Increasing thermal stability of envelope dimer proteins in Dengue virus

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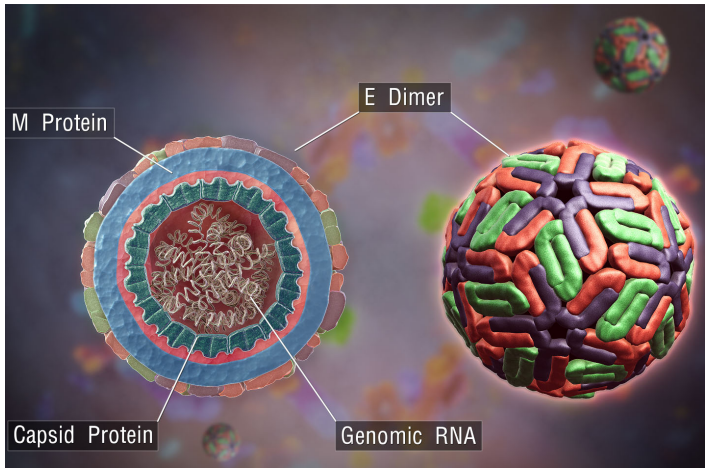
Background

- *Orthoflavivirus* causes multiple diseases (e.g., Dengue)
- Envelope protein (E), the primary component of the viral surface
  - 90 E dimers per viral particle
  - Mediate receptor binding and fusion
  - Target for neutralizing antibodies
- Vaccines target the E dimer
- E dimerization is unstable at 37°C
  - Different species are more stable



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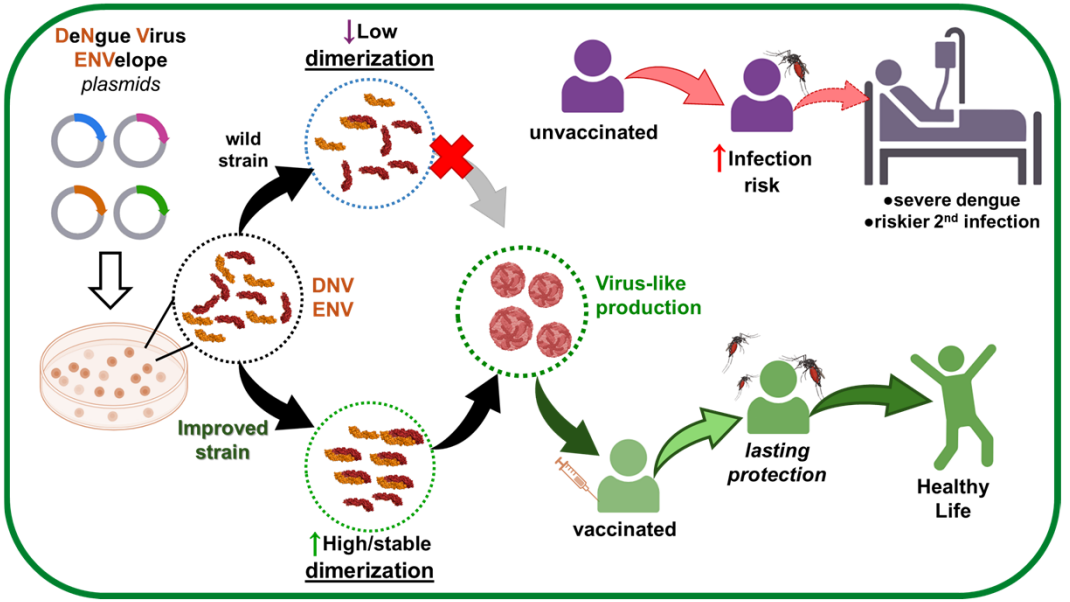
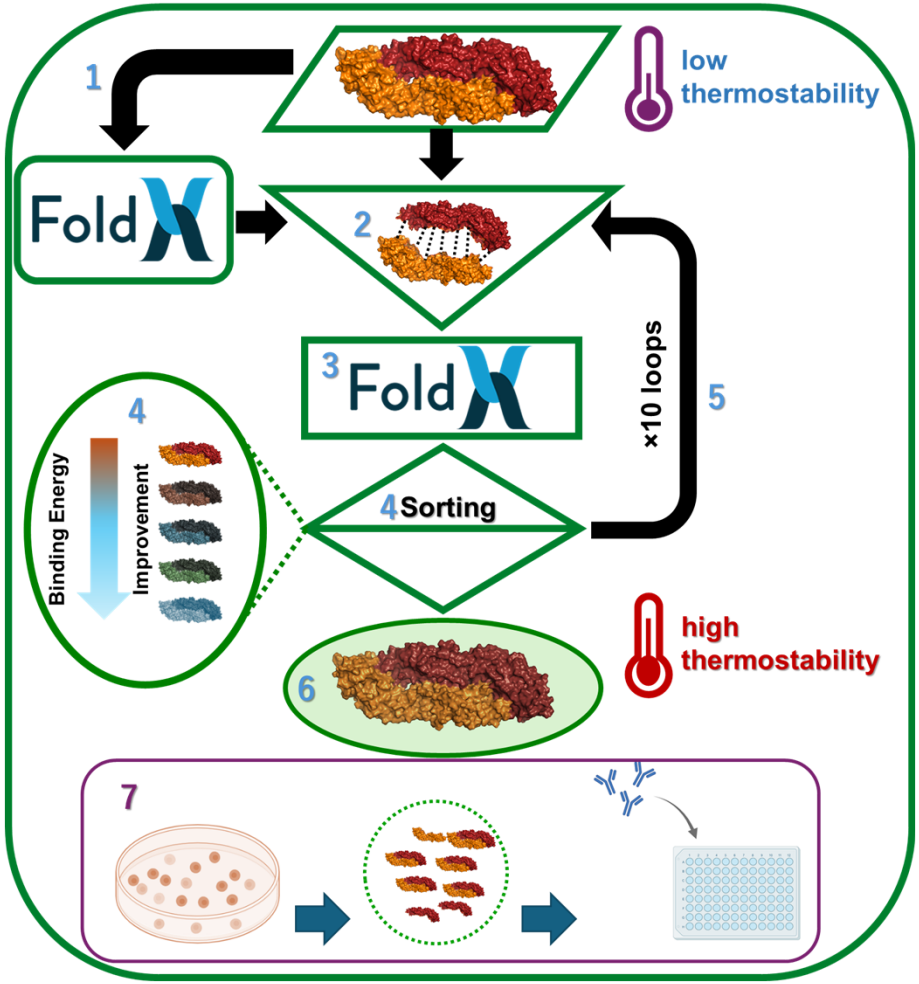


