

# Treatment of Flexibility in Protein Backbone During Steered Molecular Dynamics Simulations



Duc Toan Truong<sup>1,2</sup> Kiet Ho<sup>3</sup> Dinh Quoc Huy Pham<sup>4</sup> Mateusz Chwastyk<sup>4</sup> Thai Nguyen-Minh<sup>5</sup>  
Minh Tho Nguyen<sup>1,2</sup>

<sup>1</sup>Laboratory for Chemical Computation and Modeling, Institute for Computational Science and Artificial Intelligence, Van Lang University, Ho Chi Minh City 70000, Vietnam. <sup>2</sup>Faculty of Applied Technology, School of Technology, Van Lang University, Ho Chi Minh City 70000, Vietnam. <sup>3</sup>Institute for Computational Science and Technology (ICST), Quang Trung Software City, Ho Chi Minh City 70000, Vietnam. <sup>4</sup>Institute of Physics, Polish Academy of Sciences, Warsaw, Poland. <sup>5</sup>University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City 70000, Vietnam.

## Introduction

Steered Molecular Dynamics (SMD) simulations are widely used to study protein-ligand interactions. Traditional methods often rigidly constrain protein backbones, leading to artificial behavior. This study proposes a novel restraining approach that better mimics natural ligand unbinding processes, offering insights critical for rational drug design.

## Objectives

- Examine the impact of different restraining methods on SMD outcomes.
- Propose an optimized restraining method for accurate simulations of lig-protein unbinding.

## Methods

### Restraint Approaches:

- Mode 1: Fixing all heavy atoms.
- Mode 2: Fixing all C $\alpha$  atoms.
- Mode 3-6: Gradual flexibility by restraining C $\alpha$  atoms at specific distances from the ligand.

### Simulation Parameters:

- System: Six protein-ligand complexes: 4JNJ, 2JFZ, 1PYE, 1TSL, 2YDV and 1EVE
- Tools: GROMACS, Amber ff99SB-ILDN force field.

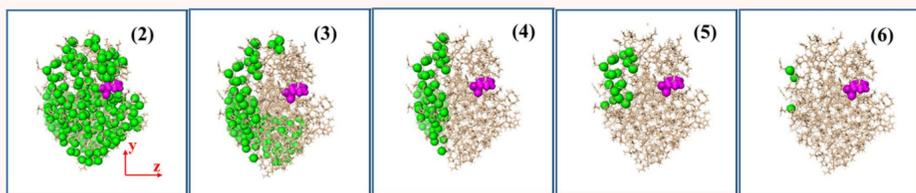


Figure 1. (2) fixing all C $\alpha$  atoms of protein; (3) fixing all C $\alpha$  atoms with a distance to ligand greater than 1.2 nm; (4) fixing all C $\alpha$  atoms with the perpendicular distance in the pulling direction to ligand greater than 1.2 nm; (5) fixing all C $\alpha$  atoms with the perpendicular distance in the pulling direction to ligand greater than 1.2 nm and all C $\alpha$  atoms with the perpendicular distance in the x-y direction to ligand smaller than 1.2 nm, and (6) fixing all C $\alpha$  atoms with the perpendicular distance in the pulling direction to ligand greater than 1.8 nm and all C $\alpha$  atoms with the perpendicular distance in the x-y direction to ligand smaller than 1.2 nm.

## Results

### Flexibility Enhances Accuracy

- Rigid modes (1 & 2) produced higher rupture forces and unbinding barriers, deviating from natural behaviours.
- Flexible modes (3-6) showed reduced barriers, aligning with physiological processes.

### Mode 3 Optimized Restraint

- Restraining C $\alpha$  atoms  $\pm 1.2$ nm from ligand offered the best balance between stability and natural motion.

### Improved Contact Sampling

- Flexible modes enables more residues to interact with the ligand, reflecting realistic dynamic.



Figure 2.

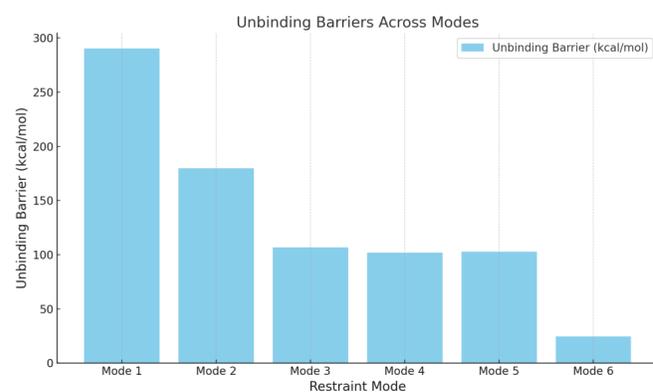


Figure 3.

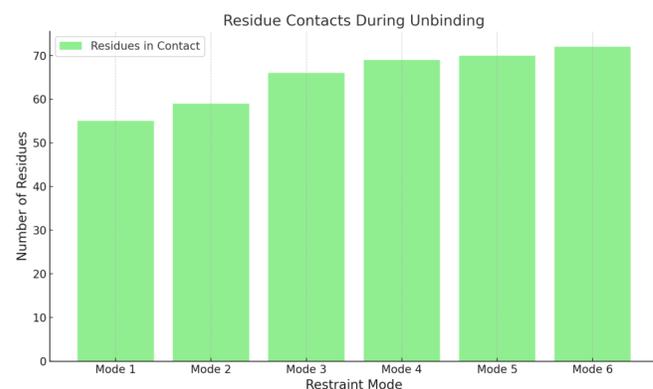


Figure 4.

## Conclusions

- Fully rigid restraints (Modes 1 & 2) are unsuitable for realistic SMD simulations.
- Flexible restraints, particularly Mode 3, provide physiologically relevant insights.
- Recommendations:
  - Use flexible restraints in SMD to ensure accurate ligand unbinding pathways.
  - Extend findings to refine drug design methodologies.

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## Reference

[1] Duc Toan Truong, Kiet Ho, Dinh Quoc Huy Pham, Mateusz Chwastyk, Thai Nguyen-Minh, and Minh Tho Nguyen. *Scientific Reports*, 14(1):10475, 2024.