Thesis progression

Title: Molecular Dynamics (MD) simulations for Antibody-Antigen interactions

Thesis title: Design and development of broad-spectrum antibodies for flavivirus therapy

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1. Introduction

Flaviviruses such as dengue virus (DENV), Zika virus (ZIKV), and Japanese encephalitis virus (JEV), are mosquito-borne pathogens responsible for widespread and recurrent outbreaks, particularly in tropical and subtropical regions (Daep et al., 2014). These viruses represent a major public health burden due to their ability to cause severe and diverse clinical manifestations, ranging from encephalitis and congenital abnormalities to hemorrhagic fever and death (Pierson et al., 2020). DENV is the most prevalent flavivirus globally, causing an estimated 390 million infections annually, with approximately 100 million symptomatic cases, and nearly 70% occurring in Asia, where JEV is also endemic (Bhatt et al., 2013). ZIKV outbreaks have further compounded this burden, particularly due to their association with neurological complications and congenital malformations, including microcephaly during pregnancy (Musso et al., 2019). Importantly, these viruses often co-circulate in the same geographic regions, leading to repeated or sequential exposure in human populations. A critical challenge arising from this overlap is the presence of cross-reactive antibodies. During recent ZIKV outbreaks in dengue-endemic areas, it became evident that pre-existing anti-DENV antibodies could enhance ZIKV infection through antibody-dependent enhancement (ADE) (Dejnirattisai et al., 2016). Numerous studies have shown that pan-flavivirus cross-reactive antibodies can enhance DENV and ZIKV infection *in vitro* (Halstead et al., 1980; Fgbami et al., 1987), and sera from DENV- or ZIKV-infected patients were also capable of enhancing infection by both viruses (Halstead et al., 1980; Fagbami et al., 1987; Dejnirattisai et al., 2016; Priyamvada et al., 2016). Furthermore, passive transfer of cross-reactive antibodies isolated from DENV- or ZIKV-infected individuals resulted in more severe disease *in vivo*, as demonstrated in DENV-infected AG129 mice and ZIKV-infected $Stat2^{-/-}$ mice (Halstead et al., 1980; Bardina et al., 2017). Despite decades of research, there are currently no approved antiviral therapeutics specifically targeting flaviviruses, and treatment remains largely supportive. While vaccines are available for JEV and DENV, outbreaks continue to

occur, reflecting limitations in vaccine coverage, variable efficacy, and safety concerns, particularly related to ADE (WHO, 2010; WHO, 2015; Kubinski et al., 2020; Waickman et al., 2023). Notably, no licensed vaccines are currently available for ZIKV, further highlighting this unmet need (Dutta et al., 2023).

The development of an effective DENV vaccine remains especially challenging due to the existence of four antigenically distinct serotypes and the risk of ADE during secondary infection (Khetarpal et al., 2016; Waickman et al., 2023). Although two vaccines, Dengvaxia®, and Qdenga®, have been approved, their use is restricted to individuals with confirmed prior DENV infection living in endemic regions, limiting their broader applicability (Mallapaty et al., 2022; Lenharo et al., 2023; Dengvaxia®, 2024). Together, these challenges underscore the urgent need for next-generation vaccine strategies that can provide broad, cross-protective immunity against DENV, ZIKV, and JEV, while minimizing the risk of ADE. Targeting conserved epitopes shared among these flaviviruses, therefore, represents a rational approach toward the development of safe and broadly effective flavivirus vaccines.

Monoclonal antibodies (mAbs) have emerged as highly promising antiviral agents due to their target specificity, favorable pharmacokinetics, and ability to neutralize viruses (Flores et al., 2025). In the context of flaviviruses, the envelope (E) protein serves as the primary antigenic target for neutralizing antibodies. This glycoprotein mediates host cell attachment and membrane fusion, comprising three structural domains: domain I (EDI), domain II (EDII), and domain III (EDIII). Most mAbs targeting the flavivirus E protein tend to be serotype-specific, often recognizing epitopes within domain III (Dejnirattisai et al., 2010; Young et al., 2020). Moreover, some cross-reactive mAbs have been identified, primarily binding to the fusion loop and bc loop regions of domain II, while a smaller subset recognizes conserved epitopes in domain III. However, designing mAbs with broad-spectrum activity across multiple flaviviruses is particularly challenging due to significant antigenic variation between and within viral species. To overcome this, rational antibody design and affinity optimization are essential.

In general, antibodies directed toward domain II form the largest proportion of cross-reactive anti-E antibodies isolated from dengue-infected individuals (Lai et al., 2008). Although these antibodies typically have low neutralizing potency, they display high cross-reactivity across multiple serotypes and often more than domain III-specific mAbs. Many of them focus on evolutionarily conserved sites within the fusion loop and bc loop of domain II and can neutralize all four DENV serotypes (Crill et al., 2009). Fusion loop-specific antibodies can sometimes exhibit both neutralizing and infection-enhancing activities. For example, the

