Transferrin binding protein-B from Neisseria meningitidis C as a novel carrier protein in glycoconjugate preparation: an in silico approach

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ABSTRACT

The linking of polysaccharide in glycoconjugate vaccine with carrier protein is an imperative step to develop a strong memory response. The excessive use of similar carrier protein known to result in bystander immunity warrants an urgent need for new carrier protein. The preparation of the glycoconjugate vaccine using cyanylation chemistry is to link the active cyanate ester site of polysaccharide with the carrier protein. In the present study, transferrin binding protein-B (Tbp-B) has been explored as a new carrier protein to develop in silico pneumococcal polysaccharide serotype-5 (PnPs-5) conjugate vaccine. The homology model of Tbp-B was constructed using the Prime module and stereo-chemically validated using ProSA, PDBsum and ProQ. The selected model revealed a Z-score of $-5.6$ within the X-ray region in ProSA analysis, LGscore: 9.776, and MaxSub: 0.8 in protein quality predictor suggesting its preferred use. Loop modeling and active site analysis followed by in silico PnPs-S activation with cyanylyzing agent CDAP was docked with Tbp-B using Glide module. The complex stability of cyanate esters with Tbp-B, analyzed by molecular dynamics (MD) simulation, revealed an average RMSD of 2.49 Å for its binding to the receptor. The RMSF values of cyanate ester-1, -2, and -3 were observed to be 1.06, 1.39 and 0.79 Å, respectively. The higher RMSF of 1.39 Å of cyanate ester-2 was further found unstable which corroborates its non-binding to the protein and also incurring conformational changes to a carrier protein. Molecular simulations revealed that cyanate ester-1 and cyanate ester-3 formed stable conjugates with carrier protein Tbp-B.

Abbreviations: CRM 197: Cross-reactive mutant; Tbp-B: Transferrin binding protein B; DT: Diphtheria toxoid; TT: Tetanus toxoid; PRP: Poly-riboysil Ribitol phosphate; CDAP: 1-cyano-4-dimethyl amino pyridine tetra fluoroborate; CPIP: 1-cyano-4-pyrrolidinopyridinium tetrafluoroborate; CnBr: Cyanogen Bromide; CE: cyanate esters; PnPs:: Pneumococcal polysaccharide

1. Introduction

Vaccines are an effective tool to prevent infectious diseases. The immunity attained by vaccination has largely suppressed several diseases like polio, measles, tetanus, meningitis. Polysaccharide vaccines in infants are poorly immunogenic as they do not develop T-cell immune responses and directly interact with B-cell/s inducing antibody production in absence of T-cell/s (T cell-independent immune response). For long-lived memory cell/s, polysaccharide (Ps) needs to be coupled with carrier protein so that it can be presented by antigen-presenting cell/s. Diphtheria toxoid (DT), tetanus toxoid (TT) and diphtheria toxoid variant CRM197 are extensively used as carrier proteins in glycoconjugate development. There is a possibility of suppression of immunological epitopes when co-administered with TT or DT as a carrier protein due to their extensive use. The repeated use of these conventional carrier proteins in glycoconjugate vaccine results in bystander interference which masks the immunological epitopes of polysaccharide (Dagan et al., 2010). Additionally, there is a need for chemical detoxification of these carrier proteins during manufacturing which may alter the immunological properties. There is also the possibility of pre-existing maternal antibodies against the conventional carrier proteins reducing polysaccharide-induced immune response.
(Gorring et al., 2001) which necessitates the need for new carrier protein.

In our study, we explored transferrin binding protein-B (Tbp-B) from Neisseria meningitidis Serogroup C as a carrier protein in glycoconjugate preparation. Previous studies showed Tbp-B is around 65–70 kilodalton (KDa) lipid anchored membrane protein which helps the mobilization of iron uptake from host cell with association with another membrane protein Tbp-A (Rokbi et al., 1997). Tbp-B has been explored for vaccine development due to its crucial iron recruiting function engendering a protective immune response against different pathogenic bacteria (Calmettes et al., 2012). Studies have shown immunogenic potential with optimal antibody titer using Tbp-B as a carrier protein in the Meningococcal C polysaccharide conjugate vaccine (Gorring et al., 2001).

