Designing peptide drug with archaeal antimicrobial peptides against multidrug resistant bacterial biofilms and human diseases: an in silico approach

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Abstract

The adverse role of multi-drug resistant bacterial biofilms accelerating human chronic diseases has posed a great risk to present world. Devising a therapy to impede such perilous effects is the need of the moment. So, in this study, we have designed a novel peptide therapy involving archaeal antimicrobial peptides. In silico predictions assign the peptide construct to be antigenic, non-allergenic, anti-cancerous and having stable physicochemical properties. Computational tools predict intracellular receptors in *Escherichia coli* and human to be possible binding targets of the construct. In silico docking of modelled peptide with targets, validated the predicted estimations. Coincidentally few human receptors binding strongly to the peptide, tend to be the widely recognized targets associated to human diseases with severe complications. Studies of conformational dynamics of peptide docked complexes with human and bacterial targets, indicated stable binding of the modelled construct. Certainly, the designed peptide could be a potent therapeutic against biofilms and human disorders.

Keywords: Bacterial biofilms, chronic diseases, peptide therapy, archaeal, potent therapeutic

Declarations

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Conflicts of interest/Competing interests

The authors declare no conflicts of interest/competing interests.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author's contributions

The authors have contributed equally to this work.

Introduction

The first conventional peptide therapy dates back to insulin as a therapeutic for diabetes in 1920s. Since then, the usage of peptides as drugs has morphed and evolved with time being continued to develop along the alterations in the modern day's therapeutic paradigms. Usage of peptides as cost effective drugs had been thwarted for a long time due to their short plasma life, challenges in oral administration, and hydrophilic nature (physiological obstacles). Despite such restraints, experimental evidences have asserted their emergence as potential alternatives showing greater efficacy in selectivity and specificity in comparison to small molecule inhibitors (Muttenthaler et al. 2021). Peptide drugs unlike their synthetic counterparts get degraded into corresponding amino acids inside the body, eschewing the formation of toxic metabolites. In addition to that, peptide drugs having short half-lives lead to less accumulation of degraded products in the body. (Hoppenz et al. 2020; Timmur and Gürsoy 2021).

The naturally occurring antimicrobial peptides (AMPs) having broad spectrum of action like cationic Polymyxin B, Gramicidin S and the cationic Lantibiotic nisin, are currently being used as efficient therapeutics to prevent the detrimental effects of antimicrobial drug resistance. AMPs are short length (ribosomally or non-ribosomally synthesized), structurally diverse, cationic peptides, present in large no. of organisms ranging from microbes to human. The list of existing effective AMPs is accessible through CAMPR3 database (Vale et al. 2014; Waghu et al. 2016). As a matter of fact, biological threats augmented by the increasing resistance to existing antimicrobial drugs has brought the usage of AMPs into light. AMPs are the need of the hour since the drug resistant bacterial infections and associated complexities have turned out to be a serious global health concern (Haney et al. 2018). The biofilm formations play a crucial role in increasing their resistance to antibiotic therapies and ability to evade host immune response by assisting the microbes to delve into persistent colonization in new niche and diverse environments (Majumdar and Pal 2017). In addition to that, biofilms ensuring survival in hostile environments has paved the way to the development of chronic infections leading to cancer predominantly gastric, pancreatic and mucinous colorectal carcinogenesis (Rizzato et al. 2019; Li et al. 2019). The most gripping standpoint of AMPs in such cases has been evinced experimentally in their ability to participate in host defence mechanism to colon carcinogenesis, bringing balance to the colon microbiome (Zhang et al. 2019).

Concomitant to the AMPs are the bacteriocins which are peptide toxins, produced by a bacterium to inhibit the spread of other bacteria. Hence bacteriocins show antimicrobial properties (Mathur et al. 2017). The most riveting features are of the AMPs produced by Archaea (a diverse and abundant family of prokaryotes, also termed as 'extremophiles' due to their drastic living habitats) called archaeosins and these have gained significant importance. Archaeosins enable archaeabacteria to colonize in harsh conditions inhibiting the growth of other bacteria. The archaeosins are the sole ribosomally synthesized positively charged AMPs (Mahlapuu et al. 2016; Candido et al. 2019) to be experimentally characterized and identified only in some species of *Halobacteria* and *Sulfolobales* (Makarova et al. 2019). Taking into account this facet of archaeal biology, this research work unfolds a new dimension of preparing potent curative with archaeal antimicrobial peptides which has not been studied before. Two peptide chains having prominent antimicrobial property were chosen from the archaeabacteria *Methanosarcina acetovorans* C2A, and *Sulfolobus acidocaldarius* DSM 639 as given by the CAMPR3 database. Using these two archaeal AMPs, this scientific endeavour focuses scrupulously on the designing of a novel peptide drug and determining its diverse therapeutic aspects against antimicrobial resistance as well as cancer.

Materials and methods

Prediction of antimicrobial motifs in selected AMPs

The CAMPR3 database (http://www.camp.bicnirrh.res.in/seqDb.php) was accessed to obtain two archaeal AMPs with peptide length in the range 0 – 200 amino acids. Among the obtained peptide sequences, one was from *Methanosarcina acetovorans* C2A and the other from *Sulfolobus acidocaldarius* DSM 639. The acquired sequences were given as input in the antimicrobial region prediction (ARP) tool (http://www.camp.bicnirrh.res.in/predict_c/) of the database to analyze the major antimicrobial motifs. The peptide length parameter in ARP tool was set to the default value of 20 and the AMP probability was chosen as result sorting option. The SVM algorithm was selected for prediction. Both the predicted sequences consisting of common Linocin M18 (bacteriocin) motif were linked with a GGSG linker to form a recombinant construct (Fig. 1).

