UNIVERSITI PUTRA MALAYSIA

BIOINFORMATIC ANALYSES OF METALLOME AND STRUCTURAL ANALYSIS OF SIRTUIN FROM Glaciozyma antarctica PI12

FOONG PIK MUN

FS 2014 39

BIOINFORMATIC ANALYSES OF METALLOME AND STRUCTURAL ANALYSIS OF SIRTUIN FROM *Glaciozyma antarctica* PI12

FOONG PIK MUN

MASTER OF SCIENCE

UNIVERSITI PUTRA MALAYSIA

2014

BIOINFORMATIC ANALYSES OF METALLOME AND STRUCTURAL ANALYSIS OF SIRTUIN FROM *Glaciozyma antarctica* PI12

By

FOONG PIK MUN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

November 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science

BIOINFORMATIC ANALYSES OF METALLOME AND STRUCTURAL ANALYSIS OF SIRTUIN FROM *Glaciozyma antarctica* PI12

By

FOONG PIK MUN

November 2014

Chairman: Professor Mohd Basyaruddin Abdul Rahman, PhD
Faculty: Science

Metal ions are essential elements that are extensively involved in many cellular activities. With rapid advancements in genome sequencing techniques, bioinformatic approaches have provided a promising way to extract the functional information of a protein directly from its primary structure. Recent studies have suggested that the metal content of an organism can be predicted from its genome sequences using bioinformatic approaches, and the variation of cellular metal contents is found to correlate with the bioavailability of metal ions in the adjacent environment. The focus of this study is targeted towards the analysis of metal composition of a psychrophilic yeast *Glaciozyma antarctica* PI12 isolated from sea ice of Antarctica. Since the cellular metal content of an organism is usually reflected in the expressed metal-binding proteins, the putative metal-binding sequences from *G. antarctica* PI12 were identified in respect to their sequence homologies, domain compositions, protein families and cellular distribution. The homologous metal-related sequences from *G. antarctica* PI12 were identified by BLAST using annotated queries available in the public database. Metal-binding protein domains in the sequences were also inspected to enhance the accuracy of prediction. Proteins with novel metal-binding sites were identified using SVMProt. All putative metal-binding proteins were assigned with Gene Ontology (GO) terms and predicted subcellular location for functional inferences. Most of the approaches have suggested that the metallome of *G. antarctica* PI12 was enriched in zinc, and descending in the order of Zn > Mg > Ca, Fe > Cu, Mn > Na, K, Co, Ni. Upon comparison, the metal composition of *G. antarctica* PI12 was found to be almost identical with those of its warm-counterparts. However, distinctive variations were noticed when compared with bacteria. The observations suggested that *G. antarctica* PI12 could have inherited a conserved trend of metal usage similar to modern eukaryotes that enriched in zinc.

Subsequently, sirtuin, a zinc-bound NAD^+-dependent deacetylase was selected for further structural investigation. A predicted model was obtained through comparative modeling and its quality was evaluated. To investigate the role of zinc, the model and its apo (with zinc removed) were independently subjected to 10 ns molecular dynamics (MD) simulations coupled at temperature 277 K, 285 K and 303 K. The results suggested that zinc may function to retain the native conformation of sirtuin and prevent it from denaturing. It was also noticed that the structural flexibility of the protein was improved by altering its amino acid composition and enhancing solvent accessibility at certain regions. In conclusion, the metal content of G. *antarctica* PI12 was depicted through an integration of multiple bioinformatic approaches and its strategy in cold-adaptation can be inferred from the structural variation and dynamics studies of sirtuin.

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

ANALISIS BIOINFORMATIK METALLOME DAN ANALISIS STRUKTUR SIRTUIN DARIPADA *Glaciozyma antarctica* PI12

Oleh

FOONG PIK MUN

November 2014

Pengerusi: Profesor Mohd Basyaruddin Abdul Rahman, PhD
Fakulti: Sains

Ion logam merupakan unsur penting yang terlibat secara meluas dalam pelbagai aktiviti sel. Dengan kemajuan pesat dalam teknik penjujukan genom, pendekatan bioinformatik telah menyediakan kaedah yang berpotensi untuk mengekstrakkan maklumat kefungsian sesuatu protein menerusi struktur primernya. Kajian-kajian terbaru telah mencadangkan bahawa kandungan logam bagi sesuatu organisma boleh diramal melalui jujukan genomnya dengan menggunakan pendekatan bioinformatik, dan variasi kandungan logam dalam sel didapati berhubung kait dengan bioketersediaan ion logam yang terdapat di persekitaran bersebelahannya. Fokus kajian ini disasarkan terhadap yis psikrofilik *Glaciozyma antarctica* PI12 yang diasingkan dari ais laut Antartika untuk menganalisis komposisi logamnya. Memandangkan kandungan logam dalam sel bagi sesuatu organisma kebiasaannya digambarkan pada protein terikat logam yang diekspresnya, jujukan protein yang terikat logam secara putatif dari *G. antarctica* PI12 telah dikenalpasti melalui jujukan homologi, komposisi domain, keluarga protein dan pengagihannya dalam sel. Jujukan homolog *G. antarctica* PI12 yang berkaitan dengan logam telah dikenalpasti melalui kaedah BLAST dengan menggunakan pertanyaan beranotasi yang diperoleh daripada pangkalan data umum. Domain-domain protein terikat logam yang terkandung dalam jujukan tersebut turut diperiksa untuk meningkatkan kejituan ramalan. Protein yang mempunyai tapak ikatan logam yang baru telah dikenalpasti dengan menggunakan perisian SVMProt. Semua protein yang terikat logam secara putatif telah ditentukan istilah ontologi gen (GO) and diramalkan lokasi subsel untuk penafsiran kefungsiannya. Kebanyakan pendekatan-pendekatan tersebut telah mencadangkan bahawa metallome *G. antarctica* PI12 diperkaya dengan zink, dan menurun secara tertib dari Zn > Mg > Ca, Fe > Cu, Mn > Na, K, Co, Ni. Secara perbandingan, komposisi logam untuk *G. antarctica* PI12 ditemui hampir serupa dengan yis yang lain. Walau bagaimanapun, perbezaan ketara telah dikesan apabila ia dibandingkan dengan bakteria. Pemerhatian-pemerhatian tersebut telah mencadangkan bahawa *G. antarctica* PI12 berkemungkinan mewarisi kecenderungan penggunaan logam yang serupa dengan eukariot moden yang diperkaya dengan zink.

Seterusnya, sirtuin, deasetilase bersandar NAD^+ yang terikat dengan zink telah dipilih bagi penyelidikan struktur yang selanjutnya. Satu model ramalan sirtuin telah diperolehi melalui pemodelan perbandingan dan kualitinya ditaksirkan. Untuk menyelidik peranan zink, model tersebut dan apo model (dengan zink disingkirkan) telah dijalankan simulasi dinamik molekul (MD) secara terpisah selama 10 ns dengan suhu yang ditetepkan pada 277 K, 285 K dan 303 K. Keputusan analisis mencadangkan bahawa zink tersebut berfungsi untuk mengekalkan susukan asli sirtuin dan mengelakkannya daripada terdenaturasi. Kajian ini juga mendapati bahawa kelenturan struktur protein tersebut telah dipertingkatkan dengan mengubah komposisi asid amino dan meningkatkan aksesibiliti pelarut pada kawasan-kawasan tertentu. Kesimpulannya, kandungan logam dalam *G. antarctica* PI12 telah digambarkan melalui integrasi pelbagai pendekatan bioinformatik dan strateginya dalam penyesuaian kesejukkan boleh dikesan daripada kajian variasi struktur dan dinamik sirtuin.

ACKNOWLEDGEMENTS

First and foremost, I must thank my advisor, Prof Dr Mohd Basyaruddin Abdul Rahman, for his guidance, advices and support over the last three years. As much as his mentorship, I appreciate his unwavering trust, which has inspired my own self-confidence as a scientist.

I wish to gratefully acknowledge my co-supervisor, Dr Roghayeh Abedi Karjiban, for her encouragement throughout these years, and her patience in enduring my questions on molecular dynamics simulation. It had made the work more enjoyable and facilitated me a clearer picture in the field of molecular mechanics.

I would like to take this opportunity to thank the committees in Enzyme and Microbial Technology (EMTech) Research Centre, especially to Prof Dato' Dr Abu Bakar Salleh, Dr Bimo Ario Tejo and Dr Normi Mohd Yahaya for their invaluable comments and guidance that have improved my work.

I thank my colleagues in Macromolecular Simulation Laboratory at Department of Chemistry, Faculty of Science for the enlightening scientific discussions, constructive advices, camaraderie and positive work environment. Their contributions during my journey are not cited in any manuscript but are invaluable nonetheless.

I thank all the members that involved in this *Glaciozyma antarctica* PI12 project (GanDB), who provided me with the detailed background of this cold-like yeast and assisted me in mining the information from the massive genome sequencing data.

This research was supported by the grant Genomics and Molecular Biology Initiatives (GMBI) of Malaysia Genome Institute (MGI) under the Ministry of Science, Technology, and Innovation Malaysia (MOSTI), and a sponsored Graduate Research Fellowships (GRF) from Universiti Putra Malaysia.

