DESIGNING OF PEPTIDE-BASED MULTI-EPITOPE VACCINE CONSTRUCT AGAINST GALLBLADDER CANCER USING IMMUNOINFORMATICS AND COMPUTATIONAL APPROACHES

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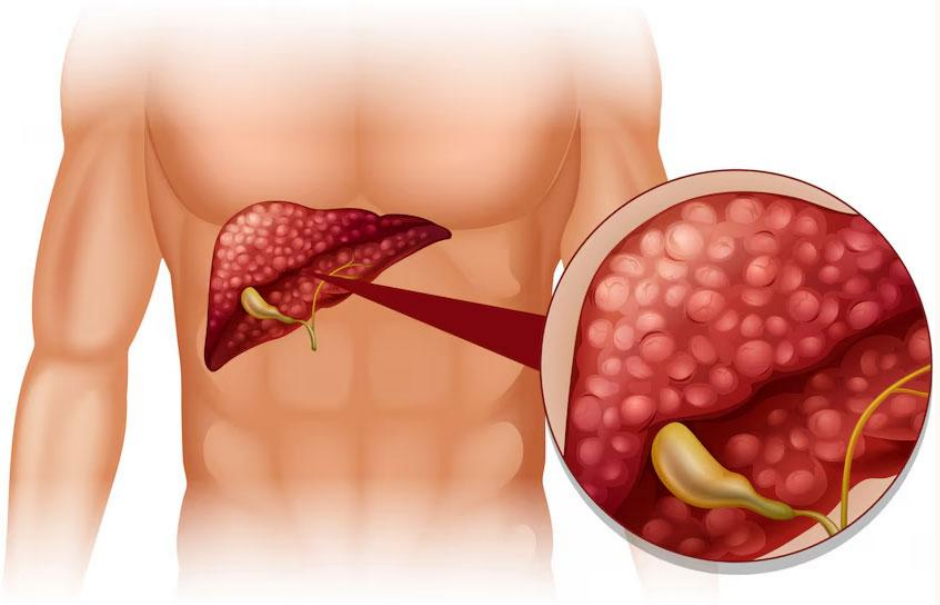
INTRODUCTION

- Gallbladder cancer is a highly aggressive form of biliary tract carcinoma, with a poor prognosis.
- In 2020, the global incidence was **115,949 cases**, with **84,695 deaths** reported.
- The five-year survival rate of GBC is **only 5–20%**, mainly due to **late-stage diagnosis** and aggressive tumor progression.
- More than **90% of GBC cases** are detected at **advanced or metastatic stages**, making treatment challenging.
- The incidence of GBC varies geographically, with the highest rates in **Asia (70.8%)**, followed by **Europe (10.8%)**, **South America (6.8%)**, and other regions. Countries like **India, Pakistan, Japan, and Chile** have particularly high rates of GBC.



CURRENT TREATMENT STRATEGIES

- **Surgical resection** is the best treatment option, but most cases are inoperable at the time of diagnosis.
- **Chemotherapy regimens** (gemcitabine + oxaliplatin or gemcitabine + cisplatin) have limited success.
- **Adjuvant radiation therapy** lacks strong evidence of improving survival rates.
- **Targeted therapies** are still under research, but there is no highly effective treatment available.

