Journal of Molecular Modeling (2022) 28: 140
https://doi.org/10.1007/s00894-022-05135-6

ORIGINAL PAPER

Rethinking the MtInhA tertiary and quaternary structure flexibility: a molecular dynamics view

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Received: 22 July 2021 / Accepted: 30 April 2022 / Published online: 10 May 2022

Abstract

Flexibility and function are related properties in the study of protein dynamics. Flexibility reflects in the conformational potential of proteins and thus in their functionalities. The presence of interactions between protein-ligands and protein-protein complexes, substrates, and environmental changes can alter protein plasticity, acting from the rearrangement of the side chains of amino acids to the folding/unfolding of large structural motifs. To evaluate the effects of the flexibility in protein systems, we defined the enzyme 2-trans-enoyl-ACP (CoA) reductase from *Mycobacterium tuberculosis*, or MtInhA, as our target system. MtInhA is biologically active as a tetramer in solution; however, computational studies commonly use the monomer justifying the independence of its active sites due to their distances. However, differences in flexibility between tertiary and quaternary structures could present impact on the size of the active site, influencing the drug discovery process. In this study, we investigated the influence of flexibility restrictions in A- and B-loops of the MtInhA in order to suggest a monomeric structure that describes the conformational behavior of the tetrameric system. Overall, we observed that simulations where restrictions were applied to the A- and B-loops present a more similar behavior to the native structure when compared to unrestricted simulations. Therefore, our work presents a monomeric model of MtInhA, which has conformational characteristics of the biologically active structure. Thus, the data obtained in this work can be applied to the MtInhA system for the generation of more reliable flexible models for molecular docking experiments, and also for the performance of longer simulations by molecular dynamics and with a lower computational cost.

Keywords Flexibility · *Mycobacterium tuberculosis* · MtInhA · Molecular dynamics simulation

Introduction

Flexibility and function are related features in the study of protein dynamics, since it reflects on the conformational range, and thus functionality [1–3]. The presence of protein-ligand and protein-protein interactions, the binding of substrates, and environmental changes can impact protein plasticity, ranging from the rearrangement of amino acid side chains to the folding and unfolding of large structural motifs [4–6]. The subject of several molecular dynamic studies, the flexibility of the enzyme 2-trans-enoyl-ACP (CoA) reductase (InhA) from *Mycobacterium tuberculosis* (Mt) or MtInhA, has been reported due to the conformational changes observed in its binding cavity [7–13]. Kumar and Shobia studied the flexibility of the substrate-binding loop (SBL) in the binding of direct InhA inhibitors [8], reporting a bonding pattern that can be used to characterize an open and closed state of the SBL in the

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MtInhA [9], and also applied computational techniques to describe hot-spot residues acting on ligands binding [10]. Tonge and coworkers explored MtInhA plasticity to explain experimental data adjustment of slow-onset inhibitors on the substrate-binding pocket (SBP) [11]. Schroeder and collaborators described how point mutations, such as I21V, lead to isoniazid resistance; they also identified considerable flexibility of the SBL motif by using the monomeric form of the MtInhA [12].

MtInha, a homotetramer in solution, is a validated target enzyme for tuberculosis treatment [13]. The enzyme active site is composed of three regions: (i) substrate-binding loop (SBL), (ii) A-loop, and (iii) B-loop. The structural configuration shows A- and B-loops towards the center of the quaternary structure, where protein-protein interactions can be observed between the subjacent subunits, whereas SBL faces the solvent, out of the tetramer contact [14]. Crystallographic structures and molecular dynamics simulations revealed that the binding of ligands on the SBP can influence the flexibility of the main structural motifs of this cavity, SBL, and A- and B-loops. However, rigid-body docking and molecular dynamics simulations have been applied to MtInhA using a monomeric structure, since it demands less computational cost compared to a tetrameric structure, and also due to the hypothesis that the distance about 40 Å apart from each binding site is sufficient to make them independent [15–17]. Recently, Tarabini and coworkers highlighted the importance of the quaternary structure to represent a dynamic ensemble of the MtInhA by computational techniques, suggesting that the monomeric form could lead to conformations that are not observed either in crystallographic structures or by simulations where the tetrameric form is used [18]. The tetrameric arrangement indicates that the flexibility of A- and B-loops is affected by the proximity of the subjacent subunit, suggesting that the quaternary structure has an impact on MtInhA plasticity, possibly restraining these motif movements [19, 20].