Objectives

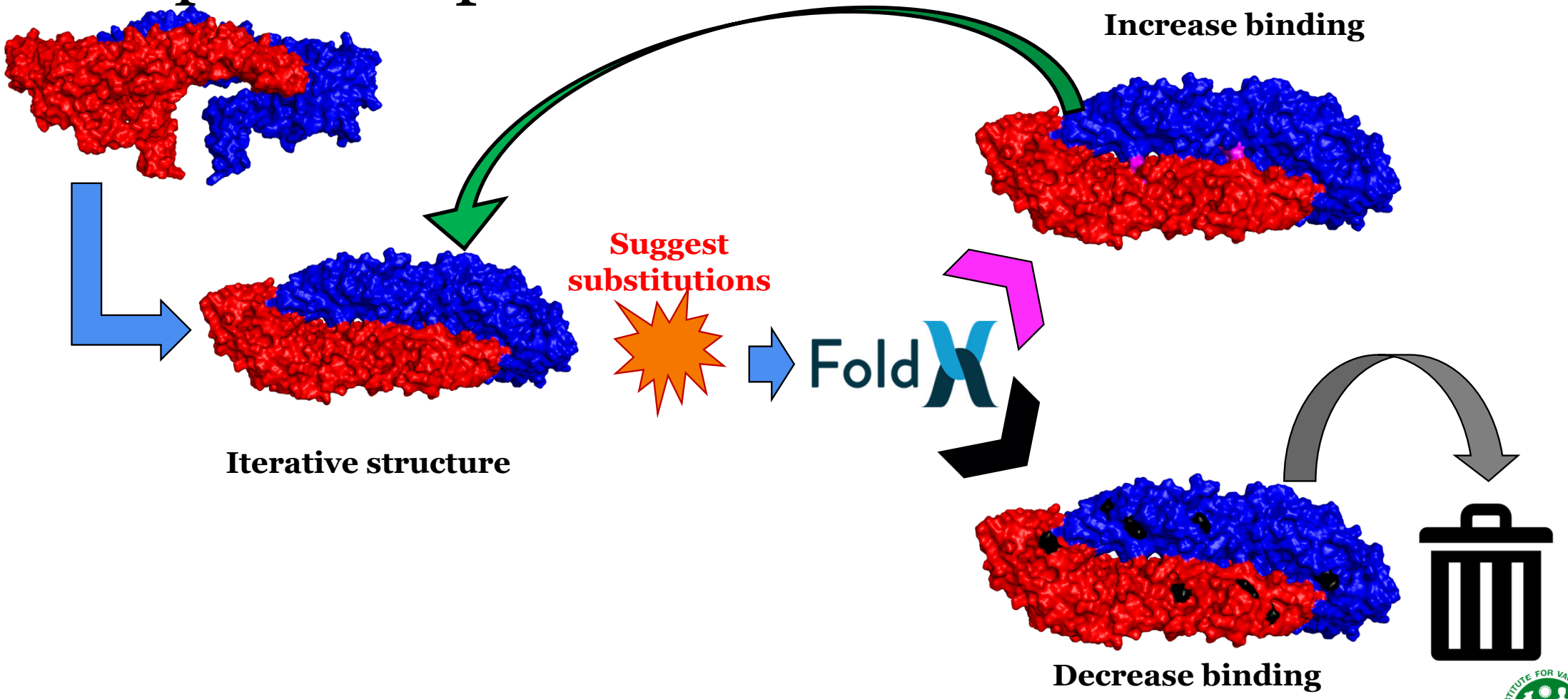
- Set of amino acid substitutions to increase E thermostability
- Minimum number of substitutions to avoid distorting the structure
- Avoid affecting the neutralizing epitopes
- Avoid using substitutions patented by similar studies

