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Design of a Chimeric Vaccine Targeting OPA Protein of *Neisseria gonorrhoeae* – An Immunoinformatics Approach

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Abstract: Gonorrhoea is a sexually transmitted disease caused by the pathogen *Neisseria gonorrhoeae*. The current treatment includes the administration of antibiotics. As the bacterium is resistant to most antibiotics, alternate methods of treatment are the need of the hour. One alternate strategy is to use vaccines. But to date, no vaccines are available to treat Gonorrhoea. In the present study, opacity proteins (Opa) present on the surface of the gonococcus were chosen to design a chimeric vaccine using the Immunoinformatics approach. Initially, ABCpred was used to predict the epitopes specific to B-cells. The epitopes were checked for antigenicity, allergenicity, toxicity, solubility, stability, and presence of transmembrane helices and signal peptides using various online tools. Epitopes DFGGWRIAADYA and RLENTRFKTHEA passed the criteria and were used to construct the vaccine. The epitopes were also checked for affinity to MHC class 1 and 2 alleles. Subunit B of the Cholera toxin was the adjuvant, and the epitopes were linked using GPGPG linkers. The vaccine construct was modeled using the Robetta server, and the model was validated using the Ramachandran plot using the Molprobity server. The physicochemical properties of the constructed chimeric vaccine were analyzed using the Protparam tool. Molecular Dynamic simulations were performed to understand the stability of the vaccine. The DNA sequence of the vaccine constructs for the expression in E.coli K-12 was reverse-translated using the online tool JCAT.

Keywords: *Neisseria gonorrhoeae*; epitope; Opa protein; peptide; vaccine; Immunoinformatics.

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1. Introduction

Globally, sexually transmitted infections (STIs) are one of the most common diseases that last a short time. Despite several efforts by local and governmental bodies, the incidence rate of these STIs remains high. STIs cause complications over the long run, such as neurological disorders, infertility, compromised immune systems, and seronegative arthritis [1]. As per the Centers for Disease Control and Prevention (CDC), drug-resistant strains of *Neisseria gonorrhoeae* are at the top of the list of critical antibiotic-resistant threats to the health of the public [2]. Gonorrhoea is a disease that has been known to man since ancient times. It is caused due to a bacterium, *N. gonorrhoeae,* which spreads quickly through unprotected sexual intercourse. *N. gonorrhoeae,* also known as gonococcus, is a gram-negative, facultative, obligate, intracellular, pathogenic diplococcus bacterium. The major sites

of the infection include the anal canal, cervix, urethral mucosa, conjunctiva, and pharynx [2-5]. Gonorrhoea was easily treatable with penicillin, tetracyclines, cephalosporins, sulfonamides, fluoroquinolones, and macrolides. Due to increased multi-antibiotic resistance strains of *N. gonorrhoeae,* treatments with antibiotics have become futile [6]. Lipooligosaccharides (LOS) on the gonococcus stimulate innate immunity, and cytokines are produced as a response. The release of cytokines favors the chemotaxis of polymorphonuclear leukocytes (PMNs) to the infected site and phagocytoses the gonococcus. PMNs release antimicrobial peptides and reactive oxygen species, but gonococcus has special efflux pumps that can export the antimicrobial peptides from its system. Thereby conferring survival and resistance to the bacterium [1]. Alternate and novel treatment methods must be developed to curb these resistant strains of *N. gonorrhoeae.* It is to be noted that currently, there are no vaccines available to treat Gonorrhoea due to the increasing number of variants [7-8].

Type IV pili are used by *N. gonorrhoeae* in order to affix themselves to the host's mucosal epithelium. Internalization is mediated by proteins present on the bacterium's outer membrane, like invasins and opacity-associated proteins (Opa) [1]. Opacity-associated proteins (Opa) are integral outer membrane-bound proteins in *N. gonorrhoeae.* Opa proteins mediate a tight interaction between the human cells and *N. gonorrhoeae.* These proteins are responsible for inter-gonococcal aggregation, making the colonies opaque, hence its name. Opa proteins are constitutively transcribed, and a single bacterium may possess up to 12 genes. Each gene contains a semi-variable, conserved, and two hypervariable regions. These hypervariable regions are located on the exterior region of the outer membrane. Also, these bacterial cells can express from none to multiple opa proteins. Opa proteins specifically bind to two types of human receptors. One is heparin sulfate proteoglycans, and the other is a carcinoembryonic antigen cell adhesion molecule (CEACAM). CEACAMs are expressed in epithelial, lymphocytes, neutrophils, and endothelial cells and are the primary receptor for Opa proteins [9-10].

