Research in Molecular Medicine

Immunoinformatics Design of a Multi-epitope-based Vaccine Against Colorectal Cancer

Zahra Yazdani[1, 2], **Alireza Rafiei**[1*]

1. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
2. Students Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Use your device to scan and read the article online

Citation Yazdani Z, Rafiei A. Immunoinformatics Design of a Multi-epitope-based Vaccine Against Colorectal Cancer. Research in Molecular Medicine. 2022; 10(3):179-192. https://doi.org/10.32598/rmm.10.3.1077.4

https://doi.org/10.32598/rmm.10.3.1077.4

Article Type:
Research Paper

Article info:
Received: 03 May 2022
Revised: 07 Jun 2022
Accepted: 02 Jul 2022

Keywords:
Colorectal cancer, Tumor biomarker, Bioinformatics analysis, Multi-epitope-based vaccine

ABSTRACT

Background: Bioinformatic approaches for designing vaccines have become a promising alternative to conventional methods. We herein designed a multi-epitope-based vaccine against colorectal cancer (CRC).

Materials and Methods: The peptides in the CRC vaccines were retrieved from PubMed, Google Scholar, Web of Science databases, and Clinical trials. The adjuvants of mycobacterial heparin-binding hemagglutinin and pan DR epitope were inserted in the vaccine sequence. Physicochemical properties, immunological characteristics, and projections of the vaccine's 2- and 3-dimensional structures were evaluated. Linear and conformational B-cells were predicted by IEDB (the Immune Epitope Database). Docking and molecular dynamics simulations were performed between the vaccine and toll-like receptor of 4 (TLR4). In silico cloning and mRNA stability were predicted to evaluate the expression of the vaccine in *Escherichia coli* (*E. coli*).

Results: The vaccine comprises 368 amino acids from peptides of OGT, FTO, CASP5, CASP8, U2SURP, MED25, FMO5, CEA, and TGFβIIR CRC antigens. It has a high-quality structure and suitable physicochemical and immunological properties. Also, it contains large and accessible B-cell epitopes. The interaction of the vaccine with TLR4 represented an appropriate and stable one between the vaccine and immune receptors. In silico cloning displayed that the vaccine can transcribe and translate in *E. coli* as a host.

Conclusion: The CRC vaccine is immunogen, non-allergen, and structurally stable. This vaccine will be verified in vitro and in vivo studies in the next step.

*** Corresponding Author:**
Alireza Rafiei, Professor.
Address: *Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.*
Phone: *+98 (11) 33543088*
E-mail: *rafiei1710@gmail.com*

Introduction

Colorectal cancer (CRC) accounts for nearly 8.5% of all cancer deaths. It is one of the most prevalent cancers worldwide [1]. The main treatments for this cancer include surgery, radiotherapy, and chemotherapy, which may cause severe side effects [2]. Immunotherapy is another approach for CRC treatment in recent years [3]. However, this treatment is only effective in 15% of patients with mutations of mismatch repair deficiency or high microsatellite instability [4]. This issue may be related to tumor-infiltrating lymphocytes (TILs) in these patients due to tumor mutational load (TMB) and higher immunogenicity. In contrast, other patients were not affected due to mild immunogenicity [5].

Cancer vaccines constitute the best approaches for treating cancer. They can stimulate a strong immune response to one or more specific tumor antigens and consequently kill the cancer cells producing these antigens. Also, vaccines lack side effects [3, 5] and can be used as a targeted cancer treatment [6]. CRC tumor antigens that are recognized by immune elements are classified according to their function or origin into one of the following groups: tumor-specific antigens which expressed in tumor tissues, not normal tissues such as YE-NEO-001, P53, and KRAS [7-9]; and the second group is associated with tumor antigens such as MAGE-1, CEA, MUC-1, VEGFR1, and VEGFR2 [10-12]. The simultaneous use of tumor-specific antigens and tumor-associated antigens can be useful in treating CRC [13]. However, procurement of a vaccine through traditional methods is time-consuming and expensive. Recently, bioinformatics tools and experimental studies have been used for biological studies. This approach can help us to select targets in a shorter time with a few sources [14, 15]. Multi-epitope vaccines have been designed against several pathogenic microorganisms such as Shigella spp. [16, 17], Helicobacter pylori [18-20], COVID-19 [21, 22], Brucellosis [23], and Mpox [24]. Also, several vaccines have been designed against some types of cancer [13, 25].

In this study, we developed a multi-epitope peptide vaccine against CRC. The vaccine used peptides in the CRC patients' treatment in clinical trials and appropriate adjuvants. The physicochemical and immunological characteristics and structural stability of the vaccine were evaluated. In silico cloning was done to show the DNA of the vaccine could be transcribed and translated into a prokaryote host.

Materials and Methods

Vaccine engineering, analysis of physicochemical characteristics, antigenicity, and allergenicity

Based on the results of the studies from databases of Web of Science, Google Scholar, PubMed, and Clinical trials, 9 peptides from OGT, FTO, CASP5, CASP8, U2SURP, MED25, FMO5, CEA, and TGFβIIR that are incorporated in the CRC vaccines were chosen to be integrated within the vaccine construction. Mycobacterial heparin-binding hemagglutinin (HBHA) and pan DR epitope (PADRE) adjuvants were integrated at the vaccine's N- and C-terminus. Different parts of the designed vaccine were joined using suitable linkers. Physicochemical parameters of the vaccine, such as molecular weight (MW), theoretical isoelectric point (pI), and total average hydrophobicity, were evaluated using online tools from Expasy ProtParam [26]. The proteins' antigenicity was assayed by VaxiJen software, version 2.0 and Antigen Pro servers [27, 28]. Allergenicity and solubility were evaluated using Allergen FP and SOLpro servers [29-31].

Prediction of 2- and 3-dimensional (3D) structures

Prediction of the 2-D structure was carried out by the SOPMA online server [32]. The 3-D structure of the vaccine was projected using the GalaxyTBM server [33]. It displayed 5 models as the predicted structures. All models were analyzed using the ERRAT server, and the highest score was chosen as the vaccine structure. The GalaxyRefine web services were used to improve the predicted model by GalaxyTBM [30]. Five structures were finally displayed by GalaxyRefine as improved models of vaccine structure. The refined 3D structures were verified using the RMSD GalaxyRefine results and servers of ERRAT, PROCHECK, and ProSA [34-36]. Finally, PyMOL software version 2.1.1 was used to view the high-quality 3-D structure of the vaccine [37].