Last but not least, I would like to express my deepest gratitude to my wonderful friends, my parent and siblings. Their love and support over the years have made this possible. Graduate school is like running a marathon. The road has been tough at times, but the amazing people who I have encountered over the years have made it worthwhile.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd Basyaruddin Abdul Rahman, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Roghayeh Abedi Karjiban, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

_____

BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Foong Pik Mun GS29204

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____

Name of Chairman of
Supervisory Committee: _____

Signature: _____

Name of Member of
Supervisory Committee: _____

LIST OF TABLES

Table		Page
3.1	The number of metal-related queries for BLAST obtained from UniProtKB/SWISS-PROT knowledgebase.	24
3.2	Organisms for comparative analysis in their metal utilization preference.	26
3.3	General details of the five sirtuins retrieved from *G. antarctica* PI12.	30
3.4	Predictive programs employed to characterize sirtuins and their respective zinc-binding sites.	30
4.1	Summary of predictive performance for the three approaches applied.	41
4.2	The number of metal-binding proteins for *G. antarctica* PI12 as predicted by the three predictive approaches is arranged in decreasing order (from Left to Right).	42
4.3 (A)	Comparison of amino acid compositions in sirtuins.	66
4.3 (B)	Comparison of amino acid properties in sirtuins.	67
4.4	Structural templates for *G. antarctica* PI12 sirtuins.	70
4.5	Representative models for each *G. antarctica* PI12 sirtuins.	73
4.6	CATH domain classification for the models of *G. antarctica* PI12 sirtuins.	76
4.7	A summary of Ramachandran plots for the psychrophilic sirtuins models calculated by PROCHECK.	77
4.8	Model assessments by VERIFY3D, ERRAT and ProSA-web.	78
4.9	Averaged R_g and SASA values.	87
4.10	Secondary structure composition of sirtuins.	91

LIST OF FIGURES

Figure		Page
2.1	The essential elements required for life showing in a periodic table.	5
2.2	Number of metal-bound structures deposited in Protein Data Bank (PDB) as of 7 January 2015.	6
2.3	Experimental approaches to metallomics.	7
2.4	Functional assignment for the zinc proteomes of (A) Archaea, (B) Bacteria, (C) Eukarya and (D) human using a combination of bioinformatic approaches.	10
2.5	*Glaciozyma antarctica* PI12	15
2.6	Acetylation and deacetylation of lysine.	17
3.1	Overall workflow of project.	20
3.2	A graphical layout of a eukaryotic cell compartments that are included for predicting the compartmentalization of metalloproteins.	29
3.3	Deprotonation of cysteine side chain by zinc ion.	33
4.1	Putative metal-binding proteins identified by BLAST.	36
4.2	Comparison of five most prevalence metal-types.	37
4.3	Number of putative metal-binding protein clustered into each metal-type using programs BLAST, Pfam-Keyword (Pfam-KW) and SVMProt.	39
4.4	Comparison between the numbers of metal-binding proteins identified.	40
4.5	The number of sequences predicted by BLAST, Pfam-Keyword (Pfam-KW) and SVMProt as metal-bound (Positive ensemble) and non metal-bound (Negative ensemble).	41
4.6	Functional classification of putative magnesium-binding proteins.	45
4.7	Functional classification of putative manganese-binding proteins.	46

4.8	Functional classification of putative calcium-binding proteins.	48
4.9	Functional classification of putative iron-binding proteins.	49
4.10	Functional classification of putative zinc-binding proteins.	51
4.11	Distribution of metal-binding proteins in cellular compartments.	53
4.12	Relative abundance of metal-binding proteins in yeast proteomes.	55
4.13	Relative abundance of metal-binding proteins in bacteria proteomes.	56
4.14	Comparison of metal composition between *G. antarctica* PI12 and the warm-adapted yeast counterparts.	58
4.15	Comparison of metal composition between *G. antarctica* PI12 and the bacteria.	59
4.16	Variation of metal preferences between the psychrophiles and thermophile in relative to their proteome size.	62
4.17	Evolution driven by the changes of chemistry in the environment.	64
4.18	Multiple sequence alignment of LAN_05_296 and its templates.	72
4.19	Zinc coordinated in a tetrahedral geometry.	75
4.20	Structural domains in sirtuin family proteins.	75
4.21	Overall structure of LAN_05_296 and its sequence alignment with its homologous template.	80
4.22	Sequence alignment in the loop regions for the model and its template.	81
4.23	Backbone RMSD of sirtuin simulated at 277 K, 285 K and 303 K.	84
4.24	Radius of gyration for sirtuin simulated at 277 K, 285 K and 303 K.	86
4.25	RMSF per residue for sirtuin simulated at 277 K, 285 K and 303 K.	89

LIST OF APPENDICES

Appendix		Page
A1	List of available databases that provide information on metal coordination in biology.	112
A2	List of bioinformatics resources for probing metalloproteins.	115
B	A modified model building script of MODELLER to include zinc ion from the template structure during model building.	118
C	Simulation boxes for solvated sirtuin in (A) apo form and (B) zinc-bound form.	119
D	Available free concentrations in the sea as varied with time (estimate).	120
E	Protein domain composition of the five psychrophilic sirtuins in *G. antarctica* PI12	121
F	Summary of sequence analyses that have been conducted to characterize the metal-binding sites for *G. antarctica* PI12 sirtuins.	122
G	Confidence limits for comparative modeling.	123
H	Template-target alignments of psychrophilic sirtuins.	124
I	Model assessment calculated from MODELLER based on molpdf, DOPE and GA341 scores.	128
J	Structural assessment for ten predicted models of LAN_05_296 sirtuin.	130
K	Structural representation of psychrophilic sirtuin.	132
L1	Summary of physical properties for simulated systems.	133
L2	Snapshots of the apo and zinc-bound sirtuin evolved at every 2 ns in the simulations conducted at temperatures of 277 K, 285 K and 303 K.	134
L3	Secondary structure changes observed during the 10 ns MD simulation at 277 K, 285 K and 303 K respectively.	135

LIST OF ABBREVIATIONS

°C	Degree Celsius
3D	Three Dimensional
Å	Angstrom
Ala (A)	Alanine
AMBER	Assisted Model Building with Energy Refinement
Arg (R)	Arginine
Asn (N)	Asparagine
Asp (D)	Aspartic acid
atm	Standard Atmosphere Pressure
BLAST	Basic Local Alignment Search Tool
BLASTp	Protein BLAST
BLOSUM	Blocks Substitution Matrix
BRP	Best Representative PSSM
CATH	Class, Architecture, Topology and Homologous Superfamily
CN	Coordination Number
Cys (C)	Cysteine
DOPE	Discrete Optimized Protein Energy
DSSP	Database of Secondary Structure of Protein
E	Energy
EBI-GOA	European Bioinformatics Institute-Gene Ontology Annotation
FASSM	Function Association using Sequence & Structure Motifs
fs	Femtosecond
Gln (Q)	Glutamine
Glu (E)	Glutamic acid
Gly (G)	Glycine
GO	Gene Ontology
GROMACS	Groningen Machine for Chemical Simulation
His (H)	Histidine
HMM	Hidden Markov model
Ile (I)	Isoleucine
K	Kelvin
Leu (L)	Leucine
LINCS	Linear Constraint Solver
Lys (K)	Lysine
MD	Molecular Dynamics
Met (M)	Methionine
MGI	Malaysia Genome Institute
NAD^+	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
nm	Nanometer
NPT	Isothermal-Isobaric Ensemble (Number of particles, Pressure, Temperature)
ns	Nanosecond

NVT	Canonical Ensemble (Number of particles, Volume, Temperature)
PBC	Periodic boundary conditions
PDB	Protein Data Bank
Pfam	Protein Families
Phe (F)	Phenylalanine
Phyre	Protein Homology/Analogy Recognition Engine
PIR	Protein Information Resource
pK_a	Acid dissociation constant
PME	Particle-Mesh Ewald
Pro (P)	Proline
ps	Picosecond
PSI-BLAST	Position-specific iterated BLAST
PSSM	Position Specific Substitution Matrix
rDNA	Ribosomal DNA
R_g	Radius of gyration
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
ROS	Reactive oxygen species
SASA	Solvent Accessible Surface Area
SAVES	Structural Analysis and Verification Server
Ser (S)	Serine
Sir2	Silent information regulator 2 (Sirtuin)
SVM	Support Vector Machine
Thr (T)	Threonine
TIP3P	Transferable Intermolecular Potential-3 Point
TM-score	Template-modeling score
Trp (W)	Tryptophan
Tyr (Y)	Tyrosine
UniProt	Universal Protein Resource
UniRef	UniProt Reference Clusters
Val (V)	Valine
Zn	Zinc

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xii
LIST OF FIGURES		xiii
LIST OF APPENDICES		xv
LIST OF ABBREVIATIONS		xvi
CHAPTER		
1	**INTRODUCTION**	
	1.1 Overview	1
	1.2 Research Objectives	4
2	**LITERATURE REVIEW**	
	2.1 Metal ions in biological system	5
	2.1.1 Metal-binding proteins, the metalloproteins	5
	2.1.2 From Bioinorganic Chemistry to Metallomics	7
	2.2 Application of bioinformatic approaches in metallomics	8
	2.3 Probing protein structure and dynamics	12
	2.4 Survivability of extremophiles in Antarctica	13
	2.5 Case study: Sirtuin, zinc-bound NAD^+-dependent deacetylase	16
3	**MATERIALS AND METHODS**	
	3.1 Overview	19
	3.2 Hardware and Software	22
	3.2.1 Computer Hardware	22
	3.2.2 Installed Software	22
	3.3 Sequence-based analyses of metalloproteins	23
	3.3.1 BLAST for metal-binding proteins using annotated query	23
	3.3.2 Functional protein domain identification	27
	3.3.3 Classification of protein functional family using SVMProt	27
	3.3.4 Predictive assessment	27
	3.3.5 Mapping of GO terms and subcellular location prediction	28
	3.4 Structural investigation on psychrophilic sirtuins	30
	3.4.1 Protein structure prediction using comparative modeling	30
	3.4.2 Validation of the modeled structures	31
	3.4.3 Molecular dynamics (MD) simulation of sirtuin	32
	3.4.4 Analysis of MD outputs	34

4 RESULTS AND DISCUSSION
4.1 Identification of the number of metal-binding proteins in *G. antarctica* PI12 35
4.1.1 Screening for homologous metalloproteins using BLAST 35
4.1.2 Putative metal-binding protein families identified using Pfam and SVMProt 37
4.1.3 Predictive performance 39
4.1.4 Functional inference for putative metal-binding proteins 43
4.1.5 Distribution of metalloproteins in cellular compartments 52
4.1.6 Comparative analysis of metal utilization preference in other counterparts 54
4.1.7 Biological significance in the variation of metal composition 62
4.2 Case study: Unravel the structural insights of psychrophilic sirtuin 65
4.2.1 Sequence analysis of psychrophilic sirtuins 65
4.2.2 Template selection and comparative modeling of sirtuins 69
4.2.3 Model validation and assessment 73
4.2.4 Structural analysis of psychrophilic sirtuin 79
4.2.5 Structural dynamics of psychrophilic sirtuins 82

