Modeller.

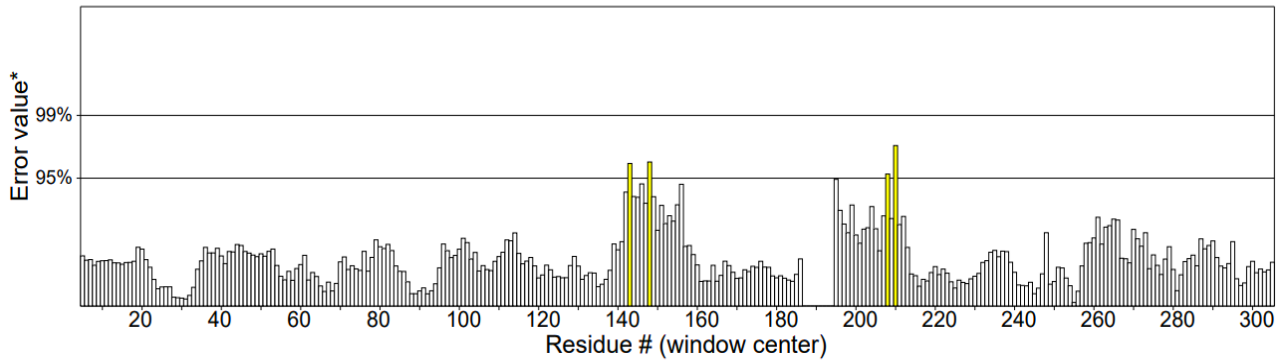


a **b** **c**

Figure 4. Ramachandran Plot for the modeled on VlsE Protein of *B. spielmanii* refinement using (**a**) trRosetta, (**b**) SWISS-MODEL, and (**c**) Modeller. The red, yellow, and white regions represent the favored, allowed, and disallowed regions, respectively.

Table III. Comparative values of PROCHECK, ERRAT, Verify3D, and PROVE in different stages of refinement used in trRosetta

Validation	After modeling	Refine loop	Minimize	Predict side chain
Ramachandran Plot				
Allowed	94.9%	90.2%	89.9%	97.2%
Disallowed	7.5%	0.7%	0.4%	0.2%
ERRAT	98.7%	97.8%	96.9%	96.4%
Verify3D	95.9%	97.1%	92.1%	93.1%
PROVE Z-score	-6.1	-4.9	-4.8	-4.8



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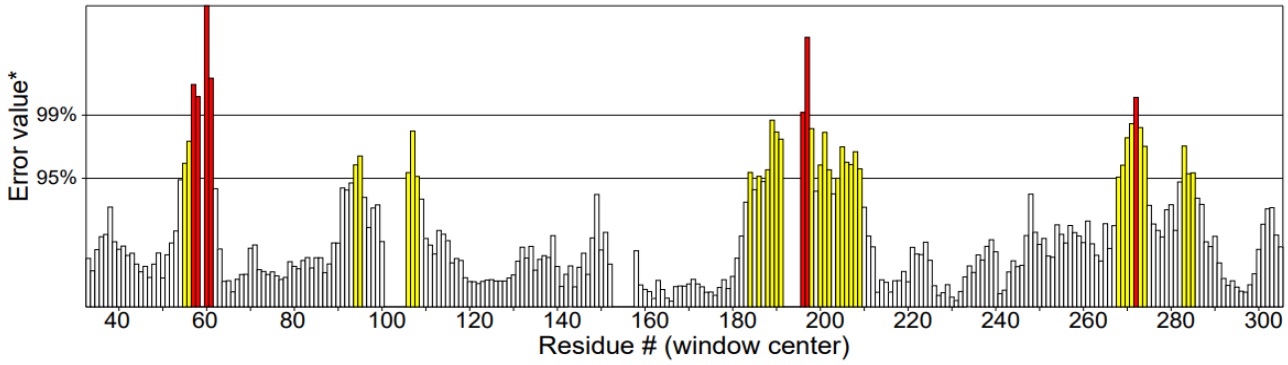