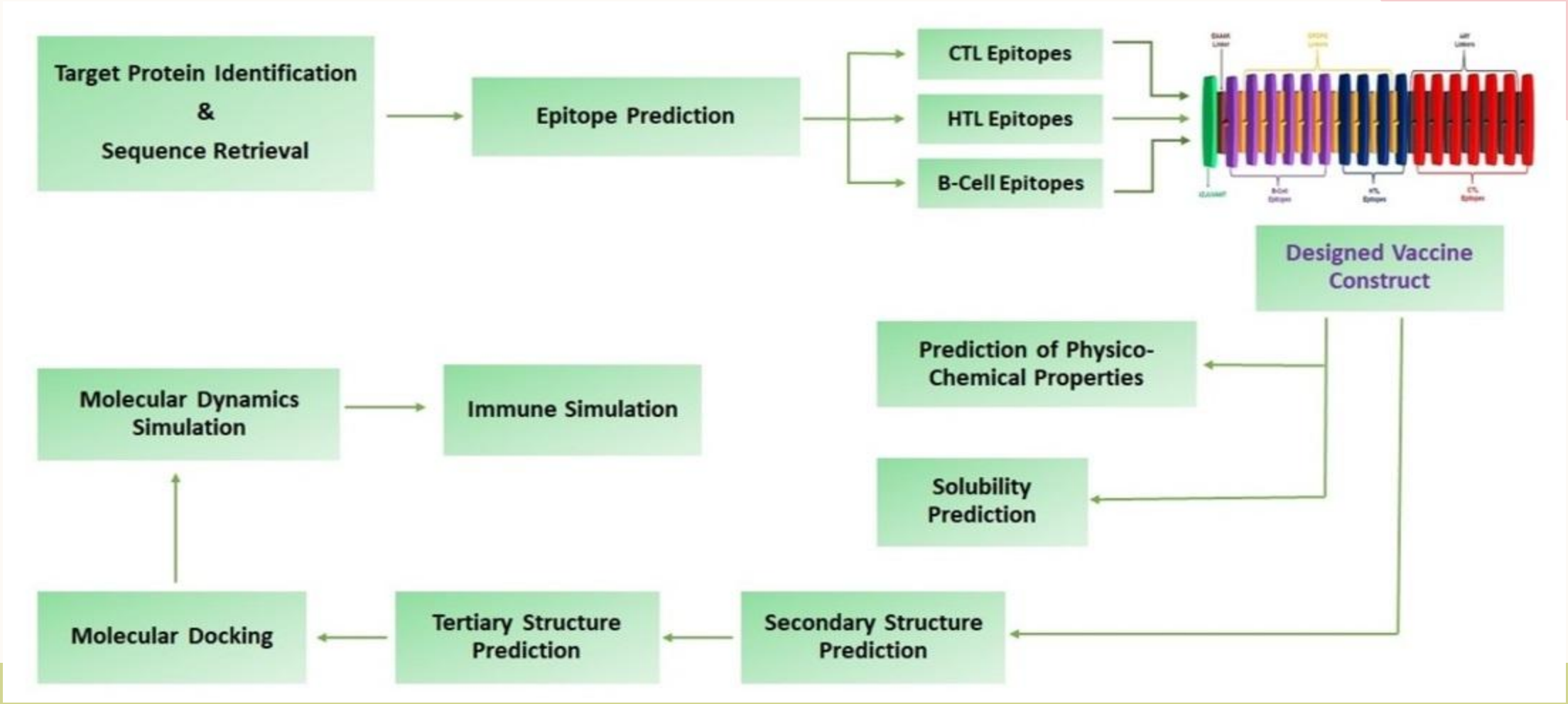
NEED FOR A GBC VACCINE

- Advances in immunotherapy suggest that cancer vaccines could stimulate the immune system to recognize and destroy GBC cells.

- Multi-epitope peptide vaccines represent a new therapeutic approach, targeting specific tumor antigens to trigger a strong immune response.

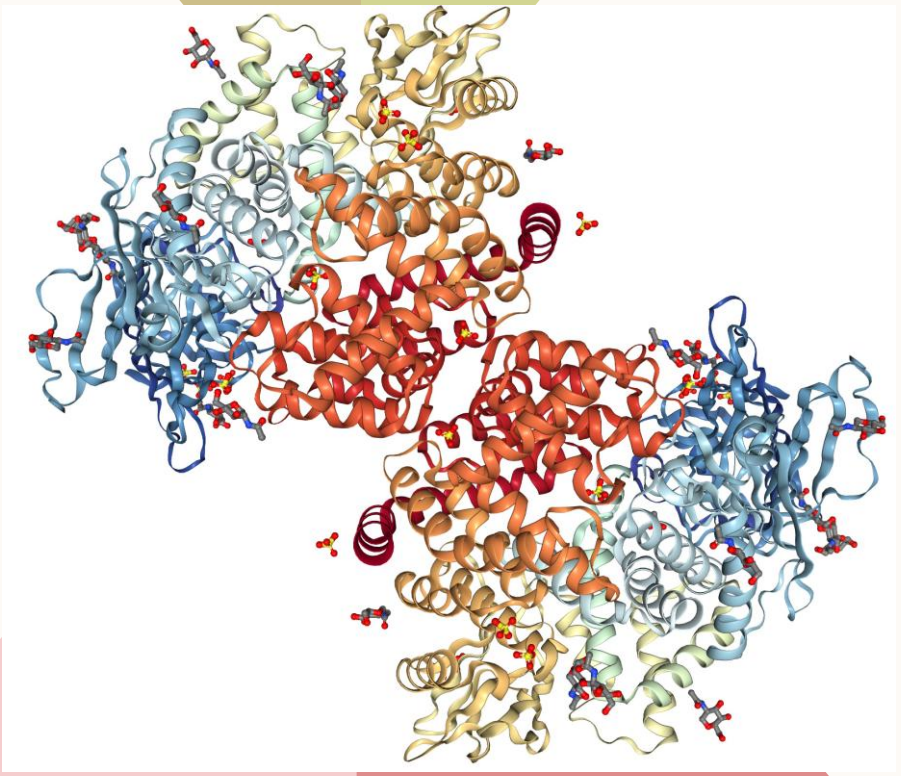
- This study aims to design a peptide-based multi-epitope vaccine using immunoinformatics and computational approaches.

MATERIALS AND METHODS



SELECTION OF TARGET PROTEINS FOR VACCINE DESIGN

- Researchers identified three **key tumor-associated proteins** that play a role in GBC:
  - **NT5E (CD73):** Converts AMP to adenosine, creating an immunosuppressive environment that helps cancer cells evade detection.
  - **ANPEP (CD13):** A membrane enzyme associated with **tumor growth and metastasis** in multiple cancers.
  - **MME (Neprilysin):** A zinc-dependent enzyme linked to **cancer progression and poor prognosis**.
- The sequences of these proteins were retrieved from **UniProtKB** in FASTA format for further analysis.