Polysaccharide (Ps) capsules play an important role as virulence factors for bacterial survival. The immunogenic nature of the polysaccharide remains the prime factor to explore Ps as a major target for vaccine production. In earlier studies, 23 pneumococcal serotypes which are known to cause invasive pneumococcal disease in 80–90% of the infected population were used in the 23-valent pneumococcal polysaccharide vaccine. The use of this vaccine has shown reduced invasive infection and mortality by 71% and 32%, respectively (Perciani et al., 2013). The first conjugate vaccine based on Hib-PRP (Poly-ribosyl Ribitol phosphate of Haemophilus influenzae type-b) conjugated to diphtheria toxoid in an attempt to improve the immune response of polysaccharides (Ps) by conjugating it to a carrier protein received FDA approval (Avery & Goebel, 1929). Subsequently, various conjugate vaccines like PRP-CRM197, PRP-TT, etc. were successfully marketed and proved effective against Hib-derived diseases (McHugh, 2020). In previous studies, several cyanating agents such as 1-cyano-4-dimethyl amino pyridine tetrafluoroborate (CDAP), 1-cyano-4-pyrrolidinopyridinium tetrafluoroborate (CPIP), cyanogen bromide (CnBr) have been used to generate highly reactive cyanate esters which further react with amine groups of the protein to form stable iso-urea derivatives, thus producing the glycoconjugate vaccine (Jin et al., 2003; Lees, 2015; Suárez et al., 2008).

An earlier study has revealed the structural confirmation of transferrin binding protein using homology modeling, molecular docking, molecular dynamic simulation and stereochemical studies (Al-Refai et al., 2020). In our study, we aimed at developing an in silico model using bioinformatics tools in which cyanate esters have been generated on hydroxyl groups of polysaccharides using cyanating agent CDAP. After stereochemical evaluation of the built protein model, the best solitary model was selected for further analysis. An approach in our study aimed at molecular docking analysis and simulation to generate the base model for the development of conjugate vaccine using new carrier protein to understand the interaction and stability of bonds between reactive groups and ligands.

2. Material and methods

2.1. Homology modeling

2.1.1. Sequence retrieval and template selection

The three-dimensional X-ray crystal structure of Tbp-B is unavailable in the PDB database; we have built the 3D structure of Tbp-B using homology modeling techniques. To construct a homology model of Tbp-B from N. meningitidis C Serogroup C18, the sequence of amino acid was acquired from the UniProt database (https://www.uniprot.org/) (UniProt Consortium, 2021) with UniProt ID: A1KVG7. The BLASTP resulted in the highest sequence similarity of transferrin binding protein B (TbpB) with serogroup B N. meningitidis (PDB ID: 4QQ1) (Adamiak et al., 2015).

Alignment analysis of query sequence i.e. transferrin binding protein B from N. meningitidis C Serogroup C18 (Tbp-B) and template sequence transferrin binding protein B from serogroup B N. meningitidis was done using Sequence Viewer of Schrodinger software. The alignment pattern for the amino acid sequence of Tbp-B (N. meningitidis Serogroup C18) and Tbp-B (N. meningitidis Serogroup B) was studied using the Prime module (Jacobson et al., 2004). From the BLASTP and alignment results, we have selected the crystal structure of transferrin binding protein B (Tbp-B) from serogroup B N. meningitidis (PDB ID: 4QQ1) as a template structure to build a homology model.

Protein Structural Alignment of the built model (Tbp-B) and template crystal structure (PDB ID: 4QQ1) were performed in Maestro v12.3.013 (Schrödinger Release 2021-1: Maestro, Schrödinger, LLC, New York, NY, 2021) which reveals the structural deviation and domain conservation within protein structures.