Prediction of linear and discontinuous B-cell epitopes

Linear B-cell epitopes in the vaccine sequence were predicted by methods of BepiPred 2.0 and Emini surface accessibility in the IEDB database. The BepiPred 2.0 predicts B cell epitopes by a random forest algorithm trained on epitopes and non-epitope amino acids detected from crystal structures. Emini surface accessibility predicted accessible B-cell epitopes [38, 39]. Discontinuous B-cell epitopes of the vaccine were predicted using ElliPro in this server [40].

In silico docking calculation and normal mode analysis (NMA) of the complex

The human toll-like receptor 4 (TLR4) tertiary structure was found in the PDB database with a PDB code of 3FXI. The HDOCK server carried out protein-protein docking of TLR4 as a receptor and the vaccine as a ligand [41, 42]. Discovery Studio software, version 4.5 was used to show the interaction between molecules. Normal mode analysis of the complex was evaluated by the iMODS server [43].

In silico cloning and expression of the designed vaccine DNA

The sequence manipulation suite reverse-translated the gene sequence of the designed vaccine [44]. Utilizing the GenScript online server, the properties of sequence genes, such as the codon adaptation index (CAI), codon frequency and distribution (CFD), and GC content were evaluated. These properties play crucial roles in achieving a high protein expression level in the host. The sequence of DNA was transformed to a potential sequence of RNA by the DNA->RNA->Protein tool to estimate the mRNA folding and secondary structure of the vaccine. Then, the secondary structure of the mRNA was predicted using the RNAfold web server [45]. This website offers minimal free energy (G kcal/mol) for structure and thermodynamically predicts the mRNA secondary structure.

Results

Selection of immunodominant epitopes and designing the vaccine structure

Using searching in Google Scholar, PubMed, Web of Science, and Clinical trials databases, 9 epitopes were selected (Table 1). These peptides were joined by repeated sequences of GPGPG, KK, and EAAAK as linkers in the vaccine sequence. After that, HBHA and PADRE sequences were added to the N and C ending of the vaccine sequence to increase the immunogenicity of the vaccine. The designed vaccine is shown in Figure 1A.

Analysis of the physicochemical features, antigenic and allergenic ability of the CRC vaccine

The physicochemical features of this vaccine were analyzed by ProtParam and SOLpro servers. Its antigenic ability was evaluated by ANTIGENpro and VaxiJen software, version 2.0. Also, the allergenic probability was assayed by AllergenFP (Table 2).

Prediction of 2 and 3-D structure and validation of the designed vaccine

Secondary structure of the vaccine was analyzed using the SOPMA online server. It comprised 62.77% helices, 8.15% strands, and 27.45% coils (Figure 1B). The GalaxyTBM was utilized to predict the 3D structure of the vaccine. The predicted models were validated by the ERRAT server. Finally, model 4, with the best score, was selected to refine using GalaxiRefine (Supplemen-

Table 1. Retrieved epitopes from PubMed, Google Scholar, Web of Science databases, and Clinical Trials selected to assemble in the CRC vaccine

No.	Protein	Peptide
1	O-GlcNAc transferase (OGT)	SLYKFSPFPL
2	Fat mass and obesity-associated (FTO)	TLSPGWSAV GGS RLSSCVPVA
3	Caspase5 (CASP5)	FLIIWQNTM
4	Caspase8 (CASP8)	ELLVRINRL
5	U2 snRNP-associated SURP motif-containing protein (U2SURP)	IQEERDERHKR
6	Mediator of RNA polymerase II transcription subunit 25)MED25(	SVDANTTL
7	Flavin containing dimethylaniline monoxygenase 5)FMO5(	RYVENQRHTI
8	Carcinoembryonic antigen (CEA)	YLSGANLNL
9	Transforming growth factor β TGFβII receptor (TGFβIIR)	RLSSCVPVA

Table 2. Evaluation of the physicochemical properties, antigenic, and allergenic ability of the colorectal cancer vaccine

Physicochemical Feature	Value
Molecular weight (Da)	38450.42
Instability index	39.76 Stable
Gravy	-0.450
Aliphatic index	77.39
Theoretical pI	7.68
Number of amino acids	368
Total number of negatively charged residues (Asp+Glu)	48
Total number of positively charged residues (Arg+Lys)	49
Number of atoms	5435
Solubility/SOLpro	Upon overexpression (0.975116)
Antigenicity Antigenicity/ANTIGENpro	0.789076
Antigenicity/VaxiJen version 2.0	0.5858 (antigenicity over 0.5 is probable antigen)
Allergenicity/AllergenFP version 1.0	Probable non-allergen

Table 3. Predicted epitopes in the colorectal cancer vaccine using the bepipred 2 method in IEDB

No.	Start	End	Peptide	Length
1	5	14	AKMAENSNID	10
2	45	92	AEETRTDTRSRVEESRARLTKLQEDLPEQLTELREKFTAEELRKAAEG	48
3	94	107	LEAATSRYNELVER	14
4	109	110	EA	2
5	113	123	ERLRSQQSFEE	11
6	130	141	GYVDQAVELTQE	12
7	152	156	AVGER	5
8	165	212	LGPGPGGPGPGSLYKFSPFPLEAAAKEAAAKTLSPGWSAVGGSRLSSC	48
9	214	223	PVAGPGPGGP	10
10	235	245	MGPGPGGPGPG	11
11	255	259	EAAAK	5
12	263	291	AKIQEERDERHKRKKKKKKSVDANTTLEA	29
13	294	333	KEAAKRYVENQRHTIGPGPGGPGPGYLSGANLNLEAAAKE	40
14	335	354	AKRLSSCVPVAGPGPGGPGP	20

