# Exploration of conformational B-cell epitopes: components to peptide-based vaccines

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**Overview:** The idea of using peptides as cost-effective vaccines is an interesting approach for the prevention and treatment of many infectious diseases and malignant disorders [1-2]. Such peptides can be designed synthetically [3] to elicit a response in immune system.

Approximately 83% of B-cell epitopes are conformational in nature with discontinuous regions of the sequence coming together in the 3D fold of the protein and it is therefore important to study how well synthetic peptides are able to mimic these conformations. Consequently, we characterized B-cell epitopes to inform improved vaccines design and allow development of peptide vaccine.

**Advantages of peptide vaccines:**

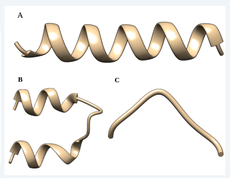
- Specificity of immune response
- Exclusion of undesirable immune response
- Improving immunity
- Cost effective
- Ease of storage/transport

**B – Cell Epitope Analysis** → **Epitope analyzed as:** 1. Regions 2. Fragments → **Epitope Regions Shape Classification** → Development of a novel algorithm

**Regions:** Continuous stretches of sequence consisting of at least 3 antibody-contacting residues each separated by <= 3 residues)

**Fragment:** Antibody-contacting residues that do not satisfy the requirements for a region)

**1. Linear**
**2. Folded**
3. Curved

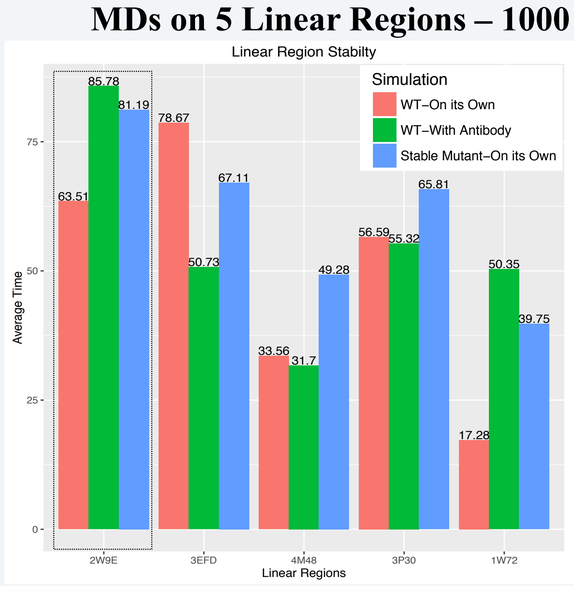


**MD simulations of wild type *Linear* and *Folded* regions** → **Mutant design of wild type region**

- End-capping
- mutations of hydrophobics to alanine and glutamine
- Stapling/cyclization

→ **MD simulations of stabilized mutant and wild type with antibody** → **MD simulations of mutant peptides** → **Regions Stability Analysis**

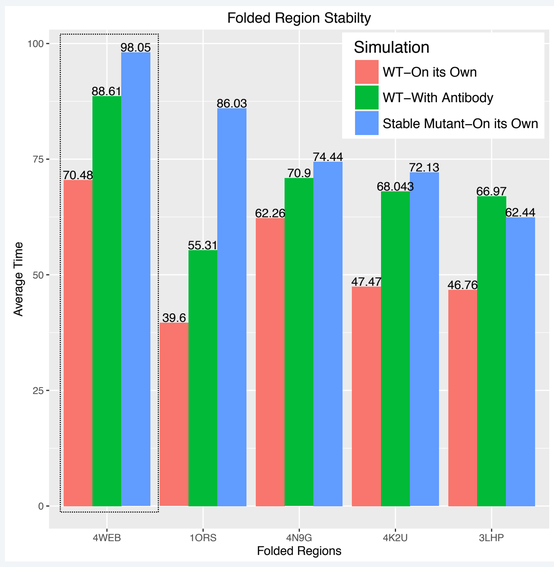
## MDs on 5 Linear Regions – 1000 ns




| PDB | Linear Peptide Sequence | Length | Possible Mutations |
|---|---|---|---|
| 2W9E | GSDYEDRYYRENMHR | 15 | 2 |
| 3EFD | RALHERFDRLERMLDD | 16 | 6 |
| 4M48 | YGTNRFSEDIRDMIGFP | 17 | 3 |
| 3P30 | HIIYELIEESQKQQEKNEQ | 19 | 4 |
| 1W72 | RNMKAHSQTDRANLGTLRGY | 20 | 4 |

- Contacting residues (with antibody)
- Contacting and hydrophobic
- Non-contacting and hydrophilic
- Non-contacting and hydrophobic (Targets for making mutations).