The recent advances in bioinformatics research with the newer techniques in omics and genome sequencing have led to new hopes in the *in silico*-based vaccine design and validation. This is achieved using the tools and techniques employed in immunoinformatics and reverse vaccinology, which deliberately increases the accuracy of the vaccine design approach from wet lab to dry lab and helps scrutinize the best vaccine candidates. The major strategy of reverse vaccinology lies behind selecting epitopes from the organisms' core proteins, which can evoke a humoral response to produce antibodies against the epitope peptides. Reverse vaccinology and immunoinformatics have paved the way for designing vaccines with minimal failures, usually encountered by conventional methods [11]. The subunit-based vaccines can elicit non-specific immune responses as they have multiple antigenic determinants and also show allergenic responses. In contrast, peptide-based vaccines lack infectious potential, which is safe for immunocompromised patients and offers chemical stability [12-13]. The present study aims to design a chimeric vaccine against Opa proteins of *N. gonorrhoeae,* employing immunoinformatics and computational tools.

2. Materials and Methods

2.1. Amino acid (AA) sequence retrieval and analysis.

The AA sequences of Opa protein in FASTA format are present in the proteome of *N. gonorrhoeae* and were retrieved from the Uniprot Proteome database [14]. BLASTp was

performed on the retrieved sequences. This was performed to check the homology between the AA sequences of *N. gonorrhoeae* with that of *Homo sapiens*. This step was performed to avoid choosing sequences that may be self-recognizing and to avoid potential unwanted immune responses [15]. The sequences retrieved from the databases were from various strains. To identify the conserved regions present across the strains for the proteins, MSA (Multiple Sequence Alignment) was performed using Clustal Omega. The conserved regions were then chosen for further studies [16].

2.2. B-cell and T-cell epitope prediction.

Identifying linear B-cell epitope is the vital step for epitope-based design of peptide vaccines. The prediction was made using the online server called ABCpred (https://webs.iiitd.edu.in/raghava/abcpred/index.html). The server predicts peptides ranging between 10-mer and 20-mer in length. In this study, epitopes of 10- mer length were predicted with a threshold set at 0.75 [17]. Major Histocompatibility Complex class I (MHC-I) binding epitopes for various alleles were predicted using the Net MHC 4.0 server, and Major Histocompatibility Complex class II (MHC- II) alleles were predicted using Net MHCII 2.3 server [18- 19].

2.3. Screening of potential epitopes.

The epitopes were then screened for various parameters such as antigenicity, allergenicity, toxicity, solubility, presence of transmembrane helices, signal peptides, and stability. VaxiJen v2.0 server was used to predict the antigenicity of the epitopes. The threshold was set at 0.4, and the organism was set to "bacteria" for predicting the antigenic epitopes [20]. The identified antigenic epitopes were then checked for allergenicity using AllergenFP v.1.0 .The resulting non-allergenic epitopes were filtered for further analysis [21]. The epitopes that are non-allergenic and antigenic were then screened for toxicity. This was checked using the ToxinPred tool using default parameters [22]. The epitopes used for the vaccine design should not possess any signal peptides, and the epitopes were screened using the Signal 4.1 server [23]. The shortlisted epitopes were predicted for their solubility using SOLpro. SOLpro checks the solubility upon overexpression and is used to check the probability of the peptide being soluble or not. Soluble peptides are preferred for the design of vaccines [24]. The presence of transmembrane helices was predicted using TMHMM Server v. 2.0 [25]. The stability of the epitopes was predicted using the Protparam server [26].

2.4. Selection of epitopes.

Based on the criteria listed, the epitopes needed for the vaccine design were chosen. Highly conserved peptides, B-cell epitopes, Antigenic, Non-allergenic, Non-toxic, Soluble, Stable, absence of transmembrane helices and signal peptides, and its affinity to bind to MHC-I and MHC-II alleles [27].