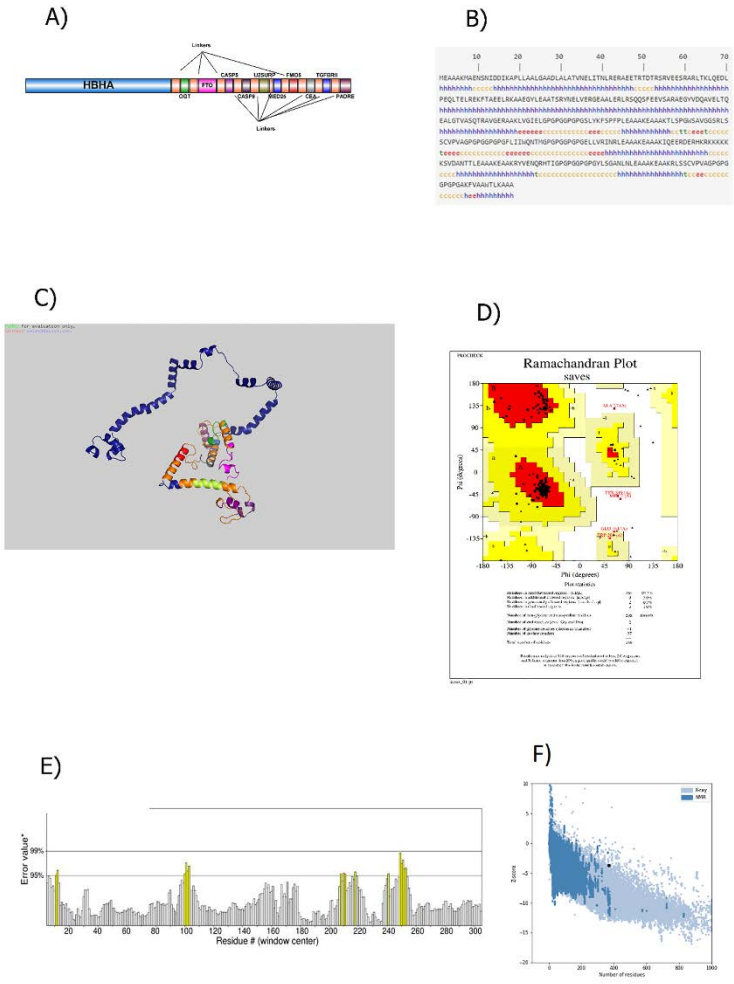


Figure 1. Refinement and validation of the final vaccine

A) Schematic showing of the CRC vaccine, B) Secondary structure of the CRC vaccine, C) 3D model of the CRC vaccine, Construct validation by D) PROCHECK, E) ERRAT, and F) ProSA web servers

tary Table 1). GalaxiRefine produced 5 models. The model with the best scores in the validation servers was chosen for the next analysis (Figure 1C). The PROCHECK results of this model showed that most residues (95.33%) were located in the highly preferred observations region, 3.0% in additional allowed regions, 0.7% in generously allowed regions, and 1% in the disallowed regions (Figure 1D). The ERRAT result indicated that

Table 4. Predicted epitopes in the colorectal cancer vaccine using emini surface accessibility method in IEDB

No.	Start	End	Peptide	Length
1	41	62	LRERAEETRTDTRSRVEESRAR	22
2	77	82	LREKFT	6
3	114	120	RLRSQQS	7
4	265	282	IQEERDERHKRKKKKKKS	18
5	299	305	RYVENQR	7

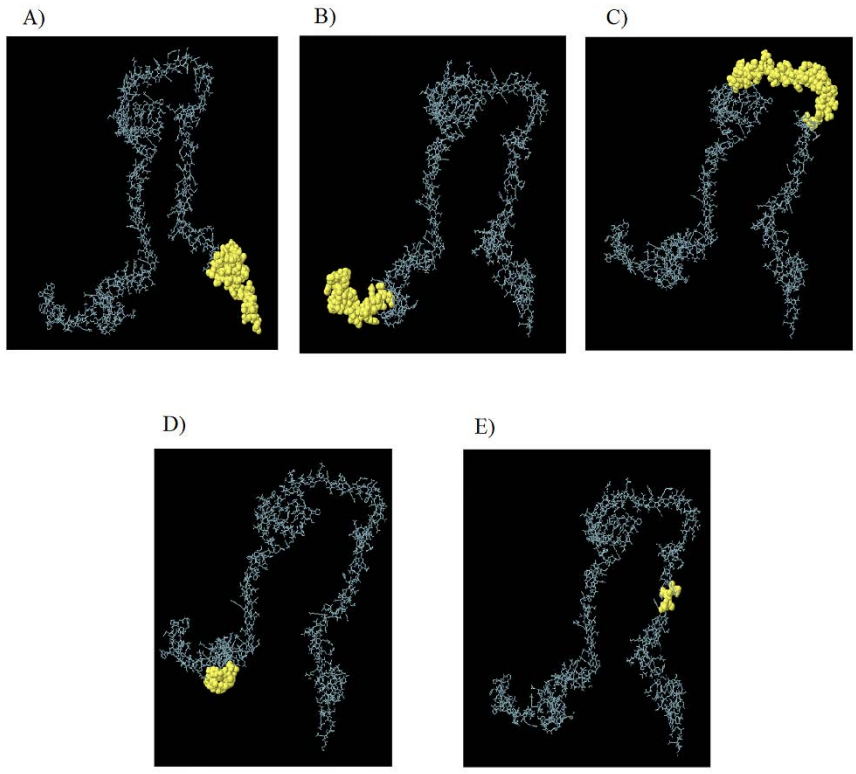


Figure 2. Conformational B-cell epitopes specified in the 3-D structure of the multi-epitope-based vaccine by ElliPro server

the vaccine model scored 92.7326 (Figure 1E). Also, the RMSD and Z-Score of the structure were 0.322 and -3.65 based on the GalaxiRefine and ProSA web servers (Figure 1F).

Identification of B-cell epitopes

The B-cell epitopes were predicted by IEDB. The Emini surface accessibility method showed the CRC vaccine has 14 accessible epitopes. Bepipred2 methods showed the vaccine has 5 linear B-cell epitopes (Table 3 and 4 and Supplementary Figure 1). The results of ElliPro showed that from 368 amino acid residues of the vaccine, 187 were specified as the conformational B-cell epitope (Figure 2).

Molecular docking and NMA analysis of the vaccine with immune cell receptors

To find the potential interactions between the CRC vaccine and immune cell receptors, molecular docking, and NMA analysis were carried out between the vaccine and TLR4. HDOCK server performed the docking of the vaccine with TLR4. This server produced 10 models for each interaction. The docked models with the maximum number of atoms interacting in the ligand and each of the receptors, the lowest docking score (-320.26), and the highest confidence score (0.9679) were chosen and observed by Discovery Studio 4.5 software (Figure 3). The iMODS server was utilized to calculate the NMA analysis of the interaction. iMODS assays the stability of a complex. It showed the interactions between the residues by lower RMSD values and areas with higher correlation. The complex of CRC vaccine-TLR4 produced an eigenvalue of 7.383627×10^{-6}. The results of this server revealed that the interacted residues in each docked complex were stable (Figure 4).

