Deep learning approach for accurate De-novo design of hyperstable Peptide Inhibitors against Class A β-lactamases

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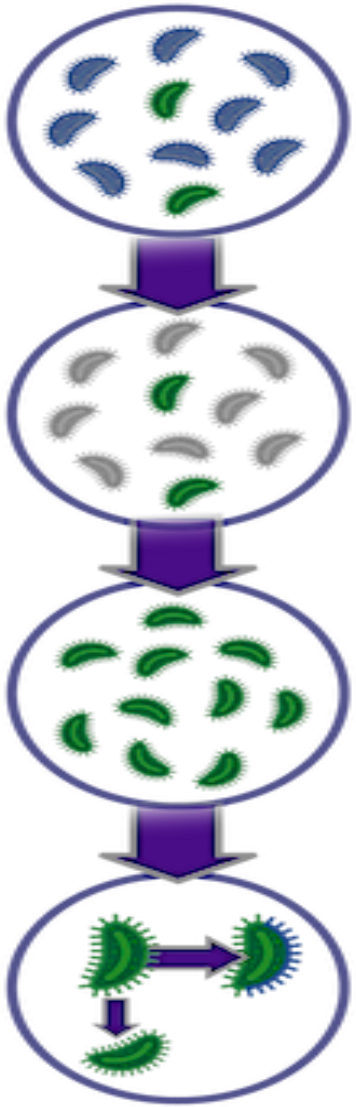
INDEX

Anti-Microbial Resistance

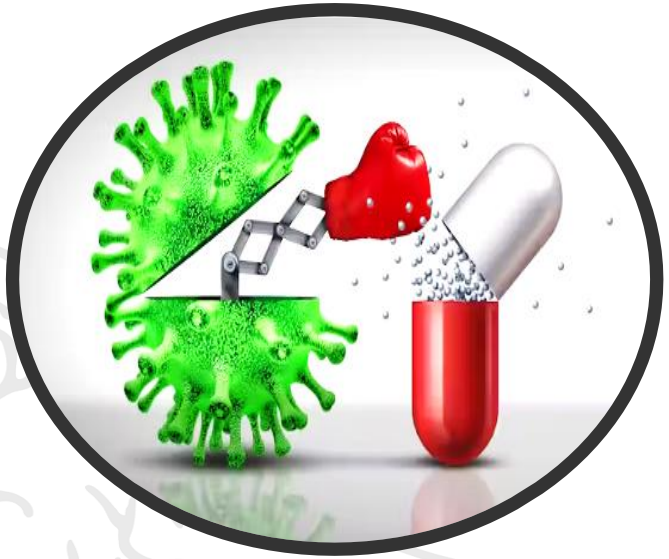


Antimicrobial products are used to kill or significantly slow the growth of disease-causing microbes.

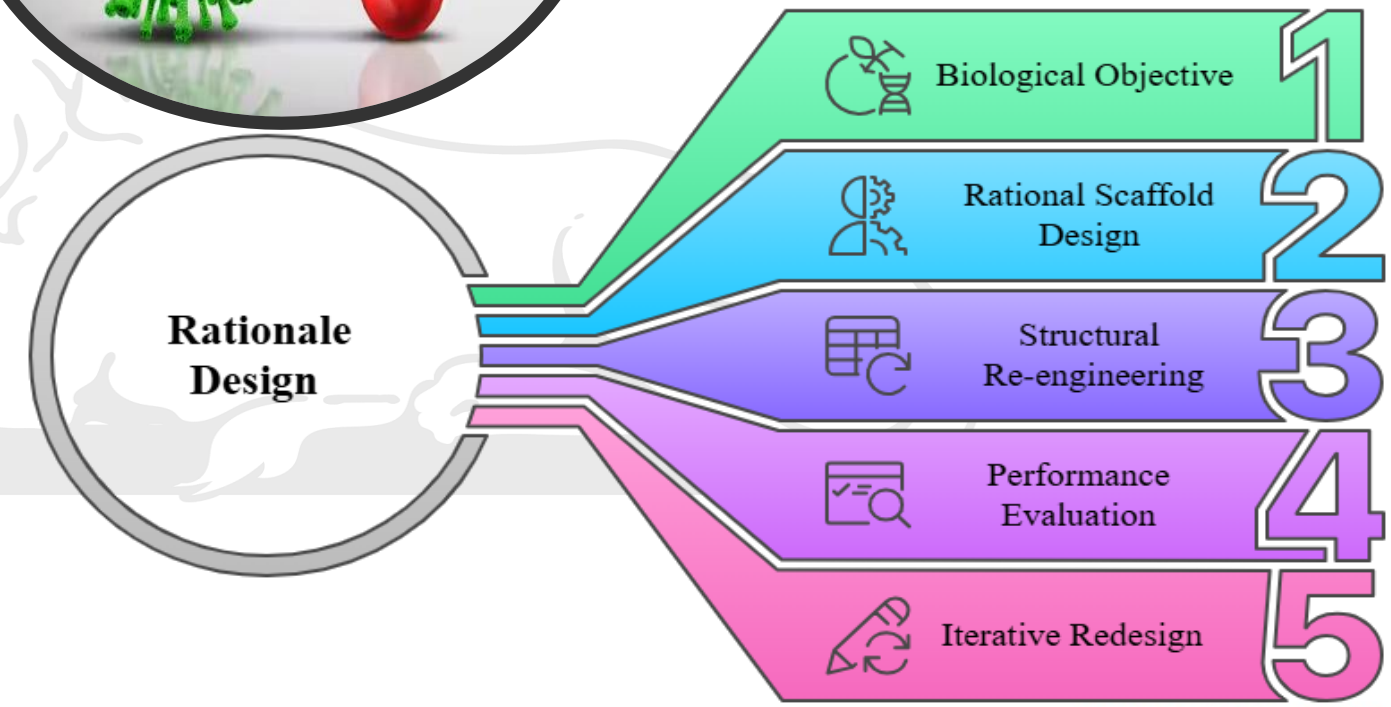
Under certain conditions, selective pressure drives evolution of mechanisms that allow some microbes to resist antimicrobial activity.

Resistant microbes are able to survive antimicrobial treatment and continue to replicate.

AMR microbes pass resistance genes to other microbes via vertical and/or horizontal transfer, increasing both the quantity and type of resistant pathogens.



