CHARMM force field parameters for the Zn^{+} centre of 6-pyruvoyl tetrahydropterin synthase enzyme

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Introduction

The malaria parasite drug-resistance has raised a great challenge in anti-malarial drug discovery and grounded the need for new treatments. 6-pyruvoyl tetrahydropterin synthase (PTPS) is the second enzyme of the malaria parasite *de novo* folate biosynthesis pathway. PTPS is responsible for the conversion of dihydroneopterin triphosphate to 6 pyruvoyl tetrahydropterin via a base catalyzed redox transfer reaction and elimination of the triphosphate tail [3]. The hexamric enzyme 3D structure comprises two symmetrical trimers, each has three monomers. The enzyme has six zinc-containing active sites, each buried in a deep pocket of 12 Å [3]. The active site Zn^{+2} ion is coordinated to three histidine residues through their NE2 atoms (Figure 2). In this study, appropriate force fields parameters describing the Zn^{+2} ion were developed, using quantum mechanics (QM) calculations and then validated through all atomics molecular dynamics (MD) simulations. The generated parameters are of important use for accurate MD simulations for future computer-aided drug discovery studies.

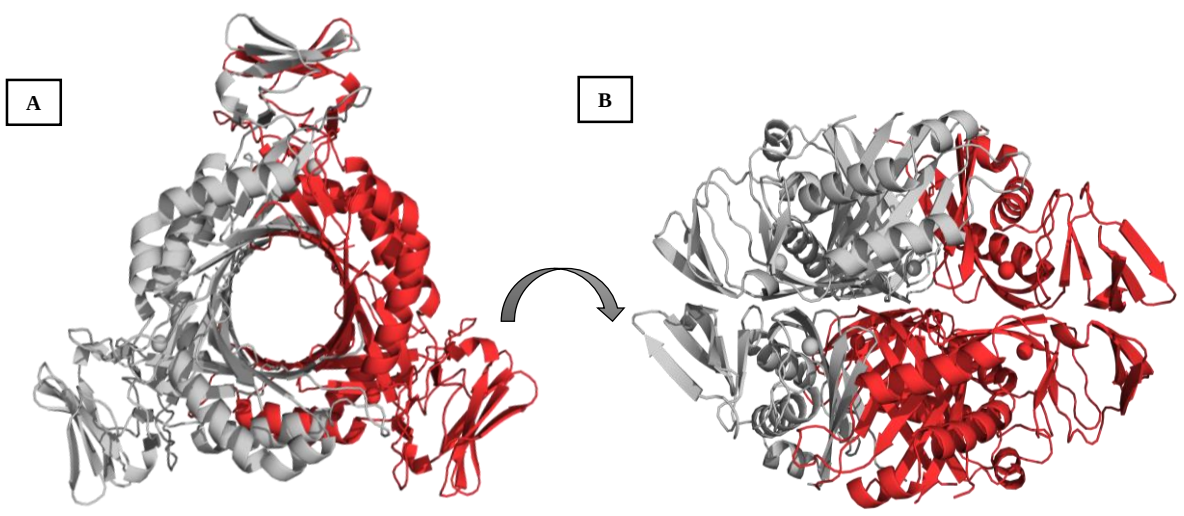
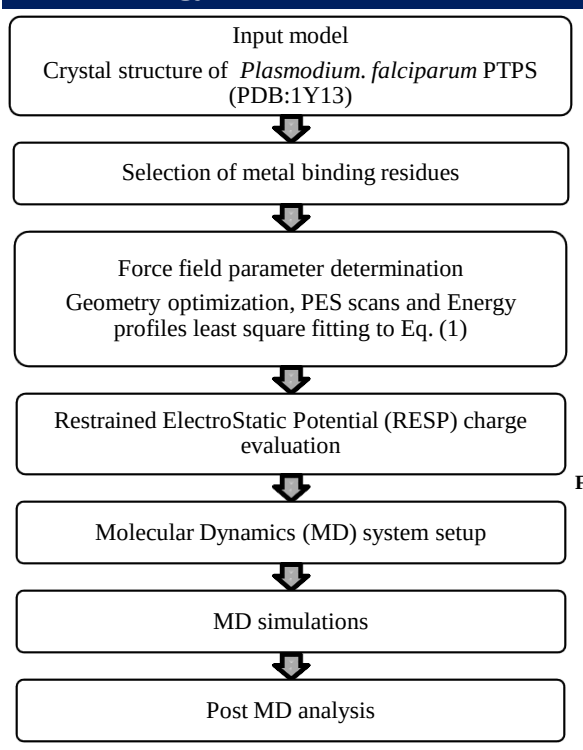