TLR4 protein is shown in blue, and the vaccine is in red. The final docked model has been observed using Discovery Studio software, version 4.5.

In silico cloning and expression of the vaccine DNA

The reverse translation of the sequence of the protein vaccine into a DNA sequence was done by the sequence manipulation suite server. CAI, CFD, and GC content for the expression of the vaccine in *E. coli* were analyzed by GenScript online server. A CAI of the DNA vaccine was 1. The GC content is 58.73% (the GC content of

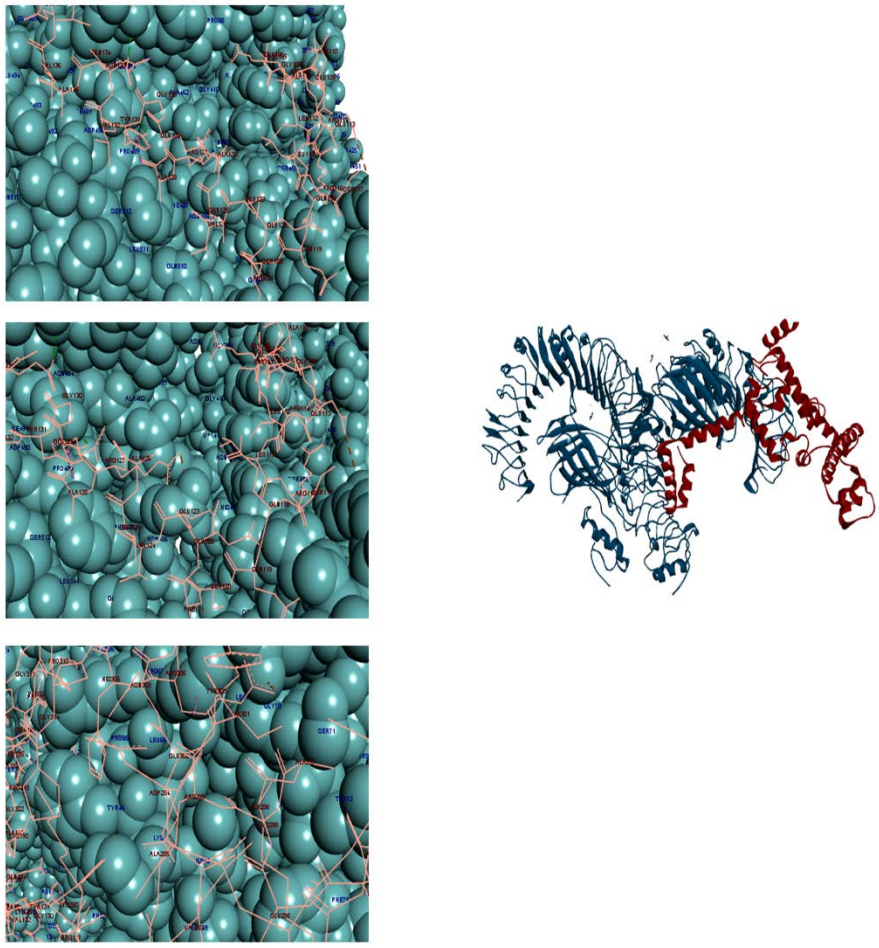


Figure 3. The interaction of TLR4-vaccine complex obtained by HDOCK server

30%–70% is ideal), and the CFD of the vaccine is 0. These results approved the effective transcription of the DNA vaccine in the host. The minimum free energy of the mRNA of the vaccine was evaluated by the RNA fold server. The optimized mRNA structure indicated an ΔG value of −573 kcal/mol (Supplementary Figure 2).

Discussion

Although surgical techniques, chemotherapy, radiotherapy, and immunotherapy have been improved to cure CRC, some challenges, including efficiency and adverse effects, still exist [2]. Cancer vaccines are new strategies for the treatment of cancer patients. However, they are in the developing stages and have not been approved [3]. Bioinformatics approaches can help design and develop vaccines promptly and safely. Several researchers, such as Nezafat et al. [46], Yazdani et al. [13], Mahdevar et al. [47], and Palladini et al. [48] have already designed bioinformatics cancer vaccines and evaluated their safety. In this study, we used an in silico analysis to design a suitable multi-epitope peptide vaccine for treating CRC patients. The vaccine consists of 368 amino acids using peptides from multiple databases. These peptides are obtained from CRC antigens of OGT, FTO, CASP5, CASP8, U2SURP, MED25, FMO5, CEA, and TGFβIIR. Therefore, the vaccine is expected to strongly activate adaptive immune systems against CRC cells that have these antigens.

Adjuvants are among the main parts of the vaccines. They heighten the immunological properties of the vaccine. The N-terminus of HBHA and the PADRE sequence are used in the N and C endings of the vaccine sequence. The N-terminus of HBHA is a TLR4 agonist, which has been approved as a potent adjuvant in several infectious diseases and cancer vaccines [13, 49]. PADRE is another potent adjuvant. It can connect to different types of MHC-II alleles. It is used in the vaccine constructs [13, 50].

