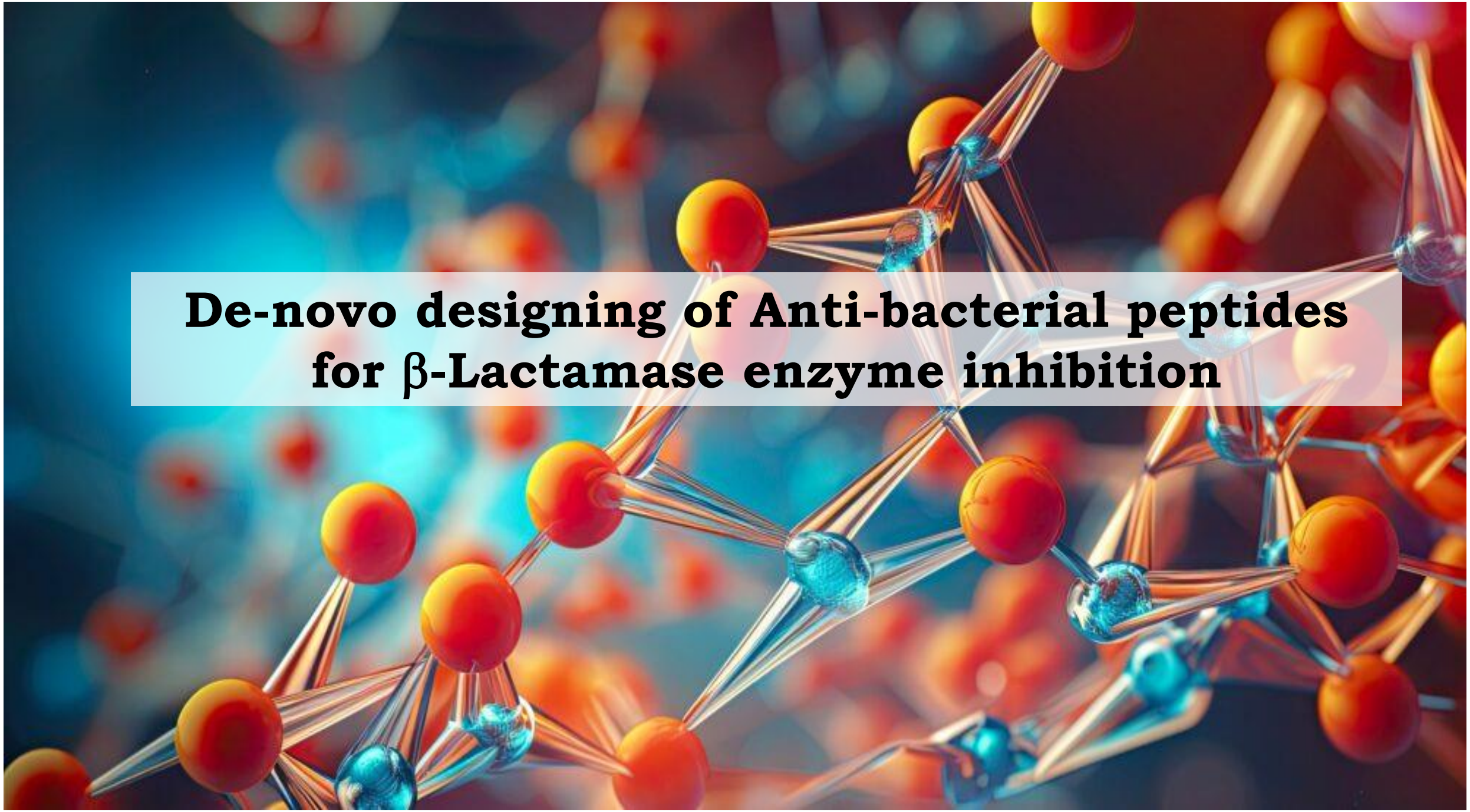
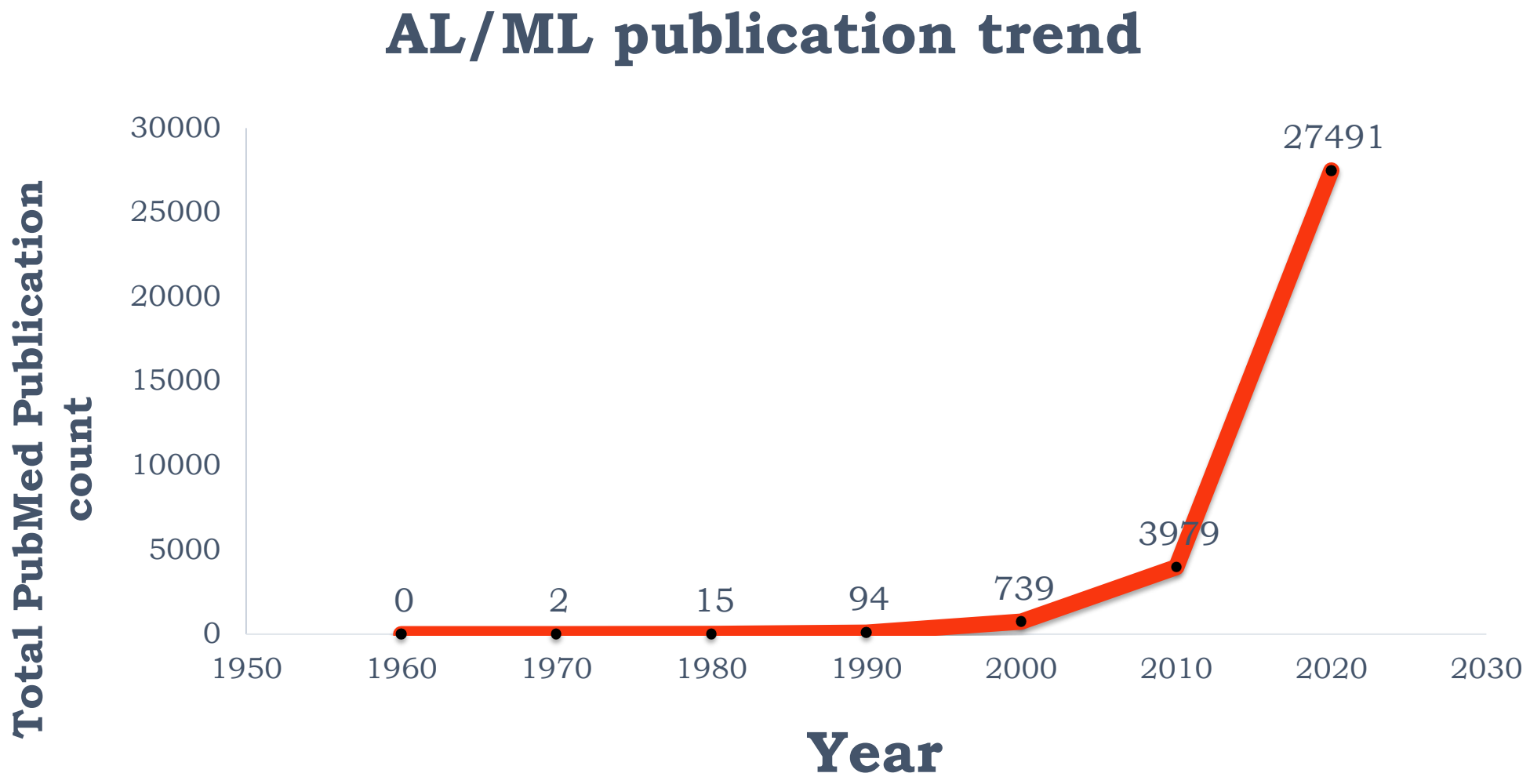
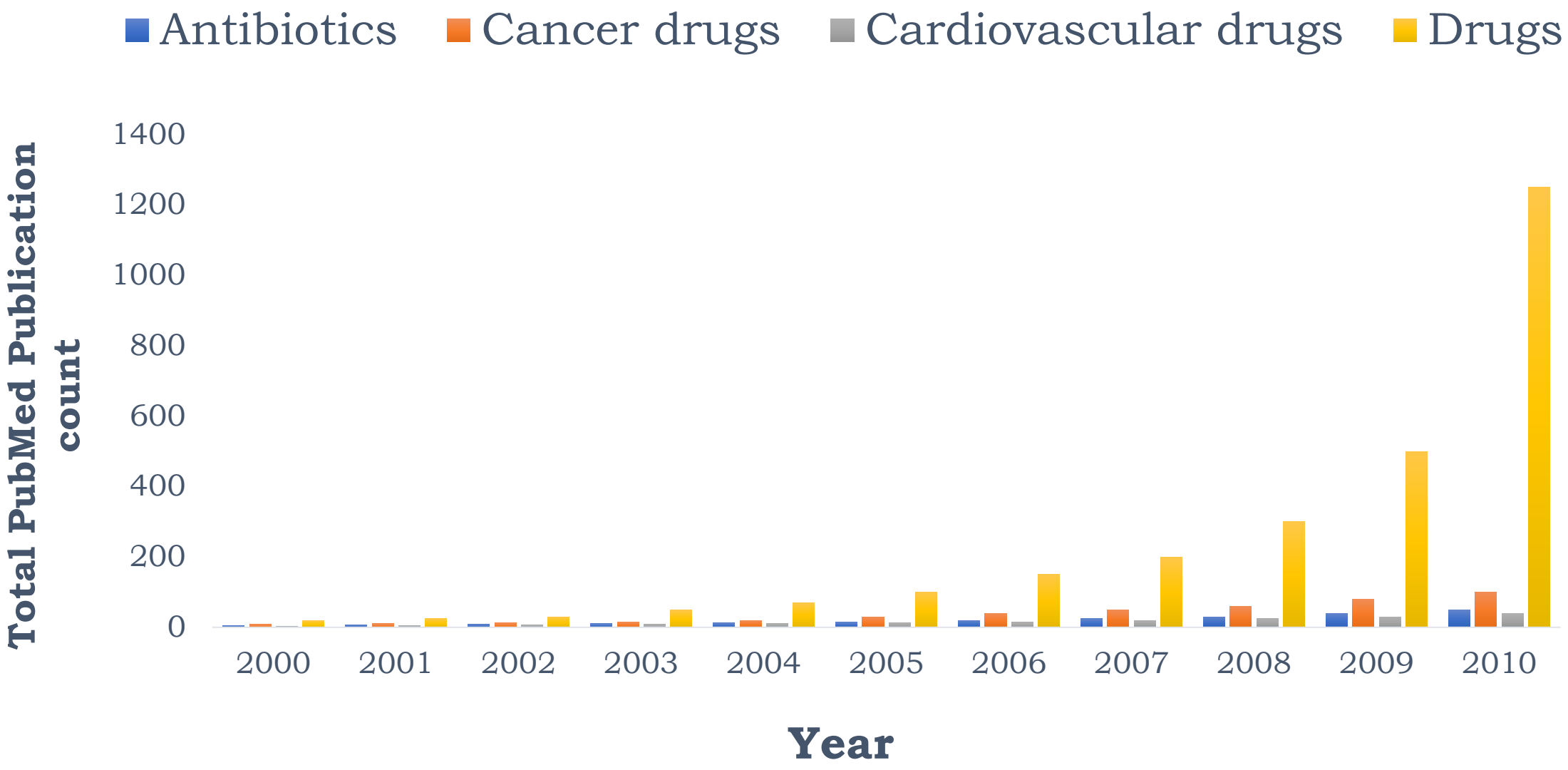