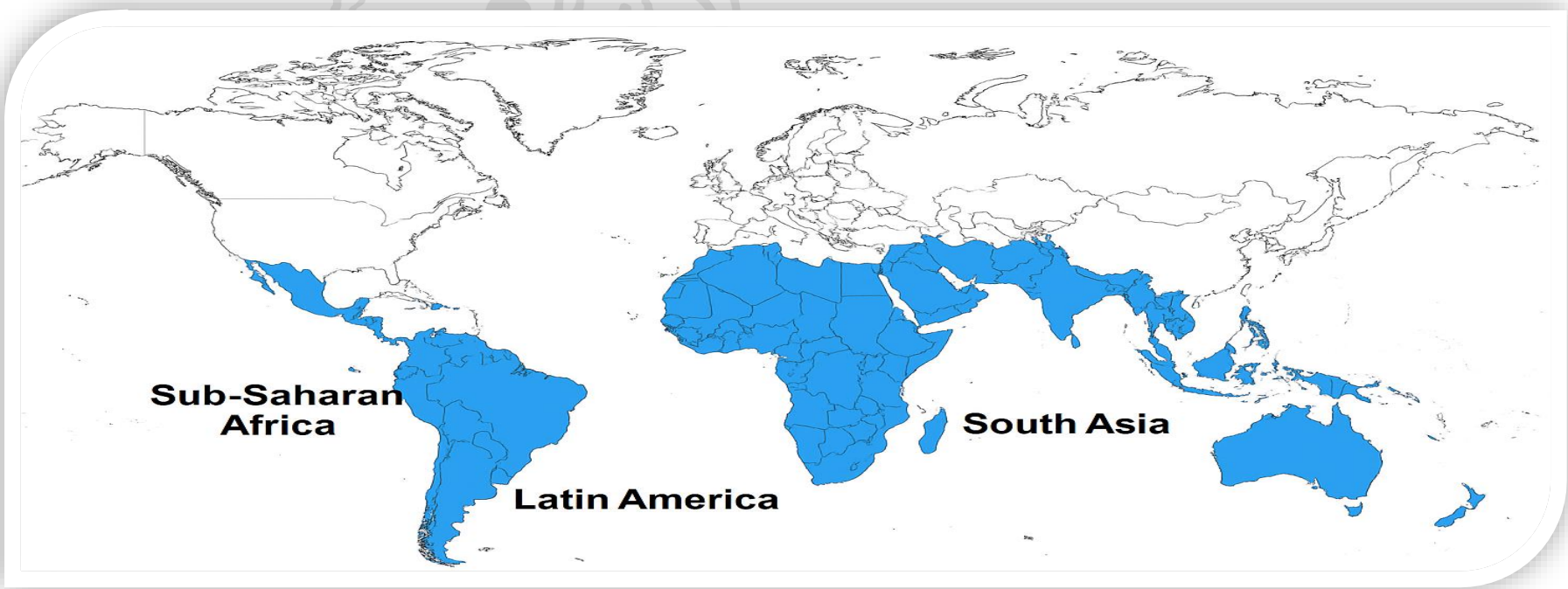
Antimicrobial resistance (AMR) is a **GLOBAL THREAT**



Global burden of Bacterial AMR

The percentage of deaths with sepsis that were associated with AMR increased from **29%** in 1990 to **35%** globally in 2019, before decreasing to **22% in 2021**.

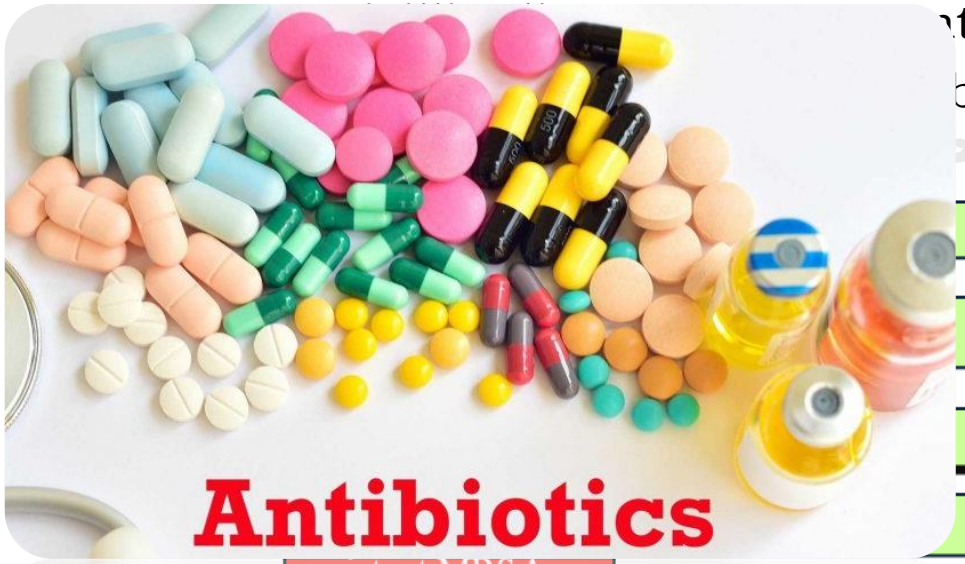
Year	Death	Most affected regions (Super-regions)
1990	1.06	Sub-Saharan Africa, South Asia
2019	1.27	Sub-Saharan Africa, South Asia
2021	1.14	Sub-Saharan Africa, South Asia
2050	**39.00**	**South Asia, Latin America, Caribbean**



Projected most Affected Regions (1990–2050) for Global Deaths Attributable to Antimicrobial Resistance (AMR)

Historical Progression of AMR

- Although there has been several drugs came into the market to combat AMR but the resistance pertains.



t MDR pathogens.

biotic introduction.

2000: Linezoid

2001: Carbapenemase Klebsiella

2003: Daptomycin

2015: Ceftazidime-avibactam

2015: Ceftazidime-avibactam resistance

resistant MRSA

2018: Pl

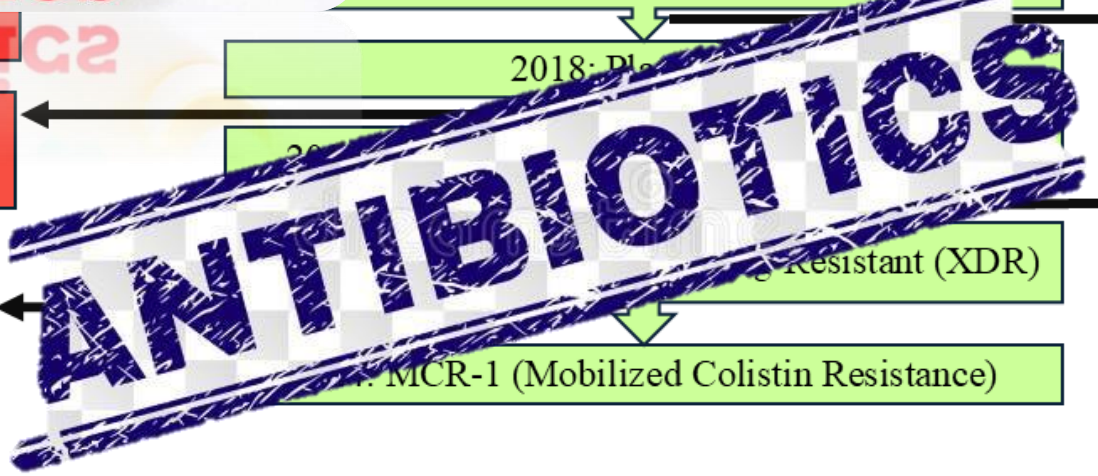
2019: Plazomicin Resistance

2020: Cefiderocol resistance

Resistant (XDR)