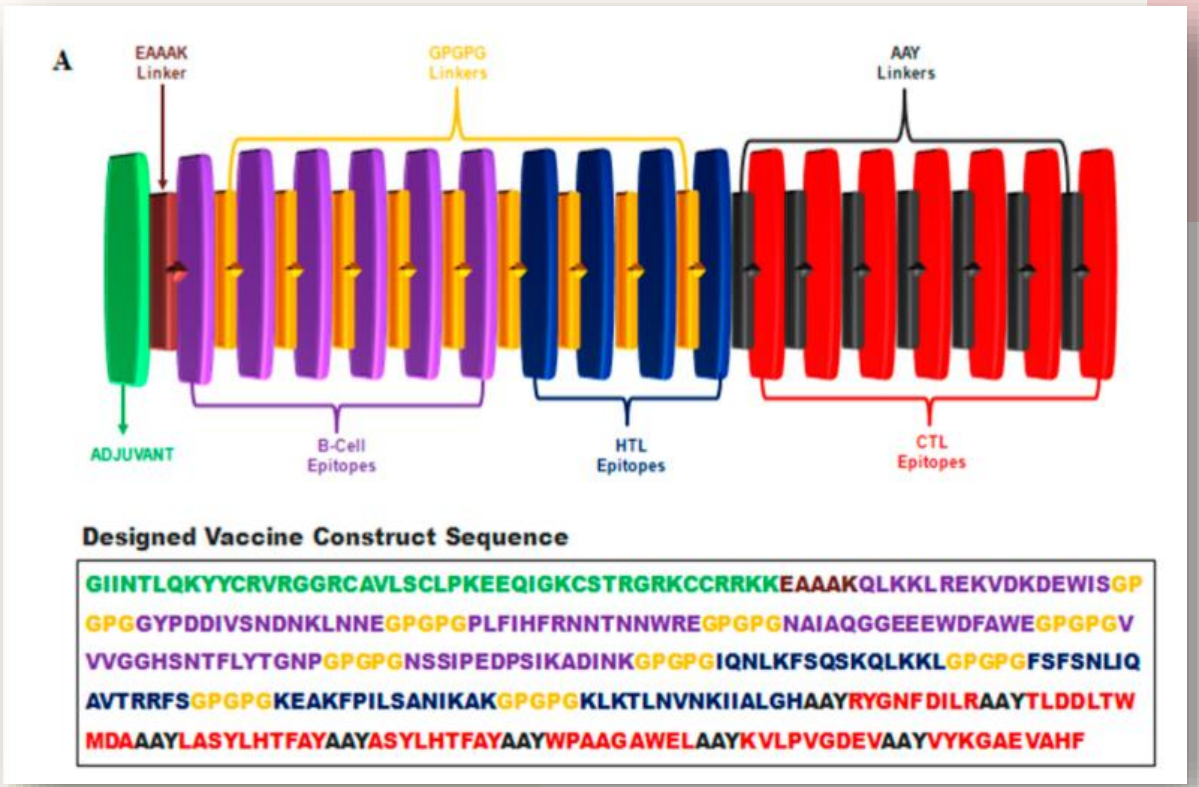
EPITOPE PREDICTION AND SCREENING

- **Cytotoxic T Lymphocyte (CTL) Epitope Prediction**
  - CTL epitopes were identified using the **IEDB MHC-I binding server.**
  - Only **high-affinity epitopes** (**IC50 < 500 nM**) were selected.
- **Helper T Lymphocyte (HTL) Epitope Prediction**
  - HTL epitopes were predicted using the **IEDB-MHCII server.**
  - Selected based on **IFN-γ induction, antigenicity, non-allergenicity, and non-toxicity.**
  - The **IEDB population coverage server** was used to examine the selected CTL and HTL epitopes.
  - **B-Cell Epitope Prediction**
  - B-cell epitopes were identified using **ABCpred and BCPred servers.**

The epitopes were further screened for immunogenicity, antigenicity, allergenicity, and toxicity.

VACCINE CONSTRUCT DESIGN

- Selected **7 CTL epitopes, 4 HTL epitopes, and 6 B-cell epitopes** to form the vaccine construct.
- Epitopes were linked with **GPGPG and AAY linkers** to ensure proper presentation to immune cells.
- A **β-defensin 3** (**hBD3**) **adjuvant** was added using an **EAAAK linker** to enhance immune response.
- The final vaccine construct was analyzed for its **physicochemical properties, including molecular weight, stability, solubility, and hydrophilicity** using the **ProtParam tool**.



STRUCTURE PREDICTION AND VALIDATION

- **2D & 3D Structure Prediction:**
  - Secondary structure was modeled using **PSIPRED server.**
  - 3D structure was generated using **RaptorX** and refined using **Galaxy Refine.**
- **Validation Tests:**
  - **Ramachandran plot analysis** confirmed a stable structure.
  - **ERRAT & ProSA tools** confirmed the model's **high structural quality.**

MOLECULAR DOCKING AND DYNAMICS

The vaccine construct was docked with immune receptors (TLR2, TLR3, and TLR4) using the **HDOCK server** to assess binding affinity.