De-novo designing of Anti-bacterial peptides
for β-Lactamase enzyme inhibition

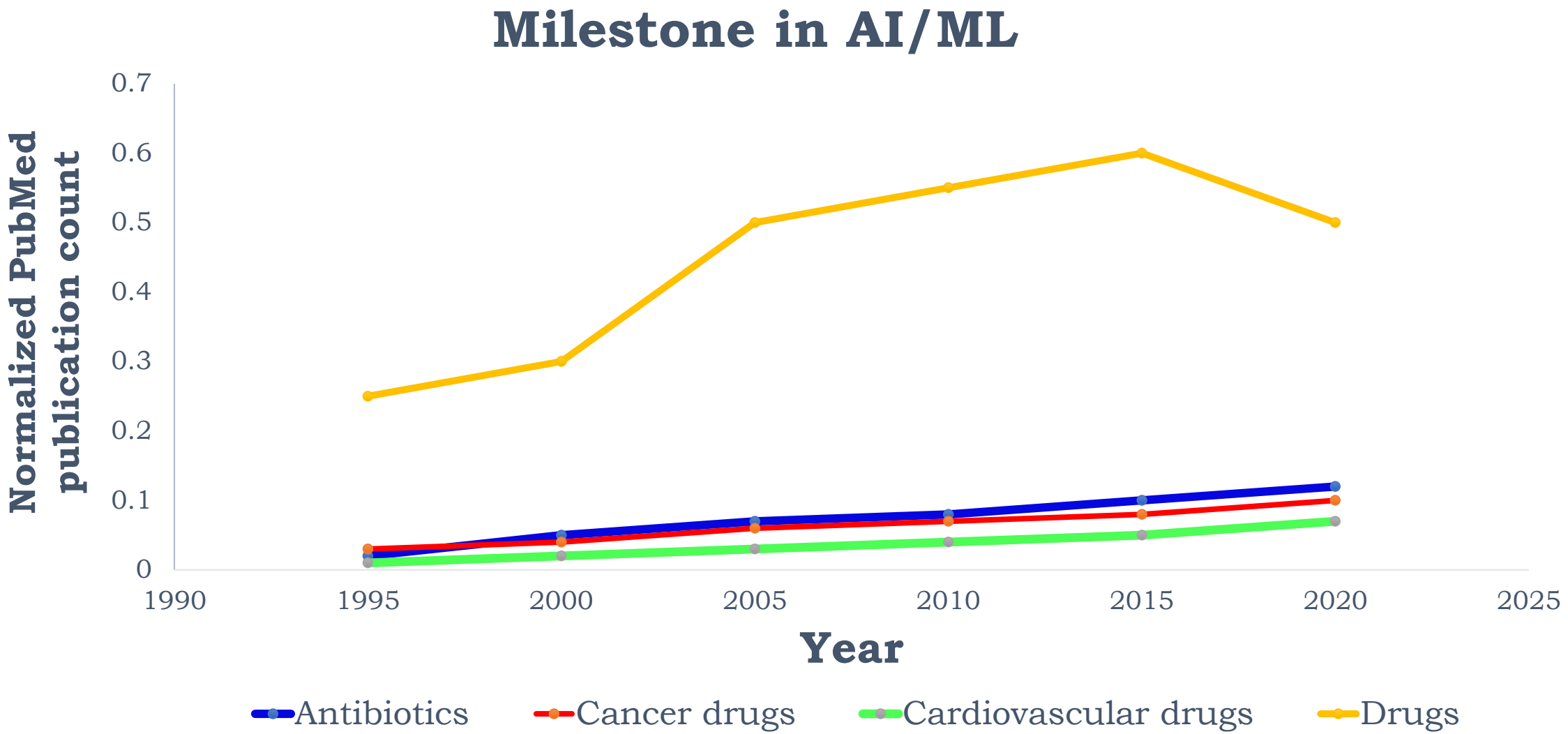
# Trends in AI/ML research over Time



# AI/ML in drug discovery research



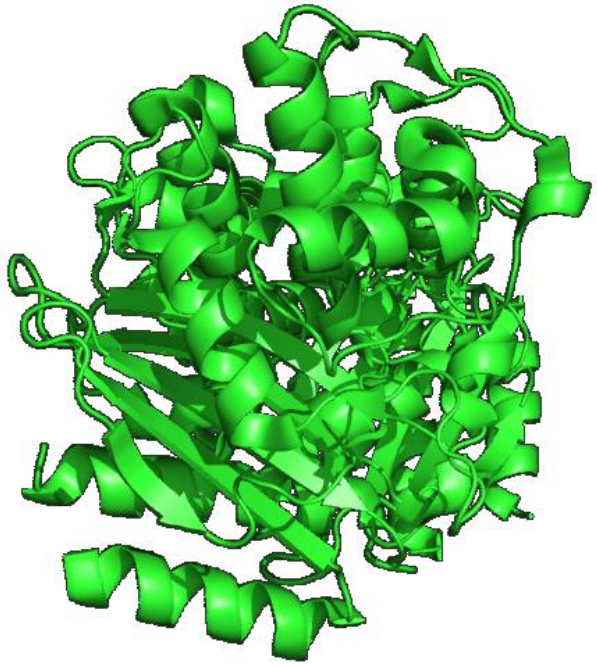
# Trends in AI/ML research



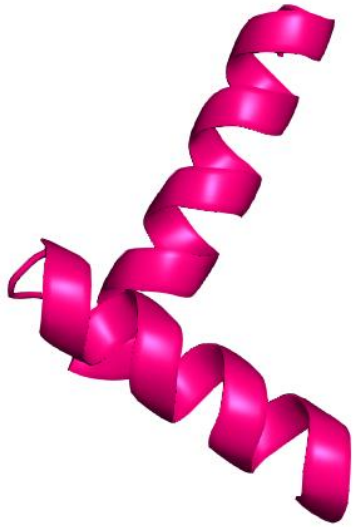
# Anti-Bacterial Peptides

- Anti-Bacterial peptides (ABPs) are a class of small peptides, usually 10-50 amino acids long, that exhibit broad-spectrum Anti-Bacterial activity.

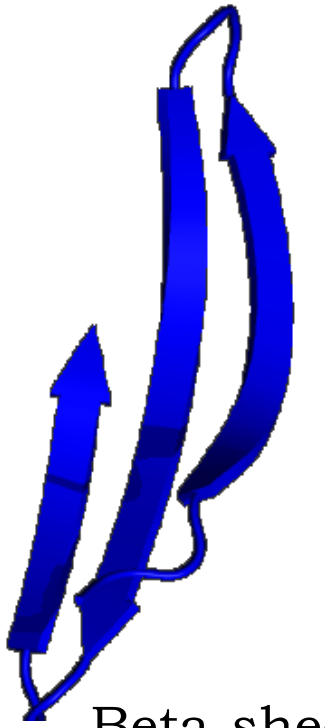
- Transforming peptides into small molecules as peptide-based molecules often present poorer bioavailability and lower metabolic stability.



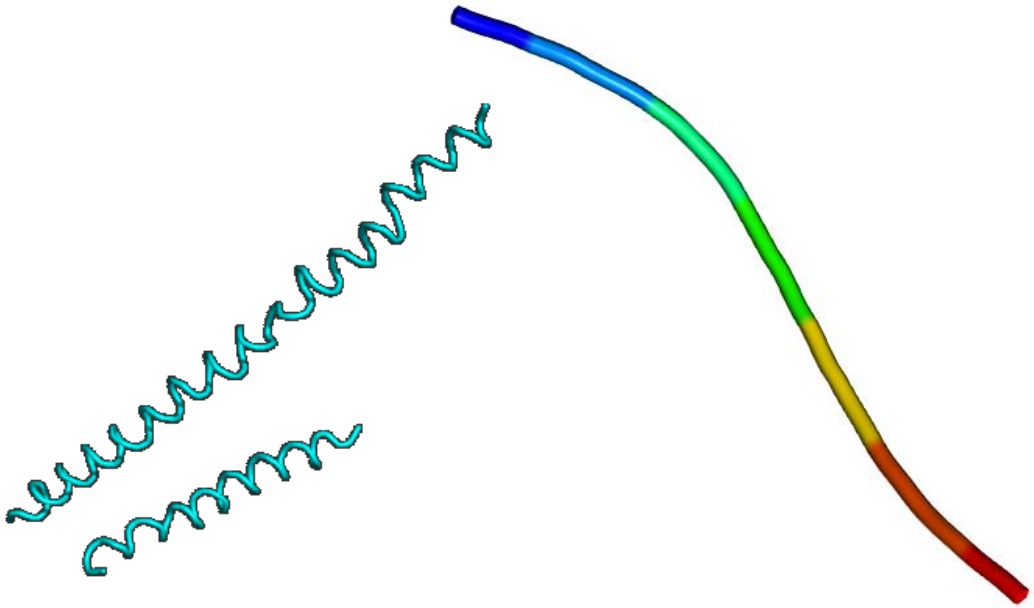
Protein Sample



α-helix



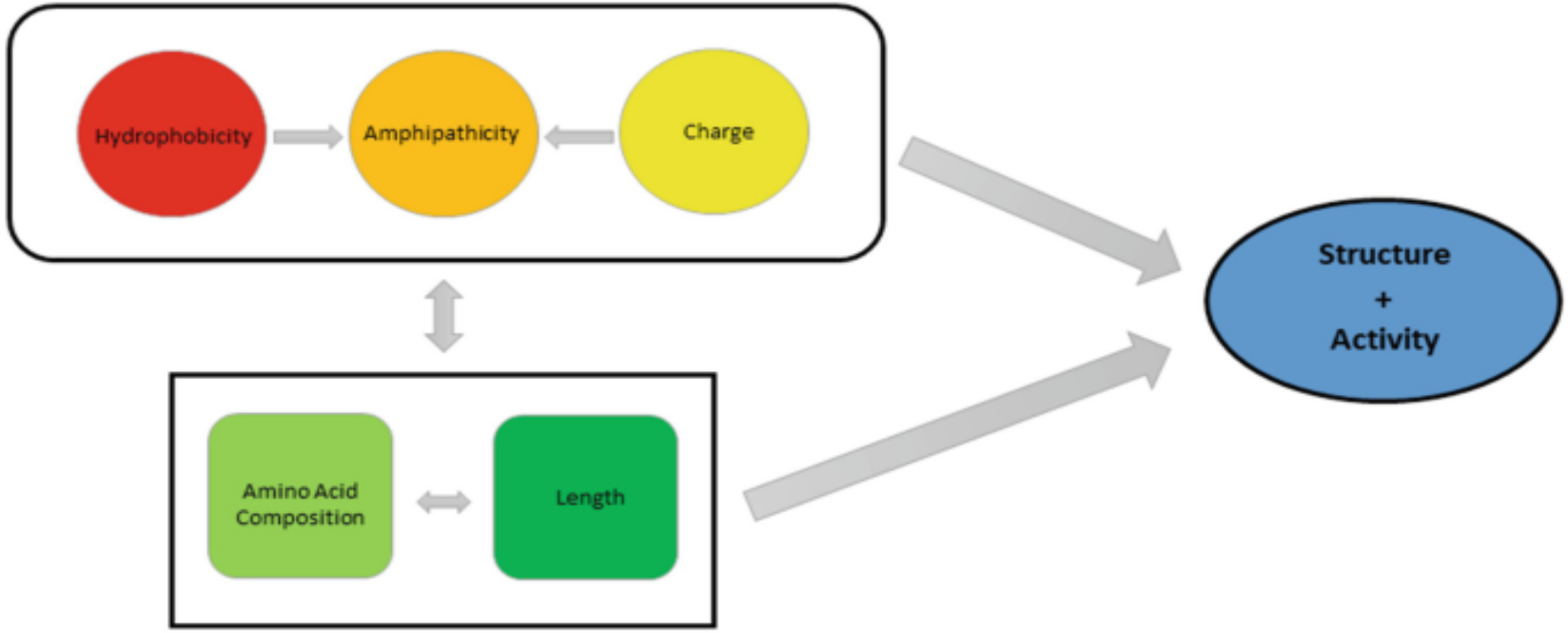
Beta-sheets



Anti-bacterial peptides

# Peptidomimetic compounds

- Peptidomimetic compounds are non-peptidic analog, synthetic molecules that mimic the structure and function of peptides.
- Improved stability, bioavailability, and pharmacological properties (such as resistant to enzymatic degradation, can more easily cross cellular membranes)
- AMP sequences can be used as templates for designing peptidomimetics.



