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# Computational Design and Analysis of a Poly-Epitope Fusion Protein: A New Vaccine Candidate for Hepatitis and Poliovirus

**Armina Alagheband Bahrami[1] · Mojgan Bandehpour[2,3] · Bahman Khalesi[4] · Bahram Kazemi[3]**

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**Abstract**
Infections with HCV, HBV and poliovirus are still considered to be substantial global health burdens. Vaccination is one of the most important preventive strategies against these infections. Multi-epitope vaccines are presented as novel strategies to circumvent the limitations associated with conventional vaccines. Given these circumstances, a multi-epitope protein was designed using the predicted high score epitopes of the antigens from HCV, HBV and Poliovirus. To this end, the sequences of HCV core protein, HBV small surface antigen and VPs of Poliovirus were collected and the consensus sequence of these antigens were obtained using BLAST and MSA analyses. Then, the physicochemical properties of these antigens along with their high score B and T-cell epitopes were predicted using various softwares. The obtained epitopes were connected with proper linkers to build the final 500 amino acids HHP protein. The secondary and tertiary structure of the HHP as well as its physicochemical properties and immunological properties were predicted using different tools. Assessment of various properties of the designed protein indicated that the HHP poly-epitope is an immunogenic and non-allergen antigen, which can derive humoral and cellular immune responses against HCV, HBV and Poliovirus infections.

**Keywords** In silico · Bioinformatic · Poly-epitope · Hepatitis · Poliovirus · Vaccination

## Introduction

Hepatitis C is still considered to be a substantial global health burden. Approximately 185 million are reported to be chronically infected with this virus worldwide. The HCV strains are classified to seven genotypes based on genome sequence and phylogenetic similarity. The structural proteins of the HCV virus are consisted of core protein, E1 and E2 glycoproteins and six none-structural (NS) proteins (Scheel and Rice 2013). The core protein (22 kD) is known to be one of the most conserved structural proteins of the HCV amongst its all genotypes. The core protein is also responsible for the formation of a viral nucleocapsid and has been involved in processes like the pathogenesis of the virus, the outspread of chronic infection and immune response compilation (McLauchlan 2000; Tan et al. 2006). These properties have provided the rationale for HCV core protein to be deemed as an appropriate target for vaccine design against HCV.

Hepatitis B is remained to be one of the most severe global health threats particularly in Asia and the third-world countries. HBV infection could lead to acute and chronic necroinflammatory disease. Cirrhosis and hepatocellular carcinoma are among the high risk diseases closely related to HBV infection. Annually one million people die from HBV-related liver diseases, mainly cirrhosis and hepatocellular carcinoma (HCC), which expresses the seriousness of the HBV infections (Kao and Chen 2002; McMahon 2014). HBV is DNA virus infectious for humans and a few animal species (duck, squirrel and woodchuck) (Rizzetto and Ciancio 2008). HBV genome is translated to several structural and non-structural peptides including hepatitis B surface

✉ Mojgan Bandehpour
Bandehpour@gmail.com; m.Bandehpour@sbmu.ac.ir

1 Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2 Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

3 Department of Biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

4 Department of Research and Production of Poultry Viral Vaccine, Razi Vaccine and Serum Research institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

antigen (HBsAg), hepatitis X protein (HBxAg), hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg) and DNA polymerase (Rui et al. 1998). These antigens have been widely used to design HBV vaccines and HBV detection antigens (Khalili et al. 2017). Although the incidence of acute and chronic HB has significantly reduce by implementation of universal HBV vaccination programs, the success of these programs is now being threatened by HBV vaccine breakthrough infection mainly due to the S gene mutants of HBV, high maternal viral load and virus-induced immunosuppression (Qin and Liao 2018).

Polioviruses are composed of a single-stranded RNA genome and a capsid protein which could lead to emergence of Poliomyelitis. Four coat protein subunits including VP1, VP2, VP3 and VP4 form Poliovirus capsid. The capsid is supposed to be responsible for the disease pathology of Polio and antigenicity of the virus. The capsid binding to the specific Poliovirus receptor (PVR: CD-155) is the key for Polio pathogenesis. VP1 is the predominantly exposed surface protein of the Poliovirus, VP3 and VP2 are exposed to a lesser extent and VP4 appears to be completely buried (Emini et al. 1984; Wetz and Habermehl 1982). The viral neutralizing epitopes are located on the three external structural capsid proteins including VP1, VP2, and VP3 (Adu 2005). These epitopes could be employed to design epitope vaccines against Polio. Although the Global Polio Eradication Initiative (GPEI) has entered its multifaceted endgame in Polio eradication (Kew and Pallansch 2018), studies are ongoing to circumvent the challenges of the Polio vaccine production. These studies are mainly focused on development of affordable and safer Poliovirus vaccines, establishment of efficient PV surveillance, development of biological tests/sero-surveillance without live PV and development of effective anti-PV drugs.

Given that the limitations of current vaccines and their continued health burden, the design and development of an efficient prophylactic or therapeutic vaccine capable of simultaneous vaccination against HCV, HBV, and Polio seems to be a compelling approach to overcome their limitation.

Contemporary, updating the design of polyvalent vaccines and combinations of multiple vaccines have become one of the priorities of the World Health Assembly (WHA). These vaccines are capable of simultaneous elicitation of immune response against several infectious diseases. Moreover, these vaccines would bring about reduced costs and inconveniency of administration schedule. In addition, these vaccines could be a solution to ease the potential risk of re-emergence infection like what is known as the vaccine-derived Polioviruses (VDPVs) from oral Polio vaccine (OPV) (Jorba et al. 2017).

Polyvalent vaccines have already been used for combined vaccination of polio with HBV (Yim et al. 1996) and HCV with HBV (Mohammadzadeh et al. 2016). Using synthetic poly-epitopes (i.e. T and B-cells epitopes) which lead to humoral and cellular immune responses (Karpenko et al. 2014) is one of the promising platforms to design these vaccines. Multiple conserved and crucial epitopes from various antigens could be co-delivered with multi-epitope vaccine modalities and the deleterious effects which are encountered using entire proteins could be avoided (Arashkia et al. 2010; Huang et al. 2013; Mishra et al. 2014). In the present study, we have used an in silico approach to design a multi-epitope vaccine candidate capable of simultaneous vaccination against HCV, Polio and HBV. To this end, potential B and T-cell epitopes of Hepatitis C, B and Poliovirus were predicted and selected using an integrated in silico approach.

## Methods

### Bioinformatics studies and Methodology

#### Amino acid sequence retrieval

In the first stage, the research for protein sequences of candidate genes was performed within the NCBI protein collection at (https://www.ncbi.nlm.nih.gov/) and the UniProt database at (https://www.uniprot.org/). Based on these searches, the reference sequences were retrieved for the surface antigen of hepatitis B, the core antigen of hepatitis C, and the VPs capsid proteins of Poliovirus.

#### Alignment of the sequences

A BLAST search was performed to collect the sequences of each antigen reported for different serotypes. The obtained sequences were used as input sequences in Multiple Sequence Alignment (MSA). All MSAs were performed by Megalign software (DNASTAR's Lasergene Suite). Multiple Sequence Alignment (MSA) was performed to obtain a consensus sequence for each antigen. The resulting consensus sequences would represent all of the viruses' main serotypes. Similar alignments were done on the Clustal algorithm at (http://www.ebi.ac.uk/Tools/ClustalX2) to verify the results.