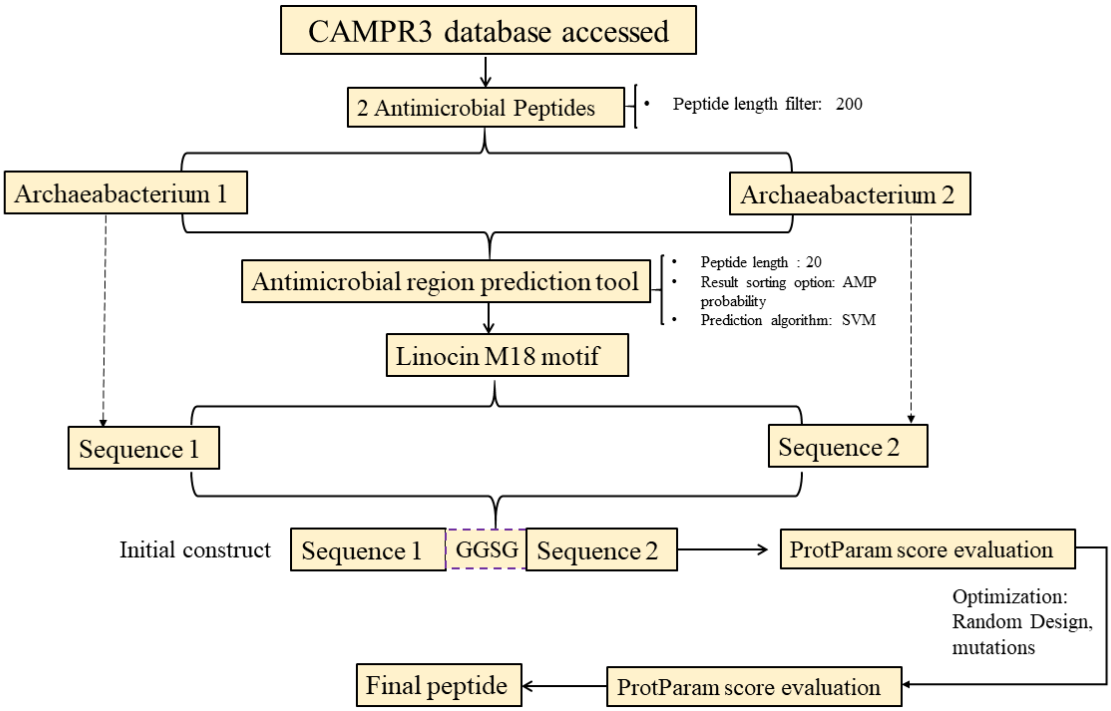

Fig. 1 Workflow for designing the peptide construct

Study of physicochemical properties of recombinant construct

The physicochemical properties of the prepared construct were analysed using the ProtParam tool (https://web.expasy.org/protparam/). The ProtParam tool takes a peptide sequence with at least 5 amino acid residues as input and computes the number of amino acids in it, its molecular weight, theoretical pI, extinction coefficient, amino-acid composition, half-life, aliphatic index, instability index, theoretical pI, grand average of hydropathicity (GRAVY) score, no. of positively charged residues, no. of negatively charged residues and atomic composition (Gasteiger et al. 2005).This tool applies N-end rule to estimate half-life, weight value of dipeptides to measure instability index, factors of relative volume occupied by aliphatic amino acids for aliphatic index and for GRAVY score, it calculates total of all hydropathy values of amino acids divided by the number of amino acids in input.

Optimization of the construct and prediction of secondary structure

Different combinations of possible mutations in the construct were tried out using the Rational Design tool (http://www.camp.bicnirrh.res.in/predict_c21/) to reach out to desired physicochemical property scores calculated with ProtParam tool. Thus, an optimized sequence was designed. The secondary structure of this peptide was predicted with PSIPRED tool (http://bioinf.cs.ucl.ac.uk/psipred/). PSIPRED allots two feed-forward neural networks to execute the prediction. (Buchan and Jones 2019; Jones 1999).

Prediction of antigenicity, allergenicity and anti-cancerous property of the final construct

The antigenicity of the final peptide was predicted with VaxiJen 2.0 tool (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). VaxiJen implements auto cross covariance (ACC) mediated transformation of amino acid sequence into the uniform vectors of prime properties of the amino acids (Doytchinova and Flower 2007, 2008). The allergenicity was determined using Allergen FP (http://ddg-pharmfac.net/AllergenFP/)and AllerTOP (https://www.ddg-pharmfac.net/AllerTOP/). AllergenFP also applies a similar method of generating vectors with ACC transformation (Dimitrov et al. 2014). The vectors are subsequently converted into binary fingerprints along with comparison in terms of Tanimoto coefficient. AllerTOP transforms protein sequences into uniform equal-length vectors applying auto cross covariance (ACC). Five E-descriptors ranging from amino acid hydrophobicity, β-strand forming propensity, helix-forming propensity to molecular size and relative abundance form the principal properties of the amino acids. The proteins

are gradually categorized with k-nearest neighbor algorithm (kNN, k=1) relying on preknown set of 2427 allergens and 2427 non-allergens. AntiCP (http://crdd.osdd.net/raghava/anticp/) was used to predict the anti-cancerous property. AntiCP predicts anticancer property with a machine learning based method of specific motif and position specific amino acid detection in peptide (Tyagi et al. 2013).

Identification of bacterial targets of the designed peptide

The tool of the Database of Antimicrobial Activity and Structure of Peptide (DBAASP) v3.0 (https://dbaasp.org/prediction/special) was used to predict peptide activity against specific bacterium. The tool predicts activity of the query peptide against microorganisms on the basis of existing informations on bioactivity, toxicity and tertiary structure of more than 15,700 AMPs in DBAASP (Pirtskhalava et al. 2021). This tool confirms a peptide to be active with positive predictive value and MIC value less than 25 µg/ml while negative predictive value and MIC greater than 100 µg/ml for non-active ones. Then, the antibacTR tool (http://bioinf.uab.cat/antibactr) was used to predict the druggable targets in the identified bacterium in which peptide shows high activity. The antibacTR expedites the essential prerequisite step of antibacterial drug discovery i.e., identification of potential antibacterial targets from the database of fully sequenced Gram-negative pathogens (Panjkovich et al. 2014). The ranking provided for each of the protein smoothens the task to discern a potential target.