2.1.2. Model building and its stereochemical validation

The Prime module of Schrodinger software (Jacobson et al., 2004) was used to build the homology model of Tbp-B. The Prime Homology Modeling workflow incorporates the complete protein structure prediction process from template identification, to alignment, and model building. The BLASTP program was used to search for suitable templates for homology modeling from the PDB database. The target (Tbp-B)-template sequences alignment was done using the CLUSTALW module of the Prime software. The homology model of Tbp-B was evaluated using protein structure Analysis (ProSA) software, this tool is extensively utilized to check the protein structures (3D models) for potential error. The resulting z-score denotes the overall quality of the built homology model and calculates the deviation in the total energy of the structure regarding an energy distribution obtained from random conformations (Wiederstein & Sippl, 2007).

Ramachandran plot analysis was applied to evaluate the model, it stipulates the overview of allowed and disallowed regions of the protein model with respect to torsion angle values and serving as an important indicator of the quality of protein 3D structures. Also, the quality of the modeled Tbp-B was evaluated by Verify 3D score (analyses the compatibility of an atomic 3D model with its amino acid sequence) and ERRAT score (evaluates the statistics of non-bonded
interactions between different atom types) using SAVES (Structure Analysis and Verification Server) v6.0 (https://saves.mbi.ucla.edu/). From the stereochemical validation study, a valid model of Tbp-B was selected for further studies. The selected valid built model of Tbp-B was prepared by Schrodinger’s Protein Preparation Wizard to refine the structure and the prepared structure was used for further analysis.

### 2.1.3. Preparation of 2D structure of cyanate esters (ligands)

The typical conjugation reaction using CDAP (1-cyano-4-dimethylaminopyridinium tetrafluoroborate) as a cyanylating agent is depicted as the following chemical reaction (Figure 1).

In brief, the pneumococcal polysaccharide Serotype-5 (PnPs-5) was activated with CDAP at pH 9.0 for 3 min which generates cyanate esters (CE) on hydroxyl groups (–OH) of PnPs-5 as shown above in Figure 1 (Lees et al., 2020). The cyanate esters are active molecules that interact with carrier protein through the amine group (–NH₂) to yield a highly stable iso-urea bond. In the present study, three types of cyanate esters are prepared in silico mimicking the above reaction considering the randomized activation of hydroxyl groups on PnPs-5 polysaccharide (e.g. –OH at position 2 and position 5 of ring 1 activated to cyanate ester refer to Figure 2).

The 2D structures of designed cyanate esters (Ligands) were drawn with the 2D sketcher of Schrodinger Maestro. All the ligands were energy minimized using the OPLS-2005 force field in Maestro to get energetically favorably conformation.

### 2.1.4. Molecular docking calculations

Molecular docking calculations were implemented to reveal the binding pattern of cyanate esters within the binding cavity of the Tbp-B protein model. The binding cavity for the Tbp-B was mapped considering the interaction of CE with the amine group (–NH₂) from the side chain of the lysine residues of Tbp-B. The binding cavity for the Tbp-B model was predicted by the SiteMap module of Schrodinger (Halgren, 2009). SiteMap resulted in five probable binding sites with a better druggability score with a good amount of hydrophobic cavity volume. The grid box for the same binding sites was set over the residues which come within the respective binding cavity with a grid box size of 10 Å. All ligands were subjected to molecular docking within predicted binding sites of the Tbp-B model using Glide software (Friesner et al., 2006). All the groups of ligands were treated as flexible during docking calculations. The Standard Precision (SP) method of Glide was used for docking. In the case of cyanate ester-2 and -3, due to a small binding cavity of the Tbp-B and complex structure of the cyanate ester-2 and -3, it fails to dock into the binding cavity using SP mode of Glide. Cyanate ester-2 and -3 were subjected to Induced Fit Docking (IFD) using Glide and Prime module of Schrodinger (Farid et al., 2006), where ligand and the binding site region (5 Å region of the binding cavity) were treated as flexible during the docking calculations. Interacting amino acids in the binding cavity were analyzed by Maestro to know the binding pattern.

#### 2.1.5. Molecular dynamic simulation

Molecular dynamic (MD) simulations were performed for cyanate esters complexed with the Tbp-B model after docking to reveal the stability of docked complexes. In this study, we have explored active site 1 (Table 1) of Tbp-B having an optimal binding affinity towards three types of cyanate esters.