2021: Linezolid resistance

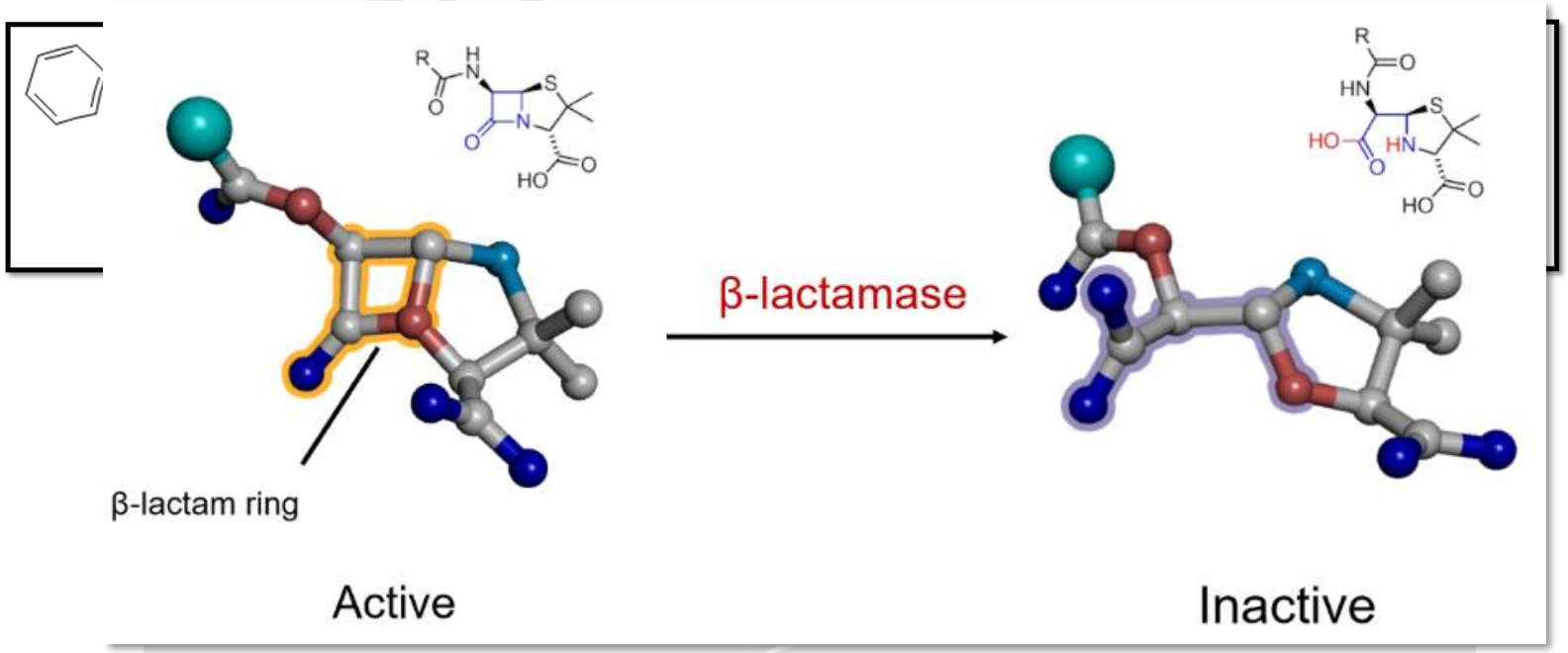
MCR-1 (Mobilized Colistin Resistance)



Introduction

β-lactam Antibiotics and Their Mechanism of Action

- β-lactam Antibiotics Includes penicillin, cephalosporins, monobactams, carbapenems.
- Target PBPs and inhibit peptidoglycan cross-linking.
- Inactivated by β-lactamases.

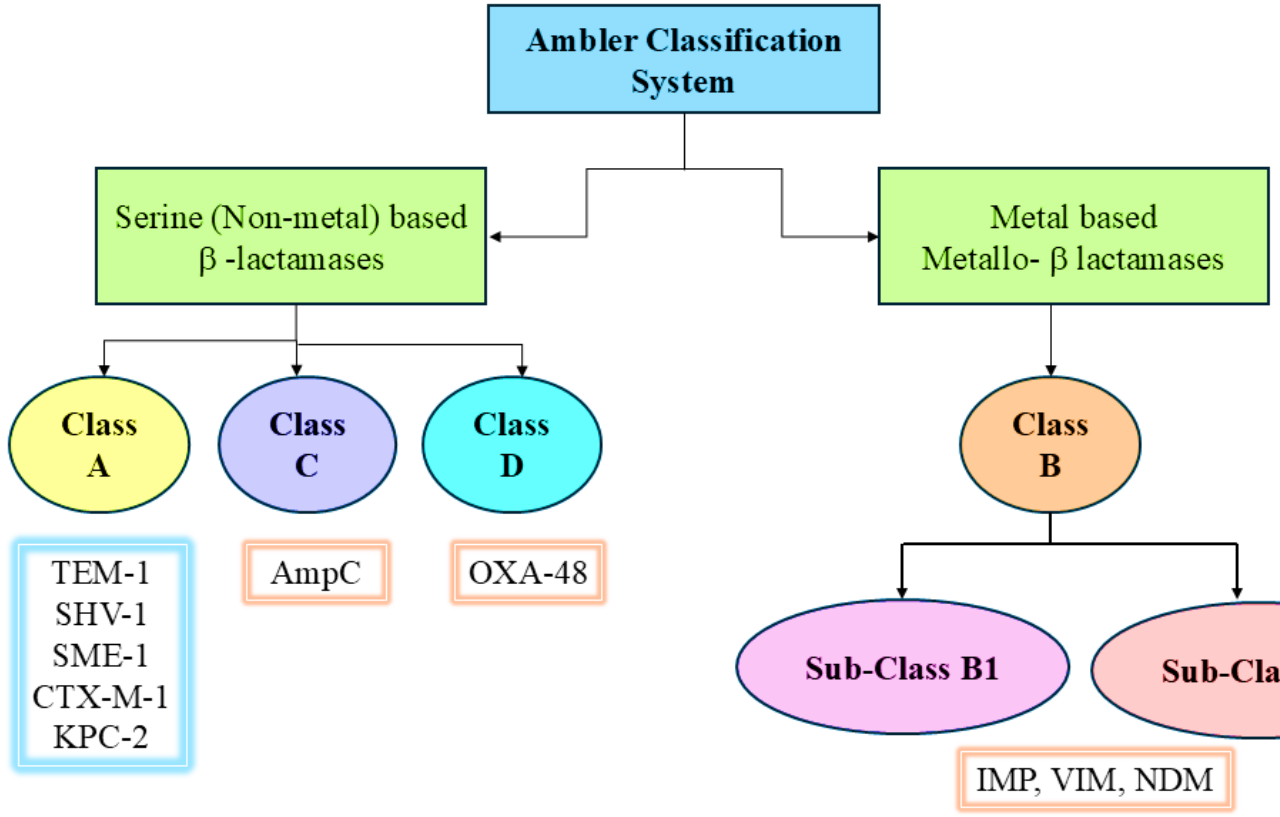


Enzymatic Cleavage of the β-Lactam Ring Leading to Hydrolysis and Loss of Drug Efficacy

Beta-lactam antibiotics for clinical use characterized due to the inclusion of a β-lactam ring in their chemical structure

Division of β-lactamases

- Ambler Classification: Class A (serine-based), B (metallo), C, and D.
- Our Focus is on **Class A serine β-lactamases**.



Flowchart represen
mode of action of β
in targeting bacteria
enzy

. Inhibit
tidoglycan
ss-Linking

ents the formation
eptidoglycan layers

Osmotic Pressure Disruption

rupts the balance of
otic pressure within
the cell

ctericidal Effect

Results in the death of bacterial cells

Representation of (A.) Beta-lactamases belonging to Class A, C, D with key residue Serine at it's active site (B.) Beta-lactamases belonging to Class B with Metal at it's active site. Blue highlights the active sites of serine-β-lactamases, and the zinc ions in metallo-β-lactamases are depicted in Green spheres.

Problem Statement

- Despite mounting attention in recent years, health threats posed by antimicrobial resistance are not new.