Figure 1: (A) top view and (B) side view of *Plasmodium. falciparum* PTPS homo-hexameric enzyme structure (PDB ID: 1Y13).

Methodology



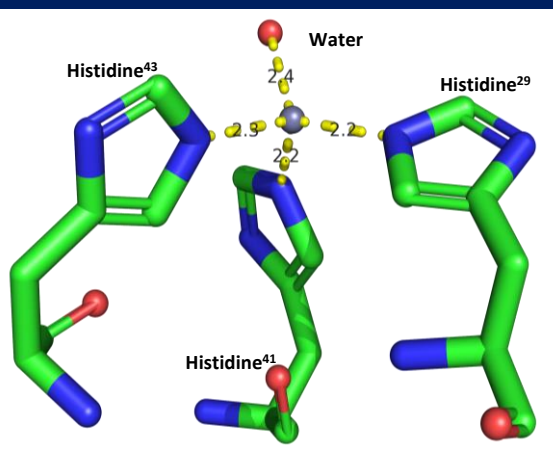


Figure 2: Active site subset selected from the x-ray structure (PDB ID: 1Y13).

$$E_{total} = \sum_{bonds} k_b(r - r_{0,b})^2 + \sum_{angles} k_a(\theta - \theta_{0,a})^2 + \sum_{dihedrals} k_{d,n}[1 + cos(n\chi - \delta_{d,n})] + \sum_{non-bonded} \epsilon \left[\left(\frac{R_{min_{ij}}}{r_{ij}}\right)^{12} - \left(\frac{R_{min_{ij}}}{r_{ij}}\right)^{6}\right] + \frac{q_i\, q_j}{\epsilon r_{ij}}$$

Equation 1: **CHARMM energy function**

Results and Discussion

Geometry optimization

PTPS initial subset structure geometry was obtained from the crystal structure and subjected to optimization via Gaussian 09 software [4] using the density functional theory (DFT) with the Becke three-parameter hybrid exchange functional and the Lee-Yang-Parr (B3LYP) correlation functional [5] [6] [7]. The LanL2DZ pseudopotential and associated basis functions were used to describe the Zn^{+2} ion and the 6-31G (d) basis set was used for the organic atoms. The optimised values of bonds distance and angles were captured and compared to the initial X-ray structure (Table.1).

Table 1. The optimised bond lengths (Å), angles (°) compared to the initial X-ray structure.

		Bond lengths (Å)			Angles (°)		
	Overlay similarity	Zn^{+2}-(NE2) HIS^{29}	Zn^{+2}-(NE2) HIS^{41}	Zn^{+2}-(NE2) HIS^{29}	HIS^{41}NE2 - Zn^{+2}- HIS^{29}NE2	HIS^{41}NE2- Zn^{+2}- HIS^{43}NE2	HIS^{29}NE2- Zn^{+2}- HIS^{43}NE2
Crystal structure 1Y13	1	2.29	2.13	2.25	91.22	94.43	97.55
QM (B3LYP/6-31G*)	0.71	2.03	2.03	2.04	115.26	114.14	117.96
MD		2.18	2.17	2.16	81.65	80.94	86.45

RESP charge evaluation

RESP Charges calculation was performed at the DFT/B3LYP level of theory using 6-31G (d) basis set for the organic atoms [5] [6] and LanL2DZ pseudopotential and associated basis functions [7] for the Zn^{+2}. The evaluated RESP potential for the Zn^{+2} was identified to be +0.80, the rest of the residues charges and their corresponding atom types are shown in Figure 3.