Genetic algorithm (GA) to simulate evolution



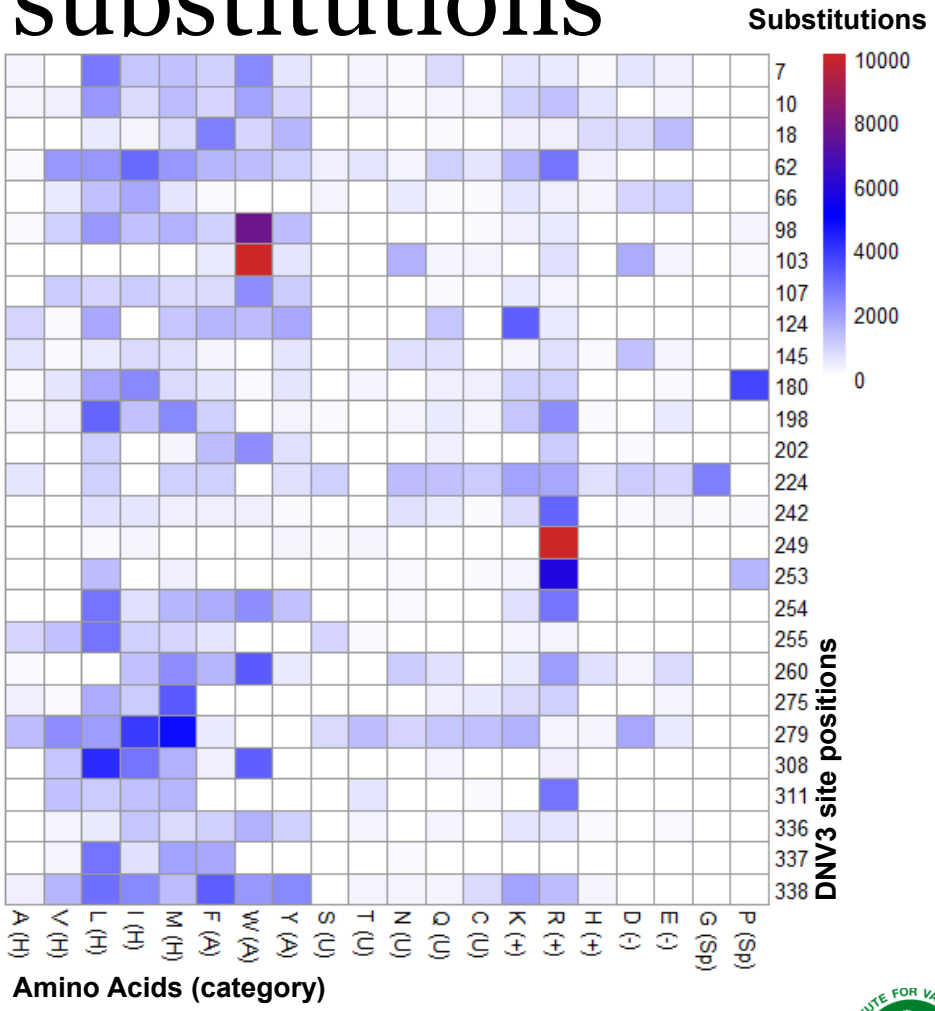
- DENV 3 structure was used as input
- GA algorithm ran 30 loops per execution with random selection of substitutions
- 100 executions were run in parallel in two PCS
- Each execution yielded ~300 models
  - → >60,000 models to analyze

Graphical representation

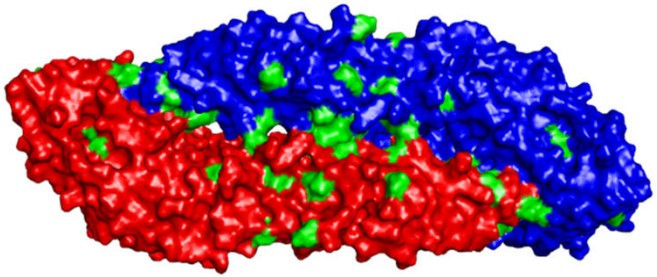


Most frequent amino acid substitutions

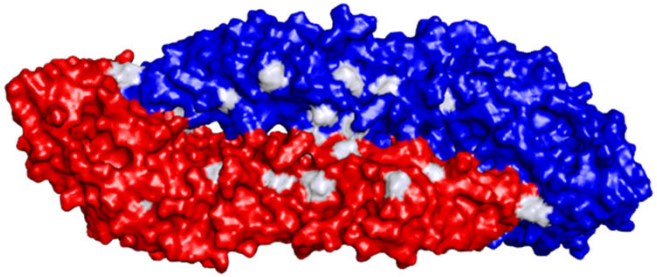
- 63K+ “improving” datasets
  - AA Substitutions from 1 to 31 sites
  - Conditions:
    - Lower binding energy
    - Lower complex energy
- Substitutions to residues
  - 38% to hydrophobic
  - *23% to aromatic*
  - 19% to positive charge
  - 9% to uncharged/polar
  - 6% to special cases/conformational
  - 5% to negative charge