# The problem we are looking upto ?

## Development of Peptidomimetic Compounds with Antibacterial Properties to Overcome Antibacterial Resistance (ABR)

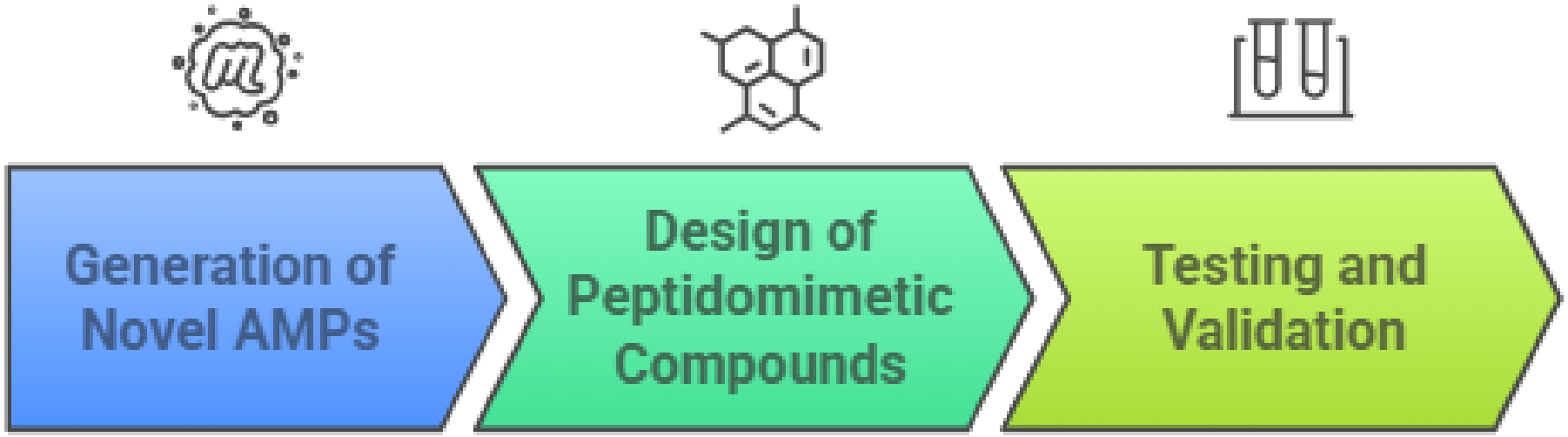
- **Mimic AMP structural motifs** - amphipathic helices, β-sheets, & positively charged.
- **Show antibacterial activity** against resistant bacterial strains
- **Improve stability** - resistance to enzymatic degradation
- **Reduce cytotoxicity** to human cells & improved specificity towards pathogens.

# Machine learning prediction of Antimicrobial peptides

| Tool name | URL | Algorithms | Features | Year | Ref |
|---|---|---|---|---|---|
| AntiBP | http://crdd.osdd.net/raghava/antibp2 | SVM,QM,ANN | Single label | 2007 | [17] |
| CAMP | http://www.bicnirrh.res.in/antimicrobial | SVM, RF, DA | Single label | 2010 | [18, 53] |
| | http://amp.biosino.org/ | BLASTP, NNA | Single label | 2011 | [54] |
| AMPA | http://tcoffee.crg.cat/apps/ampa | | AMP region scan | 2012 | [55] |
| ANFIS | | ANFIS | Single label | 2012 | [56] |
| Peptide Locator | http://bioware.ucd.ie/ | BRNN | Single label | 2013 | [57] |
| iAMP-2L | http://www.jci-bioinfo.cn/iAMP-2L | FKNN | Two-level, Multi-label | 2013 | [52] |
| DBAASP | https://dbaasp.org/prediction/general | thresholds | | 2014 | [33] |
| SVM-LZ | NG (BioMed Research International) | SVM | Single label | 2015 | [58] |
| ADAM | http://bioinformatics.cs.ntou.edu.tw/ADAM/ | SVM, HMM | Single label | 2015 | [59] |
| MLAMP | http://www.jci-bioinfo.cn/MLAMP | RF – ML-SMOTE | Multi-label | 2016 | [60] |
| *i*AMPpred | http://cabgrid.res.in:8080/amppred/ | SVM | Single label | 2017 | [61] |
| AmPEP | http://cbbio.cis.umac.mo/software/AmPEP/ | RF | Single label | 2018 | [62] |
| AMP scanner | www.ampscanner.com | DNN | Single label, Large scale | 2018 | [63] |
| AntiMPmod | https://webs.iiitd.edu.in/raghava/antimpmod/ | SVM | Single label, PTM/3D | 2018 | [64] |
| dbAMP | http://csb.cse.yzu.edu.tw/dbAMP/ | RF | Single label | 2019 | [65] |

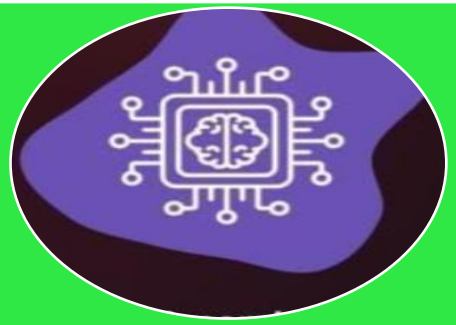
Table: Lists of some major machine learning prediction programs

# Approach to solution?



# Future Directions in Antibacterial Resistance Research Using Machine Learning


1. **Accomplished Work** in ML and Antibacterial Resistance
2. Developed **ML models** for analysing existing Antimicrobial Peptides (AMPs).
3. Investigated **patterns in AMP sequences** and their mechanisms of action.



1. **ML Model Development**: Train an ML model to generate novel AMPs.
2. **Peptide Synthesis**: Synthesize peptides in-vitro for validation.
3. **Iterative Validation**: Evaluate generated peptides for binding affinity.
4. **Selection for Synthesis**: Top 10 peptides are finalized for synthesis based on performance.


1. ML model is unable to predict the peptides that contains **non-natural amino acids**, hence complicating ML training and prediction.

2. **Lack of Repository**: No centralized, comprehensive AMP repository for training datasets.

# Target protein: BLIP-I

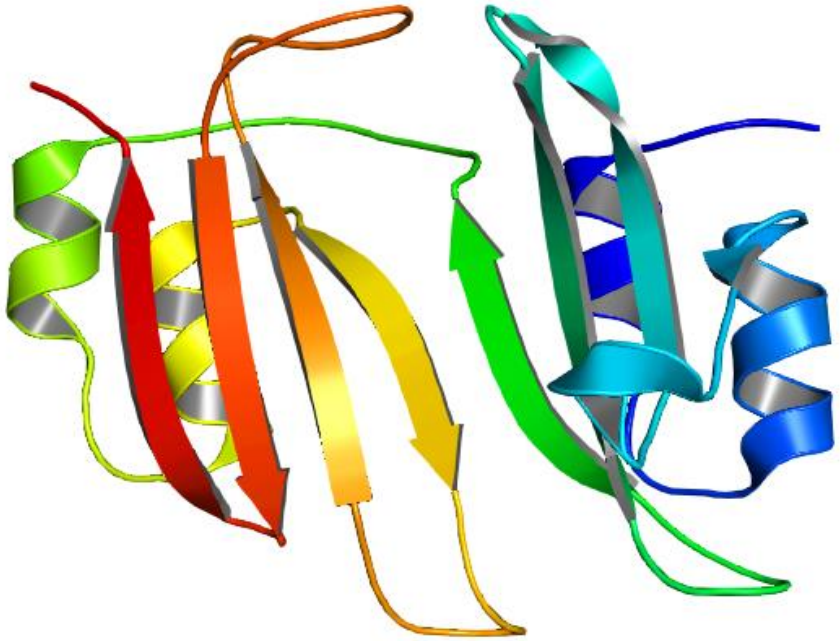


Fig: Structure of Beta-Lactamase Inhibitory Protein (BLIP-1) under PDB accession ID 3GMU

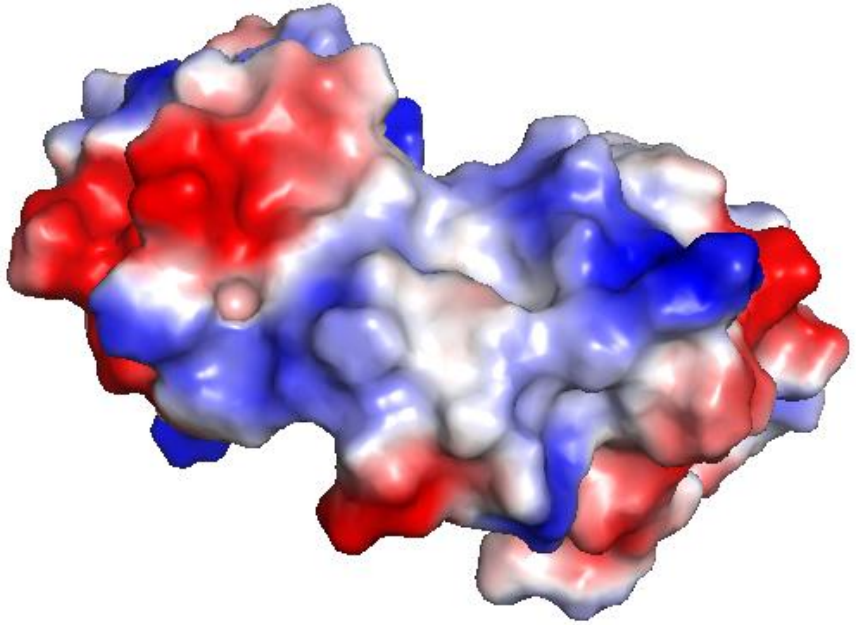
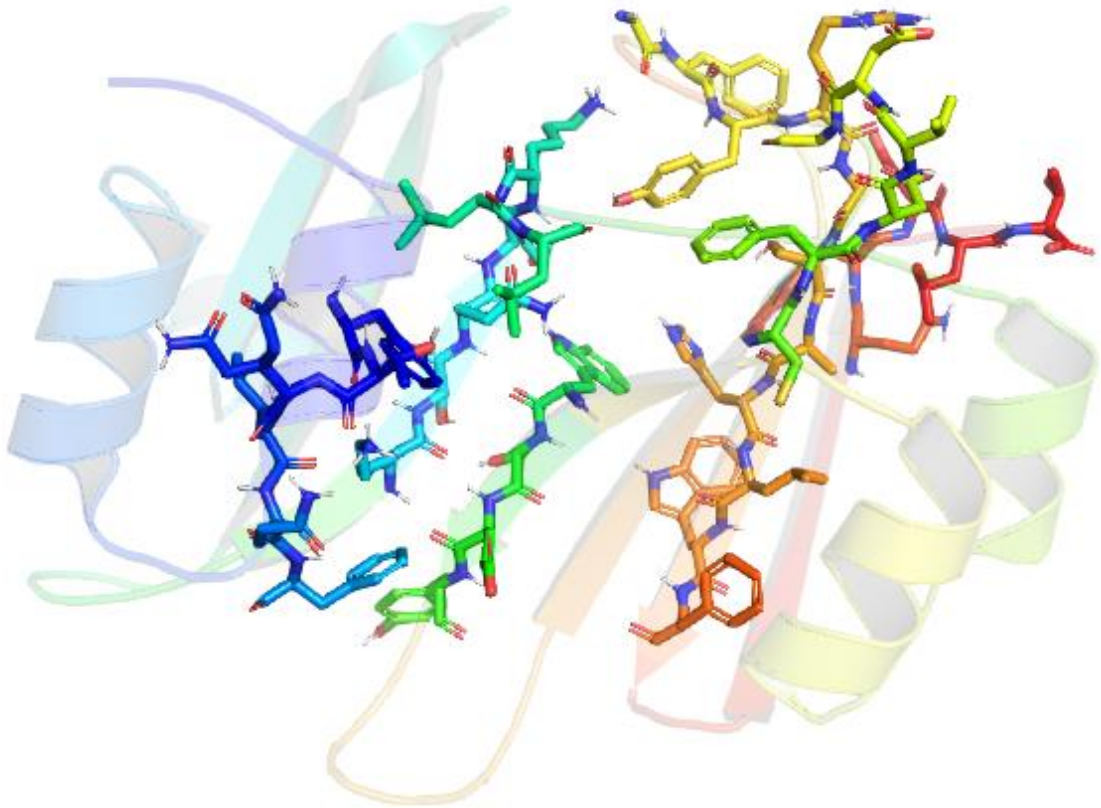