β-lactamase diversity limits current inhibitors.

Need for novel, broad-spectrum peptide-based inhibitors.

METHODOLOGY

Research Objective & Hypothesis

- **Objective:** AI-based design of inhibitors for Class A β-lactamases.
- **Hypothesis:** Rationally designed peptides can inhibit these enzymes.

Four groups of peptides were generated as follows:

S.No.	Position	Conserved Regions	Length	Shorthand
1	41-44	RLGV		
2	59-62	DERF		
3	103-106	VSPL		
4	136-139	NLLL		
5	143-146	LGGP		
6	178-183	RDTTTP		
7	251-258	NDVAILWP		
8	262-269	PLLVVIY		
9	130-132	SDN		
10	234-237	DKTG		
11	70-73	STFK	4	C11
12	166-170	EPELN	5	C12

sts of heptamer peptides based on the BLIP-1 template.

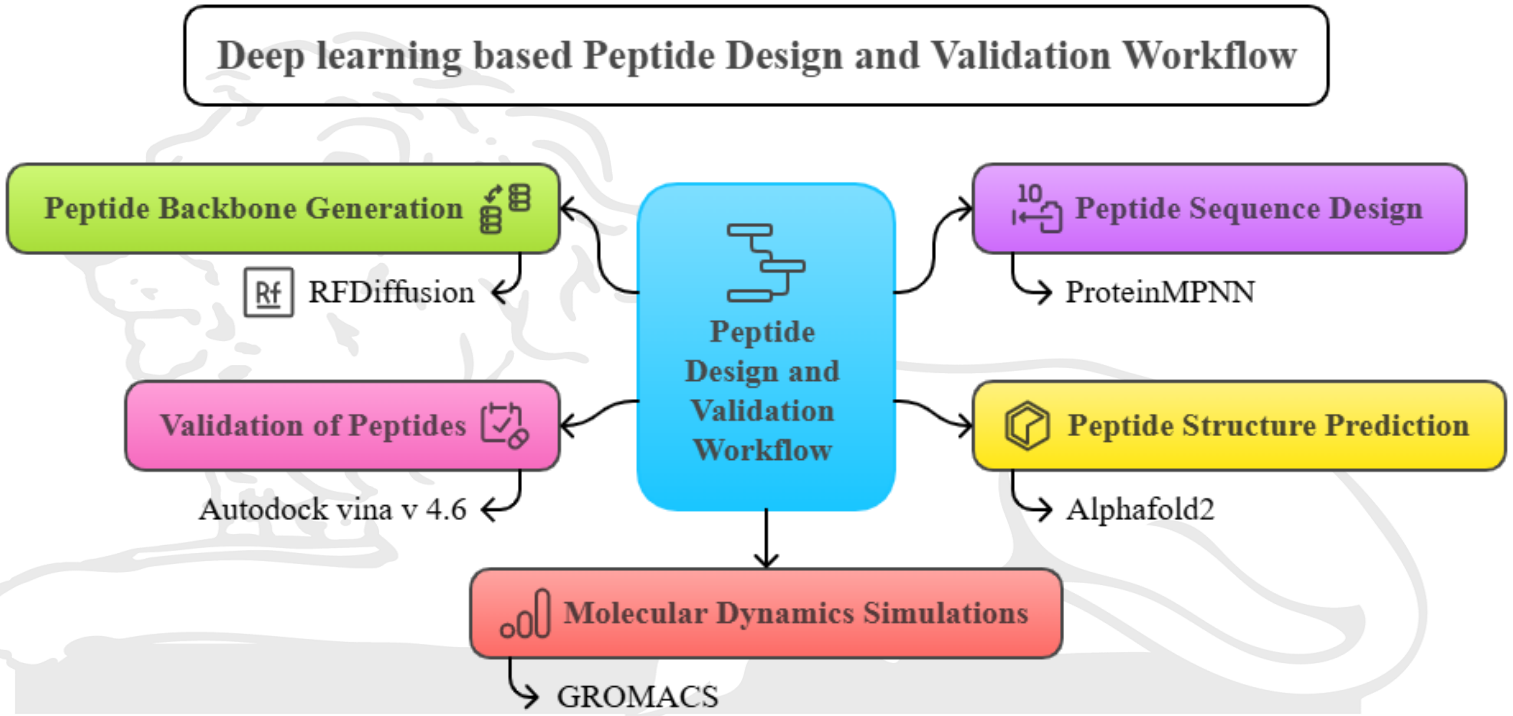
S.No.	Reference sequence	Peptide Name	Peptide Sequence	Length
1	RWEPELNEAIPNDERD	P1	EYRIR	5
2	RWEPELNEAIPNDERD	P2	TYRLR	5
3	RWEPELNEAIPNDERD	P3	TSHLR	5
4	RWEPELNEAIPNDERD	P4	TTHIR	5
5	RWEPELNEAIPNDERD	P5	ETHIH	5
6	RWEPELNEAIPNDERD	P6	TSHLH	5
7	RWEPELNEAIPNDERD	P7	ESRLH	5
8	RWEPELNEAIPNDERD	P8	ESHIH	5
9	RWEPELNEAIPNDERD	P9	ESRIH	5
10	RWEPELNEAIPNDERD	P10	TYHLH	5

4. Peptides Designed by Biswal et al. (2023) Considered as Templates for Mimicking Antimicrobial Activity

esidue (Blue) in Class A β-Lactamase (PDB ID: 8GIJ, 1.59 Å Resolution)

Workflow

- **Pipeline**: Peptide Backbone design → sequence prediction → structure validation.