This work proposes a new approach to simulate a tertiary protein structure that describes the dynamic behavior of a quaternary structure, in order to reduce the computational cost of molecular dynamics simulations without compromising the dynamic ensemble. We analyzed the dynamic ensemble of MtInhA by simulating five systems which, in three of them, were applied force constants on A- and B-loops: (i) the tetrameric structure, (ii) monomeric structure without restrictions, (iii) monomeric structure restrained at 0.25 kcal, (iv) monomeric restrained at 1.00 kcal, and (v) monomeric restrained at 5.00 kcal. To compare all simulations, we used probability density functions analysis based on the plasticity of the active site and principal component analysis to evaluate the overall flexibility of the MtInhA.

Material and methods

MtInhA crystallographic structure

All MD simulations were performed using the binary complex of the MtInhA:NADH, according to the PDB ID 1ENY [21, 22]. The tetramer (Fig. SF1) was generated using the rotational and translational matrices of the crystallographic structure.

Amber protocol

The general pipeline of the simulation consists of four basic steps, where firstly all systems were submitted to energy minimization cycles to adjust the bond length, angles, and dihedrals according to the parameters of the force field. In addition, the systems were solvated and short simulations were performed to allow the water molecules to be accommodated into the cavities of the MtInhA structure. After that, all systems were gradually equilibrated to the temperature of 298.16 K. The final step was the production phase of the simulations. All analyses were carried out using in-house python scripts.

The simulations were performed with the SANDER module of AMBER 18 [23], using the *ff14SB* force field model of Maier et al. (2015) [24]. Periodic bound conditions were applied and the NPT ensemble, where the temperature was kept to 298.16 K was used during all simulation time. All systems were solvated using the TIP3P model [25], and the constant pressure was guaranteed with an isotropic position scale, maintained at 1 bar by the Berendsen barostat [26]. The SHAKE algorithm [27], with a standard tolerance of 10^{-5} Å, was applied to restrict all bonds containing a hydrogen atom. The electrostatic interactions between unbound atoms were evaluated using the particle mesh Ewald method, with a charge network spacing of 1.0 Å. van der Waals interactions measured using a 9.0 A° atomic cutoff [28]. All hydrogen atoms, ions, and water molecules were subjected to molecular dynamics for 1.0 ns, to allow the systems to be balanced, while the protein structure was kept rigid. The systems were then submitted to 1,200 steps of steepest-descent and conjugate gradient energy minimization, up to a tolerance of 1,000 kJ mol^{-1} nm^{-1}, to remove close contacts of van der Waals forces and allow the atoms to progressively move until they were unrestrained. The temperature was gradually increased from 10 to 298.16 K in 7 steps (10 to 50 K, 50 to 100 K, 100 to 150 K, 150 to 200 K, 200 to 250 K, 250 to 298.16 K, and then kept at 298.16 K). The first six steps lasted for 200 ps each, and when the temperature reached 298.16 K, the systems were equilibrated for 8.8 ns NTP before the production phase. We performed three different simulations for each MtInhA system

by using different random seeds. The production phase of each MD simulation lasted for 90 ns and the total simulation time (heating and production) was 100 ns. All analyses were carried out using the last 90 ns of each simulation. The total simulation time was 1.5 μs.

Structural analysis of the MtInhA enzyme

To describe the influence of the quaternary structure on MtInhA plasticity, the systems were analyzed according to the distance, angle, and variation of the area based on residues present in the SBP: (i) A-loop (F97-H121), (ii) B-loop (D150-A167), and (iii) SBL (P193-D234). A pincer angle, to monitor the opening and closing of the SBP, was defined based on the center of mass of the three structural motifs. The central residue of the pincer angle was M98 (A-loop), P151 (B-loop), and A198 (SBL). All these analyses were carried out using the Geo-Measures plugin [29].

Results and discussion

MD simulations were performed to evaluate the impact of restraints on the flexibility of the MtInhA structure. Tarabini and coworkers [13] described that the mobility of A- and B-loops is restricted due to the proximity of the adjacent subunits, where approximately 40% of the A-loop and 66% of the B-loop are in contact with the adjacent subunits. It suggests that the quaternary structure implies a mechanism of flexibility regulation. According to these findings, this research aimed to mimic the dynamic behavior of the quaternary structure in a monomeric model by applying force constants to the A- and B-loops in order to restrict the flexibility of these regions.

