

Computational Design of TEM-1 β -Lactamase Using PyRosetta: Step Towards Integrating Dynamics into Computational Enzyme Design

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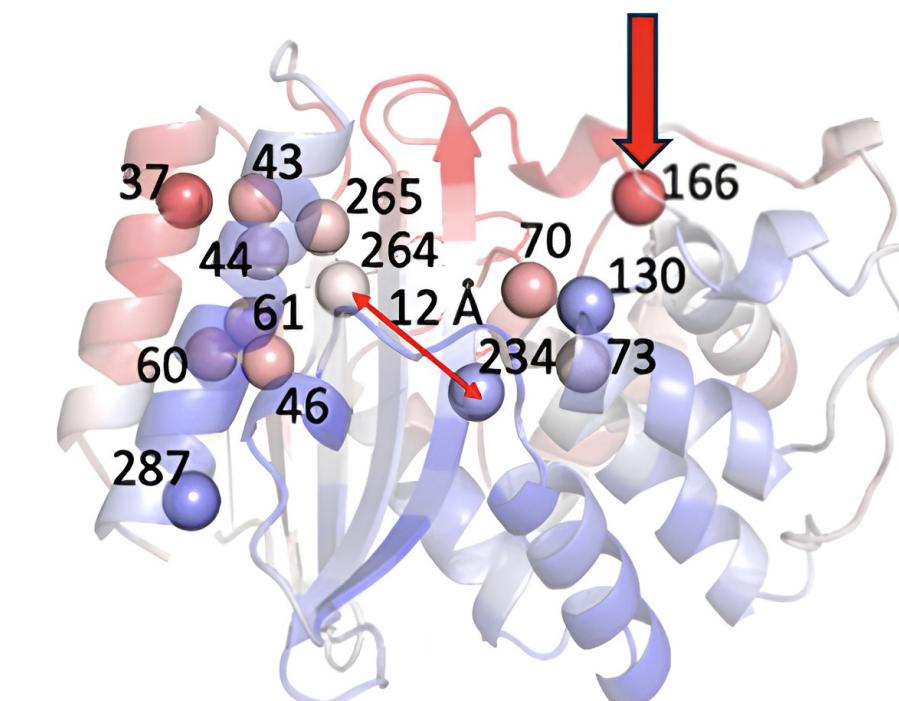
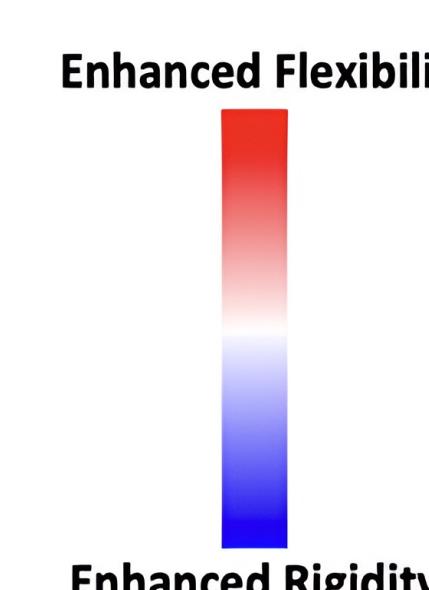
Introduction

- TEM-1 β -lactamase is a crucial enzyme in bacterial antibiotic resistance, capable of hydrolyzing β -lactam antibiotics.
- Enzyme dynamics significantly influence protein function and stability but are challenging to incorporate into design protocols.
- We developed a method to integrate dynamics into protein design using Pyrosetta and Martini simulations.
- Our approach combines computational design with rapid coarse-grained simulations to assess dynamic properties.