Prediction of possible human receptor targets of the peptide

After prediction of prokaryotic targets, it was essential to predict the human receptors of the designed peptide to estimate its therapeutic applications in human body. So, probable receptors were identified with Swiss Target Prediction tool (http://swisstargetprediction.ch/). This tool executes a prediction algorithm involving principles of similarities of input molecule in 2D and 3D aspects with a pre-existing library of large no. of known active sites on several proteins in human (Daina et al. 2019).

Selection of library of existing drugs against identified human receptors

The drugs widely used against the identified receptors were to be listed since they would act as controls for the designed test peptide. Scientific literatures and online databases like ChEMBL (https://www.ebi.ac.uk/chembl/), PubChem (https://pubchem.ncbi.nlm.nih.gov/) were the noteworthy resources to complete the list (Gaulton et al. 2012; Kim et al. 2021). The sdf files of respective ligands were collected from online databases and pdb files were obtained using the OpenBabel toolbox (O'Boyle et al. 2011).

Tertiary structure modelling and structure assessment of the construct

It is essential to predict the stable conformation of the peptide drug. So, the I-TASSER server was used to model the tertiary structure of the peptide. The Structure Assessment Swiss (https://swissmodel.expasy.org/assess), ERRAT (https://servicesn.mbi.ucla.edu/ERRAT/) and ProSA-web servers (https://prosa.services.came.sbg.ac.at/prosa.php) were used to evaluate the structures. Ramachandran plot was also generated for the structure. The I-TASSER generates models based on PDB based templates identified with a multiple threading approach in Local Meta-Threading Server (LOMETS), and iterative assembly of fragments generated from threading templates. Moreover, this tool estimates the global accuracy as well as residue specific global quality of models (Yang and Zhang 2015). ERRAT analyses the erroneous segments in the structures with the pattern of nonbonded interactions (Sippl 1993). ProSA-web highlights the quality scores and subsequent flaws in the structures (Wiederstein and Sippl 2007). The Structure Assessment provides comprehensive structural informations generating Ramachandran plot, MolProbity score, Clash score, rotamer outlier scores and residue quality scores (Kiefer et al. 2009).

Refinement and validation of the crude model

The global and local structure qualities of server generated model were improved with GalaxyRefine (http://galaxy.seoklab.org/refine) tool. The refined structure was validated again with structure assessment servers ERRAT and ProSA-web. The GalaxyRefine rebuilds sidechains followed by repackaging of sidechain leading to overall structure relaxation (Heo et al. 2013).

Molecular docking studies

The protein model was computationally docked to all the identified targets in *Escherichia coli* and human receptors using the Patchdock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) online server. The solved 3D structures of the target receptors were accessed from PDB. Patchdock applies a faster molecular docking algorithm based on matching of complementary patches. It applies geometric hashing and pose clustering to accelerate the computational processing time. Moreover, its rapid transformational search due to local feature matching leads to high docking efficiency (Duhovny et al. 2002, 2005). The obtained docking interactions were refined with Firedock (http://bioinfo3d.cs.tau.ac.il/FireDock/). The Firedock performs a high throughput refinement on protein-protein docked complexes applying side-chain optimization. It gradually provides scoring of docked solutions obtained from fast rigid-body docking (Andrusier et al. 2007; Mashiach et al. 2008). The Patchdock as well as Firedock scores were compared for each of the docking experiments performed. Similarly, all the existing drug molecules were docked with respective receptors as control docking experiments for human receptors. There was no control docking done for *Escherichia coli* K12 targets due to absence of existing drugs identified.

Interaction studies

In the cases of docking experiments in *Escherichia coli*, the complexes which had Patchdock (PD) and Firedock scores (FD) considerably higher (PD>-12000, FD>-39) than others were further selected for interaction study with Ligplot^{+} and PDBePISA (https://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver). Considering the human receptor docking, peptide docked to Cathepsin, BACE II and RNR were subjected to interaction studies. The interaction sites and residues were visualized with Pymol visualization software. Ligplot+ generates 2D interaction maps between protein and ligand (Laskowski and Swindells 2011). PDBePISA executes the analysis of the binding affinity parameters, interface study, residue interactions in binding partners of macromolecular complexes (Krissinel and Henrick 2007).

Study of conformational dynamics of chosen docked complexes

The complexes of peptide with human and *Escherichia coli* K12 targets having best docking scores were subjected to conformational dynamics studies with iMODS server (http://imods.chaconlab.org/). This tool implements Normal Mode Analysis (NMA) for computing internal coordinates and reproduces the probable trajectories of conformational changes of input macromolecules. It provides information on deformability of protein structure, eigen values and B-factor depicting disorder in protein atoms, to estimate the stability of macromolecules. It operates with rapid solutions to eigen problems extending the analysis space to larger macromolecules and applies an affine-model approach to simplify visualization of normal modes (López-Blanco et al. 2014).

Results and discussions

Preparation of the peptide construct

The AMPs found from the database belonged to Archaebacteria *Methanosarcina acetovorans* C2A and *Sulfolobus acidocaldarius* DSM 639. Selected sequences 'LLELLGTPNNPGNVFKSNTL' in *Methanosarcina acetovorans* C2A and 'IIVSPLIKGLAVVSKKGFYV' in *Sulfolobus acidocaldarius* DSM 639 have common Linocin M18 antibacterial motif (Fig. 1). These two peptides were joined with GGSG linker to form 'LLELLGTPNNPGNVFKSNTGGSGIIVSPLIKGLAVVSKKGFYV'.