Comparison of the ensembles

To evaluate the conformational changes in the SBP, we monitored the relationship between the triangle area and the pincer angle. This analysis can be used to describe the process of opening and closing the active site. According to our results, the unrestrained simulation presents the ability to sample conformations that are not accessible to other systems. Figure 1 demonstrates the conformational ensemble of the five groups based on the probability density function. The unrestrained system has a more spread plot, the most representative conformation averaging 33.5 $Å^2$ and 79.2 Å of triangle area and pincer angle, respectively. These results corroborate the hypothesis that in the absence of adjacent subunits the MtInhA presents higher flexibility; however, besides the system sample conformations not observed in other simulations, the most representative structure assumed during the simulation time is similar to the tetrameric and restricted simulations. The tetramer and restrained systems (0.25 kcal mol^{-1}, 1.00 kcal mol^{-1}, and 5.00 kcal mol^{-1}) showed a more limited area of dispersion. The most representative conformations observed for the triangle area and pincer angle in the tetrameric system were 34.0 $Å^2$ and 81.4 Å, respectively. Regarding the restrained simulations, the values of triangle area and pincer angle were 38.0 $Å^2$ and 83.3 Å (0.25 kcal mol^{-1}), 35.7 $Å^2$ and 77.7 Å (1.00 kcal mol^{-1}), and 37.8 $Å^2$ and 83.6 Å (5.00 kcal mol^{-1}). Therefore, it can be observed that the system where a force constant of 1.00 kcal mol^{-1} was applied showed a more similar distribution when compared to the tetrameric system. These data highlight the impact of the restrictions to reproduce the tetramer flexibility, and thus the importance of the adjacent subunits to the dynamics of the binding site. It is important to highlight that the relationship between the triangle area and pincer angle provides information regarding the size of the SBP, and according to our results, unrestrained simulations should be used with caution if the objective is to generate conformations for docking simulation of a flexible receptor model. Analyses monitoring the relationship between the distances of SBL:B-loop and A-loop:SBL (Figs. SF2 and SF3) also present a more spread plot for the unrestrained system when compared to restrained and tetrameric systems. These results are in agreement with Tarabini and coworkers [18], where it was described that monomeric simulations sampled conformations which are not accessible for the quaternary system.

Flexibility of the main structural motifs

The temperature factor (B-factor) is an interesting analysis to evaluate the displacement of atoms, allowing us to highlight what regions are contributing to the protein flexibility. In addition, since our goal is to propose a monomeric system that represents the dynamics behavior of the quaternary structure, it is important to observe what are the impacts of the restrictions on the A- and B-loops oN the overall structure. Figure 2 shows the difference in the B-factor between the simulations and the crystallographic structure. According to our results, we observed that the flexibility of residues composing the Rossmann fold domain is not affected by the restrictions applied to the simulations of 0.25 kcal mol^{-1}, 1.00 kcal mol^{-1}, and 5.00 kcal mol^{-1}. These observations can be confirmed by the B-factor analysis where all simulation systems present similar differences when compared to the temperature factor of the crystallographic structure. However, when analyzing regions of the SBP, the flexibility does not present the same behavior. It was observed that unrestrained simulations present higher flexibility on the A-loop, B-loop, and SBL. Moreover, it is important to highlight the differences between unrestrained and tetrameric simulations, which corroborate the hypothesis that