Molecular dynamics simulations were performed over 100 ns using **Desmond's System builder panel** to evaluate the stability of the vaccine-receptor complexes.

IMMUNE SIMULATION

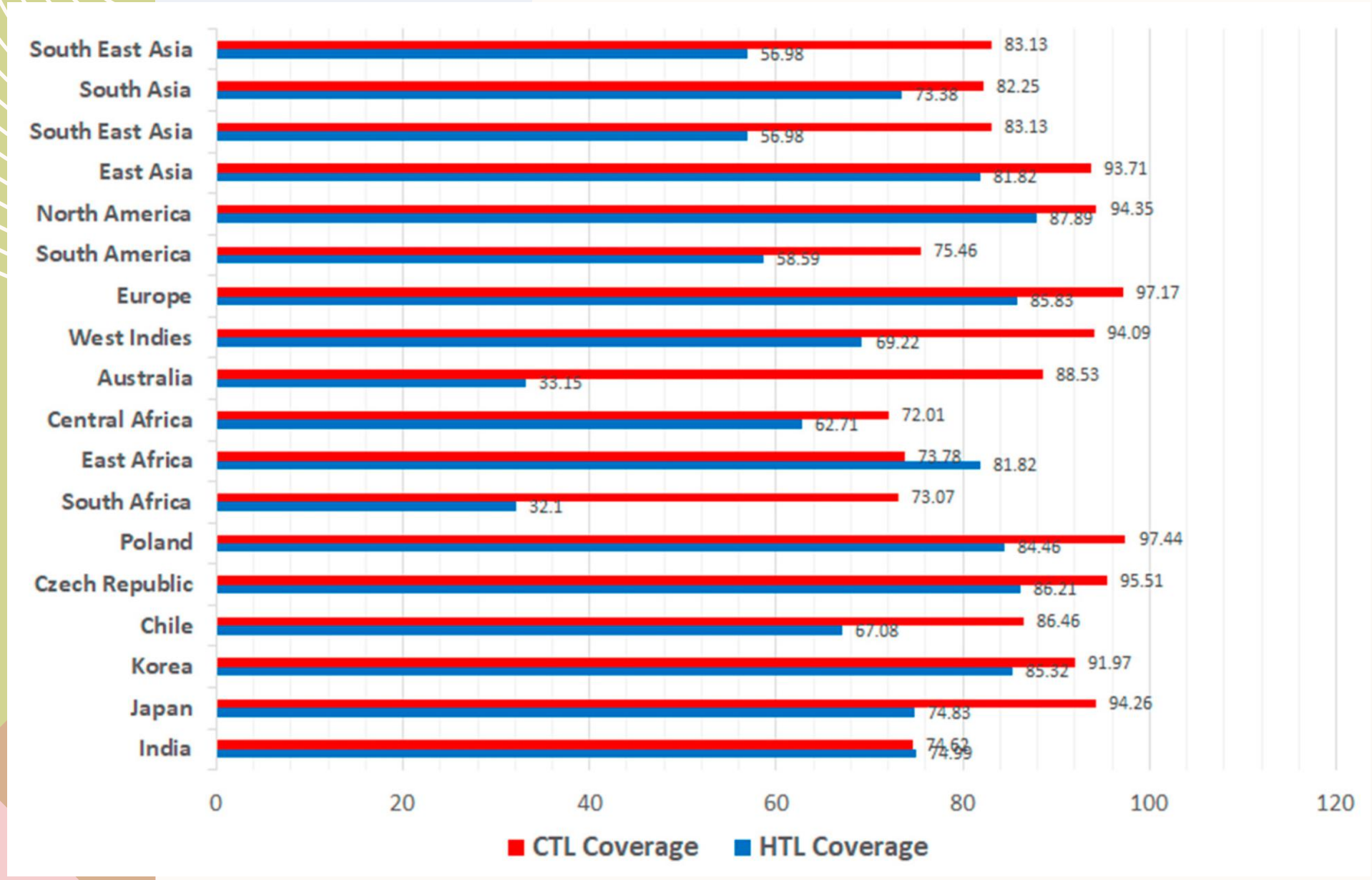
The immune response of the designed vaccine was simulated using the **C-ImmSim server**, which models the stimulation of lymph nodes, thymus, and bone marrow. Three vaccine doses were administered at intervals of 28 days (0, 28, and 56 days), and the simulation ran for 350 days, predicting the **production of immunoglobulins, cytokines, and interleukins to assess humoral and cellular immune responses.**

RESULTS

EPITOPE SELECTION

- Seven CTL, four HTL, and six B-cell epitopes were selected based on their immunogenicity, antigenicity, and non-toxicity. These epitopes were predicted to cover a large portion of the global population, with 93.78% coverage for CTL epitopes and 81.81% for HTL epitopes.