Fig: Electrostatic Potential of BLIP PDB-ID 3GMU (50.530 kcal/mol)

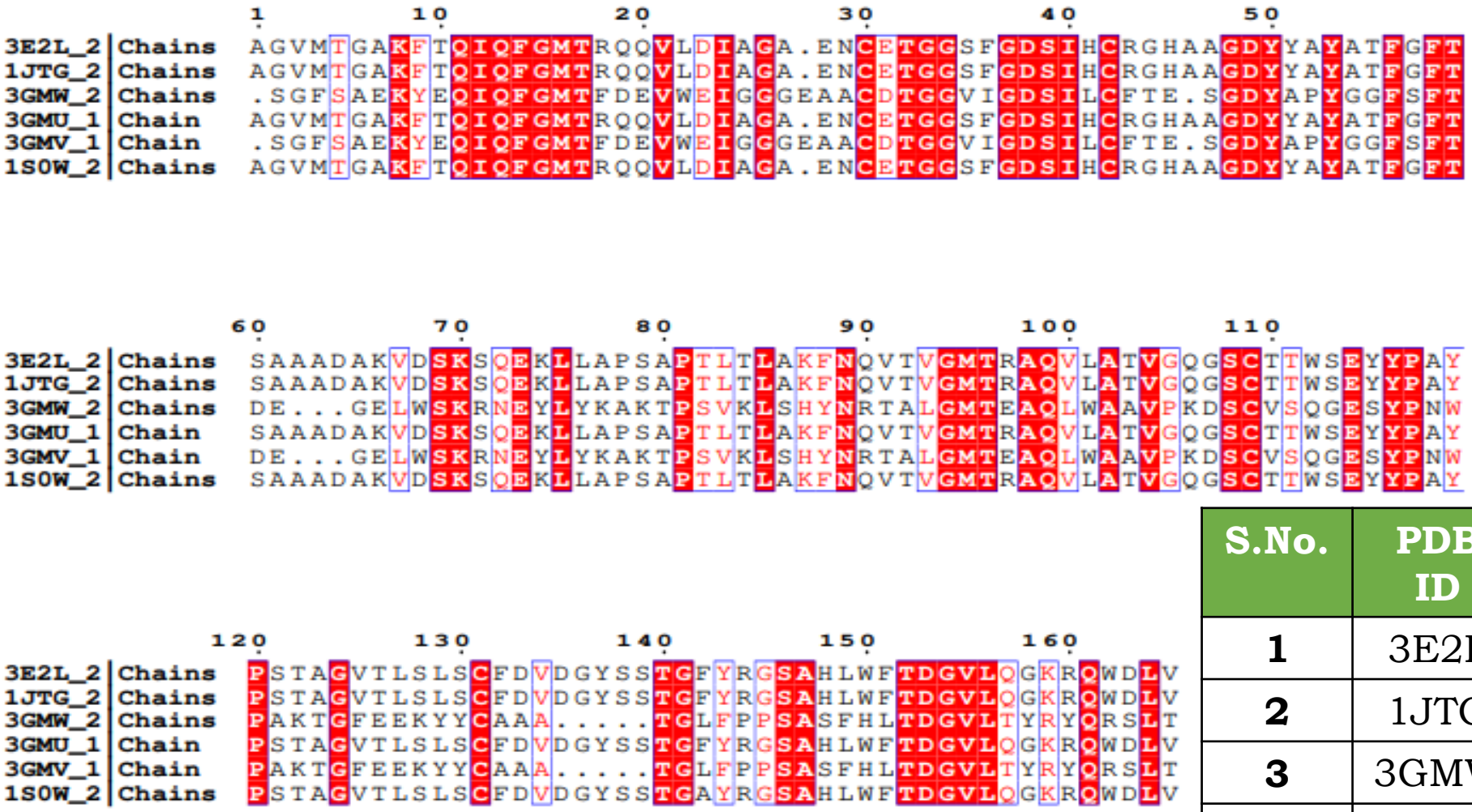
# Binding site prediction for BLIP-1

- Fixed regions for binding are: [9-14, 70-76, 111-114, 130-135, 140-150, 160-165]



| S.No. | Amino acid Residues | Residue Position | Residue Name |
|---|---|---|---|
| 1 | F | 9 | Phenylalanine |
| 2 | T | 10 | Threonine |
| 3 | I | 12 | Isoleucine |
| 4 | Q | 13 | Glutamine |
| 5 | F | 14 | Phenylalanine |
| 6 | K | 70 | Lysine |
| 7 | E | 73 | Glutamic acid |
| 8 | L | 76 | Leucine |
| 9 | T | 111 | Threonine |
| 10 | E | 114 | Glutamic acid |
| 11 | S | 130 | Serine |
| 12 | F | 132 | Phenylalanine |
| 13 | F | 142 | Phenylalanine |
| 14 | Y | 143 | Tyrosine |
| 15 | S | 146 | Serine |
| 16 | H | 148 | Histidine |
| 17 | W | 162 | Tryptophan |

# Multiple sequence alignment




| S.No. | PDB ID | Class of Beta lactamase |
|---|---|---|
| 1 | 3E2L | BLIP (KPC) |
| 2 | 1JTG | BLIP (TEM-1) |
| 3 | 3GMW | BLIP-I (TEM-1) |
| 4 | 3GMU | BLIP (Apo form) |
| 5 | 3GMV | BLIP-I (Apo form) |
| 6 | 1S0W | BLIP (TEM) |

# Target for OMEGA Loop

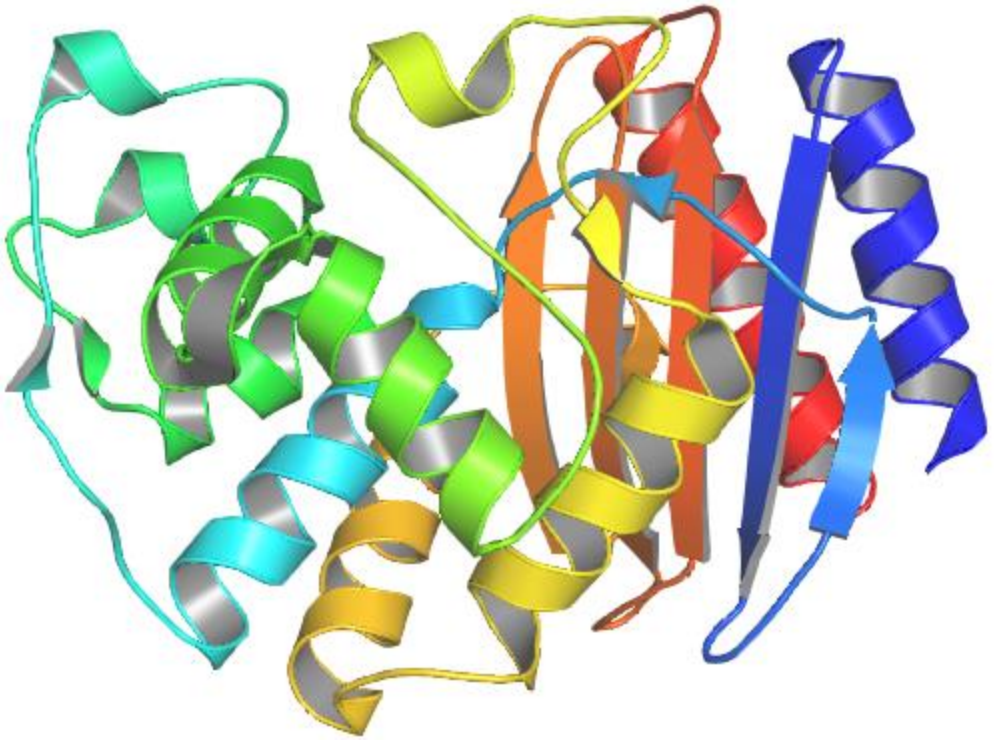


Fig. SHV-14
(Homology modeling with SwissModel)

