MD 627 102 Peer Review in Medical Microbiology Research II

Molecular Dynamics (MD) simulations for Antibody-Antigen interactions

Thesis Title: Design and Development of Broad-Spectrum Antibodies for Flavivirus Therapy

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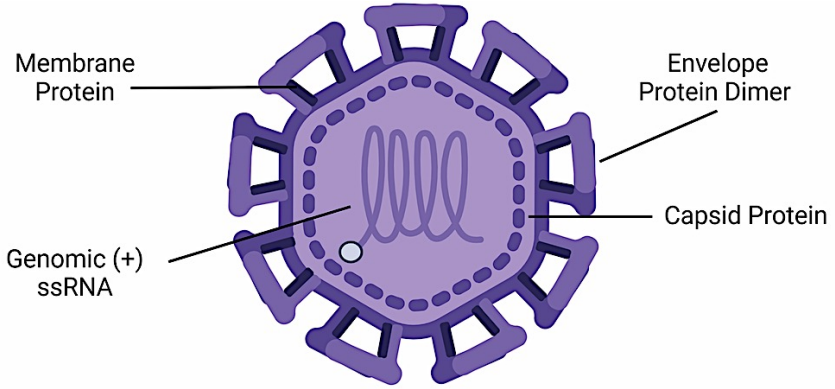
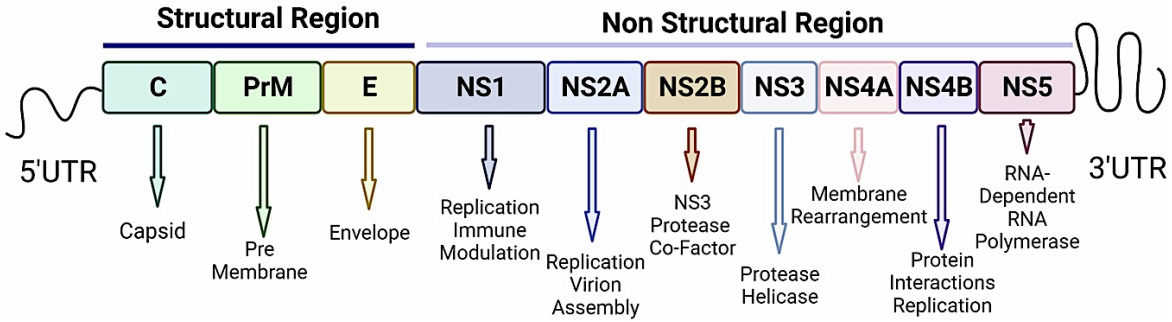
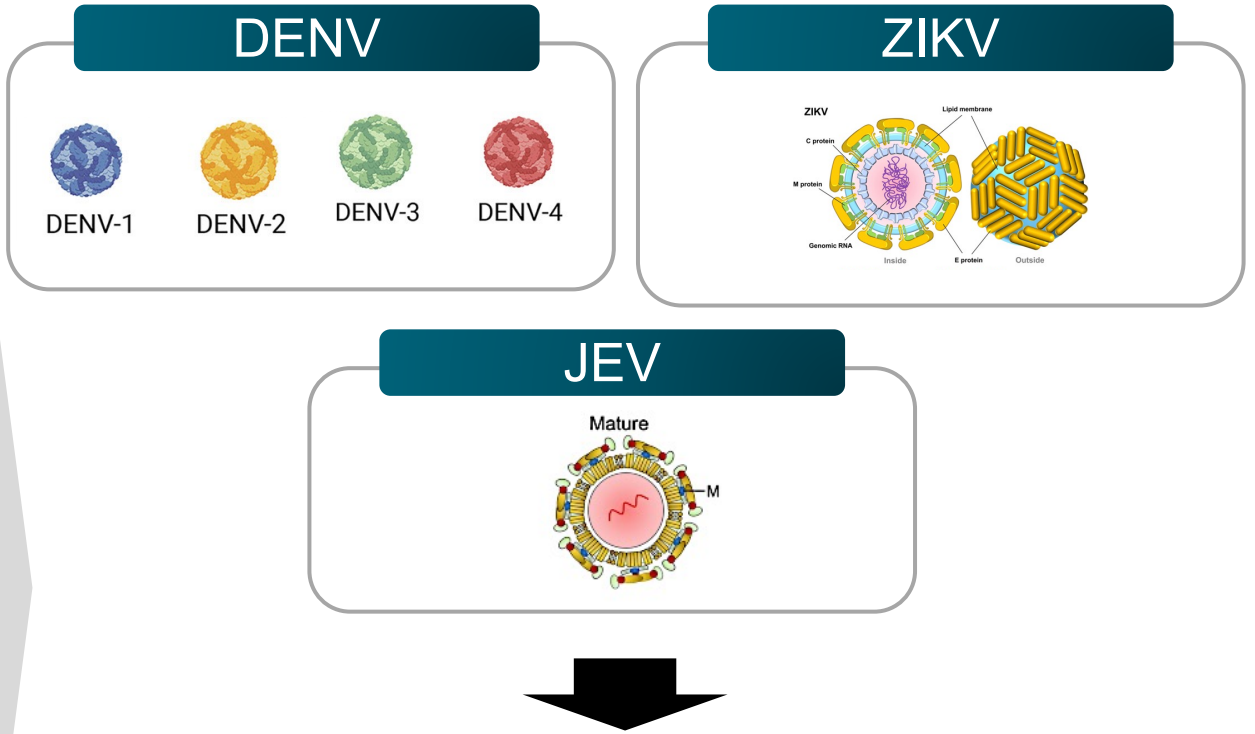
The *Flavivirus* Genome

- **Family:** Flaviviridae
- **Genus:** *Flavivirus*
- **A single positive-stranded RNA virus**
- Dengue, Zika, Japanese Encephalitis, and others.
- **Major global public health concern.**

Tripathi et al., 2025







- ✓ Shared epitopes → cross-reactive Ab design
- ✓ Cross-reactivity VS ADE
- ✓ High public health burden in overlapping regions

Lack of specific therapeutics
underscores the urgent need for
effective broad-spectrum development strategies

Current computational pipeline for antibody design

1st Pipeline

scientific reports

Check for updates

OPEN

Machine-learning-assisted high-throughput identification of potent and stable neutralizing antibodies against all four dengue virus serotypes

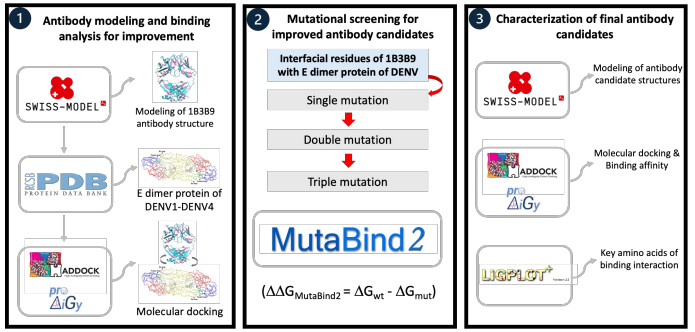
Piyatida Natsrita[1], Phasit Charoenkwan[3], Watshara Shoombuatong[4], Panupong Mahalapbutr[5], Kiatichai Faksri[1,2], Sorujsiri Chareonsudjai[1], Thanyada Rungrotmongkol[6] & Chonlatip Pipattanaboon[1]✉