#### Features evaluation of antigen determinants

To assess the physicochemical features of each antigen the ProtParam tool at (https://web.expasy.org/protparam/) (Gasteiger et al. 2005) was used. The molecular weight, isoelectric point (PI), grand average of hydropathicity (GRAVY), amino acid composition and other physicochemical features were predicted for each antigen. The secondary structure of proteins was predicted using the Self-Optimized Prediction Method with Alignment (SOPMA) server at (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/

NPSA/npsa_sopma.html) (Geourjon and Deleage 1995). SOPMA predicts four conformational states including Helix, Beta sheets and bridges, Turns and Coils for each antigen. The amino acids distribution was evaluated via LRRfinder database at (http://www.lrrfinder.com/) (Offord et al. 2010). The trans-membrane topology of proteins was predicted via TMHMM server v. 2.0 (Krogh et al. 2001) at (http://www.cbs.dtu.dk/services/TMHMM/).

#### Prediction of T-cell epitopes

The T-cell epitopes of each three antigens (MHC I, II) were predicted, using the online prediction server Immune Epitope Database (IEDB) at (http://www.iedb.org/) (Vita et al. 2014). Moreover, RANKPEP server at (http://imed.med.ucm.es/Tools/rankpep.html) was used to predict the peptide binders to MHCI and MHCII molecules from protein sequences (Reche et al. 2004). The binding affinity of the HLA haplotype to individual epitopes is the major factor related to the immune-dominance of CTL epitopes (Shahsavandi et al. 2015). According to allele frequency of different Iranian populations and among all HLA alleles, HLA-A*0201 and HLA-DRB1*01:01 are the most available ones in the world (Pelte et al. 2004; Ranjbar et al. 2013); Therefore, epitopes prediction was performed for these alleles. The MHC I (HLA-A*0201)-restricted T cell epitopes were predicted by SYFPEITHI at (http://www.syfpeithi.de/) (Schuler et al. 2007) and Bioinformatics and Molecular Analysis Section (BIMAS) at (https://www-bimas.cit.nih.gov/molbio/hla_bind/) (Taylor 2000); Furthermore, MHCpred at (http://www.ddg-pharmfac.net/mhcpred/MHCPred/) (Guan et al. 2006) and ProPred at (http://crdd.osdd.net/raghava/propred/) (Singh and Raghava 2001) servers were used to predict MHC class II (HLA-DRB1*01:01) restricted T cell epitopes.

#### Prediction of linear B-cell epitopes

The ABCpred at (http://crdd.osdd.net/raghava/abcpred/) (Saha and Raghava 2006), BCpreds at (http://ailab.ist.psu.edu/bcpred/) (Saha and Raghava 2004) and Bepipred (Jespersen et al. 2017) from IEDB were applied to predict the linear B-cell epitopes. The Ellipro server at http://tools.iedb.org/ellipro/ was also used to identify continuous epitopes in the protein regions protruding from the protein's globular surface (Ponomarenko et al. 2008).

#### Design and construction of poly-epitope protein

To design the sufficient poly-epitope protein (referred as HHP), the selected epitopes are fused together using proper amino acid linker from Linker Database at http://www.ibi.vu.nl/programs/linkerdb. The flexible Glycine-Serine rich linkers were used to join the selected epitopes. The physicochemical properties of the HHP protein were calculated by the ProtParam. After verification of the construct's properties, the polytope peptide was chemically synthesized in proper vector by GeneCreate Biological Engineering Co (Wuhan, China).

### The HHP properties evaluation

#### Antigenicity and Allergenicity

The immunogenicity of the HHP protein was predicted by VaxiJen v.2.0 server at (http://www.jenner.ac.uk/VaxiJen). To determine the antigenicity of the HHP poly-epitope "virus" option was selected as a target organism. The accuracy of this server based on the target organisms (i.e. bacterial, viral, and tumor protein datasets) varies from 70 to 89% (Doytchinova and Flower 2007). The ANTIGENpro at (http://scratch.proteomics.ics.uci.edu) was also used to predict the whole protein antigenicity (Magnan et al. 2010). Protein allergenicity was predicted exploiting the AlgPred web server at (http://www.imtech.res.in/raghava/algpred/) (Saha and Raghava 2006a, b) (near 85% accuracy with the threshold of 0.4) and the AllerTOP v.2 server at (http://www.ddg-pharmfac.net/AllerTOP) (Dimitrov et al. 2014).

#### Evaluation of the HHP secondary structure

Jpred v.4 incorporates the Jnet algorithm to predict the secondary structure of the HHP (> 80.0% accuracy) (Drozdetskiy et al. 2015). This server utilizes the hybrid method to predict the protein structure, which combines the alignment-based and single sequence-based methods. Additionally, for further verification, we used SOPMA and Psipred protein secondary structure prediction servers (McGuffin et al. 2000).

#### Prediction and analysis of the HHP tertiary structure

The tertiary structure of the HHP protein was predicted using the 3DLigandSite server at (http://www.sbg.bio.ic.ac.uk/3dligandsite/) (Wass et al. 2010). The I-TASSER at (http://zhanglab.ccmb.med.umich.edu/I-TASSER/download/) (Yang et al. 2015) was also applied to ameliorate the accuracy of tertiary structure prediction. The quality of the 3D models was measured using the QMEAN and PROSA software at (https://swissmodel.expasy.org/qmean/) (Benkert et al. 2010) and (https://prosa.services.came.sbg.ac.at/prosa.php) (Wiederstein and Sippl 2007), respectively. The best predicted 3D model was served for refinement process by GalaxyRefine server at (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) (Heo et al. 2013). To simulate the modeled structure to

the pseudo-native conformation, energy minimization was performed using YASARA Energy Minimization server at (http://www.yasara.org/minimizationserver.htm) (Krieger et al. 2009). The quality of the predicted structures was assessed by Rampage software at (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) (50). Furthermore, to evaluate the refined model we used other online validation servers such as MolProbity at (http://molprobity.biochem.duke.edu/) (Chen et al. 2010), and Verify-3D at (http://servicesn.mbi.ucla.edu/Verify3d/) (Lüthy et al. 1992). Finally, the 3D models were visually analyzed by the PyMOL software (Pymol molecular graphics system V. 1) (DeLano and Pymol 2002).

#### Prediction of cleavage sites

Netchop 3.1 at (http://www.cbs.dtu.dk/services/NetChop/) (Keşmir et al. 2002) and MAPPP online servers at (http://www.mpiib-berlin.mpg.de/MAPPP/) were used to determine the proteasome cleavage sites of the HHP protein. Peptide binding affinity to TAP protein has been computed by TAPPred server at (http://crdd.osdd.net/raghava/tappred/) (Bhasin et al. 2007).

## Results

### Sequence retrieval and collection

In current study the HBV surface antigen, HCV core protein, and Poliovirus VP proteins were selected as antigenic determinants. Amino acid sequences were retrieved in FASTA format. The sequences under GenBank accession number of **YP-009173871** for HBsAg, **NP-751,919** for HCV cp and **NC-002058** for Polio were selected for the following analyses.