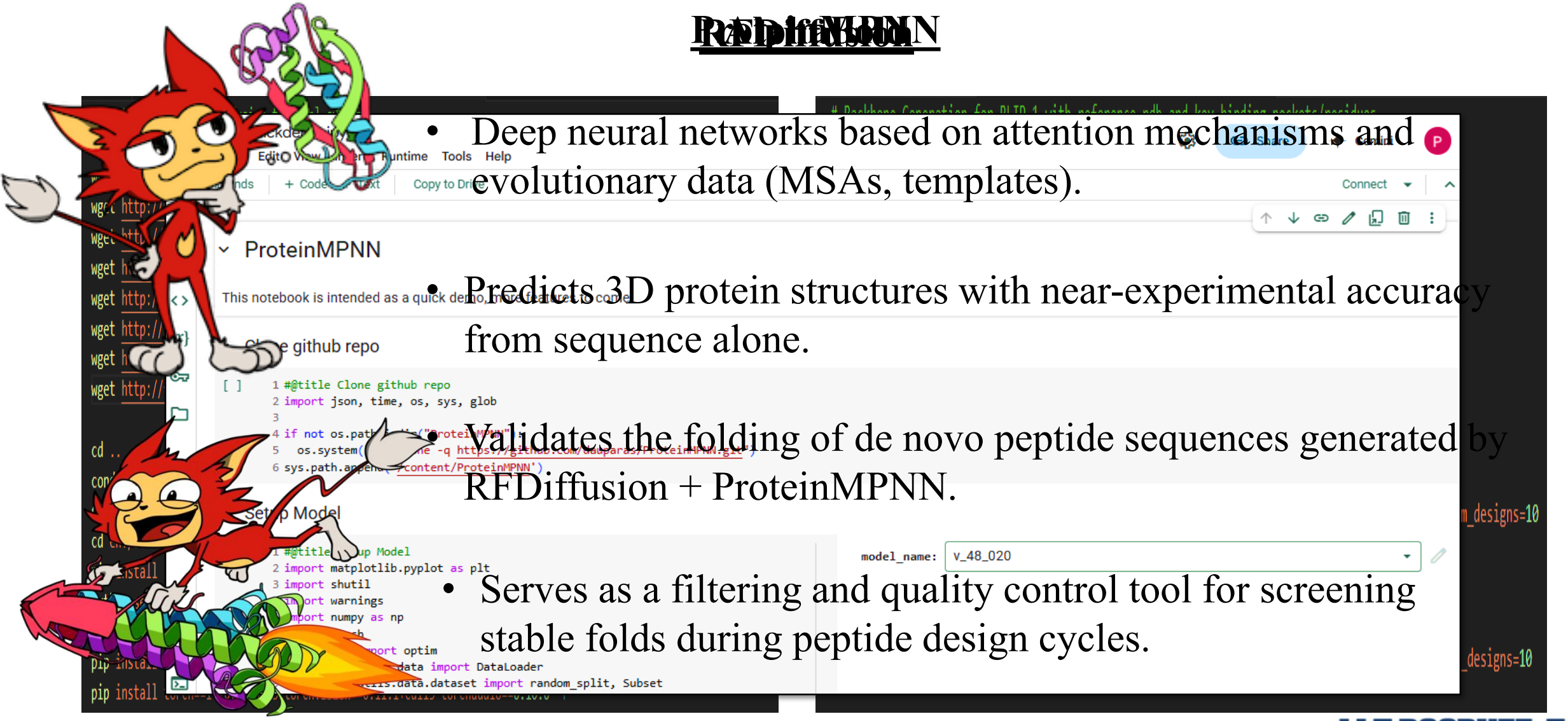


Workflow for Deep Learning-Based Peptide Design and Validation

METHODOLOGY

Material and Methods

RFDiffusion + ProteinMPNN



- Deep neural networks based on attention mechanisms and evolutionary data (MSAs, templates).
- Predicts 3D protein structures with near-experimental accuracy from sequence alone.
- Validates the folding of de novo peptide sequences generated by RFDiffusion + ProteinMPNN.
- Serves as a filtering and quality control tool for screening stable folds during peptide design cycles.

Material and Methods

Automated script for MD simulations

C: > Users > Pakhi > Desktop > $ sh

```
#!/bin/bash
# Define I/O directories
INPUT_DIR="Docked_Structures_Edited/CTXM_1_edited"
OUTPUT_DIR="Simulation_results/CTXM_1_simulation"
mkdir -p "$OUTPUT_DIR"

# Set GROMACS path
export GMX_PATH="/root/miniconda3/envs/gromacs_env/bin.AVX2_
export PATH="$GMX_PATH:$PATH"

# Ensure all required .mdp files are present
MDP_FILES=("ions.mdp" "minim.mdp" "nvt.mdp" "npt.mdp" "md.mdp")

for mdp in "${MDP_FILES[@]}"; do
    if [ ! -f "$mdp" ]; then
        echo "Error: Missing required file $mdp in the main directo
        exit 1
    fi
done

# Function to process a single PDB file
process_pdb() {
    pdb_file=$1
    filename=$(basename "$pdb_file" .pdb)
    work_dir="$OUTPUT_DIR/$filename"

    mkdir -p "$work_dir"
    cp "$pdb_file" "$work_dir/structure.pdb"

    # Copy all .mdp files into the working directory
    for mdp in "${MDP_FILES[@]}"; do
        cp "$mdp" "$work_dir/"
```

C: > Users > Pakhi > Desktop > $ sh

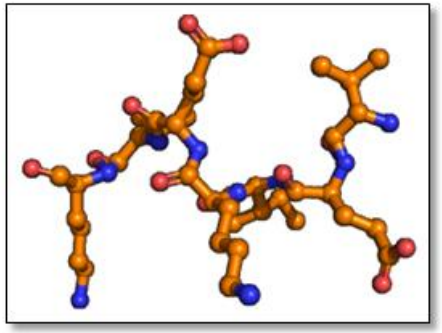
```
process_pdb() {
    "$GMX_PATH/gmx" pdb2gmx -f structure.pdb -o fnl_processed.gro -ff amber99sb-ildn -water tip3p <<EOF
6
1
EOF
    "$GMX_PATH/gmx" editconf -f fnl_processed.gro -o fnl_processed.pdb
    "$GMX_PATH/gmx" editconf -f fnl_processed.gro -o fnl_newbox.gro -c -d 1.0 -bt cubic
    "$GMX_PATH/gmx" editconf -f fnl_newbox.gro -o fnl_newbox.pdb
    "$GMX_PATH/gmx" solvate -cp fnl_newbox.gro -cs spc216.gro -o fnl_solv.gro -p topol.top
    "$GMX_PATH/gmx" editconf -f fnl_solv.gro -o fnl_solv.pdb

    "$GMX_PATH/gmx" grompp -f ions.mdp -c fnl_solv.gro -p topol.top -o ions.tpr -maxwarn 2
    echo "13" | "$GMX_PATH/gmx" genion -s ions.tpr -o fnl_solv_ions.gro -pname NA -nname CL -neutral -conc 0.15 -p topol.top

    "$GMX_PATH/gmx" grompp -f minim.mdp -c fnl_solv_ions.gro -p topol.top -o em.tpr
    "$GMX_PATH/gmx" mdrun -v -deffnm em
    "$GMX_PATH/gmx" editconf -f em.gro -o em.pdb

    "$GMX_PATH/gmx" energy -f em.edr -o pe_em.xvg <<EOF
10
0
EOF
    "$GMX_PATH/gmx" grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o nvt.tpr
    "$GMX_PATH/gmx" mdrun -nt 8 -deffnm nvt -v

    "$GMX_PATH/gmx" grompp -f npt.mdp -c nvt.gro -r nvt.gro -t nvt.cpt -p topol.top -o npt.tpr -maxwarn 2
    "$GMX_PATH/gmx" mdrun -nt 8 -deffnm npt -v

    "$GMX_PATH/gmx" grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -o md_0_1.tpr

    echo "Generated md_0_1.tpr for $filename"
    cd ../../
```

RESULTS

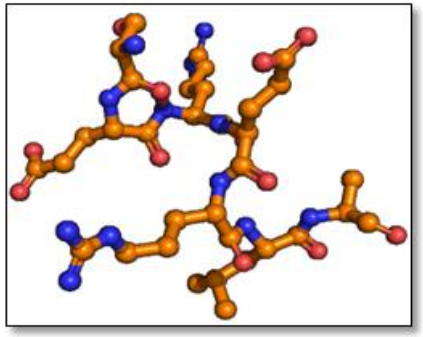
Backbone Generation with RFDiffusion

- Generated **240 p**
- Us

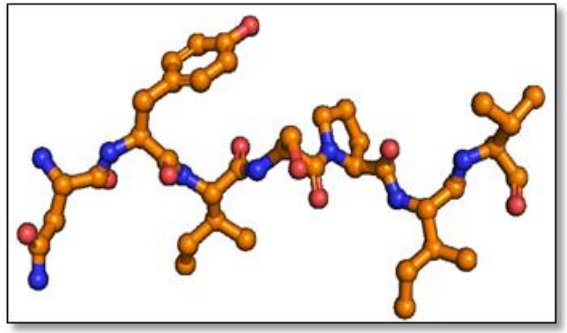
S.No	Ref peptide	Backbone	Length of	Predicted