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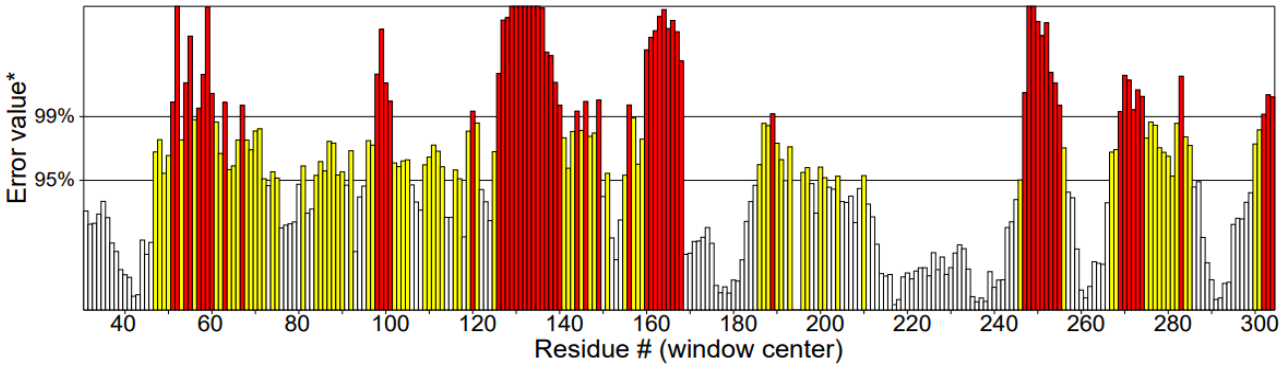
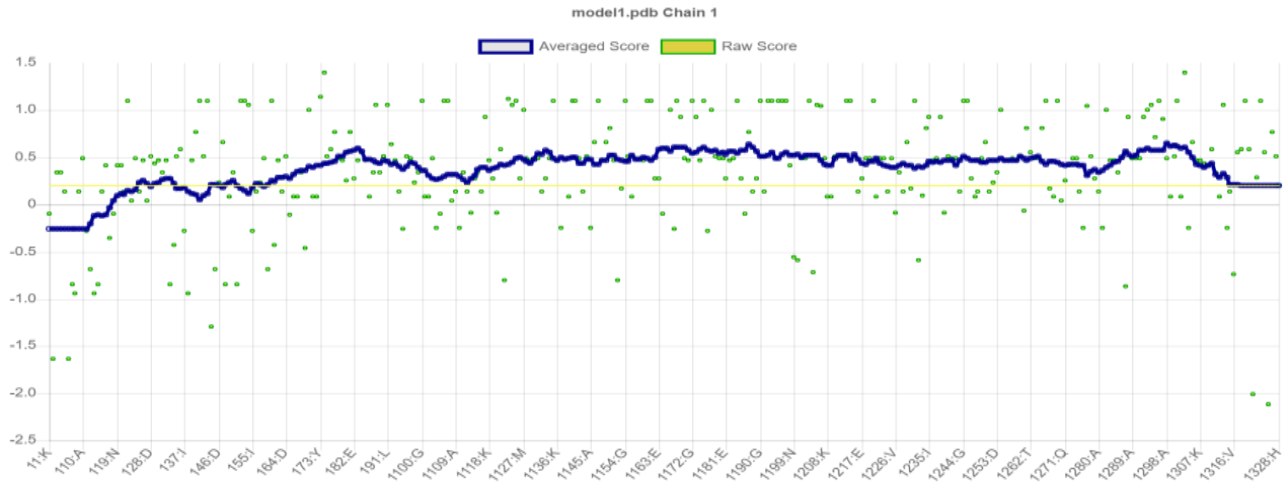


c

Figure 5. ERRAT plot on VlsE Protein of *B. spielmanii* modeled by (**a**) trRosetta, (**b**) SWISS-MODEL, and (**c**) Modeller. The red, yellow, and white regions represent the favored, allowed, and disallowed regions, respectively.