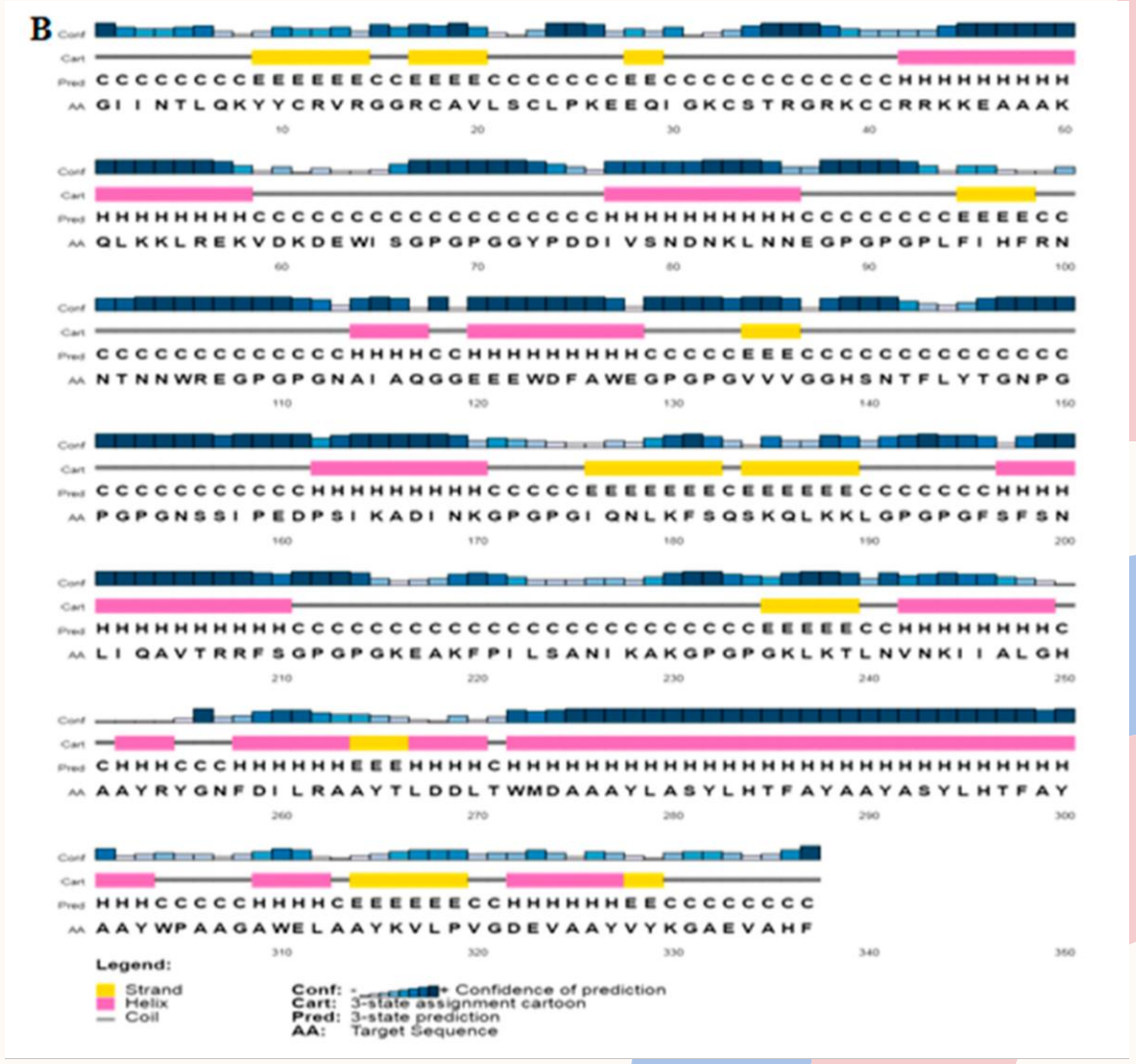
- The selected epitopes showed high population coverage in regions with high GBC prevalence, such as India, Japan, and Chile.


South East Asia
83.13
56.98
South Asia
82.25
73.38
South East Asia
83.13
56.98
East Asia
93.71
81.82
North America
94.35
87.89
South America
75.46
58.59
Europe
97.17
85.83
West Indies
94.09
69.22
Australia
88.53
33.15
Central Africa
72.01
62.71
East Africa
73.78
81.82
South Africa
73.07
32.1
Poland
97.44
84.46
Czech Republic
95.51
86.21
Chile
86.46
67.08
Korea
91.97
85.32
Japan
94.26
74.83
India
74.62
74.99
0
20
40
60
80
100
120
CTL Coverage
HTL Coverage

