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VIRTUAL SCREENING OF ANTIVIRAL PEPTIDES AS NOVEL BLOCKERS OF HUMAN PAPILLOMAVIRUS 16

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Human papillomaviruses (HPVs) contribute to 5% of cancers, yet there is a lack of specific antiviral agents targeting HPV infection. Antiviral peptides (AVPs) present a promising alternative to conventional therapeutics. This study aims to explore the use of AVPs against the HPV16 E6 oncoprotein through virtual screening. The potential binding pocket of the E6 oncoprotein was determined, and using the antimicrobial CAMPR4 database 18 AVPs were shortlisted. These AVPs were then docked to the E6 oncoprotein using the HawkDock server, followed by dynamic simulation. Among the AVPs tested, AVP18, AVP10, and AVP7 demonstrated the highest inhibitory potential against the E6 oncoprotein. AVP18 exhibited more non-bonded contacts, hydrogen bonds, and electrostatic forces. Dynamics simulation confirmed the stability of the complexes formed by these top AVPs with E6. This research suggests that AVP7, AVP10, and AVP18 are promising lead candidates for blocking HPV16 by inhibiting the E6 oncoprotein.

K e y w o r d s: human papillomavirus, E6 oncoprotein, antiviral peptides, docking, dynamics simulation.

Sexually transmitted infections (STIs) are a significant contributor to morbidity and mortality worldwide [1], affecting approximately 50-70% of sexually active individuals [2]. The human papillomavirus (HPV) is the most prevalent STI, with a wide range of types (225) categorized into five classes: α, ß, γ, μ, and ν. HPV infections can be broadly classified into two categories based on their disease burden: low-risk and high-risk types [3] (Fig. 1). Low-risk HPV is associated with the development of benign lesions such as cutaneous and anogenital warts, while high-risk HPV is linked to the onset of oropharyngeal and anogenital cancers, including cervical and penile cancers [4,5]. HPV is responsible for nearly half of all malignancies caused by infections globally [6]. In most cases, HPV infections are cleared by the immune system or enter a dormant state within a year or two, but high-risk HPV-positive women are at risk of developing cervical cancer within 3-5 years after infection [7].

HPV is a small, non-enveloped virus approximately 60 nm in diameter, with a double-stranded DNA genome belonging to the Papillomaviridae family. Its circular DNA, about 7-8 kb in size, encodes 8 functional (Early E1-E8) and 2 structural (Late L1, L2) proteins, along with a non-coding long terminal region (LTR) [8]. The assembly of HPV involves pentameric (5 copies) L1 anchored to a monomer of L2, forming 72 capsomeres [9]. The L1 protein, the major capsid component weighing 55 kDa, contains both constant and variable regions crucial for surface antigenicity, host receptor interaction, and antibody generation [10]. This variability in L1 contributes to the diverse genotypes of HPV. L1 is a key target for therapeutics and vaccines due to its high-affinity binding domains in infected hosts, triggering the appropriate immune response, and its capacity to form large, immunogenic self-assemblies that are non-infectious but potent [11].

Currently, three HPV vaccines have been approved and demonstrated a reduction in the development and progression of cutaneous and anogenital warts and cancers. The first is the bivalent vaccine Cervarix, manufactured by GSK, which targets HPV types 16 and 18, which account for approximately 70% of cervical cancers [12]. The second is the quadrivalent vaccine Gardasil, produced by Merck, which protects against HPV types 6 and 11, which cause 90% of genital warts, in addition to types 16 and 18. The third is the 9-valent vaccine Gardasil9, also manufactured by Merck, which provides immunity against the previous four types as well as HPV

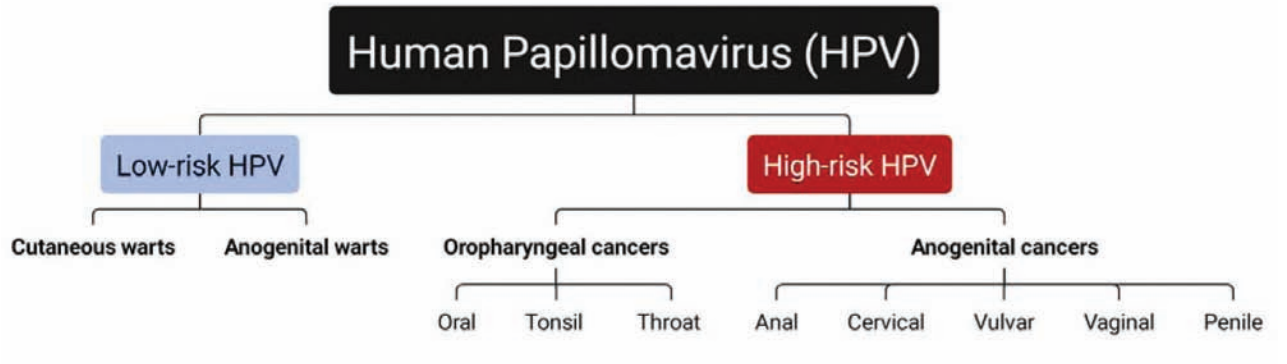

Fig. 1. Classification of HPV viral types based upon severity

types 31, 33, 45, 52, and 58, which are implicated in 18% of invasive cervical cancers [13, 14].