Natsrita et al., 2024

- ✓ CDR-H3 mutations
- ✓ Machine learning
- ✓ Simple MD simulation

Machine learning & MD simulations

2nd Pipeline

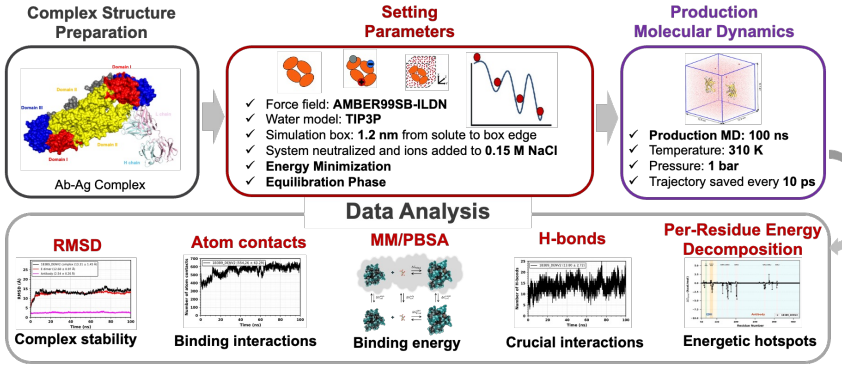


Raha et al., 2024 (Proceeding)

- ✓ Combinatorial CDR mutations
- ✓ Molecular docking & MutaBind2

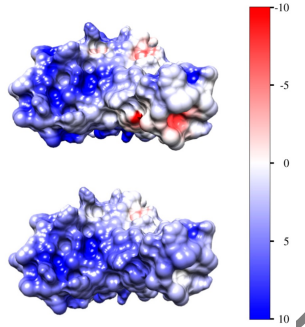
Molecular docking (MutaBind2)

Current Pipeline



Current study

- ✓ CDR structural design guided by charge-based optimization
- ✓ MD simulations



MD simulations (Gromacs)

General objective

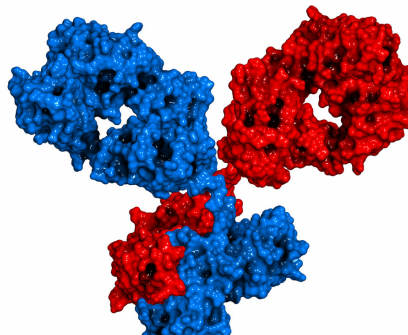
To **design and develop broadly neutralizing antibodies against multiple flaviviruses** through an integrated computational–experimental pipeline, aiming to generate safe and effective therapeutics for treatment

Specific objectives

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
2. To **evaluate antibody-antigen interactions** *in silico* through molecular dynamics simulations and refine candidates via charge optimization and stability analysis
3. To **express, purify**, and **experimentally characterize** selected antibodies for binding affinity across multiple flaviviruses
4. To **assess neutralizing activities** and **ADE risks**, establishing proof-of-concept for universal antibody-based therapeutics against flaviviral infections

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 and stability, it is possible to generate therapeutic antibodies that provide universal protection against multiple flaviviruses while minimizing the risk of antibody-dependent enhancement.

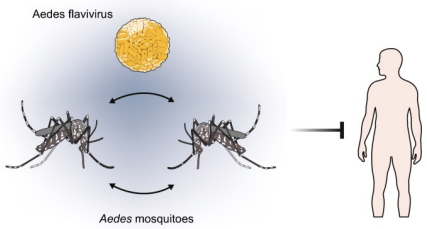


Conceptual framework

Problems → Process → Output → Outcome → Impact

Problems

Flaviviruses



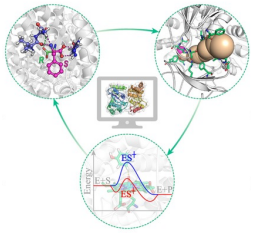
Global re-emerging threats

No approved therapeutics

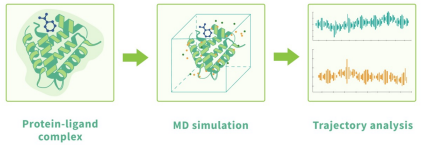
Urgent need for effective, broad-spectrum development strategies

Process

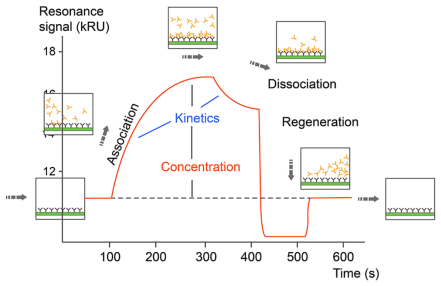
1) Antibody design & refinement



2) Computational validation

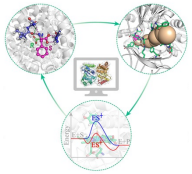


3) Experimental validation

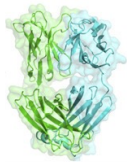


Output

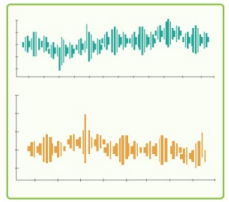
Computational design pipeline



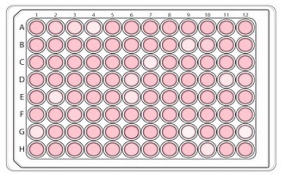
Engineered antibody variants



MD simulation and experimental data

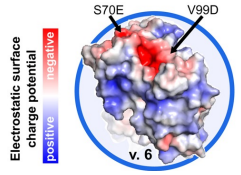


Trajectory analysis

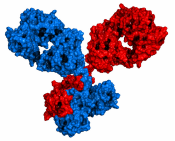


Outcome

Lead antibody identification

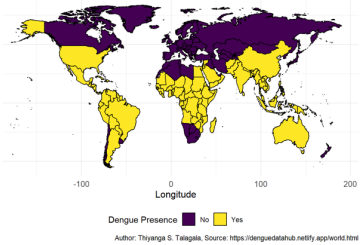


Proof-of-concept to accelerate discovery



Foundation for therapeutic development

Contribution to global virus management



Impact

Strong scientific, clinical, and public health value

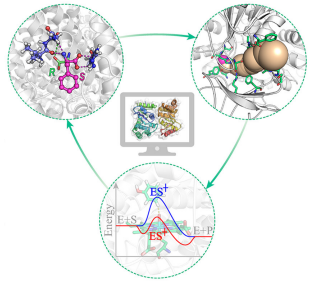
Accelerated therapeutic antibody discovery

Novel flavivirus treatments

Reduced burden of re-emerging viruses

Study Design

1) Antibody design and refinement

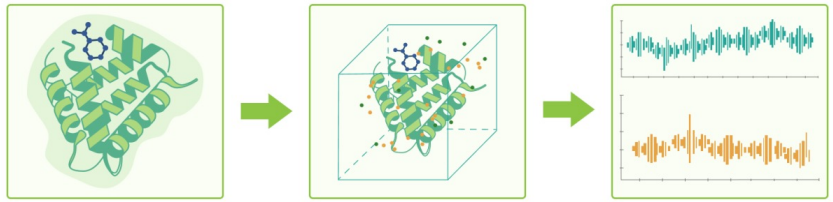


1B3B9 Ab (Template)

- Use 1B3B9 template
- Docking with target antigens (DENV-1 to DENV-4, ZIKV, and JEV)
- Identify binding interactions & optimize affinity/stability via MD simulations [charged optimization]

2) Computational validation

- Analyze binding with target antigens (DENV-1 to DENV-4, ZIKV, and JEV)