MD simulations were executed for the complexes of Tbp-B-cyanate ester-1, Tbp-B-cyanate ester-2, and Tbp-B-cyanate ester-3 using Desmond (Bowers et al., 2006) module of Schrodinger software with incorporating OPLS-2005 force field. These three complexes were solvated using an explicit solvent model (TIP3P) in a 10 Å cubic box with PBC (periodic boundary condition) box. The total charge of all the complex systems was neutralized by adding counter ions to balance the net charge of the complex system.

To balance the net charge of the system, neutralization was done by adding counter ions Na⁺ and Cl⁻. Three different MD simulations were performed for a 100 ns time scale with a 2 fs time step and energies and trajectories were recorded at 1.2 ps and 4.2 ps, respectively. An NPT (N, number of atoms; P, pressure; T, temperature) ensemble of Nose–Hoover thermostat (Martyna et al., 1994) and barostat method were implemented to balance the constant pressure (1 bar) and temperature (300 K) of all the complex systems. The hybrid algorithm of energy minimization along with the steepest descent method (1000 steps) afterwards algorithm of conjugate gradient was used.

The electrostatic interactions (long-range) were calculated using a method called PME (particle-mesh Ewald) method (Essmann et al., 1995), the SHAKE algorithm was implemented to restrain the hydrogen bond geometry (Ryckaert et al., 1977; Lokhane et al., 2020) and the cut-off for the VDW (Van der Waals) interactions was set to 9.0 Å during the simulation time.

The conformational changes and thermodynamic behavior of three different Tbp-B-cyanate ester complexes were investigated by root mean square fluctuation (RMSF) and root mean square deviation (RMSD) for each amino acid of Tbp-B, computed to probe fluctuation occurrence. Besides, protein-ligand hydrogen bond analysis was done using Desmond’s simulation event analysis (SEA) utilities.

### 3. Results and discussion

#### 3.1. Sequence analysis

The sequence alignment study of the template of 4QQ1 with A1KVGG7 was determined using blast analysis. The alignment
Figure 3 shows that Alanine (A), Cysteine (C), Aspartate (D), Phenylalanine (F), Leucine (L), Asparagine (N), Proline (P), Arginine (R), Serine (S), Tryptophan (W), Tyrosine (Y), Glutamic acid (E), Histidine (H), Methionine (M) are the conserved amino acids in the query and template sequences. Amino acid sequence from Serine(S)-51 to Arginine(R)-59, Serine(S)-395 to Alanine (A)-399 shows a barrel shape in both the sequences. Almost all the amino acids of template sequence and query sequence are in continuous and identical form except amino acid 1-6 of template sequence, which is 543 whereas query sequence is 540 amino acid long. The sequence alignment shows the 98% identity of the query sequence to the template sequence.

3.2. Homology modeling

As highlighted above, at present there are no experimental structures of Tbp-B; therefore, in order to analyze the ligand-proteins key interactions, we constructed a homology model of Tbp-B. The homology modeling was carried out using the Prime module resulting in a total of five models and the best model was considered based on the stereochemical analysis. The validation of the homology model generated from Prime was done by the ProSA, PDBsum (Laskowski et al., 2018) and protein quality predictor (ProQ), to confirm the model’s structural integrity.

The model was validated based on PDBsum generated Ramachandran plot Figure 4a shows that the maximum amino acid (AA) residues i.e. 421 (92.123%) were found in the allowed region/favorable region whereas minimal residues i.e. 33 (7.221%) were found in the additional allowed region and only 3 (0.656%) residues were found in disallowed region resulting from the good quality model. Ramachandran plot reveals psi–phi torsion angles for all residues in the structure (except glycine at chain termini). Glycine residues are separately identified as a triangle as these are not restricted to the regions of the plot appropriate to the other side chain types. The darkest region in red corresponds to the core region showing the most favorable combination of phi–psi values. Ideally one would hope to get 90% of the residues in the core region.