The application of these vaccines has significantly reduced the incidence of cervical cancers due to HPV infection in countries like Australia and Luxembourg [15,16]. However, in Sweden, the cumulative incidence of cervical cancer was 47 cases per 100,000 persons among vaccinated women and 94 cases per 100,000 persons among unvaccinated women [17]. While the currently approved vaccines have proven effective in reducing the incidence of HPV-positive cases, further improvements are needed to enhance their efficacy and mitigate any negative side effects, as some reports have indicated that the HPV vaccine may be more immunogenic than the virus itself [18].

Antiviral peptides (AVPs) are a promising class of therapeutic agents that have demonstrated potent and broad-spectrum antiviral activity against a variety of viruses, including SARS-CoV-2, influenza, and HIV. These short polycationic peptides can inhibit viral infection and replication through diverse mechanisms of action, such as disrupting viral envelopes, blocking virus-host cell interactions, and modulating host immune responses [19–21]

To the best of my knowledge, this is the first study to explore the potential of AVPs against HPV16 E6 oncoprotein via structural bioinformatics tools.

Materials and Methods

To achieve the goal of this *in silico* study, multiple successive steps and tools were used as illustrated in Fig. 2. Nonetheless, the details of each step are provided in the text below.

Receptor pre-processing. The crystal architecture of HPV16 E6 oncoprotein was fetched from the protein data bank (PDB ID: 6SJA). The crystal structure was resolved through x-ray diffraction with a resolution of 1.50 Å. Only chain B (for HPV16 E6 oncoprotein) was used (Fig. 3); otherwise, all other chains and heteroatoms were eliminated. The receptor was prepared by assigning Gasteiger charge along with polar hydrogen addition.

Prediction of the binding pocket. Before conducting molecular docking, the potential binding pocket of the HPV16 E6 oncoprotein should be predicted. Dogsite 3 [22] web portal was utilized for this purpose, and the most suggested pocket was considered the binding pocket.

Antiviral peptides selection. The antimicrobial database CAMPR4 [23] was selected for the retrieval of antiviral peptides. The retrieved peptides followed strict criteria for successful selection: (i) Antiviral peptides, (ii) against human target, (iii) 3D architecture is available in PDB, and (iv) having $\leq$ 50 amino acids. Upon applying these criteria, 18 AVPs

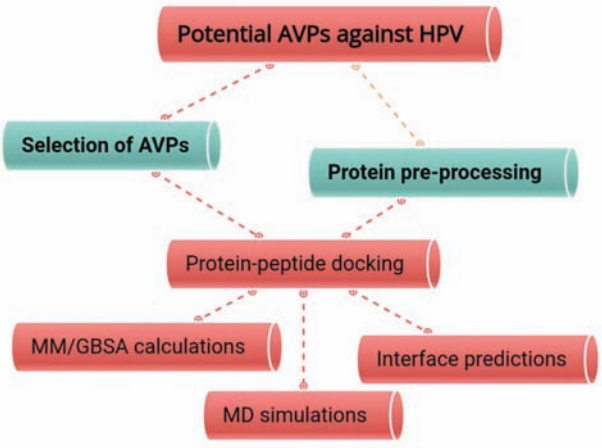

Fig. 2. General workflow employed to achieve the goal of this study

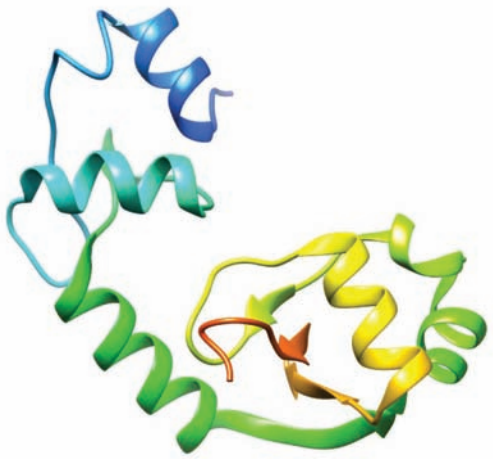

Fig. 3. HPV 16 E6 oncoprotein retrieved from PDB ID: 6SJA and colored from blue (N-terminal) to orange (C-terminal)

were obtained and chosen for further analysis. The selected AVPs were then processed the same way as the receptor (see section Receptor pre-processing).