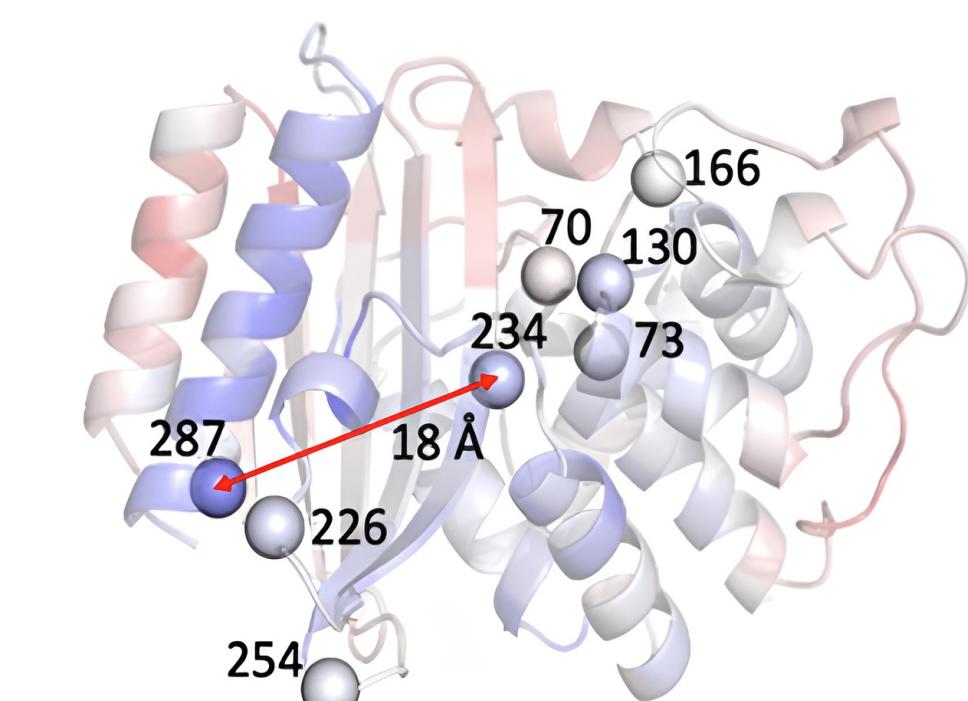


TEM-1 β -lactamase (PDB ID: 1BTL)