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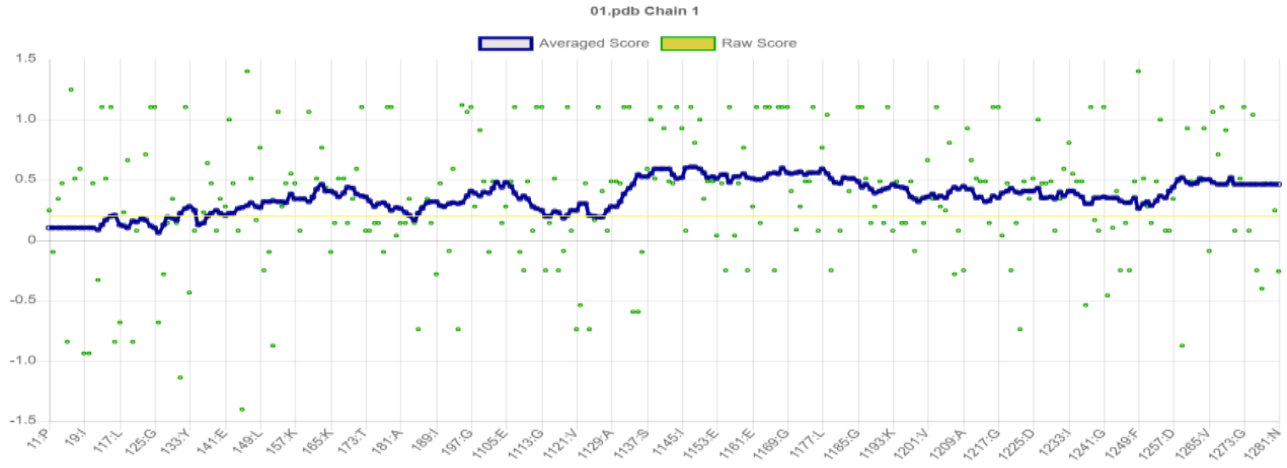

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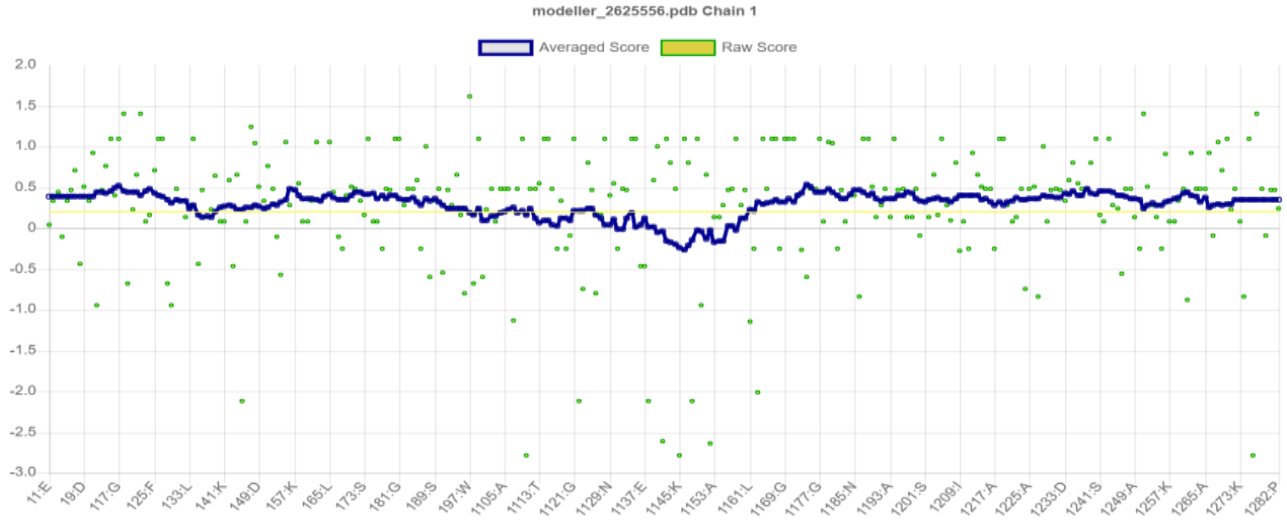