Physicochemical properties and secondary structure of the construct

The physicochemical properties were computed for initial construct and using the Rational design tool along with mutations, new construct with optimum property scores was prepared. The initial designed sequence was provided as input in the Rational Design tool with default parameters of AMP probability result sorting option, 100 best hits and SVM algorithm. The obtained construct was found to have optimum physicochemical properties. The sequence of final construct is 'LKELLGTPNNPGNVKKSNTLGGSGIIVSPLIKGLAKVSKKGFYK' (Fig. 2)

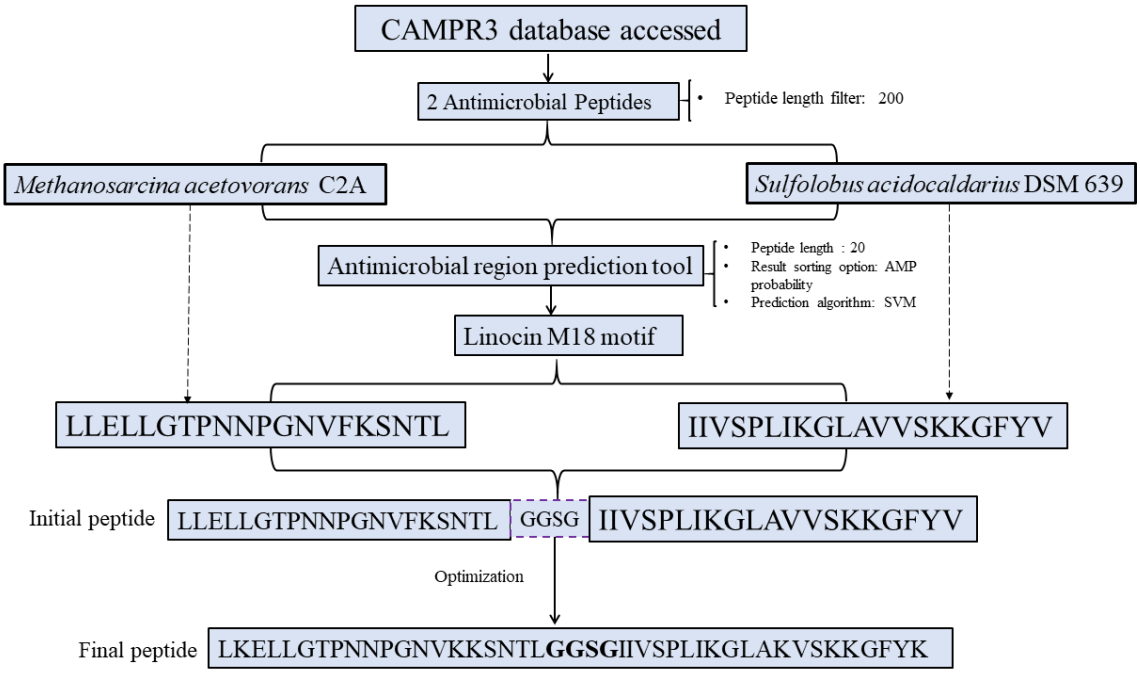

Fig. 2 Schematic diagram of the designing strategy of peptide construct

Comparison of obtained scores for the physicochemical properties of respective constructs is shown in Table 1.

Table 1 Peptide properties

Parameters	Initial peptide (Before mutation)	Final peptide (After mutation)
No. of amino acids	43	44
Molecular weight	4400.18	4.567 kDa
Theoretical pI	9.83	10.30
Extinction coefficient	1490 $M^{-1}cm^{-1}$	1490 $M^{-1}cm^{-1}$
Instability index	8.50	9.37
Aliphatic index	117.67	101.82
GRAVY score	0.421	-0.198

35% of the predicted secondary structure is helix, 5% strand and remaining 60% is coil. (Fig. 3)

Antigenicity, allergenicity and anti-cancerous property of the peptide

One of the primary criteria in peptide drug therapy is its non-toxic characteristic inside the human body. The results from the VaxiJen2.0, AllergenFP, AllerTOP, AntiCP servers confirmed the peptide to be non-allergic, non-toxic, and anti-cancerous. The VaxiJen 2.0 results were obtained for tumour as target and 0.4 as threshold.

Bacterial targets

Computational predictions estimated probable strong binding of the peptide against the following targets in *Escherichia coli* K12 – 30S ribosomal protein S18, Inorganic pyrophosphatase, Flavodoxin -1, 2,3,4,5 – tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase, 30S ribosomal protein S4, DNA directed RNA polymerase subunit α, UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase and 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase.

Human receptor targets

Cathepsin D (CTSD), Caspase-1 (CASP1), Signal transducer and activator of transcription-3 (STAT3), Beta-secretase 2 (BACE1), Beta secretase 1 (BACE2), Renin, SRC, Mitogen activated protein kinase-1 (MAPK1),

Angiotensin-converting enzyme (ACE), Angiotensin-converting enzyme 2 (ACE2), 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) and Ribonucleotide reductase (RNR) were identified as the possible targets in SwissTargetPrediction tool for the peptide drug candidate. Majority of these targets play significant role in various diseases like renal fibrosis, different carcinomas - pancreatic cancer, lymphoma; dysregulation of apoptosis, cardiovascular defects, hypertension, neuronal degeneration, and sickle-cell anemia (Fox et al. 2016, Knight and Barrett 1976; MacKenzie et al. 2010; Li et al. 2019; Chang et al. 2017, Abletshauser et al. 2002; Jackson et al. 1984; Keikhaei et al. 2016; Aye et al. 2015)

Existing drug candidates against the human receptors

Evidences from available literatures confirmed existing drugs against the receptors identified. Pepstatin inhibits Cathepsin-D (Knight and Barrett 1976; Fox et al. 2016), belnacasan and z-yad-fmk are inhibitors of Caspase-1 (MacKenzie et al. 2010; Chopra et al. 2009), crytotanshinone, niclosamide and napabucasin inhibit STAT3 (Li et al. 2019, 2013, 2020), Phenserine inhibits BACE2 (Chang et al. 2017), BACE1 inhibited by elenbecestat and umibecestat (Adewole and Ishola 2021; Tran et al. 2020), aliskiren strongly binds and inhibits Renin (Wal et al. 2011), SRC inhibited by kx-2391, bosutinib and saracatinib (Naing et al. 2013, Vultur et al. 2008; Gucalp et al. 2011), ulixertinib and ravoxertinib inhibit MAPK1 (Braicu et al. 2019), enalapril, benazepril inhibit ACE (Jackson et al. 1984; Yu et al. 2006), hydroxychloroquine inhibits ACE2 (Fu et al. 2021), HMG-CoA, rosuvastatin and simavastatin targeted against HMG-CoA (Abletshauser et al. 2002; Slater and MacDonald 1988) and hydroxyurea inhibits RDR (Keikhaei et al. 2016; Kambasu et al. 2019).