MD and DFT Calculations on the High Performance Computing System	
Number of nodes selected 4	**Queue type: Standard queue for parallel jobs**
Number of cores per node 24	**Wall time 48:00:00**
Number of MPI ranks (processes) per node 16	

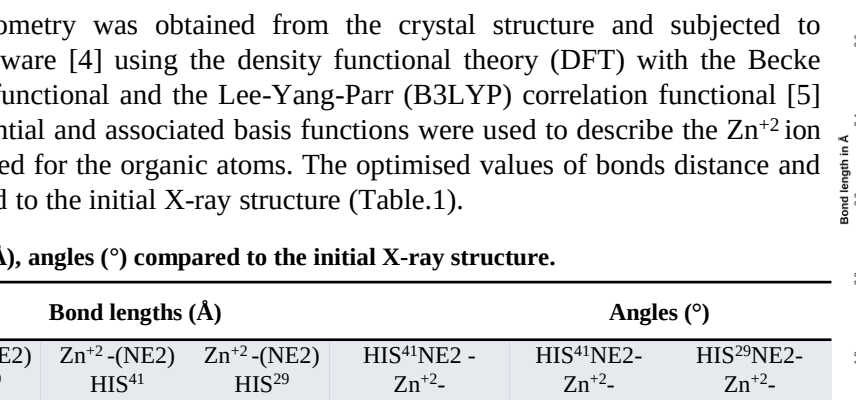


Figure 3: PTPS active site subset. (A) Atom types and (B) RESP charges

Force field parameters

The resources available at the Centre for High Performance Computing (Cape Town, South Africa) were used to accommodate the computational cost and time associated with performing such calculations. The QM calculations resulted in the generation of energy profiles for the bonds stretching, angles bending and dihedrals rotation. The energy profiles exhibited a harmonic profile and were reasonably following the trend of the theoretical (MM) data (Figure 4). Table 2 summarizes the derived force field parameters.