ProSA analysis of the selected model revealed that the model has a structure resembling of native structure with Z-Score -5.61 within the X-ray region (Figure 4(b)). Also, the analysis done by Protein Quality Predictor gives the LGscore: 9.776 and MaxSub: 0.8, suggest that the build model is an extremely good quality of the model. The Verify_3D scores which verify the compatibility of 3 D structure with its amino acid sequence were 98.13% with averaged 3 D-1D score 0.2 (Figure S1(a)), the Errat (overall quality factor) values which give statistics of non-bonded interactions between different atom types were found to be 72.366 (Figure S1(b)) which confirms the good quality if built Tbp-B model. The stereochemical analysis of Tbp-B model shows the built model using the Prime module is a good quality model and can be used for further study.

3.3. Loop modeling

The template and model structural alignment was done by using Maestro software, and the alignment score was 0.002 (smaller is better) and the Root Mean Square Deviation (RMSD) was 0.225 Å (Figure 5(a)), suggesting that the built model is of high quality with a higher confidence level. We

![Figure 2. Two-dimensional (2D) structure of (a) cyanate ester-1, (b) cyanate ester-2 and (c) cyanate ester-3.](image)

| Active site       | Binding site residues                                                                 | Site score | DScore | Size  | Volume (Å³) |
|-------------------|----------------------------------------------------------------------------------------|------------|--------|-------|-------------|
| Site 1 (Binding Pocket 1) | Lys255, Asp251, Lys563, Asp452, Ser451, Tyr427, Val450, Pro449. | 1.000      | 1.022  | 143   | 362.208     |
| Site 2 (Binding Pocket 2) | Lys585, Lys438, Lys432, Glu372, Glu373, Asp439, Lys583 | 0.941      | 0.966  | 99    | 222.950     |
| Site 3 (Binding Pocket 3) | Lys376, Lys375, Lys178, Pro322, Lys522, Asp365, Arg447, Asp523, | 0.851      | 0.849  | 74    | 327.222     |
| Site 4 (Binding Pocket 4) | Arg260, Asn271, Arg263, Arg202, Ser208, Glu204, Glu270, Glu223, Ser305, Asn303 | 0.863      | 0.830  | 73    | 217.119     |
| Site 5 (Binding Pocket 5) | Lys593, Lys404, Lys386, Lys563, Asp426, Tyr427, Ser432. | 0.832      | 0.695  | 47    | 106.330     |

*aSite score that gauges the propensity of the site to bind ligands tightly. 
*bDruggability of predicted sites, greater the value high degree of confidence (For determining whether a target pocket has drug-like physicochemical properties.) 
*cBinding pocket-size defined by the number of site points included in the site. 
*dBinding pocket volume.
found two loop regions (Lys347–Ala356 and Pro575–Glu584) of the modeled protein structurally not well aligned on the template structure, as these loop regions were missing on the template structure. In this study, two loops (Lys347–Ala356 and Pro575–Glu584) were subjected to loop modeling using the Prime module to refine the modeled loops using the OPLS-2005 force field. The resulted model with a refined loop (Figure 5(b)) was used for the docking and MD simulation studies.

3.4. Active site analysis

The validated model of Tbp-B was subjected to active site identification using SiteMap of the Schrodinger. Five active sites were predicted as mentioned in (Table 1) and Figure 6 which describes the site score, druggability score, size, and volume of the cavity. In brief, site score determines the propensity of the site bind ligand tightly, which in our case is 1 or near to 1, this is said to be a good score for ideal active site determination. The druggability of the active predicted site was good to prove the pocket/active site has drug-like physicochemical properties to which the ligand can have effective interaction. Binding pocket size and volumes are also included in Table 1.

3.5. Molecular docking analysis

The 2D and 3D structures of cyanate esters were visualized in Maestro software. Energy minimization and partial charge addition were done for all the ligands on OPLS-2005 software using the LigPrep module. LigPrep is used to prepare high quality, all-atom 3D Structure for cyanate esters, starting with 2D or 3D structures, its simplest use is to produce a single, low energy 3D conformation with the correct chirality for each molecule for further use in computational studies. To understand the binding mode, cyanate esters were docked with Tbp-B by using Glide software. Three cyanate esters (ligand) were docked with five active sites of Tbp-B by

Figure 3. The sequence alignment between Tbp-B (UniProt ID: A1KVG7) and template (PDB ID: 4QQ1). The secondary structure assessment (SSA): the strands are shown in blue colored arrow, loop presented in black line, and helix represented in orange colored cylinder.