Modelled tertiary structure of the construct and validation of refined model

For a potential drug to be prepared, essentially the tertiary structure of the peptide was modelled using I-TASSER server. I-TASSER provided top 5 models and the model with optimum C score was selected. The MolProbilty, Clash, rotamer outlier scores for the model and favoured regions were visualized in the Ramachandran plot generated in Structure assessment Swiss server. Moreover, ERRAT and ProSA-web assessed the model. The same evaluation was performed for the refined model. Promising results from Ramachandran plot, ERRAT score of 71.875 (good models have values greater than 50) and -5.1 (z score) from ProSA-web bolstered the reliability and improved quality of the predicted protein model (Fig. 3).

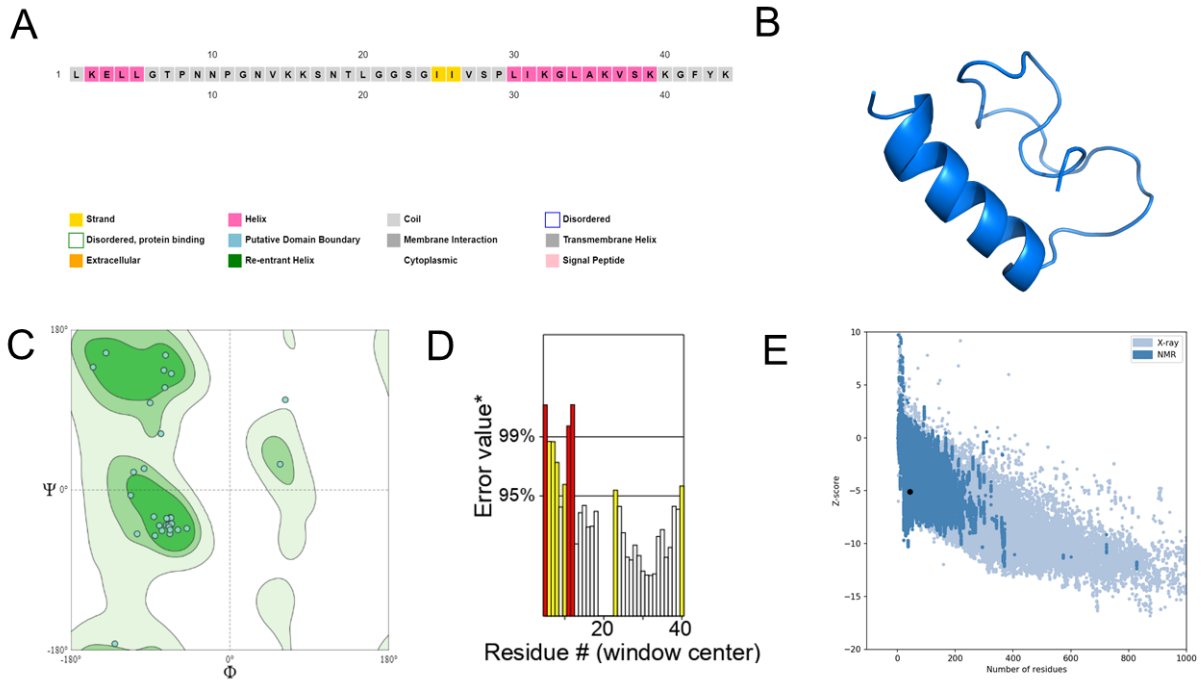

Fig. 3 Peptide secondary structure prediction and modelling its tertiary structure along with model evaluation **a** PSIPRED predicted secondary structure. **b** Refined structure of I-TASSER generated tertiary model. **c**

Ramachandran plot generated for the model from Swiss Structure Assessment server. **d** Model evaluation plot generated with ERRAT **e** Z score assessment of the model (marked as black dot) from ProSA-web

Preparation of receptors for molecular docking

The solved tertiary structures of *Escherichia coli* and human receptors were accessed from PDB for the purpose of molecular docking with peptide. Any of the bound ligands or inhibitors in receptor structures were removed before docking. The PDB ID of respective *Escherichia coli* targets are: Flavodoxin 1: 1AG9, UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase: 6P89, 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase: 4KEH, DNA-directed RNA polymerase subunit α: 3K4G, 30S ribosomal S4: 6XZB, Inorganic pyrophosphatase: 1FAJ, 30S ribosomal S18: 3JCE. PDB ID of respective human receptors: CTSD: 4OBZ, CASP1: 1BMQ, STAT3: 4ZIA, BACE2: 2EWY, BACE1: 1FKN, RENIN: 2V07, SRC: 1A07, MAPK1: 4ZXT, ACE: 1O8A, ACE2: 1R42, HMG: 1DQ8, RNR: 2WGH.

Molecular docking

The peptide model docked in silico with each of the identified receptors provided docking scores which were compared with the ones obtained from docking existing drug molecules against their respective receptors. The detailed docking scores of all docked complexes are mentioned in Table 2 and Table 3. CTSD, BACE-2 and RNR from human receptors were selected as potential peptide drug targets on the basis of docking scores. It could also be observed that these chosen targets had docking scores better than their drug counterparts. *Escherichia coli* intracellular proteins: Flavodoxin-1, UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase and 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase had relatively high docking scores than other (Threshold score- PatchDock: 12000, FireDock: -39). So, these three were selected.