5 CONCLUSION AND RECOMMENDATIONS 92
5.1 Recommendations for Future Studies 93

REFERENCES 94
APPENDICES 112
BIODATA OF STUDENT 136
LIST OF PUBLICATIONS 137

LIST OF TABLES

Table		Page
3.1	The number of metal-related queries for BLAST obtained from UniProtKB/SWISS-PROT knowledgebase.	24
3.2	Organisms for comparative analysis in their metal utilization preference.	26
3.3	General details of the five sirtuins retrieved from *G. antarctica* PI12.	30
3.4	Predictive programs employed to characterize sirtuins and their respective zinc-binding sites.	30
4.1	Summary of predictive performance for the three approaches applied.	41
4.2	The number of metal-binding proteins for *G. antarctica* PI12 as predicted by the three predictive approaches is arranged in decreasing order (from Left to Right).	42
4.3 (A)	Comparison of amino acid compositions in sirtuins.	66
4.3 (B)	Comparison of amino acid properties in sirtuins.	67
4.4	Structural templates for *G. antarctica* PI12 sirtuins.	70
4.5	Representative models for each *G. antarctica* PI12 sirtuins.	73
4.6	CATH domain classification for the models of *G. antarctica* PI12 sirtuins.	76
4.7	A summary of Ramachandran plots for the psychrophilic sirtuins models calculated by PROCHECK.	77
4.8	Model assessments by VERIFY3D, ERRAT and ProSA-web.	78
4.9	Averaged R_g and SASA values.	87
4.10	Secondary structure composition of sirtuins.	91

LIST OF FIGURES

Figure		Page
2.1	The essential elements required for life showing in a periodic table.	5
2.2	Number of metal-bound structures deposited in Protein Data Bank (PDB) as of 7 January 2015.	6
2.3	Experimental approaches to metallomics.	7
2.4	Functional assignment for the zinc proteomes of (A) Archaea, (B) Bacteria, (C) Eukarya and (D) human using a combination of bioinformatic approaches.	10
2.5	*Glaciozyma antarctica* PI12	15
2.6	Acetylation and deacetylation of lysine.	17
3.1	Overall workflow of project.	20
3.2	A graphical layout of a eukaryotic cell compartments that are included for predicting the compartmentalization of metalloproteins.	29
3.3	Deprotonation of cysteine side chain by zinc ion.	33
4.1	Putative metal-binding proteins identified by BLAST.	36
4.2	Comparison of five most prevalence metal-types.	37
4.3	Number of putative metal-binding protein clustered into each metal-type using programs BLAST, Pfam-Keyword (Pfam-KW) and SVMProt.	39
4.4	Comparison between the numbers of metal-binding proteins identified.	40
4.5	The number of sequences predicted by BLAST, Pfam-Keyword (Pfam-KW) and SVMProt as metal-bound (Positive ensemble) and non metal-bound (Negative ensemble).	41
4.6	Functional classification of putative magnesium-binding proteins.	45
4.7	Functional classification of putative manganese-binding proteins.	46
4.8	Functional classification of putative calcium-binding proteins.	48

4.9	Functional classification of putative iron-binding proteins.	49
4.10	Functional classification of putative zinc-binding proteins.	51
4.11	Distribution of metal-binding proteins in cellular compartments.	53
4.12	Relative abundance of metal-binding proteins in yeast proteomes.	55
4.13	Relative abundance of metal-binding proteins in bacteria proteomes.	56
4.14	Comparison of metal composition between *G. antarctica* PI12 and the warm-adapted yeast counterparts.	58
4.15	Comparison of metal composition between *G. antarctica* PI12 and the bacteria.	59
4.16	Variation of metal preferences between the psychrophiles and thermophile in relative to their proteome size.	62
4.17	Evolution driven by the changes of chemistry in the environment.	64
4.18	Multiple sequence alignment of LAN_05_296 and its templates.	72
4.19	Zinc coordinated in a tetrahedral geometry.	75
4.20	Structural domains in sirtuin family proteins.	75
4.21	Overall structure of LAN_05_296 and its sequence alignment with its homologous template.	80
4.22	Sequence alignment in the loop regions for the model and its template.	81
4.23	Backbone RMSD of sirtuin simulated at 277 K, 285 K and 303 K.	84
4.24	Radius of gyration for sirtuin simulated at 277 K, 285 K and 303 K.	86
4.25	RMSF per residue for sirtuin simulated at 277 K, 285 K and 303 K.	89

LIST OF APPENDICES

Appendix		Page
A1	List of available databases that provide information on metal coordination in biology.	112
A2	List of bioinformatics resources for probing metalloproteins.	115
B	A modified model building script of MODELLER to include zinc ion from the template structure during model building.	118
C	Simulation boxes for solvated sirtuin in (A) apo form and (B) zinc-bound form.	119
D	Available free concentrations in the sea as varied with time (estimate).	120
E	Protein domain composition of the five psychrophilic sirtuins in *G. antarctica* PI12	121
F	Summary of sequence analyses that have been conducted to characterize the metal-binding sites for *G. antarctica* PI12 sirtuins.	122
G	Confidence limits for comparative modeling.	123
H	Template-target alignments of psychrophilic sirtuins.	124
I	Model assessment calculated from MODELLER based on molpdf, DOPE and GA341 scores.	128
J	Structural assessment for ten predicted models of LAN_05_296 sirtuin.	130
K	Structural representation of psychrophilic sirtuin.	132
L1	Summary of physical properties for simulated systems.	133
L2	Snapshots of the apo and zinc-bound sirtuin evolved at every 2 ns in the simulations conducted at temperatures of 277 K, 285 K and 303 K.	134
L3	Secondary structure changes observed during the 10 ns MD simulation at 277 K, 285 K and 303 K respectively.	135

LIST OF ABBREVIATIONS

°C	Degree Celsius
3D	Three Dimensional
Å	Angstrom
Ala (A)	Alanine
AMBER	Assisted Model Building with Energy Refinement
Arg (R)	Arginine
Asn (N)	Asparagine
Asp (D)	Aspartic acid
atm	Standard Atmosphere Pressure
BLAST	Basic Local Alignment Search Tool
BLASTp	Protein BLAST
BLOSUM	Blocks Substitution Matrix
BRP	Best Representative PSSM
CATH	Class, Architecture, Topology and Homologous Superfamily
CN	Coordination Number
Cys (C)	Cysteine
DOPE	Discrete Optimized Protein Energy
DSSP	Database of Secondary Structure of Protein
E	Energy
EBI-GOA	European Bioinformatics Institute-Gene Ontology Annotation
FASSM	Function Association using Sequence & Structure Motifs
fs	Femtosecond
Gln (Q)	Glutamine
Glu (E)	Glutamic acid
Gly (G)	Glycine
GO	Gene Ontology
GROMACS	Groningen Machine for Chemical Simulation
His (H)	Histidine
HMM	Hidden Markov model
Ile (I)	Isoleucine
K	Kelvin
Leu (L)	Leucine
LINCS	Linear Constraint Solver
Lys (K)	Lysine
MD	Molecular Dynamics
Met (M)	Methionine
MGI	Malaysia Genome Institute
NAD^+	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
nm	Nanometer
NPT	Isothermal-Isobaric Ensemble (Number of particles, Pressure, Temperature)
ns	Nanosecond
NVT	Canonical Ensemble (Number of particles, Volume, Temperature)

PBC	Periodic boundary conditions
PDB	Protein Data Bank
Pfam	Protein Families
Phe (F)	Phenylalanine
Phyre	Protein Homology/Analogy Recognition Engine
PIR	Protein Information Resource
pK_a	Acid dissociation constant
PME	Particle-Mesh Ewald
Pro (P)	Proline
ps	Picosecond
PSI-BLAST	Position-specific iterated BLAST
PSSM	Position Specific Substitution Matrix
rDNA	Ribosomal DNA
R_g	Radius of gyration
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
ROS	Reactive oxygen species
SASA	Solvent Accessible Surface Area
SAVES	Structural Analysis and Verification Server
Ser (S)	Serine
Sir2	Silent information regulator 2 (Sirtuin)
SVM	Support Vector Machine
Thr (T)	Threonine
TIP3P	Transferable Intermolecular Potential-3 Point
TM-score	Template-modeling score
Trp (W)	Tryptophan
Tyr (Y)	Tyrosine
UniProt	Universal Protein Resource
UniRef	UniProt Reference Clusters
Val (V)	Valine
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Overview

Metal ions are one of the essential components for all life-forms. They usually present as cofactors in many proteins, and are involved in a wide-array of physiological processes by binding to substrates, regulating cellular activities, facilitating electron exchange reactions and stabilizing the protein structures (Andreini *et al.*, 2004; Degtyarenko, 2000). Lacking of these trace elements may cause the proteins to malfunction. The entire cellular metal content in a cell is coined as metallome. The comprehensive analyses in identifying these metals and how they are distributed in biological systems have emerged as a new interdisciplinary area termed as metallomics (Shi & Chance, 2008; Williams & Da Silva, 2000). Metallome is a dynamic system, and it could be influenced by any significant perturbation within a cell and its adjacent environment. This has resulted in organism-specific metalloproteome, which can be denoted as the functional division of metals in biology (Thiele & Gitlin, 2008). Each of these metals, with their unique chemistry, presents in the physiological systems with varied concentrations.

Recent studies have reported that the metal-binding proteins (i.e. proteins that recruited the metal ion for functionality) are actually widespread in almost all the living organisms, with some found to correlate with their respective habitats and metabolic preferences (Cameron *et al.*, 2012). Focusing only the prokaryotes, Zerkle *et al.* (2005) attempted to resolve the metal preference for 52 candidates with complete genomes aided by publicly available bioinformatic databases. The metal usage of prokaryotes was found to follow the trend in Fe > Zn > Mn > Mo, Co, Cu > Ni > W, V. An elevated requirement for nickel and tungsten was noticed in methanogens, which could be a biosignature for methanogenesis. Few years later, Andreini and her colleagues published their genome-wide predictions using multiple bioinformatics approaches with emphasizes on the content of zinc (2006b), non-heme iron (2007) and copper proteins (2008a) in archaea, prokaryotes and eukaryotes. They reported that the number of zinc-binding proteins was increased from prokaryotes to eukaryotes as the organismal complexity increased. In contrast, a considerable reduction was detected in the number of non-heme iron proteins, while the fraction of copper proteins was rather unchanged in these organisms.

