

# Molecular Dynamics Approach in Designing Thermostable *Aspergillus niger* Xylanase

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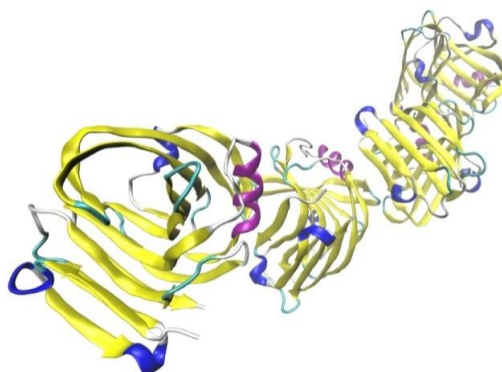
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**Abstract.** Molecular dynamics methods we have applied as a tool in designing thermostable *Aspergillus niger* Xylanase, by examining Root Mean Square Deviation (RMSD) and The Stability of the Secondary Structure of enzymes structure at its optimum temperature and compare with its high temperature behavior. As RMSD represents structural fluctuation at a particular temperature, a better understanding of this factor will suggest approaches to bioengineer these enzymes to enhance their thermostability. In this work molecular dynamic simulations of *Aspergillus niger* xylanase (ANX) have been carried at 400K (optimum catalytic temperature) for 2.5 ns and 500K (ANX reported inactive temperature) for 2.5 ns. Analysis have shown that the Root Mean Square Deviation (RMSD) significant increase at higher temperatures compared at optimum temperature and some of the secondary structures of ANX that have been damaged at high temperature. Structural analysis revealed that the fluctuations of the  $\alpha$ -helix and  $\beta$ -sheet regions are larger at higher temperatures compared to the fluctuations at optimum temperature.

## 1. Introduction

Enzyme applications in industrial processes have become very important as it is safe and environmental friendly, besides having cost advantages compared to the chemical reagents [1]. Enzymes are catalysts that are expected to reduce the impact of pollution and waste of energy because the reaction does not require high energy, be specific, and not toxic [2]. One types of enzymes that important role in the industry is the xylanase enzyme [3].



**Figure 1.** Representation of the secondary structure of *Aspergillus niger* Xilanase.

Xylanase (1,4- $\beta$ -D-xylan xilanohidrolase, E.C 3.2.1.8) is a group of enzymes that have the ability to hydrolyze xylan. This enzyme is used in pre-bleaching process chemical pulp[4]. The use of xylanase can reduce the consumption of chlorine compounds up to 20-40% and can improve the quality of the paper that produced [5-6]. In addition, the use of enzymes in pulp bleaching process is lower cost than other process. But xylanases have to be functional at 60-70° C, which is the temperature of the incoming pulp for the bleaching operation.

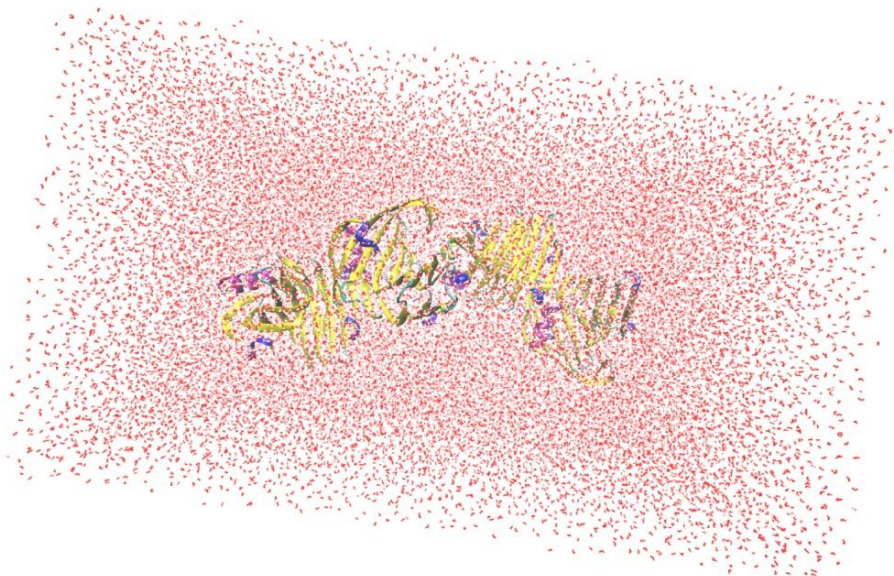
*Aspergillus niger* xylanase has all the advantages in term of size, but the optimum temperature of this enzyme is 45°C which hampers it potential use in pulp bleaching industry. Thus there is a great need to design a new xylanase enzyme from *Aspergillus niger* that has the optimum temperature of 60 - 70° C, to take advantage of its ideal size.