The sequence alignment between Tbp-B (UniProt ID: A1KVG7) and template (PDB ID: 4QQ1). The secondary structure assessment (SSA): the strands are shown in blue colored arrow, loop presented in black line, and helix represented in orange colored cylinder.
creating a grid box on binding site residues (Table 1). In molecular docking, the Tbp-B molecule was kept rigid whereas cyanate esters were kept flexible for free interaction. All the Glide docking parameters have been kept by default, and the output setting is set in such a way that, at least five docking poses per ligand will be included in the result. The resulting binding poses of each cyanate esters were analyzed with Maestro software and the top binding pose with respect to good interactions within the binding pocket of Tbp-B and its docking score were considered for further analysis. Docking analysis i.e. the binding affinity of cyanate esters with Tbp-B is mentioned in Table 2.

Three (3) cyanate esters that have activated groups were docked with active site 1 of Tbp-B (cyanate ester-1 with Standard precision glide docking, cyanate ester-2, and -3 with induced-fit glide docking). Although, several amino acids show interaction with cyanate esters, we focused primarily on the amine (–NH2) group from the side chain of the lysine

**Figure 4.** The stereochemical analysis for homology model of Tbp-B: (a) The Ramachandran plot for the Tbp-B model, allowed/favorable region, additional allowed region and disallowed region are shown in red, yellow and white colored, respectively (b) ProSA Z score graph.

**Figure 5.** (a) Model-template structural alignment, where green color represented the homology model and the template structure (PDB ID: 4QQ1) is shown by red color (b) Loop refinement, where built loop (initial loop) is represented in red color and refined loop is shown in purple color.
residues that effectively interacts with cyanate ester resulting in a stable bond formation. The docking result of cyanate ester-1 determines that Lys255, Lys252, Lys563, Asp452, Pro449 are the most interactive residues with cyanate ester. Nitrogen (N) atom of cyanate ester-1 forms one hydrogen bond with polar, basic, positively charged nucleophilic residue lysine (Lys255) with the bond distance of 2.59 Å along with a docking score of $-5.654 \text{ kcal/mol}$ (Figure 7). Cyanate ester-1 forms two hydrogen bonds with negatively charged, polar, hydrophilic, acidic amino acid Aspartate (Asp452) with the bond distance of 1.85 and 2.71 Å respectively. Cyanate ester-1 forms a one hydrogen bond with non-polar, aliphatic amino acid proline (Pro449) with a bond distance of 2.14 Å. Hydrogen bond formation was also observed between cyanate-ester-1 with polar, basic, positively charged nucleophilic residue lysine at 252 and 563 positions with a bond distance of 2.37 and 2.98 Å, respectively.

The docking result of cyanate ester-2 determines that Lys252, Tyr427, Lys563, Asp452, Val450 as the most interactive residues, in which Lys252 forms one hydrogen bond with cyanate ester with a bond distance of 2.69 Å and with a docking score of $-8.901 \text{ kcal/mol}$ by induced fit docking procedure due to the complexity of ligand and proteins. Also, Asp452 forms three hydrogen bonds at bond distances of 2.19, 1.56 and 2.56 Å. Cyanate ester-2 forms one hydrogen bond with positively charged, non-acidic, polar, hydrophilic histidine (His286) with a bond distance of 2.06 Å. Negatively charged glutamate (Glu36) was also found to form a bond with cyanate ester-2 with a bond distance of 1.74 Å. Tyrosine (Tyr427) interacted with one hydrogen bond and one aromatic hydrogen bond at a bond distance of 2.19 and 2.73 Å, respectively. Non-polar aliphatic valine (Val450) and polar, non-charged serine (Ser451) formed one hydrogen bond each with cyanate ester-2 at a bond distance of 2.26 and 2.48 Å, respectively (Figure 8).