Protein docking. To decipher the binding mode and pose of the selected AVP to the HPV16 E6 oncoprotein, HawkDock server [24] was deployed. This server is unique in its fast, accurate algorithm for docking peptides and proteins, besides the capability to perform free energy of binding (MM/GBSA) calculation. The server conducts a rigid-body docking approach of the ATTRACT algorithm, generating 10 models for each AVP-Protein pair, with optional constraints. In the present study, only the best models with the highest binding affinity into the active pocket were then selected for further free energy of binding decomposition analysis (MM/GBSA).

Protein-peptide interaction visualization. The best 3 AVPs which scored top in the molecular docking step have been considered for exploration of the type of interaction and the corresponding pose in the active site of the protein. This was accomplished by the PDBsum tool [25].

Molecular dynamics simulation. Molecular dynamics simulation (MDS) of the best 3 AVPs was conducted by the CABS-Flex 2.0 server, which depends on the coarse-grained motions of the uploaded protein [26]. Over 50 cycles and 50 trajectory frames within 10 ns each with some additional distance restraints, including a global weight of 1.0, were applied. Root-mean square fluctuations (RMSF) were used to express the complex motion.

The CABS-flex 2.0 server allows simulations of large protein systems, including multimeric proteins, and provides customizable simulation parameters such as temperature, simulation length, and distance restraints. This enables tailoring the simulations to specific requirements, such as modeling proteins with disordered regions or flexible loops.

Results

Active pocket prediction. Utilizing Dogsite 3, the active pocket of the E6 oncoprotein was determined and illustrated in Fig. 4. The determined pocket has a volume of 112.64 $Å^3$, a surface of 272.64 $Å^2$, and a depth of 7.92 Å. Accordingly, this pocket was chosen for the docking of AVPs.

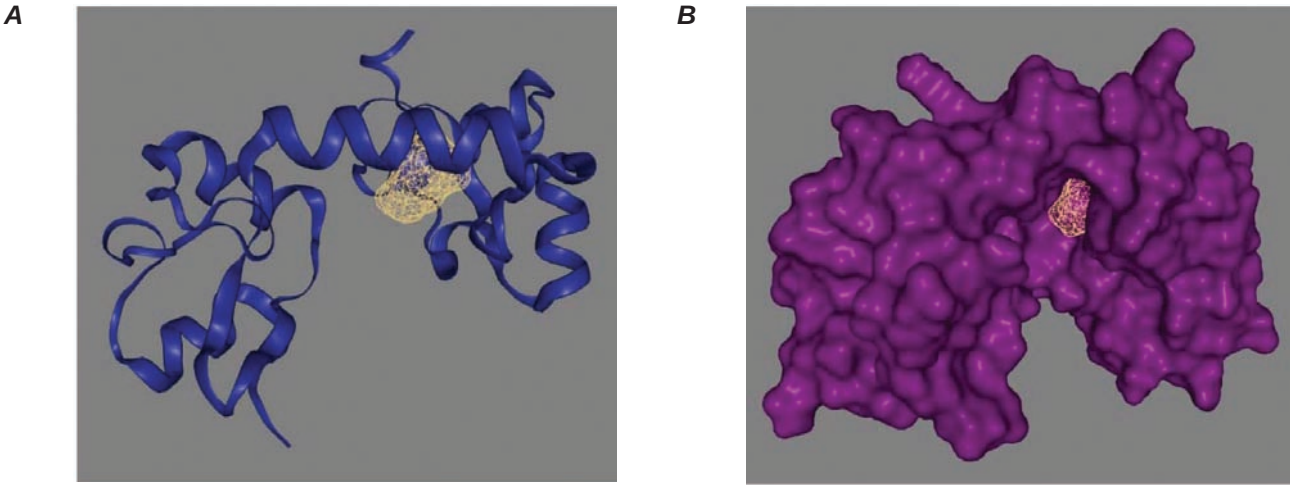

*Fig. 4. Prediction of active pocket of E6 oncoprotein by using Dogsite 3 web server shown in cartoon (**A**) and surface (**B**)*

Protein docking. The results of protein-peptide docking using the HawkDock server showed a great variance in terms of a docking score since the mean value was -3644.41 kcal/mol (in the range -5451.12 and -2619.89 kcal/mol) as provided in Table 1. Human alpha-defensin 1 (multiple Arg-Lys mutant) ranked first with docking score of -5451.12 kcal/mol, followed by kalata B8 (-4525.78 kcal/mol) and leaf-specific-expressed cyclotide vhl-1 (-4502.22 kcal/mol). These AVPs were designated as AVP 18, AVP10 and AVP7, respectively. These top 3 AVPs were analyzed for MDS, interface interactions and MM/GBSA calculation.

