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IDENTIFICATION OF ANTI-CANCER PEPTIDES INHIBITING CATK AS A POTENT PROMISING THERAPEUTIC APPLICATIONS TO COMBAT EPITHELIAL OVARIAN CANCER

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ABSTRACT
Introduction: Despite being rare, ovarian cancer is the deadliest type of gynaecological cancer and a major public health concern. The World Health Organization projects that 140,200 people will lose their lives to ovarian cancer each year, and that 225,500 new cases will be identified. Currently most common cancer in women is ovarian cancer, which is placed eighth position due to the mortality rate worldwide. Together, these figures demonstrate how significant ovarian cancer is as a global source of disease and mortality. The fifth most common cause of cancer-related deaths among women in Western nations is ovarian cancer. In this research. Cathepsin K is one cysteine protease that could be a therapeutic target for cancer treatment. Certain proteases can have their activity inhibited to decrease the progression of cancer. In terms of efficacy and adverse effects, peptide therapies are good.
Methods: We chose an anticancer peptide and screened against CatK, the Epithelial ovarian Cancer (EOC) target protein, after examining a number of parameters, VLL-28 were selected. We investigated the dock complex of VLL-28 with CatK using molecular dynamic modeling in order to confirm the interaction between the two molecules.
Result: We noticed that VLL-28 and CatK had a strong interaction, and a molecular dynamic modeling analysis supported this finding. With a binding score of -248.74 kcal/mol, docked model complex was selected due to its ability to bind to the target protein's active binding site. Using GROMCAS, MD simulation results were assessed for approximately 100 ns.
Conclusion: Our research will offer valuable information about peptide treatments for EOC. According to this study, the target protein CatK possibly inhibited by the peptide VLL28. As a CatK inhibitor, the VLL-28 peptide might be a promising treatment option for EOC.

KEYWORDS: EOC, Cancer, Peptide, In-silico, Drug, Reproductive Cancer, CatK

1.INTRODUCTION

The seventh-largest cause of death and morbidity worldwide is ovarian cancer (OC), which is regarded as one of the deadliest gynecologic cancers [1]. Because OC is asymptomatic and two-thirds of patients are not identified until the illness is in its third or fourth stage of progression, it is incredibly terrifying. After breast cancer, ovarian cancer is the second most frequent type of cancer in developed countries among women aged 40 and over [2]. Ovarian cancer is categorized into three primary types according to the cell of origin and histopathology features: epithelial (EOC), germ cell, and sex cord stromal OC. Non-epithelial ovarian cancers, which make up less than ten percent of all ovarian cancers, usually have a better prognosis [3].
EOC represents the most common type of OC, comprising nearly 90% of all ovarian cancers. It arises from the epithelial cells present on the surface of the peritoneum, Fallopian tube, or the outside surface of the ovary. The various histologic types of EOC have their own molecular, clinical, and prognostic implications. The types of EOC include serious, mucinous, endometrioid, clear cell, and transitional cell carcinomas. High-grade serious carcinomas and low-grade serious carcinomas are the two types of serious cell carcinomas. Among all the types of epithelial cell carcinomas, high-grade serious carcinomas account for 75% and are the most aggressive and deadly forms of OC.
The most common cause of gynecologic cancer-related deaths is epithelial ovarian carcinoma (EOC). Its prognosis is poor due to its often-ambiguous early symptoms, which often reflect an advanced stage of metastasized illness at presentation [8]. The transcoelomic pathway is primarily responsible for the spread of EOC, which permits

exfoliated tumor cells to go throughout the abdominal cavity and particularly to the omentum. A pool of proangiogenic chemicals that aid the malignant cells in the formation of new blood vessels must be present in the microenvironment for primary and metastatic tumors to develop. In spite of progress in surgical and chemotherapy techniques, recurrence frequently occurs, and long-term survival continues to be unsatisfactory. Enhanced early detection methods and tailored treatment strategies are critically necessary to improve results for women impacted by this condition. However, the identification of suitable targets is challenging, CatK to be suitable targets for Epithelial ovarian cancer.