There was another study conducted by Cameron *et al.* (2012) which was intended to characterize the metal usage of hyperthermophiles. The team employed the hyperthermophilic Archaea *Methanococcus jannaschii* and *Pyrococcus furiosus* as the targets of their study and the results were compared with that of the mesophilic bacterium *Escherichia coli*. They observed that the hyperthermophiles are actually demanded for more nickel, cobalt and tungsten. All these studies have suggested that zinc and iron are universal in living systems, with some scattered occurrence of other metals such as nickel and cobalt that are specifically ubiquitous in certain prokaryotes. However, the reference for metal usage in fungi examples is still missing.

The metal-binding proteins are encoded from the genes, therefore any drastic changes in the adjacent environment could impact the cellular systems that are reflected in the genetic drift (Thiele & Gitlin, 2008). As one of the greatest incidents on Earth, the rise of the atmospheric oxygen has critically influenced the acquisition of metals for biological usage, which in parallel, a driving force to the evolution of modern life. New survival mechanisms have to be developed in order to adapt the changes of the bioavailability of metal ions in corresponding to the presence of oxygen (Hong Enriquez & Do, 2012). An evolutionary shift in the trend of metal usage is therefore noticed in the speciation of metal in modern organisms as compared to primitive organisms (Dupont *et al.*, 2006; Zerkle *et al.*, 2005).

Rapid advancement of whole genome sequencing technology has geared the directions of major research towards unlocking the codes of inheritance. The explosion of high-throughput data has provided the researchers with a complete list of molecular components with detailed information on every gene present in an organism. However, regardless of the mass data generated from the protein encoding sequences, less attention have been emphasized in illustrating the relationship between metal ions and proteins (Andreini *et al.*, 2009). Meanwhile, majority of the experimental approaches that have been proposed and currently applied in metalloproteomics studies are usually laborious and complex. Some may also introduce biases and errors (Passerini *et al.*, 2007). Since the rapid advancement in genome sequencing techniques have generated mountains of sequence data each day, predictive tools that enable the scientists to sieve through an organism's genetic blueprint and subsequently analyze in detail the sequence(s) of interest are invaluable. By extracting the information directly from the sequences, bioinformatics approaches can readily solve the question of how many and which proteins may require metal ions to function properly. It is also capable to provide insights (such as the atomic details of DNA or protein structures) that are previously inaccessible by experimental approaches (Rulisek & Vondrasek, 1998; Wan & Xu, 2005). Hence, multiple bioinformatics approaches were incorporated in this study to identify the metal-binding proteins from the post-genome sequencing data.

Renowned as the coldest, windiest and driest continent on Earth, Antarctica is the fifth largest continents on Earth which is geographically isolated and permanently covered by ice and snow (Buzzini *et al.*, 2012). Although the Environment Protocol (or also known as Madrid Protocol) that aimed to protect the Antarctic environment and associated ecosystems has been implemented since 1998, this fragile region is continuously degraded at accelerated pace (Aronson *et al.*, 2011). Increased of anthropogenic waste from human activities is one of the major sources of pollution noticed in this region. Some of these toxic substances, including the heavy metals, are found to be accumulated in the food chains that have drastically affected the ecosystem nearby. The present study therefore targeted at a psychrophilic yeast *Glaciozyma antarctica* PI12 isolated from the sea ice of Antarctica, to investigate its metal composition and its possible link to the pollution issue in the respective area. Since it is also a precious source of cold-active enzymes that possess high specific activity at relatively low temperatures, this study will also provide an opportunity to explore its unprecedented biotechnology potential.

In order to investigate the role of zinc in protein, a zinc-bound NAD^+-dependent deacetylase was selected among the lists of metal-binding proteins identified in *G. antarctica* PI12 for in-depth structural analysis. This versatile silent information regulator 2 (Sir2) protein, or commonly known as sirtuin, participates in transcriptional silencing and is evolutionally conserved from bacteria to mammals (North & Verdin, 2004). It also regulates cell apoptosis, microtubule organization and cellular responses to DNA-damaging agents (North & Verdin, 2004; Sakkiah *et al.*, 2012). In some organisms, sirtuin is found to restrict the intake of calorie during adverse condition to enhance stress tolerance, and may have association with cell aging (Calvo *et al.*, 2012; Guarente, 2013). Several studies have reported the structural and dynamics perspectives of sirtuin upon interacting with cofactor NAD^+ and its potential inhibitors through molecular dynamics (MD) simulation (Hsu *et al.*, 2013; Sacconnay *et al.*, 2013; Sakkiah *et al.*, 2013a; Sakkiah *et al.*, 2013b; Sakkiah *et al.*, 2012; Shi *et al.*, 2013). However the role of zinc is often omitted, although there is an indication that the removal of zinc can discontinue the enzymatic activity (Chakrabarty & Balaram, 2010; North & Verdin, 2004). Therefore, the structural and dynamic properties of the psychrophilic sirtuin were investigated in this study with the role of zinc highlighted.

1.2 Research Objectives

The current research aimed to decipher the complete list of the metal-binding proteins in a psychrophilic yeast *G. antarctica* PI12 genome and to predict its relative cellular metal usage from the expressed metal-binding proteins. With the aid of bioinformatics tools, the comprehensive analysis on the psychrophilic metallome are hoped to outline the metal-relationship between the organism and its adjacent environment, as well as to explore the functional role of metal ions in its cellular system. Since the zinc-binding sirtuin is reported to associate with various cellular activities and is renowned to enhance stress tolerance during adverse condition, the structural investigation on the cold-adapted sirtuin from *G. antarctica* PI12 may contribute to the understanding of sirtuin function.

Therefore, these objectives will be pursued in this study:

- To identify the putative metal-binding proteins from *G. antarctica* PI12 genome.
- To infer the functional roles of the putative metal-binding proteins.
- To compare the metal usage of *G. antarctica* PI12 with selected yeast and bacteria strains.
- To investigate the stability-flexibility relationship of the psychrophilic sirtuin at various temperatures.

REFERENCES

Abedi Karjiban, R., Abdul Rahman, M. B., Salleh, A. B., Basri, M., Abdul Rahman, R. N. Z. R., & Leow, A. T. C. (2010). On the importance of the small domain in the thermostability of thermoalkalophilic lipases from L1 and T1: Insights from molecular dynamics simulation. *Protein and Peptide Letters*, 17(6), 699.

Adcock, S. A., & McCammon, J. A. (2006). Molecular dynamics: Survey of methods for simulating the activity of proteins. *Chemical Reviews, 106*(5), 1589-1615.

Ali, M., Shukuri, M., Mohd Fuzi, S. F., Ganasen, M., Abdul Rahman, R. N. Z. R., Basri, M., *et al.* (2013). Structural Adaptation of Cold-Active RTX Lipase from *Pseudomonas* sp. Strain AMS8 Revealed via Homology and Molecular Dynamics Simulation Approaches. *BioMed Research International, 2013*.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403-410.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., *et al.* (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*(17), 3389-3402.

Andreini, C., Banci, L., Bertini, I., Elmi, S., & Rosato, A. (2007). Non-heme iron through the three domains of life. *Proteins: Structure, Function, and Bioinformatics, 67*(2), 317-324.

Andreini, C., Banci, L., Bertini, I., & Rosato, A. (2006a). Counting the Zinc-Proteins Encoded in the Human Genome. *Journal of Proteome Research, 5*(1), 196-201.

Andreini, C., Banci, L., Bertini, I., & Rosato, A. (2006b). Zinc through the Three Domains of Life. *Journal of Proteome Research, 5*(11), 3173-3178.

Andreini, C., Banci, L., Bertini, I., & Rosato, A. (2008a). Occurrence of copper proteins through the three domains of life: a bioinformatic approach. *The Journal of Proteome Research, 7*(01), 209-216.

Andreini, C., Bertini, I., Cavallaro, G., Holliday, G. L., & Thornton, J. M. (2008b). Metal ions in biological catalysis: from enzyme databases to general principles. *Journal of Biological Inorganic Chemistry, 13*(8), 1205-1218.

Andreini, C., Bertini, I., & Rosato, A. (2004). A hint to search for metalloproteins in gene banks. *Bioinformatics, 20*(9), 1373-1380.

Andreini, C., Bertini, I., & Rosato, A. (2009). Metalloproteomes: A Bioinformatic Approach. *Accounts of Chemical Research, 42*(10), 1471-1479.

Andreini, C., Cavallaro, G., Lorenzini, S., & Rosato, A. (2013). MetalPDB: A database of metal sites in biological macromolecular structures. *Nucleic Acids Research, 41*(D1), D312-D319.

Aronson, R. B., Thatje, S., McClintock, J. B., & Hughes, K. A. (2011). Anthropogenic impacts on marine ecosystems in Antarctica. *Annals of the New York Academy of Sciences, 1223*(1), 82-107.

Arthur, H., & Watson, K. (1976). Thermal adaptation in yeast: growth temperatures, membrane lipid, and cytochrome composition of psychrophilic, mesophilic, and thermophilic yeasts. *Journal of Bacteriology*, 128(1), 56-68.

Atkinson, A., & Winge, D. R. (2009). Metal acquisition and availability in the mitochondria. *Chemical Reviews, 109*(10), 4708-4721.

Ayala-del-Río, H. L., Chain, P. S., Grzymski, J. J., Ponder, M. A., Ivanova, N., Bergholz, P. W., *et al.* (2010). The genome sequence of *Psychrobacter arcticus* 273-4, a psychroactive Siberian permafrost bacterium, reveals mechanisms for adaptation to low-temperature growth. *Applied and Environmental Microbiology, 76*(7), 2304-2312.

Bairoch, A., Apweiler, R., Wu, C. H., Barker, W. C., Boeckmann, B., Ferro, S., *et al.* (2005). The universal protein resource (UniProt). *Nucleic Acids Research, 33*(suppl 1), D154-D159.