Parameters

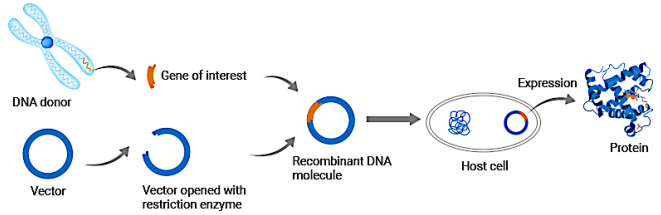
- ✓ Binding affinity
- ✓ RMSD (Binding stability)
- ✓ RMSF (Flexibility)
- ✓ Number of atom contacts
- ✓ Critical binding sites

Select final antibody candidates

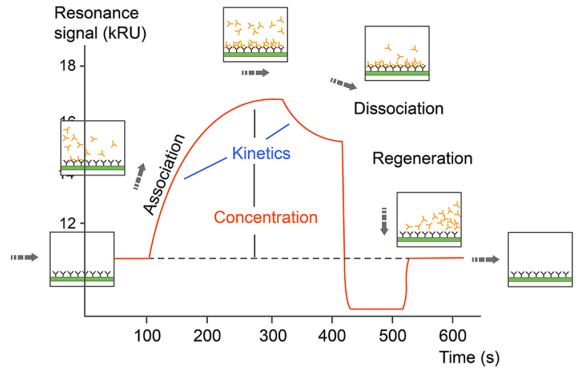
- ✓ Improved binding affinity and stability
- ✓ Potent broad-spectrum activity against all flaviviruses

3) Experimental validation

- Express/purify antibodies & antigens (DENV-1 to DENV-4, ZIKV, and JEV)



- Binding assay using surface plasmon resonance (SPR)



- Functional assays
  - NT assay
  - ADE assay

Study Design

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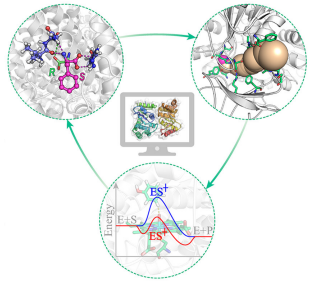

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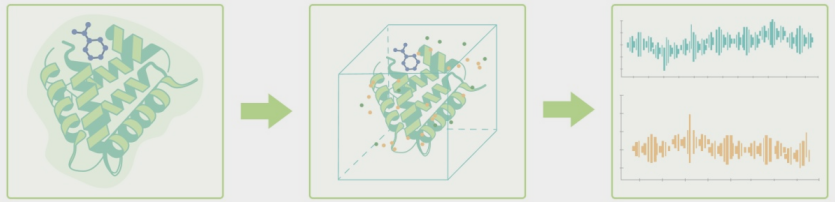

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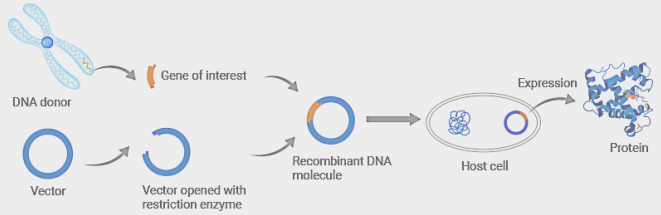

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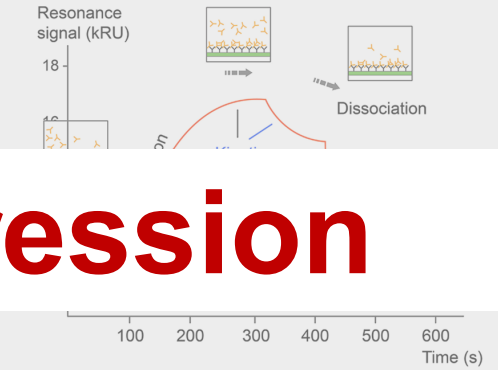

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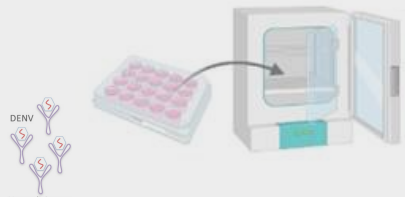
  - ADE assay



Current Progression

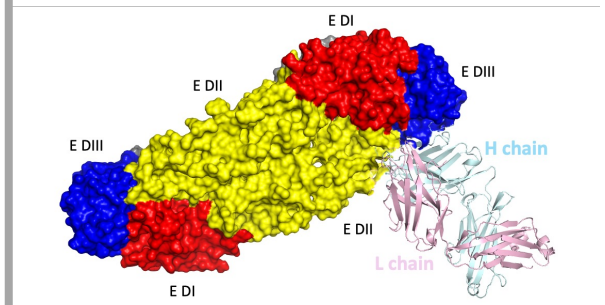
RESULT 1: Molecular Docking Analysis

Objective: To generate the complex between the antibody and target antigens for using in MD simulations

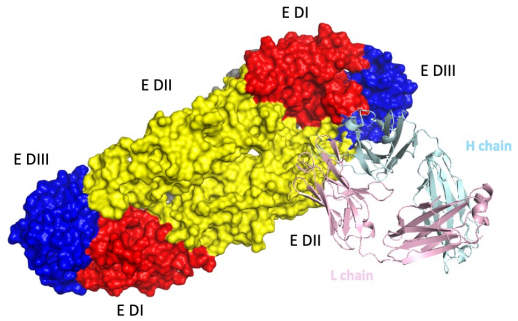
HADDOCK
High-Ambiguity Driven Docking

Ab-Ag Complexes	Docking Score
1B3B9 - DENV1	-89.2 ± 1.7
1B3B9 - DENV2	-107.8 ± 12.4
1B3B9 - DENV3	-91.4 ± 3.3
1B3B9 - DENV4	-85.8 ± 6.2
1B3B9 - ZIKV	-106.1 ± 13.1
1B3B9 - JEV	-113.7 ± 1.1