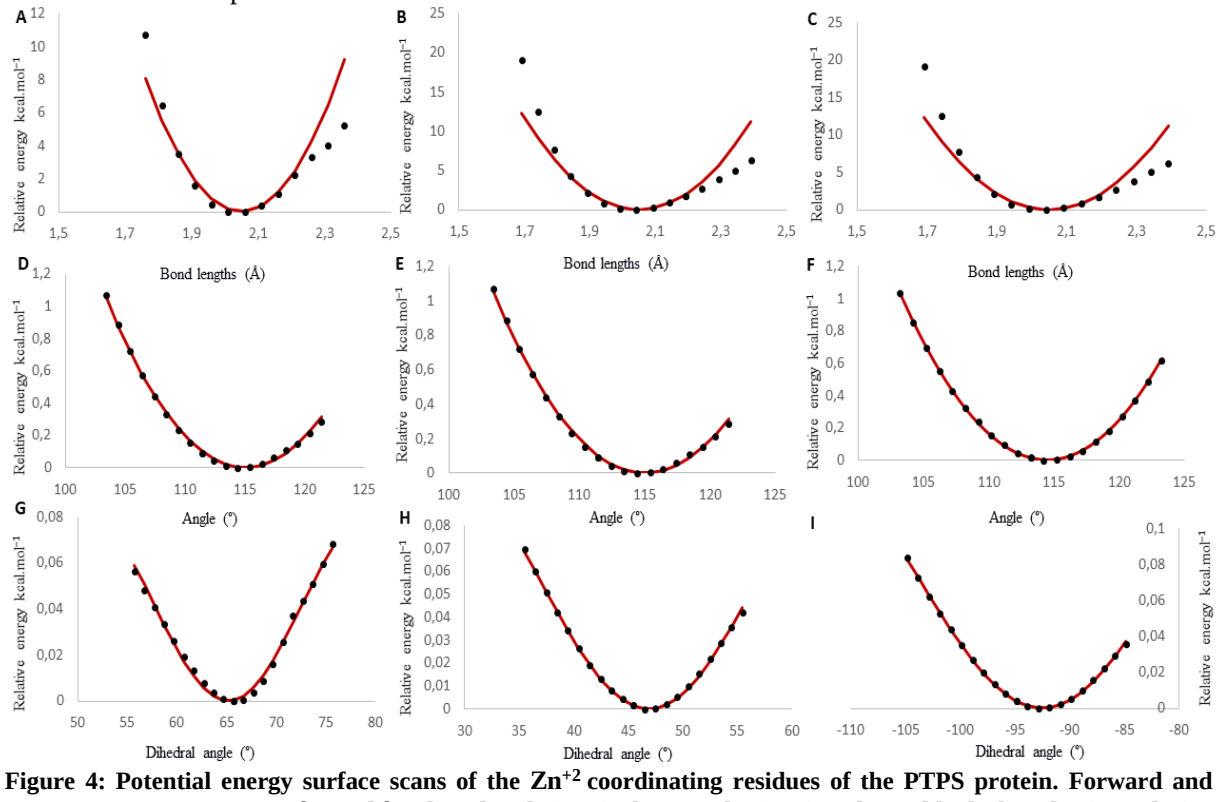


Figure 4: Potential energy surface scans of the Zn^{+2} coordinating residues of the PTPS protein. Forward and reverse PES scans were performed for three bonds (A-C), three angles (D-F) and two dihedral angles (G and H).

Table 2: Developed force field parameters of the PTPS enzyme active site.

Bonds	***Kr (kcal mol^{-1} Å^{-2})***	***r_{eq} (A)***
Zn^{+2} -$NE2^{41}$	95.86	2.01
Zn^{+2} -$NE2^{29}$	95.86	2.01
Zn^{+2} -$NE2^{43}$	95.86	2.09
Angles	***K_θ (kcal mol^{-1} rad^{-2})***	***θ_{eq} (degrees)***
$NE2^{29}$-Zn^{+2} -$NE2^{43}$	25.53	115.04
$NE2^{29}$-Zn^{+2} -$NE2^{41}$	25.53	115.04
$NE2^{41}$-Zn^{+2} -$NE2^{43}$	26.69	114.46
Dihedral	***V_n (kcal mol^{-1})***	n	γ
$NE2^{43}$-Zn^{+2} -$NE2^{29}$-$CD2^{29}$	0.08	12.41	-14.16
$NE2^{43}$ -Zn^{+2} -$NE2^{41}$-$CD2^{41}$	0.10	9.16	-14.50
$NE2^{29}$-Zn^{+2} -$NE2^{41}$-$CD2^{41}$	0.14	8.17	-3.77

MD simulations

MD simulations were performed using the Chemistry at Harvard Macromolecular Mechanics (CHARMM) molecular dynamics simulation and analysis computer software package [9].The MD trajectories of 20 ns were analysed to establish the protein stability and to deduce the validity of the integrated force field parameters (Figure 5.b). The mean distance to the Zn^{+2} from the three coordinating residues was captured and shown to be maintained throughout the simulations (Figure. 5.a).