### Structures analysis of antigen determinants

The physiochemical properties for each of the three antigens were determined and summarized in Table 1. The amino acids distribution of each antigen is illustrated in Fig. 1. The trans-membrane topology analysis of antigens showed that only HBsAg from the HBV could have transmembrane localization and the other antigens lack any transmembrane topology Fig. 2.

### Epitope identification and characterization

The performed epitope prediction analyses indicated that all of HBsAg, HCV cp, and Polio Vp antigens contain high scoring MHC I and II binding epitopes. The epitopes with highest scores which were confirmed with different software were selected as final epitopes (Table 2). Similarly, the search for B-cell epitopes within HBsAg, HCV cp, and Polio Vp antigens has resulted in several high scoring epitopes which were confirmed by more than one software as shown in Table 3.

### Construction of the HHP protein

The selected epitopes of analyzed antigens were joined to each other using suitable linkers to build the final antigen. The final construct of the HHP protein is consists of 499 amino acid residues; HBsAg contributes 6 epitopes at the N-terminal of the construct (1 – 138). Six epitopes are derived from HCV core protein and 16 epitopes are derived from Polio Vp 2,3,4,1, respectively.

### The HHP properties

#### Evaluation of physicochemical parameters

The isoelectric point (pI) and molecular weight (MW) of the HHP protein were computed to be 9.63 and 50 kDa, respectively. The protein half-life was estimated to be

**Table 1** Physicochemical parameters for each Ag candidate and HHP computed by ProtParam tool

| Physicochemical properties | HBsAg | HCV core | Polio VPs | HHP Polyepitope protein |
|---|---|---|---|---|
| Number of amino acids | 226 aa | 191 aa | 302 aa | 499 |
| Molecular weight | 25444.09 | 20765.95 | 33468.73 | 50366.11 |
| Formula | $C_{1193}H_{1782}N_{278}O_{301}S_{19}$ | $C_{918}H_{1474}N_{290}O_{250}S_6$ | $C_{1504}H_{2317}N_{403}O_{452}S_6$ | $C_{2264}H_{3584}N_{606}O_{656}S_{18}$ |
| Theoretical pI | 8.41 | 11.46 | 8.67 | 9.63 |
| Instability index | 58.50 (unstable) | 54.59 (unstable) | 33.21 (stable) | 30.68 (stable) |
| Estimated half-life (*Escherichia coli*, invivo) | > 10 h | > 10 h | > 10 h | > 10 h |
| Aliphatic index | 97.88 | 71.52 | 33.21 | 92.14 |
| (GRAVY) | 0.577 | -0.524 | 74.57 | 0.337 |

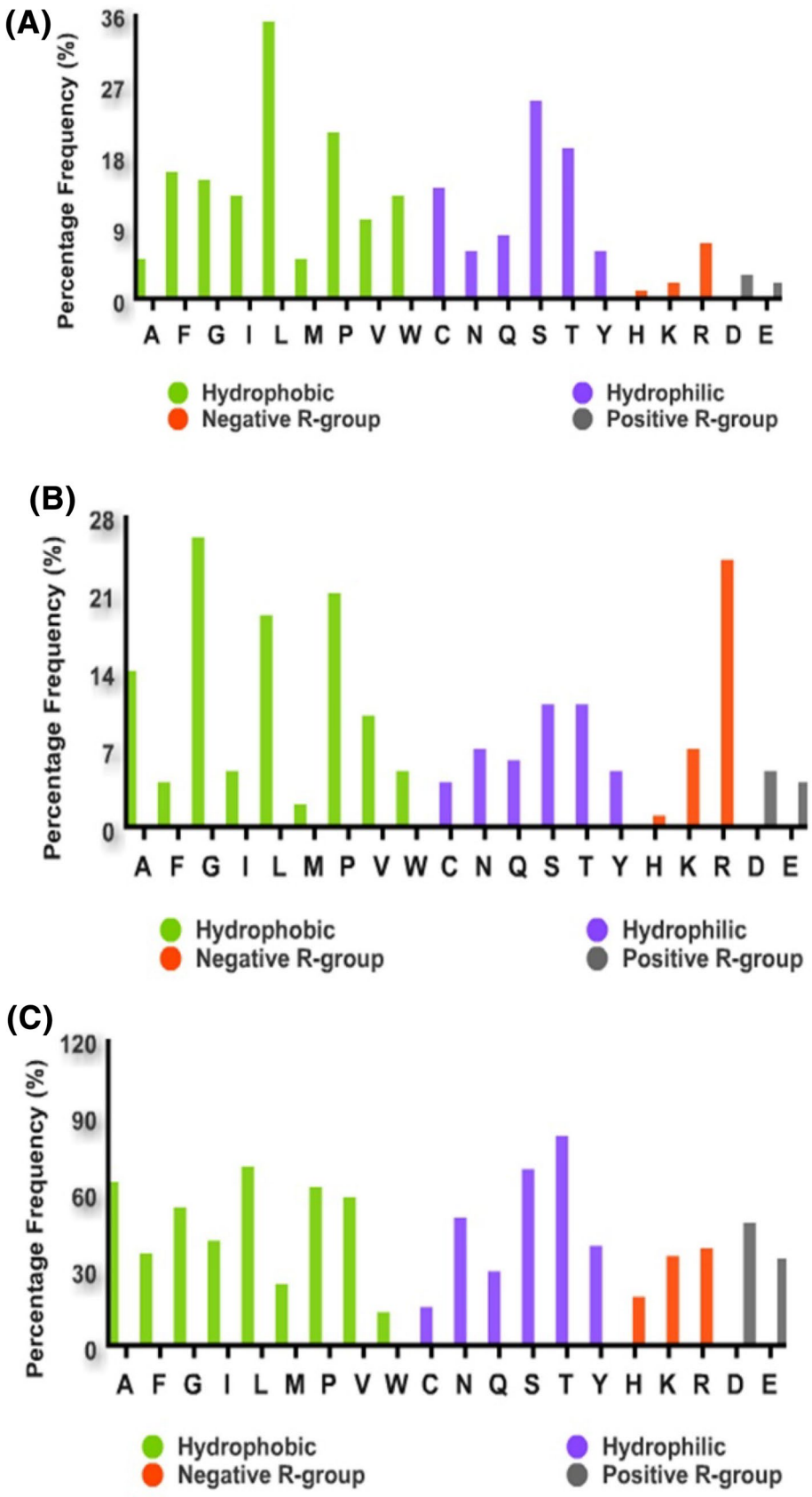


**Fig. 1** A schema of amino acid distribution of Ag candidates using lrrfinder server. **a** HBsAg, **b** HCV core protein and **c** Polio VPs