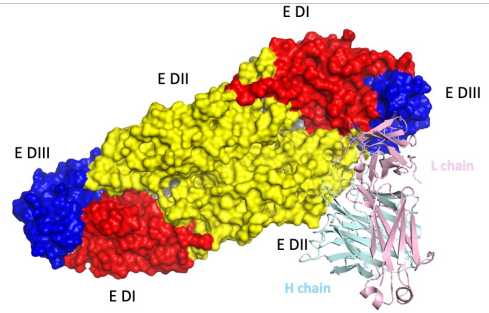
1B3B9 - DENV1



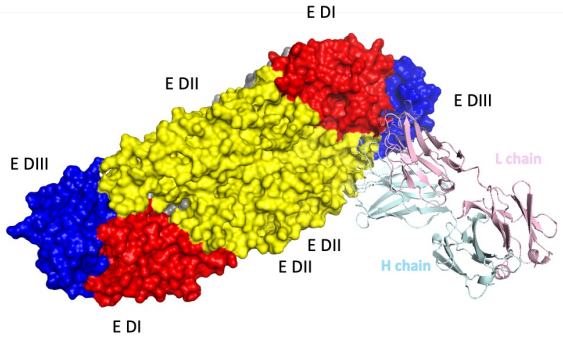
1B3B9 - DENV2



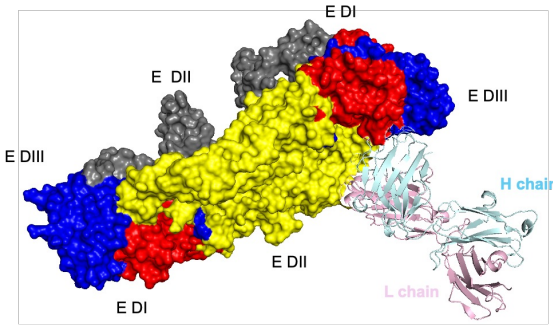
1B3B9 - DENV3



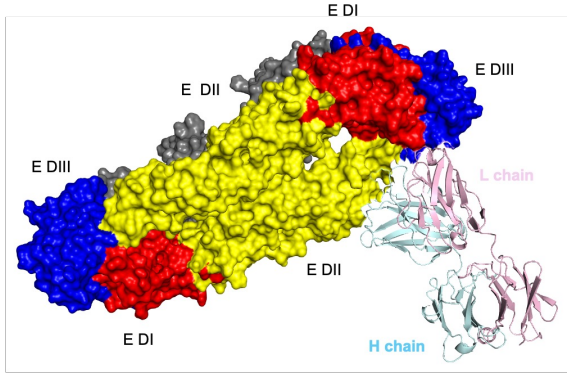
1B3B9 - DENV4



1B3B9 - ZIKV



1B3B9 - JEV



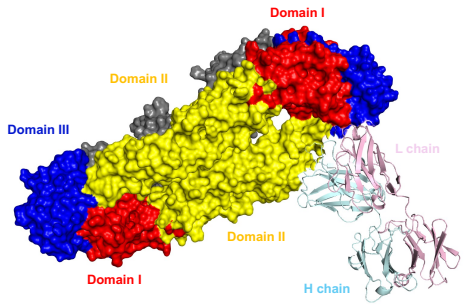
These results indicate that the 1B3B9 antibody has strong potential for cross-reactive binding to multiple flaviviruses.

RESULT 2: Molecular Dynamics (MD) Simulations for Antibody-Antigen Interactions

Objective: To create a standardized workflow of MD simulation for antibody-antigen interactions prior to experimental validation

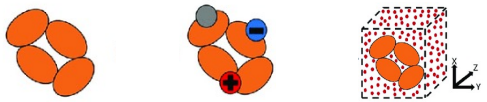
Workflow of MD simulations for Ab-Ag complex

Complex Structure Preparation



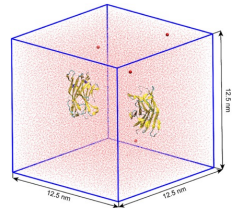
Ab-Ag Complex

Setting Parameters



- ✓ Force field: **AMBER99SB-ILDN**
- ✓ Water model: **TIP3P**
- ✓ Simulation box: **1.2 nm** from solute to box edge
- ✓ System neutralized and ions added to **0.15 M NaCl**
- ✓ **Energy Minimization**
- ✓ **Equilibration Phase**

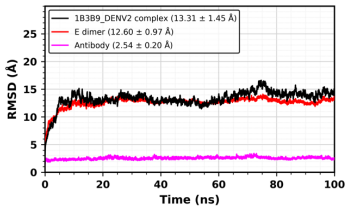
Production Molecular Dynamics



- ✓ **Production MD: 100 ns**
- ✓ Temperature: **310 K**
- ✓ Pressure: **1 bar**
- ✓ Trajectory saved every **10 ps**

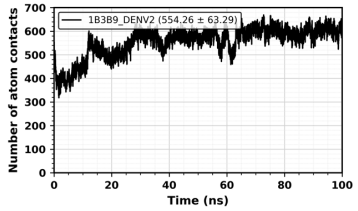
Data Analysis

RMSD



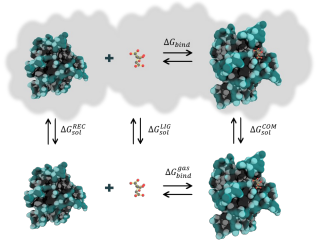
Complex stability

Atom contacts



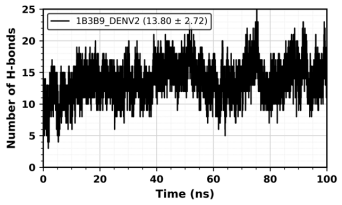
Binding interactions

MM/PBSA



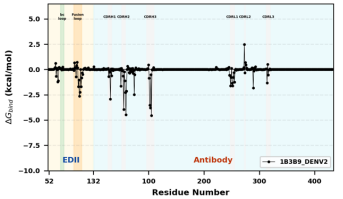
Binding energy

H-bonds



Crucial interactions

Per-Residue Energy Decomposition



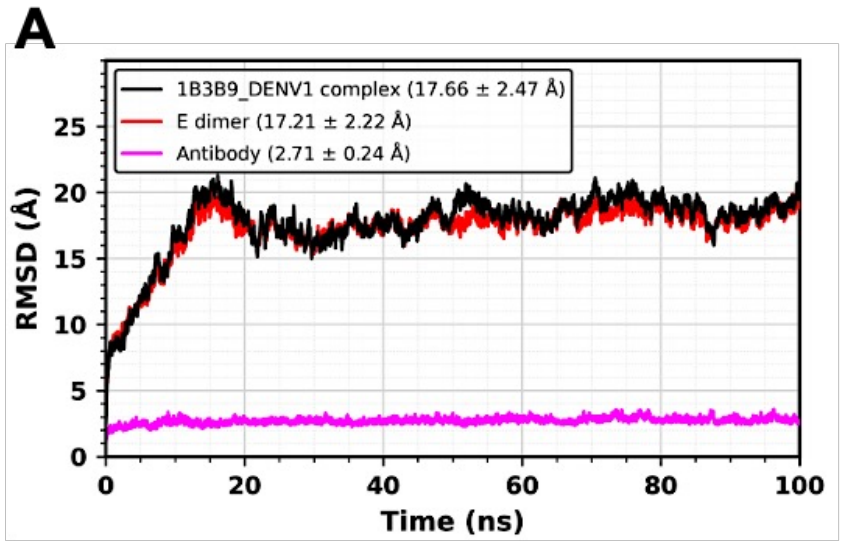
Energetic hotspots

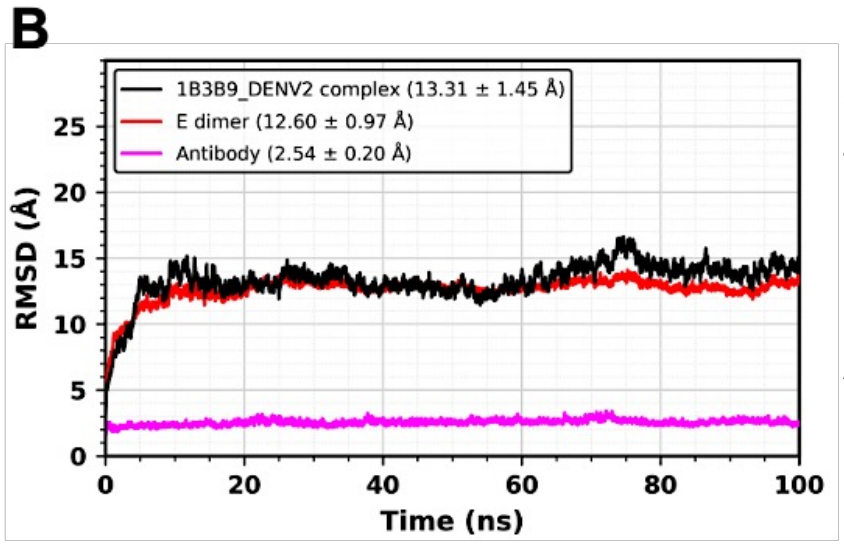
RMSD: Root Mean Square Deviation, RMSF: Root Mean Square Fluctuation; MMPBSA: Molecular Mechanics Poisson-Boltzmann Surface Area