Much work has been done to engineer enzymes to achieve the desirable state through mutations [7-8]. During past years many research groups have identified specific mutations that improve enzyme properties such as selectivity, activity, alternate catalytic route and thermal stability [9]. However, these random mutation approaches are too costly and time consuming. Computer aided design approaches offer an inexpensive means to narrow the set of possible solutions quickly, and then follow them up with experimental testing.

In this research we have carried out molecular dynamics [10] simulations and identified the region that has potential to be mutated by analyzing the root mean square deviation (RMSD) of backbone and secondary structure of *Aspergillus niger* xylanase at its optimum temperature (400 K) in comparison with its inactive temperature/ unfolding process (500 K).

## 2. Method

The crystal structure of *Aspergillus niger* Xylanase (pdb code : 1UKR) [11] used in the simulation was taken from the Protein Data Bank (PDB) [12]. This original file still contains of water molecules and 5.840 atoms. Protein must be prepared in a simulation box filled with the solvent atoms, TIP3P water box with the size of 92 x 92 x 92 Å<sup>3</sup> was used in the simulation (Figure 2). PSFgen program was used to apply a magnetic field in the system molecules. Force field that used to define the potential energy of the crystal structure is CHARMM22. To neutralize total charge system, four ions Cl<sup>-1</sup> added to water box. All those preparations were done by using Virtual Molecular Dynamics program (VMD).



**Figure 2.** Protein in the water box.

Molecules that have solvated then minimized. This stage is the initial stage of MD simulation. The purpose of minimization is to avoid the Van Der Waals contacts that do not match (bad contact) and to minimize steric effects that high energy. Each simulations get started with minimization for 100 ps to make the proteins had the lowest energy (steady state). The second stage is the stage of heating and equilibration. Protein was heated from the temperature of 0 K up to 300 K, 400 K, 450 K and 500 K to the unfolding process for 40 ps with an increase every 25 K. Equilibration was done by the langevin protocol for 10 ps. Equilibration was meant to hold protein to stay stable in simulation system. Production run is the final stage of MD simulations where the constraints which applied to the equilibration process, then removed so that protein free to move. Production run performed for 1 ns with temperature at 300 K, and 2.5 ns with temperature at 400 K, 450 K and 500 K for the unfolding. The MD simulations were performed using NAMD v.2.9 simulator [9].

Overall simulations was using parameter integration time (time step) every 2 femto seconds (fs). The simulation was done by using periodic boundary condition (PBC) method to eliminate the effect of surface tension and to achieve density and pressure conditions were more uniform. Electrostatic energy system thoroughly calculated using the particle mesh ewald (PME) method, while the van der waals interaction was calculated using the Lennard – Jones potential with each cutoff was 12 Å. All simulations was performed in single CPU powered by 3.6 GHz Intel® core i7 processor with 16 GB of RAM and using Ubuntu 12.04 Linux platform.