Background

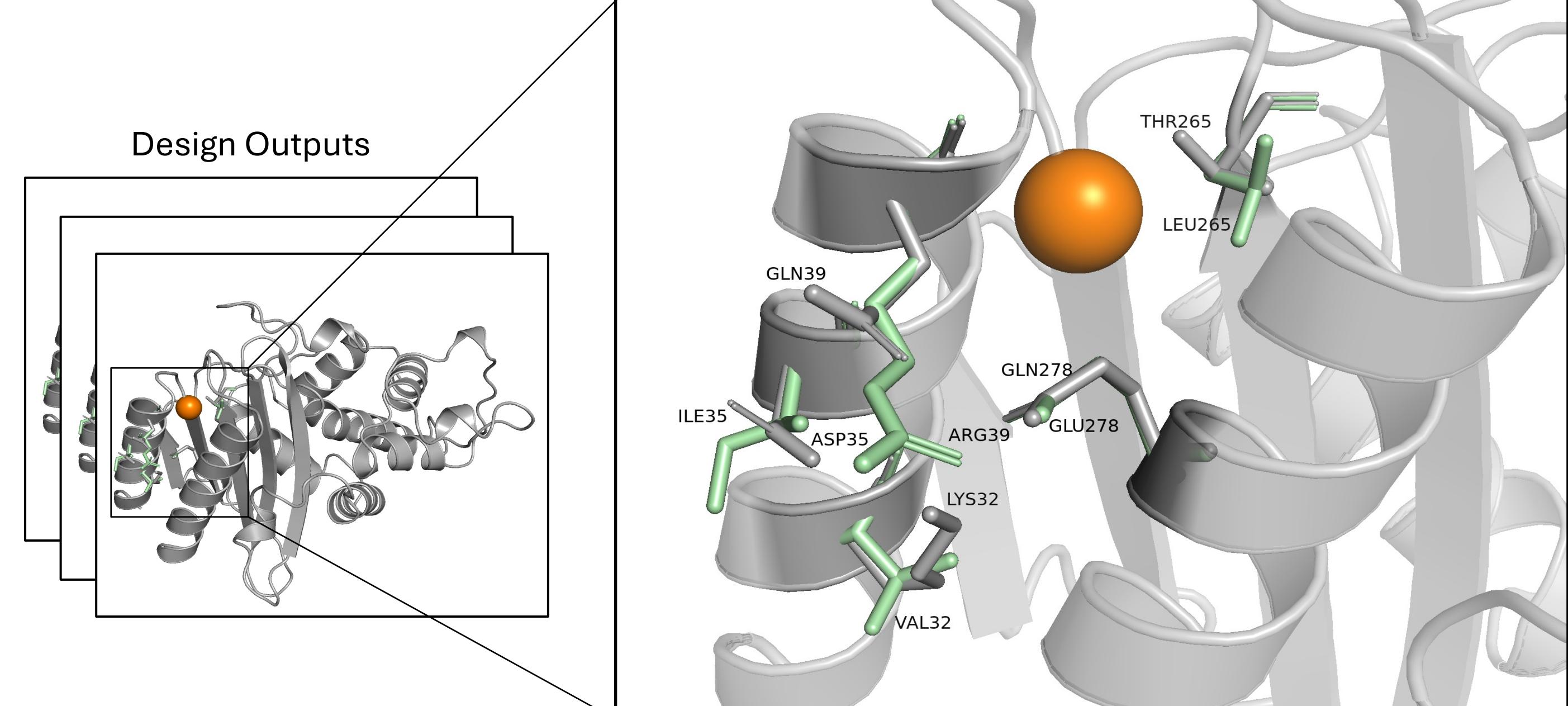