2.5. Design and modeling of the vaccine construct.

The screened epitopes were joined adjacently at the C-terminal using linkers. GPGPG Linkers were used for the construction. The linked epitopes were attached to an adjuvant. The adjuvant, Subunit B of the cholera enterotoxin (Uniprot entry: P01556) sequence, was retrieved from Uniprot [28]. Robetta server was used to model the designed vaccine construct, and the

models were refined using the Galaxy refine server and were validated using the Ramachandran plot [29-31].

2.6. Evaluation of the designed vaccine.

The designed vaccine was checked for various parameters like antigenicity, allergenicity, solubility, toxicity, signal peptide, and transmembrane helices using the same tools mentioned in 2.4. The physical and chemical parameters of the constructed vaccine were evaluated using the ProtParam tool [26].

2.7. Molecular Dynamics (MD) simulation of the vaccine construct.

Molecular Dynamic simulations were performed using the Desmond simulation package for 100 nanoseconds. The Transferable Intermolecular Interaction Potential 3 Points (TIP3P) solvation model is used for system building. Optimized Potentials for Liquid Simulations (OPLS) force field and Isothermal–isobaric (NPT) ensemble were applied during simulations. The protein's root means square deviation (RMSD) was computed and inspected [32-33].

2.8. Docking of the vaccine construct and Toll-Like Receptor – 4.

The Cluspro 2.0 server was used to dock Toll-Like Receptor – 4 (TLR-4) with the vaccine construct. Three-dimensional structure of the TLR4 (PDB ID: 3FXI) was downloaded from the PDB database (Protein data bank). The docked complex was visualized using PyMol [34].

2.9. Codon optimization of the construct.

The Java Codon Adaptation Tool (JCAT) (https://www.jcat.de/) was used to obtain the optimized codons for expression in specific organisms [35].

3. Results and Discussion

3.1. Sequence retrieval and analysis.

Traditional vaccines are prepared by either inactivating or attenuating the pathogen to induce an immunological response in the host. An issue with these vaccines arises due to the allergic or toxic nature of the proteins. Subunit and conjugate vaccines targeting a particular protein were developed to mitigate this issue. However, the design and expression of such vaccines suffer drawbacks. Nowadays, Computational techniques are employed to develop multi-epitope vaccines, and such a strategy reduces the time and expense incurred in designing such a vaccine. Chimeric vaccines are designed *in-silico* by identifying the potential epitopes within the target protein and the epitopes along with the linked adjuvants to activate the immune response. Extracellular proteins, such as the surface proteins, are used for the vaccine design. The epitopes from the proteins are screened based on parameters and are used to construct the vaccine [36-40].

N. gonorrhoeae Opa protein's amino acid sequences were obtained from the UniProt database, and BLASTp was performed against Homo sapiens to check the sequence homology between the bacteria and Humans. It was found that the sequences were not homologous, and there was no significant similarity between the organisms. Homologs of Homo sapiens are

discarded so that possible autoimmune responses can be avoided. Prediction of non-human homologous proteins can ensure that the host immune system would exclusively interact with proteins of *N. gonorrhoeae* [41]. Conserved regions were analyzed, and only those regions were used in vaccine design. This was done so that the designed vaccine could be used across different strains of *N. gonorrhoeae.*

3.2. Prediction of B-cell and T-cell epitopes.

The development of epitope-based peptide vaccines in modern times is done by the availability of epitope prediction methods apart from the experimental characterization of epitopes. Identifying B-cell epitope regions in the antigenic part of protein sequences can help produce B-cell lymphocytes that can differentiate into plasma cells and memory cells, thereby making the antibodies inhibit and clear the infection by neutralization and opsonization processes [17]. B-cell epitope analysis was performed using the ABCpred server. A total of twenty-three 10-mer epitopes were obtained and are listed in Table 1. Among the 23 epitopes, 9 were in the conserved regions, and 14 were in the partially conserved regions.

Table 1. The list of B-cell epitopes in Opa proteins predicted using the ABCpred server. The threshold value was set at 0.75.