human mAb D23-1B3B9 (B3B9) potently neutralizes all DENV serotypes but can enhance infection in Fcγ receptor-expressing cells at sub-neutralizing concentrations, limiting its therapeutic application (Setthapramote et al., 2012; add Sasaki et al., 2013). Another fusion loop-targeting antibody, 2A10G6, shows cross-reactivity not only with DENV1-4 but also with ZIKV, JEV, YFV, and WNV. It neutralizes all DENV serotypes, ZIKV, YFV, and WNV by binding to a conserved motif (98DRXW101) in the domain II fusion loop, protecting mouse models against lethal doses of DENV1-4, WNV, and ZIKV. Functional assays indicate that 2A10G6 blocks infection at a post-attachment stage of the viral life cycle (Deng et al., 2011; Dai et al., 2016). An alternative potent and broadly neutralizing human dengue virus-specific monoclonal antibody, named 1C19, reveals a unique cross-reactive epitope on the bc loop of domain II of the envelope protein (Smith et al., 2013). Thereby, EDII-targeting antibodies exhibit the potential to neutralize multiple flavivirus species by recognizing conserved structural motifs essential for viral fusion. However, such antibodies frequently display lower intrinsic neutralization potency compared to EDIII-targeting antibodies and may be associated with an elevated risk of ADE of infection under sub-neutralizing conditions (Sarker et al., 2023). These limitations pose significant challenges to the development of broadly effective therapeutic antibodies based on EDII recognition.

To overcome the limitations of EDII-targeting antibodies, a rational, engineering-driven strategy is essential. In this context, computational antibody design has emerged as a powerful complement to experimental methods, as it enables the optimization of antibody-antigen interactions entirely *in silico* before laboratory validation (Hummera et al., 2022). Leveraging recent advances in structural biology, protein design, and computational modeling, researchers can now integrate techniques such as homology modeling, molecular docking, mutational scanning, and molecular dynamics (MD) simulations to systematically design antibody variants with enhanced binding affinity, broader cross-reactivity, and a reduced risk of ADE. For example, previous studies have focused on developing broadly neutralizing antibodies effective against all four dengue serotypes by targeting conserved regions of the envelope protein. These studies have employed *in silico* analyses to identify conserved epitopes, construct scFv antibodies, and optimize their binding through mutant libraries, demonstrating enhanced stability and binding affinity of the antibody-antigen complexes (Rathore et al., 2019). And another one, they presented a computational framework combining sequence-based machine learning (ML) and MD simulations to identify potent and stable antibodies against EDII. Using a curated dataset of antibody-EDII interactions, this ML model predicted candidate antibodies, which were further validated by MD simulations for binding

stability. This approach enables the rational design of therapeutic antibodies capable of cross-reactivity across multiple dengue serotypes (Natsrita et al., 2024). Collectively, these approaches provide a clear and efficient pathway toward the development of next-generation therapeutic antibodies with the precision and breadth required to combat multiple flavivirus species effectively.

This study aims to develop broadly neutralizing therapeutic antibodies capable of targeting multiple flaviviruses by integrating computational design with experimental validation. Antibody engineering begins with the rational design and optimization of candidates using 1B3B9 as a structural template. Key residues within the binding interface are strategically mutated to enhance affinity and cross-reactivity, guided by molecular docking and binding energy calculations. Promising variants are subsequently evaluated against multiple flavivirus targets to assess broad-spectrum potential. Molecular dynamics simulations are then employed to examine the structural stability and binding behavior of antibody-antigen complexes in silico. Based on these insights, further modifications, such as charge optimization at critical binding sites are introduced to improve binding affinity. The refined antibodies are re-docked to confirm enhanced performance and shortlisted for final MD validation. Selected candidates are then expressed, purified, and experimentally characterized through binding and functional assays. The ultimate objective is to generate cross-reactive antibodies with superior binding properties and reduce risk of ADE. The outcomes of this work will contribute to the development of universal antibody-based therapeutics for the prevention and treatment of flaviviral infections, particularly in regions where multiple flaviviruses co-circulate.

2. Hypothesis

Broadly neutralizing antibodies against flaviviruses can be rationally engineered by structure-guided computational design and experimental validation. By optimizing key residues at the antibody-antigen interface to enhance affinity, cross-reactivity, and stability, it is possible to generate therapeutic antibodies that provide universal protection against multiple flaviviruses while minimizing the risk of antibody-dependent enhancement.

3. Objectives

3.1 General objective

To design and develop broadly neutralizing antibodies against multiple flaviviruses through an integrated computational-experimental pipeline, aiming to generate safe and effective therapeutics for treatment

3.2 Specific objectives

3.2.1 To rationally design and optimize antibody candidates based on 1B3B9 template by introducing targeted mutations at key binding residues, guided by molecular docking and binding energy predictions

3.2.2 To evaluate antibody-antigen interactions *in silico* through molecular dynamics simulations and refine candidates via charge optimization and stability analysis

3.2.3 To express, purify, and experimentally characterize selected antibodies for binding affinity across multiple flaviviruses

3.2.4 To assess neutralizing activities and ADE risks, establish proof-of-concept for universal antibody-based therapeutics against flaviviral infections

4. Anticipated outputs

4.1 A computational pipeline for the rational design and optimization of broadly neutralizing antibodies against flaviviruses

4.2 A set of engineered antibody variants with predicted enhanced affinity, stability, and cross-reactivity across multiple flaviviruses

4.3 Molecular dynamics simulation data supporting structural stability and binding behavior of antibody–antigen complexes

4.4 Expression and purification protocols for selected antibody candidates

4.5 Experimental data on binding affinity, neutralization activity, and ADE risk of the developed antibodies.

5. Anticipated outcomes

5.1 Identification of lead antibody candidates with broad-spectrum activity against dengue, Zika, and related flaviviruses

5.2 Proof-of-concept that computational and experimental integration can accelerate antibody discovery

5.3 A foundation for the development of universal antibody-based therapeutics to prevent and treat flaviviral infections

5.4 Contribution to the global effort in managing regions burdened by co-circulating flaviviruses, with potential translational applications in public health.