Cathepsins are strongly linked to tumor metastasis and a variety of malignancies. A, B, C, D, E, F, G, H, L, K, O, S, V, and W family members make up this superfamily [9]. Every component serves a particular purpose and contributes differently to several tumorigenic processes, including invasion, angiogenesis, metastasis, and proliferation [10]. It was recently discovered that cathepsin K (CatK), a cysteine protease was first identified as an osteoclast-produced collagenolytic protease, that was also overexpressed in a number of cancer forms [11]. About 12.1 kb of genomic DNA encode the human cathepsin K gene, which is located on chromosome 1q21 [12]. A single-copy gene of around 12.1 kb on chromosome 1q21 encodes human CatK. It has eight exons and seven introns, and it is organized similarly to CatL and CatS [13]. With 215 amino acids making up the catalytic unit, 99 amino acids composing the pro-peptides section, and 15 N-terminal amino acids, CatK has a total length of 329 amino acids. While mature CatK is different from both CatB (24% identity) and CatF (40% identity), it shares amino acid identity sequence with CatL, CatS, and CatV around 60%, which are members of the C1A family's CatL-like cluster. However, CatK is distinct from this cluster due to positively charged basic residues, which enhances the allosteric binding of negatively charged glycosaminoglycans (GAGs). These cathepsins possess a distinctively structured active groove conformation. This structure enables the enzyme and GAGs to form high-molecular-weight complexes [14, 15]. Cathepsin K, also known as CTSK, that have been linked to metastasis in a variety of cancer types. It has been linked to the peritoneal metastasis of ovarian cancer up to this point [16]. Although CatK is primarily linked to inflammatory illnesses [17], it has also been detected in several tumors at high levels [18]. TGF-β, mTOR, RANK/RANKL, and the Wnt/β-catenin signaling pathway may all be involved in the mechanism of CatK's participation in tumor cell proliferation [18, 19]. Cathepsin K mRNA has been gound in Numerous tissues, including bone, ovary, heart, placenta, lung, skeletal muscle, colon, and small intestine. Human cathepsin K has been identified and is expressed selectively in osteoclasts. The role of CTSK in OCa has been reported, and Tingting et al. [20] revealed that downregulating CTSK can inhibit cell metastasis and the proliferation of epithelial ovarian cancer cells via suppressing epithelial–mesenchymal transition (EMT). In addition, Zhao et al. [21] revealed that N-myc downstream regulatory gene 1 (NDRG1) can regulate the invasive potential of OCa cells, and their results showed that the downregulation of NDRG1 can also cause the downregulation of CTSK, MMP7and TMPRSS4. In clinical practices, OCa markers CA125 and HE4 are commonly utilized. The CA125 glycoprotein frequently found in epithelial OCa (serious cancers) and has high sensitivity, but its specificity is low. The study indicated that CTSK together with CA125 and HE4 can more specific to predict OCa with improved specificity. Clinicopathologically, CTSK expression was found to be substantially linked with metastatic and lymph node metastases. Malignant ovarian cancer exhibits overexpression of the cathepsins D, K, and L, suggesting a connection between these proteins and invasiveness, proliferation, and migration [22,23]. This study was executed using computational biology approaches like molecular modelling, molecular docking, and molecular dynamic simulation. Our study, is to focus on the selection, screening and evaluation of anticancer peptides as well as examining their patterns of interaction with CatK protein target to mitigate EOC.

2. MATERIALS AND METHOD

2.1 Target protein structure evaluation

The PDB is a repository for 3D structural data of large biological molecules like proteins and nucleic acids. The data, submitted by researchers and academics from around the world, which is typically obtained via X-ray crystallography or NMR spectroscopy methods, is in the public domain and can be freely accessed. The PDB database is maintained by the US data centre for the worldwide PDB archive and providing PDB data free of charge to all consumers without limitations on usage. The Protein Data Bank website (https://www.rcsb.org) provided the crystallographic structural information for the CatK protein; the chosen target protein's PDB ID is 6ASH. The expected active binding sites were verified using the PeptiMap server (https://peptimap.cluspro.org), guaranteeing their precision and dependability. In a carefully selected dataset of high-quality structures, our server determines the exact place where peptides bond within protein complexes, placing these predictions at the top for most situations [24]. CELLO V.2.2 (http://cello.life.nctu.edu.tw) was used for sub-cellular localisation.