Rank	Sequence	Start position	Score
1	IRTHSIHPRVSV	11	0.76
1	YDFGGWRIAADY	25	0.8
2	SDYFRNIRTHSI	6	0.79
2	THSIHPRVSVGY	14	0.79
1	SDYFRNIRTHSI	6	0.79
1	THSIHPRVSVGY	14	0.79
1	SDYFKNIRTRSV	6	0.86
2	DFGGWRIAADYA	26	0.77
2	YDFGGWRVAADY	25	0.77
2	YDFGGWRIAADY	25	0.77
1	SDYFRNIRTHSV	6	0.82
2	THSVHPRVSVGY	14	0.79
3	YDFGGWRIAADY	25	0.77
2	YDFGSWRIAADY	25	0.77
3	THSIHPRVSVGY	14	0.75
1	SDYFRNIRTRSV	6	0.87
2	TRSVHPRVSVGY	14	0.79
2	SDYFKNIRTHSI	6	0.78
1	SDYFRNVRTHSI	6	0.82
2	YDFGGWRIAADY	25	0.8
3	THSVHPRVSVGY	14	0.79
3	TRSVHPRVSVGY	14	0.79
1	RLENTRFKTHEA	4	0.78

Using Net MHC servers, the shortlisted epitopes were analyzed for their affinity to bind with various MHC class I and II alleles. The binding of peptides to MHC receptors allows the Cytotoxic and Helper T cells to identify the peptides within the target protein. MHC I class receptors are present on all nucleated cells' surfaces, which helps identify endogenous antigens and presents these antigens to Cytotoxic T cells. MHC II detects and binds exogenous antigens

and presents these antigens to Helper T cells [18-19]. The epitopes were selected based on the IC50 values ranging between 0.01 and 1000 nM. The epitopes did not bind to MHC I alleles with an affinity less than 1000nM. The epitopes recognized a wide range of MHC II alleles. The MHC-II alleles that interacted with the epitopes are DRB1_0801, DRB1_1001, HLA-DQA10101-DQB10501, HLA-DQA10102-DQB10502, HLA-DQA10201-DQB10301, HLA-DQA10301-DQB10301, HLA- DQA10303-DQB10402, HLA-DQA10501-DQB10301, HLA-DQA10501- DQB10302, HLA-DQA10501-DQB10303, HLA-DQA10501-DQB10402, DRB1_1301, DRB4_0103, and HLA-DPA10103-DPB10601.

3.3. Screening of potential epitopes.

An essential factor to look upon is antigenicity to predict the success of the vaccine construct. The designed vector should be antigenic, immunogenic, and non-allergenic to stimulate the immune response. VaxiJen server was used to predict the antigenicity of the peptides. Peptides with a score > 0.4 are considered to be antigenic. Out of the 23 epitopes, 16 epitopes were antigenic. Whereas 7 epitopes were non-antigenic. The antigenic epitopes were further screened for their allergenicity using the AllergenFP server. It was observed that only 2 epitopes, i.e., DFGGWRIAADYA and RLENTRFKTHEA were non-allergic. These two epitopes were screened for toxicity, solubility, signal peptides, and trans-membrane helices. It was observed that the epitopes, DFGGWRIAADYA and RLENTRFKTHEA, were non-toxic, soluble (probability >0.95), stable, and not a signal peptide and didn't form transmembrane helices.

3.4. Design and modeling of the vaccine construct.

The cholera enterotoxin subunit B (Uniprot ID: P01556) was chosen as an adjuvant for constructing the chimeric vaccine. The selected epitopes, i.e., DFGGWRIAADYA and RLENTRFKTHEA were linked at the C-terminal end with the GPGPG linkers. The linkers mediate the structural and functional characteristics of the chimeric vaccine as it helps represent the full structure of the epitopes in the vaccine. They help improve the flexibility of the vaccine's structure, stability, and biological activity [28, 42]. The final vaccine construct is designed as follows,

MIKLKFGVFFTVLLSSAYAHGTPQNITDLCAEYHNTQIYTLNDKIFSYTESLAG
KREMAIITFKNGAIFQVEVPGSQHIDSQKKAIERMKDTLRIAYLTEAKVEKLCVWNN
KTPHAIAAISMANGPGPGDFGGWRIAADYAGPGPGR LENTRFKTHEAGPGPG.

3D modeling of the designed vaccine gives a good picture of its important elements. It also helps in understanding the dynamic nature of the protein [43]. The Robetta server-generated models for the vaccine construct and the validation chose the best model performed using Ramachandran plot analysis (Fig. 1 and 2). The best model selected by Ramachandran plot analysis showed that 99.4% (160/161) of the residues were in allowed regions, and only a single outlier was observed.