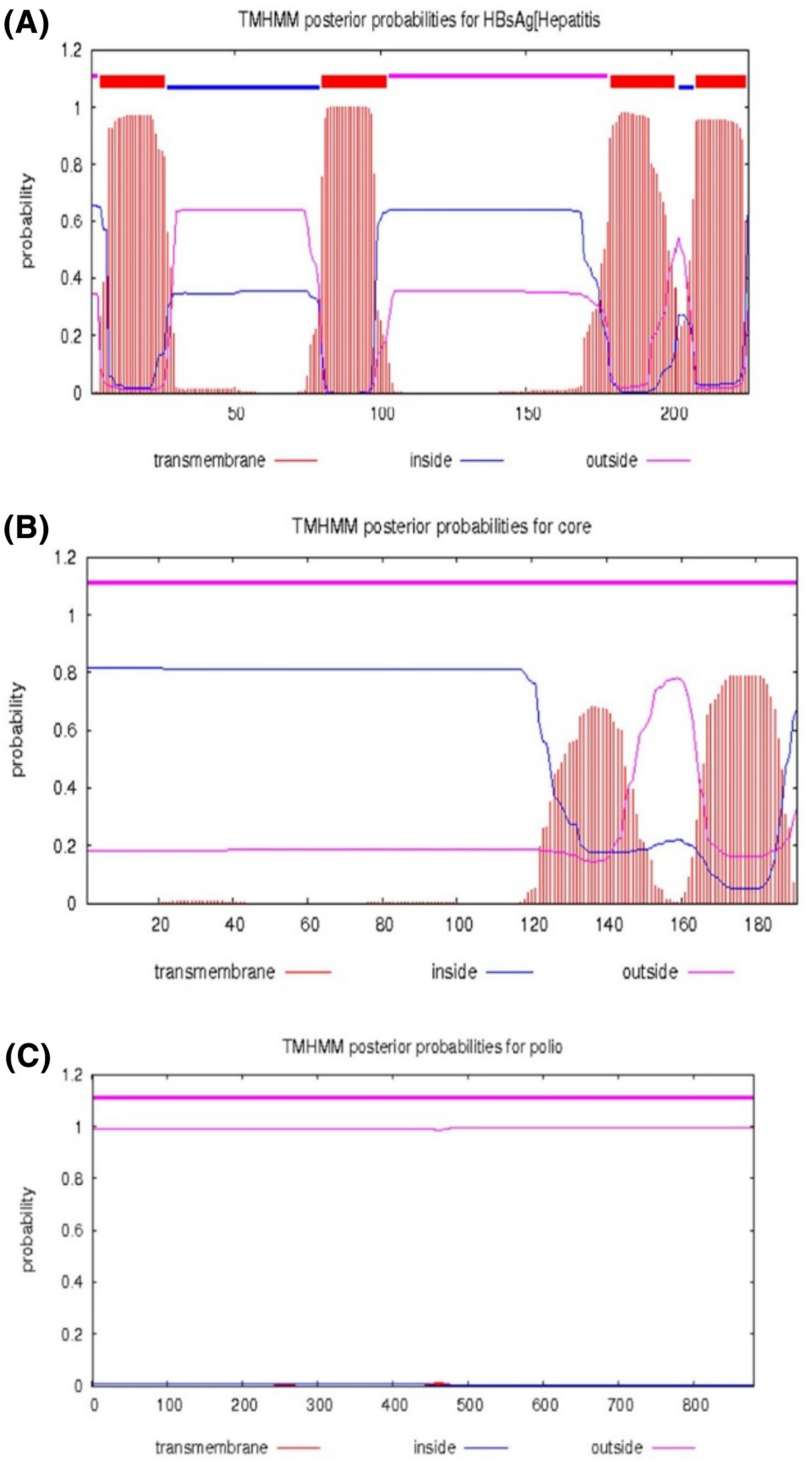


**Fig. 2** A schema of trans-membrane structure prediction of Ag candidates using TMHMM software. **a** HBsAg, **b** HCV core and **c** Polio VPs

**Table 2** The selected T-cell epitopes in design of HHP poly-epitope

| Protein | Start–end position | Epitope sequence |
|---|---|---|
| HBsAg | 14–28 | VLQAGFFLLTRILTI |
| | 81–89 | IIFLFILLL |
| | 90–98 | CLIFLLVLL |
| | 172–184 | WLSLLVPFVQWFV |
| | 208–226 | ILSPFLPLLPIFFCLWVYI |
| HCV cp | 29–36 | QIVGGVYL |
| | 37–45 | LPRRGPRLG |
| | 118–126 | NLGKVIDTL |
| | 132–148 | DLMGYIPLVGAPLGGAA |
| | 173–189 | SFSIFLLALLSCLTV |
| Polio Vp4,2,3 | 59–67 | VLIKTAPML |
| | 168–176 | YLGRSGYTV |
| | 247–255 | YLLGNGTLL |
| | 276–284 | LVLPYVNSL |
| | 464–472 | MMATGKLLV |
| | 499–507 | GLQSSCTMV |
| | 563–571 | RLLRDTTHI |
| Polio Vp1 | 579–587 | GLGQMLESM |
| | 617–625 | KEIPALTAV |
| | 699–716 | KLEFFTYSRFDMEFTFVV |
| | 762–774 | FYTYGTAPARISV |
| | 792–802 | KVPLKDQSAAL |
| | 811–819 | SLNDFGILA |
| | 838–846 | YLKPKHIRV |

3.5 h within mammalian reticulocytes (*In vitro*) and > 10 h within Escherichia coli (*In vivo*). The aliphatic index and instability index was defined to be 92.14 and 30.68, respectively. These results indicate that the HHP is a stable protein (Table 1).

### Antigenicity and Allergenicity

Based on the Vaxijen software, the antigenicity of the HHP construct with a threshold of 0.4 was predicted to be 0.4537 and 0.323% by ANTIGENpro. These results indicate that the HHP protein probably is antigenic. The AllerTope results showed that the HHP protein is probably non-allergen. Moreover, based on the Algpred server results this protein is a non-allergen with the score of -1.414.

### The HHP secondary structure

The prediction of secondary structure by SOPMA and Jpred 4 indicated that the HHP protein is consists of 19.24% α-helix (H), 26.25% extended strand (E), 16.03% beta turn (T) and 38.48% random coil (C) elements (Fig. 3). The high ratio of random coils and extended strands in the structure of HHP indicate that the designed protein is probably capable to form antigenic epitopes.

### HHP tertiary structure and refinement

The phyre and I-TASSER servers have returned one and five 3D models of the designed protein, respectively. Between the five models that were presented by I-TASSER, a model with the highest C-score (-0.77) was selected as the best model. It is worth noting the C-score is usually within the range of (McMahon 2014; McLauchlan 2000), the higher C-scores are associated with high confidence and vice-versa. The higher quality of the selected model was confirmed by QMEAN results. The ProSA result indicated that the selected model did not appear within the range of native proteins of similar size and needs to be refined. The selected primary model was refined and energy minimized. The model refinement and energy minimization run have improved the quality of the selected 3D model.

### HHP tertiary structure validation

To validate the quality of the refined model, several online validation methods like Ramachandran plot, Molprobity, ProSA and verify-3D were used. Ramachandran plot assessment of the primary model showed that 68.4%, 19.9%, and 11.7% of the residues were placed in the favored, allowed, and outlier regions, respectively (Fig. 4a). The refinement run have changed these ration to 86.9%, 10.7%, and 2.4%, respectively (Fig. 4b). The obtained results suggest that the refinement run have relocated most of the amino acids within the allowed area.