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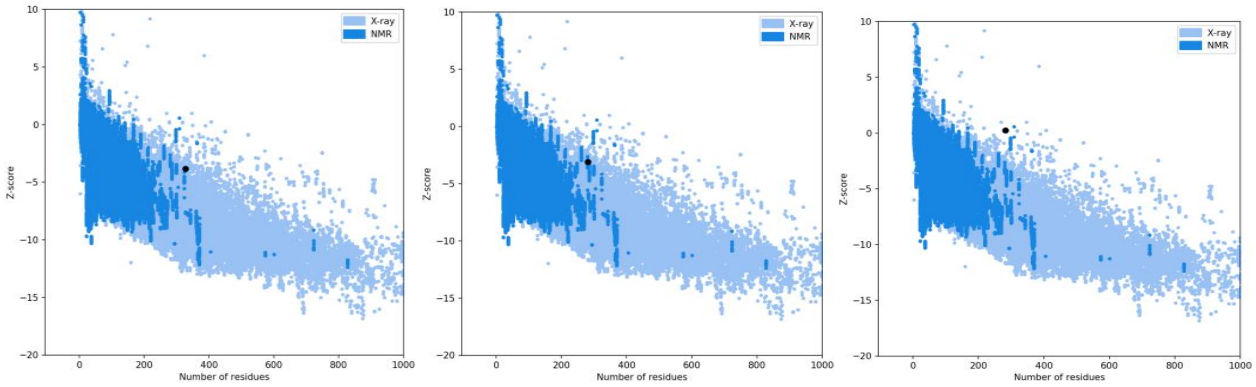
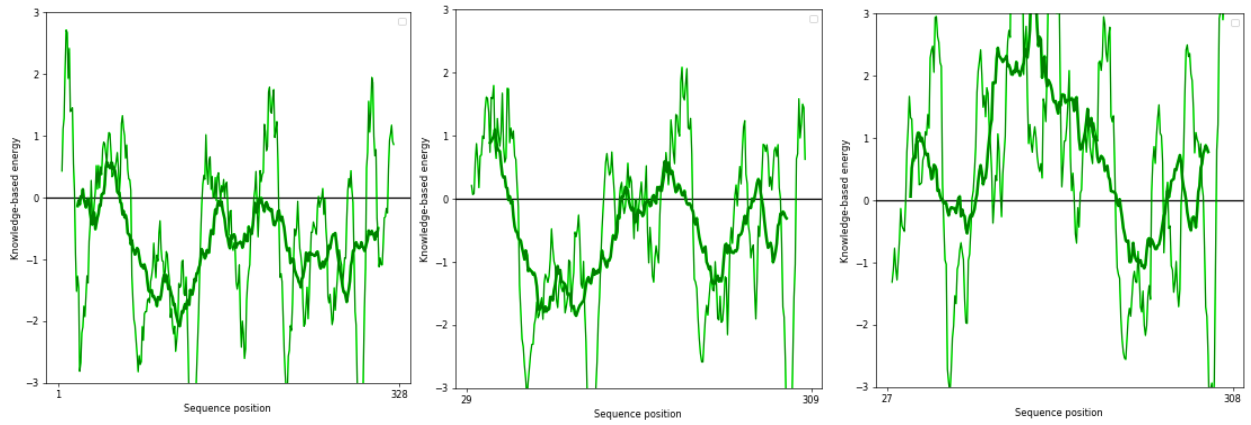
Figure 6. Verify3D plots on VlsE Protein of *B. spielmanii* modeled by (**a**) trRosetta, (**b**) SWISS-MODEL, and (**c**) Modeller.



a **b** **c**

Figure 7. ProSA-web Z-scores on VlsE Protein of *B. spielmanii* (black spot) using (**a**) trRosetta, (**b**) SWISS-MODEL, and (**c**) Modeller.



a **b** **c**

Figure 8. Verify3D plots on VlsE Protein of *B. spielmanii* using (**a**) trRosetta, (**b**) SWISS-MODEL, and (**c**) Modeller.