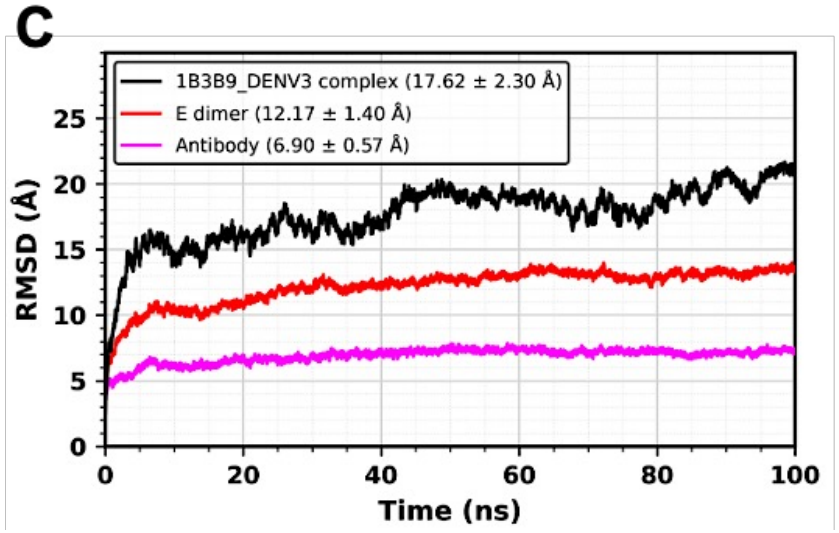
RESULT 2: Root mean square deviation (RMSD)

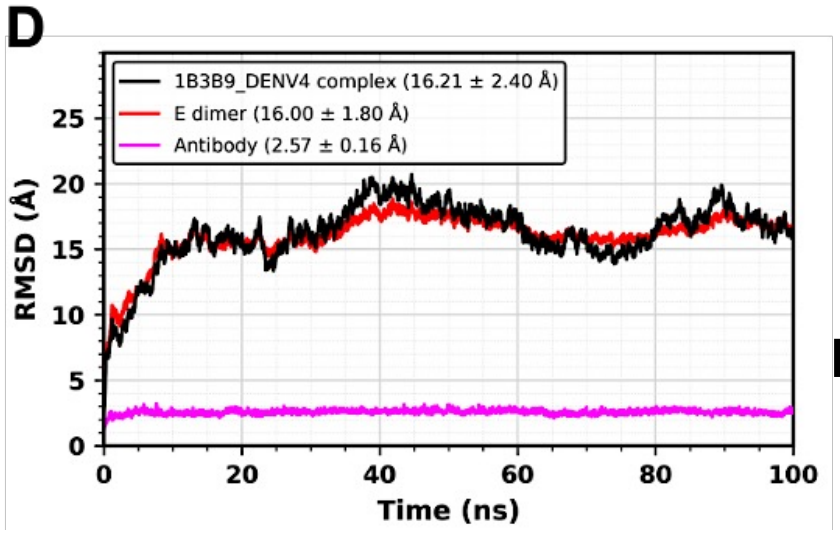
Objective: To analyze structural stability between the 1B3B9 antibody and E dimer proteins

RMSD: Total structural stability of each complex in the system









The RMSD values of each complex fluctuated within the range of 13-17 Å .

All complexes reached equilibrium after approximately 50 ns.

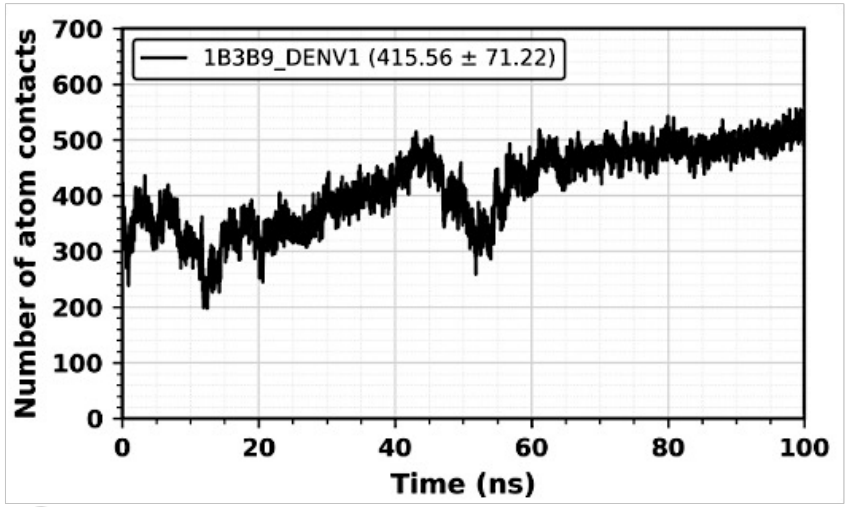
Stability by RMSD values: Complexes
DENV-2 > DENV-colo4 > DENV-3 > DENV-1

RMSD ↓ = Structural stability ↑

Objective: To further characterize the interfacial stability between the 1B3B9 antibody and E dimer proteins

Number of atom contacts: The interfacial stability between 1B3B9 and E dimer protein of DENVs

A



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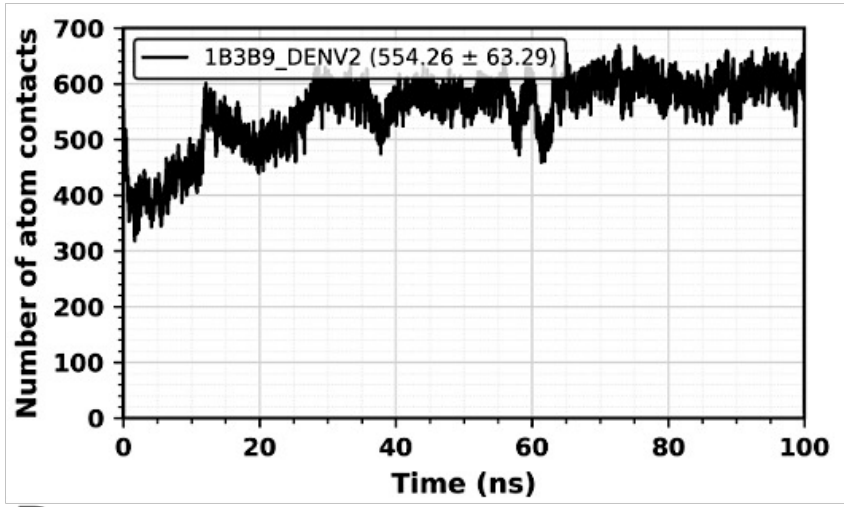