## MDs on 5 Folded Regions – 1000 ns



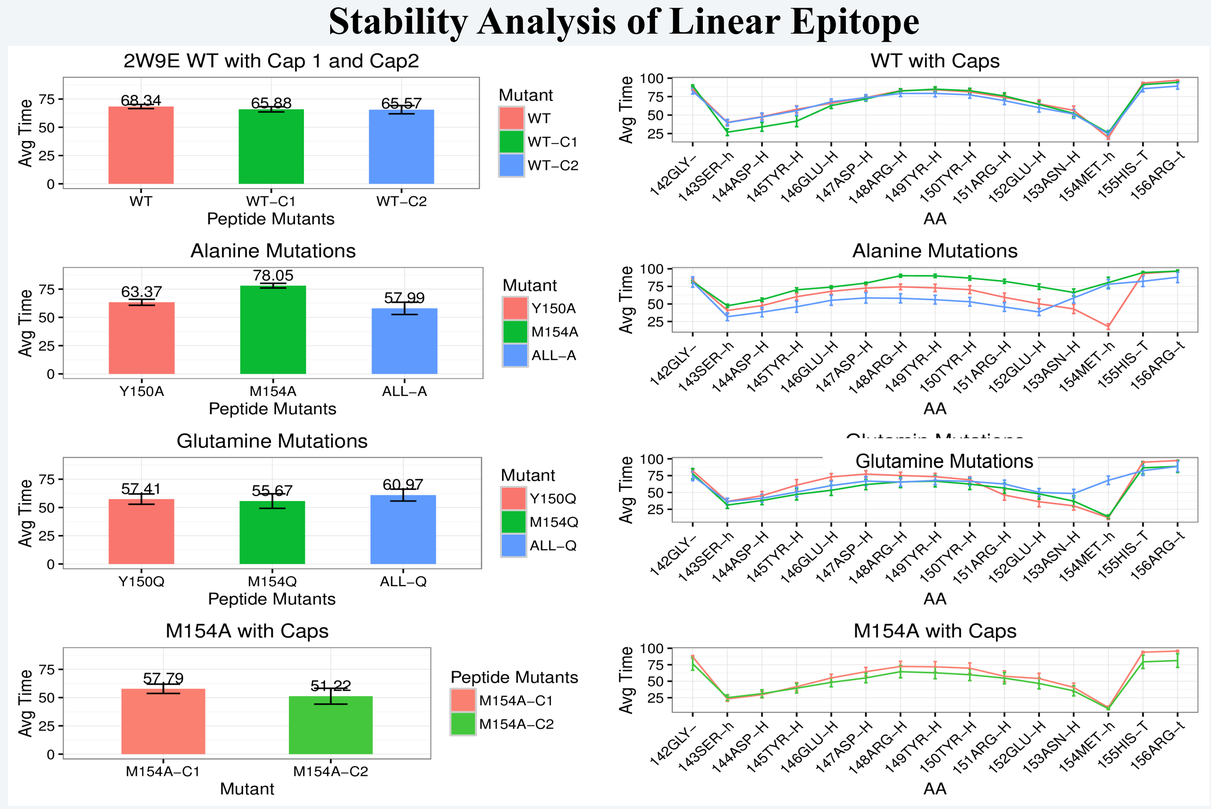

| PDB | Folded Peptide Sequence | Length | Possible Mutations |
|---|---|---|---|
| 4WEB | FKIRMYVGGVEHRLT | 15 | 3 |
| 1ORS | EGHLAGLGLFRLVRLLR | 17 | 4 |
| 4N9G | LSKINDMPITNDQKKLMS | 18 | 3 |
| 4K2U | EKLWEAMLSEHKNNINNCKNI | 21 | 4 |
| 3LHP | NDKAAALGKDKEINWFDISQSLW | 23 | 3 |

Based on the initial results, from 1000 ns simulations of :

1. wild type peptide on its own,
2. wild type peptide with antibody
3. the stabilizing mutant on its own,

We selected epitopes from 2W9E (linear) and 4WEB (folded) to perform multiple, shorter simulations (500 ns) to study the statistical significance of epitope stability.