6. Impact

The development of broadly neutralizing therapeutic antibodies against multiple flaviviruses could have a substantial scientific, clinical, and public health impact. Scientifically, the project will advance structure-guided antibody engineering and demonstrate the integration of computational and experimental approaches for rapid therapeutic discovery. Clinically, the identification of cross-reactive antibodies with high affinity and minimal ADE risk could provide a novel treatment strategy for dengue, Zika, and other flavivirus infections, addressing the lack of approved therapeutics. From a public health perspective, these antibodies could reduce disease burden in regions where multiple flaviviruses co-circulate, improving outbreak preparedness and response. Furthermore, the methodologies and insights gained could be applied to other viral families, supporting the broader goal of developing universal antiviral antibody therapeutics.

7. Conceptual framework

Flaviviruses, including DENV, ZIKV, and JEV, pose a persistent global health threat due to their expanding geographic distribution, co-circulation in endemic regions, and the lack of effective antiviral treatments. Developing broadly neutralizing therapeutic antibodies that can simultaneously target multiple flaviviruses is therefore a critical and urgent goal.

This study presents an integrative framework that combines computational and experimental approaches to guide the rational design, optimization, and validation of cross-reactive antibodies targeting conserved epitopes on flavivirus E proteins. The process begins with the selection of a known antibody template, 1B3B9, followed by rational mutagenesis at key binding interface residues to enhance affinity and cross-reactivity. Designed variants are initially assessed through molecular docking and binding energy predictions against multiple flavivirus antigens. Promising candidates are then evaluated via MD simulations to examine structural stability and binding behavior in silico, with charge-based optimization applied to refine interactions. Final designs are re-assessed through docking and MD simulations before experimental expression and purification. Binding affinity is quantified via SPR, and functional efficacy is determined using neutralization and ADE assays.

This integrative framework enables the systematic development of broadly neutralizing antibodies with reduced ADE risk. Furthermore, it provides a scalable model for the rapid engineering of antibody therapeutics against other genetically diverse and emerging viral pathogens.

8. Study design

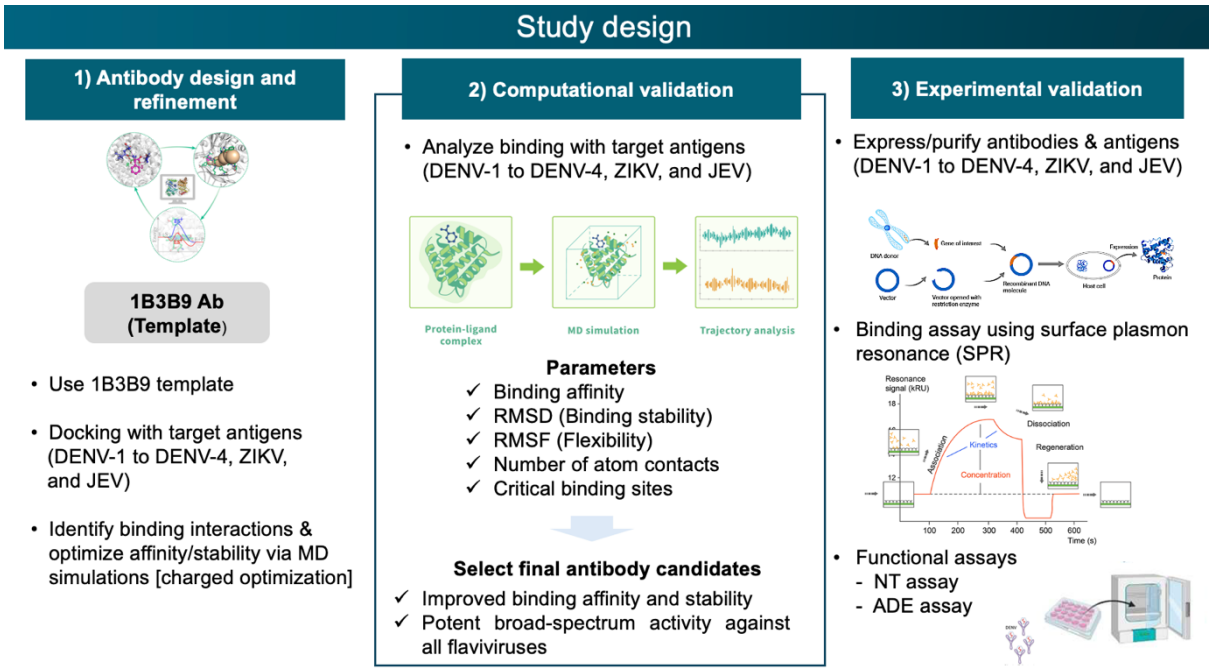

Figure 1 Study design.

This research was conducted in three major phases: (i) Antibody design and refinement, (ii) Validation by molecular dynamics simulations, and (iii) Experimental validation.

(i) Antibody Design and Refinement

The antibody template was selected, named 1B3B9. 1B3B9 is an experimentally derived antibody previously reported to exhibit antiviral activity against dengue virus. Then, the antibody template was docked with the E protein of DNEV-2. Binding interactions were analyzed, and MD simulations were employed to optimize affinity and stability. Subsequently, charge optimization was performed on selected candidates to further enhance their binding properties.