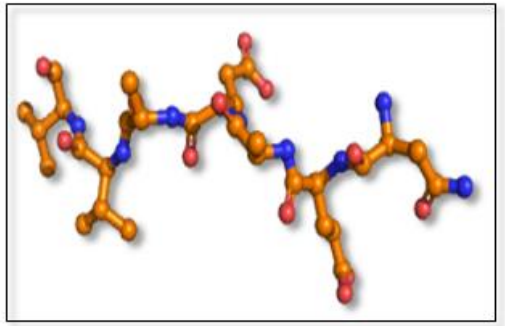
VAR_C1_0.pdb



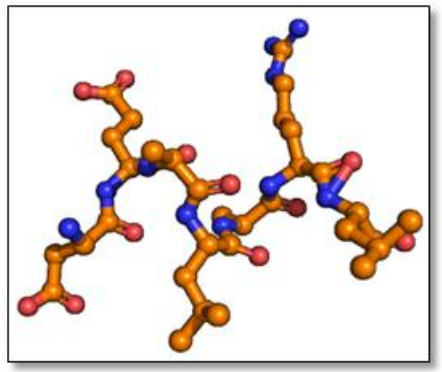
VAR_C1_1.pdb



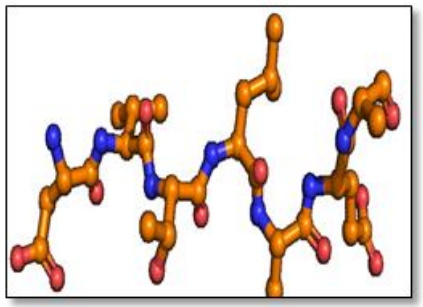
VAR_C1_2.pdb



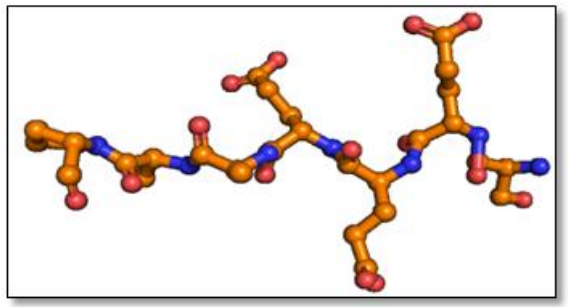
VAR_C1_6.pdb



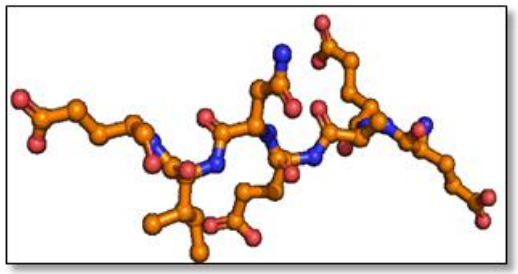
VAR_C1_3.pdb



VAR_C1_4.pdb



VAR_C1_5.pdb



VAR_C1_7.pdb

Predicted Peptide Sequences Generated from Class A β-Lactamase Structure Prediction of Peptides Using AlphaFold2 for β-Lactamase Inhibition Peptide Backbone Generated for β-Lactamase Motif Using RFDiffusion

Variant Motif-Based Scaffolds Using ProteinMPNN

Generated for β-Lactamase Motif C2 (TERT, Positions 59-62)

RESULTS

Binding affinities and docking scores with β-lactamase targets

S.No.	Predicted Peptide	KPC-2	CTX-M-1	SME-1	TEM-1	SHV-1
		Binding Energy	Binding Energy	Binding Energy	Binding Energy	Binding Energy
1	BLIP_1_P0.pdb	-5.6	-5.1	-4.8	10.6	-4.7
2	BLIP_1_P1.pdb	-5.5	-5.9	-5.7	-0.8	-6.3
3	BLIP_1_P2.pdb	-5.3	-5.4	-5.1	3.6	-5.6
4	BLIP_1_P3.pdb	-5.9	-5.3	-5.4	-0.3	-5.9
5	BLIP_1_P4.pdb	-5.7	-5.3	-5	-0.9	-5.6
6	BLIP_1_P5.pdb	-5.6	-5.2	-5.5	0.3	-5.9
7	BLIP_1_P6.pdb	-6	-5.5	-5.2	-2.7	-5.8
8	BLIP_1_P7.pdb	-5.3	-4.9	-5.5	4.2	-5.4
9	BLIP_1_P8.pdb	-5.7	-5.5	-5.1	29.7	-6
10	BLIP_1_P9.pdb	-6.1	-5.9	-5.8	-0.6	-5.6

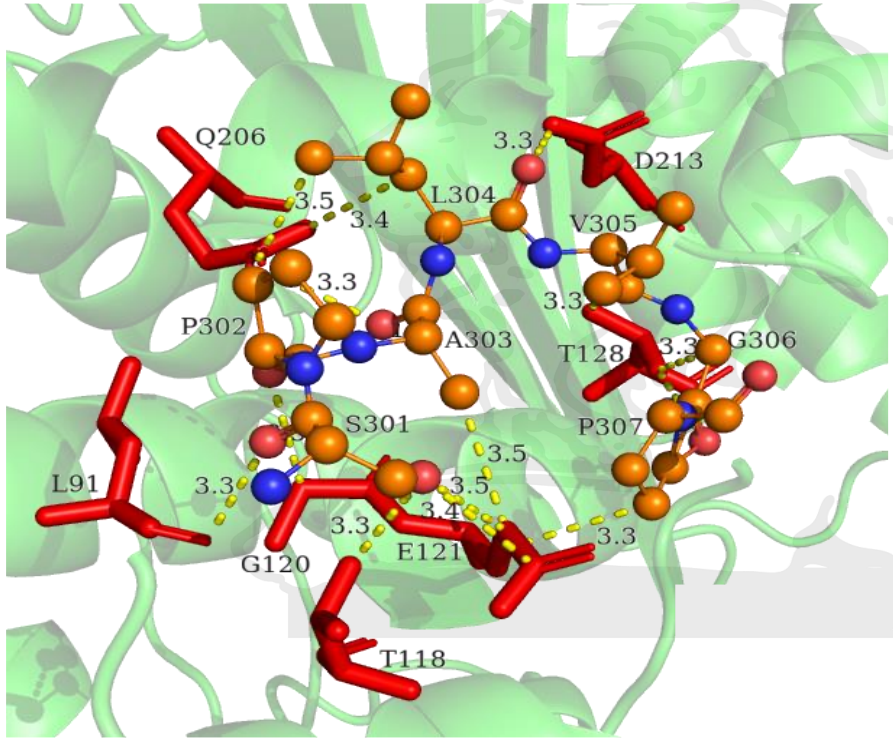
This table presents the top docking scores for the selected β-lactamase enzymes when complexed with BLIP-1 based peptides. Higher negative docking scores generally indicate stronger binding and more favorable interactions between the peptide and enzyme active sites

Representation of the top docking scores for the selected β-lactamase enzymes when complexed with Class-A β-lactamase C2 (DERF, position: 59-62) motif-based peptides.