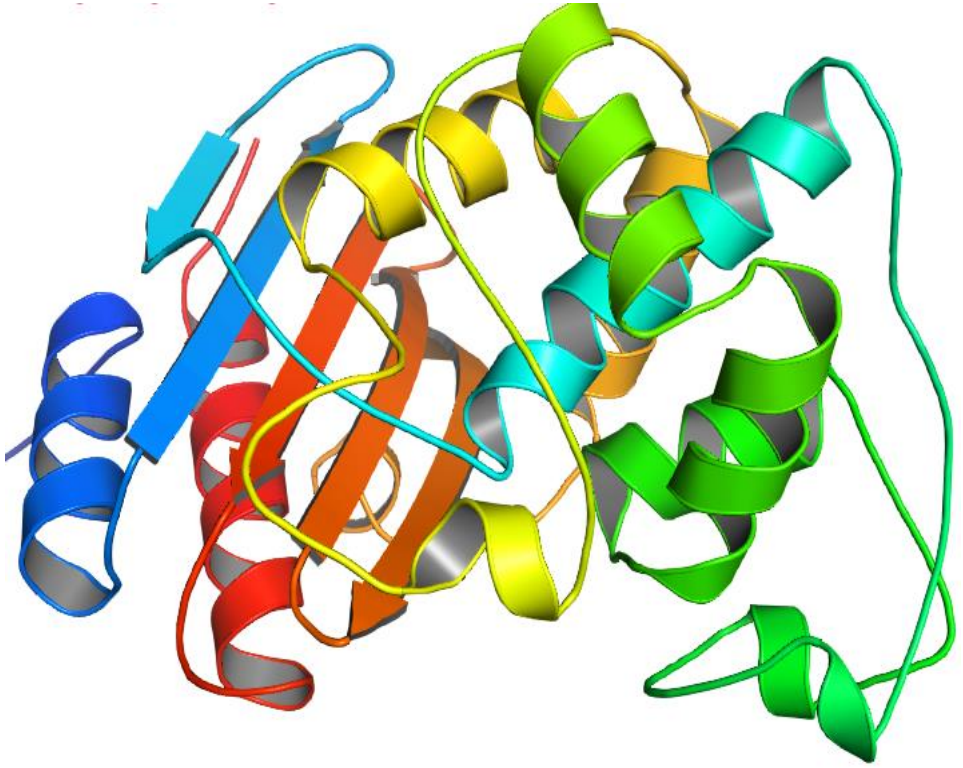
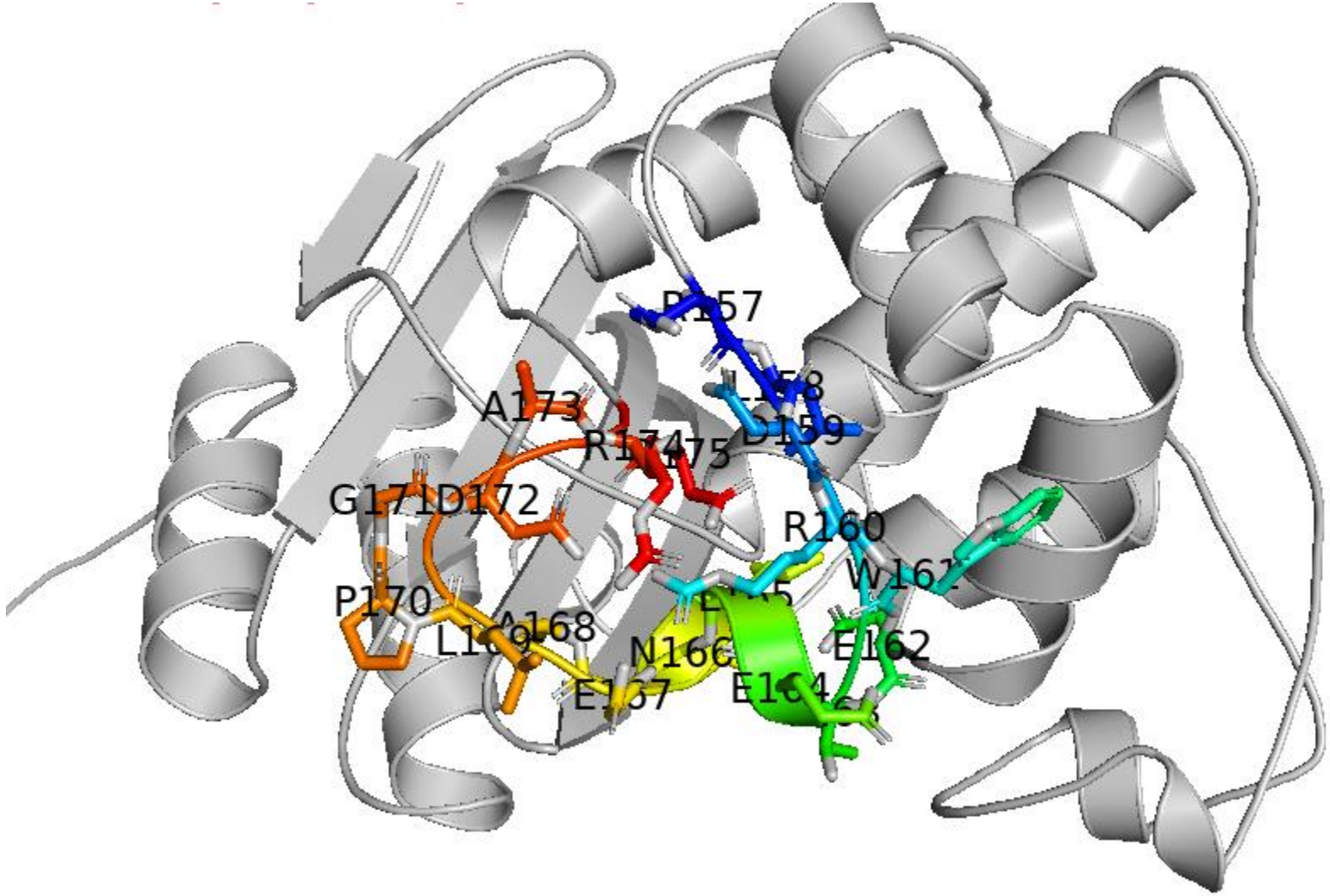