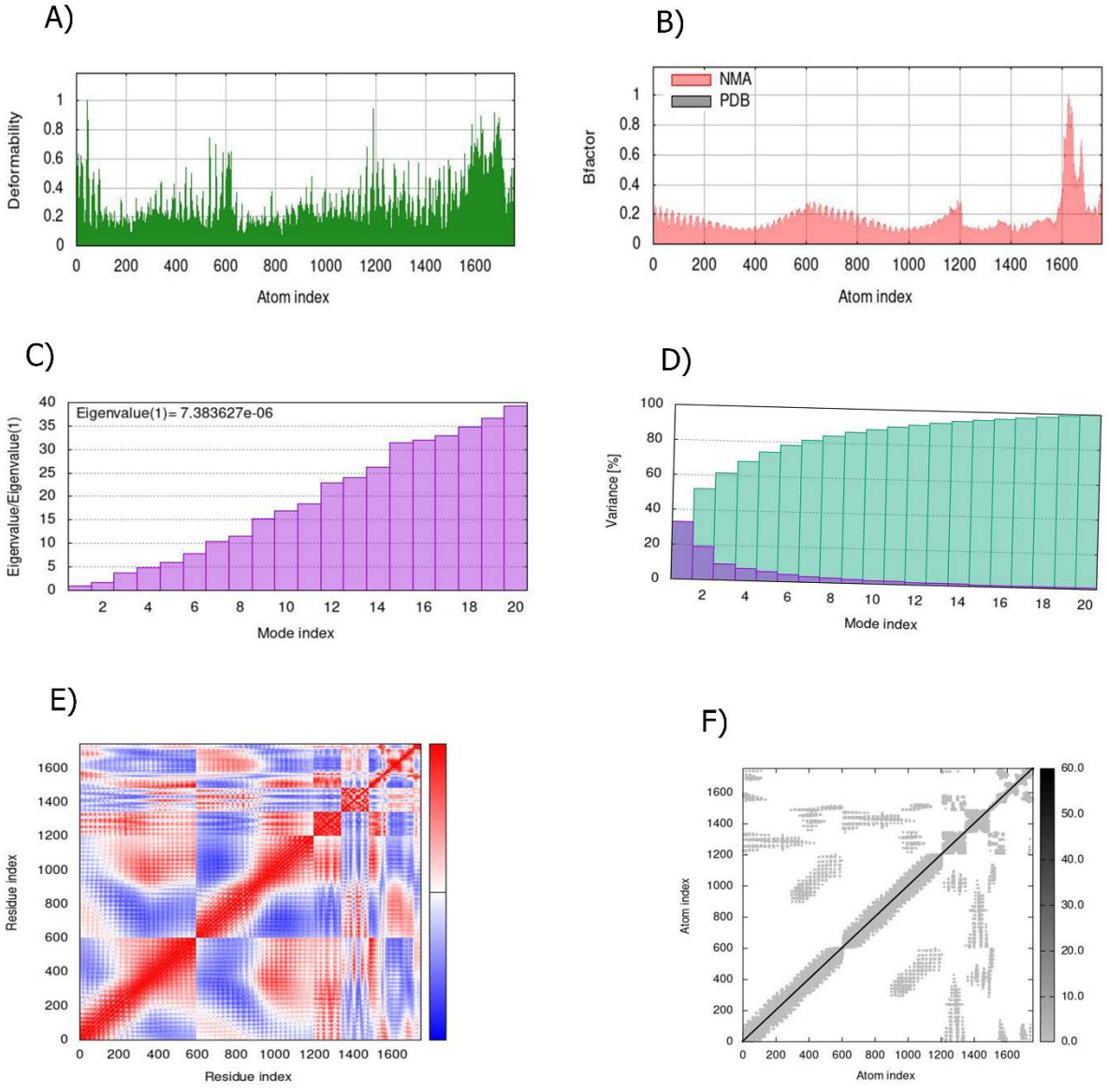


Figure 4. NMA analysis of the TLR4-CRC vaccine interaction

A) Main-chain deformability simulation, B) B-factor values that are calculated using normal mode analysis, and quantifying the uncertainty of each atom, C) The eigenvalue of the docked complex that indicates the energy required to deform the structure, D) Variance between pairs of residues, E) The covariance matrix between pairs of residues (red: Correlated, white: Uncorrelated, blue: Anti-correlated), F) The elastic network model indicates the connection between atoms and springs. The darker springs are more rigid.

The good physicochemical properties, high-quality 2-D and 3-D structure, and ability to express in *E. coli* are characteristics of an efficient vaccine. The results of our study showed that the CRC vaccine was stable, with a stability index of 39.76. It had a pI of nearly natural, non allergenicity, and antigenicity charasterictic. Also, the vaccine is soluble if it is overexpressed in *E. coli*. In addition, the results of ERRAT, PROCHECK, and ProSA servers approved the high-quality structure of the vaccine. B-cell epitopes have an important function in the stimulation of immune responses. The CRC vaccine has a large number of accessible B-cell epitopes. Therefore, it can stimulate B lymphocytes. The binding affinity of the vaccine with the TLR4 showed the vaccine can provoke antigen-presenting cells and activate T-cells against CRC tumor antigens. The expression of a vaccine in the prokaryote host is a pivotal step in designing a vaccine. GenScript was used to optimize codons

and evaluate the expression of the vaccine in *E. coli*. CAI of the DNA vaccine was 1, the average GC content of the sequence was 66.88%, and its CFD was 0. Therefore, the CRC vaccine can be expressed in *E. coli*. The secondary structure of vaccine mRNA is stable thermodynamically. The present study indicated that the CRC vaccine is immunogen, non-allergen, and has high-quality structure and physicochemical properties. However, this vaccine needs to be confirmed by laboratory and animal models.

Ethical Considerations

Compliance with ethical guidelines

The protocol of this study was approved by the Research Ethics Committee of Mazandaran University of Medical Sciences (Code: IR.MAZUMS.REC.1402.150).

Funding

This research was funded by the Research and Technology Deputy of Mazandaran University of Medical Sciences (Grant No.: 17635). The funders had no role in study design, data collection and analysis, publication decisions, or manuscript preparation.

Authors contribution's

Conceptualization, study design, supervision, data collection: Alireza Rafiei; Analysis and drafting the manuscript: Zahra Yazdani; Review, editing and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The authors thank the Mazandaran University of Medical Sciences for its assistance.