Interface interactions. The molecular docking of the top 3 AVPs revealed different patterns of binding. In other words, albeit all of them bound tightly to the active pocket of the E6 oncoprotein, AVP18 showed maximal coverage of the active pocket which account for the higher docking energy compared to the other AVPs. This is evident from the surface as well as the cartoon view of the top 3 AVPs with E6 oncoprotein in Fig. 5.

E6-AVP7 complex had 13 and 9 interface residues forming 79 non-bounded contacts and 1 H-bonds. Similarly, E6-AVP10 complex had 8 and 7 interface residues forming 61 non-bounded contacts and 1 H-bond. 21 and 16 interface residues between E6 and AVP18 forming 190 non-bounded contacts, 1 electrostatic force and 4 H-bonds. This emphasizes the superiority of AVP18 as a lead candidate when compared with AVP7 and AVP10. The interface residues of the top 3 AVPs are elucidated in Fig. 6.

MDS. After investigating the molecular docking and interface interactions, MDS of the top 3 AVPs were conducted. The mean RMSF of E6-AVP1 complex was 1.04 Å. Marked fluctuations were displayed in the regions 1094-1096 (5.74 Å) and 1136-1141 (5.08 Å) (Fig. 7). Concerning E6-AVP10, a mean value of 1.052 Å was obtained. The most prominent RMSF values were obtained in positions 1007-1010 with 4.483 Å and 1094-1096 with 3.797 Å. In addition to the E6 protein, the AVP10 exhibited marked fluctuations, particularly in positions 1 (3.998 Å) and 30 (3.57 Å). The AVP18 displayed the highest

Table 1. Molecular docking scores of all examined AVPs against E6 oncoprotein as outputted form HawkDock server

No.	PDB ID	Peptide	Docking score (kcal/mol)
1	1BDS	BDS-I from the sea anemone anemonia sulcata	-3647.03
2	1BH4	Circulin A from chassalia parviflora	-3121.95
3	1ID6	SYR6	-4076.30
4	1R1F	Cyclotide palicourein	-4118.87
5	1RPB	Tricyclic peptide active against HIV-1 virus	-3420.69
6	1W7Q	Feglymycin P65	-3162.40
7	1ZA8	leaf-specific-expressed cyclotide vhl-1	-4502.22
8	1ZMH	human neutrophil peptide 2	-3923.32
9	2ATG	Retrocyclin-2 in SDS	-2619.89
10	2B38	kalata B8	-4525.78
11	2DCX	Dermaseptin antimicrobial peptide analog	-2686.49
12	2EEM	synthetic mytilin	-3315.83
13	2KUK	vhl-2	-3934.70
14	2L6S	HIV-1 entry inhibitor targeting the GP41 fusion peptide	-2834.57
15	2LAM	cyclotide Cter M	-4175.27
16	2MXQ	DEFA1, antimicrobial peptide from the horse	-3194.16
17	2PM4	Human alpha-defensin 1	-5451.12
18	6M56	Peptide P9R	-2888.83