(ii) Computational Validation

The improved antibody-antigen complexes were subjected to extended MD simulations to evaluate their structural and functional stability. Parameters including binding affinity, root mean square deviation (RMSD) for overall stability, root mean square fluctuation (RMSF) for residue flexibility, the number of atomic contacts, and critical binding residues were analyzed to identify the most promising antibody candidates with high stability and broad-spectrum binding potential.

(iii) Experimental Validation

The top antibody candidates, together with their corresponding viral antigens, will be expressed and purified. Binding kinetics will be determined using SPR. Finally, functional assays, including neutralization tests and ADE assays, will be performed to evaluate both protective activity and the potential for enhancement of infection.

9. Methods

9.1 Computational validation by MD simulations

MD simulations were performed to validate the stability and interaction dynamics of antibody-antigen complexes computationally. The fragment antigen-binding (Fab) of the experimental 1B3B9 antibody was complexed with the E dimer protein from all four DENV serotypes. The structural templates for the E dimer proteins were obtained from the Protein Data Bank (PDB) with the following IDs: 4CCT (DENV-1), 5A1Z (DENV-2), 3J6T (DENV-3), and 4CBF (DENV-4). All system topologies and coordinate files were prepared using the pdb2gmx module of the GROMACS simulation package, applying the AMBER99SB-ILDN force field. Each antibody-antigen complex was placed in a dodecahedral simulation box under periodic boundary conditions, maintaining a minimum solute-to-box-edge distance of 1.2 nm. The systems were solvated using the TIP3P water model and neutralized with appropriate counterions, followed by the addition of NaCl to achieve a physiological ionic strength of 0.15 M. Energy minimization was first conducted to eliminate steric clashes and unfavorable contacts. This was followed by a two-step equilibration protocol. Initially, the systems were equilibrated under the NVT ensemble for 100 ps at 310 K using a 2-fs time step. Subsequently, equilibration was continued under the NPT ensemble for an additional 100 ps at 310 K and 1 atm to stabilize pressure and density under physiological conditions. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method, and all covalent bonds involving hydrogen atoms were constrained using the LINCS algorithm. Production MD simulations were then carried out for 100 ns. After that, post-simulation analyses were performed on the combined trajectories obtained from multiple replicas for each system. Structural stability and flexibility were assessed by calculating the RMSD and RMSF of individual residues. Hydrogen bond analyses were also conducted to characterize key intermolecular interactions contributing to complex stability. Hydrogen bonds were defined using standard geometric criteria (donor–acceptor distance $\leq$ 0.35 nm (3.5 Å) and hydrogen-donor-acceptor angle $\geq$ 135°).

9.2 Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) Analysis

Binding free energies (ΔG) were estimated using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) approach under the single-trajectory approximation, as implemented in the *gmx_MMPBSA* package. This method provides an efficient and widely validated framework for quantifying protein-protein binding affinities by integrating molecular mechanics energies with continuum solvation models. In this approach, the total free energy of each system was decomposed into gas-phase molecular mechanics contributions comprising bonded interactions, van der Waals forces, and electrostatic energies derived directly from the force-field parameters. Solvation effects were accounted for by calculating the polar solvation free energy using the Poisson-Boltzmann equation, while the nonpolar solvation component was estimated from the solvent-accessible surface area (SASA). Binding free energies were computed according to:

$$\Delta G_{bind} = \Delta G_{complex} - (\Delta G_{antigen} + \Delta G_{antibody})$$

where $\Delta G_{complex}$, $\Delta G_{antigen}$, and $\Delta G_{antibody}$ represent the free energies of the bound complex and the isolated antigen and antibody, respectively. To ensure statistical robustness, ΔG_{bind} values were averaged over representative configurations extracted at 1-ns intervals from the equilibrated regions of the molecular dynamics trajectories, depending on system convergence. This sampling strategy minimizes conformational bias and captures the dominant interaction energetics governing antibody-antigen recognition.

10. Results

10.1 Post MD simulations analysis

10.1.1 RMSD profiles between the 1B3B9 antibody with the E dimer protein of all four DENV serotypes

To evaluate the structural stability of the antibody-antigen complexes, the RMSD of the backbone atoms was monitored over 100 ns MD simulations for the 1B3B9 antibody bound to DENV-1 to DENV-4 E dimer proteins (Figure 2). Across all serotypes, the antibody remained highly stable, with low RMSD values ranging from 2.54 ± 0.20 Å to 6.90 ± 0.57 Å, indicating minimal conformational changes upon antigen binding. In contrast, higher RMSD values were observed for the E dimer proteins and the full complexes, reflecting the intrinsic flexibility of the viral envelope protein. Among the complexes, DENV-2 exhibited the lowest average RMSD (13.31 ± 1.45 Å), suggesting the most stable antibody-antigen complex.

The DENV-1 and DENV-3 complexes showed higher RMSD values of 17.66 ± 2.47 Å and 17.62 ± 2.30 Å, respectively, indicating increased structural fluctuations. The DENV-4 complex displayed intermediate stability, with an average RMSD of 16.21 ± 2.40 Å. Overall, these results demonstrate that while the 1B3B9 antibody structure remains stable across all four DENV serotypes, the stability of the antibody-E dimer protein complexes is serotype-dependent, with the DENV2 complex showing the most favorable structural stability during the simulation.