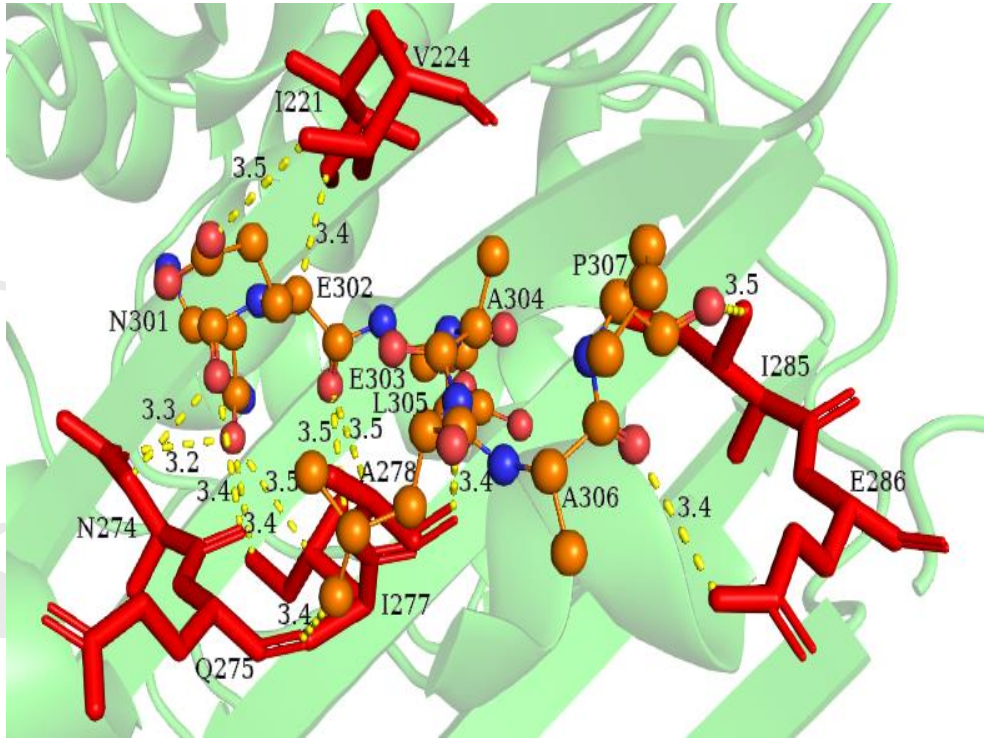
S.No.	Predicted Peptide	KPC-2	CTX-M-1	SME-1	TEM-1	SHV-1
		Binding Energy	Binding Energy	Binding Energy	Binding Energy	Binding Energy
1	VAR_C1_0.pdb	-5.2	-4.8	-4.7	-0.8	-4.8
2	VAR_C1_1.pdb	-5.6	-5.5	-4.9	1.9	-5.8
3	VAR_C1_2.pdb	-6.5	-5.8	-5.4	1.8	-5.4
4	VAR_C1_3.pdb	-6.2	-5.1	-5.2	24.4	-5.2
5	VAR_C1_4.pdb	-6	-5.7	-5.3	2.2	-5.7
6	VAR_C1_5.pdb	-5.7	-5.5	-5.6	-0.4	-6.5
7	VAR_C1_6.pdb	-6.1	-5.6	-6	-2.7	-6.5
8	VAR_C1_7.pdb	-5.5	-5.4	-5.4	-2.4	-5.3
9	VAR_C1_8.pdb	-6.2	-5.4	-5.6	5.3	-6
10	VAR_C1_9.pdb	-6	-5.3	-5.2	1.6	-6.3

Molecular Docking

- Visualization of docked interactions for Var_C11_1_SHV-1 (-7.2 A°) and OM_PAP6_1_SHV_1 (-7.3 A°), representing the peptides with the lowest binding affinities among 240 generated sequences



VAR_C11_1_SHV_1
Binding score: -7.2 A°

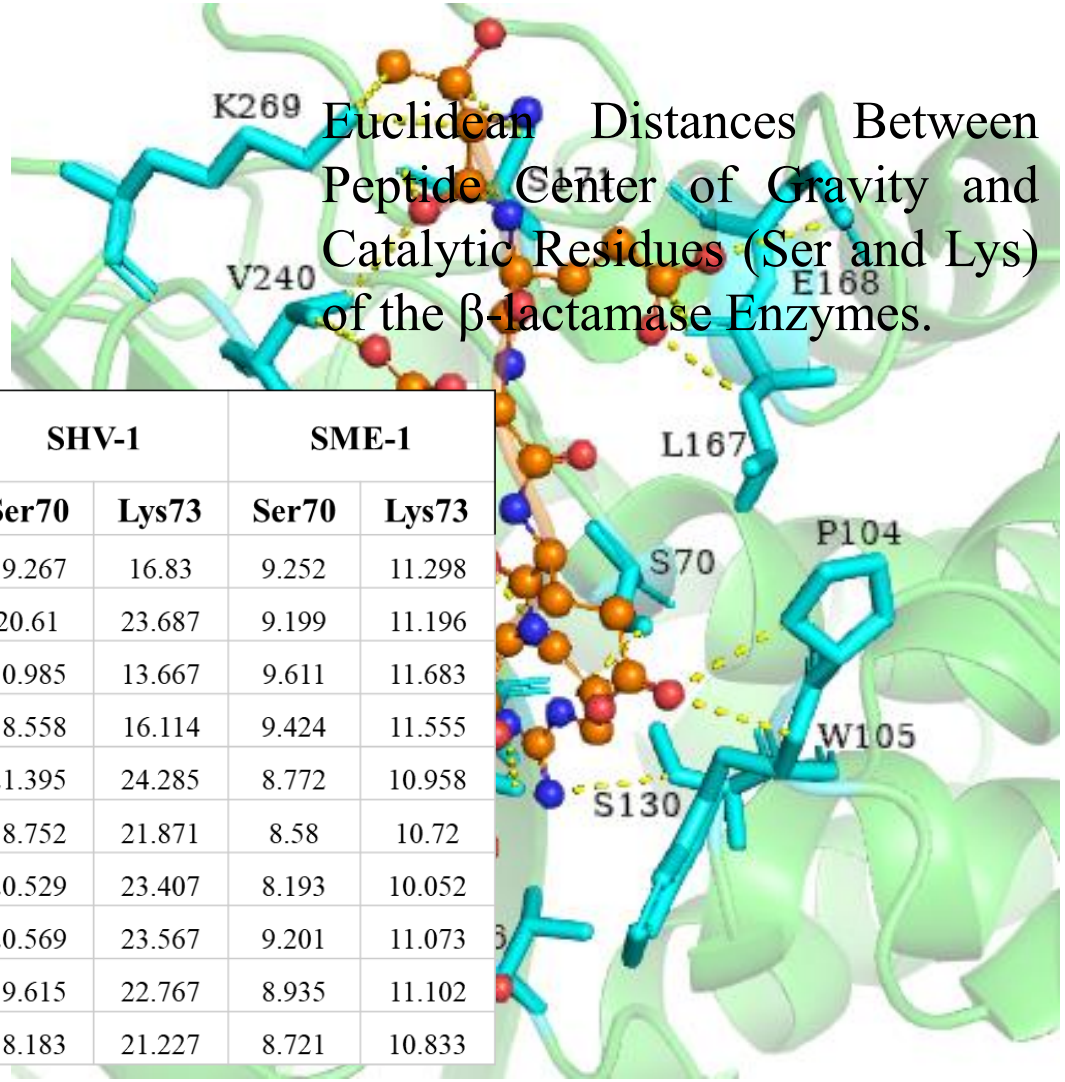


OM_PAP6_1_SHV_1
Binding score: -7.3 A°

RESULTS

Interaction visualization & interatomic distances

- Active site engagement visualized.
- Euclidean distances measured.
- MD confirms stability of peptide-enzyme complexes.