Table 2 Peptide-human receptor target molecular docking scores

SL NO.	Receptor	PEPTIDE docking score		Existing drug candidate	DRUG Docking score	
		PATCH DOCK	FIRE DOCK energy score		PATCH DOCK	FIRE DOCK energy score
1.	CTSD	13974	-55.81	PEPSTATIN	7334	-49.04
2.	CASP1	10912	-28.46	BELNACASAN	5168	-42.17
				z-yad-fmk	5940	-33.8
3.	STAT3	13088	-25.47	CRYTOTANSHINONE	4232	-38.72
				NICLOSAMIDE	4092	-35.27
				NAPABUCASIN	3622	-27.34
4.	BACE2	14030	-59.55	PHENSERINE	5444	-48.57
				POSIPHEN	5460	-53.31

5.	BACE1	13326	-21.56	ELENBECESTAT	6100	-47.84
				UMIBECESTAT	6228	-41.82
6.	RENIN	12042	-42.80	ALISKIREN	7534	-58.49
7.	SRC	11228	-22.33	Kx2-391	5688	-39.38
				BOSUTINIB	5348	-34.51
				SARACATINIB	5692	-39.50
8.	MAPK1	11892	-17.15	ULIXERTINIB	4992	-39.55
				RAVOXERTINIB	5494	-41.27
9.	ACE	10776	-16.51	ENALAPRIL	5360	-34.65
				BENAZEPRIL	6278	-44.04
10.	ACE2	15262	-1.11	HYDROXYCHLOROQUINE	4796	-37.04
11.	HMG	13326	-21.56	HMG-CoA	7758	-47.58
				SIMAVASTATIN	6168	-42.97
				ROSUVASTATIN	6170	-43.52
12.	RNR	15406	-35.07	HYDROXYUREA	1502	-12.92

Table 3 Peptide-*Escherichia coli* target molecular docking scores

SL NO.	Receptor	PEPTIDE docking score	
		PATCH DOCK	FIRE DOCK energy score
1.	30S ribosomal S18	9064	-15.58
2.	Inorganic pyrophosphatase	11218	-20

3.	Flavodoxin 1	12704	-40.36
4.	30S ribosomal S4	11568	-17.13
5.	DNA-directed RNA polymerase subunit α	13540	-31.95
6.	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	13580	-51.95
7.	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	12272	-39.66

Interactions in selected docked complexes

The selected receptors were further subjected to interaction mapping at the peptide binding interface with computational methods. The major binding sites and residues involved in hydrogen bond interactions were visualized with molecular visualization tool (Fig. 5). The residues involved in hydrogen bond interactions are listed in Table 4.

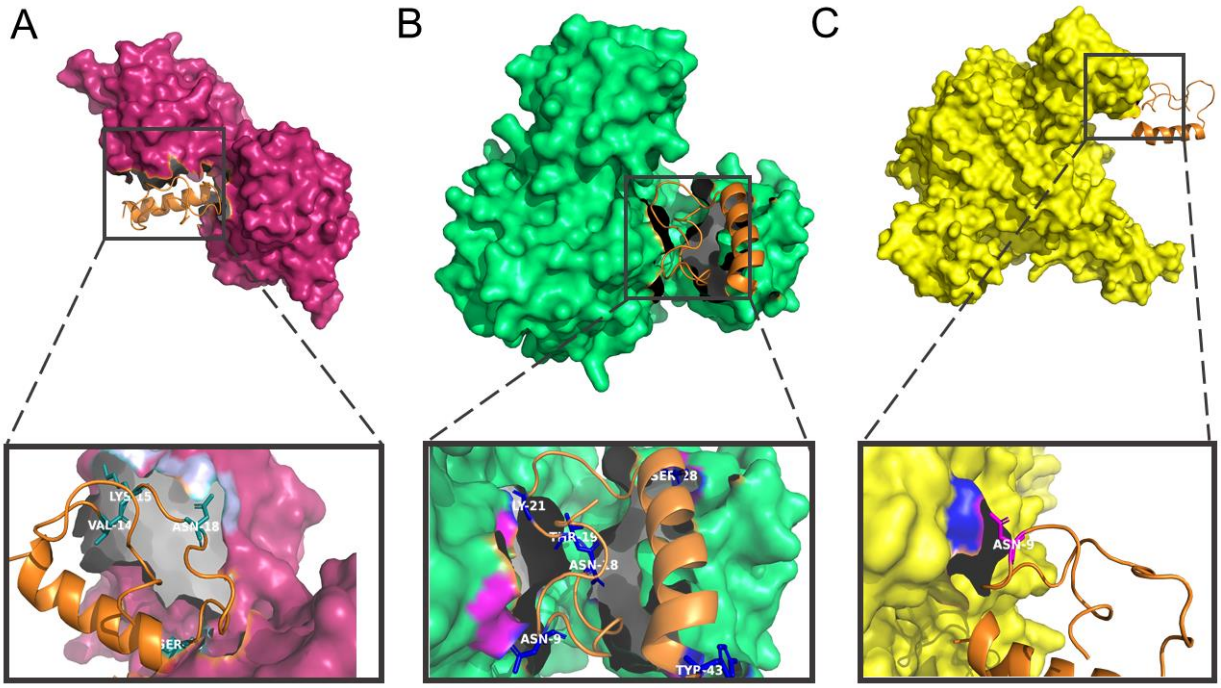

Fig. 4 Visualization of interactions in the docked complexes of peptide (orange) with **a** Flavodoxin 1 **b** 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase **c** UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase. Interacting residues labelled in white and receptor interface represented with surface.

Table 4 Residues involved in hydrogen bonding

RECEPTOR	PEPTIDE
Flavodoxin-1: Asp 77, Arg 112, Thr 72, Glu 61, Glu 75	 Asn 18, Ser 28, Lys 15, Val 14

UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase: Glu 49	Asn 9
3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase: Asn 48, Lys 112, Gln 14, Glu 49, Glu 20, Glu 110, Ser 158	Asn 9, Thr 19, Asn 18, Ser 28, Tyr 43, Gly 21
CTSD: His 57, Cys 27, Trp 54	Thr 7, Gly 21, Lys 16
BACE2: Asp 25, Lys 130, Asn 183, Pro 321, Ala 173, Gln 23	Lys 36, Tyr 43, Asn 18, Ser 28, Val 14,
RNR: Lys 316, Arg 284, Thr 741, Glu 321, Lys 320, Glu 322	Lys 2, Gly 22, Asn 9, Asn 10, Pro 11