Evaluation of the model by MolProbity indicated that 86.32% and 2.82% of all residues in the refined model as well as 66.20% and 13.08% of all residues in the initial model were in Ramachandran favored and Ramachandran outliers regions, respectively (results are not shown). To further validate the quality of the 3D model before and

**Table 3** The selected B-cell linear epitopes in design of HHP poly-epitope

| Protein | Start–end position | Epitope sequence |
|---|---|---|
| HBsAg | 109–136 | LIPGSSTTSTGPCRTCMTTAQGTSMYPS |
| HCV cp | 61–90 | RRQPIPKARRPEGRTWAQPGYPWPLYGNEG |
| Polio Vp4,2,3 | 422–435 | ILCLSLSPASDPRL |
| Polio Vp1 | 849–881 | PRPPRAVAYYGPGVDYKDGTLTPLSTKDLTT |

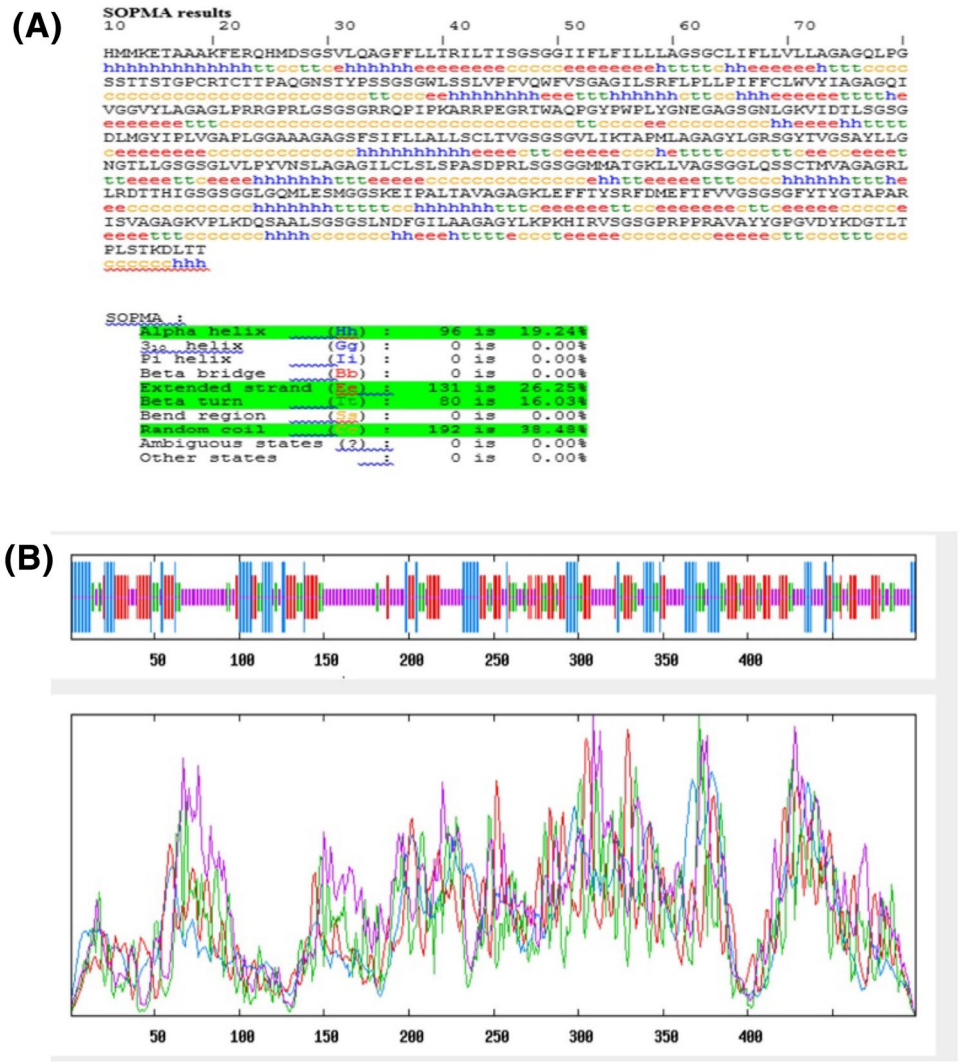


**Fig. 3** **a** The predicted secondary structure of the HHP using SOPMA software. H: Alpha helix, E: Extended strand, T: Beta-turn and C: Random coil, **b** The graphical representation of secondary elements in HHP protein (blue: alpha helix, red: extended strand, green: beta turn, yellow: random coil)

after refinement run, ProSA Z-score assessments was also considered in our study. The ProSA Z-score value for the initial model (Fig. 5a) and the refined model (Fig. 5b) was − 1.43 and − 4.93, respectively. As depicted in the Fig. 5 the structure is now more close to the range of native proteins of similar size. Accordingly, the energy plot also indicated that in the refined model most of the residues had negative values (Fig. 6). In addition, the quality of the 3D structure was assessed using Verify-3D tool. As shown in Fig. 7, 60.52% and 90.18% of the residues had a score over 0.2 in the initial model (A) and refined model (B) respectively; As validate the quality of the refined model. Generally, all of the validation assessments indicate the acceptable quality of refined model. The structure of the HHP protein is visualized in Fig. 8.

## Cleavage site

NetChop and MAPP servers have predicted 176 cleavage sites for HHP poly-epitope protein (date not shown). The HHP TAP binding affinity results, predicted by TAPPred based on SVM quantitative method, are listed in Table 4.

## HHP protein construction

The HHP gene fragment was 1500 bp in length including linkers. Then, the gene was synthesized chemically and cloned in suitable expression vector to downstream analysis. In Fig. 9 a representation of HHP sequence arrangement is shown; Linker motifs are shown with underlines.

