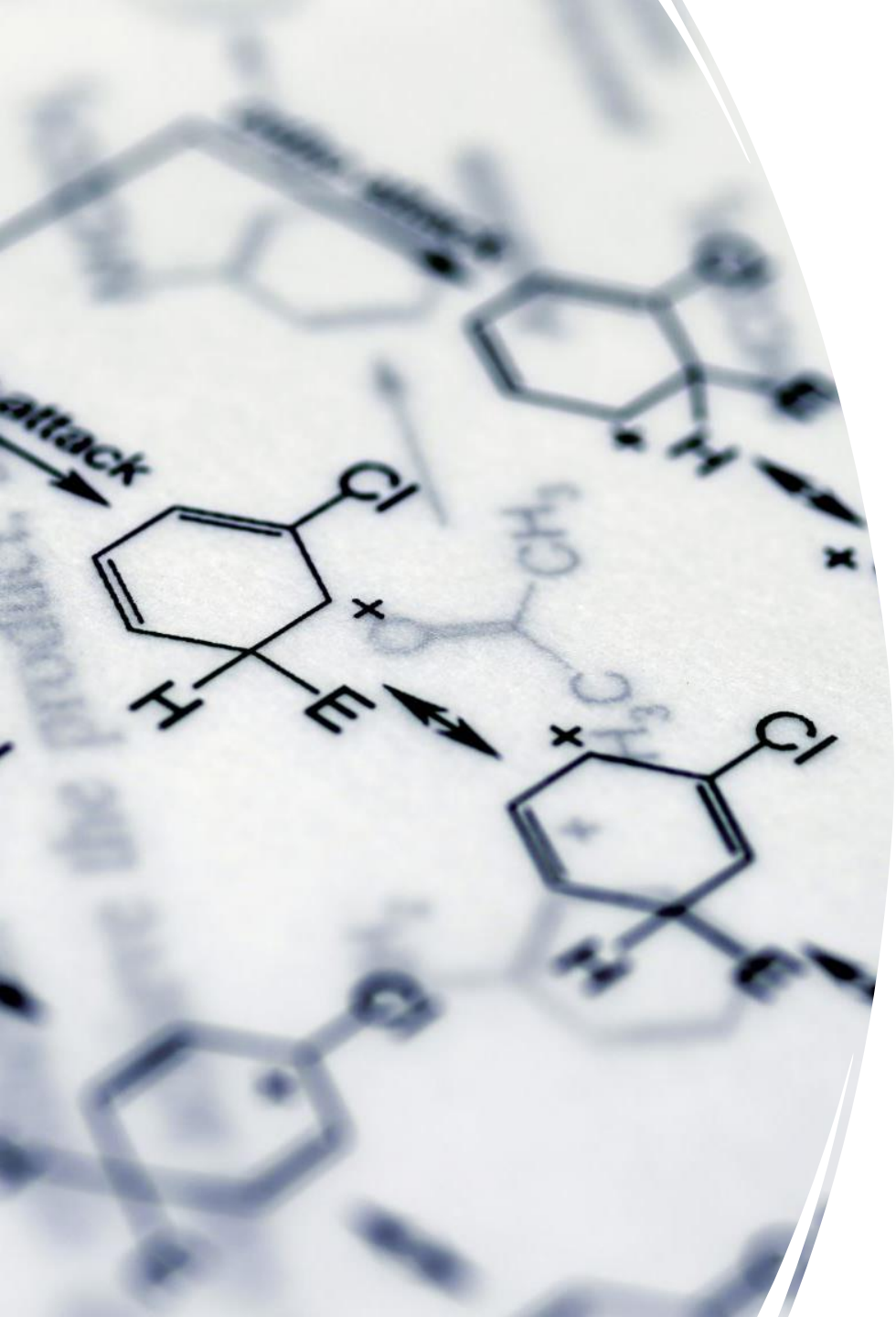


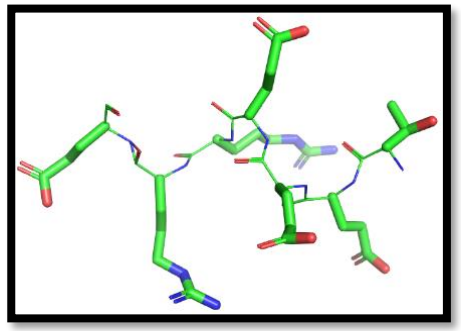
# Deep learning approach for accurate De-novo design of hyperstable peptide Inhibitors against Class A β-lactamases