Banci, L., & Bertini, I. (2013). Metallomics and the cell: some definitions and general comments *Metallomics and the Cell* (pp. 1-13): Springer.

Bateman, A., Birney, E., Cerruti, L., Durbin, R., Etwiller, L., Eddy, S. R., *et al.* (2002). The Pfam protein families database. *Nucleic Acids Research, 30*(1), 276-280.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., *et al.* (2000). The Protein Data Bank. *Nucleic Acids Research, 28*(1), 235-242.

Berridge, M. J., Lipp, P., & Bootman, M. D. (2000). The versatility and universality of calcium signalling. *Nature Reviews Molecular Cell Biology, 1*(1), 11-21.

Bertini, I., & Cavallaro, G. (2010). Bioinformatics in bioinorganic chemistry. *Metallomics, 2*(1), 39-51.

Bertini, I., Decaria, L., & Rosato, A. (2010). The annotation of full zinc proteomes. *Journal of Biological Inorganic Chemistry, 15*(7), 1071-1078.

Bertini, I., & Rosato, A. (2007). From genes to metalloproteins: A bioinformatic approach. *European Journal of Inorganic Chemistry, 2007*(18), 2546-2555.

Binder, K., Horbach, J., Kob, W., Paul, W., & Varnik, F. (2004). Molecular dynamics simulations. *Journal of Physics: Condensed Matter, 16*(5), S429.

Bitterman, K. J., Anderson, R. M., Cohen, H. Y., Latorre-Esteves, M., & Sinclair, D. A. (2002). Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. *Journal of Biological Chemistry, 277*(47), 45099-45107.

Boekhorst, J., & Snel, B. (2007). Identification of homologs in insignificant blast hits by exploiting extrinsic gene properties. *BMC Bioinformatics, 8*(1), 356.

Boo, S. Y., Wong, C. M. V. L., Rodrigues, K. F., Najimudin, N., Murad, A. M. A., & Mahadi, N. M. (2013). Thermal stress responses in Antarctic yeast, *Glaciozyma antarctica* PI12, characterized by real-time quantitative PCR. *Polar Biology, 36*(3), 381-389.

Bortolato, M., Besson, F., & Roux, B. (1999). Role of metal ions on the secondary and quaternary structure of alkaline phosphatase from bovine intestinal mucosa. *Proteins: Structure, Function, and Bioinformatics, 37*(2), 310-318.

Bowman, J. P., Abell, G. C., & Mancuso Nichols, C. A. (2005). Psychrophilic Extremophiles from Antarctica: Biodiversity and biotechnological potential. *Ocean and Polar Research, 27*, 221-230.

Bruzzone, S., Parenti, M. D., Grozio, A., Ballestrero, A., Bauer, I., Del Rio, A., *et al.* (2013). Rejuvenating sirtuins: The rise of a new family of cancer drug targets. *Current Pharmaceutical Design, 19*(4), 614.

Buljan, M., & Bateman, A. (2009). The evolution of protein domain families. *Biochemical Society Transactions, 37*(4), 751.

Bussi, G., Donadio, D., & Parrinello, M. (2007). Canonical sampling through velocity rescaling. *The Journal of Chemical Physics, 126*(1), 014101.

Buzzini, P., Branda, E., Goretti, M., & Turchetti, B. (2012). Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. *FEMS Microbiology Ecology, 82*(2), 217-241.

Cai, C. Z., Han, L. Y., Ji, Z. L., Chen, X., & Chen, Y. Z. (2003). SVM-Prot: Web-based support vector machine software for functional classification of a protein from its primary sequence. *Nucleic Acids Research, 31*(13), 3692-3697.

Calvo, S. S.-C., Navas, P., & López-Lluch, G. (2012). Sirtuin-Dependent Metabolic Control and Its Role in the Aging Process. In K. Clark (Ed.), *Bioenergetics*.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., *et al.* (2009). BLAST+: architecture and applications. *BMC Bioinformatics, 10*(1), 421.

Cameron, V., House, C. H., & Brantley, S. L. (2012). A First Analysis of Metallome Biosignatures of Hyperthermophilic Archaea. *Archaea, 2012*.

Carugo, O., & Pongor, S. (2001). A normalized root-mean-square distance for comparing protein three-dimensional structures. *Protein Science, 10*(7), 1470-1473.

Casanueva, A., Tuffin, M., Cary, C., & Cowan, D. A. (2010). Molecular adaptations to psychrophily: The impact of 'omic' technologies. *Trends in Microbiology, 18*(8), 374-381.

Casatta, N., Porro, A., Orlandi, I., Brambilla, L., & Vai, M. (2013). Lack of Sir2 increases acetate consumption and decreases extracellular pro-aging factors. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1833*(3), 593-601.

Chakrabarty, S. P., & Balaram, H. (2010). Reversible binding of zinc in *Plasmodium falciparum* Sir2: Structure and activity of the apoenzyme. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1804*(9), 1743-1750.

Cheek, S., Zhang, H., & Grishin, N. V. (2002). Sequence and structure classification of kinases. *Journal of Molecular Biology, 320*(4), 855-881.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., *et al.* (2003). Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research, 31*(13), 3497-3500.

Chi, S.-M., & Nam, D. (2012). WegoLoc: accurate prediction of protein subcellular localization using weighted Gene Ontology terms. *Bioinformatics, 28*(7), 1028-1030.

Colovos, C., & Yeates, T. O. (1993). Verification of protein structures: Patterns of nonbonded atomic interactions. *Protein Science, 2*(9), 1511-1519.

Costantini, S., Sharma, A., Raucci, R., Costantini, M., Autiero, I., & Colonna, G. (2013). Genealogy of an ancient protein family: the Sirtuins, a family of disordered members. *BMC Evolutionary Biology, 13*(1), 60.

Crichton, R. (2007). *Biological Inorganic Chemistry: An Introduction*: Elsevier Science.

Cvetkovic, A., Menon, A. L., Thorgersen, M. P., Scott, J. W., Poole Ii, F. L., Jenney Jr, F. E., *et al.* (2010). Microbial metalloproteomes are largely uncharacterized. *Nature, 466*(7307), 779-782.

Da Silva, J. J. R. F., & Williams, R. J. P. (2001). *The principles of the uptake and chemical speciation of the elements in biology* Oxford University Press.

DasSarma, S., Capes, M. D., Karan, R., & DasSarma, P. (2013). Amino acid substitutions in cold-adapted proteins from *Halorubrum lacusprofundi*, an extremely halophilic microbe from Antarctica. *PloS ONE, 8*(3), e58587.

De Castro, E., Sigrist, C. J. A., Gattiker, A., Bulliard, V., Langendijk-Genevaux, P. S., Gasteiger, E., *et al.* (2006). ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. *Nucleic Acids Research, 34*(suppl 2), W362-W365.

De Nicola, R., & Walker, G. (2009). Interaction between yeasts and zinc. *Yeast Biotechnology: Diversity and Applications* (pp. 237-257): Springer.

De Pascale, D., De Santi, C., Fu, J., & Landfald, B. (2012). The microbial diversity of Polar environments is a fertile ground for bioprospecting. *Marine Genomics, 8*, 15-22.

Decaria, L., Bertini, I., & Williams, R. J. P. (2010). Zinc proteomes, phylogenetics and evolution. *Metallomics, 2*(10), 706-709.

Degtyarenko, K. (2000). Bioinorganic motifs: towards functional classification of metalloproteins. *Bioinformatics, 16*(10), 851-864.

DeLano, W. L. (2002). The PyMOL molecular graphics system.

Derbyshire, M. K., Weinstock, K. G., & Strathern, J. N. (1996). HST1, a new member of the SIR2 family of genes. *Yeast, 12*(7), 631-640.

du Plessis, L., Škunca, N., & Dessimoz, C. (2011). The what, where, how and why of gene ontology—a primer for bioinformaticians. *Briefings in Bioinformatics, 12*(6), 723-735.

Duan, Y., Wu, C., Chowdhury, S., Lee, M. C., Xiong, G., Zhang, W., *et al.* (2003). A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. *Journal of Computational Chemistry, 24*(16), 1999-2012.

Dudev, T., & Lim, C. (2003a). Metal binding and selectivity in zinc proteins. *Journal of the Chinese Chemical Society, 50*(5), 1093-1102.

Dudev, T., & Lim, C. (2003b). Principles governing Mg, Ca, and Zn binding and selectivity in proteins. *Chemical Reviews, 103*(3), 773-788.

Dudev, T., & Lim, C. (2008). Metal binding affinity and selectivity in metalloproteins: Insights from computational studies. *Annual Review of Biophysics, 37*, 97-116.

Dupont, C. L. (2008). *Physiological, ecological, and evolutionary studies of trace metal homeostasis in marine microbes.* PhD dissertation, University of California, San Diego.

Dupont, C. L., Butcher, A., Valas, R. E., Bourne, P. E., & Caetano-Anollés, G. (2010). History of biological metal utilization inferred through phylogenomic analysis of protein structures. *Proceedings of the National Academy of Sciences, 107*(23), 10567-10572.

Dupont, C. L., Yang, S., Palenik, B., & Bourne, P. E. (2006). Modern proteomes contain putative imprints of ancient shifts in trace metal geochemistry. *Proceedings of the National Academy of Sciences, 103*(47), 17822-17827.

Eddy, S. R. (1998). Profile hidden Markov models. *Bioinformatics, 14*(9), 755-763.

Eide, D. J., Clark, S., Nair, T. M., Gehl, M., Gribskov, M., Guerinot, M. L., *et al.* (2005). Characterization of the yeast ionome: a genome-wide analysis of nutrient mineral and trace element homeostasis in *Saccharomyces cerevisiae*. *Genome Biology, 6*(9), R77.

Eisenberg, D., Lüthy, R., & Bowie, J. U. (1997). VERIFY3D: assessment of protein models with three-dimensional profiles. *Methods in Enzymology, 277*, 396.

Eissenberg, J. C., & Shilatifard, A. (2006). Leaving a mark: the many footprints of the elongating RNA polymerase II. *Current Opinion in Genetics & Development, 16*(2), 184-190.

Essmann, U., Perera, L., Berkowitz, M. L., Darden, T., Lee, H., & Pedersen, L. G. (1995). A smooth particle mesh Ewald method. *The Journal of Chemical Physics, 103*(19), 8577-8593.