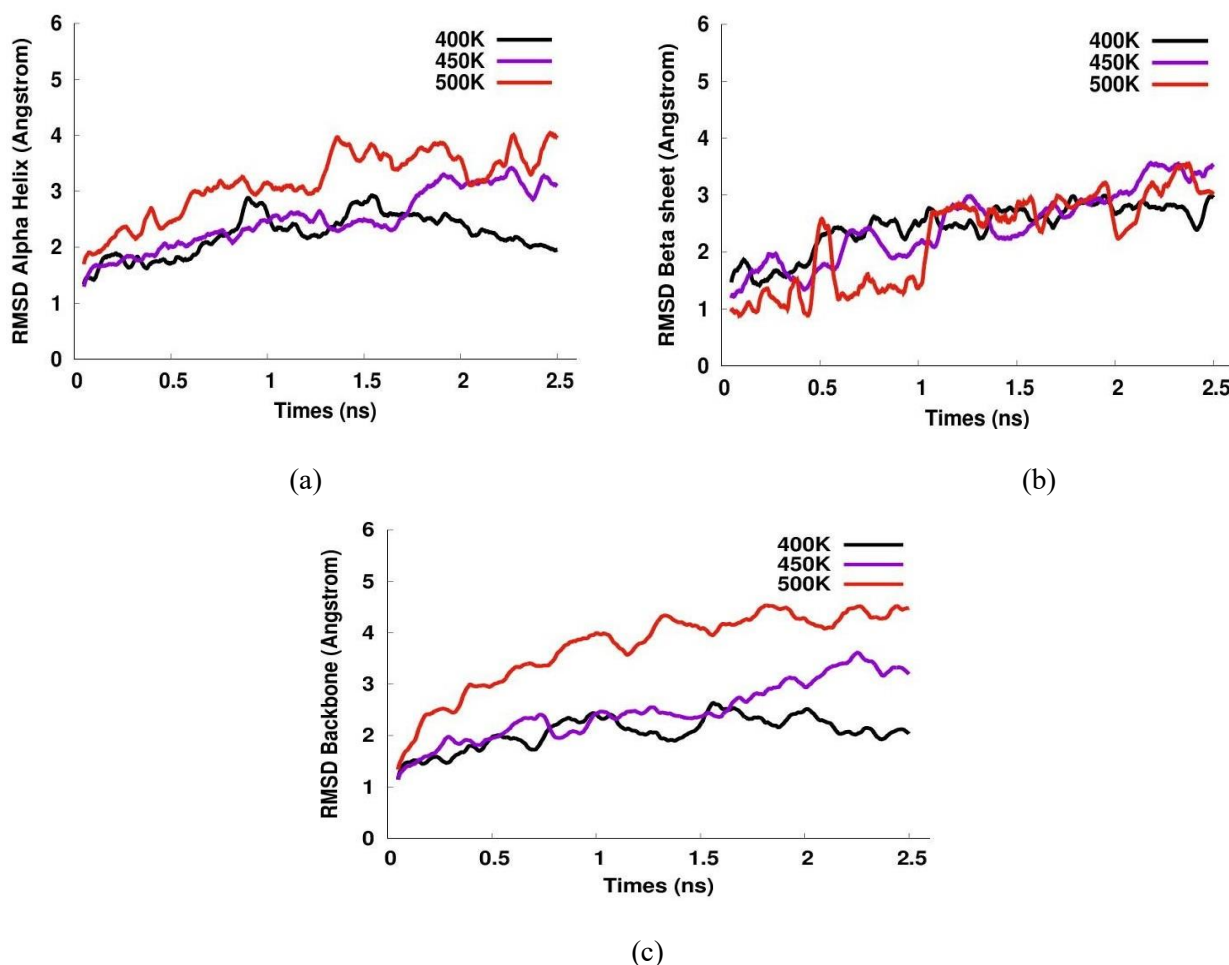
To analyze the pathway of molecules unfolding and refolding which have been simulated, was done analyze using VMD to produce the output such as secondary structure and Root Mean Square Deviation (RMSD) using VMD.

### 3. Result and Discussion

#### 3.1. Root Mean Square Deviation (RMSD)

Root Mean Square Deviation analysis is used to describe a change in the secondary structure of the protein during the simulation [13]. RMSD values that fluctuate indicate conformational change process from the beginning to the end of the simulation.

Figure 3.a and 3.b shows the fluctuations  $\beta$ -sheet and  $\alpha$ -helix RMSD values at temperatures 400 K, 450 K and 500 K for 2.5 ns. From 400 K to 500 K simulation temperature, there was no significant change in the values of RMSD. Until 2 ns the values of RMSD was constant in 2 Angstrom. An increase temperature in the system is not affecting the protein stability. There is no significant change in the  $\beta$ -sheet and  $\alpha$ -helix fluctuations of the whole enzyme.