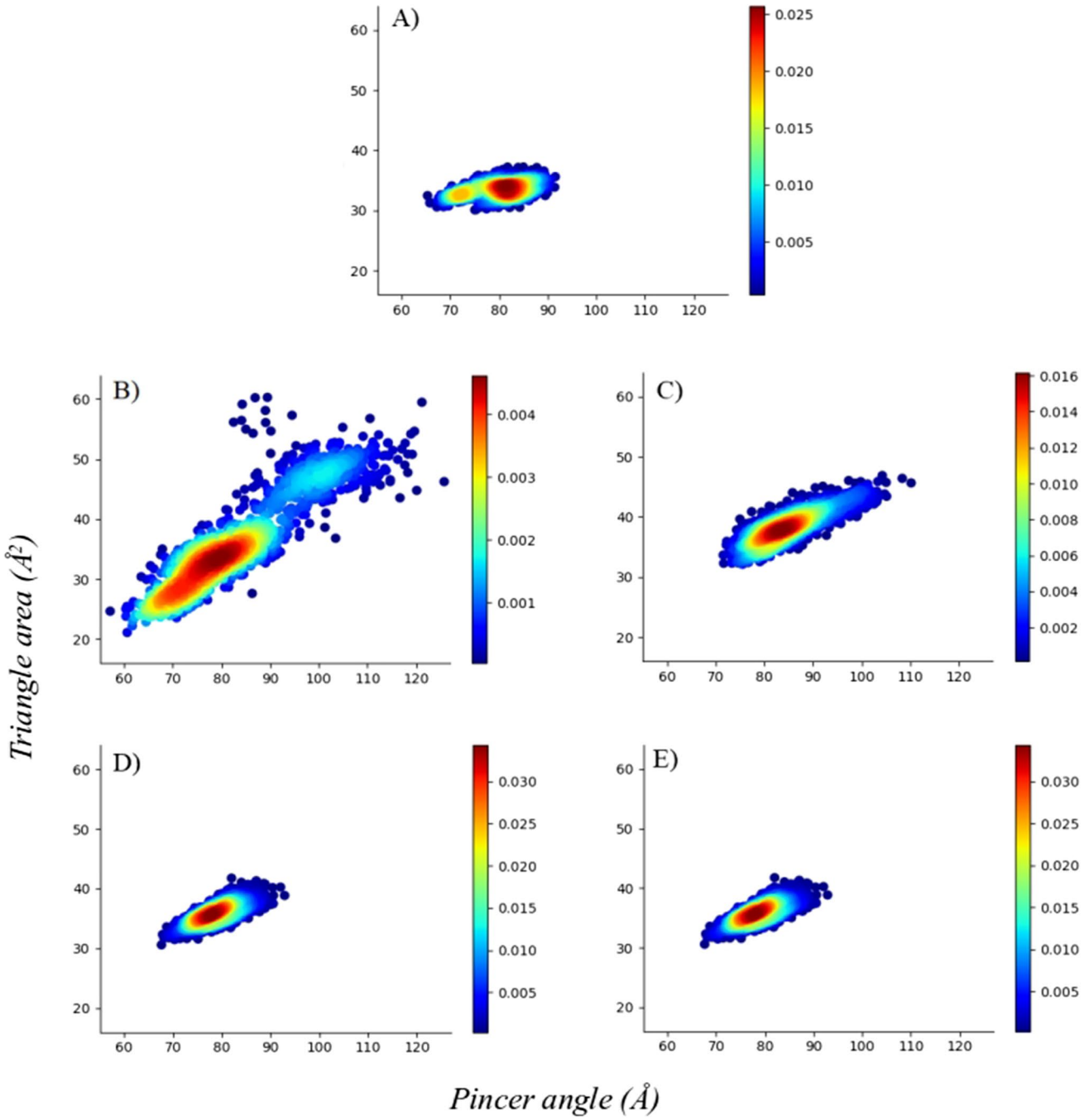

Fig. 1 Conformational ensembles of the MtInhA associated with NADH. The probability density plots of **A** tetramer, **B** unrestrained, and the restrained systems **C** 0.25 kcal mol^{-1}, **D** 1.00 kcal mol^{-1}, and **E** 5.00 kcal mol^{-1}, highlights the differences in the protein dynamics regarding the variability of the binding cavity. All data were obtained from three replicas simulations. Image generated with PyMOL [30] plugin Geomeasures [29].

the binding site conformation is perturbed by the presence of the adjacent subunits. These findings have been also observed by Tarabini and coworkers [18], where the SBP presents the largest B-factor between the monomer and tetramer simulations. Regarding the restrained simulations, the flexibility of these regions is well represented compared to the B-factor of the crystallographic structure as well as tetrameric simulations. Figure 3 presents the structure of the SBP highlighting the B-factors values. It is possible to observe that the restrictions applied to A- and B-loops can also impact the mobility of the SBL, suggesting that the 0.25 kcal mol^{-1} is sufficient to obtain values similar to the