VACCINE CONSTRUCT

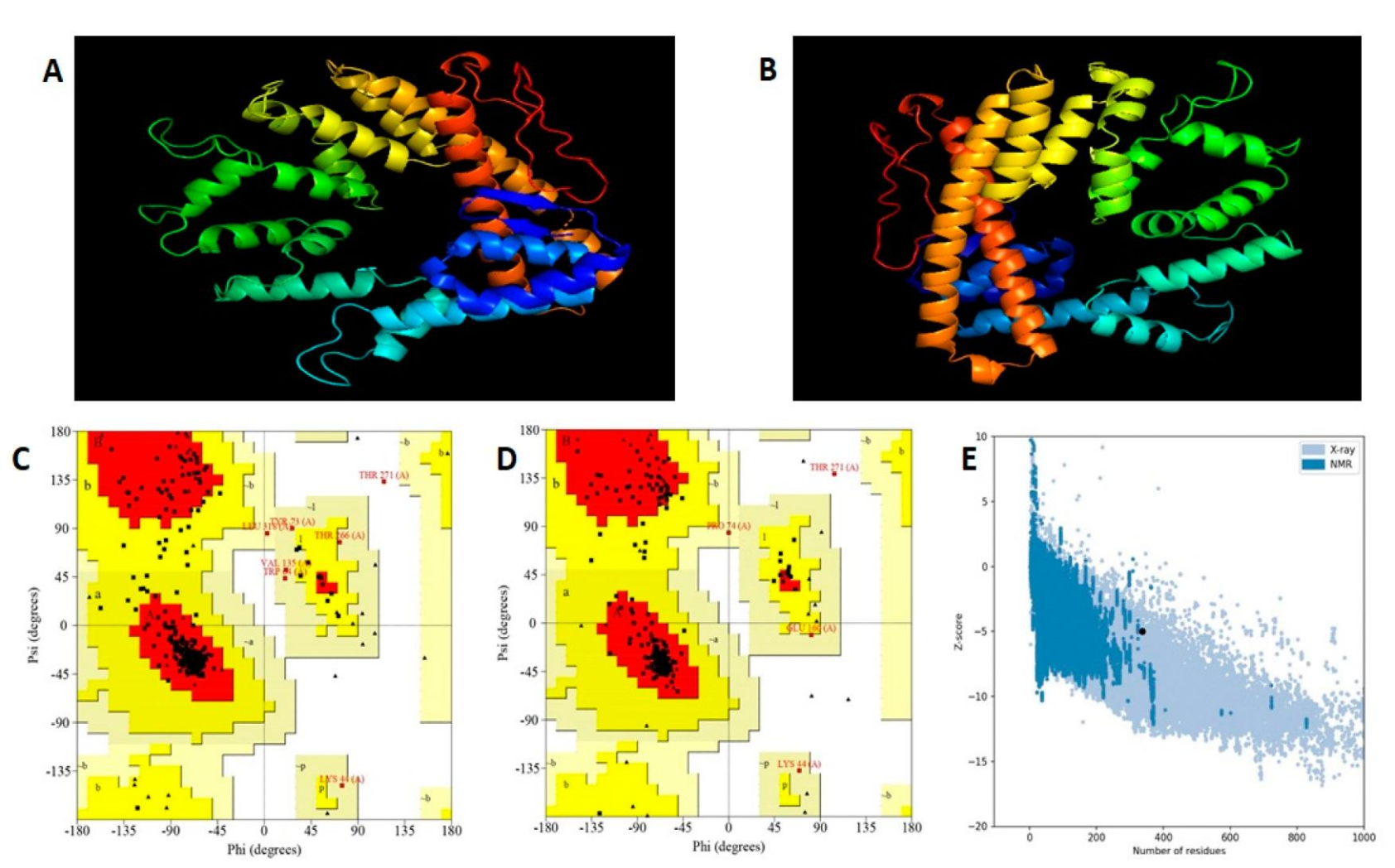
- The final vaccine construct consisted of 337 amino acids, with a molecular weight of 36.21 kDa. The construct was stable, with an instability index of 23.39, and had a high antigenicity score of 0.71 (37% alpha-helix, 14% beta-strands, 49% coils)

- The vaccine was predicted to be soluble and non-allergenic, making it suitable for further development.



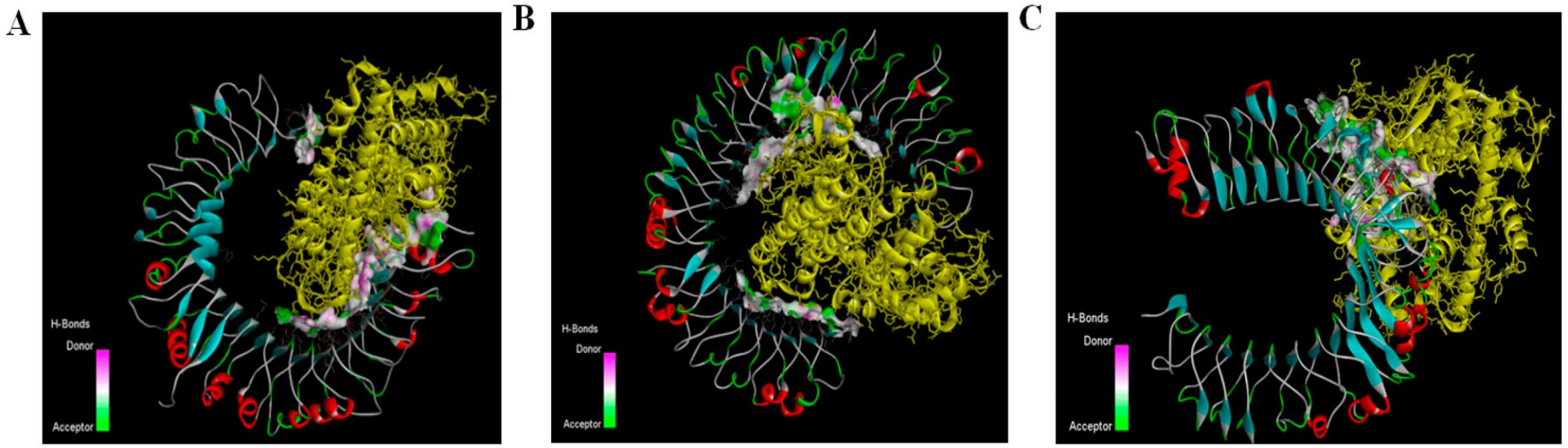
STRUCTURAL VALIDATION

The 3D model of the vaccine showed excellent structural quality, with 92.1% of residues in the most favored regions of the Ramachandran plot. The model was further validated using ProSA, which confirmed its stability.