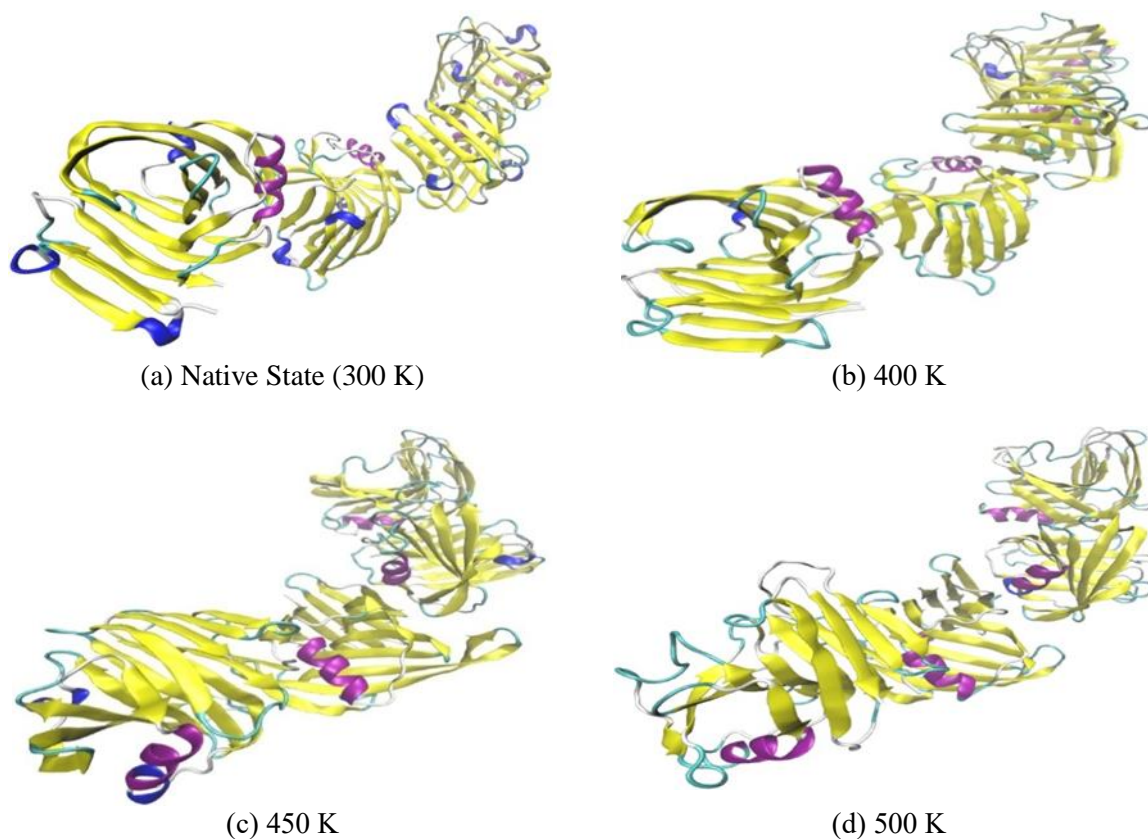


**Figure 3.** Root Mean Square Deviation (RMSD) as a function of simulation time for temperature 400 K, 450 K and 500 K (a)  $\beta$ -sheet structure (b)  $\alpha$ -helix structure (c) backbone structure.

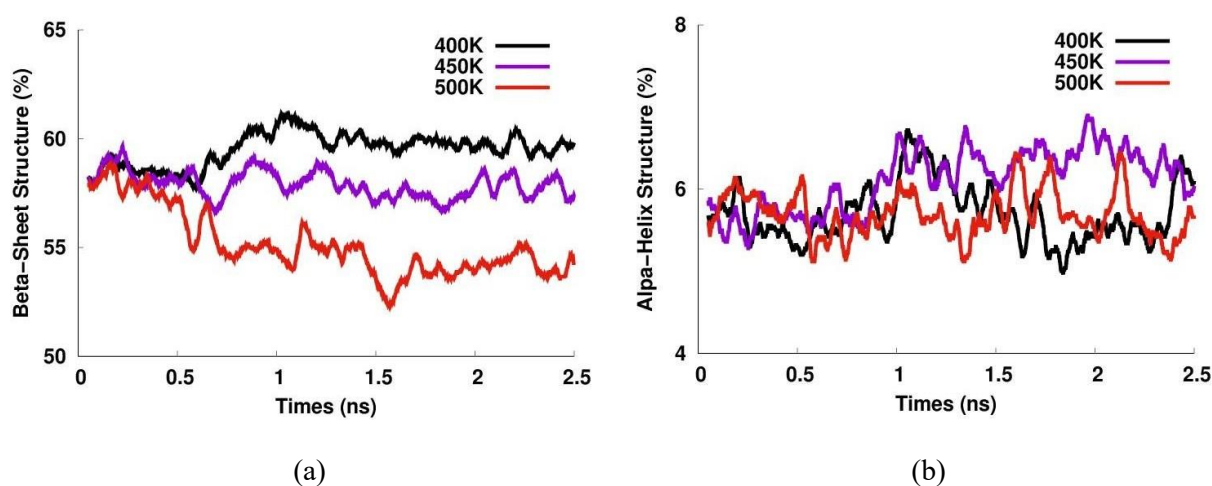
Figure 3.c shows the fluctuations Backbone RMSD values at temperatures 400 K, 450 K and 500 K for 2.5 ns. From 400 K to 450 K simulation temperature, there was no significant change in the values of RMSD. Until 2 ns the values of RMSD was constant in 2 Angstrom. An increase temperature in the system is not affecting the protein stability. Whereas the temperature 500 K, at this temperature RMSD enough constant in 2 Angstrom until 0.2 ns. The values of RMSD jumps drastically at 0.25 ns until 2.5 ns that is until 4.8 Angstrom. While still there is a need for detailed investigation of the reasons for this difference in the behavior of the fluctuations in the backbone region compared to the other region of the enzyme, we suggest that any attempt to design thermostable ANX should include mutation that could retain the backbone fluctuation of ANX at the similar level as that of fluctuations at the optimum temperature. We are currently undertaking in silico mutational studies to identify the mutations that will bring about this effect.