Cyanate ester-3 was docked by induced fit docking results Lys563, Asp452, Lys252, Tyr427, Asp251, Lys255, Ser457 as the most interactive residues with cyanate esters (Figure 9).

| Compound name | Docking score (kcal/mol) | Interacting residues | Bond type | Bond distance (Å) |
|---------------|--------------------------|----------------------|-----------|------------------|
| Cyanate ester-1 | $-5.654$ (SP) | Lys255* | H-Bond | 2.59 |
| | | Asp452 | H-Bond | 1.85, 2.71 |
| | | Pro449 | H-Bond | 2.14 |
| | | Lys252 | H-Bond | 2.37 |
| | | Lys563 | H-Bond | 2.98 |
| | | His286 | H-Bond | 2.06 |
| | | Glu36 | H-Bond | 1.74 |
| | | Lys252* | H-Bond | 2.69 |
| | | Tyr427 | H-Bond | 2.19 |
| | | Tyr427 | Ar H-Bond | 2.73 |
| | | Lys563 | 2 H-Bond | 2.25, 1.93 |
| | | Asp452 | 3 H-Bond | 2.19, 1.56, 1.89 |
| | | Val450 | H-Bond | 2.26, |
| | | Ser451 | H-Bond | 2.48 |
| | | Ser457 | H-Bond | 2.25 |
| | | Asp452 | 2 H-Bond | 1.69, 2.56 |
| | | Lys252 | H-Bond | 2.69 |
| | | Lys563* | 2 H-Bond | 2.29, 2.71 |
| | | Tyr427 | H-Bond | 1.89 |
| | | Asp251 | H-Bond | 2.04 |
| | | Lys255 | H-Bond | 2.65 |

*Lysine interacted with N atom of cyanate ester (cyanate group). SP, docking score obtained from standard precision docking; IFD, docking score obtained from induced fit docking.
In this case, Lys563 formed two hydrogen bonds with cyanate ester-3 at 1.69 and 2.56 Å, respectively with a docking score of 
\[ \text{C0} \] 6.262 kcal/mol. Aspartic acid at two different positions Asp452 (2H-bond) and Asp251 (1H-bond) individually had an affinity with cyanate ester-3 and formed hydrogen bond at a distance of 1.69, 2.56 and 2.04 Å. Serine (Ser457) at a bond distance of 2.25 Å and tyrosine (Tyr427) at a bond distance of 1.89 Å established hydrogen bond with cyanate ester-3.

Also, the molecular docking study was done on all the predicted active sites, in detailed docking analysis for the active site 2–5 are summarized in Table S1–S4, respectively.

### 3.6. Molecular Dynamic stimulation

To apprehend the effect of structural alteration of the cyanate esters and to examine the receptor-ligand contact, molecular dynamic (MD) simulation was carried out for 100 ns using the OPLS-2005 force field. Desmond MD system was used to envision the interaction and to monitor a comparative analysis between the three cyanate esters with the Tbp-B. A molecular dynamics study was done for site-1 with all three cyanate esters. The thermodynamic stability of cyanate esters with Tbp-B was scrutinized using the RMSD and RMSFs using 1000 trajectories captured during molecular dynamic simulation. The average RMSD of the trajectories for cyanate ester-1 and cyanate ester-3 and Tbp-B showed relative stability (Figure 10). The RMSD analysis of cyanate esters and the Tbp-B complex indicates that they reach equilibration and oscillate around a mean value after 40 ns. The average RMSDs in the Tbp-B structure from 30 to 100 ns for cyanate esters bound to the Tbp-B receptor was 2.49 Å.

The RMSD values for the Tbp-B when it complexes with cyanate ester-1, -2 and -3 were 1.85, 3.76 and 1.88 Å, respectively (Figure 10) suggesting that cyanate ester-1 (Movie 1) and cyanate ester-3 (Movie 3) shows insignificant variation substantiating its equilibration in the system and have stable interaction with Tbp-B receptors. The RMSD values for cyanate ester-1 and -3 were within the acceptance limit however, cyanate ester-2 reported a maximum RMSD value of 3.76 Å showing the structural deformity of Tbp-B (Movie 2), as visualized by MD trajectories in the Maestro. The higher deviation occurs in the structure of Tbp-B when it binds with cyanate ester-2, as the initially docked cyanate ester-2 displaces its position from the binding site to another site of the protein thus resulted in structural deformity of Tbp-B (Movie 2).