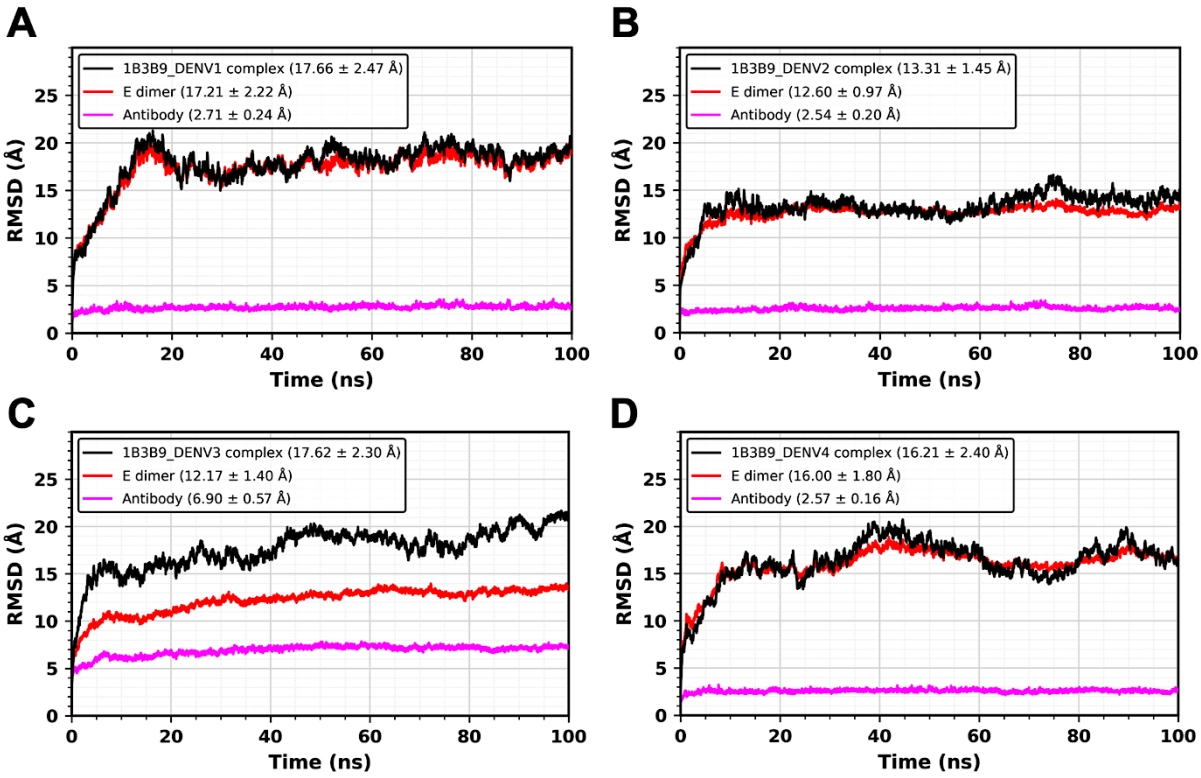

Figure 2 RMSD profiles of the 1B3B9 antibody in complex with the DENV E dimer protein complexes. RMSD profiles of the 1B3B9 complex with E dimer proteins of (A) DENV-1, (B) DENV-2, (C) DENV-3, and (D) DENV-4 over 100 ns simulations. RMSD of the complex (black), E dimers (red), and antibody (pink) are shown as mean ± SD.

10.1.2 Number of atom contacts analysis between the 1B3B9 antibody with the E dimer proteins of four DENV serotypes

To further characterize the interfacial stability of the 1B3B9 antibody-antigen complexes, the number of atom contacts between the antibody and the E dimer protein was quantified for all four DENV serotypes. The highest average number of atom contacts was observed in the 1B3B9-DENV-2 complex (554.26 ± 63.29), indicating the most extensive

intermolecular interface and strong packing interactions. In comparison, moderate contact numbers were detected for DENV-3 (423.65 ± 76.87) and DENV-1 (415.56 ± 71.22), reflecting a reduced but still substantial degree of interfacial engagement. The lowest number of atom contacts was found in the 1B3B9-DENV-4 complex (377.01 ± 38.72), suggesting a comparatively smaller and less compact binding interface. Overall, the atom contact analysis supports a serotype-dependent interaction pattern, with DENV-2 forming the most extensive antibody-antigen interface, followed by DENV-3, DENV-1, and DENV-4 (Figure 3).