### 3.2. The Stability of the Secondary Structure

Analysis of changes in the secondary structure is an analysis that can be used as a marker has been the unfolding of proteins, especially the loss/ damage of a helix and sheet structure. Secondary structural analysis may also complete understanding of structural changes during the simulation [13].



**Figure 4.** Protein secondary structure as a function of simulation time (a) for temperature 300 K, (b) for temperature 400 K (c) for temperature 450 K and (d) for temperature 500 K.



**Figure 5.** The percentage change in the composition of secondary structure during the simulation at 400 K, 450 K and 500 K for 2.5 ns for (a)  $\beta$ -sheet structure. (b)  $\alpha$ -helix structure.

The composition of secondary structures, especially  $\alpha$ -helix or  $\beta$ -sheet at temperature 400 K and 450 K for 2.5 ns did not significantly changes (Figure 4.b and Figure 4.c). Meanwhile, at temperature 500 K composition of  $\alpha$ -helix only slightly damaged while  $\beta$ -sheet had disappeared turned into coil and turn. This indicates that proteins have been in the unfolding process. So, we suggest that any attempt



to design thermostable ANX should include mutation that could retain the  $\beta$ -sheet had disappeared turned into coil and turn. We are currently undertaking in silico mutational studies to identify the mutations that will bring about this effect.

#### 4. Conclusion

The analysis have shown that RMSD of backbone showed significant difference at higher temperatures compared other regions of the enzyme, we conclude that backbone region contribute significantly to the thermostability of ANX. Mutagenesis should be targeted to amino acid residues within backbone in ANX structure with the objective of maintaining the backbone regional fluctuation to the similar level as that of fluctuations at 400 K. If that can be achieved, we propose that such mutant ANX will be the thermostable and suitable for use in the pulp industry. Further work in this direction is in progress. Analysis of changes in the secondary structure is an analysis that can be used as a marker has been the unfolding of proteins, especially the loss/ damage of a helix and sheet structure. Secondary structural analysis may also complete understanding of structural changes during the simulation. So, we suggest that any attempt to design thermostable ANX should include mutation that could retain the  $\beta$ -sheet had disappeared turned into coil and turn. We are currently undertaking in silico mutational studies to identify the mutations that will bring about this effect.

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#### References

- [1] Falch EA 1991 *Biotech Adv.* **9** 643-58
- [2] Aunstrup KO, Andressen, Falch EA and Nielsen 1979 *Microbial Technology* **1** USA
- [3] Marisa A, Lima 2013 *The Journal of Biological Chemistry* **288** 32991-3005
- [4] Kirk TK and Jeffries TW 1996 *Enzymes for Pulp and Paper Processing* Washington USA
- [5] Dhillon A, Gupta JK, Jauhari BM and Khanna S 2000 *Bioresource Technology* **73** 273 - 77
- [6] Beg QK, Kapoor M, Mahajan L and Hoondal GS 2001 *Applied Microbiology and Biotechnology* **56** 326-38
- [7] Otten LG, Sio CF, Van Der Sloot, Almer M, Cool RH and Quax WJ 2004 *Chem. Biochem.* **5** 820-25
- [8] Schmidt DMZ, Mundorff EC, Dojka M, Bermudez E, Ness JE, Govindarajan S, Babbitt PC, Minshull J and Gerlt JA 2003 *Biochemistry* **42** 8387-393
- [9] Morley KL and Kazlauskas RJ 2005 *Trends in Biotechnology* **23** 5
- [10] Noorbachia IA, Khan AM and Salleh HM 2010 *American Journal of Applied Sciences* **7** 823-28
- [11] Krengel U and Dijkstra BW 1996 *J.Mol.Biol.* **263** 70-8
- [12] Phillips JC, Braun S, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L and Schulten KJ 2005 *Comput. Chem.* **26** 1781
- [13] Day R, Bennion BJ, Ham S and Dagget VJ 2002 *Mol. Biol.* **322** 189-203