Table IV. Comparative values of PROCHECK, ERRAT, Verify3D, PROVE, and ProSA-web Z-scores between the template and modelled protein of all the three models.

Validation	trRosetta	SWISS-MODEL	Modeller
Ramachandran Plot			
Allowed	94.9%	85.9%	65.7%
Additionally allowed	7.4%	6.5%	5.5%
Generously allowed	0.7%	1.5%	2.3%
Disallowed	0.4%	0.9%	1.6%
ERRAT	98.7%	85.4%	70.5%
ProSA-web Z-score	-6.1%	-5.5%	-4%
Verify3D	93.1%	82.3%	69.4%

Figure 9. Binding site of protein.

Docking

The trRosetta output model used was analyzed for docking studies. Protein-ligand (VlsE-C14) interaction was executed that exhibits the binding energy (ΔG) of -8.09 kcal/mol (**Table V**) towards the protein and ligand with affinity score of -2032.17 was the flexible and finest interaction that was formed with the lowest binding energy. In conclusion, the tetradecane ligand (**Figures 10** and **11**) was the finest compared to the other seven ligands and their docking studies. It displayed the chemical structure of C_{14} H_{30} in **Figure 12**.

Table V. SwissDock results.

Ligand	Cluster	Element	Full fitness (kcal/mol)	Estimated ΔG (kcal/mol)
C14	0	0	-2032.17	-8.09
C15	0	1	-2031.74	-8.08
C16	0	2	-2031.74	-8.08
C19	0	3	-2031.74	-8.08
C13	0	4	-2031.74	-8.08
C12	0	5	-2031.74	-8.08
N11	0	6	-2031.74	-8.08

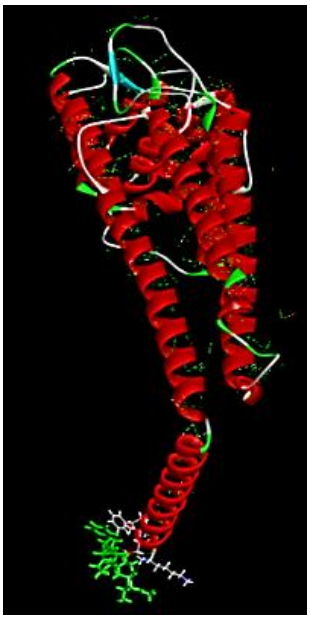


Figure 10. VlsE-C14 ligand docking results.