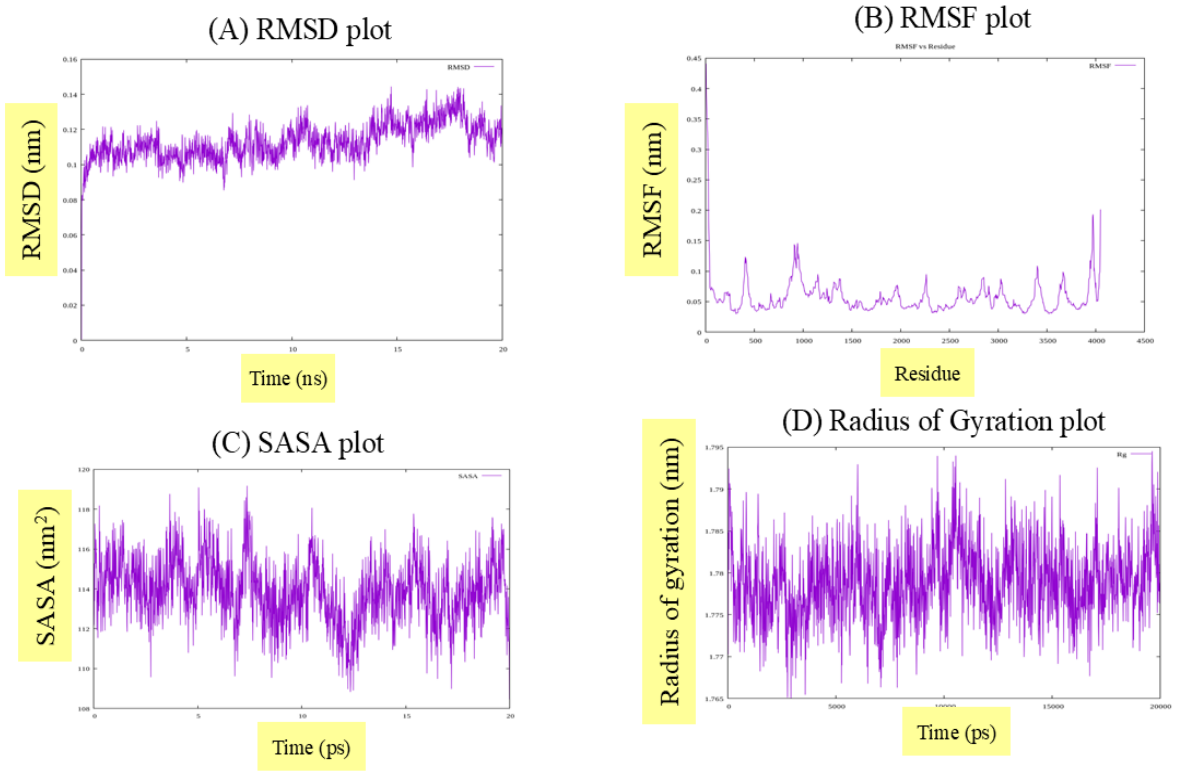
Euclidean Distances Between Peptide Center of Gravity and Catalytic Residues (Ser and Lys) of the β-lactamase Enzymes.

S.No.	Predicted Peptide	KPC-2		CTXM-1		TEM-1		SHV-1		SME-1	
		Ser70	Lys73	Ser70	Lys73	Ser70	Lys73	Ser70	Lys73	Ser70	Lys73
1	VAR_C1_0.pdb	9.165	11.063	10.157	12.117	20.512	21.42	19.267	16.83	9.252	11.298
2	VAR_C1_1.pdb	9.567	11.52	9.396	11.218	20.289	21.134	20.61	23.687	9.199	11.196
3	VAR_C1_2.pdb	8.831	10.998	9.935	11.851	19.896	20.867	10.985	13.667	9.611	11.683
4	VAR_C1_3.pdb	8.741	10.404	10.035	12.055	20.271	21.252	18.558	16.114	9.424	11.555
5	VAR_C1_4.pdb	8.401	10.195	9.243	11.346	19.755	20.584	21.395	24.285	8.772	10.958
6	VAR_C1_5.pdb	8.838	10.743	9.467	11.487	20.554	21.47	18.752	21.871	8.58	10.72
7	VAR_C1_6.pdb	8.423	10.341	9.042	10.96	19.679	20.617	20.529	23.407	8.193	10.052
8	VAR_C1_7.pdb	8.534	10.624	9.672	11.757	19.987	20.947	20.569	23.567	9.201	11.073
9	VAR_C1_8.pdb	8.647	10.751	9.24	11.091	19.789	20.688	19.615	22.767	8.935	11.102
10	VAR_C1_9.pdb	8.438	10.505	9.985	12.066	20.176	21.001	18.183	21.227	8.721	10.833

RESULTS

Molecular Dynamics Simulations

- The RMSD plot indicated complex stabilization after **~5 ns**, maintaining values between **0.12–0.14 nm**.
- RMSF analysis showed minimal residue fluctuations **(<0.1 nm)**, with higher flexibility **(~0.35 nm)** in loop and terminal regions.
- SASA remained stable (**110–125 nm²**), and
- Rg ranged from **1.775–1.790 nm**, indicating sustained structural compactness.



Assessment of VAR_C2_8–CTX-M-1 Complex using Molecular Dynamics Simulation

CONCLUSION

- Designed peptides show promise as β-lactamase inhibitors.
- Future work: Experimental validation and optimization.
- In silico results require experimental validation.
- Physicochemical Evaluation: Solubility, stability, secondary structure.
- In Vitro Assays: Nickel-NTA pull-down (binding); MIC, β-lactamase inhibition (efficacy); Hemolysis, cytotoxicity (safety)
- In Vivo Validation: Murine infection models; Pharmacokinetics, pharmacodynamics analysis; Toxicity and long-term safety profiling

REFERENCES

- Biswal, S., Caetano, K., Jain, D., Sarrila, A., Munshi, T., Dickman, R., ... & Ghosh, A. S. (2023). Antimicrobial Peptides Designed against the Ω-Loop of Class A β-Lactamases to Potentiate the Efficacy of β-Lactam Antibiotics. Antibiotics, 12(3), 553.

- Clement, C. Structure-based drug design (SBDD) and in silico pharmacophore screening approaches combined with biochemical validation enabled the discovery of di-and tetrapeptides modulators of Y-49, TEM-1 and ampC beta lactamases.

- Rettie, S. A., Juergens, D., Adebomi, V., Bueso, Y. F., Zhao, Q., Leveille, A. N., ... & Bhardwaj, G. (2024). Accurate de novo design of high-affinity protein binding macrocycles using deep learning. bioRxiv.

- Wang, B., Lin, P., Zhong, Y., Tan, X., Shen, Y., Huang, Y., ... & Wu, Y. (2025). Explainable deep learning and virtual evolution identifies antimicrobial peptides with activity against multidrug-resistant human pathogens. Nature Microbiology, 1-16.

- Wu, M., Li, Y., Shen, H., Zhang, Y., Cong, W., Hu, X., ... & Hu, H. (2024). A β-Lactamase Responsive Peptide Inhibits MRSA Infection through Self-Assembled Nanonet. Advanced Healthcare Materials, 13(31), 2402453.

Thank you