# Insights on BLIP-I based peptides

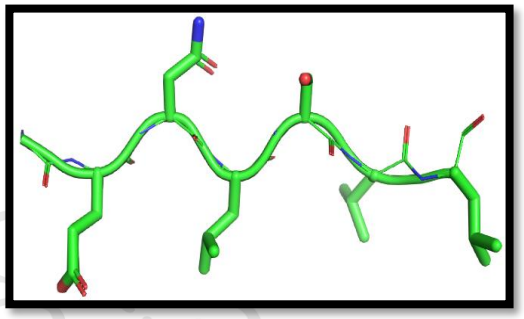
| S.No. | Reference PDB Structure | Backbone structure | Sequence Length | Predicted sequences | Predicted 3D structure | Charge (Kcal/mol) |
|---|---|---|---|---|---|---|
| 1 | BLIP_3GMU.pdb | BLIP_B0.pdb | 7 | TEEERRE | BLIP_P0.pdb | 63.309 |
| 2 | BLIP_3GMU.pdb | BLIP_B1.pdb | 7 | GENLSLL | BLIP_P1.pdb | 66.844 |
| 3 | BLIP_3GMU.pdb | BLIP_B2.pdb | 7 | ELEKLLS | BLIP_P2.pdb | 82.123 |
| 4 | BLIP_3GMU.pdb | BLIP_B3.pdb | 7 | ISEEALT | BLIP_P3.pdb | 76.202 |
| 5 | BLIP_3GMU.pdb | BLIP_B4.pdb | 7 | GLAEKLA | BLIP_P4.pdb | 71.297 |
| 6 | BLIP_3GMU.pdb | BLIP_B5.pdb | 7 | LEDLTEA | BLIP_P5.pdb | 85.967 |
| 7 | BLIP_3GMU.pdb | BLIP_B6.pdb | 7 | DVDLSAG | BLIP_P6.pdb | 69.418 |
| 8 | BLIP_3GMU.pdb | BLIP_B7.pdb | 7 | SELNIIE | BLIP_P7.pdb | 80.723 |
| 9 | BLIP_3GMU.pdb | BLIP_B8.pdb | 7 | RNRLNIT | BLIP_P8.pdb | 81.029 |
| 10 | BLIP_3GMU.pdb | BLIP_B9.pdb | 7 | IPSLLSP | BLIP_P9.pdb | 59.061 |

- We has taken BLIP-1 structure (PDB id: 3GMU) as template.
- Predicted 10 De-novo peptides as summarized above.
- Total BLIP-1 based predicted peptides: **1*10 =10 peptides**