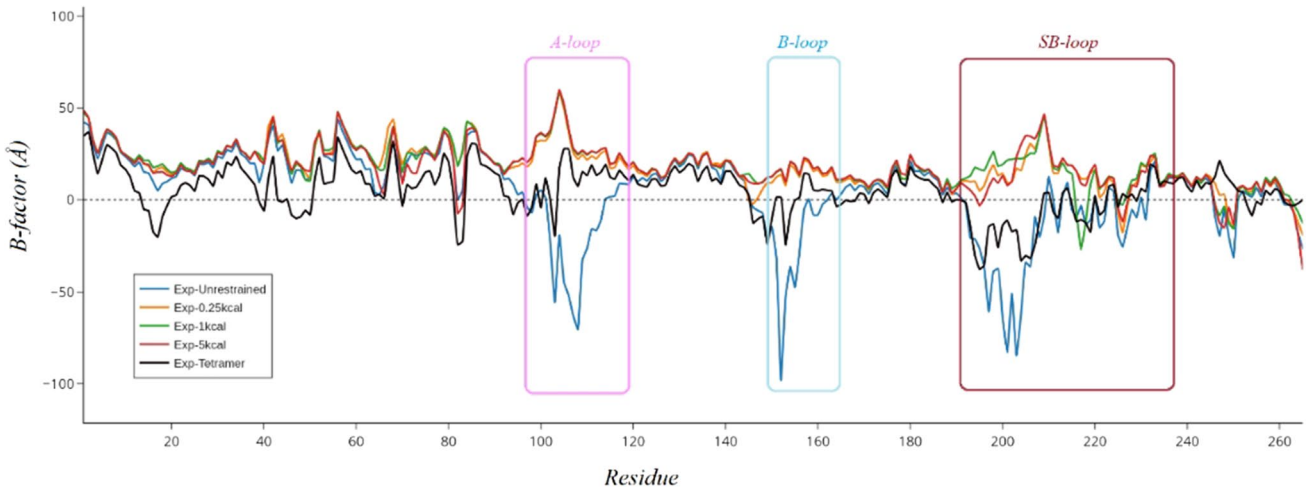

Fig. 2 Flexibility of the MtInhA structure according to different simulation systems. The figure shows the difference in the B-factors values for all systems when compared to the crystallographic structure. The regions A-loop, B-loop, and SBL are highlighted with boxes and colored in violet, blue and red, respectively. All data were obtained from three replicas simulations. Image generated with Plotly [31]