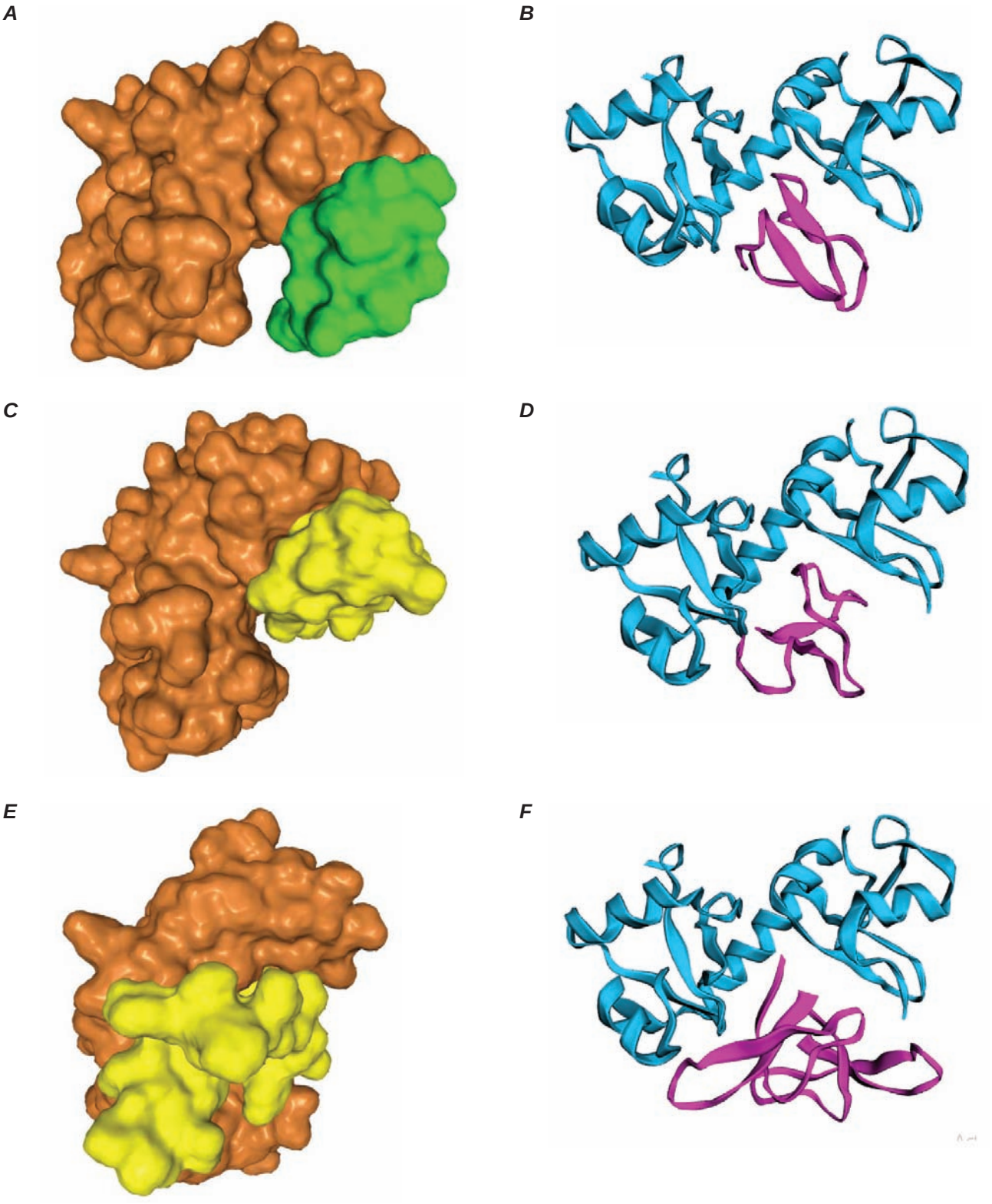

*Fig. 5. Three-dimensional visualization of AVP7, AVP10 and AVP18 in surface view (**A**, **C**, **E**) and in cartoon view (**B**, **D**, **F**)*