Eswar, N., Eramian, D., Webb, B., Shen, M.-Y., & Sali, A. (2008). Protein structure modeling with MODELLER. *Structural Proteomics* (pp. 145-159): Springer.

Eswar, N., Webb, B., Marti-Renom, M. A., Madhusudhan, M., Eramian, D., Shen, M. y., *et al.* (2006). Comparative protein structure modeling using Modeller. *Current Protocols in Bioinformatics*, 5.6. 1-5.6. 30.

Ewart, K. V., Yang, D. S. C., Ananthanarayanan, V. S., Fletcher, G. L., & Hew, C. L. (1996). Ca^{2+}-dependent Antifreeze Proteins MODULATION OF CONFORMATION AND ACTIVITY BY DIVALENT METAL IONS. *Journal of Biological Chemistry, 271*(28), 16627-16632.

Falchuk, K. H. (1998). The molecular basis for the role of zinc in developmental biology *Molecular and Cellular Effects of Nutrition on Disease Processes* (pp. 41-48): Springer.

Fell, J. W., Statzell, A. C., Hunter, I. L., & Phaff, H. J. (1969). *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie van Leeuwenhoek, 35*(1), 433-462.

Feller, G. (2013). Psychrophilic enzymes: from folding to function and biotechnology. *Scientifica, 2013*.

Feller, G., d'Amic, D., & Gerday, C. (1999). Thermodynamic stability of a cold-active α-amylase from the Antarctic bacterium *Alteromonas haloplanctis*. *Biochemistry, 38*(14), 4613-4619.

Feller, G., & Gerday, C. (1997). Psychrophilic enzymes: molecular basis of cold adaptation. *Cellular and Molecular Life Sciences, 53*(10), 830-841.

Field, L. S., Furukawa, Y., O'Halloran, T. V., & Culotta, V. C. (2003). Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria. *Journal of Biological Chemistry, 278*(30), 28052-28059.

Fields, P. A. (2001). Review: Protein function at thermal extremes: balancing stability and flexibility. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 129*, 417-431.

Finn, R. D., Clements, J., & Eddy, S. R. (2011). HMMER web server: interactive sequence similarity searching. *Nucleic Acids Research, 39*(suppl 2), W29-W37.

Finn, R. D., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J. E., *et al.* (2010). The Pfam protein families database. *Nucleic Acids Research, 38*(suppl 1), D211-D222.

Frye, R. A. (2000). Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochemical and Biophysical Research Communications, 273*(2), 793-798.

Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. *The proteomics protocols handbook* (pp. 571-607): Springer.

Gerday, C. (2002). Extremophiles: basic concepts. *Knowledge for Sustainable Development. An Insight into the Encyclopedia of Life Support Systems. UNESCO Publishing/EOLSS Publishers, Oxford, 1*, 573-598.

Glusker, J. P., Katz, A. K., & Bock, C. W. (1999). Metal ions in biological systems. *The Rigaku Journal, 16*(2), 8-16.

Goldstein, R. A. (2007). Amino-acid interactions in psychrophiles, mesophiles, thermophiles, and hyperthermophiles: Insights from the quasi-chemical approximation. *Protein Science, 16*(9), 1887-1895.

Gouet, P., Courcelle, E., & Stuart, D. I. (1999). ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics, 15*(4), 305-308.

Guarente, L. (2013). Calorie restriction and sirtuins revisited. *Genes & development, 27*(19), 2072-2085.

Han, L. Y., Cai, C. Z., Ji, Z. L., Cao, Z. W., Cui, J., & Chen, Y. Z. (2004). Predicting functional family of novel enzymes irrespective of sequence similarity: A statistical learning approach. *Nucleic Acids Research, 32*(21), 6437-6444.

Hansson, T., Oostenbrink, C., & van Gunsteren, W. (2002). Molecular dynamics simulations. *Current Opinion in Structural Biology, 12*(2), 190-196.

Haraguchi, H. (2004). Metallomics as integrated biometal science. *Journal of Analytical Atomic Spectrometry, 19*(1), 5-14.

Harding, M. M. (2004). The architecture of metal coordination groups in proteins. *Acta Crystallographica Section D: Biological Crystallography, 60*(5), 849-859.

Hartwig, A. (2001). Zinc finger proteins as potential targets for toxic metal ions: Differential effects on structure and function. *Antioxidants and Redox Signaling, 3*(4), 625-634.

Hashim, N. H. F., Bharudin, I., Nguong, D. L. S., Higa, S., Bakar, F. D. A., Nathan, S., *et al.* (2013). Characterization of Afp1, an antifreeze protein from the psychrophilic yeast *Glaciozyma antarctica* PI12. *Extremophiles, 17*(1), 63-73.

Hedlund, J. (2010). *Bioinformatic protein family characterisation.* PhD dissertation, Linköpings University (Sweden) Institute of Technology

Hess, B., Bekker, H., Berendsen, H. J. C., & Fraaije, J. G. E. M. (1997). LINCS: a linear constraint solver for molecular simulations. *Journal of Computational Chemistry, 18*(12), 1463-1472.

Hess, B., Kutzner, C., van der Spoel, D., & Lindahl, E. (2008). GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *Journal of Chemical Theory and Computation, 4*(3), 435-447.

Hong Enriquez, R. P., & Do, T. N. (2012). Bioavailability of Metal Ions and Evolutionary Adaptation. *Life, 2*(4), 274-285.

Horton, P., Park, K.-J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., *et al.* (2007). WoLF PSORT: protein localization predictor. *Nucleic Acids Research, 35*(suppl 2), W585-W587.

Hoyoux, A., Jennes, I., Dubois, P., Genicot, S., Dubail, F., François, J.-M., *et al.* (2001). Cold-adapted β-galactosidase from the Antarctic psychrophile *Pseudoalteromonas haloplanktis*. *Applied and Environmental Microbiology, 67*(4), 1529-1535.

Hsu, H.-C., Wang, C.-L., Wang, M., Yang, N., Chen, Z., Sternglanz, R., *et al.* (2013). Structural basis for allosteric stimulation of Sir2 activity by Sir4 binding. *Genes & Development, 27*(1), 64-73.

Hu, L., He, B., Wang, Y., Jiang, G., & Sun, H. (2013). Metallomics in environmental and health related research: Current status and perspectives. *Chinese Science Bulletin, 58*(2), 169-176.

Irving, H. M. N. H., & Williams, R. J. P. (1948). Order of stability of metal complexes. *Nature*, 162, 746-747.

Jacob, E., & Unger, R. (2007). A tale of two tails: why are terminal residues of proteins exposed? *Bioinformatics, 23*(2), e225-e230.

Jahandideh, M., Barkooie, S. M. H., Jahandideh, S., Abdolmaleki, P., Movahedi, M. M., Hoseini, S., *et al.* (2008). Elucidating the protein cold-adaptation: Investigation of the parameters enhancing protein psychrophilicity. *Journal of Theoretical Biology, 255*(1), 113-118.

Jahandideh, S., Abdolmaleki, P., Jahandideh, M., & Barzegari Asadabadi, E. (2007). Sequence and structural parameters enhancing adaptation of proteins to low temperatures. *Journal of Theoretical Biology*, *246*(1), 159-166.

Jensen, M. R., Hass, M. A. S., Hansen, D. F., & Led, J. J. (2007). Investigating metal-binding in proteins by nuclear magnetic resonance. *Cellular and Molecular Life Sciences, 64*(9), 1085-1104.

Jones, T., Federspiel, N. A., Chibana, H., Dungan, J., Kalman, S., Magee, B. B., *et al.* (2004). The diploid genome sequence of *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 101*(19), 7329-7334.

Joseph, B., Ramteke, P. W., & Thomas, G. (2008). Cold active microbial lipases: some hot issues and recent developments. *Biotechnology Advances, 26*(5), 457-470.

Joshi, S., & Satyanarayana, T. (2013). Biotechnology of cold-active proteases. *Biology, 2*(2), 755-783.

Kabsch, W. (1978). A discussion of the solution for the best rotation to relate two sets of vectors. *Acta Crystallographica Section A: Crystal Physics, Diffraction, Theoretical and General Crystallography, 34*(5), 827-828.

Kabsch, W., & Sander, C. (1983). Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers, 22*(12), 2577-2637.

Kaeberlein, M. (2010). Lessons on longevity from budding yeast. *Nature, 464*(7288), 513-519.

Kairys, V., Gilson, M. K., & Fernandes, M. X. (2006). Using protein homology models for structure-based studies: approaches to model refinement. *The Scientific World Journal, 6*, 1542-1554.

Karlin, S., Zhu, Z.-Y., & Karlin, K. D. (1997). The extended environment of mononuclear metal centers in protein structures. *Proceedings of the National Academy of Sciences, 94*(26), 14225-14230.

Karplus, M., & Kuriyan, J. (2005). Molecular dynamics and protein function. *Proceedings of the National Academy of Sciences of the United States of America, 102*(19), 6679-6685.

Kasana, R. C., & Gulati, A. (2011). Cellulases from psychrophilic microorganisms: A review. *Journal of Basic Microbiology, 51*(6), 572-579.

Kelley, L. A., & Sternberg, M. J. E. (2009). Protein structure prediction on the web: A case study using the Phyre server. *Nature Protocols, 4*(3), 363-371.

Konerding, D. (2004). An Essential Guide to the Basic Local Alignment Search Tool: BLAST. *Briefings in Bioinformatics*, 5(1), 93-94. doi: 10.1093/bib/5.1.93

Koonin, E. V., & Galperin, M. Y. (2003). Evolutionary concept in genetics and genomics. *Sequence-Evolution-Function* (pp. 25-49): Springer.

Kornberg, R. D., & Lorch, Y. (1999). Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell, 98*(3), 285-294.

Koubova, J., & Guarente, L. (2003). How does calorie restriction work? *Genes & Development, 17*(3), 313-321.

Kumar, A., Agarwal, S., Heyman, J. A., Matson, S., Heidtman, M., Piccirillo, S., *et al.* (2002). Subcellular localization of the yeast proteome. *Genes & Development, 16*(6), 707-719.