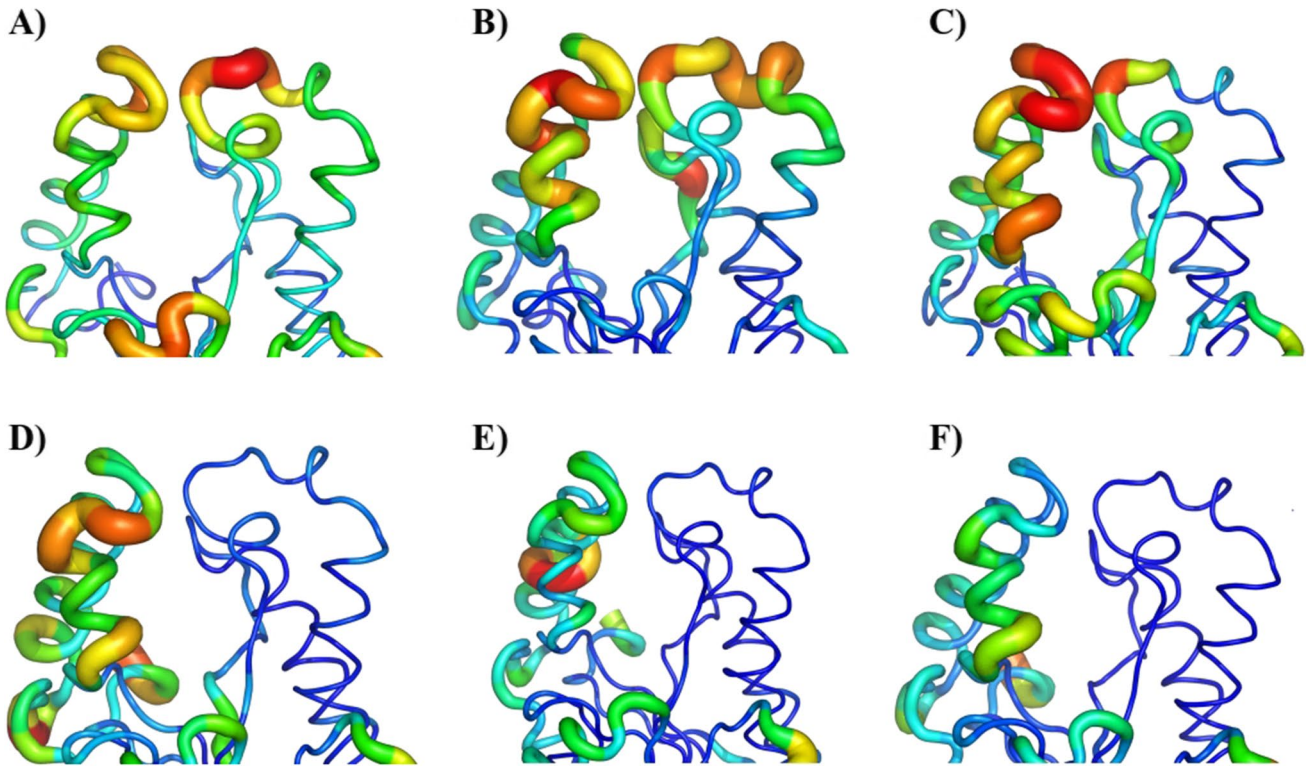

Fig. 3 B-factor values of the SBP region. **A** Crystallographic structure, **B** unrestrained, **C** tetramer, **D** 0.25 kcal mol^{-1}, **E** 1.00 kcal mol^{-1}, and **F** 5.00 kcal mol^{-1} restrained systems. All structures are represented as B-factor putty, considering the difference when compared to the crystallographic structure. All data were obtained from three replicas simulations. Image generated with PyMOL

experimental structures. However, according to these results, we should be aware that the restrictions using the same force constant to all residues of the A- and B-loops are not the best alternative to reproduce all aspects regarding the dynamic behavior of the MtInhA. An alternative could be weighting the force constant based on the proximity to the adjacent subunit. Overall, our restrained simulation model presents an interesting approach to describe the dynamic behavior of the tetrameric structure without increasing the computational cost. This is an important finding since our approach could

be applied to generate reliable confirmations for docking simulations, improving the drug discovery process.

Conclusions

Molecular dynamics simulation is a computational biophysical approach widely used to describe the dynamics of macromolecular systems. Therefore, to obtain reliable results, it is important to use the biologically active structure of the target, even when the active site is not located at the interface of the adjacent subunits. In order to reduce the computational cost, it is common to observe simulations of the tertiary structure instead of the quaternary structure. There are different explanations to carry out a system reduction, for the MtInhA is justified by the use of a monomeric system due to the distance between each binding site (≈40 Å). However, it was described elsewhere that the presence of adjacent subunits impacts the dynamic behavior of the MtInhA. Since it is a validated target to pursue new antitubercular compounds, we proposed a monomeric model system that describes the flexibility of the quaternary structure that could be used to evaluate the stability of ligands, identified by virtual screening, without losing dynamics information. The comparisons between restrained, unrestrained, and tetrameric systems allowed us to suggest a potential monomeric model that represents the conformational features of the tetrameric structure. In addition, it was possible to observe that a force constant of 0.25 kcal mol^{-1} was sufficient to mimic the interaction of A- and B-loops with the adjacent subunits of the tetrameric structure. The B-factor results indicated that the applied restrictions on the A- and B-loops do not compromise the overall flexibility of the MtInhA. In this way, our data indicates that unrestricted simulations present differences in the protein flexibility regarding the tetrameric model, whereas restrained simulations are able to better reproduce the dynamics of the tetrameric system, mainly in the SBL, A- and B-loops. We believe that this research comes up with important information to the study of the MtInhA flexibility.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00894-022-05135-6.

Acknowledgements We would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Author contribution O.N.S. and L.F.S.M.T. designed the study. L.S.C. carried out the molecular dynamics simulations. L.S.C. and L.F.S.M.T performed the structural analysis. L.S.C., L.F.S.M.T., and O.N.S. analyzed the data. L.S.C., L.F.S.M.T., O.N.S., and L.A.B wrote the manuscript. All authors reviewed the manuscript.