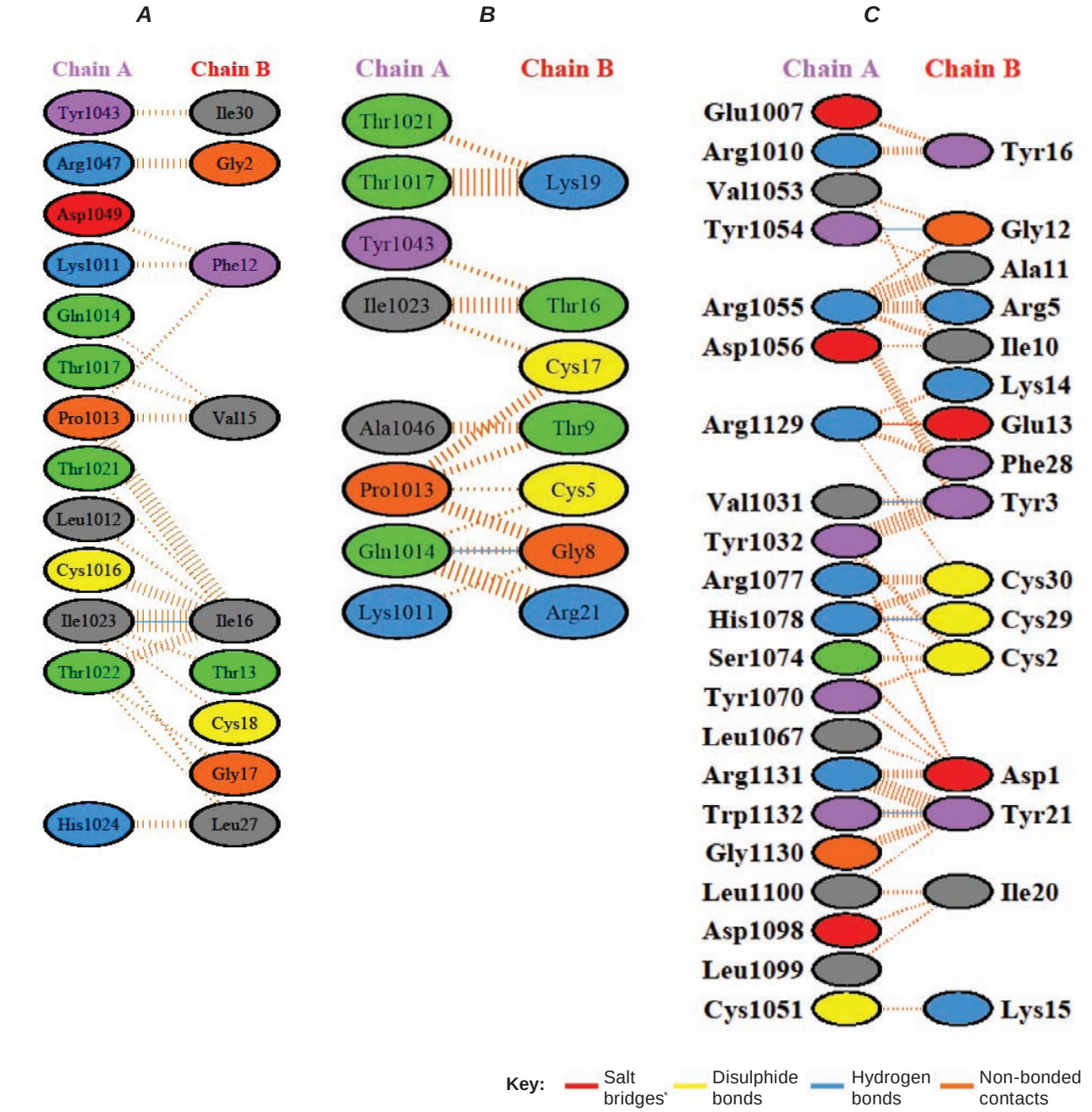

*Fig. 6. The determined interface residues of AVP7 (**A**), AVP10 (**B**) and AVP18 (**C**) with E6 oncoprotein*

RMSF among other AVPs since the mean of fluctuations was 1.08 Å. With respect to most fluctuated regions, 1094-1096 (8.49 Å) and 1136-1141 (4.288 Å) as in AVP7. These regions are responsible for binding the peptides in question. Based on the RMSF results, stability of E6-AVPs complexes was most seen in AVP7, followed by AVP10 and lastly AVP18. The superimposition of the MDS trajectories of all complexes was also shown in Fig. 7.

MM/GBSA. The trajectories of MDS were utilized for the computation of MM/GBSA to infer the free energy of binding of the top 3 AVPs. In line with MDS and RMSF findings, the AVP7 was ranked first with the total free binding energy of -51.61 kcal/mol. However, AVP18 and AVP10 ranked second and third with the free energy of -34.1 and -30.68 kcal/mol, as summarized in Table 2. Van der Waals (VDW) primarily contributed to the total free energy of binding.