On the extreme divergence, RMSF of Cα residues was computed to determine the structural changes induced by the binding pattern of the ligand. Figure 11 demonstrates the RMSF of three cyanate esters that concludes the mobility and flexibility of Tbp-B amino acids. Cyanate ester-1 and cyanate ester-3 form a stable conformation with a low RMSF value of 1.06 and 0.79 Å, respectively whereas, cyanate ester-2 deviates from site-1 and moves to site-4 without any interaction with receptors at site-1 manifests the higher RMSF value of 1.39 Å. One more interesting observation was noted during the study of the dynamics of cyanate ester-2 with the receptor at active site-1 is that ester did not bind to site-1 but also changed the conformation of protein which is very rarely observed in computational biology studies. Further studies are required to completely understand the conformational changes that occurred to protein with non-interaction of ligands. It is noteworthy that the most stabilized contact between ligand and receptor was observed in cyanate ester-1 and cyanate ester-3 with Tbp-B protein.

### 3.6.1 H-bond analysis between Tbp-B and cyanate esters

Additionally, the stability of the protein–ligand complexes during 100 ns MD simulation was examined by simulation event analysis, and the intermolecular hydrogen bond formation between ligands (cyanate ester-1, -2 and -3) and
proteins (Tbp-B) were determined. Figure 12 demonstrates the number of hydrogen bond formed between docked complexes during 100 ns MD simulation, it was observed that, the cyanate ester-1 and cyanate ester-3 forms about 2–10 hydrogen bonds constantly within the active site 1 of the Tbp-B during 100 ns MD simulation. In the case of cyanate ester-2 forms 2–12 hydrogen bonds with Tbp-B in a similar pattern as compared to cyanate ester-1 and -3, but as we observed in Movie 2, the cyanate esters-2 displace its position from the binding site to another site in the very initial phase of the MD simulation. From these studies, we can consider that cyanate ester-1 and -3 forms a stable complex with Tbp-B by showing better binding affinity with stable interactions within the binding pocket 1.

4. Conclusions
The present study focuses on the interaction of Tbp-B receptors with cyanate ester. Cyanate esters were prepared by
random activation of the hydroxyl group (−OH) present on pneumococcal polysaccharide Serogroup-5 (PnPs-5) with CDAP. The 3D structure of Tbp-B was built using homology modeling. The built model was refined by the energy minimization molecular dynamics methods. In our study residues Lys255, Asp452, Val450, Tyr427, His286, Gln372, Pro322, Arg260, Asn271 were found to be most interactive with cyanate esters, however, we explored lysine (K) which plays a vital role in the formation of isourea derivative when cyanate esters and the amino group of the protein interact with each other. Cyanate ester-1 and cyanate ester-3 were found to be more synergetic with receptors of protein when docked at active site-1 whereas cyanate ester-2 was observed to be non-reactive at the said active sites. Conformational changes for Tbp-B were also visualized during simulation of cyanate ester-2 with Tbp-B in which cyanate ester-2 did not bind to the protein and also changed the structural conformation of the protein. Such a phenomenon of protein alteration is rarely noticed in computational biology experiments.

Active site on Tbp-B receptor predicts the interactive residues present, out of which vital amino acid residues can be monitored very closely and docking can be challenged around the desired residues of the protein. Molecular docking and molecular dynamic study are useful to predict the conjugation kinetics and consequently polysaccharide activation with cyanating agents like CDAP, CnBr, etc. which will help to virtually monitor the interaction of carrier protein with the activated polysaccharide. In silico studies are helpful in designing the optimal conjugation reaction of polysaccharide and protein to construct an immunogenic glycoconjugate which will serve as an effective vaccine.

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Disclosure statement

The authors declare that there is no competing interest for the work carried out as mentioned in the manuscript.

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