Funding Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, financial code 001).

Data availability Not applicable

Code availability Not applicable

Declarations

Competing interests The authors declare no competing interests.

References

1. Tsai CJ, Kumar S, Ma B, Nussinov R (1999) Folding funnels, binding funnels, and protein function. Protein Sci 8(6):1181–1190. https://doi.org/10.1110/ps.8.6.1181
2. Bahar I, Chennubhotla C, Tobi D (2007) Intrinsic dynamics of enzymes in the unbound state and relation to allosteric regulation. Curr Opin Struct Biol 17(6):633–640. https://doi.org/10.1016/j.sbi.2007.09.011
3. Henzler-Wildman K, Kern D (2007) Dynamic personalities of proteins. Nature 450(7172):964–972. https://doi.org/10.1038/nature06522
4. Marsh JA, Hernández H, Hall Z, Ahnert SE, Perica T, Robinson CV, Teichmann SA (2013) Protein complexes are under evolutionary selection to assemble via ordered pathways. Cell 153(2):461–470. https://doi.org/10.1016/j.cell.2013.02.044
5. Marsh JA, Teichmann SA (2014) Protein flexibility facilitates quaternary structure assembly and evolution. PLoS Biol 12(5):e1001870. https://doi.org/10.1371/journal.pbio.1001870
6. Teilum K, Olsen JG (1814) Kragelund BB (2011) Protein stability, flexibility and function. Biochim Biophys Acta 8:969–976. https://doi.org/10.1016/j.bbapap.2010.11.005
7. da Costa AL, Pauli I, Dorn M, Schroeder EK, Zhan CG, de Souza ON (2012) Conformational changes in 2-trans-enoyl-ACP (CoA) reductase (MtInhA) from M. tuberculosis induced by an inorganic complex: a molecular dynamics simulation study. J Mol Model 18(5):1779–1790 https://doi.org/10.1007/s00894-011-1200-7
8. Kumar V, Sobhia ME (2013) Characterisation of the flexibility of substrate binding loop in the binding of direct InhA inhibitors. Int J Comput Biol Drug Des 6(4):318–342. https://doi.org/10.1504/IJCBDD.2013.056795
9. Kumar V, Sobhia ME (2014) Insights into the bonding pattern for characterizing the open and closed state of the substrate-binding loop in Mycobacterium tuberculosis InhA. Future Med Chem 6(6):605–616. https://doi.org/10.4155/fmc.14.27
10. Kumar V, Sobhia ME (2016) Molecular dynamics-based investigation of InhA substrate binding loop for diverse biological activity of direct InhA inhibitors. J Biomol Struct Dyn 34(11):2434–2452. https://doi.org/10.1080/07391102.2015.1118410
11. Lai CT, Li HJ, Yu W, Shah S, Bommineni GR, Perrone V, Garcia-Diaz M, Tonge PJ, Simmerling C (2015) Rational modulation of the induced-fit conformational change for slow-onset inhibition in Mycobacterium tuberculosis InhA. Biochemistry 54(30):4683–4691. https://doi.org/10.1021/acs.biochem.5b00284
12. Schroeder EK, Basso LA, Santos DS, de Souza ON (2005) Molecular dynamics simulation studies of the wild-type, I21V, and I16T mutants of isoniazid-resistant Mycobacterium tuberculosis enoyl reductase (MtInhA) in complex with NADH: toward the understanding of NADH-MtInhA different affinities. Biophys J 89(2):876–884. https://doi.org/10.1529/biophysj.104.053512