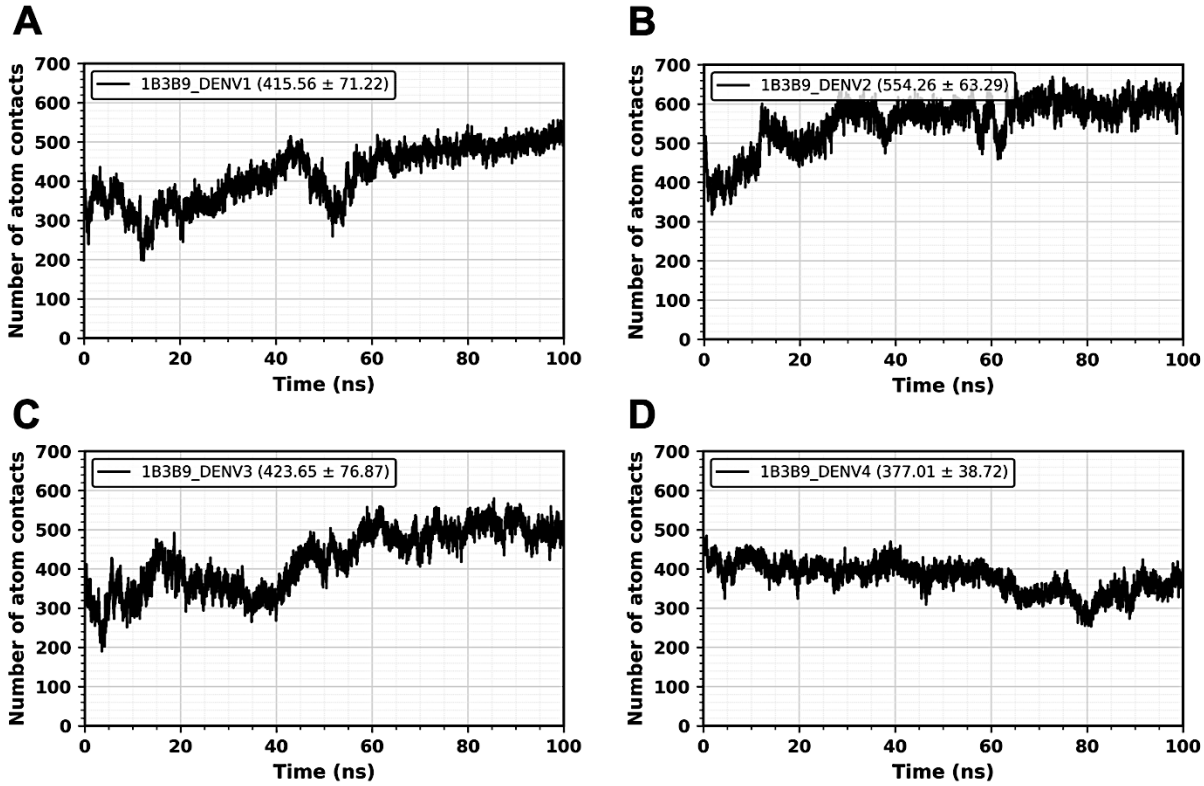

Figure 3 Number of atom contacts between the 1B3B9 antibody in complex with the DENV E dimer proteins. The number of atom contacts formed between the antibody and antigen is shown for complexes with (A) DENV-1, (B) DENV-2, (C) DENV-3, and (D) DENV-4 over 100 ns simulations.

10.1.3 Hydrogen bond analysis between the 1B3B9 antibody with the E dimer proteins of four DENV serotypes

Hydrogen bond analysis was performed to assess the stability and persistence of intermolecular interactions between the 1B3B9 antibody and the E protein of all four dengue virus serotypes. The results revealed clear serotype-dependent differences in hydrogen bond formation. Among the four complexes, the 1B3B9-DENV-2 complex exhibited the highest average number of hydrogen bonds (13.80 ± 2.72), indicating a highly stable

interaction at the antibody-antigen interface. In contrast, moderate hydrogen bond occupancies were observed for DENV-3 (10.36 ± 4.15) and DENV-1 (8.73 ± 2.77), suggesting comparatively weaker but still sustained interactions. The lowest hydrogen bond count was detected in the 1B3B9-DENV-4 complex (7.90 ± 1.88). Overall, these findings suggest that hydrogen bonding contributes most strongly to the binding stability of 1B3B9 with DENV-2, while progressively fewer hydrogen bonds are formed with DENV-3, DENV-1, and DENV-4, respectively (Figure 4).