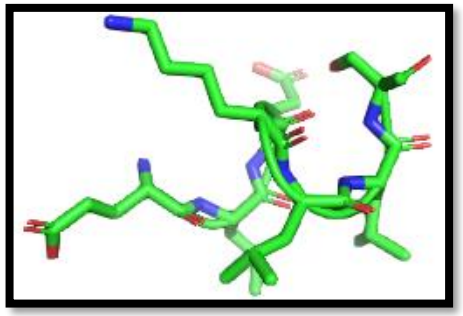
# Insights on BLIP-I based peptides



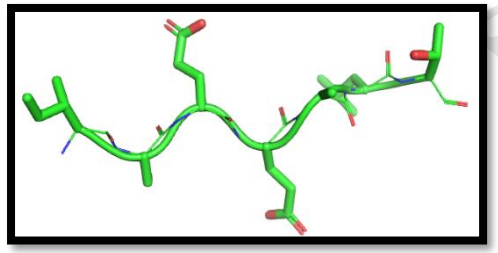
BLIP_P0



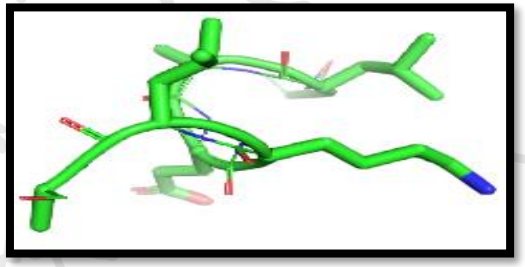
BLIP_P1



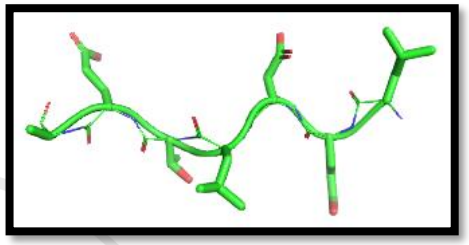
BLIP_P2



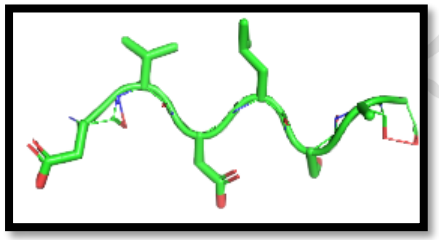
BLIP_P3



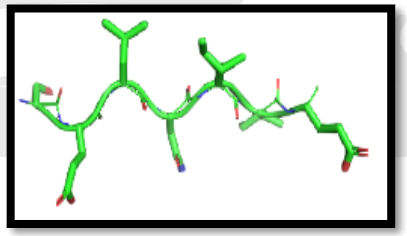
BLIP_P4



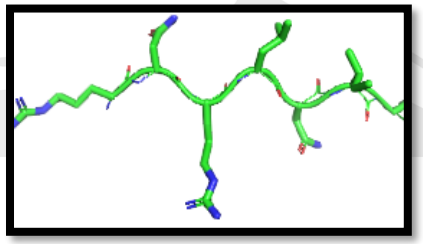
BLIP_P5



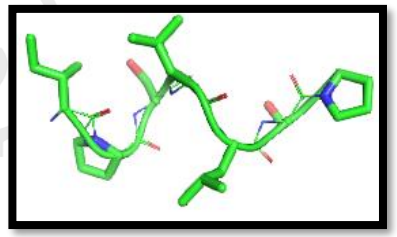
BLIP_P6



BLIP_P7



BLIP_P8



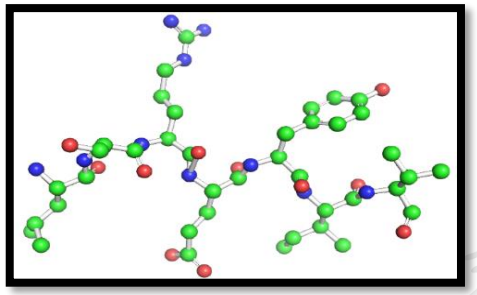
BLIP_P9

# Insights on omega loop-based peptides

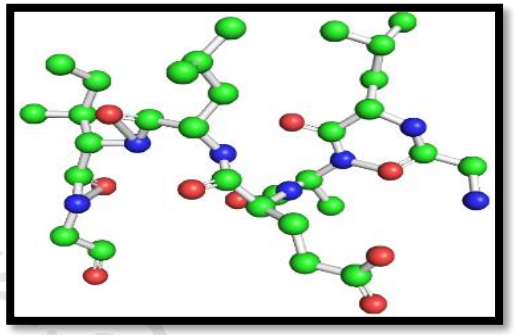
| S.No | Backbone Structure | Ref OMEGA Sequence | Predicted Sequence Length | Predicted Sequence | Predicted 3D structure | Charge (kcal/mol) |
|---|---|---|---|---|---|---|
| 1 | OM_0.pdb | RWEPELNEAIPNDERD | 7 | LSREYIV | OM_P0.pdb | 66.768 |
| 2 | OM_1.pdb | RWEPELNEAIPNDERD | 7 | GLAELIG | OM_P1.pdb | 48.636 |
| 3 | OM_2.pdb | RWEPELNEAIPNDERD | 7 | EEALLAG | OM_P2.pdb | 62.497 |
| 4 | OM_3.pdb | RWEPELNEAIPNDERD | 7 | LEEELRG | OM_P3.pdb | 78.201 |
| 5 | OM_4.pdb | RWEPELNEAIPNDERD | 7 | AERLEIS | OM_P4.pdb | 77.06 |
| 6 | OM_5.pdb | RWEPELNEAIPNDERD | 7 | SSGVLAP | OM_P5.pdb | 46.841 |
| 7 | OM_6.pdb | RWEPELNEAIPNDERD | 7 | AEAVLAA | OM_P6.pdb | 61.965 |
| 8 | OM_7.pdb | RWEPELNEAIPNDERD | 7 | GEIRLGG | OM_P7.pdb | 64.568 |
| 9 | OM_8.pdb | RWEPELNEAIPNDERD | 7 | NTAVNTA | OM_P8.pdb | 52.656 |
| 10 | OM_9.pdb | RWEPELNEAIPNDERD | 7 | DEALLAG | OM_P9.pdb | 79.412 |

- We has taken omega loop as the template.
- Predicted 10 de-novo peptides as summarized above.
- Total Omega loop-based de-novo peptides: **1*10 =10 peptides**