13. Takayama K, Wang C, Besra GS (2005) Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis. Clin Microbiol Rev 18(1):81–101. https://doi.org/10.1128/CMR.18.1.81-101.2005
14. Quémard A, Sacchettini JC, Dessen A, Vilcheze C, Bittman R, Jacobs WR Jr, Blanchard JS (1995) Enzymatic characterization of the target for isoniazid in Mycobacterium tuberculosis. Biochemistry 34(26):8235–8241. https://doi.org/10.1021/bi00026a004
15. Pauli I, dos Santos RN, Rostirolla DC, Martinelli LK, Ducati RG, Timmers LF, Basso LA, Santos DS, Guido RV, Andricopulo AD, Norberto de Souza O (2013) Discovery of new inhibitors of Mycobacterium tuberculosis MtInhA enzyme using virtual screening and a 3D-pharmacophore-based approach. J Chem Inf Model 53(9):2390–2401. https://doi.org/10.1021/ci400202t
16. Shaw DJ, Hill RE, Simpson N, Husseini FS, Robb K, Greetham GM, Towrie M, Parker AW, Robinson D, Hirst JD, Hoskisson PA, Hunt NT (2017) Examining the role of protein structural dynamics in drug resistance in Mycobacterium tuberculosis. Chem Sci 8(12):8384–8399. https://doi.org/10.1039/c7sc03336b
17. Merget B, Sotriffer CA (2015) Slow-onset inhibition of Mycobacterium tuberculosis MtInhA: revealing molecular determinants of residence time by MD simulations. PloS One 10(5):e0127009. https://doi.org/10.1371/journal.pone.0127009
18. Tarabini RF, Timmers L, Sequeiros-Borja CE, Norberto de Souza O (2019) The importance of the quaternary structure to represent conformational ensembles of the major Mycobacterium tuberculosis drug target. Sci Rep 9(1):13683. https://doi.org/10.1038/s41598-019-50213-0
19. Chollet A, Maveyraud L, Lherbet C, Bernardes-Génisson V (2018) An overview on crystal structures of MtInhA protein: Apoform, in complex with its natural ligands and inhibitors. Eur J Med Chem 146:318–343. https://doi.org/10.1016/j.ejmech.2018.01.047
20. Qiu X, Janson CA, Court RI, Smyth MG, Payne DJ, Abdel-Meguid SS (1999) Molecular basis for triclosan activity involves a flipping loop in the active site. Protein Sci 8(11):2529–2532. https://doi.org/10.1110/ps.8.11.2529
21. Dessen A, Quémard A, Blanchard JS, Jacobs WR Jr, Sacchettini JC (1995) Crystal structure and function of the isoniazid target of Mycobacterium tuberculosis. Science (New York, N.Y.), 267(5204):1638–1641 https://doi.org/10.1126/science.7886450
22. Rozwarski DA, Vilchèze C, Sugantino M, Bittman R, Sacchettini JC (1999) Crystal structure of the Mycobacterium tuberculosis enoyl-ACP reductase, MtInhA, in complex with NAD+ and a C16 fatty acyl substrate. J Biol Chem 274(22):15582–15589. https://doi.org/10.1074/jbc.274.22.15582
23. Case DA, Ben-Shalom IY, Brozell SR, Cerutti DS, Cheatham TE, Cruzeiro VWD, Kollman PA (2019) AMBER 2019. University of California, San Francisco
24. Maier JA, Martinez C, Kasavajhala K, Wickstrom L, Hauser KE, Simmerling C (2015) ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. J Chem Theory Comput 11(8):3696–3713. https://doi.org/10.1021/acs.jctc.5b00255
25. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein M (1983) Comparison of simple potential functions for simulating liquid water. J Chem Phys 79:926–935. https://doi.org/10.1063/1.445869
26. Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. J Chem Phys 81:3684–3690. https://doi.org/10.1063/1.448118
27. Ryckaert JP, Ciccotti G, Berendsen HJC (1977) Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J Comput Phys 23:327–341. https://doi.org/10.1016/0021-9991(77)90098-5
28. Norberto de Souza O, Ornstein RL (1999) Molecular dynamics simulations of a protein-protein dimer: particle-mesh Ewald electrostatic model yields far superior results to standard cutoff model. J Biomol Struct Dyn 16(6):1205–1218. https://doi.org/10.1080/07391102.1999.10508328
29. Kagami LP, das Neves GM, Timmers LFSM, Caceres RA, Eifler-Lima VL (2020) Geo-Measures: A PyMOL plugin for protein structure ensembles analysis. Comput Biol Chemhttps://doi.org/10.1016/j.compbiolchem.2020.107322
30. DeLano WL (2002) The PyMOL molecular graphics system on world wide web. https://www.pymol.org
31. Plotly Technologies Inc. Collaborative data science. Porto Alegre, BR, 2021