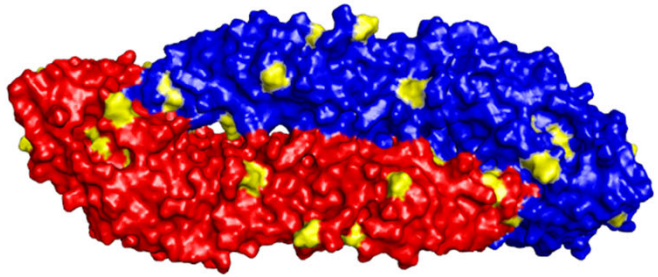
Frequently modified protein zones



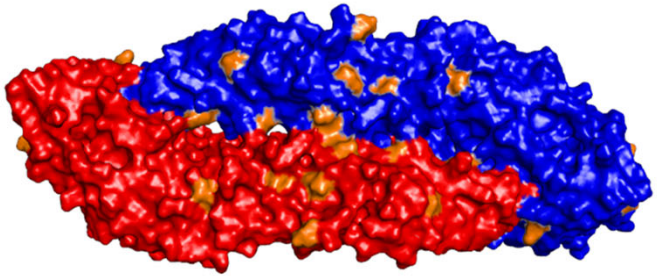
Top30 mutated sites



Top20 mutated hydrophobic sites



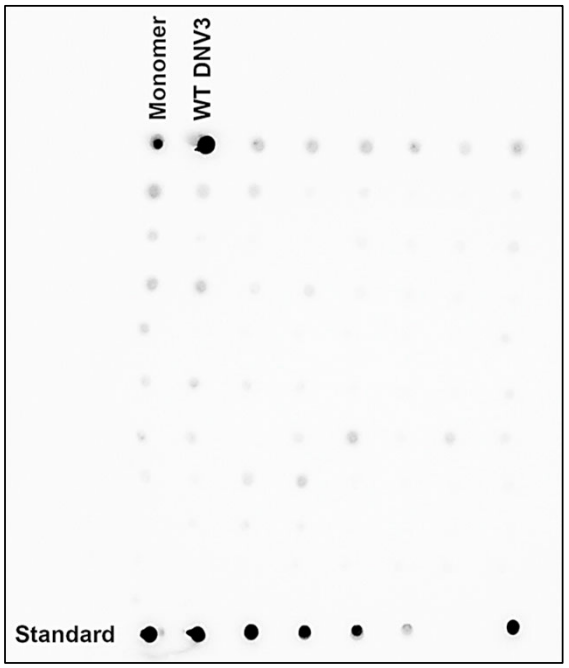
Top20 negatively charged sites

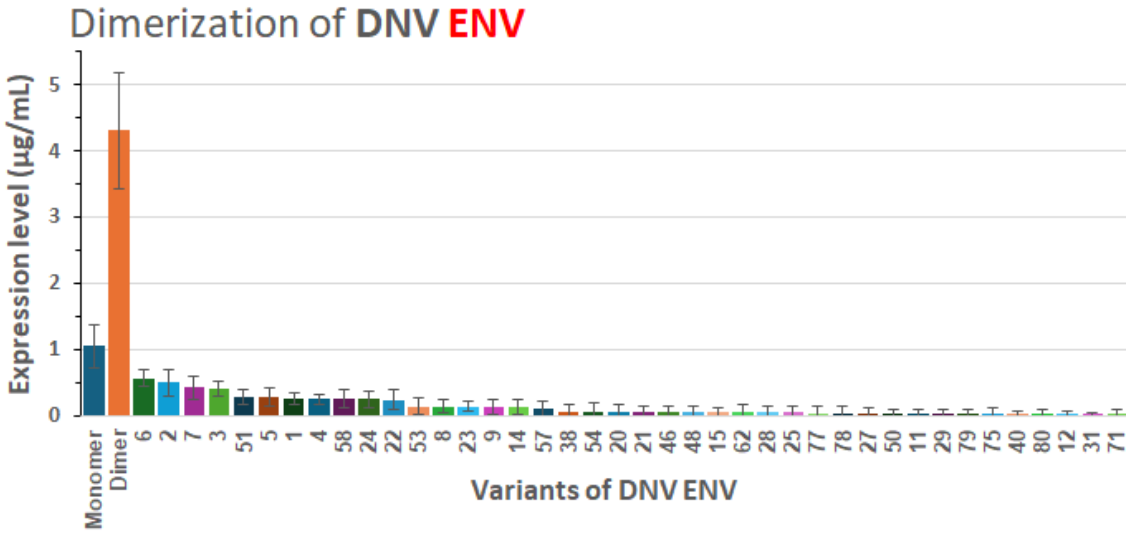


Top20 mutated positively charged sites

Candidates

- 81 “promising” AA substitution sets chosen for wet bench validation
- ENV expressed to measure the dimerization with a dot-blot test
- HEK293T cells were transfected with PEI MAX, and the culture supernatant was harvested 72 hours post-transfection. The plasmid used was a pCXSN vector carrying the E protein with a His tag.

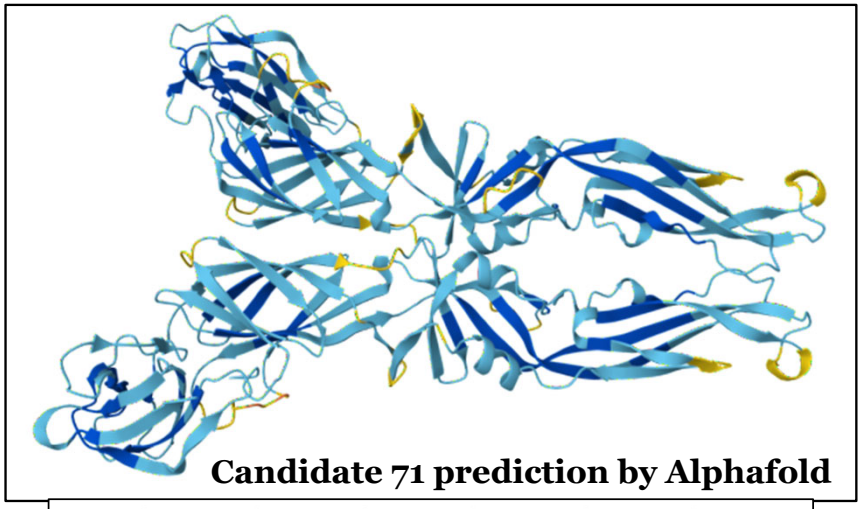




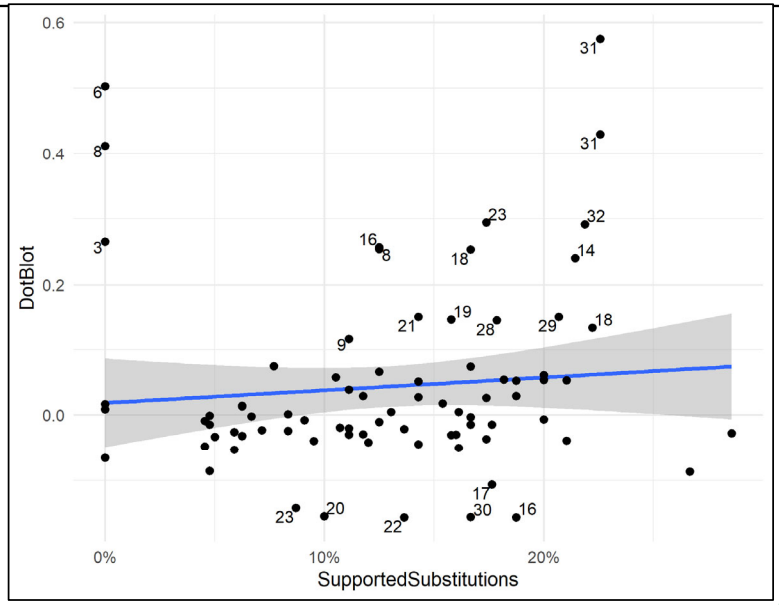
The candidates were in poorer expression/dimerization than the WT

What went wrong?