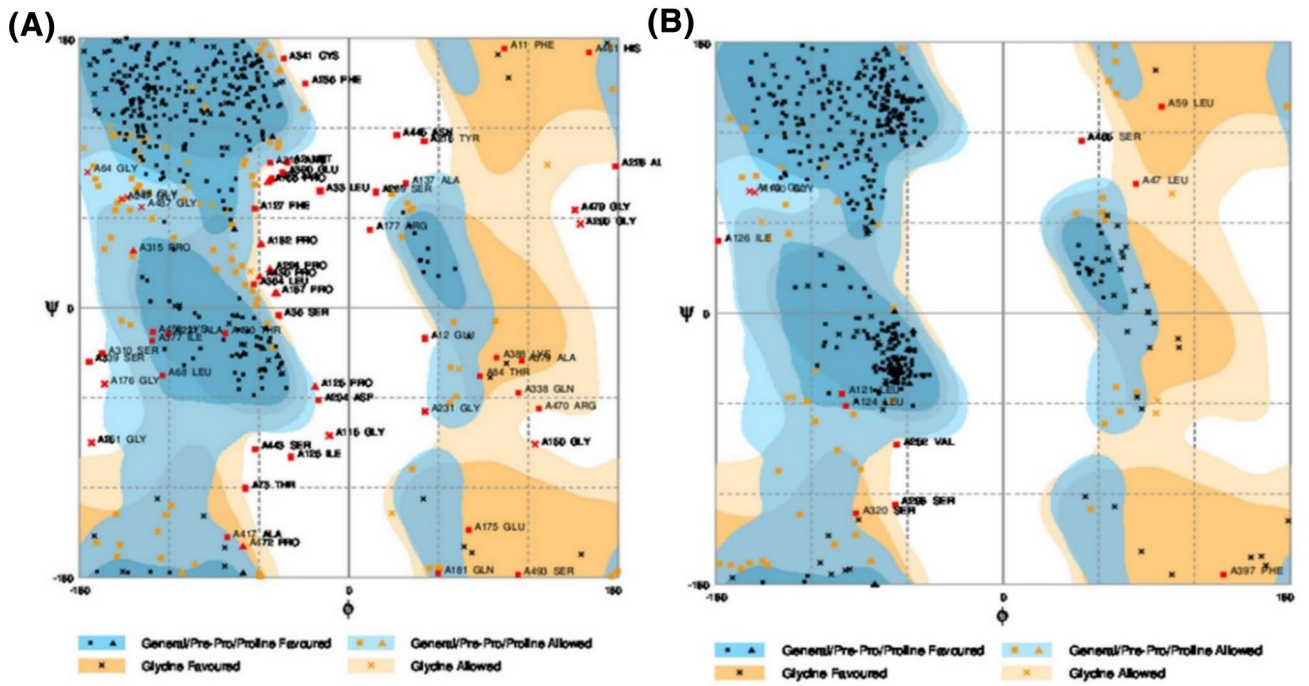


**Fig. 4** The validation of 3D protein model, using Ramachandran plot. **a** The initial model **b** The refined model

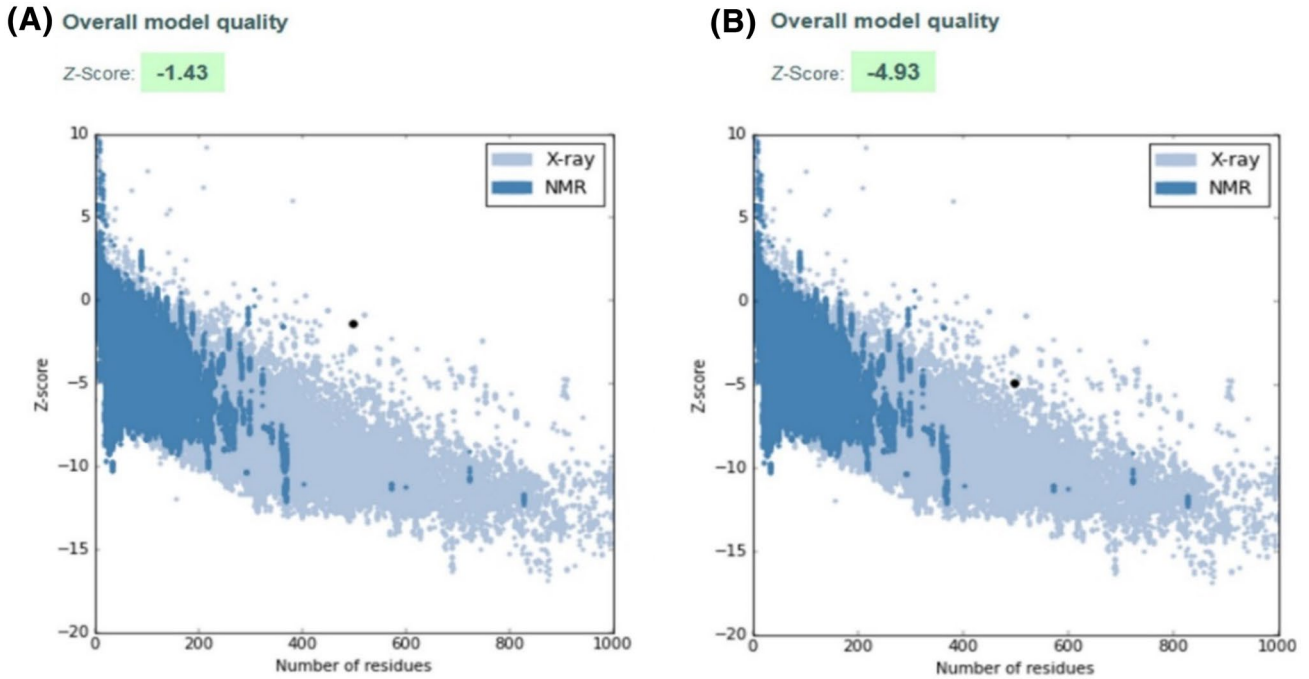


**Fig. 5** The z-Score plots for the 3D structure of construct. The z-score of **a** the initial model is −1.43 that is not in range of native protein conformation and **b** The z-score of a model after refinement processes is −4.93, which is in the range of native protein conformation. The z-Score plot contains z-scores of all experimentally protein chains in PDB determined by NMR spectroscopy (dark blue) and X-ray crystallography (light blue). The plot shows results with a z-score ≤ 10. The z-score of the protein is presented in large black dot

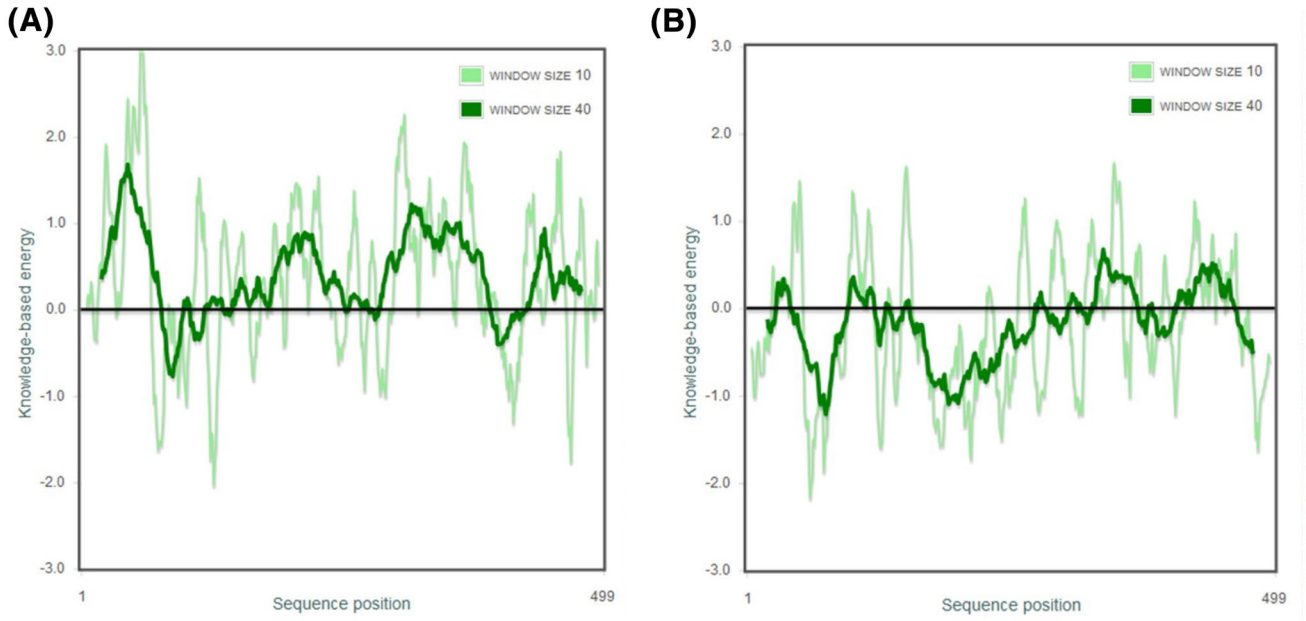


**Fig. 6** Energy plots of initial model (**a**) and refined model (**b**) obtained by ProSA server. As shown, most of the residues have negative values for the refined model