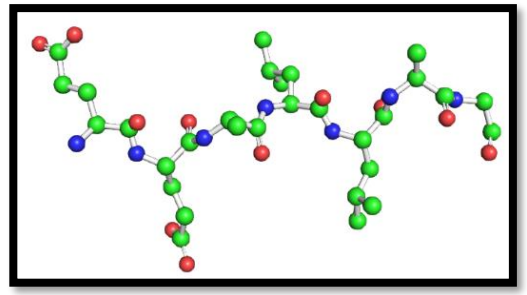
# Insights on omega loop-based peptides



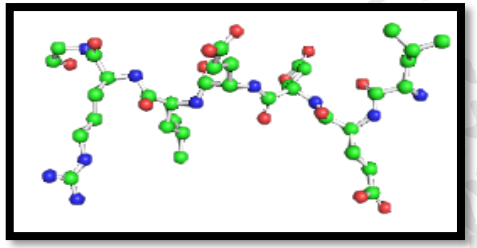
OM_P0.pdb



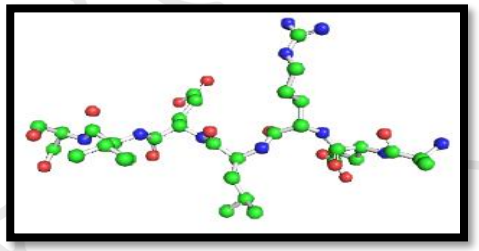
OM_P1.pdb



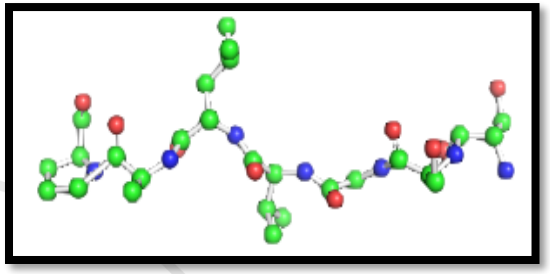
OM_P2.pdb



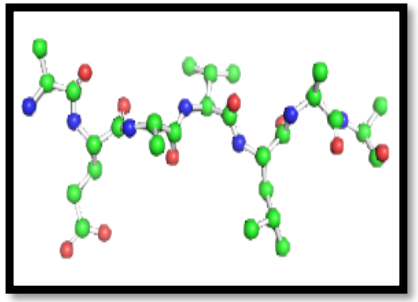
OM_P3.pdb



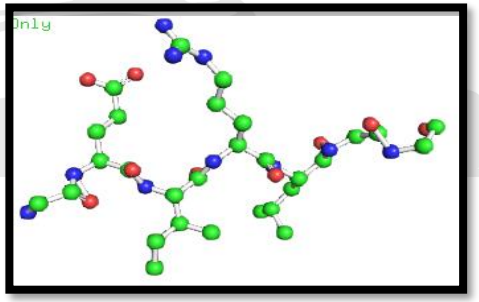
OM_P4.pdb



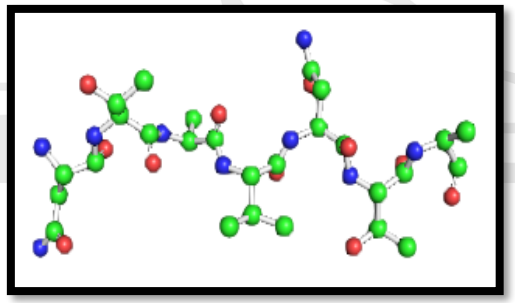
OM_P5.pdb



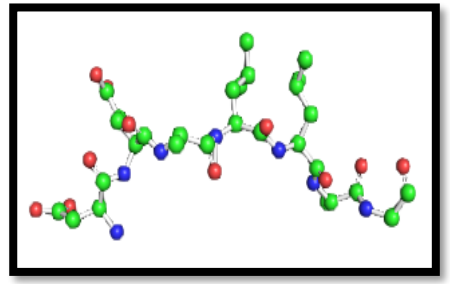
OM_P6.pdb



OM_P7.pdb



OM_P8.pdb



OM_P9.pdb

# Insights on omega loop-based peptides

| s.no | Ref P7 Sequence | Generated Backbone | Length of generated peptide | Generated AA sequence | Generate 3D structure |
|---|---|---|---|---|---|
| 1 | ESRLH | OM_PP7_0.pdb | 7 | RERELLS | OM_PAP7_0.pdb |
| 2 | ESRLH | OM_PP7_1.pdb | 7 | NLEELLS | OM_PAP7_1.pdb |
| 3 | ESRLH | OM_PP7_2.pdb | 7 | EEALRRA | OM_PAP7_2.pdb |
| 4 | ESRLH | OM_PP7_3.pdb | 7 | ALNEREA | OM_PAP7_3.pdb |
| 5 | ESRLH | OM_PP7_4.pdb | 7 | NLRELLE | OM_PAP7_4.pdb |
| 6 | ESRLH | OM_PP7_5.pdb | 7 | EGIEERG | OM_PAP7_5.pdb |
| 7 | ESRLH | OM_PP7_6.pdb | 7 | EEALIIE | OM_PAP7_6.pdb |
| 8 | ESRLH | OM_PP7_7.pdb | 7 | ADATERA | OM_PAP7_7.pdb |
| 9 | ESRLH | OM_PP7_8.pdb | 7 | TEAERLG | OM_PAP7_8.pdb |
| 10 | ESRLH | OM_PP7_9.pdb | 7 | SEALEKA | OM_PAP7_9.pdb |

- We has taken total of 10 such omega-loop based peptides.
- Each has 10 predicted de-novo peptides
- Total omega paper-based peptides: **10*10 =100 peptides**

# Conserved motifs in Class-A β-lactamase taken as template for AI-based peptide prediction

- Essential structural motifs common to subclasses of Class A beta-lactamase.

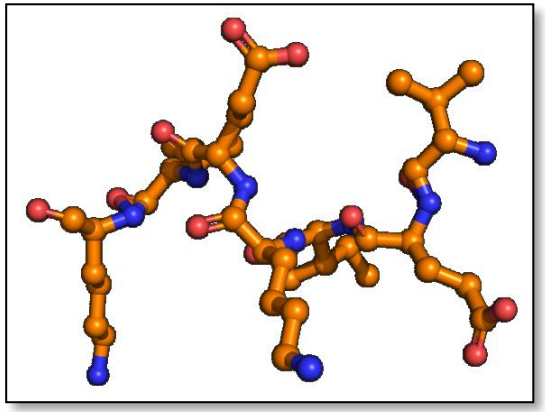
| S.No. | Position | Conserved Regions | Length | Shorthand |
|---|---|---|---|---|
| 1 | 41-44 | RLGV | 4 | C1 |
| 2 | 59-62 | DERF | 4 | C2 |
| 3 | 103-106 | VSPL | 4 | C3 |
| 4 | 136-139 | NLLL | 4 | C4 |
| 5 | 143-146 | LGGP | 4 | C5 |
| 6 | 178-183 | RDTTTP | 6 | C6 |
| 7 | 251-258 | NDVAILWP | 8 | C7 |
| 8 | 262-269 | PLLVVIY | 7 | C8 |
| 9 | 130-132 | SDN | 3 | C9 |
| 10 | 234-237 | DKTG | 4 | C10 |
| 11 | 70-73 | STFK | 4 | C11 |
| 12 | 166-170 | EPELN | 5 | C12 |