## Stability Analysis of Linear Epitope



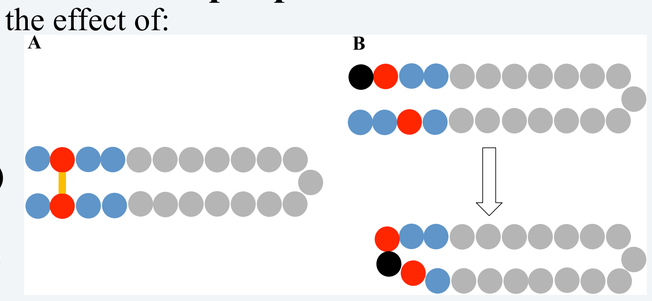

MDs were carried out on each of the mutant peptide for 500 ns. MDs were repeated 10 times to apply statistics on the measure of stability. Error bars on the data show standard error of mean.

**Conclusions:** In this linear epitope, mutation M154A in wild type epitope has stabilized the mutant to the considerable extent as shown in the graphs above. The M154A is significantly more stable than the wild type ($p = 0.0025$, Welch t-test)

## Stability Analysis of Folded Epitope

24 mutant peptides were designed to study the effect of:

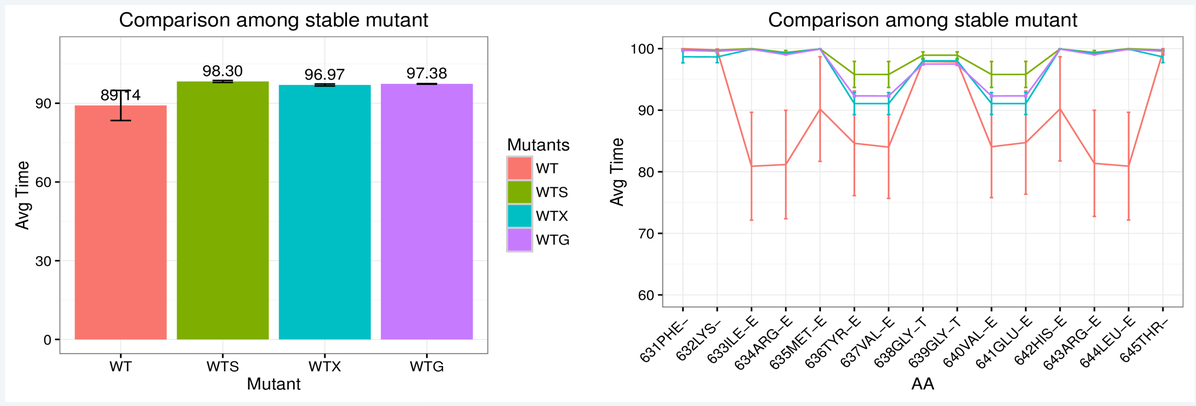
- End-capping on wild type
- Hydrophobic to alanine mutations
- Hydrophobic to glutamine mutations
- Disulphide stapling on wild type (WTS)
- Glycine linker on wild type (WTG)
- Extending the ends of wild type (WTX)




**Stapling:** A) **Disulphide stapling in a folded peptide.**
The disulphide bond between two cystiene residues is shown in yellow.

B) **Glycine linker in a folded peptide**.
The torsion angles of glycine and potential positions are adjusted to create a peptide bond between glycine and one of the potential positions. Extra residues are removed from one of the terminus.

- Epitope residues
- Non-epitope residues.
- Cystiene mutations in non-epitope/potential positions to add linker
- Glycine mutation




MDs of 500 ns were carried out for each of the mutants. The results are shown for 3 replicas of each peptide's simulation. The aim is to perform 10 replicas to provide enough sample size for statistical evaluation between wild type and mutant peptides.

**Conclusions:**

1. Disulphide bond has stabilized the wild type to its best (**WTS**)
2. Extending the ends of wild type has also stabilized the epitope (**WTX**)
3. The cyclisation of wild type by glycine linker has also improved the epitope stability (**WTG**)
4. All the mutant were stable for 70-98% of the time which suggests that the epitope is quite stable.

## Future Directions

Experimental validation of the peptide mutant which will involve:

1. Protein expression and purification of an antibody
2. Characterization of peptide structure by CD
3. Antibody peptide binding by SPR
4. Testing of peptide as immunogen

1. Berzofsky, JA. Designing peptide vaccines to broaden recognition and enhance potency. Ann. NY Acad. Sci. 1995. 754:161-168.
2. Buteau, C, Markovic, SN, Celis, E. Challenges in the development of effective peptide vaccines for cancer. Mayo Clin. Proc. 2002. 77:339-349.
3. Rothbard, J. Synthetic peptides as vaccines. Nature. 1987. 330:106-107.