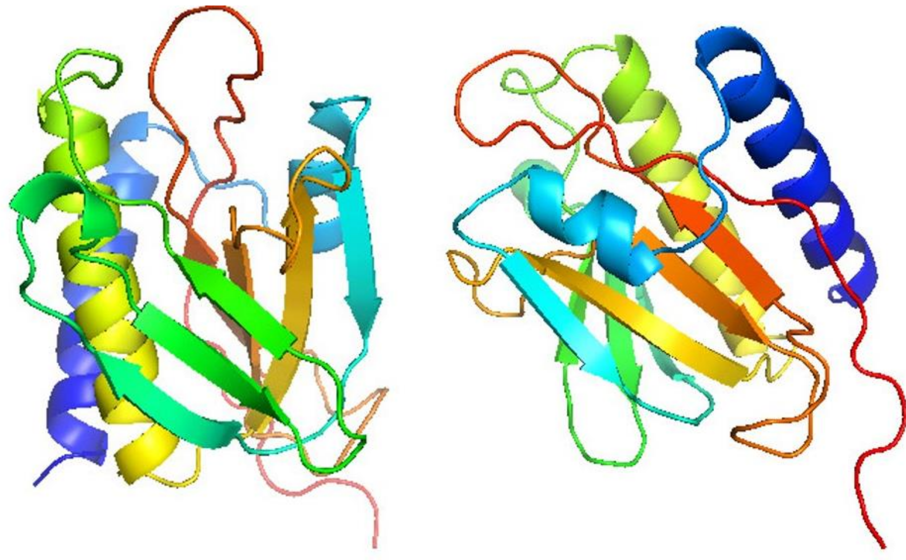

Figure 1. Model of the designed vaccine construct.

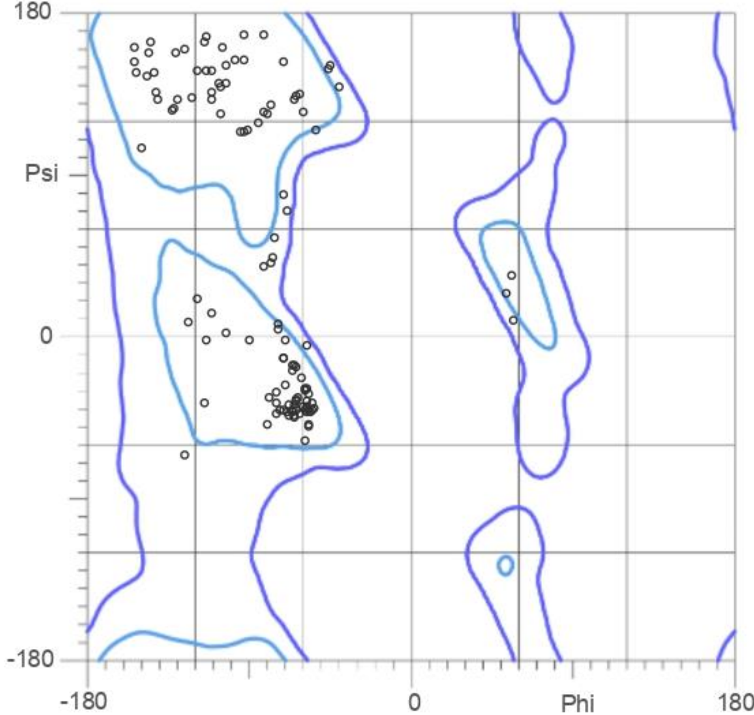

Figure 2. Ramachandran plot of the designed vaccine construct.

3.5. Evaluation of Physicochemical parameters of the constructed vaccine.

The constructed vaccine was subjected to evaluation of several parameters. The antigenicity score for the chimeric vaccine construct was 0.581, indicating it is an antigen. The allergenicity was predicted, and it showed that the vaccine is not allergenic with a Tanimoto similarity index of 0.81. The vaccine construct was non-toxic, and no transmembrane helices were found. SOLpro The server was used to indicate the solubility of the construct. The results showed that the vaccine is soluble with a probability of 0.827373. Physiochemical properties of the vaccine construct were predicted using the Protparam tool. The following results were obtained. The total aminoacid residues were 163, with an approximate molecular weight of 17.86 kDa. The Extinction coefficient of the vaccine construct, assuming all pairs of cystine residues form cystines, was predicted to be 20065 M^{-1} cm^{-1}. The Extinction coefficient of 19940 M-1 cm-1 was observed when all the cysteine residues were reduced. The instability

index was found to be 27.52, revealing that the protein is stable. The hydropathic index (GRAVY score) was found to be −0.275, and the aliphatic index was 76.13. The aliphatic index of the vaccine indicates that the protein is occupied by aliphatic side chains and thermostable. The hydropathic index indicates that the chimeric vaccine is hydrophilic in nature. The vaccine construct had an isoelectric point of 8.85. The estimated half-life was found to be 30 hours in mammalian reticulocytes (in vitro), >20 hours in yeast (*in vivo*), and >10 hours in Escherichia coli (*in vivo*).