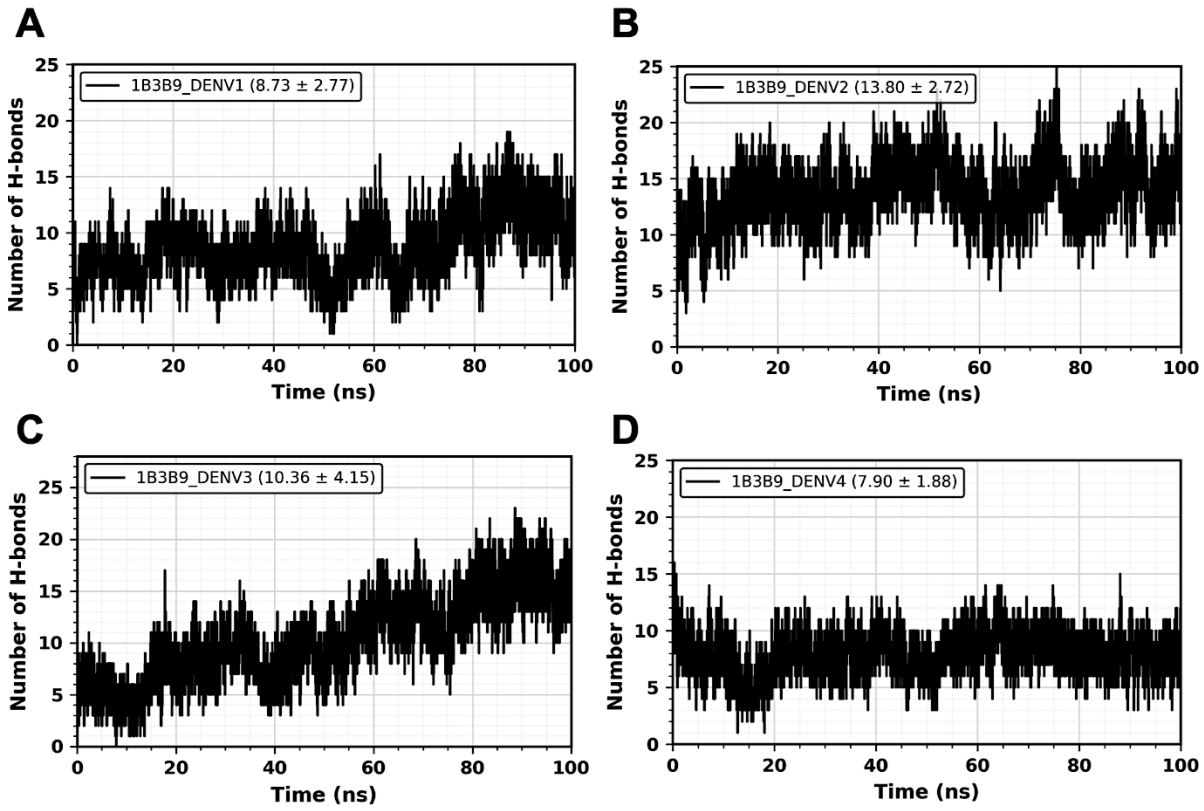

Figure 4 Number of hydrogen bonds between the 1B3B9 antibody in complex with the DENV E dimer proteins. The number of intermolecular hydrogen bonds formed between the antibody and antigen is shown for complexes with (A) DENV-1, (B) DENV-2, (C) DENV-3, and (D) DENV-4 over 100 ns simulations.

10.2 Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA)

10.2.1 Binding energy calculations between the 1B3B9 antibody with the E dimer proteins of four DENV serotypes

To characterize the binding energy of the 1B3B9 antibody in complex with the E dimer protein of all four DENV serotypes. The MM/PBSA binding free energy analysis revealed clear serotype-dependent differences in the interaction strength between the 1B3B9 antibody and the DENV E dimer proteins. Among the four serotypes, the most favorable binding free energy was observed for 1B3B9-DENV-2 (ΔG_bind = −96.33 ± 2.06 kcal/mol), indicating the strongest predicted affinity. This was followed by DENV-3 (−91.75 ± 2.31 kcal/mol), DENV-1 (−76.02 ± 2.40 kcal/mol), and DENV-4, which exhibited the weakest binding (−66.87 ± 1.21 kcal/mol). In all complexes, the binding free energy was primarily driven by favorable van der Waals and electrostatic interactions, as reflected by the strongly negative ΔE_vdW and ΔE_elec terms. Notably, the DENV-3 complex showed the most pronounced electrostatic contribution (ΔE_elec = −513.30 kcal/mol). However, this was largely counterbalanced by an equally large unfavorable polar solvation energy (ΔG_pol = 567.75 kcal/mol), resulting in a less favorable overall ΔG_bind compared with DENV-2. In contrast, the DENV-2 complex displayed a more balanced interplay between electrostatic interactions and polar solvation penalties, leading to the most favorable net binding free energy. The nonpolar solvation term (ΔG_np) consistently contributed favorably across all serotypes, although its magnitude was smaller relative to the gas-phase interaction energies. Overall, these results indicate that differences in binding affinity among DENV serotypes arise not solely from interaction strength in the gas phase, but from the balance between electrostatic attraction and solvation effects. Overall, the MM/PBSA analysis suggests a binding preference, with DENV-2 forming the most extensive antibody-antigen interface, followed by DENV-3, DENV-1, and DENV-4 (Table 1).

Table 1 Comparative MM/PBSA binding free energy analysis of 1B3B9 antibody-E dimer protein complexes across the four DENV serotypes. All values are expressed in kcal/mol.

System	ΔE_{vdW}	ΔE_{elec}	ΔG_{pol}	ΔG_{np}	ΔE_{GAS}	ΔG_{solv}	ΔG_{bind}
1B3B9-DENV1	-117.69	-274.49	329.78	-13.62	-392.18	316.16	-76.02 ± 2.40
1B3B9-DENV2	-145.17	-121.14	186.04	-16.05	-266.31	169.99	-96.33 ± 2.06
1B3B9-DENV3	-130.84	-513.30	567.75	-15.37	-644.14	552.39	-91.75 ± 2.31
1B3B9-DENV4	-84.85	-220.57	248.73	-10.18	-305.42	238.55	-66.87 ± 1.21

10.2.2 Per-residue binding free energy decomposition between the 1B3B9 antibody with the E dimer proteins of four DENV serotypes

Per-residue energy decomposition was performed to identify key antigen and antibody residues contributing to the binding of 1B3B9 with the E dimer protein of all four DENV serotypes. In all complexes, most residues exhibited near-zero contributions, indicating that binding is dominated by a limited number of energetically important hot-spot residues rather than uniformly distributed interactions. On the antigen side, several residues within the bc and fusion loop regions consistently showed strong favorable contributions across serotypes, with interaction energies reaching below −5 kcal/mol. These residues were conserved contributors in DENV-1 to DENV-4, highlighting bc and fusion loop regions as a critical recognition interface for 1B3B9 binding. Notably, the magnitude and number of favorable antigen residues were greater in DENV-2 and DENV-3 compared with DENV-1 and DENV-4, consistent with their stronger overall binding free energies. On the antibody side, dominant energetic contributions were localized primarily within the CDR regions of the heavy and light chains. Residues in CDRH2, CDRH3, and CDRL1 exhibited the most pronounced negative energy contributions, confirming their central role in antigen engagement. These CDR hot spots were preserved across all serotypes but varied in contribution strength, with DENV-2 and DENV-3 showing deeper energy minima than DENV-1 and DENV-4. Overall, the decomposition profiles reveal a serotype-dependent interaction pattern, where enhanced binding in DENV-2 and DENV-3 arises from stronger and more numerous residue-level contributions at both the bc and fusion loop regions surface and antibody CDR regions. These results support the MM/PBSA binding free energy trends and provide a structural basis for differential antibody recognition among DENV serotypes (Figure 5).