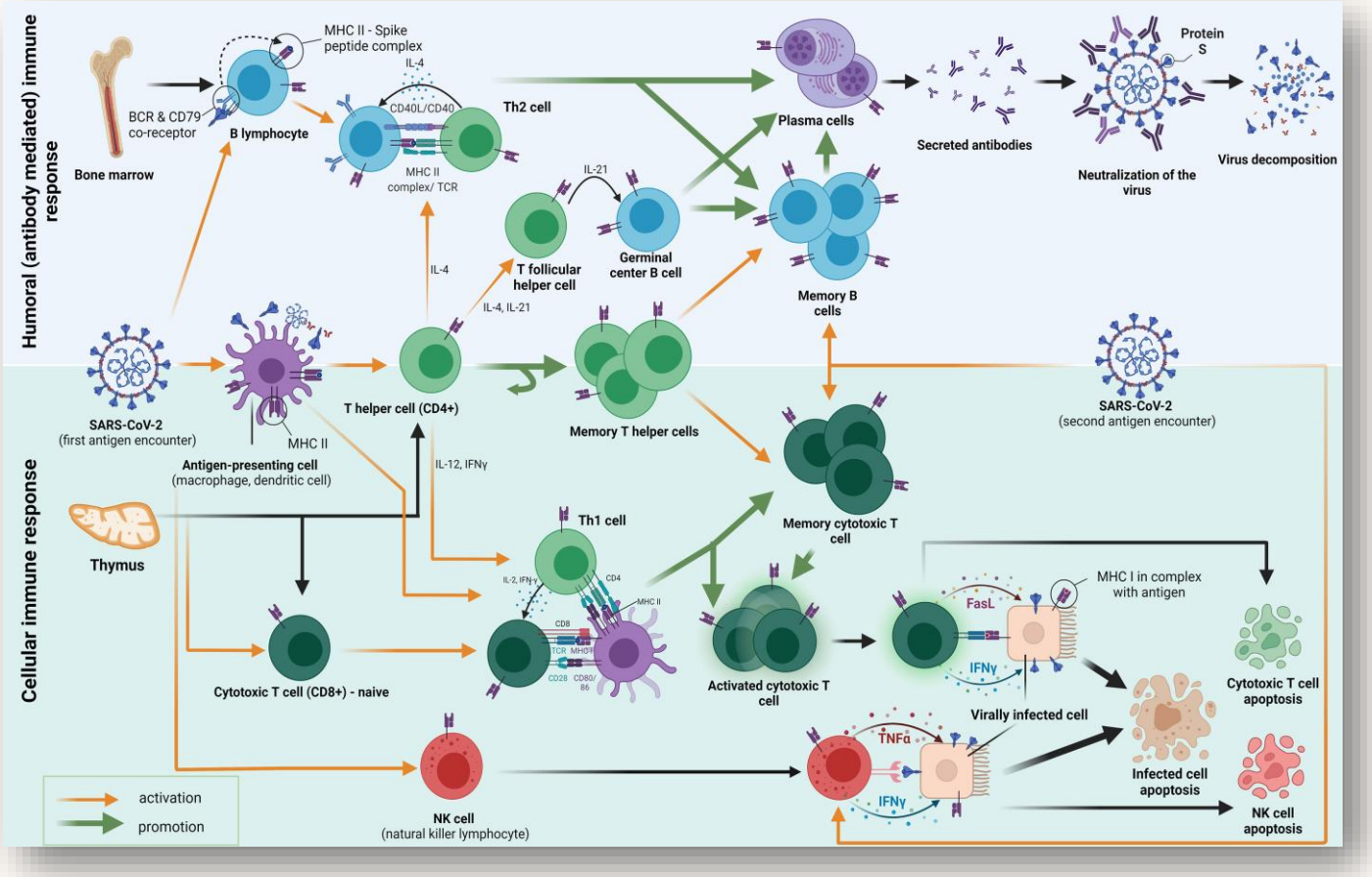
MOLECULAR DOCKING AND DYNAMICS

The vaccine showed strong binding affinity with TLR2, TLR3, and TLR4 receptors, with consistent hydrogen bonds observed. The average number of hydrogen bonds for the vaccine-TLR2, TLR3, and TLR4 complexes were 15.36, 16.45, and 11.98, respectively.



IMMUNE SIMULATION

The immune simulation showed that the vaccine induced a strong immune response, with increased levels of B-cells, T-cells, and cytokines. The response was sustained over time, with each booster dose leading to a further increase in immune activity.