3.6. MD simulation of the constructed vaccine.

The vaccine construct's dynamic behavior was analyzed through simulations for about 100 ns. MD simulations showed that the vaccine construct was stable. The atomic displacement of the complex's backbone atoms was first explored by calculating the RMSD as a function of time. The maximum RMSD value of 4.422Å was observed at a time scale of 89.2ns. The RMSD value became stable throughout the simulation with an average of 3.653Å (Fig. 3).

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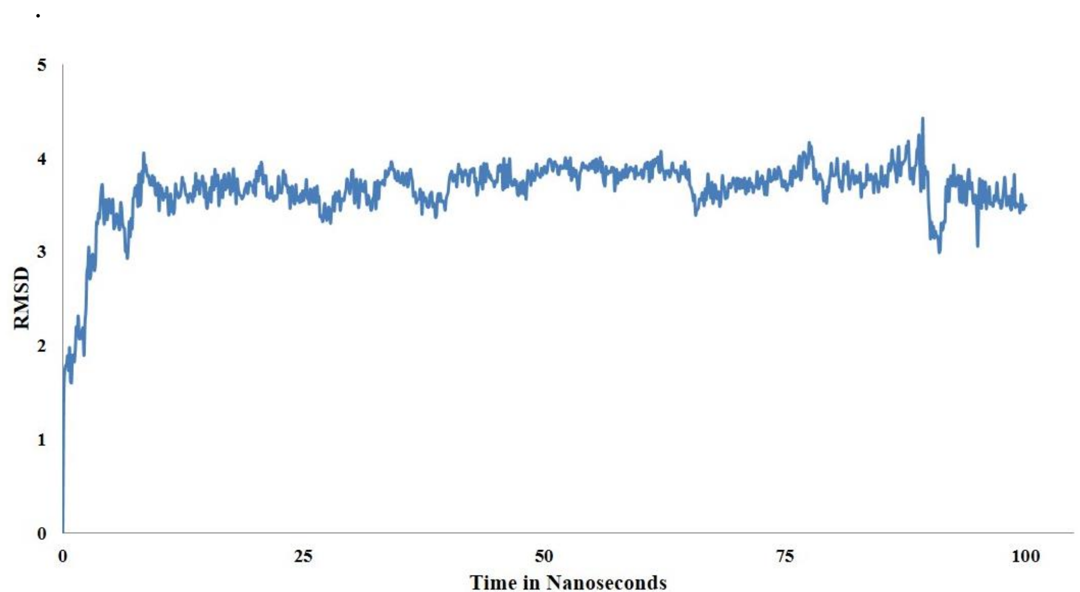

Figure 3. Root Mean Square Deviation of the designed vaccine construct when simulated for 100ns.

3.7. Interaction of vaccine with TLR4

Molecular interactions between a chimeric vaccine and TLR4 receptor were understood by docking using the ClusPro server. The affinity between the proteins is inversely related to their binding energy. The docked complex with the least energy displayed a weighted score of about -1008.6, indicating a good vaccine binding with the TLR4 receptor. Ligplot was used to view the docked model to see the interaction between TLR4 and the vaccine (Fig. 4).