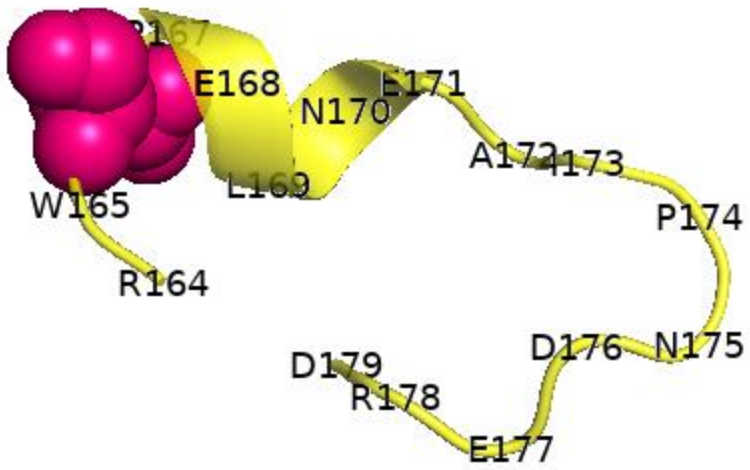
Fig. TEM-1

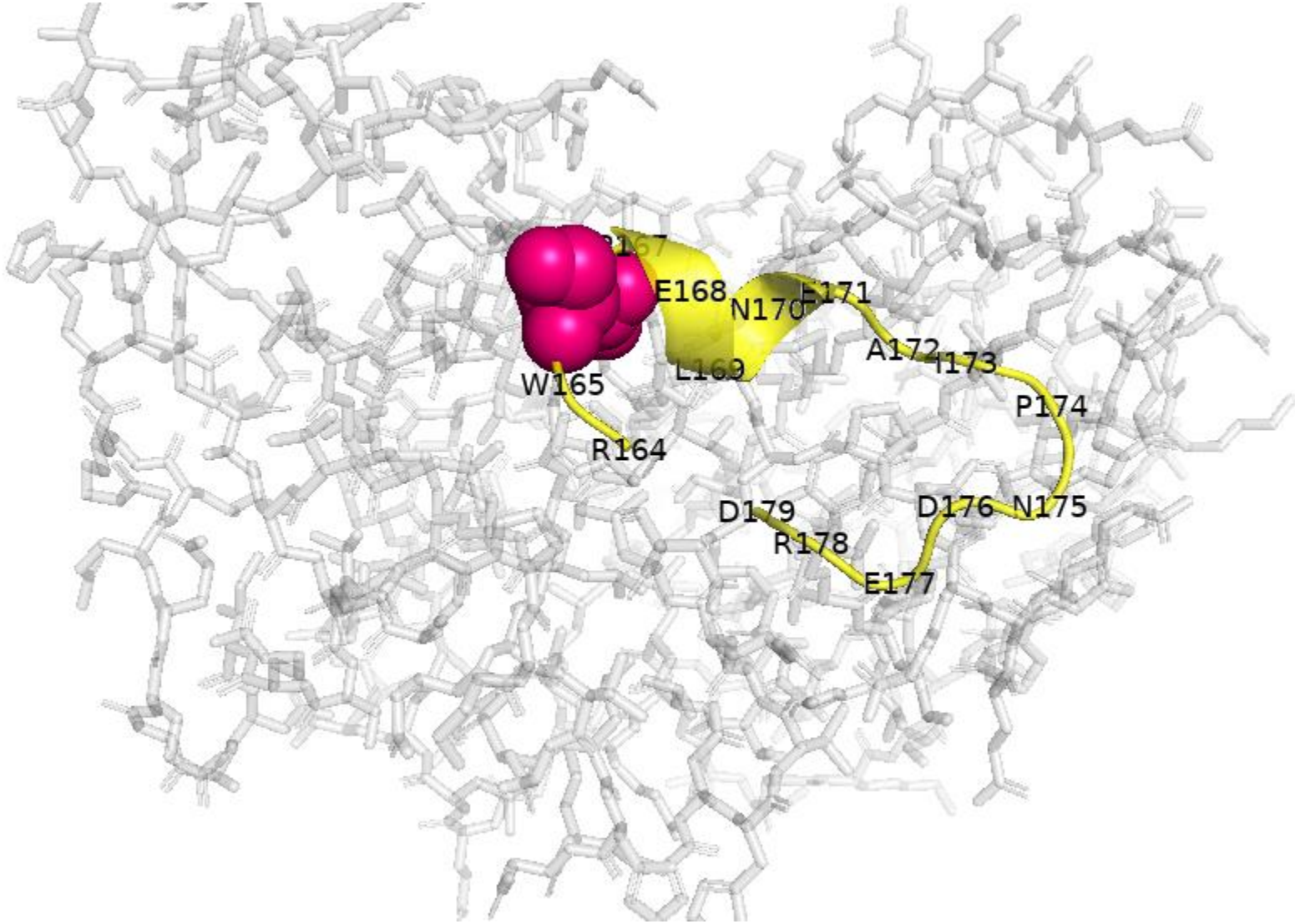
# OMEGA loop in SHV-14



# OMEGA loop in TEM-1

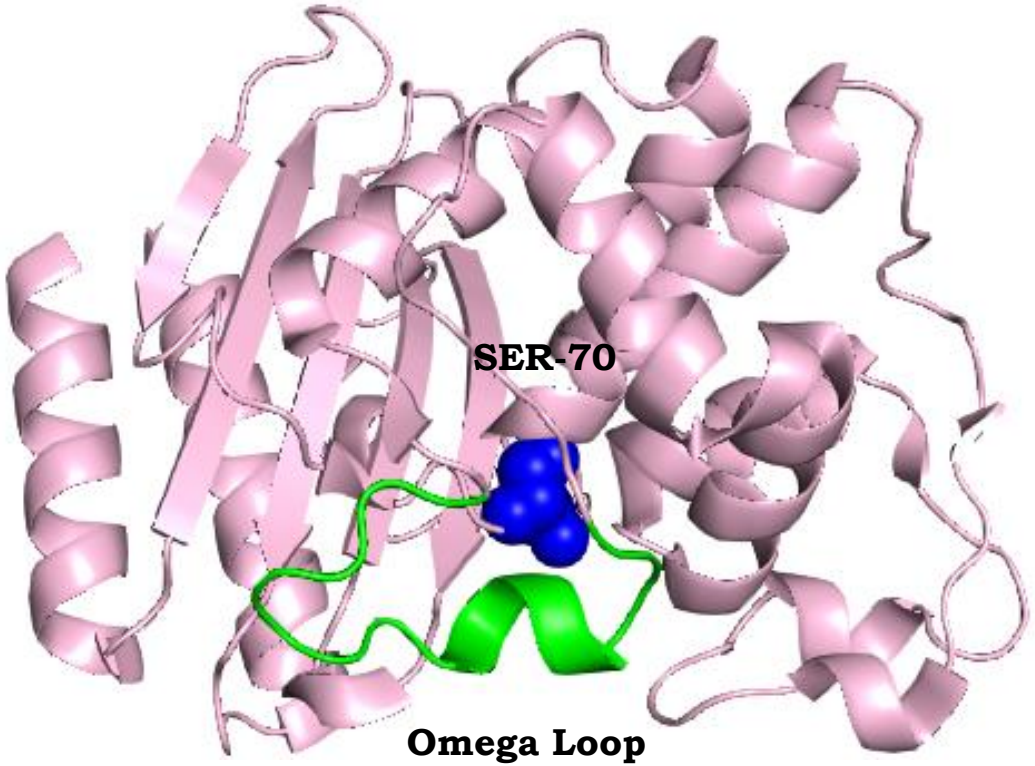
Glu166

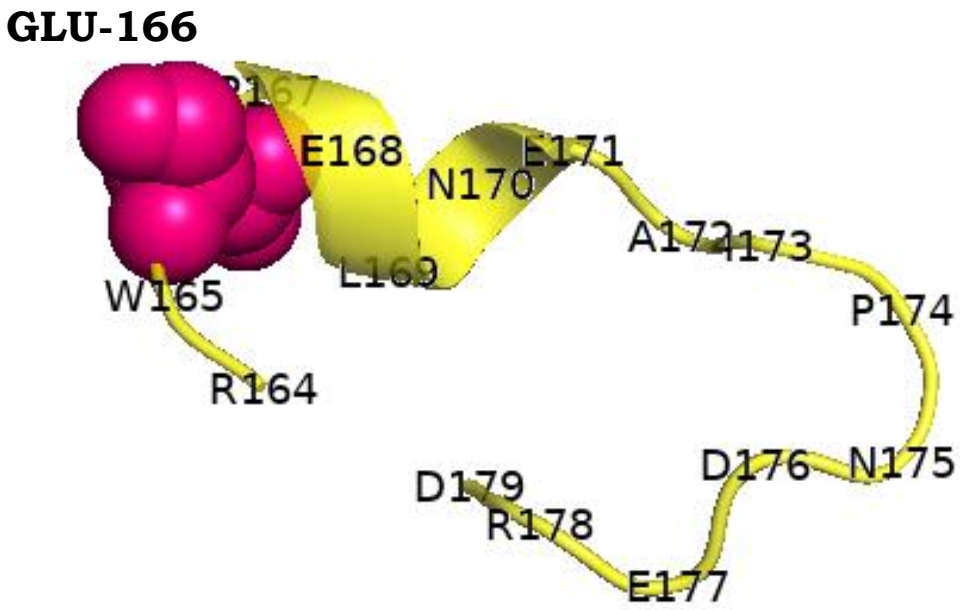


# OMEGA loop of Class-A β-Lactamase

- Ω-loop of TEM-type βLs contains **16 residues** (from Arg164 to Asp179)
- Glu166, highly conserved in class A βLs, plays a crucial role in the hydrolysis of β-lactams.



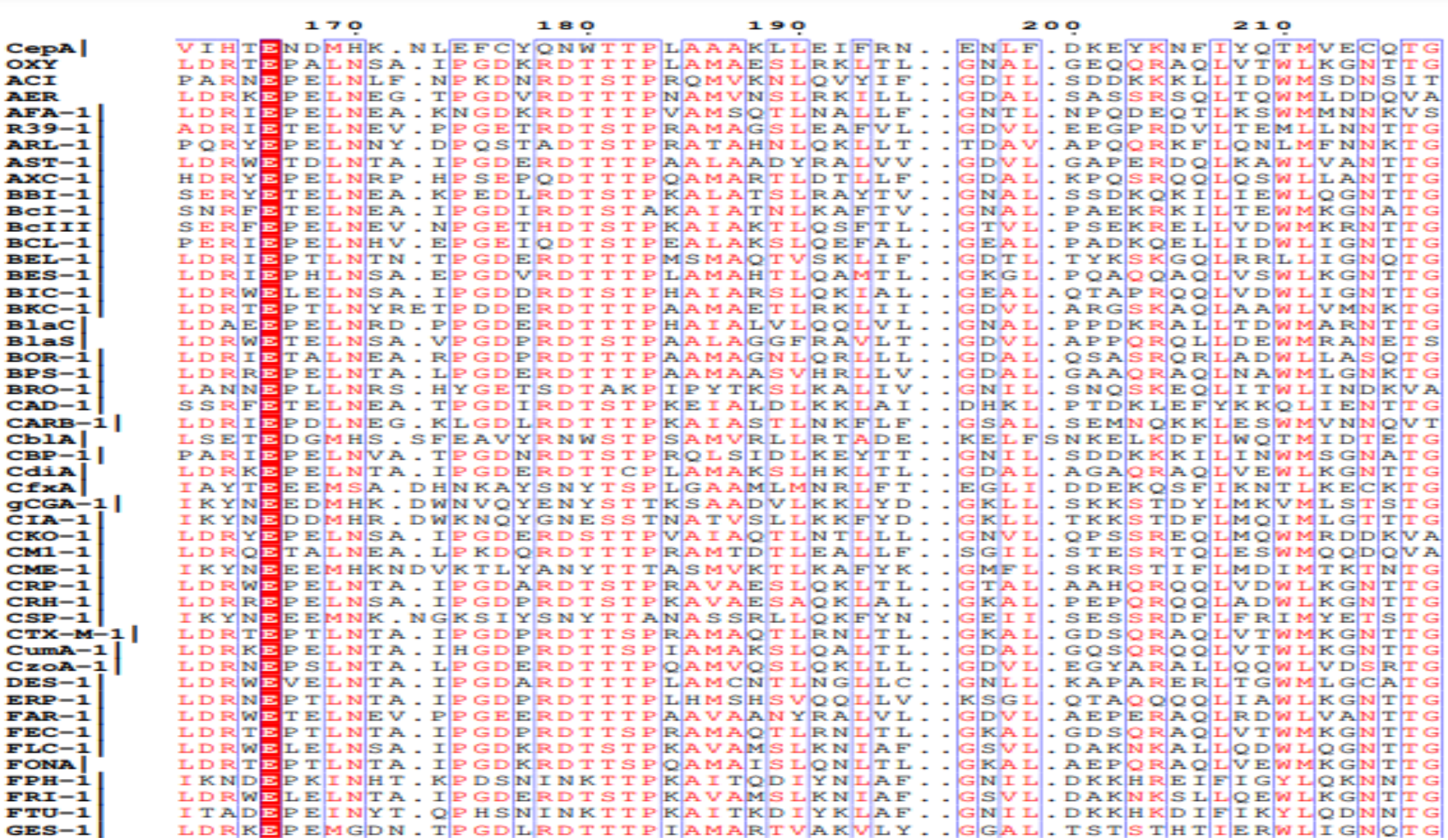

(PDB ID: 8GIJ)
Resolution: 1.59 A°

# Class-A β-lactamase variants

**Methodology**

1. performed Multiple sequence alignment using Clustal omega
2. Visualize it using Espript 3.0 web server
3. Manually identified conserved regions
4. Found 12 conserved sequences.

# Conserved motifs in Class-A β-lactamase



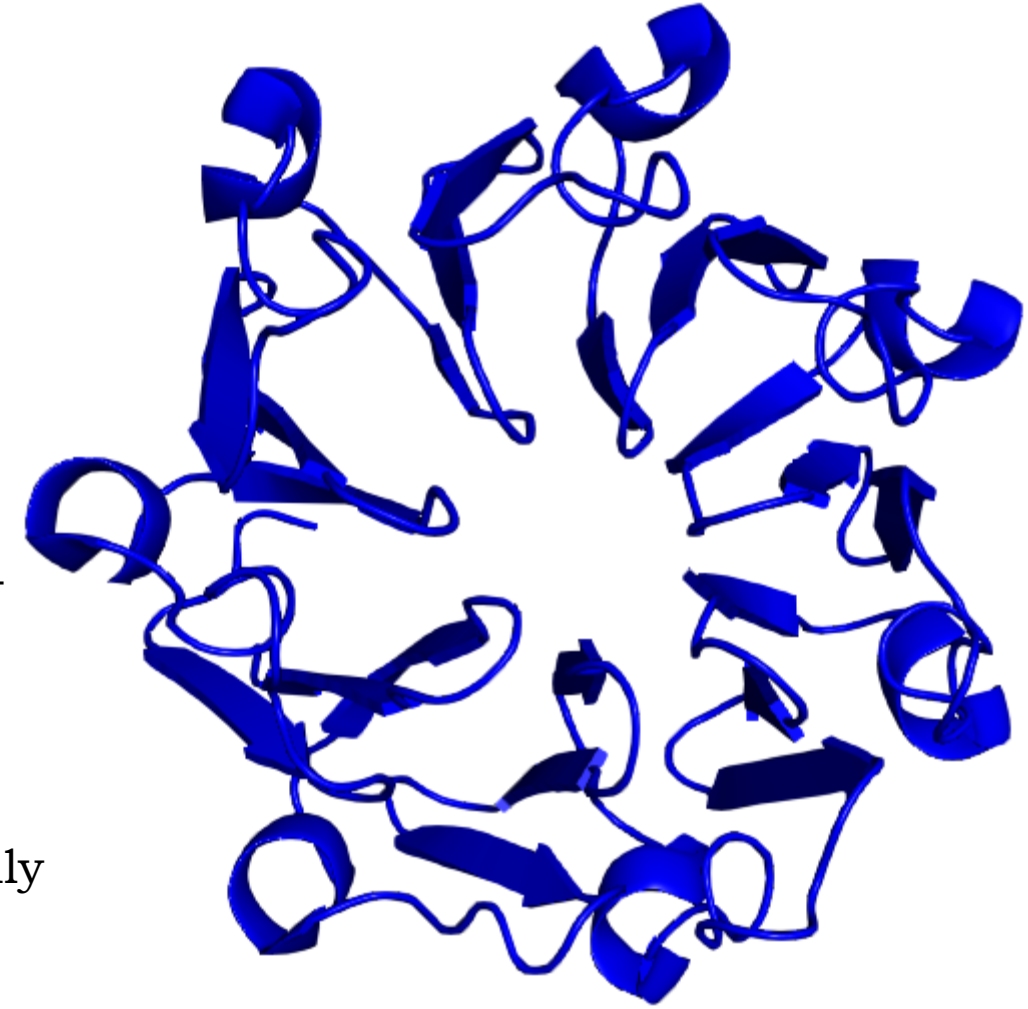
# Conserved motifs in Class-A β-lactamase

- 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 |

# BLIP-II

- Mol wt. 28KDa
- Source organism: *Streptomyces exfoliates*
- It has motif seven bladed beta-propeller contains 3-anti-parallel beta-sheets
- Blocks the beta-lactamase active site (S-70) sterically and inhibit its activity.