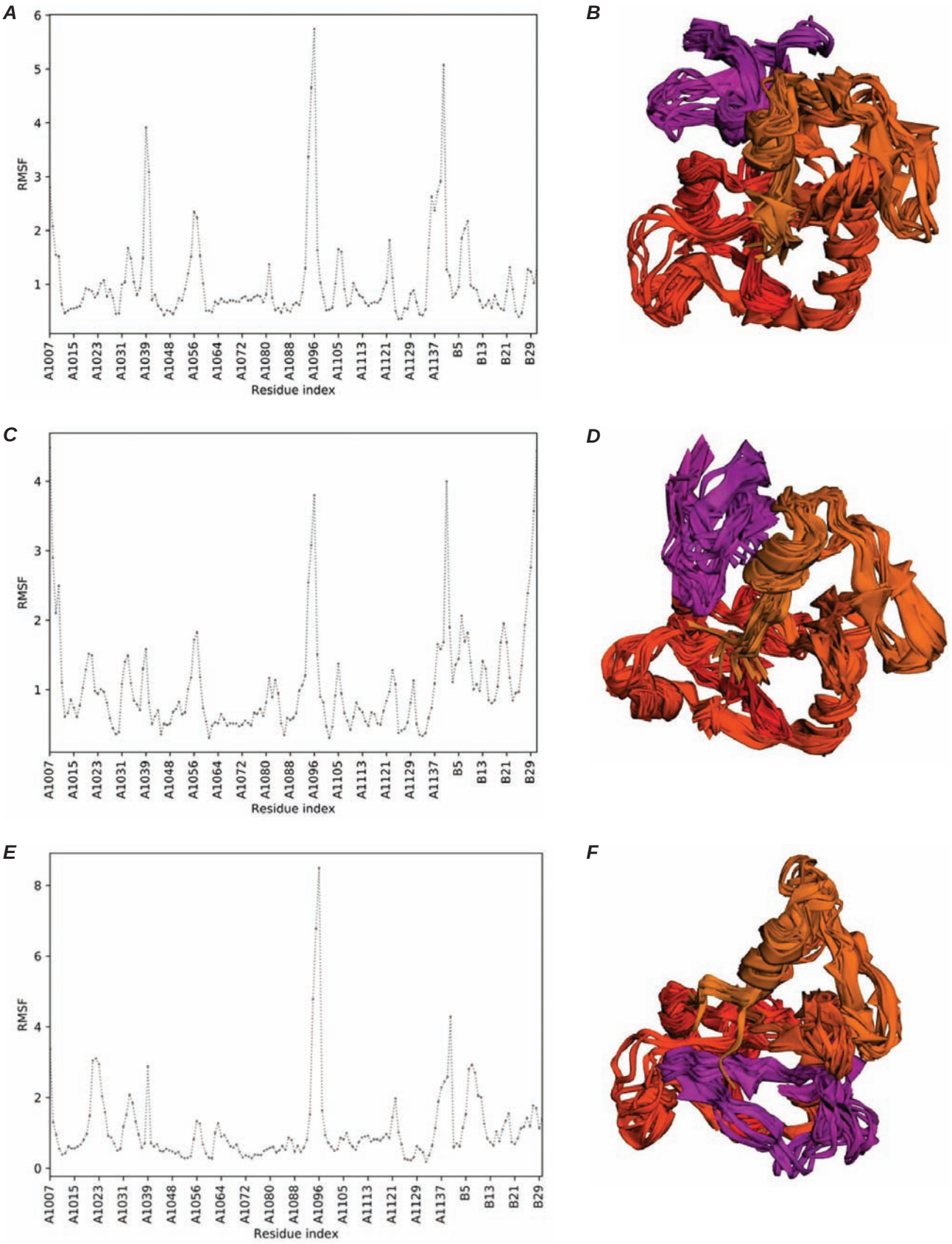

*Fig. 7. Molecular dynamics simulation output of AVP7, AVP10 and AVP18 (**A**, **C**, **E**) and the corresponding superimposition of the MDS trajectories (**B**, **D**, **F**)*

Table 2. Free energy of binding of the top 3 AVPs to E6 oncoprotein

Energy, kcal/mol	AVP7	AVP10	AVP18
VDW	-96.71	-94.08	-107.59
ELE	-244.08	17.58	195.04
GB	301.58	58.27	-107.21
SA	-12.39	-12.45	-14.33
TOTAL	-51.61	-30.68	-34.1

Note. VDW – van der Waals, ELE – electrostatics, GB – Polar Solvation free energies predicted by the Generalized Born model. SA – Nonpolar contribution to the solvation free energy calculated by an empirical model.

Discussion

High-risk HPV targets differentiating squamous epithelial tissues of digestive and urogenital systems, leading to corresponding cancer types, with uterine cervix cancer being the most common. Unsafe sexual relationships are a primary risk factor for viral transmission [27]. While sexual contact is a common mode of transmission, HPV has been found in the placental, blood, and reproductive cells of individuals without sexual activity [28]. Various HPV vaccines have been developed, tested, and proven effective in reducing HPV infections and associated cancers. However, existing vaccines do not completely eliminate the virus, and their efficacy may be lower in certain populations, including women and adults [29].

The development of effective AVPs involves identifying and validating their viral targets, which can be located on the virus or the host cell. Protein-protein interactions are crucial for many stages of the viral life cycle, making them attractive targets for AVP-based therapeutics [30]. Compared to traditional small-molecule drugs and biologics, AVPs offer advantages like high specificity, potent antiviral activity at low doses, and fewer adverse effects due to their natural or biological origins and susceptibility to host peptidases [31]. Rational design approaches, such as leveraging structural predictions and conserved AVP motifs, can aid in the discovery of lead peptides with enhanced therapeutic selectivity and potency [32]. Despite the promise of AVPs, challenges remain in addressing their sensitivity to proteolytic degradation and optimizing delivery systems to improve their bioavailability and targeting.

Therefore, the goal of this computational study was to test the potential of AVPs as possible inhibitors of E6 oncoprotein of HPV16.

The results of the present study found that many natural AVPs can block E6 oncoprotein as reflected by the molecular docking, interface interactions, MDS and MM/GBSA findings. Given that energy gained from electrostatic attractions was extremely high (-244.08 kcal/mol), AVP7 showed the strongest free energy in comparison with AVP10 and AVP18. This is in contrary to docking scores and the potential fitness to the active pocket findings of AVP18. Such discrepancy can be solved by the nature of docking versus MDS, i.e. whereas the docking process treats only peptide as flexible, MDS treats the whole complex in a flexible manner.