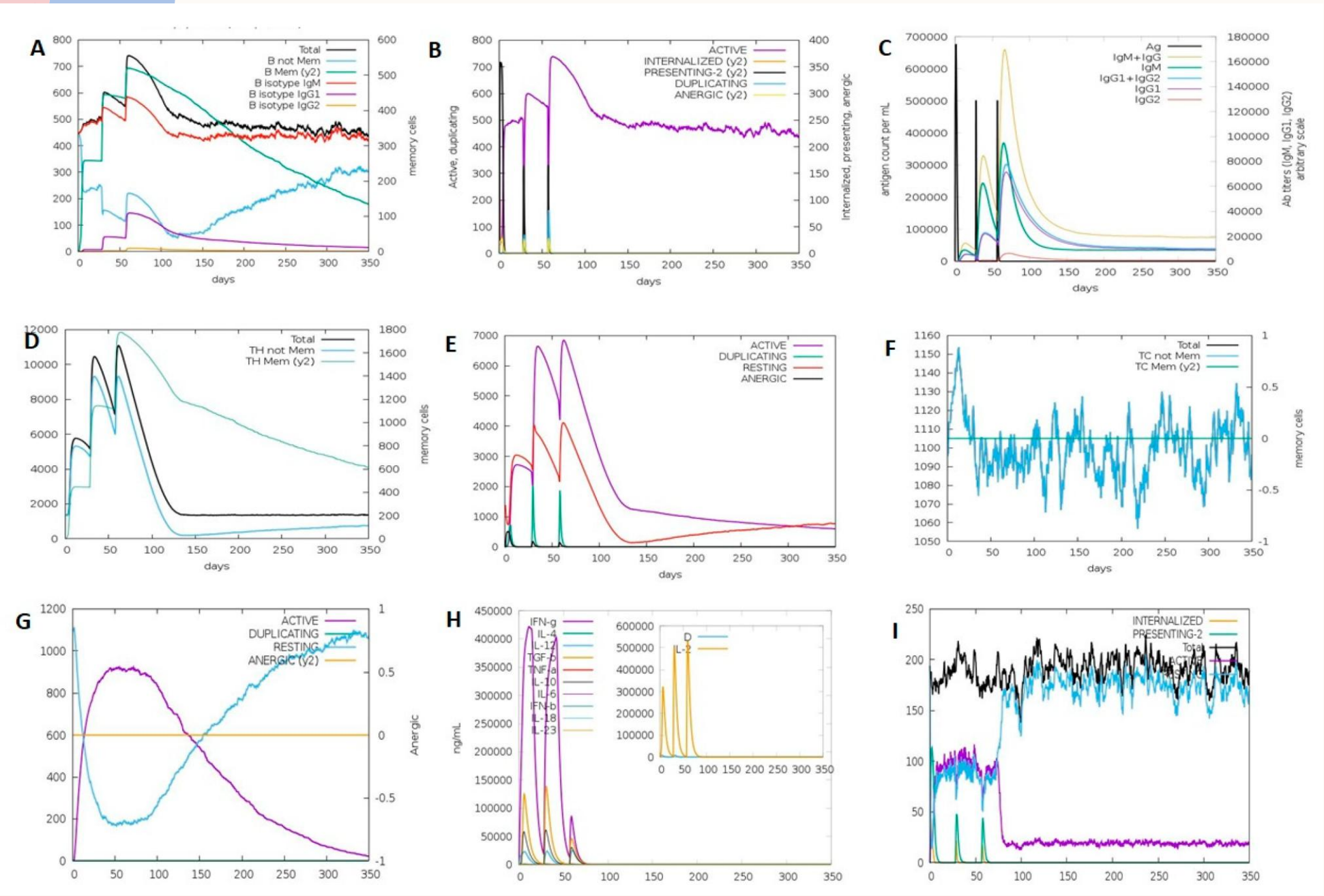


Figure 8. Demonstration of humoral and cellular immune responses using immune simulation. (**A**) B-cell population (cells per mm^3). (**B**) B-cell population per state (cells per mm^3). (**C**) Antigen, immunoglobulins and immunocomplexes. (**D**) TH cell population (cells per mm^3). (**E**) TH cell population per state (cells per mm^3). (**F**) TC cell population (cells per mm^3). (**G**) TC cell population per state (cells per mm^3). (**H**) Concentrations of cytokines and interleukins production. (**I**) Macrophage population per state (cells per mm^3).

DISCUSSION AND CONCLUSION

The study successfully designed a **peptide-based multi-epitope vaccine** using **computational immunoinformatics**.
The vaccine construct showed **strong interactions with immune receptors** (**TLR2, TLR3, TLR4**), indicating its ability to stimulate an immune response.

Advantages of this approach:

- **Personalized and cost-effective** vaccine development.
- **Minimizes allergic reactions** by excluding allergenic epitopes.
- **Increases population coverage** for effective immunization.

Limitations:

- The vaccine has only been tested **in silico** (computationally).
- **Further experimental validation (in vitro & in vivo studies) is required.**

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