



**UNIVERSITI PUTRA MALAYSIA**

***STRUCTURAL AND FUNCTIONAL PREDICTION OF  
Leucosporidium antarcticum ANTIFREEZE PROTEIN (Afp1)***

**MOHAMMAD FAIRUZ BIN ZULKIFLI**

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**By**

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**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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**STRUCTURAL AND FUNCTIONAL PREDICTION OF  
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**April 2010**

**Chairman : Professor Mohd Basyaruddin Abdul Rahman, PhD**

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Under extreme temperature of frozen state, only a few type of protein can be survived which known as antifreeze protein (AFP). The AFP can prevent and control the ice growth within the cell and avoid the cell from damage. A novel antifreeze protein (Afp1), *Leucosporidium antarcticum* with 411 base pair was expressed in pET32b and used three different *E. Coli* host strains; BL21 (DE3), Origami (DE3) and RosettaGami (DE3). The Afp1 with 177 residues was subjected to template analysis but it failed and 54 random template of AFP was chosen and aligned multiple with ClustalW but still gave poor results. The sequence was then threaded with FUGUE, mGentreader and 3DPSSM but unsatisfied score level obtained. Lastly, *ab-initio* I-TASSER (iterative-threading assembly refinement) method was applied and it produced five predicted models of Afp1; AFP1, AFP2, AFP3, AFP4 and AFP5. After evaluation process, AFP3 proposed the best results with the model of four alpha helices and two beta sheets.

Almost 80% of the residues were located in the favoured regions which strongly support this predicted model. It also showed average score between 0.30-0.60 in the Verify3D analysis which is satisfying for a low percentage of similarity protein models. In the alpha helix segments, there were five major amino acids (serine, threonine, aspartic acid, asparagine and glutamine) which had high possibility to be bonded with the water molecule at the ice surface. Molecular Dynamics (MD) simulation was applied on the AFP3 model to find the optimum temperature for the Afp1 activity. The model was repaired by using Simulated Annealing (SA) before proceed to MD simulations at 273K, 277K and 283K at 3ns. The root mean square deviation (RMSD) and radius of gyration analysis showed that the model of Afp1 was most stable at 277K. Thus, this research managed to predict the Afp1 structure via *ab-initio* I-TASSER simulations and suggest that the structure of Afp1 had optimum activity at 277K.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**RAMALAN STRUKTUR DAN FUNGSI  
*Leucosporidium antarcticum* PROTEIN ANTIBEKU (Afp1)**

Oleh

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Di bawah takat beku yang ekstrem, hanya terdapat beberapa jenis protein yang boleh mengatasi keadaan tersebut dan protein ini dikenali sebagai protein antibeku (AFP). AFP boleh mencegah dan mengawal pertumbuhan ais di antara sel dan menghalang sel tersebut daripada mengalami kerosakan. Protein antibeku yang novel (Afp1), *leucosporidium antarcticum* dengan 411 bp telah diekspreskan di dalam pET32B dengan menggunakan tiga strain hos *E. Coli* yang berbeza; BL21 (DE3), Origami (DE3) dan RosettaGami (DE3). Afp1 dengan 177 residu telah dijuruskan kepada analisis templat tetapi ianya gagal dan 54 templat rawak AFP telah dipilih dan disusun secara berlapis dengan ClustalW tetapi masih memberikan keputusan yang lemah. Jujukan tersebut kemudiannya diperbaiki dengan menggunakan FUGUE, mGenthrader dan 3DPSSM tetapi memperoleh keputusan yang kurang memuaskan. Akhirnya, kaedah *ab-initio* I-TASSER (iterative-threading assembly refinement) telah diaplikasikan dan

menghasilkan lima model ramalan Afp1; AFP1, AFP2, AFP3, AFP4 dan AFP5. Selepas proses penilaian, AFP3 mencadangkan keputusan yang terbaik dengan model yang terdiri daripada empat alfa heliks dan tiga kepingan beta. Hampir 80% residu terletak di dalam kawasan utama di mana ianya menyokong kuat model ini. Ianya juga menunjukkan takuk purata di antara 0.30-0.60 di dalam analisis Verify3D yang mana ianya memuaskan untuk model protein yang mempunyai peratusan kesamaan yang rendah. Di dalam bahagian alfa heliks, terdapat lima asid amino yang utama (Serina, Threonina, acid Aspartik, Asparagina dan Glutamina) yang mana mempunyai kebarangkalian yang tinggi untuk bercantum dengan permukaan ais. Simulasi dinamik molekul (MD) telah dilaksanakan ke atas model AFP3 bagi mencari suhu optimum untuk aktiviti Afp1. Model tersebut telah diperbaiki dengan menggunakan kaedah simulasi penguatan (SA) sebelum diteruskan dengan MD pada 273K, 277K dan 283K dalam 3ns. Analisis faktor anteseden pengupayaan psikologikal (RMSD) dan jejari putaran menunjukkan model Afp1 paling stabil pada 277K. Jadi, penyelidikan ini berjaya meramalkan struktur Afp1 melalui simulasi *ab-initio* I-TASSER dan mencadangkan struktur Afp1 mempunyai aktiviti yang optimum pada 277K.