# Insights on Class-A β-lactamases variants-based peptides

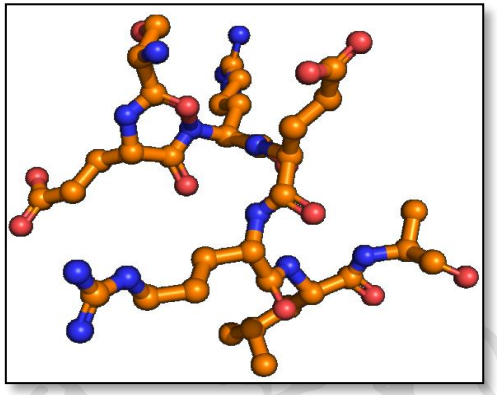
| S.No | Ref. peptide sequence | Backbone generation | Length of generated peptide | Generated AA sequence | Generate 3D structure |
|---|---|---|---|---|---|
| 1 | NDVAILWP | C7_0.pdb | 7 | TEAERLG | VAR_C7_0.pdb |
| 2 | NDVAILWP | C7_1.pdb | 7 | TLEELLK | VAR_C7_1.pdb |
| 3 | NDVAILWP | C7_2.pdb | 7 | ELEKLLA | VAR_C7_2.pdb |
| 4 | NDVAILWP | C7_3.pdb | 7 | RLAELLG | VAR_C7_3.pdb |
| 5 | NDVAILWP | C7_4.pdb | 7 | GEALRRA | VAR_C7_4.pdb |
| 6 | NDVAILWP | C7_5.pdb | 7 | EPASLTA | VAR_C7_5.pdb |
| 7 | NDVAILWP | C7_6.pdb | 7 | ELAKLTK | VAR_C7_6.pdb |
| 8 | NDVAILWP | C7_7.pdb | 7 | VLGLEKG | VAR_C7_7.pdb |
| 9 | NDVAILWP | C7_8.pdb | 7 | NLADLLA | VAR_C7_8.pdb |
| 10 | NDVAILWP | C7_9.pdb | 7 | DEAAIIP | VAR_C7_9.pdb |

- We have taken total of 12 such motifs (C1-C12).
- Each has 10 predicted de-novo peptides
- Total peptides for Class-A variants: **12*10 =120 peptides**

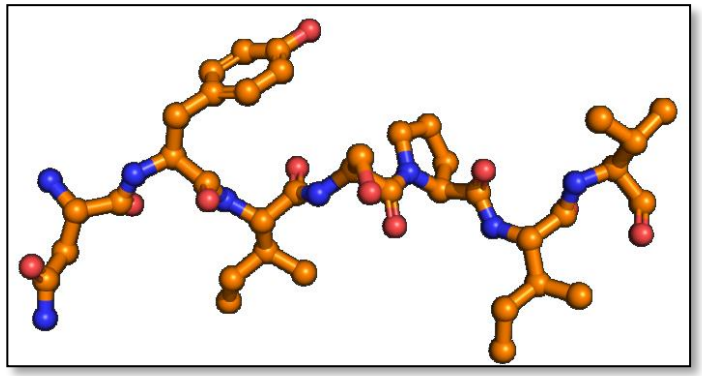
# Insights on Class-A β lactamase variants-based peptides