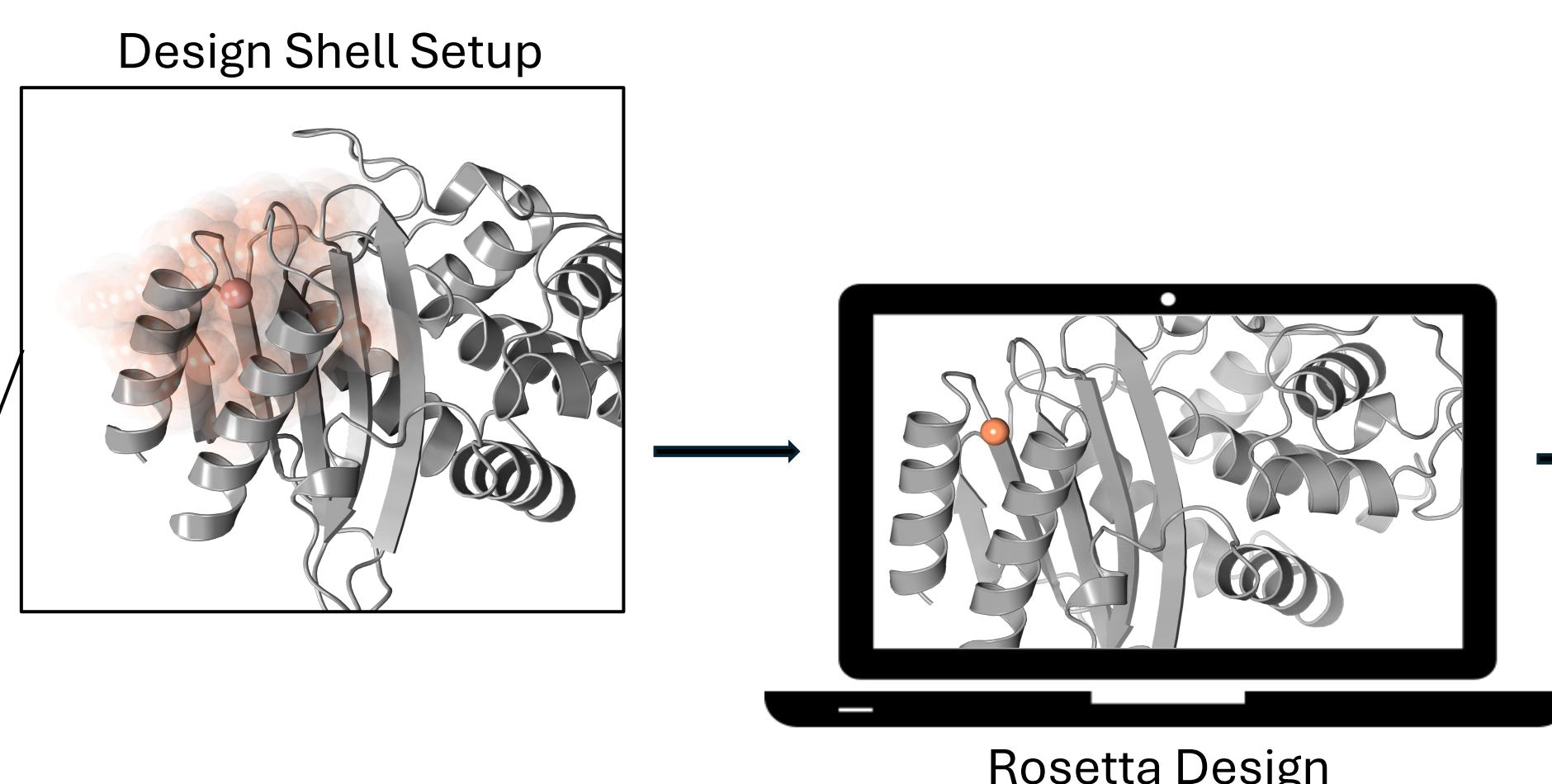
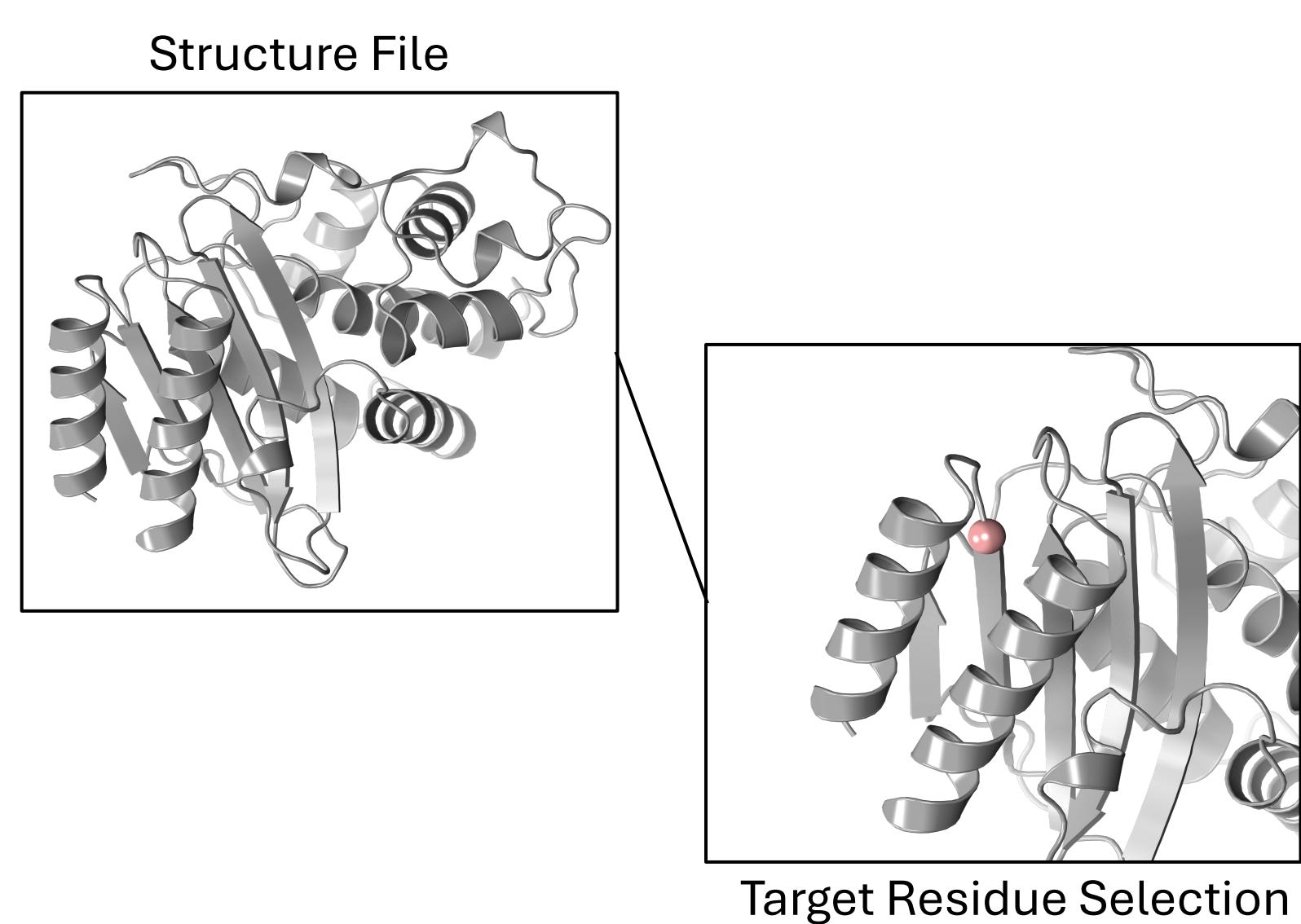