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I certify that an Examination Committee met on 12 April 2010 to conduct the final examination of Mohammad Fairuz Bin Zulkifli on her Master of Science thesis entitled “Structural and Functional Prediction of *Leucosporidium antarcticum* Antifreeze Proteins” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree.

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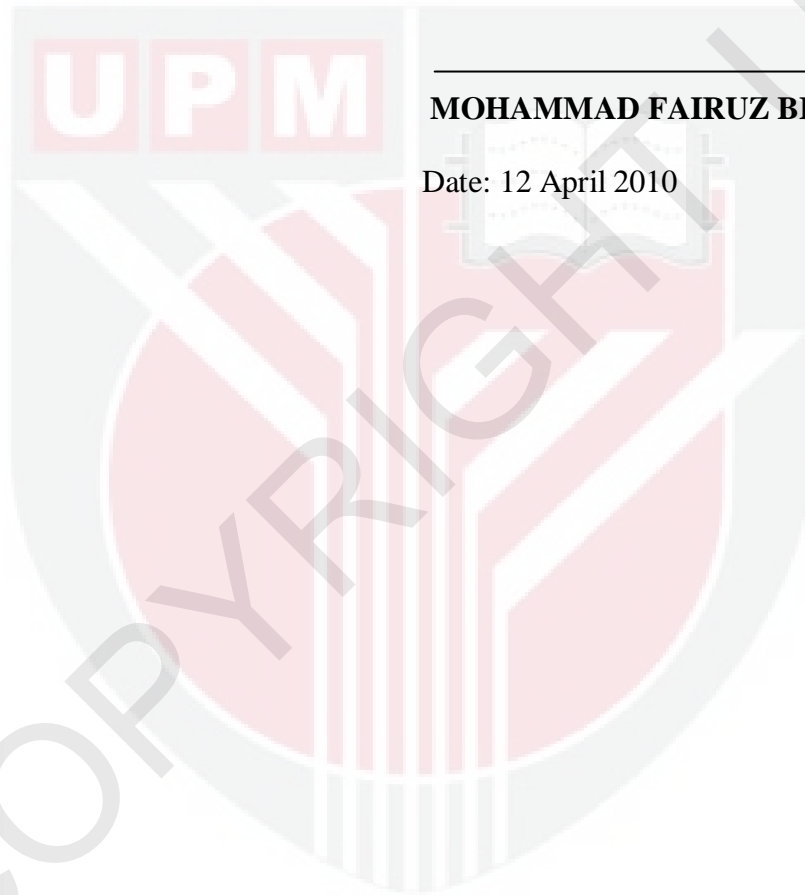
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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



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**MOHAMMAD FAIRUZ BIN ZULKIFLI**

Date: 12 April 2010



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## LIST OF ABBREVIATIONS

AFP	Antifreeze Protein
Afp1	<i>Leucosporidium antarcticum</i> Antifreeze Protein
AFP1	First Afp1 predicted structure
AFP2	Second Afp1 predicted structure
AFP3	Third Afp1 predicted structure
AFP4	Fourth Afp1 predicted structure
AFP5	Fifth Afp1 predicted structure
BLAST	Basic Local Alignment Search Tool
CASP7	Critical Assessment of Techniques for Protein Structure Prediction 7
CATH	Class, Architecture, Topology and Homologous Superfamily
DALI	Distance-matrix Alignment
FM	Free-Modeling
GROMACS	Groningen Machine for Computer Simulations
LGA	Local Global Alignment
MC	Monte Carlo
MD	Molecular Dynamics
NMR	Nuclear Magnetic Resonance
NPT	Number, Pressure, Temperature
NVT	Number, Volume, Temperature
PDB	Protein Data Base
PME	Particle Mesh Ewald.