- Poor folding of hypermutated proteins
  - up to 31 sites
- Low number of naturally occurring substitutions
  - Contrasted against GISAID sequences
  - High risk of incompatible substitutions
- Artefacts from static in silico structures
- **Conclusion: the substitution proposal phase of the algorithm was too naïve**



Candidate 71 prediction by Alphafold



Redesigning the approach

- Extending the in silico validation to estimate dissociation energies/temperatures

- Selection of sites based on the effects in charge and hydrophobic interactions

Contrasting DNV3 to higher yield viruses

Interface sites were contrasted and proposed as substitutions

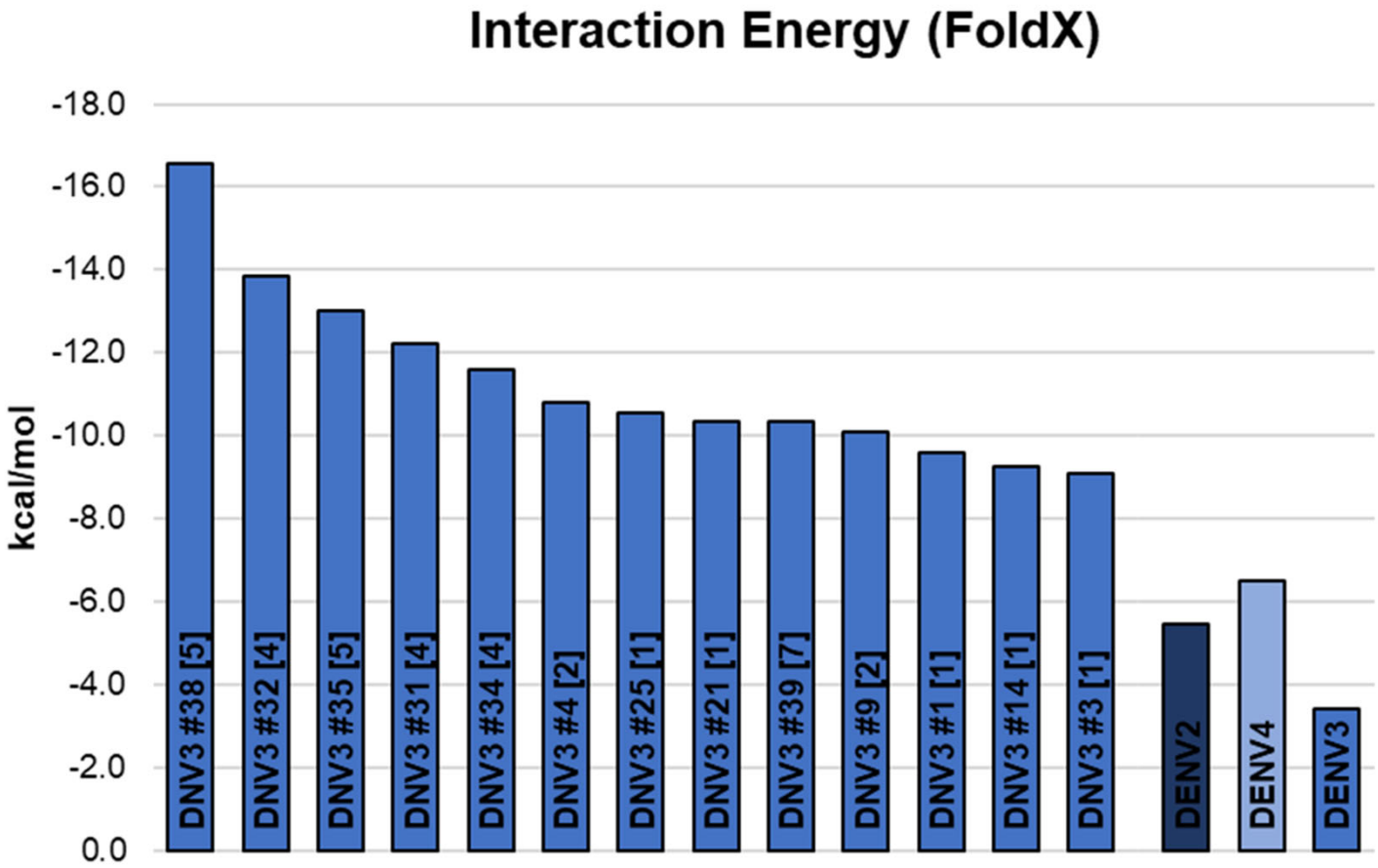
- ENV dimer proteins were simulated in water for 10 ns
  - At temperature = 300°K (~27°C) and 310°K (~37°C)
- Target sites: inter-chain contact sites that lose contact (> 4 Å = 0.4 nm) at higher temperature
- Substitutions: homologous non-conserved amino acid sites in high-yield viruses that don't lose contact

- Most changes:
  - Increased the hydrophobicity around the fusion loop
  - Complemented the charge along the interface
    - Lys→ Arg and Lys → Glu in two interacting sites, reducing electrostatic repulsion