BLIP-II
PDB ID : 1JTD

# De-novo peptides against BLIP-I

| 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 |

# De-novo peptides against OMEGA loop

| 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 |

# OMEGA loop peptide generation

| 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 |

Source: Biswal et al. (2023)

# OMEGA loop peptide generation

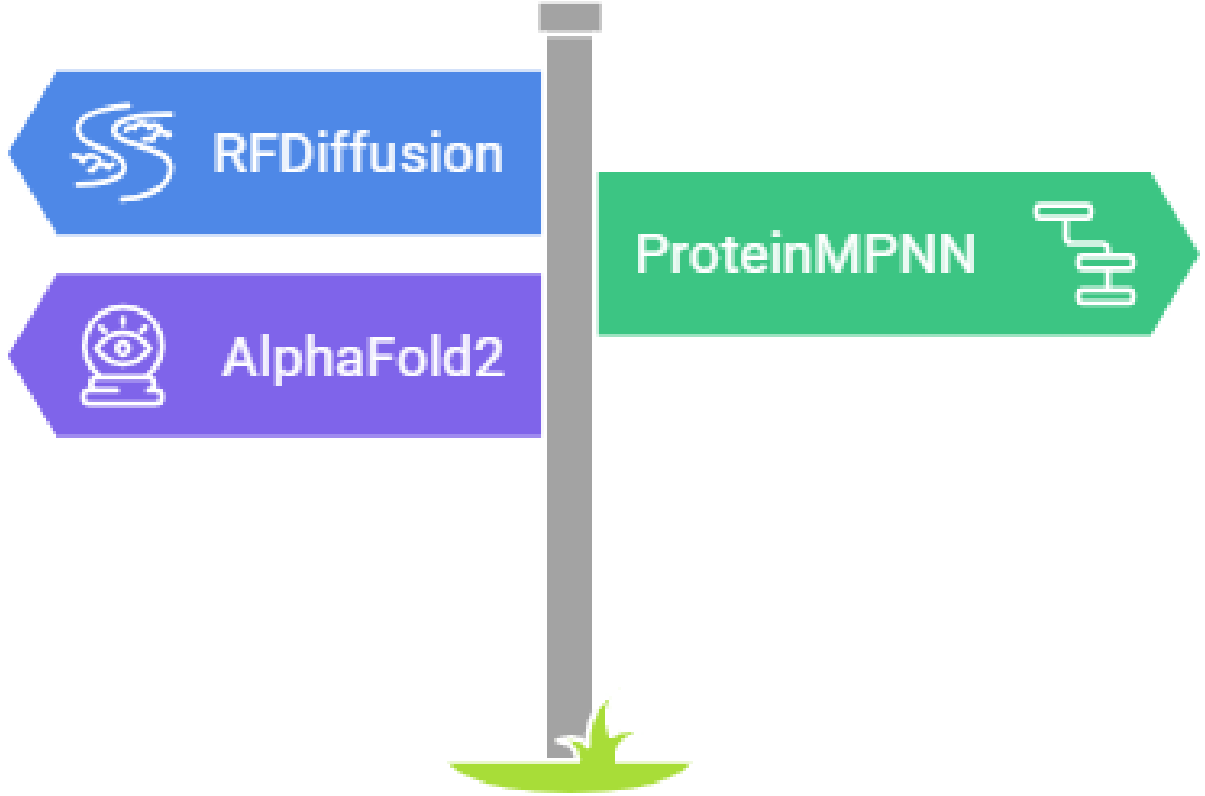
| s.no | Ref P7 Sequence | Generated Backbone | Length of generated peptide | Generated AA sequence | Generate 3D structure |
|---|---|---|---|---|---|
| 1 | ESRLH | OM_PP7_0.pdb | 7 | RERELLS | OM_PAP7_0.pdb |
| 2 | ESRLH | OM_PP7_1.pdb | 7 | NLEELLS | OM_PAP7_1.pdb |
| 3 | ESRLH | OM_PP7_2.pdb | 7 | 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 |

# Template motifs for peptide design

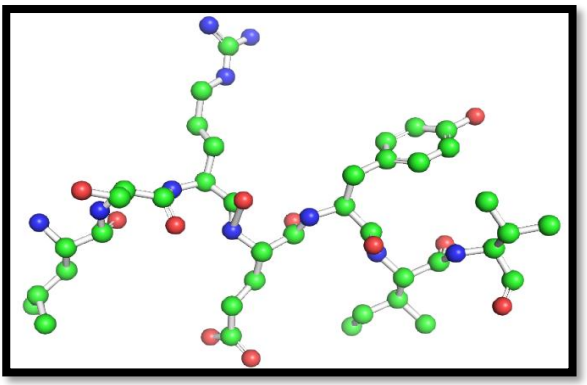
| S.No | Ref. peptide sequence | Backbone generation | Length of generated peptide | Generated AA sequence | Generate 3D structure |
|---|---|---|---|---|---|
| 1 | NDVAILWP | C7_0.pdb | 7 | TEAERLG | VAR_C7_0.pdb |
| 2 | NDVAILWP | C7_1.pdb | 7 | TLEELLK | VAR_C7_1.pdb |
| 3 | NDVAILWP | C7_2.pdb | 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 |

# Computational techniques used for peptide design

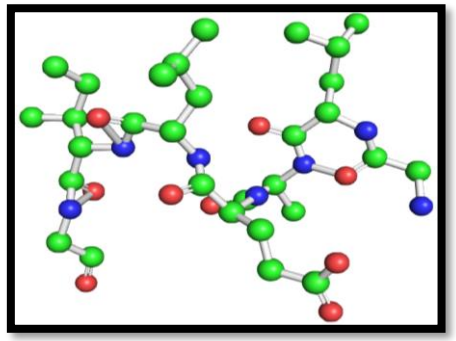
- RFDiffusion: De novo peptide generation based on protein diffusion models.
- ProteinMPNN: Optimizing sequences for structural stability and target affinity.
- AlphaFold2: Predicting peptide-target interactions and validating stability.



# OMEGA loop based peptides



OM_P0.pdb



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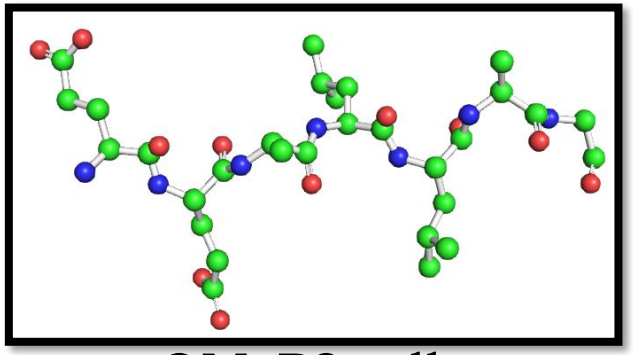

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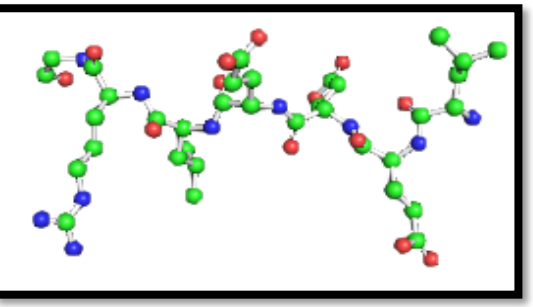

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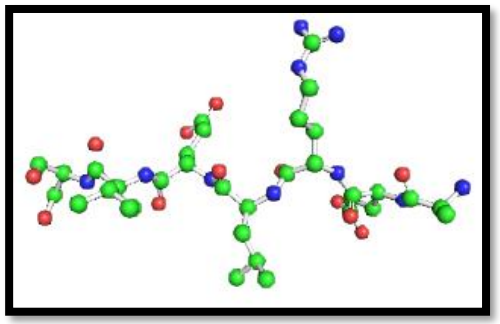

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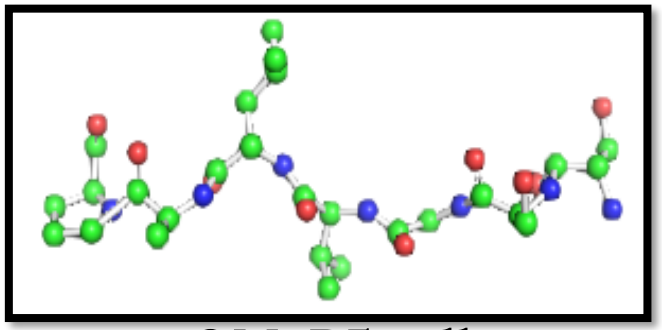

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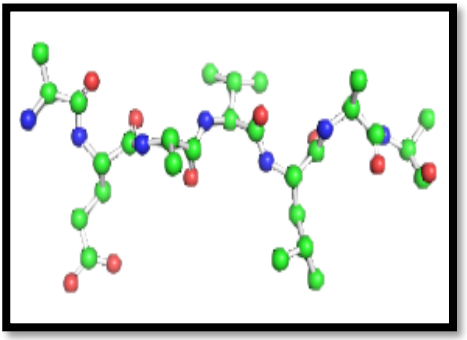

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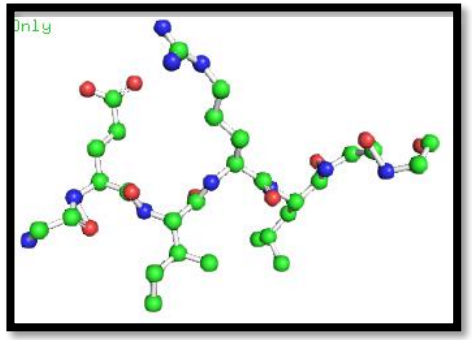

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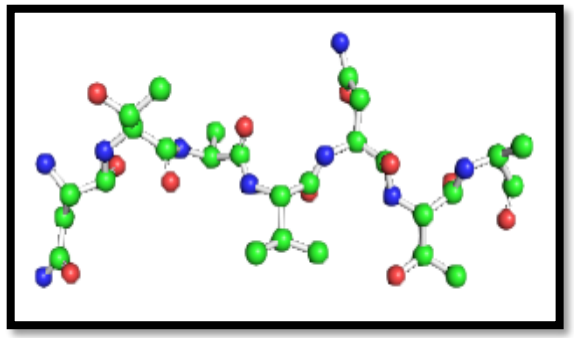

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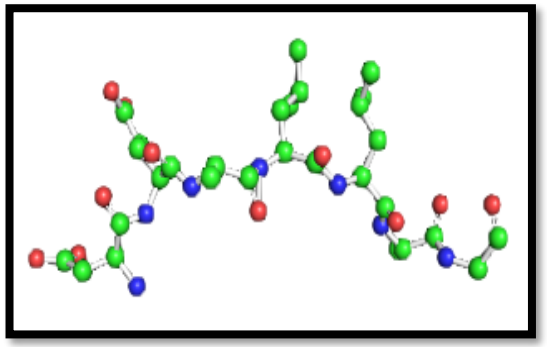

