# THE MOLECULAR BEHAVIOR OF APO FORM OF CRUZAIN ENZYME AND ITS COMPLEXES WITH NONCOVALENT INHIBITORS

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*Keyword:* Cruzain, Molecular Dynamics, Benzimidazoles.

## Introduction

Chagas disease, whose etiological agent is the parasite *Trypanosoma cruzi* (*T. cruzi*), is a tropical pathology, which affects around 6 to 7 million people all over the world, causing a huge impact on global health.[1] However, the treatment for this disease, based on nifurtimox and benznidazole, is still unsatisfactory, being ineffective during the chronic phase, presenting frequent side effects and needing long periods of administration.[1]

Recent researches have described new benzimidazole-based inhibitors (8d, 8l, and 8r) of the cruzain (CRZ) enzyme, the major cysteine protease of *T. cruzi*, presenting a powerful trypanocidal activity and an interesting therapeutic profile.[2] Benzimidazole inhibitor 8d complexed with cruzain (PDB ID: 3KKU) was used in this research. Nevertheless, the mechanism of inhibition promoted by the others compounds remains unclear.

## Objective and Methods

Our aim was to study the structural and dynamic factors related to CRZ inhibition by benzimidazole inhibitors. The inhibitor structures were built and optimized, using the Spartan'14 software, and the CRZ-inhibitor complexes were obtained using Molegro 6.0 program, with the catalytic residues (Cys25, His162 and Asn182) at default protonation state. Hence, molecular dynamics (MD) simulations of Apo system and the CRZ-inhibitor complexes were performed in aqueous mean on the GROMACS 5.1, during 50 ns. Overall, the MD trajectories were analyzed.

## Results and Discussion

Comparing our experimental data, there are differences between the obtained results through the molecular docking and MD methods, which suggest that is essential to consider the flexibility of both structures, protein and ligand, on the molecular modelling studies of this enzyme.

The results of the hydrogen bond analysis showed that the compounds 8d, 8l and 8r bind to CRZ enzyme through hydrogen bonds, mainly with the residues Gly23, Trp26, Ser64, Gly66, and Asp161.

The analysis of CRZ secondary structure content showed that there was little variation in the secondary structure of the enzyme during the simulation, even when CRZ is complexed with inhibitors (CRZ-8d, CRZ-8l and CRZ-8r).

Additionally, by comparing the conformational changes between the systems, there was a formation of a beta-sheet secondary structure between the Ala4-Asp6 (B1) residues in the presence of inhibitors 8d and 8r. The interaction with the inhibitor 8r also promoted the formation of a helix -310 between the residues Thr85-Ser88 in CRZ, which is not present to the other systems. Moreover, only in the Apo system (CRZapo), a 310-helix was formed between the residues Ser143-Thr146.

Finally, the analyses of the low frequency and high amplitude of CRZ motions in the CRZapo and CRZ-8l systems presented similar patterns of direction and extension of the terminal residues (Ala1 and Gly215). However, although the analysis of the CRZ movements, in CRZ-8d and CRZ-8r systems, showed very similar movements of the terminal residues, these motions differ from CRZ in the CRZapo and CRZ-8l systems.

## Conclusions

The results suggest that the CRZ inhibition, promoted by benzimidazole-based compounds, is a dynamic process related to the interaction, via hydrogen bond, mainly with Gly23, Trp26, Ser64, Gly66, and Asp161 residues.

## Acknowledgements

We would like to thank the Brazilian financial agencies, CNPq, CAPES, and FAPERJ.

## References

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