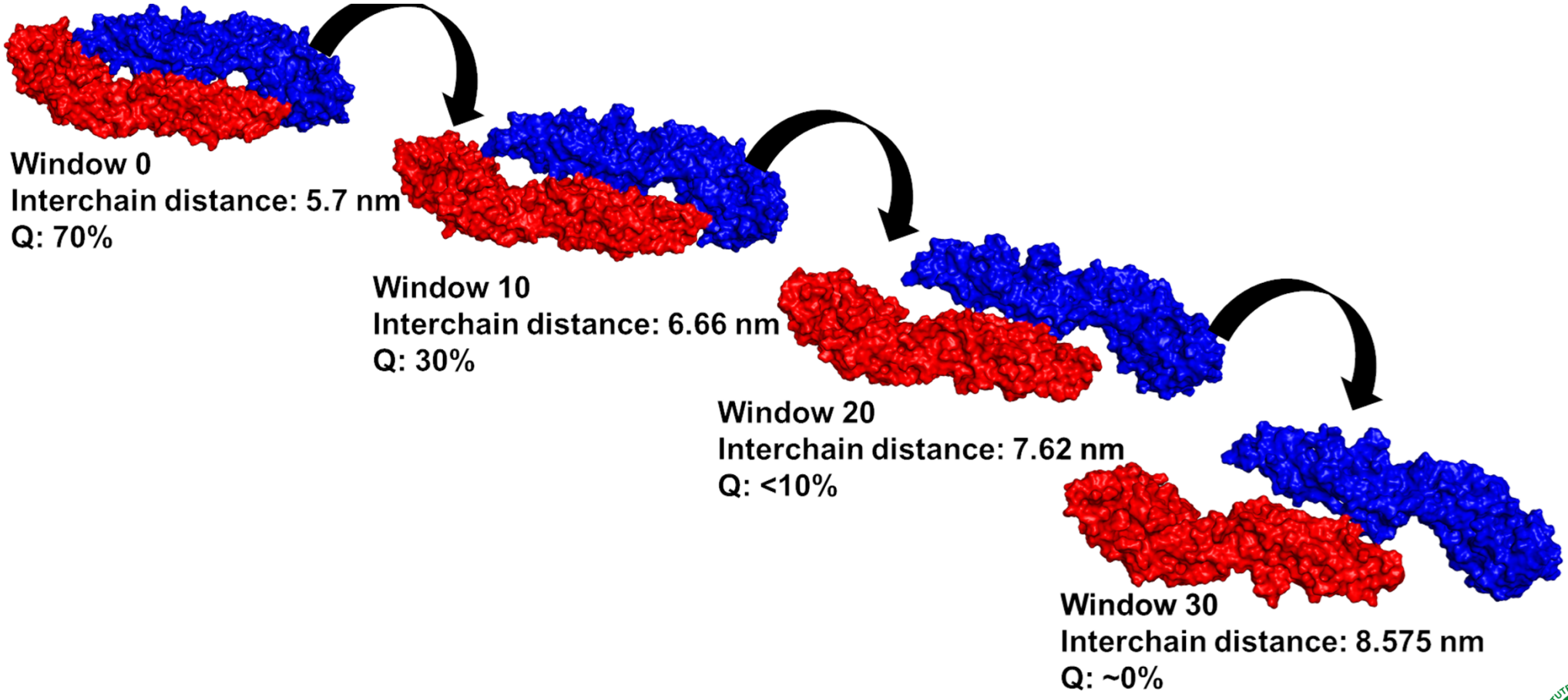
New candidates – Predicted interaction energy

Less energy needed for a dimer

No natural dimerization

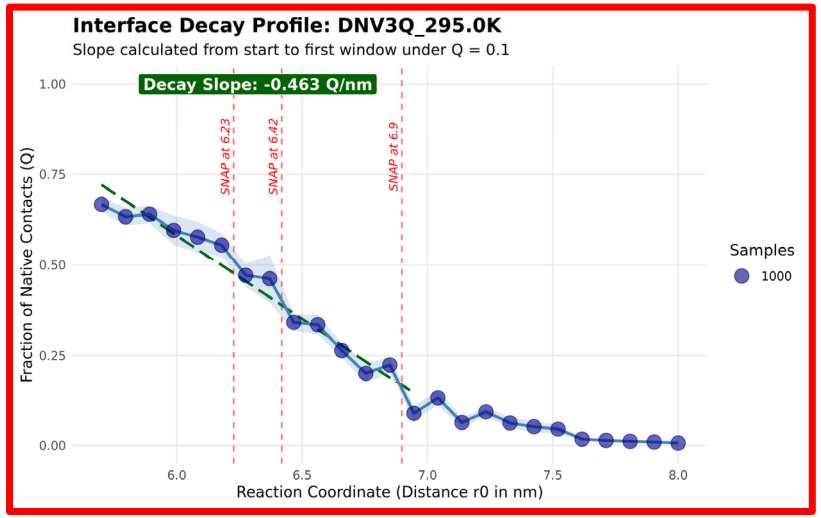


New evidence: Steered Molecular Dynamics simulation for different interchain distances