It is depends on the Support Vector Machines classification system with several classes. The one-against-one (OAO) approach of SVM classification is employed for multi-class classification. 5*(5-1)/2=10 SVM classifiers are built for the five classes of subcellular sites, and each classifier is proficient with proteins from two different subcellular locations. For each kernel and penalty parameter, cross-validation in conjunction with the one-against-one (1A1) approach is employed to assess the model's performance.

2.2 Selection of Ligand and Characterizations

The antimicrobial peptide database (https://aps.unmc.edu) contain approximately 3940 antimicrobial peptides (AMPs). As of January 2024, it list more than 3146 natural AMPs. To meet the database's rigorous standards,

peptides must fulfil specific criteria: they must originate from natural sources spanning three domains or six kingdoms, demonstrate antimicrobial efficacy (with a minimum inhibitory concentration of less than 100 μM or 100 μg/mL), have their mature amino acid sequences at least partially elucidated, and contain fewer than 100 amino acid residues [25,26]. From this APD database, retrieved 274 ACPs for further study. The physicochemical properties of the selected ACPs were evaluated using the ProtParam server (https://web.expasy.org/protparam/). The calculated parameters encompass the molecular mass, molecular formula, net charge, extinction coefficient, hydrophobicity, instability index, predicted isoelectric point (pI), aliphatic index, grand average of hydropathicity (GRAVY) and estimated half-life. Along with these descriptors, other features were also explored using in-silico tools like the ToxinPred server (http://crdd.osdd.net/raghava/toxinpred/) which is used to calculate the toxicity [27], PeptideRanker (http://distilldeep.ucd.ie/PeptideRanker/) tool was employed to examine bioactivity, water solubility was assessed using Innovagen server (http://www.innovagen.com) tool. The primary structure of the peptide sequence was predicted using the PepDraw server (https://www2.tulane.edu/~biochem/WW/PepDraw/).

2.3 Molecular docking study

This method predicts the favored alignment of the ligand with the target protein when they interact. UCSF Chimera was used to optimize and minimize the energy consumption of the structures (https://www.cgl.ucsf.edu/chimera/). By altering a number of other factors, including the addition or removal of charges, water molecules, or any other specific amino acid, it effectively removes all non-standard residues and ligand structures that are already present in the target protein. The HDOCK server (http://hdock.phys.hust.edu.cn) was used to dock the CatK target protein with the anti-cancer peptides [28]. This user-friendly server combines template-based modeling, homology search, job management for quick and reliable protein-protein docking, macromolecular docking, and structure prediction. The prediction is automatically made by the hybrid algorithm of template-based and template-free docking.

2.4 Molecular Dynamics Simulation:

Molecular dynamic simulation was performed with the GROMACS 2020 package (ftp://ftp.gromacs.org/pub/gromacs/gromacs-2020.tar.gz). The default water model was selected, and the Charmm 36 (charmm36-jul2021.ff.tgz) was used as a force field for the process. The steps were triggered by the preparation of protein topology and the ligand topology, followed by the energy minimization steps. The NVT and NPT were used to equilibrate the system, and the final production of the molecular dynamics was 100 ns. The graph of RMSD (Root-Mean-Square Deviation), RMSF (Root-Mean-Square Fluctuation), and residue of gyration [29,30,31]

3. RESULT AND DISCUSSION

3.1. Target protein structure evaluation

The identification of therapeutic candidates using computational screening is a promising and cost-effective approach that is crucial to the drug discovery and development pipeline [32]. By employing computational chemistry-based approaches for target identification and drug discovery, medicinal chemists can more effectively address the vast array of theoretical facts of modern drug development. Qian et.al, reviewed the role of CTSK in various cancers, which is expected to be a potential therapeutic target [19]. The crystallographic structure of the CatK target protein was available on the RCSB Protein Data Bank website. The PDB ID for this protein is 6ASH. The PeptiMap server's active site prediction of Chain A of the CatK target protein revealed three notable locations, which are included in Table 1. By determining the protein's subcellular location within the cell, CELLO v.2.5 predicts that the target protein resides in the cytoplasm.