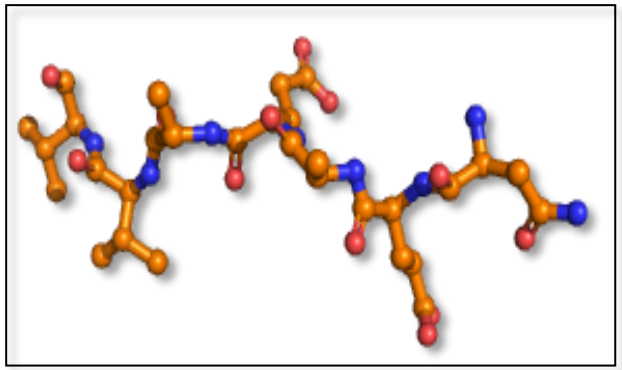
VAR_C1_0.pdb



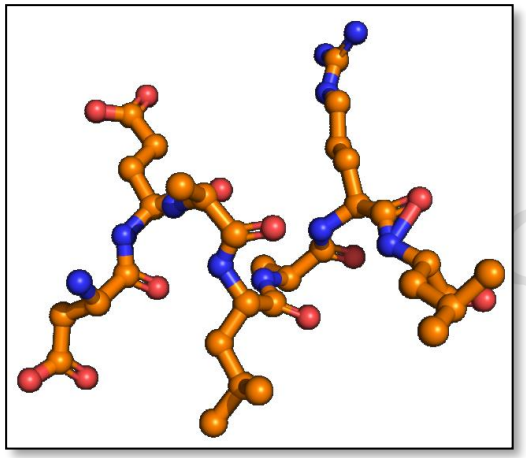
VAR_C1_1.pdb



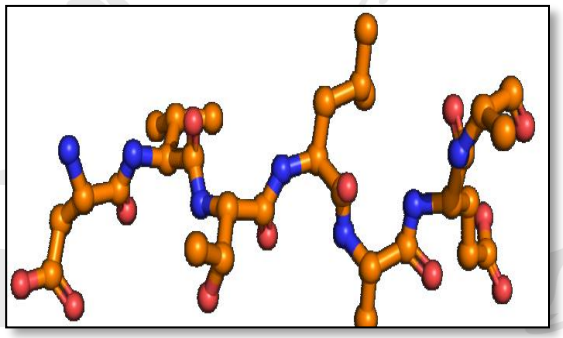
VAR_C1_2.pdb



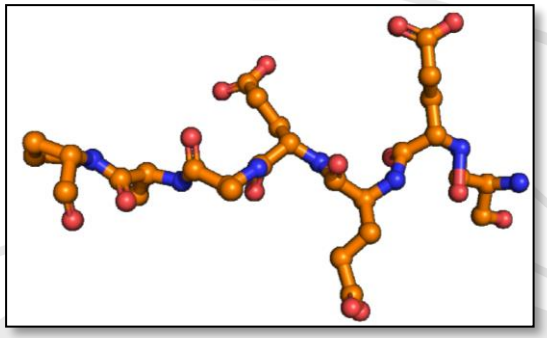
VAR_C1_6.pdb



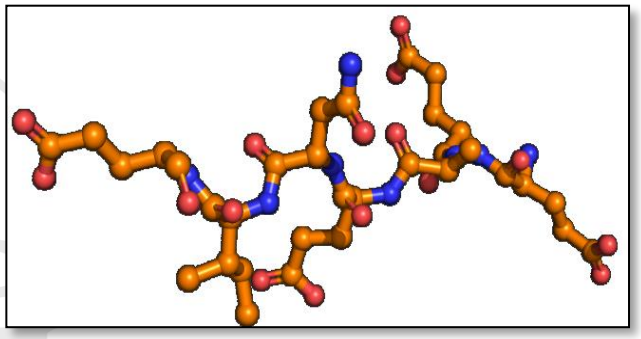
VAR_C1_3.pdb



VAR_C1_4.pdb



VAR_C1_5.pdb

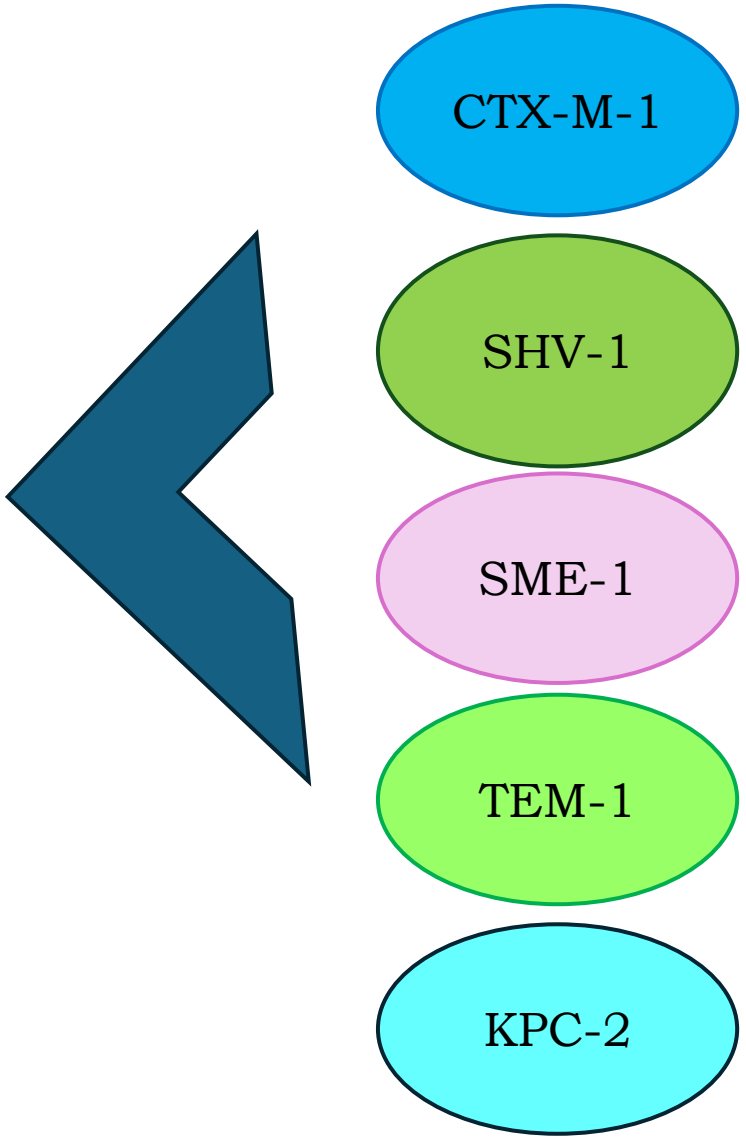


VAR_C1_7.pdb

# Number of generated peptides

| Target | Number of peptides generated |
|---|---|
| BLIP-I | 10 |
| Omega Loop | 110 |
| Class A β- lactamase | 120 |
| Total | 240 |