To the best of my knowledge, only one article examined the potentiality of AVPs against E1 and E2 of HPV 16 [33] in silico. On the other hand, in vitro studies of human and bovine lactoferrin AVPs against HPV concluded the inhibition of HPV16 virulence and cellular entry [34]. The best AVPs found in this study, including human defensin 1, are well-known to block HPV viral cycle and infection. Human α-defensins 1-3 and α-defensin 5 acted as potent antagonists of infection towards both cutaneous as well as mucosal HPV subtypes. In contrast, human β-defensins 1 and 2 displayed little or no anti-HPV activity. Indeed, human defensin 5 was extremely efficacious against sexually transmitted HPV types, with 50% inhibitory doses as small as ng/ml [35]. In their pioneering work, Zhang et al. [36] showed that short synthetic peptides derived from the HPV L2 capsid protein can enter cells and bind to the retromer protein complex, disrupting its interaction with L2 and preventing HPV from entering the retrograde transport pathway. This results in a dose-dependent inhibition of HPV infection in cultured cells and mice. By repurposing viral protein segments, these peptides can be used as rationally designed antiviral agents to target essential protein-protein interactions, potentially applicable in virology and other fields.

The findings of this study suggest the high potentiality of the top 3 AVPs (AVP7, AVP10 and AVP18) as potent inhibitors of HPV16 E6 oncoprotein. Nonetheless, the computational results may not give the same results in the experimental setting, whether *in vitro* or *in vivo,* urging the need for laboratory validations.

Conclusion. In conclusion, this study has successfully identified and characterized three potent antiviral peptides (AVPs) that exhibit significant inhibitory activity against the HPV16 E6 oncoprotein, a crucial step in the viral life cycle. The top-ranked AVPs, AVP18, AVP10, and AVP7, demonstrate remarkable binding affinity and stability in their interactions with E6, suggesting a strong potential for blocking HPV16 infection. These findings have significant implications for the development of novel antiviral therapies, as they provide a rational basis for the design of targeted interventions against HPV-mediated cancers. The discovery of these AVPs underscores the potential of repurposing natural peptides as antiviral agents, offering a promising avenue for the creation of more effective and specific treatments for HPV-related diseases.

Antiviral peptides show promise as therapeutics due to their high specificity, selectivity, and ability to be developed without prior structural knowledge of the target. However, several challenges must be addressed for their effective delivery and efficacy *in vivo.* Unfavorable conditions in the body, such as high temperature, pH, and salt concentration, can cause conformational changes and inactivation of peptides [37]. Various nanocarrier systems, including lipid-based liposomes, polymeric nanoparticles, and hybrid carriers, have been developed to improve the stability and delivery of antiviral peptides. Post-translational modifications, such as acetylation, amidation, or adding fatty acid chains, can enhance peptide stability and membrane permeability. Combining antiviral peptides with nanocarriers, antibodies, carbohydrates, or lipids can improve their quality of delivery and treatment processes. Additionally, validated characterization of antiviral peptides is essential to commence therapeutic claims against viruses [38].

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВІРТУАЛЬНИЙ СКРИНІНГ ПРОТИВІРУСНИХ ПЕПТИДІВ ЯК НОВИХ БЛОКАТОРІВ ВІРУСУ ПАПІЛОМИ ЛЮДИНИ 16

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Віруси папіломи людини (HPV) спричиняють 5% випадків раку, проте досі відсутні специфічні противірусні засоби, спрямовані на інфекцію HPV. Противірусні пептиди (AVP) є перспективною альтернативою традиційним терапевтичним засобам. Це дослідження має на меті вивчити використання AVP проти онкопротеїну E6 вірусу HPV16 за допомогою віртуального скринінгу. Було визначено потенційну ділянку зв'язування онкопротеїну E6, а з використанням бази даних антимікробних пептидів CAMPR4 відібрано 18 AVP. Ці AVP були пристиковані до онкопротеїну E6 за допомогою сервера HawkDockб після чого проведено динамічне моделювання. Серед протестованих AVP, найвищий інгібуючий потенціал проти онкопротеїну E6 продемонстрували AVP18, AVP10 та AVP7. AVP18 виявив більше нековалентних контактів, водневих зв'язків та електростатичних взаємодій. Динамічне моделювання підтвердило стабільність комплексів, що утворюються цими AVP з онкопротеїном E6. Це дослідження свідчать, що AVP7, AVP10 та AVP18 є перспективними кандидатами для блокування HPV16 шляхом інгібування онкопротеїну E6.

Ключові слова: вірус папіломи людини, онкопротеїн E6, противірусні пептиди, докінг, динамічне моделювання.

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