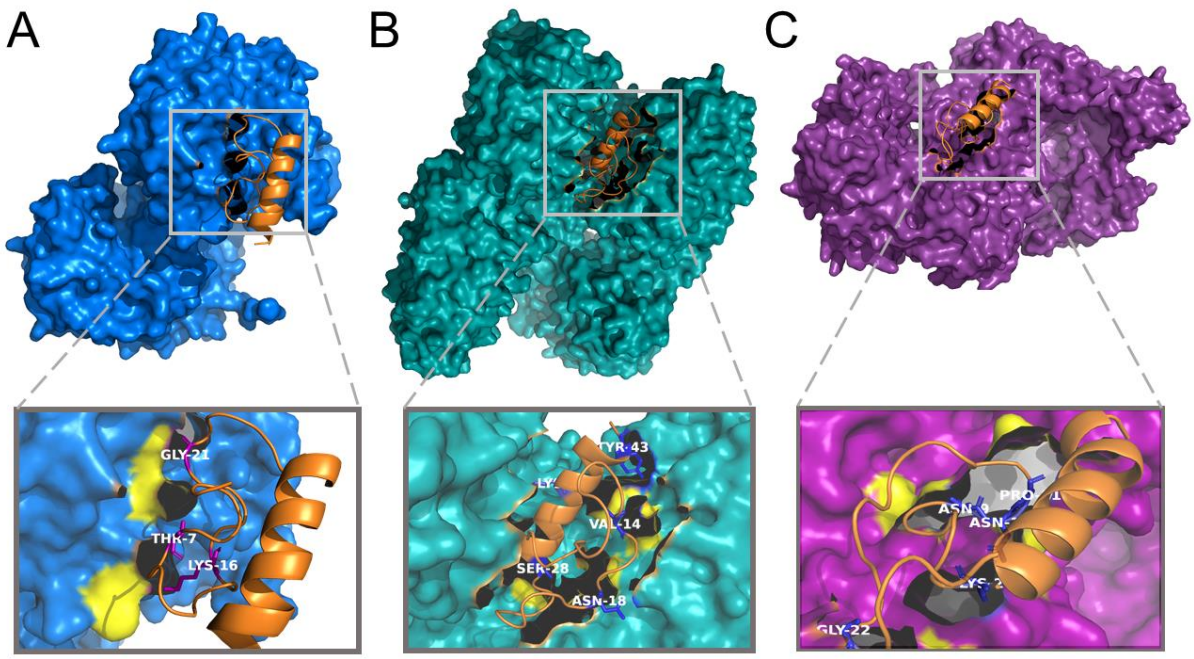

Fig. 5 3D visualization of **a** Peptide (orange)-Cathepsin D (blue) **b** Peptide (orange) – BACE2 (deep green) **c** Peptide (orange) – RDR (purple) interactions in docked complexes. Interacting residues of peptide are labelled white and that of receptor represented with yellow surfaces.

Prediction of complex dynamics from iMODS

The iMODS server predicted stability and domain movements in selected docked complexes. The deformability, NMA computed B-factor (NMB) and eigen values were computed for chosen complexes. Considering the *Escherichia coli* intracellular targets, Flavodoxin-peptide complex showed high deformability in the main chain in comparison to other targets (Fig. 6a). The UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase showed lowest deformability (Fig. 6c) and it could be confirmed that it had least no. of hinges in main chain than others. The NMA calculated B-factors indicate the extent of displacements at the atomic level. The B-factor was greater than B-factor of PDB (BPB) to a great extent with some exceptional atomic stretches in Flavodoxin-peptide complex (Fig. 6d). The NMB for 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase – peptide almost overlapped with BPB except a few regions (Fig. 6e). In case of UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase-peptide, majority of NMB was less than the BPB excluding the C-terminal atoms (Fig. 6f). The eigen values of the peptide docked with Flavodoxin-1, 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase and UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase are 1.287×10^{-4}, 8.955×10^{-5} and 7.128×10^{-6} respectively. These values provide an estimation of the energy for structure deformation. So, it could be stated that Flavodoxin-peptide complex is most stable than others since its highest eigen value correlates to more energy requirement to deform the structure, NMA-B factor values are almost overlapping with PDB B-factor and it has lowest backbone deformability characteristics. Now, flavodoxin-1 is an electron-transferase with cofactor flavin mononucleotide (FMN), vital for *Escherichia coli*. It plays an important role in global regulation of oxidative stress in bacterium accompanying other flavodoxins (Gaudu and Weiss 2000). So, if our peptide ideally binds such target, it could lead to suppression of bacterial infections and antimicrobial resistance (Salillas and Sancho 2020).