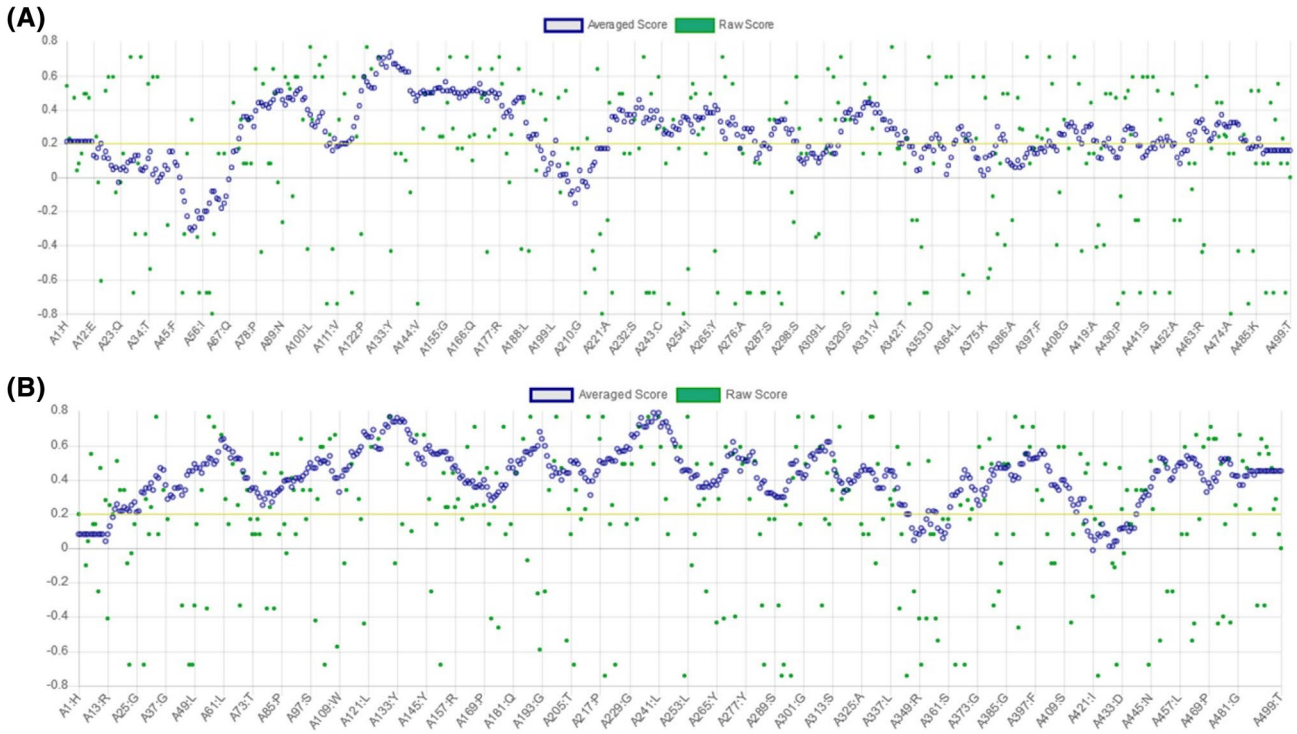


**Fig. 7** Evaluation of the quality of the 3D structures by Verify-3D tool. As shown, 60.52 and 90.18% of the residues in the initial model (**a**) and refined model (**b**) respectively have a score over 0.2

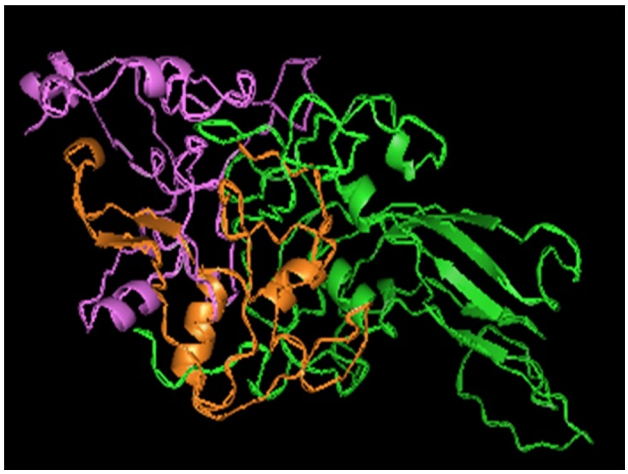

**Fig. 8** Representation of the modeled structure using (The PYMOL Molecular Graphics System). The parts in purple, orange and green color respectively show the epitopes related to HBsAg, HCV cp, and Polio $Vp_{1-4}$

**Table 4** The prediction of binding affinity of TAP binder by TAPPred in HHP poly-epitope protein

| Peptide rank | Start position | Sequence | Score | Predicted affinity |
|---|---|---|---|---|
| 1 | 395 | SRFDMEFTF | 10.337 | High |
| 2 | 389 | LEFFTYSRF | 8.483 | High |
| 3 | 118 | SRFLPLLPI | 8.244 | High |
| 4 | 476 | AYYGPGVDY | 7.982 | High |
| 5 | 171 | ARRPEGRTW | 6.969 | High |
| 6 | 164 | RRQPIPKAR | 6.601 | High |
| 7 | 125 | PIFFCLWVY | 6.596 | High |
| 8 | 299 | LAGAGILCL | 6.242 | High |
| 9 | 263 | AGYLGRSGY | 6.242 | High |
| 10 | 126 | IFFCLWVYI | 6.112 | High |
| 11 | 24 | AGFFLLTRI | 6.084 | High |

## Discussion

Despite years of relentless efforts and worldwide programs in fight against the HBV and Polio infections, they still continue to be ongoing human health challenges. Moreover, contrary to great success, broadly effective vaccines have not been developed for HCV. Utilization of multi B- and T-cell epitope based vaccines due to affordable production, safety, constancy and specificity is promising approach in control of various infections such as HCV (Nezafat et al. 2014). On the other hand, polyvalent vaccines because of simultaneous induction of humoral and cellular immune responses against several target antigens, reported to be more effectual than monovalent ones.

In this regard, herein harnessing bioinformatics tools and in silico analyses, three antigenic determinants from HBV (surface antigen), HCV (core protein), and Poliovirus (VPs) were considered to design a poly-epitope protein (HHP) as a polyvalent vaccine candidate capable of stimulating both humoral and cellular immune responses against these infections.

We have included the HBsAg within the sequence of the designed HHP protein. Aside from its ability to elicit HBV specific immune responses, inclusion of HBsAg can act as an amenable adjuvant for antigens of other pathogens. This antigen could also increase the efficiency of the target antigens to induce dendritic cells and elicit enhanced cellular and humoral responses (Guillen et al. 2010).