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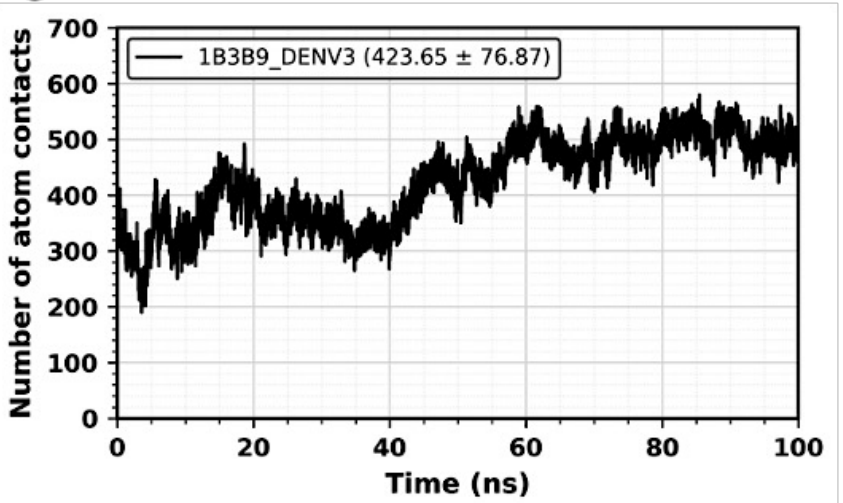
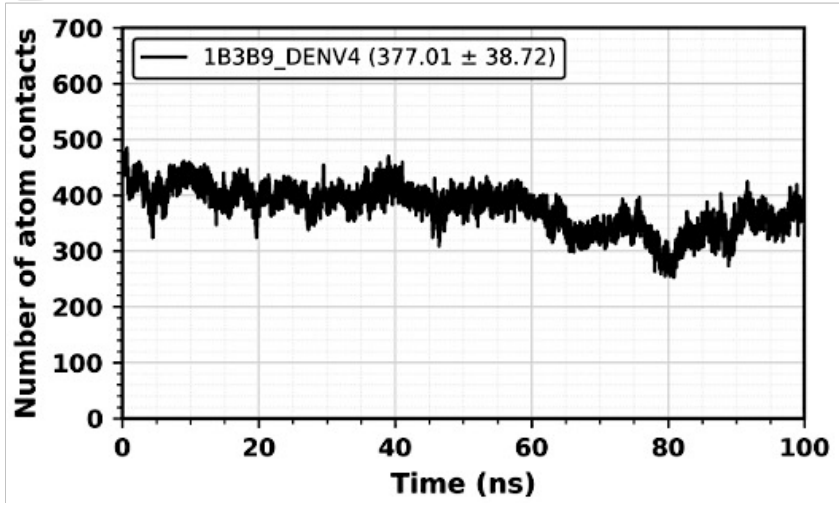


D



The number of atom contacts of all complexes involving approximately 377-554 atoms over the last 100 ns.

More number of atom contacts > More interaction between Ab-Ag complex > More binding stability.

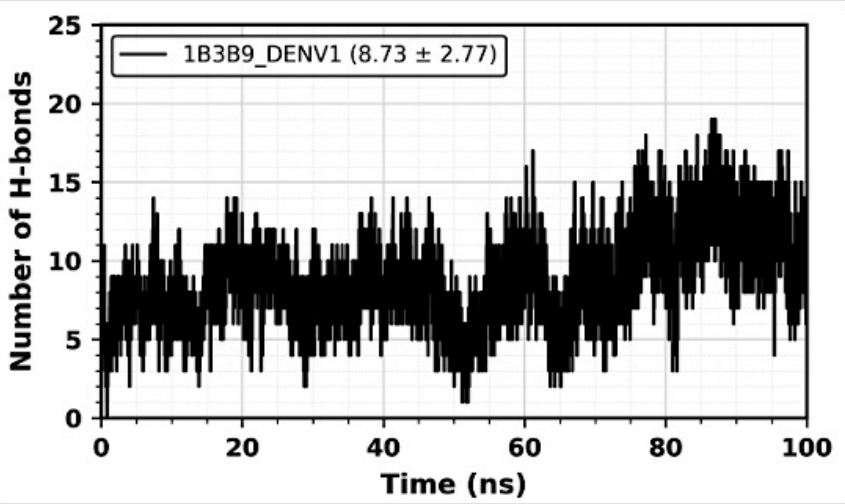
Number of atom contacts:
DENV-2 > DENV-3 > DENV-1 > DENV-4

RESULT 2: Number of H bonds

Objective: To assess the stability and persistence of intermolecular interactions between the 1B3B9 antibody and E dimer proteins

Number of H-bonds: The stability and persistence of intermolecular interactions of Ab-Ag complexes

A



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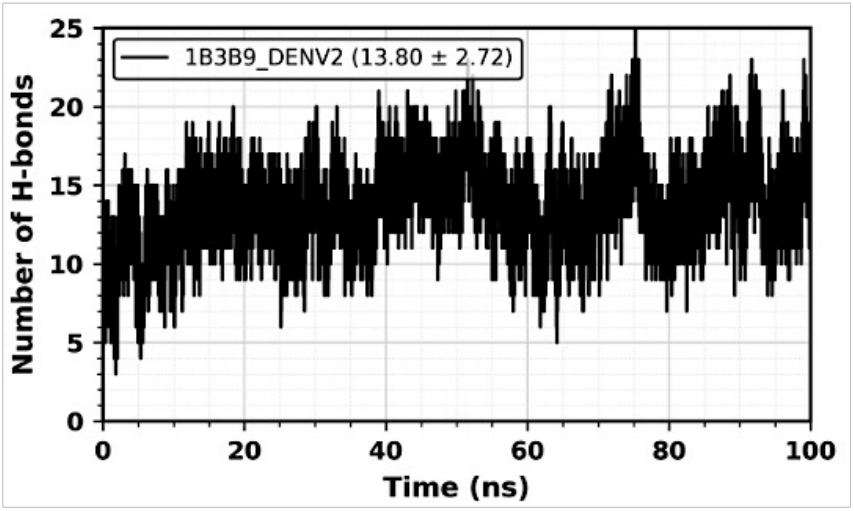

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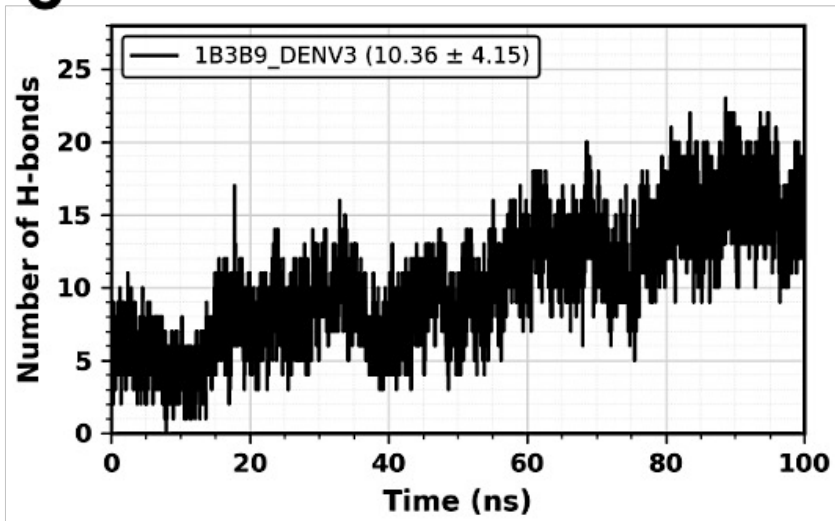

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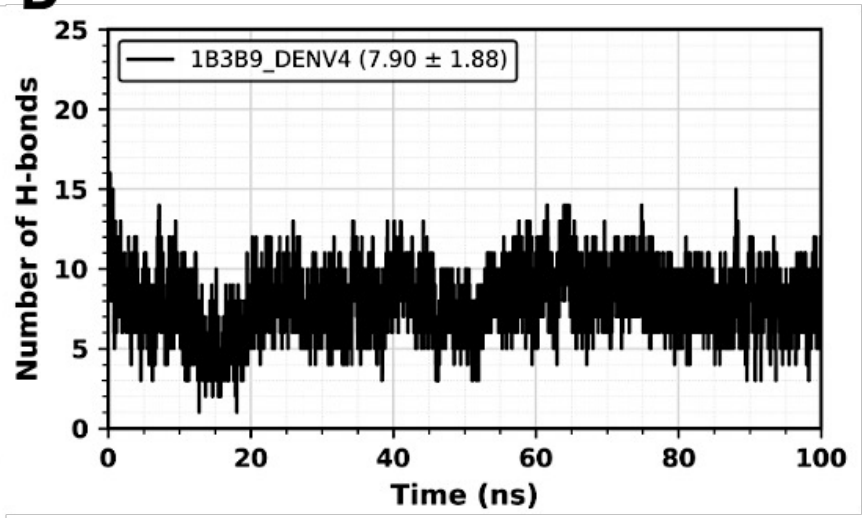


The 1B3B9 antibody formed H-bond interactions with the E dimer protein of DENV-1 to DENV-4, with numbers ranging from **8**, **13**, **10**, and **7** bonds, respectively.