References

[1] Oryani MA, Tavasoli A, Ghalavand MA, Ashtiani RZ, Rezaee A, Mahmoudi R, et al. Epigenetics and its therapeutic potential in colorectal cancer. Epigenomics. 2022; 14(11):683-97. [DOI:10.2217/epi-2022-0067] [PMID]

[2] Sharifi E, Yazdani Z, Najafi M, Hosseini-khah Z, Jafarpour A, Rafiei A. The combined effect of fish oil containing Omega-3 fatty acids and Lactobacillus plantarum on colorectal cancer. Food Sci Nutr. 2022; 10(12):4411-8. [DOI:10.1002/fsn3.3037] [PMID]

[3] Jia W, Zhang T, Huang H, Feng H, Wang S, Guo Z, et al. Colorectal cancer vaccines: The current scenario and future prospects. Front Immunol. 2022; 13:942235. [DOI:10.3389/fimmu.2022.942235] [PMID]

[4] Carlsen L, Huntington KE, El-Deiry WS. Immunotherapy for colorectal cancer: Mechanisms and predictive biomarkers. Cancers. 2022; 14(4):1028. [DOI:10.3390/cancers14041028] [PMID]

[5] Berry J, Vreeland T, Trappey A, Hale D, Peace K, Tyler J, et al. Cancer vaccines in colon and rectal cancer over the last decade: Lessons learned and future directions. Expert Rev Clin Immunol. 2017; 13(3):235-45. [DOI:10.1080/1744666X.2016.1226132] [PMID]

[6] Lin MJ, Svensson-Arvelund J, Lubitz GS, Marabelle A, Melero I, Brown BD, et al. Cancer vaccines: The next immunotherapy frontier. Nat Cancer. 2022; 3(8):911-26. [DOI:10.1038/s43018-022-00418-6] [PMID]

[7] Zheng Y, Fu Y, Wang PP, Ding ZY. Neoantigen: A promising target for the immunotherapy of colorectal cancer. Dis Markers. 2022; 2022:8270305. [DOI:10.1155/2022/8270305] [PMID]

[8] Rus Bakarurraini NAA, Ab Mutalib NS, Jamal R, Abu N. The landscape of tumor-specific antigens in colorectal cancer. Vaccines. 2020; 8(3):371. [DOI:10.3390/vaccines8030371] [PMID]

[9] Linnebacher M, Gebert J, Rudy W, Woerner S, Yuan YP, Bork P, et al. Frameshift peptide-derived T-cell epitopes: A source of novel tumor-specific antigens. Int J Cancer. 2001; 93(1):6-11. [DOI:10.1002/ijc.1298] [PMID]

[10] Wagner S, Mullins CS, Linnebacher M. Colorectal cancer vaccines: Tumor-associated antigens vs neoantigens. World J Gastroenterol. 2018; 24(48):5418-32. [DOI:10.3748/wjg.v24.i48.5418] [PMID]

[11] Shahnazari M, Samadi P, Pourjafar M, Jalali A. Therapeutic vaccines for colorectal cancer: The progress and future prospect. Int Immunopharmacol. 2020; 88:106944. [DOI:10.1016/j.intimp.2020.106944] [PMID]

[12] Hu LF, Lan HR, Huang D, Li XM, Jin KT. Personalized immunotherapy in colorectal cancers: Where do we stand? Front Oncol. 2021; 11:769305. [DOI:10.3389/fonc.2021.769305] [PMID]

[13] Yazdani Z, Rafiei A, Irannejad H, Yazdani M, Valadan R. Designing a novel multiepitope peptide vaccine against melanoma using immunoinformatics approach. J Biomol Struct Dyn. 2022; 40(7):3312-24. [DOI:10.1080/07391102.2020.1846625] [PMID]

[14] Soleymani S, Tavassoli A, Housaindokht MR. An overview of progress from empirical to rational design in modern vaccine development, with an emphasis on computational tools and immunoinformatics approaches. Comput Biol Med. 2022; 140:105057. [DOI:10.1016/j.compbiomed.2021.105057] [PMID]

[15] Soria-Guerra RE, Nieto-Gomez R, Govea-Alonso DO, Rosales-Mendoza S. An overview of bioinformatics tools for epitope prediction: Implications on vaccine development.J Biomed Inform. 2015; 53:405-14. [DOI:10.1016/j.jbi.2014.11.003] [PMID]

[16] Leow CY, Chuah C, Abdul Majeed AB, Mohd Nor N, Leow CH. Application of reverse vaccinology and immunoinformatic strategies for the identification of vaccine candidates against shigella flexneri. Methods Mol Biol. 2022; 2414:17-35 [DOI:10.1007/978-1-0716-1900-1_2] [PMID]

[17] Farhani I, Nezafat N, Mahmoodi S. Designing a novel multi-epitope peptide vaccine against pathogenic Shigella spp. based immunoinformatics approaches. Int J Pept Res Ther. 2019; 25:541-53. [DOI:10.1007/s10989-018-9698-5]

[18] Urrutia-Baca VH, Gomez-Flores R, De La Garza-Ramos MA, Tamez-Guerra P, Lucio-Sauceda DG, Rodríguez-Padilla MC. Immunoinformatics approach to design a novel epitope-based oral vaccine against Helicobacter pylori. J Comput Biol. 2019; 26(10):1177-90. [DOI:10.1089/cmb.2019.0062] [PMID]

[19] Meza B, Ascencio F, Sierra-Beltrán AP, Torres J, Angulo C. A novel design of a multi-antigenic, multistage and multi-epitope vaccine against Helicobacter pylori: An in silico approach. nfect Genet Evol. 2017; 49:309-17. [DOI:10.1016/j.meegid.2017.02.007] [PMID]

[20] Mohammadzadeh R, Soleimanpour S, Pishdadian A, Farsiani H. Designing and development of epitope-based vaccines against Helicobacter pylori. Crit Rev Microbiol. 2022; 48(4):489-512. [DOI:10.1080/1040841X.2021.1979934] [PMID]

[21] Oluwagbemi OO, Oladipo EK, Kolawole OM, Oloke JK, Adelusi TI, Irewolede BA, et al. Bioinformatics, computational informatics, and modeling approaches to the design of mRNA covid-19 vaccine candidates. Computation. 2022; 10(12):211. [DOI:10.3390/computation10070117]

[22] Yazdani Z, Rafiei A, Yazdani M, Valadan R. Design an efficient multi-epitope peptide vaccine candidate against SARS-CoV-2: An in silico analysis. Infect Drug Resist. 2020; 13:3007-22. [DOI:10.2147/IDR.S264573] [PMID]

[23] Yazdani Z, Rafiei A, Ghoreyshi M, Abediankenari S. In Silico analysis of a candidate multi-epitope peptide vaccine against human brucellosis. Mol Biotechnol. 2023; 1-15. [DOI:10.1007/s12033-023-00698-y] [PMID]

[24] Bhattacharya M, Chatterjee S, Nag S, Dhama K, Chakraborty C. Designing, characterization, and immune stimulation of a novel multi-epitopic peptide-based potential vaccine candidate against monkeypox virus through screening its whole genome encoded proteins: An immunoinformatics approach. Travel Med Infect Dis. 2022; 50:102481. [DOI:10.1016/j.tmaid.2022.102481] [PMID]