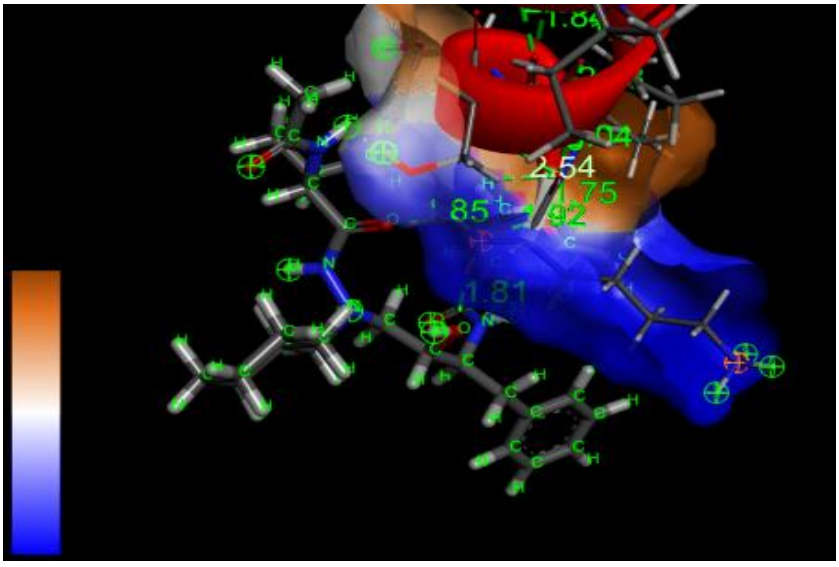


Figure 11. VlsE-C14 ligand docking results.

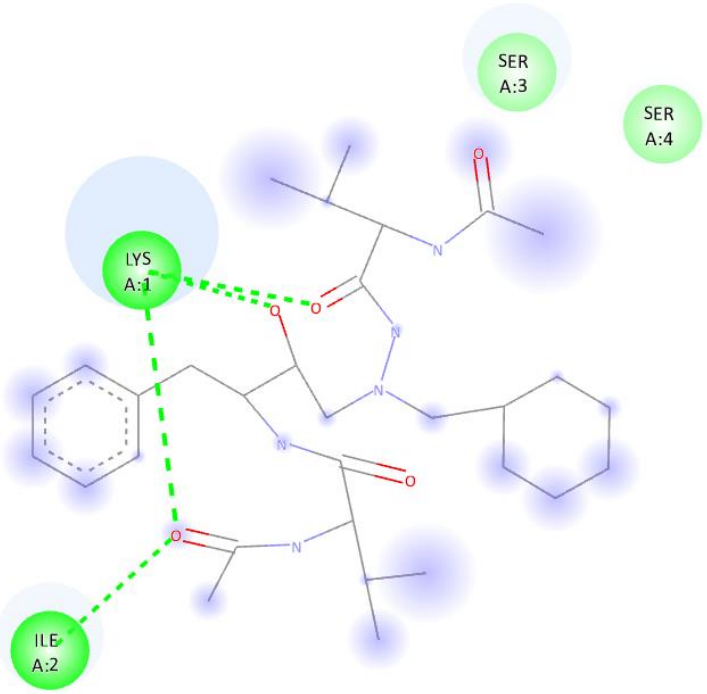


Figure 12. 2D interaction between ligand-receptor from docking results (van der Waals and conventional hydrogen bond).

CONCLUSION

VlsE is an important therapeutic target for various disorders, including Lyme disease. This study builds a homology model and docking analysis of the VlsE protein and evaluates the best model. Homology modeling experiments are conducted utilizing the three software, trRosetta, SWISS-MODEL, and Modeller. PROCHECK, ProQ, ERRAT, Verify3D, PROVE, and dDFIRE further assessed the best models produced by all software. Based on the results, trRosetta had satisfactory Ramachandran plot statistics and ERRAT plot quality factor. The online validation server (ProSA-web) revealed that the models' Z-score and protein folding energy were in good agreement with the existing protein structures in PDB, indicating that the overall quality of the structures was good. All evidence indicated that the geometric quality of the backbone conformation, initial structural conformation to its final position, and energy profile of the structure formed was substantially within the limitations. The performance of protein-ligand docking revealed that the optimum interaction is between C14 and the VlsE protein. As a result, this drug should be analyzed further for its resemblance to a lead medication.

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CONFLICT OF INTEREST

The authors declare no competing interests.

FUNDING

Not applicable.

DATA AVAILABILITY

Not applicable.

AUTHORS' CONTRIBUTIONS

Contribution of the Author **VP** and **HT** both contributed equally to this effort. **VP** and **HT** worked on molecular docking, molecular modelling, and writing – both review and editing. Ligand collection, data curation, formal analysis, validation, and visualization were all done by **TNSM**.

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