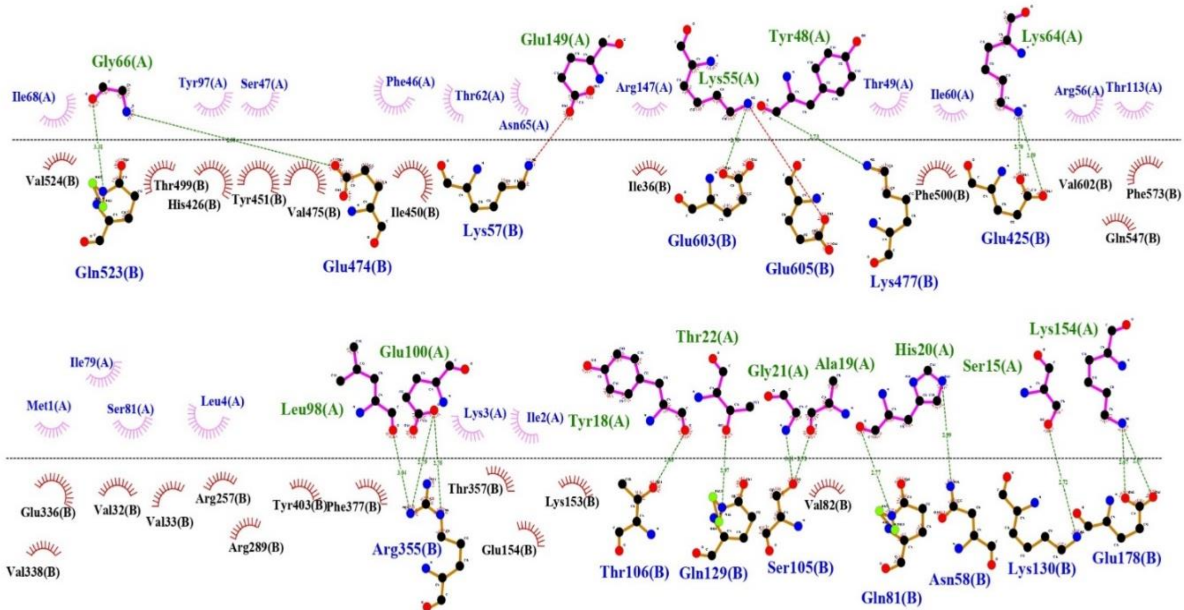

Figure 4. Interaction between the vaccine construct (A) and TLR-4 Receptor (B).

3.8. Optimization of the constructed vaccine.

An important criterion of a gene is its prowess to achieve a high protein expression level in the host. The Codon Adaptation Index value (CAI) is an indicator of the same, and CAI value of 1 is desired. While scores greater than 0.8 are generally regarded as good scores. The Guanine-Cytosine (GC) content should range between 30% and 70% [44]. For the reverse translated chimeric vaccine construct, the CAI value is 1. The GC content of the improved gene sequence for expression in *E. coli* K12 was determined to be 51.124744376278116. These results indicate that the gene sequence has the potential to express in the *E.coli* K12 host. The improved DNA sequence is shown in Fig 5.

ATGATCAAACTGAAATTCGGTGTTTTCTTCACCGTTCTGCTGTCTTCTGC 50

TTACGCTCACGGTACCCCGCAGAACATCACCGACCTGTGCGCTGAATACC 100

ACAACACCCAGATCTACACCCTGAACGACAAAATCTTCTCTTACACCGAA 150

TCTCTGGCTGGTAAACGTGAAATGGCTATCATCACCTTCAAAAACGGTGC 200

TATCTTCCAGGTTGAAGTTCCGGGTTCTCAGCACATCGACTCTCAGAAAA 250

AAGCTATCGAACGTATGAAAGACACCCTGCGTATCGCTTACCTGACCGAA 300

GCTAAAGTTGAAAAACTGTGCGTTTGGAACAACAAAACCCCGCACGCTAT 350

CGCTGCTATCTCTATGGCTAACGGTCCGGGTCCGGGTGACTTCGGTGGTT 400

GGCGTATCGCTGCTGACTACGCTGGTCCGGGTCCGGGTCGTCTGGAAAAC 450

ACCCGTTTCAAAACCCACGAAGCTGGTCCGGGTCCGGGT

Figure 5. Reverse translated gene sequence of the chimeric vaccine construct.

4. Conclusions

In the present study, we have identified epitopes from the Opa protein as a potent vaccine candidate. The novel multi-epitope chimeric vaccine construct showed appropriate

physicochemical, structural, and immunological properties. Molecular dynamics result indicates that the vaccine remains stable over time. The chimeric vaccine construct shows good binding affinity with immunoreceptor TLR4. This experimental validation of the chimeric vaccine could be done by expressing it in an appropriate host and through *in vivo* validation of the same for immunizing against *N. gonorrhoeae.*

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Conflicts of Interest

The authors declare no conflict of interest.

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