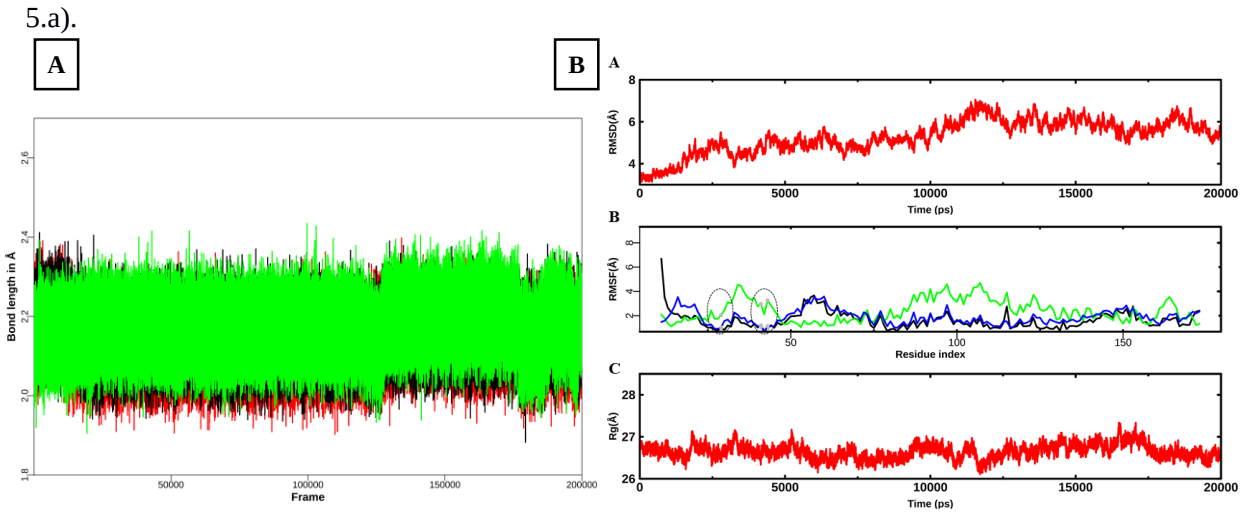


Figure.5 A: Coordination bond distance during the MD simulations. B: Stability of the PTPS protein as determined by B1: Root Mean square Deviation (RMSD), B2: Root Mean Square Fluctuation and B3: Radius of gyration (Rg).

Conclusion

In this study, QM calculations and PES scans were performed using DFT to generate Zn^{+2} force filed parameters suitable for classical MD simulations. The PES scans and the RESP atomic charge calculations were both performed at the DFT/B3LYP level of theory. The generated parameters were validated by performing a short MD simulations. The metal was shown to be stable during the simulations, suggesting that the newly generated parameters were adequately describing the coordination environment of the Zn^{+2} in the protein active site. The use of HPC was essential in obtaining these results. It has accelerated and opened new perspectives to this study, by allowing the use of parallel processor networks to study our large biomolecule system.

References

1. Müller, I. B., & Hyde, J. E. (2013). Folate metabolism in human malaria parasites—75 years on. *Molecular and Biochemical Parasitology*, *188*(1), 63–77. https://doi.org/10.1016/j.molbiopara.2013.02.008.
2. Kumar SCM. Drug resistance in malaria. In: Drug Resistance in Bacteria, Fungi, Malaria, and Cancer. ; 2017. doi:10.1007/978-3-319-48683-3_19
3. Bürgisser DM, Thöny B, Redweik U, et al. 6-pyruvoyl tetrahydropterin synthase, an enzyme with a novel type of active site involving both zinc binding and an intersubunit catalytic triad motif; Site-directed mutagenesis of the proposed active center, characterization of the metal binding site and. J Mol Biol. 1995. doi:10.1006/jmbi.1995.0558.
4. M. J. et al Frisch and et. Al, "Gaussian09 Revision A.1," Gaussian 09, Revision A.02. 2009.
5. (Becke, A. D. (1993). A new mixing of Hartree-Fock and local-density-functional theories. *J. Chem. Phys.*, *98*(2), 1372–1377. https://doi.org/10.1063/1.464304.
6. Lee, C., Yang, W., & Parr, R. G. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Physical Review B*, *37*(2), 785–789. https://doi.org/10.1103/PhysRevB.37.785.
7. T.H. Dunning Jr., P.J. Hay H.F. Schaefer III (Ed.), Modern theoretical chemistry, 3, Plenum, New York (1977), pp. 1-28.
8. Moses, V., Tastan Bishop, Ö., & Lobb, K. A. (2017). The evaluation and validation of copper (II) force field parameters of the Auxiliary Activity family 9 enzymes. Chemical Physics Letters. https://doi.org/10.1016/j.cplett.2017.04.022.
9. Brooks, B. R., Brooks, C. L., Mackerell, A. D., Nilsson, L., Petrella, R. J., Roux, B., … Karplus, M. (2009). CHARMM: The biomolecular simulation program. *Journal of Computational Chemistry*, *30*(10), 1545–1614. https://doi.org/10.1002/jcc.21287.

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