Yazdanian et al. (2015), have investigated the efficacy of HBsAg fusion to the HCVcp (amino acids 2-122) in the context of a chimeric DNA vaccine to enhance cellular response against HCV. Their results have indicated that the HBsAg fusion to HCVcp could lead to augmented Th1oriented cellular and CTL responses toward a protective epitope, comparable to that of HCVcp (subunit HCV vaccine) immunization (Yazdanian et al. 2015). In addition, similar studies have been performed to find out the potential antigens or co-vaccination agents as well as modeling of those selected epitopes for the utility as an HCV vaccine (Memarnejadian and Roohvand 2010; Patient et al. 2009; He et al. 2015).

Several studies have discussed the viral interaction and coinfection between HCV and HBV as a result of the common routes of transmission (Ende et al. 2015; Papadopoulos et al. 2018). In comparison to HBV-monoinfections, higher rates of cirrhosis (44% vs. 21%) and severe liver disease (24% vs. 6%) occurs in coinfection cases (Fong et al. 1991). Moreover, compared to HCV-monoinfections, a higher rate of cirrhosis (95% vs. 49%) was reported in HBV/

**Fig. 9** A representation of HHP sequence arrangement; Linker motifs are shown with underlines

**>HHP poly-epitope**

HMMKETAAAKFERQHMDSGSVLQAGFFLLTRILTISGSGGIIFLFILLLAGSGCLIFLLVLLAGAGQLPGSSTTSTGPCRTCTTPAQGN
STYPSSGSGWLSSLVPFVQWFVSGAGILSRFLPLLPIFFCLWVYIAGAGQIVGGVYLAGAGLPRRGPRLGSGSGRRQPIPKARRPEGR
TWAQPGYPWPLYGNEGAGSGNLGKVIDTLSGSGDLMGYIPLVGAPLGGAAAGAGSFSIFLLALLSCLTVGSGSGVLIKTAPMLAGA
GYLGRSGYTVGSAYLLGNGTLLGSGSGLVLPYVNSLAGAGILCLSLSPASDPRLSGSGGMMATGKLLVAGSGGLQSSCTMVAGAGR
LLRDTTHIGSGSGGLGQMLESMGGSKEIPALTAVAGAGKLEFFTYSRFDMEFTFVVGSGSGFYTYGTAPARISVAGAGKVPLKDQSA
ALSGSGSLNDFGILAAGAGYLKPKHIRVSGSGPRPPRAVAYYGPGVDYKDGTLTPLSTKDLTT

HCV-coinfections (Mohamed and Mesa 1997; Konstantinou and Deutsch 2015).

Furthermore, to avoid the problem of Polio vaccines in creating VDPVs, the immune epitopes of Poliovirus VP antigens were considered as the third component of the polyvalent HHP protein.

Delpeyroux et al., in order to assess the HBsAg ability to present foreign peptides, have inserted a sequence representing the amino acid residues 93–103 of capsid protein VP1 of poliovirus type 1 into the HBsAg. They have shown that expressed particles (HBsPolioAg) reacted with a poliovirus-specific monoclonal antibody, and could induce neutralizing antibodies against poliovirus and HBsAg in mice and rabbits. Their results have suggested the ability of viral envelopes HBV for presentation of peptide sequences in a biologically active form (Delpeyroux et al. 1986, 1988).

we have designed the HHP poly-epitope protein containing the linear B-cell epitopes, as well as the high scored CTL and HTL epitopes that overlap with each other. The coupling between B- and T-cell epitopes could invoke a desired increase in effectiveness of the protein. In agreement with this propose, recent insilico studies suggested the importance of both B- and T-cell epitopes to stimulate both arm of the immune system and enhanced immunogenicity of designed vaccines (Chauhan et al. 2019; Narula et al. 2018; Ali et al. 2017).

It is noteworthy that linkers could have an impact on the function and structure of poly-epitope vaccines, prevent the production of junctional epitopes (neo-epitopes) which could lead to the production of new proteins with new features as an important concern in designing epitope vaccines and facilitate the epitopes presentation and immune processing (Bai and Shen 2006). In this strategy, the final HHP protein has been designed encompassing the flexible Glycine-Serine-rich linkers between the selected epitopes that employed to connect various domains in a single protein without interfering with the function of each domain (Reddy Chichili et al. 2013). The ideal length of protein promises its presentation by DCs and induction of a robust immune response. Peptides shorter than 30 aa may be directly associated with nonprofessional APCs resulting in the stimulation of tolerance or anergy (Melief and Burg 2008). Inclusion of linker sequences has raised the poly peptide length up to 500 amino acids which is a desired length for a poly-epitope vaccine.

The physicochemical properties of the proteins play pivotal roles in various biological designs. These properties have been calculated within various in silico studies (Khatoon et al. 2017; Mard-Soltani et al. 2018). ProtParam has been used to determine the physiochemical features of the HHP protein. The computed pI value was 9.63 revealing the basic nature of the fusion protein. The instability index categorized the protein as stable. The value of aliphatic index for HHP protein was 92.14, which suggested its thermostability. The GRAVY index indicated the hydrophobicity of the peptide that describes the location of the hydrophilic residues in the amino acid sequence of the protein. The GRAVY of HHP was 0.337 which is close to 0. This property showed that hydrophilic residues could be placed on the protein surface and they are suitable for ligand binding.

The secondary structure of the predicted structure have been used to confirm the predicted 3D structure of the final antigen (Ganji et al. 2019). The secondary structure analysis revealed higher random coil content in the construct. Random coils play an important role in the high flexibility of proteins. The high coil structural percentage of the designed HHP (38.48%) was due to the high content of Glycine-rich linker sequences. The role of Glycine-rich residue in protein flexibility has been reported previously (Wriggers et al. 2005). The flexibility parameter indicates the folding valency of the protein. More flexible proteins are capable of better bending and folding into correct secondary and tertiary structures (Pan et al. 2017). Our analyses have indicated that the selected epitopes possess proper flexibility and stability which could bring about better folding of the final protein.

The tertiary structure details of proteins were of major importance in providing insights into their molecular functions. The initial 3D structure of HHP protein was modeled by I-TASSER and the best model was chosen. The evaluation of the initial model with different tools like ProSA-web and Ramachandran plot showed that the model needs the refinement process. A full atomic refinement process have already been employed for predicted protein models (Chauhan et al. 2019; Ali et al. 2017). We have adapted similar method for our model refinement. Kazemi et al. (2018) have used Ramachandran plot, Molprobity, ProSA and verify-3D to assess the quality of initial and final 3D structure. We have employed the same approach to verify the quality of the obtained structures. The performed evaluations have validated the quality improvement of HHP 3D model after refinements.

Proteasome cleavage site analysis of the protein construct was performed using Net Chop server. The predictions were based on neural network training strategy using C-term 3.0 method. The results have shown that 176 high-scored cleavage positions are located throughout the whole HHP protein (Data not shown).

The immune-stimulatory effect of designed protein needs to be investigated in future studies.

Overall, in this study employing various immunoinformatic tools, we have tried to design an effective poly-epitope vaccine candidate. The computational results indicated that the designed HHP protein is capable of simultaneous induction of immune responses against three viral diseases. It was also predicted to be immunogenic and non-allergen. We

expect that the designed HHP poly-epitope shows acceptable and hopeful results in the next *ex-vivo* step of the research.

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## Compliance with Ethical Standards

**Conflict of interest** The Authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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