PPA	Profile-Profile Alignment
PSI-BLAST	Position-Specific Basic Local Alignment Search Tool
$R_g$	Radius of Gyration
RMSD	Root Means Square Deviation
RMSF	Root Means Square Fluctuation
SA	Simulated Annealing
SCOP	Structure Classification of Proteins
SPC	Simple Point Charge
TBM	Template-Based Modeling
3DPSSM	Three-Dimensional Position-Specific Scoring Matrix

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Research background

Research on the natural antifreeze protein (AFP) that allow fish to swim in Arctic waters have urbanized a novel instrument which may assist in the making of molecules that capable to remain organ's condition and protect it from freezing at subzero temperature (Middleton *et al.*, 2009). AFP attached to the plane of ice crystals and react as a wall between the crystals and water surroundings which allows some organisms live in the extreme temperature (Graether *et al.*, 2003). AFP worked by lowering the freezing point of the body fluids without changing the colligative melting point (Nutt *et al.*, 2008; Knight *et al.*, 1993).

Thermal hysteresis (TH) is referred to an antifreeze activity and it is used to identify the comparative activities at the similar concentration of the different antifreeze peptides. The freezing point dejection is caused by straight binding of AFP to the surface of ice crystal nuclei. The AFP binding actually occurs predominately at the bipyramidal and the prism ice faces in specific orientations restricting ice growth normal to the binding surface (Cheng *et al.*, 1997). Ice cream manufacturers previously used AFP in some of their goods to get better texture of low fat ice cream. Besides, medical researchers believed that they could protect the internal organs and tissues for medical applications such as transplants (Dan and Michele, 2008).

Antarctic yeast, *Leucosporidium antarcticum* used in this research was isolated from sea ice by late Omar Pohzan (UPM) near the Casey Research Station, Antarctica in 2002. Full length of the Afp1 sequence was then isolated at Malaysia Genome Institute, Bangi and cloned it into cloning vector pGEM-T Easy. In addition, the cDNA of the gene was also isolated and cloned it into cloning vector pGEM-T Easy. The Afp1 cDNA (411 bp, without the signal peptide) was then cloned into an expression vectors pET32b (Novagen, USA). Protein expression optimization was carried out by varying the IPTG concentration (0.5-1.0mM), growth temperature (20°C, 37°C) and induction time (3, 5, 18 and 24 hours). There were a problem occurred when the protein is insoluble in water and cannot be crystallize.

The structure prediction is determined based on its function which can be applied in several of fields. Complex factor of protein sequences made it difficult to predict the 3D structures by computational methods (Raman *et al.*, 2008). This can be divided into homology modeling, threading or fold recognition and *ab-initio* methods (Bowie *et al.*, 1991; Jones *et al.*, 1992; Godzik *et al.*, 1992; Zhang and Skolnick, 2005; Sitao *et al.*, 2007; Zhang, 2007, 2008). Bowie and partners have recommended a fold-recognition/threading procedure as a explanation to homology modeling troubles in discovery the similarity of sequences (Bowie *et al.*, 1991).

The *L. antarcticum* Afp1 had gone through the homology modeling, threading and *ab-initio* methods to predict its potential structure. The predicted structure was evaluated and several alpha helices were located in it. These alpha helices had high possibility to

be attached to the surface of ice and react as the preventer to the ice growth and hence reduce the unwanted damages to the cell. The molecular dynamics simulation was also performed to investigate the stability of the protein structure at different temperatures.

By predicting and simulating the Afp1 structure, the understanding on the mechanism of ice binding between the microbial AFP and ice surface enhances which later potentially ready to be globally commercialized as a novel AFPs in its applications.

## **1.2 Problem Identification**

### **1.2.1 Low percentage of sequence identity.**

In protein structure prediction, sequence identity played a major role in getting accurate prediction model. However in this research, Afp1 *L. antarcticum* lacks of information in the protein structure library which means structure prediction of Afp1 cannot be applied by using regular method, homology modeling. All templates showed poor percentage of sequence identity which dropped in the 'twilight zone', a zone where the percentage of the template is less than 40% and it is not suitable to be used as a template in model prediction method. When the similarity between the target and the templates decreases, large number of gaps will increase in between the alignments of sequences and will resulted in inaccurate protein structure prediction.

### **1.2.2 Difficulty to Crystallize the *L. antarcticum* Afp1.**

Researchers from Malaysia Genome Institute, UKM Bangi are having problems to crystallize *L. antarcticum* Afp1 because of the recombinant protein expressed were insoluble in *Escherichia coli* and the protein cannot be purified. Without the crystal structure of the AFPs, comparison between the predicted Afp1 structure and the crystal structure cannot be done at all and directly affects the quality of the predicted structure.

### **1.3 Objectives**

This research is focused to achieve the objectives as listed below:

- i. To predict the structure of antifreeze protein from *L. antarcticum* Afp1.
- ii. To determine the potential binding site of Afp1.
- iii. To simulate the predicted Afp1 structure using molecular dynamics method.

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