Number of H bonds:
DENV-2 > DENV-3 > DENV-1 > DENV-4

H-bonds ↑ = Binding stability ↑

Objective: To characterize the binding energy between the 1B3B9 antibody and E dimer proteins

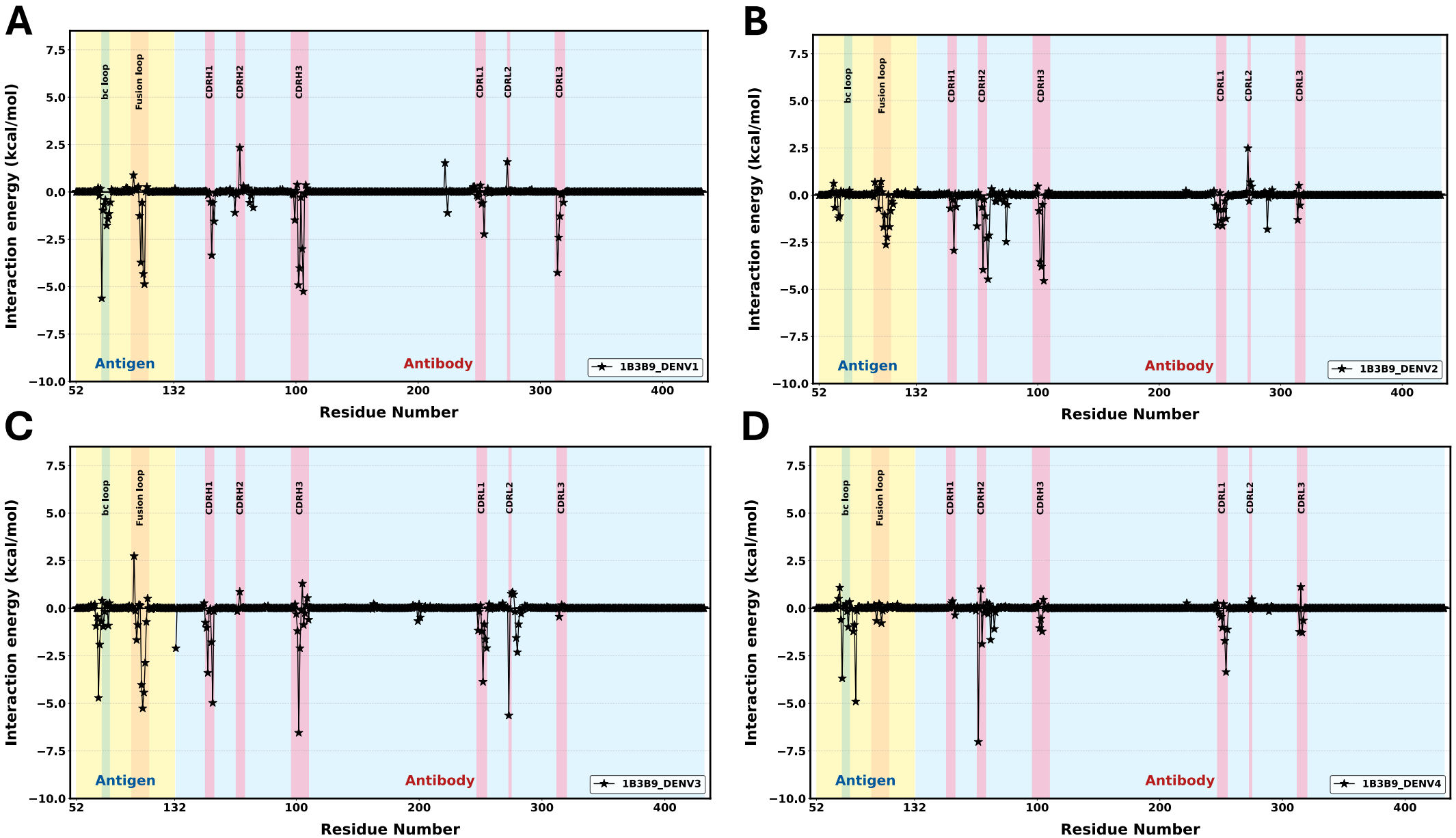
Table 1 Comparative MM/PBSA binding free energy analysis of 1B3B9 antibody-E dimer protein complexes across the 4 DENV serotypes. All values are expressed in kcal/mol.

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

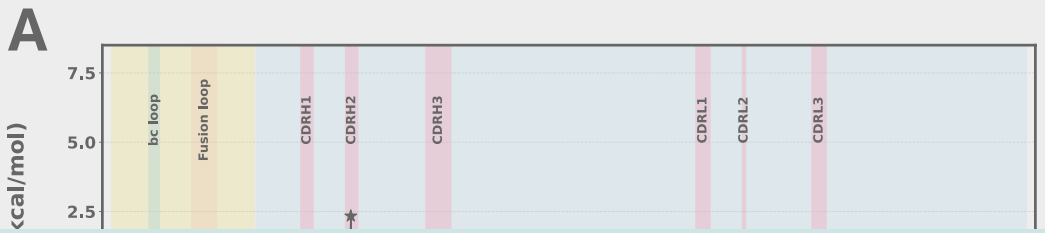
The 1B3B9 antibody showed the **highest binding affinity with DENV-2** compared to other serotypes. It ***correlates with the experimental observation*** that the neutralizing potency was found to be more favorable for DENV-2 (Sasaki et al., 2013).