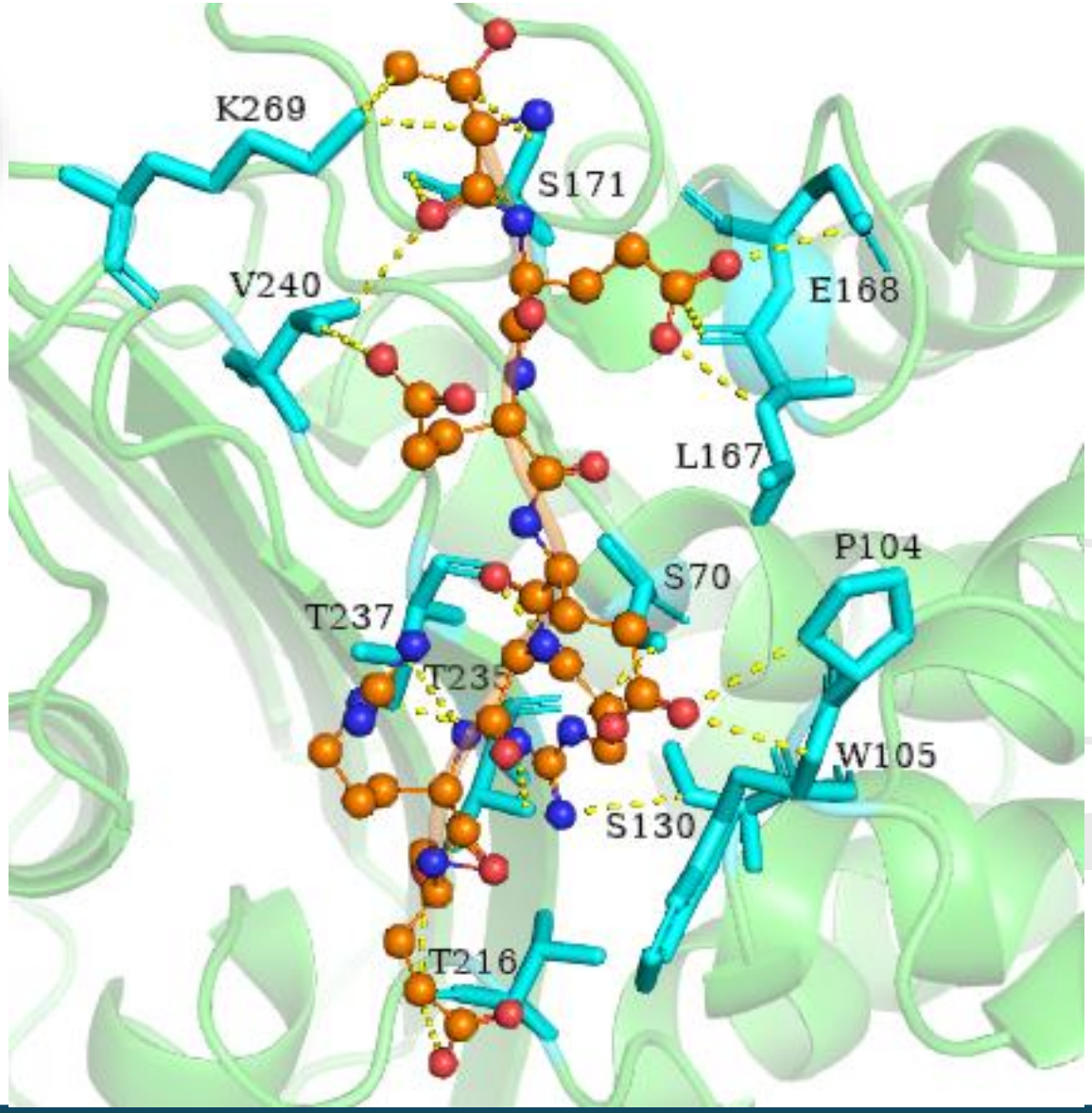
Number of peptides generated for each target.




240 peptides are docked with 5 representatives of class A beta lactamases

# Interactions of the peptide with KPC-2

Autodock 4.2




| KPC-2 | BLIP-P0 | Distance |
|---|---|---|
| K269 | T301 | 3.4 |
| K269 | T301 | 3.5 |
| V240 | T301 | 3.3 |
| T237 | E303 | 3.5 |
| T237 | R305 | 3.4 |
| T237 | E304 | 3.5 |
| T235 | E307 | 3.5 |
| T216 | E307 | 3.3 |
| S130 | R305 | 3.4 |
| S70 | R305 | 3.5 |
| W105 | E304 | 3.5 |
| P104 | E304 | 3.4 |
| L167 | E302 | 3.3 |
| E168 | E302 | 3.3 |
| E168 | E302 | 3.5 |
| S171 | T301 | 3.5 |
| S171 | T301 | 3.4 |

# Docking & evaluation

| 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 |

| 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 | OM_P0.pdb | -5.8 | -5.5 | -5.2 | 8.9 | -6.3 |
| 2 | OM_P1.pdb | -5.9 | -5 | -5.4 | 0 | -5.3 |
| 3 | OM_P2.pdb | -6.2 | -5.7 | -5.5 | -2.7 | -6.2 |
| 4 | OM_P3.pdb | -5.7 | -5.4 | -5.1 | 2.1 | -6.3 |
| 5 | OM_P4.pdb | -5.6 | -5.4 | -5 | -1.4 | -5.1 |
| 6 | OM_P5.pdb | -6.2 | -6.1 | -5.6 | -3.4 | -5.5 |
| 7 | OM_P6.pdb | -6.3 | -5.9 | -5.8 | -1.5 | -5.5 |
| 8 | OM_P7.pdb | -6 | -5.3 | -4.9 | -1.7 | -5.4 |
| 9 | OM_P8.pdb | -6.1 | -5.8 | -5.4 | -1.9 | -5.2 |
| 10 | OM_P9.pdb | -6.1 | -5.7 | -5.8 | -2.9 | -5.9 |