Table 1. Active binding site prediction of the target protein CatK of the EOC through the PeptiMap Server.

S.No.	Active binding sites	Residues present on the active binding site of CatL protein
1.	Site 1	GLN A 19,GLY A 23,CYS A 25,TRP A 26,GLY A 65,GLY A 66,MET A 68,ALA A 134,ILE A 135, ALA A 137, SER A 138, GLN A 143,LEU A 160,ASN A 161,HIS A 162,ALA A 163,TRP A 184,LEU A 209
2.	Site 2	ASN A 18,GLN A 19,GLY A 20,GLN A 21,SER A 183,TRP A 184
3.	Site 3	GLN A 19,GLY A 20,GLN A 21,CYS A 22,GLY A 23,TRP A 184

3.2. Ligand selection

For targeting the EOC CatK, 274 ACPs were retrieved from the online repository Antimicrobial Peptide Database (APD3) (https://aps.unmc.edu/home). Anticancer peptides prevent proliferation that exhibits antitumor activity by regulating the growth, apoptosis and other activities of cancer cells. Hence, therapy with appropriate peptide analogs may be able to stop tumor growth in vivo [33]. ACPs destroy cancer cells; they cause membrane instability, cytotoxicity, and cell lysis [34]. Additionally, ACPs also inhibit the proliferation of cancer cells by changing the pH inside or around the cell [35]. A total of two hundred and seventy-four anticancer peptide sequences were examined to hit the target and the selection was on the basis of physiochemical properties. Out of

these peptide sequences, VLL-28 (APD-ID: AP03049) was selected for further study. The sequence of VLL-28 is VLLVTLTRLHQRGVIYRKWRHFSGRK.

3.3 Molecular Docking

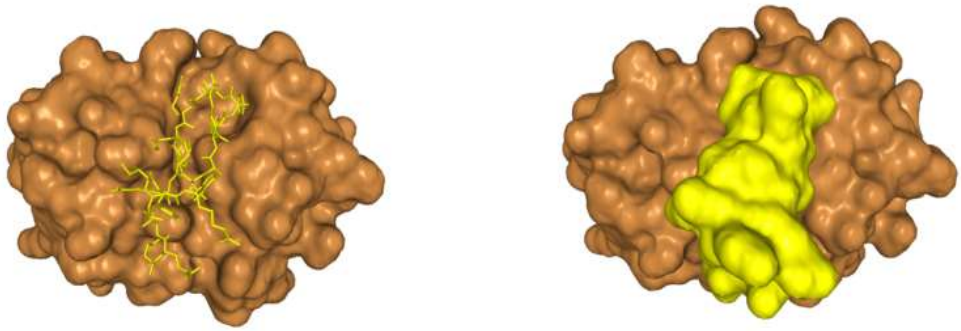
The VLL-28 peptide was docked with the CatK protein (PDB ID: 6ASH) using the HDOCK server. This server utilizes a hybrid algorithm that merges template-based and template-free docking to autonomously forecast the interaction. The server computes a binding score and a confidence score to measure the probability of binding. The docked model complex was chosen for additional analysis owing to its impressive binding score of -248.74 kcal/mol and a confidence score of 0.8781, suggesting a robust probability of molecular binding.

The results generated by the HDOCK server as output file are shown in Table 2. Based on the binding score of -248.74 kcal/mol, the docked model complex was chosen because of its capacity to bind to the binding site of the target protein.