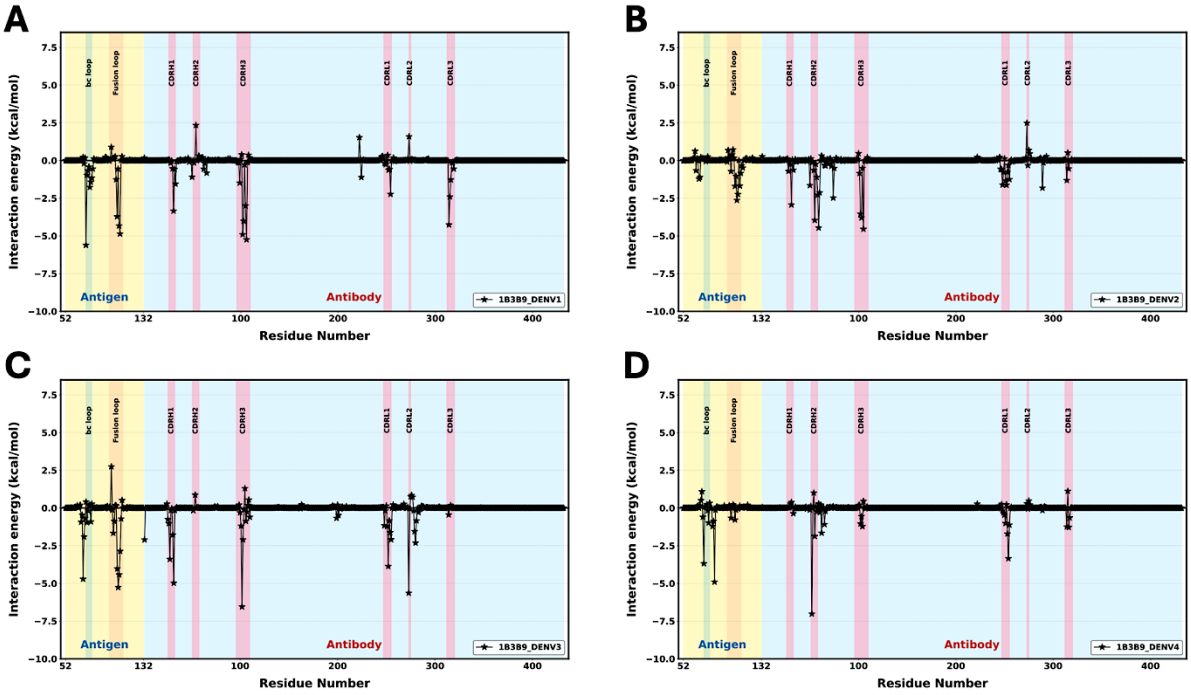

Figure 5 Per-residue binding free energy decomposition (ΔG_{bind}) between the 1B3B9 antibody in complex with the DENV E dimer proteins during 100 ns MD simulations. (A) DENV-1, (B) DENV-2, (C) DENV-3, and (D) DENV-4. Negative values indicate favorable energy contributions to the antibody-antigen interaction.

11. Conclusion

In conclusion, MD simulations and MM/PBSA analyses demonstrated stable binding of the 1B3B9 antibody to the E dimer across all four DENV serotypes. Among the complexes, DENV-2 showed the strongest binding affinity, followed by DENV-3 and DENV-1, while DENV-4 exhibited the weakest interaction. This binding trend was consistently supported by hydrogen bond numbers, atom contact analyses, and binding free energy values. Energy decomposition revealed that binding is mainly driven by van der Waals and electrostatic interactions, with key energetic contributions originating from the bc and fusion loop regions of the E protein and the CDR regions of the antibody. Although the overall binding mode is conserved across serotypes, variations in residue-level contributions explain the observed differences in binding strength. Overall, these results validate the reliability of the MD and MM/PBSA pipeline and establish 1B3B9 as a robust reference antibody for comparative analysis and future structure-guided antibody optimization against dengue virus.

9. Thesis plan

Activities	2025		2026				2027				2028	
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
1. Literature Review and Planning												
2. Quality Examination												
3. Proposal Examination												
PART 1 Antibody Design and Refinement												
4. Characterization of Antibody Candidate												
5. MD Analysis for Identification of Binding Interactions												
6. Affinity Improvement via Charged Optimization												
7. Molecular Docking with Flavivirus Target Antigens												
PART 2 Antibody Design and Refinement												
8. Antibody Candidate Validation using MD Simulations												
9. MD Analysis of Improved Antibody Candidates												
PART 3 Experimental Validation of Antibody Candidates												
10. Expression and Purification of Target Antigens												
11. Expression and Purification of Antibodies												
12. Binding Assay using SPR												
13. Neutralization Assay												
14. ADE Assay												
15. Manuscript Preparation and Submit												
16. Thesis Defense												

Work in Progress

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