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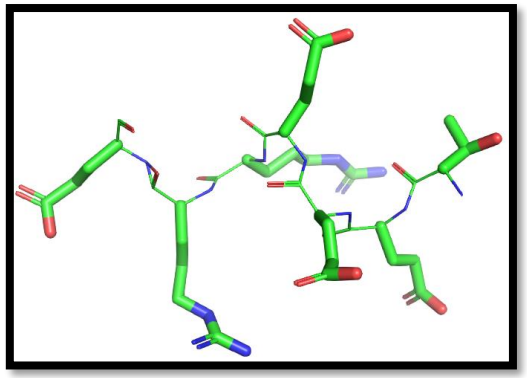
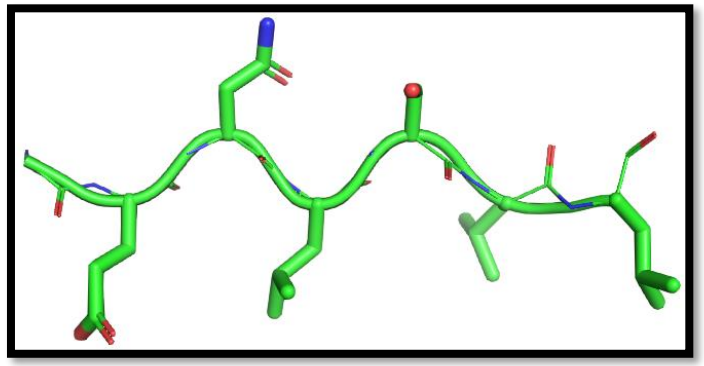
# BLIP-I based peptides



BLIP_P0



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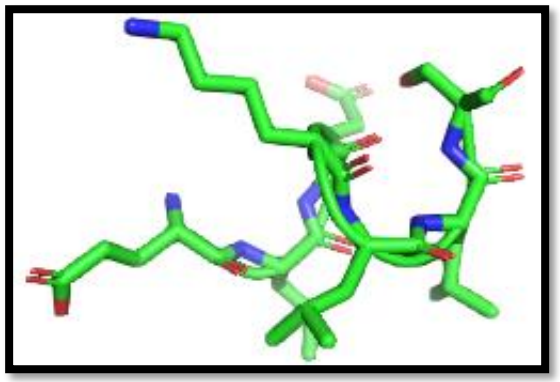
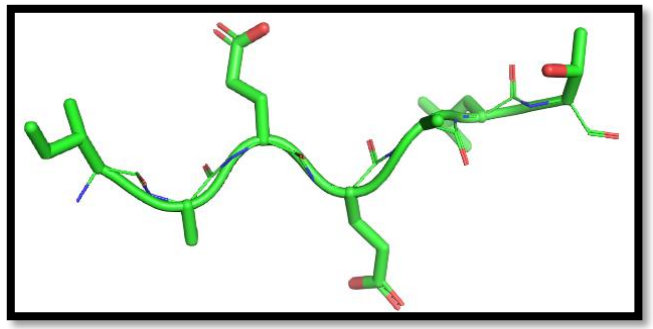


BLIP_P2



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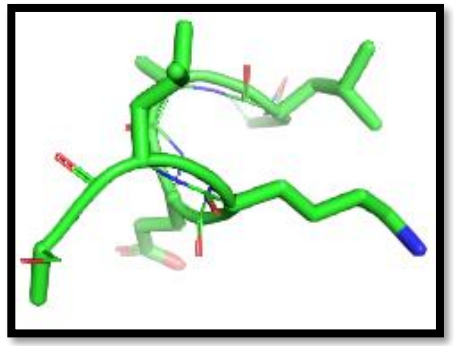

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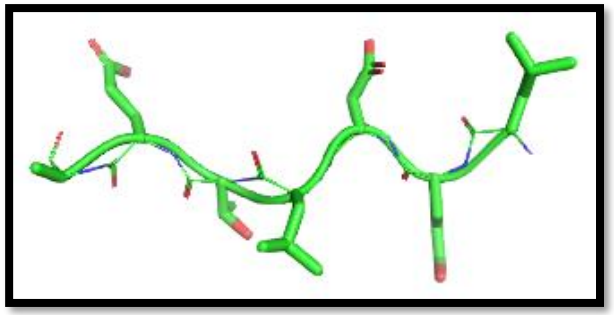

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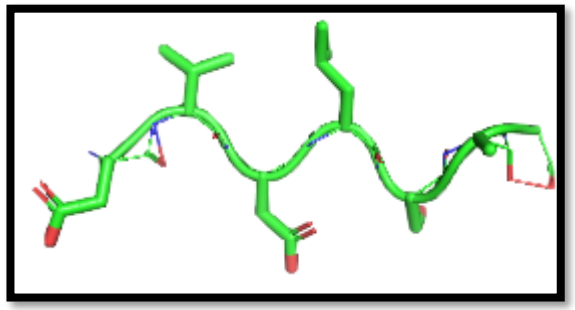

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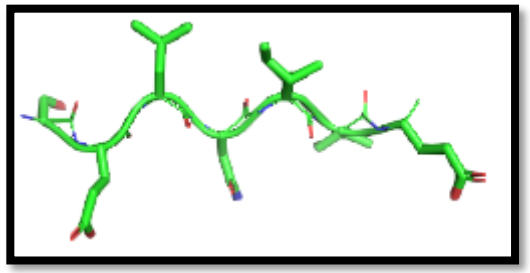

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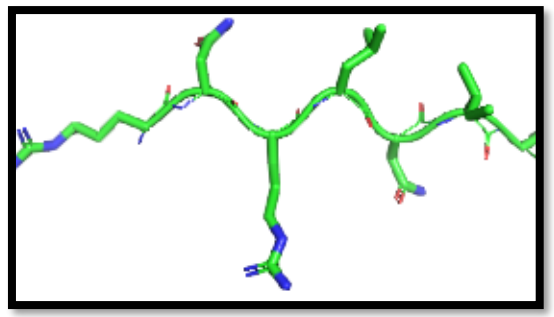

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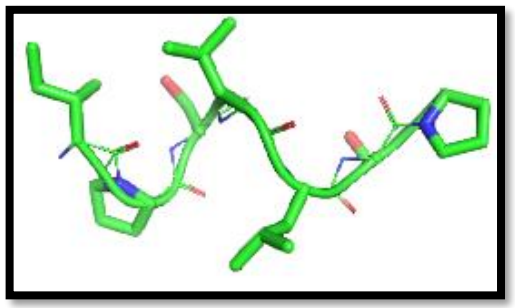


BLIP_P9

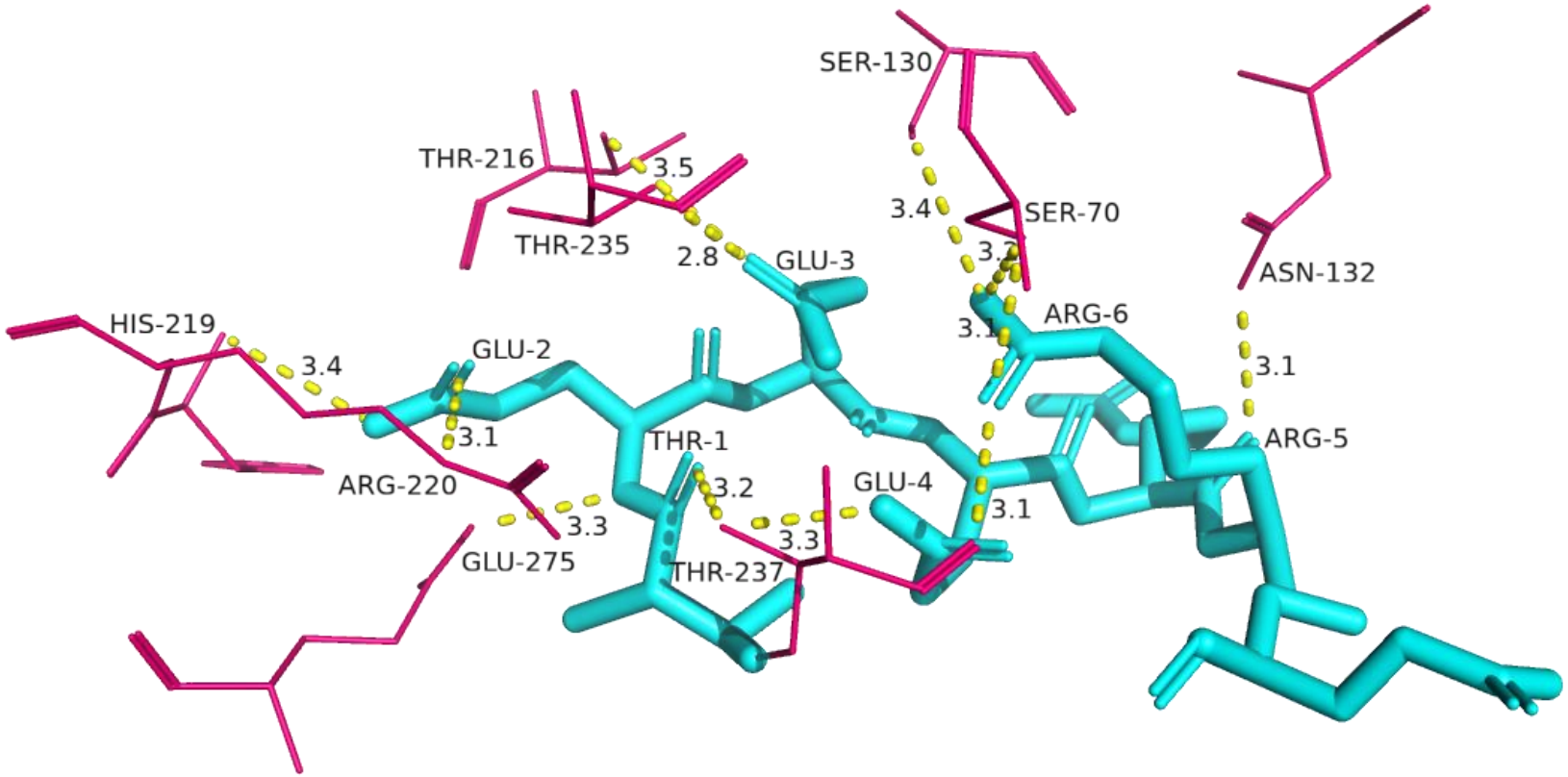
# Number of generated peptides

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

Number of peptides generated for each target.

# Docked BLIP_P0 with KPC-2

| BLIP-P0 | KPC-2 | Distance |
|---|---|---|
| THR-1 | THR-237 | 3.2 |
| GLU-2 | HIS-219 | 3.4 |
| GLU-2 | ARG-220 | 3.1 |
| GLU-2 | GLU-275 | 3.3 |
| GLU-3 | THR-216 | 3.5 |
| GLU-3 | THr-235 | 2.8 |
| GLU-4 | THR-237 | 3.3 |
| ARG-5 | ASN-132 | 3.1 |
| ARG-6 | SER-70 | 3.2 |
| ARG-6 | THR-237 | 3.1 |
| ARG-6 | SER-130 | 3.4 |



# PRODIGY analysis

| Binding Score (ΔG) kcal/mol | | | | | |
|---|---|---|---|---|---|
| | **BLIP_P0** | **OM_P0** | **OM_PAP1_0** | **VAR_C1_0** | **VAR_C12_0** |
| **CTX-M-1** | -7.5 | -8.6 | -8.4 | -8.5 | -7.7 |
| **KPC-2** | -7.5 | -11.2 | -9.2 | -8.5 | -6.6 |
| **SME-1** | -8.2 | -9.3 | -8.6 | -7.4 | -7.6 |
| **TEM-1** | -8.3 | -7.2 | -7.6 | -6.7 | -8.1 |
| **SHV-1** | -7.5 | -9.1 | -8.9 | -7.9 | -10.3 |

# Summary

- Successfully designed novel peptides targeting BLIP proteins, Omega loop, and conserved motifs.

- Computational approaches (RFDiffusion, ProteinMPNN, AlphaFold2) have successfully predicted stable and high-affinity peptide inhibitors.

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# THANK YOU!