Kolbaba-Kartchner, B. et al. (2021). "The Role of Rigid Residues in Modulating TEM-1 β -Lactamase Function and Thermostability." Int. J. Mol. Sci., 22, 2895.



- Previous work by Kolbaba-Kartchner et al. (2021) highlighted the importance of rigid residues in TEM-1's function and thermostability.
- We recreated this design protocol in PyRosetta to establish a foundation for integrating enzyme dynamics in future designs.

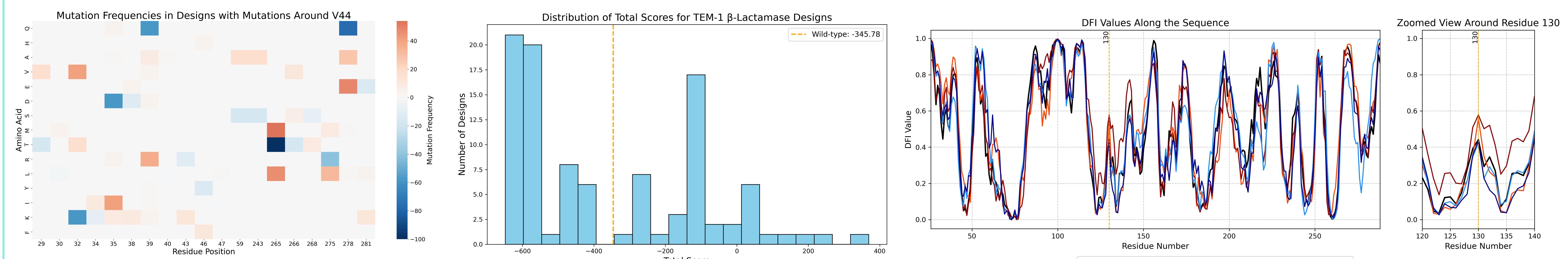
Computational Methods and Results



- Design protocol:
 - Target: Rigid residues(44 & 262) & flexible residues(55 & 226)
 - Design shell: 8-12 Å radius, Repack shell: +4 Å
 - Applied PackRotamersMover with custom TaskFactory
- Iterative design process: Random selection of design shell radius for each cycle

- Generated 100 unique designs and calculated Rosetta full-atom energy scores for each design
- Performed 100ns Martini coarse-grained simulations on each design
- Calculated DFI profiles from trajectories to assess dynamic properties

Analysis

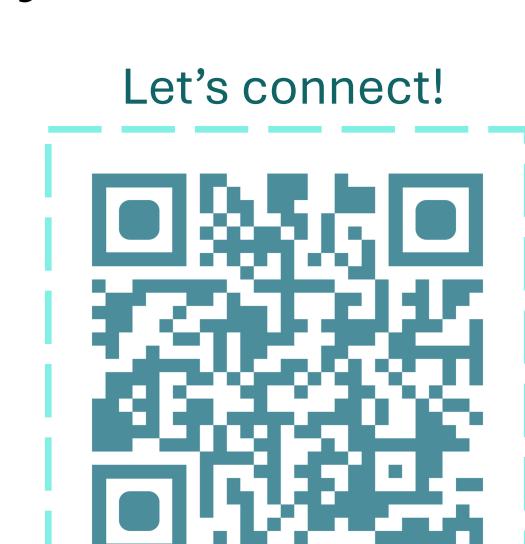


- Designs exhibit diverse mutations across various target residues
- Rosetta energy scores of designs show a wide distribution, with some designs potentially offering improved stability over wild-type

- Dynamic Flexibility Index (DFI) analysis reveals dynamics: designs targeting rigid residues changes flexibility of catalytic residues
- Designs targeting flexible residues maintain flexibility patterns more similar to the wild-type

Conclusions & Future Plans

- Successfully recreated TEM-1 β -lactamase designs using PyRosetta and performed initial Martini simulations on selected designs
- Next step: Develop automated workflow to seamlessly connect design generation, simulation, and dynamic analysis
- Refine design protocols to better control and predict dynamic properties
- Aim to extend methodology to other antibiotic resistance enzymes for broader impact



Acknowledgement

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