Kundu, S., & Roy, D. (2009). Comparative structural studies of psychrophilic and mesophilic protein homologues by molecular dynamics simulation. *Journal of Molecular Graphics and Modelling, 27*(8), 871-880.

Lahner, B., Gong, J., Mahmoudian, M., Smith, E. L., Abid, K. B., Rogers, E. E., *et al.* (2003). Genomic scale profiling of nutrient and trace elements in Arabidopsis thaliana. *Nature Biotechnology, 21*(10), 1215-1221.

Larsson-Raźanikiewicz, M. (1970). The phosphoglycerate kinase reaction and its metal ion specificity. *European Journal of Biochemistry, 17*(1), 183-192.

Laskowski, R. A., MacArthur, M. W., Moss, D. S., & Thornton, J. M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography, 26*(2), 283-291.

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling, 51*(10), 2778-2786.

Lide, D. (1996). *CRC Handbook of Chemistry and Physics, 77th Edition*: CRC Press.

Lin, H. H., Han, L. Y., Zhang, H. L., Zheng, C. J., Xie, B., Cao, Z. W., *et al.* (2006). Prediction of the functional class of metal-binding proteins from sequence derived physicochemical properties by support vector machine approach. *BMC Bioinformatics, 7*(Suppl 5), S13.

Lindh, U. (2013). Biological functions of the elements. *Essentials of Medical Geology* (pp. 129-177): Springer.

Lippi, M., Passerini, A., Punta, M., & Frasconi, P. (2012). Metal binding in proteins: machine learning complements x-ray absorption spectroscopy. *Machine Learning and Knowledge Discovery in Databases* (pp. 854-857): Springer.

Mahoney, M. W., & Jorgensen, W. L. (2000). A five-site model for liquid water and the reproduction of the density anomaly by rigid, nonpolarizable potential functions. *The Journal of Chemical Physics, 112*(20), 8910-8922.

Maret, W. (2010). Metalloproteomics, metalloproteomes, and the annotation of metalloproteins. *Metallomics, 2*(2), 117-125.

Maret, W., & Copsey, M. (2012). Metallomics: Whence and whither. *Metallomics: integrated biometal science, 4*(10), 1017-1019.

Maret, W., & Li, Y. (2009). Coordination dynamics of zinc in proteins. *Chemical Reviews, 109*(10), 4682-4707.

Margesin, R., & Schinner, F. (1994). Properties of cold-adapted microorganisms and their potential role in biotechnology. *Journal of Biotechnology, 33*(1), 1-14.

McCarthy, F. M., Bridges, S. M., Wang, N., Magee, G. B., Williams, W. P., Luthe, D. S., *et al.* (2007). AgBase: A unified resource for functional analysis in agriculture. *Nucleic Acids Research, 35*(suppl 1), D599-D603.

Melo, F., Sánchez, R., & Sali, A. (2002). Statistical potentials for fold assessment. *Protein Science, 11*(2), 430-448.

Micaroni, M. (2012). Calcium around the Golgi Apparatus: Implications for Intracellular Membrane Trafficking. *Calcium Signaling* (pp. 439-460): Springer.

Min, J., Landry, J., Sternglanz, R., & Xu, R.-M. (2001). Crystal structure of a SIR2 homolog-NAD complex. *Cell, 105*(2), 269-279.

Morita, R. Y. (1975). Psychrophilic bacteria. *Bacteriological reviews, 39*(2), 144.

Mounicou, S., Szpunar, J., & Lobinski, R. (2009). Metallomics: The concept and methodology. *Chemical Society Reviews, 38*(4), 1119-1138.

Nair, R., & Rost, B. (2005). Mimicking cellular sorting improves prediction of subcellular localization. *Journal of Molecular Biology, 348*(1), 85-100.

Nielsen, F. H. (2000). Evolutionary events culminating in specific minerals becoming essential for life. *European Journal of Nutrition, 39*(2), 62-66.

North, B. J., & Verdin, E. (2004). Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome Biology, 5*(5), 224.

Nose, S., & Klein, M. L. (1983). Constant pressure molecular dynamics for molecular systems. *Molecular Physics, 50*(5), 1055-1076.

Olufsen, M., Smalås, A. O., Moe, E., & Brandsdal, B. O. (2005). Increased flexibility as a strategy for cold adaptation a comparative molecular dynamics study of cold- and warm-active uracil DNA glycosylase. *Journal of Biological Chemistry, 280*(18), 18042-18048.

Orengo, C. A., Jones, D. T., & Thornton, J. M. (1994). Protein superfamilies and domain superfolds. *Nature, 372*(6507), 631-634.

Papaleo, E., Fantucci, P., & De Gioia, L. (2005). Effects of calcium binding on structure and autolysis regulation in trypsins. A molecular dynamics investigation. *Journal of Chemical Theory and Computation, 1*(6), 1286-1297.

Papaleo, E., Tiberti, M., Invernizzi, G., Pasi, M., & Ranzani, V. (2011). Molecular determinants of enzyme cold adaptation: comparative structural and computational studies of cold-and warm-adapted enzymes. *Current Protein and Peptide Science, 12*(7), 657-683.

Parrinello, M., & Rahman, A. (1981). Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied Physics, 52*(12), 7182-7190.

Passerini, A., Andreini, C., Menchetti, S., Rosato, A., & Frasconi, P. (2007). Predicting zinc binding at the proteome level. *BMC Bioinformatics, 8*(1), 39.

Passerini, A., Lippi, M., & Frasconi, P. (2011). MetalDetector v2.0: predicting the geometry of metal binding sites from protein sequence. *Nucleic Acids Research, 39*(suppl 2), W288-W292.

Passerini, A., Punta, M., Ceroni, A., Rost, B., & Frasconi, P. (2006). Identifying cysteines and histidines in transition-metal-binding sites using support vector machines and neural networks. *Proteins, 65*(2), 305-316.

Pearl, F. M. G., Bennett, C. F., Bray, J. E., Harrison, A. P., Martin, N., Shepherd, A., *et al.* (2003). The CATH database: an extended protein family resource for structural and functional genomics. *Nucleic Acids Research, 31*(1), 452-455.

Peitsch, M. C. (2002). About the use of protein models. *Bioinformatics, 18*(7), 934-938.

Peng, J., & Xu, J. (2010). Low-homology protein threading. *Bioinformatics, 26*(12), i294-i300.

Petukh, M., Zhenirovskyy, M., Li, C., Li, L., Wang, L., & Alexov, E. (2012). Predicting nonspecific ion binding using DelPhi. *Biophysical Journal, 102*(12), 2885-2893.

Punta, M., Coggill, P. C., Eberhardt, R. Y., Mistry, J., Tate, J., Boursnell, C., *et al.* (2012). The Pfam protein families database. *Nucleic Acids Research, 40*(D1), D290-D301.

Ramezani-Rad, M., Hollenberg, C. P., Lauber, J., Wedler, H., Griess, E., Wagner, C., *et al.* (2003). The *Hansenula polymorpha* (strain CBS4732) genome sequencing and analysis. *FEMS Yeast Research, 4*(2), 207-215.

Ramli, A. N. M., Azhar, M. A., Shamsir, M. S., Rabu, A., Murad, A. M. A., Mahadi, N. M., *et al.* (2013). Sequence and structural investigation of a novel psychrophilic α-amylase from *Glaciozyma antarctica* PI12 for cold-adaptation analysis. *Journal of Molecular Modeling, 19*(8), 3369-3383.

Ramli, A. N. M., Mahadi, N. M., Shamsir, M. S., Rabu, A., Joyce-Tan, K. H., Murad, A. M. A., *et al.* (2012). Structural prediction of a novel chitinase from the psychrophilic *Glaciozyma antarctica* PI12 and an analysis of its structural properties and function. *Journal of Computer-aided Molecular Design, 26*(8), 947-961.

Robinson, C. H. (2001). Cold adaptation in Arctic and Antarctic fungi. *New phytologist, 151*(2), 341-353.

Rothschild, L. J., & Mancinelli, R. L. (2001). Life in extreme environments. *Nature, 409*(6823), 1092-1101.

Rulisek, L., & Vondrasek, J. (1998). Coordination geometries of selected transition metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}) in metalloproteins. *Journal of Inorganic Biochemistry, 71*(3-4), 115-127.

Sacconnay, L., Smirlis, D., Queiroz, E. F., Wolfender, J. L., Soares, M. B. P., Carrupt, P.-A., *et al.* (2013). Structural insights of SIR2rp3 proteins as promising biotargets to fight against Chagas disease and leishmaniasis. *Molecular BioSystems, 9*(9), 2223-2230.

Sadraie, M., & Missirlis, F. (2011). Evidence for evolutionary constraints in Drosophila metal biology. *Biometals, 24*(4), 679-686.

Sagui, C., & Darden, T. A. (1999). Molecular dynamics simulations of biomolecules: Long-range electrostatic effects. *Annual Review of Biophysics and Biomolecular Structure, 28*(1), 155-179.

Sakkiah, S., Arooj, M., Cao, G. P., & Lee, K. W. (2013a). Insight the C-Site Pocket Conformational Changes Responsible for Sirtuin 2 Activity Using Molecular Dynamics Simulations. *PloS ONE, 8*(3), e59278.

Sakkiah, S., Arooj, M., Kumar, M. R., Eom, S. H., & Lee, K. W. (2013b). Identification of Inhibitor Binding Site in Human Sirtuin 2 Using Molecular Docking and Dynamics Simulations. *PloS ONE, 8*(1), e51429.

Sakkiah, S., Chandrasekaran, M., Lee, Y., Kim, S., & Lee, K. W. (2012). Molecular modeling study for conformational changes of sirtuin 2 due to substrate and inhibitor binding. *Journal of Biomolecular Structure and Dynamics, 30*(3), 235-254.

Šali, A., & Blundell, T. L. (1993). Comparative Protein Modelling by Satisfaction of Spatial Restraints. *Journal of Molecular Biology, 234*(3), 779-815.

Samsulrizal, N. H., Arifin, K. T., Murad, A. M. A., Noor, Y. M., Jema'on, N. A., Najimudin, N., *et al.* (2010). *Genome Sequencing and Annotation of the Alkaliphilic Bacterium Bacillus lehensis G1*. Paper presented at the Asia Pacific Bioinformatics Conference 2010.