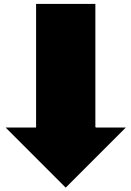
# Distance between peptides & key residues

| S.No. | Predicted Peptide | KPC-2 | | CTX-M-1 | | TEM-1 | | SHV-1 | | SME-1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ser70 | Lys73 | 0-COG Dist | 3-COG Dist | Ser70 | Lys73 | Ser70 | Lys73 | Ser70 | Lys73 |
| 1 | BLIP_1_P0.pdb | 8.979 | 11.15 | 9.549 | 11.435 | 19.741 | 20.708 | 19.442 | 17.088 | 9.392 | 11.364 |
| 2 | BLIP_1_P1.pdb | 9.057 | 10.977 | 8.812 | 10.666 | 20.758 | 21.72 | 20.648 | 23.579 | 8.543 | 10.414 |
| 3 | BLIP_1_P2.pdb | 8.89 | 10.93 | 9.99 | 11.93 | 20.379 | 21.221 | 21.602 | 24.747 | 9.139 | 10.951 |
| 4 | BLIP_1_P3.pdb | 8.293 | 10.299 | 9.643 | 11.374 | 19.987 | 20.976 | 18.845 | 21.824 | 8.763 | 10.749 |
| 5 | BLIP_1_P4.pdb | 8.49 | 10.262 | 9.436 | 11.446 | 20.128 | 21.138 | 21.069 | 24.138 | 8.7 | 10.712 |
| 6 | BLIP_1_P5.pdb | 8.892 | 10.847 | 10.506 | 12.657 | 19.979 | 20.89 | 21.036 | 24.189 | 8.632 | 10.567 |
| 7 | BLIP_1_P6.pdb | 8.179 | 10.247 | 9.384 | 11.456 | 20.314 | 21.048 | 18.009 | 20.933 | 8.399 | 10.146 |
| 8 | BLIP_1_P7.pdb | 9.431 | 11.45 | 10.619 | 12.76 | 19.01 | 19.781 | 10.18 | 13.044 | 9.206 | 11.114 |
| 9 | BLIP_1_P8.pdb | 8.806 | 11.02 | 9.589 | 11.585 | 18.71 | 19.951 | 21.954 | 25.046 | 9.02 | 11.058 |
| 10 | BLIP_1_P9.pdb | 9.101 | 11.058 | 10.082 | 12.333 | 20.847 | 21.71 | 9.493 | 11.988 | 8.874 | 10.948 |

| 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 | OM_P0.pdb | 9.525 | 11.707 | 9.824 | 11.74 | 20.086 | 20.956 | 18.639 | 21.586 | 9.305 | 11.105 |
| 2 | OM_P1.pdb | 8.197 | 9.913 | 9.09 | 11.079 | 20.134 | 20.99 | 19.293 | 22.168 | 9.341 | 10.911 |
| 3 | OM_P2.pdb | 8.486 | 10.319 | 9.044 | 11.089 | 20.197 | 21.125 | 20.942 | 23.746 | 8.414 | 10.398 |
| 4 | OM_P3.pdb | 8.996 | 11.099 | 9.432 | 11.465 | 20.311 | 21.279 | 20.075 | 23.028 | 8.873 | 11.04 |
| 5 | OM_P4.pdb | 9.274 | 11.652 | 9.247 | 11.16 | 20.39 | 21.316 | 20.871 | 23.813 | 9.347 | 11.061 |
| 6 | OM_P5.pdb | 8.161 | 10.405 | 9.595 | 11.683 | 20.717 | 21.447 | 9.066 | 11.478 | 8.487 | 10.771 |
| 7 | OM_P6.pdb | 8.086 | 10.256 | 8.649 | 10.365 | 20.553 | 21.277 | 9.04 | 11.097 | 8.167 | 10.014 |
| 8 | OM_P7.pdb | 8.472 | 10.572 | 8.793 | 10.667 | 19.818 | 20.799 | 20.454 | 23.355 | 9.111 | 11.174 |
| 9 | OM_P8.pdb | 8.442 | 10.531 | 8.946 | 10.882 | 19.9 | 20.898 | 9.19 | 11.599 | 8.529 | 10.536 |
| 10 | OM_P9.pdb | 8.098 | 10.241 | 8.726 | 10.699 | 20.83 | 21.849 | 19.283 | 22.216 | 8.27 | 10.376 |

# Ongoing Process: MD simulations

| Peptide Group | Derived From | Strategy | Purpose |
|---|---|---|---|
| Group 1 | BLIP-I | Natural protein mimic | Competitive inhibition |
| Group 2 | Omega Loop | Structural mimicry | Loop disruption |
| Group 3 | Hotspots | Conserved residue targeting | Broad-spectrum binding |
| Group 4 | AI-Engineered | De novo design | Stability & affinity |



Thermodynamic & structural stability Validation

# 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 mai
        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

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

    PATH/gmx" energy -f em.edr -o pe_em.xvg <<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 ../../
```

# Thank you