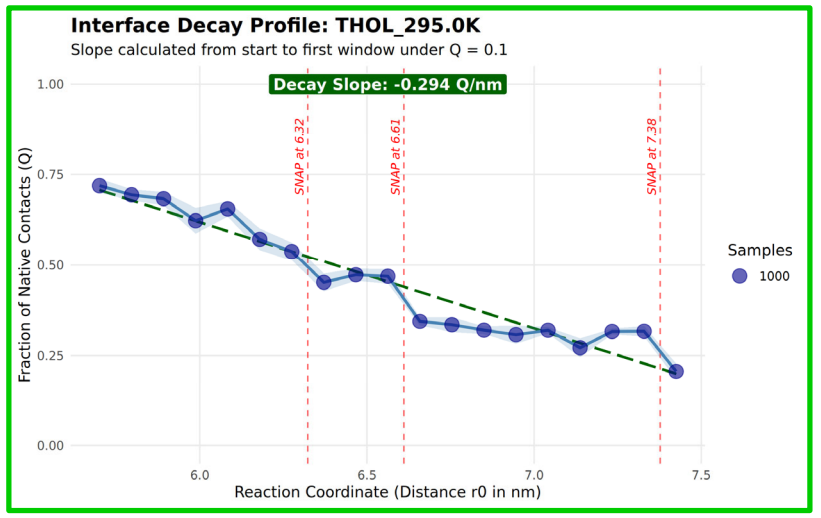


Comparing thermal stability across ENV dimers

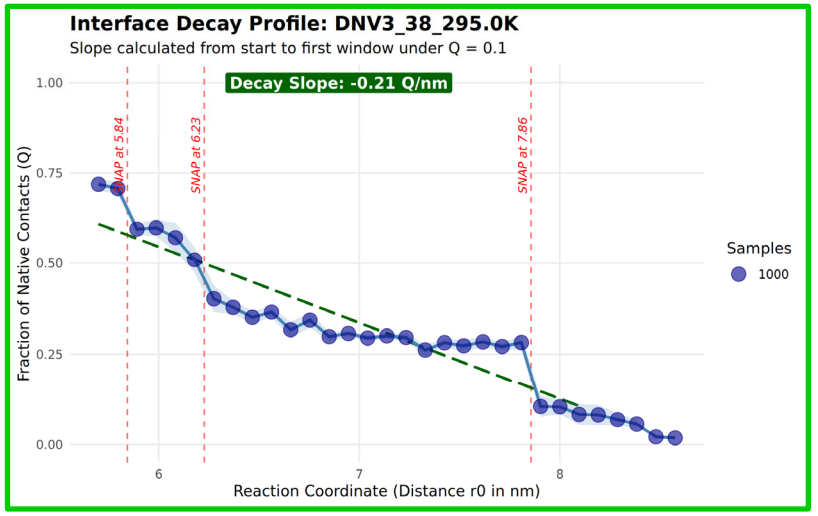
Binding energy: -3.4 kcal/mol



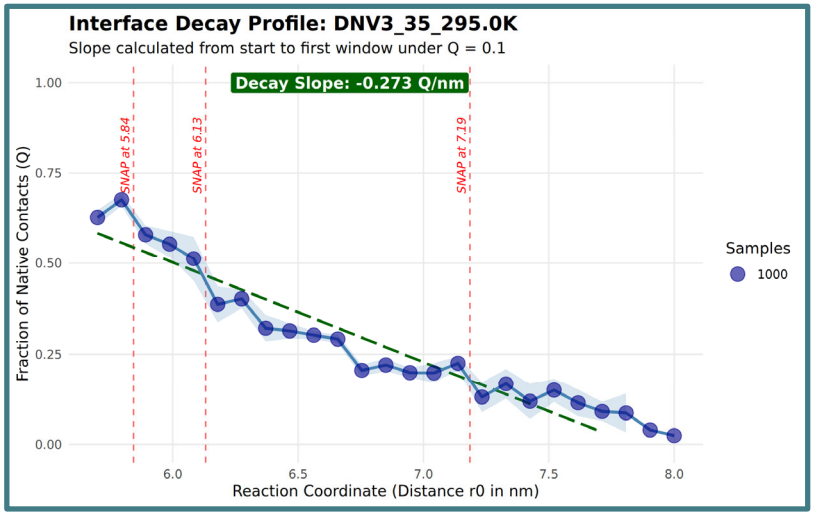
Binding energy: -16.7 kcal/mol



Binding energy: -16.6 kcal/mol

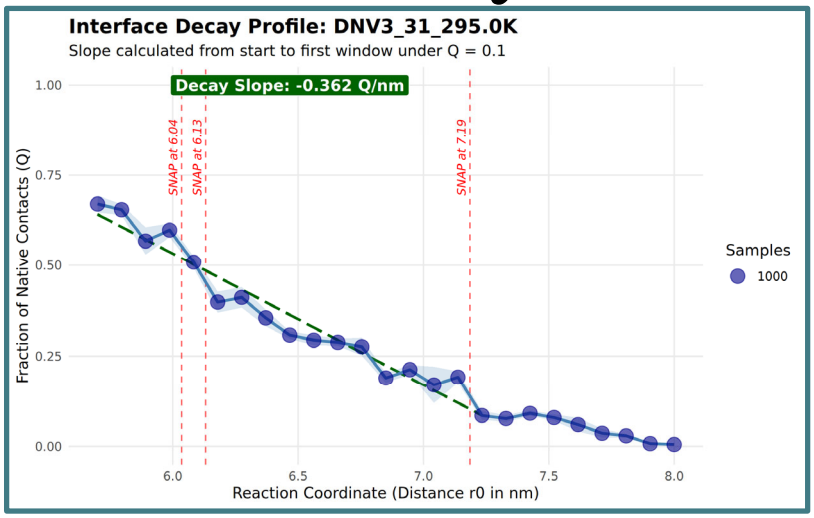


Binding energy: -13 kcal/mol

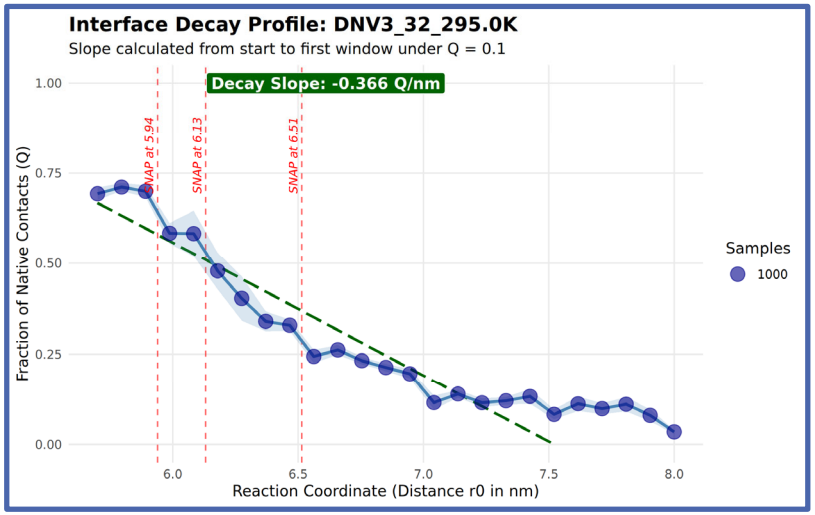


Comparing thermal stability across ENV dimers

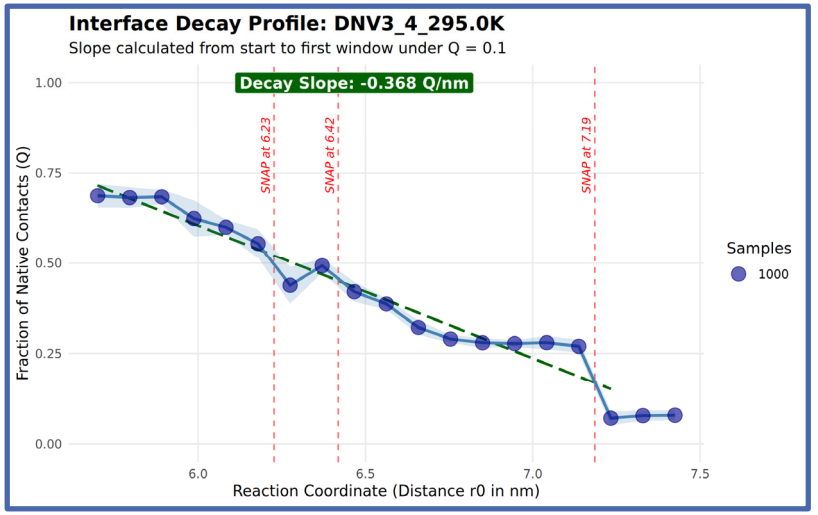
Binding energy: -12.2 kcal/mol