Sanders, B. D., Jackson, B., & Marmorstein, R. (2010). Structural basis for sirtuin function: What we know and what we don't. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1804*(8), 1604-1616.

Satyanarayana, T., & Kunze, G. (2009). *Yeast biotechnology: diversity and applications* (Vol. 78): Springer.

Schwede, T., Sali, A., Eswar, N., & Peitsch, M. C. (2008). Protein Structure Modeling. In T. Schwede & M. C. Peitsch (Eds.), *Computational Structural Biology: Methods and Applications*. Singapore: World Scientific Publishing.

Schwer, B., & Verdin, E. (2008). Conserved metabolic regulatory functions of sirtuins. *Cell Metabolism, 7*(2), 104-112.

Shameer, K., Nagarajan, P., Gaurav, K., & Sowdhamini, R. (2009). 3PFDB-A database of Best Representative PSSM Profiles (BRPs) of Protein Families generated using a novel data mining approach. *BioData Mining, 2*, 8.

Shen, M. y., & Sali, A. (2006). Statistical potential for assessment and prediction of protein structures. *Protein Science, 15*(11), 2507-2524.

Shi, T., Wang, F., Stieren, E., & Tong, Q. (2005). SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *Journal of Biological Chemistry, 280*(14), 13560-13567.

Shi, W., & Chance, M. (2008). Metallomics and metalloproteomics. *Cellular and Molecular Life Sciences, 65*(19), 3040-3048.

Shi, Y., Zhou, Y., Wang, S., & Zhang, Y. (2013). Sirtuin Deacetylation Mechanism and Catalytic Role of the Dynamic Cofactor Binding Loop. *The Journal of Physical Chemistry Letters, 4*(3), 491-495.

Shivaji, S., Prasad, G. S., Satyanarayana, T., & Kunze, G. (2009). Antarctic yeasts: Biodiversity and potential applications *Yeast biotechnology: Diversity and applications* (pp. 3-18): Springer.

Shu, N., Zhou, T., & Hovmöller, S. (2008). Prediction of zinc-binding sites in proteins from sequence. *Bioinformatics, 24*(6), 775-782.

Sillitoe, I., Cuff, A. L., Dessailly, B. H., Dawson, N. L., Furnham, N., Lee, D., *et al.* (2013). New functional families (FunFams) in CATH to improve the mapping of conserved functional sites to 3D structures. *Nucleic Acids Research, 41*(D1), D490-D498.

Sinclair, D. A., & Guarente, L. (1997). Extrachromosomal rDNA circles--a cause of aging in yeast. *Cell, 91*(7), 1033-1042.

Sonnhammer, E. L. L., Eddy, S. R., & Durbin, R. (1997). Pfam: A comprehensive database of protein domain families based on seed alignments. *Proteins-Structure Function and Genetics, 28*(3), 405-420.

Spiwok, V., Lipovová, P., Skálová, T., Dušková, J., Dohnálek, J., Hašek, J., *et al.* (2007). Cold-active enzymes studied by comparative molecular dynamics simulation. *Journal of Molecular Modeling, 13*(4), 485-497.

Suzek, B. E., Huang, H., McGarvey, P., Mazumder, R., & Wu, C. H. (2007). UniRef: Comprehensive and non-redundant UniProt reference clusters. *Bioinformatics, 23*(10), 1282-1288.

Szpunar, J. (2004). Metallomics: a new frontier in analytical chemistry. *Analytical and Bioanalytical Chemistry, 378*(1), 54-56.

Tanny, J. C., Dowd, G. J., Huang, J., Hilz, H., & Moazed, D. (1999). An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. *Cell, 99*(7), 735-745.

Thiele, D. J., & Gitlin, J. D. (2008). Assembling the pieces. *Nature Chemical Biology, 4*(3), 145-147.

The UniProt Consortium. (2014). Activities at the Universal Protein Resource (UniProt). *Nucleic Acids Research*, 42(Database issue), D191–D198. doi:10.1093/nar/gkt1140

Tin, T., Fleming, Z. L., Hughes, K. A., Ainley, D. G., Convey, P., Moreno, C. A., *et al.* (2009). Impacts of local human activities on the Antarctic environment. *Antarctic Science, 21*(01), 3-33.

Trojer, P., & Reinberg, D. (2007). Facultative heterochromatin: Is there a distinctive molecular signature? *Molecular cell, 28*(1), 1-13.

Tsuchiyama, S., Kwan, E., Dang, W., Bedalov, A., & Kennedy, B. K. (2013). Sirtuins in Yeast: Phenotypes and Tools *Sirtuins* (pp. 11-37): Springer.

Turkiewicz, M., Pazgier, M., Kalinowska, H., & Bielecki, S. (2003). A cold-adapted extracellular serine proteinase of the yeast *Leucosporidium antarcticum*. *Extremophiles, 7*(6), 435-442.

Vaquero, A. (2009). The conserved role of sirtuins in chromatin regulation. *International Journal of Developmental Biology, 53*(2), 303.

Venkatachalam, K., van Rossum, D. B., Patterson, R. L., Ma, H.-T., & Gill, D. L. (2002). The cellular and molecular basis of store-operated calcium entry. *Nature Cell Biology, 4*(11), E263-E272.

Veretnik, S., Gu, J., & Wodak, S. (2009). Identifying structural domains in proteins. In Genny Gu and Philip Bourne *Structural Bioinformatics* Second edition. Wiley-Blackwell, 485-513.

Vernon, W. B. (1987). The role of magnesium in nucleic-acid and protein metabolism. *Magnesium, 7*(5-6), 234-248.

Wan, X.-F., & Xu, D. (2005). Computational methods for remote homolog identification. *Current Protein and Peptide Science, 6*(6), 527-546.

Watson, J. D., Laskowski, R. A., & Thornton, J. M. (2005). Predicting protein function from sequence and structural data. *Current Opinion in Structural Biology*, 15(3), 275-284.

Wheeler, D. L., Barrett, T., Benson, D. A., Bryant, S. H., Canese, K., Chetvernin, V., *et al.* (2007). Database resources of the national center for biotechnology information. *Nucleic acids research, 35*(suppl 1), D5-D12.

Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Research, 35*(suppl 2), W407-W410.

Williams, R. J. P. (1984). Zinc: what is its role in biology? *Endeavour, 8*(2), 65-70.

Williams, R. J. P. (1997). The natural selection of the chemical elements. *Cellular and Molecular Life Sciences, 53*(10), 816-829.

Williams, R. J. P. (2001). Chemical selection of elements by cells. *Coordination Chemistry Reviews, 216*, 583-595.

Williams, R. J. P. (2006). The Biodistribution of Metal Ions. *Concepts and models in bioinorganic chemistry*.

Williams, R. J. P. (2013). Uptake of elements from a chemical point of view. *Essentials of Medical Geology* (pp. 75-99): Springer.

Williams, R. J. P., & Da Silva, J. J. R. F. (2000). The distribution of elements in cells. *Coordination Chemistry Reviews, 200*, 247-348.

Williams, R. J. P., & Da Silva, J. J. R. F. (2003). Evolution was chemically constrained. *Journal of Theoretical Biology, 220*(3), 323-343.

Williams, R. J. P., & Rickaby, R. (2012). *Evolution's Destiny: co-evolving chemistry of the environment and life*: Royal Society of Chemistry.

Wolf, F., & Trapani, V. (2008). Cell (patho) physiology of magnesium. *Clinical Science, 114*, 27-35.

Xie, B.-B., Bian, F., Chen, X.-L., He, H.-L., Guo, J., Gao, X., *et al.* (2009). Cold Adaptation of Zinc Metalloproteases in the Thermolysin Family from Deep Sea and Arctic Sea Ice Bacteria Revealed by Catalytic and Structural Properties and

Molecular Dynamics: NEW INSIGHTS INTO RELATIONSHIP BETWEEN CONFORMATIONAL FLEXIBILITY AND HYDROGEN BONDING. *Journal of Biological Chemistry, 284*(14), 9257-9269.

Xu, J., & Zhang, Y. (2010). How significant is a protein structure similarity with TM-score= 0.5? *Bioinformatics, 26*(7), 889-895.

Yamashita, M. M., Wesson, L., Eisenman, G., & Eisenberg, D. (1990). Where metal ions bind in proteins. *Proceedings of the National Academy of Sciences, 87*(15), 5648-5652.

Yu, D., Danku, J. M., Baxter, I., Kim, S., Vatamaniuk, O. K., Vitek, O., *et al.* (2012). High-resolution genome-wide scan of genes, gene-networks and cellular systems impacting the yeast ionome. *BMC Genomics, 13*(1), 623.

Yuan, H., & Marmorstein, R. (2012). Structural basis for sirtuin activity and inhibition. *Journal of Biological Chemistry, 287*(51), 42428-42435.

Zerkle, A. L., House, C. H., & Brantley, S. L. (2005). Biogeochemical signatures through time as inferred from whole microbial genomes. *American Journal of Science, 305*(6-8), 467-502.

Zhang, J., Lin, S., & Zeng, R. (2007). Cloning, expression, and characterization of a cold-adapted lipase gene from an Antarctic deep-sea psychrotrophic bacterium, *Psychrobacter* sp. 7195. *Journal of Microbiology and Biotechnology, 17*(4), 604.

Zhang, Y., & Gladyshev, V. N. (2010). General Trends in Trace Element Utilization Revealed by Comparative Genomic Analyses of Co, Cu, Mo, Ni, and Se. *Journal of Biological Chemistry, 285*(5), 3393-3405.

Zhang, Y., & Gladyshev, V. N. (2011). Comparative genomics of trace element dependence in biology. *Journal of Biological Chemistry, 286*(27), 23623-23629.

Zhang, Y., & Skolnick, J. (2004). Scoring function for automated assessment of protein structure template quality. *Proteins: Structure, Function, and Bioinformatics, 57*(4), 702-710.

Zhang, Y., Thompson, R., & Caruso, J. (2011). Probing the viral metallome: searching for metalloproteins in bacteriophage λ–the hunt begins. *Metallomics, 3*(5), 472-481.