[25] Yazdani Z, Rafiei A, Valadan R, Ashrafi H, Pasandi M, Kardan M. Designing a potent L1 protein-based HPV peptide vaccine: A bioinformatics approach. Comput Biol Chem. 2020; 85:107209. [DOI:10.1016/j.compbiolchem.2020.107209] [PMID]

[26] Gasteiger E, Hoogland C, Gattiker A, Duvaud Se, Wilkins MR, Appel RD, et al. Protein identification and analysis tools on the ExPASy server. In: Walker JM, editor. The proteomics protocols handbook. Springer Protocols Handbooks. New Jersey: Humana Press; 2005. [DOI:10.1385/1-59259-890-0:571]

[27] Magnan CN, Baldi P. SSpro/ACCpro 5: Almost perfect prediction of protein secondary structure and relative solvent accessibility using profiles, machine learning and structural similarity. Bioinformatics. 2014; 30(18):2592-7. [DOI:10.1093/bioinformatics/btu352] [PMID]

[28] Doytchinova IA, Flower DR. Identifying candidate subunit vaccines using an alignment-independent method based on principal amino acid properties. Vaccine. 2007; 25(5):856-66. [DOI:10.1016/j.vaccine.2006.09.032] [PMID]

[29] Dimitrov I, Naneva L, Doytchinova I, Bangov I. AllergenFP: Allergenicity prediction by descriptor fingerprints. Bioinformatics. 2014; 30(6):846-51. [DOI:10.1093/bioinformatics/btt619] [PMID]

[30] Ko J, Park H, Heo L, Seok C. GalaxyWEB server for protein structure prediction and refinement. Nucleic Acids Res. 2012; 40(Web Server issue):W294-7. [DOI:10.1093/nar/gks493] [PMID]

[31] Cheng J, Randall AZ, Sweredoski MJ, Baldi P. SCRATCH: A protein structure and structural feature prediction server. Nucleic Acids Res. 2005;33(Web Server issue):W72-6. [DOI:10.1093/nar/gki396] [PMID]

[32] Deléage G. ALIGNSEC: Viewing protein secondary structure predictions within large multiple sequence alignments. Bioinformatics. 2017; 3(24):3991-2. [DOI:10.1093/bioinformatics/btx521] [PMID]

[33] Seok C, Baek M, Steinegger M, Park H, Lee GR, Won J. Accurate protein structure prediction: What comes next. BioDesign. 2021; 9(3):47-50. [DOI:10.34184/kssb.2021.9.3.47]

[34] Colovos C, Yeates TO. Verification of protein structures: Patterns of nonbonded atomic interactions. Protein Sci. 1993; 2(9):1511-9. [DOI:10.1002/pro.5560020916] [PMID]

[35] Laskowski RA, Rullmann JAC, MacArthur MW, Kaptein R, Thornton JM. AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR. J Biomol NMR. 1996; 8(4):477-86. [DOI:10.1007/BF00228148] [PMID]

[36] Wiederstein M, Sippl MJ. ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. 2007; 35(Web Server issue):W407-10. [DOI:10.1093/nar/gkm290] [PMID]

[37] Lill MA, Danielson ML. Computer-aided drug design platform using PyMOL. J Comput Aided Mol Des. 2011; 25(1):13-9. [DOI:10.1007/s10822-010-9395-8] [PMID]

[38] Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol. 1985; 55(3):836-9. [DOI:10.1128/jvi.55.3.836-839.1985] [PMID]

[39] Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: Improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res. 2017; 45(W1):W24-9. [DOI:10.1093/nar/gkx346] [PMID]

[40] Ponomarenko J, Bui HH, Li W, Fusseder N, Bourne PE, Sette A, et al. ElliPro: A new structure-based tool for the prediction of antibody epitopes. BMC Bioinform. 2008; 9:514. [DOI:10.1186/1471-2105-9-514] [PMID]

[41] Ferrè F, Clote P. DiANNA: A web server for disulfide connectivity prediction. Nucleic Acids Res. 2005; 33(Web Server issue):W230-2. [DOI:10.1093/nar/gki412] [PMID]

[42] Remmert M, Biegert A, Hauser A, Söding J. HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. Nat Methods. 2012; 9(2):173-5. [DOI:10.1038/nmeth.1818] [PMID]

[43] López-Blanco JR, Aliaga JI, Quintana-Ortí ES, Chacón P. iMODS: Internal coordinates normal mode analysis server. Nucleic Acids Res. 2014; 42(Web Server issue):W271-6. [DOI:10.1093/nar/gku339] [PMID]

[44] Stothard P. The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. Biotechniques. 2000; 28(6):1102-4. [DOI:10.2144/00286ir01] [PMID]

[45] Mathews DH, Disney MD, Childs JL, Schroeder SJ, Zuker M, Turner DH. Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. Proc Natl Acad Sci U S A. 2004; 101(19):7287-92. [DOI:10.1073/pnas.0401799101] [PMID]

[46] Nezafat N, Sadraeian M, Rahbar MR, Khoshnoud MJ, Mohkam M, Gholami A, et al. Production of a novel multi-epitope peptide vaccine for cancer immunotherapy in TC-1 tumor-bearing mice. Biologicals. 2015; 43(1):11-7. [DOI:10.1016/j.biologicals.2014.11.001] [PMID]

[47] Mahdevar E, Kefayat A, Safavi A, Behnia A, Hejazi SH, Javid A, et al. Immunoprotective effect of an in silico designed multiepitope cancer vaccine with BORIS cancer-testis antigen target in a murine mammary carcinoma model. Sci. Rep. 2021; 11(1):23121. [DOI:10.1038/s41598-021-01770-w] [PMID]