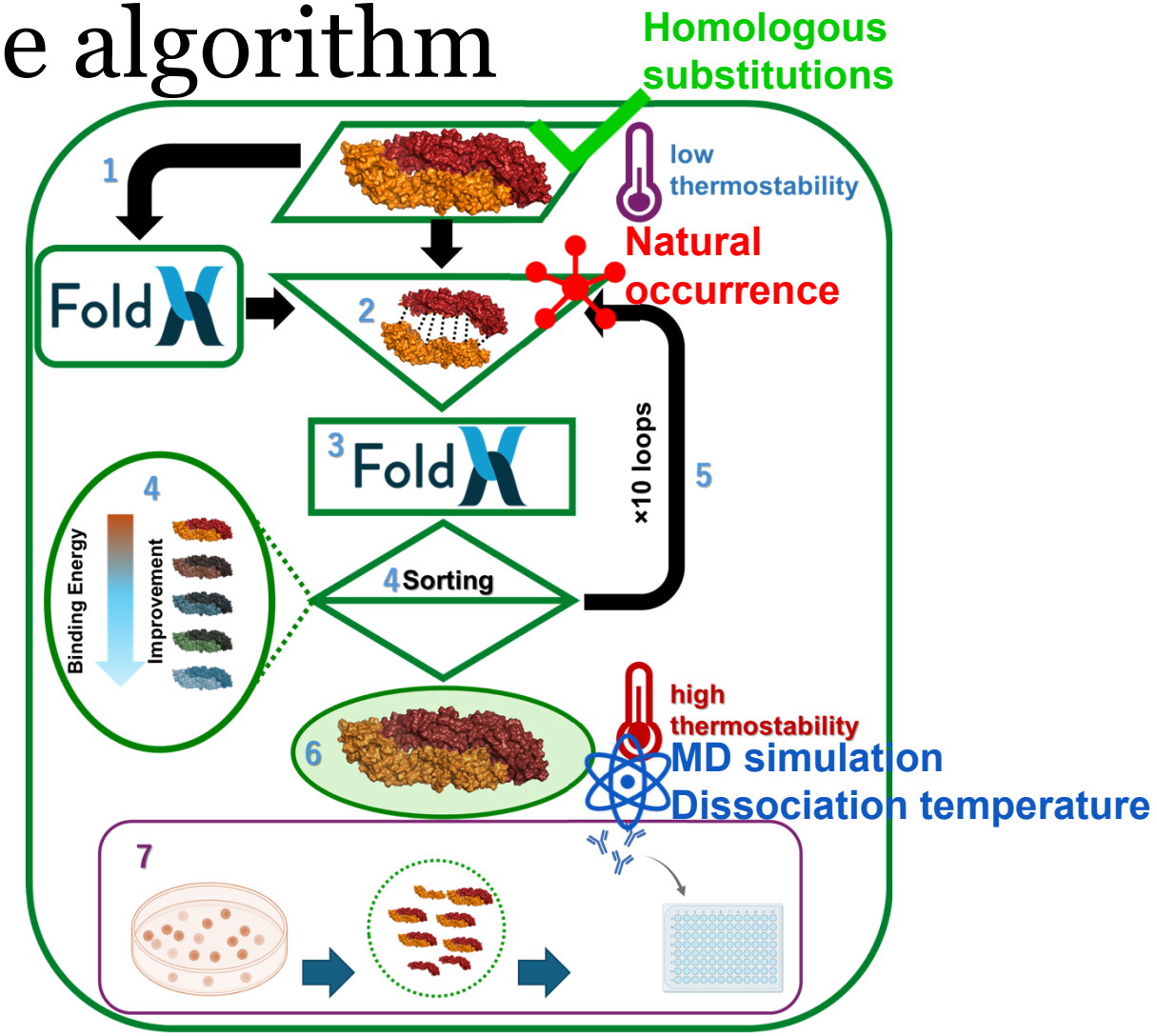
Binding energy: -13.9 kcal/mol



Binding energy: -10.8 kcal/mol



Updating the algorithm



Next steps

- Use the GA to combine effective mutations
- Providing the melting temperature for all candidates to rank the stability
- Wet bench expression of modified proteins to assess stability
- Analyzing antibodies against mutants to neutralize WT DENV3
- Analyzing the antigenicity of mutants

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