Table **2.** HDOCK output file data of the target protein CatK (6ASH) and anticancer peptide (VLL-28):

S.No.	Features	Data
1	Grid Spacing	1.200
2	Angle Step	15.000
3	Initial Rotation	0.00000 0.00000 0.00000
4	Receptor file and its center of geometry	47.682 | -2.082 | 13.771
5	Ligand file and its center of geometry	90.549 | 70.496 | -8.709
6	Three translations	3.75552 | 2.63539 | 0.27031
7	Three rotations	-33.592 | -62.299 | 15.794
8	Binding score	-248.74
9	RMSD from the initial ligand orientation	71.71
10	Translational ID for the rotation	1.00

The confidence score is calculated by the HDOCK server based on the docking score. A value greater than 0.7 shows a higher probability of molecular binding. The confidence score for the model combination 8 of the peptide VLL-28 and the target protein CatK is 0. 8781.Figure 1 shows the molecular docking of the model complex CatK-VLL-28. It has been well established that molecular docking can be employed to identify promising chemical compounds for new targets [36]. Ligands for a druggable target can be identified by a variety of computational methods. These include molecular dynamics simulations, library docking, and fragment-based synthesis [37, 38].



(a) **(b)**

Figure 1. The docked model complex of target protein CatK (6ASH) and anticancer peptide VLL-28 predicted using HDOCK server. Figure 1**(a)** shows model (in Yellow) of the ligand VLL-28 appears in licorice form and Figure 1**(b)** depicts the ligand in the surface form.

In this study, the researchers relied on "*in silico*" technology, which involves computer-based tools for simulating biological molecules. This technology is essential for discovering new drugs since it is faster and less expensive than traditional screening techniques. The computer-based tools, by modelling how the ligand, which is the VLL-28 peptide, would interact with the target protein, CatK, provide the theoretical basis for identifying potential drug candidates.

The primary limitation of the molecular docking technique is that it assumes that the receptor and the ligand molecules are rigid, which is not necessarily true. The major limitation of the technique, however, is that it only provides a static view of the interaction between the molecules, without taking into account their dynamic changes.

3.5 Molecular Dynamics Simulation:

This technique goes beyond the basic docking by observing the behavior of the protein-ligand complex over time. This technique provides an idea of the stability of the complex formed.

The molecular dynamic simulation was carried out for 100 nanoseconds using the GROMACS 2020. A CHARMM36 force field, as well as a standard water model, was used for the simulation. This process involved building the protein topology, followed by the ligand addition and energy minimization, and then equilibration in both NVT and NPT conditions. The results obtained from the simulation were analyzed critically, with several visualizations used to prove the results obtained. The complex was analyzed using various metrics to determine how it was behaving.

RMSD of protein Cα atoms when it is bound to the peptide is presented in Figure 2(a). We used the initial equilibrated structure as a reference for computing the RMSD. The RMSD increases rapidly from 0 to 0.2 nm during initial phase of the simulation, indicating the modifications in the structure of the protein-peptide complex. After the initial peak, the value of the RMSD keeps increasing and then oscillate between 0.2 and 0.25 nm. This demonstrates that the complex is still capable of structural changes and has flexibility. At around the 75 ns mark, the values start to threshold and remain around the 0.25 nm level, indicating that a stable state is being reached. At the 0.25 nm mark, there are no significant jumps or dips in the graph, implying that there are no significant disruptions or changes in the structure of the protein-peptide complex. Figure 2(b) shows a plot of the RMSF of the residues in the protein when it is bound to the peptide. The majority of the residues in the plot are relatively small, ranging from under 0.1 to 0.2 nm, apart from a significant spike in the graph at residue 100, where the value is around 0.35 nm. This implies that the majority of the regions in the protein are relatively stable and exhibit minimal fluctuations while bound to the peptide, with all residues showing minimal fluctuation and implying a relatively stable protein core. The stability of the protein-peptide interaction can influence the way the protein-peptide complex interacts with each other. In the simulation of the protein-peptide complex for 100 nanoseconds, the protein radius of gyration when bound with the peptide is shown in Figure 2(c). The protein's Rg remains constantly within the range of 1.66 to 1.7 nm, which indicates that the protein-peptide complex is constantly changing shape.