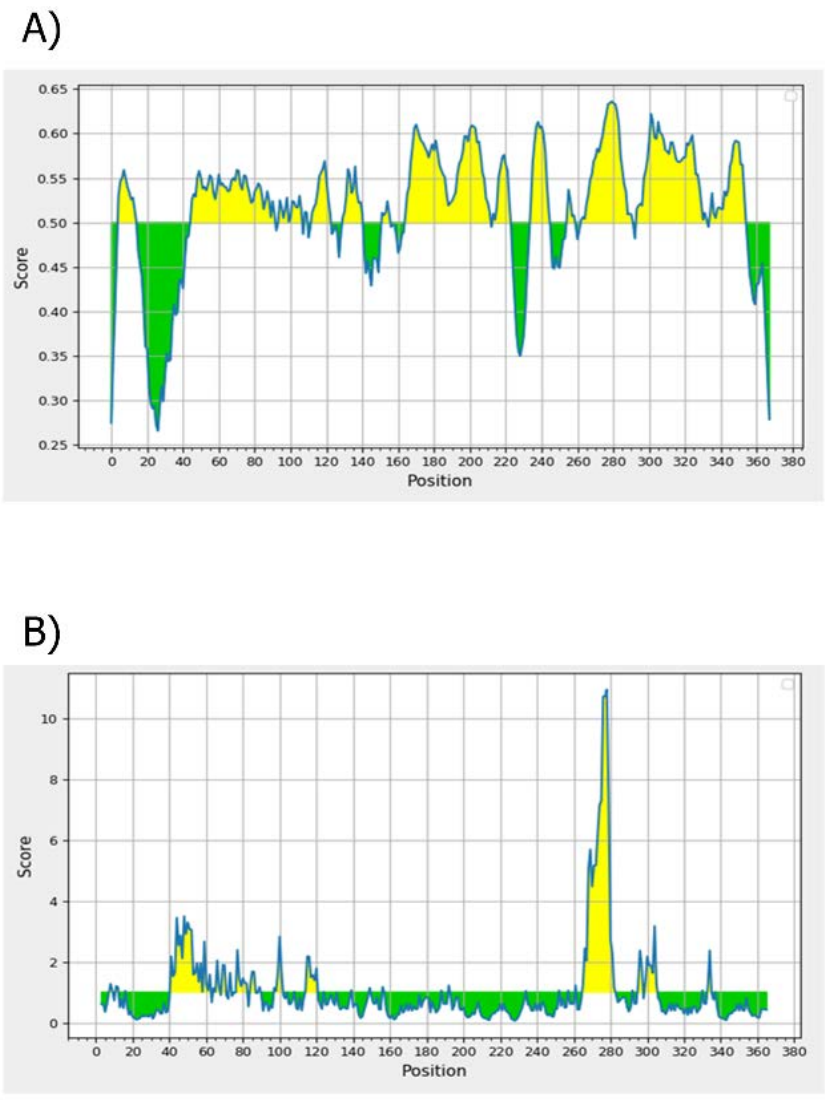
[48] Palladini A, Nicoletti G, Pappalardo F, Murgo A, Grosso V, Stivani V, et al. In silico modeling and in vivo efficacy of cancer-preventive vaccinations. Cancer Res. 2010; 70(20):7755-63. [DOI:10.1158/0008-5472.CAN-10-0701] [PMID]

[49] Nezafat N, Ghasemi Y, Javadi G, Khoshnoud MJ, Omidinia E. A novel multi-epitope peptide vaccine against cancer: an in silico approach.J Theor Biol. 2014; 349:121-34. [DOI:10.1016/j.jtbi.2014.01.018] [PMID]

[50] Bélot F, Guerreiro C, Baleux F, Mulard LA. Synthesis of two linear PADRE conjugates bearing a deca-or pentadecasaccharide B epitope as potential synthetic vaccines against Shigella flexneri serotype 2a infection. Chemistry. 2005; 11(5):1625-35. [DOI:10.1002/chem.200400903] [PMID]

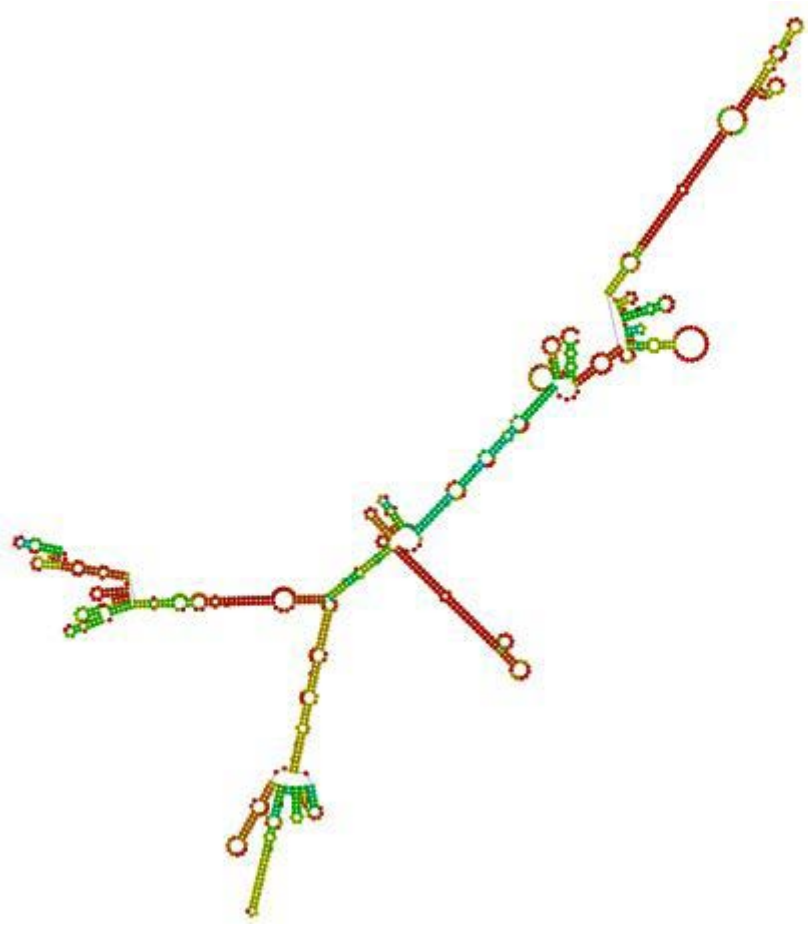
Supplementary Table 1. The ERRAT scores of the predicted models with Galaxi TBM server

Model	ERRAT
Model1	93.7853
Model2	86.5546
Mode3	89.548
Model4	92.2636
Model5	89.8017



Supplementary Figure1. The results of prediction linear B cell epitopes in the CRC vaccine by Bepipread 2 and Emini surface accessibility methods.

The exposed regions are shown in yellow and unexposed regions are shown in green colors.



Supplementary Figure 2. Secondary structure mRNA of the CRC vaccine

This Page Intentionally Left Blank