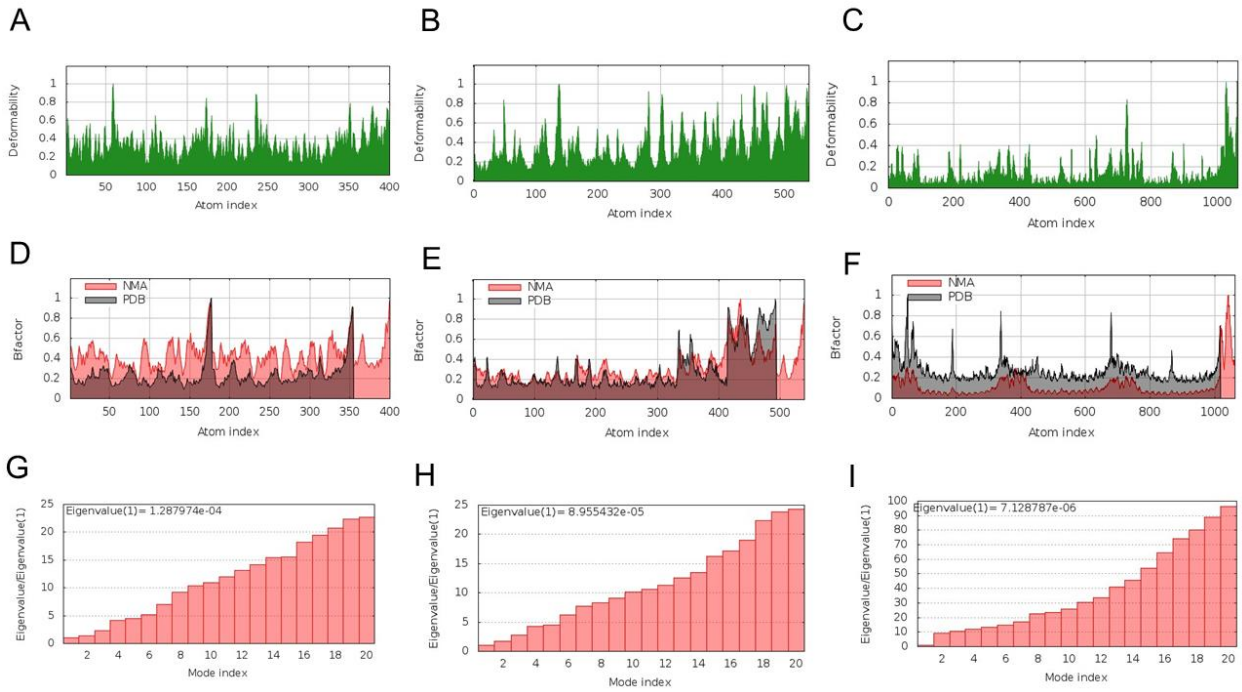

Fig. 6 Predictions of conformational dynamics from iMODS. Deformability plot for peptide complex with **a** Flavodoxin-1 **b** 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase **c** UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase. B-factor comparison for peptide complex with targets **d** Flavodoxin-1 **e** 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase **f** UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase. Eigen-values for docked complex of peptide with **g** Flavodoxin-1 **h** 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase **i** UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase

The studies of complex stability of human targets showed interesting results. The BACE-2 complex showed

highest deformability (Fig. 7b) indicating presence of more hinges in the main chain. RNR

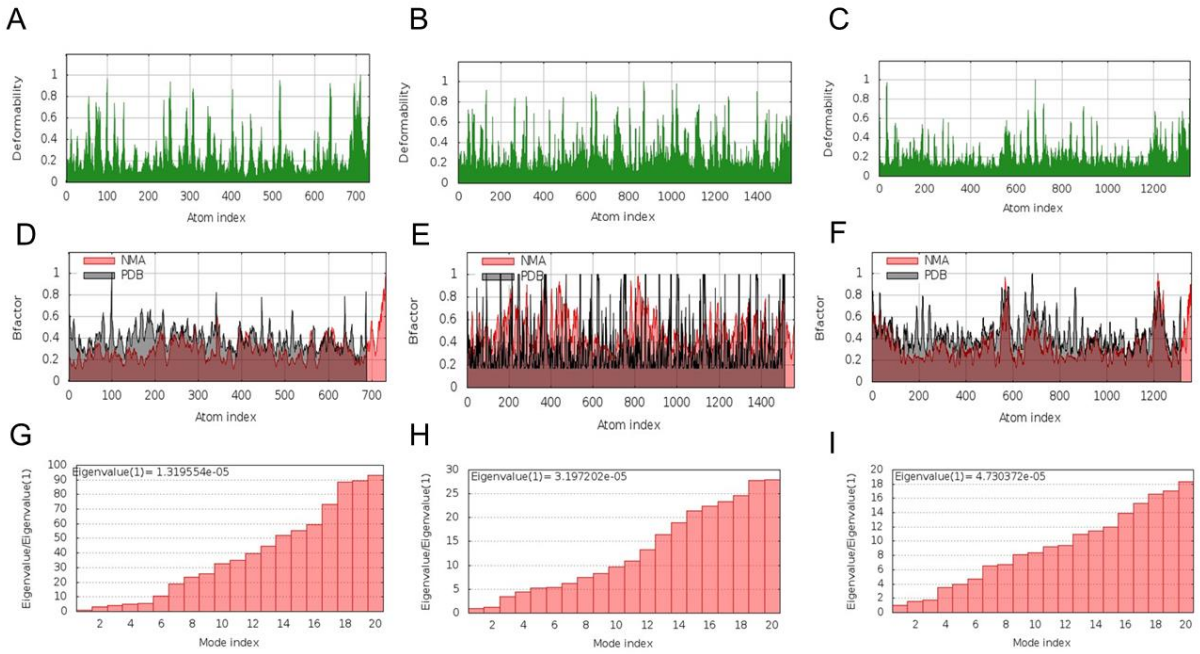

Fig. 7 Predicted conformational dynamics from iMODS. Deformability plot for peptide complex with **a** CTSD **b** BACE-2 **c** RNR. B-factor comparison for peptide complex with targets **d** CSTD **e** BACE-2 **f** RNR. Eigen-values for docked complex of peptide with **g** CSTD **h** BACE-2 **i** RNR.

complex and CTSD showed almost overlapping values of NMA B-factor with PDB B-factor (Fig. 7f) while the BACE-2 showed least common values (Fig. 7e). The eigen values of the complexes of peptide with Cathepsin D, BACE 2 and RNR are 1.319 x 10^{-5}, 3.19 x 10^{-5} and 4.73 x 10^{-5} respectively. These values provide an estimation that the RNR-peptide complex requires highest energy among others to suffer deformation. So, having an overview of all the plots, the RNR-peptide complex tends to be the most stable one with low deformability and highest eigen value. It is a well-known fact that cancer cells require RNR for synthesizing deoxyribonucleotide triphosphates de novo. So, targeting such an enzyme with this peptide drug could be an effective treatment against cancer (Aye et al. 2015).

Conclusion

Bacterial biofilm development with time has made microbes to become unassailable by multiple existing antibiotics, which in turn has led to sundry chronic infections to become ubiquitous among us. This thorough yet compendious study regarding the merits of archaeosins has shown promising results regarding the methodology and efficacies of peptide drug therapeutics involving AMPs. Using relative receptor-ligand docking studies, and computational in vivo stability characterization, it has been successfully shown that our designed peptide drug candidate can aptly act against cancer like chronic myelogenous leukemia, Alzheimer's disease, sickle cell disease etc. and even against *Escherichia coli*. All these in-silico predictions, can be further corroborated using wet lab experimental evidence which can ultimately lead to a pragmatic designing of broad-spectrum drug in future. Further experiments should be carried out to sophisticate this methodology and exploit the properties like target specificity, regioselectivity, and reify the functional effectivity of this domain of bacteriocin-peptide therapeutic constructs.

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