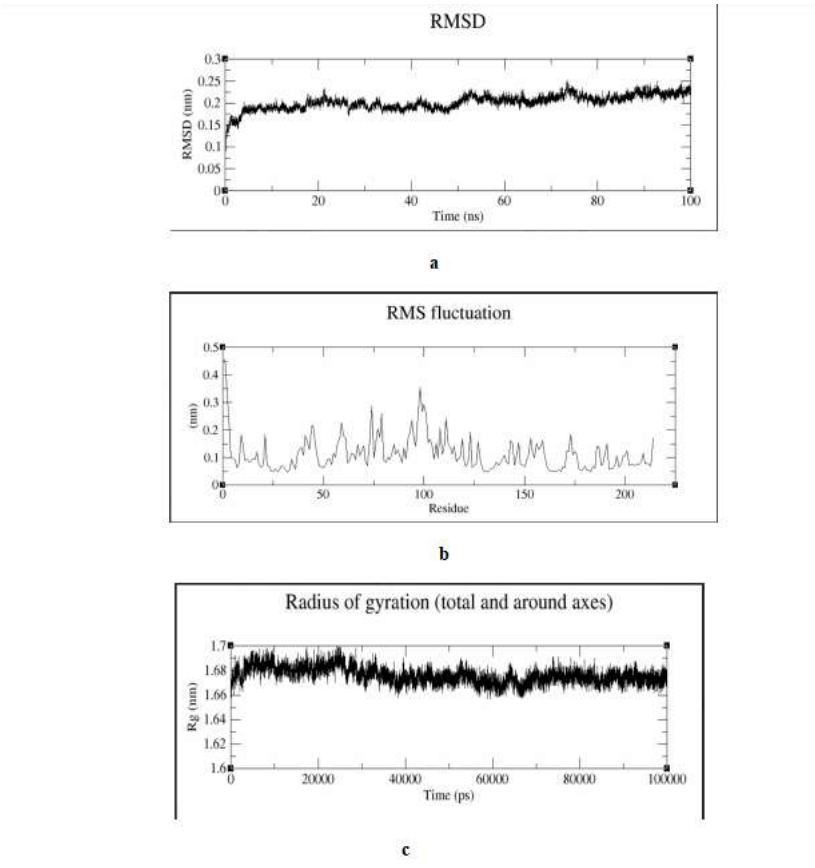


Figure 2. MD simulation of the CatK protein (6ASH) and peptide model complex VLL-28 for 100 ns: **(a)** RMS deviation, **(b)** RMS fluctuation, **(c)** Radius of gyration.

The SASA plot illustrates the degree of the biomolecules' surface exposure to the solvent. This is essential for the better comprehension of the biomolecules' interactions (see Figure 3).

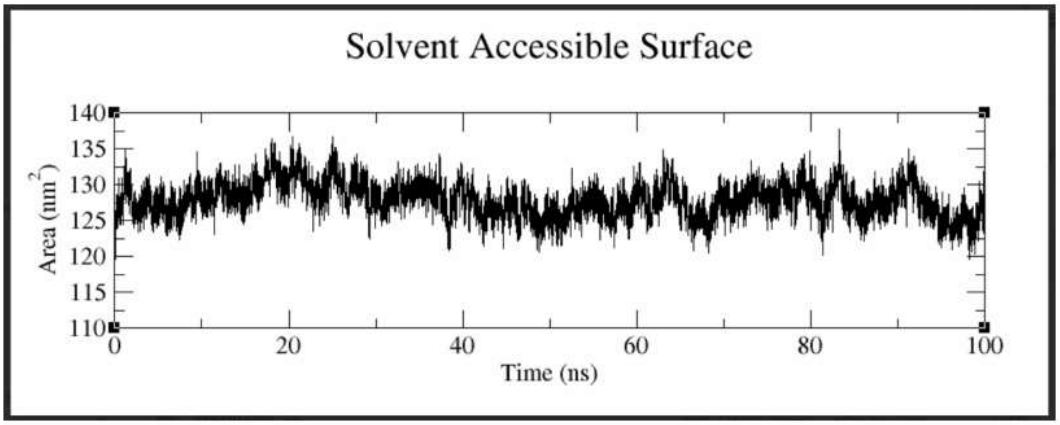


Figure **3:** Graphical representation of Solvent accessible surface area for assessing the protein-peptide interaction.