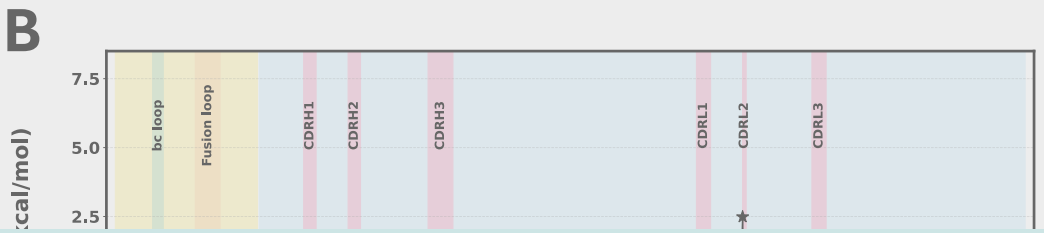
RESULT 2: Per-residue binding free energy decomposition

Objective: To identify key antigen and antibody residues contributing to the binding interactions



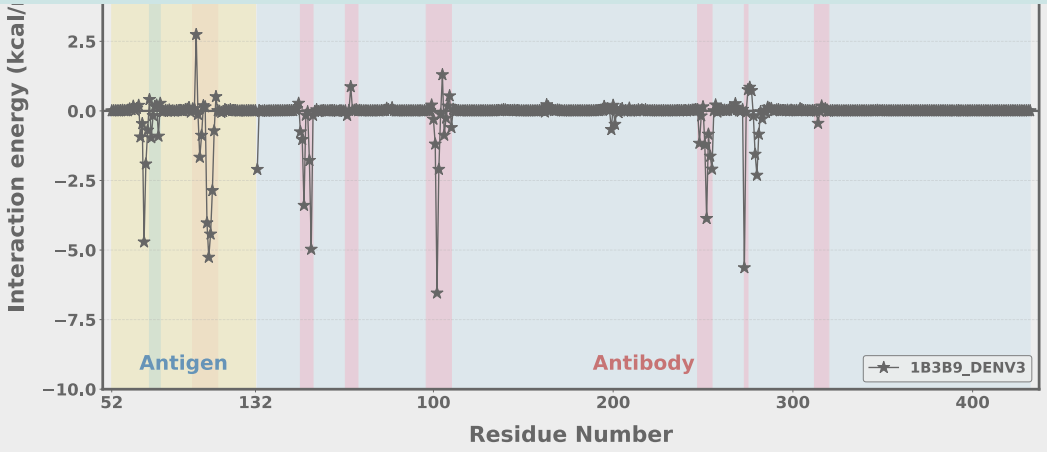
Objective: To identify key antigen and antibody residues contributing to the binding interactions

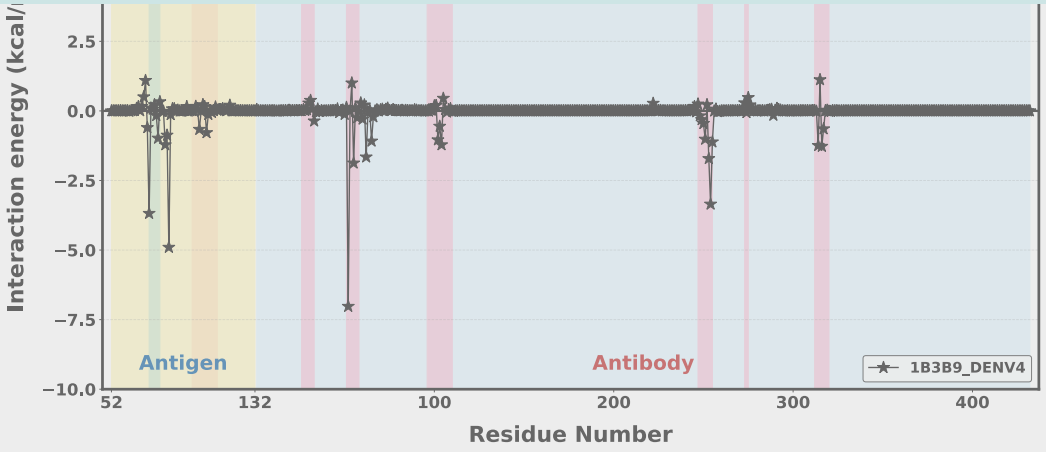




Further Plans

- ✓ Key antibody residues will be mutated to strengthen interactions with the bc and fusion loop, aiming for cross-reactive binding.
- ✓ Structure-based design (charged optimization) >> **Improve binding affinity**





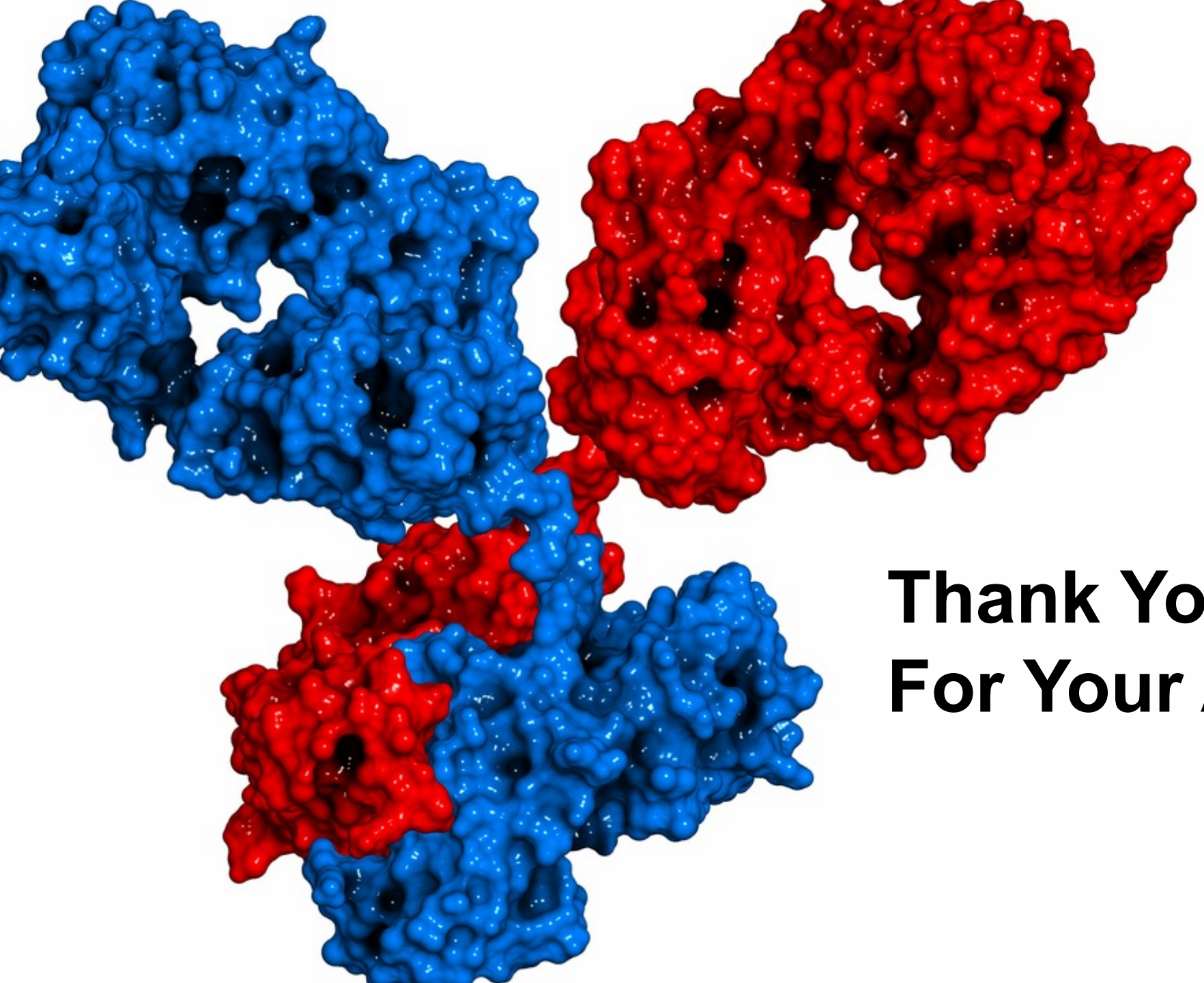
Thesis plan

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

Acknowledgement

Assist. Prof. Dr. Chonlatip Pipattanaboon (Advisor)
Department of Microbiology, Faculty of Medicine, KKU



Medical Microbiology

Thank You
For Your Attention