The analysis of hydrogen bond interactions offers an in-depth perspective on the hydrogen bonding patterns in the system, clarifying essential stabilizing interactions and their dynamics during the simulation Figure 4.

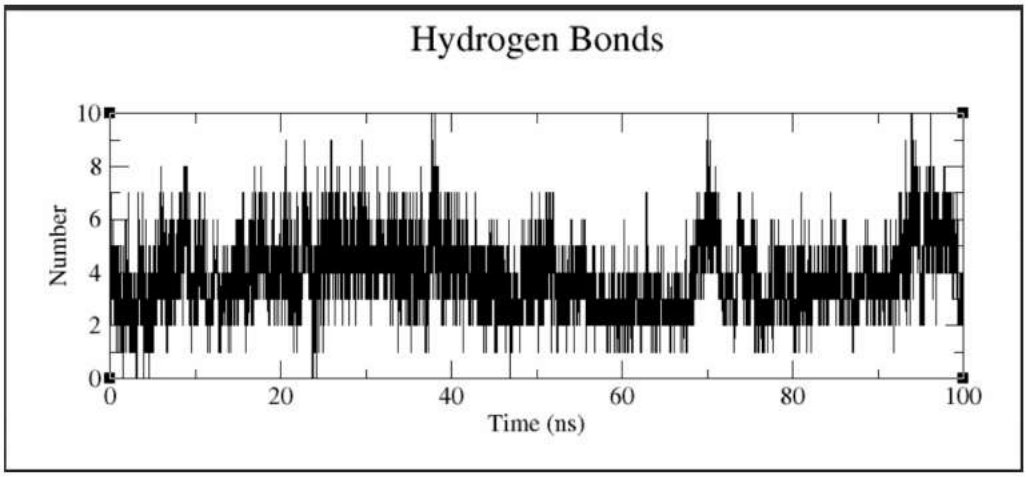


Figure **4.** Hydrogen bonds formed between protein and peptide during 100 ns of simulation.

While hydrogen bonding provides insights into the key stabilizing interactions, other potential interactions such as hydrophobic contacts or ionic interactions were not explicitly quantified in this study. Future work may include a more detailed interaction analysis to complement the dynamic stability data presented here. While the present study assessed complex stability through RMSD, RMSF, Rg, SASA and hydrogen bonding analyses, we did not include binding free energy calculations using MM/PBSA or MM/GBSA methods. Future work will incorporate such approaches to provide additional quantitative insights into the energetic stability of the VLL-28–CatK interaction.

A number of computer frameworks are available for the re-purposing of medicinal molecules *in silico* [39]. The development molecule is the outcome of the recognized benefits of repurposing to aid novel medication research initiatives and the growing interest to incorporate clinical information in regulatory approval processes. Our research will offer valuable information about peptide treatments for EOC. A limitation of this study is the absence of experimental bioassays to validate the computational predictions. Future work will include in vitro or in vivo experiments to confirm the screening results and binding efficacy of VLL-28 against CatK.

CONCLUSION

Advance bio-informatics frameworks are available for the re-purposing of therapeutic molecules using *in-silico* approaches. The development molecule is the result of the recognized benefits of re-purposing in supporting new medication research efforts and the increasing desire to incorporate clinical data in regulatory approval processes. Our research will offer valuable information about peptide treatments for EOC. According to this study, the target protein CatK possibly inhibited by the peptide VLL28. Following critical confirmation and modification in *in-vitro* investigations, this peptide may be used as a peptide therapeutic alternative for the treatment of EOC.

AUTHOR'S CONTRIBUTIONS

Study conception and design: VS. Data collection, analysis and interpretation of results, draft manuscript: AC and RRKN under the supervision of VS. All authors reviewed the results and agreed the final version of the manuscript.

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Not applicable.

CONSENT TO PARTICIPATE
Not applicable.

HUMAN AND ANIMAL RIGHTS
Not applicable.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIAL
The data and supportive information is available.

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

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